Ultraviolet and visible spectroscopy

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Lecture 2

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particular direction can distort its symmetry and thus allow all three double bonds to be involved in an excited state. If the symmetry of the benzene ring is lowered by substitution, the bands in the benzene spectrum undergo a bathochromic shift – a shift to longer wavelength. Substitution can involve either extension of the chromophore or attachment of an auxochrome (a group containing one or more lone pair of electrons) to the ring or both. Table 4.2 summarises the absorption bands found in some simple aromatic systems and these chromophore/auxochrome systems provide the basis for absorption of UV radiation by many drugs. The hydroxyl group and amino group auxochromes are affected by pH, undergoing bathochromic (moving to a longer wavelength) and hyperchromic (absorbing more strongly) shifts when a proton is removed under alkaline conditions, releasing an extra lone pair of electrons. The effect is most marked for aromatic amine groups. The absorption spectrum of a drug molecule is due to the particular combination of auxochromes and chromophores present in its structure.

Beer–Lambert Law

Figure 4.3 shows the absorption of radiation by a solution containing a UV-absorbing compound.

The measurement of light absorption by a solution of molecules is governed by the Beer–Lambert Law, which is written as follows:

$$\log I_o/I_t = A = \varepsilon bc$$

where I_o is the intensity of incident radiation; I_t is the intensity of transmitted radiation; A is known as the absorbance and is a measure of the amount of light absorbed by the sample; ε is a constant known as the molar extinction coefficient and is the absorbance of a 1 M solution of the analyte; b is the pathlength of the cell in cm, usually 1 cm; and c is the concentration of the analyte in moles litre^{-1}.

Self-test 4.1

Calculate the percentage of the incident radiation absorbed by a sample with an absorbance of (i) 2; (ii) 0.1.

Answers: (i) 99.0%; (ii) 20.6%

In pharmaceutical products, concentrations and amounts are usually expressed in grams or milligrams rather than in moles and, thus, for the purposes of the

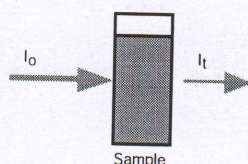

Fig. 4.3 Absorption of light by a solution.

analysis of these products, the Beer-Lambert equation is written in the following form:

$$A = A\,(1\%,\ 1\ \text{cm})\ bc$$

where A is the measured absorbance; A (1%, 1 cm) is the absorbance of a 1% w/v (1 g/100 ml) solution in a 1 cm cell; b is the pathlength in cm (usually 1 cm); and c is the concentration of the sample in g/100 ml. Since measurements are usually made in a 1 cm cell, the equation can be written:

$$\left[c = \frac{A}{A(1\%,\ 1\ \text{cm})}\right]$$

which gives the concentration of the analyte in g/100 ml.

BP monographs often quote a standard A (1%, 1 cm) value for a drug, which is to be used in its quantitation.

Self-test 4.2

What are the concentrations of the following solutions of drugs in g/100 ml and mg/100 ml?

(i) Carbimazole, A (1%, 1 cm) value = 557 at 291 nm, measured absorbance = 0.557 at 291 nm.
(ii) Hydrocortisone sodium phosphate, A (1%, 1 cm) value = 333 at 248 nm, measured absorbance = 0.666 at 248 nm.
(iii) Isoprenaline, A (1%, 1 cm) value = 100 at 280 nm, measured absorbance, = 0.500 at 280 nm.

Answers: (i) Carbimazole 0.001 g/100 ml, 1 mg/100 ml; (ii) Hydrocortisone sodium phosphate 0.002 g/100 ml, 2 mg/100 ml; (iii) Isoprenaline 0.005 g/100 ml, 5 mg/100 ml.

Instrumentation

A simple diagram of a UV/visible spectrophotometer is shown in Figure 4.4. The components include:

(i) *The light sources* – a deuterium lamp for the UV region from 190 to 350 nm and a quartz halogen or tungsten lamp for the visible region from 350 to 900 nm.
(ii) *The monochromator* – used to disperse the light into its constituent wavelengths, which are further selected by the slit. The monochromator is rotated so that a range of wavelengths is passed through the sample as the instrument scans across the spectrum.

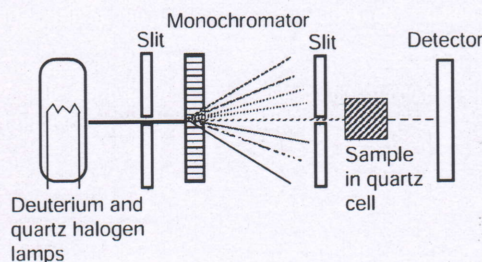

Fig. 4.4 Schematic diagram of a UV/visible spectrophotometer.

(iii) *The optics* – may be designed to split the light beam so that the beam passes through two sample compartments, and, in such a double-beam instrument, a blank solution can then be used in one compartment to correct the reading or spectrum of the sample. The blank is most commonly the solvent in which the sample is dissolved.

Diode array instruments

A photomultiplier tube is used for detection in older types of UV/visible instrument but, increasingly, photodiodes are used as detectors in spectrophotometers. A diode array consists of a series of photodiode detectors positioned side by side on a silicon crystal. The array typically contains between 200 and 1000 elements, depending on the instrument. The scan cycle is *ca* 100 ms, compared with the minute or more required to obtain a spectrum with a traditional scanning instrument. Light is passed through a polychromator, which disperses it so that it falls on the diode array, which measures the whole range of the spectrum at once. This type of instrumentation is useful in, for instance, the dissolution testing of multicomponent formulations where it is possible to select wavelengths that are specific for the different analytes of interest.

Instrument calibration

Pharmacopoeial monographs usually rely on standard A (1%, 1 cm) values in order to calculate the concentration of drugs in extracts from formulations. In order to use a standard value, the instrument used to make the measurement must be properly calibrated with respect to its wavelength and absorption scales. In addition, checks for stray light and spectral resolution are run. These checks are now often built into the software of UV instruments so that they can be run automatically, to ensure that the instrument meets good manufacturing practice requirements. Some of the practical aspects of UV/visible spectrophotometry are described in Box 4.1.

Box 4.1 Practical aspects of UV/visible spectrophotometry

- Care should be taken to avoid touching the optical surfaces of sample cells with the fingers since fingerprints can cause significant absorbance. The optical surfaces of the cell can be wiped carefully with tissue.
- The precision of the pathlength of cells is important. Tolerances for cells of good quality are ± 0.01 mm for pathlength. For maximum quantitative accuracy, the same cell should be used for measurement of both the standard and the sample. The cell should always face in the same direction in a cell holder to ensure that any cell optical effects are identical for both blank and sample measurements.
- Distilled water is the ideal solvent but is not suitable for many organic compounds. Methanol and ethanol are next best but they cannot be used below a wavelength of 210 nm.
- The solvent used to dissolve the sample, concentration, pH, and temperature can affect the position and intensity of absorption bands of molecules. These factors should be controlled as far as possible. Expansion of organic solvent with temperature can cause a change in the reading, as can evaporation; thus sample cells should have tops, particularly if an organic solvent is being used.
- Ideally absorbances measured should be in the range 0.4–1.0 to avoid being outside the linear range of the instrument.
- Scattering gives an apparent increase in absorbance and is caused by particles suspended in solution. It is important that the sample solutions are free from particles.

Calibration of absorbance scale

The British Pharmacopoeia (BP) uses potassium dichromate solution to calibrate the absorbance scale of a UV spectrophotometer; the *A* (1%, 1 cm) values at specified wavelengths have to lie within the ranges specified by the BP. The spectrum of a 0.006% w/v solution of potassium dichromate in 0.005 M H_2SO_4 is shown in Figure 4.5. The absorbance scale calibration wavelengths, with corresponding *A* (1%, 1 cm) values for 0.006% w/v potassium dichromate solution, that are specified by the BP are as follows: 235 nm (122.9–126.2), 257 nm (142.4–145.7), 313 nm (47.0–50.3), 350 nm (104.9–108.2).

Calibration of wavelength scale

The wavelength scale of a UV/visible spectrophotometer is checked by determining the specified wavelength maxima of a 5% w/v solution of holmium perchlorate. Figure 4.6 shows the spectrum of holmium perchlorate; the tolerances for calibration wavelengths specified by the BP are: 241.15 ± 1 nm, 287.15 ± 1 nm and 361.5 ± 1 nm.

The wavelength scale may also be calibrated according to the spectral lines of deuterium or mercury discharge lamps and such tests may be built into some instruments.

Determination of instrumental resolution

The resolving power of an instrument is controlled by its slit width settings. For some pharmacopoeial tests a certain resolution is specified. The resolving power of an instrument can be assessed by using a 0.02% w/v solution of toluene in hexane.

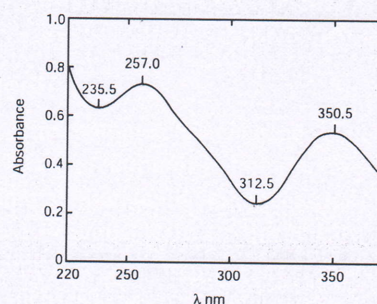

Fig. 4.5
The UV spectrum of 0.006% w/v potassium dichromate solution between 220 and 350 nm.

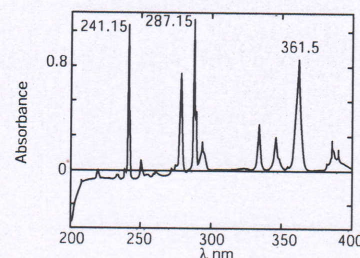

Fig. 4.6
The absorbance maxima of a 5% w/v solution of holmium perchlorate between 200 and 400 nm.