



International Journal of Pharmaceutical Sciences and Drug Analysis



E-ISSN: 2788-9254
P-ISSN: 2788-9246
IJPSDA 2025; 5(2): 16-12
www.pharmacyjournal.info
Received: 14-04-2025
Accepted: 19-05-2025

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IR spectroscopy demystified: A beginner's guide to interpretation

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DOI: <https://www.doi.org/10.22271/27889246.2025.v5.i2a.126>

Abstract

A flexible analytical technique for identifying and examining chemical substances based on their molecular vibrations is infrared (IR) spectroscopy. The basic idea is that certain frequencies that correspond to the vibrational modes of chemical bonds are absorbed when infrared light interacts with a sample. The fundamentals of infrared spectroscopy are covered in this overview, along with the key parts of an infrared spectrometer, the many kinds of molecular vibrations seen in spectra, and the connection between vibrational frequencies and molecule structure. It also emphasizes how crucial it is to identify functional groups and analyze infrared spectra for both qualitative and quantitative assessments. Giving students a basic grasp of infrared spectroscopy and its uses in a variety of fields, including biology, chemistry, and environmental science, is the goal.

Keyword: Infrared spectroscopy, fundamental vibration, FTIR, detector

1. Introduction

For material identification, quantitative analysis, and molecular structure research, infrared spectroscopy is a helpful instrument. Chemical, environmental, life, materials, pharmaceutical, and surface sciences, as well as various technical applications, all make use of infrared spectroscopy [1]. NIR, MIR, and Raman spectroscopies analytical features exhibit specific benefits that make them attractive substitutes for more difficult, time-consuming, and conventional analytical techniques [2]. As early as the 1910s, when IR spectroscopy was initially proposed for the examination of biological material, attempts were made to apply IR technology to biological sources. The method was being effectively investigated for the study of biological materials by the late 1940s. In fact, one of the recognized methods for characterizing biomolecules is now infrared spectroscopy [3]. Food analysis has seen a growing and widespread use of spectroscopic techniques, particularly near-infrared (NIR) and mid-infrared (MIR) spectroscopy [4]. The high energy of near-IR is roughly $14000\text{--}4000\text{ cm}^{-1}$. It has the ability to trigger harmonic vibrations. The far-IR has around $400\text{--}10\text{ cm}^{-1}$ of low energy, whereas the mid-IR has about $4000\text{--}400\text{ cm}^{-1}$ [5].

The infrared spectrum has a shorter wavelength and a higher energy than microwave radiation, while the ultraviolet visible spectrum has a higher wavelength and a lower energy. A molecule must be IR active to identify its functional groups. The term "IR active" refers to a molecule having a dipole moment. The molecule absorbs energy when the infrared radiation interacts with the covalent bond of materials that have an electric dipole, and the bond begins oscillating back and forth. Consequently, infrared radiation should be absorbed by the oscillation that changed the molecule's net dipole moment [6]. An interferometer, an infrared microscope, and an array detector are all used in Fourier transform infrared spectroscopic imaging, or FT-IR imaging [7]. The goal of this research is to examine the uses of functional near-infrared spectroscopy (fNIRS) for diseased populations in the healthcare business. MIR spectroscopy has been widely used over the last few decades [8]. To evaluate complicated structures as soil components, the MIR can determine the type of molecular motions, bonds, or functional groups present in the sample because each frequency correlates to a specific amount of energy and a particular molecular motion (such as stretching, bending, etc.) [9].

Today's elderly population suffers from a range of neurological and psychological conditions [10]. A variety of NIRS instruments have been produced over the last 30 years due to advancements in technology [11]. It is evident that using IR spectroscopy in routine clinical procedures has the potential to revolutionize gynecological cancer diagnostics [12].

2. The discovery of IR

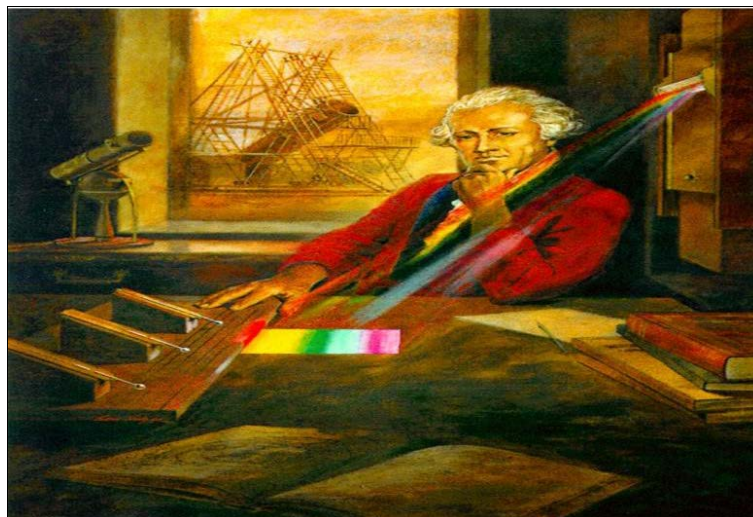


Fig 1: F.W. Herschel and the Discovery of Infrared Radiation ^[13].

In 1800, F.W. Herschel made the initial discovery of infrared (IR) radiation. There are three distinct types for infrared radiation: far-infrared (FIR), mid-infrared (MIR), and near-infrared (NIR). The electromagnetic spectrum's NIR, MIR, and FIR regions are located between 0.8 and 2.5 mm, 2.5 and 25 mm, and 25 and 100 mm, respectively ^[14]. Radiation outside of the visible red light was found by William Herschel in the 1800s. However, the near infrared (NIR) region was not thought to be relevant for spectroscopy before World War II ^[15]. Even Herschel could not have predicted that this was light, ignoring the fact that it was a significant scientific discovery. Many scientists, including Herschel's son, investigated the "heat ray" when it was discovered. Ampere verified in 1835 that the "heat ray" was invisible light with longer wavelengths than visible light. "Infrared" (IR) is the name given to the light with the longer wavelength. Maxwell established in 1864 that visible, infrared, and ultraviolet (UV) light are all electromagnetic waves. Hertz presented the experimental proof of light's electromagnetic properties in 1888 ^[16]. Vibrational spectroscopy has grown in importance as an analytical tool in study since 1905, when Coblentz obtained the first infrared spectrum. About a month after Sugano's discovery, in 1983, low-resolution spectrograms of V1143 Ori revealed a spectrum "dominated by very strong emission lines" ^[18, 17].

3. Principle of IR Spectroscopy

Three methods of measuring the interaction of infrared light with molecules absorption, emission, and reflection are an essential component of infrared spectroscopy ^[19]. Its molecules absorb photons with energies equal to the difference between two of their quantized vibrational energy levels and experience a net change in dipole moment (note: bonds that do not have an electrical dipole that is changing at the same frequency as the incoming radiation do not absorb energy). IR spectrometers can be used to measure these energy absorptions ^[20]. Using the NIR method to investigate the chemical components of wood is a common spectroscopic topic ^[21]. Numerous substances have been exposed to both quantitative and qualitative examinations using these absorption bands, and the identification and attribution of these bands to particular chemical groups

provides particular information about the product under investigation ^[22]. NIR spectroscopy, along with infrared (IR) and Raman, is an area of molecular vibrational spectroscopy, which examines the vibrational (or internal) degrees of freedom (DOFs) of molecules by interaction with electromagnetic radiation ^[23].

The following are the three Infra-red regions of interest in the electromagnetic spectrum, measured in micrometres (μm): ^[3, 24, 25]

- **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 to 300 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})

4. Fundamental of Vibration

The study of the relationship between matter and infrared light is known as IR spectroscopy. The basic idea is that when infrared light passes through a sample, its energy may cause some chemical bonds to move mechanically. This phenomenon is known as infrared absorption ^[26].

Below are examples of the several kinds of vibrations ^[27]. Vibrational or infrared spectroscopy uses diagnostic vibrational modes to provide smooth information about the three-dimensional structure of molecules as well as intra- and intermolecular interactions ^[28]. The normal vibration estimations are predicated on an accurate potential field and structure ^[29].

4.1 Vibrations come in two major types

Vibrations that stretch, characterized by bending vibrations, where the angle between two bonds changes, and a change in the interatomic distance between two atoms along the bond's axis. Rocking, scissoring, wagging, and twisting motions are the several types of bending vibrations ^[20].

Equally spaced vibrational energy levels are predicted by quantum-mechanical treatment^[30]. Conventionally, the vibrations are categorized based on their degrees of symmetry and wavenumber. Accordingly, ν_1 is the name given to the symmetric stretching vibration of water that exhibits the maximum frequency (3652 cm^{-1}). At 1590 cm^{-1} , the symmetric bending vibration is known as ν_2 , while at 3755 cm^{-1} , the antisymmetric bending vibration is known as ν_3 . These three frequencies are fundamental frequencies that are present in water's infrared spectrum^[22].

5. Instrumentation

The radiation source, monochromator and detector are the three fundamental parts of an infrared spectrometer^[21]. Research that was first developed to consolidate NIR spectroscopy as an analytical tool was made possible by the use of dispersive equipment based on diffraction gratings in the early days of NIR spectroscopy^[31].

5.1 The standard classical dispersive IR spectrophotometers^[3].

- There are four primary components that make up the basic classical dispersive infrared instrumentation.
An irradiating light source
- A prism, diffraction grating, or dispersion element
- A detector
- The optical system of mirrors method, which records the infrared spectrum by passing an IR light beam from an IR source through a sample. A monochromator aids in directing the process. Which switches wavelengths over time and is positioned before the IR detector and after the IR source^[5].

5.2 Fourier Transform spectrophotometers

The MIR region ($4000\text{--}400\text{ cm}^{-1}$) of the electromagnetic spectrum is the focus of FT-IR spectroscopy. MIR gives information on the basic vibration of the chemical functional groups, from the stable vibrational state to the first excited vibrational state^[14]. Increased sensitivity, significantly faster spectrum capture, and significantly higher energy throughput are the primary benefits of Fourier-transform instruments^[32]. One option for evolved gas analysis was the coupling of Fourier transform-infrared spectroscopy (FT-IR) to thermal analysers, such as thermogravimetry equipment^[33].

The following are the key components of an FT-IR spectrometer^{[3][6]}.

- 5.2.1. Infrared light source
- 5.2.2. Interferometer
- 5.2.3. Half-silvered mirror (beam splitter)
- 5.2.4. Moving mirror
- 5.2.5. A fixed mirror
- 5.2.6. Laser
- 5.2.7. Detector

5.2.1 Infrared light source

Fourier transform spectrometers and dispersive spectrometers use the same kinds of radiation sources. To improve power and stability, FTIR instruments often use a water-cooled supply^[27]. A silicon carbide (GlobarTM) source emits as much radiant power as any other traditional infrared source for measurements between about 400 and 100 cm^{-1} . The emissivity of Nernst glowers decreases to the point where they are not very useful as far-infrared sources,

even if they can be operated at greater temperatures than Globars. This is because they become very transparent below 200 cm^{-1} . The emissivity of a GlobarTM similarly decreases below 100 cm^{-1} , and a high-pressure mercury lamp is typically used for measurements between 50 cm^{-1} and the beginning of the microwave area of the spectrum^[34]. When infrared light sources frequently produced light with wavelengths between 800 and 900 nm at rates of 30 to 60 pulses per minute, the greatest reactions were observed^[35].

5.2.2 Interferometer

The interferometer's ability to produce interference patterns of samples that might then be transformed into spectra was acknowledged by Michelson and Lord Rayleigh later in the nineteenth century (Rayleigh, 1892). In the decades that followed, FTIR spectroscopy was developed by repurposing the use of interferometers to produce spectra. It is accurate to state that the development of interferometers transformed infrared spectroscopy. The majority of contemporary interferometer designs are still based on Michelson's original interferometer, despite the development and commercialization of numerous alternative designs^[22].

5.2.3 Half-silvered mirror (beam splitter)

The poor effectiveness of Mylar beam splitter was the main barrier to using Michelson interferometers for far-infrared spectroscopy^[34].

5.2.4. Moving mirror

The flat, highly reflecting surface of the moving mirror is supported by air bearings, which allow fast movement (movements occur once per millisecond). The beam splitter is only a few millimeters distant from the rotating mirror^[3].

5.2.5 A Fixed Mirror

A flat, highly reflecting surface makes up the fixed mirror^[3]

5.2.6 Laser: When the velocity of the moving mirror is set to generate a laser frequency of 5 kHz (the most common velocity for operation with a pyroelectric detector), the "glitch" produced by the 60-Hz interference is at -190 cm^{-1} which is approximately in the middle of the far-infrared region^[34].

5.2.7 Detector

It is necessary to determine the sensitivity of the detectors, which convert light or heat energy into an electrical signal. The FTIR spectrophotometer uses thermal and photodetectors as its two main detector types^[36]. Sensitive detection that works in tandem with the light source is essential for examining trace levels of analytes or components that have naturally low absorption cross-sections. Therefore, high quantum efficiency and, preferably, adjustable absorption band gaps are necessary for detector materials functioning in the MIR^[37].

6. Types of detectors

6.1 Photon Detectors

A type of integrated photodiode with an integrated transimpedance amplifier is called a photoelectric detector. The amplifier may operate on a single or dual power source and is suitable for battery-operated instruments^[38]. The central component of a number of gadgets that impact our

everyday lives are photodetectors (PDs), the centre of photoelectric conversion [39]. The IR spectrometer's most sensitive detectors are semiconductors, which use the intrinsic photoelectric effect to transform photon energy into electrical charge. Quantum detectors, such as liquid nitrogen cooled HgCdTe (MCT), InSb, and In (Ga) detectors, are made to function well in FTIR spectrometers [6].

6.2. Thermal detectors

For many years, research and development has focused on the application of thermal detectors for infrared imaging. Thermal detectors have, however, been used in commercial and military systems far less frequently than photon detectors. This discrepancy results from the general assumption that thermal detectors are slower and less sensitive than photon detectors [13].

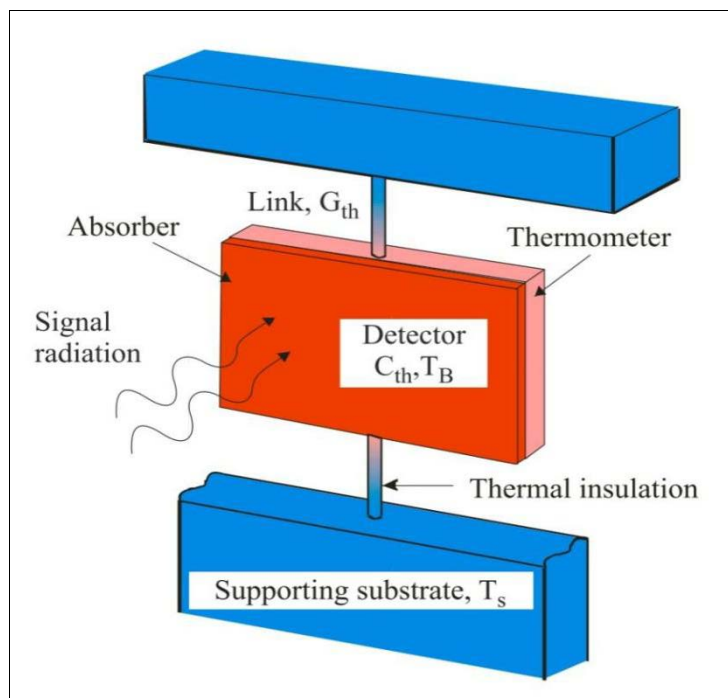


Fig 2: Thermal detector [13].

6.3 Pyroelectric Detectors

The ability to measure only modulated light is a unique benefit of interferometric systems that employ pyroelectric detectors. Consequently, an interferometric signal is not produced by other possible signals, such as heated instrument components that may be radiating onto the detector. These sources of error simply appear as a DC offset term and are not measured; therefore, they do not need to be taken into account in the instrument calibration (apart from the temperature change the environment may impact on the detector itself). This is because pyroelectric DLaTGS detectors only generate an electrical signal when the incident energy is changing levels [40].

6.4 Bolometers

Bolometers are typically thin, blackened slabs or flakes with extremely temperature-dependent impedance. There are several types of bolometers. The most widely used bolometers are semiconductor, thermistor, and metal. The superconducting bolometer is a fourth kind. This bolometer uses a conductivity transition, meaning that across the transition temperature range, the resistance varies significantly [13].

7. Sample Preparation:

These methods include sample preparation and hand sampling processes [41]. Compared to IR spectroscopy, measurements in the transmission or diffuse reflection modes without sample pre-treatment are more practical. In comparison Raman, which works well for annualizing wet

materials [23]. Sample preparation is crucial for IR spectra analysis, which involves placing the sample in a holder or cell. Because glass and plastic materials strongly absorb infrared light over the whole infrared spectrum, it is quite troublesome. Ionic substances like KBr or NaCl are used to build the cell [6].

7.1 As a Solid

An implied consequence of using isolated molecules to calculate infrared spectra is that, as an approximation for solid materials, the spectra are determined only by absorption rather than scattering [42]. To prevent scattering effects, the sample should be ground fine enough that the particle sizes are less than the radiation's wavelength, which is usually $2\ \mu\text{m}$ [20].

Since only a very tiny portion of the incoming radiation reaches the detector, stray light is a serious problem when employing transmittance via solid materials [31]. It is the most often used option for solid samples because of this characteristic as well as the fact that it does not absorb infrared light in the mid-IR band ($4000\text{--}400\ \text{cm}^{-1}$) [20]. Pellets can be substituted with mulls. To produce a two-phase mixture with a toothpaste-like consistency, the sample (1-5 mg) is mashed with 1-2 drops of a mulling agent. A thin film is created by pressing this mull between two IR-transmitting plates [27].

7.2 Liquid Samples

Liquid samples, such as calcium fluoride, potassium bromide, or sodium chloride, are sandwiched between two

salt plates in this instance. To prevent lines from being added to the spectra, the plates are typically transparent to infrared light ^[5]. Holding the plates by their edges helps prevent moisture from fingers causing damage ^[31].

8. Maintenance of Infrared Instrument

For an infrared spectrometer to be durable and available when needed, maintenance is necessary. Most of the time, the lack of a standby power stabilizer to prevent power failure hinders availability when needed. In addition, the instrument is typically exposed. Covering them will keep dust off them and ensure a positive outcome ^[5].

9. Applications of Infrared Spectroscopy

Vibrational spectroscopy has emerged as a crucial method for studying functional materials and conducting chemical and biological research ^[43]. The requirements for superior quality ^[44]. Numerous books and articles have examined the use of Raman, mid-infrared, and near-infrared (NIR) spectroscopies ^[2]. The qualitative and quantitative information provided by FT-IR spectroscopy offers unparalleled capabilities for a wide range of applications in the characterization of biological and dietary samples ^[14].

9.1 Purity Determination

The problem of values greater than 0.5% from elemental analyser samples is opening the door for infrared to be taken into consideration for sample purity assessment. Three distinct approaches the old method, the new method, and lack of reference can be seen in the use of the infrared technology to test materials for purity determination ^[5].

9.2 Adulteration detection in edible oils

The practice of partially substituting inferior oils for expensive ones is known as adulteration of edible oils. Due to their identical look, physical characteristics, and chemical makeup, traditional procedures for detecting adulteration are unsuccessful. Conversely, FT-IR has become a crucial analytical technique for identifying adulteration in food oils. An overview of the uses of FTIR in edible oil adulteration detection ^[14].

9.3 Determination of fat content

The primary ingredients of agro-food and feed products are frequently identified using infrared methods. For example, the fat content of consumables like butter, milk, wheat, or meat products, feedstuffs like fish meal or pet food, and agricultural goods like cereals may all be established using NIR spectroscopy ^[2].

9.4 Proteins Study

Protein structure has been extensively studied using infrared (IR) spectroscopy ^[45]. After astronomer Sir William Herschel discovered infrared radiation in sunlight around 1800, infrared spectroscopy was born. In 1905, William Coblentz provided a description of the infrared spectra of many samples. With the development of the double-beam dispersive infrared spectrometer following World War II, infrared spectroscopy became a standard analytical tool for researchers and was used in a variety of scientific domains, including the characterisation of protein molecules ^[6].

9.5 Bacterial Analysis

It's critical to quickly and accurately identify, separate and

quantify bacteria in a variety of matrices, including meals. Due to advancements in quick detection, microbe enumeration, and epidemiological capacities in industrialized nations, the number of reported instances of foodborne disease and other bacteria-related diseases has increased significantly in recent years (FDA 2009). Our capacity to identify and track down food and waterborne disease pathogens in the environment is partly due to the use of improved analytical techniques to study the phenotypic and genotypic characteristics of microorganisms. Recent genetic techniques, namely the examination of 16S ribosomal deoxyribonucleic acid (DNA) or 16S ribosomal ribonucleic acid (RNA), have been dubbed the "gold standard" for bacterial identification in comparison to the conventional morphological and biochemical testing. However, these genetic techniques have disadvantages particularly that they take a lot of time and ask for costly reagents and consumables ^[46].

9.6 Clinical applications

In addition to making absolute measurements of cerebral blood flow and volume, NIRS may be utilized as a continuous monitor of changes in cerebral oxygenation and blood volume by tracking variations in HbO₂ and HbT concentrations ^[47].

9.7 Miscellaneous Applications of FTIR Spectroscopy:

FTIR is a crucial instrument for detecting a wide variety of materials in many sectors. Resins, adhesives, paints, coatings, polymers, metal oxides, and several medications for example, may all be examined using this method. FTIR, for example, may be used to evaluate and identify a variety of natural samples, including gummy materials, solids, liquids, and solutions. This approach allows us to identify rubbers and filled rubbers, and FTIR allows us to assess the degrees of crystallinity in polymers, such as low-density polyethylene (LDPE) and high-density polyethylene (HDPE). Comparative chain lengths in organic compounds may also be distinguished using this technique. For the headspace analysis or environmental monitoring procedure, we may also use a gas cell to evaluate the gaseous samples ^[6]. Real-time measurements of cerebral oxygenation may be obtained using near-IR spectroscopy (NIRS), which makes it particularly appropriate for assessments in all new-borns ^[48].

NIR technology in conjunction with multivariate data analysis allows for the classification of food varieties and traceability in manufacturing history based on the chemical components and physical characteristics of muscle foods. In a broad range of muscular food items, they can also measure quality attributes including palatability and physical and chemical characteristics ^[49]. Using light from the mid-infrared spectrum, Fourier-transform mid-infrared spectroscopy scans milk samples to identify the presence of certain chemical linkages ^[50].

Milk is one of the most attractive application areas for mid-infrared spectroscopy (MIRS), one of the most pertinent technologies in the dairy sector for forecasting the technical and chemical characteristics of various food matrices ^[51].

9.8 Future

It is anticipated that computation techniques would advance to become more precise, quick, simple, and affordable. The advances in anharmonic quantum chemical computations in

particular are not enough. Applications of machine learning, artificial intelligence, and big data analysis are crucial for analytical reasons ^[16].

Future advancements in infrared spectrometry are difficult to predict, but dispersive spectrometry which combines a grating with a planar array detector may see a resurgence. Since it doesn't require a mechanism to move an optical component, such a spectrometer should be portable and compact. Spectrometers of this type are currently being manufactured and might be a useful instrument for real-world analysis in a number of domains. Finally, the development of a broadly adjustable infrared laser that is simple to use might lead to the development of an entirely new kind of spectroscopy ^[1].

Recent advancements in NIR instruments are significant. Those in PAT and NIR imaging technologies are both incredibly quick and appealing. NIR imaging applications should gain popularity, especially those that make use of portable equipment. Structure investigations, physical and chemical characterizations, quality evaluation, and field growth monitoring are all possible with NIR imaging ^[52].

10. Conclusion

Chemistry, biology, environmental research, and industrial applications are just a few of the fields in which infrared spectroscopy has shown itself to be a flexible and essential analytical instrument. It has become a crucial method for both qualitative and quantitative investigations because to its capacity to offer comprehensive molecular insights through vibrational modes and functional group identification. Instrumentation advancements like Fourier Transform Infrared (FT-IR) spectrometers and the combination of methods like Mid-Infrared (MIR) and Near-Infrared (NIR) spectroscopy have increased its usefulness. Its use in anything from bacterial identification and quality control in the oil sector to protein analysis and food adulteration detection highlights its importance. Various obstacles, guaranteeing that infrared spectrum. Further improving spectrum analysis's accuracy and usability is the integration of artificial intelligence, machine learning, and quantum chemical computations. A promising future is indicated by the development of tunable infrared lasers, advancements in small spectrometer designs, and the possible resurgence of dispersive spectrometry. Although there are still obstacles to overcome, such as the difficulty of calibration and the requirement for specialized knowledge, further research and technical developments should make infrared spectroscopy a mainstay of scientific study and industrial operations for many years to come.

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