Introduction to Molecular Spectroscopy

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Analytical Chemistry Techniques: Overview

This is part two of four separate techniques guides that provide introductions to different aspects of analytical chemistry:

Part 1: Introduction to Analytical Chemistry:
A basic introduction to analytical science in general, and analytical chemistry in particular, including an overview of the analytical process, quality assurance, and guidance on solution preparation including volumetric and concentration calculations. It also includes a bibliography of useful texts for the analytical chemist.

Part 2: Introduction to Molecular Spectroscopy:
An introduction to the different types of molecular spectroscopic analysis, including UV-Vis, fluorescence, IR, MS and NMR, describing the basic principles of each technique and practical considerations including sample preparation. It is illustrated with simple diagrams, photographs of equipment and information to aid interpretation of spectra.

Part 3: Introduction to Chromatography:
An introduction to chromatographic analysis, describing the different types of chromatography, including TLC, GC, HPLC and ion chromatography, their application areas and basic principles of operation. It is illustrated with simple diagrams, photographs of equipment and chromatograms illustrating practical aspects of the technique.

Part 4. Introduction to Atomic Spectrometry:
An introduction to the different types of atomic spectrometric analysis, including ICP-AES, ICP-MS, XRF and AAS, describing the basic principles of each technique, their application areas and modes of operation, including practical comparison of the techniques. It is illustrated with simple diagrams and photographs of equipment.

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Chapter 1
Introduction

Molecular spectroscopy investigates and quantifies the response of molecules interacting to known amounts of energy (or frequency). Molecules have certain energy levels that can be analysed by detecting the molecule's energy exchange through absorbance or emission.

Table 1: Molecular spectrometric techniques and principal applications

Technique	Property measured	Applications
Ultraviolet-visible spectroscopy	Electronic molecular absorption in solution	Quantitative determination of unsaturated organic compounds and certain coloured inorganic species.
Fluorimetry	Absorbed radiation emitted at longer wavelengths	Routine quantitative organic analysis. More sensitive at lower concentrations than UV-Vis absorption spectrometry.
Infrared spectroscopy	Vibrational molecular spectroscopy	Identification of organic compounds.
Raman spectroscopy	Vibrational molecular spectroscopy	Identification and fingerprinting of organic compounds.
Nuclear Magnetic Resonance spectroscopy	Nuclear absorption (change of spin states)	Identification and structural analysis of organic compounds.
Mass spectrometry	Ionisation and fragmentation of molecules	Identification and structural analysis of organic compounds.

Chapter 2
UV-Vis Spectroscopy

Molecules absorb radiation in the UV-visible region due to the promotion of electrons in bonding orbitals or lone-pair electrons into anti-bonding orbitals. Absorption may also be associated with charge transfer processes whereby an electron is transferred from one part of a complex molecule to another. Crystal field splitting between energy levels in transition metal ions in solution or the solid state gives rise to the transitions that lead to the colours of transition metal ions.

Molecular absorption bands in the UV-visible region are typically very broad and featureless, compared with the sharp lines observed from electronic transitions in free atoms, as in atomic spectroscopy. The reason for this is that when a molecule in solution undergoes an electronic transition it typically simultaneously undergoes one of many possible vibrational transitions (and also rotational transitions, but these latter only occur in the gas phase). Pure vibrational transitions are typically of lower energy than electronic transitions (occurring in the IR region of the spectrum).

The UV-visible spectrum of a molecule would typically consist of a large number of individual sharp lines, and this can be seen to a limited extent for some aromatic molecules in non-polar solvents. However, solvent-solute interactions cause line broadening that results in adjacent lines merging together to give the typical very broad bands that characterise the UV-visible spectra of most molecules in solution.

The electronic transitions that occur in organic molecules are transitions of valence electrons between bonding, non-bonding and anti-bonding molecular orbitals.

Table 2: The main transitions that occur in organic molecules. [Kealey and Haines (2002)]

Transition	Associated with	Wavelength range	Intensity	Example	λmax (nm)	ε mol^{-1}dm^3 cm^{-1}
$\sigma\rightarrow\sigma^*$	All covalent bonds. (i.e. all single bonds)	190 nm and below	Intense	Ethane	135	Not determined
$n\rightarrow\sigma^*$	Covalent bonds adjacent to lone pair electrons	210 nm and below	Weak	Methanol	183	500
$\pi\rightarrow\pi^*$	All multiple (double and triple) bonds	200 nm upwards	Intense	Ethene	193	10,000
$n\rightarrow\pi^*$	Multiple bonds adjacent to lone pair electrons	280 nm upwards	Weak	Acetone	279	15

The most useful transitions for analysis are the intense $\pi\rightarrow\pi^*$ transitions and the weaker, but lower energy, $n\rightarrow\pi^*$ transitions. Hydrocarbons are widely used as solvents because they are transparent over the normal analytical range, having only high energy $\sigma\rightarrow\sigma^*$ transitions. (The energy required for the $\sigma\rightarrow\sigma^*$ transition is high, putting this transition in the far-UV region of the spectrum.)

- Unsaturated groups which give rise to absorptions involving π or π^* orbitals in the near-UV/visible region are known as **chromophores**.
- Saturated groups with non-bonding electrons that can give rise to transitions involving non-bonding orbitals are called **auxochromes**.

2.1 Colour Perception

The human eye can distinguish over 10 million colours that differ in some degree of hue, lightness, or saturation. The perception of colour is based upon a variety of physical, chemical, physiological and psychological processes. All substances absorb light to some extent. The light between 400 and 700 nm that is not absorbed by the substrate is either reflected or transmitted. Upon reaching the retina of the eye, the light causes a photochemical reaction, and a series of light dependent reactions takes place. By a transfer of information between the eye and the brain, this process results in visual perception.

The human eye perceives a solid that reflects either diffusely or completely all visible light as being white. On the other hand one that absorbs all light is black. Grey is caused by a constant fraction of white light being absorbed. These are ACHROMATIC colours. CHROMATIC colours show one or more absorption bands in the visible spectrum [See Table 3]

Table 3: Chromatic colours show one or more λmax in the visible spectrum.

Wavelength (nm)	Wavenumbers (cm^{-1})	Colour Absorbed	Complementary Colour
200-300 nm	50000-33333 cm^{-1}	Far Ultraviolet	Colourless
300-400 nm	33333-25000 cm^{-1}	Near Ultraviolet	Colourless
400-435 nm	25000-23000 cm^{-1}	Violet	Greenish yellow
435-480 nm	23000-20840 cm^{-1}	Blue	Yellow
480-490 nm	20840-20410 cm^{-1}	Greenish blue	Orange
490-500 nm	20410-20000 cm^{-1}	Bluish green	Red
500-560 nm	20000-17860 cm^{-1}	Green	Purple
560-580 nm	17860-17240 cm^{-1}	Yellowish-green	Violet
580-595 nm	17240-16810 cm^{-1}	Yellow	Blue
595-610 nm	16810-16530 cm^{-1}	Orange	Greenish-blue
610-750 nm	16530-13330 cm^{-1}	Red	Bluish-green
> 750 nm	<13330 cm^{-1}	Near infrared	Green

2.2 Chromophores

Chromophores are the unsaturated groups giving rise to transitions involving π or π^* orbitals. Absorption data for a range of chromophores. [See Table 4]

Table 4: Chromophores [Denney and Sinclair (1987)]

Chromophore	Example	λmax (nm)	ε ($mol^{-1}dm^3\ cm^{-1}$)	Transition
>C=C<	pent-1-ene	190	10,000	$\pi\rightarrow\pi^*$
-C≡C-	ethyne	173	6,000	$\pi\rightarrow\pi^*$
>C=O	propanone	188	900	$\pi\rightarrow\pi^*$
		279	15	$n\rightarrow\pi^*$
-C≡N	MeCN	<160	Not determined	$\pi\rightarrow\pi^*$
-N=N-	MeN=NMe	347	4.5	$n\rightarrow\pi^*$
$-NO_2$	$MeNO_2$	278	17	$n\rightarrow\pi^*$
-ONO	$C_5H_{11}ONO$	219	1,120	$\pi\rightarrow\pi^*$
		347	Not determined	$n\rightarrow\pi^*$
$-C_6H_5$	benzene	184	60,000	$\pi\rightarrow\pi^*$
		204	7,900	$\pi\rightarrow\pi^*$
		256	200	$\pi\rightarrow\pi^*$

2.3 Factors Affecting the Position of λmax

The position of λmax depends on a number of factors, including conjugation of chromophores and auxochromes, pH and solvent.

Conjugation

Conjugation occurs when you have systems containing alternate single and double bonds. There are many examples of the effect of conjugation of chromophores, and a few examples are given below. Conjugation leads to an increase in λmax, and usually an increase in ε.

Table 5: Effects of Conjugation on λmax. [Ewing (1985)]

Compound	Double bonds	λmax nm	ε $mol^{-1}dm^3\ cm^{-1}$	Band
Ethene	1	193	10,000	π→π*
1,3-Butadiene	2	217	21,000	π→π*
1,3,5-Hexatriene	3	253	50,000	π→π*
$C_{14}H_{16}$	6	360	10,000	π→π*
β-carotene	10	452	152,000	π→π*
Propanone	1	188	900	π→π*
		279	15	n→π*
But-1-ene-3-one	2	219	3,600	π→π*
		324	24	n→π*

Auxochromes

The Absorption maxima is shifted by AUXOCHROME saturated groups with lone pairs of electrons that are adjacent to the chromophore.

- π→π* transitions show a red, or bathochromic, shift (i.e. to longer λ)
- n→π* transitions show a blue, or hypsochromic, shift (i.e. to shorter λ)

Table 6: Effects of Auxochromes on λmax. [Silverstein et al. (1997)]

Compound	Auxochrome	λmax nm	ε $mol^{-1}dm^3\ cm^{-1}$	λmax nm	ε $mol^{-1}dm^3\ cm^{-1}$
Benzene	-	204	7,900	256	200
Chlorobenzene	-Cl	210	7,600	265	240
Phenol	-ON	210	6,200	270	1,450
Aniline	$-NH_2$	234	8,600	280	1,430
Thiophenol	SH	236	10,000	269	700

Solvent

Polar solvents usually cause a red shift for π→π* transitions and a blue shift for n→π* transitions, especially for ketones.

pH

Protonation/deprotonation can alter the degree to which non-bonding electrons are involved in π systems.

> E.g. UV-visible data for acids, phenols and anilines is usually given over two pH ranges because of the different absorption characteristics of RCO_2H/RCO_2^-, $PhOH/PhO^-$ and $PhNH_2/PhNH_3^+$ respectively. Aniline has a band in water with λmax at 234 nm. The lone pair on the N of aniline is conjugated with the π system, but on protonation the lone pair is lost from the π system, and λmax is shifted to 203 nm.

2.4 Instrumentation

Single Beam Spectrophometer

The principal components of a UV-Vis Spectrometer are:

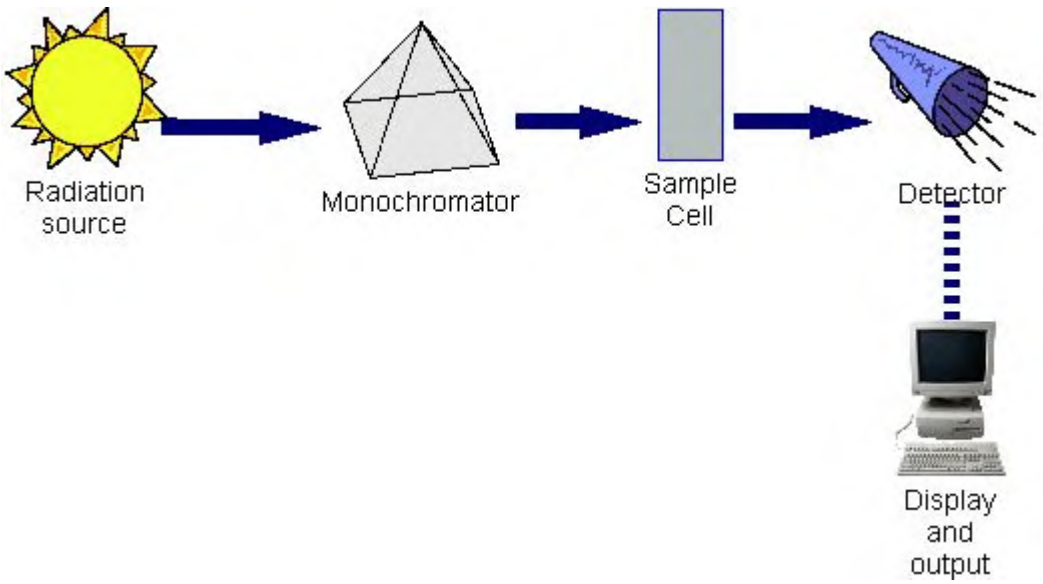


Figure 1: Basic construction of a spectrometer [Summerfield (2010)]

- Light source - either a Deuterium Lamp for the ultraviolet region (200-400 nm) or a Tungsten Lamp for the visible region (400-700 nm);
- Monochromator or filter to select the wavelength;
- Glass or plastic cells (for the visible region) or silica cells (for the UV region) of accurately known pathlength. Monochromatic light passes through the solution and the intensity of the beam through the solution is compared with a reference beam passing through air or a blank solution;
- Detector, either a photomultiplier or photodiode, which converts photons of radiation to electrical current';
- Output device to record the measured quantity.


Thermo Geneisis 10 Single Beam UV-Visible Diode Array Spectrophotometer.

Dual Beam Spectrophotometer

Shimadzu 1601 PC dual beam UV-Visible spectrophotometer

Temperature compensated sample cell with the reference (top) and sample (below)

2.5 Quantitative Analysis

Samples should be clear solutions because opaque solutions scatter the light.

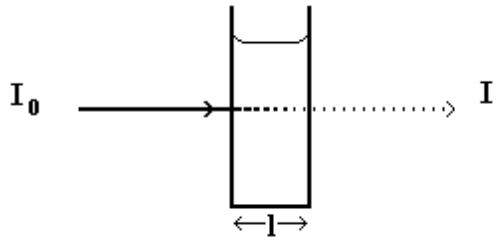


Figure 2: Light absorption through a cell.

In the diagram (Figure 2), the cell containing a solution through which a monochromatic beam of light is passing and being partially absorbed by the solution:

Where:

I_0 is the intensity of the incident beam

I is the intensity of the transmitted beam

l is the absorbing pathlength

c is the concentration of the absorbing species

Absorbance (A) and Transmittance (T) can be defined in terms of I_0 and I:

$$A = \log_{10}\left(\frac{I_0}{I}\right) \qquad T = \frac{I}{I_0} \times 100\%$$

When performing an analysis it is important that the standards and unknowns are analysed at the same wavelength, and are matched for solvent and, where necessary, pH, otherwise results will be erroneous.

The Beer-Lambert Law

The Beer-Lambert law connects concentration and light intensity as shown:

$$I = I_0 \times 10^{-\varepsilon c l}$$

Where

ε is the molar absorptivity (formerly known as the molar extinction coefficient),

l is path length.

Putting this equation together with that connecting absorbance and light intensity gives the familiar expression:

$$A = \varepsilon \times c \times l$$

Where

Molar absorptivity (ε) in $mol^{-1}\ dm^3\ cm^{-1}$,

Pathlength (l) in cm,

Concentration (c) in mol dm^{-3}.

Chapter 3
Scattering

When light of wavelength between 200 and 1000 nm is used to irradiate a sample, a number of phenomena may occur: Most of the photons pass straight through the sample but some are absorbed. Some of the photons are scattered by their collisions with other bodies.

3.1 Raman Scattering

Raman scattering involves a constant vibrational energy being added or subtracted from the incident photon and hence a subsequent shift in wavelength (Table 7). Raman scattering is sometimes confused with fluorescence. The intensity of Raman scattering is usually feeble compared with Rayleigh scattering. All solvents containing hydrogen atoms linked to either carbon or oxygen show a Raman band shifted approximately 3000cm^{-1} from the excitation radiation. Also Raman Scattering can be easily resolved at longer wavelength so there is less chance of mistaking a Raman peak as fluorescence as shown in Table 8.

Table 7: Raman scattering in various solvents at 313, 366, 405 and 436 nm.

Solvents	**Excitation Wavelength (nm)**				
	313	**366**	**405**	**436**	**NOTES**
Water	350	418	469	511	*Raman scattering*
Acetonitrile	340	406	457	504	*wavelength maximum*
Cyclohexane	344	409	458	499	*for a given wavelength*
Chloroform	346	411	461	502	

Table 8: Raman scattering in water between 200 and 750 nm.

Excitation	**Raman Scattering**
200 nm	215 nm
280 nm	310 nm
300 nm	335 nm
350 nm	398 nm
480 nm	575 nm
650 nm	838 nm
700 nm	923 nm
750 nm	1012 nm

3.2 Rayleigh-Tyndall scattering

occurs at the same wavelength as excitation and is due to elastic collisions. Rayleigh scattering is caused by solvent molecules and Tyndall scattering is caused by small-suspended particles in solution. The intensity of scatter perpendicular to the incident light beam is inversely proportional to the fourth power of the wavelength. Thus scattering of blue light (450 nm) as compared with red light (700 nm) is approximately six times greater.

Chapter 4
Fluorescence Spectroscopy

Fluorescence results from a three-stage process as illustrated by a simple electronic-state diagram (Jablonski diagram) shown in Figure 3.

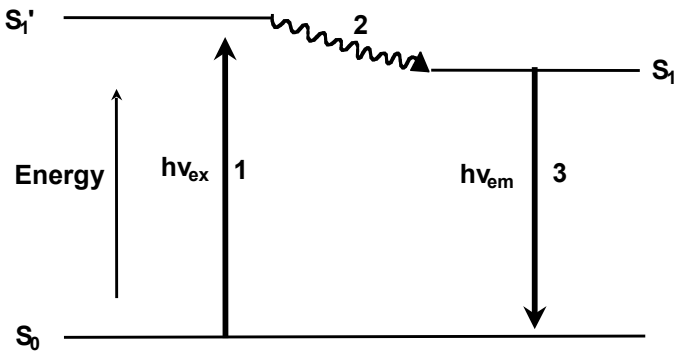


Figure 3: Jablonski Diagram of the processes involved in the creation of an excited electronic state by absorption and subsequent emission. Stages 1 to 3 are described in the text.

Stage 1: Excitation

A photon of energy ν_{ex} is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state (S_1'). When the molecule absorbs radiation, the electron is raised to an upper excited state.

Stage 2: Excited-State

This lasts typically 1-10 ns. The fluorophore undergoes conformational changes and a multitude of possible interactions with its molecular environment.

- The energy of S_1' is partially dissipated yielding a relaxed singlet excited state (S_1) from which fluorescence emission originates.
- Not all the molecules initially excited by absorption (Stage 1) return to the ground state (S_0) by fluorescence emission. Other processes such as collisional quenching, fluorescence energy transfer and intersystem crossing (see below) may also depopulate S_1.

Stage 3: Fluorescence Emission

A photon of energy $h\nu_{em}$ is emitted, returning the fluorophore to its ground state S_0. Due to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon $h\nu_{ex}$.

Other mechanisms of loss of excitation energy include photochemical decomposition (the destruction of the sample by incident radiation) and quenching.

Hitachi F4500 Spectrofluorimeter

4.1 Fluorescence Spectrum

The excitation spectrum is independent of fluorescence wavelength and should be identical to that of the absorption spectrum. However, this is seldom the case due to instrument artefacts.

The emission or fluorescence spectrum results from the re-emission of radiation absorbed by the molecule. This process is cyclical unless the fluorophore is irreversibly destroyed in the excited state by photo-bleaching. The quantum efficiency and the shape of the emission spectrum are independent of excitation. Figure 4 shows that if the exciting radiation is at a wavelength different from the absorption maxima, less radiant energy will be absorbed and hence less will be emitted.

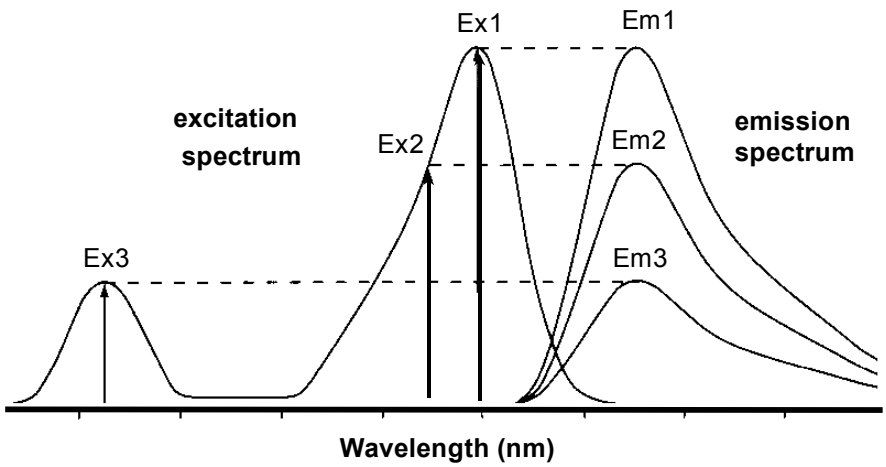


Figure 4: Excitation of a fluorophore at three wavelengths (Ex1, Ex2 and Ex3) does not change the emission profile but produces variations in fluorescence emission intensity (Em1, Em2 and Em3) that correspond to the amplitude of the excitation spectrum.

Stokes Shift

This is the difference in the excitation and emission wavelength maxima. This energy or wavelength difference [hv_{ex}-hv_{em}] is caused by the energy dissipated by non-radiative processes whilst in the excited state before its return to the ground state and is called the Stokes shift. This is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against a low background isolated from excitation photons. This contrasts with absorption spectrophotometry that requires the measurement of transmitted light relative to high incident light levels at the same wavelength.

4.2 Instrumentation

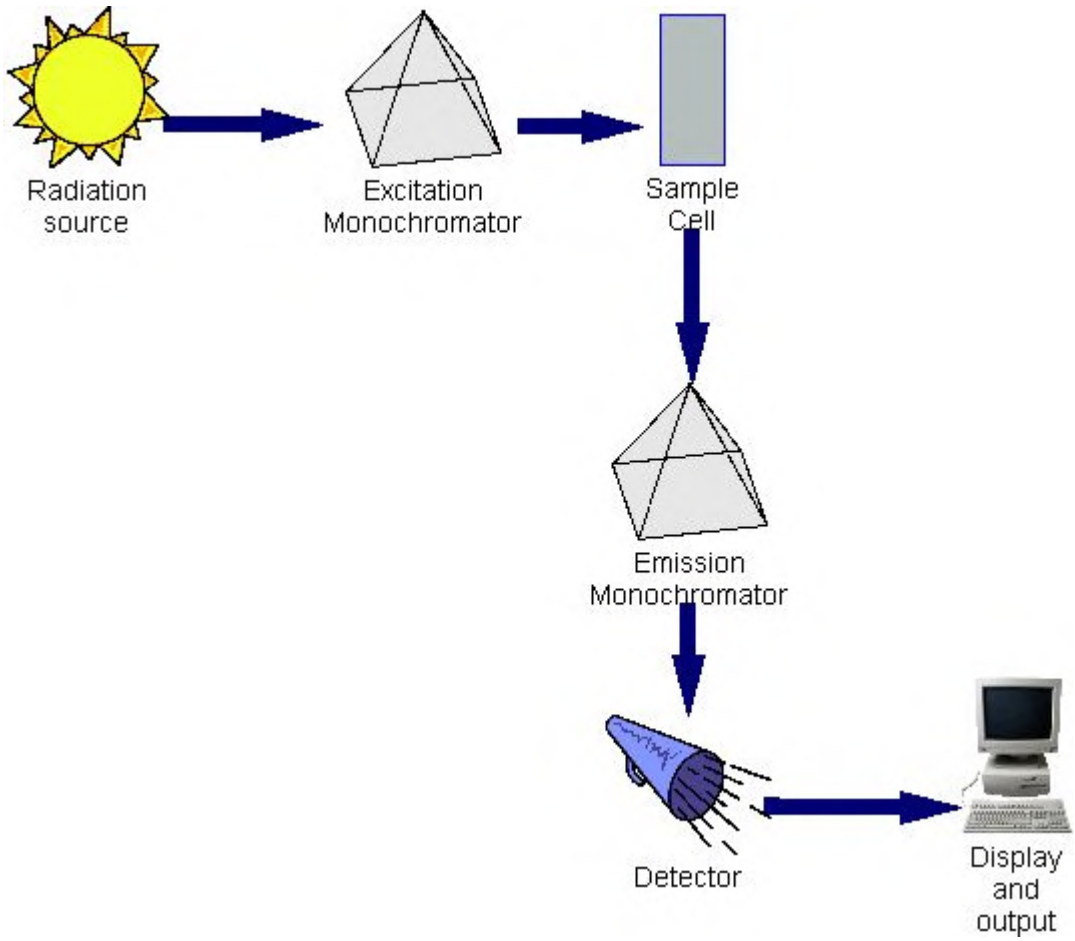
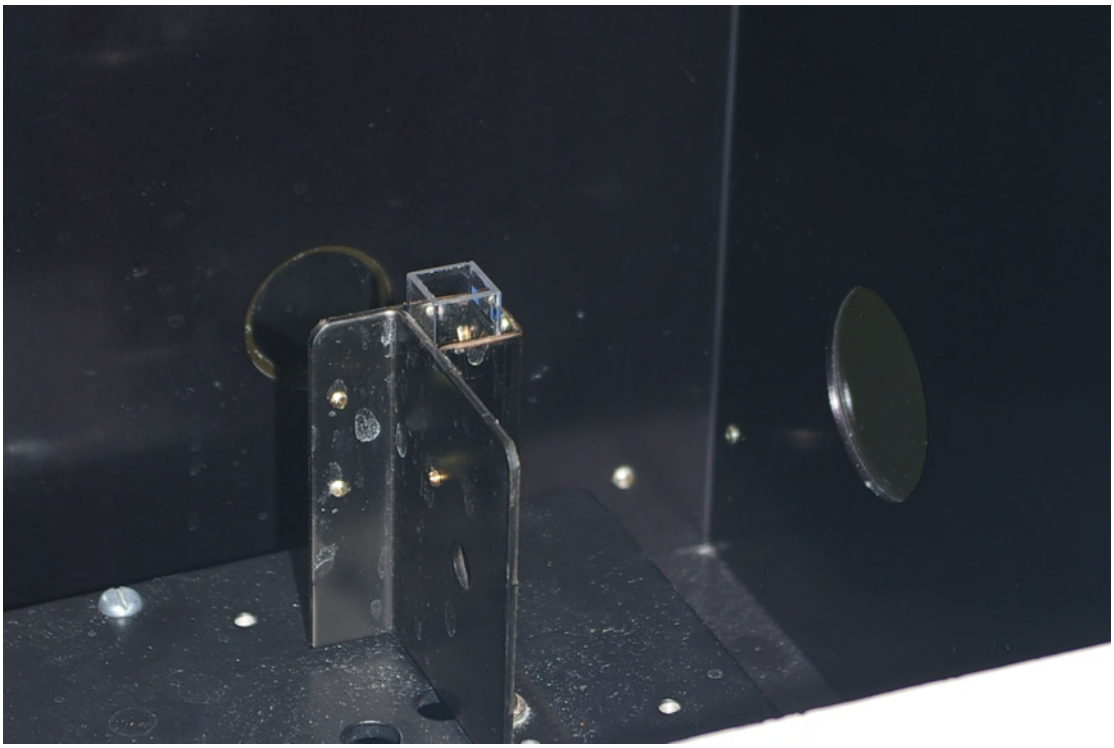


Figure 5: Schematic of a fluorimeter [Summerfield (2010)]

- Light source – Xenon Arc Lamp (200-800 nm);
- Monochromator or filter to select the wavelength;
- Glass or plastic cells (for the visible region) or silica cells (for the UV region) of accurately known pathlength.
- Monochromatic light passes through the solution and the intensity of the beam through the solution is compared with a reference beam passing through air or a blank solution;
- Detector, either a photomultiplier or photodiode, which converts photons of radiation to electrical current';
- Output device to record the measured quantity.

The measurement of emitted light (fluorescence) is always perpendicular (90 degrees) to the incident light so that the fluorescence is measured against a dark background to give maximum sensitivity.



Cell compartment with the light source (right), cell holder (centre) and detector (rear)

4.3 Characteristics of Fluorescence

Fluorescence is more sensitive than absorption methods because the signal is measured against a dark background and is proportional to the intensity of the incident radiation for dilute solutions. In solution, pg/ml levels can often be determined which are two to three orders of magnitudes better than absorption methods where the sensitivity is limited by the necessity of detecting a fractional decrease in the transmitted light.

Greater Selectivity over Spectrophometric Methods

1. A choice of both excitation and emission wavelengths permit simultaneous determinations of two constituents that emit / absorb at the same wavelength.
2. Fluorescence lifetime.
3. Fluorescence depolarisation.
4. Not all compounds that absorb light fluoresce.
5. Changing the pH would often shift the excitation and emission bands of the analytes under consideration.

Fluorescence Intensity

This is the product of the molar extinction coefficient, path-length, solute concentration, fluorescence quantum yield, intensity of light source and instrument collection efficiency. These factors combined into Equation 1 that is comparable to Beer's law if the absorptivity is below 0.05. [Equation 2]

$$F = 2.303 (\phi_f \times I_0 \times \varepsilon bc) \times \text{modular factors} \quad (1)$$

Where

F is the fluorescence intensity,

ϕ_f is the fluorescence quantum efficiency,

I_0 is incident power radiation,

b is the path length of cuvette in centimetres,

c is the analyte concentration in mol dm^{-3}

ε is molar absorptivity in $mol^{-1}cm^{-1}dm^3$.

Thus, fluorescence is increased with quantum efficiency, incident radiation, path length or molar absorptivity. Below 0.05 absorbance, the fluorescence intensity is linearly proportional to these parameters. Above 0.05 absorbance, the **inner filter effect** causes the relationship to become non-linear.

The modular factors are constant for a particular instrument. Equation 2 can be reduced to:

$$F = K \times \text{concentration} \quad (2)$$

K is a constant that includes all the other terms. This is only valid at low analyte concentrations. At higher concentrations, the inner filter effect causes the deviation from linearity.

Luminescent lifetime (τ)

This is the mean lifetime of the excited state and varies from 1-20ns for fluorescence (τ_f) and 0.001-10 seconds for phosphorescence (τ_p). The fluorescent lifetime is defined by the following.

$$F = F_o e^{t/\tau_f} \quad (3)$$

Where t is the time after removing the excitation source and F_0 is the maximum fluorescent intensity during excitation.

Fluorescence Quantum Yield

The fluorescence quantum yield (ϕ_f) is the ratio of the number of fluorescence photons emitted (Stage 3) to the number of photons absorbed (Stage 1) and is measure of the relative efficiency of the process.

$$\phi_f = \frac{\text{number of quanta emitted}}{\text{number of quanta absorbed}} \quad (4)$$

4.4 Background Fluorescence

The sensitivity of fluorescence detection is severely compromised by background signals. This originates from auto-fluorescence from endogenous sample constituents, reagent background or Raman scattering.

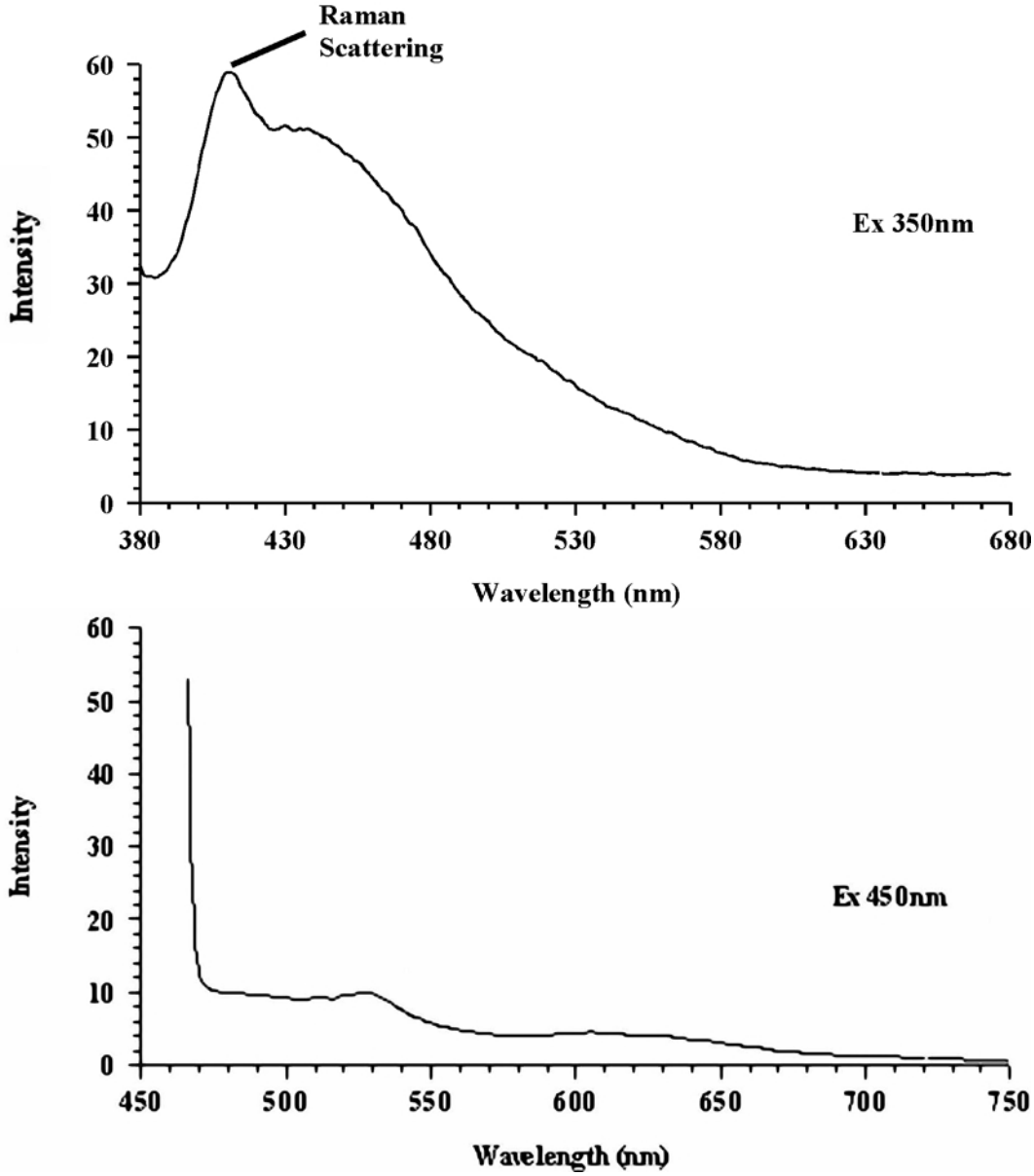


Figure 6: Background scattering and fluorescence of a 1% solution of human blood serum excited at 350 and 450 nm.
[Summerfield (1993) *Near Infrared Fluorescence,* Loughborough University.]

4.5 Fluorescence Quenching

Fluorescence quenching refers to any process, which decreases the fluorescence intensity of a given substance. Quenching may be either by a photophysical or photochemical pathway. Photochemical reaction causes photodegradation or photobleaching producing degradation products. Photophysical quenching produces new ground state products and can be divided into concentration quenching (the quenching species is itself "M"), and impurity quenching (another chemical species "Q"). Figure 4 summarises the various quenching processes.

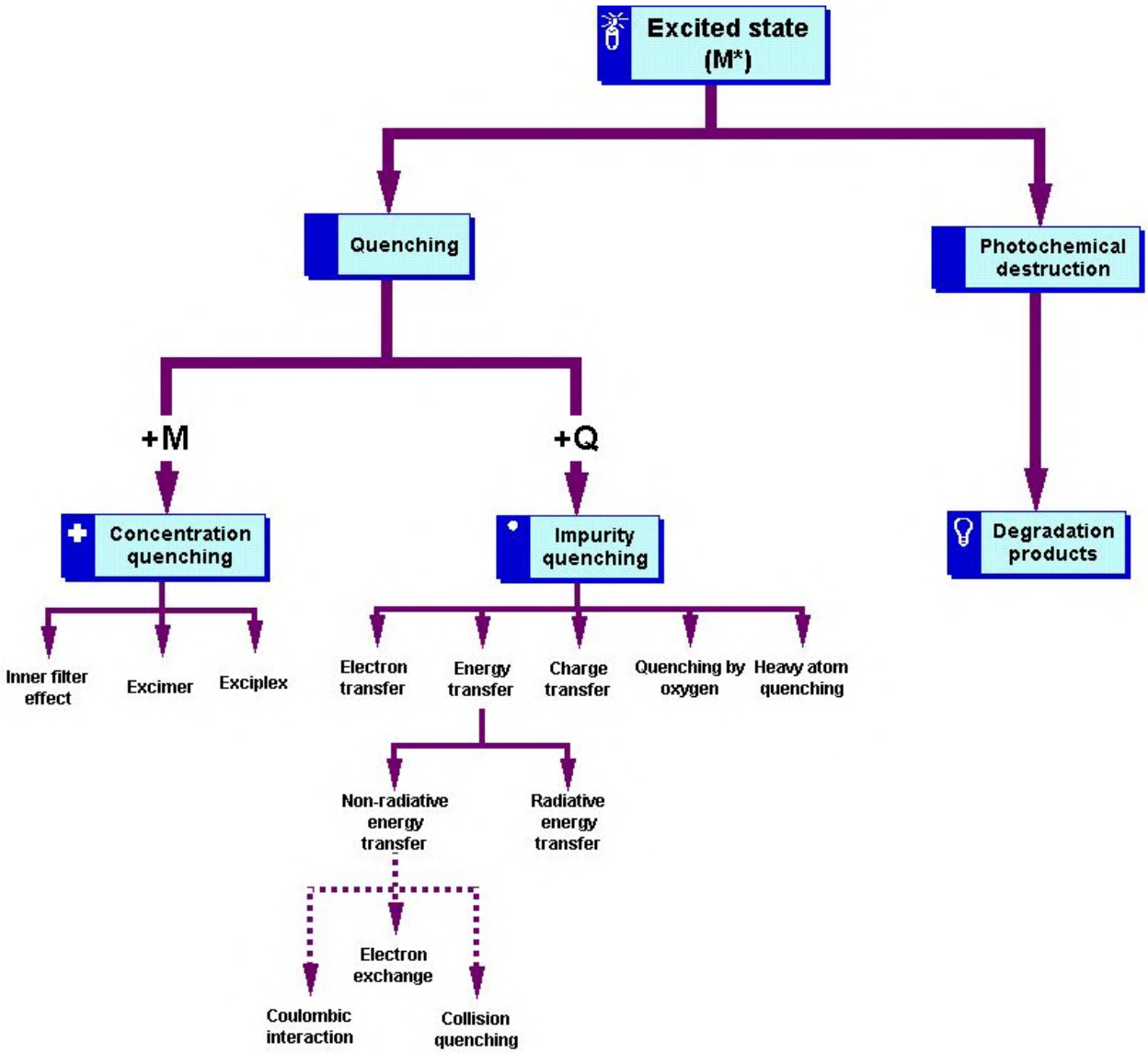


Figure 7: Quenching of the Excited state. [Summerfield (1993)]

The most important pathways are given below:
Inner Filter Effect is the re-absorption of light emitted from the fluorophore and is the major cause for the departure from the linear relationship of fluorescence intensity and concentration.

Charge Transfer takes place between the excited state species and the counter-ion. The tendency of charge transfer quenching of anions decreases as follows.

$$I^- > SCN^- > Br^- > Cl^- > ClO_4^-$$

The effect of the anion on fluorescence depends upon the concentration and polarity of the solvent.

Collisional Energy Transfer increases with increasing temperature caused by the increased collisions of the molecule in the excited state with vessel walls and other molecules.

Photobleaching or Photochemical Destruction occurs under high-intensity illumination conditions, the irreversible destruction or photobleaching of the excited fluorophore becomes the factor limiting fluorescence detectability. In all cases, photobleaching originates from the triplet excited state created from the singlet state (S1, Figure 3) via an excited-state process called intersystem crossing.

4.6 Environmental Influence on Fluorescence

Fluorescence spectra and quantum yields are more dependent on the environment than absorption spectra and extinction coefficients. A change in pH can ionise the fluorophore, commonly only one of the ionic forms is fluorescent, and so the control of pH is very important. Protonation has a higher rate constant than fluorescence so it is possible to observe the absorption spectrum of the neutral molecule and the fluorescence spectrum of the ionised molecule (e.g. α-naphthol at pH 3). Fluorophores that have strongly pH-dependent absorption and fluorescence characteristics can be used as physiological pH indicators. The pH sensitivity of the fluorophore is due to the reconfiguration of its π-electron system upon protonation.

An increase in **TEMPERATURE** usually reduces the fluorescence intensity because of the increased collisional quenching. In some cases, the temperature dependence of fluorescence is severe, as much as 5% per °C. The thermostatic control of the sample cell should be a routine precaution. An increase in viscosity of the solvent also increases the fluorescence, since the collisional interactions are reduced.

SOLVATOCHROMISM is the modification in shape, position or intensity of both the absorption and fluorescence bands by varying the solvent. The Franck-Condon principle states that an electronic transition takes place so rapidly that a vibrating molecule does not change its internuclear distance appreciably during the transition. Absorption (about 10^{-18} s) occurs before the molecule and the solvation sphere of solvent molecules arranged around it rearrange in about a picosecond from the Franck-Condon excited state to their new lower energy equilibrium excited state. Emission from this new equilibrium excited state occurs in 10ns.

- A **bathochromic (red) shift** to longer wavelength is observed, if the Franck-Condon excited state has a higher dipole than the ground state.
- A **hypsochromic (blue) shift** to shorter wavelength (also known as a blue shift) is observed, if the Franck-Condon excited state has a decreased dipole relative to the ground state. Figure 7 shows pictorially the influence of these shifts on the absorbance spectra.

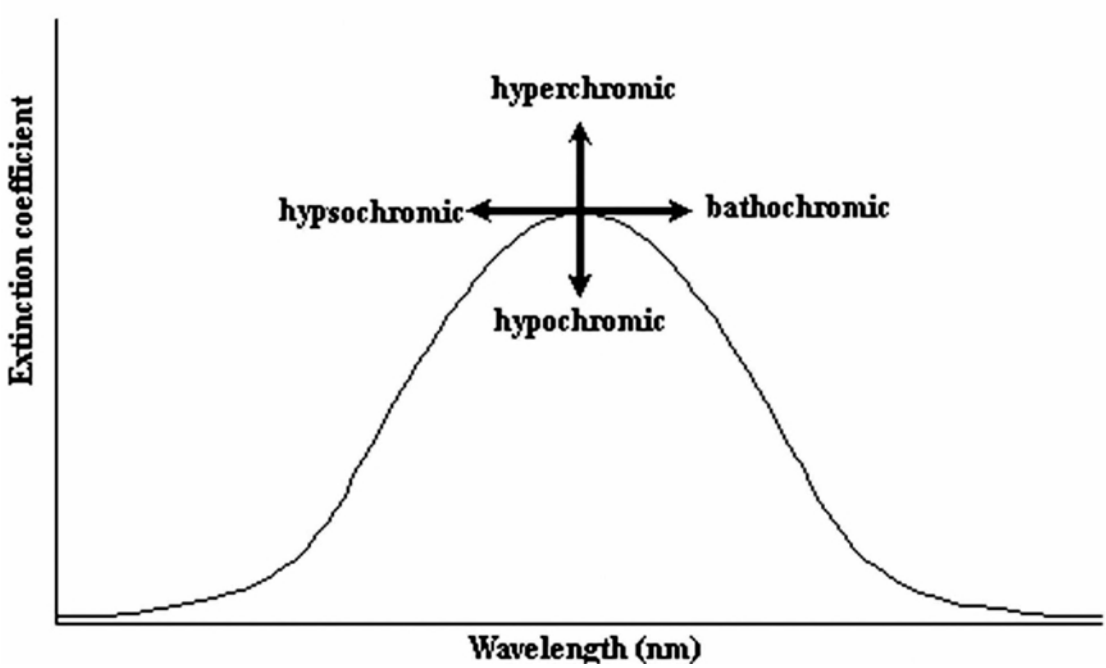
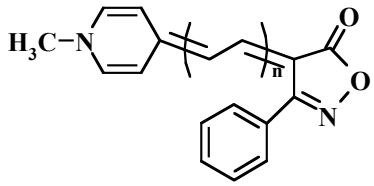


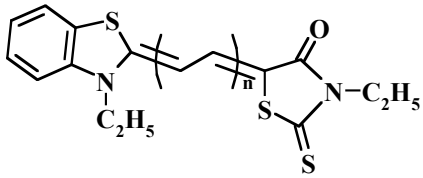
Figure 8: Shifts in maxima to longer and shorter wavelengths are called BATHOCHROMIC and HYPSOCHROMIC shifts respectively. An increase and decrease in the absorbance are termed HYPERCHROMIC and HYPOCHROMIC changes respectively.

Solvents influence the absorption spectra especially the merocyanines. Weakly polar merocyanines are red-shifted and show increased absorptivities as solvent polarity increases for example.



	n=0	n=1	n=2	n=3	pyridine:water
λabs (nm)	380	470	530	553	1:1
λabs (nm)	400	515	610	710	1:0

Highly polar merocyanines are blue shifted and show decreased absorptivities as solvent polarity increases.



	n=0	n=1	n=2	n=3	pyridine:water
λabs (nm)	432	540	633	733	1:1
λabs (nm)	432	528	605	635	1:0

Chapter 5
Infrared spectroscopy

Infrared spectroscopy permits a rapid, initial analysis of a substance sent for analysis and is an important step in the characterisation of molecules. Infrared spectroscopy is routinely used in the identification of paint and fibres, authentication of paintings, analysis of drugs, food, gemstones, ink, paper, and polymers.

IR spectroscopy is the measurement of the wavelength and intensity of the absorption of infrared radiation by a sample. Mid-infrared light (2.5 - 50 μm, 4000 - 200 cm^{-1}) does not have enough energy to bring about electronic transitions as encountered in UV-visible spectroscopy, but is energetic enough to excite molecular vibrations and rotations to higher energy levels. The wavelengths of IR absorption bands are characteristic of specific types of chemical bonds, and IR spectroscopy finds its greatest use for identification of organic and organo-metallic molecules.

There must be a change in dipole moment during the vibration or rotation for a molecule to absorb infrared radiation. For example, considering carbon dioxide:

symmetric stretch 1340 cm^{-1}		**asymmetric stretch 2350 cm^{-1}**
O=C=O		O = C=O
O==C==O	← Equilibrium position →	O==C==O
O === C === O		O=C====O
Infrared inactive		**Infrared active**

There is no change in dipole moment during the symmetric stretch vibration and the 1340 cm^{-1} band is not observed in the infrared absorption spectrum (the symmetric stretch is called infrared inactive). There is a change in dipole moment during the asymmetric stretch and the 2350 cm^{-1} band does absorb infrared radiation (the asymmetric stretch is infrared active).

Shimadzu FTIR-8300 Fourier Transform Infrared Spectrometer

5.1 Sample Preparation

The method of sample preparation is important as this can change the appearance of the spectrum. In each case an appropriate sample holder is used to place the prepared sample in the spectrometer. These methods are outlined below.

a) Liquid samples are conveniently analysed by placing a drop of the sample between a pair of NaCl or KBr discs and pressing them gently together.

b) Nujol is a saturated hydrocarbon that is a colourless, viscous liquid at room temperature. A solid sample is ground to a fine powder before mixing with Nujol to give a thick paste. A small amount of this paste is then placed between NaCl discs to give a thin film as in a). Nujol itself gives a few prominent bands in the IR spectrum, which must be allowed for in spectral interpretation.

c) A solid sample is ground to a fine powder then mixed with about a 10-fold excess of dry potassium bromide and the mixture ground to give a well-mixed powder. This is placed between two polished steel dies in a KBr disc press. Pressure of around 10 tons is applied under vacuum for about a minute to produce a thin transluscent disc. The KBr disc has the advantage that the spectrum should contain no interfering peaks (apart from water from poorly dried sample or KBr).

Infrared Press for producing KBr Disks.

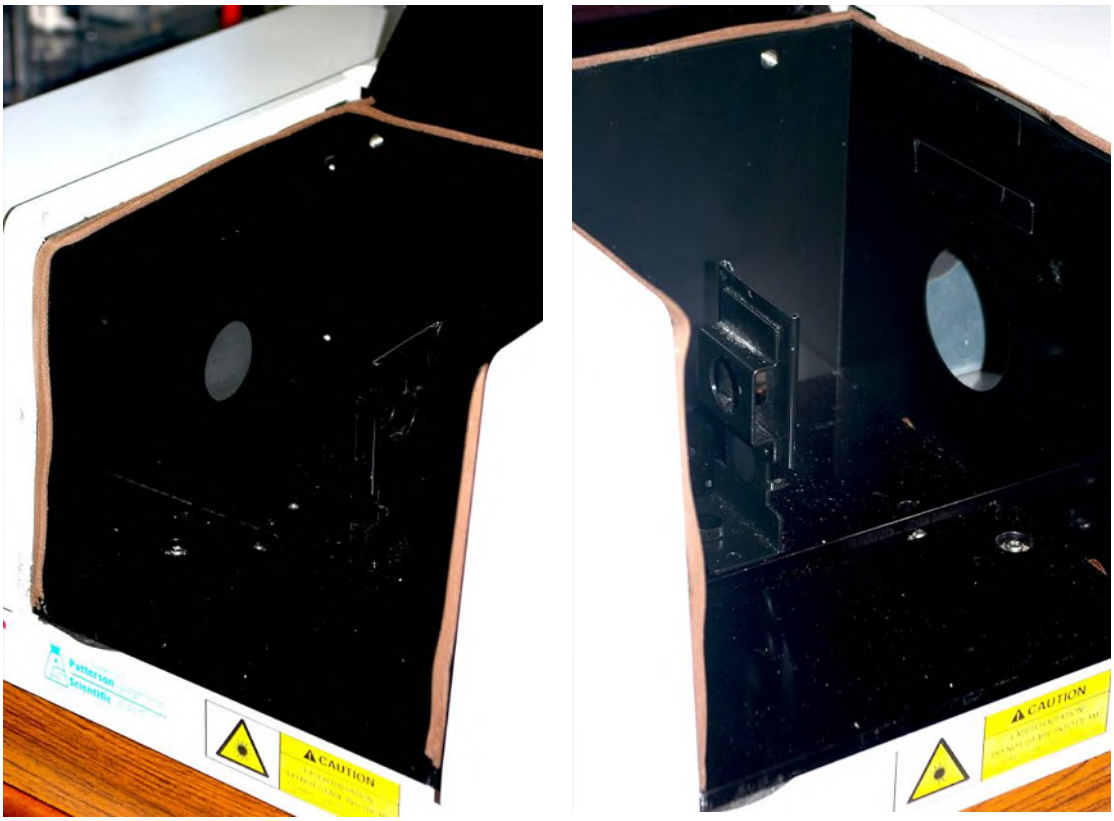
d) Photo-acoustic detection (PAS) is performed on solid (neat) samples. Useful for polymers and plastics.

e) Attenuated Total Reflectance (ATR) can be used for the direct analysis of solid samples and as a surface analysis of solid samples.

5.2 Practical considerations

A suitably prepared sample (see above) is placed in the beam of an infrared spectrometer and radiation from an electrically heated inert solid source is passed through the sample. In the case of a dispersive spectrometer, the wavelength of the radiation reaching the detector is selected by passing through a monochromator which uses a grating or prism to split the radiation into its component wavelengths - typically scanning from 4000 cm^{-1} down to 600 cm^{-1} (by contrast, Fourier-transform (FTIR) instruments contain no dispersing element, all wavelengths being detected and measured simultaneously). The intensity of the transmitted radiation at each wavelength is measured by a thermal detector, recorded and plotted as an infrared spectrum.

Infrared spectroscopy is often used for qualitative analysis, either to determine the structure of an unknown compound or to identify a compound by comparison of its spectrum with those of known compounds. Spectral databases are available on computer for searching and matching of spectra.



Cell compartment of the Shimadzu FTIR-8300 with KBr Disk holder.

5.3 Interpretation

The resulting spectrum can be interpreted in the following ways:

1. Comparing the sample and the standard spectrum of the compound e.g. pure drug or standard
2. Comparison of the fingerprint region (1500-450 cm^{-1}) to the standard or literature values
3. The principal peaks (strongest absorption) are tabulated and compared to literature values
4. The sample spectrum is compared to a library spectrum preferably by a computer but should be treated with caution and a reference spectrum should always be run.

Table 9: Some characteristic IR bands. [Williams and Fleming (1995)]

Compound Type	Bond	Wavenumber (cm^{-1})
alcohols, phenols and water	O–H	3600-3300
amines, amides	N–H	3500-3300
aromatic rings	C–H	3100-3000
alkanes	C–H	3000-2850
nitriles	C≡N	2280-2200
Aldehydes, ketones, esters	C=O	1760-1690
alkenes	C=C	1680-1610
amines, amides	C–N	1360-1180

Chapter 6
Mass Spectrometry

Mass spectrometry, in combination with chromatographic separation of the sample, is the primary tool for the determination of drugs in biological specimens. This has been important in workplace drug testing, testing motor vehicle operators for drug impairment and post-mortem toxicology.

In the mass spectrometer, the sample is ionised to produce a charged molecule (the mother ion) and a series of ionic fragments (daughter ions). The assortment of charged particles is then separated according to their mass to charge ratios (m/z) and displayed as a mass spectrum (see Figure 9).

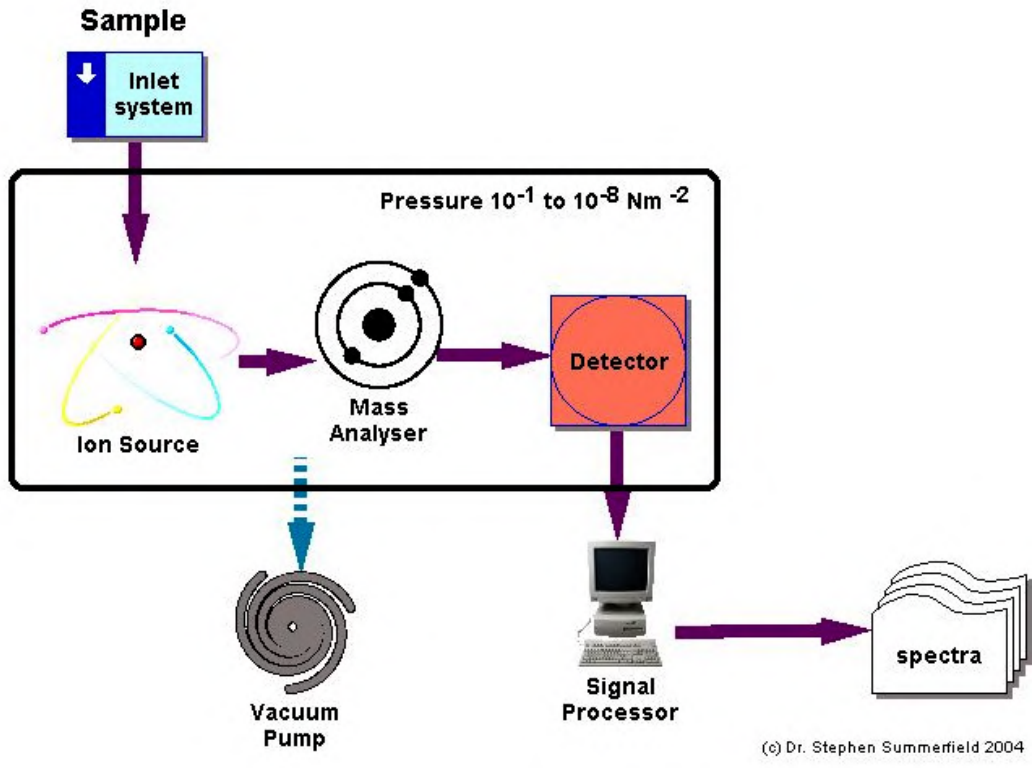


Figure 9: Diagram of a mass spectrometer

6.1 Ionisation Techniques

There are a number of means of ionising molecules or elements in a sample.

Condensed Phase Ionisation

These techniques enable the direct mass spectral analysis of untreated solids and liquids with minimal fragmentation.

- Matrix-Assisted Laser Desorption (MALDI)
- Electrospray Ionisation (ESI)

Ionisation in the gas phase.

Both Electron Ionisation (EI) and Chemical Ionisation (CI) are used for ionisation in the gas phase.

Electron Ionisation (EI) employs a high-energy electron beam (about 70 eV) to bombard the sample. Interaction between electrons and vaporised analyte molecules (M) initially results in the formation of molecular ions that then decompose into smaller fragments.

Chemical Ionisation (CI) produces less fragmentation than EI and the pseudo-molecular ion (MH^+) by collision between sample molecules and ions generated by a reagent gas (e.g. methane or ammonia).

For example, methane

a) Reagent gas ionised by EI $CH_4 + e^- \rightarrow CH_4^+ + 2e^-$
b) Secondary ion formation $CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$
c) Molecular ion formation $CH_5^+ + M \rightarrow MH^+ + CH_4$

6.2 Fragmentation

The production of a molecular ion is followed by its fragmentation into neutral species and ions of lower mass (Figure 10). The fragmentation pattern is particular to a compound. Generally, matching only the seven most abundant ions to a reference standard held in the library is sufficient for identification.

Ionisation $ABCD + e^- \rightarrow ABCD^+ + 2e^-$

Fragmentation
$ABCD^+ \rightarrow AB + CD^+$
$ABCD^+ \rightarrow AB^+ + CD$
$ABCD^+ \rightarrow ABC^+ + D$ etc…

Rearrangements
$ABC^+ \rightarrow CAB^+ + D \rightarrow C^+ + AB$
$ABC^+ + ABC \rightarrow (ABC)_2^+ \rightarrow ABCA^+ + BC$

Mass analysis & detection

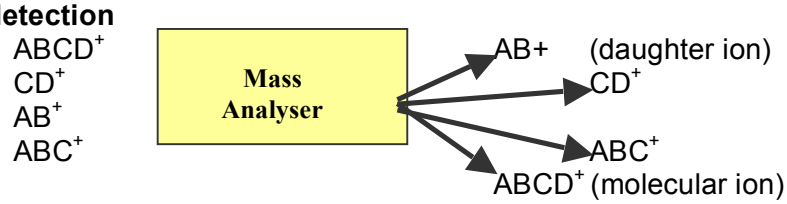


Figure 10: Summary of fragmentation caused by electron impact. [Schwedt (1997)]

6.3 Instrumentation

Quadrupole Mass Analyser

Four parallel metal rods have RF and DC voltages applied across opposite pairs of rods. For a given ratio of voltages, only ions of a certain m/z (mass to charge ratio) will reach the detector, so the mass spectrum is scanned by continuously varying the applied voltage, allowing rapid sequential detection across a range of m/z values, at a resolution of 300-1000.

TRIO-1 Quadrupole Mass Spectrometer

Double-Focussing Sector Field Mass Spectrometer

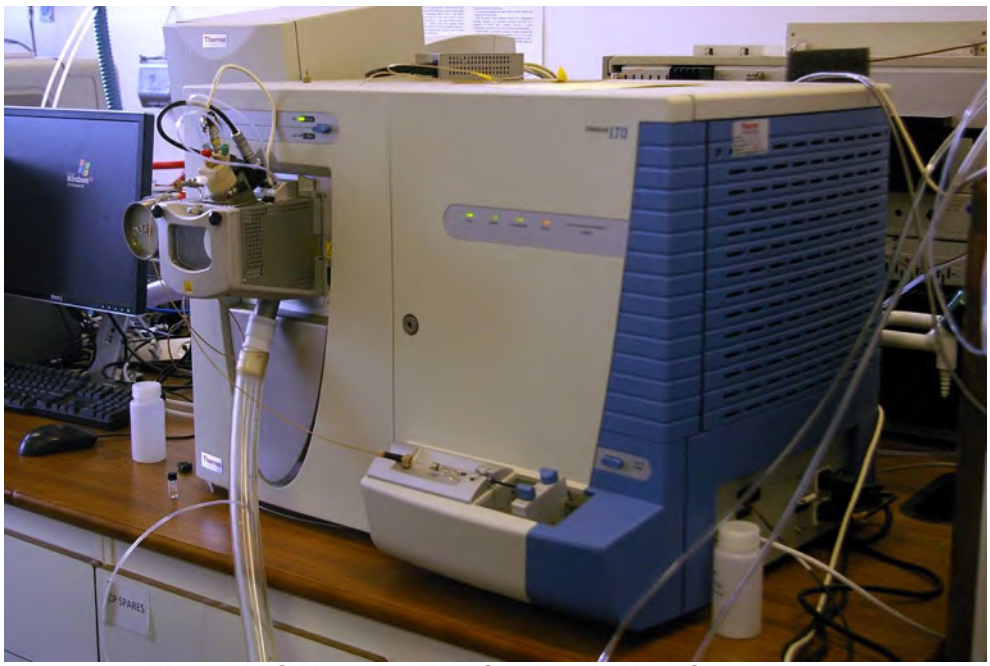
This incorporates magnetic and electrostatic analysers in sequence to separate ions of different m/z with very high resolution (~10,000), but is bigger, heavier and more expensive than the quadrupole system. Scan speeds are also slower.

Tandem Mass Spectrometry

The Tandem Mass Spectrometer (MS/MS) integrates chemical analysis, separation and identification into one instrument by using several mass analysers in series. The ions selected from the first analyser undergo collision induced dissociation (CID) with inert gas molecules within the collision cell, producing new ions that can be separated by the next mass analyser. This allows identification of drugs that traditionally has involved the screening and then confirmation, to be done simultaneously within seconds.

Quadrupole Ion-Trap Instruments

These instruments incorporate a ring electrode to trap ions then scan the mass spectrum ejecting ions from the trap by increasing the rf voltage. They have greater sensitivity than standard quadrupoles but a similar relatively low resolution (<1000).



Thermo LTQ Linear Ion Trap Quadrupole Mass Spectrometer

Time of Flight Mass Spectrometer (TOF-MS)

In this system, ions are accelerated along a flight tube towards the detector by application of an accelerating voltage. Ions are separated according to the time they take to reach the detector,

$$m/z = \frac{2\,U\,t^2}{d^2}$$

where U = accelerating voltage,
t = time
d = flight path distance.

This type of spectrometer has a resolution of 300 - 2000, giving a fast response. However, detection limits are poorer (by a factor of 10) than for a quadrupole.

6.4 Mass Spectra

Spectral data is either tabulated or shown graphically as a plot of the number of ions of each mass detected and for ease of interpretation these are represented as line diagrams. Mass spectral data can be used to provide the following information:

- Molecular mass (Mr) if the molecular ion (M) is identified
- Empirical molecular formula based on isotope peak intensities
- Fragmentation patterns gives identification of unknowns and structural features.

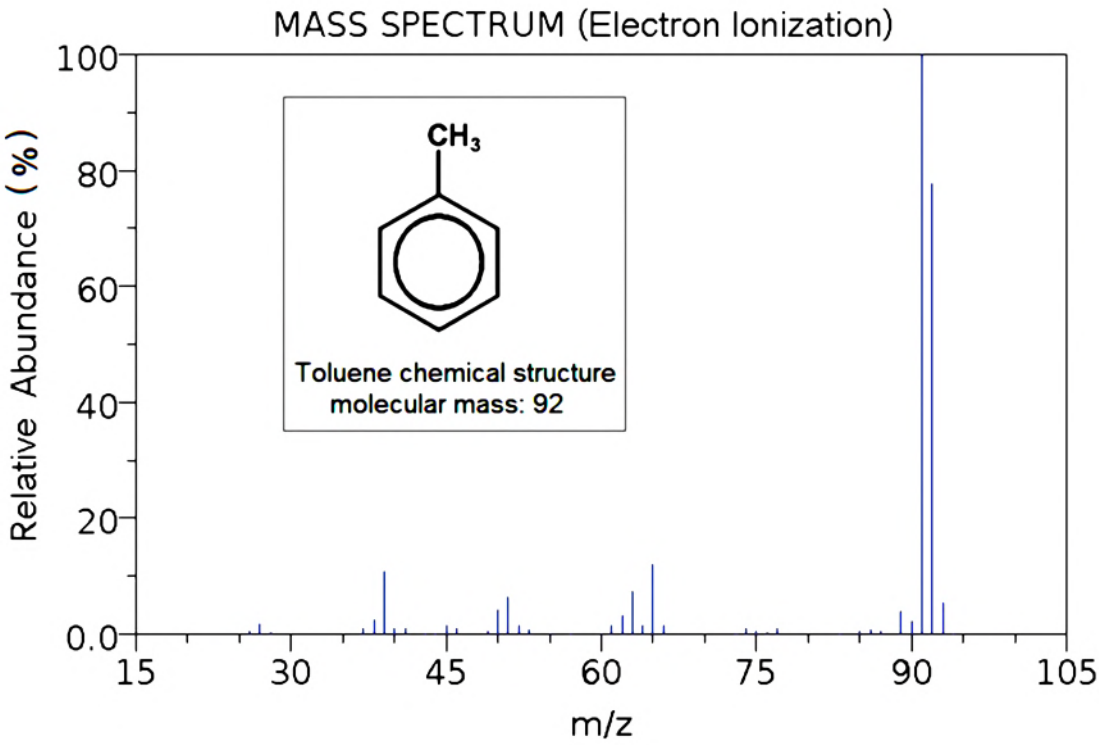


Figure 11: Mass Spectrum of toluene [after NIST]

6.5 Interpretation using empirical rules

- The intensity of the molecular ion decreases with increasing chain length and branching. An intense molecular ion indicates the presence of double bonds and ring structures tend to stabilise. Saturated rings lose side chains at the α-position.

- **Nitrogen Rule** – compounds with odd Mr have zero or even numbers of nitrogen atoms.

- **Unsaturated Sites Rule** – provides a means of calculating the degree of unsaturation.
  [No. of C atoms + ½ No. of N atoms]– [½ (No. of H + halogen atoms)] + 1

- Multiplets in the molecular ion region normally indicate the presence of heteroatoms characteristic of isotopic patterns.

	Ratio	Isotope
Silicon	100: 5: 4	^{28}Si: ^{29}Si: ^{30}Si
Sulphur	100: 4	^{32}S: ^{34}S
Chlorine	3:1	^{35}Cl: ^{37}Cl
Bromine	1:1	^{79}Br: ^{81}Br

- The C-C bond next to a heteroatom (N, O, and S) is frequently cleaved leaving the charge on the fragment containing the heteroatom whose non-bonding electrons provide resonance stabilisation.

- **McLafferty Rearrangements** in carbonyl compounds normally give rise to the elimination of a neutral molecule such as ethene.

- Small neutral molecules (including CO, C_2H_4, C_2H_2, H_2O and NH_3) are lost during fragmentation.

Table 10: Some common neutral losses from molecular ions

Mass	Associate with mass loss	Parent Compound
M-1	H	R-R, R=R, ROH
M-2	H_2	
M-14	CH_2	Homologue
M-15	CH_3	
M-16	O or NH_2	Nitro compounds, $ArCONH_2$, $ArSO_2NH_2$
M-17	OH or NH_3	ROH, ArCOOH: NH_4 salts
M-18	H_2O	Alcohol, ketone, ether
M-19	F	Fluoro compound
M-20	HF	Fluoro compound
M-26	C_2H_2	Aromatic hydrocarbon
M-27	HCN	N heterocycles
M-28	C_2H_4	Ethyl ester, aromatic ethyl ether, n-propyl ketone
M-33	SH	Thiol
M-35 & M-37	Cl	Chloro compounds
M-36 & M-38	HCl	Chloro compounds
M-45	COOH or C_2H_5O	Carboxylic acids, ethyl ester, ethyl ether
M-46	NO_2 or C_2H_5OH	Aromatic NO_2, ethyl ester, ethly ether

Table 11: Masses and possible compositions of common fragments

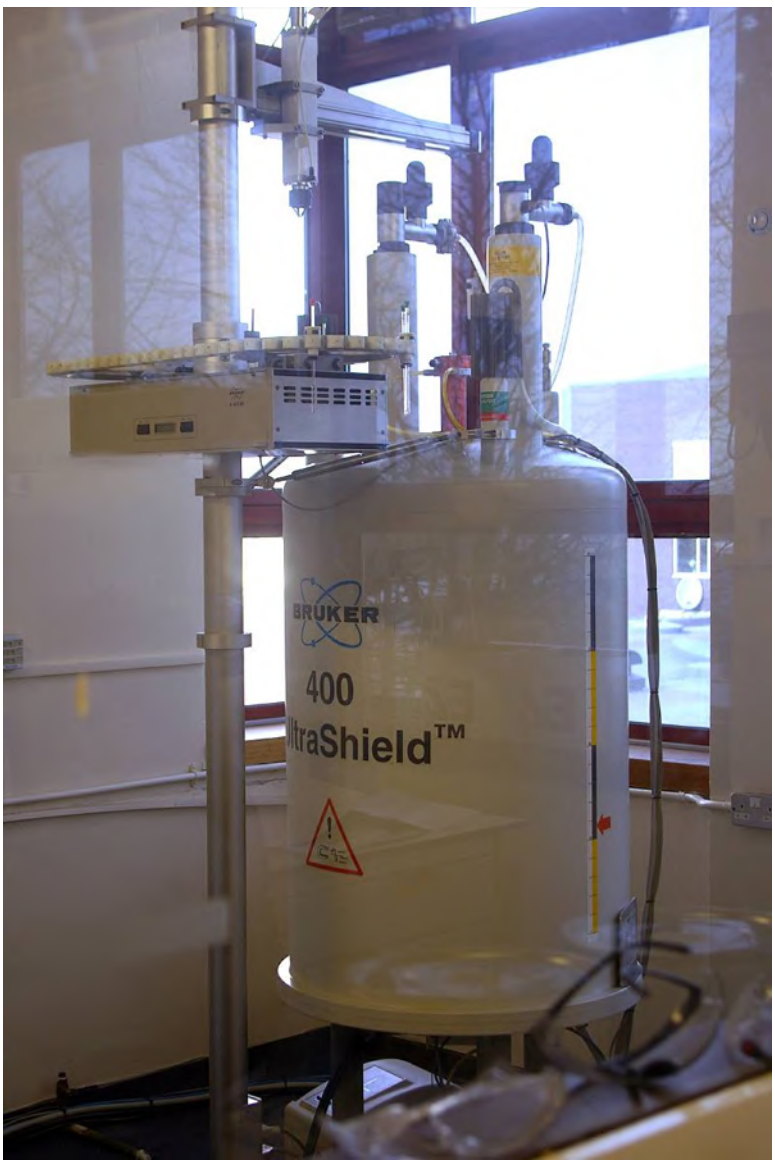
Mass	Associated with mass	Parent Compound
15	CH_3^+	
18	H_2O^+	
26	$C_2H_2^+$	
27	$C_2H_3^+$	
28	$C_2H_4^+$ or CO^+	
29	$C_2H_5^+$ or CHO^+	
49 & 51 (3:1)	CH_2Cl^+	Chloro compound
75	$C_6H_3^+$	Disubstituted benzene ring
76	$C_6H_4^+$	Benzene derivative
77	$C_6H_5^+$	Monosubstituted benzene
78	$C_6H_6^+$	Monosubstituted benzene
79 & 81 (1:1)	Br^+	Bromo Compound
80	$C_5H_6N^+$	Some pyridines, pyrroles, anilines
81 & 82 (1:1)	HBr^+	Bromo compound
83, 85 & 87 (9:6:1)	$CHCl_2^+$	Dichloro compound
91	$C_7H_7^+$	Alkyl substituted aromatic rings
91 & 93 (3:1)	$C_4H_8Cl^+$	Alkyl chloride
93 & 95 (1:1)	CH_2Br^+	Bromo compounds
106	$C_7H_8N^+$	Some pyridines or anilines
107	$C_7H_7O^+$	Some phenols
121	$C_8H_9O^+$	Some phenols and anisoles
127	I^+	Iodo compounds

- Background air shows up on the spectra at 28 (N_2^+), 32 (O_2^+), 40 (Ar^+), and 44 (CO_2^+).

Chapter 7
Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is used to determine the structures of materials such as new drugs and polymers. Magnetic resonance imaging (MRI) is also used in body scanning to diagnose and monitor conditions such as cancer and multiple sclerosis.

NMR spectroscopy gives information about the environment in which the nuclei of atoms are found in molecules. If a nucleus is in the presence of an external magnetic field it can align itself either with the external field (+) or against it (–). When a molecule is placed in a magnetic field, weak electric currents are induced in the electrons surrounding the nuclei. These currents flow in such a way as to produce a local magnetic field that reduces a fractionally smaller total field than the applied external field for the nuclei. This means that each nucleus experiences different magnetic environment and this gives rise to differences recorded in the spectra.



Bruker 400 NMR

7.1 Instrumentation

A superconducting magnet maintained at 4 K by liquid helium provides the field for the Fourier Transform (FT) NMR. The constant magnetic field excites all the nuclei at the same time by generating a range of frequencies in a pulse for several microseconds. The simultaneously excited protons emit radio frequencies and return to the equilibrium with their surroundings. The detected signal is a combination of the frequencies of each chemically distinct nucleus and is digitised on a computer. The data are Fourier transformed to extract the signal, the frequency and the intensity of each absorption to produce the NMR spectrum.

As the electron distributions around chemically different atoms in a molecule are not the same, this causes the induced fields to vary slightly in the presence of an external magnetic field. This effect is very small for hydrogen atoms and is expressed in parts per million (ppm). However, it is possible to measure these small differences, or 'chemical shifts'.

Inside an old Bruker NMR

7.2 The spectrum

An NMR spectrum does not display the absorption of energy against magnetic field or frequency. Instead, it is on a scale, δ, which increases from right to left. Peaks on this scale have the same value regardless of the magnetic field or frequency range of the instrument used because the chemical shift is induced by the applied field and is proportional to it. The δ values vary according to the chemical environment, and give an indication of the degree of shielding by the surrounding electrons experienced by the proton(s). Tetramethylsilane (TMS) is used to reference the signal to a zero and all other signals are relative to this standard.

Table 12: Typical proton shifts in an aliphatic environment

Methyl protons	δ	Methylene protons	δ	Methine protons	δ
CH_3–R	0.7–1.6	RCH_2–R	1.4	CH–R	1.5
CH_3–Ar	2.3	RCH_2–Ar	2.3–2.7	CH–Ar	3.0
CH_3–C≡N	2.0	RCH_2–C≡N	2.3	CH–C≡N	2.7
CH_3–C(=O)–R	2.2				
CH_3–C(=O)–O–R	2.0	RCH_2–C(=O)–R	2.4	CH–C(=O)–R	2.7
CH_3–C(=O)–Ar	2.6			CH–C(=O)–Ar	3.3
CH_3–C(=O)–O–Ar	2.4	RCH_2–C(=O)–Ar	2.9	CH–N–C(=O)–R	4.0
		$ArCH_2C(=O)R$	3.7		
CH_3–N–R	2.3	RCH_2–N	2.5	CH–OH	3.9
CH_3–N–Ar	3.0	RCH_2–N–C(=O)–R	3.2	CH–O–R	3.7
CH_3–N–C(=O)–R	2.9	RCH_2–Cl	3.6	CH–O–Ar	4.5
CH_3–O–R	3.3	RCH_2–Br	3.5	CH–O–C(=O)–R	4.8
CH_3–O–Ar	3.8	RCH_2–I	3.2	CH–Cl	4.2
CH_3–O–C(=O)–R	3.7	RCH_2–OH	3.6	CH–Br	4.3
CH_3–O–C(=O)–Ar	4.0–4.2	RCH_2–O–R	3.4	CH–I	4.3
		RCH_2–O–Ar	4.3		
		RCH_2–O–C(=O)–R	4.1		
		$ArCH_2$–O–C(=O)–R	4.9		

Bruker NMR

NMR controller

References

Dean J.R., Jones A.M., Holmes D., Reed R., Meyers J. and Jones A. (2002), *Practical Skills in Chemistry,* Prentice Hall.

Denney, R.C. and Sinclair R. (1987) *Visible and Ultraviolet Spectroscopy*, Open Learning.

Ewing G.A. (1985), *Instrumental Methods of Chemical Analysis,* 5th Ed, McGraw-Hill

Harris, Daniel C. (2006), *Quantitative Chemical Analysis*, 7th Edition, W. H. Freeman

Harwood L.M. and Claridge D.W. (1996), *Introduction to Organic Spectroscopy*, Oxford Chemistry Primer 43, Oxford University Press.

Kellner R., Mermet J.-M., Otto M. Widmer H.M. (1998) *Analytical Chemistry*, Wiley-VCH.

Kemp W. (1991), *Organic Spectroscopy,* 3rd Edition, MacMillan.

Lindon J.C., Tranter G.E. and Holmes J.L. (2000) *Encyclopaedia of Spectroscopy and Spectrometry*, Volumes 1-3, Academic Press.

Mermet, Jean-Michel, Otto, Matthias and Valcarcel Cases, Miguel (2005), Analytical chemistry : a modern approach to analytical science, 2nd Edition, Wiley-VCH

Meyers, R.A. (Ed.), (2000), *Encyclopaedia of Analytical Chemistry: Theory and Instrumentation,* Volumes 1-15, J. Wiley and Sons Ltd., London.

Rouessac F. and Rouessac A., (2000), *Chemical Analysis*, Wiley.

Schwedt G. (1997), *The Essential Guide to Analytical Chemistry,* John Wiley & Sons

Shugar G.J. and Dean J.A. (1990), *The Chemist's Ready Reference Handbook,* McGraw-Hill.

Silverstein R.M., Bassler G.C. and Morrill T.C. (1997), *Spectrometric Determination of Organic Compounds,* 6th Ed, Wiley, New York

Skoog, Douglas A. and Holler, F. James (2007), *Instrumental Analysis Principles*., 6th Edition, Brooks/Cole

Summerfield (1993) *Near Infrared Fluorescence*, Loughborough University.

Townshend A. (1995) *Encyclopaedia of Analytical Science*, Volumes 1-10, Academic Press, London.

Wayne R.P. (1994) *Chemical Instrumentation,* Oxford Chemistry Primers.

Whittaker D. (2000), *Interpreting Organic Spectra,* Royal Society of Chemistry.

Willard H.H., Merritt L.L., Dean J.A. and Settle F.A. (1988), *Instrumental Methods of Analysis,* 7th edition, Wadsworth Publishing Company, California.

Williams D.H. and Fleming I. (1995), *Spectroscopic Methods in Organic Chemistry,* McGraw-Hill.