



ADVANCE APPLICATIONS OF FOURIER TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

Fourier transform Infrared spectroscopy is a versatile tool in Pharmaceutical Sciences, with a wide field of applications ranging from characterization of drug formulations to elucidation of kinetic processes in drug delivery. Recent new developments in applications of these methods include study of drug delivery systems and in particular topical drug delivery system. Advance techniques of FTIR are Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR), Fourier Transform Infrared-Photo acoustic Spectroscopy (FTIR-PAS), Fourier Transform Infrared Imaging Spectroscopy, Fourier Transform Infrared Microspectrometry. FTIR-ATR method is used to study drug release in semisolid formulations, drug penetration, and influence of penetration modifiers and also in in-vivo studies. FTIR-PAS has been applied to measure drug content in semisolid and solid formulations, to determine drug penetration into artificial and biological membranes. The big advantage of this technique is the possibility of spectral depth profiling. Recently, FTIR imaging has also been used to visualize the drug and excipient distribution in pharmaceutical formulations such as tablets and therapeutic transdermal system, the mechanism of drug release, study of penetrability of cosmetic ingredients in human hair. ATR-FTIR imaging is also used to obtain images of the surface of the skin and the spatial distribution of protein and lipid rich domains. FTIR has also been used widely in disease diagnosis. Thus, the main purpose of this article is to study the varied applications of FTIR in field of pharmaceutical sciences. Further it aims to study the various advancements of FTIR like FTIR-PAS, FTIR-ATR, FTIR-Microspectrometry, FTIR-Imaging in invitro and *in vivo* studies in pharmaceutical sciences.

Keywords: FTIR, FTIR-PAS, FTIR-ATR, FTIR- Microspectrometry, FTIR-Imaging.

INTRODUCTION

Infrared spectroscopy is a standard method of analytical pharmacy and chemistry which provides the images of vibration of atoms of compound. Therefore it is also referred to as vibrational spectroscopy. IR spectrum is obtained by passing infra red radiation through the sample and determining what fraction of the incident radiation is absorbed at a particular frequency. Fourier transformation is a mathematical operation demonstrated by 'Jean Fourier' which converts the frequency domain into time domain.

This review gives a short introduction of the concept of Fourier transform (FT) technique and briefly explains the principles of attenuated total reflection (FTIR-ATR) and photo acoustic spectroscopy (FTIR-PAS) as well as microscopic methods. This review paper addresses the following areas: (1) Determination of drug content in semisolid formulations and solid dosage forms; (2) Characterization of the influence of penetration modifiers on the properties of stratum corneum (SC); (3) Non-invasive monitoring of drug penetration into membranes; (4) Study of drug release from semisolid formulations. (5) Identification and characterization of homo polymers, copolymers and polymer blends, monitoring polymerization reactions, characterization of the structural properties of polymers, including tacticity, branching, crystallinity, hydrogen bonding and orientation, investigation of the surface properties of polymers and understand polymer degradation; (6) Estimation of the secondary structure of proteins by

applying quantitative analysis of the infrared spectra of such molecules; (7) Extensive use of FTIR to characterize diseases for diagnosis.

INSTRUMENTATION

1. Fourier transform Infrared Spectroscopy^{1,2}

The term fourier transformation has originated from a mathematical operation demonstrated by 'Jean Fourier' which converts the frequency domain into time domain. Fourier transform infrared (FTIR) spectrometers have almost entirely replaced dispersive instruments because of their better performance in terms of speed and efficiency.

The instrument consists of a interferometer, fixed mirror, a movable mirror, beam splitter. A beam emitted by a source is split in two by the beam splitter, 50% of the incident radiation will be reflected to one of the mirrors while 50% will be transmitted to the other mirror. The two beams are reflected from these mirrors, returning to the beam splitter where they recombine and interfere to give constructive interference or destructive interference, depending on the difference in the optical paths between two arms of interferometer. The signal is then recorded by the detector.



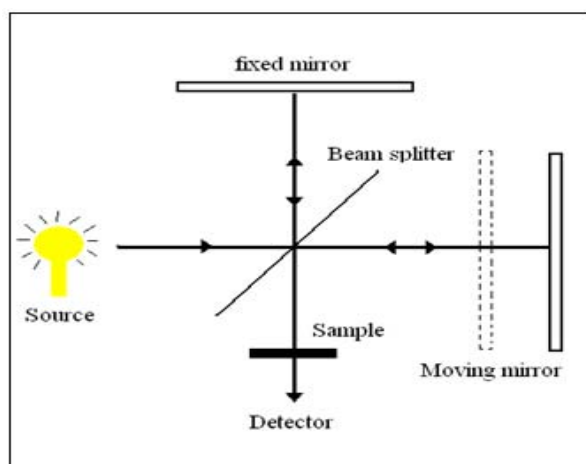


Figure1: Schematic diagram of a Michelson interferometer.

The steps for recording an FTIR spectrum are to produce an interferogram with and without a sample in the beam and then to transform these interferogram into spectra of the source with sample absorption and without sample absorption. The ratio of the former to the latter is the IR transmission spectrum of the sample. In the case of FTIR spectroscopy, the sample is usually placed between the interferometer and the detector. (**Figure1**). The recent advances of IR are FTIR-ATR, FTIR-PAS, and FTIR-Micro spectrometry.

2. Attenuated total reflection: FTIR-ATR^{2,4}

Attenuated total reflectance (ATR) spectroscopy utilizes the phenomenon of total internal reflection. A beam of radiation entering a crystal will undergo total internal reflection when the angle of incidence at the interface between the sample and crystal is greater than the critical angle, where the latter is a function of the refractive indices of the two surfaces.

In ATR, the sample is placed in contact with a special crystal, termed ATR crystal, which is composed of a material with a high index of refraction [e.g., zinc selenide (ZnSe), thallium bromide iodide (KRS-5), Ge]. The IR beam is focused onto the bevelled edge of the ATR element by a set of mirrors, reflected through the crystal, and then directed to the detector by another set of mirrors. The beam penetrates a fraction of a wavelength beyond the reflecting surface. When a material that selectively absorbs radiation is in close contact with the reflecting surface, the beam loses energy at the wavelength where the material absorbs. The resultant attenuated radiation is measured and plotted as a function of wavelength by the spectrometer and gives rise to the absorption spectral characteristics of the sample. The depth of penetration in ATR spectroscopy is a function of the wavelength, λ , the refractive index of the crystal, n_2 , and the angle of incident radiation, θ . (**Figure 2**)

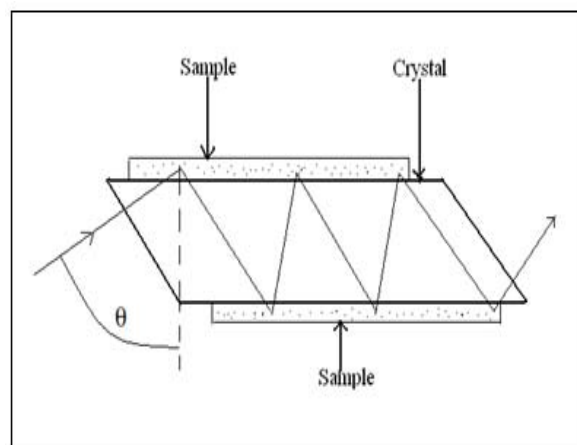


Figure 2: Schematic diagram of a typical attenuated total reflectance cell.

ATR is a quick and non-destructive sampling technique for obtaining the IR spectrum of a material's surface. Samples examined by FTIR-ATR generally require minimum, or no, sample preparation. In pharmaceutical science, the FTIR-ATR technique can be employed to (1) To characterize the structure and the properties of biological systems such as Stratum Corneum; (2) To study drug release from semisolid formulations noninvasively; (3) To measure drug diffusion from pharmaceutical systems such as polymers, films; (4) To investigate drug penetration into appropriate acceptor systems such as artificial and biological membranes; (5) To characterize interactions between drugs and synthetic, semi-synthetic, and native macromolecules.

3. Photoacoustic Spectroscopy: FTIR-PAS^{2,3}

Photoacoustic spectroscopy (PAS) is a non-invasive reflectance technique with penetration depths in the range from microns down to several molecular monolayers. Gaseous, liquid or solid samples can be measured by using PAS and the technique is particularly useful for highly absorbing samples.

The photoacoustic effect occurs when intensity-modulated light is absorbed by the surface of a sample located in an acoustically isolated chamber filled with an inert gas. A spectrum is obtained by measuring the heat generated from the sample due to a re-absorption process. The sample absorbs photons of the modulated radiation, which have energies corresponding to the vibrational states of the molecules. The absorbed energy is released in the form of heat generated by the sample, which causes temperature fluctuations and, subsequently, periodic acoustic waves. A microphone detects the resulting pressure changes, which are then converted to electrical signals. Fourier-transformation of the resulting signal produces a characteristic infrared spectrum. **Figure 3** shows a schematic diagram of a PAS cell. The step-scan technique, in conjunction with phase modulation, provides a uniform modulation frequency for all wave numbers and consequently a uniform probing depth represents the most important advantage of this method. It has a ability to carry out controllable depth

profiling of the sample. Hence, applying a sequence of several modulation frequencies in repeated manner, it is possible to obtain data from various probing depths in the course of the penetration process.

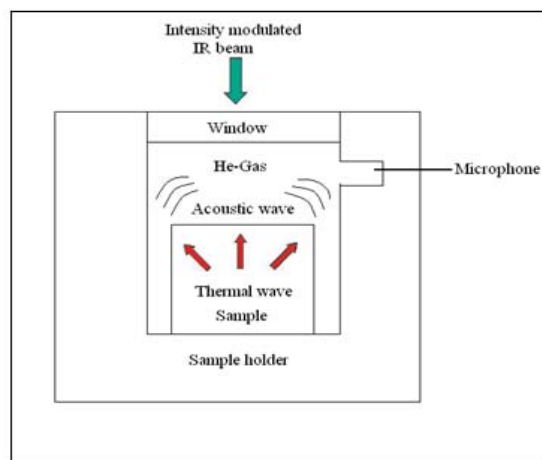


Figure 3: The scheme of a photo acoustic cell.

FTIR-PAS can be applied for; (1) Direct measurement of drug content without sample preparation in pharmaceutical formulations such as ointments and tablets; (2) non-invasive determination of drug penetration into artificial and biological acceptor systems such as membranes and isolated SC.¹

4. FTIR-Micro spectrometry¹

Infrared spectrometer with a microscope facilitates the study of very small samples. In recent years, there have been considerable advances in FTIR microscopy, with samples of the order of microns being characterized. In FTIR microscopy, the microscope is placed above the FTIR sampling compartment.

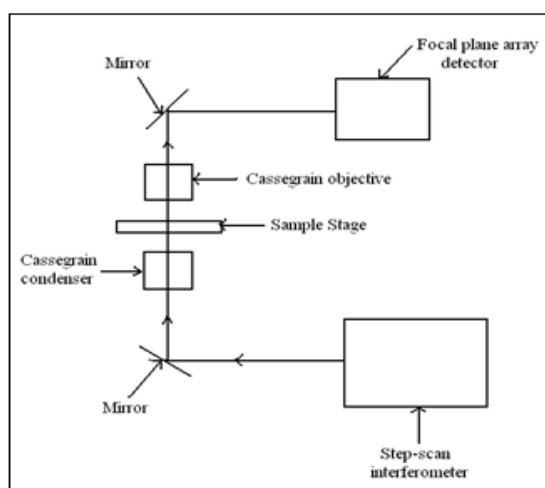


Figure4: Schematic diagram of FTIR Micro spectrometer

Infrared radiation from the spectrometer is focused onto a sample placed on a standard microscope $x-y$ stage. After passing through the sample, the infrared beam is collected by a Cassegrain objective which produces an image of the sample within the barrel of the microscope. A variable aperture is placed in this image plane. The radiation is then focused on to a small-area mercury

cadmium telluride (MCT) detector by another Cassegrain condenser. The microscope contains glass objective which allows visual inspection of the sample. In addition, by switching mirrors in the optical train, the microscope can be converted from transmission mode to reflectance mode. Microcells and diamond anvil cell (DAC) are used for handling the sample. Alternatively, a multiple internal reflectance cell may also be used as this technique can produce stronger spectra. Infrared imaging using FTIR micro spectroscopic techniques has emerged as an effective approach for studying complex or heterogeneous specimens. The technique can be used to produce a two- or three-dimensional 'picture' of the properties of a sample. This is achievable because, instead of reading the signal of only one detector as in conventional FTIR spectroscopy, a large number of detector elements are read during the acquisition of spectra and this is possible due to the development of focal plane array (FPA) detectors³

5. Diffuse Reflectance Spectroscopy²

It includes the external reflectance phenomenon. In this, the energy that penetrates one or more particles is reflected in all directions and this component is called diffuse reflectance. Diffuse reflectance (infrared) technique is commonly called DRIFT. In this technique, a powdered sample is mixed with KBr powder. The DRIFT cell reflects radiation to the powder and collects the energy reflected back over a large angle. Diffusely scattered light can be collected directly from material in a sampling cup or, alternatively, from material collected by using an abrasive sampling pad. DRIFT is particularly useful for sampling powders or fibres. **Figure 5** illustrates diffuse reflectance from the surface of a sample.

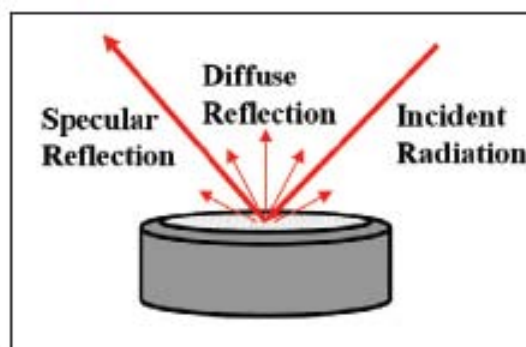


Figure 5: Schematic diagram of Diffuse Reflectance Spectroscopy

Kubelka and Munk developed a theory describing the diffuse reflectance process for powdered samples which relates the sample concentration to the scattered radiation intensity. The Kubelka–Munk equation is as follows:

$$f(R) = (1-R)^2/2R = k/S$$

Where: R is the absolute reflectance of the sampled layer, K is the molar absorption coefficient and S is the scattering coefficient.

APPLICATIONS OF FTIR

A. Content of drugs in semisolid formulations and tablets determined by FTIR-PAS^{1,5,9}

In pharmaceutical practice, the drug content in formulations is measured by analytical standard methods, such as HPLC, GC or HPTLC. But such procedure is laborious and time consuming and it has low repeatability and a low recovery rate. From this point of view, the big advantage of FTIR-PAS is that the drug content can be quantified directly with or without minimal sample preparation. Generally, the sample is only to be placed in a metal cup (maximum sample size: 10 mm diameter and 8mm height), which fits into the PAS sample holder. FTIR-PAS in conjunction with phase modulation technique has been used for the determination of brivudin (antiviral) and dithranol in a semisolid formulation (vaseline) employing step-scan. The spectrum in the fingerprint region of the mixtures vaseline/dithranol with various drug contents is recorded. These photo acoustic spectra exhibit bands belonging to dithranol at the lowest amount of drug (about 0.5 wt. %).

The quantification of drugs in solid dosage forms is also being carried out by using FTIR-PAS. The only requirement is that the tablets fit into the sample cup. As an example, the FTIR-PA spectra in the fingerprint range of model tablets of EmcocelR, microcrystalline cellulose, with various amounts of theophylline. The spectra are recorded and the spectral features belonging to theophylline is identified. Thus, it is easy to determine the amount of theophylline in tablets

B. Penetration of drug into membrane^{1,7,8}

1. Study of penetration of drug into membrane FTIR-ATR⁶

ATR is used in conjunction with FTIR to monitor the penetration of drugs into membranes and the permeation of drugs across membranes as well as to determine diffusion coefficients of the diffusants. The diffusion coefficient of several liquids in anhydrous lanolin and in polyethylene glycol ointment is determined by FTIR-ATR and also uptake of urea from a polyethylene glycol ointment into a medical grade silicon pressure-sensitive adhesive is studied. The permeation of 4-cyanophenol through both across artificial (silicone) membrane and human stratum corneum is studied using the FTIR-ATR spectroscopy.

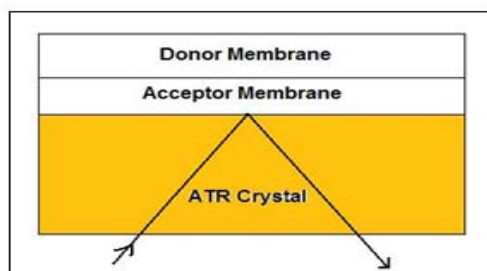


Figure 6: Sample arrangement for the drug penetration experiment using ATR technique

For studying penetration of drug through membrane, an appropriate artificial or natural membrane acting as acceptor is sandwiched between an impermeable ATR crystal and a reservoir of penetrant and the donor (**Figure 6**). The membrane is initially devoid of penetrant. As diffusion through the membrane occurs, there will be a buildup of penetrant concentration at the interface between membrane and crystal, which can be monitored online by the appearance and increase of drug-specific IR bands as a function of time

2. Study of penetration of drug into membrane by FTIR-PAS¹

FTIR-PAS has been used to study the penetration of drug into membrane. The arrangement of donor and acceptor with respect to the IR beam (**Figure 7**). The formulation is loaded in brass cup that fits into the sample holder of PAS cell and membrane is placed on the ointment surface. The penetration of many drugs like clotrimazole, dithranol, and methoxsalen from semisolid vaseline formulation into a lipophilic dodecanol–collodion membrane is studied^{7,8}. Using FTIR-PAS, the uptake of the drug in the membrane was quantified by monitoring the dependence of an appropriate drug band on the penetration time and graph of relative increase of the drug signal intensity versus the penetration time was plotted to study uptake of drug.

FTIR-PAS is also used in studying the mechanism of drug through human skin and also helps in understanding the barrier function of stratum corneum as well as for medical and cosmetic applications. The advantage of FTIR-PAS is that it used in depth profiling and for detection of weakly absorbed compound in strongly absorbing matrix.

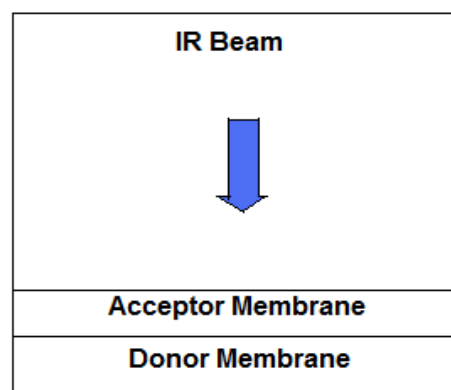


Figure 7: The Sample arrangement for Drug Penetration

C. Study of effect of penetration modifier on the properties of stratum corneum by FTIR-ATR^{1,9}

FTIR-ATR is utilized to study the structure of the stratum corneum at the molecular level and to evaluate the influence of various penetration enhancers on the SC barrier function. The advantage of ATR technique is that it enables elucidation of the extent and mechanism of percutaneous penetration enhancement in vivo in human subjects. But, because the typical range of sampling depth

is 1–2 μm , information obtained from an ATR spectrum pertains only to the immediate layer in contact with the crystal (i.e., the superficial strata of the SC). Information from the deeper regions of the SC can only be obtained through sequential tape stripping.

Penetration enhancer increases the permeation of drug through the human stratum corneum (SC). The SC bioavailability of terbinafine with four different formulations is measured by using FTIR-ATR by stripped tapes from human skin. For validation of the *in vivo* spectroscopic measurements, the amount of terbinafine extracted from the tape strips and is determined by HPLC. The concentration profiles of terbinafine were fitted to the Fick's second law of diffusion, thereby allowing the determination of the characteristic diffusion and partitioning parameters of the permeating drug. Thus, studies shows that FTIR-ATR in conjunction with HPLC allows the estimation of drug bioavailability in the SC and may be useful in the critical evaluation of bioequivalence of topical formulations. However, the disadvantage of this method is the tape stripping technique, is that it would assess only the superficial layers of the skin.

FTIR-ATR has also been used to study biophysical parameters of human SC *in vivo*, such as hydration, lipid composition, and protein composition in conjunction with tape stripping, independence of the depth into its layered structure. The spectral differences between sebum and SC were also elucidated. The level of skin hydration *in vivo* has been studied by FTIR-ATR and employed for evaluation of the occlusive properties of monolayer self-adhesive patches.

The effect of iontophoresis and permeation enhancer on the transdermal delivery of a model peptide through the human epidermal membrane *ex-vivo* is studied; the structural changes in the SC membrane were monitored by FTIR-ATR. They observed that there were shifts in the wave number positions of the asymmetric and symmetric C–H stretching vibration in the order of a few cm^{-1} . Because of the complex nature of the SC, the ATR probes only the superficial layer of the membrane, the interpretation of these findings should be done cautiously.

D. Drug release from semisolid formulations studied by FTIR-ATR^{1,10}

The drug release from semisolid is studied by FTIR-ATR. The drug release from suspensions involves the stepwise process. First, dissolution of the solid drug particles in the liquid phase of the vehicle, characterized by the dissolution coefficient, and then diffusion of the dissolved drug through the heterogeneous vehicle, described by an effective diffusion coefficient. Changing the portion of the liquid phase of the vehicle, it is possible to vary the values of these two coefficients.

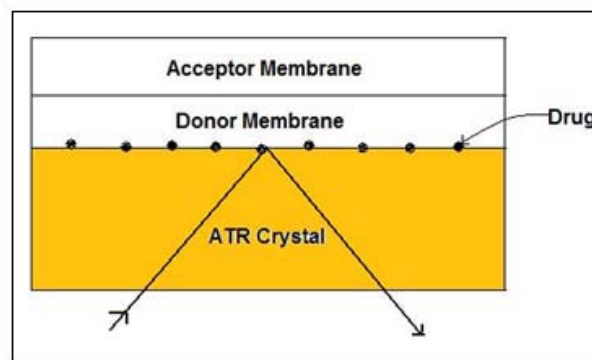


Figure 8: Sample arrangement for the drug release experiment using ATR technique

The formulation is placed on the ATR crystal and the membrane is used as acceptor compartment on the top of the ointment. In release studies, the arrangement is inverted i.e. donor compartment is in contact with IR beam (**Figure 8**). The IR beam is allowed to pass through the crystal and a change in drug content in ointment near the surface during release process is monitored. Using FTIR-ATR the release pattern of Ketoconazole is studied and dodecanol-collodion membrane is used as acceptor membrane and Vaseline as an ointment base. The FTIR-ATR spectra is recorded, the intensity of IR bands of Ketoconazole decreases in the course of release. For quantifying the release of the ketoconazole, the multivariate analysis is applied, thereby the calibration is performed with the ATR spectra of sediments of various mixtures of liquid paraffin and ketoconazole (0%, 2%, 5%, and 10% wt/wt). The decrease of the drug content near interface as function of time is measured. AUC reveals the 'dermal bioavailability' of the drug⁷.

E. Distribution of active drug and excipients is studied by FTIR^{11,12}

FTIR has wide application in visualizing the distribution of active drug and excipients. The distribution of different components (APIs and excipients) in a tablet matrix affects the physical and chemical properties of the tablet, such as hardness and robustness, adhesion to the tablet punch, dissolution properties of the tablet, and drug release. To understand the manufacturing processes and the effects of changes in a defined formulation matrix, it is important to know the distribution of each ingredient and to visualize the composition of a pharmaceutical formulation. Traditional spectroscopic techniques analyze the sample in bulk and determine an average composition across the entire sample and cannot directly determine the spatial distribution of the components in the final product. Mapping and imaging approaches have gained more importance than the traditional methods.

F. Study of Surface Cracking and Swelling Behavior of a Pharmaceutical Tablet Formulation Using FT-IR Chemical Imaging Analysis¹³

During development of a tablet dosage formulation, surface cracks were observed in experimental tablets when tablets were exposed directly to heat and high

relative humidity for an extended period of time. The nature of these surface cracks was than analysed by FTIR technique. Tablets manufactured by different processing conditions were stored at 40°C/75%RH. They were sectioned to give a smooth flat surface suitable for FT-IR imaging analysis. The samples were glued into the sample holder. The images of the distribution patterns of various excipients and active ingredient inside tablets with and without surface cracks was observed. The major difference appeared in distribution patterns of cross povidone within the tablets, suggesting that the cracking issue is related to the occurrences of large particles or aggregates of cross povidone. Therefore, the formulation and process were redesigned to improve the product quality and performance.

G. Thickness of hydrated polymer-on-polymer coatings is studied using FTIR-ATR^{14,15}

Hydrated polymer coatings are common for biomedical applications, such as tissue engineering constructs, contact lenses, and catheters. The thickness of the coatings affects the mechanical behavior of the systems and the cellular response. To measure the coating thickness is quite challenging using conventional methods. So, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is used to determine the relative thickness. This technique was successfully employed to determine the hydrated thickness of a series of cross linked tetraglyme coatings on ultrahigh molecular weight polyethylene substrates intended to reduce wear of acetabular cups in total hip replacements. The hydrated coatings ranged from 30 to 200 nm thick.

H. Blend homogeneity of drug-excipient mixtures is studied by micro spectroscopic FT-IR mapping¹⁶

A Fourier transform infrared (FT-IR) micro spectroscopic mapping system is used to assess the blend homogeneity of a pharmaceutical powder blend. The captopril as a model drug and micronized ethyl cellulose (EC) as a direct-compressible model excipient were mixed in a laboratory mill. The two-component mixture were mixed in two different weight proportions (captopril:EC, 1:1 or 10:1). Different blend mixtures sampled were determined by a reflectance FT-IR micro spectroscopic mapping system by varying the mixing time. It was observed that the blend homogeneity increased gradually with increased mixing times, but the powder began to demix or segregate as mixing continued beyond the time when homogeneity is reached. In addition, the sample mixture proportion had an effect on the uniformity of the powder blend. Thus, micro spectroscopic FT-IR mapping technique can be easily used to determine the blend homogeneity in a powder blend mixture.

I. To study the effect and penetration of Hair formulation on hair by FTIR¹⁷⁻²⁰

FTIR has profound application in studying the effect of hair formulation on hair. It is considered as the most

efficient technique to study the effect of various hair formulations on hair. The study of effect of hair care formulation containing sunscreen on hair. The extent of photo damage due to exposure to UV radiation was studied by FTIR. Hair fibers are made up of fibrous proteins known as keratin. Hair also consist minor amount of melanin pigment and lipids. Weathering of hair causes the discoloration of hair due to bleaching of melanin. This effect was due to the oxidation of cystine disulfides to cysteic acid. The most significant chromophores in proteins like Tyrosine, tryptophan and disulphide bond are absorbed at, $\epsilon = 100\text{m}^{-1}\text{cm}^{-1}$, $\epsilon = 4500\text{m}^{-1}\text{cm}^{-1}$, $\epsilon = 40\text{m}^{-1}\text{cm}^{-1}$ respectively. After exposure to UV radiation it was found that there was shift in the band. But after treatment with the hair formulation, the FTIR spectra was measured and it was found that there was again shift of band to original wave number. Thus, the effect of hair formulation is studied by FTIR. FTIR is the also used to study the effect of hair oil on hair. It was found that tryptophan decomposition rate was slower by treating with hair oil. This was found by recording the spectra of the hair.

J. FTIR – in Polymorphism²¹

Fourier Transform Infrared (FT-IR) is an important complementary tool for the solid-state characterization of pharmaceutical solids. Fourier Transform Infrared (FT-IR) is important complementary tools for the solid-state characterization of pharmaceutical solids as different polymorphs has different properties such as solubility, dissolution rate, chemical reactivity, melting point, resistance to degradation, bioavailability which dictate the biodisponibility of the active substance. The characterization is followed by monitoring of the presence of the chosen crystalline form for the active pharmaceutical ingredient and in the corresponding drug product.

K. Polymers²

FTIR is widely used in identification of polymers, study of polymerization process, to characterize polymer structure, to examine polymer surfaces and to investigate polymer degradation processes.

FTIR spectroscopy is used to investigate the miscibility of certain poly (vinyl phenol) (PVPh) ($[-\text{CH}_2-\text{CH}(\text{C}_6\text{H}_4)\text{OH}-]_n$) blends. PVPh has been blended in one case with poly(ethylene oxide) (PEO) ($[-\text{CH}_2-\text{CH}_2-\text{O}-]_n$) and in the other case with poly(vinyl isobutylether) (PVIE) ($[-\text{CH}_2-\text{CH}(\text{OC}_4\text{H}_9)-]_n$). The OH band of PVPh at 3360cm^{-1} shifts to a lower wavenumber near 3200cm^{-1} with increasing concentration of PEO. This change reflects an increase in hydrogen bonding between the PVPh hydroxyl groups and the PEO ether oxygens. Such an interaction indicates that the PVPh/PEO blend is miscible. while the O–H stretching region of the PVPh/PVIE blend. No changes to the hydroxyl group of PVPh are observed at any concentration, hence indicating that there are no interactions between these two polymers, and so this blend is immiscible.



FTIR spectroscopy is also applied for studies of the tacticity of a number of vinyl polymers. Tacticity means the orientation of each monomer adding to the growing chain. There are three stereoisomers observed for vinyl polymers: Isotactic, Syndiotactic, Atactic. The physical properties of polymers are affected by the structures of the molecular chains. Depending on the nature of the polymerization, different polymers are able to form different configurational isomers. So depending upon difference in the IR spectra it is easy to identify the particular isomer which is having good physical property.

L. Biological Applications^{2,4}

Infrared spectroscopy has proved to be a powerful tool for the study of biological molecules and the application of this technique to biological problems is continually expanding, particularly with the aid of sophisticated sampling techniques such as infrared imaging. Biological systems include lipids, proteins, peptides, biomembranes, nucleic acids, animal tissues, microbial cells, plants and clinical samples, have all been successfully studied by using infrared spectroscopy. This technique has been employed for characterization of isolated biological molecules, particularly proteins and lipids. Recently, it is used for the study of more complex systems, such as diseased tissues. Microscopic techniques, combined with other sophisticated analytical methods, allow for complex samples of micron size to be investigated.

Fourier transform infrared spectroscopy (FTIR-spectroscopy) techniques help to investigate microorganisms in biofilms. FTIR-spectroscopy is suitable for the identification of microorganisms and presents a new addition to genetic methods. The FTIR analysis of bacterial isolates provides fingerprint spectra, allowing the rapid characterization of microbial strains. The FTIR-attenuated total reflection (ATR) technique can be used for the observation of biofilms forming directly on the interface of an ATR crystal such as germanium to study interfacial process. A spectrum is acquired non-destructively, in situ and in real time. This method is suitable for fundamental biofilm research, as well as for monitoring of biofilm formation, e.g., in an ultrapure or drinking water systems. FTIR-ATR also allows the rapid analysis of deposits on surfaces, e.g., filtration membranes. The analytical discrimination between microorganisms, inorganic material can be obtained. FTIR has been used to study the secondary structure of the proteins and peptides. Thus it allows modeling the structure of the receptor that binds to it. FTIR is also used to study the protein structure in aqueous and non aqueous medium as it give characteristic IR spectra band in aqueous and non aqueous medium. It serves as a tool to study the stability of protein in both aqueous and non aqueous media²².

M. FTIR is used in Diseases Diagnosis^{2,23}

Recently, infrared spectroscopy has emerged as a powerful tool for characterizing tissue and disease diagnosis. The pressure-dependence parameters such as

wave number, intensity and band shape provides further information about the structural changes associated with malignancy. It has also been used in diagnosis of many diseases such as cervical cancer, Parkinson's disease, Alzheimer's disease, kidney stone, arthritis.

It is possible to detect the cervical cancer by using infrared band differences among normal, dysplastic and malignant cervical cells. It is also one of the approaches for diagnoses of kidney stone by examining the form of the calcium oxalate in urine. If high proportion of calcium oxalate monohydrate is obtained then the ratio of calcium/oxalate is low this indicates the high oxalate content in urine which is linked to primary hyperoxaluria, inflammatory bowel disease or high intake of oxalate-rich food. FTIR is also used to study the the metabolic changes that occur with the onset of Parkinson's. This technique is not only used as tool for diagnosis of patients of Parkinson's but also as a tool to monitor the progression of the disease during clinical trials. Microscopic FTIR is also used to study the structural changes associated with lung cancer and tuberculous cells in pleural fluid. Infrared spectra demonstrate significant spectral differences between normal, lung cancer and tuberculous cells and the ratio of the peak intensities of the 1030 and 1080 cm⁻¹ bands (mainly in glycogen and phosphodiester group so of nucleic acids) differs greatly between normal and lung cancer samples.

CONCLUSION

Fourier transform concept, special techniques such as FTIR-ATR, FTIR-PAS, and FTIR-micro spectrometry are now at the disposal of pharmacists Nowadays, FTIR-ATR is a well-established standard method used to study drug release in semisolid formulations, drug penetration, and influence of penetration modifiers and in vivo studies. FTIR-PAS has been applied to measure drug content in semisolid and solid formulations, to determine drug penetration into artificial membranes and into biological systems such as isolated stratum corneum. The big advantage of this technique is the possibility of spectral depth profiling. However, FTIR-PAS is so far limited to in vitro investigations. So, FTIR imaging shows great promise in its ability to visualize the drug and excipient distribution in pharmaceutical formulations such as tablets and therapeutic transdermal systems, as well as to reveal mechanism of drug release. Furthermore, this unique technique offers completely new possibilities to study lateral diffusion of drugs in relevant polymeric systems, in semisolid formulations, in artificial membranes, and, in the future, may be in biological systems. There is a hope that the spectroscopic techniques presented in this paper will be modified and improved in such a way that relevant processes in drug delivery can be studied *in vivo* in the near future.



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