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Review

INFRARED SPECTROSCOPY APPLICATIONS IN PHARMACY

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ABSTRACT

Infrared (IR) spectroscopy has become an indispensable analytical tool in the field of pharmaceutical science, providing non-destructive, rapid, and precise insight into the structural and physicochemical properties of pharmaceutical compounds. This review presents an examination of the various applications of infrared spectroscopy in the domain of pharmaceuticals, with a particular focus on four key areas: structural studies, solid form characterization, protein analysis, and polymorphism detection. In structural studies, the method is employed to identify functional groups and confirm the molecular structure of active pharmaceutical ingredients (APIs), thereby providing vital information for the design and development of drugs. Solid form studies benefit from the ability of IR to distinguish between crystalline and amorphous phases, thus supporting formulation optimization and stability assessment. In the domain of protein analysis, infrared spectroscopy, notably Fourier transform infrared spectroscopy (FTIR), facilitates the investigation of protein secondary and tertiary structures, which are of considerable significance for biopharmaceuticals and protein-based therapies. Furthermore, infrared spectroscopy is of significant importance in the field of polymorphism studies, a crucial part of the pharmaceutical development process and drug quality assessment, as the occurrence of several polymorphic forms can have a considerable effect on a substance's solubility, bioavailability, and compliance. Overall, IR spectroscopy remains a powerful and versatile technique that continues to evolve to support pharmaceutical research, quality control, and regulatory compliance throughout the drug development process.

KEYWORDS: infrared (IR) spectroscopy, pharmacy, polymorphism, proteins

1. Introduction

Infrared (IR) spectroscopy has a wide range of applications in the pharmaceutical field as a tool for addressing various analytical challenges. Compared to other techniques, it is a relatively established and well-understood method, based on the absorption of infrared radiation by vibrating molecules [1, 2]. The origins of IR spectroscopy date back to the 19th century, when infrared radiation was first discovered [3], with its initial practical application emerging in the early 20th century [4]. The development of Fourier transform techniques in the 1970s, along with increasing computerization of instrumentation during the 1980s, significantly enhanced interest in IR spectroscopy and contributed to its rapid advancement [2]. Infrared measurements are characterized by a broad spectrum of applications, enabling both qualitative and quantitative analysis of solid, liquid, and gaseous substances, as well as the investigation of their physical properties [5]. According to the European Pharmacopoeia, for recording by transmission or absorption, samples can be prepared in the form of gases, liquids, liquids or solids in solution, solids dispersed in a suitable liquid (mull) or in a solid (halide disc). Solids can also be analyzed by recording by diffuse reflectance, triturated with powdered and dried potassium bromide or potassium chloride. The attenuated total reflection (ATR) method allows one to examine liquid and solid samples in direct contact with a crystal. Infrared spectroscopy is referenced in many general texts and over 1200 individual pharmacopoeial monographs as one of the original cornerstone methods in pharmacopoeial testing [6].

One of the fundamental properties of chemical bonds is their ability to vibrate at frequencies characteristic of each specific bond. The vibrational frequencies of these molecules correspond to those within the infrared region of the electromagnetic spectrum [5]. Infrared absorption bands can be categorized into three distinct regions, as presented in Table 1 [2, 5].

Table 1. IR spectral regions and their corresponding wavelength ranges, wavenumbers, and frequencies.

Spectral region	Wavelength (λ) [μm]	Wavenumber (ν) [cm^{-1}]	Frequency [Hz]
near	0.8-2.5	12800-4000	1.2-3.8 $x10^{14}$
mid	2.5-50	4000-400	1.2-12$x10^{13}$
far	50-1000	400-20	6-120$x10^{11}$

In all three infrared regions, absorption occurs for a variety of molecules and chemical bonds, with each region inducing different types of molecular vibrations. For example, radiation in the far-infrared (FIR) region — characterized by the lowest energy — is absorbed primarily by heavy atoms present in inorganic and organometallic compounds, whereas the mid-infrared (MIR) region is predominantly used for the analysis of organic compounds [5]. Fig. 1 illustrates the potential applications of IR spectroscopy in the pharmaceutical field [1].

Near-infrared (NIR) spectroscopy, by contrast, did not gain widespread use until the 1960s. The current increase in interest can be attributed in part to Karl Norris, who recognized its potential for analyzing certain types of food in industrial, agricultural, and quality control applications [7]. The technique has since gained significant acceptance in the pharmaceutical industry — particularly for the analysis and monitoring of dosing and tableting processes, as well as for the examination of samples in suspension, powdered, or solid forms [8].

The near-infrared (NIR) spectral region spans from 780 nm to 2500 nm (from 12,800 cm^{-1} to 4000 cm^{-1}). NIR spectra are dominated by overtones and combination bands of the fundamental vibrations from the mid-infrared region (vibrations of C—H, N—H, O—H, and S—H groups). NIR provides complex chemical and physical information, the extraction of which generally requires appropriate mathematical treatment. The bands in the near-infrared region are much weaker than the corresponding fundamental vibration bands in the mid-infrared region. Because the values of molar absorptivity in the NIR range are low, radiation can penetrate several millimetres into materials, including solids. Many substances are relatively transparent to radiation in this region.

NIR measurements may be performed directly *in situ*, as well as under standard sampling and analytical procedures — they can be conducted off-line, at-line, in-line, and on-line, in accordance with process analytical technologies. In-line monitoring refers to measurements conducted directly within the processing vessel, whereas on-line monitoring denotes continuous control carried out until the endpoint, with the sample flowing through an external conduit. Registration of spectra performed not directly on the production line but in close proximity to it is classified as at-line analysis [5]. On-line *in situ* NIR analysis is frequently used in bioreactors — it is especially useful in the determination of substrates and metabolites, such as glucose [9, 10], ethanol, and glycerine in alcoholic fermentations [10]. Due to NIR radiation penetrating even solid samples, it can be used, for example, in in-line synthesis monitoring [11] as well as in on-line analysis of crystallization products in API production [12].

For the identification of substances, the use of suitable chemometric methods may be necessary. However, if the specificity criteria of a qualitative method are met, the identification or characterization of a solid material is possible by directly comparing the unprocessed or pre-processed spectrum of the test sample with the spectrum of a reference substance.

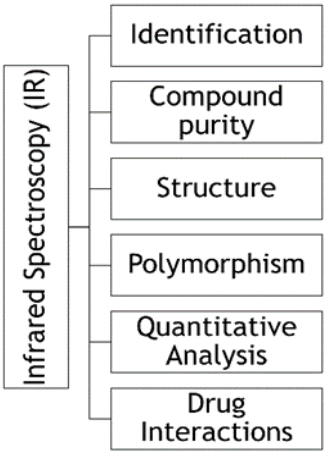


Fig. 1. Schematic representation of the potential applications of IR spectroscopy in the pharmaceutical field.

2. Development of Near-Infrared Applications

In recent years, near-infrared (NIR) spectroscopy has become one of the indispensable tools in both scientific and industrial research — used in the investigation of physicochemical properties, stability studies, quality control in synthetic chemistry, agriculture, microbiology, as well as in toxicology and forensic science, for example, in the detection of counterfeit drugs or illicit substances.

The growing application of NIR spectroscopy is primarily due to its non-invasive nature. This technique enables rapid analysis of a wide variety of materials, allowing for the acquisition of numerous physicochemical parameters from samples. This is particularly important in pharmaceutical industry research, where technological parameters of drug production processes — such as particle size, compaction, tablet hardness and dissolution rate, or water content — can be assessed using NIR spectroscopy. For instance, the harder the tablet mass, the smoother the surface of the final dosage form. Differences in material properties affect light scattering, which, in the case of fine-grained substances, results in an upward baseline shift in the recorded spectrum [5].

In February 2009, the European Medicines Agency (EMA) published a guideline on the use of NIR spectroscopy in the pharmaceutical industry, outlining the requirements for its application. Good Manufacturing Practice (GMP) guidelines recommend the implementation of NIR spectroscopy as an analytical tool within pharmaceutical production processes [5, 8, 13—15]. Due to its non-invasive nature, simplicity, and speed, NIR spectroscopy is also widely employed in the analysis of the composition of medicinal products and dietary supplements, particularly in the detection of adulteration or falsification [5, 16, 17].

3. Limitations of Near-Infrared Spectroscopy

Near-infrared (NIR) spectroscopy is a competitive analytical technique compared to other methods; however, it is important to acknowledge its limitations. One of the primary drawbacks is its limited applicability in aqueous environments. Water strongly absorbs infrared radiation, resulting in broad spectral bands that can obscure other signals and hinder the detection of additional compounds present in the sample — especially those occurring in lower concentrations. This also restricts the ability to perform quantitative analysis of certain substances [5].

Spectra obtained via NIR spectroscopy are often complex and difficult to interpret, requiring knowledge of advanced chemometric methods. They are typically affected by photometric noise due to the interference of various chemical and physical factors. A low signal-to-noise ratio can degrade spectral quality and reduce the precision and accuracy of the analysis. Numerous factors influence both the shape and intensity of the NIR spectrum, including temperature, humidity, sample thickness, and the sample's ability to scatter light. This variability often necessitates the use of complex calibration models and can lead to erroneous analytical results. Therefore, NIR spectra are commonly pre-processed prior to analysis to minimize baseline shifts and improve spectral interpretation [18]. Table 2 presents a comparison of the advantages and disadvantages of using NIR spectroscopy [5].

Table 2. Comparison of the advantages and disadvantages of NIR spectroscopy.

Advantages	Disadvantages
Non-invasive method	Low sensitivity for detecting substances at concentrations below 0.1% (w/w)
Rapid measurements (<1 minute)	Relatively high cost of instrumentation
Capability to analyze a large number of samples in a short time, enabling batch analysis	Requires continuous monitoring, calibration sets, and precise chemical and physical analysis of reference samples
Minimal sample preparation required (e.g., drying and grinding) or no sample preparation needed (allows analysis of insoluble samples)	Limited quantitative analysis capabilities (often necessitates the use of complex calibration models)
Availability of portable instruments	Requirement of a reference method for quantitative determination

4. Attenuated Total Reflectance Infrared Spectroscopy

The ATR-IR technique (Attenuated Total Reflectance Infrared Spectroscopy) is a variant of infrared spectroscopy that enables rapid and non-destructive analysis of the chemical composition of solid, liquid, and semi-solid samples without the need for complex preparation. In this method, IR radiation passes through a crystal — commonly made of diamond, germanium, or ZnSe — upon whose surface the sample is placed. The radiation undergoes multiple internal reflections within the crystal, generating an evanescent wave at the interface with the sample. This evanescent wave penetrates the sample surface, allowing the collection of the sample's IR spectrum (Fig. 2). ATR-IR is distinguished by its ease of use, high reproducibility, and the capability to analyze even small sample amounts without special preparation [19, 20].

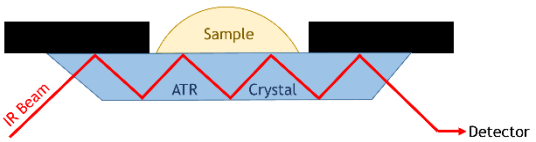


Fig. 2. Schematic representation of ATR-IR spectroscopy.

The ATR-IR spectroscopy technique serves as a rapid and non-invasive analytical tool widely applied in the analysis of biological, pharmaceutical, and food samples. It has been demonstrated that ATR-IR effectively enables phytochemical differentiation of species within the same genus [21], highlighting its utility in plant composition analysis. The method's efficacy has been confirmed in detecting synthetic adulterants in herbal medicines [22], as well as in identifying unwanted substances in food products (e.g., ethanol in non-alcoholic beverages) [23]. Furthermore, ATR-IR can be applied to the study and differentiation of polymorphic forms of compounds [24, 25].

One of the most recent applications of this technique involves combining ATR-FTIR with machine learning algorithms to enable rapid and automated bacterial strain typing [26], underscoring the potential of ATR-IR in microbiological diagnostics. Collectively, these studies demonstrate that ATR-IR combines high sensitivity, rapid analysis, and minimal sample preparation requirements, making it a versatile tool in modern chemical analysis.

From a pharmaceutical perspective, the use of ATR-IR spectroscopy in skin testing and the study of substance penetration through the skin is particularly interesting. One study used this technique for the characterization of structural skin barrier defects in atopic dermatitis [27]. Spectral analysis showed a reduction in the 1465 cm^{-1} and 1340 cm^{-1} peak areas, indicative of less lipid ordering and reduced carboxylate functional groups. It was associated with reduced hydration, elevated transepidermal water loss, and redness. Due to the complex structure of the skin and the different properties of its successive layers, quantitative analysis of substance penetration is difficult. However, using chemometric and machine learning methods, it is possible to quantitatively assess the penetration of substances through individual layers of the skin [28]. This method effectively links stratified quantitative analysis of transdermal penetration with the visualization of active ingredient distribution within the skin.

5. Analysis of the Structure of Organic Compounds

Since the 1940s — over 80 years ago — infrared spectroscopy has enabled the structural analysis of compounds exhibiting pharmacological activity. The earliest reports of such applications come from a British-American team during World War II, who proposed three possible structures of penicillin based on its IR spectrum [1].

The analysis of an IR spectrum primarily relies on assigning absorption bands to vibrations of bonds within specific functional groups, based on the characteristic wavenumber (v) and the bandwidth of these bands. For this purpose, fundamental tables of spectral band ranges for individual chemical groups (Table 3) can be utilized. However, it should be noted that these are average ranges and do not account for unique spectral variations arising from molecular chirality, geometric isomerism, hydrogen bonding, and other effects influencing bond angles and, consequently, observed vibrations.

Table 3. Characteristic infrared absorption of selected functional groups in organic molecules [2].

Functional group	Wavenumber [cm^{-1}]	Intensity
C-H stretching vibrations		
Alkanes	2960-2850	m, s
Alkenes	3095-3010	m
Aromatic compounds	3030	v
C=C stretching vibrations		
Alkenes	1680-1600	v
Aromatic compounds	1600, 1580, 1500, 1450	v, v m, m
C=O stretching vibrations		
Ketones	1725-1540	s
Aldehydes	1740-1680	s
Esters	1780-1717	s
Carboxylic acids	1725-1680	s
Anhydrides	1850-1740	s
Amides	1690-1630	s
O-H stretching vibrations		
Non-associated OH group	3650-3590	v, sh
Associated OH group (intermolecular hydrogen bonding)	3550-3450	v, sh
Associated OH group (intramolecular hydrogen bonding)	3570-2450	v, sh
N-H stretching vibrations		
Primary amines	3400	m
Secondary amines	3500-3310	m
Stretching vibrations		
C-F	1110-1000	m
C-Cl	800-600	s
C-Br	540-510	m
C-I	520-490	m
Organic sulfur-containing compounds		
C-S	705-570	v
C=S	1200-1050	s
S-H	2590-2550	v
R=SO_4	1070-1030	s
R-SO_3H	1260-1150	s

Intensity: s — strong, m – medium, v — variable, sh — sharp band

Currently, spectral libraries are most commonly used, and automated methods compare the recorded sample spectrum with reference spectra in the database — either created in-house or commercially acquired — enabling rapid identification of unknown compounds.

Using Fourier-transform infrared spectroscopy (FTIR), it is possible to distinguish isomers — such as tautomers and cis/trans isomers — for example, different isomers of curcumin, including those adsorbed on other particles [29].

Stereoisomerism can also influence the vibrational bands observed in IR spectroscopy. As presented in Table 4 on the example of ezetimibe, (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one — a selective cholesterol-absorption inhibitor, differences in IR spectra of stereoisomers may be noticeable and allow one to identify optical isomers. As stereoisomers might be impurities in API production (synthesis- and process-related byproducts or degradation products) — a rapid and replicable analytical method with the possibility of on-line application, such as IR, can be a valuable tool in quality control in the synthesis of active ingredients [30].

6. Assessment of Purity

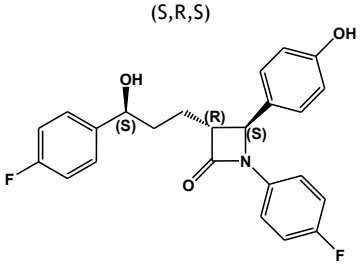
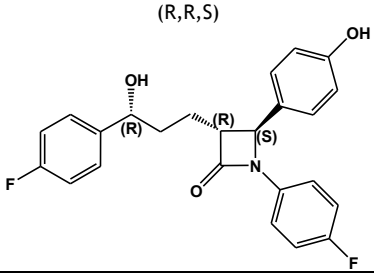
IR spectroscopy allows for simultaneous confirmation of a compound's structure and assessment of sample purity. In the process of registering a pharmaceutical product, it is mandatory, according to Good Manufacturing Practice (GMP) requirements, to identify potential impurities in the product. These include residues of reagents and solvents used in the synthesis of the active pharmaceutical ingredient (API), by-products of ongoing reactions, degradation products, and other possible contaminants.

In practice, after identifying potential impurities, batch analyses are conducted to confirm the structure of the active compound and its purity based on characteristic spectral bands. For example, in chloramphenicol production, one specified impurity is dichloroacetic acid, for which the analytical absorption band at 1745 cm^{-1} has been assigned [1]. Thus, IR spectroscopy enables both qualitative and quantitative analysis of impurities, including batch testing, at low cost and within a short time frame.

7. Analysis of Plant-Derived Substances Using Near-Infrared Spectroscopy

Over the past forty years, near-infrared (NIR) spectroscopy has become one of the most valuable methods for analyzing proteins, carbohydrates, dry matter in plant extracts, and other plant materials [5, 31-36]. Plants, as primary producers in the food chain, play a significant role in many industries and, due to their medicinal properties, are also important in the pharmaceutical field [37]. Plants are frequently analyzed, classified, and standardized based on the presence of specific secondary metabolites. For example, NIR spectroscopy enables the differentiation of three closely related species from the *Orchidaceae* family, which are used, among other applications, for treating digestive system issues and reducing fever. It has been demonstrated

Table 4. Infrared bands for ezetimibe (S,R,S) and its stereoisomer (R,R,S) [30].

Typical IR bands (cm^{-1})		
Stereoisomers of ezetimibe		
(S,R,S) 	(R,R,S) 	**Vibration type**
3436, 3278	3561, 3313	-O-H stretching vibrations
3073-3015	3017	-C_{Ar}-H stretching vibrations
2927	2928	Aliphatic –C-H stretching vibrations
1616-1597	1720	Aromatic –C=C stretching vibrations
1731	1617, 1603	Amide –C=O stretching vibrations
1509	1511	Amide –C-N stretching vibrations
1399	1396	-C-H deformation vibration
1224	1222, 115	-C_{Ar}-O, -C_{Ar}-F stretching vibrations

that these plants can be successfully distinguished based on the intensity of specific band patterns in the spectrum at 5180 cm^{-1} and within the 4900—4800 cm^{-1} range [5].

Profiling primary and secondary metabolites is particularly useful for estimating the therapeutic efficacy of herbal medicines by ensuring consistent quality and reproducibility between batches, including those from different harvests [38, 39]. Another example where NIR spectroscopy was applied for classification and quality control of plant substances is the analysis of *Ginkgo biloba* extract. The NIR method proved sensitive to qualitative changes in the extract caused by variations in the manufacturing process. Since some quality specifications of *G. biloba* extract are inherently linked to production processes, these products often exhibit uncontrolled variability affecting subsequent dosage forms (e.g., during granulation or tableting). Some of these variations cannot be detected by conventional quality control tests, which highlights the great utility of NIR spectroscopy, allowing evaluation of the impact of individual manufacturing steps as well as the quality of the resulting preparations [18, 40].

Currently, various plant-based preparations are produced under stringent quality control, where each production stage is verified using different Process Analytical Technology (PAT) tools, including NIR spectroscopy [5].

8. Identification of Polymorphic Structures Using IR Spectroscopy

Polymorphic forms of an active pharmaceutical ingredient (API) exhibit different properties during stability studies due to variations in their internal structure. Crystallization conditions, drying, milling, and storage under varying temperature and relative humidity conditions influence the stability of a given polymorphic form and may induce polymorphic transformations. Therefore, continuous monitoring of these transformations is required at every stage of API manufacturing, from laboratory-scale synthesis to industrial production [41]. Currently, there is no universal method for polymorphism analysis that can be applied to all existing drugs [1]. Commonly used techniques for distinguishing and characterizing internal polymorphic forms include X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), optical and electron microscopy, Raman spectroscopy (RS), and infrared spectroscopy (IR), including near-infrared spectroscopy (NIR) [42]. Among these, IR spectroscopy plays a primary role and, when often combined with complementary Raman spectroscopy, provides comprehensive information about the structure of the studied compounds [1]. Differences in the internal structure of polymorphic forms, resulting from various intermolecular interactions, cause shifts in spectral bands, enabling identification of specific forms [41], including cocrystals [43].

IR spectroscopy is complemented by X-ray diffraction (XRD) [1]. Different crystallographic structures produce distinct diffraction patterns and characteristic spectra. Therefore, this technique allows the identification of even minor crystalline impurities (e.g., those formed due to degradation of undesired polymorphic forms, solvates, or intermediates) and enables unambiguous differentiation between crystalline and amorphous forms [1, 41].

9. Analysis of the Drug Dosage Form

Infrared spectroscopy in various configurations provides a comprehensive tool for the characterization of drug dosage forms — from API quantification and physical property assessment to detailed investigation of drug release mechanisms. Their integration into continuous manufacturing systems and Process Analytical Technology (PAT) frameworks significantly enhances production efficiency, product quality, and patient safety, as the qualitative and quantitative analysis of a drug is a key element of pharmaceutical quality assurance and is crucial for ensuring the safety and efficacy of pharmacotherapy. This technique facilitates real-time

observation of processes such as polymer swelling, water penetration, API diffusion, and matrix erosion, thus it is particularly advantageous for studying release mechanisms in complex formulations, including polymer-based matrices and modified-release systems. When combined with UV/Vis detection, the technique allows simultaneous visualization of internal transformations and quantification of API release kinetics. The combination of IR with other methods commonly used in the pharmaceutical industry, such as Raman spectroscopy and high-temperature X-ray diffraction, allows for the assessment of dosage forms both during the development phase and in quality control. In recent years, the development of advanced spectroscopic, chemometric, and imaging techniques has significantly enhanced the ability to accurately and rapidly characterize pharmaceutical products, particularly in continuous manufacturing environments.

One of the fundamental parameters in drug dosage form analysis is the quantification of API and its uniformity within individual units and across production batches. Although conventional reference techniques such as high-performance liquid chromatography (HPLC) exhibit high precision, they are often time-consuming and less suited to real-time monitoring in manufacturing settings. Modern analytical tools, including near-infrared (NIR) spectroscopy, enable rapid and nondestructive API determination. In studies involving tablets produced via wet granulation, a chemometric calibration model was developed to accurately predict API content. The method demonstrated a high coefficient of determination (R^2 = 0.9979) and a low root-mean-square error of prediction (RMSEP = 1.09%), indicating excellent agreement with the HPLC reference method. Importantly, analytical accuracy was maintained even at inspection speeds of 250,000 tablets per hour, fully meeting content uniformity requirements (±15%) [44]. Beyond chemical composition, tablet evaluation requires characterization of physical attributes such as hardness, porosity, friability, and their behavior under dissolution conditions. Standard dissolution tests provide quantitative information on drug release but offer limited insight into the chemical and structural mechanisms occurring within the tablet matrix. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic imaging overcomes this limitation by enabling in situ monitoring of structural and compositional changes during tablet dissolution [45]. ATR-FTIR imaging has proven especially valuable in the investigation of advanced and unconventional solid dosage forms. It allows detailed analysis of multilayer tablets, swelling hydrogels, high-throughput dissolution systems, and microfluidic-based release studies. The ability to observe localized chemical and structural changes — such as concentration gradients, formation of diffusion pathways, or degradation of polymer networks — makes ATR-FTIR an indispensable tool in the development of innovative drug delivery systems. [46]. NIR spectroscopy is also widely applicable for assessing mechanical and biopharmaceutical tablet parameters, such as crushing strength and dissolution behavior. In formulations containing sulfamethazine, simple linear regression applied to NIR data yielded a correlation coefficient of r = 0.986 for content uniformity. Partial least squares (PLS) models further enabled reliable prediction of tablet hardness (r = 0.84) and dissolution at 120 minutes (Q120; r = 0.92) [47]. These results demonstrate that NIR, combined with chemometric modelling, can successfully predict critical quality attributes even in formulations with varying API levels. Fourier transform infrared derivative spectroscopy (FTIR-DS), a spectroscopy method which uses first or higher derivatives of spectral data, has been used in the analysis of furosemide tablets. The method demonstrated excellent linearity (R^2 = 0.9998), low overall variability (<2%), and recoveries within 100 ± 3% across a wide concentration range. As results were consistent with pharmacopeial methods, FTIR-DS can be considered a rapid and reliable alternative for routine quality control of solid dosage forms [48].

IR advantages in comparison to traditional analytical approaches in the analysis of inhalation aerosols, particularly dry powder inhalers (DPIs), includes foremost the ability to resolve micro- and nanoscale chemical heterogeneity within individual particles. Recent advances in IR techniques, particularly optical photothermal infrared spectroscopy (O-PTIR) and atomic force microscopy–infrared spectroscopy (AFM-IR), have enabled unprecedented insight into the solid-state chemistry and spatially resolved composition of inhalation aerosols [49]. DPI particles typically range from a few nanometers to several micrometers in size and often represent complex mixtures of one or more active ingredients with excipients such as lactose. At this scale, phase separation, heterogeneous distribution of components, and drug–excipient interactions can significantly influence aerosol performance, including emitted dose, fine particle fraction, and deposition in the respiratory tract. Conventional bulk analytical methods are incapable of characterizing the nanochemical composition of individual particles or distinguishing drug and excipient domains within heterogeneous matrices. As a result, there has been a longstanding need for analytical platforms capable of providing spatially resolved chemical information at submicrometer resolution [49]. O-PTIR was applied to characterize size-segregated particles. Formulations containing fluticasone propionate, salmeterol xinafoate, and lactose — prepared either by spray drying solutions or suspensions — were analysed. Spatially resolved O-PTIR spectra confirmed the presence of diagnostic vibrational bands associated with fluticasone propionate (1746, 1702, 1661, and 1612 cm^{-1}), salmeterol xinafoate (1582 cm^{-1}), and lactose (1080 cm^{-1}). For solution-spray-dried powders, the ratio of drug to lactose peaks remained consistent across particle size fractions, indicating compositional homogeneity throughout the aerosol. By contrast, suspension-spray-dried formulations exhibited progressive enrichment of drug relative to lactose, highlighting significant heterogeneity in drug–excipient distribution. Comparisons between O-PTIR results and conventional wet assays demonstrated strong qualitative agreement, supporting the suitability of O-PTIR as an analytical screening platform for DPI formulations [50]. The integration of O-PTIR with atomic force microscopy-infrared spectroscopy further extends the spatial resolution and sensitivity of infrared-based aerosol analysis, enabling direct nanochemical mapping of individual particles. Studies involving three powder formulations — commercial Seretide (a physical blend of

fluticasone propionate, salmeterol xinafoate, and crystalline lactose), and two spray-dried powders containing either amorphous or crystalline lactose — demonstrated the ability of these techniques to resolve drug and excipient domains with unprecedented precision. For the first time, it was possible to map the distribution of APIs within single aerosol particles, revealing formulation-dependent patterns of drug localization. Such insights are critical for understanding the mechanisms underlying aerosol performance, including particle dispersibility, deposition efficiency, and dose uniformity [51].

The analytical characterization of semi-solid dosage forms such as gels, creams, and ointments increasingly relies on advanced spectroscopic techniques that enable rapid, non-destructive, and formulation-specific assessment. In gel formulations, transmission FT-NIR spectroscopy has been shown to provide highly accurate quantification of active pharmaceutical ingredients, as demonstrated by the near-perfect calibration ($r = 0.9996$) achieved for ketoprofen in Carbopol-based gels, confirming the technique's suitability as an efficient alternative to more time-consuming analytical methods [51]. Beyond quantification, vibrational spectroscopic imaging methods such as microscopic ATR-IR and confocal Raman microscopy offer deeper insight into the microstructure of creams, revealing the spatial distribution of oily domains and active ingredients, thereby supporting formulation design and confirming phase behavior within semi-solid matrices [52]. The applicability of NIR spectroscopy has also been extended to quality control of both creams and ointments in pharmacy settings, where transflectance-based models enabled accurate quantification of several active ingredients in hydrophilic and lipophilic bases, meeting regulatory requirements for linearity, precision, and robustness despite challenges posed by certain low-concentration analytes [53].

Transdermal drug dosage forms have also been successfully analysed using IR methods, as research on transdermal patches has increasingly focused on optimizing polymer matrices, permeation enhancers, and analytical methods to improve drug delivery efficiency and structural stability. For example, electrospun nanofibers have emerged as a promising platform for transdermal diclofenac delivery, as they exhibit improved homogeneity, mechanical strength, and enhanced skin permeation, while also inducing a crystalline-to-amorphous transformation of the drug that favors controlled release. FTIR analysis was performed to identify diclofenac diethylamine (DDA) and ethylcellulose (EC) functional groups and to investigate their possible interference in electrospun fibers. In IR spectra, the peak bandwidth of the 2500—3500 cm^{-1} region of DDA is due to the salt form of this compound. The broadening in the FTIR spectrum was reduced for two samples of fibers obtained from different formulations, indicating that DDA may no longer be in salt form in the presence of popular penetration enhancers such as farnesol and nerolidol. Also, the absorption band for the C=O group in the DDA shifted from 1628 cm^{-1} to 1721 cm^{-1} in the range of the two mentioned samples, which is another confirmation of the protonation of the carboxylate group of DDA as a result of adding farnesol and nerolidol. No change in the diagnostic peaks of the drug (the removal of the peak or the appearance of a new peak) indicated that no specific reaction had occurred [54].

10. Investigation of Protein Structure and Stability

Infrared (IR) spectroscopy is one of the most versatile analytical techniques, providing valuable information regarding the structure of amino acids, peptides, isolated proteins, and enzymes. It enables the observation of protein secondary structure and is highly specific and sensitive to changes in bonding. Measurement conditions can be tailored to allow analysis in organic solvents, thin films or deposits, and, in certain cases, even in aqueous solutions. The characteristic IR bands of proteins are listed in Table 5.

Table 5. Typical IR bands in protein analysis [55—57].

Wavenumber [cm^{-1}]	Vibrational assignment
~3300	N-H stretching (amide A band)
~3080	N-H stretching (amide B band)
1600—1700	C=O stretching (amide I)
1500—600	C-H stretching (amide II)
1200—1350	N-H bending and C-N stretching (amide III)
625—770	O-C-N deformation (amide IV)
640—800	N-H bending (amide V)
540—600	C=O bending (amide VI)
500—550	S-S stretching

IR spectroscopy cannot determine the primary structure of peptides; however, a major advantage of this technique is its capability to analyze poorly soluble compounds. The sensitivity of this method decreases with increasing protein size, though analyzing proteins with molecular weights of 150—200 kDa does not pose significant challenges.

Proteins exhibit nine characteristic absorption bands that facilitate their identification, among which the most significant are the amide I band (~1700—1600 cm^{-1}), the amide II band (~1600—1500 cm^{-1}), and, to a lesser extent, the amide III band (~1340—1200 cm^{-1}). IR spectroscopy enables the study of conformational changes induced by variations in temperature, pH, pressure, or steric constraints, including the hydrophobic or hydrophilic nature of amino acid side chains [58].

Proteins possess secondary structures such as α-helices and ß-sheets, which are critical for characterizing proteins of unknown structure. Therefore, evaluating the secondary structure is one of the primary applications of FTIR spectroscopy. This analysis is based on the assumption that any protein can be considered as a linear combination of several basic secondary structural elements, with the relative proportion of each element corresponding to the spectral intensity (the molar absorptivity of C=O stretching vibrations is essentially constant across different secondary structural elements).

Spectra of protein structures are typically complex due to the variety of overlapping secondary structure bands, complicating spectral interpretation. The amide bands consist of numerous overlapping sub-bands specific to individual secondary structures. Techniques that enhance spectral resolution can be applied to extract information on band intensity, position, and the relative proportion of different secondary structures.

Table 6. Examples of the use of IR spectroscopy in protein studies.

Application	Description	Reference
Secondary structure analysis	Determining α-helix and ß-sheet content from amide I and II bands.	[60]
Protein folding and unfolding	Monitoring structural transitions under pH or temperature stress using FTIR.	[61]
Protein aggregation detection	Identifying ß-sheet-rich aggregates via characteristic amide I shifts.	[61]
Protein–ligand interaction studies	Detecting conformational or binding-site changes upon drug binding.	[62]
Protein hydration studies	Examining hydrogen bonding between proteins and water molecules.	[63]
Protein–excipient interactions	Analyzing interactions between proteins and stabilizers (e.g., sugars, PEG).	[64]
Quality control	FTIR fingerprinting	[65]
Protein adsorption on surfaces	ATR-FTIR reveals conformational changes on nanoparticle or implant surfaces.	[66]
Enzyme catalysis mechanism	Time-resolved IR detects bond vibrations during enzymatic reactions.	[67]
Effect of lyophilization and storage	Studying protein structure before and after freeze-drying via amide I region.	[68]

Protein stability studies provide insights into folding/unfolding processes and the structural stability of protein molecules. A key advantage of FTIR over other techniques is its ability to monitor the folding/unfolding of all protein regions simultaneously and in real time.

Obtaining high-quality IR spectra of denatured proteins requires using high concentrations of chemical denaturants to ensure complete unfolding. Consequently, an IR cell with a short optical path length is necessary to prevent detector saturation by the strong absorption of the chemical denaturant. This limitation restricts the applicability of this method for proteins highly resistant to denaturation.

FTIR has been widely used in protein aggregation studies. Current theoretical and experimental evidence suggests that protein aggregates arise from partially folded intermediate species. Protein aggregation presents serious medical and economic challenges, motivating efforts to elucidate its mechanisms and pathways and to characterize protein aggregates. A successful approach involves combining thermal and chemical denaturation to characterize aggregation intermediates. This method is valuable for investigating aggregation processes across a wide range of proteins. Identification and characterization of aggregation intermediates can aid in developing novel therapeutic strategies for diseases associated with protein aggregate accumulation, such as ß-amyloid deposits in Alzheimer's disease. IR spectroscopy can thus provide critical information for therapeutic targets and facilitate improvements in manufacturing and quality control processes, enabling the development of novel therapeutic proteins, underscoring the remarkable potential of this technique [58, 59]. Table 6 summarizes and provides examples of the applications of IR spectroscopy in protein research.

11. Summary

This review article explores the broad applications of infrared (IR) spectroscopy in the pharmaceutical sciences. It focuses on the structural analysis of drug molecules, the characterization of solid forms, the study of protein structures, and the detection of polymorphisms. It highlights the importance of the IR spectroscopy, particularly Fourier-transform IR (FTIR), as a non-destructive, rapid, and reliable tool for identifying functional groups, monitoring drug stability, and distinguishing between crystalline and amorphous forms. It also plays a pivotal role in characterizing protein therapeutics and identifying polymorphic variations that impact drug performance. Recent advancements, such as IR microscopy and chemometric methods, have significantly improved analytical sensitivity and data interpretation.

Looking to the future, integrating IR spectroscopy with machine learning and artificial intelligence is expected to further improve spectral analysis and predictive modeling in drug development. Miniaturised and portable IR devices are also anticipated to facilitate real-time, on-site quality control in manufacturing processes. Furthermore, developments in hyphenated techniques that combine IR with thermal or chromatographic methods may provide deeper insights into complex pharmaceutical systems. Together, these innovations suggest an increasingly important and evolving role for IR spectroscopy in modern pharmaceutical research and industry.

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