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M.Sc. CHEMISTRY

III SEMESTER

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SPECTROSCOPY-APPLICATIONS IN ORGANIC AND INORGANIC CHEMISTRY

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SYLLABI – BOOK MAPPING TABLE
SPECTROSCOPY-APPLICATIONS IN ORGANIC AND INORGANIC CHEMISTRY

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UNIT I Basic Principles – electronic excitations-solvent effects - factors affecting position and intensity of absorption bands - instrumentation	**Pages 1-12**
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UNIT: I APPLICATIONS OF UV-VISIBLE SPECTROSCOPY

Structure

1.0 Introduction:

The molecular spectroscopy is the study of the interaction of electromagnetic waves and matter.

The scattering of sun's rays by raindrops to produce a rainbow and appearance of a colorful spectrum when a narrow beam of sunlight is passed through a triangular glass prism are the simple examples where white light is separated into the visible spectrum of primary colors. Thisvisible light is merely a part of the whole spectrum of electromagnetic radiation, extending fromthe radio waves to cosmic rays. All these apparently different forms of electromagnetic radiations travel at the same velocity but characteristically differ from each other in terms of frequencies and wavelength (Table 1).

Table1:The electromagneticspectrum

Radiationtype	Wavelength λ,(Å)		Frequency ν= c /λ, (Hz)	Applications
radio	$_{10}14$		$3 x10^4$	
Nuclearmagneticr esonan ce	10^{12}		$3 x10^6$	
Television	10^{10}		$3 x10^8$	Spinorientation
Radar	10^8		$3 x10^{10}$	
Microwave	10^7		$3 x10^{11}$	Rotational
Farinfrared	10^6		$3 x10^{12}$	Vibrational
Nearinfrared Visible	10^4 $8 x 10^3$-	$4 x 10^3$	$3 x10^{14}$ $3.7x10^{14}$-	
			$7.5x10^{14}$	
Ultraviolet	$3 x10^3$		$1 x10^{15}$	Electronic
X-rays	1		$3 x10^{18}$	
Gammarays	10^{-2}		$3 x10^{20}$	Nucleartransiti ons

	Cosmicrays	10^{-4}		3×10^{22}	

1.1Objectives

After going through this unit, you will be able to:

- To understand about the basic principles of UV-Visible spectroscopy
- To learn about various electronic transitions
- To understand the methods of determining the various factors affecting the position intensity of absorption band
- To learn about the basic principles and instrumentation

1.2. Basic principles

UV-Visible spectra arise from the transition of valency electrons with in a molecule or ion from a lower electrons energy level (Ground state E_0) to higher electronic energy level (Exited state E_1). This transition occurs due to the absorption of UV wavelength from 100 to 400 nm or visible wavelength from 400 to 750 nm region of the electronic spectrum by a molecule or ion.

The actual amount of energy required depends on the difference in energy between the ground state and the excited state of the electrons.

$$E_1 - E_0 = h\nu$$

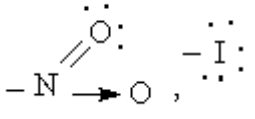
1.2.1 Color and light absorption- the Chromophore concept

Compounds that absorb light of wavelength between 400 to 800 nm (visible light) appear colored to the human eye, the precise color being a complicated function of which wavelengths the compounds subtract from white light. Many compounds have strong ultraviolet absorption bands, the shoulders of which may tail into the visible spectrum absorbing the violet end of the white-light spectrum. Subtraction of violet from white light leaves the complementary colors, which appear yellow/orange to the human eye, and for these reasons yellow and orange are the most common colors among organic compounds. Progressive absorption from 400 nm upward leads to progressive darkening through yellow, orange, red, green, blue, violet and ultimately black.

1. **Chromophores**

The presence of one or more unsaturated linkages (π-electrons) in a compound is responsible for the color of the compound, these linkages are referred to as Chromophores.

Example: C=C, - C ≡ C-, - C ≡ N-, - N=N-, C=O,



etc.,

Chromophores undergo $\pi \rightarrow \pi^*$ transitions in the short wavelength regions of UV-radiations.

2. Auxochrome

It refers to an atom or a group of atoms which does not give rise to absorption band on its own, but when conjugate to chromophore will cause a red shift.

Examples – OH, - NH_2, - Cl,- Br, - I etc.,

Laws of Light Absorption

(i) Lambert's Law

Lambert's law states that, "when a beam of monochromatic radiation is passed through a homogeneous absorbing medium the rate of decrease of intensity of the radiation 'dI' with thickness of absorbing medium 'dx' is proportional to the intensity of the incident radiation 'I".

It is mathematically expressed as

$-dI/dx = kI$ --------- (1)

Where, k = absorption coefficient.

On integrating the equation (1) between limits $I=I_0$ at x=0 and I=I at x=x, we get,

$$\int_{I_0}^{I} dI/I = -\int_{0}^{x} k dx$$

$$\mathbf{\ln I/I_0 = - kx}\text{---------} (2)$$

The above equitation (2) is known as Lambert's Law.

(ii) Beer's Law (or) Beer-Lambert Law

Beer extended the above equation (2) to solutions of compound in transparent solvent.

According to this law, " when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation 'dI' with thickness of the absorbing solution 'dx' is proportional to the intensity of incident radiation 'I' as well as the concentration of the solution 'C'

It is mathematically represented as,

$$-dI/dx = kIC \text{ ---------} (1)$$

Where, k= molar absorption coefficient.

On integrating the equation (1) between limits $I=I_0$ at x=0 and I=I at x=x, we get,

$$\int_{I_0}^{I} dI/I = -\int_{0}^{x} kC dx$$

I_0 0

$$\ln I/I_0 = - k Cx \text{ (or) } 2.303 \log I/I_0 = - kCx$$

$$\text{(or) } \log I_0/I = k/2.303 \text{ X } Cx$$

$$\text{(or) } A = \varepsilon Cx \text{--------- (2)}$$

where, $\varepsilon = k / 2.303$ = molar absorptivity coefficient

$\log = I_0/I = A$ = absorbance

This above equation (2) is called Beer-Lamber's law.

Electronic excitation

It is known that a bond formation between two atoms involves the overlap of two atomic orbitals each containing one electron leading to new molecular orbitals. One of them, which are lower in energy, is bonding molecular orbital and higher energy one is known as antibonding orbital. The former is filled with two paired electrons and the latter is supposed to be vacant. Some molecules have non-bonding orbital with valence electrons. The respective orbitals can be energy wise arranged as shown in figure 1.2.1.

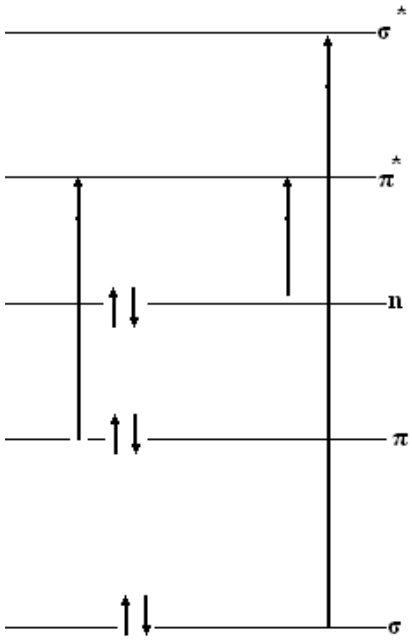


Figure 1.2.1: Energy levels of bonding, non-bonding and anti-bonding orbitals

σ, π and non bonding electrons are the occupying the respective bonding/non- bonding orbitals. On absorption of energy from UV or visible light, changes are produced in the electronic energy due to the transitions of electrons from one energy level to another. Thus promotion of an electron from π orbital to π* orbital is

designated by the notation: $\pi \rightarrow \pi^*$ likewise $\sigma \rightarrow \sigma^*$ etc can be defined. The most important transitions would appear to involve the promotion of one electron from the highly occupied molecular orbital to lower unoccupied orbital. But in many cases, several other transitions can also be observed.

In alkanes, the only possible transition is the promotion of electron from σ orbital to σ^* orbital. This is a high energy process and requires very short wavelength (140-150 nm). In simple alkenes, several transitions are available, but the lowest energy transition of $\pi \rightarrow \pi^*$ is important. This occurs around 170-190 nm in unconjugated olefin. The above two transitions occur out of UV radiation range and hence can not be studied.

In saturated aliphatic ketones and molecules with C=S and –N=N- functional groups, the lowest energy transition is n to π^* occurring around 280 nm. $n \rightarrow \sigma^*$ of saturated alcohols occurs at 180-185 nm, saturated amines at 190-220 nm, chlorides at 170-175 nm, bromides at 200-210 nm and iodides at 257 nm.

In conjugated dienes, the orbitals of the separate double bonds combine to form new orbitals, two bonding π_1, π_2 and two anti-bonding π_3 and π_4 (Fig. 1.2.2).

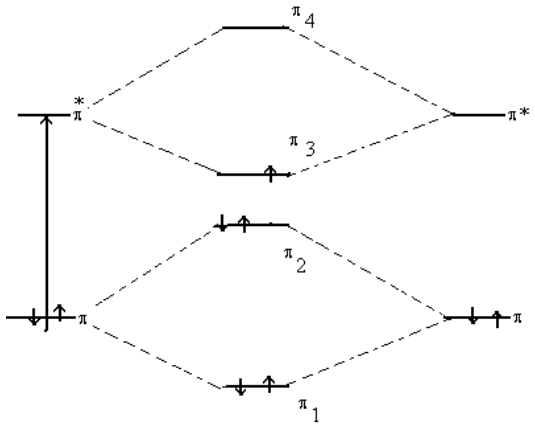


Figure 1.2.2: Comparison of transitions in ethylene and dienes

It is apparent that a new π_2 to π_3 transition is now possible as a result of this conjugation. Conjugated dienes therefore show absorption at much lower energy, at higher wavelength than the isolated alkenes, typical value being 215 nm. The same is the case with conjugated ketones for its $\pi \rightarrow \pi^*$.

1.3. Solvent effects

A most suitable solvent is one which does not itself absorb in the region under investigation. A dilute solution of the sample is always prepared for the spectral analysis. Most commonly used solvent is 95% ethanol. Ethanol is a best solvent as it is cheap and is transparent down to 210 mμ. Commercial ethanol should not be used as it contains benzene which absorbs strongly in the ultraviolet region. Some other solvents which are transparent above 210 mμ, are

n-hexane, methyl alcohol, cyclohexane, acetonitrile, diethyl ether etc. Some solvents with their upper wavelength limit of absorption are given in table 1.3.1.

3. Table 1.3.1: Solvents used in UV- spectroscopy

Solvent	Upper wavelength limit (mμ)
Ethanol	210
Hexane	210
Methanol	210
Cyclohexane	210
Diethyl ether	210
Water	205
Benzene	280
Chloroform	245
Tetrahydrofuran	220
Carbon tetrachloride	265

Hexane and other hydrocarbons can be used as these are less polar and have least intersections with the molecule under investigation. For ultra-violet spectroscopy, ethanol, water and cyclohexane serve the purpose best.

The position and the intensity of absorption maximum is shifted for a particular chromophore by changing the polarity of the solvent. By increasing the polarity of the solvent, compounds like dienes and conjugated hydrocarbons do not experience any appreciable shift. Thus, in general, the absorption maximum for the non-polar compounds is the same in alcohol (polar) as well as in hexane (non-polar). The absorption maximum for the polar compounds is usually shifted with the change in polarity of the solvents. α, β- unsaturated carbonyl compounds show the different shifts (Fig 1.3.1).

$n \rightarrow \pi^*$ **transition** (less intense).

In such a case, the absorption band moves to shorter wave-length by increasing the polarity of the solvent. In $n \rightarrow \pi^*$ transition, the ground state is more polar as compared to the excited state. The hydrogen bonding with solvent molecules takes place to lesser extent with the carbonyl group in the excited state. For example, adsorption maximum of acetone is at 279 mμ hexane as compared to 264 mμ in water.

$\pi \rightarrow \pi^*$ **transition** (intense). For such a case, the absorption band moves to longer wavelength by increasing the polarity of the solvent.

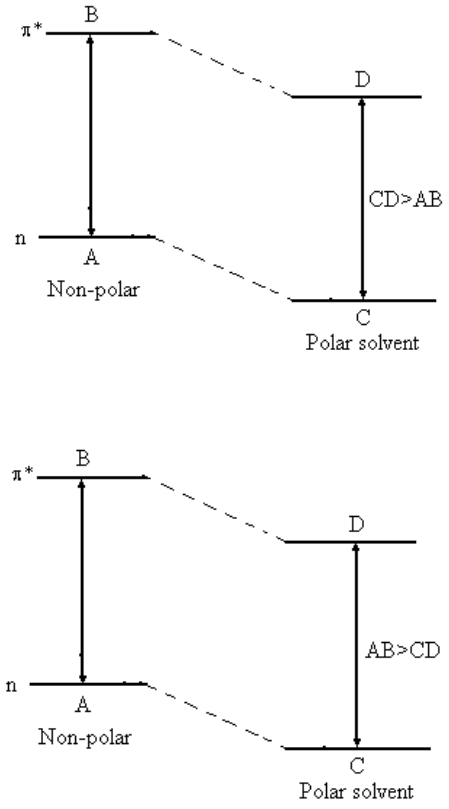


Figure 1.3.1: Absorption shift with change in polarity of the solvent

The dipole interactions with solvent molecules lower the energy of the excited state more than that of the ground state. Thus, the value of absorption maximum in ethanol will be greater than that observed in hexane.

In short, π^* orbitals are more stabilized by hydrogen bonding with polar solvents like water and alcohol. It is due to greater polarity of π^* orbital compared to π orbital. Thus, small energy will be required for such transition and absorption shows a red shift.

$n \rightarrow \sigma^*$ transitions are also very sensitive to hydrogen bonding. Alcohols as well as amines from hydrogen bonding with the solvent molecules. Such associations occur due to the presence of non-bonding electrons on the hetero atom and thus, transition requires greater energy.

In general, we say that

If the group (carbonyl) is more polar in the ground state in the excited state, then increasing polarity of the solvent stabilizes the non-bonding electron in the ground state due to hydrogen bonding. Thus, absorption is shifted to lower wavelength.

If the group is more polar in the excited state, then absorption is shifted to longer wavelength with increase in polarity of the

solvent which helps in stabilizing the non-bonding electrons in the excited state.

It has been found that increase in polarity of the solvent generally shifts $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ bands to shorter wavelengths and $\pi \rightarrow \pi^*$ bands to longer wavelengths. The following points may also be noted in connection with the effect of solvent polarity on the types of bands.

K-bond. The k-bond absorption due to conjugated 'enes' and 'enones' are effected differently by changing the polarity of the solvent. Usually, K-bonds due to conjugated dienes are not effected by changing the polarity of the solvent while these bands due to 'enones' show a red shift by increasing the polarity of the solvent.

R-band. The absorptions shift to lower wavelength (blue shift) with the increase in polarity of the solvent.

B-band. The position as well as the intensity of the B-band is not shifted increasing the polarity of the solvent. But in heterocyclic aromatic compounds, a marked hyperchromic shift (increase in ε_{max}) is observed by increasing the polarity of the solvent.

1.4. Factors affecting position and intensity of absorption bands

1. Bathochromic effect

It is an effect by virtue of which the absorption maximum is shifted towards longer wavelength due to presence of an auxochrome or by the change of solvent (Fig. 1.4.1). Such an absorption shift towards longer wavelength is called **Red shift** or bathoochromic shift. The $n \rightarrow \pi^*$ transition for carbonyl compounds experiences bathochromic shift when the polarity of the solvent is decreased.

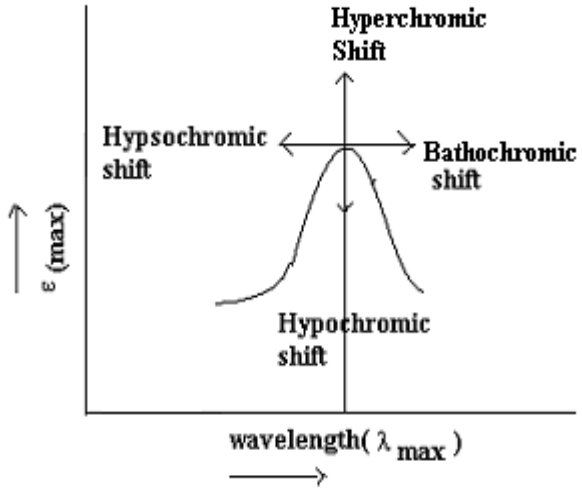


Figure 1.4.1: Absorption and intensity shifts.

2. Hypsochromic shift

It is an effect by virtue of which the absorption maximum is shifted towards shorter wavelength. The absorption shifted towards shorter wavelength is called **Blue shift** or hypsochromic shift.

It may be caused by the removal of conjugation and also by changing the polarity of the solvent. In the case of aniline, absorption maximum occurs at 280 μm because the pair of electrons on nitrogen atom is in conjugation with the π-bond system of the benzene ring. In acidic solutions, a blue shift is caused and absorption occurs at shorter wavelength (~ 203μm). In $C_6H_5\ NH_3^+$ ion formed in acidic solution, the electron pair is no longer present and hence conjugation is removed.

3. Hyperchromic shift

It is an effect due to which the intensity of absorption maximum increases i.e.,ε_{max} increases. For example, the B-band for pyridine at 257μm $\varepsilon_{max\ 2750}$ is shifted to 262μm ε_{max} 3560 for 2-methyl pyridine (i.e., the value of ε_{max} increases). The introduction of an auxochrome usually increases intensity of absorption.

4. Hypochromic shift

It is defined as an effect due to which the intensity of absorption maximum decreases, i.e., extinction coefficient, ε_{max} decreases. The introduction of group which distorts the geometry of the molecule causes hypochromic effect. For example, biphenyl absorbs at 250μm ε_{max} 19000 whereas 2-methyl biphenyl absorbs at 237 μm ε_{max} 10250 [ε_{max} decreases]. It is due to the distortion caused by the methyl group in 2-methyl biphenyl.

1.5. Instrumentation

The various components of a UV-visible spectrometer are given in the figure 1.5.1.

1. Radiation source

In UV-visible spectrometers, the most commonly used radiation sources are hydrogen (or) deuterium lamps.

Requirements of a radiation source

i. It must be stable and supply continuous radiation

ii. It must be of sufficient intensity

2. Monochromator

The monochromator is used to disperse the radiation according to the wavelength. The essential elements of a monochromator are an entrance slit, a dispersing element and an exit slit. The dispersing element may be a prism or grating (or) a filter.

3. Cells (Sample cell and Reference cell)

The cells, containing samples or reference for analysis, should fulfill the following conditions.

They must be uniform in construction

i. The material of construction should be inert to solvents

ii. They must transmit the light of the wavelength used

4. Detectors

There are three common types of detectors used in UV- visible spectrophotometers. They are Barrier layer cell, photomultiplier tube, and photocell. The detector converts the radiation, falling on which, into current. The current is directly proportional to the concentration of the solution.

5. Recording system

The signal from the detector is finally received by the recording system. The recording is done by recorder pen.

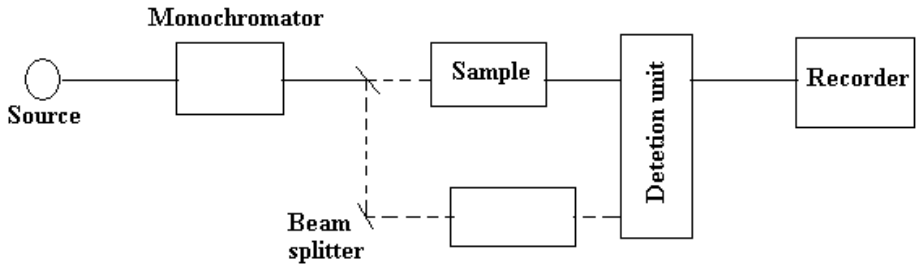


Figure 1.5.1: Block diagram of UV-Visible spectrophotometer

i. Working of UV-Visible spectrophotometer

The radiation from source is allowed to pass through the monochromator unit. The monochromator allows a narrow range of wavelength to pass through an exit slit. The beam of radiation coming out of the monochromator is split into two equal beams. One-half of the beams (the sample beam) are directed to pass through a transparent cell containing a solution of the compound to be analyzed. The another half (the reference beam) is directed to pass through an identical cell that contains only the solvent. The instrument is designed in such a way that it can compare that intensity of the two beams.

If the compound absorbs light at a particular wavelength, then intensity of the sample beam (I) will be less than that of the reference beam (I_0). The instrument gives output graph, which is a plot of wavelength Vs absorbance of the light. This graph is known as an absorption spectrum.

1.4 Check your progress questions:

1. Define Beer lambert law.
2. Discuss the basic principle and instrumentation of UV-Visible spectroscopy.
3. Explain how various solvents affects the UV-visible spectrum.
4. Discuss the various transition occurred in organic molecules.
5. Discuss the various factors affecting position and intensity of absorption bands

1.5Answers To Check Your Progress Questions

1. Beer Lambert Law

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:

$A = \lambda \times b \times c$

where A is the measured absorbance, λ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:
A = *epsilon* * b * c

where *epsilon* is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

2. Limitations of the Beer-Lambert law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:
deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximityscattering of light due to particulates in the sample fluorescence or phosphorescence of the samplechanges in refractive index at high analyte concentrationshifts in chemical equilibria as a function of concentrationnon-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption bandstray light

1.6 Summary

1. Inorganic chemists always using UV-Visible spectroscopy for analyzing the photophysical properties of inorganic complex/organic molecules.
2. It helps to understand the interaction between the molecule and solvents.
3. *UV-Vis* is a fast, simple and inexpensive method to determine the concentration of an analyte in solution. It can be used for relatively simple analysis, where the type of compound to be analyzed ('analyte') is known, to do a quantitative analysis to determine the concentration of the analytes.

1.7 Keywords

Solvent Effect: Highly pure, non-polar solvents such assaturated hydrocarbons do not interact with solute moleculeseitherinthegroundorexcitedstateandtheabsorptionspectrumofacompoundin thesesolventsissimilartotheoneinapuregaseousstate**.**

Beer Lambert Law: The attenuation of light to the properties of the material through which the light is travelling. The law is commonly applied to chemical analysis measurements and used in understanding attenuation in physical optics, for photons, neutrons, or rarefied gases.

1.8 Self-assessment questions and exercises

1. What is the basic principle of UV-Visible spectroscopy?
2. What is the range of UV visible spectroscopy?

3. Who discovered UV spectroscopy?
4. Discuss in detail about basic principle, instrumentation and applications of UV-Visible spectroscopy.

1.9 Further readings

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UNIT: II: APPLICATIONS OF UV-VISIBLE SPECTROSCOPY

Structure

2.0 Introduction:

Derivative spectra can be used to enhance differences among spectra, to resolve overlapping bands in qualitative analysis and, most importantly, to reduce the effects of interference from scattering, matrix, or other absorbing compounds in quantitative analysis. Although UV-visible spectra do not enable absolute identification of an unknown, they frequently are used to confirm the identity of a substance through comparison of the measured spectrum with a reference spectrum. Where spectra are highly similar, derivative spectra may be used. This increase in complexity of the derivative spectra can be useful in qualitative analysis, either for characterizing materials or for identification purposes.

2.1 Objectives:

- Understand about the applications of UV-visible spectroscopy and their qualitative and quantitative analysis
- Calculate the maximum absorption of UV-visible in organic molecules.
- Understand about various spectra of dienes, unsaturated ketones and aromatic compounds
- Understand the Woodward Fisher rule and their uses.
- Understand the charge transfer spectra.

2.2 Applications of UV-Visible Spectroscopy

UV-visible spectroscopy has been mainly applied for the detection of functional groups (chromophore), the extent of conjugation, detection of polynuclear compounds by comparison etc. Some important applications of ultraviolet spectroscopy are as follows:

1. Qualitative analysis

Ultra violet visible spectra provide a useful source of supporting evidence in the elucidation of structure of organic compounds. Moreover, selective absorption also serves as an identifying finger print for a particular structure in many cases.

Example: If there is no appreciable absorption in the region 270 to 280 nm, then the compound does not contain a benzene ring. Similarly, the compound contains no conjugated unsaturation, if there is no absorption from about 210 nm to the visible. Isolated double bonds must be absent if transparency extends down to 180 nm.

The pK_a value of indicator can be determined successfully spectrum of an acid base indicator as a function of pH.

In figure 1.6.1 are plotted the absorption curves for phenol red at a series of pH values. It is evident that absorption at λ 615 nm increases with increasing pH, while lesser absorption at λ 430 decreases. It is also evident from the plot that various curves cross very nearly at a common point at λ 495. This point is called isoabsorptive point or isobestic point and is characteristic of a system containing two chromophores which are interconvertible.

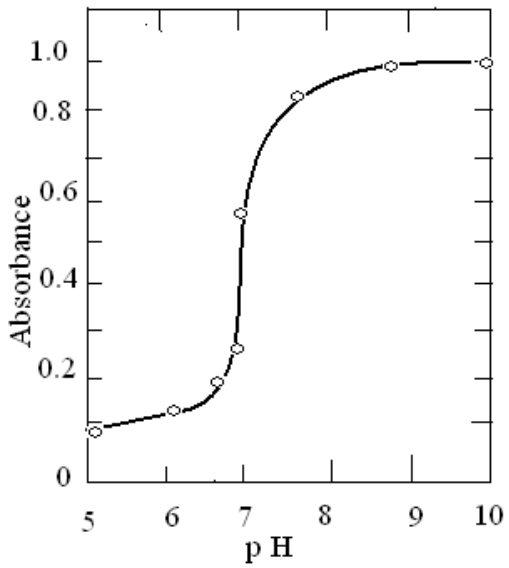


Figure 2.2.1.1 Absorption curves for qualitative analysis.

The S-shaped curve is obtained if we plot absorbance at λ 615 against pH. The horizontal portion to the left corresponds to the acidic from of phenol red indicator, while the upper portion to the right corresponds to nearly complete conversion to the basic form.

Since pK may be defined the pH value for which one half of the indicator is in the acid form and one half in the basic form, it may be determined by a point mid-way between the left and right horizontal segments. The dissociation constants of compounds with absorption in the ultra violet rather visible can often be determined similarly.

Examples: Theobromine (λ 240 nm) and benzotrizole (λ 274). Data in such cases are usually plotted in three dimensions and the presentation is called stereospectrogram in which three axes refer to wavelength, pH and absorbance.

The application of ultra violet and visible spectroscopy to qualitative analysis is more limited because the absorption bands tend to be broad and hence details are lost. However, special investigations in this region provide useful qualitative information regarding the presence or absence of functional groups such as carbonyl, nitro, aromatic, aldehydes, or conjugated dienes in organic compounds. Highly absorbing impurities in non-absorbing media can also be detected. For example, if an absorption peak for the contaminant has a sufficiently high absorptivity, the presence of trace amounts can be readily established.

Certain non-absorping organic functional groups are also determined by absorption methods. For example, low molecular weight aliphatic alcohols react with cerium (IV) to produce a red 1:1 complex that can be saved used for analytical purposes.

2. Quantitative analysis

Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis. The most important characteristics of photometric and spectrophotometric methods are their wide applicability, high sensitivity, moderate to high selectivity, good accuracy and ease of convenience. A wide variety of inorganic and organic species absorb in the ultra violet and visible region and thus susceptible to quantitative determinations. Even many non-absorbing species can be analyzed after converting them to absorbing species by making use of suitable chemical treatment. Molar absorptivities in the range if 10,000 to 40,000 are common specially for charge transfer complexes of inorganic species. Thus absorption methods are very sensitive and analysis in the concentration range of 10^{-4} to 10^{-5} can be easily performed. The range can be extended to 10^{-6} to 10^{-7} M by modifying the procedure. It is also possible to locate a wavelength region in which the only absorbing component in the sample is the substance being determined. Corrections based on additional measurements at other wavelengths are also possible in those cases where overlapping absorption bands occur.

Thus absorption measurements are moderate to highly selective. In the case of photometric and Spectrophotometric methods, the relative error in concentration measurements lies in the range of 1-3%. The error can be reduced to few tenths of a percent by using special techniques. This shows that procedure has a good accuracy. Spectrophotometric and photometric measurements can be easily and readily performed because of commercial availability of the modern instruments**.** Thus absorption measurements are easy as well as convenient.

There are numerous of applications of quantitative absorption methods. Spectrophotometric analysis for any organic compound

containing one or more of the chromophoric groups are potentially feasible. A number of inorganic species also absorb and thus susceptible to direct determination. We have already discussed the absorption behavior of some organic compounds and inorganic compounds such as lanthanides, actinides and other transition metals. A number of other species such as permanganate, nitrate, and chromate ions, ozone, osmium and ruthenium tetraoxides, molecular iodine etc have also been found to show characteristic absorption.

A large number of reagents react with non-absorbing species to yield that absorb strongly in the ultra violet and visible regions. Thus substances that do not show useful absorption can also be determined by adding a reagent to form an absorbing complex or other chromophore. The successful application of such reagents to quantitative analysis needs that the color forming reaction be forced to near completion. These reagents are frequently used for the determination of an absorbing species, such as transition metal ion. The molar absorptivity of the completing agents has been used for the determination of inorganic species. The molar absorptivity of the product will frequently be greater in magnitude than that of the uncombined species. A large number of typical inorganic reagents are thiocyanate for Fe, Co and Mo: the anion of H_2O_2 for Ti, V and iodide ion for Bi, Pd and Te. Organic chelating agents which form stable, colored complexes with cations are o-phenanthroline for Fe, dimethyl glyoxine for Ni, diethyl dithio carbonate for Cu, and diphenyl thio carbazone for lead. Dithizone is a good reagent for this purpose and is soluble in chloroform. It gives red complex when reacts with cations of transition metals. The reagent can be made specific by adjusting the pH.

Another example is the determination of trace amounts of Hg (II) with the dye 4,4-bis (dimethylamino) diphenylamine, known as Binds Chelder's green in citrate solution. The complex, extracted into 1, 2 dichloroethane follows Beer's law from 8×10^{-7} to 4×10^{-6}M. Tin has been found to interface out of 21 metals checked by Tsubouchi (1970).

Analysis of an absorbing substance can be carried out directly thoroughly Beer's law in the absence of any other absorbing material. For example, the concentration of ozone in urban smog was measured by setting up a high pressure mercury arc lamp on the roof of a building and its radiation was then received with a prism spectrophotometer placed on another building several hundred feet distant. Because of the impracticability of a double beam system, zero calibration was made at night, when atmospheric ozone was known to drop to negligible values. The effect of other oxidants, such as NO_2 was eliminated by determining the ratio of the absorbance at the ozone maxima of λ 313 and 165 nm. Ozone was found to rise to about 22 parts per 10^8 at noon, as the average of 50 days.

3. Detection of functional groups

The technique is applied to detect the presence or absence of the chromophore. The absence of a band at a particular wavelength may be regarded as an evidence for the absence of a particular group in the compound. A little information can be drawn from the UV spectrum if the molecule is very complicated. If the spectrum is transparent above 200 mμ, it shows the absence of (i) conjugation (ii) a carbonyl group (aldehydes and ketones) (iii) benzene or aromatic compounds and also (iv) bromo or iodo atoms. An isolated double bond or some other atoms or groups may be present.

4. Extent of conjugation

The extent of conjugation in polyenes R-$(CH=CH)_n$-R can be estimated. Addition in unsaturation with increase in the number of double bonds (increase in the value of n) shifts the absorption to longer wavelength. It is found that the absorption occurs in the visible region. i.e., at about 420 mμ. If n=8 in the above polyene. Such an alkene appears colord to the human eye.

5. Distinction in conjugated and non-conjugated compounds

It also distinguishes between a conjugated and non-conjugated compound. The following isomers can be readily distinguished since one is conjugated and the other is not (Fig.1.6.2).

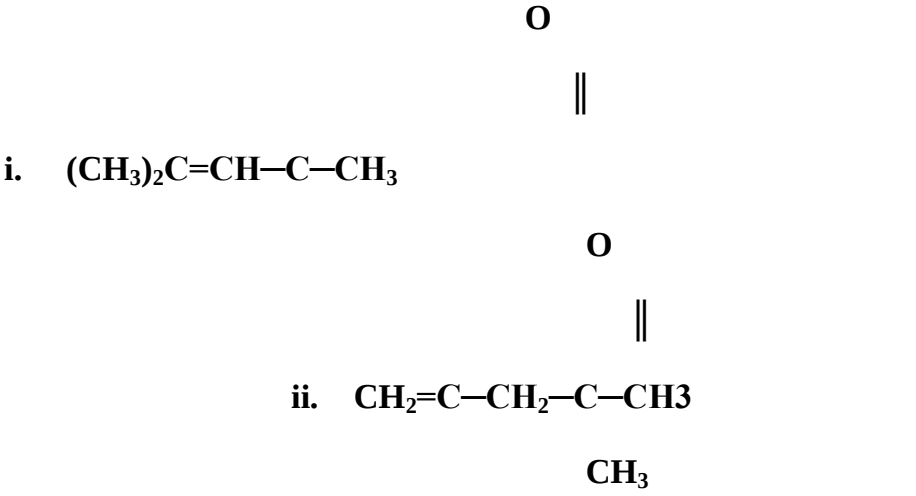


Figure 2.2.5.1 Conjugated and non-conjugated isomers

The forbidden n→π* band for the carbonyl group in the compound (i) will appear at longer wavelength compared to that for the compound (ii).

The alkyl substitution in an alkene causes a bathochromic shift. The technique is not much useful for the identification of individual alkenes.

6. Identification of an unknown compound

An unknown compound can be identified by comparing its spectrum with the known spectra. If the two spectra coincide, the two compounds must be identical. If the two spectra do not coincide, then the expected structure is different from the known compound.

7. Examination of polynuclear hydrocarbons

Benzene and polynuclear hydrocarbons have characteristic spectra in the ultra-violet and visible-region. Thus, the identification of the polynuclear hydro-carbons can be made by comparison with the spectra of known polynuclear compounds. The presence of substituents on the ring, generally, shifts the absorption maximum to longer wavelength.

8. Elucidation of the structure of vitamins A and K

It is useful for the elucidation of the structures of vitamins K_1 and K_2 and also those of A_1 and A_2. The ultraviolet spectra of vitamins K_1 and K_2 are due to the presence of the same chromophore, i.e., 2, 3 dimethyl naptha-quinone. The absorption maxima of this compound are 243, 249, 260, 269 and 330 mμ.

The elucidation of the structures of vitamins A_1 and A_2 are possible by this technique. Vitamin A_1 absorbs at 325 mμ and absorption maxima for vitamin A_2 appear at 287 and 351 mμ. The absorption maxima appear at longer wavelength for vitamin A_2 due to the presence of additional ethylenic bond.

9. Preference over two Tautomeric forms

If a molecule exits in two tautomeric forms, preference of one over the can be detected by ultra-violet spectroscopy. Consider 2-hydroxy pyridine which exists in equilibrium with its tautomeric form, pyridine-2. The spectra of these two compounds were found pyridine-2 which is an α , β-unsaturated ketone and clearly, the equilibrium is shifted towards the right, i.e., phyridine-2.

10. Identification of a compound in different solvents

Sometimes, the structure of the compound changes with the change in the solvent. Chloral hydrate shows an absorption maximum at 290 mμ in hexane while the absorption disappears in the aqueous solution. Clearly, the compound contains a carbonyl group in hexane solution and its structure is $CCl_3.CHO.H_2O$ whereas in aqueous solution it is present as $CCl_3.CH_9(OH)_2$.

11. Determination of configurations of geometrical isomers

The results of absorption show that cis-alkenes absorp at different wavelengths as compared to their corresponding trans isomers. The distinction becomes possible when one of the isomers is forced to be non-coplanar by steric hindrance. Thus, cis forms suffer distottion and absorption occurs at lower wavelength. For example, consider the spectra of cis-and trans stilbenes as shown above.

12. Distinguishes between equatorial and axial conformations

This technique also distinguishes between equatorial between and axial conformations. The $n\rightarrow\pi^*$ (R-bond) which appears at longer wavelength in α , β-unsaturated ketones is influenced by the presence of polar group in the γ-position. It has been noted that the

effect of an axial substituent to displace the R-bond to longer wavelength is greater compared to that observed in its equatorial isomers.

2.2.2 Spectra of dienes

The spectrum of 2,5 dimethyl 2,4 hexadiene compound shows (Fig.2.2.2.1) a broad absorption band in the region of 210-260 mm with a maximum at 241.5 mm. It is the wavelength of maximum absorption (λ_{max}).

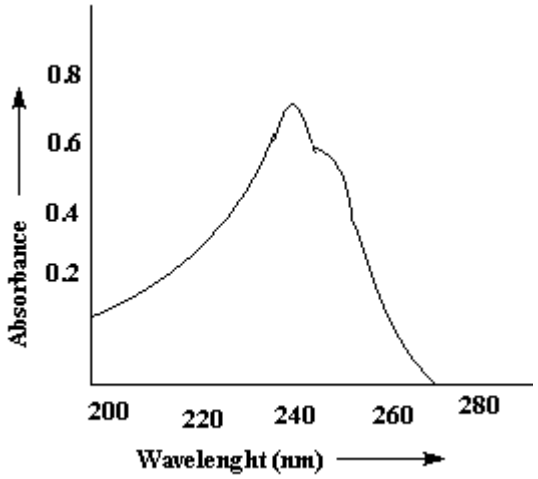


Figure 2.2.2.1 Spectra of diene compound

2.2.3α-β-unsaturated carbonyl compounds (Woodward-Fisher rule)

Woodward and Fieser framed certain empirical rules for estimating the absorption maximum for α-β-unsaturated carbonyl compounds. The rules are as follows:

1. The basic value α-β-unsaturated ketone is taken as 215mμ. The α-β-unsaturated ketone may be a cyclic or six membered.

For a compound, = CH-COX, basic value is 215 mμ, if X is an alkyl group.

If X=H, basic value becomes 207mμ. The basic value is 193 mμ. If x is -OH or OR.

2. If the double bond and the carbonyl group are contained in a five membered ring (cyclopentenone), then for such an α-β-unsaturated ketone, the base value becomes 202 mμ. The ε_{max} for such compounds are generally above 10,000.

The structural increments for estimating λ_{max} for a given α-β-unsaturated carbonyl compound as follows,

i. For each exocyclic double bond +5 mμ

ii. For each double bond endocyclic in

five or seven membered ring except

cyclo-pent-2 enone +5 mμ

iii. For each alkyl substituent or ring residue at the

α-position +10 mμ

β-position +12 mμ

γ-or δ- or higher position +18

iv. For each double bond extending conjugation +30 mμ

v. For a homoannular conjugated diene +39 mμ

vi. Increments for various auxochromes in the various α-,β-,γ- etc., positions are given in the table 2.2.3.1.

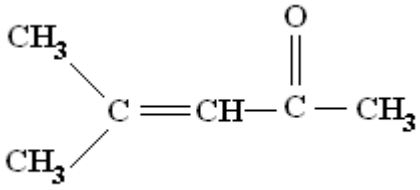
Table 2.2.3.1: Chromophore increment in mμ for position with respect the carbonyl group

Chromophore	α-	β-	γ-	γ-or δ- or higher position
-OH	+35	+30	-	+50
-OAc	+6	+6	+6	+6
-Cl	+15	+12	-	-
-Br	+25	+35	-	-
-OR	+35	+30	17	31
-SR	-	+85	-	-
$-NR_2$	-	+95	-	-

Making use of this above rules, the absorption maximum for the various α-β-unsaturated compounds can be estimated

Example 1: Calculate λ_{max}(Ethanol) for the given structure:

The basic value for a cyclic α,β-unsaturated ketone is 215 mμ. In this structure, we see two β-alkyl substituents. The value of absorption maximum is thus calculated as:



Basic value = 215 mμ

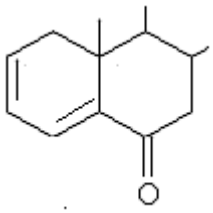
2β-alkyl substituents (2x2) = 24 mμ

———

Calculated value = 239 mμ.

The observed value is found to be λ_{max} 237 mμε_{max} 12,500.

Example 2: Calculate $\lambda_{max\ for}$ the given structure



Base value = 215 mμ

α-ring residue = 10 mμ

β-ring residue = 18 mμ

1 exocyclic = 5 mμ

Homoannular conjugated

diene = 39 mμ

1 double bond extending

conjugation = 30 mμ

Calculated value = 317 mμ

Observed value = 319 mμ

2.2.4. Charge-transfer complexes

Iodine imparts violet color in hexane while it is brown in benzene. When aniline is dissolved in chloroform and tetracyanoethylene (colorless) is added to it, a deep blue solution results. These color shifts are due to the formation of complexes between the pairs of molecules. As a result, two new molecular orbitals are formed which undergo new electronic transition.

The formation of these complexes involves the transfer of electronic charge from an electron rich molecule to an electron deficient molecule with molecular orbitals of suitable energy and symmetry. These complexes are called transfer complexes. Some charge transfer donors and acceptors are given in figures 2.2.4.1 and 2.2.4.2 respectively.

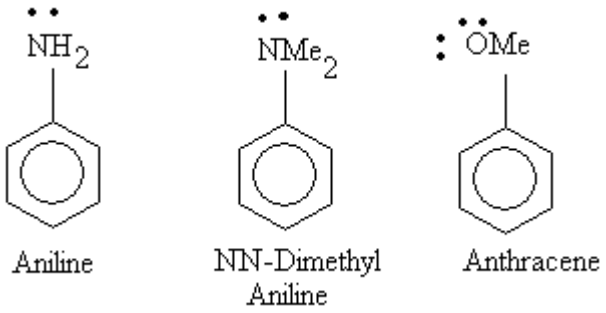


Figure 2.2.4.1: Charge transfer donors

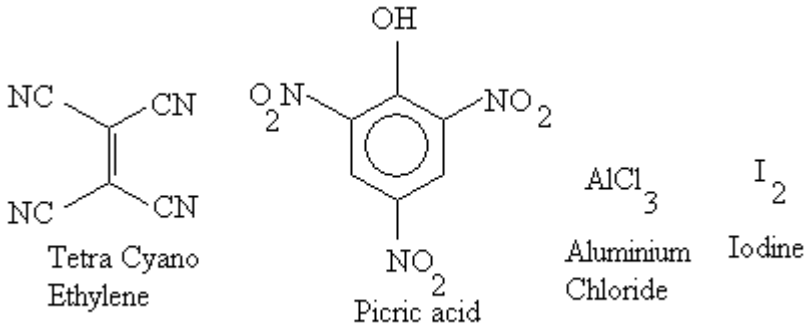


Figure 2.2.4.2: Charge transfer acceptors

The filled π-orbitals (A) in the donor molecule overlap with depleted orbitals (B) in the acceptor molecule. Due to this, two new molecular orbitals are formed. These are (i) the low energy molecular orbital (occupied) in the ground state (A_1) (ii) the upper molecular orbital (B_1). The transitions from A1 to B1 result in the formation of

new absorption bands. Some charge transfer complexes are shown in figure 2.2.4.3.

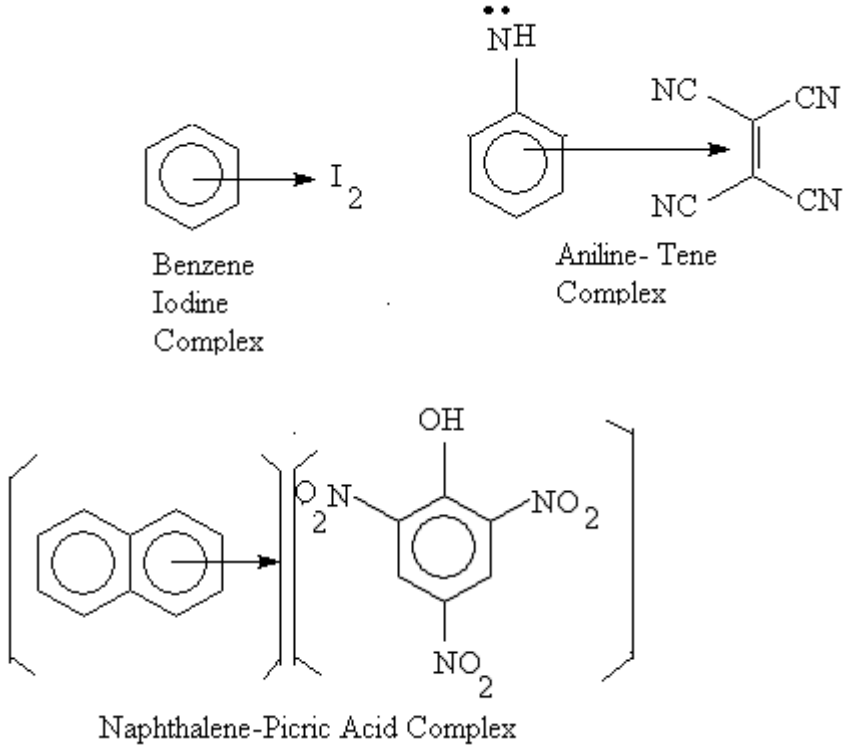


Figure 2.2.4.3: Examples for charge transfer complexes

The electronic transitions for charge-transfer complexes. Donor and acceptor orbitals combine to form two new orbitals (A and B) for the complex. New electronic transitions for long λ are then possible between A and B. In the benzene-iodine complex, λ_{max} for benzene is 255 nm but for molecular iodine in hexane, the absorption occurs in the visible region around 500 nm. This charge transfer complex has an intense additional band around 300 nm but this tails into the visible region and modifies the violet color of molecular iodine to brown. The λ_{max} value for aniline is 280 nm and that for tetracyanoethylene is at 300 nm. But the complex of aniline with tetracyanoethylene absorbs in the visible region at 610 nm. (Fig.2.2.4.4).

2.3 Check Your Progress

1. Define Woodward fisher rule? How can be used for determining the maximum wavelength of organic molecules?

2. Write short note on charge transfer spectra.

3. Based on the Woodward Fisher rule calculate the maximum λ_{max} for unsaturated carbonyl compounds and aromatic carbonyl compounds.

4. List out the differentiate MLCT and LMCT.

5. What is mean by inter ligand charge transfer?

2.4 Answers To Check Your Progress Questions

1. Woodward Fieser rule:

Woodward–Fieser rules are several sets of empirically derived rules which attempt to predict the wavelength of the absorption maximum (λmax) in an ultraviolet–visible spectrum of a given compound. Inputs used in the calculation are the type of chromophores present, the substituents on the chromophores (known as auxochromes), and shifts due to the solvent.

2. LMCT: If the migration of electron is from ligand to the metal, then the cahrgetranser is called ligand to metal charge transfer (LMCT). To make the electron transfer from ligand to metal more favorable, we require a metal with a relatively high ionization energy so that it would have empty orbitals at fairly low energies. The metals would be transition or posttransitions metals, or metals of main group with low ionisation energy, especially in higher oxidation states. An ideal ligand would be a nonmetal with a relatively low electron affinity, which would mean that it would have filled orbitals of fairly high energy and would be readily oxidizable.

3. MLCT: If the migration of electron is from metal to ligand, then charge transfer is called metal to ligand charge transfer (MLCT).

2.5 Summary

- Using Woodward Fieser'srule, we can calculate the maximum UV-absorption of organic/inorganic molecules.
- An electronic transition between orbitals that are centred on different atoms is called charge transfer transition and absorption band is usually very strong. These transitions involve electron transfer from one part of a complex to another. More specifically, an electron moves from an orbital that is mainly ligand in character to one that is mainly metal in character (ligand-to-metal charger transfer, LMCT) or vice versa (metal-to-ligand charger transfer, MLCT).
- Further, based on charge transfer complexes, we can easily understand what kind of transitions happened in the molecules such as CT and inter ligand charge transfer and intra ligand charge transfers from lined itself.

2.6 Keywords

Woodward Fisher's rule: Theoretically we can calculate the maximum UV-absorption of organic molecules.
MLCT: Metal to ligand Charge transfer
LMCT: Ligand to metal Charge transfer

2.7 Self-assessment questions and exercises

1. Based on Woodward Fieser's rule how can you calculate the maximum absorption of organic molecule.
2. Write short note on Woodward Fieser's rule.
3. Explain with suitable examples of LMCT and MLCT.
4. Write short note on charge transfer spectra

2.8 Further readings

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UNIT III : IR SPECTROSCOPY

Structure

3.0 Introduction:

The two atoms joined together by a chemical bond (may be single, double or triple bond), macroscopically can be composed as two balls joined by a spring. The application of a force like (i) stretching of one or both the balls (atoms) away from each other or closer to each other (ii) bending of one of the atoms either vertically or horizontally and then release of the force results in the vibrations on the two balls (atoms). These vibrations depend on the strength of the spring and also the mode (stretching or bending) in which the force is being applied.

Similarly, at ordinary temperatures, organic molecules are in a constant state of vibrations, each bond having its characteristic stretching and bending frequencies. When infrared light radiations between 4000-400 cm-1 (the region most concerned to an organic chemist) are passed through a sample of an organic compound, some of these radiations are absorbed by the sample and are converted into energy of molecular vibrations. The other radiations which do not interact with the sample are transmitted through the sample without being absorbed. The plot of % transmittance against frequency is called the infrared spectrum of the sample or compound.

This study of vibrations of bonds between different atoms and varied multiplicities which depending on the electronegativity, masses of the atom and their geometry vibrate at different but specified frequencies; is called infrared spectroscopy. The presence of such characteristic vibrational bands in an infrared spectrum indicates the presence of these bonds in the sample under investigation.

3.1 Objectives

- Understand to recognize which bonds give useful bands on an IR spectrum
- To list out the bands that you should look for in the spectrum of each functional groups.
- To identify the area of the spectrum where you should look for a particular band.
- To identify the important bands and functional group of the spectrum of an unknown compound
- To identify important differences between the spectra of compounds with different functional groups.
- Understand the use of IR spectra to evaluate the success of a reaction.

3.2 Introduction to IR spectroscopy

Spectroscopy can be defined as the interaction between matter and light. Infrared spectroscopy is a very powerful technique which uses electromagnetic radiation in the infrared region for the determination and identification of molecular structure as well as having various quantitative applications within analytical chemistry (Figure 1).

We do not aim to provide a mechano-quantic description of light and its interaction with atoms, as this is out of the scope of this module. However, it is important to note that atoms can absorb energy from electromagnetic radiation; this absorbed energy alters the state of the atoms within the molecule. These changes are usually manifest in alterations to the frequency and amplitude of molecular vibrations, which may be measured and plotted to produce an infrared spectrum.1-4

Infrared spectrometers use optical devices for dispersing and focusing electromagnetic radiation of IR frequency which is passed through the sample and any changes in absorbance measured against a reference beam.

There are three well defined IR regions (near, mid and far). The boundaries between them are not clearly defined and debate still persists, but broadly they are defined as:

• Near infrared (12820-4000 cm-1): poor in specific absorptions, consists of overtones and combination bands resulting from vibrations in the mid-infrared region of the spectrum.
• Mid-infrared (4000-400 cm-1): provides structural information for most organic molecules.
• Far Infrared (400-33 cm-1): has been less investigated than the other two regions; however,
it has been used with inorganic molecules.

The low energies, typically encountered within the infrared region, are not sufficient to cause electronic transitions; however, they are large enough to cause changes in the frequency and amplitude of molecular vibrations.

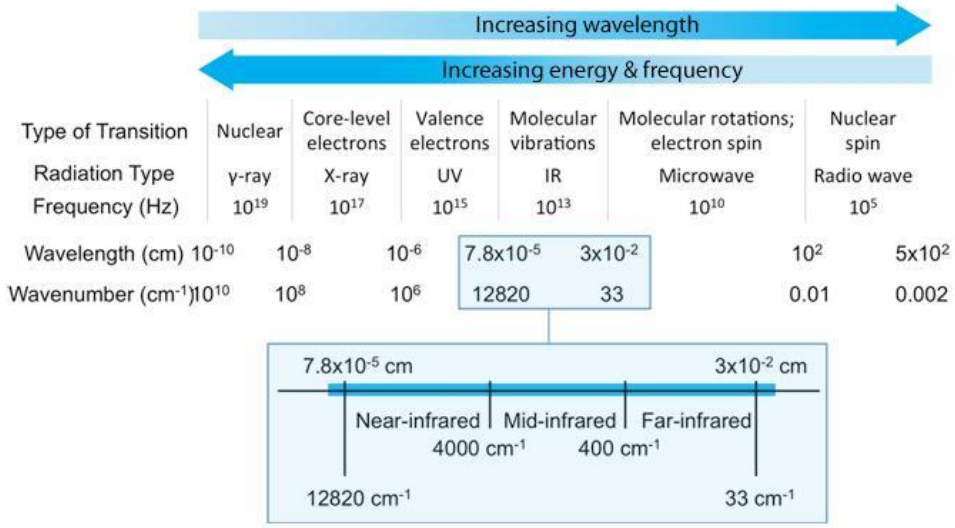

Figure1:Theelectromagneticspectruman

dtheinfraredregion.

2. Electromagnetic Spectrum

The electromagnetic spectrum is the range of all possible frequencies of electromagnetic radiation, each of which can be considered as a wave or particle travelling at the speed of light, often referred to as a photon. These waves differ from each other in length and frequency

Frequency ν - the number of wave cycles that pass through a point in one second. Measured in

Hertz (Hz).

Wavelength λ - The length of one complete wave cycle (cm). Frequency and wavelength are inversely related (Equation 1):

Where:

c = speed of light 3 x 1010 cm/sec

The energy of a photon (E in Joules) is related to wavelength and frequency as follows (Equation 2):

Where:

h = Planck's constant 6.6 x 10-34 Joules-sec

Energy is directly proportional to frequency; therefore, high energy radiation will have a high frequency.

Energy is inversely proportional to wavelength, hence, short wavelengths are high energy and vice versa (Figure 2).

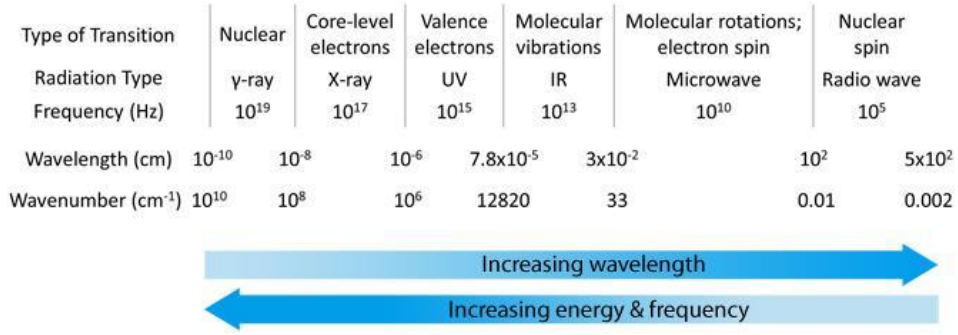

Figure 2: Electromagnetic spectrum

Electromagnetic Radiation and Spectroscopy

The frequency and wavelength of electromagnetic radiation varies over many orders of magnitude. The electromagnetic spectrum is divided according to the type of atomic or molecular transition that gives rise to the absorption or emission of photons; UV, IR, microwave, radio wave etc. (Table 1).

Absorption spectroscopy relies on the absorption of energy from a photon which subsequently promotes the analyte from a lower-energy state to a higher-energy, or excited, state. As the energy of the photon changes the type of transition that the analyte undergoes will change. For example in IR spectroscopy, the absorption of relatively low IR radiation results in the vibration of chemical bonds within the analyte; a process which requires a fairly low energy input. Whereas, higher energy photons, such as those found in the UV-visible region of the electromagnetic spectrum, promote valence electrons to move from their ground state to excited state energy levels within the atoms of an analyte; a process that requires a much greater energy input.

Infrared Regions

Infrared spectroscopy can be rationalized as the spectroscopy that deals with electromagnetic radiation of infrared frequency. As previously explained, there are three well defined infrared regions; each of them has the potential to provide different information: (Figure 4)

•Far-Infrared (400-33 cm-1): vibrations of molecules containing heavy atoms, molecular skeleton vibrations and crystal lattice vibrations

• Mid-Infrared (4000-400 cm-1): useful for organic analysis

• Near Infrared (12820-4000 cm-1): overtones; very useful for quantitative analysis

Infrared spectroscopy is one of the most useful and widely used methods to perform structural analysis.

Given that the molecule under investigation is infrared active, (i.e. it absorbs Infrared radiation), then different types of structural information can be obtained.

Information achievable with Infrared spectroscopy includes:

1. The type of atoms within the molecule.

2. The type of bonds between atoms.

3. The molecular structure. More often than not, infrared spectroscopy is insufficient to determine the complete structure and additional techniques (such as NMR, mass spectroscopy, etc.) are used to solve the puzzle.

Both structures have the molecular formula C2H4O

4. From a quantitative point of view, infrared spectroscopy has a very well gained reputation for its power, flexibility, and reliabi

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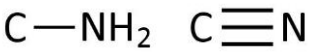

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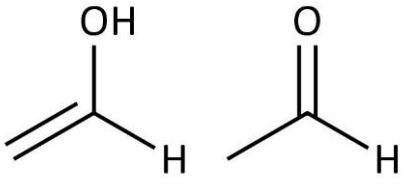

Bothstructures havethemolecularformulaC_2H_4O

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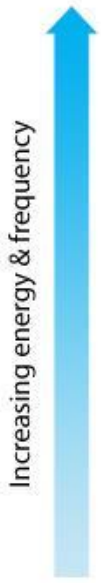


Typeof EnergyTransfer	Region of the Electrom	SpectroscopicTechnique
Absorption	γ	Mossbauer
	X	X-rayabsorption
	U	UV-Vis
		Atomicabsorption
	Inf	Infrared(IR)
		Raman
	Microwave	Microwave
		Electronspinreso
	Radiowaves	Nuclearmagnetic
Emission(t	UV-Vis	Atomicemission
Photoluminesce	X-ray	X-ayfluorescence
	UV-Vis	Fluorescence
		Phosphorescence
		Atomicfluorescence

Table1:Electromagneticspectrumregion,typeofenergytransfer,andtheassociatedspectroscopic technique.

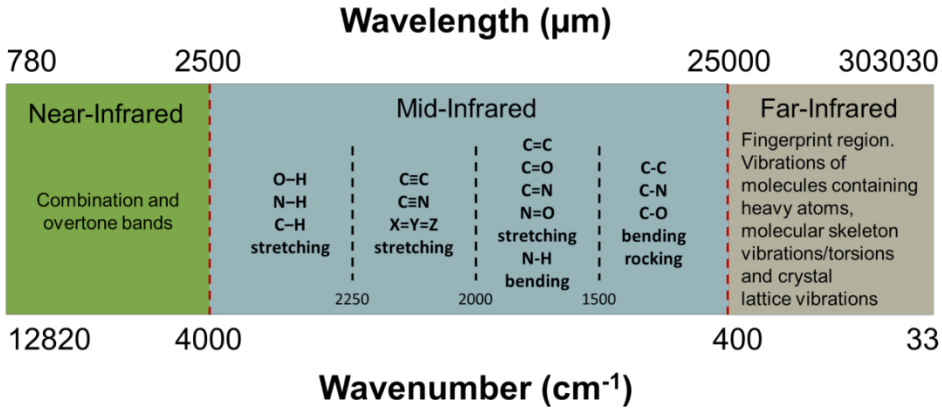
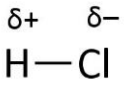


Figure 4: Infrared spectroscopy regions (oversimplified).

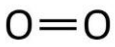
5. Molecular Vibrations

The absorption of light will increase both amplitude and frequency of molecular vibrations. When the radiant energy matches the energy of a specific molecular vibration, absorption occurs. Molecules with a permanent dipole moment, such as water, HCl, and NO, are infrared active.

The HCl molecule possesses a permanent dipole moment, so it is infrared active.



The O2 molecule does not possess a permanent dipole moment, so it is not infrared active.



In the case of alkenes (C=C) and alkynes (C≡C) if the bond is symmetrically substituted no band will be seen in the IR spectrum, however, if the bond is asymmetrically substituted a stretching frequency corresponding to the alkene or alkyne bond will be present (Table 2).

Oscillator	Wavenumber(cm^{-1})
C-H	3320-2700
-C=C-	1690-1590
C=O	1870-1590
C-O	1300-1050
C≡C	2250-2150
C-Cl	800-600

Table 2:Wavenumbersforselecteddiatomicoscillators.

In order to understand molecular vibrations, a bond can be treated as a simple harmonic oscillator composed of two masses (atoms) joined by a spring. Figure 6 depicts a diatomic molecule with two generic atoms (of masses m1 and m2) connected by a spring.

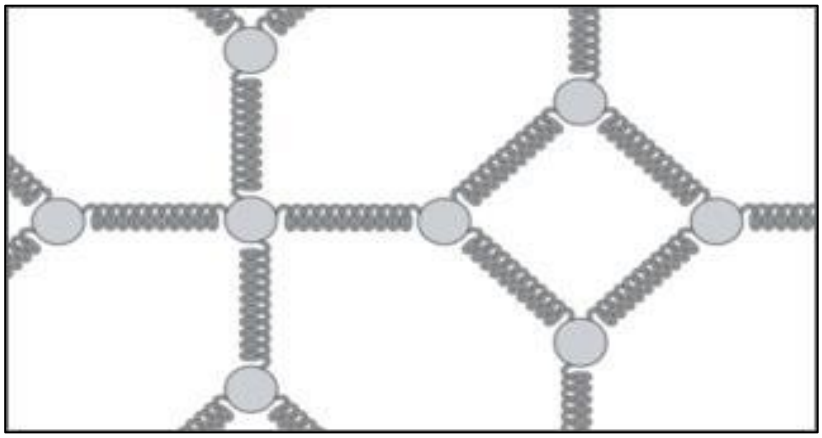


Figure5:Representationofapolyatomicmolecule.

Figure 6: Representation of a diatomic molecule. If masses m1 and m2 are equal, no change in the dipole moment will occur as the molecule vibrates.

The classical vibrational frequency for a diatomic molecule (with force constant k and masses m1 and m2) has been derived from Hooke's Law (Equation 3):

Where:

μ = reduced mass

In terms of the wavenumber ( ) (Equation 4):

Where:

c = speed of light = 3 x 10^{10} cm/sec

3.3 Hooke`s law and absorption of radiations.

The band positions in the IR spectrum are presented in wave numbers (ν) whose unit is the reciprocal centimeter (cm-1). ν is proportional to the energy of vibration.

$\Delta E = h\upsilon = hc / \lambda = hc\,\nu$

Therefore, in principle, each absorption of radiation in the infrared region is quantized and should appear as sharp line. However, each vibrational transition within the molecule is associated with number of rotational energy changes and thus appears as combination of vibrational-rotational bands.

The analogy of a chemical bond with two atoms linked through a spring can be used to rationalize several features of the infrared spectroscopy.

The approximation to vibration frequency of a bond can be made by the application of Hooke's law. In Hooke's law, two atoms and their connecting bond are treated as a simple harmonic oscillator composed of two masses joined by a spring and frequency of vibration is stated as

$$\bar{\nu} \propto \sqrt{\frac{\text{bond strength}}{\text{mass}}}$$

$$\text{then} \quad \bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{(m_1 m_2)/(m_1 + m_2)}} \qquad \text{eq. 1}$$

Where $\bar{\nu}$ = the vibrational frequency (cm^{-1})
c = velocity of light (cm/s)
K = force constant of the bond (dyne/cm)
m_1 and m_2 = masses of the two atoms

The quantity $(m_1 m_2) / (m_1 + m_2)$ is often expressed as μ, the reduced mass of the system.

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}} \qquad \text{eq. 2}$$

Since, according to equation 2

$$\bar{\nu} \propto \sqrt{K}$$

Therefore, the vibrational frequency of a bond would increase with the increase in bond strength. Consequently, we can expect that C=C and C=O stretching will have higher frequencies than C-C and C-O stretching, respectively.

$$\text{also} \quad \bar{\nu} \propto \sqrt{\frac{1}{\mu}}$$

Therefore, the vibrational frequency of a bond would increase with the decrease in reduced mass of the system. It implies that C-H and O-H stretching absorptions should appear at higher frequencies than C-C and C-O stretching frequencies. Similarly, O-H stretching should appear at higher frequency than O-D stretching. Further, in parallel with the general knowledge that the stretching of the spring requires more energy than to bend it, the stretching absorption of a band always appear at higher energy than the bending absorption of the same band.

The Hooke's law can be used to theoretically calculate the approximate stretching frequency of a bond. The value of K is approximately $5x10^5$ dyne/cm for single bonds and approximately two and three times this value for the double and triple bonds, respectively

Let us calculate the approximate frequency of the C-H starching vibration from the masses of carbon and hydrogen

mC = mass of carbon atom = 20x10-24 g

mH = mass of hydrogen atom = 1.6x10-24 g

$$\nu = \frac{\cdot\cdot}{24}\sqrt{\frac{7}{2 \times 22} \times \frac{1}{3 \times 10^8} \quad \frac{5 \times 10^5}{(20 \times 10^{-24})(1.6 \times 10^{-24}) / (2.0 + 1.6)10^{-}}}$$

$$= \sim 3100\ cm^{-1}$$

Let us consider how the radiations are being absorbed.

We know that at ordinary temperature, molecules are in constant state of vibrations. The change in dipole moment during vibration of the molecule produces a stationary alternating electric field. When the frequency of incident electromagnetic radiations is equal to the alternating electric field produced by changes in the dipole moment,

the radiation is absorbed and vibrational levels of the molecule are excited.

Once in the vibrationally excited state, the molecules can loose the extra energy by rotational, collision or translational processes etc. and come back to ground state. Therefore, only those vibrations which result in a rhythmical change in the dipole moment of the molecule absorb infrared radiations and are defined as IR active. The others which do not undergo change in dipole moment of the molecule are IR inactive e.g. the stretching of a symmetrically substituted bond, viz C C in acetylene and symmetrical stretching in carbon dioxide (figure 13) – a linear molecule, produce no change in the dipole moment of the system and these vibrations cannot interact with infrared light and are IR inactive. In general, the functional groups that have a strong dipole give rise to strong absorption bands in the IR.

Modes of molecular vibrations

Molecules with large number of atoms possess a large number of vibrational frequencies. For a non-linear molecule with n atoms, the number of fundamental vibrational modes is (3n-6); linear
molecules have 3n-5 fundamental vibrational modes. Therefore, water - a non-linear molecule
theoretically possesses 3 fudamental vibrations – two stretching and one bending (figure 7); whereas carbon dioxide - a linear molecule possess 4 fundamental absorption bands involving two stretching and two bending modes (figure 8).

Amongst these theoretically possible vibrations, a stretching vibration is a rhythmical movement along the bond axis such that interatomic distance is increasing or decreasing. A bending vibration consists of a change in bond angle between bonds with a common atom or the movement of a group of atoms with respect to remaining part of the molecule without movement of the atoms in the group with respect to one another

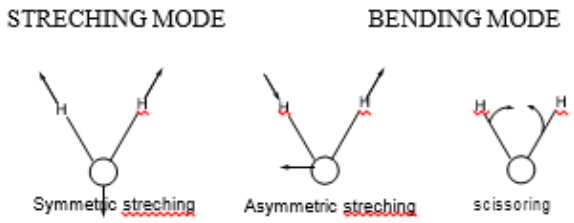


***Figure 7**: Vibrational modes for water molecule*

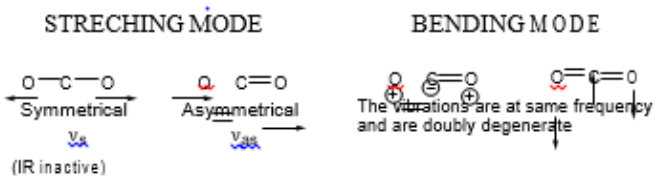


***Figure 8**: Vibrational modes for carbon dioxide molecule*

The various stretching and bending modes can be represented by considering an AX_2 group appearing as a portion of molecule, for example, the CH_2 group in a hydrocarbon molecule (figure 14). Any atom joined to two other atoms will undergo comparable vibrations for example NH_2 or NO_2. Each of different vibration modes may give rise to a different absorption band so that CH_2 groups give rise to two C-H stretching bands i.e. υ_{sym} and $\upsilon_{antisym}$. Some of the vibrations may have the same frequency i.e. they are degenerate and their absorption bands will appear at same position (for CO_2, see figure 9).

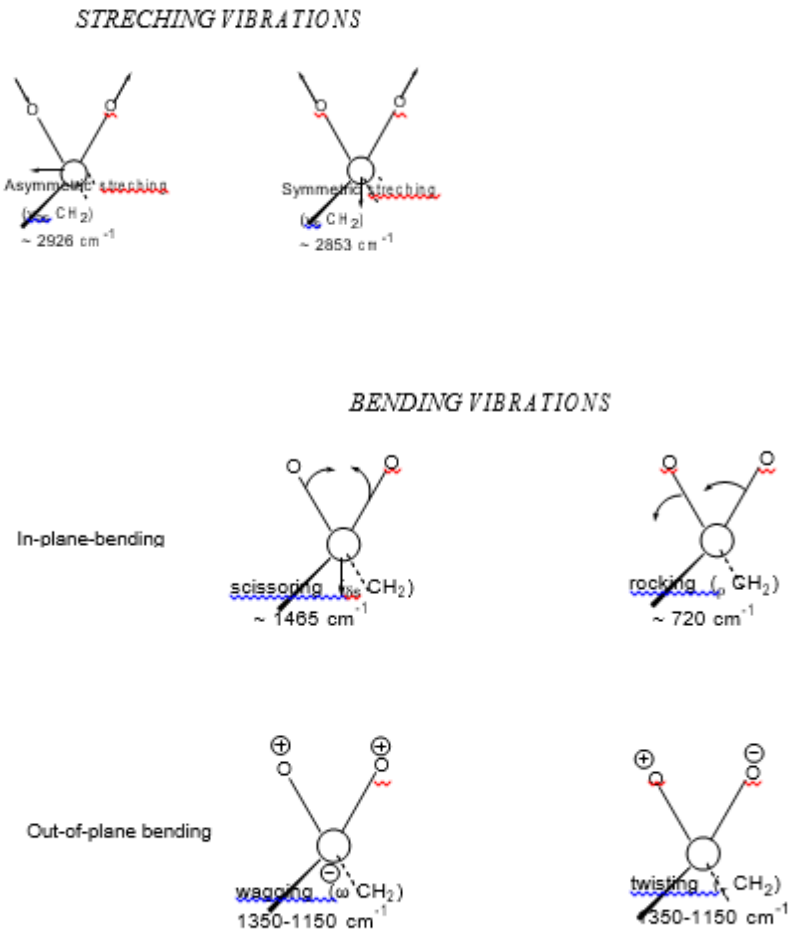


Figure 9: *Vibrational modes of a CH_2 group.*
[⊕ and ⊖ indicate movement above and below the plane of page]

In addition to the fundamental vibrations, other frequencies can be generated by modulations of the fundamental bands. Overtone bands appear at integral multiples of fundamental vibrations. Therefore, the strong absorptions at say 800 cm-1 and 1750 cm-1 will also give rise to weaker absorptions at 1600 cm-1 and 3500 cm-1, respectively. In the IR spectra of benzaldehyde (figure
30) and acetophenone (figure 31), due to C=O stretching vibration a weak overtone can be seen at 3400 and 3365 cm-1, respectively. Two frequencies may interact to give beats which are combination or difference frequencies. The absorptions at x cm-1 and y cm-1 interact to produce two weaker beat frequencies at x + y cm-1 and x – y cm-1.

Therefore, whereas the factors like degeneracy of bands from several absorptions of the same frequency, lack of change in molecular dipole

moment during vibration and fall of frequencies outside the 4000-400 cm-1 region decrease the number of bands whereas the overtone and beats increase the number of bands actually appeared in IR spectrum. Therefore, theoretical numbers of fundamental frequencies are seldom observed.

Other Factors Influencing Vibrational Frequencies

The vibrational frequency of a bond, being part of a molecule, is significantly affected by the electronic and steric factors of the surroundings, in addition to the bond strength and atomic masses discussed above. When two bond oscillators share a common atom, they seldom behave as individual oscillators where the individual oscillation frequencies are widely different. The mechanical coupling interactions between two oscillators are responsible for these changes.

For example, the carbon dioxide molecule, which consists of two C=O bonds with a common carbon atom, has two fundamental stretching vibrations – an asymmetrical and a symmetrical stretching mode. The symmetrical stretching mode produces no change in dipole moment and is IR inactive. Asymmetric stretching mode is IR active and appears at a higher frequency (2350 cm-1) than observed for a carbonyl group in aliphatic ketones (1715 cm-1).

The carbonyl stretching frequency in RCOCH3 (~1720 cm-1) is lower than acid chloride RCOCl (1750-1820 cm-1). This change in frequency of the C=O stretching may be arising due to (i) difference in mass between CH3 and Cl (ii) the inductive or mesomeric influence of Cl on the C=O bond (iii) coupling interactions between C=O and C-Cl bonds (iv) change in bond angles arising due to steric factors etc. It is usually impossible to isolate one effect from the other. However, the appropriate emphasis can be placed on those features that seem to be most responsible in explaining the characteristic appearance and position of group frequencies.

Sample Preparation

For recording an IR spectrum, the sample may be gas, a liquid, a solid or a solution of any of these. The samples should be perfectly free of moisture, since cell materials (NaCl, KBr, CsBr etc.) are usually spoiled by the moisture.

Liquids are studied neat or in solution. In case of neat liquid, a thin film of < 0.01 mm thickness is obtained by pressing the liquid between two sodium chloride plates and plates are subjected to IR beam. Spectra of solutions are obtained by taking 1-10 % solution of the sample in an appropriate solvent in cells of 0.1-1 mm thickness. A compensating cell, containing pure solvent is placed in the reference beam of the instrument. The choice of solvent depends on the solubility of the sample and its own minimal absorption in IR region. Carbon tetrachloride, chloroform and carbon disulfide are preferred solvents.

The spectrum of a solid can be obtained either as a mull or as an alkali halide pellet. Mulls are obtained by thoroughly grinding 2-5 mg of a solid sample with a drop of mulling agent usually Nujol (mixture of parafinic hydrocarbons) or fluorolube (a completely fluorinate polymer). The suspended particles must be less than 2 µM to avoid excessive scattering of

radiations. The mull is placed between two sodium chloride plates and plates are subjected to IR beam.

For preparing, an alkali halide pellet, 1-2 mg of dry sample is grinded with ~ 100 mg of KBr powder. The mixture is then pressed into a transparent pellet with a special die under a pressure of 10,000-15,000 psi. KBr pellet is then mounted on holder and is placed in sample beam of IR spectrophotometer.

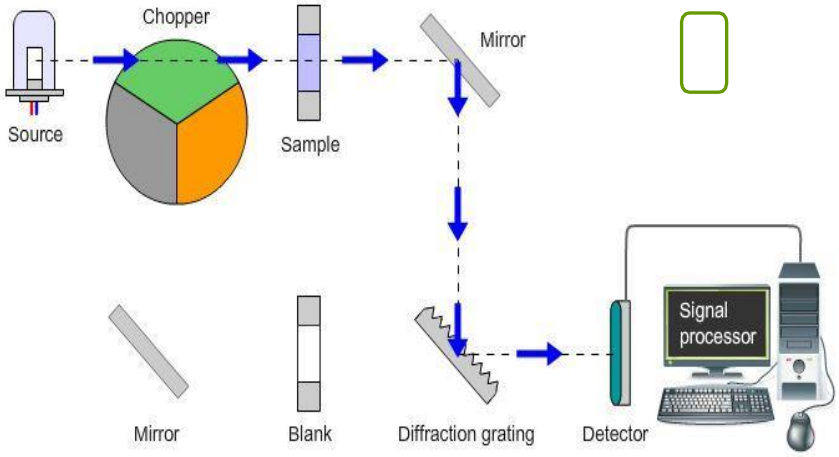
Characteristic Group Vibrations of Organic Molecules

An infrared spectrum of an organic compound comprises many bands and assigning each band to a particular mode of vibration is practically impossible but two non-identical molecules generally

have different IR spectra. An IR spectrum, therefore, is a fingerprint of the molecule. The region most useful for the purpose of "fingerprinting" of the compound is 650-1350 cm-1. This region comprises a large number of bands due to skeletal vibrations and when the spectrum we are studying coincides exactly with the spectrum of a known compound, it can be safely assumed that the two compounds are identical.

The region above 1350 cm-1 often provides easily recognizable bands of various functional groups and thus much valuable structural evidence from relatively few of theses bands is

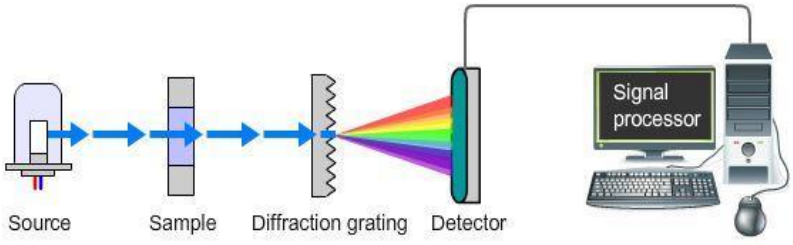
obtained and total interpretation of the complete spectrum is seldom required. In the following sections, the basic information about the vibrational modes in basic functional groups has been discussed.



Dispersive IR Instruments

Most IR spectrometers can be categorized into two classes: dispersive and Fourier Transform instruments.

The basic design of a dispersive single beam instrument includes a source of infrared radiation, a monochromator, and the detector (Figure 10).



After interacting with the sample (or the blank), infrared radiation is dispersed by a monochromator into its individual frequency components and information on which frequencies were absorbed can be obtained using a photodiode array detector. Sources and detectors for infrared radiation have limited stability; with light intensity and detector sensitivity changing over time, or with fluctuations in temperature etc. The blank (reference or background) and sample measurements should be made one after the other to ensure they are made under the same analytical conditions. This limitation is minimized by the use of double beam instruments which are capable of measuring the sample and reference simultaneously. Double beam instruments use 'choppers' to control the path of the radiation, alternating between the sample and the reference (Figure 11). These instruments use the known speed of rotation of the beam chopper to compare and resolve the information reaching the detector. The use of an opaque surface provides the means for adjusting the 0% transmittance response of the detector.

Finally, it is easier to correct for absorption of infrared radiation by carbon dioxide and water (present within the instrument background) with double beam instruments than with their single beam counterparts.

FTIR Instruments

FTIR stands for Fourier Transform Infrared. FTIR spectrometers consist of an IR source, interferometer, sample cell or chamber, detector and a laser. A schematic of an FTIR instrument is shown below (Figure 12).

IR source

IR radiation is emitted from a glowing black body source. IR radiation passes through an aperture which controls the amount of radiation that reaches the sample, and therefore, the detector.

Common IR sources are:

1. Silicon carbide rods which are resistively heated and commonly known as a Globar. An electric current is passed through the rod which becomes very hot (1300 K) and emits large amounts of IR radiation. Previously, cooling with water was required to avoid damaging electrical components; however, advances in metal alloys have led to the production of Globars that do not require cooling by water.
2. Nichrome and Kanthanl wire coils were once popular IR sources and did not require cooling as they ran at lower temperatures than Globars, however, this also resulted in lower amounts of IR radiation being emitted.
3. Nernst Glowers are manufactured from a mixture of refractory oxides and are capable of reaching hotter temperatures than a Globar; however, they are not capable of producing IR radiation above 2000 cm-1.

Interferometer

The first interferometer was invented by Albert Abraham Michelson, who received a Nobel Prize for his work in 1907. Without this essential piece of optical equipment the modern day FTIR system would not exist.

The interferometer consists of a beam splitter, a fixed mirror, and a moving mirror.

Beam Splitter

The beam splitter is made of a special material which transmits half of the incident radiation and reflects the other half. IR radiation from the source strikes the beam splitter and is separated into two beams. One beam is transmitted through the beam splitter to the fixed mirror while the other beam is reflected from the beam splitter to the moving mirror. Both mirrors reflect the radiation back to the beam splitter where the two beams interfere to produce an interferogram.

M**oving Mirror**

The moving mirror is a flat highly reflective surface mounted on air bearings that allow for high speed movement of the mirror (movements are made once every millisecond). The moving mirror only moves a few millimeters away from the beam splitter.

Fixed Mirror

The fixed mirror is a flat
highly reflective surface.

3.5 Characteristic Group Vibrations of Organic Molecules

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Fixed Mirror:

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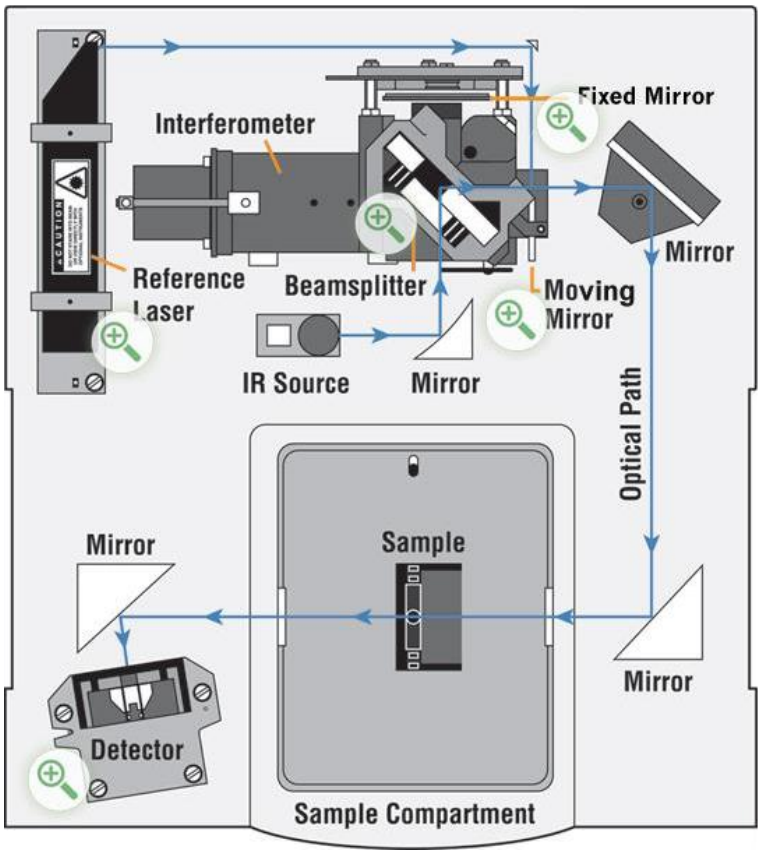


Figure 12: Operational Schematic of a Thermo Nicolet FTIR Instrument. Imagere produced with permission from Thermo Fisher Scientific(Madison,WI,USA).

Laser

Many instruments employ a Helium-Neon laser as an internal wavelength calibration standard. It is imperative that the position of the moving mirror is known at any given moment. The moving mirror moves back and forth at a precise constant velocity that is timed using a very accurate laser wavelength.

The intensity of the laser beam is measured at two points in the interferometer. As the mirror moves the intensity at these two points will rise and fall due to the enhancement and cancellation of the HeNe beam paths, producing a sine wave of intensity vs. mirror position. The number of "fringes" in the sine wave allows the instrument to know exactly how far the mirror has moved, and the relative phase of the sine wave tells the instrument in which direction the mirror is moving

Detector

There are two classes of infrared detectors; thermal and photonic detectors. Thermal detectors use the IR radiation as heat; whereas, quantum mechanical (photonic) detectors use the IR radiation as light which results in a more sensitive detector.

Thermal detectors: detect changes in temperature of an absorbing material (lithium tantalate ($LiTaO_3$), lead selenide (PbSe), germanium etc.). Many temperature dependent phenomena can be followed to measure the effects of the incident IR radiation. Bolometers and

microbolometers use changes in resistance, while thermocouple and thermopiles use the thermoelectric effect. Golay cells monitor thermal expansion.

Photonic Detector: exhibit faster response times and higher sensitivity in comparison to their thermal counterparts, therefore, they are much more prolific in FTIR instruments. The materials used in these detectors are semiconductors with narrow band gaps. The incident IR radiation causes electronic excitations between the ground and first excited states, which in photoconductive detectors result in a change in resistivity which is monitored.

FT IR Operation:

Prior to the development of FTIR spectrometry, the limitation within IR was the slow scanning process. FTIR allows for all the infrared frequencies to be scanned simultaneously, allowing for data to be collected in a matter of seconds rather than several minutes. This is achieved through the use of an optical device called an interferometer which produces a signal which is made up of all of the infrared frequencies.

Most interferometers consist of a beam splitter which splits the incident infrared beam into two separate optical beams. One beam is reflected from a fixed mirror, while the other beam is reflected from a mirror that is constantly moving in the instrument. The moving mirror typically moves by only a few millimeters from the beam splitter (Figure 13).

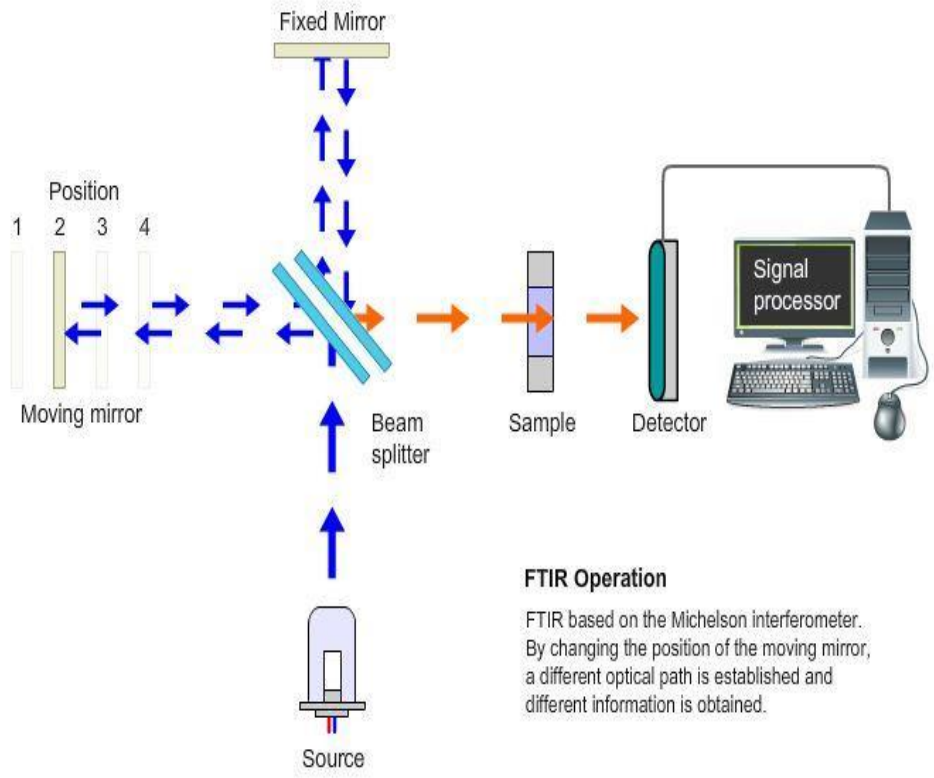


Figure 13: FTIR based on the Michelson interferometer. By changing the position of the moving mirror, a different optical path is established and different information is obtained.

The two beams are reflected from their respective mirrors and recombine at the beam splitter. The path length of the beam that is reflected from the fixed mirror remains constant, while the path length of the beam that is reflected from the moving mirror is constantly changing as the mirror moves. The signal that exits the interferometer is the result of these two beams interfering with each other, and is called an interferogram (Figure 14).

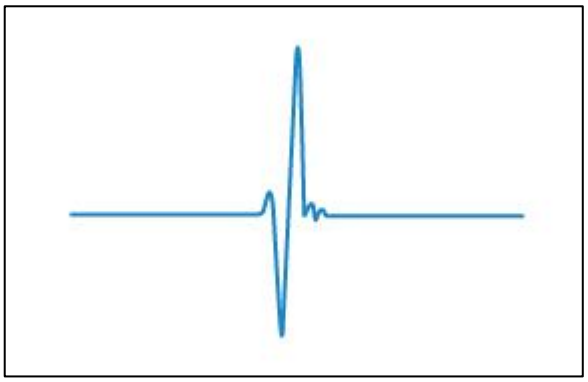


Figure14:Interferogram.

The interferogram is unique in that every data point, which is a function of the moving mirror position, has information about every infrared frequency emitted from the source. This allows for all frequencies to be measured simultaneously.

The interferogram is converted to a more familiar IR spectrum (wavenumber vs. % transmittance) using the well-known mathematical technique called Fourier transformation. The transformation of the interferogram is carried out by the instrument software.

IR spectra are presented on a relative scale (%T), therefore, a background spectrum must be measured. A background spectrum is taken with no sample in the beam and is then subtracted from the sample spectrum to remove artifacts generated by the instrument or air (i.e. water, carbon dioxide, etc.).

FTIR Advantages

FTIR instruments have several advantages over dispersive IR instruments including:

Speed

All IR frequencies are measured simultaneously, resulting in measurements being taken in seconds rather than minutes. This is often referred to as the Felgett Advantage.

Sensitivity

The detectors utilized in FTIR instruments are highly sensitive which results in lower signal to noise ratios. This is known as the Jacquinot Advantage.

Simplicity

The only moving part in an FTIR instrument is the mirror in the interferometer; therefore, there is very little need for mechanical maintenance.

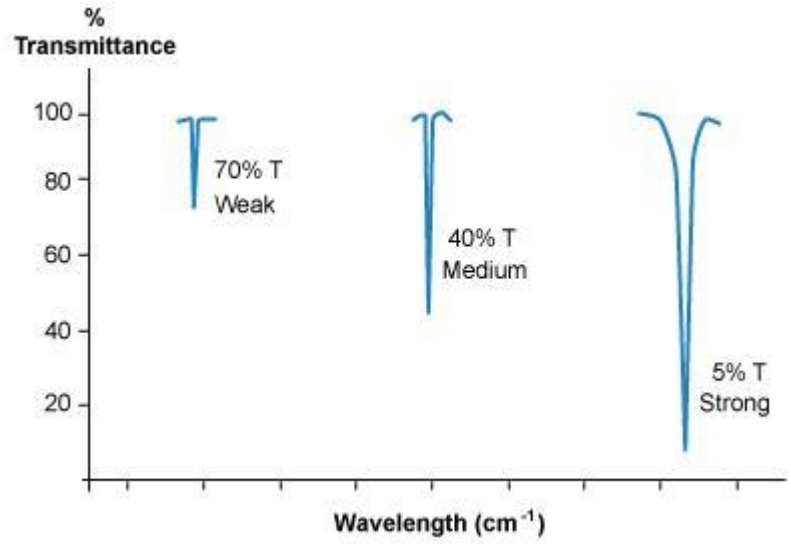
Internal calibration

The internal laser is used to self-calibrate the moving mirror in the FTIR instrument negating any need for timely or complicated external calibration. This is denoted as the Connes Advantage.

14. Sample Preparation

Proper sample preparation is required to obtain meaningful spectra with sharp peaks, which have good intensity and resolution. Ideally the largest peaks should be attributable to the compound being analyzed opposed to the background or sample matrix (water, CO_2, solvent etc.) and should ideally have an intensity of 2-5 %T for the strongest peaks in the spectrum (Figure 15). A transmission of 5 % is equivalent to an absorbance (A) = 1.3 (i.e. the amount of light that is absorbed by the sample), which is the upper detection limit for most detectors. The equation above is worth remembering as it allows the absorbance of a sample to be calculated from the percentage transmittance data.

Peaks that are of higher intensity will be cut off and the sample will need to be prepared again. Compounds can be analyzed in the vapor phase, as pure liquids, in solution, and as solids.



As a Liquid

A drop of the liquid is squeezed between two sodium chloride (NaCl) plates, which are transparent in the 4000-625 cm-1 region (Figure 16). The plates are then placed in a holder and a spectrum is taken. If the peaks in the spectrum are too intense the liquid can be wiped from one plate, then the spectrum taken again.

NaCl plates are very fragile and sensitive to water. Samples should never be dissolved in water and placed on a NaCl plate as it will fog up or dissolve. The plates should be held by the edges to avoid moisture from fingers damaging them. After a sample has been run, ethanol can be used to clean the plates. Moisture in the air can also damage NaCl plates; therefore, they should be stored in a desiccator. Cloudy or damaged plates (pitted, fingerprints etc.) will result in **poor spectra** with broad bands and spectra with less than optimum transmission (Figure 17). Cloudy plates can be restored by polishing.

Liquids can also be placed directly on an Attenuated Total Reflectance (ATR) plate which will be discussed later.

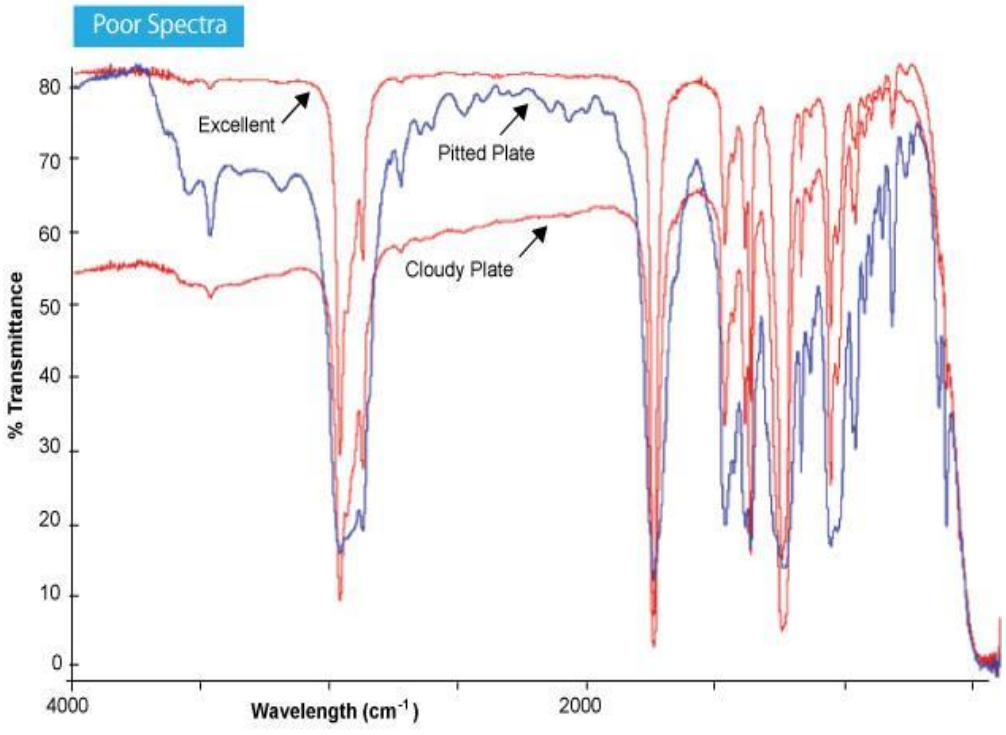


Figure 17: Representative spectra obtained with sodium chloride (NaCl) plates in various conditions.

As a Solution

Samples can be dissolved in an appropriate solvent to give a solution. The spectrum is then taken by placing a drop on a NaCl plate or by using a sodium chloride solution cell (Figure 18). Solvents should be free of water to avoid damaging the sodium chloride cell surfaces. A reference spectrum of the blank solvent should be obtained and subtracted from the sample spectrum.

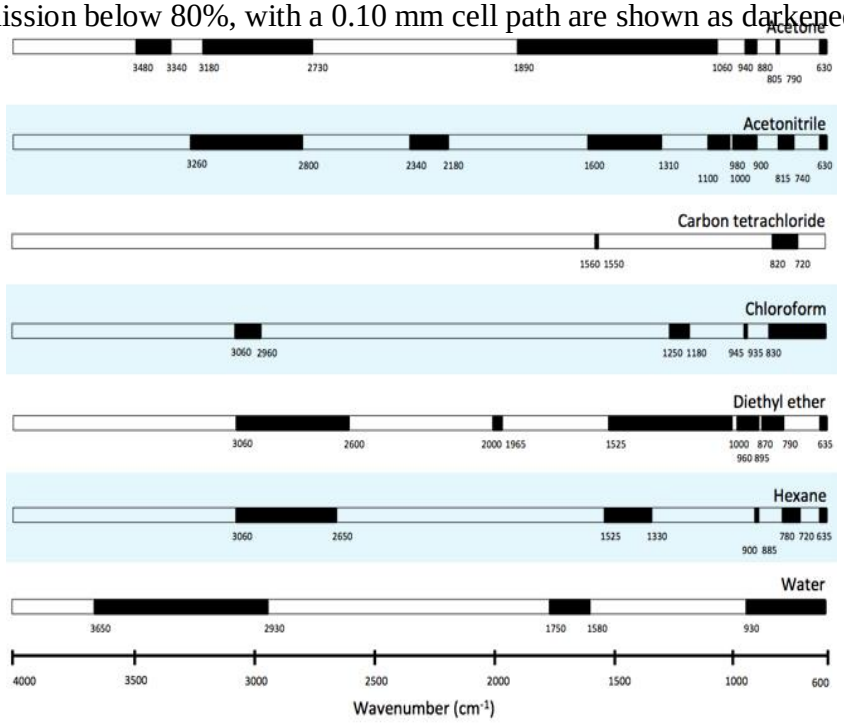
When solvents absorb ~80**%** of the incident light, spectra cannot be obtained because insufficient light will be transmitted and detected. The regions in which common solvents absorb too strongly to give meaningful spectral information from a sample are shown in the table below.

If aqueous solvents must be used for solubility, special calcium fluoride cells can be used.

Figure 18: Sodium chloride solution cell. Image reproduced with permission from International Crystal Laboratories (Garfield, NJ, USA).

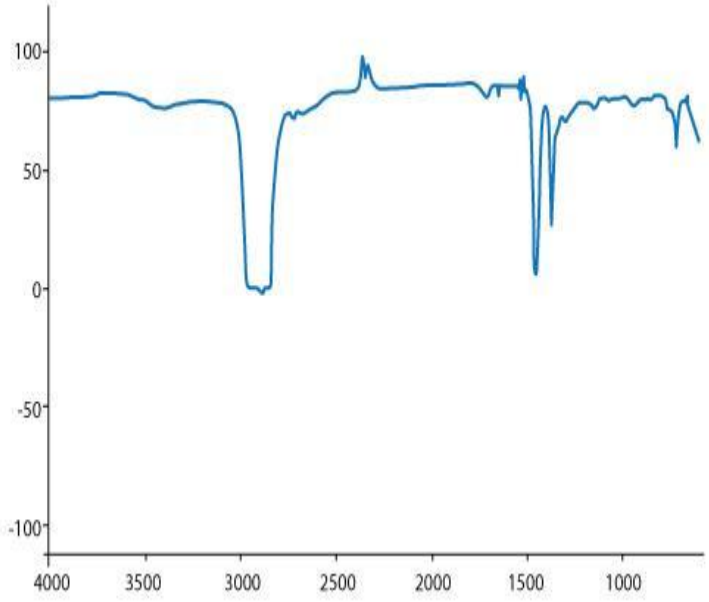
Transmission Characteristics of Common Solvents.

Transmission below 80%, with a 0.10 mm cell path are shown as darkened areas



As a Nujol Mull

IR spectra of solid samples can be obtained using a Nujol mull. Nujol is a mineral oil which itself has an IR spectrum (Figure 19).



A small amount of sample is ground using a small agate mortar and pestle and a drop of Nujol (Figure 20). The mull is then pressed between two NaCl plates and the spectrum obtained. The mull should appear transparent and free of bubbles when properly prepared. If the peaks in the spectrum are too strong one plate can be wiped clean and the spectrum re-run.

Figure 20: Agate mortar and pestle. Image reproduced with permission from Cole-Parmer (Hanwell, London, UK).

As a KBr Disc

A solid sample can be ground with 10-100 times its mass of pure potassium bromide (KBr). Solid samples should be finely ground before adding the KBr. This is then pressed into a disc using a special mold and a hydraulic press (Figure 21). The use of KBr eliminates any bands that may obscure analyte signals when using a Nujol mull. A band at 3450 cm^{-1} will often be present and is attributable to the OH group from traces of water. Water can be minimized by drying the KBr in an oven. Excessive grinding of the hygroscopic KBr can increase the water content.

Solid state spectra can differ greatly from solution state spectra due to intermolecular interactions between functional groups, i.e. hydrogen

bonding. Conversely, solid state spectra will often exhibit a greater number of resolved bands which can aid in compound identification.

Material	Wavelength Range	Wavelength Range (cm^{-1})	Refractive
NaCl	0.25-17	40,000-590	1.52
KBr	0.25-25	40,000-400	1.53
KCl	0.30-20	33,000-500	1.5

Table 3: Material used for obtaining solid state IR spectra. Note these materials can also be used to produce plates and solution cells for obtaining spectra with liquids and mulls.

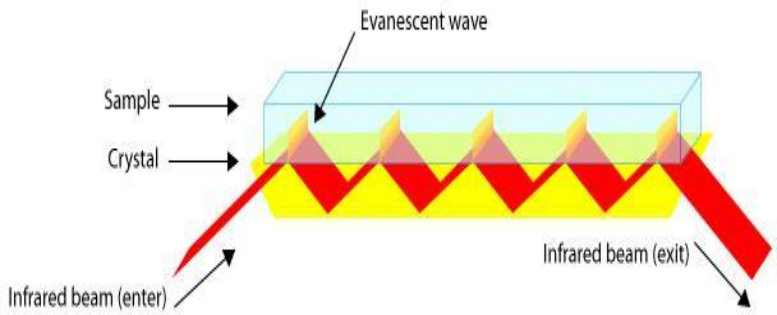
Reasons for Cloudy Discs

- KBr mixture not properly ground
- Sample was not dry
- Sample:KBr ratio too high
- Disc too thick
- Sample has a low melting point

15. Attenuated Total Reflectance (ATR)

As has been discussed previously, IR spectra can be obtained from samples as liquids, solids, or mulls; however, the primary drawback is the sample preparation that is required to obtain good quality spectra. IR instruments which utilize an attenuated total reflectance (ATR) stage negate the necessity for complex and timely sample preparation resulting in good quality, reproducible spectra.

With traditional means of IR spectroscopy the IR radiation is passed through the sample and the resulting radiation which is transmitted is measured. Attenuated total reflectance measure the changes which occur in a totally internally reflected IR beam when it is in contact with a sample (Figure 22).



The infrared beam enters the crystal which is made of an optically dense material (i.e. it has a high refractive index) at a particular angle of incidence, the IR beam is internally reflected (usually between five and ten times), this internal reflectance results in the production of an evanescent wave which can extend beyond the crystal surface and into the sample itself. The wave will usually penetrate into the sample with a depth of 0.5-2 µm. The depth to which the wave penetrates is dependent on the angle of the incident IR beam and the refractive index of the crystal material and sample itself.

When a samples absorbs the infrared radiation there is a change in the evanescent wave; in other words the wave is attenuated. The attenuated

energy from each of the evanescent waves is then transferred back to the IR beam which exits the crystal and is measured by the detector to produce an IR spectrum.

Typical ATR Crystal Materials

In ATR instruments the crystal is an optically dense material which has a refractive index that is greater than the sample. Common ATR crystal materials are listed in Table 4. The most common are zinc selenide (ZnSe) and Germanium (Ge).

Zinc selenide is applicable to the analysis of liquids and non-abrasive pastes. It has a working pH range of 5 – 9. Germanium is more robust with a working pH range of 1 – 14 and can be used to analyze weak acids and alkalis. For a greater initial cost, instruments which utilize diamond as the ATR crystal material exhibit greater durability and robustness, with the crystal having to be replaced less in comparison to ZnSe and Ge.

Materials such as ZeSe and Ge can scratch easily; therefore, care must be taken when cleaning crystal surface. It is recommended that crystal surfaces are cleaned with lint free tissues soaked with solvents such as water, methanol or isopropanol.

Material	Wavelength Range	Refractive
ZnS	20,000-500	2.43
Zn	22,000-750	2.25
Ge	5,000-600	4.01
Si	10,000-100	3.42
Diamond	45,000-10	2.40

Table 4: Attenuated total reflectance (ATR) crystal materials.

ATR Instrument

In traditional ATR instruments the sample was clamped against the vertical face of the crystal. This design has now been replaced by horizontal ATR stages where the upper surface of the crystal is exposed (Figure 26). ATR accessory kits can be purchased which can be used to modify existing IR instruments.

Similarly to FT-IR instruments a background spectrum must be collected; this is taken from the clean ATR crystal. The background spectrum which is obtained can be a useful indication of the cleanliness of the ATR crystal; a line at 100% T should be obtained with no spectral features. In order for total internal reflectance to occur there must be good contact between the sample and crystal surface. Liquid samples can be placed directly onto the ATR crystal; the whole crystal must be covered. Similarly pastes or other viscous substances can be spread onto the crystal. In the case of solids, these are more readily analyzed on single reflection ATR instruments which are often made of diamond. High quality spectra can be obtained directly from powder samples placed on the ATR crystal. The amount of sample should entirely cover the crystal and does not need to be more than a few millimeters thick. In order to ensure that there is good contact with the crystal surface the instrument pressure arm is positioned over the sample and tightened; it may be necessary to apply greater pressure

when analyzing high density polymers or coatings on metal surfaces, however, the user manual should always be consulted for optimum operating parameters.

The major advantages of ATR instruments are the lack of sample preparation, the ability to obtain high quality reproducible spectra, and due to their ease of use, the variation between users is minimized.

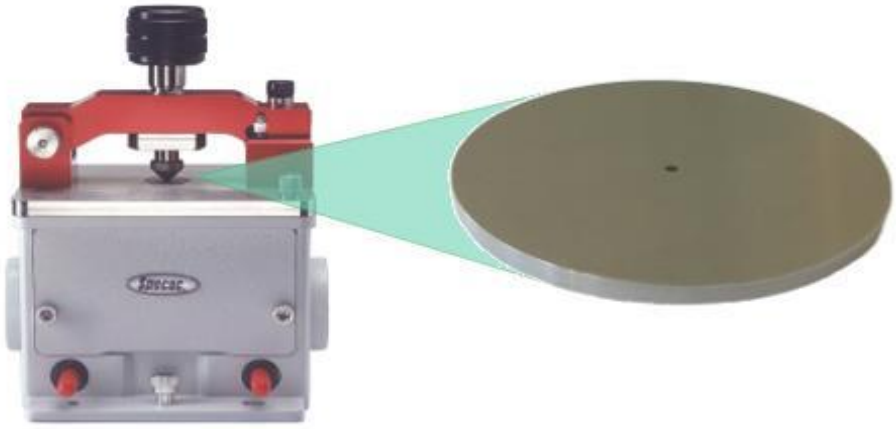


Figure 26: ATR Instrument. Image reproduced with permission from Specac (Orpington, Kent, UK).

16. Applications of IR Spectroscopy

IR spectroscopy has primarily been used for structural elucidation and identification of unknowns (by comparison with a spectrum of a standard).

Modern advances have seen the development of 2D IR techniques which have been applied to a myriad of different applications including isotope labelling studies of biological species, the investigation of proteins, peptides, and hydrogen bond dynamics,and also the study of nanocrystalline thin films.

Other areas of note where IR spectroscopy is being utilized are in stem cell studies, materials science, catalysis, and reaction kinetics. Which demonstrates the applicability and flexibility of this analytical technique.

3.7 Check Your Progress

1. Discuss the basic principle of IR spectroscopy.

2. Define Hook's law.

3. Explain with suitable examples of stretching and bending vibrations with suitable examples.

4. What is mean by Fermi resonance? Explain.

5. Discuss the basic principle, instrumentation and applications of IR spectroscopy.

3.8 Answers To Check Your Progress Questions

1. Hooke`s Law: Hooke's law is a law of physics that states that the force needed to extend or compress a spring by some distance x scales linearly with respect to that distance. That is, where k is a constant factor characteristic of the spring: its stiffness, and x is small compared to the total possible deformation of the spring.

2. **Stretching and bending vibrations**

3**. Fermiresonance** : It is the shifting of the energies and intensities of absorption bands in an infrared or Raman spectrum. It is a consequence of quantum mechanical mixing.

4.**Overtones** : An overtone is any frequency greater than the fundamental frequency of a sound. Using the model of Fourier analysis, the fundamental and the overtones together are called partials. Harmonics, or more precisely, harmonic partials, are partials whose frequencies are numerical integer multiples of the fundamental.

3.9 Summary

1. From this unit we can easily understand the basic principles of IR spectroscopy and its various vibration modes.

2. Further, the shifting of energies and their intensities of absorption bands in an IR as well as Raman spectrum.

3. Also we studied the instrumentation of IR spectroscopy and various parts of instruments.

3.10 Keywords

Hooke`s law, Fermi resonance, stretching and bending vibrations, overtones.

3.11 Self-assessment questions and exercises

1. Explain the basic principle of IR spectroscopy.

2. Derive Hook’s law.

3. Discuss elaborately about the stretching and bending vibrations of molecules with suitable examples.

4. What is mean by Fermi resonance? Explain.

5. Discuss the basic principle, instrumentation and applications of IR spectroscopy.

3.12 Further readings

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UNIT IV: APPLICATIONS OF IR SPECTROSCOPY

Structure

4.0 Introduction

Infrared spectroscopy is the study of interaction of infrared light with matter, which can be used to identify unknown materials, examine the quality of a sample or determine the amount of components in a mixture. Infrared light refers to electromagnetic radiation with wavenumber ranging from 13000 – 10 cm^{-1} (corresponding wavelength from 0.78 – 1000 μm). Infrared region is further divided into three subregions: near-infrared (13000 – 4000 cm^{-1} or 0.78 – 2.5 μm), mid-infrared (4000 – 400 cm^{-1} or 2.5 – 25 μm) and far-infrared (400 – 10 cm^{-1} or 25 – 1000 μm). The most commonly used is the middle infrared region, since molecules can absorb radiations in this region to induce the vibrational excitation of functional groups. Recently, applications of near infrared spectroscopy have also been developed.

By passing infrared light through a sample and measuring the absorption or transmittance of light at each frequency, an infrared spectrum is obtained, with peaks corresponding to the frequency of absorbed radiation. Since all groups have their characteristic vibrational frequencies, information regarding molecular structure can be gained from the spectrum. Infrared spectroscopy is capable of analyzing samples in almost any phase (liquid, solid, or gas), and can be used alone or in combination with other instruments following different sampling procedures. Besides fundamental vibrational modes, other factors such as overtone and combination bands, Fermi resonance, coupling and vibration-rotational bands also appear in the spectrum. Due to the high information content of its spectrum, infrared spectroscopy has been a very common and useful tool for structure elucidation and substance identification.

4.1 Objectives

- Recognize which bonds give useful bands on an IR spectrum.
- List the bands that you should look for in the spectrum of each functional group.
- Identify the area of the spectrum where you should look for a particular band.
- Identify the important bands and functional group of the spectrum of an unknown compound.

4.2 Applications of organic compounds

1. Hydrocarbons C-H and C-C stretching and bending vibrations

(i) Alkanes: In simple hydrocarbons, only two types of atoms - C and H and only two types of bonds – C-C and C-H are present. The C-H starching vibrations usually occur in the general region between 3300 cm-1 (in alkynes) and 2700 cm-1 (in aldehydes).

A hydrocarbon containing a methyl group usually shows two distinct bands, one at 2960 cm-1 due to asymmetric stretching and the other at 2870 cm-1 due to symmetric stretching. The C-H bonds in methylene group undergo number of stretching and bending vibrations as shown in figure 14. The two stretching vibrations – asymmetrical and symmetrical occur at 2925 cm-1 and appear in the spectrum within a range of + 10 cm-1.The C-H bending vibrations of the methyl groups in the hydrocarbons normally occur at 1450 and 1375 cm-1. The band at 1375 cm-1 is due to methyl on the carbon atom and is quite sensitive to the electronegativity of the substituent present at the methyl group. It shifts from as high as

1475 cm-1 in CH3-F to as low as 1150 cm-1 in CH3-Br. However, this band is extremely useful in detecting the presence of methyl group in a compound because it is sharp and of medium intensity and is rarely overlapped by absorptions due to methylene or methine deformations. The intensity of this band usually increases with the number of methyl groups in the compound. However, the presence of two or more methyl groups on one aliphatic carbon atom (isopropyl or t-butyl groups) results in splitting of this band due to in-phase or out-of phase interactions of the two symmetrical methyl deformations.

In case of methylene group, C-H bending vibrations such as scissoring, twisting, wagging and rocking normally appear at fairly different frequencies. If two or more CH2 groups are present, the usually strong scissoring and rocking absorption bands appear at 1465 and 720 cm-1,respectively. Whereas weak bands due to twisting and wagging vibrations appear at 1250 + 100 cm-1. So, the scissoring absorption band of methylene around 1465 cm-1 often overlaps with asymmetric bending vibration of methyl at 1450 cm-1.

In cyclic aliphatic hydrocarbons, the C-H stretching frequencies are the same (2800 – 3000 cm-1) as in the case of acyclic compounds, if the ring is unstrained. However, methylene scissoring bands shift slightly to smaller wavenumber (1470 cm-1 in hexane and 1448 cm-1 in cyclohexane, see figure 15). In sterically strained cyclic compounds, the C-H stretching normally occurs at slightly higher wavenumber e.g. 3080 -3040 cm-1 in cyclopropane.

The C-C bond vibrations appear as weak bands in 1200-800 cm-1 region and are seldom used for structural study. Whereas the C-C bending absorptions occur at < 500 cm-1 and are usually below the range of IR – instrument.

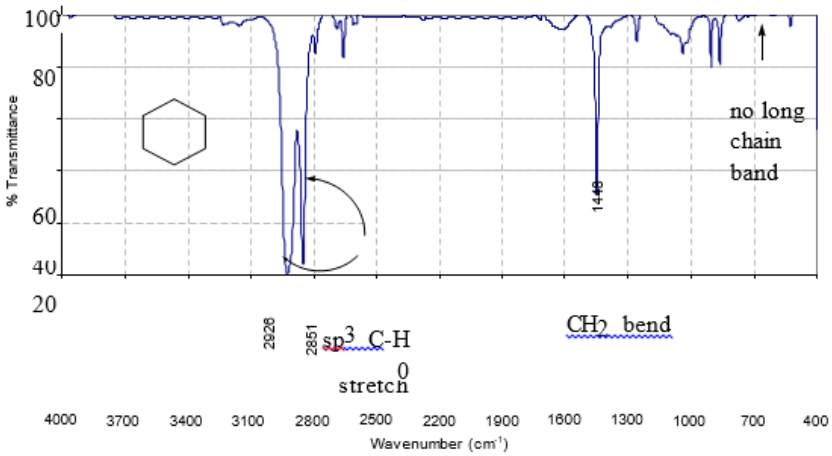


Figure 15 : *The infrared spectrum of cyclohexane (neat liquid)*

(ii) Alkenes: The carbon-carbon double bond has a higher force constant than a C-C single bond and in a non-conjugated olefin, C=C stretching vibrations appear at higher frequency (1680-1620 cm-1) than that of the C-C starching vibrations (1200-800 cm-1).

In completely symmetrical alkenes, such as ethylene, tetrachloroethylene etc., C=C stretching band is absent, due to lack of change in dipole moment in completely symmetrical molecule. On the other hand, non-symmetrically substituted double bonds exhibit strong absorption bands. The absorption bands are more intense for cis isomers than for trans isomers; for mono or tri substituted olefins than for di and tetra substituted ones. Also, terminal olefins show stronger C=C double bond stretching vibrations than internal double bonds. Similarly C=C groups conjugated with certain unsaturated group show stronger band than for non-conjugated ones. In
case of olefins, conjugated with an aromatic ring, the C=C stretching appears at 1625 cm-1 (s)
and an additional band at ~1600 cm-1 is observed due to aromatic double bond. In compounds containing both olefinic and alkyl C-H bonds, the bands above 3000 cm-1 are generally attributed to aromatic or

aliphatic C-H stretching, whereas between 3000-2840 cm-1 are generally assigned
to the alkyl C-H stretching.

The absorption frequency of a double bond in a cyclic ring is very sensitive to ring size (figure
16). The absorption frequency decreases as the internal angle decreases and is lowest in cyclobutene (90o angle). The frequency increases again for cyclopropane.

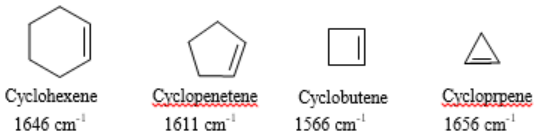


Figure 16 : *C=C vibration frequencies of cycloalkenes*

The exocyclic double bonds exhibit an increase in frequency with decrease in ring size (figure 17). The exocyclic double bond on six-membered ring absorbs at 1651 cm^{-1} and it is shifted to
1780 cm^{-1} in case of exocyclic double bond on cyclopropane. The allenes show the highest double bond absorptions at 1940 cm^{-1}.

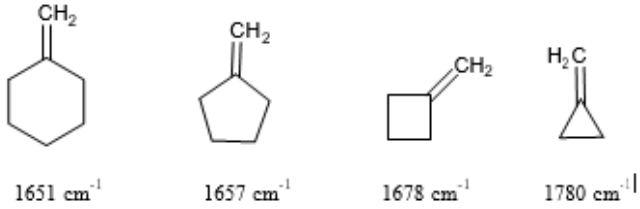


Figure 17 : *Exocyclic C=C double bond frequencies in various ring sizes*

(iii) Alkynes : All alkynes both terminal (R– C≡ CH) or non-terminal (R– C ≡ CR) contain carbon
– carbon triple bond but the non-terminal alkynes also contain a C – H bond. The force constant for a triple bond is grater than that for a double bond. Consequently, whereas a C-C stretching vibrations occur between 1300-800 cm^{-1} and the C=C stretching vibration occur in the region 1700-1500 cm^{-1}, the C ≡ C vibrations are observed at significantly higher frequencies in the region of 2300 to 2050 cm^{-1}.

The terminal alkynes show weak triple bond stretching vibration at 2140-2050 cm^{-1}, whereas the unsymmetrically disubstituted alkynes show a triple bond absorption at 2260-2190 cm^{-1}. The acetylene C-H stretching vibrations are normally observed as a sharp characteristic band in the region 3310-3200 cm^{-1} and the acetylenic C-H bending vibrations occur in the region 600-650 cm^{-1}.

Therefore the frequency of the absorption of C-H bond is a function of the type of hybridization of the carbon to which hydrogen atom is attached. While moving from sp^3 to sp^2 and sp hybridized carbons, the s-character increases and so is the bond strength (force constant0 of C-H bond and the frequency of absorption (Table 7).

(iv) Aromatic Hydrocarbons: In the aromatic compounds, the most prominent bands are due to out-of-plane bending of ring C-H bonds in the region of 900-650 cm^{-1}. These bands can be used to assign the ring substitution pattern in mono substituted benzenes and 1,2-, 1,3-, and 1,4- substituted benzene derivatives. Mono substituted benzene derivatives exhibit strong absorption band near 690 cm^{-1} (see IR spectrum of toluene, figure 18). The absence of this band indicates the absence of mono substituted phenyl rings in the sample. A second strong band appears at
~750 cm^{-1}. 1,2-Disubstituted benzenes give one strong absorption band at ~ 750 cm^{-1}. 1,3- Disubstituted rings give three absorption bands at ~690, ~780 and ~ 880 cm^{-1}. 1,4-Disubstituted
rings give one strong absorption band in the region 800-850 cm^{-1} (strong absorption band at 831
cm^{-1} is seen in IR spectrum of t-butylphenol, figure 22). The spectra of aromatic compounds typically exhibit many weak or medium intensity C-H stretching vibrations in the region 3100-
3030 cm^{-1}, the
region of olefinic
compounds.

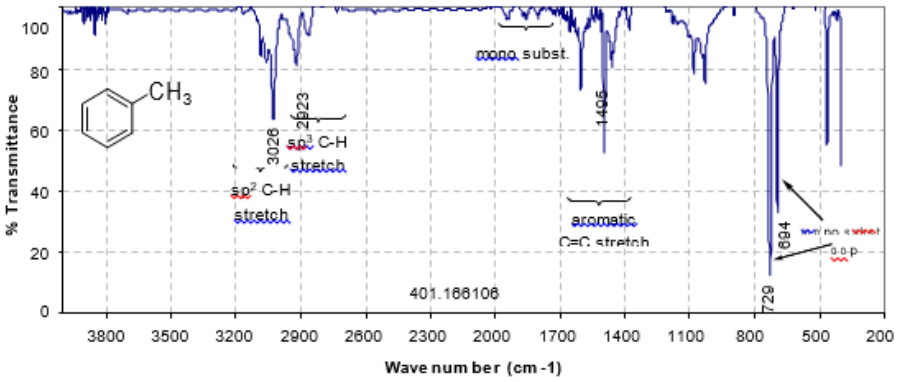


Figure 18.: The infrared spectrum of toluene (neat liquid)

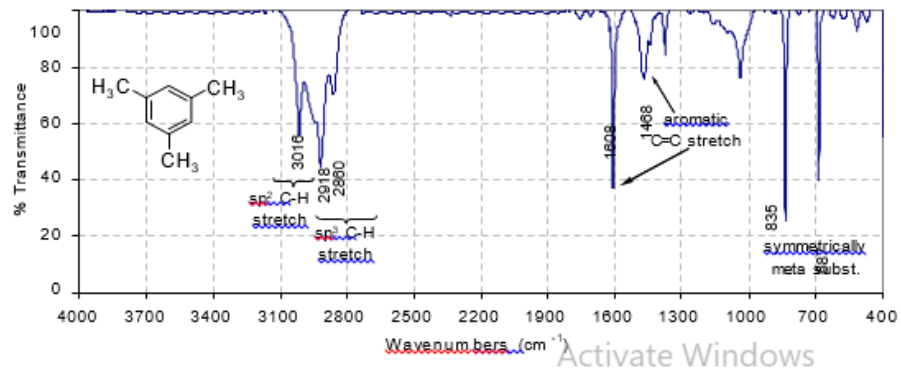


Figure 19.: The infrared spectrum of mesitylene (neat liquid)

The bands considered to be of most help in diagnosing the aromatic character of the compound appear in the region 1650-1400 cm-1. There are normally four bands in this region at about 1600,
1585, 1500 and 1450 cm-1 and are due to C=C in-plane vibrations (see spectra in figures 18 and
19). The combination and overtone bands in 2000-1650 cm-1 region are also characteristics of aromatic rings. Moreover, they are very weak and are observed only in the case of concentrated
solutions of highly symmetric benzene derivatives.

2. Alcohols and Phenols

When a hydrogen atom from an aliphatic hydrocarbon is replaced by an OH group, new bands corresponding to new OH and C-O band absorption appear in the IR spectrum. A medium to strong absorption band from 3700 to 3400 cm-1 (see IR spectra of 1-butanol and t-butylphenol in figures 21 and 22) is a strong indication that the sample is an alcohol or phenol (The presence of
NH or moisture causes similar results). The exact position and shape of this band depends largely on the degree of H-bonding. A strong, sharp peak in the region as higher 3700 cm-1 in gaseous or extremely dilute solutions represents unbounded or free OH group(s).

Alcohols and phenols in condensed phases (bulk liquid, KBr discs, concentrated solution etc.) are strongly hydrogen bonded, usually in the form of dynamic polymeric association; dimmers, trimers, tetramers etc. (Figure 20) and cause broadened bands at lower frequencies. The hydrogen bonding involves a lengthening of the original O-H bond. This bond is consequently weakened, force constant is reduced and so the stretching frequency is lowered.

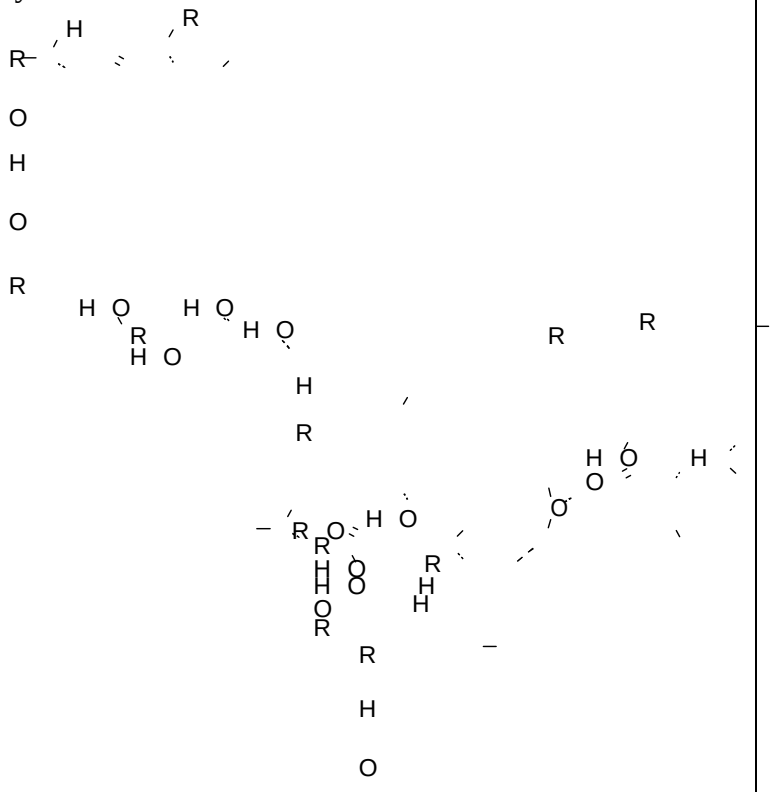


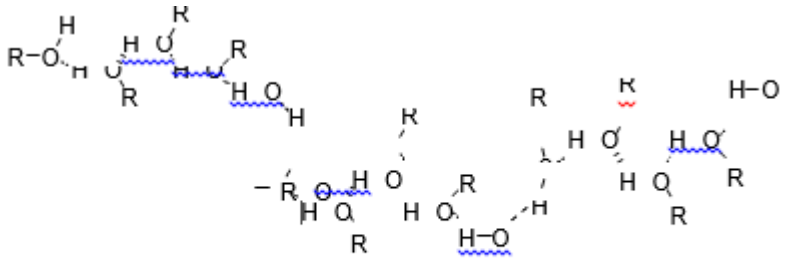
Figure 20 : Polymeric association of ROH

The C-O stretching in phenols / alcohols occurs at a lower frequency range 1250-1000 cm-1. The coupling of C-O absorption with adjacent C-C stretching mode, makes it possible to differentiates between primary (~1050 cm-1), secondary (~1100 cm-1) and tertiary (~1150 cm-1) alcohols and phenols (~1220 cm-1).

3 Enols and Chelates

Hydrogen bonding in enols and chelates viz. acetyl acetone and methyl salicylate (figure 23), is particularly strong and the observed O-H

stretching frequency may be very low (2880 cm-1). Since these bonds are not easily broken on dilution by an inert solvent, free O-H may not be seen at low concentrations. In structures, such as 2,6-di-t-butylphenol, in which steric hindrance prevents hydrogen bonding, no bounded O-H band is observed, not even in spectra of neat samples.



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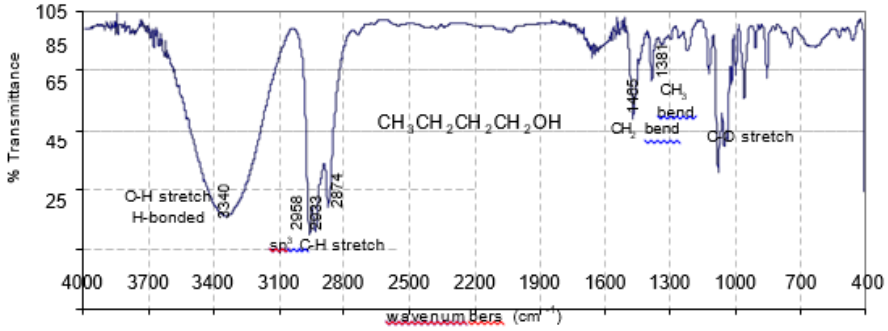

Figure 21 : *The infrared spectrum of 1-butanol (neat liquid)*

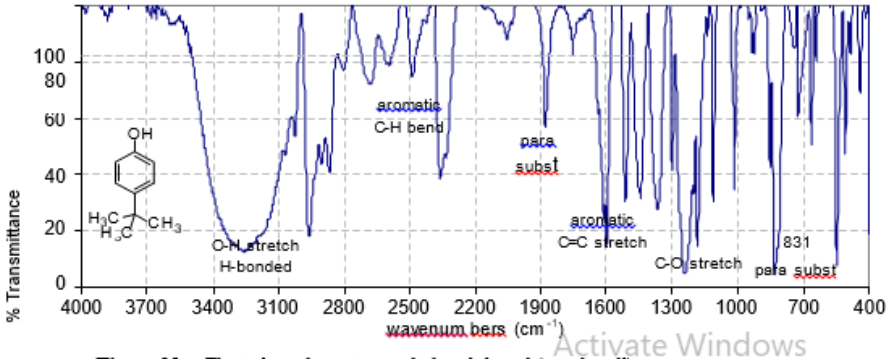

Figure 22 : *The infrared spectrum of t-butylphenol (nujol mull)*

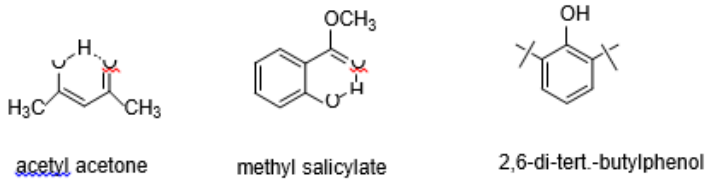

Figure 23 : H-boned structures of acetyl acetone and methyl salicylate and sterically hindered 2,6-di-tert.-butylphenol

4. Ethers and Epoxides

In the spectra of aliphatic ethers, the most characteristic absorption is a strong band in the 1150-1000 cm-1 region because of asymmetric C-O-C stretching, but a band in this region is also observed in other oxy compounds like alcohols, aldehydes, ketones, acids etc. Therefore, we consider the possibility that a compound is ether or an epoxide only if the unknown oxy compound shows no absorption bands in O-H (3750-3000 cm-1) or carbonyl (1850-1550 cm-1)regions.

The conjugation of ether with carbon-carbon double bond or phenyl ring shifts the C-O-C symmetric stretching to ~1250 cm-1. The resonance increases the bond order from single to partial double bond and so higher the force constant and higher the absorption frequency (figure 24).

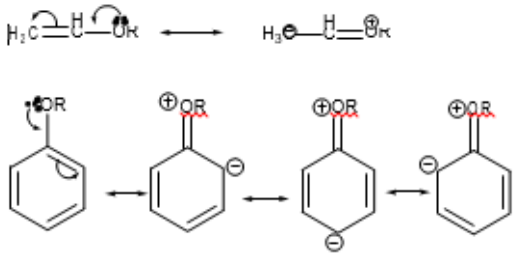

Figure 24 : The resonance effects in vinyl ethers and aryl alkyl ether

5.Carbonyl Compounds

The absorption peak for C=O stretching in the region 1870 to 1600 cm-1 is perhaps the easiest band to recognize in IR spectrum and is extremely useful in analysis of carbonyl compounds. The changes in C=O stretching frequency in various carbonyl compounds viz. aldehydes, ketones, acids, esters, amides, acid halides, anhydrides etc. can be explained by considering (i) electronic and mass effects of neighboring substituents (ii) resonance effects (both C=C and heteroatom lone pair) (iii) hydrogen bonding (inter and intramolecular) (iv) ring strain etc. It is customary to refer to the absorption frequency of a saturated aliphatic ketone at 1715 cm-1 as normal value and changes in the environment of the carbonyl group can either lower or raise the absorption frequency from the "normal" value.

(i) Inductive and Resonance Effects: The replacement of an alkyl group of the saturated aliphatic ketone by a heteroatom (O, N) shifts the C=O stretching frequencies due to inductive and resonance effects. In esters, the oxygen due to inductive effect withdraws the electrons from carbonyl group (figure 25) and increases the C=O bond strength and thus the frequency of absorption. In amides, due to the conjugation of lone pair of electrons on nitrogen atom, the resonance effect increases the C=O bond length and reduces the C=O absorption frequency. Therefore, C=O absorption frequencies due to resonance effects in amides are lowered but due to inductive effect in esters are increased than those observed in ketones.

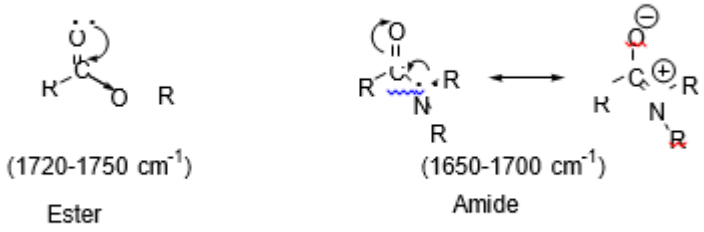

Figure 25 : *Inductive and resonance effects in ester and amide groups*

In acid chlorides, the halogen atom strengthens the C=O bond through inductive effect and shifts the C=O stretching frequencies even higher than are found in esters. The acid anhydrides give two bands in C=O stretching frequency region due to symmetric (~1820 cm-1) and asymmetric (~1760 cm-1) stretching vibrations (f

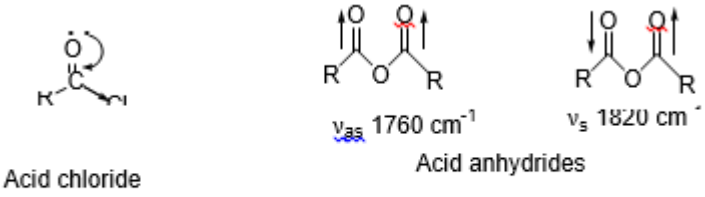

Figure 26 : *Inductive effect in acid chloride and C=O stretch in anhydride*

(ii) Conjugation Effects: The C=O stretching frequencies for carbon-carbon double bond conjugated systems are generally lower by 25-45 cm-1 than those of corresponding non- conjugated compounds. The delocalization of π-electrons in the C=O and C=C bonds lead to partial double bond character in C=O and C=C bonds and lowers the force constant (figure 27). Greater is the ability of delocalization of electrons, the more is lowering in C=O stretching frequency. In general s-cis conformations absorb at higher frequency than s-trans conformations.

A similar lowering in C=O stretching frequency occurs when an aryl ring is conjugated with carbonyl compound.

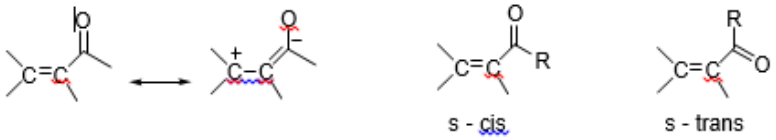

Figure 27 : *Resonance effects and s-cis and s-trans structures in enones*

(iii) Ring Size Effects:

Six-membered rings with carbonyl group e.g. cyclohexanone absorb at normal value i.e. 1715 cm-1. Decrease in ring size increases the C=O stretching frequency. Smaller rings require the use of more p- character to make C-C bonds for the requisite small angles. This gives more s character to the C=O sigma bond and thus results in strengthening of C=O double bond.

The comparison of C=O stretching frequencies of various compounds in figure 28 shows that in ketones and esters, ~ 30 cm-1 increase in frequency occurs on moving to one carbon lower ring.

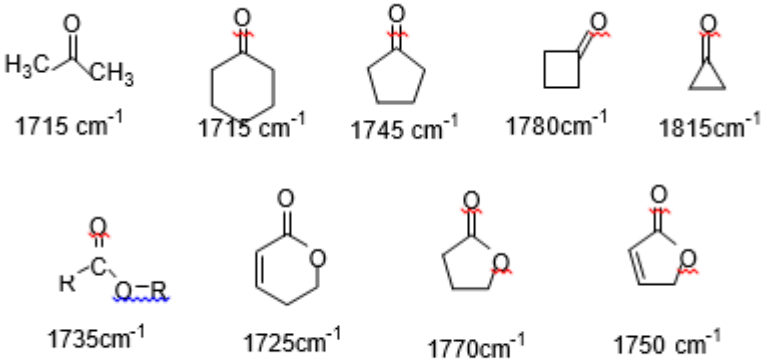

Figure 28 : *C=O stretching frequencies in various compounds*

(iv) Hydrogen Bonding Effects: Hydrogen bonding to a C=O group withdraws electrons from oxygen and lowers the C=O double bond character. This results in lowering of C=O absorption frequency. More effective is the hydrogen bonding, higher will be the lowering in C=O absorption frequencies.

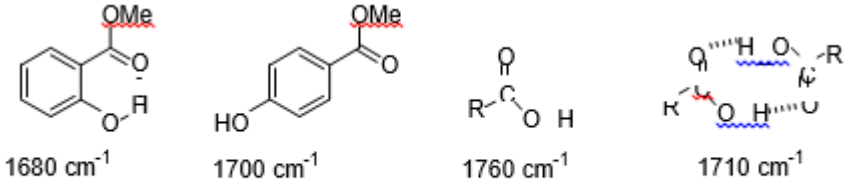

Figure 29 : *H-bonding effects on C=O stretch*

The monomeric carboxylic acids (in very dilute solutions) absorb at ~1760 cm^{-1}. The dimerization of carboxylic acids in their concentrated solutions or in solid state lowers the carboxyl carbonyl frequency to 1710 cm^{-1}. The more effective intramolecular hydrogen bonding in methyl salicylate lowers the C=O stretching frequency to 1680 cm^{-1} than observed at 1700 cm^{-1} in case of methyl *p*-hydroxybenzoate.

a. Aldehydes and Ketones

Aliphatic aldehydes show strong C=O stretching in the region of 1740 – 1725 cm^{-1}. The conjugation of an aldehyde to a C=C or a phenyl group lowers C=O stretching by ~ 30 cm^{-1}. This effect is seen in benzaldehyde in which aryl group is attached directly to the carbonyl group and shifts C=O stretch to 1701 cm^{-1} (see IR spectrum of benzaldehyde, figure 30). Aldehyde C-H stretching vibrations appear as a pair of weak bands between 2860-2800 and 2760-2700 cm^{-1}. The higher C-H stretching band (2860-2800 cm^{-1}) of aldehyde is often buried under aliphatic C- H band. But the lower C-H band at 2760-2700 cm^{-1} is usually used to distinguish aldehydes from ketones. The C-H bending vibrations appear between 945-780 cm^{-1}.

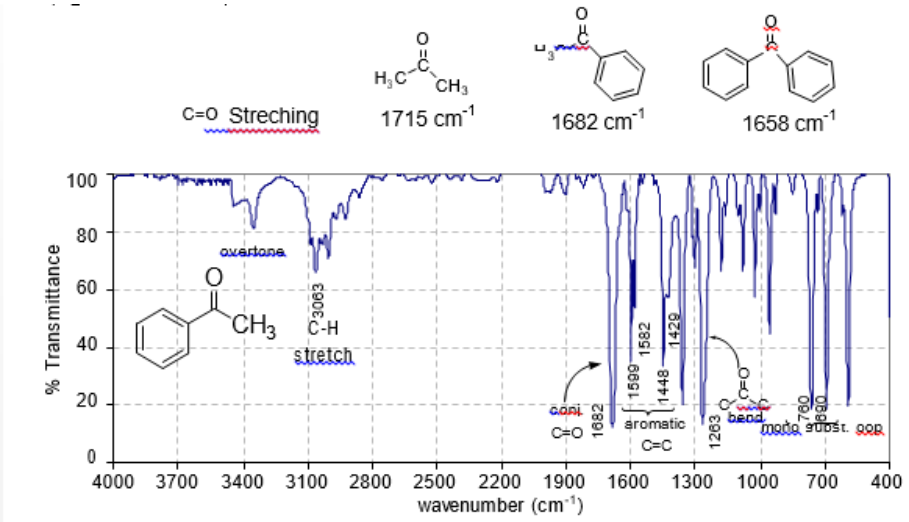

Figure 31 : *The infrared spectrum of acetophenone (neat liquid)*

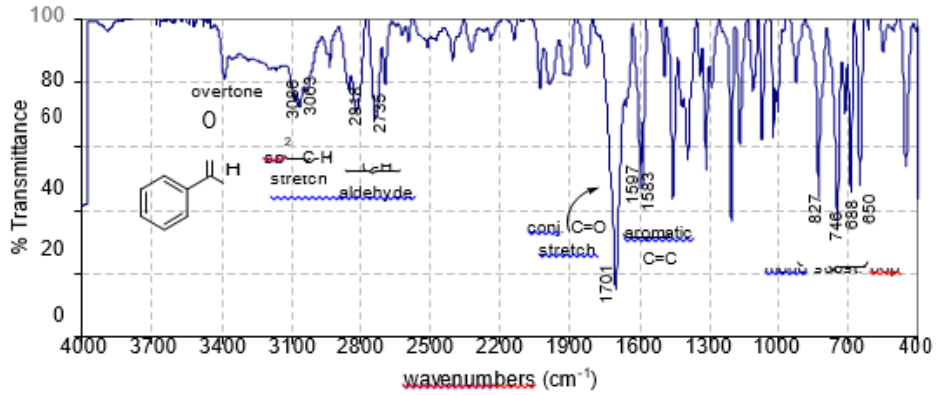

Figure 30 : *The infrared spectrum of benzaldehyde (neat liquid)*

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The aliphatic acyclic ketones show C=O stretching between 1720 to 1700 cm-1 which is shifted to lower frequencies by 20-30 cm-1 on conjugation with C=C or phenyl ring. The presence of two conjugated groups as in benzophenone further lowers the C=O stretching frequency to 1665 cm-1 (figures 31 and 32).

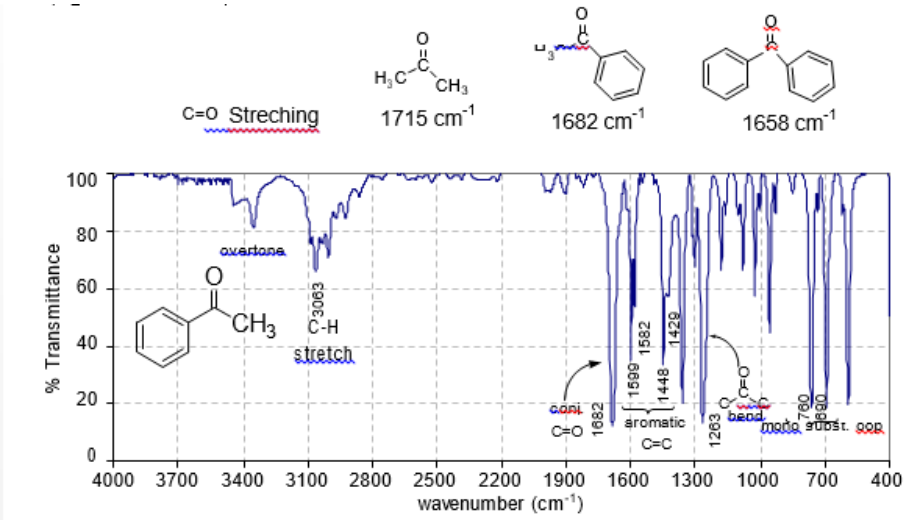

Figure 31 : *The infrared spectrum of acetophenone (neat liquid)*

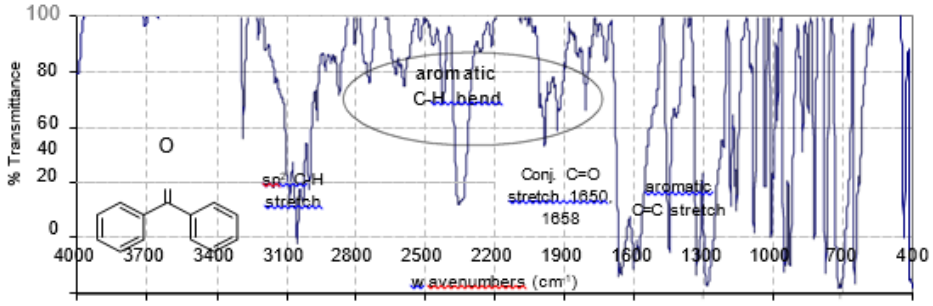

Figure 32 : *The infrared spectrum of benzophenone (nujol mull)*

In case of cyclic ketones, the coupling between C=O stretching and C(=O)-C single bond causes increase in C=O stretching frequency as the C-C(=O) angle decreases (figure 28).

b. Carboxylic Acids, Esters and Carboxylates

In case of carboxylic acids, in solid state or pure liquid state, the intermolecular hydrogen bonding weakens the C=O bond and thus lower the stretching frequency to ~1720 cm-1. The O-H stretch appears as a very broad band between 3400 – 2500 cm-1 (see IR spectrum of benzoic acid, figure 33). The appearance of strong C=O stretching along with broad hydroxyl peak centered at

~ 3000 cm-1 in an IR spectrum certainly shows the presence of carboxylic acid. In addition a medium intensity C=O stretch appears between 1320 – 1260 cm-1. In dilute solutions, the

carboxylic acids attain monomeric structures and the inductive effect of oxygen shifts the C=O

absorption band to higher values (1760 – 1730 cm-1) than observed in ketones.

c. Acid Chlorides and Anhydrides

Both carboxylic acid halides and anhydrides show strong C=O absorptions at characteristically high frequencies > 1800 cm-1 and are easily differentiated form other carbonyl compounds.

The acid anhydrides show two absorption bands in carbonyl region at 1820 cm-1 due to symmetric and at 1760 cm-1 due to asymmetric stretching vibrations. In case of anhydrides of conjugated carboxylic acids, the frequencies due to these bands are shifted to 1775 and 1720 cm-1. The effect of conjugation is clearly visible in IR spectrum of benzoyl anhydride in figure 37. The strong and broad C-O stretching vibrations appear in the region 1300 – 900 cm-1. In case of acid chlorides, the C=O stretching frequencies appear at 1810-1790 cm-1 which is attributed to high electronegativity of chlorine.

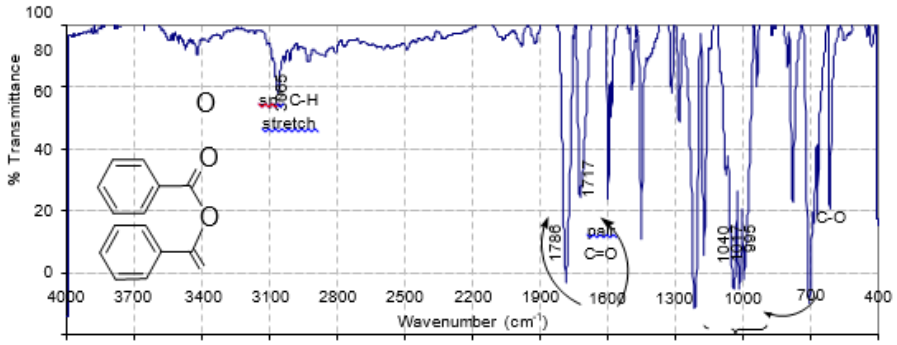

Figure 37 : *The infrared spectrum of benzoyl anhydride (nujol mull)*

d. Amides

In case of amides strong resonance participation of lone pair of electrons by amide nitrogen weakens the carbonyl bond. Consequently, the C=O stretching frequency in amides appears in the range 1680-1630 cm-1 i.e. 20-50 cm-1 lower than that of C=O stretching of ketones. The C=O stretching band in IR spectra of amide is called amide I band. In primary and secondary amides, NH deformation band appears in the region 1655 - 1595 cm-1 and is called amide II band.

In the solid or pure liquid state, primary amides, which are highly hydrogen bonded, exhibit two N-H stretching bands, one at 3550 cm-1 due to N-H asymmetric stretching and other at 3180 cm-1 due to N-H symmetric stretching. In dilute solutions, due to lowering in degree of hydrogen bonding, the absorption bands shift to higher frequencies at 3500 and 3400 cm-1, respectively. The secondary amides show only one N-H band at ~ 3300 cm-1. The comparison of double and triple bond stretching vibrations has been given in table 8.

Thus, the vibrational frequencies provide important structural information about a compound and since two same type of bonds in two different compounds would vibrate at different frequencies and so no two compounds can have exactly same infrared spectrum especially in the finger printing region. This makes IR spectroscopy a simple and versatile tool for identification of samples.

4.4 Check Your Progress

1. Explain how the size of the atoms in the bond, the bond order, and the type of vibration affect the frequency of light absorbed by a bond vibration.

2. Explain how dipole moment and number of bonds will affect the amount of light absorbed by a bond vibration.
3. Demonstrate the four bending vibrations of a CH_2 group.
4. Explain what causes organic molecules to absorb IR light.
5. Explain why some bonds do not absorb IR light.

4.5 Answers To Check Your Progress Questions

1. **Infrared Spectroscopy:** Triggering molecular vibrations through irradiation with infrared light. Provides mostly information about the presence or absence of certain functional groups.

2. **Vibrational modes:** Covalent bonds can vibrate in several modes, including stretching, rocking, and scissoring. The most useful bands in an infrared spectrum correspond to stretching frequencies, and those will be the ones we'll focus on.
3. **IR active bonds:** Strongly polar bonds such as carbonyl groups (C=O) produce strong bands. Medium polarity bonds and asymmetric bonds produce medium bands. Weakly polar bond and symmetric bonds produce weak or non observable bands.

4.6 Summary

- Using IR spectroscopy, specific bands may fall over a range of wavenumbers, cm^{-1}. Specific substituents may cause variations in absorption frequencies.
- Absorption intensities may be stronger or weaker than expected, often depending on dipole moments. Additional bands may confuse the interpretation.
- In very symmetrical compounds there may be fewer than the expected number of absorption bands (it is even possible that all bands of a functional group may disappear, i.e. a symmetrically substituted alkyne!).
- Infrared spectra are generally informative about what functional groups are present, but not always.

4.7 Keywords

1. **Bond angle:** Bond angle is simply the angle between two bonds or two bonded electron pairs in a compound

2. **Vibrational frequencies:** The typical frequencies of molecular motions, known as the vibrational frequencies, range from less than 10^{13} Hz to approximately 10^{14} Hz, corresponding to wavenumbers of approximately 300 to 3000 cm^{-1}. A fundamental vibration is evoked when one such quantum of energy is absorbed by the molecule in its ground state.
3. **Hydrogen bond:** A weak bond between two molecules resulting from an electrostatic attraction between a proton in one molecule and an electronegative atom in the other

4.8 Self-assessment questions and exercises

1. Identify important differences between spectra of compounds with different functional groups.

2. Use IR spectra to evaluate the success of a reaction.

3. List the bands that you should look for in the spectrum of each functional group.

4. Identify the area of the spectrum where you should look for a particular band.

4.9 Further readings

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BLOCK II: NMR SPECTROSCOPY

UNIT – V: ^{1}H NMR SPECTROSCOPY

Structure

5.0 Introduction:

Theoretical principles:

Nuclear Magnetic Resonance spectroscopy is a powerful and theoretical complex analytical tool. In NMR, the experiments are performing on the nuclei of atoms, not the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei.

Nuclear spin and the splitting of energy levels in a magnetic field

Subatomic particles (electrons, protons and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C) these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in some atoms (such as ^{1}H and ^{13}C) the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows;

1. If the number of neutrons and the number of protons are both even, then the nucleus has NO spin.
2. If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2).
3. If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3)

The overall spin, I, is important. Quantum mechanics tells us that a nucleus of spin I will have 2I + 1 possible orientation. A nucleus with spin 1/2 will have 2 possible orientations. In the absence of an external magnetic field, these orientations are of equal energy. If a magnetic field is applied, then the energy levels split. Each level is given a magnetic quantum number, m.

When the nucleus is in a magnetic field, the initial populations of the energy levels are determined by thermodynamics, as described by the Boltzmann distribution. This is very important, and it means that the lower energy level will contain slightly more nuclei than the higher level. It is possible to excite these nuclei into the higher level with electromagnetic radiation. The frequency of radiation needed is determined by the difference in energy between the energy levels.

Calculating transition energy:

The nucleus has a positive charge and is spinning. This generates a small magnetic field. The nucleus therefore possesses a magnetic moment, m, which is proportional to its spin, I. The constant, g, is called the gyromagnetic ratio and is a fundamental nuclear constant which has a different value for every nucleus. h is Planck's constant.

The energy of a particular energy level is given by;

$$E = \frac{\gamma h}{2\pi} mB$$

Where B is the strength of the magnetic field at the nucleus. The absorption of radiation by a nucleus in a magnetic field.

Imagine a nucleus (of spin 1/2) in a magnetic field. This nucleus is in the lower energy level (i.e. its magnetic moment does not oppose the applied field). The nucleus is spinning on its axis. In the presence of a magnetic field, this axis of rotation will precess around the magnetic field. The frequency of precession is termed the Larmor frequency, which is identical to the transition frequency.

The potential energy of the precessing nucleus is given by;

$$E = - m B \cos q$$

Where, q is the angle between the direction of the applied field and the axis of nuclear rotation.

If energy is absorbed by the nucleus, then the angle of precession, q, will change. For a nucleus of spin 1/2, absorption of radiation "flips" the magnetic moment so that it opposes the applied field (the higher energy state).

It is important to realize that only a small proportion of "target" nuclei are in the lower energy state (and can absorb radiation). There is the possibility that by exciting these nuclei, the populations of the higher and lower energy levels will become equal. If this occurs, then there will be no further absorption of radiation. The spin system is saturated. The possibility of saturation means that we must be aware of the relaxation processes which return nuclei to the lower energy state.

5.1 Objectives

- To Know how nuclear spins are affected by a magnetic field, and be able to explain what happens when radiofrequency radiation is absorbed and also be able to predict the number of proton and carbon NMR signals expected from a compound given its structure.

Relaxation processes:

Emission of radiation is insignificant because the probability of re-emission of photons varies with the cube of the frequency. At radio frequencies, re-emission is negligible.

If the relaxation rate is fast, then saturation is reduced. If the relaxation rate is too fast, line-broadening in the resultant NMR spectrum is observed.

There are two major relaxation processes;

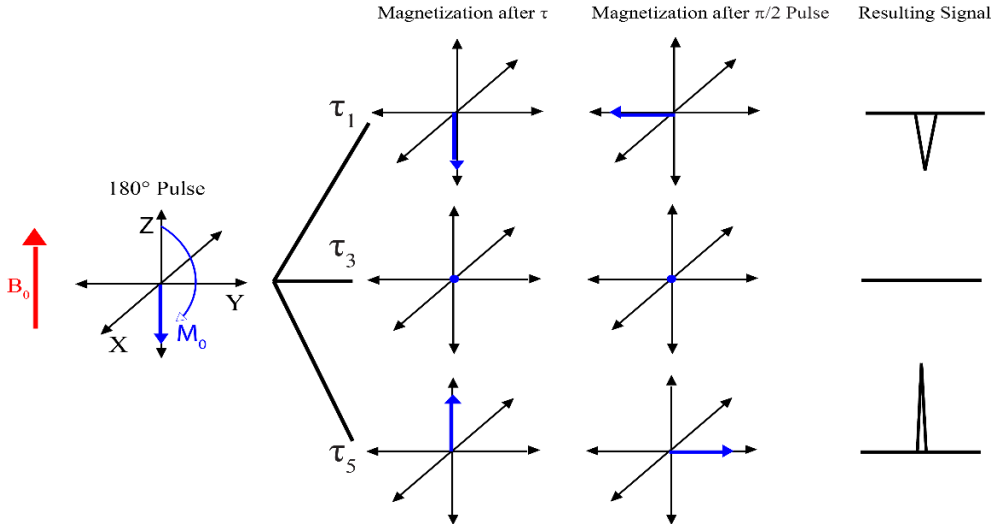
Spin - lattice (longitudinal) relaxation

Spin - spin (transverse) relaxation

Spin - lattice (longitudinal) relaxation:

Nuclei in an NMR experiment are in a sample. The sample in which the nuclei are held is called the lattice. Nuclei in the lattice are in vibrational and rotational motion, which creates a complex magnetic field. The magnetic field caused by motion of nuclei within the lattice is called the

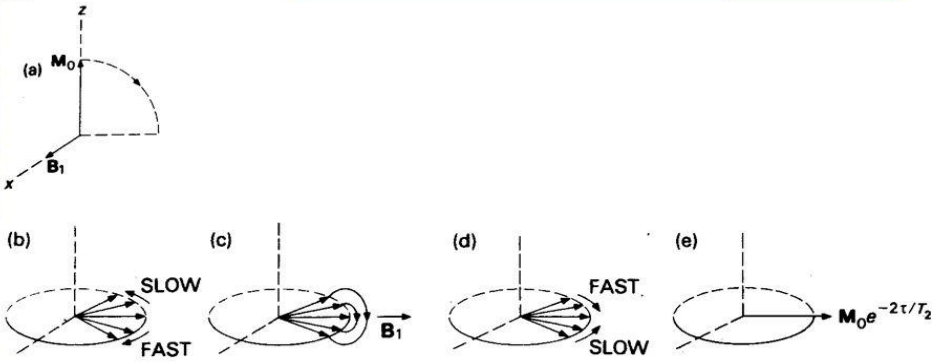
lattice field. This lattice field has many components. Some of these components will be equal in frequency and phase to the Larmor frequency of the nuclei of interest. These components of the lattice field can interact with nuclei in the higher energy state, and cause them to lose energy (returning to the lower state). The energy that a nucleus loses increases the amount of vibration and rotation within the lattice (resulting in a tiny rise in the temperature of the sample). The relaxation time, T1 (the average lifetime of nuclei in the higher energy state) is dependent on the gyromagnetic ratio of the nucleus and the mobility of the lattice. As mobility increases, the vibrational and rotational frequencies increase, making it more likely for a component of the lattice field to be able to interact with excited nuclei. However, at extremely high mobility, the probability of a component of the lattice field being able to interact with excited nuclei decreases.



Spin - spin relaxation:

Spin - spin relaxation describes the interaction between neighbouring nuclei with identical precessional frequencies but differing magnetic quantum states. In this situation, the nuclei can exchange quantum states; a nucleus in the lower energy level will be excited, while the excited nucleus relaxes to the lower energy state. There is no net change in the populations of the energy states, but the average lifetime of a nucleus in the excited state will decrease. This can result in line-broadening.

Spin-spin relaxation (Transverse) T_2

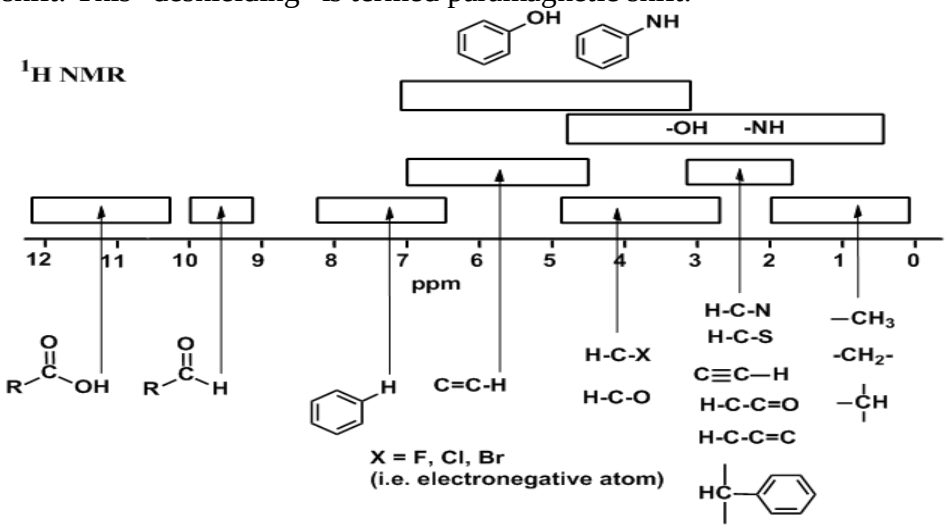


Chemical shift:

The magnetic field at the nucleus is not equal to the applied magnetic field; electrons around the nucleus shield it from the applied field. The difference between the applied magnetic field and the field at the nucleus is termed the nuclear shielding.

Consider the s-electrons in a molecule. They have spherical symmetry and circulate in the applied field, producing a magnetic field which opposes the applied field. This means that the applied field strength must be increased for the nucleus to absorb at its transition frequency. This upfield shift is also termed diamagnetic shift.

Electrons in p-orbitals have no spherical symmetry. They produce comparatively large magnetic fields at the nucleus, which give a low field shift. This "deshielding" is termed paramagnetic shift.



Chemical shift:

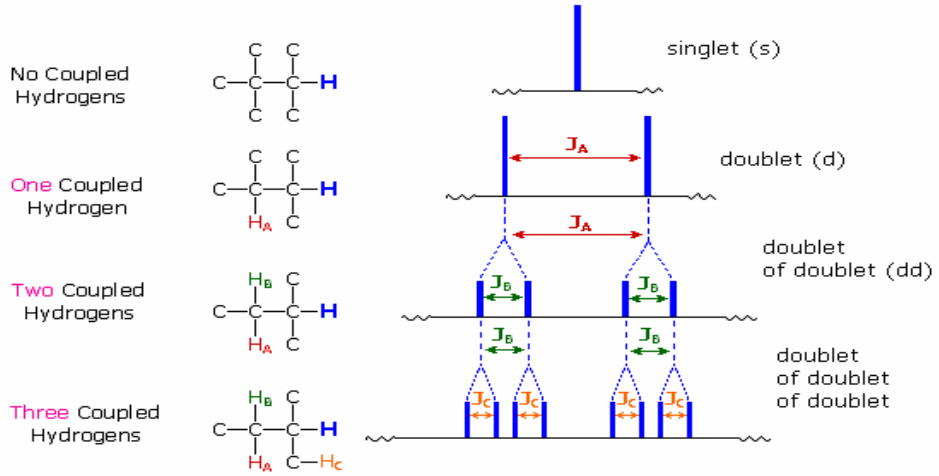
$$\delta = \frac{\text{(Frequency shift from } Me_4Si \text{ in Hz)}}{\text{(Spectrometer frequency, MHz)}}$$

In proton (^1H) NMR, p-orbitals play no part (there aren't any!), which is why only a small range of chemical shift (10 ppm) is observed. We can easily see the effect of s-electrons on the chemical shift by looking at substituted methane, CH_3 X. As X becomes increasingly electronegative, so the electron density around the protons decreases, and they resonate at lower field strengths (increasing dH values).

Chemical shift is defined as nuclear shielding / applied magnetic field. Chemical shift is a function of the nucleus and its environment. It is measured relative to a reference compound. For ^1H NMR, the reference is usually tetramethylsilane, Si $(CH_3)_4$.

Spin - spin coupling:

Consider the structure of ethanol, the ^1H NMR spectrum of ethanol (below) shows the methyl peak has been split into three peaks (a triplet) and the methylene peak has been split into four peaks (a quartet). This occurs because there is a small interaction (coupling) between the two groups of protons. The spacing between the peaks of the methyl triplet are equal to the spacing between the peaks of the methylene quartet. This spacing is measured in Hertz and is called the coupling constant, J.



To see why the methyl peak is split into a triplet, let's look at the methylene protons. There are two of them, and each can have one of two possible orientations (aligned with or opposed against the applied field). This gives a total of four possible states;

In the first possible combination, spins are paired and opposed to the field. This has the effect of reducing the field experienced by the methyl protons; therefore a slightly higher field is needed to bring them to resonance, resulting in an upfield shift. Neither combination of spins opposed to each other has an effect on the methyl peak. The spins paired in the direction of the field produce a downfield shift. Hence, the methyl peak is split into three, with the ratio of areas 1:2:1.

Similarly, the effect of the methyl protons on the methylene protons is such that there are eight possible spin combinations for the three methyl protons;

Out of these eight groups, there are two groups of three magnetically equivalent combinations. The methylene peak is split into a quartet. The areas of the peaks in the quartet have the ration 1:3:3:1.

In a first-order spectrum (where the chemical shift between interacting groups is much larger than their coupling constant), interpretation of splitting patterns is quite straightforward;

The multiplicity of a multiplet is given by the number of equivalent protons in neighbouring atoms plus one, i.e. the n + 1 rule

Equivalent nuclei do not interact with each other. The three methyl protons in ethanol cause splitting of the neighbouring methylene protons; they do not cause splitting among themselves

The coupling constant is not dependant on the applied field. Multiplets can be easily distinguished from closely spaced chemical shift peaks.

Chemical Shifts

The NMR spectra is displayed as a plot of the applied radio frequency versus the absorption. The applied frequency increases from left to right, thus the left side of the plot is the low field, downfield or deshielded side and the right side of the plot is the high field, upfield or shielded side.

NMR spectra:

The position on the plot at which the nuclei absorbs is called the chemical shift. Since this has an arbitrary value a standard reference point must be used. The two most common standards are TMS (tetramethylsilane, $(Si(CH_3)_4)$ which has been assigned a chemical shift of zero, and $CDCl_3$ (deuterochloroform) which has a chemical shift of 7.26 for ^1H NMR and 77 for ^{13}C NMR.The scale is commonly expressed as parts per million (ppm) which is independent of the spectrometer frequency. The scale is the delta (δ) scale.

Delta scale:

The range at which most NMR absorptions occur is quite narrow. Almost all ^1H absorptions occur downfield within 10 ppm of TMS. For ^{13}C NMR almost all absorptions occurs within 220 ppm downfield of the C atom in TMS.

Shielding in NMR:

Structural features of the molecule will have an effect on the exact magnitude of the magnetic field experienced by a particular nucleus. This means that H atoms which have different chemical environments will have different chemical shifts. This is what makes NMR so useful for structure determination in organic chemistry. There are three main features that will affect the shielding of the nucleus, electronegativity, magnetic anisotropy of π systems and hydrogen bonding.

Factors affecting proton NMR:

Electronegativity:

The electrons that surround the nucleus are in motion so they created their own electromagnetic field. This field opposes the applied magnetic field and so reduces the field experienced by the nucleus. Thus the electrons are said to shield the nucleus. Since the magnetic field experienced at the nucleus defines the energy difference between spin states it also defines what the chemical shift will be for that nucleus. Electron with-drawing groups can decrease the electron density at the nucleus, deshielding the nucleus and result in a larger chemical shift. Compare the data in the table below.

Compound,	CH_3X	CH_3F	CH_3OH	CH_3Cl	CH_3Br	CH_3I	CH_4	$(CH_3)_4Si$
Electronegativity of X		4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift δ (ppm)		4.26	3.4	3.05	2.68	2.16	0.23	0

As can be seen from the data, as the electronegativity of X increases the chemical shift, δ increases. This is an effect of the halide atom pulling the electron density away from the methyl group. This exposes the nuclei of both the C and H atoms, "deshielding" the nuclei and shifting the peak downfield.

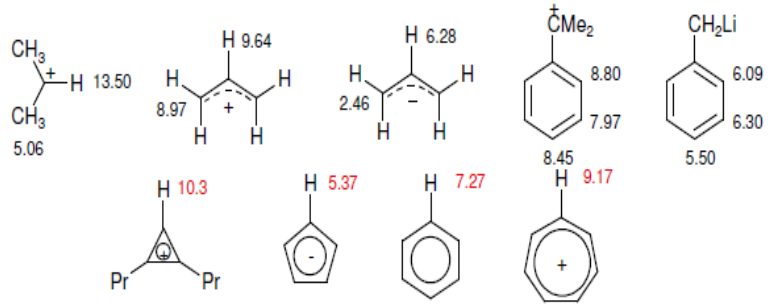
The effects are cumulative so the presence of more electron withdrawing groups will produce a greater deshielding and therefore a larger chemical shift, shown figure

These inductive effects are not only felt by the immediately adjacent atoms, but the deshielding can occur further down the chain, shown figure

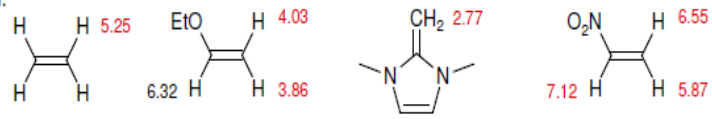
This is especially useful in the interpretation of the NMR chemical shift of protons in aromatic systems. The protons ortho and para to electron donating and electron withdrawing substituents show distinct upfield and downfield shifts.

CH_3F	CH_3Cl	CH_3Br	CH_3I	CH_3CH_3	CH_4	CH_3SiMe_3	CH_3Li
4.26	3.05	2.69	2.19	0.96	0.2	0.0	-2.1

The chemical shifts of protons attached to sp^2 hybridized carbons also reflect charges within the π system (approximately 10 ppm/unit negative or positive charge).



Even without formal charges, resonance interactions can lead to substantial chemical shift changes due to π polarization.



Magnetic Anisotropy: π Electron Effects

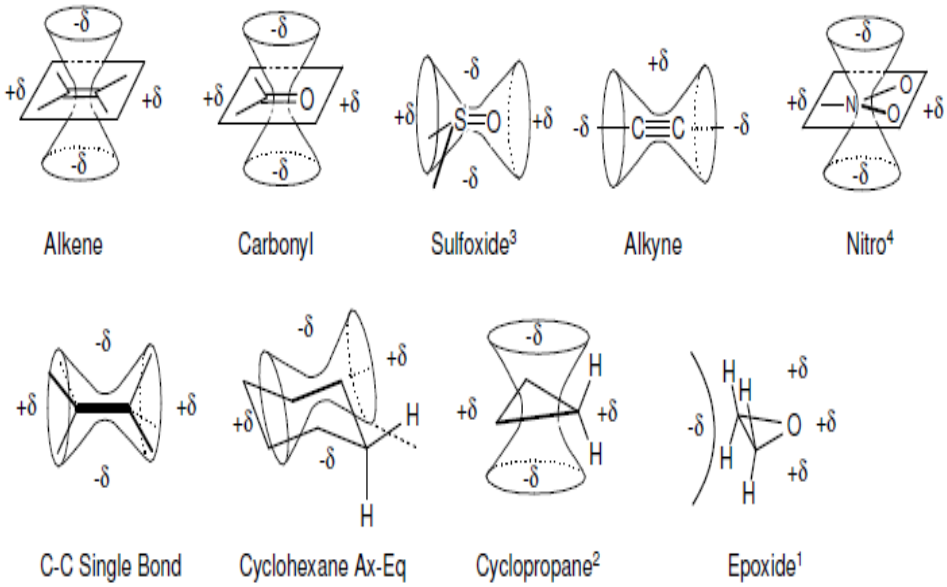
The π electrons in a compound, when placed in a magnetic field, will move and generate their own magnetic field. The new magnetic field will have an effect on the shielding of atoms within the field.

Magnetic anisotropy of benzene:

This effect is common for any atoms near a π bond, i.e.

Proton Type	Effect	Chemical shift (ppm)
C_6H_5 -H	highlydeshielded	6.5 - 8
C=C-H	deshielded	4.5 - 6
C≡C-H	shielded*	~2.5
O=C-H	very highly deshielded	9 - 10

* the acetylene H is shielded due to its location relative to the π system.



Hydrogen Bonding Effects on Chemical Shifts - OH, NH and SH Protons:

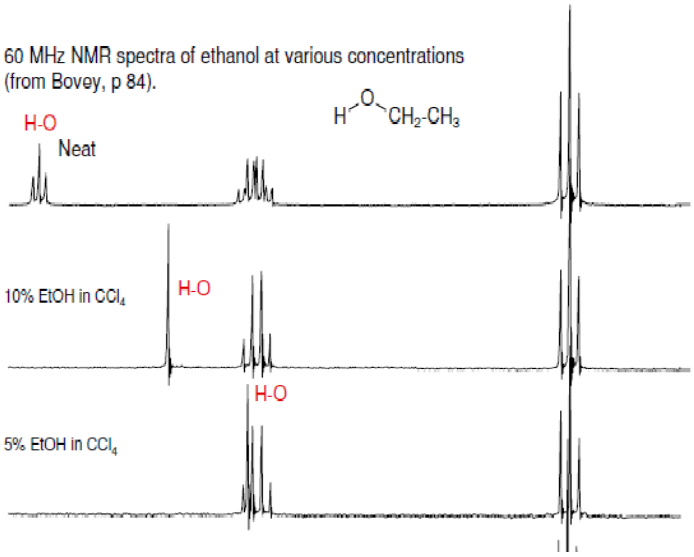
The chemical shifts of OH and NH protons vary over a wide range depending on details of sample preparation and substrate structure. The shifts are very strongly affected by hydrogen bonding, with strong downfield shifts of H-bonded groups compared to free

OH or NH groups. Thus OH signals tend to move downfield at higher substrate concentration because of increased hydrogen bonding. Both OH and NH signals move downfield in H-bonding solvents like DMSO or acetone.

There is a general tendency for the more acidic OH and NH protons to move further downfield. This effect is in part a consequence of the stronger H-bonding propensity of acidic protons, and in part an inherent chemical shift effect. Thus carboxylic amides and sulfonamides NH protons are shifted well downfield of related amines, and OH groups of phenols and carboxylic acids are downfield of alcohols.

Alcohol OH Protons. In dilute solution of alcohols in non-hydrogen-bonding solvents (CCl_4 , $CDCl_3$, C_6D_5) the OH signal generally appears at δ 1-2 At higher concentrations the signal moves downfield, e.g. the OH signal of ethanol comes at δ 1.0 in a 0.5% solution in CCl_4 , and at δ 5.13 in the pure liquid.

Hydroxyl OH Protons. In dilute solution of alcohols in non hydrogen-bonding solvents (CCl_4, $CDCl_3$, C_6D_5) the OH signal generally appears at δ 1-2 At higher concentrations the signal moves downfield as a result of increased fraction of H-bonded alcohols, e.g. the OH signal of ethanol comes at δ 1.0 in a 0.5% solution in CCl_4, and at δ 5.13 in the pure liquid (from Bovey).



Spin-spin coupling:

Indirect spin-spin coupling (indirect dipole-dipole interaction, J-coupling) - a magnetic interaction between individual nuclear spins transmitted by the bonding electrons through which the nuclear spins are indirectly connected.

Chemically and magnetically equivalent nuclei:

Magnetically equivalent nuclei possess the same resonance frequency and only one characteristic spin-spin interaction with the nuclei of a neighboring group.

The spin-spin coupling between magnetically equivalent nuclei does not appear in the spectrum. Nuclei with the same resonance frequency are called chemically equivalent or isochronous. Chemically equivalent nuclei will not be magnetically equivalent if they have different couplings to other nuclei in the molecule.

Magnetic equivalence causes great simplification in the resulting NMR spectra, but those cases where nuclei are chemically equivalent but not magnetically equivalent give complicated spectra in which second-order effects are prevalent.

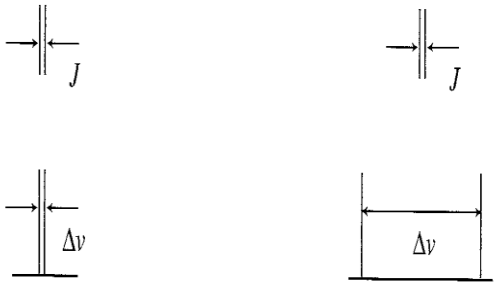
Notation for spin systems:

A spin system includes nuclei between which spin-spin interaction exists and defines the number and type of magnetic nuclei and the relationship between them. Each nucleus (spin ½) is assigned a capital letter of the Roman alphabet:

- If the chemical shift difference between a pair of nuclei is much greater than the coupling constant between them, they are assigned letters well apart in the alphabet (AX, AA'XX', etc.)
- if a chemical shift difference is of the order of or less than the corresponding coupling constant, adjacent letters of the alphabet are used for the two nuclei involved (AB, AA'BB', etc.)

First-order rules ($\Delta\nu$/ J >> 1):

- For nuclei with I=1/2 the multiplicity of the splitting equals n+1, where n is the number of nuclei in the neighbouring group (for I > 1/2, 2nI+1).



Strong coupling, second-order spectra ($\Delta v/J$ small)

Weak coupling, first-order spectra ($\Delta v/J$ large)

Pascal triangle:

Singlet	1
Doublet	1 1
Triplet	1 2 1
Quartet	1 3 3 1
Quintet	1 4 6 4 1
Sextet	1 5 10 10 5 1
Septet	1 6 15 20 15 6 1

3 Pascal's triangle.

Second-order (or strong coupling) effects:

Chemical shift difference Δv

Spin-spin coupling constant J

Zero-order spectrum (no coupling) $\Delta v/ J = \infty$

First-order spectrum ("weak" coupling) $\Delta v/ J >> 1$

Second-order spectrum ("strong" coupling) $\Delta v / J \approx 1$

When $\Delta v / J \approx 1$, the effects due to J-coupling and chemical shift have similar energies. This leads to alterations in relative line intensities and in line positions. The intensity of the lines nearest to the multiplet of the neighbouring group is greatly enhanced while that of other lines decreases ("roof' effect). Generally, more lines are observed in the second-order spectrum than one would expect for the corresponding first-order spectrum. The perturbation of the spectra from the first-order appearance is a function of the ratio $\Delta v /J = v\partial /J$ and at a high enough frequency many second-order spectra approach their first-order limit.

Factors affecting spin-spin coupling:

Spin-spin coupling over one bond 1J

Geminal spin-spin coupling 2J

Vicinal spin-spin coupling 3J

Long-range spin-spin coupling nJ, $n \geq 4$

Spin-spin coupling constants are not easy to predict theoretically, and depend on a number of factors:

(i) the hybridization of the atoms involved in the coupling;

(ii) the bond angles;

(iii) the dihedral angles;

(iv) the C - C bond length;

(v) substituent effects (electronegativity, neighbouringπbond and lone pair effects);

Instrumentation:

Some types of atomic nuclei act as though they spin on their axis similar to the Earth. Since they are positively charged they generate an electromagnetic field just as the Earth does. So, in effect, they will act as tiny bar magnetics. Not all nuclei act this way, but fortunately both ^{1}H and ^{13}C do have nuclear spins and will respond to this technique.

NMR Spectrometer

In the absence of an external magnetic field the direction of the spin of the nuclei will be randomly oriented (see figure below left). However, when a sample of these nuclei is place in an external magnetic field, the nuclear spins will adopt specific orientations much as a compass needle responses to the Earth's magnetic field and aligns with it. Two possible orientations are possible, with the external field (*i.e.* parallel to and in the same direction as the external field) or against the field (*i.e.* antiparallel to the external field). See figure below right.

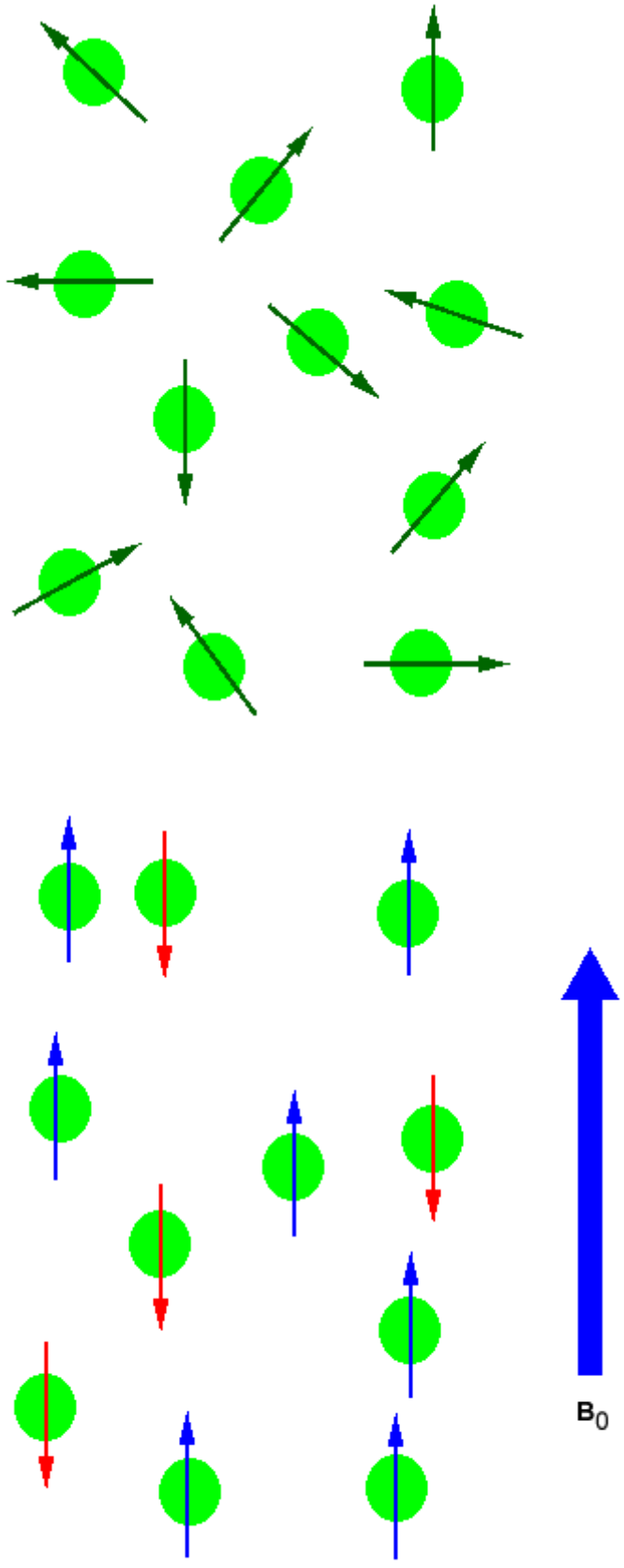
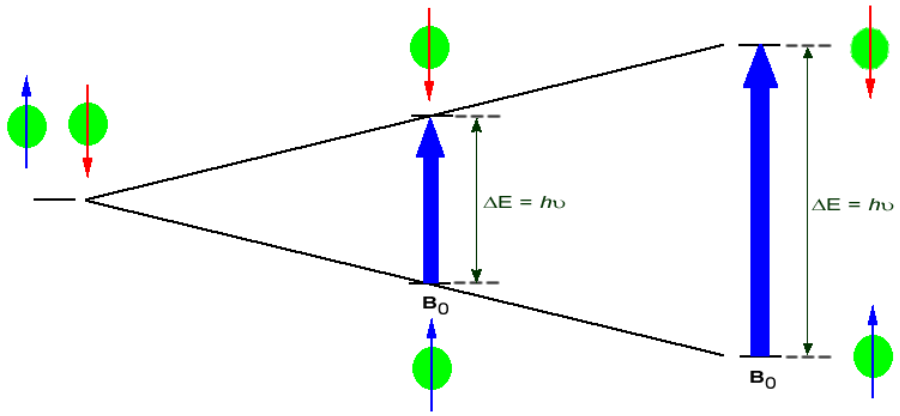


Figure 1: (Left) Random nuclear spin without an external magnetic field. (Right)Ordered nuclear spin in an external magnetic field

If the ordered nuclei are now subjected to EM radiation of the proper frequency the nuclei aligned with the field will absorb energy and "spin-flip" to align themselves against the field, a higher energy state. When this spin-flip occurs the nuclei are said to be in "resonance" with the field, hence the name for the technique, **N**uclear **M**agentic **R**esonance or NMR.

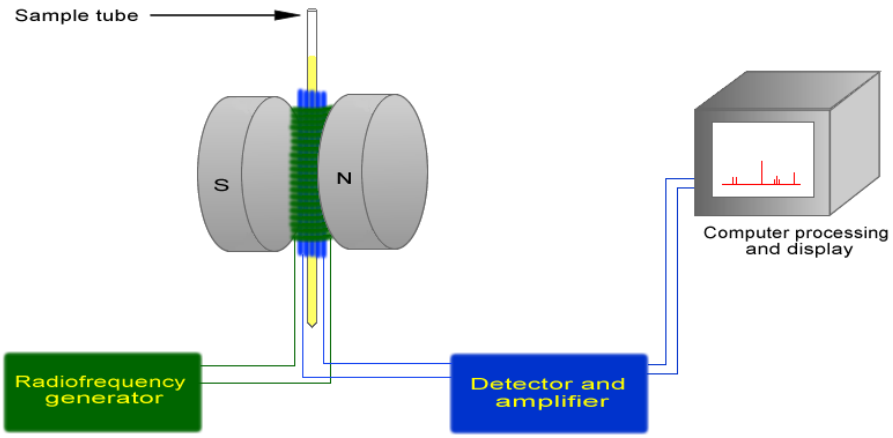
The amount of energy, and hence the exact frequency of EM radiation required for resonance to occur is dependent on both the strength of the magnetic field applied and the type of the nuclei being studied. As the strength of the magnetic field increases the energy difference between the two spin states increases and a higher frequency (more energy) EM radiation needs to be applied to achieve a spin-flip (see image below).



Superconducting magnets can be used to produce very strong magnetic field, on the order of 21 tesla (T). Lower field strengths can also be used, in the range of 4 - 7 T. At these levels the energy required to bring the nuclei into resonance is in the MHz range and corresponds to radio wavelength energies, *i.e.* at a field strength of 4.7 T 200 MHz bring ^{1}H nuclei into resonance and 50 MHz bring ^{13}C into resonance. This is considerably less energy then is required for IR spectroscopy, ~10^{-4} kJ/mol versus ~5 - ~50 kJ/mol.

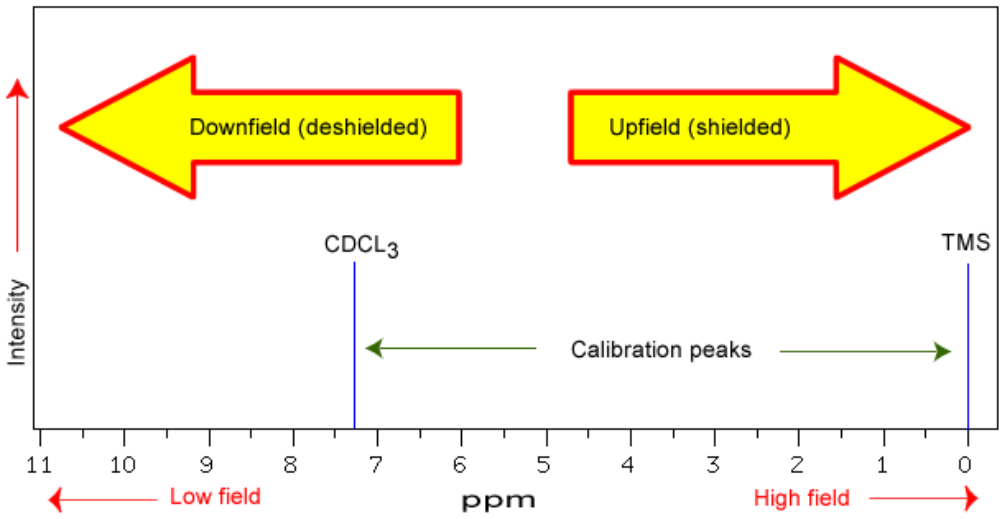
^{1}H and ^{13}C are not unique in their ability to undergo NMR. All nuclei with an odd number of protons (^{1}H, ^{2}H, ^{14}N, ^{19}F, ^{31}P ...) or nuclei with an odd number of neutrons (*i.e.* ^{13}C) show the magnetic properties required for NMR. Only nuclei with even number of both protons and neutrons (^{12}C and ^{16}O) do not have the required magnetic properties.

The basic arrangement of an NMR spectrometer is displayed below. A sample (in a small glass tube) is placed between the poles of a strong magnetic. A radio frequency generator pulses the sample and excites the nuclei causing a spin-flip. The spin flip is detected by the detector and the signal sent to a computer where it is processed.



Chemical Shifts

The NMR spectra is displayed as a plot of the applied radio frequency versus the absorption. The applied frequency increases from left to right, thus the left side of the plot is the low field, downfield or deshielded side and the right side of the plot is the high field, upfield or shielded side (see the figure below). The concept of shielding will be explained shortly.



The position on the plot at which the nuclei absorbs is called the **chemical shift**. Since this has an arbitrary value a standard reference point must be used. The two most common standards are TMS (tetramethylsilane, $(Si(CH_3)_4)$ which has been assigned a chemical shift of zero, and $CDCl_3$ (deuterochloroform) which has a chemical shift of 7.26 for ^{1}H NMR and 77 for ^{13}C NMR.

The scale is commonly expressed as parts per million (ppm) which is independent of the spectrometer frequency. The scale is the **delta (δ) scale**.

$$\delta = \frac{\text{frequency of signal - frequency of standard}}{\text{spectrometer frequency}} \times 10^6$$

The range at which most NMR absorptions occur is quite narrow. Almost all ^{1}H absorptions occur downfield within 10 ppm of TMS. For ^{13}C NMR almost all absorptions occurs within 220 ppm downfield of the C atom in TMS.

Shielding in NMR

Structural features of the molecule will have an effect on the exact magnitude of the magnetic field experienced by a particular nucleus. This means that H atoms which have different chemical environments will have different chemical shifts. This is what makes NMR so useful for structure determination in organic chemistry. There are three main features that will affect the shielding of the nucleus, electronegativity, magnetic anisotropy of π systems and hydrogen bonding.

Electronegativity

The electrons that surround the nucleus are in motion so they created their own electromagnetic field. This field opposes the the applied magnetic field and so reduces the field experienced by the nucleus. Thus the electrons are said to **shield** the nucleus. Since the magnetic field experienced at the nucleus defines the energy difference between spin states it also defines what the chemical shift will be for that nucleus. Electron with-drawing groups can decrease the electron density at the nucleus, deshielding the nucleus and result in a larger chemical shift. Compare the data in the table below.

Compound, CH_3X	**CH_3F**	**CH_3OH**	**CH_3Cl**	**CH_3Br**	**CH_3I**	**CH_4**	**$(CH_3)_4Si$**
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift δ (ppm)	4.26	3.4	3.05	2.68	2.16	0.23	0

As can be seen from the data, as the electronegativity of X increases the chemical shift, δ increases. This is an effect of the halide atom pulling the electron density away from the methyl group. This exposes the nuclei of both the C and H atoms, "deshielding" the nuclei and shifting the peak downfield.

The effects are cumulative so the presence of more electron withdrawing groups will produce a greater deshielding and therefore a larger chemical shift, *i.e.*

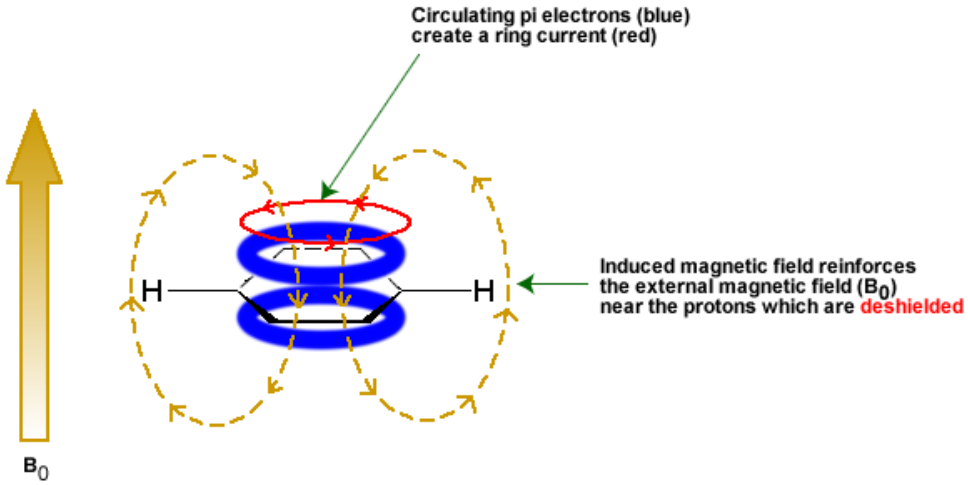
Compound	CH_4	CH_3Cl	CH_2Cl_2	$CHCl_3$
δ (ppm)	0.23	3.05	5.30	7.27

These **inductive effects** are not only felt by the immediately adjacent atoms, but the deshielding can occur further down the chain, *i.e.*

NMR signal	$-CH_2-CH_2-CH_2Br$
δ (ppm)	1.25 1.69 3.30

Magnetic Anisotropy: π Electron Effects

The π electrons in a compound, when placed in a magnetic field, will move and generate their own magnetic field. The new magnetic field will have an effect on the shielding of atoms within the field. The best example of this is benzene (see the figure below).



This effect is common for any atoms near a π bond

Shift reagent:

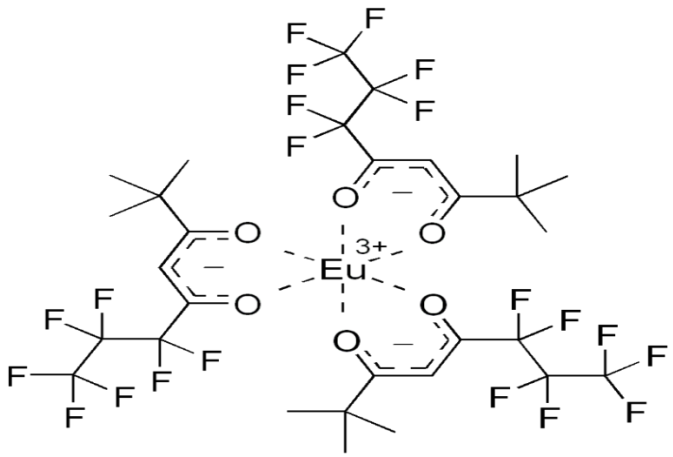
Shift reagents are used in NMR. Spectroscopy to reduce the equivalence of nuclei by altering their magnetic environment, and are of two types:

Aromatic solvents such as benzene or pyridine, and paramagnetic metal complexes. The latter function by coordinating to suitable donor atoms in the compound understudy, thereby expanding their co-ordination shell and

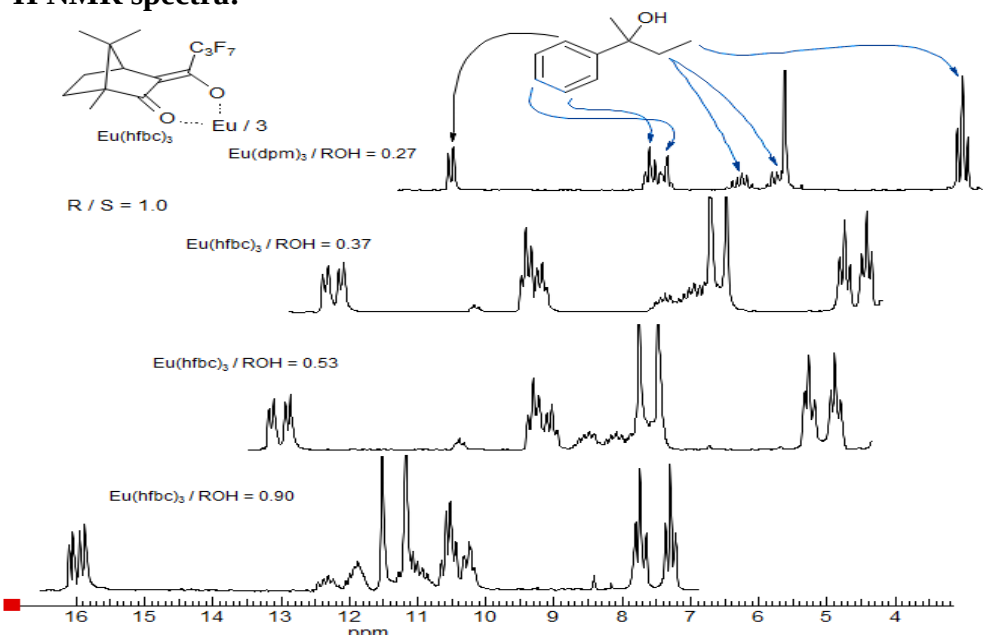
forming a new complex in solution. Apart from effects due to shielding by bonding electrons, the chemical shifts are altered by the paramagnetic metal ion by a transfer of electron spin density, via covalent bond formation, from the metal ion to the associated nuclei (contact shift), or by magnetic effects of the unpaired electron magnetic moment (pseudo contact shift). First-row transition-metal complexes can be used as shift reagents and operate by both contact and pseudo contact mechanisms, although the former predominates owing to the covalent character of these compounds. Unfortunately, these shift reagents exhibit an adverse effect on the resolution of the NMR spectra by causing severe line-broadening. In 1969 Hinckleyl initiated a major advance in this field by introducing the use of a

Lanthanide -metal complex as a shift reagent and since then it has become established that lanthanide complexes produce far less linewidth broadening and give shifts which are caused virtually exclusively by the pseudo contact mechanism. The complexes found most useful are lanthanide acetylacetonate derivatives, some of which are fluorinated and exhibit greater shifting power.The most common practice is to successively add known amounts of the lanthanide shift reagent (LSR) to the compound under study (substrate) and record the n.m.r. spectrum after each addition. The chemical shift of each proton in the substrate alters, to a greater or lesser degree, with each addition of shift reagent and the extent of this lanthanide induced shift (LIS) is measured.

EuFOD is the chemical compound with the formula $Eu(OCC(CH_3)_3 CHCOC_3F_7)_3$, also called $Eu(fod)_3$. This coordination compound is used primarily as a shift reagent in NMR spectroscopy. The structure of lanthanide shift reagent.



^{1}H NMR spectra:



The proton NMR spectra shows the upper spectra is normally record, the lower spectra is recorded after the addition of lanthanide shift reagent. The spectra is pulled over a much wider range of frequencies, so that is simplified almost to first order. The paramagnetic of europium complex induces enormous shift to higher frequency in the resonance. The use of europium and other lanthanide derivatives a chemical shift reagent or lanthanide shift reagent.

GUIDELINES FOR PREDICTING NUMBER OF SIGNAL:

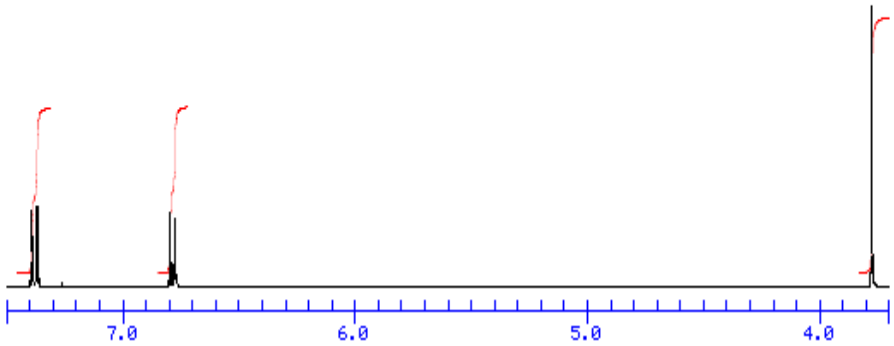
- Proton present in different environment it give different signal.
- Proton which are related by an axis of symmetry are in same environment and give same signal. Because they are homotopic protons. Example **benzene**.
- Protons that are related by plane of symmetry are said to be equivalent proton, they will give one signal. Example **acetone.**
- Protons of enantiomers that will give same signal.
- Enantiotopic proton will give same signal in achiral environment.
- Two protons of CH_2 having diasterotopc relationship with each other when the protons are neither related by plane or axis of symmetry it give different signal.
- NMR predicting signal mainly depend on concentration, temperature, solvent.
- Equivalence in chemical environment due to the rotation of single bond. It give same signal.

5.8 Check Your Progress

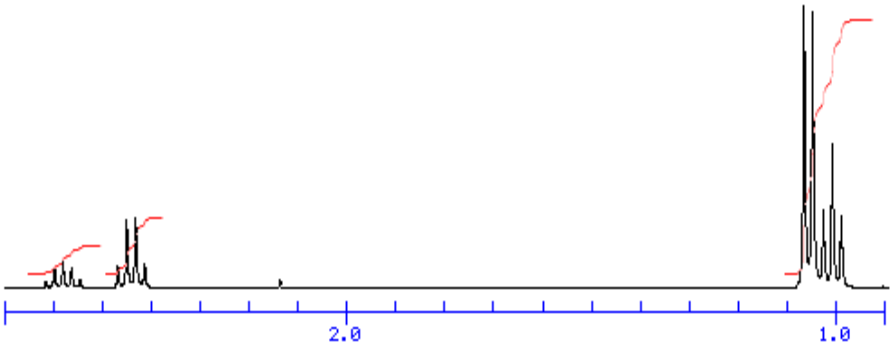
1. What is the purpose of the Fourier transformation?
2. What is FID (free induction decay)?
3. Give a list of main parts of a pulse NMR instrument.
4. What is the spectrum of higher order?
5. What is the zero-order spectrum?
6. Write short note on spin-spin coupling.
7. Discuss the principle and instrumentation of NMR spectroscopy.

5.9 Answers To Check Your Progress Questions

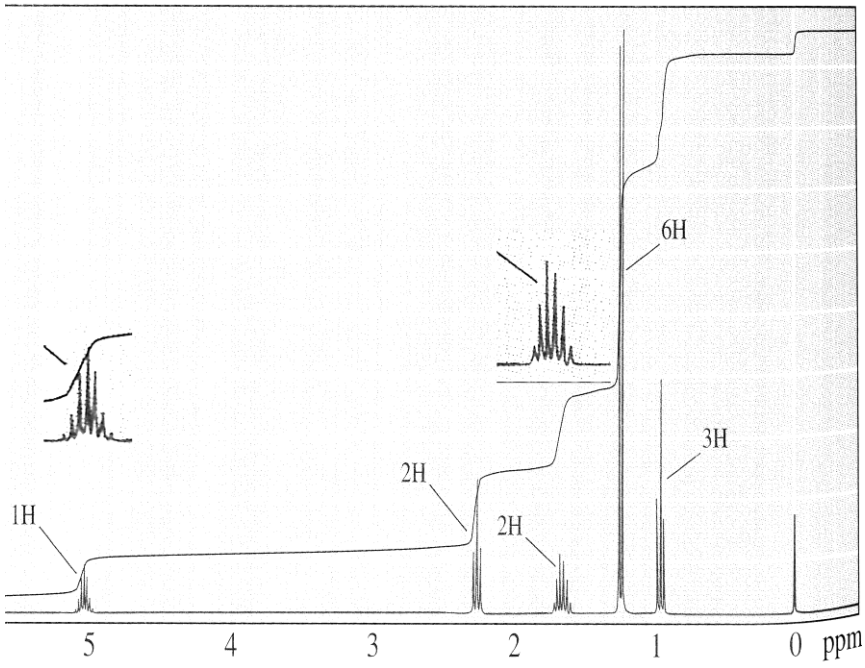
1. Analyze the following NMR spectrum for a molecule with the formula C_7H_7OBr.



2. Analyze the following NMR spectrum for a molecule with the formula $C_6H_{12}O$.



The ^1H NMR spectrum for a compound with the molecular formula $C_7H_{14}O_2$, is shown below. Determine the structure of this compound.



4. What is the connection between a nuclear angular momentum and a nuclear spin quantum number? Write down the equation. The nuclear angular momentum is given by the nuclear spin quantum number.
   $P = p\, I(I+1)\hbar$
   P = Nuclear angular moment
   I = Spin quantum number
   $\hbar = h/2$,
   h = Planck's constant

5. How many values can the magnetic quantum number have? The magnetic quantum number m can have the following values: m = I, I - 1, ... , -I A sum of (2I+1) different values of m.

6. What is the relaxation?
   Immediately after a pulse the spin system will start to revert to its equilibrium state. This is relaxation. Relaxation occurs as transverse (spin-spin) and longitudinal (spin-lattice). The dipoles will dephase, reducing My until zero, restoring their random distribution in precession around the z-axis. Nα and Nβ return to their equilibrium state, gradually increasingM_z

.

5.10 Summary

- First, the chemical shift or location of the peak (in ppm) tells you how deshielded the protons are and hence their "local chemical environment", *i.e.* what possible deshielding groups maybe adjacent to the protons.
- Second, the integration ratios tell you the number of each type of proton in the simplest ratio.
- Third, the spin-spin splitting (coupling pattern or multiplicity) tells you the number of protons on the adjacent C atom. It will be one less than the number of peaks.

- This information combined gives us the basic skeletal structure of the molecule.

5.11 Keywords

Chemical Shift: The effect of the magnetic field (Be f f) on a specific molecule is always less than the applied field (B0). This is due to shielding effect (σ) on the nuclei which is analyzed. As nuclei are shielded differently due to their molecular environment they will give separate resonance signals in the spectrum, creating a specific chemical shift for a specific nuclei. The different Larmour frequencies of the nuclei result in different chemical shifts.

Acquisition time: The time used to obtain the FID per scan (time of relaxation between each pulse).

Fourier transformation: Its purpose is to transform the time domain spectrum, the FID, recorded during analysis into readable data in the frequency domain.

5.12 Self-assessment questions and exercises

1. Identify important differences between spectra of compounds with different functional groups.
2. Use IR spectra to evaluate the success of a reaction.
3. List the bands that you should look for in the spectrum of each functional group.
4. Identify the area of the spectrum where you should look for a particular band.

5.13 Further readings

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UNIT – VI ^{1}H- NMR SPECTRAL TECHNICS

Structure

6.0 Introduction

Over the past fifty years nuclear magnetic resonance spectroscopy, commonly referred to as nmr, has become the preeminent technique for determining the structure of organic compounds. Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Although larger amounts of sample are needed than for mass spectroscopy, nmr is non-destructive, and with modern instruments good data may be obtained from samples weighing less than a milligram. To be successful in using nmr as an analytical tool, it is necessary to understand the physical principles on which the methods are based.

The nuclei of many elemental isotopes have a characteristic spin (I). Some nuclei have integral spins (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. ^{12}C, ^{16}O, ^{32}S, ...). Isotopes of particular interest and use to organic chemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have I = 1/2. Since the analysis of this spin state is fairly straightforward, our discussion of nmr will be limited to these and other I = 1/2 nuclei.

6.1 Objectives:

1. Know how nuclear spins are affected by a magnetic field, and be able to explain what happens when radiofrequency radiation is absorbed.
2. Be able to predict the number of proton and carbon NMR signals expected from a compound given its structure.
3. Be able to predict the splitting pattern in the proton NMR spectrum of a compound given its structure.
4. With the aid of a chart of chemical shifts from 1H and 13C NMR, be able to assign peaks in an NMR spectrum to specific protons in a compound.
5. Be able to interpret integration of NMR spectra.

6. Be able to use NMR spectra to determine the structures of compounds, given other information such as a molecular formula

Double resonance:

Nuclear magnetic resonance decoupling (NMR decoupling for short) is a special method used in nuclear magnetic resonance (NMR) spectroscopy where a sample to be analyzed is irradiated at a certain frequency or frequency range to eliminate fully or partially the effect of coupling between certain nuclei. NMR coupling refers to the effect of nuclei on each other in atoms within a couple of bonds distance of each other in molecules. This effect causes NMR signals in a spectrum to be split into multiple peaks. Decoupling fully or partially eliminates splitting of the signal between the nuclei irradiated and other nuclei such as the nuclei being analyzed in a certain spectrum. NMR spectroscopy and sometimes decoupling can help determine structures of chemical compounds.

Homo nuclear decoupling:

Homo nuclear decoupling is when the nuclei being radio frequency (rf) irradiated are the same isotope as the nuclei being observed (analyzed) in the spectrum. Heteronuclear decoupling is when the nuclei being rf irradiated are of a different isotope than the nuclei being observed in the spectrum. For a given isotope, the entire range for all nuclei of that isotope can be irradiated in broad band decoupling, or only a select range for certain nuclei of that isotope can be irradiated.

Main problems:

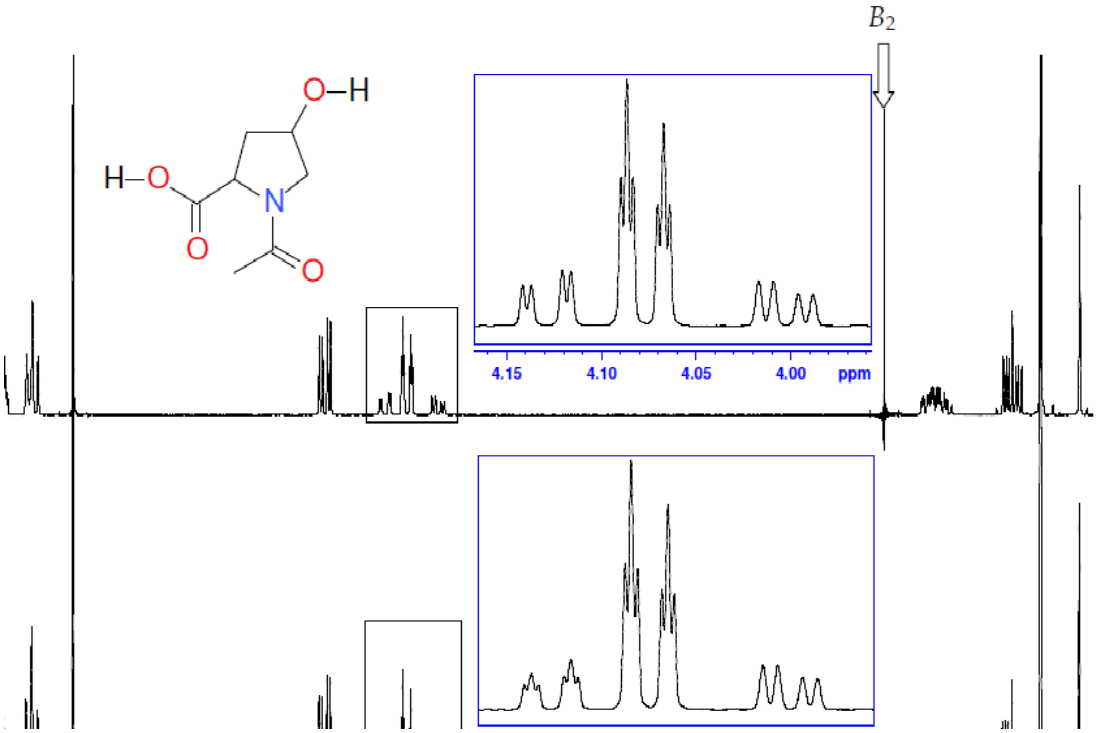
(i) suffers from difficulties associated with selective irradiatiation of only those spins which are desired;

(ii) Bloch-Siegert shift

If several spin-decoupling experiments are desired, it is generally more time-efficient to perform a two dimensional

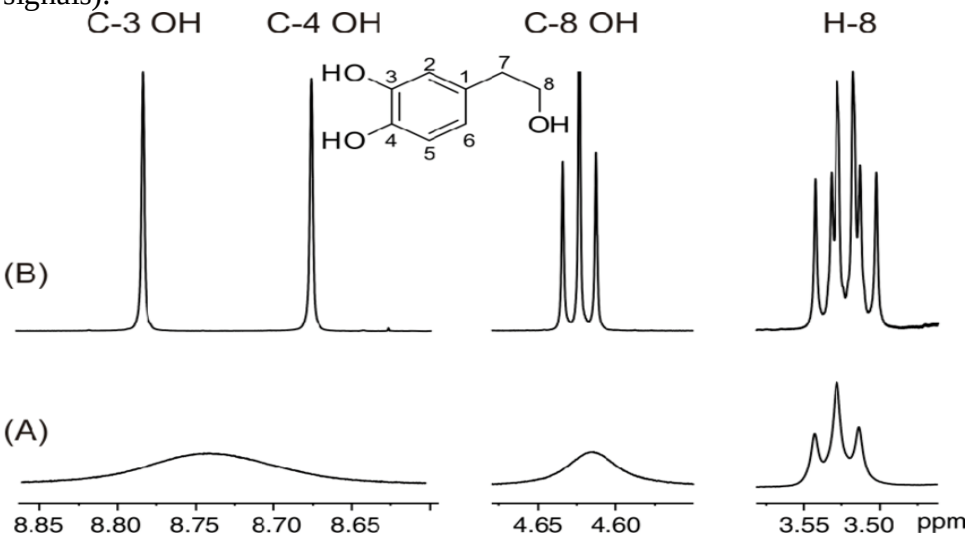
COSY experiment in order to establish the connectivity.

For simple spectra, the results are straightforward: record two spectra, one with and another without selective decoupling and check which multiplets change. For more crowded spectra, it is common to take the difference between the two spectra in order to observe changes that would otherwise be difficult to detect. The spectra shows



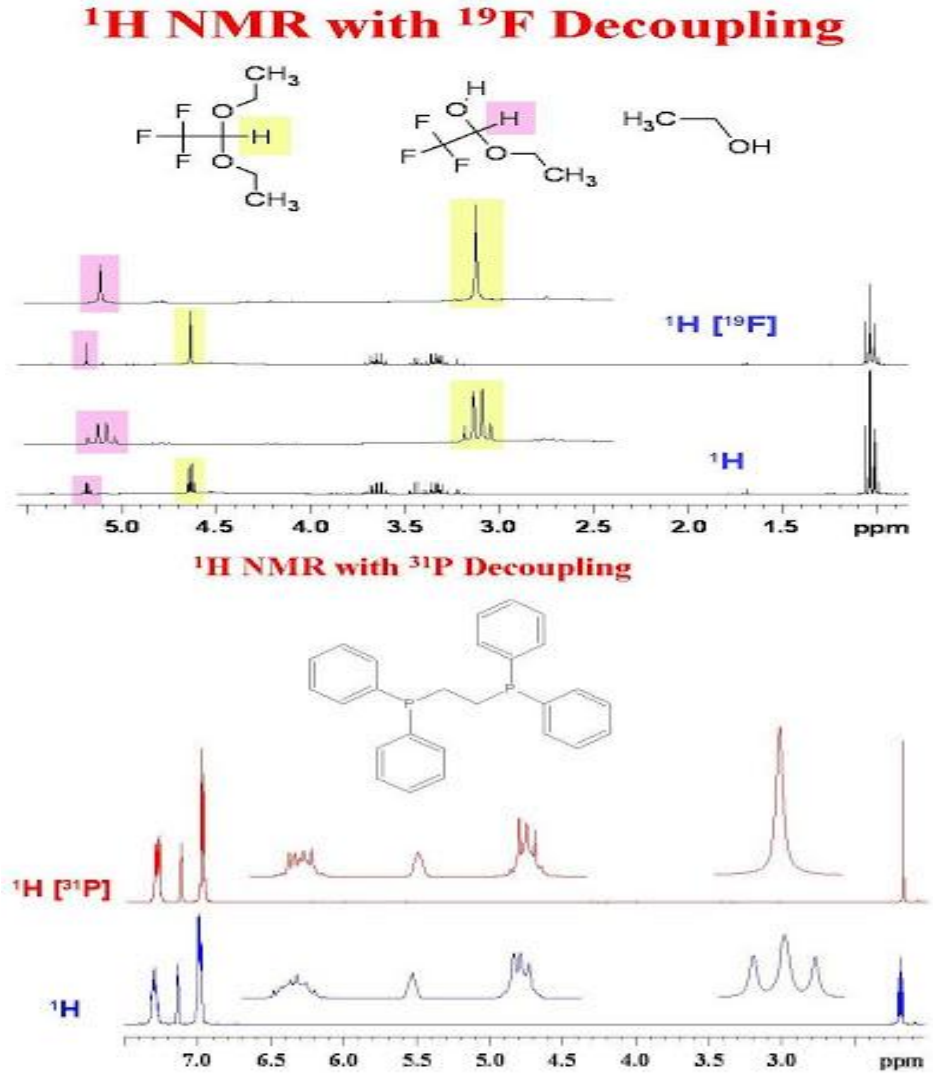
Off - resonance decoupling:

Off - resonance decoupling of ^{1}H from ^{13}C nuclei in ^{13}C NMR spectroscopy, where weaker rf irradiation results in what can be thought of as partial decoupling. In such an off-resonance decoupled spectrum, only ^{1}H atoms bonded to a carbon atom will split its ^{13}C signal. The coupling constant, indicating a small frequency difference between split signal peaks, would be smaller than in an undecoupled spectrum. Looking at a compound's off-resonance proton-decoupled ^{13}C spectrum can show how many hydrogens are bonded to the carbon atoms to further help elucidate the chemical structure. For most organic compounds, carbons bonded to 3 hydrogens (methyl) would appear as quartets (4-peak signals), carbons bonded to 2 equivalent hydrogens would appear as triplets (3-peak signals), carbons bonded to 1 hydrogen would be doublets (2-peak signals), and carbons not bonded directly to any hydrogens would be singlets (1-peak signals).



Another decoupling method is **specific proton decoupling:**

specific proton decoupling (also called band-selective or narrowband). Here the selected "narrow" ^{1}H frequency band of the (soft) decoupling RF pulse covers only a certain part of all ^{1}H signals present in the spectrum. This can serve two purposes: (1) decreasing the deposited energy through additionally adjusting the RF pulse shapes/using composite pulses, (2) elucidating connectivity of NMR nuclei (applicable with both heteronuclear and homo nuclear decoupling). Point 2 can be accomplished via decoupling e.g. of a single ^{1}H signal which then leads to the collapse of the J coupling pattern of only those observed heteronuclear or non-decoupled ^{1}H signals which are J coupled to the irradiated ^{1}H signal. Other parts of the spectrum remain unaffected.



SPIN TICKLING:

The most common experiment of this type is homo nuclear decoupling in proton NMR spectra (HOMODEC), which is a simple and effective technique for establishing coupling relationships among protons. ... In spin tickling experiments one of the lines in a coupled multiplet is irradiated with very weak power.

Nuclear Over Hauser Effect:

Nuclear over Hauser Effect, which can be used to determine intra- (and even inter-) molecular distances. The NOE effect is the change in

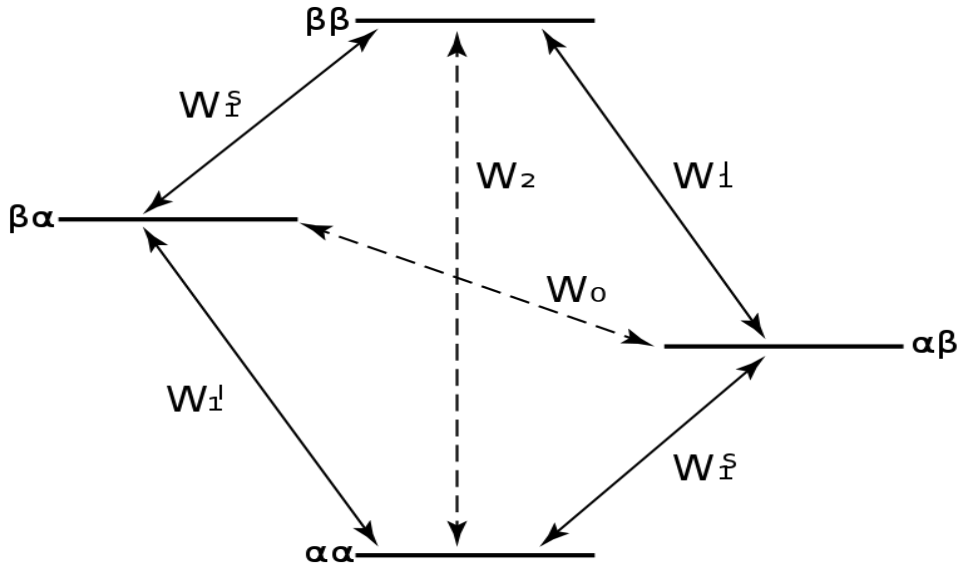
population of one proton (or other nucleus) when another magnetic nucleus close in space is saturated by decoupling or by a selective 90 or 180 degree pulse. To understand this effect, we have to first consider the consequences of applying a second radio-frequency during an NMR experiment (decoupling).

Definition of NOE:

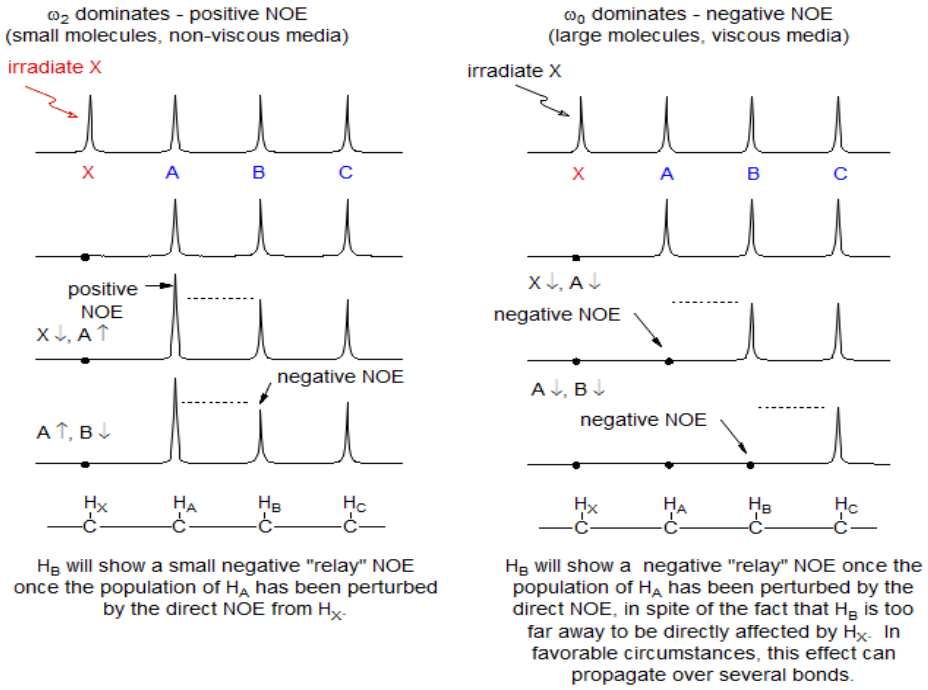
The alteration of normal spin population of a nucleus X by irradiation will cause the populations (and hence signal intensities) of other (non-irradiated) nuclei (A) to change provided that X is causing T1 relaxation of A by the dipole-dipole mechanism. This is known as the Nuclear over Hauser Effect (NOE).

Distinction between Decoupling and the NOE experiment. In a decoupling experiment (HOMODEC) the B1 irradiation must be on during acquisition of the FID (but not necessarily otherwise), and in an NOE experiment the decoupler is on during a delay period, but may be turned off during the acquisition of the FID.

Nuclear spin energy level diagram is,



Origin of the NOE Effect. When a proton is close in space to another proton (or any other nucleus with spin > 0), their magnetic dipoles interact (Dipole-Dipole interaction, DD). This interaction is distinct from J coupling, which is not a through space effect, but is mediated by polarization of bonding electrons in the molecule. The effects of DD interactions on the appearance of NMR spectra is completely averaged by the normal tumbling of molecules in solution if the medium is isotropic and viscosity is low enough to allow sufficiently fast molecular motion (short enough correlation time, τc). The DD interactions between protons do, however, dominate the ^{1}H T1 relaxation processes in most molecules that contain more than one proton.

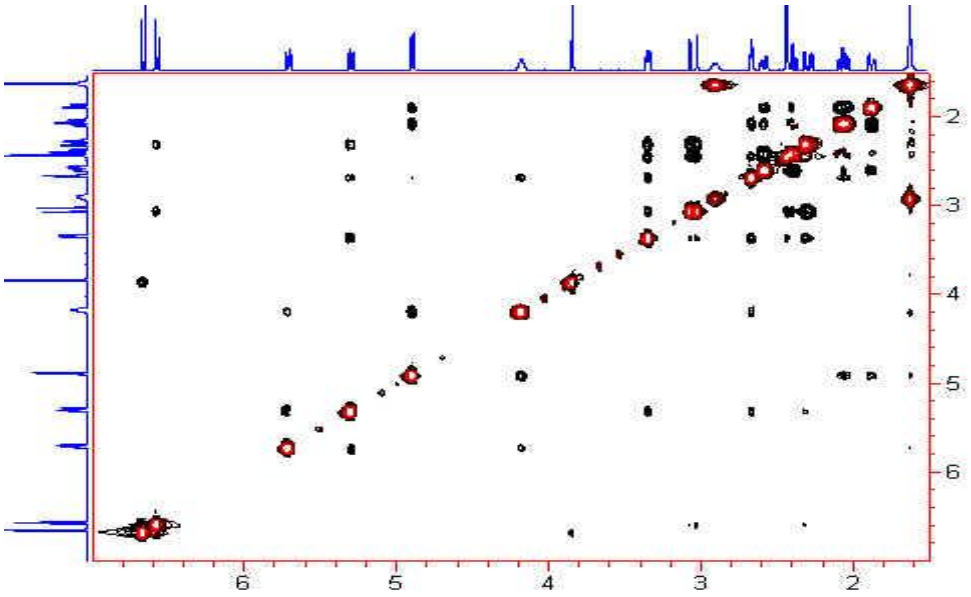


To understand the NOE effect, consider a pair of protons AX, close in space, but not J coupled to each other (J coupling is unrelated to the NOE effect, but complicates the discussion). Such a system has four energy states, corresponding to the αα, αβ, βα, and ββ spin states. The DD interaction of the protons will cause T1 relaxation between the spin states with the transition probabilities ω1 (for the single quantum relaxation αα/αβ, αα/βα, αβ/ββ and βα/ββ), ω2 (for the double-quantum relaxation αα/ββ) and ω0 (for the zero-quantum relaxation αβ/βα). In the graphic below there will be an excess population of Δ in the αα state, and a deficiency of -Δ in the ββ state.

Two-Dimensional NMR

Pulse sequence for the standard two-dimensional NOESY experiment 2D NOESY spectrum of codeine.

The motivations for using two-dimensional NMR for measuring NOE's are similar as for other 2-D methods. The maximum resolution is improved by spreading the affected resonances over two dimensions, therefore more peaks are resolved, larger molecules can be observed and more NOE's can be observed in a single measurement. More importantly, when the molecular motion is in the intermediate or slow motional regimes when the NOE is either zero or negative, the steady-state NOE experiment fails to give results that can be related to internuclear distances.



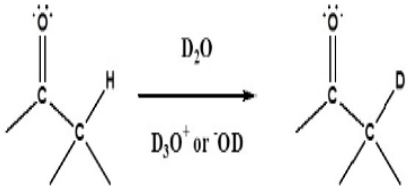
Nuclear Overhauser Effect Spectroscopy (NOESY) is a 2D NMR spectroscopic method used to identify nuclear spins undergoing cross-relaxation and to measure their cross-relaxation rates. Since ^{1}H dipole-dipole couplings provide the primary means of cross-relaxation or organic molecules in solution, spins undergoing cross-relaxation are those which are close to one another in space. Therefore, the cross peaks of a NOESY spectrum indicate which protons are close to each other in space. In this respect, the NOESY experiment differs from the COSY experiment that relies on J-coupling to provide spin-spin correlation, and whose cross peaks indicate which ^{1}H's are close to which other ^{1}H's through the chemical bonds of the molecule.

Deuterium exchange reaction:

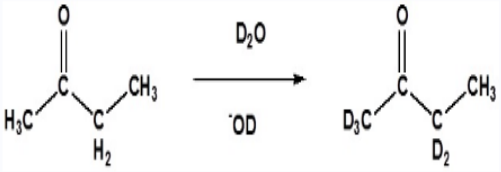
Hydrogen–deuterium exchange (also called H–D or H/D exchange) is a chemical reaction in which a covalently bonded hydrogen atom is replaced by a deuterium atom, or vice versa. It can be applied most easily to exchangeable protons and deuterons, where such a transformation occurs in the presence of a suitable deuterium source, without any catalyst. The use of acid, base or metal catalysts, coupled with conditions of increased temperature and pressure, can facilitate the exchange of non-exchangeable hydrogen atoms, so long as the substrate is robust to the conditions and reagents employed. This often results in perdeuteration: hydrogen-deuterium exchange of all non-exchangeable hydrogen atoms in a molecule.

Due to the acidic nature of α hydrogens they can be exchanged with deuterium by reaction with D_2O (heavy water). The process is accelerated by the addition of an acid or base; an excess of D_2O is required. The end result is the complete exchange of all α hydrogens with deuterium.

General reaction:

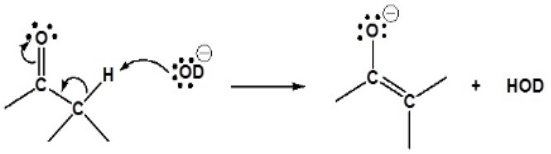


EXAMPLE 1: DEUTERIUM EXCHANGE

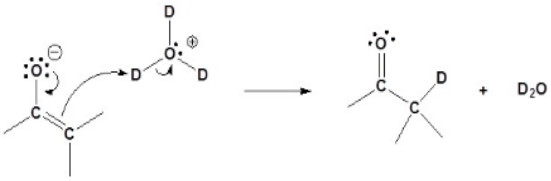


Mechanism in basic conditions

1) Enolate Formation



2) Deuteration



Detection:

H–D exchange was measured originally by the father of hydrogen exchange KajUlrikLinderstrøm-Lang using density gradient tubes. In modern times, H–D exchange has primarily been monitored by the methods: NMR spectroscopy, mass spectrometry and n **NMR spectroscopy:**

Hydrogen and deuterium nuclei are grossly different in their magnetic properties. Thus it is possible to distinguish between them by NMR spectroscopy. Deuterons will not be observed in a ^{1}H NMR spectrum and conversely, protons will not be observed in a ^{2}H NMR spectrum. Where small signals are observed in a ^{1}H NMR spectrum of a highly deuterated sample, these are referred to as residual signals. They can be used to calculate the level of deuteration in a molecule. Analogous signals are not observed in ^{2}H NMR spectra because of the low sensitivity of this technique compared to the ^{1}H analysis. Deuterons typically exhibit very similar chemical shifts to their analogous protons. Analysis via ^{13}C NMR spectroscopy is also possible: the different spin values of hydrogen (1/2) and deuterium gives rise to different splitting multiplicities. NMR spectroscopy can be used to determine site-specific deuteration of molecules.

Another method uses HSQC spectra. Typically HSQC spectra are recorded at a series of time points while the hydrogen is exchanging with the

deuterium. Since the HSQC experiment is specific for hydrogen, the signal will decay exponentially as the hydrogen exchanges. It is then possible to fit an exponential function to the data, and obtain the exchange constant. This method gives residue-specific information for all the residues in the protein simultaneously. The major drawback is that it requires a prior assignment of the spectrum for the protein in question. This can be very labor-intensive, and usually limits the method to proteins smaller than 25 kDa. Because it takes minutes to hours to record a HSQC spectrum, amides that exchange quickly must be measured using other pulse sequences.

Applications of proton NMR:

- Proton NMR widely used for structural elucidation.
- Inorganic compounds are investigated by solid state NMR.
- Solid state proton NMR constitute a powerful approach to investigate hydrogen bonding and ionization state of small organic compounds.
- Application of NMR in medicine MRI specialists' application of multidimensional Fourier transformation NMR such as anatomical imaging, tumors and tissue perfusion studies.
- Determine the enantiomeric purity.
- NMR is used in pharmaceutical chemistry, to study pharmaceutical and drug metabolism.

6.8 Check Your Progress

1. What is mean by double resonance?
2. Write short note on NOE.
3. List out the various applications of NMR spectroscopy.

6.9 Answers To Check Your Progress Questions

1. Deduce the identity of the following compound fromthe 1H NMR data given.C_4H_7BrO: δ 2.2 (3H, singlet), 3.5 (2H, triplet), 4.5 (2H, triplet) (ppm)
2. Deduce the identity of the following compound fromthe 1H NMR data given.$C_3H_6Br_2$: δ 2.4 (2H, quintet), 3.5 (4H, triplet) (ppm)
3. Deduce the identity of the following compound fromthe spectral data given.C_3H_4BrN: 1H NMR, δ 2.98 (2H, triplet), 3.53 (2H, triplet); 13C NMR, δ 21.05 (triplet), 23.87 (triplet), 118.08 (singlet) (ppm); IR, 2963, 2254 cm-1

3. The ^1H NMR spectrum for a compound with the molecular formula $C_7H_{14}O_2$, is shown below. Determine the structure of this compound.

6.10 Summary

- Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure.
- NMR can quantitatively analyze mixtures containing known compounds.
- This information combined gives us the basic skeletal structure of the molecule.

6.11 Keywords

Double resonance: When the protons are irradiated, the Boltzman distribution of spin states is perturbed, resulting in more H in the excited

state than usual; if we apply Le Chatelier's principle, the system responds to minimize the perturbation.

Spin tickling : One of the lines in a coupled multiplet is irradiated with very weak power. Lines in multiplets of other nuclei coupling to the irradiated one show additional splitting of individual lines in the multiplet which can be used to determine the relative signs of coupling constants.

Nuclear Overhauser Effect: The alteration of normal spin population of a nucleus X by irradiation will cause the populations (and hence signal intensities) of other (non-irradiated) nuclei (A) to change provided that X is causing T1 relaxation of A by the dipole-dipole mechanism. This is known as the Nuclear Overhauser Effect (NOE).

6.12 Self-assessment questions and exercises

1. Why is it necessary to use deuterated solvents for NMR experiments?.
2. Which tasks an NMR probe has to perform?
3. When and why are paramagnetic compounds deliberately added to the sample before running an NMR experiment?

6.13Further readings

1. High Resolution NMR Techniques in Organic Chemistry. Timothy D. W. Claridge. Pergamon Press 1999. An excellent explanation of the many experiments useful for structure elucidation. Also a good introduction to spin physics and NMR instrumentation. Highly recommended.

2. A Complete Introduction to Modern Nmr Spectroscopy. Roger S. Macomber A text on fundamentals of nuclear magnetic resonance (NMR) spectroscopy, using a straightforward approach that develops all concepts from a rudimentary level without using heavy mathematics. Assuming only a knowledge of basic chemistry, it provides an understanding of all the techniques needed to solve molecular structures from 1D and 2D NMR spectra with hundreds of worked out examples.

3. The Basics of NMR. Joseph P. Hornak, Ph.D. An interactive web based textbook found at http://www.cis.rit.edu/htbooks/nmr/nmr-main.htm. Excellent illustrations, making full use of color and animation. Highly recommended. See particularly Chapter 7, NMR Hardware, and Chapter 8, Practical Considerations.

4. 150 and More Basic NMR Experiments. S. Braun, H. O. Kalinowski, S. Berger. Wiley-VCH, Weinheim. 1998. A wealth of practical information on setting up and running a wide variety if NMR experiments. A copy is kept in the NMR lab.

5. Modern NMR Spectroscopy : A Guide for Chemists. Jeremy K. M. Sanders, Brian K. Hunter. Oxford University Press, 1993. This book provides a non-mathematical, descriptive approach to modern NMR spectroscopy. It contains much practical advice on the acquisition and use of spectra.

6. A Handbook of Magnetic Resonance. Ray Freeman. John Wiley & sons, New York, 1987. A small encyclopedia of NMR. Insightful and sometimes entertaining explanations of NMR concepts. It assumes a basic knowledge of the subject. There are no entries under chemical shift or spin-spin coupling, for instance.

7. NMR Data Processing. Jeffery C. Hoch and Alan S. Stern. John Wiley & sons, New York, 1996. Examines and explains the techniques used to process, present and analyze NMR data. Standard techniques such a apodization, zero filling and Fourier transform; as well as advanced techniques such as multi-dimensional processing, linear prediction, maximum entropy.

8. Principles of nuclear magnetic resonance in one and two dimensions . Richard R. Ernst, Geoffrey Bodenhausen, and Alexander Wokaun. Oxford University Press, 1987. If you want the theoretical and mathematical rigor, it's all here.

9. NMR: The Toolkit. P.J. Hore, J. A. Jones, S. Wimperis. Oxford University Press, 2000. A short book the focuses on the mathematical and quantum mechanical tools need to completely understand modern multi-dimensional NMR.

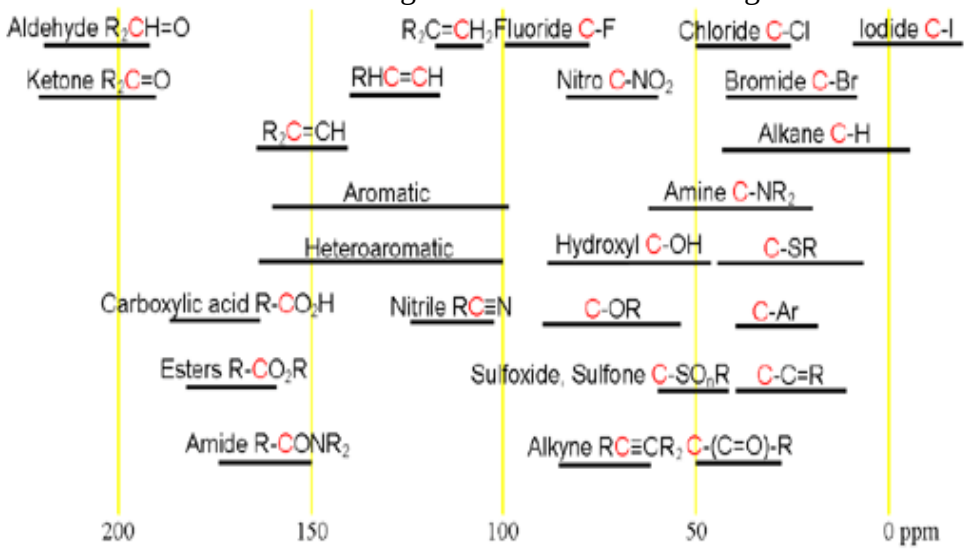
UNIT – VII ^{13}C- NMR Spectroscopy

Structure

7.0 Introduction

The 1D ^{13}Carbon NMR experiment is much less sensitive than Proton (^{1}H) but has a much larger chemical shift range. Its low natural abundance (1.108%) and proton decoupling means that spin-spin couplings are seldom observed. This greatly simplifies the spectrum and makes it less crowded. ^{13}C is a low sensitivity nucleus that yields sharp signals and has a wide chemical shift range.

A typical analysis of a ^{13}C NMR spectrum consists of matching expected chemical shifts to the expected moieties. Our NMR service provides ^{13}C NMR along with many other NMR techniques. Each type of signal has a characteristic chemical shift range that can be used for assignment.



(Choose the structure that most closely represents the hydrogen in question. R = alkyl or H, Ar = aryl).

Objectives

- Know how nuclear spins are affected by a magnetic field, and be able to explain what happens when radiofrequency radiation is absorbed.
- Be able to predict the number of proton and carbon NMR signals expected from a compound given its structure.
- Be able to predict the splitting pattern in the proton NMR spectrum of a compound given its structure.
- With the aid of a chart of chemical shifts from ^{1}H and ^{13}C NMR, be able to assign peaks in an NMR spectrum to specific protons in a compound.
- Be able to interpret integration of NMR spectra.

- Be able to use NMR spectra to determine the structures of compounds, given other information such as a molecular formula.

Integration is almost useless in a regular ^{13}C NMR spectrum because of uneven nuclear Overhauser effect (NOE) enhancement of the signals by decoupling and long longitudinal relaxation times (T1's). Quantitative spectra may be obtained by inverse gated decoupling and long delays in the region of 10 minutes between pulses. However, this is very insensitive for ^{13}C and is rarely a realistic option. The signal enhancement due to NOE of the decoupled spectrum of ethylbenzene under comparable conditions to the quantitative spectrum. The enhancement is much greater under routine conditions where much shorter repetition times and sensitivity enhancing window functions are used.

Requirement of carbon NMR:

- Proton NMR used for study of number of non-equivalent proton present in unknown compound.
- Carbon NMR can used to determine the number of non-equivalent carbons and to identify the types of carbon atoms which may present in the compound.
- Carbon-13signals are spread over a much wider range than proton signals making it easier to identify and count individual nuclei.

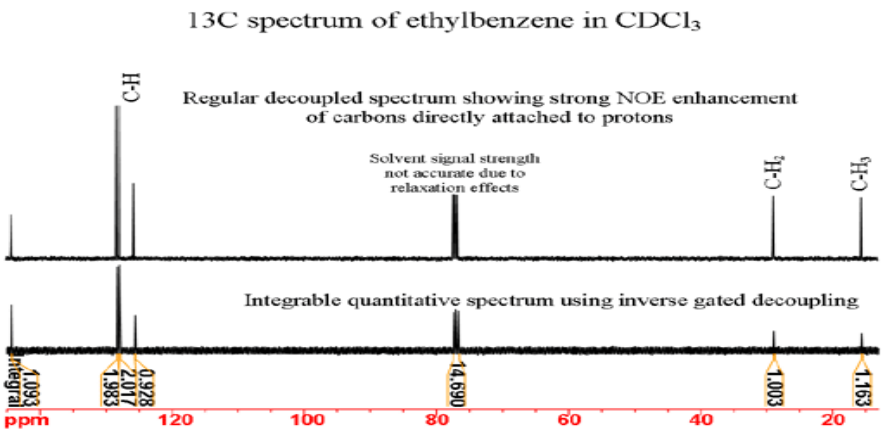
^{13}C interpretation:

- Check the chemical shift window.
- Count the resulting lines and related to how many types of carbon.
- Check the splitting pattern.
- Symmetry duplicates give same line- if there are more carbons in your spectrum – symmetry.

Characteristic features:

- Chemical shift of carbon NMR is wider compare to proton NMR.
- ^{13}C - ^{13}C Coupling is negligible because low abundance of the material.
- Spectrum each magnetically non-equivalent carbon give a single sharp peak that does undergo further splitting.
- Chemical shift is high in carbon NMR compared to proton NMR.

NOE enhancement in a ^{13}C NMR:

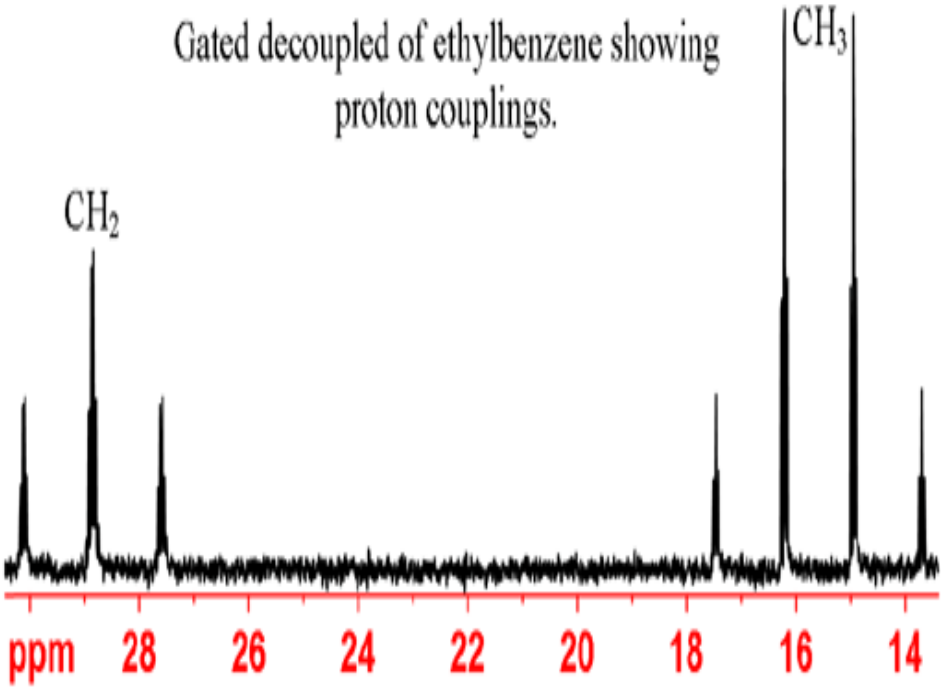


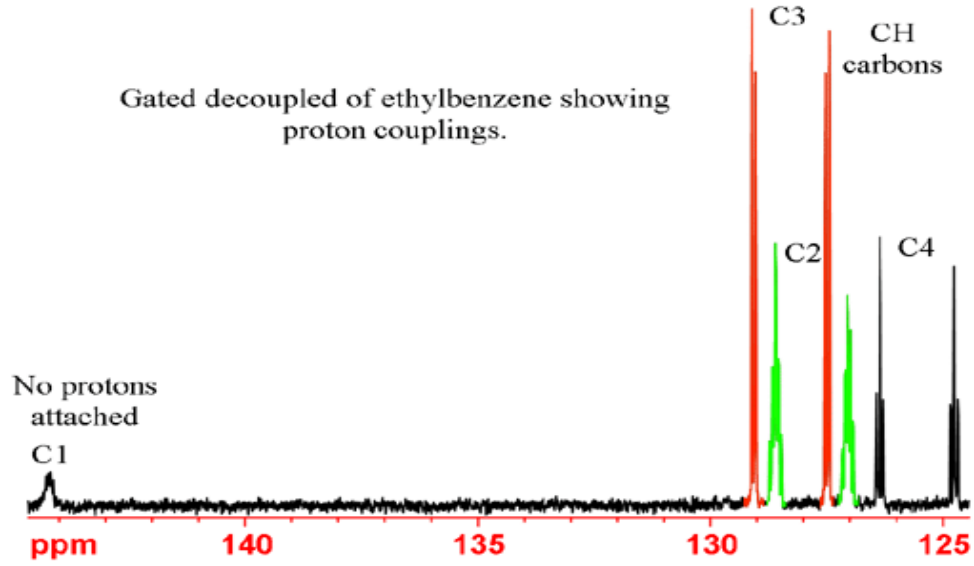
The chemical shifts are used for the initial assignment of the spectrum. For ethylbenzene, we see that the signals at 15.6 and 28.9 ppm fall in the aliphatic region and therefore belong to the CH_2 and CH_3 carbons. Usually CH_3 has a lower chemical shift than CH_2 so can be provisionally assigned to 15.6 and 28.9 ppm, respectively. The remaining signals are in the aromatic region at 125.6, 127.8, 128.3 and 144.2. The carbon not attached to any protons is called 'quaternary' (four-fold) even though this is a misnomer for unsaturated carbons such as in our case where it is only attached to only three other carbons. Quaternary carbons usually give sharper signals than other carbons and usually give weaker signals (fig. 3) under normal acquisition conditions – decoupling and relatively short repetition times. This is because of their slow relaxation and lack of NOE enhancement. The chemical shifts of aromatic carbons not attached to protons (for ethylbenzene C1) are generally higher than for those attached (for ethylbenzene C2, C3 and C4). Therefore the signal at 144.2 ppm is provisionally assigned to C1.

Gated decoupled 13C NMR:

Gated decoupling may be used in order to observe proton couplings. Gated decoupling is preferable to no decoupling because it preserves the NOE sensitivity enhancement. In the coupled spectrum (fig. 4) methine (CH) carbons appear as doublets, methylene (CH_2) carbons as 1:2:1 triplets and methyl (CH_3) carbons as 1:3:3:1 quartets.

Figure shows Gated decoupled ^{13}C spectrum of ethylbenzene showing CH_3 quartet, CH_2 triplet, CH doublets and a carbon that has only long-range proton couplings.



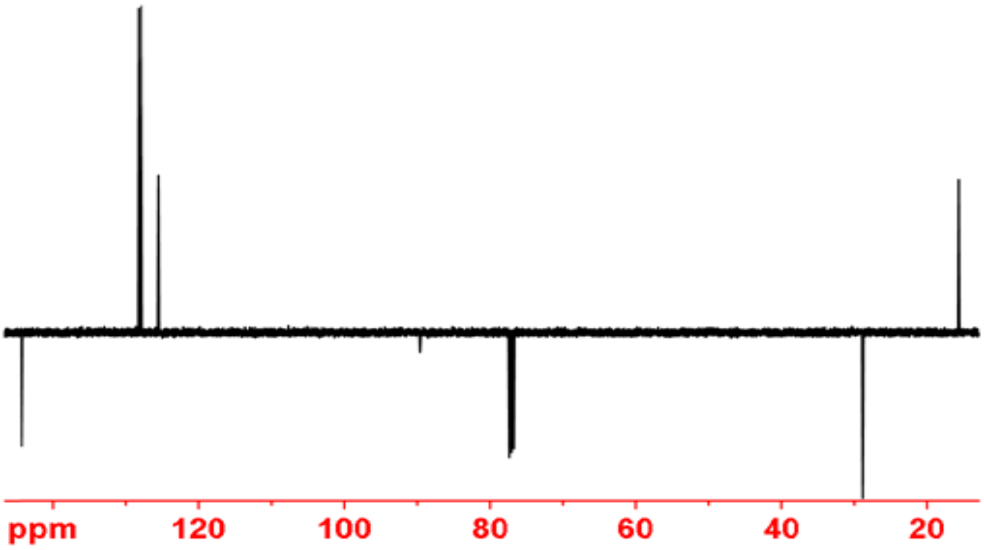


The coupling constants are typically 125 Hz for sp3 carbons, 160 Hz for sp2 carbons and 200 Hz for sp carbons. However, electronegative substituents increase the coupling constants, e.g., 214 Hz for chloroform, while electropositive ones reduce the coupling, e.g., 117 Hz for TMS. The one-bond coupling constant can be used as a measure of hybridization when there are no strongly electropositive or electronegative substituents. Long range couplings (predominantly 3-bond that are usually paradoxically stronger than 2-bond) of up to about 10 Hz can be observed but may be difficult to assign. In the case of ethylbenzene (fig. 5), the long-range couplings can be used to assign the aromatic CH carbons. C4 can be assigned using the intensity arguments above. The long-range couplings visible in the gated decoupled experiment are mostly three-bond (this is usually but not always the case) and can be used to differentiate C2 and C3. In the diagram below, C3 (and its coupled protons in red) is coupled to one proton and is therefore a double doublet in the gated decoupled spectrum. C2 (and its coupled protons in green) would be expected to be a double triplet but is more complex, due to second order coupling between protons. Three-bond couplings display a Karplus type relation ($^3J_{CH}=9\cos 2\theta-\cos\theta+0.3$ in Hz) with torsion angle, θ, and can sometimes be used to estimate the torsion angle. Couplings may be observed with other nuclei such as 19Fluorine or 31Phosphorus.

Attached proton test (APT)

However, if only multiplicity is important rather than coupling constant then the attached proton test (APT) and distortion less enhancement by polarization transfer (DEPT) experiments are more sensitive than gated decoupling. The APT experiment yields methine (CH) and methyl (CH_3) signals positive and quaternary (C) and methylene (CH_2) signals negative (fig. 6). It is slightly less sensitive than DEPT but shows all carbon signals at once unlike DEPT that supresses quaternary carbons.

Figure shows, APT spectrum of ethylbenzene showing CH and CH_3 positive while CH_2, is negative.



DEPT

The DEPT experiment is slighlty more sensitive than APT and can fully separate the carbon signals. However, it has to be run three times with different final pulse angles and compared with the regular decoupled ^{13}C spectrum in order to provide a full analysis. The DEPT experiment requires at least four scans in order to cancel out the quaternary signals although many more scans are usually acquired so this is not a problem. DEPT 45 (figure1) yields CH, CH_2 and CH_3 signals positive, DEPT 90 (fig. 2) yields only CH signals and DEPT 135 (fig. 3) yields CH and CH_3 positive while CH_2 s negative.

Figure 1 shown, DEPT 45 spectrum of ethylbenzene showing only carbons attached to protons: CH, CH_2 and CH_3 all positive.

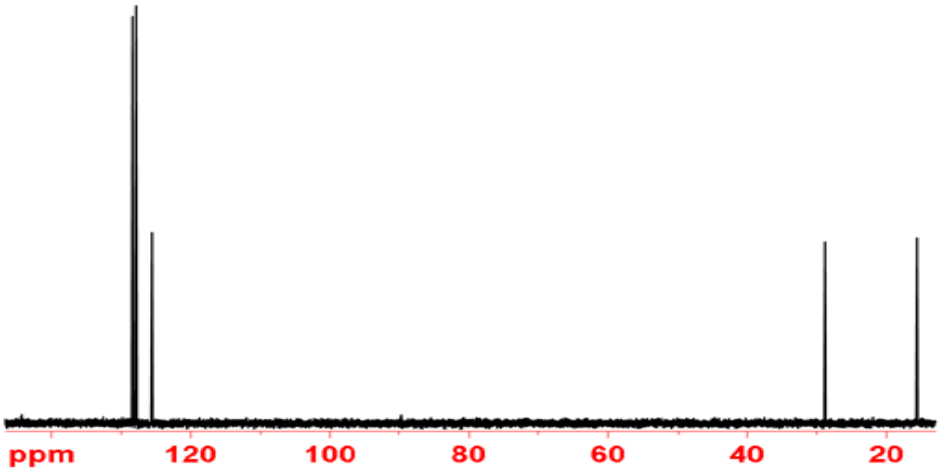


Fig. 2. DEPT 90 spectrum of ethylbenzene showing only CH carbons. Suppression of attached CH_2 and CH_3 is not complete.

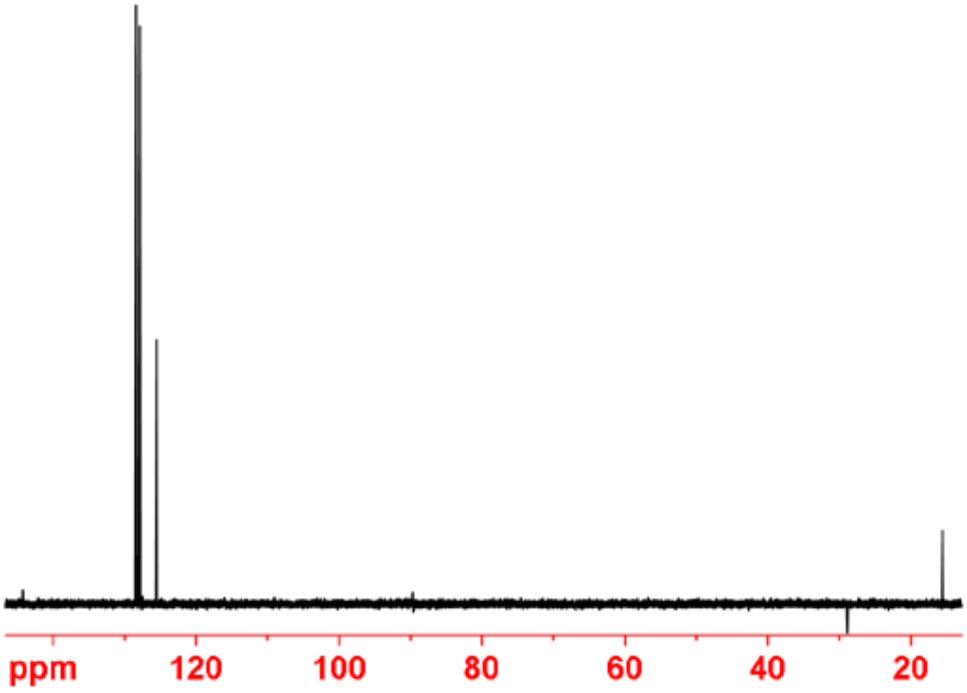
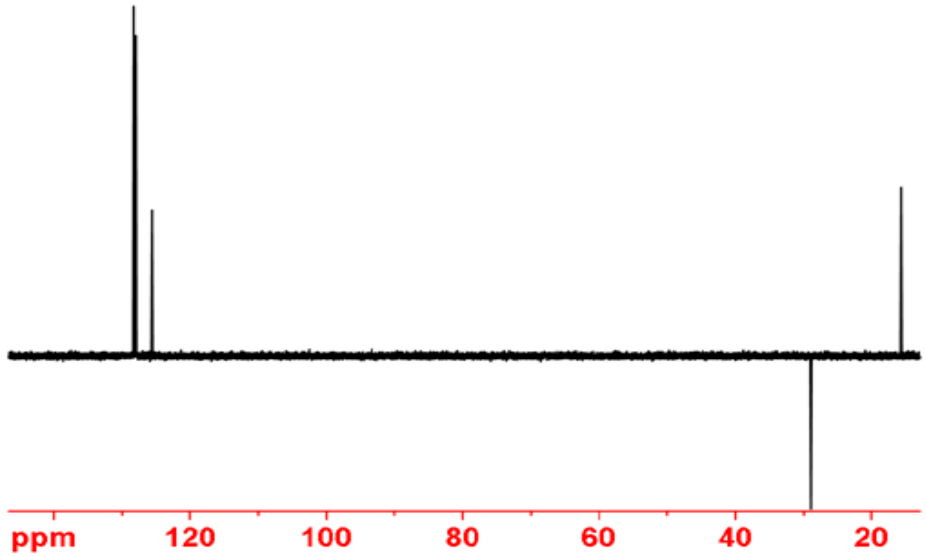


Fig. 3. DEPT 135 spectrum of ethylbenzene showing only CH, and CH_3 positive and CH_2 negative.



In theory, adding DEPT 45 – 0.08 DEPT 90 - 1.2 DEPT 135 yields CH_2 only (fig. 10) and DEPT 45 -1.52 DEPT 90 + 1.2 DEPT 135 yields CH_3 only (fig. 11). However, slight adjustments to these factors are required by trial and error in practice. In principle, subtracting DEPT 45 from the regular spectrum yields the quaternary carbons only. However, suppression is poor because of slight changes in temperature of the sample due to decoupling that shift the peaks slightly.

Simplest NMR experiment:

- Exposing the proton in an organic molecule to a powerful external magnetic field.
- The proton will precess at same frequency.
- We irradiate these precessing proton with appropriate radio frequency energy.
- Promote protons from lower energy (aligned state) to higher energy (opposed state).

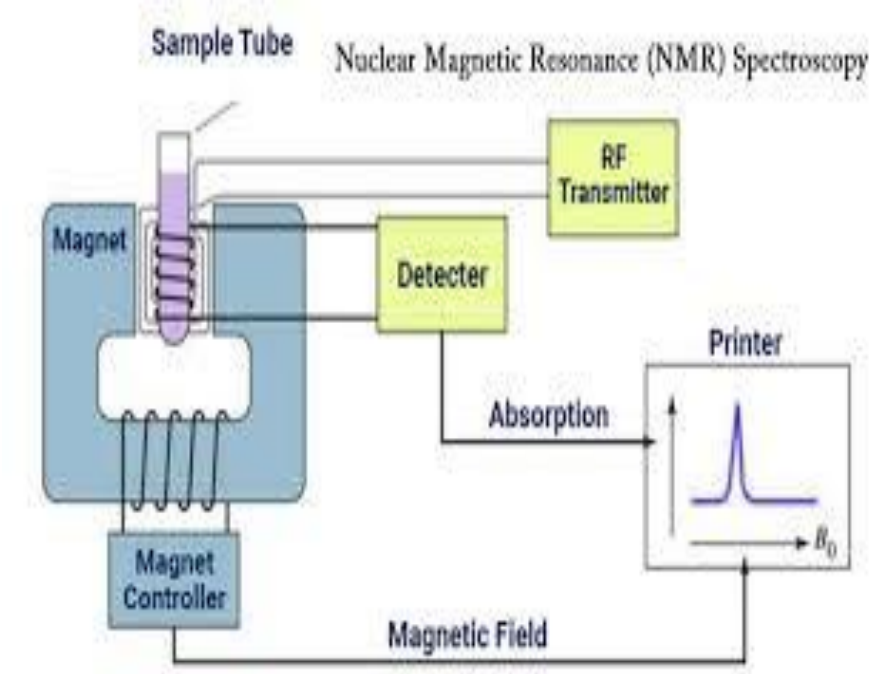
- We record this absorption of energy in the form of NMR spectrum.

7.2INSTRUMENTATION:

The basic feature of the instrumentation needed to record an NMR spectrum are a

- Magnet
- Radiofrequency source
- Sample holder
- Detection system
- Computer display

To indicate the energy is being transferred from the radiofrequency beam to the nucleus. The schematic representation is



APPLICATION:

- Carbon-13 which the signal intensities and help in the tracing the cellular metabolism.
- Carbon-13 nuclei are stable isotope and hence not subjected to dangers related to radiotracers.
- CMR technique is used for quantification of drug purity to determination of the composition of high molecular weight synthetic polymer.
- Other application fields are medicine, chemistry (to detect compound), purity determination, non-destructive testing.
- Acquisition of dynamic information, data acquisition in the petroleum industry.
- Flow probes for NMR spectroscopy, process control, earth field NMR, Zero field NMR and quantum computing.

7.3 Check Your Progress

1. How does ^{13}C NMR work?
2. What is DEPT NMR spectroscopy?
3. How many 13C NMR signals are there in benzene solvent.
4. List out the various applications of NMR spectroscopy.

5. Discuss the factors affecting vicinal coupling constant. Give appropriate examples.

6. Explain anisotropic effect with suitable examples.

(i) Discuss the utility of HMBC spectrum.

(ii) A doublet and a quartet with intensity 3:1 are observed in the ^{31}P NMR of P_4S_3. Predict the structure and geometry of the compound.

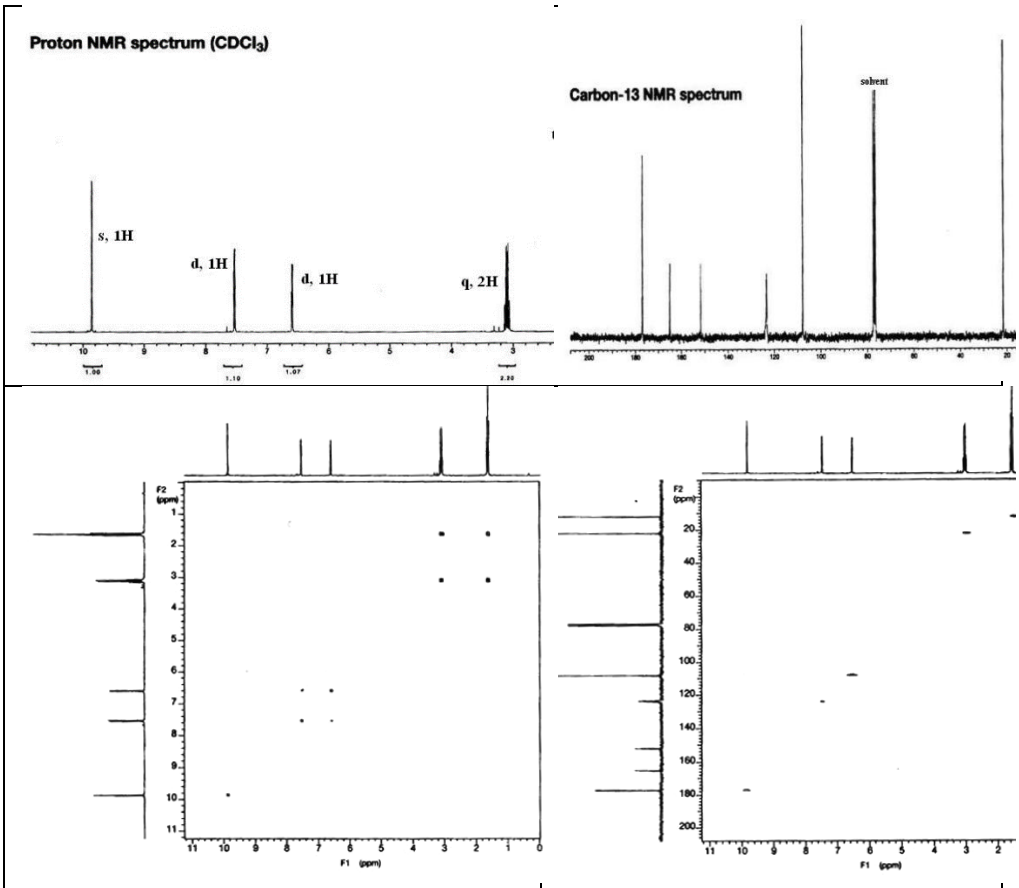
(iii) In the complex $[TiF_5(C_2H_5OH)]^-$, an octahedrally coordinated Ti was proposed. Predict the number of signals, intensity and the splitting pattern of each signal in the ^{19}F NMR.

7.4Answers To Check Your Progress Questions

1. The two isomers of C_2H_6O are ethanol, CH_3CH_2OH, and methoxymethane, CH_3OCH_3. Describe as fully as you can what the C-13 NMR spectra of the two compounds would look like.

Answer: The ethanol spectrum would have two lines because of the two carbons in different environments. The line for the carbon with the oxygen attached would be in the region 50 - 90 ppm, and the other one due to the CH_3 group in the 10 - 15 region. (In fact, it is slightly higher than this. The effect of the oxygen atom is still felt slightly.) The methoxymethane spectrum will consist of a single line, because both CH_3 groups are in exactly identical environments. The presence of the C-O single bond would mean the line would be in the 50 - 90 ppm region.

2. Predict the structure of a compound with the following data. Assign the ^1H and ^{13}C chemical
shifts. Molecular formula:$C_7H_8O_2$;FT IR strong absorption ~1700–1690 cm^{-1}.



3. The ^{1}H NMR spectrum for a compound with the molecular formula $C_7H_{14}O_2$, is shown below. Determine the structure of this compound.

7.5 Summary

- ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure (especially total number of carbon present).
- NMR can quantitatively analyze mixtures containing known compounds.
- This information combined gives us the basic skeletal structure of the molecule.

7.6 Keywords

Gated decoupled ^{13}C NMR: Appropriate to complete the unambiguous chemical shift assignment and the conformational condition of molecules.
DEPT NMR : This experiment allows to determine multiplicity of carbon atom substitution with hydrogens.

7.7 Self-assessment questions and exercises

1. Why is it necessary to use deuterated solvents for NMR experiments?.
2. Which tasks an ^{13}C NMR probe has to perform?

7.8Further readings

Techniques in Organic Chemistry. Timothy D. W. Claridge. Pergamon Press 1999. An excellent explanation of the many experiments useful for structure elucidation. Also a good introduction to spin physics and NMR instrumentation. Highly recommended.

1. A Complete Introduction to Modern Nmr Spectroscopy. Roger S. Macomber A text on fundamentals of nuclear magnetic resonance (NMR) spectroscopy, using a straightforward approach that develops all concepts from a rudimentary level without using heavy mathematics. Assuming only a knowledge of basic chemistry, it provides an understanding of all the techniques needed to solve molecular structures from 1D and 2D NMR spectra with hundreds of worked out examples.

2. The Basics of NMR. Joseph P. Hornak, Ph.D. An interactive web based textbook found at http://www.cis.rit.edu/htbooks/nmr/nmr-main.htm. Excellent illustrations, making full use of color and animation. Highly recommended. See particularly Chapter7, NMR Hardware, and Chapter 8, Practical Considerations.

3. 150 and More Basic NMR Experiments. S. Braun, H. O. Kalinowski, S. Berger. Wiley-VCH, Weinheim. 1998. A wealth of practical information on setting up and running a wide variety if NMR experiments. A copy is kept in the NMR lab.

4. Modern NmrSpectroscopy : A Guide for Chemists. Jeremy K. M. Sanders, Brian K. Hunter. Oxford University Press, 1993. This book provides a non-mathematical, descriptive approach to

modern NMR spectroscopy. It contains much practical advice on the acquisition and use of spectra.

5. A Handbook of Magnetic Resonance. Ray Freeman. John Wiley & sons, New York, 1987. A small encyclopedia of NMR. Insightful and sometimes entertaining explanations of NMR concepts. It assumes a basic knowledge of the subject. There are no entries under chemical shift or spin-spin coupling, for instance.

6. NMR Data Processing. Jeffery C. Hoch and Alan S. Stern. John Wiley & sons, New York, 1996. Examines and explains the techniques used to process, present and analyze NMR data. Standard techniques such a apodization, zero filling and Fourier transform; as well as advanced techniques such as multi-dimensional processing, linear prediction, maximum entropy.

7. Principles of nuclear magnetic resonance in one and two dimensions . Richard R. Ernst, Geoffrey Bodenhausen, and Alexander Wokaun. Oxford University Press, 1987. If you want the theoretical and mathematical rigor, it's all here.

8. NMR: The Toolkit. P.J. Hore, J. A. Jones, S. Wimperis. Oxford University Press, 2000. A short book the focuses on the mathematical and quantum mechanical tools need to completely understand modern multi-dimensional NMR.

BLOCK III: ESR, MASS SPECTROSCOPY AND ORD AND CD

UNIT VIII : ESR SPECTROSCOPY

Structure

8.1 Introduction

ESR is a method for observing the behavior (dynamics) of the electrons within a suitable molecule, and for analyzing various phenomena by identifying the electron environment. ESR measurements afford information about the existence of unpaired electrons, as well as quantities, type, nature, environment and behavior. Electron Spin Resonance is a branch of absorption spectroscopy in which radiation having frequency in the microwave region is absorbed by paramagnetic substances to induce transitions between magnetic energy levels of electrons with unpaired spins. The magnetic energy splitting is done by applying a static magnetic field.

8.2 Objectives

1. To learn some properties of a simple microwave reflection spectrometer.
2. To calibrate the magnetic field using DPPH.
3. To measure the g factor, nuclear spin, and hyperfine coupling constant of the $_{55}Mn^{2+}$ ion.

8.3 Instrumentation:

The instrumentation of ESR spectroscopy consists of

1) Klystrons
    - Klystron tube acts as the source of radiation.
    - It is stabilized against temperature fluctuation by immersion in an oil bath or by forced air cooling.
    - The frequency of the monochromatic radiation is determined by the voltage applied to klystron.
    - It is kept a fixed frequency by an automatic control circuit and provides a power output of about 300 milli watts.
2) Wave guide or wavemeter
    - The wave meter is put in between the oscillator and attenuator.
    - To know the frequency of microwaves produced by klystron oscillator.

- The wave meter is usually calibrated in frequency unit (megahertz) instead of wavelength.
- Wave guide is a hollow, rectangular brass tube. It is used to convey the wave radiation to the sample and crystal.

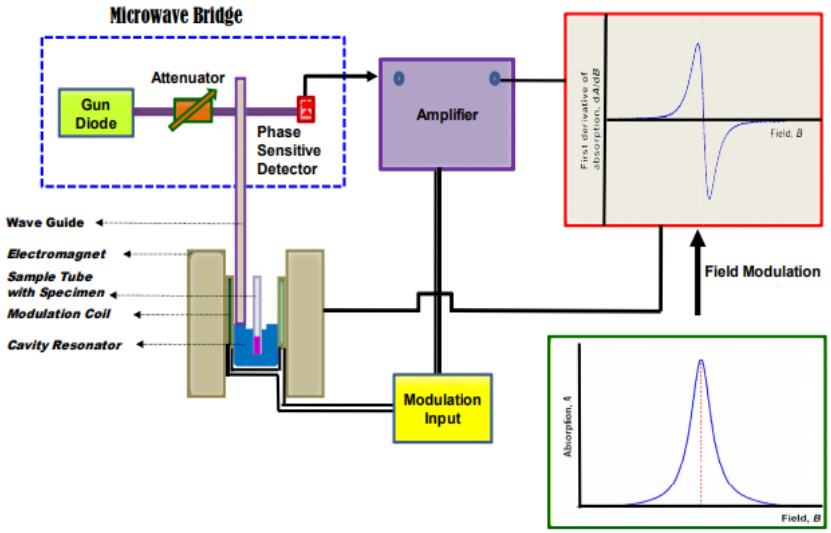
3) Attenuators
   - The power propagated down the wave guide may be continuously decreased by inserting a piece of resistive material into the wave guide. This piece is called variable attenuator.
   - It is used in varying the power of the sample from the full power of klystron to one attenuated by a force 100 or more.
4) Isolators
   - It's device which minimizes vibrations in the frequency of microwaves produced by klystron oscillator.
   - Isolators are used to prevent the reflection of microwave power back into the radiation source.
   - It is a strip of ferrite material which allows micro waves in one direction only.
   - It also stabilizes the frequency of the klystron.
5) Sample cavities
   - The heart of the ESR spectrometer is the resonant cavity containing the sample.
   - Rectangular TE120 cavity and cylindrical TE011 cavity have widely been used.
   - In most of the ESR spectrometers, dual sample cavities are generally used. This is done for simultaneous observation of a sample and a reference material.
   - Since magnetic field interacts with the sample to cause spin resonance the sample is placed where the intensity of magnetic field is greatest.
6) Couplers and matching screws
   - The various components of the micro wave assembly to be coupled together by making use of irises or slots of various sizes.
7) Crystal detectors
   - Silicon crystal detectors, which converts the radiation in D.C has widely been used as a detector of microwave radiation.
8) Magnet system
   - The resonant cavity is placed between the poles pieces of an electromagnet.
   - The field should be stable and uniform over the sample volume.
   - The stability of field is achieved by energizing the magnet with a highly regulated power supply.
   - The ESR spectrum is recorded by slowly varying the magnetic field through the resonance condense by sweeping the current supplied to the magnet by the power supply.
9) Modulation coil
   - The modulation of the signal at a frequency consistent with good signal noise ratio in the crystal detector is

accomplished by a small alternating variation of the magnetic field.

- The variation is produced by supplying an A.C. signal to modulation coil oriented with respect the sample in the same direction as the magnetic field.
- If the modulation is of low frequency (400 cycles/sec or less), the coils can be mounted outside the cavity and even on the magnet pole pieces.
- For higher modulation frequencies, modulation coils must be mounted inside the resonant cavity or cavities constructed of a non-metallic material e.g., Quartz with a tin silvered plating.

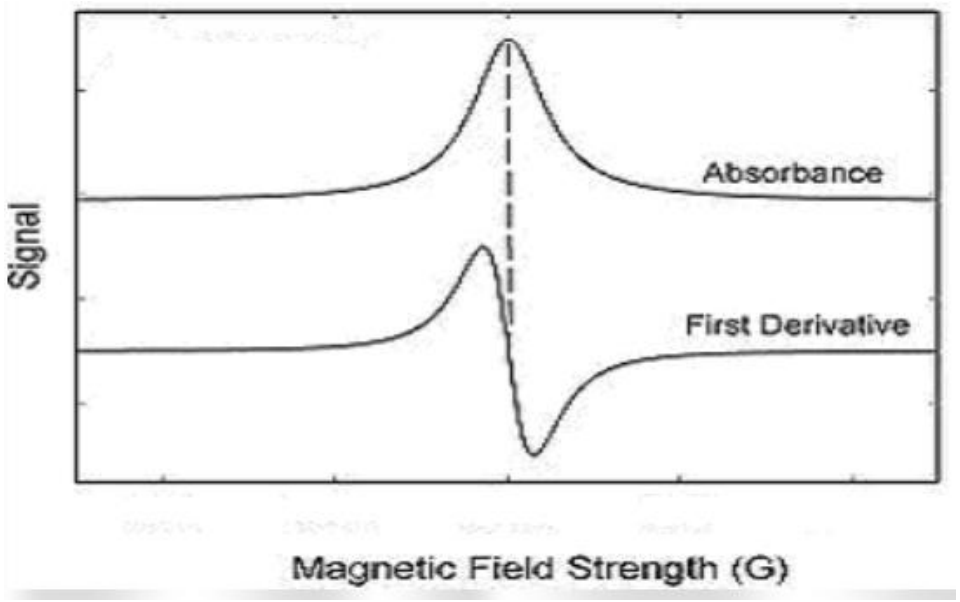
10) Display devices

- In order to observe the signal a system is connected different devices can be used.



Diagrammatic representation of ESR spectroscopy.

Presentation of spectra



For absorption: Intensity against strength of magnetic field.
First derivative curve: First derivative (slope) of absorption curve vs strength of magnetic field.

Comparision between NMR and ESR:

- NMR deals with nuclear spin resonance but ESR deals with electron spin resonance.
- The difference in two energy levels (ΔE) in ESR spectrum is greater than that of NMR spectrum.
- In NMR spectroscopy, the absorption is caused by radiation of radiowave frequency while in ESR spectroscopy it is caused by radiation of microwave frequency.
- To obtain ESR spectrum, it is necessary that the substance must have atleast one unpaired electron but for NMR, the nucleus should have definite nuclear spin value.

g- value:

The value of g for an unpaired electron in a gaseous atom/ ion is given by the expression

$$g = 1 + \frac{J(J+1) + S(S+1) - L(L+1)}{2J(J+1)}$$

where S= sum of the spin quantum number of all the unpaired electrons, J = total angular momentum of the ground state, L= total orbital angular momentum. g is proportionality factor/ spectroscopic splitting factor/ Lande`s splitting factor. It is a measure of ratio between frequency and magnetic field. The value of g for free electrons is 2.0023 which may vary by 0.0003. In ionic crystals, value of g vary from 0.2- 0.8. The reason is that unpaired electrons are localized in a particular orbital of the atom and orbital angular momentum couples with spin angular momentum giving rise to a low value of g in ionic crystal. The g- factor in ESR is analogous to chemical shift in NMR.

Applications to Organic and Inorganic compounds:

- In Biological Systems:

  The presence of free radicals in healthy and diseased tissues has been studied by ESR. Transition metal ion if present, can also be studied. Some typical systems which have been

studied by ESR are hemoglobin, nucleic acids, enzymes, irradiated chloroplasts, riboflavin and carcinogens. The role of free radical in photosynthesis has been provided by the observation of a sharp ESR resonance line.

- Study of free radicals:

A free radical is a compound which contains an unpaired spin such as methyl radical produced through the breakup of methane.

$$CH4 \rightarrow °CH3 + H°.$$

Methyl radical, has three 1H nuclei each with I = 1/2, and so the number of lines expected is,

$$2nI + 1 = 2(3)(1/2) + 1 = 4$$

4 peaks are observed in the proportion of 1:3:3:1.

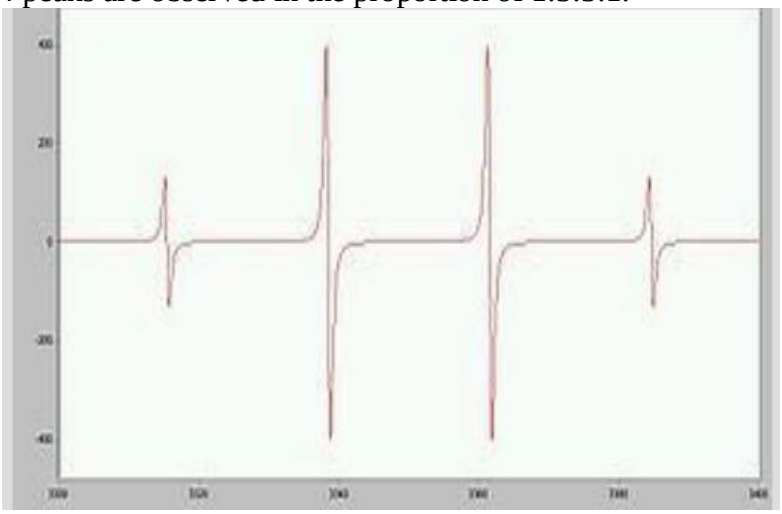
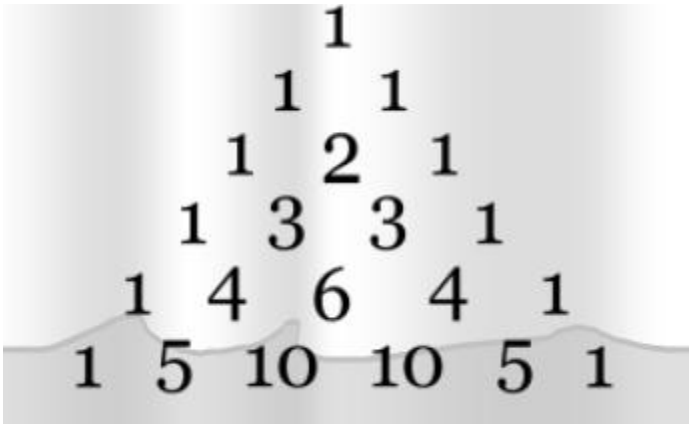


Figure: ESR Spectrum (First Derivative) for methyl radical .

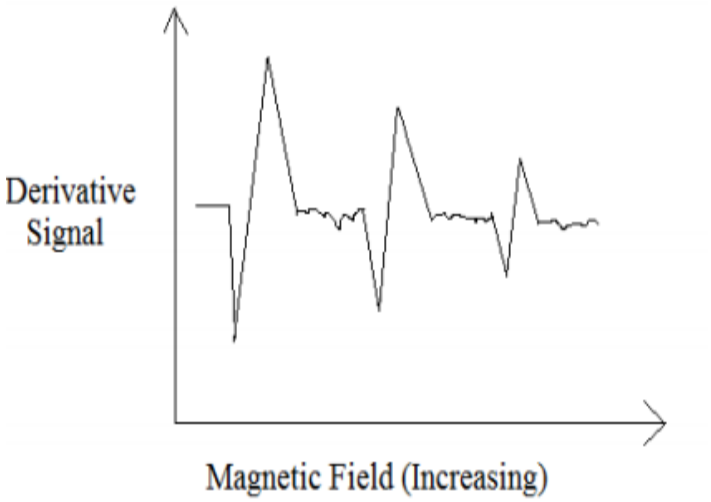
In case of I = 1/2 nuclei (e.g., ^{1}H, ^{19}F, ^{31}P), the line intensities produced by a population of radicals, each possessing N equivalent nuclei, will follow Pascal's triangle.



- Study of inorganic compounds:

E.g; $[NO(SO_3)_2]^{2-}$ yields a triplet in its ESR spectrum in chloroform. This arises from the interaction between the spin of the unpaired electron and the spin of a ^{14}N nucleus (I=1), conforming that this electron is mainly localised on the nitrogen atom.

$$(2I + 1) = 2 \times 1 + 1 = 3$$



- Study of catalysts:

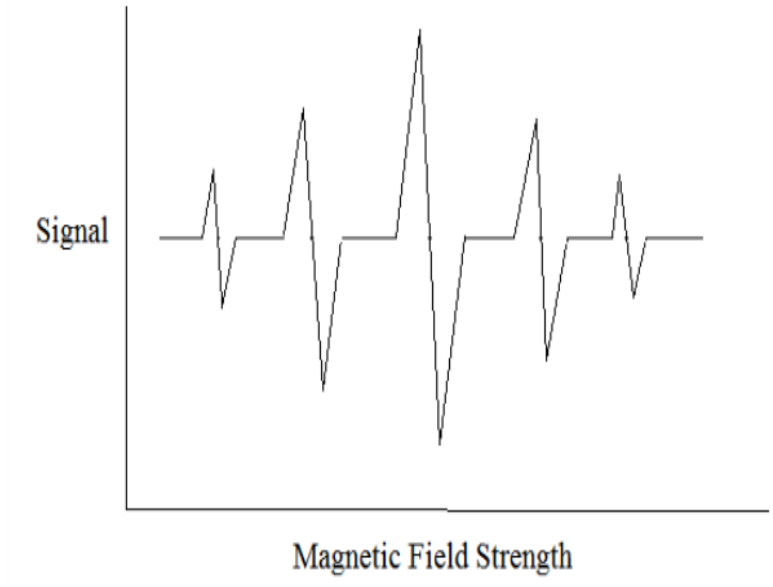
  In a study of heterogeneous catalysis, the spin trapping technique has been used to prove the presence of radical species on a catalyst surface. e.g; In a study of palladium metal catalyst supported on alumina, it was shown that hydrogen is dissociatively chemisorbed by trapping hydrogen atoms with PBN (α-phenyl-N-t-butyl nitrone).

- Conducting electrons:

  ESR spectroscopy has been used to detect conduction electrons in solutions of alkali metals in liquid ammonia, alkaline earth metals, alloys (e.g; small amounts of paramagnetic metal alloyed with another metal).

- Reaction velocities and mechanisms:

  ESR is also found to be useful for determination of mechanisms and kinetics of reaction. The molecular interactions that exist e.g; between solvent and solute (environment) can also be studied by ESR spectroscopy. Special cells have been used in ESR spectroscopy, in which radicals are produced by irradiation with UV, gamma or X-rays or by electrolytic redox reactions.



E.g; Ethyl radicals are produced when ethyl alcohol is irradiated with X-ray radiation. Spectrum shows five lines which conforms the formation of ethyl radicals. The 5 lines thus obtained are in the proportion of 1 : 4 : 6 : 4 : 1. This technique can also be used to study very rapid electron exchange reactions. e.g; addition of naphthalene to a solution of naphthalene radical anion. This causes the broadening of the hyperfine component of ESR resonance line, which can thus be employed to calculate the rate constant for the exchange between naphthalene and naphthalene radical anion.

- Analytical applications:

Determination of Mn^{2+}:

ESR spectrum of Mn2+ ionsshows six lines. The multiplicity is given by 2I + 1, where I is 5/2. i.e; 2 x 5/2 + 1 = 6. This ions can be measured and detected even when present in trace quantities.

Determination of Vanadium:

Traces of vanadium in petroleum oils cause corrosion in combustion engines and furnaces and alter the catalytic cracking of petroleum during processing. ESR spectrum shows an 8 line spectra (I is 7/2); 2I + 1 = 2 x 7/2 + 1 = 8.

Determination of Polynuclear hydrocarbons:

ESR spectroscopy has been used to estimate polynuclear hydrocarbons which are first converted into radical cations and then absorbed in the surface of an activated silica-alumina catalyst. Free radicals so formed are then analysed. E.g; Naphthalene, anthracene, dimethylanthracene, perylene, etc. In case of Naphthalene negative ion, there are two sets (alpha & beta) of 4 equivalent protons each. (2nI+1)(2nI+1) = (4+1)(4+1) = 25 lines.

8.6 Check Your Progress

1. Explain effects of the crystal lattice on the magnitude of e2Qq.
2. Compare the e_2qQ values of $^{35,37}Cl$ and $^{79,81}Br$ in the compounds NaCl, FCl, BrCland NaBr.
3. Discuss the applications of ESR spectroscopy.
4. List out the difference between NMR and ESR.
5. Brief on the apparent frequency due to Doppler Effect for different cases.

Answers To Check Your Progress Questions

1. What can we learn from temperature dependent EPR spectra?

The increment of the intensity of EPR is associated with the magnetic transition phase may be from para to antiferromagnetic because the spin system in magnetic materials are highly depend on the temperature of the system (curie and niel temperature)"

2. What we can learn from ESR

ESR measurements afford information about the existence of unpaired electrons, as well as quantities, type, nature, surrounding environment, and behavior.

- The ‘g’ value, which reflects the orbit level occupied by the electron
- Line width, which is related to the transverse relaxation time
- Saturation characteristics, which are related to the longitudinal relaxation time
- Number of unpaired electrons
- Hyperfine structure :hfs, which represents the interactions between electrons and nuclei
- Fine structure: fs, which represents the interactions between electron and electron
- Exchange interactions reflecting the exchanges between electrons

3. List out the various applications of EPR spectra

- Electron state, such as magnetic materials and semiconductors
- Electron state of semiconductor lattice defects and impurities (dopants)
- Structure of glass and amorphous materials
- Tracking of catalytic reactions, changes in charge state
- Photo-catalytic reactivity and photochemical reaction mechanisms
- Radicals of polymer polymerization processes (photo-polymerization, graft polymerization)
- Polymer resolution (photolysis, radiolysis, pyrolysis, chemical decomposition)
- Active oxygen radicals related to aging in disease in living organisms
- Oxidative degradation of lipids (food oils, petroleum, etc.)
- Detection of foodstuffs exposed to radiation
- Measurement of the age of fossils and geological features using lattice defects

8.7 Summary

- In the EPR experiment the sample is placed in a magnetic field, which removes the degeneracy of the various spin states of the paramagnetic center. Transitions between the different spin states can then be induced by irradiation at the appropriate microwave frequency.
- The registration of the absorption of the microwave by the sample produces the EPR spectrum. This spectrum is highly sensitive to the physical and chemical environment of the unpaired electrons and therefore it is very useful for the characterization of paramagnetic centers.
- There are different ways for carrying out EPR measurements, such as continuous irradiation of the microwaves, at a fixed frequency, while changing the magnetic field or by application of series of microwave pulses at a fixed magnetic field. Like NMR, EPR is a very rich spectroscopy, including many different experimental techniques, relying on well established theoretical foundations based on both quantum and statistical mechanics. Nonetheless, the

challenge for devising new experimental techniques is still there to improve resolution and sensitivity.

8.8 Keywords

Homogeneous broadening and Heterogeneous broadening
Zeeman effect, g-factor and hyperfine interaction

8.9 Self-assessment questions and exercises

1. What are the difference between NMR and EPR?.
2. Discuss the theory of EPR instrument.
3. Explain with suitable examples of homogeneous and heterogeneous broadening.
4. Discuss the basic principle and instrumentation of EPR.

8.10 Further readings

1. Pake G. E., *Paramagnetic Resonance*, (W. A. Benjamin, 1962).
2. Wertz J.E., Bolton J.R. *Electron spin resonance.* - New York, McGraw-Hill Book Company, 1972.
3. C. P. Slichter, *Principles of Magnetic Resonance* (CLAS), 3rd ed., (Springer-Verlag, 1992 and Harper & Row, 1963), p. 65.
4. Van Vleck J. H., *Phys. Rev.*, **74**, 1168, (1948).
5. Feynman, Leighton, and Sands, *Lectures on Physics*, (Addison-Wesley Publishing Company, 1965), Vol. II, Chap. 23 & 24.
6. McConnell, H.M. J. Chem. Phys. **24**, 764 (1956)

UNIT IX: MASS SPECTROSCOPY

Structure

9.0 Introduction:

Mass spectrometry's characteristics have raised it to an outstanding position among analytical methods: unequalled sensitivity, detection limits, speed and diversity of its applications. In analytical chemistry, the most recent applications are mostly oriented towards biochemical problems, such as proteome, metabolome, high throughput in drug discovery and metabolism, and so on. Other analytical applications are routinely applied in pollution control, food control, forensic science, natural products or process monitoring. Other applications include atomic physics, reaction physics, reaction kinetics, geochronology, inorganic chemical analysis, ion–molecule reactions, determination of thermodynamic parameters (ΔG°_f, Ka, etc.), and many others. Mass spectrometry has progressed extremely rapidly during the last decade, between 1995 and 2005. This progress has led to the advent of entirely new instruments. New atmospheric pressure sources were developed, existing analysers were perfected and new hybrid instruments were realized by new combinations of analysers.

9.1 Objectives:

The learner will be able to,

- Mass spectrometry (MS) is the technique for protein identification and analysis by production of charged molecular species in vacuum, and their separation by magnetic and electric fields based on mass to charge (m/z) ratio.
- MS has increasingly become the method of choice for analysis of complex protein samples in proteomics studies due to its ability to identify thousands of proteins.
- Define Fundamentals of Mass Spectrometry Describe Ionization techniques.
- Recall Mass Analyzers, and, Recall Tandem mass spectrometry

Introduction

Mass spectroscopy is a quantitative and qualitative analytical technique by which we can measure the molecular mass and formula of a compound and the record is known as mass spectra.

Mass spectra is useful

To establish the structure of a new compound

To give the exact molecular mass

To give the molecular formula

To indicate the presence of functional group in a compound

Principle/function:

The mass spectrometer is designed to perform four basic functions

• To vaporize the compound by increasing volatility.

• To generate the ions from the neutral compound in resulting vapor pressure

• To separate the ions according to their mass to charge ratio (m/z) in a magnetic field.

• To collect the mass and record.

Parent ion:

An electrically charged molecular moiety which may dissociate to form fragments, one or more of which may be electrically charged, and one or more neutral species. A parent ion may be a molecular ion or an electrically charged fragment of a molecular ion.

Metastable ion:

Fragment of a parent ion will give rise to a new ion either a neutral molecule or a radical.

$$M_1^+ \rightarrow M_2^+ + \text{non charged particle.}$$

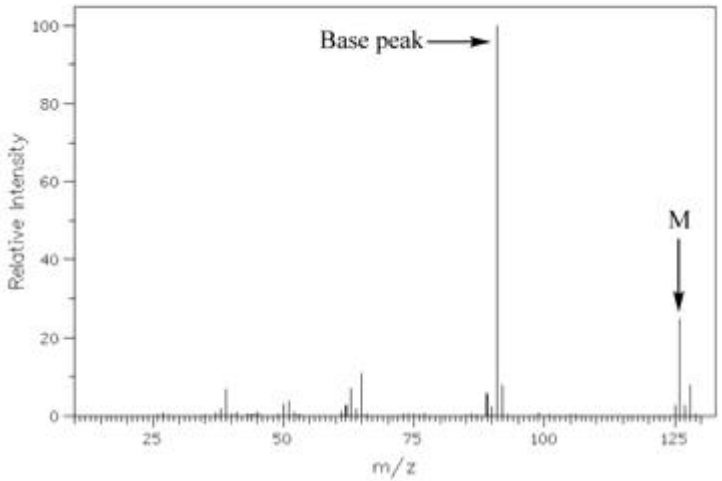
An intermediate situation is possible. M_1^+ may decompose to M2+ while being accelerated. The resultant daughter ion M2+ will not be recorded at either M1 or M2, but at a position M* as a rather broad, poorly focused peak. Such an ion is called a meta stable ion.

Isotopic ion

Any ion containing one or more of the less abundant naturally occurring isotopes of the elements that make up its structure. For example, CH_2D^+.

Base peak

The most intense (tallest) peak in a mass spectrum due to the ion with the greatest relative abundance is called base peak. Base peaks are not always molecular ions and molecular ions are not always base peaks.

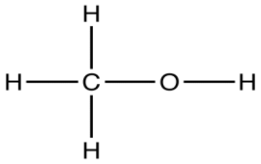


The electron impact ionization mass spectrum of $PhCH_2Cl$ is shown above in which the base peak is a fragment ion m/z = 91.

Nitrogen rule

The nitrogen rule states that a molecule that has no or even number of nitrogen atoms has an even nominal mass, whereas a molecule that has an odd number of nitrogen atoms has an odd nominal mass.

Examples:



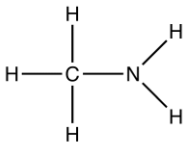
molecular formula = CH_4O

nominal mass = (1x12) + (4x1) + (1x16)

= 32

N atoms = 0

nominal mass = 32 (even #)



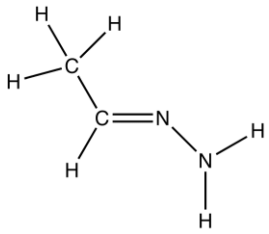
molecular formula = CH_5N

nominal mass = (1x12) + (5x1) + (1x14)

= 31

N atoms = 1 (odd #)

nominal mass = 31 (odd #)



molecular formula = $C_2H_6N_2$

nominal mass = (2x12) + (6x1) + (2x14)

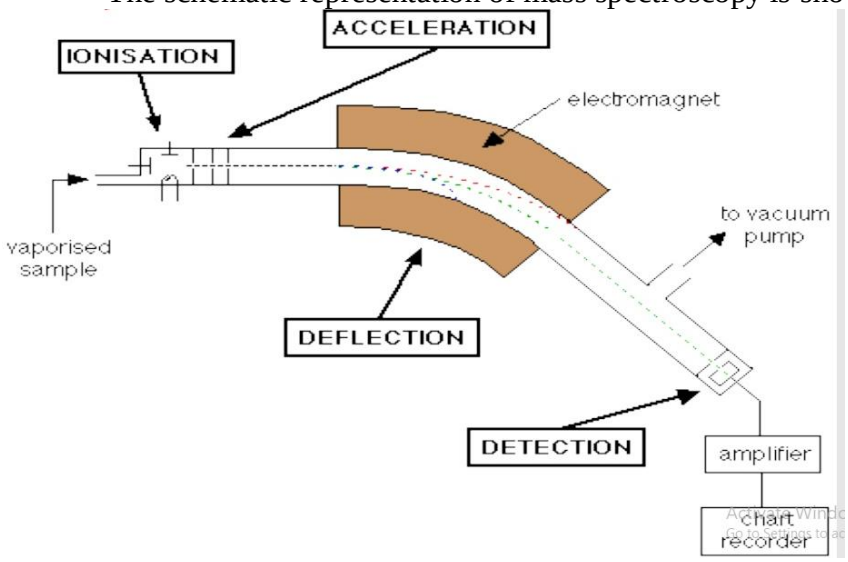
= 58

N atoms = 2 (even #)

nominal mass = 58 (even #)

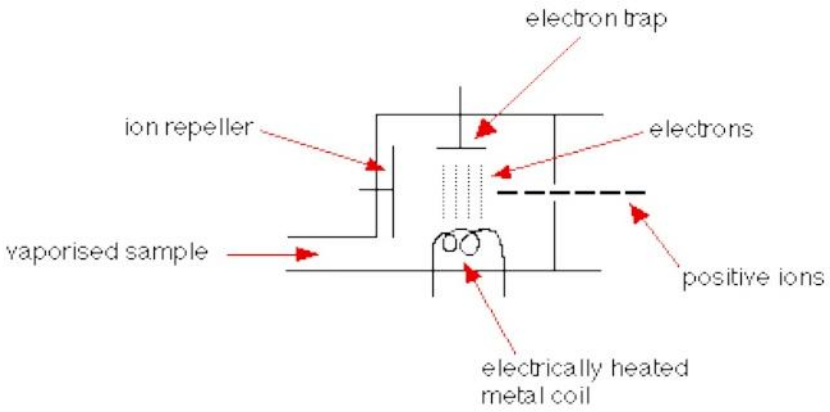
Instrumentation:

The schematic representation of mass spectroscopy is shown below



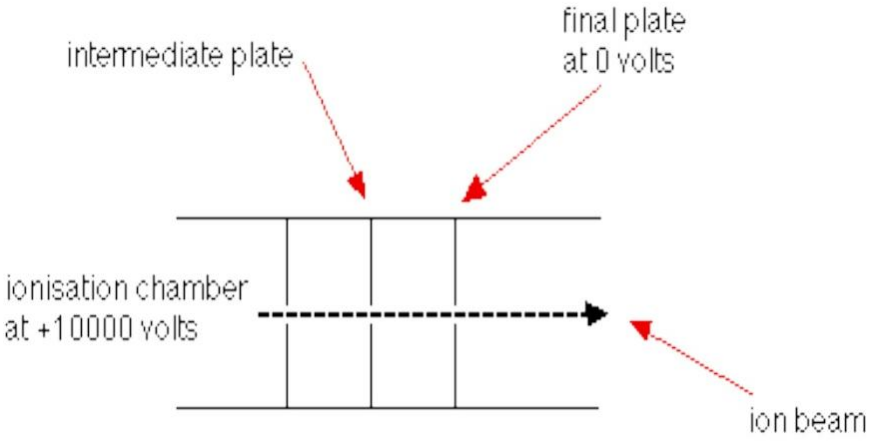
Ionisation:

The atom is ionized by knocking one or more electrons off to give a positive ion. The particles in the sample are bombarded with a stream of electrons to knock one or more electrons out of the sample particles to make positive ions. Most of the positive ions formed will carry a charge of + 1. These positive ions are persuaded out into the rest of the machine by the ion repeller which is another metal plate carrying a slight positive charge.



Acceleration:

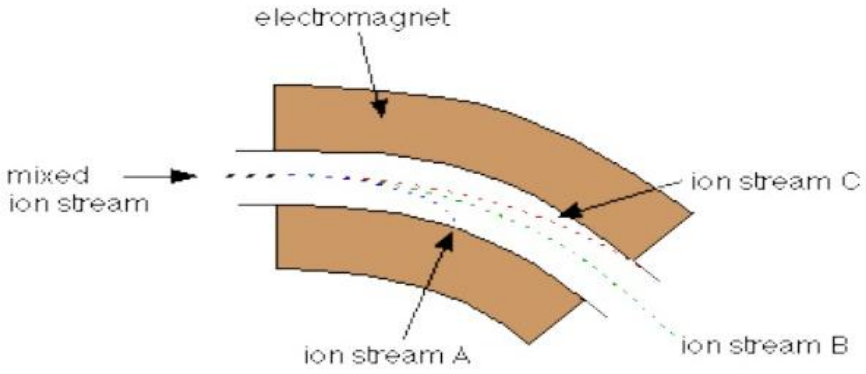
The ions are accelerated so that they all have the same kinetic energy. The positive ions are repelled away from the positive ionization chamber and pass through three slits with voltage in the decreasing order. The middle slit carries some intermediate voltage and the final at 0 volts. All the ions are accelerated into a finely focused beam.



Deflection:

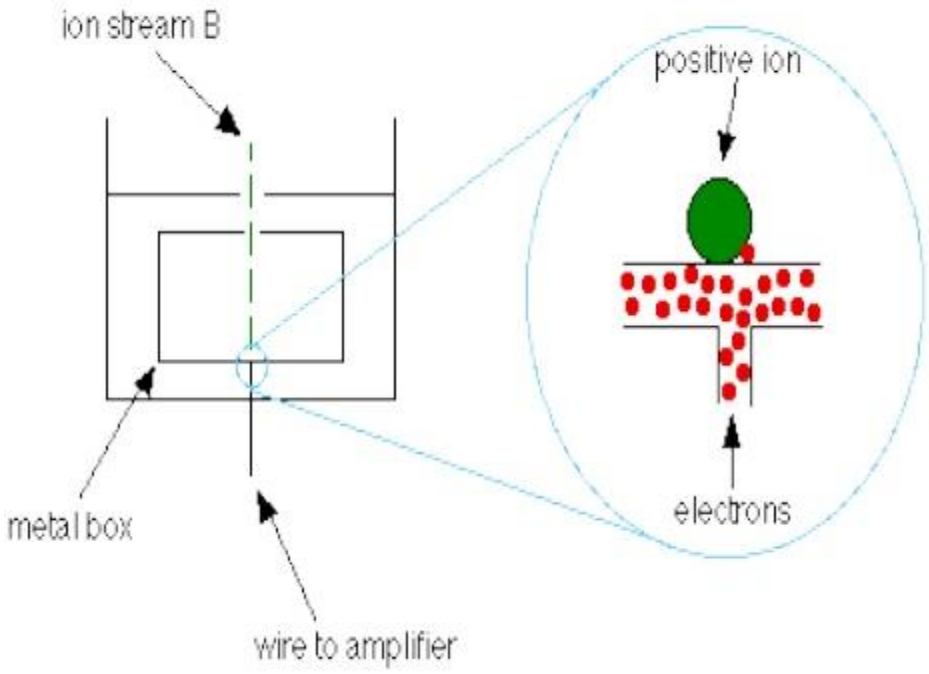
The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they deflected. The amount of deflection also depends on the number of positive charges on the ion. The more the ion is charged, the more it gets deflected. The different ions are deflected by the magnetic field by different amounts. The amount of deflection depends on

- Mass of the ion and
- Charge of the ion.



Detection

The beam of ions passing through the machine is detected electrically. When an ion hits the metal box, its charge is neutralized by an electron jumping from the metal on to the ion. That leaves the space amongst the electrons in the metal and the electrons in the wire shuffle along to fill it. A flow of electrons in the wire is detected as an electric current which can be amplified and recorded. The more ions arriving, the greater the current.



General rules for fragmentation

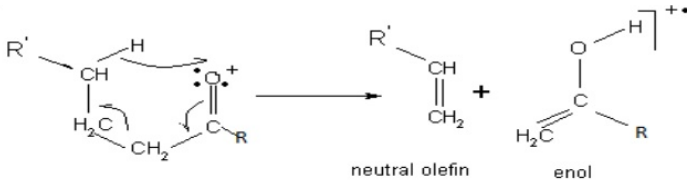
1) The relative height of the molecular ion peak is greatest for the straight chain compound and decreases as the degree of branching decreases.
2) The relative height of the molecular ion peak usually decreases with increasing molecular weight in a homologous series.
3) Cleavage is favored at alkyl substituted carbon atoms, the more substituted, the more likely is cleavage. This is a consequence of the increased stability of a tertiary carbon atom over a secondary, which in turn is more stable than a primary.

$CH_3^+ < RCH_2^+ < R_2CH^+ < R_3C^+$.

Mclafferty rearrangement

Fragmentation due to rearrangement of parent ion: The cleavage of bonds in molecular ions is due to the intramolecular atomic rearrangement. This leads to fragmentation whose origin cannot be described by simple cleavage of bonds. When fragments are accompanied by bond formation as well as a bond breaking, a rearrangement process is said to have occurred. Such rearrangement involves the transfer of hydrogen from one part of the molecular ion via a six membered cyclic transition state. This process is favoured energetically because as many bonds are formed as are broken.

Compounds containing hydrogen atom at position gamma to carbonyl group found to a relative intense peak. This is probably due to rearrangement and fragmentation accompanied by a loss of water molecule. This rearrangement is known as Mclafferty rearrangement. This rearrangement results in the formation of charged enols and a neutral olefins.



To undergo Mclafferty rearrangement, a molecule must posses

1) An appropriately located hetero atom (expect oxygen)
2) A double bond.
3) An abstract able hydrogen atom which is gamma to C=O system.

Table for the common Mclafferty peak in the spectra of carbonyl group.

Compound type	Substituent (R)	McL peak
Aldehyde	-H	44
Methyl ketone	$-CH_3$	58
Amide	$-NH_2$	59
Acid	-OH	60
Ethyl ketone	$-CH_2CH_3$	72
Methyl ester	$-OCH_3$	74

Structural elucidation

1. Saturated hydrocarbons:

a. Straight chain compounds: Following are the features of the mass spectra of alkanes.

- The relative height of the parent peak decreases as the molecular mass increases in the homologous series.
- The molecular ion peak is normally present
- The spectra generally consists of clusters of peaks separated by 14 mass units corresponding to differences of CH_2 groups.
- The largest peak in each cluster represents C_nH_{2n+1} fragment. This is accompanied by C_nH_{2n} and C_nH_{2n-1} fragment corresponding to the loss of one and two H atoms respectively.

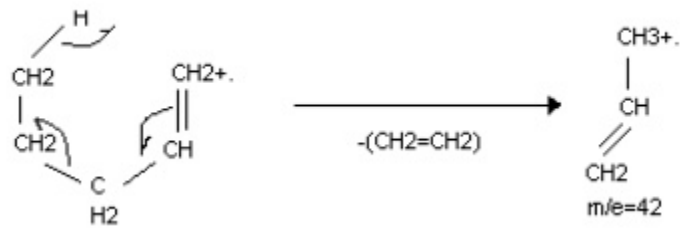
b. Branched chain Hydro carbons:

- Greater the branching in alkanes less is the appearance of the molecular ion and if it appears, intensity will be low.
- Bond cleavage takes place preferably at the site of branching. Due to such cleavage, more stable secondary or tertiary carbonium ion results.
- Greater number of fragments results from the branched chain compound compared to the straight compound. This is due to greater pathways available for cleavage.

2. Alkenes

- The molecular ion of alkene containing one double bond tends to undergo allylic cleavage i.e. at the beta bond without the double bond and gives resonance structure.
- The molecular ion peak in the spectra of unsaturated compounds is more intense than the corresponding saturated analogues. The reason is the better resonance stabilization of the charge on the cation formed by the removal of one of the π-electrons.
- The relative abundance of the molecular ion peak decreases with increase in molecular mass.
- The general mode of fragmentation is the allylic cleavage.
- The fragments formed by Mclafferty rearrangement are more intense.

Ex: 1- pentene.



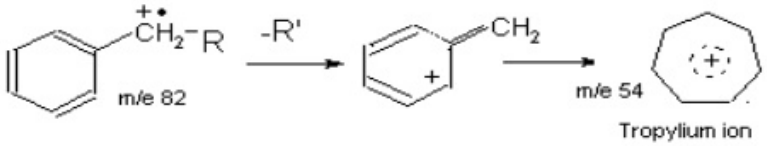
3. Aromatic compounds:

- It shows prominent molecular ion peak, as compared to the alkanes and alkenes containing same number of C

atoms. This is as a result of the stabilizing effect of the ring.

- In these compounds $M^+ + 1$ and $M^+ + 2$ are also noticed, due to ^{13}C. If aromatic ring is substituted by an alkyl groups a prominent peak is formed at m/z 91. Here, benzyl cation formed rearranges to tropylium cation. This may eliminate a neutral acetylene molecule to give a peak at m/e 65.

Ex:



4. Alcohols:
   - The molecular ion peak of 1^0 and 2^0 alcohol is usually of low abunordance. It is not detected in 3^0 alcohols.
   - The fragmentation modes in alcohols depend upon the fact whether it is 1^0, 2^0 or 3^0 alcohols.
   - The fragmentation of C-C bond adjacent to oxygen atom is preferred fragmentation mode i.e. alpha cleavage.
   - 1^0 alcohols shows M-18 peaks, corresponding to the loss of water
   - Long chain members may show peaks corresponding to successive loss of water.
   - The $–CH_2OH$ is the most significant peak in the spectra of 1^0 alcohols.
   - 2^0 alcohols cleave to give prominent peaks due R-CH=OH at m/z= 45, 59, 73.
5. Aromatic alcohols:
   - The relative abundance of the parent ion of aromatic alcohols is large.
   - Some of the fragment modes of benzyl alcohol are loss of one, two or three hydrogen atoms.
   - M+H fragment of benzyl alcohol also rearranges to form hydroxyl tropylium ion.
   - The OH group in the benzyl positions fragments in a way, which favors charge retention on the aryl group.

6. Phenols:
   - The molecular ion peak is intense
   - The peak due to the loss of hydrogen radical, M^+ - H is small.
   - The fragment ion due to the loss of carbon monoxide is significant.
   - Cresols form very intense peak due to the formation of hydroxyl tropylium ion.
7. Aldehydes:
   - The molecular ion peak of aliphatic aldehydes is week.
   - Aromatic aldehydes shows moderate intense peak.

$$R\text{-}CH{=}O^{+} \rightarrow R\text{-}CO^{+} + H^{+}$$

- The characteristic feature of aromatic aldehyde is loss of α hydrogen.
- The common feature of aliphatic aldehyde is loss of β hydrogen.
- For ex, Aldehyde with $–CH_2CHO$ end groups gives rise to characteristic M-43 peaks.

8. Ketones:
   - Molecular ion peaks are intense than aldehyde. Most of the abundant ions in the mass spectra of ketones can be accounted by α cleavage and Mclafferty rearrangement.
9. Nitro compounds:
   - Aliphatic nitro compounds fragment by loss of NO_2 to give strong carbonium ion.

$$R\text{-}NO_2^{+} \rightarrow R^{+} + NO_2.$$

   - A Mclafferty rearrangement occurs but such type of peaks are weak.
   - Nitrites show β cleavage
10. Aliphatic acids:
    - The molecular ion peak in aliphatic acids is less intense as compared to that of aromatic acids. Carboxyl group is directly eliminated by α cleavage and a signal is formed at m/e 45.
    - If α carbon atom is not substituted in aliphatic acids containing a gamma hydrogen, Mclafferty rearrangement ion is formed at m/e 60. It is often the base peak.
    - In short chain acids, M-OH and M-COOH peaks are prominent.
11. Halogen compounds:
    - A compound with 1 chlorine atom gives a M+2 peak, which is one third the intensity of the molecular ion peak due to the presence of Molecular ion containing ^{37}Cl isotope.
    - In mono bromo derivative the M+2 peak is almost of equal intensity to the molecular ion and due to the presence of molecular ion containing ^{81}Br isotope.
    - Fluorine and iodine being mono isotopic do not give these patterns.
    - Aliphatic chlorine compounds fragment mainly by the loss of HCl to give peaks at M-36 and M-38. HCl peaks can also be seen at m/z 36, 38.
    - The relative abundance of the molecular ion decreases with increase in chain length and increase in branching.
12. Ethers:
    - Aliphtic ethers undergo facile fragment fragmentation and exhibit a weak molecular ion peak because the resultant ion is highly stabilized by resonance.
    - The major fragmentation modes occur through α and β cleavages.
13. Aromatic ethers:

The molecular ion peak of aromatic ether is prominent. 1^0 cleavage occurs at the bond β to the ring and first formed ions can decompose further.

9.8 Summary

- All mass spectrometers combine ion formation, mass analysis, and ion detection.
- This chapter discussion is concerned with how various mass analyzers are used to separate ions according to their massto-charge ratio.
- Each mass analyzer has its own special characteristics and applications and its own benefits and limitations.
- The choice of mass analyzer should be based upon the application, cost, and performance desired. There is no ideal mass analyzer that is good for all applications.

Keywords:

Meta stable ion: Metastable ions are those ions that have internal energy in excess of that required to break chemical bonds but are sufficiently long lived to fragment only after leaving the source.

Isotopic ions: Isotope Peaks of Ionic Fragments in Mass Spectrometry. Theory. Mass Spectrometry is based on the formation of a beam of ionic fragments by bombardment of test molecules, usually with energetic electrons. The generated ions are then separated by application of electrostatic or magnetic fields or by a combination of both.

Nitrogen rule: The nitrogen rule states that a molecule that has no or even number of nitrogen atoms has an even nominal mass, whereas a molecule that has an odd number of nitrogen atoms has an odd nominal mass.

MacLafferty Rearrangement : The McLafferty rearrangement is a characteristic fragmentation of the molecular ion of a carbonyl compound containing at least one gamma hydrogen

9.9 Self-assessment questions and exercises

1. What is meta stable ion peak?
2. What is McLafferty rearrangement?
2. Discuss the principle, instrumentation and applications of mass spectroscopy.
3. Define basic peak nitrogen rule.
4. What is mean by general rule of fragmentation. Explain with suitable example.
5. List out the various types of mass spectroscopy.
6. How can mass spectrometric data be used for structure analysis?

1. How large a molecule can be analyzed?
2. What other techniques are usually combined with mass spectrometry?
3. How is mass spectrometry used for quantitative analysis?

Answers To Check Your Progress Questions

1. What is isotope peak?

This probability is the sum of probabilities of all combinations resulting in the same nominal mass. The most intense peak is called base peak and the

relative intensities of the other peaks are commonly reported as % of base peak (blue numbers).

2. What is metastable ion in mass spectrometry?

   An ion which is formed with sufficient excitation to dissociate spontaneously during its flight from the ion source to the detector.
3. What is nitrogen rule in mass spectrometry?
   The nitrogen rule states that organic compounds containing exclusively hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens either have 1) an odd nominal mass that indicates an odd number of nitrogen atoms are present or 2) an even nominal mass that indicates an even number of nitrogen.
4. What is the M+ peak in mass spectrometry?
   In the mass spectrum, the heaviest ion (the one with the greatest m/z value) is likely to be the molecular ion. A few compounds have mass spectra which don't contain a molecular ion peak, because all the molecular ions break into fragments. That isn't a problem you are likely to meet at A' level.
5. What is meant by McLafferty rearrangement?
   The McLafferty rearrangement is an organic reaction seen in mass spectrometry. ... The McLafferty rearrangement is an example of a hydrogen atom jumping to the other fragment as a part of the process of the bond breaking. It happens in an organic molecule containing a keto-group.

9.10 Further readings

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3. Cotter, R.J. (ed.) (1994) Time-of-Flight Mass Spectrometry, American Chemical Society, Washington, DC.

4. March, R.E. and Todd, J.F.J. (2005) Quadrupole Ion Trap Mass Spectrometry, 2nd edn, John Wiley & Sons, Inc., New York.

5. Korfmacher, W.A. (2005) Using Mass Spectrometry for Drug Metabolism Studies, CRC Press, Boca Raton, FL.

UNIT X: ORD AND CD

Structure

10.1 Introduction:

Optical Rotatory Dispersion (ORD)

ORD is the variation in the optical rotation of the substance with a change in the wavelength of light. It can be used to find the absolute configuration of metal complexes. The measurement of optical rotation as a function of wavelength is termed as optical rotatory dispersion spectroscopy. The fundamental principles of ORD are

- Plane/ linearly polarized light
- Optical activity
- Specific rotation
- Circular Birefringence/ optical rotation

Circular Dichromism (CD)

Circular dichroism is the difference in the absorption of left- handed circularly polarised light (L- CPL) and right- handed circularly polarised light (R- CPL) and occurs when a molecule contains one or more chiral chromophores (light- absorbing groups).

Circular dichroism = $\Delta A\ (\lambda) = A(\lambda)LCPL - A(\lambda)RCPL$,

Where, λ is the wavelength

Circular dichroism (CD) spectroscopy is a spectroscopic technique where the CD of molecules is measured over a range of wavelengths. CD spectroscopy is used extensively to study chiral molecules of all types and sizes, but it is in the study of large biological molecules where it finds its most important applications. A primary use is in analyzing the secondary structure or conformation of macromolecules, particularly proteins as secondary structure is sensitive to its environment, temperature or pH, circular dichroism can be used to observe how secondary structure changes with environmental conditions or on interaction with other molecules. Structural, kinetic and thermodynamic information about macromolecules can be derived from circular dichroism spectroscopy. Measurements carried out in the visible and ultra- violet region of the electro- magnetic spectrum to monitor electronic transitions. A circular dichroism signal can be positive or negative, depending on whether L- CPL is absorbed to a greater extent than R- CPL (CD signal positive) or to a lesser extent (CD signal negative).

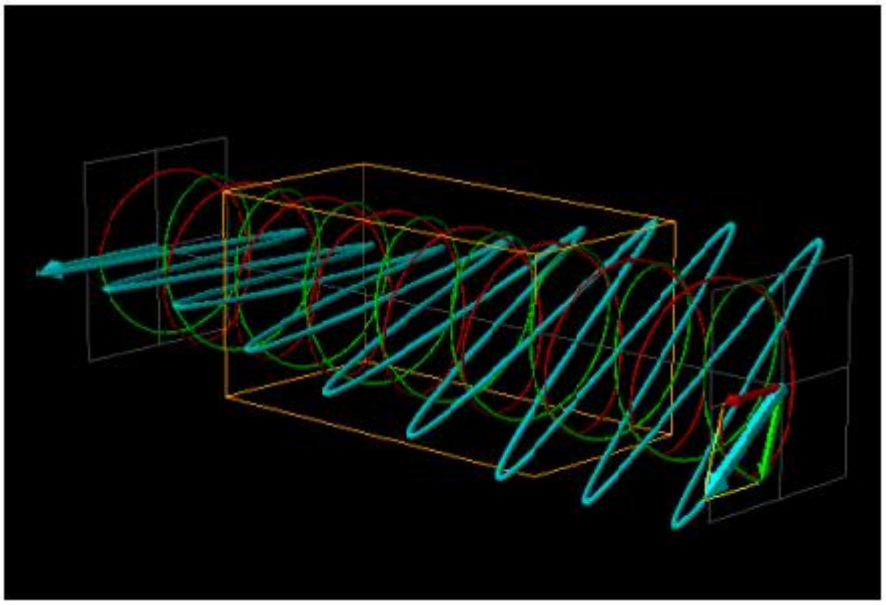
Objectives:

The learner will be able to,

- To understand about assigning R and S configuration to chiral structures.
- To learn and Relate plain ORD curves and their sign with absolute configuration of sample.
- To understand why anomalous ORD or Cotton effect is observed.
- To understand about the electronic or vibrational transistions associated with a chiral center in a molecule absorb right and left circularly polarized light differently.
- To study about a plot of this ORD vs change of wavelength gives rise to CD curve.

Circular birefringence

Chiral molecules exhibit circular birefringence, which means that a solution of a chiral substance presents an anisotropic medium through which left circularly polarised (L- CPL) and right circularly polarised (R- CPL) propagate at different speeds. A linearly polarised wave can be thought of as the resultant of the superposition of two circularly polarised waves, one left- circularly polarised, the other right- circularly polarised. On traversing the circularly birefringent medium, the phase relationship between the circularly polarised waves changes and the resultant linearly polarised wave rotates. This is the origin of the phenomenon known as optical rotation, which is measured using a polarimeter. Measuring optical rotation as a function of wavelength is termed optical rotatory dispersion (ORD) spectroscopy.

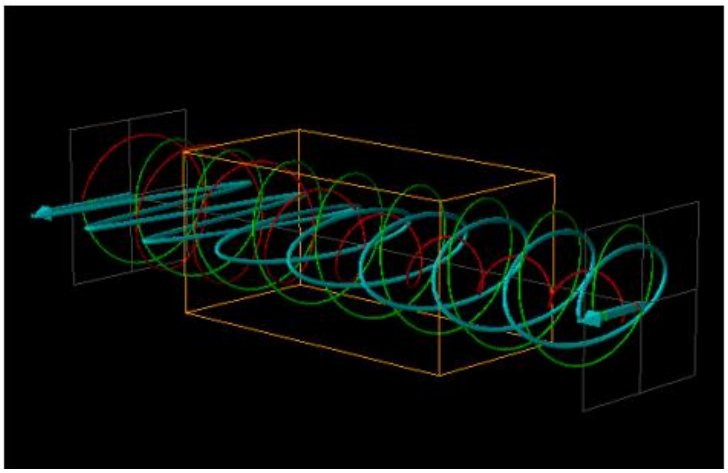


Circular birefringence - the orange cuboid represents the sample.

Circular dichroism

Unlike optical rotation, circular dichroism only occurs at wavelengths of light that can be absorbed by a chiral molecule. At these wavelengths Left- and right- circularly polarised light will be absorbed to

different extents. For instance, a chiral chromophore may absorb 90% of R- CPL and 88% of L- CPL. This effect is called circular dichroism and is the difference in absorption of L- CPL and R- CPL. Circular dichroism measured as a function of wavelength is termed circular dichroism (CD) spectroscopy and is the primary spectroscopic property measured by a



circular dichroism spectrometer such as the Chirascan.

Circular dichroism - the orange cuboid represents the sample

Optical rotation and circular dichroism stem from the same quantum mechanical phenomena and one can be derived mathematically from the other if all spectral information is provided. The relationship between optical rotatory dispersion, circular dichroism, absorption spectra and chirality are shown below, with a comparison of the two enantiomers of camphor sulphonic acid.

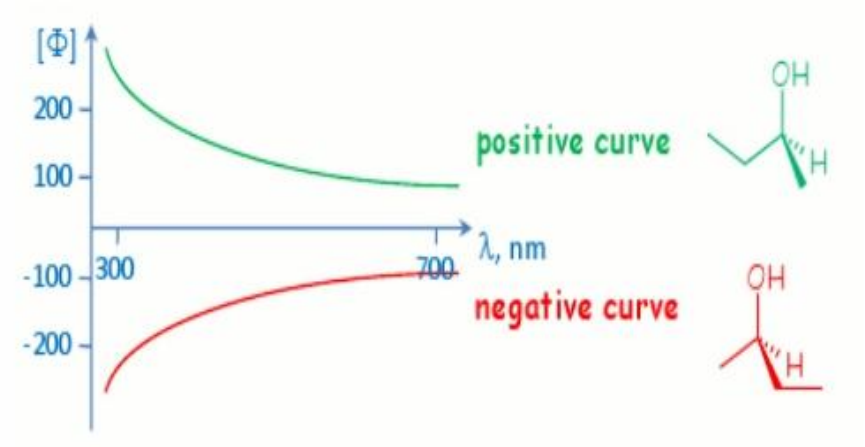
Cotton effect:

The Cotton effect is the characteristic change in optical rotatory dispersion and/or circular dichroism in the vicinity of an absorption band of a substance. The combination of both circular birefringence and circular dichroism effects in the region in which the optically active bands are observed give rise to a phenomenon called cotton effect. This phenomenon was discovered in 1895 by the French physicist Aimé Cotton.

Djerassi and Klyne suggested that the rotatory dispersion curves classified into 2 types. They are

- Plain curves
- Cotton effect curves

Plain curves: These are normal curves occurs at absorption maximum. These curves obtained for compounds which don`t have absorption in wavelength where optically active compounds are examined.

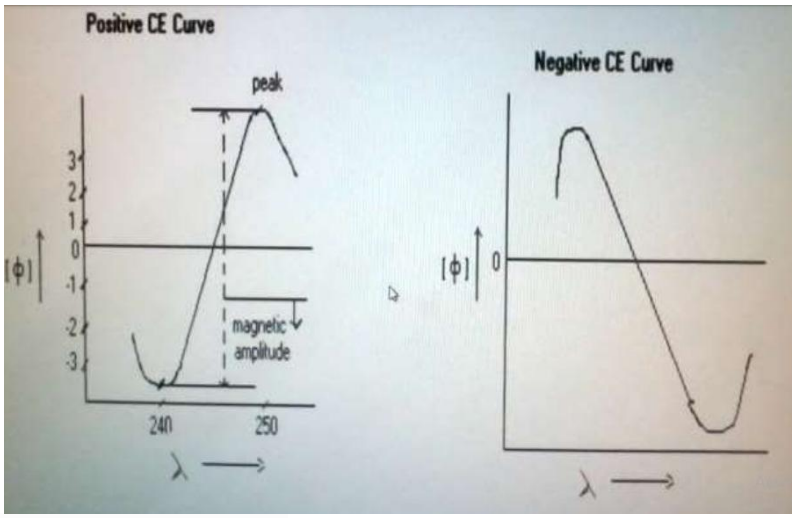


Cotton effect curves:

Cotton effect curves show high peaks and troughs which depends on the absorbing groups. These curves will obtain for the compounds having asymmetric carbon and chromophore which absorbs near UV region. These also again divided into two types, they are a) single cotton effect curves b) multiple cotton effect curves.

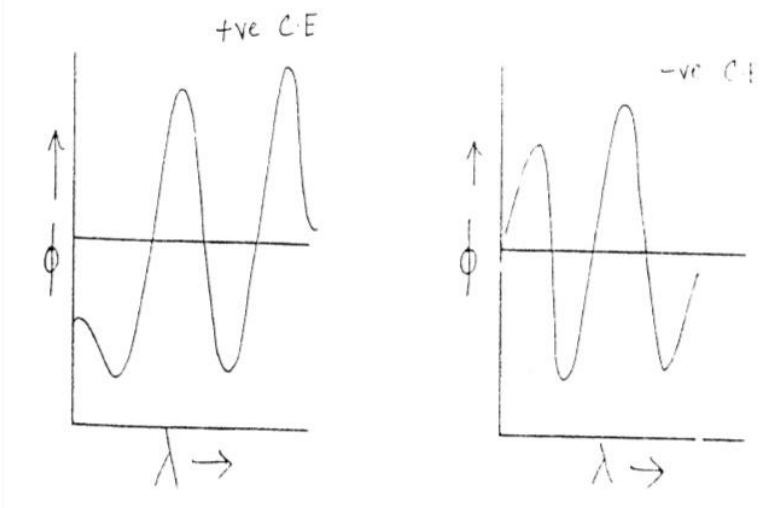
Single cotton effect curves:

These single cotton curve will show both maximum and minimum curves at maximum absorption. The Cotton effect is called positive if the optical rotation first increases as the wavelength decreases, and negative if the rotation first decreases. Protein structure like beta sheet shows positive Cotton Effect.



Multiple cotton effect curves:

These are little different from single cotton effect curves. Here more than two crest or trough is obtained. Ex. Camphor.



10.8 Summary

- Light is an electro-magnetic wave and interacts with matter. Classically speaking the electrons are forced into an oscillation. A forced oscillation can be modelled as a spring with an inert mass coupled to a (mechanical) oscillator
- Easily understand the theory and applications of ORD and CD.
- These spectroscopic techniques are suited to determine chiral structures. In particular in proteins that means helical and leaf-type structures.
- The sign of Cotton effect gives information about the stereochemistry in the nearby environment of the chromophore, i.e., the carbonyl group (n→ π* absorption of the carbonyl group around 280 nm) acts as a probe of the chirality of its environment.

Keywords: Circular birefringence, circular dichromism, Cotton effect

10.9 Self-assessment questions and exercises

1. Discuss the principle of ORD and CD?
2. Explain with suitable examples of Cotton effect.
2. What is the main role of cotton effect?
3. What are the causes of cotton effect?
4. What is the cotton effect in chemistry?
5. List out the difference between the circular birefringence and circular dichromism.

Answers To Check Your Progress Questions

1. Explain the basic principles of ORD and CD?

   The variation of optical rotation as a function of wavelength is called optical rotary dispersion (ORD). Right- and left-circularly polarized light will also be absorbed to different extents at some wavelengths due to differences in extinction coefficients for the two polarized rays called circular dichroism (CD).
2. What is the Octant rule?

Octant rule is very simple and basic rule of chemistry. This is used to find the no. Now any atom tends to stay in state of rest by making an octant in outermost shell, so it can either donate or accept electrons.

3. What are the causes of cotton effect?

   The Cotton effect is called positive if the optical rotation first increases as the wavelength decreases (as first observed by Cotton), and negative if the rotation first decreases. A protein structure such as a beta sheet shows a negative Cotton effect.

4. What is circular dichroism used for?

   Circular Dichroism (CD) is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light. Optically active chiral molecules will preferentially absorb one direction of the circularly polarized light.

10.10 Further readings

1. Huheey, J.E., E.A. Keiter and R.L. Keiter. 2002. Inorganic Chemistry: Principles of Structure and Reactivity, 4th Edition. New York: HarperCollins Publishers.

2. Gary L. Miessler., Paul J. Fischer., Donald A. Tarr. Inorganic Chemistry, 5th ed : Pearson

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UNIT XI: APPLICATIONS OF ORD AND CD

Structure

11.1 Introduction:

Circular dichroism (**CD**) is dichroism involving circularly polarized light, i.e., the differential absorption of left- and right-handed light. Left-hand circular (LHC) and right-hand circular (RHC) polarized light represent two possible spin angular momentum states for a photon, and so circular dichroism is also referred to as dichroism for spin angular momentum. This phenomenon was discovered by Jean-Baptiste Biot, Augustin Fresnel, and Aimé Cotton in the first half of the 19th century. It is exhibited in the absorption bands of optically active chiral molecules. CD spectroscopy has a wide range of applications in many different fields. Most notably, UV CD is used to investigate the secondary structure of proteins. UV/Vis CD is used to investigate charge-transfer transitions. Near-infrared CDisusedtoinvestigate geometric and electronicstructure by probing metal d→d transitions. Vibrational circular dichroism, which uses light from the infrared energy region, is used for structural studies of small organic molecules, and most recently proteins and DNA.

11.2 Objectives

After going through this unit, you will be able to:

- Understand about the basic principles of ORD and CD
- Understand the different configuration methods
- Explain the configurational analysis with different rules

11.3. Application of cotton effect curves

Cotton effect curves have been mainly applied to structural determination in two important fields.

(i) Amino acids, polypeptides and proteins, and

(ii) Complex natural products, such as steroids, terpens, antibiotics etc.,

(iii) The curves can provide information concerning the configuration of angular substituents at ring junctions, the location of ketone groups, conformational analysis of

substituents, the degree of coiling of protein helices and the type of substitution in amino acids. The applications of CD are less developed than ORD but CD provides much useful structural information regarding organic and biological systems and also metal-ligand complexes.

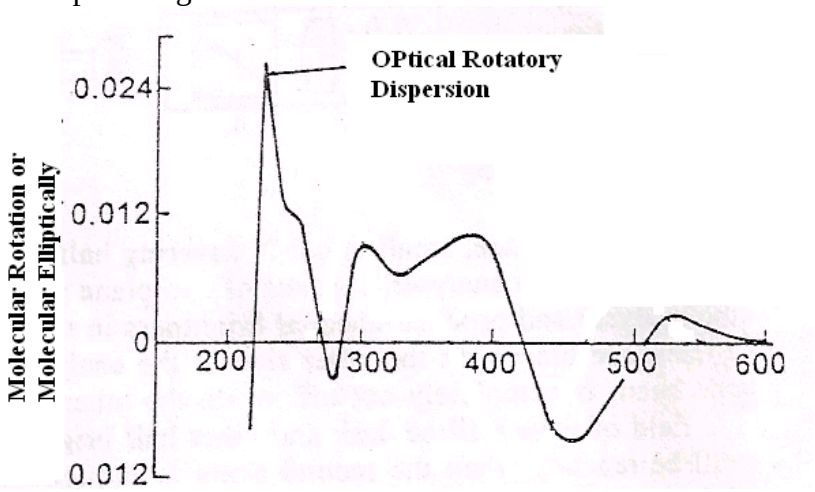
ORD curves

(i) The optical rotatory dispersion (ORD) curves consist of a plot of optical rotation as a function of wavelength. The types of curvature can be discerned.

(ii) The normal dispersion range, is a region in which [α] changes gradually with wavelength,

(iii) The region of anomalous dispersion which occurs near an absorption peak.

(iv) If one peak is isolated from others, the anomalous part of the dispersion curve will have the appearance of the curve (n_1-n_d) shown in figure 3.13.1. This indicates that rotation undergoes rapid change to some maximum (or minimum) value, alters direction to values corresponding to normal dispersion. As indicated in the figure a change in sign of the rotation may accompany these changes. If molecules have overlapping absorption peaks, the overlapping regions of anomalous dispersion give to ORD curves.

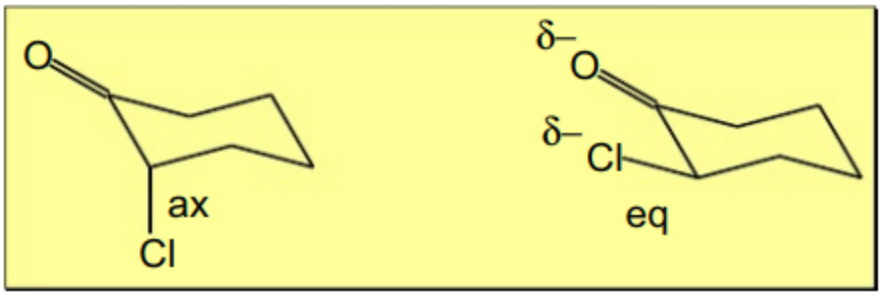


ORD curves

11.4 α- haloketone rule and Octand Rule

α- haloketone :

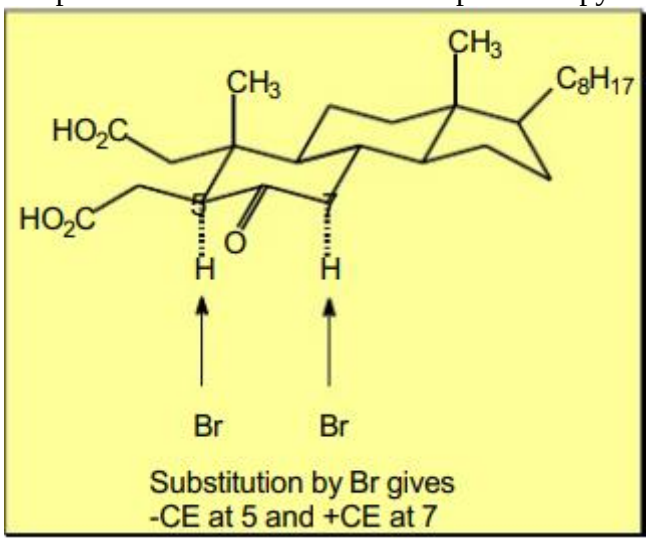
The α- haloketone rule is the most widely applied sector rule based on ORD measurements carried out on steroidal ketones that had been (axially) substituted with a halogen atom at the α-carbon. Axial substitution is often preferred because of the dipole-dipole repulsions in the equatorial isomer.



The following examples illustrate applications of the axial haloketone rule in structure determination.

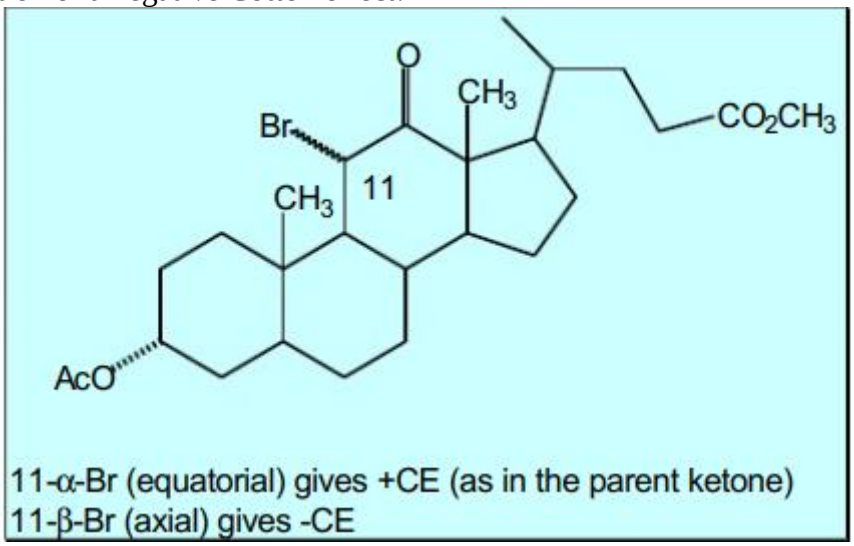
1. **Determination of Position of Halogen Substitution (Constitution)**

In the example below, a negative Cotton Effect is seen upon bromination of the cyclic fused ring ketone. Therefore, substitution must have occurred predominantly at the 5 position. The axial nature of bromine atom in the product was deduced from IR spectroscopy.



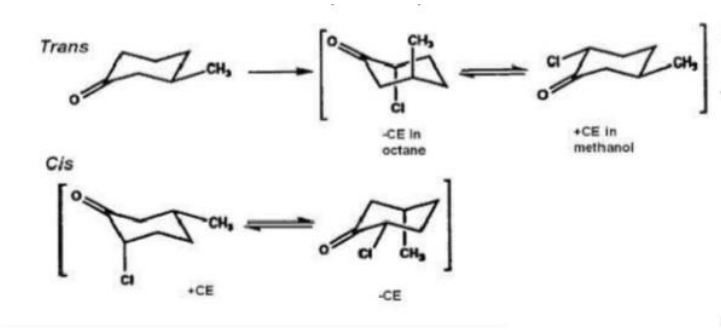
2. **Determination of Absolute Configuration**

The configuration of the 11-bromo-12-ketosteroid product from the bromination of the parent 12-ketosteroid was deduced to be (R) from the observation of a negative Cotton effect.



3. Demonstration of conformational mobility

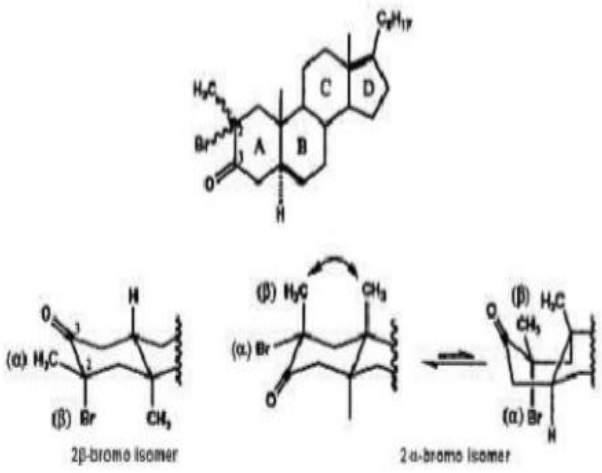
On chlorination of (R) - (+)-3- methylcyclohexanone, a crystalline 2-chloro-5-methyl product is isolated that shows a negative Cotton effect in octane, but a positive one in methanol. The negative CE is consistent only with trans stereochemistry, with independent evidence for axial Cl (in octane")



The change in sign of the CE on changing the solvent to (more polar) methanol is presumably a reflection of the greater stability of the equatorial conformer in that solvent.

4. Demonstration of the existence of a boat conformer

Of the 2α and 2β-bromo isomers of 2-bromo-2-methylcholestane-3-one, (with axial Br established by IR spectroscopy) the latter displays a positive CE as expected. The 2α-bromo isomer unexpectedly shows a negative CE. This is best explained by supposing the boat conformer is significant in ring A of this isomer, because of steric hindrance between the (axial) methyl groups in the chair conformer.



Octant rule:

The axial haloketone rule is a special case of the octant rule for saturated ketones. A set of left-handed Cartesian coordinates is drawn through the carbonyl groupwith its origin at the center of the bond and with the z axis collinear with the bond,as shown below. The coordinate system divides the space around the carbonyl group into 8 sectors or octants (diagram (a)). The effect on the CE associated with the n-$\pi^{\square}$ transition of the carbonyl group is given by the position of a substituent (as a product of its coordinates) in these segments. Thus, a substituent in the bottom right rear sector (diagram (b)) would have coordinates -x, +y, -z and so would

give a positive CE. Substituents located on or near nodal planes make no contribution to the Cotton effect.

The octant rule was first applied to fused cyclohexanone ring systems, such as those in steroids, because of their conformational rigidity. The cyclohexanone skeleton is placed in the coordinate system as shown below, with the 2 and 6 carbon atoms in the yz plane and the carbonyl at the head of the chair.

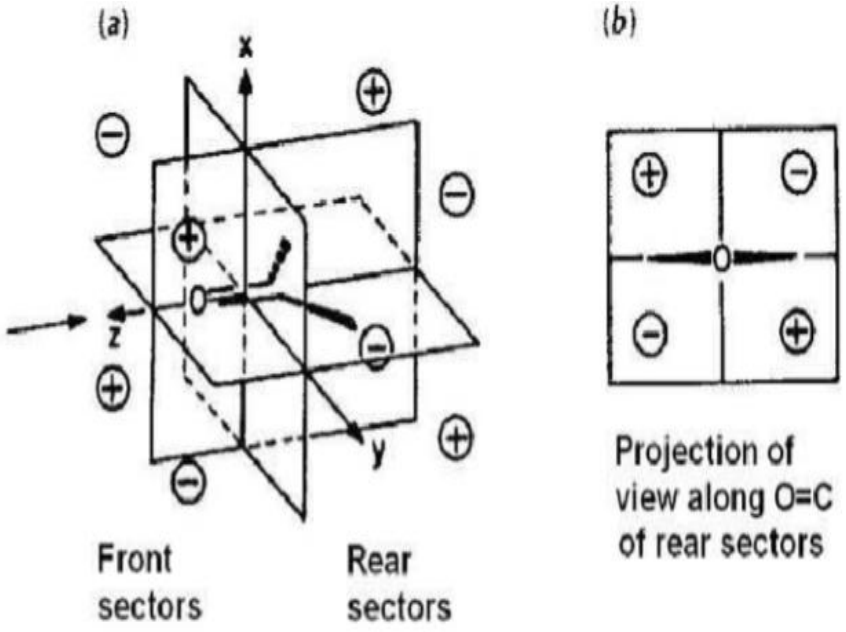


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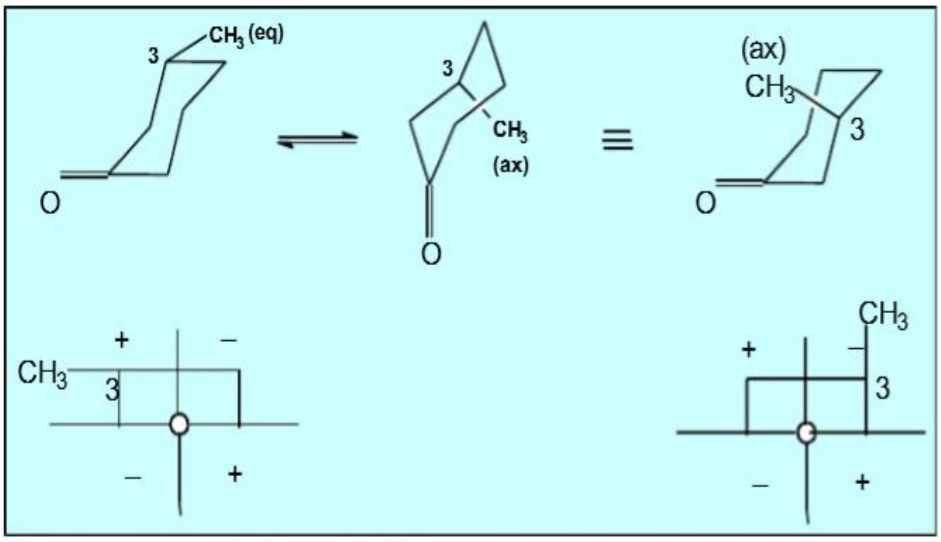
Diagram (b) shows the projection of the view along O=C with the signs of the rear octants. Contributions from hydrogens in the simple cyclohexanone skeleton are usually ignored, being assumed to more or less cancel. Substituents at position 4 will have no effect on the CE, since either equatorial or axial groups here in the nodal xz plane. Likewise, equatorial groups at positions 2 and 6 will make only small contributions to the CE, because of their proximity to the yz plane.

The working of the octant rule is illustrated by the following examples.

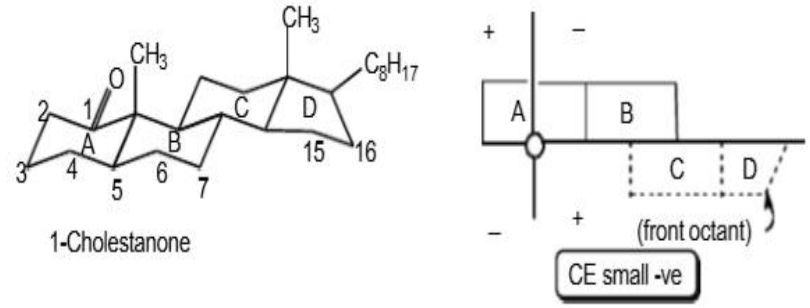
1) Determination of preferred conformation of a cyclohexanone of known configuration

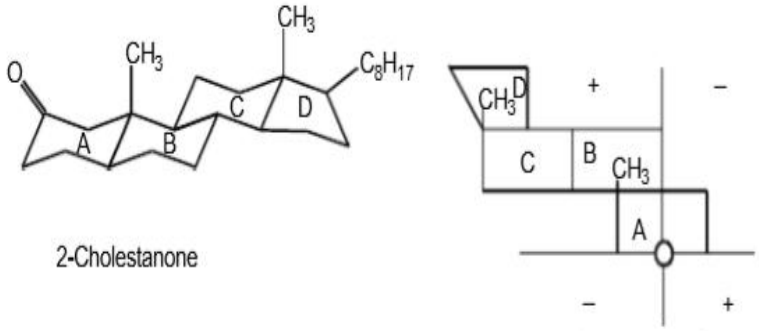
The compound (R)-(+)-3-methylcyclohexanone exhibits a positive Cotton effect. Application of the octant rule to the projections of the equatorial and axial conformations (below) indicate clearly that the preferred conformer is the equatorial one.

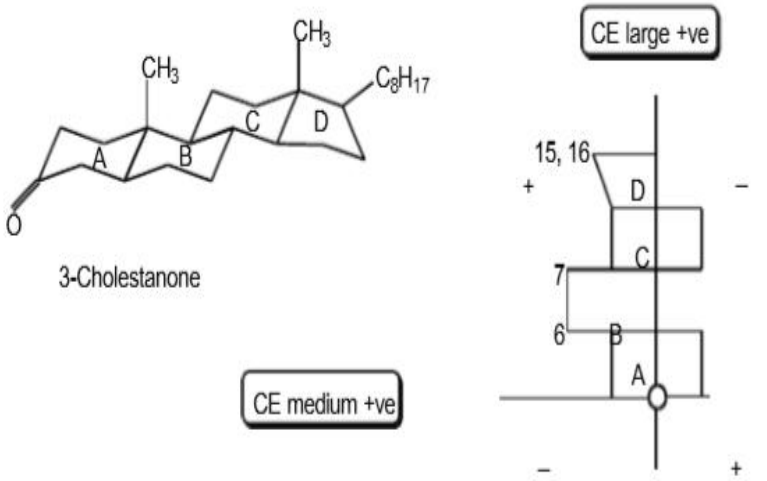


2) Estimation of the magnitude of CE in Ketosteroids

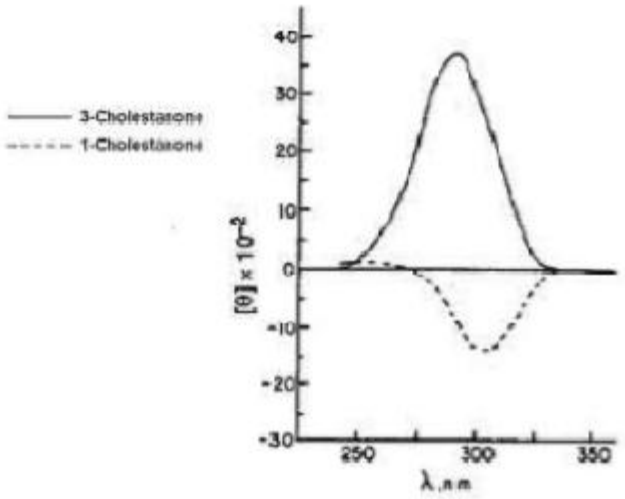
When applying the octant rule to ketosteroids, the sector with most carbons in it will make the biggest contribution to the sign of the Cotton Effect. Hence, the octant rule can be used to estimate the relative magnitudes of the CE for isomeric 1-, 2- and 3-cholestanones. The three isomers and their octant rule projections are shown below, where it can be seen that for the 1-keto isomer, the balance of carbons in negative sectors is greater, indicating a moderate negative CE. The 2-keto isomer projection shows a majority of carbons in the + sector indicating a large positive CE, whereas that of the 3-keto isomer has a small majority of carbons in the + sector, suggesting a very small positive Cotton Effect.







The CD spectra of 1- and 3-cholestanone are in agreement with this prediction, as can be seen below. The (positive) CD spectrum of 2-cholestanone would be off-scale.



11.5 Applications for determination of conformation and configuration

1. Conformational analysis

ORD curves are also used in the determination of conformation, i.e., special arrangement of a known organic structure or configuration. For example, the chair and boat conformation of cyclohexane, the two chair forms which are inconvertible in mobile cyclohexanes, and the axial and equatorial conformation of substituents in steroid chemistry have been well studied by making use of ORD curves. Moffitt (1961), also developed octant rule in this connection. The technique has further been refined and tested with a large number of ketones by Djerassi, Klyne and Ourission (1963).

When a carbonyl group in cyclohexane gives rise to ultraviolet absorption (in the range of 280-330mμ), asymmetry in the molecule will cause a Cotton effect ORD curve. Qualitatively, and semiquantitatively, the contribution of the substituents to the rotatory dispersion can be interpreted, and their special arrangement can be assigned. For example, in a normal chair steroid cyclohexane ring four octants in space (beyond the C=O bond) generally fall away because no substituent will extent beyond. The other four define space are shown in figure 3.16.1.

The upper left quadrant and the lower right contribute to positive Cotton effect at carbon-2 and carbon-5, and equatorial substituents at carbon-5. The contributions of the other two quadrants have the opposite sign. This technique has been refined and tested by a number of workers with a large number of ketones, Because of facility of interpretation and the favorable spectral conditions. It is advantageous to introduce chemically a carbonyl group when studying configuration or conformation.

An interesting example of the application of ORD curves in conformational analysis is in proteins and polypeptide chemistry. It has been found that α-helix and the percentage of alpha-helical conformation of the long chain molecule can be redetermined from the ORD curves. Earlier interpretation was based on plain curves in the near ultraviolet region, and more recently, on the Cotton effect region in the far ultraviolet region. An interesting technique used before the availability of spectropolarimeters reading down to 200 μm, was the use of optically active acriflavine dye. When this dye was coupled to polypeptides, Cotton effect ORD curves were obtained in the visible region, allowing conformational interpretation of the chain structure.

2. Determination of relative and absolute configuration

We have seen that ORD curves can be used to determine the configuration, i.e., the special relationship of substituents around an asymmetric carbon atom. The L-α-amino acids are by no means all laevorotatory at the medium D line which has been used. Consequently unattractive symbol L (+) and L(-) have been used. Using copper complexes, which have absorption in the visible region, it has been observed that L (+) valine and L (-) phenyl alanine are essentially identical, despite the change opposite rotation at 589mμ of the parent compounds. This proves identical configurations at the α-carbon atom. However, this approach is not of much significance because the Cotton effects of the free amino acids around 225 mμ are now accessible with modern instruments.

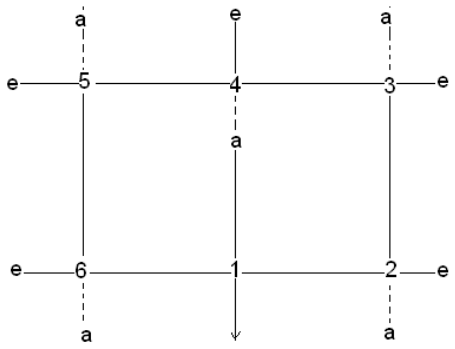


Figure 3.16.1: Two-dimensional schematic diagram of the operation of the octant rule.

Much of the recent work is now related with steroids which are of great importance in pharmaceutical research, and which represent particularly difficult stereo chemistry problems. An example is the beta configuration of the hydrogen at C-10 in 19-nor-Δ^4-3-oxosteroids. This configuration could be assigned by anology of the ORD curves to the Δ^4-3- oxo-steroids.

The only direct method of obtaining absolute configuration i.e., actually which of the two mirror image representation is a true compound, is a X-ray analysis. However, once the absolute configuration of a typical compound is known, it can be used as a reference. Using ORD curves, which are very sensitive to steric differences, the configurational features can be peeled off by working through analogies.

11.7 Answers to check your progress questions

1. Define α-halo ketone rule:In ketone formation of a strong and inherently dissymmetric chromophore due to the interaction between lone pair electrons of the axial halogen substituents at Ca associated with a strong molecular rotation and red shift at the extremum has been known as the axial- haloketone rule.

2. Cotton effect: The Cotton effect is the characteristic change in optical rotatory dispersion and/or circular dichroism in the vicinity of an absorption band of a substance
3. Conformational change : conformational change is a change in the shape of a macromolecule, often induced by environmental factors

11.8 Summary

- α- haloketone rule mainly based on ORD measurements in steroidal ketones that had been (axially) substituted with a halogen atom at the α-carbon.
- Main role in determination of configurational analysis in different halo substituted compounds.
- The axial haloketone rule is a special case of the octant rule for saturated ketones and the working of the octant rule is illustrated by the following examples.Determination of preferred conformation of a cyclohexanone of known configuration, The compound (R)-(+)-3-methylcyclohexanone exhibits a positive Cotton effect. Application of the octant rule to the projections of the equatorial and axial conformations (below) indicate clearly that the preferred conformer is the equatorial one.

11.9. Keywords

Cotton effect, haloketone rule conformational change

11.9 Self-assessment questions and exercises

1. What is meant by α-halo ketone rule?
2. What is the main role of cotton effect?
3. Explain conformational mobility?
4. Give in detail octant rule with suitable examples?

11.10 Answers To Check Your Progress Questions

1. What are the causes of cotton effect?
   The Cotton effect is called positive if the optical rotation first increases as the wavelength decreases (as first observed by Cotton),

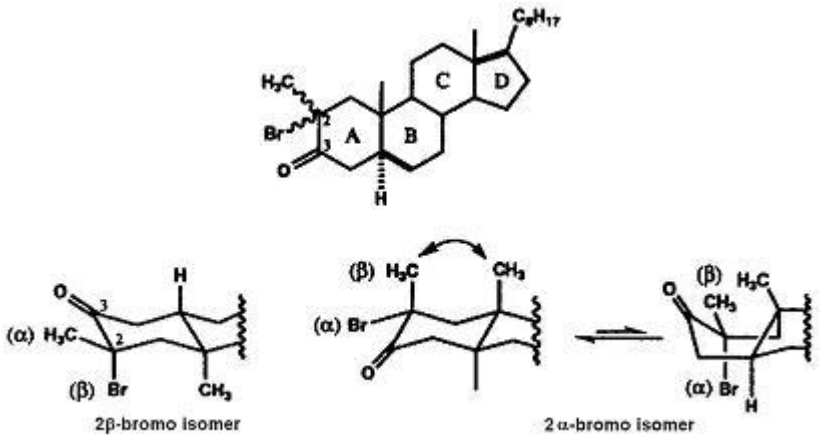
and negative if the rotation first decreases. A protein structure such as a beta sheet shows a negative Cotton effect.

2. Discuss the axial haloketone rule and the octant rule for saturated ketones

   The octant rule is the most widely applied sector rule. It was developed from an earlier rule, known as the "axial haloketone rule", based on ORD measurements carried out on steroidal ketones that had been (axially) substituted with a halogen atom at the α-carbon. Axial substitution (conformation) is often preferred because of the dipole-dipole repulsions in the equatorial isomer.

3. How will you demonstrate the existence of a boat conformer?

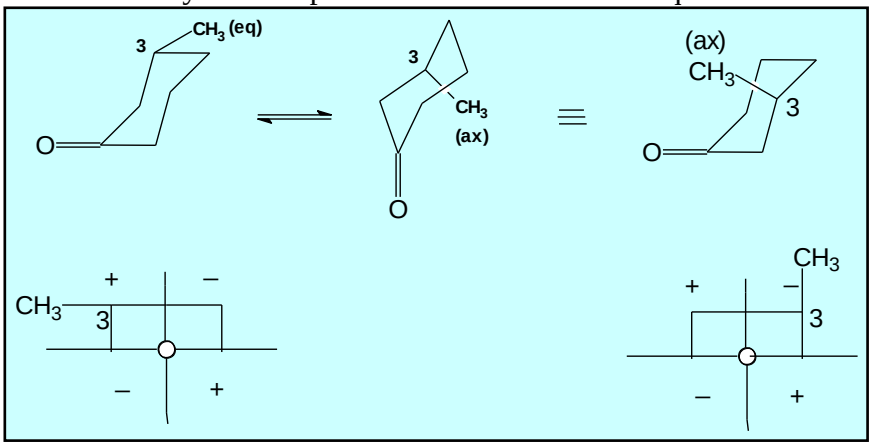
   The 2α- and 2β-bromo isomers of 2-bromo-2-methylcholestane-3-one, (with axial Br established by IR spectroscopy) the latter displays a positive CE as



   expected. The 2α-bromo isomer unexpectedly shows a negative CE. This is best explained by supposing the boat conformer is significant in ring A of this isomer, because of steric hindrance between the (axial) methyl groups in the chair conformer.

4. Draw the preferred conformation of a cyclohexanone configuration.

   The compound (R)-(+)-3-methylcyclohexanone exhibits a positive Cotton Effect. Application of the octant rule to the projections of the equatorial and axial conformations (below) indicate clearly that the preferred conformer is the equatorial one.



11.11 Further readings

1. Huheey, J.E., E.A. Keiter and R.L. Keiter. 2002. Inorganic Chemistry: Principles of Structure and Reactivity, 4th Edition. New York: HarperCollins Publishers.
2. Gary L. Miessler., Paul J. Fischer., Donald A. Tarr. Inorganic Chemistry, 5th ed : Pearson
3. Shriver and Atkins., Inorganic Chemistry, 5th ed : W. H. Freeman and Company New York.

BLOCK- IV: THERMAL AND SPECTROMETRIC METHODS OF ANALYSIS

UNIT XII: THERMAL ANALYSIS

STRUCTURE

12.1 Introduction

Thermal analysis refers to any technique for the study of materials which involves thermal control. Measurements are usually made with increasing temperature, but isothermal measurements or measurements made with decreasing temperatures are also possible. Table 1 shows a selection of thermal analysis techniques, illustrating the breadth of the field. In fact, any measuring technique can be made into a thermal analysis technique by adding thermal control. Simultaneous use of multiple techniques increases the power of thermal analysis, and modern instrumentation has permitted extensive growth of application. The basic theories of thermal analysis (equilibrium thermodynamics, irreversible thermodynamics and kinetics) are well developed, but have to date not been applied to actual experiments to the fullest extent possible.

12.2 Objectives

After going through this unit, you will be able to:

- Understand about the basic principles of Thermogravimetry
- Understand the Instrumentation, types, Interpretation
- Understand the factors influence on the TGA curve
- Understand the Differential thermal analysis and applications
- Explain in detail differential scanning calorimetry, types and applications
- Explain the basic principles of thermometric titrations and it's merits

12.3 THERMOGRAVIMETRY:

Thermogravimetric Analysis is a technique in which the mass of a substance is monitored as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere. The changes in the mass of a sample due to various thermal events (desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition) are studied while the sample is subjected to a program of change in temperature. Therefore, it is used in the analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibers, coatings, paints, etc.

Types of TGA:

The different types of TGA are,

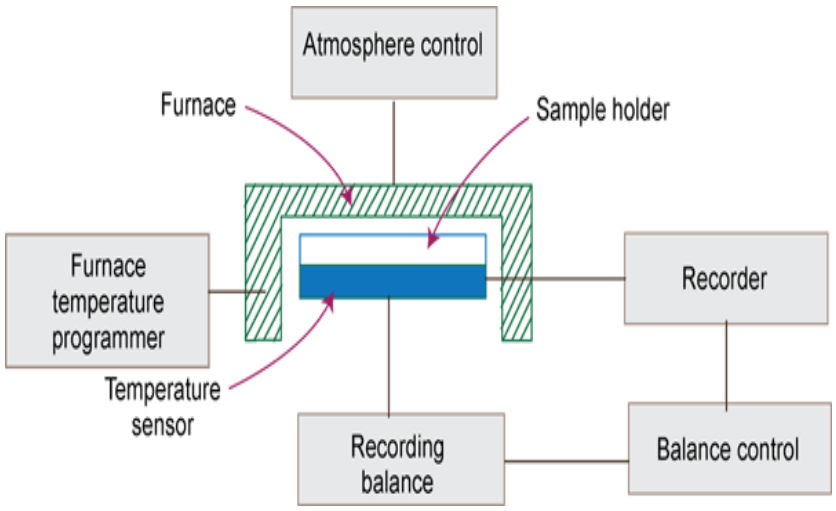
1. Isothermal or Static TGA: In this case, sample is maintained at a constant temperature for a period of time during which change in weight is recorded.

2. Quasi-static TGA: In this technique, the sample is heated to a constant weight at each of a series of increasing temperature.

3. Dynamic TGA: In this type of analysis, the sample is subjected to condition of a continuous increase in temperature at a constant heating rate, i.e., usually linear with time.

Instrumentation:

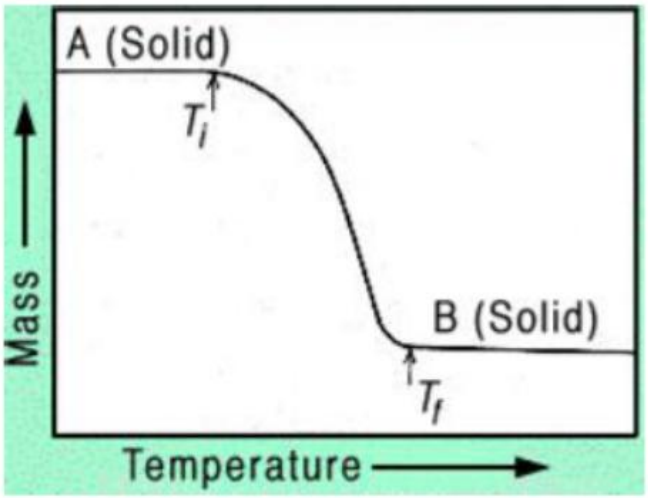
The instrument used for TGA analysis is a programmed precision balance for a rise in temperature (called as Thermobalance). Thermobalance consists of an electronic microbalance (important component), a furnace, a temperature programmer and a recorder.



The Block Diagram of a Thermobalance.

TGA curve

The plot of mass change in percentage versus temperature or time known as TGA curve is shown as

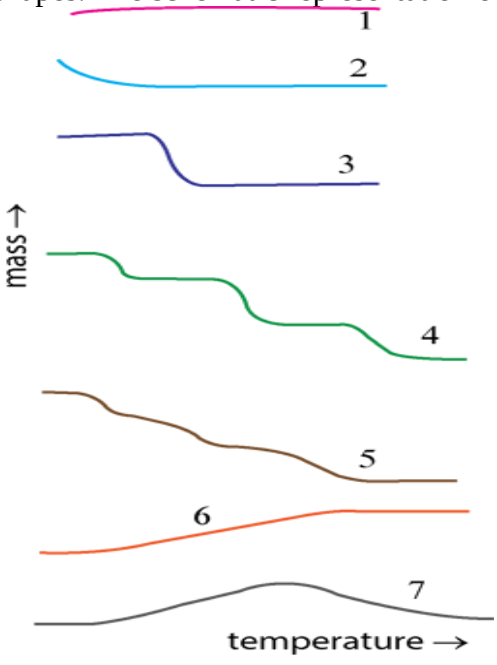


The plot of mass change with temperature.

There are two temperatures in the reaction. They are the T_i (starting of decomposition temperature) and T_f (final temperature) representing the lowest temperature at which the onset of a mass change is seen and the lowest temperature at which the process has been completed, respectively. The reaction temperature and interval (T_f - T_i) strongly depend on the conditions of the experiments. Hence, they cannot have any fixed values.

Interpretation of TGA Curves:

TGA curves are typically classified into seven types according to their shapes. The schematic representation of various types of TGA curves are



Schematic representation of various types of TGA curves.

- Curve 1: No change: This curve depicts no mass change over the entire range of temperature, indicating that the decomposition temperature is greater than the temperature range of the instrument.
- Curve 2: Desorption / drying: This curve shows that the mass loss is large followed by mass plateau. This is formed when evaporation of volatile product(s) during desorption, drying or polymerization takes place. If a non-interacting atmosphere is present in the chamber, then curve 2 becomes curve 1.
- Curve 3: Single stage decomposition: This curve is typical of single-stage decomposition temperatures having T_i and T_f.
- Curve 4: Multistage decomposition: This curve reveals the multi-stage decomposition processes as a result various reactions.
- Curve 5: Similar to 4, but either due to fast heating rate or due to no intermediates.
- Curve 6: Atmospheric reaction: This curve shows the increase in mass. This may be due to the reactions such as surface oxidation reactions in the presence of an interacting atmosphere.
- Curve 7: Similar to curve 6, but product decomposes at high temperatures. For example, the reaction of surface oxidation followed by decomposition of reaction product(s).
-

Factors affecting TGA:

1) Effect of changing air buoyancy and convection
    - Apparent change in weight gain
    - Decreased air buoyancy
    - Increased convection
    - Effect of heat on balance mechanism
2) Measurement of temperature
    - Change in measurement of temperature
    - Partly due to thermal lag, finite time required to cause a detectable change in weight
3) Effect of atmosphere
    - Atmosphere near the sample surface is modified continuously

- Small changes in composition of this atmosphere affects thermogram
- Maintain constant atmosphere

4) Effect of heating rate
    - Affect thermogram appreciably
    - Important for kinetic analysis
5) Sample characteristics
    - Weight of the sample
    - Particle size of the sample
    - Compactness of the sample
    - Previous history of the sample

Example for TGA curve:

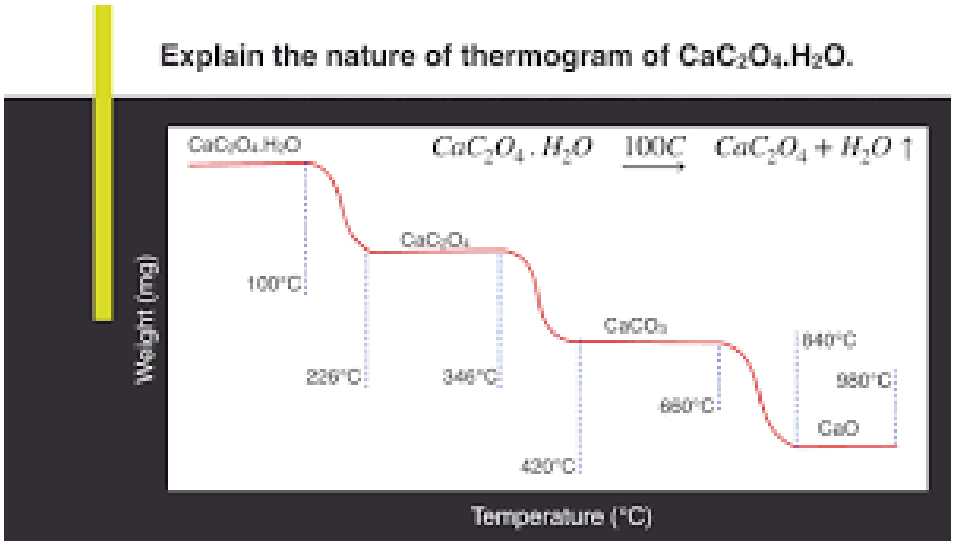
The thermal decomposition of calcium oxalate monohydrate studied by TGA.

(a)Ca (COO) $_2$ $H_2O \rightarrow$ Ca $(COO)_2 + H_2O$ (g);

(b)Ca (COO) $_2 \rightarrow CaCO_3 + CO$ (g); and,

(c)$CaCO_3 \rightarrow CaO + CO_2$ (g).

The TGA curve depicts the mass change corresponding to each reactions of calcium oxalate monohydrate is given as below,



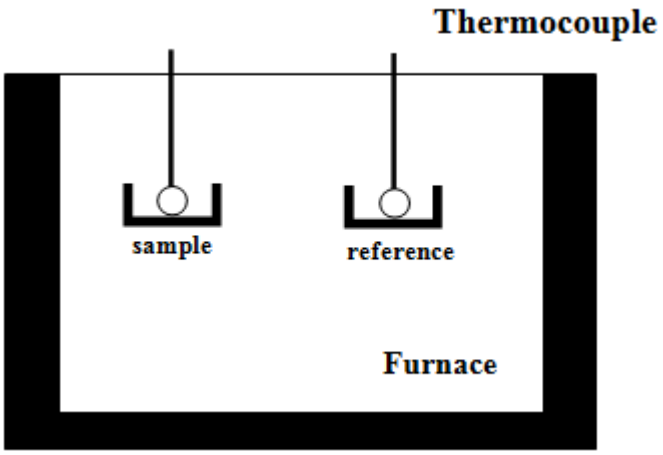
Applications of TGA:

a) Thermal stability of the related materials can be compared at elevated temperatures under the required atmosphere. TGA curve helps to explicate decompositionmechanisms.
b) Materials Characterization: TGA curves can be used to fingerprint materials for identification or quality control.
c) Compositional analysis: By a careful choice of temperature programming and gaseous environment, many complex materials/ mixtures can be analyzed by decomposing or removing their components. For example: filler content in polymers; carbon black in oils; ash and carbon in coals, and the moisture content of many substances.
d) Kinetic studies: A variety of methods can be used to analyze the kinetic features of weight loss or gain through controlling the chemistry or predictive studies.
e) Corrosion studies: TGA provides a means of studying oxidation or some reactions with other reactive gases or vapors.

12.4 DIFFERENTIAL THERMAL ANALYSIS:

DTA consists of heating a sample and reference material at the same rate and monitoring the temperature difference between the sample and reference.

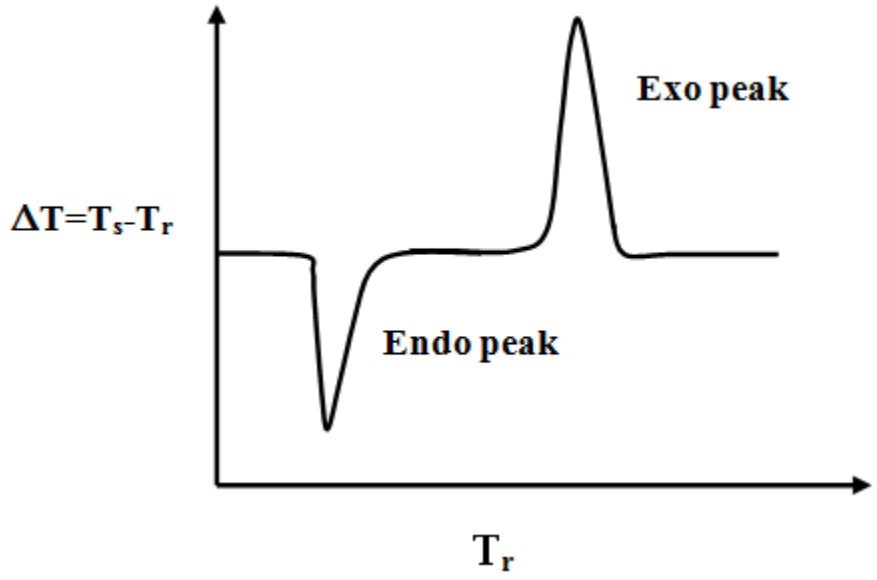
In this method, the sample is heated along with a reference standard under identical thermal conditions in the same oven. The temperature difference between the sample and reference substance is monitored during the period of heating. As the samples undergo any changes in state, the latent heat of transition will be absorbed/ evolved and the temperature of the sample will differ from that of the reference material. This difference in temperature is recorded. Hence, any change in state can be detected along with the temperature at which it occurs.



Schematic diagram for differential thermal analysis technique.

When an endothermic process occurs (ΔH positive) in the sample, the temperature of sample (T_s) lags behind the temperature of reference (T_r). The temperature difference $\Delta T = (T_s - T_r)$ is recorded against reference temperature T_r and the corresponding plot is shown in Fig 12. In DTA, by convention, endothermic response is represented as negative that is by downward peaks. When an exothermic process (ΔH negative) occurs in the sample, the response will be in the reverse direction and the peaks are upward. Since the definition of $\Delta T = T_s - T_r$ is rather arbitrary, the DTA curves are usually marked with endo or exo direction.

It is essential that reference sample must not undergo any change in state over the temperature range used and both the thermal conductivity and heat capacity of reference must be similar to those of samples. Both sample and reference materials should be also inert towards sample holder or thermocouples. Alumina or silicon carbide are most commonly used standard reference samples. DTA profiles are affected by heating rate, sample size and thermocouple position within the sample.



Typical exo and endo peak in a DTA profile.

Application of DTA:

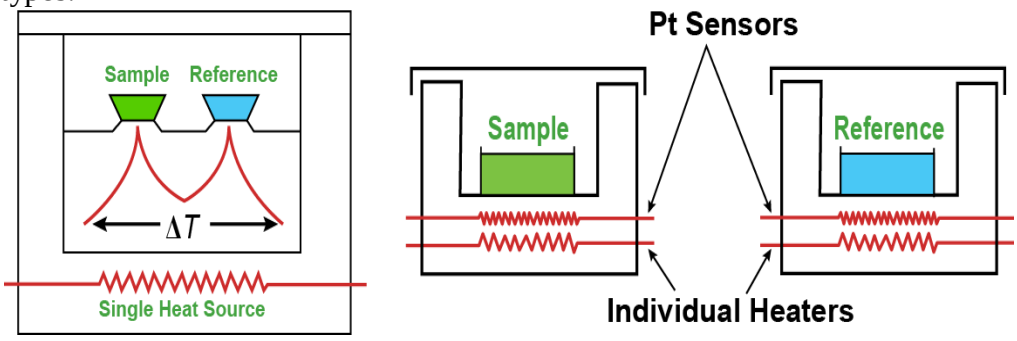
Any change associated with enthalpy change can be studied by DTA. In general DTA curves are used to get information about temperature and enthalpy changes for decomposition, crystallization, melting, glass transition etc. In solid catalysis it is particularly useful to detect phase changes associated with calcination process. For example change of aluminum hydroxide to alumina can be easily detected by DTA.

12.5 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) technique was developed by E.S. Watson and M. J. O'Neill in 1962 and commercial introduction was done at 1963 in Pittsburgh conference. It is a thermo-analytical technique in which the differences in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned and analyzed. In general, the temperature program of the DSC is designed to increase the sample holder temperature linearly as a function of time. The main application of DSC is in studying phase transitions such as melting point, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity.

Types of DSC:

There are two types of DSC commercially available: Heat Flux (HF) Type and Power Compensation (PC) Type. The block diagram for HF and PC types.



Schematic diagram for HF and PC types DSC.

In HF type DSC:

Both sample and reference pans are heated by a single furnace through heat sink and heat resistor. Heat flow is proportional to the heat difference of heat sink and holders. The temperature versus time profile through a phase transition in a heat flux instrument is not linear. At a phase transition, there is a large change in the heat capacity of the sample, which leads to a difference in temperatures between the sample and reference pan.

A set of mathematical equations convert the signal into heat flow

information. By calibrating the standard material, the unknown sample quantitative measurement is achievable.

In PC type DSC:

Both sample and reference pans are heated by a different furnaces. When an event occurs in the sample, sensitive Platinum Resistance Thermometer (PRT) detects the changes in the sample, and power (energy) is applied to or removed from the sample furnace to compensate for the change in heat flow to or from the sample. As a result, the system is maintained at a "thermal null" state at all times. The amount of power required to maintain system equilibrium is directly proportional to the energy changes occurring in the sample. No complex heat flux equations are necessary with a power compensation DSC because the system directly measures energy flow to and from the sample.

In addition, PC type DSC has enhanced modulated temperature DSC (StepScan) technique and fast scan DSC (HyperDSC) for dramatic improvements in productivity, as well as greater sensitivity.Furthermore, the heating and cooling rate of PC types DSC can be as high as 500°C/min.

Detection of phase transitions:

The underlying principle is that when the sample undergoes a physical transformation (phase transitions, etc), more or less heat will be needed to flow to it as compared to the reference to maintain both of them at the same temperature. This certainly depends on whether the process is exothermic or endothermic.

For example:

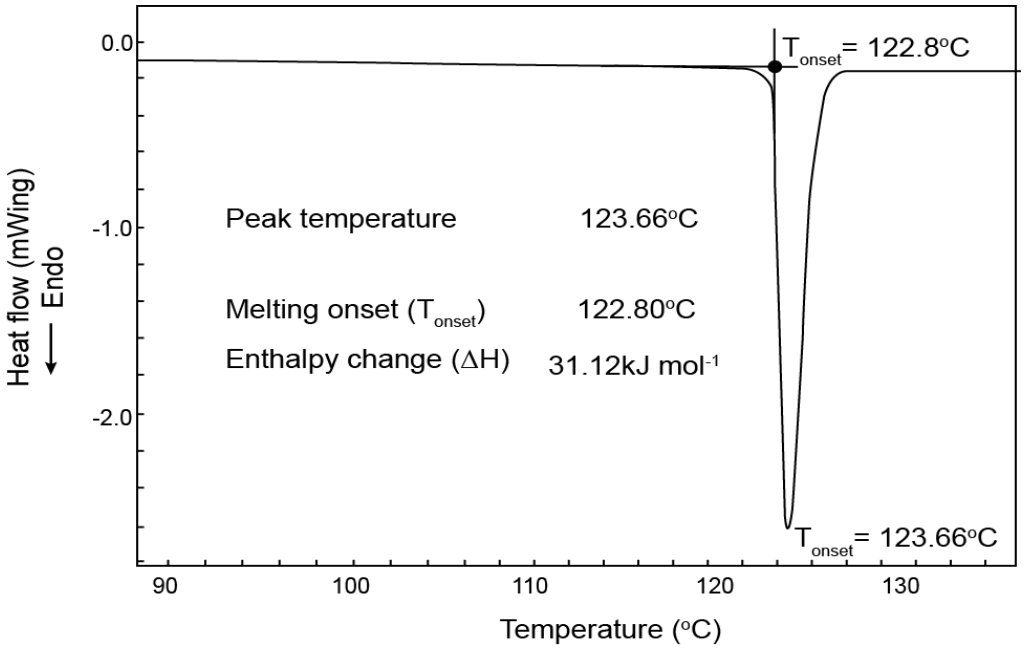
When a solid sample melts into a liquid, then it requires more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Similarly, when the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature.By observing the difference in heat flows between the sample and reference, DSC is able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions.

Information about the DSC curves:

In general, the result of a DSC experiment is a curve of heat flux versus temperature or versus time. This curve can be used to calculate enthalpies of transitions, i.e., $\Delta H = kA$ (where, H is the enthalpy of transition, k is the calorimetric constant, and A is the area under the curve), which is done by integrating the peak corresponding to a given transition. The value of k is typically given by the manufacturer for an instrument or can generally be determined by analyzing a well-characterized sample with known enthalpies of transition.

Evaluation and interpretation of DSC curve

The typical DSC curve for a sample exhibiting endotherm of melting at a particular heating rate is explained below, the onset of melting (122.8°C) and peak temperature of melting (123.66°C) can be determined by extrapolation technique and peak values, respectively.

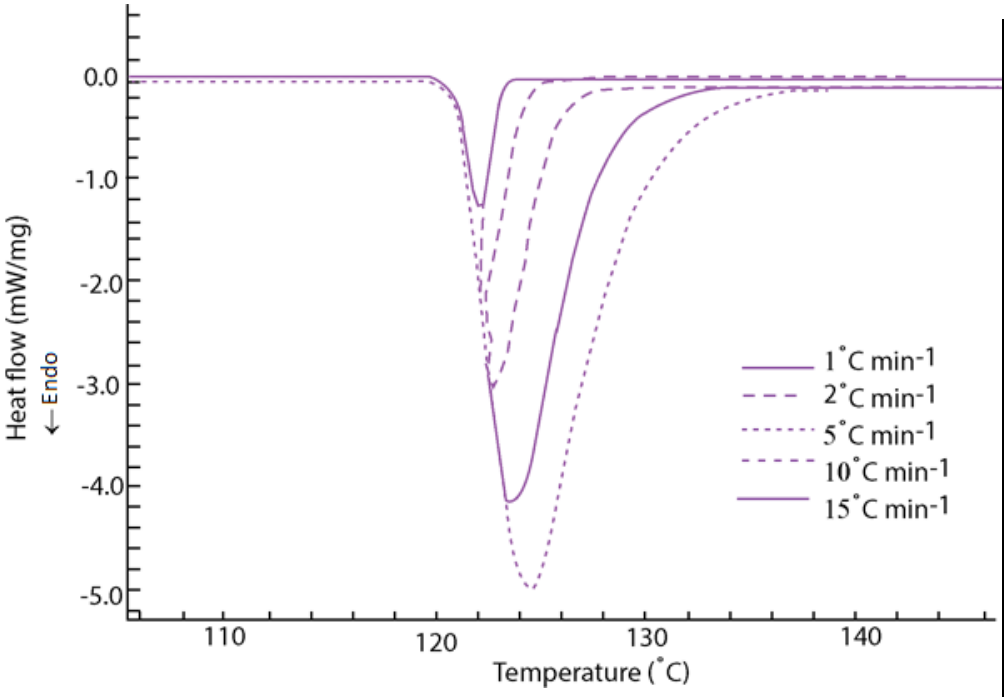


Typical DSC curve of a sample.

The enthalpy change can be calculated by integrating the area under the curve. The unit can be either J/g or J/mole depending on the nature of the sample.

Effect of heating rate:

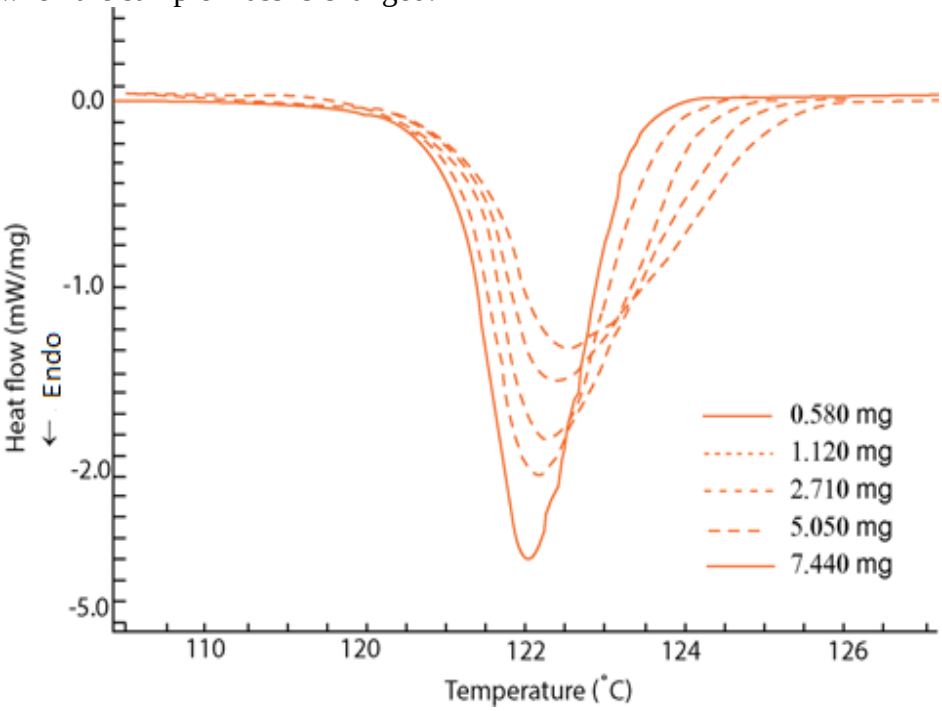
Heating rate affects the melting point and enthalpy of melting. The figure shows the typical DSC curves taken at different heating rate. With increasing heating rate, the onset of the melting does not change significantly, but the peak point of melting shifts slowly to higher temperature.



Typical DSC curves taken at different heating rates.

Effect of sample weight:

The sample weight also affects the thermal properties significantly. The figure below shows the typical DSC curves taken at a constant heating rate for different mass of the samples.It could be clearly seen that the onset of melting, peak point of melting and enthalpy undergo small variations when the sample mass is changed.



Typical DSC curves taken for different weighed samples.

Applications of DSC:

DSC technique can be used to obtain glass transition, melting points, crystallization times and temperatures, heats of melting and crystallization, percentage of crystallinity, oxidative stabilities, heat capacity,

completeness of cure, purities, thermal stabilities, polymorphism, recyclates or regrinds.

12.6 THERMOMETRIC TITRATIONS:

The basic principle of thermometric titrations is based on the change in temperature with the addition of titrant and determine the end point from a plot of temperature vs. volume of titrant. The titrant is added to an isothermal titrate in an adiabatic titration calorimeter. In most instances there occurs a change in enthalpy concomitantly, yielding a corresponding heat of reaction. As a result these are also called enthalpy titrations and the titration curves are called enthalpograms. It can be exothermic or endothermic. In thermometric titration, change in temperature occurs only when titration is in progress and sample reactant is present. Thus, start and end point of a titration are readily observed and the number of moles titrated is calculated as in regular titration.

In the thermometric titration, titrant is added at a known constant rate to a titrand until the completion of the reaction is indicated by a change in temperature. The endpoint is determined by an inflection in the curve generated by the output of a temperature measuring device.

Consider the titration reaction:

$$aA + bB = pP \quad (3)$$

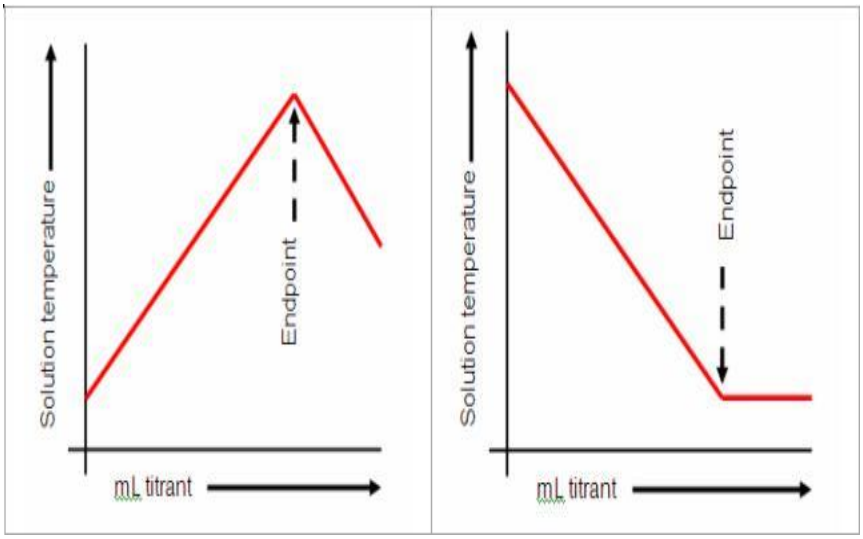
Where:

A = the titrant, and a = the corresponding number of moles reacting

B = the analyte, and b = the corresponding number of moles reacting

P = the product, and p = the corresponding number of moles produced

At completion, the reaction produces a molar heat of reaction ΔH_r which is shown as a measurable temperature change ΔT. In an ideal system, where no losses or gains of heat due to environmental influences are involved, the progress of the reaction is observed as a constant increase or decrease of temperature depending respectively on whether ΔH_r is negative (indicating an exothermic reaction) or positive (indicating an endothermic reaction).



Idealized thermometric titration plots of exothermic (left) and endothermic (right) reactions.

A suitable setup for automated thermometric titrimetry comprises the following:

- Precision fluid dispensing devices – "burettes" – for adding titrants and dosing of other reagents
- Thermistor-based thermometric sensor
- Titration vessel
- Stirring device, capable of highly efficient stirring of vessel contents without splashing
- Computer with thermometric titration operating system
- Thermometric titration interface module – this regulates the data flow between the burettes, sensors and the computer

Advantages of thermometric titrations:

The most salient advantages of thermometric titration are

- ❖ Easy-to-learn and carry out, and is completely supported by the tiamo™ titration software
- ❖ Results can be obtained rapidly
- ❖ Solves the issue of titrating difficult samples that cannot be titrated potentiometrically
- ❖ Single sensor for all applications
- ❖ Sensor calibration is not required
- ❖ Sensor is maintenance-free
- ❖ No membrane or diaphragm issues
- ❖ Robust technique for routine work
- ❖ Highly suitable for aggressive media.

Applications of thermometric titrations:

Thermometry titrimetry can be used for the following reaction types.

- ✓ Acid – base (acidimetry and alkalimetry)
- ✓ Redox titrations

- ✓ Precipitation and
- ✓ Complexometric titrations.

12.7 Summary

- Thermogravimetric Analysis is a technique in which the mass of a substance is monitored as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere. The changes in the mass of a sample due to various thermal events (desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition) are studied while the sample is subjected to a program of change in temperature. Main role in determination of configurational analysis in different halo substituted compounds.

- DTA consists of heating a sample and reference material at the same rate and monitoring the temperature difference between the sample and reference. Here, the sample is heated along with a reference standard under identical thermal conditions in the same oven. The temperature difference between the sample and reference substance is monitored during the period of heating. As the samples undergo any changes in state, the latent heat of transition will be absorbed/ evolved and the temperature of the sample will differ from that of the reference material. This difference in temperature is recorded.

- Differential scanning calorimetry technique in which the differences in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment.

- Thermometric titrations is based on the change in temperature with the addition of titrant and determine the end point from a plot of temperature vs. volume of titrant. The titrant is added to an isothermal titrate in an adiabatic titration calorimeter. In most instances there occurs a change in enthalpy concomitantly, yielding a corresponding heat of reaction. As a result these are also called enthalpy titrations and the titration curves are called enthalpograms. It can be exothermic or endothermic. In thermometric titration, change in temperature occurs only when titration is in progress and sample reactant is present. Thus, start and end point of a titration are readily observed and the number of moles titrated is calculated as in regular titration.

12.8 Self-assessment questions and exercises

1. What is the basic principles of thermo gravimetric analysis?
2. Explain in detail different types of TGA curves and its applications?
3. What is the main role of differential thermal analysis?

4. Explain in detail about differential scanning calorimetry technique and its applications??

4. Give in detail Thermometric titrations and its merits?

12.9 Further readings

1. Huheey, J.E., E.A. Keiter and R.L. Keiter. 2002. Inorganic Chemistry:Principles of Structure and Reactivity, 4th Edition. New York: HarperCollins Publishers.
2. Gary L. Miessler., Paul J. Fischer., Donald A. Tarr. Inorganic Chemistry, 5th ed : Pearson
3. Shriver and Atkins., Inorganic Chemistry, 5th ed : W. H. Freeman and Company New York.

UNIT XIII FLAME PHOTOMETRY

Structure

13.1 Introduction

Atomic spectroscopy is thought to be the oldest instrumental method for the determination of elements. These techniques are introduced in the mid of 19th Century during which Bunsen and Kirchhoff showed that the radiation emitted from the flames depends on the characteristic element present in the flame. The potential of atomic spectroscopy in both the qualitative as well as quantitative analysis were then well established. The developments in the instrumentation area led to the widespread application of atomic spectroscopy. Atomic spectroscopy is an unavoidable tool in the field of analytical chemistry. It is divided into three types which are absorption, emission, and luminescence spectroscopy. The different branches of atomic absorption spectroscopy are (1) Flame photometry or flame atomic emission spectrometry in which the species is examined in the form of atoms (2) Atomic absorption spectrophotometry, (AAS), (3) Inductively coupled plasma-atomic emission spectrometry (ICP-AES).

13.2 Objectives

- Understand about the basic principles of flame photometry
- Understand the Instrumentation, types and Interpretation of FP spectra
- Understand the various applications of FP

13.3 Flame Photometry:

Flame photometry is a process wherein the emission of radiation by neutral atoms is measured. The neutral atoms are obtained by introduction of the sample into flame. Hence the name flame photometry. Since radiation is emitted, it is also called as flame emission spectroscopy.

Principle:

The solution of metallic salt is sprayed as fine droplets into a flame. Due to the heat of the flame, the droplets dry leaving a fine residue of salt. This fine residue converts into neutral atoms. Due to the thermal energy of the flame, the atoms get excited and after that return to ground state. In this process of return to ground state, excited atoms emit radiation of specific wavelength. This wavelength of radiation emitted is specific for every element. This specificity of the wavelength of light emitted makes it a qualitative aspect. While the intensity of radiation depends on the concentration of element. This makes it a quantitative aspect. The process

seems to be simple and applicable to all elements. But in practice, only a few elements of Group IA and group IIA (like Li, Na, k & Ca, Mg) are only analyzed. The radiation emitted in the process is of a specific wavelength. Like for Sodium (Na) 589nm yellow radiation, Potassium 767nm range radiation.

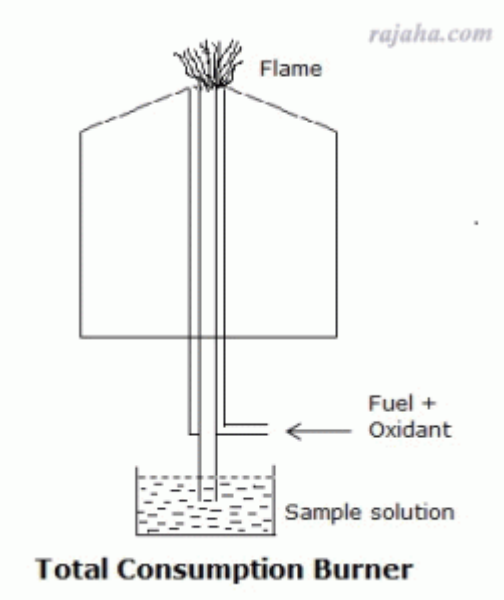
Instrumentation:

The instrumentation of flame photometer comprises of

1. Burner
2. Monochromators
3. Detectors
4. Recorder and display.

Burner:

This is a part which produces excited atoms. Here the sample solution is sprayed into fuel and oxidant combination. A homogenous flame of stable intensity is produced. There are different types of burners like Total consumption burner, Laminar flow and Mecker burner.



Total Consumption Burner

Fuel and oxidants:

Fuel and oxidant are required to produce the flame such that the sample converts to neutral atoms and get excited by heat energy. The temperature of flame should be stable and also ideal. If the temperature is high, the elements in sample convert into ions instead of neutral atoms. If it is too low, atoms may not go to excited state. So a combination of fuel and oxidants is used such that there is desired temperature.

Monochromators:

Filters and monochromators are needed to isolate the light of specific wavelength from remaining light of the flame. For this simple

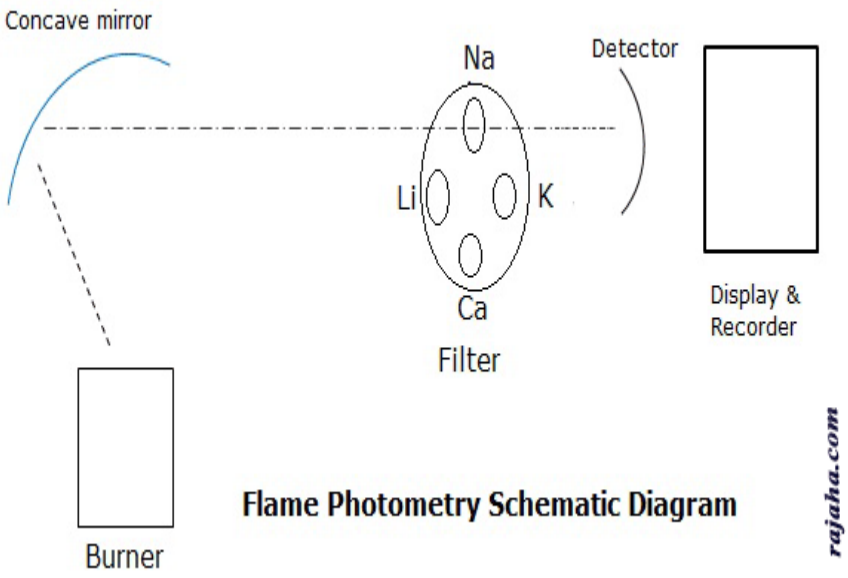
filters are sufficient as we study only few elements like Ca, Na, K and Li. So a filter wheel with filter for each element is taken. When a particular element is analyzed, the particular filter is used so that it filters all other wavelengths.

Detector:

Flame photometric detector is similar to that used in spectrophotometry. The emitted radiation is in the visible region, i.e., 400nm to 700nm. Further, the radiation is specific for each element, so simple detectors are sufficient for the purpose of photovoltaic cells, phototubes, etc.

Recorders and display:

These are the devises to read out the recording from detectors.



Flame photometer Applications:

1. For qualitative analysis of samples by comparison of spectral emission wavelengths with that of standards.

2. For quantitative analysis to determine the concentration of group IA and IIA elements. For example,

- Concentration of calcium in hard water.
- Concentration of Sodium, potassium in Urine
- Concentration of calcium and other elements in bio-glass and ceramic materials.

13.5 Check Your Progress

1. Why flame photometry is also called atomic flame emission spectrometry?
2. Write about the principle underlying the quantitative analysis by flame photometry?
3. Enlist the advantages of flame photometry?
4. Explain the major components of a flame photometer and draw a block diagram of the equipment.

13.6 Answers To Check Your Progress Questions

1. As we measure the emission spectrum of the atoms that were excited using the thermal energy of flame, the technique is called flame atomic emission spectroscopy (FAES)
2. In flame photometry, the thermal energy from flame is utilized to convert the analyte into gaseous atoms and then to excite them to higher energy level. As the excited atoms return to a state of lower energy, radiation of wavelength characteristic of the element is emitted. The intensity of the emitted radiation is related to the concentration of the element present, forming the basis of the quantitative analysis by flame photometry.
3. The following are the major advantages of flame photometry.

It provides high sensitivity and high reliability for the determination of elements like, sodium, potassium, lithium, calcium, magnesium, strontium, and barium. These determinations are useful in medicine, agriculture and environmental science.

It is also successful in determining certain transition elements such as copper, iron and manganese.

By making wavelength scan of the emission spectrum, it is possible to do qualitative analysis by employing flame photometer, but the application is severely limited.

4. The major basic components of instrumentation for flame photometry are:

- Flame atomiser (consisting of nebuliser and burner)
- Monochromator
- Detector
- Amplifier and Readout device

The block diagram of the instrumentation involved in flame photometry is given below:

13.7 Summary

- Atomic spectroscopy is the oldest instrumental method of elemental analysis.
- The atomic spectroscopic methods are based on the transitions amongst the quantized electronic energy levels caused by the

absorption of radiation by the atoms in vapor phase or by the emission of radiation by the excited atoms.

- The type of atomic spectroscopic method is determined both by the method of atomization as well as the nature of the analyte radiation interaction.
- The sensitivity of the flame photometric method depends on the number of excited atoms, which in turn depends on the flame temperature. The flame temperature is a function of the type of fuel and oxidant used.
- The quantitative analysis can be carried out using standard calibration curve, by standard addition or internal addition method. The method is subject to various interferences such as spectral, ionisation, and chemical interferences.
- The major applications of flame photometry include qualitative and quantitative analysis especially of Group I metals (Li, Na, K) and Group II metals (Mg, Ca, Sr, Ba). It is very useful in routine determination of these metals in medicinal, biological, agricultural and industrial fields.
- Merits of the technique include high sensitivity and reliability, inexpensive instrumentation and advantages in analysis of alkali and alkaline earth metals. On the other hand, the method is chiefly restricted to these elements, liquid samples, and is subject to different types of interferences, and gives no information on chemical form of the element present.

13.8 Keywords

Flame photometry: A process wherein the emission of radiation by neutral atoms is measured.
Monochromator: An optical device which works as narrow band wavelength filter with mechanically adjustable transmission wavelength.
Atomisation: A process of breaking bulk liquids into small droplets.

13.9 Self-assessment questions and exercises

1. What is the basis of qualitative and quantitative analysis by flame photometry?
2. What are the different methods for quantitative analysis?
3. What are the different kinds of interferences possible in quantitative analysis by flame photometry?
4. What are the applications of flame photometry?

13.10 Further readings

1. “Principles of Instrumental Analysis” by Douglas A Skoog and James Holler.

2. “Instrumental Methods of Chemical Analysis” by Galen W Ewing.

3. “Principles of physical chemistry” by M.S.Patania, B.R. Puri, L.R. Sharma.

UNIT XIV: TURBIDIMETRY AND NEPHELOMETRY

Structure

14.1 Introduction:

Turbidimetry measures the intensity of a beam of light transmitted through the sample, and nephelometry measures the light that is scattered at an angle away from the beam. Nephelometry is more sensitive but is more subject to interference from particulate matter in the sample.

14.2 Objectives:

- Understand the amount of transmitted light (and calculating the absorbed light) by particles in suspension to determine the concentration of the substance by turbidimetry
- Understand the measurement of scattered light from a cuvette containing suspended particles in a solution by Nephelometry

TURBIDIMETRY

Turbidimetry is the measurement of the transmitted light by the suspended particles to the incident beam. This is used for the determination of the high concentration suspensions.

Principle:

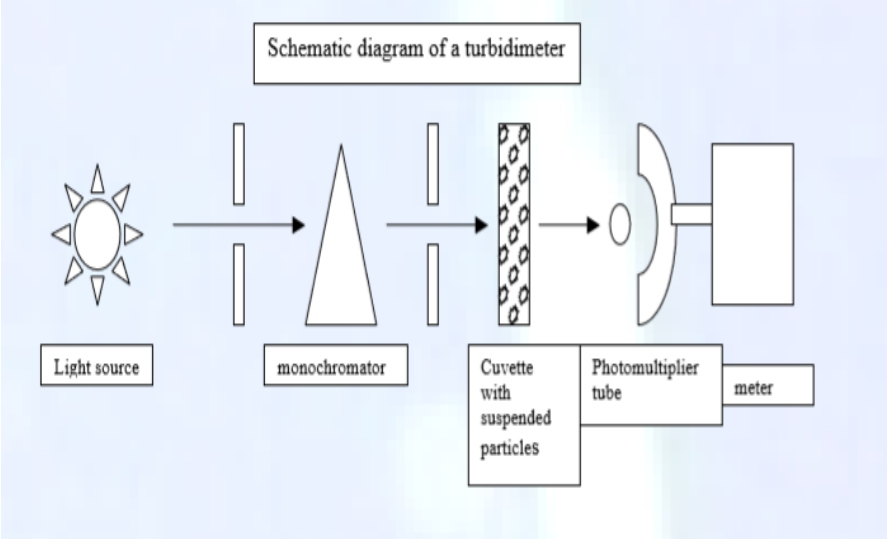
The basis of turbidimetric analysis is the measurement of the intensity of transmitted light as a function of the cone of the suspended particles.

Instrumentation:

1. Sources:

    - Mercury is lamp: Under light pressure, the excitation of mercury atoms is done by electric discharge.
    - Tungsten lamp: It contains a piece of tungsten wire which is heated in a controlled atmosphere.

2. Filters: Filters will convert the polychromatic light to monochromatic light. Generally filters are used for this purpose.
3. Fitters are of two types:
    - Absorption filters
    - Interference filters
4. Sample cells: In general, a cell with a rectangular cross-section is preferred, where measurements are to be made at angles other than 90°. Semi-octagonal cells are widely used.
5. Detector:Most commonly used detectors in turbidimetry are photomultiplier tubes.
6. Turbidimetres: In most turbidimeters, ordinary calorimeters (or) spectrophotometers may be used. The schematic representation of a turbidimeter is as follows



Working :

The photodetector is placed such that it is in direct line with the incident light and the solution, usually referred to as either a 0° or 180° angle. The light source should emit a wavelength in the near ultraviolet range (290–410 nm). The photodetector must be aligned with the incidence source and collect the beam after passage through the solution, therefore measuring a decrease in signal or the reduction in light intensity that occurs as a result of the combination of reflection, absorption, or scatter of incident light. Turbidimetric analysis is influenced by both Rayleigh and Mie light scattering.

Applications:

1) Determination of the concentration of total protein in biological fluids such as urine and CSF which contain small quantities of protein (mg/L quantities) using trichloroacetic acid.
2) Determination of amylase activity using starch as substrate. The decrease in turbidity is directly proportional to amylase activity.
3) Determination of lipase activity using triglycerides as substrate. The decrease in turbidity is directly proportional to lipase activity.

NEPHELOMETRY

Nephelometry is the measurement of the scattered light by the suspended particles at right angles to the incident beam. This method is mainly used for the determination of the low concentration suspensions.

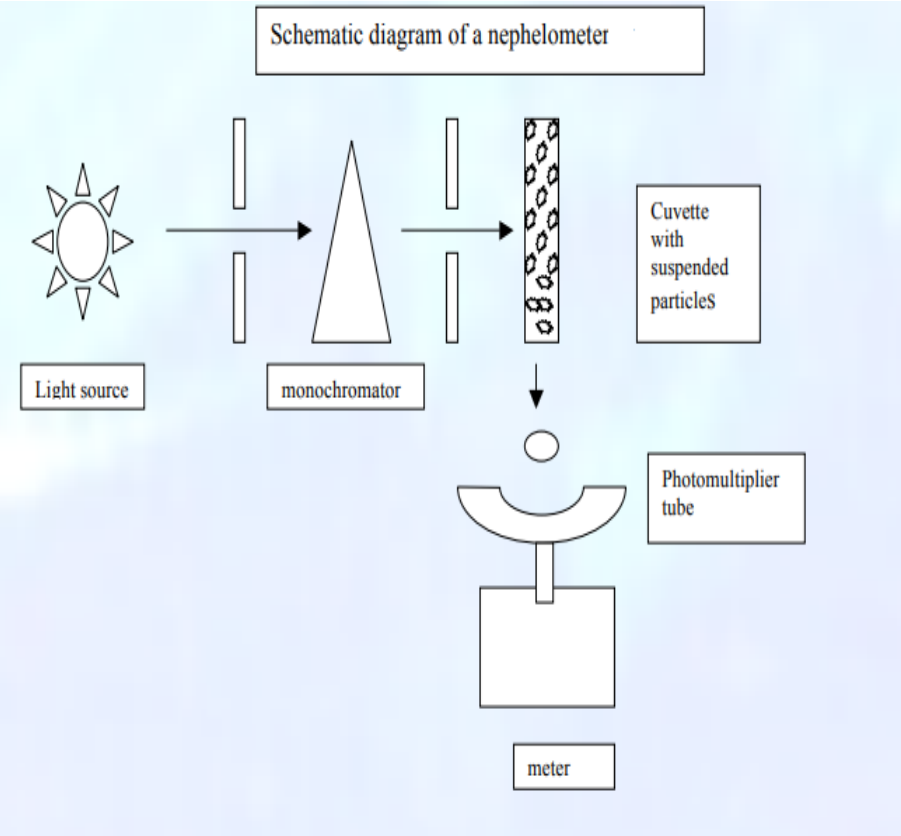
Principle:

In nephelometry, the basic principle involved is the measurement of the intensity of the scattered light as a function of the concentration of the dispensed phase.

Instrumentation:

1. Sources:
   - Mercury is lamp: Under light pressure, the excitation of mercury atoms is done by electric discharge.
   - Tungsten lamp: It contains a piece of tungsten wire which is heated in a controlled atmosphere.
2. Filters: Filters will convert the polychromatic light to monochromatic light. Generally filters are used for this purpose. Fitters are of two types:
   - Absorption filters
   - Interference filters
3. Sample cells: In general, a cell with a rectangular cross-section is preferred, where measurements are to be made at angles other than 90°. Semi-octagonal cells are widely used.
4. Detector:The photo-multiplier tube detector is used as a receiver which is mounted on a turn table and may be positioned at any desired angles from 0° to 180° relative to the

exit beam. The schematic representation of nephelometer is as follows



Applications:

1. Determination of immunoglobulins (total, IgG, IgE, IgM, IgA) in serum and other biological fluids.
2. Determination of the concentrations of individual serum proteins; hemoglobin, haptoglobin, transferring, c-reactive protein, 1-antitrypsin, albumin (using antibodies specific for each protein)
3. Determination of the size and number of particles (laser-nephelometer}

14.5 Check Your Progress

1. State the principle of turbidimetry?
2. Write about the principle underlying the quantitative analysis by nephelometry?
3. List out the applications of nephelometry?
4. Explain the instrumentation of turbidimetry?

14.6 Answers To Check Your Progress Questions

1. The basis of turbidimetric analysis is the measurement of the intensity of transmitted light as a function of the cone of the suspended particles.

2. The principle involved is the measurement of the intensity of the scattered light as a function of the concentration of the dispensed phase.

3. The following are the major applications of nephelometry,

4. Determination of immunoglobulins (total, IgG, IgE, IgM, IgA) in serum and other biological fluids.
5. Determination of the concentrations of individual serum proteins; hemoglobin, haptoglobin, transferring, c-reactive protein, 1-antitrypsin, albumin (using antibodies specific for each protein)
6. Determination of the size and number of particles (laser-nephelometer}

4. The major basic components of instrumentation for turbidimetry are:

- Light source
- Filter
- Sample cell and
- Detector.

14.7 Summary

- Turbidimetry is the measurement of the transmitted light by the suspended particles to the incident beam.
- This is used for the determination of the high concentration suspensions.
- The basis of turbidimetric analysis is the measurement of the intensity of transmitted light as a function of the cone of the suspended particles.
- Nephelometry is the measurement of the scattered light by the suspended particles at right angles to the incident beam.
- This method is mainly used for the determination of the low concentration suspensions.
- In nephelometry, the basic principle involved is the measurement of the intensity of the scattered light as a function of the concentration of the dispensed phase.

14.8 Keywords

Turbidimetres:To measure the relative clarity of a fluid by measuring the amount of light scattered by particles suspended in a fluid sample.
Detector: A machine that responds to particular substances in a consistent way.

Scattering: Atoms or molecules which are exposed to light absorb light energy and re-emit light in different directions with different intensity.

14.9 Self-assessment questions and exercises

1. What is the basis of qualitative analysis by turbidimetry?
2. What is the basis of quantitative analysis by nephelometry?
3. What are the different types of filters used in turbidimetry?
4. What are the applications of turbidimetry?

14.10 Further readings

1. "Principles of physical chemistry" by M.S.Patania, B.R. Puri, L.R. Sharma.

2. "Principles of Instrumental analysis" by Douglas A Skoog and James Holler.