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INFRARED SPECTROSCOPY: A REVIEW

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ABSTARCT

Infrared (IR) spectroscopy is a vibrational spectroscopic technique which is based on the absorption of infrared radiation by matters which results into excitation of vibration of molecular band. It is a powerful as advantageous method for investigating functional, structural, and compositional changes in cells, biomolecules, and tissues. In the recent years, (vibrational spectroscopies) infrared spectroscopy have been developed for all types of analysis in microbiology. Important characteristics of these methods are the relative ease with which measurements can be performed. Fourier transform infrared spectroscopy (FT-IR) is a technique that has been used over the years for the identification of substances in chemical analysis and is one that may be applied to the characterization of microorganisms. This paper intends to review the basic theory of Infrared Spectroscopy, Principle, Instrumentation, Sampling Techniques and its applications in the field of Analytical Science. It can be concluded that vibrational spectroscopy shows high potential as novel methods.

KEYWORDS: Infrared spectroscopy, Near Infrared, Mid Infrared, Far Infrared, molecular vibrations, Fourier Transform spectrophotometers, Instruments, Sampling Techniques, Detector.

INTRODUCTION

Spectroscopy is a term which describes the interaction of electromagnetic radiation with matter. Several forms of interaction therefore exist such as impedance, resonance, absorption, emission, diffraction and inelastic scattering of radiation. Therefore, and as a big science,

spectroscopy is used to characterize/detect matter like atoms, molecules, and nuclei depend on the produced spectra and following their interaction with radiation. The electromagnetic spectrum, and as the word *spectrum* implies, is a sequence of frequencies of the electromagnetic radiation and the corresponding photon energies and wavelengths.[1,2] Attempts to use IR technology to biological source began as early as the 1910s, when the use of IR spectroscopy was first suggested for the analysis of biological samples. By the late 1940s, the technique was being successfully explored for the study of biological materials and, in fact, IR spectroscopy has become one of the accepted tool for the characterization of biomolecules.[3] Infrared spectroscopy is one of the Advantageous methods for structure determination of small molecules. This is due to its sensitivity to the architecture of molecules and chemical composition. The large information content in an infrared spectrum carries over also to biological systems. This is the reason which makes infrared spectroscopy a valuable and important tool for the investigation or determination of protein structure.[4-12]

The concept of IR-spectroscopy as a means for characterizing microbial samples was initiated after the development of modern interferometry IR spectroscopy, powerful new algorithms for multivariate statistical analysis, the availability of low-cost mini computers, powerful new algorithms for multivariate statistical analysis and pattern recognition methodologies.[13] William Herschel in 1800 discovered the Infrared radiation.[14] The energy levels were investigating by Herschel, which is associated with the wavelengths of light in the visible spectrum. Sunlight was directed through a prism and showed the well-known *rainbow colored* visible spectrum, i.e., the visible spectrum from blue to red with the analogous wavelengths or frequencies.[15,16]

In the ultraviolet region, the IR radiations are invisible to the human eye. IR radiations observed at a longer wavelength compared to the visible region. Accordingly, and since electromagnetic radiations travel which is the speed of light (c, 2.997 924 58 × 10^8 m s^{-1}), therefore the frequency of IR radiations is lower compared to that of the visible light, applying the formula $\upsilon = c/\lambda$ where υ = the frequency of light, λ = the wavelength of light and c = the speed of light. This is nothing but that energy associated with the IR radiations is inferior to that of the visible light and longer than that of microwaves, for instance.[17-20]

PRINCIPLE

IR light or “Infrared rays” is not visible to human eye. The IR spectrum starts at 0.75 nm. Wavelengths from 390 to 750 nm, typical human eye will respond. One nanometer (nm) is

10^{-9} m. The Infrared spectrum is divided into, Near Infrared (NIRS), Mid Infrared (MIRS) and Far Infrared (FIRS).[21-23] Ranges given between this spectrum are identified on the wavelength (λ) and wavenumber (ῡ) scales.[24]

The three Infra red regions of interest in the electromagnetic spectrum in terms of wavelengths the three regions in micrometers (μm) are the following:

- Near Infrared Spectroscopy (NIRS): (0.7 μm to 2.5 μm)
- Mid Infrared Spectroscopy (MIRS): (2.5 μm to 25 μm)
- Far Infrared Spectroscopy (FIRS): (25 μm to300 μm)

In terms of wavenumbers the three regions in cm^{-1} are:

- Near Infrared Spectroscopy (NIRS) : (14000-4000 cm^{-1})
- Mid Infrared Spectroscopy (MIRS) : (4000-400 cm^{-1})
- Far Infrared Spectroscopy (FIRS) : (400-10 cm^{-1})

The first region NIRS allows the study of overtones and combination or harmonic vibrations. The MIRS region is to study the rotation-vibration structure of small molecules and the fundamental vibrations, whereas the FIRS region is for the low heavy atom vibrations (metal-ligand or the lattice vibrations). Infrared (IR) light is electromagnetic (EM) radiation with a longer wavelength than that of visible light: ≤0.7μm. One micrometer (μm) is 10^{-6}m.[15]

MOLECULAR VIBRATIONS

Vibrational spectroscopy is important to measure *molecular* vibrations which results from absorption of light/photons. Therefore, absorption of energy, E, that matches the vibration frequency (υ) and because of the change in the dipole moment would trigger molecular vibration. Both techniques give spectral sketches that express the chemical temperament of a sample.

Two main modes of vibrations are commonly known

1. *Stretching* (where the distance between the two atoms and hence the bond length is affected)
2. *Bending* (where the slant between the two bonds is altered)

Stretching vibrations include two types of motions

a. *Symmetric* (where the two atoms *simultaneously* move toward and away from the central atom)
b. *Anti-Symmetric* (where one of the atoms move toward the central atom, while the second moves away from the central atom).

Bending vibrations include four types of motions:

a. *Rocking* (the two atoms moving in-plane either clockwise or anti-clockwise),
b. *Scissoring* (also in-plane, both atoms are simultaneously moving either toward each other or away from each other),
c. *Wagging* (out-of-plane, where both atoms simultaneously move like a V sign back and forth), and
d. *Twisting* (out-of-plane, where one atom moves forward while the other moves backward).[25]

INSTRUMENTATION

In general, this field has seen a extensively progress especially after grating was first introduced in 1823, and after the first commercial IR spectrometer came to the scene. The conventional IR spectrophotometer, firstly introduced in the 1940s, was a dispersive instrument. Rudimentary parts of this instrument were *a monochromator, a radiation source*, and *a detector,* most frequently in a double-beam setup. Monochromator, serves to separate the broad spectrum of IR radiations into a continuous sequence of IR bands with resolved frequencies and is nothing but the dispersive device. These instruments work by tracing only a single frequency at a time. Consequently, a whole spectrum needs a long time to be recorded.[18-20, 26,27]

We have two types of IR spectrophotometers: The **classical** and the **Fourier Transform spectrophotometers** with the interferometer.

The standard classical dispersive IR spectrophotometers[17,21]

The main elements of the standard classical dispersive IR instrumentation consist of 4 parts

1. A light source of irradiation
2. A dispersing element, diffraction grating or a prism
3. A detector
4. Optical system of mirrors

The infrared radiation passes through the sample from the source by reflecting to a flat mirror and reference monochromator then through the sample. On a rotating mirror, the beams alternate the mirror, rotates slowly and different frequencies of infrared radiation pass to detector.

Fourier Transform spectrophotometers

It was in the 1960s when Fourier transform (FT) instruments firstly introduced. *Interferometers* was the key difference between FT and dispersive instruments. Hence the output of the FT-IR instrument is then called an *interferogram.* FT-IR instruments, though were intended to extend the use of IR, had limited applications and were used only for advanced research. This was mainly because of the need for supercomputers to record the generated data and the expensive electronics component.[18-20,26-29]

FT-IR spectroscopy is a form of vibrational spectroscopy, and the FT-IR spectrum reflects both molecular environment and molecular structure. In this technique, from an infrared source the sample is irradiated and the absorption of this radiation stimulates vibrational motions by depositing quanta of energy into vibrational modes.[30,31] Therefore, a molecule, when exposed to radiation produced by the thermal emission of a hot source (a source of IR energy), absorbs only at frequencies which are corresponds to its molecular modes of vibration in the region of the electromagnetic spectrum between visible (red) and short waves (microwaves).[3] These changes in vibrational motion results into appearance of bands in the vibrational spectrum; each spectral band is characterized by its frequency and amplitude.[31]

Michelson FT-IR Spectrometer consist of the following main parts[32-34]

1. Light source (IR Source)
2. Interferometer
3. Beam splitter (half silvered mirror)
4. Moving mirror
5. fixed mirror
6. Laser
7. Detector

1. Light source (IR source)

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

Common IR sources are as follows

- Silicon carbide rods which are resistively heated and commonly known as a Globar source. An electric current is passed through the rod which becomes very hot (1300 K) and emits excess 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 which does not require cooling by water.
- Nichrome and Kanthanl wire coils were once popular IR sources and as they ran at lower temperatures than Globars, they did not require cooling, however, this also resulted in lower amounts of IR radiation being emitted.
- Nernst Glowers are manufactured from a mixture of refractory oxides and are able to reach hotter temperatures than a Globar; however, they are not capable of producing IR radiation above 2000 cm^{-1}.

2. Interferometer

The first interferometer was introduced by Albert Abraham Michelson, who received a Nobel Prize for his work in 1907. Without this required piece of optical equipment, the modern day FTIR system would not exist. The interferometer mainly consists of a beam splitter, a fixed mirror, and a moving mirror.

3. Beam Splitter

The beam splitter is made up of a special material which plays an important role in transmission of half of the incident radiation and reflects the other half. IR radiation from the source strikes the beam splitter and is separates into two beams. First beam is transmitted through the beam splitter to the fixed mirror while the Second 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 in order to produce an interferogram.

4. Moving Mirror

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

5. Fixed Mirror

The fixed mirror is a flat and highly reflective surface.

6. Laser

Many instruments use 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 particular constant velocity that is timed using a very accurate laser wavelength.

The intensity of the laser beam is measured in the interferometer at two points. The intensity at these two points will rise as the mirror moves and fall due to the enhancement and cancellation of the He-Ne 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 allows the instrument to which direction the mirror is moving.

7. Detector

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

- **Thermal detectors**: detect changes in temperature of an absorbing material (lead selenide (PbSe), lithium tantalate ($LiTaO_3$), germanium etc.). Many temperature dependent phenomenon can be followed to measure the effects of the incident IR radiation. Microbolometers and Bolometers use changes in resistance, while thermopiles and thermocouple use the thermoelectric effect. Golay cells monitor thermal expansion.

- **Photonic Detector:** exhibit faster/longer response times and higher sensitivity in comparison to their thermal counterparts, therefore, they are much more prolific in FTIR instruments. The materials which are used in these detectors are semiconductors with

narrow band gaps. The incident IR radiation responsible for electronic excitations between the ground and first excited states, which in photoconductive detectors result in a change in its resistivity which is monitored.

FTIR ADVANTAGES

This method can be applied to dehydrated, powdered or aqueous samples. Also, the FT-IR spectrometer can be modified in order to make the study of very small samples, such as single colonies and tissue sections, a possibility. The technique is extensively sensitive.[31]
FTIR instruments have many advantages over dispersive IR instruments including.[32-34]

Speed

Simultaneously all IR frequencies are measured, resulting in measurements being taken in seconds rather than minutes.

Sensitivity

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

Simplicity

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

Internal calibration

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

SAMPLE PREPARATION

1. As a Solid

Nujol Mull Method[32-34]

- Using a Nujol mull, IR spectra of solid samples can be obtained.
- Using a small agate mortar and pestle and a drop of Nujol, a small amount of sample is get grounded.
- Then the spectrum is obtained by pressing mull between two NaCl plates.
- The mull should be transparent and free of bubbles when properly prepared.

- One plate can be wiped clean if the peaks in the spectrum are too strong and the spectrum re-run.

KBr Disc Method[32-34]

- A solid sample will ground with 10-100 times its mass of pure potassium bromide (KBr).
- Solid samples should be finely ground before adding the potassium bromide (KBr).
- This is then pressed into a disc using a special mold and a hydraulic press.
- The use of KBr is useful to eliminate 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.
- By drying the KBr in an oven water can be minimized. Excessive grinding of the hygroscopic KBr can increase the water content.

2. As a Liquid[32-34]

- A drop of the liquid is squeezed between two sodium chloride plates, which in region 4000-625 cm^{-1} is transparent.
- NaCl plates are very sensitive and fragile to water. Samples should never be dissolved in water and placed on a NaCl plate as it will dissolve or fog up.
- The plates should be held by the edges which helps to avoid moisture from fingers damaging them.
- Ethanol can be used to clean the plates after a sample has been run.
- Moisture in the air can also damage sodium chloride plates; therefore, they should be stored in a desiccator.

APPLICATIONS OF IR SPECTROSCOPY

- IR spectroscopy (Vibrational spectroscopy) is used in industry and chemistry for characterization and identification of molecules. Since an IR spectrum is the "fingerprint" of each molecule IR is used to characterize substances.[35,36]
- Fourier Transform infrared spectroscopy of the whole cells is a valuable/ important tool for the identification of microorganisms and is used, e.g. in medical applications, in strain collections, in the pharmaceutical industry and in drinking water control.[37]
- IR spectroscopy has been highly Advantageous in measuring the degree of polymerization in polymer manufacture.[38]

- IR spectroscopy is important for characterizing and identifying substances and confirming their identity since the IR spectrum is the "fingerprint" of a substance. Therefore, IR also has a forensic purpose and IR spectroscopy is used to analyze substances, such as, drugs, alcohol, fibers, paints and bloods.[39-48]
- IR spectroscopy is being utilized are in stem cell studies,[49] materials science,[50] catalysis,[51] and reaction kinetics.[52] Which explains the applicability and flexibility of this analytical technique.
- Commercial instruments are also available for measuring the fat content of emulsified meat samples by IR spectroscopy. A large number of additional applications have been reported in recent years, particularly with respect to the characterization of fats and oils, including determination of *cis* and *trans* contents, measurement of the degree of unsaturation, and identification of the source of olive oils.[53, 54]

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