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XRD (X Ray Diffraction) Study of Tofacitinib Citrate

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Abstract
Although various researched works have been carried out in x-ray crystallography and its applications, but there are still limited number of researches on crystallographic theories and industrial application of x-ray diffraction. The present study reviewed and provided detailed discussion on atomic arrangement of single crystals, mathematical concept of Bravais, reciprocal lattice, and application of x-ray diffraction. Determination of phase identification, crystal structure, dislocation density, crystallographic orientation, and gran size using x-ray diffraction peak intensity, peak position, and peak width were discussed. The detailed review of crystallographic theories and x-ray diffraction application would benefit majorly engineers and specialists in chemical, mining, iron, and steel industries.

Keywords: Crystal structure, X-ray diffraction, Wavelength, Light

Introduction

X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. X-ray diffraction peaks are produced by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample. The peak intensities are determined by the distribution of atoms within the lattice. Consequently, the X-ray diffraction pattern is the fingerprint of periodic atomic arrangements in a given material. This review summarizes the scientific trends associated with the rapid development of the technique of X-ray diffraction over the past five years pertaining to the field of pharmaceutical industry, forensic science, geological applications, microelectronics and glass industry, as well as in corrosion analysis.

XRD is a non-destructive technique and helps scientists to understand how atoms are arrangedand how such arrangements can affect the behavior of materials or can be used to determine the structure of complex molecules. XRD was developed at the beginning of the 20th century and its application to the analysis of crystals led to the award of the Nobel Prize in Physics to Sir William Henry and William Lawrence Bragg in 1915 – one of the many Nobel Prizes associated with XRD.

X-rays were discovered by Wilhelm Roentgen who called them x-rays because the nature at first was unknown so, x-rays are also called Roentgen rays. X-ray diffraction in crystals was discovered by Mas von Laue. The wavelength range is 0.01 to about 10 mm. X-rays are short wave length electromagnetic radiations produced by the deceleration of high energy electronsor by electronic transitions of electrons in the inner orbital of atoms.

The penetrating power of x-rays depends on energy abo, there are two types of x-rays

i) Hard x-rays: which have high frequency and have more energy.

ii) soft x-rays, which have less penetrating and have low energy.

The radiation is employed within the kind of X-ray radiation, electrons, and neutrons. X-ray may be a gauge hosan with high energy that includes a wavelength startingfrom zeru.5 up to 2.5 Armstrong once Associate in Nursing X-ray beam interacts with a fabric,a number of the beams are going to be absorbed, transmitted, and a few of it's scattered diffracted. This scattered optical miracle is what XRD detects. The scattered X-ray beam is canceled one another out as a result of the sections square measure completely different and afew square measure reciprocally reinforcing as a result of the phase is that the same.

X-ray beams that reinforce one another square measure referred to as optical phenomenon beams, once X rays square measure discharged at the Bravais lattice of the fabric to be tested, the rays square measure diffracted and kind bound patterns referred to as fingerprints (Hakimetal, 2019: Bunaciu et al.. 2015). The application of this XRD instrument is extremely wide used. This XRD instrument is most frequently used for the identification ofunknown crystalline resources (e.g,, minerals, inorganic compounds). one amongst them is that the characterization of unknown. supplies victimization XRD tools is extremely helpful for studies in earth science, ecology, materials science, engineering, and biology, different applications of XRD instruments square measure the characterization of crystalline materials, identification of fine-grained reserves, determination of unit dimensions, and measure of sample purity (Bunaciu et al, 2015; Alexander & Klug, 1948, Zhou et al.

Diffraction occurs when light is scattered by a periodic array with long-range order, producing constructive interference at specific angles. The atoms in a crystal are periodically arranged thus diffract light. The wavelength of X-ray are similar to the distancebetween atoms, Powder X-ray Diffraction (PXRD) techniques uses this principle to elucidate the crystalline nature of materials. The scattering of X-rays from atoms produce a diffraction pattern that contains information about the atomic arrangement in crystal. Amorphous materials like glass do not have periodic array with long-range order so; they do not produce any significant peak in diffraction pattern.

Applications

- Many of these techniques can also be used for polycrystalline layered materials such as coatings and thin films using a method called grazing incidence XRD (GIXRD). Studies ofsmall areas in polycrystalline materials employ a method called micro-diffraction.
- Other X-ray diffraction techniques for materials that are not polycrystalline (for example single crystal semiconductor wafers or epitaxial layers) include high-resolution analysis ofhetero-epitaxial layers (HR-XRD). The analysis of these make use of Bragg's Law,dynamical diffraction theory, and single crystal orientation, for both wafer as well as ingots.
- Differentiation between crystalline and amorphous materials.
- Determining the grain/particle size of the material.
- Determining the degree of texture (preferred orientation of grains) in material.
- Determination of the texture of poly-grained materials.
- Measurement of sample purity.
- Determination of orientation of single crystals.
- Determination of electron distribution within the atoms and throughout the unit cell.
- Determination of molecular structure and characterization of proteins and nucleic acid.
- Determination of unit cell dimensions.

- Whether the sample is a composite material consisting of multiple crystallographic phasesand, if so, determine the fraction of each phase.
- Qualitative and quantitative phase analysis of pure substances and mixtures. The mostcommon method for phase analysis is often called 'X-ray powder diffraction' (XRPD).
- Analysis of phase changes under other special conditions such as temperature, humidityand applied pressure (non-ambient studies).
- Analysis of physical properties such as crystallite size (diameter), crystal orientation, andresidual stress, which together are called the 'microstructure' of polycrystalline materials.

Prepare a sample for XRD

The three things you need for preparing a sample for x-ray diffraction are: The sample substance Equipment or a tool to grind it with A sample holder First, take a few tenths of agram of the sample substance. Grind it to a fine powder. Typically, this should be done in a fluid to minimize any extra surface energy, which might otherwise randomise the sample.The optimum size of powder is less than 10μm (micrometers). Next, place this ground powder onto a sample surface, or within a holder. It is important to create a flattened uppersample surface, with a random distribution of lattice orientations.

The Advantages of XRD

- You can use XRD to determine the orientation of the individual grains of a crystal and to identify crystal structures in unknown substances.
- This technique will measure the internal stress, the size and shape of small crystalline areas,and measure the average spacing between the layers of rows of atoms in samples. It can determine their minerology.
- XRD will produce clear, unambiguous results, and as a technique it is both powerful and rapid.
- Preparation only involves minimal sample quantities. These you grind into a fine powder,with an optimum size that is less than 10μm (micrometers).
- Interpreting the results of XRD is relatively straightforward. The x-ray diffractometer continuously records data during the process, and presents peak positions and x-ray countsin a table.
- In the table, the Bragg equation calculates the d-spacing of each peak. Once you have all the d-spacing for the sample, you can compare these readings to d-spacing of known materials, to identify the sample.
- X-ray diffractometers are widely available testing instruments, both as benchtop and portable models.
- Nondestructive, fast, and easy sample preparation
- High-accuracy for d-spacing calculations
- Can be done in situ
- Allows characterizing single crystal, poly, and amorphous materials
- Standards are available for thousands of material systems

Disadvantages of XRD

- XRD is a specialist technique and has limitations in what you can apply it to.
- If you are using it to identify a substance that is not known to you, then the sample must besingle phase and homogeneous.
- The sample must also be very small, in quantities of tenths of a gram. Proper preparation of samples is crucial.
- If the sample is a non-isometric crystalline structure, then indexing its patterns can be complex. Where there are high-angle reflections, peak overlay can occur and worsen.
- Despite requiring small sample sizes, XRD is much more accurate measuring largecrystalline structures rather than small ones. Small structures present only in trace elementswill often go undetected. If the sample consists of mixed materials, the detection limit is 2% of the sample.

XRD Instrumentation

Max von Laue and Co., in 1912, discovered that crystalline substances act as three-dimensionaldiffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice (Friedrich et al., 1912). X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode raytube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample.

X ray Diffraction Principle

X-ray diffraction is based on constructive interference of monochromatic X- rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample.When a monochromatic x-ray incident occurs on a crystal. The atomic electrons in the Crystalare sent into vibration. With the same frequency as that of the frequency of the incident ray andare accelerated. These Accelerated electrons then emit the radiation of the same frequency as that of incident x-rays in all directions.

X-rays have wavelengths in the range of 10−10 m, the same order of magnitudeas the distance between atoms . In a typical XRD experiment, thesample is illuminated with a beam of X-rays. The X-ray source and the detector move at different angles in a synchronized motion. Most materials consist of many small crystals. Thesecrystals have a regular arrangement of atoms, each surrounded by a cloud of electrons. When an X-ray encounters an atom, its energy is absorbed by the electrons, and is then released in the form of a new X-ray in a phenomenon called “elastic scattering”.

Scattered waves are subject to interference. When the waves align in what is called “constructive interference” the detected signal is amplified and a reflection is observed. Conversely, in case of destructive interference – signals out of alignment – the signal is destroyed.

If the wavelength of incident radiation is large compared to the dimensions of the Crystal. Thenthe radiated X-ray are in phase with each. But since the atomic dimension are nearly equal to the wavelength of X-Ray. The radiation emitted by the electrons is out of phase with each other. These radiations may interfere constructively or destructively producing a diffraction pattern (i.e. maxima and minima) in certain directions.

Bragg’s law:

Bragg’s law is a special case of Laue diffraction, which determines the angles of coherent andincoherent scattering from a crystal lattice. When X-rays are incident on a particular atom, theymake an electronic cloud move like an electromagnetic wave. The movement of these chargesradiates waves again with similar frequency, slightly blurred due to different effects, and this phenomenon is known as Rayleigh scattering. Basically, this law explains the relationship between an x-ray light shooting and its reflection from a crystal surface.

Brag’s Law states the following:

When the X-ray is incident onto a crystal surface, its angle of incidence, θ, will reflect with thesame angle of scattering, θ. And, when the path difference, d is equal to a whole number, n, ofwavelength, λ, constructive interference will occur. The exact process takes place uponscattering neutron waves via nuclei or a coherent spin interaction with an isolated electron. These wave fields that are re-emitted interfere with each other destructively or constructively, creating a diffraction pattern on a film or detector. The diffraction analysis is the resulting waveinterference, and this analysis is known as Bragg diffraction.

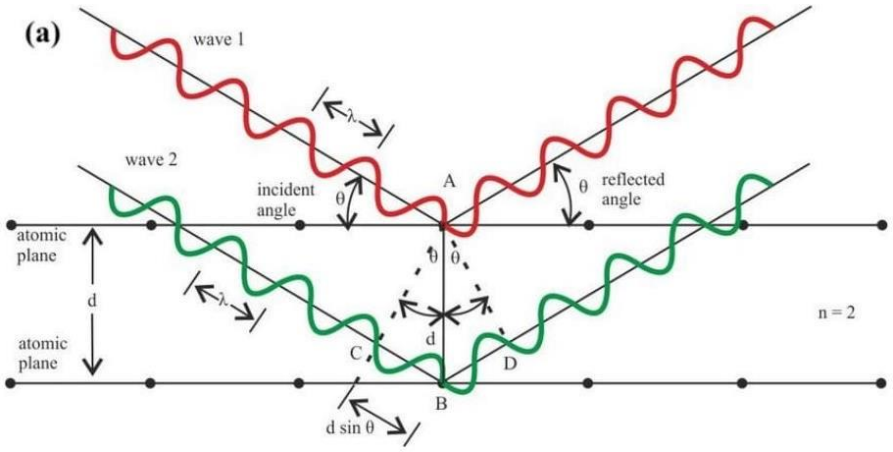

Fig.No.:1.1 Bragg's Law

This observation illustrates the X-ray wave interface, called X-ray diffraction (XRD) andproof of the atomic structure of crystals.

Bragg was also awarded the Nobel Prize in Physics for identifying crystal structures starting with NaCl, ZnS, and diamond. In addition, to understand the structure of every state of matterby any beam, e.g., ions, protons, electrons, neutrons, with a wavelength similar to the length between the molecular structures, diffraction was developed.

Derivation of Bragg's Law

Consider the following figure of beams in which the phases of the beams coincide when the incident angle is equal to the reflecting angle. The incident beams are parallel to each other until they reach point z. When they are at point z, they strike the surface and travel upwards.At point B, the second beam scatters. AB + BC is the distance travelled by the second beam.The extra distance is known as the integral multiple of the wavelength.

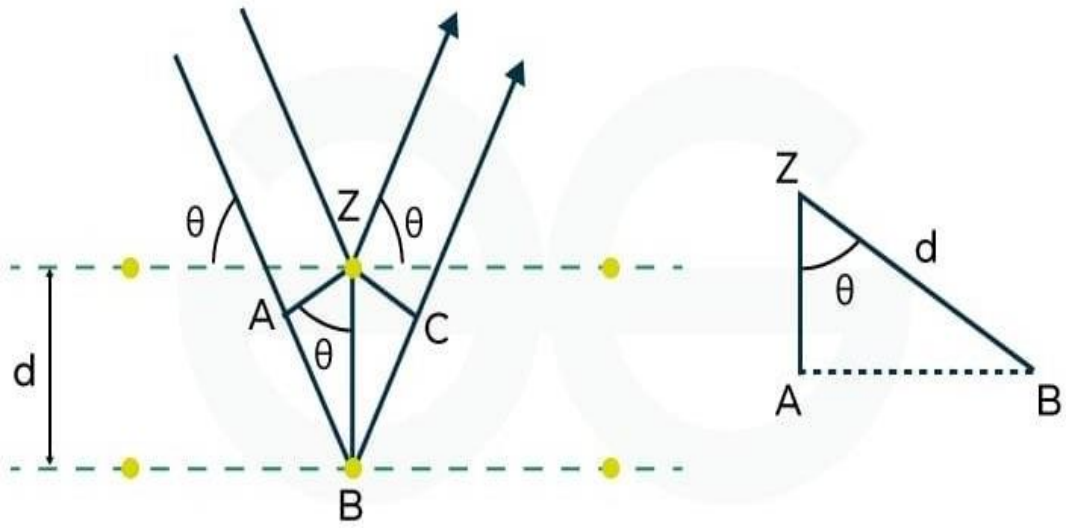

Fig.no:1.2: Bragg's Law Of Reflection

nλ = AB + BC

We also know that AB = BCnλ = 2AB (equation 1)

d is the hypotenuse of the right triangle Abz. Ab is the opposite of the angle θ.AB = d sinθ (equation 2)

Substituting equation 2 in equation 1

nλ = 2d sinθ

The above equation is Bragg's law expression.Where,

A = wavelength of the x-ray

d= spacing of the crystal layers (path difference)

θ= incident angle (the angle between incident ray and the scatter plane)n= integer

λ=x-ray Wavelength

XRD INSTRUMENTATION CONSIST OF FOLLOWING PARTS :-

1) X-ray source/ X-ray tube
2) Collimator
3) Monochromator

A) Filters monochromator
B) Crystal monochromator

4) Detectors

A) Photographic detector
B) Counter detector

a) Geiger Muller tube detector
b) Proportional counter detector
c) Scintillator counter detector
d) Semiconductor detector

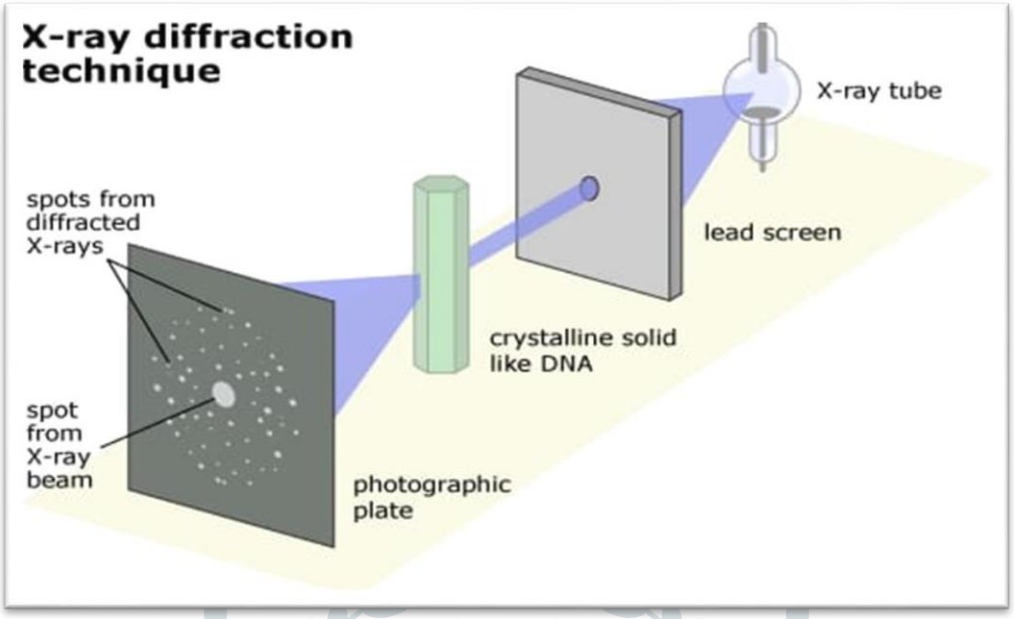

Fig.1.3:- XRD INSTRUMENTATION

1) X-ray tube

X-rays are produced whenever high-speed electrons collide with a metal target. A source of electrons - hot W filament, a high accelerating voltage between the cathode (W) and the anodeand a metal target, Cu. Al. Mo. Mg. The anode is a water-cooled block of Cu containing desiredtarget metal.

Features & Functioning of XRT

* Composed of evacuated tube possessing cathode (tungsten filament) at one end & anode(metal target) at another end.
* Passage of current through tube causes tungsten filament to glow & emits electron.
* Among the two electrodes large voltage difference is applied, causing electrons to move at high velocity from filament and strike to anode. X rays are generally produced in XRT.

Due to high velocity impact of electrons on the target, inner shell electrons of metal get dislodge, which causes the outer shell electrons to jump to a lower energy shell to replace the dislodge electrons. These electronic transitions results in the generation of X-rays. The produced X-rays are allowed to move through a window of X-ray tube.

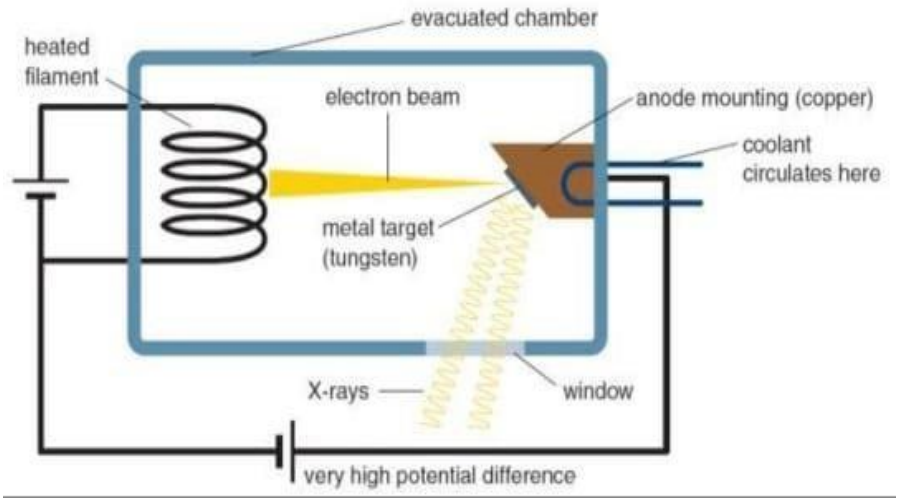

Fig.1.4:- X-ray tube

1. Collimator

A device that is used for narrowing of a beam of particles or waves.
Collimator makes, random directional X-rays to be narrow and parallel. These are usually madeup of tungsten, stainless steel and ceramics. Pitch ranges from 400microns to 6 microns.

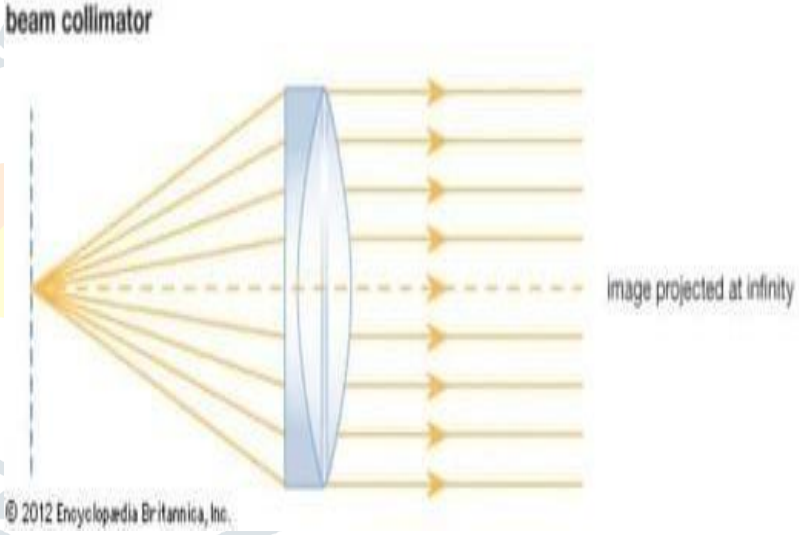

Fig.1.5:- Collimator

2. Monochromator

A device that is used for removal of unwanted radiation.

1. Filters Monochromators
2. Crystal Monochromators

1. Filters Monochromators:-

Made up of specialized material that absorb unwanted radiation and passes the desiredradiation.
e.g X-rays produced from molybdenum are monochromatized by zirconium filters. These zirconium filters absorb the molybdenum and allows to pass the k(beta) lines. For target elementlike copper, iron, cobalt, nickel the filters like Ni, Mn, & Co are commonly used.

2. Crystal Monochromators:-

These are the device that partially polarize a non polarized X-ray beam on the basis of Bragg'sdiffraction.
For broad band use: pyrolytic graphite materialFor narrow band use: Si, Ge or quartz
Types:

1. Flat Crystal
2. Curved Crystal

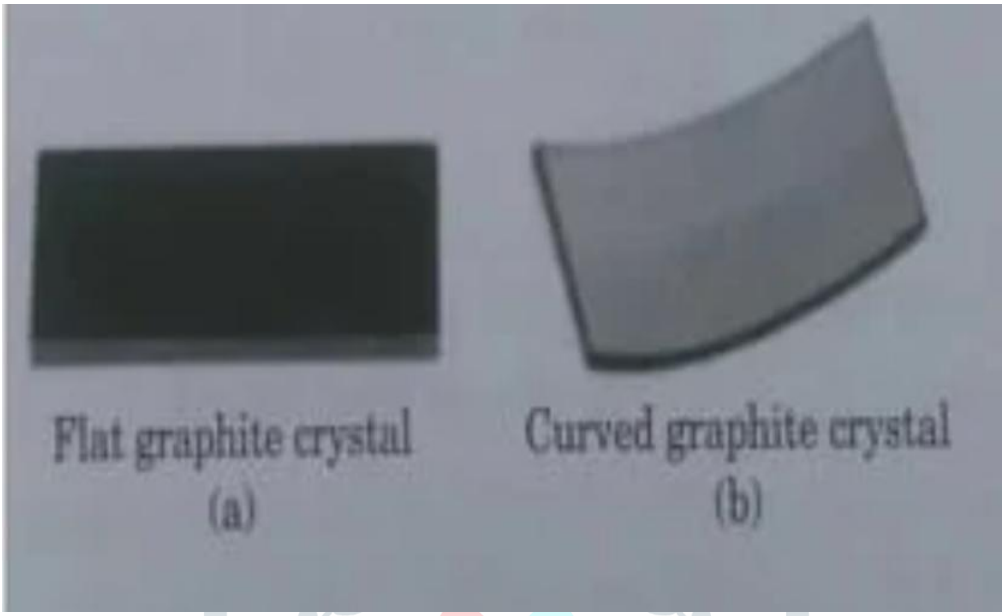

Fig.1.6:- Images For Crystal Monocromaters

4. Detectors

X-rays can be detected using two types of detectors:

1. Photographic Detectors
2. Counter Detectors

a) Geiger Muller Tube Counter
b) Proportional Counter
c) Scintillator Counter
d) Semiconductor Detector

1. Photographic detectors

A plane or cylindrical film is used to determine the position & intensity of X-rays. Cylindricalfilms are developed by exposing the detectors to X-rays. The extent of blackening of developedfilm is expressed as density. Density is the direct measurement of X-ray energy which causes blackening of photographic film.
D = log.I/I

2. Counter Detectors

a. Geiger muller Tube Counter :-

It is composed of glass tube (19 mm diameter). The tube is comprised of a half metal cylinder of about 4 inches length, made up of copper. Along the axisof cylinder a thin metal wire of tungsten is tied. The cylinder & wire are connected to an

electrical voltage source. The tube is filled with gas, usually Argon at a low pressure .a voltageis set up between the cathode and anode.

Working:-

When X-rays enters the Geiger tube, a collision occurs between the gas molecule and X-rays. Thereby electrons are ejected out of atoms of neutral molecules of argon gas. This causes production of positive molecular ions and free electrons. These electrons being negatively charged, moves towards anode and positively charged argon ions moves toward cathode. A potential gradient is applied to accelerate electrons. This causes electrons to pick much energyto eject more electrons out of atom.

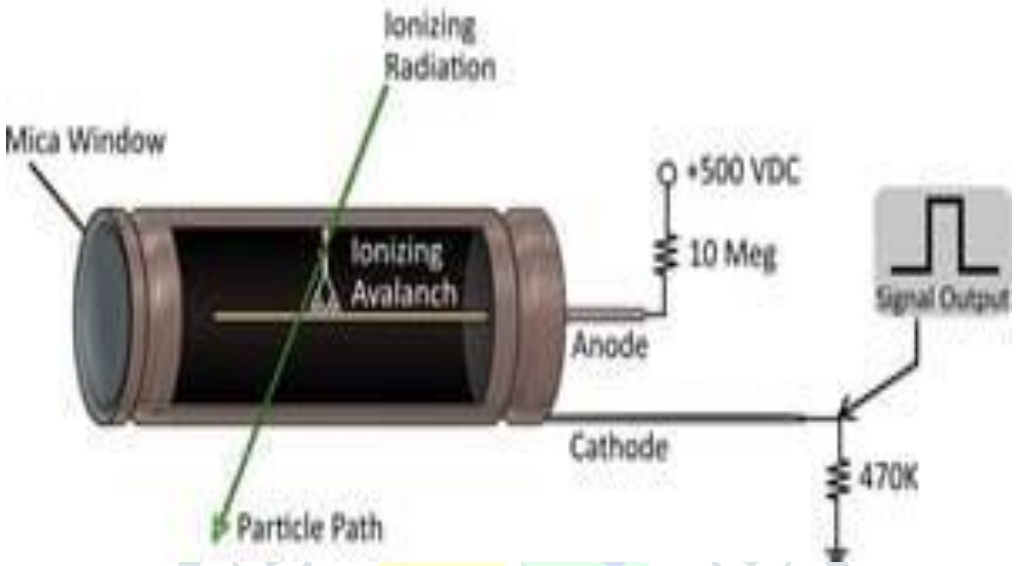

Fig.1.7:- Geiger muller Tube Counter

Merits:

1. significant signals are obtained for a given X-ray intensity
2. Economical
3. Requires less Maintenance

Demerits:

1. Used for measuring low rate X-rays.
2. Low efficiency below 1angstrom
3. Unable to measure energy of ionizing radiation

Application:

1. To detect alpha, beta and gamma radiation from given sample
2. To check for environmental levels of radioactivity.

In risk assessment in various working places. To identify radioactivity in rocks and minerals.

b) Proportional Counter:-

Similar in construction to Geiger-Muller Counter Heavy gas like P-10 or xenon or krypton isused.
Proportional counter is a combination of two ionization regions

a) lon drift region: region that exist in outer volume of the chamber.

b) Avalanche region: region that exist in the immediate vicinity of the anode.

A specialized circuit is connected to tube so that X-rays of particular energy can be measured.Therefore the output of proportional counter apparatus depends upon incident X-ray intensity.This in turn picks up further energy and liberate even more electrons. Such a progressive pro is called avalanche. Positive ions hit the cathodic half cylinder with enough energy to eject further more electrons. Therefore avalanche of electrons incline on wire which is detected as apulse of electric current. The electric pulse so generated indicates passing of a charged particlethrough the tube. This pulse can be read or measured through a meter.

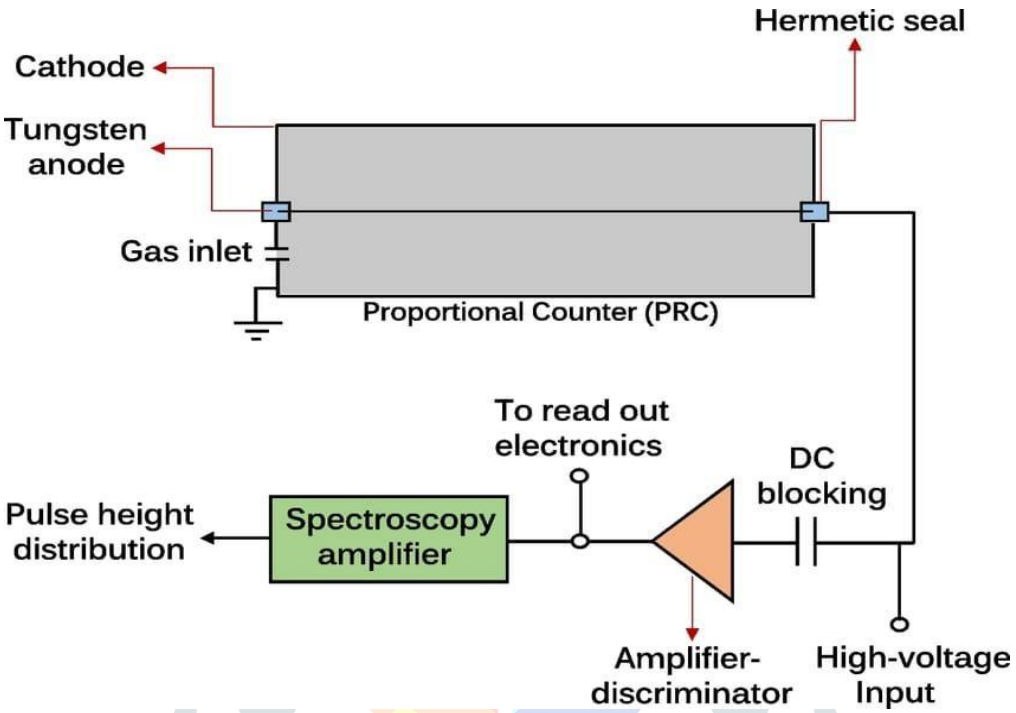

Fig.1.8:- Proportional Counter

Merits: count high rates without error good sensitivity and efficiency.

The ability to measure energy of radiation and provide spectrographic information, discriminate between alpha and beta particles, and that large area detectors can be constructed.**Demerits:** expensive complex circuit.

That anode wires delicate and can lose efficiency in gas flow detectors due to deposition, theefficiency and operation affected by ingress of oxygen into fill gas, and measurement windows easily damaged in large area detectors.

Applications:-

- Commonly used in standards laboratories, health physics laboratories, and for physicsresearch.
- Seldom used in medical centers.
- The wire chamber is a multi-electrode form of proportional counter used as a researchtool.

- The proportionality between the energy of the charged particle travelling through thechamber and the total charge created makes proportional counters useful for chargedparticle spectroscopy.
- Proportional counters are also useful for high energy photon detection, such as gamma- rays, provided these can penetrate the entrance window. They are also used for the detection of X-rays to below 1 Kev energy levels, using thin walled tubes operating at oraround atmospheric pressure.
- These are used extensively to check for radioactive contamination on personnel, flatsurfaces, tools and items of clothing.

c) Scintillator Counter:-

Scintillator: a material that exhibits the property of luminescence on excitement by ionizing radiation. A scintillator detector is a combination of scintilla tor with an electric light sensor such as PMT or photodiode. PMT absorbs the light emitted by the scintillator and further re- emits in the form of electrons. The subsequent multiplication of those electrons result in an electrical pulse which on analysis provides information about incident radiation particles. There are different scintillation materials avail such as sodium iodide, anthracene and naphthalene etc. A scintillator counter possesses many advantages such as resolution of gammarays for Co & Cs, linearity, density, speed, transparency and also manufacturing cost.

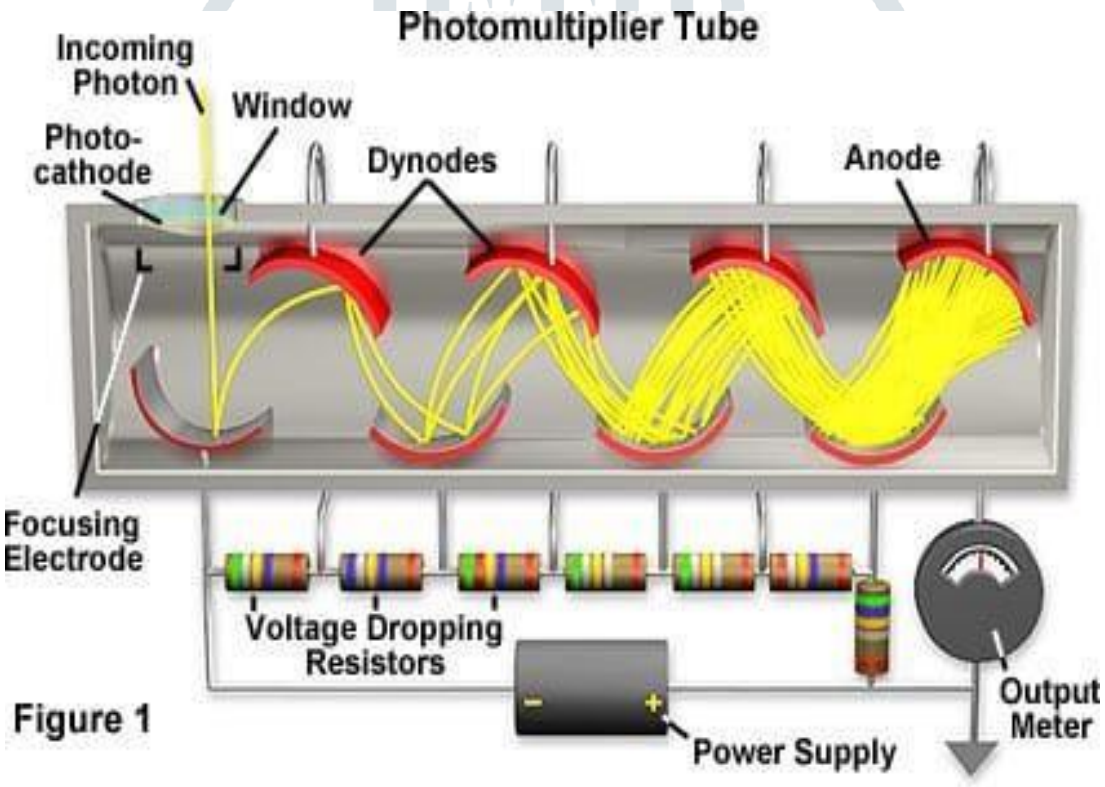

Fig.1.9:- Scintillator Counter

d) Semiconductor Detector:-

These detectors assists in promotion of X-rays through generated electrons into conduction bands. Therefore the current so generated is a direct measurement of X-rays intensity. These semiconductor detectors commonly makes use of Lead telluride or Mercury cadmium telluride.In these detectors the thin layer of p-type of semiconductor is kept over the n-type surface to make a diffused p-n junction. The p-type surface is exposed to radiation w generates hole andelectron pairs. These holes and electron pairs are separated by internal field existing on p-n junction. As a result voltage is generated. This assembly of semiconductor is arranged into a vacuum bottle cooling unit. The cooling is done through liquid nitrogen or Joule Thomson coolers of compressed nitrogen gas or liquid helium.

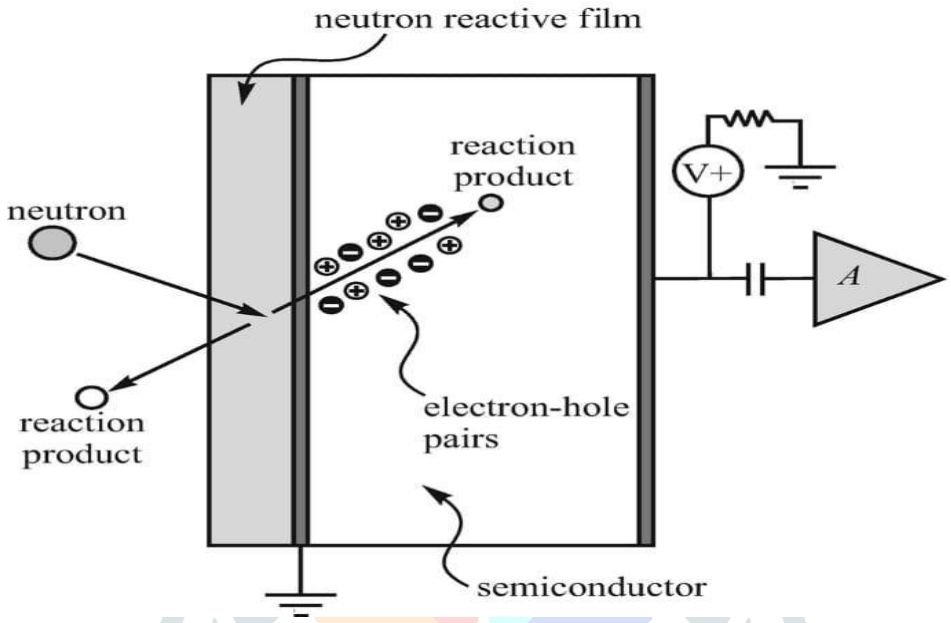

Fig.1.10:- Semiconductor Detector

2. Objectives And Aim:

1. Relationship between structures of engineering materials.
2. To understand the classification of crystals.
3. To understand mathematical description of ideal crystal.
4. To understand Miller indices for directions and planes in lattices and crystals.
5. To understand how to use X-Ray Diffraction for determination of crystal geometry.
6. Understand the concept of diffraction in crystals.
7. Be able to derive and use Bragg's law.
8. Know how X-rays are produced.
9. Know the typical emission spectrum for X-rays, the source of white radiation and the Kand KB lines.
10. Know about Compton scattering. understand the concept of diffraction in crystals

3. Plan of work:

1) Literature Survey
2) Selection of Tofacitinib citrate API
3) Compatibility study of Tofacitinib citrate
- XRD(X-ray diffraction)

4) Identification of Tofacitinib citrate API:- XRD
5) Evaluation Of Tofacitinib citrate
- Appearance
- IUPAC Name
- Colour
- Molecular formula
- Melting point
- BSC class
- Dissolution constant
- Half life
- Solubility
- Dose
- XRD Graph
- Percentage of compound present

6) Conclusion

4. Material and Method:-

Sr.no.	Properties	Tofacitinib Citrate
1	IUPAC Name	3-[(3R,4R)-4-Methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxopropanenitrile
2	Appearance	White to off white powder
3	Colour	
4	Molecular formula	C22H28N6O
5	Molecular weight	312.377 g/mol
6	Melting point	198-202° C (dec.)
7	Log p	1.15
8	Category	Janus kinase (JAK) inhibitor
9	Dissociation constant	8.46
10	BSC class	Class III (high aqueous solubility and moderate permeability)
11	Half life	3 hours.
12	Synonyms	CP-690550, Xeljanz, Tasocitinib, (3R,4R)-4-Methyl-3-(methyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino)-ß-oxo-1-piperidinepropanenitrile citrate salt
13	Bioavailability	74% oral absorption (absolute bioavailability), with peak plasma concentrations (T max) achieved in 0.5-1 hour.
14	Solubility	Freely soluble in N,N-Dimethylacetamide, slightly soluble in water, and very slightly soluble in ethanol (99.5% ethanol)

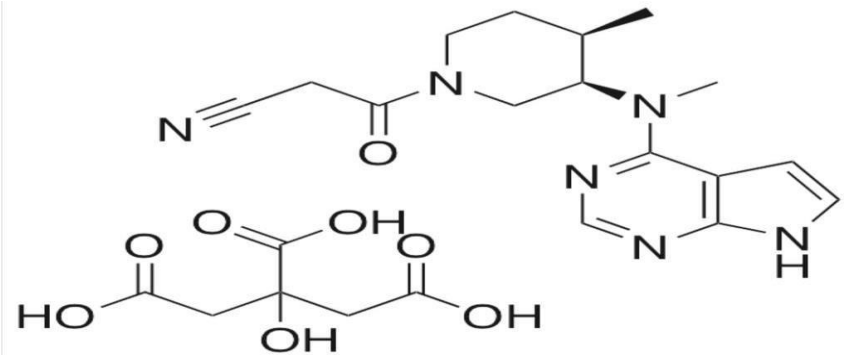

Fig.1.11:- Tofacitinib Citrate

Background :-

Tofacitinib is an inhibitor of Janus kinases, a group of intracellular enzymes involved in signalling pathways that affect hematopoiesis and immune cell function. It is approved by theFDA for treatment of moderate to severe rheumatoid arthritis that responds inadequately to methotrexate or in those who are intolerant to methotrexate. Besides rheumatoid arthritis, tofacitinib has also been studied in clinical trials for the prevention of organ transplant rejection, and is currently under investigation for the treatment of psoriasis. Known adverse effects include nausea and headache as well as more serious immunologic and hematological adverse effects. Tofacitinib is marketed under the brand name Xeljanz by Pfizer.

Indication

Tofacitinib is indicated for the treatment of adult patients with moderately-to-severely active rheumatoid arthritis (RA), active psoriatic arthritis, active ankylosing spondylitis, or moderately-to-severely active ulcerative colitis who have had an inadequate response or intolerance to one or more TNF blockers.5 It is also indicated as an oral solution in patients ≥2years of age for the treatment of polyarticular course juvenile idiopathic arthritis who have hadan inadequate response or intolerance to one or more TNF blockers.
Tofacitinib is not recommended to be used in combination with other biologic disease-modifying anti-rheumatic drugs (DMARDs) or potent immunosuppressive agents such as azathioprine or cyclosporine.

Pharmacodynamics

Tofacitinib targets inflammation present in rheumatoid arthritis by inhibiting the janus kinasesinvolved in the inflammatory response pathway. In placebo controlled trials of rheumatoid arthritis patients receiving 5mg or 10mg of tofacitinib twice daily, higher ACR20 responses were observed within 2 weeks in some patients (with ACR20 being defined as a minimum 20%reduction in joint pain or tenderness and 20% reduction in arthritis pain, patient disability, inflammatory markers, or global assessments of arthritis by patients or by doctors, according to the American College of Rheumatology (ACR) response criteria list), and improvements inphysical functioning greater than placebo were also noted. Common known adverse effects oftofacitinib include headaches, diarrhea, nausea, nasopharyngitis and upper respiratory tract

infection. More serious immunologic and hematological adverse effects have also been noted resulting in lymphopenia, neutropenia, anemia, and increased risk of cancer and infection.

Before initiations of tofacitinib patients should be tested for latent infections of tuberculosis, and should be closely monitored for signs and symptoms of infection(fungal, viral, bacterial, or mycobacterial) during therapy. Therapy is not to be started in the presence of active infection, systemic or localized, and is to be interrupted if a serious infectionoccurs. Tofacitinib has been associated with an increased risk of lymphomas, such as Epstein-Barr virus associated lymphomas, and other malignancies (including lung, breast, gastric, andcolorectal cancers). It is recommended to monitor lymphocytes, neutrophils, hemoglobin, liverenzymes, and lipids.

Tofacitinib use is associated with a rapid decrease in C-reactive protein (CRP), dose dependent decreases in natural killer cells, and dose dependent increases in B cells. Depression in C-reactive protein levels continue after 2 weeks of tofacitinib discontinuation and suggest that pharmacodynamic activity last longer than pharmacokinetic half life

Fig.1.12: Tofacitinib citrate

API PowderMechanism of action

Rheumatoid arthritis is an autoimmune disease characterized by a dysregulation of pro-inflammatory cytokines including IL7, IL15, IL21, IL6, IFN-alpha, and IFN-beta. (3) Cytokines signalling results in tissue inflammation and joint damage by stimulating the recruitment and activation of immune cells via the janus kinase signalling pathway.

Tofacitinib is a partial and reversible janus kinase (JAK) inihibitor that will prevent the body from responding to cytokine signals. By inhibiting JAKs, tofacitinib prevents thephosphorylation and activation of STATs. The JAK-STAT signalling pathway is involved in the transcription of cells involved in hematopoiesis, and immune cell function. Tofacitinib works therapeutically by inhibiting the JAK-STAT pathway to decrease the inflammatory response. However, there is evidence to suggest that it may also achieve efficacy via other pathways as well.

Absorption

74% oral absorption (absolute bioavailability), with peak plasma concentrations (T max) achieved in 0.5-1 hour. Administration with fatty meals does not alter AUC but reduces Cmaxby 32%.

Toxicity

Minimum lethal dose in rat: 500 mg/kg. Maximum asymptomatic dose in non human primate:40 mg/kg. Lymphatic, immune system, bone marrow and erythroid cell toxicity was seen in animal studies involving rate and monkeys. Doses used in these studies ranged from 1mg/kg/day to 10mg/kg/day, over a duration of 6 weeks to 6 months. Lymphopenia, neutropenia, and anemia is seen in human subjects and may call for an interruption or discontinuation of therapy if severe.

Reduced female fertility in rats was seen at exposures 17 times the maximum recommended human dose. Fertility may be impaired in human females and harm may be caused to unborn child. Carcinogenic potential is seen, however evidence for dose dependency is lacking. Because the janus kinase pathway plays a role in stimulating the production of red blood cells and is involved in immune cell function, inhibition of this pathway leads to increased risk of anemia, neutropenia, lymphopenia, cancer and infection. Lymphopenia, neutropenia, and anemia in human subjects may call for an interruption or discontinuation of therapy if severe. Role of JAK inhibition in the development of gastrointestinal perforation is not known.

Volume of distribution

Vd= 87L after intravenous administration. Distribution is equal between red blood cells and plasma.

Protein binding

40%, mostly bound to albumin.

Metabolism

Metabolized in the liver by CYP3A4 and CYP2C19. Metabolites produced are inactive.

Route of elimination

70% metabolized in the liver by CYP3A4 (major) and CYP2C19 (minor). Metabolites produced are inactive. 30% renally eliminated as unchanged drug.

5. Result and Discussion:-

XRD Interpretation of Tofacitinib

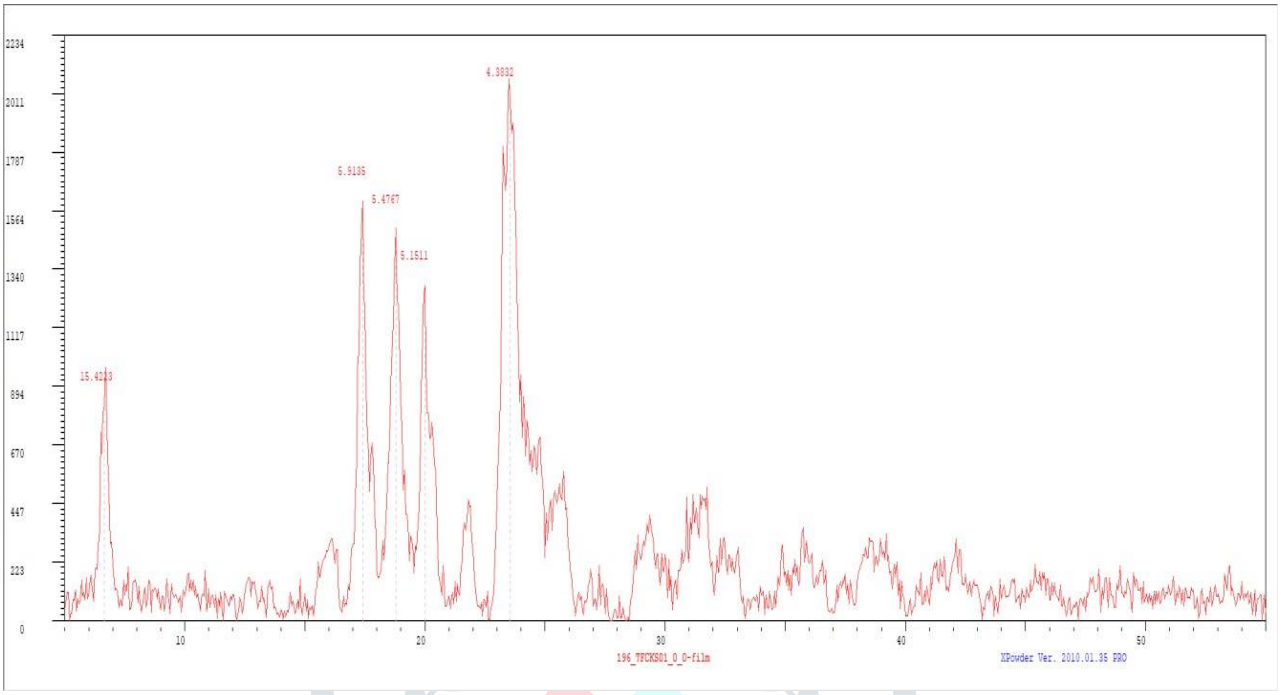

Fig.:1.13:XRD Interpretation of Tofacitinib graph

Line	2-theta angle	d-spacing	Intensity	H K L
1	6.65	15.4223	412.90	h k l
2	17.40	5.9135	808.25	h k l
3	18.80	5.4767	754.76	h k l
4	20.00	5.1511	644.59	h k l
5	23.55	4.3832	1000.00	h k l

Interpretation:

The XRD diffractogram of API Tofacitinib Citrate was obtained using Cu-K -alpha rays. The diffractogram Shoed the prominent peaks at 2Ɵ values listed in the above table confirming thecrystallinity of the drug, with **Orthorhombic** crystal shape.

6. Conclusion

X-ray diffraction (XRD) is an analytical technique used to characterize crystalline phases of a wide variety of materials, typically for mineralogical analysis and identification of unknown materials. Powder diffraction data are fundamentally derived by the atomic and molecular arrangements explained by physics of crystallography.

In the past few years, powder XRD systems have become more and more efficient for the pharmaceutical industry due to innovations and improvements in detection and source emission technology. X-ray diffraction methods are especially significant for theanalysis of solid materials in forensic science. They are often the only methods that allow a further differentiation of materials under laboratory conditions. Minerals are the building blocks of the solid Earth. Some minerals are readily recognized by their distinctive colors or crystal forms, but in most cases, powder X-ray diffraction is the primary and most definitive method used to identify minerals. The high flux and density of X-rays produced at synchrotrons provide the microelectronics industry with a powerful probe of the structure and behavior of awide array of solid materials that are being developed for use in devices of the future. X-ray diffraction studies are also used to obtain information on the short and intermediate range structure of glasses.

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