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 11 – Spectroscopic techniques based upon the absorption or emission of electromagnetic radiation for the measurement of molecular species

Contents

Comparison of spectroscopic techniques

Table (11.1) below, summarises the spectroscopic techniques, which provide valuable analytical information. With the exception of Mass Spectrometry, all utilise to radiation which forms part of the electromagnetic spectrum. Those starred in ★ are solely or partly emission techniques, the remainder rely to the absorption of radiation.

Spectroscopic technique	Molecular transitions and mode of application	Applications
UV/visible	Electronic transitions Absorption of radiation	Technique mostly used for quantitative analysis and as a basis for detection in HPLC, CE and FIA. Visible spectroscopy mostly applicable to transition metal complexes and complex ions.
Infrared	Vibrational transitions Absorption of radiation	Powerful technique for identifying molecular structure particularly of organic compounds. Can also be used for quantitative measurement. It is particularly useful for continuous air quality monitoring
Near infra-red	Vibrational transitions Absorption of radiation	Complex spectra requiring computational analysis in order to elicit useful data. Very useful for the rapid measurement of the protein contents of cereals.
Raman	Vibrational and electronic transitions. Emission of radiation	Complements IR for identifying molecular structure but can offer higher sensitivities than IR. Finding increased application for examination of biological systems and in combination with optical microscopy. Applicable to process analysis
Fluorescence & luminescence ★	Electronic transitions Vibrational transitions Emission of radiation	Very sensitive quantitative technique particularly applicable to biological systems. Can be used as a basis of detection in HPLC, CE and FIA.
Mass spectrometry	Ionisation	Although MS may be applied directly as an analysis tool, it is mostly used as part of a tandem technology (eg: LCMS or ICPMS). Very powerful technique for both identification and quantification of drugs and drug metabolites.
X-ray methods ★	High energy electronic transitions	Elemental analysis by XRF, sometimes without sample pretreatment. Crystal structure determination by XRD, also identification of crystalline compounds in complex matrices.

Table 11.1 – Brief summary of spectroscopic techniques

Generic instrumentation for use within the UV/visible and infra-red spectral regions

A spectrometer or spectrophotometer is an instrument that will separate polychromatic radiation into separate wavelengths. The main components are:

- Source of radiation
- Monochromator to separate the radiation
- Associated optics
- Detectors
- Read out devices

Although some aspects of spectroscopic instrumentation are covered in this teaching and learning programme, a more extensive discussion of instrumentation may be found in the following two websites:

http://en.wikipedia.org/wiki/Ultraviolet-visible_spectroscopy
http://en.wikipedia.org/wiki/IR_spectroscopy

One important parameter in the successful acquisition of spectra is the selection of spectral slit-width.

UV/Visible spectrometers

Spectrometers for use in this part of the electromagnetic spectrum are either: **Single beam** or **Double beam.** Double beam instruments are spectroscopically more complex than single beam instruments, and they were mainly devised as recording instruments (instruments that were able to automatically vary the wavelength and produce a record of Absorbance *versus* Wavelength, over a chosen wavelength range). They were also able to compensate for optical drift and electronic noise. Early single beam instruments, were developed mainly to record absorbances at fixed chosen wavelengths, appropriate to a target analyte.

Modern single beam instruments can now however perform the same functions as double beam spectrometers, due to the use of newer spectroscopic components and more reliable electronics. Figure (11.1) is a block diagram of a typical single beam UV/visible spectrometer.

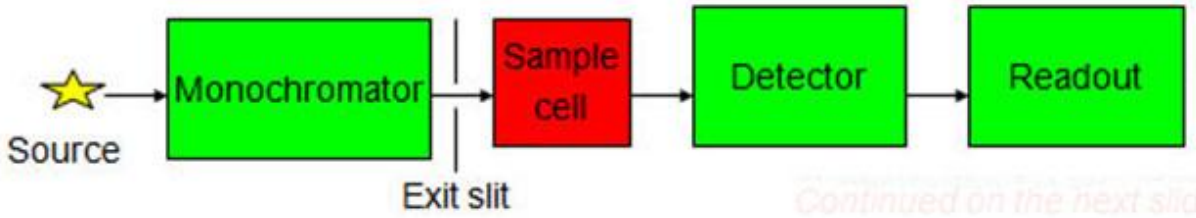

Figure 11.1 – block diagram of a single beam spectrometer

Note: this type of instrument is known as a **dispersion spectrometer**, as the source radiation is separated (dispersed) before impinging on the sample.

Continued on the next slide

Although figure (11.1) on the previous slide shows the monochromator separating the radiation before it is incident on the sample solution, this is not always the case. Figure (11.2) shows the alternative arrangement whereby all of the radiation emitted by the source is passed through the sample. This allows a total spectrum to be obtained more rapidly than with a typical dispersion instrument.

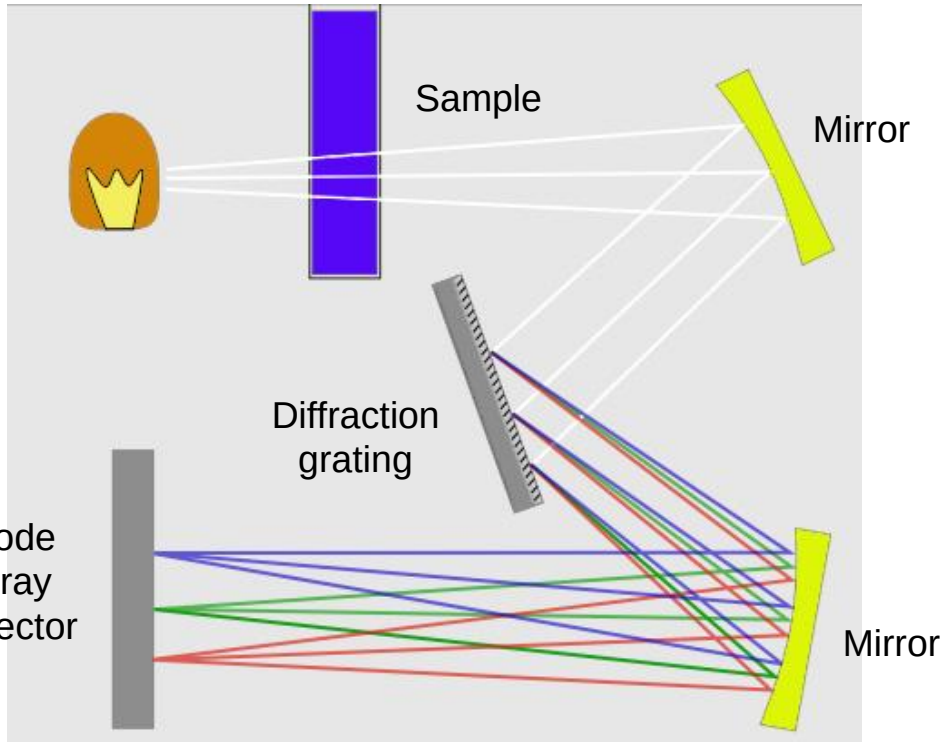

Figure 11.2 – spectral layout of a diode array spectrometer

In traditional spectrometers the monochromator is rotated in order to direct the separated radiation sequentially and selectively onto the detector.
In figure (11.2) however all of the emitted radiation is passed to the detector, which consists of an array of diodes each chosen to measure a particular small bandwidth.
This is known as a **Diode Array Detector**

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Obtaining UV/Visible spectra

Many organic and inorganic compounds can produce electronic spectra which can be used both for qualitative and quantitative application. As has been indicated already, the poor resolution of spectra in this region means that qualitative identification using this technique is rarely applied. However, for compounds, with large molar absorptivities, the technique offers potential for the development of sensitive quantitative methods of analysis. Although there are a very few examples where measurements can be made by using reflective techniques, the majority of applications within the UV and visible regions of the electromagnetic spectrum are made using transmission modes on solution spectra. When making measurements three decisions need to be made:

- Choice of cell materials
- Cell pathlengths
- Choice of solvents

Cell materials and sizes

The cell materials normally available for use in these regions are summarised in table (11.2) below. Cells normally have two transparent faces and two opaque faces. Basic cells are commercially available in 0.5, 1.0, 2.0 and 4.0 cm pathlengths. A typical 1.0 cm pathlength cell is shown in figure (11.3)

Cell material	Application for UV region 200 - 370 nm	Application for Visible region 370 - 850 nm
Silica	√	√
Quartz	√	√
Glass	X	√
Plastic	X	√

Table 11.2 – cell materials for use in the UV/visible spectral regions

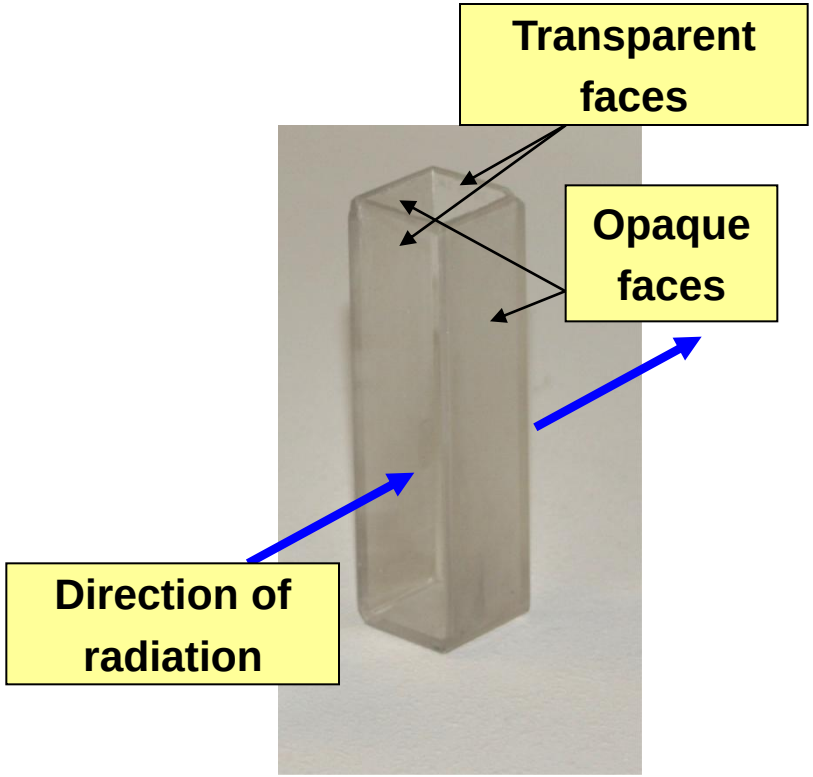


Figure 11.3 – typical 1 cm pathlength cell

Further cell designs are shown on the next slide.

Alternative cell designs

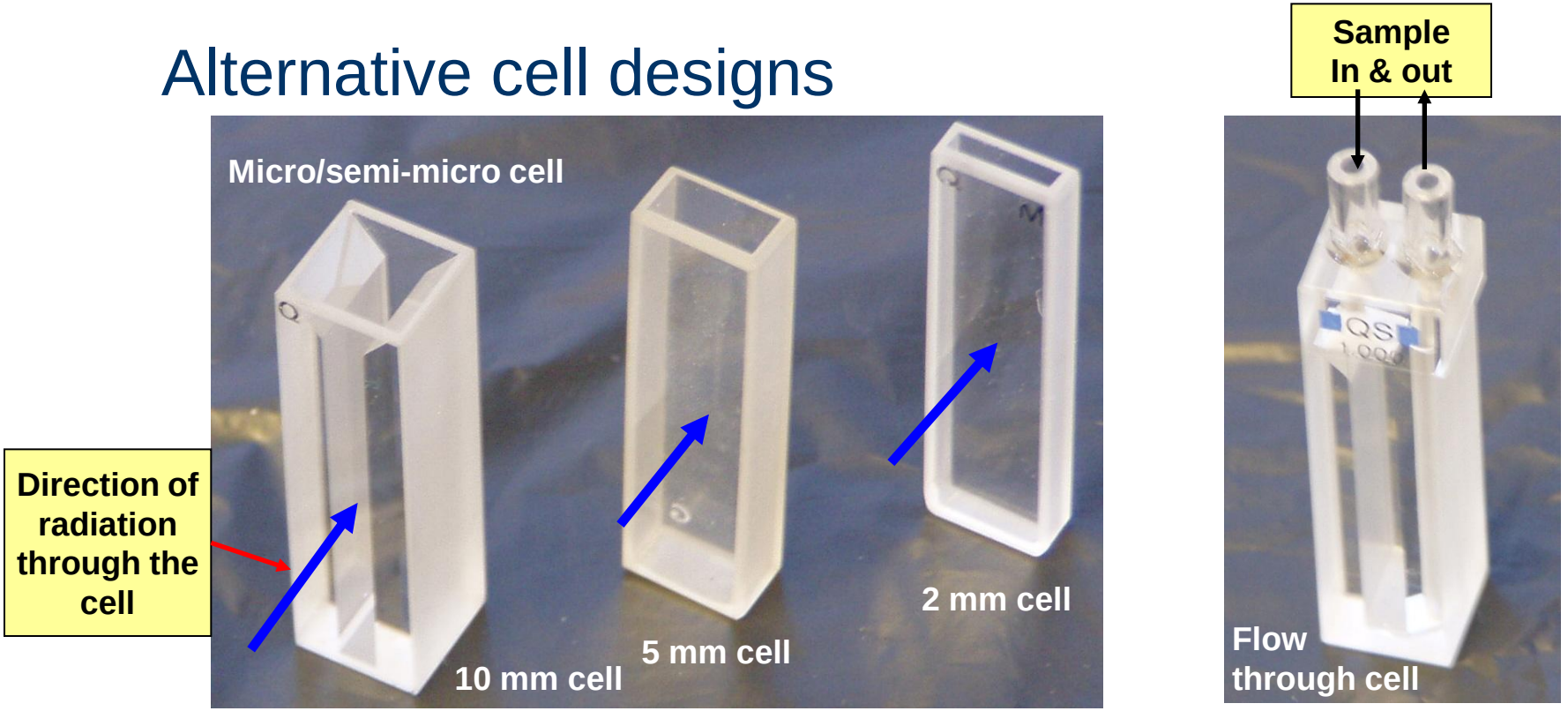


Figure 11.4 – alternative cell designs

The cells shown in figure (11.4) represent some alternative cell designs. Cells can be obtained in a range of pathlengths – typically 40, 20, 10, 5 and 2 mm. Semi-micro or micro cells are useful when only a small quantity of sample is available and cells also may be found with stoppers, which are useful when handing volatile solvents . Flow cell are widely used in HPLC and automated methods such as Flow Injection Analysis (**see Chapter 14 of this teaching & learning programme**), however these 'user' techniques tend to have purpose-made cell designs

Solvents for use in the UV-visible spectral regions

Any solvent chosen for use within these spectral regions will need to be transparent over the spectral region of interest. This restricts considerably the number of popular solvents that are available for use. Water is applicable to both of these regions, however there are relatively few organic compounds that are water soluble. The main points in solvent choice are:

- Any colourless solvent may be used for measurements within the visible region;
- No solvents containing aromatic groupings, C=O groupings (ketones, aldehydes, carboxylic acids or esters) may be used in the UV region;
- The most popular solvents for measurements within the whole of the UV region are water, ethanol (95%), propan-2-ol, acetonitrile, cyclohexane, heptane.

Table (11.3) shown on the next slide shows the spectral transparency limits for arange of popular solvents for use in UV spectroscopy.

Solvent	Cut off point/nm
Water	200
Ethanol / Methanol / Propan-2-ol	205 / 210 / 210
Acetonitrile	210
Cyclohexane	210
Hexane & Heptane	210
Dioxane	220
1,2-dichloroethane / Dichloromethane / Chloroform	230 / 233 / 245
Benzene / Toluene/ mXylene	280 / 285 / 290
Acetone	330

Table 11.3 – Lower transparency limits for solvents in the UV spectral region

Comments on the choice of solvent

- As indicated, any solvent that is transparent to the radiation over the spectral region of interest may be employed. Dependent upon the polarity of the analyte under investigation the most popular choices are 95% ethanol (not absolute ethanol which contains traces of benzene) or cyclohexane.
- Spectra recorded in solvents of low polarity reveal more of the fine structure which tend to be obscured by the more polar solvents. Some references suggest that cyclohexane may be used down to 195 nm [lower than the 210 nm given in table (11.3) shown on the previous slide].
- In general, polar compounds are more sensitive to change in solvent than compounds that are non-polar and care should always be exercised when comparing spectra measured in solvents of differing polarities.

Application of UV-visible spectroscopy

As indicated in earlier chapters (**please refer to Chapter 7 of this teaching & learning programme**), the technique is widely applied as a mode of detection in HPLC. However, where separation is not required prior to analysis, this technique offers itself as a fairly inexpensive and versatile tool, particularly for quantitative measurement. Due to its relative simplicity, the technique is particularly applicable for 'on-site' analyses (ie: analyses carried out outside the laboratory environment). Typical spectra as shown in figure (11.5 A&B) indicate that the spectra, when obtained in solution, are relatively simple in structure and exhibit poor selectivity.

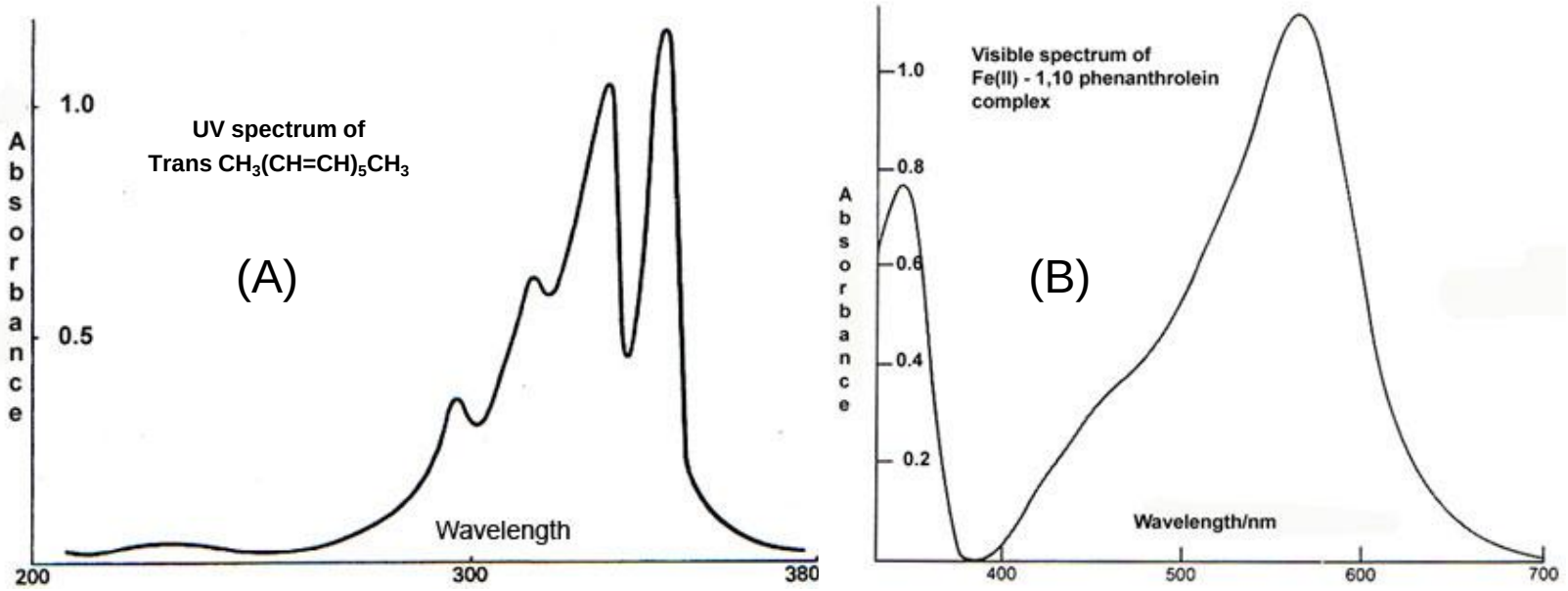


Figure 11.5 – typical UV and visible spectra

Continued on the next slide

Despite its limitations (**see chapter 10 of this teaching and learning programme**), the simplicity and universal availability of UV- visible absorption spectrometry ensures its continued widespread use in many fields.

- One of the most common uses is as a detection method for high performance liquid chromatography and other chromatographic separations. Spectrometers are provided with carefully designed low volume flow cells for this application, so that the resolution of the separation is not degraded in the detection step. Diode array spectrometers (slide 6) allow such detection systems to be used at more than one wavelength simultaneously, and/or provide a full absorption spectrum for each separated component as it passes through the flow cell.
- Many biochemical analyses routinely use absorption spectrometry. Enzymatic reactions involving the formation or oxidation of the co-factors NADH and NADPH can be followed at 340 nm, and colorimetric assays are available for many other enzymes. Proteins absorb radiation at 280 nm because of their aromatic amino-acid (tyrosine and tryptophan) content, and at ca. 210 nm, where peptide bonds provide the **chromophore**. Nucleic acids absorb radiation at ca. 260 nm.
- The flow analysis systems widely used in clinical chemistry and environmental studies normally use UV-visible detectors. In environmental work simple and robust systems for field use (**see Flow Injection Analysis in Chapter 14 of this teaching and learning programme**) are popular.

Quantitative analysis using UV-visible spectroscopy

The technique is generally only applied where the compound being measured exhibits a very large molar absorptivity (extinction coefficient). [Note: a few compounds have molar absorptivities exceeding 90,000, which enables the technique to be applied for analysis at trace levels]

Example (11.i)
For a compound with a molar absorptivity of 90,000, we can calculate the molar concentration of a solution giving an absorbance of say 0.05, when measured in a 1 cm pathlength cell:
From Beer's Law, A = εbC [where ε = molar absorptivity, b = cell pathlength, C = molar concentration]
0.05 = 90000 X 1 X C
C = 0.05/90000 = **5.5×10^{-7} molar**

In order to achieve maximum sensitivity and maximum linearity for the calibration graph, measurement should be made at the position of maximum absorbance (λ_{max}) on the absorption spectrum. In figure (11.5B) [**copied here**] this relates to about 565 nm. Quantitative measurements could be made at alternative wavelengths (eg: 465 nm), but with loss of both sensitivity and linearity

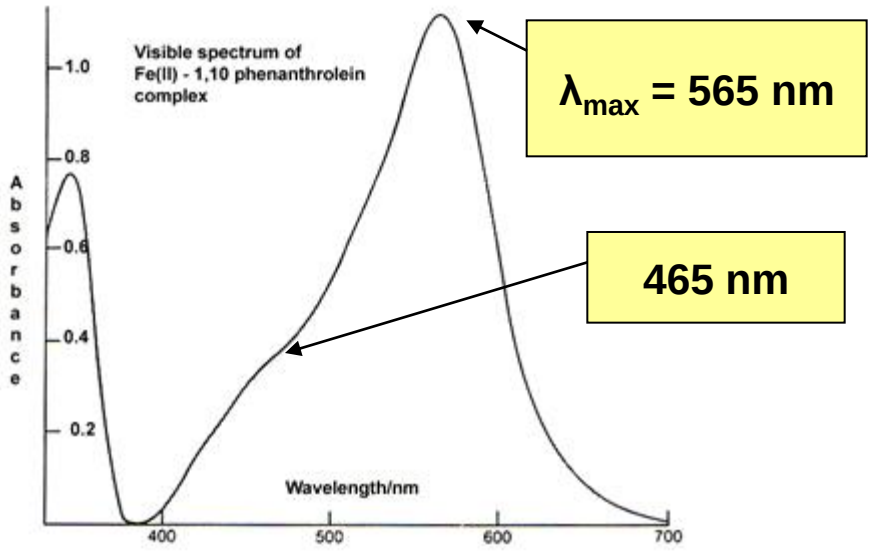


Figure 11.5B

Limitations of absorption spectroscopy

Despite their widespread use in almost every area of the chemical sciences, UV-visible absorption spectroscopy methods have some limitations.

- Many molecules have low molar absorptivities, resulting in poor analytical sensitivities. This is illustrated in example (11.ii) below
- Difficulty in accurately measuring small differences in transmission signals
- Poor selectivity when measuring mixtures of absorbing compounds
- Limited linearity

Example (11.ii).

Suppose that we study a compound with ε = 5,000 M^{-1} cm^{-1} in a cuvette with a 1 cm path-length and a spectrometer which can detect an absorbance change of 0.002 units. An estimate for the limit of detection for the compound, c_0, is then given by –

$A = \varepsilon bc$, so in this case: $0.002 = 1 \times 5000 \times c_0$

So $c_0 = 0.002/5000 = 4 \times 10^{-7}$ M.

If the compound has a molecular weight of, say, 250, then this concentration is equal to 10^{-4} g/dm^3 = 10^{-7} g/cm^3, or 0.1 parts per million.

While it is true that some compounds have far higher ε values than 5000 and some spectrometers may give better repeatability than 0.002 absorbance units, limits of detection in the parts-per-billion to parts-per-million range are typical for molecular absorption measurements.

Continued on the next two slides

In an absorption experiment with very low solute concentrations a comparison is made of the intensities of two light signals. One is the signal that reaches the detector when there is no sample in the light beam (often only solvent), and the other is the very slightly reduced signal that reaches the detector when a minute amount of sample is present. (Double-beam spectrometers make this comparison directly and more or less instantly). But it is always difficult to detect a small difference between two large signals, numbers, etc.

To give a non-chemical analogy. Suppose that you had two piles of pound coins, one containing 1000 pounds, the other 999 pounds. How could you tell which was which? You could weigh each pile, but the precision of your balance might well be poorer than the difference between the two weights. Or you could count the coins; but counting up to 1000 without error is very difficult. Distinguishing between the two very similar signal intensities in absorption spectroscopy presents exactly analogous problems.

Far better (lower), limits of detection are attainable using fluorescence spectroscopy (see slides 22 - 40).

Although absorption spectroscopy methods are very widely used in many fields of application, they have further limitations, over and above the issue of relatively *modest limits of detection* discussed in the previous two slides.

It has been shown already that deviations from Beer's Law can occur for both instrumental and sample-related reasons. The result is that these methods often have a **fairly limited linear range**, i.e. the range over which absorbance is directly proportional to concentration.

In addition the uv-visible absorption spectra of many compounds are rather broad and featureless. [Figure (11.6) is an example of a spectrum of a polynuclear aromatic hydrocarbon - relatively unusual in showing several distinct absorption maxima]. While the spectra of fairly simple mixtures can often be resolved using statistical methods a more typical sample will contain many components with overlapping spectra, so the spectroscopic determination will have to be preceded by one or more separation steps such as solvent extraction, chromatography, etc. This limitation is generally described as **poor selectivity**. Again fluorescence methods may be superior in this respect.

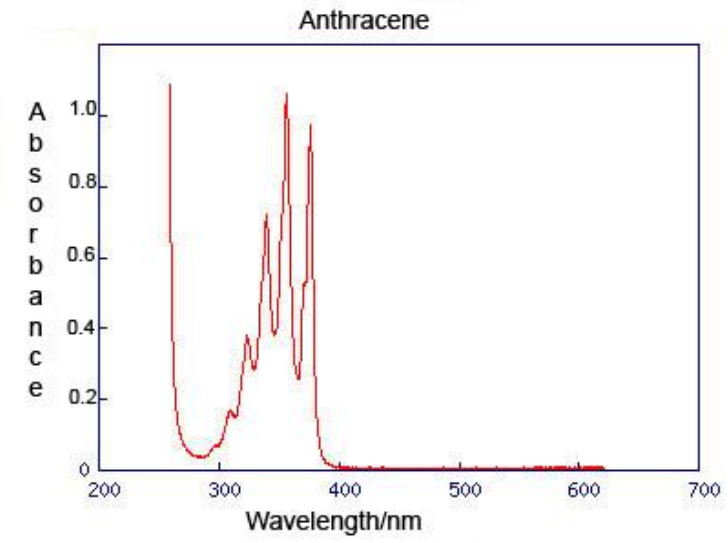


Figure 11.6

What happens to the absorbed energy?

It has been shown in Chapter 10 of this teaching and learning programme that when molecules absorb photons in the UV and visible spectral regions, quite large amounts of energy are involved: a wave-length of 400 nm at the blue end of the visible spectrum for instance, corresponds to an energy of around 300 kJ $mole^{-1}$.

It is worth remembering that in an everyday spectroscopic experiment with a conventional light source, only a few of the molecules studied are actually excited by light absorption at any one moment. Nonetheless, each of those molecules does gain a lot of energy – so what happens to that energy once the absorption process is complete?

In answering this question, two important points should be remembered.

- The first is that in most experiments each solute [sample] molecule will be at least loosely associated with a group or cage of solvent molecules which surround it, so it is the properties of this solvated sample molecule that must be studied.
- The second is that the time taken for the actual photon absorption process to occur is very short indeed. This can be shown by using Heisenberg's famous **Uncertainty Principle** which is considered on the next slide

Applying the Uncertainty Principle

As applied to the photon absorption process, the **Uncertainty Principle** tells us that the product of the energy change involved, and the time taken for the transition from the molecule's ground state to its excited state, should be approximately equal to Planck's constant, *h*. That is:

$$\Delta E.\Delta t \sim h$$

Where ΔE is the energy change and Δt is the time interval. In Chapter 10 (slide 11) it was shown that the energy associated with each 400 nm photon was about 5×10^{-19} Joules. Since Planck's constant is 6.62×10^{-34} J s, it is clear that:

$\Delta t = 6.62 \times 10^{-34}/5 \times 10^{-19}$ s $= 1.3 \times 10^{-15}$ s, or just over one femtosecond (fs).

This extremely short time interval is significantly less than any normal molecular motion. For example typical bond vibrations take $10^{-13} – 10^{-14}$ s. Bimolecular collisions occur with a frequency of $10^{10} – 10^{11}$ s^{-1} at room temperatures. So it can be assumed that a sample molecule and its surrounding solvent molecules are motionless and undergo no other changes during the more or less instantaneous absorption of the photon. This very important result is known as the **Franck-Condon Principle**. Its significance will become apparent later.

Photodecomposition

We can now return to the issue of possible mechanisms for the dissipation of the energy of molecules in their excited states. Firstly, it is important to note that the energies (in kJ mole^{-1}) of some typical covalent bonds are:

CH_3 – H: 440 CH_3 – NH_2: 365 CH_3 – OH: 382

and the energies of some photons in the same units (**the choice of these wavelengths will become clearer later**) are:

280 nm: 427 365 nm: 328 400 nm: 299 488 nm: 245

Since the photon energies and the bond energies are similar, it is evident that one way in which an excited molecule might lose its energy is by the process of **photodecomposition**: the molecule is changed by the rupture of one or more of its bonds into a new chemical species. In most experiments this phenomenon is regarded as undesirable, as it clearly makes the sample unstable, so repeated measurements (for example) are impossible or inaccurate. (When fluorescence is studied (**slides 22 - 40**) intense light sources are desirable, so the problem is often particularly acute). But in a few applications, the photodecomposition effect is valuable, as the products of the reaction may absorb or fluoresce more strongly than the original solute: this effect is sometimes used in the detection of chromatographic separations.

Fluorescence – a major analytical method

An excited molecule at the lowest vibrational level of the S_1 electronic state can lose its excess energy by emitting a photon and returning to one of the vibrational levels of the ground state S_0 [see figure (11.7)]. This process, which is the basis of one of the most important modern spectroscopic methods, is called **fluorescence**. As the molecule has already lost some of its excitation energy, the emitted photon has lower energy, i.e. a longer wavelength, than the absorbed photon. So all fluorescent molecules have two characteristic wavelengths, **those of absorption and those of fluorescent emission**: this is a major and important feature of fluorescence methods (**see slide 39**).

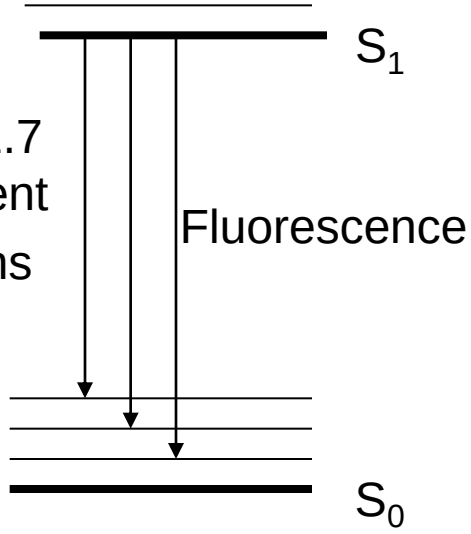


Figure 11.7 Fluorescent transitions

Two other important properties of fluorescence are its **lifetime** and its **quantum yield**. The lifetime is the time taken for the intensity of the emitted light to fall to 1/e of its previous value. (This is just like a half-life in radioactivity, except that it uses 1/e instead of ½). For many organic fluorophores, a typical lifetime lies in the range 1-10 nanoseconds. The quantum yield is the fraction of the excited molecules that lose their energy by the emission of fluorescence: it is usually expressed as a number between 0 and 1, but percentages are also used.

Why is fluorescence so useful?

In analytical work, fluorescence has a number of major characteristics, leading to many crucial advances in biochemical and environmental science. The major advantages are:

- **Extreme sensitivity**: Most of the experiments in which **single molecules** have been detected in free solution, have used fluorescence. In more routine work, limits of detection in the picomolar and femtomolar ranges are accessible. **The reason for this sensitivity is discussed in more detail in slide 32.**
- **Enhanced selectivity**: As we have seen, fluorescent molecules have two distinct characteristic wavelengths, those of **absorption** and **emission**. This helps in the study of complex mixtures: two molecules with similar absorption wavelengths may have different fluorescence wavelengths. Fluorescence lifetimes and other properties such as fluorescence polarisation are also used to provide selectivity.
- **Ease of sample handling**: Fluorescence instruments can study solid surfaces, suspensions and concentrated solutions just as easily as dilute solutions, and in recent years intra-cellular fluorescence studies have become commonplace.
- **Environmental effects**: Fluorescence is very susceptible to changes in molecular environment. You might think that this could give problems, but in practice it has been put to good use in many applications.

Molecules that exhibit fluorescent properties

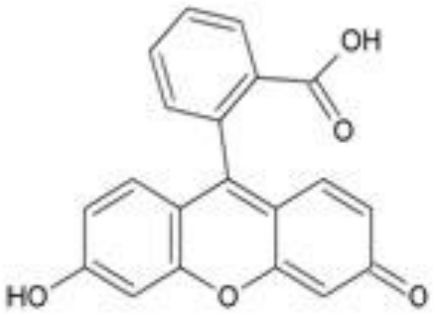
Most molecules absorb light in readily accessible parts of the UV and visible spectral regions. But only a small proportion of them are fluorescent, and even fewer are strongly fluorescent. Interestingly, this has not greatly hampered the use of fluorescence as an analytical technique. The principal reason for this, is the extensive use of covalent fluorescent labels and non-covalent probes. By using such fluorophores, non-fluorescent species can be converted to strongly fluorescent ones: hundreds of labels and probes have been produced (many for specific applications), covering a wide wavelength range.

In general, fluorescent molecules feature delocalised π-orbital systems, so:

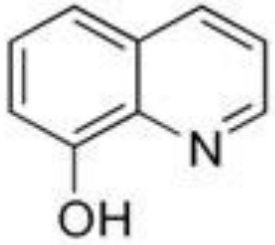
- Most aliphatic molecules are not fluorescent, whereas aromatic ones are;
- The larger the π-orbital system the longer the wavelength of fluorescence. For example the emission wavelengths of benzene, naphthalene, anthracene and tetracene are ca. 278, 322, 400 and 512 nm respectively.;
- Electron donating substituents such as NH_2, OCH_3 usually enhance fluorescence;
- Electron withdrawing ones, e.g. NO_2 reduce it.

Predicting the fluorescence properties of complex molecules is often difficult, as several structural and substituent effects may be competing with each other. The structures of some well-known fluorophores are shown in the next slide.

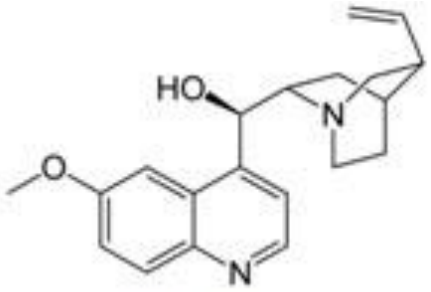
Some typical fluorophores



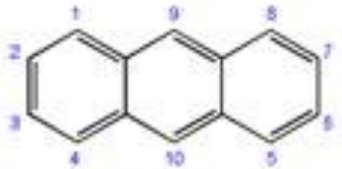
Fluorescein



8-Hydroxyquinoline

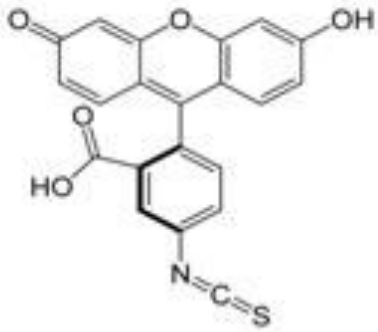


Quinine

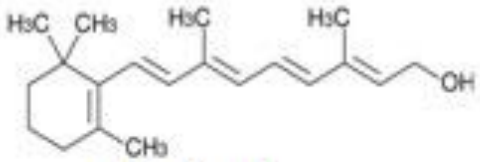


Anthracene

Nucleus of "BODIPY" dye fluorophores



Fluorescein isothiocyanate



Retinol

Note: comments on the properties, importance and uses of these compounds are provided in next few slides.

Some Applications of Fluorescence

- Fluorescence is the main method used to detect trace concentrations of the very dangerously carcinogenic polynuclear hydrocarbons (PNHs). These compounds, containing many conjugated aromatic rings (see the structure of anthracene on the previous slide), are intensely fluoresecent, and often occur in complex mixtures, so fluorescence is used to detect them after they have been separated by high performance liquid chromatography.
- The well known street drug LSD (lysergic acid diethylamide – “acid”) contains an indole group and has an intensely blue fluorescence, which is often used as a quick visual method for checking street samples: the blue emission shows up when the sample is illuminated with an ultra-violet light source.
- 8-hydroxyquinoline (see previous slide) is not very strongly fluorescent by itself, but it forms an uncharged chelate complex with Al(III) ions, $Al(8\text{-}HQ)_3$, which is very fluorescent and can be extracted into organic solvents. Trace levels of Al(III) can be detected in this way. Similar methods, with other chelators, have been used to analyse many metal ions. Quinine (see previous slide - it is added to tonic water to make it taste bitter) also contains a quinoline group, and has a bright fluorescence at 450 nm. It is widely used as a fluorescence quantum yield standard.

Fluorescence in biochemistry

It is in biochemistry that the most important applications of fluorescence certainly arise. Apart from their intrinsic sensitivity, fluorescence methods have the great advantage that, because of the relatively long time (~10^{-8} s) for which a molecule is in its excited state, its fluorescence behaviour is strongly dependent on the molecular environment. Two major application areas utilise this characteristic:

In **fluorescence polarisation studies** polarising filters are added to the excitation and emission beams of the spectrometer. The sample is excited with vertically polarised light, and the emission intensity is measured twice, through vertically ($I_{\parallel}$) and horizontally ($I_{\perp}$) oriented polarisers. The difference between the two intensities divided by their sum is the fluorescence polarisation, *p*:

$$p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$$

Theory shows that *p* ~0 for small fluorescent molecules, but takes values up to 0.5 for large fluorophores or for small fluorophores bound to larger molecules. This result is widely used to study the binding of small molecules to large ones, for example in fluorescence immunoassays, where large antibody molecules bind small fluorescent species. Such assays use analyte analogues which have been labelled with fluorescent groups. Fluorescein isothiocyanate (see slide xx) has been much used in this way, but modern methods use other fluorophores such as those based on the boron-containing BODIPY group.

Energy Transfer Methods

The second fluorescence phenomenon widely used in biochemistry, both for analysis and for studying molecular structure and interactions, is that of **energy transfer**. In a typical energy transfer system a fluorescent group, A, absorbs a photon and is promoted to its first excited singlet state, A*. Instead of emitting a fluorescence photon A* (the “donor”) transfers its excess energy to a second molecule or chromophore group, B (the “acceptor”). If B is a fluorescent molecule the result may be that B fluorescence is seen instead of A fluorescence; if B is not fluorescent (it is then called a “dark acceptor”), then the only observed result of the energy transfer is that A fluorescence is quenched (reduced). For reasons connected with the underlying theory of the phenomenon the process is properly known as **Fluorescence Resonance Energy Transfer (FRET).**

The key feature of this phenomenon is that when singlet states of A* and B are involved the efficiency of the FRET process is very strongly distance dependent:

Efficiency $= Kr^{-6}$ Equation (11.1)

- where r is the donor acceptor distance, and K a constant that depends on the electronic properties of A and B and their orientation.

The inverse 6^{th} power relationship effectively means that FRET does not occur unless A and B are extremely close to each other or part of the same molecule or complex. This is obviously extremely valuable in a great range of studies.

A fluorescence spectrometer

A typical layout of a fluorescence spectrometer is shown in figure (11.8) below. A comparison between fluorescence and absorption spectrometers is given on the next slide.

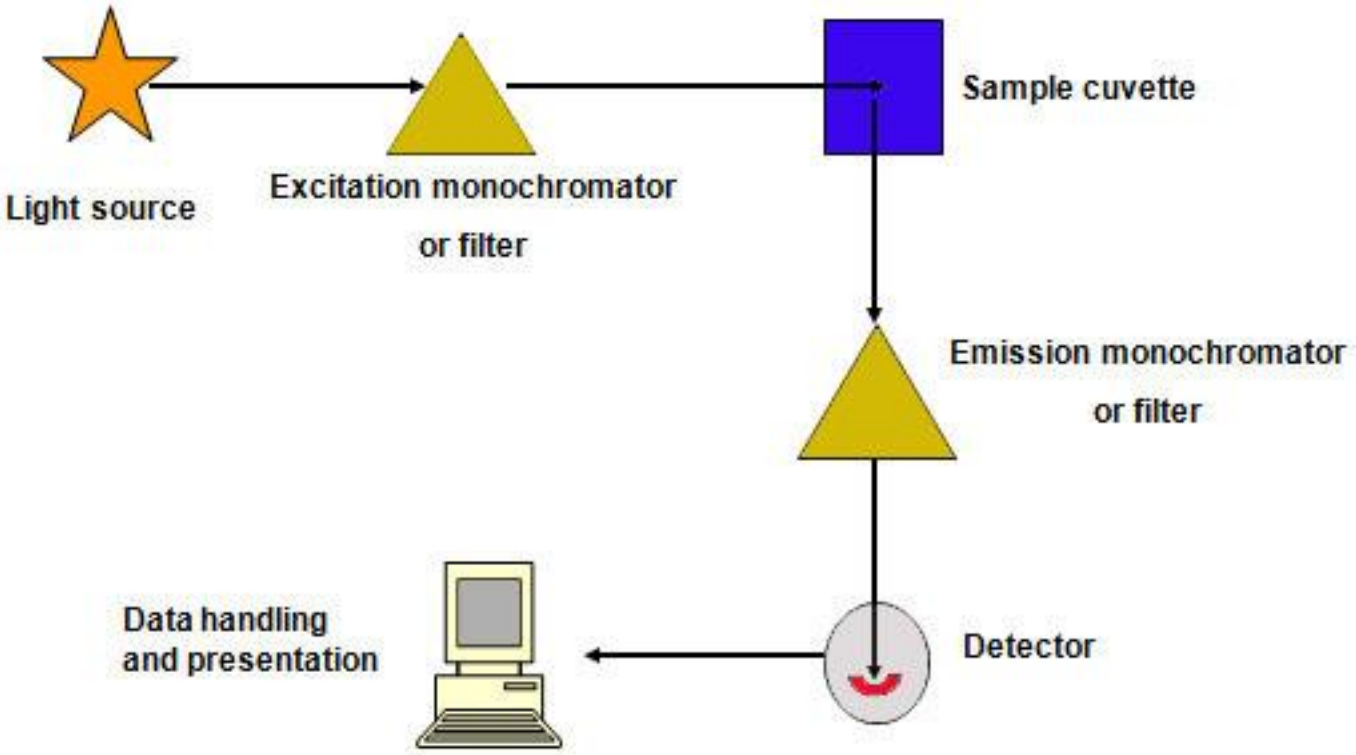


Figure 11.8 - spectral layout of a fluorescence spectrometer

Continued on the next slide

Fluorescence instruments differ in several ways from instruments used for absorption spectrometry.

- **The fluorescence intensity from a sample is proportional to the intensity of the exciting (incident) light** (**see slide 32**). So very bright light sources (often lasers) are used in fluorescence measurements. (Note that this increases the chance of damaging the sample by photodecomposition). In absorption spectrometry there is usually no advantage in using a very bright light source.
- **Fluorescent samples are excited at one wavelength and emit fluorescence at a second, longer, wavelength**: so the instrument needs two monochromators or filters to isolate these two wavelengths.
- **Almost all fluorescence spectrometers have a characteristic right-angled optical pathway**, i.e. the fluorescence is observed at an angle of 90° to the incident light beam. (For liquid samples, this means that we need a cuvette with four polished faces). This arrangement has two benefits:
  - Photons that are transmitted by the sample, which are of no interest to us, are not detected;
  - Scattered light, which might interfere with the detection of the fluorescence, has a minimum intensity in this 90° direction.

Fluorescence intensity

For analytical work it is necessary to have an equation that relates fluorescence intensity with concentration, just as Beer's Law relates absorbance and concentration. Since fluorescence from a molecule is preceded by the absorption process, we can use Beer's Law as the starting point. We first find the amount of light *absorbed*, P_A, which is the difference between the incident light intensity, P_0, and the transmitted intensity, P. Beer's Law can be written:

$P = P_0 \times 10^{-\varepsilon bc}$ Equation (11.2) So P_A is given by:

$P_A = P_0 - P = P_0(1 - 10^{-\varepsilon bc})$ Equation (11.3)

Since only a fraction (the **quantum yield**, given the symbol φ_f) of the molecules that absorb light emit fluorescence, the fluorescence intensity, I_f, is given by:

$I_f = \varphi_f P_0(1 - 10^{-\varepsilon bc})$ Equation (11.4)

Expansion of the exponential term gives:

$I_f = \varphi_f P_0(1 - [1 + 2.303\varepsilon bc - (2.303\varepsilon bc)^2/2 + (2.303\varepsilon bc)^3/3 - \ldots])$ Equation (11.5)

Neglecting the squared and higher terms we finally get the simple equation……

$\boldsymbol{I_f = \varphi_f P_0 \times 2.303\varepsilon bc}$ **Equation (11.6)**

Why are fluorescence methods so sensitive?

Equation (11.6), developed on the previous slide is: $\boldsymbol{I_f = \varphi_f P_0 \times 2.303 \varepsilon b c}$

So the **fluorescence intensity** from a compound of given quantum yield, φ_f, and molar absorptivity, ε, is **proportional to** both the incident light intensity, $\boldsymbol{P_0}$, and the concentration, $\boldsymbol{c}$. However, some assumptions have been made to arrive at this result. These include;

- $P_A = P_0 - P$ (i.e. we have neglected scattered and stray light);
- Beer's Law is applicable;
- εbc is small, so that the higher terms in the expansion of $10^{-\varepsilon bc}$ can be neglected.

The absorbance of the sample in fluorescence should be <ca. 0.02 to make this last assumption safe. This is not a big practical disadvantage, as fluorescence is often used at concentrations where absorption methods are insensitive.

Continued on the next slide

What gives fluorescence this extreme sensitivity?

The main reason is that, in a fluorescence experiment in very dilute solution, we are trying to detect a *small light signal against a zero background*. From the optical diagram shown as figure (11.8) in slide 29 it is clear that if the sample cuvette contains no fluorescent material, no signal should reach the detector. If there is a trace of a fluorophore, present a few photons are available for detection.

By contrast, absorption spectroscopy involves detecting a small difference between the two large light signals, P_0 and P, which reach the detector when a small amount of sample is absent or present respectively: such small differences are always hard to detect, as can be easily demonstrated in terms of the simple analogy given on the next slide

Continued on the next slide

In slide 17 we introduced the problem of two piles of coins, one containing 1000 coins, and the other 999. How could we find out which is which? The simplest possibility was to weigh each pile, but our balance might not be precise enough to distinguish between them. We might count the coins individually (NB: photon counting is an established and sensitive spectroscopic detection technique), but it is very hard to count up to 1000 without making a mistake (the same is true for photons). So distinguishing between 1000 and 999 coins is really difficult: this is analogous to what we attempt in the *absorption spectroscopy* of dilute solutions.

Suppose now that we are given two new piles of coins, one containing one coin and the other zero coins. Even though the difference in sizes is again just one coin, there is no problem at all in distinguishing them! – that is effectively what we try to do in the **fluorescence spectroscopy** of dilute solutions. This is why ultra-trace analysis is often possible using fluorescence methods.

This extreme sensitivity evidently works only if the background signal, i.e. in the absence of the fluorophore, really is zero or nearly so. So we must take extreme precautions to minimise scattered and stray light, use scrupulously clean vessels and cuvettes, very pure non-fluorescent solvents, and so on. Fluorescence in practice is often used in the detection of previously-separated molecules, or of a labelled molecule, in which case other fluorophores may not be present and the background will indeed be close to zero.

Solvent effects in fluorescence

Slides 19 & 20 showed that the process of a molecule absorbing a photon is a more or less instantaneous process, taking ~10^{-15} seconds; this is the Franck-Condon Principle.

It means that when a solvated molecule reaches its excited electronic state, the solvent molecules surrounding it are still in the same positions as they were before the photon absorption took place. But the excited sample molecule is in effect a new molecular species, i.e. it has a new arrangement of electrons in molecular orbitals. (It often has a higher dipole moment than the ground state one). The solvent cage surrounding it will probably not be the optimum (lowest energy) solvent arrangement, so over a very short period (ca. 10^{-11}s) the solvent molecules will **relax** to form a new coordination arrangement, the optimum one for the excited molecule.

This effect naturally contributes to the fact that the emitted fluorescence has a lower energy (longer wavelength) than that of the exciting light. The energy difference associated with the solvent relaxation tends to be higher in polar solvents than in non-polar ones, because of the interaction with the highly polar excited solute molecule. **Many fluorescent molecules thus show an emission wavelength that increases as solvent polarity increases.**

Continued on the next two slides

When the fluorescence photon is emitted and the molecule returns to the ground electronic state a similar (though usually smaller) effect occurs, as the newly-formed ground state molecule will still have the solvent coordination appropriate to the excited state. Again a rapid solvent relaxation takes place, with a further loss of energy. These phenomena are shown diagrammatically in figure (11.9) in the next slide.

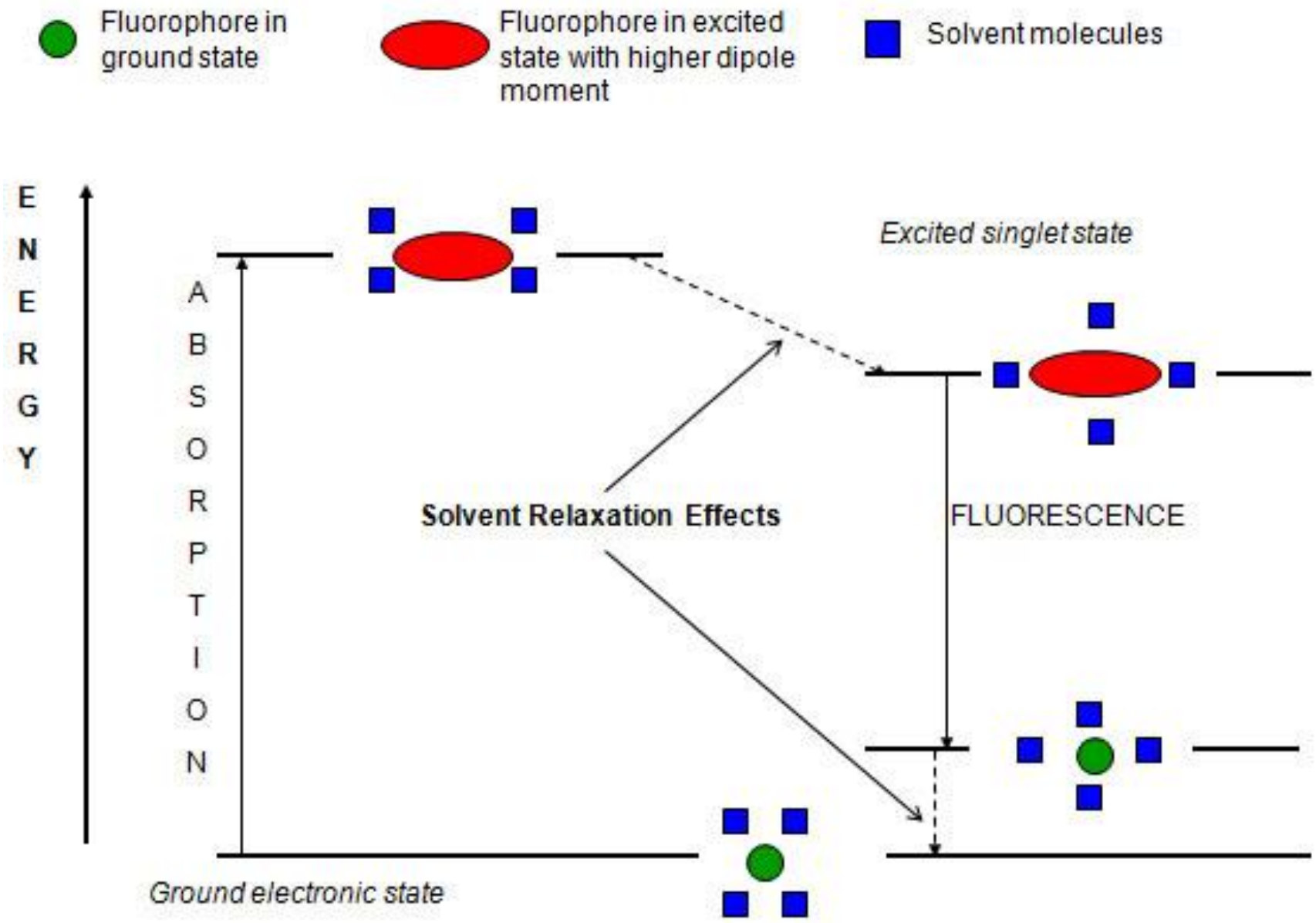


Figure 11.9 – solvent relaxation in fluorescence

Sample cells for fluorescence

The characteristic right-angled optical pathway in fluorescence (**see slide 39**) means that for solution studies we need to use sample cells/cuvettes that are transparent on two adjacent sides. In practice fluorescence cells are normally **transparent on all four faces**, so that except for work of the highest precision it does not matter much which way round the cell is placed into the sample holder.

Cells are also available for the study of solid surfaces and suspensions, as are flow cells for use in capillary electrophoresis and as hplc detectors. Much ingenuity has been used in designing micro-liter and nano-liter flow cells for use with “lab on a chip” systems

Cells are usually made of silica or glass, but for routine work, some disposable polystyrene or acrylic ones have been found satisfactory. Only silica is suitable for work using excitation wavelengths below ca. 300 nm. Whichever material is chosen, it is essential that a cell has a **very low background fluorescence** when used with the chosen excitation wavelength.

The fluorescence intensity of many materials is **markedly dependent on the temperature** in the cell. Many light sources in common use, emit heat as well as light, thus warming the cell during an experiment. It is wise therefore to use the cell with a temperature-controlled holder.

Practical Aspects of Fluorescence

Previous slides have suggested some of the precautions needed to obtain good results in fluorescence spectroscopy in solution. In particular these are:

- **Ensure the solutions are very dilute**: the linear relationship between the intensity of fluorescence and concentration, only holds if the absorbance of the sample is low, preferably <ca. 0.02. At higher absorbance values the intensity of fluorescence detected is lower than it should be.
- A second and quite separate problem arises if the absorbance is too high, that of **inner filter effects**.

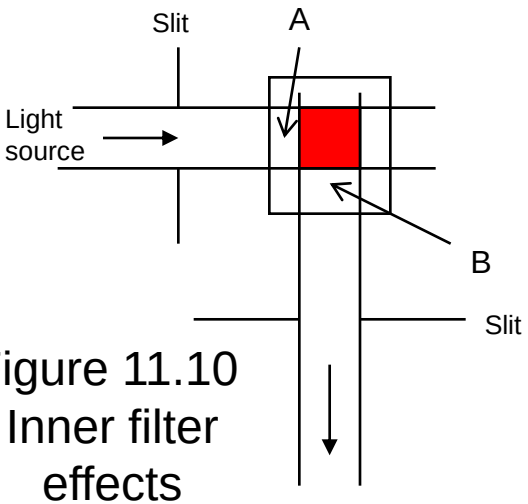


Figure 11.10 Inner filter effects

The paths of the incident and fluorescence light beams in the sample cell are restricted by the monochromator slits, as shown in figure (11.10). So only the **central** part of the cell is illuminated by the source *and* observed by the detector. In the region marked 'A', light is absorbed, reducing the intensity falling on the **central** region, but the resulting fluorescence is not detected. In region 'B', some of the fluorescence from the **central** region may be re-absorbed before leaving the cell, either by other fluorophore molecules or by other sample components. In either case the observed fluorescence intensity is reduced. These artifacts are minimised by keeping the absorbance of the sample very low.

Fluorescence Spectroscopy – Keep it Clean!

The following important practical points need to be taken into account to maximise sensitivity and reliability when using fluorescence as a quantitative tool:

- It has been shown that the exceptional sensitivity of fluorescence arises because the emitted light is detected against a zero or near-zero background. it follows that if the background is not very low, sensitivity is lost. There are many simple experimental precautions available to ensure that the background is as low as possible: taking such precautions routinely brings great benefits!
- Sample cells must be made of non-fluorescent materials, and must be scrupulously cleaned inside and out to prevent sample carry-over.
- The solvents used in fluorescence measurements must be as free as possible of fluorescent contaminants. This may mean using water distilled several times, and choosing organic solvents that contain no intensely fluorescent aromatic hydrocarbons. In principle solvent background signals can be subtracted from the emission intensity of the sample studied, but it is much better to have little or no background to start with!
- Dust, hairs and other suspended material in a sample greatly increase unwanted scattered light signals, and should be removed by centrifugation etc.
- Reagent containers and stoppers made of synthetic polymers often contain fluorescent materials – using very clean glass vessels is usually best.

Other emission techniques

Despite its extreme importance, fluorescence is not the only application of the emission of radiation used in molecular spectroscopy. Other emission techniques which offer analytical measurement applications are:

- Phosphorescence;
- Chemiluminescence;
- Bioluminescence.

These techniques together with possible applications, are discussed on the next few slides

Phosphorescence

On slide 20 in Chapter 10, it was shown that an excited molecule might move from a singlet state to a triplet state by the process of **inter-system crossing**. In principle this could then emit a photon from the lowest triplet state, T_1, to return to the ground state S_0 as shown in figure (11.11). **This is the process of phosphorescence**. But just as in the case of inter-system crossing, this triplet-singlet transition is "forbidden" as it involves a change of electron spin. Since in this case there is a substantial Energy change also, the probability of the transition is usually low. The lifetimes of phosphorescence signals are often in the milliseconds to seconds range – a very long period in molecular terms – and ***quenching*** of the emission (especially by oxygen) is very likely. Phosphorescence can only be observed at room temperatures in rare cases or in very specialised experimental conditions, so despite its potential benefits (great sensitivity as with fluorescence, extra and easily gained selectivity on the basis of lifetime differences) its applications have been limited.

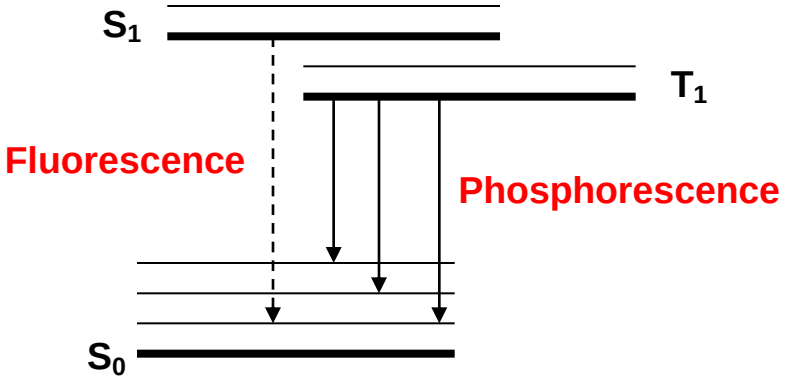


Figure 11.11 - showing the difference between fluorescence and phosphorescence.

Since T_1 has a lower energy than S_1, the phosphorescence wavelength of any molecule is **longer** (i.e. less energetic) than its fluorescence wavelength.

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Chemiluminescence and bioluminescence

Much more important in practice are methods in which excited molecules are produced, not by illumination by an external source, but in a chemical reaction. Such phenomena, are called **chemiluminescence** (CL). In some cases the reactions occur in living systems as a result of enzyme catalysis, and they are then referred to as **bioluminescence** (BL).

Almost all CL reactions can be written in the general form:

A (sample) + B (oxidising agent) $\xrightarrow[\text{CATALYSIS}]{\text{OXIDATION}}$ P☼

where the ☼ symbol indicates that the product molecule P is formed in an excited electronic state, before emitting a photon to return to the ground state.

The intensity of CL/BL emission is proportional to:

- The rate of formation of P☼ (and hence under suitable conditions, to the concentration of A)
- To the quantum yield of P☼, i.e. the proportion of the excited molecules that lose their energy by emitting a photon.

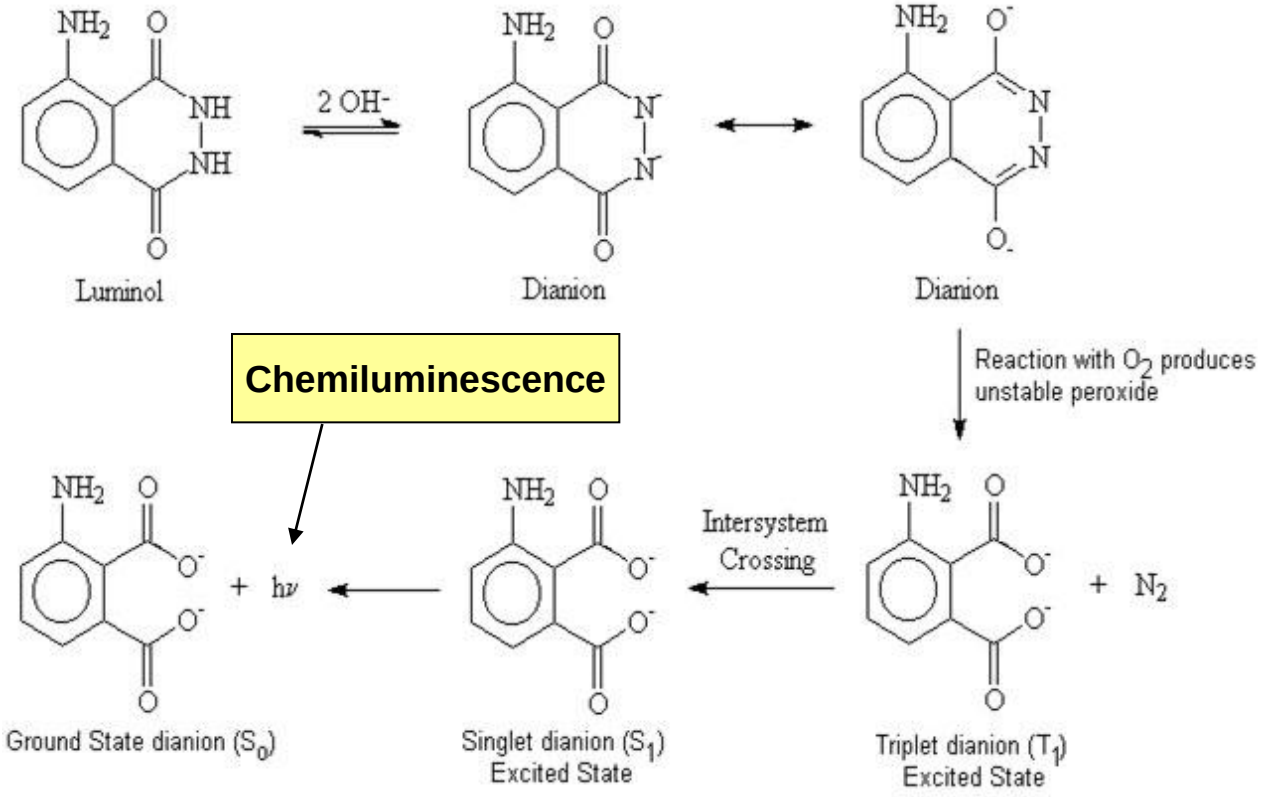
The experimental conditions can sometimes be controlled so that the light is emitted either as a brief intense pulse (allowing rapid and frequent measurements, but possibly with modest precision) or less intense light signals emitted over tens of minutes, thus allowing replicates and more precise studies. BL reactions may have higher quantum yields than CL ones, but the reagents, including enzymes, are less robust so these methods are less used in practice.

Chemiluminescence in action

CL measurements have two major advantages – very simple equipment, and excellent sensitivity. These two benefits are closely linked. A typical CL detector is simply a vessel in which the CL reaction can be started in controlled conditions, placed very close to a sensitive detector such as a photomultiplier tube (even simpler systems have been used, such as polaroid cameras!). Usually no attempt is made to achieve wavelength discrimination by using filters or monochromators: the aim is to collect as much light as possible, hence the simplicity and sensitivity. Just as in fluorescence measurements, no CL signal should be seen when there is no CL reaction, so the detection of the light should take place against a dark or almost dark background. Once again this means that attention must be paid to the purity of the reagents, the use of non-luminescent containers etc, otherwise the sensitivity advantage is lost.

As with fluorescence, most applications of CL reactions involve labelling a target molecule with a CL reagent, so the fact that only a very few CL systems have found practical use is not a disadvantage.

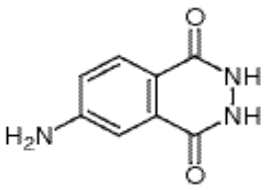
Many analytical methods are based in practice on the molecule **luminol**, which was first synthesised 80 years ago. The proposed mechanism for the production of the chemiluminescence is shown as figure (11.12) on the next slide.



Oxidation of luminol in mildly alkaline solution, for example with hydrogen peroxide, produces 3-amino-phthalate ions in an excited state. The blue emitted light has a λ_{max} of ca. 425 nm. A catalyst such as a metal ion may be needed: so the reaction can be used to detect these ions, or hydrogen peroxide.

Figure 11.12 - proposed mechanism for Luminol light producing reaction

Note, in some applications *Isoluminol* (see right) is used. Isoluminol can be used to label other molecules through its $-NH_2$ group, and is preferred to Luminol in such applications, as this group is further from the luminescent part of the molecule, which is formed from the phthalazine-dione function.



Isoluminol

Example (11.iii) – A question of sensitivity

Some molecules, e.g. 3-amino-phthalic acid, can be excited both in a CL system (i.e. from luminol) or in a fluorescence system by direct irradiation. In each case, as we know, the emitted photons should be detected against a dark background, and we can use a similar photomultiplier or other light detection device. The quantum yield of the emission is presumably also the same (provided that the solvent, temperature, and other experimental conditions are the comparable). So which of the two methods would be expected to be more sensitive?

This is a good question, well worth thinking about, even though there is no very simple answer. The main differences between the two methods seem to lie in (a) the rates at which the excited molecules can be produced; and (b) the efficiency with which the emitted photons can be detected. CL methods may have the advantage over fluorescence ones in terms of (b). Moreover if the experimental conditions are right almost all the luminol molecules in a CL experiment could be oxidised to give the excited product, whereas in a fluorescence experiment only a small proportion of the sample molecules are excited at any single instant. On the other hand we note that in CL each excited molecule is only produced once from a luminol molecule, whereas in fluorescence a single molecule could be excited, emit a photon to return to the ground state, be excited again, emit again etc many times in a fluorescence experiment. (Only photodecomposition would limit this). Overall the two methods are probably comparable in sensitivity.

Applications of chemiluminescence

Determination of oxides of Nitrogen in the atmosphere

The photograph shown in figure (11.13) is of one of the Air Quality monitoring stations spread throughout the UK to monitor air quality on a regular basis and to provide data for the UK database. Substances that are routinely measured are: CO, SO_2, NOX (oxides of nitrogen), PM10s. NO and NO_2 are measured by using chemiluminescence (CL).

Figure 11.13 – air quality monitoring in Norwich

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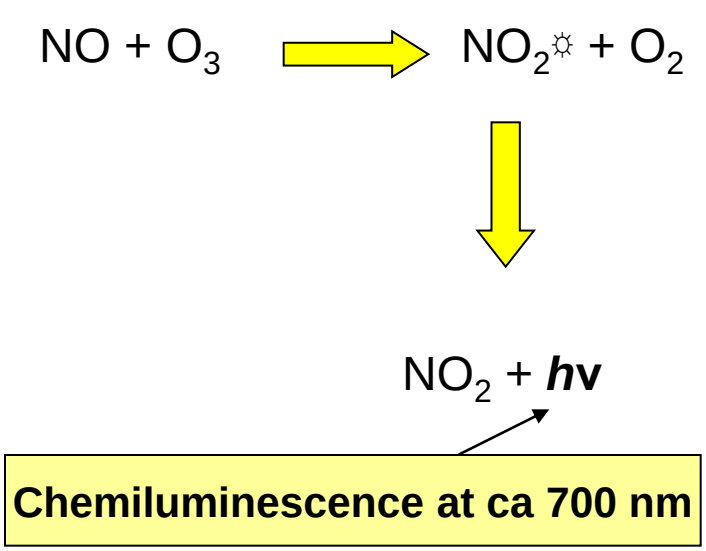
A important and unusual application for CL is in gas detection. Nitrogen oxides in the atmosphere can be detected with the aid of the reaction:



Ozone is generated within the instrument and mixed with the atmospheric sample. The emitted radiation is measured using a photomultiplier, which gives the NO concentration in the sample. Total NOX (oxides of nitrogen), can also be measured by thermally decomposing the NO_2 in the atmosphere to NO before analysis. The difference between the measured values relates to the NO_2 content. The detection limit of the method is approximately 18 ppb w/v (18 $\mu g/m^3$).

Ozone itself can be determined using CL methods, and phosphorus compounds can be determined in a hydrogen flame using the 520 nm emission of transient HPO☼ species. The latter application, which involves a reduction reaction, finds use in gas chromatography detection

Applications in biochemistry

CL in solution has many applications in biochemistry. Luminol derivatives can be used to label antibodies for immunoassays. Oxidase enzymes, reagents in many analyses, generate hydrogen peroxide which can be measured with the luminol reaction. These approaches can be combined: antibodies can be labelled with peroxidase enzymes, whose reactions can also be monitored using luminol.

Application in trace metal analysis

Since the luminol reaction is catalysed by transition metal ions, such ions can be determined at very low concentrations. Co(II), Cu(II) and Cr(III) are amongst the species analysed in this way at sub-nanomolar levels. Other transition metal ions actually inhibit the generation of luminol CL, and the reduced signal can be used to analyse them.

Use of Luminol in forensic science

Perhaps the best-known application of CL, and of luminol in particular, is in forensic science, where it is applied to the detection of traces of blood at crime scenes and on possible weapons etc.

If a mixture of Luminol and hydrogen peroxide (aerosol based preparations are commercially available) is sprayed on to a surface that may carry traces of blood, the iron-porphyrin component of hemoglobin acts as a catalyst in the luminol reaction. The Luminol emission can then be seen in a darkened room, and photographed. Since this iron component of blood is very stable, even quite old blood-stains can be detected in this way.

It is important to note that this use of Luminol has some disadvantages. The most obvious one is that the spray mixture may dilute, damage or destroy some other sources of evidence, the all-important DNA in particular. Moreover the Luminol reaction is also promoted by some common household products such as bleaches (which usually contain hypochlorite), so the test is not a perfectly specific one for bloodstains. For these reasons it is often regarded as a method of last resort – not quite the impression often given by several television programmes!

Infra-Red Spectroscopy

Introduction

The infra-red (IR) region of the spectrum covers a wide range of wavelengths (and hence techniques). Its lower end adjoins the high wavelength end of the visible spectrum, at about 750 nm, but its upper wavelength limit extends to 25,000 nm or even higher. In practice we can roughly divide this large range into three regions, summarised in table (11.4) below

Wavelength Range (nm)	Molecular Transitions
750 - 1000	Electronic absorption or emission
1000 – 2500	Vibrational overtones and harmonics
2500 – 25000	Fundamental vibrational frequencies

Table 11.4 – Infra-red spectral regions

Continued on the next slide

The very near IR region, up to ca. 1000 nm, is closely related to the UV-visible region previously described. The energies of these photons still correspond to **electronic transitions**, and fluorescence methods in this range are particularly useful as the background signals from scattered light and matrix fluorescence are usually low – perfect conditions for ultra-sensitive fluorescence measurements.

However this section will concentrate mainly on the longer wavelength IR methods, in which **molecular vibrations** are the cause of the interactions of molecules and radiation. This interest in the study of the longest wavelength range (2500 – 25000 nm), often termed **the mid-IR region**, has attracted analytical interest for over half a century, due to its strength as an interpretative analytical tool, capable of differentiating not only between molecular species but also structural isomers (for instance propan-1-ol and propan-2-ol, spectra of which are shown as figures (11.14 & 11.15) on the next slide).

Figure 11.14 - propan-1-ol (CH_3-CH_2-CH_2OH)

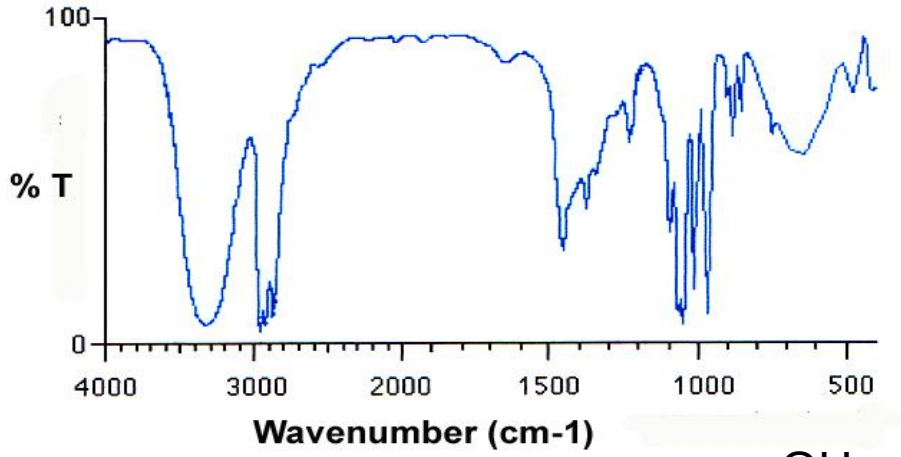
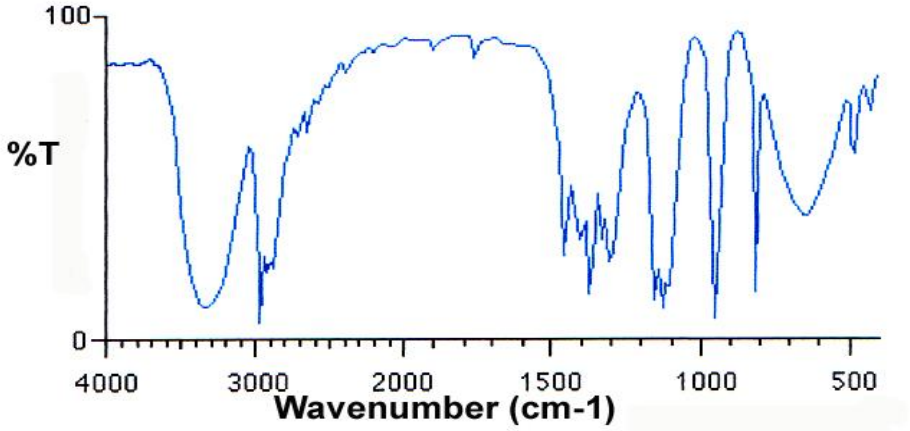


Figure 11.15 – propan-2-ol (CH_3-$CH(OH)$-CH_3)



The figures (11.14 & 11.15) show distinct differences in their IR spectra due to their differing molecular structures. The only band that remains constant is the O-H stretching band shown at approximately 3350 cm^{-1}

%T refers to the % of the incident radiation that reaches the detector and is referred to as % transmittance

Molecular Vibrations

Classical (or mid-) IR spectroscopy characterises molecules on the basis of their fundamental vibrations. A molecule containing *n* atoms has 3*n* – 6 vibrations, except in the case of a linear structure, where the number is 3*n* – 5. This can be illustrated by using a simple example of a triatomic molecules - water.

The structure of **water** is shown in the box below. The molecule is effectively triangular, with an angle of ca. 105˚ between the 2 O-H bonds, each of which has a length of just under 1Å, or 0.1 nm. The three fundamental modes of vibration are simple to deduce. They are (1) **symmetrical stretching** of the two bonds, i.e. they lengthen and shorten in phase, the bond angle being maintained; (2) **asymmetrical bond stretching**, i.e. one bond contracts while the other extends; and (3) the **bending vibration**, in which the angle between the two bonds opens and closes. The results of these vibrations are simply illustrated as shown in figure (11.16) below

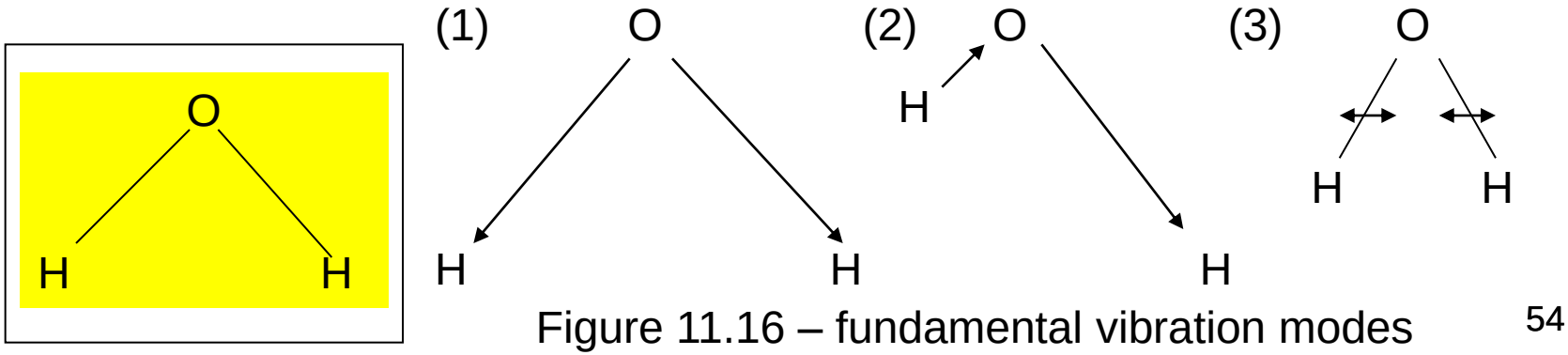


Figure 11.16 – fundamental vibration modes

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Using cm^{-1} units

In this region of the IR spectrum the use of **wavenumber, i.e. cm^{-1} units** (see slide 103) is commonly applied. **These simply represent the reciprocals of wavelengths**.

Using this approach the **symmetrical stretching vibration** of liquid water is found at ca. 3280 cm^{-1}, corresponding to a wavelength of:

$1/3280\ cm = 3.05 \times 10^{-4}\ cm = 3.05 \times 10^{3}\ nm$ or 3050 nm.

Note: The value 3280 cm^{-1} is quite often referred to loosely as a frequency, although clearly it is not.

The "frequency" of the **asymmetrical stretching vibration** for water is fairly similar, at 3490 cm^{-1} (i.e. 2865 nm), while the **bending mode** is found at 1644 cm^{-1} (6061 nm), i.e. this mode has a significantly lower energy.

Continued on the next slide

Why do molecules absorb infra-red radiation?

The absorption of infra-red radiation by a molecule occurs when the frequency of the radiation exactly matches the energy required to produce a change in the vibrational energy of a molecule or polyatomic ion.

Do all molecular vibrations lead to IR absorption?

To answer this question it is necessary to look at radiation as a form of wave motion. The oscillating radiation is accompanied by an oscillating electric (and magnetic) field of the same frequency (hence the name “electromagnetic radiation”) [see figure (11.17)]. If a molecular vibration results in **a change in the molecule’s dipole moment**, the oscillating dipole moment interacts with the electric field of radiation of the same frequency, and leads to the absorption of that radiation.

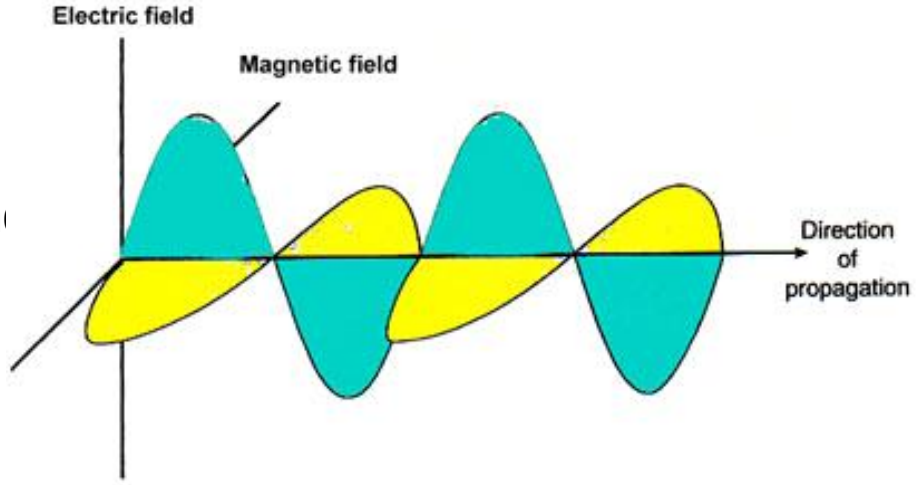
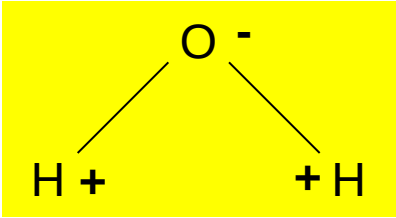


Figure 11.17 – oscillating electric an
magnetic field

Continued on the next slide

Water has a substantial permanent dipole moment, the oxygen “end” of the molecule carrying a net negative charge and the hydrogen atoms a net positive one. It is fairly evident that each of the three fundamental modes of vibration will cause this dipole moment to change, so all three modes are infra-red active.”



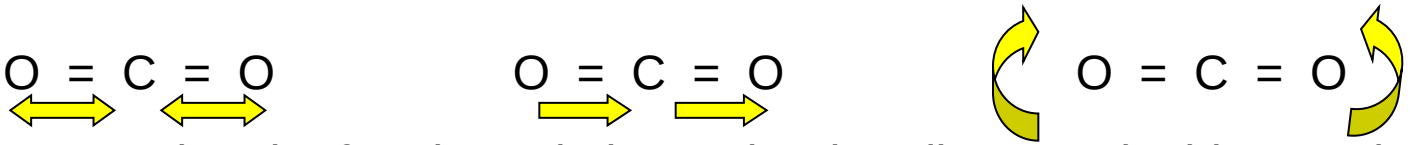
Some molecules however, especially highly symmetrical ones, may have modes of vibration that do not generate a changing dipole moment and are hence “**infra-red inactive**”. A good example of this is carbon dioxide

The case of carbon dioxide

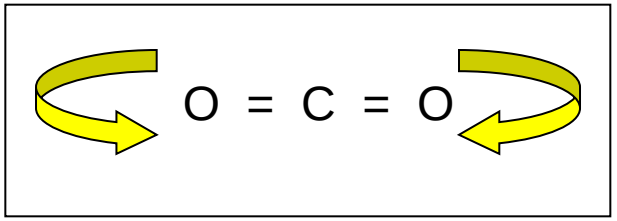
In the study of the IR absorption properties of carbon dioxide, CO_2, several new features arise. This is because the molecule is linear and symmetrical:

O = C = O

Since the molecule is linear, it has $3n - 5$, i.e. **four modes of vibration**. It is fairly easy to see that, as with water, three of these modes will be the symmetrical and asymmetrical stretching of the two C = O bonds, and a bending mode that will produce an O=C=O bond angle of less than 180°. But where is the fourth mode?



It turns out that the fourth mode is another bending one, in this case in a plane at right angles to the first. So a bending vibration in the plane of this slide image counts as a separate one from a bending mode that moves the two oxygen atoms in and out of the plane of the image. Of course the two bending modes have the same frequency, and so do not give separate IR absorption bands: they are said to be **degenerate** modes.



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Since the CO_2 molecule is intrinsically symmetrical, it has no ground state dipole moment. Its symmetrical stretching vibration will clearly not generate a changing dipole moment, so this mode of vibration is **IR inactive**. The asymmetrical stretch and bending modes occur at ca. 2330 and 667 cm^{-1} respectively.

Why do IR frequencies seem to vary?

If you look up the infra-red absorption frequencies of water and carbon dioxide in books, research papers, Web sites etc, you may well find that different numbers are given by these different sources. In all probability this is not the result of mistakes, as the wave-number scales of the spectrometers used will be accurate to within a few cm^{-1} or better. So why the differences?

The most likely answer is that the samples have been studied under different experimental conditions. The spectrum of water vapour is significantly different from that of liquid water, for example. So the physical state of the sample is liable to be crucial. Gas-phase spectra will also depend on temperature and pressure, and samples in solution will be affected by temperature and by the solvent used – is it polar or non-polar, does it participate in hydrogen bonding, etc? Concentration effects may also occur if a solute is inclined to aggregate.

These effects may be important when trying to compare an experimental spectrum with one in a reference data base for identification purposes, or if spectra are to be used for structural studies.

More complex molecules and IR spectra

The vibrations of the water and carbon dioxide molecules are fairly easy to understand, because the molecules themselves are so simple. But what about a larger molecule, such as **benzene**, C_6H_6?

Since benzene has 12 atoms, the $3n - 6$ formula shows that it has no less than 30 fundamental vibrational modes. However, because the molecule is highly symmetrical many of these modes will be degenerate and/or infra-red inactive, suggesting that the spectrum should be fairly simple. It is evident that the symmetry of a molecular structure will have a strong influence on its absorption spectrum. For example the isomeric benzene derivatives 1,3-dichlorobenzene and 1,4-dichlorobenzene have rather different degrees of symmetry, so their spectra are not identical, even though they contain the same atoms.

On the other hand the infra-red absorption spectra of even quite simple molecules often turn out to be more complex than we might expect. This is because of the occurrence of **overtones** and **combination bands** (**which are especially important in near ir spectrometry – see slides 88 & 89**). These absorption bands are discussed briefly in the following slides.

Overtone bands in IR spectra

Absorption transitions in IR spectrometry are governed by the rules of quantum mechanics, as are the transitions in electronic spectra. Each electronic state of a molecule has a series of quantised vibrational levels associated with it. **(*See Chapter 10 slide 18*).** (Note: previously we described the radiation as a wave, but now we begin to regard it instead as a stream of photons).

Absorption of a photon of IR radiation is expected to excite the molecule from one of these vibrational levels to the next one above it. In other words, if the vibrational levels are called v = 0, 1, 2, 3 etc.(v = 0 being the lowest vibrational level associated with a given electronic state), then in simple theory the absorption and emission of radiation are governed by the selection rule $\Delta v = \pm 1$. However in practice this selection rule is obeyed only approximately, so transitions involving $\Delta v = \pm 2$, 3 etc also occur, though usually with a low intensity. Since the levels v = 0, 1, 2, 3 etc are approximately equally spaced in energy terms, this gives rise to extra absorption bands at about twice, three times etc the frequency (or wave-number) of the fundamental frequency. These bands are referred to as **overtones.**

A simple example is provided by the spectrum of HCl gas. This linear diatomic molecule has $3n - 5 = 1$ vibration, the stretching of the H-Cl bond. The molecule has a dipole moment, so the vibration is IR-active. The fundamental frequency is at ca. 2880 cm-1 and the first overtone is at ca. 5670 cm-1, i.e. nearly but not quite twice the frequency.

Combination bands in IR spectra

Another phenomenon that often increases the complexity of IR spectra is the occurrence of **combination bands**. A simple example from the spectrum of liquid water will illustrate the principle. We have seen that the symmetric and asymmetric stretch frequencies of water are found at ca. 3280 and 3490 cm^{-1} respectively. The spectrum also shows an absorption band at ca. 6800 cm^{-1}, i.e. at an energy corresponding to the sum of the two individual fundamental modes. In simple physical terms we can imagine this band as arising when a photon has enough energy to excite both symmetric and asymmetric vibrations simultaneously. This band is quite strong, though not so intense as the strong absorption of the two closely overlapping fundamental bands. Another quite strong combination band occurs at about 5250 cm^{-1}: this arises mainly from the fundamental asymmetric stretching vibration plus the bending vibration found at 1645 cm^{-1}.

It will be seen that both these combination bands occur in the region that we have previously described as the near infra-red region of the spectrum: 6800 cm^{-1} is equivalent to a wavelength of 1470 nm, and 5250 cm^{-1} is equivalent to 1900 nm. Virtually all the absorption phenomena in the near IR region are due to overtones and combination bands. As will be seen this area of the spectrum has become a major field of applications in recent years.

Mid-IR Instrumentation

The Mid IR region refers to the wavelength range of around 2.5 – 15 μm (4000 – 625 cm^{-1}). To measure the mid-IR spectrum of molecules, we need:

- A source of IR radiation;
- Cells suitable for holding a wide variety of samples;
- A means of separating IR radiation of different frequencies prior to measurement;
- A sensitive detector and its associated electronics;
- A computer-based system to record, process and present the results.

Although IR spectra in this region used to be obtained from a **dispersive** double beam IR spectrometer, nowadays most spectra are obtained by using small bench-top Fourier Transform instruments (FTIR)

Note: FTIR is covered later in this Chapter (see slides 106 - 109)

Sample Cells – cell materials

Samples in mid-IR spectroscopy may be in solid, liquid, or gas form, and a range of sample cells has therefore been developed to accommodate this range of sample types. **Cell materials chosen to hold the samples have to be transparent to IR radiation in this region**. Table (11.5) below shows some typical Cell materials together with frequencies over which they can be employed

Cell material	Frequency range/cm^{-1}
NaCl	4000 - 625
KBr	4000 - 400
CsI	4000 - 200
BaF_2	4000 - 850
AgCl	4000 - 350
KRS5	4000 - 250

NaCl, KBr and CsI are all crystalline materials that are very soluble in water. BaF, AgCl and KRS5 (mixture of thallium bromide and iodide) are only very sparingly soluble in water and thus may be used with aqueous solutions

Table 11.5 – cell materials for IR measurements

Of the cell materials shown in table (3a.3), the most important are **NaCl** and **KBr** for routine infra-red studies. **KRS5** is normally used as the crystal material of choice when using ATR (attenuated total reflectance spectroscopy)

Sample cells – cell design and sample handling

Figure (11.18) below shows a typical small laboratory Fourier Transform infra-red (FTIR) spectrometer, with the cell compartment opened.

Figure 11.18 – typical FTIR spectrometer

The cell holder is universal for the acquisition of infra-red spectra of solids, liquids and gases. Each of the types of sample require separate sample methods, all of which are described in the next few slides.

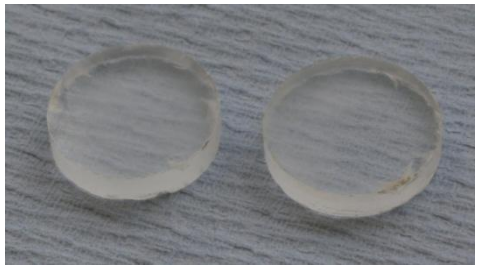
Sampling of gases

As gases show relatively weak absorbances, gaseous samples require little preparation beyond purification, but a sample cell with a long pathlength (typically 5-10 cm) is normally needed. A typical cell is illustrated as figure (11.19). Because the intensity of the spectrum is related to the quantity of gas held in the cell, it is normal to make all measurements at atmospheric pressure. This can be achieved by initially pressurising the sample gas in the cell and then opening one of the taps to allow gas to escape until atmospheric pressure is established.

Figure 11.19
Typical IR
gas cell

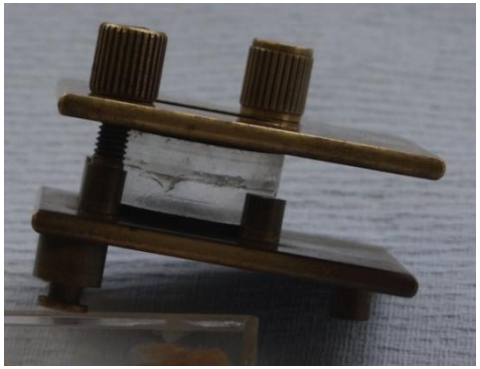
Sampling of liquids

Liquid samples can be sandwiched between two plates of a high purity salt. Sodium chloride is by far the most popular, however potassium bromide could also be used if it was necessary to make measurements at lower Wavenumbers [see table (11.5)]. Other materials are available for use with aqueous samples. The simplest sampling procedure involves placing a few drops of the sample onto one of the plates and placing the other plate on top. This is then placed in the sample holder. The thickness of the sample films areof the order of a few microns, too thick a film will produce too strong a spectrum with loss of resolution and fine structure. Figure (11.20 A, B & C) shows the NaCl plates in the sample holder, ready to be placed in the spectrometer housing.



NaCl plates (A)

NaCl plates with sample sandwiched In between (B)



NaCl plates in a sample holder (C)

Figure 11.20 – NaCl with sample placed into the sample holder

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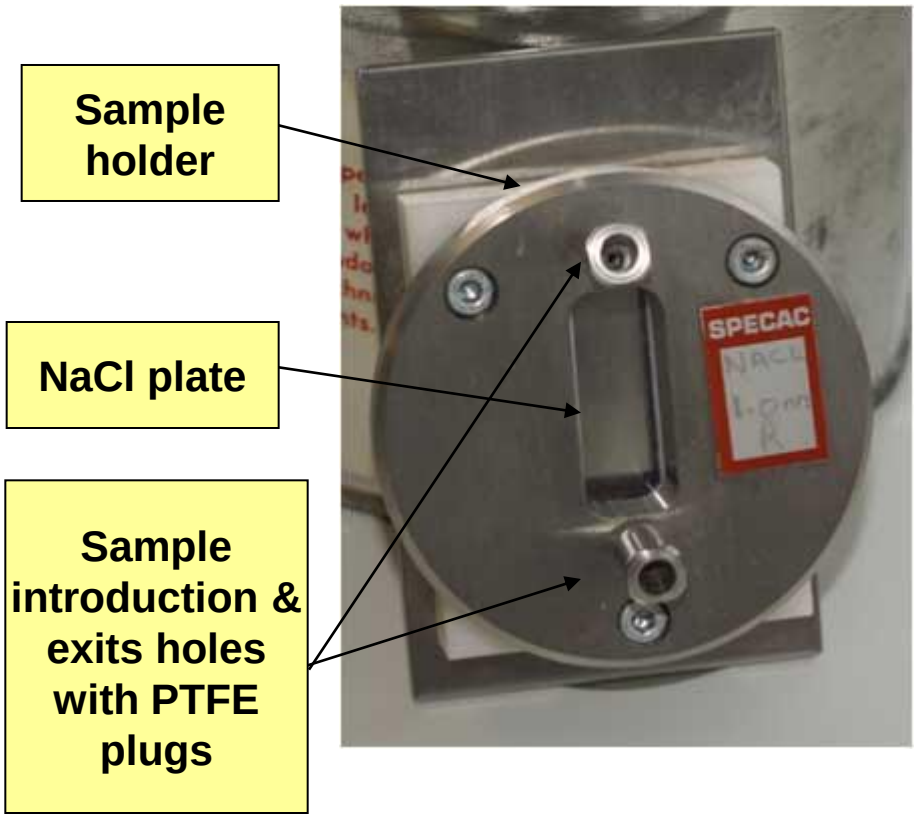
When handling dilute solutions of solid or liquid analytes, or when quantitative measurements are required a fixed pathlength cell is used. The cell consists of two NaCl plates separated by a thin PTFE spacer of nominally 5 or 10 microns thickness. A typical liquid sampling cell is shown in figure (11.21) below. The



sample is added to the cell compartment (the space between the plates) by using a Luer fitting syringe. Once the sample cell is filled and all air removed (visual inspection), small Teflon plugs are inserted to seal the sample cell. The cell can then be inserted into the spectrometer. Given that this cell has a fixed pathlength, it can be used to for quantitative measurements using any of the quantitative methodologies described in Chapter 4 of this teaching and learning programme.

Figure 11.21 – 1 mm fixed pathlength IR liquid cell

Sampling of solids

Solid samples can be prepared for IR measurement in four major ways.

1 Making a mull

Solid materials are not normally placed on their own between two NaCl plates, as the differences in refractive indices between the NaCl, the organic sample and air, will cause some of the incident radiation to be lost due to refraction. The sample is therefore crushed with a pestle in an agate mortar together with a mulling agent (usually Nujol). A portion of the resultant 'mull' is placed between the two NaCl plates to form a thin film. The Nujol has a refractive index similar to that of the NaCl and the sample matrix and thus avoids the loss of radiation by refraction. The sample between the two plates is placed in the sample holder and transferred to the IR spectrometer.

Nujol is sometimes called liquid paraffin (a C_{22} long chain aliphatic hydrocarbon), and is unsuitable as a matrix when (C-H) transitions are of interest. Under these circumstances hexachloro butadiene may be used as a mulling agent. Figure (11.22) shown on the next slide is an IR spectrum of Nujol.

Continued on the next slide

The spectrum of Nujol exhibits strong bands in the 3000 – 2800 cm^{-1} and the 1500 – 1300 cm^{-1} spectral regions, which can make it very difficult to acquire useful interpretative information from samples run in Nujol as a mulling agent.

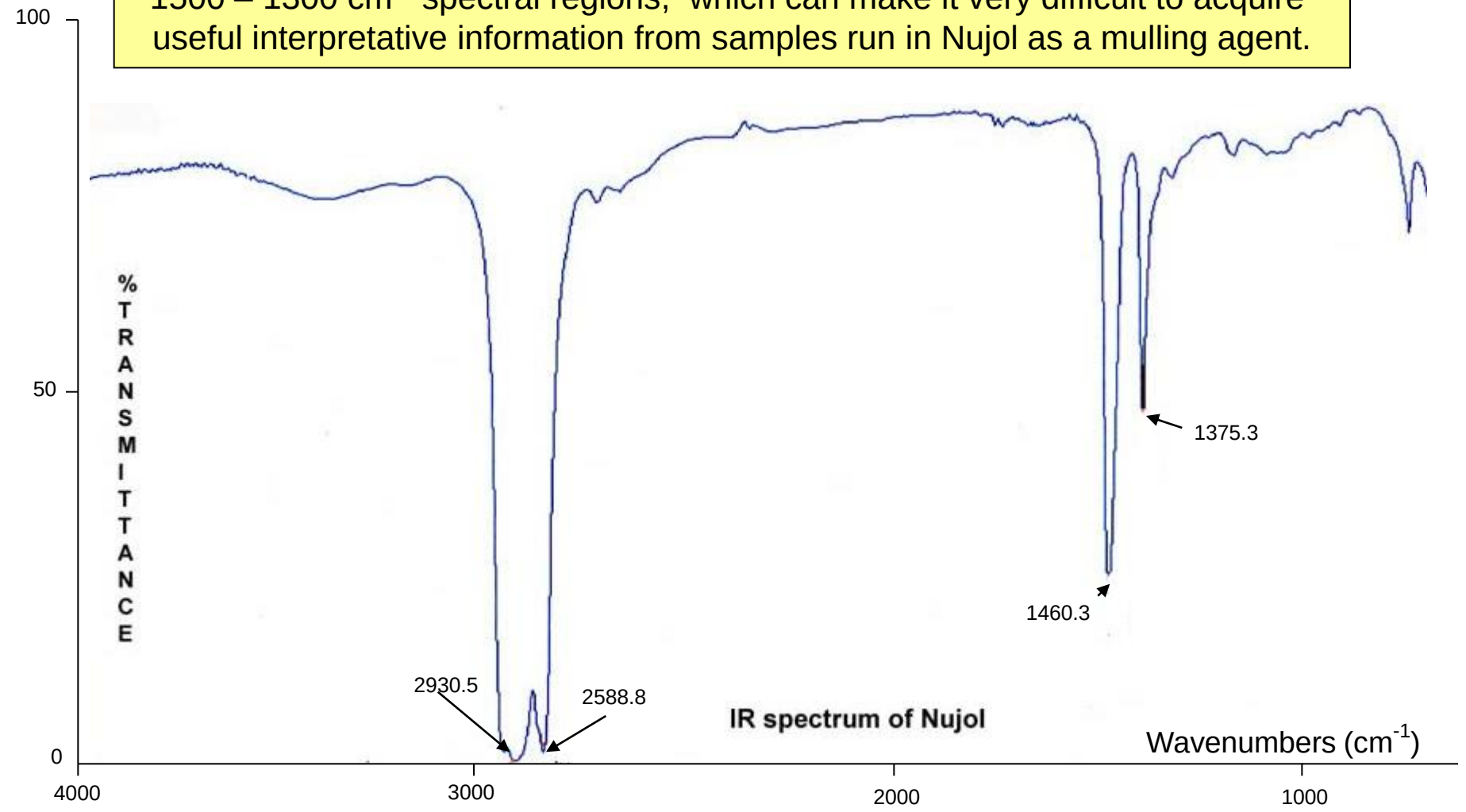


Figure 11.22 – IR spectrum of Nujol

2 Making a KBr disc

A portion of the ground sample (1-2 mg) is mixed with dry KBr (200mg) and the mixture is further ground to produce a fine powder. The powder mixture is then transferred to a mechanical die press and compressed to form a translucent pellet, through which the radiation beam from the spectrometer can pass.

The whole process is shown diagrammatically as Figure (11.23) on the next slide

Because NaCl and KBr are so hydroscopic, it is important to keep these materials at all times under desiccated conditions.

Components required to make a KBr disc

Ground mix of sample and KBr, transferred to die

Second die then placed on top of first followed by the plunger

The sample sandwiched between the dies is then compressed at 10000 kg pressure

The resultant disc placed In holder, before being Itself placed in the IR sample holder

Sample in the IR spectrometer

Figure 3a.41 – Figure 11.23 – making a KBr disc

3 The cast film technique

The cast film technique is used mainly for polymeric materials.

The sample is first dissolved in a suitable, non hygroscopic solvent. A drop of the resultant solution is deposited on surface of KBr or NaCl plate and the solvent evaporated under an IR lamp or by blowing a stream of air over the plate. The resultant polymeric film is then analysed directly by placing the plate in a typical IR sample holder. Care is important to ensure that the film is not too thick otherwise the incident radiation cannot pass through. This technique is suitable for qualitative analysis only.

Alternatively it may be possible to physically remove the film from the NaCl plate and attach it, by the use of Sellotape, above and below the opening of an IR sample holder.

4 Use of a microtome knife

The final method is to use microtomy to cut a thin (20-100 micron) film from a solid sample. This is one of the most important ways of analysing failed plastic products for example, because the integrity of the solid is preserved. Figure (11.24) below is a low-cost instrument which can produce thin slices of polymeric materials between 20 µm to 3 mm. These instruments are capable of taking thin slices at right angles to the sample (HS-1) or at a chosen angle to satisfy the objectives of the analysis. This equipment could therefore be used to carry out depth profiling of a composite polymeric sample. The example shown on the next slide, illustrates how this technique may be applied.

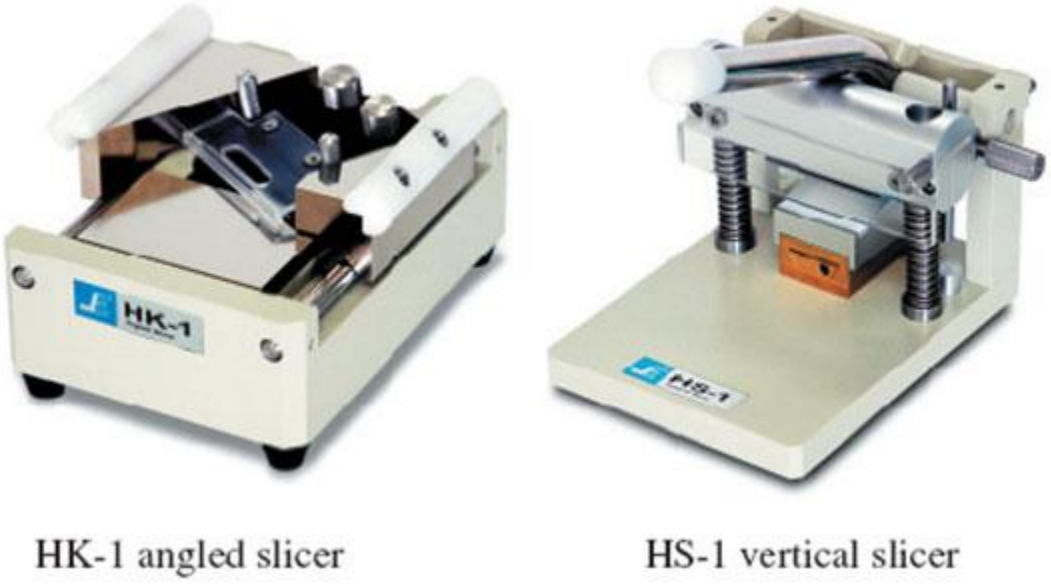


Figure 11.24 – device for producing thin samples of polymeric materials

(Image reproduced by permission of Jasco International Co Ltd)

Example (11.iv) - the use of thin sections (*Jasco International Co Ltd*)

A thin cross section of a commercial food packaging laminate was taken using the HS-1 slicer shown on the previous slide. The sample was analysed using the conventional transmission mode and the resultant spectra are shown in figure (3a.43). These spectra were identified as:

Polyethylene (PE)
Polyvinyl alcohol (PVA)
Polyethylene terephthalate (PET)

The main bands used in the identification are shown in table (11.6) below:

Band identity	Frequency/cm^{-1}	Vibration
A	3500	- OH stretching
B	2920	C-H stretching
C	1440	C-H bending
D	1730	C=O stretching
E	1250	Aromatic

Table 11.6

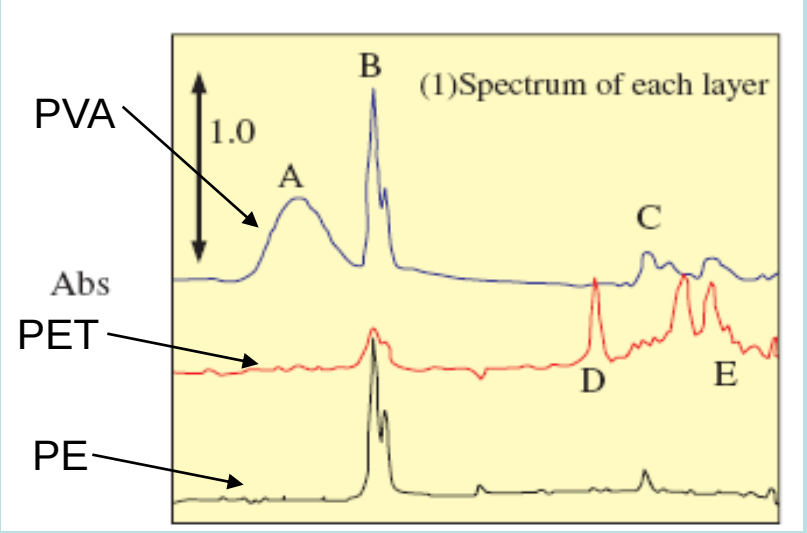


Figure 11.25

The IR spectrum shown in figure (11.25) has been recorded in the Absorbance rather than the more usual transmittance mode

Use of ATR to obtain IR spectra

ATR (**Attenuated Total Reflectance**) is a sampling technique used in conjunction with infrared radiation which enables samples to be examined directly in the solid or liquid state with little or no sample preparation.

ATR uses a property of **total internal reflection** called the **evanescent wave**. A beam of infrared light is passed through the ATR crystal in such a way that it reflects at least once off the internal surface in contact with the sample. This reflection forms the **evanescent wave** that extends into the sample, normally by a few micrometres. Whilst in the sample, the beam is attenuated by absorption of selective bands by those functional groups of molecules that are IR active. The beam then exits the crystal and is measured by the detector. The resultant spectrum is very similar to that of a transmission IR spectrum. Figure (11.26) below, illustrates the effect

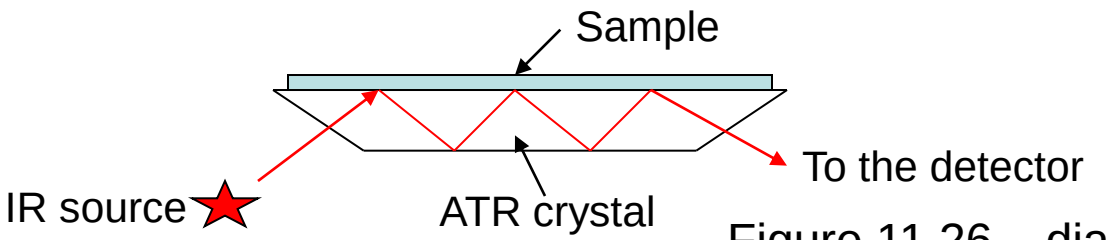


Figure 11.26 – diagrammatic representation of the ATR effect

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This effect works best if the crystal is made of an optical material with a higher refractive index than the sample being studied. Typical materials for ATR crystals include germanium, KRS-5 and zinc selenide. Diamond makes an ideal material for ATR, particularly when studying very hard solids, but its much higher cost means that it is less widely used.

Although the technique originally found applicability in obtaining spectra of polymers, modern developments have produced ATR equipment which is capable of obtaining rapid spectra of both liquids and solids. In the case of a liquid sample, pouring a shallow amount over the surface of the crystal is sufficient. In the case of a solid sample, it is pressed into direct contact with the crystal. Because the evanescent wave into the solid sample is improved with a more intimate contact, solid samples are usually firmly clamped against the ATR crystal, to avoid the presence of trapped air which would distort the spectrum. This technique is now the recommended method for obtaining rapid IR spectra, and figures (11.27) shown on the next slide illustrate how the technique is currently applied.

Sample placed in the hole directly onto the crystal

The arm of the device is now brought across and screwed down to ensure good contact between the sample and the crystal

Figure 11.27 – sampling equipment for use with ATR technology

Early applications of ATR were carried out by placing the ATR cell into the sampling compartment of a double beam dispersive IR spectrometer. Because the level of energy incident on the cell was low, it was almost as much of an art as a science to generate usable ATR spectra. Modern equipment is now used with FTIR spectrometers (slides 106 - 109) where all of the energy emitted from the source is incident on the sample cell. Reproducible spectra are now obtainable and the sampling system shown in figure (11.27) above, now represents 'state-of-the-art' technology for the production of IR spectra of both solids and liquids, to be used for interpretative analysis.

Applications of IR spectroscopy

Although often considered as essentially an interpretative technique, IR can also be used for quantitative measurement both in the laboratory and more importantly for routine environmental monitoring and for process control on chemical plants.

IR spectra and molecular structure

A very important qualitative use of infra-red spectra is in the establishment or verification of the structures of organic and organo-metallic compounds, usually of relatively low molecular weight. It is more or less obligatory for researchers who claim to have synthesised a new compound to demonstrate *via* the infra-red spectrum (along with other methods, such as nmr, elemental analysis, mass spectrometry etc) that their material has the expected structure.

This approach is possible because the vibrational frequencies of many of the groups that commonly occur in organic and organo-metallic molecules are about the same, i.e. they are independent of the other structural features present. Thus most carbonyl groups absorb strongly at ca. 1600 cm^{-1} in many such compounds; -C≡C- bonds absorb at ca. 2200 cm^{-1}; the >N-H group in amides and amines absorbs at ca. 3400 cm^{-1}; and so on.

Continued on the next slide

These and other **group frequencies** are well known and published in many texts. Naturally the principle is not perfect: in many cases these frequencies are **coupled** to some extent with those of neighbouring groups in a molecule, so the exact frequencies seen, vary somewhat. And unless the molecule is fairly simple one, IR methods alone cannot be expected to reveal its complete structure. But an IR spectrum incompatible with an expected or proposed structure suggests that the latter is incorrect

An IR spectrum is recorded usually between 4000 – around 650 cm^{-1}, the most important part of that spectrum from the interpretative aspect, is that between around 1600 – 650 cm^{-1} which is termed the **fingerprint region**. This is the part of the spectrum that displays the finest structure, enabling detailed analysis of the spectra to be related to chemical structure. Each molecule has a complete spectrum unique to that chemical structure, hence the term **fingerprint** that is frequently applied. Conversely, the region between 4000 – 1600 cm^{-1} is sometimes referred to as the **group frequency** region. Very large libraries of spectra of known compounds are available, and allow their speedy identification, often with the aid of rapid computer software

Figure (11.28) on the next slide shows where some of the main vibrational bands are observed and figure (11.29) on slide 83 is an IR spectrum of 2-butanone where some of the major bands have been identified.

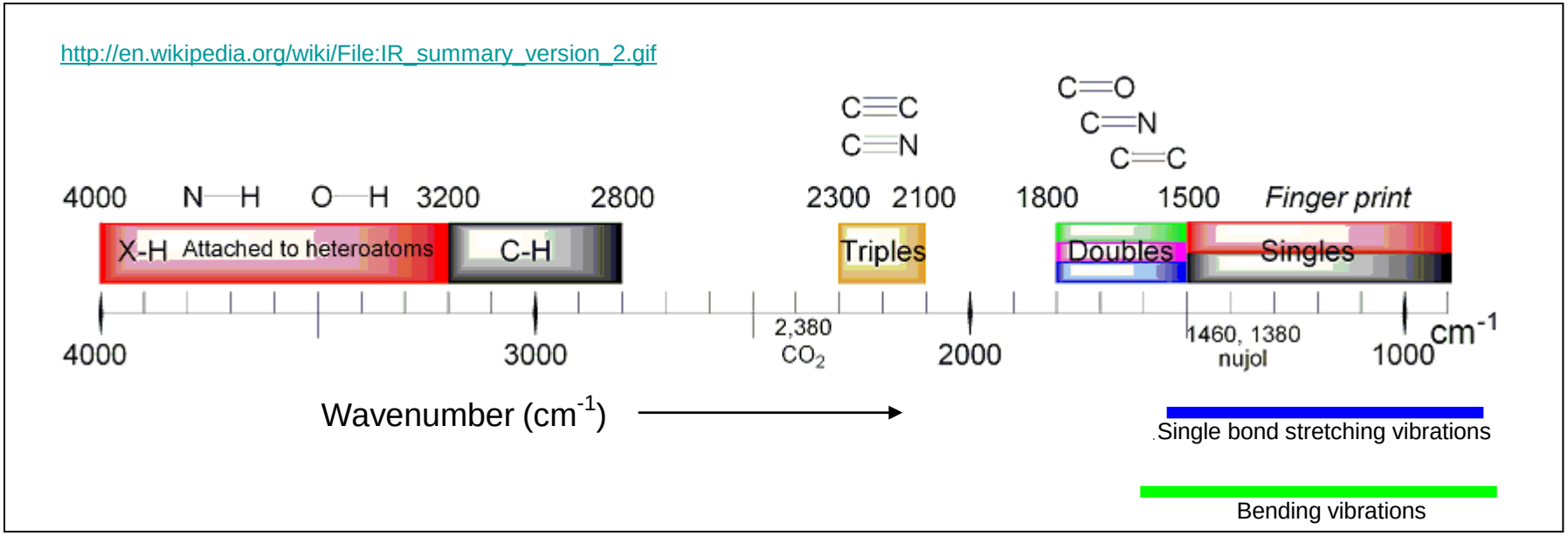


Figure 11.28 – main areas of stretching and bending vibrations in mid IR

A detailed list of the main absorption bands for organic functional groups is outside the remit of this teaching and learning programme. Anyone wishing to identify individual bands within the IR spectral region should consult specific texts on interpretative spectroscopy. Also, all modern instruments capable of producing Mid IR spectra, will have extensive databases containing many thousands of individual spectra. Computer identification of an unknown is suggested based upon a comparison between the spectra of the analyte and those stored in the database. Mixtures of absorbing compounds will produce additive spectra, however it is often still possible to identify components of the mixture from specific bands present in the combined spectra.

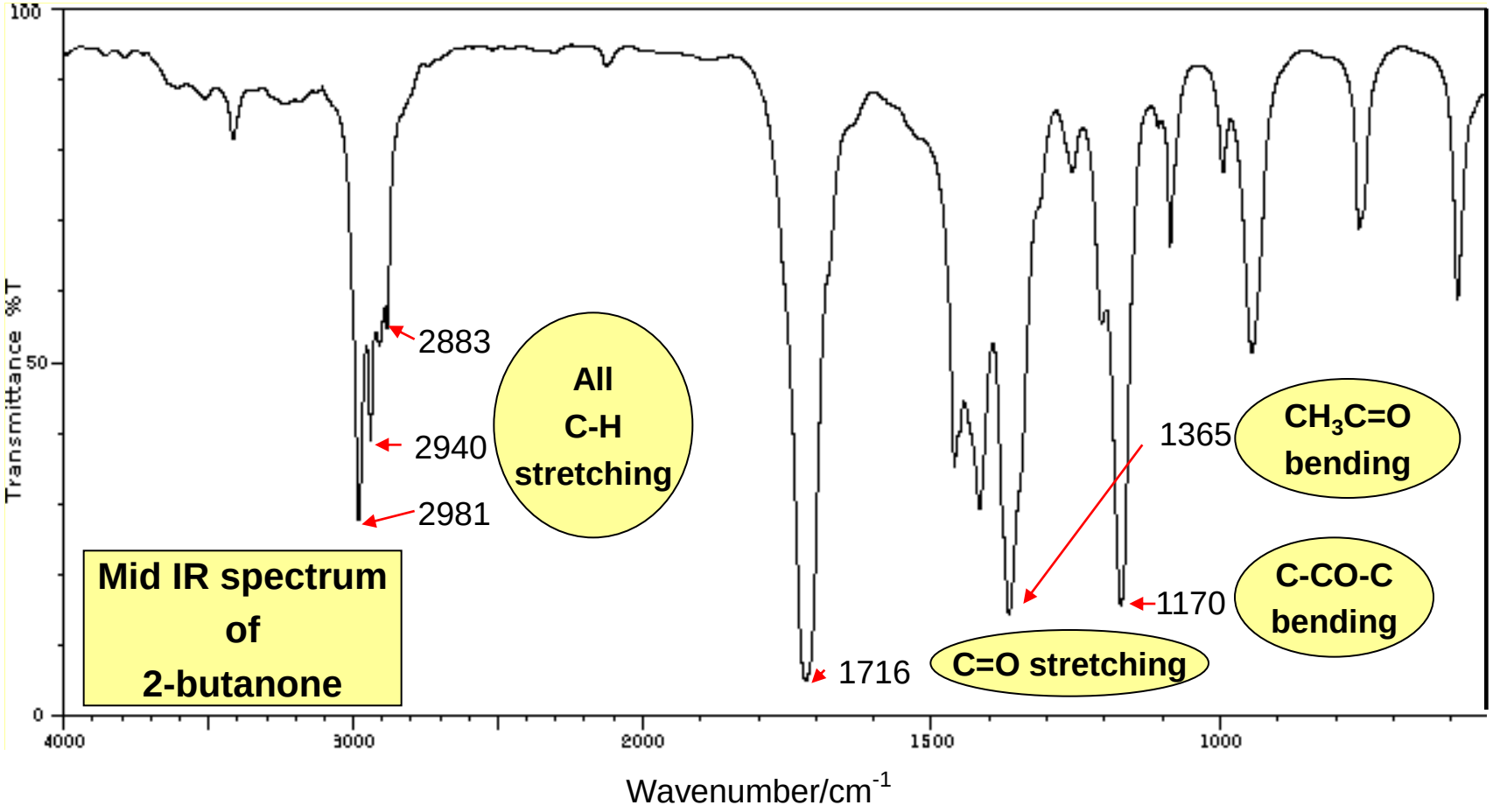


Figure 11.29 – mid IR spectrum of 2-butanone

Quantitative analysis

Beer's law may be applied in the IR spectral region, in the same way as indicated in the earlier discussion of UV-visible spectroscopy. Equations introduced originally in Chapter 10 of this teaching and learning programme are reproduced below:

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

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

Older IR spectrometers only provided a displayed spectrum in terms of %Transmittance *versus* wavenumber, as shown in figure (11.29) on the previous slide. In this situation it is necessary to calculate the absorbance using the equations shown above, following the measurement of the P/P_0 ratio as indicated in figure (11.30). In this example the ratio of:

$P/P_O = 0.5/9.7$

Thus $\log (P/P_O) = -1.288 = \log T$

Thus absorbance = - log T = 1.288

Newer spectrometers with a computer read-out are capable of presenting the spectrum as Absorbance *versus* Wavenumber, making direct measurements of absorbance a much simpler task. and thus enhancing the technique as a quantitative tool.

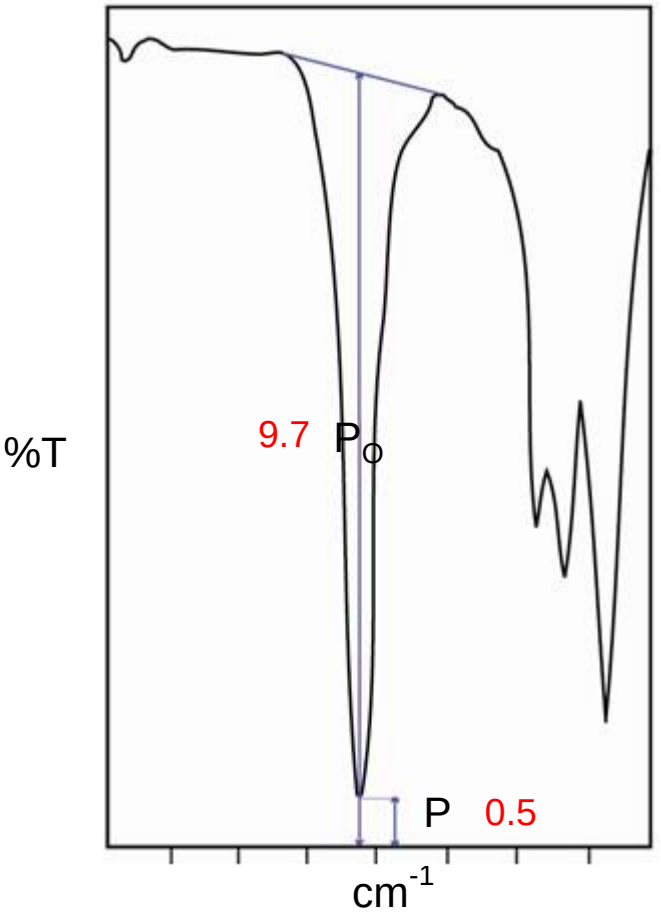


Figure 11.30 – calculating absorbance from %T scale

Continued on the next slide

As shown on the previous slide, quantitative measurements in IR spectroscopy are based upon a single band in the IR spectrum. Although the band of choice is that offering maximum sensitivity and resolution from other interfering bands, there is no reason why any other band relating to the analyte molecule's IR spectrum should not be used. Often in the case of mixtures, it is necessary to select a band that has the minimum overlap with other bands present in the mixture.

It is important to remember that cell pathlength is one of the terms in Beer's law and thus when attempting to use IR spectra quantitatively it is imperative to keep the **cell pathlength constant**, by using a fixed pathlength cell of the type illustrated in figure (11.21) on slide 69. These pathlengths are generally less than 1 mm and under these circumstances the technique does not offer the sensitivity of the comparable UV-visible technique. It can however be useful for the determination of an analyte's present at low % (eg: 1-10 %) concentration levels. The exception to this, is when the technique is used for the continuous monitoring of gases present in workspace atmospheres or in the air quality monitoring.

Application of IR spectroscopy to environmental monitoring

Although pathlengths used in making IR measurements within the laboratory are generally extremely narrow, much longer pathlengths may be used for Environmental monitoring. Figure (11.19) on slide 67 showed a typical gas cell of 5 to 10 cm pathlength, however for environmental monitoring, pathlengths of 1 m are not uncommon and by the use of mirrors to pass a beam radiation through a sample several times, pathlengths of 50 m can be achieved. Under these circumstances it is possible to achieve high levels of sensitivity.

Although it is possible to measure a number of gases and vapours using a **dispersive** or Fourier Transform spectrometer, most instruments are dedicated to the measurement of a single analyte and use the simpler optics associated with a non-dispersive technology. A typical non-dispersive IR gas analyser is described on the next slide

Continued on the next slide

Figure (11.31) shows a diagrammatic representation of a non-dispersive IR analyser specific for carbon monoxide. The IR radiation passing through the reference cell is absorbed by the CO present in the detector cell, causing the gas in the left hand detector to be heated and to expand. If there is no CO passing through the sample cell, then the heating effect is mirrored in the right hand detector cell and the diaphragm separating these cells remains in its original position. However, if the sample contains CO,then some of the IR energy is absorbed within the sample cell, such that less is absorbed by the detector cell. The pressure in the left hand detector cell is now greater than that in the right hand cell causing the diaphragm to be distorted as indicated in the diagram. By chopping the beams, an oscillation will be produced The size of which is related to the CO concentration present in the sample. Many other analytes can be measured using this technology including methane, sulphur dioxide, carbon dioxide and water vapour.

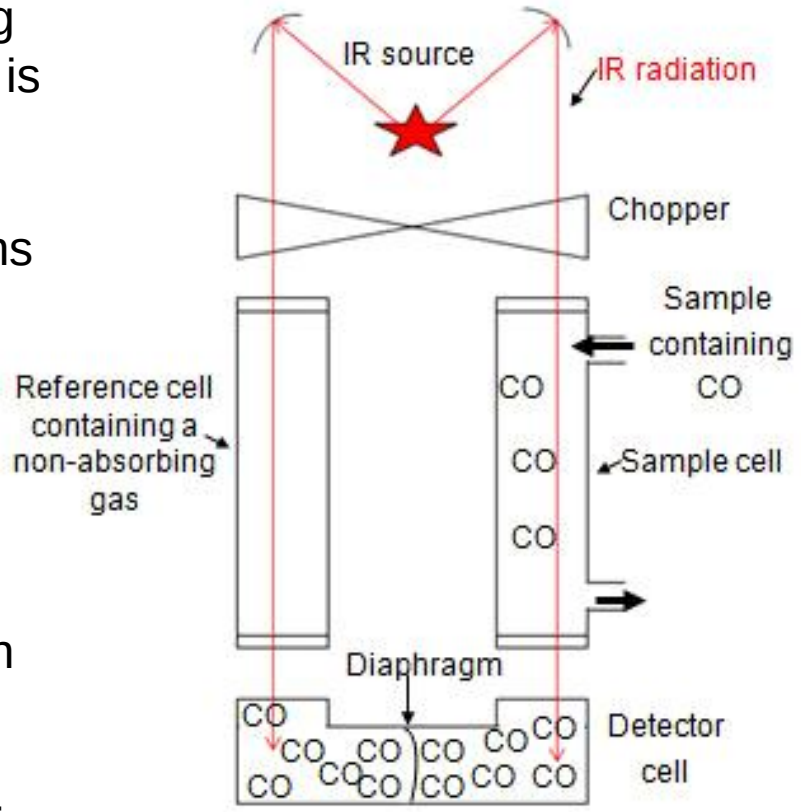


Figure 11.31 – non-dispersive IR analyser for CO

Near-IR (NIR) Spectroscopy

The near infra-red region of the spectrum, extending from ca. 800 – 2500 nm, i.e. from ca. 13000 – 4000 cm^{-1} has become the focus of many analytical applications in recent years. In many respects it is a sort of hybrid region; the instrumentation used often bears much resemblance to that used in UV-visible spectroscopy (some spectrometers operate over the whole range ca.180 – 2,500 nm), but the absorption bands studied are overtones and/or combination bands from molecular vibrations (**see slides 61-63**). Thus the first overtone of the O-H stretching frequency in alcohols, phenols organic acids etc occurs at ca. 1400 nm. Because these bands are so numerous and overlap so strongly, it is not normally possible to use near-IR methods for the identification of compounds or their constituent groups. NIR spectra are often rather broad and superficially similar to each other, but they contain a great deal of information that can be extracted using modern data handling methods (**chemometrics**); NIR methods would be of little practical value without the use of such additional techniques. An example of an NIR spectrum is shown as figure (11.32) on the next slide

Many organic solvents are optically transparent over most or all of the NIR region, but in practice the most common and useful applications are those in which **diffuse reflectance** NIR methods are used to study materials with no solvent present. An established example is the determination of the protein content of wheat. A more extensive coverage may be found at:

http://en.wikipedia.org/wiki/Near_infrared_spectroscopy

http://en.wikipedia.org/wiki/File:Dichloromethane_near_IR_spectrum.png

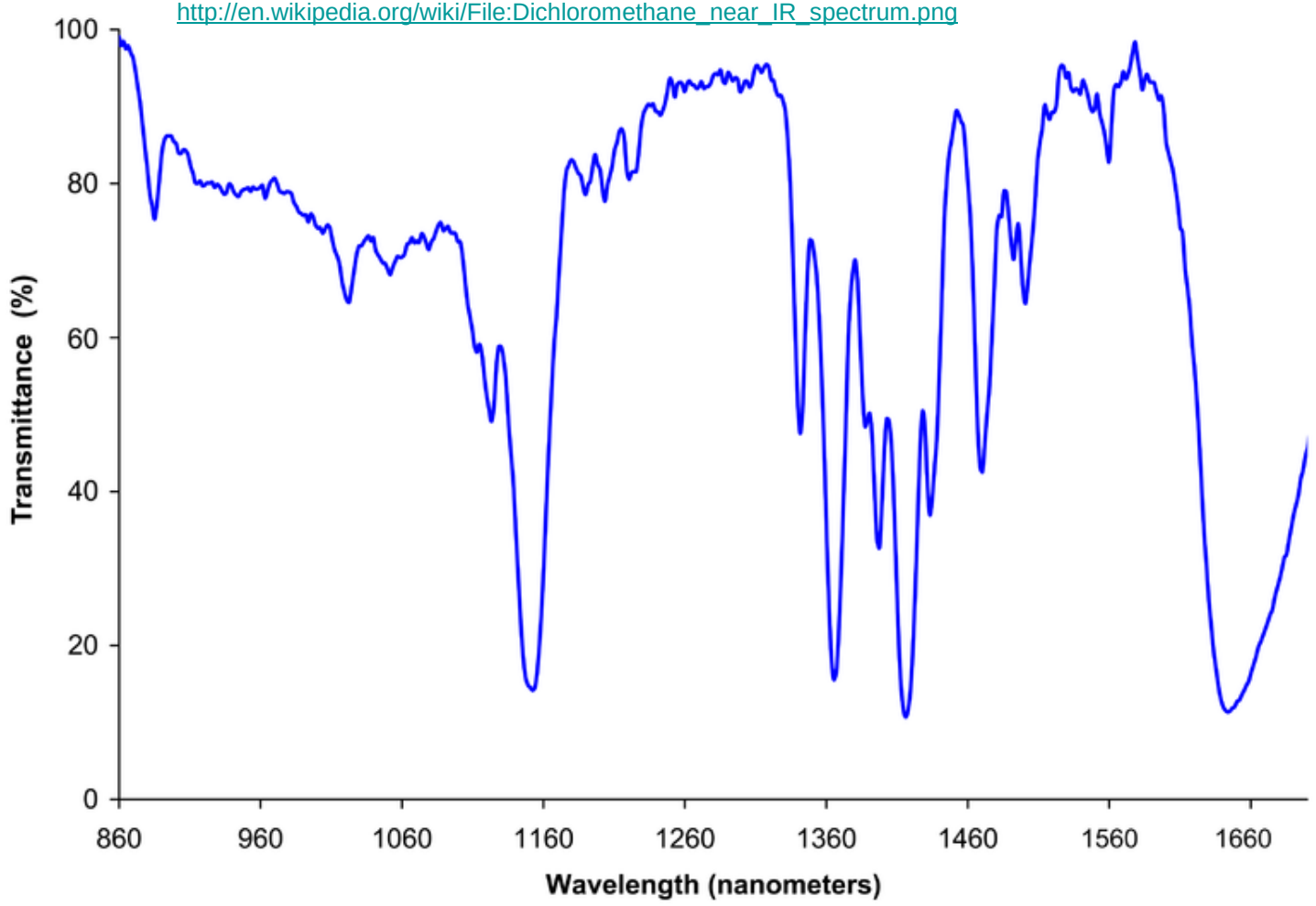
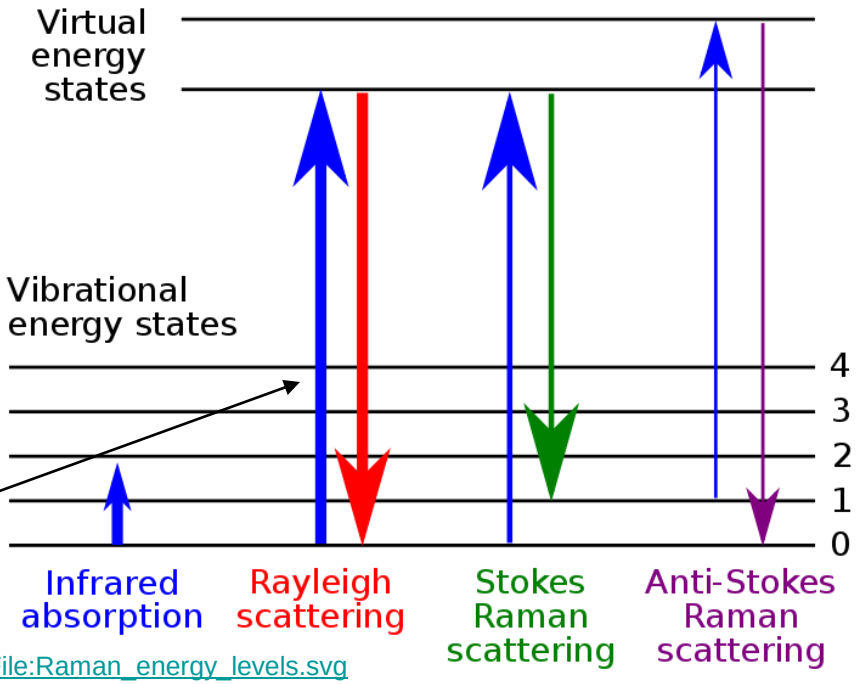


Figure 11.32 – Near IR absorption spectrum of dichloromethane showing complicated overlapping of mid IR absorption features.

Raman Spectroscopy - Introduction

Infra-red spectroscopy is not the only method by which molecular vibrations can be studied. This is important, because not all such vibrations are IR active as they do not all involve a change in dipole moment (**see slides 54 - 59**). An alternative approach is provided by **Raman spectroscopy**. This method was first used about 80 years ago, but it is only in Recent years that technical advances (particularly the development of high powered lasers), have turned it into an extremely powerful and popular technique. Figure (11.33) shows the energy levels Involved In the production of Raman spectra.

Figure 11.33 – energy level diagram showing the states involved in Raman signal



http://en.wikipedia.org/wiki/File:Raman_energy_levels.svg

Note: the line thicknesses are roughly proportional to the signal strength from the different transitions

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Continued on the next slide

Raman spectroscopy is a **scattering** method. The scattering of light, i.e. collisions between photons and molecules so that the direction of travel of the photons is changed, causes problems in electronic spectroscopy and in reality occurs whenever photons interact with molecular samples. Most scattering proceeds *via* the **Rayleigh** mechanism, in which the photon–molecule collisions are **elastic**, i.e. do not involve any exchange of energy. As a result, the scattered photons have the same wavelength (i.e. energy) as the incident photons – see figure (11.33) on the previous slide. But in normal conditions a very small proportion of the photons (<<1%) are scattered **inelastically** - some energy is exchanged between a photon and the molecule it collides with. The quanta of energy involved correspond to the vibrational energy levels of the molecule involved. In most cases the molecule will be in its ground vibrational state, so will accept energy from the photon rather than *vice-versa*. The Raman scattered light thus has a higher wavelength (lower energy) than the incident light. Figure (11.34) illustrates scattered radiation from a water molecule.

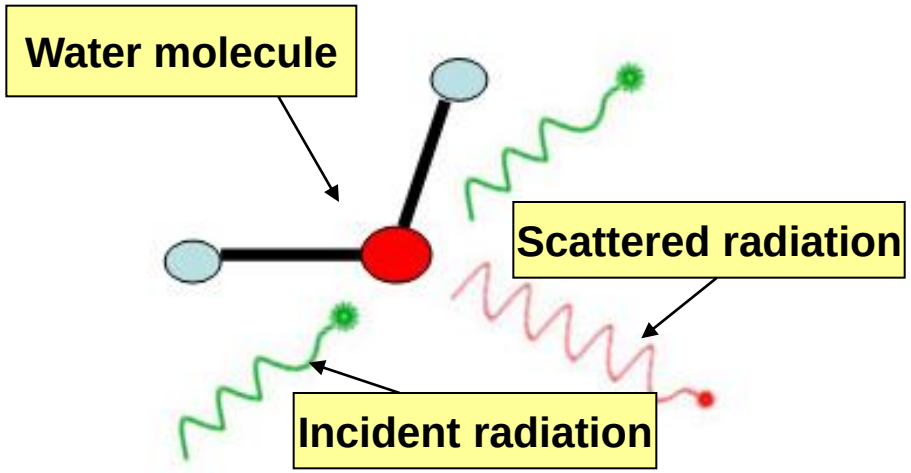

Figure 11.34 – scattered radiation from a water molecule

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Raman Scattering: More on the Mechanisms

In figure (11.33) on slide 90 the upper energy levels to which a molecule is excited by the incident light, which lie between the ground electronic state and the first excited singlet state, are referred to as "virtual levels." The excitation in this case is (unlike the situation in fluorescence) *not quantised*, i.e. in principle the incident light might have **any** UV-visible or near infra-red wavelength. Nonetheless the method gives information on the quantised vibrational levels of the molecule's ground state, as the molecule must return to one of those levels when the scattering event is over.

As we have seen, most of the photons scattered by the Raman mechanism have a higher wavelength (lower energy) than the incident light. These are called the "**Stokes**" lines, after a famous 19th century physicist, Sir George Stokes. (*The same terminology is used in fluorescence, a field in which Sir George did much pioneering research, where the term "Stokes Shift" describes the difference between the incident light wavelength and the [longer] fluorescence wavelength*).

However as the same slide shows, it is also possible for Raman scattering to give lines at shorter wavelengths (higher energy) than the wavelength of the incident light. These lines are rather curiously but understandably called "**Anti-Stokes**" lines. Anti-Stokes Raman lines are normally even weaker in intensity than the Stokes lines, so are only used for special purposes in structural studies, and are not of general value in analytical work.

The Basis of Raman Spectroscopy

We have seen that when using infra-red spectroscopy, absorption only occurs when a vibration causes a change in **dipole moment**. The mechanism in Raman scattering is different, even though the same bonds and vibrations are involved.

When the electric field associated with the incident radiation interacts with a bond, it causes a momentary distortion in the associated electron cloud and a short-lived **induced dipole moment**, which disappears when the radiation is re-emitted. The magnitude of this induced dipole moment is proportional to the **polarisability** of the bond, i.e. the extent to which the electron cloud is distorted by the electric field. As bonds lengthen they become more polarisable.

Raman scattering only occurs when a molecular vibration is accompanied by a change in polarisability. CO_2 provides a good example of how the different mechanisms of the IR and Raman methods complement each other (**see next slide**).

Continued on the next slide

The symmetrical stretching mode of CO_2 involves a polarisability change in both C=O bonds as they lengthen and contract, so this vibration is Raman active (at ~1480 cm^{-1}). By contrast in the asymmetrical stretching mode one C=O bond lengthens as the other contracts, so there is no overall change in polarisability, hence no Raman activity. Similarly the bending mode of the molecule involves no bond length changes, so again not Raman activity. So in a simple linear centrosymmetric molecule such as CO_2, IR-active modes are Raman-inactive, and *vice-versa*. [See figure (11.35) below]

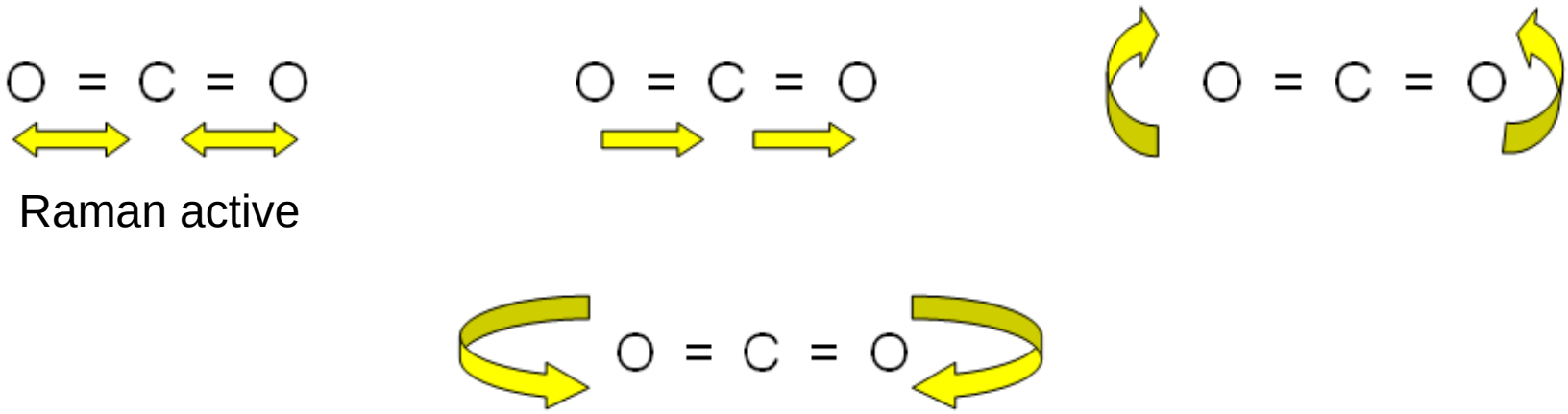

Figure 11.35 – stretching and bending vibration modes of CO_2

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Raman Spectrum Intensities

The intensity of Raman scattering is normally proportional to the concentration of the species being studied. In this respect the technique is similar to fluorescence spectroscopy, but differs from the absorption spectroscopic techniques, where there is a logarithmic relationship (**slide 84**).

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

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

Where: A = absorbance; T = transmittance; a = absorptivity; b = cell pathlength; c = concentration

Raman intensities are, like fluorescence signals, **proportional to the power of the incident light beam** (hence the normal use of laser sources) **and to the polarisability of the sample molecule.**

Continued on the next slide

As with **Rayleigh** scattering, the intensity of Raman scattering from a particular sample varies with the **inverse fourth power** of the incident light wavelength, i.e. UV light is scattered much more strongly than visible or infra-red wavelengths. In practice, however, the use of low incident light wavelengths has been limited, as UV sources also generate much fluorescence, and may also photo-decompose the sample. Early commercially available Raman spectrometers used **argon ion lasers at a wavelength of ~488 nm**, but most recent measurements are made with **lasers emitting in the red and near infra-red** regions of the spectrum. Their main advantages are:

- Less risk of photodecomposition;
- The Raman spectral shifts when measured in wavelength terms are large, so there is no significant fluorescence interference.

For example if a diode laser operating at 670 nm is used as the light source, the main water Raman band, shifted by 3400 cm^{-1}, appears at ~877 nm, whereas any fluorophores excited at 670 nm normally emit at ca. 700-720 nm.

Modern Quantitative Raman Spectroscopy

Introduction

Under normal conditions, the Raman scattering effect is so weak that there is no real possibility of using it for trace analysis: applications are limited to fundamental studies of molecular structures, vibrations etc.

However in the last 30 years or so, the widespread availability of tunable laser sources has permitted the development and use of two separate but complementary methods of obtaining tremendously enhanced intensities, facilitating trace analysis at the very low levels.

Raman spectroscopy has thus recently joined the small and select group of techniques capable of **detecting and studying single molecules in solution.**

Resonance Raman Spectroscopy

One technique for enhancing Raman intensities is to use so-called Resonance Raman Spectroscopy (RRS). The key feature of this approach (and the one requiring the use of tunable lasers) is to **excite the sample at a wavelength at or near an electronic absorption maximum of the analyte**.

Sample molecules are excited to a higher electronic state, and very rapidly (10^{-14} s) lose energy to return to an upper vibrational level of the lower electronic state. The crucial difference from fluorescence is that the latter emission only takes place after a much longer period (~10^{-8} s), which allows vibrational relaxation to occur at the upper electronic level (**slide 18 & 19 in Chapter 10 of this teaching and learning programme**).
Practical problems of RRS include the intense heat generated by the laser beam, and – again! – the dangers of interfering fluorescence signals and of photodecomposition of the sample.

Surface Enhanced Raman Spectroscopy

A second technique that produces greatly enhanced Raman intensities is called **Surface Enhanced Raman Spectroscopy (SERS).** The crucial feature of this method is that the sample is not studied in free solution, but instead **adsorbed on to the surface of a colloidal preparation of (usually) silver or gold**. A great many explanations have been offered for the high Raman intensities observed, but the practical benefits of the approach are remarkable, not least because the colloidal metal surfaces can be generated in a great variety of ways. For example:

- In suspension in solution,
- Attached to electrode surfaces,
- Covalently bonded to nanoparticles that also contain bio-sensing molecules such as antibodies.

As a result the method is now finding many applications in biochemistry and molecular biology: these methods make good use of the fact that Raman spectroscopy in aqueous systems is relatively straightforward, **in contrast to infra-red studies.**

Surface Enhanced Resonance Raman Spectroscopy

SERS can be combined with Resonance Raman Spectrometry, generating **Surface Enhanced Resonance Raman Spectrometry (SERRS)**, in which the sensitivity gains of the two techniques are effectively combined.

Comment on the sensitivity achievable with modern Raman Spectroscopy

In Raman spectroscopy and other techniques for studying single molecules in solution, this ultimate goal of molecular analysis is achieved by combining high sensitivity in molar terms with very low volume measurement systems. Thus if an analyte is detected at the 10^{-14} M level in a volume of 10^{-9} L, the number of moles interrogated is 10^{-23} – just 6 molecules!

Raman Instrumentation

Figure (11.36) below, shows the schematic spectral arrangement of a typical Raman spectrometer.

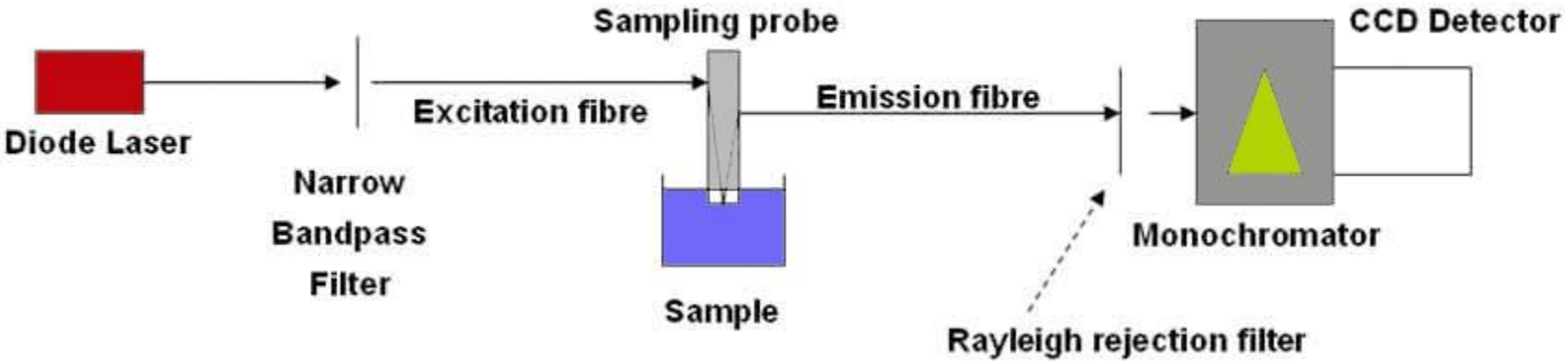


Figure 11.36 – spectral arrangement of a typical Raman spectrometer

Raman spectrometers have some requirements and some optical components in common with fluorescence instruments (**slide 29**). In each case there is the need for an intense light source (Diode laser), and in each case the sample is irradiated at one wavelength and the signal detected at a second (usually longer) wavelength. In both methods the Rayleigh scattered light is eliminated by using a filter or grating monochromator and a sensitive detector (charge coupled device multi-channel) is needed.

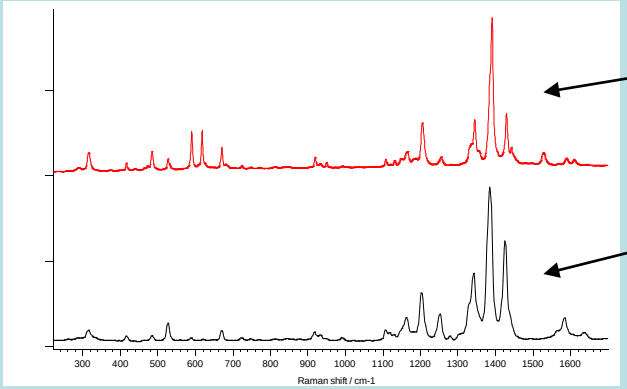
Raman microscopes

A further area of interest for Raman measurements is in the development and commercial availability of Raman microscopes. [Note: this is another similarity between Raman and fluorescence technologies]. Figure (11.37) below is a photograph of a Raman microscope (*with permission of Renishaw inc.)*

Figure 11.37 – Renishaw's inVia Raman Microscope with Smiths Detection FT-IR accessory

Example (11.v)

The Raman microscope shown as figure (11.37) was used in the forensic examination of a very small chip of paint transferred from one vehicle to the other in an automobile collision. The examination was conclusively able to identify the paint via a standard sample of paint pigment.



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Some Applications of Raman Spectroscopy

Applications of Raman scattering can be divided broadly into two categories – structural studies, and quantitative analysis.

For structural studies the method frequently complements infra-red spectroscopy or accesses regions that are problematical for infra-red work. A particular advantage is the ability to study very low frequency vibrational bands, with Raman shifts as small as only a few tens of cm^{-1}. Many organo-metallic complexes have bands in the range ca. 250 – 800 cm^{-1}, and their symmetry and structures can be readily studied.

Areas of application include:

- Examination of minerals and other inorganic compounds
- Examination of small samples of valuable art and antiquities
- Examination of polymers and emulsions
- Examination of electronic components for contamination
- Examination of pharmaceuticals
- Forensic examinations

Advantages of Raman spectroscopy

- Non-destructive analysis
- No sample preparation
- Ability to collect information at low (100 cm^{-1}) wavenumbers
- Fast collection of data
- High spectral resolution is possible (typically 1 cm^{-1})
- High spatial information (< 1 µm)
- Line width of spectral features generally sharp offering good chemical distinguishing power
- Water is a weak Raman scatterer and does not mask the spectrum, enabling the analysis of aqueous samples.

Raman *versus* Fluorescence

Raman spectroscopy involves illuminating the sample with light at one wavelength and observing the scattered light signal at longer wavelengths. In spectroscopic terms this clearly implies an optical system similar to those used in fluorescence (**slides 22 - 40**). Indeed Raman scattering can significantly interfere with fluorescence studies, and fluorescence is the bane of the life of a Raman spectroscopist. In practice the two phenomena can be readily distinguished as illustrated in the text box below

- If the wavelength of the incident light is increased (e.g. by 10 nm), the wavelength of any fluorescence signal will not change (though its intensity probably will). But any Raman scattering signal will occur at a new (and higher) wavelength, as the molecules remove **fixed** quanta of vibrational energy from the photons.
- Raman signals are so weak in fluorescence work that they are only detectable from the solvent. The Raman properties of the commonly used solvents are well known (e.g. in water the main Raman peak is separated from the incident light by a wavelength equivalent to ca. 3400 cm-1) so such signals are easily identified.
- The wavelength used to excite Raman scattering is not critical, so it is common now to use long wavelength (>600 nm) lasers as light sources. Fluorescence at such long wavelengths is relatively rare, so interferences are reduced.
- Peak shape helps; Raman peaks are usually narrower than fluorescence ones.

Fourier Transform Spectroscopy

In the last few decades a major change in the way in which spectra are obtained has revolutionised the value and applicability of several spectroscopic techniques. **Fourier Transform (FT)** techniques are now more or less universal studies of the spectra of distant stars. They have also been much used in Raman, nmr and mass spectrometry, and in plasma emission and microwave procedures. Two related areas where FTs have made little impression are in UV-visible and fluorescence spectroscopies, where the problems of sensitivity and spectral overlap are very significantly different.

Fourier Transform principles have also been used in the digital filtering of "noisy" data, producing significant improvements in signal-to-noise (S/N) ratios with the aid of rapid routines in widely available programs such as Excel®.

The Fourier Transform, named after J.B. Fourier (1768 – 1830, a French baron, mathematician and associate of Napoleon Bonaparte) essentially allows a signal obtained in the **frequency domain** to be converted to one in the **time domain**, but in optical spectroscopy the advantages of using the time domain (as opposed to the use of the frequency domain by dispersive instruments) are very formidable.

Fourier Transform Spectra – Spectacular Benefits!

FT optical spectrometers claim three major advantages:

- The throughput or **Jacquinot advantage**;
- Their **high resolving power and the reproducibility** of their wavelengths or frequencies;
- The multiplex or **Fellgett advantage** resulting from the very rapid acquisition of the spectra.

The throughput or **Jacquinot advantage** arises because FT instruments have very few optical components, and in particular they have no slits such as those used in conjunction with the monochromators of dispersive instruments. The radiant power reaching the detector is thus significantly greater than in dispersive systems, and the S/N ratios are correspondingly improved.

The **high resolution** of the instruments results from their optical design: they are interferometers (see next slides) and the time domain spectra they give are interferograms. Resolutions of a fraction of 1 cm^{-1} are very feasible.

Interferometers measure all parts of the spectrum simultaneously. It is thus possible to measure a spectrum extremely quickly, and also to obtain the average of many such spectra, with a great gain in S/N. In theory the S/N gain is proportional to the square root of *n*, the number of spectra averaged. This, *the* **Fellget** *or multiplex advantage*, is critical in infra red spectroscopy, where the limit of detection is usually set by the level of detector noise.

Optical Time-Domain Spectra

How can we obtain these advantages of FT spectroscopy in practice? The direct measurement of time-domain spectra is not possible using conventional systems because the radiation frequencies of interest (e.g. slide xx) are in the range 10^{12} – 10^{15} Hz: available detectors cannot respond at such high frequencies. It is thus necessary to *modulate* the high frequency signal to give an output of measurable frequency, while maintaining the information inherent in the original signal.

This can be done using a **Michelson interferometer**, a device was invented in the 1890s by the American physicist and Nobel Prize winner AA Michelson (1852 -1931), and used by him and EW Morley in a famous experiment that involved measuring the velocity of light, which led to Einstein's theory of Special Relativity.

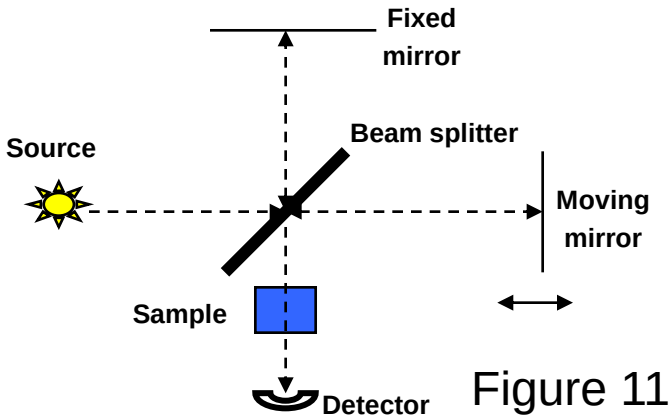
A simple schematic diagram of the interferometer is shown in the next slide [figure (3a.55). The main optical components are a **conventional infra-red light source, a beam splitter, and fixed and movable mirrors**. The beam splitter divides the source output into two roughly equal components, one of which strikes the fixed mirror and the other the movable mirror. These beams return to the beam splitter, half of each of them then passing through the sample and on to the detector.

Additional information on FT spectroscopy and how it works may be found at:

http://en.wikipedia.org/wiki/Fourier_transform_spectroscopy;
http://scienceworld.wolfram.com/physics/FourierTransformSpectrometer.html;
http://www.ijvs.com/volume5/edition5/section1.html#Feature.

Interferometry for FT spectrometry

The simple diagram below [figure (11.38)] summarises the main features of an interferometer typical of those used in infra-red spectroscopy. Light from the source is divided by a beam splitter and the two resultant beams travel on to a fixed mirror and to a moving mirror. After reflection at the mirrors, one of these beams is slightly out of phase relative to the other as a result of the mirror movement, so when the beams re-combine an interference pattern is produced (from all the light source wavelengths). This pattern then passes through the sample (which thus "sees" all wavelengths simultaneously) to the detector. As the mirror moves the interferogram changes with time, and absorbance of wavelengths by the sample produces a spectrum in the time domain that has to be converted to the wave-length domain using the Fourier transform, i.e. by an attached computer.



Fourier transform methods are now applied to a very wide range of spectroscopic techniques, including infra-red, Raman and nmr. Indeed conventional (dispersive) infra-red instruments are now only rarely used

Figure 11.38 – schematic diagram of an interferometer as used for IR spectroscopy

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X-Ray Methods – An Introduction

X-rays are a form of electro-magnetic radiation with extremely high energies. Most practical X-ray experiments use wavelengths in the range ca. 0.01 – 2.5 nm. A wavelength of 1 nm corresponds to an energy of about 100 million Joules per mole: this is of course why X-ray experiments require a range of safety precautions. Such energies are usually obtained by the interactions of extremely high energy electrons with electrons in the inner orbitals of atoms, the latter having very high binding energies. X-rays produced in this way can be used to generate **secondary** X-rays from other atoms. In practice X-rays are also obtained from radioactive materials or, more rarely, from synchrotrons and similar high energy beam sources. Like other forms of electromagnetic radiation, X-rays can be absorbed or scattered, or generate fluorescence. In the analytical sciences X-rays thus have a range of applications, of which two are of outstanding importance:

- **X-Ray Fluorescence**, in which a sample irradiated by X-rays emits X-rays with longer wavelengths, the latter being characteristic of the atoms involved. **This technique is related to elemental analysis and is thus covered in Chapter 12 of this teaching and learning programme**
- **X-Ray Diffraction**, which results from the scattering of X-rays by crystals, and thus gives information about the structures of the latter.

Note: X-ray methods are also covered in chapter 12 of this teaching and learning programme, particularly with respect to elemental analysis.

X-Ray Diffraction

As already noted, X-rays can be scattered according to their wavelengths in the same way as UV-visible or infra-red light is diffracted by gratings, by using the regular lattice structure of crystals. This principle can also be used in reverse – the structures of crystals can be determined by measuring the effects they have on the diffraction of X-rays. This was discovered by Max von Laue in 1912, and is now the basis of a crucial method of determining molecular structures.

The fundamental equation in X-ray scattering is the **Bragg equation** [equation (11.7) below]. If an X-ray beam strikes a crystal at an angle θ to the surface, constructive interference, leading to intense scattering of the radiation, occurs when:

$$2d\sin\theta = n\lambda \qquad \text{Equation (11.7)}$$

Where n is an integer, λ is the X-ray wavelength, and d is the spacing between the successive layers of atoms in the crystal.

In the simplest approach to structure determination a crystalline sample is ground to a fine homogeneous powder. In an X-ray beam the randomly oriented particles will allow the fulfilment of the Bragg condition for all the different regular planes in the crystal structure, allowing d values to be found from observed θ values and the crystal structure deduced. This is known as **X-ray powder diffraction**.

The main components of a powder diffraction XRD spectrometer is shown as figure (11.39). A typical instrument is shown as figure (11.42) on the slide 114.

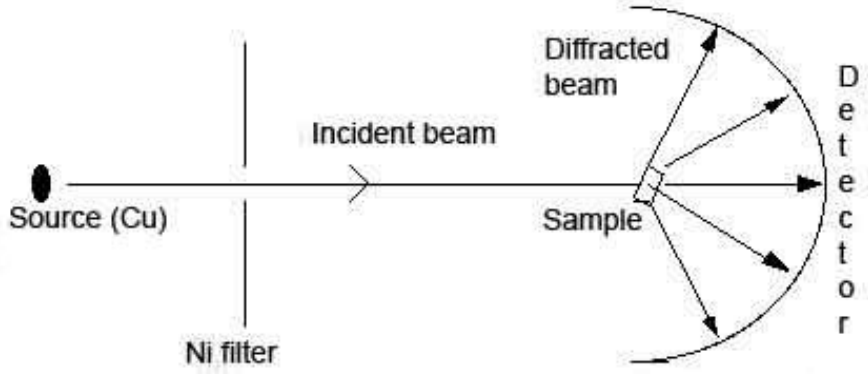

Figure 11.39 – schematic diagram of a basic XRD spectrometer

The X-ray radiation most commonly used is that emitted by copper, whose characteristic wavelength for the K radiation (see next slide) is =1.5418Å. When the incident beam strikes a powder sample, diffraction occurs in every possible orientation of 2theta. The diffracted beam may be detected by using a moveable detector such as a **Geiger** or **Scintillation** counter, which is connected to a chart recorder. In normal use, the counter is set to scan over a range of 2theta values at a constant angular velocity. Routinely, a 2theta range of 5 to 70 degrees is sufficient to cover the most useful part of the powder pattern. The scanning speed of the counter is usually 2theta of 2 degrees min^{-1} and therefore, about 30 minutes are needed to obtain a trace. A typical output from this analysis is shown as figure (11.41) on slide 114.

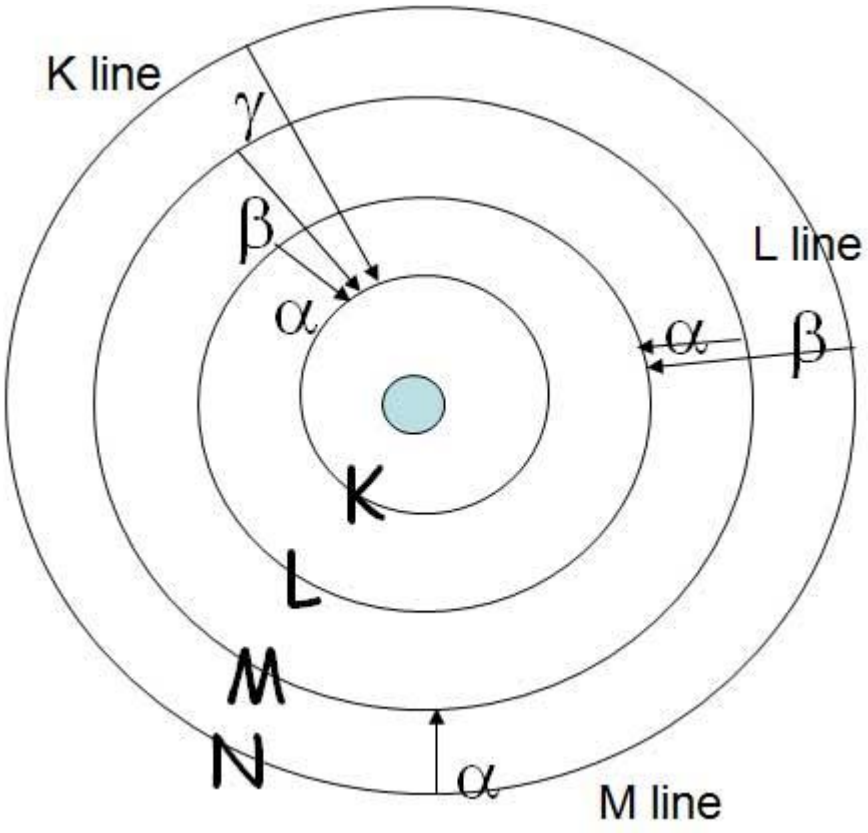

Figure 11.40 – nomenclature of X-ray lines

Nomenclature:

Transitions of electrons to the K shell are identified as Kα, Kβ, Kγ for L→K, M →L, N →M, respectively.

Whereas for the L shell are identified as Lα, Lβ for M →L, N →M, respectively.

And, for the M shell are identified as Mα for N →M.

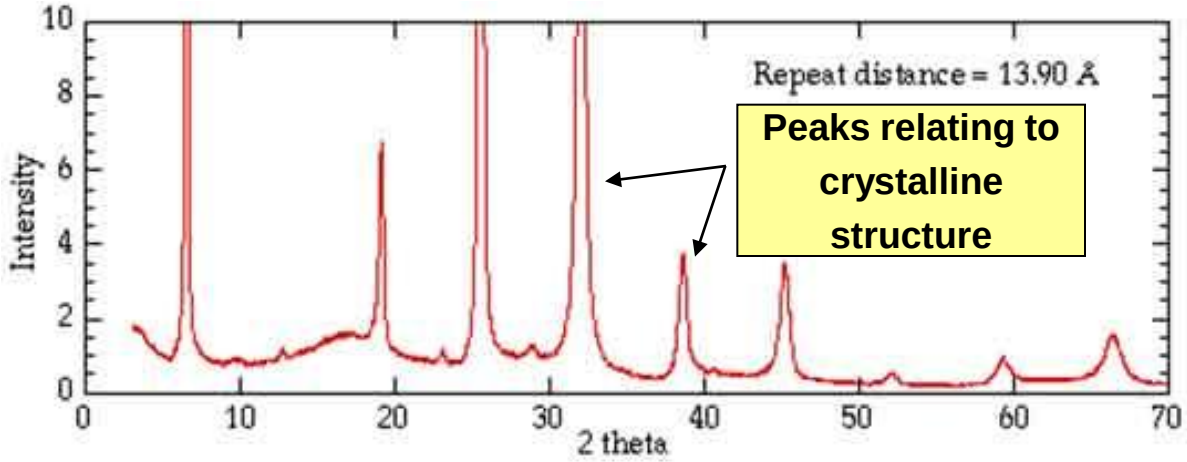

Figure 11.41 – recorded output from a powder XRD of a clay mineral

The diffraction pattern as illustrated in figure (11.41) is typical of that obtained by subjecting a crystalline powder to XRD examination. Each peak relates to a different diffraction of the incident beam and from a set of diffraction tables may be used to identify crystalline materials present in the sample. A typical XRD instrument is shown as figure (11.42) below.

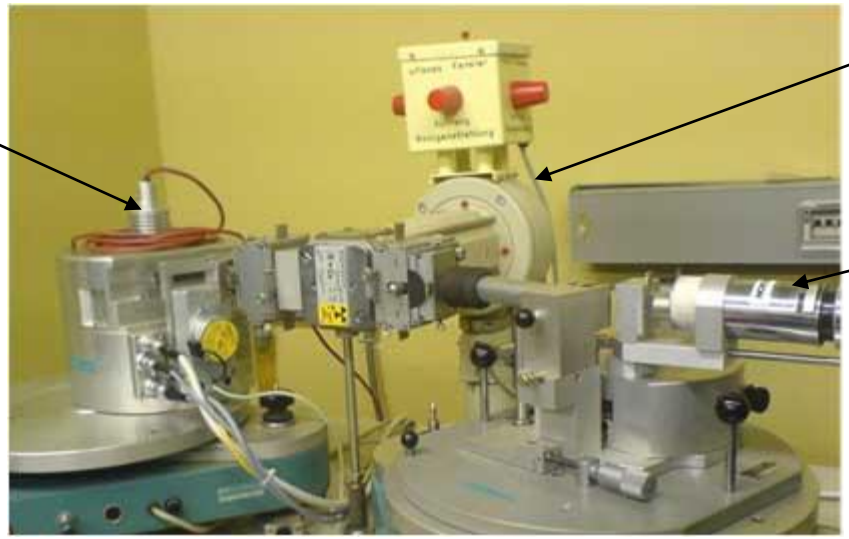

Figure 11.42 – powder diffraction spectrometer

X-Ray Diffraction Applications

X-ray diffraction can be used simply to identify low molecular weight species by comparing their powder diffraction patterns with structures stored in computer databases, which now contain information on many thousand compounds, and provide fast and efficient computer software to automate searches.

The speed with which X-ray data can be processed means that, if a new compound is synthesised, its crystals can often be studied and used alongside mass spectra, infra-red, nmr and other spectroscopic data to confirm its structure.

In the last 50 years, and especially in the last two decades, X-ray crystallography has been used to study proteins and other biological macromolecules, which contain thousands or millions of atoms in complex 3-dimensional structures. The techniques used are as expected very different. Single crystals of [e.g.] a protein are needed (the first protein was crystallised successfully in ca. 1920) and the data capture and collection are huge tasks. Until quite recently it was estimated that several man-years (and good equipment!) were required to determine the structure of even a modest-sized protein, but again modern automation and data processing methods have made such tasks much simpler and quicker.

One obvious question that arises with such molecules! - is the structure of [e.g.] a protein in a crystal the same as it is when the protein in operating normally in aqueous solution? Fortunately the answer to this question is – generally yes!

Question 11.1 Slide 20 shows that light scattering effects can be measured in an absorption spectrometer, a technique known as *turbidimetry*. Light scattering can also be measured *directly* rather than indirectly, using another useful method known as *nephelometry*. Both of these methods have many analytical uses, for example in measuring the formation of complexes between antibodies and other molecules. Which of them do you think would normally be the more sensitive? (Hint: think about the sensitivity of fluorescence measurements compared with absorption measurements).

Question 11.2 A sample studied in solution in a conventional fluorescence spectrometer has an absorbance of 0.10. Using the equations in slides 31 and 32, estimate the loss of fluorescence intensity that arises from studying a sample having this excessive absorbance.

Question 11.3 Comment on whether the following compounds are likely to exhibit any useful fluorescence properties (look up their structures if you do not know them):
(a) tryptophan;
(b) propan-2-ol;
(c) 1-nitronaphthalene;
(d) rhodamine B;
(e) pyrene;
(f) vitamin A.

Question 11.4 How many modes of molecular vibration does ethyne (acetylene) have? Comment on your result in relation to the actual infra-red spectrum of ethyne

Question 11.5 A given sample is irradiated with a UV lamp at 280 nm, by an argon-ion laser at 488 nm, or by a 670 nm diode laser in a Raman or fluorescence spectroscopy experiment. Assuming that the incident light intensity is the same in each case compare the intensities of the scattered light signals at the three wavelengths. What are implications of your results for the practice and application of these spectroscopic methods?

Question 11.6 In a sample that shows both Raman and fluorescence spectra when excited by a suitable light source it is found that, as the temperature rises, the relative intensities of *anti-Stokes* Raman lines increase (compared with Stokes lines), but fluorescence intensities decrease. Can you explain these results?

Question 11.7 The reaction shown in slide 48 for the chemiluminescence determination of nitrogen oxides in environmental samples is also the basis of a specific method of detecting nitrogen containing compounds in gas chromatography separations. Can you see how such a detector might work? What would be its most desirable properties?

Outline answer to question 11.1

The answer to this question may be found in slides 33/4 in Chapter 10 and 22 - 40

- In a nephelometer the light scattered by a sample is detected directly by placing a photomultiplier tube, photocell, etc at angle to the incident light beam. An angle of less than 90° should normally be used as the scattering intensity is actually weakest at that position (though in practice fluorescence spectrometers with the usual 90° optics have been used as nephelometers, both the monochromators being set to the same wavelength to detect Rayleigh scattered light). Commercial nephelometers have a variety of laser, diode laser and l.e.d. light sources.
- The difference between turbidimetry and nephelometry is exactly analogous to the difference between absorption and fluorescence spectroscopy. Absorptiometry and turbidimetry both effectively measure the photons that are *not* transmitted by the sample, so in trace analysis they attempt to distinguish between two large and similar light signals received by the detector: this is a serious limitation on their sensitivity. By contrast both nephelometric and fluorescence measurements measure photons emitted by the sample, so at trace concentrations they aim to detect a small number of photons above a zero or near-zero background. This is a much easier process, so these two methods are each significantly more sensitive than turbidimetry and absorptiometry respectively.

Outline answer to question 11.2

- If we look at the equation (11.5) at the bottom of slide 31, the first term in the squared brackets after the initial 1 is 2.303A, which in this case is 0.2303 [A = εbc]. So if we ignore all the subsequent terms (and note that the two 1s cancel each other out!) the intensity of fluorescence according to the equation is then given by:

$$I_f = \varphi_f P_0 \times 0.2303$$

- However if we include the next two terms in the exponential expansion, we find that we get:

$$\begin{aligned} I_f &= \varphi_f P_0 [0.2303 - (0.2303)^2/2 + (0.2303)^3/6] \\ &= \varphi_f P_0 [0.2303 - 0.0265 + 0.0020] \\ &= \varphi_f P_0 \times 0.2058 \end{aligned}$$

  So if we include the extra terms in the equation we find that, at this quite high absorbance the intensity is ca. 10% lower than the approximate equation would suggest. Similarly a solution with an *A* value of 0.05 has ca. 5% less fluorescence than the approximate equation suggests.

- Note that in all these equations the instrument cannot collect all the fluorescence emitted in all directions, so the measured intensity is less than the equation would show; of course this factor is true for all samples, so cancels out in an analysis.

Outline answer to question 11.3

Help with this answer may be found on slides 24/5

- Tryptophan is an indole, and like most such compounds is fluorescent, with optimum excitation and emission wavelengths of ca. 280 and 360 nm respectively in water. Tryptophan is a good example of a compound in which (a) the fluorescence intensity is highly temperature dependent; and (b) the emission wavelength is very solvent dependent (see slide 35); the emission maximum in non-polar solvents may be 330 nm or even lower.

- Propan-2-ol is entirely aliphatic, and so not fluorescent at any normal wavelength.

- The nitro-group in 1-nitronaphthalene is strongly electron-withdrawing in its effect on the aromatic nucleus, so this compound is also not fluorescent (though it may show some phosphorescence).

- Rhodamine B is a xanthene dye like fluorescein, and strongly fluorescent: it has amine as opposed to hydroxyl substituents, so its excitation and emission wavelengths are longer than that of fluorescein (ca. 535, 560 nm respectively).

- Pyrene is a polynuclear aromatic hydrocarbon, and strongly fluorescent, with excitation and emission wavelengths of ca. 315 and 390 nm respectively.

- Vitamin A is retinol (see slide 25); its extended conjugated (but not aromatic) system ensures that it is fluorescent (excitation ca. 320 nm, emission ca. 410 nm)

Outline answer to question 11.4

Help with this answer may be found in slides 54 - 58

- Ethyne (acetylene) is a linear molecule with 4 atoms, and thus has $3n - 5 = 7$ modes of vibration:

  H–C≡C–H

  However its symmetry ensures that, as in the case of CO_2, the number of observed frequencies is less than this.

- In this case only 5 fundamental frequencies are found; see if you can find their values from the published literature or from the internet. Additional combination bands can also be seen in the spectrum – can you identify these? Note also that as a linear symmetrical molecule ethyne should exhibit the same behaviour as CO_2, i.e. its infra-red and Raman bands should be mutually exclusive.

- It is worth noting that in recent decades infra-red methods have detected ethyne molecules in the heads of several comets, probably to the extent of <1% of the total volatile material present.

Outline answer to question 11.5

Help with this question may be found on slides 33/4 in Chapter 10 and in slides 95/6, 101 & 105

- Scattering intensities are inversely proportional to the fourth power of the incident light wavelength. So we can be sure that in this example the scattering is less intense at 670 nm than at the other two wavelengths.
- If we arbitrarily assign a value of 1 to the scattering intensity at 670 nm, the relative intensity at 488 nm is given by $(670/488)^4 = 3.55$. Similarly the intensity at 280 nm, relative to that at 670 nm, will be $(670/280)^4 = 32.78$.
- From these results it is clear that scattering phenomena become immensely greater as we move from the red end of the visible spectrum to the near ultra-violet. In fluorescence measurements scattering signals are undesirable, so the use of long excitation wavelengths is highly advantageous. Note also that the longer wavelength light sources in this question are lasers, with extremely narrow emission bandwidths; this makes the reduction or removal of scattered light using filters much easier than with broad-band uv sources. Longer wave-length sources have the further advantage of minimising photodecomposition.
- In theory uv-excited Raman spectroscopy ought to be very sensitive, and it has indeed been used, but the photodecomposition problem, along with the need to use safety precautions in the uv, and the ease with which long-wavelength filters can be used, encourages both fluorescence and Raman spectroscopists to use long-wavelength sources wherever possible in modern practice.

Outline answer to question 11.6

Help with this question may be found in slides 90 – 96 and 38

- These two temperature effects are really more or less unrelated, but each reminds us of the need to control temperatures in high quality spectroscopic studies.

- In Raman spectroscopy, most of the lines seen at room temperatures are Stokes lines, i.e. they have higher wavelengths, lower energies, than the incident light. This is because most of the molecules will be in their ground *vibrational* state, so it is only possible for the incoming photons to give up vibrational energy to the molecules, not *vice-versa*. But as the temperature rises, more molecules gain vibrational energy, so it is possible for them to give up vibrational quanta to the scattered photon, giving more intense anti-Stokes lines.

- In fluorescence spectroscopy a molecule excited by absorption of a uv-visible photon acquires a lot of excess *electronic* energy, and several mechanisms are available by which this energy can be lost rapidly, including energy transfer, inter-system crossing, photodecomposition and also *internal conversion*. In the latter process all the excess energy is lost as heat, a process which is facilitated by molecular collisions and larger amplitude vibrations. So as the temperature rises these non-fluorescent pathways become more likely and less fluorescence is observed. In some cases the effect can be quite large amounting to a 2 or 3% loss of fluorescence intensity for every ° C rise in temperature.

Outline answer to question 11.7

- A detector for nitrogen in gas chromatography eluants uses a temperature of 1800° C to convert all nitrogen compounds to NO, which is then oxidised by ozone to give chemiluminescent excited NO_2 molecules, as described in slide 48, with emission in the red and near infra-red spectral regions. Such detectors may be used in conjunction with a flame ionisation detector (f.i.d.), which provides the initial high temperature.

- The most desirable features of such detectors are that they respond equally to all nitrogen containing compounds, i.e. that they effectively respond to the mass of N present; and that their response is far greater towards nitrogen than to any other element. In practice a good nitrogen detector responds to N atoms at least 10 million times more strongly than to C atoms.

- A not dissimilar detector is available for sulfur atoms. In this case the flame ionisation detector (see Chapter 7) converts the sulfur-containing compounds to SO radicals, which are again oxidised, this time to excited SO_2 molecules which emit blue and ultra-violet chemiluminescence.