Prepare a standard solution of magnesium (125 $\mu g\,ml^{-1}$) by dissolving oxide-free magnesium ribbon (125 mg) in 5 ml hydrochloric acid (S.G. 1.18) and diluting to 1 l with *water*. Dilute 5 ml of this solution to 100 ml, giving a standard solution of magnesium (6.25 $\mu g\,ml^{-1}$)

Method Prepare a series of standard solutions of calcium (0, 0.5, 1.0, 1.5, 2.0 and 2.5 $\mu g\,ml^{-1}$) by appropriate dilutions of the standard solution (10 $\mu g\,ml^{-1}$). Also prepare a series of solutions of magnesium (0, 0.125, 0.25, 0.375, 0.50 and 0.625 $\mu g\,ml^{-1}$) by appropriate dilutions of the standard solution (6.25 $\mu g\,ml^{-1}$).

To 1 ml of the serum add 10 ml of 1% lanthanum solution (Experiment 6) and dilute to 50 ml with *water*. Record the absorbances at 422.7 nm of the diluted serum samples and the calibration series of standard solutions of calcium in a 10 cm air–acetylene flame. Similarly record the absorbances at 285.2 nm of the diluted serum sample and the calibration series of standard solutions of magnesium. Calculate the concentrations of calcium and magnesium in the serum with reference to the appropriate calibration graphs.

Experiment 7 *Determination of thiomersal (0.002%) as mercury in a solution for contact lenses*

The direct examination of the solution by atomic absorption is complicated by (a) the relatively low sensitivity of the method for mercury when using a flame, (b) the different response given by $Hg^{+}$and Hg^{2+} and (c) the organic combination of mercury in the bacteriostat (*Thiomersal*). A considerable increase in sensitivity is obtained by converting organically combined mercury to the mercury (II) salt followed by reduction to Hg and sweeping this as vapour through a gas cell with end-windows of quartz. Absorption of 254 nm radiation gives adequate response for less than 0.1 μg of mercury. The method is the basis for the determination of the very low levels of mercury encountered in pollution studies.

Apparatus For the Pye-Unicam, EEL 240 and Perkin-Elmer instruments the gas cell may be purchased to fit into the space occupied by the burner, but, if no such accessory is available, it may be easily prepared from glass tubing and two end-windows of quartz or silica. Figure 8.5 illustrates the cell and general arrangement. The pathlength of the cell is about 11 cm with end-windows 22 mm diameter fixed with any convenient resin, which also serves to hold the glass tube for locating the cell in position in the place normally occupied by the burner.

The coarse sinter-glass filter should be fairly large but capable of being covered by 5 ml of solution. A 50 ml Quickfit boiling tube is convenient for holding the sample. The delivery of air should be about 1000 ml min^{-1} as supplied by a small pump. The system as described is open-ended but the vapour from the sample may be vented to the atmosphere through dilute HNO_3. A closed-circuit system is also possible but a more elaborate pump is required and all leaks must be eliminated.

Reagent Tin(II) chloride solution made by dissolving tin(II) chloride A.R. (20 g) in hydrochloric acid (20 ml) and water (20 ml), boiling in the presence of tin granules and diluting to 100 ml with *water*.

Method Evaporate the sample (5 ml) to dryness on a boiling water-bath, add sulphuric acid (1 ml) and continue heating with the occasional addition of a few drops of hydrogen

quinine sulphate may be assayed with good accuracy and precision in Quinine Sulphate Tablets (300 mg) by measuring the absorbance at 348 nm of a filtered extract of the tablet powder in 0.1M hydrochloric acid. However, low dosage drug formulations containing less than 1 mg per dose unit and biological samples (blood, urine etc.) containing low concentrations of drugs, may require the high sensitivity of spectrofluorimetry, which is the spectrophotometric method of choice for many hormones, alkaloids and vitamins in formulation or biological samples.

Selectivity

Two factors confer on spectrofluorimetry a greater selectivity than that given by ultraviolet–visible absorption spectrophotometry. First, not all substances that absorb in the ultraviolet–visible region fluoresce. In non-fluorescent molecules, absorbed energy is lost by alternative radiationless pathways, principally by internal conversion (p.263). Molecules require, in addition to a chromophore (p.315), a degree of rigidity in their structure to reduce the dissipation of absorbed energy by internal conversion.

Substances that are fluorescent are characterised by their wavelengths of maximum excitation and maximum emission. Different fluorescent species may show different wavelengths of maximum excitation and/or emission. The facility to vary independently the wavelength of excitation and the wavelength of fluorescence allows the analyst to select the optimum combination of wavelengths for the analyte and to reduce interference from other fluorescing species in the sample.

Quantitative aspects

Many of the quantitative aspects of spectrofluorimetry may be understood by reference to the fundamental equation for the intensity of fluorescence emitted. This equation may be derived from that of the Beer–Lambert Law

$$\frac{I_0}{I_T} = 10^{abc} \qquad \text{(Chapter 7, p.277)}$$

$$\therefore \quad I_T = I_0 \times 10^{-abc}$$

But fluorescence $(F) = (I_0 - I_T)\Phi$ where $\Phi =$ quantum yield of fluorescence

$$\therefore \quad F = (I_0 - I_0 \times 10^{-abc})\Phi$$
$$= I_0(1 - 10^{-abc})\Phi$$
$$= I_0(1 - e^{-2.3abc})\Phi$$

Now

$$e = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \ldots$$

Experiment 4 *To determine the concentration of quinine sulphate in Ferrous Phosphate Syrup with Strychnine and Quinine*

Method Dilute the syrup (about 2 g, accurately weighed) with 0.05M sulphuric acid to 100 ml. Dilute this solution further to give a concentration of quinine sulphate in 0.05M sulphuric acid of about 0.4 $\mu g\,ml^{-1}$. Measure the fluorescence (λ_{ex} = 350 nm; λ_{em} = 450 nm) of this solution and of a series of standard solutions of quinine sulphate (0.0 to 0.5 $\mu g\,ml^{-1}$) in 0.05M sulphuric acid. Read the concentration of quinine sulphate from a calibration graph or calculate it by using linear regression analysis (p.280). Determine the weight per ml of the syrup and calculate the concentration of anhydrous quinine (as % w/v) in the sample.

Experiment 5 *To determine the concentration of quinine in urine*

The assay may be applied to urine spiked with quinine (0.2 to 0.4 $\mu g\,ml^{-1}$) or to a sample of urine collected over 24 h after drinking 100–200 ml of tonic water. The latter sample may require a modification to the procedure, e.g. a further dilution, to cope with concentrations outside the 0.2 to 0.5 $\mu g\,ml^{-1}$ range. The method is based upon that of Mulé and Hushin (1971).

Method Transfer 5 ml of urine to a 35 ml stoppered centrifuge tube, adjust the pH to 9–10 with drops of dilute ammonium hydroxide solution and extract with 10 ml of chloroform : propan-2-ol (3:1 v/v) for 1 min. Allow the phases to separate, with centrifugation if necessary. Transfer 5 ml of the organic phase to a dry centrifuge tube, extract with 5 ml of 0.05M sulphuric acid for 1 min and centrifuge. Measure the fluorescence of the acidic extract (λ_{ex} = 350 nm; λ_{em} = 450 nm) and of a standard solution of quinine (0.5 $\mu g\,ml^{-1}$) and blank carried through an identical procedure.

Experiment 6 *To show quenching of fluorescence*

(a) *Inner-filter effect* Dissolve 25 mg quinine sulphate in sufficient 0.05M sulphuric acid to produce 500 ml of solution. By dilution with 0.05M sulphuric acid, prepare two series of solutions of quinine sulphate so that the concentrations are (i) 0.1, 0.2, 0.3, 0.4, and 0.5, and (ii) 10, 20, 30, 40, and 50 $\mu g\,ml^{-1}$ respectively. Measure the fluorescence (λ_{ex} = 350 nm; λ_{em} = 450 nm) of the first series setting the sensitivity with the 0.5 $\mu g\,ml^{-1}$ solution and of the second series setting the sensitivity with the 50 $\mu g\,ml^{-1}$ solution. Plot the measurements against concentration in the normal manner (see Fig. 9.2).

(b) *Collisional quenching* Using the 50 $\mu g\,ml^{-1}$ standard solution of quinine sulphate, prepared above, and a solution of 0.1M potassium iodide, prepare solutions of quinine sulphate (0.5 $\mu g\,ml^{-1}$) in 0.05M sulphuric acid containing 0, 0.0005, 0.001, 0.0015, 0.002, 0.0025, 0.005, 0.0075, 0.01 and 0.015M potassium iodide. Plot the fluorescence against concentration of potassium iodide.

The fluorescence of the weak solutions shows a linear relationship with concentration. The stronger solutions show marked quenching effects due in this instance to the inner-filter effect (p.362). It is thus possible for a particular intensity of fluorescence to correspond to two concentrations of the substance. In fluorimetric assays, therefore, it is

10
Infrared Spectrophotometry

A.G. DAVIDSON

Introduction

The infrared (IR) region of the electromagnetic spectrum extends from 0.8 μm (800 nm) to 1000 μm (1 mm) and is subdivided into near infrared (0.8 to 2 μm), middle infrared (2 to 15 μm), and far infrared (15 to 1000 μm).

The **fundamental region** between 2 and 15 μm is the region that provides the greatest information for the elucidation of molecular structure and most IR spectrophotometers are limited to measurements in this region.

Absorption of IR radiation causes changes in vibrational energy in the ground state of the molecule. The transition from vibrational level 0 to vibrational level 1 (Fig 6.5(a)) gives rise to the fundamental absorption of the molecule, and overtones or harmonics are caused by the transitions 0–2, 0–3 and so on, though the intensity of absorption for these overtones is very much less than that for the fundamental frequencies. For energy to be transferred from the light source to the molecule the frequency of vibration of each must coincide and, moreover, must be accompanied by a change in the dipole moment of the molecule. Certain symmetrical molecules, e.g. ethene, show no change in dipole moment during a stretching vibration and such vibrations do not result in the absorption of IR radiation. The C=C stretching vibration of ethene is described as **infrared inactive**.

Just as ultraviolet absorption spectra are strictly caused by changes in electronic, vibrational and rotational energy, so IR absorption spectra are due to changes in vibrational energy accompanied by changes in rotational energy. For simple molecules in the gas phase this leads to results from which information may be obtained on force constants, bond lengths, moments of inertia and, where adequate resolution is available, on isotopes in an easily accessible region of the spectrum. Experiment 1 for hydrochloric acid illustrates some of these points. For non-linear polyatomic molecules the number of normal modes of vibration is given by the expression $3n - 6$ ($3n - 5$ for a linear molecule) where n = number of atoms. All these vibrations occur at the same time and an IR spectrophotometer has been likened to a stroboscope in that it enables the particular frequencies of vibration to be recorded.