

Standards & Best Practice in  
Absorption Spectrometry

# Standards and Best Practice in Absorption Spectrometry

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This book was published to celebrate the 50th anniversary of the UV Spectrometry Group (UVSG) and is dedicated to all the many people who have given their support to the group over the last 50 years.

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# Preface

The UV Spectrometry Group (UVSG), originally called the Photoelectric Spectrometry Group, was founded in 1948 as a forum for people working with UV-visible spectrophotometry. Some of the background to the formation of the group is given in the Appendix. In the 1980s and early 1990s, the group produced four definitive monographs on UV spectrometry. Volume 1, *Standards in Absorption Spectrometry*, was followed by a similar volume on *Standards in Fluorescence Spectrometry*. Volumes 3 and 4 addressed practical aspects of absorption spectrometry [1-4].

Part 1 of this book comprises a revised and updated version of Volume 1 that takes account of the work that has been carried out and the changes in the regulatory environment since its publication in 1981.

Because Volume 3 is out of print, the opportunity has also been taken to include in Part 2, without revision, some of the chapters on best practice from Volume 3. Although much work, particularly on the chemometric aspects of UV spectrometry, has been published since the first edition of Volume 3, it was felt that it would be useful to draw together the relevant material from that volume.

In the years since the first volumes were published by the UVSG, instruments and instrumentation have progressed far more rapidly than anyone could have predicted. UV spectrometry has become the main detection technique for high-performance liquid chromatography. The introduction of diode array technology and computer-controlled instrumentation has resulted in instruments that are cheaper and more reliable than 20 years ago. The experience of the group in running training courses would suggest that the converse might be true of the instrument operators! The need for standards that provide the 'link with sanity' is as great as ever.

Much of the material from Volume 1 remains intact, and we fully acknowledge the work of the many people involved with that volume. Where only minor changes to the original chapters have been made, we have added the new contributor's name to the contents page. The chapter on stray light has undergone extensive revision and we acknowledge the original work of G.J. Buist, who wrote the original chapter. Much dichromate has flowed since the original chapter on liquid absorbance standards and we would like to acknowledge the work of Elaine Vinter

who wrote the chapter on liquid standards in Volume 1. Chapters have been updated where necessary to reflect recent work and the changes in instrumentation. An additional chapter on regulatory compliance has been added and the chapters on cells rationalized into one chapter. The recommended procedures chapter has undergone a significant overhaul and numerous spectra and references have been added. The UVSG references in the Appendix have also been updated and a section documenting the history of the group has been added to these.

We are grateful for the efforts of all the contributors to this book. As with the first volume, all the proceeds from the sale of the book will go to the support of continued research and training in UV spectrometry. The publication of this book is the swan song of the UVSG, as the group has become too small to be viable and is sadly being wound up. The assets are being transferred to the Association of British Spectroscopists Trust to continue the work of the group.

C. Burgess and T. Frost

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## Part 1

# Standards in Absorption Spectrometry



# **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 reproductibility 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 book. 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 and in the references. 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 fingerprint on the front surface of a cuvette. The fundamentals which concern us here are those which bear upon the best use of the available equipment to achieve a spectroscopic measurement. We have assumed that 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 [1-10].

## **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 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 a vacuum and  $\lambda$  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:

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

and although unlike wavelength it is directly proportional to energy, it has no particular spectroscopic significance. Visible light is generally considered to extend from 370 nm to 680 nm, and the near-UV region from 200 nm to 370 nm. Like the other defining wavelengths, the 200 nm limit is arbitrary in that for many instruments, particularly old ones, the stray-light performance rapidly deteriorates with further decreasing wavelength. Oxygen, gaseous and dissolved, and solvent absorption exacerbate the problem even more in the region below 200 nm.

As the use of halographic gratings in spectrophotometers is now widespread, a lower wavelength limit of 185 nm for solution work might be more realistic. However, special precautions need to be observed when working in this region, see later for more details. 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 as systematic errors of several nm are not unknown.

### 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 is the subject of more detailed consideration in the companion monograph on fluorescence spectrometry [7, 10, 11].

## 1.3 Absorption

### 1.3.1 Attenuation of radiation

When a beam of radiation of specific wavelength impinges upon a substance, the energy associated with the beam may be altered by one of four processes; reflection, refraction, absorption and transmission. Most experimental measurements are concerned with elimination of, or corrections for, effects other than absorption.

Usually such measurements are carried out using a spectrophotometer, more correctly called a spectrometer. This consists of a number of basic elements; a source of radiant energy, a wavelength selection device, a sample compartment and a detector. For more details, the reader is directed to the references, in particular [3, 8, 12-14].

The simplest situation with respect to the intensity of absorption is that in which the system obeys the Beer-Lambert 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,  $I$ , is:

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

where  $\epsilon$ , the molar absorptivity ( $\text{litre mole}^{-1} \text{cm}^{-1}$ ), is independent of  $c$  but is a function of wavelength,  $\lambda$ , 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 more familiar logarithmic 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  $T$ :

$$T = \left( \frac{I}{I_0} \right) \quad \text{and} \quad A = -\log_{10} T$$

Transmittance is usually expressed as a percentage, i.e.  $\%T = 100 \left( \frac{I}{I_0} \right)$ , and this convention will be followed in this book. Spectroscopic nomenclature is a never-ending source of confusion, especially in older works. The conventions followed in this book are detailed in Refs [9, 15].

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 for our purposes. 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.

#### *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 (chapter IV in Ref. [1]). Using their terminology, for parallel radiation of intensity  $I_i$  falling normally on a cuvette containing a solvent and a solute:

$I_r$  = reflection losses at cuvette interfaces;

$I_s$  = scattering losses at cuvette 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.

### *Reflection losses*

If sample and reference cuvettes are made to a sufficiently high specification, the outer face reflections will cancel. Also, so 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 cuvette filled with water is only about 0.4% of the incident light energy at 589.3 nm [16]. 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 on the solute absorbance measurement is exceedingly small, being of the order of 0.001%. 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.

### *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.

### *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 practices will reduce the gratuitous introduction of scattering errors, see Section 1.5 for more details.

## **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.