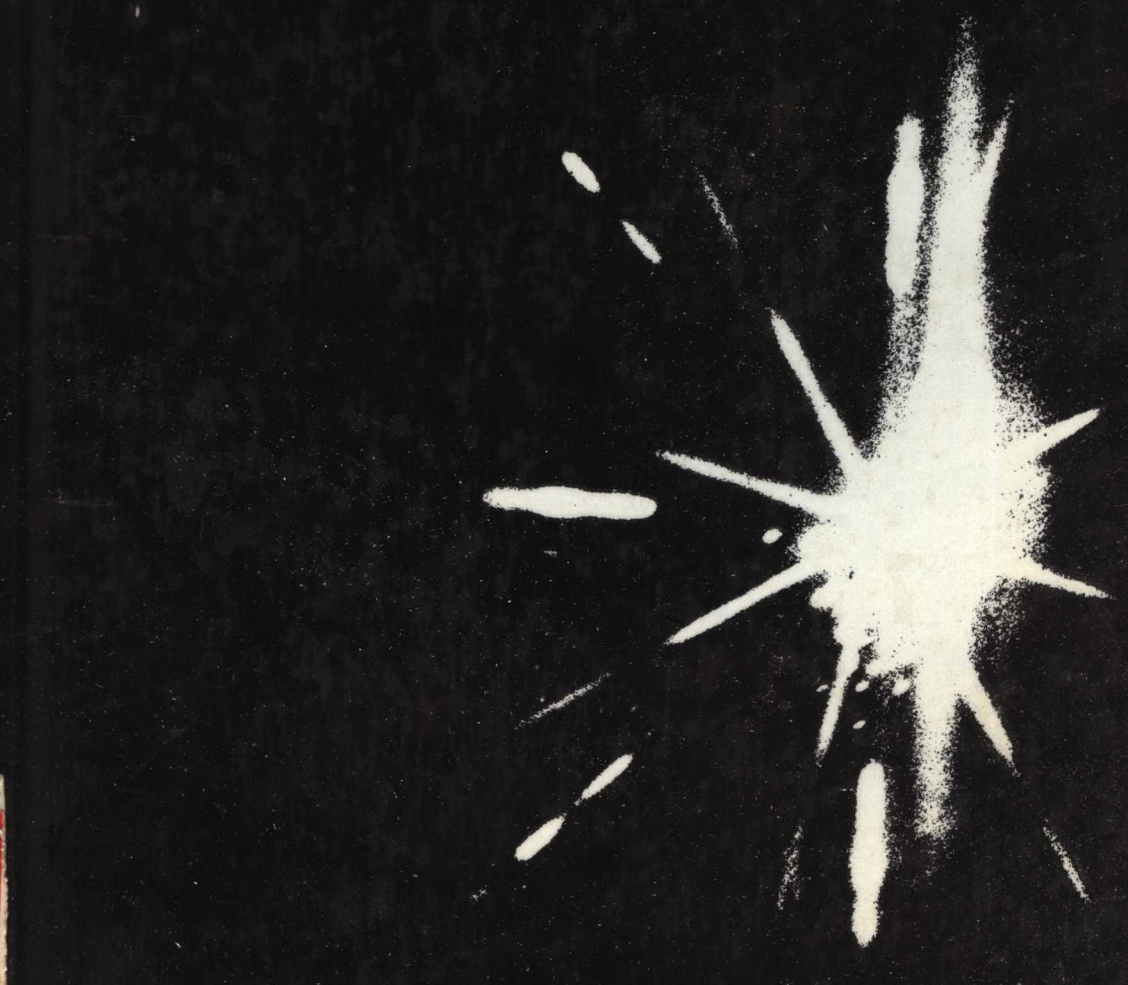
TECHNIQUES IN VISIBLE AND ULTRAVIOLET SPECTROMETRY VOLUME 1

Standards in Absorption Spectrometry

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



Standards in Absorption Spectrometry

ULTRAVIOLET SPECTROMETRY GROUP

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LONDON NEW YORK
CHAPMAN AND HALL

First published 1981
by Chapman and Hall Ltd.
11 New Fetter Lane, London EC4P 4EE

Published in the U.S.A. by
Chapman and Hall
in association with Methuen Inc.
733 Third Avenue, New York NY 10017

Typeset by Tek-Art, Croydon, Surrey
and printed in Great Britain
at the University Press, Cambridge

ISBN 0 412 22470 4

British Library Cataloguing in Publication Data

Standards in absorption spectrometry. – (Techniques in visible and ultraviolet spectrometry; Vol. I).
1. Absorption spectra
I. Burgess, C II. Knowles, A III. Ultraviolet Spectrometry Group IV. Series
543'.085 QD96.A2 80-41128

ISBN 0-412-22470-4

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Preface

The Photoelectric Spectrometry Group was formed in July 1948 in Cambridge. The Group was born out of a need for a forum of users to discuss mutual problems and methodology associated with the new era of photoelectric spectrometers heralded by the Beckmann DU absorptimeter. Over the years the aims and objectives of the Group have been broadened to include many aspects of ultraviolet and visible spectrometry. In 1973, the Group renamed itself the UV Spectrometry Group. The techniques of fluorescence, diffuse reflectance and to a lesser extent ORD and CD were included in the Group's interest. In 1979, the UVSG became a registered charity. The present Group membership is some 200 practising spectroscopists, many from the UK plus a small but growing overseas membership.

The Group's active interest in standards and standardization over the last thirty years is readily seen from our Appendix. In August 1977, the committee under the chairmanship of Dr A. J. Everett, Wellcome Research Laboratories, set up three working parties: Cells for UV-Visible Spectrophotometers; Photometric and Wavelength Standards; and the Calibration of Fluorimeters. It was felt that a wealth of information and expertise in the practice of UV spectrometry was available within the Group and that it was appropriate for this to be gathered together in the form of a number of monographs. Initially the intention was that these should be limited to circulation amongst the Group membership. However, the suggestion was made that these monographs would be of interest to other scientists outside our specialist Group.

This monograph is a combination of the first two working party reports and attempts to cover those areas of UV-visible spectrophotometry which are vital to production of accurate and precise

data. This is essentially a practising chemists' manual and in no way claims theoretical rigour. Notwithstanding, theoretical aspects have been covered and appropriate references made to more fundamental works in those sections where the reader may require a deeper insight, for example in the chapter on Stray-Light.

We have set out a series of recommended standards for cells and procedures for instrumental standardization. We regard these as much akin to the late J. R. Edisbury's 'Links with Sanity'. The recommendations are a consensus of informed user opinion and do not reflect any commercial interest or bias. An introduction to the topic of UV-visible spectrophotometry has been supplied by Dr Everett in his own highly individualistic style and sets the scene for the later more specialized chapters.

The Cell Working Party discussions were based on British Standard Specification No. 3875 (1965) which led to a unification of cell design and a raising of manufacturing standards. The recommendations of the Deutsches Institut für Normung and the Institut für Standisierung und Dokumentation im Medizinischem Laboratorium contained in DIN 32 635 and DIN 58 963 (Parts 1 and 2) have also been taken into account.

It should be emphasized that this monograph is a product not only of the working parties concerned but also of the 'workshops' which followed the issue of the first drafts. As chairmen of the working parties we are grateful for the interest and expertise displayed at those subsequent meetings in helping to produce the final article. Special mention of individuals is not usual in this type of group effort. However, we wish to acknowledge the extensive assistance of Professor D. Thorburn Burns, Queen's University, Belfast, in providing the Standards Working Party with data and in his critical appraisal of the first draft.

Thanks are due also to the Committee and our present Chairman, Dr M. A. Russell, B.D.H. Chemicals Ltd, who have guided our efforts so ably and to our publishers Chapman and Hall.

The Editors would welcome any comment or criticism concerning this monograph. All proceeds accruing from sales will go to Group funds for the furtherance of UV spectrometry.

February 1980

C. BURGESS

A. KNOWLES

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1 General considerations on UV–visible spectrometry

1.1 Introduction

Ultraviolet spectrophotometry, as opposed to spectroscopy, has been generally available since about 1943 when it became possible with manual photoelectric spectrophotometers to make reasonably quantitative measurements of the amount of energy absorbed as a function of the wavelength of the incident radiation. Since then a wide range of manual and recording spectrophotometers has become available, but sadly there is no compelling evidence that the reproducibility of measurements between laboratories approaches that from within a given laboratory. Reasonably competent operators seem able to achieve acceptable precision but often only with rather poor accuracy. Many publications have dealt with this problem which of course is the starting point for this Volume. The UV Group has played an active part in the quest for the optimum performance of instruments and a selection of the Group's publications is given in the Appendix.

It is not within our scope to discuss the fundamentals of UV-absorption spectroscopy in terms of the electronic phenomenon. The ramifications of quantum mechanics have little impact upon the finger print on the front surface of a cell. The fundamentals which concern us here are those which bear upon the best use of the available equipment to achieve a spectroscopic measurement. We assume that most readers will have a spectroscopic background and that the following notes will merely serve to jog the memory as well as bring to mind key references [1-4].

1.2 Radiant energy

Three properties of electromagnetic radiation are necessary to specify it. The quantity or intensity is specified in units of energy or

power. The quality is defined by the frequency or the vacuum wavelength. Finally, the state of polarization should be specified.

1.2.1 *Wavelength*

In general, the frequency of UV radiation is too high for direct measurement (about 10^{15} Hz) so that experimental measurements must be in terms of wavelength. Frequency is then derived from:

$$\text{frequency} = \frac{c}{\lambda}$$

where c is the velocity of light in vacuum and λ is the wavelength of the radiation.

It is important not to confuse frequency with wavenumber. The latter is the number of wave maxima per unit length, being given by:

$$\text{wavenumber (cm}^{-1}) = \frac{10^7}{\lambda\,(\text{nm})}$$

and although, unlike wavelength it is directly proportional to energy, it has no particular spectroscopic significance.

Visible light is generally considered to extend from 680 nm (14 706 cm^{-1}) to 370 nm (27 027 cm^{-1}) and the near-UV-region from 370 nm to 200 nm (50 000 cm^{-1}). Like the other defining wavelengths, the 200 nm limit is arbitrary in that for old instruments the scattered-light performance rapidly deteriorates with further decreasing wavelength, and oxygen and solvent absorption exacerbate the problem.

Today with the new generation of holographic grating spectrometers a limit of 185 nm might be more realistic. In most instances instrument manufacturers provide spectrophotometers whose precision and accuracy of wavelength read-out are adequate, but this must not dissuade the spectroscopist from simple checks of calibration.

1.2.2 *Intensity*

It cannot be said that the same confidence in wavelength accuracy applies to the measurement of intensity, the second defining property of the radiation. Fortunately for the UV spectroscopist, absolute light intensity measurement rarely arises. It is the attenuation of the light beam which is of more interest to the majority, who are concerned with absorption spectrometry and, here, intensity is loosely equated to absorbance as defined below. If the need does arise, the absolute intensity of the light beam may be expressed

in convenient energy units per unit time. The latter aspect will be the subject of detailed consideration in the companion monograph on Fluorescence spectrometry.

1.3 Absorption

1.3.1 *Absorbance*

When a beam of radiation of specific wavelength impinges upon a substance, the energy associated with the beam may be altered by reflection, refraction, absorption and transmission processes.

Most experimental measurements are concerned with elimination of, or corrections for, effects other than absorption. The simplest situation with respect to the intensity of absorption is that in which the system obeys the Lambert-Beer Law. In this case if I_0 is the intensity of a parallel beam of radiation incident normally on a layer of thickness b cm and molar concentration c, the intensity of the emergent beam is:

$$I = I = I_0 \ 10^{-\epsilon cb}$$

where ϵ, the molar absorptivity (litre mole^{-1} cm^{-1}), is independent of c but is a function of wavelength, temperature and solvent.* Of course this implies that each layer, or indeed each molecule, of the absorbing substance absorbs a constant fraction of the incident radiation. The above equation can be expressed in the form:

$$\log_{10} \left(\frac{I_0}{I}\right) = \epsilon cb$$

or

$$A = \epsilon cb$$

where A is the absorbance of the sample in the beam. The ratio of the light intensity transmitted by the sample to the light intensity incident on the sample is the transmittance T:

$$T = I/I_0 \qquad \text{and} \qquad A = -\log_{10} T$$

In this Volume, transmittance will be expressed as a percentage, i.e. $T = 100\,I/I_0$ per cent.

*The nomenclature used throughout is based on the recommendations of the American Chemical Society [5], the American Society for Testing and Materials [6], and the British Standards Institution [7].

Absorbance is more simply related to concentration and absorptivity than are I, I_0 or T. Strictly, absorbance is only applicable to solutions, the more general term 'optical density' applying to solids and homogeneous liquids as well. However, absorbance will be taken to be synonymous with optical density.

The attenuation of a beam of radiation in passing through a sample is due in part to absorption within the sample and in part to reflection and scatter at the external surfaces. The transmission of the material itself, without the external losses, will be termed the 'internal transmission', and is thus defined as that percentage of the radiant flux leaving the entry surface which eventually reaches the exit surface.

1.3.2 *Sources of absorbance error*

It is convenient to consider two categories of absorbance error. The first originates with the spectrophotometer and the second directly or indirectly with its use. In practice this dichotomy is not so clearly defined.

(a) *Spectrophotometer limitations*

At the outset it is desirable to distinguish between the working definition of transmittance or absorbance and the true transmittance or absorbance as outlined by Jones and Sandorfy [2]. Using their terminology, for parallel radiation of intensity I_i falling normally on a cell containing a solvent and a solute:

I_r = reflection losses at cell interfaces
I_s = scattering losses at cell surfaces and from the solution
I_b = absorption losses by the solvent
I_a = absorption by the solute.

The true transmittance of the solute is:

$$T = \frac{I_i - (I_a + I_b + I_r + I_s)}{I_i - (I_b + I_r + I_s)}$$

On the other hand the working definition of transmittance, T', generally using a double-beam technique, is:

$$T' = \frac{I}{I_0} = \frac{I_i - (I_a + I_b + I_r + I_s)}{I_i - (I'_b + I'_r + I'_s)}$$

It follows that T and T' are only identical when:

$$I_b + I_r + I_s = I'_b + I'_r + I'_s$$

Deviations from this condition are most likely to occur for a sample with low molar absorptivity and high molecular weight.

(b) *Reflection losses*

If sample and reference cells are made to a sufficiently high specification the outer face reflections will cancel. So also will the reflections at the liquid-to-fused-silica interfaces if, as is usual in UV spectrophotometry, the solute concentration is very low. To put the matter into perspective, the loss from internal reflections in a fused silica cell filled with water is only about 0.4 per cent of the incident light energy at 589.3 nm [8].

Even on passing through an absorption band where the solution refractive index and hence the reflectance loss (see Section 1.4.2) is rapidly changing, the effect being on the solute absorbance measurement is exceedingly small, being of the order of 0.001 per cent. Measurable effects arising from refractive index imbalance between reference and sample do arise, but they are essentially of an instrumental nature and may be responsible for some of the difficulties associated with the use of potassium nitrate solutions as absorbance standards where the concentrations are as high as 0.15 M.

(c) *Solvent absorption*

Usually in UV spectrometry the mole fraction of the solute is so low that $I_b = I'_b$, i.e. the numbers of absorbing solvent molecules in each beam are almost identical.

(d) *Scattering losses*

Small non-conducting particles will, when present as a cloudy sample, exhibit Tyndall scattering whose intensity is proportional to the fourth power of the frequency. This can give rise to very serious problems which lead to apparent deviations from the Beer-Lambert Law, particularly at short wavelengths. Good working practice will reduce the gratuitous introduction of scattering errors.

1.4 User limitations

The following factors are ones which should be considered when attempting to obtain the greatest precision and accuracy from a

spectrophotometer. In some instances the instrument design will dictate procedure; in others the user can have a marked influence on the quality of the result.

1.4.1 *Gravimetric and volumetric accuracy*

This is not the place to deal with these factors *in extenso*. Suffice to note that in most instances weighing errors certainly ought not to exceed 0.1 per cent and volumetric errors should be little more, unless very small volumes are to be handled in which case the solvent should be weighed and corrections applied for solvent density. Solvation, particularly hydration, is a frequent source of error associated with the measurement of molar absorptivity. It must also be borne in mind that appreciable temperature changes, besides affecting volumetric equipment, will frequently lead to actual changes in the molar absorptivity of the sample. For example it has been suggested that the latter is partly responsible for the apparent variability of the molar absorptivity of aqueous potassium nitrate. Particulate matter can be removed by using a proprietary membrane filter on a plastic hypodermic syringe. The syringe should be washed before use, as it may be coated with a lubricant. Glass hypodermic syringes may introduce fine particles of glass and should never be used for transferring solutions. Small bubbles of air adhering to the window surfaces are a source of error exacerbated by greasy surfaces but alleviated by solvent degassing and careful manipulation of the solutions.

1.4.2 *Cell handling*

An obvious first requirement of any photometric measurement of solutions is that the effect of the container should be measurable or compensated. Ideally, the sample and reference cells should be optically identical. Apart from the identity of the window geometries and the consistent orientation of the cells with respect to the light beams, it is an elementary requirement that they be clean. Recommended cleaning procedures are given in Chapter 8.

The ratio of the reflected light intensity I_r to the incident light intensity I on a surface is governed by the Fresnel relationship:

$$\frac{I_r}{I} = \left(\frac{n_1 - n_2}{n_1 + n_2}\right)^2$$

where n_1 and n_2 are the refractive indexes of the two media. Other than at short wavelengths the transmittance of empty synthetic

fused silica will be governed by reflectance losses. For example, the theoretical transmittance of an empty cell at the wavelength of the sodium D line (589.3 nm) is 0.933 (A = 0.0301) whereas on filling with water† the transmission rises to 0.996 (A = 0.018) because the internal reflection losses are decreased as a consequence of the nearer matching of the refractive index of water to that of fused silica. However in practice an empty cell need never be used in the reference beam of a spectrophotometer. Ideally, cells should only be handled with tongs or hands covered by clean cotton gloves.

Accuracy is required in setting the cell in the beam [3]: For an absorbing medium of refractive index n and for an angle of a radians from the normal to the cell with respect to the incident beam, the fractional error in path length δ, is given by:

$$\delta = \left(\frac{0.0123a}{n}\right)^2$$

For many advanced instruments an absorbance of 2 can be read to at least 0.001 A (δ = 0.0005). Even simpler instruments can approach this precision when properly used in the difference mode. An alignment error of just over 3° would introduce an error of this magnitude. To put the matter in perspective there would have to be a side play of 1 mm in a 1 cm cell-holder to introduce this error. Nonetheless the hazard of using cells with non-standard external dimensions should be recognized. It is also wise to check that the mounting of short cells is such as to give adequate reproducibility and that micro cells and long cells do not attenuate either by vignetting the beam at the front window or by the light beam grazing the cell walls.

In most spectrophotometers the cells are mounted symmetrically with respect to the focus of the beams and pathlength. Errors arising from beam divergency are usually negligible.

For the most precise work with dilute solutions it is probably best to use only one cell and leave it in position for both solvent and solute readings. A hypodermic syringe with a plaster catheter tube is then used for filling, emptying and washing the cell. The solvent spectrum readings are subsequently subtracted from those of the solution. Spectrophotometers interfaced to computers now make this approach quite attractive to the user if not to the cell manufacturer. The cell 'blank' can be stored so that the experiment time remains the same

†Water must be stored in fused silica vessels or, failing that, polyethylene which contains neither antioxidant nor plasticizer.