IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Study Of Tofacitinib Citrate By Using Fourier Transform Infrared

Dindule Ashok Shrikisan

Prof Bhosale.B.S
Dr.Prachi Udapurkar

Department of Pharmacy,Kishori college Of Pharmacy

ABSTRACT:

Tofacitinib citrate, a Janus kinase inhibitor used in the treatment of rheumatoid arthritis and other inflammatory conditions, was analyzed using Fourier-transform infrared (FTIR) spectroscopy to elucidate its structural characteristics and confirm its chemical composition. The FTIR spectra of tofacitinib citrate were recorded in the range of 4000–400 cm^-1, revealing distinct absorption bands corresponding to various functional groups present in the molecule. The characteristic peaks at 3310 cm^-1 (N-H stretching), 2920 cm^-1 (C-H stretching), 1650 cm^-1 (C=O stretching), and 1240 cm^-1 (C-N stretching) were identified, confirming the presence of amide, aromatic, and aliphatic structures. This spectroscopic analysis provided a non-destructive and rapid method for the qualitative assessment of tofacitinib citrate, ensuring its purity and aiding in the quality control of pharmaceutical formulations. The results demonstrated the utility of FTIR spectroscopy in the pharmaceutical analysis of complex molecules like tofacitinib citrate.

INTRODUCTION

FTIR spectroscopy works by measuring how much infrared radiation is absorbed by a sample at different wavelengths. The resulting spectrum represents a molecular fingerprint of the sample,with distinct absorption peaks corresponding to different functional groups within the molecule.This makes FTIR an invaluable tool for identifying unknown compounds, studying molecular ineractions, and monitoring the purity and stability of pharmaceuticals.

Fourier transform infrared (FTIR) spectroscopy is a form of vibrational spectroscopy that is useful in the study of a variety of soil chemical processes. In the mid-infrared (mid-IR) range, vibration sarise from many environmentally important molecules such as organic acids, soil organic matter,mineral phases, and oxyanions. It is possible to utilize FTIR spectroscopy as a quantitative analytical method and also as a tool to determine bonding mechanisms in solids andon surfaces.Molecular vibrations can be related directly to the symmetry of molecules, and so it

is often possibleto determine precisely how a molecule is bonding on surfaces or asacomponent in asolidphase from its infrared spectrum. Many experimental methods exist for probing samples of various statesandat different spectral regions

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Spectroscopy is a powerful analytical tool used for identifying and characterizing chemical compounds. It works by measuring how infrared radiation is absorbed by a sample, providing information about the molecular composition and structure of the material.

KeyAspectsofFTIR:

Principle:

Infrared light is passed through a sample.Molecules in the sample absorb specific wavelengths of the IR light, causing vibrations within the molecule.The resulting spectrum shows absorption peaks corresponding to different vibrational modes of the molecules, which are characteristic ofspecific functional groups.

Fourier Transform: A mathematical process (Fourier Transform) is used to convert the raw data(interferogram) into an actual spectrum.This process enhances the speed and accuracy of spectral data collection.

Components of an FTIR Spectrometer:

Source: Infrared light source. Interferometer: Splits the IR light into two beams, modulates them, and recombines them to createan interferogram.

Sample Holder :Holds the sample in place (can be A kbr pellet, ATR crystal, etc.).

Detector: Detectsthetransmittedorreflectedlightandgeneratesaninterferogram.

Applications of FTIR:

- ❖ Chemical Identification: Identifying unknown compounds by comparing their spectra with reference spectra.
- ❖ Functional Group Analysis: Determining the functional groups present in a molecule.
- ❖ Material Characterization: Analyzing polymers, coatings, pharmaceuticals, and othermaterials.
- ❖ Quality Control: Ensuring the consistency and purity of products in manufacturing.
- ❖ Environmental Analysis: Detecting pollutants and analyzing environmental samples.

FTIR is widely used in various fields, including chemistry, biology, materials science, and environmental science, due to its ability to provide detailed information about the molecular composition of samples.

Application of FTIR Spectroscopy to Tofacitinib Citrate

Tofacitinib citrate is a Janus kinase (JAK) inhibitor used primarily to treat rheumatoid arthritis andother autoimmune conditions. The detailed molecular structure of tofacitinib citrate can be characterized using FTIR spectroscopy to ensure its quality and consistency during manufacturingandformulation.

Stepsin FTIR Analysis of Tofacitinib Citrate:

Sample Preparation: Solid-state samples like tofacitinib citrate can be prepared by grinding thedrug to a fine powder and pressing it into a pellet with potassium bromide (KBr). Alternatively, asmall amount of the drug can be dispersed in a liquid or directly applied onto the ATR (AttenuatedTotalReflectance)crystal.

Data Collection:

The prepared sample is exposed to an infrared beam, and the transmitted or reflected light is collected and analyzed.The FTIR instrument generates an interferogram, which is then mathematically transformed into a spectrum via Fourier Transform. Spectrum Interpretation: Theresulting spectrum is analyzed to identify characteristic absorption peaks. For tofacitinib citrate,the sepeakscorrespondtovariousfunctionalgroupslikeamides,aromatic rings,andnitrile groups.

Quality Control:

FTIR spectracan be compared against reference spectrato verify the identity and purity of the tofacitinib citrate. It can also detect any potential contaminant degradation products.

FTIR spectroscopy,which stands for Fourier Transform Infrared Spectroscopy ,is a technique used to obtain the infrared spectrum of absorption or emission of a solid, liquid, or gas sample. It works by collecting an interferogram of the sample signal using an interferometer, and then performing a Fourier Transform (FT)on thisinterferogram to obtain thespectrum. **FTIR spectroscopy hasseveral key applications and benefits:**

Versatility: It can be used to analyze a wide variety of materials, including organic and inorganic compounds, polymers, and biological samples (Agilent) (Thermo Fisher). Applicable to various forms of the drug(solid, liquid, and gas).

if needed. **Quick and Efficient**: Rapid data collection and analysis provide timely results.

High Sensitivity: Capable of detecting evenminor changes in the chemical composition

Speed and Sensitivity: FTIR spectrometers are known for their speed and sensitivity, making them suitable for both qualitative and quantitative analysis (ThermoFisherScientific).

SamplePreparation:Differentsamplingtechniquessuchastransmission,AttenuatedTotalReflect ance (ATR),and diffuse reflectance can be employed depending on the nature of thesample.ATRisparticularlypopularbecauseitrequiresminimalsamplepreparation(HighValSci MatResDxSol) (Thermo Fisher)Overall, FTIR spectroscopy is a powerful andversatiletoolwidelyusedinvariousfieldsincludingpharmaceuticals,materialsscience,environ mentalmonitoring,andforensics.Tofacitinibcitrate,amedicationusedprimarilyfortreatingrheumat oidarthritisandotherautoimmunediseases,canbeanalyzedusingFourierTransform Infrared (FTIR) spectroscopy. The FTIR spectrum of tofacitinib citrate would provid evaluable information regarding its molecular structure and the functional groups present within the compound. Here's how FTIRspectroscopy is typically applied to tofacitinib citrate:

SamplePreparation:Thetofacitinib citrate sample is prepared, often by grinding it with potassium bromide (KBr) and pressing it into a pellet, or by using other suitable methods like ATR(Attenuated Total Reflectance) if the sample preparation allows.

Spectrum Acquisition: The FTIR spectrometer records the spectrum by passing infrared radiation through the sample. The molecule absorbs specific wavelengths of the IR radiation, corresponding to its vibrational modes.

Spectrum Analysis: The resulting FTIR spectrum displays characteristic absorption peaks thatcorrelate with different functional groups in the tofacitinib citrate molecule. These peaks can

C-HS tretching: Typically observed around2800-3000 cm^{-1}.

N-HS tretching: Peaksaround 3200-3400cm^{-1},indicativeofamine groups.

C=OStretching: Characteristic absorption near1650-1750cm^{-1},indiative of carbonyl groups.

C-NStretching: Peaks typically appear around1200-1350cm^{-1}.

Comparative Analysis: The obtained spectrum is compared with reference spectra or literature data to confirm the identity of the compound and ensure its purity.

groups present in tofacitinib citrate, such as aromatic rings, amides, amines, and other relevantstructures.

Analyzing the FTIR spectrum of tofacitinib citrate helps in:

Verification of Compound: Ensuring the compound is indeed tofacitinib citrate.

Quality Control: Assessing the purity and identify in ganypotential impurities.

Structural Information: Confirming the presence of specific functional groups and understanding the molecular interactions within the compound.

Material Characterization: FTIR is widely used to analyze polymers, composites, and thermaterials to determine their composition and assess their quality.

Monitoring Chemical Reactions: It can be used to monitor the progress of chemical reactions inreal-time by observing changes in the IR spectrumas reactants are converted to products.

Non-Destructive Testing: FTIR spectroscopy is non-destructive, meaning the sample remains intactand unaltered, which is essential for precious or limited samples.

Environmental Analysis:It is used to detect pollutants and analyzes amples from the environment, such as water, air, and soil, for contaminants.

INSTRUMENTATION

FIG.1.FTRISPECTROSCOPY

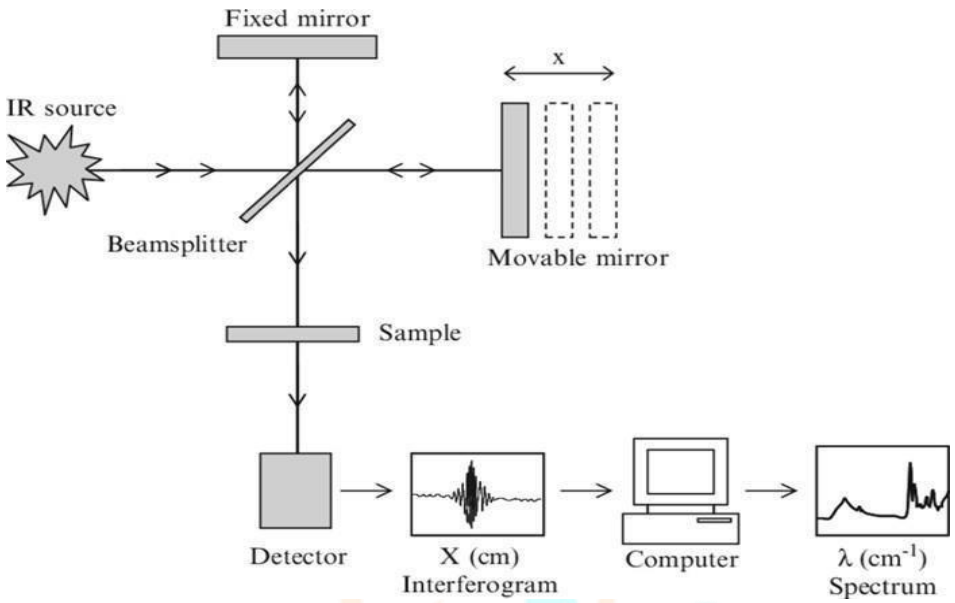


FIG1.2.INSTRUMENRATION OF FTIR

Infrared spectrometers are composed of the same basic components as in the ultraviolet and visible regions, although the source of radiation, detectors and the materials used in the fabrication of theoptical components are different. The standard infrared spectrophotometer is a filter-grating or prism-grating instrument covering the rangefrom4000-650cm[21] (02.5-15.4 μ).The grating instruments offer high resolution that permits separation of closely spaced absorptionbands, accurate measurements of band positions and intensities and high scanning speeds for agivenresolution and noise level.

The radiation from a source emitting in the infrared region is interrupted (ie. chopped, pulsed ormodulated) at a low frequency level (10-26 Hz) and is passed alternately through the sample andthe reference. This minimizes the effect of stray radiations emerging from the sample and

cellbefore it reaches the detector. The temperature and humidity affects the performance of infraredspectrophotometer. The following are the essential components of an infrared spectrophotometer.

1. Light source
2. Mono chromator and optical materials
3. Sample holder
4. Detector and
5. Instrument for recording the response (Recorder)

Infrared Radiation Sources

The infrared radiation sources are the hot bodies, continuously emitting the radiations, which approximate a black body radiator in their emission properties.

(a) Incandescent lamp:

A closed wound nichrome coil can be raised to incandescence by resistive heating. A black oxidefilm formed on the coil give acceptable emissivity. In this, the temperature can be reached up1100°C. The nichrome coil does not require water cooling. It requires little or no maintenance andgives long service. This source is recommended where reliability is essential. Though this source issimpleand rugged, it is less intensethan some other infrared radiation sources.

A rhodium wire heater sealed in a ceramic cylinder has also been used as a source of infrared radiations. **(b)**

Nernst glower:

In IR spectroscopy. Nernst glower is the most commonly used source of radiation. It is constructed by fusing a mixture of oxides of metals like zirconium, yttrium and thorium. They are moulded inthe form of hollow tubes or rods about 1-3 mm in diameter and 2-5 cm in length. The ends of therods are cemented to short ceramic tubes for mounting and short platinum leadsare provided for power connections. Nernst glowers are fragile.

Theyhavenegativecoefficientofresistanceandtheyarepreheatedtobeconductive.Thus,theyare provided with auxiliary heaters. To prevent overheating they are provided with ballast, but theyshould also be protected from draught even as ventilation is needed to remove surplus heat. Theenergy output of Nernst glower is predominantly concentrated between 1-10 μ with relatively lowenergy beyond 10 μ. Radiation intensity is approximately thrice that of nichrome and globarsources, except in the near infrared region. The main advantages of Nernst glower are that

it emitsinfrared radiations over wide wavelength range and the intensity of radiation remains steady and constant over along period oftime; second, it canbeusedin airas it is not oxidized.

(c) Globar Source:

It is a rod of sintered silicon carbide 6-8 mm in diameter and 50 mm in length. It is self starting and is electrically heated. The operating temperature is about 1300°C. It has a positive coefficient of resistance and can conveniently be controlled with a variable transformer. It is often enclosed in a water cooled brass tube, with a slot provided for the emission of radiations.

It emits maximumradiationat5200cm³IncomparisonwithNernstglowertheGlobarisalessintensesourcebel ow 10.Thetwo sourcesarecomparable to about15 μ,and the Globaris superior beyond about 15 μ.

(d) Mercury Arc:

Intheveryfarinfraredregion;ie.beyond50μ(200cm³),blackbodytypesourcesloseeffectiveness as their radiations decrease with the fourth power of wavelength. Mercury arc givesintense radiation in this region. It is enclosed in a quartz jacket to reduce loss. The output frommercury arc is similar to that of black body sources, but additional radiation is emitted from plasmawhichenhances the longwavelength output.

(e) Tungsten Filament Lamp:

This source is useful for near infrared region only.

Monochromator

The radiation source emits radiations of various frequencies. As the sample in IR spectroscopy absorbs only at certain frequencies, it is therefore necessary to select desired frequencies from the radiation source and reject the radiations of other frequencies. This selection is achieved by means of mono chromator .Themonochromators are of two types(a) Prism monochromator and(b) Grating monochromator

Prism Monochromator.

These are favoured because of greater range and simplicity. Neither glass nor quartz is sufficiently transparent to infrared radiations and therefore other materials like halogen salts are used in prism monochromators as they are transparent to infrared radiations. The bulk of

All the commonly used prism materials except quartz are water soluble and are easily scratched.These materials must be protected from moisture either by using desiccants or by placing in a sealed housing which is evacuated.In the infrared spectrometers the focusing of the radiations isachieved by using concave mirrors rather than prisms. These mirrors can be prepared from variousmaterials like metals or glass coated aluminium. The main advantage of these materials is thatmaterials have no chromatic aberration and are sturdy. Besides concave mirrors plane reflectingmirrorsarealso used.

The prism monochromator may be a single pass monochromator or a double pass monochromator as shown in Figs. 3 and 4respectively.

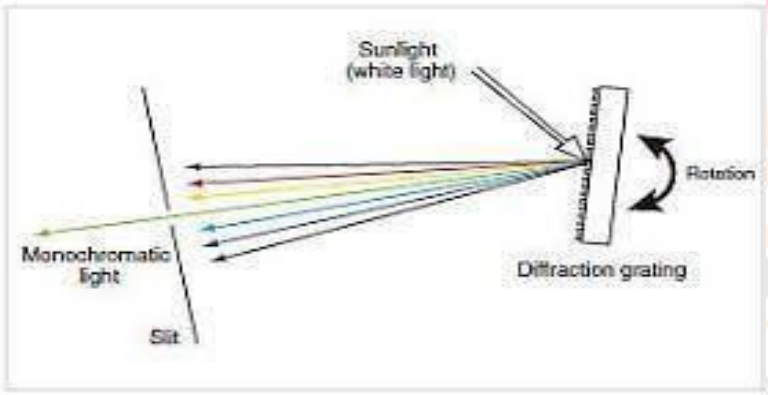


FIG1.3.SINGLEPASSMONOCHROMOMETER

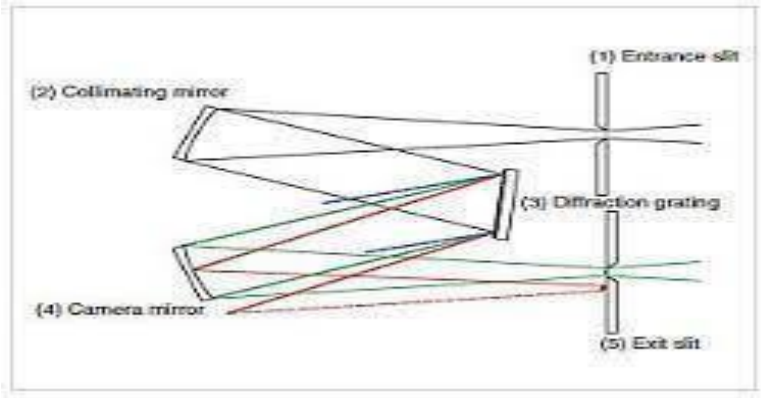


FIG1.4.DOUBLE PASS MONOCHROMOMETER

1)Single pass monochrashator.

The sample iskeptator near thefocusof the beam,justbefore theentrance slitA tothemonochromator. The radiation from the source after passing through the sample and the slit strikesthe off-axis parabolic Littrow mirror 3. This renders the radiation parallel and is transmitted to theprism 'C'. The dispersed radiation after reflecting from a plane mirror 'D' returns through the prismsecond time and focuses into the exit slit of the monochromator and then to the detector part of theinstrument.

(ii)Double pass monochromator:

In the double pass monochromator, there occurs a total of four passes of radiation through prism as shown (1) (2) (3) and (4) in the Fig. 1.4 The double pass monochromator produces more resolution of radiation than single pass monochromator.

In both single and double pass monochromators, sodium chloride (rock salt) prisms are employed for the entire region from 4000-650 cm^3 (2.5 to 15.4 μ). Prisms of lithium fluoride and calcium fluoride give more resolution in the region where the significant stretchingvibrations are located.

(a) Grating monochromator:

The grating is essentially a series of parallel straight lines cut out into a plane surface. It is usually constructed from glass or plastic which is coated with aluminium. To minimize greater amounts of scattered radiations and the unwanted radiations of other spectral orders, the gratings are blaze to concentrate the radiation into a single order. A grating is generally used In combination with a small prism which acts as order sorter. Sometimes filters transparent over a limited wavelength range are incorporated with gratings. Grating monochromator has certain advantages over prismmonochromator as (a) the grating construction material is not attacked

Sample Holders (SampleCell) and Sampling of Substances

As solvents used to prepare sample solutions have the tendency to absorb the infrared radiation, sample cells or sample holders are usually of much narrower (0.1-01 mm) than the one used invisible or ultraviolet region. The sample cells are usually constructed using pickle salt (sodium chloride). The sample cells are demountable and teflon spacers are used along with sample cell to adjust the path lengths. Fixed path length cells are also available and they can be filled or emptiedwithhypodermicsyringe.Asthesamplecellsaremadeofalkalimetalsalts,theybecomefoggy due to moisture and thus they need polishing with buffing powder to render them useful again.

The sampling of the substance in infrared spectrophotometry depends upon the state of the sample,ie whether it is gas, liquid or solid. Depending upon the nature, various sampling techniques have been developed and used. The inter-molecular forces of attraction are more operative in solid phase than in gases. The sample of the same substance shows shift in the frequencies of absorption as it passes from the solid to the gaseous state. In some cases, additional bands are also observed with the change in the state of the sample. Therefore, it is always important to mention the state of the sample and the solvent to be employed for scanning in the infra region for correct interpretation of spectra. The samples whose spectra are to be recorded must be pure and free of water.

Sampling of solids:

Solid whose infrared spectra are to the recorded can be sampled invarious ways:

1. Solid dissolved in solvent:

The solid samples are usually dissolved in a suitable solvent and this solution is used in one of thecells. This method cannot be used for all solids because suitable solvents are limited in number andgenerally no single solvent is transparent throughout the infrared region. The commonly usedsolventsarecarbontetrachloride,chloroform,alcohols,acetone,cyclohexaneandcarbondisulp hide. Sometimes

two solvents have complementary absorption region are used to cover the complete wavelength region. When the solutions of solids are used for scanning in the infrared region, the absorption due to solvent has to be compensated by keeping the solvent in a cell of same thickness as that of sample in that path of reference beam of a double beamspectrophotometer.

2. As solid film:

In this technique, sample solution is placed on the surface of a potassium bromide or sodiumchlorideandthesolventisallowedtoevaporate.Thus,thesolidsampleformsathinfilmonthe

3. Mulltechnique

In this technique, the solid sample is mixed with heavy mineral oil (Nujol) to form a paste. Thispasteisthensandwichedbetweentwosaltplatesandthenusedforspectralmeasurement.Althoug h Nujol is transparent in most parts of the infrared region but it has absorption maxima at2915, 1462, 1376 and 719 cm^3 This is the drawback in using Nujol for certain compounds which may have absorption in the region similar to Nujol. This technique Is mostly used for qualitativework and not for quantitative estimations.

3. Pressed pellet technique (Disk method):

This technique is frequently used for the qualitative work. In this, a small amount of finely ground solid sample (dried) is intimately mixed with about 100 times its weight of powdered potassium bromide (IR grade and thoroughly dried) in a small agate pestle mortar. This mixture is pressed under a high pressure (25000 psi/g) in an IR tablet press to form a small pellet or tablet. There sulting pellet is transparent to infrared radiation and can be used as Such This technique has some advantages over the Nujol Mull method. It eliminates the problem of bands which appear inIR spectrum due to use of Nujot () The potassium bromide pellet if preserved properly can be reused for recording the spectra if required again, (ii) The resolution of spectrum in potassiumbromidepelletissuperiortotheoneobtainedinNujolmulltechnique.Onedisadvantageass ociated with this technique is that as high pressure is involved in the preparation of pellet, there could be polymorphic changes in the crystalline of samples like inorganic complexe that cancause complications in the IR spectrum.

The sample free from moisture in the form of thin layers in variety of absorption cells. Various types of cells like sandwich cell demountable cell and cavity cells are available for handling liquid samples. These cells are made up of sodium bromide, potassium bromide or thallium bromide. In demountable cell the salt plates are usually separated by a gasket and held together by a clamp The thickness of liquid layer can adjusted by spacers. The sample
thickness should be such that thetransmittanceliesbetween15-70percent.Usuallyformostoftheliquidslayerthicknessof0.01- mm is quite satisfactory. Sometimes the liquid samples can be dissolved in a suitable solventand scanned in the infrared region using any suitable cell. In double beam spectrophoto meter,'matched cells are generally employed. In one cell sample solution is placed while in other the solvent employe dis placed. The cells usd in this must have the same thickness. These sample cells must be protected from moisture.

DETECTORS

Infrared Spectroscopy

Instrumental Methods of Analyst

There are two types of detectors used in infrared spectrophotometry (thermal detectors and (b)photo-detectors.

(a)Thermal detectors:

When the infra radiations falls on these detectors, they cause heating which gives rise to a potentialdifference which is measured. This potential difference depends upon the amount of radiation. Thethermal detectors commonly used are thermocouples, bolometer and thermistors and Golay cell orGolaydetector

(1)Thermocouple:

It is the most commonly used detector in fred spectrophotometry. Thermocouples are basically thedissimilar strips of metals joined together at one end. Thermocouples are constructed in variouswaysinoneofthethermocoupledetectorstwofinewiresofmetalswhichhavedifferentthermo electrical properties are welded with blackened gold foil and which absorbs the radiations.One welded joint (cold junction) is kept at constant temperature and the other welded joint (hotjunction) is exposed to radiations. This exposure of not junction causes a thein is temperature.Thus, as the two junctions are at different temperatures, it causes a potentialdifference which

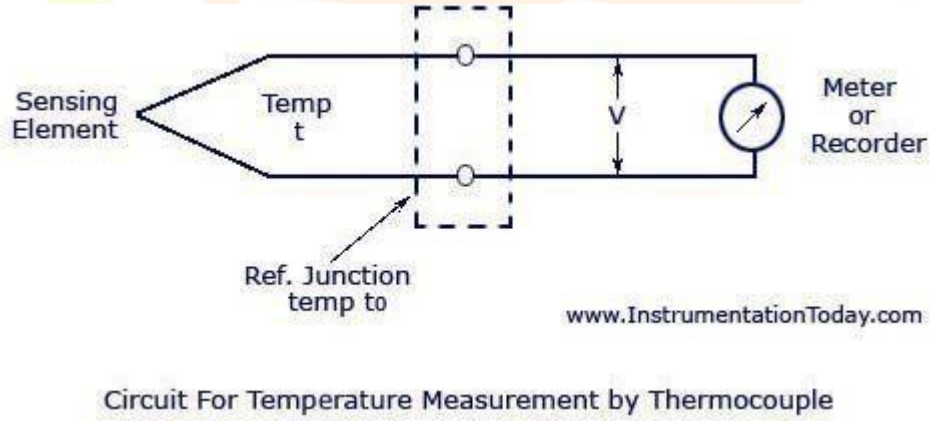


FIG 5 THERMOCOUPLE

(ii) Bolometer:

They are constructed from metals or semiconductors. In this large change of electrical resistance depends on temperature When the radiations fall on bolometer, there is temperature change which causes change in the resistance of the conductor. This change in resistance depends upon theamount of radiations falling on the bolometer.Bolometer is made in one arm of the Wheat stonebridge and a similar stof metal is used as balancing arm of the bridge, which is not exposed to infrared radiations when no infrared radiations fall on the bolometer, the bridge remains balanced .As the radiations fall on the bolometer, the bridge becomes unbalanced due to change in electrical resistance and thus the electrical current flows through galvano meter G. The Lourt of current flowing

through galvanometer is a measure of the intensity of the radiations falling on the detector There sponse time for boiometeris 4 msec. The schematic representation is givenbelow

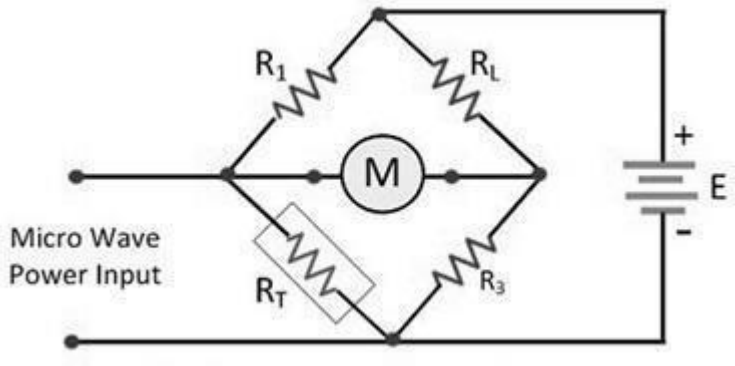


FIG:6 BOLOMETER

Gas samples are examined in infrared region after removing the moisture or water vapours. Thedriedgasesareintroducedviaastopcockandasystemwherebyapartialpressureofabout5-50mm of mercury can be applied. The gas sample is introduced into the gas cell which is made up ofglass or a metal cylinder of about 10 cm long. The end walls of the gas cell are made of sodiumchloride. For measuring very low concentration of gases long path cells are required. However, thesampling area of most spectrophotometers is restricted in length. The gas cell is equipped withmirrorsandusedtobringaboutmultiplereflectionstoincreasetheeffectivepathlength.Sometimes the GLC is coupled with IR spectrophotometers to analyse elutes from GLC, for this purpose special cells are designed Infrared Spectroscope instrumental Methods of Analyste

There are two types of detectors used in infrared spectrophotometry (thermal detectors and(b)photo-detectors.

(A) Thermal detectors:

When the infra radiations falls on these detectors, they cause heating which gives rise to a potential difference which is measured. This potential difference depends upon the amount of radiation. Thethermal detectors commonly used are thermocouples, bolometer and thermistors and Golay cell or Golay detector

(1) **Thermocouple**:

It is the most commonly used detector infred spectro photometry. Thermocouples are basically the dissimilar strips of metals joined together at one end. Thermocouples are constructed in variouswaysinoneofthethermocoupledetectorstwofinewiresofmetalswhichhavedifferentthermo electricalproperties are welded with blackened gold foil and which absorbs the radiations.One welded joint (cold junction) is kept at constant temperature and the other welded joint (hot junction) is exposed to radiations. This exposure of not junction causes a thein is temperature.Thus, as the two junctions are at different temperatures, it causes a potentialdifference which isproportionaltodegreeofheatingofhotjuncionforamountofradiationsfallingonthehotjunction)

(III) Thermistors:

These function similar tobolometer.They are the resisters made by fusing several metallic coxides. These show a negative thermal coefficient of electrical resistance.

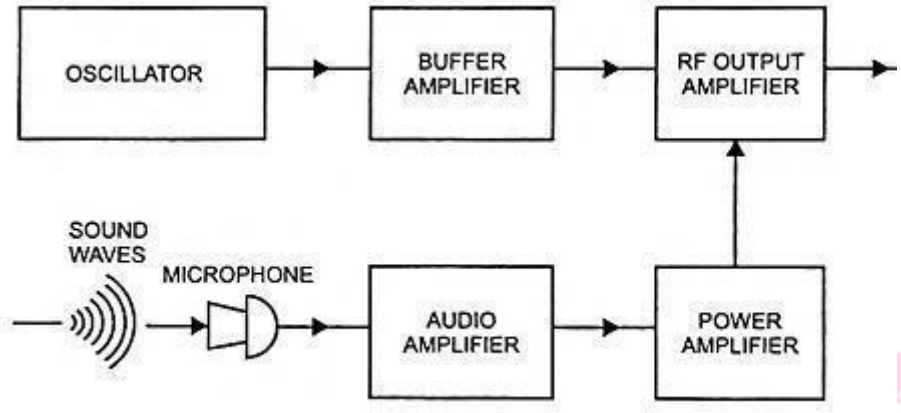


FIG.

(iv) Golaycellor Golay detector:

Golay cell is now-a-days used in several commercial spectrophotometers. It consists of a small metal cylinder, one end of which closed by blackened metal plate and the other with a metalized diaphragm. A light bearn falls on the diaphragm which reflects to phototube. The cylinder is filled with non-absorbing gas like xenon. When the radiations fall on blackened metal plate, it is heated, which causes the expansion of gas, this in tum affects the diaphragm (motion of the diaphragm).This causes the change in the output of cell received by the phototube, which can be modulatd according to the power of the falling radiations on Golay cell. Thermocouples and Golay detectors possesses similar sensitivity in the mid infrared region.

(a) Photon detectors:

Photon detectors are widely used in near infrared region. They consist of suitable semiconductors like lead sulphide, lead telluride or germanium which are non-conducting at lower energy state. When the radiations fall on these they are raised to higher level which can conduct and produce a signal which is proportional to the amount of radiation. In these there is a drop of electrical resistance and if small voltage is applied there is a large increase in current which can be amplified and indicated on a meter or recorder.

Recorder

In infrared recording spectrophotometers as the sample absorbs some energy, the sample beam and reference beam differ in their radiant energies. Then detector system generates the signal which is normally amplified and goes to servo meter. The servo meter which is connected to attenuator comb blocks the part of reference beam till energies of reference and sample beams

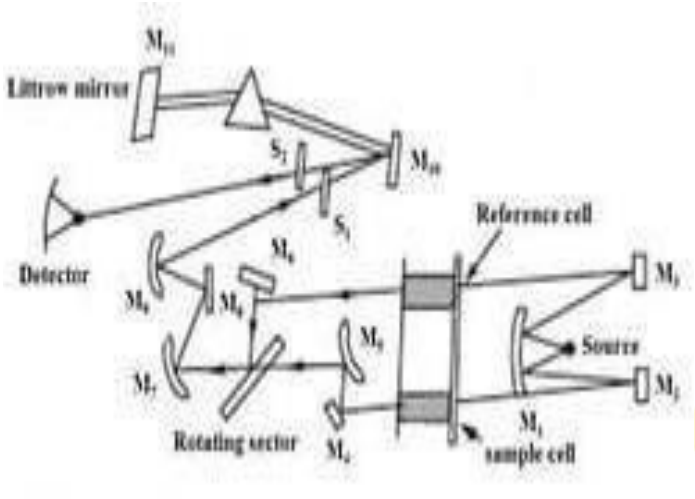


FIG .7BLOCK DIAGRAM OFINFRARED IRSPECTROPOTOMETER

AIM AND OBJECTIVES

Fourier Transform Infrared (FTIR) spectroscopy is a powerfull analytical technique used to obtain the infrared spectrum of absorption, emission, and photo conductivity of a solid ,liquid, or gas. The main objectives of FTIR spectroscopy include:

Molecular Identification :Identifying organic and inorganic compounds by their characterist infrared absorption spectra, which provides a molecular fingerprint unique to each substance.

· Functional Group Analysis: Determining the presence of specific functional groups in a molecule, as different functional groups absorb infrared radiation at characteristicwavelengths.

· Quantitative Analysis: Measuring the concentration of a substance in a mixture by analyzing the intensity of its characteristic absorption bands.

· Structural Elucidation: In ferring the molecular structure of a compound,including information about molecular symmetry ,geometry, and bonding.

· Qualitative Analysis: Providing qualitative information about complex mixtures, helping to identify the components and understand the sample's composition.

· Monitoring Chemical Reactions: Observing changes in the infrared spectrum over time to study reaction kinetics and mechanisms, and to monitor the progress of a chemical reaction.

· Material Characterization: Characterizing materials in various fields, such as polymers ,pharmaceuticals, and biological samples, to determine purity, composition, and structural integrity.

· Environmental Analysis: Detecting and quantifying pollutants and contaminants in environmental samples such as air, water,and soil.
· Sample Preparation: Obtainand prepare samples for analysis.
· Instrument Setup: Ensure the FTRI spectrometeris properly calibrated and ready for use. Data Analysis: Process and interpret the spectral data.

· Report and Documentation: Compile the finding to a comprehensive report.
· Identify Samples: Determine the samples to be analyzed.
· Preparation: Clean and,if necessary, grind samples to ensure homogeneity.
· Standard Preparation: Prepare calibration standards if required.
· Labeling: Clearly labelall samples and standards.
· Baseline Measurement: Runa blank sample to establish a baseline.
· Validation: Perform a quick validation run using a known sample to ensure the.

LITERATURE REVIEW

1. Fourier transform infrared (FTIR) spectroscopy

R. Hienerwadel et al Fourier transform infrared (FTIR) spectroscopy probes the vibrational properties of amino acids and cofactors, which are sensitive to minute structural changes. The lack of specificity of this technique, on the one hand, permits us to probe directly the vibrational properties of almost all the cofactors, amino acid side chains, and of water molecules. On the other hand, we can use reaction-induced FTIR difference spectroscopy to select vibrations corresponding to single chemical groups involved in a specific reaction. Various strategies are used to identify the IR signatures of each residue of interest in the resulting reaction-induced FTIR difference spectra.

2. Topical Tofacitinib: A Janus Kinase Inhibitor for the Treatment of Vitiligo in an Adolescent Patient

Rachel Berbert Ferreirab et al Abstract Vitiligo is an autoimmune skin disease presenting with areas of de pigmentation. Recent reports suggest that Janus kinase (JAK) inhibitors may be an effective therapy. In this case report, we show our experience with an adolescent patient with a long history of generalized and refractory vitiligo, for which treatment with topical tofacitinib, a JAK inhibitor, associated with phototherapy for 9 months, resulted in near complete repigmentation**.**

3. Formulation And Evaluation Of A Topical Gel Containing Minoxidil And Tofacitinib Citrate For Alopecia Areata

Dr. V. S. Mannura et al ABSTRACT Objective: The objective of the present study is to formulate and evaluate a topical gel containing Minoxidil and Tofacitinib citrate for alopecia areata. Methods: Six gels were formulated using the direct-dispersion method by using polymers in the ratio of Carbopol 934: HPMC and Carbopol 934: HPC in three different concentrations each. All the prepared gels were then characterized for its drug content, pH, Rheological measurement, Spreadibility, skin adhesion study, In vitro drug release, Ex-vivo skin permeation study and stability study.

4. Formulation Development and Evaluation of Tofacitinib Citrate Effervescent Floating Tablet

R. G. Sapkal et al The objective of the present study was to formulate and evaluate Effervescent Floating tablets of Tofacitinib citrate for the treatment of Antirheumatic agent. Tablets were prepared by direct compression using directly compressible polymers such as HPMC K4M, and Carbopol 934 and were evaluated for drug-excipients compatibility, density, buoyancy test, drug content, and In-Vitro release profile. Sodium bicarbonate and citric acid were used to produce the base for the buoyancy of tablets. Analysis of drug release from tablet indicates drug release by zero order, first order rate kinetics. No significant change was observed in physical appearance, drug content, floatability, or in-vitro dissolution pattern after storage at 450C/750C RH for three months

5. Formulation and Quality Control Tests for Nanoemulsion of Tofacitinib: A Novel Approach

Singh S et al ABSTRACT Received 02 September 2021 Accepted 11 November 2021 Published 16 November 2021 Background: Tofacitinib (TFB) is a pioneer JAK (Janus kinase) inhibitor mainly employed to treat rheumatoid arthritis. It has proven efficacy for the treatment of rheumatoid arthritis in the oral dosage form. Oral TFB exhibited several toxic effects. Current research aims to develop a topical formulation of TFB to achieve effective treatment without any adverse effects. Study Design: Ultrsonication Methods. Place and Duration of Study: Sample: Swami Dayanand Postgraduate Institute of Pharmaceuticals Sciences, University of Health Sciences, Rohtak; 2020-2021. Methods: Oleic acid, tween 80, and propylene glycol were selected as oil, surfactant, and cosurfactant, respectively. The ratio of oil:surfactant:co-surfactant was selected based on a ternary phase diagram using the aqueous titration method. The selected ratio was employed to develop eight formulations of TFB by ultra-sonication. The formulations (F1- F8) were characterized using several physicochemical methods like pH, viscosity, particle size distribution, zeta potential, drug content, and in vitro release. Results: The formulations (F1-F8) were formulated by using the ultrasonication (high energy) method. The optimized formulation selected on the basis of characterization methods for instance.

6. Controlled Release Formulation of Tofacitinib Citrate Tablets Evaluated Using Quality by Design (QBD) Approach

Patel B. A et al The goal of the project is to use the quality-by-design (QbD) method to create and optimise Tofacitinib citrate matrix tablets with controlled release excipients. The drug's physicochemical parameters, reference product characterisation, QTPP (Quality Target Product Profile), and CQAs were used to start product development (Critical Quality Attributes). Hypromellose (Methocel K100 premium LV CR), Polyethylene Oxide (Sentry Polyox WSR N80 LEO), and Magnesium Stearate were used as formulation variables and were optimised together. The complete factorial design of experiment (DOE) with three centre points was used to investigate traditional monolithic controlled release matrix tablets. Using Design-expert12 programme, dissolution was assessed as CQA. At acidic pH, hypromellose with a higher viscosity grade provides a regulated release pattern by retaining the integrity of the medication and preventing rapid drug release. Due to the nonionic nature of the polymer, drug release from the polymer matrices is pH independent. Present monolithic controlled release matrix system the extensive degradation of Tofacitinib Citrate in the acidic condition can be avoided with desired in- vitro drug release

PLANOFWORK

Tofacitinib citrate is a medication used primarily for the treatment of rheumatoid arthritis and the immune conditions. To plan a work protocol for testing tofacitinib citrate using the Ftri (Fourier Transform Infrared)test, follow these steps:

1. **Objective**
2. **Sample Preparation**:
3. **Instrument Calibration**:
4. **Sample Analysis**: **5. Data Interpretation**:.

1. Objective Definition

Determine the goal of the FTIR analysis (e.g., identifying functional groups, studying molecular interactions, or characterizing materials). Verify the identity and purity of tofacitinib citrate using FTIR spectroscopy.

2. Sample Preparation

Solid Samples: Grind the sample to a fine powder if necessary. Mix with potassium bromide (KBr)and press into a pellet, or use an ATR(attenuated total reflectance)accessory.

Liquid Samples :Use a liquid cell with appropriate epathlength. Alternatively, place a drop of liquid on the ATR crystal.

Gaseous Samples: Use a gas cell designed for FTIR analysis. Weigh an appropriate amount of tofacitinib citrate (typically 1-2 mg).Grind the sample with KBr (200-300 mg) to form a fine, homogeneous mixture. Compress the mixture into a transparent pellet using a pellet press

3. Instrument Setup

Calibration: Ensure the FTIR spectrometer is calibrated according to the manufacturer's instructions Run a background scan with a KBr pellet to ensure the baseline is clear. Turn on theFTIRspectrometerandallowittostabilize.Performabackgroundscanwithnosampleinplace.

Parameters: Set the appropriate resolution (typically 4 cm^-1 for general purposes).Select the spectral range (usually 4000-400 cm^-1). Choose the number of scans to average (higher numberfor better signal-to-noise ratio).

4. Data Acquisition Background Scan: Perform a background scan to establish a baseline.

Sample Scan: Place the sample in the sample compartment or on the ATR crystal. Collect the sample spectrum.

2. Data Analysis

Baseline Correction: Adjust the baseline to remove any offsets

Comparison: Compare the obtained spectrum with reference spectra from data base so literature Identify functional groups based on absorption peaks. Place the tofacitinib citrate pellet in the sample holder.Scan the sample over the mid-infrared range (typically 4000-400 cm^-1).Record the spectrum.

2. Interpretation and Reporting

Interpretation : Functional Group Identification: Assign absorption bands to specific functionalgroups.
Quantitative Analysis: If quantification is required, use Beer-Lambert law and calibration curves. Compare the obtained spectrum with the reference spectrum of pure tofacitinib citrate. Identify characteristic peaks corresponding to functional groups in tofacitinib citrate

Report Preparation:

Summarize findings with spectra, tables of peak assignments, and interpretations. Include experimental details and any observed anomalies.

3. Cleanup and Maintenance

Sample Disposal: Dispose of samples according to safety guidelines.

MATERIALS AND METHOD

Performing an FTIR spectroscopy test on tofacitinib citrate involves several steps to ensure accurate and reliable results. Here'sa detailed procedure:

Materials Needed : Tofacitinib citrate sample Potassium bromide (KBr) (for pellet method) orATRaccessory(forATRmethod)Mortarandpestle(ifusingKBrpelletmethod)FTIRspectrometerS ampleholder or ATR crystal

METHODS

1. Sample Preparation:

KBr Pellet Method: Grind a small amount of tofacitinib citrate with dry potassium bromide (KBr) powder in a mortar and pestle until a fine, homogeneous mixture is obtained. The ratio is

typically around 1 part sample to 100 parts KBr by weight. Place the mixture into a pellet press and apply high pressure to for math in, transparent pellet.

ATR Method: Ensure the ATR crystal is clean .Place a small amount of tofacitinib citrate directly on to the ATR crystal. Ensure good contact between the sample and the crystal.

2. Instrument Setup: Turn on the FTIR spectrometer and allow it to warm up according to the manufacturer's instructions. Set the instrument to collect background spectra with no sample in the beam path. This background spectrum will be subtracted from the sample spectrum to eliminate the effects of atmospheric moisture and CO_2.

3. Data Acquisition:

For KBr Pellet Method: Insert the KBr pellet containing tofacitinib citrate in to the sample holder. Collect the sample spectrum over the desired range (typically4000to 400cm^{-1}).

For ATR Method: Place the sample (tofacitinib citrate) on the ATR crystal. Collect the sample spectrum over the desired range(typically4000 to 400 cm^{-1}).

4. Data Processing:

Subtract the background spectrum from the sample spectrumto obtain a corrected spectrum. Analyze the spectrum to identify characteristic peaks corresponding to different functional groups in tofacitinib citrate.
Analysis and Interpretation: Identify key peaks in the FTIR spectrum:O-H/N- HStretching:3200-3500cm^{-1}C-HStretching:2800

Compare the peaks with standard reference spectra of tofacitinib citrate to confirm its identity.Evaluatetheintensityandpositionofpeakstoassessthepurityandpossiblepresenceofimpuri ties.Fourier Transform Infrared (FTIR) spectroscopy is a powerful technique used to obtain the infrared spectrum of absorption or emission of a solid, liquid, or gas. Here's an overview of the key methods and principles involved in FTIR spectroscopy:

1. Basic Principle

FTIR spectroscopy works on the principle of measuring the absorption of infrared radiation by the sample material as a function of wavelength. The resulting spectrum represents the molecularfingerprint of the sample.

2. Fourier Transform

The core of FTIR spectroscopy is the Fourier transform, a mathematical process that converts the raw data (interferogram) obtained from the instrument into a usable spectrum. The interfero

gram isa signal obtained as a function of time (or optical path difference), which is then transformed to provide a spectrum as a function off requency (wavelength).

3. Interferometer

The key component of an FTIR spectrometer is the Michelson interferometer, which splits a beam of infrared light into two paths ,reflects them back, and then recombines them to create an interference pattern. The interferometer modulates the light over a range of wavelengths, allowing for the simultaneous collection of data a cross the spectrum.

4. Sampling Techniques

❖ Transmission: The sample is placed in the path of the infrared beam, and the amount of light absorbed by the sample at each wavelength is measured. This method is suitable for gases
,liquids, and thin films.

❖ Attenuated Total Reflectance (ATR): A sample is placed in contact with a crystal with a high refractive index. Infrared light passes through the crystal and is internally reflected, creating an evanescent wave that penetrates the sample. This method is particularly useful for solid and liquid samples without extensive preparation.

❖ Diffuse Reflectance: This method is used for powders and rough surfaces. Infrared light is scattered off the sample, and the reflected light is collected and analyzed. Reflectance (Specular): This technique measures the light reflected directly off a smooth, reflective surface, providing

information about surface properties

5. Data Analysis

The resultant spectra are analyzed to determine the molecular composition and structure of the sample. Peaks in the spectrum correspond to vibrational modes of the molecules, and their positions and intensities provide qualitative and quantitative information.

6. Applications

FTIR spectroscopy is widely used in various fields including:

Chemistry: Identification of chemical compounds and functional groups. Pharmaceuticals: Characterization of active ingredients and excipients. Materials Science: Analysis of polymers, coatings, and composites.

Infrared spectrophotometer is a very important tool used in qualitative identification and quantitative estimation of many drugs and chemicals. The instrument is particularly useful In pharmaceutical industry in identification of drugs and detection of impurities.

Qualitative analysis: It is clear from earlier discussion that each compound or substance gives a characteristic IR spectrum. Thus, for identification, IR spectrum of a substance is compared with the IR spectrum of the authentic sample of the same substance.

The sample spectrum is superimposed on the spectrum of authentic sample and if the spectra of both are identical then substance under examination and the authentic sample are the same.

Various pharmacopoeias like IP, BP and USP have included "IR spectra" as one of the test for identification of many drugs and substances.

Detection and identification of impurity in pharmaceutical substances can be ascertained by IR spectro photometry. When a compound contains impurity, it reduces sharpness of individual bands, causes appearance of extra band or peak, Conditions for detection of impurity are most favourable when impurity possesses a strong band in IR region where main substance does not possess absorption band in that region, e.g. small quantity of ketone in hydrocarbon can be detected as a band near 1720 cm^3, characteristic of ketone.

IR spectrophotometer is also useful in determining shape or symmetry of molecule, e.g. NO,(nitrogen dioxide) if linear, should show two bands and if non-linear three bands.IR spectra of NO,gives threebandsin750, 1323,1616 cm region showing it is a bend structure and not a linear.**RESULT:**

Performing FTIR (Fourier Transform Infrared) spectroscopy on tofacitinib citrate will yield a spectrum that provides detailed information about its molecular structure and functional groups.Below is an outline of what you might expect in the results and how to interpret them:

Expected FTIR Spectrum for Tofacitinib Citrate

Key Functional Groups and Their Characteristic Absorption Peaks:Steps to Obtain and Analyze FTIR Results:

Sample Preparation:

KBr Pellet Method: Mix small amount of tofacitinib citrate with KBr and press into a pellet.ATR Method: Place a small amount of tofacitinib citrate directly on to the ATR

crystal.

Collecting the Spectrum:

Record the background spectrum.

Insert the sample (KBr pelletor ATR)and record the sample spectrum.

Data Interpretation:

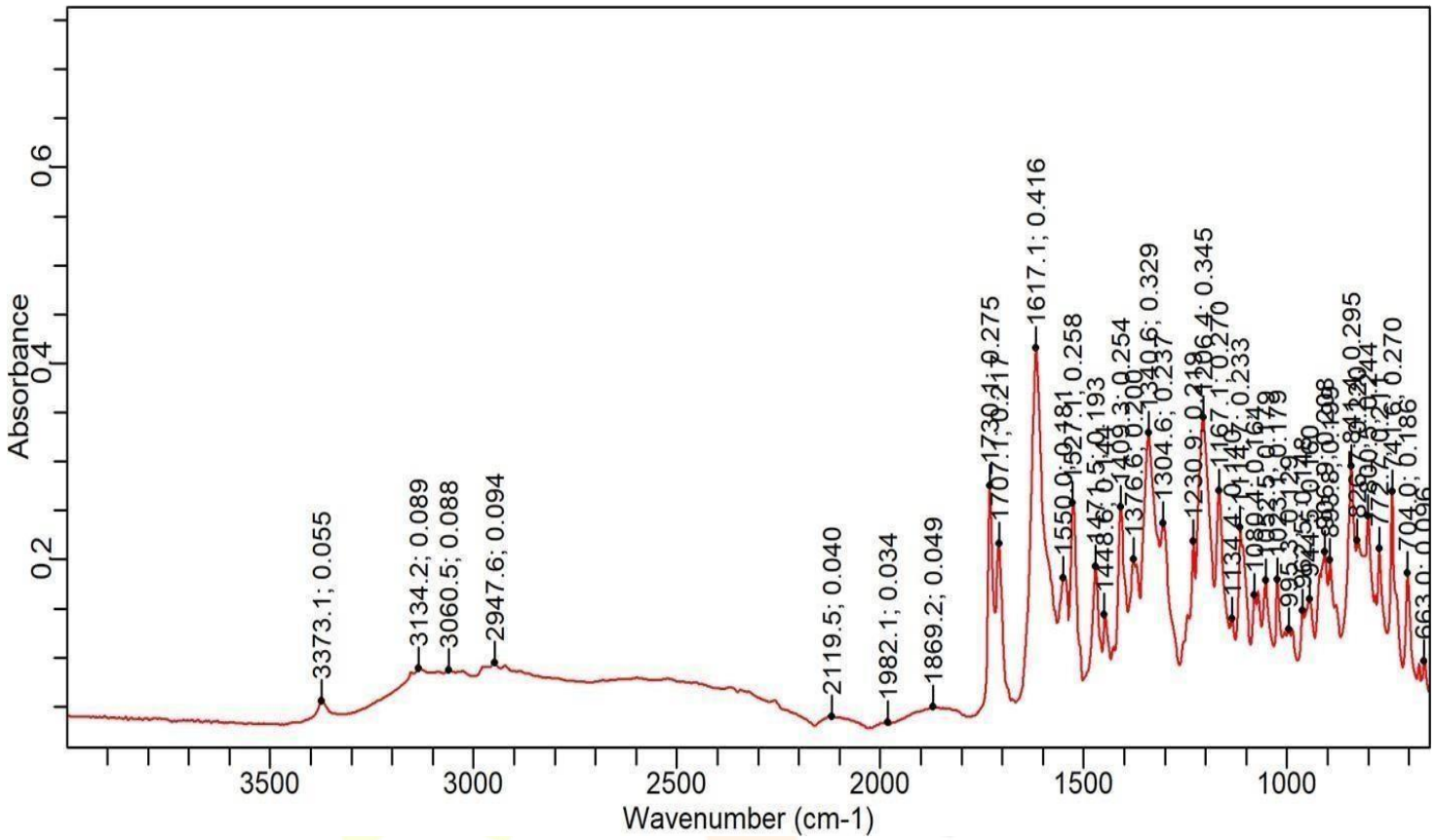
Analyze the peaksin the FTIRspectrum:

O-H/N-HS tretching:Broadpeaksaround3200-3500cm^{-1}.

C-H Stretching: Peaks around 3000-3100 cm^{-1} for aromatic C-H.C=O Stretching: Strong peaks around 1650-1750cm^{-1}.

C=C Stretching: Peaks around1400-1600cm

SampleID:TFCKS01	**Method**
	Name:C:¥Users¥Public¥Documents¥Agilent¥MicroLab¥ Methods¥JR_ATR.a2m
SampleScans:140	User:Admin
BackgroundScans:140	Date/Time:05/17/202412:56:57PM
Resolution:4	Range:4000-650
System Status:Good	Apodization:Triangular



FTIRINTERPRETATION TOFACITINIB CITRATE

Sample name: TFC/KS/01		
peaks	**actual**	**Functional groups**

710-690	663	C-H mono (phenyl)
700(broad)	703	C is-C-H out of plane bend
750-0720	741	Methylene –$(CH^2)_n$rocking (n≥3)
670-900	772,800,826,841,893 ,906	Aromatic CH In plane bend
1055-1000/1005-925	944,962,995,1023,10 52	Cyclo hexenring vibretation
1140-1070	1080,1114,1134	Cyclicethers, large ringC-Ostretch
1159-1164	1167,1206	C-Ostretching,tertiaryalcohol
1233	1230	StretchingPo-2asym
1310-1390	1304,1340,1376	OH bonding(phynol)
1470-1430	1409,1448,1471,	Methyl C-Hasym/symbend
1510-1410/1580-1615	1527,1617	C=CCaromaticringstretch
1725-1705	1707,1730	Ketone
1870-1820	1869	5memberedringanhydride
2100-1800	1982,2119	Transition metel carbonyls
2970-2950	2947	Methylene C-Hsym/asymstretch
	3060,3134,3373	OH group

CONCLUSION

We have shown in this review that infrared spectroscopy provides a means to have a look at structural details for proteins in solution with spatial and temporal resolution greater than in most of the available crystallographic data. Infrared spectroscopy is unique to probe amino acid side chains and water molecules, as well as cofactors in redoxstates, which are silent in other spectroscopic techniques. Therefore, although this technique requires well defined experimental conditions and the use of site directed mutants or isotope labeling, it will continue to play a key role in the analysis of photo systems and other proteins, and notably to unravel the mechanism of water oxidation in PSII. The remarkable increase in the number of publications on proteins involving (FTIR) spectroscopy shows the important and specific role of this technique. The continuous development of new and complementary experimental strategies and theoretical approaches opens its field of application in various research areas

REFERENCES

1) Ayala I, Range K, York D, Barry BA (2002) Spectroscopic properties of tyrosyl radicals indipeptides. JAm ChemSoc 124:5496–5505 Barth A (2000) The infrared absorption ofaminoacid sidechains

2) . ProgBiophysMolBiol 74:141–173 Barth A (2007) Infrared spectroscopy of proteins.BiochimBiophysActa 1767:1073–1101 Barth A, Corrie JE (2002) Characterization of a newcagedprotoncapableof inducinglargepH jumps.

3) Biophys J 83:2864–2871 Barth A, Mantele W, Kreutz W (1990) Molecular changes in thesarcoplasmic reticulum Ca2? ATPase during catalytic activity: a Fourier transform infrared(FTIR)studyusingphotolysisofcagedATPtotriggerthereactioncycle.FEBSLett277:14 7–150BauscherM,NabedrykE,BagleyK,BretonJ,Ma¨nteleW(1990)Investigationofmodels forphotosyntheticelectronacceptors.

4) Infraredspectroelectrochemistryofubiquinoneanditsanions.FEBSLett261:191– 195Berthomieu C, Boussac A (1995) FTIR and EPR study of radicals of aromatic amino acids,4-methylimidazoleand phenol generated byUV-irradiation.

5) Biospectroscopy1:187–206BerthomieuC,HienerwadelR(2001)IroncoordinationinphotosystemII:interactionbetwee nbicarbonateandtheQBpocketstudiedbyFouriertransform infrared spectroscopy. Biochemistry 40:4044–4052 Berthomieu C, Hienerwadel R(2005)

6) Vibrational spectroscopy to study the properties of redox-active tyrosines in photosystem IIand other proteins. BiochimBiophysActa 1707:51–66 Berthomieu C, Boussac A, Ma¨nteleW, Breton J, Nabedryk E (1992) Molecular changes following oxidoreduction of cytochromeb559 characterized by Fourier transform infrared spectroscopy and electron paramagneticresonance: photooxidation in photosystem II and electrochemistry of isolated cytochromeb559and iron protoporphyrinIX-bisimidazole model compounds.

7) Biochemistry 31:11460–11471 Berthomieu C, Boullais C, Neumann JM, Boussac A (1998a)Effect of 13C, 18O, and 2H labeling on the infrared modes of UV-induced phenoxyl radicals.BiochimBiophysActa1365:112–116BerthomieuC,HienerwadelR,BoussacA,BretonJ,DinerBA(1998b) Hydrogen bonding of redox-active tyrosine Z of photosystem IIprobedbyFTIRdifferencespectroscopy.

8) Biochemistry37:1054710554 Berthomieu C, MarboutinL, DupeyratF,Bouyer P(2006)Electrochemically-induced FTIR difference spectroscopy in the mid to far infrared (200 lm)domain: a new setup for the analysis of metal-ligands in redox-proteins.Biopolymers 82:363–367 1

9) Breton J, Burie J-R, Berthomieu C, Berger G, Nabedryk E (1994a) The binding sites ofquinonesinphotosyntheticbacterialreactioncentersinvestigatedbylight- inducedFTIRdifference spectroscopy: assignment of the QA vibrations iRhodobactersphaeroides using18O-or13C-labeled ubiquinones andvitamin K1.

10) Biochemistry 33:4953–4965 Breton J, Boullais C, Burie JR, Nabedryk E, Mioskowsky C(1994b) Binding sites of quinones in photosynthetic bacterial reaction centers investigated bylight-induced FTIR difference spectroscopy: assignment of the interactions of each carbonylofQAin Rhodobactersphaeroides usingsite-specific 13Clabeledubiquinone.

11) Biochemistry 33:14378–14386 Breton J, Boullais C, Mioskowski C, Nabedryk E (1995)Binding sites of quinones in photosynthetic bacterial reaction centers investigated by light-induced FTIR difference spectroscopy: symmetry of the carbonyl interactions and closeequivalence of the QB vibrations in Rhodopseudomonassphaeroides and Rhodobacterviridisprobed byisotopelabeling.

12) Biochemistry34:1160611616BretonJ,XuW,DinerBA,ChitnisPR(2002)Thetwohistidine axial ligands of the primary electron donor chlorophylls (P700) in photosystem Iaresimilarlyperturbed uponP700?formation.

13) Biochemistry 41:11200–11210 Brudler R, de Groot HJM, van Liemt WBS, Steggerda WF,Esmeijer R, Gast P, Hoff AJ, Lugtenburg J, Gerwert K (1994) Asymmetric binding of the 1-and 4-C=O groups of QA in Rhodobactersphaeroides R26 reaction centers monitored byFouriertransforminfra-redspectroscopyusingsite-specificisotopicallylabelledubiquinone-

10. EMBO J 13:5523–5530 Brudler R, de Groot HJM, van Liemt WBS, Gast R, Hoff AJ,LugtenburgJ,GerwertK(1995)FTIRspectroscopyshowsweaksymmetrichydrogenbonding of the QB carbonyl groups in Rhodobactersphaeroides R26 reaction centers. FEBSLett 370: 88–92 Buchet R, Jona I, Martonosi A (1991) Ca2? release from caged-Ca2? altersthe FTIR spectrum of sarcoplasmicreticulum.

14) BiochimBiophysActa 1069:209–217 Chu HA, Hillier W, Law NA, Babcock GT (2001)Vibrationalspectroscopyoftheoxygenevolvingcomplexandofmanganesemodelcompo unds.BiochimBiophysActa1503:69– 82ChuHA,HillierW,DebusRJ(2004)Evidence that the C-terminus of the D1 polypeptide of photosystem II is ligated to themanganese ion that undergoes oxidation during the S1 to S2 transition: an isotope-editedFTIRstudy.

15) Biochemistry43:3152– 3166ColthupNB,DalyLH,WiberlySE(1975)IntroductiontoinfraredandRaman spectroscopy.Academic Press,New York DeaconGB,PhillipsRJ(1980)Relationshipsbetweenthecarbonoxygenstretchingfrequenciesof carboxylatocomplexesandthe typeof carboxylate coordination.

16 CoordChemRev33:227– 250DebusRJ(2008)Proteinligationofthephotosyntheticoxygenevolving center. CoordChemRev 252:244–258 Deng H, Callender R (1999) Ramanspectroscopic studies of the structur

16) MethodsEnzymol308:176–201DiDonatoM,CohenRO,DinerBA,BretonJ,vanGrondelle R, Groot ML (2008) Primary charge separation in the photosystem II core fromSynechocystis:acomparisonoffemtosecondvisible/midinfraredpumpprobespectraofwil d-type and two P680 mutants. Biophys J94:4783–4795

17) DupeyratF,VidaudC,LorphelinA,BerthomieuC(2004)Longdistancechargeredistributionupo nCu,Zn-superoxidedismutasereduction— significanceofdismutasefunction.JBiolChem279:48091–

48101FallerP,DebusRJ,BrettelK,SugiuraM,RutherfordAW, BoussacA (2001)
18) Rapid formation of the stable tyrosyl radical in photosystem II. ProcNatlAcadSci USA98:14368–14373 Gourion-Arsiquaud S, Chevance S, Bouyer P, Garnier L, Montillet JL,BondonA,BerthomieuC(2005)IdentificationofaCd2?andZn2?bindingsiteincytochrome c using FTIR coupled to an ATR micro-dialysis set-up and NMR spectroscopy.Biochemistry 44:8652–8663 Griffith PR, de Haseth JA (1986) Fourier transform infraredspectroscopy.
19) Wiley,NewYorkHasegawaK,OnoT,NoguchiT(2000)VibrationalspectraandabinitioDFTcalc ulations of 4-methylimidazole and its different protonation forms: infrared and Ramanmarkers of the protonation state of a histidine side chain. J PhysChem B 104:4253–4265HasegawaK,OnoT,NoguchiT(2002)AbinitioDFTcalculationsandvibrationalanalysisof zinc-bound 4-methylimidazole as a model of a histidine ligand in metalloenzymes. J PhysChem A 106:3377–3390 Hastings G, Ramesh VM, Wang R, Sivakumar V, Webber A (2001)Primary donor photo-oxidation in photosystem I: a re-evaluation of (P700(?)- P700)Fouriertransforminfrared differencespectra.
20) Biochemistry 40:12943–12949HauserK,KrejtschiC,HuangR,Wu L,KeiderlingTA(2008)Sitespecificrelaxationkineticsofatryptophanzipperhairpinpeptideusin gtemperature-jump IR spectroscopy and isotopic labeling. J Am ChemSoc 130:2984–2992HienerwadelR,BerthomieuC(1995)Bicarbonatebindingtothenonhemeironofphotosyste m II investigated by Fourier transform infrared difference spectroscopy and 13Clabeledbicarbonate
21) Biochemistry 34:16288–16297 Hienerwadel R, Thibodeau D, Lenz F, Nabedryk E, Breton J,Kreutz W, Ma¨ntele W (1990) Time-resolved infrared spectroscopy of electron transfer inbacterial photosynthetic reaction centers: dynamics of binding and interaction upon QA and Q Breduction.
22) Biochemistry 31:5799–5808 Hienerwadel R, Grzybek S, Fogel C, Kreutz W, Okamura MY,Paddock ML, Breton J, Nabedryk E, Ma¨ntele W (1995) Protonation of Glu L212 followingQB- formation in the photosynthetic reaction center of Rhodobactersphaeroides: evidencefromtime-resolvedinfraredspectroscopy.Biochemistry34:2832– 2843HienerwadelR,Boussac A, Breton J, Berthomieu C (1996) Fourier transform infrared difference study of tyrosine D oxidation and plastoquinoneQAreduction inphotosystemII.
23) Biochemistry 35:15447–15460 Hienerwadel R, Boussac A, Breton J, Diner BA, BerthomieuC(1997)Fouriertransforminfrareddifferencespectroscopyofphotosystem IItyrosi