Ulf Ritgen

Analytical Chemistry I

Springer

Analytical Chemistry I

Ulf Ritgen

Analytical Chemistry I

Springer

Ulf Ritgen
FB 05 – Angewandte Naturwissenschaften
Hochschule Bonn-Rhein-Sieg
Rheinbach, Nordrhein-Westfalen, Germany

ISBN 978-3-662-66335-6 ISBN 978-3-662-66336-3 (eBook)
https://doi.org/10.1007/978-3-662-66336-3

This Springer imprint is published by the registered company Springer-Verlag GmbH, DE, part of Springer Nature.
The registered company address is: Heidelberger Platz 3, 14197 Berlin, Germany

Preface

Some students wonder why they should actually use a textbook, when everything relevant to the exam is covered in the lecture, while others struggle with attending a lecture *at all,* when they can do self-study with a suitable textbook. A good reason for the combination of these two media is that in the context of a lecture, which cannot possibly cover the entire material of a book with 500 pages (or even more ...), the explanation of important principles with the detailed treatment of *selected* focal points provides a structure for the overall material. Accordingly, the book *Analytical Chemistry I* is intended as a kind of "read-along lecture", whereby—as is usual in many lectures—a textbook *relevant to* the respective *module* is recommended, to which the lecture refers again and again. For the "Read-along Lecture Analytical Chemistry" the textbook in question is "the Harris", *Quantitative Chemical Analysis,* in which each of the topics addressed in this book is treated much more extensively and in more detail. You will therefore find plenty of additional information there; accordingly, it is recommended that you always have "the Harris" at hand when working with *Analytical Chemistry I*.

At the same time, analytical chemistry makes use of all the principles that you have learned in introductory courses on general and inorganic chemistry, which is why occasionally—for example, to clarify *connections even* more clearly—reference is also made to the "Binnewies" *(General and Inorganic Chemistry)*. There you can look up again what atoms do all day long, how they form bonds with each other and interact in other ways ... and what the consequences of these interactions are (keywords: molecules and ion lattices, inter- and intramolecular interactions). Also regarding the different forms of chemical reactions (acid/base-, redox-, complex-, etc.) you can get good information there. Why is all this mentioned here, when this book is about *analytical chemistry*?—Because *all* those principles of general chemistry are also indispensable in analytics; they are used here constructively and creatively, so to speak.

Since this book is intended as a "lecture to read" and a lecture is usually characterised by the proverbial "guiding thread", the individual parts of this book build on each other:

Part I deals first of all with how one actually obtains more precise information about a substance or a mixture of substances of any kind. *Two different forms of analysis have* to be considered, which differ in the question that is to be answered.

- *Qualitative* analysis is concerned with *what* is contained in a mixture of substances.

  Typical questions are: "Does this drinking water contain traces of toxins? And if so, what are they?"
- In *quantitative* analysis, one usually already knows which substances are (or can be) present, and now the task is to determine their quantity (or optionally: mass, volume fraction, etc.).

  Here a typical question would be: "How many µg/mL lead(II) ions does this drinking water contain? Is this still acceptable or is this amount already considered a problem?"

In both cases, it is indispensable to deal not only with the basic concepts of analytics but also with sampling and sample preparation, to know the (often even internationally) binding standards and norms and also to have the necessary tools to estimate or even quantify the reliability and accuracy of one's respective measurements or measured values. Part I therefore deals with the general *principles of analytics*.

Part II deals with volumetric analysis. In the so-called wet chemical analysis, chemical reactions play an important role:

- You do not know whether a solution to be examined contains silver(I) ions or not?—If upon adding a solution containing chloride ions a fine-crystalline white precipitate is formed, then this is very much in favour of the working hypothesis "yes, there are Ag^+ ions!" This is where the solubility product and thus the law of mass action have their say.
- A solution reacts acidically (pH < 7), but *how much* acid does it contain?—This can be determined with an acid/base titration, and there you will encounter all aspects of (Brønsted) acids and bases again, acid and base strength (keyword: pK_S and pK_B values), pH value calculations, and the like (and thus ultimately the law of mass action again).
- Analogously, redox processes can also be used in analytics. Accordingly, it is necessary that you can set up redox equations (stoichiometrically correct) (are the mass balance and charge balance correct?) and also know how to calculate electrochemical potentials (using the Nernst equation, among others). (In case of doubt, *the Binnewies* will help).
- The same applies to reactions from the field of complex chemistry. Here, it is not only necessary to keep the acid/base concept according to Lewis in mind (including the aspects of nucleophilicity and electrophilicity), but also to determine stability constants—in which the law of mass action again plays a major role. (It goes without saying that one should also be able to name the resulting complexes correctly in the sense of IUPAC).

So in this part, as promised above, you will re-encounter many already familiar concepts.

In chromatographic *methods,* which we deal with in *Part III,* the *intermolecular* interactions are of particular importance (basic question: "What dissolves in what? And why?"), especially the interactions between analytes and one or the other column material and/or the solvent (mixture) used. The actual structure of the analytes under consideration is usually less important than whether functional groups or other structural elements are present that have a significant effect on the nature and extent of any intermolecular interactions. This part of the book deals primarily with *principles*; with regard to the basic mathematical-physical formulae and the interrelationships of selected aspects of analytics—qualitative or quantitative—which would also go beyond the scope of a lecture, reference is then made to *the Harris* as required.

Part IV deals with the basics *of molecular spectroscopy*. Here, we are mainly interested in (covalent) binding relationships: Thus, it is the actual molecular (three-dimensional) structure of our analytes that matters—we are concerned with *intramolecular* interactions. By letting interact the individual molecules with electromagnetic radiation, depending on their energy content, electrons are excited to occupy energetically less favourable levels (in order to later return to the energetically more favourable ground state), or we cause atoms to vibrate, tremble, and wobble, so that bond distances and bond angles change. In doing so, of course, we also change the energy content of our analyte. In all cases, energy must be absorbed accordingly. However, we will see that with some of the analytical methods that are now to come, the analyte can even be made to emit electromagnetic radiation itself; in other words, we make it "glow". In explaining the corresponding phenomena, we resort to atomic and molecular orbitals. If so far you have not yet been able to relate this subject area (which is often perceived as being very abstract) to all the other materials in various chemistry courses, this should change abruptly with this part of the book: At the latest now, considerations of energy levels and the like make real, analytically useful sense.

Finally, *Part V* deals with *atomic spectroscopic methods* of analysis. In these methods, individual atoms are usually considered in isolation from one another—thus, intra-*atomic* processes are dealt with here. However, the associated techniques and methods are often based on principles with which you are already familiar and/or which you have even used yourself, for example, this can explain the characteristic flame coloration of alkali metals and their ores. And because atoms of the same element behave in the same way when excited in the same way, the methods presented in this booklet generally allow not only the clear *identification of* the type of atom present, but also its *quantification*. The corresponding quantification procedures are, of course, again based on principles you already know—from previous parts of this book or from other courses and/or materials. Also, what actually happens in each of the excitations presented to you here is thus merely a continuation of already known concepts.

Speaking of "continuation": There is also a continuation—a "sequel", if you wish—to this introduction to the fundamentals of *analytical chemistry*. Many of the concepts presented here will be taken up and expanded upon in the textbook *Analytical Chemistry II*. So please do not be surprised if this book refers to its "sequel" from time to time. (Of course, I cannot and do not want to force you to busy yourself with it, but it is worthwhile—and I would be pleased if you were also with me for "Volume II").

Ultimately, in *Analytical Chemistry I* you will gradually come across things that you "actually" already know—just in a slightly different context. In other words: You will not only get to know one or the other fundamentally new thing here, but in addition you will also be able to connect already familiar things in a new way—it is precisely this "new linking" that actually makes up *the process of studying.* And when it comes to recognizing already familiar concepts (also known as an "Aha! moment"), studying becomes even more fun, and *that's* exactly what I hope you will have!

Ulf Ritgen
Rheinbach, Germany

Contents

Basics

Requirements

Even though we will not look at too many chemical aspects in detail in this first part on the subject of "*Analytical Chemistry*", you should know the basics of *general chemistry* (from corresponding introductory books or courses), because from Part II onwards we will need them again and again; the same applies to the basics of *inorganic chemistry.* But already in Part I of particular importance are:

- the difference between pure substances and mixtures,
- the difference between solutions and suspensions.

What you should also be familiar with:

- the concept of polarity,
- hydrogen bonds, van der Waals forces and other inter- and intramolecular interactions
- the basic principles of all chemical reactions:
  - acid/base reactions,
  - redox reactions,
  - complex reactions.

Of course, you should also be familiar with the nomenclature of at least inorganic compounds as you have learned it in *General and Inorganic Chemistry.*

In short, everything you have learned so far you will encounter again sooner or later in analytical chemistry.

1.1 Learning Objectives

In "Analytical Chemistry I", you will gradually gain an overview of the most important and common analytical methods and techniques used in chemistry, but also in "related disciplines" such as biology, pharmacology, etc.

Classical *wet-chemical* methods, in which one may/must "still do it yourself", are addressed here as well as more modern methods, in which the analyst must resort to "heavy equipment" and leave the actual work to sophisticated (usually large and expensive) machines. But also in the field of *instrumental analysis* (which is dealt with in this book primarily in Parts IV and V, and which you will encounter again in "Analytical Chemistry II", if you are interested), the measuring instruments used are not intended to be an end in themselves or to be treated as "black boxes" in which mysterious and incomprehensible things go on that ultimately lead to a measured value—of whatever kind—, that then "only" has to be interpreted. On the one hand, you will be introduced to the technical structure of the individual instruments, and on the other hand, for each individual method of instrumental analysis, it should become clear to you which (physico-) chemical properties of the substance to be analysed form the basis for the mea-

sured values ultimately obtained and which physical and/or chemical processes are involved in the respective analytical procedure.

In this first part, you will mainly learn basic terms of trade and selected technical terms of analytics, which will enable you to communicate more or less without misunderstanding with other (prospective) experts. You will learn what needs to be considered before you can carry out analysis actively, and how the material to be examined must be prepared for the actual analysis. You will get an overview of generally accepted standards for describing the analyte content of a sample, and you will learn which considerations have to be made in order to get from an observation/measurement to an actual qualitative or quantitative (ideally even useful) statement.

Contents

1 Basic Concepts of Analytics

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_1

Before you can begin to perform experiments in analytical chemistry, you should make a basic consideration:

- **What Is the Question, Anyway?**

The aim of analytical chemistry is to find out more about a substance/mixture of substances/solution/whatever. It is important to consider *what* you actually want to find out—and *how* this is to be done. You can imagine that haphazard trial and error is probably not very promising.

1.1 Design of the Experiment

The firstquestion is whether you want to do **qualitative** or **quantitative** analysis:

- Do you want to *identify* a substance or prove the presence of a certain substance?—Then you are in the field of *qualitative* analysis.

  Such questions are asked, for example, in the search for valuable ores ("Does this rock contain rare earths such as yttrium or europium, which are needed for LEDs?") or (in suspected cases) for illicit, performance-enhancing substances in the blood (or other body fluids) of athletes.
- You already know *that* your sample contains one substance or another, and now you need to find out *how much?* This is a case for *quantitative* analysis.

  Qualitatively, for example, it has been proven that seawater contains gold. But is the amount dissolved in seawater large enough to make the extraction and purification of this gold worthwhile in purely economic terms?—Before you get your hopes up: According to current estimates/measurements, there is more than 15,000 tonnes of gold dissolved in all the world's oceans, but there is also plenty of seawater. At best, 0.03 mg is found per cubic meter—that's *not* worth the effort. The costs for gold extraction would be significantly higher than the profit that could be made with the gold. (But maybe the price of gold will rise so drastically some time, so that the whole effort will be worth it in the end.)

Another question is: "How should my sample to be examined fare *afterwards*?" In principle, one distinguishes between two approaches:

- If the material to be examined is available in sufficiently large quantities so that it does no harm if a small sample of it is *physically* and/or *chemically altered* in one way or another (i.e., to put it bluntly, is broken in the end), one can make use of appropriate **destructive examination methods**.

  This category includes the classical qualitative wet-chemical analysis of ion mixtures, which is still carried out by students in the laboratory today, even if the working methods there have little to do with the "real existing analytics" of current research, industry, or the like. (The qualitative **separation procedure** is and remains a wonderful way to get a good feel for "chemistry itself".) Even with the various methods of *volumetric analysis* (more on this in Part II), the substances to be analysed are all forced to undergo chemical reactions and, of course, undergo changes in the process.
- If, on the other hand, one is dealing with sample material that should (or must) be remain unchanged after completion of the analysis, **non-destructive examination methods** are preferable.

  If, e.g., you bought a ring, and you are not quite sure whether it really consists of sterling silver or only of a cheap nickel alloy, you will hardly be prepared to dissolve this ring in nitric acid. (This way you will sooner or

later get a clear result regarding the silver content, but the ring will be gone.) Thanks to various spectroscopic methods (more on this in Part IV), you can also answer the question "silver or nickel?" *without* losing the piece of jewellery. And if, for example, after months of painstaking laboratory work, you finally succeeded in isolating a certain protein on a microgram scale, you will hardly want to destroy it chemically first to test its purity, will you?

Many of the non-destructive methods fall into the field of *spectroscopy*. You will learn more about this from Part IV onwards, but the basic principle can be outlined right here and now: In spectroscopy, one allows the sample substance to interact with electromagnetic radiation of one or another wavelength range (or possibly also of a single precisely defined wavelength) and can then draw conclusions about certain properties of the sample material on the basis of the resulting measurement results. Even if you already know this from *general chemistry:* It can't hurt to remind yourself of the connection between wavelength and energy content ($E = h \times v$) (this will be indispensable in Part IV at the latest). When in doubt, Binnewies will help.

Binnewies, Section 2.3: The structure of the electron shell

1.2 And How Do I Get a Useful Answer?

Once you know what question you actually want to answer, the next hurdle to overcome in chemical analysis is the fact that atoms, molecules, and ions are so tiny that they cannot be observed directly with all the methods available to us so far, so all the information can only be gathered *indirectly:*

In *volumetric analysis*, for example, classical colour reactions are often used to find out something about the given conditions:

- In redox reactions, for example, an ion in solution can be oxidized (or reduced) in such a way that a **colour change** occurs.

  Thus, permanganate ions MnO_4^-, which have a strong oxidizing effect, are dark red to deep purple in aqueous solution, depending on the concentration, while manganese(II) ions (Mn^{2+}) present after the reduction of permanganate in an acidic medium are almost colourless.
- Ligand exchange can also cause a clearly recognizable colour change in complex reactions.

  Cu^{2+} ions in aqueous solution (present there as the tetraaqua complex $[Cu(H_2O)_4]^{2+}$) are pale blue in colour. When ammonia is added, the deep blue tetraammine copper(II) complex ($[Cu(NH_3)_4]^{2+}$) is formed. If the bidentate **chelate ligand** ethylenediamine ($H_2N–CH_2–CH_2–NH_2$, abbreviated as en) is then added, all ammine ligands are exchanged and the deep blue-violet coloured bisethylenediamine copper(II) chelate complex ($[Cu(en)_2]^{2+}$) is obtained. (Again, you know from *general chemistry* that chelate complexes are more stable than complexes with monodentate ligands.)
- In the case of acid/base reactions, very few participants do us the favour of showing a characteristic colour, but there is a remedy for this: **indicators** are used, which change colour in a pH range typical of the indicator used in each case.

Binnewies, Section 12.5: Chelate complexes

Read more about these different volumetric analysis methods in Part II.

1

For the Nitpicky

Yes, of course the description "Cu^{2+} ions (...) are coloured pale blue" is linguistically a bit ... non-scientific, after all, individual ions are so tiny that they have no colour of their own at all. When talking about "coloured" or "colourless" ions in the context of analytics, what is actually meant is: "Ions in solution that interact with just that part of the electromagnetic spectrum that is commonly referred to as" light "(with the wavelength range λ = 400–800 nm) in such a way that the human eye perceives a colour (or not)". But I'm sure you'll agree that this is just talking your head off or typing your fingers sore. I trust that you understand what is meant.

Spectroscopic methods also frequently make use of electromagnetic radiation, but often from wavelength ranges that humans are unable to perceive with the unaided eye (see, for example, Binnewies, Fig. 26.20 or Harris, Fig. 17.2): If no "biological-natural measuring instruments" such as the human eye can be used, then suitable detectors are used. (You will learn more about these in Parts IV and V; we will return to spectroscopic methods in Part I of "Analytical Chemistry II".)

Binnewies, Section 26.5: Optics
Harris, Section 17.1: Properties of light

An important aspect of any form of analysis is that the results obtained must be **reproducible.** This means that the test in question (whether qualitative or quantitative, destructive or not) should be carried out *several times* and ideally the same result should be obtained each time. (Of course, within the scope of **measurement accuracy,** certain deviations can always occur; in this case, *statistics* also play an important role—you will learn some basics on this subject in this part; more will follow in Part V of "Analytical Chemistry II".) In order to obtain truly *comparable results*, you must distribute the substance to be analysed over several identical portions (so that ideally there is always exactly the same sample quantity and/or exactly the same concentration). Such a sample portion is called an **aliquot**.

In order to avoid misunderstandings (or even confusion) in the laboratory when comparing different measurement results, things such as quality assurance (more on this in ► Sect. 3.5) and, above all, unambiguous ways of expression and notation (more on this in ► Sect. 3.1) play an important role.

Questions

1. You are asked to determine the lead content of a coin from the Bronze Age. Which type of analysis and which procedure do you decide on?
2. You are to aliquot 100.00 mL of a Cu^{2+} solution with concentration $[Cu^{2+}]$ = 0.10 mol/L into ten portions. Say something about each of these aliquots:
   (a) What is its volume?
   (b) What concentration does it have?
   (c) What amount of Cu^{2+} does each of these aliquots contain?
   (d) How many grams of copper are in each of these subsamples?

Sampling and Sample Preparation

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_2

Summary

First of all, *anything* can be considered a "sample"—whether it is the blood sample of an athlete, a few millilitres of an aqueous solution that may (or may not) contain toxic cyanide ions, or a piece of rock that is said to contain precious rare earths.

But in the beginning a completely different question arises: Is the sample to be examined **homogeneous** or **heterogeneous**?

- A sample is said to be *homogeneous* if it has a *uniform composition.* There are several possibilities:
  - the **solution** of a solid in a (liquid) solvent.
  - liquids that can be mixed together (such as water and ethanol; many people like to consume that recreationally).
  - a mixture of different gases.

An example of a homogeneous sample is the (aqueous) solution of a salt. If you take several samples, you will find the same concentration in each aliquot. (What is really meant here is *exactly* the same concentration, apart from purely statistical fluctuations. But I don't want to get too hung up on statistics right now: You will learn a few basics in this part, and the whole thing will be dealt with in more detail in Part V of "Analytical Chemistry II".)

- In *heterogeneous* samples, the composition *varies.* There are many different forms of heterogeneous mixtures (which are sometimes also referred to as **inhomogeneous**):
  - A mixture of several *liquids that* are not soluble in each other is called an **emulsion** (i.e.: liquid/liquid).

    An example from everyday life is olive oil in wine vinegar, as in the preparation of salad dressings.
  - One speaks of a **suspension** when insoluble *solids* are mixed with a solvent (i.e. a solid/liquid mixture is present).

    Most wall paints, for example, are suspensions in which the colour pigments are finely dispersed in the solvent concerned.
    - In the case of a suspension with the solvent *water* (which is *often* the case, but not always), one sometimes also speaks of a **slurry**. (This is not necessarily common in technical terms, but you should not be surprised if this term appears somewhere in the literature.)
  - Solids can also form a heterogeneous mixture.

    An example is the rock granite, which is effectively an (inhomogeneous) mixture of different minerals.

Important

If you send a *solution* that deserves this name through a filter, nothing remains in this filter. *Cave*:

Not everything passing completely through the filter is a solution, (—that works with some suspensions, too—) ***but*** **if something** ***remains in the filter, it was*** **not a** ***solution***.

And while we're on the subject: *Solutions are always clear.* They may be colourless or coloured, even so dark as to appear opaque, but they *must be* clear: solutions never show a **Tyndall effect**. (If this term means nothing (any more) to you: see Binnewies, Fig. 24.46.)

Binnewies, Section 24.9: The elements of group 11: copper, silver and gold

If a *heterogeneous* sample is present, the first thing to do is to try to **homogenise** it. In the case of solids, it helps to grind them as finely as possible or (on a laboratory scale) to rub them finely with a mortar and a pestle. (With some

samples, such as animal or plant tissue, this can be a somewhat squishy affair, in which case prior freezing is recommended.)

After that, it's time to separate the different substances present (if there weren't different substances, it wouldn't be a heterogeneous mixture, would it?). For this, you look for a suitable solvent (if you have no idea what to use, water is always a good start—but sometimes you need something else). And then you will find out whether the sample is completely soluble in the chosen solvent or not.

If the sample dissolves completely in the chosen solvent: Congratulations, you now have a homogeneous sample that should be decent to work with. This sample undoubtedly contains the substance to be analysed, commonly referred to as the **analyte**.

In addition, however, this solution probably also contains a lot of stuff that is *not* the analyte, but something else—this is generally referred to as **impurities**, even if the sample to be examined contains only a minimal amount of the analyte sought. (Sometimes the technical language of analytics has a certain unintentional comedy: When a real analyst talks about the initial example of gold dissolved in seawater, at the end one has the feeling that actually the whole sea consists of pure gold, which is only contaminated by larger amounts of water as well as some salts etc.). Of course, these impurities have to be removed, because they do not only lead to having to work with "unnecessarily large" sample quantities, but may even interfere with the actual analysis processes.

In addition, it is also quite possible that your mixture to be analysed does *not* dissolve completely. In this case, too, the analyte and the impurities must be separated from each other. We will come to that in a moment.

Questions

3. Characterise each of the following mixtures according to whether they represent heterogeneous or homogeneous samples:
   (a) Tap water
   (b) Blood
   (c) White wine vinegar
   (d) Vinegar/oil mixture
   (e) Gin
   (f) Milk
   (g) Gold ore
   (h) A mixture of 100 mL copper(II) choride solution (with $[CuCl_2] = 0.23$ mol/L) and 42 mL sodium sulfate (with $[Na_2SO_4] = 0.10$ mol/L)

2.1 Separation Process

Sooner or later, a substance separation is practically always required in analysis (unless you are in the—extremely rare—fortunate position of having your analyte available as a pure substance). If, for example, the sample material to be analysed dissolves only *incompletely in* the chosen solvent, you will have to answer further questions and carry out additional steps until you can turn to the actual analysis. The most important question first: Where *is* the substance you are actually interested in, i.e. the **analyte,** to be detected (in the case of qualitative analysis) or quantified (in the case of quantitative analysis)? Has it gone into solution or is it part of the undissolved solid?

Filtration

If you are lucky, the analyte turns out to be *well soluble* in the chosen solvent, and what remains undissolved contains no trace of your analyte at all (which, of course, must be checked experimentally, at least for quantitative analysis!). To continue working with your analyte, you only need to **filter** the mixture.

- The **filtrate** then contains the analyte. This filtrate represents a homogeneous mixture and the work could begin. *Could*, because the filtrate, in addition to the analyte, can of course contain lots of other stuff that has also proven to be highly soluble. If we're unlucky, these impurities (there they are again!) will interfere with further analytical steps. (You'll find out what to do then in later parts, at the respective analysis methods. Here we will first deal with the basics.)
- If the **residue** actually no longer contains any analyte, it can be **discarded.**

Hint for the Lab

In a purely qualitative analysis it is not necessary to "catch" the total amount of the analyte, because it is only a question of "Is the analyte present at all or not?" In this case, it may even be possible to dispense with filtration, since a small amount of solution is sufficient for the analysis. Accordingly, the solution can be decanted, i.e. the **supernatant** can be " poured off, taking care that none of the insoluble residue, which usually collects at the bottom of the vessel used (and is referred to as the **precipitate**), slides over the casting edge". This is sometimes quite practical in the laboratory (particularly under time pressure).

Centrifugation

While filtration only allows the separation of the liquid and solid phases of a suspension, the use of a centrifuge also allows the separation of the different components of an emulsion. The principles at work here are centrifugal force (the sample container is continuously moved in a circle at high speed) and mass inertia: the higher the density of the substance in question, the faster it moves "outwards". Ultimately, after slow braking of the centrifuge, the various substances are present in the sample tube sorted from top to bottom according to increasing density.

For example, ordinary cow's milk can be separated by centrifugation into the (lighter) cream on the one hand and low-fat milk (with higher density) on the other. This happens all the time in the food industry.

Of course, suspensions can also be separated this way if one wishes to refrain from filtering; after all, the mixture of substances to be separated may well be air-sensitive: Centrifugation rapidly transports the suspended solid to the bottom of the centrifuge tube, and one can safely remove the supernatant. And with appropriately designed centrifuges, even a gas mixture can be separated.

You might also use the principle of the centrifuge in everyday life—at least if you use a salad spinner.

Extraction Procedure

Sometimes it is just the other way round: only some (or with very, very good luck actually all) impurities go into solution. Then you can filter off again, but this time you will find your analyte (and possibly other, also non-soluble impurities) in the (filtration) residue. At least you have already found an effective method to get rid of at least some impurities (namely all the non-analytes that were soluble in the solvent in contrast to the aalyte).

Harris, Section 22.1: Solvent extraction

Now you just have to find another solvent in which your analyte will also dissolve. Occasionally, such a thing amounts to a real *trial-and-error game* ... definitely based on *systematic(!)* trial and error. (Depending on the type of analyte, one or the other "try-this-first-and-then-this-and-then-that-solvent" list might be found in the literature.) The term "solvent" is much broader than one might think: In addition to the usual suspects (water, ethanol, various other organic solvents such as diethyl ether, one hydrocarbon or another, or even hydrocarbon mixtures such as petroleum ether), changing the pH value can also be effective: Some substances dissolve only in an acidic or only in a basic medium.

► Example

Aluminium(III) hydroxide ($Al(OH)_3$) is practically insoluble in water, whereas it dissolves almost immediately in both acidic and basic media:

- Free Al^{3+} ions are formed in the acidic medium:

$$Al(OH)_3 + 3\,H^+ \rightarrow Al^{3+} + 3\,H_2O$$

- In the basic medium, the complex tetrahydroxidoaluminate(III) anion is formed:

$$Al(OH)_3 + OH^- \rightarrow \left[Al(OH)_4\right]^-$$

But of course you know the amphoterism of Al^{3+} from the *basics of inorganic chemistry*. ◄

Now and then you might have to work with a creative/constructive mixture - even rather unusual mixtures like "70 parts water by volume, 25 parts methanol, 5 parts 10% ammonia solution" can be the solution to the puzzle. The only way to come up with a result like this is generally through trial and error. *A lot of* trial and error. Sometimes until it's no fun anymore. I'm speaking from first-hand experience.

And of course there are also much more "exotic" solvents, such as supercritical carbon dioxide (CO_2). Those are of course very useful, especially suitable for special requirements ... and go far beyond the basics that will be addressed in this first part.

All the techniques mentioned so far have involved transferring the analyte from one phase to another (in the previous examples: from a solid phase to a liquid phase), i.e. *extracting* it. This process is called **solvent extraction**, often simply called **extraction.**

Important

The principle of extraction *is, of* course, an old (laboratory) wisdom that you (should) know from *general chemistry*, among other things: ***like dissolves like.***

- Polar substances (including *charged* particles, i.e. ions) dissolve much better in polar solvents (such as water or ethanol) than in non-polar solvents: Anyone who has ever tried to dissolve normal table salt in olive oil when preparing a salad dressing knows the meaning of frustration.

- Conversely, non-polar substances dissolve more easily in non-polar solvents such as diethyl ether, tetrahydrofuran, or petroleum ether, than in water.

This principle is also exploited in the regular use of the separating funnel in the laboratory: Here, two different, immiscible solvents are filled into this glass vessel; the solvents in question undoubtedly differ in polarity (otherwise they would hardly be immiscible!). If a mixture of substances is now added, the more polar substances will dissolve accordingly in the more polar solvent, while the less polar components of the mixture will end up in the less polar solvent.

► Example

The fact that substances can also be separated this way is not only used in the laboratory for analytical chemistry, but also in the sophisticated kitchen: If, for example, olive oil and water are poured into a separating funnel, a sprig of rosemary is added and you then shake the funnel (ideally with a cap on top ...), you will observed that the aqueous phase and the oil phase almost immediately separate again—the difference in polarity is simply too great. (Because of the lower density, the olive oil naturally collects at the top after the phase separation is complete.) If you then first drain the water and taste it (we are in the kitchen here, you are allowed to do that—in the chemistry lab, taste tests *never* take place!), and then repeat the taste experiment with the oil phase, you will find that the different dissolving properties have released completely different aroma substances from the rosemary twig—and of course you can then use them separately in the kitchen. Such chemical subtleties belong to the "secret working techniques" of many a star chef.

But you also use the principle "like dissolves like" in everyday life: when doing the laundry as well as when washing dishes. With the polar solvent water alone, only polar or easily polarizable "dirt" can be removed, e.g. residues of table salt or sugar when washing dishes; fats (butter, oil, etc.) and other non-polar substances, on the other hand, can hardly be removed with water alone. This is exactly why detergents and dishwashing liquids are used: amphiphilic compounds (detergents, soap) with a polar and a non-polar part of the molecule. With their non-polar molecule part they interact with non-polar substances, and the hydrophilic molecule part ensures that such detergents are also soluble in water (or similar solvents)—and thus also the *non*-polar substances which just interact with the detergent: They are practically "pulled into" the (polar!) solvent by the attachment to the detergent molecules. ◄

Cave

Please note: The statement "not soluble in solvent X" is about as absolute as the statement "this salt is poorly soluble". First of all, this only means that the K_L value of the substance in question is very small (and thus the pK_L value quite large). Corresponding tables (see for example Binnewies, Table 9.1) show that some substances are really only very poorly soluble in water. But "poorly soluble" does not mean "*in*soluble": if you mix a poorly soluble salt with water and then filter it, you will be able to detect at least small amounts of the ions of the salt in question in the filtrate.

Binnewies, Section 9.2: Quantitative description of the chemical equilibrium

The same applies to liquids: Even if, for example, water (H_2O) and diethyl ether (C_2H_5–O–C_2H_5) are considered to be "immiscible" (which is always used in the laboratory when using the separating funnel), a certain amount of water can be detected in the ether phase once the two phases have separated, and vice versa: At room temperature, just under 70 g (i.e. about 1 mol) of diethyl ether dissolve in 1 L of water, while about 20 g of water still dissolve in 1 L of diethyl ether. As you can see, "insoluble" and "immiscible" are relative.

Accordingly, this relativity must also be taken into account when answering the question in which of the two phases one or the other substance (and thus also our analyte) will dissolve: Even of a less polar substance that dissolves primarily in the less polar solvent, at least a measurable portion will also be present in the more polar phase. This phenomenon is described quantitatively by **Nernst's distribution law**. This law states that when a substance is **shaken out** with two immiscible solvents, a (dynamic) equilibrium is established in which the ratio of the concentrations the substance in question is present in in one solvent and in the other is constant:

$$K = \frac{c_{(\text{in Solvent 1})}}{c_{(\text{in Solvent 2})}} \tag{2.1}$$

The relevant K-value, which is referred to as the **distribution coefficient**, depends primarily on *which* solvents are used (for this one must generally consult tables), not on *how much of* the respective solvent was used. If the substance to be extracted is initially present in *one* solvent, but you want to transfer it to the *other* solvent, it follows from Eq. 2.1 that it is more efficient to extract several times with smaller portions of the desired solvent than once with a large portion, because said equilibrium is naturally re-established each time. (If you want to follow the whole thing mathematically: The corresponding calculation steps can be found in Chapter ► 11 of Binnewies and Chapter ► 22 of Harris.)

Binnewies, Section 8.11: Modern separation methods, chromatography
Harris, Section 22.1: What is mass spectrometry?

▪ Chromatographic Methods

In many respects, **chromatography** corresponds to extraction: here, too, it is primarily a matter of intermolecular interactions, but with one difference (due purely to the experimental set-up): Unlike extraction, in chromatography one of the phases used is immobile, while the second phase passes the first more or less slowly, i.e. flows past or through it. Accordingly, a distinction is made between the **stationary phase** (which does not move or is held in place) and the **mobile phase** (which moves). The decisive factor is then how strongly (or weakly) the analyte dissolved in the mobile phase interacts with the stationary phase.

Harris, Section 22.2: What is chromatography?

- As a mobile phase we can use:
  - a *liquid* (then we deal with **liquid chromatography**, usually abbreviated to **LC**) or
  - a *gas* (**gas chromatography** , **GC**)
- A *solid* is used as the stationary phase. This usually has a relatively large surface area so that the analyte dissolved in the mobile phase can also interact well with it.

A very nice schematic representation of the principle of chromatography is given in Fig. 8.34 in Binnewies.

Binnewies, Section 8.11: Modern separation methods, chromatography

▪▪ And How Does Something Like That Work?

Let us take a look at the basic design of a chromatograph: The solid, which serves as the stationary phase, is usually contained in a (relatively narrow) tube (of varying length) through which the stationary phase flows at (approximately) constant velocity. This tube is commonly referred to as a (chromatography or separation) **column.**

- In front of the front end of the column is the **sample inlet** through which the mixture to be analysed is introduced into the stationary phase.
- At the rear end of the column is a **detector** that "reports" when the analyte has passed through the entire tube and "exits" the column.

Binnewies, Section 8.11: Modern separation methods, chromatography

Figure 8.38 in Binnewies shows schematically how a gas chromatograph works; as already mentioned above, liquid chromatography is based on the same principle, except that a liquid serves as the mobile phase.

This interaction can be visualised in the way that the single molecules/ions of the analyte are adsorbed on the surface of the (stationary) column material for a certain time, and then, after a continuous flow of further solvent (= mobile phase), finally reach the end of the tube—and at this moment the detector reports how long it took for the analyte to migrate through the stationary phase.

A Remark on the Technical Language
Please note: This is about the phenomenon of **A_d_sorption of** the analyte *to the surface of* the column material. Do not confuse this with A_b_sorption, which is the phenomenon whereby a substance penetrates *the interior of* a solid.

Now the similarity to extraction should become apparent: Here, too, the principle "like dissolves like" is in effect, only slightly reinterpreted to "like *interacts* more strongly with like", because in this form of chromatography it is important that mobile and stationary phases differ in their **polarity:**

A non-polar solvent ***(mobile phase) is combined with a moderately to strongly polar stationary phase*** *(occasionally vice versa).*

This principle also allows the separation of different substances. This applies just as much to a mixture in which the desired analyte has to be separated from all conceivable impurities as it does to a mixture of diverse analytes, *all of* which are of interest to the analyst.

► Example

For the sake of simplicity, let us take as an example a mixture of two generic analytes that differ in polarity. Based on the distinctive interactions of the individual substances with the stationary phase, it should be understandable that with a polar column (= stationary phase) the more polar analyte will spend longer time "on the column" than its non-polar counterpart (and vice versa). In other words, with more polar analytes, it takes more solvent (mobile phase) to "flush it off the column" than with less polar substances; accordingly, the less polar analyte arrives at the detector earlier than the more polar one.

Let us return to washing dinner plates: Even *without* detergents you *can* clean a butter-smeared plate—you just need *considerably* more water. This is exactly the parallel to (column) chromatography: If a non-polar analyte (in this analogy: butter) has been adsorbed onto the non-polar stationary phase (here: the dinner plate), then significantly larger amounts of polar solvent (water) are required to detach the analyte—so it reaches the detector at the end of the column comparatively late. ◄

Of course, these few keywords only offer a first insight into the large, colourful world of chromatographic separation methods; you will find much more on this topic in Part III. There we will also come to the properties that are essential for a detector (or that an analyte must possess in order to be detected by one or the other type of detector) and the like.

■ Derivatisation

In some forms of analysis (the methods mentioned so far are only the tip of the proverbial iceberg!) it may be necessary to chemically modify the analyte in question, i.e. to **derivatise** it. Here are just two examples of frequently occurring cases:

- Many analytes cannot be detected in their "natural state" by the usual detectors. Often this unfortunate circumstance can be eliminated by synthetically equipping the analyte with an active group of one kind or another, which the detector then responds to.

  If, for example, an otherwise "inconspicuous" analyte is made part of a complex, this of course changes its entire way of interacting with the various electromagnetic wavelengths. You will encounter a typical example of this in Part IV of this series on the subject of *fluorescence detectors*.
- In other cases, the problem does not arise during detection after separation of any impurities, but because the analyte itself interacts too strongly with the material used for substance separation—but this can be prevented by careful chemical modification (i.e. derivatisation).

  If, for example, a free carboxylic acid (any molecule with the general molecular formula R–COOH, as you have either already learned or will certainly soon learn in *organic chemistry*) is to be chromatographically separated from all impurities, the strong positive polarization of the acidic H atom ensures that the analyte will interact strongly with a polar stationary phase (see ► Sect. 2.1) almost indefinitely long—getting it off the column again could be a serious problem. (Of course, you can rinse the whole column with an even more polar solvent until it's clean again, but I wonder if the column will like that. Actually, such chromatography columns are reused many times after (thorough) cleaning, otherwise this would become decidedly too expensive...).

  If, on the other hand, the free acid function is esterified, a much *less polar derivative* of the carboxylic acid is obtained. This ester will certainly still interact with the stationary phase to some extent (after all, thanks to the electronegativity differences between C, O and H, there are other serious polarizations in the molecule), but it will no longer adhere to it for all time.

Now you have already become acquainted with a whole range of ways and means of separating the desired analyte from as many (or even all) impurities as possible—only one impurity, which is usually present in considerable quantities, has not really been taken care of so far: the solvent. It is difficult to weigh out an analyte dissolved in a solvent in order to determine its mass, for example, and perhaps you have (instrumental) procedures in mind for the further investigation of your analyte in which you need the pure substance and nothing but the pure substance. In this case, of course, the solvent must be removed in advance.

2.2 Sample Drying

In principle, just about anything that is a liquid under the selected extraction or isolation conditions can serve as a solvent (as is well known), but of particular importance is undoubtedly the most common solvent (in the laboratory as well as in everyday life): water. For example, if the analyte of interest has first been brought into solution, then extracted as a precipitate from the solution using a suitable precipitation reagent, and largely freed from the solvent by filtration, there will of course still be considerable amounts of water attached to it. The standard method of drying consists of storing the precipitate at 110 °C in a drying oven until repeated reweighing always leads to the same result, i.e. **mass constancy** is achieved. (This is particularly important for gravimetry, which we will discuss in more detail in Part II.) However, some analytes, especially substances that are classified as "organic chemistry", do not tolerate such tem-

2

Harris, Section 2.8: Drying

peratures very well (or not at all). In this case, other ways and means must be found to remove the excess or unwanted water. It is helpful to use a desiccator in which the substance to be dried is kept near a water-absorbing agent. In addition to the desiccants listed in Table 2.6 of Harris, concentrated sulfuric acid should not remain unmentioned, which, due to its strongly **hygroscopic behavior**, is also very suitable for drying substances that are not excessively sensitive to acid.

Questions

4. A slurry of sand and saline ($NaCl_{(aq)}$ with [NaCl] = 1.42 mol/L) is present in a beaker. After filtration, what will the filtrate consist of, and what will the residue consist of?
5. You have a mixture of sand and household sugar, to which a small quantity of margarine was added. The analytes of interest are to be household sugar and margarine. The solvents available to you are water (H_2O), diethyl ether ($H_3C–CH_2–O–CH_2–CH_3$), ethanol (C_2H_5OH), and hexane ($H_3C–(CH_2)_4–CH_3$).
   (a) Which solvent do you choose to extract the sugar?
   (b) Which solvent do you choose for the extraction of the margarine?
   In both cases, the answer should be explained.
6. A mixture of margarine and sugar is to be separated using a polar chromatography column; diethyl ether ($C_2H_5–O–C_2H_5$) is used as the mobile phase. Even though chromatography is not actually covered until Part III: Which of the two substances is more likely to be purged from the column? Again, an answer without explanation will not be accepted. (You could just guess there! Where would that lead to?)

Quality Assurance and Calibration

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_3

Summary

If only for the sake of *reproducibility* of your results, you should always know (as precisely as possible) the sample quantity with which you are currently working. You should specify this as *mass* (i.e. in grams with all its SI-compliant prefixes), as *volume (*litres) or as *amount of substance* (mole).

Of course, you can theoretically use other measurements, but these three are clearly the most common.

In any case, it is important that you use the base quantities and units of the international system of units, the SI *(Système International d'unités)*. There are seven base physical quantities, you will already know from the basics of physics, from which all other units are derived: Mass (in kilograms), length (in meters), time (in seconds), temperature (in Kelvin), amount of substance (in moles), and electrical current and luminous intensity (but we won't deal with those any further here).

Important

Admittedly, the **Kelvin scale** has not quite caught on yet for temperature measurements in the "everyday laboratory"—most analysts still tend to think in terms of the *Celsius scale*, which is much more familiar from everyday life. Fortunately, the distance between two temperature steps in the Kelvin and Celsius scales is identical, so it is easy to convert:

$$\textbf{Temperature in}\left[\textbf{Kelvin}\right] = \textbf{Temperature in}\left[^{\circ}\textbf{C}\right] + \textbf{273.15} \tag{3.1}$$

Just be glad that the Kelvin scale has been agreed upon worldwide in the natural sciences. This saves you the trouble of converting °C to °F (Fahrenheit)—for these two scales the temperature steps are *not* identical. The conversion factor required for this (times 9/5 + 32) is actually only required if you want to follow a US weather report.

As soon as you deal with relatively large or relatively small quantities, (SI-compliant) prefixes for powers of ten, which you will also already know from the basics of physics, are helpful. These prefixes are commonly used for mass, length or substance quantities. A temperature of 1.2 kK (i.e. 1200 K or 1473.15 °C) is rarely used. And please note that the prefix T is really "Tera-", not "Terra-" (even though practically *everyone* pronounces it that way ...).

Of course, these prefixes also allow the "conversion" of units of the same size into each other. So you can either buy 1 kg of bread or 1000 g of bread (or even 10^{-3} tons of bread) and get exactly the same quantity. (Whereby we will see in ▶ Sect. 3.6 that from the point of view of analytics the statement "1 kg" is not exactly (!) the same as "1000 g"—more on this later.)

In ◘ Table 3.1 three additional powers of ten are listed, which are *not* SI-compliant but nevertheless so common that one simply *has* to know them:

You will certainly be familiar with the prefix "centi-" from the centimetre, and you will probably have come across the "deci-" mainly when measuring volume: In old cookbooks, you'll often find "two decilitres of milk" in the list

◘ **Table 3.1** Non-SI compliant prefixes and units

Potency	Symbol	Prefix	Unit
10^{-1}	d	Deci-	
10^{-2}	c	Centi-	
10^{-10}	Å		Ångström; independent unit (10^{-10} m)

of ingredients, and friends of high-proof alcoholic beverages will know that whisk(e)y, for example, is preferably served in 2-cL portions (even if the litre-L is usually written in lower case).

There is a third non-SI-compliant power of ten, which is extremely popular in certain circles: statements based on 10^{-10} are, however, only common for *length specifications*. The **Ångström** is a unit of its own (not SI-compliant and thus not "official"), namely 10^{-10} m; the associated unit symbol is the Å. This unit deviates from the SI rules ("for exponents, always use a multiple of 3"), and yet you will encounter this unit occasionally—especially if you are dealing with crystallographers or physicists, because the Ångström has a considerable advantage: 10^{-10} m is exactly in the order of magnitude of atomic radii and bond lengths. The typical C–C single bond with its length of 154 pm, for example, can be described accordingly as 1.54 Å. This is not in the SI sense, but it is still tacitly tolerated.

■ The Scientific Notation

As is well known, any (rational) number can also be written as a multiple of a power of ten. Especially with very large or very small numbers, this approach certainly leads to much more easily manageable results.

► Example

If, for example, one wants to describe the mole itself, it is certainly easier to recognise the order of magnitude of this number of particles if one writes 6.022×10^{23} than if one uses a numerical monstrosity such as 602 200 000 000 000 000 000 000 (which would, of course, be even more confusing without order-creating blanks).

In turn, the typical C–C bond distance mentioned above in the presence of a single bond is 154×10^{-12} m (i.e. 154 pm) or 1.54×10^{-10} m (i.e. 1.54 Å) or 0.154×10^{-9} m (i.e. 0.154 nm), etc. ◄

Of course, you are by no means forced to always choose your powers of ten so that they belong to the SI-compliant prefixes. But this is always recommended if you

- work with units *and*
- also want to use the corresponding prefixes.

Otherwise, you can describe numbers using any power of ten: 1.0 is the same as 0.10×10^{1} or 0.00010×10^{4}. (But watch out: 1000×10^{-3} *strictly speaking* means something different. See ► Sect. 3.6 for the difference.) Such numbers must then be linked to a (base) quantity and a unit, with mass being particularly popular in analytics.

The practical thing about mass is that (unlike volume, for example) it is *independent of temperature.* This is just as true for the mole (which, as we know, is just a "number of pieces"), but first, the corresponding numbers are sometimes a bit unwieldy (1 kg of water at 20 °C is pretty much equal to 55.555 ... mol), and second, most non-chemists run away screaming as soon as they hear the word "mole". For whatever reason.

It is therefore particularly helpful if the sample can be *weighed out*. (This is even the basic principle of a whole analytical technique: we will turn to ***gravimetry*** in Part II.)

Sometimes, however (actually: almost always), one does not deal with a (weighable) pure substance, but with an (ideally homogeneous) mixture of substances, the composition of which one should be able to describe as precisely as possible. This involves questions such as:

- How much of the relevant substance does the mixture contain? *or optionally:*
- How much analyte is mixed with how much impurity?

Such statements should of course be clear and unambiguous, so one has to agree with their colleagues (or even better: with all scientists worldwide) on a **standard of presentation**.

3.1 Everything Must Have Its Order: The Norm

However, before one can make such a statement, one must agree on generally valid standard conditions to describe the experimental conditions in question—after all, the volume of a gas depends on pressure and temperature (you have certainly already encountered Boyle-Mariotte's law in both *general chemistry* and *physics*), and the fact that the prevailing temperature also has an influence on the volume of liquids has just been mentioned. Thus, it is essential to at least keep an eye on the conditions (pressure, temperature) under which all experiments were carried out.

see for example Binnewies, Section 8.1: Ideal and real gases

■ Norm and Standard Conditions

Unfortunately, there is not one valid standard, but (at least) two that coexist on an equal footing.

For chemistry, **IUPAC** has established the following **standard conditions:**

- Temperature: 0 °C (= 273.15 K)
- Pressure: 1000 hPa (= 1 bar = 0.986 atm)

On the other hand, many experiments in chemistry are related to the temperature standard "room temperature", i.e. to 20 °C (= 293.15 K), and if you ask an engineer, chances are good that he will tell you that the room temperature is now clearly fixed at 25 °C (i.e. 298.15 K).

In addition, there is (how could it be otherwise in Germany?) an official DIN standard—which unfortunately does *not* correspond to the IUPAC recommendations. According to DIN 1343, the following **norm conditions** apply:

- Temperature: 0 °C (= 273.15 K)
- Pressure: 1013 hPa (= 1.013 bar = 1.0 atm)

The argument about which is better can begin.

For you, this means that you have to find out *each time* which of the standards had been used—and in your own documentation (more on this in ► Sect. 3.5) this must of course also be clearly stated.

Of course, there is also a standard (valid for all analysts in Germany) on how the *content information* required above is to be provided—and this time, fortunately, it is *not* undermined by a second opinion:

■ Content Statements According to DIN 1310

In principle, three different types of content statements can be distinguished:

- Concentration statements
- Proportion statements
- Ratios

Binnewies, Fig. 1.3, Section 1.1: Reaction equations and reaction schemes

For all these data there is an official **formula symbol,** a unique unit and a likewise unique **definition equation;** these can be taken from the respective tables. In the analytical laboratory, it is mainly the concentration statements that are important, as you will see in Part II.

■■ Concentration Statements

The question here is how much of the substance to be analysed (i.e. the analyte, generally abbreviated as *index i* in DIN 1310) can be found in a precisely defined quantity of the solvent used. In the case of concentration statements, the amount of solvent is always given in the form of its *volume* (even if this volume, as mentioned above, is temperature-dependent, which is why it is always necessary to keep the above-mentioned conditions in mind). The question now is: Do you state the analyte content as amount of substance, as mass, or as volume? Or even with the absolute number of particles?

	Formula symbol	Unit	Definition equation
Substance concentration	c_i	$\frac{mol}{m^3}$	$c_i = \frac{n_i}{V_{tot}}$
Mass concentration	β_i	$\frac{kg}{m^3}$	$\beta_i = \frac{m_i}{V_{tot}}$
Volume concentration	σ_i	$\frac{m^3}{m^3}$	$\sigma_i = \frac{V_i}{V_{tot}}$
Particle number concentration	C_i	$\frac{1}{m^3}$ (i.e. "pieces per volume")	$C_i = \frac{N_i}{V_{tot}}$

V_{tot} always refers to the *total volume of* the solution; V_i is the intrinsic volume of the analyte i in question.

You may have noticed that the volume here is given in m^3, i.e. in *cubic metres*. For the "normal laboratory routine" this is usually a little oversized, which is why one almost always works with the litre as the "base volume". But the base unit for length of the SI units is the *metre*, so the cubic metre actually is correct. After all, the litre is a cubic *deci*metre, and you know that the prefix "deci-" is *not* SI-compliant since ◘ Table 3.1. You can still work with it. But be careful when calculating and documenting!

■■ Proportion Fractions

Here one usually ends up with *percentages*, because it is ultimately a question of how large the proportion of the analyte is in the total mixture. Here again, the amount of substance, mass, volume, or the number of particles play the main role. (To avoid confusion, percentages are then written as vol.-% or mass-percent or similar. DIN does not provide for this kind of information, but you should at least have passive knowledge about this kind of **laboratory jargon**. Besides, one can see indications in percent by volume on gin bottles and similar nice things.)

	Formula symbol	Unit	Definition equation
Substance amount fraction	χ_i	$\frac{mol}{mol}$	$\chi_i = \frac{n_i}{n_{tot}}$
Mass fraction	ω_i	$\frac{kg}{kg}$	$\omega_i = \frac{m_i}{m_{tot}}$
Volume fraction	φ_i	$\frac{m^3}{m^3}$	$\varphi_i = \frac{V_i}{V_0}$
Particle number fraction	X_i	–	$X_i = \frac{N_i}{N_{tot}}$

Cave: V_0 represents a special feature here: What is meant here is the *theoretical total volume* that would result from purely mathematical calculations if the individual volumes of all the components involved were added together. From *general chemistry,* you are certainly already familiar with the phenomenon of volume contraction: Some components (molecules) can interact differently with each other in the mixture than they do in the pure substance itself—and increased interactions can lead to a decrease in the resulting total volume. For example, if you add 600 mL of water (H_2O) to 400 mL of ethanol (C_2H_5OH), you will get slightly less than 1000 mL of mixture.

■■ Ratios

Here, too, dimensionless numbers result. It is important to note that not only the amount of substance, mass, or volume of the analyte i is considered here, but also a concrete information about the residue k. Here k stands for another specific substance: This might be just used for a statement about the analyte being present in a solvent and one wants to know how much of the total mass of this mixture comes from the analyte and how much from the solvent, or it might be anyone of a large number of different components accompanying the analyte—and each of these different components then of course can (or even might have to) be considered individually. For the time being, however, we will restrict ourselves to two-component mixtures.

	Formula symbol	Unit	Definition equation
Substance amount ratio	$r_{(i,k)}$	$\frac{mol}{mol}$	$r_{(i,k)} = \frac{n_i}{n_k}$
Mass ratio	$\zeta_{(i,k)}$	$\frac{kg}{kg}$	$\zeta_{(i,k)} = \frac{m_i}{m_k}$
Volume ratio	$\psi_{(i,k)}$	$\frac{m^3}{m^3}$	$\psi_{(i,k)} = \frac{V_i}{V_k}$
Particle number ratio	$R_{(i,k)}$	–	$R_{(i,k)} = \frac{N_i}{N_k}$

■■ Not a Typo: The Molality

The problem that the volume of a substance (whether an analyte or a solvent) is temperature-dependent has already been addressed several times. This may not be of excessive importance in the laboratory, but it is when you have to work on a large scale: If, for example, you work in *technical chemistry* with several thousand liters of a solution, it is quite a considerable computational challenge to determine the substance concentration of the dissolved substance each time the temperature (and thus: the volume) changes. For this reason, the temperature independence of the mass (also mentioned above) is exploited, especially in large-scale technology:

1000 g of water do have a different volume at different temperatures, but the mass remains the same.

Of course, this also applies if you are not dealing with pure water (or any other solvent), but with a solution—and so we come to the **molality,** which is also precisely defined according to DIN 1310:

	Formula symbol	Unit	Definition equation
Molality	$b_{(i,\,k)}$	$\frac{\text{mol}}{\text{kg}}$	$b_{(i,k)} = \frac{n_i}{m_{\text{Solvent}}}$

If, therefore, when processing larger quantities of solutions at different temperatures, their *mass* is measured, the molality can also be used to make precise statements about the quantities of substance i used.

> The temperature-dependence of the volume may be regarded as the scourge of every motorist: If you buy the same volume of petrol in summer, i.e. at generally higher temperatures, as in winter, you will get slightly less fuel even if the price is exactly the same—because the price to be paid is determined by the *volume,* not by the *mass of* the petrol filled up. At higher temperatures, however, the same amount of petrol takes up a slightly larger space, so you effectively get less for your money.

Questions

7. Convert the mass fraction of a solution (according to DIN 1310) into a substance concentration *without using concrete numbers,* but only using the units/variables.

3.2 Quantity Ranges

If a mixture of substances, for example a solution, is to be analysed in one way or another, it must first be ensured that the chosen analytical method is appropriate for the quantity of analyte present in said mixture. It is important to find the **working range,** i.e. the concentration range that still permits precise and correct *quantitative* statements.

This **working range A** is commonly described as:

$$A = m_i \tag{3.2}$$

The question is therefore what minimum quantity of analyte i must be present in order to be *reproducibly* recovered by the analytical system used.

This work area is inseparably linked to the **sample range P:**

$$P = m_i + m_0 \tag{3.3}$$

The sample range describes the quantity range of component i to be determined and the total mass of all other components of the mixture (i.e.: impurities)—just as you already know from the definition equations from DIN 1310 (and ► Sect. 3.1).

From these two terms, the **content range G** can be constructed. It holds:

$$G = \frac{m_i \cdot 100}{m_i + m_o}\left(\text{specified in\%}\right) \tag{3.4}$$

Accordingly, these three areas (A, P, G) are in the following relationship:

$$P = \frac{A}{G} \cdot 100 \tag{3.5}$$

For example, if the working range and content range are known (i.e. the approximate order of magnitude of the concentration of analyte i in the mixture), it is easy to estimate the minimum sample quantity required to obtain a reasonably reliable result.

Technical Language Tip

In the case of analytes which are present only in minute quantities in the total mixture, it admittedly sounds a little silly to speak of "impurities" which account for more than 99% of the total mass: Here the term **matrix** is recommended. Of course, this still refers to everything in a sample mixture that is *not* the analyte in question.

Let us return to the gold in seawater from the example in ► Sect. 1.1: In view of the extremely low concentration, it would probably be a hopeless undertaking to simply try to convert the gold into a poorly soluble solid form by means of a precipitation reaction, which would then be weighed out in order to make more precise statements about the gold content. This method of analysis is decidedly too inaccurate for that—unless you were to condense really *enormous* amounts of water first until the gold concentration became sufficiently high—but then, of course, you would have changed the *sample range.* To what extent you introduced new errors into your analysis system by changing the sample (and you will have done so in any case changing its concentration) is another matter ...

Questions

8. The relevant analyte is contained in a sample at about 10% by mass. The method used for purely qualitative analysis is sensitive enough to detect analyte amounts of 0.5 mg. What is the minimum sample quantity that must be analysed?
9. What would be the minimum sample size required for the above task if the analyte content was only 1 mass percent?

The terms **limit of detection** and **limit of quantification** also refer to the working range, sample range and content range. Without wanting to go too far into the mathematical description of statistics here (a little more about this in ► Sect. 3.7, and much more—if you are interested—in the last part of "Analytical Chemistry II"), the *detection limit* is the amount of analyte that can still be found more or less reliably with the aid of the selected analytical method: At even lower analyte concentrations, the measurement signal disappears, so to speak, in the (statistical) background noise, and one can no longer be sure whether a measured value is really the result of the analyte being present, or whether a false alarm has occurred for purely statistical reasons. Although the quantification limit is related to the limit of detection, there is nevertheless a different idea behind it: While the limit of *detection* refers to *qualitative* statements ("Can I still be sure that my analyte is really present?"), the *limit of quantification is* concerned with whether the amount of analyte present is large enough to also permit a more or less reliable *quantitative* statement; after all, in view of the statistical background noise, such statements become increasingly imprecise as the sample size decreases.

It should be self-evident that significantly larger quantities are required for quantitative determination than for qualitative statements. This brings us to the field of **trace analysis**. Here the analyte content lies far below the percentage range, often even below the per mille range. Depending on the analytical method chosen, one or the other analyte can still be reliably detected (the content of the analyte in question is therefore well above the detection limit), but if the content is particularly low, any quantitative statements are already afflicted with an error that can no longer be neglected, because the analyte content can already be quite close to the limit of quantification. (If this is the case, one is dealing with real **ultratrace analysis**.)

3.3 Calibration

If, for example, the concentration of a solution is to be determined with a measuring instrument of any kind, this inevitably requires the **calibration** of the instrument in question: After all, it is necessary to first determine how the analytical method in question responds to the analyte in question, i.e. what the relationship is between the physical quantity being measured and what the measuring instrument used indicates.

For this purpose, **standard solutions** of different concentrations are prepared, i.e. solutions with a precisely defined analyte content which also contain all the reagents etc. required for the analytical method in question. (We will come back to the standards in ► Sect. 3.4.)

In addition, one must also find out to what extent these reagents and possibly other method-specific additives influence the measurement result, so one also measures a **blank sample** that resembles a standard solution in every respect, except that it reliably does *not contain any analyte*.

In order to find out how the measuring instrument in question responds to solutions of different concentrations or generally different amounts of analyte in relative terms, it is always useful to draw up a **calibration curve.** The different concentrations/analyte quantities are plotted on the x-axis, and the respective instrument- and method-specific measured values (i.e. generally the measured signal of the detector or similar) are plotted on the y-axis. Look at Fig. 4.12 in Harris (and ignore any statements about questionable values for the time being; we will come to such things later):

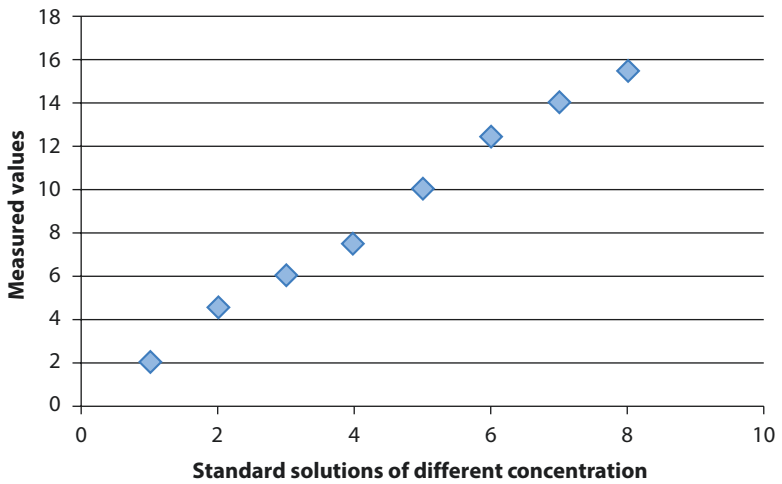
Harris, Fig. 4.12, Section 4.8: Calibration curves

Each measured amount of analyte (in this case a protein, but it could also be any other analyte) leads to a more or less characteristic measured value. If several standard solutions are measured—and this is indispensable for the creation of such a calibration curve—a *correlation* between the amount of analyte and the corresponding detector signal should be recognizable.

Let's first look at a few (in this case, completely abstract and arbitrary) values:

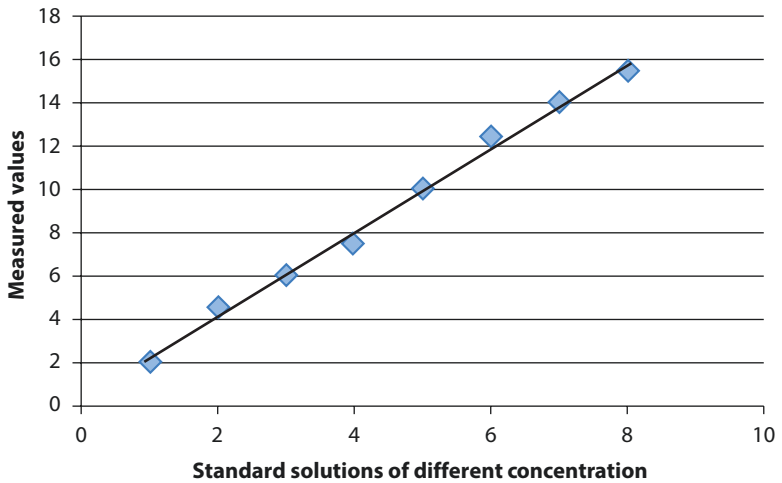
x value	1	2	3	4	5	6	7	8
y-value	2	4.5	6	7.5	10	12.4	14	15.5

Even without graphical application, one thing becomes apparent: higher concentrations (x-value) lead to higher measured values (y-value). A corresponding graph then shows that the curve is *approximately* linear:



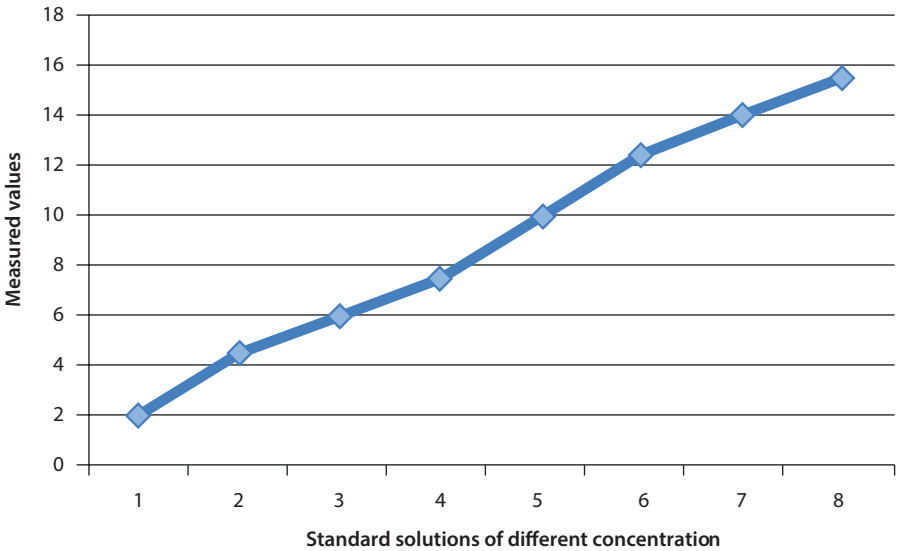
Graphical application of the measured data from the above table

However, it can also be seen that at least some fluctuations have occurred. Nevertheless, the linear trend is quite recognizable. Accordingly, it is recommended to draw a **trend line** (a compensation line):



New! Now with a compensation line

Please refrain from simply connecting the points so that a zigzag line is created. Such **fever curves** have no scientific value whatsoever—they are not only nonsensical, but simply useless in the context of analytics, after all, compensation lines are supposed to facilitate **interpolation**.



Nonsensical fever chart—don't ever do that!

If you look at the second figure again, you can now determine the concentration of an unknown sample (which of course must contain the same analyte and must have been prepared in the same way as the standard solutions) by **interpolation** within this linear range: If a measured value is obtained that lies *between* those of two standard solutions, you can use this calibration curve not only to roughly estimate that the concentration of the unknown sample must probably also lie between those of those two standard solutions, but even to make fairly accurate statements: Fig. 4.13 from Harris shows how the measured y-value can be interpolated to the corresponding x-value.

Harris, Fig. 4.13, Section 4.8: Calibration curves

It seems reasonable to assume that it would still be possible to draw conclusions about the analyte content of a solution even if its associated measured value lies beyond that of the highest-concentration standard solution. In principle, such an **extrapolation** is also possible—but it is not always useful: sometimes it leads to incorrect results. One problem is that the relationship between concentration (or analyte content) and measured value does not always remain linear for all concentrations. Above a certain concentration (which of course depends on the system under consideration), one leaves the *linear range* of the calibration curve and reaches its *dynamic range* (Harris, Fig. 4.14). We will encounter this "problem" again in Part IV, Molecular Spectroscopy I, for example, when we want to determine the concentration of a solution on the basis of the absorption of light according to Lambert-Beer's law. Here, uncontrolled extrapolation leads straight into a hell of a mess.

Harris, Fig. 4.14, para. 4.8: Calibration curves; ► http://xkcd.com/605/

When you create your first calibration curves yourself, you may notice that some of the solutions used for this are already too highly concentrated, so that a linear relationship can no longer be recognised over the entire curve. In this case, the calibrated measuring instrument is only suitable for samples whose concentration really lies in the *linear* range of the calibration curve—for samples with higher concentrations, you may have to come up with something new. And there is another potential problem: What if one or the other impurity contained in the solution to be analysed can interfere with the measurement

of the analyte itself?—Then one must address the issue of the **specificity** of the analytical method used (sometimes called **selectivity**). The more specific a method, the less sensitive it is to any interferences. In this introductory part, we will refrain from giving concrete examples; for the respective methods presented in the upcoming parts, we will also consider their versatility, sensitivity and selectivity, if necessary.

3.4 Not All Standards Are Created Equal

In the last section, you learned how standardised solutions are used to create a calibration curve, for example. But what defines this standard?—In principle, there are three different forms of the standard:

■ The Dilution Series

Harris, Section 1.3: Preparation of solutions

For a dilution series, a relatively highly concentrated solution of the analyte in question must first be prepared, whereby the concentration of this **stock solution** must of course be determined as precisely as possible.. Subsequently, precisely defined quantities of this stock solution are taken and diluted with the solvent to obtain a volume which must again be determined exactly.

> If you fill 58.44 g of the salt sodium chloride (NaCl) into a 1.000-litre volumetric flask and then fill it up to the mark with water, you will obtain a solution of the concentration c(NaCl) = 1.000 mol/L, because M(NaCl) = 58.44 g/mol. If you now take 10.00 mL of this solution with an appropriately accurate pipette and transfer it to a 100.00 mL volumetric flask and then fill it up to the calibration mark, you will have exactly 100.00 mL of a solution with the concentration c(NaCl) = 0.100 mol/L; if you take 20.00 mL of the stock solution for a second standard solution and fill it up to 100.00 mL, the resulting solution will have a concentration of c(NaCl) = 0.200 mol/L, etc.

If, starting from the stock solution, several solutions of different concentrations are prepared in this way, a dilution series is obtained which has been created by **parallel dilution.**

If you want to cover a much larger concentration range, you can also dilute **serially.** For this purpose, further solutions are prepared, which are not based on the *original* stock solution, but on solutions *already produced* from the stock solution.

> Let us return to the above example: If you now take 10.00 mL again from the first diluted solution (with c(NaCl) = 0.100 mol/L), transfer it to another 100.00 mL volumetric flask and then make up to 100.00 mL again, you will obtain a solution with the concentration c(NaCl) = 0.010 mol/L and so on.

Thus, several orders of magnitude of concentration can easily be covered by **serial dilution.** (The extent to which all the resulting solutions still lie in the linear range for the selected analytical method must be determined on the basis of the corresponding calibration curve.)

In addition to the possibility described above of first creating a dilution series of the analyte, measuring different concentrations and then interpolat-

ing the samples of unknown concentration in the linear range of the resulting calibration curve, there are also other possibilities for using standards.

The Specifically Added Standard

If you deal with a sample that contains the analyte but also interfering substances of whatever kind, the separation of which would be too time-consuming in the run-up to the analysis or is not practicable for other reasons, you can also add a precisely defined amount of the analyte to a sample of unknown analyte content. This makes much more sense than it might seem at first glance: It is quite possible that the other ingredients mentioned make the measuring instrument used (whatever it may be) respond differently to the analyte content than would be the case with a "pure" solution containing only the analyte of interest in exactly the same concentration. This is very nicely illustrated in Fig. 5.4 of Harris. If one now adds to aliquots of the sample in each case different, but exactly defined amounts of the analyte, this naturally leads, depending on the amount of analyte added, to stronger signals than when measuring the sample *without* the added standard. Correspondingly, the extent of the signal increase, which is due to the precisely known amount of the added standard, allows a direct conclusion to be drawn by extrapolation (to smaller values) as to how large the analyte content of the sample must have been in the beginning. Fig. 5.6 of Harris also provides a very informative illustration of this.

Harris, Section 5.3: Standard Supplement

Harris, Section 5.4: Internal standards

The Internal Standard

The use of an internal (or inner) standard offers a completely different benefit. This is particularly useful in two cases:

- If the composition of the samples to be compared varies—for example, because sample losses are to be expected in the course of sample preparation (i.e. before measurement).
- If the measuring instrument used does not always respond to the analyte in the same way due to technical or other variations—this happens particularly often in chromatographic substance separations.

In the former case, an internal standard added to the sample material before sample preparation would presumably be lost to a similar extent as the analyte itself; the ratio "internal standard to analyte" would thus remain (largely) constant, and the direct comparison of the resulting measured value of the lossy sample with the measured value obtained by measuring the "original quantity" of the internal standard then allows conclusions to be drawn as to what the *actual* analyte content of the sample must have been.

Now we come to the second quite common method of using the internal standard: In the case of chromatographic separation methods, one of the issues was how long the various substances/analytes remain on the column or how long it takes for them to be washed off the column again. (You remember the principle of chromatography from ► Sect. 2.1, don't you? We will go into this in more detail in Part III, but it would be nice if you could at least remember the principle.) If there are now different flow rates (for whatever technical reasons), the measurement results ("delay time until the desired analyte actually arrives, not some impurity") would be very difficult to compare with each other (especially if the desired analyte and one or the other impurity do not differ drastically in their residence time on the column). If, on the other hand, one adds an internal standard whose reaching the detector is unmistakable (e.g., because it is present at a significantly higher concentration than any analyte or impurity), one has a relative reference point, because even though

Table 3.2 A snake experiment

Animal no.	1	2	3	4	5	6	7	8	9	10
Temperature (in ° C)	2	4	6	8	10	12	14	16	18	20
Heart rate	5	11	11	14	22	23	32	29	32	33

the flow rate and thus the *absolute delay* times may vary from measurement to measurement, the *relative* delay times of the various substances (analyte, impurities, internal standard) are largely constant. So if you know, say, that the analyte takes twice as long to pass the column as the internal standard, and its signal is reported by the detector after 100 s, you know that the signal the detector then reports after a total of 200 s probably belongs to the analyte of interest. (As already mentioned: we will return to the principle of chromatography in Part III.)

Questions

A snake physiologist wants to determine the influence of temperature on the heart rate of pythons. For this purpose, ten animals of the same sex and approximately the same age and size are selected (after all, one needs comparable initial conditions!) and brought into appropriately tempered rooms. After the animals have adapted to the new environmental temperature, the following measured values are obtained (Table 3.2):

Display these measured values graphically. What conclusions can be drawn from them?

3.5 Analytical Quality Assurance (AQA) and Good Laboratory Practice (GLP)

Harris, Section 5.1: Fundamentals of Quality Assurance; Section 5.2: Method Validation

Entire textbooks are devoted to the topic of quality assurance—to go into it in the appropriately epic broadness would by far exceed the scope of this book. Chapter 5 of Harris, for example, provides a brief introduction. Therefore, we will confine ourselves here to the most important aspects of what is referred to as *Good Laboratory Practice* (GLP): the guidelines designed to ensure the reproducibility of any scientific results.

Two things are essential here:

1. In no single step of the process may chance play more than a statistically unavoidable role.
2. Any analytical result must be fully re-traceable by means of complete documentation.

Appropriate Documentation

This documentation serves the following purposes:

- First of all, the analytical method used should be described in a way that is comprehensible to an outsider. This includes the explanation of the respective type of detection as well as which solvents, reagents, and also measuring devices were used (up to manufacturer and series etc.).
- This is to avoid any form of misunderstanding, which occurs all too easily in informal oral communication.
- At the same time, GLP-compliant documentation also ensures that not only the analysts themselvel, but also their staff and others involved in the analysis in question are familiar with all the details of the entire analysis process.

- Last but not least, only appropriate documentation makes it possible to verify the results obtained. Only then is it also possible to trace back the relevant thought processes/reasoning of the experimenter(s) concerned and to identify any errors in thinking or measurement.

There is also another important aspect that speaks in favour of complete documentation: In the course of scientific work (the bachelor's or master's thesis or even the doctorate), there is usually really *a lot of* measurement data that accumulates over the course of weeks and months (or even years). Even if Thursday you might still know the difference between the series of measurements taken Monday and Tuesday: Do you really trust your memory so much that you believe you can still remember this difference two and a half years later? After you have performed hundreds of other, presumably often very similar measurements? It's very easy to overestimate yourself ...

3.6 Significant Figures or: When Does "Exact" Become "*Too* Exact"?

Exactly, Precisely, Correctly: What Does That Mean?

You'll find that analytics is all about accuracy and precision: "Is my result reliable? Is it accurate enough?" The precision required does not even stop at the language itself: many terms that are also commonly used in everyday language are much narrower in the context of analytics jargon: For example, the terms **precision, trueness,** and **accuracy** are used (practically) synonymously in everyday life, whereas in analytics, although they are certainly related, they describe rather different things:

Harris, Section 5.2: Method validation

Important

- *Correctness* says something about the extent to which the measured value approximates the "true" value (i.e., the value that would be obtained if one could know "the Truth™").
- *Precision*, on the other hand, describes the *reproducibility* of individual measured values or how closely the results of different repeat measurements agree. Precisely reproducible, but nevertheless nonsensical or otherwise unusable data understandably do not help at all.
- Finally, *accuracy* says something about *data quality*: How small is the error inherent in *each* measurement?

An illustrative example of these two terms is a rifle that—assuming accurate aim—always hits exactly 3 cm to the left of the intended target. The *precision of* this weapon is undoubtedly commendable, but *correct* is another kettle of fish alltogether.

Accuracy and precision also include dealing with an **outlier,** i.e. a measured value that differs significantly from all others. In a first approximation, one can (and should) ignore such a measured value, where obviously "something must have gone wrong". Sometimes, however, supposed outliers turn out to be much more significant (in the sense of: more meaningful) than one might have assumed in the first place, and furthermore, especially with a relatively wide range of values, it is not so easy to decide what is already an outlier and what is "only" a "normal" measured value that is relatively far away from the "true value". (You will learn ways and means of making this decision in Part V of "Analytical Chemistry II", for example.)

Of course, there may be *more* than one outlier in a series measurement, especially if there is a really large number of measurements. If they are all really outliers, one should not be afraid to classify more than one measured value as "clearly wrong".

One must not fall into the (extremely convenient) trap of simply branding *all* measurement results that do not really suit one's purposes as "outliers! That way, you get exactly the data you want, but the extent to which a study conducted this way is still relevant to reality is another matter entirely.

As important as precision, correctness, and accuracy are (especially in analytics): Accuracy recorded in documents may well be deceptive: This is where **significant figures** come into play (occasionally they are called *significant digits*). Suppose you had determined a mass of 1.8 mg for a sample of whatever kind (i.e. 1.8×10^{-3} g, 1.8×10^{-6} kg or even 1.8×10^{6} ng): Would you be allowed to record this figure as 1800 µg your laboratory notebook or database?

"Common sense" would certainly allow you to do this at first, after all, 1 mg corresponds by definition 1000 µg—but here said "common sense" falls into the trap of **significance**, which must be taken into account *with all experimentally determined* numerical values:

- There is *always* some measurement uncertainty.
- This measurement uncertainty is reflected in the *last* digit *of* the numerical value.

Harris, Section 3.1: Significant figures

Look at Fig. 3.1 in Harris and read the reading symbolised by the vertical line from the linear percentage scale shown there (top; looks like a ruler).

Even at first glance you can see that the reading is between 50 and 60, and if you look at the fine graduation it will be equally undoubtedly obvious that it is greater than 58 but less than 59. With a bit of eyeballing, you'll notice that the vertical line between the two smaller scale marks is slightly closer to the left than to the right, so you're probably estimating a reading of 58.4. Or maybe it's more like 58.3? In any case, it would undoubtedly be plain nonsense to try to take a reading of 58.392 from the "Harris ruler": All digits after the decimal point would simply be (educatedly) guessed. But estimating "58.4", with some uncertainty to *the last digit of the numerical value* would certainly be reasonable. But this also goes for "58.3".

Important

This brings us to the number of significant figures of a measured value:

1. All digits *including* the *one* uncertain digit are considered significant.
2. This is true even if there is a zero at the end of the number (in the "uncertain place").
3. Zeros *between* other significant digits are also significant.

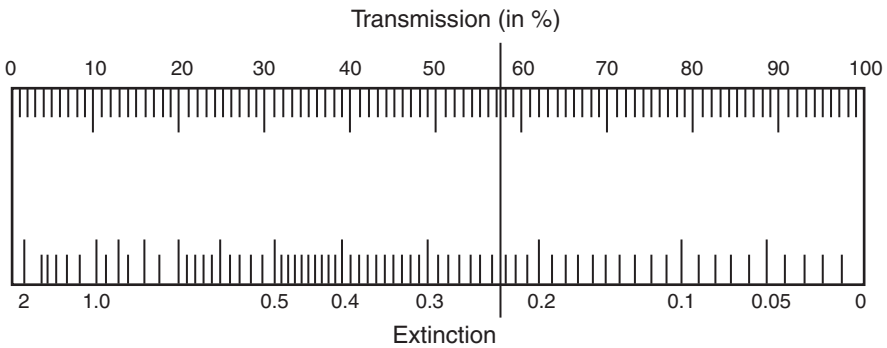


Fig. 3.1 How to determine measuring data

4. "Leading" zeros (i.e., zeros placed at the *beginning of* a number) are *not significant*.

Let us apply *item 1 of* this list to the example measurement value "1.8 mg" mentioned above: Here we have *two* significant figures, the last of which (the "8") has some measurement uncertainty, so it could also be, e.g., 1.78 or 1.83 mg. There are no other zeros here at the moment, so we will return to that later.

Especially *point 4 of* this list often leads to confusion, but the scientific notation from the beginning of ► Chap. 3 can help here. The now familiar 1.8 mg, for example, corresponds to 0.0018 g or 0.000 001 8 kg—the three or six *leading* zeros, however, carry no information whatsoever with regard to *accuracy*, because these digits have been created by simple "shifting along the potency scale" and can also be removed again the same way.

Now to the conversion 1.8 mg = 1.8×10^3 µg, thus 1800 µg (?). The first two digits are undoubtedly correct, the number of significant figures has not changed (the $\times 10^x$-expressions have no effect here). By the (purely mathematically correct) change from "scientific notation" to "normal numbers", however, you apparently increased the number of significant figures: According to point 2 of the above enumeration, the number 1800 has *four* significant figures, because a *terminal* zero is considered significant (see *point 2*), so of course the two "new" zeros are as well *(point 3),* and only the last of the two zeros would be subject to error: the range of variation at 1800 lies roughly between 179x and 180x µg, so it totals just under 20 µg. On the other hand, for the (actual) statement 1.8×10^3 µg, which just allows also the "true values" $1.7x \times 10^3$ µg and $1.8x \times 10^3$ µg, the error is (see above) in the range of *almost 200* µg. In short:

Please do not simulate accuracy that you cannot vouch for.

► Example

Imagine you are in a natural history museum and overhear one visitor whisper to another, "Look, that dinosaur skeleton over there is seventy million and two years old." No doubt you're dying to know how said person knows that for a fact, and when you ask, you're told, "I was here two years ago, and one of the museum staff told me that skeleton was seventy million years old."

The first of all following zeros probably (!) already holds an uncertainty: Whether the death of the primeval animal occured "only" 69 million or "already" 71 million years ago, may not make a big difference, but a fluctuation range of two million years is no small feat, either. With the statement "7.0×10^7 years" according to the rules of scientific notation, one certainly comes closer to the actual facts than with all the zeros falsely promising accuracy. However, should you really *know* the age of the dinosaur to the year, and it is indeed *exactly* 70 million, then this accuracy can also be accounted for with scientific notation. Then you write "$7.000\,000\,0 \times 10^7$ years", and suddenly *all the* given zeros are significant. But you should only do this if you really *know* it so precisely. ◄

■ Arithmetics with Significant Figures

When calculating with measured values (i.e. adding, subtracting, multiplying, dividing, etc.), you must of course take into account where the greatest *measurement uncertainty* lies. For example, if you are to add the numbers 1.983, 0.896 and 17, you have the problem that these values have different numbers of significant figures:

- 1.983 has *four*
  (the uncertainty is 3, so the "true value" could also be 1.9826 or 1.9834)
- 0.896 has only *three*
  (remember: leading zeros are not significant, here the number is 8.96×10^{-1}!)
- 17 has *two* significant figures (for this reading, the "true value" could also be 16, or even 18).

Any calculator will of course report 19.879 as the result of the addition—and this calculation result even has *five* significant figures.

If we were to add more numbers, sooner or later we would even get a six-digit result, but it can't be that the accuracy of a result depends on its absolute magnitude, can it?—We must not forget that *every single* measured value added here carries an uncertainty at its last digit—and these uncertainties propagate further and further in the calculations. What to do?

If different measurement results with different numbers of significant figures have to be combined, the accuracy of the final result is determined by the *accuracy of the most inaccurate value* (i.e. by the value with the highest measurement uncertainty).

In the above example, this is 17 with its two significant figures, so the final result must also have only two significant figures. It is therefore necessary to *round the* calculated final result sensibly.

▪▪ Rounding and Rounding Errors

The principle of rounding is actually quite simple: When you know how many digits the number to be rounded should have, you move from the left in the direction of the digit that must remain last, and look at which digit is next to it on the right:

- If the right neighbouring digit is *smaller* than 5, the last remaining digit remains unchanged.

  The rest of the number is simply truncated, this is called *rounding off.* (If there are two significant figures, 4.23 then becomes 4.2).
- If the right neighbouring digit is greater than 5, the last remaining digit is increased by 1.

  This is *rounding up.* (2.28 to be expressed with two significant figures becomes 2.3).

  If the last remaining digit is a 9, it then is rounded up to zero and the left neighbouring digit is also increased by 1: If, for example, the number 41.98 is to be given with three significant figures, the result is 42.0. *But don't forget this zero then:* It is significant here!

And if the right neighboring digit is now just a 5? Then the discussion begins, because here there are different opinions:

In analytics, the general rule is:

If the right neighbouring digit is a 5, rounding takes place in such a way that an even *last significant digit results.*

With two significant figures, 41.5 is *rounded up* to 42 accordingly, while 42.5 is *rounded down* to 42. (But this *only* applies if the digit to the right of the last significant digit is a 5! You are *not* supposed to round around mercilessly until there are only even numbers left!)

Two Warnings for the Price of One

The point about "rounding to an even number at 5" is not seen that way by all analysts! If in doubt, avoid encouraging your examiner to give marks carelessly by strictly insisting on this rule of five.

And if you have to perform several arithmetic operations in succession with your measured values, you may *round only after the very last calculation.* For all preceding calculations, it is advisable to include at least one, or even two or three, additional (and thus actually non-significant) digits—simply so that the rounding *errors* that inevitably occur during rounding do not continue to propagate.

What you have just learned about addition (and accordingly also subtraction) is in principle also valid for multiplications and divisions.

► Example

If, for example, you want to determine the volume of a rectangular hollow body whose edge lengths are 23, 42 and 666 mm, the result is mathematical:

$$23\ \text{mm} \cdot 42\ \text{mm} \cdot 666\ \text{mm} = 643\,356\ \text{mm}^3$$

This calculation result has six significant digits. But here numbers with only *two* (23, 42) resp. *three* (666) significant figures were used for calculation, and once again no increased accuracy can be achieved by calculation alone. Here, too, the number with the smallest number of significant figures determines the achievable accuracy, and accordingly it is necessary to round to *two* places:

The last significant digit is the 4, the right neighbour (a 3) leads to rounding off—so we end up with 640 000 mm^3 for the time being, but this number still has *six* significant figures. (What was that again with the following zeros?).

Within the limits of measurement accuracy, we can therefore only reasonably state the result as follows: $6.4 \times 10^5\ \text{mm}^3$. ◄

Now the addition example with the numbers 1.983, 0.896 and 17 is a little unfair or at least unrealistic in that these numbers are in *different orders of magnitude*, so in scientific notation they are sensibly described with different powers of ten:

- 1.983 is really simple 1.983×10^0
- 0.896 should usefully be written as 8.96×10^{-1}
- 17 is 1.7×10^1

With different orders of magnitude, it *can* get a bit confusing if you look strictly at the significant figures.

Tip

If you work with different measured values in a series of measurements or similar in analytics, these will generally be of the *same order of magnitude*. If this is the case, it is recommended to deviate from the strict consideration of all significant digits and to consider *only the decimal places* instead.

Suppose you were to weigh out the same piece of iron five times in succession. The scale reports on its display with three decimal places:

- 9.982 g (four significant figures)
- 9.991 g (four significant figures)
- 9.979 g (four significant figures)
- 9993 g (four significant figures) and
- 10.001 g (five significant figures)

10.001 is, of course, 1.0001×10^1, but why bother to account for the occurrence of different magnitudes here? After all, the number of decimal places (reliable according to the balance) remains the same. Now determine the arithmetic mean (simply add up all the measurement results and then divide by the number of measurements—there are certainly other methods of determining such values; you will learn about the most important ones, for example, in Part V of "Analytical Chemistry II") and obtain as a mathematical result

(9982 + 9991 + 9979 + 9993 + 10,001)/5 = 99,892

But that's *five* significant figures again—or optionally: *one decimal place too many.* So it's a matter of rounding until the number of decimal places is correct again.

The last usable digit is the final 9, the digit after that (the 2) leads to rounding down, so the final result is 9.989.

It can be that simple if you stay in the same ballpark.

How Reliable Are Integers?

That always depends on what kind of integers we are talking about. Actually, whole numbers do *not* have any decimal places, but if they are ***exact numbers***, then they do not have *any measurement uncertainty at all*:

► Example

If there are 23 people in a room, then they are not *approximately* 23, who in reality could just as well be 22.93 or 23.08 people, but *exactly* 23.

And even *defined* numbers (for example, the conversion factor from kg to g ($\times$-10^3, i.e. "times 1000") are *not* subject to measurement uncertainty. ◄

Accordingly, exact numbers are to be treated as if they had an *infinite* number of decimal zeros (i.e. 23.000 00... persons or a conversion factor of 1000.000 000...).

So, if integers appear, you have to check *before* calculating whether they are

- an *experimentally determined* number (with a predetermined number of significant figures) or
- an *exact* number (with, in principle, an infinite number of significant figures).

Accordingly, exact numbers do not play a role in the question of how many significant figures the final result has.

- If you multiply the number 23.426 669 (eight significant figures) by the *exact* number 3 (infinite number of decimal zeros), you get a result that must also have eight significant figs. (70.280007).
- But if you are to calculate the area of a paper strip whose edge length has been experimentally determined as 23.426 669 mm and 3 mm, the statement "about 70 mm^2" would be too precise, since there are already *two* significant digits here, and the experimentally determined "3 mm" permits the specification of only *one* significant digit. So you have no choice but to state the result as 7×10^1 mm^2. (Perhaps you should then have a serious word with the experimenter who was so careless in measuring the shorter edge of said paper strip.)

FAQ Significant Figures: Are Decimal Places and Significant Figures the Same Thing?

One encounters this false (!) assumption over and over again. For some reason, the decimal point seems to be something "especially meaningful™" for most students. But you do know from scientific notation that the position of a decimal point is freely movable: 6.0 is also 0.60×10^1 or 60×10^{-1}. And yet this masically false assumption has a *core of truth*, because with the help of scientific notation the number of significant figures can be made clear at a glance, even with (very) large numbers.

If you don't use the scientific notation (with powers of ten and a decimal point), but the everyday notation, it can be a bit tricky to make statements about the accuracy.

Think back to the example with the dinosaur skeleton: The number 70 million (years) mentioned *could* well have eight significant figures—even if this is rather unlikely. At least theoretically, it is conceivable that the uncertainty in the number 70 000 000 lies in the last digit, i.e. the good dinosaur may have passed away exactly 69 999 995 or about 70 000 004 years ago. If one introduces the decimal point here (which is possible at any time, see above), in order to clarify this accuracy, one would have to write "7,000 000 0 $\times 10^7$ years". (We already had that.)

If, on the other hand, one wants to express unambiguously that the uncertainty in this number begins much earlier, one must reduce the number of significant figures accordingly—in the above notation, the number of decimal places. If the uncertainty were already at the second digit (so the true age could also be 69 or 71 million years), one should write "7.0×10^7 years". So everything depends on the decimal places?—This questions warrants a very definite "well, maybe ...":

One could just as well give this value as "70×10^6 years" or as "0.70×10^8 years". So it is *not the number of decimal places that* is decisive, but simply the *total number of significant figures.* These can also all be placed *before* the decimal point, as is the case with "70×10^6 years".

Abstracts:

- If decimal places are present, they are all significant (in scientific notation)—but this also applies to all digits *before* the decimal point.
- If you work with experimentally determined values that are *in the same order of magnitude*, then you can also simplyconcentrate on the number of decimal places. Sometimes that's easier.

Questions

11. Say something about each of the following series of measurements in terms of accuracy and precision:
    (a) "true" value: 3.0; measured: 3.1, 3.2, 3.2, 3.3, 4.8
    (b) "true" value: 23; measured: 19, 21, 21, 22, 22, 23, 24, 24, 25, 25, 28
    (c) "true" value: 42; measured: 1, 17, 23, 42, 93, 110, 112, 666
12. How many significant figures do the following numbers have?
    (a) 23,42
    (b) 23,420
    (c) 0,42
    (d) 0.420
    (e) 0.004 20
    (f) 66.800 000 03
13. Round:
    (a) 23.666 to three significant figs.
    (b) 23.666 to four significant figs.
    (c) 187 582 to two significant figs.
    (d) 0.000 000 372 to two significant figs.
14. Calculate the volume of a rectangular hollow body with dimensions height = 10 mm, width = 0.23 cm, depth = 23.0 mm and state the result with the correct number of significant figs.
15. Naturally occurring chlorine on Earth consists of 75.78% isotope ^{35}Cl with molar mass M(^{35}Cl) = 34.968 85 g/mol and 24.22% isotope ^{37}Cl with M(^{37}Cl) = 36.965 90 g/mol. Calculate the molar mass of elemental chlorine.

3.7 A Brief Foray into Statistics

When dealing with significant figures (► Sect. 3.6), it has been pointed out again and again that measurement uncertainties arise, and if you look again at the ruler of Fig. 3.1 in Harris, it should immediately become clear how such errors occur: Different people estimate distances differently. So *chance* plays a role here.

Harris, Section 3.1: Significant figures

Harris, section 4.1: Gaussian distribution

■ Random Error

How much the estimates differ, and whether this is significant, becomes quite clear if one approaches the whole thing *statistically* and has the readings taken by as large a number of different people as possible. It is then worthwhile to compare the "readings" obtained in each case by first determining the **mean value ($\bar{x}$).** This is done according to a simple formula:

$$\text{Mean value } \bar{x} = \frac{\sum_{i=1}^{n} x}{n} \tag{3.6}$$

This says nothing more than simply adding up all the measurements x_i and dividing the result by the number of measurements n. (This is the *arithmetic mean*, which you most likely already know). This way, if there is a sufficiently large number of measurements (i.e. in this case: a sufficiently large number of "test persons"), it *statistically* compensates the fact that there is certainly one or the other person who is really bad at estimating and therefore considers a "much too low" value to be correct, because if really enough people are questioned, it is to be expected that there will also someone be who estimates badly "exactly in the other direction". Thus, these extreme values are simply "averaged out".

Now, of course, it is interesting to see how far the various measured values are from this (purely mathematically determined) mean $\bar{x}$ value. This is revealed by the **standard deviation (*s*)**. This is calculated according to:

$$\text{Standard deviation } s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n-1}} \tag{3.7}$$

The smaller the standard deviation *s* obtained in this way, the less the individual measured values deviate from the mean (and vice versa).

Here, one can easily walk into a trap, because with the standard deviation, large absolute measured values result in correspondingly larger values for *s* than with smaller absolute measured values. From this alone, one should not necessarily draw hasty conclusions regarding the accuracy or precision of a measurement series!

▶ Example

Let's compare two series of measurements with five measurements each (this is not exactly statistically robust, but the principle should become clear):

1. Measurement series: 593, 623, 642, 666, 668
2. Measurement series: 5.93, 6.23, 6.42, 6.66, 6.68

For the first series of measurements the following results

$$\bar{x}_1 = \frac{593 + 623 + 642 + 666 + 668}{5} = 638.4$$

and thus

$$s_1 = \sqrt{\frac{(593-638.4)^2 + (623-638.4)^2 + (642-638.4)^2 + (666-638.4)^2 + (668-638.4)^2}{4}}$$

$$= \sqrt{\frac{(-45.4)^2 + (-15.4)^2 + (3.6)^2 + (27.6)^2 + (29.6)^2}{4}}$$

$$= \sqrt{\frac{2061.16 + 237.16 + 12.96 + 761.76 + 876.16}{4}} = \sqrt{\frac{3949.2}{4}} = \sqrt{987.3} = 31.42$$

for the second, on the other hand:

$$\bar{x}_2 = \frac{5.93 + 6.23 + 6.42 + 6.66 + 6.68}{5} = 6.384$$

and thus

$$s_2 = \sqrt{\frac{(5.93-6.384)^2 + (6.23-6.384)^2 + (6.42-6.384)^2 + (6.66-6.384)^2 + (6.68-6.384)^2}{4}}$$

$$= \sqrt{\frac{(-0.454)^2 + (-0.154)^2 + (0.036)^2 + (0.276)^2 + (0.296)^2}{4}}$$

$$= \sqrt{\frac{0.2061 + 0.0237 + 0.0013 + 0.0762 + 0.0876}{4}} = \sqrt{\frac{0.3949}{4}} = \sqrt{0.09873} = 0.3142$$

As you can see, s_2 and two powers of ten are smaller than s1—but only because the measured values themselves differ by two powers of ten. ◄

It seems as if the deviation in the second series of measurements is much smaller than in the first, and thus the second series of measurements should be "significantly more correct", more precise and more accurate. In terms of absolute numbers, this is certainly true: If you measure a length of 1 m by 1 mm, this is clearly more in *absolute* terms than if you measure a length of 1 mm by 1 µm. But in *relative terms,* it is exactly the same. In this respect, you are well advised to also determine the **coefficient of variation** (also referred to as **relative standard deviation**): For this, you only need to convert the standard deviation in relation to the mean value into a percentage, and the measured values become much easier to compare:

$$\text{Relative standard deviation} = \frac{s}{\bar{x}} \cdot 100 \tag{3.8}$$

► Example

For our examples of just now, it follows:

- for the first series of measurements: $\frac{31.42}{638.4} \cdot 100 = 0.0492$
- for the second series of measurements: $\frac{0.3142}{6.384} \cdot 100 = 0.0492$

Well, that probably doesn't really come as a surprise. But now you have it *mathematically confirmed*. ◄

But not all errors can be attributed to chance or statistical fluctuations: There are also *systematic* errors, which can be attributed in one way or another to the (measuring) equipment used.

■ Systematic Errors

You have already encountered such a systematic error at the very beginning of this section: in the case of the rifle that reproducibly missed the intended target by 3 cm each time, and in the same direction each time. It is not so easy to say exactly where the systematic error lies: perhaps the sight is misaligned, or the barrel has a flaw, etc.

Harris, Section 3.3: Error types

Comparable situations can also be found in analytics, whether it is a detector that reproducibly reports only 90% of the "actual" measured value (a systematic *relative* error—it could, for example, be due to the fact that the detector optics are dirty), or a pH meter that reproducibly shows a value that is 0.1 pH units too low for each measurement (a systematic *absolute* error).

What is decisive here, however, is that if one *knows* about the systematic error, effective countermeasures can be taken relatively easily: The error can be

taken into account when evaluating the measurement results. (Trying to *correct* it, on the other hand, can turn into a lengthy undertaking. Sometimes it is easier to leave it as it is, because you know how to deal with it.)

3.8 Summary

3.8.1 Basic Terms

In *qualitative* analysis, the presence of an analyte is checked.

In *quantitative* analysis, the quantity of the analyte is determined.

In both cases, a distinction is made between *non-destructive* and *non-destructive* methods.

3.8.2 Sampling and Separation Methods

Is the sample *homogeneous* or *heterogeneous*? In the latter case, the sample must be *homogenised.*

When filtering a heterogeneous mixture, the *filtrate* passes through the filter into a new vessel, and the *residue* remains in the filter.

If an analyte has to be *extracted*, the solvent chosen for this purpose is adapted to the solvent properties of the analyte concerned:

- For *non-polar* analytes, select a *non-polar* extraction solvent,
- for *polar* analytes correspondingly a *polar* one.

The old adage is that ***like dissolves like.***

In chromatographic separation processes, a distinction is made between the *stationary* phase (the column material; immobile and usually solid) and the *mobile* phase, i.e. the solvent (mixture). Depending on the method, a liquid or a gas is used as the mobile phase.

In some analytical procedures, the analyte cannot be determined directly, but must first be subjected to a specific chemical modification, a *derivatisation.*

3.8.3 Quality Assurance and Calibration

Any measured values are to be reported with SI compliant prefixes and/or in the scientific notation.

DIN 1310 applies to content specifications.

In order to find out whether an analytical method is suitable for the mixtures present in each case, the quantity ranges must be taken into account:

It applies:

- Working range $A = m_i$
- Sample range $P = m_i + m_0$ (where m_0 = total mass of all impurities)
- Content range $G = \dfrac{m_i \cdot 100}{m_i + m_0}$

This results in the relationship: $P = \quad P = \dfrac{A}{G} \cdot 100$

Every measuring instrument must be *calibrated.* For this purpose, *calibration curves* must be generated with the aid of *standard solutions* (several blank and at least one empty sample); such curves can only be used reliably in their *linear* range. A compensation line drawn in this range allows interpolation.

Note the *significant figures* when specifying measured values.

Answers

1. When it comes to a content, quantitative analysis is always called for. And with a historical find like a coin, a destructive analysis is undoubtedly out of the question, so you will need to find a non-destructive method for the quantitative determination of lead. And there is one. But we won't go into X-ray fluorescence analysis until Part V.
2. First of all, they should aliquot the initial copper(II) solution into 10 identical portions.
   (a) Ideally, each of these aliquots would have a volume of 10.0 mL. However, this actually only works on paper. If you have to divide 100 mL of a solution among ten 10.00 mL volumetric flasks in a real laboratory, you will find that the last flask will *not* be filled up to the mark. A small portion of the initial solution will invariably remain in the initial flask. Accordingly, you should use this last (incomplete) sample. After all, you still have nine aliquots to work with.
   (b) For each of these aliquots, of course, $[Cu^{2+}] = 0.1$ mol/L still applies: the concentration of a solution does not depend on its volume. And that is exactly the point of aliquoting: to obtain smaller portions with still exactly given concentration. (Each aliquot can then be further diluted as required, thus creating a **dilution series**.)
   (c) To determine the amount of copper(II) ions present, you need a formula that you undoubtedly already know from *General Chemistry* (Binnewies, Section 1.2):

Binnewies, Section 1.2: Sizes and units

$$c = \frac{n}{V};\ \text{with}\ n = \text{Amount of substance}\left(\text{in mol}\right);\ V = \text{Volume}\left(\text{in L}\right)$$

   We are looking for the amount of substance, so we have to convert to

$$n = c \cdot V$$

   This gives 0.1 [mol/L] × 10.0 [mL] = 0.1 [mol/L] × 0.010 [L] = 0.001 [mol] Cu^{2+} ions present in each aliquot.
   (d) Once again, you should fall back on a formula that Binnewies has already supplied you with:

$$n = \frac{m}{M};\ \text{with}\ m = \text{Mass}\left(\text{in g}\right);\ M = \text{Molar mass}$$
$$\left(\text{in g / mol;can be found in any good periodic table}\right)$$

   What we are looking for is the mass of the copper(II) ions in solution, so once again we have to convert:

$$m = n \cdot M$$

   Here you should arrive at 0.001 [mol] × 63.5 [g/mol] = 0.0635 [g] = 63.5 mg Cu per aliquot.

Tip

For calculations such as 2c and 2d, it is always advisable to include the units and look at them. This way, you can always check right away whether the way of calculation *can be* correct at all. If, for example, you want to calculate a concentration, but the units come out as L/mol^2 or a similar monstrosity, something *must* have gone wrong.

Cave:

If the units "fit", that doesn't mean the calculation is necessarily correct. But if the units are wrong, it *cannot* be correct.

3. For liquids, the question of whether a residue remains in the filter during filtration provides a good first clue. Now one step after the other:
   (a) Tap water should obligingly be a homogeneous sample (lots of water and some salts dissolved in it). If your tap water has to be filtered before drinking, I would seriously think about having the water pipes replaced ...
   (b) Blood is a colourful mixture of water-soluble substances (various salts etc.) and water-*in*soluble things such as cell fragments. Plenty of solid matter would remain in the filter. Thus: a heterogeneous sample.
   (c) White wine vinegar is an aqueous solution of numerous easily water-soluble compounds, including acetic acid (CH_3-COOH), various easily water-soluble aromas and several salts. This homogeneous mixture should actually pass completely through the filter. (If something remains in the filter, then *mycoderma aceti* bacteria in the bottle have formed *mother of vinegar*. Might look a little gross, but it's completely harmless.)
   (d) The fact that a vinegar/oil mixture is an emulsion was even explicitly mentioned in the text—even if *some* claim that this is not quite correct, because in a *true* emulsion no segregation takes place, whereas in any salad dressing that has not been stabilised by an emulsifier (such as mustard or similar), phase separation will eventually occur again, with the oil phase floating on the aqueous vinegar phase due to its lower density. In any case, it is a *hetero*geneous mixture.
   (e) Like many other clear alcoholic beverages, gin is an aqueous solution with an alcohol content in the region of 40%, in which numerous aromatic substances are also dissolved. Unlike some herbal liqueurs, gin is filterable *without* decomposition.
   (f) Milk is an emulsion, i.e. a liquid-liquid mixture of water and milk fat, in which some proteins and lactose are also dissolved. In a first approximation, one can discribe milk as "fine milk fat droplets in aqueous solution". The demixing/phase separation takes much longer than with a vinegar/oil mixture, but milk still *is* a heterogeneous mixture. (This also applies to so-called *homogenised* milk! In this case, the fat droplets have merely been mechanically broken down further, so that the phase separation takes even longer. But also homogenised milk is a *heterogeneous* mixture. What non-scientists sometimes do to scientific jargon ...)
   (g) The extremely noble metal gold often occurs in nature in *pure form* (i.e. actually as elemental gold, not in any kind of compounds)—but mostly enclosed in other minerals. Thus, gold ores also belong to the heterogeneous mixtures.
   (h) Here, two homogeneous solutions (in each case dissolved salt in the solvent water) are mixed together without precipitation or any other chemical reaction occurring. Accordingly, a homogeneous mixture of freely floating cations and anions is formed.
4. Obviously, the sand has not dissolved in the solvent water, otherwise it would not be a slurry. Accordingly, when filtered, the sand remains in the filter and thus forms the residue, while the saline solution is the filtrate.

   No doubt *you* already have enough chemistry experience not to come up with such an idea, but some laymen seriously believe that if you filter a saline solution, the salt must remain in the filter, and the filtrate is then completely saltless. It is very difficult to explain to such people that (and why) the desalination of seawater is *somewhat* more complex.
5. Let us first order the available solvents according to their polarity: Here it is advisable to think again about things like hydrogen bonds, which are very characteristic for polar compounds, and otherwise to keep an eye on elec-

tronegativity differences. The most polar compound is water: With two H atoms and two free electron pairs each, water can be both donor and acceptor in hydrogen bonds. In ethanol, we have an OH group capable of hydrogen bonding, but at the same time we have the ethyl radical, which can be considered nonpolar due to the extremely small difference in electronegativity between C and H. In diethyl ether, we have two of these (nonpolar) ethyl radicals bridged by a significantly more electronegative oxygen atom. This O-atom is surrounded by two bonding partners (one ethyl-C each) and two free electron pairs, which leads to an angular structure according to the VSEPR model (Binnewies, section 5.4). This results in a permanent, albeit very moderate, dipole moment—and thus diethyl ether is still significantly more polar than the hydrocarbon hexane, which can do practically nothing except van der Waals interactions. So the polarity order is Water > Ethanol > Diethyl ether > Hexane

Now let's look at the analytes under consideration:

(a) Household sugar is extremely polar (otherwise it would hardly dissolve in tea in serious quantities, and tea, as you know, consists mainly of water). At the same time, you know that neither sand nor margarine is well soluble in water. So the sugar should be easily extracted from the mixture with water.

(b) Margarine, on the other hand, is a mixture of various extremely nonpolar fats. According to the principle "like dissolves like", one should choose a solvent that is as nonpolar as possible, and neither sugar nor sand dissolves in hexane.

Bonus info: What would happen if you chose ethanol as the extractant? Sand is not soluble in ethanol either, but because ethanol is *amphiphilic*, due to the polar OH group and the nonpolar ethyl residue, both sugar *and* margarine will dissolve in it to some extent. Thus, if the analyte content is not too large, you could extract both analytes from the mixture at the same time. However, it would remain to be seen whether this really would be advantageous in any further analysis steps ...

6. Again, polarity is crucial: margarine is significantly less polar than sugar. The stationary phase is polar, the mobile phase largely non-polar. It follows: The polar sugar will interact more strongly with the stationary phase, so a relatively large amount of solvent will be needed to wash it off the column. The non-polar margarine cannot interact very strongly with the polar stationary phase anyway, and moreover it interacts quite splendidly with the solvent, which is also not very polar. So the margarine arrives much earlier at the other end of the column.

7. To do this, first of all, you need the equations of definition of the proportion statement and the concentration statement from the tables above:

The proportion specification $\omega_i = \frac{m_i}{m_{(Solution)}}$ is to be converted to the concentration specification $c_i = \frac{n_i}{V_i}$

From *general chemistry* you know that

$$n_i = \frac{m_i}{M_i}$$

This already gets you somewhere with the *numerator* of the equation you're aiming for. But how do you get from the mass of the solution (m(solution))

to the volume?—The connection results from the equation of definition for the *density*:

$$\rho_{(\text{Solution})} = \frac{m_{(\text{Solution})}}{V_{(\text{Solution})}}$$

Now you can insert the information:

$$c_i = \frac{n_i}{V_{\left(\overline{Solution}\right)}} = \frac{m_i \diagup M_i}{m_{(\text{Solution})} \diagup \rho_{(\text{Solution})}} \quad \text{and that's equal to} \quad \frac{m_i}{M_i} : \frac{m_{\left(\overline{Solution}\right)}}{\rho_{\left(\overline{Solution}\right)}},$$

which is: $\frac{m_i}{M_i} \cdot \frac{\rho_{(\text{Solution})}}{m_{(\text{Solution})}}$

Since $\frac{A}{C} \cdot \frac{B}{D} = \frac{A}{D} \cdot \frac{B}{C}$, we can say: $\frac{m_i}{M_i} \cdot \frac{\rho_{(\text{Solution})}}{m_{(\text{Solution})}} = \frac{m_i}{m_{(\text{Solution})}} \cdot \frac{\rho_{(\text{Solution})}}{M_i}$

And because $\frac{m_i}{m_{(\text{Solution})}} = \omega_i$, we obtain:

$$c_i = \omega_i \cdot \frac{\rho_{(\text{Solution})}}{M_i} = \omega_i \cdot \frac{m_{(\text{Solution})}}{V_{(\text{Solution})} \cdot M_i}$$

The answer to the question then is:

$$c_i = \frac{\omega_i \cdot m_{\left(\overline{\text{Solution}}\right)}}{V_{\left(\overline{\text{Solution}}\right)} \cdot M_i}$$

You don't always *have to* do it that way. But first of all, this is a good exercise, and secondly, many examiners react remarkably allergic to students trying to solve any arithmetic problems by stringing together rule-of-three statements. It *can be done*, but it is ... inelegant. Apart from that, the rounding errors, which are unavoidable when calculating all *intermediate results*, can add up quite a bit. More on this in ► Sect. 3.6.

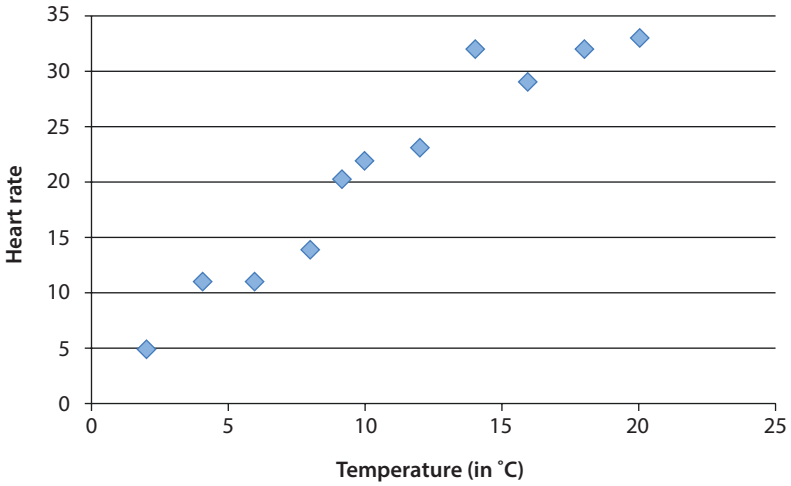
8. Yes, this simply *begs* for a simple rule-of-three approach. But we want to handle this like a *real analytics question*. So let's reformulate the question to quantity ranges and the official formula symbols of DIN 1310: About the analyte x, we know that ω(x) = 10% = 0.1. Its mass m(x) must be at least 0.5 mg to ensure unambiguous detection. Which sample mass m(sample) is required?

$$\omega(x)\text{Sample} = 0.1 = \frac{m_x}{m_{(\text{Sample})}}$$

Therefore, m(x) = 0.1 − $m_{(\text{sample})}$ or m(sample) = = $\frac{m_x}{0.1} = \frac{0.5[\text{mg}]}{0.1}$ 5 mg

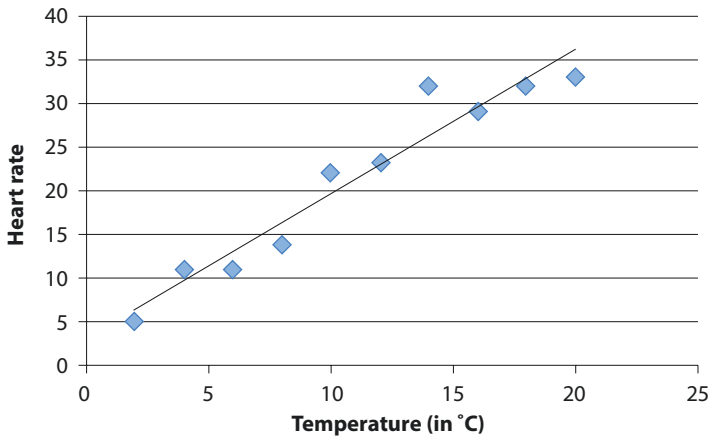
The corresponding quantity ranges? The content range from Eq. 3.4 is nothing else than ω, but already multiplied by 100, the working range A is m(x) (or m_i), and the sample range P, i.e. the amount of sample required for an unambiguous result, is simply m(x) + m(all impurities), often called m_0.

9. Quick answer: If the sensitivity of the measurement method has not changed, the sample quantity must probably be increased tenfold, i.e. in this case 50 mg, if the content has dropped to one tenth. But you should really recalculate that with ω_i, A, P and G.
10. If we plot the heart rate of the individual animals against the temperature (I hope you have not plotted the number of the animals on the x-axis, have you?), we get the following picture:



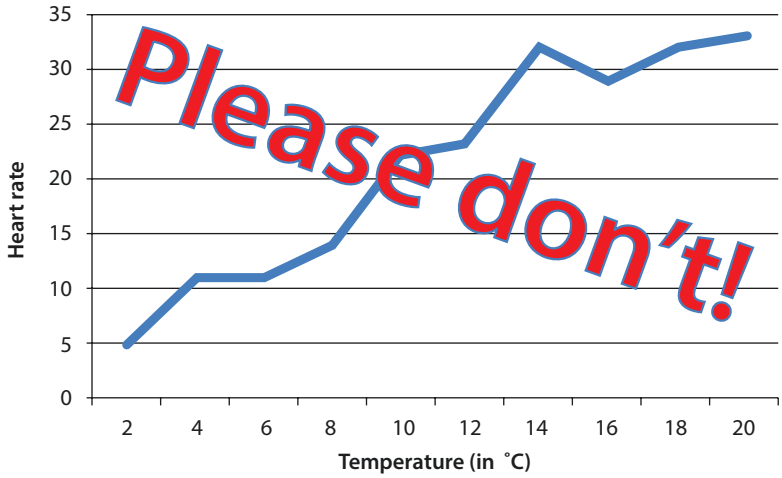
The bare data

Even if obvious fluctuations can be observed, a trend can be clearly identified and a trend line drawn accordingly:



... with trend line

Again, of course, it would be completely out of place to simply connect the dots to obtain a fever curve:



No fever curves! Ever!

The trend line (but *not* the fever curve!) suggests a direct (linear?) relationship between temperature and heart rate: The higher the temperature, the faster the hearts of the test animals beat. But that is about all that can be said more or less reliably: For more accurate data, the number of test animals per temperature zone alone would have to be drastically increased. (If the general tendency is indeed correct, it is hard to see why there should be a constancy of heart rate in the temperature range between >2 and <8 °C.) However, the trend line at least allows (within the limits of measurement accuracy) the interpolation to heart rates at *odd* temperature values, even though we only used even oves.

What this graph does *not* allow at *all,* however, is *extrapolation*: Although the trend line suggests that with increasing temperatures higher heartbeat rates would also be expected, on the one hand the question really arises whether at significantly increased temperatures there is still a more or less *linear* relationship between temperature and heartbeat rate, or whether sooner or later a maximum plateau is reached (because the hearts of the animals simply cannot go any faster). On the other hand at some point you will inevitably reach the temperature above which the heartbeat rate abruptly drops to zero. It's considered common knowledge that cooked snake tastes about like chicken.

Extrapolation to lower temperatures also is certainly more than just questionable: Following the trend line in the direction of decreasing temperatures, purely graphically one quickly ends up with negative heartbeat rates. It stands to reason that something like this is physiologically ... interesting. And even if a tendency should still be recognizable with sinking temperatures, this will certainly no longer be linear. Instead the curve will asymptotically approach the x-axis, i.e. the measured value "0". Infinitely long?—No, at some point even pythons are frozen stiff, and then you will definitely get the specific measured value "0".

11. (a) Apart from the obvious outlier "4.8", all measured values are quite close to each other, so the *precision* is not bad at all. However, all measured values are above the "true" value. There seems to be a *systematic* error here that affects the *accuracy*. (b) The accuracy is commendable, but the individual readings are quite widely scattered, so the *accuracy* is poor at best. (c) "Gone with the wind" applies equally to accuracy and precision. The fact that a "bull's eye" was achieved once can confidently be regarded as pure coincidence.

12. The rules of the game set out in ► Sect. 3.6 apply:
    (a) 23.42: All digits are significant, so: *four* significant figs.
    (b) 23,420: Subsequent zeros are significant, i.e.: five significant figs.
    (c) 0.42: Leading zeros are not significant (the number can also be represented as 4.2 × 10−1), so only two significant figs.
    (d) 0.420: Leading zeros are still not significant, but trailing zeros are, so three significant figs.
    (e) 0.00420: The same applies as for (d), here the number 4.20 × 10−3, i.e. again three significant figs.
    (f) 66,800 000 03: Zeros between significant digits are significant, of course, so we have a full *ten* significant figures here.
13. Round:
    (a) 23.666 to three significant figures: The first 6 is significant, the "neighbour-6" leads to rounding up, so 23.7.
    (b) 23.666 to four significant figures: The same shifted one place further to the right results in 23.67.
    (c) 187 582 to two significant figures: Only the 1 and the 8 are significant, the following 7 leads to rounding up to 190 000, but this has decidedly too many significant figures, i.e. 1.9×10^5 or 19×10^4.
    (d) 0,000 000 372 to two significant figures: All leading zeros are not significant, but only the 3 and the 7. The following 2 leads to rounding; the result is 0.000 000 37 or (better) 3.7×10^{-8} (or also 37×10^{-9}).
14. This problem contained a small trap: The width is given in cm, the other two dimensions in mm; so please convert appropriately. Then the dimensions height x width x depth lead to the pure calculation result 529 mm^3. But only the depth was really given with *three* significant figures, the height, you could see at first sight, only with two, and the width (the one with the cm) had a leading zero. So 529 mm^3 must now be rounded to *two* significant figs. 5 and 2 are significant, the 9 leads to rounding up, so mathematically we get 530, but that is again one figure too many with respect to significant figures, so the correct answer is: 5.3×10^2 mm^3.
15. First some pure arithmetic: (0.7578 × 34.968 85 g/mol) + (0.2422 × 36.965 90 g/mol) = 35.452 535 51 g/mol. What do the significant figures say about this? The atomic masses of the individual chlorine isotopes are given to "five decimal places", for a total of *seven* significant figures. The pure calculation result simulates an accuracy of *ten* significant figures. Therefore, at least five digits behind the decimal point should be rounded, which would be 35.452 54 g/mol. Behind 3, the last significant digit, there is a 5, and if you apply the "analyte's rule of five", you should round up to an (even!) 4 ... but this consideration was completely in vain, because the two percentages from the problem are *also* determined experimentally, so only a total of *four* significant figures are legitimate here. Let's start again: The calculated 35.452 535 51 g/mol then becomes 35.45 g/mol (the 2 after the 5 leads to rounding down).

    And yet the final result as the answer to the question is *wrong* (you didn't expect that, did you?), because the question asked for the molar mass of *elemental* chlorine, and from *General Chemistry* (or *Fundamentals of Inorganic Chemistry*) you certainly know that chlorine in its elemental state occurs *di*atomically, i.e. as Cl_2 molecule. Thus, of course, the molar mass of elemental chlorine is 2 × 35.45 g/mol, or 70.90 g/mol. Since the 2 used here mathematically is an *exact* number (there are, after all, not "approximately 2" chlorine atoms per molecule, but "*exactly* 2"), this does not influence the number of significant figures. And please don't forget: *The zero at the end is significant again*.

Literature

Binnewies M, Jäckel M, Willner, H, Rayner-Canham, G (2016) Allgemeine und Anorganische Chemie. Springer, Heidelberg

Brown T L, LeMay, H E, Bursten, BE (2007) Chemie: Die zentrale Wissenschaft. Pearson, München

Harris DC (2014) Lehrbuch der Quantitativen Analyse. Springer, Heidelberg

Jander G, Blasius, E (1983) Lehrbuch der analytischen und präparativen anorganischen Chemie. Hirzel, Stuttgart.

Mortimer CE, Müller U (2015) Chemie: Das Basiswissen der Chemie. Thieme, Stuttgart

Ortanderl S, Ritgen U (2018) Chemie—das Lehrbuch für Dummies. Wiley, Weinheim

Riedel E, Janiak C (2007) Anorganische Chemie. de Gruyter, Berlin

Schwister K (2010) Taschenbuch der Chemie. Hanser, München

Of course, there are many other textbooks on analytical chemistry: this list does not claim to be exhaustive.

In addition, it is advisable, once basic knowledge has been acquired, to look into further literature. In particular, the (really considerable) bibliography of Harris will help you further.

Volumetric Analysis

- **Requirements**

In this study section, "wet chemical" quantitative analytical methods are dealt with. Behind this are the usual reactions that you will already know from *General and Inorganic chemistry*.

For the quantitative consideration of *acid/base titrations,* we should know how to deal with the following terms, concepts, and principles:

- acids and bases according to Brønsted,
- acids and bases according to Lewis,
- acid/base pairs,
- mono- and polyprotonic acids,
- the law of mass action (LMA),
- dynamic balance,
- equilibrium concentrations,
- autoprotolysis of water,
- pH value,
- ampholyte/amphoteric,
- buffer, *and*
- the Henderson-Hasselbalch equation.
- The "proper names" of at least extremely common acids (hydrochloric acid, sulfuric acid, etc.) and bases (ammonia, caustic soda) are also assumed to be known.

When *complexes* are used to analyse a solution, we need:

- ligands,
- chelate ligands,
- the chelation effect,
- stability constants, *and*
- a certain general understanding of what complexes as a whole are all about.

If *reduction and oxidation processes* play a role in the chosen analytical method, there are also some technical terms and concepts that you should be familiar with:

- oxidation/oxidising agent,
- reduction/reducing agent,
- redox couples,
- oxidation numbers (and how to determine them!),
- disproportionation/synproportionation (= comproportionation),
- partial equations,
- redox potentials,
- the (electrochemical) voltage series,
- the Nernst equation,
- galvanic elements,

- half cells,
- salt bridges,
- cathode/anode, *and*
- concentration cells.
- Furthermore, you should be able to set up reaction equations for redox reactions (balanced—keyword: *substance balance*/*charge balance*).

Finally, if a (poorly soluble) solid is *precipitated* from a solution during analysis, then the following terms should not be new to you:
- saturated solution,
- solubility product (closely related to the LMA!),
- solubility.

In addition, there are the usual concentration specifications (compliant with DIN 1310 from Part I): We should therefore be able to calculate with (molar) concentrations and the like, and it must also be clear what is meant when two reactants are present "in equivalent quantities". The technical language from Part I is therefore also presumed.

1.1 Learning Objectives

The term "volumetric analysis" covers various methods of quantifying analytes present in a homogeneous solution by wet chemical means. Practically all principles of chemistry can be used in one way or another here: acid/base reactions, redox reactions, precipitation reactions, and complex compound chemistry. These then form the basis for a titration, for example. The fact that in the context of volumetric analysis we work not only qualitatively but also quantitatively requires, as is to be expected, dealing with the laws and formulas behind the principles, which—as should become clear in the course of this part—are by no means an end in themselves but make such statements possible in the first place. (Without the law of mass action, for example, it is simply not possible to make any quantitative statements about acid/base reactions.)

In addition to the listed methods of quantitative analysis, which ultimately boil down to titration, we will also look at various detection methods in this part. These detection methods, in turn, are based on principles that we will encounter again in the following parts, especially in the topic of *instrumental analysis*. However, the foundations for all this will already be laid in this part.

Because this part takes a closer look at the various types of reactions that can be used in volumetric analysis, this text also provides a concise summary of the most important principles of chemistry in this regard and shows how they are related:
- In *acid/base volumetry*, the (mass) content of an analyte acting as an acid or base can be determined with remarkable accuracy—either by *direct* titration (if the acid or base behaviour of the analyte is sufficiently pronounced) or *indirectly*, by subsequent further treatment of the titration solution obtained.

- A particularly frequently used technique in this case is *back titration*: in this case, the analyte is mixed in excess with a precisely defined quantity of a reagent which reacts stoichiometrically with the analyte. However, since the reagent mentioned is present in excess, a residue will remain after the reaction has been completed; this is then quantified via a *second* titration and allows corresponding conclusions to be drawn about the original analyte content.
- In *complexometry*, quantitative analysis is based on the interaction of the analyte with molecules/ions that are or become part of a complex. Thereby, the analyte can
  - either be complexed themselves, *or*
  - prevent or promote the formation of a complex.
- In the same way, redox reactions can also be used for volumetric analysis; the principle of titration remains unchanged.
- The poor solubility of some compounds also permits *precipitation titrations*: In this case, the analyte is removed from the homogeneous solution by forming a precipitate.
  - The determination of the mass of the precipitated material by weighing then leads to the method of *gravimetry*.

In addition to the different reaction types, we also need to explore how to obtain usable measurement results at all. Accordingly, we will not only address selected detection methods in general, but also go into what actually happens at the molecular or atomic level.

Contents

General Information on Volumetric Analysis

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_4

Summary

Volumetric analysis is also referred to as **volumetry**—this term makes it much easier to see the principle behind this method of analysis: A solution of a reagent is added to a solution containing the analyte (and possibly other things as well, more on this later), which reacts with the analyte in a characteristic way so that one or another (microscopic or macroscopic) property of the analyte solution changes in a quantifiable way. The decisive factor in volumetric analysis is that the *volume of the reagent solution added to the analyte solution* is determined as accurately as possible, and conclusions can be drawn about the concentration of the analyte solution on the basis of this reagent volume (i.e. the "reagent consumption").

This leads us first to three questions:

- **First: What Are We Dealing with?**

In volumetrics, the following collective terms have become common:

- The **analyte** is not only the substance to be quantified but also the (homogeneous) solution containing it.
- **Titrant** is the name for both the substance that is brought to react with the analyte and the (homogeneous) solution of this substance.

- **Second: What Exactly Are We Doing?**

A large number of different volumetric methods uses the principle of finding out something about the analyte itself on the basis of changes in specific properties of an analyte solution via the interaction (usually: reaction) of analyte and titrant. The most important methods here are:

- the acid/base titration; we will come to this in ► Chap. 5,
- volumetric analysis with complexes, also known as **complexometry**; you can find out more about this in ► Chap. 6,
- redox titration, in which the oxidisability or reducibility of the analyte is exploited (more on this in ► Chap. 7),
- precipitation titration, in which the solubility product plays an important role, and the basic principles of which we consider in ► Chap. 8.

- **Third: What Properties Are Changing?**

In principle, every solution has a large number of characteristics that could (at least theoretically) be changed, but usually one restricts oneself to a few properties or phenomena:

- the *colour change*: a change in the analyte solution's colour, which can often be observed at least qualitatively with the naked eye after the addition of a sufficient quantity of titrant. In this case, the observed colour must be compared with a reference scale tailored to the analyte in question—this is **colorimetry**, about which you will learn a little more in ► Sect. 10.1.

  In many cases, such colour changes are due to one or another indicator that is often added to the analyte solution in such analyses. (We will return to these indicators in the sections on the individual volumetric analysis techniques, because even though they all may change the colour, they differ considerably in their chemical behavior.)

  If necessary, the different colourations can also originate from the analytes themselves; this is particularly common in redox titrations.
- the *absorption behaviour for electromagnetic radiation,* whereby usually not only a single wavelength is considered, but a certain wavelength *range*.

  This can refer to visible light, i.e. electromagnetic radiation in the wavelength range 380–780 nm, but also radiation from the (higher-energy) ultraviolet range or (lower-energy) infrared radiation; even much more extreme ranges can be used in analytics.

If radiation from the *VIS* range—i.e. the radiation visible to the human eye—is used, we speak of **photometry**, which we will examine in more detail in ► Sect. 10.2. (We will limit ourselves to these wavelengths for the time being; we will look at the application of other wavelength ranges in Parts IV and V, and you will encounter them again in "Analytical Chemistry II" if you are interested.)

- the *electrochemical* behaviour: Two different techniques are worth mentioning here:
  - In **potentiometry**, changes in the electrochemical potential are observed; more on this in ► Sect. 10.3.
  - **Conductometry**, to which we turn in ► Sect. 10.4, allows conclusions to be drawn about the analyte on the basis of the conductivity of an analyte solution.

Volumetric Analysis with Acids and Bases

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_5

Binnewies, Section 10.1: The Brønsted-Lowry concept

Summary

Behind the technique of volumetric analysis, in which acids and bases are made to react with each other, primarily are the acid/base concepts that you already know from *general chemistry* and about which you should, if necessary, read up again in Binnewies before we move on to consider these concepts *quantitatively*, which have so far been treated largely qualitatively. Particularly important here is the concept of Brønsted and Lowry.

Important

Because it is so important, the core statements of this concept and the principles derived from it (and relevant for analytics) are summarised here once again:

- No substance *is* an acid or base by itself, it only *acts* as such if necessary, provided a corresponding reaction partner is available.
- **Acids** are substances that release H^+ ions, i.e. act as *proton donors*; **bases** absorb H^+ ions and thus act as *proton acceptors.*
- No substance *can* act as an acid, i.e. give off a proton, if there is no reaction partner that can also take up the proton, i.e. act as a base (and vice versa).
- If a molecule (ion) has acted as an acid, i.e. has split off a proton, the conjugate base of the acid in question is present after deprotonation. Similarly, bases are converted to the conjugate acid by protonation. (In older textbooks you will occasionally encounter a "corresponding acid/base pair", but in the meantime "conjugate" is considered correct.)
- The reference medium for describing acids and bases is always the solvent *water*: A substance is commonly referred to as an acid if it *acts as an acid towards water*, i.e. protonates water (H_2O) to form hydroxonium ions (H_3O^+); correspondingly, any compound that acts as a base towards water and thus deprotonates water molecules to hydroxide ions (OH^-) is considered a base.
- Water itself performs **autoprotolysis**, so even in the absence of any substances dissolved in water, the following dynamic equilibrium always occurs:

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

- Although the autoprotolysis equilibrium is far to the left (the reactant side), the cations and anions formed in the process must still not be neglected. Under standard conditions, the concentration of H^+ ions (hydroxonium ions) and OH^- ions (hydroxide ions) formed by autoprotolysis is 10^{-7} mol/L each.
- According to the law of mass action, the **ion product of water** is obtained:

$$K_W = \left[H_3O^+\right] \times \left[OH^-\right] = 10^{-14}\ \text{mol}^2/\text{L}^2 \qquad (5.1)$$

- This ion product does not change even if "additional" H_3O^+ or OH^- ions are introduced into the aqueous solution by adding acids or bases. Accordingly, the concentration of free OH^- ions in a solution with "excess H_3O^+ ions" (i.e. an *acidic* solution) decreases drastically (and vice versa).
- In general, the H_3O^+ ion concentration of an aqueous solution is not expressed in mol/L in the "usual" laboratory way. Instead, the negative logarithm (to the base 10) of the concentration is used because this gives clearer figures: this is the **pH value**.
  - The concentration of OH^- ions can be expressed accordingly by the **pOH value** (even if this is much less common than the pH value).
  - Accordingly, autoprotolysis results in pH = 7 for water in which no other substances are dissolved (and thus also: pOH = 7, because the number of hydroxonium ions produced by autoprotolysis corresponds exactly to the number of hydroxide ions produced in the process).
  - Acids *lower* the pH value of an aqueous solution, bases *increase* it.

- Using "small p" is also helpful when dealing with the ionic product of water. It applies:

$$\mathbf{pH + pOH = 14}. \tag{5.2}$$

- The tendency of a substance to act as an acid in aqueous solution is expressed by the **pK_A value**, which is ultimately derived from the associated law of mass action, and where the "small p" again has exactly the same meaning. Thereby applies:
  The smaller the value, the more acidic the substance in question.
  Looking at it at the molecular level:
  The smaller the value, the more easier it is to split off the hydrogen atom in question.
- The same applies vice versa for bases: Here, the **pKB value** is to be considered, for which analogous applies.

Regarding the LMA, there is a very useful simplification to consider for acid/base reactions in aqueous solution:

From a purely "chemical point of view" it is indeed reasonable to set up the corresponding reaction equation for the dissociation of an acid HA in the sense of Brønsted and Lowry, that is:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

(The commonly used general formula HA for any acid and A^- for its conjugate base of course derive from the word *acid*.)

In the LMA, however, we always work with concentrations of quantities of substances (for acid/base reactions: in aqueous solution), and in the reaction equation in the sense of Brønsted and Lowry, water is not only a solvent, but also a *reaction partner*. So we need a statement about "the concentration of water in itself". Let us look again at the definition of the amount of substance and the equation for determining the concentration of the amount of substance from DIN 1310:

$$n = \frac{m}{M} \text{ and } c = \frac{n}{V}$$

Here, as usual: n = amount of substance (in mol), m = mass (in g), M = molar mass (in g/mol); c = molar concentration (in mol/L), and V = volume (in L).

Let's look at the corresponding concrete numbers: When we specify concentrations of quantities of substances in the laboratory, we usually do so with the unit mol/L. (As you will no doubt remember from Part I, this is not entirely in the spirit of DIN 1310, but in everyday laboratory work it is very tedious to always convert all the volumes used into cubic metres.) Since the density of water at room temperature is about ρ = 1 g/cm^3 and 1 L corresponds to 1000 cm^3, the approximate mass of 1 L of water is $m(H_2O)$ = 1000 g.

If we now consider the molar masses of the atoms involved ($M(H) \cong 1$ g/mol, $M(O) \cong 16$ g/mol), we get $M(H_2O) \cong 18$ g/mol.

What we are looking for is $c(H_2O)$, so according to the formula we get

$$c = \frac{\frac{m}{M}}{V} = \frac{m}{M \cdot V} = \frac{1000\ [g]}{18\left[\frac{g}{mol}\right] \cdot 1\ [L]} = 55.555\ldots\ \ [mol/L]$$

1 L of water thus corresponds to a quantity of substance of more than 55 mol—so it makes no appreciable difference to the total number of H_2O molecules present if one or the other molecule is protonated and thus "consumed"—not even if the acid is used in high concentrations such as 1 mol/L (or even 10 mol/L).

Learning Tip

If you lack some experience with such calculations, it is recommended to perform a **dimensional analysis** every time. Before inserting any values into an existing (or self-generated) formula, check whether the formula in question (or the concatenation of several formulas) *can* lead to a meaningful result at all. If, for example, you want to calculate a concentration, but your "formula" leads to a calculation result with the unit "mol/m" (i.e. amount of substance per distance), something has gone thoroughly wrong.

Accordingly, it simplifies life (and calculation!) immensely if one assumes that the concentration of the reactant water (which acts as a base to the acid HA) in aqueous solutions is constant. (This is, strictly speaking, *not quite* correct, but it does not affect the calculation result appreciably—at least in the range of any significant digits. Apart from that, if we wanted to be "even more price", we would have to introduce the concept of "activity" here, but this goes beyond the scope of "Analytical Chemistry I": You will find out more about the difference of molar concentration (c) and activity (a) in Chapter 4 of "Analytical Chemistry II".)

If $[H_2O]$ is constant this way, one can include this value in the LMA in the associated reaction constant, and the reaction equation of dissociation simplifies to:

$$HA \rightleftharpoons H^+ + A^- \tag{5.3}$$

The corresponding law of mass action is then:

$$K_A = \frac{[H^+] \cdot [A^-]}{[HA]} \tag{5.4}$$

Since for each H^+ released by dissociation of the molecule HA, exactly one A^- is also produced, $[H^+] = [A^-]$ applies. Thus, Eq. 5.4 can be further simplified to:

$$K_A = \frac{[H^+]^2}{[HA]} \quad \left(\text{or also } K_A = \frac{[A^-]^2}{[HA]}, \text{ if you prefer that} \right) \tag{5.5}$$

From here to the pK_A value it is then only a small step. (Of course, we could also perform analogous calculations for bases. In this case, the K_B or pK_B value must be used accordingly—you know the principle now.)

Since the strength of an acid (or base) certainly affects its chemical behaviour in equilibrium (see above), a distinction is made between different types of acids (or bases):

- Substances HA, which (practically) completely dissociate to $H^+ + A^-$ in water, have negative pK_A values due to the large equilibrium constant (LMA!). Such substances are called **strong acids.**
- If the pK_A value is between 0 and 4, the dissociation is incomplete but still "serious". This is referred to as **medium strength acids.**
- For **weak acids**: $pK_A > 4$. Here, the vast majority of molecules in aqueous solution are undissociated, and only a very small fraction dissociates.
  - The same statements and categorisation can be made about bases based on the pK_B value.
    (This admittedly somewhat arbitrary distinction becomes important when calculating the respective pH of aqueous solutions of acids (or bases) of different strength).

Important

For conjugate acid/base pairs, the following is always true:

$$pK_A\ (\text{Acid}) + pK_B\ (\text{Conjugated base}) = 14 \qquad (5.6)$$

This relationship, which is once again ultimately due to the ion product of water and thus to Eq. 5.1, makes dealing with conjugate acid/base pairs immensely easier.

A fact that you will certainly still know from *general chemistry* should nevertheless be expressly emphasised here once again, because many students, at least initially, tend to getting confused here:

The pK_A or pK_B value of a substance is substance-specific*: it says something about how "willingly" the acid or base in question enters into the corresponding reaction.*

As just mentioned, the lower the pH of a solution, the more acidic it is. (Likewise, the lower its pOH value, the more basic it is, but the pOH value is rarely used to describe aqueous solutions.)

The pH value only tells you how many free H_3O^+ ions are in the solution. You should always keep in mind that every pH value is really a *concentration statement:*

$$pH = -\lg\left[H_3O^+\right]$$

So this only tells you something about the *number* of hydroxonium ions present, not where they come from. Thus, if you have an aqueous solution with a low pH value, then this only allows the statement that it obviously contains more free H_3O^+ ions than this is normally the case with water—but not who or what is responsible for this:

- There could be a relatively small amount of very acidic molecules (with a low pK_A value) behind it, where (almost) every single molecule has willingly given up "its" proton to the water (complete dissociation).
- However, the solution could also contain a much larger quantity of much less acidic molecules, of which only a certain proportion was prepared to give off an H^+. (In this case, obviously incomplete dissociation occurred, i.e. the compound in question has a significantly higher pK_A value.)

If this concept is not yet completely clear to you, it might be advisable to take another look at Binnewies. If, on the other hand, you already have the basics down pat "per se", but want to look at the whole thing again specifically from the analytical point of view, Harris will help you again.

Binnewies, Section 10.2: Quantitative description of acid/base equilibria in aqueous solution
Harris, Section 8.1: Strong acids and bases
Harris, Section 8.2: Weak acids and bases
Harris, Section 8.3: The equilibria of weak acids
Harris, Section 8.4: The equilibria of weak bases

▶ Example

Let's go through the related considerations with more manageable numbers:

Whether the solution contains 100 molecules of the substance HA, each of which has split off its proton (i.e. it is completely dissociated into H^+ and A^-), or whether there are 1,000,000 molecules of the compound HZ in the solution, but only one in ten thousand of which dissociates according to $HZ \rightleftharpoons H^+$ und Z^-, makes no difference whatsoever to the number of free H_3O^+ ions in solution. Accordingly, these two hypothetical solutions will have exactly the same pH value. ◀

Attention

From chemistry beginners one will hear sentences like: "Hydrochloric acid has pH = 0, therefore it is a strong acid." So a direct connection between pH and pK_A values is constructed. You should *absolutely refrain from doing this*, because:

The pH value is a concentration value, the pK_A value is a substance-specific constant.

Any substance with $pK_A < 0$ is a strong acid—this is simply a matter of definition.

Let us briefly calculate again: Hydrochloric acid is the aqueous solution of hydrogen chloride (HCl), and $pK_A(HCl) < 0$, so we may assume complete dissociation of all HCl molecules that have entered the water:

Therefore, in the reaction $HCl + H_2O \rightleftharpoons H_2O^+ + Cl^-$. the equilibrium lies practically completely on the right side.

If at the beginning 1 mol HCl was dissolved in water, this results in an initial concentration for hydrogen chloride (before equilibrium has been established!) of $[HCl]_{\text{at the beginning}} = 1$ mol/L. Then, however, equilibrium is established (very quickly), and as the equilibrium lies indeed almost completely on the right side, this results in $[HCl]_{\text{at the beginning}} = [H_3O^+]_{\text{at equilibrium}} = 1$ mol/L.

Thus, the introduction of one mole of hydrogen chloride into 1 L of water results in a concentration of free H_3O^+ ions of 1 mol/L—that is ten million times what results from autoprotolysis (which was known to be 10^{-7} mol/L, or only one ten-millionth of a mole—not the animal, of course). Under such circumstances, we may neglect the contribution of autoprotolysis to the total concentration of H_3O^+ ions. Accordingly, for the 1-molar hydrochloric acid, we can say:

$$pH_{(\text{HCl–Solution, after the equilibrium has been established})} = -\lg\left(1 \text{ mol/L}\right) = 0.$$

In the case of a less concentrated solution of hydrogen chloride in water, complete dissociation still occurs; after all, the pK_A value is substance-specific and not concentration-dependent. *The pH value of the resulting solution, on the other hand, is very much dependent on the concentration;* after all, it is a concentration statement:

If, for example, "only" 0.1 mol HCl are dissolved in 1 L water, the equilibrium results in

$$[HCl]_{\text{beginning}} = \left[H_3O^+\right]_{\text{at equilibrium}} = 0.1 \text{ mol/L, so:}$$

$$pH_{(\text{HCl–Solution, after the equilibrium has been established})} = -\lg(0.1 \text{ mol/L}) = -(-1) = +1$$

Because of the lower concentration, the pH value of this solution is higher than in our initial example. However, this does not change the acid *strength* of this compound (described by the pK_A value). Even at higher dilution (or lower concentration) hydrogen chloride remains a *strong acid,* because the acid strength is *substance-specific.*

If we add only a minimal amount of a strong acid to a very large amount of water (e.g. a drop of 1-molar hydrochloric acid to a fully-filled swimming pool), this will hardlychange the pH of the aqueous solution (i.e.: the pool water)—because there were too few acid molecules in total to release a sufficiently large number of H_3O^+ ions so that a change in the pH would be measurable.

A second popular rookie mistake:

A pH value is the *indication of a concentration in an aqueous solution:* It is completely nonsensical to want to assign a pH value to a solid. Nevertheless,

one hears again and again that a salt which *reacts* basic (i.e. when added to water leads to a solution with pH >7) "is" basic or even that "the salt has pH >7". Please consider:

A solid does *not* have a *pH value.*

The specification of a pH value is only possible and meaningful for an *aqueous solution*.

Let us now turn to the various ways of letting acids react with bases in the context of analysis—and observe what happens step by step in each case. Let's start with unambiguous ratios and use both acids and bases that (almost) completely dissociate in the aqueous medium, i.e. have $pK_A < 0$ and $pK_B < 0$ respectively.

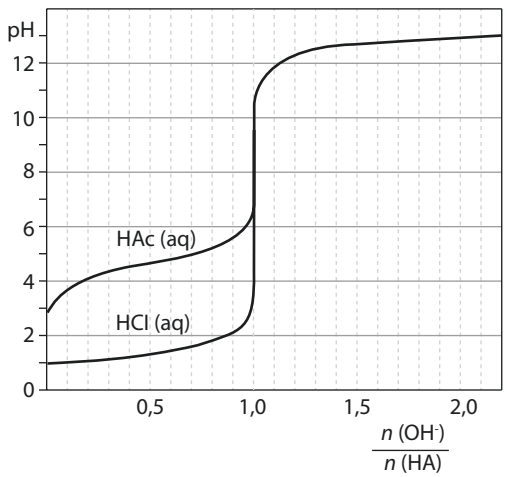
5.1 Strong Acid with Strong Base (and Vice Versa)

If you dissolve a strong acid—i.e. a substance HA that dissociates almost completely to form H^+ and A^- in the aqueous medium (or reacts with water almost completely according to $HA + H_2O \rightleftharpoons H_3O^+ + A^-$), you get a solution for which the following is undoubtedly true: pH < 7. If you then add the aqueous solution of a strong base drop by drop, the pH value of the reaction solution changes hardly noticeably at first—which should not be surprising, after all the pH scale is *logarithmic.* (You should not forget that either!)

This can be seen particularly clearly if the volume of the base added (on the x-axis) is plotted against the pH value measured in each case (on the y-axis). Such a **titration curve** is shown in ◘ Fig. 5.1. (Should the picture look familiar to you: This is Fig. 10.3 from Binnewies; for the time being, please concentrate only on the curve marked $HCl_{(aq)}$.)

Some change will still be noticeable, after all the hydroxonium ions produced by the acid are **neutralised** by the hydroxide ions coming from or produced by the base:

$$H_3O^+ + OH^- \rightleftharpoons 2\ H_2O$$



◘ **Fig. 5.1** Titration curves of various acids. (Binnewies et al., General and Inorganic Chemistry, Fig. 10.3, 3rd edition, 2016, © Springer-Verlag GmbH Berlin Heidelberg. With permission of Springer)

▶ Example

Accordingly, the hydroxonium ions present in the solution (responsible for the lower pH value) are gradually consumed. However, the change in pH is not particularly rapid at first, precisely *because* the pH scale is logarithmic.

As a reminder, a solution with pH = 4 contains *ten times* as many free hydroxonium ions as a solution with pH = 5. Accordingly, ten times more base must be added to the acid if you want to raise the pH from 4 to 5 than if you want to change the pH from 5 to 6. ◀

5

Harris, Section 10.1: Titration of a strong acid with a strong base

However, if one comes close to the **equivalence point**, i.e. the point at which identical (= equivalent) amounts of hydroxonium ions as hydroxide ions are present in the reaction mixture, the pH value of the solution changes drastically "within a few drops of base addition". ◘ Figure 5.1 clearly shows that the *titration curve* not only has a clear inflection point, but also that this is exactly at pH = 7. Upon further addition of the base (i.e. when **over-titrating)** the pH value of the solution continues to rise sharply for "a few more drops" until one has arrived in the recognisably alkaline pH range. After that, the pH of the solution continues to rise only very, very slowly—please note the similarity to the shape of the curve in the recognisably acidic pH range, long *before* the equivalence point is reached. (The individual Sections of a titration curve are discussed in a little more detail using Fig. 10.1 Harris, but there "in reverse": a strong base is titrated against a strong acid.)

The titration curve discussed here should also immediately clarify the principle behind any volumetric analysis:

If, for example, you want to determine the mass content of an acidic solution (i.e.: of your *analyte*) as accurately as possible, a simple measurement of the pH value is understandably not sufficient. However, if you follow the (stepwise) change of the pH value during a titration of this acid againstt the solution of a base of precisely defined concentration (i.e.: the *titrant*) and this way precisely determine the equivalence point, this allows a much more precise statement about the initial concentration of your analyte.

Technical Language Tip

In analysis, the analyte is **titrated *against*** the titrant. If, for example, the solution of an acid is gradually reacted with the solution of a base, then the acid is titrated *against* the base. (To outsiders, the correct technical terminology may occasionally seem peculiar.)

Please note that behind the acid/base titration there is primarily the (mutual) neutralisation of hydroxonium and hydroxide ions. Accordingly, for the course of such a titration curve it is only of interest that *equal amounts of acid and base* are present at the *equivalence point.* The *concentrations* of the solutions used in each case may well differ considerably—understandably, this must then be taken into account.

If, for example, you titrate 10 mL of 1-molar hydrochloric acid against 1-molar caustic soda solution, you will only have to add 10 mL of caustic solution until the equivalence point is reached; if, on the other hand, you let the 1-molar hydrochloric acid react with 0.1-molar caustic soda solution, you will need ten times the titrant volume. It should also be obvious that the use of a more dilute titrant solution enables you to determine the inflection point of the titration curve much more precisely.

Important

Since the absolute titrant consumption is concentration-dependent, but ultimately only the molar ratio of titrant and analyte is behind this, it has become generally accepted to use the (relative) **titration grade (τ)** instead of (absolute) volume specifications (in mL or similar, where the concentration of the titrant must then also be explicitly stated). The following applies:

$$\tau = \frac{n(\text{Titrant})}{n(\text{Analyt})} \tag{5.7}$$

Sometimes the degree of titration is also given as a percentage; then:

$$\tau[\%] = \frac{n(\text{Titrant})}{n(\text{Analyt})} \times 100) \tag{5.8}$$

Harris, Section 10.1: Titration of a strong acid with a strong base

The principle described here is also applicable to the case where we start with a strong base as the analyte solution, i.e. at a rather *high* pH value, which we then gradually *lower* by successive addition of a strong acid (= titrant). This is shown, for example, in Fig. 10.1 of Harris.

Let's look again at the three regions of the resulting titration curve:

- *Before* the equivalence` point is reached (τ <1), the ions responsible for the pH value of the solution and produced by the interaction of the solvent with the analyte (in the acidic case: H_3O^+ ions, in the basic case: OH^- ions) are gradually consumed by the added titrant, but the *analyte* still clearly determines what happens. To calculate the initial pH value, one takes advantage of the fact that strong acids (or bases) dissociate completely and thus each acid (base) molecule protonates (deprotonates) exactly *one* solvent molecule. Accordingly

$$\left[H_3O^+\right]_{\text{at equilibrium}} = [\text{Acid}]_{\text{at the beginning}} \quad \text{or} \quad \left[OH^-\right]_{\text{at equilibrium}} = [\text{Base}]_{\text{at the beginning}}$$

And since each titrant equivalent neutralises one analyte equivalent, the titrant consumption can be counted towards the analyte consumption by a factor of 1:1.

- Once the equivalence point has been *exceeded* (τ >1), the H^+ or OH^- ions originating from the titrant have neutralised all hydroxonium or hydroxide ions that were there due to the analyte; from now on, therefore, the titrant alone is responsible for the pH value of the solution: Each titrant equivalent then yields exactly one equivalent of base/acid.
- And what exactly happens *at* the equivalence point (at τ = 1)?—If the analyte is a strong acid and the titrant a strong base (or vice versa), then the hydroxonium ions and the hydroxide ions have neutralised each other, so that only the autoprotolysis of the water itself determines the pH value. And for "neutral" water pH = 7 applies; you know that from the ion product of water (cf. Eq. 5.1). Accordingly, the equivalence point of the titration curve (mathematically: the *inflection point of* this curve) coincides exactly with the **neutral point** of the solution.

If, on the other hand, only one of the two reactants (i.e. either the analyte or the titrant) falls into the "strong" category (regardless of whether it is an acid or a base), but the other does not, the shape of the curve changes and new things have to be taken into account.

5.2 Weak Acid with Strong Base/Weak Base with Strong Acid

Again, the degree of titration τ from Eq. 5.7 or Eq. 5.8 is important here. And yet other factors obviously come into play, because if we return once again to ◘ Fig. 5.1 and this time concentrate on the curve marked $HAc_{(aq)}$ (HAc = the abbreviated notation for acetic acid, CH_3–COOH, used in analytical chemistry, with $pK_A(HAc) = 4.75$), it can be seen that although there is also a recognisable inflection point of the curve here (i.e. the equivalence point), this *no longer coincides with the neutral point*, but lies in the basic range, at pH >7. Let us derive the reason for this together:

First, we have to consider what "incomplete dissociation" actually means. For this purpose, let us consider an arbitrary acid HA with $pK_A > 0$. If we add a precisely defined amount of substance (so that we have a concrete example with which we can also calculate well, let us now arbitrarily say: 1 mol) of the acid HA to a likewise precisely defined volume of water (for the sake of simplicity: 1 L), then a completely dissociating acid (i.e. a strong acid) would correspondingly protonate exactly 1 mol of water molecules to exactly 1 mol of H_3O^+ ions, and in addition the solution would contain exactly 1 mol of A^- ions (the conjugate base). Thus:

$$\text{Complete dissociation}: HA + H_2O \rightarrow H_3O^+ + A^-$$

Here, therefore, the reactant side of the reaction equation is of no further concern.

But for acids with $pK_A > 0$, at least *some HA molecules remain undissociated* in aqueous solution, and the greater the pK_A value of the acid in question, the more so: These undissociated acid molecules are present in solution, but do not give up their proton to the solvent. Accordingly, *both* sides of the reaction equation describing the associated dissociation equilibrium are important here:

$$\text{Incomplete dissociation}: HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

Note that the *equilibrium arrow* has been chosen here. In the case of incomplete dissociation, when the equilibrium of the reaction has been established, a serious amount of reactants (HA and H_2O) is present in addition to the products (H_3O^{+-} and A^- ions)—depending on how far the equilibrium lies on the reactant side, even (significantly) more than of the products.

Harris, Section 10.2: Titration of a weak acid with a strong base
Binnewies, Section 10.3: Acid/base titration and titration curves

This has several interesting consequences. Let us have a look at the shape of such a titration curve (weak acid with strong base) in Fig. 10.2 from Harris:

1. After the partial protolysis of the weak acid has produced at least some hydroxonium ions to lower the pH of the solution accordingly, small amounts of added base are sufficient to cause a noticeable increase in pH.
2. Subsequently, there is a range in the titration curve in which the pH value of the solution increases only very slowly *despite* the constant addition of bases. (You are certainly already familiar with the phenomenon underlying this **buffer range** from Binnewies.) Here the curve shows a *first turning point.*
3. If further base is added until the buffer range is exceeded, the curve shows the course already familiar from ► Sect. 5.1: The equivalence point of the curve corresponds to the *second inflection point,* but it lies now in the basic range (at pH >7).
4. Beyond the equivalence point, further base addition then rapidly leads to the same higher pH values as in ◘ Fig. 5.1.

Let's have a look at these four areas in turn and pay attention to what determines the course of the curve in each case—and what is chemically behind it:

In the Beginning

Before even the first drop of titrant has been added to the acidic analyte solution, the (weak) acid HA understandably guarantees pH < 7. But since weak acids do not dissociate completely (that is how they are defined, remember?), we know:

$$\left[H_3O^+\right]_{\text{at equilibrium}} < \left[\text{Acid}\right]_{\text{at the beginning}}$$

How big the difference is depends in each case on the strength of the acid under consideration, i.e. on its pK_A value. Let's take a look at a concrete example:

For the acetic acid in ◘ Fig. 5.1, $pK_A(HAc) = 4.75$.

So general: in this example becomes $K_A = \dfrac{\left[H^+\right]_{\text{at equilibrium}} \cdot \left[Ac^-\right]_{\text{at equilibrium}}}{\left[HAc\right]_{\text{at the beginning}}}$

$$K_S\left(HAc\right) = 10^{-pK_S(HAc)} = \frac{\left[H^+\right]_{\text{at equilibirum}} \cdot \left[Ac^-\right]_{\text{at equilibrium}}}{\left[HAc\right]_{\text{at equilibrium}}} = 10^{-4.75}$$

Suppose we were dealing with a 0.1 molar solution: $[HAc]_{\text{at the beginning}} = 0.1$ mol/L.

The concentration of the H^+ and Ac^- ions produced by protolysis (i.e. $[H^+]_{\text{at equilibrium}} = [Ac^-]_{\text{at equilibrium}}$) is initially unknown (i.e.: x), but since for each H^+ exactly one Ac^- is also produced (we know this from Eq. 5.3), we can say: $[H^+]_{\text{at equilibrium}} = [Ac^-]_{\text{at equilibrium}} = x$.

Accordingly, $[HAc]_{\text{at equilibrium}} = ([HAc]_{\text{at the beginning}} - x)$, so here $(0.1 - x)$.

When inserted into the LMA, the following is obtained:

$$K_A\left(HAc\right) = 10^{-4.75} = \frac{x^2}{0.1 - x}$$

This is a "completely normal" quadratic equation, to which, once it has been converted to 0, the pq-formula can be applied. (The fact that *two different* results are possible with this formula from a purely mathematical point of view should not worry us here, because negative concentrations understandably make no sense.)

$$\text{From } K_A = \frac{x^2}{0.1 - x} \text{ it becomes } x^2 + xK_A - 0.1\ K_A = 0.$$

Accordingly, we can now insert into the pq formula:

- for p: $K_S = 10^{-4.75}$
- for q: $0.1\ K_S$

That results in:

$$x = -\frac{10^{-4.75}}{2} \pm \sqrt{\left(\frac{10^{-4.75}}{2}\right)^2 + \left(0.1 \cdot 10^{-4.75}\right)}$$

$$= -\frac{10^{-4.75}}{2} \pm \sqrt{\left(\frac{10^{-4.75}}{2}\right)^2 + 10^{-5.75}} = 1.325 \cdot 10^{-3}$$

This gives x, i.e. the concentration of H^+ ions released by protolysis, and if you then calculate the logarithm to base 10 of this and invert the sign at the result, you get the pH value:

$$pH = -\lg\left[H^+\right] = -\lg\left(1.325 \cdot 10^{-3}\right) = +2.878.$$

Since it is extremely costly in terms of equipment (and, moreover, usually hardly sensible) to determine pH values with an accuracy of more than two digits behind the decimal point, the result obtained purely by calculation should be rounded off accordingly in a sensible manner. Thus: pH = 2.88.

The general formula for calculating the pH of weak acids (with a known K_S value) is then (with c_0 = initial concentration and c = concentration at equilibrium):

$$x = -\frac{K_A}{2} \pm \sqrt{\left(\frac{K_A}{2}\right)^2 + c_o \cdot K_A} \tag{5.9}$$

You will agree that this is quite a lot of calculating. But with a simple approximation we can make our whole laboratory life easier:

In the case of a weak acid, it is known that only (very) few acid molecules dissociate in aqueous solution—a few are, of course, otherwise weak acids in aqueous solution would not lead to pH <7, but by far the majority are *undissociated* in solution. This fact allows the simplifying assumption that the number of actually dissociating molecules is negligibly small compared to the number of *un*dissociated molecules.

If however $[H^+]_{\text{at equilibrium}} \ll [HAc]_{\text{at the beginning}}$, then one may simplifyingly assume that the concentration of the undissociated molecules HA also corresponds to the initial concentration of this acid at equilibrium (so : $[HAc]_{\text{at equilibrium}} = [HAc]_{\text{at the beginning}}$).

This gives the following approximate formula:

$$K_A\left(HAc\right) = 10^{-pK_A(HAc)} = 10^{-4.75} = \frac{\left[H^+\right]_{\text{at equilibrium}} \cdot \left[Ac^-\right]_{\text{at equilibrium}}}{\left[HAc\right]_{\text{at the beginning}}}$$

Accordingly, by rearranging (and using the general formula HA for all arbitrary *weak* acids) one obtains:

$$\left[H^+\right] = \sqrt{K_A \cdot \left[HA\right]_{\text{at the beginning}}}, \text{ and thus } pH = \tfrac{1}{2}\left(pK_A - \lg\left[HA\right]_{\text{at the beginning}}\right)$$

Our example with $[HAc]_{\text{at the beginning}}$ = 0.1 mol/L then leads to pH = ½ (4.75 – lg 0.1) = ½ (4.75 – (−1)) = ½ (5.75) = 2.88, which is *exactly* the result we obtained using the general pq formula, the Eq. 5.9 derived from it, and sensible rounding.

Thus, for weak acids, the approximate formula gives sufficiently accurate results.

Important

The general formula for the simplified calculation of the pH of the aqueous solution of **weak acids** is:

$$\mathbf{pH} = \tfrac{1}{2}\left(\mathbf{pK_A} - \lg \mathbf{c_0}\right) \tag{5.10}$$

(with c_0 = concentration of the acid at the beginning *and* c = concentration of the acid at equilibrium)

For weak bases, the pOH value can be determined accordingly via the pK_B value, and once you have that, you only need to consider that $pH + pOH = pK_W$ (Eqs. 5.1 and 5.2 apply to *any* aqueous solutions).

Thus, by rearranging, we obtain $pH = pK_W - pOH$, and under standard conditions:

(Yes, both books actually cover this topic in the same subchapter.)

$$pH = 14 - pOH$$

Harris, Section 10.2: Titration of a weak acid with a strong base
Binnewies, Section 10.2: Quantitative description of acid/base equilibria in aqueous solution.

Studying Tip

When using formulas such as Eq. 5.10, it is always advisable to carry out a **plausibility check**: *Can the results obtained with them be correct at all?* To illustrate this, let us compare the pH values obtained at the (initial) concentrations $c_{0(1)} = 1$ mol/L and $c_{0(2)} = 0.1$ mol/L for acetic acid with $pK_A = 4.75$:

- With $c_{0(1)} = 1$ mol/L, we arrive at $pH = ½ (4.75 - \lg 1) = ½ (4.75–0) = ½ (4.75) = 2.4$.
- For $c_{0(2)} = 0.1$ mol/L, this gives $pH = ½ (4.75 - \lg (0.1)) = ½ (4.75 - (-1)) = ½ (5.75) = 2.88$.

This makes perfect sense, after all the pH of the higher concentrated acid will be lower than that of its more dilute (less highly concentrated) counterpart.

If you keep an eye not only on the calculation results but also on the *chemical* conditions, the danger of performing nonsensical and wrong calculations is drastically reduced.

If the strength of the acid under consideration is too large to allow the above-mentioned assumption—i.e. if the number of dissociated molecules ($H^+ + A^-$) is *not* negligibly small compared to the number of undissociated molecules—this approximation formula is *no longer applicable*: When dealing with **moderately strong acids** ($0 < pK_A < 4$), only the use of the pq formula leads to sufficiently accurate results.

In the Buffer Area

After the first drops of base added to the aqueous solution of a weak acid have caused a slight, yet noticeable increase in the pH value (look again at the corresponding curve from Fig. 10.2 of Harris!), the pH value changes only insignificantly for quite a large amount of further acid added.

Harris, Section 10.2: Titration of a weak acid with a strong base

Responsible for this is again a *chemical equilibrium*:

- Prior to the addition of the base (and thus hydroxide ions which then enter the aqueous solution of the weak acid) the reaction mixture contained only the solvent water (H_2O), many undissociated acid molecules HA and, in addition, negligibly few actually deprotonated acid molecules A^- (i.e. the conjugate base to the acid HA) as well as the associated protons (which are naturally present in aqueous solution as H_3O^+).
- However, the newly added hydroxide ions from the base added now deprotonate further acid molecules: Further ions of the conjugated base (i.e.: A^-) are formed. Thus, both the undissociated acid itself (HA) and its conjugate base (the anion A^-) are now present in the reaction mixture.

- Such a mixture of a weak acid and its conjugate base (or a weak base and its conjugate acid) is called a **buffer,** because such solutions keep their pH practically constant upon *moderate* addition of further hydroxonium ions (e.g. by adding a strong acid) or further hydroxide ions (e.g. by adding a strong base).

Behind this buffer effect is a very simple system: If an aqueous solution contains such a buffer acid/base pair (HA/A^-), the following happens when further acid or base is added:

- If additional H^+ are added by acid addition, they react with the A^- to form HA, so that the molar ratio HA/A^- changes, but there are no additional *free* H_3O^+ ions in the solution, which would lower the pH value.

  (The added hydrogen cations protonate the conjugated base and not a solvent molecule, because the A^- ions are more basic (they have a smaller pK_B value) than the water molecules.)
- If one tries to increase the number of free OH^- ions in the solution by adding a strong base and thus increase the pH value, each newly added OH^- ion instead deprotonates a previously undissociated acid molecule HA to A^-. This way, too, only the molar ratio HA/A^- changes, but not the number of hydroxide or hydroxonium ions in solution.

The ratio of the concentrations of acid HA and its conjugate base A^- can be easily determined using the simplification from the previous Section: If we assume that a weak acid practically does not dissociate at all in water (i.e. it exists only as $HA_{(aq)}$), but on the other hand each HA molecule that comes into contact with a (far more basic!) hydroxide ion (added by the addition of a strong base) immediately splits off its proton, so that A^- ions are formed, then when a weak acid reacts with a strong base, the amount of substance of free A^- ions corresponds exactly to the

Amount of Substance of Added Hydroxide Ions

If you start with 1000 mL of a 1-molar solution of acetic acid (i.e. assumed initial concentrations: [HA] = 1.0 mol/L, $[A^-]$ = 0 mol/L) and then add 400 mL of 1-molar sodium hydroxide solution ($NaOH_{(aq)}$), approximately 40% of all acetic acid molecules are converted to acetate ions, while 60% of all acetic acid molecules remain unchanged. Accordingly, the ratio HA/A^- is 0.600 mol/0.400 mol = 6/4 or 3/2.

There is also a simple formula for calculating the pH value of such a buffer:

Important

The **Henderson-Hasselbalch equation:**

$$\mathbf{pH} = \mathbf{pK_A} - \lg\frac{[\mathbf{HA}]}{[\mathbf{A^-}]} \quad \textbf{or} \quad \mathbf{pH} = \mathbf{pK_A} + \lg\frac{[\mathbf{A^-}]}{[\mathbf{HA}]} \left(\textbf{this is a matter of taste}\right) \quad (5.11)$$

Again, [HA] is the concentration of the acid itself, and $[A^-]$ is the concentration of the corresponding conjugate base. Here you can also see how helpful the above-mentioned approximation with the HA/A^--quotient is—you can insert it directly into the equation.

■■ At What pH Does Such An Acid/Base Pair Do "the Buffering Thing"?

The Henderson-Hasselbalch equation leads to two other important insights about buffers:

Harris, Section 10.2: Titration of a weak acid with a strong base

1. At which pH value the buffer effect of a suitable acid/base pair is maximised depends on the pK_A value of the acid used (and thus also the pK_B value of its conjugate base). If you look again at Fig. 10.2 from Harris, you will see that the first inflection point of the curve, which lies exactly the middle of the buffer range, corresponds exactly to the pK_A value of the acid used. At this point, [HA] = [A⁻]. Thus, we are at the **half-equivalence point**. Here we know pH = pK_A, because once again Eq. 5.11 applies, and
$$\lg\left(\frac{x}{x}\right) = \lg 1 = 0.$$
2. The equation is concerned with the molar ratio of the concentrations of acid used and its conjugate base: An equivalent change of *both* concentrations (e.g. by dilution) does not change the pH range in which this buffer solution works.

► Example

Let us return again to acetic acid solution from the above example (with pK_A(HAc) = 4.75)—with [HA] = 0.600 mol/L and [A⁻] = 0.400 mol/L results according to the Henderson-Hasselbalch equation:

$$\mathrm{pH} = 4.75 - \lg\frac{[0.600]}{[0.400]} = 4.75 - \lg 1.5 = 4.75 - 0.176 = 4.57$$

(or, if you like that better, pH

$$= 4.75 + \lg\frac{[0.400]}{[0.600]} = 4.75 + \lg 0.666 = 4.75 + (-0.176) = 4.57)$$

If this solution is diluted to ten times its volume by adding water, the concentrations of both the acid (HA) and the base (A⁻) are correspondingly reduced to one tenth (i.e. [HA] = 0.060 mol/L and [A⁻] = 0.040 mol/L), but the quotient HA/A⁻ (or A⁻/HA) logically remains the same: 6/4 (4/6). ◄

A buffer buffers (that's really how you say that!) most efficiently in both pH directions when the amounts of the acid and its conjugate base are not only equal (i.e. exactly around the pK_A value of the acid used), but also as large as possible.

Important

A logical consequence of the Henderson-Hasselbalch equation:
As long as the molar ratio HA/A⁻ does not change, the pH value of the buffer solution does not change either.

■■ Why Are Higher Concentrations "Better" Than Lower Ones for Buffering Purposes?

For a solution to actually buffer, both a certain amount of free acid molecules HA and a certain amount of the conjugate base A⁻ are needed. The former can intercept newly added hydroxide ions, the latter react with newly added H^+ ions. However, with excessive amounts of H^+ or OH^- ions, HA or A⁻ will eventually be used up. And if no more acid or base molecules are available, the buffering effect ends.

Preparing Buffers Yourself

Assume you need a buffer which has an optimum buffering effect at pH = 4.75: Here you need equimolar amounts of an acid with $pK_A = 4.75$ and its conjugate base. What could be more obvious than to take acetic acid and an acetate salt (ideally one that is readily soluble in water)? You put both substances into a volumetric flask in the desired (ideally precisely determined) amount in each case, fill up to the calibration mark and then know not only that the solution really buffers at pH = 4.75, but also what its concentration is.

And if the buffer area doesn't quite match what you need?—Again, a look at Fig. 10.2 from Harris and the Henderson-Hasselbalch equation will help:

The figure shows that there is no buffer *point*, but a **buffer area**, and as a rule of thumb we can say:

A weak acid and its conjugate base buffer by $pK_A \pm 1$ pH unit.

If the solution is now to buffer at pH = 5.75, for example, you julz need ten times as much of the conjugate base as of the acid, according to Eq. 5.11. (Perhaps you would like to calculate this yourself?)

In the case of such buffer solutions with a *shifted* range of action, it should be borne in mind that if they contain, for example, much more base A^- than acid HA, the pH value of the solution is kept almost constant with moderate acid addition, but the buffering effect is *much weaker against bases*. The reverse is also true if the buffer range is shifted *below* $pH = pK_A$ by an excess of acid.

If the molar ratio HA/A^- becomes even more extreme than 10:1, the buffer effect is effectively lost. It is not advisable to try to shift the buffer range further by adding even more extreme amounts of acid or base. When in doubt, use a different acid/base pair.

■ With $\tau = 1$

At the equivalence point, exactly equimolar amounts of base were added to the acid, i.e. the analyte, present in the solution at the beginning. However, since the acid HA contained in the analyte solution is weak ($pK_A > 4$), its conjugate base A^- is a weak base, as well (remember: $pK_A(HA) + pK_B(A^-) = 14$), so the neutral point (pH = 7) does *not* coincide with the equivalence point. Instead, it lies in the basic range (i.e. pH > 7). Why?—Because the conjugate base of the analyte acid is itself basic enough to raise the pH value.

Attention

Please avoid misinterpretation of the terms "strong" and "weak":

1. *By definition,* an acid with $pK_A < 0$ or a base with $pK_B < 0$ is called strong. All other acids are "medium" or "weak".
2. The conjugate base of a weak acid is *relatively strongly* basic, so it does exhibit basic behaviour, but it is *by no means a strong base.*

Let's look at two important examples: Acetic acid (CH_3–COOH, HAc) and ammonia (NH_3):

- $pK_A(HAc) = 4.75$; correspondingly, for the conjugate base of this acid $pK_B(Ac^-) = 14 - pK_A(HAc) = 9.25$.
- According to the tables, $pK_B(NH_3) = 4.75$ for ammonia, and $pK_A(NH_4^+) = 9.25$ for the conjugate base of ammonia, the ammonium ion NH_4^+, according to Eq. 5.6.

Let us now consider the behaviour of the two substances in aqueous solution:

- Undoubtedly, acetic acid acts as an acid towards water: even for a very dilute solution of it, we know pH < 7.
- Only small amounts of ammonia (gaseous at room temperature) need to be dissolved in water to reach pH >7: Ammonia reacts unmistakably basic.
- On the other hand, the aqueous solution of the salt sodium acetate (consisting of Na^+ and Ac^- ions) also reacts clearly basic. The conjugate base of acetic acid thus possesses basic properties—only its basic character is less pronounced than the acid character of the acid.
- Ammonium salts, on the other hand (e.g., ammonium chloride, NH_4Cl), react acidic thus providing pH < 7 in aqueous solution. Again, we see: The base ammonia (NH_3) is more basic than its conjugate base, the ammonium ion, is acidic, but *both* properties play an important role in chemical behaviour.

If one compares the pK_A values of the two acids (HAc and NH_4^+) directly with each other (4.75 and 9.25), one comes to the conclusion that acetic acid, whose acid strength is really not overly impressive, is still more than *four orders of magnitude* more acidic than the ammonium ion, but nevertheless the conjugate acid to the base ammonia also shows undeniably acidic behaviour.

The conjugate acid of a weak base is of course not a "strong acid" in the sense of the definition, but it is still "quite strong".

▪ When Exceeding the Equivalence Point

Beyond the equivalence point, the acid that was present in the analyte solution at the beginning is completely used up: Now only the titrant affects the pH of the solution. Since a weak acid has reacted with a strong base in this case, exactly what was said about "beyond the equivalence point" in ► Sect. 5.1 applies.

▪▪ Weak Bases Titrated Against a Strong Acid

Everything that has been said so far regarding weak acids and their conjugate bases is completely transferable to weak bases and their conjugate acids. It should be understandable that the corresponding titration curves look somewhat different if you start in the basic range and gradually lower the pH value by successive additions of acid, but in principle they have the same shape and the same course. If necessary, look at the illustration on the inside back cover of the book at the bottom of Harris; it is explained again in detail in the associated chapter.

Harris, Section 10.3: The titration of a weak base with a strong acid

5.3 Polyprotic Acids

From *general* and/or *inorganic chemistry*, you are also familiar with *polyprotic* acids, i.e. molecules that—assuming the presence of a sufficiently basic reaction partner—can also split off more than one H^+ ion. For such polyprotic acids (in older terminology: polybasic acids—nowadays, such substances should no longer be called that, but at least passively you should be familiar with this term), there is a separate pK_A value for each deprotonation step (pK_{A1}, $pK_{A2,}$ ...). It should be understandable that the multiple deprotonated forms become correspondingly less and less acidic with increasing negative charge, resulting in correspondingly increasing pK_A values. Let us take a look at two important examples:

▪▪ The Sulfuric Acid (Diprotic)

In the case of sulfuric acid, there are two deprotonation steps:

$$H_2SO_4 \rightleftarrows HSO_4^- + H^+ \qquad pK_{A1} = -3 \qquad \text{strong acid}$$

$$HSO_4^- \rightleftarrows SO_4^{2-} + H^+ \qquad pK_{A2} = 1.92 \qquad \text{medium strength acid}$$

The Phosphoric Acid (Triprotic)

Accordingly, there are three deprotonation steps here:

$$H_3PO_4 \rightleftarrows H_2PO_4^- + H^+ \qquad pK_{A1} = 1.96 \qquad \text{medium strength acid}$$

$$H_2PO_4^- \rightleftarrows HPO_4^{2-} + H^+ \qquad pK_{A2} = 7.21 \qquad \text{weak acid}$$

$$HPO_4^{2-} \rightleftarrows PO_4^{3-} + H^+ \qquad pK_{A3} = 12.32 \qquad \text{acid is so weak,}$$

that the *conjugate base* is of medium strength again

Harris, Section 9.1: Diprotic acids and bases
Harris, Section 9.2: Two-proton buffers
Harris, Section 9.3: Polyprotic acids and bases
Harris, Section 9.4: What is the main species?
Harris, Section 9.5: Equations for calculating the proportions of individual shapes

Accordingly, for the medium-strong or weak acids there are several buffer regions (shown schematically in Fig. 9.2 from Harris), and for these again everything that we have already discussed in ► Sect. 5.2 applies. Accordingly, this topic should not be given too much space here; in Harris you will find detailed explanations of diprotic and polyprotic acids, including all regions in the associated titration curves.

Polyvalent Bases

Harris, Section 10.4: Titration in diprotic systems

Again, what has been said about polyprotic acids also applies analogously to compounds/ions that can take up more than one hydrogen cation. A corresponding example for the titration of a diprotic base can also be found in Harris.

Questions

1. Calculate the pH value of:
   a) 0.1 molar hydrobromic acid (pK_A (HBr) = −8.9)
   b) 0.01 molar hydrochloric acid (pK_A (HCl) = −6.2)
   c) 0.2-molar acetic acid (pK_A (HAc) = 4.75)
2. Find the molar ratio of acid and conjugate base for the following buffers:
   a) Acetic acid/sodium acetate buffer at pH = 5.05
   b) Acetic acid/potassium acetate buffer at pH = 3.87
   c) Ammonium chloride/ammonia buffer at pH = 10.0

5.4 Endpoint Determination

To draw a titration curve, it is necessary to have ways and means of checking the pH value of the solution under investigation at regular intervals or (ideally) even continuously in the course of the associated titration. The latter can be done quite easily with the aid of a pH electrode. However, if the aim is merely to determine the *end point* of a titration, the use of an *indicator* is usually sufficient. Let's take a brief look at both techniques.

pH Electrodes

Harris, Section 10.5: Determining the end point with a pH electrode

These electrodes determine the pH value *electrochemically*: different concentrations of hydroxonium ions cause different electrochemical potentials (see the Nernst equation, known from *general chemistry* and/or *inorganic chemistry*); this concentration-dependent potential is measured against a reference electrode. (However, this already brings us to the subject of *potentiometry,*

which has its own subchapter in ► Sect. 10.3. Therefore, we will leave it at this point; of course, you can already take some first remarks on this from Harris.)

pH Indicators

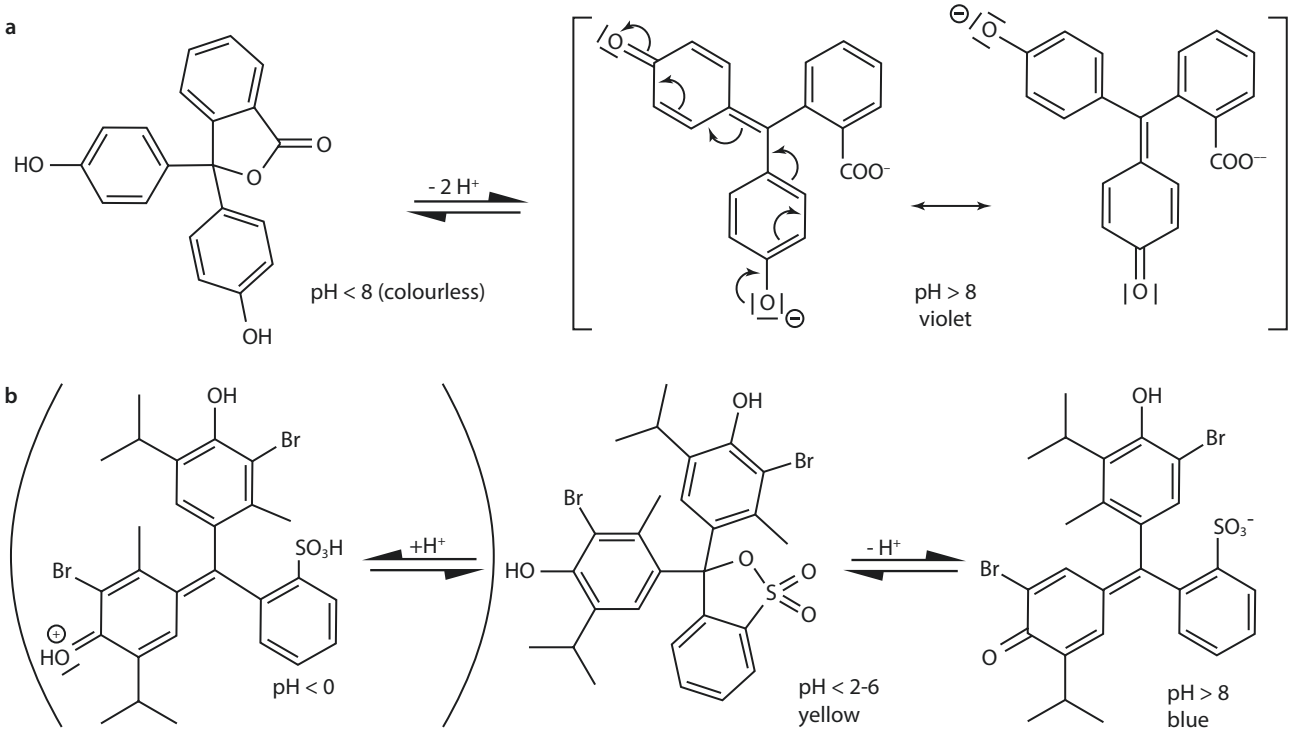
Chemically, the principle of pH indicators is very simple: they are (mostly organic) compounds which themselves represent a Brønsted acid or base and produce different colorations in aqueous solution depending on the degree of (de)protonation: A **colour change** results (optionally from one colour to the other, occasionally also from colourless to one or the other colour).

- A typical example of a "from colourless to ..." indicator is *phenolphthalein* (◘ Fig. 5.2a), which is colourless in aqueous solution below pH = 8.2, but turns violet above this pH.

  Behind this is the simple deprotonation of the weak acid phenolphthalein (pK_A = 9.7). The protonated form is colourless, the deprotonated form is violet. (By the way: Please pronounce this substance "phenol-phthalein").
- Bromothymol blue (◘ Fig. 5.2b, pK_A = 7.1) is a little more versatile: Between pH = 2 and pH = 6, the aqueous solution of this indicator is yellow; if the pH is increased further (by base addition), the solution turns green, and at pH > 8 it becomes blue.

  The green shade can be explained as the mixed colour of the non-deprotonated (yellow) and deprotonated (blue) forms; conveniently, this shade of green is most apparent just at pH = 7, giving us an excellent neutral point indicator.

  Interestingly, in a really strongly acidic medium (pH < 0, achievable e.g. with concentrated sulfuric acid or perchloric acid), the indicator turns *red* due to further protonation. (However, this can hardly be exploited for analytical purposes.)



◘ **Fig. 5.2** The acid/base behaviour of **a** phenolphthalein and **b** bromothymol blue

Harris, Section 10.6: Endpoint determination with indicators

Each indicator is therefore associated with the pH range for which it is particularly suitable. Accordingly, for practical application it is necessary to select a suitable indicator for the pH range to be considered. The use of phenolphthalein, for example, would hardly be effective if the end point of your titration were to be expected in a weakly acidic medium at pH = 4–6. A selection of commonly used indicators together with their characteristic colours and their respective ranges can be found in Table 10.3 of Harris.

Attention

There are two things to strongly consider when using indicators:

The colour change does not happen abruptly

Please keep in mind that every (de)protonation reaction represents a chemical equilibrium: As is well known, a pK_A value of 9.7 does not mean that the corresponding (weak) acid molecules in an aqueous solution of pH = 9.7 will abruptly all split off their acidic proton, but that at this pH value (statistically) exactly half of all acid molecules HA in solution are deprotonated to their conjugate base A^-. (This is exactly what the Henderson-Hasselbalch equation says, i.e. Eq. 5.11.) Since this is the case, it should not be surprising that the colour change does not occur abruptly at the pK_A value of the indicator, but "creeps in", so to speak. Accordingly, it is relatively difficult to pinpoint the change point really *unambiguously with* the help of an indicator. (However, this is also possible: with the help of the specialist field of colorimetry, to which we turn in ► Sect. 10.1.)

"A lot helps a lot" does not apply to indicators

If you want to "observe" an acid/base equilibrium with the help of a pH indicator, you should not forget that the indicator molecules themselves are also acids or bases in Brønsted's sense. Therefore the following equilibrium reactions can be found again and again in the literature:

$$H-Ind \rightleftarrows H^+ + Ind^-, \quad \text{if the indicator represents a } \textit{Brønsted} \text{ acid, or}$$

$$Ind + H^+ \rightleftarrows H-Ind^+, \quad \text{when the indicator acts as a } \textit{Brønsted} \text{ base.}$$

Accordingly, with each indicator molecule you in fact interfere with exactly the acid/base reaction that you actually only wanted to "observe". However, as long as you hold back on adding the indicator, there should be no excessive deviation from the "true" measured value. However, it is really advisable to use your indicator acid or base only in as small quantities as possible (the quantities should, however, be large enough to be able to detect anything *at all*). The (often wrong) maxim "a lot helps a lot!" is definitely no good advice for titrations.

How to Get the Desired Accuracy

"[HAc] = 0.1 mol/L"—how accurate is that? This is not only about the (supposed) accuracy of the specification (keyword: significant figures, from Part I), but actually also about the accuracy with which the described solution was prepared:

What is written on a bottle or flask label does not necessarily have to be correct (anymore)! When the solvent gradually evaporates from a solution, the concentration of the solution gradually increases, and aqueous solutions of substances that are gaseous at room temperature may over time *gas out* of the solute at least partially (causing the concentration of the solution to gradually decrease).

If one wants to work accurately, one must therefore add a correction factor that indicates the difference between the actual concentration—the "actual concentration" c_{is}—and the originally desired concentration—the "target concentration" $c_{desired}$:

$$f = \frac{c_{is}}{c_{desired}} \tag{5.12}$$

This correction factor f is called the **titre**, sometimes also called the *normal factor*.

However, this poses a problem: To determine the titre of a solution, a solution with a precisely defined concentration is required. But there is a solution (no pun intended) to this supposed vicious circle: One uses an **primary standard**—a substance with which solutions of very precisely defined concentrations can be prepared (so-called **standard solutions**).

Such a primary standard must have some special properties:

- It must have a well-defined chemical composition and react completely.
- It must dissolve well in the respective solvent (usually: water).
- It should have an indefinite shelf life and not change its composition over time:
  - It should not react with atmospheric oxygen.
  - It should not mind any humidity either.
  - It should not show any hygroscopy (i.e. it should not "draw water", as they say in laboratory jargon); after all, the mass would gradually change due to the attached water molecules.
  - And if life were perfect, any solutions made up of these primitives would also have *all* of these properties.
- In addition, an original titre should have as high a molar mass as possible in order to minimise the weighing error: For example, if you want to weigh exactly 1.00 mol of a substance, and the molar mass is, say, 15 g/mol, you will get a larger (percentage) weighing error than would be the case with a molar mass of, say, 250 g/mol.

Questions

3. What is the factual concentration of an ostensibly 0.23-molar acetic acid with f = 1.09?
4. Find the titre of a 0.25-molar sodium hydroxide solution with the actual concentration [NaOH] = 0.26 mol/L.

Volumetric Analysis with Complexes (Complexometry)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_6

Summary

In case you want to brush up on your basic knowledge of "complexes in general", I recommend taking a look at Binnewies, especially the sections about stability constants and the special features of chelate complexes. (By the way, said book also offers a short first insight into complexometry in Section 12.6.)

Binnewies, Section 12.4: Description of ligand exchange reactions by stability constants
Binnewies, Section 12.5: Chelate complexes

Especially the Sections on stability constants (Section 12.4) and the special features of chelate complexes (Section 12.5)

6.1 Combination of Two Principles

Two basic principles are used in complexometry:

1. Most metal atoms (especially in ionic form), particularly metals from the d- or f-block of the periodic table, can be regarded as electrophiles, i.e. as Lewis acids, which form complexes with correspondingly sufficiently nucleophilic Lewis bases by forming coordinative bonds. These electron-donating Lewis bases can interact with the metal, designated as the central particle of the complex, via a single atom (in which case they are referred to as monodentate ligands), or via *more* than one such coordination site (in which case a monocyclic or oligocyclic structure results). In the latter case, one is dealing with a multidentate ligand or chelate ligand; one then also speaks of a chelate complex. Whether monodentate or multidentate ligands L are involved: Behind the formation of a complex there is always a (multi-stage) equilibrium reaction:

$$\mathrm{M}^{x+} + \mathrm{n\ L} \rightleftarrows \left[\mathrm{ML}\right]^{x+} + \left(\mathrm{n}-1\right)\ \mathrm{L} \rightleftarrows \left[\mathrm{ML_2}\right]^{x+} + \left(\mathrm{n}-2\right)\ \mathrm{L}$$
$$\rightleftarrows \left[\mathrm{ML_3}\right]^{x+} + \left(\mathrm{n}-3\right)\ \mathrm{L} \rightleftarrows \left[\mathrm{ML_4}\right]^{x+} + \left(\mathrm{n}-4\right)\ \mathrm{L}\ \text{etc.}$$

2. For statistical and thermodynamic reasons, chelate complexes are usually much more energetically favourable (and therefore more stable) than comparable complexes with monodentate ligands. Therefore, monodentate ligands on a central particle can often be easily displaced by chelate ligands.
   - Once a chelate complex has been formed (in this way or otherwise), a comparable *ligand exchange* can only take place if the second, third, fourth etc. bond of the chelate ligand is also broken practically simultaneously after the first one. This is, from a purely statistical point of view, improbable.
   - A chelating ligand releases *several* monodentate ligands previously bound to the central particle; thus, after exchanging several monodentate ligands for a chelating ligand, there are *more* free particles in solution than before. Consequently, the entropy in the system increases ("more disorder"), and this is thermodynamically favourable.

Understandably, the stability of those chelate complexes increases with increasing **denticity** of the chelate ligand. ◘ Figure 6.1 shows an example of the bidentate ligand ethylenediamine (a), once in the non-complexed state, once in interaction with any metal ion of unspecified (positive) charge (M^{x+}), the tridentate ligand diethylenetriamine (b) and the tetradentate ligand nitrilotriacetic acid (c), shown here in the triply deprotonated form, i.e. the nitrilotriacetate ion.

Basically, there are numerous ways to use (chelate) complexes in analytical chemistry; here are the most common ones:

- Some multidentate ligands practically "do not let go" of a metal ion once it has been complexed: Metal ions in solution react quantitatively (i.e. com-

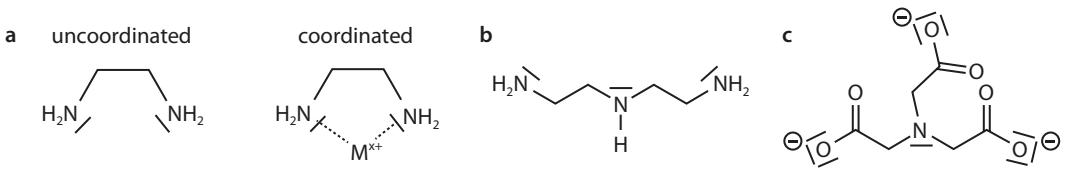


Fig. 6.1 Selected muldidentate ligands: (**a**) ethylenediamine; (**b**) diethylenetriamine; (**c**) nitrilotriacetate ion

pletely) with such complexing agents; side reactions are practically impossible. Accordingly, volumetry can be carried out with a complexing agent solution of precisely defined concentration, and the concentration of the metal ion (= analyte) present can be deduced from the consumption of the titrant.

- Some metal ions form such poorly soluble compounds with one or the other polydentate ligand that the resulting complexes are even used in *gravimetry* (more on this topic in ► Chap. 9).
- Many (especially transition metal) complexes show characteristic colours whose intensity correlates more or less linearly with their molar concentration. Of course, this can also be exploited—depending on which technique is chosen, *colorimetrically* (► Sect. 10.1) or *photometrically* (► Sect. 10.2).

The first application of complex chemistry mentioned here is by far the most common in analytics, therefore we will largely confine ourselves to it in this Section.

The multidentate ligand that is by far the most frequently used (and therefore also discussed here) is EDTA. Even Harris does not delve much deeper into the topic of complexometry.

Harris, Section 11.7: Titration methods with EDTA

What This Part Does *Not* Offer

Complex chemistry is a rather complex subject: Even if it is quite helpful in analytical chemistry to assume that a central particle interacts with six monodentate ligands (or a correspondingly reduced number of multidentate ligands), this simplified view of things simply does not do justice to the whole complex subject. For example, it is not easy to predict *which* central particle interacts with *which* (and how many) ligands and which spatial structure the resulting complex then has.

Just one example: While silver cations (Ag^+) form linear diammine silver(I) cations ($[Ag(NH_3)_2]^+$) with two ammonia molecules each, the copper(II) cation (Cu^{2+}) reacts in the presence of ammonia to form the square-planar tetraammine copper(II) cation ($[Cu(NH_3)_4]^{2+}$)—which, to be *even more* precise, would be better described as a tetraammin*diaqua*copper(II) cation ($[Cu(NH_3)_4(H_2O)_2]^{2+}$), in which the two water molecules acting as ligands represent the two tips of a tetragonal bipyramid above and below the square-planar tetraammine copper(II) base. It hopefully goes without saying that the VSEPR model cannot be used to predict the three-dimensional structure of such complexes—as is well known, this model *only* works if the central particle comes from the periodic table's main groups, but not for representatives of the d- or f-block.

In short: Since this chapter is "only" about the *applicability* of complexes in the context of analytical chemistry, not about "complex chemistry itself", you will have to just accept the complexes that appear here. The question of *why* which structures form in each case will be addressed in (advanced) courses or textbooks of *inorganic chemistry*.

6.2 Versatile and Widely Used: EDTA

Of particular importance are the hexadentate ligand ethylenediaminetetraacetic acid and its tetra-anion ethylenediaminetetraacetate, **EDTA** for short, derived from it (◘ Fig. 6.2a). The latter coordinates via the free electron pair of each of the two nitrogen atoms and via the four deprotonated carboxyl groups, and it does so with practically every conceivable metal cation, almost irrespective of its charge or size. This results in an oligocyclic structure (as mentioned above): The two nitrogen atoms form a five-membered ring with the central particle of the complex via the CH_2–CH_2 (ethylene) bridge connecting them, and basically the same goes for any coordination between one of these nitrogen atoms and one of the four deprotonated carboxyl groups. The complexed Lewis acid (M^{x+}) is octahedrally surrounded by the six coordination centres (◘ Fig. 6.2b).

However, before we turn to the usability of EDTA in analytics, some basics should first be touched upon:

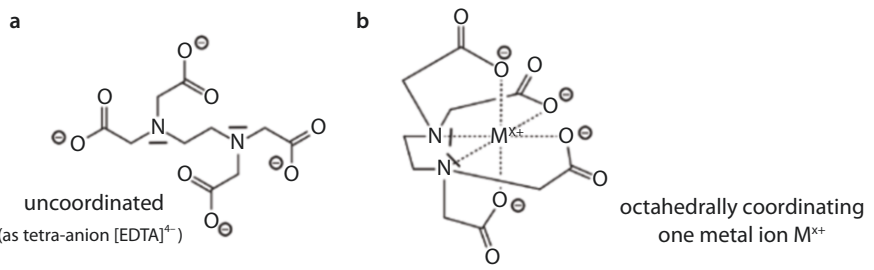
If you look again at ◘ Fig. 6.2, you will see that ethylenediaminetetraacetic acid is present in such complexes as a tetra-anion, so strictly speaking one would always have to speak of "$EDTA^{4-}$"—but nobody does that. Nevertheless, it is important to consider that the neutral molecule ethylenediaminetetraacetic acid can act both as a (tetraprotic) acid and as a base (the latter even twice: due to the two N atoms). In addition, the carboxylic acid groups of this molecule are acidic enough to protonate the molecule's "own" nitrogen atoms, so that ethylenediaminetetraacetic acid exists as a **zwitterion** (German for: "*hermaphrodite ion*") in aqueous solution (◘ Fig. 6.3).

Since the four carboxyl groups are equivalent, it is irrelevant which two of the total of four have now acted as an acid towards the nitrogen atoms. Furthermore, this acid can still split off a total of four protons: It remains tetraprotic, since the (now) protonated nitrogen atoms, which are the conjugate acid of the base "nitrogen atom with free electron pair", also have a pK_A value (we had this in ► Sect. 5.2).

Thus, one would actually always have to consider the following acid/base equilibria:

$$H_4EDTA \rightleftarrows H_3EDTA^- + H^+ \rightleftarrows H_2EDTA^{2-} + 2\ H^+ \rightleftarrows HEDTA^{3-} + 3H^+ \rightleftarrows EDTA^{4-} + 4\ H^+$$

But again, nobody does this; it is tacitly assumed that the system already adapts itself accordingly to the pH value present in each case (which is not discussed any further). Accordingly, "EDTA" in complexometry almost always means: "The particle that forms stable chelate complexes with metal ions and whose effective total charge is currently not of importance."



◘ **Fig. 6.2** The hexadentate Ligand EDTA; (**a**) uncoordinated and (**b**) octahedrally coordinating a central particle

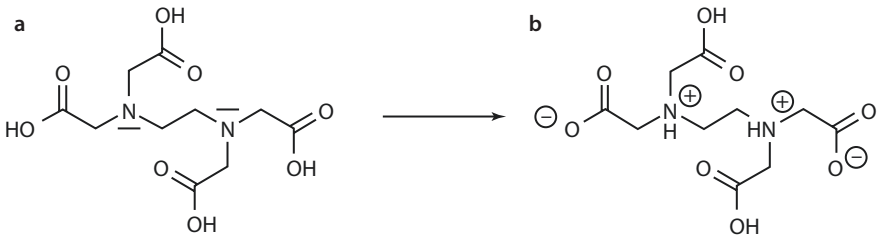


Fig. 6.3 Ethylenediaminetetraacetic acid as a zwitterion

A Simple Ratio

One of the reasons why EDTA is so popular as a complexing agent in analytical chemistry is that the central particle and the ligand practically *always* react with one another in a ratio of 1:1—the stoichiometry of such reaction equations thus remains pleasingly manageable. However, the vast majority of EDTA complexes are colourless, so that the *end point* of the titration cannot be observed while simply adding the titrant drop by drop to the analyte solution. For this reason, one or the other **metal indicator** is frequently used in complexometry (especially with EDTA). Basically, this is "just" another (complexing) ligand ... which, however, must have two special properties:

1. It must form an only *moderately stable* complex with the metal ion to be quantified (i.e. the analyte)—in any case, the interaction between the central particle and the metal indicator ligand must be weak enough that a stronger ligand (namely EDTA) easily displaces it from the complex, so that the EDTA, as mentioned above, quantitatively complexes the analyte.
2. The metal indicator must show a colour in solution both in the free state (i.e. uncomplexed) and in the interaction with the analyte ion—but show *different* colours in each case. In addition, the colour of the metal indicator-analyte complex should be more intense than the colour of the free metal indicator:
   - Metal indicator (uncomplexed): *Colour 1*
   - Metal indicator (complexed): *Colour 2*

As is usual with indicators, the metal indicator is not used in stoichiometric quantities: after all, it is only intended to serve as an indicator. Therefore, minimal amounts should be sufficient—just enough to allow the colour of the analyte-indicator complex to be clearly recognised.

If both criteria are met, complexometry works according to the following principle:

First, the (as yet uncomplexed) metal indicator (Ind for short) is added to the analyte solution (which contains M^{x+} ions):

$$Ind\ \left(Colour\ 1\right) + M^{x+} \rightleftarrows \left[M - Ind\right]^{x+}\ \left(Colour\ 2\right)$$

Subsequently, the thus characteristically coloured analyte-metal indicator solution is titrated against EDTA. Here, two different reactions must be taken into account, which take place one after the other. Initially, the solution still contains plenty of uncomplexed analyte ions M^{x+} despite the addition of the metal indicator. These are now reacted with EDTA. This is usually done at pH values at which the free ethylenediaminetetraacetic acid (H_4EDTA) is already at least doubly deprotonated, i.e. H_2EDTA^{2-} is present. Accordingly, the following schematic reaction equation is obtained (which also shows why it is advisable to *buffer* the analyte solution during complexometry):

$$M^{x+} + H_2EDTA^{2-} \rightleftarrows \left[M - EDTA\right]^{(x-2)+}\left(colourless\right) + 2\ H^+$$

With further EDTA addition, the point is finally reached at which (practically) no more free M^{x+} ions are present. (Strictly speaking, the EDTA-complexation of the free analyte ions shifts the M^{x+}/[M-Ind]$^{x+}$-equilibrium in the direction of the free M^{x+}-ions, in the sense of Le Chatelier, but we may ignore this fact at this point, since we have added only minimal amounts of metal indicator anyway).

Finally, criterion 1 for metal indicators comes into play: they must be displaceable by EDTA, according to the following schematic reaction equation:

$$\left[\mathrm{M}-\mathit{Ind}\right]^{x+}\left(\mathit{Color}\ \ 2\right)+\mathrm{H_2EDTA}^{2-}\rightleftarrows\left[\mathrm{M}-\mathrm{EDTA}\right]^{(x-2)+}\left(\mathit{colourless}\right)+\mathit{Ind}\left(\mathit{Color}\ \ 1\right)+2\ \mathrm{H}^{+}$$

Since EDTA is colourless both in its uncomplexed form and in interaction with the analyte ion M^{x+}, the equivalence point of this reaction can be recognised by the colour change:

- Before reaching the equivalence point, the reaction mixture shows *colour 2*—the colour caused by the analyte-metal indicator complex.
- At the equivalence point all metal indicator molecules Ind have been displaced by EDTA, i.e. all analyte ions are "taken out of" the metal indicator complex. Accordingly, at the equivalence point the colour of the free metal indicator, *colour 1,* can be seen.
- During further titration (i.e. overtitration) only further colourless EDTA titrant is added to the analyte solution, so that no further colour change can be observed (except that the colour of the free metal indicator is further diluted, due to further EDTA titrant addition). Accordingly, the colour change from colour 2 to colour 1 allows a fairly precise determination of the equivalence point.

Lab Tip

However, as already mentioned in ► Sect. 6.1, behind every formation of a complex there is also a (multistage) equilibrium reaction, and this equilibrium can react quite touchy to changes in pH value;, after all, in a sufficiently acid medium the equilibrium

$$\mathrm{M}^{x+}+\mathrm{H_2EDTA}^{2-}+2\ \mathrm{H_2O}\rightleftarrows\left[\mathrm{M}-\mathrm{EDTA}\right]^{(x-2)+}+2\ \mathrm{H_3O}^{+}$$

will shift with decreasing pH value further and further to the *reactants* side. For this reason, it is strongly recommended to protect the analyte solution from excessive pH changes by means of a suitable buffer system.

Harris, Section 11.7: Titration methods with EDTA

In addition to the very elegant use of an appropriate metal indicator for the *direct titration* of the analyte against EDTA, as we just had in ► Sect. 6.2, Harris knows several other ways to determine the endpoint of a complexometric titration:

- In *back titration,* which becomes necessary when the analyte forms a poorly soluble compound with EDTA (i.e. precipitates), EDTA is used in excess. (Again, a precisely defined amount of EDTA is used!) Then a second reagent is used to determine the amount of EDTA that is still free and has *not* precipitated with the analyte.
- When dealing with an analyte that, one knows, is capable of quantitatively displacing another metal ion from the metal-EDTA complex because the analyte-EDTA complex is significantly more stable, one can perform a corresponding *displacement titration.* Nevertheless, the principle remains the same.

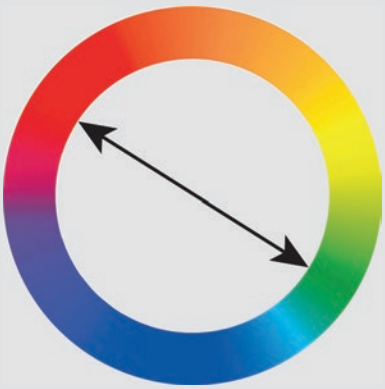
Where Does the Colourfulness Come From?

No doubt you are familiar with the electromagnetic spectrum from *general chemistry* and/or *physics*. (If not, take another look at Binnewies (Fig. 26.20) or Harris (Fig. 17.2). That should help.) *Visible light* is only the comparatively tiny part of the whole spectrum, with a wavelength of 380–780 nm.

Binnewies, Section 26.5: Optics

Harris, Section 17.1: Properties of light

Now the peculiarity of our biological detectors for such electromagnetic radiation (we call them: the eyes) comes into play: We perceive light that contains *all of* these wavelengths as *white* (colourless). However, if certain wavelengths are *removed* from this light (for example, by interaction with a substance that is capable of absorbing precisely these wavelengths), so that the human eye only catches the remaining part of the spectrum, the human brain reports the *complementary colour to the missing wavelengths*. The respective complementary colour to a wavelength can be determined using the colour wheel.



If, for example, a certain red component (i.e. rather long-wave light from the 650–800 nm range) is removed from white light, the object that absorbs this wavelength to the human eye appears to have the corresponding complementary colour, i.e. green. (If more than one wavelength is absorbed at the same time, it becomes a bit more complicated to determine the complementary colour, but the principle remains the same.)

And what decides which wavelengths can be absorbed by a molecule or complex or the like (and which cannot)?—We will deal with this topic in slightly more detail in Part IV, but you will already get a first insight in ► Sect. 10.2.

Questions

5. What is present in solution upon the direct complexometric titration of an analyte cation with EDTA the moment you reached the equivalence point?
6. In the direct titration of your analyte solution (V(analyte) = 20.00 mL) against EDTA solution, 10.42 mL of ($c_{desired}$ (EDTA) = 0.1 mol/L, f = 1.03) this was consumed until the equivalence point was reached. Report the concentration of your analyte in mmol/L.

A Combination with Considerable Potential: Redox Titrations

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_7

Summary

The principle of volumetry can also be combined with redox reactions: This procedure is particularly useful if the analyte can be easily oxidised or reduced. In this case, "only" an oxidising or reducing agent solution is selected as the titrant, and the consumption of reagent is determined volumetrically until the analyte has been completely oxidised (or reduced). This way, redox titration corresponds in principle to acid/base titration.

In principle, redox titrations can be carried out with practically any reducing or oxidising agent, as long as the concentration of the titrants to be used can be determined with appropriate accuracy (*see* standard solutions) and one is properly informed about the respective redox behavior.

The redox behavior of oxidising or reducing agents always depends on their electrochemical potential, which is primarily determined by two factors:

- the standard potential (E^0) of the corresponding redox pair, i.e. where it is located in the voltage series,
- the respective concentrations of oxidised and reduced (or better: less highly oxidised) form. The concentration dependence of potentials is described by the Nernst equation.

Binnewies, Section 11.3: Voltage series and standard electrode potential
Binnewies, Section 11.4: The Nernst equation

You have learned the basics of redox reactions in *General and Inorganic Chemistry*; if necessary, you will find a summary of the most important aspects in Binnewies.

Some redox pairs that are particularly popular in analytical chemistry should be known:

- The redox pair iodine/iodide (I_2/I^- with $E^0 = 0.54$ V) represents the basis of **iodometry**; it is particularly versatile because it can be used for both oxidisable and reducible analytes.
- The standard potential of the redox couple dichromate/Chom(III) (Cr_2O_{72}-/Cr^{3+}) is $E^0 = 1.23$ V.
- Chemically similar, but with an even slightly higher standard potential, is the chromate/chromium(III) pair ($E^0(CrO_4^{2-}/Cr^{3+}) = 1.40$ V).
- **Permanganometry** exploits the strong oxidation effect of the permanganate ion (MnO_4^-): The standard potential of this redox pair is $E^0(MnO_4^-/Mn^{2+}) = 1.51$ V. (Please note that the permanganate is only reduced to the oxidation number +II in a (strongly) acidic medium: In a basic medium, you get manganese dioxide (MnO_2) instead, where the manganese has oxidation number +IV.)
- Finally, **cerimetry** takes advantage of the fact that tetravalent cerium can be easily reduced to the oxidation state +III: E^0 (Ce^{4+}/Ce^{3+}) = 1.61 V.

Harris, Section 15.1: The shape of the redox titration curve

Table 15.1 from Harris offers you a wide range of other reducing and oxidising agents that are also highly valued in analysis.

7.1 Short-Circuited

Ultimately, every redox reaction is a short-circuited battery (as in the case of a **local cell**): there is a direct transfer of electrons from the reduced (base) reaction partner to the oxidised (noble) reaction partner. This can also be used in analysis: The analyte is oxidised (or reduced), i.e. it donates electrons (or accepts them), while the titrant represents the oxidising or reducing agent. In this way, the electrochemical processes in the reaction mixture can be described with the usual partial equations—without any usable electric current flowing.

The stoichiometry of such redox reactions is generally quite easy to understand, and so redox titrations, if the endpoint of such a titration can be determined

well (more on this in Sect. 7.3), can obviously be used similarly well for e.g. the quantification of a corresponding analyte, as is the case with acid/base titrations.

However, just as with the acid/base reactions, the determination of the end point alone provides only part of the achievable information. Accordingly, it is advisable to draw up the corresponding titration curves. In order to obtain such curves, one must "measure" an easily quantifiable characteristic of the reaction solution at regular intervals.

For acids and bases, the pH-value was chosen for this (obviously), but what do you take for redox-reactions?—Even though no usable current flows in these "short-circuited batteries", it is quite easy to determine what current *would* flow based on the potential difference of the electron-donating and electron-accepting "half-cell". In other words, one need only determine the electrochemical potential within the cell.

7.2 Reference Values

Here, too, electrodes help us. In ► Sect. 5.4 you learned that pH electrodes do not actually measure the pH value itself, but rather the electrochemical potential of the H_3O^+ ions present in the solution (the concentration of which is known to be very, very closely related to the pH value). However, the measurement only works with a reference electrode, precisely for comparison purposes. What this all means and which redox pairs can be used here belongs to the field of *potentiometry,* which you will encounter again in ► Sect. 10.3. For the time being, we assume for simplicity's sake that we would use as reference electrode the **normal hydrogen electrode** (NHE) known from *general chemistry* and/or *physical chemistry*, so that our measured values can be compared directly with the galvanic series. (We will briefly look at why this is not likely to be the case in actual measurement setups in the laboratory in ► Sect. 10.3.)

Let us construct selected measuring points from such a titration curve using our own arbitrarily chosen example:

20.0 mL of a solution of 5.00 mmol Sn^{2+} in 1-molar nitric acid are to be examined *cerimetrically.* A solution with $[Ce^{4+}]$ = 0.0200 mol/L serves as titrant. The aim is to find out what the electrochemical potential of the analyte solution to which the titrant has been added is after the addition of:

(a) 0.100 mL, (b) 1.00 mL, (c) 5.00 mL, (d) 10.00 mL, (e) 10.10 mL, (f) 12.00 mL and (g) = 20.00 mL titrant.

■■ Setting Up the Equation(s)

The first thing to do is to set up the corresponding partial equations and to assemble a redox equation that reaolly deserves this name (keywords: **mass balance, charge balance**).

The tin is *oxidised* here:

$$\mathrm{OX:}\quad Sn^{2+} \rightarrow Sn^{4+} + 2\ e^-$$

The cerium(IV) ions act as oxidising agents, i.e. they are being *reduced* themselves:

$$\mathrm{RED:}\quad Ce^{4+} + e^- \rightarrow Ce^{3+}$$

This leads to the following overall equation:

$$Sn^{2+} + 2\ Ce^{4+} \rightarrow Sn^{4+} + 2\ Ce^{3+}$$

▪▪ Preliminary Considerations

Before you can start calculating the potentials that result from adding x mL of titrant to the analyte solution with the help of the Nernst equation, you should think about a few things:

- The electrochemical potential of an ion alone cannot be measured. In order to say something about a potential, the "counterpart" of the redox pair in question must also be present. In other words: *No* electrochemical potential can be determined *for the starting point of the titration,* before the first drop of titrant was added.
- *Before* the equivalence point is reached—i.e. before enough cerium(IV) ions have been added to convert all the tin(II) ions to tin(IV) ions—all the Ce^{4+} ions added in the form of the titrant are being reduced to cerium(III). Accordingly, the redox potential of the analyte solution before reaching the equivalence point depends *exclusively* on the redox pair Sn^{2+}/Sn^{4+}.
- *At* the equivalence point, just enough cerium(IV) solution was added that all tin(II) ions were oxidised to Sn^{4+} and all Ce^{4+} were reduced to trivalent cerium. Accordingly, the electrochemical behavior of the analyte solution at this point is determined by the Sn^{4+} and Ce^{3+} ions. (We will come to that soon.)
- *Beyond* the equivalence point, the cerium(IV) ions from the titrant have oxidised all the tin(II) ions to $Sn^{4+;}$ thus, there are plenty of Ce^{3+} ions present. However, after all the Sn^{2+} ions have been consumed, the over-titrated Ce^{4+} ions can no longer be reduced, so that the redox potential of the solution now depends on the redox pair Ce^{3+}/Ce^{4+}.

So before one could start calculating any potentials, one should find out where (i.e.: at which titrant volume) the equivalence point actually lies in this experimental setup.

▪▪ The Equivalence Point

We know:

- V(analyte solution) = 20 mL = 0.020 L
- $[Sn^{2+}]$ = 5 mmol/L = 0.005 mol/L
- $[HNO_3]$ = 1 mol/L (i.e.: pH = 0)
- $[Ce^{4+}]$ = 0.0200 mol/L

Since two Ce^{4+} ions are required to oxidise one Sn^{2+}, the amount of cerium(IV) ions added at the equivalence point must be exactly twice the initial amount of tin(II) ions in the analyte solution.

In order:

That is, $n(Sn^{2+})$ = 0.1 mmol.

$$n\left(Ce^{4+}\right) = 2n\left(Sn^{2+}\right) = 2 \cdot 0.1 \text{ mmol} = 0.2 \text{ mmol} = 0.0002 \text{ mol}.$$

What $V(Ce^{4+})$ is now required to reach the equivalence point?

Using $[Ce^{4+}]$ = 0.0200 mol/L and rearranging the above equation, we get:

$$V = \frac{n}{c}, \quad \text{also } V\left(Ce^{4+}\right) = \frac{0.0002}{0.0200} L = 0.010\,L = 10\,mL.$$

The equivalence point is therefore reached at (d).

In other words, points (a)–(c) lie *before,* (e) and (f) are *after* the equivalence point. For these five measuring points, the electrochemical potential is obtained according to the **Nernst equation:**

$$E = E^0 + \frac{0.059\ V}{z} \cdot \lg \frac{[Ox]}{[Red]} \qquad (7.1)$$

Here, z stands for the number of electrons exchanged in the corresponding redox reaction, [Ox] for the concentration of the *oxidised* form of the redox pair in question, and [Red] for the concentration of the *reduced* form (or better: the *not so strongly oxidised* form).

Before the equivalence point (see above) the redox potential of the analyte solution depends exclusively on the redox pair Sn^{2+}/Sn^{4+}, so you only need the value $E^0(Sn^{2+}/Sn^{4+})$. Tables can help here—or also the corresponding table from Harris. This tells us: $E^0(Sn^{2+}/Sn^{4+}) = 0.139$ V, and z = 2.

Harris, Appendix H (Standard Reduction Potentials)

With each drop of titrant added to the analyte solution, the total volume changes (for a) V_{total} = 20.1 mL, for b) 21.0 mL and for c) 25.0 mL). Do we really need to calculate the current concentrations of all ions involved each time?—Fortunately, no. Since the absolute concentrations are not important in the Nernst equation, but only the concentration ratio (this is exactly like with the Henderson-Hasselbalch equation, GI. 5.11!), life can be simplified immensely if one does not performs concentration calculations, but instead considers what *proportion* (in percent, for example) of the starting substance has already reacted.

■■ (a) *Before* the Equivalence Point: With an Addition of 0.10 mL Titrant

If the equivalence point is reached with 10.0 mL titrant, then exactly 1% of all tin(II) ions have been converted to Sn^{4+} when 0.10 mL titrant has been added—and every particle which has been converted from the reduced form [Red] to the oxidised form [Ox] is now correspondingly "missing" in the denominator of the Nernst equation. Thus the ratio [Ox]/[Red], i.e. $[Sn^{4+}]/[Sn^{2+}]$, is here 1/99, and with $E^0(Sn^{2+}/Sn^{4+}) = 0.139$ V, we get:

$$E_{(a)} = 0.139\ V + \frac{0.059\ V}{2} \cdot \lg \frac{1}{99} = 0.080\ V$$

■■ (b) For an Addition of 1.00 mL Titrant

With 1.00 mL titrant, 10% of all tin(II) ions are then converted to tin(IV). Thus:

$$E_{(b)} = 0.139\ V + \frac{0.059\ V}{2} \cdot \lg \frac{10}{90} = 0.111\ V.$$

■■ (c) With an Addition of 5.00 mL Titrant

With 5.00 mL titrant we have reached exactly the *half-equivalence point*. Accordingly, 50% of all tin(II) ions have been oxidised to tin(IV) here. This simplifies the Nernst equation even more, because:

$$E_{(c)} = E^0 + \frac{0.059\ V}{2} \cdot \lg \frac{50}{50} = E^0 + \left(\frac{0.059\ V}{2} \cdot \lg 1 \right) = E^0 + \left(\frac{0.059\ V}{2} \cdot 0 \right)$$
$$= E^0 \left(Sn^{2+} / Sn^{4+} \right) = 0.139\ V.$$

Parallels

Do you see the similarity to acid/base reactions?—For weak acids, at the half-equivalence point pH = pK_A, for redox reactions, at the half-equivalence point E = E^0.

■■ (d) *At the* Equivalence Point: First Attempt

Here we are not dealing with a redox pair in the proper sense (i.e.: with the oxidised and the reduced form of *one kind of* atom, molecule or ion, which can be converted into each other accordingly by reduction or oxidation), but rather with tin(IV) ions on the one hand and cerium(III) ions on the other—and these cannot be converted into each other at all by redox processes. The Nernst equation is simply not designed for such a situation. We will return to this question when we have completed all calculations based solely on the Nernst equation, then.

■■ (e) After the Equivalence Point (EP): With an Addition of 10.10 mL Titrant

As already stated above, all tin(II) ions have been used up by now, so the tin(IV) ions formed by the oxidation have no counterpart. At the equivalence point, all cerium(IV) ions were reduced to Ce^{3+}, but now we have "over-titrated", i.e. additional cerium(IV) ions are present in solution. The crucial redox pair is therefore Ce^{4+}/Ce^{3+}. Now all we have to do is determine the concentrations of the two ions—or better yet, the ratio of their *amounts of substance*, since we don't need the absolute concentrations after all. We know:

- At the EP all were reduced Ce^{4+} to form Ce^{3+} At the EP applied $n(Ce^{3+}) = 2 \cdot n(Sn^{2+}) = 0.2$ mmol = 0.0002 mol = $2 \cdot 10^{-4}$ mol.
- In addition, 0.10 mL (= 0.0001 L) of cerium(IV) solution containing $[Ce^{4+}] = 0.0200$ mol/L was added. Via $n = c \cdot V$, we get:

Harris, Appendix H (Standard Reduction Potentials)

Now we only need to consider that $E^0(Ce^{3+}/Ce^{4+}) = 1.61$ V (again Harris helps us—there you can also see why the information in which acid the analyte was dissolved was important!) and z = 1. Accordingly we get

$$E_{(e)} = E^0\left(Ce^{3+}/Ce^{4+}\right) + \frac{0.059\ \text{V}}{1} \cdot \lg \frac{2 \cdot 10^{-6}\ \text{mol}}{2 \cdot 10^{-4}\ \text{mol}}$$

$$= 1.61\ \text{V} + \frac{0.059\ \text{V}}{1} \cdot \lg \frac{2 \cdot 10^{-6}\ \text{mol}}{2 \cdot 10^{-4}\ \text{mol}} = 1.492\ \text{V}$$

■■ (f) For an Addition of 12.00 mL Titrant

The calculation remains the same: Of the 12.00 mL titrant we may consider (thanks to the ÄP) 10.00 mL as cerium(III) solution, the remaining 2.00 mL as cerium(IV) solution:

$$E_{(f)} = 1.61\ \text{V} + \frac{0.059\ \text{V}}{1} \cdot \lg \frac{2}{10} = 1.569\ \text{V}$$

■■ (g) For an Addition of 20.00 mL Titrant

Here, $V(Ce^{3+}) = V(Ce^{4+})$, so even in the total volume (V_{total} = 40 mL), it can be said that $[Ce^{3+}] = [Ce^{4+}]$. Thus:

$$E_{(g)} = E^0 + \frac{0.059\ \text{V}}{1} \cdot \lg \frac{x}{x} = E^0 + \left(\frac{0.059\ \text{V}}{1} \cdot \lg 1\right)$$

$$= E^0 + \left(\frac{0.059\ \text{V}}{1} \cdot 0\right) = E^0\left(Ce^{3+}/Ce^{4+}\right) = 1.61\ \text{V}.$$

■■ Now What About the Equivalence Point Itself?!

As we have just seen: The Nernst equation is not designed for such "ion mixtures". For this we need **Luther's rule**. It says:

When calculating the equivalence point of a redox titration, both the standard redox potentials of the ions involved and the number of electrons involved in the redox equilibria in question are included in the calculation according to the following formula:

$$E_{\text{at equilibrium}} = \frac{z_1 \cdot E^0(\text{redox pair 1}) + z_2 \cdot E^0(\text{redox pair 2})}{z_1 + z_2} \quad (7.2)$$

With $z_1 = 2$ and $E^0(Sn^{2+}/Sn^{4+}) = 0.139$ V and $z_2 = 1$ and $E^0(Ce^{3+}/Ce^{4+}) = 1.61$ V, the result is:

$$E_{(d)} = \frac{z_1 \cdot E^0(Sn^{2+}/Sn^{4+}) + z_2 \cdot E^0(Ce^{3+}/Ce^{4+})}{z_1 + z_2}$$
$$= \frac{2 \cdot (0.139\ \text{V}) + 1 \cdot (1.61\ \text{V})}{2+1} = 0.629\ \text{V}.$$

The graphical plotting of these seven measuring points (a–g) has been omitted, because seven points do not give a decent curve and we do not want to overdo it with calculation examples here. But a certain tendency is certainly already recognisable, and so it should be understandable that the course of the curve is quite similar to that of Fig. 15.3 from Harris. (And if you choose another example where the stoichiometry of the two redox pairs is 1:1 (i.e., $z_1 = z_2$), you even get symmetrical curves where the equivalence point lies exactly in the middle of the steeply rising part of the curve, as is the case in Fig. 15.2. If you just recognised certain similarities with acid/base titration curves, you are on exactly the right track.)

Harris, Section 15.1: The shape of the redox titration curve

7.3 The End Point (Almost Like with Acids and Bases)

In order to follow the course of a redox titration, it is necessary to determine the resulting redox potential at the shortest possible intervals (using appropriate measuring electrodes; this has already been mentioned in ► Sects. 1.1 and 1.2). If, however, only the end point is concerned, this can be determined most simply with the aid of an indicator, as is the case with acid/base titrations. And just as acid/base indicators themselves represent acids or bases which, when used in excess, influence/falsify the actual measurement result, redox indicators must themselves be oxidisable or reducible and thus, strictly speaking, also interfere with the behavior of the analyte solution. Here, too, "a lot helps a lot" is definitely *not a* good idea.

Analogous to the acid/base indicators, a redox indicator must also have some characteristics:

- Its redox potential must not only be known (you should be able to look it up in data bases!), but should ideally lie close to the equivalence point of the redox titration in question.
- It must show different colours depending on the reduction or oxidation state, (whereby there are again the possibilities "colourless/coloured" or "colour 1/colour 2").

An overview of common redox indicators together with the corresponding E^0 value (which ultimately decides where the indicator changes over) is given in Table 15.2 from Harris.

Harris, Section 15.2: Determining the end point

Questions

7. Covering cerimetry, it was pointed out that a graphical application makes little sense with so few measuring points. Therefore, state the expected values at
   (a) V(titrant) = 7 mL and
   (b) V(titrant) 13.5 mL
   and enter them into the graphic. Do the results match any roughly estimable trends?

Poor Solubility Can Be Advantageous: Precipitation Titration

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_8

Harris, Appendix F (Solubility Products)

Summary

The poor solubility of one substance or another can also be used to quantify corresponding analytes. From *general* and/or *physical chemistry* you know that the solubility of a substance is described by its **solubility product** (K_{sp}) and there are (once again) databases on this subject. (The solubility products of some compounds that appear particularly frequently in analytics can also be taken from Harris.)

The principle of these titrations remains the same: The analyte is made to react with a corresponding titrant, whereby one or another physicochemical property of the analyte solution is changed—and ideally particularly clearly so at the equivalence point (or in its immediate vicinity), i.e. at the point at which an amount of the titrant equivalent to the amount of analyte present has been added. (Some remarks on which properties this can be and how to determine the end point can be found in ► Sect. 8.3.)

8.1 The Importance of the Solubility Product

8

Although you are undoubtedly already familiar with the solubility product in principle, we will nevertheless briefly review the most important basics here:

- The dissolution of a solid in water is regarded as a chemical reaction: A solid, which of course is present in a more or less ordered crystal lattice (keyword: lattice energy), dissolves if the energy released by the interaction between the individual lattice constituents (cations, anions) and the solvent molecules (here: H_2O) as well as by the entropy gain (keyword: Gibbs-Helmholtz equation) is greater than the lattice energy of the solid present.

  Correspondingly, a reaction equation can also be drawn up for each dissolving process; here as an example with common salt:

  $$NaCl \rightleftarrows Na^+ + Cl^-$$

  Since this dissolving process, like all dissolving processes, represents a dynamic equilibrium, the corresponding mass action law comes into force again, which leads to the $\mathbf{K_{sp}}$ **value**:

  $$K_{sp} = \frac{\left[Na^+\right]\left[Cl^-\right]}{\left[NaCl\right]_{undissolved}}$$

  (If the sum formula of the salt under consideration contains stoichiometric factors >1, these are included in the LMA as exponents, as usual.)
- Even if sodium chloride is actually quite well-soluble in water: the amount of NaCl going into solution will *not be unlimited*. Sooner or later, a **saturated solution** is present; upon further addition of substance, a **precipitate** of undissolved salt forms at the bottom of the reaction vessel. This leads to the question of how this undissolved salt should enter (as a denominator) into the LMA: *What is the concentration of an* undissolved *substance in solution?*—The part of the salt that is not dissociated into cations and anions *must be* undissolved, because NaCl (or other ionic compounds) are not present as "NaCl molecules". However, "$[NaCl]_{undissolved} = 0$" would not be a good idea for mathematical reasons alone; after all, one should *never* divide by 0. Besides, the undissolved substance *could* go into solution at any time, if only more solvent were added. In addition, this undissolved substance also interacts with the solution: After all, there is a *dynamic* equilib-

rium, in which there is a constant exchange of substances between the precipitate and the solution. (Should this confuse you, please have another look at Binnewies).

Binnewies, Section 9.2: Quantitative description of the chemical equilibrium

Since the undissolved substance is clearly *present* (in the form of the precipitate), but one cannot say any more about its actual concentration, the concentration of an undissolved substance that is in contact with a solution (and *could* therefore be involved in any reactions at any time) is always treated and given as *[undissolved substance] = 1.* (This should sound familiar from electrochemistry. Important keywords here: Daniell element and electrodes.)

Cave

If the concentration of an undissolved substance in contact with a solvent is given as "1", this 1 is dimensionless—it has *no unit*.

Please do not provide this "solid-1" with concentration units like "mol/L" or similar.

- Thus, the denominator of the LMA does not contribute to the unit of the total expression, and for 1:1 compounds the unit mol^2/L^2 results for the K_{sp} value. For salts with a different stoichiometry (e.g. $CaCl_2$), "even higher dimensional" units result (here correspondingly: mol^3/L^3); therefore, the solubility products of different salts are not necessarily directly comparable with each other: One must convert from the respective solubility product to the (molar) **solubility** (in mol/L).

  However, since this is not absolutely necessary for precipitation titrations, we will not deal with it here.
- Many tables do not list K_{sp} values, but instead **pKsp values**—where the "small p" again plays the same role as with the pH value. If the tables state $pK_{sp}(PbS) = 27.5$ for the extremely poorly soluble compound lead(II) sulfide, this means that the corresponding K_{sp} value is $10^{-27.5} = 3.16 \times 10^{-28}$ $[mol^2/L^2]$.

8.2 Graphical Representation

Before we turn to the course of a precipitation titration curve, a preliminary remark: If the solubility product is of importance, then it is not only a question of the *amounts of substance* present, but also of the actual *concentrations* concerned—and these are known to vary greatly: from "extremely dilute" (in the µmol range or even below) to "concentrated solutions" with one to even two-digit moles per litre. It is therefore helpful when drawing up corresponding titration curves to proceed in a similar way as when considering the concentration of hydroxonium ions in solution. There, only very rarely the actual concentration was given, but much more frequently the corresponding pH value, i.e. the *negative decadic logarithm* of this concentration.

Corresponding statements can be made about the concentration of any other ions: with the help of the p-function.

The p-Function

It simply is: $pX = -\lg [X]$, where X can be a cation or also an anion.

For example, if you let react an analyte solution containing chloride ions (Cl^-) with a silver ion titrant, you can determine the titrant's actual concentration present (using an electrode, *see* ► Sect. 10.3) and report it as a pAg value, where pAg is just-lg $[Ag^+]$.

And because silver chloride is quite poorly soluble in water ($K_{sp}(AgCl) = 1.8 \times 10^{-10}$ mol^2/L^2), a large proportion of these ions, depending on the real concentration of chloride ions present, precipitate immediately after their addition.

This brings us a second time to the important role of the solubility product:

Please note that the K_{sp} value indicates the **ion product** in solution. If the analyte is the aqueous solution of a readily soluble compound containing chloride ions, then [Cl^-] in this solution is correspondingly considerably high. Thus, even minimal amounts of silver(I) ions added in the form of the titrant are sufficient to exceed the solubility product: Precipitate formation occurs (AgCl↓).

If the concentration of the analyte ions, which form a poorly soluble compound with the "titrant ion", is sufficiently high, the precipitation will proceed almost quantitatively with each drop of titrant. However, the closer you get to the equivalence point, the fewer analyte ions are still in solution, which means that the quantity of titrant ions (here: Ag^+) that can remain in the solution *without* precipitating increases. At the equivalence point (practically) all analyte ions have precipitated—apart from the (extremely) few that are actually still in solution according to the solubility that can be determined from the solubility product.

Harris, Section 26.5: Precipitation titration curves

Once the equivalence point has been *exceeded*, there is a corresponding excess of titrant ions. These now cause some physicochemical characteristics of the solution to change considerably—and it is precisely this change that can be used to determine the end point (and thus the equivalence point) of the titration (we will come to this in ► Sect. 8.3). Overall, if the concentration of the analyte ions is given by the **p-function**, the result is a curve as in Figs. 26.8 and 26.9 of Harris.

8.3 Determination of the End Point

Harris, Section 26.8: End point determination

The most common method for endpoint determination in precipitation titration is also the use of electrodes, so that ultimately *potentiometry* is used again (► Sect. 10.3). Alternatively, it is also possible to work with indicators—but these must then be adapted to the titrant used. Even though silver(I) ions are anything but rare as titrants (in Harris you will find two exemplary indicator methods for this purpose), it should be noted that there are many other ions used in precipitation titrations. In this respect, if you should work with this technique later on, you will have to look around in the technical literature for suitable indicators for the titrant used.

Or you *could* use an electrode.

Questions

8. For silver bromide, we know $pK_{sp}(AgBr) = 12.3$.
   (a) Determine the K_{sp} value.
   (b) Specify its unit.
   (c) Say something about the solubility of silver bromide (in mol/L and in g/L).
9. At what titrant volume does the equivalence point lie if you titrate 10 mL of an analyte solution containing [Br^-] = 0.23 mol/L against the following silver nitrate titrant (with f = 1.02): [$AgNO_3$] = 0.1 mol/L.

In ► Chap. 8 you learned that one can precipitate the analyte from the solution in which it was present. This can also be used in other ways—and this brings us to the principle of *gravimetry*.

Gravimetry

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_9

Summary

The fact that an analyte can be precipitated as a poorly soluble compound is something you know from *general chemistry* (or at the very latest ever since ► Chap. 8). So it seems obvious that the solid obtained by precipitation could simply be weighed out and the mass of the solid used to infer the mass content of the analyte. This is precisely the principle of gravimetry.

However, there are a few things to keep in mind here, because the form in which one precipitates the analyte in question does not always really meet the criteria that need to be taken into account if one wants to make a reliable statement about the mass. Or to put it briefly:

The precipitation form of the analyte does not necessarily have to correspond to the weighing form.

If the precipitation form corresponds to the weighing form, this is of course rather nice—but you don't get to have that luck very often.

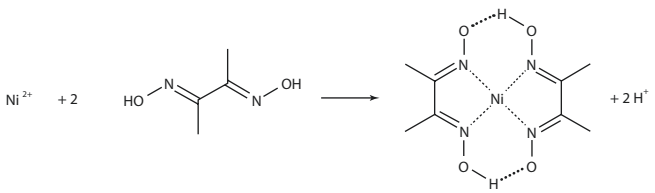
9.1 Precipitation Form

If an analyte is to be precipitated from an (aqueous) solution, a suitable precipitation reagent is required—which ideally forms a precipitate *exclusively* with the analyte and thus completely ignores any impurities. And there really are numerous precipitation reagents suitable for gravimetry in which the "interfering impurities" are at least kept within limits.

Let us look at a good example of a suitable precipitation reagent: Nickel(II) ions form a characteristically pink precipitate with diacetyldioxime (also known as dimethylglyoxime) (◘ Fig. 9.1).

That this chelate complex is quite stable should come as no surprise:

- In the course of complexation, the two bidentate chelate ligands each split off *one* of their (OH-acidic) hydrogen cations, so that the two ligands are not only in contact with the central particle, but are also connected to each other via hydrogen bonds.
- The coordination centers (the nitrogen atoms) then form a total of two five-membered and two six-membered rings with the central particle, and you certainly know from *organic chemistry* (keyword: cycloalkanes) that it is precisely at these ring sizes that the ring tension is minimised.
- The resulting chelate complex can precipitate because the double positive charge of the central particle and the double deprotonation of the ligands result in a net charge of ±0. (Reminder: A precipitate must *always* be electrically neutral, otherwise you would have "charge separation in the flask". This won't happen.)
- It should be understandable that the precipitation of nickel(II) ions with this precipitation reagent proceeds better in a slightly basic medium than in a weakly (or even strongly) acidic medium.



◘ **Fig. 9.1** Precipitation of nickel(II) ions as a chelate complex

Fortunately, there are not too many ions that interfere with this reaction as impurities; only the higher homologues of divalent nickel (Pd^{2+} and Pt^{2+}) precipitate in the same way. Some other transition metals also form coloured complexes with this reagent (which can even be used photometrically, *see* ► Sect. 10.2), but precipitation does *not* occur here. A first overview for further (organic) precipitation reagents is given in Table 26.2 of Harris.

Harris, Section 26.1: Examples of gravimetric analyses

However, if you want to precipitate the analyte, you must always make sure that the solid does not form/precipitate excessively quickly: The less "cleanly" it crystallises, the greater the risk of co-precipitation of impurities. The slower you let the crystal grow, the more time the individual lattice constituents have to move into the most energetically favourable position and prevent foreign atoms or ions from being incorporated/enclosed into the lattice. It is therefore advisable, for example, to add the precipitation reagent rather slowly to the analyte solution. However, as this section is only intended to provide a first insight into the various techniques of analytical chemistry, we will not go into further detail on this and other subtleties that still need to be taken into account when precipitating analytes. Harris offers a whole lot more information on this, of course.

Harris, Section 26.2: Precipitation

9.2 Weighing Form

Unfortunately, as mentioned above (and unlike in the case of nickel with the precipitating reagent diacetyldioxime), the precipitation form (i.e. the compound in which your particular analyte is precipitated from solution) is not necessarily suitable for determining the total mass and thus the analyte mass. What problems can occur?—There is always the question of what criteria a compound must (or at least *should*) exhibit to be useful in gravimetry:

- The precipitation form must have a *precisely defined chemical composition*.
- It should have an *indefinite shelf life* and not change its composition over time:
  - It should not react with atmospheric oxygen (and accordingly should not change mass).
  - It should not be hygroscopic and should not react with the humidity of the air.
- In addition, the weighing form should have as *high* a *molar mass* as possible to minimise the weighing error.

  (This reminds you of a rather similar sentence from ► Sect. 5.4?—Very good, you have recognised a principle.)

Table 26.1 of Harris provides you with the preferred precipitation and weighing forms for a whole series of important analytes. Sometimes the two are identical (as in the case of divalent nickel), sometimes the precipitation form is "simply" richer in water than the weighing form, so that extensive drying at sufficiently high temperatures is sufficient. (This is the case, for example, with analytes that are precipitated in the form of phosphates—the fact that in such cases the molar mass of the precipitation form is higher than that of the weighing form is regrettable, but cannot be changed, because these more water-rich forms do not necessarily contain (completely) stoichiometric amounts of water and thus have a disproportionately greater influence on the weighing result.) There are also cases in which the precipitation form must subsequently be chemically processed in a more complex manner, but fortunately these are quite rare and will therefore not be discussed further here.

Harris, Section 26.1: Examples of gravimetric analyses

9.3 Special Case: Electrogravimetry

In electrogravimetry, a solution of metal (= analyte) ions is electrolysed in such a way that the analyte ions are deposited in elemental form on the surface of an electrode—a particularly elegant (and rather fast) method, because if the analyte solution does not also contain other metal ions, the mass of the analyte can simply be determined from the difference in mass (mass of the electrode *after completion of electrolysis* minus mass of the electrode *before the start of the experiment*). This assumes two things:

1. use of an electrode that does not interact in any way with the analyte (solution) (except to act as a "capture surface").

   Even if it is expensive: Platinum electrodes are a good choice here—preferably as a platinum mesh to increase the surface area so that electrolysis is completed even more quickly.
2. a quantitatively proceeding electrolysis—whether this really happens one has to check during the experiment (you might already be finished, after all!).

   If you are dealing with analyte ions with a characteristic colour when present in the elemental state (copper, for example), the simplest method is to immerse the platinum electrode (which is expected to be platinum-coloured, i.e. silver) in the electrolyte solution for only about two thirds of its total length at the start of the experiment. Once the electrolysis has run long enough, or if you otherwise have good reason to believe that you might already be finished, you just immerse the electrode a little deeper into the (now hopefully analyte-free) electrolyte solution and continue electrolysis for a few more minutes. If the colour of the electrode does not change any more, i.e. no more elemental (red) copper is deposited on the platinum-coloured surface of the electrode, you may probably stop. Now you only have to dry the electrode until the mass is constant before you determine the mass difference (after—before, see above).

If the mass (and thus the amount of substance) of the analyte is known, the corresponding concentration of the amount of substance can also be determined if the volume of the analyte solution has been determined with sufficient accuracy at the start of the experiment.

Questions

10. What is the mass fraction of chromium(III) ions in a sample if 23.0 mg lead(II) chromate is precipitated from 50.0 g sample material after the necessary work-up (dissolving, etc.)?
11. How many mg of lead(II) sulfate can be expected gravimetrically from 25 g of sample material if $\omega_{Pb} = 42\%$?
12. 666 mg of copper were electrolytically deposited from 42.23 mL of solution on a platinum electrode. Determine the concentration of the substance and the mass concentration.

Selected Detection Methods

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_10

Summary

As already mentioned in ► Chap. 4, the (quantitative) investigation of analyte solutions is usually based on the fact that one or other property of the respective solution changes in a characteristic way after the titrant has been added.

10.1 The Colour Change: Colorimetry

If the colour change of an analyte solution to which a corresponding indicator has been added (and it is initially irrelevant whether this is an acid/base, a complex or a redox indicator!) is used to infer its substance content (usually: the molar concentration), then strictly speaking one is in the specialist field of **colorimetry,** because the evaluation is carried out on the basis of a colour scale prepared in advance, which is based on precisely known concentrations of the analyte under consideration in a comparable medium (= solvent).

Harris, Section 10.6: Endpoint determination with indicators

You have undoubtedly already made use of this principle yourself—for example, when you have used universal indicator paper to at least estimate the pH value of a solution/(aqueous) reaction mixture by applying a drop of this solution to the pH paper and matching the resulting colour with the associated pH colour scale. This is nothing more than *crude qualitative* colorimetry. (The same applies to any other comparison of the colour of a reaction mixture with the various hues of an indicator table, i.e., among other things, also to all pH indicators—a good overview of the transition ranges of the most important ones can be taken from Tabler 10.3 of Harris; for thymol blue *see also* Colour Plate 3 of the book.)

In most cases, such a "measurement" is really only roughly qualitative. If you want to do the whole thing quantitatively, you usually need not only *significantly more*, but above all *precisely defined* reference samples whose composition does not correspond "only rudimentarily" to that of your analyte solution (e.g.: "is also an aqueous solution with a pH ~ 5"), but which, in addition to the analyte to be considered (for pH indicators, these are of course H^+ ions!), also contain any interfering "non-analytes" in at least comparable quantities.

And even then it is still questionable whether one really obtains reproducible-precise *quantitative* results, after all, the human eye serves as the measuring instrument—which is, in each case, the eye of real, existing, individual person. However, the human perception of colour is deeply subjective.

Fancy to Have a Go Yourself?

If you have the opportunity, try out the precision of the measuring instrument "human eye" yourself together with some fellow students of your choice: To do this, first agree on the indicator to be used and then find out in which pH range you can expect which colour change, for example: "Indicator methyl red; turns from red to yellow in the pH range 4.8–6.0." (The mere fact that a *range of change* is given should not lead you to expect excessive precision, but this has already been discussed in ► Sect. 5.4.) Then take a small amount of water, add the indicator and some acid to make sure you are in the sufficiently acidic range, and drop soda lye into the analyte solution one drop at a time (using a burette is a good idea). You will be surprised when the first fellow students already shout "There was the transition!", while others will vehemently deny it.

And if you now think: "Yes, from red to yellow, that *can't be* anything, there's still orange in between, so it's no wonder that the transition is fluid!", then try the same thing with an indicator whose transition is more "unambiguous"—phenolphthalein (see ► Fig. 5.2) is a good choice. (Below the

pH range 8.9–9.6 this indicator is colourless, above it is purple.) Even if you hold a white sheet behind the beaker you are using to better see the colour change, you will get a Gaussian distribution in your fellow students regarding the "There was the colour change!" moment.

In the meantime, however, there are systems available that do *not* use the human eye with its subjective colour perception as a measuring instrument, but instead make use of (supposedly?) more objective systems such as computer-controlled sensors. Nevertheless, quantitative colorimetry is not an everyday laboratory technique—unlike its "roughly qualitative" counterpart (especially when pH paper of any kind is involved).

Nitpickers' Corner

As already mentioned, the comparison of colours with a reference scale is commonly referred to as **colorimetry**—but there are also those who say that this is actually a special case of **photometry,** because the colour of a solution is, as is well known, nothing other than the result of the interaction of the substances in the solution with electromagnetic radiation (see "Where does the colour come from?" in ► Sect. 6.2).

This "interaction" can usually be equated with "absorption", and in photometry one measures the extent to which the intensity of electromagnetic radiation of a certain wavelength (from the visible light range) is absorptively reduced when passing through a solution.

Nevertheless, claiming colorimetry and photometry (we will discuss the latter in ► Sect. 10.2) to be synonyms is not quite correct, because there is *one huge* difference:

- Any solution that is to be examined photometrically *must* obey the Lambert-Beer law (you will find out what this is in a moment—in any case, you always need a *homogeneous solution*).
- Colorimetry also allows quantitative statements to be made (by direct comparison) about analytical mixtures which do *not* follow this law—e.g. because there is no homogeneous solution, but a suspension or a colloidal solution or some such.

10.2 Altered Absorption Behaviour: Photometry

Photometry measures the extent to which n analyte solution absorbs electromagnetic radiation of one wavelength or another. (This energetically excites the analyte, but that is less important here and now than the fact *that* photons are absorbed. We will deal with the questions of what exactly happens during this excitation, what is particularly easily excited at which wavelength, and why, in Parts IV and V. However, if your curiosity gets the better of you, you are welcome to read the relevant basics in Harris.)

Harris, Section 17.1: properties of light

In "conventional" photometry, only a single, pre-selected wavelength is generally considered—which may well depend on the analyte in question. In photometry, one usually uses a wavelength from the range of visible light (VIS, see ► Chap. 4); some analytes, however, require somewhat higher-energy radiation for excitation, so that UV radiation is also occasionally used. (Strictly speaking, one has then already left the field of photometry and is performing *UV/VIS spectroscopy*.)

In any case, it is crucial that the analyte absorbs radiation of the selected wavelength. If a wavelength to which the analyte responds is known, measurements with **monochromatic light** of just this wavelength are recommended. In order to obtain *quantitatively* useful results that can be compared (e.g. as part of a series of measurements), this radiation is directed through vessels (made of a material that is transparent to the radiation in question; simple glass is often sufficient) with a precisely defined volume or layer thickness (a *cuvette*):

- Such a vessel contains the solution of the analyte (with all reagents that were or are necessary to bring or keep the analyte in solution).
- A second vessel contains the analyte-free solvent, which should also contain all the reagents mentioned above, otherwise it would not be a true **blank** (see Part I).

Subsequently, the intensity of the radiation that has passed through is compared. (The basics of photometry have already been summarised compactly but clearly in Binnewies, *see* in particular Fig. 12.11.)

Binnewies, Section 12.5: Chelate complexes (Excursus: Fundamentals of photometry)

Intensity losses can also occur in the blank sample (e.g. because the solvent or one or other of the reagents contained in the blank sample absorb part of the light of the selected wavelength—this *should* not be the case, but it *can* happen)—but this loss would then also be expected to the same extent in the analyte solution. Therefore, one equates the intensity (I) of the radiation passing through the blank sample with the initial intensity (I_0). The analyte will absorb radiation of the chosen wavelength much more strongly (otherwise you have chosen the wrong wavelength and have to start all over again!), accordingly I_{sample} will be much smaller than I_0. One can describe the difference in two different ways:

- One can specify the **transmission T,** i.e. the permeability—preferably in %. The corresponding formula is:

$$T = \frac{I_{sample}}{I_0} \times 100\ [\%] \tag{10.1}$$

- More commonly used, however, is the **absorbance E,** which has the great advantage that it correlates linearly with the layer thickness of the cuvette:

$$E = \lg \frac{I_0}{I_{sample}} \tag{10.2}$$

The extinction is also found in the most important formula for photometry, **Lambert-Beer's** law:

$$E = \varepsilon_\lambda \times c \times d \tag{10.3}$$

Harris, Section 17.2: Light absorption

Here ε_λ is the (substance-specific and wavelength-dependent) *extinction coefficient* of the analyte under consideration, c is the concentration of the analyte solution and d is the optical path length (i.e. layer thickness through which the relevant light beam of wavelength λ has passed).

10.3 Changes in Electrochemical Potential: Potentiometry

In ► Chap. 7, we have dealt quite extensively with methods of analysis in which the electrochemical potential is changed. in In the course of this (keyword: *before* addition of the titrant), something was also addressed once again which you already know from *general chemistry* and/or *physical chemistry*:

Only a potential difference *can be measured, i.e. a* relative *value, but not the* absolute *potential.*

For this reason, a reference electrode is required for every potentiometric test, which means nothing more than: "The potential is being measured here".

Actually, we are already used to this: We know that the standard potentials E^0 from the electrochemical voltage series have obtained their numerical values from direct comparison with the normal hydrogen electrode (NHE) or standand hydrogen electrode (SHE), to which the standard potential $E_0(H_2/2\ H^+) = 0.00$ V has been assigned (purely arbitrarily). Now, an NHE is a bit impractical for everyday use in the laboratory; after all, one needs, among other things, a platinum electrode that is not only continuously immersed in an aqueous solution with $c(H_3O^+) = 1$ mol/L (i.e., a solution with pH = 0, which should not be a problem), but which is also constantly surrounded by elemental hydrogen with the partial pressure $p(H_2) = 1$ atm — which, in view of the moderate solubility of hydrogen in water, would be a little complicated (and also not entirely harmless, because the undissolved hydrogen would gas out again, and hydrogen is, after all, very flammable).

Instead, however, one can use other standardised electrodes whose own potential (again, compared to the normal hydrogen reference electrode) is well known. There are sereval. Particularly common are:

- the *Ag/AgCl electrode* (redox couple: elemental silver/silver(I) chloride)
- the *calomel electrode* (redox couple: elemental mercury and mercury(I) chloride)

If these (or other) reference electrodes are used in potentiometry, and if (e.g. in the case of computer-aided processing of the measured values) the relative potentials of these electrodes are not "calculated out" of the measured values as constants in advance, one only has to consider that the measured values obtained are shifted by the "own potential" of the reference electrodes used in comparison to those which would result from the use of the NHE instead. However, since this applies to each individual measured value of the entire potentiometric test series, the curve (or similar) does not actually change—it is simply *shifted*.

Harris, Section 14.1: Reference electrodes

10.4 Changes in Electrical Conductivity: Conductometry

In conductometry, too, the change in a physical quantity is observed as a function of the interaction of the analyte concerned with the titrant used: Here we are talking about conductivity, usually abbreviated by the symbol κ and expressed in units of µS/cm, or microsiemens per centimeter. (Although you certainly know this from *physics*: The *electrical conductivity,* given in Siemens, is the reciprocal value of electrical resistance, which is given in Ω. Thus, 1 S = 1 A/V.)

The basis of this technique is the electrical conductivity of aqueous electrolyte solutions, which can, however, differ considerably between different solutions. Three factors in particular affect the conductivity of electrolyte solutions:

- the number of charge carriers in the solution, which of course depends on the *concentration* of the solution used.
- the number of charges carried by each individual cation or anion in solution (i.e. the *ionic valence*).
- the *mobility* of the ions concerned—which in turn depends on the one hand on the size (and in the case of polyatomic molecular ions possibly even on the spatial structure) and the charge density of the electrolytes, and on the other hand also on the viscosity of the solvent. (If you ever tried to pull a spoon first through water, then through honey, you know that for the same

distance this takes quite a bit more time and effort ...). Since the viscosity of any solvent is temperature-dependent, this is another factor when deviating from standard conditions.

Of course, there are also many reference books on the subject of conductometry, in which one can look up the conductivities (λ) of various ions under one or the other set of standard conditions (e.g. at 25 °C) (usually given in the unit S cm^2/mol).

These conductivity measurements can be combined particularly well with acid/base titrations, since hydroxonium ions and hydroxide ions are particularly suitable electrolytes (keyword: *proton jumping*, a.k.a. **Grotthuss mechanism**). Before we start looking at a specific conductometric titration curve, we should—on the basis of the titration of the strong acid hydrochloric acid against the strong base sodium hydroxide—give some thought to what the conductivity will be like *before, at* and *after* the equivalence point.

Since we are concerned here with the principle itself, not with concrete conductivity calculations, the concentrations of analyte solution and titrant as well as the ever-increasing total volume in the course of the titration (which then understandably affects the concentration present in each case) are not of interest here and now. Instead, let us first consider which ions contribute to the conductivity in each of the mentioned areas of the curve:

■■ Before the Equivalence Point

At the very beginning, only an aqueous solution of hydrogen chloride is present. Since HCl is a strong acid that dissociates (almost) completely, we have plenty of H^+ (more precisely: H_3O^+) and Cl^- ions present. Since hydroxonium ions are far better electrolytes than chloride ions and the total conductivity of a solution is *additive* from the sum of the individual conductivities of all electrolytes present, a high conductivity results ...

... which, however, decreases with increasing amounts of added titrant (NaOH, which itself dissociates into Na^+ and OH^- ions). Because even though hydroxide ions are almost as good electrolytes as hydroxonium ions, in the course of these acid/base titrations the most effectively conducting ions react to form uncharged and therefore *non-conducting* water molecules:

$$H_3O^+ + OH^- \rightarrow 2\ H_2O$$

On the other hand, with each equivalent of hydroxide ions that neutralizes one equivalent of hydroxonium ions, the reaction mixture is also enriched by one equivalent of sodium ions, which in turn are of course also electrolytes—but conduct the current much less effectively than H_3O^+ and OH^- ions.

■■ At the Equivalence Point

Minimum conductivity is to be expected here:

- When equivalent amounts of base have been added to the acid, all hydroxonium ions originating from the acid were neutralised. The most efficient known electrolytes are thus (practically) completely eliminated. (We will not discuss autoprotolysis, which provides pH = 7 here, even though, strictly speaking, it also makes some contribution to the total conductivity—but this contribution is within very narrow limits. Please recall the concentration of H^+ and OH^{--} ions produced by autoprotolysis.)
- The conductivity is now only caused by the *counterions of* the acid (i.e. the chloride ions in our example) and the counterions of the Titrant base (here: sodium ions).

  Na^+ and Cl^- are certainly good electrolytes, but they cannot compete with hydroxonium or hydroxide ions.

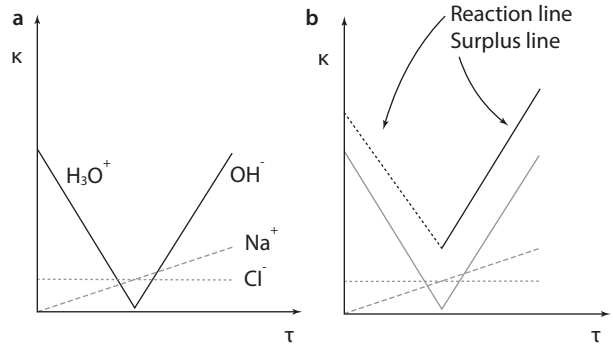


Fig. 10.1 Conductometry titration curve (schematic): (**a**) relative contribution of the ions; (**b**) total result

After the Equivalence Point

Logically, the number of chloride ions in solution does not change at all, but the number of sodium and hydroxide ions does, and since the latter are very good electrolytes, the total conductivity will increase considerably once the equivalence point has been exceeded. Figure 10.1a shows the relative contribution of the individual ions to the conductivity of the entire mixture, and Fig. 10.1b schematically shows the resulting total conductivity.

It should be obvious that the curve is different if, for example, a weak acid reacts with a strong base.

Questions

13. What colour does the sample of an analyte excited by relatively high-energy blue light (λ = 450 nm) show?
14. What colour does the human eye perceive in a sample whose analyte requires even higher-energy light (λ < 380 nm) for excitation?
15. Which curve do you expect to see when titrating a sodium chloride solution against a silver nitrate solution? (The concentrations present are irrelevant.) The ionic conductivity (in each case in S cm^2/mol) is:

$$\lambda\left(Na^+\right) = 50.1; \lambda\left(Cl^-\right) = 76.4; \lambda\left(Ag^+\right) = 62.2; \lambda\left(NO_3^-\right) = 71.1$$

10.5 Summary

Basic Terms

In volumetric analysis and the analytical methods derived from it, the analyte in solution (or to be brought into solution) is reacted with a precisely defined, (at least) (molar) equivalent quantity of a suitable reagent, so that (physico-)chemical properties of the resulting reaction solution change. The quantification of this change then allows conclusions to be drawn as to the amount of substance/concentration (etc.) in which the respective analyte was initially present.

Volumetric Analysis

In volumetric analysis, various reaction types are used to bring about the changes mentioned in ► Chaps. 5–8 within the framework of reactions that are usually carried out and monitored titrimetrically:

- In *acid/base titration*, an unknown quantity of an analyte with acidic or basic behaviour is reacted with a solution of a precisely defined concentration of a titrant with a corresponding basic or acidic behaviour; the original concentration of the analyte is then deduced from the titrant consumption once the equivalence point is reached, at which equivalent quantities of acid and base have been present.

- In *complexometry*, the analyte is quantified titrimetrically, either directly or indirectly, as part of a complex reaction. In most cases, this method is used with analytes that tend to act as the central particles of a complex, i.e. primarily with metal cations.
- In *redox titrations,* an oxidisable or reducible analyte is titrated against an oxidising or reducing agent. Again, the consumption of the titrant is used to infer the molar content of the analyte.
- In *precipitation titrations,* the consumption of the reagent with which the analyte forms a poorly soluble compound is determined.

Common to all methods of volumetric analysis is that a suitable detection method must be chosen to determine the equivalence point.

Gravimetry

If the quantification of the analyte is based on the determination of a mass (i.e., something is "weighed"), one deals with the field of gravimetry. It should be borne in mind that an analyte which upon reacting with a suitable reactant precipitates out of a solution as a poorly soluble compound (► Sect. 8.1) cannot necessarily be weighed in the form of the precipitated compound inself; further treatment might be necessary. Some metallic analytes can be obtained in their elemental form by electrolysis; this is the special form of *electrogravimetry*.

Selected Detection Methods

Colorimetry compares the colour depth of a sample mixture of unknown concentration with corresponding reference samples.

Photometry can be used to determine the concentration of a sample using a calibration curve based on Lambert-Beer's law.

In *potentiometry*, the course of a volumetric measurement is followed based on the electrochemical potential in comparison with a reference electrode.

Conductometry exploits the different conductivities of different electrolytes to determine the equivalence point of titrations.

Answers

1. The decisive factor here is whether the acids are strong, medium or weak. If $pK_A < 0$, complete dissociation may be assumed, so the same applies to hydrogen bromide as to hydrogen chloride dissolved in water: the original concentration at the beginning $[HA]_0 = [H_3O^+]_{\text{at equilibrium.}}$ And if you know the concentration of hydroxonium ions, you know the pH. Thus:

$$\left[H_3O^+\right]_{\text{at equilibrium}} = 0.1 \text{ mol/L, thus: } pH(HBr)$$

$$= -\lg(0.1) = -\lg\left(\frac{1}{10}\right) = -\lg\left(\frac{1}{10^1}\right) = -\lg 10^{-1} = -(-1) = 1$$

(a)

$$\left[H_3O^+\right]_{\text{at equilibrium}} = 0.01 \text{ mol/L, thus } pH(HCl)$$

$$= -\lg(0.01) = -\lg\left(\frac{1}{100}\right) = -\lg\left(\frac{1}{10^2}\right) = -\lg 10^{-2} = -(-2) = 2$$

Acetic acid with $pK_A(HAc) = 4.75$, on the other hand, is a *weak* acid, so the appropriate approximate formula (known as ► Eq. 5.10) is needed here:

(b) With $[HAc]_0 = 0.2$ mol/L and $pK_A(HAc) = 4.75$, we get:

$$pH = \tfrac{1}{2}(4.75 - \lg(0.2)) = \tfrac{1}{2}(4.75 - (-0.7) = \tfrac{1}{2}(4.75 + 0.7)$$

$$= \tfrac{1}{2}(5.45) = 2.73.$$

Can that be true? —Let us carry out the plausibility check: In ► Sect. 5.2, we had gotten pH = 2.88 for 0.1-molar acetic acid using the approximate formula. Our acetic acid here had a concentration twice as high, so it is understandable that the pH value here is somewhat lower.

2. First of all, the counter ions are not important in all three subtasks: Whether sodium or potassium is the counter ion of the conjugate base of acetic acid is just as irrelevant as whether the chloride ion is the counter ion of the conjugate acid of the base ammonia. So in Tasks 2a and 2b we need only consider the ratio [HAc]/[Ac⁻], and in Task 2c we need only consider the ratio $[NH_3]/[NH_4^+]$. In all three cases we should immediately think of the Henderson-Hasselbalch equation (► Eq. 5.11), which then has to be rearranged according to the molar ratio:

   $pH = pK_S - \lg\frac{[HA]}{[A^-]}$ This results in: $\lg\frac{[HA]}{[A^-]} = pK_A - pH$. If you know the pK_A values, you only have to delogarithm afterwards:

   $\frac{[HA]}{[A^-]} = 10^{(pK_A - pH)}$ This leads to: acetic acid with $pK_A = 4.75$:

   (a) $[HAc]/[Ac^-] = 10^{(4.75 - 5.05)} = 10^{(-0.3)} = 0.5$, so the ratio must be [HAc]/[Ac⁻] = 0.5 : 1, i.e. 1:2. *Plausibility check:* At this ratio, there is more of the conjugate base present than of the acid. It stands to reason that the resulting pH value is higher than the pK_A value.

   (b) $[HAc]/[Ac^-] = 10^{(4.75 - 3.87)} = 10^{(0.88)} = 7.6$. In this case, there are almost eight times as many acid molecules in the solution as there are molecules of the corresponding conjugate base. *Plausibility check:* Then it is not surprising that the pH value is clearly below the pK_A value.

   (c) Note that the ammonium ion (NH_4^+) is the acid here, so we are looking for $[NH_4^+]/[NH_3]$. From ► Sect. 5.2 we know that $pK_A(NH_4^+) = 9.25$. With $[NH_4^+]/[NH_3] = 10^{(9.25-10.0)} = 10^{(-0.75)} = 0.2$. the mass ratio is about 1:5, so we have much more base than acid—and this is again plausible, because the pH-value is higher than the pK_A-value of the acid used. Fits like a glove!

3. According to ► Eq. 5.12, with f = 1.09 and $c_{desired}$ = 0.23 mol/L, we get $c_{is} = f \times c_{desired}$ = 1.09 ×0.23 mol/L = 0.25 mol/L.

4. With $c_{desired}$ = 0.25 mol/L and c_{is} = 0.26 mol/L, again according to ► Eq. 5.12, the titre is f = 1.04.

5. At the equivalence point, the amount of EDTA added to the analyte solution is equal to the amount of analyte, i.e. n(EDTA) = n(analyte). Ideally, EDTA and analyte form a stable chelate complex here. (In fact, dissociation also occurs to a minimal extent, so that really tiny amounts of free analyte particles and free EDTA are present in solution. Under most conditions, however, this dissociation can be neglected.)

6. The actual concentration of the EDTA solution used can once again be determined according to ► Eq. 5.12: $c_{is}(EDTA) = f \times c_{desired}(EDTA)$ = 1.03 ×0.1 mol/L = 0.103 mol/L. According to task 5 the following is then obtained

   And with c(analyte) = n(analyte)/V(analyte) an analyte concentration of 1.07 [mmol]/20.00 [mL] = 53.5 mmol/L.

7. First, it must again be taken into account whether the measured values lie *before* or *after* the equivalence point.

   (a) V(titrant) = 7.00 mL is before the EP, so the potential depends on tin(II) and tin(IV): With 7.00 mL titrant, 70% of all tin(II) ions are then converted to tin(IV). So

$$E_{(new\,1)} = 0.139\ V + \frac{0.059\ V}{2} \cdot \lg\frac{70}{30} = 0.150\ V.$$

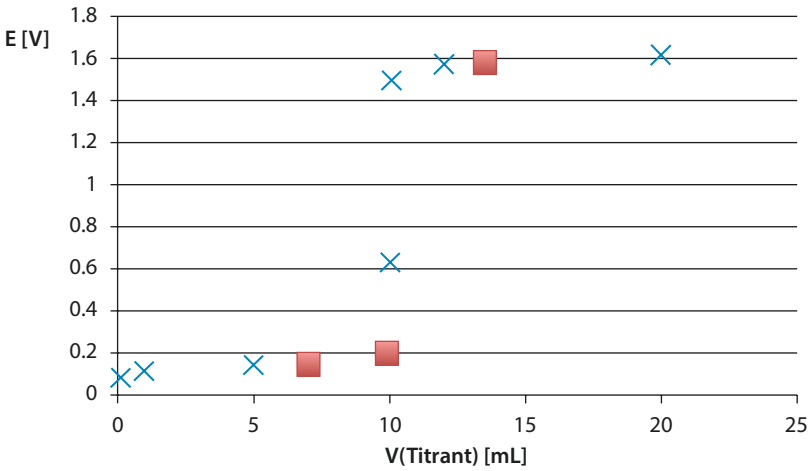
(b) V(titrant) = 9.90 mL is still (very slightly) *before* the EP. Here 99% of all tin(II) ions have been converted to tin(IV):

$$E_{(new\,2)} = 0.139\ V + \frac{0.059\ V}{2} \cdot \lg\frac{99}{1} = 0.198\ V.$$

(c) 13.00 mL titrant are *after* the EP, so we may consider 10.00 mL as cerium(III) solution, the remaining 3.00 mL as cerium(IV) solution:

$$E_{(new\,3)} = 1.61\ V + \frac{0.059\ V}{1} \cdot \lg\frac{3}{10} = 1.58\ V.$$

Let's look at the graphical plot (the three "new" readings are marked as squares):



Graph of cerimetry from 5.2

This is quite consistent with the trend indicated by the readings marked with diamonds.

8. Here we must first remember that the pK_{sp} value is the negative decadic logarithm of the K_{sp} value, so it holds:

$$K_L = 10^{-pK_{SP}}$$

(a) This results in: $K_{sp}(AgBr) = 10^{-12.3} = 5.01 \times 10^{-13}$.

(b) Since the dissociation equation is: $AgBr \rightleftarrows Ag^+ + Br^-$, the $([Ag^+] \times [Br^-])$ unit mol^2/L^2 is obtained for the solubility product.

(c) Since in this solution, $[Ag^+] = [Br^-]$ (there are exactly as many cations as anions in solution), following also applies

$$K_{sp} = \left[Ag^+\right]^2 = \left[Br^-\right]^2, \text{ so is } n\left(Ag^+\right) = n\left(Br^-\right) = n(AgBr).$$

$$\text{in solution} = \sqrt{K_{sp}} = \sqrt{10^{-12.3}} = 7.077.07 \times 10^{-7}\ \text{mol/L}$$

To get from the amount of dissolved substance (i.e. the concentration of the amount of substance, which you have learned from Part I according to DIN 1310 to abbreviate c_i) to the mass concentration β_i (actually to be given in kg/m^3, but we stick to kg/L here), we still need the molar mass of our substance. But we can get that from any useful periodic table; after all, all we have to do is look up the masses of the atoms involved. With M(Ag) = 107.86 g/mol

and M(Br) = 79.90 g/mol, we get M(AgBr) = 187.76 g/mol. According to the formula n = m/M (known from part I) and the equation for the mass concentration (known from the same part) we arrive at a volume $V_{solution}$ = 1.0 L:

$$\beta_i = \frac{m_i}{V_{Lsg}} = \frac{n_i \times M_i}{V_{Lsg}} = \frac{7.07 \times 10^{-7}\,[\text{mol}] \times 187.76\left[\frac{\text{g}}{\text{mol}}\right]}{1\,[\text{L}]}$$
$$= 0.000132\ \text{g/L} \quad = \quad 0.132\ \text{mg/L}$$

That's not much.

9. We are looking for V(titrant), where n(titrant) = n(analyte). Accordingly, it is worthwhile to first determine n(analyte) and n(titrant).

   We know: V(analyte) = 10.0 mL, c(analyte) = 0.23 mol/L.

   By converting c = n/V one arrives at

$$(\text{analyte}) = c(\text{analyte}) \times V(\text{analyte}) = 0.23\ [\text{mol/L}] \times 0.010\ [\text{L}]$$

   (The dimensional analysis scans!) = 0.0023 mol (= 2.3 mmol)

   Furthermore we know: $c_{desired}$(Titrant) = 0.1 mol/L, f(Titrant) = 1.02. With the same formula, extended by the correction factor f, we obtain

$$n(\text{titrant}) = V(\text{titrant}) \times c_{is}(\text{titrant}) = V(\text{titrant}) \times f(\text{titrant}) \times c_{desired}(\text{titrant}).$$

   But what we are looking for now is V(titrant), and we know that at the equivalence point we have:

   n(titrant) = n(analyte). The total is thus:

   (Dimensional analysis scans again!) = 0.02.255 L = 22.55 mL.

   Plausibility check: The analyte solution is more than twice as concentrated as the titrant solution, so more than twice the titrant volume is also required to reach the equivalence point.

10. Here one could think very extensively " outside the box" and bring in many, many formulas, but ultimately there are some much simpler considerations behind it.

    Firstly, the (molar) amount of lead(II) chromate must be identical to the amount of chromium(III) ions contained in the sample. Thus: $n(PbCrO_4) = n(Cr^{3+})$. So also applies: $m(PbCrO_4)/M(CrO_4) = m(Cr^{3+})/M(Cr^{3+})$. In other words:

    It is easy to determine the mass of chromium contained in the precipitate:

$$m(Cr^{3+}) = M(Cr^{3+}) \times m(PbCrO_4) / M(PbCrO_4).$$

    With $M(Cr^{3+}) = M(Cr) = 51.99$ g/mol and $M(PbCrO_4) = 323.14$ g/mol *(the periodic table helps!)* we come, since $m(PbCrO_4)$ is given, to

    Second, the precipitation must have occurred quantitatively, so one can use this mass of chromium right away in the equation of determination for the mass fraction ω_{Cr} (from Part I).

    This results in

$$\omega_{Cr} = m(Cr^{3+}) / m_{total} = 3.70\ [\text{mg}] / 50.0\ [\text{g}]$$
$$= 3.70\,[\text{mg}] / 50.000\,[\text{mg}] = 7.4 \times 10^{-5} \quad \text{or} \quad 0.0074\%$$

11. A mass fraction of ω_{Pb} = 42% means (rearrange the corresponding equation of determination!) that $m(Pb) = \omega_{Pb} \cdot m_{total} = 0.42 \cdot 25$ [g] = 10.5 g. According to n = m/M, this gives

$$n(Pb) = 10.5\ [g] / 207.19\ [g / mol] = 0.051\ mol.$$

Since $n(Pb) = n(PbSO_4)$, we can state in one step:

$$m(PbSO_4) = M(PbSO_4) \cdot m(Pb) / M(Pb)$$
$$= 303.22\ [g / mol] \cdot 10.5\ [g] / 207.29 [g / mol] = 15.36\ g.$$

12. We know: m(Cu) = 666 mg; V(solution) = 42.23 mL. For the molarconcentration c(Cu), c = n/V and n = m/M can be summed up to c = m/MV. Thus:

$$c(Cu) = 0.666\ [g] / 63.55\ [g / mol] \times 0,04.223\ [L]$$

(The dimensional analysis therefore already tells us that it correctly boils down to the unit mol/L!) = 0.25 mol/L. And for the mass concentration?—There it is even simpler, after all the equation of determination for this is $\beta_i = m_i/V_{total}$. m_i here means m(Cu), and V_{total} = V(solution), so it is β_i = 666 [mg]/42.23 [mL] = 15.77 (or 0.1577 %).

13. The specific wavelength is not overly important here: What we need here is once again the colour wheel (► Sect. 6.2): the complementary colour to blue is orange (the mixed colour resulting from "the other two primary colours"), so the sample of an analyte that can be excited by this wavelength and therefore "cuts it out" partly or completely from the spectrum of visible light will appear orange in aqueous solution.

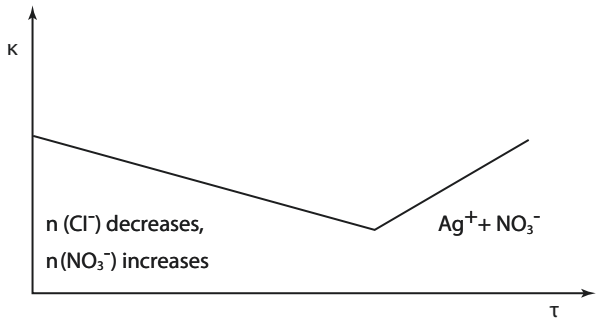
14. Electromagnetic radiation with $\lambda < 380$ nm is no longer visible to the human eye (we are in the near UV range); accordingly, there is also no complementary colour: If radiation from this wavelength range is "cut out" of the spectrum, this does *not* lead to a colour impression that can be perceived by the human eye. If the analyte does not also absorb electromagnetic radiation from the part of the spectrum known as VIS (i.e., from the 380 nm < λ < 780 nm range), the sample appears colourless. To humans, at least. Some butterflies, for example, also perceive a part of the UV radiation, which is why these animals recognise remarkable patterns in flowers that are "boringly white" for us, which for the human eye can only be perceived via false colour images.

15. Initially, i.e. before the addition of the silver nitrate solution, only sodium and chloride ions are present, which accordingly both contribute to the conductivity. But as soon as the first drop of titrant has been added, it is necessary to consider what actually happens chemically in this conductometric titration: While NaCl and $AgNO_3$ are quite soluble in water, this is not at all true for silver chloride (AgCl): the solubility product of this compound is quite low with $K_{sp}(AgCl) = 2 \times 10^{-10}\ mol^2/L^2$ ($pK_{sp}(AgCl) = 9.7$). Accordingly, the following reaction equation results:

$$Na^+_{(aq)}Cl^-_{(aq)} + AgNO_{3(aq)} \rightarrow Na^+_{(aq)} + NO^-_{3_{(aq)}} + AgCl\downarrow$$

In other words: While the newly added nitrate ions contribute their part to the conductivity of the reaction mixture, one chloride ion precipitates for each NO_3^- ion added. Nothing changes for the sodium ions, and the silver ions have no chance at all to contribute their part to the conductivity, since they precipitate immediately. But since nitrate ions are less conductive than chloride ions (the question itself told you that!), the conductivity of the mixture as a whole will drop. At least up to the equivalence point, and there everything changes: at the EP, exactly as many silver cations as chloride ions have met, so virtually all the chloride ions have precipitated. (AgCl is so poorly soluble in water that the few Ag^+ and

Cl^- ions that remain in solution hardly contribute anything to the total conductivity.) After the EP, new silver and nitrate ions are now added, and from now on the Ag^+ cations also contribute to the conductivity, after all the reaction mixture now no longer contains ions with which they would be forced to precipitate immediately. Accordingly, the conductivity increases significantly after the EP:



Conductometric titration of a saline solution with silver nitrate

Cave

Two popular mistakes:

- People who have *not* understood at *all* what the pH value (which is known to be logarithmic) is all about, seriously come up with the following kind of calculation:

  "If a 0.1 molar solution has the pH value x, then a 0.2 molar solution must have the pH value 2x". The fact that this inevitably results in a *higher* value (i.e. the solution is supposedly *less* acidic despite the higher acid concentration) is simply ignored. In brief: don't.
- Those who have understood *a little,* but not enough, like to abbreviate their reasoning as follows:

  "If a 0.1 molar solution has pH x, then a 0.2 molar solution, which of course should have a lower pH, has pH x/2." This actually almost makes sense at first glance — but it's still completely wrong.

Further Reading

Binnewies M, Jäckel M, Willner, H, Rayner-Canham, G (2016) Allgemeine und Anorganische Chemie. Springer, Heidelberg

Brown T L, LeMay, H E, Bursten, BE (2007) Chemie: Die zentrale Wissenschaft. Pearson, München

Harris DC (2014) Lehrbuch der Quantitativen Analyse. Springer, Heidelberg

Jander G, Jahr KF (1989) Maßanalyse. de Gruyter, Berlin

Mortimer CE, Müller U (2015) Chemie: Das Basiswissen der Chemie. Thieme, Stuttgart

Kunze UR, Schwedt G (2002) Grundlagen der qualitativen und quantitativen Analyse. Wiley, Weinheim

Ortanderl S, Ritgen U (2018) Chemie—das Lehrbuch für Dummies. Wiley, Weinheim

Ortanderl S, Ritgen U (2015) Chemielexikon kompakt für Dummies. Wiley, Weinheim

Schwister K (2010) Taschenbuch der Chemie. Hanser, München

Some of the works mentioned here are more suitable for revising or deepening the "chemical basics", others go beyond "the Harris" with regard to selected areas of analysis.

Chromatographic Methods

Requirements

Chromatography is primarily based on the interaction of the analytes we are interested in with the chromatographic material used in each case, so intermolecular interactions in particular are of indispensable importance in this part. The following terms should therefore be second nature to you:

- ion-dipole and dipole/dipole interactions,
- hydrogen bonds,
- van der Waals forces.

Accordingly, you should not only be familiar with terms like polar/non-polar, but (very important!) have internalised that these two terms are not to be treated "digitally" ("Polar? yes/no"), but that there is a continuous transition from "completely non-polar" to "extremely polar". It should also be self-evident that the polarity of a molecule (or molecular ion) depends on the electronegativity difference of the atoms involved as well as the spatial structure of the respective compound (here the VSEPR model from *General and Inorganic Chemistry* comes in handy, of course).

In addition, there are various aspects of extraction processes covered in Part I, in particular:

- *"Like dissolves like."*,
- the Nernst distribution rate/coefficient of distribution,
- the terms *stationary phase* and *mobile phase*,
- adsorption and desorption.

1.1 Learning Objectives

The collective term "chromatography" covers numerous separation methods which make use of the fact that the individual analytes are adsorbed to different extents on the surface of one or the other solid, can be brought into solution with different degrees of ease, etc. The term "chromatography" is also used to describe the whole process of separation based on these phenomena.

In this part, you will get to know various chromatographic methods in which the analytes are present in solution, or in gaseous form, and interact with solids or liquids. You will find that the underlying fundamentals have already been covered in Part I and Part II, but this time the aim is to make not only roughly qualitative but also *quantitative* statements, in particular with regard to the quality of the separation obtained (= resolution). Various (statistical) effects come into play, which have also already been dealt with in Part I and Part II (and to

some extent in other courses or textbooks). Various aspects of *general and inorganic chemistry* on the one hand and *physical chemistry* on the other hand will go hand in hand here.

As in Part II, in addition to the various separation methods, various detection methods will again be considered, at least some of which are based on principles you are already familiar with, while others already offer an outlook on subsequent parts (especially on the subject of *instrumental analysis*).

Part I dealt with the qualitative fundamentals of extraction, which ultimately already enabled the first substance separations on the basis of (physico-)chemical properties, such as polarity, and which are based on distribution equilibria. We will again devote ourselves to these fundamentals of adsorption and desorption here with regard to different chromatographic processes, and we will look at them a little more closely, occasionally up to the level of quantification (or at least quantifiability).

- In liquid chromatography, in particular the variant in which *chromatography columns* are used, the analytes are present in solution, and they are, depending on their physicochemical properties, adsorbed more strongly or more weakly onto a solid, from which they must subsequently be desorbed again, so that suitable detectors can ultimately state their presence.
- In gas chromatography, gaseous analytes are either adsorbed onto a solid in an analogous manner or dissolved in a liquid film wetting the surface of a solid, and are subsequently released.
- In more specific forms of chromatography, forces other than polarity—and thus possibly hydrogen bonding or other dipole-dipole interactions—come into play between the analyte and the adsorbent material.
- Selected forms of electrophoresis, which involves the migration of charged particles in an electric field, only at first glance seem to have nothing to do with chromatography.

The more precisely you understand the principles behind all of these techniques, the more likely you are to develop an eye for possible problems or new kinds of problems and thus move from pure knowledge reproduction to independent thinking (in your field of expertise or even beyond) ... and that exactly is what ultimately makes the difference between “learning by heart” and “studying”.

Contents

General Information on Chromatography

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_11

Summary

The generic term *chromatography* covers a whole range of different processes for separating substances, all of which have one thing in common—the principle behind them:

All chromatography is based on the fact that different substances present in a *mobile* phase (liquid or gaseous) (usually: the analytes) interact differently with a *stationary* phase (usually a solid).

Harris, Section 22.2: What is chromatography?

There are numerous concepts behind this, which we in part already dealt with in the section "Extraction" in some detail in Part I, whereby—as already mentioned in said Part—in chromatography, unlike in extraction, one of the phases used is *immobile* (the **stationary phase,** which does not move or is specifically held in place), while the second phase passes the first more or less quickly (flows past it or through it) and is therefore referred to as the **mobile phase.** The desired separation effect then results from how strongly (or weakly) the analyte dissolved in the mobile phase interacts with the stationary phase and is accordingly hindered/restrained when flowing through the apparatus (usually referred to as a **column**).

The extent of the interactions considered is determined almost exclusively by polarity: The individual analyte particles (neutral compounds or ions of whatever charge) are adsorbed on the surface of the stationary phase for a certain period of time, and then finally desorbed after further solvent (i.e. the mobile phase) has continuously flowed in.

It is important to note that the mobile and stationary phases differ in polarity:

- Often a non-polar (or very low polar) mobile phase is combined with a (moderately to strongly) polar stationary phase. This is called ***normal phase*** **chromatography (NP-)**.
- Depending on the analytes to be separated from each other, it is sometimes done the other way round; then it is called ***reversed phase*** **chromatography** (usually abbreviated **RP-**).

Cave

It should be emphasised once again that the terms "polar" and "non-polar" must always be considered to be *relative:* Two substances classified as "polar" may still differ immensely in their polarity. Accordingly, in the case of a sufficiently polar stationary phase, for the mobile phase a solvent may also be required that would by no means be called "non-polar" outside this specific context or without direct comparison with its counterpart.

Ethanol, for example (C_2H_5OH), is undoubtedly "less polar than water", but if one considers the electronegativity differences present there and determines the spatial structure of this molecule using the VSEPR model, it becomes immediately apparent that this molecule doubtlessly still has a dipole moment and is therefore very much "polar"—just not as polar as H_2O is.

Depending on what is used as the mobile phase and what is used as the stationary phase, a distinction is made between different techniques:

- In **liquid chromatography** (**LC** covered in ► Chap. 12), which belongs to the *adsorption* chromatography methods, a solid is usually used as the stationary phase, while a liquid (a solvent or a solvent mixture) serves as the mobile phase.
  - **Thin-layer chromatography (TLC)** is such a classic example of liquid **chromatography,** that is difficult to imagine everyday laboratory life without it. (We will deal with this in ► Sect. 12.1.)

  - LC, which uses the kind of **columns** already mentioned in Part I, also falls into this area (► Sect. 12.2), as does the **high-performance liquid chromatography** (commonly abbreviated **HPLC;** ► Sect. 12.3) based on it.
- **Gas chromatography (GC)** is also based on the adsorption of the analyte(s) onto the stationary phase, which is also generally present as a solid. The big difference is that in GC the mobile phase is *gaseous* and the analytes thus first must be brought into this state of aggregation (more on this in ► Chap. 13).
  - In addition, there are numerous more specialised forms of chromatography (we will discuss some of these in ► Chap. 14). Examples of these are:
  - **ion chromatography** based on ion exchange columns;
  - **ion pair chromatography,** which can also be regarded as a special form of HPLC;
  - **exclusion chromatography,** in which the absolute size of the analytes is of primary importance; *and*
  - **affinity chromatography,** in which the interaction between the analyte and the two separation phases is not based exclusively on the classical intermolecular interactions (polar/non-polar) and is much more specific.
  - Finally, various forms of (gel) **electrophoresis** can also be regarded as chromatography. For this reason, they have also been included in this part; the most important fundamentals on this subject are dealt with in ► Chap. 15.

A good overview of the different forms or basic types of chromatography is provided (of course!) by Harris.

Harris, Fig. 22.2, Section 22.1: Solvent extraction

Liquid Chromatography (LC)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_12

Summary

In **liquid chromatography**, usually referred to simply as **LC**, the separation of different analytes is based on the interaction between the analyte particles in solution and the solvent molecules (the mobile phase) on the one hand and the interaction between the analytes and the stationary phase on the other. In principle, a distinction is made between two cases:

- In *thin-layer chromatography*, the stationary phase is deposited on a carrier material.
- In *column chromatography*, a hollow body (usually column-shaped or wire-like) is filled with the solid serving as the stationary phase.

The separation principle—the better/stronger the analyte can interact with one or the other phase, the faster or slower it will move to the other end of the stationary phase—in both cases remains unchanged.

12.1 Thin Layer Chromatography (TLC)

Thin layer chromatography (usually abbreviated **TLC**), already known for a long time, is still part of everyday life in many laboratories today because it can be used to achieve at least rough qualitative separations of different analytes very easily and within a short time (usually only a few minutes).

In this process, one drop of each of the different samples is applied to a starting line near the lower end of a carrier plate coated with silica gel (silicon dioxide, SiO_2), aluminium oxide (Al_2O_3), or cellulose (a polysaccharide with the general molecular formula $(C_6H_{10}O_5)_n$). A glass plate or a relatively thick aluminium foil often serves as the carrier material.

Lab Tip

In the latter case, the size of the TLC plate can be individually determined with the help of simple scissors, which is of course very practical for everyday routine examinations. However, cutting the plates requires a little skill: if the cutting tool is held at the wrong angle, two problems can occur:

- If the scissors are held at to flat an angle, the coating may crumble off—more or less completely.
- If the scissors are held too steeply, there is a risk that stress cracks will appear in the coating. Those are not necessarily visible to the naked eye, but lead to—clearly unintentional—additional capillary effects (see below).

As is so often the case in the lab, practicing helps.

The plate is then placed in a vessel whose bottom is covered with the mobile phase—a (rather) non-polar solvent or solvent mixture. Thanks to the capillary action of the fine crystals on the carrier plate, the solvent immediately migrates upwards—and depending on how pronounced the non-polar behaviour of the respective analytes is, those are "carried along" by the mobile phase to a greater or lesser extent. ◘ Figure 12.1 shows an example of the thin-layer chromatographic separation of two (arbitrary) analyte mixtures.

When the mobile phase, rising due to capillary forces, has almost reached the upper end of the TLC plate (in ◘ Fig. 12.1b the solvent **front** is indicated by a dotted line), the plate is removed from the solvent container, and the dis-

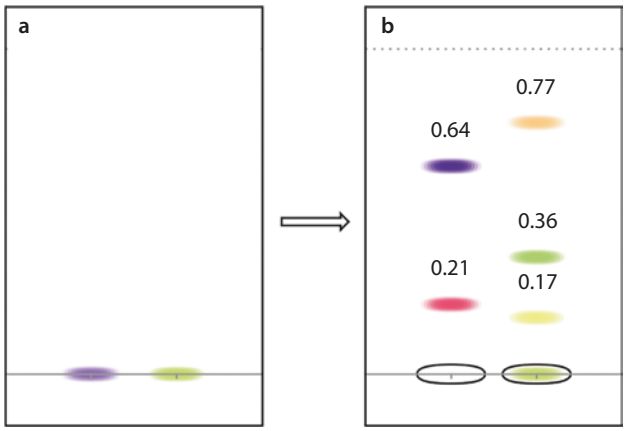


Fig. 12.1 TLC separation of two analyte mixtures: (**a**) at the start; (**b**) in the end

tance of the respective substance spots from the starting line is determined. The ratio of the distance migrated by the respective analyte to the distance migrated by the solvent front is referred to as the **retention factor,** often just called the **R_f value:**

$$R_f = \frac{\text{Distance migrated by the analyte}}{\text{Distance migrated by the solvent}} \tag{12.1}$$

Since two distances are related to each other here, i.e. this is a *relative measurement*, the retention factor R_f of each analyte is *dimensionless* and substance-specific for the same solvent and the same TLC plate coating (provided the conditions, such as temperature, etc., are otherwise identical, as well). (In Fig. 12.1b, the respective R_f values of each analyte are given individually. Of course, $0 \leq R_f \leq 1$ *always* applies. How could it be otherwise?)

Lab Tip

Since, as mentioned above, capillary forces are responsible for the rising of the solvent (mixture), it becomes understandable how the above-mentioned stress cracks can extremely falsify TLC results: Should any additional capillary effects arise locally due to fine cracks, the solvent front will migrate much faster at the corresponding sections of the plate than elsewhere—but, since most solvent (mixtures) are colorless, this will hardly be seen at first. The alleged R_f values obtained this way are then hardly comparable.

Following the principles repeated in ► Chap. 11, it should be understandable that if a nonpolar liquid phase and a polar stationary phase are used (and all the coating materials stated above are strongly polar!), the R_f value of each analyte *decreases* with increasing polarity.

However, an excessively polar analyte would interact so strongly with the stationary phase that it would *not migrate at all* under the influence of a nonpolar mobile phase. For such cases or analytes, there is then the **reversed-phase thin-layer chromatography** (RP-TLC for short), in which the analytes are applied to a nonpolar-coated TLC plate, and a *polar* solvent then serves as the mobile phase. In this form of *reversed-phase chromatography*, the R_f value *increases* accordingly with the polarity of the respective analyte.

Lab Tip

Thin-layer chromatography is often used in synthesis chemistry, for example, to determine whether the reaction solution already contains the desired product—or at least some substance that was not yet present in the reaction mixture at the start of the reaction. (Then one still has to consider whether the increased or decreased polarity of this new substance compared to the reactants also matches the desired product. Occasionally it can be noticed by thin layer chromatography if the synthesis has taken a completely unexpected/undesired turn.)

Questions

1. On a TLC plate of 10.0 cm length, a substance mixture was applied on the starting line located 1.0 cm above the lower edge and then the separation was initiated. After the plate was removed from the solvent container when the solvent front was still 4 mm from the top edge of the plate, three discrete spots of substance could be detected on the plate, centered at 4.2 cm, 5.8 cm, and 7.3 cm, respectively, above the lower edge of the plate. Determine the R_f values of the three substances.
2. Why is it inadvisable to use water as the mobile phase for TLC plates coated with alumina?
3. An allegedly pure substance deposited on a silica TLC plate showed an R_f value not differing from 0 when using diethyl ether (C_2H_5–O–C_2H_5) as a solvent. A lab colleague recommends to try a diethyl ether/ethanol mixture instead of pure diethyl ether for a second attempt. What are your thoughts on this?

12.2 Column Chromatography

When columns come into play, we still deal with *adsorption* chromatography: It is again a matter of the interaction of the analyte(s) with the stationary and the mobile phase, respectively. The weaker the interaction with the column material (i.e. the stationary phase), the faster the analyte will be "rinsed off the column". Accordingly, two analytes of different polarity will also have different *retention times on the column* (mostly just called **retention times t_r**).

Unlike thin-layer chromatography, column chromatography is not concerned with *how far* the analyte migrates within a certain time, but *how long* it takes for one or the other analyte to leave the column again. The principle of column chromatography is beautifully illustrated in Fig. 22.5 of Harris.

Harris, Section 22.2: What is chromatography?

Since thin-layer chromatography not only permits the more or less efficient separation of various analytes of different polarity, and since the (substance-specific) R_f value associated with the respective analyte permits not only relative but, under constant conditions, even at least roughly quantitative statements, the same should apply to column chromatography, which is, after all, based on the same principle.

Yes, it should. The problem is that in column chromatography, in addition to the three parameters of relevance—column material used (i.e. the *stationary* phase), solvent used (the *mobile* phase), and temperature—, there is a large number of further parameters to consider:

- the *column length:* Since here, after all, no relative measurement takes place, but the retention time is measured in *absolute terms*, a change in the column

length (understandably) leads to different results even with unchanged stationary and mobile phases (and constant temperature).
- the *flow velocity of the mobile phase:* The faster the mobile phase moves, the faster it should carry the analytes along with it.—But how do we specify this velocity? Should we perhaps note in our lab log that the *volume flow rate of* our column is, for example, 23 mL/min? Does this statement help at all? After all, in order to be able to do something with such a statement, something else would have to be taken into account:
- the *column diameter:* The internal volume of a wider column is undoubtedly larger than that of a narrower column. Accordingly, a wider column will also require more solvent/mobile phase from the outset. So we should rather resort to the *linear flow velocity*, which tells us how many centimetres (or millimetres) of the total column length the "solvent front" covers within a given time.
- the pure "distance": Even an analyte that does not interact with the stationary phase at all, but is simply "carried along with the solvent front", requires a certain amount of time to reach the other end of the column.

In order to take all these aspects into account, some simple considerations and calculations are required—which are understandably easier and more plausible the more you know what you are actually doing here.

Chromatograms: What They Look Like ...

Harris, sec. 22.3: Chromatography from a pipelayer's point of view

Let us first look at a stylised chromatogram with four analytes with the following retention times:

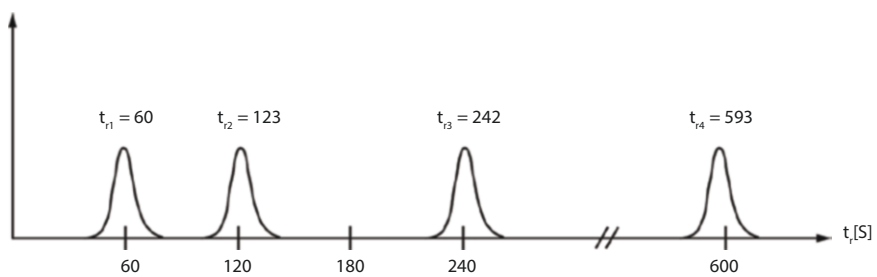
$t_1 = 60$ s; $t_2 = 123$ s; $t_3 = 242$ s; $t_4 = 593$ s.

Certainly, it is useful to plot the (retention) time on the x-axis and the strength of the detector signal obtained for each analyte on the y-axis (◘ Fig. 12.2).

Apart from the fact that we already know which of the four analytes interacted most strongly with the stationary phase (this is, of course, analyte 4, precisely because it took the longest to reach the detector), this stylised chromatogram immediately leads us to at least three further questions:

(a) What kind of detector was actually used?
(b) Do the detectors respond to each analyte in exactly the same way? (This would at least be very useful if one wants to *quantify* the different analytes at the same time.)
(c) Do all signals (usually called **peaks** in chromatography) really always look the same?

Let's go through these questions in order:
- To (a) we can say: Basically one here uses mainly the same detectors which were already mentioned in part II. (In addition, there are also more special cases, but these will not be discussed further here.) Many of the detectors



◘ **Fig. 12.2** Chromatogram of an analyte mixture

commonly used in chromatography are based on the absorption of electromagnetic radiation of one or another wavelength (often from the UV/VIS range).

- As for (b): Occasionally you either have to try out a little longer until you find the right detector (or even just the right measuring wavelength) ... or else you have to think about how to solve or otherwise circumvent the problem if the chosen detector responds differently to different analytes. (But we are actually already familiar with this problem, too: Isn't it strikingly reminiscent of the calibration problem [from Part I]? For the time being, however, we are not yet interested in the problem of quantification: For the moment, we assume that all analytes are present in the same concentration and that the detector responds to them all in the same way.)
- And to (c): a firm *no.* In fact, with a little expertise and skill, a great deal of additional information can be gleaned from the individual shape of each peak. You will encounter some of this information later in this part (in ► Sect. 12.3), but most of it is beyond the scope of any basic introduction. All that remains for me to do here is to refer you to the more detailed technical literature.

So for now, let's make it a bit simpler: We just consider four *peaks* that differ only in their respective retention times ($t_{r1–4}$).

To be able to compare the measured values, we must first find out what the "supposed retention time" is (depending on column length and linear flow velocity) for a substance that spends a *minimum of time on the column*, i.e. passes through it practically without any interaction with the stationary phase. The following question must therefore be answered:

How long would it take a substance to simply pass through the column, even if the stationary phase did not hold it back by interactions of any kind?

In our example, this is exactly the case for the substance with $_{tr1}$ = 60 s, our (supposed) analyte 1. In fact, we have added this to our actual analytes (2–4) as an *internal standard* (just as you already know them from Part I!) in order to determine the *minimum dwell time* (the **dead time**) of any substance on the column.

Cave

For such a procedure, one must of course be able to rely on the fact that the substance selected as the internal standard (whatever it may be) will *really* pass through the column without interaction. Accordingly, one should inform oneself extensively in advance. *"Trial and error"* would probably be the wrong approach here, but there is a technical aspect that can occasionally be exploited in these cases: The analyte mixture must, after all, be applied to the column in the first place at the start of the separation; this is usually done by one form of injection or another, and this often leads to an *injection peak*. This is commonly all the more noticeable,

- the more the refractive index of the analyte mixture differs from the refractive index of the mobile phase used,
- the more sensitive the detector used, and
- the shorter the wavelength to which the detector has been set.

If all goes well, the injection peak is clearly visible, but it may also be limited—according to the above criteria—to a minor and not very meaningful baseline disturbance.

Lab Tip

The use of an internal standard is not absolutely necessary—especially with a well-known pair of mobile and stationary phase and with continuous use of the same column (with correspondingly known constant volume and constant length) one can possibly do without it. But it is undoubtedly *safer* (and also more comprehensible/transparent for others—think of the quality assurance from Part I!) to work with an internal standard.

However, an internal standard that is used exclusively to indicate the dead time is only used extremely rarely in everyday laboratory work (in this text it was introduced specifically to explain the concept of dead time). It is more common to use an internal standard that has a certain similarity to the actual relevant analytes in terms of its polarity (and thus in terms of its interaction with the column material) and—as far as possible—also in terms of its structure (functional group/s or the like). If the chromatographic behaviour of this internal standard is known, the retention times obtained for the respective analytes can be evaluated much better. If, in addition, the internal standard is used in a precisely known concentration, this also permits—by comparison with the peak height (or peak area) obtained—statements about any substance losses during the work-up or about the sensitivity of the detector used.

If we then subtract this minimum retention time ($\mathbf{t_m}$, for *minimum*) from the actually measured retention times t_r of the other analytes, we end up with the **reduced retention times $\mathbf{t_r'}$**—and those are considerably more informative:

$$t_r' = t_r - t_m \tag{12.2}$$

Retention times (t_r) and reduced retention times (t_r')

	Retention time [s]	Reduced retention time [s]
Analyte 1 (internal standard)	60	$\mathbf{t_m}$ **(= 0)**
Analyte 2	123	63
Analyte 3	242	182
Analyte 4	593	533

Now, for a mixture of different analytes (here: those to which the peaks 2 to 4 respectively belong), their respective *reduced* retention times can indeed be compared—with the help of the **separation factor α**, which is just the ratio of the relative reduced retention times (i.e. the *relative retention*):

$$\alpha = \frac{t_{r(b)}'}{t_{r(a)}'} \tag{12.3}$$

By definition, one always handles it in such a way that the larger of the two corrected retention times ($t_{r(b)}$) acts as the numerator, the smaller ($t_{r(a)}$) as the denominator. In this way, $\alpha > 1$ always holds, and the larger the separation factor, the better the separation. (If it were exactly 1, there would obviously be no separation at all, and that is not exactly the idea ...)

Depending on the additional literature you might use, it is quite possible that you will encounter the term *"corrected retention time"*. Please remember that **reduced retention time** and **corrected retention time** are synonyms. (The same applies, of course, to *unreduced* and *uncorrected* retention time.)

Please note that by using the reduced retention time, something is already stated about the minimum retention time on the column. In some cases, however, it is more useful not to indicate the corrected relative retention, but to determine the *ratio of the relative unreduced retention times* (i.e. the *uncorrected relative retention*), usually abbreviated with the Greek letter γ:

$$\gamma = \frac{t_{r(b)}}{t_{r(a)}} \tag{12.4}$$

Figure 12.2 already clearly shows that the separation has proceeded cleanly, after all, the individual peaks do not overlap (which need not always be the case, as you will soon see!). Nevertheless, we should take a look at the separation factors in question (it should be obvious that one always compares only immediately adjacent peaks with one another ...):

$$\alpha_{3/2} = 182/63 = 2.89;\ \alpha_{4/3} = 533/182 = 2.93$$

However, the separation factor again only leads to a *relative* statement, as two analytes are always compared with one another. If *only one* analyte is to be described, the use of the **retention factor k** is recommended, in which the minimum retention time of an analyte on the column is also taken into account:

$$k = \frac{t_r - t_m}{t_m} = \frac{t_r'}{t_m} \tag{12.5}$$

Thus, for each individual analyte, one obtains the column chromatographic counterpart to the R_f value from thin-layer chromatography, quite independent of any other analytes that may also have passed through the column; in this case: $k_2 = 1.05$; $k_3 = 3.03$; $k_4 = 8.88$. (The question of why no *k* value was given for analyte 1 should definitely not arise ...)

Questions

4. A chromatogram, obtained using a column with a minimum retention time of 27 s determined in advance, shows the following retention times: $t_{r1} = 42$ s, $t_{r2} = 93$ s, $t_{r3} = 142$ s, $t_{r4} = 169$ s and $t_{r5} = 267$ s.
   a) What are the reduced retention times?
   b) Say something about the associated retention factors.
   c) Determine the uncorrected relative retention and the separation factor for peaks 3 and 4 as an example.

... and How to Evaluate Them

The individual peaks from our stylised chromatogram (Fig. 12.2) are cleanly separated, but what happens when the retention times (corrected or uncorrected) are closer together? And how *wide* are peaks in chromatograms in general?

It would certainly be desirable if the peak profiles were always as pleasingly narrow as shown in Fig. 12.3a, so that even closely spaced peaks (i.e. peaks whose [un]corrected retention times are very similar) would be clearly separated from each other. In real life, however, one often does not get such narrow peaks, but experiences an overlapping of the individual peaks if the t_r differences are too small, as shown schematically in Fig. 12.3b.

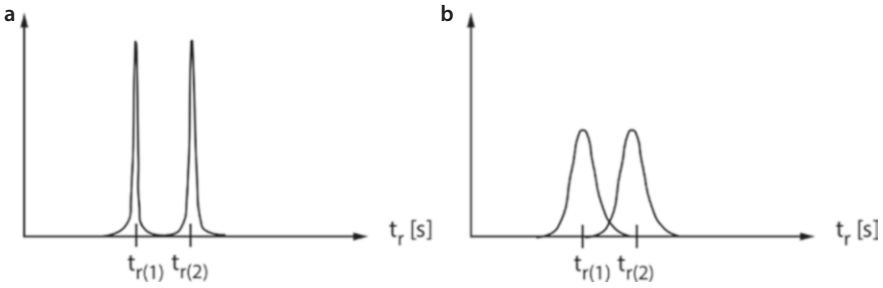


Fig. 12.3 **a** Near-ideal and **b** more realistic *peak shapes*

Please note that the difference in the retention times of the two analytes in Fig. 12.3a, b is identical, and this thus also applies to the uncorrected relative retention γ and the separation factor α. Obviously, the separation factor alone does not determine the *resolution of* a chromatogram.

Peaks as Gaussian Curves

If we take a closer look at the two peak shapes in Fig. 12.3, we come to the conclusion that both largely correspond to a Gaussian curve, the middle of which is regarded as t_r in each case—which is why the two retention times in Fig. 12.3a, b also correspond. Nevertheless, we need a tool to describe the *shape* of the real peaks, as well. In principle, one could determine the **base width w** for this purpose. However, it is less common to measure the width of the corresponding peak at the *base* (Fig. 12.3b shows us that this can be difficult or even impossible if two or even more peaks overlap, i.e. merge into each other), than to choose $\mathbf{w_{½}}$, the width of the peak *at half height* (increasingly called **half-width**), as shown in Fig. 22.9 from Harris.

Harris, para. 22.4: Efficiency of separation

For a peak corresponding to the Gaussian ideal, the ratio of $w_{½}$ to w is constant—although it should be pointed out at this stage that not all peaks get even close to this ideal: Possibilities for corresponding perturbations abound (you will encounter one of them in the very next section). But before we deal with non-ideal peak shapes, we should address the question of how an (ideal) Gaussian curve can actually be described mathematically.

Here a formula helps, to whose understanding certainly also fig. 4.3 from Harris contributes:

Harris, Section 4.1: Gaussian distribution

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\bar{x})^2}{2\sigma^2}} \tag{12.6}$$

Let's look at what each variable stands for:

- σ is the *standard deviation*, which we already dealt with in Part I.
- e is *Euler's number* (2.718281...), on which, among other things, the natural logarithm (ln) is based.
- $\bar{x}$ is the *mean value* of all measured values of the respective measurement series (you already know this from Part I). Then it is immediately understandable that the curves become broader and flatter (and thus, as in Fig. 12.3b, less useful in terms of analysis), the more the individual measured values deviate from each other.

  Applied to $w_{½}$ and w, the Gaussian expression is then given by
  - $w_{½} = 2.3548\ \sigma$ (however, for simplicity, one usually calculates with $w½ = 2.35$);
  - $w = 4\ \sigma$
- Again, we see: larger fluctuations lead to wider and flatter peaks.

For Those Who Want to Know More

This mystery number 2.3548 does not come out of nowhere, but is calculated:

$$2\sqrt{(2\ln 2)} \tag{12.7}$$

To deduce this, however, would go beyond the scope of this introduction.

■■ Fluctuations

Harris, Section 22.2: What is chromatography?

Please do not forget what we actually deal with here: In a solution that contains "one analyte only" (i.e. more precisely: only particles of one type of analyte), there is still a (hugely large) number of individual analyte particles (molecules or ions). These will hardly all reach the detector at exactly the same time: bundled, so to speak. The closer the individual particles remain to their peers, the sharper the detector signal will be, because then practically all the analyte particles really do have approximately the same retention time. If this is the case, you get a very narrow and sharp peak. But if you look again at Fig. 22.5 from Harris, it should immediately become clear that it is simply illusory to want to let all analyte particles "start the run to the detector" at exactly the same time: The wider the "starting zone" on the column, the more the individual retention times of the individual analyte particles will differ, if only because the retention time is based on the (erroneous) assumption that all analyte particles started running at exactly the *same time* and exactly the *same point* at the beginning of the column. The larger the difference in the individual retention times on the column, the broader the peak will ultimately be.

Actually, however, we want to look at how *different peaks* relate to each other, i.e. how well the different analytes were separated from each other and to what extent one can also rely on the measured results obtained. (Please do not lose sight of the fact that, with all the mathematical considerations here, it is not about "the calculation itself": We still want to perform one or the other chemical analysis!)

The resolution of a chromatogram is inextricably linked to the individual Gaussian curves of the respective analytes. In general:

$$\text{Resolution} = \frac{\Delta t_r}{w_{(av)}} = \frac{0.589\Delta t_r}{w_{\frac{1}{2}(av)}} \tag{12.8}$$

To determine the resolution, we must consider both the retention times of the various analytes (adjacent in the chromatogram) and their respective *peak widths*:

- Δt_r is simply the difference between the respective retention times.
- $w_{(av)}$ is the average *of* the width of the two peaks under consideration.
- $w_{½\ (av)}$ is then—this will hardly surprise you—correspondingly the mean value of the two peak widths at *half peak height*.

Look at ◘ Fig. 12.4 (which is, of course, based on ◘ Fig. 12.3): For the almost ideal peak shapes a), the base width w is already so small that $w_{½}$ can hardly be measured. For the much more realistic stylised peaks b), the situation is quite different.

The width of a peak, regardless of whether it is w or $w_{½}$, is also usually given in time units. This may sound a little odd, but the half-width corresponds to the retention time $t_r \pm x$ seconds; after all, on the x-axis we tend to plot the time. Let's take a closer look at a single peak: In ◘ Fig. 12.5, the retention time is $t_r = 35$ s.

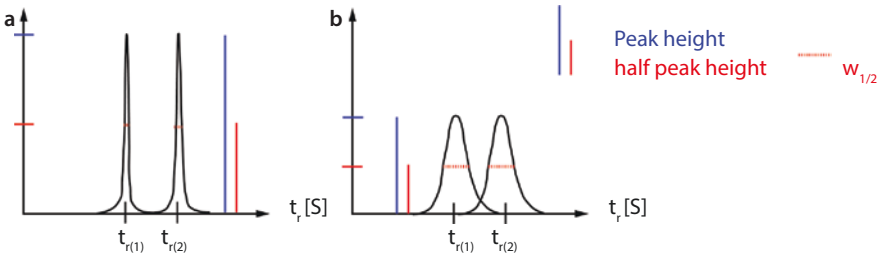


Fig. 12.4 The width at half peak height

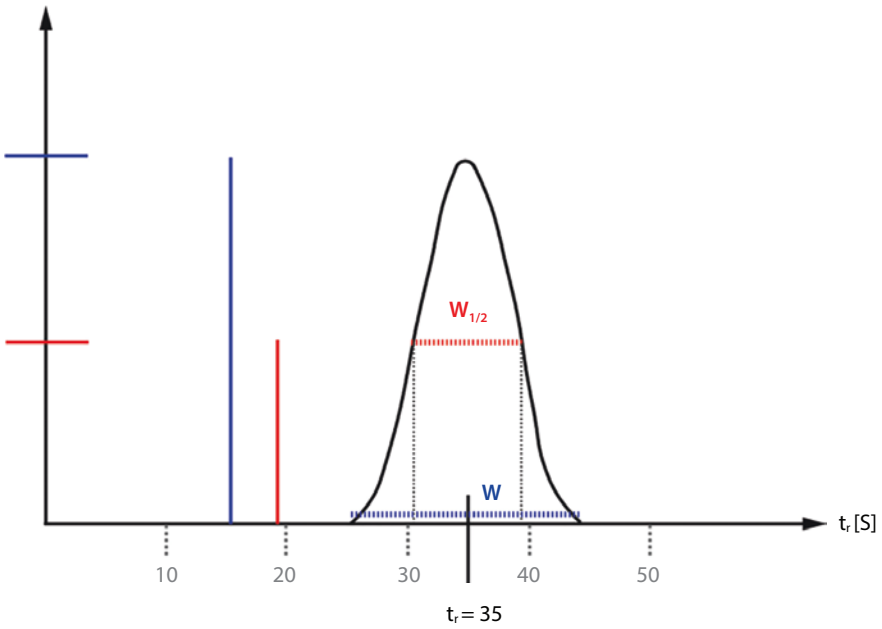


Fig. 12.5 Determination of w and $w_{½}$

If we look at the time value at which the peak begins (about 26 s) and where it ends (44 s), we get a *base width* of 44–26 s, i.e. w = 18 s. To determine the peak width at half height, the values 30 s and 40 s are significant—easily read off by the vertical dashed lines—, so $w_{½}$ = 10 s.

Plausibility Check

Do these values correspond to our idea of a Gaussian curve?

From Eq. 12.6 it could be deduced that for a "correct" Gaussian curve the ratio $w_{½}$: w corresponds to the ratio 2.3548: 4. This ratio is also in Eq. 12.8: 2.3548/4 = 0.5887, meaning 0.589 when sensibly rounded.

And what does it look like here?—With $w_{½}$ = 10 s and w = 18 s, we get: $w_{½}$/w = 0.555. That's not perfect, but it comes pretty close.

Again, it turns out that for a peak close to the Gaussian ideal, the base width and the width of a peak at half-height are related by a constant (0.589)—and $w_{½}$ in many cases is *decidedly* easier to measure than w.

In general we can say: *A resolution* > 1.*50 is desirable.* What happens if the resolution is lower (i.e., to put it bluntly: worse) is shown very nicely in Fig. 22.10 from Harris.

Harris, para. 22.4: Efficiency of separation

? Questions

5. A chromatogram with three peaks shows the following properties: $t_{r(1)}$ = 23 s, $w_{½(1)}$ = 6; $t_{r(2)}$ = 42 s, $w_{½(2)}$ = 12; $t_{r(3)}$ = 72 s, $w_{½(3)}$ = 10. Give a statement on the resolution of each peak pair.

■ Influence of the Column

The fact that the retention time of any analyte depends to a large extent on how much it interacts with the stationary phase, i.e. the column material, has already been discussed at length—and equally, that these interactions, at least in ordinary chromatography, are based on the polar/non-polar interplay. (Selected other interactions will be discussed in ► Chap. 14.) But in order to quantify the *separation efficiency* of a column (and that is exactly what we just did with regard to resolution), it is above all useful to deal with the (purely theoretical!) construct of the **theoretical plates** and the resulting (equally theoretical) **plate height of the column** in question.

In order to be able to address this topic adequately, we need to recall what we know ever since at least Part I about the (dynamic) distribution equilibrium of analytes in/at mobile and stationary phases.

Here, the characteristics of both the column material and the mobile phase used come into play, as do the (physico-)chemical properties of the respective analytes, but ultimately, when considering the *theoretical plates,* it boils down to how often, when analyte and mobile phase flow through the entire length of a column, the distribution equilibrium mentioned above is re-established.

Illustration

Very simplified, the whole thing can be understood a little like a multiple distillation: Each time a substance is distilled, the equilibrium between the composition of a mixture of substances in the gas phase and in the liquid phase is re-established. Understandably, with repeated distillation, one gets closer and closer to the pure substance of the lighter boiling component. In short, the more often you distill, the "purer" the result. Because of this analogy between chromatography and the theory behind such distillation processes, the corresponding technical terms are also used in column chromatography.

However, the plate level of a column cannot simply be measured: Only retrospectively, after a separation has taken place (more or less successfully), statements can be made about how many theoretical plates the column must have had in order to have produced the separation performance just obtained. The formula symbol for this **number of theoretical plates of a column** is **N.** (You will soon find out why this is of interest to us.)

If one has determined this way how many theoretical plates the column under consideration must have had, one can also determine the (equally theoretical) height of the individual plates, i.e. the **plate height H** (whereby one—it stands to reason!—assumes that these theoretical plates are equidistant in each case): It simply corresponds to the ratio of column length (L) and number of plates (N), i.e.

$$H = L / N.$$

Illustration

If you know that a staircase covers a height difference of 3 m and that each step is 20 cm higher than the previous one, how many steps do you have to climb to reach the top of the stairs?—The answer "all" will not be accepted.

And now we take recourse to statistics—and with it to all the considerations about the standard deviation σ ... which we do not want to go into further right now, because the decisive thing here is also the *principle,* and by now it should have become clear that the smaller the standard deviation σ, the more efficiently the separation proceeds. We have just dealt with the connection between σ, peak width, and retention time, so let us summarise the whole thing:

From Eq. 12.6, we know that for the base width of a peak, $w = 4\sigma$, in other words, $\sigma = w/4$. Using considerations that are a bit beyond the scope of these remarks (but are discussed well comprehensibly in Harris), one then comes to:

Harris, para. 22.4: Efficiency of separation

$$N = \frac{t_r^2}{\sigma^2}$$

It follows that:

$$N = \frac{16 t_r^2}{w^2} \tag{12.9}$$

And since the width of the peak at half height $w_{½}$ is easier to determine than the base width (you remember?), but the two are related (according to Eq. 12.8) by a constant, we can also say about the base number of a column:

$$N = \frac{5.55 t_r^2}{w_{1/2}^2} \tag{12.10}$$

The larger the number of (theoretical) plates in a column, the better, because (just to make it clear that this theoretical construct allows direct statements about the separation performance!) the more plates a column has per unit length, the more frequently the distribution equilibrium is established, the smaller the standard deviation σ, and the narrower the peaks become:

- In gas chromatography, plate heights between 0.1 mm and 1.0 mm should be obtained.
- In liquid chromatography, the range is commonly between 30 and 50 µm, but values <10 µm are also achievable in higher resolution HPLC (more on this in ► Sect. 12.3).
- With the help of capillary electrophoresis (which we will discuss in ► Sect. 15.1), even values <1 µm can be obtained.

The plate height H also directly correlates with the resolution of a chromatogram: As long as the individual peaks of a chromatogram are symmetrical enough to be more or less fittingly described in Gaussian terms, two adjacent peaks 1 and 2 (with the uncorrected retention times $t_{r2} > t_{r1}$) it can be said that:

$$\text{resolution} = \left(1 - \frac{t_{r(2)}}{t_{r(1)}}\right)\frac{\sqrt{N}}{4}, \quad \text{thus}: (1-\gamma)\frac{\sqrt{N}}{4} \tag{12.11}$$

However, corresponding measurement series' show that how much you might rack your brain about potentially suitable stationary and/or mobile phases in advance: *The longer an analyte remains on the column, i.e. the longer the retention time, the broader the associated peak becomes.*

■ Dependence of Peak Width on Retention Time

This is due, among other things, to the *diffusion* of the individual analyte particles: What you know as **Brownian molecular motion** also applies to the movement of the individual analyte particles in the direction of the "column outlet":

The longer the individual particles remain on the column, the more each of them moves back and forth in all three spatial directions—purely statistically—and thus in the process can also move away from the others. This then means that even an originally very compact group of analyte particles is "driven apart" further and further over time: σ increases more and more—as a function of time: σ = f(t).

This brings yet another aspect: The larger and/or heavier and thus more inert the analytes are, the less drastic the peak broadening will turn out to be. However, in the gas phase (we already discussed the basics of gas chromatography in Part I, more on this in ► Chap. 13), where the individual particles can move practically undisturbed from one another in all spatial directions, the diffusion-related broadening of the analyte peaks is *ten thousand times more pronounced* than in liquids. (Harris provides a graphical illustration of the phenomenon in question with Fig. 22.12.)

Harris, para. 22.4: Efficiency of separation

Without going into too much of the mathematics behind it, the phenomenon of diffusion can be summarised by a **diffusion coefficient D**, which is directly related to Eq. 12.6 describing the Gaussian curve of the peak under consideration. (Harris goes into a little more detail; here we are again interested only in the general principle.) Finally, based on this diffusion coefficient D, the *standard deviation of a band* can be described:

Harris, para. 22.5: Why bands get wider

$$\sigma = \sqrt{2Dt}$$

We have just seen that the extent of diffusion increases over time. But in fact there is even more to this diffusion coefficient.

The van Deemter Equation

12

This equationfor determining the plate height H breaks down the diffusion that actually occurs into three different individual factors, two of which depend on the flow velocity (*u*) of the column under consideration, while the third of them does not. In general, this (proportionality) equation, which is extremely important in chromatography, can be stated as follows:

$$H \sim A + \frac{B}{u} + C \cdot u \tag{12.12}$$

Let us look at the three terms of this equation, which are also often referred to in the literature as the *A through C terms of the van Deemter equation*, in their alphabetical order:

- **A**—First of all, you have to take into account the "additional distance" that the particles have to cover in order to reach the detector at all. Please recall in your mind's eye what such a column actually looks like: a tube filled with the stationary phase (which you are welcome to imagine, for the time being, as small solid particles in simplified terms), through which the mobile phase flows. If an analyte is to migrate from one end of the tube to the other, it cannot simply "run straight through" because the particles of the stationary phase are in their way. Some analytes will then turn to the left to avoid them, others to the right, etc. In other words, each analyte particle takes a slightly different path, and the individually chosen paths may well differ slightly in length. All in all, this results in a *scattering diffusion* that corresponds to a vortex movement (so to speak, "stirring at the molecular level"; in the technical literature, the term **eddy diffusion** increasingly gains acceptance). The difference in path length of the individual

particles is certainly not excessive, but it should be understandable that the resulting path differences of the individual analyte particles are completely independent of the flow velocity of the mobile phase: The distance must be covered either way. (Figure 22.20 from Harris illustrates this phenomenon quite vividly.)

Harris, para. 22.5: Why bands get wider

- **B**—This is where the already mentioned Brownian molecular motion in all three spatial directions comes into play. Some of these motions can certainly also be described as "back-and-forth twitching", because if a particle under consideration is moving in one direction for now, but the very next moment might move in exactly the opposite direction, a lot of things average out statistically—but it is to be expected (just as statistically) that at least *some* analytes twitch more frequently in the "target direction" than others (i.e. along the long axis of the column they are migrating through), while others tend to moving exactly in the opposite direction. Thus, the analyte particles diffuse apart one another not only due to the *Eddy diffusion* from term A of Eq. 12.12, but also because of the Brownian molecular motion. For the B term of the van Deemter equation, **longitudinal diffusion** is of particular importance, i.e. the effect that, due to the statistical motions, some analytes run more towards the target than others—and accordingly reacht the column's end (somewhat) earlier. (*Transversal diffusion,* i.e. movement at right angles to the direction of flow within the column, also takes place, of course, but for the detector it generally makes no difference whether the analyte to be detected is located more at the edge or more in the middle of the column at the time of its arrival.) Here, as already indicated above, it should become clear that longer dwelling on the column results in a broadening of any Gaussian curves, because the longer a statistical system exists, the more "outliers" (in the sense of "more improbable events") one will observe. (This term of the van Deemter equation may become even better comprehensible with Fig. 22.17 from Harris.)

Harris, para. 22.5: Why bands get wider

Illustration

Because there are so many misconceptions about statistics and "probability", please allow me a brief interjection at this point:

If you roll the dice three times in a row and get a 6 each time, you should at least have some degree of suspicion about the dice in question. But if you roll the dice a zillion times in a row and afterwards look at the resulting sequence of numbers (...121635243...), you will most likely also come across (...666...) somewhere. What you should never forget: Even a very improbable event, if it truly is purely statistically determined, *will* occur sooner or later, if you only give the associated system enough time. There is no purposeful intention behind it (by whom- or whatsoever), but simply the law of probability. So if an improbable event (or, happening even more often, an event *estimated as* improbable) occurs, it has no deeper meaning than the occurrence of any other equally improbable event ... even if any observers may initially see it differently from their point of view:

Imagine, for intance, a monkey that has been taught to randomly pull three tablets, each labeled with a letter, from a bag, and each letter occurs exactly once. (The emphasis is on *random.* This is not about teaching a monkey to write!) If said monkey does this often enough, sooner or later he will also get the letter sequence Y-O-U—which would by no means mean that the animal has finally become aware of the person observing it, or that some other somehow special event has occurred: The result is simply *one possible* letter sequence.

Please keep in mind: This sequence of letters *per se* is without any intrinsic meaning. For someone who does not speak English, this letter sequence would be just as meaningless as, say, E-W-P or Z-R-K, which the animal is exactly as likely to retrieve from the bag.

Do not *ever* confuse "statistically improbable but possible and purely statistically determined things" with things that are *not* exclusively statistically determined! No matter how many times you might try: When you jump up, you *will* not simply float in the air, let alone fly like a bird, because in this case gravity also has something to say about it.

- **C**—If the flow velocity of the column is too high, the problem arises that the system no longer has enough time to reach a new distribution equilibrium (this is also referred to as the **mass transfer term**). It should be obvious that too high a flow velocity leads to an (undesired) increase of the theoretical plate height, and thus finally to suboptimal measurement results (in the sense of: broadened peaks).

Please remember: In chromatography, "the faster, the better" is by no means a helpful rule. Rather, one should heed the maxim: **As fast as possible, as slow as necessary.** Every chromatographic system should be optimised.

The total interplay of the different terms of the van Deemter equation, some of which also run in opposite directions, is excellently illustrated in Fig. 22.16 from Harris.

Harris, para. 22.5: Why bands get wider

With the van Deemter equation, the difference mentioned in the previous paragraph with regard to the achievable theoretical floor heights of the various chromatographic techniques should become a little more comprehensible: In gas chromatography, for example (► Chap. 13), we are dealing with analytes in gaseous form, and with gaseous particles, the (longitudinal) diffusion is much more pronounced (B-term). If one wants to minimise this, one has to increase the flow rate significantly. In the other methods mentioned, different columns are used, and the column material used in each case also has an effect on the plate height/separation efficiency that can actually be achieved.

12.3 High Performance Column Chromatography (HPLC)

A particularly powerful method of liquid chromatography is **HPLC**, with this (globally used) abbreviation nowadays standing for *high performance liquid chromatography*. (Since in most HPLC systems the (liquid) mobile phase is pumped through the column under sometimes considerable pressure, it used to be read as *high pressure* ..., which, however, HPLC experts generally dislike.)

The principle behind this is exactly the same as that described in ► Sect. 12.2. Nevertheless, we will devote a little more space to this special form of LC, simply because it has now become a real "standard method".

Ultimately, it is once again a matter of "flushing" an analyte, which interacts to a greater or lesser extent with the stationary phase of a column, off said column with the aid of a mobile phase (i.e. a solvent of whatever type—the eluent)—although it is clearly better in technical terms to speak here of **eluting.**

In principle, there are various possibilities for elution:

1. You can simply select a suitable solvent. However, it is quite possible that a single solvent will not lead to ideal results because, for example, it is not

polar enough to get even the most polar analyte off the column. (Or perhaps it would simply take far too long, and the consumption of solvent would no longer be justifiable—think back to Part I: Even with water alone, without any detergent at all, you *can* clean greasy plates, but the water consumption will be considerable, and it will take *plenty* of time ...)

If this is the case, option 2 is recommended:

2. You decide not to use only a single solvent, but instead a mixture of solvents. (This is exactly what your lab colleague had already suggested to you in Task 3, remember?) Diethyl ether, for example, is much less polar than ethanol, but a (volume) mixture of 95% diethyl ether and 5% ethanol (or generally: x% A and y% B) may then have exactly the required degree of polarity.

In both cases you would carry out an **isocratic elution,** and depending on the solvents A and B used (or if necessary also C, D, E, ...) the result may already be quite satisfactory. Look at Fig. 24.12 from Harris:

Harris, Section 24.1: The chromatographic process

It shows how a different proportion of B in an isocratic procedure leads to clearly different chromatograms for the same mixture of substances.

However, such an isocratic procedure often leads to analytes with similar elution properties being eluted in excessively close succession, i.e. the corresponding peaks in the associated chromatogram follow each other very closely or even overlap (keyword: resolution, ► Sect. 12.2). Better results can usually be achieved by other means:

3. Select an **elution gradient,** i.e. change the solvent during the elution process. To do this, start with a solvent mixture of components A and B and increase—step by step or continuously—the B-portion of the mixture. If necessary, further solvent (mixtures) C, D, etc. can be added.

Compare the various chromatograms from Fig. 24.12 of Harris with Fig. 24.13 from the same book: it is very clear that the gradient chosen there has led to a chromatogram which is incomparably more satisfactory.

Harris, Section 24.1: The chromatographic process

■ Solvent (Mixtures)

In HPLC, too, (more or less) polar column material is usually used as stationary phase, in most cases (modified) silica (i.e. silica gel; we will look at some other column materials in ► Sect. 13.1 and in ► Chap. 14). Accordingly, it is useful to sort potentially useful solvents (= eluents) according to their polarity (more precisely: according to their *increasing adsorption energy;* see Fig. 24.11 from Harris).

Harris, Section 24.1: The chromatographic process

This way the **elutropic series** of solvents is obtained. (Table 24.2 of Harris gives the relative eluting power of the most common solvents for columns packed with pure silica gel.)

Cave

Please do not forget that this relative elution capacity of the different solvents, which increases with increasing polarity of the solvent, always refers to a *polar* stationary phase, as is usual in so-called *normal phase* chromatography (NP-HPLC).

Of course, it is also possible to perform *reversed phase chromatography* (**RP-HPLC**) and combine a non-polar stationary phase with one or the other polar solvent (mixture). (You already know this from RP-TLC in ► Sect. 12.1.) In this case, as probably expected, the elution capacity of the various solvents increases with *decreasing* polarity.

Special Features of HPLC

Perhaps the greatest advantage of this separation method is that under the selected conditions (column material, pressure, etc.—we will go into this in a little more detail a bit later) the distribution equilibrium between stationary and mobile phase (as known from Part I and already mentioned again in ► Sect. 12.2) is established particularly quickly. In addition, the van Deemter equation comes into play again, which (you certainly remember) describes the theoretical plate height H of a chromatography system (in brief: the smaller, the better):

- Since we are dealing with solids (stationary phase) on the one hand and liquids (mobile phase) on the other, diffusion (the B term from Eq. 12.12) takes place at about only *one hundredth* of the speed it has in the gaseous mobile phase (more on gas chromatography in ► Chap. 13).
- In addition, in high performance liquid chromatography, the mobile phase is passed through the stationary phase at considerable pressure and accordingly quite rapidly, so there is a considerable flow velocity. You will certainly remember that in the B term of the van Deemter equation, the flow velocity (u) appears as denominator, thus higher flow velocities lead to a *lower* value for the B term—which ultimately then results in a reduced plate height H and thus an *increased separation efficiency*.

The stationary phase (i.e. the column) should obviously also be able to withstand these kind of pressure. This now raises the question of what kind of column material is used in HPLC at all.

Column Material

As long as we are in the field of NP-HPLC, the basis of any column material is first of all high-purity silica gel (silica gel, amorphous silicon dioxide, SiO_2)—mostly in the form of microporous spheres, resulting in a considerable surface area.

As a Reminder

That maximised surface areas are desirable is once again almost self-explanatory, after all the whole adsorption chromatography is about the interaction of the analytes to be separated with the solvent on the one hand and the surface of the column material on the other. More surface area means, in short, more interactions.

It is precisely this surface that we should now take a closer look at, because after all we deal here with the possible interactions of the atoms (molecules, ions) involved in this surface with our analytes. (Perhaps you would like to read up on the chemistry of [amorphous] silicic acid anhydride again in Binnewies or another textbook of your choice?)

Binnewies, Section 18.15: Silicon dioxide

Binnewies, Section 18.16: Silicates and aluminosilicates

In amorphous silica, as in its crystalline counterparts (quartz and the like), corner-linked SiO_4 tetrahedra are present:

- the silicon atoms are tetrahedrally surrounded by four oxygen atoms,
- the oxygen atoms bridge—more or less linearly—two silicon atoms each.

However, in contrast to the crystalline form, the *amorphous* SiO_2 is not particularly stable under basic conditions: An aqueous solution with pH > 8 is able to dissolve this material to form various silicates within a very short time.

If we now want to take a closer look at the structure of amorphous silicon dioxide, we are confronted with a general problem that arises always and everywhere when describing *any* solid (whether only crystalline or amorphous): For the sake of simplicity, one always assumes that such a system is infinite in all three spatial directions, but *exactly this is not the case*: At the surface, the system must have "defects".

► Example

The crystalline structure of common salt (NaCl) may certainly be assumed to be generally known:

- Each sodium cation is octahedrally surrounded by six chloride ions.
- Each chloride ion is octahedrally surrounded by six sodium cations.

But what happens at the *surface of* such a salt crystal? A chloride ion there *can* non-not possibly *be* octahedrally surrounded by six sodium atoms, otherwise it wouldn't be on the surface. So *each* crystal is no longer perfect at its surface. Corresponding defects then can be compensated (to a certain extent) by adsorption of other molecules (H_2O from humidity, or O_2-molecules etc.). ◄

And what about the surface of amorphous silicon dioxide (i.e. silica gel)?—At the end of the –Si–O–Si–O–Si chain, instead of a oxygen or silicon radical, there is always an OH, a hydroxy group, bonded to the silicon atom in question. In analogy to the carbon-OH compounds, which are known to be called "alcohols", such Si–OH groupings are referred to as **silanol** groups.

Against this background, it should then also become understandable why silica gel as a material is so polar:

- The difference in electronegativity between silicon (EN_{Si} = 1.9) and oxygen (EN_O = 3.5) is considerable, but if both are part of a crystal lattice, there is hardly any possibility for interaction with foreign atoms.
- In the case of the silanol groups, on the other hand, the polarisation between O and H (EN_H = 2.2) is the same as in the water molecule ($H^{\delta+}$, $O^{\delta-}$); accordingly, formation of hydrogen bonds and the like are to be expected here as well.
- And another parallel to water and alcohols must be taken into account: Both show OH-acidity, so they can also be deprotonated—assuming a sufficiently basic reaction partner is present. In the case of otherwise untreated amorphous silica gel—which, due to its large surface area, has *abundant* silanol groups—this occurs at pH = 2–3. Thus, if one does not work in a strongly acidic medium (and this happens rather rarely), the silanol groups of the silica gel are deprotonated, i.e. they are *anionic*. Under neutral to moderately acidic conditions (the fact that at least untreated silica gel is easily hydrolysed under basic conditions has already been mentioned), deprotonated silanol groups are present, i.e. $–Si–O^{\ominus}$. It should be obvious that these provide plenty of polarisation.

However, the columns filled with this unmodified silica gel, which is partially deprotonated under the selected conditions, do not exactly lead to optimal chromatograms—especially when the analytes to be separated contain individual atoms with a relatively high positive charge density (such as protonated amines with $R–NH_3^+$): In this case, a deviation of the peak shape from the Gaussian curve occurs, known as **tailing**: It is distorted *asymmetrically*, so that the side with longer retention time is slightly flattened.

Illustration

The reason for the phenomenon known as *tailing* is actually quite obvious. Let us recall again how the Gaussian curve in itself comes about: "Ideally", all analytes should arrive at the detector at the same time, except that—for (various) statistical reasons—there are always a few somewhat faster and a few somewhat slower. However, if excessively strong interactions occur between the analyte and the column material, this leads not only to a *general* slowing down of the analytes (in other words: to a extended retention time), but also (and above all) to a certain slowing down of the analytes in the process of diffusing *away* from the column material itself.

Here, additional forces come into play, which ensure that there still are, purely statistically, a few *faster* candidates, but an above-average number of *slower ones* (precisely because they are additionally retained). Accordingly, the peak, which from a purely statistical point of view should actually be mirror-symmetrical, is distorted in the direction of a *longer retention time.*

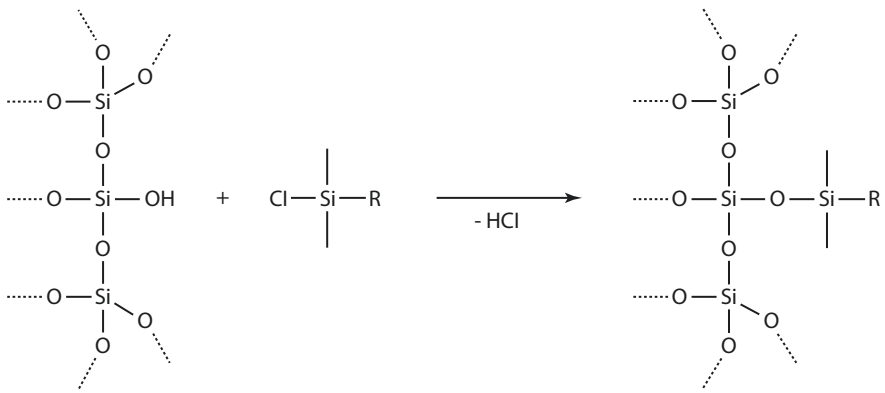
For this reason, in addition to the "normal" silica gel, various variants have been developed in which the silanol groups have been partially modified by chemical means (generally referred to as **endcapping**), so that the number of free Si–OH groups is significantly reduced. This is particularly easy with compounds of the general molecular formula $R(CH_3)_2SiCl$ (i.e. derivatives of trimethylsilyl chloride $(CH_3)_3SiCl$) (◘ Fig. 12.6).

Typical residues –R for NP columns are e.g. $–CH_2–CH_2–CH_2–NH_2$ and $–CH_2–CH_2–CH_2–C{\equiv}N$. (Note that the amino group or the nitrile function at the end of the $–(CH)_2$-chain provides a similar polarity as would be the case with a free silanol group.)

The resulting polarities are similar, but by no means identical. As expected, the electronegativity difference between C and N in the C≡N group leads to positive polarisation of the carbon and negative polarisation of the nitrogen atom, but the resulting negative partial charge on the N is not of the same order of magnitude as the negative charge density on the oxygen atom of a deprotonated silanol $–Si–O^{\ominus}$.

If these kinds of modified silica gels are used as the stationary phase, this at least largely prevents the aforementioned tailing; occasionally this even results in at least as good a separation (keyword: dissolution) with even reduced retention times (see, for example, Fig. 24.7 from Harris).

Harris, Section 24.1: The chromatographic process



◘ **Fig. 12.6** End capping

Bonus Information
Incidentally, the base stability of silica gel can also be increased in a similar way: If at least some of the free silanol groups are forced to undergo a condensation reaction with ethylene glycol (1,2-ethanediol, HO–CH_2–CH_2–OH), corresponding Si–O–CH_2–CH_2–O–Si cross-links are obtained, which provide significantly increased stability (see Fig. 24.6b from Harris).

Harris, Section 24.1: The chromatographic process

In normal-phase HPLC, the analytes interact with the (strongly) polar solvent; accordingly, strongly polar analytes are retained more strongly than less polar ones, while completely non-polar or only very moderately polar analytes are practically eluted from the column together with the solvent front. In other words, NP-HPLC is primarily suitable for the separation of *polar* analytes.

However, a considerable proportion of all compounds, especially those classified as "organic", are significantly less polar or even non-polar. Here, *phase inversion* is recommended, as we already know from *reversed-phase TLC* in ▶ Sect. 12.1.

▪ RP-HPLC

In fact, reversed-phase HPLC is now by far the more common variant of high-performance liquid chromatography. It is used, among other things:

- for doping tests in competitive sports,
- in drug screening, and
- generally in the investigation of pharmaceuticals and their biological degradation products.

As an example, we will look at some substances that are isolated and/or quantified by RP-HPLC (often in combination with other methods of instrumental analysis, especially mass spectrometry, which we will deal with in "Analytical Chemistry II"; ◘ Fig. 12.7).

- ◘ Figure 12.7a shows the testosterone-derived anabolic steroid hormone nandrolone, which still shows some degree of polarity due to the free OH group (although the nonpolar moiety far predominates in this molecule).
- ◘ Figure 12.7b shows nandrolone decanoate (b), in which the hydroxy group of (a) has been esterified with decanoic acid, so that this analyte can be regarded as almost nonpolar. In this form, nandrolone is also administered as a doping agent (usually by intramuscular injection).
- Stimulants can also be readily isolated and quantified by (RP-)HPLC: ◘ Fig. 12.7c shows the structure of amphetamine, ◘ Fig. 12.7d that of methamphetamine; both are commonly consumed for performance enhancement. Methamphetamine, which is less polar because of the additional methyl group on the nitrogen, can cross the blood-brain barrier even more easily, precisely *because* of the reduced polarity, and therefore has additional euphoric and psychotropic effects; as *'crystal meth',* it is currently (as of 2022) mentioned quite frequently in the media.
- Structurally closely related and, despite the theobromine ring system, similarly nonpolar is fenetylline (◘ Fig. 12.7e), which was widely used in competitive sports until the 1980s because of its performance-enhancing effects; although now banned, it is still occasionally detected in doping tests.

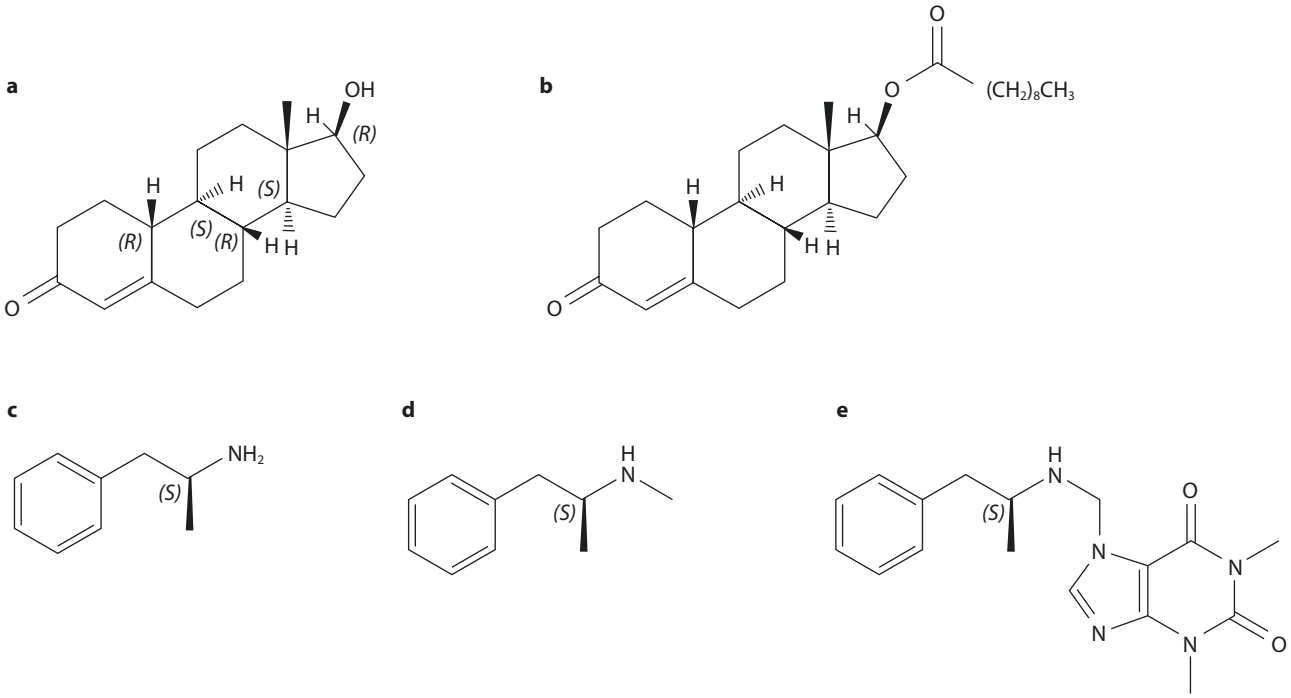


Fig. 12.7 Selected analytes for RP-HPLC: (**a**) nandrolone; (**b**) nandrolone decanoate; (**c**) amphetamine; (**d**) methamphetamine; (**e**) fenetylline

12

These analytes with extremely limited polarity are typical for an investigation or separation using an RP-HPLC column. The remarkable thing here is that the columns required for reversed phase chromatography are also based on silica gel.

The desired inversion of polarity is again achieved by *endcapping*. The principle of the modification corresponds exactly to that shown in Fig. 12.6, except that long-chain hydrocarbon derivatives or other significantly less polar molecular fragments are used as residues –R here. Typical residues –R here are, for example, the octyl residue ($–CH_2–(CH_2)_6–CH_3$) and the (of course even less polar) octadecyl residue ($–CH_2–(CH_2)_{16}–CH_3$). (Such columns are then designated RP-C8 or RP-C18; most of the columns described here are commercially available.)

It should be understandable that the type of "less polar chains" bound to the column backbone via endcapping has a decisive influence on the scope of the column in question:

- The octyl residue (RP-C8) is particularly well suited for the separation of *moderately* polar analytes,
- the octadecyl residue (RP-C18) is optimised for *moderately polar to completely non-polar* analytes.
- Endcapping with the propylphenyl group ($–CH_2–CH_2–CH_2–C_6H_5$) results in a column suitable for *moderately polar* compounds, with the interaction between analyte and stationary phase increased when the analyte is *mono- or polyunsaturated.*
- A propylphenyl moiety perfluorinated on the aromatic system ($–CH_2–CH_2–CH_2–C_6F_5$) allows particularly efficient separation of *halogenated* analytes, etc.

Of course, there are other less-polar residues that are used in RP-HPLC; further literature should provide you with plenty of material.

A Little More on Organic Chemistry

If you look closely at ◘ Fig. 12.7, you will notice that the absolute configuration according to Cahn, Ingold, and Prelog (CIP) is given for all stereocentres (which you certainly from Fundamentals of *Organic Chemistry*), because stereochemistry can also be of considerable importance in analysis. In ► Sect. 15.1, for example, you will learn about a separation method that also allows the differentiation of stereoisomers, even enantiomers (!).

Questions

6. You have decided to use an isocratic method with 50% ethanol and 50% diethyl ether for the separation of your analytes via NP-HPLC, but during the first test run you notice that your analytes not only all have very short retention times, but also elute so closely one after the other that the resolution of the resulting chromatogram is more than modest. How do you go about achieving better separation performance?
7. The task is to separate all isomers of octanol (1-octanol, 2-octanol, 2-methyl-1-heptanol, etc.) via HPLC. Do you decide to use normal phase chromatography or to use a PR-HPLC column? Please explain your answer.

12.4 Detection Methods

The most common method of detecting analytes is once again photometry, which we already know from Part II. As a reminder, one determines the extent to which the constituents of a solution absorb electromagnetic radiation of one or the other wavelength, with radiation commonly being chosen from the UV/VIS range.

However, it must be taken into account that *the solvent molecules themselves* may also be excited by one or the other wavelength, if the corresponding photons are only energy-rich (i.e. short-wave) enough. For this reason, a UV cut-off wavelength (λ_{CutOff}) is specified for each solvent, below which any measurement simply does not make sense anymore—after all, the detector cannot logically distinguish whether any absorption is due to the analyte or to the solvent itself. (Again, reference is made here to Table 24.2 from Harris.)

Harris, Section 24.1: The chromatographic process

Outlook

Solvents with a C–O double bond (such as acetone, $(CH_3)_2C{=}O$) or an aromatic system (such as toluene, $C_6H_5{-}CH_3$) are particularly easy to excite, therefore the corresponding cutoff wavelengths of 330 nm (acetone) and 284 nm (toluene) lie clearly above those of hydrocarbons such as heptane, hexane, or pentane, with λ_{CutOff} = 200, 195 and 190 nm, respectively.

But what *exactly* happens with this stimulation, and why multiple bonds are obviously of special importance here, you will find out in Part IV. Stay tuned!

As long as the cut-off wavelength of the components in question is taken into account, even solvent mixtures and/or solvent gradients do not pose a problem for a UV/VIS detector.

However, the situation is different for other detection methods, which we have also already discussed in Part II:

- Understandably, *potentiometric* detectors based on redox processes are also conceivable in principle. On the other hand, they require very precise knowledge of the electrochemical behavior of one's solvent(s). And once e.g. a solvent *gradient* comes into play, the number of variables becomes hardly calculable.
- The same applies to detection by means of *conductivity measurements:* This is also possible, but not (or at least not particularly well) in combination with a solvent gradient.

Harris, Section 24.2: Injection and detection in HPLC

In addition, there are several other detection methods that have not yet been addressed:

- If the analytes have been sufficiently investigated or if the number of substances to be detected is (rather to very) limited (ideally, the question should be: "Does the solution to be investigated contain anything else at all besides the solvent, and if so: how much of it?"), one can, on the basis of a calibration curve, also use the *refractive index* as the basis for measurement—which, understandably, will hardly work for gradients whose different components are most likely to have differing refractive indices.
- If necessary, *fluorescence detectors* can also be used; you will learn more about them in Part IV.
- The signal delivered by a *light scattering detector* depends only on the actual (absolute) *total overall mass* of the analyte in question, but not on its structure or the mass of the *individual* analyte particles. This kind of detector is not interested in whether the analyte was eluted isocratically or with the aid of a gradient: The detection method is based on the fact that in the first step, all solvent molecules are evaporated, and then one examines how much the radiation is scattered by the remaining amount of analyte. Obviously, this method is only suitable for analytes that are less readily vaporisable than the solvent(s) used.
- HPLC can be wonderfully combined with *mass spectrometry* (MS)—HPLC/MS is currently becoming a new standard in many laboratories. However, as we will deal with mass spectrometry) in "Analytical Chemistry II", I must ask you for a little patience.

Gas Chromatography (GC)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_13

Summary

Behind gas chromatography lies (once again) the same principle as behind liquid chromatography: adsorption of the analytes to the stationary phase and the question of how long the mobile phase needs to desorb the analyte again.

In GC, one or the other *carrier gas* serves as "solvent". Helium, nitrogen, and hydrogen are most often used; it is important that the analytes do not react with this gas and are therefore not changed during the course of separation.

On the other hand, the column material used in GC (i.e. the stationary phase) differs considerably from the (modified) silica gel used in liquid chromatography.

Harris, Section 23.1: The separation process in gas chromatography

13.1 Columns in GC

For a start, GC almost exclusively uses very thin **capillary columns** (with an inner diameter of 100–500 µm), i.e. columns with a disproportionately smaller diameter than their LC equivalents (whose diameter in principle is unlimited; see, for example, Fig. 22.8 from Harris).

Harris, Section 22.3: Chromatography from a pipelayer's point of view

In addition, the columns used in GC must ideally be heated homogeneously over their entire length (of 10–100 m; about 30 m is a "standard" length) so that the vapour pressure of all components to be separated (analytes, solvents, etc.) remains constant over the entire length. (In addition, substance separation would simply take far too long at too low a temperature.)

Depending on the gas chromatography method used, different distribution equilibria play a role:

■■ Distribution Equilibrium Analyte$_{\text{adsorbed on the surface of the solid}}$/Analyte$_{\text{in the gaseous phase}}$

Here, the analyte is adsorbed onto the surface of a *solid* (as we are already familiar with). In GC, quartz (i.e. SiO_2) again is the basis of the column material; however, the inner wall of this quartz tube (i.e. the column) is coated with another solid—ideally a solid with a maximised surface. Typical representatives are:

- Activated carbon (good for separating various non-polar or low polar compounds).
- Aluminosilicates such as the various zeolites (those are clearly more polar and therefore more suitable for polar analytes); some molecular sieves are also based on them (but this brings us to a topic that belongs more to ► Chap. 14).
- Cyclodextrins—these cyclic polysaccharides (you should know sugars and the like from *fundamentals of organic chemistry*) are (like so many natural products) chiral and, unlike all the column materials mentioned so far, also allow the separation of enantiomers. (Please have a look at Excursus 23.1 from Harris.)

Harris, Section 23.1: The separation process in gas chromatography

Open capillary columns are usually used here. "Open" in this context means that the columns, apart from the wall covering, are *not* filled with column material of any kind. This has an immense advantage: The entire flow velocity-independent term of the van Deemter equation (the A term from ► Eq. 12.12—remember?), which takes into account the different path lengths of the individual analyte particles, is omitted.

■■ Distribution Equilibrium Analyte$_{\text{dissolved in the liquid stationary phase}}$/Analyte$_{\text{in the gas phase}}$

The alternative is to use a *liquid* as the stationary phase. For this type of GC, different types of columns are available:

- In *packed columns,* fine-grained packing material is used, on the surface of which the liquid serving as the stationary phase is located; the packing material is basically wetted (more or less homogeneously) with this liquid.
- Alternatively, *wall-covered open capillary columns* can be used, in which the inside of the capillary itself is covered with a thin film of liquid.
  - Various polysiloxanes of varying polarity are used as liquids; they should be selected individually according to the physicochemical properties of the analytes. (Table 23.1 from Harris provides a selection.)
- Some of the substances forming the basis for solid adsorption as column coatings in the previous section can also be used in the form of a solution (such as the cyclodextrins mentioned in that section, which we will encounter again in ► Sects. 14.3 and ► 15.1).
- Some ionic liquids, which have also recently come to use in GC, have some truly remarkable properties. (However, this is a fairly new field of research, so new knowledge about this is currently gained almost on a daily bases.)

Harris, Section 23.1: The separation process in gas chromatography

Different columns are also suitable for different purposes:
- *Packed* columns are capable of holding (and thus separating) significantly larger amounts of substance; thus this type of column is mainly used for *preparative* purposes.
- *Open* capillaries, on the other hand, lead to much cleaner separations; they are also much more sensitive. On the other hand, they can only be used to separate smaller quantities of analytes, which is why their preparative use is practically ruled out. However, if you want to carry out really clean (trace) analysis, they are clearly preferable.

Since it is well known (see ► Eq. 12.11) that the resolution of a column increases with the number of its theoretical plates N and that the number of theoretical plates of a column is related to its length, column length and separation efficiency are also closely related. (Infinitely long columns simply are not in use because analysts want to *finish* their work at some point.)

Technical Aspects

If open capillary columns are actually nothing more than extremely thin tubes made of silicon dioxide (and glass is made of nothing else) with a length of dozens of meters, why don't they break immediately?—The reason is simply that these hair-thin glass tubes are coated with plastic: usually a polyimide that can withstand the required heating temperatures, in some cases >300 °C. This coating ensures that the tubes do not break, and apart from providing the necessary stability, it also protects the column material from (air) moisture. (A good overview of the structure of corresponding capillaries is provided by Fig. 23.2 from Harris.)

Harris, Section 23.1: The separation process in gas chromatography

Sample Preparation

Not every compound can be analysed "just like that" via gas chromatography:
- Some solutions are too concentrated and would hopelessly overload the column. (This happens in the analyst's everyday life much more often than one would like!)
- Other solutions are decidedly too dilute, accordingly it is then necessary to look for suitable methods to increase their concentration—here the study of further literature is recommended. (Some specific suggestions on the subject of sample preparation are already offered by Harris.)

Harris, Section 23.4: Sample preparation

However, there are other "problems" that may arise prior to gas chromatographic analysis of a substance (or mixture of substances):

- Please do not forget that the principle of GC is based on bringing the various analytes *into the gas phase.*

In other words: Anything that cannot be evaporated without decomposition will also be difficult to be examined via GC. A complete DNA strand will hardly want to play along, and the same applies to glasses and comparable solids (as often as this supposedly happens according to numerous TV series, whose names shall remain mercifully unmentioned here).

- Some substances are difficult to bring into the gaseous phase despite their moderate molar mass: Think, for example, of a wide variety of alcohols or carboxylic acids, which, due to pronounced intermolecular interactions, have much higher boiling points than anyone without chemical knowledge might expect.
- And then there are those analytes that would react with the column material itself: Free carboxylic acids, for example, would either attack the polysiloxanes, or they would combine with the silica "backbone" of the column to form a mixed acid anhydride. In the latter case, you would then have achieved two things at once:
  - Your analyte is no longer coming off the column.
  - Your column can no longer be used.

Now you know why practically all gas chromatographers react downright allergic to free carboxylic acids. But it is actually quite easy to get an acid (which is aggressive and much too difficult to transport into the gas phase) to cooperate: You only have to *esterify* it, i.e. *derivatise* the free acid to an ester (this has already been briefly touched upon in Part I).

13

This works particularly well using diazomethane (CH_2N_2). Under release of elemental nitrogen (N_2), carboxylic acids are thus rather easily converted to their methyl ester (◘ Fig. 13.1).

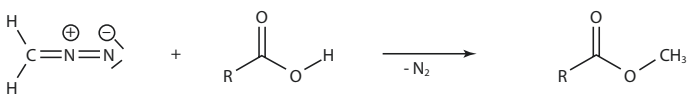
This way, the polarity of the carboxyl group is significantly reduced (which lowers the boiling point considerably: while e.g. acetic acid boils at 118 °C, its methyl ester passes into the gas phase at 57 °C, despite its molar mass being about 14 g/mol higher), and at the same time we also prevent any chemical reaction between the (now derivatised) analyte and the column material.

Once the analyte is available in a GC-compatible form (i.e. derivatised, if necessary, and in an appropriately-concentrated solution), it must be brought into the capillary—which is anything but trivial, since ideally all analyte particles of the same type should reach the detector as simultaneously as possible, so all of them should also start at the same time. For this purpose, take another look at Fig. 22.5 from Harris.

Harris, Section 22.2: What is chromatography?

(even if it is about liquid chromatography):

A certain peak broadening is of course unavoidable (keyword: van Deemter equation!), but the narrower the sample zone in the column, the narrower the corresponding peak should be. The same is true in GC, so the various technical possibilities to keep the sample zone as small as possible are literally a science



◘ **Fig. 13.1** Esterification of free carboxylic acids with diazomethane

in themselves—which is why we will not go into those details here. (A little cynically, one could claim: This has nothing to do with "chemistry as such", it belongs to the field of engineering sciences ...) The basics on this topic can, of course, be found in Harris.

Harris, Section 23.2: Sample injection

13.2 Detectors

Since the analytes in the gas chromatographic separation reach the detector in gaseous form, we (unfortunately) won't get any information using photometry this time. Instead, there are numerous other detectors—which will only be mentioned briefly here (a little more on this can be found in Harris):

Harris, Section 23.3: Detectors

- The standard method of detection in gas chromatography has long been (basically since the development of GC itself) to determine the thermal conductivity of the gas mixture present: The thermal conductivity of helium (the standard carrier gas) is remarkably high; however, when a helium-analyte mixture reaches the associated *thermal conductivity detector,* the conductivity inevitably decreases.
- In the meantime, however, the combination of gas chromatography and mass spectrometry (GC/MS for short) has become practically a lab standard—quite analogous to LC/MS from ► Sect. 12.4. That way, detection and further analysis are meaningfully linked with each other (but mass spectrometry does not come until "Analytical Chemistry II").
- *Flame ionisation detectors*, FID for short, (indirectly) detect carbon atoms of the analyte by thermally inducing a reaction of the mixture of carrier gas and analyte with hydrogen and atmospheric oxygen. Under those conditions, carbon atoms that were not already part of a C–O double bond in the analyte react with the oxygen to form *radical cations* (of the molecular formula CHO^+) and free electrons. The latter then cause an electrical current to flow—and *this electrical current* then is detected. Even though only about every ten thousandth C atom actually releases detectable electrons (which drastically reduces the sensitivity of this method), the resulting current is proportional to the analyte mass.
- In addition, there is a large number of more specialised detectors, each of which is only suitable for specific analytes, which have to be determined precisely in advance. When in doubt, having a look at much more specialised literature is absolutely indispensable.

Questions

8. Why does overloading the column by injecting an analyte solution that is too concentrated lead to unsatisfactory separation results?
9. Why does the use of elemental nitrogen (N_2) instead of helium (He) as a carrier gas decrease the dissolution?

Specialised Forms of Chromatography

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_14

Summary

Even though adsorption chromatography is certainly by far the most widely used chromatographic separation method—not least because it is so versatile and allows the separation of an almost infinite variety of substances—it should not be remain unmentioned that there are other analytical methods, which are based on a similar principle but are considered to be much more specialised—either because the respective separation method is only suitable for a relatively small group of analytes (which then have to exhibit one or the other special characteristic) or because the technology behind it is (still) considered to be quite complex.

14.1 Ion Exchange Columns

Harris, Section 25.1: Ion exchange chromatography

The principle of ion exchange chromatography is that charged particles (i.e. cations or anions) are embedded or covalently fixed in the cavities of a three-dimensional network/mesh, which then undergo electrostatic interaction with oppositely charged analytes. It should be understandable that under such circumstances said analytes can no longer simply flow through the column unhindered but are (electrostatically) retained. And quite in analogy to adsorption chromatography, a larger amount of solvent—which understandably must also contain charge carriers—is required in order to elute the analyte, which so far still strongly interacts with the "column contents". Let us go through the various questions that inevitably arise, one after the other:

■ Columns Used

The most important component of ion exchange columns once again is the *column material*, i.e. the stationary phase. There are two major kinds of column material:

- (organic) resins: Mostly polystyrene resins (polymerised styrene C_6H_5-CH=CH_2; more about polymers can be found in *organic chemistry* or *macromolecular chemistry*) are used, whose degree of crosslinking can be relatively finely adjusted by the amount of crosslinking agent added (usually: divinylbenzene), so that the desired pore size is obtained. (In principle, such columns can be used for analytes with a molar mass < 500 g/mol; the pore size can have a direct effect on the separation performance, depending on the size of the relevant analytes.)

  or
- (also organic) gels: These usually consist of polysaccharides (please think back to the carbohydrates from *organic chemistry*) and have significantly larger pores/cavities/interstices, so that *large* molecules (e.g. protein fragments or possibly even complete proteins) can also be separated.

Understandably, neither gel material is designed to withstand extremely rigid conditions (high temperatures, strong oxidation or reduction effects of analytes and/or solvents, extreme pH values, etc.).

- For such (really special) cases, one falls back on (inorganic) ion exchanger materials whose basic frameworks consist of more or less covalently built-up metal oxides. But this is really quite special.

■ The Extent of Interactions

The column material alone (whether made of polystyrene or polysaccharides of one kind or another) does not yet contribute (or at least not significantly) to the interactions desired here. In order to obtain acceptable separation efficiencies, appropriately fixed negative or positive charges must still be introduced into the stationary phase. This happens

- in the form of (strongly or moderately acidic) sulfonic or carboxylic acid groups (-SO_3H or -COOH), which are deprotonated above a certain pH and therefore then are anionic
  or
- by tertiary or quaternary amines (–NR_2H^+ or –NR_3^+), which provide positive charges—in the case of tertiary amines below a certain pH, in the case of quaternary amines permanently (because deprotonation is simply not possible here).

(It is recommended to look at Fig. 25.1 from Harris for this.)

Harris, Section 25.1: Ion exchange chromatography

The degree to which oppositely charged analytes are attracted to the fixed charges in the column material depends on several factors:

- On the one hand, the **charge density** plays an important role.
- It has to be taken into account that we mostly work in one or the other solvent, insofar a *solvate shell* will be formed for all charge carriers—whereby this can become quite massive with increasing charge density of the considered particle. For this reason, it can also happen that a *small* cation or anion with its solvate shell is overall larger than an *actually larger* ion with a lower charge density "in its free state".
- Polarisability (along the lines of the HSAB concept from *General and Inorganic Chemistry*) is important here, as well.

The whole thing can even be quantified (to a certain extent), and the resulting selectivity ("What interacts with what particularly effectively?") can be translated into formulas or at least key figures. However, since this section is again primarily concerned with the underlying principle, we will not go into further detail here; if you are interested, Table 25.3 from Harris, for example, will help you out.

Harris, Section 25.1: Ion exchange chromatography

Elution of the Analytes

Before we can answer the question of how the interactions affect the elution of the analytes, we should once again consider the initial situation: We deal with (positively or negatively) charged analytes, which, of course, must have possessed a counter ion before coming into contact with the chromatography column. (It cannot be emphasised enough that permanent macroscopic charge separation simply is not possible.)

Similarly, the fixed (positive or negative) charges of the column material must have brought a counter ion along. This technique then takes advantage of the fact that the interaction between analyte and column material is stronger than the interaction between

(a) the analyte and its (original) counter-ion on the one hand, *and*
(b) the column material and its original counter ion on the other hand.

Thus, *ionic partner exchange* takes place—which is why this process is also called *ion exchange chromatography*.

Once it is clarified that column material and analyte interact stronger with each other than the two interaction pairs initially present do, there are basically two possibilities:

- If the interaction between analyte and stationary phase is only moderately pronounced, it may be sufficient to offer the stationary phase its *original counter-ion* in increasingly high concentrations as an **elution gradient.** For this purpose, a mixture of two liquids is dosed onto the column:
  - Vessel 1 contains only the solvent that has been used from the beginning.
  - Vessel 2 contains a highly concentrated solution of the original counter ion of the column material (with a "counter-counter ion", which should of course be different from the analyte ...).

Elution then begins using only the free solvent, which is unlikely to suffice to flush the analytes from the column (otherwise the interaction between column-fixed charge carriers and analytes would be arguably weak). The amount of solution from vessel 2 is then gradually increased until the concentration of the original counter ion of our analyte is high enough to elute the analyte from the stationary phase.

- If the interaction between stationary phase and analyte is very strong, even a highly concentrated solution of the original counter ion may no longer be sufficient to effect the desired elution. In this case, the only solution (no pun intended!) is to introduce a new substance (an alternative partner) that has the same charge as our analyte but interacts *even more strongly* with the column material. If this happens, the analyte will elute accordingly. This has a certain disadvantage: This new substance is now quite firmly attached to our column. After use, the ion exchange column must be **renatured,** i.e. returned to its original state. This can be done by rinsing the column with a solution of its original counter ions. Certainly, one has to rinse for *quite a long time,* and the concentration of this rinsing solution should be quite high, but it works.—Why does it work?

A Recourse to General and Inorganic Chemistry

Why is it sufficient to rinse the column extensively with a "counter ion solution"?—Behind this ultimately once again lies the law of mass action. For the sake of illustration, let us look at what happens on the column with much smaller but more descriptive numbers than if we really had to work on the scale of Avogadro's number:

If the column material (for example) has 1000 anionic binding sites (yes, of course, there will be more in reality!), correspondingly 1000 (cationic) analyte particles may have attached to it. If we elute this with another substance (we call it X), up to 1000 cationic particles X will subsequently be attached to the stationary phase.

If (for example) the original counter ion to the column material was a sodium cation, it is usually sufficient to rinse extensively (!) with saline solution because here we offer the 1000 binding sites not only 1000 sodium ions but a much larger number. (Now, you may bring out the Avogadro number again, if you like.) Now, the law of mass action tells us how much an equilibrium is shifted to one side or the other, but—and this is where we bring Le Chatelier on board—we also know that the position of a chemical equilibrium can very well be shifted by further addition of a reactant or product. And that is exactly what is happening here.

The interaction ratios between analyte and column material can also be modified by changing the pH value and other aspects. In ion exchange chromatography, too, many different parameters have to be taken into account. But here, once again we are predominantly interested in the underlying principle.

Special Application 1: Ion Chromatography

Just as HPLC is the high-performance variant of liquid chromatography, ion chromatography may be regarded as its counterpart to classical ion-exchange chromatography. It is mainly used for the analysis of anions (i.e. working with cationic stationary phases) and gradually becomes a genuine laboratory standard—once again in full analogy to HPLC.

The detection of the analytes under consideration using this technique is usually carried out conductometrically, i.e. in the same way we already know from Part II. Accordingly, it must be ensured, especially in the case of changes

in the pH value during the process, that any hydroxonium or hydroxide ions do not mask any measurement reading.

The underlying aspects of this *suppression,* the technical requirements of the separations themselves, and also the consideration of possible operating variants would once again go beyond the scope of this brief introduction, so reference is made here only to Section 25.2 of Harris.

Harris, Section 25.2: Ion chromatography

■ Special Application 2: Ion Pair Chromatography

Here, perhaps unexpectedly, the concept of ion-exchange chromatography and the principle of *reversed-phase* HLPC meet. By adding an anionic surfactant (i.e. an intrinsically non-polar molecular ion that carries a negative charge on a functional group, cf. Fig. 25.12 from Harris) a corresponding RP-HPLC column, which is actually also non-polar, is converted into an ion exchange column on which the original counter ions of the surfactant now compete with the analytes.

Harris, Section 25.2: Ion chromatography

As elegant as this concept is: Because the "adsorption/interaction equilibrium" here is extremely pH-sensitive, it does not cope very well with temperature fluctuations and also only sets in only very slowly, what we are dealing here with is a real special application, which is generally only used when the analytes in question cannot be separated by anything else.

Questions

10. Why does the selectivity of a polystyrene-based ion exchange column increase with decreasing pore size?
11. Why is ion pair chromatography so sensitive to changes in pH?

14.2 Exclusion Chromatography

This process is sometimes referred to as **gel filtration**—and conveniently, the term 'filtration' pretty much describes the principle behind it ... albeit recognising this requires a bit of "thinking outsite the box":

The principle of an "ordinary" filter is at first hardly distinguishable from that of a colander or any other sieve (remember Part I):

Sufficiently small things (e.g. water molecules or even ions of the salt used in cooking dissolved in water—or do not you salt your pasta water?!) pass through the sieve practically unhindered, larger things (mainly the pasta) remain in the sieve, which here fulfils the function of a filter.

In exclusion chromatography, columns are again used whose stationary phase has numerous pores, i.e. which, in addition to the "outer surface", also have a large "inner surface" (as we already know from ► Chap. 11). The pores of the column material are large enough that small analytes (or impurities) can penetrate them more or less effortlessly, while larger molecules are prevented from doing so. They can only glide *past* them—which means that they cover a much *shorter* distance than the smaller particles, migrating *through* the labyrinthine interior of the column material, do. Thus, larger analytes reach the end of the column sooner than smaller ones (well illustrated in Fig. 25.14 of Harris): They are eluted earlier.

Harris, Section 25.3: Molecular exclusion chromatography

The column material used either consists of the same polysaccharides we already learned about in the ion-exchange columns in ► Sect. 14.1, or of polyacrylamide: also a polymer that (just like polystyrene) forms a three-dimensional network by the addition of a network former. (Look at Fig. 25.15 from Harris. This is the network we will need again in ► Sect. 15.2.) And just as with cross-linked polystyrene, we can also create smaller or larger pores in polyacrylamide by selectively increasing or decreasing the proportion of network former in the overall mixture. This makes columns of this kind very versatile: they can be tailored practically exactly to the specific analytical needs.

Harris, Section 25.3: Molecular exclusion chromatography

One of the applications of exclusion chromatography is the determination of the molar mass of quite large to really large molecules (which brings us back to proteins and other natural products with a large molar mass).

However, one must bear in mind that there is a certain size, above which one can (or at least should) no longer describe corresponding molecules as "primarily radially symmetrical": That the three-dimensional shape can (and will) play a role. A truly (approximately) spherical large molecule e.g. will not pass through the gel shown in Harris's Fig. 25.14 with the same speed as an approximately rod-shaped particle of the same molar mass. But for a first bit of knowledge to be gained the gel filtration is often sufficient anyway.

The practical aspect of this "inverted filtration" is that salts present in a solution can also be removed very easily in exactly the same way. Any (usually much larger) analytes come off the column more or less quickly, and the salts present in the analyte solution are left behind for the time being and can, if necessary, be eluted separately at a later time (or they are discarded as part of the column renaturation). This process, known as **desalting**, is of immense importance, especially in bioanalysis.

Questions

12. What could be the reason if a mixture of analytes cannot be separated via exclusion chromatography ?

14.3 Affinity Chromatography

In this form of substance separation, the aim is not so much to completely separate a complex mixture into its individual components as to extract a single analyte (or a series of chemically very similar compounds) from a more complex mixture. To do this, similar to ion-exchange chromatography, one uses a column material with which the relevant analyte interacts much more strongly than any other. (Since the reason for this stronger interaction may well be found in one or the other functional group, affinity chromatography occasionally also permits the isolation of an entire *family* of substances.) Accordingly, all other components of the substance mixture will pass through the column more rapidly than the analyte, which is subsequently removed from the column by changing the elution conditions (different solvent, different pH value, etc).. (If you are clever, you could claim that when you try to analyse free carboxylic acids by gas chromatography, you are also using affinity chromatography: No one else interacts with the column material as beautifully as free carboxylic acids. However, on the one hand, this is not desired there, and on the other hand, the elution of the carboxylic acids is a completely different kettle of fish ... usually very closely associated with frustration.)

Harris, Section 25.4: Affinity chromatography

Since it is possible to produce the column material required in a very targeted manner by mechanically or covalently binding practically any substance imaginable to the carrier material, the possibilities of what can be specifically "captured" with it are almost endless—in addition to the functional groups already mentioned for the separation of entire classes of substances, there is also nothing to prevent the use of, for example, a specific protein, an antibody, an antigen, or any other substance.

- This way, for example, proteins can be isolated (which only under very specific physiological conditions actually enter into exactly the desired type of interaction).

- As is well known, the separation of enantiomers (or other stereoisomers) is not trivial. In ► Chap. 13, however, we already mentioned that the use of chiral column material (keyword: cyclodextrins) allows corresponding separations. Clever choice of (also chiral) column material for affinity chromatography also allows the differentiation of (*R*)- and (*S*)-enantiomers, etc.
- And that is just the beginning.

■ Chiral Chromatography

Enantiomers, as you will have learned in *organic chemistry*, basically have the same structure and differ "only" in the configuration at the stereocenter(s). (Please do not forget that a molecule can also have *more than one* center of chirality; in the case of enantiomers, the configuration is then inverted at *all* stereocenters.) Thus, the two compounds of a pair of enantiomers interact in exactly the same way, and this also means that their melting and boiling points, their density and all other physical properties have exactly the same value (apart from the angle of rotation when interacting with linearly polarised light). For this reason, enantiomers cannot be separated from each other by distillation or similar physical processes.

However, separation can well be achieved if the two enantiomers interact with another chiral compound of which *not* both configurations are present, but only one of the two—and this is exactly the case in chiral chromatography: Here, enantiomerically pure cyclodextrins (or other enantiomerically pure chiral column material) are used.

If the (*R*)-configured isomer of the analyte then interacts with the column material—which here, purely arbitrarily, is also assigned (*R*)-configuration—the result is an adduct which now has (*R,R*)-configuration, while the (*S*)-configured analyte with the (*R*)-configured column material has correspondingly (*S,R*)-configuration. However, this means that the analyte-column material adducts no longer represent *enantiomers* but *diastereomers,* and these by no means always show the same physical properties. Accordingly, enantiomeric separation is very well possible here, precisely through the formation of chiral adducts.

We will encounter this principle again (briefly) in ► Sect. 15.1.

Especially when dealing with chiral compounds (natural products, pharmaceuticals, substances otherwise synthesised under consideration of stereochemistry) the principle of enantiomer separation is of immense importance.

Electrophoresis

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_15

Summary

This chapter may seem surprising at first because electrophoresis seems to be based on a completely different principle than chromatography. After all, this field of analysis deals with the migration of charged particles (i.e. ions) in an electric field. However, you will soon see why this topic is nevertheless dealt with here.

In order to separate different (charged) particles efficiently by electrophoretic means, the different analytes must differ in their **electrophoretic mobility (μep)**. This characteristic follows the following formula:

$$\mu_{ep} = \frac{qE}{f} \tag{15.1}$$

If a particle with the general charge q (given in Coulomb; whether the particle in question is positively or negatively charged is irrelevant for the time being) is located in an electric field E (with the unit volt per meter), a force acts on it: This force can be stated as qE (with the unit newton).

Under electrophoretic conditions, however, the charged particle (i.e.: the ion) is in solution, so this force qE, responsible for the movement (technically speaking: the **migration**), is counteracted by a *friction force* f, which (of course) depends on the velocity of the moving particle (ion). (Do you still remember the spoon you were supposed to drag through honey in the topic "Conductometry" from Part II? The faster the spoon is supposed to move, the more you will notice how much the honey opposes your efforts.) And the *viscosity* of the solution in question also plays a role (which again brings us back to the honey). On top of that, the viscosity of any liquid is *temperature dependent.*

Illustration

The fact that the viscosity of water is temperature-dependent is something everyone *has* experienced at least once, who only noticed a crack in a teacup when using it: If you fill it with normal cool tap water, almost nothing happens (at most you may notice a small drop forming at the bottom of the cup, so that you wonder whether you were perhaps a little clumsy when filling it); if, however, you pour a tea bag with *boiling* water, the contents of the cup escape practically over the entire length of the crack.

15

Because of the numerous parameters to be taken into account, tabulated values of the electrophoretic mobility of a wide variety of ions can only be compared with each other at all if *all* parameters are taken into consideration. However, this does not change the *principle of electrophoresis* itself.

15.1 Capillary Electrophoresis (CE)

In capillary electrophoresis (CE), open capillaries are used, which, in terms of their structure, have considerable similarities with the columns used in gas chromatography (► Fig. 13.1)—however, they are not nearly as long (a typical CE capillary is between 30 and 100 cm long) but a good deal thinner: An internal diameter of 20–80 μm is the rule, although a smaller diameter also contributes to better resolution in CE.

Said capillaries are once again made of amorphous silica, once again the thin "glass tubes" are protected from breakage and moisture by a polyimide layer, and once again the problem of free silanol groups arises (especially since the capillaries used have not been modified in any way beforehand, so these –Si–OH groups are really *abundant* here).

But do the free –Si–OH groups lead to a problem here at all?—You will notice immediately that behind the experimental setup, which currently looks tremendously like gas chromatography in disguise (which should explain why it appears here and now), there lies a very elegant idea that turns CE into something completely self-contained ... and an extremely powerful tool for analysis.

■ Special Features of CE

As in gas chromatography, the open columns of capillary electrophoresis ensure that the flow rate-independent term of the van Deemter equation (► Eq. 12.12)—the A term with the different path lengths—is completely omitted. Accordingly in comparison to HPLC a disproportionately lower bottom height H results and thus a significantly higher number of theoretical bottoms (N)—in other words: a better resolution.

But it gets even better:

The principle behind capillary electrophoresis is by no means that of adsorption chromatography (however much the idea may seem obvious!). It is *not* about the analytes interacting with the wall of the capillary. As abstruse as it may sound:

In capillary electrophoresis, there is no stationary phase *at all!*

For Nitpickers

For this reason, language purists who define "chromatography" as the separation of substances on the basis of the different interactions of various analytes with mobile and stationary phases (as we did in ► Chap. 11) place immense emphasis on the fact that CE is not a chromatographic process at all.

This is why **electropherograms** are obtained instead of **chromatograms.**

For some examiners, such linguistic subtlety may make the difference between one or the other mark and a "Fail!".

This means that there is no need for an adsorption or solution equilibrium, which, in turn, means that *the C term of* the van Deemter equation also does not apply.

Thus, for the CE, the theoretical bottom height H is only proportional to the term of the longitudinal diffusion (van Deemter's B term)—and thus inversely proportional to the flow velocity: The *larger the* latter is, the *more* theoretical bottoms result for a given column length H:

$$H = \frac{B}{u} \tag{15.2}$$

But if the separation is not based on the interaction of the analytes with the column material, then what *is it* based on?

■ Very Thin Capillaries

As mentioned above, there are abundant silanol groups on the inner surface of the column that, as mentioned in ► Sect. 12.3, show OH-acidity. Their pK_A value is low enough that they can be considered completely deprotonated above pH = 3.

- As long as we are not in a really extremely acidic medium (pH < 3), the surface of such a CE-capillary shows numerous negative charges—to which corresponding counter-ions belong, of course. In contrast to the negative charges, which are now clearly localised at precisely defined oxygen atoms, this does *not* apply to the (positively charged) counter-ions: those will certainly remain in the *vicinity* of one or the other negative charge but can still change their position relative to the negatively charged surface of the capillary quite easily—at least theoretically.

This results in an *electrochemical double layer:* A layer of positive charge carriers lies more or less loosely on a stationary layer of negative charges. (Look at the whole thing in Fig. 25.24a from Harris. Please look closely, we are going to need exactly that in just a moment.)

Harris, Section 25.6: Basics of capillary electrophoresis

- Now let us take into account that this capillary is not used in a "dry" state but in the presence of a solution that, in addition to (uncharged) solvent molecules, also contains one or the other charge carrier (which might be "only" our analytes, but since CE is also very popular in bioanalysis, the chances are not bad that our analyte solution also contains other ions, e.g. cations and anions which are part of a buffer or similar).

And now, please consider Fig. 25.24b from Harris to see what happens when the electric field is applied, without which electrophoresis (understandably) does not work:

- The negative charges on the capillary surface (shown as small white circles in Fig. 25.24) cannot move in the electric field, as already mentioned; they are *stationary*.
- Their positively charged counter-ions (marked in grey in Fig. 25.24) will not all move towards the cathode, because at least some of them are more or less firmly adsorbed to the surface, but some of them will certainly start moving—whereby the electrophoretic mobility from Eq. 15.1 again plays a certain role ... but only a *certain one* because the electrostatic interaction between the stationary negative charges and the partially mobile positive charges undoubtedly restricts the freedom of movement of these cations.
- However, the same does not apply to the next "layer", which arises here because according to the old law *opposite charges attract each other, equal charges repel each other,* the rest of the cations and anions in the capillary will also sort themselves, so that immediately above the *conditionally mobile* positive layer there arises a *freely mobile* negative layer, and above it again a positive layer which is just as mobile.

Since there are effectively more positive than negative free-moving charge carriers here, the electric field leads to a *net driving force* of the whole system *in the direction of the cathode*: An **electroosmotic flow** results within the capillary.

This electroosmotic flow provides a uniform direction and profile of motion throughout the capillary—including a "clear anterior front," as can be seen very well in Harris' Fig. 25.25a (cross-section of a capillary).

Harris, Section 25.6: Basics of capillary electrophoresis

- The electroosmotic flow now not only ensures that the *positive* charge carriers migrate in the electric field due to their own electrophoretic mobility (μ_{ep}), but that their velocity is increased even further by the electroosmotic effect: They receive a velocity increase of μ_{eo}.
- The direction of movement of the electroosmotic flow also affects the *negative* charge carriers: While any negatively charged particle should *actually* move to the anode (in Fig. 25.24b of Harris on the left), the electroosmotic flow at least neatly stops it from doing so—if not completely. Its own μ_{ep} (which, of course, then points in the other direction and therefore has a different sign) is reduced by μ_{eo} (with opposite sign). If $\mu_{eo} < \mu_{ep}$, the negative charge carrier in question will never arrive at the anode; if $\mu_{eo} > \mu_{ep}$, it will at least take recognisably longer (the longer, the larger μ_{ep} is).

For all charge carriers, there then results a **total mobility (μ_{total})**, which is the sum of the two mobility components, as Eq. 15.3 shows:

$$\mu_{total} = \mu_{ep} + \mu_{eo} \tag{15.3}$$

Again, it should be noted that in the case of negative charge carriers μ_{eo} and μ_{ep} have different signs so that the electroosmotic flow results in at least a deceleration of the analyte particle.

It should be understandable that *non-charged* particles, for whose electrophoretic mobility $\mu_{ep} = 0$ logically always applies, are very well carried along by the electroosmotic flow: All uncharged components of the analyte mixture move with exactly the same velocity (namely μ_{eo}) towards the cathode. It should be understandable that *no separation whatsoever* is to be expected for uncharged components.

As with gas chromatography, there are various ways of injecting or otherwise introducing the analyte into the capillary for CE. (Since at least the analytes to be separated must all be charged, there is, for example, the possibility of *electrokinetic injection,* in which the analytes are "sucked" into the capillary by an electric field, and the like.) In addition, there are still a variety of elegant methods to optimise the resolution of a capillary electropherogram by working with sample solutions/buffers of different concentrations or by varying the pH during the measurement, etc. Again, also on the subject of CE, this section can only provide a rough first insight: The technical literature on this is remarkably extensive—CE is, in fact, gradually becoming a serious standard as well, especially in the life sciences.

And since—by means and methods that would once again go beyond the scope of this article—it is also possible to use *chiral* column material (particularly cyclodextrins come into play once again), enantiomer separation by capillary electrophoresis is now not only possible but also routinely used, especially in pharmaceutical research.

Questions

13. Assuming exactly identical ionic radius: Would μ_{ep} of a divalent cation X^{2+} actually be twice as high as μ_{ep} of a monovalent cation Y^{+}? (Give reasons for your answer.)
14. Why, again by analogy to gas chromatography, is it also necessary in CE to keep the temperature of the column used constant over its entire length?

15.2 Gel Electrophoresis

As the name of this method suggests, a gel rather than a column is used here: usually a more or less tightly cross-linked polyacrylamide gel is used, as we already know from the exclusion chromatography in ► Sect. 14.2—and just as there, the pore size/mesh size of the network can be specifically influenced by varying the proportion of cross-linking agent.

Although gel electrophoresis is also possible with other network-forming agents than polyacrylamide (e.g. with various agarose gel variants), acrylamide is so common as a starting material for such gels (although monomeric acrylamide is anything but healthy!) that the terms gel electrophoresis and **PAGE** (polyacrylamide gel electrophoresis) are sometimes used synonymously. This form of gel electrophoresis is used particularly frequently in bioanalysis, especially for the separation of macromolecules, such as proteins or protein subunits. (This is discussed in detail in *biochemistry* courses or textbooks.)

However, there is a subtle but decisive difference between gel *filtration* and gel *electrophoresis*. With the latter, one can dispense with "thinking outside the box" because when it comes to separating macromolecules from one another, even the smallest ones are still decidedly too large to fit into any cavities and

migrate different distances on their way to the other end of the gel. Instead, a simple *sieve effect is* used here:

- The smaller the analytes, the easier it will be for them to migrate through the gel network (again, the analyte *must have a net charge*; more on that in a moment).
- Depending on the mesh size, it is very well possible that above a certain analyte size, separation can no longer be effected—then you just have to add less crosslinking agent to the next gel you cast in order to obtain a gel that is not quite as tightly meshed.

■ SDS-PAGE

As with any form of electrophoresis, the analytes *must* have one charge or the other. In bioanalytics, especially in the analysis of proteins and DNA or RNA fragments, the addition of an anionic detergent, which interacts optimally with all non-polar molecular components, ensures a homogeneous coating of all analytes. After all, anionic detergents not only have a long, non-polar molecular tail but also an anionic, polar head (and a corresponding counter ion, of course).

■■ Proteins: A Brief Glimpse into Biochemistry

When examining proteins, the analytes are then let to react with a reducing agent as part of the sample preparation. Just in case you do not deal with biochemistry yet, it should be briefly explained here why this is the case: Biochemically/biologically active proteins are quite large molecules that, in order to be able to fulfil their biological function, must adopt a protein-specific three-dimensional spatial structure (= *native folding*). This spatial form is held together by the typical inter- or intramolecular interactions that we already know (hydrogen bonds, dipole-dipole interactions, van der Waals forces, etc.). In addition, there are almost always disulfide bridges (–S–S–) newly formed during folding, which then—after all, these are *real covalent* bonds!—quite efficiently prevent further changes of the folding state.—This is a good thing, because without their respective native folds, the proteins would lose their biological effectiveness. If the interactions or bonds responsible for the native folding are disturbed or broken, this leads to the *denaturation* of the protein, which then has completely different properties—also macroscopically. Protein denaturation can be caused by an increase in temperature (this is one of the reasons why human biochemistry gets seriously out of control when body temperature approaches 42 °C) or by sufficiently aggressive chemicals.

Some natural scientists consistently say ‘ovalbumin thermally denatured in lime shell’ when they mean a hard-boiled egg, and you will certainly agree with me that raw (still native) chicken egg white really has different (even purely physical) properties than cooked (denatured) chicken egg white. (By the way, if you treat native chicken egg white with hydrochloric acid, it very quickly also looks like cooked. You *could* even eat it.) The decisive thing about denaturing, i.e. the destruction of the intramolecular interactions mentioned above, is that an energetically more favourable state with minimised surface area is assumed. Denatured proteins are approximately radially symmetric.

If denaturation is desired in the context of analysis—for example, in order to determine the molar mass of proteins (or their subunits, but that go too far here) by PAGE—one plays it safe and also destroys the disulfide bridges via the reducing agent added, which are then converted to thiol groups (–S–H, i.e.: “thio-alcohol”) in the process.

Since a different number of detergent molecules can attach to different analytes depending on their size, and since the relationship between the number of detergent molecules (i.e. the number of negative charges present) and the spatial size (and thus the molar mass) of the analyte is approximately linear, nega-

tively charged analytes of different sizes but with approximately identical charge density or approximately *identical mass/charge ratio* are obtained.

Because the standard detergent used in PAGE is *sodium dodecyl sulfate* (CH_3-$(CH_2)_{11}$-OSO_3^- Na^+), which is usually abbreviated SDS, this variant of gel electrophoresis is almost exclusively referred to as **SDS-PAGE**.

In the end, such a gel has striking similarities to a thin-layer chromatogram (► Sect. 12.1), because here we again look at how far the respective analytes migrated on the gel towards the anode: The heavier the (denatured) proteins are, the less far they travel. For comparison, and in order to obtain a reference value (analogous to the solvent front) which allows the determination of the respective R_f values, the mixture to be analysed is usually mixed with an internal standard: a small, light molecule (ion) that is sufficiently well recognisable on the gel (often dyed) and moves quickly enough through the gel to be able to be regarded as the counterpart to the "solvent front" from thin-layer chromatography (► Sect. 12.1).

■ Native PAGE

PAGE can also be helpful when it comes to separating proteins in their natural (non-denatured, i.e. *native*) folding state because the vast majority of proteins possess at least one or the other charge under physiological conditions (pH ~ 7) and therefore also move in an electric field. (Occasionally detergents are also used here to increase the number of charges—but these are so mild that they do not cause denaturation.) However, since in native folding the proteins may have entirely different spatial structures than just the (approximate) spherical shape, native PAGE is not suitable for the absolute determination of molar masses. However, the analysis of proteins while *maintaining their native folding* is really quite a special case.

■ Combined with the CE

Even though gel electrophoresis is often still performed on a medium scale (with gels of 10–20 cm edge length), (SDS-)PAGE can also be performed within CE capillaries. Such a procedure is particularly recommended for routine investigations and *high-throughput measurement series.* (With this method, it is no longer a question of R_f values but of *retention times.* The principle is nevertheless the same.) Fig. 25.22 from Harris shows a schematic diagram of a capillary electrophoresis apparatus; further details are given in Section 25.7.

Harris, Section 25.7: Performance of capillary electrophoresis

Lab Tip

One must not forget that for each new measurement, new gel must be introduced into the capillary beforehand—after use, the capillaries are simply washed out again.

However, it also does not take long to introduce new gels: Please consider the minimal internal volumes of CE capillaries: The mixture of acrylamide and crosslinking agent is thoroughly polymerised and thus cured within a very short time.

Questions

15. When performing a capillary gel electrophoresis experiment using SDS as detergent, does the polarity of the electric field need to be reversed? (As always with this kind of yes/no questions: Please state reasons for the answer you chose.)

Choice of Methodology

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_16

Summary

In the previous chapters you have become acquainted with various, sometimes very different, methods for separating a wide variety of analytes—and undoubtedly all of them have their advantages and certain disadvantages. This inevitably leads to the question:

- **What Criteria Are Used to Decide on the Analytical Method?**

Of course, which method you choose (NP- or RP-HPLC or rather molecular exclusion chromatography?) and which solvent(s) you should use depends largely on the analytes to be separated. In principle, it is advisable to first make a rough subdivision of the (potential) analytes:

- Are they rather small molecules with a molecular mass < 2000 g/mol (approximately)?
- Or is it a question of macromolecules whose molar masses may lie far above this (more or less arbitrary) limit? In the latter case, it is often not so much the individual interactions of the molecules, i.e. effectively the individual atoms within them, that are of concern, but rather the size, mass or even the spatial shape of these significantly larger molecules.

Harris, Fig. 24.15, Section 24.1: The chromatographic process

Once this question has been answered, it remains to be clarified in which solvent(s) the sample is best soluble (i.e. is it more polar or more nonpolar?). It is then helpful to use the appropriate decision tree from Harris as a guide.

16.1 Summary

16.1.1 Liquid Chromatography (LC)

Liquid-chromatographic substance separation based on adsorption takes advantage of the fact that analytes of different polarity interact with the column material to different extents and therefore take different lengths of time to migrate a certain distance. In thin-layer chromatography, one then measures how far different analytes have migrated on the adsorbent material within a certain period of time, while in column chromatography one determines how long different analytes need to cover the entire column length.

The more an analyte resembles the stationary phase in polarity, the more it is retained by the adsorbent; the more its properties resemble those of the mobile phase, the more rapidly it moves.

- *Normal phase* chromatography (NP-) combines a polar stationary phase with a non-polar mobile phase.
- In *reversed phase* chromatography (RP-), a non-polar stationary phase meets a polar mobile phase.

In addition to the intermolecular interactions between analyte and stationary or mobile phase, the various aspects of *diffusion* described by the van Deemter equation must also be taken into account in column chromatography, which affect the elution process.

The resolution of a chromatogram represents a measure of the quality of substance separation; various aspects of statistics come into play here.

The detection of the analytes is preferably carried out UV/VIS-photometrically; for detection in the UV range, the cut-off wavelength of the respective solvent (or solvent mixture) used must be taken into account. Other detectors are also commonly used, but some are not suitable for solvent mixtures or gradients. Of particular importance is the combination of high performance liquid chromatography (HPLC) with mass spectrometry (HPLC-MS).

16.1.2 Gas Chromatography (GC)

In GC, analytes interact with a solid stationary phase or (more commonly) with a liquid phase that wets the surface of the column material.

Only analytes that can be transferred to the gas phase without decomposition can be analysed via GC; carboxylic acids and other extremely polar and/or aggressive functional groups that may attack the column material may require derivatisation.

Since longitudinal diffusion is disproportionately more pronounced in the gas phase than in the liquid phase, GC requires a significantly higher flow velocity according to the van Deemter equation.

The detection of the analytes is preferably carried out via thermal conductivity measurement; occasionally other detectors are also used. Analogous to HPLC/MS, gas chromatography is also increasingly linked to mass spectrometry.

16.1.3 More Specific Forms of Chromatography

Here, the separation of substances is not based on the interplay of adsorption and desorption:

- In *ion-exchange chromatography*, the column is provided with fixed positive or negative charges; if the analyte has a correspondingly opposite charge, it is retained electrostatically.
  - This variant of *ion chromatography* is mainly used for anionic analytes.
  - *Ion pair chromatography* is derived from reversed phase chromatography; it is extremely sensitive to interfering influences and is therefore only used in special cases.
- In *exclusion chromatography,* also known as gel filtration, finely porous column material is used: Sufficiently small analytes (or also impurities) can penetrate these pores and thus have to cover a longer distance when passing through the column than larger analytes, which can only glide between the individual column filling particles. Therefore, they reach the end of the column more quickly; the principle of exclusion chromatography also allows the desalting of solutions.
- In *affinity chromatography,* interactions other than polarity or van der Waals forces between analyte and column material are utilised. This might involve complex functional groups but also antigen-antibody interactions (cf. life sciences!). Accordingly, this method is very special but, if often also remarkably specific.

16.1.4 Electrophoresis

In electrophoresis, the migration of charged particles in an electric field is considered. Accordingly, the various electrophoretic separation methods are also only suitable for charged analytes; non-charged particles of any kind are generally *not* separated by this (although separation *can* be achieved if necessary, but this, then, is *very* special).

- The crucial aspect of *capillary electrophoresis* is the electroosmotic flow resulting from the stationary negative charges of the silica gel capillary, deprotonated at pH > 3, and the mobility of the positive counter ions, which starts the moment an electric field is applied. It accelerates cationic analytes (or other sample components) towards the cathode, while anionic

components are significantly slowed down, but eventually they also reach the cathode (and the detector located there).

- The actual separation of the different (cationic and anionic) analytes is based on their different electrophoretic *mobility*.
- In gel electrophoresis, a *sieve effect* is used which allows smaller (in the sense of "less massive") analytes to pass through the gel rather than larger analytes. By clever choice of the amount of cross-linking agent, the "mesh size" of the resulting gel can be adapted very precisely to the respective requirements of the analytes to be separated.
  - Analogous to thin layer chromatography, R_f values are determined for the various analytes on the gel.
  - The combination of gel electrophoresis and CE leads to *capillary gel electrophoresis*, in which the different analytes are again distinguished on the basis of their retention times.

Answers

1. First, the actual running distance of the solvent must be determined: 10 cm = 100 mm. The distance between the starting line and the solvent front is 100 – 10 – 4 mm = 86 mm. Now, one only has to determine the respective migration distances and then divide these by the migration distance of the solvent (front) as described by ► Eq. 12.1:
   (a) The spot, which is 4.2 cm above the lower edge, has thus moved 32 mm, resulting in $R_{f(1)} = 32/86 = 0.37$.
   (b) For the spot at 5.8 cm height, $R_{f(2)} = 48/86 = 0.56$.
   (c) For the substance that has migrated to a height of 7.3 cm, i.e. has risen 6.3 cm, we get $R_{f(3)} = 63/86 = 0.73$.
   Specifications with more than 2 digits after the decimal point are rarely useful.
2. Alumina (Al_2O_3) is similarly polar to silica (SiO_2), and water (H_2O) is also polar, so a usable separation is unlikely to result. In TLC, a (more or less) polar stationary phase should always be used with a (more or less) non-polar mobile phase, or vice versa. Only with the analytes being so polar that they interact extremely strongly with the stationary phase and the solvent being too non-polar to obtain a separation, it makes sense to increase the polarity of the mobile phase carefully. (For a non-polar stationary phase and non-polar analytes, the exact reverse is true accordingly.) This is exactly what the following task is about.
3. This poses exactly the problem already mentioned in the previous task (or solution): If the interaction between analyte and stationary phase is very strong, a solvent (mixture) must be used that comes somewhat closer to the properties of the stationary phase. (Yes, occasionally *trial-and-error* is required to find a suitable solvent mixture.) Since ethanol is significantly more polar than diethyl ether, simply because of the OH-group and its ability to form hydrogen bonds, your colleague's suggestion makes sense in any case. (Depending on the nature of the analytes, it is quite possible that even this will not be sufficient, but it is definitely a sensible approach.)
4. The minimum dwell time $t_m = 27$ s was specified.
   (a) According to ► Eq. 12.2, this leads to the the respective reduced retention times: $t'_{r(1)} = (42\text{ s} - 27\text{ s}) = 15$ s, $t'_{r(2)} = 66$ s, $t'_{r(3)} = 115$ s, $t'_{r(4)} = 142$ s, and $t'_{r(5)} = 240$ s. (The fact that here $t'_{r(4)}$ and $t_{r(3)}$ are identical is pure coincidence.)
   (b) According to 7 Eq. 12.5, the following then applies accordingly to the retention factors: $k_1 = (42 - 27)/27 = t'_{r(1)}/27 = 0.555$; $k_2 = 66/27 = 2.444$,

$k_3 = 115/27 = 4.259$; $k_4 = 142/27 = 5.259$; and $k_5 = 240/27 = 8.888$. (So you see that although $t_{r(3)}$ has the same numerical value as $t'_{r(4)}$, k_3 and k_4 are very different.)

(c) The uncorrected retention for peaks 3 and 4 is given by ► Eq. 12.4 as 169/142, so $\gamma = 1.190$; the associated separation factor is then calculated by ► Eq. 12.3 as 142/115, so $\alpha = 1.235$.

5. Understandably, with regard to the resolution, it is always useful to consider only immediately adjacent peaks. Accordingly, we need $\text{resolution}_{\text{Peak1/Peak2}}$ and $\text{resolution}_{\text{Peak2/Peak3}}$ here. According to ► Eq. 12.8, in each case we need the difference in retention time (Δt_r) and the corresponding mean value of the peak widths at half height ($w_{½av}$). For peak 1 and peak 2, $\Delta t_r = (42 - 23) = 19$ and $w_{½av} = (6 + 12)/2 = 9$. Thus, $\text{resolution}_{\text{Peak1/Peak2}} = 0.589 \times 19/9 = 1.243$. For peak 2 and peak 3, $\Delta t_r = (72 - 42) = 30$ and $w_{½av} = (12 + 10)/2 = 11$; i. e., $\text{resolution}_{\text{Peak2/Peak3}} = 0.589 \times 30/11 = 1.61$. Peak 1 and peak 2 are too close to each other to speak of satisfactory resolution, while peak 2 and peak 3 are clearly enough separated.
6. NP-HPLC means that the column material (the stationary phase) is polar and a significantly less polar mobile phase is used. If the analytes elute very rapidly with the selected solvent mixture, this means that the eluent has too high an elution capacity, i.e. its properties are too similar to those of the stationary phase. Accordingly, it would be advisable to first drastically reduce the polar fraction of the solvent mixture used for elution. If the use of an ethanol/diethyl ether mixture of the ratio 10:90 should cause the retention times of the individual analytes to become too long (which, on the one hand, leads to a significantly larger difference in the individual retention times but, on the other hand, also to a peak broadening due to the long residence time on the column), the polarity of the mixture could then be carefully increased via a gradient.
7. Even if one should be able to estimate polarities of rather simple compounds, it is always advisable to find out more about the analytes to be expected. A glance at the relevant reference book (or access to a corresponding database) tells us that 1-octanol, for example, is only very poorly soluble in water (in the order of 0.5 g/L); accordingly, this analyte is only very slightly polar despite the hydroxy group: A separation via NP-HPLC should, therefore, be ruled out since the interaction between analyte and stationary phase would only be minimally pronounced, so that correspondingly short retention times would result. For the other isomers of octanol, the same is likely to be true. In this respect, RP-HPLC would certainly be the better choice. However, whether a C8 or a C18 column is better cannot yet be judged on the basis of the information presented in this part. For this, it would be necessary to deal with the relevant technical literature.
8. There are two reasons for this: Firstly, the analytes must all interact with the column, and especially with open columns the surface available for this is quite limited (in order to overload a packed column in this respect, considerably more substance is required); if the amount of analyte is too large, a part will simply pass through the column without the desired adsorption-desorption equilibrium being achieved. On the other hand, a column (whether open or packed) can also be overloaded if, in principle, every analyte particle interacts with the column material, simply because the sample zone is too wide and the analytes do not reach the detector more or less simultaneously. (To illustrate this, take another look at Fig. 22.5 from Harris, even though it deals with liquid chromatography.) If the difference in the retention times of the individual analytes is then also too small, it is only too understandable that the broadened bands will merge into one another.

9. Nitrogen is much heavier than helium ($M(N_2)$ = 28 g/mol, M(He) = 4 g/mol), and accordingly it is much more difficult for the analytes to diffuse through the more massive gas. This slows down establishing the adsorption-desorption equilibrium (quite in the sense of the C term of the van Deemter equation, ► Eq. 12.12), and thus the theoretical bottom height increases (or the number of theoretical bottoms decreases). This definitely reduces the resolution.
10. The smaller the pores, the more difficult it is for the analytes, as well as for all other particles of the same charge, to reach the respective counter-charge bearing places of the column material in the first place. Reduced pore size, thus, slows down the interaction process, and—you will know this from *organic chemistry*—the slower a process proceeds, the more specific it is.
11. If you look again at Fig. 25.12 from Harris, you will see that (before interaction of the column material with the analytes) the negative charges of the functional group of the anionic surfactants form a comparatively close ion pair with their original counter ions, i.e. remain spatially close to each other. If the concentration of free H_3O^+ or OH^- ions increases, these will interact with the anionic group (and thus protonate it to neutrality) or interact with its counter ion so that this close ion pair no longer exists. In addition, the counter ions of the acid or base responsible for the change in pH (which are also necessarily present) will also interact.
12. The reason might be: to too small a pore size of the column material used. If all analytes are too large to diffuse into the voids, they will all pass through the column without separation.
13. No, it would not. Please keep in mind that an ion with the same radius but increased charge will also have increased *charge density*. Correspondingly, the interaction with the solvent molecules will also lead to a more extensive solvate shell, so here a particle would be brought to migrate that has the same ionic radius *when unsolvated* but is significantly larger *when solvated.* Analogous to what has already been said about the charge density of solvated ions in ► Sect. 14.1, it is even possible that the increase in radius due to the solvation shell ultimately leads to a *reduced* electrophoretic mobility compared to the singly charged ion, despite the higher factual charge.
14. Consider the importance of the viscosity of the solvent: As the temperature increases, the viscosity commonly decreases, which in turn increases the mobility of the analytes. If you were to perform the same experiment twice (same solvent, same analytes, same capillary and capillary length, etc.) but changed the temperature, the resulting electropherograms would be drastically different. This temperature sensitivity is also a reason why the resolution decreases as the inner diameter of the capillary used increases. Any movement of a charged particle in the electric field results in heating. If this heat cannot be dissipated equally and uniformly over the entire column diameter (which is why the capillaries are cooled with the aid of a thermostat), this leads to an internal temperature gradient with a maximum in the center of the capillary—and there the viscosity of the solvent is then significantly reduced. Accordingly, the mobilities of analytes even of the same type would then change depending on their relative position within the capillaries—which would lead to a considerable broadening of the resulting peaks. And the thicker a capillary, the more difficult it is to dissipate the heat generated by electrophoresis.
15. One might think that polarity reversal is indeed necessary (or at least reasonable) because if the analytes are effectively negatively charged several times by the detergent SDS, they would "actually" migrate in exactly the wrong direction with the usual polarity (the cathode is located at the "exit"

of the capillary). However, this is not correct because the electroosmotic flow also has an effect in capillary gel electrophoresis, and electrophoretic mobility (μ_{ep}) is much smaller than movement by μ_{eo}, especially for large/heavy analytes (such as proteins or DNA/RNA fragments). This means that although analytes *should* tend to migrate in the opposite direction due to their own charge, they are only slowed down when migrating to the other pole (because for anions, μ_{eo} and μ_{ep} have opposite signs)—which ultimately only increases separation efficiency; different analytes will also have different values of μ_{ep}, after all. Were the polarity reversed, on the other hand, μ_{eo} and μ_{ep} would complement each other. This would make the experiment much faster but the separation efficiency would be drastically reduced, if not completely eliminated (all analytes would reach the detector practically simultaneously) .

Further Reading

Binnewies M, Jäckel M, Willner, H, Rayner-Canham, G (2016) Allgemeine und Anorganische Chemie. Springer, Heidelberg
Cammann K (2010) Instrumentelle Analytische Chemie. Spektrum, Heidelberg
Hage DS, Carr JD (2011) Analytical Chemistry and Quantitative Analysis. Prentice Hall, Boston
Harris DC (2014) Lehrbuch der Quantitativen Analyse. Springer, Heidelberg
Langford A, Dean J, Reed R, Holmes D, Weyers J, Jones A (2010) Practical Skill in Forensic Science. Prentice Hall, Pearson Education Ltd, Harlow
Lottspeich F, Engels JW (2012) Bioanalytik. Springer, Heidelberg
Ortanderl S, Ritgen U (2018) Chemie—das Lehrbuch für Dummies. Wiley, Weinheim
Ortanderl S, Ritgen U (2015) Chemielexikon kompakt für Dummies. Wiley, Weinheim
Schwister K (2010) Taschenbuch der Chemie. Hanser, München
Skoog DA, Holler FJ, Crouch SR (2013) Instrumentelle Analytik. Springer, Heidelberg
Unger KK (1989) Handbuch der HPLC, Teil 1. GIT, Darmstadt
Some of the works mentioned here go far beyond "the Harris" with respect to selected areas of analytics.

Molecular Spectroscopy

Requirements

The term "spectroscopy" covers various analytical methods based on the interaction of the substance(s) to be analysed with electromagnetic radiation of various wavelengths. Electrons can be excited in such a way that other energy levels are occupied than the ones normally occupied in the ground state, or molecular components or the entire molecular structure are made to oscillate. It should be understandable that a closer look at the respective processes requires certain prior knowledge.

More generally, we need:

- the quantisation of energy,
- the concept of the photon *per se*,
- the electromagnetic spectrum,
- the relationship between wavelength and energy content,
- colours and complementary colours,
- the blank sample (and what that actually means), and
- Lambert-Beer's law.

When it comes to excitation of electrons, the following terms should be more than just "roughly familiar":

- atomic orbitals (and their spatial shape),
- the concept of molecular orbitals,
- σ- and π-interactions,
- bonding/anti-bonding interaction,
- non-bonding interactions.

In vibrational spectroscopy, you need:

- the VSEPR model and/or other methods to predict the spatial structure of molecules (or ions) based on the corresponding structural formula,
- enough practice to always keep an eye on free electron pairs and the like and to be able to estimate bond angles,
- an eye for dipole moments (which can change drastically when there are changes in bond lengths or angles),
- a sense of the polarisability of bonds, *and*
- at least a rough idea of the field of molecular symmetry.

1.1 Learning Objectives

In this part, you will learn about the influence of electromagnetic radiation of different wavelengths on polyatomic systems: Depending on the energy content, such radiation can cause a change in the occupation of orbitals as well as a dynamic, time-limited deviation from the respective spatial structure to be expected according to the models for the prediction of structures discussed in previous learning units.

Based on the molecular orbital theory, you will recognise the connection between microscopic objects (polyatomic molecules and their bonding properties) and macroscopic phenomena (perceivable colour, absorption and emission of electromagnetic radiation/light) and learn not only to "read" but also to *understand* different types of spectra.

Taking symmetry considerations into account, you will become acquainted with a powerful analytical tool in the form of vibrational spectroscopy, which allows sufficient conclusions about the bonding situation present in a compound on the basis of absorption or emission spectra, in order to unambiguously identify previously unknown compounds if necessary.

As with Parts I, II, and III, the main purpose of this text is to emphasise and focus on the importance of certain topics covered in the Harris. However, the Harris deals only very briefly with vibrational spectroscopy, which is one of the main topics of this part.

Harris, Chapter 17: Fundamentals of spectrophotometry

For this reason, ► Chap. 19 offers a general introduction, while in ► Sect. 19.2 infrared spectroscopy and in ► Sect. 19.3 Raman spectroscopy are presented in more detail. A recommended literature for further reading is Hesse/Meier/Zeeh. (This book will undoubtedly serve you well again if you also deal with "Analytical Chemistry II").

Hesse/Meier/Zeeh, Spectroscopic Methods in Organic Chemistry, **1**: UV/Vis Spectra and **2**: Infrared and Raman spectra

Contents

General Information About Spectroscopy

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_17

Summary

The term "spectroscopy" covers a wide range of analytical methods based on the interaction of the substance to be analysed with electromagnetic radiation of various wavelengths.

Binnewies, Section 2.3: The structure of the electron shell

From *General Chemistry* and *Physics* (as well as from e.g. Binnewies), you surely know the relation between wavelength λ, frequency ν, and energy content E:

$$E = h \times \nu = \frac{hc}{\lambda} \tag{17.1}$$

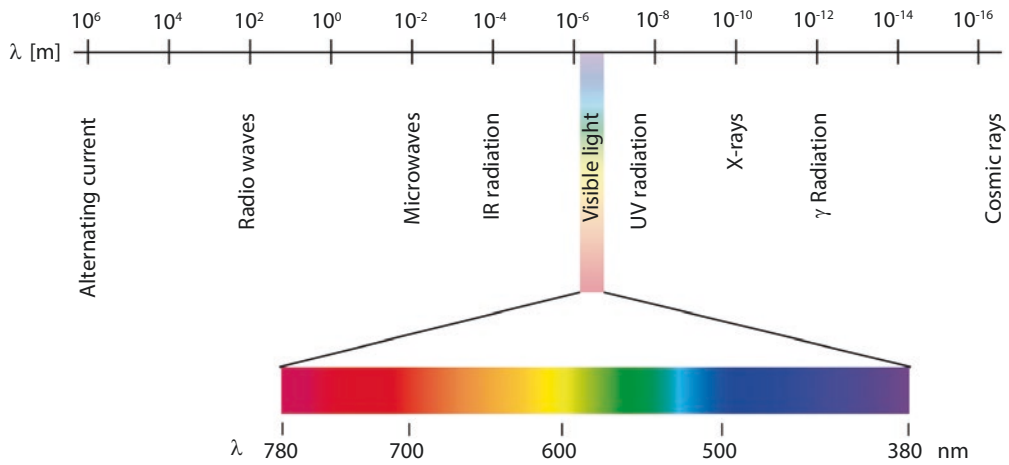
The following applies:

- h is the Planck quantum of action: h = 6.626 × 10^{-34} Js
- c is the speed of light; m vacuum is c = 2.998 × 10^{8} m/s.

> In by far the majority of cases, one may confidently calculate with c = 3 × 10^{8} m/s; methods of analytics that require calculating with the numerical value of the speed of light, do not necessarily take place in a vacuum, and in other media, the numerical value for c is *somewhat* different.

Equation 17.1 states: The shorter the wavelength of the electromagnetic radiation under consideration, the more energetic said radiation is. This applies (of course) to *all* electromagnetic radiation of the entire spectrum, which you can admire in all its splendour in ◘ Fig. 17.1—whether it concerns the range of visible light (with wavelengths λ from 380 to 780 nm), the clearly lower-energy infrared radiation or also more energetic photons, for example, from the UV, the X-ray, or the γ-ray range.

If an analyte then absorbs electromagnetic radiation, completely different things happen to it depending on the radiation's energy content—in any case, however, first of all the analyte's own energy content is increased by exactly the energy which the photon in question possessed and thus transmitted.



◘ **Fig. 17.1** The electromagnetic spectrum (S. Ortanderl, U. Ritgen: Chemie—das Lehrbuch für Dummies, p. 137. 2018. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

Important

It should be explicitly pointed out once again that the energy transmitted or transmittable by electromagnetic radiation is **quantised.** As Max Planck discovered around the turn of the last century, energy can only be transmitted in the form of "energy packets" whose energy content corresponds to a multiple of Planck's quantum of action (h).

This also means that visible light, for example, which with the aid of a prism can be split (dispersed) into the various wavelengths, is in fact a **quasi-continuum,** which should rather be understood as a very narrow sequence of individual, strictly separated wavelengths. (If necessary, take another look at Fig. 26.24 from Binnewies.)

Binnewies, Appendix A (Some basic concepts of physics)

At the same time, this also means that a theoretically excitable analyte, whose different energy states are also quantised (!), can only be excited by *precisely defined* wavelengths, while the analyte will *not* absorb all other wavelengths.

This is one of the fundamental differences between the **microscopic** and the **macroscopic** world:

- If you want to overcome a difference in energy in the *macroscopic* world, the only thing that matters to you is the *minimum* effort required to do so. For example, if you want to climb a stair step (and thus increase your potential energy by the amount of energy resulting from the difference in height of the two steps), you must lift your foot *at least* far enough to reach the next higher step. But you can also lift it much higher and then drop it onto the target step. (This would certainly look odd, but it *would* work.)
- If there is an energy difference of (arbitrarily) 100 energy units for a *microscopic* system between the current and the next higher possible energy level, this corresponding excitation is also only caused by absorption of a photon with *exactly* the energy content required. The fact that a photon with too little energy (of e.g. only 99 energy units) achieves nothing may perhaps still be compatible with the much-cited common sense, but if the system in question encounters a photon that would transfer e.g. 101 energy units, then—unlike in the macroscopic world—we do *not* get excitation after which only the surplus energy would be released (because it is not required for excitation) in one form or another. In fact, *nothing* happens*:* in the microscopic world, "a bit too much energy" is just as useless as "a bit too little".

This should *always* be kept in mind when doing spectroscopy.

If a photon is absorbed by an atom or molecule, this increases the respective particle's energy content. But what exactly changes here?—Let us go through the entire electromagnetic spectrum—starting with the "lower energy end" (in Fig. 17.2 from Harris or ◘ Fig. 17.1 from right to left): You will see that actually *every* wavelength range can be used for one or another form of analysis:

Harris, Section 17.1: Properties of light.

- The very modest energy content of **radio waves** is still sufficient to change the *spin state of atomic nuclei.* This is the basis of nuclear magnetic resonance spectroscopy (NMR), which we will discuss in some detail in Part I of "Analytical Chemistry II".
- The field of **microwave radiation** offers two attractive possibilities:
  - With comparatively long-wave microwave radiation, electrons can be excited in a similar way as is possible with atomic nuclei using radio

waves. However, since the **electron spin resonance** (ESR) method is only suitable for very special analytes (they must have at least one *unpaired* electron, i.e. show **paramagnetic behaviour**), it will not be discussed further here.
  - If an analyte molecule absorbs short-wave microwave radiation, it is brought to **rotation**; the resulting absorption lines then allow conclusions about details concerning the structure of the analyte. This is referred to as **microwave spectroscopy.**

What Should Not Be Forgotten

The rotation of a molecule (or molecular ion), i.e. its movement around its own centre of gravity, is a **microscopic event:** Even if one certainly imagines that the rotation of a molecule around its own axis is continuous, one should keep in mind that we are looking at objects for which the laws of the *quantum world* apply, including the quantisation of *all* possible energy states. A molecule can not turn "steplessly" and thus at any conceivable angle. As also rotation is quantised, only selected wavelengths are suitable to cause such a rotation. With other wavelengths, molecule can do nothing. Correspondingly, there are also *rotation levels*, analogous to the different energy levels, which we know from the electrons, and which, as we know, can differ in their energy content.

- Infrared radiation can induce *internal molecular oscillations* (**vibrations** in technical terms)—although it should be noted that these vibrations are also *quantised* (just like the rotations): When corresponding IR photons are absorbed, the energy input causes different *vibrational levels* to be adopted, changing bond *lengths* or bond *angles*. We will return to the vibrational spectroscopic methods based on this in ► Chap. 19. These include:
  - **IR spectroscopy,** in which an absorption spectrum is recorded, and
  - Raman spectroscopy, in which the relevant data are taken from an **emission spectrum.**
- Even some photons from the visible light range (VIS) carry / transfer enough energy to make electrons change from an energetically more favourable orbital to an energetically less favourable orbital, i.e. to put the molecule in question into an (electronically) **excited state.**
  - This applies all the more to **UV radiation**, which is even more energetic. The principle of **UV/VIS spectroscopy** has already been briefly discussed in Part II, but we will return to it in ► Sect. 18.1.
- X-rays are more energetic by several orders of magnitude, and they also excite electrons—but with a crucial difference: While UV/VIS spectroscopy deals with *valence electrons* or the electrons in the **frontier orbitals**, the energy content of X-ray photons is sufficient to excite **core electrons.** One of the most important analytical techniques that make use of X-rays is X-ray fluorescence analysis, which we will discuss in Part V.

Bonus Information

Yes, X-ray **structure analysis,** also called **X-ray diffraction**, also uses X-rays, as the names certainly suggest. However, this method of **solid state analysis** is not based on the *absorption of* this radiation but rather on its *diffraction at the crystal lattice.* And since this part will deal with spectroscopic methods, we will not go into this topic in any further detail here.

- Even with the really extremely energetic **γ-radiation,** analytics can still be carried out, though the energy of these photons is more than enough to break covalent bonds within a very short time (which is why it is not exactly beneficial to health to be exposed to such radiation for a longer period of time). The most important analytical technique using this radiation is **Mößbauer spectroscopy,** in which atomic nuclei (!) firmly bound in a crystal lattice are energetically excited. From the resulting absorption or **transmission spectrum,** not only individual atoms can be identified, but also relative atomic positions within the existing lattice, charge density distributions and much more can be determined. But we will not go into this any further here.

Excitation of Electrons

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_18

Summary

The principle of any analytics based on the excitation of electrons by absorption of (UV/VIS) photons we already covered in Part II, because *photometry* is nothing else. (Moreover, we also returned to this topic in Part III for detectors commonly used in chromatography.) A brief review:

In photometry, we observe wether the analyte under consideration absorbs electromagnetic radiation from a particular wavelength range:

- If one restricts oneself to the range of visible light (VIS), one sometimes also speaks of **spectrophotometry.**
- Much more common, however, are measurement setups that search not only for visible light but also for absorption by the analyte in the UV range: The term **UV/VIS spectroscopy** is then used.

Since spectrophotometry can be regarded as a "special form" of UV/VIS spectroscopy—after all, it is based on exactly the same principle, except that UV/VIS spectroscopy covers a larger wavelength range—we will treat both techniques together.

18.1 Spectrophotometry and UV/VIS Spectroscopy

Once an analyte interacts with electromagnetic radiation from the UV/VIS range, several things happen at once:

- When a suitable photon hits the analyte, the energy of this photon (whose energy content can be calculated according to ► Eq. 17.1) is transferred to the molecule.
- The energy content of the analyte increases by exactly this amount. (Take a look at Fig. 17.3 from Harris; we will look at exactly what this energy absorption means at the microscopic level in ► Sect. 18.2.)

Harris, Section 17.2: Light absorption

- Considering the fact that for this kind of energy transfer the energy quantisation (mentioned again in ► Chap. 17) applies—i.e. only certain photons can be absorbed, others cannot—, it can be determined by means of corresponding spectra which wavelength(s) (there can also be more than one) was/were absorbed—and to what extent. (In this way, not only qualitative but also *quantitative* spectra can be created.)
  - Should the electromagnetic radiation originate from the range of visible light (VIS), it would be expected that the human eye (or better: the corresponding brain) sees (respectively perceives/reports) the complementary colour of the absorbed photons because a part of this radiation has been removed from the quasi-continuous VIS spectrum by the absorption—just as we already covered it in Part II (think of the colour wheel). With this, the *colorimetry*—already discussed there—can then also be operated.

Similar to photometry from Part II, spectrophotometry or UV/VIS spectroscopy is also concerned with the *extent* to which the analyte absorbs electromagnetic radiation—depending on its wavelength, of course. However, there is a huge difference between photometry and spectrophotometry:

- In *photometry*, one prefers to work with **monochromatic** light of exactly the wavelength that one knows the analyte absorbs. (Of course, for that you have to know your analyte quite well.)
- In *spectrophotometry* or *UV/VIS spectroscopy,* the analyte is irradiated with different wavelengths one after the other (or simultaneously, this is a question of the equipment and/or the experimental set-up), and then it is determined for *each individual wavelength* whether and, if so, to what extent the analyte absorbs it (or not)—preferably across the entire quasicontinuum of the UV and VIS range.

Since, as you will see in the upcoming ► Sect. 18.2, the wavelength range in which absorption occurs already allows certain conclusions to be drawn about the analyte, spectrophotometry—or the even more informative: UV/VIS spectroscopy—can be used to even identify a previously unknown substance. (This would be rather tricky with UV/VIS alone, but in combination with various other analytical methods—many of which we will cover in "Analytical Chemistry II"—UV/VIS spectroscopy is a very efficient tool.)

■ Transmission and Extinction Spectra

It should come as no surprise that exactly the same laws apply to spectrophotometry and UV/VIS spectroscopy that we have already discussed dealing with "ordinary" photometry in Part II. However, because these laws are so important here as well, they shall be reviewed/repeated once again.

Ultimately, behind both techniques lies **Lambert-Beer's** Law:

$$A = \varepsilon_\lambda \times c \times d \tag{18.1}$$

ε_λ is the *extinction coefficient* (substance-specific and wavelength-dependent (!), hence the subscript λ) of the analyte under consideration; c is the concentration of the analyte solution; and d is the thickness of the cuvette used, i.e. the layer thickness through which all rays of the respective wavelength λ have passed.

Important

It should be emphasised once again that Lambert-Beer's law also has its limits:

- Firstly, it is only suitable for *real solutions*, i.e. not for suspensions, emulsions, or other mixtures that exhibit a **Tyndall effect.**
- On the other hand, the relationship between absorption and concentration is only really linear for very dilute solutions (as a rule of thumb: <0.01 mol/L): *Solutions that are* too *highly concentrated no longer follow Lambert-Beer's law.*

On this basis, the intensities of the wavelengths passing through the samples are compared. Together, they give the **absorbance**.

In order to be able to carry out quantitative measurements, the loss of intensity due to solvent, cuvette material, etc. must of course be taken into account—just as in photometry. Therefore, a **blank sample** must also be measured, and the intensity (I) of the radiation that has passed through the blank sample must be set equal to the initial intensity (I_0). Subsequently, the intensity I_{Sample} obtained in each case is plotted graphically for each individual wavelength:

- Many wavelengths will simply not be absorbed by the analyte, i.e. these wavelengths will pass through the analyte solution unhindered in the same way as in the blank sample.
- Some wavelengths, however, are absorbed by the analyte, so I_{Sample} will be much smaller than I_0. (This is really *just* like the photometry from Part II.)

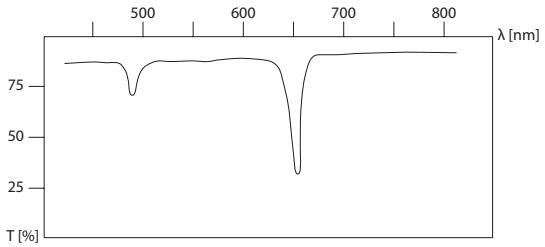
Of course, you do not measure every single wavelength by hand in the laboratory and then laboriously record the photometric data obtained on graph paper. This would also be possible but would take far too long. Nowadays, spectra are recorded almost fully automatically and within a very short time. (You will learn the absolute basics of the construction of the equipment used here in ► Sect. 18.5.)

There are two fundamentally different methods of visualising the respective measurement results—and again, from Part II you already know the underlying principles:

For **transmission spectra,** the wavelength (λ) is plotted on the x-axis and the **transmission T**, i.e. the relative transmittance (for the respective wavelength), is plotted on the y-axis, the latter readily given as a *percentage*. The corresponding formula is:

$$T = \frac{I}{I_0} \times 100\ [\%] \tag{18.2}$$

Since the blank sample ideally should not absorb a single photon of any relevant wavelength, a transmission of 100% would be expected there: therefore, it has become common practice to label the x-axis at the *top of* transmission spectra. The result looks something like this:



Stylised transmission spectrum

It can clearly be seen that the sample—whatever it may be—absorbs radiation of wavelengths 480 nm and 650 nm, with the absorption being more pronounced for the longer wavelength radiation. In other words:

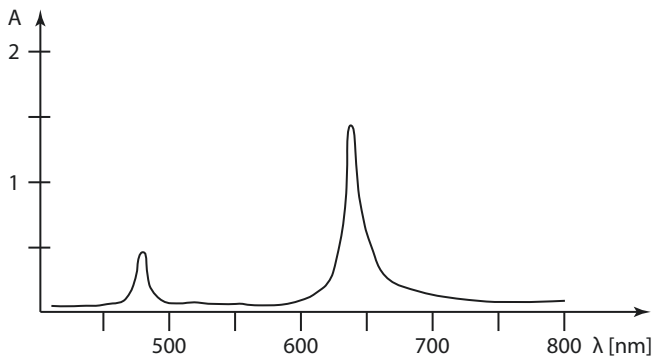
The spectrum shows two absorption bands (or **bands** for short).

Such transmission spectra are not very common in UV/VIS spectroscopy, because the absolute transmission depends on the optical path length, in brief: the *size of the cuvette used.* (However, you will encounter very similar spectra in IR spectroscopy in ► Sect. 19.2.)

It is much more usual to indicate the **absorbance** on the y-axis, which we already know from photometry (from Part II) and which has the great advantage of correlating linearly with the size of the cuvette:

$$A = \lg \frac{I_0}{I} \tag{18.3}$$

The resulting spectra will of course look a little different (the *position of* the bands will not change, though):



Stylised extinction spectrum

The relationship between these two spectra should be recognisable.

Attention

Please note that here not only simply "the spectrum was turned upside down": For absorption spectra *logarithmic values* are plotted on the y-axis, after all (because of Eq. 18.2):

$$A = -\lg T \tag{18.4}$$

Thus, it is no wonder that the absorption bands are not as steep as the bands in the transmission spectrum.

Questions

1. A UV/VIS transmission spectrum shows three bands: For λ_1 = 280 nm, T_1 = 70%; for λ_2 = 440 nm, T_2 = 52%; and for λ_3 = 713 nm, T_3 = 23%. Say something about which A_1–A_3 are to be expected in the corresponding *absorbance* spectrum.
2. Assuming your sample still obeys Lambert-Beer's law even if its concentration were doubled: What absorbance value E_2 would you obtain for a sample of concentration c_2 if a sample of concentration c_1 at a given wavelength λ leads to an absorbance value of E_1 = 0.21 (assuming a measurement at the same wavelength and the assumption $c_2 = 2 \cdot c_1$)?

18.2 What Is the Effect of the Energy Transmitted by the Photon?

From *General Chemistry*, you probably know that some metals can be identified by the flame colouration that results when salts of said metals are introduced into a burner flame. (Important examples are the alkali metals and most of the alkaline earth metals.) You probably also remember where these characteristic colours come from in each case (if not, you may want to look again in Binnewies) but just to be on the safe side, let us briefly review:

The thermal energy of the flame is sufficient to remove a valence electron (possibly even more than one) from its ground state—in which said electron is located in the very **atomic orbital** into which it belongs according to the periodic table of the elements—and to transport it into another, energetically less favourable atomic orbital. This, of course, increases the total energy of the atom under consideration, and when that (currently excited) electron falls returns to its ground state, the energy released in the process is emitted in the form of a photon of electromagnetic radiation. If the associated wavelength lies in the VIS range, it has a characteristic colour for the human eye.

► Example

For example, the characteristic yellow colouration of sodium salts in the burner flame is due to the fact that the valence electron of sodium is initially transferred from the 3 s orbital, its ground state orbital, to the energetically less favourable 3 p orbital by thermal excitation. When the currently excited atom falls back to its ground state, light of wavelength λ = 589 nm is **emitted** (nicely illustrated in Fig. 15.1 of Binnewies).

Binnewies, Section 15.2: Properties of alkali metal compounds

Accordingly, the energy difference between the 3 s orbital and the (triply degenerate) 3 p orbital set can be calculated according to ► Eq. 17.1:

$$E = = \frac{hc}{\lambda} = \left(6.626 \times 10^{-34}\left[\mathrm{J \times S}\right] \times 2.98 \times 10^{8}\left[\mathrm{m \times s^{-1}}\right] / 589 \times 10^{-9}\left[\mathrm{m}\right]\right)$$
$$= 3.35 \times 10^{-19}\left[\mathrm{J}\right]$$

(At the same time, you can use Avogadro's number (N_A = 6.022 × 10^{23} mol^{-1}) to calculate the energy required to excite one mole of sodium atoms accordingly: E = 201.7 kJ/mol). ◄

Important

To allow you to at least place the amounts of energy involved in orders of magnitude: The energy content (in kJ/mol) of visible light lies between:

- long-wavelength red (any weaker is not possible within the VIS range): $\lambda = 790$ nm ⇒ 150 kJ/mol
- shortest wavelength violet (the most energetic photons the VIS range has to offer): $\lambda = 380$ nm ⇒ 315 kJ/mol

Attention

Of course, assuming a much stronger energy input, the valence electron of sodium can also be transferred from its "normal" 3 s orbital into a 3 d orbital (which is of course also unoccupied in the ground state), or into the 4 p orbital, or even into one or the other 5 f orbital, etc. It should be understandable that a return from these "dizzying heights" releases significantly more energy—so much that the corresponding photons can no longer be located in the VIS region of the electromagnetic spectrum. But if one extends the measurement range into the UV region or even beyond, the **spectral lines** belonging to these electron transitions can also be found.

In a similar way spectroscopic investigation of coloured flames deals with the transitions of electrons from their ground state to higher, energetically less favourable orbitals *in individual atoms* (we will return to this type of *atomic spectroscopy in* Part V), UV/VIS spectroscopy deals with electron transitions *within molecules*. Understandably, there is also more than one possibility where an electron then can be transported.)

Theoretically, in order to keep track of the possible electron transitions, one would first have to draw up a complete **molecular orbital diagram** for each molecule, as shown, for example, for formaldehyde ($H_2C{=}O$) in Fig. 17.12 from Harris.

Harris, Section 17.6: Light absorption processes

You will certainly agree that this four-atom molecule is really not overly complicated, and yet there are already *six* molecular orbitals to be considered here that are occupied in the ground state—and, in addition, there are all the molecular orbitals that are *not* occupied in the ground state (a total of four). Figure 17.12 from Harris shows only the most energetically favourable of these.

Some Important Things Regarding Molecular Orbitals

Do not worry, we will not go into detail here about how to draw up molecular orbital diagrams and how to know what approximate shape the resulting molecular orbitals have. But we should nevertheless make a few considerations, so that the whole subject does not remain completely abstract and thus becomes difficult to understand (and learn).

1. In molecular orbital theory, the fundamentals of which you certainly know from *General Chemistry,* the concept of hybridisation and/or aspects derived from it (such as the VSEPR model) are dispensed with completely. Instead, the *wave nature of electrons* is considered at far greater length, and the corresponding three-dimensional standing waves, which we call *orbitals*, are also allowed to *interfere with each other*. In principle, *all orbitals of each individual atom* must be taken into account in a polyatomic molecule.
2. But only in principle.
   - For the sake of simplicity, we restrict ourselves to the *valence orbitals* of each atom: MO theory assumes, as a first approximation, that any core electrons are not involved in the interactions responsible for the bonding between two (or more) atoms.

- Nevertheless, the number of orbitals to be considered can grow quite rapidly, because for each atom *all* orbitals of the valence shell have to be taken into account—even those that were already doubly populated/occupied beforehand or are **vacant** (i.e. unoccupied) in the ground state.

▶ Example

Let us take as a first example the molecule borane (BH_3), which admittedly is not stable in the free state, but still:

- Each of the three hydrogen atoms has the valence electron configuration $1s^1$, i.e. contributes an s-orbital, each of which is occupied by *one* electron.
- The boron atom has the valence electron configuration $2s^2 2p^1$, which means that it contributes:
  - the 2s-orbital, which is occupied by *two* electrons,
  - a 2p-orbital, in which (in the ground state) *one* electron resides, and
  - two 2p-orbitals, which are *vacant* (again: in the ground state).

Thus, we are dealing with seven atomic orbitals (a total of three from the H atoms, four from the B), which are occupied by a total of six valence electrons.

In comparison, let us look at the molecule water (H_2O):

- Here, too, each of the two hydrogen atoms has the valence electron configuration $1s^1$, i.e. contributes an s orbital, each occupied by *one* electron.
- The oxygen has the valence electron configuration $2s^2 2p^4$, which means it contributes:
  - the 2s-orbital, which is occupied by two electrons (i.e. contains an electron pair),
  - two 2p-orbitals, in each of which there is *one* electron, and
  - one 2p-orbital, which again contains an *electron pair*.

Thus, there are a total of six atomic orbitals in which there are a total of eight valence electrons.

In both cases, the task now is to combine these atomic orbitals into **molecular orbitals** and then to occupy the resulting molecular orbitals, according to their respective relative energy content, with the available electrons.

3. The combination of the involved atomic orbitals follows the basic rules of ***LCAO theory***. Again, it is beyond the scope of this paper to discuss this in detail, although the basic concept is quite simple. Two things in particular are crucial:
   (a) If two or more atomic orbitals interact, resulting in a (logically polyatomic) molecule, there are initially two possibilities as to the nature of this interaction:
   - If the interaction of the atomic orbitals leads to *constructive* interference, a **bonding molecular orbital** results, i.e. an MO that contributes to the stability of the resulting molecule and causes shortened bond lengths/distances. (A prerequisite here is, of course, that this molecular orbital, when the MO diagram is finally fully constructed, is also occupied by at least one electron. *Vacant orbitals in themselves have* no *influence on molecular stability*.)
   - Destructive interference results in an **antibonding** molecular orbital that, when occupied, destabilises the molecule and provides increased bond distances/bond lengths.
   - *In such cases, the* bonding *interaction is always energetically more favourable than the* antibonding *interaction.*

- The linear combination of two s-orbitals to form a bonding orbital (which is energetically *more* favourable than the atomic orbitals from which it emerged) and an antibonding molecular orbital (which is energetically *less* favourable) is shown graphically in Fig. 5.36 of Binnewies.

Binnewies, Section 5.11: Introduction to molecular orbital theory (MO theory)

(b) The number of molecular orbitals obtained by linear combination is *always and inevitably* equal to the number of atomic orbitals to be considered (see above). ◄

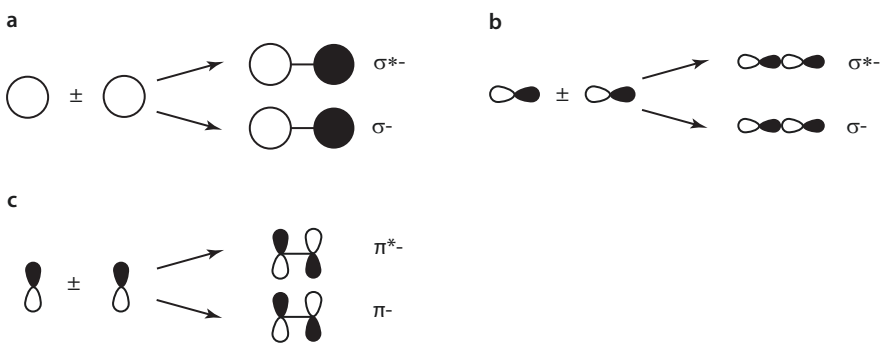
► Example

For the borane molecule (BH_3) (which, just to point that out again, is *not* stable in the free state, but still a very good example for the MO theory ...), the MO diagram must accordingly contain seven molecular orbitals, while the MO diagram of water (H_2O) gets by with six molecular orbitals.

Please do not be surprised that the corresponding MO diagrams are neither given here nor can be found in Harris or Binnewies, either. In order to draw up such diagrams for molecules consisting of more than two atoms, extensive symmetry considerations are also required for all resulting molecular orbitals, and this would go far beyond the scope of this introduction. On the other hand, we will not be able to make do without statements about molecular symmetry at the latest when we want to turn to vibrational spectroscopy, so it could at least do no harm to sooner or later have a look at the corresponding chapter in Binnewies. But the basics of this topic have certainly already been dealt with in *General* and in *Inorganic Chemistry*. ◄

Binnewies, Section 5.12: Molecular symmetry

4. The linear combination of two s-orbitals leads to one bonding σ-orbital and one antibonding σ*-orbital each (◘ Fig. 18.1a), the same applies to two p-orbitals oriented "head to head" to each other (i.e. along the connection axis; ◘ Fig. 18.1b). The linear combination of two p-orbitals oriented *parallel to each other*, on the other hand, leads to one bonding π-orbital and one antibonding π*-orbital each (◘ Fig. 18.1c).
5. There are of course more possible interactions to consider:
   - Please do not forget that—assuming correct spatial orientation—a bonding σ- and/or an antibonding σ*-interaction is also possible between an s- and a p-orbital (◘ Fig. 18.2a).
   - Depending on the spatial orientation, there is the possibility that an (occupied or vacant) orbital can interact with other orbitals neither in a bonding nor in an antibonding way, i.e. neither contributes to stability nor has a destabilising effect: Such **non-bonding interactions** (**n,** ◘ Fig. 18.2b; the two shown here are of course energetically degenerate) must also be considered occasionally. Occupied orbitals can be involved here as well as vacant ones. The orbitals responsible for the non-bonding interactions—in short: the n-orbitals—can usually be regarded as localised at *one* atom, i.e. as a ***lone pair.***



◘ **Fig. 18.1** Bonding and antibonding interactions

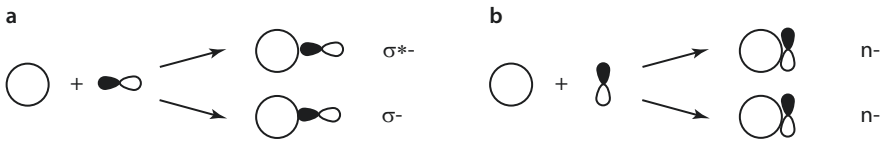


Fig. 18.2 Further interactions

6. Not all of the resulting molecular orbitals need necessarily be occupied: The very fact that preferentially the more energetically favourable orbitals are occupied (which commonly belong to bonding rather than antibonding interactions) is the reason for the stability or even the existence of molecules. Compare, for example, the MO diagram of the H_2 molecule, in which only the bonding σ-orbital is occupied but not the antibonding σ*-orbital (Fig. 5.39 from Binnewies), with the corresponding diagram for the non-existent dihelium molecule (He_2; Binnewies—Fig. 5.41).

Binnewies, Section 5.11: Introduction to molecular orbital theory (MO theory)

7. Since not only a large part of chemistry, in general, can be traced back to the interaction of the **frontier orbitals** of participating molecules (comparatively *high-energy occupied* orbitals transfer the charge density of their electrons to comparatively *low-energy unoccupied* orbitals of the reaction partner), but also UV/VIS spectroscopy is based on the transfer of electrons from *high-energy occupied* orbitals to comparatively *low-energy unoccupied* orbitals (in this case, however, of the same molecule), it makes sense to give the relevant frontier orbitals catchy names:
   - The **HOMO** *(highest occupied molecular orbital)* of a molecule (or molecular ion) is the energetically *least favourable molecular orbital still occupied*. A HOMO can be single or double occupied; **degeneracy** is also possible, so that there can be two or even more HOMOs with the same energy content.
     - If a HOMO is occupied only by a *single* electron and not by a pair of electrons, it is sometimes called a **SOMO** *(singly occupied molecular orbital)*.
   - The energetically *next higher* molecular orbital is then called **LUMO** *(lowest unoccupied molecular orbital)*; this LUMO can also be degenerate.
     - That the LUMO (degenerate or not) of a molecule must always be energetically less favourable than its HOMO hopefully goes without saying.
     - That there can be no "SUMO" should also be understandable.
   - Occasionally it is necessary to also consider the next-lowest molecular orbital in energy (energetically below the HOMO). For the sake of simplicity, this is called HOMO-1. (Of course, degeneracy is also possible here if necessary.)
   - The molecular orbital above the LUMO, which of course is also unoccupied in the ground state, is then called LUMO+1. You get the gist.

► Example

The "ordinary" dioxygen (O_2) is an example of a simple diatomic molecule whose HOMO is not only doubly degenerate but whose HOMOs also represent SOMOs because the two orbitals of the doubly degenerate HOMO set are single occupied spin-parallelly according to **Hund's rule**; the corresponding MO diagram can be found in Fig. 5.45 of Binnewies.

Binnewies, Section 5.11: Introduction to molecular orbital theory (MO theory)

There you will also see that the HOMO set is the (antibonding) π*-orbitals. The LUMO of this molecule, thus, represents the σ*-orbital. ◄

UV/VIS spectroscopy (including spectrophotometry) is based on the fact that electrons can be excited from the HOMO (or another molecular orbital occupied in the ground state) into the LUMO (or another, energetically even less favourable molecular orbital, i.e. located above the HOMO): If the wavelength required for such transitions is known, the energy difference of the two orbitals involved (i.e. the **frontier orbital distance**) can be calculated according to ► Eq. 17.1.

Glancing at Inorganic Chemistry

Such frontier orbital observations are by no means restricted to spectroscopy but are also used in other areas of chemistry. You already know complex compounds from *General* and/or *Inorganic Chemistry,* so you probably also know that many complexes show a (often characteristic) colour in aqueous solution. As an example, consider trivalent titanium (Ti^{3+}), which is present in aqueous solution as an octahedral hexaaqua complex cation $[Ti(H_2O)_6]^{3+}$:

The octahedral coordination of the six aqua ligands splits the d-orbital set of the metal, so that three of the five d-orbitals (d_{xy}, d_{xz}, d_{yz}—the t_{2g}-orbital set; you will certainly come across this name in advanced courses or textbooks of inorganic chemistry; let us just take it as a proper name for now) become somewhat more favourable energetically, while the energy content of the other two d-orbitals ($d_{x^2-y^2}$, d_{z^2}, i.e. the e_g set; this is also just a name for now, so that we do not have to explicitly name all the d-orbitals involved each time) increases a bit. However, since the trivalent titanium has the electron configuration d^1, only one of the three more favourable orbitals is singly occupied, the other two, as well as the two energetically less favourable ones, remain vacant.

Accordingly, we can say:

- The t_{2g} orbital set corresponds to the (triply degenerate) HOMO of this complex,
- the e_g orbital set corresponds to the (doubly degenerate) LUMO.

Analogous to the electron transitions described in this section, this complex can also be excited accordingly to a HOMO→LUMO transition—and this is exactly the reason why titanium(III) ions turn their aqueous solution red-violet. Kindly think back again to the colour wheel from Part II:

- The complementary colour to red-violet is blue-green.
- Table 17.1 from Harris tells us that blue-green light lies in the 470–500 nm wavelength range.

To calculate the energy difference between the HOMO and the LUMO of this complex—i.e. the energy needed to transport an electron from the HOMO into the LUMO—we simply refer to ► Eq. 17.1 and insert these two values (470 nm, 500 nm). (Please calculate it yourself!)

Using other experiments, the frontier orbital distance was determined, and the result $\Delta E_{HOMO/LUMO}$ = 243 kJ/mol was obtained. You will notice that this value fits the wavelength λ = 490 nm.

Harris, Section 17.2: Light absorption Harris, Section 17.2: Light absorption

However, it should be emphasised again: The electron transitions observable with UV/VIS are not necessarily limited to the transition HOMO→LUMO. Electrons that populate the HOMO-1 in the ground state can also change to the LUMO, and with sufficiently strong excitation, transitions from the HOMO (or the HOMO-1) to the LUMO+1 are also possible, etc.

- It should be understandable that such larger energy jumps also require photons of a shorter-wavelength.
- Accordingly, transitions with *even* larger energy differences (e.g. from HOMO-2 to LUMO+3 or similar) can no longer be excited by UV/VIS radiation; *even higher-energy* radiation would then be required.

For any molecule in whose MO diagram σ- and π-interactions are to be considered, the following points apply:

- Because of the better overlap of the atomic orbitals from which they stem, σ-orbitals are commonly more energetically favourable than π-orbitals simply because more binding energy is released.
- Similarly, π*-orbitals are energetically more favourable than σ*-orbitals because the suboptimal overlap of the associated atomic orbitals has a correspondingly less antibonding/repelling effect.
- If non-bonding orbitals occur, mostly in the form of free electron pairs that do not interact with neighboring atoms (for spatial or other reasons), they are usually (!) energetically located between the π-orbitals and the π*-orbitals.

The relative position of the individual molecular orbital species is shown schematically in ◘ Fig. 18.3.

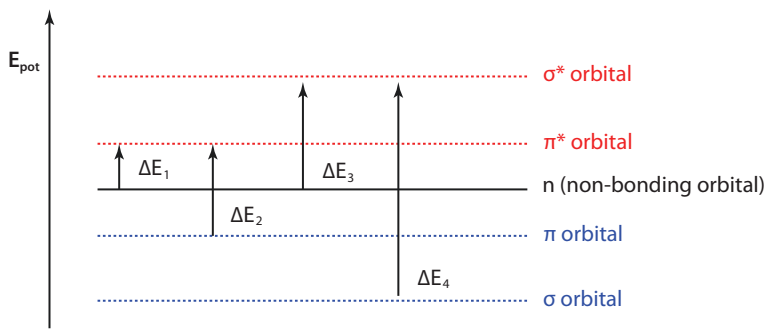
Attention

Please note that ◘ Fig. 18.3 is really only a *schematic* representation! Even though it may seem so here for the sake of clarity, the individual energy levels are *by no means* **equidistant.**

Furthermore, please consider that there is by no means always exactly *only one* π- or σ*-orbital. ◘ Figure 18.3 is only meant to show the relative energetic position of different *energy levels:*

- A sufficiently extended π-electron system (more precisely, *any* system with two or more conjugated multiple bonds) has *multiple* π- and π*-molecular orbitals that differ (at least slightly) in their energy content.
- The different π-molecular orbitals (with slightly different energy content) are often grouped together to form the π-level, and the π*-molecular orbitals are correspondingly grouped together to form the π*-level.
- (If you spontaneously thought of the electronic band structure used to describe the metallic bond, you have again recognised a connection because in the end the electronic bands are nothing else than the extension of the molecular orbital theory to metals.)

Of course, the energy difference between different orbitals of the *same* energy level (say two—non-degenerate—π*-molecule orbitals) is nowhere nearly as large as the energy difference between orbitals of *different* energy levels (say π* and σ*).



◘ **Fig. 18.3** Energy transitions in UV/VIS spectroscopy

The various transitions are then denoted, for example, as an $n \rightarrow \pi^*$ transition, when an electron changes from an occupied nonbonding orbital to a (vacant) π^* orbital, and so on.

But UV/VIS spectroscopy is not limited to moving electrons from one (more energetically favourable) orbital to another (less energetically favourable) orbital:

■ Spin Also Plays a Role

From an energetic point of view, it makes a difference whether the electron, when excited from the ground state into a higher orbital, in the process changes its spin or not, since this has a direct effect on the **multiplicity state.**

■■ The Multiplicity ...

You will also encounter this term again in the topic of nuclear magnetic resonance spectroscopy (for example in "Analytical Chemistry II"), where the term will be used in a *somewhat* broader sense. However, since UV/VIS spectroscopy only deals with electrons, we will limit ourselves here to the **electron spin**.

As you know from *General Chemistry*, with respect to spin there are, fundamentally, only two possibilities for each electron within an orbital, and these possibilities differ slightly in their energy content:

- Energetically somewhat more favourable is the spin state described with the spin quantum number $m_s = +½$ and—especially in chemistry—is often abbreviated as (↑).
- Correspondingly, the spin state with spin quantum number $m_s = -½$, or (↓) for short, is a little less favourable.
- There are *no other options*.

The multiplicity becomes noticeable only within a magnetic field—but since in everyday life we are surrounded by the magnetic field of the earth as well as by a high-frequency *electro*magnetic field (we call it "light"), *all* atoms we want to observe are *always* in a magnetic field (unless we would specifically shield the atoms under consideration from it, and that would be ... costly). So, firstly, we have to look at how many different ways there are theoretically for the electrons to align themselves within the magnetic field. This is exactly what **multiplicity** describes.

■■ 1-Electron Systems

If we have a 1-electron system (for example, a hydrogen atom with the electron configuration $1s^1$, this electron can assume the (energetically more favourable) spin state $m_s = +½$ (along the magnetic field, which can be described by (↑) or something like that) or the energetically somewhat less favourable state $m_s = -½$ (visualised by (↓), the electron is aligned opposite to the magnetic field). There are exactly *two* possibilities which are open to the single unpaired electron, thus a doublet (abbreviated: **D**) is present here as multiplicity, which just states the number of possible alignments. (For this, the usual Greek numerical words are used.)

■■ Multi-electron Systems

In the case of multi-electron systems, there should theoretically be considerably more possibilities but it is also important whether some or all of the electrons in question are *spin-paired:*

- If two electrons occupy the same orbital, according to the **Pauli exclusion** they *must* differ in their spin quantum number, which can be symbolised by (↑↓). So there are *no* different possibilities here because even if one electron "wanted" to change its spin (for whatever reason), its partner electron would

have to do the same instantly because of the Pauli exclusion principle, so overall *nothing* would change. If there is only one possibility for the electrons to orientate themselves in the magnetic field, this is called a **singlet (S).**

The situation is different if the two unpaired electrons occupy *different* orbitals, each of which is then to be represented by a bracket. Here, several possibilities arise:

- The two electrons can easily occupy their respective orbitals in a spin-parallel fashion—in the spirit of **Hund's rule:** (↑)(↑)
- One of the two electrons can reverse its spin (which is energetically a little less favourable than the previous case, but it is certainly *possible*). In this case, therefore, if one electron is oriented parallel to the magnetic field and the other antiparallel to it, the orbital occupation is (↑)(↓). Which of the two electrons ("left" or "right") performs the spin reversal is irrelevant for our purposes, (↑)(↓) and (↓)(↑) are therefore equivalent.
- Energetically even more unfavourable but still possible, would also be the orientation of *both* electrons antiparallel to the magnetic field: (↓)(↓)

Since, for this system, with two unpaired electrons there are *three* possibilities for the electrons to orient themselves in the magnetic field, it is called a **triplet (T).**

If, in addition to the unpaired electrons, one or the other electron pair (↑↓) is also present, this does not change the multiplicity of the system at all, since a change is not possible with electron pairs. (Remember the Pauli exclusion principle!) So we see that *only the unpaired electrons* are relevant here. If the number of unpaired electrons continues to increase, the number of possible orientations increases accordingly. This leads to a simple rule for calculating the multiplicity M for systems with one or more unpaired electrons:

$$M = \left(\text{Number of unpaired electrons}\right) + 1 \tag{18.5}$$

- For a system with three unpaired electrons, the corresponding result is M = 3 + 1 = 4, i.e. a *quartet.*
- With four unpaired electrons, M = 5, so we are dealing with a *quintet.*
- If there are five unpaired electrons (M = 6), there is a *sextet,* etc.

To Calculate the Multiplicity

Formula Eq. 18.5 is applicable to all systems in which the individual participants have a spin of ±½. For others, it becomes a little more complicated, but we will confine ourselves here to electrons. We will deal with systems in which the spin quantum number m_s can take other values in Part I of "Analytical Chemistry II". You will recognise the designation for the resulting multiplets there, as well.

So far, we have only turned to the question which *possible* orientations of the electrons there are, and we have found that these different possibilities certainly differ in their energy content. Accordingly, it must also be possible to describe these different states as unambiguously as possible.

■■ ... and What It Means

In order to be able to "use" the multiplicity, one must find out which **total spin** $\mathbf{S_{total}}$ results for the system in each case. To do this, you have to take a closer look at *all* the electrons: One determines how much each electron contributes

to the total spin of the system. To do this, one simply adds up their respective spin quantum numbers m_s. The formula for calculating the total spin is:

$$S_{total} = \left| \sum_{i=1}^{i} m_{s(i)} \right| \tag{18.6}$$

The total spin is therefore simply the sum of the m_s-values of all electrons to be considered.

In many textbooks, the formula symbol for the total spin is simply the S—and this can of course be magnificently confused with the symbol for the singlet, for which reason we will consistently stick to S_{total} in the context of this book. (But I wanted to point out this sneaky pitfall to you in any case.)

Equation 18.6 looks worse than it is: The **modulus** (the absolut value) must be determined after the individual spin quantum numbers of the electrons under consideration have been added together:

- For all *paired* electrons-represented by (↑↓), after all, the parenthesis says that the two electrons are in the same orbital-there is a contribution to the total spin of 0, because +½ + (−½) = 0.
- For all *unpaired* electrons, their respective spin is decisive:
  - Each (↑)-electron has a spin of +½.
  - Each (↓)-electron has a spin of −½.

▶ Example

As an example, consider the triplet of electron orientation of our system with two electrons populating different orbitals:

- For the most energetically favourable case described by (↑)(↑), we get S_{total} = +½ + ½ = 1
- For the case (↑)(↓) or (↓)(↑), we get +½ + (−½) or −½ + ½ = 0.
- For the energetically most unfavourable case, (↓)(↓), we initially arrive at −½ + (−½) = −1 but since in the calculation of the total spin according to Eq. 18.6 the *modulus* is considered at the end, here again we get S_{total} = 1

We already described such a system with two unpaired electrons, a **diradical,** even in this section: the "ordinary" dioxygen (O_2), whose MO diagram you will find as Fig. 5.45 in Binnewies. ◀

Binnewies, Section 5.11: Introduction to molecular orbital theory (MO theory)

However, it is rather unusual to describe the behaviour of corresponding systems in the magnetic field via the total spin (although this can also be found in the technical literature). Much more common is the specification in terms of the **multiplet state (M_Z)**. The corresponding formula is:

$$M_Z = 2S_{total} + 1. \tag{18.7}$$

The resulting multiplet states are then labeled with the same number prefixes that we already know from the multiplet itself.

▶ Example

Let us return once again to the three states of our two-electron system with M = 3 (and thus to the HOMO of the dioxygen diradical):

- At (↑)(↑), with S_{total} = 1, a *triplet* state is obtained according to Eq. 18.7 with $M_Z = 2 \times 1 + 1 = 3$.
- For the case (↑)(↓) or (↓)(↑), S_{total} = 0 and hence M_Z = 1 yields a *singlet* state.
- For the least energetically favourable orientation (↓)(↓), Eq. 18.7 leads again to a triplet state—but it is an *excited* one because of the modulus used in the for-

mula for calculating S_{total} (Eq. 18.6), and thus $M_Z = 3$ again. (If one adds the information of this state being *excited*, not the ground state, the statement becomes at least clearer.)

For a one-electron system, on the other hand—such as a single hydrogen atom with valence electron configuration $1s^1$, which obviously has an unpaired electron—a *doublet* results according to 18.5, so there are two possibilities for orientation: the electron can align itself (somewhat more energetically favourable) along the magnetic field (↑), or exactly in the opposite direction (↓), which is slightly less energetically favourable.

If we now determine the multiplicity state M_Z for these two possibilities, we get

- for (↑) on $M_Z = (2 \times S_{total}) + 1 = \left(2 \times \left(\left|+\frac{1}{2}\right|\right)\right) + 1 = 1 + 1 = 2$

and

- for (↓) to $M_Z = \left(2 \times \left|-\frac{1}{2}\right|\right) + 1 = 1 + 1 = 2$

With $M_Z = 2$, therefore, a *doublet* state is present in both cases. If one describes the second one again as the "excited doublet state", there is no longer any danger of confusion. ◀

Attention

Again, there is a popular pitfall: Many teaching texts unfortunately do not distinguish (or do not distinguish clearly enough) between **multiplicity M** and **multiplet state MZ**, even though they are two different things:

- The multiplicity M indicates the number of *possible* different orientations of the electrons in the magnetic field. It is calculated according to Eq. 18.5.
- The multiplet state M_Z (also called **multiplicity state**) refers to *each of* the possible states according to multiplicity and depends on the total spin *actually present in* each case, which is given by the spin quantum numbers of all electrons considered according to Eq. 18.6. M_z is then calculated according to Eq. 18.7.

However, if a multiplet state is abbreviated with M just like multiplicity, which unfortunately happens in numerous textbooks, the confusion is naturally preprogrammed.

But the confusion can even be maximised: In many texts, instead of the multiplet state of multi-electron systems, the **spin state** of the system under consideration is referred to, and this is then abbreviated as ***S*** instead of M or M_Z explained above. However, since S stands for a multiplicity of 1 (i.e. a *singlet*) or for the *singlet state* (when it comes to the multiplicity state), depending on the context, it is also easy to get confused. The fact that in such texts the formula sign of the spin state is an *S set in italics is* usually not enough to make things clear. In the context of this book, we stick to the distinction M and M_Z for the sake of clarity, but since the formula sign *S* is also used in some texts, you should at least have seen it before and be able to classify it.

▪ Excitation of Electrons, a Closer Look

Before something goes fundamentally wrong here:

Please keep in mind that the two electrons in the HOMO of the dioxygen molecule occupy *two separate* orbitals (of the same energy content)! (This is what the respective brackets around the arrows are supposed to symbolise.)

By no means this is supposed to mean a single orbital in which both electrons have the same spin. That would be a blatant violation of the Pauli exclusion principle (and thus would be absolutely impossible according to the entire current understanding of physics).

Suppose we were dealing with a system whose HOMO belongs to a free electron pair and in which there are also π-interactions (and hence likewise π*-interactions). Here, an electron transition from the HOMO to the LUMO, i.e. an n → π* transition, which is quite in agreement with Fig. 17.13 from Harris, offers two possibilities:

Harris, Section 17.6: Light absorption processes

(a) The slightly less energetic of the two electrons (↓), which together represented the free electron pair prior to excitation (↑↓), completes the n → π* transition while retaining its original spin (−½) (shown in Fig. 17.13a; stylised here as $_{(\uparrow)}^{(\downarrow)}$) to show that here the two orbitals involved—hence the brackets—do *not* have the same energy content, i.e. are *not degenerate*.

(b) An alternative would be for the same electron to make exactly the same n → π* transition, but *change* its spin in the process (as in Fig. 17.13b; i.e. $_{(\uparrow)}^{(\uparrow)}$). This state is slightly more favourable energetically, which should be quite understandable; after all, (↑) *is* slightly more favourable energetically than (↓). And, in general, "Mother Nature" is always interested in with saving as much energy as possible.

So how do the two states differ? Do they differ at all?—Yes, they do! Let us look at the resulting *total spin* S_{total} *of* this system:

(a) In the case of $_{(\uparrow)}^{(\downarrow)}$, there are two unpaired electrons but they are oriented spinantiparallel. The overall result is $S_{total(a)} = 0$. Thus, according to Eq. 18.7, $M_{Z(a)} = 1$, so this is a **singlet state**, and yet it is a diradical.

(b) $_{(\uparrow)}^{(\uparrow)}$ is also a diradical, but since $S_{total(b)} = 1$, it gives a multiplicity of $M_{Z(b)} = 3$, so it is a **triplet state**.

■■ And Which of These Transitions Is Taking Place Now?

The fact that **molecular symmetry** also plays an important role in analytical chemistry has already been mentioned at the beginning of this section. Since this topic is usually already dealt with in the context of *General* and/or *Inorganic Chemistry*, you will certainly be able to follow me when I remark, by way of example, that for a (radially symmetric) s-orbital it makes no difference whatsoever whether it has been rotated by 180° or not, whereas a rotation by the same angle for any (dumbbell-shaped) p-orbital would lead exactly to a *phase reversal*—which, of course, has quite a drastic effect on any interactions with other orbitals. Against this background, you can certainly at least imagine why some transitions are called **symmetry-forbidden** or **symmetry-allowed:**

Harris, Section 17.6: Light absorption processes

We already find an example of a symmetry-forbidden transition in the n → π* transition that led to a *triplet* state (Fig. 17.13b from Harris): a change of spin quantum number (from −½ to +½) is not always allowed, but this also depends on what symmetry properties the source and target orbitals have. As already mentioned, symmetry is really a science in itself. But one thing should be noted: *Such a symmetry prohibition does not mean that such prohibited transitions do* not *occur* at all *but only much less frequently.* (Just like humans, not all atoms or molecules necessarily adhere to every prohibition—most do, but there are always exceptions.)

? Questions

3. (a) How many molecular orbitals are to be considered for the Li_2 molecule (which actually occurs in the gas phase, i.e. when elemental lithium evaporates)?
   (b) How many molecular orbitals are to be considered for the HCl molecule?

4. (a) What is the multiplicity of a single fluorine atom? What is the multiplicity of a single nitrogen atom in the ground state?
   (b) Which multiplet states are possible in each case?
5. (a) What is the term used to describe the transition in a molecule, each with fully occupied bonding σ- and π-interactions and a non-bonding electron pair, when an electron changes from a π-orbital (the HOMO-1) to the LUMO? What is the transition from the HOMO to the LUMO+1 called?
   (b) Which multiplicity states would result in each case if the excited electron were to perform a spin reversal in the two transitions from 5a? Which multiplicity results if the original spin state were kept?
6. What multiplicity states are possible for the O_2 molecule? How do they differ in their relative energy content?

18.3 Who Does What?

It was already hinted at in Part II (on the subject of colour) that certain structural elements or functional groups can be excited particularly easily by radiation from the UV/VIS spectrum. Let us look at what is particularly worth mentioning there (please keep ◘ Fig. 18.3 in mind):

▪▪ Only σ-Bonds Present

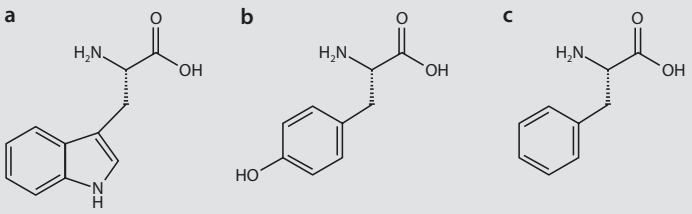
If there are only σ-bonds in a molecule, the HOMO also represents a σ-orbital. The distance between the HOMO and the LUMO (which will then be a σ*-orbital) is correspondingly large, so a considerable amount of energy is required to bring about the associated $\sigma \rightarrow \sigma^*$ transition. This commonly requires photons with a wavelength $\lambda < 150$ nm, and this is still below the common UV radiation.

▪▪ π-Bonds Also Present

If the analyte additionally has π-electrons (and thus π and π* orbitals, where the latter need not be (fully) occupied), $\pi \rightarrow \pi^*$ transitions are also possible. From the schematic representation of the relative energy content of different orbital levels alone (◘ Fig. 18.3), it should be obvious that the energy difference between π and π* orbitals will not be as large as that between σ and σ*. Accordingly, the $\pi \rightarrow \pi^*$ transition requires recognisably less excitation energy, and can thus be brought about by photons of a longer wavelength: Very generally speaking, wavelengths of $\lambda = 200–700$ nm are to be mentioned here. (Admittedly, this is a rather wide window but we will see in a moment that a whole series of other factors must be taken into account.) Such statements are not limited to C=C double bonds: C=O or C=N multiple bonds also ensure that $\pi \rightarrow \pi^*$ transitions exist. (We will come back to the C=O bond in a moment.)

Lab Tip

Since they can be excited comparatively easily, aromatic compounds can be detected very well via UV/VIS spectroscopy and, using a corresponding calibration curve, can also be quantified. In protein analysis, for example, one often looks for the three aromatic amino acids tryptophan (a), tyrosine (b) and phenylalanine (c), whose π-electron systems absorb at $\lambda_{(Trp)} = 281$ nm (a), $\lambda_{(Tyr)} = 276$ nm (b) and $\lambda_{(Phe)} = 258$ nm (c), respectively.



The aromatic amino acids: (**a**) Tryptophan, (**b**) Tyrosine, (**c**) Phenylalanine

On the other hand, this also means that aromatic solvents (such as benzene (C_6H_6), toluene (C_6H_5–CH_3), etc.) are of extremely limited suitability for use in UV spectroscopy because they too have an absorption maximum in the range of $\lambda_{(Ar\text{-}R)}$ = 250–280 nm. (It should be understandable that the nature of any substituents -R on the aromatic ring affects the position of the absorption maximum.)

Attention

Even though it should be common knowledge, perhaps it will not hurt to make this point again:

- *Every* molecule of whatever kind has σ-electrons (i.e. σ- and σ*-orbitals) in *any case* because there is *always* at least one σ-bond in every molecule.
- π-electrons (i.e. π- and π*-orbitals) *will be* added, whenever multiple bonds are present, since—you will know this from *General Chemistry*—every multiple bond consists of *one* σ-part, to which is then added *one* π-part in the case of a double bond, or *two* π-partis in the case of a triple bond.

And there is something else to consider: According to current knowledge, two (or even more) σ-bonds between the same two atoms is just as impossible as the occurrence of a π-bond between two atoms that are *not* also connected to one another via a σ-bond.

▪▪ With Free Electron Pairs

If the analyte has one or more free electron pairs, this offers the possibility of further transitions. Two possibilities are to be distinguished here:

1. The system to which said free electron pair belongs otherwise has *only* σ-electrons. In this case, in addition to the above-mentioned σ → σ* transitions originating from the σ-electron framework, energetically more favourable n → σ* transitions are also possible.

   Free electron pairs (in short: n-electrons) are (naturally) found on oxygen and nitrogen atoms (as well as their higher homologues), but also in halogens. n → σ* transitions still require quite a lot of energy (although already much less than σ → σ* transitions): systems that have a free electron pair (or even several) absorb in the range of 150–250 nm, although absorptions with λ > 200 nm are rather rare. Typical representatives for the n → σ* transition are the substance classes of alcohols (R-OH) and thiols (R-SH), amines (R-NH_2, R-NHR′ and R-NR′R″) and (saturated!) halogenated hydrocarbons.

Lab Tip

Alcohols such as methanol (CH_3OH) and its homologues or also water (H_2O) are *quite* suitable as solvents for UV/VIS spectroscopy because in this energy range both can only be excited to n → σ* transitions, and this requires comparatively short-wave UV radiation, i.e. their **cut-off wavelength** (which you already know from Part III) is quite low:

- $\lambda_{CutOff}(CH_3OH) = 185$ nm
- $\lambda_{CutOff}(H_2O) = 166$ nm

2. The analyte has, in addition to the free electron pair(s) and in addition to the σ-electron skeleton, one or more *multiple bonds,* i.e. in addition to σ- and n-electrons, it also has one or more π- and π*-levels. This way, understandably, n → π* transitions also become possible, and they are particularly easy to effect energetically. (Feel free to compare this again with the schematic representation from ◘ Fig. 18.3.) This is exactly why systems with (at least) one free electron pair and (at least) one double bond can be easily excited in the UV range (as you have already learned in Part II).

   Particularly noteworthy here is the *carbonyl group* (the C=O double bond): The oxygen provides the free electron pair, the C=O double bond provides π- and π*-MOs. Here, wavelengths λ > 200 nm are sufficient for excitation.

   If a heteroatom with a free electron pair is added to the carbonyl carbon in the α-position, as is the case with the peptide bond of proteins (–C(=O)–NH–), the absorption maximum shifts even further in the direction of less energetic photons: For this n → π* transition, λ_{max} (proteins) = 220 nm. Therefore, if the aim of analysis is to determine the occurrence of proteins in a solution, a UV/VIS investigation is recommended, in which the analyte is looked for specifically in this wavelength range.

Lab Tip

However, this also means that solvents with a carbonyl group, such as acetone, $(CH_3)_2$ C=O, *only to a certain degree* are suitable for UV/VIS spectroscopy—especially if one really wants reach the higher-energy-UV range. Radiation from the VIS range, on the other hand, is not energetic enough to cause excitation here—as can be seen from the fact that acetone appears colourless to the human eye. In *spectrophotometry*, acetone therefore can be used safely as a solvent.

▪ Some Technical Terms

A functional group that ensures that the electrons of a polyatomic system can be excited more easily (i.e. already by longer-wavelength photons: early UV or even VIS) is called a **chromophore** (from Greek *chromos* = colour, *phorein* = to carry, i.e. colour carrier). These chromophores shift the UV/VIS absorption maximum (λ_{max}) of a compound in the direction of longer wavelengths and, as soon as λ_{max} lies in the VIS range, ensure one or the other colour.

- The most important representative of the chromophores is probably the carbonyl group already discussed (◘ Fig. 18.4a), but other multiple bonds (which accordingly allow π → π* transitions) can also be regarded as chromophores—especially if at least one heteroatom with (at least) one free

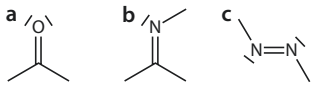


Fig. 18.4 Selected chromophores

electron pair is involved in this multiple bond, which accordingly (see above) also allows the energetically favourable n → π* transitions: Then λ_{max} shifts even further towards the VIS region. For this reason, the imino group (Fig. 18.4b) and the azo bridge (Fig. 18.4c), for example, also represent good chromophores.

A chromophore *enables* the colourfulness of a compound, but this does not mean that every compound that has such a functional group inevitably has an absorption maximum in the range of visible light. As already mentioned: Despite the carbonyl group, acetone appears colourless to the human eye.

Functional groups that cannot be considered chromophores themselves but which are responsible for a shift of the absorption maximum *in interaction with* a chromophore, are called **auxochromes.**

- If an auxochrome shifts λ_{max} towards lower-energy radiation, this is called a **bathochromic effect.**
- The opposite of this, i.e. the shift to *reduced* wavelengths also exists, this is a **hypsochromic effect.**

A bathochromic effect is possessed, for example, by heteroatoms that have at least one free electron pair. Let us compare benzene (C_6H_6) with aniline (C_6H_5–NH_2) as an example: λ_{max}(benzene) = 254 nm, λ_{max}(aniline) = 280 nm; the latter is already coloured (brownish).

Conjugated double or triple bonds also represent auxochromes—which should not be surprising, since with each double bond comes one new π- and one new π*-molecular orbital (of which only the bonding one is occupied); correspondingly there are further energetic possibilities for both the π- and π*-levels.

This Time It's True: A Lot Helps a Lot

The more extended a conjugated π-electron system is (i.e.: the more atoms are involved in it), the more π- and π*-molecular orbitals there are, and the smaller the energy distance between the individual molecular orbitals becomes. For a system that does not have additional non-bonding electrons, this means that the distance between the most energetically unfavourable π-MO (the HOMO) and the most energetically favourable π*-MO (the LUMO) becomes smaller and smaller as the number of conjugated multiple bonds increases—and thus λ_{max} shifts further and further to longer wavelengths ... to the point where colouration occurs *without* a chromophore being present. Let us look at three examples:

- In β-carotene (Fig. 18.5a), 11 double bonds are conjugated with each other, but we will not find a "proper" chromophore. Strictly speaking, this dye, which is largely responsible for the characteristic colour of carrots, is merely a polyunsaturated hydrocarbon without any functional group.

The 11 double bonds are—consider this a brief review of the atomic orbitals from which this π-electron system emerged—the consequence of the interaction of a total of 22 p-orbitals.

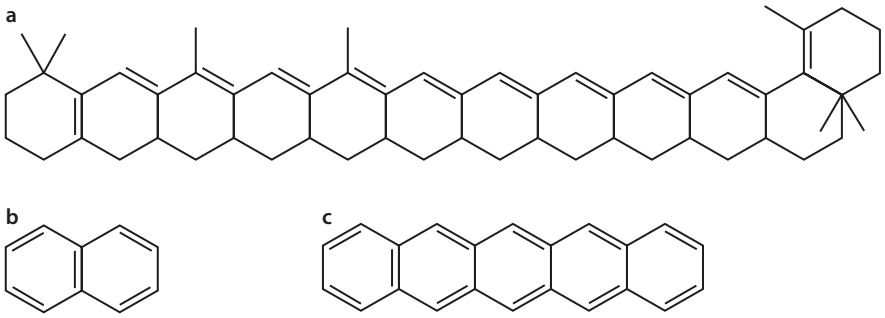


Fig. 18.5 Extended conjugate π-systems

Accordingly, both the π- and π*-levels consist of 11 molecular orbitals each.

The energy difference between the energetically least favourable still occupied (π-)orbital, i.e. the HOMO, and the energetically most favourable *not yet* occupied (π*-)orbital, i.e. the LUMO, has decreased so much that λ_{max}(β-carotene) = 450 nm. So this compound absorbs blue light, which is why we see the typical carrot orange.

It becomes even clearer when we come to aromatic systems:

- Naphthalene (Fig. 18.5b) exhibits a system of π- and π*-molecular orbitals originating from altogether 10 p-orbitals, thus both the π-level and the π*-level consist of 5 molecular orbitals. To the human eye, naphthalene appears colourless; λ_{max} = 286 nm. (Note that compared to benzene with only 3 molecular orbitals each in the π- and π*-levels, this already represents a shift of more than 30 nm!)
- The π- and π*-levels of pentacene (Fig. 18.5c), on the other hand, consist of 11 molecular orbitals each: The HOMO-LUMO distance here is already so small that *yellow* light is sufficient for excitation: λ_{max}(pentacene) = 580 nm; we perceive this compound as violet.

A larger π-electron system can be excited by photons of a longer wavelength—which, in turn, means that the absorption maximum shifts more and more towards the VIS range, until it reaches colouration perceptible to the human eye.

The different possible electron transitions in UV/VIS spectroscopy should not be considered quite so strictly separated from one other, however, because the example of the carbonyl group already shows that it is very easy for the individual molecular orbitals to interact. In fact, the UV/VIS spectrum of acetone does not only show *one* absorption maximum, but two:

- 186 nm (the π → π* transition; significantly weaker) and
- 266 nm (the more easily excitable n → π* transition because of the smaller energy difference).

An Important Aspect

We should always keep in mind that molecular orbitals always extend over the *entire* molecule. Even two functional groups located "at opposite ends" of a molecule may very well interact with each other. Let us look at *p-dimethylamino cinnamaldehyde* as an example (Fig. 18.6).

Here, there is a fully-conjugated π-electron system extending beyond the aromatic ring, which is a **push-pull system** because of the negative polarisation

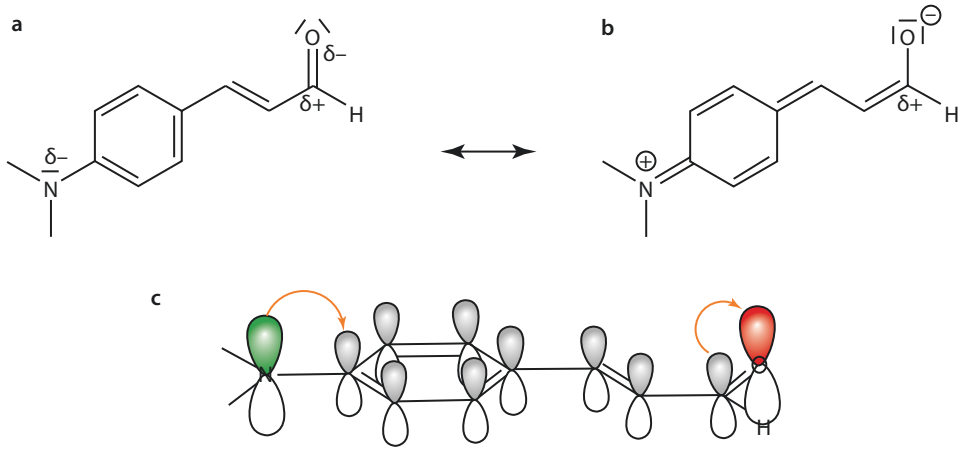


Fig. 18.6 *p-Dimethylamino cinnamaldehyde*

on the dimethylamino nitrogen and the positive partial charge on the "opposite" carbonyl carbon:

- Nitrogen clearly possesses a + M effect, which should initially provide increased π-electron density in the aromatic ring: It "*pushes*" π-electrons. In other words: It constitutes an *auxochrome*.
- The oxygen, on the other hand, because it is connected to the carbonyl carbon by a double bond, has an -M effect; overall, of course, this carbonyl group is the *chromophore.*
- Since the carbonyl group is also conjugated to the aromatic system via the vinyl group (-CH=CH-), the π electron charge density in the aromatic ring is again somewhat reduced. Effectively, the carbonyl carbon "*pulls*" on the π-electrons there *(pull)*.

In this way, the centre of the π-electron density shifts, as the resonance structures (Fig. 18.6a, b) should make clear (the interplay of the p-orbitals responsible for the π- and π*-molecular orbitals is indicated in Fig. 18.6c). This leads to the fact that the system can be excited quite easily. The corresponding UV/VIS spectrum shows two relative maxima:

- λ = 255 nm (with an extinction of 0.2) and
- λ = 395 nm (with an extinction of 0.58)

Again, the transition more easily induced (i.e. by less energetic photons) is unmistakably dominant.

Question: If p-dimethylamino cinnamaldehyde absorbs electromagnetic radiation of the wavelength λ = 395 nm, should it not show a colour?—Exactly. This wavelength belongs to violet light so the human eye should perceive the corresponding complementary colour. According to the colour wheel from Part II, this is definitely a yellow hue, Table 17.1 of Harris is a bit more precise and describes this hue as green-yellow ... and indeed, p-dimethylamino cinnamaldehyde is present as a fine crystalline, beige powder at room temperature.

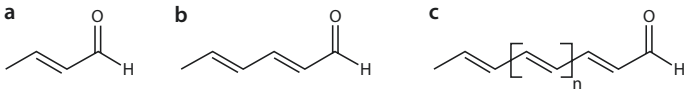
Harris, Section 17.2: Light absorption

The connection between absorption (or extinction) maximum and colour is shown very nicely in colour plate 15 from Harris.

Harris, colour plates

Questions

7. In which of the following compounds do you expect the extinction maximum to be shifted furthest towards longer wavelengths?



Polyunsaturated conjugated aldehydes

8. Find the *push-pull systems* in the pH indicators from Part II, whose structures are given in Fig. 5.2?

18.4 Returning to the Ground State

Whichever electron transfer may be caused by the radiation from the UV/VIS range: In any case, the analyte is energetically excited this way. However, since in nature it is always a question of avoiding excessive (energy) effort (everything that happens spontaneously only happens if energy can be released in one way or another or "saved" in some other way), this excited system will sooner or later return to the ground state.

It would stand to reason that the whole thing could be summed up easily: The excited electron should simply return to exactly where it came from. For that, it would of course, have to somehow release the previously absorbed energy to do so. First of all, there are two ways to do this:

- The energy emission could take the form of a photon. It should then be possible to detect this photon again—it should, therefore, be possible to record an *emission spectrum* of the excited analyte. However, since the return to the ground state can also occur with a time delay, i.e. not all analytes return from their respective excited state to the ground state at the same time, this is usually not very useful: The photon yield is (very) limited.
- Alternatively, the excited particle could simply release the absorbed energy in the form of *heat* and return to the ground state this way. This would then be a **radiationless transition** that returns the particle to its initial state.

And in fact both ways happen—and others, as well, as there are, in fact, other possibilities: Switching electrons from one orbital to another (with a different energy content) is by no means the only thing a molecule can do when excited: After all, it has already been suggested in ► Chap. 17 that the energy content of longer-wavelength radiation (such as from the IR and microwave regions) is not sufficient for this. Instead, the molecules in question are "just" excited to **vibrations** and **rotations,** which—it cannot be stressed often enough—are *also quantised.* In other words:

There are energy levels not only

- for electrons (we usually call these electron levels "orbitals"),

but also

- for vibration movements (the so-called *vibration levels*), and
- for rotary movements (*rotation levels*).

We will not go into the difference between these two here; for the sake of simplicity, let us summarise them as **vibration/rotation levels** (in short: vib./rot. levels). Thus, just as a system can be excited electronically, it can also be excited vibrationally/rotationally (in short: vib./rot.-excited).

The fact that we have to take these additional levels into account for molecules (and also polyatomic ions etc.) means that we should first take a closer look at the *excitation of* our system. What exactly is happening there?

■ Excitation of a Molecule: A Closer Look

Just as an electron needs a well-defined amount of energy (always a multiple of Planck's quantum of action) to change from one state to another (another shell/another orbital), the same is true for vibrations and rotations—only the amounts of energy needed for that are much smaller. Now, however, there is nothing to prevent a system in an *electronically* excited state from also possessing a precisely defined (vibration or rotation) energy surplus (which, of course, must still be a multiple of Planck's quantum of action): So these additional vib./red. levels are used.

Without now considering specific energy values and/or wavelengths suitable for excitation, let us look at the influence these vib./red. levels have on the possible energy state of the system under consideration. Please note: Here, the excitation of the system can no longer be induced with only a single wavelength but by several wavelengths close to each other from a (narrowly limited) wavelength range, resulting in a **quasi-contiuum** (a very close succession of several *still strictly separated* wavelengths).

Important

Please do not fear that we have to say goodbye to a cherished (and important) basis of quantum mechanics now that wavelength *ranges* are mentioned! As a reminder: It is already completely true that *individual atoms* can really only be excited by exact, precisely defined wavelengths—just as you learned about this in *General Chemistry* (and in the repetition also in ► Chap. 17 and ► Sect. 18.2). In the case of atoms, as you will recall, electrons were moved from an energetically more favourable to an energetically less favourable (quantum) state, that is, they were moved to a higher shell or orbital. After that, the system (the atom) was in an *electronically excited* state. If, however, the wavelength with which this excitation was to be brought about did not correspond *exactly* to the energy difference between the respective shells or orbitals, the excitation failed to occur at all.

But that only applies to individual atoms.

As soon as we are dealing with a multi-atomic system (i.e. a molecule or an ionic compound), the *interatomic* interactions (i.e. the bonds) must also be taken into account. This in turn means that *vibrations* (i.e. oscillations; more on this in ► Sect. 19.1) and *rotations* must also be considered, which—as mentioned above—are also quantised. This means that in multi-atomic systems there is no longer just one single wavelength by which the system can be excited, but that (unlike in the case of individual atoms) an excitation can also occur if *somewhat more* than the energy required for the pure electron transition is involved.

Illustration with Simplified Figures

Let us first make several assumptions:

- The ground state of the particle is a singlet state, which we will denote as S_0: $\underset{(\uparrow\downarrow)}{()}$; the empty bracket represents a higher-energy, currently vacant orbital.
- The first (electronically) excited state is also a singlet—$\underset{(\uparrow)}{(\downarrow)}$—which, because of its higher energy content, shall be called S_1 (much as with the $n \rightarrow \pi^*$ transition in Fig. 17.13a from Harris).

Harris, Section 17.6: Light absorption processes

- The relative energy difference between S_0 and S_1 shall be considered to be (arbitrarily) 1000 energy units; it can be overcome with electromagnetic radiation of wavelength λ_1. In other words, a photon with wavelength λ_1 causes the transition $S_0 \rightarrow S_1$—in our simplified orbital scheme: $\underset{(\uparrow\downarrow)}{(\,)} \rightarrow \underset{(\uparrow)}{(\downarrow)}$.

If there were only electronic excitation, and if the excitation had really taken place with a photon of wavelength λ_1, the S_0 ground state must be taken again after the excess energy has been released (either as a photon with wavelength λ_1 or as heat).

However, since there are also vibrational and rotational levels, which lie energetically much closer together (i.e. differ much less in their energy content than the various *electron levels*), a polyatomic system, unlike a single atom, can also be excited by slightly higher-energy radiation λ_2 ($\lambda_2 < \lambda_1$).

- For the sake of simplicity, let us assume that the energy difference between the individual vib./red. levels were 2 energy units each. (In fact, they are not equidistant, but that would leads too far now.)

This would mean that this system could *also* be excited with a wavelength belonging to the energy content "1002 energy units". With this wavelength, the system would then not simply be put into the excited state S_1 (as above) but into an *excited* state S_1*, which would—as the term "excited state" implies—additionally also be vib./red. excited ... and thus be still somewhat more energetic than "just" the electronically excited state S_1 (which is, after all, at its lowest possible oscillation level).

Likewise, the system could be excited with 1004 energy units: Here, an electronic excitation state would subsequently be present, which would additionally be somewhat more energetic in a vibratory/rotatory manner, i.e. S_1** etc.

The same could be done—assuming that the vibration levels were indeed equidistant—with 1006, 1008, etc. energy units. But it is important that the wavelength belonging to an energy content of 1003 or 1005 energy units would still *not* lead to an excitation of the system. Even it has been said or written really often now: The oscillation-states (vibration/rotation) are also *quantised.*

The fact that "in truth" not only *one* ground state and equally well-defined excited states (of whatever multiplicity) exist but that *for each electron level there* is a multitude of vib./red. levels, is also the reason why the absorption bands of the UV/VIS spectra are *relatively broad:*

It is quite possible that the *electronically not yet* excited analyte is already more or less strongly *excited with respect to vibration and rotation* (S_0* or S_0** etc.). If this is the case, a slightly less energetic photon suffices for this system to reach the electronically excited state S_1 (with minimal vib./red. excitation, hence no asterisk). Correspondingly, such a system, already excited to oscillations, can be electronically excited by a somewhat longer wavelength λ_2 (with $\lambda_2 > \lambda_1$).

Likewise, a photon that is "actually" a little too energetic can be absorbed to push the analyte out of the electronic ground state (whether without additional vib./red. excitation S_0 or out of a vibrationally/rotationally already excited electronic ground state S_0* or S_0** etc.) into a more or less vib./red. excited electronic excitation state S_1*, S_1** etc.).

(Quantitative statements about relations between the different (quantised!) electron excitation states and vibrations—just on the basis of quantum mechanics—can be made with the **Franck-Condon principle** ... which, however,

would again clearly go beyond the scope of this introduction. What one can find out about analytes, however, if one concentrates *exclusively* on their vibrations, you will learn in ► Chap. 19.)

▪ Relaxation

If a system is in an electronically and/or vib./red. excited state, this is naturally unfavourable from a purely energetic point of view; accordingly, the system will try to return to the (lower-energy) ground state (or at least to assume a state that is no longer quite so strongly excited), whereby a certain degree of vib./red. excitation may well remain. This reduction in the energy content of any system is called **relaxation.** Let us now look in turn at the various ways in which a system can decrease its energy content after it has been brought into an excited state by the application of energy. (For this section, you should have Fig. 17.15 from Harris handy.) Again, we assume that the ground state of the system is a singlet state, which we shall again denote as S_0; let it be represented graphically again by $\underset{(\uparrow\downarrow)}{()}$.

Harris, Section 17.6: Light absorption processes

As explained in the previous sections, an electronically (and preferably also vibrationally/rotationally) as yet *unexcited* system S_0 can not only be placed in the purely electronically excited state S_1, graphically represented as $\underset{(\uparrow)}{(\downarrow)}$, but can also be additionally vib./rot. excited, so that actually is in the state S_1* or S_1** etc. (which of course does not change the distribution/orientation of the electrons). This "excess" rotational/vibratory energy can first be dissipated by the system, usually by collisions with other molecules (either other analytes or also, if present, the solvent). This leads to a (slight) heating of the analyte mixture—which may even be observable macroscopically, but it *need* not *be.*

- This way, the system transitions from its electronically *and* vibrationally/rotationally excited state S_1* to the electronically excited *vib./rot. ground state* S_1—which is, of course, still energetically less favourable than the ground state S_0 *without* vib./rot. excitation. This relaxation process is referred to as R_1 in Fig. 17.15 from Harris.
- If the system then returns from this electronically excited state S_1—$\underset{(\uparrow)}{(\downarrow)}$—(which is now no longer additionally excited to oscillation) back to the (electronic) ground state—$\underset{(\uparrow\downarrow)}{()}$—and thereby emits the energy released in the form of a photon, its wavelength obviously no longer corresponds to the wavelength of the *initial* radiation because part of the excitation energy (which has led to the increased rotation or vibration of the system in the first place) has been "used up". Accordingly, one observes/measures a slight increase of the wavelength (larger wavelength means lower energy content, as you certainly remember), thus one might see a different colour than that of the excitation radiation. This phenomenon—that a system excited with a wavelength λ_1 emits light of wavelength λ_2, where $\lambda_2 > \lambda_1$—is called **fluorescence.** (In the next section, we will discuss this in a little more detail.)

However, as already mentioned, this is not the only possibility. If the system has relaxed from the electronically *and* vibrationally/rotationally excited state S_1* into the only electronically excited state S_1 (i.e. has carried out the relaxation described as R_1 in Fig. 17.15 from Harris), there are two further alternatives:

- You will have recognised that the y-axis (only indicated) in Fig. 17.15 from Harris is intended to illustrate the relative energy content of the various electron or vib./red. levels. Thus, there are not only vibratory/rotatory completely unexcited states S_0 or S_1 but in addition to the already familiar moderately vib./red. excited states S_0* and S_1*, there are also much more strongly vib./red. excited states S_0*** or S_1***. (*** here is generally meant to mean

that we are talking about unspecified vib./red. levels, which are significantly more energetic.) Furthermore, you see that a vibrationally/rotationally strongly excited state S_0*** may well have exactly the same energy content as the only electronically but not vib./red. excited state S_1 (or also the *additionally* vib./red. excited state S_1* etc.). If this is the case, so that *energetically degenerate* levels are present, an **internal conversion** can occur (usually abbreviated **IC**), in which the multiplet state of the system does not change ($S_1 \rightarrow S_0$***) but the population of the orbitals does: $\overset{(\downarrow)}{(\uparrow)} \rightarrow \overset{()}{(\uparrow\downarrow)}$ However, the resulting electronic ground state S_0*** is clearly vib./red.-excited (exactly that is what *** means).

This excess energy can now be released again by *collisions* with other molecules, leading to further heating of the mixture; in Fig. 17.15 from Harris, this relaxation path is marked R_2.

Overall, this ultimately leads to the *radiationless relaxation* to the ground state S_0.

There is, however, a second path which, although it bears some resemblance to internal conversion, is not called so because there is a fundamental difference:

- In this relaxation path, *the multiplicity state* of the system *changes*: The electronically excited singlet system S_1(*) (with or without additional vib./rot. excitation, hence the brackets), whose electron population can again be represented by $\overset{(\downarrow)}{(\uparrow)}$, can transition to the electronically still excited triplet state T_1* *with spin reversal* of the electron in the energetically less favourable of the two orbitals: $\overset{(\uparrow)}{(\uparrow)}$. Such a change of the multiplicity state of a system is called ***intersystem crossing*** **(ISC)**.

In ► Sect. 18.2 and in connection with Fig. 17.13 from Harris, you learned that the spin state +½ (i.e.: ↑) is energetically somewhat more favourable than the spin state −½ (i.e.: ↓); accordingly the *intersystem crossing* should actually proceed preferentially. However, you were also told in the same section that such a simple change of spin quantum number is *symmetry-forbidden* in most cases, and that means: It happens much less frequently. (But it just does *not* mean that it does not occur *at all.* It just occurs *less often*.)

Harris, Section 17.6: Light absorption processes

The vib./red.-excited triplet state T_1* created by *intersystem crossing* (which is of course also electronically excited, because there *is* no electronic triplet ground state for our system) can now again dissipate the vibrational/rotational energy by **collision** with other molecules and pass radiationlessly (giving up the energy to the environment in the form of heat) into the no longer vib./red.-excited state T_1. The associated relaxation process is denoted as R_3 in Fig. 17.15 from Harris.

Important

Unfortunately, Fig. 17.15, which is so indispensable for this topic, contains a small error: First, the system T_1 present after the *intersystem crossing* (which is also vib./red. excited, so that strictly speaking it should be called the T_1*** state) relaxes radiationless (via R_3), so that the no longer vib./red. excited state T_1 is reached. Subsequently, a second *intersystem crossing* occurs, where the system transitions from the vib./red. non-excited state T_1 to a vib./red. excited state S_0***. The corresponding arrow from T_1 to S_0 should not say "to T_0" but "to S_0" because at the beginning we found out that the *electronic ground state* (and nothing else is meant by the subscript 0) is a *singlet state,* and therefore *every* triplet state must automatically be electronically excited, which is symbolised by subscripts >0.

Yes, that is *very* nitpicky, but since it is easy to get confused on this topic anyway, I would like to spare you the frustration of dealing with a simply incomprehensible and impossible state, which is supposed to be assumed here.

Once a vibratory/rotatory state T_1 is *no longer* excited, the system has two options for further relaxation:

- On the one hand, another *intersystem crossing* can occur: $T_1 \rightarrow S_0$***, because it is most likely that there will again be a vib./red. excited electronic *ground state* that has exactly the same energy content as the vibrationally/rotationally non-excited state T_1. Again, the population of the involved orbitals changes (otherwise one would call it an ISC) : $\overset{(\uparrow)}{(\uparrow)} \rightarrow \overset{()}{(\uparrow\downarrow)}$

  The excess energy is then released again without radiation (i.e. by collision), so that at the end the system is in the vibrationally/rotationally no longer excited state S_0; the corresponding relaxation process is marked R_4 in Fig. 17.15 from Harris.
- Alternatively, the system can also transition "directly" to the ground state S_0. The previously surplus energy is then emitted again in the form of a *photon*, which is correspondingly less energetic and thus longer-waved than the excitation radiation; after all, the system has already lost part of its energy content since its excitation. This luminous phenomenon is called **phosphorescence**; it will be briefly discussed again in just a moment.

Graphic representations of the possible transitions of multi-atomic systems to the various excited states (both electronic and vibrational/rotational, as in Fig. 17.15 from Harris) are called **Jablonski diagrams** or **Jablonski term diagrams.**

The different possibilities of an analyte to release absorbed energy in the form of electromagnetic radiation lead to two important luminous phenomena (besides the flame colouration, of course, which we have already discussed). Just because those phenomena are so important (and also because they are often wrongly (!) regarded as synonyms beyond the physico-chemical terminology), they shall be considered once again in a little (!) more detail.

▪ Fluorescence and Phosphorescence

In both cases, a system is initially **photochemically** excited, but what follows is quite different:

- In **fluorescence**, there is initially an almost instantaneous radiationless transition (*without a* change in the multiplet state, i.e. without an internal conversion*,* IC), so that part of the excitation energy is converted into rotational and/or vibrational energy; the remaining energy is emitted in the form of a photon, which is lower in energy than the excitation photon. So a system capable of fluorescence is induced to emit longer wavelength radiation by shorter wavelength radiation. (You are certainly familiar with such fluorescence phenomena: In clubs, discotheques, etc., you can occasionally observe some items of clothing, which are printed with fluorescent colours and are excited by "black light"—i.e. comparatively long-wave UV radiation—, to glow in a completely different colour.) Fluorescence phenomena end practically instantaneously at the moment the excitation ceases; the associated transitions are usually completed within 10^{-7} to 10^{-10} s.
- The phenomenon of **phosphorescence** also involves radiationless transitions, which ultimately lead to the emission of photons of increased wavelength. Unlike fluorescence, however, *phosphorescence changes the multiplicity state,* i.e. an *intersystem crossing* (ISC) takes place. Such symmetry-forbidden transitions take much longer, and thus the excitation energy remains "stored" in the system for a certain time; accordingly, the luminescence phenomenon known as phosphorescence may well persist for several minutes or (in systems specially optimised for this purpose) even

hours after the photochemical excitation has ended. (You have certainly seen this kind of "luminescent paint" in action: Occasionally it is used to mark emergency exits, e.g. in aircraft or in usually illuminated tunnels. Even in the case of an electricity cut due to an emergency or some such, the markings are still clearly visible for a long time due to the afterglow.)

These two phenomena, which are based on energy absorption and more or less immediate re-emission, should not be confused with **chemiluminescence:** This is the term used for chemical reactions accompanied by luminescent phenomena, such as the gradual oxidation of white phosphorus (P_4) by atmospheric oxygen. Although the accompanying *luminescence* has given *phosphorescence* its name, it would simply be wrong to speak of "phosphorescence" in the case of this oxidation reaction, because in the case of the luminescent phenomena described above (fluorescence and phosphorescence) the substance under consideration (the analyte or similar) does *not* change chemically, and so the luminescent phenomena in question can theoretically be repeated as often as desired. The situation is quite different with chemiluminescence: Once the white phosphorus has been completely converted to phosphorus(III) oxide (P_4O_6), for example, the beautiful glow stops.

Questions

8. Which type of non-radiative transition must have occurred when an electronically *and* vib./red. excited system T_2** relaxes to the S_1* state?

18.5 Technical Aspects of (Spectral) Photometers

Without devoting too much space to the technical aspects of these measuring instruments, the most important components shall be at least briefly outlined (see Fig. 19.1 from Harris).

Harris, Chapter 19: Spectrophotometer

Light Sources

First of all, a light source is needed that is ideally capable of covering the entire UV/VIS range—reproducible, continuous, without fluctuations or "missing" areas ... and preferably also inexpensive. As is so often the case, this is not so easy to obtain, but two important light sources should nevertheless be mentioned:

- If deuterium (heavy hydrogen: $^2H_2$, i.e. D_2) is made to dissociate in a corresponding arc lamp by an electric charge, this lamp emits UV radiation with λ = 200–400 nm.
- Glowing tungsten wire covers the entire VIS spectrum with its emission, plus the longer wavelength UV and the first regions of the infrared: λ = 315–2400 nm.

If both are combined, any wavelengths from the entire UV/VIS range are available. The radiation intensity depends on the wavelength (see Fig. 19.4 from Harris) but since this is known, one can work with calibration curves in case of doubt or—more common nowadays—have a computer-assisted calibration carried out.

- If you need only one wavelength but this wavelength is really exclusive, you usually use a **laser**—but the technical aspects of this would really go beyond the scope of this book.

■ Monochromators

For photometric investigations, it is essential to be able to irradiate the sample specifically with one (or more) wavelength(s) that are as precisely defined as possible. To do this, one must, of course, isolate the wavelength in question from the electromagnetic spectrum (unless one is working with a laser that emits only monochromatic radiation). There are several possibilities:

- Light can be *refracted* by a prism (or other suitable dispersing body), taking advantage of the fact that the refractive index depends not only on the material but also on the wavelength.
- One can also *diffract* the electromagnetic radiation at a grating. Here, too, the wavelengths differ in their behaviour.

By using concave mirrors, the different wavelengths can individually be focused accordingly. With a sufficiently narrow exit slit, one can then direct extremely narrow wavelength ranges in the desired direction. (For the beam path in a grating monochromator, please refer to Fig. 19.6 from Harris.)

Harris, Section 19.2: Monochromators

■ Detectors

Detectors were already mentioned occasionally in Parts I, II and III,. Now, it is time to cover this term in some more detail:

As long as we deal with the interaction of our analytes with electromagnetic radiation—i.e. with photons—, the basic concept of any detector suitable for this purpose is based on registering the impact of photons. If a photon (possibly also of a precisely defined wavelength) hits the mark, the detector generates an electrical signal.

- An example of such a structure is the *photocell*, which is based on the **photoelectric effect:** Here, a (sufficiently energetic, i.e. short-wave) photon releases an electron when it hits a metal surface with a negative charge (i.e. a cathode). This electron then moves (usually in a vacuum) to the anode and thus provides a measurable current flow. It should be understandable that the resulting current becomes stronger the more photons arrive and accordingly release more electrons.

The Photoelectric Effect

Behind the photocell is the photoelectric effect, which was already discovered in the nineteenth century and has since been extensively studied. Elaborate measurements initially led to the following results (expressed in the technical language commonly used today—please remember: in the nineteenth century, the quantisation of energy was not yet known!):

- If sufficiently energetic electromagnetic radiation (we are still in the UV/VIS range!) hits a cathodic metal surface in a vacuum, electrons can be released which then move away from the metal surface.
- The speed at which the released electrons move can be measured.
- If the *radiation intensity* is intensified (i.e. if *more photons* hit the metal surface), the number of electrons released increases. It was shown that even the most intense radiation does not increase the *speed of* the released electrons.
- If, on the other hand, the *energy content* of the radiation used to obtain the electrons is *increased* (i.e. if its wavelength is shortened), this leads to a constant number of photons being extracted—the intensity of the radiation remains the same—, but with increasing energy content of the radiation, the electrons driven out of the metal move through the vacuum with an *increased speed*.

The wavelength of the light used thus only affects the speed *of the electrons released,* not their number*; the intensity of the radiation, in turn, only affects the* number *of electrons released,* not their speed.

It was not until 1905 that a young doctoral student in physics presented a conclusive explanation for this, which, however, required a certain departure from any previous ideas about the nature of light itself (which is why he was promptly awarded the Nobel Prize in 1922): The interpretation presented by the aforementioned young doctoral student—his name was Albert Einstein—presupposed that electromagnetic radiation is only capable of transmitting precisely defined amounts of energy, in short: that light is *quantised*—thus, Einstein introduced the concept of the *photon.*

This quantisation of the energy transmitted by light (or generally: electromagnetic radiation) leads to the notion of a "light particle" knocking out an electron when it hits the cathode surface, just as one hitting billiard ball sets another in motion (perhaps a little strange-seeming, but not entirely false). In this case it becomes also nderstandable, that a faster billiard ball (i.e. a higher-energy, shorter-wavelength photon) transfers more kinetic energy to the target object than a slower billiard ball, whereas changing the *number* of billiard balls used will not affect the speed obtained.

(The fact that the electron in this picture is regarded as a "pure particle" and thus its **wave-particle duality** is ignored is unfortunate but makes it more illustrative.)

However, since the number of electrons extracted in this way is quite small, only a very weak current results—which at the same time means that fluctuations are hardly noticeable: Slight differences in the number of incident photons hardly lead to noticeable differences in the measurement results. Such a photocell is correspondingly insensitive.

More effective (and more sensitive) are *photomultipliers,* where the electrons released by the photoelectric effect are only the beginning of a whole cascade: They, in turn, release further electrons, which then also induce the release of further electrons, etc. This way, a single photon can lead to several hundred thousand electrons being released—which, of course, increases the sensitivity of the associated measurements. (For the technical aspects of such a photomultiplier, please refer to Fig. 19.14 from Harris.)

Harris, Section 19.3: Detectors

■ One After the Other, or at the Same Time?

While classical spectrophotometers for measuring absorption/extinction make the analyte interact with the different wavelengths of the spectrum one after the other, a *photodiode array* consisting of a large number of diodes can pass through the entire spectrum simultaneously:

- The sample is irradiated with white (i.e. **polychromatic)** light; depending on the UV/VIS behaviour of the analyte in question, one or other wavelength is then at least partially absorbed.
- The remaining "residual radiation" is split into its spectral components by a *polychromator*, and the individual wavelengths (ranges) will then each hit a different diode, so that effectively each wavelength is examined individually after all.

Figure 19.15 of Harris shows a schematic representation of the operation of such a photodiode array. In addition, ► Sect. 19.3 of this book offers numerous other detection techniques, but their respective modes of operation are all of a physical rather than chemical nature; for this reason, they will not be discussed further here.

Harris, Section 19.3: Detectors

18.6 Applications

In Part II, you already learned that photometry can be used to determine the concentration of solutions of known composition—in most cases on the basis of a *calibration curve* (which we already had in Part I). Spectrophotometry is of course also suitable for this purpose; after all, the *one* major difference between the two techniques is that one of them works with only a single, precisely defined wavelength, while the other can, at least theoretically, sample the entire (UV/)VIS spectrum. Whether this is necessary in each case depends, of course, entirely on the specific task:

For example, if one wants to determine the equilibrium constant of a reaction, one must be able to track the change in concentration of at least *one* reaction participant. If one knows at which wavelength the (UV/)VIS absorptions of the various participants differ, one can determine the increase in concentration of a product or the decrease in concentration of a reactant by measuring the absorbance and, if necessary, plot it graphically. This technique is immensely popular in *biochemistry,* particularly in the interaction between antibody and antigen. (The keyword **Scatchard plot** shall just be mentioned at this point; you can find out more about it in Harris.)

Harris, Section 18.2: Determination of equilibrium constants: the Scatchard plot

Similarly, **Job's method** can be used to determine the stoichiometric ratio of central particles and ligands in a complex by measuring the absorbance of a solution at exactly the wavelength corresponding to the absorbance maximum of the complex under investigation. (Of course, this only works if neither the free ligand nor the free central particle in solution also exhibit an absorbance maximum at exactly that wavelength. Fortunately, complexes often show more than one absorption band, so sooner or later a suitable wavelength certainly *will* be found.) Subsequently, a series of measurements is made in which the amount-of-substance ratio of the central particle and the ligand is successively changed; this is why Job's method is also called the method of *continuous variation.* (You once again can find more information on this topic in Harris.)

Harris, Section 18.3: Method of continuous variation

Vibrational Spectroscopy

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_19

Summary

It has already been mentioned in ► Chap. 17 that infrared radiation, which is known to be less energetic than the radiation from the VIS range, is not sufficiently energetic to excite *electrons*. However, the energy content of IR photons is ideally suited to excite the analyte molecules to *vibrations.*

19.1 Vibration Modes

There are two fundamentally different types of oscillations:

- During **valence vibrations** (ν), the *distances* between the atoms involved in a bond change.
- During the **deformation vibrations** (δ or γ) bond *angles* change.

There are a total of six vibration modes, shown in ◘ Fig. 19.1. These are commonly further differentiated:

In the case of *valence* oscillations, a distinction is made between

- *symmetrical* stretching (ν_s; ◘ Fig. 19.1a), in which the centre of charge of the bonds involved (and thus the dipole moment of the entire analyte) *does not* change,

and

- *asymmetric* stretching (ν_{as}; ◘ Fig. 19.1b), in which the shift of the center of charge changes the dipole moment of the analyte or an analyte that has *no* dipole moment in the "vibrational ground state" (e.g. carbon dioxide, CO_2) *develops* such a dipole moment.

In the case of *deformation* vibrations, there are even two subcategories:

- On the one hand, one considers oscillations in which the atoms involved remain within the same plane and which are, therefore, called **in-plane oscillations** (abbreviated: δ). Here one distinguishes:
  - *scissoring* or *bending,* in which the opposite direction of movement of two bond partners causes a narrowing or widening of the bond angle (◘ Fig. 19.1c), and
  - *rocking,* in which the molecule "tilts to one side" due to the aligned movement of two bonding partners, without the bonding angle between the atoms in motion changing noticeably (◘ Fig. 19.1d); of course, the angle between each of the atoms involved and the other neighbours, which are *not* in motion, changes in each case.
- On the other hand, there are also ***non-planar*** oscillations (***out-of-plane* oscillations**; abbreviated γ), in which the motions of the binding partners involved can only be described in three-dimensional space. These include:
- *twisting,* which cause parts of the molecule to twist relative to each other (◘ Fig. 19.1e; here, too, the angle between the affected atoms themselves does not change but the one between them and their other neighbours does), *and*
- *wagging,* in which individual planes of the molecule move relative to each other as if that part of the molecule were "tilting to one side" (which, in turn, does not then lead to altered bond angles between the atoms in motion themselves; ◘ Fig. 19.1f).

Beautiful animations of these oscillations are offered by the "ChemgaPedia":
► http://www.chemgapedia.de/vsengine/vlu/vsc/de/ch/3/anc/ir_spek/molekuelschwingungen.vlu/Page/vsc/de/ch/3/anc/ir_spek/schwspek/mol_spek/ir3_1/dreiatomlinear_m19ht0300.vscml.html

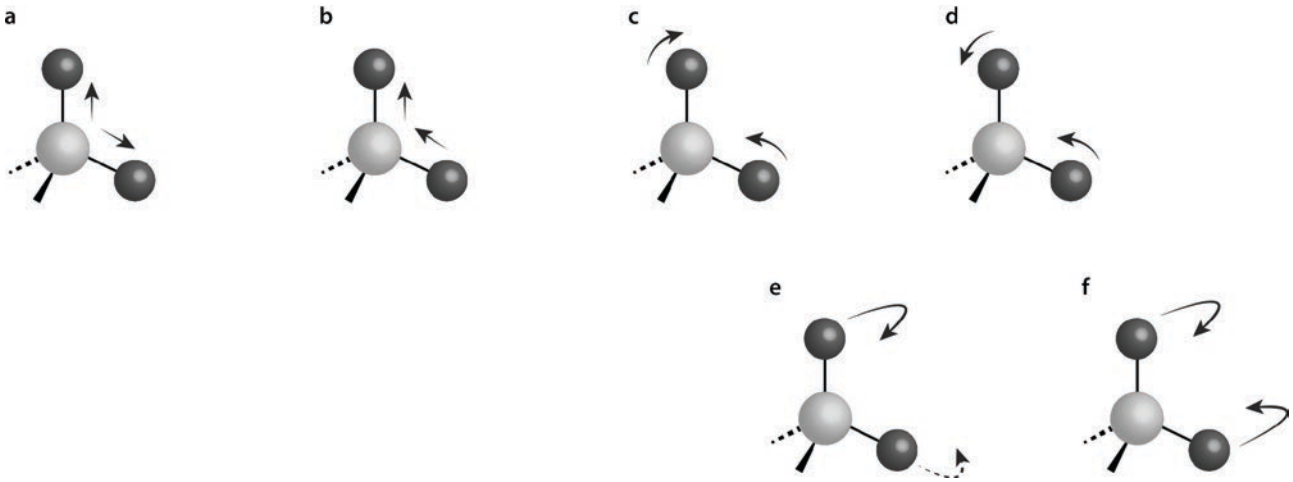


Fig. 19.1 The six modes of vibration: (**a**) symmetrical and (**b**) asymmetric stretching; (**c**) scissoring/bending, (**d**) rocking, (**e**) twisting, (**f**) wagging. (S. Ortanderl, U. Ritgen: Chemie—das Lehrbuch für Dummies, p. 979. 2018. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

For the excitation of the different vibrational modes, photons of different energy content are required—of course, always depending on the analyte under consideration. Accordingly, absorption spectra are obtained in vibrational spectroscopy, in which the extent of the absorption of different wavelengths is graphically represented.

Two fundamentally different forms of vibrational spectroscopy will be discussed separately here. They differ in the type of vibrations that can be observed in each case:

- **Infrared spectroscopy** (which is almost exclusively called **IR spectroscopy**) can only be used to observe vibrations in which the **dipole moment** changes.
- **Raman spectroscopy** only allows the study of vibrations that affect the **polarisability** of the analyte.

These *can be* mutually exclusive, but they do not *have to be*—as you will soon see.

How Many (Different) Vibrations Can a Single Molecule Actually Perform?

Understandably, larger molecules, i.e. molecules consisting of more atoms, can perform significantly more different oscillations than smaller molecules do. In fact, it is even possible to calculate exactly how many different oscillations a molecule can perform. For this purpose, let us consider which individual possibilities of motions each atom has within a molecule (without being torn off the atomic compound by this motion and thus breaking bonds, of course):

Theoretically, every single atom can move in all three directions of space at any time—not unlimitedly, but as long as it does not move too far away from its bonding partner(s) (i.e. as long as no bonds are broken), it has *some* room to move. As there are only *three* mathematically independent directions of motion in three-dimensional space (exactly along the three axes of the Cartesian coordinate system; all other directions can be described by the combination of motions along these three axes), the theoretical number of **degrees of freedom** of motion for each atom is 3.

ChemgaPedia also has something for you for deformation vibrations:
► http://www.chemgapedia.de/vsengine/vlu/vsc/de/ch/3/anc/ir_spek/molekuelschwingungen.vlu/Page/vsc/de/ch/3/anc/ir_spek/schwspek/mol_spek/ir3_3/methylengruppe_m19ht0300.vscml.html

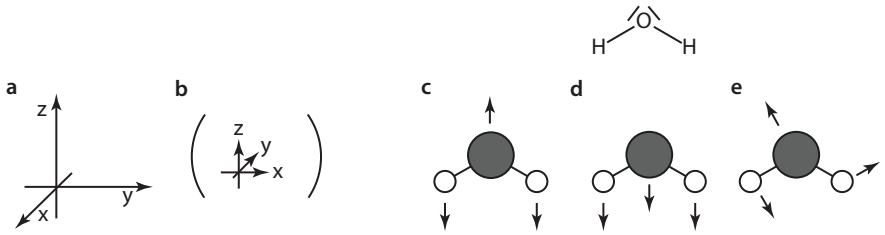


Fig. 19.2 The coorditate system (as it is commonly used in (**a**) chemistry, (**b**) crystallography) and three examples of permissible atomic movements: (**c**) symmetric stretching, (**d**) movement and (**e**) rotation of the entire molecule

This applies, as just said, to *each individual atom* within the molecule in question. Thus, for a molecule consisting of N atoms, a total number of $3N$ degrees of freedom results—purely mathematically. But you will see in a moment that not all of these are *vibrational degrees of freedom*. For the sake of illustration, let us consider a simple molecule with whose structure you are doubtlessly familiar: H_2O.

To do this, we lay out the axes of a Cartesian coordinate system (a right-handed one, of course) as it is very often done in chemistry (shown graphically in Fig. 19.2a):

- The z-axis corresponds to the *vertical* on the paper plane.
- The y-axis represents the *horizontal*.
- The x-axis perpendicularly comes out of the paper plane towards the viewer (positive direction of this axis) or disappears behind it (negative direction).

Of course, you can also *rotate* your reference frame: The variant of axis orientation shown in Fig. 19.2b is common, especially in crystallography-it still cannot hurt to have both options in mind. (Make no mistake here, however: It must still remain a *right-handed* system.)

And now we look at three different motions of atoms involved, relative to each other, permitted by previous rules for degrees of freedom:

- In the variant in Fig. 19.2c, the two H atoms move equally in the −z direction, while the O atom oscillates in the +z direction. At first glance, this looks like exactly the same scissoring that we already had in Fig. 19.1c. But consider this: the oxygen atom is much heavier than the two hydrogens (compare the mass numbers of ^{1}H and ^{16}O!), so it is more likely that only the two hydrogen atoms move away from or return to the O atom along their bonding direction, while the oxygen remains virtually motionless in place and the HOH angle hardly changes. So this is, in fact, *symmetric stretching* ν_s as known from Fig. 19.1a.
- But what happens if the O atom also oscillates in the −z direction (as indicated in Fig. 19.2d)?—Then, in reality, there is no oscillation at all but a *movement of the entire molecule* in this direction. Exactly the same can of course happen in the ±x- or ±y-direction. So from the possible 9 degrees of freedom of this triatomic molecule (i.e. $N = 3$) we have to subtract three **translational degrees of freedom**.
- A seeming oscillation, where the directions of motion of the individual atoms of the molecule add up to a *rotation of the whole system*, is shown to you in Fig. 19.2e. Correspondingly, because these movements also can be described by linear combination of movements along the three spatial directions of the coordinate system, three **rotational degrees of freedom** must also be subtracted.

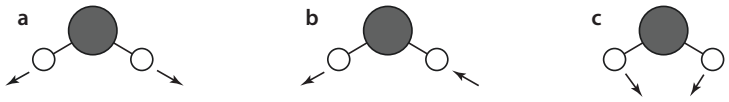


Fig. 19.3 Possible vibration modes for the water molecule: (**a**) symmetrical stretching; (**b**) asymmetric stretching; (**c**) in-plane scissoring

This leaves *only three vibrational degrees of freedom for this molecule*. Another look at Fig. 19.1 tells us: The three possible modes of vibration for H_2O are:

- symmetrical stretching (ν_s; Fig. 19.3a),
- asymmetric stretching (ν_{as}; Fig. 19.3b),
- the *in-plane scissoring* (δ, Fig. 19.3c)—but now please note the direction of motion of the two H atoms, while the oxygen will shift its position only slightly (quite as its mass would lead us to expect), so that the centre of mass of the whole molecule remains in place.

From this, a general rule can be established:

Important

- For all *non-linear* molecules consisting of N atoms, there are a total of *3N–6* vibrational degrees of freedom F:

$$F_{(\text{non-linear})} = 3N - 6 \tag{19.1}$$

- For linear molecules, which can be rotated arbitrarily about their long axis (every linear molecule is, after all, rotationally symmetric about one of the three spatial axes), *one* rotational degree of freedom is accordingly omitted. Thus for *linear N-atomic* molecules it is:

$$F_{(\text{linear})} = 3N - 5 \tag{19.2}$$

Questions

10. How many degrees of vibrational freedom do the molecules (a) SO_2; (b) SO_3; (c) NO; (d) H_2S possess?
11. What are the possible vibrational modes of the carbon dioxide molecule?
12. Why is it not possible to observe the *in-plane rocking* (Fig. 19.1d) for the water molecule?

19.2 Infrared Spectroscopy (IR)

Unfortunately, Harris only deals with infrared spectroscopy to a very limited extent. On the other hand, the basics of both IR and Raman spectroscopy (we will come to the latter in ► Sect. 19.3) can be found very briefly but clearly in Binnewies.

Binnewies, excursus in Sect. 5.12: Molecular symmetry.

As already mentioned in ► Sect. 19.1, the analytes are excited to different vibrations by IR photons of different wavelengths; the bands resulting from the absorption give the corresponding IR spectrum. Among the most informative bands (especially for the structural elucidation of organic compounds) are wavelengths from the range of λ = 2–15 µm. However, in the course of time, it has become common practice in IR spectroscopy not to describe the respective bands on the basis of the associated wave*length* (λ) but rather on the basis of the associated *wavenumber*.

▪ Wavenumbers

The wavenumber ($\bar{\nu}$ pronounced "nu transverse") is the reciprocal of the wavelength—but with the unit cm^{-1}, i.e. "per *cent*imeter". IR spectroscopy has been practiced for quite some time (until the sixties of the last century, it was still called "ultra-red spectroscopy" but it was already used), and thus a non-SI unit has been able to hold its own (internationally). Please note:

$$\bar{\nu} = \frac{1}{\lambda} \tag{19.3}$$

But the conversion factor should also be kept in mind:

$$\text{Wavenumber}(\bar{\nu})\left[\text{in cm}^{-1}\right] = \frac{10^4}{\text{Wavelength}(\lambda)[\text{in }\mu m]} = \frac{10^7}{\text{Wavelength}(\lambda)[\text{in nm}]} \tag{19.4}$$

Accordingly, ► Eq. 17.1 can be extended for IR spectroscopy:

$$E = h \times v = \frac{hc}{\lambda} = h \times c \times \bar{\nu} \tag{19.5}$$

This also explains why it is often easier to work with wave*numbers* than with wave*lengths:*

The energy content of a photon is directly proportional to its wavenumber (no matter in which unit this is given): $\mathbf{E \sim \bar{\nu}}$

Questions

13. Which wavenumber $\bar{\nu}$ belongs to the wavelength λ = 299 nm?
14. Which wavelength corresponds to the wavenumber $\bar{\nu} = 2342\ cm^{-1}$?

▪ IR Spectra

In an infrared spectrum, the wavenumber is plotted on the x-axis (with increasing wavenumber, i.e. increasing energy content, from right to left, which might take a bit of getting used to); on the y-axis you will find either the *transmission* (usually given in %), occasionally also the *absorption*, but the recording of a transmission spectrum is much more common. Since, of course, the vast majority of wavelengths are *not* absorbed, the "zero line", i.e. our spectrum's baseline, is at the very *top in* IR spectroscopy, and the various absorption bands then all point "downwards". As an example, ◘ Fig. 19.4 shows the IR spectrum of acetone, CH_3–C(=O)–CH_3).

Since infrared radiation stimulates individual atoms to vibrate within their shared bond, it should be understandable that the energy required for this increases the stronger the bond between the atoms involved:

- A C–C single bond will undoubtedly be easier to excite to stretching than a C=C double or even C≡C triple bond.
- Accordingly, the band belonging to $\nu_{(C-C)}$ will occur at smaller wavenumbers (= lower energy content) than that of $\nu_{(C=C)}$, and the wavenumber of $\nu_{(C\equiv C)}$ will be largest. In fact, for the stretching of the C–C single and multiple bonds there are "key figures" which at least give the order of magnitude of the wavenumbers to be expected in each case (◘ Table 19.1)

Attention

- Such values are not to be understood as "set in stone". but really only as approximate values: Depending on the substituents carried by the atoms involved in the respective bond and whether the π-part of a double or triple

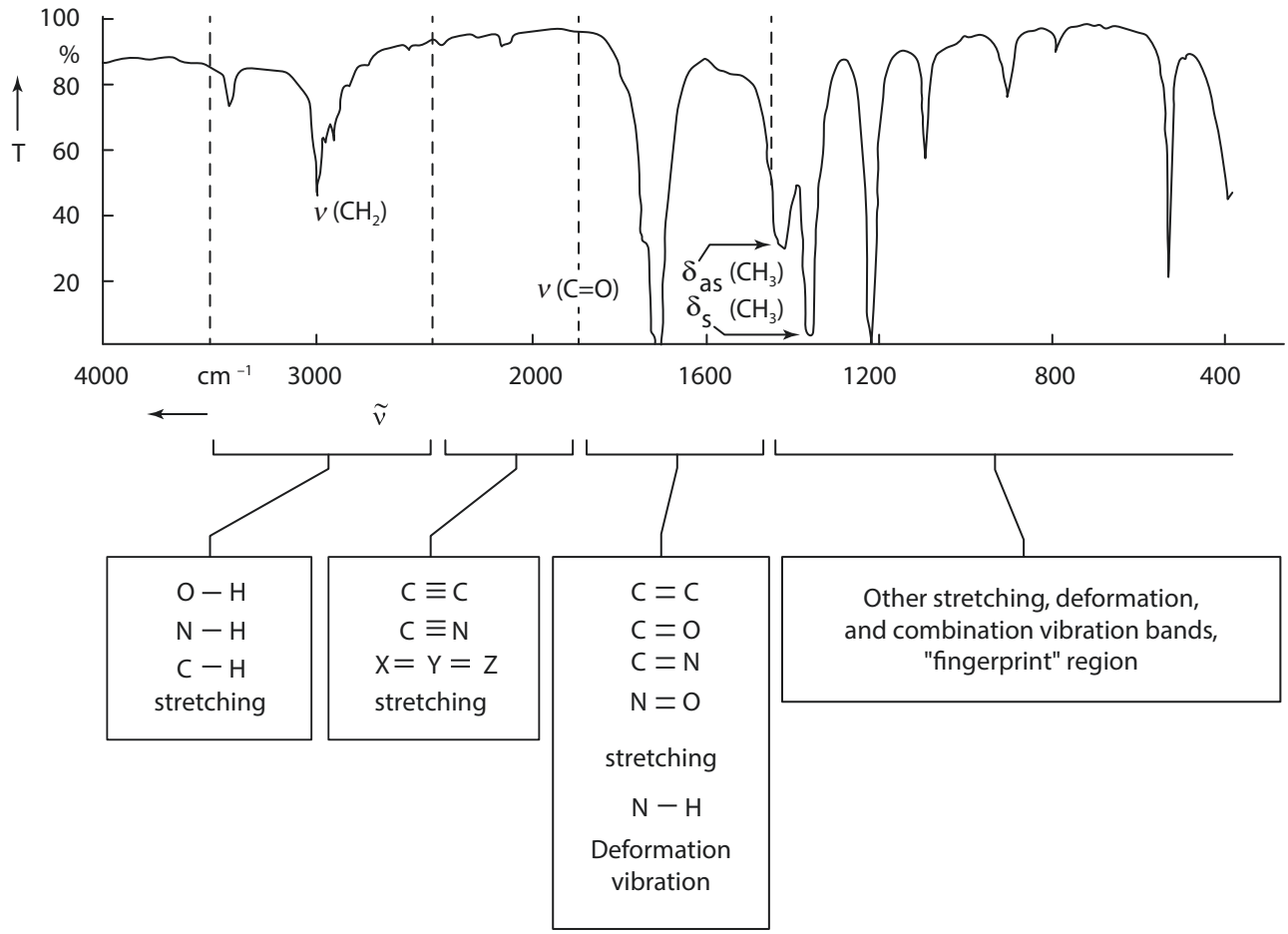


Fig. 19.4 IR spectrum of acetone. (Courtesy of: S. Bienz, L. Biegler, T. Fox, H. Meier, Spektroskopische Methoden in der organischen Chemie, Georg Thieme Verlag, 2016, Stuttgart, Fig. ► 2.9, Courtesy of Georg Thieme Verlag KG)

Table 19.1 Characteristic wavenumbers for the stretching vibration of C–C bonds

Stretching vibration	Wavenumber [in cm^{-1}]
$\nu_{(C-C)}$	1000
$\nu_{(C=C)}$	1650
$\nu_{(C\equiv C)}$	2200

Of course, those are only ballpark figures.

bond is in conjugation with further π-systems (conjugated bonds, aromatic systems, free electron pairs), the value may well shift towards higher or lower wavenumbers.

- Stretching vibrations involving a *hydrogen atom* (–O–H, –N–H, –C–H etc.) absorb at *particularly high* wavenumbers—which should not be surprising given the low mass of the hydrogen atom; after all, this low mass enables a remarkably high vibrational frequency. As the mass of the atoms involved increases, the wavenumber of the associated absorption frequency decreases:

while $\bar{\nu}(\mathrm{C-H}) = 3000\ \mathrm{cm}^{-1}$, the wavenumber of an ordinary C–Cl bond lies of the order of 700 cm^{-1}.

The dependence on the mass of the atoms involved becomes particularly clear in the case of hydrogen's heavier isotope deuterium (^{2}H): Compared to hydrogen-1, whose nucleus consists of only one proton, this hydrogen isotope is twice as heavy because of the additional neutron, and the corresponding wavenumber drops drastically: $\bar{\nu}\left(\mathrm{C-}^{1}\mathrm{H}\right) = 3000\ \mathrm{cm}^{-1}$; $\bar{\nu}\left(\mathrm{C-}^{2}\mathrm{H}\right) = 2100\ \mathrm{cm}^{-1}$.

It should be emphasised once again that IR-radiation does not motivate the analyte molecules to "something unnatural" but only induces completely natural oscillatory movements of the molecule. Please never forget that above the (not attainable) absolute zero (0 K, −273.15 °C) *all* atoms of a multiatomic association of any kind are constantly moving at least a little bit in all directions of space, so there are always certain fluctuations of the bond lengths and angles anyway. The IR-photons, therefore, only "support an already established behaviour".

The labeling of the x-axis of an IR spectrum is usually not linear because bands with $\bar{\nu} > 2000$ are significantly rarer than bands with lower wavenumbers. Thus it is common to display this "high energy part" of IR spectra in *compressed form* for reasons of space. (However, this is only *common,* not obligatory. In some databases, this is no longer the case.)

In this diagram, the *transmission* is shown on the y-axis, abbreviated as T.

■■ Subdivision of the Spectrum into Different Ranges

Although in the graphical representation of such a spectrum there seems to be something special about the wavenumber 2000 cm^{-1}, as it the spectrum often is compressed from then on, it is customary to make a somewhat different subdivision in *terms of content:*

- In the range $\bar{\nu} > 1500\ \mathrm{cm}^{-1}$, there are mainly bands which can (quite easily) be assigned to individual functional groups (e.g. the carbonyl band of acetone with $\bar{\nu} = 1745\ \mathrm{cm}^{-1}$). This is called the ***functional group region***.
  - In ◘ Fig. 19.4, you can see this range to be further subdivided to indicate the orders of magnitude of the wavenumbers at which characteristic X-H stretching, triple bonds, double bonds, etc. can be found.
- At smaller wavenumbers (below $\bar{\nu} = 1500\ \mathrm{cm}^{-1}$), significantly more bands appear. Those are often no longer due to a single molecular region that has been made to oscillate (such as the methyl groups of acetone—where one can even distinguish the modes of oscillation; we will come back to this in a moment), but can be explained by the complex interaction of several molecular moieties. Of course, this has disadvantages ... but also advantages:
  - Bands there can usually not be assigned specifically to one or the other vibration (or even to a vibration *mode*),
  - but the bands observed in this part of the spectrum, which are due to the **framework vibrations**, are extremely characteristic for the respective compound. In fact, they are as individual as a human fingerprint, which is why this part of the IR spectrum is often referred to as the ***fingerprint region***.

■ Assigning the Bands

Since the acetone molecule has a mirror plane (it has already been noted that some prior knowledge on the subject of "symmetry" would really not be bad—

we refer once again to Sect. 5.12 of Binnewies or to *General and Inorganic Chemistry*!), the left and right parts of the molecule are identical: The methyl group on the left will undoubtedly require exactly the same amount of energy as the one on the right to be excited to the same vibration. Accordingly, only *one* band results for the associated vibrational mode.

Binnewies, Section 5.12: Molecular symmetry

▪ Degenerate Vibrations

Let us look at the number of possible vibrations in the acetone molecule:

- If we apply Eq. 19.1 (acetone is definitely not linear!), with the molecular formula C_3H_6O, i.e. with a total of 10 atoms, we get 24 possible oscillations (30–6).
- On the other hand, the six hydrogen atoms are chemically indistinguishable: Two of the three H atoms on each of the two methyl carbons, for example, will be excited to symmetric stretching, etc., by exactly the same energy (i.e. at the same wavelength and thus, of course, at the same wavenumber). Those are called **degenerate oscillations.**

Of course, this also means that the—theoretically conceivable—bands for all of these degenerate oscillations will coincide and thus by far not as many bands will appear in the IR spectrum as the number of oscillation degrees of freedom would initially suggest.

If you go back again to ◘ Fig. 19.4, you will see three more clearly identifiable bands to the left of the *fingerprint region*, i.e. at higher wavenumbers, in addition to the carbonyl band (at 1745 cm^{-1}):

- The slightly smaller band at about 3000 cm^{-1}, which has a **shoulder** at slightly smaller wavenumbers, belongs to the two *stretchings* (asymmetric and symmetric) of the CH_2 group. (Do not forget: A methyl group also *contains* a CH_2 group, even—so to speak—three at once!)
- To the left and right of 1380 cm^{-1} lie the asymmetric and symmetric *deformation vibrations* of the methyl group.

A helpful rule of thumb can be derived from this as well:

In the case of deformation oscillations (whether *in-plane* or *out-of-plane*), only bond angles change, but not bond lengths. That is why they are much easier to excite, so that they are (mostly) found at smaller wavenumbers: Commonly, they are part of the *fingerprint region*.

Analogous to ◘ Table 19.1, textbooks (and especially reference works) on vibrational spectroscopy contain characteristic wavenumbers for almost all conceivable functional groups or substitution patterns on the aromatic compound: Of course, an *ortho*-disubstituted benzene, for example, can vibrate quite differently from its *para*-disubstituted counterpart!

A popular mistake: The fact that *two* bands appear for the methyl group in ◘ Fig. 19.4 is not due to the fact that the analyte—acetone—actually contains two methyl groups! Please do not forget that you are not spectroscoping *single* analyte molecules, but always a huge number of them. (One should never lose sight of how large Avogadro's number is, after all.) Of course, not every molecule will perform every single possible vibrational mode (that would simply not be possible—how could a methyl group perform a symmetric *and* an asymmetric stretching vibration at the same time?), but because (significantly) more than one molecule is examined at the same time, a statistical average of *all* vibrational modes is obtained. Larger bands, therefore, also allow conclusions to be drawn, at least to a limited extent, as to which vibrations the ana-

lyte in question can be excited more frequently and which are of more secondary importance.

Description of IR Spectra

Nowadays—a consequence of the digital revolution—databases with IR spectra (and many more data including other spectra etc.) of numerous compounds are generally accessible. At least one of these databases should be mentioned explicitly; we will refer to it later in this part (and also in "Analytical Chemistry II"): The *National Institute of Standards and Technology* (NIST) provides a wealth of physicochemical data on numerous compounds. It can be easily searched by name and molecular formula (as well as other criteria).

However, before these technical possibilities existed, i.e. just a few decades ago, it would have been simply unthinkable to print complete IR spectra in technical articles or textbooks: On the one hand, the printing effort for this was enormous at that time, on the other hand, there was the problem of space requirements alone. (Unlike with a website, for a printed work it *does* make a difference whether a text becomes a little longer or not.)

► http://webbook.nist.gov/
The following mask can be used for a specific search:
► http://webbook.nist.gov/chemistry/form-ser.html

For this reason, a descriptive *shorthand notation for IR* spectra was established, which you should at least passively master—in case you work with original publications or similar in due time. In principle, the information is limited to specifying the intensity of the individual characteristic (!) bands in addition to the wavenumber of the absorption maximum. The common abbreviations are:

Abbreviation	Meaning	Notes
vs	*Very* strong	Transmission <20% (sometimes abbreviated ss)
s	*Strong*	Transmission<40%
m	*Medium*	Transmission <60%
w	*Weak*	Transmission <80%
vw	*Very weak*	Still clearly different from the background noise
If no specific measured values are given in tables but just guide values, the abbreviation v (for varying) is also given.		

As far as possible, the bands should then be assigned to the appropriate vibration modes using the abbreviations given above.

► Example

The IR spectrum of acetone would then be described as:

3000/2980 m, $\nu(CH_2)$; 1720 vs, $\nu(CO)$; 1400 s, $\delta_{as}(CH_3)$; 1360 vs, $\delta_s(CH_3)$; 1060 (m); 870 (w); 740 (vw); 540 (s).

It has already been mentioned that it is usually not possible to make assignments in the *fingerprint area.* ◄

Not All Vibrations Appear in the IR Spectrum

It should be emphasised once again that not all oscillations are detectable by infrared spectroscopy: there are IR-active and IR-inactive vibrations.

Important

IR-active are only those vibrations in which the *dipole moment* of the analyte changes.

It is not necessary for this analyte to have a (permanent) dipole moment in the ground state; if it is generated by a symmetric or (more likely) asymmetric vibration, this is sufficient.

And one more thing should be mentioned: The examples mentioned so far should not give you the impression that IR spectroscopy is only suitable for "organic molecules": "Inorganic" molecules or molecular ions can also be studied by IR spectroscopy (in Binnewies, for example, IR spectroscopy is explained using the nitrate ion (NO_3^-) as an example). All that is needed are covalent bonds that can be excited into corresponding vibrations.

Questions

15. Which oscillations of CO_2 (see last block of questions) are IR-active, which are not?
16. Can nitric oxide (NO) be detected via IR or not? (Please also consider a rationale for the answer.)

▪ Device Setup

As in photometry (which we already dealt with in Part II), the sample must interact with electromagnetic radiation of the corresponding wavelength; the subsequent quantifying measurement then allows statements to be made about which (percentage) proportion of the respective wavelength was absorbed. Since there can of course also be instrumental intensity losses or the like, a reference beam again is required. This is why IR spectrometers use the two-beam principle, in which a beam splitter splits the relevant light beam (yes, actually one should not speak of "light" in IR, but "radiation beam" would be a peculiar word monstrosity!), so that half of it passes through the sample, the other half through an identically constructed cuvette filled with the same solvent (and any other additives), but *not* the analyte (thus it is an ideal *blank sample*, as we have already discussed in Part II).

In classical, conventional IR spectrometers, the sample is gradually scanned over the entire (relevant) wavelength range—which, of course, takes time: The average measurement takes about a quarter of an hour.

Thanks to the ubiquity of powerful computers, this has changed tremendously:

▪▪ FT-IR

Instead of a time-consuming *wavelength scan*, where the wavelength used is successively changed, but basically monochromatic in every step, it is also possible to work with polyfrequency IR radiation and thus scan all wavelengths simultaneously—provided that the computer has sufficient computing capacity. The actual work is then done by an interferometer and the computer:

- The interferometer converts the IR radiation of the light source into an interferogram.
- This modified beam is then brought into interaction with the sample.
- The computer then performs a **Fourier transformation** on the interferogram, which is now altered by the absorption, so that a wavelength spectrum is again obtained.

Using this technique, a corresponding measurement can be completed within a few seconds. The resulting FT-IR spectra do not differ in shape or information content from spectra obtained the old-fashioned way—except that they are even clearer: The signal-to-noise ratio of FT-IR spectra is significantly better. (The reasoning for this is so physical, however, that it lies once again beyond the scope of this brief introduction. The same applies, of course, to a more detailed consideration of the Fourier transformation itself, etc.)

A Brief Reflection
Is computing capacity still an obstacle these days?—No, not at all. Even a commercially available smartphone from 2014 had more computing capacity than the entire computer arsenal used on the Apollo moon mission in 1969. That is why FT-IR spectrometers have all but replaced their classic *wavelength-scanning* predecessors.

▪ Samples

IR spectroscopy can be carried out in all three states of aggregation of the analyte in question (provided, of course, that the analyte can assume all three aggregate states at all, i.e. can also be brought into the gas phase without decomposing ...):

- A standard method of sample preparation for *solids* is to grind a sample of the analyte with ten to fifty times the amount of potassium bromide (KBr); under fairly high pressure a KBr glass is then pressed from the mixture. (Meaning: The cations and anions of the salt do not form a crystalline but an *amorphous* solid, in which the analyte is enclosed.) KBr glass is pleasingly transparent to IR radiation: So if any radiation is absorbed, this absorption is entirely due to the analyte.
- Solutions can also be investigated by IR spectroscopy.
- The same is true for gases: Especially in environmental analysis, nitrogen oxides (NO_x, i.e. also the nitrogen monoxide from question 16) and the like are often detected by infrared spectroscopy.

When investigating solids in IR (e.g. as components of the KBr compacts just mentioned), it is important that the samples are as dry as possible, because if solvent molecules get into the compact, they are of course *also* excited to vibration, which leads to corresponding bands: If, for example, you detect a—usually broad—band in the range of 3100–3600 cm^{-1} in the spectrum of a compound that does not itself have an OH group (this is the range in which OH stretching (ν) appears), then this indicates that traces of water (or ethanol or similar) are present.

And since you have already learned in this section that bands can often be assigned to exactly those vibrational modes that you learned about in ► Sect. 19.1, it seems only reasonable to take a look at the IR spectrum of the water molecule, after all, the three possible vibrational modes of this triatomic molecule have been discussed in detail (◘ Fig. 19.3). Here, again the already mentioned NIST database helps us out:

► http://webbook.nist.gov/cgi/cbook.cgi?ID=C7732185&Units=SI&Type=IR-SPEC&Index=1#IR-SPEC

There we find two bands:

- 3600/3200 ν_s, $\nu_{as}(H_2O)/\nu_s(H_2O)$
- 1600 m $\delta(H_2O)$

Thus, in the extremely broad band above 3000 cm^{-1}, the symmetric and asymmetric stretching of this molecule coincide.

19.3 Similar, Yet Different: Raman Spectroscopy

The spectra belonging to Raman spectroscopy have striking similarities with the IR spectra from ► Sect. 19.2: The x-axis also shows wavenumbers (in cm^{-1}) (again with increasing energy content from right to left), and again bands are observed. At first glance, there seems to be only one real difference:

- Compared to IR spectra, Raman spectra are "upside down", i.e. the baseline (the background noise) represents the "bottom edge" of the spectrum and the bands point "upwards".

In fact, however, there lies a completely different principle behind the Raman spectra: If one directs intense light of a precisely defined wavelength (i.e. monochromatic light), which is *not energetic enough to induce electron transitions* (as in ► Sect. 18.2), onto a concentrated analyte solution or onto the pure analyte in liquid form, various phenomena can be observed:

- A large part of the light passes through the sample unhindered. This type of *transmission* was certainly to be expected.
- About 1 per thousand of the light (i.e. every thousandth photon) interacts with the analyte in the form of *elastic collisions.* This means that the radiation is scattered without any transfer of energy (in any direction). If there is no energy transfer, it should be understandable that the photons scattered this way have exactly the same wavelength as the radiation used to excite the analyte. This radiation is called **Rayleigh scattering** (named after its discoverer).
- In rare cases (about every hundred millionth photon, i.e. with a probability of $1{:}10^8$) **Raman scattering** occurs, which is based on *inelastic collisions* between analytes and photons. There are two different forms here:
  - A large proportion of these photons involved in Raman scattering transfer a certain part of their energy (which is of course still quantised!) to the analyte. This puts the analyte into an excited (vibrational/rotational) state. The energy content of the photon present after the interaction with the analyte is correspondingly lowered by exactly this amount of energy, and thus has a *longer* wavelength than the radiation used for excitation. This is referred to as **Stokes radiation**.
  - A much smaller fraction of these photons (which are already not very numerous, remember?) hits analyte molecules that already had been in a vibrationally excited state to begin with. Upon interaction with the photon, this excess vibrational energy on the analyte is transferred to the photon—which thus becomes *more energetic* and has a *smaller* wavelength than before. This is the **anti-Stokes radiation**.
  - The energy difference between the excitation radiation and the radiation resulting from Raman scattering corresponds (of course) exactly to the energy that would be needed to excite one or the other vibrational mode.

Harris discusses Raman radiation in Excursus 17.3. *Raman spectroscopy*, however, is mentioned only briefly in Harris. Instead, the basics of this technique, together with those of infrared spectroscopy, are summarised very clearly in Binnewies.

Harris, Section 17.7: Luminescence
Binnewies, excursus in Sect. 5.12: Molecular symmetry

Although the phenomenon of *anti-Stokes radiation* is only very weakly pronounced (after all, it requires analyte molecules that are already in a vibrationally/rotationally excited state!), Raman spectroscopy is based on precisely this radiation. Here, therefore, no absorption spectroscopy is performed but rather *emission spectroscopy*: The radiation *emitted* by the sample is examined.

The fact that Raman spectroscopy is about emissions may explain why the y-axis of the Raman spectra has been "turned upside down" compared to the IR spectra: Here, the intensity of the *emitted* radiation is plotted on the ordinate.

However, this also reveals the two major disadvantages of Raman spectroscopy:

- The excitation radiation should be of really considerable intensity. The best results so far have been achieved with laser radiation—and not every analyte can withstand *that.*
- Because the information is obtained from the *anti-Stokes radiation*, which occurs in almost negligible amounts compared to the other two types of radiation (Rayleigh and Stokes radiation), Raman spectroscopy is not particularly sensitive.

This is why Raman spectroscopy is not one of the usual routine analytical laboratory techniques. Nevertheless, it is really useful and is also used under special conditions (more on this in a moment), since this method has some undeniable advantages:

- Raman spectroscopy can also be carried out with aqueous solutions, whereas in IR spectroscopy water is not used as a solvent, since this molecule itself shows far too strong absorption in the IR range. (Moreover, IR spectroscopy often uses IR-transparent cuvettes made of sodium chloride (NaCl), and this material is highly soluble in water.)
- In contrast to IR spectroscopy, a molecular vibration is Raman-active *only and precisely* when the **polarisability** changes within the scope of this vibration.

Thus, it should be understandable that some IR-inactive oscillations are Raman-active and vice versa. For which oscillations exactly this applies can be determined with the help of **selection rules,** which, however, require quite solid knowledge of group theory, which is why we do not want to go into this further here.

Please do not make the mistake of assuming that a vibration can only ever be *either* IR *or* Raman active. This **alternative prohibition** *only* applies to analytes that have a *center of inversion.* (If necessary, please have another look at your material on *General and Inorganic Chemistry.*)

However, since this way some oscillations cannot be found in the IR spectrum, while they can be observed very well in the Raman spectrum, the two techniques in many respects are complementary.

Applications

Raman spectroscopy is mainly used to investigate special material properties (e.g. pigments or semiconductors; in general, "inorganic" analytes are often easier to investigate via Raman than via IR spectroscopy); no sample preparation of any kind is required, and the spectra obtained in each case are as characteristic and unique as fingerprints. However, this method is not suitable for metallic materials—not least because the sample can heat up considerably due to the extremely high-energy excitation radiation. In addition, Raman spectroscopy is not overly sensitive, as already mentioned, precisely *because* the Raman effect is not very intense.

Questions

17. Are there Raman-active oscillations in the CO_2 molecule?

19.4 Summary

19.4.1 Spectrophotometry and UV/VIS Spectroscopy

In spectrophotometry and UV/VIS spectroscopy, electrons of the analytes are excited by absorption of photons of visible light (spectrophotometry) or of the entire UV/VIS range in such a way that they populate energetically higher molecular orbitals. The excitation to the electron transitions in question can only take place by photons whose energy content corresponds exactly to the energy difference between the source and target orbitals, so that the energy difference of the orbitals involved can be directly inferred from the absorption of the wavelength in question.

In spectrophotometry, the absorption of wavelengths visible to the human eye results in a characteristic coloration of the analyte solution.

From the excited state induced by the absorption of the photon in question, the analyte can relax in various ways:

- The excess energy can be released radiation-free in the form of heat.
- The excess energy can be released again in the form of a photon of exactly the same wavelength that caused the excitation.

Part of the excess energy can be "consumed" by the analyte entering a vibrationally or rotationally excited state, and only the "rest" of the excess energy is then emitted in the form of electromagnetic radiation. Because of the now reduced energy content, this photon then has a longer wavelength than the excitation energy. Depending on which transition has led to the excited state, and whether—depending on the spin state of the electron concerned—a permitted or a forbidden electron transition has occurred, the emission of the excess residual energy leads to fluorescence or phosphorescence.

19.4.2 For Vibrational Spectroscopy

Photons that are too low in energy to cause electron transitions can put the analyte into different excited vibrational states. Since the vibration of microscopic objects (such as molecules) is also quantised, this excitation can also only occur through precisely defined wavelengths. In absorption spectra (in IR spectroscopy) or emission spectra (Raman spectroscopy), suitable wavelengths or wavenumbers (reciprocal of the wavelength; indicated with the unit cm^{-1}) can be plotted against the corresponding extent of absorption/emission.

Depending on the number of atoms involved and the spatial structure of the polyatomic system, the number of theoretically possible vibrations/vibrational modes can be calculated via the theoretical degrees of freedom, whereby not all vibrations are detectable by every vibrational spectroscopic method:

- IR-active, i.e. to be found via IR spectroscopy, are only vibrations in which the dipole moment of the analyte changes.
- In Raman spectra, only the vibrational modes that affect the polarisability of the analyte can be observed.

19.4.3 IR Spectroscopy

Absorptions of photons from the IR spectrum can often be assigned to characteristic molecular moieties—multiple bonds, ring systems, functional groups, etc.—and often even to specific vibrational modes of these molecular components.

Such oscillations are commonly found in the IR spectrum at wavenumbers $>1500\ cm^{-1}$, the *functional group region*.

Below these wavenumbers lies the *fingerprint region*, in which mainly substance-specific framework vibrations lead to a large number of absorption bands. These can no longer be assigned to individual vibration modes, but, precisely because the framework vibrations are substance-specific, they represent a kind of "molecular fingerprint" which—assuming an appropriate comparison spectrum is available—usually enables the analyte to be clearly identified.

19.4.4 Raman Spectroscopy

The analyte is irradiated with intense monochromatic light. In addition to Rayleigh scattering, Raman scattering occurs, which leads to the emission of characteristic photons:

- A photon of excitation radiation can put an analyte into a vibrationally excited state. This leads to Stokes radiation.
- When a photon hits an analyte that is already in a vibrationally excited state, the analyte can transfer its excess energy to the photon. The anti-Stokes radiation emitted in this process leads to the Raman spectrum.

Raman and IR spectroscopy are complementary in many respects; the alternative prohibition states that for analytes with inversion centers (and only for such analytes!) vibrations are either IR or Raman active.

Appendix: Answers

1. For $\lambda_1 = 280$ nm with $T_1 = 70\%$ the value 0.155 results according to $A_1 = -\lg T_1$; for $\lambda_2 = 440$ nm with $T_2 = 52\%$, $A_2 = -\lg T_2 = 0.284$; and for $\lambda_3 = 713$ nm with $T_3 = 23\%$, A_3 is calculated via $-\lg T_3$ to 0.638. So, where the transmission band points further and further "downwards", the extinction band rises more and more steeply "upwards" but not to quite to the same extent due to the logarithm.
2. Lambert-Beer's law states that (within its scope) doubling the concentration always leads to a doubling of the absorbance, so here $E_2 = 0.42$ is to be expected.
3. (a) There is a simple answer here ... and a slightly more complex one. Let us start simple: The bonding between the two lithium atoms occurs via the 2s orbitals; after all, the valence electron configuration of lithium in its ground state is $2s^1$. Accordingly, we obtain one (energetically more favourable) σ_{2s} orbital and one (energetically less favourable) σ_{2s}* orbital. In the ground state, only the bonding of these two molecular orbitals is occupied (after all, we only need to distribute two electrons), with one electron pair (↑↓). This brings us to two molecular orbitals: the σ_{2s} orbital represents the HOMO, and the σ_{2s}* is vacant in the ground state ( ). But *actually*, one should/must consider *all* orbitals of the valence shell, i.e. also the three 2p orbitals of each of the two Li atoms, and thus we would be dealing with a total of *eight* molecular orbitals (two 2s and three 2p orbitals each per lithium atom). However, in a first approximation we can safely ignore this, since the LUMO already originates from the 2s orbitals. (If, on the other hand, we wanted to consider the *electronic band structure* for lithium, we would also have to take into account the 2p band that is vacant in the ground state, but that is not necessary here.) (b) Hydrogen has the valence electron configuration $1s^1$; for the chlorine atom it is $3s^2p^5$. Thus, since there is no 1p orbital, hydrogen really contributes only one atomic orbital, while chlorine provides a fully occupied 3s orbital (↑↓) and three 3p orbitals, two of which are also fully occupied, whereas is populated by only one single electron,: (↑↓)(↑↓)(↑) This gives a total of five molecular orbitals, four of which are each spinantiparallelly

occupied (i.e. they contain a pair of electrons each), while the most energetically unfavourable one (the one yielded by the antibonding interaction of the 1s orbital from hydrogen with the 3p orbital of chlorine, which is only singly occupied) remains unoccupied in the ground state.

4. (a) In the fluorine atom with the valence electron configuration $2s^2 2p^5$, the 2s orbital and two of the three 2p orbitals are spinantiparallel doubly occupied, only the third 2p orbital has an unpaired electron: $\underset{(\uparrow\downarrow)}{}^{(\uparrow\downarrow)(\uparrow\downarrow)(\uparrow)}$ Here, the result is a multiplicity of M = 2, i.e. a *doublet*. The nitrogen atom, on the other hand, has the valence electron configuration $2s^2 2p^3$, i.e. the 2s orbital is again fully occupied, and the three 2p orbitals are each single-occupied spin-parallelly (entirely in accordance with Hund's rule): $\underset{(\uparrow\downarrow)}{}^{(\uparrow)(\uparrow)(\uparrow)}$ because of the three unpaired electrons in the 2p orbital set, the multiplicity M = (number of unpaired electrons) + 1 = 4, i.e. a *quartet*.
(b) For fluorine, it is simple: the electron from the only *singly* occupied 2p orbital can assume the energetically more favourable state (↑) or the energetically somewhat less favourable state (↓). For (↑), a multiplicity state $M_z = \left(2\times\left|\frac{1}{2}\right|\right)+1 = 1+1 = 2$ then results according to ► Eq. 18.5. The state (↓) also leads with $\left(2\times\left|-\frac{1}{2}\right|\right)+1 = 1+1$ to $M_z = 2$, but the latter would again be an *excited* state. For the nitrogen atom, things get funnier: After all, M = 4 means that there must be *four different* possibilities of electron orientation, which differ accordingly in their total spin S_{total} and thus in their multiplicity state. If we ignore the fully occupied 2s orbital, we are left with the most energetically favourable p-electron arrangement (↑)(↑)(↑) with three unpaired electrons of orientation +½, and we get to $M_z = \left(2\times\left|\left(+\frac{1}{2}+\frac{1}{2}+\frac{1}{2}\right)\right|\right)+1 = \left(2\times\left|\left(+\frac{3}{2}\right)\right|\right)+1 = 3+1 = 4$; thus, a *quartet* state is present. If one of these three electrons reverses its spin (it does not matter *which* of the three does so, we do not want to go that *deep* into the nitty gritty here!), we get (↑)(↑)(↓): $M_z = \left(2\times\left|\left(+\frac{1}{2}+\frac{1}{2}-\frac{1}{2}\right)\right|\right)+1 = \left(2\times\left(+\frac{1}{2}\right)\right)+1 = (+1)+1 = 2$, thus a *doublet* state. The next least energetic option would then be something like (↑)(↓)(↓). This yields a multiplicity state of $M_z = \left(2\times\left|\left(+\frac{1}{2}-\frac{1}{2}-\frac{1}{2}\right)\right|\right)+1 = \left(2\times\left(+\frac{1}{2}\right)\right)+1 = 1+1 = 2$, also a *doublet* state (which would be more excited, however). Accordingly, the least energetic orientation would be (↓)(↓)(↓). The associated multiplicity state is then: $M_z = \left(2\times\left|\left(-\frac{1}{2}-\frac{1}{2}-\frac{1}{2}\right)\right|\right)+1 = \left(2\times\left(+\frac{3}{2}\right)\right)+1 = 3+1 = 4$; this is again an (excited) *quartet*.

5. (a) The transition from HOMO-1 to LUMO, which in the presence of a nonbonding electron pair must be a π*-orbital, would correspondingly be a π → π*-transition; the transition from HOMO (i.e. the free electron pair) to LUMO+1, which would then be a σ*-level, would be an n → σ*-transition.
(b) For both the π → π* transition and the n → σ* transition, a spin reversal of the excited electron would yield the orientation $\underset{(\uparrow)}{}^{(\uparrow)}$, so that a *triplet* state would be present. If the excitation takes place while preserving the original spin, the result would be $\underset{(\uparrow)}{}^{(\downarrow)}$, so that a (excited) *singlet* state would be present.

6. From the MO diagram of the dioxygen molecule O_2 (see Fig. 5.45 of Binnewies) you can see that—as already mentioned in ► Sect. 18.2—the HOMO is the (doubly degenerate) π*-level, in which each of these

π*-orbitals—according to Hund's rule—is occupied spin-parallelly singly. Accordingly, the multiplicity is M = 3. The most energetically favourable state here is the *triplet* with $M_z = (2 \times (+½ + ½)) + 1 = (2 \times 1) + 1 = 3$, which can be represented schematically as follows: (↑)(↑) At the first excited state, one of the two electrons has its spin reversed (which one, for now, does not matter): (↑)(↓), and thus we oxcair cue multiplicity state $M_z = (2 \times (½ - ½)) + 1 = (2 \times 0) + 1 = 1$, so there is a *singlet* state. With even greater excitation (which is not yet sufficient to transport one of the electrons into the LUMO!), the spin reversal of both electrons of the HOMO is then obtained: (↓)(↓) here applies $M_z = \left(\left(2 \times \left|-\frac{1}{2} - \frac{1}{2}\right|\right) + 1 = (2 \times 1) + 1 = (2) + 1 = 3\right.$, thus, again a *triplet* state (albeit excited).

7. In general, the more extended the π-electron system, the easier it is to excite. Compound (a) is *(E)*-2-butenal, also known as crotonaldehyde. Its λ_{max} is 220 nm. If we extend the chain by one –CH=CH unit, we arrive at *(E,E)*-2,4-hexadienal with λ_{max} = 270 nm (b); thus, we already observe a clear shift to longer wavelengths, even though this compound is still colorless to the human eye (and smells pleasantly fruity-spicy, just by the way). Further extension by an unsaturated C_2 unit then leads to the *(E,E,E)*-octatrienal (c with n = 1), whose absorption maximum with λ_{max} = 312 nm lies still in the UV range but has again shifted to longer wavelengths compared to its predecessor. This trend continues: compounds from (c) with n = 2, n = 3 and n = 4 include λ_{max} = 343, 370 and 393 nm, respectively.
8. If we look at Fig. 5.2a from Part II, we can clearly see that in this compound in the deprotonated state (right), the negative charge on the (obviously deprotonated) oxygen atom donates electron density into the π-electron system (*push),* while the carbonyl group on the ring "top left" can "take over" the electron density (and the negative charge) *(pull)*. In the molecule of Fig. 5.2b, there are several possibilities: In the very strongly acidic medium (pH < 0), the five-membered heterocycle is broken, and a protonated carbonyl group is present at the "left" ring, which accordingly attracts electron density to itself (*pull),* while the OH group at the "middle" ring can push electron density into the π-electron density thanks to the free electron pairs at the oxygen atom (*push)*. In a slightly to strongly basic environment (pH > 8), the sulfonic acid group (–SO_3H) is present in a *deprotonated* form; the corresponding negative charge then provides a *push effect,* as do the free electron pairs of the oxygen of the OH group as well as the bromine atoms, while the carbonyl group (again on the "left" ring) is again responsible for the *pull effect*.
9. First of all, this system must have dissipated at least part of the vibratory/rotatory energy surplus (by collision or the like); this is exactly what the indices ** and * mean. At the same time, however, the multiplicity state has also changed: from $_{(\uparrow)}{}^{(\uparrow)}$ (a triplet) to $_{(\uparrow\downarrow)}{}^{(\,)}$ (a singlet). Accordingly, not only vib./red. relaxation occurs here, but also *intersystem crossing*.
10. Please look at the spatial structure of the molecules:

Structural formulae of the compounds concerned: (**a**) sulfur dioxide; (**b**) sulfur trioxide; (**c**) nitrogen monoxide; (**d**) hydrogen sulfide

The sulfur dioxide molecule (a) is by no means linear but angled because of the free electron pair at the sulfur atom. Accordingly, Eq. 19.1 applies here, so there are (3 × 3) − 6 = 3 degrees of freedom. For sulfur trioxide (b), which is composed of four atoms, there are a total of (3 × 4) − 6 = 6 degrees of

freedom. Since nitric oxide (c) is linear, Eq. 19.2 applies here, so we arrive at $(3 \times 2) - 5 = 1$. This molecule is capable of only one stretching vibration. For the hydrogen sulfide molecule (d), which is also angled, we get, now again according to Eq. 19.1, the same number of degrees of freedom as for the analogously constructed water molecule: $F(H_2S) = (3 \times 3) - 6 = 3$.

11. The carbon dioxide molecule (O=C=O) is linear, so according to Eq. 19.2 it has $(3 \times 3) - 5 = 4$ degrees of freedom. So, at least theoretically, there are also four modes of oscillation:

Vibrational modes of the carbon dioxide molecule: (**a**) symmetric and (**b**) asymmetric strechting; (**c**, **d**) deformation vibrations (S. Ortanderl, U. Ritgen: Chemie—das Lehrbuch für Dummies, p. 1079. 2018. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

These are:

- the symmetric stretching (ν_s, a),
- their asymmetric counterpart (ν_{as}, b),
- and the deformation vibration
- within the paper plane (c),
- and twisted or tilted to it (d).

These two δ-oscillations are, of course, energetically degenerate because they are the same thing, just rotated by 90° to each other. Accordingly, only three oscillations can be distinguished from each other in the spectra.

12. The hypothetical *in-plane rocking* (from ◘ Fig. 19.1d) would correspond to a *rotation of the entire molecule* on the plane of the paper.
13. According to Eq. 19.4, the wavelength $\lambda = 299$ nm includes the wavenumber $\bar{\nu} = 33.445$. This wavelength or wavenumber does not lie in the energy range that is relevant in IR spectroscopy. Of course, this does not change the calculation itself.
14. The wavenumber $\bar{\nu} = 2342\ \mathrm{cm}^{-1}$ corresponds, again according to Eq. 19.4, which had to be rearranged accordingly, to the wavelength 4269.8 nm (or 4.27 μm).
15. Although the two oxygen atoms are negatively polarised due to their higher electronegativity, while the carbon has a corresponding positive polarisation, the carbon dioxide molecule in the ground state has no dipole moment because the center of charge of the two oxygen atoms coincides with the center of charge of the positively polarised carbon atom. This does not change with the symmetric stretching of the CO_2 molecule (a): the center of mass of the two oxygen atoms still coincides with the center of mass of the carbon atom. Thus, $\nu_s(CO_2)$ is IR inactive. The situation is different for asymmetric stretching (b): Due to the fact that the two oxygen atoms oscillate in the same direction, the center of their polarisation comes to lie next to the center of charge of the carbon atom, so a dipole moment is induced in the carbon dioxide molecule; $\nu_{as}(CO_2)$ is IR-active. The same is true for the (degenerate) deformation oscillations δ: in (c) it is shown that the two oxygen atoms oscillate "down" while the carbon moves "up", so that the center of positive polarisation is above and the center of negative polarisation is below the center of gravity of the molecule. Thus, $\delta(CO_2)$ is IR-active.
16. The only vibration that the (obviously linear) NO is capable of (see Eq. 19.2) is symmetric stretching. However, since the molecule has a dipole moment due to the difference of electronegativity between nitrogen and oxygen (O

is negatively polarised, N is positively polarised), and since the movement of the atoms slightly shifts the centers of charge, the dipole moment also changes: ν(NO) is IR-active, so this gas can be detected via IR.

17. The symmetric stretching $\nu_s(CO_2)$, shown in (a), is known to be IR-inactive because the dipole moment does not change. However, as the differently polarised atoms (C and O) move away from each other in this oscillation, the charge density of the C–O bonds is stretched, and this means that their polarisability changes. Thus, this vibration is Raman-active. The situation is different for $\nu_{as}(CO_2)$ from (b): Here the polarisability does not change. This may seem surprising at first sight, since the charge density distribution of the C–O bond "stretched" in this oscillation is different from that of the "compressed" bond but on *average* nothing changes. The same applies to the deformation vibrations $\delta(CO_2)$: Here, too, the polarisability does not change: Only $\nu_s(CO_2)$ is Raman-active.

Further Reading

Bienz S, Biegler L, Fox T, Meier H (2016) Spektroskopische Methoden in der organischen Chemie. Georg Thieme Verlag KG, Stuttgart

Binnewies M, Jäckel M, Willner, H, Rayner-Canham, G (2016) Allgemeine und Anorganische Chemie. Springer, Heidelberg

Cammann K (2010) Instrumentelle Analytische Chemie. Spektrum, Heidelberg

Ebbing DD, Gammon SD (2009) General Chemistry. Brooks/Cole, Belmont, CA

Hage DS, Carr JD (2011) Analytical Chemistry and Quantitative Analysis. Prentice Hall, Boston

Harris DC (2014) Lehrbuch der Quantitativen Analyse. Springer, Heidelberg

Ortanderl S, Ritgen U (2018) Chemie—das Lehrbuch für Dummies. Wiley, Weinheim

Ortanderl S, Ritgen U (2015) Chemielexikon kompakt für Dummies. Wiley, Weinheim

Reichenbächer M, Popp J (2007) Strukturanalytik organischer und anorganischer Verbindungen. Teubner, Wiesbaden

Riedel E, Janiak C (2007) Anorganische Chemie. Walter de Gruyter, Berlin.

Schröder B, Rudolph J (1985) Physikalische Methoden in der Chemie. VCH, Weinheim

Skoog DA, Holler FJ, Crouch SR (2013) Instrumentelle Analytik. Springer, Heidelberg

As with the other parts, "the Harris" is again the reference textbook. Nevertheless, for a more in-depth study of this topic, we would like to refer to the Spectroscopic Methods by Hesse/Meier/Zeeh: Here, not only application possibilities in the context of analytics are treated, but also the underlying fundamentals are explained in an extremely comprehensible way.

Atomic Spectroscopy

- **Requirements**

This part once again deals with the excitation of the analytes with electromagnetic radiation. Therefore, you should be familiar with the relationship between wavelength or frequency and energy content, which has already been discussed in Part IV.

You should also be familiar with the quantification of analytes using Lambert-Beer's law.

The same applies to the energetic excitability of electrons and subsequent relaxation. However, atomic spectroscopy is not (only) about valence electrons, but also about the atomic core (not the nucleus!). The Bohr atomic model with its different shells is usually used to describe the conditions there. It, of course, should also be known.

As always: You should be (more or less) familiar with the contents of Parts I–IV.

1.1 Learning Objectives

In this part, we will deal with the effect electromagnetic radiation has on atomic analytes. You will learn that—in analogy to molecular spectroscopy—information can also be obtained from the investigation of atoms through the absorption of energy as well as through the emission of excited analytes. Similarities and differences to selected analytical methods presented so far will be pointed out.

In addition to the basic understanding of the intra-atomic conditions during the excitation and relaxation processes, you will also learn about various possible applications for the different analytical methods and be made aware of possible measuring difficulties.

In atomic spectroscopy, detection limits and potential measuring errors are inextricably linked to the technical aspects of the respective analytical methods used. Therefore, in addition to the "chemical" aspects ("What happens to the analyte during the respective measurements?"), selected instrument-specific conditions are also considered.

The aim of this part is still to summarise principles concisely and to emphasise selected fundamental aspects. Unfortunately, the qualitative and quantitative use of X-ray fluorescence for analysis is not covered in Harris. Therefore, the principle behind it is described in somewhat more detail in ► Chap. 23. Cammann and Skoog are expressly recommended for further study:

Cammann, Instrumentelle Analytische Chemie (Instrumental Analytical Chemistry), Section 4.7: X-ray fluorescence analysis

Skoog/Holler/Crouch SR, Instrumental Analysis, Section 12.3

Both books deal with the theoretical background and the fundamentals as well as with apparative aspects.

Contents

General Information on Atomic Spectroscopy

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_20

Summary

Just like molecular spectroscopy (the basics of which you learned about in Part IV), atomic spectroscopy is also based on excitation of the analyte (using various methods, which we will look at in turn). Starting from this, we can then obtain information on the nature and/or quantity of the analyte present

- on the basis of the extent of the absorption of excitation energy,

and/or

- based on the behavior of our analyte in the excited state.

In the former case, we are dealing with *absorption spectroscopic* methods, which will be discussed in ► Chap. 21. The principle behind this is the same as for IR spectroscopy from Part IV:

- Which wavelength is absorbed? The answer to this question allows a *qualitative* statement about the type of analyte present, i.e. its identification.

respectively

- To what extent is energy absorbed? Since it is possible to infer the amount of analyte present from the extent of absorption, a corresponding measurement (calibrated, of course, in accordance with Part I) also allows *quantitative* statements to be made.

If, on the other hand, we obtain our information by observing what wavelength is emitted by an analyte that returns to the ground state (or at least to a somewhat less excited state—this is generally referred to as **relaxation**), we perform *emission spectroscopy*, which we look at in ► Chap. 22. And just as atomic absorption spectroscopy resembles IR spectroscopy in a certain way, atomic emission spectroscopy has at least a certain resemblance to Raman spectroscopy from Part IV: Here, too, we are dependent on first obtaining a sufficiently large number of analytes (in this case not molecules but atoms) that are actually present in the excited state.

Harris, Section 20.1: Atomic spectroscopy, overview

A good overview of the similarities and differences between these two techniques is provided in Fig. 20.1 from Harris.

- In atomic absorption spectroscopy, we take advantage of the fact that our analytes can assume one excited state or another, each requiring precisely defined amounts of energy (Fig. 20.1, bottom center).
- In atomic emission spectroscopy, we look at what amounts of energy are released or what wavelengths are emitted when the analyte returns to the ground state from the current excited state (or relaxes otherwise) (Fig. 20.1, bottom left.).
- In Fig. 20.1, bottom right, a special case of emission spectroscopy—called atomic fluorescence—is discussed (the basics of fluorescence should already be known from Part IV). Here again, a precisely defined amount of energy (E_1) is required to bring the analyte from the ground state (GS) to an excited state (ES*), whereupon the analyte does not return "directly" to the ground state but first releases part of the excess (excitation) energy radiation-free and thus assumes a second excited state (ES_2*) that is energetically slightly more favourable. (Thus, after this relaxation, the following holds: energy content ES_2* < ES*) From this energetically somewhat more favourable second excited state, the analyte then returns to the ground state; accordingly, the amount of energy E_2 released during the transition ES_2* → GS is smaller than the excitation energy E_1. This means:
- If electromagnetic radiation of wavelength λ_1 was required for excitation GS → ES*, *and*
- the transition ES* → ES_2* takes place radiation-free,
- the photon of the energy released at the transition ES_2* → GS will have a wavelength λ_2 for which holds: $\lambda_2 > \lambda_1$.

Of course, this again corresponds to what we have already discussed in Part IV on the subject of "fluorescence". We will not go into atomic fluorescence any further in this part (but we will return to it in "Analytical Chemistry II").

The fundamental difference between atomic and molecular spectroscopy should already have become clear. We can only select

- the nature *or*
- the quantity at hand

Of the respective atomic species under consideration but say nothing about the state in which the analyte atoms were *before* the atomic spectroscopic investigation was carried out, because for this purpose the analyte atoms must first be transferred into the gas phase in *atomic* form.

Both atomic absorption spectroscopy (► Chap. 21) and atomic emission spectroscopy (► Chap. 22) are anything but non-destructive. Maybe you still remember the example of the putative silver ring from Part I: Of all the analytical methods presented in this part, only X-ray fluorescence analysis (► Chap. 23) allows the determination of its actual silver content without the ring losing its shape.

20.1 Atomisation

In the introduction to this chapter, it was already pointed out that the sample must first be "prepared" for atomic spectroscopic examination—and that this analytical procedure is not non-destructive. It could hardly be *any more* destructive, because the sample is literally broken down into its individual atoms: It is *atomised*.

Three different procedures were or are common:

- the use of open flames,
- a (graphite) furnace,
- the use of plasma.

Nowadays, flames are hardly ever used. However, since the first atomic spectroscopic investigations were based on the excitation by open flames, we will also have a look at this historically important atomisation method.

20.1.1 Flames

In principle, the three aspects of the **combustion triangle** are always required to generate flames:

- combustible material,
- a suitable oxidising agent (for most flames this is oxygen; often the oxygen content of the air is already sufficient),
- the required ignition energy.

Harris, Section 20.2: Atomisation: flames, furnaces and plasmas

In AAS, a mixture of ethyne (acetylene, HC≡CH) and air is usually used for flame generation, which allows flame temperatures of 2300–2700 K to be achieved. (Table 20.1 from Harris shows you even more possibilities, which lead to sometimes drastically higher temperatures.)

The analyte solution is introduced into this flame with the aid of an atomiser (which should work as efficient as possible). In view of the high temperatures, not only does the solvent used—atomised/sprayed to ideally *very* small drops—evaporate practically instantaneously: The solid that then remains—now very finely distributed—is broken down into its atoms. And these analyte

atoms are now excited by the (monochromatic) radiation of the corresponding lamp (a hollow cathode lamp or similar—more on this in ► Sect. 21.2).

Warning

Because this often leads to misunderstandings: If the analyte is ionic, for example, in the form of a salt, the high flame temperature leads to a *homolytic* cleavage of any interactions, so that there are actually metal *atoms* in the gas phase (plus the atoms that were previously part of the counter ion).

For this type of bond cleavage, the ordinary burner flame is already sufficient for some compounds (with the propane gas commonly used in the laboratory, a temperature about 2200 K is reached): The characteristic flame colourations of the alkali metal or alkaline earth metal salts, which you will certainly remember from *inorganic chemistry,* are due to the thermal excitation and the subsequent relaxation under emission of the characteristic photons of the metal atoms formed in the flame. Thus, the *valence electron* (in the case of alkali metals) or the *valence electrons* (in the case of alkaline earth metals) of the respective atoms are excited to the higher energy levels.

▪ Some Thoughts on Temperature

Again, one might suspect: The higher the temperature, the better. This is also not completely wrong because if the temperature of the flame is not high enough, some of the analyte particles are not *atomised* but are present in the gas phase in the form of oxide or hydroxide molecules (!). These also absorb electromagnetic radiation to some extent, of course, but just not discrete wavelengths. *Indeed, only analytes present* atomically *lead to clear lines.* But this is not the whole truth.

If the temperature is *too* high, ionisation is to be expected (as in the interior of a hollow cathode lamp—again we refer to ► Sect. 21.2—in which noble gas atoms are ionised to the corresponding cations by splitting off an electron, if sufficient energy is supplied). These ions then also absorb, of course, but again do not produce absorption lines but absorption *bands.*

In view of the prevalent temperatures there, the analyte atoms within the flame are certainly no longer all in the ground state but undoubtedly in one or other *excited* state. In other words, the flame itself also emits light—which, of course, must first be subtracted when determining the **absorbance**. Therefore, the quantification of the analyte on the basis of the absorption of the excitation radiation (according to Eqs. 21.2 and 21.3) is also not possible *directly* but only on the basis of corresponding calibration curves prepared beforehand (as we already know from Part I).

Then, the question arises whether the ratio of fuel and oxidant is balanced:

- If there is an excess of fuel, this is referred to as a **rich flame.** For some elements, such a procedure increases the detection strength (i.e. lowers the *detection limit*) because any oxides or hydroxides are then reduced by the excess fuel (via the carbon of the hydrocarbons or the elemental hydrogen used), so that the analyte is ultimately present in its atomic form again.
- For analytes that are difficult to transfer to the gas phase and atomise there, a **lean flame** may be desirable. Given the excess oxidant present here, a higher flame temperature results.

The preferred method depends on the analyte in question. In the laboratory, AAS often involves a fair amount of *trial and error.*

Harris, Section 20.1: Atomic spectroscopy, overview

The schematic experimental setup of an AAS with flame ionisation is shown in Fig. 20.2 from Harris.

This also gives an idea of why this historical variant of the AAS is hardly ever used nowadays:

- The analyte usually remains in the flame for less than 1 s. Such a short residence time of the analyte in the measuring region also means that the measuring time is reduced accordingly. And a short measuring time is often accompanied by reduced sensitivity.
- In addition, such a measurement requires a comparatively large sample volume (in the order of magnitude of 1–2 mL).

Nowadays, thermal excitation in a (graphite) furnace is much more common.

20.1.2 Oven

Harris, Section 20.2: Atomisation: flames, furnaces and plasmas

With an electrically heated graphite furnace (such as the one shown in Fig. 20.6 of Harris and schematically illustrated in Fig. 20.8), the analytes can be detected much better (the detection limits are thus drastically reduced)—which also has a direct effect on the required sample volumes. Assuming the same concentration of the analyte solutions as in ► Sect. 20.1.1, a two-digit µL volume is usually sufficient; for some analytes even less than 10 µL.

The reason for the lowered detection limit is that the analytes remain in the oven for several seconds: This longer measuring time causes increased sensitivity.

If a graphite platform, *which is not heated itself*, is used inside the furnace, the measurement efficiency can be increased even further: The sample material is applied (in solution or as a solid) to this graphite surface, which is often called the **L'vov platform**, in honor of its developer (Boris V. L'vov from Leningrad). If the internal temperature of the furnace is subsequently increased, the platform, which is *not actively* heated, heats up uniformly, resulting in the sample material also being uniformly vaporised/transferred into the gas phase.

A Technical Aspect

It is important that the furnace is operated under an argon (or other inert gas) atmosphere, otherwise the elemental carbon of the L'vov platform would be oxidised. For the same reason, the maximum temperature of such a furnace is also limited to about 2250 K.

What Is Happening to the Matrix?

The term "matrix" for everything in a sample that is *not* the analyte is already known from Part I. Actually, the matrix should not cause any problems in the AAS, because not only the emission but also the absorption of the relevant wavelengths is element-specific. However, it is quite possible that the matrix (or a part of it) evaporates together with the analyte and remains in the gas phase in molecular form—and the fact that molecules do not show absorption lines but absorption *bands* has already been mentioned in ► Sect. 20.1.1. In this respect, there would be a risk of a misleadingly increased absorbance/extinction value.

If necessary, *matrix modifiers* are used to convert any interfering matrix components to highly volatile compounds so that they have already evaporated and left the (graphite) furnace before the actual analytes enter the gas phase and can be measured.

20.1.3 Plasma

From appropriate *physics courses* and/or teaching materials, you are certainly familiar with plasma as the fourth state of matter, in addition to solid, liquid, and gas. At a sufficiently high temperature, a superheated gas changes into the plasma state, in which cations and free electrons are present in addition to the atoms or molecules from the gas phase due to the strong thermal excitation, so that this supposed "superheated gas" also shows electrical conductivity.

In atomic spectroscopy, argon is again used for this purpose, whereby significantly higher temperatures are reached than in the atomisation processes presented so far: up to 10,000 K. (These extreme temperatures are also the reason why plasma is rarely used in atomic absorption spectroscopy, but it has become practically the standard in atomic emission spectroscopy, which we will discuss in ► Chap. 22. But since plasma is also an important basis for atomisation in general, it shall be treated here and now anyway.)

Harris, Section 20.2: Atomisation: flames, furnaces and plasmas

Inductively coupled plasma (**ICP**) is used for this purpose, in which the plasma flame is stabilised by the high-frequency magnetic field of an induction coil: The electrons present in the plasma are immensely accelerated in this field and, through collisions with the gas atoms, within a very short time transfer part of their energy homogeneously to the entire gas/plasma, which can remain stable for a practically unlimited period of time when contained by the induction field (assuming efficient cooling of the quartz burner used). This also results in an increased residence time of the analytes. The schematic construction of such a plasma torch can be seen in Fig. 20.12 of Harris.

Lab Note

Using this technique, the argon throughput is considerable: If such a plasma flare is used in continuous operation, a consumption of one compressed gas cylinder of argon per day is not uncommon. Despite the rather moderate price of this noble gas, the operation of an ICP flare is expensive in the long run.

In combination with an *ultrasonic atomiser,* which ensures even smaller analyte solution droplets and thus even smaller analyte particles in the gas phase, which in turn can be atomised almost quantitatively, the sensitivity of this method can be improved by another order of magnitude.

In particular, excitation by ICP can be combined very well with another analytical technique which you are already familiar with, at least by name, from Part III: If a mass spectrometer (MS) is used to detect the analyte ions instead of using optical detection as described so far in this chapter—i.e. if ICP/MS is used—the detection limit can be reduced by more than another power of ten. (Even though we will deal with mass spectrometry in general in more detail in "Analytical Chemistry II", you will already learn the first basics of this method in ► Sect. 22.3.)

Questions

1. Why do molecules (originating from the matrix) not lead to lines but to bands in atomic spectroscopy?
2. Why is the combination of a L'vov platform with an ICP flare not useful?

Atomic Absorption Spectroscopy (AAS)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_21

Summary
Atomic absorption spectroscopy is based on the law that any atom of any element can not only be made to *emit* radiation of element-specific wavelength(s) by sufficient (thermal or photochemical) excitation but can also *absorb* radiation of exactly the same wavelength very effectively (recognised as such by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff).

21.1 Excited States of the Atoms

Harris, Section 17.1: Properties of light

Behind this lies a principle that you probably already know from *General Chemistry* (and certainly from Part IV): the fact that electrons, as components of an atom or even a molecule, can only assume certain energy states, while others are simply unattainable. So it is about the **quantisation** of the energy content of the respective states—think for instance of UV/VIS spectroscopy and the possible transitions from HOMO (or HOMO-1 etc.) to LUMO (or LUMO+1, etc.). The same is true for transitions of electrons of single atoms:

- An electron can pass from the energetically least favourable occupied atomic orbital or—in the case of sufficiently strong excitation—also from one of the lower energy levels of the atom into one of the energetically higher atomic orbitals that are not yet occupied in the ground state.
  - According to Bohr's atomic model, this can be described by the fact that an electron changes from the valence shell (or one of the underlying shells) into an energetically less favourable higher shell.
  - The larger the energy difference for this electron transition, the more energetic (and thus: of shorter wavelength) is the photon that can cause such a transition.

Important
Even though this has already been addressed in *General Chemistry* and in Part IV: Again, the crucial thing here is the connection between wavelength λ, frequency ν, and energy content E:

$$E = h \times \nu = \frac{hc}{\lambda} \tag{21.1}$$

with h (Planck's quantum of action) = 6.626×10^{-34} J s; c (speed of light) = 2.998×10^{8} m/s

Binnewies, Section 2.3: The structure of the electron shell

- If an atom that was excited this way falls back into its ground state (i.e. if the electron returns to exactly the atomic orbital from which it was "taken out" by the absorption of energy), the atom emits a photon of precisely the wavelength which, according to Eq. 21.1, belongs to this energy content.

This is exactly what is exploited in atomic absorption spectroscopy (AAS for short):

- By thermal or photochemical excitation of a sample of one or the other metal, individual atoms are induced to emit the respective element-characteristic radiation. This results in an "atomic species-specific lamp" that emits only photons with *element-specific* wavelengths.
- The intensity of the received light radiation can be measured. (Again, parallels arise with topics already discussed in previous parts of this book: Now we are back to photometry from Part II, and in a moment we will also need Lambert-Beer's law again.)
- If this radiation (with known intensity I_0) is now directed onto a (suitably prepared) sample containing atoms of the same species, these analyte

atoms, since they are—being non-excited—currently still in the ground state, will absorb part of the photons emitted by the "atomic species lamp". Thus, after the light from this lamp has passed through the sample, the intensity of the radiation will be attenuated: $I < I_0$.

- The extent of the weakening of the intensity, indicated as **extinction**, then allows a conclusion to be drawn about the analyte atom content of the sample under investigation:

$$E = \lg \frac{I_0}{I} \left(\text{or } E = -\lg \frac{I}{I_0} \right) \tag{21.2}$$

At the same time, according to Lambert-Beer's law, the extinction can be described as:

$$E = \varepsilon_\lambda \times c \times d \tag{21.3}$$

Here ε_λ is again the (wavelength-specific) extinction coefficient (with the unit m^2/mol), c the concentration (commonly in accordance with the DIN 1310 known from Part I with the unit mol/m^3, *not* mol/L), and d the layer thickness of the irradiated sample. (If you think back to Sect. 18.1, where you learned how samples are prepared for analysis, it should be clear why the term "cuvette thickness" is not used here.)

The principle briefly described here (which was also touched upon in Binnewies) should inevitably lead to two questions:

Binnewies, Section 2.3: The structure of the electron shell (excursus)

■■ Do the "Atom-Specific Lamps" Emit Monochromatic Radiation?

Binnewies, Section 2.3: The structure of the electron shell

Not necessarily. After all, various electron transitions are possible, because the relaxation of an excited atom does not necessarily have to lead (directly) back to the *ground state* (we already know this from Part IV). Most of these transitions may be so energetic that the corresponding photons lie in the UV range or have even shorter wavelengths (or, conversely, they are so low in energy that they fall into the IR range), but there may well be more than one transition that lies in the VIS range.

Just think of the hydrogen atom and its characteristic emission spectrum (to be found as Fig. 2.14 in Binnewies). In principle, the one electron of the H-atom, which in the ground state populates the K-shell, i.e. the (s-)orbital with the principal quantum number n = 1, can be promoted to any higher shell (n = 2, 3, ...). Once this has happened, sooner or later the electron will "drop" back to the ground state, or at least to a less excited state, and the energy released in the process will be emitted in the form of one photon each at the wavelength corresponding to the energy difference between the different energy levels involved (◘ Fig. 21.1). Let us take a closer look at the various possibilities:

- In the ground state, the electron of the hydrogen atom is in the 1s orbital, i.e. in the "shell" with n = 1. Now, in principle, it can be transported into any conceivable orbital that is energetically less favourable, i.e. 2s, 2p, 3s, etc.

Important

In the case of the hydrogen atom, which is known to have only a single electron, the situation is somewhat simplified because here the orbitals of the same main quantum number, but differing in their secondary and/or magnetic quantum number do *not* differ in their energy content. Since they are therefore **degenerate**, these (actually different) energy levels need not be considered separately.

But this only *applies to the hydrogen atom! For all other atoms - i.e. for all multi-electron systems -, there is* indeed *a difference in energy e.g. for 2s and 2p orbitals!*

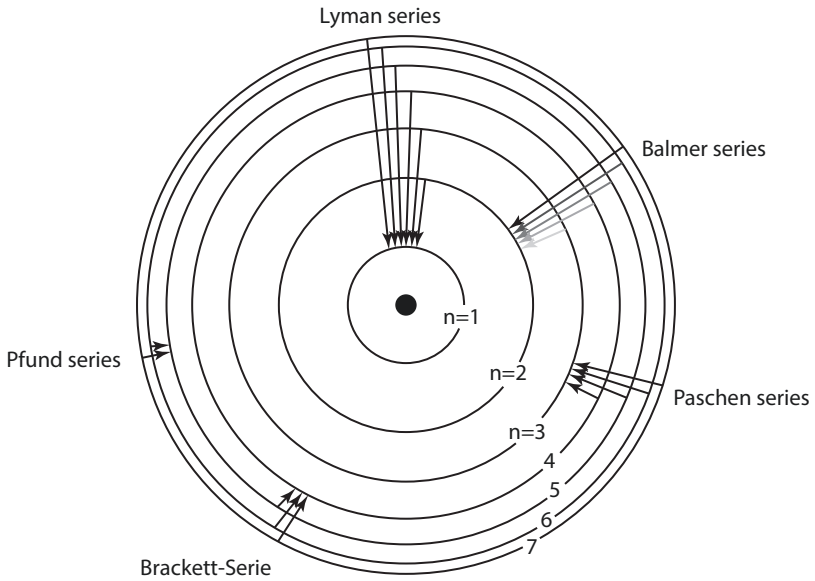


Fig. 21.1 Emissions of the excited hydrogen atom. (S. Ortanderl, U. Ritgen: Chemie—das Lehrbuch für Dummies, p. 139. 2018. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

- Thus, in the excited state, the electron can then return to the orbital with n = 1 (the K shell). These transitions (from n = 2 → n = 1, n = 3 → n = 1, n = 4 → n = 1, etc.) are called the **Lyman series** (Fig. 21.1). For all of these transitions, λ < 130 nm applies, so they all lie in the (high-energy) UV range.
- However, as mentioned above, it is not absolutely necessary that the electron immediately returns to its *ground* state. Relaxation to a *less excited* state is also possible. This opens up numerous other possibilities:
  - If the electron initially falls back to the energy level with n = 2, we are dealing with the **Balmer series**. This is responsible for the part of the hydrogen emission spectrum from Fig. 2.14 of the Binnewies, which lies in the range of *visible light* (Table 21.1).
  - The transitions in which the excited electron pauses intermediately at the energy level with n = 3 (i.e. n = 4 → n = 3, n = 5 → n = 3, etc.) are grouped together in the **Paschen series.** All wavelengths lie at λ > 820 nm, i.e. in the infrared range of the electromagnetic spectrum.
  - The photons emitted during the transition from *even* higher energy levels to n = 4 belong to the **Brackett series**. Here, λ > 1450 nm applies, so we are still in the IR range.
  - The transition to n = 5 also occurs with photon release. The wavelengths assigned to the **Pfund series** are even longer than all those mentioned so far: Even longer wavelength IR radiation is emitted.

Except for the transitions assigned to the Balmer series, none of this takes place in the VIS range, so the number of different wavelengths emitted by such an "atom-specific lamp" is at least very manageable.

Why Is This (polychromatic) Radiation Element-Specific?

This is where the fact comes into play that—as mentioned above—*only* in the case of the hydrogen atom the secondary and magnetic quantum numbers do

Table 21.1 The Balmer series

Transition	Wavelength [nm]	Color
n = 3 → n = 2	656	Red
n = 4 → n = 2	486	Green
n = 5 → n = 2	434	Blue
n = 6 → n = 2	411	Purple
n = 7 → n = 2	397	Purple

not have an influence on the relative position of the corresponding energy levels.

- For every other atom, therefore, there are first of all considerably more characteristic transitions (e.g. 3s → 2s, 3p → 2s etc.; the question which of those are symmetry-prohibited and which are permitted will not be considered further here). To each of these transitions a precisely defined wavelength can be assigned.
- In addition, a multi-electron atom (and they *all* are, except for hydrogen) has more electrons that can be excited—provided the right energy is supplied—, and that increases the number of individual spectral lines even more.
- All in all, this results in a kind of "spectral line fingerprint".

The respective position of the individual energy levels (1s, 2s, 2p etc.) of different atoms depends decisively on two factors:

- The **atomic number** (Z) of the atom under consideration, i.e. the number of protons in the nucleus, leading to the overall charge of the nucleus,
- The extent to which the orbitals farther from the nucleus are *shielded* by the underlying (more energetically favourable) orbitals occupied in the ground state.

The stronger the shielding, the weaker the electrostatic interaction between the positive nuclear charge and the negative charge of the electrons located in the orbitals under consideration. This is all the more true the further away from the nucleus the electrons under consideration are, i.e. the larger their *principal quantum number n* is. On the basis of these two factors, a screening factor σ can be determined, at least for the principal quantum numbers of the energy levels considered, with the help of **Slater's rules,** which (once again) go beyond the scope of this introduction. This then leads to the **effective nuclear charge (Z_{eff}):**

$$Z_{eff} = \sigma \times Z \tag{21.4}$$

This effective nuclear charge describes the positive charge density that *actually* provides the electrostatic attraction between the atomic nucleus and the electron under consideration.

It should be emphasised once again that the effective nuclear charge with which an electron interacts depends on its respective principal quantum number (and thus on its distance to the nucleus): Different electrons of the same atom "feel" different effective nuclear charges.

One Remark

As you can see, in atomic spectroscopy the principal quantum number is clearly more important than the other quantum numbers. For this reason, the Bohr atomic model with its shells (K, L, M, N ...) still enjoys great popularity in atomic spectroscopy. (So there *was* a reason why in *General Chemistry* any and all explanations were not exclusively based on orbitals, but also on shells.)

We will meet the effective nuclear charge (Z_{eff}) again in ► Chap. 23.

All in all, this means that the energy differences of the respective energy levels are slightly different for each type of atom, so corresponding electron transitions (whether in the UV, VIS, or IR range) will also result in slightly different wavelengths each time.

Questions

3. For which transition more energy has to be absorbed: To excite an electron from the K-shell to the M-shell, or (assuming the same type of atom) for a transition from the L-shell to the N-shell? At which relaxation to the respective initial state of this model atom is longer-wavelength radiation emitted?

Having just explained why the element-specific lamps used in atomic absorption spectroscopy do not emit monochromatic light, but several discretely separated wavelengths, the question arises how to select *one* (namely the desired) wavelength from them. For this purpose, a monochromator is used—exactly the same device that has already been discussed in Part IV. Whether the monochromator used is based on *refraction* or *diffraction of* the polychromatic light is irrelevant.

21.2 Light Sources

Now let us take a closer look at the "element-specific lamps" required in the AAS.

The Hollow Cathode Lamp (HCL)

Hollow cathode lamps are frequently used as gas discharge tubes in which there is a cathode and an anode in addition to the gas filling and which emit light when the minimum voltage required to ionise the gas is applied. Such a hollow cathode lamp (which is almost always just abbreviated to **HCL**) is filled with a noble gas, usually neon or argon.

The crucial thing is that the cathode *used must consist of exactly the element that is to be quantified by AAS.* (The approximate pot-shape of this cathode then also explains its name.)

- The applied voltage causes the ionisation of the filling gas; thus, noble gas cations are formed.
- These are accelerated in the electric field due to their (positive) charge (we already know this from Part III), then hit the cathode with considerable energy and knock out atoms of the respective metal used there. (In this context, one speaks of **sputtering**.)
- These knocked-out metal atoms then, in turn, meet the extremely energetic electrons created during ionisation.
- The transfer of part of their energy causes the excitation of the knocked-out metal atoms, as already described at the beginning of this section—and this excitation energy is then released again in the form of element-specific photons.

The schematic structure of such a hollow cathode lamp can be seen in Fig. 20.16 of Harris.

Harris, Section 20.4: Atomic spectroscopy, apparatus

An Alternative: The EDL

An alternative to the HCL is the **electrodeless discharge lamp, EDL** for short. Here, a sealed quartz tube is used, which is also filled with a noble gas and which additionally contains traces of the desired metal—optionally elemental or in the form of a salt. (The fact that the metal atoms are present in the form of their (cat)ions without excitation is irrelevant in view of the homolytic bond cleavage that occurs as a result of the excitation: If there is sufficient excitation, *atomisation* does occur.) In this case, the energy is not supplied by means of cathode and anode but by a high-frequency field (which can also be generated by a microwave field). Again, with sufficient excitation, ionisation of the noble gas occurs; the (high) frequency of the field causes acceleration of the resulting ions, which then enable *sputtering*.

In principle, the (more modern) EDL is superior to the HCL: The radiation emitted by it is more intense and thus allows the detection of even smaller amounts of analyte. However, there are (still) two reasons why the use of such **induction lamps** is often dispensed with:

- Up to now, on the market there are not yet suitable light sources available for all elements.
- The acquisition costs are (still) significantly higher than those of a standard HCL.

This should not only explain the principle of AAS and the rough technical aspect of the excitation lamps used but also the biggest *disadvantage* of this analytical method: Since the atoms of the investigated element are quantified on the basis of their absorption of the radiation of an element-specific lamp, one can only look for *one* element at a time, because the excitation lamp must be based on exactly the respective element. A "broadband investigation", which would allow the detection of several different elements, is thus impossible. (The situation is quite different with atomic emission spectrometry, which we will discuss in ► Chap. 22.)

Atomic Emission Spectrometry (AES, OES)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_22

Summary

As an alternative to letting a well-defined amount of light pass through a sample and measuring the resulting absorbance or extinction, as described in ► Chap. 21, one can also do the reverse: The analyte is excited sufficiently so that it itself emits light (or other electromagnetic radiation). Since the quantisation of energy also plays a role here, the energy content of the resulting wavelength(s) can be calculated according to ► Eq. 21.1.

Harris, Section 20.4: Atomic spectroscopy, apparatus

However, in order to excite a sufficient number of atoms to the point of emission, a not inconsiderable amount of energy is required. In the case of alkali and alkaline earth metals, the 2000 °C of the standard laboratory propane gas flame suffices (this has already been mentioned in ► Sect. 20.1.1), but for all other metals a quantifiable level of emission is only achieved at much higher temperatures—which is why the plasma from ► Sect. 20.1.3 is very popular here.

22.1 The Historical Precursor: Flame Photometry

The term "flame photometry" may already suggest it: The first attempts to make quantitative statements on the basis of flame colourations had striking similarities with photometry, which we have already discussed in Part II: Depending on the analyte content, the flame colouration characteristic of the respective (alkaline earth) metals is more or less pronounced. The "non-luminous burner flame" is usually chosen as the excitation source, i.e. a *lean flame*, which is abundantly supplied with oxygen and in which no incompletely reacted combustion intermediates occur (which are otherwise responsible for the orange-yellow hydrocarbon flame typical of the laboratory).

Due to the moderate excitation temperature, the emission spectra obtained by this method, known as *flame atomic emission spectrophotometry* (**F-AES**), show a relatively small number of line, so that it is quite easy to "cut out" the desired wavelength—in most cases, this does not even require a dispersing monochromator: A simple optical filter may be sufficient (of course, this also depends on the accuracy desired), and a photometer and a corresponding calibration curve already should lead to useful results.

However, the method using a simple burner flame is not particularly sensitive. If more precise measurements are to be made, the use of a plasma torch is also recommended for the (earth) alkali metals.

22.2 ICP-OES

Harris, Section 20.5: Interference

There are different variants of plasma emission spectrometry; the most important of these is again based on the use of *induced coupled plasma*. This allows—due to the very element-specific photon emission—the quantification of several elements within one single measurement: Depending on the experimental setup chosen, more than 60 analytes can be determined simultaneously.

There are two things to specifically mention about this process:

- Unlike AAS, *no* element-specific excitation lamps are required here.
- The (polychromatic) emission is split into its corresponding wavelengths by a diffraction grating (or a similar device) and then analysed separately—usually using a photomultiplier. (The basics of the photomultiplier were at least roughly touched upon in Part IV.)

Because an *optical* separation of the wavelengths obtained is performed here, this method is generally referred to as **ICP-OES** *(inductively coupled plasma optical emission spectrometry)*. Terms such as "atomic emission" are commonly avoided because the emission spectra obtained by ICP-OES are not exclusively due to intra*atomic* excitations: *Ions* also contribute to them.

- **MPT-AES**

A variant of ICP-OES is *microwave plasma torch atomic emission spectrometry* (**MPT-AES**). The main difference is that the containment field required to stabilise the necessary plasma is generated by microwaves. MPT-AES is used primarily in trace analysis and is somewhat less complex in terms of equipment; in addition, argon consumption is more moderate with this technique. (Since temperatures of 10,000–12,000 K are reached here, the analytes are largely *atomised*, so that one can actually use the term "*atomic spectroscopy*" here.)

An Application Example

AAS is ideally suited for trace analysis, for example for the analysis of water samples: The detection limits for the majority of the elements lie in the range of 0.001–0.025 ppm in the "classical" AAS or $2 \cdot 10^{-3}$ to $1 \cdot 10^{-2}$ ppb (that is 2–10 ppt!) in the ICP-OES, i.e. even the tiniest amounts can be detected.

However, before you can get down to work, you must—understandably—also draw up a calibration curve for each element to be quantified in order to ensure that the concentration of the analyte solution still lies in the concentration range in which there is a linear relationship between concentration and absorbance in the sense of Lambert-Beer's law. If one wants to be absolutely sure that the measurement results are really comparable with each other, it is recommended to measure a reference sample (i.e. solutions of known analyte concentration) immediately *before and after* the analysis of the analyte solution because the number of variables that are difficult or impossible to control is enormous both during atomisation and during the actual measurement. If deviations then occur in the measurement results of the reference solutions, these can be correlated with the calibration curve via a *correction factor*, and then statements can also be made about the actual analyte content of the sample to be measured.

The results of such an experiment are shown very clearly—albeit on the basis of a variant of AAS in which atomic fluorescence is used, which will not be discussed further in this introduction—in Fig. 20.4 from Harris: In this, using a sample of drinking water whose lead content is to be determined via AAS, it also becomes clear how the different standard solutions lead to signals of different heights.

Harris, Section 20.1: Atomic spectroscopy, overview

22.3 In Combination with Mass Spectrometry

The methods described in this chapter can also be combined with other analytical procedures. The interaction with **mass spectrometry** (MS) is particularly important. We will discuss this in more detail in Part I of "Analytical Chemistry II" but the underlying principle shall be already explained here:

Harris, Section 20.6: Inductively coupled plasma mass spectrometry (ICP-MS)

The analytes are first ionised—in ICP-MS by the argon cations (Ar^+) from the plasma. The resulting analyte ions then break down into individual fragments which, supported by a magnetic field, are immediately separated according to their mass (or to be more precise: their *mass to charge ratio* m/*z*) and observed separately. The fragments obtained this way allow conclusions to be drawn about the overall structure of the analyte in question.

A Thought Experiment

Suppose you had quite a few puppets of the same kind but did not yet know *what* form they were (a human being, an animal—and if the latter: horse or fish?). These "analyte puppets" are now in large numbers fed to a large apparatus which more or less at random cuts one or the other of the connecting cords of the individual joints: perhaps only one, perhaps several at a time. This way you get a large number of "puppet fragments", which you first look at separately, drawing initial conclusions from them, and then relate the information obtained to each other:

- If you find single legs (or even larger puppet fragments with one or more (more or less complete) legs attached to them), but nothing that would correspond to or contain a fin, you can already rule out the working hypothesis "it is a fish".
- Once you have discovered the first puppet fragment that has *three* legs, the same applies to any *two-legged* animal or even a humanoid puppets.
- If the fragment you assume to be a head has antlers, you know you cannot possibly be dealing with a puppet of a horse, and so on.

It is, therefore, a matter of linking the individual pieces of information that you have gained from the respective fragments in a meaningful way. It is a bit like putting together a jigsaw puzzle whose overall motif you do not yet know—challenging, but entertaining at the same time.

Let us leave it at that for the moment. So now you know that your analytes—in this case, the puppets of initially unknown shape—are fragmented and that you can draw conclusions from the resulting fragments. In real-world mass spectrometry, of course, real-world analytes are fragmented, usually in molecular form, with some bonds (in our thought experiment: the strings of the individual puppet joints) being severed/broken. What the resulting spectra look like and how they are to be interpreted, you will learn in Part I of "Analytical Chemistry II".

When initially dealing with a mixture of different analytes, those must, of course, be separated from one another prior to fragmentation (for example, chromatographically, as described in Part III).

With regard to mass spectrometry, two basic things should be kept in mind:

1. The respective analytes are mostly molecules that are fragmented *individually*—but in each case in (very) large numbers. (Please never forget how large **Avogadro's number** is!) The analytes are each converted to (radical) cations in the course of ionisation, which in the vast majority of cases are not stable and therefore decay further. This decay can occur in different ways (with some bond breaks being much more likely than others—this makes interpreting the resulting spectra much easier). Of course, after ionisation, each individual analyte molecule can only follow *one* of the (often numerous) possible decay paths, but at the same time a (really large) number of analyte molecules are fragmented simultaneously. This results in a purely statistically determined mixture of diverse, which are detected and analysed in parallel.
2. *Each individual fragment* has a characteristic mass—depending on which atoms it is composed of. Here, it must be noted that the fragments obtained in each case are actually considered (or detected) *individually:* Thus, the individual masses of the individual *atoms* contained therein are responsible for the respective masses. Accordingly, two "identical" fragments, which consist of the same atoms but contain different **isotopes**, differ in their effective mass—or, to be more precise, in their **mass/charge ratio** m/z,

because in the course of ionisation, multivalent cations (X^{2+} or even X^{3+}) may well be formed, even though singly ionised fragments usually are the vast majority. (The role of isotopes in mass spectrometry is also discussed further in "Analytical Chemistry II".)

The mass spectrometric analysis of *ionic* compounds (i.e. analytes consisting of ions) is also possible (although less common); these analytes are then separated into the respective cations and anions. In the case of polyatomic molecular ions (such as nitrate or sulfate, and even more so in the case of more complicatedly structured cations and anions, such as alkylammonium ions, cationic or anionic metal clusters or similar), fragmentation (see above) again is to be expected.

The fact that via mass spectrometry actually *individual* atoms or ions are detected already indicates that this is an extremely sensitive detection method: Depending on the analyte, the detection limit lies in the **ppt range**, i.e. in a dilution or content range of $1{:}10^{12}$. Just to illustrate what this means: Based on a sample volume of 1 L, analyte quantities in the nanogram range can still be detected this way, making ICP-MS ideal for many analytes, especially in trace analysis.

Lab Tip

The high sensitivity of the ICP-MS technique naturally also has a certain disadvantage: One must work *extremely* cleanly. The slightest contamination—for example through insufficiently cleaned glassware or similar—immediately leads to significant falsification of the measurement results.

Despite the high performance of ICP-MS, there are three (potential) problems with this method:

- If parts of the matrix get into the mass spectrometer together with the analyte, this naturally leads to massive interferences.
- If the analyte is an organic compound, presumably one that is comparatively rich in carbon, there is a risk that at least some of the carbon atoms will be converted to *soot* (i.e. elemental carbon)—which not only will falsify the measurement result, but also harbours the risk of purely mechanical sticking to the measuring equipment. However, this can usually be prevented by a targeted supply of oxygen, because the soot is then converted into carbon according to the equation

  $$C + O_2 \rightarrow CO_2$$

  and correspondingly passes into the gas phase.
- If the sample contains chlorine, things get particularly interesting—regardless of whether the halogen originates, for example, from the hydrochloric acid with which the sample was brought into solution or whether the analyte itself contains one or the other chlorine atom. Here, we should definitely keep in mind that in the plasma flare there is no longer any difference between covalently bonded chlorine atoms and chloride ions, after all both are atomised to chlorine *atoms* ($Cl^{\cdot}$)—and chlorine atoms are **isoelectronic** with the argon cations ($Ar^{\cdot+}$) from the plasma. Accordingly, these two particles show similar chemical behavior, and thus $ArCl^+$ is formed, among other things.
- Of course, the argon cations originating from the plasma can also react with other atoms in the gaseous phase: ArO^+, ArC^+, ArN^+, etc. are obtained.

Chemically speaking, noble gas *atoms* are practically inert—but this does not apply to any *ions* resulting from them: An argon cation, for example, no longer fulfils the octet rule and will therefore easily enter into chemical reactions—with practically all atoms within the effective range of the plasma flare.

Of course, these ions also each have an individual mass, and this (or their mass/charge ratio, m/z) can certainly have a disturbing effect—especially when it is (almost) identical to the mass of the currently relevant analyte. Thus, m/z(ArO^+), if the isotopes ^{40}Ar and ^{16}O are involved, corresponds to that of the iron isotope ^{56}Fe, etc., unless one also consides the *second digit after the decimal* point. Accordingly, the associated m/z peak in the mass spectrum will be unduly large—unless the resolution of the mass spectrometer is large enough to detect mass differences <0.02: If the resolution is *not* large enough, the resulting problem is called **isobaric interference**.

Questions

4. What could be done to ensure that the matrix does not interfere with ICP-MS?
5. In the analysis of which element does the $^{40}Ar_2^+$ cation formed from the plasma cause isobaric interference?

X-Ray Fluorescence Analysis (XRF)

Contents

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3_23

Summary

As already mentioned in ► Chap. 20: Of all the analytical methods presented in this section, only ***X-ray fluorescence* analysis** (**XRF** for short) is non-destructive. This makes it suitable for examining the composition of materials or components that are ready for use, as well as works of art (e.g. "Which pigments were used?"), which should be reason enough to take a closer look at this method.

You already know the basics of fluorescence from Part IV, but this time the resulting data are not a consequence of the excitation and relaxation of *valence electrons*.

Unfortunately, Harris does not address X-ray fluorescence analysis; an excellent overview is provided in:
Cammann E, Instrumentelle Analytische Chemie, Section 4.7: X-ray fluorescence analysis
Skoog DA, Holler FJ, Crouch, SR, Instrumental analysis, Section 12.3: X-ray fluorescence methods

23.1 The Basic Principle

As in "ordinary" fluorescence, electrons are excited in X-ray fluorescence analysis by electromagnetic radiation—however, the corresponding X-ray photons are energetic enough to have an effect not on valence electrons but on **core electrons.** Such a high-energy X-ray photon then leads to the **photoelectric effect**, so that the cation present after interaction with this photon has a gap in the population of one *inner* electron shell (◘ Fig. 23.1 *left*). The resulting non-population of an energetically more favorable energy level with simultaneous population of a higher level leads to a more nucleus-distant electron (from one of the outer shells) moving into this gap. This naturally reduces said electron's potential energy, so energy is released.

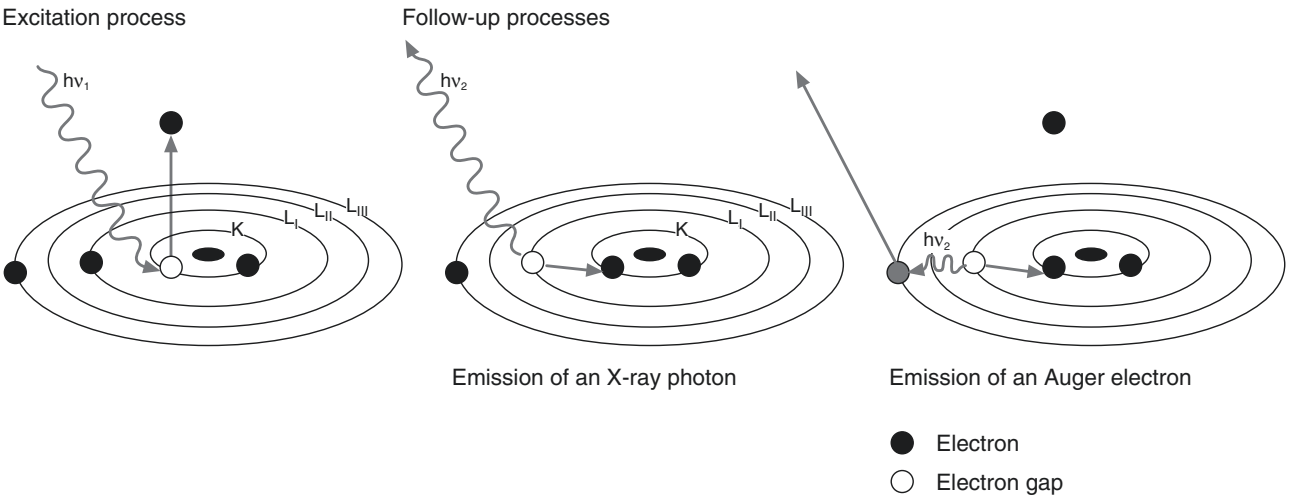
This energy released is emitted in the form of a new photon (◘ Fig. 23.1 *centre*), whose wavelength is again *element-specific.*

The wavelength of the photon in question depends on two factors (in addition to the type of element, i.e. its atomic number, i.e. the number of positive charges in its nucleus):

- From which (core) shell was the first electron knocked out?
- From which (core or valence) shell does the electron come, which changes its potential energy accordingly?

(The relationship between the energy difference and the resulting wavelength is summed up in ► Eq. 21.1.)

Since the analyte atoms thus have several possibilities for fluorescence (with correspondingly different resulting wavelengths), one again obtains an X-ray



◘ **Fig. 23.1** Induced X-ray fluorescence and the Auger effect . (K. Cammann (Ed.): Instrumentelle Analytische Chemie, pp. 4–80, Fig. 4.49, 2010 Copyright Spektrum Akademischer Verlag Heidelberg. With permission of Springer)

fluorescence *spectrum*, not just a single fluorescence line. However, some basic facts facilitate the handling of the measurement results immensely:

- In most cases, the electron knocked out by the interaction with an X-ray photon comes from the 1s orbital of the atom under consideration, i.e. from the **K shell.**
- The (quantum-mechanically) most probable result is the moving "downwards" of an electron from the immediately neighbouring L shell. The energy difference between the K and L shells of the atom under consideration then leads to the wavelength to be calculated according to ► Eq. 21.1; in the resulting X-ray fluorescence spectrum, this line is called the **K_α line.** In most cases, this element-specific line is already sufficient to identify an element unambiguously.
  - Possible, *but much less probable,* is an advancement from the M shell into the K shell (in this case the L-shell is "skipped"). This leads to the (because of the energy difference between K and M shells being larger clearly more energetic) **K_β line** . However, since this relaxation is (*vide supra*) less probable than the one leading to the K_α line, the corresponding line is recognisably less pronounced and plays only a minor role in analytics. Only in the (few) special cases in which the K_α line alone is not meaningful or unambiguous enough, one pays attention to the K_β line.
  - For sufficiently large atoms, where the N shell is also (partially or completely) occupied in the ground state, a *K_γ line* can also be observed, but this is of no further importance in analytics.
- Somewhat more likely than the N→K transition, which leads to the K_γ line, is the ionisation of the analyte by knocking out an electron from the L shell. If an electron from the M shell then follows "downwards", the (much less energetic) L_α line results.
- If the analyte in turn has a partially or fully occupied N shell, an L_β line is also possible, analogous to the K_β line, which, however, is usually at best *distinguishable* from the background noise of the spectrum but hardly usable.

Actually

For quantum mechanical reasons, there are *two* K_α, K_β and K_γ lines each; in the case of the (less pronounced) L lines, the system has even more energetically slightly different relaxation possibilities. This can be explained in more detail by corresponding **selection rules** based on the quantum numbers of the electrons involved in each case. Here, phenomena such as *spin-spin* and *spin-orbit coupling* have an effect, which, however, would go far beyond the scope of this introduction.

■ Energy Data

The photons emitted in X-ray fluorescence analysis are significantly more energetic than the (familiar) UV/VIS range, i.e. the associated wavelengths are much shorter. Kindly remember:

- The wavelength of radiation from theUV/VIS range lies between 200 and 800 nm. This corresponds (in reverse order, after all the wavelength of a photon is *inversely* proportional to its energy content!) to an energy content of 150–600 kJ/mol.
- X-rays are λ = 5–250 pm, i.e. 0.005–0.250 nm, which corresponds to an energy content of 480,000–24,000,000 kJ/mol , i.e. 480×10^3–24×10^6 kJ/mol.

Because such large numbers are slightly unwieldy, it is common practice when dealing with X-rays to express the energy content not in kJ/mol but in electron volts (eV) or, for even larger numbers, in kiloelectron volts (keV).

Here, 1 eV is defined as the increase in energy (due to acceleration) of an elementary charge in an electric field of the strength 1 V. The conversion factor is quite simple:

$$1\ \text{eV} = 1.602 \times 10^{-19}\ \text{J} \tag{23.1}$$

Important

Please note: This is for *a single particle*. Converted to a mole, the result is:

$$\begin{aligned} 1.602 \times 10^{-19}\,\text{J} \times \text{N}_\text{A}\,\text{mol}^{-1} &= 1.602 \times 10^{-19}\,\text{J} \times 6.022 \times 10^{23}\,\text{mol}^{-1} \\ &= 96472.44\ \text{J / mol} \\ &= 96.5\ \text{kJ / mol} \end{aligned}$$

Thus the UV/VIS range lies in the order of 1.5–6 eV, while the X-ray range is to be found between 4960 eV = 4.96 keV and 280 keV. (If, after reading Part IV, you prefer **wavenumbers** ($\bar{\nu}$) instead of **wavelengths,** you are, of course, free to convert them accordingly—you learned how to do this in Part IV, but it is not common practice in XRF ...)

Such conversions take a little practice (and getting used to).

An example of a unit converter:
► http://cactus2000.de/de/unit/masswav.shtml

The Internet offers numerous unit converters that can do the work for you.

The photon yield in fluorescence increases with the atomic number of the atoms involved, while atoms with too low a nuclear charge are no longer detectable: If an atom does not have enough electrons to "move downwards" at all, fluorescence understandably fails to occur. The first element that can be detected *at all* by XRF—and, except with the most modern, highly sensitive instruments, mainly only *qualitatively*—is beryllium (with the electron configuration $1s^2 2s^2$); beginning with fluorine ($1s^2 2s^2 2p^5$) XRF works reasonably well also *quantitatively*. Only from atomic number 15 (i.e. phosphorus) upwards does XRF allow *serious* quantitative statements to be made, even with somewhat older instruments. For heavier atoms, on the other hand, the detection limit lies (for some elements even significantly) below 20 µg/g sample substance; here, the **ppm range** is already reached.

▪ Quantitative Considerations

The relationship between the wavelength of a fluorescence photon obtained by the photoelectric effect and the atomic number of the element under consideration is described by **Moseley's law**. Commonly it is formulated as follows, related to the energy content of the photon in question according to ► Eq. 21.1:

$$\text{E} = \text{h}\nu = \text{R}_\infty \text{hc}\left(\text{Z} - \sigma\right)^2 \left(\frac{1}{\text{n}_1^2} - \frac{1}{\text{n}_2^2}\right) \tag{23.2}$$

The symbols are:

- R_∞ is the Rydberg constant (with the value 109,737 cm^{-1}),
- h is the (now familiar) Planck' quantum of action (6.626×10^{-34} Js),
- c is the speed of light (2.998×10^8 m/s),
- Z is the atomic number of the element under consideration,
- σ is the *shielding constant* (which can be calculated according to the Slater rules) (we already know this from ► Sect. 21.1) *and*
- n are the principal quantum numbers of the shells involved; n_1 is smaller (i.e. closer to the atomic nucleus) than n_2.

Conversion by wavelength and using ► Eq. 21.4 then leads to:

$$\lambda = \frac{1}{R_\infty Z_{eff}^{\ 2}} \left(\frac{n_1^2 n_2^2}{n_2^2 - n_1^2} \right) \tag{23.3}$$

This way, you can immediately see that the larger the difference between n_2 and n_1, the smaller the wavelength emitted. (Which brings us back to question 3 of this part of the book ...)

■■ A General Comment

Since all intraatomic events caused by the interaction with X-ray photons take place in the atomic core, the *bonding conditions* (ionic, polar or nonpolar covalent, metallic) of the atoms under consideration are irrelevant for X-ray fluorescence analysis. Therefore, it is also not necessary to first detach the atoms from their respective molecular or crystal bond—which, of course, explains why XRF is *non*-destructive.

■ Auger Electrons

A possible difficulty arises if the photon emitted when the electron (from whichever shell) moves "downwards" is energetic enough to knock *another* electron out of the (already ionised) atom (shown schematically in ◘ Fig. 23.1 on the *right*). The additional electrons knocked out by these fluorescence photons are called **Auger electrons** (named after the French physicist Pierre Auger, so this name should be pronounced accordingly). However, the kinetic energy of such an electron is just as element-specific as the wavelength of the fluorescence photon in question and can therefore also provide analytical information—in this case, one performs **Auger electron spectroscopy** (occasionally abbreviated **AES,** which creates ideal conditions for confusion with atomic emission spectrometry (from ► Chap. 22), also known to be abbreviated AES). However, the *Auger effect* does not reach very far (only a few atomic layers); in this respect Auger electron spectroscopy is particularly suitable for the analysis of material *surfaces*.

23.2 Detection of the Resulting Photons

Of course, the photons emitted in X-ray fluorescence and/or the Auger effect must also be detected. In principle, a distinction is made here between two different detection methods:

- If the *energy content of* the fluorescence photons is measured, **energy dispersive X-ray fluorescence analysis (EDRFA)** is used.
- If, on the other hand, the resulting photons are split up according to their *wavelength* (the same way as in Part IV for electromagnetic radiation of much longer wavelengths: with a dispersion element, such as a diffraction grating), we speak of **wavelength-dispersive X-ray fluorescence analysis (WDXRF)**.

■ EDRFA—Energy Dispersive

The detectors used determine the energy content of the detected X-ray photons. The photon in question hits the detector material and causes excitation there which is (of course) proportional to the energy content of the photon. However, since certain (statistical) fluctuations have to be taken into account (e.g. the detector atom which has interacted with the X-ray photon may also

have been in a (rotationally) excited state—we already dealt with this problem in Part IV—), no "sharp lines" are obtained but peaks with a certain resolution (i.e.: selectivity of neighbouring peaks).

■ WDXRF—Wavelength Dispersive

Alternatively, the resulting polychromatic fluorescence radiation can also be broken down into its respective wavelengths; this way, the individual photons obtained can be described. For this purpose, the resulting fluorescence radiation is diffracted at an analyser crystal—the extent of diffraction (the diffraction angle) allows direct conclusions to be drawn about the wavelength of the respective photons. (Already the breaking down of white light on a prism shows that radiation of shorter wavelength is refracted more strongly than radiation of longer wavelength; for electromagnetic radiation of higher energy the same applies, of course.) The selectivity here is generally much better than with energy-dispersive detection.

■■ Advantages and Disadvantages

Both detection methods, of course, have their advantages and disadvantages:

- The resolution of the WDRFA is significantly better than that of the EDRFA:
  - For the WDXRF, it is of the order of 10 eV.
  - With EDXRF, the selectivity of 100–500 eV is weaker by more than one order of magnitude.
- On the other hand, the energy dispersive detection is much more sensitive; accordingly this detection method can be better used in trace analysis.
- In addition, the optics responsible for a correspondingly high resolution in the WDXRF and also the corresponding analyser crystals used are quite expensive.
- The EDRFA requires much shorter measurement times.

The faster and less expensive energy dispersive detection is, therefore, undoubtedly more suitable for "everyday laboratory use", while the more cost-intensive wavelength dispersive detection is mainly used for high-performance analyses.

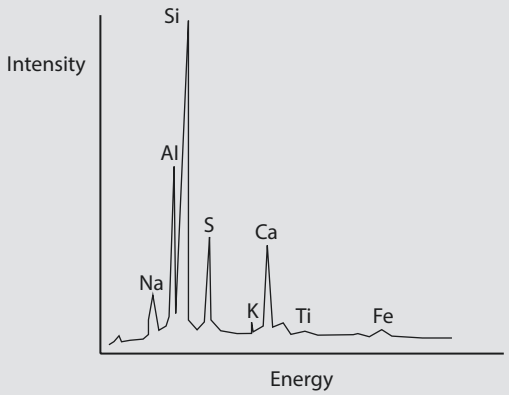
An Sample Application

Since XRF enables the non-destructive analysis of materials, it is often used e.g. to check the composition of components made from one alloy or another. However, the scope of this method is by no means limited to alloys: For example, during the restoration of the famous Raphael painting "Madonna del Prato" (also known as "Madonna of the Meadow", 1505/1506; an illustration of this painting can be found in the English-language Wikipedia), the problem arose which pigment was used for the striking blue colouring of the Marian vestment at the time. The options were

- azurite (a basic copper carbonate of the composition $2CuCO_3 \cdot Cu(OH)_2$) *and*
- Ultramarine blue (an aluminosilicate with polysulfide inclusions; the general formula is $Na_{8-10}Al_6Si_6O_{24}S_{2-4}$).

In the energy-dispersed XRF spectrum obtained from the examination of the painting, the characteristic K_α and K_β lines of copper were not found, but corresponding lines for sodium, aluminum, silicon and sulfur were clearly visible: Thus the question was unambiguously answered.

► https://en.wikipedia.org/wiki/Madonna_del_Prato_(Raphael)#/media/File:Raphael_-_Madonna_in_the_Meadow_-_Google_Art_Project.jpg



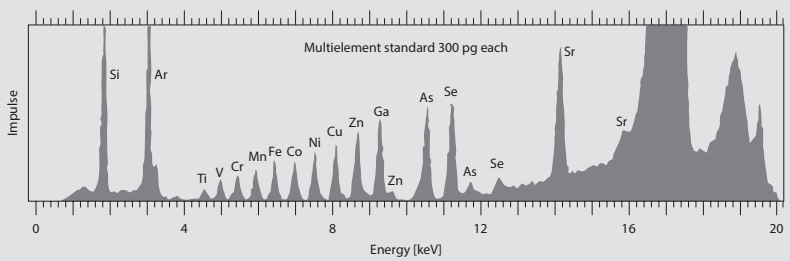
Energy-dispersed XRF spectrum of an art-historically important sample (M. Otto: Analytische Chemie, p. 195, Fig. 3.47. 2010. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

However, the use of standards is also essential in such analyses. As is so often the case, a distinction can be made between the use of external or internal standards. External standards are of course much more convenient, because for the use of an *internal* standard, two further things have to be considered:

- Either the sample would have to be changed by adding the reference substance—which must not be part of the sample to be measured!—in a precisely known quantity. In this case, however, it would obviously no longer be possible to take advantage of the fact that XRF in itself is *non*-destructive.
- With sufficiently well-known samples, one could—if applicable—make use of the fact that those contain an element suitable as a reference in exactly known quantity. But, of course, this must first be the case.

In the case of *external* standards, another problem arises: The fluorescence processes allowing measured values do not only take place on the *surface* of the material to be investigated but also *inside* it. In this respect, only substances whose overall composition is as similar as possible to that of the analyte are suitable for use as external standards; otherwise, *matrix effects* occur, which, however, will not be discussed further here.

However, once a suitable matrix has been found for the reference sample, it is easily possible to produce corresponding multi-element standards in which a large number of elements are present, each with a precisely known content. The measurement curve of such a standard is shown in the exemplary figure below. (The remarkably high values above 15 keV are due to the molybdenum tube used for the measurement.



Characteristic curves of various metals contained in a multi-element standard (K. Cammann (ed.): Instrumentelle Analytische Chemie, p. 4–88, Fig. 4.55b. 2010. Copyright Spektrum Akademischer Verlag Heidelberg. With permission of Springer.)

Questions

Why are M-lines not important in XRF?

Why is it not to be expected that the photon released via the Auger effect causes further ionisation of the analyte atom (or ion)?

23.3 Summary

In atomic spectroscopy, the atoms of the element to be quantified are

- either excited with suitable electromagnetic radiation, whereby the quantification is carried out via the absorption—following Lambert-Beer's law—of the excitation radiation (*absorption spectroscopy*),
- or on the basis of the radiation emitted during the relaxation that occurs after excitation (*emission spectroscopy*).

In both cases, the bonding state of the analyte atoms (ionic, covalent, metallic) is irrelevant, because the samples to be investigated are atomised prior to the measurement.

Atomisation takes place

- by means of gas flames (which, except for the classical flame photometry of alkali and alkaline earth metals, is mainly of historical interest),
- in the (graphite) furnace, which provides even better results using a L'vov platform, *or*
- by plasma. Nowadays, the plasma torches used in this process are usually stabilised by a high-frequency induction field, so that one speaks of induction-coupled plasma (ICP).

23.3.1 Atomic Absorption Spectroscopy (AAS)

The atoms to be quantified are excited with element-specific radiation. This radiation stems from discharge lamps (hollow cathode or induction lamps are used), each of which contains the element to be quantified. For this reason, with the AAS only *one* element can be quantified at a time.

- During excitation, electrons of the analyte "move upwards" to energetically less favorable shells not occupied in the ground state. Not all conceivable transitions can be effected with radiation from the VIS range, but it is precisely these transitions, of which the corresponding absorption reduces the intensity of the excitation radiation, which form the basis of this technique.

23.3.2 Atomic Emission Spectroscopy (AES)

In atomic emission spectrometry (AES) , whose historical precursor is flame photometry (F-AES), the analyte is excited to emit element-specific radiation. The resulting emission spectra also allow the quantification of different elements in parallel (more than fifty, depending on the technique used).

- The measurement methods commonly used today are based almost exclusively on induction-coupled plasma; since not only atoms but also ions contribute to the resulting emission spectrum, this is referred to as *optical emission spectrometry* (OES).

- An alternative to ICP-OES is *microwave plasma torch atomic emission spectrometry* (MPT-AES), in which the plasma is contained by a microwave field; this technique is particularly common in trace analysis.
- The combination of OES with other analytical techniques can dramatically increase the sensitivity of this method; especially important is the combination with mass spectrometry (ICP-MS).

23.3.3 X-Ray Fluorescence Analysis (RFA)

High-energy X-rays can cause a photoelectric effect in the core electrons of the affected atom. The resulting electron gap on an energetically more favorable energy level than the valence shell leads to the "moving downwards" of a more energetic electron, with the energy released in the process being emitted in the form of a fluorescence photon.

- The wavelengths of the fluorescence photons obtained are element specific.
  - The energy content of the fluorescence photons obtained is usually expressed in eV or keV.
  - The detection of the photons can be energy dispersive (EDRFA) or wavelength dispersive (WDXRFA) .
- Since XRF is based on the excitation of core electrons, the bonding state of the atoms concerned is also irrelevant here.

The fluorescence photons can cause a new ionisation of the affected atom; the Auger electrons knocked out this way can also be used analytically in *Auger electron spectroscopy*.

23.4 Appendix: Answers

1. In the case of molecules (or in general: polyatomic systems), in addition to electronic excitation, excitation to vibrations and rotations must also be taken into account. Accordingly, vibration and rotation levels also come into play here, which—in contrast to pure electron transitions—can be caused by a large number of closely spaced wavelengths. The fact that, in addition, radiationless transitions from more strongly vibrationally/rotationally excited systems to less strongly excited ones are possible (key words: *internal conversion* and *intersystem crossing*), also ensures a (limited) quasi-continuum for emission processes instead of discrete emission lines.
2. If a L'vov platform is used, it is imperative to use inert gas to prevent oxidation of the elemental carbon to CO and CO_2 (or similar). But the high temperatures of an ICP flare, even in the presence of one or the other inert gas, would destroy the platform itself.
3. From *General Chemistry*, you know that the energy difference between different shells of the same atom becomes smaller and smaller as the principal quantum numbers increase: Accordingly, more energy is required for the excitation of an electron from the K-shell to the M-shell than for a transition from the L-shell to the N-shell: $\Delta E(K \rightarrow M) > \Delta E(L \rightarrow N)$, Accordingly, less energy is released during the relaxation N→L; the associated emitted radiation, therefore, has a longer wavelength than for the relaxation M→K.
4. In principle, there are two possibilities, and the simplest solution to this problem is almost trivial: if it is possible, one should try to use the analyte

as a pure substance. Alternatively, the evaporation of the matrix can be accelerated by the use of matrix modifiers (Sect. 20.1.2), so that the matrix enters the gas phase (long) before the analyte does and thus cannot enter the mass spectrometer together with the analyte.

5. A look at the corresponding isotope tables tells us: The $^{40}Ar_2^+$ cation mentioned has approximately the same m/z ratio as the $^{80}Se^+$ cation. Accordingly, attention must be paid to such interferences when quantifying selenium. (► Table 21.1 from the Harris (Sec. 21.1: What is mass spectrometry?) contains the masses of selected isotopes; unfortunately, selenium is not among them. But if you perform mass spectrometry yourself, you will most likely use much more comprehensive tables anyway.)
6. For any M-lines, first an electron would have to be removed from the M-shell and then an electron from one of the more distant shells would have to move "downwards" to it (which in itself, of course, would have to be occupied first). The resulting photon would not be very energetic (see question and answer 3: The more distant the shells are from the nucleus, the smaller the energy difference to the next-higher shell), so that a corresponding photon would no longer be within the energy range considered in XRF (too few eV).
7. There are two reasons for this: First, the Auger photon released by this effect is in any case far less energetic than the photon emitted in "normal" X-ray fluorescence, so it is unlikely to be sufficiently energetic to bring about ionisation anyway. On the other hand, an atom which has not only shown a "normal" X-ray fluorescence appearance (which lead to it already having been ionised to form the cation M^+ according to the photoelectric effect), now even has a *double* positive charge (M^{2+}) after knocking out a *second* electron (precisely by the Auger effect, i.e. a new photoelectric effect). Correspondingly, all the electrons remaining in this doubly positively charged cation are bound much more strongly to the nucleus than is the case with the cation M^+ (and even more so with the uncharged atom M), so much more energy would be required for a *third* ionisation.

Literature

Bienz S, Bigler L, Fox T, Meier H (2016) Hesse—Meier—Zeeh: Spektroskopische Methoden in der organischen Chemie. Thieme, Stuttgart

Binnewies M, Jäckel M, Willner, H, Rayner-Canham, G (2016) Allgemeine und Anorganische Chemie. Springer, Heidelberg

Cammann K (2010) Instrumentelle Analytische Chemie. Spektrum, Heidelberg

Harris DC (2014) Lehrbuch der Quantitativen Analyse. Springer, Heidelberg

Huheey J, Keiter E, Keiter R (2014), Anorganische Chemie. deGruyter, Berlin

Ortanderl S, Ritgen U (2018) Chemie—das Lehrbuch für Dummies. Wiley, Weinheim

Otto M (2011) Analytische Chemie. Wiley, Weinheim

Riedel E, Janiak C (2007) Anorganische Chemie. de Gruyter, Berlin

Schröder B, Rudolph J (1985) Physikalische Methoden in der Chemie. VCH, Weinheim

Schwister K (2010) Taschenbuch der Chemie. Hanser, München

Skoog DA, Holler FJ, Crouch SR (2013) Instrumentelle Analytik. Springer, Heidelberg

While the Harris is also the reference textbook this time, three other works from this (of course, again not complete) list should be explicitly highlighted:

- The basics of X-ray fluorescence analysis are explained very well in Cammann, while Skoog deals with the apparative conditions (and, if necessary, also difficulties) of this technique.
- For those of you who would like to deal with the selection rules mentioned in Sect. 23.1, I strongly recommend Huheey.

Otherwise, I would like to refer again to the bibliography in the Harris.

Supplementary Information

U. Ritgen, *Analytical Chemistry I*, https://doi.org/10.1007/978-3-662-66336-3

Glossary

α *see separation factor*

Absorbance (A) A strictly speaking wrong, but increasingly common term for *Extinction.*

Absorbance spectrum Any spectrum in which the absorbance/extinction is plotted on the y-axis; *see Extinction spectrum*.

Absorption/absorb There are two definitions: Either it means the absorption of a substance into another substance, e.g. the insertion of elementary hydrogen into the interior of a (transition) metal, or the absorption of an electromagnetic quantum, so that electrons are brought into an energetically less favorable (excited) state. Please do not confuse a_*b*_sorption with a_*d*_sorption.

Absorption bands Primarily the "peaks" appearing in UV/VIS spectra that indicate in which wavelength ranges and to what extent an analyte absorbs electromagnetic radiation (absorbance).

Absorption spectrum *See Extinction spectrum*

Accuracy The extent to which measurements that should lead to identical results in purely mathematical terms (such as the repeated measurement of the same or identical samples) differ in their results. In natural sciences, the accuracy of a series of measurements is indicated by the number of significant digits.

Acid/base pair, conjugate Any substance that, according to Brønsted-Lowry, having acted as an acid, i.e. split off a hydrogen cation, can then act as a base and reabsorb this (or another) proton. The sum of the pK_A value of an acid and the pK_B value of the conjugate base (at 25 °C, with water being the solvent) is always 14.

Acids and bases according to Brønsted or Brønsted-Lowry Acids are proton donors, i.e. molecules or molecular ions that can split off one or more H^+ ions (examples: HCl, H_2SO_4); bases are correspondingly proton acceptors, i.e. molecules/molecular ions that can accept protons (e.g. NH_3).

Acids and bases according to Lewis Acids are electron-acceptors (i.e. electrophilic), bases however are electron-donors (i.e. nucleophilic). Thus, according to Lewis, every complex reaction between a central particle and a ligand can also be understood as an acid/base reaction ... which makes chemistry all in all *much* simpler.

Acids, strong/medium/weak Substances with a pK_A value <0/between 0 and 4/>4.

Adsorption Adhesion of a substance to the surface of a solid, e.g. in chromatographic separation processes. Not to be confused with a_*b*_sorption.

AES *See Atomic Emission Spectroscopy*, *see also Auger Electron Spectroscopy*

Affinity chromatography Chromatography with specially treated column material that interacts specifically with only one analyte (or one substance class/family of analytes): also of immense importance in the life sciences due to e.g. the use of antibodies or antigens. Very effective, very special and at the same time versatile due to the variability of the column material.

Aliquot Partial portion of a sample (usually a solution).

Amphiprotic *See Ampholyte*

Ampholyte Substances that can act as both an acid and a base in the Brønsted-Lowry sense because they have both at least one sufficiently positively polarised, covalently bonded hydrogen atom and at least one free pair of electrons. The associated phenomenon is called amphoterism. (Occasionally, such compounds or ions are also called amphi*protic*, because they can *accept* protons, but also *release* them.)

Amphoteric *See Ampholyte*.

Analysis, qualitative Any method used to determine the identity of analytes present or to prove the presence of an analyte in question. In short, "*what* does my sample *consist of*?".

Analysis, quantitative Any method of investigation by which the quantity (mass, volume, amount of substance) of the analytes of interest can be determined. In short, "*How much of the* analyte(s) is in my sample?"

Analyte The substance that is the subject of an analysis: the substance relevant to an investigation or the relevant constituent of a mixture, for example when searching for certain ions whose counterions are not relevant. In volumetry, often the entire solution containing the analyte, including all its other components, is referred to as the analyte (or analyte solution).

Analytical methods, destructive Any analytical method in which the substance to be examined is, once the measurements have been completed, no longer in the same micro- and macroscopic state as it was before. It is irrelevant whether the sample was "only" mechanically stressed (e.g. crushed in a mortar) or whether the analyte was chemically changed in the course of the measurements (e.g. by a redox process or other reactions).

Analytical methods, non-destructive Analytical methods by which a sample can be examined without physically or chemically altering the sample itself.

Ångström Non-SI unit of length: 10^{-10} m (i.e. 0.1 pm or 100 nm); used mainly in crystallography because the Ångström is in the order of bond lengths and atomic distances in the crystal lattice.

Anode The part of an electrochemical system at which oxidation takes place; in spontaneous redox reactions, the negative pole; in electrolysis, the anode serves as the positive pole.

Atomic emission spectroscopy (AES) Method of spectroscopy based on exciting atoms and identifying the analyte by the wavelengths emitted while the atom(s) return to the ground state.

Atomic orbital Any orbital of an atom that has not (yet) interacted (bonding or antibonding) with the orbital(s) of another atom.

Auger electron In XRF, the (fluorescence) photon emitted when a higher-energy electron moves into the gap in the core electrons created by X-rays in accordance with the photoelectric effect can cause a renewed ionisation of the atom concerned; in other words, a second electron is knocked out of the (already ionised) atom. This is called an Auger electron and can also be analytically evaluated within the framework of Auger electron spectroscopy.

Auger electron spectroscopy (occasionally abbreviated AES) Method of analysis in which the kinetic energy of Auger electrons is used to obtain information.

Autoprotolysis (of water)—Dynamic equilibrium in which two water molecules act as acid and base towards one another. This produces one hydroxonium ion (H_3O^+), the conjugate acid of the base water (H_2O), and one hydroxide ion (OH^-), the conjugate base of the acid water (H_2O). Note that autoprotolysis is in principle possible for all ampholytes.

Avogadro's number (N_A) Number of particles corresponding to one mole: 6.022×10^{23} pieces; this results in the unit 1/mol (reciprocal mole, i.e.: "per mole") or mol^{-1} for N_A.

Balmer series The series of transitions of excited electrons in which relaxation causes the originally excited electron to populate an orbital with principal quantum number n = 2. Forthe hydrogen atom, the wavelengths emitted in the course of this relaxation lie in the range of visible light.

Band Correct term for a "peak" in an IR or UV/VIS spectrum.

Base width (w) Width of a peak at its base; occasionally somewhat difficult to determine.

Base, strong/medium/weak A substances with a pK_B value <0/between 0 and 4/>4.

Bathochromic effect The shift of the absorption maximum of a compound by an auxochrome towards longer wavelengths.

Blank sample Sample which definitely does not contain the analyte to be determined (qualitatively or quantitatively), but which, for the sake of comparability with a *positive* blank sample, has been mixed with all the other substances also contained in the positive blank sample, in order to compensate for any instrumental conditions.

Blank sample, positive Conducting an experiment with a sample (solution) that contains the analyte to be determined (qualitatively or quantitatively) with certainty, in order to check how the analytical method in question indicates the presence of the analyte, i.e. for example, how a positive qualitative detection reaction proceeds, which colour change can be expected when the analyte is present, or which measured values a corresponding instrument designed for quantitative measurements delivers at a precisely defined concentration of the analyte. The opposite of the positive blank sample is just called *blank sample*.

Bohr's atomic model Simplified description of the atom, in which the electrons orbit the atomic nucleus like planets orbit their central star, except that these "planetary orbits" are three-dimensional and are therefore called "shells". It is of utmost importance that the electrons can only stay on energetically exactly defined shells; all other (theoretically conceivable) states are by definition not possible. The further away from the nucleus a shell is, the more energetic the electrons in it are; for historical reasons, the shells are labelled alphabetically "from the inside out", i.e. according to increasing energy content, starting with K. (Here, the K shell of Bohr's atomic model corresponds to the principal quantum number n = 1 from the quantum mechanical model, the L shell to the principal quantum number n = 2, etc.).

Brackett series The series of transitions of excited electrons in which relaxation causes the originally excited electron to populate an orbital with principal quantum number n = 4. For the hydrogen atom, the wavelengths emitted in the course of this relaxation lie in the infrared region.

Brønsted acid/base *See Acids and bases according to Brønsted and Brønsted-Lowry* respectively

Brownian molecular motion Chaotic heat-movement of all particles, also at molecular or atomic level.

Buffer Solution of a weak acid and its conjugate base (or of a weak base with its conjugate acid, which is exactly the same thing). Near the pK_A value of the acid used (±1 pH unit), a buffer keeps the pH value largely constant even when acid or base is added.

Buffer equation *See Henderson-Hasselbalch equation*

Buffer range The pH range around which a buffer maintains a largely constant pH, even upon moderate addition of acid or base; in acid/base titration curves, the range in which the slope of the curve is minimised. The midpoint of the buffer range coincides with the pK_A value of the acid.

Calibration Determining how the selected analytical method responds to the analyte in question: What is the relationship between the physical quantity measured and the values indicated by the measuring instrument?

Calibration curve Graphical plot of analyte concentrations (on the x-axis) and the corresponding measured values (on the y-axis). Which property is used in each case to obtain the measured values in question depends on the analysis method used. If it is possible to indicate a trend line on the basis of the available measured values (which is very desirable!), this graph also enables the conclusion to be drawn about their respective analyte content by interpolation on the basis of the measured values obtained from samples of unknown concentration. Extrapolation is also permissible, but only within the linear range of corresponding graphs.

Cathode The part of an electrochemical system where reduction occurs; in spontaneous redox reactions, the positive pole; in electrolysis, the cathode serves as the negative pole.

CE Abbreviation for *capillary electrophoresis*.

Central particle The atom/ion that is the center of a complex compound and acts as a Lewis acid when interacting with its ligands.

Cerimetry Redox titrations based on the redox pair Ce^{4+}/Ce^{3+}.

Charge balance When drawing up a reaction equation worthy of the name, care must be taken to ensure that the sum of the charges appearing on both sides of the reaction or equilibrium arrow is identical: charges cannot simply disappear into the void or be pulled out of thin air as required; *see also Substance Balance*. Please note: It is a question of the sum of the charges. If, for example, common salt is dissolved in water ($NaCl \rightarrow Na^+ + Cl^-$), positive and negative charge carriers are of course present in the resulting solution, whereas the initial substance was electrically neutral, but since the sum of the occurring charges (so to speak "1 time +, 1 time −") exactly cancels each other out, the solution as a whole is also uncharged. (It could obviously not be any other way.)

Charge density To determine the charge density of an ion, one compares its volume (or its surface area) with its total charge: Two particles of the same charge but different size have different charge densities: The charge density of Li^+ is far higher than that of K^+. (Similar charge density of different ions can lead to similar chemical behavior: Consider, for example, the diagonal relationship between Li^+ and Mg^{2+}).

Chelate effect Generic term for the various factors which ensure that chelate complexes are generally significantly more stable than comparable complexes with monodentate ligands.

Chelate ligand Any ligand of a complex that is coordinated to the central particle of the complex via more than one atom. In most cases, complexes of chelate ligands are far more stable than comparable counterparts with monodentate ligands.

Chemiluminescence One speaks of chemiluminescence when a chemical reaction is accompanied by luminescent phenomena; not to be confused with phosphorescence or fluorescence.

Chromatogram Graphical representation of the results of a chromatographic analysis; usually the retention time of the individual analytes is plotted on the x-axis, while the peak height is indicated on the y-axis.

Chromatography Method of substance separation based on the fact that different substances dissolved in a mobile phase (liquid or gaseous) interact in different ways with a stationary phase (usually a solid).

Chromophore Functional group that causes easier excitability of an electron system. Typical chromophores are the carbonyl group or other multiple bonds involving at least one heteroatom with (at least) one free electron pair.

Coefficient of variation/relative standard deviation Specification of the standard deviation as a percentage value; facilitates the comparison of different measurement series whose measured values lie in different orders of magnitude.

Collision When two molecules collide, vibrational or rotational energy can be transferred from one to the other; basis of non-radiative relaxation.

Colorimetry Method of determining the analyte content of a (usually liquid) mixture of substances by comparison with a colour scale based on blank samples of precisely known concentration of the same analyte (in the same medium). (For the particularly meticulous: Although the principle of colorimetry is very similar to that of photometry, the two techniques should be distinguished from each other because photometry can only be used with homogeneous analyte solutions that obey Lambert-Beer's law.)

Column, chromatography or separation The stationary phase in any form of chromatography.

Combustion triangle Common visual representation of the conditions that must be met for a fire to occur: combustible material, oxidant, and sufficient excitation energy (usually referred to as ignition energy). The mixing ratio also plays an important role, but is not commonly integrated into the combustion triangle in combustion theory.

Comma ,

Complexometry Any titration ultimately based on the analyte entering into a complex reaction, either as a ligand or as a central particle.

Concentration cells (also: concentration chain) Two half-cells of a galvanic element containing the same electrolytes but differing in concentration, resulting in a potential difference.

Conductometry Volumetric analytical method in which the course of titration is traced on the basis of the change in the electrical conductivity of the analysis solution.

Conjugate acid/base pair *See acid/base pair*

Content range Relative content of the analyte within a mixture of substances; usually given as a percentage.

Conversion, *internal* (IC) Radiationless transition of a system from one state to another without changing the multiplicity state of the system; in contrast to *internal system crossing* usually symmetry-permitted.

Core electrons Electrons of an atom (or molecule) that do not belong to the valence shell or do not populate the frontier orbitals.

Corresponding acid/base pair Obsolete term for conjugate acid/base pair; *see acid/base pair*

Cutoff wavelength (λ_{CutOff}) Substance-specific value for solvents; the wavelength below which UV/VIS photometric measurement of the analytes dissolved in the solvent in question (or their detection) is no longer possible because the solvent itself absorbs below this wavelength.

D *See Multiplicity*

Dead time The time required for a substance to pass through the column without any (restraining) interaction with the column material; minimum residence time of any analyte on the column.

Decanting Laboratory jargon for "careful pouring off of the supernatant of a liquid/solution with precipitate".

Definition equation Equations for the precise specification of concentrations, proportions, or ratios. (The equations binding in analytics together with the official variables, formula symbols, and units to be used are specified for Germany in DIN 1310; this forms the basis of this text book, as well. Most of those variable, symbols etc. are considered internationally valid, though.)

Deformation vibrations Vibrations in which the bond angles of the analyte change. A distinction is made between *in-plane* and *out-of-plane vibrations*.

Degeneracy The term "degenerate" is used to describe anything that has exactly the same energy content or can be excited by exactly the same amount of energy. Degenerate orbitals, for example, have exactly the same energy content. The simplest example is the three p-orbitals of the same principal quantum number: The p-orbital set is threefold degenerate. Correspondingly, all d-orbital sets are each fivefold degenerate, etc. Molecular orbitals can also be degenerate, regardless of whether they are bonding, non-bonding or antibonding. One speaks of degenerate vibrations (especially in IR spectroscopy) when two or more vibrations of a molecule can be excited by the same wavenumber, so that the number of bands in the spectrum is (occasionally significantly) smaller than the number of vibrational degrees of freedom would lead one to expect.

Degree of titration Substance ratio of titrant and analyte, given as a dimensionless number between 0 and (theoretically) infinity; usually abbreviated as τ. Before the equivalence point is reached: $\tau < 1$: at the EP: $\tau = 1$; with $\tau > 1$ one has over-titrated (which, however, is not necessarily a bad thing).

Degrees of freedom Number of possible movements/directions of movement of each individual atom, also within multi-atomic assemblies. A distinction is made between translational, rotational, and vibrational degrees of freedom.

Deprotonation Splitting off of a hydrogen cation from a molecule or molecular ion, which thus acts as an acid in the Brønsted-Lowry sense.

Derivatisation The conversion of, for example, an analyte into a new compound that has different properties; may be required for the separation or detection of analytes.

Desalination The exclusion chromatographic removal of cations, anions, and other particles with comparatively small molecular mass; essential in bioanalysis.

Detection limit If the analyte content of a sample lies below the detection limit, it can no longer be stated with certainty that the analyte is present in the sample at all; then only statistical probabilities can be considered. Where the detection limit lies depends, of course, on the analyte under consideration and on the analytical method used.

Diamagnetism If all electrons in a substance are spin-paired, a sample of this substance is slightly pushed out of a magnetic field; opposite: paramagnetism.

Dilution, parallel Creating a dilution series starting from a stock solution.

Dilution, serial Preparation of a dilution series with each solution obtained being used as stock solutions for a subsequent dilution.

Dilution series Samples of a precisely defined volume are taken from a solution with a precisely known substance content and then filled up with the selected solvent to a final volume, which is also precisely defined. This way, different dilutions of the same substance with a very precise content can be achieved, which can be used, for example, to create calibration curves.

Dimensional Analysis Logical thinking through of an "arithmetical problem" in which different units come into play: One checks whether the interaction of the different units ultimately leads to a result with the correct unit or not. If it does not, something went awry.

Dipole moment If in the ground state of a molecule (or molecular ion) the centers of positive and negative partial charges do not coincide, the corresponding compound exhibits a dipole moment.

Diradical Atom, molecule, or molecular ion with two unpaired electrons. (The multiplicity of this system does not necessarily have to be M = 3.)

Discard Lab jargon for "dispose of in the trash can" or "throw away".

Disproportionation Redox reaction in which two (or more) atoms of the same kind of element, which have the same oxidation number at the beginning of the reaction, are present with different oxidation numbers after the reaction's completion. The opposite of this is synproportionation.

Doublet (D) *See Multiplicity State*.

Dynamic equilibrium When two processes running in opposite directions occur at the same speed, so that no macroscopic change is perceptible, a dynamic equilibrium has established. In the chemical sense, a dynamic equilibrium is established when the forward and the reverse reaction take place at the same speed, and no further product is produced (or reactant consumed). Such dynamic equilibria can be described using the law of mass action.

Eddy diffusion Increasingly common term for scattering diffusion described by the A term of the Van Deemter equation.

EDL *See Electrodeless Discharge Lamp*

EDRFA *See X-ray fluorescence analysis*

EDTA Abbreviation for the tetra-anion of ethylenediaminetetraacetic acid (ethylenediaminetetraacetate), which is important as a six-dentate ligand, particularly in complexometry.

Electrochemical series A list of the electrochemical standard potentials of various redox pairs. The corresponding values are determined experimentally; so far there is no conclusive theory as to *why* which redox pair has which potential. Maybe it will found one day.

Electrodeless discharge lamp (EDL); *also:* Induction lamp The energy required is supplied by means of a high-frequency field; as an element-specific excitation light source, it is an alternative to the hollow cathode lamp.

Electron spin Quantum mechanics dictate that electrons populating the same orbital must differ in their spin. There are two possibilities (therefore an orbital, however spatially extended, can only be populated with a maximum of two electrons according to the Pauli principle): $m_s = +½$ (usually symbolised by ↑) or $m_s = -½$ (short: ↓).

Electron spin resonance (ESR) Analysis method based on the excitation of unpaired electrons by long-wave microwave radiation. Because the occurrence of unpaired electrons inevitably leads to paramagnetic behavior, ESR is also referred to as *electron paramagnetic resonance* (EPR).

Electroosmotic flow The "pumping motion" in the direction of the cathode that occurs inside a CE capillary upon application of an electric field, because the number of freely movable, positively charged carriers is larger than the number of freely movable, negatively charged carriers (the negative charges of the deprotonated silanol groups on the capillary wall are stationary).

Electropherogram Graphical representation of the results of an electrophoretic analysis; in CE, quite analogous to most chromatograms, the retention time of the individual analytes is plotted on the x-axis, while the peak height is indicated on the y-axis.

Electrophoresis Separation of substances according to the different migration behaviour of charged particles in an electric field.

Elution Washing out of an adsorbed substance with the aid of a solvent or solvent mixture.

Elution gradient The elution of analytes using a solvent mixture of time-dependent composition; in normal phase chromatography, when an A/B mixture is used in which B is the more polar component of the mixture, the B component is gradually increased.

Elutropic series Ordering of solvents according to increasing polarity/increasing eluting capacity for normal phase chromatography; for reversed phase chromatography it is inverted accordingly.

Emission spectrometry In principle, any form of spectrometry in which the analyte content is inferred from the element-specific emission caused by the corresponding excitation of the analyte. In ICP-OES *(inductively coupled plasma optical emission spectrometry)* the excitation is carried out by means of a plasma torch, in F-AES *(flame atomic emission spectrophotometry)* by a gas flame.

Emission spectrum Spectrum obtained after excitation of the analyte: Spectrum of energy released upon return to the ground state (or a less excited state), some or all of which is emitted in the form of electromagnetic radiation.

Emulsion More or less stable dispersion of two liquids that are not soluble in each other; after a longer waiting time (sometimes even days), at least partial phase separation can be observed.

Endcapping Chemical modification of the free silanol groups in silica gel; reduces (or prevents) tailing of analyte peaks.

EP *See Equivalence point*

Equidistant Scientific term for "at the same distance from one another".

Equilibrium concentrations The concentrations of reactants and products of a chemical (equilibrium) reaction that are actually present when dynamic equilibrium has been established. Please never confuse the initial concentrations and concentrations at equilibrium.

Equivalence Point (EP) The point in a volumetric analysis where equivalent amounts of titrant have been added to the analyte solution. Note that equivalent amounts does not necessarily mean equi*molar* amounts: If a diprotic acid is titrated against a base, the EP is reached when *double* amounts of base have been added to the acid, etc..

ESR *See Electron Spin Resonance*

Exact numbers Numbers that have no measurement uncertainty, i.e. in the context of dealing with significant digits, can be regarded as numbers with an infinite number of significant digits/decimal places. Examples are precisely defined (conversion) factors or precisely determined integers (e.g. number of marbles in a bag, as there cannot be "half a marble").

Excited state Any state of an atom or molecule in which not all electrons have the lowest possible energy content: *Any deviation* from the ground state of a system leads to an excited state (accordingly, the number of possible excited states is in principle unlimited). In molecular spectroscopy, a distinction is made between whether a (polyatomic) system is electronically or vibrationally/rotationally (vib./rot.) excited. In an electronically excited system, (at least) one electron is in an energetically less favorable orbital than it would occupy in the ground state; in vib./rot. excitation, energetically different vibrational levels are reached. For the latter, much smaller amounts of energy are sufficient, so that even an electronically non-excited system (a system that, with respect to its electrons, is in the ground state) can very well be vib./red. excited.

Exclusion chromatography (gel filtration) Separation of different analytes according to their sizes; in this process, larger analytes pass through the column used more quickly than smaller ones, because the latter also penetrate into column material cavities and thus have to migrate a larger distance; also suitable for desalting (analyte) solutions.

Extinction (E) Quantity commonly used in photometry to describe the ratio of analyte solution and blank sample (directly compatible with Lambert-Beer's law); increasingly referred to as *absorbance*.

Extraction Removing a solute from its solvent; usually the analyte in question is transferred to another solvent.

Extrapolation The estimation of expected measured values beyond the (experimentally) secured range. This might work well, but does not have to.

F-AES *See Flame Atomic Emission Spectrometry*

Fever curves The result of the bad habit—popular with many students—of thoughtlessly connecting the individual measuring points with their respective neighbours by straight lines when graphically plotting various measured values, instead of creating a trend line/compensating straight line, if possible, starting from the totality of all measuring points, which allows interpolation

(and possibly even extrapolation). In short: *Don't do it.* (Unless the goal is really just to plot graphically what an individual patient's body temperature was at each measurement time. But the resulting zigzag line does not allow any further statements.)

Filtering The separation of finely distributed suspended substances via a filter. The residue remains in the filter, the filtrate is then free of suspended matter.

Filtrate That which remains in the liquid phase when filtering an inhomogeneous mixture, i.e. "comes out of the bottom of the filter".

Fingerprint region (or range) IR-Bands occurring in the region $\bar{\nu} < 1500\ \mathrm{cm}^{-1}$ can rarely be assigned to individual vibrations or vibrational modes because the associated IR photons excite the entire molecule to more complex framework vibrations. Accordingly, the bands occurring in this region of the spectrum form a kind of "molecular fingerprint" that is usually characteristic for the substance in question.

Flame atomic emission spectrophotometry (F-AES) *See emission spectrometry*

Flame, fat *In atomic spectroscopy:* an atomising flame with excess fuel; has a reducing effect.

Flame, lean *In atomic spectroscopy:* an atomisation flame with oxidant excess; leads to higher flame temperature.

Fluorescence In fluorescence (*not* to be confused with phosphorescence), a substance is excited by electromagnetic radiation of wavelength λ_1 to emit electromagnetic radiation of wavelength λ_2 after a radiationless (intramolecular or intraatomic) transition, for which the following holds: wavelength $\lambda_2 > \lambda_1$. The cause behind this is the photochemical excitation of the substance and one or the other radiationless symmetry-allowed transition (without changing the multiplicity state). Any fluorescence appearance ceases within a time span of about 10^{-8} s after termination of the excitation.

Forbidden *See symmetry-permitted/forbidden*

Formula symbols Generally accepted symbols for physical quantities within the relevant scientific field; often backed up by one or other DIN or other standard. Only when the corresponding formula symbols have been agreed upon, everyone know that e.g. c_i stands for the concentration of substance i (in whatever solvent), etc.

Framework Vibration *See Fingerprint Region*

Frank-Condon principle Quantum mechanical description of the connection between vibrational and electronic excitation states of a polyatomic system.

Frontier orbital distance Energy difference between HOMO and LUMO of a molecule or molecular ion.

γ-radiation Electromagnetic radiation with a wavelength <10–1 nm; extremely energetic.

Galvanic element Two electrochemical half-cells separated from each other, in which a potential difference builds up when they are conductively connected,

so that an electrical current flows (with oxidation and reduction taking place in the respective half-cells).

Gamma radiation *See γ-radiation*

Gas chromatography (GC) Chromatographic separation process in which a gas is used as the mobile phase.

GC *See Gas Chromatography*

Gel electrophoresis Electrophoretic separation of charged analytes based on the sieving effect caused by the gel used, which allows smaller analytes to move more rapidly towards the cathode or anode than larger ones; can be combined with capillary electrophoresis.

Gel filtration Alternative name for exclusion chromatography; considered "too casual" by some, but capturing the essence of the matter quite well.

Gravimetry Analytical method in which conclusions are drawn about the mass of the analyte based on the readout of a balance.

Ground state (of an atom or molecule) The state in which all electrons have the lowest possible energy content; addition of energy then leads to excited states.

Half-cells Part of a galvanic element in which either reduction or oxidation takes place.

Half-equivalence point The point in a titration at which exactly half the amount of titrant required to reach the equivalence point has been added to the analyte; particularly important when considering buffers (*see Henderson-Hasselbalch equation*).

Half-width ($w_{½}$) Increasingly common term for the width of a (chromatography) peak at its half height.

HCL (not to be confused with HCl) *See Hollow Cathode Lamp*

Henderson-Hasselbalch equation Equation for calculating the pH value of a buffer solution using the pK_A value of the weak acid and the individual concentrations of acid and conjugate base used; ►Eq. 5.11. (By the way: Don't you dare to omit Mr. Hasselba_l_ch's L—even though you will hear it wrong all the time and it is even mis-spelled in some textbooks.)

Heterogeneous Samples with a varying composition are called heterogeneous or inhomogeneous. If a heterogeneous sample were to be aliquoted, it were to be expected that the individual subsamples would differ in their analyte content.

High performance liquid chromatography (HPLC) Form of liquid chromatography in which the mobile phase is pumped through quite narrow chromatography columns at considerable pressure; leads to significantly higher resolutions than classical liquid chromatography.

Hollow cathode lamp (HCL) Element-specific excitation light source for atomic spectroscopy; a gas discharge tube filled with a noble gas, the cathode consisting of the element to be detected.

HOMO *(highest occupied molecular orbital)* The most energetic populated orbital of an atom or molecule.

HOMO-1 The orbital which energetically lies directly below the HOMO, i.e. is energetically (somewhat) more favorable.

Homogeneous Sample with uniform composition. Aliquoting a homogeneous sample results in aliquots with identical analyte content.

Homogenise To convert a heterogeneous sample into a homogeneous sample. (Yes, homogenisation is also used for milk, but that is not what is meant here.)

HOMO-LUMO distance The difference in energy between the energetically highest occupied and the energetically lowest *un*occupied orbital of an atom or molecule; the energy that must be expended to reach the excited state at which an electron has "jumped" from the HOMO to the LUMO. Also referred to as the *frontier orbital distance*.

HPLC *(High performance liquid chromatography)* *See High performance liquid chromatography*

Hund's rule Degenerate orbitals are subsequently occupied in a spin-parallel single manner.

Hydroxonium ion H_3O^+ ion; formed by the reaction of a water molecule with a reactant that has acted as an acid towards it. The hydroxonium ion is the conjugate acid of the base water (H_2O); sometimes it is called oxonium ion or hydronium ion.

Hydroxide ion OH^- ion; formed by the reaction of a water molecule with a sufficiently strong base so that deprotonation occurres; also occurs in the autoprotolysis of water. The hydroxide ion is the conjugate base of the acid water (H_2O).

Hygroscopy "Hygroscopic" is the term used to describe substances that bind/attract water from the environment (usually atmospheric humidity). This can be desirable (for example, in the case of hygroscopic substances that serve as desiccants) or annoying: Hygroscopic analytes, for example, seem to inexplicably increase in mass on the balance.

Hyperchromic effect Anything that has a colour-deepening effect, i.e., increases the amount of absorption of a given wavelength without causing a change in the wavelength absorbed.

Hypsochromic effect Shift of the absorption maximum of a compound to shorter wavelengths by an auxochrome; the opposite of the *bathochromic effect*.

IC *See Conversion*, internal

ICP *See Plasma, inductively coupled*

ICP-OES *See emission spectrometry*

Ignition energy The energy required to ignite a mixture of combustible material and oxidant.

Impurities *In an analyte sample:* Anything that is not analyte. Impurities can reduce measurement accuracy or even prevent measurement altogether. (If you wish, you can also consider the matrix of any sample an impurity.)

In principle In the natural sciences, no "not *per se*, but ... ", but actually a categorical "No!".

Indicator, pH Substance which shows different colours depending on the pH value of the solution it is used in, because it interacts differently with visible light depending on its degree of (de)protonation.

Indicator, redox Substance which shows different colours depending on its oxidation or reduction state.

Induction lamp *See Electrodeless discharge lamp*

Infrared radiation (IR radiation) Electromagnetic radiation, significantly less energetic than visible light, with a wavelength of approximately 10^3–10^6 nm. This range is often further subdivided:– Far IR: λ = 0.5 µm to 10 000 µm (= 10 mm or 1 cm, but "centi-" is not SI-compliant). – mid-wave IR: λ = 3–50 µm – near IR: λ = 800 nm–3000 nm (= 3.0 µm)

Infrared spectroscopy (IR spectroscopy) Spectroscopic method in which analytes are excited to various vibrations by comparatively low-energy infrared radiation. If the dipole moment of the respective analyte changes, this vibration is IR-active and will lead to a band in the corresponding spectrum.

Inhomogeneous *See heterogeneous*

In-plane deformation vibrations (δ) Vibrations in which bond angles change; a distinction is made between bending and rocking vibrations.

Internal conversion *See Conversion*

Internal standard *See Standard*

Interpolation If the measured values y for varying analyte contents x allow the creation of a trend line, this allows the expected measured values y to be estimated for x values that have not already been measured; at the same time, the resulting y value can be used to infer the x value belonging to a newly measured sample—the idea behind every calibration curve.

Intersystem Crossing (ISC) Radiationless transition of a system from one state to another, changing the multiplicity state of the system; often symmetry-forbidden.

Iodometry Redox volumetry based on the redox pair I_2/I^-.

Ion chromatography Variant of ion exchange chromatography used primarily for anionic analytes.

Ion pair chromatography Variant of ion exchange chromatography in which additional aspects of reversed phase chromatography come into play; used for very special applications.

IR *See Infrared…*

ISC *See Intersystem Crossing*

Isobaric interference In ICP-MS, isobaric interference occurs when reactions of analyte atoms with plasma cations lead to the formation of particles with a mass/charge ratio whose m/z value is almost the same as that of the analyte and thus, if the resolution is not sufficient, cause interference.

Isocratic elution Elution with only one solvent or solvent mixture without a gradient.

Isoelectronic Two atoms/ions are isoelectronic if they have the same number of (valence) electrons.

Isotopes Atoms of the same kind that differ in their mass number but not in their atomic number. Due to the different number of neutrons, different masses result for different isotopes; of immense importance in mass spectrometry.

IUPAC *(International Union for Pure and Applied Chemistry)* Generally recognised institution for all questions concerning the designation of chemical compounds (nomenclature), natural constants to be used in chemistry, etc. The recommendations of the IUPAC are not legally binding, but are usually followed.

Jablonski diagram/Jablonski term diagram Graphical representation of the different possible excitation states as well as possible relaxation paths of molecules; electronic excitation states as well as vibrational or rotational states are taken into account.

Job's method Method for determining the stoichiometric composition of a complex (ZL_x). In this method, different mixtures of central particle and ligand solutions are examined (spectro-)photometrically at the wavelength corresponding to the absorption maximum of the complex in question.

k *See retention factor*

Kelvin scale Temperature scale that uses the absolute (thermodynamic) zero of temperature (−273.15 °C) as the zero value. The spacing of the temperature steps in the Kelvin scale is identical to that of the (presumably more familiar) Celsius scale; the conversion therefore is: – Temperature in °C = Temperature in K − 273.15 and – Temperature in K = Temperature in °C + 273.15 (By the way, the unit of the Kelvin scale is just called "Kelvin". Please avoid the term "degrees Kelvin".)

K_{SP} value Description of the solubility of an ionic substance or its ion product based on the law of mass action. Often given in the form of the pK_{SP} value.

K_α line The emission line that results from an electron "moving" from the L-shell into the gap in the K-shell created by ionisation via X-ray photons. In most cases, the K_α line is already sufficient for the unambiguous identification of an element.

K_β line The emission line that results from an electron "moving" from the M-shell into the gap in the K-shell created by ionisation via X-ray photons; also element-characteristic, but much less pronounced than the K_α-line; therefore, it is usually used for analytical purposes only in rather special cases.

λ_{CutOff} *See Cutoff wavelength*

Lab jargon Technical jargon that is usually not entirely precise (or not in the sense of IUPAC), but has become accepted among experts and is therefore still used. This includes the use of obsolete/historically conditioned substance names (e.g. "soda lye" instead of "aqueous solution of sodium hydroxide") or a substance class generic term for a concrete substance class representative (when asked for "ether" in the laboratory, "diethyl ether" is practically always meant) as well as the use of at least ... questionable terms. (Chemists, for instance, tend to "filter off" suspensions instead of simply filtering them.) Ideally, one should not actively use laboratory jargon, but at least have a sufficient passive command of it to understand what the colleagues might mean.

Lambert-Beer's law Describes the relationship between the concentration of an analyte solution and the absorbance at a precisely defined wavelength; basis of *photometry*.

Laser (acronym from Light Amplification by Stimulated Emission of Radiation) The radiation of a (nearly) monochromatic generator of electromagnetic radiation ("laser light"); based on the electronic excitation of suitable systems, which are then specifically induced to release the energy stored in the excited state in the form of photons.

Law of mass action (LMA) Quantitative description of a chemical equilibrium: ratio of the mathematical product of the concentration of the products to the mathematical product of the concentration of the reactants. (Stoichiometric factors enter as exponents.) The value of this ratio is sstated as the equilibrium constant K.

LC (liquid chromatography) *See Liquid Chromatography*

LCAO theory (linear combination of atomic orbitals) Basis of molecular orbital theory; based on constructive and destructive interference of the wave functions (= orbitals) involved; leads to bonding and antibonding interactions. (Non-bonding interaction is also possible.)

Lewis acids/bases *See Acids and bases according to Lewis*

Ligands Mono- or polyatomic molecules or ions which interact via at least one atom with the central particle of a complex as an electron donor in the sense of a Lewis base. If a ligand interacts with the central particle via more than one coordination site, it is referred to as a multidentate or chelating ligand.

Light, visible (VIS) Electromagnetic radiation from the wavelength range 380–800 nm.

Limit of determination (LOD) If the analyte content of a sample lies above the limit of detection, but below the limit of quantification, the presence of the analyte can only be determined qualitatively. Quantitative statements are no longer possible at such low contents. The limit of determination is, of course, always dependent on the analyte under consideration and the analytical method used.

Liquid chromatography (LC) Chromatographic separation process in which a liquid serves as the mobile phase.

LMA *See Law of Mass Action*

Local element If a metal that is conductively connected to a less noble other metal (for example, by touching each other) is oxidatively attacked, unhindered electron transfer occurs from the less noble metal, which is then oxidised accordingly instead of the more noble metal; ultimately, a local element is a "short-circuited battery".

lg Logarithm to base 10; please do not use the abbreviation "log", even though this is—incorrectly—written on almost every pocket calculator.

Lone pair A pair of spin-paired electrons populating the same orbital, not directly involved in a chemical bond.

Longitudinal diffusion Effect of Brownian molecular motion on the relative migration velocity of identical analyte particles along the direction of flow of a chromatography column; considered as the B term in the van Deemter equation.

LUMO + 1 The next higher (i.e. energetically a little less favorable) molecular orbital above the LUMO.

LUMO (lowest unoccupied molecular orbital) The lowest-energy orbital of an atom or molecule that is not occupied by electrons.

Luther's rule At the equivalence point of a redox titration, both the standard redox potentials of the ions involved and the number of electrons involved in both redox equilibria in question must be taken into account. The corresponding formula can be found as ▶ Eq. 7.2.

L'vov platform Graphite platform used in the graphite furnace to uniformly transfer the analyte to the gas phase.

Lyman series The series of transitions of excited electrons in which relaxation causes the originally excited electron to populate an orbital with principal quantum number n = 1; for the hydrogen atom, the wavelengths emitted in the course of this relaxation lie in the high-energy UV range.

L_α/L_β line The emission line that results from the "migration" of an electron from the M-shell or N-shell into the gap in the L-shell created by ionisation via X-ray photons. Significantly less energetic than the K lines.

M *See Multiplicity*

m/z *See mass/charge ratio*

Macroscopic properties Properties of numerous (not to say: practically countless) atoms/ions/molecules in a compound, which show certain properties only when being together; those include characteristics such as colour (you already know from Part I of this book that individual ions have no colour of their own) as well as, for example, ferromagnetism (a single iron atom is by no means ferromagnetic) and the like.

Mass constancy One speaks (especially in gravimetry) of mass constancy if the balance shows (almost) the same result when weighing a sample several times after it has been dried in the drying oven.

Mass spectrometry (MS) Analytical method in which the analyte is ionised and fragmented in a magnetic field; the mass-to-charge ratio (m/z) of the fragments obtained then allows conclusions to be drawn about the structure of the analyte in question.

Mass transfer term The C term of the van Deemter equation (► Eq. 12.12)

Mass/charge ratio/mass-to-charge ratio (m/z) The ratio of the mass of a particle to its total charge; of crucial importance in mass spectrometry.

Matrix That which is present in the sample apart from the analyte: the sum of all impurities. A matrix is mainly referred to when the analyte is present in a relatively small (substance) quantity.

Matrix modifiers Substances that convert any interfering matrix components to volatile compounds in atomic absorption spectroscopy.

Mean value The sum of all individual measured values, which must then be divided by the number of measured values.

Measurement accuracy A measure of the "reliability" of a measured value, the difference between the measured value and the "true" value. When dealing with significant digits, the following applies: The last digit of the number as viewed from the left (i.e. the last digit) is considered uncertain; all digits to the left of it are considered reliable. For a reading of 10.42, the digits 1, 0, and 4 are considered "accurate," while 2 is subject to measurement uncertainty (so it could be 10.41 or 10.43), while the reading 10.4 is an order of magnitude less accurate. (Here the "true" value could also be 10.3 or 10.5.)

Metal indicator Lewis base acting as a complex ligand, which (especially in complexometry with EDTA) forms a complex with the analyte metal ion and shows a different colour than in the uncomplexed state. It is important that the metal indicator-analyte complex must be less stable than the EDTA-analyte complex.

Microscopic event Any event that can no longer be described by classical Newtonian physics because the particles involved are too small and therefore obey the laws of the quantum world.

Microscopic properties Properties of individual atoms, molecules, or ions, such as: Molecular mass, spatial structure (bond distances and angles), dipole moment, etc.

Microwave plasma torch atomic emission spectrometry (MPT-AES) Variant of ICP-OES in which the plasma is stabilised by a microwave field.

Microwave spectroscopy Spectroscopic method for the investigation of molecular rotations.

Migration Technically correct term for the movement of a particle (especially in electrophoresis: migration in the electric field).

MO Short for "molecular orbital".

Mobile phase The "mobile" adsorbent in a chromatographic substance separation. If a liquid is used as the mobile phase, it is liquid chromatography; if a gas is used as the mobile phase, it is gas chromatography.

Mobility (μ_{total}) The speed at which a charged particle moves/migrates in an electric field; sum of electrophoretic and electroosmotic mobility.

Mobility, electroosmotic (μ_{eo}) The acceleration (positively charged particles) or deceleration (negatively charged particles) effect experienced by a particle in capillary electrophoresis due to electroosmotic flow.

Mobility, electrophoretic (μ_{ep}) Mobility of a charged particle in the electric field; depends on its charge density and polarisability, as well as on the properties of the solvent used, the temperature (and thus the viscosity), etc.

Molality Content parameter for describing a solution: The amount of solute × per mass of solvent is given (with the usual unit mol/kg). Please do not confuse with mola_r_ity!

Molarity Lab jargon for the molar concentration c and its unit mol/L resulting from the associated definition equation (substance quantity per volume). If one speaks of a 1-molar solution of substance x, this means a solution that contains substance x in a concentration of 1 mol per litre.

Molar Solubility *See Solubility*

Molecular orbital (MO) An orbital that "includes" all the atoms involved in the bond in question. (If one interprets the MO theory consistently: An orbital that "includes" all atoms that are part of the whole molecule under consideration.) A distinction is made between bonding, antibonding and non-bonding molecular orbitals.

Molecular orbital theory (MO theory for short) Model of the chemical bond based on the linear combination of atomic orbitals, according to which bonding and antibonding interactions result between the atomic orbitals involved. According to the MO theory, the number of resulting molecular orbitals (the number of bonding and antibonding ones added) must be identical to the number of atomic orbitals involved.

Molecular orbital, antibonding (ψ^*) Molecular orbital that was "created" by destructive interference of atomic orbitals. Antibonding molecular orbitals are always energetically less favorable than the atomic orbitals from which they originated and therefore do not contribute to the stability of the molecule when populated with electrons. They are indicated by an asterisk (*) next to the Greek symbol standing for the symmetry of the orbital in question.

Molecular orbital, bonding (ψ) A molecular orbital that was "created" by constructive interference of atomic orbitals. Bonding molecular orbitals are always energetically more favorable than the atomic orbitals from which they originated and thus, when populated, contribute to the stability of the molecule and increase the bond order. They are labelled with a Greek symbol standing for the symmetry of the orbital in question (σ,π, ...).

Molecular orbital, non-bonding Molecular orbital that is neither bonding nor antibonding in nature—for example due to the spatial orientation of the atomic orbitals involved—and thus neither increases nor decreases the molecule's stability. Mostly, non-bonding molecular orbitals originate from free electron pairs that cannot enter into constructive or destructive interactions with other orbitals.

Molecular symmetry The systematic consideration of all symmetry properties of a molecule; please refert to textbooks and/or courses on General and/or Inorganic Chemistry and/or Section 5.12 of Binnewies.

Mono- and polyprotic acids A monoprotic acid is any acid according to Brønsted or Brønsted-Lowry that has only *one* acidic hydrogen atom. In the case of polyprotic acids, the number of acidic hydrogen atoms is correspondingly larger.

Monochromatic light Light of only a single wavelength (for technical reasons often: light whose photons originate only from a very narrow wavelength *range*).

Monochromator A device that isolates one wavelength (or a narrow range of wavelengths) from the electromagnetic spectrum.

Moseley's law Describes the dependence of the energy content of a fluorescence photon on the nuclear charge/atomic number of the atom from which it was emitted and on the principal quantum numbers of the shells involved.

Mößbauer spectroscopy (often erroneously spelled Mössbauer spectroscopy) Method for the analysis of solids in which the analyte is excited by γ-radiation; the resulting transmission spectrum allows, among other things, conclusions to be drawn about crystal structures and charge density distributions.

Multiplet state, also: Multiplicity state (M_Z, also: M) While the multiplicity M indicates how many different possible states a system of one or more particles with a spin >0 can assume in the magnetic field, the multiplicity state M_Z describes which state was actually assumed in any specific case. In general, $M_Z = 2S_{total} + 1$. For this, one adds the respective concrete spin quantum numbers (+½ or −½) of all *unpaired* electrons. The result is then used in the formula to calculate the total spin or multiplicity. If $M_Z = 1$, it is called a singlet state (S); if $M_Z = 2$, a doublet state (D); if $M_Z = 3$, a triplet state (T); if $M_Z = 4$, a quartet state, and so on.

Multiplicity (M) Term describing the possible behavior of a particle in the magnetic field. For multi-electron systems, the determination of the multiplicity is very simple: Since the contribution of paired electrons to the total spin is always omitted (+½ + (−½) = 0) and for all unpaired electrons the amount of spin (i.e. |±½| = ½) is to be counted, the multiplicity M = (number of unpaired electrons) +1.

Nernst equation Equation for determining the redox potential of a redox pair as a function of the concentrations present of each under standard conditions.

Nernst's distribution theorem If a substance is shaken out with two mutually immiscible solvents, an equilibrium results according to which the ratio of the concentrations of the substance in the two phases is always constant.

Neutral point In any acid/base titration, the point at which exactly the amount of titrant has been added to the analyte so that the solution is exactly neutral (i.e. pH = 7). This neutral point can be important for the curve—for example, it coincides exactly with the equivalence point when reacting a strong analyte (acid or base) with a strong titrant (base or acid)—but it does not have to.

Neutralisation The reaction of an acid with a base (or vice versa) so that the H_3O^+ ions of the acid present react with the OH^- ions of the base to form H_2O.

NIST *(National Institute of Standards and Technology)* NIST, founded in 1901, is one of the oldest physics laboratories in the United States. On its website, it offers an almost unmanageable variety of information on practically all scientific fields (physics, chemistry, biology, and everything even remotely related, such as materials science and the like). For the content of this book, the (infrared) spectra from the associated database are of particular relevance.

NMR *See Nuclear Magnetic Resonance Spectroscopy*

Normal factor *See Titre*

Normal hydrogen electrode Reference electrode for measuring the normal potentials (E^0) of other redox pairs. The redox potential of the associated reaction ($H_2 \rightleftarrows 2H^+ + 2e^-$) is thereby (arbitrarily) set as 0.00 V.

Normal phase chromatography (NP-HPLC etc.) Chromatography with polar stationary and non-polar mobile phase.

Nuclear charge number (Z) Number of protons in the nucleus of an atom; identical to the atomic number.

Nuclear charge number, effective (Z_{eff}) In any multi-electron system, the electrostatic attraction of particles with opposing charges is affected by the interaction of the electrons with each other. For electrons "farther away" from the nucleus (i.e. populating orbitals with a higher principal quantum number), the shielding effect of more energetically favorable electrons (closer to the nucleus) leads to a reduced electrostatic attraction—as if the charge of the nucleus were slightly lower. The extent of shielding thus depends on the number of electrons present and also on the principal quantum number of the electron under consideration in each case. Quantitative statements can be made with the help of Slater's rules.

Nuclear magnetic resonance (NMR) spectroscopy Important analytical method for structure elucidation, based on the interaction of (suitable) atomic nuclei with radio waves under the influence of a (very) strong magnetic field.

Outlier A measured value within a series of measurements that differs significantly from the other measured values in the same series.

out-of-plane deformation vibration (γ) Deformation vibration in three-dimensional space; a distinction is made between twisting and wagging vibrations.

Overtitrating Addition of the titrant beyond the equivalence point. A certain amount of overtitration is usually necessary to be sure that you actually found the equivalence point. (It is imperative to meticulously record the consumption of the titrant, obviously.)

Oxidation Reaction in which one or more electrons are released. An oxidation reaction can only take place if a reduction also takes place and vice versa.

Oxidation numbers (and how to determine them!) Helpful construct for understanding redox reactions. For monoatomic ions, the oxidation number is identical to the charge. In the case of covalent bonds (molecules or molecular ions), one pretends that the electrons of the respective bond belong to the more

electronegative bond partner and the bond thus splits heterolytically. (In the case of atoms of the same kind, homolytic cleavage is necessary.)

Oxidising agent Substance that oxidises another and is itself reduced in the process.

Oxonium ion *See Hydroxonium ion*

π-orbital Molecular orbital (bonding or antibonding) in which the electron density is maximised above and below the bond axis, while a nodal plane runs along the bond axis, hence is antisymmetric with respect to rotation along the bond axis.

ψ/ψ* General sign for a wave function whose symmetry is not (or is not supposed to be) explicitly stated. A superscript * indicates that it is an antibonding wavefunction.

PAGE Abbreviation for polyacrylamide gel electrophoresis; although electrophoresis is also possible with other gels, the terms "PAGE" and "gel electrophoresis" are used *almost* synonymously, particularly in the field of life sciences.

Paramagnetism If a substance has at least one unpaired electron, a sample of that substance will be pulled into a magnetic field (making paramagnetism the opposite of diamagnetism); the larger the number of unpaired electrons, the more pronounced the paramagnetic behavior of the substance in question.

Partial equations Reaction equations used to formally separate the two processes of a redox reaction, i.e. reduction and oxidation; here, too, substance balance and charge balance must be taken into account.

Partition coefficient The equilibrium constant for the distribution of a substance dissolved in two different, immiscible solvents according to Nernst's distribution theorem.

Paschen series The series of transitions of excited electrons in which relaxation causes the originally excited electron to populate an orbital with principal quantum number n = 3. For the hydrogen atom, the wavelengths emitted in the course of this relaxation lie in the infrared region.

Pauli exclusion principle All electrons of a multi-electron system must differ in at least one quantum number. If electrons match in their first three quantum numbers, i.e. if they populate the same orbital, they *must* differ in their spin quantum number m_s, i.e. their electron spin.

Peak Substance signal in a chromatogram

Permanganometry Redox volumetry based on the redox pair MnO_4^-/Mn^{2+}. Only in the acidic medium does the reduction to divalent manganese occur; in the basic medium the oxidation effect of the permanganate is not quite so pronounced. (Permanganate is then only reduced to form manganese dioxide, MnO_2.)

Permitted *See symmetry-permitted/forbidden*

p-function The concentration of ions in solution can always be given in the form of the negative decadic logarithm of their concentration; prominent of course for its use for H_3O^+ ions (*see pH value*). Apart from that, using the p-function is common practice for the titrant ions used in the context of a precipitation titration.

pH value Negative decadic logarithm of the concentration of H_3O^+ ions in an aqueous solution; a special case of the p-function, but so common that hardly anyone thinks about it. Please note that stating a pH value is effectively stating a *concentration*.

Phosphorescence If an energetically excited system can "store" part of the excitation energy and only gradually release it in the form of photons, this is known as phosphorescence; the reason for this behaviour is symmetry-forbidden internal transitions (change in multiplicity state). In contrast to fluorescence, this luminous phenomenon can last for several minutes to several hours.

Photochemical Technical term for: by supplying energy in the form of photons (i.e. electromagnetic radiation).

Photoelectric effect Sufficiently energetic photons release (valence or core) electrons from an atom when they strike the surface of a cathodic metal; amongst other things, the basis of XRF.

Photometry Analytical method in which the analyte content of a solution can be determined on the basis of the absorption of a precisely defined wavelength from the visible light range.

Photon "Light particle" with a precisely defined wavelength and thus (via $E = h \cdot \nu$) precisely defined energy content.

pK_A value/pK_B value The negative decadic logarithm of the K_A or K_B value of a substance; measure of the tendency of a substance to act as an acid or as a base. The smaller the pK_A value, the stronger the acid; the smaller the pK_B value, the stronger the conjugate base. The following always applies: pK_A (acid) + pK_B (conjugate base) = 14.

pK_{SP} value Negative decadic logarithm of the K_{SP} value (the solubility product) of an ionic compound.

pK_W value Negative decadic logarithm of the K_W value of a substance; sum of pH and pOH value. As a rule of thumb (because actually only applicable at 25 °C, and only in water): pH + pOH = 14; *see* ► *Eqs.* 5.1 *and* 5.2.

Planck's quantum of action (h) The natural constant determining the amount of energy that can be transmitted by a photon. The transmission of an amount of energy E, which cannot be described according to the formula $E = h \cdot v$ by an integer multiple of Planck's quantum of action, is *in principle* impossible.

Plasma, inductively coupled plasma (ICP) A plasma (usually argon) stabilised by the high-frequency magnetic field of an induction coil.

Plate height, theoretical (H) The (theoretical) distance that is calculated between the theoretical plates of a chromatography column; provides information about the separation efficiency of the column in question under the

conditions selected (including flow rate, etc.). The theoretical plate height of a column is described quantitatively by the van Deemter equation.

Plate number of a column (also: number of theoretical plates) (N) Key figure for quantifying the separation efficiency of a chromatography column; is related to the theoretical plate height H via the relationship $H \times N = L$ (length of the column).

Plausibility check With every calculation in which one deals with abstract numbers, one should make sure at the end whether the resulting calculation result is also meaningful. For example, if you have determined the pH of an acidic solution with a given concentration x mol/L and now have to find out what the pH of the solution will be if the concentration of the acid is doubled, the result should preferably be a noticeably lower pH. Just like the *dimensional analysis*, recommended for*every* calculation. Really.

Polarity Shift in the charge density of electrons caused by differences in electronegativity, so that a (permanent) dipole moment can result, depending on the spatial structure of the compound in question. Polarity plays a major role in chemical reactivity as well as in the separation of different substances: Polar substances interact quite efficiently with other polar substances and are therefore often readily soluble in each other, while non-polar substances tend to be insoluble in polar substances, etc.

Polarisability The polarisability of a molecule (ion) describes the extent to which the particle in question, in the case of an interaction with another particle (or also with electromagnetic radiation), can shift the charge density along the individual bonds, resulting in a difference from the original charge (density) distribution.

Polychromatic light Light consisting of electromagnetic radiation of different wavelengths; the opposite oof monochromatic. The VIS range, i.e. the light perceivable by the human eye, consists of the quasi-continuum of the entire corresponding wavelengths; the human eye perceives this polychromatic light as “white”.

Positive blank sample *See Blank sample*

Potentiometry Analysis of a solution or a titration process on the basis of the electrochemical potential present or changing; for this, a reference electrode is always required.

Pound series The series of transitions of excited electrons in which relaxation causes the originally excited electron to populate an orbital with principal quantum number n = 5; for the hydrogen atom, the wavelengths emitted in the course of this relaxation lie in the long-wave infrared region.

ppm The abbreviation ppm, used as a unit, stands for parts *per million,* i.e. “millionths”; analogous to the indication of an analyte content in percent, only four powers of ten smaller: 1% corresponds to a ratio of $1{:}10^{-2}$, i.e. one hundredth, one ppm corresponds to $1{:}10^{-6}$. In relation to the mass content, 1 ppm means that 1 g of sample contains 10^{-6} g (i.e. 1 μg) of the analyte; those stamenets are particularly important in trace analysis. For lower analyte contents the unit ppb, *parts per billion,* is used, meaning $1{:}10^{-9}$, i.e. one billionth. For even lower analyte content, i.e. in ultra-trace analysis, the unit ppt, *parts per trillion*, is in use. It stands for $1{:}10^{-12}$ and is to be used analogously. Using

these "units" is not in the sense of the SI (and certainly not in the sense of DIN 1310, which is authoritative in Germany), and the fact that in British English "ppt" is occasionally also read as "part per thousand", i.e. corresponds to what in Germany is called "Promill" (‰), does not exactly simplify the situation.

Precipitate General term for the solid part of a liquid/solid mixture after (partial or complete) phase separation, such as the undissolved residue at the bottom of the vessel containing a saturated solution.

Precipitation form In gravimetry, the form in which an analyte is precipitated from solution using an appropriate precipitation reagent. The precipitation form need not be identical to the weighing form.

Precision A measure of the reproducibility of measurement results when using the same or an identical sample: How accurately do the results match?

Proton Commonly the positively charged nuclear building block p^+; in chemistry also—admittedly slightly impure—lab jargon for the hydrogen cation H^+, because the nucleus of the most common hydrogen isotope in the universe (^1H) consists of only one single proton.

Protonation Transfer of a hydrogen cation to an atom or molecule(-ion); the latter then has acted as a base in the Brønsted-Lowry sense.

Push-pull system An aromatic system having at least two substituents, one of which has a +M effect, another of which has an −M effect; the substitution pattern of the system must permit resonance shifting of the center of charge.

Quantisation *See* quantised

Quantised Property of any system that is no continuum: Within a quantised system, only certain values (or states) can be assumed, while other values are principally impossible-not "hard to achieve" or "quite improbable," but *principally impossible*. (An important example is the possible energy levels an electron can take: Between the 1s and the 2s orbitals, there simply *is* no possible "place" for the electron to "go" to.) Quantisation is a characteristic of the microscopic world (which is therefore also called the quantum world), but there are analogues of it in the macroscopic world as well: For example, you can only ever buy whole screws at the hardware store, either individually or in larger packs, but you won't find a half-screw—or any other non-integer number of pieces—there. If the difference between the individual possible values or states is so small that it cannot be observed without elaborate aids (like the electromagnetic spectrum), it is called a quasicontinuum: It seems to be a continuum, even though ertain imaginable wavelengths λ (all those which do not correspond to $E = h \cdot \nu = h \cdot c/\lambda$ and thus belong to a non-integer multiple of Planck's quantum of action according to the relation) are *in principle* impossible and do not exist.

Quasi-continuum A quantised system in which the difference between the individual possible values is so small that at first glance they seem indistinguishable, although in reality there is a *great* deal of difference.

Radiationless transition The relaxation of an excited system to a less excited state (which may or may not be the electronic and/or vibrational/rotational ground state) without the energy being released appearing in the form of a photon.

Radical Atom or molecule (molecular ion) with at least one unpaired electron. If the number of unpaired electrons is >1, one indicates their number with the usual Greek number prefixes (diradical, triradical etc.).

Radio waves Electromagnetic radiation with a wavelength of about 10^8–10^{12} nm.

Raman scattering Scattered radiation resulting from inelastic collisions of molecules with photons. The wavelength of the scattered radiation can be larger (= lower energy) than that of the excitation radiation, in which case it is called *Stokes radiation*, or smaller (= higher energy). In the latter case, one speaks of *anti-Stokes radiation*. It is precisely this type of radiation Raman spectroscopy is based on.

Raman spectroscopy Spectroscopic method in which molecules or molecular ions are excited by monochromatic radiation to absorb or release (quantised amounts of) vibrational energy. When a vibrationally excited analyte interacts with a photon of the excitation radiation, the molecule transfers this vibrational energy to the photon and itself relaxes to the vibrational ground state. The energy transferred to the photon allows—analogous to IR spectroscopy—conclusions to be drawn about the vibrational behavior and thus the structure of the analyte. However, only vibrational modes in which the polarisability of the analyte changes are Raman-active.

Rayleigh radiation Scattered radiation resulting from elastic collisions of molecules with photons. The wavelength of the scattered radiation is identical to the irradiated wavelength.

Redox pairs If an atom/ion/whatever is oxidised, the resulting oxidation product can also be reduced back to the starting material; the redox counterpart to conjugate acid/base pairs.

Redox potentials The actual redox potential of a half cell (in a galvanic element) depends on both the standard potential and the concentration of the electrolyte solution used. The Nernst equation is used to calculate the actual potential of a half-cell.

Reducing agent Substance that reduces another; is itself oxidised in the process.

Reduction Reaction in which one or more electrons are taken up. A reduction reaction can only take place if oxidation also takes place and vice versa.

Relative standard deviation *See coefficient of variation*

Relaxation Any process in which an (electronically or vibrationally/rotationally) excited system assumes a more energetically favorable state; this may be the ground state or merely a less excited state.

Renaturation *In connection with (chromatography) columns:* The return to the initial state.

Residue That which remains in the filter when a solid/liquid mixture is filtered.

Resolution *In chromatography:* A measure of the efficiency of a separation, determined from various key figures/properties of different analyte peaks.

Retention factor in column chromatography *(k)* Ratio of the corrected retention time of an analyte to the (column-dependent) minimum retention time; the column chromatographic counterpart to the R_f value of the TLC.

Retention factor in thin layer chromatography (R_f value) Ratio of the distance migrated by the analyte to the distance the solvent front covered.

Retention time (t_r) The time an analyte spends on a chromatography column or that elapses between the application of the analyte to the column and its detection. Since no considerations of the (column-dependent) minimum retention time have been taken into account here, the retention time is also referred to as the *unreduced* or *uncorrected retention time*.

Retention time, minimum (t_m) Retention time of an analyte not interacting with the stationary phase on a chromatographic column; the minimum time in which an analyte can pass through a column at all; required to determine the *reduced retention time*.

Retention time, reduced/corrected (t'_r) Retention time of an analyte in chromatography corrected by the measurement technique-dependent minimum retention time (t_m).

Retention time, unreduced/uncorrected (t') *See Retention time*

Retention, relative *See Separation factor α*

Reversed phase chromatography (RP-HPLC etc.) Chromatography with inverted polarity (hence: *reversed* phase): Here a non-polar stationary phase is combined with a polar mobile phase. Accordingly, the inversion of the elution capacity of the solvents used must be taken into account.

R_f value *See Retention factor*

RP-(TLC/HPLC or similar) In reversed phase chromatography, the polarity of the stationary and mobile phases are exactly reversed in comparison to the usual conditions (normal phase chromatography), i.e. stationary phase = non-polar; mobile phase = polar.

Rule of mutual exclusion For a molecule with a center of inversion, a vibration is either IR or Raman active.

Rydberg constant (R_∞) Natural constant: 109 737 cm^{-1}; calculated from the ionisation energy of the hydrogen atom.

S *See Multiplet State*

σ-Orbital Molecular orbital (bonding or antibonding) in which the electron density is maximised along the nuclear bond axis; symmetric with respect to rotation along the nuclear bond axis.

Sample range The sum of the mass of analyte and all non-analytes of a sample, i.e. the mass of analyte and matrix together. There is a fixed relationship of the sample range with working range and content range.

Saturated solution A solution in which the ion product of the dissolved particles has been reached or even exceeded; *see solubility product*.

Scatchard plot Graphical plotting of concentrations or concentration ratios (usually determined photometrically) used primarily in biochemistry to determine equilibrium constants.

Scattering diffusion (increasingly also: Eddy diffusion) Phenomenon in column chromatography; considered as the A term in the van Deemter equation.

SDS-PAGE Gel electrophoresis based on polyacrylamide gels, in which the desired homogeneous charge density of the various analytes is achieved by using the detergent *sodium dodecyl sulfate* (SDS).

Selection rules Quantum mechanical rules which allow statements about which changes of state (under excitation as well as under relaxation) are (symmetry) allowed and which are forbidden.

Selectivity, *also* specificity The more accurately an analytical method is able to determine the desired analyte even in the presence of impurities, the more selective it is. (Strictly speaking, selectivity and specificity are not *entirely* synonymous. In abstract terms, a selective method *favors* analyte A over analyte B, while a specific method responds *exclusively* to analyte A.)

Separation factor (α) The ratio of the relative, reduced retention times of two adjacent peaks in chromatography; also referred to as *relative retention*.

Shaking out Lab jargon for a liquid-liquid extraction based on the different polarities of two immiscible liquids/solvents. In which solvent which substances of a mixture of substances will preferentially accumulate can be roughly estimated on the basis of the polarity; for finer (quantitative statements) one must fall back on the Nernst distribution theorem.

Shell, K-, L-, M-, N- ... *See Bohr's atomic model*

Shielding factor σ The shielding of the positive charge of an atomic nucleus with which a more distant electron (in a shell with $n > 1$) interacts, which can be calculated according to the *Slater rules*; with the aid of this shielding factor, the effective nuclear charge number Z_{eff} can be determined for each shell of an atom with the atomic number Z.

Shoulder *In spectra and chromatograms:* A seeming "kink" in a curve, which is usually due to the fact that a second, smaller band/peak is "hidden" underneath one band (or peak), i.e. two bands/peaks of different sizes run into each other.

Significance *When stating measured values in analytics:* the number of significant digits (or places). Please do not confuse with "statistical significance".

Significant figures / digits All digits of a measured value that can be specified "with certainty", plus a last digit (the digit "on the far right") that is subject to a certain measurement uncertainty. If a measured value is given with fewer digits than the number of significant digits, this reduces the precision.

Silanol group OH group bonded to a silicon atom; the silicon counterpart of an alcohol.

Singlet (S) *See multiplicity state*.

Slater rules Rules for determining the effective nuclear charge acting on electrons in orbitals with different principal quantum numbers in multi-electron systems.

Slurry Laboratory jargon for a suspension based on the solvent water.

Solid state analysis Generic term for all analytical methods in which the analyte is present as a solid, i.e. does not have to be brought into solution or transferred to the gas phase for analysis.

Solubility Indication of the amount of a substance that can be dissolved in a given amount of solvent before the solubility product is exceeded; usually given in g/L or mol/L; in the latter case, it is often called *molar solubility*.

Solubility product (SP) The mathematical product of the concentrations of the various ions of a salt, described (once again) via the LMA. Again, stoichiometric factors enter as exponents. Because the solubility products of salts with different stoichiometries (1:1, 1:2, etc.) have different units, solubility products often cannot (or should not ...) be directly compared.

Solution Homogeneous mixture of at least two substances, usually in liquid form. Standard meaning: A substance that is solid at room temperature, dissolved in a substance that is liquid at room temperature, e.g. table salt in water (yes, you cook your noodles in a homogeneous mixture of substances). But hydrochloric acid is also a solution: The substance hydrogen chloride (HCl), gaseous at room temperature, dissolved in water (H_2O).

Solvent Designation for the substance in which another substance is dissolved. Most solvents are liquids, but in gas chromatography the gaseous mobile phase may also be regarded as a solvent.

Solvent extraction *See Extraction*

Solvent front The height to which the mobile phase has risen on the stationary phase in thin-layer chromatography; the distance between the starting line and the solvent front is essential for determining the *retention factor*.

SOMO *(singly occupied molecular orbital)* Molecular orbital which is populated with only a single electron, not with an electron pair.

Specificity *See selectivity*

Spectral lines If a thermally excitable analyte is brought into a burner flame and the (often characteristically coloured) flame is examined with the aid of a spectroscope, element-specific sharp emission lines become visible, which are the result of the relaxation of excited electrons to their ground state (or at least a less excited state).

Spin state Specifies the spin quantum number m_s used to describe his electron: If $m_s = +½$, the electron is often represented graphically by (↑); if $m_s = -½$, by (↓). A spin reversal represents a change in the spin state. However, especially in physics textbooks, the term spin state is also used to describe the multiplicity state (M_Z) of multi-electron systems; it is then abbreviated as S. This is quite obvious (after all, the multiplicity of a system depends on the respective spin state of all electrons involved), but it easily leads to confusion, especially since

the abbreviation for the general spin state, *S,* can easily be confused with the singlet state S; *see Multiplicity State*.

Sputtering The ejection of individual atoms from a metal by means of sufficiently strongly accelerated ions (of whatever kind) striking the surface of the metal.

Stability constant Value derived from the law of mass action which says something about the stability of a complex.

Standard *In chromatography:*a substance that is applied to a chromatography column independently of the analytes in order to determine the reproducibility of their properties (in rare cases: to determine the *dead time*). It is also possible to add the standard (preferably with a well-defined concentration) to the analyte mixture; in this case it is called an *internal standard*. Such an internal standard facilitates the comparison of different chromatograms, because one can fall back on a (ideally very well known) reference value.

Standard conditions Standard values for temperature T and pressure p. The standard conditions commonly used in physics are T = 0 °C, p = 1013 mbar, while in chemistry the standard conditions T = 0 °C (i.e. 273.15 K), p = 1.000 bar = 1000 hPa are more common. Add to this, however, that not only are there some other standardised combinations of these variables, but tabulated values are sometimes also related to room temperature, which is occasionally considere to be 20 °C, but sometimes 25 °C, you see: When using tabulated values, it is better to check three times which conditions have been chosen.

Standard deviations A measure of how much the individual measured values of a series of measurements deviate from their mean value. The smaller the standard deviation, the less error-prone (i.e. more accurate) the measurement. However, the standard deviation indicates *absolute* values: Small measurements then automatically result in a smaller value for s than large measurements. Using the *coefficient of variation* makes it easier to compare different series of measurements.

Standard solution A solution with a precisely known composition (the concentration being as precisely defined as possible; indispensable in volumetry). In analytics, this also means that the solution not only contains the analyte in a precisely known concentration, but may also contain additional reagents, also in precisely known quantities, which are required for the selected analytical procedure. A *blank sample* is a standard solution with the analyte concentration $c_i = 0$, i.e. it contains all additional reagents, but *not* the analyte.

Stationary phase The "immobile" adsorbent in a chromatographic substance separation; the column material.

Stock solution Solution with a well-defined concentration (or otherwise well-defined content) which serves as a starting material for further solutions derived from it in the context of a dilution series or similar.

S_total *See Total spin*

Stretching (ν) *From IR spectroscopy:*molecular vibrations in which bond distances change. This can happen in a symmetrical (ν_s) or asymmetrical way (ν_{as}).

Substance balance In a reaction equation, on both sides of the reaction arrow, all atoms of whatever kind must agree in number, even if they may differ so much in their bonding state, their oxidation number, etc.. Atoms do not simply disappear into nothing, nor do they appear "just like that"; *see also charge balance*.

Suspension Inhomogeneous mixture of liquid(s) and solid(s).

Symmetry-allowed/forbidden Certain electron transitions in UV/VIS spectroscopy (and also other excitation processes) can be energetically (unexpectedly) unfavorable due to the symmetry properties of the orbitals involved, so that these transitions occur only very rarely. Those are called symmetry-forbidden (or just forbidden, for short) transitions. Please note that the following not only applies to humans, but also to atoms and molecules: *"Just because something is forbidden does not mean that we do not do it at all. We just don't do it that often."* So, occasionally, symmetry-forbidden transitions *can* be observed.

Synproportionation *See Disproportionation*

T *Depending on the context:* in photometry: *see Transmission*; in spectroscopy it stands for Triplet*: see Multiplicity*

τ (tau) *See degree of titration*

t'_r *See Retention time,* reduced

Tailing Deviation of a peak shape from the Gaussian curve with distortion to increased retention times.

Theoretical plates, number of *See Plate number of* a *column*

Thermal (excitation) Supply of energy in the form of heat.

Thin layer chromatography (TLC) Adsorption chromatography in which the different polarity of different analytes leads to different retention values.

Titre (also called *normal factor*) Correction factor that facilitates the handling of (standard) solutions whose actual concentration (c_{is}) deviates from the desired concentration ($c_{desired}$).

Titre, Primary Substance suitable for preparing a standard solution used predominantly for titrations; must have certain properties.

Titrant The substance that is added to the analyte solution during a titration.

Titration curve Graphical plot of the titrant volume added to the analyte solution, sketched against the characteristic of the solution observed in the course of the respective titration (for acid/base titrations: the pH value, for (potentiometric) redox titrations the electrochemical potential, etc.).

TLC (thin layer chromatography) *See Thin Layer Chromatography*

t_m *See Retention time*, *minimum*

Total spin (S_{total}) Since we only want to consider electrons: The sum of the actual spin quantum numbers of all the electrons involved in a system. Spin-paired

electrons (↑↓) together contribute 0 to the total spin, because +½ + (−½) = 0. The total spin of a system thus results from the sum of the actual spin quantum numbers of all *unpaired* electrons.

t_r *See Retention time*

Trace analysis The examination of analytes whose content in the sample lies clearly below "percentages", but rather in the ppm range (or even below).

Translation Movement of a system along a precisely defined direction so that all (mass) points involved experience exactly the same displacement.

Transmission (T) *In (spectro-)photometric analysis:* the ratio of the light intensities of analyte solution and blank sample; given in %; unlike absorbance, not overly common.

Transmission spectrum A spectrum based on the measurement of how large the portion of the irradiated electromagnetic wavelengths is that passes through the sample unobstructed/unchanged.

Trend line Line to be drawn in the graphical application of measured values, which illustrates a generally recognisable tendency (e.g. the greater the x-value, the greater the y-value, etc.).

Triplet (T) *See Multiplicity state*.

Tyndall effect If one illuminates a heterogeneous mixture of finely distributed solid particles or liquid droplets suspended or emulsified in a solvent, the beam path can be traced with the naked eye because the light is scattered by the surface of the suspended particles. This effect does not occur in *real* (= homogeneous) solutions.

Ultra trace analysis The analysis of analytes whose content in the sample is below the ppm range. Here chances are one will reach the limit of quantification.

Ultraviolet radiation (UV radiation) Electromagnetic radiation with a wavelength of about 100–380 nm; not visible to the human eye.

UV *See Ultraviolet…*

UV/VIS range Electromagnetic radiation of the wavelength range 100–800 nm, i.e. ultraviolet radiation and light visible to the human eye.

Vacant *In technical terms referring to orbitals:*not occupied by an electron or a pair of electrons. (By definition, every LUMO *must* be vacant (i.e. _*u*_noccupied) in the ground state, otherwise it would not be an L_*U*_MO.)

Validity A measure of how closely measured values correlate with the value that would ideally have been obtained, i.e. if the measurement had been completely free of error.

van Deemter equation Formula describing the dependence of the theoretical plate height of a chromatographic column on the various diffusion phenomena in column chromatography; since—depending on the column material and

separation method—up to three different phenomena have an effect, the van Deemter equation consists of three terms to be treated separately, commonly called the A through C terms.

Vib./Rot. excitation If a multi-atomic system is excited to a higher energy level of vibration or rotation, this is called vibratory/rotatory excitation, abbreviated as vib/rot excitation (with or without the period).

Vibration modes Different types of (quantised!) vibrations that can be induced in vibrational spectroscopy: A distinction is made between stretching and deformation vibrations.

Vibration/rotation levels Collective term for the different energy levels of vibrations and rotations.

Vibrational degrees of freedom (F_S) Number of vibrations that are theoretically possible in a molecule (or molecular ion) consisting of a number of N Atoms. The number of vibrational degrees of freedom of an N-atomic molecule is calculated to be $F_S = 3N - 6$ for all non-linear molecules; for linear molecules the number of degrees of freedom increases to $F_S = 3N - 5$.

Vibrational spectroscopy Generic term for spectroscopic methods in which molecules or molecule moieties are excited to vibrations by electromagnetic radiation; absorption spectra are produced in the process.

VIS range Electromagnetic radiation from the wavelength range 380–780 nm; perceived by the human eye as light of wavelength-dependent colour.

VIS *(visible)* *See Light*, visible.

Volumetric flask Glass instrument for the measurement of a precisely defined volume. Unlike graduated cylinders, volumetric flasks do not have a scale, but a ring mark that facilitates reading. Such volumetric flasks are then calibrated to the respective volume at a temperature of 20 °C. Volumetric flasks with volumes from 5.00(0) mL to 5.000 L are commonly used in the laboratory and are commercially available.

Volumetry Any form of analysis in which the concentration of the analyte is inferred from the consumption of a reagent added to the analyte solution.

w *See Base width*

$w_{½}$ Width of a peak at half height, also called *half width*; easier to determine than the corresponding base width.

Wavenumber ($\tilde{\nu}$) Alternative way of specifying the energy content of an electromagnetic wave or photon; reciprocal of the wavelength. In spectroscopy, the wavenumber is usually given in the unit cm^{-1} (even if this is not strictly SI compatible).

Wave-particle duality Some microscopic objects (amongst others: electrons and photons) can neither be described completely as particles nor as waves: They always seem to have "something of both". Ever since quantum mechanics was developed, the attempt was made to interpret contradictory, occasionally even absurd, measurement results in a meaningful way. So far, however, a

clear explanation is not yet available. *We see: The natural sciences are not yet "finished".*

WDXRF *See X-ray fluorescence analysis*

Weighing form Form in which the amount of substance of an analyte can be determined gravimetrically; must have special properties.

Weighing out Laboratory jargon for "using a balance to determine the mass of a substance as accurately as possible".

Working range The specification of the working range provides information about the minimum amount of the substance to be analysed that must be present in a sample in order to make reproducible statements with the aid of the selected analysis method.

X-ray diffraction/X-ray structure analysis Solid-state analytical method for the investigation of crystal structures based on the diffraction of the X-rays used on the crystal lattice.

X-ray fluorescence analysis (XRF) Non-destructive analysis technique in which the sample material is excited to fluorescence by high-energy X-ray photons (which can lead to the release of Auger electrons). The detection of the fluorescence photons can be energy dispersive (EDXRF) or wavelength dispersive (WDXRF). In the English-language literature referred to as XRF, *x-ray fluorescence.*

X-rays Electromagnetic radiation with a wavelength of about 10^{-3} to 10 nm.

XRF (x-ray fluorescence) *See X-ray fluorescence analysis*

Z *See Nucleus charge number*

Z_{eff} *See Nucleus charge number*, effective

Zwitterion A molecule that has both one (or more) positive and one (or more) negative charge(s) such that it is *outwardly hinelectrically* neutral (i.e. behaves as if it were uncharged); actually a German word, meaning "Hermaphrodite ion".

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X