Ultraviolet and visible spectroscopy

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Lecture 4

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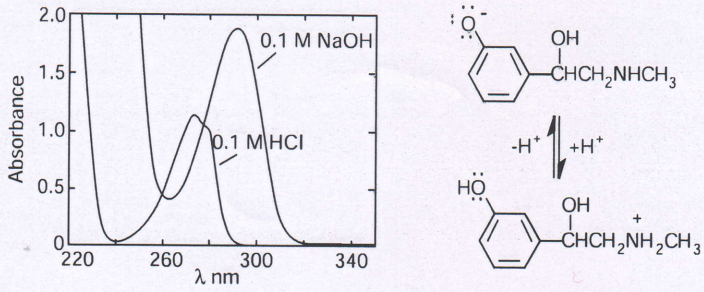


Fig. 4.11
UV spectrum of phenylephrine under acidic and basic conditions.

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:

$$\text{p}K\text{a} = \text{pH} + \log \frac{A\text{i} - A}{A - A\text{u}}$$

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.

$$\text{p}K\text{a} = 8.5 + \log \frac{1.224 - 0.349}{0.349 - 0.02} = 8.5 + 0.402 = 8.902.$$

Self-test 4.4

Calculate the p*K*a value of the weakly 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

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

Furosemide (frusemide) in tablet form

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

(i) Tablet powder containing *ca* 0.25 g of furosemide (frusemide) is shaken with 300 ml of 0.1 M NaOH to extract the acidic furosemide (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 furosemide (frusemide) tablets:

- Stated content per tablet: 40 mg of furosemide (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.

(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 4.2

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/100 ml

Volume of original extract: 500 ml

Therefore, amount of furosemide (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 4.5

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 = 99.3

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 is 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 4.6

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.

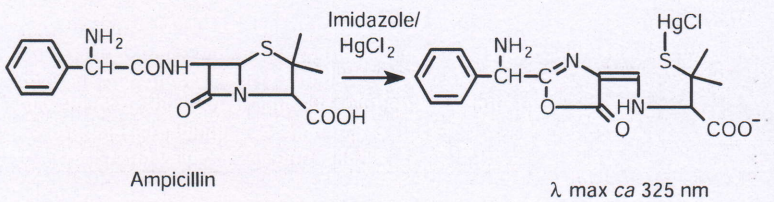
Haloperidol injection:

(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)

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



Ampicillin

λ max *ca* 325 nm

Fig. 4.12
Reaction of penicillins with mercury imidazole reagent.

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, amoxicillin, carbenicillin, cloxacillin, flucloxacillin and phenoxymethylpenicillin. The assay is not used for the closely related cefalexins.

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 lidocaine (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.