# 4 Ultraviolet and visible spectroscopy



## KEYPOINTS

**Principles**

Radiation in the wavelength range 200-700 nm is passed through a solution of a compound. The electrons in the bonds within the molecule become excited so that they occupy a higher quantum state and in the process absorb some of the energy passing through the solution. The more loosely held the electrons are within the bonds of the molecule the longer the wavelength (lower the energy) of the radiation absorbed.

**Applications in pharmaceutical analysis**

- A robust, workhorse method for the quantification of drugs in formulations where there is no interference from excipients.
- Determination of the p*K*a values of some drugs.
- Determination of partition coefficients and solubilities of drugs.
- Used to determine the release of drugs from formulations with time, e.g. in dissolution testing.
- Can be used to monitor the reaction kinetics of drug degradation.
- The UV spectrum of a drug is often used as one of a number of pharmacopoeial identity checks.

**Strengths**

- An easy-to-use, cheap and robust method offering good precision for making quantitative measurements of drugs in formulations
- Routine method for determining some of the physico-chemical properties of drugs which need to be known for the purposes of formulation
- Some of the problems of the basic method can be solved by the use of derivative spectra.

**Limitations**

- Only moderately selective. The selectivity of the method depends on the chromophore of the individual drugs, e.g a coloured drug with an extended chromophore is more distinctive than a drug with a simple benzene ring chromophore
- Not readily applicable to the analysis of mixtures.

## Introduction

The interaction between radiation and matter is a fascinating area in its own right. Most drug molecules absorb radiation in the ultraviolet region of the spectrum although some are coloured and thus absorb radiation in the visible region, e.g. a substance with a blue colour absorbs radiation in the red region of the spectrum. The absorption of UV/visible radiation occurs through the excitation of electrons within the molecular structure to a higher energy state; Figure 4.1 illustrates the nature of the transitions taking place. These transitions occur from the bottom vibrational state in the electronic ground state of the molecule to any one of a number of vibrational levels in the electronic excited state. The transition from a single ground state energy to one of a number of excited states gives width to UV spectra. Figure 4.1 shows a UV spectrum in which individual bands for different Vo to Vn transitions can be seen. Vibrational fine structure can be seen although the bands overlap extensively; the vibrational bands themselves have width due to rotational transitions which are intermediate in energy between each vibrational transition. The relative energy of electronic:vibrational:rotational transitions is 100:1:0.01. In most molecules the vibrational behaviour is complex and the degree of overlap of the different energies of the vibrational transitions is too great for vibrational fine structure to be observed.

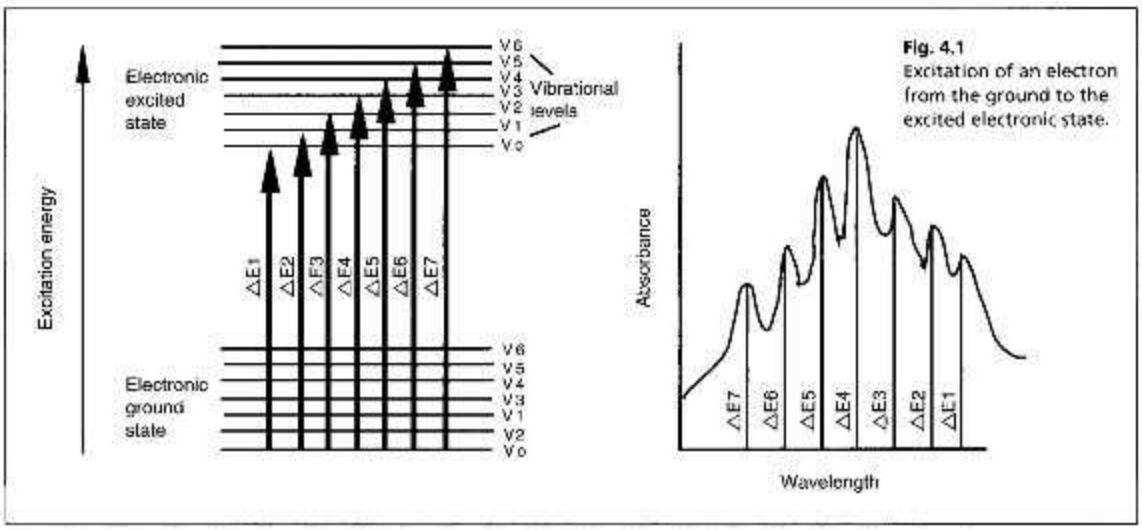



**Fig. 4.1**
Excitation of an electron from the ground to the excited electronic state.

## Factors governing absorption of radiation in the UV/visible region

Radiation in the UV/visible region is absorbed through excitation of the electrons involved in the bonds between the atoms making up the molecule so that the electron cloud holding the atoms together redistributes itself and the orbitals occupied by the bonding electrons no longer overlap. Short wavelength UV radiation < 150 nm (> 8.3 eV) can cause the strongest bonds in organic molecules to break and thus is very damaging to living organisms. It is the weaker bonds in molecules that are of more interest to analysts because they can be excited by longer wavelength UV radiation > 200 nm (> 6.2 eV), which is at a longer wavelength than the region in which air and common solvents absorb. Examining a very simple organic molecule such as ethylene (Fig. 4.2) it can be seen that it contains two types of carbon–carbon bonds, a strong σ bond formed by extensive overlap of the $sp^2$ orbitals of the two carbons and a weaker π bond formed by partial overlap of the p orbitals of the carbon atoms. The σ bond would become excited and break when exposed to radiation at *ca* 150 nm. The weaker π bond requires less energetic radiation at *ca* 180 nm to produce the π* excited state shown in Figure 4.2. This excitation can occur without the molecule falling apart since the σ orbitals remain unexcited by the longer wavelength radiation at 180 nm. However, a single double bond is still not useful as a chromophore for determining analytes by UV spectrophotometry since it is still in the region where air and solvents absorb.

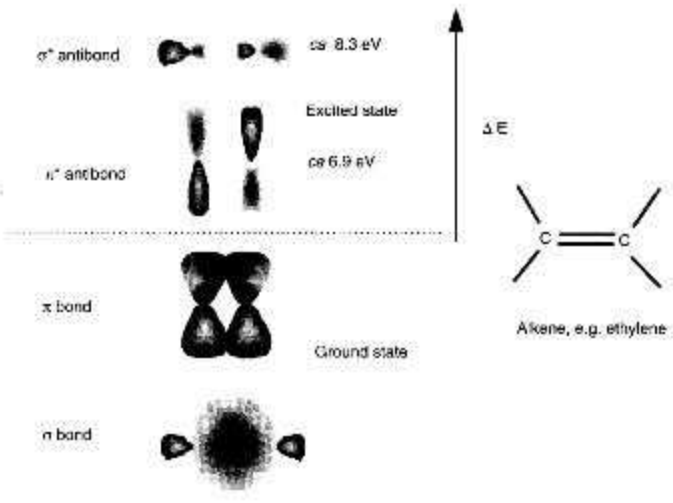



**Fig. 4.2**
Excitation of the carbon–carbon bonds in ethylene by radiation in the short wavelength UV region.

If more double bonds are present in a structure in conjugation (i.e. two of more double bonds in a series separated by a single bond) absorption takes place at longer wavelengths and with greater intensity as detailed in Table 4.1 for a series of polyenes. The *A* (1%,1 cm) value, which is described later, gives a measure of the intensity of absorption. The type of linear conjugated system which is present in polyenes is not very common in drug molecules.

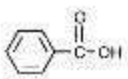
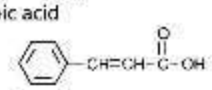
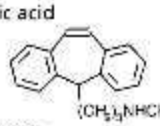
Such extended systems of double bonds are known as 'chromophores'. The most common chromophore found in drug molecules is a benzene ring (Table 4.2).

**Table 4.1** Longest wavelength maxima and absorption intensities of some polyenes

| Polyene | λ max | *A* (1%, 1 cm) |
|---|---|---|
| $CH_3(CH{=}CH)_2CH_3$ | 275 | 2800 |
| $CH_3(CH{=}CH)_3CH_3$ | 310 | 6300 |
| $CH_3(CH{=}CH)_4CH_3$ | 342 | 9000 |
| $CH_3(CH{=}CH)_5CH_3$ | 380 | 9800 |

Benzene itself has its λ max at a much shorter wavelength than a linear triene such as hexatriene (λ max 275 nm) and its strongest absorbance is at the wavelength of absorption of an isolated double bond at 180 nm. It also has a strong absorption band at 204 nm. This is due to the symmetry of benzene; it is not possible to have an excited state involving all three bonds in benzene because this would mean that the dipole (polarisation of the chromophore), a two-dimensional concept which is created in the excited state, would be symmetrical and thus would have to exist in three dimensions rather than two. There is a weak absorption in the benzene spectrum close to the λ max for hexatriene and this can occur because vibration of the benzene ring in a 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 either involve 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.

**Table 4.2** The UV absorption characteristics of some chromophores based on the benzene ring

| Chromophore | Longest wavelength λ max | (*A* 1%, 1 cm) |
|---|---|---|
|  Benzene | 255 nm | 28 |
|  Benzoic acid | 273 | 85 |
|  Cinnamic acid | 273 | 1420 |
|  Protriptyline | 292 | 530 |
|  Phenol | 270 nm ⇌ 287 nm<br>Bathochromic | 172 ⇌ 271<br>Hyperchromic |
|  Aniline | 255 nm ⇌ 286 nm<br>Bathochromic | 16 ⇌ 179<br>Hyperchromic |

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.

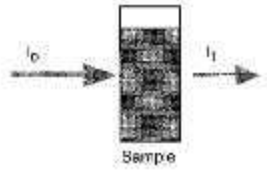



**Fig. 4.3**
Absorption of light by a solution.

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

$$\log I_0/I_t = A = \varepsilon\, b\, c$$

where $I_0$ 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; $\varepsilon$ is a constant known as the molar extinction coefficient and is the absorbance of a 1M 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 liter$^{-1}$.

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

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

$A$ 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:

$$c = \frac{A}{A\,(1\%,\ 1\ \text{cm})} \quad \text{which gives the concentration of the analyte in g/100 ml}$$

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; isoprenaline: (iii) 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:

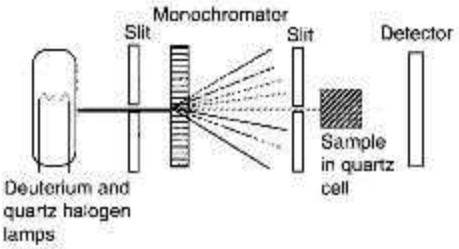



**Fig. 4.4** Schematic diagram of a UV/visible spectrophotometer.

(i) *The light sources* – a deuterium lamp for the UV region from 190–350 nm and a quartz halogen or tungsten lamp for the visible region from 350–900 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.
(iii) *The optics* – may be designed to split the light beam so that the beam passes though 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.

## 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.

### 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 0.0065% 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 0.0065% w/v potassium dichromate solution which 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).

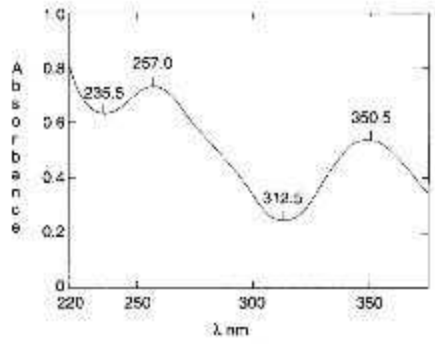



**Fig. 4.5**
The UV spectrum of 0.0065% w/v potassium dichromate solution between 220 and 350 nm.

## 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.

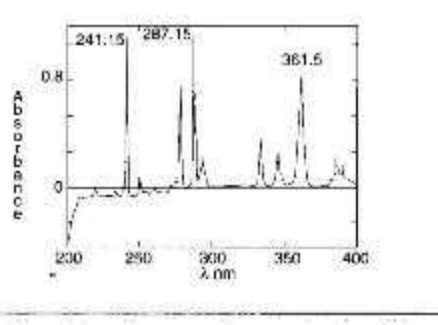



**Fig. 4.6**
The absorbance maxima of a 5% w/v solution of holmium perchlorate between 200 and 400 nm.

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. The BP specifies that the ratio of the absorbance for this solution at 269 nm to that at 266 nm should be at least 1.5.

## Determination of stray light

Stray light is light which falls on the detector within a UV instrument without having passed through the sample. It can arise either from light scattering within the instrument or by entry of light into the instrument from outside. It gives a false low absorbance reading for the sample since it appears as though the sample is absorbing less light than it actually is. This is most serious where the sample has a high absorbance, e.g. at an absorbance of 2 the sample is absorbing most of the light passing through it and thus it would only require very low intensity stray light to lower the reading substantially. Stray light is checked by measuring the absorbance of a 1.2% solution of KCl in water against a water blank at a wavelength of 200 nm. If the absorbance of the sample is < 2 then stray light is present and the instrument needs to be serviced.

# UV spectra of some representative drug molecules

## Steroid enones

The chromophores of most drugs are based on a modification of the benzene ring chromophore. One large class of drugs that do not fit into this category are steroidal androgens and corticosteroids. The spectra of hydrocortisone and betamethasone are shown in Figure 4.7. These spectra are common to many steroids and all have absorbance maxima of similar intensity at around 240 nm. The extra double bond in betamethasone as compared with hydrocortisone does not make a great difference to the wavelength of maximum absorption since it does not extend the original chromophore linearly. However, the shape of the absorption band for betamethasone is quite different from that for hydrocortisone. Such differences in the spectra can be be employed in qualitative identity tests; these are used particularly in conjunction with HPLC identification checks where the method of detection is by diode array UV spectrophotometry (Ch. 12 p. 250).

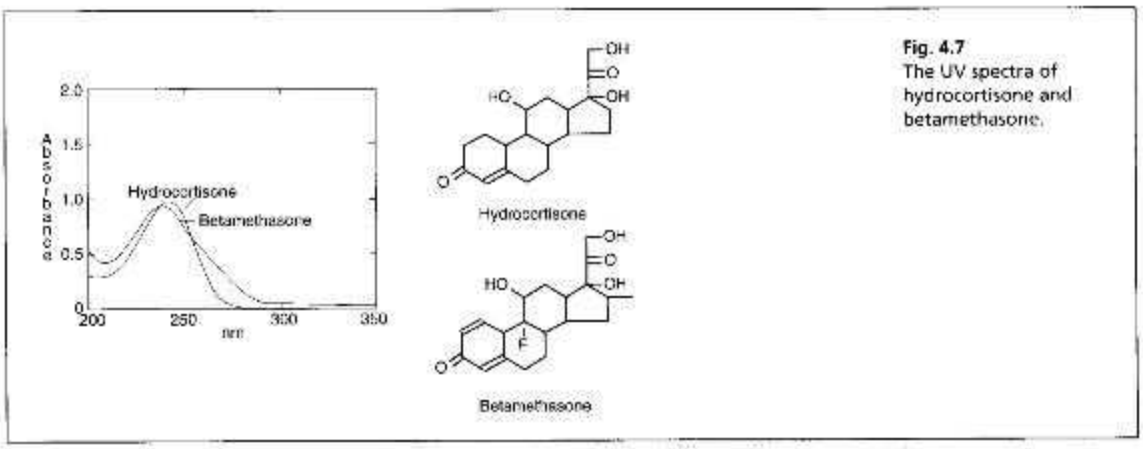



**Fig. 4.7**
The UV spectra of hydrocortisone and betamethasone.

Table 4.3 summarises the absorption data for some steroid structures and illustrates the effect of molecular weight on the $A$ (1%, 1 cm) value. The strength of the enone chromophore is similar for all the steroids since the $A$ (1%, 1 cm) value is based on the absorption of a 1% w/v solution; it will thus decrease as the molecular weight of the steroid increases. This is of course true for all molecules.

**Table 4.3** Absorption maxima for some corticosteroids

| Steroid | Molecular weight | λ max | *A* (1%, 1 cm) value |
|---|---|---|---|
| Hydrocortisone | 362.5 | 240 | 435 |
| Betamethasone | 392.5 | 240 | 390 |
| Clobetasol butyrate | 479.0 | 236 | 330 |
| Betamethasone sodium phosphate | 516.4 | 241 | 296 |

## Ephedrine: the benzoid chromophore

Figure 4.8 shows the UV absorption spectrum of a 100 mg/100 ml solution of ephedrine. Ephedrine has the simplest type of benzene ring chromophore, which has a spectrum similar to that of benzene with a weak symmetry forbidden band *ca* 260 nm with an *A* (1%, 1 cm) value of 12. Like benzene its most intense absorption maximum is below 200 nm. There are no polar groups attached to or involved in the chromophore so that its vibrational fine structure is preserved because the chromophore does not interact strongly with the solvent.

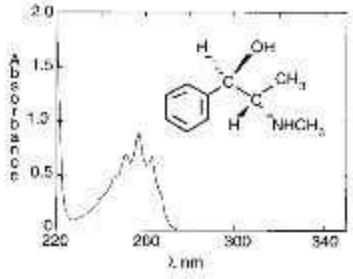


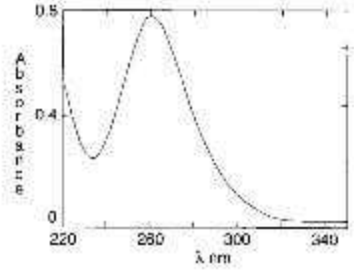

**Fig. 4.8**
The UV spectrum of ephedrine, a simple benzoid spectrum.

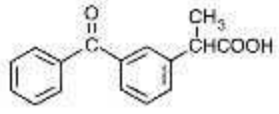
Drugs having a chromophore like that of ephedrine include: diphenhydramine, amphetamine, ibuprofen and dextropropoxyphene.

## Ketoprofen: extended benzene chromophore

The spectrum of ketoprofen is shown in Figure 4.9. In this case the simple benzoid chromophore has been extended by four double bonds and thus the symmetry of the benzene ring has been altered. In addition, the strong absorbance band present in benzene at 204 nm has undergone a bathochromic shift giving a λ max for ketoprofen at 262 nm having an *A* (1%, 1 cm) value of 647.

**Fig. 4.9**
UV absorption spectrum of ketoprofen.



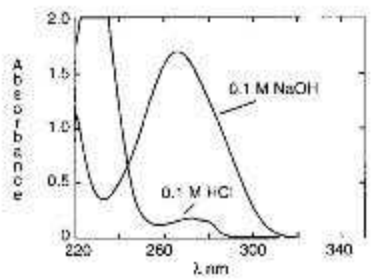




Ketoprofen

Other drugs which have an extended benzoid chromophore include: cyproheptadine, dimethindine, protriptyline, zimeldine.

## Procaine: amino group auxochrome

Figure 4.10 shows the UV absorption spectra of a solution of procaine in 0.1 M HCl and 0.1M NaOH. In procaine, the benzene chromophore has been extended by addition of a C = O group and under acidic conditions, as in Figure 4.10, the molecule has an absorption at 279 nm with an $A$ (1%, 1 cm) value of 100. In addition to the extended chromophore, the molecule also contains an auxochrome in the form of an amino group, which under basic conditions has a lone pair of electrons that can interact with the chromophore producing a bathochromic shift. Under acidic conditions the amine group is protonated and does not function as an auxochrome but when the proton is removed from this group under basic conditions a bathochromic shift is produced and an absorption with λ max at 270 nm with an $A$ (1%, 1 cm) value of 1000 appears.



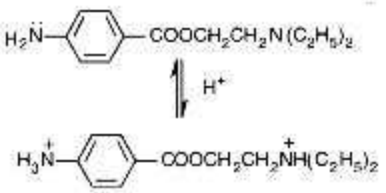




**Fig. 4.10**
UV spectrum of procaine under acidic and basic conditions.

Drugs with a chromophore such as that of procaine include: procainamide and proxymetacaine. It should be noted that local anaesthetics such as bupivacaine and lignocaine do not fall into this category since they are aromatic amides and the lone pair on the nitrogen atom is not fully available due to electron withdrawal by the adjacent carbonyl group.

## Phenylephrine: hydroxyl group auxochrome

The chromophore of phenylephrine is not extended but its structure includes a phenolic hydroxyl group. The phenolic group functions as an auxochrome under both acidic and alkaline conditions. Under acidic conditions it has two lone pairs of electrons, which can interact with the benzene ring and under basic conditions it has three. Figure 4.11 shows the bathochromic and hyperchromic shift in the spectrum of phenylephrine, which occurs when 0.1 M NaOH is used as a solvent instead of 0.1 M HCl. Under acidic conditions the λ max is at 273 and has an $A$ (1%, 1 cm) value of 110 and under alkaline conditions the λ max is a 292 nm and has an $A$ (1%, 1 cm) value of 182.

The types of shifts observed for procaine and phenylephrine can be exploited in order to achieve analysis of mixtures. Two examples of this are given later in the chapter.

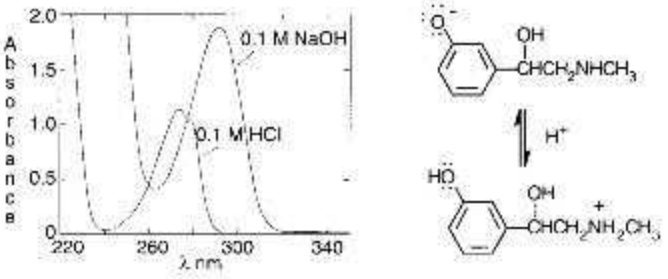



**Fig. 4.11**
UV spectrum of phenylephrine under acidic and basic conditions.

## Use of UV/visible spectrophotometry to determine p*K*a values

Where a pH-dependent UV shift is produced, it is possible to use it to determine the p*K*a of the ionisable group responsible for the shift. In the case of phenylephrine, the p*K*a value of the phenolic group can be determined conveniently from the absorbance at 292 nm since the absorbance of the molecular species where the phenolic group is un-ionised is negligible at this wavelength. This is not the case for all molecules. A general equation for determination of p*K*a from absorbance measurement at a particular wavelength is given below.

The following equation can be used for an acid (for a base the log term is subtracted) where increasing pH produces a bathochromic/hyperchromic shift:

$$pKa = pH + \log \frac{Ai - A}{A - Au}$$

where $A$ is the measured absorbance in a buffer of known pH at the wavelength selected for analysis, $Ai$ is the absorbance of the fully ionised species and $Au$ is the absorbance of the un-ionised species.

The wavelength used for analysis is one where there is the greatest difference between the ionised and un-ionised species. An approximate knowledge of the p*K*a value is required to select a suitable pH value, within ± 1 of the p*K*a value, for measurement of $A$. For accurate determination measurement is made at a number of closely spaced pH values.

It should be noted that if the acid or base undergoes a shift to lower absorbance and shorter wavelength with increasing pH the log term above is subtracted; this situation is less common in drug molecules.

### Calculation example 4.1

The absorbance of a fixed concentration of phenylephrine at 292 nm is found to be 1.224 in 0.1 M NaOH and 0.02 in 0.1 M HCl. Its absorbance in buffer at pH 8.5 is found to be 0.349. Calculate the p*K*a value of its acidic phenolic hydroxyl group.

$$pKa = 8.5 + \log \frac{1.224 - 0.349}{0.349 - 0.02} = 8.5 + 0.402 = 8.902.$$

**Self-test 4.3**

Calculate the pKa value of the weekly basic aromatic amine in procaine from the data given below. Absorbance of a fixed concentration of procaine in: 1 M HCl at 296 nm = 0.031; absorbance in 0.1 M NaOH = 1.363; absorbance in buffer at pH 2.6 = 0.837.

Answer: 2.41

# Applications of UV/visible spectroscopy to pharmaceutical quantitative analysis

## Introduction

Pharmacopoeial methods rely heavily on simple analysis by UV/visible spectrophotometry to determine active ingredients in formulations. These methods are usually based on the use of a standard $A$ (1%, 1 cm) value for the active ingredient being assayed and this relies on the UV spectrophotometer being accurately calibrated as described earlier in the chapter. Such methods also presume that there is no interference from excipients (preservatives, colourants, etc.) present in formulations and that the sample is free of suspended matter, which would cause light scattering.

## Assay examples

### *Frusemide in tablet form*

A typical example of a straightforward tablet assay is the analysis of frusemide tablets:

(i) Tablet powder containing *ca* 0.25 g of frusemide is shaken with 300 ml of 0.1 M NaOH to extract the acidic frusemide.
(ii) The extract is then made up to 500 ml with 0.1 M NaOH.
(iii) A portion of the extract is filtered and 5 ml of the filtrate is made up to 250 ml with 0.1 M NaOH.
(iv) The absorbance of the diluted extract is measured at 271 nm.
(v) The $A$ (1%, 1 cm) value at 271 is 580 in basic solution.

From the data below calculate the % of stated content in a sample of frusemide tablets:

- Stated content per tablet: 40 mg of frusemide
- Weight of 20 tablets: 1.656 g
- Weight of tablet powder taken for assay: 0.5195 g
- Absorbance reading: 0.596 (see Calculation example 4.2).

### *Assay of cyclizine lactate in an injection*

The steps in this assay are more difficult to follow since a number of extractions take place prior to preparing the final dilution in order to remove excipients:

(i) Dilute 5 ml of injection to 100 ml with 1 M sulphuric acid.
(ii) Add 2 g of sodium chloride to 20 ml of this solution and shake with two 50 ml quantities of ether.

**Calculation example 4.2**

Expected content in tablet powder taken: $\frac{0.5195}{1.656} \times 40 \times 20 = 251.0$ mg.

Dilution factor

5 – 250 ml = 50.

Concentration in diluted tablet extract: $\frac{0.596}{580} = 0.001028$ g/100 ml = 1.028 mg/100 ml.

Concentration in original tablet extract: $1.028 \times 50 = 51.40$ mg/100ml.

Volume of original extract: 500 ml.

Therefore amount of frusemide in original extract: $51.40 \times 5 = 257.0$.

Percentage of stated content: $\frac{257.0}{251.0} \times 100 = 102.4\%$.

**Self-test 4.4**

Calculate the percentage of stated content of promazine hydrochloride in promazine tablets from the following information:

(i) Tablet powder containing *ca* 80 mg of promazine hydrochloride is ground to a paste with 10 ml of 2 M HCl.
(ii) The paste is then diluted with 200 ml of water, shaken for 15 min and finally made up to 500 ml.
(iii) A portion of the extract is filtered.
(iv) 5 ml of the filtrate is taken and diluted to 100 ml with 0.1 M HCl.
(v) The absorbance is read at a wavelength of 251 nm.

- *A* (1%, 1 cm) value of promazine.HCl at 251 nm = 935
- Stated content of promazine.HCl per tablet = 50 mg
- Weight of 20 tablets = 1.667 g
- Weight of tablet powder taken for assay = 0.1356 g
- Absorbance reading = 0.755.

Answer Percentage of stated content = 99 %

(iii) Add 20 ml of 5 M sodium hydroxide and extract with three 50 ml quantities of ether.
(iv) Combine the ether extracts and then wash with two 10 ml quantities of a saturated solution of sodium chloride.
(v) Extract the ether layer with two 25 ml quantities of 0.05 M sulphuric acid and then with two 10 ml quantities of water.
(vi) Combine the acidic and aqueous extracts and dilute to 100 ml with water.
(vii) Dilute 5 ml of this solution to 200 ml with 0.05 M sulphuric acid and measure the absorbance of the resulting solution at 225 nm.

Calculate the percentage of w/v of cyclizine lactate in the injection from the following information:

- *A* (1%, 1 cm) of cyclizine lactate at 225 nm = 331
- Volume of injection assayed = 5 ml
- Measured absorbance = 0.413
- Measurements were made in a 1 cm cell.

### Calculation example 4.3

The first dilution is 5 ml to 100 ml (× 20). Then 20 ml of this dilution is taken and extracted with ether to remove excipients, the cyclizine remains in the acidic water layer since it is a base. After extraction with ether the acidic layer is basified and the cyclizine is extracted into ether; it is then back extracted into 0.1 M sulphuric acid and made up to 100 ml, thus the dilution factor in the second step is 20 to 100 ml (× 5). Finally a third dilution is carried out in which 5 ml of the second dilution are diluted to 200 ml (× 40).

Total dilution: $20 \times 5 \times 40 = 4000$.

For the diluted injection c: $\frac{0.413}{331} = 0.001248$ g/100 ml.

Concentration in original solution: $0.001248 \times 4000 = 4.992$ g/100 ml.

Concentration of injection = 4.992% w/v.

### Self-test 4.5

Determine the concentration of the following injections:

*Isoxsuprine injection* is diluted as follows:

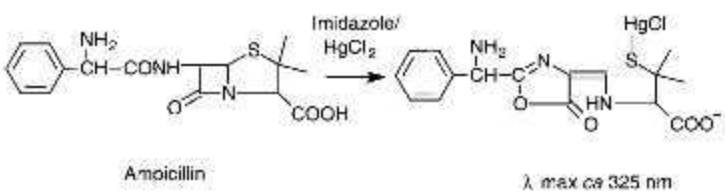
(i) Diluted 10 ml of injection to 100 ml and then 10 ml of the dilution to 100 ml.
- Absorbance reading at 274 nm = 0.387
- *A* (1%, 1 cm) value at 274 nm = 73.

*Haloperidol injection*:

(i) Add 15 ml of 1 M HCl to 5 ml of injection.
(ii) Extract three times with ether washing the ether extracts with 10 ml of water.
(iii) Combine the aqueous layers and dilute to 100 ml.
(iv) Take 10 ml of the diluted aqueous solution and dilute to 100 ml.
- Absorbance reading at 245 nm = 0.873
- *A* (1%, 1 cm) value at 245 nm = 346.

Answers: Isoxsuprine injection = 0.530% w/v; haloperidol injection = 0.505 % w/v

### *Assay of penicillins by derivatisation* (Fig. 4.12)




Amoicillin

λ max *ca* 325 nm

**Fig. 4.12** Reaction of penicillins with mercury imidazole reagent.

The BP utilises formation of a derivative in order to quantify penicillins in formulations. Some penicillins do not have distinctive chromophores; a further problem with these molecules is that when they are in suspensions they are not readily extracted away from excipients since they are quite insoluble in organic solvents which are immiscible with water. Using the formation of a complex with the mercuric ion in the presence of imidazole as a catalyst, a derivative of the penicillin structure is produced, which has an absorption maximum between 325 and 345 nm. In the assay, comparison with pure standard for the particular penicillin is carried out rather than relying on a standard *A*(1%, 1 cm) value. This assay is used by the BP for

analysis of preparations containing ampicillin, amoxycillin, carbenicillin, cloxacillin, flucloxacillin and phenoxymethylpenicillin. The assay is not used for the closely related cephalexins.

### Calculation example 4.4

Cloxacillin injection is assayed using the mercury–imidazole reaction in comparison with a cloxacillin standard. The sample and standard were both diluted in 500 ml of water and then 25 ml was taken from each of the solutions and was made up to 100 ml. 2 ml of the sample and standard solutions were then reacted with mercury–imidazole reagent. From the data below calculate the amount of cloxacillin per vial.

Weight of the content of 10 vials = 2.653 g.

Weight of injection powder used for assay = 0.1114 g.

Weight of cloxacillin sodium standard used in calibration solution = 0.1015 g.

Absorbance of sample solution = 0.111.

Absorbance of standard solution = 0.106.

In this calculation the dilutions can be ignored since:

$$\text{Weight of cloxacillin in sample} = \frac{\text{Absorbance sample}}{\text{Absorbance of standard}} \times \text{weight of standard}$$

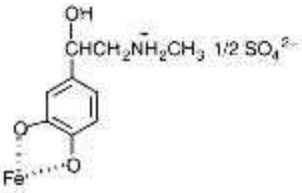
$$\text{Weight of cloxacillin in sample } \frac{0.111}{0.106} \times 0.1015 = 0.1063 \text{ g.}$$

$$\text{Contents of 1 vial } \frac{2.653}{10} = 0.2653 \text{ g.}$$

$$\text{Amount of cloxacillin in 1 vial } \frac{0.2653}{0.1114} \times 0.1063 = 0.2532 \text{ g.}$$

### *Assay of adrenaline in lignocaine adrenaline injection*

Adrenaline is present as a vasoconstrictor in some local anaesthetic injections in a much smaller amount than the local anaesthetic itself, which obscures the absorption of adrenaline in the UV region. The selectivity of UV/visible spectroscopy for the analysis of adrenaline can be increased by complex formation, which occurs between iron (II) and molecules containing a catechol group (Fig. 4.13). These complexes are purple in colour and absorb at *ca* 540 nm at much longer wavelengths than for instance local anaesthetics, which do not form such complexes. The adrenaline in the injection is quantified against a standard solution of adrenaline.




Adrenaline iron (II) complex

**Fig. 4.13**
The complex formed between adrenaline and iron, which is used to analyse adrenaline at low levels in an injection.

### Self-test 4.6

Adrenaline in bupivacaine/adrenaline injection is assayed by complex formation with iron (II). 20 ml of the injection is mixed with 0.2 ml of reagent and 2 ml of buffer and a reading is taken in a 4 cm pathlength cell. A reading of a solution containing 5.21 µg/ml of adrenaline is taken under the same conditions.
The following results were obtained:

- Absorbance of sample = 0.173
- Absorbance of standard solution = 0.181

Calculate the percentage of w/v of adrenaline in the injection.

Answer 0.00048% w/v

## Difference spectrophotometry

In difference spectroscopy, a component in a mixture is analysed by carrying out a reaction which is selective for the analyte. This could be simply bringing about a shift in wavelength through adjustment of the pH of the solution in which the analyte is dissolved or a chemical reaction such as oxidation or reduction. In the following example the selective alkaline shift of aspirin is used to determine it in a preparation also containing dextropropoxyphene, naphthalene sulphonic acid and caffeine. Caffeine, dextropropoxyphene and the naphthalene sulphonic acid anion do not undergo appreciable alkaline shifts whereas aspirin does. Figure 4.14A shows the spectrum of the extract from tablets in 0.1 M HCl – in fact there is relatively minor interference at the wavelength used for the determination of aspirin but by using the sample in HCl in place of a blank in the reference cell one can be sure that interference from the excipients is eliminated. Figure 4.14B shows the difference spectrum with the capsule extract in 0.1 M HCl in the reference cell and the capsule extract in 0.1 M NaOH in the sample cell. The absorbance at 299 nm is thus wholly due to aspirin. The problem remains of how to quantify the analyte in such a sample. This can be readily carried out using standard additions which involves adding a known amount of aspirin standard to the sample and comparing the absorbance of the original sample extract with the absorbance of the spiked sample.

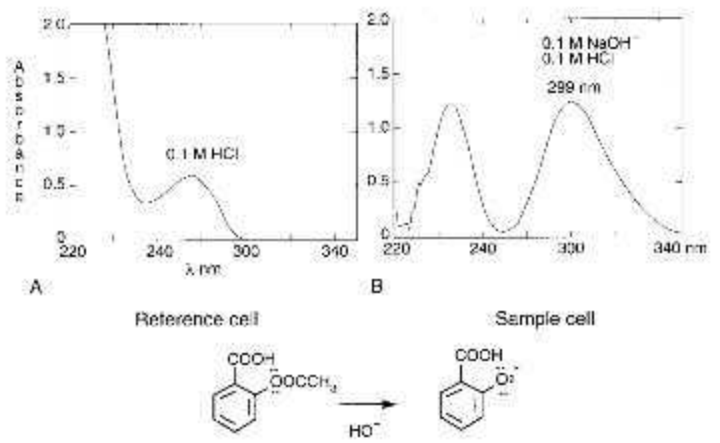



**Fig. 4.14**
UV difference spectrum used in the quantification of aspirin in dextropropoxyphene capsules.

## Analysis of aspirin in dextropropoxyphene compound tablets

Analysis was carried out by difference spectrophotometry. A one-point standard calibration for the determination of aspirin in dextropropoxyphene compound capsules was prepared by adding a known amount of aspirin to the sample from a standard stock solution. Stated content in the capsules:

- Aspirin 250 mg
- dextropropoxyphene napsylate 100 mg
- caffeine 30 mg.

(i) 5 ml of the solution of sample in methanol is diluted to 500 ml with 0.1 M HCl: Reference solution.
(ii) 5 ml of the solution of sample in methanol is diluted to 500 ml with 0.1 M NaOH.
(iii) 5 ml of the solution of sample solution in methanol and 5 ml of aspirin standard solution were mixed and then diluted to 500 ml with 0.1 NaOH.

Readings were taken at 299 nm of the sample solutions with and without standard addition against the reference solution prepared by diluting the sample extract with 0.1 M HCl.

The following data were obtained:

- Weight of contents of 20 capsules = 10.556 g
- Weight of capsule content analysed = 0.1025 g
- Capsule contents were dissolved in methanol and adjusted to 100 ml
- Concentration of aspirin standard solution = 50.4 mg/100 ml
- Absorbance of sample at 299 nm in 0.1 M NaOH without standard addition = 0.488
- Absorbance of sample at 299 nm in 0.1 M NaOH with standard addition = 0.974.

### Calculation example 4.5

In dilution (iii) aspirin standard solution is diluted 5 ml to 500 ml (× 100).

Concentration of aspirin standard in standard addition solution: $\frac{50.4}{100} = 0.504$ mg/100 ml.

The difference between the absorbance with standard addition and that without represents the absorbance due to a 0.504 mg/100 ml solution of aspirin.
Absorbance difference: 0.974 − 0.488 = 0.486.

Therefore concentration of aspirin in the sample solution = $\frac{0.488}{0.486} \times 0.504 = 0.506$ mg/100 ml.

Dilution factor for sample = 5 ml to 500 ml (× 100).
Concentration of aspirin in undiluted sample solution = 0.506 × 100 = 50.6 mg/100 ml.
Volume of initial extract = 100 ml.
Therefore amount of aspirin extracted from the capsule powder = 50.6 mg.

Amount expected in capsule powder analysed = $250 \times 20 \times \frac{0.1025}{10.556} \times 1000 = 48.6$ mg.

Therefore percentage of stated content = $\frac{50.6}{48.6} \times 100 = 104.1$.

## Derivative spectra

Derivative spectra can be used to clarify absorption bands in more complex UV spectra. The technique is used extensively in the rapidly developing field of near infrared spectrophotometry (see Ch. 5) and can also be applied in the determination of the purity of chromatographic peaks when they are monitored by diode array detection. The main effect of derivatisation is to remove underlying broad absorption bands where there is only a gradual change in slope. The first derivative spectrum is obtained by plotting, for instance, the slopes of 2 nm segments of the spectrum and this results, as shown for a Gaussian band in Figure 4.15, in a spectrum where the slope is zero at the maximum of the peak and the slope is maximum at approximately half the peak height. In the second derivative spectrum the slopes of adjacent 2 nm segments are compared and this gives the points of maximum curvature of the spectrum. The rate of curvature of a spectrum has its greatest negative value at its maximum and the greatest rates of curvature are observed for narrow absorption bands. Figure 4.15 shows the first, second, third and fourth derivatives of a Gaussian band.

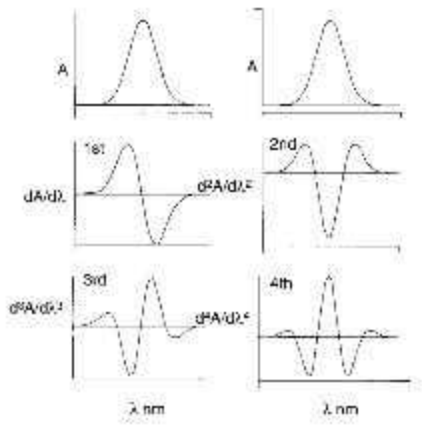



**Fig. 4.15**
Derivatives of a Gaussian absorption band.

As would be expected, the first order spectrum of pseudoephedrine, shown in Figure 4.16, gives maxima at the points where the slope is at a maximum in the zero order spectrum. In addition, the second order spectrum gives minima corresponding to the maxima in the zero order spectrum, i.e. where the negative curvature of the spectrum is at its maximum.

By examining the UV spectrum of an elixir containing pseudoephedrine, dextromethorphan and triprolidine (30 mg, 10 mg and 1.25 mg, respectively) shown in Figure 4.17, it can be seen that the pseudoephdrine spectrum lies on top of a large background due to dextromethorphan and triprolidine, which have much stronger chromophores than pseudoephedrine. However, the underlying slope of the absorption curve due to contributions from dextromethorphan and triprolidine is shallow. The steepest underlying increase is due to dextromethorphan, which reaches a maximum at 278 nm. When the second derivative spectrum is examined it can be seen that the only peaks derive from pseudoephedrine and even where the dextromethorphan makes its maximum contribution at 278 nm there is little absorption in the second derivative spectrum. Thus it would be possible to use the

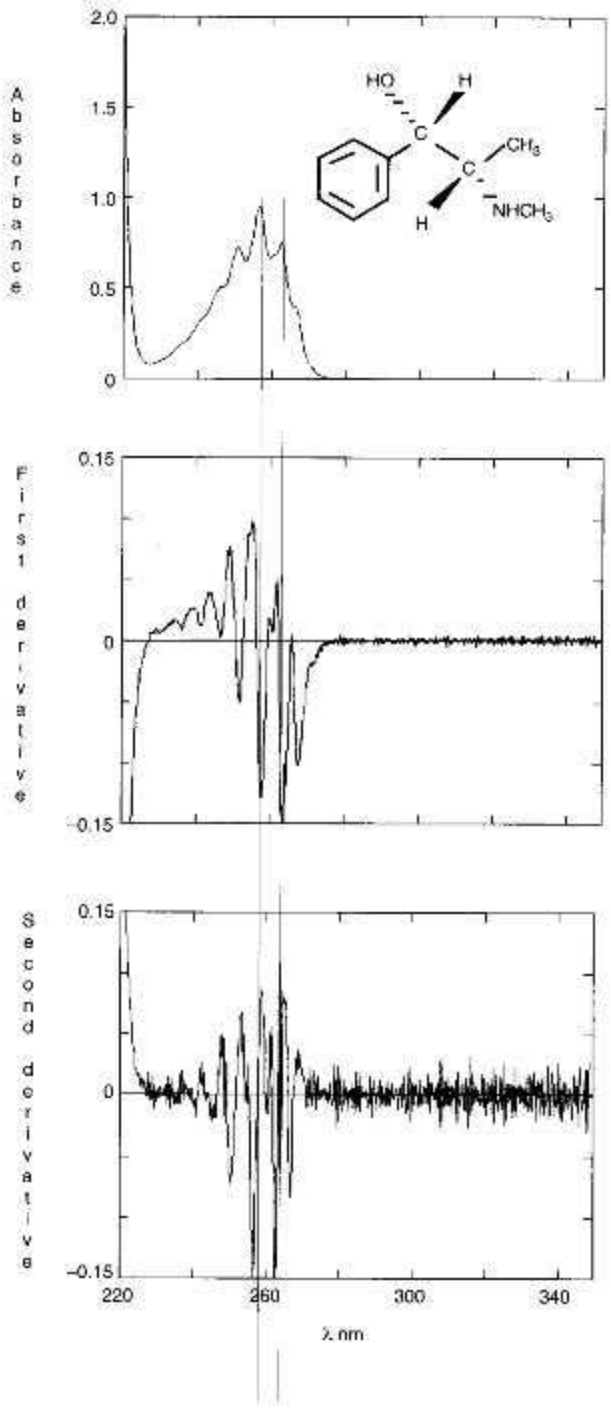



**Fig. 4.16**
UV spectrum of pseudoephedrine with its first and second derivative spectra. The minima correspond to the maxima in the absorbance spectrum.

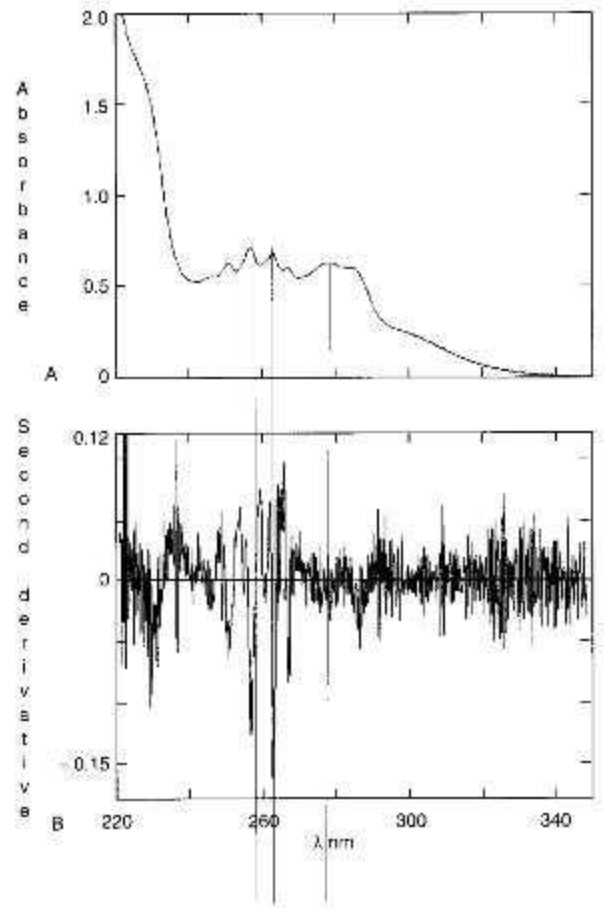



**Fig. 4.17**
(A) UV spectrum of an extract elixir containing pseudoephedrine, dextromethorphan and triprolidine. (B) Second derivative spectrum of the extract. Note the absorbance maximum of dextromethorphan disappears.

height of the pseudoephedrine peak to determine the amount of pseudoephedrine in the elixir with suitable calibration, e.g. standard additions of pseudoephedrine to the sample extract.

The signal:noise ratio is poorer in the second derivative spectra because through dividing the spectrum into segments in order to calculate the derivative the underlying noise is less efficiently averaged out, which occurs when the spectrum is scanned in much narrower segments.

## Applications of UV/visible spectroscopy in preformulation and formulation

UV/visible spectrophotometry is a standard method for determining the physicochemical properties of drug molecules prior to formulation and for measuring their release from formulations. The type of properties which can be usefully determined by the UV method are listed as follows.

## Partition coefficient

The partition coefficient of a drug between water and an organic solvent may be determined by shaking the organic solvent and the water layer together and determining the amount of drug in either the aqueous or organic layer by UV spectrophotometry. If buffers of different pH values are used, the variation of partition coefficient with pH may be determined and this provides another means of determining the p*K*a value of a drug.

## Solubility

The solubility of a drug in, for instance, water may be simply determined by shaking the excess of the drug in water or buffer until equilibrium is reached and then using UV spectrophotometry to determine the concentration of the drug that has gone into solution. Another method for determining solubility, where an ionisable group is present in the drug, is to dissolve varying concentrations of the salt of the drug in water and then add excess acid to a solution of the salt of an acidic drug or excess base to a solution of the salt of the basic drug, thus converting the drugs into their un-ionised forms. When the solubility of the un-ionised drug in water is exceeded, a cloudy solution will result and UV spectrophotometry can be used to determine its degree of turbidity by light scattering, which can be measured at almost any wavelength, e.g. 250 nm.

## Release of a drug from a formulation

UV spectrophotometry is used routinely to monitor in vitro release of active ingredients from formulations. For simple formulations the drug is simply monitored at its λ max. In the example shown in Figure 4.18 the rate release of pseudoephedrine from a controlled release formulation was monitored.[1] The release of the drug was followed by monitoring its release into distilled water using a UV spectrophotometer set at 206 nm. In the example given in Figure 4.18 the particle size of the ethylcellulose used in the formulation affected the rate of release.

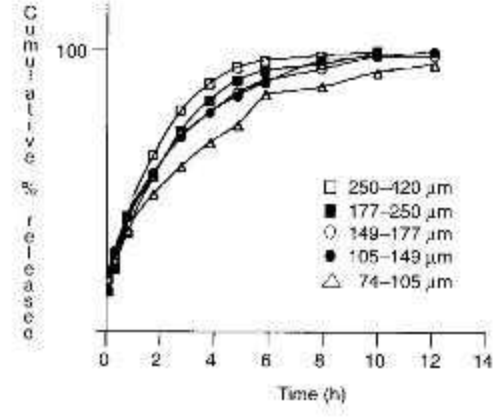



**Fig. 4.18**
Release of pseudoephedrine from a controlled release formulation. Reproduced with permission Int. J. Pharmaceutics (see Reference 1).

If UV-absorbing excipients were present in such a formulation the UV wavelength used for monitoring release would need to be selected carefully or high-pressure liquid chromatography (HPLC) coupled to UV detection might be used. For such studies the sampling of the dissolution medium may be fully automated so that the medium is filtered and pumped through to the UV spectrophotometer at set time intervals in order to take a reading.

### References

1. P.R. Kantikaneni, S.M. Upadrashta, S.N. Neau and A.K. Mitra. Int. J. Pharmaceutics 123, 119–125 (1995).

### Further reading

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