



College of Pharmacy-University of Mustansiriyah
5Th. Year- Practical advance pharmaceutical analysis – 2025 -

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. **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 ($\tilde{\nu}=\text{cm}^{-1}$)** 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:-

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 resulting to be normally rich in peaks because of several vibration modes ($3n-6$ 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 **(2.5-8 μm)** and a wavenumber from **(4000-1300 cm^{-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**



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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	$\text{C}_2\text{H}_2\text{Cl}_2$	3089	2	CH_3CHO	1729
3	$\text{CH}_2 = \text{CH}_2$	3105—2990	3	$\text{H}_5\text{C}_2\text{COC}_2\text{H}_5$	1720
4	C_6H_6	3099	4	HCOOH	1729
5	CH_3OH	2977	5	CH_3COOH	1718
6	$\text{CH} \equiv \text{CH}$	3287	6	CF_3COOH	1776

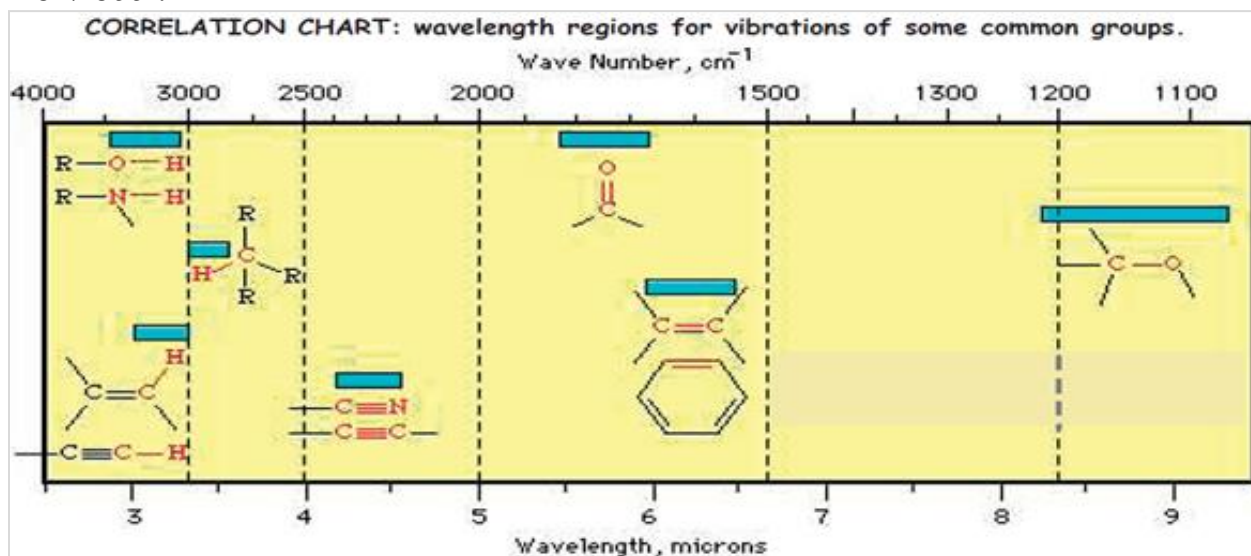
(b) Fingerprint region: -

Having a wavelength ranging from **(8.0-25 μm)** 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 no two '**fingerprints**' could be identical in human beings, exactly in a similar manner no two compounds may have the same 'fingerprint region', thus, every molecule essentially gives rise to a unique spectrum that offers a characteristic feature of the same.

IR may 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 the IR of a pure compound bears the same relationship to that compound as fingerprints do to an individual.





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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:

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

ν 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:

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

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

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

Solution: -

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

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

$$c = \lambda \nu, \frac{1}{\lambda} = \frac{\nu}{c} = \hat{\nu} \text{ (wavenumber)}$$

$$\hat{\nu} = \frac{9.247 \times 10^{13} \text{ s}^{-1}}{2.998 \times 10^{10} \text{ cm.s}^{-1}} = 3084 \text{ cm}^{-1}$$

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**, the **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 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 ν , $\hat{\nu}$, and λ with atom mass? (H.W.)



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Determination of IR Spectrum of a Solid Pharmaceutical Substance: -

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

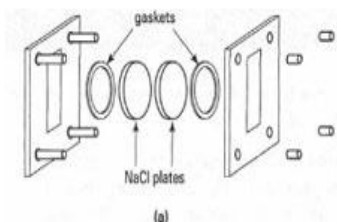
(a). Mull Technique

Procedure: Take 15-20 mg of the 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 Fluorolube 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, put 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, and finally, introduce the cell to the respective cell compartment.

Salient Features: -

- (i) The particle size of the sample has to be reduced below 200 mesh or 3 μm 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 a 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 mold 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 1 minutes, carefully, release the pressure and dismantle the dies, now, remove the disc from the mold and keep it in position onto the sample holder.



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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, and 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 free from any solvent peaks (unlike the Mull Technique) and it is employed extensively in routine analysis.

Internal Standard for KBr-Disc Technique:

In quantitative analysis it is essential to examine 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.

All IR-spectroscopes need to be calibrated periodically, per the specific instructions to ascertain their accuracy and precision.



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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; Cimetidine; Clofazimine; Clofibrate; Clonidine hydrochloride; Cloxacillin sodium; Colchicine; Cyclophosphamide; Cyproheptadine hydrochloride; Dexamethasone; Activated dimethicone; Diphenylpyraline hydrochloride; Erythromycin ; Ethambutol hydrochloride; Ethirylestradiol; Ethiosuximide; Fludrocortisone acetate; Fluphenazine hydrochloride; Ibuprofen; Diluted isosorbide dinitrate; Lincomycin hydrochloride; Mebendazole; Metoformin hydrochloride; Methdilazine hydrochloride; Methotrexate; Nalidixic acid; Nandrolone decanoate; Nandrolone phenylpropionate; Niclosamide; Nitrofurantoin; Nitrofurazone ; Norethisterone; Oxprenolol hydrochloride; Pentazocine hydrochloride; Pentolamine hydrochloride; Phentolamine mesylate; Primidone; Prochlorperazine mesylate; Proguanil hydrochloride; Pyrazinamide; Pyrimethamine; Rifampicin; Spironolactone; Stilbesterol 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 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



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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 tertiary 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) and 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.



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