10.3.5.2 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.

10.3.5.2.1 Qualitative analysis

The identification of an analyte may be achieved by comparing the absorption spectrum of the unknown substance with the spectra of known substances.

10.3.5.2.2 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*.

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 λ_a (a = 1,2 ... k-1, k) the total absorbance is given by

$$A(\lambda_a) = b\sum_{i=1}^{n}\varepsilon_i(\lambda_a)c_i$$

where $\varepsilon_i(\lambda_a)$ is the *molar (decadic) absorption coefficient* of analyte i at the wavelength λ_a and c_i its molar concentration. The ratio $A(\lambda_a)/b$, where b is the absorption path length, is called the *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.

10.3.5.2.3 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 made by subtracting it from the analyte absorbance by graphical or algebraic methods.

10.3.5.2.4 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.

10.3.5.2.5 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.

10.3.5.2.6 Derivative spectroscopy

The *first (second, ...) derivative absorption spectrum* of a molecule is defined as first (second, ...) derivative, $\mathrm{d}A(\tilde{\nu})/\mathrm{d}\tilde{\nu}$, $(\mathrm{d}^2A(\tilde{\nu})/\mathrm{d}\tilde{\nu}^2, \ldots)$ of the absorbance A as a function of wavenumber . Wavelengths may be used in place of wavenumbers but the shape of the derivative spectra will be slightly different.

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

$$\mathrm{d}A(\tilde{\nu})/\mathrm{d}\tilde{\nu} = (-\lambda^2)\mathrm{d}A(\lambda)/\mathrm{d}\lambda$$

$$\mathrm{d}^2A(\tilde{\nu})/\mathrm{d}\tilde{\nu}^2 = (\lambda^4)\mathrm{d}^2A(\lambda)/\mathrm{d}\lambda^2 + 2\lambda^3\,\mathrm{d}A(\lambda)/\mathrm{d}\lambda$$

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. In addition 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 $d\phi_o/d \equiv 0$),

$$\frac{d\phi_t / d\tilde{\nu}}{\phi_t} = -2.303cb\frac{d\varepsilon}{d\tilde{\nu}}$$

$$\frac{d^2\phi_t / d\tilde{\nu}^2}{\phi_t} = [2.303cb\frac{d\varepsilon}{d\tilde{\nu}}]^2 - 2.303cb\frac{d^2\varepsilon}{d\tilde{\nu}^2}$$

Second derivative spectra are predominantly used in quantitative analysis.

The possible methods for the quantitative evaluation of derivative spectra are illustrated in Fig. 10.15 and are called *tangent* (Fig. 10.15a), *peak-peak* (Fig. 10.15b) and *peak-zero* (Fig. 10.15c) methods.

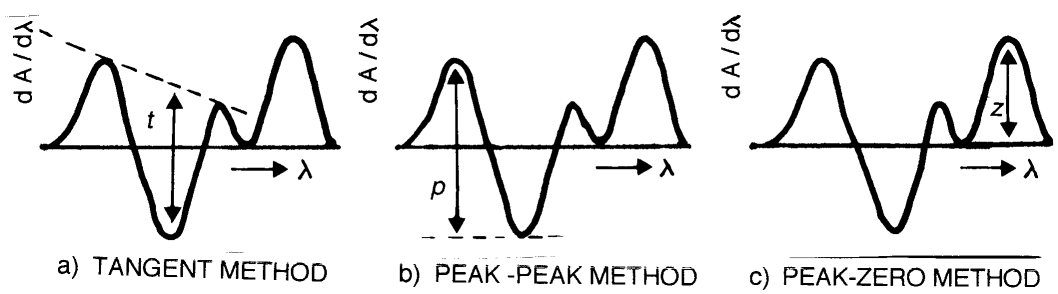

Fig.10.15 Quantitative evaluation of first derivative spectra, the quantitative measure being t in Fig 10.15 (a), p in (b) and z in (c)

10.3.5.2.7 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.

10.3.5.2.8 Random fluctuations

Random errors limit the precision of analysis. 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. a photomultiplier.

10.3.5.2.9 Other sources of imprecision

Temperature variations may give rise to *temperature errors*. Errors due to inhomogeneous distribution of absorbing species in the analytical sample are called *inhomogeneity errors*.

10.3.5.2.10 Factors influencing accuracy of absorbance measurements

Accuracy, discussed in this section, refers to systematic errors which introduce bias in the measurement. 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. 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*. To measure the true shape, particularly the true maximum of an absorption band, the spectral bandwidth $\Delta\lambda$ of the instrument 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.

TABLE 10.12 Factors other than instrumental that influence absorption spectra

Factor	Cause	Information	Process*
(1) concentration	self-association	association equilibrium constant	$nA \leftrightarrow A_n$ ($n \geq 2$)
(2) pH	acid/base (protolytic) equilibrium	protonation equilibrium constant	$A+H_3O^+ \leftrightarrow AH^++H_2O$
(3) time	thermal chemical reaction (including solvolysis)	rates of reaction	$A \rightarrow B$
(4) (radiant) power	photochemical reaction	rates of reaction	$A \xrightarrow{h\nu} B$
	photochromism	equilibrium constant and reaction rates	$A \xrightarrow{h\nu} B$
(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

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*. 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. 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, can also result in an increased measured absorbance. These errors are collectively termed *optical beam errors*. The presence of particulate matter may cause attenuation of the transmitted beam giving rise to *scattering errors*.

Fluorescence due to the incident radiation enhances the radiation received by the detector and gives rise to *fluorescence errors*. *Cell errors* can arise from contamination of the cell windows or if the incident beam does not fall perpendicularly on to the cell. The absorbance of some samples may change with time, for example as a result of photochemical reactions, the formation of aggregates or adsorption on cell walls. Such changes give rise to *sample errors*.

10.3.5.2.11 Factors other than instrumental that affect absorption spectra

The main factors affecting absorption spectra, often resulting in deviations from the Beer-Lambert law are listed in Table 10.12. These attenuations can appear as a *wavelength shift* or as changes in band shape and position.

The terms, symbols and units used in molecular absorption spectroscopy are summarised in Table 10.3. See in section 10.2.1.3.