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IR Spectroscopy in Qualitative and Quantitative Analysis

Nabeel Othman

Abstract

The infrared technique is one of the oldest techniques; it deals with the frequencies of bond vibration in a molecule. The main uses of this technique are to identify and determine components in various organic or inorganic compounds. In this technique, a part of the incident infrared radiation is absorbed by the molecules of the sample and the other is transmitted. The favorite method of infrared spectroscopy is FTIR (Fourier transform infrared). There have been many developments in using IR technique in qualitative and quantitative analyses, including the first and second derivatives of the infrared spectrum. IR rays do not damage the exposed skin like other rays such as ultraviolet light. It must be mentioned that the IR technique was used in hyphenated techniques (instead of the detector in chromatographic device), for example, after separation by gas chromatography detected by IR. Also, this chapter contains essential information about Raman spectroscopy. Infrared spectroscopy is a technique that has acceptable accuracy and sensitivity to be one of the most important analytical techniques used in the qualitative analysis, and also, it is used in the quantitative estimation of compounds through measuring the transmitted or absorption intensity of the active groups.

Keywords: infrared, Raman, first and second derivatives, qualitative and quantitative estimations, hyphenated techniques

1. Introduction

The introduction included the followings below:

1.1 Infrared spectroscopy

Spectroscopy is the branch of science contracts with learning about the interaction of the radiation of electromagnetic rays with substances.

Electromagnetic Radiation (EMR) is a type of energy that is around us and taking various forms, these types included radio waves, microwaves, infrared, visible light, ultraviolet X-rays, and gamma-rays. Sunlight is also considered a form of EMR, with Vis light only a minor share of the EM spectrum, which covers a wide range of wavelengths. Visible light has high energy compared with IR light [1, 2].

Infrared Spectroscopy (IRS) deals with the frequencies of bond vibration in a molecule. The main use is to identify the functional groups in many samples. The most covalently bonded compounds, whether organic or inorganic compounds, absorb electromagnetic radiation in the region of infrared. This IR region lies between the visible light and the microwaves region. IR radiation mainly considers thermal energy, in covalent bonds it gives stronger vibrations to molecules. Near-IR can be used in direct determination (nondestructively) of protein present in feeds, and this type of IR region is increasingly used in analytical chemistry for quantitative analysis of various compounds [1].

IR can be divided into main three different bands:

1. Near-Infrared (NIR, 0.78~3.0 μm).
2. Mid-Infrared (MIR, 3.0~50.0 μm)
3. Far-Infrared (FIR, 50.0~1000.0 μm) [3].

In UV and Vis. of the spectrum, the unite of wavelength is nanometer (nm), while in the infrared region wavenumbers are used, and cm^{-1} is the unit [2, 4]. The IR spectrum is drawn via a plot of absorbed or transmittance% (T%) against the wave-number (**Figures 1 and 2**).

1.2 Fourier transform infrared spectroscopy (FTIR)

The favorite method of IRS is FTIR (Fourier Transform infrared), in IRS the infrared radiation is passed through the investigated sample. A part of the incident infrared radiation is absorbed by the sample and the other is transmitted. The resulting spectrum represents the absorption molecules. FTIR spectrophotometers have many advantages when compared with the older techniques IR, the FTIR instruments are more accurate, and more sensitive, all frequencies of functional groups are estimated simultaneously compared with an individual estimation of functional groups in IR, and they are fast in performance as was in the case of older IR instruments.

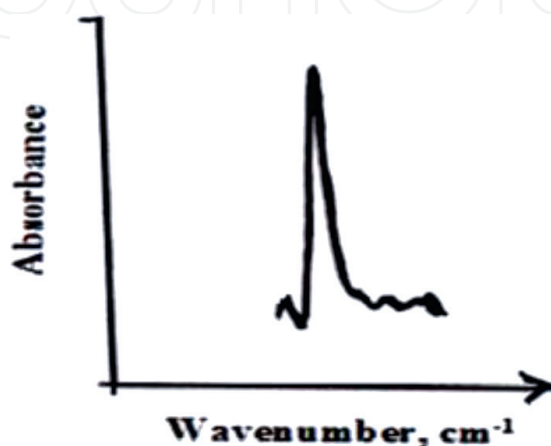


Figure 1.
The spectrum of an absorption mode.

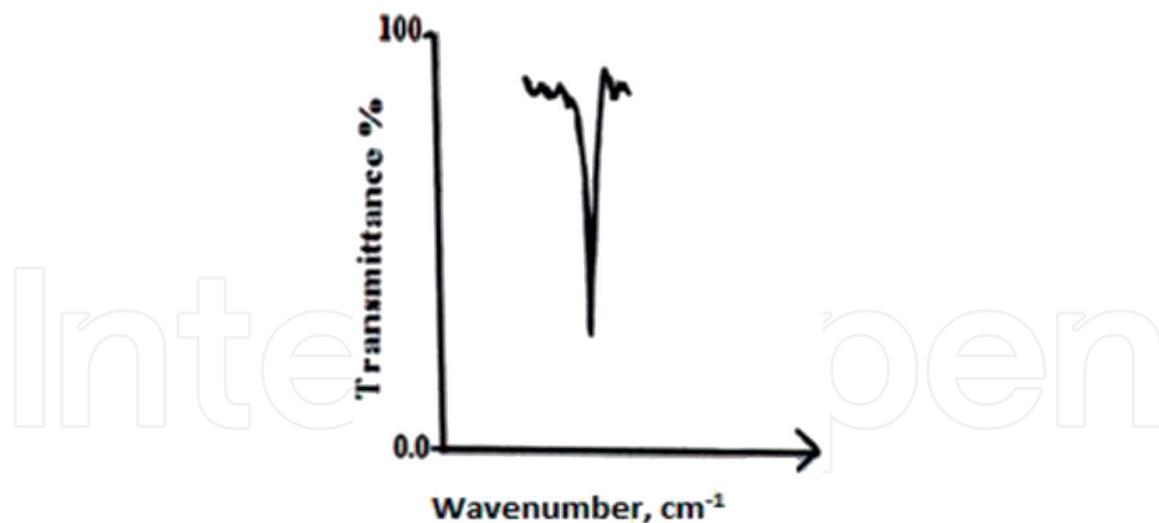


Figure 2.
The spectrum of T% mode.

1.3 Classification of IR bands

Figure 3 shows the main three types of IR bands they classified according to their relative intensities in the IR spectrum.

An increase in the dipole moment according to the increase in the distance between atoms caused an increase in the intensity of the absorption peak [5].

1.4 IR peaks shapes

Two main types of IR band shapes are narrow (thin and pointed) and broad (wide and smoother). An example for broad is the O-H peak in alcohols and carboxylic acids, as shown below in **Figure 4** [5].

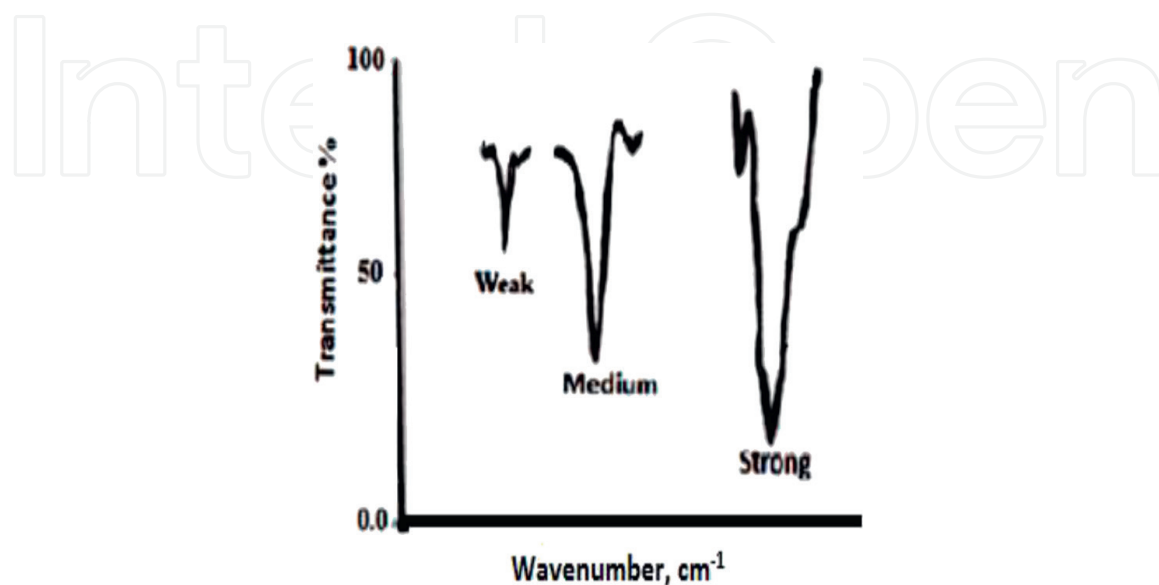


Figure 3.
The types of IR bands according to their relative intensities.

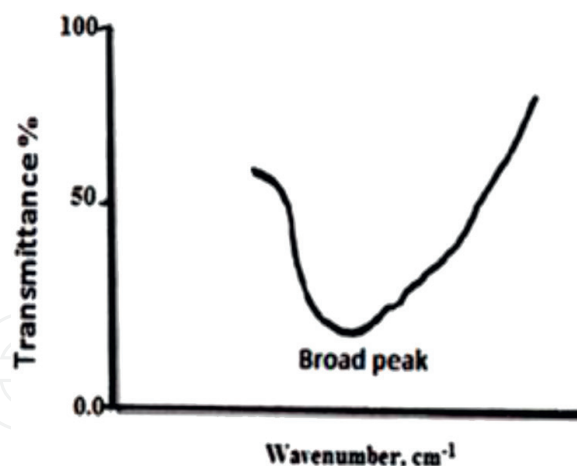


Figure 4.
The broad peak of the hydroxyl group.

1.5 Range of IR absorption

The typical IR absorption range to covalent bonds in molecules is from 600 to 4000 cm^{-1} . The graph shows the regions of the spectrum where the following types of bonds normally absorb. For example, the sharp band around 2200–2400 cm^{-1} would designate the possibility of the presence of a C-N or a C-C triple bond, and other ranges in IR-absorption for other types of bounds.

1.6 Overtones and combination bands

When a molecule absorbed electromagnetic radiation in the IR region, then the molecule is promoted from the ground state to the second, third, or even fourth vibrational excited state. These bands are known as Overtones. The intensity of these bands is very weak. It is helpful in the characterization of aromatic compounds.

When two fundamental vibrational frequencies ($\nu_1 + \nu_2$) in a molecule couple give rise to a new vibrational frequency within the molecule, it is known as a combination band.

1.7 Coupled vibrations

The coupled vibrations are observed in groups such as $-\text{CH}_2$, NH_2 , etc. In these groups, the same atoms are attached to the central atom. When $-\text{CH}_2$ undergoes vibration, vibrational frequencies for the $-\text{CH}_2$ group are observed at 2950 cm^{-1} (asymmetric stretching) and 2860 cm^{-1} (symmetric stretching). A number of molecules contain the same functional group and show a similar peak above 1500 cm^{-1} , but they show a different peak in the fingerprint region. Therefore, we can say that each and every molecule has a unique peak or band, which is observed in the fingerprint region; it is just like the fingerprint of a human.

1.8 The functional groups and fingerprint regions

IR spectrum can be separated mainly into two regions. Most of the functional groups show absorption bands at the wavelength (4000–1200 cm^{-1}) region, which is

called the functional group region. Will the second region from 1200 to 400 cm^{-1} is called the fingerprint region. Fingerprint region is characteristic of the compound as a whole. An example is 2-pentanol and 3-pentanol, the two compounds with similar absorption in the functional group region. However, their fingerprint regions are different, because the two compounds differ, and to accurately identify the compound by comparing the fingerprint area with the fingerprint area of a standard or known sample of this compound [6].

1.9 Factors affecting the vibrational frequency

The main factors affecting the vibrational frequency are listed below:

- A. Conjugation: As the conjugation increases, stretching frequency decreases, because force content decreases due to conjugation.
- B. Inductive effect and resonance effect: Oxygen is more electronegative than nitrogen; therefore, nitrogen easily donates electron or ion pair of nitrogen undergoes delocalization with a C=O bond. Due to delocalization double bond of a C=O change into a partial double bond, therefore force constant decreases, which decreases the C=O stretching frequency.
- C. Hydrogen bonding: Intermolecular hydrogen bonding weakens the O-H bond, thereby shifting the band to a lower frequency. For example, in a clear solution O-H stretching vibration of phenol was observed in the range from 3400 to 3300 cm^{-1} . When the solution is diluted the O-H frequency shifted toward a higher frequency at 3600 cm^{-1} . Whereas in the case of methyl salicylate, intramolecular hydrogen bonding lowers the stretching frequency of O-H at 3200 cm^{-1} . Intramolecular hydrogen bonding does not change its frequency even in a very dilute solution because upon dilution structure of the compound does not change.
- D. Ring strain: As the size of the ring decreases, the vibrational frequency of C=O increases. For example [5]:

Cyclohexanone	Cyclopentanone	Cyclobutanone
1710 cm^{-1}	1745 cm^{-1}	1780 cm^{-1}

An increase in wavenumber of the carbonyl group

1.10 General uses of IR

- One of the most important uses of infrared rays is for military purposes, and one of these uses is in binoculars for night vision in case of difficulty in seeing and observing hostile targets.
- Use in remote sensing, astronomy, and space in planetary detection, radio communications, spectroscopy, and weather forecasting.

- Infrared radiation, which is the oldest technique used in wireless communication, and is used in remote control and TV or recorder, as it is used in calculators, one of disadvantages is the speed offered is slow compared with other wireless technologies.
- Spectroscopy Infrared is a widely used technique to help identify carbon-containing organic compounds. Only the polar molecules are active because they have a permanent dipole moment.

A molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The principle of action is to shine infrared light so that it passes through the organic compound to be identified; absorption occurs for some of the frequencies by the model. The different precise frequencies of absorption can be used to identify the different groups in the unknown compound, which represent specific groups of atoms within the molecules over a period of time. We can identify the compound because each group has an absorption frequency that differs from the other. Using a detector to determine the different absorbance, which records the amount of infrared light that passes through the compound. Some frequencies pass without being fully absorbed, while others will be greatly absorbed due to the special chemical bonds in the molecules. This leads to obtaining a spectrum containing different selves expressing the totals in the model [7].

- Infrared therapy numerous studies have been described that IR can recover the healing of skin wounds, relieve pain, psychiatric disorders, and cardiac stem cells. There are two types of treatments:
 1. Low-level light therapy (LLLT) using light of low power intensity and the effects are not a response to heat but to the light. The popular light sources used are low-power lasers.
 2. Photobiomodulation (PBM) therapy uses non-ionizing types of light sources, including lasers, it is a non-thermal process.

It is now approved that the PBM therapy is an extra accurate and exact term for the therapeutic application of low-level light compared with “LLLT.” A basic principle called the biphasic dose-response included that the large doses of light were found to be less actual than smaller doses. The human skin is reliably exposed to environmental IR radiation, which indirectly or directly stimulates the manufacture of free radicals or reactive oxygen species (ROS). 8~12 μm IR radiation is almost used on full-thickness skin wound therapeutic in rats.

IR light crosses the outer layers of the skin and reaches the tissues of the body. The good thing about using infrared light in therapy is that IR rays do not damage the exposed skin like other rays such as ultraviolet light. An advantage of exposure to IR ray that it improves the circulation of blood and promotes cell regeneration [8–11].

1.11 Raman spectroscopy

Raman scattering firstly was observed by Raman and Krishnan (Indian physicists) in 1928. It is an analytical technique where the scattered light is used to measure the vibrational energy styles of molecules. Raman spectroscopy can offer chemical

structural information, as well as identify the substances to be studied through their characteristic Raman “fingerprint.” Raman spectroscopy extracts the information over the detection of Raman scattering from the investigated sample. After the light is scattered via molecule, the oscillating electromagnetic field of the photon persuades a polarization of the molecular electrons cloud. The photon is transported to the molecule, due to the formation of a very short-lived complex (photon-molecule), and it is called commonly the virtual state. It is not stable and the photon can be re-emitted immediately as scattered light. Approximately 1/10 million photons Raman scattering occurs. The transfer of energy between the scattered photon and molecule and if the molecules gain energy from the photon according to the scattering (an excitation to a higher vibration level) and after that, the scattered photon loses energy, and this phenomena is called Stokes Raman, included an increase in wavelength. If the molecule loses energy by transferring to a lower vibrational level the scattered photon gains energy, inversely, the wavelength decreases, which is called Anti-Stokes Raman. Finally, if most of the molecules are in the ground vibrational level (Boltzmann distribution) and as a result, the Stokes Raman scatter is a continuously more probable process and intense than the anti-Stokes; for this reason, it is approximately always the Stokes Raman scatter used in Raman spectroscopy.

The main differences between IR and Raman scattering are listed in **Table 1**.

As a common rule included that everything that does not seem in the IRS is taken in Raman (bond of molecule either be with Raman active or be IR active but it not with be both). H_2 or CCL_4 doesn't have spectrum in IR; but they give spectra in Raman. Also nitrogen-nitrogen, carbon-carbon, and sulfur-sulfur bonds have a change in polarizability, the incident photons interact with these models, these are examples of bonds that give rise to Raman active spectrum bands, but it is difficult to get spectrum in FTIR [12, 13].

Raman spectroscopy has several applications, such as the identification of materials and identification of different minerals ranging from iron oxy (hydroxides) to rare minerals. Study of the crystallinity, the composition, and uniformity, and also measurement of local temperature and stress. Raman spectroscopy is nondestructive, and the technique has a good resolution [14].

Recently, Raman spectroscopy has been used in blood identification and distinguishing between human and nonhuman blood using a portable Raman spectrometer, which can be used at a crime division, and the bloodstain of human could be

No.	IR	Raman
1.	The principle based on the light absorption.	The principle based on scattering of light.
2.	To appear the spectrum the variation in the polar moment of the molecule to be study must not be equal to zero.	To achieve the Raman spectrum it is not important to have dipole moment or the change of polarity, the bonds of molecular have specific transition energy in which cause a change of polarizability to give a rise to Raman active.
3.	The source of light used depend on the region of electromagnetic spectrum, tungsten filament lamp in Near-infrared, coil of Nichrom wire in Mid- infrared and high pressure mercury-arc lamp in Far infrared.	laser was the excitation source. Almost , solid state lasers types are used in Raman tools with general wavelengths of 532, 785, 830 and 1064 nm.

Table 1.
The main differences between IR and Raman spectroscopy.

distinguished from the non-human ones via using a principal component analysis, and also this analysis is useful for forensic [15].

2. Application

2.1 Qualitative analysis

Example 1:

FTIR spectroscopy is the most reliable tool for identifying bone types and can also be widely used in forensic medicine. Identification of human and non-human skeletal remains unknown to investigators and is of great interest in forensic and anthropological procedures. Especially when the traditional morphological methods for diagnosing and differentiating between these types of bones took a long time. Therefore, the use of infrared spectroscopy and chemical measurement methods to determine the spectral differences between these two types of bones, human, and non-human bones (such as pigs, goats, and cows). The results showed that pig bone is not suspicious of human bone in the study of changes after death because it is more sensitive to environmental conditions than human bone [16].

Example 2:

The micro-FTIR technique was used to characterize the components of a dye painted on the walls of a church in Cyprus. The product was copper-based and the dye contained hydrated copper oxalate. Reflective imaging of the localization sites for the presence of copper and calcium oxalate within the layers of the plate. We conclude from this study that imaging calcium oxalate within different layers of paint samples is very important for studying copper-based pigments in general, and in particular for analyzing pigments used in coatings on different external surfaces [17].

Example 3:

Different heterocyclic compound derivatives have been synthesized via the reaction of ortho-Carboxybenzaldehyde with various aromatic amines (using six amino compounds) to produce Schiff bases (**Figure 5**).

The Schiff bases compounds gave FTIR spectra with an absorption appeared at wavenumber between 1602 and 1614 cm^{-1} this peak belongs to the new C=N group, and also carbonyl of carboxyl group gave absorption appeared at (1741–1766) cm^{-1} , and the absorption at (3306–3462) cm^{-1} for OH group of carboxylic acid. The authors noticed that the carbonyl of aldehyde disappeared, therefore our conclusion that FTIR proves the suggested mechanism and helps to suggest the structure of the product using the absorption of selective functional groups (**Table 2, Figure 6**) [18].

2.2 Quantitative analysis

Example 1:

Fourier transform infrared (FTIR) is used in numerous areas of industrial pharmacy with satisfactory results. The technique's characteristic and nature tolerate unequivocally bright forecasts for quantitative analysis. FTIR is considered a green analytical chemistry technique. It is very easy, fast to work by a temperately knowledgeable technician, covers a large range of spectra to analyze the pharmaceutical formulations, the main advantages are that it has a good resolution and is considered nondestructive

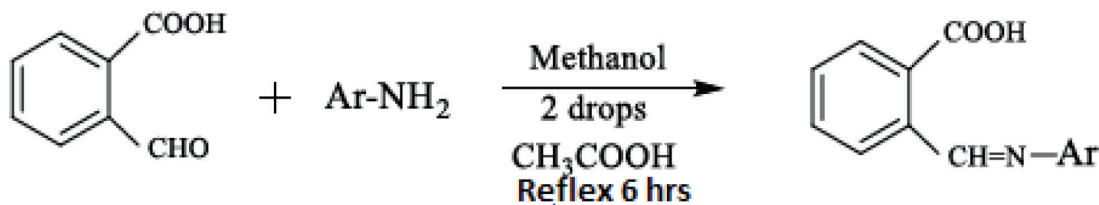


Figure 5.
The reaction of Schiff base preparation.

Compound No.	1	2	3	4	5	6
Ar Structure	<chem>c1ccc(OC)cc1</chem>	<chem>c1ccc(C)cc1</chem>	<chem>c1ccc(C(=O)O)cc1</chem>	<chem>c1ccc(Cl)cc1</chem>	<chem>c1cc[nH]s1</chem>	<chem>c1ccc(cc1)-c2ccccc2</chem>

Table 2.
The aromatic part of amine.

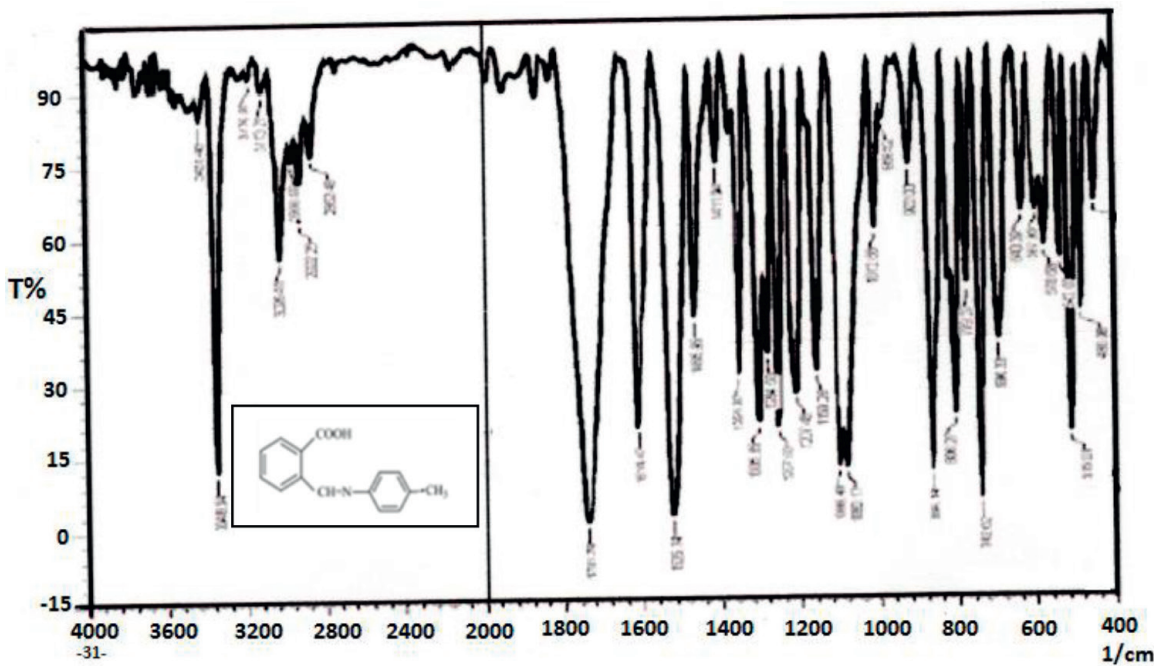


Figure 6.
FTIR spectrum of the product resulted from the reaction of p- toluidine with ortho-carboxybenzaldehyde [from reference 18].

device, and it is also friendly to the environment because in procedure no use of a dangerous organic solvent or any harmful reagents is required for the analysis. Many attempts were suggested for using derivative IR in determination diclofenac sodium in its formulations, but the results indicated that the first derivative spectra are the best technique for determination of diclofenac sodium. The first derivative spectra deleted IR band overlapping with the band understudies and increased sensitivity without any interference of the other band's [19].

Example 2:

Abdulhameed and Nabil (2022) developed a simple and rapid method for the determination of ketoprofen. The method is based on normal and infrared derivative (first derivative) spectroscopy. The results of the study found that the method is accurate

and there is the possibility of its application in quality control to determine ketoprofen in pharmaceutical formulations. Ketoprofen was quantified in a range of estimation from 1000 to 4000 $\mu\text{g/ml}$. This range was based on measuring the T% of the normal spectrum and its first derivative spectrum versus the concentration of ketoprofen in the solution (**Figure 7**). The results prove the validity of the method, as the relative errors were +4.33% and 4.78% and the RSD% values were 1.15% and 1.37%, respectively, and since the values are less than $\pm 5\%$, the method is considered accurate and precise. The research also included the application of the two methods to estimate the compound under study in its different pharmaceutical preparations with a comparison of the results obtained with the results obtained via using high-performance liquid chromatography technique and calculating the t-student and F tests at $P = 0.05$.

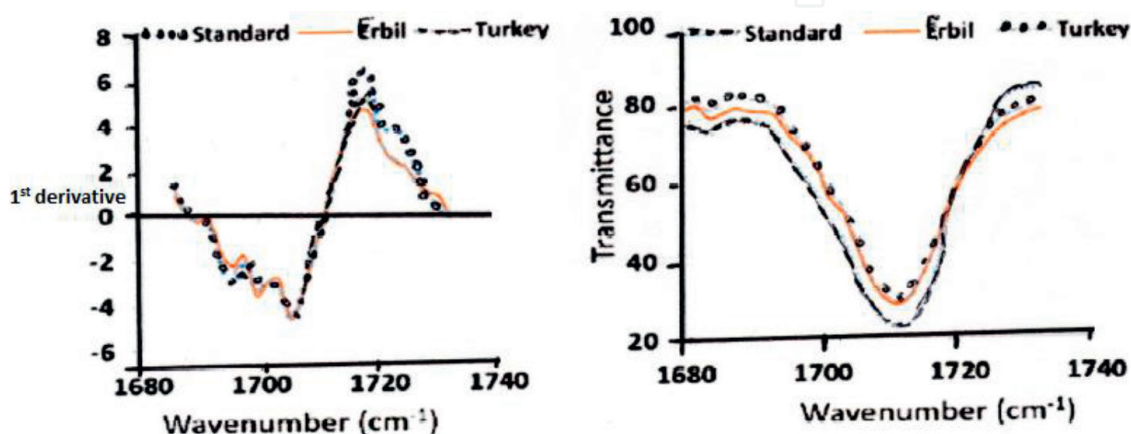


Figure 7.

The first derivative and the normal spectra of two standard ketoprofen from two companies Erbil and Turkey [from reference 20].

Figure 7 shows the derivative spectra of Standard, Erbil, and Turkey ketoprofen solutions, CCl_4 was the solvent used. The two individual peaks of carbonyl groups at 1718 cm^{-1} as a positive peak and at 1705 cm^{-1} as a negative peak, and these peaks gave two calibration curves as various concentrations analyses, there is a reverse proportional relationship between the concentration and the percentage of transmittance(T%) (**Figure 8**) and the other indirect proportion. The reverse proportional relationship is according to decreases in the transmittance% of the solution with an increase in concentration (as shown in **Figure 8**), will in **Figure 9** there is a direct proportion or positive relationship for the first derivative IR according to the peak chosen (peaks of carbonyl groups at 1718 cm^{-1} as a positive peak) [20].

Example 3:

Michael et al (1995) used second derivative IR spectroscopy as a non-destructive tool to assess the purity and structural integrity of various samples such as proteins. Spectroscopy using second derivative infrared is a fast, easy, reproducible, cost-effective, and nondestructive method for assessing the purity of samples of some proteins (water-soluble) extracted from a diversity of sources. The 2ed IR spectra were calm under the lab-proven conditions of aqueous (D_2O) solutions of seven different commercial samples for the same enzyme, porcine pancreatic elastase (2.0–3.8 mg protein/100 μl D_2O , pD = 5.4–9.1)., the amide at the region defined by I ($1700\text{--}1620\text{ cm}^{-1}$) from the IR spectra using the 2ed derivative for each of the seven elastase

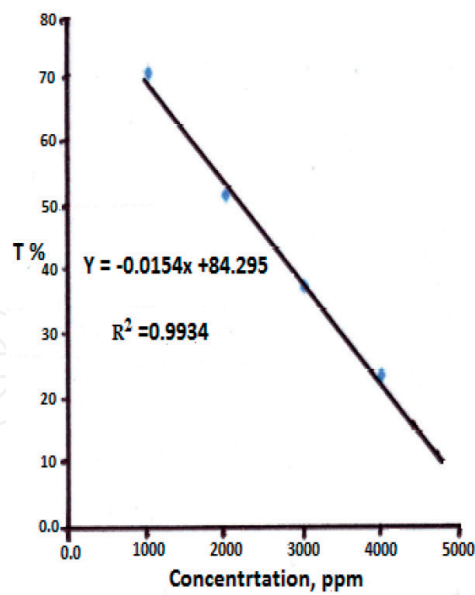


Figure 8.
Calibration curve via normal IR method via first derivative IR.

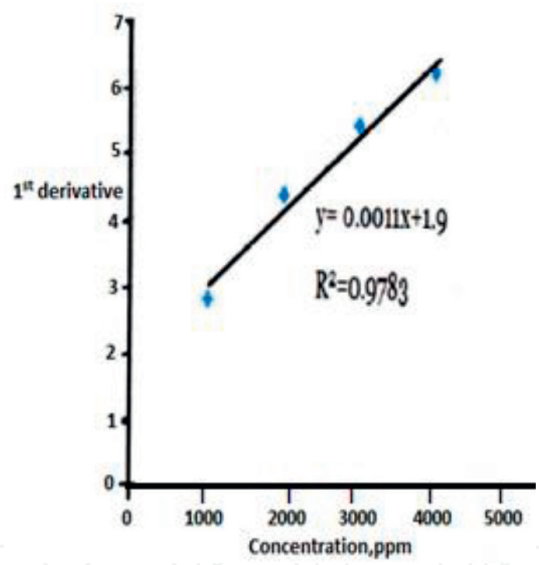


Figure 9.
Calibration curve [from reference 20]. method [from reference 20].

samples displays a characteristic pair of bands: one of them is very weak showing intensities near to 1684 cm^{-1} ; the other is close to 1633 cm^{-1} is moderate to strong. While one of the 7 samples under study shows a striking decrease in the noted density of amide I bands relative to the 1516 cm absorbance, along with the appearance of a new strong band at 1614 cm^{-1} . That the seventh sample is of much lower quality than the other samples and sure contains a quintile of the protein present in the non-native state. In addition, the apparent slight changes in the relative location, and intensity of a section of the separate amide I band among the seven spectra indicate slight differences in the formation of the amount of the peptide support of the samples under study. From the results of two samples, it seems that these few changes, sample purity, and identification of non-protein contaminants [21].

3. Hyphenated techniques

During the past five decades, hyphenated techniques developed rapidly and seemed to dominate many analyzes by introducing them to solve many problems related to complex analyzes, as they were widely used in the pharmaceutical industries from the stage of discovery to human use and the study of its concentration in living body fluids. Accuracy and high sensitivity, and one of the most important disadvantages are the high costs of the devices, and they need maintenance and accurate knowledge while working on the device. Liquid chromatography-mass spectroscopy (HPLC -MS) is one of the most widely applied hyphenated techniques because MS is more compatible with high performance-liquid chromatography (HPLC), and has good sensitivity compared with nuclear magnetic resonance (NMR) or IR. It is also possible to connect infrared spectrometers with thermal analyzers, the methods used by thermal analysis give information about the important temperature to study the physical properties of different materials. However, it is not always possible to obtain information about the chemical changes that occur as a result of changes in temperature through the literature. We note that it is possible to link the thermal analyzer with an infrared spectrometer in order to obtain information about the chemical and physical changes that occur at different and more appropriate temperatures. More suitable is the connection between thermogravimetric analysis (TGA) and FTIR spectroscopy. However, there are limitations in its analytical use. The more advantages of the hyphenated technique include sensitivity, accuracy, speed, and applicability [7, 22].

3.1 Gas chromatography–infrared (GC-IR)

3.1.1 Difficulties in the combination of GC-IR

In the development of joining the IR technique with GC, the speed of the IR must be changed to a high speed so that the unknown components can enter at the same speed from the GC column, in this case, there is a loss of efficacy and the results are not complete. The best way to solve the connecting problem is that the condensation of the gas that comes out from the column and the process is not easy, it must collect all gas eluted because it contains the component and the gas is collected in a cooled part that converts the gas into a liquid because the infrared technique deals with the liquid solutions. Reentry GC technique combined with Fourier transform infrared to give faster and more accurate technique.

3.1.2 Application

Salerno, et al (2020) suggest an accurate method for determination of illicit drugs via gas chromatography–Fourier transform infrared spectroscopy. According to the increasing number of synthetic molecules that can be used in the illicit drug market, correspondingly they require strong separation and sophisticated analytical techniques. It can be achieved by spectroscopic measurements, using firstly a gas chromatography (GC) technique as the separation device. Then the GC is coupled with FTIR to give a powerful tool. In the current study, the efficacy of GC-FTIR, in achieving elucidation of the structure of 1-pentyl-3(1-naphthyl) indole, known as JWH-018, a synthetic cannabinoid whose components have been identified as being a component of non-incense “incense blends” have been demonstrated in the current

study. Moreover, it was quantified with an estimation range on the nano-gram scale. It was obtained in the range of 20–1000 ng, the detection limit and the quantification limit were evaluated to be 4.3 and 14.3 ng, respectively. Finally, the new technique was applied to quantify the activity in the “ST” sample." A real drug seized by law enforcement officers, consisted of a herbal collection containing four types of industrial cannabis belonging to the JWH class. Correct estimation of this type of compound showed that they are chemically similar to each other. The usefulness of the proposed method of analysis using related techniques. It obtains reliable results for complex mixtures of illegal drugs and is a widely applicable alternative to measurement using mass spectrometry [23].

4. Conclusions

Infrared is an important technique and its main application at the beginning to identify polar organic compounds that have a dipole moment. The infrared device has been developed, and we have obtained Fourier transform infrared (FTIR) technique, which is characterized by high accuracy, high sensitivity, and speed of analysis of the compound as a whole. The uses of the technique in the qualitative analysis are identifying the effective groups and the type of bonds between the different atoms constituting the molecule. The technique is used in the quantitative analysis through measurement of absorption or percentage of transmittance (proportional with concentration). The researchers used the first and second derivatives of the infrared spectrum in quantification research and also linked the infrared device with separation devices (for example, GC) to form a new technique called hyphenated techniques, and used in many studies with high sensitivity and precision compared with using each technique individually.

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I must appreciate thanks and gratitude to the researchers who preceded us for their efforts to reach the information that enriches the subject of infrared techniques, which is the topic of the chapter.

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Author details

Nabeel Othman

Chemistry Department, College of Science, University of Mosul, Mosul, Iraq

*Address all correspondence to: nsn20002004@uomosul.edu.iq

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