Experiment- 6

Infrared Spectroscopy Experiment

Outcomes:-

After completing this experiment, the student should be able to:

- The basic principles of vibration spectroscopy.
- Determine the relationship between molecular structural features and absorptions in the IR spectrum.
- Mull technique and disc technique of solid pharmaceutical substance.
- Calibration of IR spectroscopy.

Introduction:-

The **infrared spectrum** provides the largest number of characteristic properties of a compound. It also serves as a powerful '*analytical tool*' for the extensive and intensive study of molecular structure, in fact, **infrared absorption spectra** are due to changes in vibration energy accompanied by changes in rotation energy, broadly speaking, the range in the electromagnetic spectrum that extends from **(0.8 -200 µm)** is referred to as the infrared region.

In usual practice, however, either the **wavelength (λ)** or the **wavenumber(ῡ=cm^{-1})** is employed to measure the position of a given infrared absorption, more precisely, the infrared regions may be categorized into three distinct zones based on their respective wavenumber and wavelength as stated below:-

S. No.	Region	Wavenumber(cm^{-1})	Wavelength(µm)
1.	Near I.R.	12500-4000	0.8-2.5
2.	Ordinary I.R.	4000-667	2.5-15
3.	Far I.R.	667-50	15-200

Besides, the infrared region is found to be normally rich in peaks by virtue of the fact that there exist a number of vibration modes (3*n*-6 for any nonlinear molecule, 3n-5 for any linear molecule, where, *n* = number of atoms).

Another school of thought advocates that there are two general regions in the infrared spectrum, namely:-

(a) **Group frequency region:-**

Having a wavelength ranging from **(2.5-8µm)** and a wavenumber from **(4000-1300cm^{-1})** Here, the stretching and bending vibration bonds associated with specific structure or function groups are observed frequently.

Example : The C = O stretching vibration is about 1700 cm^{-1}; whereas the C—H stretching vibration is about 3000 cm^{-1} and both of them are almost independent of

the rest of the molecule as depicted:- **Stretching vibration found in Group Frequency Region**

C—H Stretch			C = O Stretch		
S. No.	**Molecule**	**Wavenumber(cm^{-1})**	**S. No.**	**Molecule**	**Wavenumber(cm^{-1})**
1	**$CHCl_3$**	**3019**	**1**	**CH_3COCH_3**	**1715**
2	**$C_2H_2Cl_2$**	**3089**	**2**	**CH_3CHO**	**1729**
3	**$CH_2 = CH_2$**	**3105—2990**	**3**	**$H_5C_2COC_2H_5$**	**1720**
4	**C_6H_6**	**3099**	**4**	**HCOOH**	**1729**
5	**CH_3OH**	**2977**	**5**	**CH_3COOH**	**1718**
6	**CH ≡CH**	**3287**	**6**	**CF_3COOH**	**1776**

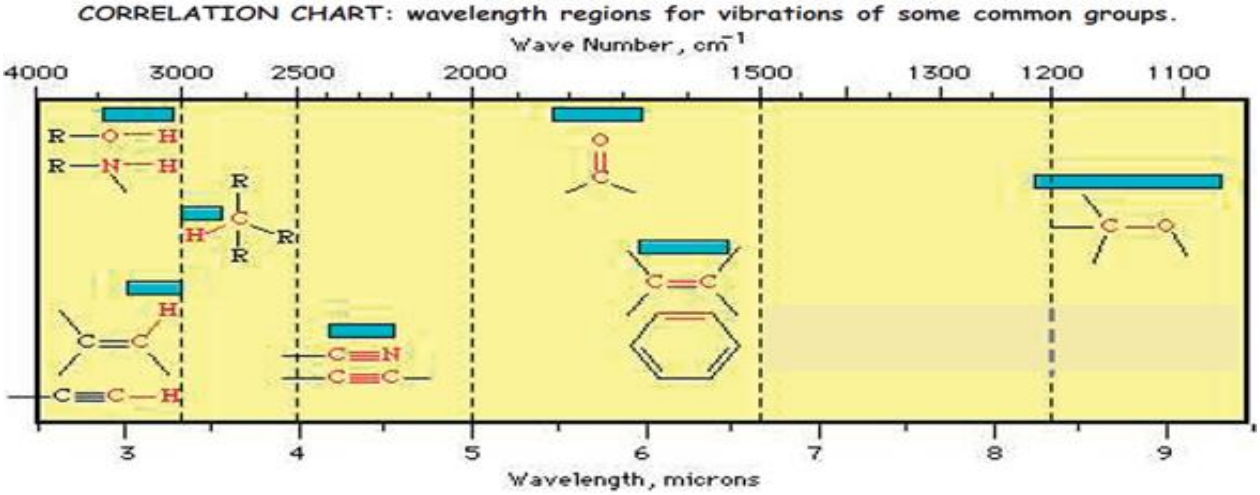
(b) **Fingerprint region:-**

Having a wavelength ranging from **(8.0-25µm)** and a wavenumber from 1300-400 cm^{-1} Here, the vibration modes depend solely and strongly on the rest of the molecule.

Example:-The C—C stretching vibration depends largely on what else is bonded to the carbon atoms, it is interesting to observe here that this particular region of the spectrum is densely populated with bands.

As we know that no two **'fingerprints'** could be identical in human beings, exactly in a similar manner no two compounds may have the same 'fingerprint region', thus, each and every molecule essentially gives rise to a unique spectrum which offers a characteristic feature of the same.

IR may actually be thought of as ***a Function Group detector***, the ***quickest and easiest way*** to determine the presence of "Function Groups" is to take the IR spectrum of the compound; the technique is simple and can often provide a definitive answer in less than ten minutes, evidence provided by IR is widely respected, it is commonly used in judicial proceedings as much as fingerprints are used, in fact, the IR of a pure compound bears the same relationship to that compound as fingerprints do to an individual.



Calculation of Vibration Frequencies:-

The vibration frequency may be calculated with fairly remarkable accuracy by the help of Hooke's Law **"**The vibration frequency of a bond is expected to increase when the bond strength increases, and also when the reduced mass of the system decreases**",** and is expressed as:

$$\upsilon = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}} \qquad \& \qquad \mu = \frac{m_1 m_2}{m_1 + m_2}$$

υ Frequency, K Force constant of the bond, m_1 and m_2 = Masses of two atoms, μ the reduced mass of the bond system.

Example*:* Calculate the approximate frequency of the C—H stretching vibration from the following data:

K = 500 Nm^{-1} = 5.0 × 10^5 g s^{-2} (since 1 Newton = 10^3 gm.s^{-2});

m_C = mass of the carbon atom = 20 × 10^{-24} g ; **How?(H.W.)**

m_H = mass of the hydrogen atom = 1.6 × 10^{-24} g ; **How?(H.W.)**

Solution:-

$$\mu = \frac{m_1 m_2}{m_1 + m_2} = \frac{20 \times 10^{-24}\ \text{g} \times 1.6 \times 10^{-24}\ \text{g}}{20 \times 10^{-24}\ \text{g} + 1.6 \times 10^{-24}\ \text{g}} = \mathbf{1.48 \times 10^{-24}\ g}$$

$$\upsilon = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}} = \frac{7}{2 \times 22}\sqrt{\frac{5.0 \times 10^5\ g.s^{-2}}{1.48 \times 10^{-24}\ g}} = \mathbf{9.247 \times 10^{13}\ s^{-1}}$$

$$c = \tilde{\lambda}\,\upsilon\ ,\ \frac{1}{\tilde{\lambda}} = \frac{\upsilon}{c} = \acute{\upsilon}\ \text{(wavenumber)}$$

$$\acute{\upsilon} = \frac{9.247 \times 10^{13}\ s^{-1}}{2.998 \times 10^{10}\ cm.s^{-1}} = \mathbf{3084\ cm^{-1}}$$

Infrared spectroscopy measures the frequencies of IR light absorbed by a sample and the intensities of the absorptions, the vibration **frequencies** depend on the **nature of the vibration** (bending & stretching), **bond strengths,** and the **masses of the atoms involved in the vibration, t**he **intensities** depend on the **change in dipole moment** that accompanies the vibration as well as the **number of bonds involved.**

The energy of infrared radiation is sufficient to change the vibration energy states of a molecule, if the dipole moment of a molecule changes as it vibrates, infrared radiation can interact with the molecule**.**

When the frequency of the radiation matches the frequency of a particular vibration, energy is transferred to the molecule, increasing the amplitude of the vibration. One observes the transfer of energy **because light equal in energy to the molecular vibration** is absorbed from the beam of incident infrared light.

The important point is that the energy involved in a vibration is inversely related to the masses of the atoms involved, that is, the heavier the atoms involved, the lower the energy, What are the relating between **υ, $\acute{\upsilon}$ and λ with mass of atom? (H.W.)**

Determination of IR Spectrum of a Solid Pharmaceutical Substance:-

The determination of IR spectrum of a solid pharmaceutical substance is invariably accomplished by any one of the *two* following techniques namely:-

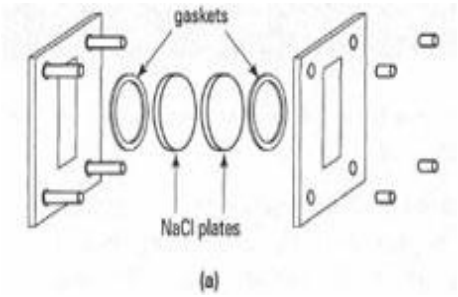
(*a*). Mull Technique

Procedure: Take about 15-20 mg of sample in a previously cleaned small agate mortar and powder it thoroughly (about 200 mesh). Add to it 2 drops of purified paraffin (Nujol–a hydrocarbon liquid, or Flourolube 1370-4000 cm^{-1}) or any other suitable mulling liquid and continue the trituration until a very smooth paste of uniform consistency is achieved.

Now, transfer the slurry to a sodium chloride plate, placing it carefully into the cavity made by the spacer, consequently, place the other plate of NaCl on top and thus assemble the cell. With the help of a clean piece of tissue-paper wipe out the excess paste that has squeezed out from the cell plates, finally, introduce the cell in the respective cell-compartment.

Salient Features:-

(*i*) Particle size of the sample has got to be reduced below 200mesh or 3 µm so as to avoid scattering of radiation thereby causing poor absorption spectrum.
(*ii*) Hydrogen bonding and crystal forces usually influence the trace obtained.
(*iii*) Paraffin itself gives rise to strong band either at 1460-1380 cm^{-1} or at 2820-2850 cm^{-1}.



Clean the salt plates with CCl_4 moistened paper towel and dry them with lint-free paper towels after use.

(*b*).Potassium Bromide Disc Technique:-

For a disc of diameter (1-1.3 cm), take 100 mg of spectroscopic grade KBr in a previously cleaned agate pestle and mortar and grind it thoroughly with **(0.05-0.5mg)** of the sample, now carefully place the sample mixture into the pressing chamber of the mould in such a manner that it is held between the polished surfaces of the bottom and top pressing dies, finally, enhance the pressing force to 100,000 lb/in^2 or 10-12 $tons/in^2$ for a period of 1 minutes, carefully, release the pressure and

dismantle the dies, now, remove the disc from the mould and keep it in position onto the sample holder.

Salient Features:-

(*i*) There exists a possibility of interaction between vibrations of the sample and the potassium bromide lattice.

(*ii*) It is considered to be the most suitable method for other screening of very minute quantities of substances being eluted from the columns in Gas Liquid Chromatography (GLC), in actual practice, about 300 mg of the spectroscopic grade KBr is placed in a short column immediately after the detector.

Consequently, the solid is powdered, pressed into a disc in the normal procedure and ultimately the absorption spectrum of the trapped substance is studied.

(*iii*) It enjoys the advantage of producing spectra absolutely free from any solvent peaks (unlike Mull Technique) and hence it is employed extensively in routine analysis.

Internal Standard for KBr-Disc Technique:

In quantitative analysis it is essential to examine absolutely uniform discs of identical weights, to achieve this, known weights of both KBr and analyte are required in the preparation of the KBr-disc and finally from the absorption data a calibration-curve may be obtained, in this process, it is a must to weigh the discs and also to measure their thickness at different points

Calibration of Infrared Spectroscopy:-

The wavelength (or wavenumber) scale calibration of infrared spectroscopy is usually carried out with the aid of a strip of **polystyrene film** fixed on a frame; it consists of several sharp absorption bands, the wavelengths of which are known accurately and precisely.

Basically, all IR-spectroscopes need to be calibrated periodically as per the specific instructions so as to ascertain their accuracy and precision.

Applications of IR Spectroscopy in the Analysis of the Pharmaceutical Substances, Determination of Aspirin, Phenacetin and Caffeine in Tablets

Outcomes:-

After completing this experiment, the student should be able to:

- Explain the IR spectrum of some pharmaceutical substances.
- Identification of some pharmaceutical substances by IR spectrum.

Introduction:-

A host of pharmaceutical substances can be identified and critically examined with the help of infrared spectroscopy; hence, the latest versions of British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) contain the complete IR-spectrum of such pure pharmaceutical substances that are essentially included in the respective *official compendium.*

Example: The infrared absorption spectrum of the following pharmaceutical substances, namely:

Ampicillin sodium; Amylobarbitone; Betamethasone; Betamethasone valerate; Carbenicillin disodium; Chloroquine phosphate; Chloroquine sulphate; Cemetidine; Clofazimine; Clofibrate; Clonidine hydrochloride; Cloxacilline sodium; Colchicine Cyclophosphamide; Cyproheptadine hydrochloride; Dexamethasone; Activated dimethicone; Diphenylpyraline hydrochloride; Erythromycin ; Ethambutol hydrochloride; Ethirylestradiol; Ethiosuximide; Fludrocortisone acetate; Fluphenazine hydrochloride; Iburprofen; Diluted isosorbide dinitrate; Lincomycin hydrochloride; Mebendazole; Metoformin hydrochloride; Methdilazine hydrochloride; Methotrexate; Nalidixic acid; Nandrolone decanoate; Nandrolone phenylpropionate; Niclosamide; Nitrofurantoin; Nitrofurazone ; Norethisterone; Oxprenololhy drochloride; Pentazocine hydrochloride; Pentolamine hydrochloride; Phentolamine mesylate; Primidone; Prochlorperazine mesylate; Proguanil hydrochlorde; Pyrazinamide; Pyrimethamine; Rifampicin; Spironolactone; Stilbosterol diphosphate; Sulphadimethoxine; Sulphalene; Sulphamethizole; Testosterone propionate; Thiabendazole; Trifluoperazine hydrochloride; Triflupromazine hydrochloride.

The quantity is solely based on the intensities of the carbonyl bands at 1764, 1511 and 1665 cm^{-1} for aspirin, phenacetin and caffeine respectively.

Materials and Equipment: APC-Tablets; Chloroform and IR Spectroscopy.

Procedure:

The drug contents of an appropriate number of tablets are directly extracted into chloroform, filtered if necessary so as to remove the insoluble tablet components, and the final concentration of chloroform solution is made in such a way so that it should contain: 90 mg ml^{-1} of aspirin; 64 mg ml^{-1} of phenacetin, and 134 mg ml^{-1} of caffeine. The IR-spectrum is now recorded in a 0.1 mm NaCl –cell between (1400--2000 cm^{-1}).

Table of IR Absorptions

Functional Group	Characteristic Absorption(s)(cm^{-1})	Notes
Alkyl C-H Stretch	2950 - 2850 (m or s)	Alkane C-H bonds are fairly ubiquitous and therefore usually less useful in determining structure.
Alkenyl C-H Stretch Alkenyl C=C Stretch	3100 - 3010 (m) 1680 - 1620 (v)	Absorption peaks above 3000 cm^{-1} are frequently diagnostic of unsaturation
Alkynyl C-H Stretch Alkynyl C=C Stretch	~3300(s) 2260 - 2100 (v)	
Aromatic C-H Stretch Aromatic C-H Bending Aromatic C=C Bending	~3030 (v) 860 - 680 (s) 1700 - 1500 (m, m)	
Alcohol/Phenol O-H Stretch	3550 - 3200 (broad,s)	See "Free vs. Hydrogen-Bonded Hydroxyl Groups" in the Introduction to IR Spectra for more information
Carboxylic Acid O-H Stretch	3000 - 2500 (broad,v)	
Amine N-H Stretch	3500 - 3300 (m)	Primary amines produce two N-H stretch absorptions, secondary amides only one, and tetriary none.
Nitrile C=N Stretch	2260 - 2220 (m)	
Aldehyde C=O Stretch Ketone C=O Stretch Ester C=O Stretch Carboxylic Acid C=O Stretch Amide C=O Stretch	1740 - 1690 (s) 1750 - 1680 (s) 1750 - 1735 (s) 1780 - 1710 (s) 1690 - 1630 (s)	The carbonyl stretching absorption is one of the strongest IR absorptions, and is very useful in structure determination as one can determine both the number of carbonyl groups (assuming peaks do not overlap) but also an estimation of which types.
Amide N-H Stretch	3700 - 3500 (m)	As with amines, an amide produces zero to two N-H absorptions depending on its type.

Note:- http://www.chem.ucla.edu/~webspectra/#Problems