Chapter 2. **Principles of Spectrophotometry**

2.1. Introduction

Spectrophotometric methods are among the oldest methods of analytical chemistry. The absorption of visible light by certain chemical substances has long been used for visual determination of their concentration. As early as in the middle of the XIX century methods were known for the determination of bromide in natural waters by oxidation and extraction of the resulting bromine into ether, of ammonia by Nessler's method, of titanium by the peroxide method, and of molybdenum by the thiocyanate method. The term "colorimetry" was used for those analytical methods, in which chemical elements were determined by comparing the colour of unknown samples with appropriate standards, either in graduated cylinders or in visual comparators. The use of photoelectric instruments has given rise to measurement of the absorption of radiation as it passes through the analysed samples and has enabled us to extend the useful radiation range outside the visible region.

Nowadays, spectrophotometry is regarded as an instrumental technique, based on the measurement of the absorption of electromagnetic radiation in the ultraviolet (UV, 200–380 nm), visible (VIS, 380–780 nm), and near infrared region. Inorganic analysis uses UV–VIS spectrophotometry. The UV region is used mostly in the analysis of organic compounds. Irrespective of their usefulness in quantitative analysis, spectrophotometric methods have also been utilized in fundamental studies. They are applied, for example, in the determination of the composition of chemical compounds, dissociation constants of acids and bases, or stability constants of complex compounds.

Spectrophotometry as a measuring technique has developed enormously as a consequence of the progress in technology, and in the development of new materials and of methods of data processing. The development of specialized optics, and of spectrophotometers coupled with microprocessors controlling their operation, has extended considerably the possibilities of using these instruments, the recording of absorption spectra, and the treatment of the data collected. Spectrophotometric methods have proved to be particularly suitable for automation, both in analytical procedures and in the treatment of data. They belong to the detection techniques most frequently used in automatic flow injection analysis (FIA).

2.2. Absorption and molecular structure

Spectrophotometric methods of identification and determination of substances are based on the existence of relationships between the position and intensity of absorption bands of electromagnetic radiation, on the one hand, and molecular structure on the other. Electronic spectra result from changes in the energy states of electrons [σ, π, and free electron pairs (n)] in a molecule as a result of absorption in the UV–VIS region. The changes depend on the probability of electronic transitions between the individual energy states of the molecule. The number of absorption bands, and their positions, intensities and shapes are the spectral parameters utilized in qualitative and quantitative chemical analyses [1–3].

The positions of individual absorption bands recorded in the spectra depend on the energy of the absorbed radiation. Radiation from the near-infrared region gives rise to changes in the rotational and oscillation energy states in a molecule. Narrow bands that are due to small

changes in wave number are connected with characteristic groups of atoms (functional groups) and are used for identification of such groups.

The UV–VIS radiation gives rise to changes in the energy of electronic states of a molecule. The probability of electronic transitions in a molecule depends on the presence of multiple bonds in the molecule and on the kind, number and positions of the substituent groups. Determination of the kind of transitions corresponding to the observed bands of absorption spectra enables one to determine the structure of the molecule.

Spectral transitions of electrons associated with absorption of radiation correspond to transitions from binding orbitals (σ, π, n) to anti-bonding orbitals of higher energy state (σ^*, π^*). The energy of the respective transitions decreases in the following order:

$$\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$$

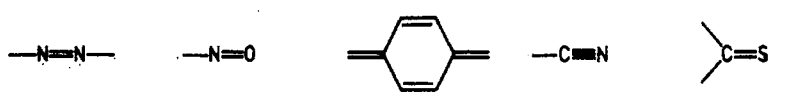
The $\sigma \rightarrow \sigma^*$ transitions may take place in the far ultraviolet, which is generally not recorded in spectrophotometers. Other transitions occur in the near ultraviolet and visible regions. The $n \rightarrow \pi^*$ transitions are characterized by high intensity which varies depending on the number and kind of multiple bonds in the molecule. An increase in the number of conjugated bonds results in a reduction of the distance between the π and π^* levels, an increase in the probability of transition, and increase of intensity of the spectrum recorded.

The considerable bandwidth and the high intensity are characteristic for absorption bands due to intermolecular charge transfer without ionization. They appear when an electron is transferred, under the effect of radiation energy, from a donor molecule to a free orbital of another molecule (acceptor). The charge transfer bands occur in the near ultraviolet and are broad in general.

The visible and the near UV regions are characterized by absorption bands owing to intra-atomic $d - d$ transitions. This kind of transition is specific for ions of transition metals with an incomplete d shell. Splitting of the d sub-levels in the ligand field creates a possibility of transitions between the levels due to absorption of appropriate radiation quanta. The colour of solutions of transition metal ions is connected with the intra-atomic $d - d$ transitions. The transition metals are capable of forming coloured complexes even with colourless reagents, which do not contain chromophoric groups.

Changes in the energy of electrons within the ligands, charge-transfer bands, and bands owing to intra-atomic transitions determine finally the shape of the absorption spectrum of the given compound. The position and the intensity of electronic spectra may change when substituents are introduced into the molecule or when its environment is changed.

The colour of a molecule is an effect of the presence of **chromophoric groups**. A chromophore may be a group of atoms containing easily excitable π electrons (formula 2.1), including the most important groups for the visible region: the azo group –N=N– and the *p*-quinonoid system.



(2.1)

The changes in the energy levels of these electrons owing to the absorption of radiation quanta give rise to characteristic bands in the absorption spectrum. The more easily the bond electrons in a molecule are excited, the more intense is the colour of the compound. The shape of the spectrum and the intensity of the absorption band depend on the position of the

chromophores in the molecule. In isolated systems, where multiple bonds are separated from each other by at least two single bonds, the spectra contain absorption bands characteristic for individual chromophores. Where double bonds are present in conjugated systems, intense absorption bands, shifted towards longer wavelengths, appear in most cases.

The features of the absorption spectra change if the so-called **auxochromes** (*e.g.*, $-NH_2$, $-NR_2$, -SH, -OH, -OR) are introduced into the molecules. The presence of free electron pairs in the auxochromic group, that interact with π- electrons of the chromophoric group (*e.g.*, the free electron pair at nitrogen in the $-NH_2$ group) leads to a state of conjugation which may result in formation of a new absorption band in the spectrum.

An action of a substituent or a solvent may give rise to a shift of absorption band towards longer wavelengths – the bathochromic effect, or towards shorter wavelengths – the hypsochromic effect. An increase or a decrease of band intensity is referred to as the hyperchromic or the hypsochromic effect, respectively.

The shape of a spectrum and its intensity depend on the positions of substituents in the molecule and on their inter-relationships, as in *cis-* and *trans-* isomers. In general, higher band intensities, and shifts to the higher wavelength part of the spectrum, are characteristics of the *trans* isomers, in which the coupling of π- electrons is stronger for spatial reasons.

The intensities and the positions of absorption bands may also be influenced by solvent molecules. Electrostatic dipole interactions, and specific interactions that lead to formation of complexes based on hydrogen bonds and of *charge-transfer* type complexes, result in changes in the spectra of chemical compounds.

As the pH is increased, spectrophotometric reagents ionize and their electronic structure becomes deformed, which often leads to a bathochromic shift of the absorption maximum. Ionization causes polarization of the chromophoric system. The formation of a chelate complex disturbs the electronic state of the organic molecule to produce, as a rule, a bathochromic shift.

2.3. Absorption laws

Spectrophotometric measurements are generally made on solutions, either in water or in organic solvents, contained in a measuring cell which is placed in the path of a beam of monochromatic radiation of chosen wavelength.

From the total radiation of intensity I_0 that impinges upon a layer of solution, one fraction of the beam I_a is absorbed on passing through the solution, another fraction I_t is transmitted, and still another fraction I_r is reflected by the cell walls and scattered:

$$I_0 = I_a + I_t + I_r$$

As absorption measurements are always made by comparison with a standard solution, and both the sample and the standard are placed in identical cells, the part of radiation denoted as I_r is constant and may be neglected.

The amount of radiation absorbed depends on the thickness of the absorbing layer and on the concentration of the solution [4,5]. In 1729 Bouguer established the relationship between the amount of absorption (the absorbance) and the thickness of the absorbing layer. A mathematical formulation of this relationship was given by Lambert in 1769. In 1852, Beer settled a relationship between the absorbance and the concentration of coloured solutions. In the formula derived (the Bouguer–Lambert–Beer law) both the solution concentration and the layer thickness are taken into account.

When a parallel beam of monochromatic radiation of intensity I_0 impinges upon a layer of solution of thickness dl, a part of the radiant energy is absorbed. If the layer thickness changes, the absorption changes proportionally. The fraction absorbed increases exponentially with linear increase of the layer thickness:

$$\frac{dI}{I} = -kdl$$

where k is a constant, and the minus sign denotes that the intensity of the radiation transmitted decreases as the thickness of the layer increases.

Integration of the above equation gives the following expression:

$$\ln \frac{I_0}{I} = kl$$

where I_0 denotes the initial beam intensity (for l = 0).

By conversion of natural logarithms to decimal ones the above equation assumes the following form:

$$\log I_0/I_t = 0.434 \ln I_0/I_t = 0.434 \text{ Kl} = A$$

where K is a new constant, and A is the absorbance.

The **absorbance** is a logarithm of the ratio of incident beam intensity, I_0 to the intensity of the beam transmitted, I_t.

If the concentration, c, of the absorbing species is doubled and the absorbing layer thickness is reduced by a factor of two, then the total number of absorbing molecules remains the same, hence the absorbance A will also remain the same. Therefore the absorbance is a function of the number of absorbing centres in the light-beam, *i.e.*, of the product cl, and the above equation can be given the form:

$$\text{A} = \log I_0/I_t = \varepsilon cl$$

where ε is a new constant called the molar absorptivity (or absorption coefficient), c is the concentration of absorbing species (M, in moles per litre), and l is the layer thickness (in cm).

The equation is a mathematical expression of a fundamental law of spectrophotometry, the **Bouguer–Lambert–Beer** law, which states that absorption of radiation depends on the total number of absorbing centres, *i.e.*, on the product of concentration and layer thickness of the solution.

In spectrophotometric measurements the thickness of the sample layer is usually identical to that of the reference solution, and only Beer's law, which relates the absorbance with the concentration of the sample solution, is of practical significance.

If a solution contains more than one absorbing species and there is no interaction between the components, the total absorbance of the solution is equal to the sum of all the component absorbances. The **law of additivity** of absorbance (providing the optical path length is constant) is expressed by the formula:

$$\text{A} = (\varepsilon_1 c_1 + \varepsilon_2 c_2 + \ldots + \varepsilon_n c_n)l$$

The additivity of absorbance constitutes the basis for studies of multicomponent systems.

If a coloured solution obeys Beer's law, the graph of $A = f(c)$ is a straight line passing through the origin.

From a practical point of view it is desirable that the solution should follow **Beer's law** for the concentration range corresponding to absorbances not exceeding 1 (unity).

Deviations from Beer's law may result from either chemical reasons connected with the sample, or physical ones connected with the instruments involved [6–8]. In the former case

the deviations are due to changes in the form of the determined component as a consequence of chemical reactions (*e.g.*, hydrolysis, solvation, association, polymerization) associated with changes in the analyte concentration. Any change in the form of the substance being determined gives rise, as a rule, to changes in its optical properties. Thus, for example, dichromate ions (orange) are transformed, on dilution, into yellow chromate ions: $Cr_2O_7^{2-} + H_2O \rightarrow 2CrO_4^{2-} + 2H^+$. In the case of weak complexes, a dilution of the solution leads to a dissociation and decomposition of the complex thus giving rise to deviations from Beer's law.

Beer's law is not obeyed in systems where complexes are formed in a stepwise manner. The reaction constants and the quantitative ratios of individual complexes depend on the concentration ratio of the reactants and the pH of the reaction medium.

The optical medium must be homogeneous. Turbid solutions give deviations from Beer's law. Such deviations occur in two-phase systems insufficiently homogenized by protective colloids.

Deviations from Beer's law may also arise from insufficient quality of measuring instruments, mainly from the use of non-ideal monochromatic light, improper width of the spectral band, or scattering of radiation. The detector signal should be proportional, over a wide range, to the intensity of the radiation recorded.

Despite the many possibilities of deviation from Beer's law, in the absorbance range of practical interest for analytical purposes, colour systems not conforming to Beer's law are fairly rare.

2.4. Spectrophotometric apparatus

The quality of the measuring instrument has a strong influence on the reliability of the results obtained. The standard spectrophotometric apparatus used in modern analytical laboratories is very different from the equipment used in the initial period of application of this technique. The progress in the development of spectrophotometric apparatus up to the middle of the 1980s has been described [9–11]. The trends in the development of UV–VIS spectrophotometry, with special consideration to improved detection, and modern methods of data treatment, have been discussed [12]. The following discussion will be devoted to apparatus installed in most analytical laboratories.

The set of components that enable us to record a radiation absorption spectrum consists of: radiation source, monochromator, cuvette, and detector with the data treatment system (Fig. 2.1).

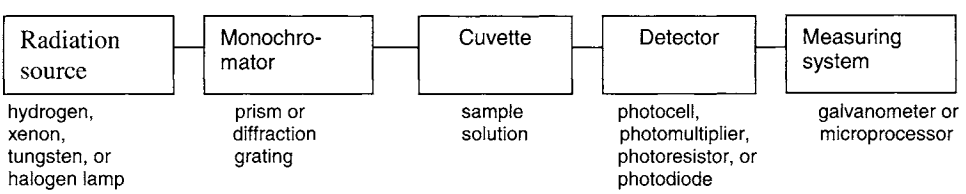


Fig. 2.1. Block diagram of spectrophotometer

The intensity of light emitted by the source, the effectiveness of its monochromatization, and the sensitivity of the detector are decisive for the quality of the spectrophotometer.

2.4.1. Radiation sources and monochromators

In most cases spectrophotometers are equipped with two independent radiation sources: UV and VIS. The UV source is usually a deuterium- or xenon lamp that emits radiation in the range of 180–400 nm or 190–750 nm, respectively. The development of the UV radiation sources has been reviewed [13].

The sources emitting visible light are tungsten- and halogen lamps. A feature of the halogen lamps is their wider spectral range, higher radiation intensity, and longer lifetime. In modern spectrophotometers the exchange between the UV and VIS proceeds automatically. The increasing use of lasers as high intensity sources of monochromatic radiation is observed [14].

The principal element of the spectrophotometer is the monochromator which serves for dispersion of the radiation emitted by the source and isolation of a beam of monochromatic radiation of definite wavelength. The monochromator comprises a system of slits, a collimator, a light-dispersing element, and lenses or mirrors to focus the dispersed radiation. The dispersing system is the essential part of the monochromator. The degree of monochromatization is an important feature of the dispersing element.

Beams of monochromatic radiation or radiation of wavelength comprised within a specified narrow range are isolated by means of filters, prisms, or diffraction gratings. The beams of radiation of a limited range of wavelength are separated from the continuous spectra by means of properly selected colour filters.

Modern spectrophotometers are equipped with diffraction gratings, whose dispersion is independent of the kind of material used and the wavelength of radiation applied. Gratings of 1,800 and 2,400 grooves/mm are used for the UV region, and those with 600 and 1,200 grooves/mm are applicable for the visible light. The separation of the grooves, denoted as the grating constant, is the parameter characteristic for the given grating. The high precision of forming the grooves and the regularity of their separations are characteristic for holographic diffraction gratings having up to 6,000 grooves/mm. The substitution of diffraction gratings for prisms enabled researchers to increase the spectral resolution and to extend the measuring range from 1 to 4 in the absorbance scale.

To record a diffraction spectrum in a required wavelength range it is necessary to change the position of the grating to isolate the beam of a given wavelength. The manual method of changing the position, used in former instruments, has been replaced by mechanical systems. Quick and precise changes in position of the diffraction grating may be obtained by means of a laser beam.

2.4.2. Measuring cuvettes

Measuring cuvettes, in which sample solutions are placed, are made of various materials depending on the range of radiation used in the measurement. Measurements in the UV are performed with the use of quartz cuvettes. Synthetic quartz, which is less contaminated with traces of metals, has better optical properties. Measurements in the VIS range are made using quartz, glass, or plastic cuvettes.

The cuvette should provide maximum transmission of radiation and definite, precisely known thickness of the light-absorbing layer. Cuvettes of different thicknesses within the range 5 μm – 10 cm are produced. Small cuvettes capable of accepting samples of volumes down to 100 μl are also available. Small volume cuvettes that enable multiple passage of the beam of radiation are of special interest [15].

The cuvette material should be resistant to the action of chemicals. The cuvettes are placed in measuring chambers in special holders that provide accurate and reproducible location of the sample in the path of the radiation beam.

Cuvettes of special design are used for measurements over wide ranges of temperature and pressure or under conditions of permanent flow. An automatic method for cleaning the cuvettes has been proposed [16].

2.4.3. Detectors

After traversing the measuring cuvette the radiation impinges on the detector. The role of the detector is to convert the energy of the incoming electromagnetic radiation into electrical energy. The signal transformation should be linear, which means that the electrical signal generated should be proportional to the optical signal received. This condition is successfully fulfilled by photocells, photomultipliers, photoresistors and photodiodes. A comparison of various detectors used in UV and VIS spectrophotometry has been given [17].

The operation of **photocells** and **photomultipliers** is based on the external photoelectric effect. Photons impinging on the surface of a photosensitive cathode (photocathode) knock out electrons which are then accelerated in the electrical field between the cathode and the anode and give rise to electric current in the outer circuit. The spectral sensitivity of a photocell depends on the material of the photocathode. The photocathode usually consists of three layers: a conductive layer (made, *e.g.*, of silver), a semiconductive layer (bimetallic or oxide layer) and a thin absorptive surface layer (a metal from the alkali metal group, usually Cs). A photocathode of the composition, Ag, Cs–Sb alloy, Cs (blue photocell), is photosensitive in the wavelength range above 650 nm; for longer wavelengths the red photocell with Ag, Cs–O–Cs, Cs is used. The response time of the photocell (the time constant) is of the order of 10^{-8} s.

Photomultipliers are equipped with several supplementary diodes (dynodes) to which the electrons emitted from the photocathode are directed. The electrons impinging on the dynodes give rise to the emission of secondary electrons from the successive dynodes and they thus amplify the signal generated by a factor of up to 10^8.

In the **photoresistors** and **photodiodes** use is made of the internal photoelectric phenomenon and of specific properties of semiconducting materials. Photons impinging on the photosensitive element generate an electrical current, which flows through the photoconductor and is amplified by the effect of a small applied voltage. The increase of the current intensity is proportional to the intensity of photons that strike the photosensitive element.

The microcrystalline layer of lead(II) sulphide deposited on a dielectric (glass or quartz) plate may serve as an example of a photoresistor applied in the wavelength range above 700 nm. Photodiodes are made of two or three layers of semiconducting materials containing suitable admixtures. Silicon photodiodes are used in the UV–VIS range

Modern spectrophotometers are equipped with multichannel detecting devices that contain a large number of photodiodes (a photodiode array) and enable simultaneous detection over the whole range of the spectrum. Details of the design and the advantages of using such detectors in spectrophotometric measurements have been presented [17–20].

2.4.4. Data recording and processing

The application of microprocessors and the rapid development of computer techniques has made it possible to automate the analytical operations from the step of sampling up to

full processing of the data obtained. In modern spectrophotometers, microprocessors are applied to control many operations that were formerly operated manually.

The functions now realized by microprocessors include the control of the optical system (lamp and analytical wavelength selection), selection of the kind of data collected (*e.g.*, absorbance, concentration), zero-adjustment, autocalibration and control of measurement parameters [21]. The microprocessor determines the equation of the regression curve and provides statistical processing of the results. It can also be programmed to measure the absorbance, the % transmittance at a selected wavelength, or the concentration based on the relationship (linear or non-linear) established between the measured absorbance and the concentration.

The advanced spectrophotometers are coupled with computers that facilitate the recording of results and the processing of the data obtained. Appropriate software enables the presentation of results on the display, smoothing of the obtained spectrum, calculation of peak heights with respect to the base-line, and mathematical processing of the results that provides the possibility of, *e.g.*, resolving signals owing to individual components of the sample analysed (see "Derivative Spectrophotometry" in Section 2.5). The development of the computer techniques has facilitated the identification of the structures of chemical compounds by enabling quick and easy access to catalogues of UV–VIS spectra.

The data recorded and the results obtained can be stored in the computer memory. This gives the possibility of comparing the obtained results and evaluating their quality by rapid comparison with greater numbers of data. A critical evaluation of the obtained results always remains the task of the analyst.

2.5. Spectrophotometric techniques

If the value of the molar absorptivity, ε, for the wavelength used in measurement of absorbance of the given system is known, it is possible to determine directly the concentration of the analyte by means of an equation based on Beer's law. The value of ε is determined from the measurement of absorbance of several solutions containing precisely known amounts of the analyte under conditions identical to those used in the measurement of the sample solution.

In analytical practice, the concentration of the given analyte is, in most cases, determined by the **standard curve** technique. The technique is based on the determination of the relationship between the absorbance and the analyte concentration under the measuring conditions. The relationship is given in terms of the regression equation, or graphically in the form of a standard curve. For systems that obey Beer's law this curve is a straight line.

The determination error is smaller if the absorption of radiation is a consequence of the nature of the analyte itself, as with the coloured ions of transition metals. Conversion of the analyte into a form capable of absorbing radiation in proportion to its concentration requires some additional procedures (such as the use of a chromogenic reagent, pH adjustment, or addition of masking agents), that must be identical in the treatment of standard solutions and of the sample solution.

The absorbance measurements must be carried out after the equilibrium has been settled in the system. If the absorbance varies with time, the time of measurement should be strictly specified.

In analytical practice, use is sometime made of standard curves in which the changes in absorbance are inversely proportional to changes in the analyte concentration. The analyte concentration is found from the reduction of absorbance of the system, which is proportional to the amount of the analyte. The accuracy and the precision of determination depend on the

precise knowledge of the initial reagent concentration and on the reproducibility of the reaction conditions for different concentrations of the analyte.

Differential spectrophotometry [22,23] consists in the measurement of the absorbance of a solution of the given element, not with reference to the solvent used, but with reference to a solution of this element (in the form of a coloured complex) of known concentration, slightly lower than the concentration of the solution studied. In this technique, the measuring error is in proportion to the difference of concentrations (and not to the concentration of the analyte in the solution under test), which enables one to reduce the relative error. Grey filters of appropriate absorbance have been proposed as references [24].

The theoretical bases of differential spectrophotometry have been presented [22]. The relative error of absorbance measurement is 0.2–0.5%, and is less in differential spectrophotometry [25–28] than in the regular method. Hence, the precision of differential spectrophotometry is comparable with that of gravimetric and titrimetric methods. This fact enables the technique to be applied in the determination of higher contents of the analytes.

In definite coloured systems the concentrations of reference solutions are selected with a view to obtaining maximum precision of the measurements. Particular attention is required in the preparation of standard solutions. In cases where temperature variations may influence the absorbance measurements, thermostating of the system is required.

Spectrophotometric titration [29–32] consists in repeated measurement of an absorbance which changes in the course of titration of the sample solution. The use of this method depends on the existence of a linear relationship between the absorbance measured and the concentration of the absorbing substance in the solution being titrated. The course of the titration is represented graphically by two intersecting straight lines. To find the titration end-point it is necessary to determine the absorbance at two points before and two points behind it. The graphs are drawn in the system of A (absorbance) *versus* v (volume of titrant solution). To increase the accuracy of determination, corrections are made for the dilution caused by the addition of the titrant solution.

The sample solution is titrated, at a definite wavelength, in a titration vessel placed inside the spectrophotometer. For this reason the spectrophotometers commonly applied require some adaptations that enable one to place a suitable titration vessel, the tip of the burette, and a mixer inside it. In this technique, the parameter of primary importance is not the absolute value of the absorbance measured but its changes during the course of titration. To reduce the effect of dilution on the absorbance one is recommended to use concentrated titrant solutions and micrometric syringes.

Spectrophotometric titrations are used in cases where it is difficult to determine the end-point visually as, for example, when there is a permanent change in the colour of the system. Good results are obtained in titrations of rather dilute solutions, of the order of 10^{-5} M. Spectrophotometric titrations are often performed in automatic systems.

Dual-wavelength spectrophotometry [33–36] is applied in systems where the difference in absorbance of two absorbing components at definite wavelengths may be used for determination of their concentrations in the given solution. The choice of the wavelengths depends on the system studied. Usually one wavelength corresponds to the maximum absorption of the analyte, and the other may correspond to an absorption maximum of the reagent or of an interfering species. The highest sensitivity is obtained when the absorption is measured at λ_{max} of the analyte. A necessary condition is that the

individual components obey Beer's law. The errors associated with the use of the dual-wavelength technique have been estimated [34–36].

Derivative spectrophotometry [37–44] is an analytical technique that uses the 1st to 5th (I–V) -order derivatives of absorption spectra in the VIS and UV ranges. The recorded curve of the derivative of the spectrum (the derivative spectrum) represents the values of absorbance differentials as a function of wavelength (wave-number) according to the following equation:

$$\frac{d^n A}{d\lambda} = {}^nD_{X,\lambda} = f(\lambda)$$

where n denotes the order of derivative and ${}^nD_{X,\lambda}$ is the value of the n-th derivative of absorption spectrum of the substance X at the given wavelength, λ.

The height of the signal of the respective derivative is proportional to the analyte concentration:

$${}^nD_{X,\lambda} = \frac{d^n \varepsilon}{d\lambda_n} \cdot l \cdot c$$

and is additive in cases where the system contains more than one component absorbing in the radiation range studied. The shape of the derivative spectrum depends on the shape of the zero-order spectrum. The width of the half-height band (L- band-width in the middle of its height) is an important parameter characterizing the system in derivative spectrophotometry.

The derivative spectrophotometry methods provide higher selectivity and higher sensitivity than do the methods based on normal (zero-order) absorption spectra. The increase in selectivity (with reduction or elimination of the effect of the spectrum of one substance on the spectrum of another one) results from reducing the band-width in the derivative spectra. An appropriate order of derivative spectrum may give complete separation of the spectra owing to the corresponding components of the system).

The increase of selectivity in the derivative spectrophotometry methods results from the fact that the values of derivatives increase, in the case of basic spectra characterized by sharp peaks, and decrease in cases of broad-band zero-order spectra (Fig. 2.2). The sharp-peak spectra enable one to make determinations of analytes in the presence of considerable excess of elements having flat spectra. An example may be the direct determination of traces of manganese (as MnO_4^-) in nickel salts, based on the fourth-order derivative spectrum [45]. An increase of selectivity may also be obtained by proper selection of the instrument setting parameters in recording the derivative spectra.

The derivative spectra are obtained in spectrophotometers fitted with microprocessors which enable digital processing of the spectra recorded [10,44–46]. Derivative spectra may also be obtained using spectrophotometers coupled with analogue differentiating systems. The instrument parameters affecting the shape of the obtained spectra are: scanning rate, integration time, distance between measurement points, and degree of amplification. The Savitzky-Golay algorithm [47,48] is that used most frequently in the treatment of basic spectra aiming at obtaining suitable derivatives.

The analytical value of the derivative is determined mostly by the zero-crossing method (determination of the derivative at the zero point of the derivative for the interfering component), by the peak-to-peak method (determination of the amplitude of the derivative spectrum in a point corresponding to the maximum difference between the derivatives of the

analyte and the interfering component), and by the baseline-to-peak method (determination of the derivative of the analyte spectrum at its maximum). The sources of errors in determinations by derivative spectrophotometry have been discussed [48].

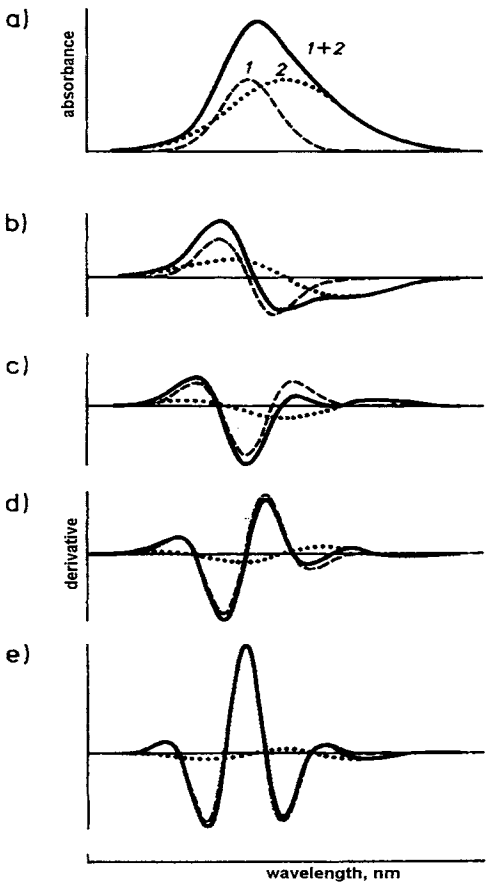


Fig. 2.2. Zero-order absorption spectra (a) and their derivatives: 1st order (b), 2nd order (c), 3rd order (d), and 4th order (e). Broken lines - substance 1 and substance 2; continuous line - mixture of substances 1 and 2.

Derivative spectrophotometry is applied more and more widely in the determination of inorganic and organic substances without preliminary separation. It is also used for the identification of organic substances.

The dependence of the recorded signal upon the instrument parameters is a disadvantage of this technique. Reproducible results are obtained on using one — and the same — type of spectrophotometer and identical conditions of spectra recording, or by adaptation of a definite method to the available apparatus.

Flow injection analysis (FIA) is an automated method which consists in the injection of the sample solution to a continuous stream of an inactive carrier (*e.g.*, a pH buffer or water) [49–51]. The diluted analyte is transported to a reaction spiral where a chromogenic reagent is added to the mixture. The dimensions of the spiral, the volume of the sample injected, and

the flow rate are optimized to provide proper reaction conditions, sensitivity, and selectivity. The liquid zone formed in the spiral and carrying the analyte is transported, by means of a pump, to the detector. The signals from the detector, which are proportional to the analyte concentration in the sample injected into the carrier stream, are recorded continuously.

Spectrophotometry is a technique most frequently applied in flow injection analysis, mainly owing to the easy coupling of the two methods, and good reproducibility of the measuring conditions. Practically all rapid reactions suitable for use in spectrophotometric determinations may be utilized under conditions of flow injection analysis. FIA coupled with spectrophotometry is a rapid (several dozens of determinations per hour) and sufficiently precise analytical technique [50–54].

The method uses small sample volume (10–100 μl), which is the basis of high sensitivity and is particularly useful in cases where small amounts of sample material are available (*e.g.*, physiological fluids). The automation includes often also the sample injection.

The spectrophotometry coupled with FIA has found numerous applications in determinations of chemical elements in environmental and clinical samples, especially in laboratories involved in rapid serial analyses.

Turbidimetry is a technique based on measurement of the absorbance by suspensions of sparingly soluble compounds. The media determined should be turbid systems, and not colloidal solutions stabilized by protective colloids. Turbidimetric determinations are often realized by visual methods or by comparison with standards in measuring cylinders, *e.g.*, the determination of sulphate as $BaSO_4$· or chloride as AgCl. The absorption of light by a suspension depends on the dispersion of the suspended solid which depends, in turn, on the concentration of the ions determined, the rate of adding the reagents, the temperature, and the ionic strength of the solution, and the presence of organic solvents miscible with water. Turbid solutions used in this technique do not generally obey Beer's law and the precision of determinations is rather low, since it is difficult to keep experimental conditions strictly reproducible. Higher sensitivity is attained in the case of coloured suspensions, such as metal sulphides.

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