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Instrumentation and Application of Infrared Spectroscopy In Pharmaceutical Industry

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ABSTRACT

This review mainly focuses on the principal, instrumentation, interpretation, application of infrared spectroscopy. Infrared (IR) radiation refers broadly to that part of the electromagnetic spectrum between the visible and microwave region. Of greatest practical use to the organic chemist is the limited portion between 4000 and 400cm -1. Infrared spectroscopy monitors the interaction of functional groups in chemical molecules with infrared light resulting predictable vibrations that provides a “fingerprint” characteristic of chemical or biochemical substances present in the sample. Infrared spectroscopy is a technique that probes the vibrations within a material. Infrared spectroscopy has always been a powerful tool for the identification of organic materials. The development of Fourier transform infrared (FTIR) spectroscopy has introduced a popular method for the quantitative analysis of complex mixtures, as well as for the investigation of surface and interfacial phenomena.

Keywords: Infra-red, Spectroscopy, Stretching, Instrumentation.

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INTRODUCTION

The measurement of the absorption, emission, or reflection of infrared radiation by matter is known as Infrared spectroscopy, sometimes known as Vibrational spectroscopy or IR spectroscopy. It's use involves examining and recognizing chemical compounds or functional groups in solid, liquid, or gaseous states. An Infrared spectrometer is used to perform the procedure or technique of infrared spectroscopy. It generates an infrared spectrum. A graph with infrared light transmittance (or absorbance) on the vertical axis and frequency or wavelength on the horizontal axis can be used to display an IR spectrum. Reciprocal centimeters, often known as wave numbers, are commonly used units of frequency in infrared spectra. They are denoted by the notation cm-1. Infrared wavelength units are often represented by the symbol µm, or micrometers (previously known as "microns").[1]

The portion of the electromagnetic spectrum between the visible and microwave domains is commonly referred to as infrared (IR) radiation. The narrow range of 4000–400 cm-1 is most useful to organic chemists in practice. The near-IR has drawn a lot of curiosity. As well as the far-IR region (700-200 cm-1). Even if the molecule as a whole is represented in the IR spectrum, specific atomic groups give rise to bands at or near the same frequency with little consideration to the remainder of the molecule's structure. The enduring nature of these traits indicates that allows the chemist to consult generalized charts of characteristics group frequencies and perform a quick inspection to gain valuable structural information.[2]

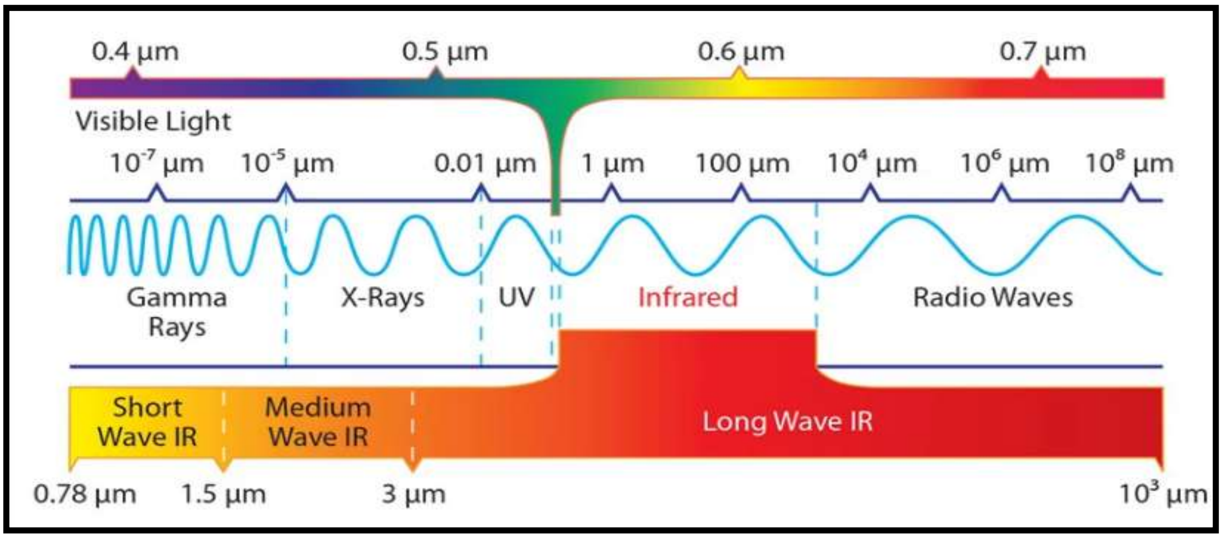


Figure 1:Electromagnetic Spectrum

Principle

Infrared radiation of frequencies less than about 100cm-1 is absorbed and converted by an organic molecule into energy monocular rotation. These absorptions is quantized; thus a molecular rotation spectrum consist discrete lines.

IR radiation in the range from about 10000 -100cm-1 is absorbed and converted by an organic molecule into energy of molecular vibration. This absorption is also quantized, but vibration spectra appear as bands rather than as lines because a single vibrational energy change is accompanied by a number of rotational energy changes. It is with these vibrational rotational band, particularly those occurring between 4000 and 400cm-1. The frequency or wavelength of absorption depends on the relative masses of the atoms, the force constants of the bonds, and the geometry of the atoms.

This technique is based upon the simple fact that a chemical substance show marked selective absorption in the infrared region. after absorption of IR radiation, the molecule of the chemical substance vibrates at many rates of vibration, giving rise to close packed absorption bands, called an IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in the IR spectrum which will corresponding to the characteristic functional group and bond present in the chemical substance. Thus, an IR spectrum of a chemical substance is a fingerprint for its identifications.

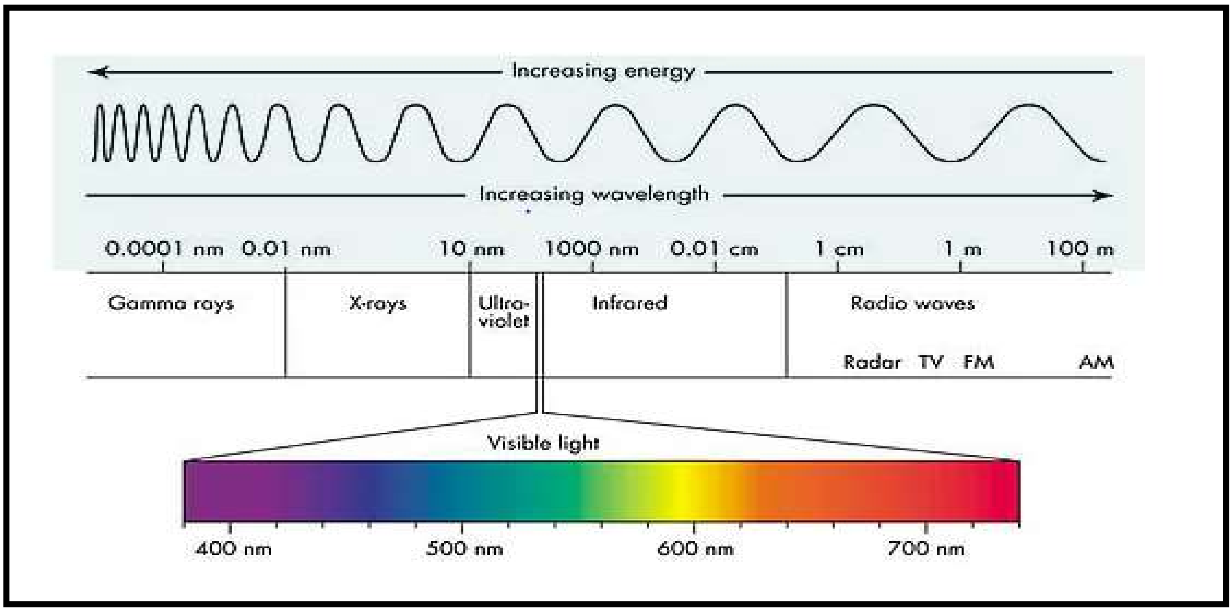


Figure 2: Electromagnetic Spectrum

Theory

Infrared spectroscopy (IR spectroscopy) deals with the interaction of infrared radiation with matter. It is used to identify the functional groups present in an organic compound. In this technique, an instrument called infrared spectrometer is used to produce an infrared spectrum which gives characteristic absorbance (or transmittance) bands to get information about the sample under observation. To analyze a sample by IR spectroscopy, the IR spectrum of the sample is recorded by passing IR radiation through the sample. A silicon carbide rod (5-10 mm width and 20- 50 mm length), known as Globar (Glow and bar) is usually used as thermal light source for infrared spectroscopy. It is electrically heated upto 1000-16500 C to emit radiation. An IR spectrum is basically a graph of infrared light absorbance (or transmittance) on the Y- axis vs. wavenumber (or frequency) on the X- axis. Fourier Transform Infra-Red (FT-IR) spectrometer is usually used in the laboratories for the analysis of different samples.[3]

In electromagnetic spectrum the infrared portion is usually divided into three regions –

- **Near Infrared region**: The wavenumber in this region lies in the range 13000– 4000 cm−1 (corresponding wavelength range 0.8–2.5 um) (approximately). The IR radiation in this region has high energy and are suitable to excite overtone or harmonic vibrations.
- **Mid Infrared region**: The wavenumber in this region lies in the range 4000– 400 cm−1 (corresponding wavelength range 2.5–25 um) (approximately). The energy of the IR radiation lying in this region is suitable for the study of fundamental vibrations**.**
- **Far Infrared region**: The wavenumber in this region lies in the range 400– 10 cm−1 (corresponding wavelength range 25–1000 um) (approximately). The IR radiation in this region has low energy and may be used for rotational spectroscopy. Samples having covalent bonds are mainly analyzed by IR spectroscopy.[4]

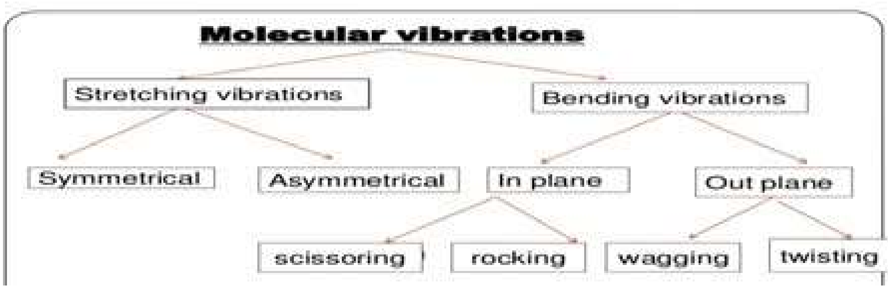
Samples having covalent bonds are mainly analyzed by IR spectroscopy. During analysis the sample should be used with high level of purity. This is because samples with high purity will have fewer IR active bonds and as a result the spectra observed will be simple. On the other hand, if the sample is impure then due to the presence of higher IR active bonds the spectra will be complex, which will be less useful for a synthetic Chemist.

RANGES OF IR SPECTROSCOP

IR Region: 12800 – 10 cm-1

Regions	**Wavelength λ(µm)**	**Wavenumber υ (cm-1)**
Near	0.78-2.5	12800-4000 Carbohydrates and Protein
Middle	2.5-50	4000-200 Organic Molecules and Functional Groups
Far	50-1000	200-10 Inorganic Co-Ordination Bond and Quaternary Ammonium Compounds.
Most Used	2.5-15	4000-670

Molecular Vibrations



Molecular Vibrations

The atoms in a molecule encounter a number of different types of vibrations, as a result the positions of atoms in a molecule are not fixed but changes periodically.

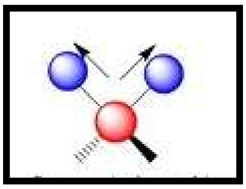
molecular vibrations can be classified into two categories – [5]

1.Stretching Vibrations –

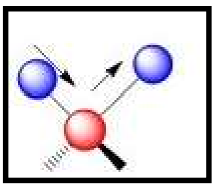
In this type of vibrations, the distance between the two atoms changes i.e. the distance between the atoms either increases or decreases but the atoms remain in the same bond axis.

There are two types of stretching vibrations –

➢ **Symmetric Stretching:** In this type of stretching vibrations, the movement of the atoms with respect to a particular central atom takes place in the same direction.[6]



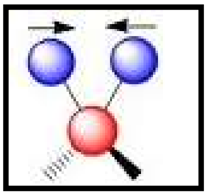
➢ **Asymmetric Stretching:** In this type of stretching vibrations, the movement of the atoms with respect to a particular central atom takes place in opposite direction i.e. one atom approaches towards the central atom while the other atom moves away from it.



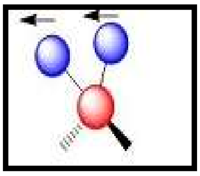
2. Bending Vibrations: In this type of vibrations, the angle between the bonded atoms changes i.e. the positions of the atoms changes with respect to the original bond axis. Bending vibrations are of four.[7]

***Bending vibrations are of four types**

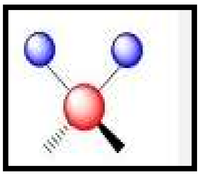
➢ **Scissoring:** In this type of bending vibrations, two atoms either move away from each other or move towards each other with respect to a central atom i.e. they operate like a scissor. In this type of bending vibration, the operation takes place in plane.



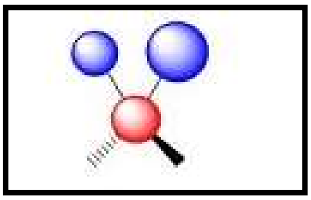
➢ **Rocking:** In this type of bending vibrations, two atoms move in the same direction with respect to a central atom. In this type of bending vibration, the operation takes place in plane.



➢ **Wagging:** In this type of bending vibrations, two atoms either move up of the plane or move down of the plane with respect to the plane containing the central atom. In this type of bending vibration, the operation takes place out of-plane.



➢ **Twisting:** In this type of bending vibrations, one atom move up of the plane while other atom move down of the plane with respect to the plane containing the central atom. In this type of bending vibration, the operation takes place out of-plane.



Instrumentation Of IR

The main compoonents of IR-

- IR radiation sources
- Monocromators
- Sampling techniques
- Sample cell
- Detector
- Ampliflier

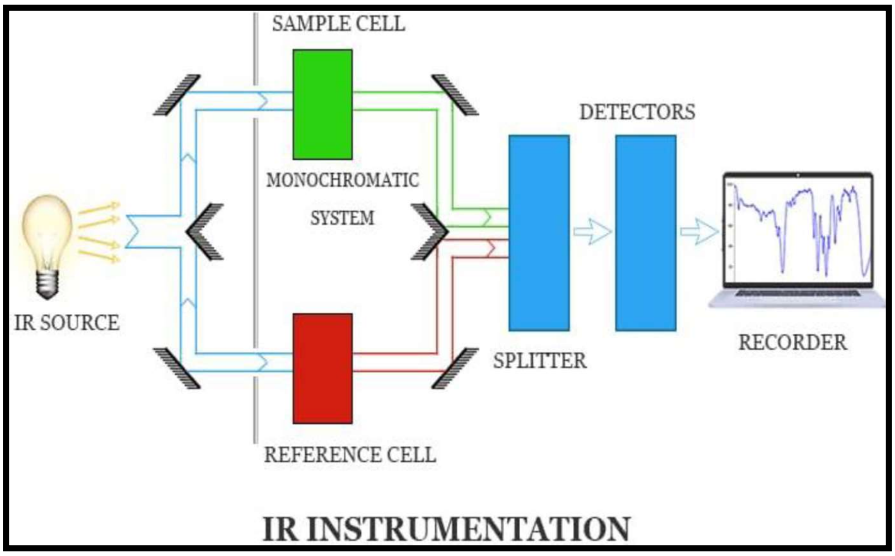


Figure 3: Instrumentation of IR Spectroscopy

Infrared Instrumentation is divided into two classes, Dispersive and Non –dispersive . The dispersive instruments use a priam or gratig and are similar to ultarviolet visible spectrometer except in the IR region ,different sources and different detectors must be used. [8]

Non-dispersive spectrometer may use interferences filters, tunable laser sources, an intrferometer used in very popular Fourier transform Infrared(FTIR)spectrometer .For IR region front surfaced mirrors are used , since glass and quartz used in lenses are opaque to IR radiation.

1. IR Radiation Source:

In common with other types of absorption spectrometers, infrared instruments require a source of radiant energy which provides a means for isolation narrow frequency bands. The radiation source must emit IR radiation which must be

- Intensive enough for detection
- Steady
- Extend over the desired Wavelength
- Although these radiations are continuous, only selected frequencies will be absorbed by the sample.

The various popular sources of IR radiations are:

a. Incandescent lamp: An everyday incandescent light is typically utilized in near-infrared devices. Unfortunately, because to its low spectral emissivity and glass enclosure, this fails in the far infrared

b. Mercury Arc: The sources mentioned above become ineffective in the far infrared spectrum (wave number <200 cm-1), hence high-pressure mercury arc lamps are employed instead. In a novel way, Beckman invented the quartz mercury lamps for the same area. At shorter wavelengths, the radiation is emitted by the heated quartz envelope, whereas at longer wavelengths, the quartz is penetrated by the mercury plasma

c. Globar source: This is a 50 mm long by 4 mm diameter rod made of sintered silicon carbide. It emits a lot of radiation in the infrared spectrum when heated to temperatures between 1300 and 17000C. At 5200 cm-1, it emits its maximum radiation.

2. Monocromator: The radiation source emits radiation of various frequencies. The sample in IR spectroscopy absorbs only at certain frequencies, it therefore becomes necessary to select desired frequencies from the radiation source and reject the radiation of other frequencies. the selection has been achieved by means of monochromators.[9]

The monochromator used to make this selection are primarily of two types

Prism Monochromator:

Any prism that is employed as a dispersive element needs to be made of infrared-transmitting materials, including different metal halide salts. Despite being used in the visible and ultraviolet, glass and quartz absorb and perform poorly in the far-infrared. Sodium chloride is most likely the most often used prism salt due to its strong dispersion in the 4 to 15cm range, a region that is particularly important for students in functional groups. It is regrettable that a large number of these salt materials are water soluble, mechanically and/or thermally unstable. Damage prevention measures must be taken consistently.[10]

Grating Monochromator:

Greater dispersion can be obtained by substituting grating for a prism in a prism monochromator. Reflection gratings come in a multitude of materials and provide linear dispersion.[11]

Multiple rulings, each with a distinct outcome. To cover the large wavelength (energy) range associated with infrared light, (lines/cm) are required. There are several combinations of grating-equipped or grating-free transmission or interference filters used. [12]

In essence, the grating is a set of straight, parallel lines that have been carved into a flat surface. The law of diffraction governs dispersion by gratings. It adheres to the subsequent mathematical relationship.[13]

$$d (\sin i \pm \sin 0) = n \lambda$$

Where,

n is the order (a whole number)

λ is the radiation wavelength

d is the distance between the grooves

i is the angle at which the IR radiation beam is incident

0 is the angle at which the wavelength of light is dispersed

3. Sampling Techniques and Sample cell:

Since solid, liquid, and gas samples have all been characterized using infrared spectroscopy, handling samples in multiple phases is obviously necessary. However, these samples require a different approach. But the only thing that unites the sampling of various phase is that the sample-containing material needs to be IR radiation-transparent. This constraint limits the salts we can choose from, such as KBr or NaCl. Nevertheless, the wavelength range to be examined will determine the ultimate salt selection.

Sampling of Solid	Sampling of Liquid	Sampling of Gases
a. Attenuated total reflectance (ATR) This is the fastest and most widely used method for solids and powders, requiring minimal preparation. **Procedure:** Clean ATR crystal → Place solid sample on crystal → Apply pressure with clamp → Collect spectrum → Clean crystal **b. KBr pellet technique** This transmission method is suitable for powdered samples and requires a hydraulic press. **Procedure:** Solid sample -> Grind into fine powder with mortar and pestle -> Mix with dry KBr powder -> Place mixture in pellet die -> Apply high pressure with hydraulic press -> Transparent KBr pellet -> Mount pellet in spectrometer -> Collect spectrum **c. Mull technique This** method is an alternative to KBr pellets, especially if a press is unavailable. **Procedure:** Solid sample -> Grind to fine powder -> Mix with a few drops of mulling agent (e.g., Nujol) -> Create a thick paste (mull) -> Sandwich mull between two salt plates -> Mount plates in spectrometer -> Collect spectrum	**a.Neat liquid film** This technique is used for pure, non-viscous liquids. **Procedure:** One drop of liquid sample -> Place between two IR-transparent salt plates -> Press plates together to form a thin film -> Mount plates in spectrometer -> Collect spectrum **b. ATR** ATR is a very convenient method for liquid samples as well. **Procedure:** Clean ATR crystal -> Place a drop of liquid directly onto the crystal -> Collect spectrum -> Clean crystal **c. Liquid cell** This method is used for viscous or dilute liquids. **Procedure:** Assemble liquid cell (salt windows + spacer) -> Inject sample with a syringe -> Mount sealed cell in spectrometer -> Collect spectrum	**a.Gas cell analysis** Due to low concentration, gaseous samples require a longer pathlength. **Procedure:** Evacuate and purge gas cell with dry nitrogen -> Introduce gaseous sample into cell -> Mount cell in spectrometer -> Collect spectrum

SAMPLE CELLS-

Material Composition:

The sample cell windows are predominantly made from alkali halide salts, most commonly sodium chloride (NaCl) or potassium bromide (KBr).

Reason for Selection:

These salts are chosen because they are transparent (non-absorbing) to the infrared radiation across the mid-IR region, ensuring the cell material does not create interfering peaks in the spectrum.[14]

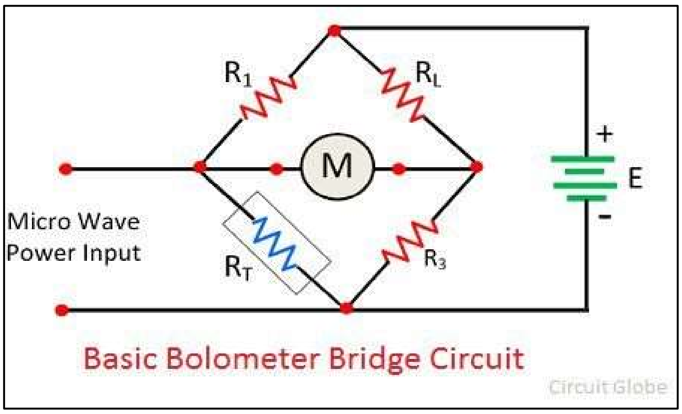
Handling Caution:

A crucial limitation is that these salt plates are highly sensitive to water and moisture (hygroscopic) and will dissolve or fog if exposed to aqueous samples or high humidity.

Detector

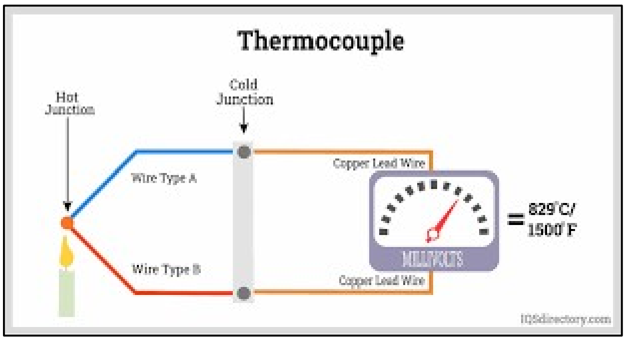
Bolometer

A bolometer usually consists of a thin metal conductor. When radiation, such as IR, falls on this conductor, its temperature changes. As the resistance of a metallic conductor changes with [temperature, the degree of change in resistance is regarded as measure of the amount of radiation that has fallen on the bolometer. A bolometer is made one arm of the Wheatstone bridge. A similar strip of metal is used the balancing arm of the bridge. This strip is not exposed to IR radiation. When no radiation falls on the bolometer, the bridge remains balanced If IR radiation falls on the bolometer, the bridge becomes unbalanced due to change in the electrical resistance which causes a current to flow through the galvanometer (G). The amount of current flowing through the galvanometer is a measure of the intensity of the radiation falling on the detector. The response time for a bolometer is 4 m sec.



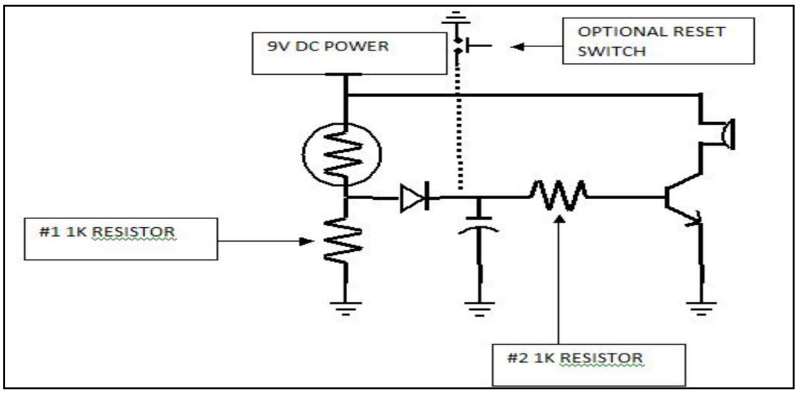
Thermocouple

The Thermocouple detector is based upon the fact that an electrical current will flow when two dissimilar metal wires are connected together at both ends and a temperature differential exists between the two ends. The end exposed to the infrared radiation is called the "hot junction”. To increase the energy gathering efficiency, it is usually a "black body." The other connection, the "cold junction," is thermally insulated and carefully screened from stray light. The electricity which is directly proportional to the energy differential between the two connections. If two welded joints are kept at different temperatures, a small electrical potential is developed between the joints. A thermocouple is closed in an evacuated steel casing With a KBr window to avoid losses of energy by convection. In the IR spectroscopy, one welded joint (called cold junction) is kept at a constant temperature and is not exposed to IR radiation, but the other welded joint called hot junction is exposed to the IR radiation which increases the temperature of the junction. The temperature difference between the two junction generates potential difference which depends on how much IR radiation falls on the hot junction. The response time of a thermocouple is about 60 m sec.



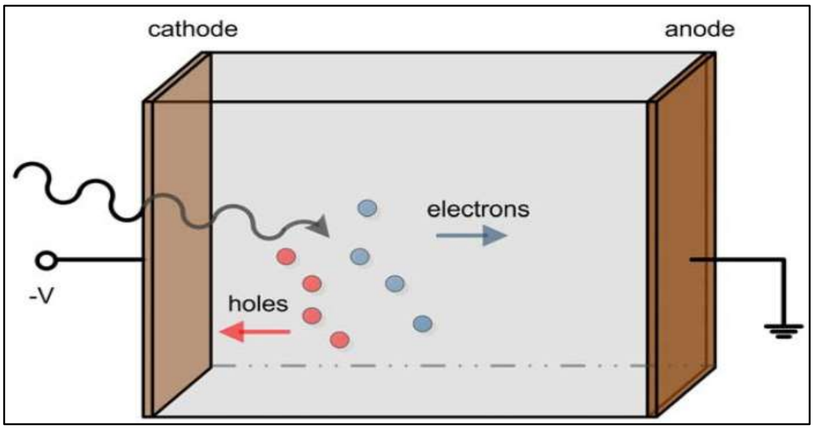
Thermister

A fused combination of metal oxides makes up a thermistor. In contrast to the bolometer, the mixture's electrical resistance reduces as temperature rises. This partnership Thermistors can be utilized as infrared detectors in the same manner as bolometers due to the relationship between temperature and electrical resistance. Usually, the thermistor alters resistance by 5% for every degree Celsius. It responds slowly as well. [14]



Semiconductor detector

Semiconductors are materials that are insulators when no radiation falls on them, but which become conductors when radiation falls on them. Exposure to radiation causes a very rapid change in their electrical resistance and therefore a very rapid response to the IR signal. The basic concept behind this system is that an IR photon displaces an electron in the detector, changing conductivity greatly .materials, such as each telluride, indium antimonide, and germanium doped with copper or mercury, have been used as semiconductor detectors. In general, a semiconductor detector is fabricated with the semiconductor material deposited on glass in a sealed, evacuated envelope. Exposure to radiation causes a rapid change in the material conductivity. The response time of this detector is the time required to change the semiconductor from an insulator to a conductor, which is frequently as short as 1 sec. Semiconductor detectors are very sensitive, very fast, and are finding wide acceptance in the field of IR spectroscopy [15]



Amplifier-

The amplifier converts the weak electrical signal from the detector into a stronger, measurable signal, allowing for sensitive detection and analysis of molecular vibrations. Different types of amplifiers exist, such as lock-in amplifiers for noise-sensitive measurements and femtosecond amplifiers for ultrafast pulsed IR spectroscopy, all serving the essential function of boosting the signal for subsequent digital processing and spectrum generation.

Signal Enhancement:

Detectors in IR spectrometers generate very small electrical signals in response to the absorbed or transmitted infrared radiation. The amplifier's primary role is to boost these weak signals to a level that can be reliably processed by the instrument's electronics and analog-to-digital converters (ADCs).

Noise Reduction:

Amplifiers, particularly lock in amplifier are designed to improve the signal-to-noise ratio by selectively amplifying signals at a specific, narrow frequency band while rejecting noise at other frequencies.

Signal Conditioning:

Some amplifiers also condition the signal to make it compatible with the subsequent processing stages, such as digital signal processing.

APPLICATIONS

In the pharmaceutical industry, infrared (IR) spectroscopy is an essential analytical technique used for quality control, research, and manufacturing. By measuring how molecules absorb infrared light, it produces a unique vibrational "fingerprint" that can identify and quantify chemical compounds. The technique is valued for being non-destructive, rapid, and versatile for analyzing various materials.

1. **Identification of Functional Groups:** This is the primary application of IR spectroscopy in organic chemistry. Different functional groups (like C=O carbonyls, O-H hydroxyls, and C-H bonds) vibrate and absorb IR radiation at characteristic frequencies. By analyzing the unique "fingerprint" region of the spectrum, the presence or absence of specific functional groups can be determined.
2. **Identification of Unknown Compounds:** The IR spectrum of a pure compound is unique, much like a fingerprint. By comparing the spectrum of an unknown sample to a library of reference spectra, the substance can be identified. This is widely used in quality control and forensic analysis.[16]
3. **Monitoring Chemical Reactions:** The progress of a chemical reaction can be studied by examining the reaction mixture at different times. The disappearance of characteristic reactant peaks and/or the appearance of product peaks indicates the reaction is occurring.
4. **Detection of Impurities:** The presence of extra peaks in a sample's IR spectrum, when compared to a standard reference spectrum of a pure compound, can indicate the presence of impurities. This is vital for quality assurance in pharmaceuticals and materials production.
5. **Quantitative Analysis:** IR spectroscopy can be used to determine the concentration of a substance within a mixture, based on the intensity of its characteristic absorption bands and the principles of the Beer-Lambert law. This is useful for environmental monitoring of pollutants, such as detecting automobile emissions.[17]
6. **Studying Isomerism and Hydrogen Bonding:** IR spectroscopy can help distinguish between certain types of isomers (e.g., cis/trans isomers, some positional isomers) and study intramolecular and intermolecular hydrogen bonding by observing shifts in absorption frequencies.
7. **Forensic Analysis:** Due to its non-destructive nature and ability to work with very small samples, IR spectroscopy is a crucial tool in forensic science. It is used to analyze evidence like paint chips, fibers, illicit drugs, and ink on questioned documents.
8. **Material and Polymer Analysis:** In industry, IR spectroscopy is used for quality control, failure analysis, and research involving various materials like plastics, paints, and semiconductors. It helps determine the chemical composition and structure of polymers and other complex materials.[18]

9. **Pharmaceutical and Biomedical Applications:** It is extensively used in the pharmaceutical industry for drug identification, testing for purity, characterizing drug crystalline structures (polymorphism), and studying interactions between active ingredients and excipients. In biomedical research, it can be used for protein characterization and even early disease detection through tissue or body fluid analysis.
10. **Environmental Analysis and Atmospheric Studies:** FT-IR can analyze substances in solid, liquid, or gaseous states, making it ideal for monitoring air and water quality. It is used to determine the concentration of gases in the atmosphere and study atmospheric pollutants.

Advantages

1. Non-destructive: The sample is not consumed or damaged during analysis and can be recovered for further testing.
2. Fast analysis time: Spectral data can be collected in seconds, allowing for high throughput screening.
3. Versatility: It can be used to analyze almost any type of sample, including solids, liquids, and gases.
4. Functional group identification: The technique is excellent for identifying the functional groups present in a molecule, which is crucial for structural analysis.
5. High sensitivity: Modern Fourier Transform Infrared (FTIR) instruments are highly sensitive, capable of detecting even very small amounts of a substance.
6. Qualitative and quantitative analysis: It can be used for both identifying substances (qualitative) and determining their concentration in a mixture (quantitative).

Disadvantages

1. 1Water interference: Water absorbs strongly in the infrared region, which makes analyzing aqueous samples very difficult.
2. Incomplete structural information: It identifies functional groups but does not provide complete structural detail, such as the relative positions of groups.
3. Complex spectra: Large or complex molecules can produce crowded spectra with overlapping peaks, making interpretation difficult.
4. Symmetry limitations: Symmetrical molecules or bonds with no change in dipole moment during vibration are IR-inactive and will not produce a signal.
5. Requires pure samples: Impurities introduce extra peaks in the spectrum, which can complicate analysis and lead to inaccurate results.

6. Low sensitivity for trace analysis: While modern FTIR is sensitive, it is not always effective for detecting substances in very low concentrations compared to other techniques.

CONCLUSION

It was concluded that the IR spectroscopy it is an analytical instrument. It is based on the principle of vibrational and rotational energy of a molecule. It is used for qualitative analysis by constructing a Calibration curve. It is used in conjunction with UV spectroscopy, chromatography, mass spectroscopy and nuclear magnetic resonance spectroscopy.

IR spectroscopy provides a unique chemical fingerprint for a molecule, allowing for the identification of functional groups and the determination of its structure and concentration. The technique is valuable across many fields, including pharmaceutical analysis, material science, and the study of biological molecules, though its effectiveness can be limited by sample complexity and requires careful data interpretation and validation.

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