

17.2 THEORY OF SPECTROPHOTOMETRY* AND COLORIMETRY

When light (monochromatic or heterogeneous) falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium, and the remainder is transmitted. If the intensity of the incident light is expressed by I_0 , that of the absorbed light by I_a , that of the transmitted light by I_t , and that of the reflected light by I_r , then:

$$I_0 = I_a + I_t + I_r$$

* Spectrophotometry proper is mainly concerned with the following regions of the spectrum: ultraviolet, 185–400 nm; visible 400–760 nm; and infrared, 0.76–15 μm . Colorimetry is concerned with the visible region of the spectrum. In this chapter attention will be confined largely to the visible and near ultraviolet region of the spectrum.

For air-glass interfaces arising from the use of glass cells, it may be stated that about 4 per cent of the incident light is reflected. I_r is usually eliminated by the use of a control, such as a comparison cell, hence:

$$I_0 = I_a + I_t \quad (1)$$

Credit for investigating the change of absorption of light with the thickness of the medium is frequently given to Lambert,¹ although he really extended concepts originally developed by Bouguer.² Beer³ later applied similar experiments to solutions of different concentrations and published his results just prior to those of Bernard.⁴ This very confusing story has been explained by Malinin and Yoe.⁵ The two separate laws governing absorption are usually known as Lambert's Law and Beer's Law. In the combined form⁶ they are referred to as the Beer-Lambert Law.

Lambert's Law. This law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light. This is equivalent to stating that the intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically, or that any layer of given thickness of the medium absorbs the same fraction of the light incident upon it. We may express the law by the differential equation:

$$-\frac{dI}{dl} = kI \quad (2)$$

where I is the intensity of the incident light of wavelength λ , l is the thickness of the medium, and k is a proportionality factor. Integrating equation (2) and putting $I = I_0$ when $l = 0$, we obtain:

$$\ln \frac{I_0}{I_t} = kl$$

or, stated in other terms,

$$I_t = I_0 \cdot e^{-kl} \quad (3)$$

where I_0 is the intensity of the incident light falling upon an absorbing medium of thickness l , I_t is the intensity of the transmitted light, and k is a constant for the wavelength and the absorbing medium used. By changing from natural to common logarithms we obtain:

$$I_t = I_0 \cdot 10^{-0.4343kl} = I_0 \cdot 10^{-Kl} \quad (4)$$

where $K = k/2.3026$ and is usually termed the **absorption coefficient**. The absorption coefficient is generally defined as the reciprocal of the thickness (l cm) required to reduce the light to $\frac{1}{10}$ of its intensity. This follows from equation (4), since:

$$I_t/I_0 = 0.1 = 10^{-Kl} \quad \text{or} \quad Kl = 1 \quad \text{and} \quad K = 1/l$$

The ratio I_t/I_0 is the fraction of the incident light transmitted by a thickness l of the medium and is termed the **transmittance** T . Its reciprocal I_0/I_t is the **opacity**, and the **absorbance** A of the medium (formerly called the **optical density**

D or extinction E) is given by:*

$$A = \log I_0/I_t \quad (5)$$

Thus a medium with absorbance 1 for a given wavelength transmits 10 per cent of the incident light at the wavelength in question.

Beer's Law. We have so far considered the light absorption and the light transmission for monochromatic light as a function of the thickness of the absorbing layer only. In quantitative analysis, however, we are mainly concerned with solutions. Beer studied the effect of concentration of the coloured constituent in solution upon the light transmission or absorption. He found the same relation between transmission and concentration as Lambert had discovered between transmission and thickness of the layer [equation (3)], i.e. the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically. This may be written in the form:

$$\begin{aligned} I_t &= I_0 \cdot e^{-k'c} \\ &= I_0 \cdot 10^{-0.4343k'c} = I_0 \cdot 10^{-K'c} \end{aligned} \quad (6)$$

where c is the concentration, and k' and K' are constants. Combining equations (4) and (5), we have:⁶

$$I_t = I_0 \cdot 10^{-acl} \quad (7)$$

or

$$\log I_0/I_t = acl \quad (8)$$

This is the fundamental equation of colorimetry and spectrophotometry, and is often spoken of as the **Beer-Lambert Law**. The value of a will clearly depend upon the method of expression of the concentration. If c is expressed in mole L^{-1} and l in centimetres then a is given the symbol ϵ and is called the **molar absorption coefficient** or molar absorptivity (formerly the molar extinction coefficient).

The specific absorption (or extinction) coefficient E_s (sometimes termed absorbancy index) may be defined as the absorption per unit thickness (path length) and unit concentration.

Where the molecular weight of a substance is not definitely known, it is obviously not possible to write down the molecular absorption coefficient, and in such cases it is usual to write the unit of concentration as a superscript, and the unit of length as a subscript. Thus

$$E_{1\text{cm}}^{1\%} 325 \text{ nm} = 30$$

means that for the substance in question, at a wavelength of 325 nm, a solution of length 1 cm, and concentration 1 per cent (1 per cent by weight of solute or 1 g of solid per 100 mL of solution) $\log I_0/I_t$ has a value of 30.

It will be apparent that there is a relationship between the absorbance A , the transmittance T , and the molar absorption coefficient, since:

$$A = \epsilon cl = \log \frac{I_0}{I_t} = \log \frac{1}{T} = -\log T \quad (9)$$

The scales of spectrophotometers are often calibrated to read directly in absorbances, and frequently also in percentage transmittance. It may be mentioned that for colorimetric measurements I_0 is usually understood as the intensity of the light transmitted by the pure solvent, or the intensity of the light entering the solution; I_t is the intensity of the light emerging from the solution, or transmitted by the solution. It will be noted that:

- (a) The **absorption coefficient** (or extinction coefficient) is the absorbance for unit path length:

$$K = A/t \text{ or } I_t = I_0 \cdot 10^{-Kt}$$

- (b) The **specific absorption coefficient** (or absorptancy index) is the absorbance per unit path length and unit concentration:

$$E_s = A/cl \text{ or } I_t = I_0 \cdot 10^{-E_s \cdot cl}$$

- (c) The **molar absorption coefficient** is the specific absorption coefficient for a concentration of 1 mol L^{-1} and a path length of 1 cm.

$$\varepsilon = A/cl$$

Application of Beer's Law. Consider the case of two solutions of a coloured substance with concentrations c_1 and c_2 . These are placed in an instrument in which the thickness of the layers can be altered and measured easily, and which also allows a comparison of the transmitted light (e.g. a Duboscq colorimeter, Section 17.5). When the two layers have the same colour intensity:

$$I_{t_1} = I_0 \cdot 10^{-\varepsilon l_1 c_1} = I_{t_2} = I_0 \cdot 10^{-\varepsilon l_2 c_2} \quad (10)$$

Here l_1 and l_2 are the lengths of the columns of solutions with concentrations c_1 and c_2 respectively when the system is optically balanced. Hence, under these conditions, and when Beer's law holds:

$$l_1 c_1 = l_2 c_2 \quad (11)$$

A colorimeter can, therefore, be employed in a dual capacity: (a) to investigate the validity of Beer's Law by varying c_1 and c_2 and noting whether equation (11) applies, and (b) for the determination of an unknown concentration c_2 of a coloured solution by comparison with a solution of known concentration c_1 . It must be emphasised that equation (11) is valid only if Beer's Law is obeyed over the concentration range employed and the instrument has no optical defects.

When a spectrophotometer is used it is unnecessary to make comparison with solutions of known concentration. With such an instrument the intensity of the transmitted light or, better, the ratio I_t/I_0 (the transmittance) is found directly at a known thickness l . By varying l and c the validity of the Beer-Lambert Law, equation (9), can be tested and the value of ε may be evaluated. When the latter is known, the concentration c_x of an unknown solution can be calculated from the formula:

$$c_x = \frac{\log I_0/I_t}{\varepsilon l} \quad (12)$$

Attention is directed to the fact that the molar absorption coefficient ε depends

upon the wavelength of the incident light, the temperature, and the solvent employed. In general, it is best to work with light of wavelength approximating to that for which the solution exhibits a maximum selective absorption (or minimum selective transmittance): the maximum sensitivity is thus attained.

For matched cells (i.e. l constant) the Beer–Lambert Law may be written:

$$c \propto \log \frac{I_0}{I_t}$$

$$c \propto \log \frac{1}{T}$$

or

$$c \propto A \tag{13}$$

Hence, by plotting A [or $\log(1/T)$] as ordinate, against concentration as abscissa, a straight line will be obtained and this will pass through the point $c = 0$, $A = 0$ ($T = 100$ per cent). This calibration line may then be used to determine unknown concentrations of solutions of the same material after measurement of absorbances.

Deviation from Beer's Law. Beer's Law will generally hold over a wide range of concentration if the structure of the coloured ion or of the coloured non-electrolyte in the dissolved state does not change with concentration. Small amounts of electrolytes, which do not react chemically with the coloured components, do not usually affect the light absorption; large amounts of electrolytes may result in a shift of the maximum absorption, and may also change the value of the extinction coefficient. Discrepancies are usually found when the coloured solute ionises, dissociates, or associates in solution, since the nature of the species in solution will vary with the concentration. The law does not hold when the coloured solute forms complexes, the composition of which depends upon the concentration. Also, discrepancies may occur when monochromatic light is not used. The behaviour of a substance can always be tested by plotting $\log I_0/I_t$, or $\log 1/T$ against the concentration: a straight line passing through the origin indicates conformity to the law.

For solutions which do not follow Beer's Law, it is best to prepare a calibration curve using a series of standards of known concentration. Instrumental readings are plotted as ordinates against concentrations in, say, mg per 100 mL or 1000 mL as abscissae. For the most precise work each calibration curve should cover the dilution range likely to be met with in the actual comparison.

PHOTOELECTRIC PHOTOMETER METHOD

Photoelectric colorimeters (absorptimeters). One of the greatest advances in

the design of colorimeters has been the use of photoelectric cells to measure

the intensity of the light, thus eliminating the errors due to the personal

characteristics of each observer.

The photovoltaic or barrier-layer cell, in which light striking the surface of

a semiconductor such as selenium mounted upon a base plate (usually iron)

leads to the generation of an electric current, the magnitude of which is governed

by the intensity of light beam, has been widely used in many absorptimeters.

It, however, suffers from the defects that (1) amplification of the current produced

by the cell is difficult to achieve which means that the cell does not have a very

high sensitivity for low levels of light and (2) the cell tends to become fatigued.

For these reasons it has now been largely superseded by the

photomultiplier

and by silicon diode detectors.

Photo-emissive cells. In the simplest form of photo-emissive cell (also called

phototube) a glass bulb is coated internally with a thin, sensitive layer, such as

caesium or potassium oxide and silver oxide (i.e. one which emits electrons

when illuminated), a free space being left to permit the entry of the light. This

layer is the cathode. A metal ring inserted near the centre of the bulb forms the

anode, and is maintained at a high voltage by means of a battery.

The interior

of the bulb may be either evacuated or, less desirably, filled with an inert gas

at low pressure (e.g. argon at about 0.2 mm). When light, penetrating the bulb,

falls on the sensitive layer, electrons are emitted, thereby causing a current to

flow through an outside circuit; this current may be amplified by electronic

means, and is taken as a measure of the amount of light striking

the

photosensitive surface. Otherwise expressed, the emission of electrons leads to

a potential drop across a high resistance (R) in series with the cell and the battery;

the fall in potential may be measured by a suitable potentiometer (M), and is related to the amount of light falling on the cathode. The action of the photo-emissive cell is shown diagrammatically in Fig. 17.5.

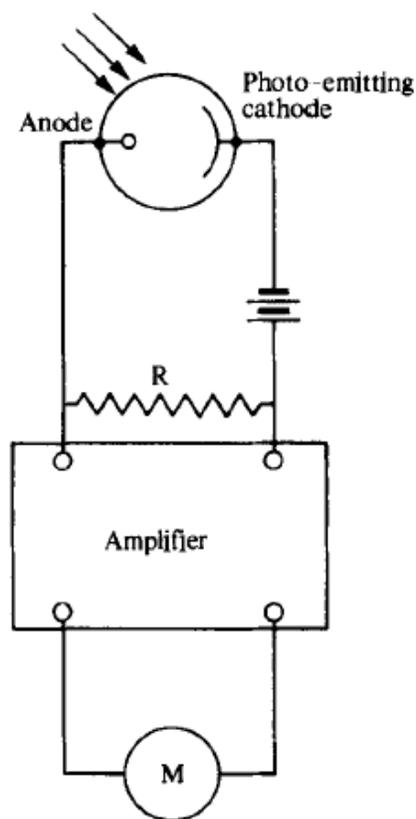


Fig. 17.5

The sensitivity of a photo-emissive cell (phototube) may be considerably increased by means of the so-called **photomultiplier tube**. The latter consists of an electrode covered with a photo-emissive material and a series of positively charged plates, each charged at a successively higher potential. The plates are covered with a material which emits several (2–5) electrons for each electron collected on its surface. When the electrons hit the first plate, secondary electrons are emitted in greater number than initially struck the plate, with the net result of a large amplification (up to 10^6) in the current output of the cell. The output of a photomultiplier tube is limited to several milliamperes, and for this reason only low incident radiant energy intensities can be employed. It can measure intensities about 200 times weaker than those measurable with an ordinary photoelectric cell and amplifier.

Phototube detectors are normally sensitive either to radiation of wavelength 200 nm to 600–650 nm, or of wavelength 600–1000 nm. To scan a complete spectral range an instrument must therefore contain two photocells; a 'red' sensitive cell (600–800 nm) and a 'blue' cell (200–600 nm).

The **silicon diode** (photodiode) detector consists of a strip of *p*-type silicon on the surface of a silicon chip (*n*-type silicon). By application of a biasing potential with the silicon chip connected to the positive pole of the biasing source, electrons and holes are caused to move away from the *p*-*n* junction. This creates a depletion region in the neighbourhood of the junction which in effect becomes a capacitor. When light strikes the surface of the chip, free

electrons and holes are created which migrate to discharge the capacitor:
the

magnitude of the resultant current is a measure of the intensity of the light.
Such a detector has a greater sensitivity than a single phototube, but less
than
that shown by a photomultiplier.

With modern technology it is possible to form a large number of such
photodiodes on the surface of a single Silicon chip. This chip also contains
an
integrating circuit which can scan each photodiode in turn to give a signal
which
is transmitted to a microprocessor. Each photodiode can be programmed
to
respond to a certain small band of wavelengths so that the complete
spectrum
can be scanned virtually instantaneously.'

When using a spectrophotometer which is equipped with a diode array, an
absorption spectrum is obtained by electronic scanning rather than the
mechanical scanning which occurs in a conventional spectrophotometer.
This

results in what is virtually instantaneous recording of the absorption curve;
it

may be accomplished in 1-5 seconds. In consequence, samples are
exposed to

radiation for such short periods of time that there is little possibility of
photochemical reactions taking place, and the effects of fluorescence in
the

samples are minimised. The speed of operation makes such instruments useful for the investigation of fast chemical reactions, and for monitoring the eluate from liquid chromatographs. Diode detectors do however suffer from somewhat limited resolution; about 1 nm in the ultraviolet, and about 2 nm in the visible region.

17.7 WAVELENGTH SELECTION

Bearing in mind that the colour of a substance is related to its ability to absorb selectively in the visible region of the electromagnetic spectrum, it follows that having achieved the ability to measure the intensity of light with a high degree of accuracy, if we wish to analyse a solution by measuring the extent to which some coloured component absorbs light, the accuracy will be improved if measurements are made at the wavelength which is being absorbed. In this connection, it must be borne in mind that the observed colour is due to the radiation which is *not* absorbed, or in other words by the radiation which is transmitted by the coloured solution: the colour corresponding to this radiation is said to be complementary to the colour corresponding to that of the radiation which is being absorbed. Complementary colours are listed in Table 17.2.

Procedures which can be used to select specified regions of the visible part of

Table 17.2 Complementary colours

Wavelength (nm)	Hue (transmitted)	Complementary hue
400–435	Violet	Yellowish-green
435–480	Blue	Yellow
480–490	Greenish-blue	Orange
490–500	Bluish-green	Red
500–560	Green	Purple
560–580	Yellowish-green	Violet
580–595	Yellow	Blue
595–610	Orange	Greenish-blue
610–750	Red	Bluish-green

the electromagnetic spectrum include the use of (1) filters, (2) prisms and (3) diffraction gratings.

Light filters. Optical filters are used in colorimeters (absorptimeters) for isolating any desired spectral region. They consist of either thin films of gelatin

containing different dyes or of coloured glass.

Interference filters (transmission type). These have somewhat narrower transmitted bands than coloured filters and are essentially composed of two

highly reflecting but partially transmitting films of metal (usually silver separated

by a spacer film of transparent material). The amount of separation of the

metal

films governs the wavelength position of the pass band, and hence the colour

of the light that the filter will transmit. This is the result of an optical interference

effect which produces a high transmission of light when the optical separation

of the metal films is effectively a half wavelength or a multiple of a halfwavelength.

Light which is not transmitted is for the most part reflected.

The wavelength region covered is either 253-390nm or 380-1100nm, peak transmission is between 25 and 50 per cent and the bandwidth is less than 18 nm

for the narrow-band filters suitable for colorimetry.

Absorptiometers equipped with filters are now rarely used but they are inexpensive, and for certain specified measurements can be very satisfactory.

Prisms. To obtain improved resolution of spectra in both the visible and ultraviolet regions of the spectrum it is necessary to employ a better optical system than that possible with filters. In many instruments, both manual and

automatic, this is achieved by using prisms to disperse the radiation obtained

from incandescent tungsten or deuterium sources. The dispersion is dependent

upon the fact that the refractive index, n , of the prism material varies with

wavelength, λ , the dispersive power being given by $dn/d\lambda$. The separation

achieved between different wavelengths is dependent upon both the dispersive

power and the apical angle of the prism.

In instruments in which the radiation is only passed through the prism in a single direction it is common to use a 60° prism. In some cases double dispersion

is achieved by reflecting the radiation back through the prism by placing a mirrored surface behind the prism, as in the Littrow mounting, Fig. 17.6.

Monochromatic radiation of different wavelengths is brought to focus on the

instrument slit by rotation of the prism.

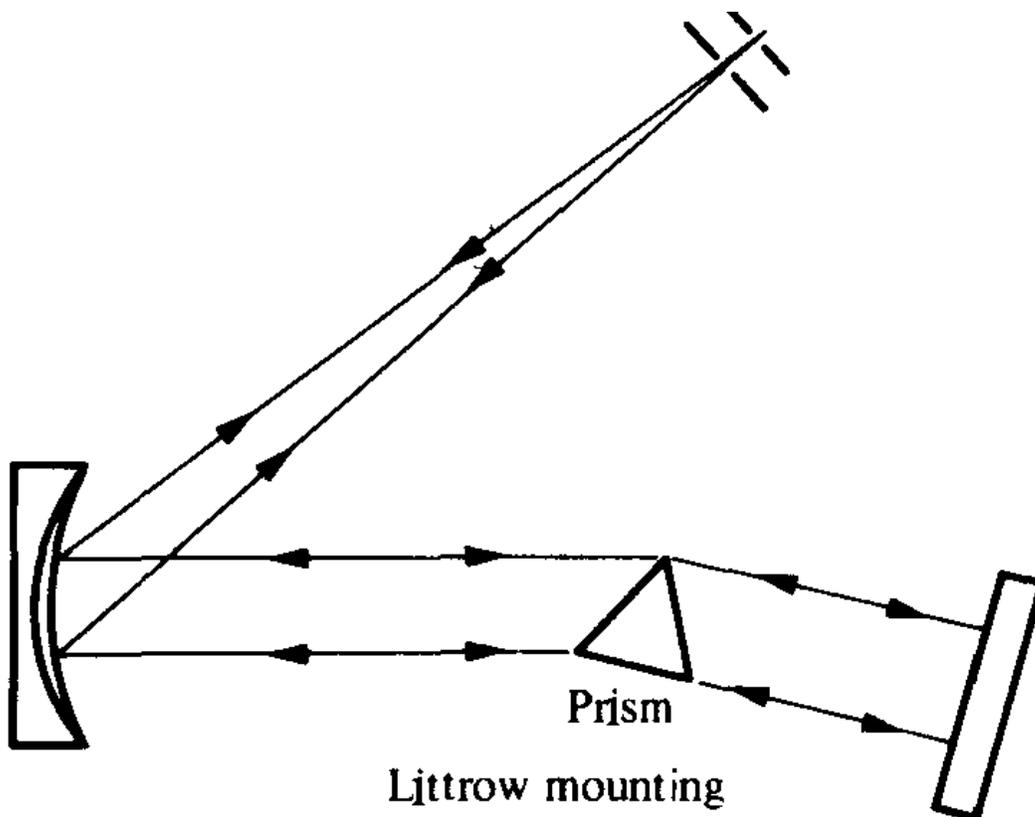


Fig. 17.6

Unfortunately no single material is entirely suitable for use over the full range

of 200-1000 nm, although fused silica is the favourite compromise material. Glass prisms can be employed between 400 and 1000 nm for the visible region,

but are not transparent to ultraviolet radiation. For the region below 400nm quartz or fused silica prisms are required. If quartz is employed for a 60°

single pass prism it is necessary to make the prism in two halves, one half from right-handed quartz and the other from left-handed quartz in order that polarisation effects introduced by one will be reversed by the other.

Prisms have the advantage that, unlike the diffraction gratings described below, they only produce a single-order spectrum.

Diffraction gratings. This alternative method of dispersion uses the principle

of diffraction of radiation from a series of closely spaced lines marked on a surface. Early diffraction gratings were made of glass through which the radiation

passed and became diffracted; these are known as transmission gratings.

To

achieve the diffraction of ultraviolet radiation, however, modern grating spectrophotometers employ metal reflection gratings with which the radiation

is reflected from the surfaces of a series of parallel grooves. These are often

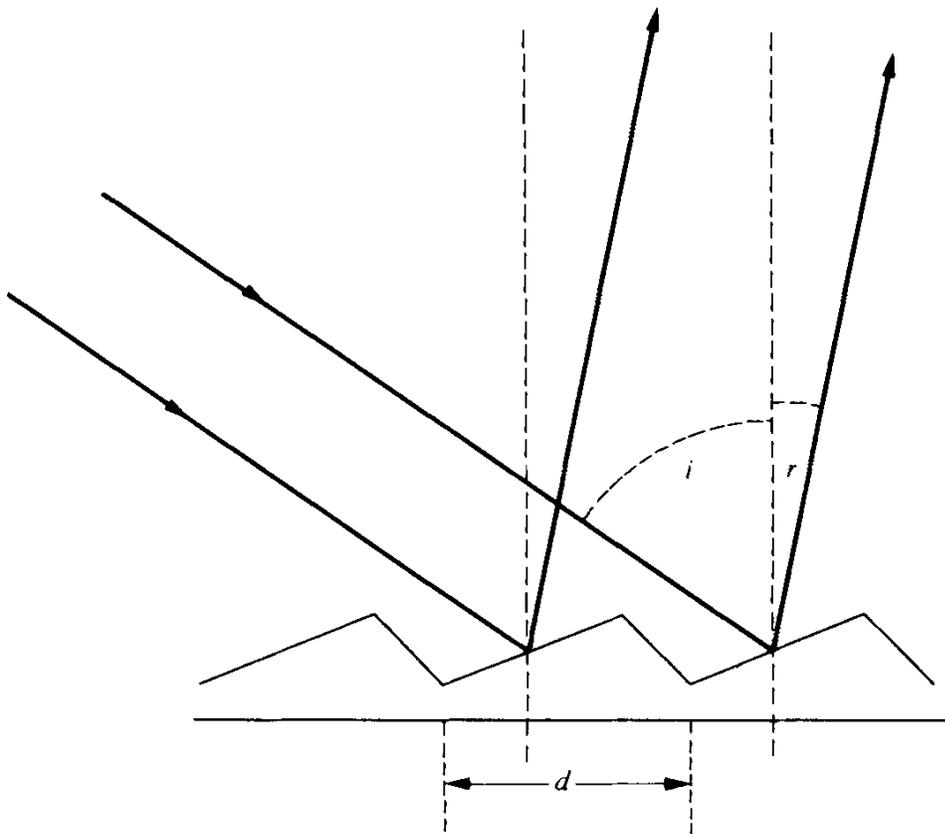
known as echelette gratings.

The principle of diffraction is dependent upon the differences in path length

experienced by a wavefront incident at an angle to the individual surfaces of the grooves of the grating. If i is the angle of incidence and r the angle of reflection the path difference between rays from adjacent grooves is given by

$$d \sin i - d \sin r$$

where d is the distance between the grooves, Fig. 17.7. Because of the path difference that is created, the new wavefronts interfere with each other except



Monochromation and bandwidth. From the foregoing discussion it is clear that for simple absorptimeters (colorimeters) where only the visual spectrum is involved, filters or prisms will suffice for selecting the appropriate spectral region for a given determination. On the other hand, with diffraction gratings, which are employed in spectrophotometers, a much wider range of wavelengths extending into the ultraviolet may be examined, and the instrument is of much greater sensitivity. The portion of the instrument which enables selection of an appropriate spectral region is referred to as the monochromator: it contains, in addition to the prism or diffraction grating, an entrance slit which reduces the incident beam of radiation to a suitable area, and also an exit slit which selects the wavelength of the radiation which is to be presented to the

sample

under investigation. An important feature is the range of wavelengths present

in the beam to which the sample is subjected; this is measured by what is termed

the spectral half-bandwidth, which is the wavelength range embraced by the

beam at the point where the intensity of the beam is one-half of its maximum

value (Fig. 17.8). The width of the slits in the monochromator can be adjusted,

and the narrower the slit the better the resolution of an absorption band, but

decrease in slit width necessarily reduces the intensity of the beam reaching the

detector. In practice, a compromise may need to be made between resolution

and adequate intensity of radiation to permit accurate absorption readings.

17.8 RADIATION SOURCES

For simple absorptiometers a tungsten lamp is the usual source of illumination.

With spectrophotometers, however, to cover the full wavelength range of the

instrument, two lamps are provided. The first is usually a tungsten-halogen (or

quartz-iodine) lamp which covers wavelengths ranging from the red end of

the visible spectrum (750-800 nm) to the near-ultraviolet (300-320 nm): the lamp is provided with a quartz outer sheath to permit use of the ultraviolet part of the emission. For measurements in the far-ultraviolet (down to 200 nm), a hydrogen or a deuterium lamp is used: the latter is preferred on account of the greater intensity of the radiation. This must also be provided with a quartz envelope. Xenon arc lamps with a spectral range of 250-600nm may also be used.

17.9 CELLS

To investigate the absorption of radiation by a given solution, the solution must be placed in a suitable container called a cell (or cuvette) which can be accurately located in the beam of radiation. The instrument is provided with a cell-carrier which serves to site the cells correctly. Standard cells are of rectangular form with a 1 cm light path, but larger cells are available when solutions of low absorbance are to be examined, and likewise for solutions of high absorbance, cells of short path length can be obtained (semimicro or micro cells). For aqueous solutions it is possible to obtain comparatively cheap cells made

of polystyrene. Standard cells are made of glass to cover the wavelength range 340-1000 nm, but for lower wavelengths (down to 220 nm) they must be made of silica, and for the lowest wavelength (down to 185 nm) a special grade of silica must be used. All standard cells are supplied with a lid to prevent spillage, but if volatile solvents are to be used, special cells with a well-fitting stopper should be employed. In addition to the usual rectangular-pattern cell, continuous flow cells (which are useful as chromatographic detectors) and sampling cells (which are fitted with tubes making it possible to empty and fill the cells without removing them from the spectrophotometer) are also available. Standard cells are produced in three grades: Grade A have a path length tolerance of 0.1 per cent; Grade B have a tolerance of 0.5 per cent and are regarded as suitable for routine use; Grade C can have a tolerance of up to 3 per cent. Even the highest quality cells differ slightly from each other and in work of the highest accuracy it is usual to select a matching pair of cells, one to hold the test solution and the other, a blank or reference solution. If unmatched cells are used, then a correction must be applied for the differing

transmissions,

but with many modern spectrophotometers which embody microprocessor control, the necessary correction can be done automatically.

17.1 0 DATA PRESENTATION

The most common method of recording the absorbance of a solution was to

use a micro-ammeter to measure the output of the photoelectric cell, and this

method is still applied to the simplest absorptimeters. The meter is usually

provided with a dual scale calibrated to read (1) percentage absorbance and

(2) transmission. For quantitative measurements it is more convenient to work

in terms of absorbance rather than transmittance; this is emphasised by reference

to the two graphs shown in Fig. 17.9.

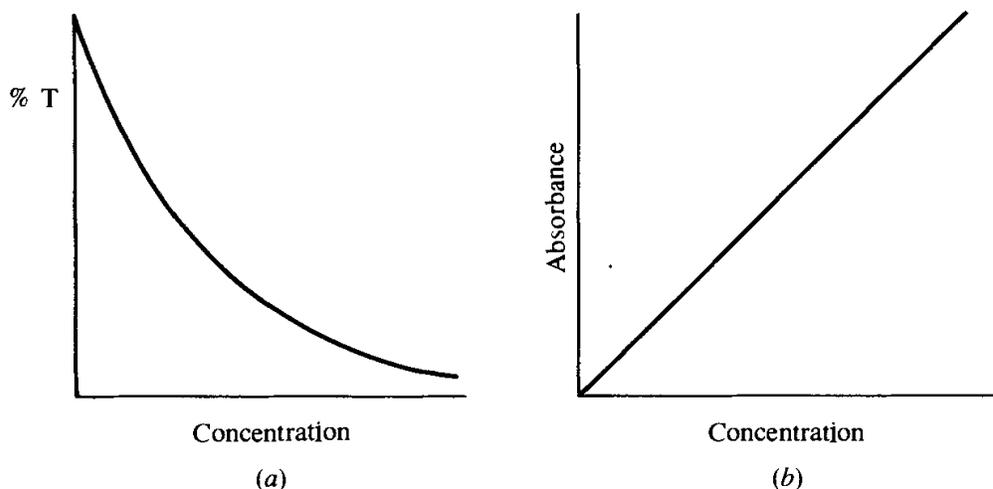


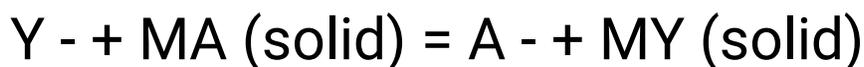
Fig. 17.9 Reproduced with permission from J. E. Steward (Ed.), *Introduction to Ultraviolet and Visible Spectrophotometry*, 2nd edn, Philips/Pye Unicam, Cambridge, 1985.

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SULPHATE determination spectrochemically.

Discussion. The barium Salt of chloranilic acid (2,5-dichloro-3,6-dihydroxy-pbenzoquinone) illustrates the principle of a method which may find wide

application in the colorimetric determination of various anions. In the reaction



where Y^- is the anion to be determined and A^- is the coloured anion of an

organic acid, MY must be so much less soluble than MA that the reaction is quantitative. MA must be only sparingly soluble

so that the blanks will not be too high. Sulphate ion in the range 2-400 mg L⁻¹ may be readily determined by utilising the reaction between barium chloranilate with sulphate ion in acid solution to give barium sulphate and the acid-chloranilate ion:

The amount of acid chloranilate ion liberated is proportional to the sulphate-ion concentration. The reaction is carried out in 50 percent aqueous ethanol buffered at an apparent pH of 4. Most cations must be removed because they form insoluble chloranilates: this is simply effected by passage of the solution through a strongly acidic ion exchange resin in the hydrogen form (see Section 7.2).

Chloride, nitrate, hydrogencarbonate, phosphate, and oxalate do not interfere at the 100mg L⁻¹ level. The pH of the solution governs the absorbance of chloranilic acid solutions at a particular wavelength; chloranilic acid is yellow, acid-chloranilate ion is dark purple, and chloranilate ion is light purple. At

pH 4 the acid-chloranilate ion gives a broad peak at 530 nm, and this wavelength is employed for measurements in the visible region. A much more intense absorption occurs in the ultraviolet: a sharp band at 332 nm enables the limit of detection of sulphate ion to be extended to 0.06 mg L^{-1} .

Procedure. Pass the aqueous solution containing sulphate ion ($2\text{-}400 \text{ mg L}^{-1}$) through a column 1.5 cm in diameter and 15 cm long of Zerolit 225 or equivalent cation exchange resin in the hydrogen form. Adjust the effluent to pH 4 with dilute hydrochloric acid or ammonia solution. Make up to volume in a graduated flask. To an aliquot containing up to 40 mg of sulphate ion in less than 40 mL in a 100 mL graduated flask, add 10 mL of a buffer (pH = 4; a 0.05M solution of potassium hydrogenphthalate) and 50 mL of 95 per cent ethanol. Dilute to the mark with distilled water, add 0.3 g of barium chloranilate and shake the flask for 10 minutes. Remove the precipitated

barium sulphate and the excess of barium chloranilate by filtering or centrifuging. Measure the absorbance of the filtrate with a filter colorimeter or a spectrophotometer at 530nm (green filter) against a blank prepared in the same manner. Construct a calibration curve using standard potassium sulphate solutions prepared from the analytical grade salt.

DETERMINATION OF ARSENIC(III) AND ANTIMONY(III) IN A MIXTURE

Discussion. In acid solution arsenic(III) can be oxidised to arsenic(V) and antimony(III) to antimony(V) by the well-established titration with a solution of potassium bromate and potassium bromide (Section 10.133). The end point for such determinations is usually observed indirectly, and very good results have been obtained by the spectrophotometric method of Sweetser and Briker. No change in absorbance at 326nm

is obtained until all the arsenic(III) has been oxidised, the absorbance then decreases to a minimum at the antimony(III) end point at which it rises again as excess titrant is added.

Reagents. Bromate/bromide solution. Prepare a standard bromate/bromide solution by dissolving 2.78 g potassium bromate and 9.9 g potassium bromide in water and diluting to 1 L in a graduated flask. This solution is 0.017M

potassium bromate with a slight excess of the theoretical amount of potassium bromide. Analytical grade reagents should be employed.

Arsenic/antimony solution. Prepare a mixed solution containing approximately 115 mg arsenic and 160mg antimony in 1 L by dissolving about 150mg arsenic(III) oxide and 300 mg antimony(III) chloride in 6M hydrochloric acid.

Procedure. Place 80 mL of the arsenic/antimony solution in the titration cell of the spectrophotometer. Titrate with standard bromate/bromide solution at

326nm taking an absorbance reading at least every 0.2mL. From the curve obtained calculate the concentration of arsenic and antimony in the solution.

DETERMINATION OF COPPER(II) WITH EDTA

Discussion. The titration of a copper ion solution with EDTA may be carried out photometrically at a wavelength of 745nm. At this wavelength the copper-EDTA complex has a considerably greater molar absorption coefficient than the copper solution alone. The pH of the solution should be about 2.4.

The effect of different ions upon the titration is similar to that given under iron(II) (Section 17.57). Iron(II) interferes (small amounts may be precipitated with sodium fluoride solution): tin(IV) should be masked with 20 per cent aqueous tartaric acid solution. The procedure may be employed for the determination of copper in brass, bronze, and bell metal without any previous separations except the removal of insoluble lead sulphate when present.

Reagents. Copper ion solution, **0.04M**. Wash analytical grade copper with petroleum spirit (b.p. 40-60 °C) to remove any surface grease and dry at 100 °C.

Weigh accurately about 1.25 g of the pure copper, dissolve it in 5 mL of concentrated nitric acid, and dilute to 1 L in a graduated flask. Titrate this standard copper solution with the EDTA solution using fast sulphon black as indicator (Section 10.55), and thus obtain a further check on the molarity of the EDTA.

EDTA solution, 0.10M, and buffer solution. pH 2.2. See Section 17.57.

Procedure. Charge the titration cell (Fig. 17.24) with 10.00 mL of the copper ion solution, 20 mL of the acetate buffer (pH = 2.2), and about 120 mL of water.

Position the cell in the spectrophotometer and set the wavelength scale at 745 nm. Adjust the slit width so that the reading on the absorbance scale is zero. Stir the solution and titrate with the standard EDTA: record the

absorbance every 0.50 mL until the value is about 0.20 and subsequently every 0.20 mL. Continue the titration until about 1.0 mL after the end point; the latter occurs when the absorbance readings become fairly constant. Plot absorbance against mL of titrant added; the intersection of the two straight lines (see Fig. 17.23 C) is the end point. Calculate the concentration of copper ion (mg mL^{-1}) in the solution and compare this with the true value.

DETERMINATION OF IRON(III) WITH EDTA

Discussion. Salicylic acid and iron(II) ions form a deep-coloured complex with a maximum absorption at about 525 nm: this complex is used as the basis for the photometric titration of iron(II) ion with standard EDTA solution. At a pH of ca 2.4 the EDTA-iron complex is much more stable (higher stability constant) than the iron-salicylic acid complex. In the titration of an ironsalicylic

acid solution with EDTA the iron-salicylic acid colour will therefore gradually disappear as the end point is approached. The spectrophotometric end point at 525 nm is very sharp. Considerable amounts of zinc, cadmium, tin(IV), manganese(II), chromium(III), and smaller amounts of aluminium cause little or no interference at pH 2.4: the main interferences are lead(II), bismuth, cobalt(II), nickel, and copper(I).

Reagents. **EDTA** solution, 0.10M. See Section 10.49. Standardise accurately (Section 10.49).

Iron (III) solution, 0.05M. Dissolve about 12.0 g, accurately weighed, of ammonium iron(II) sulphate in water to which a little dilute sulphuric acid is added, and dilute the resulting solution to 500mL in a graduated flask. Standardise the solution with standard EDTA using variamine blue B as indicator (Section 10.56).

Sodium acetate-acetic acid buffer. Prepare a solution which is 0.2 M in sodium

acetate and 0.8M in acetic acid. The pH is 4.0. Sodium acetate-hydrochloric acid buffer. Add 1 M hydrochloric acid to 350 mL of 1 M sodium acetate until the pH of the mixture is 2.2 (pH meter).

Salicylic acid solution. Prepare a 6 per cent solution of salicylic acid in acetone.

Procedure. Transfer 10.00mL of the iron(II) solution to the titration cell (Fig. 17.24), add about 10mL of the buffer solution of pH = 4.0 and about 120 mL of water: the pH of the resulting solution should be 1.7-2.3. Insert the titration cell into the spectrophotometer; immerse the stirrer and the tip of the 5 mL microburette (graduated in 0.02 mL) in the solution. Switch on the tungsten lamp and allow the spectrophotometer to 'warm up' for about 20 minutes. Stir the solution. Add about 4.0 mL of the standard EDTA (note the volume accurately). Set the wavelength at 525 nm, and adjust the slit width of the instrument so that the reading on the

absorbance scale is 0.2-0.3. Now add 1.0 mL of the salicylic acid solution; the absorbance immediately increases to a very large value (> 2). Continue the stirring. Add the EDTA solution slowly from the microburette until the absorbance approaches 1.8; record the volume of titrant. Introduce the EDTA solution in 0.05 mL aliquots and record the absorbance after each addition. Continue the titration until at least four readings are taken beyond the end point (fairly constant absorbance). Plot absorbance against mL of titrant added: the intersection of the two straight lines (see Fig. 17.23 A) gives the true end point. Calculate the concentration of iron(II) (mg mL^{-1}) in the solution and compare this with the true value.

Determination of iron(II) in the presence of aluminium. Iron(II) (concentration ca 50 mg per 100 mL) can be determined in the presence of up to twice the amount of aluminium by photometric titration with EDTA in the presence of

5-sulphosalicylic acid (2 per cent aqueous solution) as indicator at pH 1.0 at a wavelength of 510nm. The pH of a strongly acidic solution may be adjusted to the desired value with a concentrated solution of sodium acetate: about 8-10 drops of the indicator solution are required. The spectrophotometric titration curve is of the form shown in Fig. 17.23.

17.48 SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION (CHROMIUM AND MANGANESE)

Discussion. This section is concerned with the simultaneous spectrophotometric determination of two solutes in a solution. The absorbances are additive, provided there is no reaction between the two solutes. We may write:

$$A_{\lambda_1} = \epsilon_{\lambda_1} A_1 + \epsilon_{\lambda_1} A_2 \quad (14)$$

$$A_{\lambda_2} = \epsilon_{\lambda_2} A_1 + \epsilon_{\lambda_2} A_2 \quad (15)$$

where A_{λ_1} and A_{λ_2} are the *measured* absorbances at the two wavelengths λ_1 and λ_2 ; the subscripts 1 and 2 refer to the two different substances, and the subscripts λ_1 and λ_2 refer to the different wavelengths. The wavelengths are selected to coincide with the absorption maxima of the two solutes: the absorption spectra of the two solutes should not overlap appreciably (compare Fig. 17.20), so that substance 1 absorbs strongly at wavelength λ_1 and weakly at wavelength λ_2 , and substance 2 absorbs strongly at λ_2 and weakly at λ_1 . Now $A = \epsilon cl$, where ϵ is the molar absorption coefficient at any particular wavelength, c is the concentration expressed in mol L⁻¹, and l is the thickness (length) of the absorbing solution expressed in cm. If l is 1 cm:

$$A_{\lambda_1} = \epsilon_{\lambda_1} c_1 + \epsilon_{\lambda_1} c_2 \quad (16)$$

$$A_{\lambda_2} = \epsilon_{\lambda_2} c_1 + \epsilon_{\lambda_2} c_2 \quad (17)$$

Solution of these simultaneous equations gives:

$$c_1 = \frac{\epsilon_{\lambda_2} A_{\lambda_1} - \epsilon_{\lambda_1} A_{\lambda_2}}{\epsilon_{\lambda_1} \epsilon_{\lambda_2} - \epsilon_{\lambda_2} \epsilon_{\lambda_1}} \quad (18)$$

$$c_2 = \frac{\epsilon_{\lambda_1} A_{\lambda_2} - \epsilon_{\lambda_2} A_{\lambda_1}}{\epsilon_{\lambda_1} \epsilon_{\lambda_2} - \epsilon_{\lambda_2} \epsilon_{\lambda_1}} \quad (19)$$

The values of the molar absorption coefficients ϵ_1 and ϵ_2 can be deduced from measurements of the absorbances of pure solutions of substances 1 and 2. By measuring the absorbance of the mixture at wavelengths λ_1 and λ_2 , the concentrations of the two components can be calculated.

The above considerations will be illustrated by the simultaneous determination of manganese and chromium in steel and other ferro-alloys. The absorption spectra of 0.001 M permanganate and dichromate ions in 1 M sulphuric acid, determined with a spectrophotometer and against 1 M sulphuric acid in the reference cell, are shown in Fig. 17.20. For permanganate, the absorption maximum is at 545 nm, and a small correction must be applied for dichromate absorption. Similarly the peak dichromate absorption is at 440 nm, at which permanganate only absorbs weakly. Absorbances for these two ions, individually and in mixtures, obey Beer's Law provided the concentration of sulphuric acid is at least 0.5 M. Iron(III), nickel, cobalt, and vanadium absorb at 425 nm and 545 nm, and should be absent or corrections must be made.

Reagents. *Potassium dichromate.* 0.002 M, 0.001 M, and 0.0005 M in 1 M sulphuric acid and 0.7 M phosphoric(V) acid, prepared from the analytical grade reagents.

Potassium permanganate. 0.002 M, 0.001 M, and 0.0005 M in 1 M sulphuric

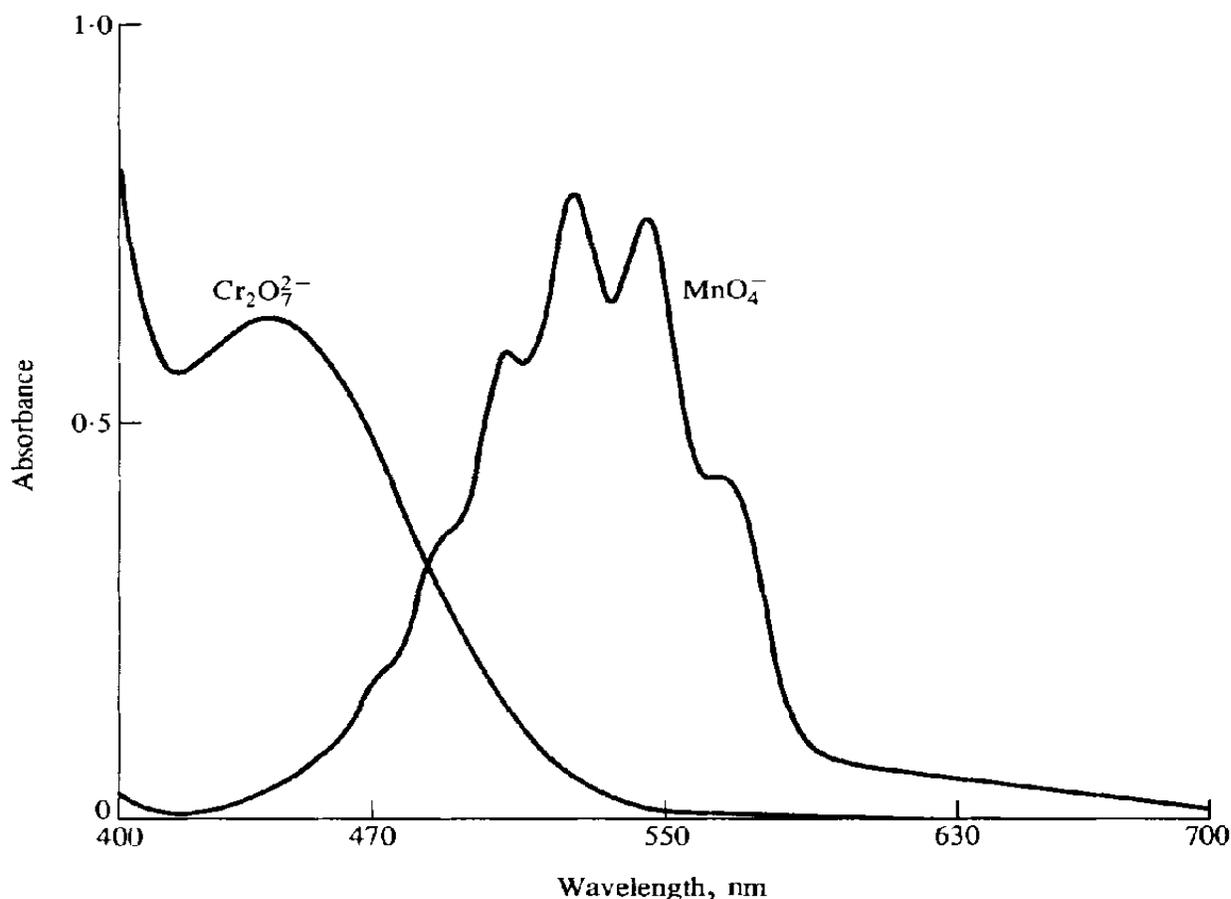


Fig. 17.20

acid and 0.7 M phosphoric(V) acid, prepared from the analytical grade reagents. All flasks must be scrupulously clean.

Procedure. (a) Determination of molar absorption coefficients and verification of additivity of absorbances. The molar absorption coefficients must be determined for the particular set of cells and the spectrophotometer employed. For the present purpose we may write:

$$A = \epsilon cl$$

where ϵ is the molar absorption coefficient, c is the concentration (mol L^{-1}), and l is the cell thickness or length (cm).

Measure the absorbance A of the above three solutions of potassium dichromate and of potassium permanganate, each solution separately, at both 440 nm and 545 nm in 1 cm cells. Calculate ϵ in each case and record the mean values for $\text{Cr}_2\text{O}_7^{2-}$ and MnO_4^- at the two wavelengths.

Mix 0.001 M potassium dichromate and 0.0005 M potassium permanganate in the following amounts (plus 1.0 mL of concentrated sulphuric acid), and prepare a set of results similar to those in Table 17.5, which is a set of typical results included for guidance only. Measure the absorbance of each of the mixtures at 440 nm. Calculate the absorbance of the mixtures from:

$$A_{440} = \epsilon_{\text{Cr}} \cdot c_{\text{Cr}} + \epsilon_{\text{Mn}} \cdot c_{\text{Mn}}$$

Table 17.5 Test of additivity principle with $\text{Cr}_2\text{O}_7^{2-}$ and MnO_4^- mixtures at 440 nm

$\text{K}_2\text{Cr}_2\text{O}_7$ solution (mL)	KMnO_4 solution (mL)	A observed	A calculated
50	0	0.371	—
45	5	0.338	0.340
40	10	0.307	0.308
35	15	0.277	0.277
25	25	0.211	0.214
15	35	0.147	0.151
5	45	0.086	0.088
0	50	0.057	—

(b) Determination of chromium and manganese in an alloy steel.* Weigh out accurately about 1.0 g of the alloy steel into a 300 mL Kjeldahl flask, add 30 mL of water and 10 mL of concentrated sulphuric acid [also 10 mL of 85 per cent phosphoric(V) acid if tungsten is present]. Boil gently until decomposition is complete or the reaction subsides. Then add 5 mL of concentrated nitric acid in several small portions. If much carbonaceous residue persists, add a further 5 mL of concentrated nitric acid, and boil down to copious fumes of sulphuric acid. Dilute to about 100 mL and boil until all salts have dissolved. Cool, transfer to a 250 mL graduated flask, and dilute to the mark.

Pipette a 25 mL or 50 mL aliquot of the clear sample solution into a 250 mL conical flask, add 5 mL concentrated sulphuric acid, 5 mL 85 per cent phosphoric(V) acid, and 1–2 mL of 0.1 M silver nitrate solution, and dilute to about 80 mL. Add 5 g potassium persulphate, swirl the contents of the flask until most of the salt has dissolved, and heat to boiling. Keep at the boiling point for 5–7 minutes. Cool slightly, and add 0.5 g pure potassium periodate. Again heat to boiling and maintain at the boiling point for about 5 minutes. Cool, transfer to a 100 mL graduated flask, and measure the absorbances at 440 nm and 545 nm in 1 cm cells.

Calculate the percentage of chromium and manganese in the sample. Use equations (18) and (19) and values of the molar absorption coefficients ϵ determined above: these will give concentrations expressed in mol L^{-1} , from which values the percentages can readily be calculated. Each value will require correction for the amounts of vanadium, cobalt, nickel, and iron which may be present, using Table 17.6. The values listed are the equivalent percentages of the respective constituent to be subtracted from the apparent Cr and Mn percentages for each 1 per cent of the element in question. It can be shown that utilising the known (or determined) molar absorption coefficients ($_{545}\epsilon_{\text{Mn}}$ 2.35; $_{545}\epsilon_{\text{Cr}}$ 0.011; $_{440}\epsilon_{\text{Cr}}$ 0.369; $_{440}\epsilon_{\text{Mn}}$ 0.095):

$$\text{Mn, per cent} = \frac{0.00549V}{W}(0.426A_{545} - 0.013A_{440})$$

$$\text{Cr, per cent} = \frac{0.01040V}{W}(2.71A_{440} - 0.110A_{545})$$

for a sample of W grams in a volume of V mL.