Ultraviolet and visible spectroscopy

David G. Watson

Lecture 3

EMAD ALSAMARRAI

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 does not fit into this category is 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 employed in qualitative identity tests; these are used particularly in conjunction with high-pressure liquid chromatography (HPLC) identification checks where the method of detection is by diode array UV spectrophotometry (Ch. 12, p. 322).

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

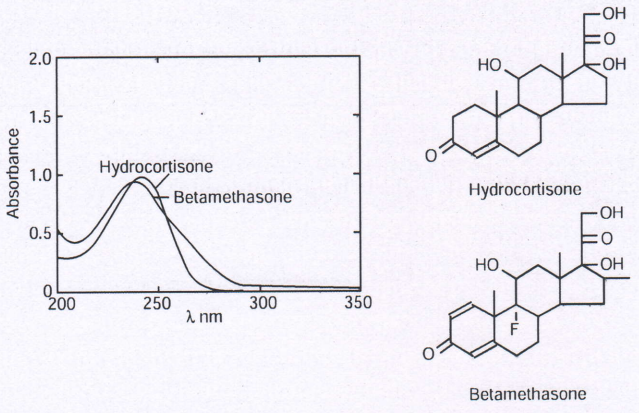


Fig. 4.7
The UV spectra of hydrocortisone and betamethasone.

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

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.

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 its vibrational fine structure is preserved because the chromophore does not interact strongly with the solvent.

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.

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

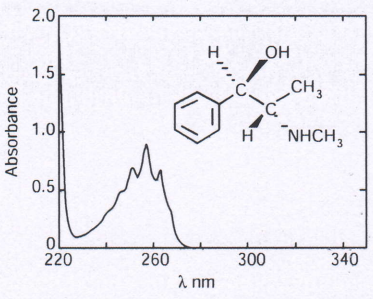


Fig. 4.8
The UV spectrum of ephedrine, a simple benzoid spectrum.

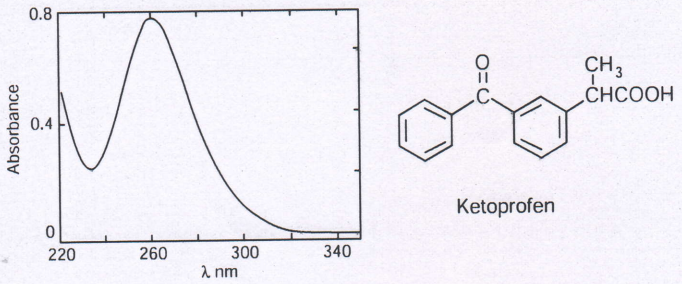


Fig. 4.9
UV absorption spectrum of ketoprofen.

Procaine: amino group auxochrome

Figure 4.10 shows the UV absorption spectra of a solution of procaine in 0.1 M HCl and 0.1 M NaOH. In procaine, the benzene chromophore has been extended by the 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.

Drugs with a chromophore such as that of procaine include: procainamide and proxymetacaine. It should be noted that local anaesthetics such as bupivacaine and lidocaine (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

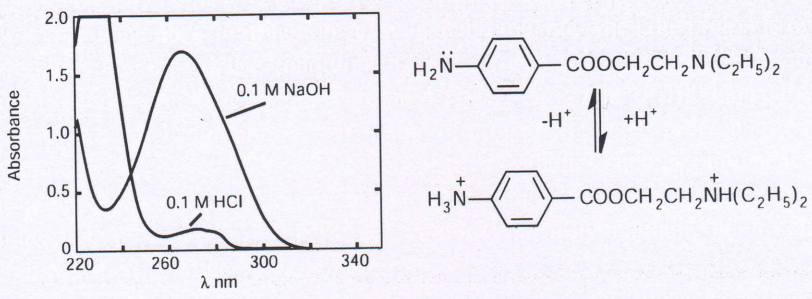
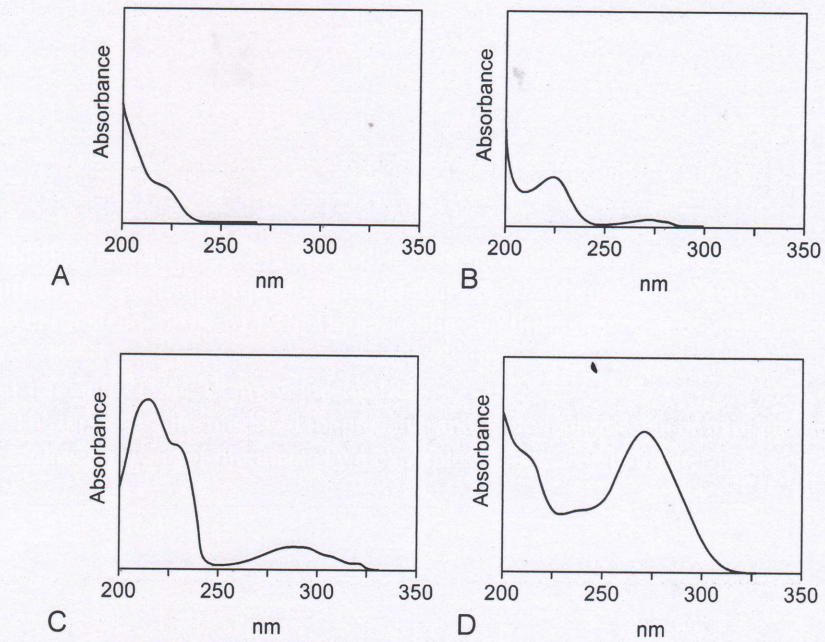


Fig. 4.10
UV spectrum of procaine under acidic and basic conditions.

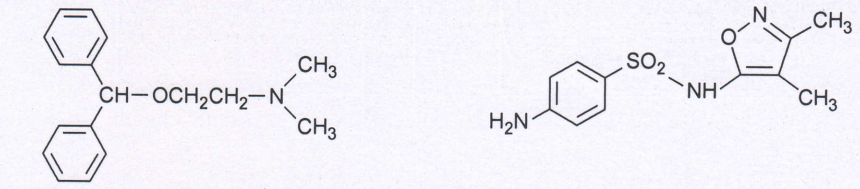
conditions it has three. Figure 4.11 shows the bathochromic and hyperchromic shift in the spectrum of phenylephrine that occurs when 0.1 M NaOH is used as a solvent instead of 0.1 M HCl. Under acidic conditions the λ max is at 273 and has an *A* (1%, 1 cm) value of 110 and under alkaline conditions the λ max is at 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.

Self-test 4.3

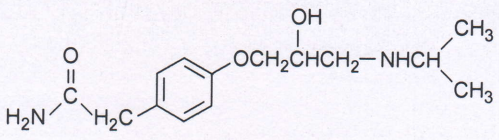


Link the structures to the UV spectra A–D.

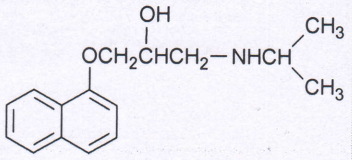


A Diphenhydramine

B Sulfurazole



C Atenolol



D Propranolol

Answer: A diphenhydramine, B sulfurazole C atenolol D propranolol.