Ultraviolet and visible spectroscopy

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Lecture 1

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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.

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KEYPOINTS *(Continued)*

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 that 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.

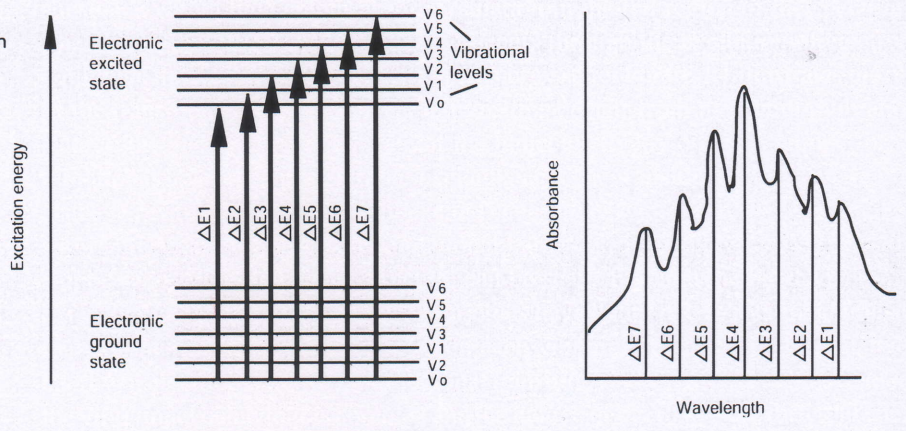


Fig. 4.1
Excitation of an electron from the ground to the excited electronic state.

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.

If more double bonds are present in a structure in conjugation (i.e. two or 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).

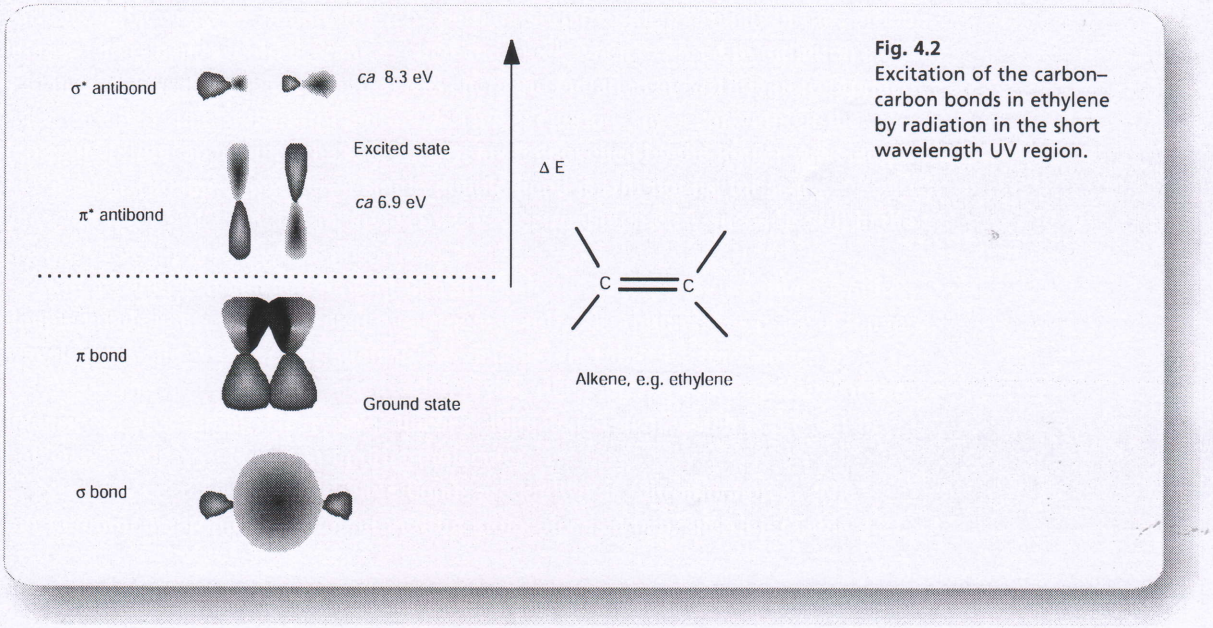
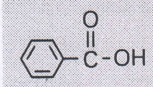
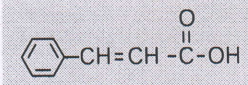
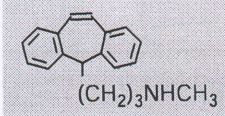
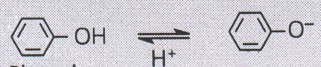
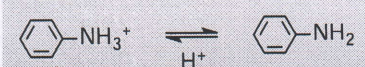


Fig. 4.2
Excitation of the carbon–carbon bonds in ethylene by radiation in the short wavelength UV region.

Table 4.1 Longest wavelength maxima and absorption intensities of some polyenes

Polyene	λ max	*A* (1%, 1 cm)
$CH_3(CH=CH)_3CH_3$	275	2800
$CH_3(CH=CH)_4CH_3$	310	6300
$CH_3(CH=CH)_5CH_3$	342	9000
$CH_3(CH=CH)_6CH_3$	380	9800

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 → Bathochromic	72 ⇌ 271 → Hyperchromic
 Aniline	255 nm ⇌ 286 nm → Bathochromic	16 ⇌ 179 → Hyperchromic

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