Ultraviolet and Visible Solution Spectrophotometry and Colorimetry 1980

An Essay Review

Methods for the Examination of Waters and Associated Materials

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Ultraviolet and Visible Solution Spectrophotometry and Colorimetry 1980 Version

An Essay Review

K C Thompson

Methods for the Examination of Waters and Associated Materials

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Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; and 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About This Series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analyticals techniques of interest to the water and sewage industries. This booklet is one such review. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users – the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis
9.0 Radiochemical methods.

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occuring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

T A DICK
Chairman

L R PITWELL
Secretary

4 December 1980

Summary

This essay review is intended as an introduction to solution spectrophotometry and its application to general water analysis. Solution spectrophotometry is inherently a simple technique and analysis at mg/litre or even lower determinand levels can be carried out on relatively simple instrumentation. The technique can be applied to the analysis of water, sewage effluents, sewage sludges and trade wastes for a wide variety of determinands. In fact, there are few determinands of interest to the water chemist that have not been successfully determined by solution spectrophotometry.

This review outlines theoretical aspects of solution spectrophotometry, basic instrumentation, safety considerations, performance, operating characteristics and method development, applications in the water industry and conclusions. Although this review is primarily concerned with solution spectrophotometry and colorimetry, some other related techniques are described in Section 8. It is felt that these techniques could be of interest to many readers, and many can be carried out on a suitably modified solution spectrophotometer.

Forty six references are cited in order to allow the interested reader to pursue specific applications or techniques.

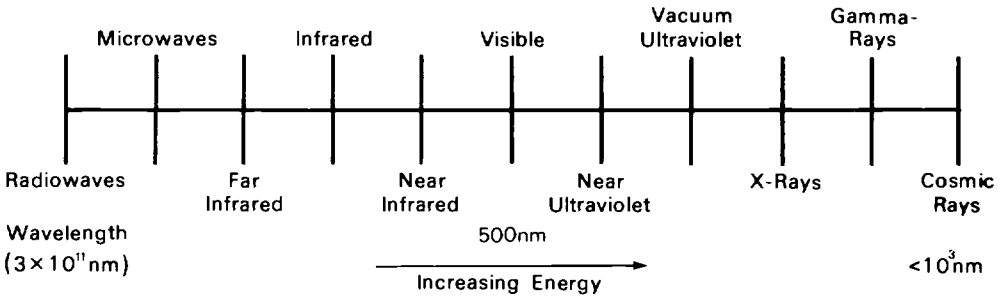

Figure 1 The electromagnetic spectrum

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1 Introduction

Solution spectrophotometry is concerned with the measurement of the absorption or transmission of light of various wavelengths by homogenous solutions*. The analytically useful wavelength range normally available on commercial instrumentation extends from about 190 to 1000 nm. When light (electromagnetic radiation) passes through a homogenous, transparent medium containing a dissolved substance, some of the light may be absorbed by the substance, and the amount absorbed is related to the concentration of the substance. The wavelengths at which absorption occur are characteristic of the absorbing substance.

The first recorded observations of absorption spectra were made by Brewster in 1833 and the first work in the ultraviolet region of the spectrum was independently communicated to the Royal Society in 1862 by Miller and Stokes. Miller studied the transparency of various substances in the ultraviolet region using a quartz spectrograph with photographic detection, whereas Stokes detected the ultraviolet radiation by viewing the visible fluorescence emitted from a suitable substrate placed in the light beam. Hartley[1] (1872) attempted to establish a relationship between the chemical nature of an organic moleçule and its absorption spectrum, by measuring the thickness of the solution required to completely absorb light of a given wavelength. Baly[2] extended this work using photographic detection for monitoring the degree of absorption and it was established that similarity in absorption spectra often correlated with similarity in structure. The first systematic investigation of ultraviolet absorption spectra of simple organic compounds was made by Victor Henri and his pupils from about 1919. They demonstrated that the absorption spectrum of a compound is not specifically characteristic of the whole molecule but only particular colour absorbing chromophores. For example, simple ketones exhibit a weak absorption band between 270 and 300 nm and for a homologous series of ketones the wavelength of maximum absorption gradually increases with increasing molecular weight.

Widespread use of the technique was restricted because of lack of commercially available instrumentation. However, filter colorimeters (see section 3.3) became generally available from about 1934 with the Hilger and Watts H290 Spekker. Soon after that spectrophotometers with monochromators that functioned in the visible region appeared, whilst the first widely available ultravolet visible spectrophotometer (see section 3.4) was the famous Beckmann Model DU which first appeared in 1941[3]. This unit consisted of a prism monochromator, a source unit containing both a hydrogen arc lamp and a tungsten filament lamp, and photocell detection of the radiation. Thirty thousand of these units were produced before production finally ceased in July 1976. The first ultraviolet visible spectrophotometers to be manufactured in the United Kingdom were the Hilger and Watts Uvispek and the Unicam SP500 which both appeared in 1947.

Solution spectrophotometry is an analytical technique that can be used to determine a very wide range of substances provided that they are in a homogenous solution, and is very well suited to water analysis. Used with care and careful method development the technique can have good specificity, consequently sample preparation can be relatively simple and thus handling errors are minimal. The vast majority of routine auto-analysis methods ultimately rely on colorimetric estimation of a suitable reaction product of the determinand. This testifies to the widespread use and acceptance of the technique[4].

Detection limits of many determinands of interest in the water industry are generally considered adequate for routine analysis.

---

* It should be noted that some of the complementary techniques described in Section 8 can be applied to suspensions or even solids.

2 Theory

2.1 The Electromagnetic Spectrum

The electromagnetic spectrum is depicted in Figure 1 and the wavelength range is from $>3 \times 10^{11}$ nm to $<10^{-3}$ nm. The effect of electromagnetic radiation of various wavelengths upon a typical molecule is given in Table 1. This table shows that solution spectrophotometry is only concerned with the ultraviolet, visible and near infra-red regions of the spectrum.

At wavelengths below 190 nm, oxygen in the air and most solvents completely absorb radiation and this severely limits the use of these wavelengths for routine analytical use[5,6]. At wavelengths above 1000 nm there are few species that exhibit electronic absorption bands (see section 2.2).

Table 1 The Effect of Electromagnetic Radiation upon Molecules

Wavelength nm	Description	Typical Reaction after Absorption by matter
$<10^{-3}$	Cosmic Rays	Shatter nuclei
$10^{-3}-10^{-2}$	γ rays	Cause nuclear transitions
$10^{-2}-1$	X rays region	Excite inner electrons
1–190	Vacuum ultra violet region	Excite outer or bonding electrons*. (Electronic transitions).
190–380	Ultra violet region	
380–750	Visible region**	
750–1000	Near Infra-red region	
$10^3-5 \times 10^4$	Infra-red region	Excite vibrational modes
$5 \times 10^4-10^6$	Far infra-red and micro-wave region	Excite rotational modes

* This region of the spectrum is also capable of exciting free atoms as in atomic absorption.

** The shortest wavelength detectable by the human eye tends to increase with age.

2.2 Factors influencing the Absorption Characteristics of Molecules

Figure 2 depicts the potential energy curves for the case of a simple diatomic molecule and typical energy levels for a polyatomic molecule. It can be seen that only a small quantum of electromagnetic energy is required to effect a rotational energy transition, a larger quantum is required to effect a vibrational energy transition, whilst a considerably larger quantum (corresponding to ultra-violet or visible light) is required to effect an electronic energy transition.

The absorption spectrum of a simple electronic transition would be expected to be a sharp band less than 0·1 nm in half-width, but in practice bands with half-widths of 50 nm or even more are normally observed*. This is because it is impossible to effect an electronic transition without concurrent vibrational and rotational energy level changes. At room temperature an appreciable number of vibrational and rotational ground electronic states are populated and all of these will absorb radiation thus severely broadening the absorption band. A further significant broadening effect occurs in solutions because of interaction between neighbouring molecules and because of solvation effects. In fact for many absorption bands measured in liquids the fine vibrational-rotational structure is not observable and each electronic transition results in a single wide continuous absorption band of the type normally associated with solutions of organic compounds. Gas phase absorption spectra (eg benzene vapour), however, exhibit considerable fine structure.

After a molecule absorbs a quantum of radiation, the excited molecule returns to the ground state by giving up its excess energy as heat (by collision with other unexcited molecules) or in a few cases as fluorescent radiation.

* Some rare earth compounds exhibit much sharper absorption bands (see section 5.6.2).

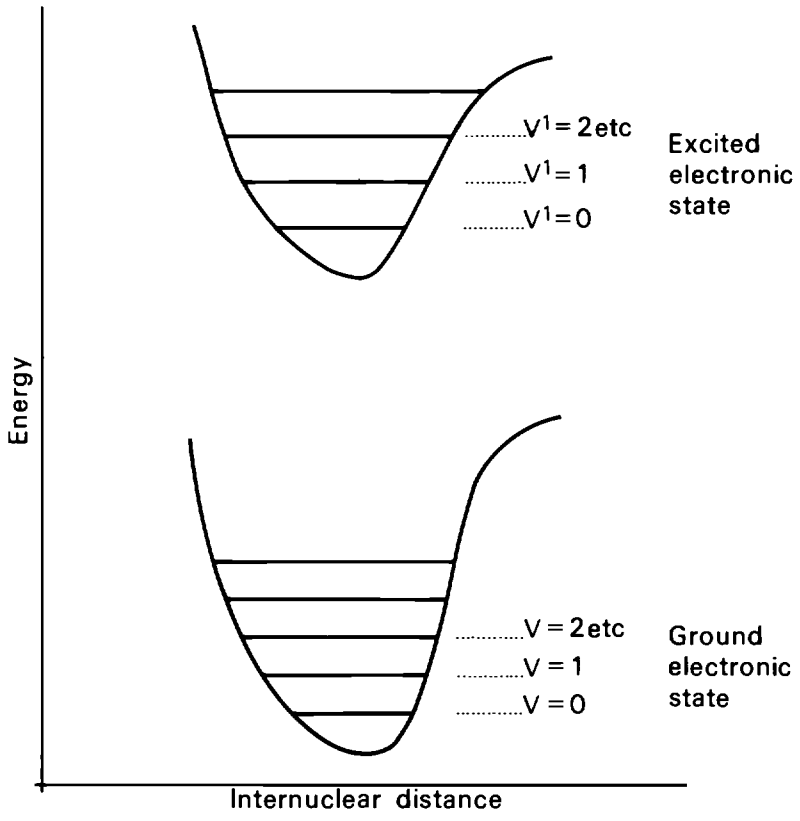


Figure 2a Potential energy curves for a diatomic molecule

Note: Horizontal lines represent vibrational levels. Each of these vibrational levels is split into a number of rotational levels.

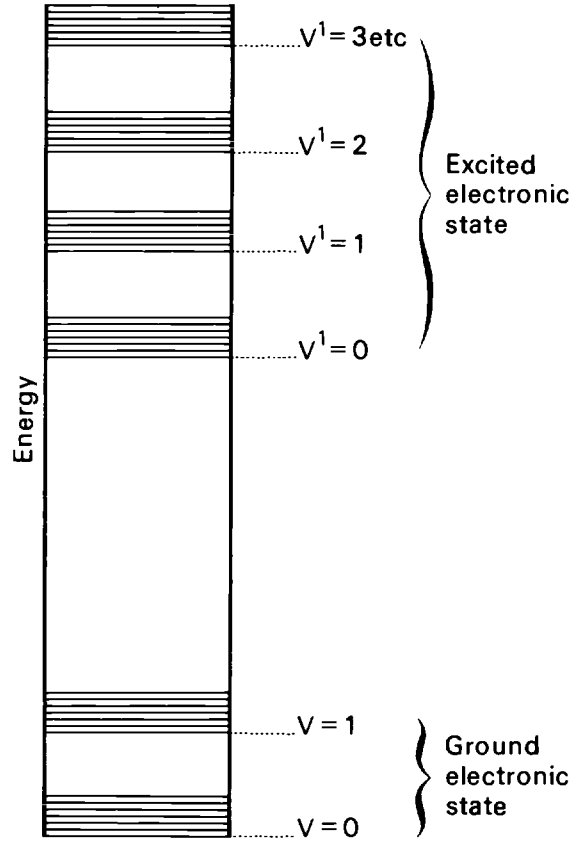


Figure 2b Typical energy levels for a polyatomic molecule

Note: Horizontal lines represent rotational levels.

V = 0, 1, 2, etc. correspond to the various vibrational levels.

2.3 Types of Absorbing Molecules

2.3.1 *Organic Molecules*

Compounds with only single bonds involving bonding electrons and no lone pair electrons (eg aliphatic alkanes) only exhibit absorption bands below 190 nm and are completely transparent from 190–1000 nm. The fact that these substances sometimes appear to absorb strongly at wavelengths below about 230 nm is due to traces of impurities present. The highly purified n-alkanes do not exhibit electronic absorption bands above 190 nm.

Substances that absorb in the wavelength range 200–900 nm owe their absorption to the presence of one or more unsaturated linkages involving electrons (ie double or triple bonds) or to the presence of lone pair electrons. In the visible region of the spectrum only conjugated unsaturated linkages can result in absorption. These linkages or groups which absorb the light are called chromophores, some typical chromophores and their approximate wavelengths of maximum absorption are given in Table 2.

A molecule which contains two or more chromophoric groups, provided they are well isolated will show the absorption bands characteristic of each group. However, if a new chromophoric group is conjugated with another such group already present, the electronic structure of both groups may be significantly altered. In this case the absorption of the resulting compound will differ greatly from a simple summation of the absorbances of the two isolated chromophores. For instance, conjugated dienes and the corresponding alkene; nitrobenzene and nitromethane. In this latter example the nitro-group absorption wavelength is shifted from 270 to 330 nm because of conjugation with the benzene ring (see Table 2). Certain groups which do not confer absorption by themselves, but enhance the absorbing power of a chromophore are called auxochromes and some examples of these are C-I, C-Br, C-OH, C-NH_2.

Table 3 lists the approximate ultra-violet cut-off wavelengths for a number of solvents.

2.3.2 *Inorganic Ions*

2.3.2.1 Transition Metal Ions

These absorptions involve d-d or f-f transitions of transition metal ions normally in aqueous solutions (eg Ti $(6H_2O)^{3+}$, Cr $(6H_2O)^{3+}$, Cu $(H_2O)_6^{2+}$ etc). They are usually very weak and not much use analytically.

Table 2 Some Typical Chromophores and Their Wavelength of Maximum Absorption

Substance		Wavelength of Maximum Absorption (nm)
Alkene	$>C=C<$	190
Conjugated Diene	$>C=C-C=C<$	220
Conjugated Triene	$>C=C-C=C-C=C<$	260
Azo	$>N=N<$	290–400
Ketone	$>C=O$	195, 275
Alkyl-nitro	$-CH_2-N\begin{matrix}\nearrow O\\ \searrow O\end{matrix}$	270
C_6H_5-R		
R =	H	205, 255
R =	COOH	230, 270
R =	C_6H_5	245
R =	$-N\begin{matrix}\nearrow O\\ \searrow O\end{matrix}$	250, 280, 330

Table 3 Approximate Ultraviolet Cut-off Wavelengths for Some Common Solvents

Solvent	Cut-off Wavelength (nm)
Water	<195
Methanol	210
Ethanol	210
Hexane	212
Ethyl Acetate	255
1, 4 Dioxan	220
Acetone	330
Acetonitrile	<210
Chloroform	250
Carbon Tetrachloride	265
Benzene	280
1, 1, 1, 3, 3, 3, Hexafluoropropan-2-ol	<195

Note. The cut-off wavelengths correspond to approximately 25% transmission of radiation in a 1 cm path length cell. These wavelengths given are for spectroscopic grade solvents. Typical analytical reagent grade solvents may display significantly higher cut-off wavelengths.

2.3.2.2 Charge Transfer Spectra

When the absorption of radiation causes an electronic transition between two molecular orbitals such that one is more heavily concentrated on one atom while the other is more heavily concentrated on another different atom, this is known as a charge transfer transition. The bands tend to be rather broad and exhibit high extinction coefficients (see section 2.4.1) often greater than 10^4 litres $mole^{-1}$ cm^{-1}. Typical examples of charge transfer absorbing species are $Cr\ O_4^{2-}$, $Mn\ O_4^{2-}$, $Fe\ (CNS)_6^{3-}$ and $Fe\ (o\text{-phenanthroline})_3^{2+}$. Many of these bands are analytically useful.

2.3.2.3 Common Inorganic Ions that Absorb in the Ultraviolet Region

Hydroxide, carbonate, bicarbonate, nitrate, nitrite, iodide, bromide and to a much lesser extent chloride, exhibit significant absorption in parts of the ultraviolet regions of the spectrum[5].

2.4 Relationship between Absorption, Concentration and Path Length

2.4.1 *Beer – Lambert – Bouguer Relationship*

The Lambert – Bouguer Law (1729) states that the fraction of radiation absorbed by a given amount of substance under fixed conditions is independent of the intensity of the incident radiation. It also can be expressed that each successive layer of thickness dl absorbs equal fractions $(\frac{dl}{I})$ of the radiation of intensity I incident upon it. This can be mathematically expressed as:

$$\text{Log}_{10}\frac{I_O}{I_T} = kl \qquad (1)$$

Where I_O = the incident radiation intensity
I_T = the transmitted radiation intensity
l = the path length over which the absorption occurs
k = a constant

Beer's Law (1852) states that successive increments in the number of identical absorbing molecules in a given path length of a beam of monochromatic radiation absorb equal fractions $(\frac{dI}{I})$ of the intensity I upon it. This can be mathematically expressed as:

$$\text{Log}_{10}\frac{I_O}{I_T} = k'c \qquad (2)$$

where c = concentration
k' = a constant.

Hence the Beer – Lambert – Bouguer Relationship can be obtained by combining Equations (1) and (2)

$$A = \text{Log}_{10}\frac{I_O}{I_T} = Ecl \quad (3)$$

Where A = The absorbance or optical density
E = Molar extinction coefficient (litres mole^{-1} cm^{-1})
c = Concentration (moles litre^{-1})
l = Path length (cm)

The larger the value of E the greater the absorbance of a given molar concentration of a substance at the wavelength corresponding to maximum absorption.
Note: If the molecular weight of the absorbing substance is not known the absorption coefficient E (1%, 1 cm) is used.

$$E\ (1\%\ 1\,\text{cm}) = \frac{\text{Absorbance}}{\text{Path length (cm)} \times \text{Concentration (\% m/V)}}$$

It can be demonstrated that the theoretical maximum value of E for any substance is 10^5 litres mole^{-1} cm^{-1}. Thus from equation (3) assuming that the detection limit corresponds to an absolute absorbance of 0·002 and using a 2 cm path length cell, the concentrational detection limit (c) is given by:

$$c = \frac{A}{E \times 1} = \frac{0.002}{10^5 \times 2} = 1 \times 10^{-8}\text{M} \quad (4)$$

Thus concentrations below 10^{-8}M of any absorbing substance are unlikely to be detected.

The total absorbance of a sample which contains two or more absorbing species is given by the equation:

$$A_T = A_1 + A_2 + \ldots\ldots A_n \quad (5)$$
$$= 1(E_1C_1 + E_2C_2 + \ldots\ldots E_nC_n)$$

Where A_T = the total absorbance of the sample solution at a given wavelength etc
A_1, A_2 are the respective absorbances contributed by each substance present.

2.4.2 Deviations from Beer's Law

It is sometimes found that the absorbances (optical densities) of a set of standards are not directly proportional to their concentrations, ie the molar extinction coefficient appears to vary with the concentration of the absorbing species. In most, but not all cases, it appears to decrease with increasing concentration. There are a number of causes of this behaviour[5,8]:

1. The light is not sufficiently monochromatic; that is E is not constant over the spectral range of the light reaching the detector. This can often be observed when a filter rather than a monochromator is used to isolate the wavelength range of interest (see section 3.3.2). A typical calibration graph exhibiting this effect is shown in Figure 3.
2. The light reaching the detector contains a significant proportion of stray light at other wavelengths. This is often a problem at wavelengths below 230 nm especially with older instruments. It can also be observed when tungsten filament lamp sources are used at wavelengths below about 350 nm. In the presence of stray light similar calibration graphs to those given in Figure 3 are observed.
3. Errors due to non-linearity of the amplifier and/or readout device. These tend to be more significant at high absorbances and should not be apparent on modern instrumentation used within its specification.
4. Interaction between the absorbing species and other molecules in the solution can result in a linear graph up to a certain concentration, and then at concentrations above this the graph bends towards the concentration axis. (See Figure 3). This effect is still observed with effectively monochromatic light and is independent of the spectral bandpass.

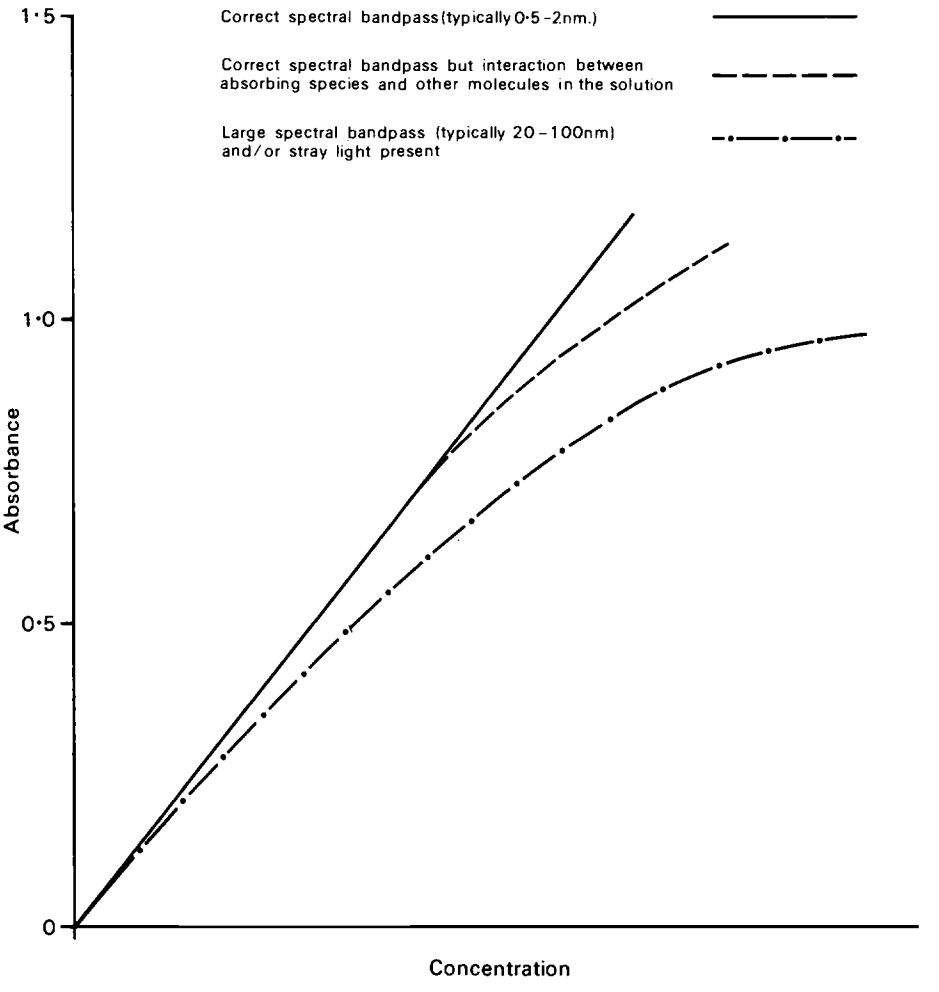


Figure 3 Typical calibration graph shapes

5. The absorbance is critically dependent upon pH.
   eg:

   a. $Cr_2O_7^{2-} + H_2O \rightleftharpoons 2\,CrO_4^{2-} + 2H^+$
      (orange) (yellow)

   b. Picric acid $\rightleftharpoons$ picrate ion + H^+
      (colourless) (yellow)

   Thus if measurements are carried out in unbuffered solutions, Beer's Law will appear not to be obeyed. This is because the ratio of the amount of actual absorbing species to the calculated theoretical amount present, (ie the sum of the concentrations of the two species) is dependent upon the concentration of the absorbing species. Positive or negative deviations can be observed. For these type of systems the solutions should be buffered so that only one species effectively exists in the solution. This usually requires buffering to either a high or a low pH.

   However, there is an alternative method that can be used for the determination of the total concentration of a substance that exists in two forms that both absorb radiation such that the absorption bands partially overlap (eg the indicator bromothymol blue). This method is derived from Bolzano's theorem which states that if two interconvertible species exhibit a continuous absorption spectrum between two given wavelengths, then there is a wavelength at which the absorbance is only dependent upon the total molar concentration of the two species. Figure 4 illustrates this behaviour for an indicator such as bromothymol blue. It can be seen that at one wavelength designated the 'isobestic point' the absorbance is completely independent of pH, does not depend upon the individual concentrations of the two interconvertible species and only depends upon the total molar concentration of the two species.

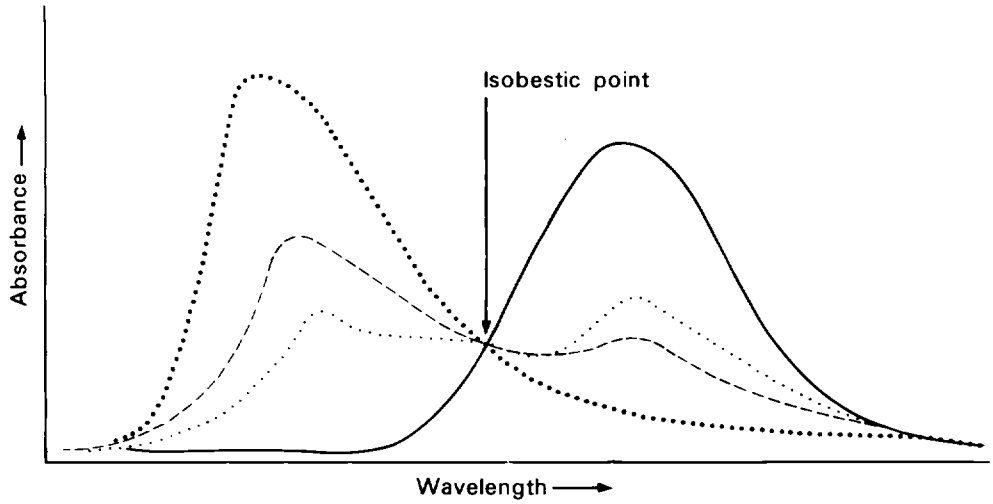


Figure 4 Typical wavelength/absorbance curves for an indicator at four different pH values

Note: All four curves correspond to the same total molar concentration of the two interconvertible forms of the indicators

6. If a metal ion forms a number of successive moderately dissociated complexes with a reagent (eg Cu – NH_3 system) Beer's Law will only apply if the ligand is present in a large excess (10^3-10^4 times) over the metal ion.
7. Some of the solutions or measuring cells or cuvettes containing an absorbing impurity or extraneous matter (eg colloids, dust, gas bubbles etc).
8. Changes of E caused by variations in temperature or time of standing. The performance characteristic data of an analytical method, such as the optimum colour development time, are normally determined at concentrations significantly above (typically 20–100 times) the detection limit. For a few methods, longer colour development times can be required at concentrations close to the detection limit. Where much routine analytical work is carried out, a low concentration standard at 4–5 times the concentration of the quoted detection limit should always be run.
9. If the absorbing species undergoes polymerization, association, hydrolysis or condensation reactions, the rate and equilibrium point of these reactions will be dependent upon the absorbing species concentration. eg the association of benzyl alcohol in carbon tetrachloride which is markedly dependent upon the concentration of benzyl alcohol.

   It is important to stress that strict adherance of data to Beer's Law is not essential for performing precise quantitative analysis provided adequate calibration graphs are plotted.

2.4.3 Physical Light Losses in the Sample Cell or Cuvette

A typical absorption cell (cuvette) containing a sample solution is depicted in Figure 5.

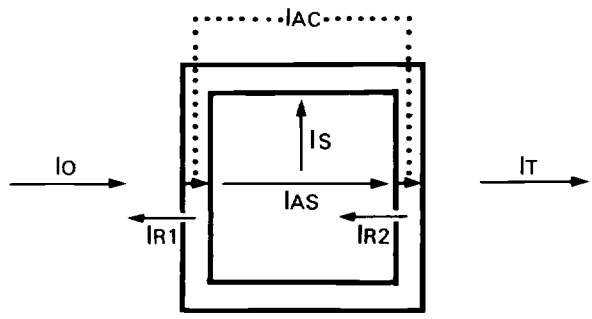


Figure 5 Depiction of physical light losses in the sample cuvette

From this Figure it can be seen that:

$$I_O = I_{R1} + I_{R2} + I_S + I_T + I_{AC} + I_{AS} \quad (6)$$

Where I_O = the intensity of the incident radiation*

I_{R1} and I_{R2} = Losses due to reflection, (typically 3–4%)

I_S = the intensity loss due to scattering of the incident radiation. For true homogenous solutions this term is negligible, but in the presence of colloidal matter (turbid samples) it can be significant.

I_T = the intensity of the transmitted radiation

I_{AC} = the intensity loss due to absorption of light by the optical windows of the absorption cell

I_{AS} = the intensity loss due to absorption of light by the solution in the cell.

For a given absorption cell (cuvette) and solvent in the absence of any turbidity, I_{R1} and I_{R2} should be constant and I_S should be negligible. The reason why matched cells tend to cost significantly more than unmatched cells is because it is difficult to ensure that the ratio $\frac{I_{AC}}{I_O}$ varies in an identical fashion with wavelength for the matched cells. A particular problem is matching silica cells in the ultraviolet below about 240 nm. The ratio $\frac{I_{AC}}{I_O}$ can become quite significant and increases with decreasing wavelength; I_{AC} is mainly caused by impurities in the silica. Good matched cells require the use of high grade silica, and also good quality control to ensure reproducible cell wall thickness and optical surface finish. When purchasing matched cells, users would be wise to ensure that the cells are matched for both absorbance and path length.

3 Instrumentation

3.1 Introduction

Figure 6 illustrates the main components of a system for measuring or comparing absorbances of solutions. A light source, a wavelength discriminator (eg monochromator or filter), an absorption cell or cuvette, a light detector and a readout system are required.

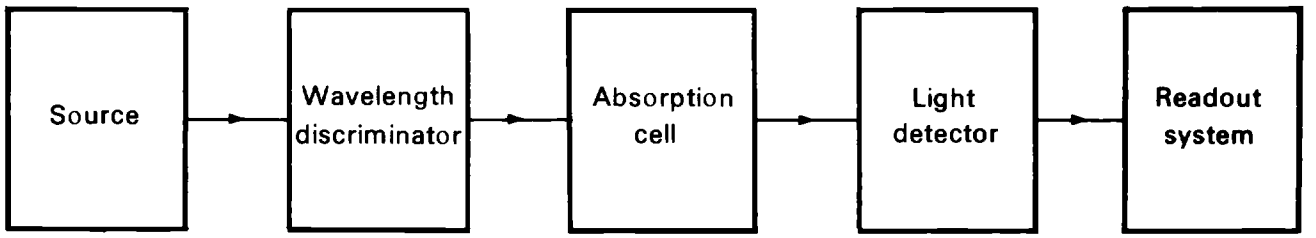


Figure 6 Typical lay-out of a photometer for measuring or comparing absorbances of solutions

Note: Colorimetric measurements will be designated as those where the radiation passing through the absorption cell comprises a relatively broad band of wavelengths isolated with a simple gelatin, coloured glass or an interference filter. In some instances the entire visible spectrum (ie daylight or diffused white light from a tungsten filament lamp) is used directly, (see section 3.2).

Spectrophotometric measurements will be designated as those where the radiation passing through the absorption cell is essentially monochromatic and derived from the use of a monochromator or in a few isolated cases from a good quality interference filter.

It should be noted that most instruments position the wavelength discriminator between the source and the absorption cell. This ensures that only the radiation of interest passes through the absorption cell. If all the source radiation (especially that

* Although the term 'intensity' is used, a more accurate description would be 'radiant power' or 'radiant flux'.

from a hydrogen or deuterium lamp) passes through the sample, prior to isolation of the radiation of interest, photodecomposition and/or fluorescence of the sample can sometimes occur.

3.2 **Visual Colorimetric Measurements**

Typical visual comparators are depicted in Figure 7.

3.2.1 *Light Source*

This is normally daylight or white light radiation from a tungsten filament lamp after passage through a diffuser.

3.2.2 *Wavelength Discriminator, Light Detector and Readout System.*

The human eye functions as the wavelength discriminator, light detector and readout system. Unfortunately the human eye, unlike photoelectric devices, cannot accurately estimate absolute intensities, differences in or the ratio of the intensities of two light beams. It is only capable of comparing the intensities at the same time. (ie a sample and an appropriate standard).

3.2.2.1 Advantages of the Human Eye as a Wavelength Discriminator and Light Detector

1. Relatively rapid analysis can be carried out once all the samples and standards are prepared.
2. Can be used under field conditions.

3.2.2.2 Disadvantages of the Human Eye as a Wavelength Discriminator and Light Detector

1. The sensitivity of the human eye is very dependent upon wavelength, it is most sensitive in the green and yellow regions of the spectrum and least sensitive in the blue-violet and red regions of the spectrum. The eye is not sensitive to radiation in the ultraviolet region of the spectrum, ie wavelengths below about 380–400 nm.
2. The average observer can only just detect a ± 3% change of intensity in the most sensitive yellow-green region. A typical photoelectric detector (see 3.4.4) can detect much smaller differences (typically better than ± 0·3%). Thus the precision and sensitivity of the human eye are considerably inferior to that of a photoelectric detector. If the minimum difference between two intensities in the yellow-green region that can be detected is much greater than ± 5% it would suggest that some degree of colour blindness in the individual concerned exists.
3. Visual and mental fatigue can be a problem with large numbers of samples.
4. It is impossible to plot a calibration graph, thus each sample must be compared with the appropriate standards.
5. Any naturally coloured or turbid samples will result in a significant positive bias in the results. This is because the non-specific absorption caused by the natural colour or turbidity in the samples will not be present in the standards and blanks. Interference effects from other substances reacting with reagents to form a coloured or turbid product can be a problem. For these reasons the technique is mainly limited to analyses of non-turbid samples of low colour intensity that have a relatively constant matrix, (eg potable waters), or for the analysis of determinands that can be readily separated from the sample (eg the distillation of ammonia, hydrogen cyanide or volatile phenols).

3.2.2.3 The Precision of the Human Eye as a Detector

The precision achievable with the human eye can be calculated using the Weber-Fechner Law which states that 'the smallest change of intensity that can be detected by the eye is a constant fraction of the total intensity (I_T) observed'.

$$\text{ie } \frac{\Delta I_T}{I_T} \approx 0{\cdot}03 \text{ (yellow-green region)} \qquad (7)$$

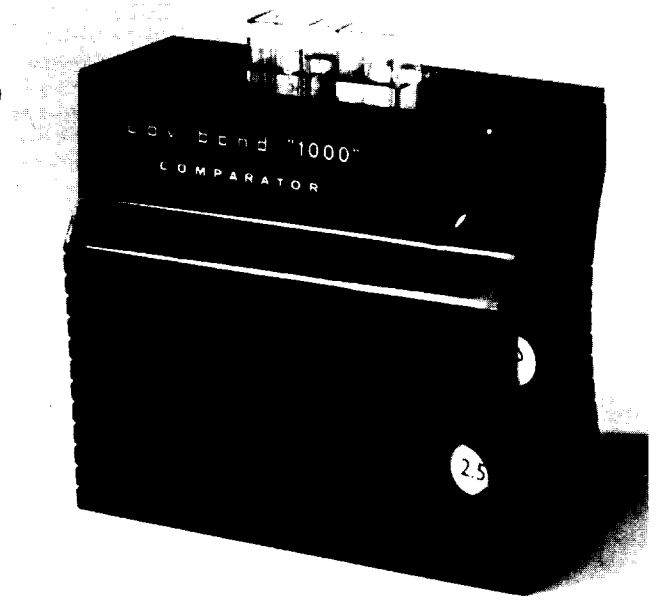


Figure 7a Visual Comparator for use with 13.5 mm Path Length Cells

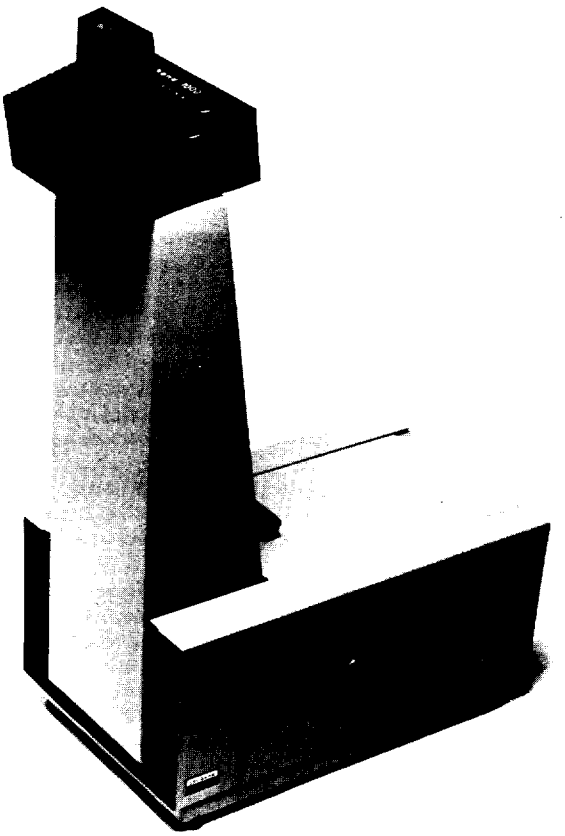
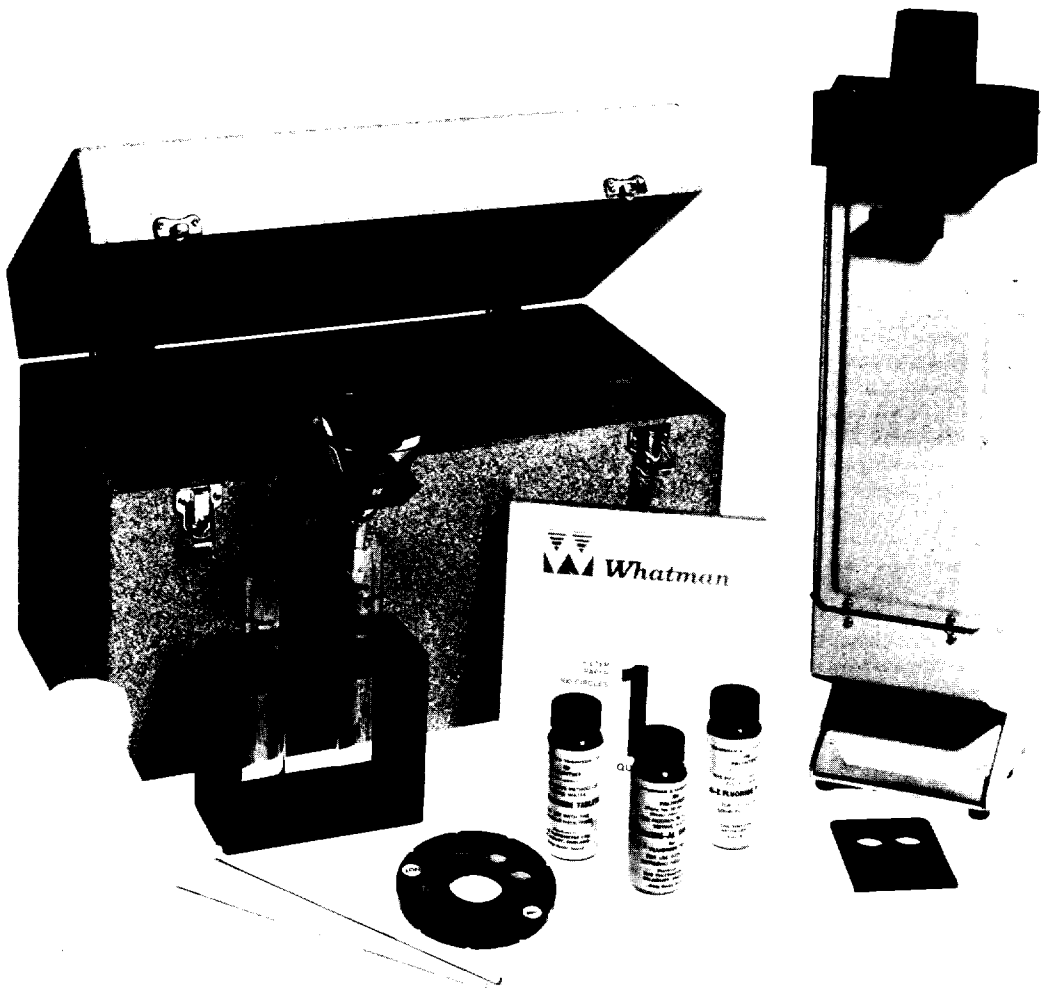


Figure 7b(i) Visual Comparator with Nesslerizer Attachment

Figure 7b(ii) Visual Comparator with Nesslerizer Attachment showing Nessler Tubes and Coloured Glass Filters



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Now from equation (3) Concentration α Absorbance = A

$$A = \log_{10}\frac{I_O}{I_T} = 0{\cdot}434\,(\log_e I_O - \log_e I_T) \tag{8}$$

$$\therefore \frac{dA}{dI_T} = -0{\cdot}434\frac{1}{I_T} \tag{9}$$

$$\therefore \Delta A = -0{\cdot}434\,\frac{\Delta I_T}{I_T} \tag{10}$$

Where Δ A = the error expressed as an absorbance which is proportional to the error expressed as a concentration of the determinand.

From equation (7) it can be seen that the right hand side of equation (10) is a constant. Thus the concentration error is effectively independent of the absolute absorbance of the solution. For instance if Δ A = 0·02

At A = 0·05, Relative error = 40%

At A = 1·00, Relative error = 2%

Hence the relative error can be minimized by working at high optical densities.

3.2.3 *The Absorption Cell*

Cuvettes with path lengths of 10–40 mm are normally used. However, when weakly coloured solutions are being compared, a longer path length is necessary for adequate precision. The typical absorption cell for this type of work is the 'Nessler Tube' which consists of an optically clear glass tube of uniform bore with a flat bottom and calibrated at one or more points to facilitate dilution to a given volume (typically 50 or 100 ml with a path length of 100–250 mm).

3.2.4 *Typical Visual Comparators*

A pocket-sized comparator that accepts 13·5 mm path length cuvettes is shown in Figure 7a. The same unit can be adapted to accept Nessler tubes and this mode of operation is depicted in Figure 7b. The two cuvettes or Nessler tubes are placed in a suitable holder and light (either daylight or diffused light from a tungsten filament lamp) is reflected from an evenly illuminated surface so that it passes through the tube contents and is then viewed. The holder prevents light from entering the sides of the tubes.

The colour of each sample can then be compared with that of suitable standards until the sample is estimated to have an absorbance falling between two standards. An interpolation is then made. In practice this laborious technique is seldom used, because of the requirement to prepare freshly a large number of standards for each batch of samples. To overcome this, a number of coloured glass filters which are mounted around the edge of a plastic disc can be purchased for most methods.[9]

The disc is mounted so that it can be rotated until the colour of the cuvette or Nessler tube containing the sample matches that of the cuvette or Nessler tube containing the blank solution in conjunction with the appropriate filter. The discs containing the coloured glass filters are designed to be used under closely controlled and specified reagent and reaction conditions. Each filter corresponds to a particular determinand concentration or amount. Some visual comparators have prisms to bring together the field of view from each tube, thus making visual comparison easier. Discs containing coloured glass filters are designed to be used under closely controlled and specified interest to the water chemist, eg ammonia, chlorine, iron, nitrate, nitrite, dissolved oxygen, phosphate, silica and colour. For some of the determinands, where a wide range of concentrations are often encountered, more than one disc is available. For instance one commercially available system[9] offers four discs each containing nine coloured glasses for the determination of ammonia. The discs cover the ranges 0·001–0·01, 0·01–0·026, 0·028–0·06 and 0·06–0·1 mg ammonia per tube. For a 50 ml volume contained in a standard Nessler Tube this is equivalent to a total range of 0·02–2 mg/litre ammonia.

3.3 **Simple Colorimeters**

A typical single beam colorimeter is depicted in Figure 8. The differences between single and double beam instruments are discussed in Section 3.4.5.

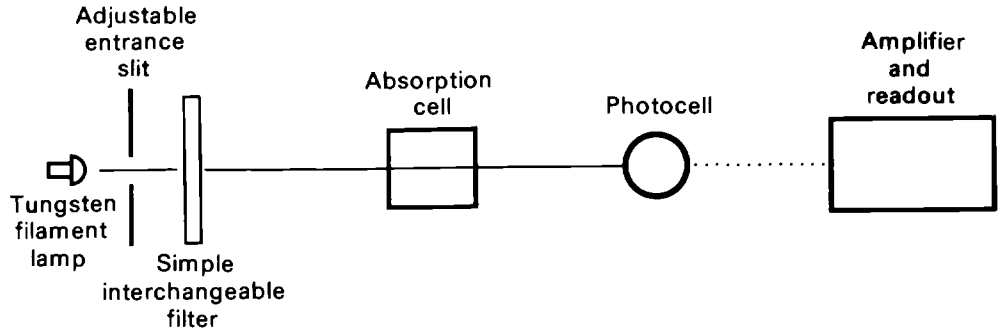

Figure 8 Simple single beam colorimeter

3.3.1 *Light Source*

This is normally a tungsten filament lamp with an adjustable diaphragm to control the amount of light reaching the detector. The shortest usable wavelength is about 325–350 nm.

3.3.2 *Wavelength Discriminator*

Most colorimeters are supplied with a set of interchangeable coloured glass filters with typical spectral bandpasses of 50–150 nm. Information concerning transmission characteristics of glass filters can be obtained from the following sources:

1. Kodak Filters for Scientific and Technical Uses. Kodak Ltd. ISBN 879850299 (Distributed by Patrick Stephens Ltd, Bar Hill, Cambridge CB3 8EL)
2. Precision Optical Instruments (Fulham) Ltd, 158 Fulham Palace Road, Hammersmith, London W6.
3. Ilford Ltd, Technical Service Dept, Basildon, Essex.

Interference filters[11], can also be used, these consist of multilayers of semi-aluminised coated transparent dielectric, transmitting over a small wavelength band typically 2–30 nm by constructive interference. Other wavelengths are rejected by destructive interference. Unfortunately a comprehensive set of these filters to adequately cover the spectral range 325–1,000 nm can cost more than an average monochromator. However, if the instrument is to be used for a limited number of determinands, the purchase of the requisite interference filters can be justified. The improved spectral purity and better matching of peak absorption wavelength with filter maximum transmission wavelength will result in both improved sensitivity and more linear calibration graphs, especially at higher absorbance readings. (See Figure 3).

On some instruments the filters are mounted between the absorption cell and the detector. This minimizes the exposure of the photocells to direct daylight whilst removing and replacing the cuvettes. However, this arrangement could result in photochemical decomposition of some samples.

3.3.3 *Detector*

Most modern instruments use photocells. A photocell consists of an evacuated glass or silica envelope which contains a photocathode and an anode. (Figure 9a). When exposed to light the photocathode liberates electrons which are attracted to an anode, maintained at a positive potential with respect to the photocathode. Some instruments employ two photocells; one for the 325–600 nm and another for the 600–1,000 nm spectral region.

3.3.4 *Readout System*

This can consist of a direct readout on a meter, galvanometer or digital display unit. Some units have additional facilities to monitor the output on a pen recorder. Both single and double beam systems (see section 3.4.5) are commercially available, the single beam instruments usually cost significantly less than an equivalent double beam instrument.

3.3.5 *Absorption Cell (or Cuvette) Compartment*

Most modern units will accept up to 5 or 10 cm path length glass cuvettes. Various other attachments (section 3.4.7) may be compatible with the instrument.

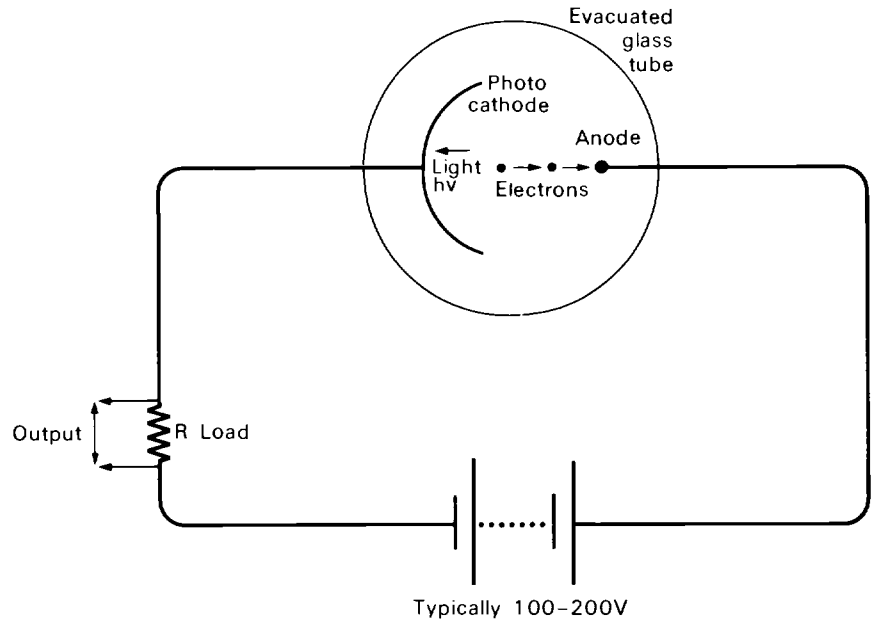

Figure 9a Typical phototube

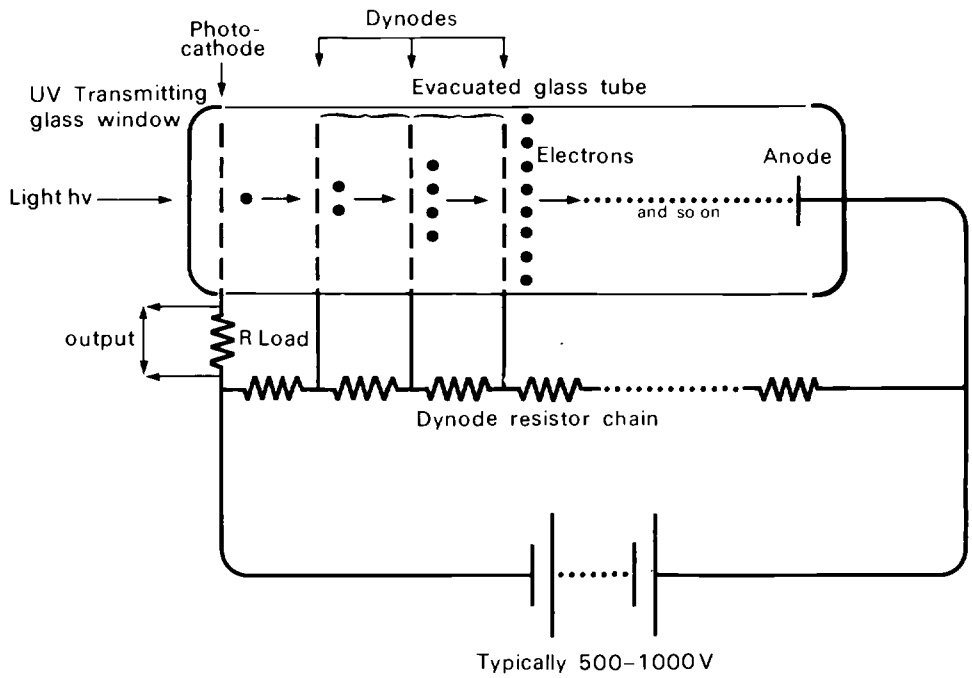

Figure 9b Typical photomultiplier

Note: Only 3 dynodes shown. Typical photomultipliers have 8–12 dynodes

3.4 **Spectrophotometers incorporating Monochromators**

Typical single and double beam spectrophotometers are depicted in Figure 10.

3.4.1 *Light Sources*

Almost all instruments utilize a tungsten (or tungsten halide) filament lamp for the spectral region 325–900 nm and a hydrogen (or deuterium) arc lamp for the 190–325 nm region. Some units will automatically change source lamps at about 325 nm when automatic wavelength scanning is used.

In theory a xenon arc lamp could be used as a single source for the wavelength range 190–900 nm. However, stray light is a particular problem in the ultraviolet region below about 240 nm because of the intense emission in the visible region of the spectrum from these lamps. In addition the stability of xenon arc lamps is somewhat inferior to tungsten or hydrogen lamps. To the best of the author's knowledge no commercial unit utilizes a xenon arc lamp source.

3.4.2 *Wavelength Discriminator*

A monochromator is universally used. The monochromator isolates the required spectral region from the light source.[11]

3.4.2.1 Prism Monochromators

Silica prism monochromators used to be popular but have the disadvantage that the optical dispersion of a prism varies with wavelength, so that resolution for a fixed slit width setting decreases markedly with increasing wavelength. Thus a prism will have good resolution below 250 nm but relatively poor resolution above 400 nm. To the best of the author's knowledge, prism monochromators are no longer incorporated into commercial solution spectrophotometers.

3.4.2.2 Diffraction Grating Monochromators

Diffraction grating monochromators are universally used in modern instruments and have the advantage that the optical dispersion of a grating is virtually independent of the wavelength; thus resulting in a linear wavelength readout and a fixed resolution throughout the spectrum for a given slit width setting. Modern blazed diffraction gratings where the reflecting surface of each groove on the grating lies at a fixed angle to the grating surface concentrate about 60–80% of the incident light energy into the first order spectrum. One grating, normally blazed at about 300 nm, can satisfactorily be used to cover the wavelength range 190–900 nm. However, some higher priced instruments are fitted with two gratings; one is blazed at about 250 nm for use in the ultraviolet region of the spectrum, whilst the other is blazed at about 500 nm for use in the visible and near infrared region of the spectrum. The gratings are automatically interchanged at a wavelength of about 400 nm.

Adjustable slit widths are situated along the optical path of the monochromator so that the optimum compromize may be made between spectral purity and energy reaching the photodetector. In practice spectral bandpasses of 1–4 nm are normally used for the vast majority of routine spectrophotometric analyses of natural water samples. In order to measure high absorbances (>1·0) with good precision, it is essential that the stray light reaching the detector is minimal. Stray light is radiation at wavelengths other than the selected spectral bandpass. For example, if the stray light reaching the detector corresponds to 0·2% of the incident light intensity, at a given wavelength, reaching the detector, this would normally result in a 4% negative error in the measurement of a true absorbance of 2·00.

Stray light can be minimized by using high quality gratings. Recently, relatively inexpensive holographic gratings have been developed and these exhibit very low stray light levels.[12] Many modern instruments can reliably monitor absorbances around 2·50.

Some higher priced instruments actually use a double monochromator (two monochromators mounted in series). Thus if each monochromator has a stray light figure at a given wavelength of 0·1%, the combination will have a stray light figure in the region of 10^{-4}%. These instruments can reliably monitor absorbances around 3·50.

For routine water analysis, stray light should not represent a serious problem on any modern instrument because methods involving measurements of absorbances in excess of 1·00 are seldom encountered. However, it is a wise precaution to test regularly for stray light especially at the wavelength extremes because of instrument malfunction (see section 5.6.3).

For improved lifetime of optical components, especially in corrosive atmospheres, the optical surfaces of many modern instruments are now coated with silicon dioxide.[12]

3.4.3 *Detector*

Photomultipliers are invariably used, the main requirements being an adequate response over the spectral range 190–900 nm and low dark current. A photomultiplier (Figure 9b) is similar to a photocell (see section 3.3.3) except that a series of dynodes (electron multipliers) are positioned between the photo-cathode and the anode. An electron liberated from the photocathode by a light photon strikes the first dynode and liberates more electrons which then strike the second dynode liberating further electrons and so on. A typical nine stage photomultiplier can exhibit a significant output current for a very low incident light intensity. Some older instruments are fitted with photomultipliers that will not respond above 700–750 nm, however, with recent

developments in photocathode materials, almost all modern instruments will function up to 900 nm. Photomultipliers are easily damaged if exposed to excessive light intensities. Thus elaborate precautions are taken in the design of the instrument so that when the cell compartment is uncovered (eg removing or replacing cuvettes), daylight does not reach the photomultiplier photocathode when the high tension voltage is applied to the dynodes.

3.4.4 *Readout Systems*

Most modern spectrophotometers have a digital display unit and many have an associated pen recorder, this is especially useful for automatic plotting of absorption spectra. Some older instruments use a manually operated circular absorbance/transmittance scale which is rotated until a null point is observed on a meter whilst others use a simple meter to display the actual absorbance/transmittance. The advantage of a digital display is that operator interpretation is obviously more reliable and accurate than with an analogue meter or scale. Another advantage is that assuming a linear calibration graph, direct concentration readout can be obtained by adjustment of a sensitivity (ie scale expansion or contraction) control.

For the automatic operation most modern units can be operated with a suitable printer that can also identify samples, standards and blanks.

3.4.5 *Single and Double Beam Systems*

The output signal level of a single beam instrument (Figure 10a) will vary as the source intensity varies whilst that of a double beam instrument is effectively independent of the source intensity. In this latter instrument the light beam leaving the monochromator (see Figures 10b and 10c) is split into two; one beam is passed through the sample cell or cuvette, whilst the other passes through a reference cell or cuvette. (Matched cells or cuvettes should obviously be used). The two beams are then detected either using two photomultipliers (Figure 10b) or recombined using a mechanical chopping device and detected using a single photomultiplier. (Figure 10c). The mechanical chopper allows the amplifier to resolve the signals from the two beams. The ratio of the intensities of the two beams is taken and will be independent of any variation in light intensity. The latter method utilising a single photomultiplier in conjunction with a mechanical chopper (Figure 10c) is invariably used in commercial instruments. This is because it is very difficult to obtain two photomultipliers with identical photometric sensitivity at all wavelengths. If the photometric sensitivity varies with wavelength it is then very

Table 4 Comparison of Single and Double Beam Instruments

Single Beam Instrument	
Advantages:	
(1)	Relatively simple optics.
(2)	Less expensive than comparable double beam instruments.
(3)	Very high photometric accuracy is possible.
Disadvantages:	
(1)	Longer source warm-up time is required.
(2)	Impossible to automatically plot absorption spectra.
Double Beam Instrument	
Advantages:	
(1)	Short source warm-up time required.
(2)	No significant baseline drift.
(3)	Automatic plotting of absorption spectra is relatively easy.
Disadvantages:	
(1)	More expensive than a comparable single beam instrument.
(2)	Luminous throughput may be noticeably poorer in the ultraviolet region below 220 nm, this is caused by attenuation of the light beam by the additional optical components required.
(3)	The cuvettes or cells in the reference and sample beams should be optically matched.

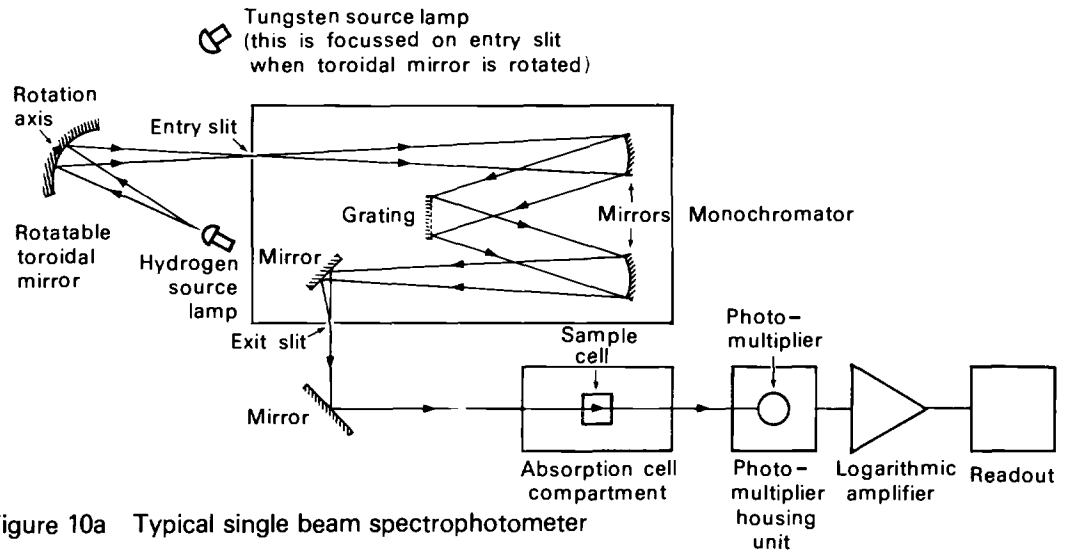

Figure 10a Typical single beam spectrophotometer

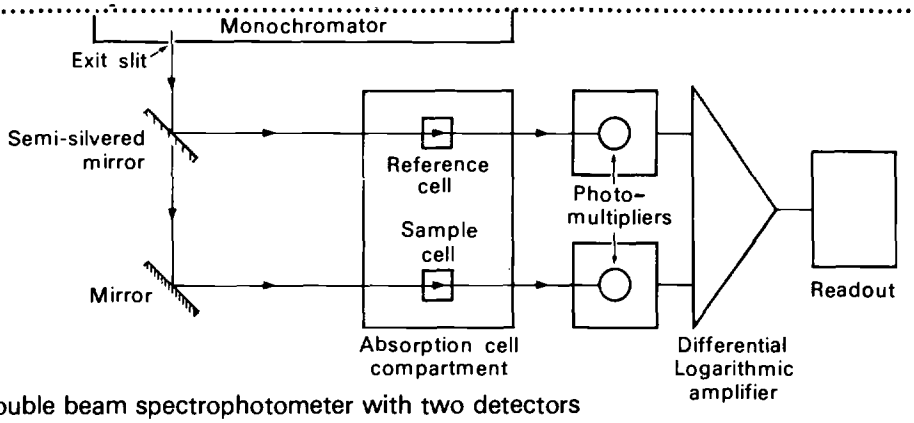

Figure 10b Double beam spectrophotometer with two detectors

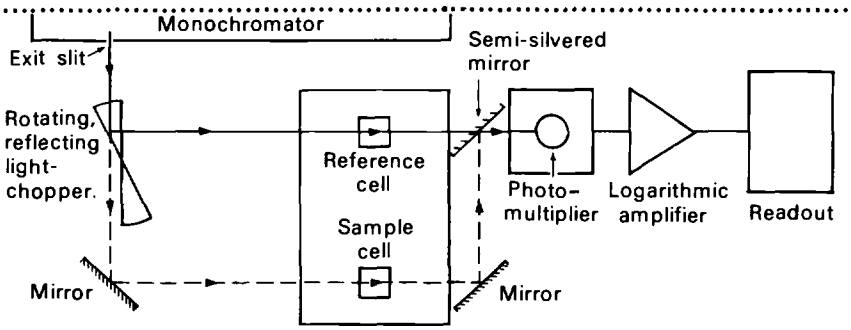

Figure 10c Double beam spectrophotometer with single detector

Note: Sources and monochromator are common to all units.

difficult to automatically plot accurate absorption spectra. The advantages and disadvantages of single and double beam operation are summarised in Table 4.

Most commercially available scanning ultraviolet-visible spectrophotometers are double beam instruments and one important reason for this is that absorption spectra (ie the trace showing absorbance versus wavelength) can be automatically plotted by mechanically rotating the wavelength drive mechanism at a constant speed and monitoring the output on a pen recorder. Although most double beam instruments can be operated in a single beam mode, the disadvantages (1) and (2) given above will still apply.

3.4.6 *Microprocessors*

Microprocessors are now being incorporated into some ultraviolet-visible spectrophotometers. These devices act on input data and also process output data upon instructions from a keyboard. For example, the wavelength, the spectral bandpass, the scale expansion, the wavelength scan speed, the recorder chart speed and the initial and final wavelength settings can simply be entered through the keyboard. The microprocessor will also automatically compute a calibration graph, even if the absorbing

species under examination exhibit significant deviations from Beer's Law,* the final displayed readout can be in any desired concentration units. The incorporation of microprocessors can also make interfacing to computers and other peripherals relatively easy.

3.4.7 *Absorption Cell (or Cuvette) Compartment*

Most units will accept cells with path lengths up to 5 or 10 cm. Microcells with total cell volumes of about 10–100 µl can also be used. These are useful when only limited volumes of sample are available. Automatic samplers in conjunction with flow through cells can also be used. In this mode of operation the sample absorbances at a fixed wavelength can be recorded and some instruments will automatically perform a wavelength scan and plot the absorption spectrum for each sample. Thermostatic control of the cuvette contents is also possible on many instruments, this is especially important when studying reaction kinetics.

Various accessories for applications such as gel scanning, diffuse reflectance measurements, monitoring the effluent of a high pressure liquid chromatograph (see section 3.6), nephelometry etc are available.

It is very important to keep the absorption cell compartment clean and immediately mop up any spillages.

3.5 Colorimeters for Automatic Analysis Equipment

It is thought that for most laboratories engaged in routine water analysis, more absorption measurements are made using these devices than any other type of photometer. A typical instrument is depicted in Figure 11. These units are normally double beam in order to ensure long term stability and minimize baseline drift (see section 3.4.5). Most units contain two detectors (Figure 10B) and incorporate interference filters for the reasons given in section 3.3.2. The cuvette is a flow through cell with a typical cell volume of 50–500 µl and a path length of 5–50 mm. By passing a blank sample stream (with the colour reagent replaced by water) through the reference cell, automatic correction for sample colour and/or turbidity can be obtained, (see section 6.1).

3.6 Detectors for High Pressure Liquid Chromatography (HPLC)

The vast majority of substances separated by HPLC do not absorb in the visible region of the spectrum, however, many absorb in the ultraviolet region at wavelengths around 250 nm. Fixed wavelength HPLC detectors are constructed on a similar basis to the colorimeters depicted in Figure 11, the main difference is that the source is a mercury lamp and the wavelength discriminator is an interference filter that will transmit the very intense 253·7 nm mercury line. The cuvette is a flow-through cell with silica windows. In order to minimize loss of resolution the cell volume must be kept as small as possible. Typical cell volumes are 10–100 µl whilst typical path lengths are 5–20 mm. Thus the cross-sectional area of the flow cell is very small and point light sources are required for high luminous throughput.

Although many substances absorb at 253·7 nm, a large number of substances either only absorb at other (usually) lower wavelengths or only weakly absorb at 253·7 nm, the wavelength of maximum absorption being at another wavelength. Thus a variable wavelength detector is far more versatile for the detection of a wide range of compounds.[10] Although a standard ultraviolet visible spectrophotometer can be used, it is not very cost effective if it is just dedicated to HPLC work. However, there are a number of commercially available, less expensive, dedicated variable wavelength (190–350 nm) HPLC detectors. These are miniaturized forms of standard spectrophotometers that are specifically designed for HPLC work, the cell compartment is specifically designed to accept flow through cells. In most of these units the tungsten light source is omitted as few substances of interest absorb in the visible region of the spectrum.

4 Safety Considerations

The instrument manufacturer's handbook should be read carefully and any safety procedures given should be adhered to strictly. Some general precautions are given below.

* The important criterion is that the calibration graph is reproducible.

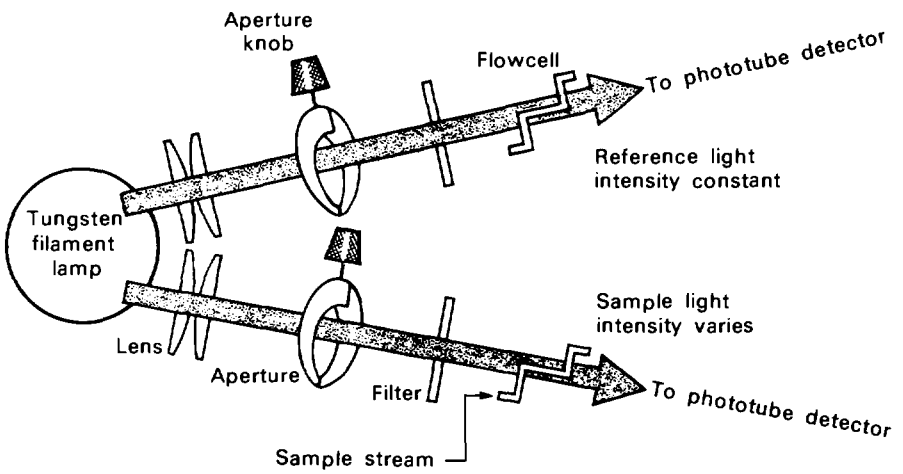

Figure 11 An automatic analysis colorimeter layout

Note: The output from the two detectors is passed to a differential logarithmic amplifier and then to a control module assembly and readout system.

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4.1 Ultraviolet Radiation

Never directly view the hydrogen (or deuterium) arc lamp as this emits strongly in the ultraviolet region of the spectrum. For almost all instruments it is not possible to view this lamp without removal of a safety cover. However, if a hydrogen (or deuterium) lamp has to be replaced, the new lamp usually requires positional adjustment for maximum light throughput. When this adjustment is carried out it is essential to wear ultraviolet absorbing glasses. Ordinary crown glass spectacles do not give full protection against near ultraviolet radiation.

4.2 Organic Solvents

Many methods require the use of organic solvents (eg chloroform, ethyl acetate etc). Organic solvents should only be manipulated in a well ventilated area (eg a fume cupboard or fume hood). Certain toxic solvents (eg benzene) can be absorbed through intact skin whilst almost all solvents can cause dermatitis. The fire risk from the use of organic solvents should always be considered when devising safe operating procedures.

5 Performance, Operating Characteristics and Method Development

This section gives an indication of the analytical performance data, a general description of the main operating parameters, their optimization, and a typical method development technique.

5.1 Sensitivity for 1% Absorption

This is defined as the concentration of the determinand in a solution that results in an absorbance of 0·0044 under the operating conditions used. (See section 2.4). This is equivalent to a 1% decrease in the transmitted radiation. It can be readily calculated by determining the concentration of the analyte that will give an absorbance value of 0·1 and dividing that concentration by 22·7. This figure corresponds to the analytical sensitivity but it does not directly relate to the limit of detection as it can give no indication of the signal to noise ratio.

5.2 Limit of Detection

Until fairly recently the limit of detection has been defined as that concentration of the analyte that gives an absorption signal equivalent to twice the within-batch standard deviation of the noise fluctuations of the blank signal (σ_w). The limit of detection, unlike the sensitivity for 1% absorption, is very dependent on the instrumental stability. In practice, a more realistic definition of limit of detection (as recommended by the Standing Committee of Analysts) when an analytical result is obtained by subtracting a single blank result from a single sample result, is given by $4 \cdot 65\sigma_w$[13], where σ_w is the within-batch standard deviation of a single blank determination expressed as a concentration. This limit of detection corresponds to that of a determinand concentration for which there is only a 5% probability that the result will be less than half of this limit of detection value. It is important to stress that even then the limit of detec-

tion refers to a pure aqueous solution of the analyte; if the samples contain appreciable quantities of matrix elements (eg estuary samples), the limit of detection can be significantly worse.

5.3 Precision and Accuracy of Measurement

The precision of measurement, defined as the within-batch relative standard deviation of a number of measurements on the same solution, should be better than 0·5% at concentrations greater than twenty times the limit of detection. The accuracy of measurement can be defined as the total error of a result[17], that is the combined systematic and random error of results.

If a solution containing an unknown amount of the determinand is analysed on a number of different days the relative standard deviation for a determinand concentration greater than twenty times the limit of detection is typically 1–3%. It is important to stress that high precision does not imply high accuracy. Many interlaboratory trials have shown that many laboratories obtain very precise but highly inaccurate results.[15] (Significant systematic errors but insignificant random errors). Control solutions should be prepared on a regular basis; these solutions should contain determinand levels in the range of typical samples. Some of the control solutions should be prepared by spiking previously analysed samples with the determinand, this procedure will help to indicate whether interelement effects causing a change in analytical sensitivity are significant for typical samples.

The variation in the precision of a measurement with absolute absorbance is difficult to quantify[6,8,14] but it is generally agreed that optimum precision is obtained over the absorbance range 0·2–0·8.

5.4 Selection of Operating Parameters

5.4.1 *Wavelength of Measurement*

In the case of a filter instrument the operator has little choice other than to utilize the available filter. However, if the calibration graph is markedly non-linear and a large number of measurements at a particular wavelength are used, the purchase of a suitable interference filter to replace a simple coloured glass filter should be considered (see section 3.3.2). In the case of a spectrophotometer the wavelength of maximum absorption is normally selected. However for some methods, a wavelength slightly displaced from the wavelength of maximum absorption is used in order to minimize interelement effects (see section 6.2).

5.4.2 *Spectral Bandpass*

In the case of a filter instrument the operator has little choice other than to utilize the available filter. In the case of a spectrophotometer a low spectral bandpass, corresponding to a narrow slit width, will allow resolution of complex absorption bands. However, the narrow slit width will limit the amount of light reaching the detector and consequently result in a degradation of the signal to noise ratio. If a very wide slit width (large spectral bandpass) is used, this could result in deviations from Beer's Law (see section 2.4.2). For the vast majority of analytical work on a modern spectrophotometer a spectral bandpass of 1–4 nm is thought to be perfectly satisfactory.

5.4.3 *Path Length of the Absorption Cell (Cuvette)*

Ideally the path length of the cell should be chosen so that the majority of samples give a final absolute absorbance within the range 0·2–0·8. However, many determinands (eg cyanide, phenol) are normally present at levels close to, or below the detection limit. For these cases the maximum allowable path length cell (typically 4–5 cm) should be used. It should be noted that for ultra-violet region measurements below about 240 nm, there is not much advantage in using path lengths greater than 1 cm. This is because the detection limit is usually governed by variation in the absorption of impurities present in the measured solutions.

5.4.4 *Construction of Calibration Graph*

A calibration graph should be regularly plotted. Often the time for optimum colour development significantly increases at low determinand concentrations, for this reason a standard containing the determinand at a concentration of four or five times the expected detection limit should always be run. It is essential to plot a calibration graph whenever a new batch of colour forming reagent is used (see section 5.5.1).

5.4.5 *Temperature of Measurement*

The temperature of the solutions is not thought to be very critical over the range 18–25°C for most methods, but variations can be significant for some methods, especially at low determinand concentrations. For this reason standards and samples should be prepared and stored under identical conditions so that temperature variations in the final measured solutions are not significant.

5.5 Development of an Analytical Method

A few hints and suggestions are now given for the development of a new analytical method. For the following example it is assumed that the determinand reacts with a colour forming reagent to form a light absorbing species. In this context the term colour denotes any wavelength from 250–900 nm. There are very few routine methods that utilize wavelengths below 250 nm for this type of system.

5.5.1 *Colour Forming Reagent*

The sensitivity can significantly vary with the batch (and occasionally with storage) of the reagent. (eg diphenylcarbazide for the determination of chromium).[16] Sometimes this variation can be minimized by purifying the reagent (eg by recrystallization). Variations in the residual determinand concentration in the reagent can also occur, thus when developing a new method it is wise to test various batches (preferably from different manufacturers) of the colour forming reagent.

5.5.2 *Absorption Spectra*

Plot the absorption spectra of the colour forming reagent and the reaction product of the determinand with excess of the colour forming reagent at various pH's and select both a wavelength of measurement and a pH. Ideally the reagent should not significantly absorb at the measurement wavelength but the determinand—colour reagent complex should exhibit significant absorption. It is possible to elucidate the formula of the complex by the method of continuous variations[8] or using the molar ratio method.[8] These involve plotting the variation of absorbance at various determinand: colour forming reagent concentration ratios.

5.5.3 *Optimum Colour Forming Reagent Concentration*

A plot of absorbance versus colour forming reagent concentration should be made at the highest and lowest determinand concentration of the proposed calibration graph and a suitable reagent concentration should be selected, such that the absorbance is not dependent on the reagent concentration.

5.5.4 *Interference Tests*

Interference tests for the common matrix elements and any other substance likely to be encountered in typical samples should be made.[13] The concentrations used should represent the maximum level of each interfering element likely to be found in the samples. If significant interelement effects are found, a method should be sought to over come this (see Chapter 6).

5.5.5 *Time of Colour Development*

The time of colour development and the stability of the colour should be tested both at high and low determinand concentrations. The effect of temperature upon the time of colour development should also be tested. It must be stressed that some methods require a significantly longer time of colour development at low determinand concentrations.

5.5.6 *Testing of Final Method*

The final proposed method should then be tested preferably by more than one laboratory and the performance characteristics determined as outlined by the Department of the Environment, Standing Committee of Analysts.[17] The following information is required:

1. Substance determined.
2. Types of samples (eg potable waters, river waters etc).
3. Basis of method (a brief description of the method should be given).
4. Range of application (the concentration range of the method should be given).

5. Calibration graph shape (the linear range of the calibration graph should be given).
6. Total standard deviation (This should be determined for a number of solutions on at least five days. The solutions should contain a range of concentrations of the determinand and should include some spiked typical samples. There should be nine or more degrees of freedom for each standard deviation measurement).
7. Limit of detection (4·65 times the within batch standard deviation of the blank).[13] This corresponds to the subtraction of a single blank result from a single sample result (see section 5.2).
8. Sensitivity (mg/litre for a stated absorbance).
9. Bias.
10. Interferences (A list of the interference effects observed at zero determinand and one or two other determinand concentrations should be given).
11. Time required for analysis. (The total analytical and operator times for a batch of typical samples should be given).

Finally the method should be written up giving comprehensive details of all necessary operating procedures and pointing out any safety hazards. It is recommended that the format outlined by the Department of the Environment should be adhered to.[16,17]

5.6 **Routine Maintenance**

5.6.1 *General Maintenance*

Spectrophotometers and colorimeters require little maintenance. The instruments ideally should be positioned in a clean dry area and should not be placed in a corrosive environment. This is especially important for a spectrophotometer as the replacement of corroded optical parts can prove very expensive. The instrument dust cover should be replaced when the instrument is switched off.

The cell compartment should be kept scrupulously clean and any spillages should be mopped up immediately.

5.6.2 *Photometric Checks*

The cells should be cleaned regularly by soaking in laboratory detergent and then thoroughly rinsed with tap water and finally deionized water. At wavelengths below 240 nm apparently clean cells can often exhibit spurious absorption bands which can be caused by fingerprints, trace of grease, oil or other substances on the silica surface. An adherent deposit on internal surfaces can be removed by partially filling the cuvette with wet paper pulp and shaking vigorously. Paper pulp can be prepared by agitating a mixture of shredded soft filter paper and hydrochloric acid (36% m/m). Matched cells should be checked for cleanliness prior to each run by running a wavelength scan with each cell filled with deionized water. A straight line trace should be observed over the range 200–700 nm with a maximum deviation for a good spectrophotometer of less than ± 0·02 Absorbance Units.

Occasionally it is worthwhile to check the wavelength accuracy using a holmium or didymium glass filter. Most manufacturers are able to supply these filters. Holmium exhibits well defined absorption bands[14] with useful peaks at 241·5 ± 0·2 nm, 279·4 ± 0·3 nm, 287·5 ± 0·35 nm, 333·7 ± 0·55 nm, 360·9 ± 0·75 nm, 418·4 ± 1·1 nm, 453·2 ± 1·4 nm, 536·2 ± 2·3 nm and 637·5 ± 3·8 nm, whilst didymium (a neodymium-praesodymium mixture) exhibits slightly broader absorption bands[14] at 573 ± 3 nm, 586 ± 3 nm, 685 ± 4·5 nm, 741 ± 5·5 nm, and 803 ± 6·3 nm. It is a good idea to actually run a wavelength scan if possible and compare the trace with the previously obtained trace and if significant loss of wavelength accuracy or reduction in the resolution of the absorption bands is observed, the service engineer should be summoned. In fact, an annual service visit is worth considering if the spectrophotometer work load is significant. This should ensure clean and accurately aligned sources and optical components. The accuracy of the absolute absorbance readings can be tested by measuring the absorption of known path length cells containing accurately prepared solutions of acidified potassium dichromate. The absorption coefficient E (1%, 1 cm) (see section 2.4.1) of 0·006% m/V potassium dichromate in 0·04% V/V sulphuric acid (98% m/m) is given as follows:

Wavelength (nm)	235	257	313	350
Max or Min	min	max	min	max
E (1%, 1 cm)	125	145	49	107

The above data are reproduced with permission from Reference 14.

Alternatively neutral thin-film metal coatings on fused silica blanks can be purchased from the National Physical Laboratory, Teddington, Middlesex TW11 0LR. The coatings cover a range of absorbance from 0·1 to 4·0 and any specified wavelength from 200–1,000 nm. It is a good idea to keep spare hydrogen and tungsten light sources as these have an annoying habit of burning out at the start of a run when the results are urgently required. The shelf life of modern hydrogen lamps should be in excess of three years. For emergency situations it is worth remembering that some atomic absorption manufacturers offer a cell holder accessory that can be clamped to the top of a standard burner. This allows spectrophotometric measurements to be obtained on atomic absorption instruments. For measurements at wavelengths below 350 nm a hydrogen or deuterium hollow-cathode lamp source can be used. The lamp should run at a low current and a spectral bandpass of 1–2 nm should be used. This will minimize photo-chemical decomposition of the sample (see section 3.1). For wavelengths above 350 nm an iron or tungsten hollow cathode lamp, can be used as these lamps emit a very large number of atomic lines over the wavelength range 350–900 nm. A low operating current and a wide spectral bandpass should be used for the reasons given.

5.6.3 *Stray Light Checks*

The stray light figures for a modern spectrophotometer for typical water analysis should be less than 0·25%. Many instruments quote figures of less than 0·1%. The stray light figure can be calculated by placing a solution that completely absorbs at the wavelength of interest, but transmits at most other wavelengths. Table 5 lists three solutions that can be used.

Table 5 Solutions for Testing Stray Light[18]

Note: These solutions completely absorb (absorbance >4) at the indicated wavelength in a 1 cm path length cell but transmit at other wavelengths. A reference blank of deionized water should be used to set the zero absorbance level.

Wavelength (nm)	Substance	Concentration g/litre	Light Source
220	NaI	10	H_2 or D_2 arc
370	K_2CrO_4	0·25	W Lamp
	KOH	2·8	
680	$CuSO_4$	350	W Lamp

When the absorbance of a cell containing these solutions is measured at the indicated wavelength, a reading in excess of 2·6 should be obtained if the stray light figure is below 0·25%. When comparing stray light figures for different instruments it is important that the method of testing, the wavelength of measurement and the light source should be identical.

Note: Some older spectrophotometers will not read above an indicated absorbance of 2·000. The unit should then be operated in the single beam mode and the zero absorbance should be set with a wire gauze, which exhibits an absorbance of 1·0, placed in the sample beam. The gauze should then be removed and the appropriate absorbing solution inserted in place of the deionized water blank. A reading of greater than 1·6 should be observed if the stray light figure is below 0·25%.

If the stray light figure is significantly higher than the manufacturers quoted figure a service visit should be arranged.

6 Interference Effects

6.1 Some Common Types of Interference

Spectrophotometric methods are often subject to various interference effects. Some examples of common types of interference are listed below:

1. Other components in the sample absorb at the wavelength of interest.
2. The sample is turbid.
3. Other components in the sample also react with the added reagents to form species that absorb at the wavelength of interest.
4. Other components react with the added reagent(s), not to cause absorption at the wavelength of interest, but to reduce the amount of reagent available to react with the determinand.
5. Other components in the sample react with the determinand and prevent quantitative reaction of the determinand with the added colour forming reagent.

6.2 Methods of Minimizing Interference Effects

Assuming that the determinand undergoes a reaction with a colour forming reagent, the effect of natural absorption or turbidity of the sample can often be compensated by running a sample blank with each sample. These blanks are prepared under identical conditions to the real samples except that the colour forming reagent is replaced with an equal volume of deionized water. The absorbance reading from the sample blank solution is then subtracted from the corresponding sample reading. It should be noted that if the sample and reference cuvettes are not exactly matched, a further correction is necessary to allow for this non-matching. Interferences from reactions of the colour forming reagent with other matrix components to form light absorbing species (see section 6.1.3) are more difficult to overcome and various methods (A–D) given below have been successfully used. Method E, also given below, is normally used to overcome reaction of metallic determinands with other sample components.

A. The Use of Complexing Reagents

The addition of complexing reagents that will specifically react with the interfering species in order to prevent reaction with the colour forming reagent. Obviously the complexing reagent should not affect the determinand colour reagent reaction. Examples of commonly used complexing reagents are ethylenediaminetetraacetic acid disodium salt, sodium citrate, sodium dihydrogen monophosphate, potassium cyanide.

B. Wavelength of Measurement

Measurement at a wavelength displaced from that corresponding to maximum absorption.

It is sometimes possible to minimize interference effects by sacrificing sensitivity. The interfering substance may exhibit negligible absorption at a wavelength where the determinand colour forming reagent absorbing species still exhibits appreciable absorption.

C. Adjustment of the pH

It may be possible to select a pH where the interelement effect is insignificant. Again as in B. this may lead to a loss in sensitivity.

D. Selective Extraction of the Determinand or Interfering Species

It may be possible to extract the determinand selectively, the final determinand reaction product or the interfering species from the sample solution. If the extract contains the determinand, a useful increase in sensitivity can be observed by using a smaller volume of extraction solvent than aqueous sample.

E. Pre-treatment of the Sample

It is essential that the determinand must be present in the sample in a form capable of reacting with the added colour forming reagents. For example many trace metals naturally present in a river or a sewage works final effluent exist in a complexed form associated with naturally occurring organic species. (See section 6.1.5). Pretreatment is required in order to release the metals so that they can quantitatively react with the desired colour forming reagents. A common

pretreatment is digestion with nitric-sulphuric acids until sulphuric acid fumes are observed. The sample is then carefully diluted with water and adjusted to a suitable pH.[19-22]

A good example of procedures A. C. D. and E. is in the determination of lead using dithizone.[22] The sample is digested with nitric-sulphuric acid to destroy any organic matter that could prevent the lead reacting with the dithizone and/or result in a light absorption at the wavelength of interest in the final measured solution. The digested sample is then made strongly alkaline in the presence of potassium cyanide and the lead dithizone complex extracted into chloroform. Lead, unlike most other toxic metals, does not form a stable complex cyanide and at pH 12, it can then be selectively extracted as its dithizone complex into chloroform. Another example is one of the DOE-SCA recommended methods for arsenic[21] where non-saline samples are digested with nitric-sulphuric acid, and saline samples are irradiated with intense ultra-violet light in order to destroy organic matter and ensure that the arsenic is present in a suitable form for the next stage of the procedure. This involves reducing the arsenic to arsine using sodium borohydride, the liberated arsine is collected in an iodine-potassium iodide solution which converts it to arsenate. This is then reacted with ammonium molybdate and reduced with ascorbic acid to form an arseno-molybdenum blue complex.

It is impossible to deal comprehensively with interelement effects in this essay review, but it should be appreciated that unsuspected interelement effects are probably the biggest drawback of many colorimetric methods. The effect is usually far more significant for low determinand concentrations (eg lead and cadmium in natural waters) than for relatively high determinand concentrations (eg chloride or total hardness in natural waters). For instance in the DOE-SCA recommended method for manganese[19] the presence of 5 mg/litre iron (III) was found to result in a +0·030 mg/litre bias in the manganese result at manganese concentrations of 0·000 and 0·500 mg/litre. Whilst the relative error at the 0·500 mg/litre level of +6% is not highly significant in routine water analysis, the relative error (+500%) at the quoted manganese detection limit of 0·005 mg/litre is highly significant. This is one of the reasons for the popularity of the atomic absorption technique for the routine determination of trace metals as the bias effect, described above, is not observed when the background corrected atomic absorption technique is used.[7]

7 Applications

It is impossible to deal with the very large number of applications of solution spectrophotometry in this essay review. Some indication of the very large number of available methods for each determinand is given in a paper by Pittwell[30] where 25 methods for the spectrophotometric determination of copper are compared and contrasted. All that can be done is to give the reader some indication of the wide variety of possible applications. Table 6 gives details of some references to various aspects and applications of the technique.

Table 6 Index of References to Various Applications and Aspects of Solution Spectrophotometry

Accuracy and Precision of Measurements	13, 17
Applications—Inorganic	22–30, 32
Organic	22, 24, 27, 32
Visual Comparator	22, 24
Books—Theory	2, 5, 6, 8, 14
Optical Components	11, 12, 14
Sampling Considerations	17
Spectra of various substances	35–37
Standing Committee of Analysts Methods	16(Cr), 19(Mn), 20(Fe), 21(As), 31(SO_4^{2-}), 33(NO_3^-)

7.1 Inorganic Determinands

7.1.1 *The Determination of Trace Inorganic Species by Reaction with Colour-Forming Reagents*

Solution spectrophotometry and colorimetry has now been superseded by atomic absorption[7] for the routine determination of many trace metals. Three very important advantages of atomic absorption are:

1. Sample preparation for all routinely determined elements simply requires digestion of the sample with acid. This predigestion is also required for many samples when solution spectrophotometry is used (see Chapter 6).

2. The rate of analysis is more rapid than solution spectrophotometry.

3. Interelement effects, especially bias effects, are much less significant using atomic absorption (see section 6.2).

However, for certain substances solution spectrophotometry (or colorimetry) has certain advantages:

1. Some elements and species cannot readily be determined with adequate sensitivity by direct flame atomic absorption methods[7], but can be both sensitively and accurately determined by solution spectrophotometry[22-28] (eg aluminium, boron, cyanide, iron, phosphorus (as phosphate), silicon (as molybdate reactive silicate), sulphur (as sulphate), nitrogen (as ammonia, nitrite and nitrate), chlorine and chloride).

2. Colorimetric methods of analysis can be carried out on portable inexpensive instrumentation that does not require either gas supplies or a mains electricity supply. Sensitive methods are available for most elements.[24]

3. The relative amounts of the various oxidation states of the element can often be determined. For example iron (II) and (III), manganese (II) and (VII), chromium (III) and (VI) can readily be differentiated, but the problem of stabilizing the samples, so that changes in the relative amounts of different oxidation states of an element at trace levels during storage has not been satisfactorily solved for many elements.

7.1.2 *The Determination of Certain Trace Elements Utilizing their Natural Absorption Spectrum*

It is possible to determine nitrate, chromate and permanganate at trace levels using the natural absorption spectrum of the species. The direct determination of nitrate by monitoring the absorption of the sample at 210 nm would appear to be an ideal method for the determination of nitrate in many non-saline waters. A solution containing 1 mg/litre nitrate (as nitrogen) gives an absorbance of approximately 0·5 in a 1 cm path length cell. Thus, most routine samples will require a dilution of about ten times. The only sample preparation other than dilution and filtration is the addition of sulphamic acid to remove nitrite (as nitrogen) and sulphuric acid to remove bicarbonate which also absorb at 210 nm. The biggest drawback of the method is that any organic matter and some metal irons (eg Fe^{3+}) absorb at 210 nm. This can be minimized by measuring the absorbance of each sample at 275 nm, where nitrate does not significantly absorb and then multiplying this absorbance by a suitable factor, which for many natural water samples has been found to be about 4·0.[33] The resulting correction factor is then subtracted from the gross absorbance observed at 210 nm and gives a corrected absorbance from which the nitrate concentration is calculated. If the measured absorbance at 275 nm exceeds 10% of that at 210 nm the method is of doubtful validity. For many potable waters the correction factor calculated from the measured absorbance at 275 nm is negligible. Recently a method that removes the interfering substances prior to measurement has been reported.[34] The sample is initially made alkaline and passed through an activated charcoal filter. This removes the interfering organic matter and any interfering metal irons. The sample is then acidified with sulphuric and sulphamic acids and the absorbance at 210 nm is monitored. The method has been successfully applied to raw, potable and waste waters.[34] It is also possible to utilise the gas phase absorption spectrum of ammonia and hydrogen sulphide (derived from sulphide) to determine trace levels of these species in natural waters (see section 8.8).

7.2 **Organic Substances**

7.2.1 *The Determination of Organic Species by Reaction with Colour Forming Reagents*

A large variety of organic substances can be readily determined by solution spectrophotometric techniques. In fact there are few substances of interest to water chemists that cannot be determined by some means or other using this technique[22,24,27,32]. Examples of routinely determined organic substances are formaldehyde, phenols, anionic detergents, non-ionic detergents, and carbohydrates. Obviously for the latter

four determinands there are large numbers of possible substances within each class of compounds. For instance the term 'phenols' covers a wide range of compounds and two basic types of determination are carried out routinely.[22] The first is for total phenols that is the total of the monohydric, dihydric and possibly some of the polyhydric phenols present, the second is for monohydric phenols after separation from the sample by steam distillation from the acidified sample. For both types of determination phenol standards are used to construct the calibration graph. Thus the quoted result will only be approximate as the photometric sensitivity will be dependent to some degree on the secondary chemical structure of the determinand. For instance some phenols (eg pentachlorophenol and certain sterically hindered phenols) do not react or only partially react with many chromogenic reagents. Even the phenols that undergo quantitative reaction with the chromogenic reagent will give somewhat differing absorption spectra. Thus colorimetric analysis of samples containing more than one phenol will obviously only give an approximate result of the total phenol present. Although chromatographic techniques are capable of separating and measuring all the component phenols present, this complex procedure is not suited to routine analysis of large numbers of samples, mainly because of the time and skill required to complete the analysis. It is also necessary to prepare suitable standards for all the phenols of interest. For certain classes of substances (eg pesticides and herbicides), other techniques such as gas-liquid chromatography are far more sensitive, specific and cost effective.

7.2.2 *The Determination of Certain Organic Substances Utilizing their Natural Absorption Spectrum*

Most substances that are likely to be found in natural waters cannot be directly determined by monitoring their absorption spectrum. The amount of absorption at concentrations likely to occur (even in pollution incidents) is too low. In addition naturally occurring organic matter will modify the observed absorption spectrum. However, if the determinand exhibits a characteristic absorption spectrum and forms an ionic species in acid conditions but exists as a neutral extractable species under alkaline conditions or vice versa, a very rapid screening procedure can readily be devised. For example strychnine can be rapidly detected by taking 500 ml of the sample, acidifying with sulphuric acid and extracting the solution with two 50 ml aliquots of chloroform. This removes most of the interfering organic matter but leaves the strychnine in the aqueous phase. The sample is then made alkaline with sodium hydroxide and the strychnine extracted with two 20 ml aliquots of chloroform. Unfortunately chloroform does not transmit at wavelengths below about 250 nm (see Table 3) so the combined chloroform extracts are then shaken with 20 ml of 0·1 M sodium hydroxide in order to bring the strychnine back into the aqueous phase. The absorption spectrum of the aqueous phase is then plotted over the wavelength range 220–320 nm. An absorption band with a maximum at 255 nm would indicate the presence of strychnine. For quantification, two other 500 ml aliquots of the sample should be spiked with strychnine to give concentrations of 0·5 and 1 mg/litre and the procedure repeated. A blank should also be run using 500 ml of distilled water. A detection limit of less than 0·02 mg/litre can be readily achieved. (See Figure 12). If a positive result is obtained, confirmatory tests using another technique should be carried out. A similar screening procedure can be used for penatchlorophenol except that the initial extraction with chloroform to remove interfering organic matter is carried out from an alkaline solution, the next extraction to remove the pentachlorophenol is carried by extracting out from acid and finally the pentachlorophenol is extracted from the chloroform into 0·1 M sodium hydroxide. Sodium pentachlorophenate exhibits two absorption peaks, a weak peak at 320 nm and a strong peak at 250 nm. A detection limit of less than 0·05 mg/litre can readily be achieved.

7.3 **Automatic Analysis of Natural Waters**

Alkalinity, aluminium, ammonia, anionic detergents, chloride, nitrate, nitrite, phosphate, silicate, total hardness and many other species can all be readily determined in natural water samples by automatic analysis techniques with minimal interelement effects[32]. In the author's opinion this surely represents the most cost effective method of analysis for a busy routine water laboratory.[4]

8 Complementary Techniques

This section describes techniques that are related to solution spectrophotometry. Many of these techniques can be carried out on a simply modified solution spectrophotometer. Some of the techniques such as photometric titrations, turbidimetry and spec-

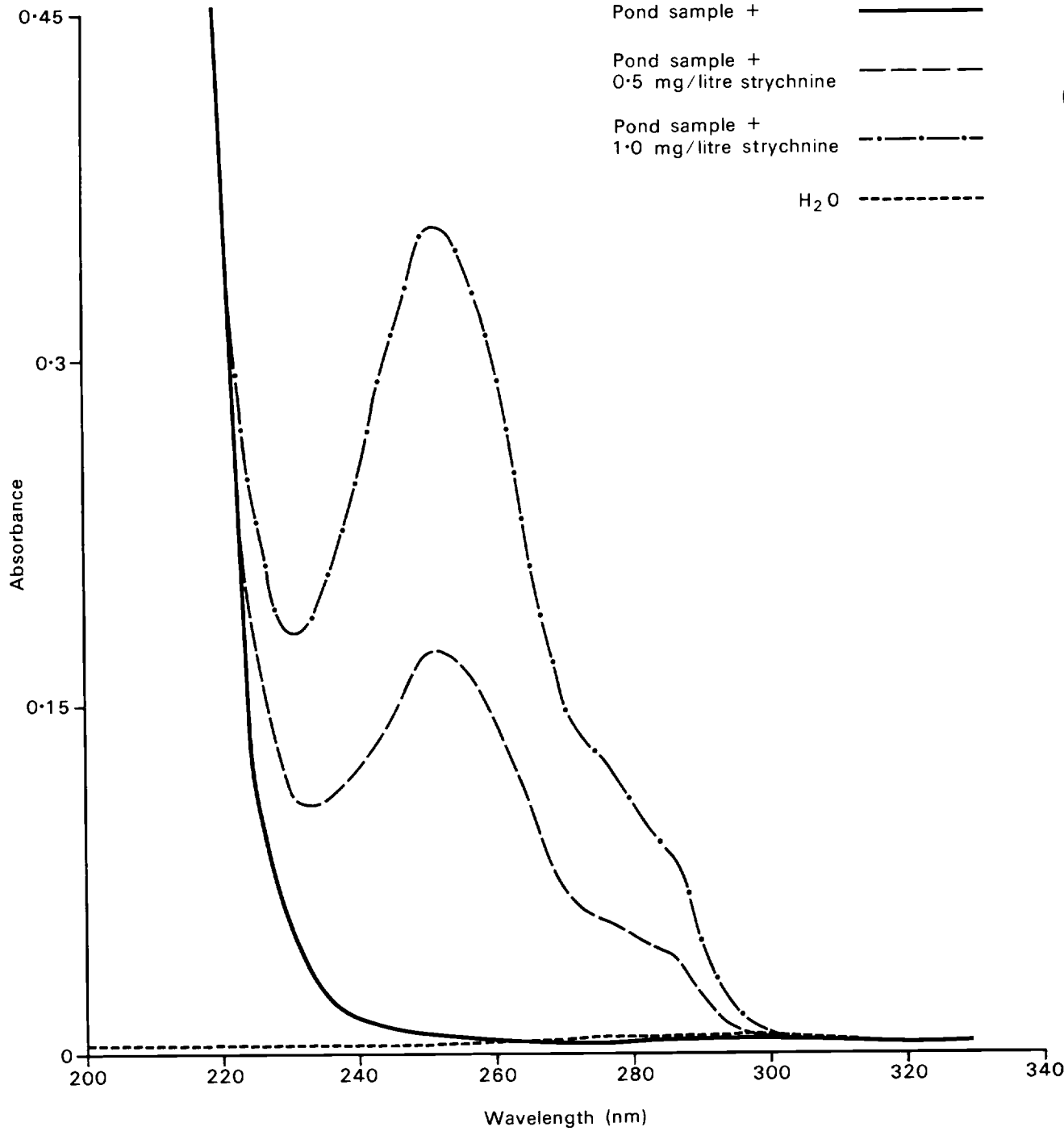

Figure 12 Rapid screening method for detecting strychnine

trofluorimetry have been in use for a considerable time whilst others such as optoacoustic spectrophotometry and vapour phase ultraviolet absorption spectrophotometry for the determination of ammonia and sulphide are relatively new. Although most of these techniques are not routinely used by the majority of water chemists, this could in part, be due to lack of awareness of such techniques. For this reason it was felt worthwhile to include these complimentary techniques in this essay review.

8.1 **Photometric Titrations**

A photometric titration is a titration where the end point is calculated from data on the absorbance of the solution during the actual titration. A suitable transparent titration vessel, containing the solution to be titrated and a magnetic stirrer, is placed in the light path of the spectrophotometer and a wavelength appropriate to the particular titration

is selected. A measured volume of the titration reagent is added and the absorbance monitored at several points before and after the expected end-point. The end-point is then found graphically. For example the titration of ferrous ions with standard permanganate would be carried out at 525 nm which is the wavelength of maximum absorption for the permanganate ion. Manganous, ferrous and ferric ions do not absorb at this wavelength. So the absorbance will remain constant until excess permanganate is present. The titration graph, which is a plot of absorbance, corrected for dilution, against the volume of permanganate, will be constant at a negligible absorbance until the end point and then rise linearly with excess titrant. It is not necessary to take a reading at the actual end point. Photometric titrations can usually be carried out at significantly lower determinand concentrations than conventional visual titrations.

8.2 Turbidimetry

The turbidity of a filtered solution of the sample is monitored after the addition of an excess of a suitable precipitation reagent that will react specifically with the analyte. In order to stabilize the precipitate a conditioning reagent is normally added prior to the addition of the precipitating reagent. A well known example of this technique is the determination of sulphate by adding excess barium chloride to the sample plus conditioning agent and monitoring the turbidity of the resulting colloidal barium sulphate precipitate.[22] The conditioning reagent in this case consists of sodium chloride, hydrochloric acid, isopropanol and glycerol.

The disadvantages of the technique are:

1. It is prone to unsuspected interference effects.
2. It requires very strict control of operating conditions (eg rate of stirring, elapsed time prior to measurement, temperature etc).
3. It cannot readily be automated.
4. It is not very sensitive.

For these reasons turbidimetry is seldom used today.

8.3 Solution Spectrofluorimetry

Whilst a relatively large number of molecules exhibit electronic absorption bands in the wavelength range 190–1,000 nm, relatively few molecules exhibit analytically useful fluorescence spectra. Those that do fluoresce consist of a few highly conjugated aliphatic substances, such as vitamin A and a much larger number of substances containing one or more aromatic and/or heterocyclic ring systems, such as fluorescein, indole, polycyclic aromatic hydrocarbons etc. Under ideal conditions, those substances that do exhibit useful fluorescence spectra can frequently be detected at ultratrace levels (10^{-12} M) using spectrofluorimetry.[38] Typical monochromator and filter spectrofluorimeters are depicted in Fig 13.

The sample cuvette is normally of square cross section, (typically 1 cm path length) and optically ground on all four sides. The sample solution is irradiated by light of a chosen wavelength band emitted by a high intensity source such as a xenon-arc lamp or a high-pressure mercury discharge lamp. The wavelength band selected normally corresponds to that of maximum absorption of the fluorescent species and is isolated either using an interference filter or a monochromator. The resulting fluorescence radiation is emitted in all directions and the detector views the radiation emitted at right angles to the excitation radiation. The wavelength of measurement normally corresponds to that of maximum fluorescence emission and is isolated either using an interference filter or a monochromator. Instruments that incorporate monochromators invariably have wavelength scanning facilities on both monochromators. This allows the excitation spectrum of the determinand to be plotted by setting the fluorescence monochromator to the wavelength of maximum fluorescence emission of the determinand fluorescence species and mechanically scanning the wavelength of the excitation monochromator. The resulting spectrum, after correction, corresponds to the normal absorption spectrum of the fluorescent species. Correction is required to allow for the variation of source intensity and instrumental sensitivity with wavelength. Similarly the fluorescence spectrum can be plotted by setting the excitation monochromator to the wavelength corresponding to maximum absorption and mechanically scanning the wavelength of the fluorescence monochromator. Stokes' Law states that for excitation

Figure 13 Typical spectrofluorimeters

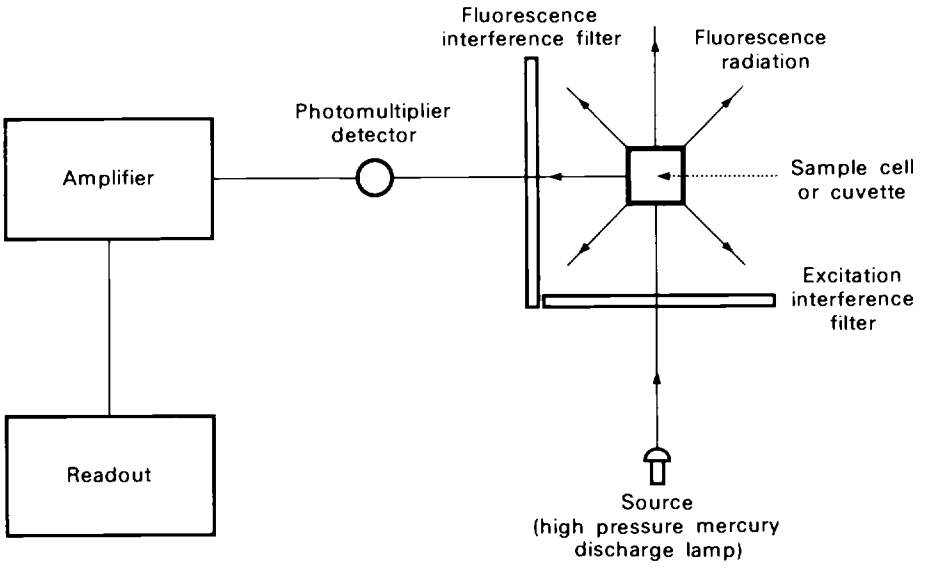

Figure 13a Spectrofluorimeters containing two interference filters

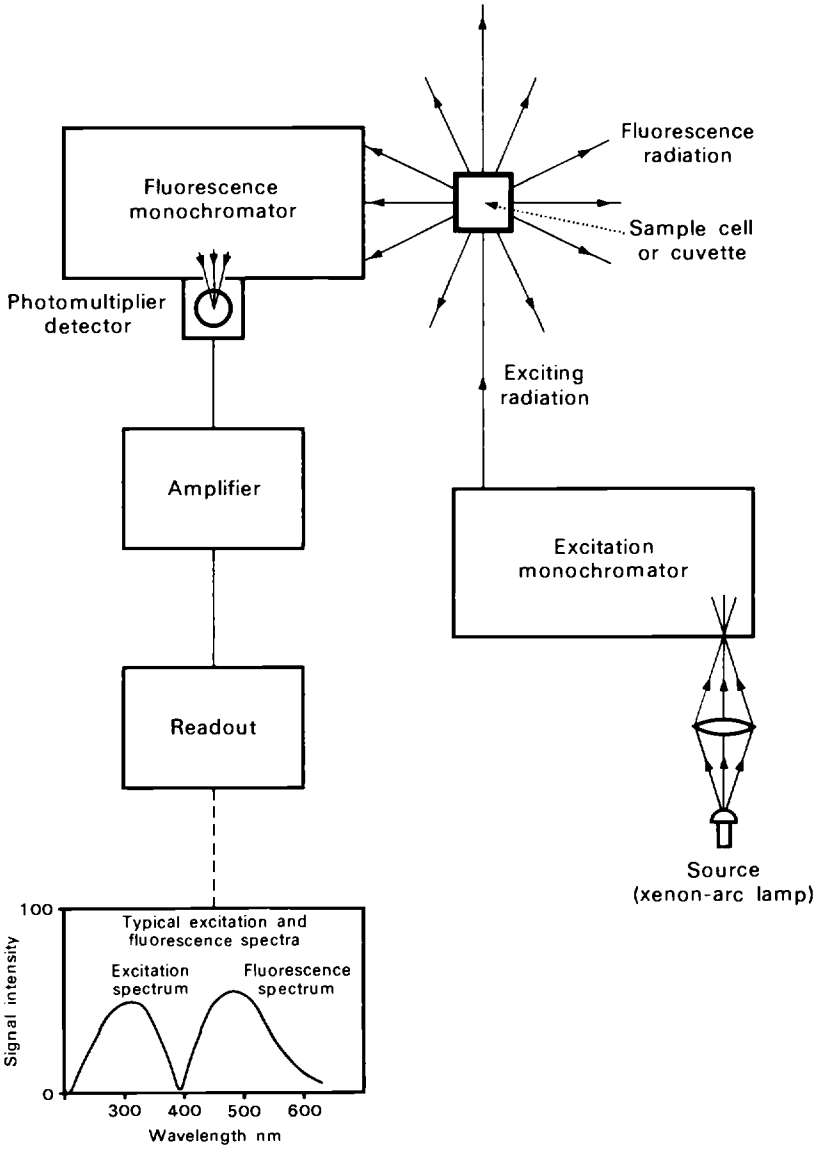

Figure 13b Spectrofluorimeters containing two monochromators

from the lowest vibrational energy level of the ground state (see Fig 2b) the wavelength of fluorescence is always greater than that of the exciting light.

The magnitude of absorbance signals, observed in solution spectrophotometry, is independent of the source intensity (see equation 3). The fluorescence signal is directly proportional to the source intensity, therefore in principle the instrumental sensitivity should only be limited by the intensity of the exciting light source. It has been shown (see section 2.4) that the ultimate detection limit for solution spectrophotometry is of the order of 10^{-8}M, whilst for solution spectrofluorimetry it is possible to detect 10^{-12}M levels of certain substances (eg quinine bisulphate, fluorescein, certain polycylic hydrocarbons).

Spectrofluorimetry is seldom used routinely in general water analysis for the following reasons:

1. There are few determinands of interest to the water chemist that fluoresce. Although it is possible to form a fluorescent species by reaction of many determinands with suitable reagent(s), most of these determinands can be more readily determined using other simpler techniques.
2. Background fluorescence is often observed from other trace substances (eg humic and fulvic acids) present in many natural water samples. This background fluorescence signal will vary from sample to sample and can be difficult to differentiate from the determinand fluorescence signal.
3. A spectrofluorimeter incorporating two monochromators costs considerably more than an average solution spectrophotometer and, as mentioned just above, the technique can only be applied to relatively few determinands although it has been used to characterise oil spills.[39] Filter spectrofluorimeters cost significantly less than monochromator instruments, but are much less versatile as spectra cannot be plotted. They are much more prone to spectral interference effects (eg background fluoresence).

Spectrofluorimetry is often used in clinical analysis because many clinical and pharmaceutical determinands are naturally fluorescent or are easily converted to useful fluorescent species. Most tissue samples exhibit very little matrix variation and consequently interference effects are relatively easily to minimize for this type of analysis.

Spectrofluorimetric HPLC detectors are commonly used to detect ultra trace levels of polycyclic aromatic hydrocarbons (PAH's). Without prior chromatographic separation it is virtually impossible to determine individual PAH's in a mixture by conventional spectrofluorimetry because of overlapping absorption and/or fluorescence spectra.

8.4 **Reflectance Spectrophotometry**

Reflectance spectrophotometric techniques are used mainly for the measurement of colours and glosses of materials. There are two distinct processes for reflection of radiant energy at boundary surfaces:

1. Specular reflection – This is mirror type reflection which normally exhibits marked plane polarization.
2. Diffuse reflectance – This originates from penetration of a part of the incident radiation into the interior of the sample. Part of this radiation is returned to the surface of the sample following partial absorption and multiple scattering at the boundaries of the individual particles. The diffuse reflectance radiation exhibits little polarization, but has widely differring spacial scatter characteristics dependent upon the substance being examined and for this reason an expensive integrating sphere usually coated with magnesium oxide or barium sulphate is required for the collection of the diffusely reflected radiation.

Reflectance spectrophotometry is commonly employed in the paint, paper, textile, glass and food industries. For instance good quality tomato juice exhibits a high reflectance at wavelengths above 600nm, and a low reflectance at 550nm. Juice from unripe tomatoes is characterised by a high reflectance at 550nm. Reflectance spectrophotometry is seldom used for water industry applications. Instrumentation is uncommon, relatively expensive and requires highly skilled operation.

8.5 Optoacoustic Spectrophotometry

Optoacoustic spectrophotometry[40, 41] is a relatively simple technique that can be used for the optical examination of solids. It can also be applied to liquids and gases. When light energy is allowed to fall on a suitable absorbing material, in most cases the absorbed energy is converted into heat. This conversion takes place extremely rapidly. If the incident radiation is modulated by the use of a mechanical light chopper, the energy absorption is also modulated at a similar frequency and consequently the liberated heat energy also exhibits this modulation frequency. In a closed sample enclosure of constant volume, containing an absorbing gas, the periodic heating will produce a periodic increase in pressure in phase with the light source modulation. At modulation frequencies from about 30 Hz to 15 KHz the varying pressure will result in an acoustic signal (ie soundwaves) whose amplitude can be measured with a simple microphone. With solid samples, in a closed system, the heat generated by the sample is detected by monitoring the acoustic signal generated by heat transfer to the gaseous atmosphere surrounding the sample. The energy of the optoacoustic signal is directly proportional to the energy of the source, so intense sources are used (eg xenon arc lamps). The absorption cell is of small volume (approximately 10 ml) and can be constructed from stainless steel with a silica window and a sample holder directly opposite the window. A condenser microphone is mounted at right angles to the window-sampler holder axis. A typical experimental set-up is depicted in Figure 14. The technique has the following advantages:

1. The output signal to noise ratio can be improved by increasing the source intensity, thus substances with low absortivities can be examined.
2. Although radiation must be absorbed by the sample as in conventional absorption spectrophotometry, there is no need to detect the radiation transmitted by the sample.
3. Unlike diffuse reflectance spectrophotometry only absorbed energy is detected, hence problems from scattered source radiation do not arise (ie the microphone does not respond to light).
4. Very small samples can be examined.

Possible envisaged future applications could include monitoring in vivo spectra of species such as chlorophyll, algae or even bacterial colonies; a rapid screening technique for estimating certain impurities in bulk chemicals such as lime, alum or ferrous sulphate; identification of scale or deposits in pipelines; examination of various types of sludges.

8.6 Monitor for Organic Matter in Natural Waters

It has been demonstrated that the absorbance of natural water samples at 253·7 nm is often related to the permanganate value (ie organic matter present). Simple, portable instrumentation has been devised to monitor the absorbance of natural waters at this wavelength[42, 43]. Briggs and Melbourne[43], have constructed, a battery operated instrument, suitable for continuous water monitoring. A collimated light beam from a small low pressure 12 volt mercury lamp is interrupted by a rotating disc in which alternate sectors contain visible and ultraviolet light filters. The light passing through a flow cell (containing the sample) is detected by a vacuum photocell. The signal from the photocell is passed through a logarithmic amplifier with the result that alternating component of the output is a function of the difference of the logarithms (that is the logarithm of the ratio) of the absorbance of the samples at the two wavelengths (254 and 546 nm). This simple arrangement will compensate for drift in the surface intensity and fouling of the absorption cell window provided the absorbance of the fouling material is identical at the two wavelengths. The presence of substantial quantities of suspended matter should have little effect on the output. However, a few inorganic species (eg iron, chromate) can interfere. This inherently simple system is a very neat method for detecting significant organic pollution in many rivers and watercourses.

8.7 Vapour-phase Ultraviolet Absorption Spectrophotometry for the Characterization of Organic Compounds

A difficult problem in many water laboratories is the rapid characterization of gross pollution in watercourses by oil and related substances. Often it is necessary to compare the sample from a pollution incident with a large number of samples from nearby watercourses. Gas-liquid chromatographic techniques are normally used for

Figure 14 Optoacoustic Spectrophotometers

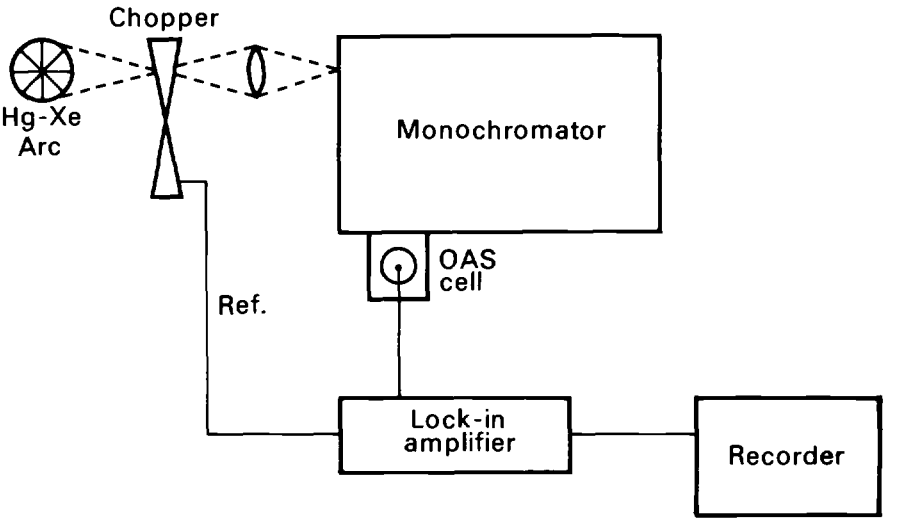

Figure 14a Schematic diagram of a singlebeam optoacoustic spectrophotometer

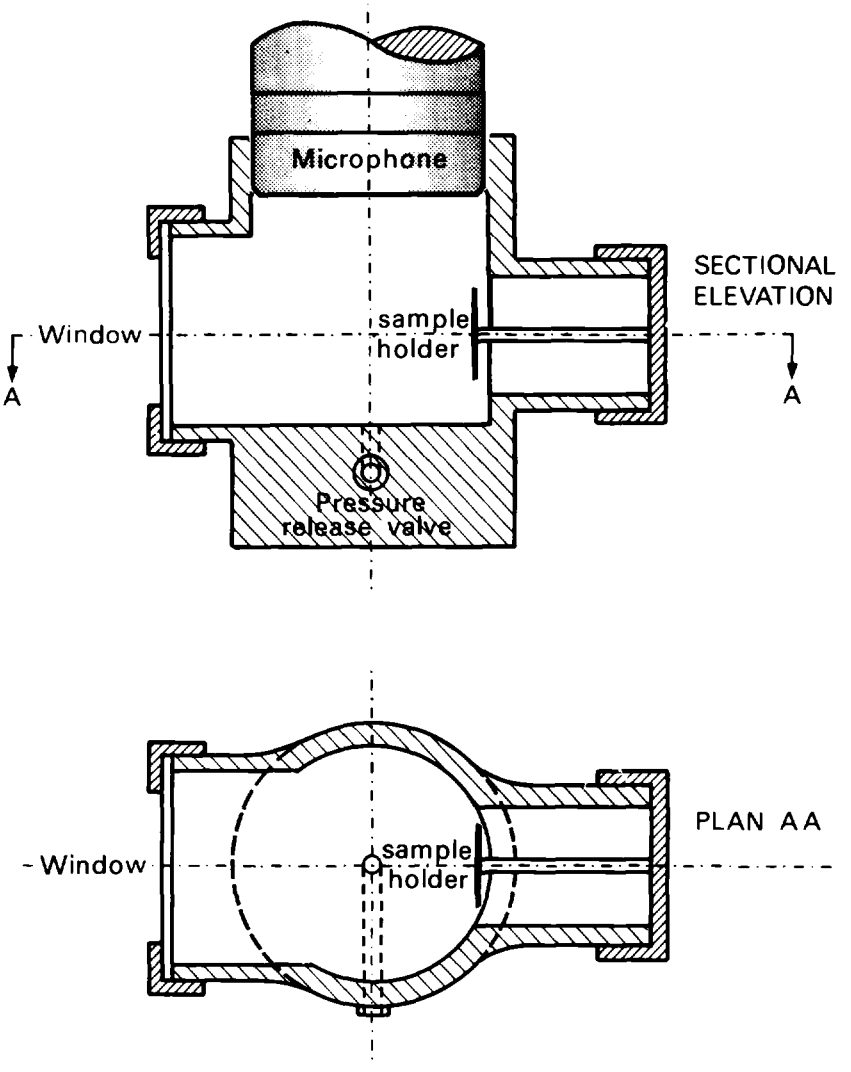

Figure 14b A typical optoacoustic cell

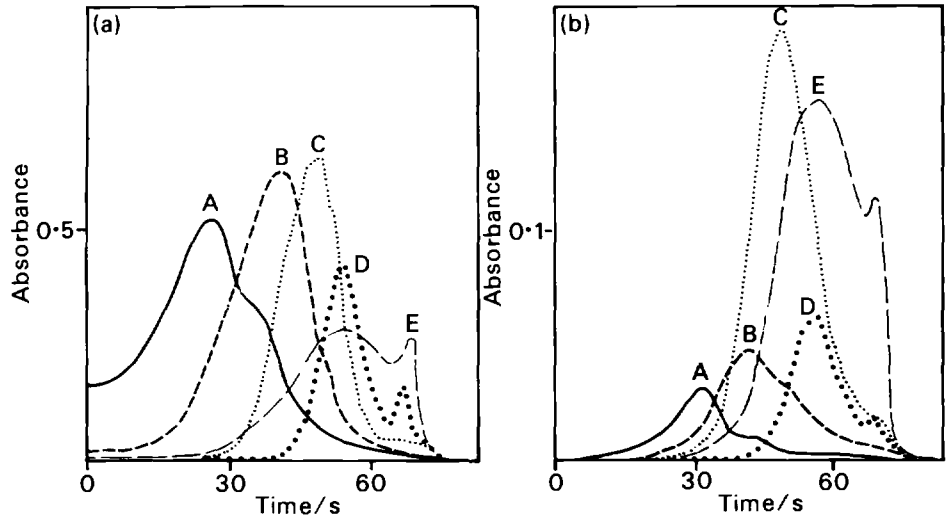

Figure 15 Typical oil traces obtained using vapour phase ultraviolet absorption spectrophotometry

All solutions 10% *V/V* of oil in hexane; injection volume 0·5μl; wavelength (a) 190 nm and (b) 253·7 nm; ramp rate 11°Cs^{-1}. A, 28s fuel oil, B, 35s fuel oil; C, light lubricating oil; D, SAE 20/50 lubricating oil; and E, 1500s fuel oil.

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this type of analysis after extraction of the sample into a suitable solvent such as hexane. However, this is a rather time consuming procedure if a large number of samples are involved. An alternative technique is to inject a small amount (1–10 μl) of the extract into a small graphite tube that is resistively heated at a controlled rate of about 10°C/s in an atmosphere of flowing nitrogen. Some commercially available flameless electrothermal atomizers are suitable[44]. The vapour phase ultraviolet absorption along the tube axis is then monitored with respect to time at a preselected wavelength. For maximum sensitivity a wavelength of 190 nm is normally used, further traces made at other wavelengths can give additional information. It should be noted that it is difficult to make absorbance measurements in liquids at 190 nm because most solvents (see Table 3) are opaque at this wavelength and also many common ions, eg nitrate, chloride, bicarbonate absorb strongly at this wavelength.

Figure 15 shows some typical vapour-phase ultraviolet spectra of a range of oils obtained by the author and co-worker[44]. Each trace takes less than two minutes to run. The technique has also been adapted for screening for trace levels of non-specific extractable organic matter, non-ionic detergents, pentachlorophenol and certain herbicides in natural waters.[44]

8.8 Gas-phase Ultra-Violet Absorption Spectrophotometry for the Determination of Ammonia and Sulphide

The characteristic ultraviolet absorption spectrum of gaseous ammonia can be used to determine trace levels of ammonia in a wide variety of samples.[45] An aliquot of the sample solution is made strongly alkaline with sodium hydroxide or potassium carbonate and air is then passed through the solution contained in a small Dreschel bottle, maintained in a thermostatted bath at a temperature of about 40°C. The liberated ammonia is passed into the 15 cm length, 8 mm id silica absorption cell and the absorption due to ammonia is monitored at 201 nm on a pen recorder.[45] The author has obtained a detection limit of about 0·05 mg/litre using this technique.

It is also possible to determine sulphide[46] by a similar technique. In this case the sample is acidified and the liberated hydrogen sulphide absorption at 200 nm is monitored.

9 Conclusions

Solution spectrophotometry is a well tried and tested technique that is commonly used for routine chemical analysis. The instrumentation of the technique, especially the simpler forms, has not radically altered over the last ten years. The main changes have been improvements in the individual components of spectrophotometers, for example silica coated optics, improved diffraction gratings, photomultipliers with a very wide

wavelength response and improved semiconductor circuit technology. The only really significant development is the advent of the microprocessor which has noticeably improved the control and data handling facilities of spectrophotometers. In the author's opinion, instrumentation will not change much over the next ten years. The use of laser technology does not appear to be particularly advantageous and the cost of wide range tuneable wavelength lasers, even if their output stability improves, is unlikely to be favourable for incorporation into future spectrophotometers. In the author's opinion, vidicon or diode array (multi-wavelength) detection devices have little advantage for routine spectrophotometric measurements, although they are invaluable for rapidly recording absorption spectra of transient species (eg enzyme activity or flash photolysis studies) or simply recording large numbers of absorption spectra.

Unlike many other spectroscopic techniques, solution spectrophotometry is not a 'black box' instrumental technique with the simple operating philosophy of 'sample in – short delay – result out'. The successful development of solution spectrophotometric measurements requires a fundamental knowledge of basic analytical chemistry. The cost of a simple visual comparator, capable of analysing many determinands at sub mg/litre levels is of the same order as a small electrically heated hotplate. Even a fairly sophisticated ultraviolet-visible spectrophotometer costs less than many other analytical spectroscopic instrumentation. The routine use of solution spectrophotometry is likely to continue well into the foreseeable future.

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