# Analytical Science

A course (in 15 Chapters), developed as an Open Educational Resource, designed for use at 2nd year England & Wales undergraduate level and as a CPD training resource

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# Chapter 10 – Introduction to and theoretical principles underlying spectroscopic measurements

## Contents

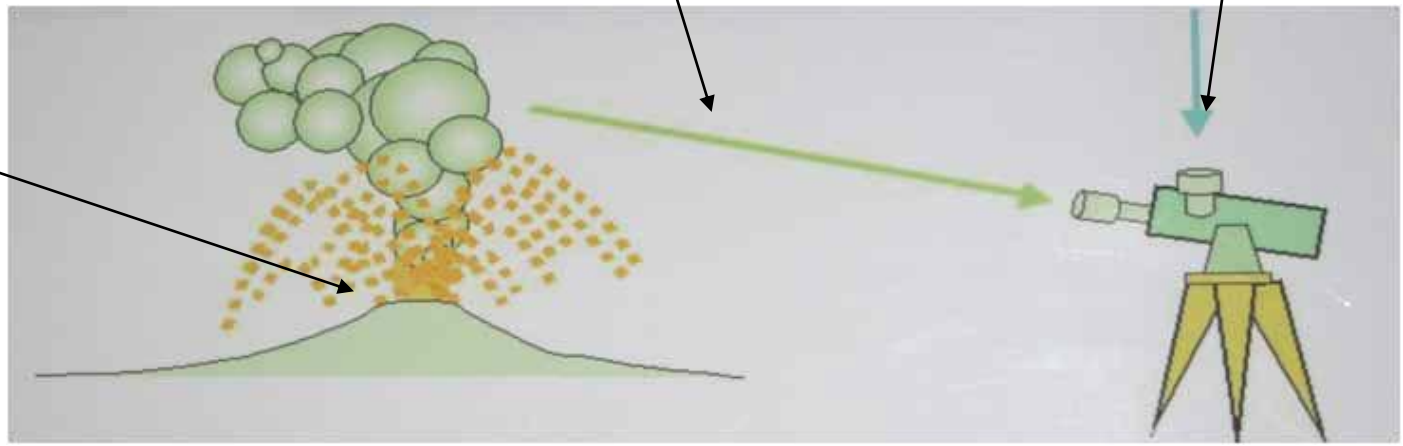
# Spectroscopic methods - introduction

Spectroscopy certainly provides the most important modern techniques for the laboratory analysis of atomic and molecular species of every kind – organic and inorganic, large and small, synthetic and naturally occurring. Its applications include qualitative and quantitative analysis over an extremely wide range of concentrations. Spectroscopy also plays a crucial role in the determination of molecular structures, including those of naturally occurring macromolecules such as proteins, and hence in providing a full understanding of chemical and biochemical reactions.  It is thus a key tool for chemists, biochemists and many other physical and biological scientists.

Spectroscopic methods are by no means confined to the laboratory. Portable and miniaturized detection systems, many of them extremely simple to operate, are now in common use for field studies (e.g. in atmospheric and environmental chemistry), and also in clinical chemistry and related biomedical studies. Figure (3a.1) shown on the next slide, shows an early instrument used to measure gaseous emission from volcanic eruptions.


Figure 10.1 - early emission spectrometer

Figure (10.1) shows an early emission spectrometer used for remote measurement of the flux and concentrations of certain gases within volcanic plumes



Astronomy and cosmology rely on spectroscopy, at many different wavelengths, for almost all studies of the structures of the solar system and of our galaxy, and of the history of the universe since the Big Bang! The instrumentation may be earth-based or flown on satellites (e.g. the Hubble Space Telescope) or on inter-planetary probes such as the current Cassini mission to Saturn and its moons

The cosmological image shown in Figure (10.2) shows distinct areas of hydrogen (red) and oxygen (green). These have been identified by using emission spectroscopy



Figure 10.2 – Cosmological image

*"This is the golden age of astronomy" – thanks to advances in spectroscopy*

# Electromagnetic radiation – wave motion

Most spectroscopic methods, with the major exception of mass spectrometry, rely on the interaction of atoms or molecules with electromagnetic radiation, the critical features of which are summarized here and in the following slides.

For many purposes electromagnetic radiation can be regarded as radiant energy that moves as a wave. (In some applications it is convenient to think of light as a stream of photons – energy quanta – a topic that will be considered later in this element). The vibrations of the wave are transverse (i.e. at 90˚ ) to the direction of its motion, and are characterized by one or more of three properties.

- **Wavelength**
- **Frequency**
- **Wavenumber**

Definitions of these three parameters are given on the next slide

**The following website shows the typical wave motion of an electromagnetic wave:**

*http://en.wikipedia.org/wiki/Electromagnetic_radiation*

## Wavelength

This is defined as the distance from one maximum of the vibrational amplitude to the next: it is always given the Greek letter λ ("lamda").

## Frequency

This is defined as the number of cycles of the wave passing a given point in a fixed time (often 1 second, in which case the units would be $s^{-1}$, also called Hertz, Hz). Frequency is given the Greek symbol ν ("nu").

**The frequency and the wavelength are related by the velocity of light 'c' thus:**

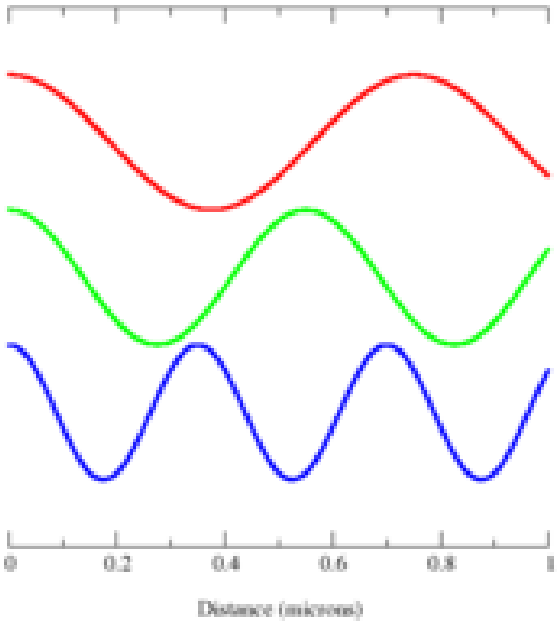
$$\lambda = c/\nu$$

## Wavenumber

This is defined as the reciprocal of the wavelength, i.e. the number of vibrations in a given distance (usually 1 cm), in which case the units are $cm^{-1}$. These units are mostly used in infra-red spectroscopy). Its symbol is (confusingly, perhaps) ("nu-bar").

**Since $\lambda = c/\nu$ then**

$$= 1/\lambda = \nu/c.$$

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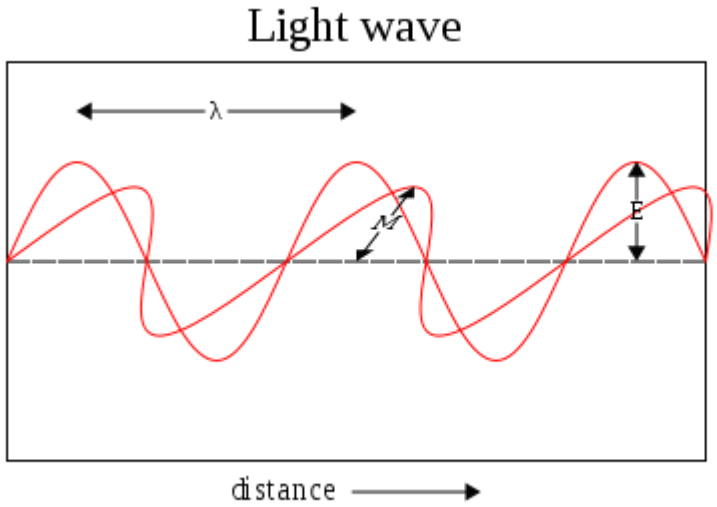


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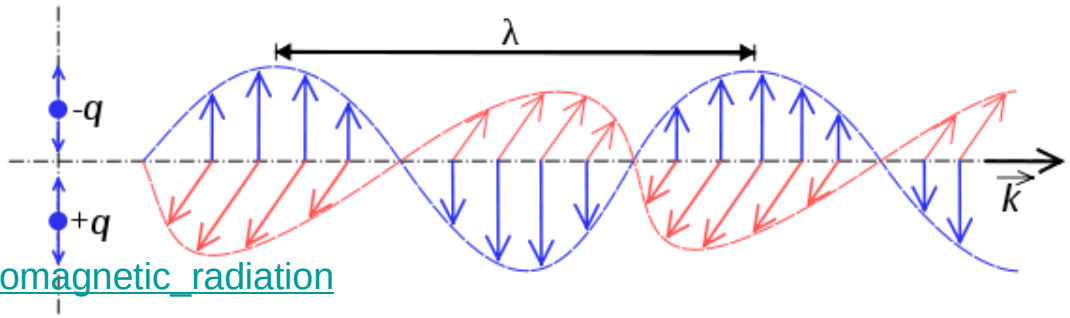


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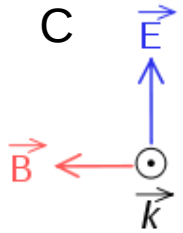



Figure 10.3 – wave Propogation diagrams as illustrated in:
http://en.wikipedia.org/wiki/Electromagnetic_radiation

In strict terms, electromagnetic radiation has two components – an electric and a magnetic component, which propogate at 90$^{o}$ to one another. These are illustrated in figures (10.3B) and (10.3C) above, however for most analytical technologies, it is only the electric component which is of interest. Fig (10.3A) shows 3 different waves, the blue wave being the higher frequency and thus the more energetic.

**Example (10.i)**

*The strong yellow coloured radiation produced when sodium salts are introduced into a Bunsen burner flame is mainly due to the Sodium D line that emits radiation at 589 nm (589 X $10^{-9}$ m). Express the wavelength of the sodium D line as both frequency and wavenumber. The speed of light 'c' is 3.0 X $10^{8}$ m $s^{-1}$*

$$\text{Frequency } (\nu) = \frac{c}{\lambda} = \frac{3.0 \times 10^{8} \text{ m s}^{-1}}{589 \times 10^{-9} \text{ m}} = \mathbf{5.09 \times 10^{14}\ s^{-1}}$$

$$\text{Wavenumber } (\text{cm}^{-1}) = \frac{1}{\lambda} = \frac{1}{589 \times 10^{-9} \text{ m}} \times \frac{1 \text{ m}}{100 \text{ cm}} = \mathbf{1.70 \times 10^{4}\ cm^{-1}}$$

Note: in example (10.i) above, units for 'c' and 'λ' have been quoted and then some are cancelled out, resulting in the '$s^{-1}$' and '$cm^{-1}$' for frequency and wavelength respectively.

Try converting these wave properties from one to another, making sure that you use the correct units: for example if the wavelength is in centimetres, then 'c' must be $3 \times 10^{10}$ cm $s^{-1}$.

# Electromagnetic radiation: photons

The energy associated with a beam of radiation is best characterized by regarding it as a stream of energy quanta, called **photons**.

**Photon**

The energy of a unit of radiation. It is related to both to frequency & wavelength

The idea of a photon as an energy packet is a useful concept, when considering the interaction of a light beam with single molecules or atoms .

The energy '*E* ' of a quantum of radiation of wavelength λ is given by the Planck Equation shown as equation (10.1)

$$E = hcl\lambda = h\nu \qquad \text{Equation (10.1)}$$

In this equation *h* is Planck's constant, with a value of $6.62 \times 10^{-34}$ Joule seconds.

**From this equation it is clear that the *shorter* the wavelength (higher the frequency) of a photon, the *greater* its energy**. So in the visible and ultra-violet regions of the spectrum, blue and ultra-violet photons are more energetic than red ones – one good reason for the importance of sun-tan lotions in preventing ultra-violet photons from penetrating your skin!

Again it is a good idea to practise using the Planck equation with the right units. In example (10.ii) below the energy of 400 nm photons (i.e. blue visible light) is calculated in kJ/mole. It is important to remember to multiply the energy of one photon by Avogadro's number (6.023 X 1023) in order to find the amount of energy that would be gained by one mole of a substance if each of its molecules absorbed one photon. Verify that this answer, in kJ $mole^{-1}$, is similar to the energy of many common chemical bonds (see also slide xx). Try calculating in the same way the energies of photons at wavelengths of 280 nm, 365 nm, and 488 nm.

**Example (10.ii)**

Calculate the energy of 400 nm photons, presenting the answer in kJ/mole

From equation (xx) $E = hc/\lambda$

If $h = 6.62 \times 10^{-34}$ Js; $c = 3.0 \times 10^{8}$ $ms^{-1}$; Avogadro's number $6.023 \times 10^{23}$

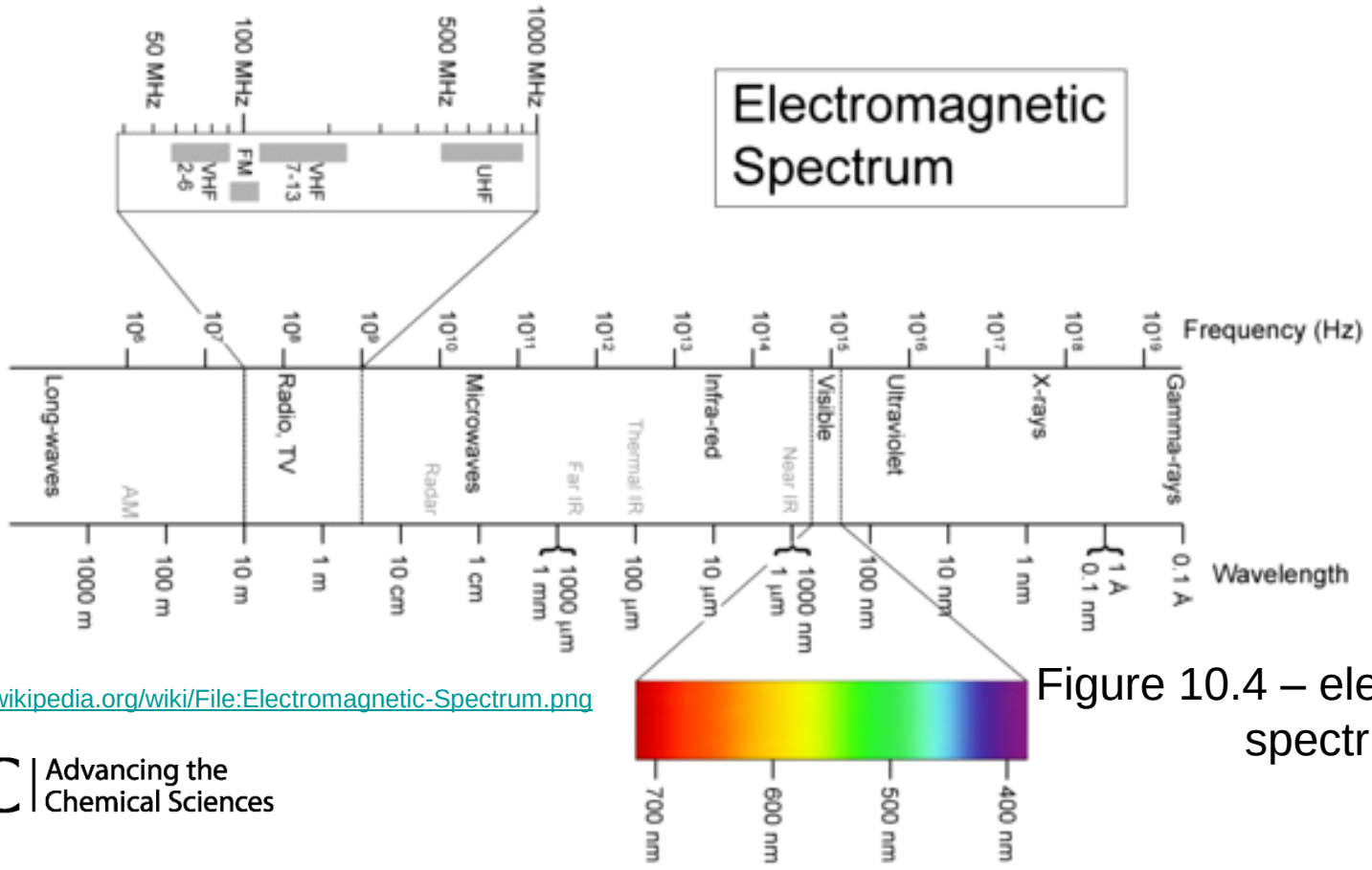
$$E = \frac{6.62 \times 10^{-34} \times 3.0 \times 10^{8} \times 6.023 \times 1023}{4.00 \times 10\text{-}9} \text{ J/mole}$$

$$= 0.299 \times 106 \text{ J/mole}$$

$$= \mathbf{299\ kJ/mole}$$

# The electromagnetic spectrum

Chemists and other scientists use electromagnetic radiation over a wide range of frequencies, from extremely energetic radiation in the gamma-ray and x-ray regions, to very low energy radiation in the microwave and radio-wave regions. Figure (10.4) below shows the Electromagnetic spectrum arranged in terms of wavelength, frequency and energy




http://en.wikipedia.org/wiki/File:Electromagnetic-Spectrum.png

Figure 10.4 – electromagnetic spectrum

Each region of the spectrum provides information on different aspects of atoms and molecules. When electrons are moved into or out of energy levels close to the nucleus of an atom, the energy changes are large, corresponding to the X-ray region of the spectrum. Analytical information can be gained by observing radiation either being absorbed or emitted by atoms and molecules

A typical X-ray wavelength of about 0.1 nm ($10^{-10}$ m) corresponds to a frequency of $3 \times 10^{18}$ Hz and an energy of a billion J $mol^{-1}$**.** By contrast the energy changes involved in molecular rotations, often detected by using microwave radiation of wavelength about 1 cm, have a frequency of $3 \times 10^{10}$ Hz, and so are 100 million times smaller.

# Ultra-violet and visible spectroscopy

These regions of the electromagnetic spectrum cover the wavelength range from around 200 – 750 nm. Within this range the ultra-violet region extends from 200nm to around 380 nm, and the visible region from around 380 nm (violet), through indigo, blue, green, yellow and orange, to 750 nm (red). (Note: The wavelengths detectable visually as colours, vary slightly from one individual to another). Figure (10.5) shows the visible spectrum from 380 – 750 nm

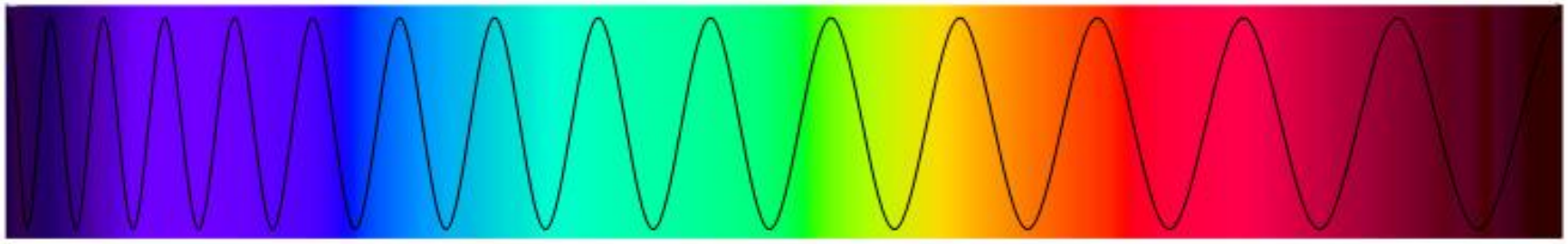



Figure 10.5 - UV/visible region of the electromagnetic spectrum http://en.wikipedia.org/wiki/File:Spectre.svg

Molecules absorb light in these wavelength regions because of **electronic transitions** from lower to higher energy levels.



**Continued on the next slide**

# Electronic transitions

The electrons involved in UV-Visible spectroscopy are those most distant from the nuclei of the atoms in the molecule. They may be:

- Pairs of electrons directly involved in bond formation between two atoms;
- Non-bonding (*n*-) electrons, mostly localised near a single atom in the molecule.

Bonding orbitals (energy levels) are of two types:

- $\sigma$ ("sigma") orbitals have their maximum electron density on the axis of the bond between the two atomic nuclei;
- $\pi$ ("pi") orbitals have their lowest electron density along the bond axis, and their maximum electron density on either side of that axis. These $\pi$-orbitals may be *delocalised* across three or more atoms in a molecule (see next slide).

In the *ground state* (i.e. normal lowest energy state) of most molecules, all the electrons are *paired*, the electrons in each pair having opposite spins. Two well-known small molecules, oxygen and nitric oxide, are exceptions.

# Electron orbitals and transitions

The different types of molecular orbital summarised in the previous slide, provide a variety of possible electronic transitions. Generally, $\sigma$ -electrons can only be promoted by absorption of radiation to $\sigma^*$-orbitals, i.e. higher energy or *excited* $\sigma$ – orbitals, and $\pi$-electrons can only be promoted to $\pi^*$ or excited $\pi$-orbitals. These excited orbitals are sometimes called *anti-bonding* orbitals. Non-bonding electrons can be promoted to either type of excited orbital, so $\sigma - \sigma^*$, $\pi - \pi^*$, $n - \sigma^*$, and $n - \pi^*$ transitions are all possible (but not equally likely). These transitions are summarised in the figure (10.6) below.

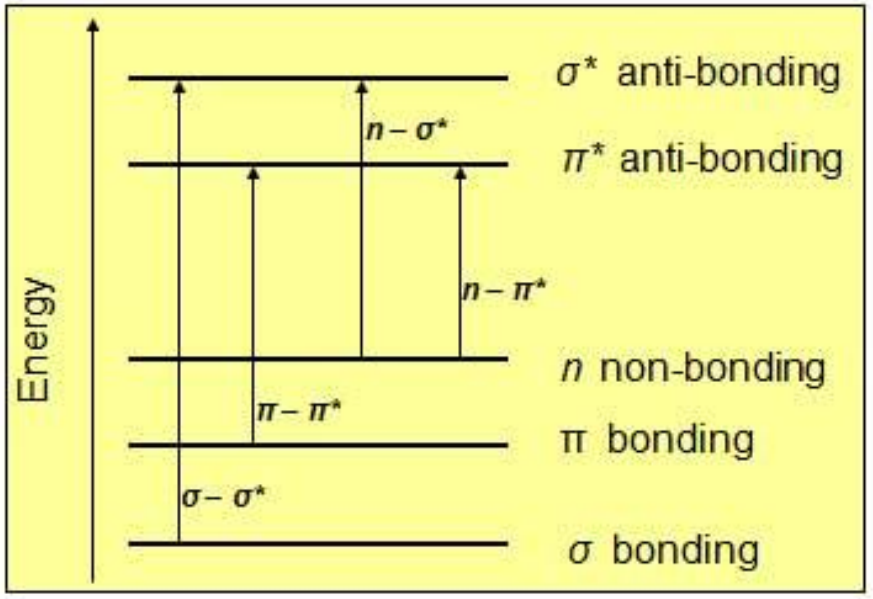



Figure 10.6 - possible orbital transitions

Since the energies of the molecular orbitals are *quantised*, i.e. can only take certain values, the transition energies, which must match the energies of the absorbed photons, can also only take certain values. You might therefore think that molecular absorption spectra should consist of a series of sharp lines, the wavelengths of which would correspond to the energy differences between the orbitals. In practice, however, this is usually not true in studies of molecules in solution: indeed many solution spectra in the ultraviolet and visible regions are broad and rather featureless [see figure (10.7)]. This is because:

- Some vibrational (and rotational) energy levels – not shown in the figure (10.6) on the previous slide – are super-imposed on the electronic energy levels;

- Interactions between solute molecules and surrounding solvent molecules "smooth" the spectral profile. It is very important to remember that solution spectra are really studies of a solute-solvent complex.

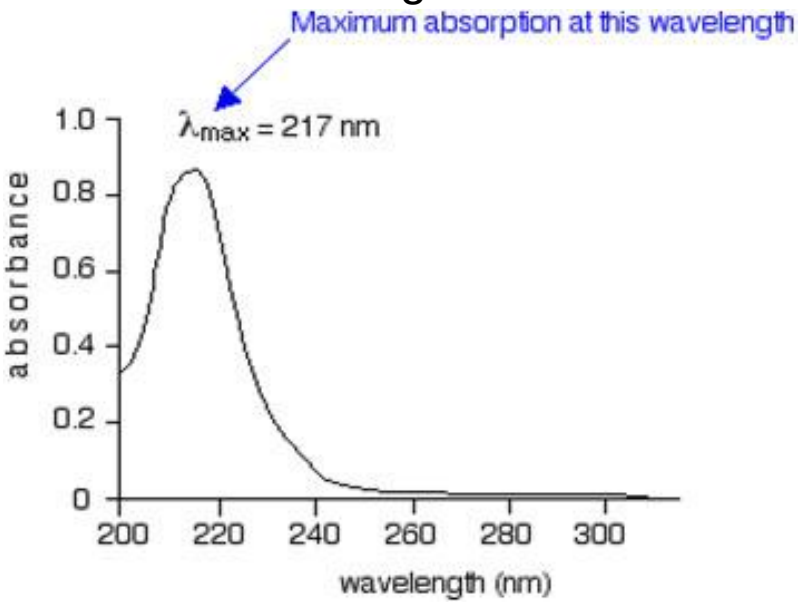



Figure 10.7 - absorption spectrum of buta-1,3-diene

# Vibrational energy

When a molecule absorbs UV/visible light it may gain vibrational energy as well as electronic energy. This is illustrated in Figure (10.8), which shows the electronic states (bold lines) and their associated vibrational levels (fainter lines) for a simple molecule.

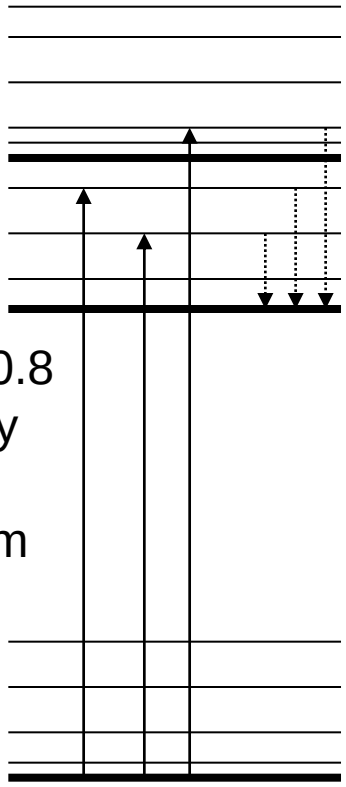



Figure 10.8 Energy level diagram

The electronic states $S_0$, $S_1$, $S_2$ etc are **singlet** states, which means that they have no unpaired electrons. $S_0$ is called the **ground state** and this is where the molecule normally resides. The vibrational levels are much closer to each other than the electronic ones: moreover the vibrational levels of the excited states may overlap, so the upper vibrational levels of $S_1$ overlap the lower vibrational levels of $S_2$. When the molecule absorbs a photon it may gain vibrational energy as well as electronic energy as shown by the arrows in the figure: the exact level reached after photon absorption depends on the photon's energy and on the molecule's properties.

# Vibrational energy losses

When an excited molecule gains additional vibrational energy as well as moving to a new electronic state, often the first energy dissipation process that occurs is that this vibrational energy is lost. The process is often called **vibrational relaxation**, and is typically complete within about $10^{-11}$ seconds. (The energy lost appears in the sample as heat, but is so small as to be extremely hard to detect). As is shown by the lines in figure (10.8) on the previous slide, the result is that the molecule's energy falls to the lowest vibrational level of $S_1$, the first excited singlet state. Even when the initial photon absorption results in the molecule being excited to $S_2$ or a higher electronic level, because of the overlap of the vibrational levels of $S_1$, $S_2$ etc, there is a smooth pathway down to the lowest vibrational state of $S_1$. Such a transition, e.g. from $S_2$ to $S_1$, is known as **internal conversion**.

However, once the molecule has reached this energy state, there is usually a much bigger energy gap to be crossed before it can return to the ground state, so it may remain in $S_1$ for an extended period (in molecular terms!) of $10^{-9}$ – $10^{-8}$ s, i.e. 1-10 ns. This period allows several competing energy loss processes to occur, including some involving **triplet** electronic energy levels. These are discussed in the next slide.

# Triplet states

Figure (10.9), shows molecular electronic and vibrational energy levels again (for simplicity only the first excited singlet state, $S_1$, is shown) but now with the addition of the molecule's lowest **triplet** state. Triplet states have two unpaired electrons, i.e. two electrons with parallel spins (as a result they are paramagnetic and can be directly detected in this way). So the lowest triplet state, $T_1$, can be regarded as similar to $S_1$ except that a pair of electrons has become unpaired. As a result the energy of the $T_1$ state is slightly lower than that of the $S_1$ state, i.e. it lies between that of $S_1$ and that of $S_0$.

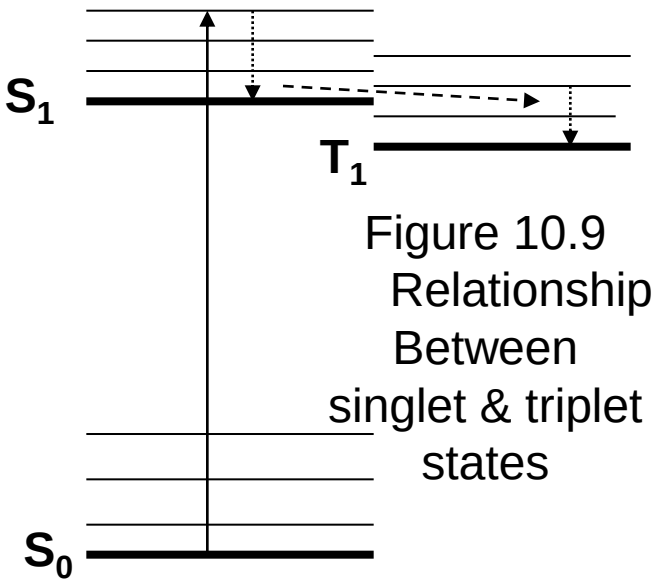



Figure 10.9 Relationship Between singlet & triplet states

Because their energy levels overlap strongly it might be thought that it would be very easy for a molecule to pass from $S_1$ to $T_1$. This process, shown by the broken arrow, is called **inter-system crossing**, and occurs with no significant change in the molecule's energy, but in fact it typically takes $10^{-7}$ – $10^{-8}$ s, because under the laws of quantum mechanics it is a **forbidden transition**, as it involves a change of electron spin. Such transitions are not really wholly forbidden, they are simply less likely, and thus occur at lower rates, than transitions between one singlet state and another, or one triplet state and another. Once in the $T_1$ state the molecule can again easily lose any excess vibrational energy.

# Using UV-visible absorption spectrometry

The wavelengths at which compounds absorb most light in the UV-visible regions (e.g. 217 nm in the ultra-violet region in the case of buta-1,3-diene) give some information about the **chromophores**, i.e. light-absorbing groups, that they contain. But other methods, e.g. **vibrational** spectroscopy, nuclear magnetic resonance, and mass spectrometry – tell us so much more about molecular structure, that the use of UV-visible spectrometry for this purpose is now relatively infrequent.

The main modern uses of UV/visible methods are in **quantitative analysis**, i.e. The determination of **how much** of a given chemical a sample contains. The terms and symbols used in such experiments are summarised in figure (10.10) below.

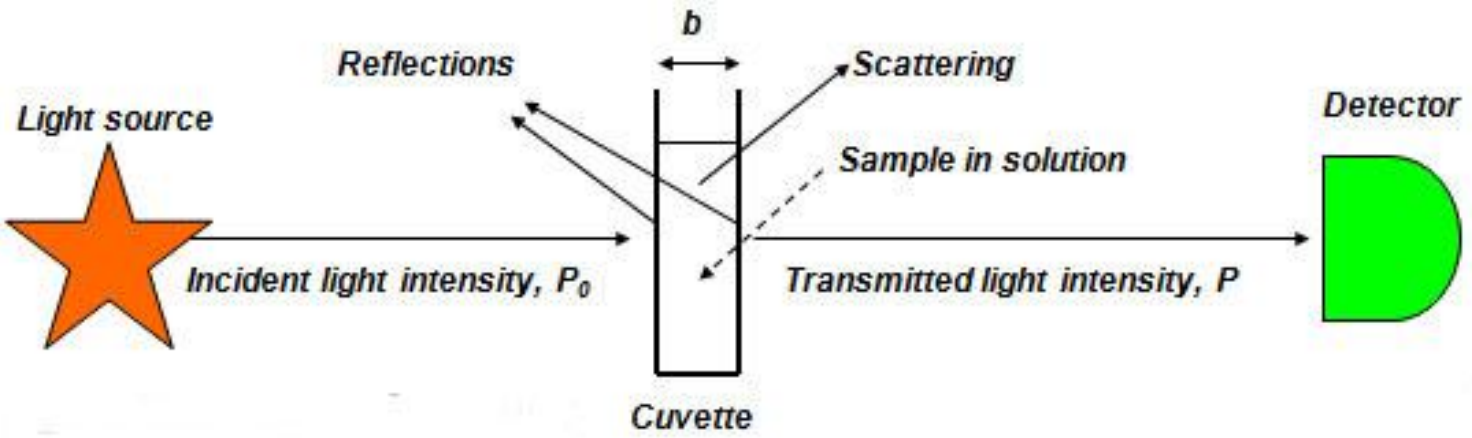



Figure 10.10 – terms & symbols

# Quantitative absorption spectroscopy

As shown in the previous slide, in a quantitative analysis experiment, light from a suitable source, with a radiant power $P_0$, is shone on to the sample in a silica, glass, or plastic cuvette. (Usually there is a filter or monochromator between the light source and the sample so that the latter is illuminated by only a limited range of wavelengths – we consider several aspects of the instrumentation later (**see chapter 11 of this teaching and learning programme, slides 4 – 6 & 64 - 66**). The sample may absorb some of this light as the electrons in the sample molecules are promoted to excited states, so the radiant power, $P$, of the light leaving the sample and falling on a suitable detector is less than the incident radiant power $P_0$. The ratio of these two light intensities, $P/P_0$, is known as the **transmittance**, $T$, of the sample. We would expect that $T$ should depend in some way on the amount of absorbing material in the sample cuvette, and on the path-length, $b$, through which the light travels in passing through the cuvette. About 280 years ago it was shown that this relationship is given by the equation (10.2)

**Transmittance, $T = P/P_0 = 10^{-kb}$** Equation (10.2)

- where $k$ is a constant depending on the sample concentration and properties.



**Continued on the next slide**

The constant *k* was shown by Beer to depend on the sample concentration, *c*, and on the intrinsic ability, *a*, of the sample to absorb light at the incident wavelength. If the transmittance is expressed logarithmically, and we also replace *k* with $a \times c$, we can re-write the equation (10.2) on the previous slide as:

$$\text{Log } T = \log (P/P_0) = -abc$$

Equation (10.3)

In practice it is convenient to replace the logarithmic function and the negative sign with a new function called the absorbance, *A*, given by –

$$A = -\log T = abc$$

Equation (10.4)

This is the conventional form of the law frequently called **Beer's Law** (though in practice several other scientists contributed to its discovery). The value of using the absorbance to describe the behaviour of the sample is that *A* is directly proportional to the concentration *c*. Absorbance was formerly known as *optical density* but the use of this term is now discouraged. The constant *a* is called the **absorptivity** of the sample material. For a single solute, if *c* is a *molar* concentration, *a* is replaced by ***ε***, the **molar absorptivity**, with units of $cm^{-1}$ $mole^{-1}$ L. Tables of ***ε*** values for many compounds are widely available.

**Example (10.iii)**

A sample in a 1 cm cuvette transmits 90% of the light incident on it at a given wavelength. Find its concentration, given that it has a molar absorptivity of 5,000 and a relative molecular mass (molecular weight) of 250.

Since $T$ = 90%, i.e. 0.90,
$A = \log 1/T = \log 1/0.90 = \log 1.11 = 0.0458$

Using molar units, $A = \varepsilon bc$, where $b = 1$ and $\varepsilon = 5{,}000$

Thus $c = 0.0458/5{,}000 = 9.15\ 10^{-6}$ M.
The molecular weight of the solute is 250, so the concentration is:
$250 \times 9.15 \times 10^{-6}$ M $= 2.29 \times 10^{-3}$ g/dm$^3$
This can also be represented as $2.29 \times 10^{-6}$ g/cm$^3$ or 2.29 µg/cm$^3$ [ppm (w/v)]

Note:The first line of this answer can be replaced by a similar calculation.
Since $T$, as used in that line, is a percentage divided by 100, i.e. $T = \%T/100$,
we can write $A = \log (100/\%T) = \log 100 - \log \%T = 2 - \log \%T = 2 - 1.9542 = 0.0458$

You should practice calculations of this kind, so that you can convert absorbance and transmittance easily, and use different concentration units.

# Absorption of light by mixtures

If a sample contains two absorbing solutes, *x* and *y*, with concentrations $c_x$ and $c_y$ and molar absorptivities at a particular wavelength of $\varepsilon_x$ and $\varepsilon_y$, the Absorbance (*A*) of this mixture at that wavelength is normally expected to be:

$$A = \varepsilon_x b c_x + \varepsilon_y b c_y \qquad \text{Equation (10.5)}$$

i.e. the absorbances of the two components will be additive. This means that, if the molar absorptivities of the two components at *two* separate wavelengths – often the wavelengths at which the two components have their absorption maxima – are known, the concentrations of the two solutes can be determined in a single experiment by solving two simultaneous equations. (**An example calculation is shown on the next slide**) Using modern spectrometers that acquire spectra over a range of wavelengths very quickly (diode array and Fourier Transform instruments), and with the aid of statistical techniques implemented on a PC, this principle can now be extended to the analysis of mixtures containing several components.

However it is important to realise that this approach to multi-component analysis is only valid if the sample components **do not interact or react** with each other. If that happens, the analysis of such mixtures is more complex as the interactions may alter the spectroscopic properties of the solutes.

**Example (10.iv)**

Potassium dichromate & potassium permanganate have overlapping spectra in 1M $H_2SO_4$. Both substances exhibit complex spectra with overlapping bands. For analysis of these two substances measurements at 440 & 545 nm are to be preferred. Given the following data it is possible to determine the composition of a mixture of the two substances:

$K_2Cr_2O_7$ ($1.00 \times 10^{-3}$) – $A_{440} = 0.374$, $A_{545} = 0.0009$
$KMnO_4$ ($2.00 \times 10^{-4}$) – $A_{440} = 0.019$, $A_{545} = 0.475$

A mixture of the two substances measured at these two wavelengths gave the following absorbance data: $A_{440} = 0.405$; $A_{545} = 0.712$

The pathlength of the cell is not know precisely, however as all measurements were made in the same cell, the pathlength term is now constant.

$0.374 = k_{Cr,440} \times 1.00 \times 10^{-3}$ Thus $k_{Cr,440} = 374$
$0.009 = k_{Cr,545} \times 1.00 \times 10^{-3}$ Thus $k_{Cr,545} = 9$
$0.019 = k_{Mn,440} \times 2.00 \times 10^{-4}$ Thus $k_{Mn,440} = 95$
$0.475 = k_{Mn,545} \times 2.00 \times 10^{-4}$ Thus $k_{Mn,545} = 2.38 \times 10^{3}$

From equation (xx) on the previous slide, the measured absorbance at each wavelength is a combination of the individual absorbances of the two components of the mixture. Thus:
$0.405 = 374 [Cr_2O_7^{2-}] + 95[MnO_4^-]$ : $0.712 = 9[Cr_2O_7^{2-}] + 2.38 \times 10^3 [MnO_4^-]$
Solving these equations simultaneously gives:

**$[Cr_2O_7^{2-}] = 1.01 \times 10^{-3}$ M and $[MnO_4^-] = 2.95 \times 10^{-4}$ M**

# Beer's Law in quantitative analysis

In practice the $\varepsilon$ values for the solute or solutes being analysed are often not known – the compounds may be new or poorly characterised, and in any case molar absorptivities depend on the solvent used, the temperature and so on. But this does not stop quantitative analyses being carried out, as the calibration approach can be used. Properly used, this method (which is outlined in the next slide and described in more detail in Chapter 4 of this teaching and learning programme can provide accurate and precise analyses, in many types of spectroscopic measurement. A calibration experiment involves measuring the absorbance at a suitable wavelength of a series of standards, (solutions with *known* concentrations), and using the results to plot a calibration graph of absorbance (*y*-axis) against concentration (*x*-axis). Test materials, i.e. those with *unknown* concentrations of solute, are studied in the same experimental conditions, and their concentrations found from the graph by interpolation.

Benefits of the calibration approach include:

- The ability to measure many test materials with a range of concentrations using just one calibration graph;
- The elimination of some sources of systematic error. For example if the wavelength used was not that of exactly $\lambda_{max}$, any errors should cancel out between the standards and the test materials, giving results that are still valid.

# How calibration works

In the diagram [figure (10.11)] the points marked by open circles show the Absorbance (or other instrument response) values, for a set of calibration materials with known concentrations (including a blank). The statistically calculated best straight line through those points is shown in black, and the estimation by interpolation of the concentration of a test, (i.e. “unknown”) sample is shown in red.

In this simple method it is assumed that all the errors are in the *y*-direction, i.e. in the instrument response, and that all the calibration points have equal weight, i.e. equal importance in calculating the best straight line.

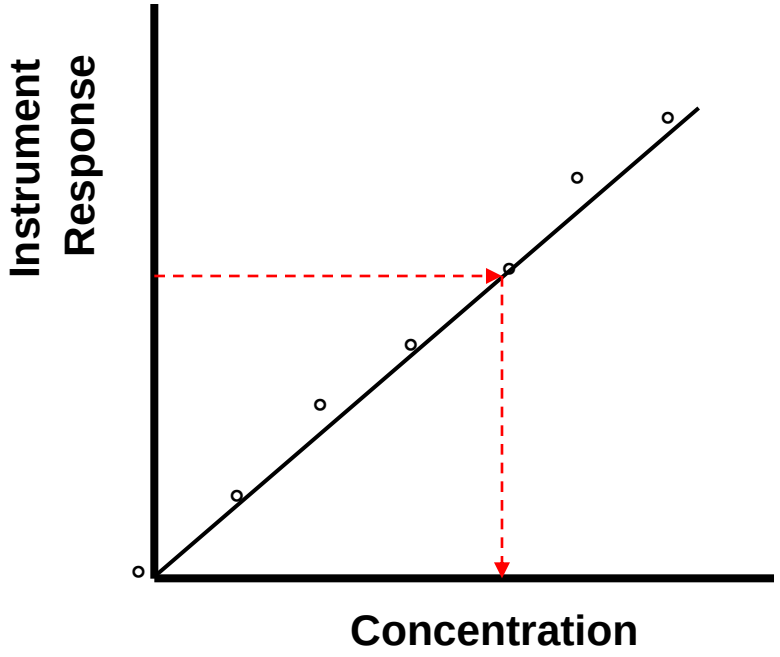



Figure 10.11 – typical calibration plot

# Does Beer's Law always work?

Although Beer's Law is extremely useful in many situations, it is wrong to think that it can always be applied. Deviations from the law occur for a variety of reasons. These may be categorised as:

- Deviations due to chemical properties of the samples studied;
  - Monomer-dimer equilibrium;
  - Metal ion complexes;
  - Chemical species in equilibrium.
- Deviations due to solution and sample properties;
  - Scattered light;
  - Solvent properties at low wavelengths.
- Deviations due to instrumental effects.
  - Use of incident radiation that is not monochromatic.;
  - Effects of stray light;
  - Non-linearity at high absorbances

These effects are considered in detail in the next 7 slides

# Deviations due to chemical effects

Chemical causes of Beer's Law deviations often arise when the absorbing species studied are part of an equilibrium system. Some compounds have a tendency to associate to form dimers. This association increases as the concentration rises - as collisions between monomer molecules in solution become more frequent. The effect of the monomer-dimer equilibrium on the absorbance may vary. Often the dimer itself absorbs less strongly than the monomer at the maximum absorption wavelength of the latter: in that case there will be **negative deviations from Beer's Law** as the concentration rises. But if the equilibrium is studied with the aid of a coloured reagent that reacts more strongly with the dimer than the monomer, then **positive deviations from the Law** will be seen.

Metal ions in solution are often analysed with the aid of complexing agents that combine with them to give coloured derivatives. In such cases excess reagent should be used. Otherwise Beer's Law deviations might be caused by the dissociation of the complexes at low concentrations.



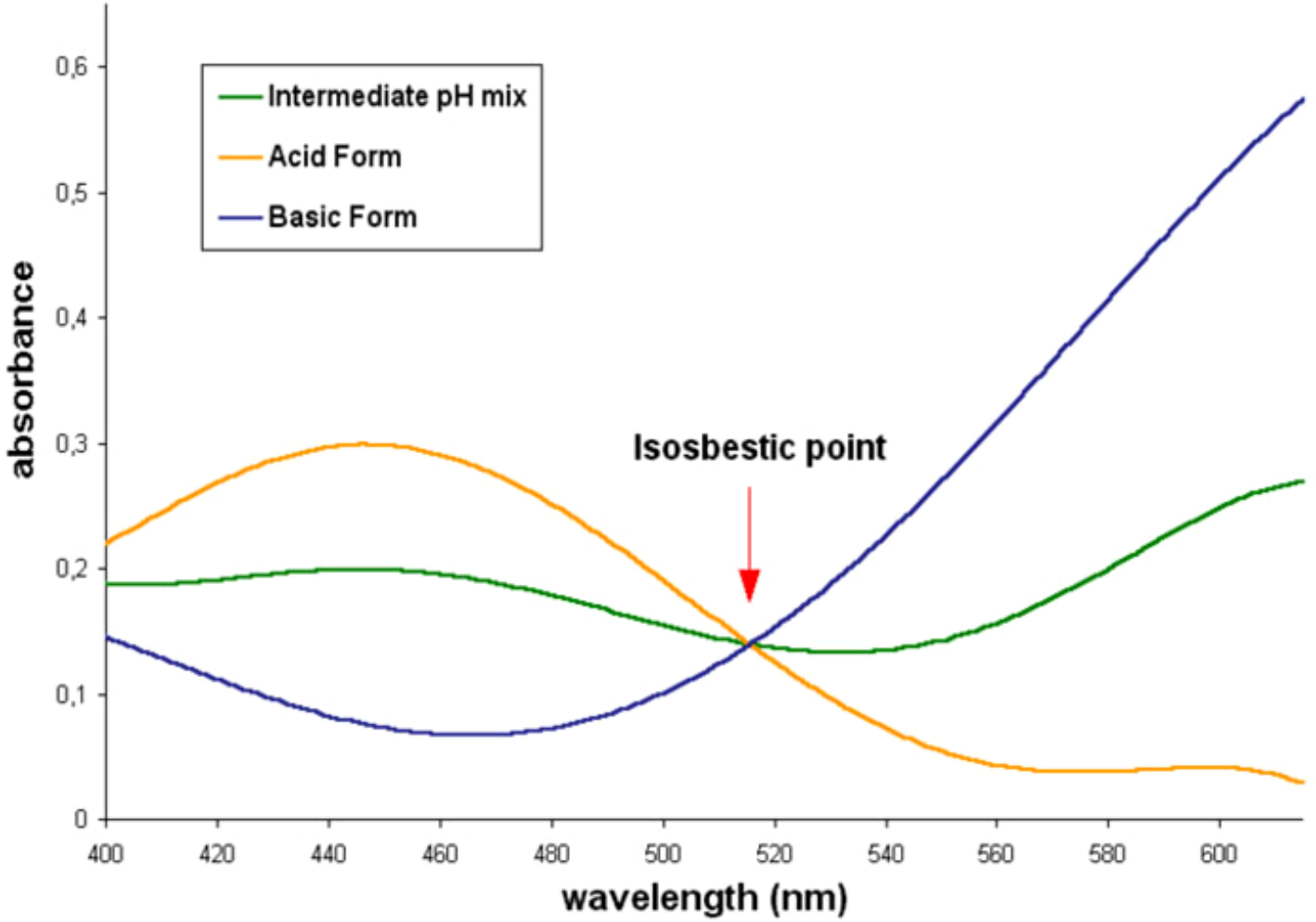
**Continued on the next slide**

Absorbance measurements are often used to study chemical systems in which two species are present in equilibrium. These systems include:

- Those in which monomer-dimer interactions occur;
- Those involving the combination of a metal ion and a chelating agent designed to combine with it, to yield a coloured product. Use excess reagent to minimise deviation effects.
- The pH-dependent colour changes of indicator dyes. Use buffer solutions to minimise deviation effects

If the spectra of the two components in an equilibrium system are measured separately, they are often found to overlap, and there may be a wavelength at which they both have the same $\varepsilon$ (molar absorptivity) values. Then any mixtures of the two components will have the same absorbance and pass through the same point, an **isosbestic point**, at that particular wavelength in the spectrum. The presence of such a point is very good evidence that two species are present in the samples (though there may be other components, e.g. with $\varepsilon = 0$, at that wavelength). An example of such a system is shown as figure (10.12) on the next slide. In favourable cases such spectra can be used to estimate the association constant for complex formation, and the composition (molar ratio) of the complexes.

Figure 10.12 spectra of bromocresol green showing an Isosbestic Point




The figure shows the absorption spectra of bromocresol green in acidic, basic, and neutral solutions. The acidic and basic forms of this dye have quite different spectra, but at about 515 nm their $\varepsilon$ values are equal, so the absorbance of a given solution at that wavelength is the same at all pH values. http://en.wikipedia.org/wiki/File:Bromocresol_green_spectrum.png

# Deviations due to sample & solution effects

Sample characteristics can affect absorption measurements. One of these is the problem of **scattered light**. Scattering refers to the phenomenon (**see slide 21**) in which an incident light photon collides with a molecule in the sample cuvette, and as a result emerges in a new direction, so that it does not reach the detector. From a quantitative point of view the result is just as if the sample had absorbed the photon - the apparent absorbance is increased. Light scattering can in principle be detected in an absorption spectrometer. The technique is called **turbidimetry**) and can be applied to measure solutions containing low levels of suspended particles.

In many experiments on dilute solutions of low molecular weight solutes, almost all the scattering comes from the solvent (there are not sufficient sample molecules present to make a difference) so the effect is the same for all the standards and test samples in a calibration experiment.



**Continued on the next slide**

Macromolecules such as proteins scatter much more light (again the method is routinely used in the analysis of such samples) so their solutions might exhibit more apparent absorbance than is truly due to the absorption phenomenon. **The scattering intensity from a given sample varies as $1/\lambda^4$, so is most severe at short wavelengths**.

Solvent properties can also cause problems, especially at low wavelengths. Even solutions of common salts such as NaCl absorb light below about 210 nm, and oxygen dissolved in aqueous solutions gives problems in the same region. Absorbance measurements at these low wavelengths are clearly problematical!

# Deviations due to instrumental effects

Apparent deviations from Beer's Law can occur for instrumental as well as chemical reasons. Several distinct factors are involved. The most important one is probably the Law's **assumption that monochromatic light is used to irradiate the sample**. In practice the incident light wavelengths, isolated by a grating monochromator in most instruments, have a band-width of a 1-2 nm. If the $\varepsilon$ value of the compound studied varies significantly over this range, e.g. for a polynuclear aromatic hydrocarbon (PAH), which may have a very sharply featured spectrum [see figure (10.13) below], then the observed absorbance will depend on the bandwidth used, and will depend critically on accurate agreement between the monochromator and the wavelength of maximum absorbance. The result of such errors is a negative deviation from the true absorbance.

If the sample has a broad spectral maximum this is not such a critical problem.

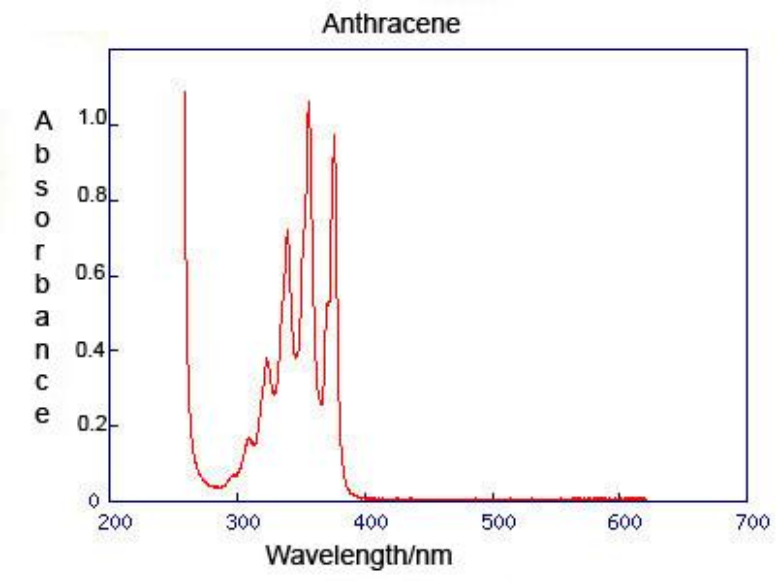



Figure 10.13 – absorption spectrum of anthracene, a typical PAH



**Continued on the next slide**

A further problem is that of **stray light** photons reaching the detector, when they should not do so. Any such photons increase the detector output, thus giving a falsely low absorbance value. They come from the monochromator in the spectrometer, or as a result of sample scattering, photons reflected from cuvette walls (see slide 21), unwanted ambient light, and so on. In a modern instrument levels of stray light should be less than the equivalent of 0.1% transmission. This would give an error of 0.4% for a sample with $A = 1$ (see below): such an error is probably small compared with other errors in a typical experiment.

**Examples (10.v)** If A = 1, $T = 10^{-A} = 0.1$. This is the ratio $P/P_0$, the transmitted radiant power divided by the incident radiant power. Since S = 0.1% = 0.001 the apparent T value is (0.1 + 0.001)/(1 + 0.001) = 0.1009. The log of this number is -0.996, so A = +0.996, 0.4% lower than the true value of 1.000

All spectrometers have a limited range over which they respond linearly to changes in sample concentration. In a modern instrument this limit could be as high as $A = 4$. Since the transmittance, $T$, is given by $T = 10^{-A}$, this corresponds to a transmittance of $10^{-4}$; that is, only 0.01% of the incident light is reaching the detector. This therefore requires good instrument design if accurate measurements are to be made with such low light levels

**Question 10.1** Benzene absorbs ultra-violet light at a wavelength of about 254 nm in the gas phase. Convert this wavelength into a frequency and into wave-numbers. (b) proteins show a strong absorption band in the mid-infra-red spectrum at ca. 1650 $cm^{-1}$, mainly due to their -NH-C=O ("amide") groups. Convert this wave-number into a wavelength and a frequency.

**Question 10.2** If a sample has an absorbance of 0.5, what fraction of the incident light does it transmit? (Light scattering can be ignored). (b) Calculate the absorbance (*A*) of a 1 micromolar solution of a compound with a molar absorptivity (ε) of 90,000 $cm^{-1}$ $mole^{-1}$ L in a 1 cm cuvette (you may assume that the solvent does not absorb radiation at the wavelength in question).

**Question 10.3** In slide 29, some assumptions used in the calibration approach to the evaluation of instrumental analysis results are summarised. How valid do you think these assumptions might be in practice?

# Outline answer to question 10.1

The answer to this question may be found on slides 7 - 9

- (a) 254 nm = 2.54 x $10^{-5}$ cm, so the wave-number result is the reciprocal of this, i.e. 39370 $cm^{-1}$. Frequency is given by $v = c/\lambda$, where $c$ is the velocity of light and $\lambda$ the wavelength. So here $v$ = 3 x $10^{10}$/2.54 x $10^{-5}$ = 1.18 x $10^{15}$ Hz.

- (b) The reciprocal of 1650 $cm^{-1}$ gives the wavelength value as 6.06 x $10^{-4}$ cm, or 6.06 μm. Using the same equation as in (a) the frequency is 3 x $10^{10}$/6.06 x $10^{-4}$ = 4.95 x $10^{13}$ Hz.

# Outline answer to question 10.2

The answer to this question my be found on slides 22 - 24

- a. $A = -\log T$ (see slide 17), so $T = 10^{-A}$. In this case $T = 10^{-0.5} = 0.316$. So 31.6% of the light is transmitted in this example. Best results in uv-visible absorption spectroscopy are normally obtained at $A$ values between ca. 0.4 and 0.7, i.e. when 20 – 40% of the light is transmitted.
- b. The absorbance of a sample, $A$, is given by $A = \varepsilon bc$, where the three latter terms are respectively the molar absorptivity of the solute, the cuvette pathlength in cm, and the molar concentration of the solute. In this case they take values of 90,000, 1 and $10^{-6}$ respectively, giving an $A$ value of $90{,}000 \times 10^{-6} = 0.09$. Note that even though this solute has a high $\varepsilon$ value, its absorbance at the 1 micromolar level is small, emphasising again that nanomolar and lower concentrations are very difficult to measure using absorption spectrometry.

# Outline answer to question 10.3

The answer to this question may be found in slides 28 - 36

- The methods normally used to evaluate data from calibration experiments use a number of assumptions, some more easily justified than others. The two listed in slide 28 are perhaps the most important in practice.
- The first assumption is that, when the instrument response is plotted on the *y*-axis of a calibration graph and the standard concentrations on the *x*-axis, errors only occur in the *y*-direction, i.e. the concentrations are known exactly (or in practice, have negligible errors). This is often a reasonable view: random errors in many measurement methods are ca. 2-3% (relative standard deviation, rsd) while standards can be made up with an accuracy of 0.1% or better. However some modern automated methods (e.g. hplc, flow injection) have RSDs of less than 1%, so in such cases it may be wise to take extra precautions to minimise errors in the standards, e.g. by making them up using only gravimetric methods rather than volumetric ones.
- The second assumption is that the *y*-direction error is the same for all values of *x*. This means that all the points on the graph have equal importance – equal *weight* so that simple *unweighted* regression equations can be used to give the best line. However it frequently turns out that the *y*-direction errors increase as *x* increases. This means that more complex *weighted* regression calculation methods should be used.