PART VII: MOLECULAR ABSORPTION SPECTROSCOPY, ULTRAVIOLET AND VISIBLE (UV/VIS)

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1. INTRODUCTION

This document, Part VII, is concerned with molecular absorption spectroscopy (UV/VIS).

In the IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units, 2nd revision [Pure Appl. Chem., 51, 1-41 (1979)] section 2.8 deals with quantities related to spectroscopy; see also IUPAC Manual "Quantities, Units and Symbols in Physical Chemistry" 1988 (pub.. for IUPAC by Blackwell Scientific Publications, Oxford 1988) pp. 22-28.

Molecular absorption spectroscopy in the ultraviolet (UV) and visible (VIS) is concerned with the measured absorption of radiation in its passage through a gas, a liquid or a solid. The wavelength region generally used is from 190 to about 1000 nm, and the absorbing medium is at room temperature; however, in some cases measurements at temperatures above (e.g. in enzyme assays) or below room temperature may be advantageous or necessary. This document is restricted to the conventional means for measuring UV/VIS spectra, i.e. transmission of radiation as a function of wavelength.

Not included in this document are terms relating to other methods for obtaining molecular spectra such as by measuring reflectance or by the variation of radiant power (e.g. fluorescence, phosphorescence) as a function of the excitation wavelength *(excitation spectrum,* see Part VI). Similarly the measurement of the heat generated in the vicinity of absorbing molecules as a result of the dissipation of excitation energy, which is a measure of the absorbed radiation, *(photo-acoustic spectroscopy)* is not included.

Although molecular absorption spectra are more meaningfully presented as a function of wavenumber (cm^{-1}), the more commonly used quantity is wavelength (nm) and this quantity will accordingly be used for this document. Where wavenumber is used in special cases, this will be indicated.

Many of the terms relating to instrumental factors in absorption spectroscopy are covered in Parts I, III and VI.

2. FUNDAMENTALS OF MOLECULAR ABSORPTION SPECTROSCOPY (UV/VIS)

2.1 The UV/VIS absorption spectrum

Molecules which absorb photons of energy corresponding to wavelengths in the range 190 mm to about 1000 nm exhibit *UV /VIS absorption spectra*. The *quantized internal energy* E_{int} of a molecule in its electronic ground or excited state can be approximated, with sufficient accuracy for analytical purposes, by

$$E_{int} = E_{el} + E_{vib} + E_{rot} \qquad \text{(VII.1)}$$

where E_{el} is the electronic, E_{vib} the vibrational and E_{rot} the rotational energy, respectively. Absorption of a photon results in a change of the electronic energy accompanied by changes in the vibrational and rotational energies. Each *vibronic transition,* i.e. a particular electronic plus vibrational transition, corresponds to an *absorption band* consisting of rotational lines. In liquids

and solids the rotational lines are broad and overlap so that no rotational structure is distinguishable.

The UV/VIS absorption spectrum of a molecular species is normally represented as a graph of some characteristic for the radiation absorbed as a function of wavelength. The graph is representative for that species, solvent, concentration and temperature. If a linear energy scale for the abscissa is preferred, then wavenumber, u is used instead of wavelength, l.

When the absorption of UV/VIS radiation by a solute is measured in a highly viscous or solid matrix at low temperature (less than about 100 K) a *low temperature UV/VIS absorption spectrum* results.

Highly structured spectra can be obtained when the UV/VIS absorption of a solute in certain *polycrystalline matrices* (e.g. n-alkanes, cycloalkanes, inert gases such as rare gases) is measured at low temperatures. For all spectra the solvent, solvent temperature and solute concentration should be specified (see Note [1]).

Direct recordings of these low temperature UV/VIS spectra are very useful for the identification of compounds; they are less useful in quantitative analysis because of the difficulty in measuring the true absorbance (see Section 2.2) of the sample, which usually exhibits high radiation scattering.

2.2 The Beer-Lambert-Bouguer law

The *Beer-Lambert-Bouguer law*, generally called the *Beer-Lambert law*, may be written for a single absorber either gaseous or in solution,

$$-\log_{10}\left(F_t / F_0\right) = -\log_{10} t_i = e\,c\,b = A \qquad \text{(VII.2)}$$

where F_t is the monochromatic radiant power transmitted by the absorbing medium, F_0 is the monochromatic radiant power incident on the medium, t_i is the *internal transmittance* (= F_t/F_0), e is the *molar (decadic) absorption coefficient, c* is the *amount concentration, b* the *absorption path length* and *A* the (decadic) *absorbance.* These terms and their preferred units have already been defined in Parts I, III, and VI (Table VI.4).

Internal transmittance t_i, i.e. transmittance of the medium itself disregarding boundary effects, has to be distinguished from the *total transmittance* t. The difference, which is mainly due to reflection losses associated with cell windows, can be compensated by using *matched cells (* see Section 3.2.1).

Absorptance (or *absorption factor) a* is defined by $a = 1 - t$ where reflection is assumed to be negligible.

The Beer-Lambert law holds only if the absorbing species behave independently of each other, and if the absorption occurs in a uniform medium. Further, the incident radiation must be parallel, monochromatic (see Note [2]) and there should be no measurable *saturation effect* due to depletion of the ground state molecules. Causes for deviations from the Beer-Lambert law are listed in Section 7.

3. INSTRUMENTAL FACTORS

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[1] Terms such as Shpol'skii or quasi-linear spectra should not be used.

[2] In practice parallel, monochromatic radiation is not always used. Convergence or divergence of the light beam as found in practice, will cause only minor deviations from the Beer-Lambert law. Section 6.3 discusses errors due to non-monochromatic radiation.

An instrument for measuring molecular absorption spectra (UV/VIS) usually consists of a *radiation source,* an optical system including a *spectral apparatus,* a *sample compartment,* a *radiation detector* and a system for *data acquisition* and *data processing.* Means for *amplitude modulation* and/or *wavelength modulation* may also be part of the instrument.

The classification of instruments according to single beam or double beam operation, recording, non-recording etc. is covered in Part IX, which also contains definitions and nomenclature on optical systems relevant to molecular absorption spectroscopy. Radiation detectors and modulators are treated in Parts VI and XI. Part V (radiation sources) does not deal with sources used in molecular spectroscopy. These will be covered in this section.

3.1 Radiation sources (see Note [3])

Pertinent factors relating to the properties of continuum radiation sources are

the *spectral distribution* defined as the variation of the *spectral radiance* L_l, with wavelength,

the *maximum spectral radiance,* $L_l(\max)$ within the usable wavelength range,

the wavelength at this maximum, $l_{\max}$,

the *usable wavelength range,* defined by the lower limit l_1 and the upper limit l_u, at which the spectral radiance is a specified fraction of $L_l(\max)$.

Continuum sources are normally used for molecular absorption measurements, whereas *spectral-line sources* (see Part V) are employed for wavelength calibration of a spectrometer. Examples of continuum sources commonly used are

tungsten-halogen lamps, which have a radiance temperature (see Part V) T of ~ 3000 K and therefore have maximum spectral radiance at a wavelength of about 1.2 µm; they are widely used in the visible and near ultraviolet spectral region (above 330 nm),

deuterium lamps (gas-discharge lamps) which emit strongly in the UV region below 330 nm; the continuous spectrum has deuterium atomic emission lines superimposed on it,

xenon arc lamps which give a continuum from below 190 nm to above 1000 nm.

Examples of spectral-line sources are

low-pressure mercury-discharge lamps, which are sometimes used for measuring absorbances at fixed wavelengths. Such lamps are also useful for wavelength calibration,

Tunable lasers with and without frequency doubling and/or Raman shifting are high intensity sources with narrow spectral bandwidths. Their use may enable the spectral apparatus to be omitted. They may be either continuous (cw) or pulsed in nature.

3.2 Sample compartment

3.2.1 Liquid samples

Liquid samples are usually contained in *sample cells* which are placed in *sample cell holders.* Cell holders may be heated or cooled in order to control the temperature of the liquid in the sample cell.

The important characteristics of sample cells are,

cell shape (e.g. rectangular, cylindrical),

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[3] Radiative properties of sources should not be described by photometric units (e.g. candela m) (see Part I).

absorption path length, b, defined as the length of the radiation path through the absorbing medium; it is equal to the *cell path length, l,* in the case of single-pass cells at normal incidence of radiation,

volume and cross section,

window material (window thickness and degree of deviation from parallelism of the windows are also important).

A pair of cells with closely similar optical properties are called matched cells. One cell is the sample cell while the other, the *reference (or blank) cell* contains the solvent or a reference solution (see Section 4.4). In double beam spectrometers, radiation is passed either simultaneously or alternately through the cells. In single beam instruments the cells are moved sequentially into the radiation beam.

3.2.2 Gaseous samples.

Gases and vapours are measured in gas cells similar to those used for liquids. Generally the cell pathlength is much greater. Gases at any pressure are contained in *closed cells* for measurement.

3.2.3 Solid samples.

Solid samples are held in *solid-sample holders.* When solid samples are measured, difficulties may be experienced e.g. in matching the sample and reference pathlengths.

3.2.4 Special cells.

Low temperature cells are required for certain applications. These may include *cooled cells, thermally insulated cells,* and *cold-finger cells.*

A stopped-flow cell comprises a small-volume absorption cell connected to a rapid mixing chamber.

A multiple-pass cell is constructed in such a way that mirrors either form part of the cell or are mounted in the sample cell holder. It permits multiple passage of radiation to increase the absorption pathlength.

A continuous-flow cell allow the liquids (or gaseous) sample to pass through the cell continuously while absorption measurements are made.

A variable pathlength cell is a cell whose pathlength can be varied either continuously or in steps by means of spacers.

3.3 Data acquisition and data processing

The equipment used for data acquisition and data processing can be classified according to the form of the information required. In the simplest case the output may present the transmittance or absorptance in an analog or digital form. Further processing of the output signal enables various functions of the original signal to be obtained. These include

- absorbance
- log absorbance
- averaged spectra (with enhanced signal-to-noise ratio)
- *derivative spectra* (see Section 4.6)
- with additional processing, background correction (see Section 4.3)

For the fast acquisition of data, a *polychromator* (see Part III) fitted with a detector that enables spatial resolution, e.g. a *vidicon tube* or *silicon photodiode array,* (see Part XI) together with a

multichannel analyser may be used. The whole detection system is called an *optical multichannel analyser*.

4. MEASURING TECHNIQUES

The sample type and the information required determine which of the various techniques available will be used when conducting a molecular absorption analysis. The following are some of the more important methods.

4.1 Qualitative analysis

The identification of an analyte may be achieved by comparing the absorption spectrum of the unknown substance with graphs or tables of the spectra of known substances. In some cases two or more analytes may be recognized.

4.2 Quantitative analysis

A known analyte can be determined by measuring the absorbance at one or more wavelengths and using the Beer-Lambert law and the molar absorption coefficient to calculate its amount concentration. If the concentration is expressed in other units (e.g. g/L) the corresponding *specific (decadic) absorption coefficient* has to be used. Concentrations can also be determined from an *analytical function* or *analytical calibration curve* where the latter is obtained by plotting the measured absorbance of different *reference solutions* (or gases or solids) against their known concentrations. A graph of absorbance against concentration is a straight line passing through the origin if the Beer-Lambert law applies (see Section 7 for causes of deviations).

Multicomponent analysis of a mixture consisting of *n* absorbing analytes is possible provided the Beer-Lambert law holds, by measuring the absorbance at *k* suitable wavelengths with $k > n$. For each wavelength l_a (a = 1,2 ... *k*-l, *k*) the total absorbance is given by

$$A(l_a) = b \sum_{i=1}^{n} e_i(l_a) c_i \qquad \text{(VII.3)}$$

where $e_i(l_a)$ is the molar (decadic) absorption coefficient of analyte i at the wavelength l and c_i its molar concentration. The ratio $A(l_a)/b$ is called *linear (decadic) absorption coefficient.* This quantity is also called *linear absorbance.*

The concentrations of the *n* analytes may then be calculated from the *k* simultaneous equations.

4.3 Spectral background correction

The *spectral background absorbance* of a sample arising, for example, from the presence of an impurity with a weak, broad absorption band, can be subtracted from the analyte absorbance by graphical or algebraic methods when the spectral background absorbance is known. In practice, however, this applies only when the spectral background absorbance varies in a simple way (e.g. as a linear or quadratic function of wavelength; for other methods see Sections 4.5 and 4.6).

4.4 Difference absorption spectroscopy

A highly concentrated analyte in the analytical sample can be determined with better precision by replacing the blank (reference) cell by one containing a solution of the analyte or other absorber of known concentration *(difference absorption spectroscopy).* Difference spectra can also be obtained by computer or other subtraction methods.

4.5 Double-wavelength spectroscopy

The effect of spectral background due to impurities, solvent or radiation scattering may be reduced if the difference in the absorbances of a sample measured at two selected wavelengths is obtained. This is often achieved by repetitively switching from one wavelength to the other. *Double-wavelength spectroscopy* does this automatically by allowing two beams of radiation of different wavelength to pass through the cell. One beam is fixed at a longer wavelength and the other measures absorbance while being scanned over a limited wavelength range at shorter wavelengths.

4.6 Derivative spectroscopy

The *first (second, ...) derivative absorption spectrum* of a molecule is defined as first (second, ...) derivative, $dA(\tilde{u})/d\tilde{u}$, ($d^2A(\tilde{u})/d\tilde{u}^2$, ...) of the absorbance A as a function of wavenumber $\tilde{u}$. Wavelengths may be used in place of wavenumbers but the shape of the derivative spectra will be slightly different. When derivative spectra are obtained at low temperature, they are called *first (second, ...) derivative low temperature absorption spectra,* (specifying the solvent, temperature and solute concentration).

Derivative spectra are usually obtained by digital differentiation or by wavelength modulation of the radiation entering the sample cell. The *wavelength modulation interval* has to be much less than the bandwidth of any absorption band in the spectrum. The derivative spectrum in terms of wavenumber can be calculated from the spectrum in terms of wavelength by

$$dA(\tilde{u})/d\tilde{u} = \left(-l^2\right) dA(l)/dl \qquad \text{(VII.4)}$$

$$d^2A(\tilde{u})/d\tilde{u}^2 = \left(l^4\right) d^2A(l)/dl^2 + 2l^3\, dA(l)/dl \qquad \text{(VII.5)}$$

The use of derivative spectroscopy decreases the signal-to-noise-ratio. However, it can improve the detection of a sharp band in a broad background, or a narrow shoulder on a broad main band. Also gradual changes in spectral background or source flux will be less pronounced in derivative spectroscopy.

The first and second derivatives of the Beer-Lambert law are (assuming $dF_0/d\tilde{u} \equiv 0$),

$$\frac{dF_t/d\tilde{u}}{F_t} = -2.303cb\frac{de}{d\tilde{u}} \qquad \text{(VII.6)}$$

$$\frac{d^2F_t/d\tilde{u}^2}{F_t} = \left[2.303cb\frac{de}{d\tilde{u}}\right]^2 - 2.303cb\frac{d^2e}{d\tilde{u}^2} \qquad \text{(VII.7)}$$

Second derivative spectra are predominantly used in quantitative analysis.

The possible methods for the quantitative evaluation of derivative spectra are illustrated in Fig. 1 and are called *tangent* (Fig. la), *peak-peak* (Fig. lb) and *peak-zero* (Fig. lc) methods.

4.7 Absorbance matching

Absorbance matching is a procedure where the concentration of a known analyte may be determined by diluting the sample with solvent until the absorbance matches the absorbance of a known concentration of the analyte in the reference cell. This method is particularly useful if the Beer-Lambert law does not hold.

5. FACTORS INFLUENCING PRECISION OF ABSORBANCE MEASUREMENTS

5.1 Random fluctuations

Random errors limit the precision of analysis (see Part II). When measuring very large or very small absorbances, uncertainties due to *random fluctuations* become particularly large. To obtain better precision in the absorption measurement it is necessary to adjust the sample concentration or cell pathlength to bring the absorbance into the range 0.1 to 1.0. The theoretical minimum error occurs at a *best precision absorbance* of about 0.43 for a spectrometer with a detector which is thermal noise limited, e.g. phototube or photodiode; and of about 0.86 for a spectrometer with a detector which is shot noise limited, e.g. photomultiplier.

5.2 Temperature effects

Temperature variations will to a greater or lesser extent affect all instrument functions. The absorbance of the sample, as well as the sample factors considered in section 6.7, may also depend on temperature. All of these effects give rise to *temperature errors.*

5.3 Inhomogeneous samples

Errors due to inhomogeneous distribution of absorbing species in the analytical sample are called *inhomogeneity errors.* They may be observed by probing the sample volume with an incident beam of radiation of reduced cross-section.

6. FACTORS INFLUENCING ACCURACY OF ABSORBANCE MEASUREMENTS

Accuracy, discussed in this section, refers to systematic errors which introduce bias in the measurement. Additional measurements must be made before errors such as those specified below can be detected and corrected.

6.1 Spectrometric factors

Any deviation from linearity of the response of the detector to the measured radiant power may cause a *non-linearity error.*

If insufficient time is allowed for the reading to settle or if an absorption peak is scanned too rapidly a *settling error* results.

If the two beams in a double-beam spectrometer are not fully equivalent in transmitted power nor are corrected to be so, a *base-line error* occurs, e.g. due to unmatched cells.

6.2 Wavelength accuracy

If there is a difference between the (mean) wavelength of the radiation entering the sample cell and the indicated wavelength on the spectrometer scale, an error in absorbance may occur. This error is called a *wavelength error.*

6.3 Spectral bandwidth

To measure the true shape, particularly the true maximum of an absorption band, the spectral bandwidth Δl of the instrument (see Part I) must be much less than the width of the absorption band. A *spectral bandwidth error* results from using too large a bandwidth relative to the absorption band being measured.

6.4 Stray radiation

A spectrometer set to pass radiation of a particular wavelength band always has a small amount of *stray radiation* of other wavelengths. Since the sample may absorb more (or less) of this stray radiation than of the radiation at the selected wavelength an error can occur. This error is called *stray radiation error.*

6.5 Polarization (for definitions see Part IX)

The radiation leaving the monochromator will rarely be completely unpolarized. *Polarization errors* may arise from the fact that the absorbance of a sample (especially a solid) could depend on the polarization of the incident radiation and from the polarization dependent response of the photodetector.

6.6 Optical beam effects

For wavelength-scanning spectrometers, an error may be introduced if the image position on the photodetector changes. Reflection of the incident radiation, e.g. between the cell walls, is another source of error and results in a measured absorbance slightly higher than the true absorbance. These errors are collectively termed *optical-beam errors.*

6.7 Scattering

The presence of particulate matter, emulsions, micelles, etc. may cause radiation scattering. This will result in further attenuation of the transmitted beam and the measured absorbance will be too high. This error is called the *scattering error.*

6.8 Fluorescence effects

Some samples are excited to fluoresce by the incident radiation beam. Added to the transmitted radiation beam, the fluorescence results in an erroneous reading, called the *fluorescence error.* It is particularly noticeable if the photodetector is situated close to the sample.

6.9 Cell factors

An error in the measured absorbance of a sample can occur if the incident beam does not fall perpendicularly to the windows of the cell, or if the cell windows are contaminated or have some other imperfection. These factors result in *cell errors.*

6.10 Sample stability

The absorbance of some samples may change with time, e.g. as a result of photochemical reaction, formation of aggregates or adsorption on the cell wall. Errors due to these changes are called *sample errors (* see Section 7).

Table X.1 Factors other than instrumental that influence absorption spectra

Factor	Cause	Information	Process*
(1) concentration	self-association	association equilibrium constant	$nA \leftrightarrow A_n$ (n ³ 2)
(2) pH	acid/base (proto-lytic) equilibrium	protonation equilibrium constant	$A+H_30^+ \leftrightarrow AH^+ +H_20$
(3) time	thermal chemical reaction (including solvolysis)	rates of reaction	A -> B
(4) (radiant) power	photochemical reaction	rates of reaction	$A \xrightarrow{h\tilde{n}} B$
	photochromism	equilibrium constant and reaction rates	$A \xleftrightarrow{h\tilde{n}} B$
(5) presence of component B	complex formation	equilibrium constant	$A + B \leftrightarrow AB$
(6) temperature	thermochromism	equilibrium constant	$A \leftrightarrow B$
(7) solvent	solute-solvent interactions	transition type	

* A and B are reacting species

7. FACTORS OTHER THAN INSTRUMENTAL THAT INFLUENCE ABSORPTION SPECTRA

The main factors that may affect the absorption spectrum and often result in deviations from the Beer-Lambert law are listed in Table X.1. These alterations in the spectrum may appear either as a *wavelength shift* with little change in band shape, or as major alterations in band shape and position. Often more than one of the factors listed below are relevant as causes for changes in absorption spectra.

8. TERMS, SYMBOLS AND UNITS USED IN MOLECULAR ABSORPTION SPECTROSCOPY

Term	Symbol	Practical Unit	Notes
radiant energy	Q	J	see Parts I and II
radiant power	$F = \mathrm{d}Q/\mathrm{d}t$	W	see Part I
radiant intensity	I	W sr^{-1}	see Parts I and II
spectral radiance	$L_\lambda(B_\lambda)$	W m^{-1} sr^{-1} nm^{-1}	
radiant power incident on absorbing medium	F_0	W	
radiant power transmitted by absorbing medium	F_t	W	
radiant power reflected by sample	F_r	W	
radiant power absorbed by medium	F_a	W	
transmittance or transmission factor	t	1	$= F_t/F_0$
reflectance or reflection factor	r	1	$= F_r/F_0$
absorptance or absorption factor	a	1	$= 1-t$
internal transmittance	t_i	1	
internal absorptance	a_i	1	
(decadic) internal absorbance	A	1	$= -\log_{10} t_i$
linear (decadic) absorption coefficient	a, K	cm^{-1}	$= A/b$
molar (decadic) absorption coefficient	e	L mol^{-1} cm^{-1}	$= A/(c\,b)$
specific (decadic) absorption coefficient	d	L g^{-1} cm^{-1}	
molar concentration of absorber	c	mol L^{-1}	
wavelength	λ	nm	
wavenumber	n	cm^{-1}	$= 1/\lambda$
spectral bandwidth of spectrometer	$D\lambda$	nm	see Part I
wavelength modulation interval	$D\lambda_{mod}$	nm	wavenumber may also be used
absorption pathlength	b	cm	
cell pathlength	l	cm	