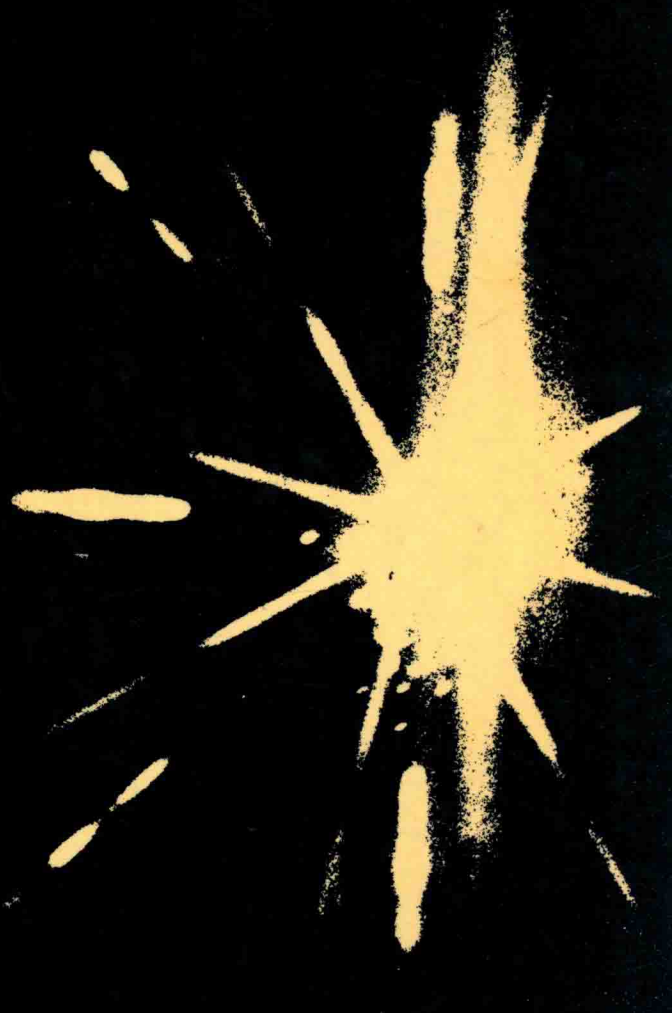
TECHNIQUES IN VISIBLE AND ULTRAVIOLET SPECTROMETRY VOLUME 3

Practical Absorption Spectrometry

UV SPECTROMETRY GROUP
edited by A. Knowles and C. Burgess



Practical Absorption Spectrometry

ULTRAVIOLET SPECTROMETRY GROUP

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TECHNIQUES IN VISIBLE AND
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VOLUME THREE
PRACTICAL ABSORPTION SPECTROMETRY

Preface

The inspiration for this volume lies in Edisbury's *Practical Hints for Absorption Spectrometry* which was published 17 years ago. Dr Edisbury was a founding member of the Photoelectric Spectrometry Group, served as its first Secretary and edited the Bulletin for many years. His wisdom, humour and pragmatism was evident in early meetings of the Group and in the first issues of the Bulletin, and these qualities were distilled in the writing of *Practical Hints*.

In 1977, the Committee of the Group, which by then had been re-named The UV Spectrometry Group, decided to make use of the expertise available amongst the members of the Group in writing some monographs on the practice of UV and visible spectrometry. Working parties were set up which formulated and produced the first two volumes of the series on *Standards in Absorption Spectrometry* and *Standards in Fluorescence Spectrometry*.

The success of these volumes lead the present Committee of the Group to set up a new Working Party in 1981 to plan a modern version of Edisbury's book. The idea really caught fire at the first meeting of the Working Party, when ideas sufficient to fill ten volumes were put forward. We would not pretend to emulate Edisbury's unique style, but hoped to produce a readable book for the newcomer to UV–visible absorption spectrometry, and perhaps to improve the technique of more experienced users.

The Editors had then to tackle the task of selecting from the variety of topics proposed by the Working Party and assigning the various sections of the book to its members. We hope that the result is a coherent whole that provides the kind of information that is not found in standard texts or in instrument manufacturers' literature. We have attempted to survey the types of instrument that are currently in use, and hope that this provides assistance in the selection of an instrument from the bewildering range that is now available.

We decided that it would be invidious to provide a comprehensive *Which* report and have as far as possible avoided mentioning instruments by name: this is in accord with the Group's policy of impartiality. Where instruments or components are named, this does not imply that the UV Spectrometry Group in any way endorses that particular instrument or component.

The euphoria of completing the manuscript has been marred by the untimely death of Dr Brian Chadburn, who was a member of the Working Party and Honorary Treasurer of the UV Group. We gratefully acknowledge his enthusiastic support of the project, and will miss his participation in the future activities of the Group.

We have received support and assistance from many people outside the Working Party. In particular, we would like to thank Messrs Bausch and Lomb, Beckman, Hewlett Packard, Perkin–Elmer, Pye Unicam, Shimadzu, Starna and Thermal Syndicate for permission to reproduce diagrams. Our thanks are due to Anne Grundy, Benita Hall, Wendy McElroy, Jillian Wearmouth and Jennifer Wood for help with the typing, and to Bob Bourne and Linda Stanley for running spectra. We also thank Dr T.L. Threlfall for assistance with Chapter 1, and Susan Donaghy and Mary Ann Ommanney of Chapman and Hall for their support and encouragement. All proceeds from sales of this book will go to Group funds for the furtherance of UV spectrometry.

February 1983

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Glossary

Terms and abbreviations used in absorption spectrometry

Full definitions and examples of most of the terms will be found by reference to the index. In general, SI units are employed, but in several instances older units are still used by most spectroscopists. Some abbreviations used in the book are not listed here, but will be found in the Index.

A. See Absorbance.

$A_{1\,cm}^{1\%}$. See $E_{1\,cm}^{1\%}$

Å. See Ångstrom unit.

Absorbance. Quantity expressing the absorption of radiation by a solution at a specified wavelength. It is given by:

$$A = \log 1/T = -\log T$$

and is linearly related to the pathlength and concentration of the solution. It is dimensionless but is expressed in absorbance units (A) so that a solution of $T = 0.1$ has an absorbance of 1 A.

Absorption. The process by which radiation is attentuated on passing through a substance. The term implies that the radiant energy is converted into some other form, e.g. heat, fluorescence etc., as distinct from losses by scattering or refraction.

Absorption band. See Band.

Absorption spectrum. A plot of the absorption of radiation by a sample against the wavelength of the radiation.

Absorptivity. The absorbance of a solution of a compound in unit concentration measured in unit pathlength at a specified wavelength. See Molar absorptivity.

Ångstrom unit (Å). A unit of wavelength, now rarely used in absorption spectrometry. 1 Å = 0.1 nm = 10^{-10} m.

b. See Pathlength.

Band. A general term describing a maximum in a plot of some quantity against wavelength. An absorption band is a broad maximum in an absorption spectrum and may comprise a number of minor peaks. An emission band might be the profile of an intensity versus wavelength plot of the spread of wavelengths leaving a monochromator.

Band pass. See Passband

Beer's Law (Beer–Lambert–Bouguer Law). This relates the absorbance of a solution to the pathlength of the cell and the concentration of the solute. The absorbance of a solution at a specified wavelength is given by:

$$A = \epsilon bc$$

where ϵ is the molar absorptivity at that wavelength, b is the pathlength in cm and c is the molar concentration.

Blazed grating. A diffraction grating made with a particularly high reflectivity in a particular spectral region.

c. Velocity of light.

cm^{-1}. See Wavenumber.

cps. Cycles per second; see Hertz.

Cell. A container to hold solutions for the measurement of their absorption spectra. This should have two parallel optical windows fixed at a specified distance apart.

Coated optics. The coating of optical components to improve their optical performance and protect them against atmospheric attack.

Cuvette. See Cell.

Dark current. The background signal from a detector due primarily to the thermal emission of electrons.

Derivative spectra. A plot of the first, second or higher derivative of an absorbance spectrum with respect to wavelength in order to correct for background absorption and to accentuate minor features in the spectrum.

Detector. The device in a spectrometer which measures the intensity of light transmitted by a sample.

Deviation. The displacement of the spectrometer measuring beam due to optical defects in the cell, or in its alignment.

Difference spectrum. Small changes in the absorbance spectrum of a sample can be more readily detected if the spectrum is measured using the original sample as a reference solution.

Diffraction grating. A device used to resolve radiation into its component wavelengths by an interference process. See Grooves.

Dispersion. The power of a prism or grating to separate radiation of different wavelengths.

Double-beam spectrometer. An instrument in which the measuring beam is split into two equivalent paths, one passing through the sample and one through a reference cell.

Dual-wavelength spectrometry. Measurement of the absorbance of a sample at two wavelengths simultaneously in order to compensate for background absorption, etc.

$E^{1\%}_{1\,cm}$. An expression of the absorptivity of a solute of unknown molecular weight. It is the absorbance of a 1% w/v solution of a compound measured in 10 mm pathlength. It is related to molar absorptivity by:

$$E^{1\%}_{1\,cm} = 100 \times \epsilon/M.$$

ESW. See Effective spectral slitwidth.

Effective spectral slitwidth (ESW). The band of wavelengths emerging from a monochromator, expressed as the bandwidth at half-peak height.

Electromagnetic radiation. Radiation which can be regarded as wave motions of characteristic wavelength, including γ-radiation, X-rays, UV, light, IR and radio waves.

Emission. The process by which electromagnetic radiation is radiated from a substance.

Emission line. Emission of radiation in a very narrow wavelength band characteristic of the output of gas discharge lamps.

Excited state. A molecule that has absorbed UV or VIS radiation is said to have been raised from its ground state to an excited state.

Extinction. See Absorbance.

Extinction coefficient. See Molar absorptivity.

Far stray-light. The component of the monochromator stray-light lying at wavelengths well away from the passband. This may be of low intensity but can be a major factor in the problem of instrumental stray-light.

Far-ultraviolet. Radiation at the shortwave end of the UV region. There are no agreed limits, but the far-UV is generally taken to lie between 190 and 250 nm.

First order. See Grating order; Derivative spectra.

Flow cells. A cell designed for the measurement of a flowing stream of solution.

Fluorescence. A process in which radiation absorbed by a molecule is instantaneously re-emitted as UV or VIS radiation. This is usually of longer wavelength than the exciting radiation.

Fluorimeter. An instrument specifically intended for the measurement of fluorescence.

Frequency (ν). A specification of electromagnetic radiation by the number of waves per unit time. It is related to wavelength by:

$$\nu = \lambda/c \text{ Hz}$$

where λ is the wavelength in m and c is the velocity of light in m s^{-1}.

Gaussian. Most absorption bands when plotted on a frequency scale have a shape equivalent to a Gaussian or normal distribution, and can be fitted by:

$$A = A_m \exp -2.773((\nu - \nu_m)/w)^2$$

where A is the absorbance at frequency ν, A_m is the absorbance at the maximum, frequency ν_m, and w is the width of the band at $0.5\,A_m$. Also see Lorentzian.

Grating. See Diffraction grating.

Grating order. A diffraction grating will reflect a given wavelength of light at more than one angle. Most of the incident energy undergoes first-order reflection, the second-order reflection is weaker and so on.

Grooves. A diffraction grating works by the interference of light rays reflected from the faces of grooves ruled in its surface. The spacing

of these grooves determines the optimum wavelength range for the grating.

Hz. See Hertz.

Hertz. Unit of frequency equivalent to one wave or cycle per second.

Holographic grating. A diffraction grating produced photographically by the interference of beams of light.

IR. See Infrared.

ISL. See Instrumental stray-light.

Incident beam. The radiation directed into the entrance window of the cell in the spectrometer.

Infrared. The region of electromagnetic radiation of wavelength greater than the red end of the VIS region, i.e. from 800 nm to 1000 μm.

Instrumental stray-light (ISL). The overall effect of stray-light in a spectrometer manifested as detector output. Thus it is the signal generated in the detector by all wavelengths outside the monochromator passband that reach it.

Isosbestic point. If a sample contains two compounds in equilibrium, then at any wavelength at which their molar absorptivities are the same, the absorbance of the sample will be independent of the relative amounts of the two species. When the spectra of a series of mixtures of differing compositions are plotted, these will be seen to cross at these wavelengths, forming isosbestic points.

Light. See Visible light.

Lorentzian. Some absorption bands when plotted on a frequency scale have a shape equivalent to a Lorentzian or Cauchy distribution and can be fitted by:

$$A = A_m / \{1 + [2(\nu - \nu_m)/w]^2\}$$

where A is the absorbance at frequency ν, A_m is the absorbance at the maximum frequency ν_m, and w is the width of the band at $0.5\,A_m$. Also see Gaussian.

Luminescence. A general term for the emission of UV or VIS radiation from a molecule. In most cases, it can be regarded as the sum of the fluorescence and the phosphorescence emissions.

MSL. See Monochromator stray-light.

mμ. Millimicron; see Nanometre.

Manual spectrometer. An instrument without a wavelength drive mechanism.

Mask. An opaque screen with an aperture designed to limit the cross-section of a light beam.

Microcell. A cell with a narrow chamber requiring the minimum volume of sample for a given pathlength.

Micron. Micrometre (μm), 10^{-6} m.

Millimicron. Nanometre (nm), 10^{-9} m.

Molar absorptivity (ϵ). The absorbance at a specified wavelength of a solution of a compound of unit molar concentration measured in a 10 mm pathlength. It has dimensions of $\text{M}^{-1}\ \text{cm}^{-1}$.

Monochromator stray-light (MSL). Radiation emerging from a monochromator of wavelengths outside the passband to which the monochromator is set.

NBW. See Natural bandwidth.

NIR. See Near-infrared.

Natural bandwidth (NBW). The width of an absorption band of a particular substance, measured at half-peak height.

Near-infrared. The part of the infrared region closest to the VIS region, generally taken to be between 800 nm and 2 μm.

Near stray-light. The component of the monochromator stray-light lying within a few nanometres of the monochromator passband.

Near-ultraviolet. The part of the UV region closest to the VIS region. There are no agreed limits, but the range is generally taken to be 250 to 400 nm.

OD. See Optical density.

Optical density. This is a general term for the absorbance of any material: absorbance should only be applied to solutions.

Passband. The band of wavelengths emerging from a monochromator, generally centred on the indicated wavelength.

Pathlength. The thickness of a sample solution traversed by the beam as it passes through a cell.

Phosphorescence. A process in which radiation absorbed by a molecule is re-emitted as UV or VIS radiation, generally of longer wavelength than the exciting radiation. Unlike fluorescence, there is a delay between absorption and emission.

Photon. A quantum of UV or VIS radiation.

Phototube. A vacuum tube (valve) detector.

Photovoltaic detector. A detector which generates a voltage in response to light.

Quantum. A fundamental unit of radiation. The energy of a quantum is related to the frequency of the radiation by:

$$E = h\nu$$

where h is Planck's constant.

Radiation. See Electromagnetic radiation.

Reference cell. A cell identical to that used for the sample solution, but containing only solvent. The difference between the absorbances of sample and reference cells is thus a true measure of the absorbance of the solute.

Resolving power. The ability of an instrument to distinguish between two closely-spaced maxima.

SSW. See Spectral slitwidth.

Sampling cell. A cell designed to measure a number of discrete samples in succession, each sample displacing its predecessor.

Scan. To drive a spectrometer through its wavelength range in order to measure an absorption spectrum.

Scatter. See Scattered light.

Scattered light. Radiation which is reflected or refracted out of the measuring beam during its passage through the spectrometer. This may occur due to optical defects, dust or contamination of the optical surfaces in the instrument, or due to particles or inhomogeneities in the sample solution.

Second order. See Grating order; Derivative spectra.

Semi-micro cell. A cell with chamber of reduced width and thus requiring less sample solution to fill it.

Single-beam spectrometer. An instrument with a single optical path. This means that for each wavelength setting, the sample cell must be moved out of the beam so that the transmission scale can be set to unity.

Sipper system. A manual or electric pump system used to draw sample solutions into a sampling cell.

Slew rate. A term used to describe both the rate of wavelength scan of a spectrometer and the rate of movement of the recorder pen.

Slit. An opaque screen with a fixed or adjustable aperture designed to limit the width of a light beam.

Slitwidth. The width of the aperture of a slit. Monochromator slits determine both the amount of light and the spread of wavelengths transmitted by the monochromator.

Spectral line. Generally synonymous with emission line, but can be used for very narrow absorption maxima.

Spectral slitwidth (SSW). The range of wavelengths emerging from a monochromator when it is set to a particular wavelength and slit opening. Effective spectral slitwidth is the preferred means of expressing this.

Spectrometer. An instrument for measuring the transmittance or absorbance of a solution at different wavelengths.

Spectrophotometer. See Spectrometer.

Spectrum. See Absorption spectrum.

Stray-light. Radiation present in a spectrometer beam of wavelengths outside the monochromator passband. This may be due to optical defects, dust etc.

T. See Transmittance.

Transmission. The process by which radiation passes through a material. It therefore represents radiation that is not absorbed, scattered or otherwised dispersed by the material.

Transmittance. The proportion of light that is transmitted by a sample:

$$T = I/I_0 = 10^{-A} = \text{antilog}(-A)$$

where I_0 and I are the intensities of the light falling on the sample and that emerging from it. Thus T lies in the range from 0, for an opaque material, to 1 for a transparent one. T has no units, but is sometimes expressed as a percentage, i.e.

$$T\% = 100 \times I/I_0$$

UV. See Ultraviolet.

UV–VIS spectrometer. An instrument designed to operate through the UV and VIS regions, i.e. from 180 to 800 nm.

Ultraviolet (UV). The region of the electromagnetic spectrum lying at shorter wavelengths than the blue end of the visible region, and generally taken to be between 100 and 400 nm.

VIS. See Visible light.

Vacuum ultraviolet. The shortwave part of the UV region where oxygen and other gases absorb strongly, lying between 100 and 180 nm. Spectrometers for this region must be housed in evacuated chambers.

Visible light. The region of electromagnetic radiation that can be seen by the human eye, and generally taken to extend from 400 nm (violet) to 800 nm (red).

Wavelength (λ). A measure of electromagnetic radiation, being the length of the waves associated with the radiation. In the UV–VIS region, these waves are very small being of the order of 10^{-7}–10^{-6} m in length; about 150 waves of green light would span the thickness of this page.

Wavenumber ($\bar{\nu}\,\text{cm}^{-1}$). An alternative measure of frequency, expressed as the number of waves cm^{-1}. This can be derived from wavelength without knowledge of the velocity of light:

$$\bar{\nu} = 10^7/\lambda\ \text{cm}^{-1}$$

where λ is the wavelength in nanometres.

Window. The optical faces of a cell.

Working area. That part of the window of a cell that is up to optical specification and through which the measuring beam can pass without risk of interference with the walls or floor of the cell, or with the meniscus of the sample.

Zero order. In discussing derivative spectra, zero order is used to describe the fundamental absorption spectrum.

α. Sometimes used for absorptivity or molar absorptivity.

ϵ. Molar absorptivity.

ϵ_{max}. The maximum absorptivity of an absorbance band.

λ. Wavelength.

λ_{max}. The wavelength of maximum absorbance of an absorption band.

μm. Micrometre.

ν. Frequency.

$\bar{\nu}$. Wavenumber.