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Infrared spectrum of organic compounds: A review

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Abstract
Infrared radiation (IR; or mid-infrared, miR; 4000-400 cm-1; 2500-25000 nm) has become one of the most powerful tools in the analysis process of chemicals. Due to its high molecular peculiarities, application to a wide range of samples, rapid measurement and unambiguity, infrared spectroscopy forms a powerful approach to clarifying qualitative and quantitative information from various types of biological and chemical substances. For these reasons, it has become a bioanalytical technique with diverse applications. This work aims to be a comprehensive and critical review of recent achievements in the field of biomolecular infrared spectroscopy and analysis.

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1. Introduction

Furthermore, the technique can be cheap and is considered the most important method of routine used in laboratories of chemical analysis, including those that count with equipment of high resolution (Ivleva, 2021) [25].

An extensive production of spectroscopic data is found in the literature, and the method can be applied to practically all types of organic compounds. The experimental practice is simple and allows us to make use of this data in a qualitative or quantitative form in optimizations of reaction conditions (documentation of formation intermediates or a phase selector of reagents), in the characterization of a final product of a chemical reaction (demonstration of purity, presence of impurities or isomeric forms), and to control atomic reactivity (bonding perturbations caused either by the environment that originates the change in the conditions, or by mutation-induced changes in the active site of biological macromolecules), among others. (Tanykova *et al*.2021) [72] (Johnson *et al*., 2023) [28].

One of the main characteristics of chemical bonds is the absorption in the infrared spectrum. The importance of the latter was recognized and reaffirmed many times in the literature, demonstrating that the experimental study of vibrations in the infrared region allows relevant structural information (Dereka *et al*.2021) [13] (Guerrero-Pérez and Patience 2020) [20].

With the increasing interest in organic compounds in the chemical and biochemical fields, the necessity of knowledge of the supermarkets and possible interactions that can happen between them in diverse situations becomes more and more necessary. In this regard, we can find, for example, the pharmaceutical industry, the industry of insulator materials, materials for fiber optics, etc. (Mei *et al*.2020) [40] (Weisskopf *et al*., 2021) [81].

1.1. Principles of Infrared Spectroscopy

The family of normal vibrations is divided into two classes. One is obtained if the position vector of the atoms in the system is represented by a distance matrix and constant of the system only hold. These are the so-called normal modes of a linear molecule. The other class is obtained regardless of any geometrical consideration. Their wave numbers correspond to the non-zero EIchally-strierent constants associated with the force constants. Each of these wave numbers corresponds to the frequency associated with a wavelength or a partial vibration of the atoms in the molecule. The vibration of one or more of these atoms can, however, produce a dipole moment change along its path and therefore create an absorbable IR radiation. This is why the fundamentalaires of vibrations are called infrared frequencies, or simply infrared-active fundamentals. The vast majority of the normal vibrations in a molecule are, however, infrared-inactive. The strictly forbidden transitions occur only if the vibration

creates a partial charge separation in the atoms in the molecule. (Cui & Terentjev, 2020) [11] (Ji *et al*., 2021) [26].

To understand the infrared (IR) spectrum of a molecule, one must first look at its atomic structure and how a vibration of the molecule can produce an absorption. The mechanism behind IR absorption is a harmonic vibration of the molecule. Vibration can indeed take place in the molecule if at least two or more atoms are assumed to be radially bound to a center of mass. A system of m point masses can, however, have only 3n-6 independent vibrations. It is also possible to have free rotations around the ωi-vectors that are some of the 3n-6 independent normal coordinates of the molecule, and this increases the degree of freedom of the system. The number of normal vibration modes of the molecule increases with the complexity of the atomic structure. (Campanella *et al*., 2021) [9] (Yi *et al*., 2020) [84] (Lemmens *et al*.2020) [31].

1.2. Instrumentation and Techniques

In the development of IR, after the radiation advances to an atom or molecule, the energy level of both of them will change, resulting in a change of both energy forms. However, several criteria need to be considered before IR light can be absorbed. Generally, an IR instrument is a spectrometer used to detect the IR radiation that the substance can absorb. The SM instrument is an example of a unique instrument that measures the IR signal of any substance at the IR part of the EM spectrum. Specifically designed for interaction, semiconductors are used as the material for their construction, i.e., Si, Ge, Hg1 x Znx Te, and BaF2 (these are only some of the materials that can be used in the construction of SM instruments). (Wang *et al*., 2020) [79] (Phal *et al*., 2021) [53]

Infrared (IR) spectroscopy covers the frequency range from 1 cm-1 to 4000 cm-1. Infrared spectroscopy involves the interaction of infrared radiation with matter and can be used to identify organic and inorganic compounds. The IR part of the electromagnetic spectrum has been divided into: near IR 14000–4000 cm-1, mid IR 4000–400 cm-1, and far IR 400–1 cm-1. Near infrared 14000–4000 cm-1, mid IR 4000–400 cm-1, and far IR 400–1 cm-1. The likely possibility of most organic molecules to have a mode that resonated and hence required them to interact with light in that region. What made these instruments unique was that they only illuminate matter where electromagnetic rotation (microwave) frequencies do not have a wavelength that can interact, enabling electrons to be excited and IR radiation to be absorbed. (Stuart, 2021) [71] (Ozaki, 2021) [50] (Mikhailenko and Barbe 2020) [43].

2. Functional Groups and Infrared Absorption Bands

Several functional groups or types of bonds are traditionally considered in the first classes of Organic Chemistry. The carbonyl group, the precursor of the carbonyl ion, of the carboxyl ion, and of the oxonium ion, was created to covalently link the carbon and oxygen of the formaldehyde and carboxylic groups in their respective alcohols. Covalent bonds between carbon and nitrogen, nitrogen lone pair, and π electrons linked to nitrogen are found in the amine group, almost always covalently linked to some other functional groups. Propensity towards redox reactions and hydrogen bonding, chemical analyses in organic and biological substances may take advantage of the recognition that just a spectrum of infrared absorption can provide. (Deng *et al*.2021) [12] (Cesari *et al*. 2021) [10] (Loh & Aldridge, 2021) [34].

The concept of a functional group is of paramount importance for the prediction and interpretation of the infrared absorption spectra of organic molecules. The infrared spectra of an organic substance present a number of absorption bands due to energy absorptions of several types. In organic compounds dissolved in a solvent or in the vapor state, these absorptions occur in absorption intervals varying from about 3800 to 650 cm^{-1} of wave length. Popularly, the interpretation of the absorptions focuses on the aforementioned absorption intervals, regrouping the vibrations that lead to each absorption band into eight regions. The atoms contributing to the vibrations that lead to absorption into one of these intervals are denominated functional groups and the interval itself is known as the spectral region attributed to the absorption caused by the existence of these atoms, also known as the type of bond. (Jin *et al*.2020) [27].

2.1. Alkanes and Alkenes

The fluorinated alkenes are those where one or both hydrogen atoms of the alkene are replaced by fluorine. They have a stronger C-F bond with almost double bond strength, and the bands due to the C-F stretches, which are observed within the 1300-1150 cm range, are relatively stronger than those due to the C-H stretching vibration. In the alkyne molecules, there is a presence of a carbon-carbon triple bond. (Yan *et al*., 2021) [82] (Zhou *et al*.2021) [86] (Zhu *et al*.2021) [87].

The C-H stretching vibrations absorb in the 3000-2800 cm zone, while the C-H deformation vibrations are seen below 1500 cm. Primary and especially the secondary alkyl groups are responsible for most of the bands due to the alkanes in a mixture. Their position also depends on the individual alkyl group. The position of 730-720 cm bands is stable and common to both the liquid film and the solid alkanes. Alkenes are compounds containing a carbon-carbon double bond of C=C type. There is an appearance of a new strong band between 1680 and 1620 cm due to the C=C stretching vibrations. The other characteristic C=C deformation bands are seen between 1670 and 1500 cm at their thumb nail identification. The peak at about 908-905 cm may be somewhat useful to indicate the alkene as well as the side chain structure in some alkenes. (Ahluwalia, 2023)(Volkov *et al*., 2021)(Pejcic *et al*., 2021)

2.2. Alkynes

Almost the same, the bonding pair of the sulfur and extra-substituted E- and Z-selectivity factors are compared.

Compounds with finitely conjugated alkynes: ENU, ethynyl group in 1-(2-(dimethylamino) ethynyl-9-ethenyltioxanthone; EDS, ethynyl group in 1-(2-(dimethylamino)thiophen-5-yl)ethynyl-9-ethenyltioxanthone-4,4-dioxide; and EAS, ethynyl group in 1-(2-(dimethylamino)anenyl)-diselanyl-9-ethenyltioxanthone. (Streckaite *et al*.2020) (Gao & Tykwinski, 2022).

Thioethmerged. The ethylenic Δs is well described for alkenes, but quite poor in the case of alkynes. Two peaks due to the C≡C group are quite attractive to specify it. One peak is often observed at about 2250 cm^-1 in cases of terminal alkynes, and that peak is quite related to terminal alkynes. It is important to distinguish 2250 and 3300 cm^-1, the peak due to terminal alkynes and the broad peak due to alcohols or carboxylic acids, because 3300 cm^-1 is used as a criterion of recognition of biochemical reaction like a fusion of bioactive and functional molecules. (Romei *et al*.2022)(Dong

et al., 2022)

2.3. Aromatics

Several alkyl-substituted aromatics, or more correctly the products of alkylation with formed have been investigated very thoroughly. Some of the basic features of the substituent effect can be summarized. Methyl substituents cause a red shift for the CH stretching vibration by ca. 5 cm-1. The shift is more significant when the methyl group is attached to an ortho- or para-position. Entropy increase in the CH vibration, changes in the absolute intensity and half bandwidth values are correlated to the increase in the red shift. Such correlations do not hold for grafted CH2 groups. However, the CCH stretching vibrations do not exhibit any distinguishable addition artifacts. (Bhattacharyya *et al*.2021)(Saito *et al*., 2022)

Most of the common aromatics, including their alkylated derivatives, which contain radicals, exhibit a sharp CH stretching band at 3000-3060 cm-1, which is red-shifted 10-20 cm-1 in comparison with saturated hydrocarbons. The CH aromatic and CCH stretching vibrations are found from 3100 to 3020 cm-1 and from 1580 to 1450 cm-1, respectively. Most likely, the position and intensity of bands in the aromatic region and particularly the latter two bands are quite characteristic and allow us to identify substitution patterns. (Maurya *et al*., 2023)(Naganandhini *et al*.2021)

2.4. Alcohols and Phenols

The wave number for the phenolic group of phenols in solvents is found to be related to the ionization potential of the solvent. A slight upshifting of the phenolic OH band is also observed upon phenol complex formation with different solutes. The phenolic hydroxyl bond of simple phenols occurs in the range 3700 to 3600 cm-1, the values of low frequency for simple phenols with intermolecular associations are within the ranges 3650 to 3450 cm-1. The highest frequencies are observed for phenolics with strong intramolecular hydrogen bond constraints, while the lowest frequencies are observed for phenolics with weak intramolecular hydrogen bond constraints. (Rajhard *et al*.2021)(Baltacıoğlu *et al*.2021)

The presence of an alcoholic hydroxyl hydrogen-bonded is indicated by a frequency shift of this band of approximately 200–300 cm-1 as compared with the 3600–3000 cm-1 frequency interval, where the band of the free OH group occurs. In the alcohols, the OH stretching frequency is sensitive to hydrogen bond donor and acceptor strength, peak separation, and the structure of the alcohol. Hydroxyl groups in solution may also give bands which correspond to both the free and hydrogen-bonded species. Alcohols may be either primary, R1R2CH(OH), or secondary, RR1CH(OH)R2, in structure (though tertiary alcohols do not exist). These primary alcohols have the strongest hydroxyl stretching mode, while the secondary alcohol has a slightly weaker one. The difference in these frequencies may be 20–40 cm-1. The hydroxyl band has a symmetrical shape and appears between 3700 and 3300 cm-1. However, in mixtures such as phenols, the association of phenol molecules may also involve proton transfer or formation of a true hydrogen bond, thus the phenolic hydroxyl may also appear to some extent as a broad band on the low-frequency side of the aromatic OH stretching. When phenols are dissolved in solvents of lower basicity, the hydroxyl stretching frequency downshifts and the hydrogen bond strength of phenol, defined as indicated previously, increases. Phenolate ions are observed by using solvents of higher basicities. (Mitani *et al*., 2022)(Fu *et al*.2022)

Both simple and complex alcohols give rise to an intense broad hydroxyl band that can be considered characteristic of this functional group. In the case of aliphatic compounds, this band occurs in the 3650–3200 cm-1 frequency range, weakening as the number of structural alterations to the straight chain alcohol increases. The phenomenon is originated by the O-H stretching vibration when it is participated by hydrogen bonding. For this reason, the normal O-H stretching vibration of an alcohol is found at a position noted as free frequencies of the alcohols, thereby being shifted to the low frequencies. This shift is, of course, inversely related to both the bond strength and the intermolecular bond assisted and keeps constant as far as the alcoholic group takes part in the hydrogen bond synthetic series are measured. Studies have been carried out with binary mixtures of one of the alcohols and aliphatic or aromatic compounds, chosen at random, in order to investigate the possibility of using this shift for identifying an alcoholic group in an unknown compound. (Sahana *et al*.2023)(Tsigoias *et al*.2023)(Nandiyanto *et al*.2023)

2.5. Aldehydes and Ketones

The Centers for Carbonyl Bending Vibration in Aldehydes: The range of these bending vibrations takes place with the following values: (a) approximately 1210–1230 cm−1 (lower than that of the azomethine group, <1250 cm−1, and acyclic anhydride <1240 cm−1), (b) slightly drifted as 1250–1300 cm−1, and (c) slightly drifted to higher values, above 1300 cm−1. The difference in alcohol, phenol, enol–π, and pyrrole hydrogen bonded medicating systems is that the center of the hydrogen-deprotated bond and the primary amide of the ROS type are completely mixed, qualitatively discriminated by primary amide and C=O stretching frequencies. The mixture of the O–H bond and the amide bond shows the highest stability of the primary amide hydrogen donor. (Trends, 2023)(Ahluwalia, 2023)

It can be stated that the C=O stretching, COH bending, and CH3 rocking vibrations take place at very definite regions of the infrared spectrum. Consequently, the presence of carbonyl stretching is 1710–1715 cm−1, carbonyl bending is at 1000–1300 cm−1, and CH3 stretching and bending vibrations occur in the ultraviolet (UV) region at 2950–2850 cm−1. CH3 rocking frequencies are between 1330 and 1450 cm−1. In the infrared spectrum of CH3 stretching, the coordination of the substances with the most important H2O stretching vibration (3450–3400 cm−1) is decreased in the presence of a very intense C=O stretching vibration. (Asemani & Rabbani, 2020)(Paladini *et al*.2021)(Rachid *et al*.2020)

2.6. Carboxylic Acids and Derivatives

In the first IR report, discussed the vibrations for carboxylic acids R-COOH. visited the same IR and Raman. Charge transfer was observed for the νasym CO group. But, only a few groups, including and H.J. Bernstein and Zwier's lab at Columbia, are working at this time. They updated the IR and Raman for several-bond dissociation reactions. In the universal pattern, we need to know not only R-COOH but also its derivatives RCOX, RCOOH, RCONH2, CH3COOH, and some compounds with OCH2 bridges such as glycolic acid. First, we made all the R-COOH singly, converted to its

derivatives, and then studied the spectra. At this time we limited our samples to carboxylic acids and the alkyl esters of p-hydroxybenzoic acid, m-fluorobenzoic acid, p-methoxybenzoic acid, p-iodobenzoic acid, and finally, p-chlorobenzoic acid. In the future, we will study other members of this series. Based on the experimental data, all of the ν(CO) was down rather than 1724 cm−1, part of the eacetonide form and other νs, ρ(C centered dot O.C hydrophobe), and O-CH2 dissociation direction rather than ν, R1 = CF3∼CH3∼C∞H. First complexation was detected for the CCF3 symmetric O-C-F.N combination. Other observed effects were an antiresonance between the six-member ring and a methoxy group close to the O-selective patch. (Meyer & Nejad, 2021)(Lang *et al*.2020)(Bedran *et al*.2021)

Carboxylic acids (I) and their derivatives are important as intermediates and final products in the syntheses of many compounds. The general formula R-COOH has always been modified to R-COOR, RCOX, RCOCl, RCONH2, and others. They are recognized easily by groups attached directly or indirectly to the keto group. But, reactivity depends on the various compounds and new methods and techniques are needed. Spectroscopy can be useful to understand stereostructure and reactivity in carboxylic acids and derivatives. Raman and infrared spectra show characteristic bands which shed light on the keto group and would help to associate stereostructure with reactivity in the keto group. (Leech & Lam, 2020) (Pongpamorn *et al*.2021)

2.7. Esters

Sound identification requires more care with the carbocatechol esters, such as ethyl acetate and its hydrolysis product, which both have three alpha-hydrogens R'–CHR–CO–OR. When there is benzene ring resonance stabilization, an ester with two alpha-hydrogens absorbs at about 1730 and 1250 cm–1, and one with only one alpha-hydrogen at about 1750 and 1200 cm–1. In phenyl acetate, interestingly, the analogous hydrogen-bonding energy shift of the ester's alcohol band from 3400 to 3280 cm–1 also prohibits any doubling of the band from the intramolecularly hydrogen-bonded carboxylic acid, and so neither substituent nor hydrogen-bonded dimer is self-evident. (Sakpal *et al*.2024)(Qamar *et al*.2022)

Esters resemble acid halides in general reaction behaviors and are closely related to amides and hydroxamic acids, which we have discussed at length. For example, the reactive ester group (R–CO–OR') gives a strong band at about 1750 cm–1, but only when it is bonded to one oxygen, such as in phenyl carbonodithioate S-phenyl amide. While bonding to both oxygens, as in tetraethyl pyrophosphate P(–O–OPhEt)O, causes a marked energy shift to 1240 cm–1. Also, symmetrical esters (–CO–O–R–CO–) give an appreciably weaker absorbance only when the compound is a solid, such as in ethyl valerate. Likewise, energy shifts to 1745 and 1030 cm–1 will occur when the carbon is replaced by sulfur to form the identical ester functional group. In particular, energy shifts reflecting the relevance of increased periodicity or crystal structure lead to acceptable distinguishability, especially those to values (at IR energy) clearly outside the range of most fingerprint interferences. (Thirunavukkarasu *et al*.2021)(Ban *et al*., 2023)

2.8. Amines

Under this review for the amines, the definitions, isolations, and characteristics of amines are presented firstly, and then the IR spectra assignments of amines are summarized. The application and purpose of this review is to help researchers recognize the functional properties and conduct further determination of the amines from their compounds, creating good use of FTIR spectroscopic techniques, etc., to enable the fast and cheap analyses of samples. (Hu *et al*.2020) (Murugesan *et al*.2020)

The N-H stretching frequencies of primary amines are in the range 3450-3300 cm-1, and for secondary amines, in the range of 3320-3120 cm-1. The second band is generally the most intense primary amine N-H stretching band, whereas in secondary amines, the first N-H stretching band is normally the most intense. The N-H bending vibrations of primary amines range from 1000 cm-1 to 1235 cm-1, and for secondary amines, the range is 995-1010 cm-1. The first N-H bending vibration band is generally more intense than the other one, and its intensity decreases as well as shifts to lower wavenumbers with increasing molecular weight or molecular size. The stretching and bending region of amines can be analyzed by using almost all commercial Fourier transform infrared (FTIR) instruments when O, C, S, etc. decorate the molecule groups. (Ahluwalia, 2023) [1] (Nemeş and Negrea 2023) [48] (Selvakumari *et al*.2021) [67].

3. Quantitative Analysis Using Infrared Spectroscopy

Quantitative analysis using infrared (IR) spectroscopy, due to the weak absorption of organic compounds, is often discouraged. Nevertheless, low concentrations of over 10 ppm can still give strong infrared bands, and a much higher detection limit can be obtained by using an IR cell of long path length. In addition, when the concentration of the functional group increases or when key functional groups do not overlap with other strong IR bands, the concentration information of the functional group might still be able to provide the quantitative analysis. Besides simply preparing sample spikes, another common way is to make the functional group react with reagents that will simplify the IR spectrum. After that, the peaks can be integrated directly. Another method requiring an ANC-800 preparative fractionation apparatus is used to measure the IR absorption spectra of the coating materials of each component in the coating liquid mixture, analyze the characteristic peak of the functional group absorption band, and then calculate the mass percentages of the organic components in each fraction. (Johnson *et al*., 2023) [28] (Valand *et al*.2020) [77] (Zhao *et al*., 2021) [85].

3.1. Beer-Lambert Law

Understanding the spectral properties and how to control the factors that violate or change the basic radiation properties that make the Beer-Lambert law apply with high accuracy for infrared spectral analysis is a key factor to improve the quantitation accuracy of analysis obtained from the FTIR-ATR, which is the primary infrared spectral analysis instrument prevalent today. (Mamouei *et al*. 2021) [36] (Li *et al*.2022) [32].

The dependence of radiation absorption on the population of the vibrational state is the basis for studying the concentration of different states of the vibrational mode of the molecule responsible for the change in the population of the vibrational mode. The Beer-Lambert law is not applied to electronic transitions, but it is nearly temperature independent in the window of wavelength where the spectral distribution of the light allows the change in radiation absorption. This is the reason for developing and discovering the modern instrument

of IR analysis used today. (Mallet *et al*.2021) [35] (Pipa *et al*.2023) [54].

Applying the Beer-Lambert law to the infrared spectrum analysis, two facts must be noticed as follows: - The absorption coefficient is determined by some basic radiation absorption properties like natural lifetime and the strength of the oscillator. - The concentration term, C, also represents the population of the vibrational states of the molecule and the radiation absorption depends on the population of the vibrational state and the oscillator strength of the mode. (Aldhuhaibat *et al*. 2021) [2].

If the absorption coefficient is independent of concentration, we call ζ an absorption coefficient constant. Therefore, if only one absorbing species is present, it defines a relationship between absorption, concentration, thickness of the solid or liquid medium, and radiation properties of the light. (Qasem *et al*.2020) [58].

Let A be the absorption of the light, C is the molecular concentration, ζ is the absorption coefficient, I0 is the incident beam intensity, and It is the transmitted beam intensity after it passes through the medium of thickness L. Then the Beer-Lambert law can be written as $A = \log10(I0/I) = \zeta CL$.

Many radiation absorption phenomena can be explained using the Beer-Lambert law, which is one of the basic laws in the study of radiation absorption. The Beer-Lambert law states that the absorption of a monochromatic beam of light passing through a transparent medium is directly proportional to the thickness and concentration of radiatively absorbing species. (Oshina & Spigulis, 2021) [49].

3.2. Applications in Pharmaceutical Analysis

In the field of pharmaceutical analysis, the use of FTIR for quality analysis and stability studies is increasing. Infrared spectrophotometry allows the identification of functional groups and is applicable for quality control. Furthermore, it is able to discriminate between polymorphs, mainly through the use of the fingerprint region where the interactions of the spectrum are maximized. FTIR in Diffuse Reflectance mode (ICDD database), Differential Scanning Calorimetry-FTIR and Transmission-FTIR spectroscopy were used to corroborate or to complete comments made by the IR method such as thermogram and calorimetric details for the determination of amorphous content. More specifically, the mentioned techniques are also vital to understand drug disordered structures, amorphous compounds with fixed mole ratio and to provide information about drugs and excipients behavior at increasing concentration weight. Otherwise, FTIR is very useful for pharmaceutical formulations providing valuable information about material characteristics such as chemical identification (foreign polluters), crystallinity and integrity regions. (Song *et al*. 2020) [68] (Tiernan *et al*. 2020) [74].

4. Recent Advances in Infrared Spectroscopy

The methods that use the difference of the reflection coefficients or depend on the reflection coefficient value at some specific frequencies are unprecedentedly sensitive to the local mechanical and electrical properties of solids. Evanescent wave techniques are perspective for the investigation of 1 monolayer adsorption, compact small molecules, and mechanical properties of the surface. Near-IR or surface-enhanced IR methods at flow-through cell are unique in protein-ligand studies. The search for the minimal range of the applied IR or Stokes field is prospective for increasing the signal-to-noise ratio and applying the technique to the small amount of poorly absorbing or dichroic materials or I-layer substances. (Hwang, 2021) [24] (Yi *et al*.2023) [83] (Qin *et al*.2021) [59].

In the review, the aspects and achievements of modern IR spectroscopy are under consideration. The historical background is briefly described. The set of achieved data is classified first in accordance with the leading physical phenomenon and energy of incident quanta: absorption, Raman effect. The main part of the review is devoted to the novel and very prospective techniques, such as near-IR and far-IR, as well as surface- and evanescent wave IR spectroscopies, which have become the unique tools for the study of various practical problems. The list of studied systems, including biomaterials, polymers, solutions, and adsorbed compounds, is reviewed. The results are summarized in a table. The IR methods MIEDR and ISSQ, based on the inter subharmonic difference of the IR reflection coefficients, are the most perspective in the study of biological molecules and drugs. Near-IR and far-IR are the unique methods in the study of proteins, lipids, hydroxyapatite, and DNA. (Wang *et al*. 2021) [80].

4.1. Infrared Microscopy

In the middle of the 1980s, the research output of IR microscopy became insufficient. AASW thought that interest in sample preparation methods with IR microprobe might be unfavorable for the development and dissemination of IR microscopy. AASW also recognized that the burden of this method was greater than one might think because the spectrum of an IR microprobe measured directly from a thin slice of a substance was affected by the change of environment from its bulk and that more skill was required to interpret the spectra. However, the ready measurement of a real IR chart of a piece and the spectrum's sharpness persisted as powerful means that could compensate for these disadvantages. Furthermore, the viewpoints and techniques provided the basis for important contributions to the advancement of new relevant fields (namely, liquid crystal polymers, multilayer samples, biomaterials, and forensic medicine) and directions (contribution in the micro destructive analysis methods for minute samples). (Mayerhöfer *et al*. 2021) [39] (Mathurin *et al*. 2022) [37].

Infrared (IR) microscopy, as an enhancement of point measurement, was first reported by K. A. Ruben. The combination of an ordinary optical microscope and an IR spectrometer with an IR-transparent microscope stage allowed the characteristic IR spectrum to be obtained from an amount as small as 30 μg of solid sample. After the introduction of the Infrared Analyscope by Perkin-Elmer Corp., IR microprobe techniques were developed rapidly. In 1971, AASW developed a confocal beam splitter that could reduce the influence of scattered light from the sample when a long slit was used or when the attenuated total reflection mode was adopted. The beam splitter enjoyed great popularity all over the world with continual improvements. (Meyvisch *et al*.2022) [42] (Pirutin *et al*.2023) [55].

4.2. Attenuated Total Reflectance (ATR)

Both simplified handling and the possibility to directly measure the bitter aqueous and other liquor samples are the origin of the wide application of this technique. Also, in comparison with other ATR procedures, the method is

relatively easy to use. With modern automatic recording equipment, it is possible to measure any type of them at any given moment. The important disadvantage of ATR lies in the limited penetration of the radiation (1–2 µm, in some cases even more, and in some cases even less). The second disadvantage is the domination of the measured band intensities by the interferents. (Estupiñán Méndez & Allscher, 2022) [15] (Liu & Kazarian, 2022) [33].

Attenuated total reflectance (ATR) is often the method of choice for solids or liquids. This technique has been available since 1933 and is based on the principle of sharpened total reflection by an internal reflection prism (Fig. 4). This method can also be applied to small sizes of the solid samples, which are held in close contact with the sensitive crystal that is often made from either natural diamond or various artificial crystals (sodium halide-NaCl, cesium iodide-CsI, germanium-Ge, etc.). (Riswahyuli *et al*., 2020) [62] (He *et al*. 2020) [22].

5. Challenges and Limitations in Infrared Spectroscopy

As was mentioned in the introduction of this paper, interpretation of an infrared spectrum is the process of determining which wavenumber (invariant with the path length) absorbs light for the isolated molecule under investigation. The majority of bioorganic-associated modes are grasped by the most general force fields available, principally by molecular mechanics and cis-trans isomerization mainly for canals, to which reason a careful search for these conformations might be a smart strategy. Frequent problems related to assignments respecting these structures are the strategic isomer inclusion and the refined existing force fields, which do not exist in many cases or electronic densities are not correctly parameterized by specialized researchers. Complete literature or database searches are thus usually recommended in some manuals/guidelines prior to doing any assignment of the vibrational modes occurring in the organic molecule under investigation. (Stuart, 2021) [71] (Beć *et al*., 2022) [6].

Incoherent and unidentifiable IR spectra are usually one of the problems encountered by students during their first laboratory sessions, especially in the fourth and fifth year of studies. This is due to the fact that even senior students dealing with the research did not manage to interpret their own peaks, or the assignments were made on these bad quality peaks. This situation - and its resistance to improvement - may cause replication of poor assignments and bad practices from one class to another; consequently, students may initiate plagiarism in the worst case. It is thus important to point out to our colleagues where the common problems encountered with IR spectra are, and students must know how to identify these inconveniences. (Fakayode *et al*.2020) [16] (Spector, 2020) [69].

6. Conclusion and Future Perspectives

It is expected that real-time and early-stage disease diagnosis or monitoring will be ringside results when *in vivo* applications of IR, and especially NIR, technologies are easily and consistently realized. *In vivo* and ex vivo reflectance, transmission, and fluorescence results showed promising potential but are limited to localized applications because it uses invasive probe such as fiber optic. During this course, the class trial spectra of the diseased and found tissues could be collected and classified by IR transmission, which were chopped, and downsized, and miniature IRTAs and algorithm development software could be combined. Similar results were used to define the coupling positions and spray pattern of the active agents in the floor equipment prototype. Upon gradual and improved sensor sensitivity, future sensor objectives are a remote, portable and non-invasive mode of operation. IR-photonic nanostructures have been developed for sensor applications at the transmissive or surface-plasmon mode in the mid-infrared region for efficient, selectivity, and reflectance sensor use for on-the-spot, continuous or periodic leak detection and environmental monitoring.

In conclusion, among the various methods available, FTIR spectroscopy still remains a powerful tool for qualitative, quantitative, and/or comparative analysis of organic compounds. Research studies showed that the fingerprint region and characteristic peaks could be clearly defined, and various methods such as manipulation, preprocessing, classification techniques, as well as data modeling could be implemented for the interpretation of complicated IR signals. Unlike in UV-Vis spectroscopy, there are several SMEs that can be used with FTIR, and ambient conditions such as temperature, humidity, and concentration must not be as precisely controlled as in mass spectrometry. For all these reasons, IR spectroscopy has yielded promising results for *in vivo* and ex vivo tissue analysis both in the laboratory and in the hospital. Despite all its advantages, it is sometimes disadvantageous because sample preparation, homogenization, and transportation problems may occur and result in inaccurate and inconsistent results. Thus, future perspectives of IR spectroscopy on difficult problems or systems are discussed in the context of various applications of tissue analysis (*in vivo* real time and homogeneous or essentially rigorous accuracy), modern artificial intelligence methods, infrared sensors, nanotechnology, and matrix/analytes.

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