

1

Introduction to Optical Spectroscopy

Bene docet, qui bene distinguit
(Horace)

Good choices means good teaching

1.1

Overview

The term *Optical Spectroscopy* (OS) in this book covers all types of qualitative and quantitative analytical methods that are based on the interaction of light with living and non-living matter.

Here the term “light” includes electromagnetic radiation covering the spectral range from the far-ultraviolet (UV), followed by visible light (VIS) up to the near-infrared region (NIR). For more than 200 years optical spectroscopy has been utilized in various fields of science, industry and medicine, particularly in (bio-) chemistry, biology, physics and astronomy. OS is highly specific, each substance is discernible from all others by its spectral properties. Samples can be qualitatively and quantitatively analyzed. In contrast to other spectroscopic methods such as NMR (nuclear magnetic resonance), ESR (electron spin resonance), Mößbauer or mass spectroscopy, the requirements of the samples to be analyzed are not particularly restrictive. Measurements of various optical parameters as a function of wavelength/energy (“spectrum”) or time (“kinetics”) provide valuable insights that are not, or not readily, attainable by other analytical methods.

Optical spectral analysis is a well developed method. However, there is still a rapidly growing market for spectrophotometers and in addition to routine analyses many novel applications are being continuously reported. Depending on their required specifications, spectrophotometers differ markedly in size, form and usability and, last but not least, in price. They can be specifically adapted to the envisaged purpose. Therefore, the current trend is to use highly specific, moderately priced spectrophotometers rather than installing and using the most expensive “all-purpose machines” that have the best specifications available for more diverse applications.

The specifications of modern optical spectrophotometers are now approaching the limits set by physics; the advantages compared with other analytical methods are many, as follows.

- OS is not destructive or invasive.
- Remote measurements are feasible, i. e., measurements ranging from distances of a few millimeters up to long distances, such as from airplanes or satellites, without any physical contact with the sample. Hazardous or unattainable objects are easily analyzed.
- In principle liquid samples as well as solids and gases are acceptable, independent of their “optical quality”. Transparent samples as well as highly light scattering, turbid and opaque samples are measurable.
- The photometric as well as kinetic time scales covered are extraordinarily wide and are unmatched by other analytical methods.
- OS (for example, femtosecond laser spectroscopy) allows measurements of extremely fast reactions down to a femtosecond time scale (10^{-15} s).
- Minute samples in the micrometer range (microspectroscopy) or very rare events can be investigated using single photon counting (measurement of a few photons per second).
- Extremely low concentrations down to 10^{-18} moles are detectable by luminescence methods.
- Radioactive markers for the investigation of biochemical and molecular-biological processes are increasingly being replaced by cheaper and easy to use luminescence markers and low cost luminescence spectrometers.

In this book we will discuss the essential features of optical spectroscopy with minimum descriptions of complex theories, various spectrophotometric procedures and apparatus. This is not an easy task in light of the significant progress that has been made in optoelectronics, laser and microcomputer techniques in recent years. However, the book is not intended to be a “how to use” manual for a specific spectrophotometer. For this purpose the reader should consult the manufacturer’s manual. In addition, spectrophotometer manufacturers offer practical and theoretical introductory seminars; however, they are often rather expensive, time consuming and only focus on their own products.

After a short journey through the history of optical spectroscopy in the current chapter, Chapter 2 offers a short orientation and introduction to quantum theoretical concepts that are a prerequisite to the understanding of the spectroscopic properties of matter. Starting with the hydrogen atom as the simplest quantum mechanical system we then focus on the spectroscopic properties of small, diatomic molecules and finally turn our attention to complex (bio-)molecules.

Optical spectroscopy is intimately connected to optical physics. Thus, in Chapter 3 we will establish the necessary fundamental knowledge and become acquainted with the diverse optical elements: starting with the radiometric and

photometric definition of light units, the basics of geometric, wave and particle optics, and finally the generation of light and its measurement will be described. We will discuss the properties of optical components such as filters, mirrors, lenses, optical fibers, integrating spheres as well as methods for dispersing light by prisms, gratings and interferometers.

In the fourth chapter we will utilize the theoretical and practical knowledge obtained from the first three chapters to describe atomic absorption (AAS) and atomic emission spectrometry (AES) as two variants of atomic spectroscopy for theoretical and practical discussions.

In the fifth chapter we will discuss in more detail the theoretical and practical aspects of molecular absorption spectroscopy, the most widely utilized analytical tool in various fields. We will discuss different versions of absorption spectrophotometers and also focus on diverse methods of spectral evaluation in order to extract “hidden” information from spectra that may not be immediately apparent at first glance.

The sixth chapter deals with luminescence spectroscopy. While absorption or extinction is the only measured parameter to be determined in absorption spectroscopy, luminescence spectroscopy provides a number of measurable parameters for the molecular system of interest.

Photoacoustic spectroscopy (PAS), where heat emission rather than light is detected, will be discussed in the seventh chapter. Supplementing absorption and luminescence spectroscopy, PAS allows conclusions to be drawn about thermodynamic relationships, particularly of complex molecular systems in biology, chemistry and medicine. Moreover, it allows spectral scans to be made of non-visible, subsurface layers of sample materials such as skin or fruit shells.

Measuring methods and phenomena that depend on various scattering effects such as Rayleigh, Mie, Fraunhofer or Raman scattering, including the related reflection and ATR spectroscopy (*attenuated total reflection*), will be discussed in the eighth chapter.

Because of the importance of the chiral and stereochemical properties of biomolecules, we introduce ORD (*optical rotational dispersion*) and CD (*circular dichroism*) spectroscopy in the ninth chapter, even though both methods are derivatives of absorption spectroscopy and are related to Chapters 5 and 8. Finally, ellipsometry utilizes the phase shift from the surface reflection of polarized light, an effect that is applicable for the highly sensitive analysis of biologically relevant molecules.

With the availability of low cost, fast, on-line microcomputers with large memories, near-infrared spectroscopy (NIR) has experienced an unprecedented boom in recent years, particularly in routine industrial analysis. NIR spectroscopy has become indispensable in the pharmaceutical industry, medicine and the food industry, and in petrochemical, geological and environmental analysis. Further technical developments in these areas are anticipated, also extending into other application areas of industrial research. Therefore, this topic is treated in Chapter 10.

The most important physical constants as used in this book can be found in the Appendix. The Periodic Table of the elements, including detailed electron distributions, may serve as a quick overview. Another table allows the easy conversion

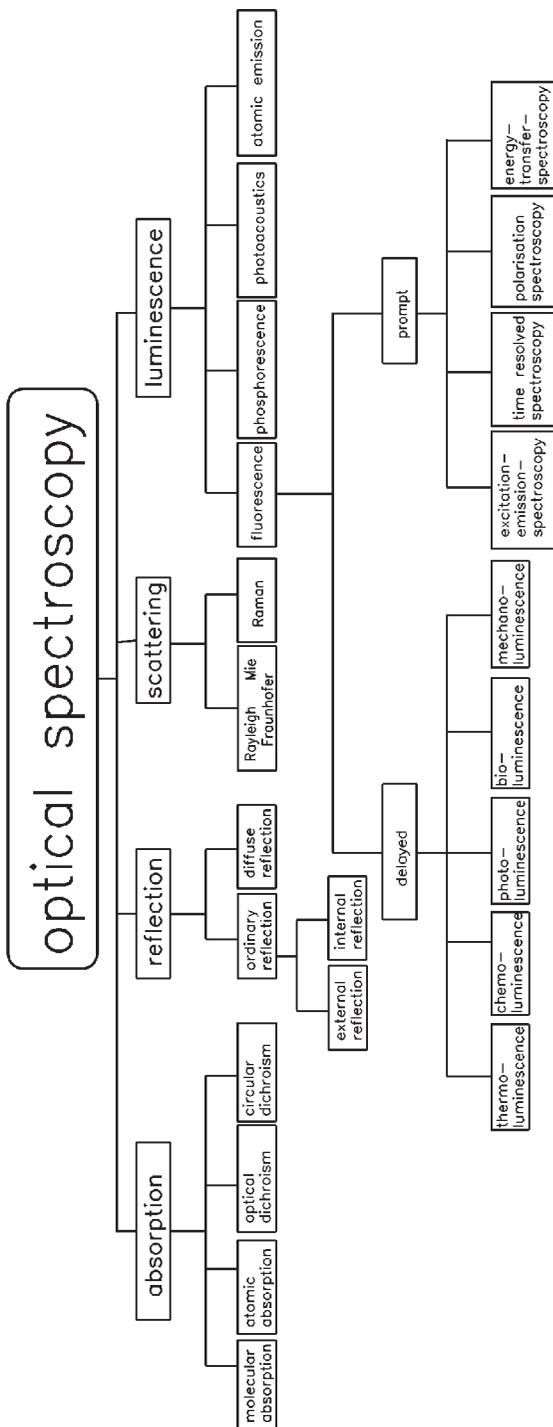


Figure 1.1 Overview of optical spectroscopy as discussed in the present book. We distinguish absorption, reflection, scattering and luminescence spectroscopy, with secondary disciplines as shown. For simplicity, photoacoustic spectroscopy, a type of emission spectroscopy (emission of heat), is attributed here to luminescence spectroscopy.

of various units of energy. As an “interface” between the reader and various manufacturers and distributors of optical spectrophotometers and components of all types, important addresses including email and URL addresses are listed. Supplementary literature is given at the end of each chapter. Figure 1.1 depicts the whole field of optical spectroscopy as discussed in the present book.

1.2

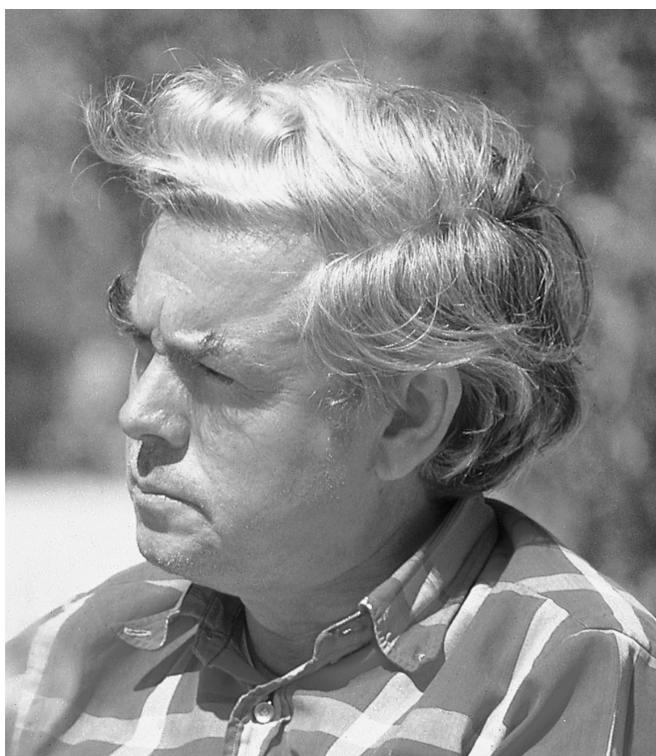
History of Optical Spectroscopy

Table 1.1 presents the highlights in the history of optical spectroscopy.

| | |
|------|--|
| 1666 | Sir Isaac Newton is the first to use a glass prism for spectral dispersion |
| 1675 | Newton's famous book on Optics (“Optiks”) |
| 1758 | Markgraf: Analysis by flame coloration (without spectral dispersion) |
| 1800 | Herschel discovers IR radiation (England) |
| 1801 | Ritter discovers UV radiation (Jena, Germany) |
| 1802 | Wollaston uses a slit and lens in flame spectroscopy for the first time |
| 1807 | Young: Experiment with double slits to prove wave-nature of light, interprets as the first “colors of thin layers” and action of optical gratings properly |
| 1808 | Malus recognizes polarization of light (by reflection) |
| 1814 | Fraunhofer: Invention of the grating (1500 lines in the sun's spectrum) |
| 1817 | Young solves the problem of “polarization by reflection”: united wave- and corpuscle picture; transversal polarization |
| 1818 | Fresnel and Huygens unite wave character and Huygens' principle |
| 1834 | Talbot distinguishes spectrally “strontium-red” and “lithium-red”, birth of analytical optical spectroscopy |
| 1848 | Foucault: sodium in the electrical arc (absorption/emission) |
| 1859 | Kirchhoff: absorption = emission wavelength, discovers sodium in the sun's spectrum |
| 1860 | Kirchhoff and Bunsen discover cesium and rubidium |
| 1868 | Ångström lists lines of the sun's spectrum |
| 1868 | Kirchhoff and Bunsen discover helium in the sun |
| 1885 | Balmer discovers Balmer series lines in hydrogen spectrum |
| 1887 | Michelson and Morley experiment, end of ether-theory |
| 1887 | Henry A. Rowland revolutionizes experimental spectroscopy with his concave grating; improvement of Ångström's experiments of 1868 |
| 1888 | Rowland publishes the first sun atlas (“Rowland circle”) |
| 1891 | Rowland compares spectra of all known elements with the sun's spectrum and finds several new lines |
| 1893 | Rowland publishes the standard spectral atlas (reference: sodium) |
| 1894 | Helium discovered on earth (1868 in the sun's spectrum) |
| 1896 | Balmer develops his first series formula |

| | |
|------|---|
| 1897 | Thompson discovers the electron |
| 1897 | Rubens succeeds with the first isolation of an infrared band utilizing multiple reflections at metal layers |
| 1911 | Rutherford discovers the atomic nucleus |
| 1920 | H. v. Halban: first determination of concentration of molecules utilizing “light electric apparati” |
| 1925 | G. Scheibe, W. Gerlach and E. Schweitzer: first real quantitative spectral analysis |
| 1926 | G. Hansen builds the first recording double beam spectrophotometer following the plans of P. P. Koch (1912) |
| 1941 | The famous prism photometer, Model DU, from Zeiss comes to the market; for the first time this spectrophotometer covers the UV range, resolution 1 nm, measured values are read from a scale with a needle pointer |
| 1972 | W.L. Butler uses a monochromator (Cary 14) on-line with a minicomputer (PDP11, Digital Equipment Corp. AF-01) thereby pioneering optical spectroscopy in biological/biochemical research (see photograph of Butler) |

Table 1.1 History of optical spectroscopy



Warren Lee Butler (28.1.1925–21.6.1984), co-discoverer of the plant pigment phytochrome and one of the pioneers of optical spectroscopy in biological/biochemical research (photograph taken by the author)

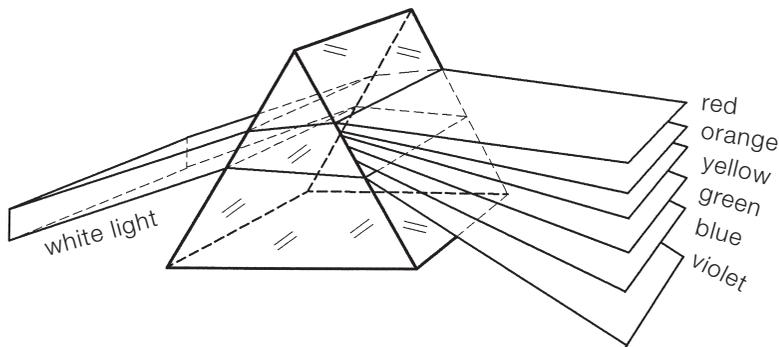


Figure 1.2 Newton was the first to discover that a glass prism will disperse parallel sunlight into its components, i. e., its spectral colors.

The word “spectrum” is derived from the Latin and implies “apparition” or “scheme”. It was first introduced, in the sense of today’s science, by Isaac Newton in 1666 in his classic volume “Optiks”. He was the first to use a prism to disperse sunlight into its spectral colors (Figure 1.2). In 1758 Markgraf was the first to use the coloration of flames to identify substances by visualization with the bare eye. In 1802 the English physicist William H. Wollaston resumed Newton’s prism experiment, improved the set-up and thus provided the first observation of numerous dark lines in the sun’s spectrum. In the same way J. Herschel and W. H. T. Talbot analyzed the light from a flame. In 1834 Talbot, using the same method, succeeded in distinguishing unequivocally the red flames of lithium and strontium on a spectral basis, which could be regarded as the birth of spectral chemical analysis.

With this new technique, which has enriched optical spectroscopy up to the present day, an apprentice who originally learned to grind mirrors, who was self-taught and later became Professor of Physics in Munich, Josef Fraunhofer, succeeded in the dispersion of sunlight (1814).

Fraunhofer’s family were very poor and as a young boy he became an “apprentice”. For three years he was taught how to grind lenses and mirrors, and thus he became very interested in optics; but as a boy from the lowest social class he had no chance or opportunity to study, and his application to study was turned down. Thus, he taught himself secretly, and invented the optical grating, which later “enriched” science tremendously. At the beginning of these activities he was not accepted by the German scientific/academic community. Nevertheless, after many years they had to accept his work and finally he even became Professor of Physics in Munich and a highly respected person.

On the basis of the optical grating he invented – and which is still used in modern spectral apparatus today – Fraunhofer developed a high dispersive optical spectroscopy, which can now be found in the Deutsches Museum in Munich, Germany (Figure 1.3). Using such an apparatus, in 1859 Kirchhoff detected sodium in the sun’s spectrum (Figure 1.4), and on this basis derived the famous *Kirchhoff law*:



Joseph von Fraunhofer (1787–1826)

“At a given wavelength and temperature the magnitude of the spectral emission of any object equals the magnitude of its absorption”.

Prior to the 20th century there were no theories that could explain satisfactorily the complex behavior exhibited by all substances. The most important founda-



Figure 1.3 First grating spectroscope, Fraunhofer, 1821 (Deutsches Museum, Munich, Germany).

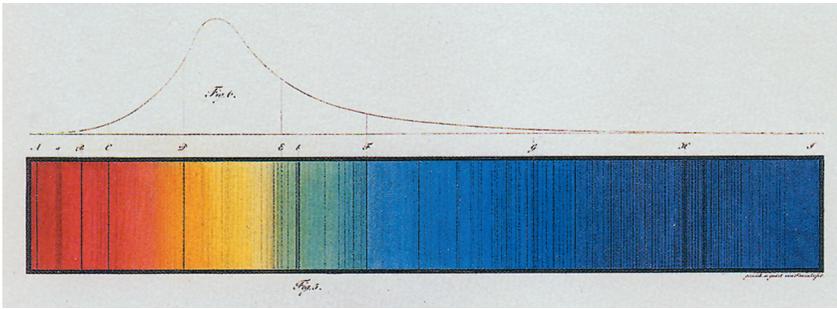
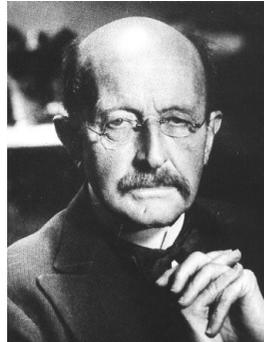


Figure 1.4 Fraunhofer lines in the sun's spectrum. Copper engraving, 1814, taken from: *Denkschriften der kgl. Akademie der Wissenschaften zu München*, 1814 and 1815,

Vol. 5, Table II, Munich 1817. The solid line (cf. Figure 3.4) shows the sensitivity curve of the human eye (Deutsches Museum, Munich, Germany).



Gustav Robert Kirchhoff (1824–1887)



Max Planck (1858–1947)

tions which led to today's understanding of spectra were laid down by the following scientists.

In 1885 the Swiss scientist J. J. Balmer discovered the “Balmer” series of lines in the spectrum of hydrogen. In 1897, the English physicist J. J. Thomson discovered the electron. In 1911 his fellow countryman Ernest Rutherford discovered the atomic nucleus. In 1900 *Max Planck* formulated the first principles of quantum theory. Werner Heisenberg (1932) and Erwin Schrödinger (1933) won the Nobel Prize for their pioneering work on quantum mechanics. These early quantum theory concepts were further advanced by Paul A. M. Dirac and Wolfgang Pauli (1945), for which both researchers were honored with the Nobel Prize (Dirac in 1933, together with Schrödinger). Today quantum theory is well established and its development is in a sense complete.

As the history of science is entwined with the history of measurements and analysis, the history of optical spectroscopy is largely reflected in the history of



Werner Karl Heisenberg
(1901–1976)



Erwin Schrödinger
(1887–1961)



Wolfgang Pauli
(1900–1958)

astronomy and thus in the history of atomic spectroscopy. Only at the end of the 19th century did *molecular spectroscopy* become important as a powerful analytical method. For example, forensic investigations could now solve murders through just tiny spots of blood, using spectrophotometers that were capable of measuring the characteristic “bands” of hemoglobin and thus distinguishing between blood and red dyes.

For many decades, as a result of the simple tungsten glow lamps, prisms, gratings and light detectors, optical spectroscopy was confined to the narrow visible wavelength range between 500 and 700 nm.

In the 40 years at the beginning of the 20th century only a few commercial spectrophotometers were available as they were manufactured in limited number, and, in addition, were difficult to operate (General Electric Hardy Spectrophotometer, Cenco “Spectrophotometer”, Coleman Model DM). At that time the “measurement” of extinction for the determination of concentration was performed visually by comparison of two visual fields next to each other (similar to the Nagel anomaloscope used today for testing color vision) by the naked eye. The famous Pulfrich-Photometer (some thousand units were produced by Zeiss in Jena, Germany) also worked in this way, relying heavily on a series of so-called S-filters in the visible range (interference filters with half-widths of only 15–20 nm). By 1941 there were already more than 800 publications on the determination of the concentration of the clinically important components of blood and other body fluids using this very spectrophotometer.

However, even by the 1930s the great significance of the ultraviolet spectral range (UV) had been recognized, particularly for the quantitative determination of vitamin A (which has an absorption at 320–330 nm). Indeed, this turned out to be the key factor in the subsequent development of UV–VIS (visible) spectrophotometers. Under the leadership of H. H. Cary and A. O. Beckman, the famous Model D spectrophotometer, with a selling price in 1941 of US\$ 723, brought about a breakthrough in optical spectroscopy, as it was the first commercial instrument to include the UV region. A hydrogen lamp as the only UV light

source was developed for this purpose. The quartz glass required for the manufacture of UV prisms was difficult to obtain, as it was used primarily by the military in quartz-stabilized oscillators for radio transmitters. A photomultiplier for the UV range down to 220 nm had to be developed (the available cesium oxide types were only good for wavelengths >600 nm). The DU/DU1 series of instruments from Beckman, probably the most successful series worldwide ever, was developed continuously up till 1976, and all together more than 30000 units were sold.

In parallel with the development of UV-VIS spectrophotometers, infrared spectrometers were developed. However, only after World War II did the market for spectroscopic analytical instruments widen rapidly, and various manufacturers developed the techniques further. Owing to the better resolution and low stray light compared with a prism, dispersing gratings were chosen, and automatic scanning double monochromators yielded baseline-corrected spectra, thereby leading to use for routine analytical work. Stray light was lowered dramatically thereby increasing the detection capability of spectrophotometers by 4–5 orders of magnitude. Specialized photometers, e.g., for radiometry, colorimetry or dual wavelength analysis, were developed for the market. A significant push came at end of the 1970s with the advent of powerful, low cost minicomputers, which accelerated still further in the early 1980s with the appearance of the first microcomputers leading to today's PC (personal computer) based technology. The PC not only lightens the routine workload, but in addition, enables on-line measurements and complex analyses to be made, which had previously only been possible with large computers. Old theories such as that of Kubelka–Munk on scattering analysis, Fourier analysis or diverse correlation and fitting procedures or chemometrics as utilized in near-infrared spectroscopy, which depend heavily on calculation power, are now available to everyone and are performed readily.

1.3

Selected Further Reading

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