# STUDIES ON THE CRYSTAL FORMS OF SELECTED FLUOROQUINOLONES

**A thesis submitted to**

**THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI-32**

**As a partial fulfillment of the requirements for the award of the degree of**

**DOCTOR OF PHILOSOPHY**
**IN**
**PHARMACY**

**Submitted by**
**Mr. T. ETHIRAJ, M.Pharm.,**
**Ex-II(1)/21837/2010**

**Under the guidance of**

**Dr. V.GANESAN, M.Pharm., Ph.D.,**

**Principal, Professor and Head**

**Department of Pharmaceutics**


**THE ERODE COLLEGE OF PHARMACY**
**AND RESEARCH INSTITUTE,**
**VEPPAMPALAYAM,**
**ERODE – 638 112, INDIA**

**JANUARY-2016**

# DECLARATION

I, hereby declare that the thesis entitled **"STUDIES ON THE CRYSTAL FORMS OF SELECTED FLUOROQUINOLONES"** submitted herewith to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for the fulfillment for the award of Degree of DOCTOR OF PHILOSOPHY in PHARMACY, is the result of work carried out by me in the Department of Pharmaceutics, under the guidance and supervision of Professor Dr. V.GANESAN., M. Pharm., Ph.D., Head, Department of Pharmaceutics and Principal, The Erode College of Pharmacy and Research Institute, Erode.

The result of this work has not been previously submitted for any Degree or fellowship.

Date: T.ETHIRAJ

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# *ACKNOWLEDGEMENT*

*With deep devotion and great reverence I bow my head with all humility at the feet of* ***Lord Ganesh*** *who is supposed to be the first manuscript writer and I submit my sincere and respectful pranams to the lotus feet of* ***God saraswathi*** *for seeking her blessings which has been provided to me.*

*It is my great privilege, pride and honor in expressing my humble thanks to my esteemed research guide* ***Dr. V.Ganesan, M.Pharm., Ph.D.,*** *Principal, The Erode College of Pharmacy & Research Institute, Erode, for his sustained enthusiasm, motivation, exemplary guidance and constant encouragement throughout the course of this investigation. Without his valuable advice and deep rooted knowledge, this work would not have reality.*

*I express my profound indulge to the management Personale* ***Dr. K. R. Paramasivam,*** *President* ***Mr. A. Natarajan, B.A, H.D.C,*** *Secretary and Correspondent and Lion* ***Sri R. Kolandaisamy,*** *Treasurer for providing me the required facilities and infra structure to undergo my research work.*

*I express my loyal thanks to* ***Dr. R. Anandan, M.Pharm., Ph.D.,*** *Professor and Head, Department of Pharmacology,* ***Dr. M. Periasamy, M. Pharm., Ph.D., Mrs. G. Sumithra, M. Pharm., Mr. P. Royal Frank M. Pharm., Mrs. H. Rajamathanky, M.Pharm.,*** *The Erode College of Pharmacy, Erode.*

*I wish to thank* ***Dr. R. Sivakumar M. Pharm., Ph.D.,*** *Grace College of Pharmacy, Palakkad for his ultimate driving forces in completing this work.*

*I would like to express my sincere thanks to* ***Dr. V.S. Saravanan, M. Pharm., Ph.D., Dr. P Sivakumar, M.Pharm., Ph.D., Dr. T. Sudha, M. Pharm., Ph.D., Mr. P. Siva Subramanian M. Pharm., Mrs. P. Nirmala, M. Pharm., Mrs. P. Hemalatha, M. Pharm.***

*I sincerely acknowledge my colleagues* ***Dr. S.P. Senthil, M. Pharm., Ph.D., Mrs. T. Sudhamani, M. Pharm.,( Ph.D)., Mrs. P. Jeyaprabha M. Pharm.***

*I owe my warm and humble thanks to* ***Dr. V. R. Ravikumar, M. Pharm., Ph.D., Dr. S. Manokaran, M. Pharm., Ph.D.,*** *Department of Pharmacognosy. The Erode College of Pharmacy, Erode.*

*I take immense pleasure to express my sincere and deep sense of gratitude to* ***Dr. R. Senthil selvi, M. Pharm., Ph.D., Dr. J. Nandhakumar, M. Pharm., Ph.D., Mr. S. Rajarajan, M. Pharm.,*** *Department of Pharmacy Practice and* ***Mr. T.P. Sugumar, M.Sc., PGDCA.*** *The Erode College of Pharmacy, Erode.*

*I take this opportunity with great pride and immense pleasure to express my deep sense of gratitude to my research colleagues* ***Dr. S.R. Senthil Kumar, M. Pharm., Ph.D., Mrs. S. Allimalarkodi, M. Pharm., (Ph.D).,*** *for their active suggestion to improve my work for the successful completion of this thesis.*

*I also express my thanks to non teaching staffs* ***Ms. R. Kanimozhi, Mrs. S. Chithra, Mr. D. Kannan, Mr. D. Velmurgan, Mr. S. Muthukumar*** *Office Supdertent* ***Mrs. R. Madheswari Senior*** *Clerk,* ***Mr. P. Sadhasivam*** *Cashier,* ***Mr. S. Prakash, S.A.Sridhar, Mrs. M. Shanmugapriya and Mr. K. Varadharajan,*** *The Erode College of Pharmacy, Erode.*

*The completion of this thesis is not only fulfillment of my dreams, but also the dreams of my beloved parents* ***Thiru V. Thiruvenkadam*** *and* ***T. Sagunthala*** *whose unconditional support was a good strength for me throughout my studies. It is my great pleasure to acknowledge my dear in-laws* ***Thiru. L. Ramadoss*** *and* ***R. Santha*** *who believed me and stood with me in good and bad times and whose constant encouragement given success every time. I am very happy to express my love and greatful to my sons* ***Master E.Yuvaraj & Master E. Sibi Krishnaa*** *and my wife* ***Mrs. R. Revathi*** *for their unconditional love, endurance, support and encouragement.*

*I kindly thankful to all the gentle people associated with this work directly or indirectly whose names have been unable to mention here who gave constant encouragement and help throughout my educational career.*

## ABBREVIATIONS

| | | |
|---|---|---|
| % | - | Percentage |
| $^{o}$C | - | Degree Celsius |
| μm | - | Micrometer |
| μl | - | Microliter |
| cm | - | Centimeter |
| A | - | Absorbance |
| API | - | Active Pharmaceutical Ingredient |
| AFM | - | Atomic Force Microscope |
| AR | - | Analytical Reagents |
| λ max | - | Absorption Maxima |
| AUC | - | Area under Curve |
| BA | - | Bioavailability |
| BCS | - | Biopharmaceutics Classification System |
| BE | - | Bioequivalence |
| CSD | - | Crystal Size Distribution |
| Conc. | - | Concentration |
| CSF | - | Cerebrospinal Fluid |
| $C_{max}$ | - | Concentration of peak plasma |
| $cm^{-1}$ | - | Centimeter inverse |
| CL | - | Clearance |
| CNS | - | Central Nervous System |
| DSC | - | Differential Scanning Calorimetry |
| DTA | - | Differential Thermal Analysis |

| | | |
|---|---|---|
| DMSO | - | Di methyl Sulfloxide |
| DCM | - | Di chloromethane |
| DMF | - | Dimethyl Formamide |
| DNA | - | Deoxyribonuclic Acid |
| DRIFT | - | Diffuse Reflectance Infrared Fourier Transform |
| EDS | - | Energy Dispersive X-ray Spectroscopy |
| EDTA | - | Ethylene Diamine Tetra Acetic acid |
| ENT | - | Ear Nose Throat infection |
| ESEM | - | Environmental Scanning Electron Microscopy |
| FBRM | - | Focused Beam Reflectance Measurement |
| FT-IR | - | Fourier Transform Infrared Spectrophotometer |
| Fig | - | Figure |
| FDA | - | Food and Drug Administration |
| GMP | - | Good Manufacturing Practice |
| gm | - | Gram |
| GC | - | Gas Chromatography |
| hrs | - | Hours |
| HCL | - | Hydrochloric Acid |
| HSM | - | Hot Stage Microscopy |
| HS-GC | - | Headspace Gas Chromatograpy |
| HPLC | - | High Performance Liquid Chromatography |
| HPMC | - | Hydroxy Propyl Methyl Cellulose |
| IDR | - | Intrinsic Dissolution Rate |
| IP | - | Indian Pharmacopiea |

| | | |
|---|---|---|
| ICH | - | International Conference on Harmonisation |
| IR | - | Infrared Spectroscopy |
| IVIVC | - | *In-vitro – In-vivo* Corrleation |
| KF | - | Karl-Fischer Aqumetry |
| Kg | - | Kilogram |
| KBr | - | Potassium Bromide |
| $K_{el}$ | - | Elimination rate constant |
| kV | - | kilovolt |
| L | - | Litre |
| ml | - | Milli liter |
| mol | - | Molecule |
| min | - | Minute |
| mg | - | Milligram |
| sec. | - | Second |
| SMPT | - | Sovent-Mediated Phase Transformation |
| mp | - | Melting Point |
| mcg | - | Microgram |
| NMR | - | Nuclear Magnetic Resonance |
| nm | - | Nanometer |
| PMR | - | Proton Magnetic Resonance |
| PK | - | Pharmacokinetic |
| pKa | - | Acid dissociation constant |
| pH | - | Potential Hydrogen |
| PEG | - | Poly Ethylene Glycol |

| | | |
|---|---|---|
| PVA | - | Poly Vinyl Alcohol |
| R | - | Corrleation coefficient |
| RPM | - | Revolutions Per Minute |
| RNA | - | Ribonucleic acid |
| PXRD | - | Powder X-Ray Diffraction |
| QT | - | Quality Time |
| RH | - | Relative Humidity |
| SD | - | Standard Deviation |
| TLC | - | Thin Layer Chromatography |
| TEM | - | Transmission Electron Microscope |
| TGA | - | Thermal Gravimetric Analysis |
| $t_{1/2}$ | - | Biological half-life |
| $T_{max}$ | - | Time to achieve peak plasma |
| SEM | - | Scanning Electron Microscopy |
| US | - | United States |
| Vss | - | Volume steadystate |
| Vd | - | Volume of distribution |
| w/v | - | Weight by Volume |
| NMR | - | Nuclear Magnetic Resonance |
| UTI | - | Urinary Tract Infection |
| UV | - | Ultra Violet |
| USP | - | United States Pharmacopoeia |
| VIS | - | Visible |

# 1. INTRODUCTION

Most pharmaceuticals are fundamentally organic compounds and they can often exist in a variety of solid forms. A drug will remain as the same chemical entity and have the same physico-chemical properties like stability, reactivity, etc., but it may not always act in the same way in all the solid states. These differences may or may not cause a difference in bioavailability, absorption and pharmacological effect.

Pharmaceutical solids can exist in two general forms: Crystalline and non-crystalline forms. Crystalline solids are usually highly stable and have a well-established solubility and dissolution rate. Non-crystalline solid for is referred to as the amorphous form. This type of solid is randomly arranged with a high degree of disorder in the molecular arrangement. The amorphous form is often less stable than the crystalline form and is usually hygroscopic[1].

Many organic and inorganic compounds can exist in different solid forms. They can be in the amorphous, i.e., disordered or in the crystalline, i.e., ordered, state. Pharmaceutical polymorphic solids of the same chemical compound differ in internal solid state structure and, therefore possess different chemical and physical properties, including packing, thermodynamic, spectroscopic, kinetic, interfacial and mechanical properties. These properties can have direct impact upon drug substance processability, drug product manufacturability and drug product quality such as stability, dissolution and bioavailability. As a result, pharmaceutical solid polymorphism has received more importance throughout various stages of drug development, manufacturing and regulation[2].

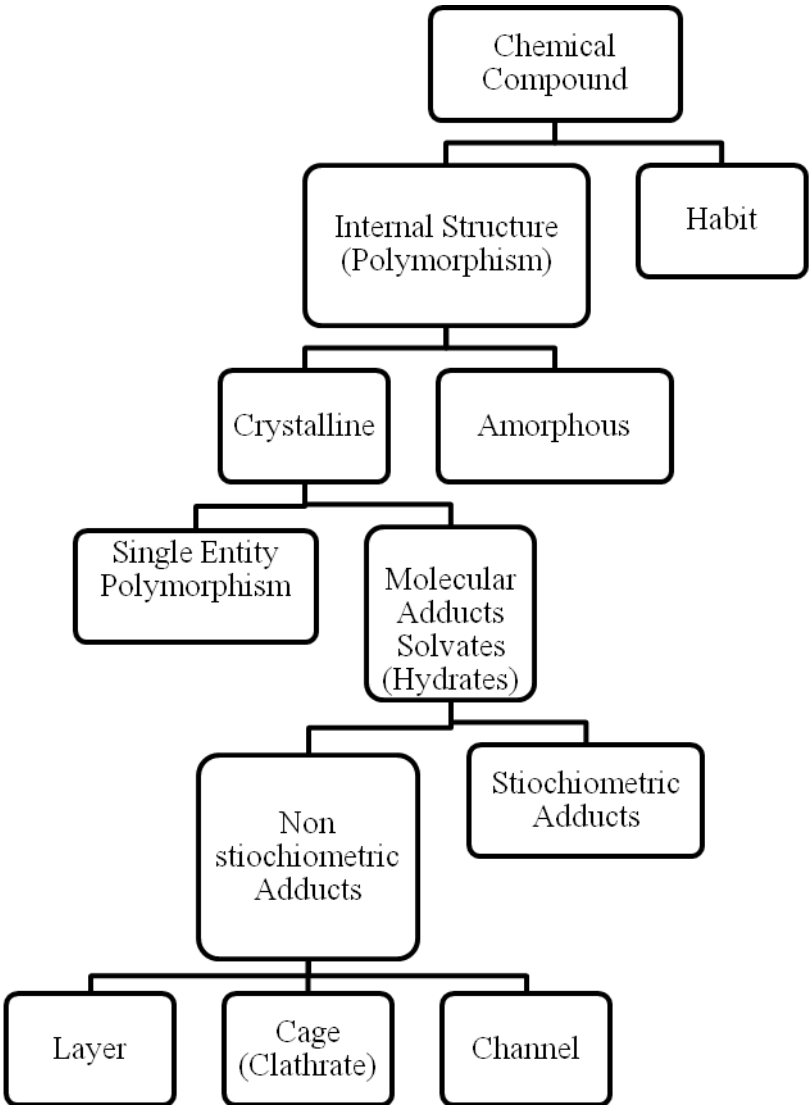



**Fig. 1: Schematic representation of pharmaceutical solid habit and polymorphism of a chemical compound.**

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms, when applied to solids, the adjective, crystalline, implies an ideal crystal in which the structural unit, termed unit cell, are repeated regularly and indefinitely in three dimensions in space. The unit cell has a definite orientation and shape defined by the translational vectors, a, b, and c and hence has a

definite volume, V, that contains the atoms and molecules necessary for generating the crystal. Many drugs exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. It is desirable to choose the most suitable and stable form of the drug in the initial stages of drug development[3].

For the problems that can be caused by changes in solid form, it is imperative polymorphism is to be thoroughly investigated early in the drug development process. So, the investigation about the potential of multiple polymorphs, the physical and chemical properties of the individual polymorphs, the suitable polymorph for development and its stability are very essential. This information will be of service throughout the pharmaceutical product lifecycle from API and product development. If the adequate information regarding polymorphs is not known and polymorphic changes occur, the following problems may arise during the process of manufacturing.

- Difficulty to decide the suitable dosage forms during formulation development process due to changes in physical properties of polymorph
- A clinical study may be affected due to solubility and bioavailability changes
- Stability problems such as precipitation from solution may occur in the commercial dosage form
- Problems in various manufacturing processes such as tablet compressing, colour changes in liquid orals
- Product quality changes such as grittiness in a topical cream formulation
- Solidification of ointments and/or suppositories over time.

A good development process should address the solid state properties of the API. The API manufacturing process should be well controlled to yield the intended crystal polymorph. Product stability studies should assure that there are no polymorphic changes during the product shelf life of the commercial package. If here is any change in the manufacturing process of drug substances and drug product it should address the potential for polymorphic changes to the API crystal[3].

Many drugs are poorly soluble or insoluble in water, which results in poor bioavailability because the solubility of a drug is an important factor in determining the rate and extent of its absorption. Numerous approaches have been employed to enhance the dissolution profile and, in turn, the absorption efficiency and bioavailability of poorly water soluble drug. Use of water soluble salts, polymorphic forms, water soluble molecular complexes, solid dispersion, co precipitation, lyophilisation, micro encapsulation, and inclusion of drug solutions of liquid drugs into soft gelatin capsules are some of the major formulation tools which have been shown to enhance the dissolution characteristics of water insoluble drugs.

Active pharmaceutical ingredients (API) are frequently delivered in the solid state as a part of an approved dosage form (such as tables, capsules etc) to the patient for treatment. Solid state of API or a drug product provides a suitable, convenient, compact and more stable format to store for long period. Studying and controlling the physic-chemical properties of APIs in solid state, both as pure drug and formulated products, is therefore an important aspect of the drug development process. APIs can be present in a variety of distinct solid crystal forms, including polymorphs, solvates, hydrates, salts and co-crystals. Each solid state form of API displays unique physicochemical properties that can be profoundly influence the bioavailability,

solubility, chemical and physical stability, moisture uptake, manufacturability and other performance characteristics of the drugs[4].

## 1.1 CRYSTALLIZATION

Crystallization is the spontaneous arrangements of the particles into a repetitive orderly array, i.e. regular geometric patterns. During the development of the pharmaceutical industry, crystallization has been engaged more and more extensively for the purification, separation of impurities, particle size reduction, ease of handling, solubility and stability of pharmaceutical materials. Active pharmaceutical ingredients (APIs) can be present in a variety of distinct solid crystal forms, including polymorphs, solvates, hydrates, salts and co crystals[5].

The two or more crystalline arrangements are chemically identical, physically different. Steroids are 67% polymorphs, Sulphonamides are 40% polymorphs and Barbiturates are 63% polymorphs. Chloramphenical palmitate exists in four polymorphs, three crystalline (A, B, and C) and an amorphous one. Novobiocin has been identified in two forms, one of which is crystalline and other amorphous. Methyl prednisolone exhibits of two polymorphs (Form I and Form II).[6]

## 1.2 POLYMORPH SCREENING[7]

In the absence of solvents and humidity, the thermodynamically stable polymorph is the only one that is guaranteed which cannot be converted into another polymorphic form. The disadvantage of the thermodynamically stable form is, of course, that it is always the least soluble polymorph and therefore has the lowest bioavailability. The kinetic of interconversion from one form into the other and the

reproducibility of producing consistently the same ratio of polymorphs are important. A metastable form might be preferable normally for one of the following reasons:

Too low solubility (and bioavailability) of the stable form

a) High dissolution rate needed for quick-relief formulations
b) Manufacturing difficulties
c) Chemical instability of the thermodynamically stable form due to topochemical factors.

- If the solubility of the stable polymorph is critically low and no salt is feasible, several options exist. Liquid-like formulations (emulsions, micro-emulsions, liposomal formulations) or soft gelatin capsules filled with solutions of the drug in a non-aqueous solvent may be used. Alternatively, a metastable solid form, a solvate or co-crystal might be selected for development.
- In some instances, quick onset of action of a drug is of particular importance. In such cases, metastable forms with a higher dissolution rate may accelerate the uptake of the drug and may therefore act faster.
- Different polymorphs will also have different mechanical properties, such as hardness, powder flow properties, compressibility and bonding strength.
- If the thermodynamically stable polymorph is protected by patents, while other forms are free, the respective drug substance can be marketed as metastable form without obtaining a license from the patent owner.
- Generally, thermodynamically most stable polymorph is also the most stable chemically. This has been attributed to the fact that its density is typically higher, but it could also be explained by its lower free energy.

When a polymorphism screening should be carried out, the choice of form to develop should be made. Since different solid forms have different properties and may have different bioavailability, it is advisable to select the final form together with the accompanying formulation before carrying out pivotal clinical studies. Accordingly, by that time a polymorphism screening that is primarily designed to identify these forms with a large probability should have been completed. Polymorphism screening that is performed with an early batch of drug substance still containing many impurities may provide different results from a screening performed with a later, purer batch. It is highly advisable to repeat at least a limited polymorphism screening with a batch of drug substance produced with the final GMP procedure, which has the impurity profile of the product to be marketed.

## 1.3 METHODS FOR PREPARATION OF CRYSTALS [8,9]

Organic drug substance that can exist in two or more solid state often can provide some distinct advantages during manufacturing of dosage forms. Factors related to processing like powder flow characteristics, compressibility, filterability and hygroscopicity, may decide the use of one polymorph in reference to another. It is essential to ascertain whether the crystalline material that results from a synthetic procedure is thermodynamically stable before conducting the trails, since a more stable form may be obtained subsequently. It is necessary to prepare different polymorphic forms of various organic drug molecules to provide the relative stability, solubility, oral absorption and effective drug release. Following are the different methods employed for the preparation of different crystal forms:

### 1.3.1 Sublimation

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of the crystals produced. The occurrence of polymorphic modifications depends on the temperature of sublimation. In general, unstable crystals form preferentially at lower temperatures, while at higher temperatures stable forms are to be expected. Nevertheless, mixtures consisting of several modifications are frequently found together. The sublimation technique is applicable only to those compounds that are thermally stable.

### 1.3.2 Crystallization from a single solvent

Crystallization from a single solvent by slow solvent evaporation is a valuable method for producing crystals. Solutions of the material being crystallized, preferably saturated, are filtered and then left undisturbed for a reasonable period of time. For the solvent to be useful for recrystallization purposes, the solubility of the solute should be on the order of 5-200 mg/mL at room temperature. If the solubility exceeds 200 mg/mL, the viscosity of the solution will be high and a glassy product is likely to be obtained. A useful preliminary test can be performed on 25-50 mg of sample, adding few (5-10) drops of solvent. If the entire solid dissolves, the solvent will not be useful for recrystallization purposes. Similarly, high viscous solvents and those having low vapour pressure (such as glycerol or dimethyl sulphoxide) are not usually conductive to efficient crystallization, filtration and washing operations.

The solvents selected for recrystallization should include any with which the compound will come into contact during synthesis, purification and processing, as

well as solvents having a range of boiling points and polarities. The process of solution mediated transformation can be considered as the result of two separate events, (a) Dissolution of the initial phase (b) Nucleation/growth of the final, stable phase. If crystals do not grow as expected from saturated solution, the interior of the vessel can be scratched with a glass rod to induce crystallization by distributing nuclei throughout the solution. Alternatively, crystallization may be promoted by adding nuclei, such as seed crystals of the same material.

A commonly used crystallization method involves controlled temperature change. Slow cooling of a hot, saturated solution can be effective in producing crystals if the compound is more soluble at higher temperatures, alternatively slow warming can be applied if the compound is more soluble at higher temperatures. Sometimes it is preferable to heat the solution to boiling, filter to remove excess solute and then quench cool using an ice bath or even a dry ice acetone bath. High boiling solvents can be useful to produce meta stable polymorphs. Some solvents favor the crystallization of a particular form or forms because they are selectively absorbed to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to advantage of others.

Among the factors affecting the types of crystals formed are:

- The solvent composition or polarity
- The concentration or degree of supersaturation
- The temperature, including the cooling rate and the cooling profile
- Additives
- The presence of seeds
- pH, especially for salt crystallization
- Agitation

In determining which solvents are to be used for crystallization, one should be careful to select those likely to be encountered during formulation and processing, typically these are water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate and hexane

### 1.3.3 Evaporation from a Binary Mixture of Solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation method depends on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to the saturated solution of the compound in good solvent. Often a solvent system is selected in which the solute is more soluble in the component with the higher vapour pressure. As the solution evaporates, the volume of the solution is reduced and, because the solvents evaporate at different rates, the composition of the solvent mixture changes.

### 1.3.4 Vapour Diffusion

In the vapour diffusion method, a solution of the solute in good solvent is placed in a small, open container which is then stored in a larger vessel containing a small amount of miscible, volatile nonsolvent. The larger vessel (often desiccators) is then tightly closed. As solvent equilibrium is approached, the nonsolvent diffuses through the vapour phase into the solution, and saturation or Supersaturation is achieved. The solubility of the compound in a precipitant used in a two-solvent crystallization method such as vapour diffusion should be as low as possible (much less than 1 mg/mL) and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution. The most

frequent application of this technique is in the preparation of single crystals for crystallographic analysis.

### 1.3.5 Thermal Treatment

Frequently when using differential scanning Calorimetry as an analysis technique, one can observe an endothermic peak corresponding to the phase transition, followed by a second endothermic peak corresponding to melting. Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases, it is often possible to prepare the higher melting polymorph by thermal treatment.

### 1.3.6 Crystallization from the Melt

In accordance with Ostwald's rule, the cooling of melts of polymorphic substances often first yields stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that super cooling is necessary to crystallize it from the melt. After melting, the system must be super cooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can sometimes result in the formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization.

### 1.3.7 Rapidly changing solution pH to precipitate Acidic or Basic substances

Many drug substances fall in the category of slightly soluble weak acids or slightly soluble weak bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of a weak acid or upon

addition of an alkali to an aqueous solution of a soluble salt of weak base, crystals often result. These crystals may be different from those obtained by solvent crystallization of a weak acid or weak base. Nucleation does not necessarily commence as soon as the reactants are mixed, unless the level of super saturation is high and the mixing stage may be followed by an appreciable time lag before the first crystals can be detected. Well-formed crystals are likely to result in these instances than when rapid precipitation occurs.

### 1.3.8 Thermal desolvation of crystalline solvates

The term "desolvated solvates" has been applied to compounds that were originally crystallized as solvates but from which the solvent has been removed generally by vaporization induced by heat and vacuum. Frequently, these "desolvated solvates" retain the crystal structures of the original solvate form and exhibit relatively small changes in lattice parameter. For this reason, these types have been referred to as pseudo polymorphic solvates. However, in instance where the solvent serves to stabilize the lattice, the process of desolvation may produce a change in lattice parameters, resulting in the formation of either a new crystal form or an amorphous form. These solvates have been referred to as polymorphic solvates. The process of desolvating pseudo polymorphic solvates is similar, involving only two steps of

- Molecular loosening
- Breaking of host-solvent hydrogen bonds or association

Among the factors that influence the desolvation reaction are the appearance of defects, the size of tunnels in the crystal packing arrangement and the strength of hydrogen bonding between the compound and its solvent of crystallization.

### 1.3.9 Growth in the Presence of Additives

The presence of impurities can have a profound effect on the growth of crystals. Some impurities can inhibit growth completely and some may enhance growth. Still others may exert a highly selective effect, acting only on certain crystallographic faces and thus modifying the crystal habit. Some impurities can exert an influence at very low concentrations (less than 1 part per million), whereas others need to be present in fairly large amounts to have any effect. Crystallization in the presence of additives like surfactants and polymers is a relatively less explored area but is important for polymorphic screening of a compound during its developmental stage. Surfactants and polymers act by various mechanisms to influence either the growth or the nucleation phase, resulting in modification of either the polymorphic form or the crystal habit.

### 1.3.10 Grinding

Polymorphic transformations have been observed to occur in grinding of certain materials, such as sulfathiazole, barbital, phenylbutazone, cephalexin, Chloramphenicol palmitate, indomethacin and chlorpropamide by,

- Molecular loosening (nucleation by separation from the lattice)
- Solid solution formation
- Separation of the product (crystallization of the new phase).

Depending on the material of the conditions employed, grinding can result in convertion in to an amorphous substance. With the exercise of care, different polymorphic forms can be obtained. It is known that the polymorphic form of a drug

substance is produced by the grinding of a single component, grinding it with some excipients.

### 1.3.11 Application of crystallization process[10]

The use of drugs in crystalline solid state form has several advantages such as,

- **Purification of drugs**

  Crystallization is used as a purification process. It is used for removing impurities from pharmaceutical products i.e. recrystallization technique.

- **Better processing characteristics:**

  Crystallization technique is used to change the micromeritics of drugs such as compressibility and wettability

- **Ease of handling:**

  Crystallization facilitates various operations such as transportations and storage.

- **Better chemical stability:**

  Crystallization increases the stability of drugs for example, amorphous penicillin G is less stable than crystal. Amitryptyline is more stable in crystalline form than in amorphous form.

- **Improved physical stability:**

  Crystallization forms play an important role in product properties such as suspension stability and hardness of a tablet. Using dehydrating materials such as dehydrated alcohol and glycerol, the stability of hygroscopic substance can be enhanced.

- **Improved bioavailability:**

  Some drugs are more effective in their crystalline form. For example, penicillin G does not dissolve immediately in the gastric fluids, so its degradation decrease. Hence, bioavailability of penicillin G enhances.

- **Sustained release:**

  Drug substances with different sizes of crystals can be used in the production of sustained release dosage forms. For example, protamine zinc insulin in crystalline form slowly and continuously releases insulin from the site of injection for prolonged periods.

## 1.4 CHARACTERISTICS OF CRYSTAL[10]

### 1.4.1 Crystal lattice

A crystal can be defined as a solid particle, which is formed by the solidification (crystallization) process (under suitable environment) is which structural units are arranged by a fixed geometric pattern of lattice.

### 1.4.2 Crystal systems or Forms

A finite number of symmetrical arrangements are possible for a crystal lattice and these may be termed as crystal forms or crystal system. They are designated as cubic, hexagonal, tetragonal, orthorhombic, monoclinic and triclinic.

### 1.4.3 Crystal habit

Crystal is a polyhedral solid with number of planar surfaces. The following different type of crystal habits, columnar, blade, plate, tabular, equant, acicular.

## 1.5 THEORY OF CRYSTALLIZATION[10]

The formation of crystals from solution involves three steps, which include

- Super saturation
- Nucleus formation
- Crystal growth

### 1.5.1 Super saturation

When the solubility of a compound in a solvent exceeds the saturation solubility, the solution becomes super saturated and the compound gets precipitated or crystallized. Super saturation can be achieved through,

- Evaporation of solvent from the solution
- Cooling of the solution, if the solute is a positive heat of solution
- Formation of a new solute as a result of chemical reaction.
- Addition of a substance, which is more soluble in solvent than the solid to be crystallized.

### 1.5.2 Nucleation

Nucleation refers to the birth of very small bodies of a new phase within a homogenous super saturated liquid phase. Initially, several molecules or ions or atoms associate to form clusters. These are loose aggregates, which usually disappear quickly. The initially formed crystals are of molecular size, which are termed as nuclei. Several methods are available for nucleation. These are,

- Soft or weak crystals on impact with moving parts in a crystallizer can break into fragments such as nuclei.

- Small crystals which are formed in the previous process are added to act as nuclei.
- In a supersaturated or under poor mixing, needle like structures are observed on the ends of crystals. These structures grow faster than the sides of the crystals and come out to give crystals of poor quality.

### 1.5.3 Crystal growth

Crystal growth is a diffusion process and a surface phenomenon. From solution, solute molecules or ions reach the faces of a crystal by diffusion. On reaching the surface, the molecules or ions must be accepted by the crystal and organized into the space lattice. This phenomenon continues at the surface at a finite rate. Neither the diffusion nor the interfacial step will proceed unless the solution is super saturated.

## 1.6 TYPES OF POLYMORPHISM

'Polymorphism' comes from the Greek word, Polus = many and morph = shape. Thus, it is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in the crystal lattice.

Polymorphs are categorized into two types, namely monotropes and enantiotropes, depending upon their stability with respect to the range of temperatures and pressures. If one of the polymorphs is stable over a certain temperature range and pressure, while the other polymorph is stable over a different temperature range and pressure, then the two polymorphs are said to be enantiotropes. On the other hand, the

polymorphs in which only one polymorph are stable at all temperatures below the melting point, with all the other polymorphs being unstable said to be monotropes.

Hence, polymorphism refers to different structural forms of a chemical substance. It is defined as the ability of a solid material to exist in two or more crystalline forms with different arrangements or conformations in the crystal lattice. More than 50% of APIs are estimated to have more than one polymorphic form. Polymorphism and pseudomorphism are very common types of crystalline form which are also responsible for differences in many properties. Commonly, polymorphism is classified into two types, such as monotropic systems and enantiotropy systems. In monotropic system, only one polymorphic form is stable at the temperatures below the melting point of the solid drug. If each crystalline form is the more stable within a given temperature range, the change from one form to another is reversible and is said to be enantiotropy. The change from one phase to another is univariate equilibrium, so that at a given pressure this state is characterized by a transition temperature. However, in the latter, no such phenomenon is observed, though different forms can be stable than the others irrespective of any noticeable temperature pattern[11, 12]

### 1.6.1 Pseudo polymorphism

The term pseudo polymorphism is used to describe solvates (including hydrates), where a solvent is present in the crystal matrix in stoichiometric proportions or the solvent is trapped in the matrix in variable proportions. However, the term pseudo polymorphism is ambiguous because of its use in different circumstances. It is therefore preferable to use only the terms solvates and hydrate[13]

Different crystalline forms like polymorphs, solvates or hydrates may be produced by varying the crystallisation conditions such as temperature, pressure, solvent, concentration, rate of crystallisation, seeding of the crystallisation medium, presence and concentration of impurities, etc. Polymorphs and hydrates differ in internal solid-state structure and possess different physical properties like melting point, crystal habit, colour, density, compressibility, packing, thermodynamic, spectroscopic, kinetic, interfacial, and flow properties[14,15]. Pharmaceutical solvates, resulting from the incorporation of one or more solvent molecules in the crystal lattice of the drug, are important products in drug development. Their ability to disrupt the hydrogen bonding patterns would directly affect the physicochemical properties of the parent molecule[16].

Solvates are crystalline solid substance containing solvent molecules within the crystal structure. If the incorporated solvent is water, a solvate is termed a hydrate. They are usually significant differences in their physical properties, such as density, hardness, compressibility, refractive index, melting point, enthalpy of fusion, vapour pressure, solubility and dissolution rate. For approval of a new drug, Food and Drug Administration (FDA) states that "appropriate" analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance. It is also very important to control the crystal form of the drug substance during the various stages of product development, because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystalline can alter the bioavailability of the drug.

Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. The crystal forms are more stable in the presence of water and/or solvents. The common methods for the characterization of hydrates and solvates are polarized light microscopy and hot stage microscopy, DSC, TGA, Karl Fischer titrimetry, single-crystal X-ray diffractometry, Powder X-ray diffractometry and infrared spectroscopy. The combined physical analytical techniques of thermo gravimetry and infrared spectroscopy (TG/IR) can permit identification of the solvent incorporated into the crystal lattice. [3,17].

### 1.6.2 Differences in polymorphs in their physical properties[9]

Packing properties

- Density
- Refractive index
- Conductivity (electrical & thermal)
- Hygroscopicity

Thermodynamic properties

- Melting temperature
- Vapor pressure
- Solubility

Spectroscopic properties

- Electronic transition (UV spectra)
- Vibrational transitions (IR spectra)
- Nuclear spin transition (NMR spectra)

Kinetic properties

- Dissolution rate
- Stability

Surface properties

- Surface free energy
- Interfacial tension
- Habit (i.e. shape)

Mechanical properties

- Hardness
- Tensile strength
- Compatibility (Tableting)
- Handling, flow & blending properties.

## 1.7 INFLUENCE OF SOLUBILITY, DISSOLUTION, BIOAVAILABILITY AND STABILITY ON POLYMORPHISM[2, 18, 19]

As the solid-state properties of a drug substance are known to potentially exert a significant influence on the solubilization of drugs and as polymorphic forms differ in internal solid-state structure, polymorphs of a drug substance may have different apparent aqueous solubilities and dissolution rates. When the differences in the solubilities of the various polymorphs are sufficiently large, this may have an effect upon drug product bioavailability. Nevertheless, whether differences in the solubilities of the various polymorphs will have an effect upon drug product bioavailability and bioequivalence (BA/BE) is also dependent upon other factors that

govern the rate and extent of drug absorption, including gastrointestinal motility and intestinal permeability.

For a drug whose rate and extent of absorption is limited by its dissolution, large differences in the solubilities of the various polymorphic forms are likely to affect BA/BE. On the other hand, for a drug whose rate and extent of absorption is only limited by its intestinal permeability, differences in the solubilities of the various polymorphs are less likely to affect BA/BE. Furthermore, when the solubility of the polymorphic forms are sufficiently high and drug dissolution is rapid in relation to gastric emptying, differences in the solubilities of the various solid-state forms are unlikely to affect BA/BE.

The enhancement of aqueous solubility is very essential to make faster dissolution rate, in order to achieve rapid absorption and efficacy or to achieve acceptable systemic exposure for a low solubility drug. A significant solubility difference between two polymorphs is likely to occur due to a difference in oral absorption rate, reflected in a difference in $C_{max.}$

Polymorphs of a pharmaceutical solid may have different physical and solid-state chemical (reactivity) properties. The most stable form of the drug substance is often chosen during development, based upon its minimal potential for conversion to another form and upon its greater chemical stability. However, in some instances a metastable form may be chosen because of various reasons, including bioavailability enhancement. The drug product stability is affected by a multitude of other factors, including formulation, manufacturing process and packaging, it is the stability of the drug product, not the drug substance, that is the most relevant measure of the drug

quality. Polymorphs may also undergo phase conversion, when exposed to a range of manufacturing processes, such as drying, milling, micronization, wet granulation, spray-drying and compaction. Exposure to environmental conditions such as relative humidity and temperature may also induce phase conversions. The extent of polymorphic form conversion depends upon the relative stability of the polymorphs, on the kinetic barriers for phase conversions and upon the stress applied. Based upon the potential for such conversions, the most stable crystalline form is often selected and controlled during the entire manufacturing process. It should generally not be of serious concern provided they occur consistently and reproducible as a part of a validated manufacturing process, where critical manufacturing process variables are well understood and controlled.

## 1.8 IMPACT OF POLYMORPHISM ON DRUG PRODUCT DEVELOPMENT[20]

Highly water-soluble compound have no effect on bioequivalence since the first step in the bioavailability of any drug is solubilization. The only effect that polymorphism could have in the case of a highly water-soluble compound is with respect to manufacturability. The particle shape and powder density is dependent on polymorphism. Both of these physical parameters can affect manufacturability resulting in poor flowability and compaction. The tabletting properties were related to the water content and solid state structure of these materials. Common techniques such as wet and dry granulation can be used to improve the physical characteristics of the powder so that tablets and or capsules can be effectively manufactured.

## 1.9 CHARACTERIZATION OF POLYMORPHIC FORMS[21-35]

**Table 1: Methods for characterizing pharmaceutical solid polymorph**

| S.No. | Methods | Usage |
|---|---|---|
| 1 | Microscopy | Morphology |
| 2 | X-Ray Powder diffraction (XPRD) | Crystallography |
| 3 | Thermal Analysis:<br>Differential Scanning Calorimetry (DSC)<br>Thermal Gravimetric Analysis (TGA)<br>Hot-stage microscopy | Polymorphic transition |
| 4 | Spectroscopy:<br>Infra Red (IR)<br>Raman Spectroscopy<br>Solid state Nuclear Magnetic Resonance | Molecular motion |
| 5 | Densimeter | Solubility studies |
| 6 | Nuclear Magnetic Resonance | Chemical environment |

### 1.9.1 Spectroscopic methods

#### 1.9.1.1 Powder x-ray diffraction (PXRD)

PXRD is a non destructive analytical technique which reveals information about the crystallographic structure, chemical composition and physical properties of pharmaceutical substances and polymer films. These techniques are based on observing the intensity of scattered x-ray beam radiation after hitting a sample as a function of incident and a scattered angle, wave length and energy.

X-ray analysis has been successfully employed for obtaining a detailed picture of the thermal vibrations of each atom in the crystal information about the environment of the molecule and intermolecular interactions in order to obtain the complete electron distribution in the molecule.

**Bragg's law**

When a beam of x-ray radiation is incident upon a sample, it is reflected in all directions at the same frequency from each crystal plane. Every crystalline substance scatters the x-rays in its own unique diffraction pattern producing a fingerprint of its atomic and molecular structure. The relationship between the wavelength of the x-ray beam λ, the angle of diffraction and distance between each set of atomic planes of the crystal lattice d, is governed by the Bragg's condition.

Now days, the x-ray study may be viewed as a routine procedure in structural investigation of crystals and many polymers.

### 1.9.1.2 Infra red spectrophotometry

Electromagnetic radiation ranging between 400 $cm^{-1}$ and 4000 $cm^{-1}$ (2500 and 20,000 nm) is passed through a sample and is absorbed by the bonds of the molecules in the sample causing them to stretch or bend. The wave length of the radiation absorbed is characteristic of the bond absorbing it.

**Fourier transform infrared spectrometer (FT-IR**)

Two types of instrument are commonly used for obtaining IR spectra,

- **Dispersive type**: It uses a mono chromator to select each wave number in turn in order to monitor its intensity after the radiation has passed through the sample.
- **Fourier transform:** It uses interferometer in which individual wave numbers can be monitored with a pulse of radiation without dispersion being required.

**Principle of interferometry**

Infrared radiation consisting of all the wavelength which is splitted into two beams and recombines after a path difference has been introduced (one beam is of fixed length and the other is of variant path length).

The result of a complete variation of wave lengths is a series of destructive and constructive combinations. This is called interference, Fourier transformation converts this interferogram from the time into one spectral point and these type of similar transformation at successive points throughout this variation gives rise to the complete IR spectrum.

The method is used in a qualitative finger print check for the identity of new material and for identifying drugs, films, coating and packing materials. It can also be used to detect polymorphs of drugs (polymorphs are different crystal forms of a molecule that have different physical properties such as solubility and melting point, which may be important in the manufacturing process and bio-availabiity)

**1.9.1.3 Nuclear magnetic resonance spectroscopy (NMR)**

Radiation is the radiofrequency region which is used to excite atoms, usually protons or carbon-13 atoms, so that their spins switch from being aligned with the being aligned against an applied magnetic field. The range of frequencies required for excitation and the complex splitting patterns produced are very characteristic of the chemical structure of the molecule.

It is a powerful technique for the characterization of the exact structure of raw material in crystalline form as well as in amorphous form. Chemical shift scale of the NMR spectrum provides much more related information about the proton

environments in each functional group. $^{19}F$, $^{13}C$ NMR studies also show the relationship of proton with other atoms in the molecule.

### 1.9.2 Thermal methods

Thermal methods of analysis comprise a group of techniques in which the physical properties of a substance is measured as a function of temperature while the substance is subjected to a controlled temperature programme. The most commonly used techniques are:

- Thermo microscopy (Hot-stage microscopy)
- Differential Scanning Calorimetry (DSC)
- Differential Thermal Analysis (DTA)
- Thermo Gravimetry (TG)

These techniques are widely used for the determination of

- Identity and purity of drugs
- Melting and crystallization characteristics of drugs and polymers
- Phase transformation
- Dehydration and desorption
- Crystalline transition

#### 1.9.2.1 Thermo microscopy

It involves the observation of a sample through a microscope fitted with a stage that can be heated or cooled at a controlled rate. It can cover the range of -180$^{o}$c to + 600$^{o}$c and particularly useful for the examination of liquid crystals and phase transformation.

#### 1.9.2.2 Differential scanning calorimetry (DSC)

In this method, the energy necessary to establish a zero temperature difference ($\Delta T=0$) between a sample and a reference material is recorded as a function of temperature or time when both are subjected to controlled temperature programme. Here, sample and reference materials are heated with two individual heaters. DSC curves show endothermic and exothermic peaks depending on the changes in the physico-chemical property of the drug, crystalline material and polymer.

#### 1.9.2.3 Differential thermal analysis (DTA)

The temperature difference ($\Delta T$) between the sample and reference material is recorded against time or temperature when two specimens are subjected to an identically controlled temperature regime. DTA allows the detection of every physical and chemical change including crystalline transition whether (or) not it is accompanied by a change in weight.

#### 1.9.2.4 Thermo gravimetry (TG)

In this technique, the change in mass of the sample is monitored while it is being subjected to a controlled temperature program. This method determines the temperature at which the material loses its weight. This loss indicated about the process whether decomposition or evaporation. The temperature at which no weight loss takes place is also revealed, which indicates about stability of the material.

### 1.9.3 Microscopic method

#### 1.9.3.1 Optical microscopy

It is a type of microscope which uses visible light and a system of lenses to magnify images of small crystals. The image from an optical microscope can be captured by normal light-sensitive cameras to generate a micrograph.

### 1.9.3.2 Scanning electron microscope (SEM)

It uses a focused beam of high energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron sample interactions reveal information about the sample including external morphology (texture), chemical composition and crystalline structure and orientation of materials making up the sample. The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons depends on the angle at which beam melts surface of specimen, i.e. on specimen topography. By scanning the sample and collecting the secondary electron with a special detector, an image displaying the topography of the surface is created.

## 1.10 ANTIBIOTIC - FLUOROQUINOLONE

Antibiotics or antibacterial' are a type of anti microbial used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. Several antibiotics are also effective against fungi and protozoan and some are toxic to humans and animals, even when they are given in therapeutic dose.

The newer fluoroquinolones have broad-spectrum bactericidal activity, good tissue penetration and favorable safety and tolerability profiles. A new four-generation classification of the quinolone drugs takes into account the expanded antimicrobial spectrum of the more recently introduced fluoroquinolones and their clinical indications. Enhanced antimicrobial activity has extended the use of the fluoroquinolones beyond the traditional indications for quinolone antibiotics in the treatment of urinary tract infections. The fluoroquinolones are effective in a wider variety of infectious diseases, including skin and respiratory infections. Because of

their excellent safety and tolerability, they have become popular alternatives to penicillin and cephalosporin derivatives in the treatment of various infections.

**Table 2: Classification of fluoroquinolones**

| S.No. | First Generation | Second generation | Third generation | Fourth generation |
|---|---|---|---|---|
| 1. | Nalidixic acid, | Ciprofloxacin, | Sparfloxacin, | **Prulifloxacin,** |
| 2. | Cinoxacin, | Lomefloxacin, | Levofloxacin, | **Gemifloxacin,** |
| 3. | Oxolinic acid, | Norfloxacin, | Temafloxacin, | Moxifloxacin, |
| 4. | Priomidic acid, | Ofloxacin, | Tosufloxacin, | Sitafloxacin, |
| 5. | Pipemidic acid, | Pefloxacin, | Pazufloxacin. | Trovafloxacin, |
| 6. | Rosoxacin. | Rufloxacin. | | Gatifloxacin. |

### 1.10.1 Mechanism of action of fluoroquinolones [37,38]

The main mechanism of action of quinolones involves interactions with both DNA gyrase, the originally recognized drug target and topoisomerase IV, a related type II topoisomerase. Commonly, DNA gyrase is more sensitive in gram-negative bacteria and topoisomerase IV more sensitive in gram-positive bacteria. Usually the more sensitive enzyme represents the primary drug target determined by genetic tests, but poorly understood exceptions have been documented. The formation of the ternary complex of quinolone, DNA, and either DNA gyrase or topoisomerase IV occurs through interactions in which quinolone binding appears to induce changes in

both DNA and the topoisomerase that occur separately from the DNA cleavage that is the main objective of quinolone action.

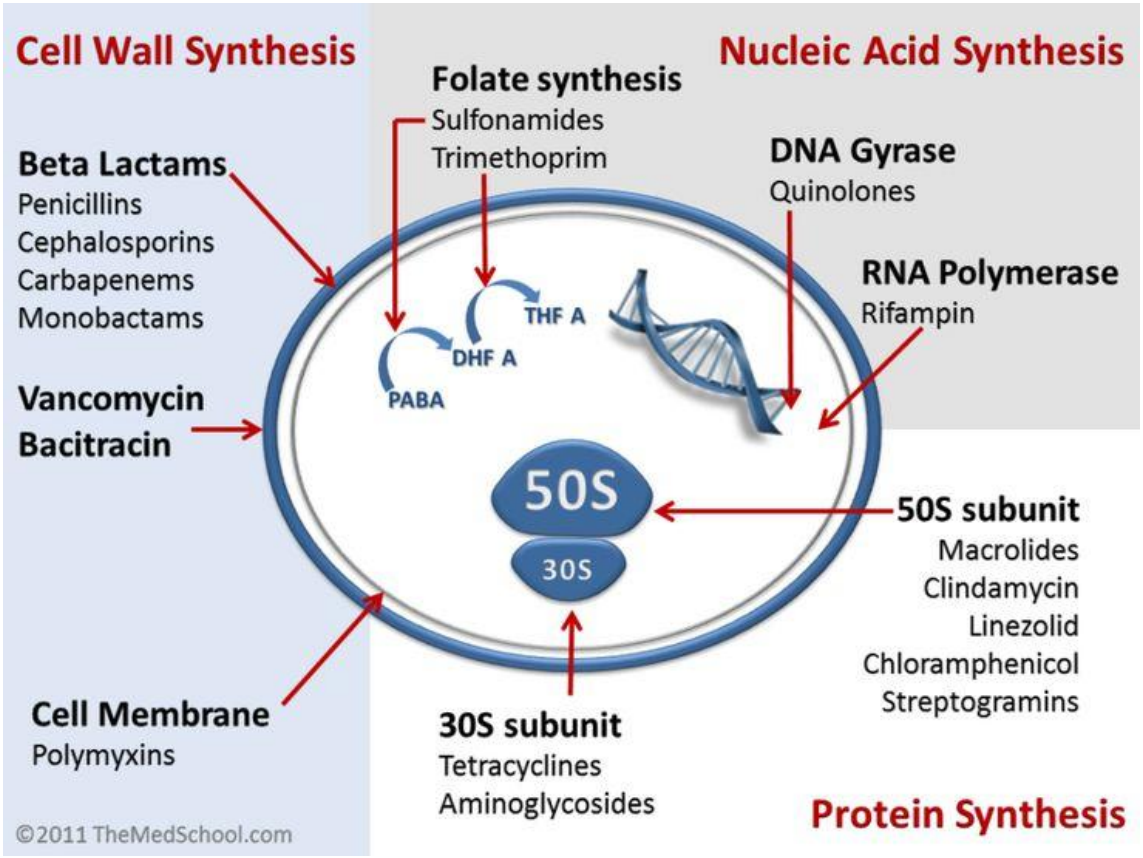



**Fig. 2: Mechanism of action of antibiotics**

The fluoroquinolones are broad-spectrum antibiotics with particular activity against the following gram-negative and gram-positive organisms.[39]

- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Mycoplasma sp*
- *Chlamydia sp*
- *Chlamydophila sp*
- *Legionella sp*

- *Enterobacteriaceae*
- *Pseudomonas aeruginosa*
- *Mycobacterium tuberculosis*
- Some atypical *mycobacteria*
- *Methicillin-sensitive staphylococci*

Nosocomial methicillin-resistant *staphylococci* are usually resistant. Older fluoroquinolones have poor activity against *streptococci* and anaerobes. Newer fluoroquinolones have reliable activity against *streptococci* (including *Streptococcus pneumoniae* with reduced penicillin sensitivity) and some anaerobes; As use has increased, resistance, particularly to older fluoroquinolones, is developing among *Enterobacteriaceae, P. aeruginosa, S. pneumoniae,* and *Neisseria sp.* Fluoroquinolones are no longer recommended for treatment of gonorrhea in the US because of increasing resistance.

### 1.10.2 Indications for fluoroquinolones[40]

- Urinary Tract Infection
- Prostatitis
- Acute pyelonephritis
- Epididymitis
- Perioperative antibiotic prophylaxis for transurethral surgery
- Bronchitis
- Pneumonia

- Sinusitis
- Gastrointestinal infections
- Soft tissue infections
- Bone and joint infection.

## 1.11 *IN-VIVO* PHARMACOKINETIC STUDIES[41-49]

Clinical pharmacokinetic studies are performed to examine the absorption, distribution, metabolism and excretion of a drug under investigation (investigational drug and approved drug) in healthy volunteers and/or patients. Data obtained from such studies are useful for the design and conduct of subsequent clinical trials. They are also necessary for appropriate analysis and evaluation of the efficacy and safety data obtained in clinical trials for new drug development and in post-marketing clinical trials.

### 1.11.1 Application of pharmacokinetic studies

The principal objectives are to estimate the factors involved in the absorption, distribution and elimination (metabolism and excretion) and to compare the bioavailability of the active ingredient from two or more product formulations or various routes of administration (bioequivalence studies).

**Bioavailability**

The rate and extent of absorption of the amount of unchanged drug from its dosage form is termed as bioavailability.

**Objective of bioavailability studies**

- Primary stages of development of a suitable dosage form for a new drug entity
- Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption
- Development of new formulations for the existing drugs
- Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption.

**Measurement of bioavailability**

- **Pharmacokinetic methods**
    a. Plasma level-time studies
    b. Urinary excretion studies
- **Pharmacodynamic methods**
    a. Acute pharmacologic response
    b. Therapeutic response

Pharmacokinetic data can also be used to predict plasma concentrations, target tissue doses and the fate of the administered dose. This information can then help to achieve the following,

1. To decide which toxicity studies should be conducted,
2. To select doses for chronic toxicity and carcinogenicity studies,
3. To determine the mechanism of toxicity and assist in the interpretation of toxicity data,
4. To improve the risk assessment process.

### 1.11.2 ADME studies

#### 1.11.2.1 Absorption

Both the rate and extent to which the active ingredient or active moiety are available systemically should be determined. Generally, the rate of absorption can be determined only from plasma/blood curve-time data; urine data can be used only to measure the extent of absorption. Whenever possible, comparison with an equivalent intravenous dose should be made, as only intravenous (bolus or infusion) data permit the evaluation of the extent of absorption (absolute systemic availability). If a systemic effect is expected (e.g. an active ingredient with high intrinsic activity) the degree of systemic absorption should be quantified. Systemic availability is usually determined by measurement of the concentration of the active ingredient (therapeutic moiety or metabolite) in plasma as a function of time. Urinary excretion (active ingredient, metabolites) and acute pharmacological effects may also be considered. Systemic availability is a comparative measurement with respect to either an intravenous (absolute) or extravascular (relative) reference product.

#### 1.11.2.2 Distribution

The extent of distribution will often be reflected in the volumes of distribution (Vc, Vss, and Vz). If appropriate, these distribution parameters should be estimated. When relevant to the claims, distribution in body fluids (CSF, synovial fluid, mucus, milk, etc.) and in body tissues (lung, liver, kidney, skeletal muscle, etc.) should be determined. This constitutes the distribution pattern of the active ingredient. The percentage and, in certain cases, characteristics of binding of the active ingredient to plasma proteins should be studied over the anticipated range of plasma concentrations obtained after administration of the proposed dose(s).

### 1.11.2.3 Metabolism

If there is an indication that pharmacologically active metabolites are formed and if there is reason to suspect that they contribute significantly to the therapeutic activity and/or adverse reactions, the metabolite plasma profile should be studied.

### 1.11.2.4 Excretion

The relative contribution of the different routes of excretion of the total substance (active ingredient + metabolite), if relevant to the claimed effect, should be quantified. It is useful to know the fraction of the dose subjected to renal elimination in order to predict the influence of renal disease on elimination of the active ingredient from plasma. Another reason to estimate urine and fecal elimination is to assess the potential environmental load from the active ingredient and its metabolites.

### 1.11.3 Selection of animals

Metabolic and pharmacokinetic data from two rodent species (usually the rat and mouse) and a non rodent species (usually the dog) are recommended. If a dose dependency is observed in metabolic and pharmacokinetic or toxicity studies with one species, the same range of doses should be used in metabolic and pharmacokinetic studies with other species. If human metabolism and pharmacokinetic data also are available, this information should be used to help in selecting the test species for the full range of toxicity tests and may help to justify using data from a particular species as a human surrogate in safety assessment and risk assessment.

Studies should be performed in representative animals under well defined and controlled conditions. The animal species, group size, age (adult, young, neonate) and sex should be defined. Basic pharmacokinetic studies should be carried out in a

sufficient number of healthy animals of the target population. The number of animals used should be justified.

### 1.11.4 Route of administration

For the administration of an active ingredient to individual animals, dose should be expressed on a body weight basis and body area basis. The procedure used to estimate body area should be described and justified. In the case of tablets for administration to dogs (and cats) fixed dose (based on tablet strength) can be administered but weights of dogs should be standardized. When the exact dose per animal cannot be determined (feed medication, veterinary medicinal product administered by inhalation etc.), the dose should be expressed using the most appropriate reference (e.g. concentration in food and quantity of ingested food for medicated feeds). For substances or active ingredients intended to be administered via food or drinking water, special attention should be given to the behavior of the animals (daily intake of food and water, delay between intake of active ingredient and sampling, etc.). If radioactive isotopes are used, the tracer dose may be combined with a quantity of non-labeled substance/active ingredient to attain the proposed dose range.

### 1.11.5 Selection of dose

Selection of the dosing regimen for metabolism and pharmacokinetic studies depends on the type of information that is needed. Metabolic and pharmacokinetic parameters are usually determined following a single administration of the test compound. Comparing parameters obtained from studies in which a range of single doses have been administered can be used to determine the doses at which saturation of absorption, distribution, metabolism or excretion occurs. Multiple dosing studies

can be used to determine the potential of a compound to induce or inhibit its absorption, distribution, metabolism or excretion. Identification and quantification of the major metabolites following administration of single and multiple doses may indicate whether saturation or induction of a particular biotransformation pathway can occur. *In vitro* experiments may be useful in screening for dose dependencies, and provide more accurate descriptions of the enzyme kinetics or other processes underlying dose dependencies observed in the whole animal. *In vitro* studies usually indicate identical metabolic pathways and metabolic rates comparable to those obtained from whole animal studies but require fewer animals to perform and can be completed in less time with fewer resources.

For a new active ingredient, kinetic studies using at least three different doses should be performed, the central dose being the expected recommended dose. Omission of the data should be justified. Appropriate statistical tests should be carried out to determine linearity. In the case of non dose-linearity or a very steep dose/effect curve, studies using three different dose levels may be necessary if a range of therapeutic doses is recommended, the central dose being the median of the dose range. If the active ingredient is used only at one dose level, a single dose study may be sufficient, but should be justified.

### 1.11.6 Sampling method

Suitable biological fluids (blood, plasma, serum, urine, etc.) and tissues should be selected for pharmacokinetic investigation. Plasma is generally considered to be the most useful biological fluid for such studies.

### 1.11.6.1 Blood sampling

Attention should be given to the site of blood collection, sampling procedure, material used for sampling, blood collecting tubes, anticoagulant, delay and conditions of centrifugation to obtain plasma. The stability of the substance during sampling and under conditions of storage pending analysis should be assessed. The number of blood samples and the timing of sampling should be appropriate to allow an adequate determination of absorption, distribution and elimination. Blood samples in the post-absorption phase should be obtained over as long a period as is necessary for the purpose of the investigation (sometimes up to four "half-lives").

### 1.11.6.2 Sampling of other biological fluids and tissues

Other biological fluids may be of general interest (e.g. if analytical constraints limit the usefulness of blood samples, urine samples may be used to determine the terminal disposition slope) or of particular interest (e.g. local distribution to support a claim). Collection of some of these fluids requires special attention (e.g. immediate pH measurement of urine, conditions of storage, etc.). For tissues, repeated biopsies using local anesthesia may be considered for scientific reasons as this conserves animals and allows an individual-based analysis. However, special attention should be paid to ascertain absence of pain and discomfort when using a biopsy method.

### 1.11.7 Method of bio analysis

Active substance concentration should be determined using appropriate analytical methods. The use of a chemical assay method is generally preferable (e.g.) UV, HPLC LC-MS/MS method. The selected method should be accurately described in an internationally accepted standard or validated properly and the following item should be reported:

- Purpose and scope
- Reagents and apparatus
- Collection and storage of samples
- Preparation (and eventual clean up) of samples
- Procedure for the measurement of plasma or serum concentration
- Calculation of results (e.g. method of standardization, mathematical model, calibration curves)
- Statistical analysis
- Quality control (internal)
- The method chosen should be adequately validated

### 1.11.8 Experimental design

Single dose studies should be performed within the recommended dose range. Multiple dose and steady-state experiments are necessary if therapeutic use of the substance/active ingredient relies on steady-state conditions. Multiple dose studies and steady-state experiments should be conducted with the recommended dosage regimens (dose, dosing interval, number of administrations). Comparison of plasma concentration profiles after administration of the first and last dose is highly desirable. Multiple dosing followed by examination of the washout period may elucidate the existence of a slow elimination phase which might not be detected following a single dose.

The ADME parameters obtained from *in-vitro* and *in-vivo* models, which provide information about the prediction of drug behaviors in patients, are important for the decision to approve or terminate a drug substance. These studies use

compartmental and non-compartmental methods to determine multiple parameters, including maximum concentration ($C_{max}$), time of maximum concentration ($T_{max}$), area under the concentration time curves from time zero to infinity ($AUC_{0-\infty}$), volume of distribution (Vd), clearance (CL), terminal elimination half life ($T_{1/2}$), and bioavailability (F), thereby define the PK profiles of a compound.

**$C_{max}$**: The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.

**$T_{max}$**: The peak time that gives an indication of the rate of absorption

**AUC**: The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

### 1.11.9 Measurement of pharmacokinetic parameters and interpretation

Appropriate mathematical methods should be used to generate basic parameters (compartmental and/or "non-compartmental" analysis). Appropriate pharmacokinetic computer programs should be used under specified conditions (regression methods, weighting factor, etc.). Whenever possible, plasma concentration time data should be analyzed for individual animals. Standard equations or equivalent calculations should be used to calculate pharmacokinetic parameters and interpretation provided of the values obtained. Dosage regimen determination (to include the size of dose, dosing interval and route of administration) should be take into account, the range of therapeutic plasma concentrations, systemic availability of active ingredient from the dosage form (veterinary medicinal product) administered

and values of pharmacokinetic parameters in the target species. Special approaches (simultaneous modeling of pharmacokinetics and pharmacodynamics, population kinetics, deconvolution for the study of controlled-released formulations, etc.) are encouraged, if applicable.

## 2. AIM AND OBJECTIVES

Crystal forms of drug substances are important in pharmaceutical manufacturing. Physical state of the active constituent has paramount importance in preformulation studies and also for getting better bioavailability of the active ingredients. Different crystal forms of any pharmaceutical entity with attendant variation in physicochemical and biological activity should be prepared.

The present work was undertaken with the aim to study crystal forms of some fluoroquinolones, a new class of widely used synthetic chemotherapeutic agents.

The objective of the project was to make different crystals forms of commonly used fluoroquinolones namely prulifloxacin and gemifloxacin mesylate. It was planned to prepare crystal form using solvents of varying polarity and by change of phase. It was planned to characterize the crystal form using techniques like melting point, scanning electron microscopy, infrared spectroscopy, differential scanning calorimetry and X-ray diffractometry. Dissolution and solubility studies were planned to be carried out for the crystal forms of these fluoroquinolones. It was also thought to corroborate dissolution study result by carrying out *in-vivo* studies of these crystal forms at varying time interval of their dissolution study.

## 2.1. PLAN OF WORK

### 2.1.1 Literature review

### 2.1.2 Procuring of drugs and analytical grade solvents for the preparation of crystal forms

### 2.1.3 Preformulation studies

2.1.3.1 Solubility studies

2.1.3.2 Melting point determination

### 2.1.4 Preparation of different crystal forms using various solvents

### 2.1.5 Characterization of prepared crystals

2.1.5.1 Melting point determination

2.1.5.1 Scanning Electron Microscopy (SEM)

2.1.5.2 Fourier Transform Infrared Spectroscopy (FT-IR)

2.1.5.3 Differential Scanning Calorimetry (DSC)

2.1.5.4 Powder X-Ray Diffractometry (PXRD)

### 2.1.6 Solubility and *In-vitro* dissolution study

2.1.6.1 Preparation of buffer solution for performing dissolution

2.1.6.2 $\lambda_{max}$ determination

2.1.6.3 Construction of standard curve using buffer solution

2.1.6.4 Determination of percentage drug release

2.1.6.5 Stability studies

### 2.1.7 *In-vivo* pharmacokinetic (PK) study

2.1.7.1 Animal selection

2.1.7.2 Drug administration

2.1.7.3 Blood collection and plasma separation

2.1.7.4 Bio analysis

### 2.1.8 Determination of PK parameters

2.1.8.1 Maximum plasma concentration ($C_{max}$)

2.1.8.2 Peak time ($T_{max}$)

2.1.8.3 Half life ($T_{1/2}$)

2.1.8.4 Area under curve (AUC)

2.1.8.5 Elimination rate (Kel)

## 3. LITERATURE REVIEW

**Ibrahim H G et al.,** (1977)[50] examined the four crystalline modification of phenylbutazone. It was prepared by isobutyl alcohol (Form-I); cyclohexane (Form-II); n-heptane (Form-III); 2-propanol (Form-IV). It was identified by FT-IR, SEM, X-RPD, solubility and dissolution study. The thermal behavior of the polymorphs under different treatment conditions also was investigated. Compression of the thermodynamically unstable forms, at a compression force of 1590-2040 kg, induced polymorphic changes in the crystals. The apparent equilibrium solubility of polymorphs and the dissolution of the polymorphs as compressed disks in an aqueous medium were determined. The small effective surface area possessed by one polymorph resulted in slow dissolution.

**Walking W D et al.,** (1979)[51] described the methods for the preparation of two difenoxin hydrochloride polymorphic forms. Solubility studies demonstrated that among the two difenoxin hydrochloride polymorphs, Form I was more soluble than Form II. Difenoxin hydrochloride Form I dissolved in 3: 1 isopropanol: water by refluxing. Form II dissolved in 9: 1 ratio of methanol: dimethyl formamide on refluxing. The above prepared crystals were identified by X-ray diffraction, IR spectra, and dissolution rate.

**Lagas M et al.,** (1981)[52] reported three polymorphic Forms I, II and III of sulphathiazole. Polymorphic form I was obtained by crystallization from propanol at 80$^{o}$C. Polymorph II was prepared by boiling a supersaturated solution of sulphathiazole in water (boiling temperature about 100$^{o}$C) until all the solvent was evaporated. Polymorph III was obtained by very slow crystallization from water or ethanol or water-ethanol mixture or the chloroform-acetone mixture. All the polymorphic forms were characterized by DSC, TG, XR-PD, FT-IR and dissolution

study. The melting points of polymorphs I, II and III were 201.0ºC, 196.5ºC and 173.6ºC respectively. Complete melting of the polymorphs I and II were only observed if the crystalline modifications were very pure. The amorphous form showed a glass transition at 62ºC. Dissolution studies have shown that the polymorphs I and II were very unstable in water and were rapidly converted into polymorph III of sulphathiazole.

**Laszlo Borka et al.,** (1983)[53] studied two polymorphic forms of triamcinolone diacetate. The Form-I was obtained by slow evaporation of dichloromethane solution which left long plates, up to 1 mm. Form-II was obtained by chloroform solvent, as micro crystalline powder with particles of 1-2 micro meters in diameter. Third crystalline form obtained was solvates of N, N-dimethylacetamide (DMA) which was rod shaped. All the above crystals such as two polymorphic modifications and one solvate were characterized by FT-IR, DTA and Thermo microscopy. Examination of the material in triamcinolone diacetate suspension for injection showed it to be Form II. However, in one batch, a significant amount of Form I was found in addition to Form II. IR spectroscopy and melting point determination proved to be the simplest analytical methods for the identification of formation of different crystal forms.

**Siegfried Lindenbaum et al.,** (1985)[54] have been identified two crystalline forms of auranofin, which were designated as A and B. Form A has the lower apparent equilibrium solubility and has low melting temperature. The two crystals were characterized by DSC, solution calorimetry, FT-IR and dissolution study. Two Forms of auranofin, designated as A and B, differ also in their crystalline morphology. This apparent anomaly was rationalized in terms of greater cooperatives of interaction between molecules of A in the crystal lattice, although the strength of the individual interactions is weaker as evidenced by the lower melting point. DSC

and heat of solution measurements show that Form A, although lower melting is the more stable by 3 kcal/mol in terms of the energy required to break up the crystal in lattice.

**Satoshi Kitamur et al.,** (1994)[55] investigated about eight polymorphic forms of mefloquine hydrochloride. The polymorphic Forms of A, B, E and I were prepared by saturated solution of various solvents as follows, Form A from acetonitrile; Form B from acetone; Form E from ethanol; Form I from isopropanol; Form D was prepared by 20 ml of distilled water, Form C was obtained by 500 ml of distilled water. Form B' was obtained by heating Form B on a hot stage to 190$^{o}$C. Form M was obtained by heating Form I on a hot stage to 175$^{o}$C. The prepared crystals were characterized by DSC, PXRD, FT-IR, Hot stage microscopy, solubility, gas chromatography, stability TLC. A study of the effect of various excipients on the solid-state crystal transformation revealed that microcrystalline cellulose promoted the transformation from Form E into Form D. However, methylcellulose, hydroxyethylcellulose, β- cyclodextrin, crospovidone and hydrous lactose had no effect on the crystal transformation. It was concluded that mefloquine hydrochloride can be crystallized into at least eight different forms.

**Martinez-Oharriz M C et al.,** (1994)[56] developed a new crystalline form of Form-IV of diflunisal. This new crystal (Form-IV) of diflunisal was prepared from toluene, Form-II from distilled water, Form I from carbon tetra chloride or chloroform and Form III from ethanol or methanol. It was characterized by DSC, IR, X-PRD and Hot stage microscopy and dissolution study. According to the different X-ray diffractions profiles, an identification system for the polymorphs can be developed based on the different peak positions of the diffraction patterns. All forms first recrystallized to the more stable form (Form I) and then melted at 210ºC; only one

weak transition peak was detected corresponding to transformation of Form III to Form I. Difference observed in IR spectra indicated that intermolecular hydrogen bonding occured between hydroxyl and carbonyl groups and/or between fluorine atoms. The intrinsic dissolution rates were determined from compressed disks in an aqueous medium. Unexpectedly the dissolution rate of Form IV was lower than that of the most stable modification Form I.

**Hiroaki Kitaoka et al.,** (1995)[57] studied about the differential scanning calorimetry (DSC) curves of levofloxacin hemihydrates which were measured under various conditions and they showed different thermograms. These phenomena were attributed to be the dehydration. Dehydration caused a multiple-phase transition. Dehydration at a higher temperature gave a sharp endothermic peak on the DSC curve due to the melting of the γ Form and at a lower temperature gave a sharp endothermic peak due to the melting of the α Form. In contrast, the thermal behavior of levofloxacin monohydrate was not affected by dehydration. The difference in the thermal behavior between the hemihydrates and the monohydrate might be attributed to a difference in the interaction between levofloxacin and crystal water. Observation by thermo microscopy, the changes in powder X-ray diffraction patterns during heating and single X-ray analysis all supported the above interpretation.

**Yuji Chikaraishi et al.,** (1996)[58] performed the work on preparation of piretanide in amorphous form and polymorphic form. A new polymorph, form C was formed at an acid-base molar ratio of 1:1. In X-ray powder diffractometry, the amorphous form showed no diffraction peak and form C showed significantly different peaks from forms A and B. Various techniques were used for the identification of these polymorphs like FT-IR, DSC, SEM, water determination, solubility study using the dispersed amount method.

**Renli teng et al.,** (1996)[59] had studied about the pharmacokinetics of trovafloxacin in rats, dogs and monkeys following oral and intravenous administration. After intravenous dosing, the systemic clearances of trovafloxacin in rats, dogs and monkeys were 12.5, 11.1, and 7.2 ml/min/kg of body weight, respectively and the respective volumes of distribution were 0.9, 1.7, and 4.3 liters/kg, with corresponding elimination half-lives of 0.7, 1.8, and 7.0 h. After the administration of oral doses of 50, 20 and 20 mg/kg to rats, dogs and monkeys serum trovafloxacin concentrations reached a maximum at 0.6, 2.3, and 2.3 h, respectively, with respective maximum concentrations of trovafloxacin in serum of 11.5, 3.5 and 5.2 mg/ml; the corresponding elimination half-lives were 2.2, 2.5 and 7.5 h. The oral bioavailability of trovafloxacin was 68, 58, and 85% in rats, dogs and monkeys, respectively.

The binding of trovafloxacin to serum proteins was concentration independent, averaging 92, 75 and 66% for rats, dogs and monkeys, respectively. Trovafloxacin penetrated well into tissues in dogs. The urinary recoveries of unchanged drug were less than 5% in dogs and monkeys, with or without incubation with alkali or Glusulase (b-glucuronidase and sulfatase). In rats, 99.8% of the orally administered radioactivity was recovered in feces, while 20.6, 3.4 and 67.1% of the radioactive dose in bile duct-cannulated rats were recovered in feces, urine and bile, respectively. These results suggested that the elimination of trovafloxacin from rats and possibly from dogs and monkeys was primarily through biliary excretion.

**Kristl A et al.,** (1996)[60] explained about the existence of acyclovir in hydrated form and that the ratio between acyclovir and water molecules in the crystal structure was 3: 2. The anhydrous crystalline form of acyclovir was also prepared.

It was found that acyclovir existed as a pseudo polymorphic and polymorphic solvate. It was examined by means of thermal analysis, X-ray powder diffraction, infrared spectroscopy, solubility and dissolution rate studies. It was found, that besides hydrate, two anhydrous forms of acyclovir were present: the unstable one, obtained at a drying temperature below 150ºC (which was converted to the hydrate almost immediately in the atmosphere) and the stable one, obtained at drying temperature above 150ºC (which showed, on heating to 172ºC, the solid-solid transition).

**Kakkar A.P et al.,** (1997)[61] had prepared three crystalline and one amorphous form of ciprofloxacin hydrochloride and characterized them by various instrumental techniques. The hygroscopicity, stability and solubility of all the forms were also determined. Results indicated that all three crystalline and one amorphous form had distinctly different properties. Form I and II were identified as hydrates and Form III as dimethyl formamide (DMF) solvate. Stability determination of various forms under different conditions indicated that Form II was most stable against temperature and Form I against humidity. Among crystal forms, Form I was found to possess maximum aqueous solubility and was expected to exhibit maximum bioavailability.

**Mohamad A Hassan et al.,** (1997)[62] have studied the characterization of three polymorphic forms of famotidine (Forms A, B, C). These crystals were obtained from acetonitrile, methanol, THF respectively and they were confirmed by DSC, FT-IR, X-RPD, SEM, Hot stage microscopy, solubility measurement. The Forms A, B, and C melted at 171.3ºC, 166.4ºC and 160.9ºC, respectively. The Form B, which is the commercial form of famotidine, is probably most stable and has therefore the lowest aqueous solubility (0.55mg/ml). The solubility of the A Form (0.82 mg/ml) was comparable to that of the C Form (0.85 mg/ml).

**Dilraj singh et al.,** (1998)[63] has reported a novel crystal form II of the benzodiazepine chlordiazepoxide. The formation of form II was dependent on the crystallizing solvent, being the predominant form isolated from methanol. Recrystallization from other alcoholic solutions (ethanol, propanol and butanol) and toluene yielded form I. The new polymorphic phase was characterized and distinguished from the standard form (form I) by X-ray diffractometry, Differential Scanning Calorimetry, Infrared spectroscopy, solution calorimetry and solid-state Nuclear Magnetic Resonance. Differences were observed in the packing arrangement of the dimmers in the polymorphs.

**Llacer J M et al.,** (1999)[64] reported the formation of three polymorphs of ondansetron. These compounds showed different physico-chemical and structural characteristics and were confirmed by DSC, FT-IR, $^{1}H$ NMR. Samples were tested under different conditions of temperature, pulverization and pH and in different solvents. The factors identified were able to cause the formation of polymorphs which were heated to different temperatures for different times with the use of ethanol and methanol as solvents.

**Jan-Olav Henck et al.,** (1999)[65] demonstrated the thermodynamic terms of enantiotropy and monotropy by means of solid-state analytical results of polymorphous flurbiprofen (FBP). Vibration spectra, Differential scanning calorimetry (DSC), Raman spectroscopy, Thermo microscopy investigations as well as X-ray powder patterns for three modifications of FBP were described. The thermodynamic relationships between the three modifications were demonstrated by a semi schematic energy/temperature diagram. Theoretical vapor pressure/temperature diagrams and energy/temperature diagrams were compared and briefly discussed.

**Zornoza A et al.,** (1999)[66] investigated the polymorphism of glisentide. Three polymorphs (I, II, III) had been prepared by recrystallisation from different solvents and other polymorphic form (IV) was obtained by heating polymorph III at 100$^{o}$C. It had been observed that the polarity of the recrystallisation solvent and its ability to from hydrogen bonds had a great influence on the polymorphism of glisentide. The different solid forms of glisentide had been characterized using X-ray diffraction analysis, differential scanning calorimetry (DSC), Thermo gravimetric analysis (TGA), IR spectroscopy and optical microscopy.

**Rustichelli C. et al.,** (2000)[67] described that the polymorphs of a compound had same solid crystalline phases with different internal crystal lattice in pharmaceuticals. Difference due to polymorphism and pseudo-polymorphism had affected the bioavailability and their effective clinical use. The work was to obtain the different polymorphic modifications of the anticonvulsant drug carbamazepine. The results confirmed the existence of three different polymorphic forms for anhydrous carbamazepine. Form III was considered as commercial one. Form I was obtained by heating the Form III and Form II was crystallized from ethanolic solution of form III. These were characterized by means of typical structure-sensitive analytical techniques such as FT-IR spectroscopy, XRPD and DSC. Further investigations were also performed by hot stage FT-IR thermo microscopy, which permitted the visible and spectroscopic characterization of polymorphic forms during heating.

**Vrecer F et al.,** (2003)[68] investigated the three polymorphic forms of piroxicam crystal. The crystal (Form-1) was prepared from benzene saturated solution; Form II (needles) was crystallized from saturated solution of absolute alcohol; Form-III was obtained by pouring the hot saturated piroxicam solution in absolute ethanol on dry ice. The characterization of piroxicam crystal forms were

studied by PXRD, $^{13}C$ NMR, DSC, FT-IR, Raman spectrum and dissolution studies. A new polymorphic Form designated as Form III was obtained by forced crystallization using dry ice. Differences in IR spectra were attributed mainly to the difference in the number and positions of H-bonds in all the piroxicam crystal forms. Slow crystallization of piroxicam from absolute ethanol solution resulted in a mixture of Form II and monohydrate. Crystal structure analysis proved that Form II represents Form $\alpha_{2.}$ Difference in dissolution rates among crystal forms of piroxicam were attributed to differences in their wettability, where highest wettability was obtained from monohydrate and the lowest from Form III.

**Rahul v et al.,** (2004)[69] studied the formation of crystal hydrates of niclosamide. The hydrates obtained by the process of recrystallization from acetone (Form-I) and obtained from ethyl acetate (Form-II) were classified based on the differences in their dehydration profile, crystal structure, shape and morphology. Crystals obtained in the absence of moisture were unstable, and when exposed to the laboratory atmosphere transformed to their corresponding hydrates. Differential scanning calorimetry thermo grams indicated that Form I changed into an anhydrate at temperatures below 100ºC, while Form II dehydrated in a stepwise manner at above 140ºC. This finding was further confirmed by thermo gravimetric analysis. It was characterized by DSC, XRPD, Optical microscopy, moisture sorption analysis. Form I, Form II and the anhydrate of Form II showed no significant moisture sorption over the entire range of relative humidity. Although the anhydrate of Form I did not show any moisture uptake at low humidity, it was converted into the monohydrate at elevated relative humidity (>95%). All forms could be interconverted depending on the solvent and humidity conditions.

**Elqidra R et al.,** (2004)[70] had investigated the effect of polymorphism on *in-vitro, in-vivo* properties of carbamazepine (CBZ). For this purpose, three difference polymorphs and a dihydrate of CBZ were obtained and the conventional tablets of these crystalline forms at the dose of 200 mg were prepared. The polymorphs were examined by the IR and DSC analysis. The tablets were investigated by the *in-vitro* dissolution test. Tegretol was selected as a reference. The tablets of β CBZ demonstrated the lowest dissolution rate, while the α form tablets exhibited the highest. Six healthy volunteers particiated in an open-randomized cross-over designed clinical trial. After a total blood sampling peroid of 96 h, plasma levels of CBZ were determined by HPLC analysis using a CN column and a acetonitril-water (30:70) mobile phase. There were no significant differences between the plasma concentration-time curves of tegretol, bulk powder and β CBZ tablets. The only marked difference was the time required to reach plasma peak concentration.

**Teofilo Mazon Cardoso et al.,** (2005)[71] studied about two crystal forms of nimodipine (sample I and sample II). Morphological differences concerned with the shape, size and crystalline of particles were visualized by Scanning Electron Microscopy (SEM) and X-ray powder diffraction. The techniques used in this study can be said to be efficient in the characterization and evaluation of quality control of the raw material. The other physico-chemical properties of the substances were evaluated by thermal analysis (DSC and TG/DTG), HPLC, crystallography, and microscopy.

**Barbas R et al.,** (2007)[72] discovered a new polymorph of norfloxacin Form C and characterized by DSC, thermo gravimetry, PXRD, Raman spectroscopy, FT-IR, Solid state $^{13}C$ NMR spectroscopy. New Form C shows a high similarity with Form B regarding their molecular spectroscopic properties such as FT-IR, Raman spectrum,

NMR but not with form A. The relationship between the new Form C and the previously known Forms A and B have been studied and reported. Moreover, the crystal structure of the known form has been solved by single-crystal method.

**Mange Ram Yadev et al.,**(2008)[73] investigated five different crystals forms of pefloxacin using solvents of varying polarity. The polymorphs differed in their dissolution profile and all of them showed unusual behavior of highest dissolution in the first 15 min. The rate of dissolution went on decreasing and got stabilized to a constant value after 4 hours. Five different polymorphs of pefloxacin were characterized by PXRD, FT-IR, microscopy, thermal analysis, GC, Karl-Fischer (KF) aquametry, micro calorimetry methods.

**Antonio Llinas et al.,** (2008)[74] had applied the potentiometric cycling for the polymorphic creation (PC) to sparfloxacin. Two different trihydrate phases precipitated from aqueous solution; X-ray data indicated that one of these was a previously unknown polymorph of sparfloxacin trihydrate. Because both forms crystallized from solution at the same time, the two crystalline forms were concomitant polymorphs that precipitate in a thermodynamically controlled ratio.

**Nighute A B et al.,** (2009)[75] developed micro crystals of cefuroxime axetil. It was prepared by emulsion solvent diffusion method with various surfactants to faster its solubility and dissolution rate. Micro crystals were screened for apparent solubility, SEM, drug content, dissolution study, PXRD, DSC, stability studies, wettability. Microcrystal prepared with HPMC E-15LV showed highest solubility and dissolution rate than the untreated drug and the crystals prepared with other surfactants. The microcrystals were passed through accelerated stability and wettability studies and observed to be improved.

**Mahalaxmi R et al.,** (2009)[76] identified the recrystallization of carbamazepine. Recrystallization of carbamazepine was done by using two solvents of ethanol, acetone at different cooling conditions. The various solvent and cooling conditions could modify the size, shape, polymorphs and thereby the dissolution of original drug. In this work, carbamazepine crystals were modified by using ethanol and acetone under four different cooling conditions. The samples recrystallized from ethanol and acetone solution as well as the original carbamazepine samples exhibited identical IR spectra, indicating that there was no change at the molecular level. The above study indicated that the size, shape and the type of polymorphs in the sample had affected its dissolution behavior. It was evaluated by microscopic method, FT-IR, DSC, dissolution study and stability studies.

**Maria Klimakow et al.,** (2010)[77] had studied about the crystallization of nifedipine by means of synchrotron X-ray diffraction, single-crystal X-ray structural analysis and Raman -spectroscopy. The surface morphology of each crystal was followed by means of light microscopy and Environmental Scanning Electron Microscopy (ESEM) coupled with Energy-Dispersive X-ray Spectroscopy (EDS) analysis. Fast crystallization processes (typically minutes) in different solvents always lead to the final formation of the thermodynamically most stable α – polymorph of nifedipine. They observed a novel pseudo-polymorphism due to slow crystallization from DMSO. The single-crystal X-ray structure of the solvated species nifedipine, DMSO (1:1) was reported for the first time. Different diffractions pattern and Raman spectra were observed for crystals grown from stock solution and those obtained by drying the solution on soda lime silicate surfaces.

**Alok Tripathi et al.,** (2010)[78] performed a study on rabeprazole sodium, a proton pump inhibitor, which exhibited various polymorphic forms and they were

evaluated by various analytical techniques. The X-ray diffraction pattern of amorphous form, Form II, Form X, Form Y, Form Z, α Form, β Form, γ Form, Form V, Form VI, Form F were compared and they had different peaks and intensities also.

**Renu Chandha et al.,** (2010)[79] identified and characterized the different crystal modifications of 5-flurouracil. The drug was found to exist in five polymorphic and one solvatomorphic form. The above said crystals were prepared by using various boiling solvents like methanol, ethanol, isopropyl alcohol, butanol and mixture of 1, 4 dioxane and acetonitrile (85:15). SEM was used to clarify the characteristic crystalline shapes of the drug and its crystal modifications. The melting temperature determined from DSC studies suggested that they were different crystal forms while TGA revealed that Form III was acetonitrile solvate. Some major differences were found in the FTIR spectra of various Forms. Further differentiations of polymorphic forms were performed by determining the enthalpy of solution in the given solvent which was based on difference in the lattice energy. The obtained crystals were evaluated under the thermal methods of analysis, PXRD, FT-IR, Calorimetric study, aqueous solubility measurement, SEM and dissolution study.

**Modha N B et al.,** (2010)[80] investigated three different types of crystals of fluconazole. It was prepared from single solvent of methanol, acetone and isopropyl alcohol. Polymorphs of fluconazole lead to higher dissolution rate and improved bioavailability. The discriminatory dissolution criteria described under biopharmaceutical classification of solvent guidelines were studied using 0.1N HCL, distilled water, acetate buffer pH 5.0 and phosphate buffer pH 6.8. The accelerated stability study was subjected to potential formulation in accordance with stability guidelines and characterized by FT-IR, DSC, PXRD and dissolution study.

**Mohd R Abu Bakar et al.,** (2011)[81] investigated riddle of sulfathiazole polymorphism. The pure forms of different polymorphs were obtained by using various crystallization conditions such as different cooling rates and initial solute concentrations. They were examined using various characterizations methods such as optical microscopy, SEM, DSC, TGA, FT-IR spectroscopy, XRPD.

**Pandu Ranga Rao et al.,** (2011)[82] prepared the stable new polymorphic form of atorvastatin calcium. The new polymorphic form of atorvastatin calcium (I) was obtained by suspending the drug in a mixture of 10 % v/v aqueous ethanol. An efficient simple, consistent and economic process for stable new polymorphic form of atorvastatin calcium (I) was described. The new polymorphic form of atorvastatin calcium (I) was characterizated by PXRD.

**Wei Du et al.,** (2013)[83] had prepared various polymorphs of Form I, Form II and Form III of agomelatine and the variability of polymorphs were investigated and then the *in-vitro, in-vivo* correlations were established. The presence of three polymorphs of agomelatine was corroborated through studies by XRPD, TGA and DSC. All the forms obtained were then subjected to the powder and intrinsic dissolution tests. The IDR ranked in the order of Form III > Form I > Form II. Form I and Form III both underwent solvent-mediated phase transformation (SMPT) to Form II during dissolution and the transition points were 62 and 45 min, respectively. Pharmacokinetic profiles were acquired after oral administration of tablets, showing that the $k_a$ and $AUC_{0-12}$ h of Form I, Form II and Form III were 0.58 ± 0.11, 0.34 ± 0.05, 0.74 ± 0.07 $h^{-}1$ and 296.25 ± 49.39, 186.05 ±45.93, 331.16 ± 54.74 ng h/ml, respectively. Good linearity between IDR and ka, IDR and AUC were established, suggesting that the agomelatine polymorphic forms with faster dissolution rates by *in-vitro* increased the rate and extent of oral absorption by *in-vivo*. These results

demonstrated that IDR was predictive in estimating the relative bioavailability of agomelatine polymorphic forms.

**Anchal Kulshrestha et al.,** (2013)[84] designated three polymorphic modifications of fexofenadine hydrochloride as Form A1, Form B1 and Form C1 which had been obtained by recrystallisation in organic solvents such as n-propanol, ethanol and methanol under variable conditions. Different polymorphs of fexofenadine hydrochloride were characterized by powder X- ray crystallography diffractometry (PXRD), differential scanning calorimetry (DSC), and thermo gravimetric analysis (TGA).

**Marco Farias et al.,** (2014)[85] investigated the occurrence of polymorphic transitions which is a serious problem for pharmaceutical companies, because it can affect the bioavailability of the final product. With several known polymorphic forms carbamazepine is one of the most problematic drugs in this respect. Raman spectroscopy is a vibrational technique that is becoming very important in the pharmaceutical field, mainly due to its highly specific molecular fingerprint capabilities and easy use as a process analytical tool. However, multivariate methods are necessary for both identification and quantification. In this work, an analytical methodology using Raman spectroscopy and interval Partial Least Squares Regression (iPLS), were developed in order to quantify mixtures of carbamazepine polymorphs in the presence of the most common excipients. The three polymorphs CBZ I, CBZ III and CBZ DH (which was a dihdyrate) were synthesized and characterized by PXRD and DSC. Subsequently, tablets were manufactured using excipients and 15 different mixtures of carbamazepine polymorphs. The iPLS model presented average prediction validation errors of 1.58%, 1.04% and 0.22% wt/wt, for CBZ I, CBZ III and CBZ DH respectively, considering the whole mass of the tablet.

The model presents a good prediction capacity and the proposed methodology could be used to perform quality control in final products.

**Aline Quelian Penna Garbuio et al.,** (2014)[86] studied the dissolution of different polymorphs of drug, which may have different solubilities. Importantly, the pharmacopoeia monographs, usually not have tests for the characterization of these polymorphic forms of a drug. The study was performed with polymorphic forms of mebendazole present in raw materials and also pills available in the Brazillian pharmaceutical market through the techniques of infrared (FTIR), Differential Scanning Calorimetry (DSC), dissolution, solubility and X-ray Diffraction pattern (XRPD). The analysis of FTIR and DSC curves showed that there were three main polymorphic forms of mebendazole present in raw materials and tablets. The data obtained in the dissolution and solubility tests showed that Form A was less soluble than Form B which was less soluble than the C Form, when using a dissolution medium without added surfactant. It has been found that in some tablets of mebendazole there was a mixture of polymorphic forms, and that the raw materials present two major polymorphic forms. Then it was suggested the need of quality control regarding the type of polymorph used in the production of mebendazole tablets to ensure greater therapeutic efficacy.

**Natalia L Calvo et al.,** (2015)[87] reported the identity of the polymorphic form of an active pharmaceutical ingredient. It is an important parameter that may affect the performance of the drug formulation. This calls for special techniques, able to classify crystal forms or assign the polymorphic identity to given solid in mixture. In order to develop a method to determine the relevant polymorphs of Cimetidine (CIM) which was present in commercial tablet samples, authentic forms A, B, D and M1 of the drug were prepared, structurally characterized and employed as standards.

Thus attenuated total reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) was coupled to Principal Component Analysis (PCA) and used for the classification of physical mixtures of CIM and excipients, as well as laboratory-made and commercial tablets, according to their polymorphic composition. It was demonstrated that two Principal Components (PCs) suffice to classify the samples of the four forms of CIM into distinct groups, and that method performance was optimum when the second and third PCs were used for the classification process. The application of the method to commercial tablets of CIM also gave good results, confirming they were prepared employing the correct polymorph (Form A).

**Rajamma A.J. et al.,** (2015)[88] reported the introduction of gliclazide in the pharmaceutical industry, a large number of research groups have been engaged in various investigations aiming to enhance its biomedical application. But, very limited efforts have been to study polymorphism of gliclazide. Therefore, this study focuses on solvent-induced polymorphism of gliclazide and its characterization by thermal methods. Three polymorphs namely, Form-I, II and III and an amorphous powder were produced from different solvents and solvent mixtures. Crystals were analyzed using infrared spectroscopy, Differential Scanning Calorimetry, X-ray powder diffraction and single crystal x-ray diffraction. Polymorph Form-1 was found to exist in centro-symmetric triclinic P-I space group and has endothermic peak at 162.93ºC. Form-11 has endothermic peak from 171.2ºC to 172.35ºC and exists in centro-symmetric monoclinic $P2_1$ la space group while Form-III has endothermic peak from 168.93ºC to 169ºC and exists in centro-symmetric monoclinic P2, in space group. The equilibrium solubility values of Form-I, II, III and the amorphous form were 0.4825±0.025, 0.2341±0.042, 0.2581±0.038 and 0.5213±0.072 mg/ml, respectively. The Form-I has relatively higher solubility and similar to that of amorphous

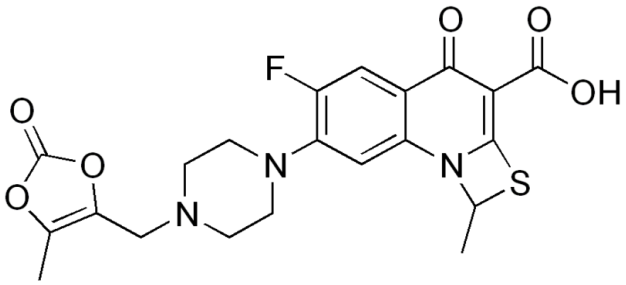
gliclazide. Form-II and Form-III were relatively most stable and least soluble. However, there was no remarkable difference in their aqueous solubility under the conditions in which study was conducted.

# 4. DRUG PROFILE

## 4.1 PRULIFLOXACIN[89-91]

**Drug Name** **: PRULIFLOXACIN**

**Structure** **:**




**Molecular Formula** **:** $C_{21}H_{20}FN_30_6S$

**Molecular Weight** **:** 461.463 g/mol

**Chemical Name** **:** 6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4- yl) methyl-1-piperazinyl]-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid

**Category** **:** Antibacterial/Antibiotic

**Description** **:** Light yellow crystalline powder, with funk.

**Solubility** **:** Insoluble in water and methanol but soluble in DMF & DMSO

**pKa value** **:** pKa 5.85

**Melting point** **:** 211-214$^0$C

## ANTIMICROBIAL ACTION

Prulifloxacin is a broad-spectrum antibiotic that is active against both gram positive and gram-negative bacteria. Like other Fluoroquinolones, Prulifloxacin prevents bacterial DNA replication, transcription, repair and recombination through inhibition of bacterial DNA gyrase. Quinolones and Fluoroquinolones are bactericidal drugs, eradicating bacteria by interfering with DNA replication.

Quinolones are synthetic agents that have a broad spectrum of antimicrobial activity as well as a unique mechanism of action, resulting in inhibition of bacterial DNA gyrase and topoisomerase IV. Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. For many gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria. It is believed that eukaryotic cells do not contain DNA gyrase or topoisomerase IV.

## PHARMACOKINETICS

Prulifloxacin 600 mg achieves peak plasma concentration (C max) of ulifloxacin (1.6 µg /mL) in a median time to C max (T max) of 1 hour. Ulifloxacin is ≈45% bound to serum proteins *in vivo*. It is extensively distributed throughout tissues and shows good penetration into many body tissues. The elimination half-life ($t_{1/2}$) of ulifloxacin after single-dose of Prulifloxacin 300–600 mg ranged from 10.6 to 12.1 hours. After absorption from the gastrointestinal tract, prulifloxacin undergoes extensive first-pass metabolism (hydrolysis by esterase, mainly paraoxonase to form ulifloxacin, the active metabolite). Unchanged ulifloxacin is predominantly eliminated by renal excretion.

## INDICATION

- Urinary Tract Infection
- Prostatitis
- Acute pyelonephritis
- Epididymitis
- Perioperative antibiotic prophylaxis for transurethral surgery
- Bronchitis
- Pneumonia
- Sinusitis
- Gastrointestinal infections
- Soft tissue infections
- Bone and joint infection.

## ADVERSE EFFECTS

In one review article it has been stated that prulifloxacin has a similar tolerability profile to that of ciprofloxacin. In another study, it was found that prulifloxacin patients experienced a similar number of adverse reactions compared to those in the ciprofloxacin group (15.4% vs. 12.7%). There were four serious adverse events in each treatment arm, including 1 death in the prulifloxacin arm. None were considered treatment related by the investigator. If approved in U.S., prulifloxacin may have carried a black box warning for tendon damage, as the FDA has determined that this is a class effect of fluoroquinolones.

Prulifloxacin has a reduced effect on the QT interval when compared to other fluoroquinolones and may be a safer choice for patients with pre-existing risk factors for arrhythmia.

## CONTRAINDICATIONS

The contraindications of prulifloxacin are:

- Prulifloxacin is contraindicated in elderly population due to the risk of Tendon damage.
- Prulifloxacin is contraindicated in patients with anamnesis of tendon diseases related to the administration of quinolones.
- Prulifloxacin is contraindicated in persons with a history of hypersensitivity to Prulifloxacin any member of the quinolone class of antimicrobial agents or any of the product components.
- Prulifloxacin is contraindicated in subjects with celiac disease.
- Prulifloxacin is contraindicated in the children, pregnant women, nursing mothers and in patients with epilepsy or other seizure disorders.
- **Pregnancy**
- The fluoroquinolones rapidly cross the blood-placental and blood-milk barrier. They are extensively distributed into the fetal tissues. The fluoroquinolones have also been reported as being present in the mother's milk and are passed on to the nursing child.
- **Pediatric population –** Fluoroquinolones are not licensed by the U.S. FDA for use in children due to the risk of permanent injury to the musculoskeletal system, with two exceptions. However, the

fluoroquinolones are licensed to treat lower respiratory infections in children with cystic fibrosis in the UK.

**DRUG INTERACTION**

Probenecid : Prulifloxacin urinary excretion decreases when concomitantly administered with probenecid.

Fenbufen : The concomitant administration of fenbufen can cause increased risk of convulsions.

Hypoglycemic agents : May cause hypoglycemia in diabetic patients under treatment with hypoglycemic agents.

Theophylline : May cause a decreased theophylline clearance.

Warfarin : May enhance the effects of oral anticoagulants such as warfarin and its derivatives.

Nicardipine : May potentiate the phototoxicity of prulifloxacin.

**DOSAGE & ADMINISTRATION**

- Uncomplicated lower urinary tract infections. Adult: In acute cases (simple cystits): Single dose of 600mg (oral).
- Acute exacerbations of chronic bronchitis. Adult: 600mg daily up to 10days (oral)
- Complicated lower urinary tract infections. Adult: 600mg daily up to 10days (oral)

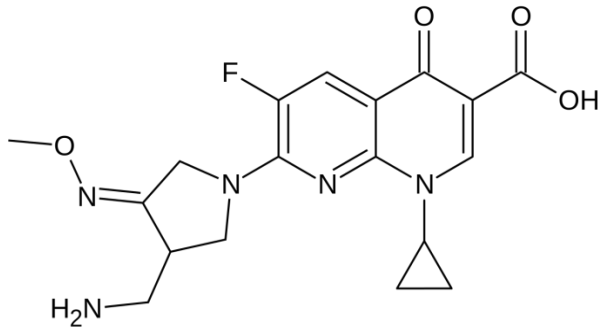
**STORAGE**

Stored at -20$^{o}$C; Protected from light and moisture.

## 4.2. GEMIFLOXACIN MESYLATE[92-94]

**Drug Name** **: GEMIFLOXACIN MESYLATE**

**Structure** **:**




**Molecular formula** **:** $C_{18}H_{20}FN_5O_4$

**Molecular Weight** **:** 389.381 g/mol

**Chemical Name** **:** 7-[(4*Z*)-3-(Aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo- 1,8-naphthyridine-3-carboxylic acid

**Functional category** **:** Antibacterial/Antibiotic

**Description** **:** White crystalline powder.

**Solubility** **:** Soluble in DMS, methanol (hot) and water.

**pKa value** **:** pKa 6.02

**Melting point** : 235-237$^0$C

## ANTI MICROBIAL ACTION

Gemifloxacin mesylate is a broad-spectrum antibiotic and it is active against both gram positive and gram-negative bacteria. Like other Fluoroquinolones, it prevents bacterial DNA replication, transcription, repair and recombination through inhibition of bacterial DNA gyrase. Quinolones and fluoroquinolones are bactericidal drugs, eradicating bacteria by interfering with DNA replication.

Quinolones are synthetic agents that have a broad spectrum of antimicrobial activity as well as a unique mechanism of action, resulting in inhibition of bacterial DNA gyrase and topoisomerase IV. Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. For many gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria. It is believed that eukaryotic cells do not contain DNA gyrase or topoisomerase IV.

## PHARMACOKINETICS

Bioavailability of gemifloxacin is 71%, Protein binding 60-70 %, Limited metabolism by liver to minor metabolites. It is excretion in feces 61% and urine 36%.

Absorption : Rapidly absorbed from the GI tract; absolute bioavailability: About 71%.

Distribution : Widely distributed into body tissues including bronchial mucosa and lungs. 55-73% bound to plasma proteins.

Metabolism : Limited hepatic metabolism.

Excretion : Elimination half-life: 7 hr. As unchanged drug and metabolites in the faeces and urine.

## INDICATIONS

Gemifloxacin is indicated for the treatment of infections caused by susceptible strains of the designated microorganisms in the following conditions:

- Acute bacterial exacerbation of chronic bronchitis caused by *S. pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae or Moraxella catarrhalis*
- Community-acquired pneumonia (of mild to moderate severity) caused by *S. pneumoniae* (including multi-drug resistant strains,*Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae, or Klebsiella pneumonia.*

## ADVERSE EFFECTS

Fluoroquinolones are generally well tolerated with most side effects being mild and serious adverse effects being rarely. Some of the serious adverse effects which occur more commonly with fluoroquinolones than with other antibiotic drug classes include CNS and tendon toxicity. The currently marketed quinolones have safety profiles similar to that of other antimicrobial classes.

The serious events may occur with therapeutic or with acute overdose. At therapeutic doses they include: central nervous system toxicity, cardiovascular toxicity, tendon / articular toxicity and rarely hepato toxicity. Events that occur in acute overdose are rare and include: renal failure and seizure. Children and the elderly are at greater risk. Tendon damage may manifest during, as well as up to a year after fluoroquinolone therapy. The FDA ordered black box warnings on all fluoroquinolones advising consumers of the possible toxic effects of fluoroquinolones

on tendons. On August 15th, 2013 the FDA issued a Safety Announcement where they described that they are requiring the medication guides and drug labels for all fluoroquinolones to be updated and better describe the risk for peripheral neuropathy. The peripheral neuropathy may occur very quickly and may be irreversible. This warning applies to fluoroquinolones taken by mouth and injection, but does not apply to fluoroquinolones taken topically.

**CONTRAINDICATIONS**

- **Gemifloxacin and Pregnancy**

  Caution when used during pregnancy Category C: Either studies in animals have revealed adverse effects on the foetus (teratogenic or embryocidal or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the foetus.

- **Gemifloxacin and Lactation**

  Caution when used during lactation

- **Gemifloxacin and Children**

  Safety and efficacy not established

- **Gemifloxacin and Other Contraindications**

  Hypersensitivity. Patients receiving class IA or III antiarrhythmics.

**DRUG INTERACTION**

Lower bioavailability if coadministered with multivalent cation preparations e.g. aluminum, magnesium, or iron salts. May potentiate QT prolongation when used with drugs that affect the QT interval e.g. cisapride, erythromycin, antipsychotics and

TCAs. May increase prothrombin time when used with warfarin or its derivatives. Increased plasma levels when used with probenecid.

**DOSAGE & ADMINISTRATION**

- Acute bacterial exacerbation of chronic bronchitis Adult: 320 mg once daily for 5 days.
- Community-acquired pneumonia Adult: 320 mg once daily for 7 days.

**STORAGE**

Stored at -20$^{o}$C; Protected from light and moisture.

# 5. MATERIALS AND METHODS

## 5.1 MATERIALS

The materials and reagents used in the present investigation were either AR/LR grade or the best quality pharma grade.

**Table 3: List of chemicals**

| S.No | MATERIALS | SUPPLIED BY |
|---|---|---|
| 1 | Prulifloxacin | Hetero Drugs, Hyderabad |
| 2 | Gemifloxacin mesylate | Yarrow chem. Products, Mumbai |
| 3 | Acetone | S.D.Fine Chemicals Ltd, Mumbai |
| 4 | Acetonitrile | S.D.Fine Chemicals Ltd, Mumbai |
| 5 | Acetic acid | S.D.Fine Chemicals Ltd, Mumbai |
| 6 | Benzene | Merck, Pvt. Ltd, Mumbai |
| 7 | Chloroform | Chemepure, Laboratories, Chennai |
| 8 | Cyclohexane | Chemepure, Laboratories, Chennai |
| 9 | Dichloromethane | S.D.Fine Chemicals Ltd, Mumbai |
| 10 | Dimethyl sulfoxide | Chemepure, Laboratories, Chennai |
| 11 | Dimethyl formamide | Merck, Pvt. Ltd, Mumbai |
| 12 | Diethyl ether | S.D.Fine Chemicals Ltd, Mumbai |
| 13 | Ethyl acetate | Merck, Pvt. Ltd, Mumbai |
| 14 | Ethanol | Chemepure, Laboratories, Chennai |
| 15 | n-Hexane | Merck, Pvt. Ltd, Mumbai |
| 16 | Methanol | Chemepure, Laboratories, Chennai |
| 17 | Isopropanol | S.D.Fine Chemicals Ltd, Mumbai |

| | | |
|---|---|---|
| 18 | Toluene | S.D.Fine Chemicals Ltd, Mumbai |
| 19 | Distilled water | Pharm. Chem. Laboratory |
| 20 | Potassium dihydrogen phosphate | Lobachem, Mumbai |
| 21 | Disodium hydrogen phosphate | Lobachem, Mumbai |

## 5.2 INSTRUMENTS USED

**Table 4: List of instruments**

| S.No | NAME OF THE EQUIPMENT/ INSTRUMENTS | SUPPLIER/COMPANY NAME |
|---|---|---|
| 1 | Centrifuge Apparatus | Remi, India |
| 2 | Differential Scanning Calorimeter | DSC-6300SSI Nano Tech, NETZCHSTA 449F3 |
| 3 | Digital balance | Schimadzu, Japan |
| 4 | Dissolution apparatus | Lab India disso2000, Mumbai |
| 5 | Double beam UV-VIS Spectrometer | Systronics, Mumbai |
| 6 | Desiccator | Tarsons, Durga Co., Pvt. Ltd., Chennai |
| 7 | Digital pH meter | Systronics, Mumbai |
| 8 | Electrically heated water bath | Guna Enterprises, Chennai |
| 9 | FT-IR spectroscope | Shimadzu,Japan |
| 10 | Hot air oven | Sigma Scientific Private Ltd, Chennai |
| 11 | HPLC | Shimadzu, Japan |
| 12 | Melting point apparatus | In Lab equipment Ltd, Madras |
| 13 | Refrigerator | LG |

| | | |
|---|---|---|
| 14 | Scanning Electron Microscope | Jeol JSM-6100 |
| 15 | Sieve (100) | Jay & Sieves |
| 16 | Stability chamber | LabTop, Mumbai |
| 17 | Vacuum Pump | Superfit, Mumbai |
| 18 | X-ray Diffractometer | Ricnaku Miniflex 2c |

## 5.3 METHODS FOR PRULIFLOXACIN

### 5.3.1 PREPARATION OF CRYSTAL FORMS FROM DIFFERENT SOLVENTS (BY COOLING CRYSTALLIZATION METHOD)[8]

#### 5.3.1.1 From acetonitrile (P-I)

The drug (0.5gm) was dissolved in acetonitrile (5 ml) to check its solubility. To this solution, another weighed amount of Prulifloxacin (2.5 gm) was added and refluxed with acetonitrile (30 ml) for 2.5 hours. The solution was filtered through Whatmann filter paper and the filtrate was concentrated by recovery of the solvent to one third of its original volume and kept at room temperature to afford well defined crystals of Prulifloxacin (P-I). The crystal were collected by filtration, dried under vaccum at room temperature for 48 hours and stored in well closed container. Yield, 2.4gm (80.00%), melting point: 226.44ºC.

#### 5.3.1.2 From acetone (P-II)

The drug (0.5 gm) was dissolved in acetone (5 ml) at its boiling point to check its solubility. To this solution, another weighed amount (3.5 gm) of Prulifloxacin was added and refluxed with acetone (30 ml) for 3 hours. The solution was filtered through Whatmann filter paper and concentrated by recovery of solvent to one third of its original volume and kept for crystallization at room temperature to get well defined crystals of Prulifloxacin (P-II). The crystal were collected by filtration, dried under vaccum at room temperature for 24 hours and stored in well closed container. Yield, 3.00gm (75.00%), melting point: 222.33ºC.

#### 5.3.1.3 From dichloromethane (P-III)

The drug (0.5 gm) was dissolved in dichloromethane (5 ml) at its boiling point to check it solubility. To this solution another weighed quantity (2.5 gm) of Prulifloxacin was added and refluxed with dichloromethane (25 ml) for 2.5 hours. The solution was filter through Whatmann filter paper and the filterate was concentrated by recovery of the solvent to one third of its original volume and kept at room temperature until it was formed as well defined crystals of prulifloxacin (P-III). The obtained crystals were collected by vacuum filtration, dried for 24 h in desiccators and stored in well closed container. Yield, 2.00gm (66.67%), melting point: 224.91ºC

### 5.3.2 CHARACTERIZATION OF PREPARED PRULIFLOXACIN CRYTALS

#### 5.3.2.1 Determination of melting point [95]

The melting point of prulifloxacin was determined by capillary method using Remi melting point apparatus. Capillary tube was filled with prulifloxacin amorphous drug about 3 mm height and tube was put into the sample and it was tapped on the bottom of the sample dish to get the drug into the tube. Filled capillary tube was placed in the melting point apparatus and the temperature range was set at high degree to get approximate melting range of the drug. Melting process was observed through magnifying lens. Once the melting point range was determined, again the exact melting point was observed by placing another drug filled capillary tube in the apparatus and setting the increment in temperature was not more than 2ºC per minute. Then, the temperature was recorded at which it melted.

#### 5.3.2.2 Scanning Electron Microscopy (SEM) [96,97]

Scanning electron microscopic study was performed to characterize and differentiate the surface morphology of the crystals with its amorphous form. The

morphology of prepared crystals of prulifloxacin and pure drug was investigated by the use of scanning electron microscope (Jeol JSM-6100 instrument). All the samples were mounted on a metal stub with an adhesive and coated under vacuum with gold.

### 5.3.2.3 FT-IR Spectroscopy [98,99]

The FT-IR spectrum of prulifloxacin pure drug and its crystals were analyzed by mixing these separately with AR grade potassium bromide. Then, each sample was pressed into fine pellets and scanned with FT-IR spectrophotometer (Shimadzu, Japan) equipped with pyroelectric detector using dispersion method. The FT-IR measurements were performed in the scanning range of 4000-400 $cm^{-1}$ at ambient temperature and recorded.

### 5.3.2.4 Differential Scanning Calorimetry (DSC) [100,101]

The DSC thermo gram for pure drug of prulifloxacin and its polymorphs were obtained using a DSC-6300SSI Nano Tech and the temperature range of scan was set from 100 to 400ºC at a rate of 10ºC/min. The sample (50-100 mg) was purged under a flow of nitrogen at a flow rate of 50 ml/min. The exact peak temperature, melting point and heat of fusion were determined and their corresponding thermograms were recorded.

### 5.3.2.5 Powder X-Ray Diffractometry (PXRD) [102,103]

The powder x-ray diffraction patterns of the samples were recorded using a Ricnaku Miniflex 2C. The operating condition were as follows: target, cu, voltage 40kV, current 30mA, receiving slit,0.3mm, preset time, 0.60 sec, scan speed 10 (deg/min), sampling pitch 0.1º . The divergent slit and scatter slit 1º and auto slit were not used

### 5.3.3 DISSOLUTION AND SOLUBILITY STUDIES

#### 5.3.3.1 Preparation of phosphate buffer pH 6.8[95]

About 28.8 gm of anhydrous disodium hydrogen phosphate and 11.44 gm of potassium dihydrogen phosphate were weighed individually and dissolved in distilled water to produce 1000 ml.

#### 5.3.3.2 Determination of $\lambda_{max}$ by UV Spectroscopy

About 20 mcg/ml concentration containing solution was prepared accurately by dissolving the active prulifloxacin drug in phosphate buffer pH 6.8. UV spectrum of the above solution was scanned between **200- 400 nm** by using UV-Visible double beam spectrophotometer (SYSTRONICS, DOUBLE BEAM UV-VISIBLE Spectrophotometer: 2201, Mumbai) and the wavelength maximum ($\lambda_{max}$) was observed.

#### 5.3.3.3 Preparation of standard curve in phosphate buffer pH 6.8

Accurately 100 mg of prulifloxacin pure drug was weighed, dissolved in DMSO and diluted to the mark with phosphate buffer pH 6.8 in a 100 ml volumetric flask. This was named as primary stock solution that contained concentration of 1000 mcg/ml. From this primary stock solution, a series of aliquots were pipetted out and diluted with phosphate buffer pH 6.8 to get the concentration of 5-50 mcg/ml of prulifloxacin. The absorbances of these solutions were measured against the phosphate buffer pH 6.8 as blank at **272nm** using UV-Visible double beam spectrophotometer. Then, a calibration curve was plotted taking concentration in mcg/ml on X- axis and absorbance on Y- axis.

### 5.3.3.4 Solubility assessment [104,105]

The solubility of all the prepared crystalline samples in buffer was determined by taking excess quantity of samples in 50 ml screw- capped glass vials filled with **phosphate buffer pH 6.8**. The vials were shaken for 24 hours on mechanical shaker. Then, the solution was filtered through Whatmann filter paper No.1 and drug concentration was determined at **272 nm.**

### 5.3.3.5 *In-vitro* Dissolution study [106- 108]

The *in-vitro* dissolution profiles of pure drug and obtained crystals were carried out by using eight stations of LAB INDIA DISSO 2000 dissolution test apparatus with USP type II (paddle). The temperature and paddle speed were maintained at 37±2ºC with a constant speed of agitation at 100 rpm. The samples of prulifloxacin crystals (100 mg) were placed in the dissolution medium of phosphate buffer (pH 6.8). The fixed volume (1 ml) of the sample was withdrawn (with replacement) at 15, 30, 45, 60, 120 and 240 min time intervals and diluted suitably. Then, the solution was analyzed spectrophotometrically at **272 nm** and the percentage drug release was calculated.

### 5.3.3.6 Drug content [109]

. Drug content of each crystalline form of prulifloxacin (P-I, P-II and P-III) was carried out by dissolving 50 mg of each with 10 ml of **phosphate buffer pH 6.8.** The prepared solutions were shaken well and allowed to stand for 10 min with occasional swirling and the same solvent was added to produce 100 ml. After suitable dilution, absorbance of samples was measured at **272 nm**. Drug content was determined from standard curve graph.

### 5.3.3.7 Stability studies [110]

Accelerated stability study of these three crystals was carried out as per the ICH guidelines. The stability samples were (n=3) kept at 40±2ºC and 75±5% RH in stability testing chamber (LabTop Mumbai, India) for a period of 30, 60 and 90 days. After completion of the above said period, samples were taken out and analyzed for drug content.

## 5.3.4 PHARMACOKINETIC STUDY IN RATS [111-113]

*In-vivo* evaluation and protocol for pharmacokinetic studies on the crystal forms of selected fluoroquinolones were approved by Institutional Animal Ethical Committee (IAEC/XXXXIV/SRU/435/2015). The *in-vivo* kinetic study was performed by using Male Sprague – Dawley rats. The experiment was carried out by administering the pure and crystalline form of prulifloxacin samples to rats by oral route and blood samples were collected by retro-orbital plexus method at regular time intervals to calculate the concentration of metabolites in rat plasma and other pharmacokinetic parameters.

### 5.3.4.1 Animals used

Male Sprague – Dawley rats (weighing approximately 230 g) were used for the study. Rats were randomly divided into two groups of six rats per group. All the animals were observed for mortality/viability and other clinical signs daily, during acclimatization period, before and after the administration of test dose on test day and twice daily till they were sacrificed. Body weight of each animal was weighed prior to drug administration, before and after blood collection.

### 5.3.4.2 Drug administration

The overnight fasted male rats were divided into two groups and all the rats from each group were received the following test dose of 10 mg/kg body weight through oral route. Samples used were prulifloxacin pure drug and optimized crystal form (P-II).

### 5.3.4.3 Blood sampling

The blood samples were collected at predetermined time intervals of 0 (initial), 0.5, 1, 2, 4, 8, 12 and 24 h into heparinized tubes. At pre-determined time intervals approximately 0.4 ml of blood samples were collected from the retro-orbital plexus under Isoflurane anesthesia into labeled tubes containing K2 EDTA (4 mM per ml of blood) as an anticoagulant. The blood samples were mixed by manual inversion for 4-5 times and were kept chilled on ice until centrifugation. Plasma were separated by centrifuging the whole blood sample at 5000 rpm for 5-10 minutes at 2-8$^{o}$C. The plasma were separated within 1hr from time of blood collection and plasma samples were placed in labeled tubes and stored below -70$^{o}$C until their analysis.

### 5.3.4.4 Bioanalysis by HPLC method

Concentration of metabolite of administered drug in plasma at various time points were analysed by a sensitive High Performance Liquid Chromatographic (HPLC) method. The HPLC system (Shimadzu LC solution) equipped with UV-VIS detector and C18 column (Hypersil) was used for the experiment.

### 5.3.4.5 Preparation of mobile phase

Phosphate buffer (pH 3.2) was prepared by dissolving 6.056 gm of potassium dihydrogen ortho phosphate in 445 ml of HPLC grade water, which was mixed with 55 ml of 0.1M phosphoric acid and the pH was adjusted to 3.2 using triethylamine. A

mobile phase used for the study was prepared by mixing acetonitrile and phosphate buffer in the ratio of 40:60 v/v. The above said mobile phase solvent system was freshly prepared for each run, filtered through 0.45 µm filter and degassed for 30 min using ultra sonicator before use.

**5.3.4.6 Other chromatographic condition**

Flow rate was set at 1 ml/min and column used was Hypersil C18 (4.6x250mm, 5µm). About 20 µl of samples were injected into the chromatographic system with a running time of 15 min. The chromatogram was recorded at ambient temperature and the eluent was monitored at **285 nm.**

**5.3.4.7 Preparation of working standard solution**

Stock solution of 1µg/ml of prulifloxacin was prepared separately by dissolving 100 mg of pure drug in 100 ml of mobile phase solvent. From the stock solution, working standard solutions were prepared to contain 25, 50, 100, 150, 200, 250 and 300 µg/ml of prulifloxacin by diluting suitably with mobile phase solvent as diluents.

**5.3.4.8 Preparation of calibration standard for linearity graph**

About 10µl of each working standard solutions of prulifloxacin were picked freshly with aliquots of 90µl of plasma and diluted up to 1.0 ml with diluent to produce final concentration of 0.25, 0.50, 1, 1.5, 2, 2.5, 3µg/ml and then vertexed for 60 s. Then, these plasma samples were centrifuged at 10,000 rpm for 1 min at 10 ± 2ºC. After centrifugation, the supernatant layer was separated and 20µl of sample solutions were injected into HPLC system. Standard calibration curves were plotted from the linear square regression analysis using peak area on y-axis and theoretical concentration on x-axis.

### 5.3.4.9 Sample extraction from rat plasma

Accurately about 100 µl of rat plasma was diluted with mobile phase solvent (diluents) up to 1.0 ml and mixed well for one minute. The resultant solution was vertexed for 60 sec. and centrifuged at 10,000 rpm for 10 min. 20 µl of supernatant liquid was separated and injected into HPLC system to determine the concentration of drug in plasma sample. The concentration of metabolite of prulifloxacin (ulifloxacin) present in plasma samples was calculated from the calibration curve. Prior to the analysis of standard and samples of prulifloxacin, the blank plasma was injected for specificity study which confirmed that there was no interference of blank plasma with drug.

### 5.3.4.10 Pharmacokinetic parameters

The most common pharmacokinetic parameters such as peak plasma concentration ( $C_{max)}$, time to reach maximum plasma concentration ($T_{max)}$, Area under the plasma concentration-time curve (AUC0-α), Elimination rate constant (Kel) and elimination half-life ($T_{1/2}$),were calculated from the plasma concentration – time curve using PK Solution 2.0, non compartmental pharmacokinetic analysis software.

## 5.4 METHODS FOR GEMIFLOXACIN MESYLATE

### 5.4.1 PREPARATION OF CRYSTAL FORMS FROM DIFFERENT SOLVENTS (BY EVAPORATION CRYSTALLIZATION METHOD) [8]

#### 5.4.1.1 From isopropanol (G-I)

The drug (0.5 gm) was dissolved in isopropanol (5 ml) at its boiling point to check it solubility. To this solution, another weighed amount (2.5 gm) of gemifloxacin mesylate and hot solvent of isopropanol (30 ml) were added, under constant stirring. The above solution was saturated and supernatant liquid was poured into petri dish. Then, it was kept at room temperature for about 24 h to produce the fine crystals of gemifloxacin mesylate (G-I). The obtained crystals were collected immediately and dried in vaccum desiccators at ambient temperature using silica gel for at least 24 h. The resultant product was sieved and stored in a well closed container. Yield, 2.5gm (83.33%), melting point: 206.6ºC

#### 5.4.1.2 From chloroform (G-II)

The drug (0.5 gm) was dissolved in chloroform (5 ml) at its boiling point to check it solubility. To this solution, another weighed amount (2.5 gm) of gemifloxacin mesylate and hot solvent chloroform (30 ml) were added, under constant stirring. The above solution was saturated and supernatant liquid was poured into petri dish. Then, it was kept at room temperature for about 24 h to produce the fine crystals of gemifloxacin mesylate (G-II). The obtained crystals were collected immediately and dried in vaccum desiccators at ambient temperature using silica gel for at least 24 h. The resultant product was sieved and stored in a well closed container. Yield, 2.1gm (70.00%), melting point: 208.6ºC

#### 5.4.1.3 From dichloromethane (G-III)

The drug (0.5 gm) was dissolved in dichloromethane (5 ml) at its boiling point to check it solubility. To this solution, another weighed amount (2.5 gm) of gemifloxacin mesylate and hot solvent of dichloromethane (30 ml) were added, under constant stirring. The above solution was saturated and supernatant liquid was poured into petri dish. Then, it was kept at room temperature for about 24 h to produce the fine crystals of gemifloxacin mesylate (G-III). The obtained crystals were collected immediately and dried in vaccum desiccators at ambient temperature using silica gel for at least 24 h. The resultant product was sieved and stored in a well closed container. Yield, 2.0gm (66.66%), melting point: 209.8ºC

#### 5.4.1.4 From benzene (G-IV)

The drug (0.5 gm) was dissolved in benzene (5 ml) at its boiling point to check it solubility. To this solution, another weighed amount (2.5 gm) of gemifloxacin mesylate and hot solvent of benzene (30 ml) were added, under constant stirring. The above solution was saturated and supernatant liquid was poured into petri dish. Then, it was kept at room temperature for about 24 h to produce the fine crystals of gemifloxacin mesylate (G-IV). The obtained crystals were collected immediately and dried in vaccum desiccators at ambient temperature using silica gel for at least 24 h. The resultant product was sieved and stored in a well closed container. Yield, 2.2gm (73.33%), melting point: 214.2ºC.

## 5.4.2 CHARACTERIZATION OF PREPARED GEMIFLOXACIN MESYLATE CRYSTAL FORMS

### 5.4.2.1 Determination of melting point [95]

The melting point of gemifloxacin was determined by capillary method using Remi melting point apparatus. Capillary tube was filled with gemifloxacin amorphous drug about 3 mm height and tube was put into the sample and it was tapped on the bottom of the sample dish to get the drug into the tube. Filled capillary tube was placed in the melting point apparatus and the temperature range was set at high degree to get approximate melting range of the drug. Melting process was observed through magnifying lens. Once the melting point range was determined, again the exact melting point was observed by placing another drug filled capillary tube in the apparatus and setting the increment in temperature was not more than 2ºC per minute. Then, the temperature was recorded at which it melted.

### 5.4.2.2 Scanning Electron Microscopy (SEM) [114, 115]

A Jeol JSM-6100 Scanning electron microscope was used to obtain photomicrographs of crystals of gemifloxacin mesylate. Samples were mounted on a metal stub with an adhesive and coated under vaccum with gold.

### 5.4.2.3 FT-IR Spectroscopy [116,117]

The FT-IR spectrum of gemifloxacin mesylate and crystals were analyzed with FTIR spectrophotometer (Shimadzu, Japan) equipped with pyroelectric detector using dispersion method. The FT-IR measurements were performed in the scanning range of 4000-400 $cm^{-1}$ at ambient temperature and recorded**.**

#### 5.4.2.4 Differential Scanning Calorimetry (DSC) [118,119]

DSC thermograms of gemifloxacin mesylate and crystals were recorded using differential scanning colorimeter NETZCH STA 449F3 Instruments, each sample was scanned in pierced A1 pans. The measurement was performed between room temperature to 400ºC/min under an inert atmosphere flushed with nitrogen at a rate of 50ml/min at heating rate 50ºC/min.

#### 5.4.2.5 Powder X-Ray Diffractometry (PXRD) [120-122]

The powder x-ray diffraction patterns of the samples were recorded using a Ricnaku Miniflex 2C. The operating conditions were as follows: target, cu, voltage 40kV, current 30mA, receiving slit, 0.3mm, preset time, 0.60 sec, scan speed 10 (deg/min), sampling pitch 0.1° . The divergent slit and scatter slit 1° and auto slit were not used.

### 5.4.3 DISSOLUTION AND SOLUBILITY STUDIES

#### 5.4.3.1 Preparation of phosphate buffer pH 7.0

About 0.50 gm of anhydrous disodium hydrogen phosphate and 0.301 gm of potassium dihydrogen phosphate were weighed separately and dissolved in distilled water to produce 1000 ml.

#### 5.4.3.2 Determination of $\lambda_{max}$ by UV Spectroscopy

UV spectrum of 10 mcg/100 ml solution of gemifloxacin in phosphate buffer pH 7.0 was scanned between **200 - 400 nm** by using UV-Visible double beam spectrophotometer (SYSTRONICS, DOUBLE BEAM UV-VISIBLE Spectrophotometer: 2201, Mumbai) and the $\lambda_{max}$ observed.

### 5.4.3.3 Preparation of standard curve in phosphate buffer pH 7.0

About 100 mg of gemifloxacin mesylate was accurately weighed, dissolved and made upto the mark with phosphate buffer pH 7.0 in a 100 ml volumetric flask which was marked as primary stock solution, contained concentration of 1000 mcg/ml. From this primary stock solution, 1ml was pipetted out and transferred into 100 ml volumetric flask. Then, volume was made upto the mark with phosphate buffer pH 7.0 and denoted as secondary stock solution.

From this secondary stock solution, 1-10 ml were pipetted out into a series of 10 ml volumetric flask and volume was made up to mark with phosphate buffer pH 7.0. These stock solutions contained 1-10 mcg/ml. The absorbances of these solutions were measured against the phosphate buffer pH 7.0 as blank at **271nm** using UV-Visible double beam spectrophotometer. Then, a calibration curve was plotted taking concentration in mcg/ml on X-axis and absorbance on Y- axis.

### 5.4.3.4 Solubility assessment[123]

The solubility of all the prepared crystalline samples in buffer was determined by taking excess quantity of samples in 50 ml screw- capped glass vials filled with **phosphate buffer pH 7.0**. The vials were shaken for 24 hrs on mechanical shaker. Then, the solution was filtered through Whatmann filter paper No.1 and drug concentration was determined at **271 nm**.

### 5.4.3.5 *In-vitro* Dissolution study [124, 125]

The *in-vitro* dissolution studies were carried out using eight stations of LAB INDIA DISSO 2000 dissolution test apparatus. The samples of crystals (100 mg) were placed in dissolution medium of phosphate buffer (pH 7.0) at 37±2°C with a

constant speed of agitation at 50 rpm using USP type II (paddle). The fixed volume (1 ml) of sample was withdrawn (with replacement) at 15, 30, 45, 60, 90, 120 and 240 min time intervals and diluted appropriately. The percentage drug release was determined by VU spectroscopic method using **271 nm** as the λ max of gemifloxacin mesylate.

### 5.4.3.6 Drug content [126]

Drug content of each crystalline form of gemifloxacin (G-I, G-II, G-III and G-IV) was carried out by dissolving 50 mg of each with 10 ml of **phosphate buffer pH 7.0.** The prepared solutions were shaken well and allowed to stand for 10 min with occasional swirling and the same solvent was added to produce 100 ml. After suitable dilution, absorbance of samples was measured at **271 nm**. Drug content was determined from standard curve graph.

### 5.4.3.7 Stability studies [110]

Accelerated stability study of these three crystals was carried out as per the ICH guidelines. The stability samples were (n=3) kept at 40±2ºC and 75±5% RH in stability testing chamber (LabTop Mumbai, India) for a period of 30, 60 and 90 days. After completion of the above said period, samples were taken out and analyzed for drug content.

## 5.4.4 PHARMACOKINETIC STUDY IN RATS [127-129]

Protocol for pharmacokinetic studies on the crystal forms of selected Fluoroquinolones were approved by Institutional Animal Ethical Committee (IAEC/XXXXIV/SRU/435/2015). The *in-vivo* kinetic study was performed by administering the pure and crystalline form of gemifloxacin mesylate to rats orally

and blood samples were collected at regular time intervals to calculate the concentration of drug in plasma.

#### 5.4.4.1 Animals used

Male Sprague – Dawley rats (weighing, approximately 230 g) were used for the study. Rats were randomly divided into two groups of six rats per group. All the animals were observed for mortality/viability and other clinical signs daily, during acclimatization period, before and after the administration of test dose on test day and twice daily till they were sacrificed. Body weight of each animal was weighed prior to drug administration, before and after blood collection.

#### 5.4.4.2 Drug administration

All the male rats from each group were received the following test dose of 10 mg/kg body weight through oral route. Samples used were gemifloxacin mesylate pure drug and optimized crystal form (G-I).

#### 5.4.4.3 Blood sampling

The blood samples were collected at the time points of 0 (initial), 0.5, 1, 2, 4, 8, 12 and 24 hrs and stored at -80ºC. As per above mentioned time points, blood samples were collected from rats. At pre-determined time intervals approximately 0.4 ml of blood samples were collected from the retro-orbital plexus under Isoflurane anesthesia into labeled tubes containing K2 EDTA (4 mM per ml of blood) as an anticoagulant. The blood samples were mixed by manual inversion for 4-5 times and were kept chilled on ice until centrifugation. Plasma were separated by centrifuging the whole blood sample at 5000 rpm for 5-10 minutes at 2-8$^{o}$C. The plasma were separated within 1 hr from time of blood collection and plasma samples were placed in labeled tubes and stored below -70$^{o}$C until their analysis.

#### 5.4.4.4 Bioanalysis by HPLC method

Concentration of metabolite of administered drug in plasma at various time points were analysed by a sensitive High Performance Liquid Chromatographic (HPLC) method. The HPLC system (Shimadzu LC solution) equipped with UV-VIS detector and $C_{18}$ column (Hypersil) was used for the experiment.

#### 5.4.4.5 Preparation of mobile phase

About 28.80 gm of disodium hydrogen phosphate and 11.45 gm of potassium dihydrogen phosphate were weighed and transferred into 1000 ml flask. The above said substances were dissolved in sufficient water to produce 1 litre to phosphate buffer pH 6.8. A mobile phase used for the study was prepared by mixing acetonitrile and phosphate buffer pH 6.8 in the ratio of 20:80 v/v. The above said mobile phase solvent system was freshly prepared for each run, filtered through 0.45 µm filter and degassed for 30 min using ultrsonicator before use.

#### 5.4.4.6 Other chromatographic condition

Flow rate was set at 1 ml/min and column used was Hypersil $C_{18}$ (4.6 X 250 mm, 5µm). About 20 µl of samples were injected into the chromatographic system with a run time of 15 min. The chromatogram was recorded at ambient temperature and the eluent monitored at **265nm.**

#### 5.4.4.7 Preparation of working standard solution

Stock solution of 1µg/ml of gemifloxacin was prepared separately by dissolving 100 mg of pure drug in 100 ml of mobile phase solvent. From the stock solution, working standard solutions were prepared to contain 25, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg/ml of gemifloxacin by diluting suitably with mobile phase solvent as diluent.

### 5.4.4.8 Preparation of calibration standard for linearity graph

About 10µl of each working standard solutions of gemifloxacin were picked freshly with aliquots of 90µl of plasma and diluted up to 1.0 ml with diluent to produce final concentration of 0.25, 0.50, 1, 1.5, 2, 3, 4 and 5 µg/ml then vortexed for 60 s. Then, these plasma samples were centrifuged at 10,000 rpm for min at 10 ± 2ºC. After centrifugation, the supernatant layer was separated and 20 µl of sample solutions were injected into HPLC system. Standard calibration curves were plotted from the linear square regression analysis using peak area on y-axis and theoretical concentration on x-axis.

### 5.4.4.9 Sample extraction from rat plasma

Accurately about 100 µl of rat plasma was diluted with mobile phase solvent (diluent) up to 1.0 ml and mixed well for one minute. The resultant solution was vortexed for 60 sec. and centrifuged at 10,000 rpm for 10 min. About 20 µl of supernatant liquid was separated and injected into HPLC system to determine the concentration of drug in plasma sample. Prior to the analysis of standard and samples of gemifloxacin, the blank plasma was injected for specificity study which confirmed that there was no interference of blank plasma with drug.

### 5.4.4.10 Pharmacokinetic parameters

The most common pharmacokinetic parameters such as peak plasma concentration ($C_{max}$), time to reach maximum plasma concentration ($T_{max}$), Area under the plasma concentration-time curve (AUC0-α), Elimination rate constant (Kel) and elimination half-life ($T_{1/2}$),were calculated from the plasma concentration-time curve using PK Solution 2.0, non compartmental pharmacokinetic analysis software.

# 6. RESULTS AND DISCUSSION

The work carried out has been discussed under the headings of individual drugs prulifloxacin and gemifloxacin mesylate. Based on the nature of the work each heading has been sub-categorized under the sub-headings viz: preparation & characterization of crystal forms, solubility & dissolution studies and *in-vivo* pharmacokinetic studies.

## 6.1 PRULIFLOXACIN

### 6.1.1 PREPARATION AND CHARACTERIZATION OF CRYSTAL FORMS

#### 6.1.1.1 Preparation of crystal forms from different solvents[8]

Prulifloxacin was procured as raw material from Hetero drugs, Hyderabad and it was used as such for the preparation of crystal forms. Prulifloxacin crystal forms were tried to prepare from different solvents viz: dimethyl formamide, methanol, ethanol, acetonitrile, chloroform, ethyl acetate, acetone, isopropanol, dichloromethane, n-hexane, benzene, toluene. But the pure drug of prulifloxacin had shown appreciable solubility only in acetonitrile (P-I), acetone (P-II), dichlormethane (P-III) and these solvents only gave different types of crystals.

#### 6.1.1.2 Characterization of crystal forms

Melting points of all prepared crystal forms of P-I, P-II and P-III were found 226ºC, 222ºC and 224ºC respectively. It was found that the melting point P-III similar to pure drug of prulifloxacin (P) 224ºC. The melting point of P-I was higher than that of pure form and it may be the stable polymorph. The percentage yield of prepared crystal forms such as P-I, P-II and P-III were 80.00%, 75.00% and 66.67% respectively (Table 5).

**Table: 5 Crystal yield and melting point of crystal forms of prulifloxacin**

| S.No. | Crystal Forms | Crystal yield (%) | Melting point (°C) |
|---|---|---|---|
| 1 | Prulifloxacin (P) | -- | 224 |
| 2 | P-I | 80 | 226 |
| 3 | P-II | 75 | 222 |
| 4 | P-III | 66 | 224 |

### 6.1.1.3 Scanning Electron Microscopy (SEM) [130]

SEM photographs of three crystal forms of prulifloxacin were reported that these clearly demonstrated the difference in the morphology of each crystalline form. From the results obtained it was concluded that P-I, P-II and P-III crystals were plate, rod or needle and prismatic shaped particles (Table 6) respectively which clearly indicating the formation of different types of crystals. The particles present in the pure drug have irregular shape and also have smaller particle size. Based on the photographs obtained from scanning electron microscopy, homogeneity was observed in the surface of each crystalline form with different particle size. The SEM images for prepared crystals had shown in Fig. 3-6.

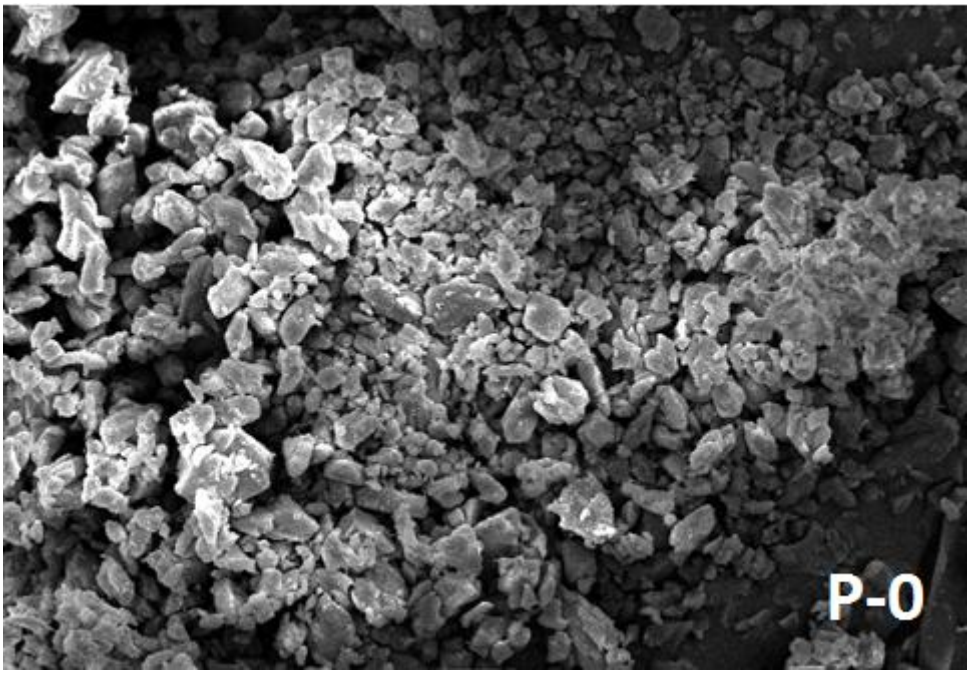



**Fig. 3: SEM photograph for prulifloxacin (P)**

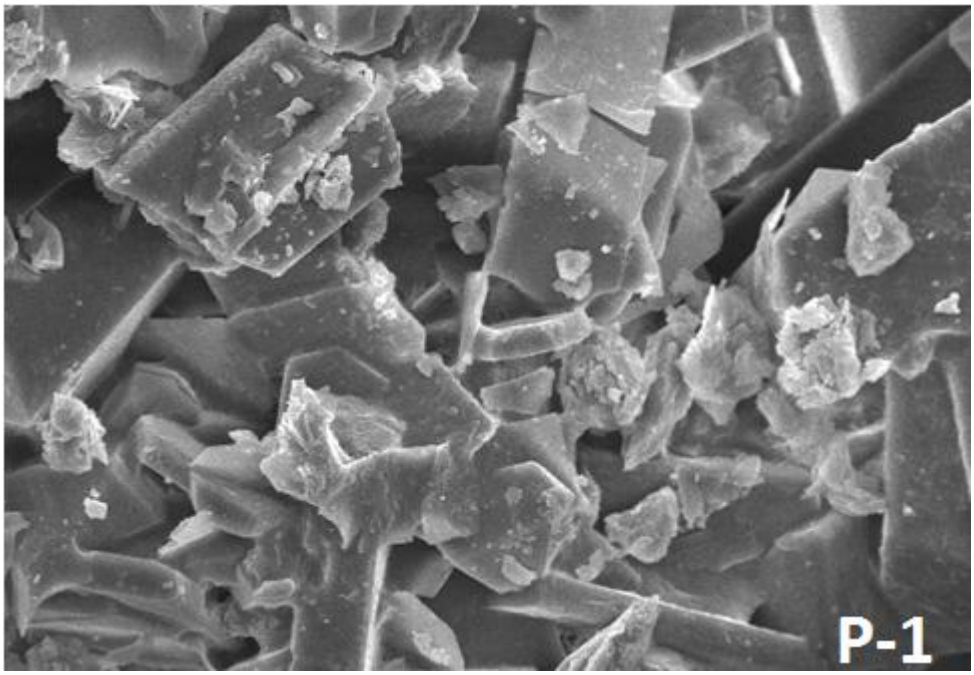



**Fig. 4: SEM photograph for P-I**

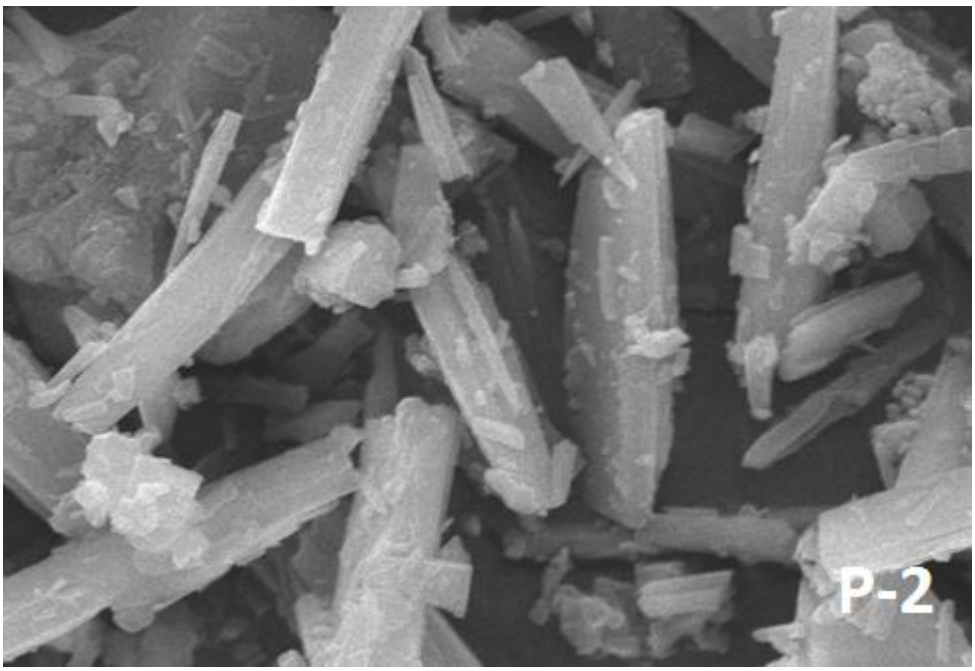



**Fig. 5: SEM photograph for P-II**

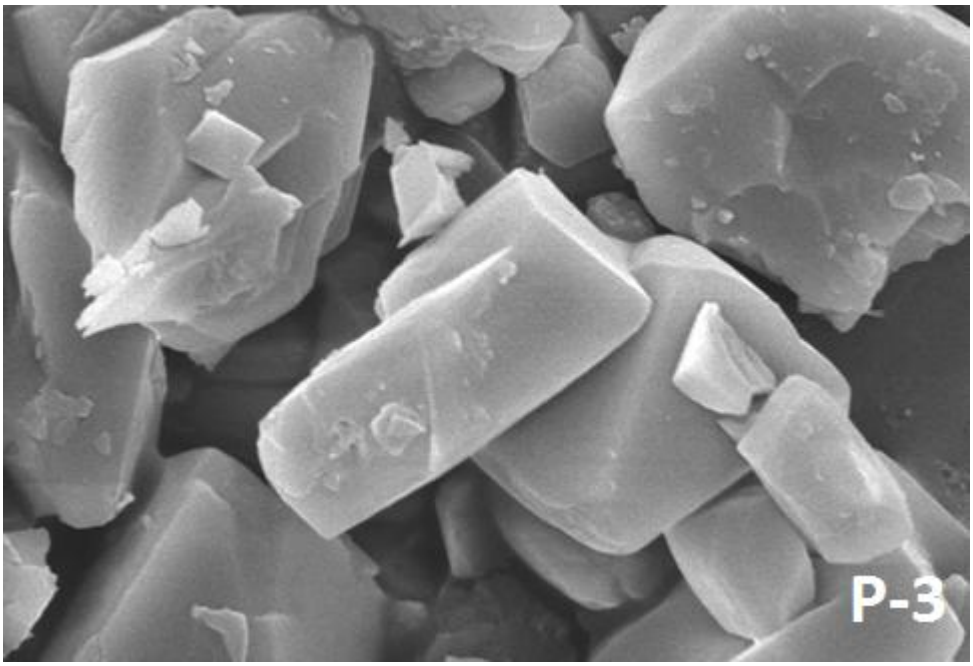



**Fig. 6: SEM photograph for P-III**

**Table: 6 SEM analysis of crystal forms**

| S.No. | Crystal Forms | Shape |
|---|---|---|
| 1 | Prulifloxacin (P) | Smoky quartz |
| 2 | P-I | Plate like |
| 3 | P-II | Rod |
| 4 | P-III | Prismatic |

### 6.1.1.4 FT-IR Spectroscopy [131]

FT-IR analysis was carried out to confirm the purity and quality of drug. Infrared spectroscopy is another important technique for characterization of polymorphs. On comparing the spectra of pure and its crystals (Fig. 7-11), differences in the peak pattern were observed in the region of 3000 to 1100 $cm^{-1}$. There was a small shift in the peaks, but no considerable changes in position of the peaks. It indicated that there was no chemical change in crystal forms when compared to pure form. The IR Spectra of the three crystal forms were showed the strong bands due to C-F stretching which appeared between 1250 and 1100 $cm^{-1}$. The peaks of crystals of P-I, P-II, P-III showed at 1157.33, 1130.32, 1118.75 $cm^{-1}$ respectively. The N-H stretching showed the peak ranges from 1400-1300 $cm^{-1}$ and P-I, P-II, P-III showed peaks at 1348.29, 1377.72, 1386.16 $cm^{-1}$. There was not much significant difference in the absorption band due to N-H stretching vibration. The broad bands of COOH group had peaks in range of 3000-2800 $cm^{-1}$ and peaks for P-I, P-II, P-III appeared at 2976.26, 2972.40, 2762.16 $cm^{-1}$ (Table 7).

This shifting could not alter the functional group i.e., there was no difference in chemical nature and pharmacological action which indicated there was no interaction between the drug and solvents during the crystallization process.

**Table 7: Interpretations of FT-IR peaks for prulifloxacin and its crystal forms**

| S.No. | Sample | COOH-Broad band ($cm^{-1}$) | C-F Stretching ($cm^{-1}$) | N-H Stretching ($cm^{-1}$) | C=0 Keto group ($cm^{-1}$) |
|---|---|---|---|---|---|
| 1 | Frequency range for functional Groups | 3400-2800 | 1400-1000 | 1400-1300 | 1708.99 |
| 2 | Prulifloxacin P | 2937.68 | 1134.18 | 1371.43 | 1708.99 |
| 3 | P-I | 2976.26 | 1157.33 | 1348.29 | 1708.77 |
| 4 | P-II | 2972.40 | 1130.32 | 1377.22 | 1708.99 |
| 5 | P-III | 2762.16 | 1118.75 | 1386.16 | 1708.99 |

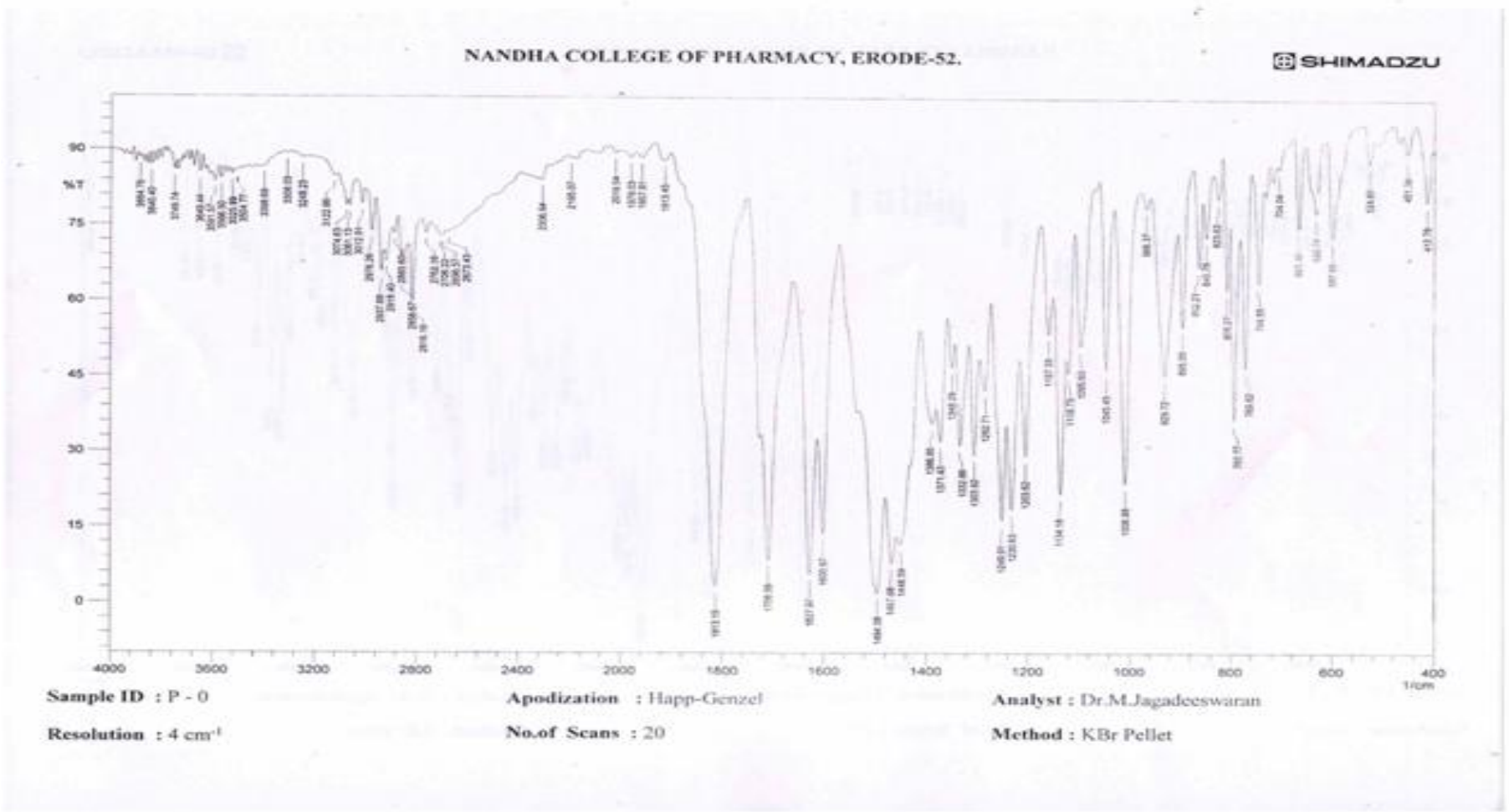



Fig. 7: FT-IR spectra of prulifloxacin (P)

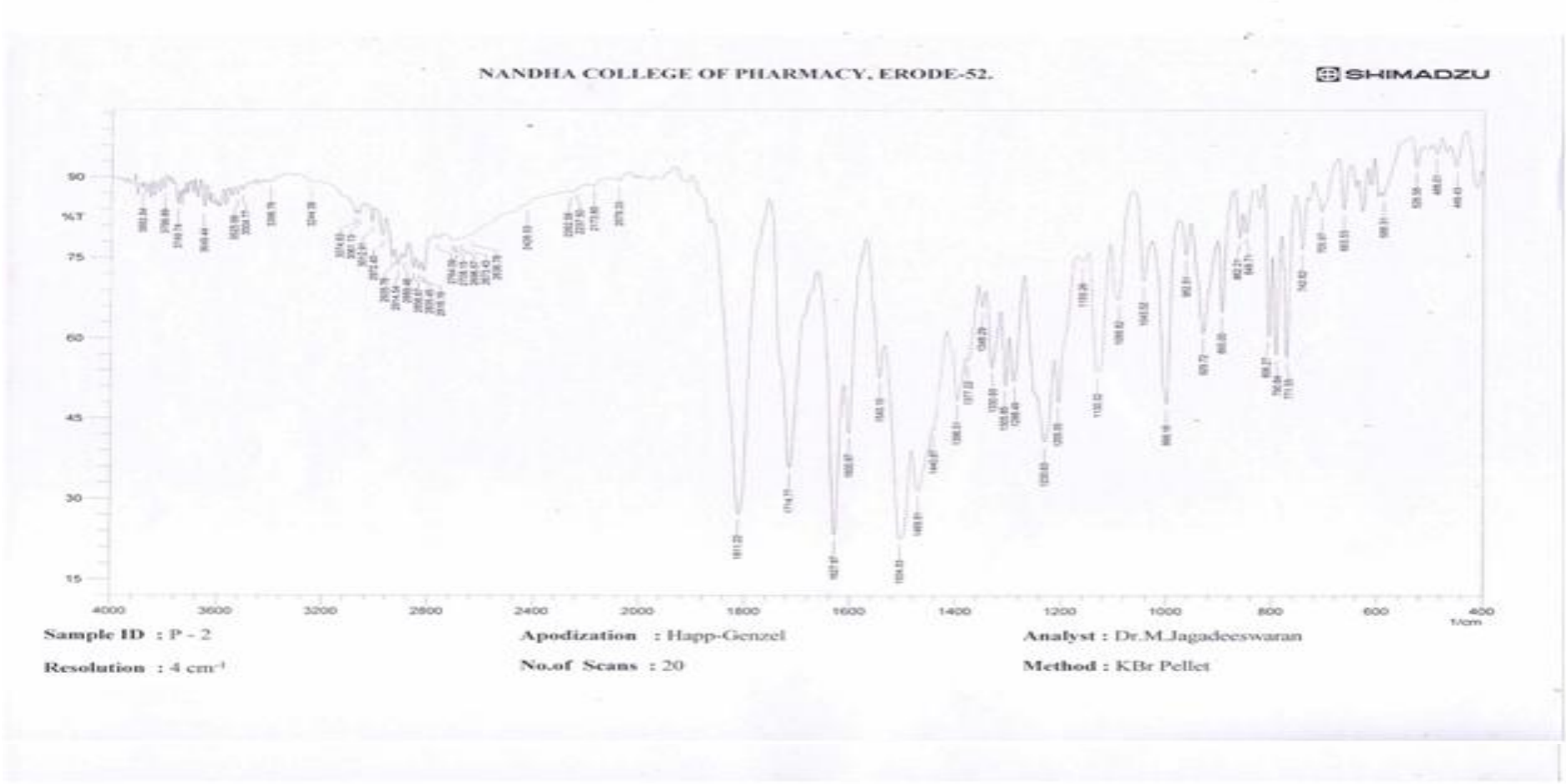



**Fig. 8: FT-IR spectra of P-I**

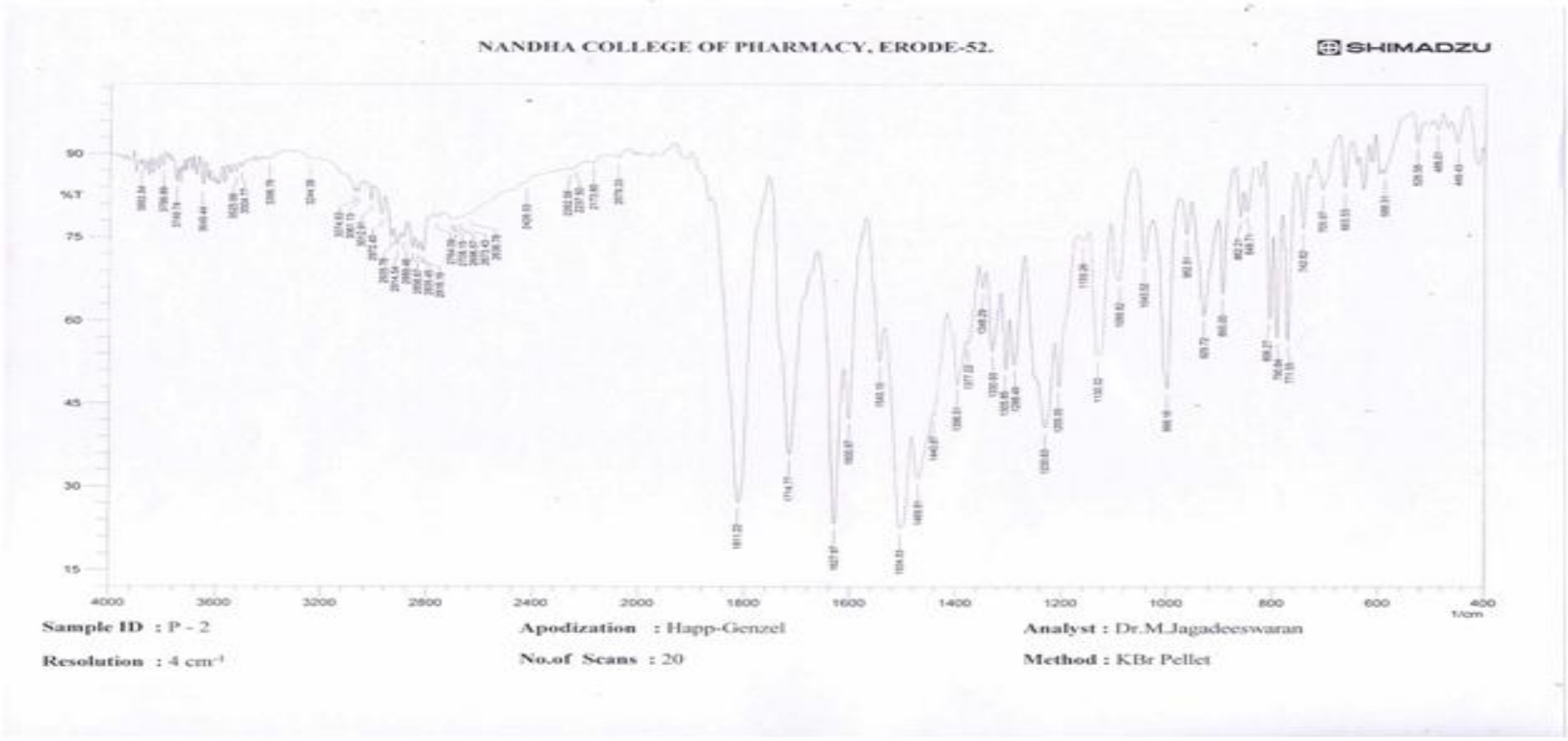



**Fig. 9: FT-IR spectra of P-II**

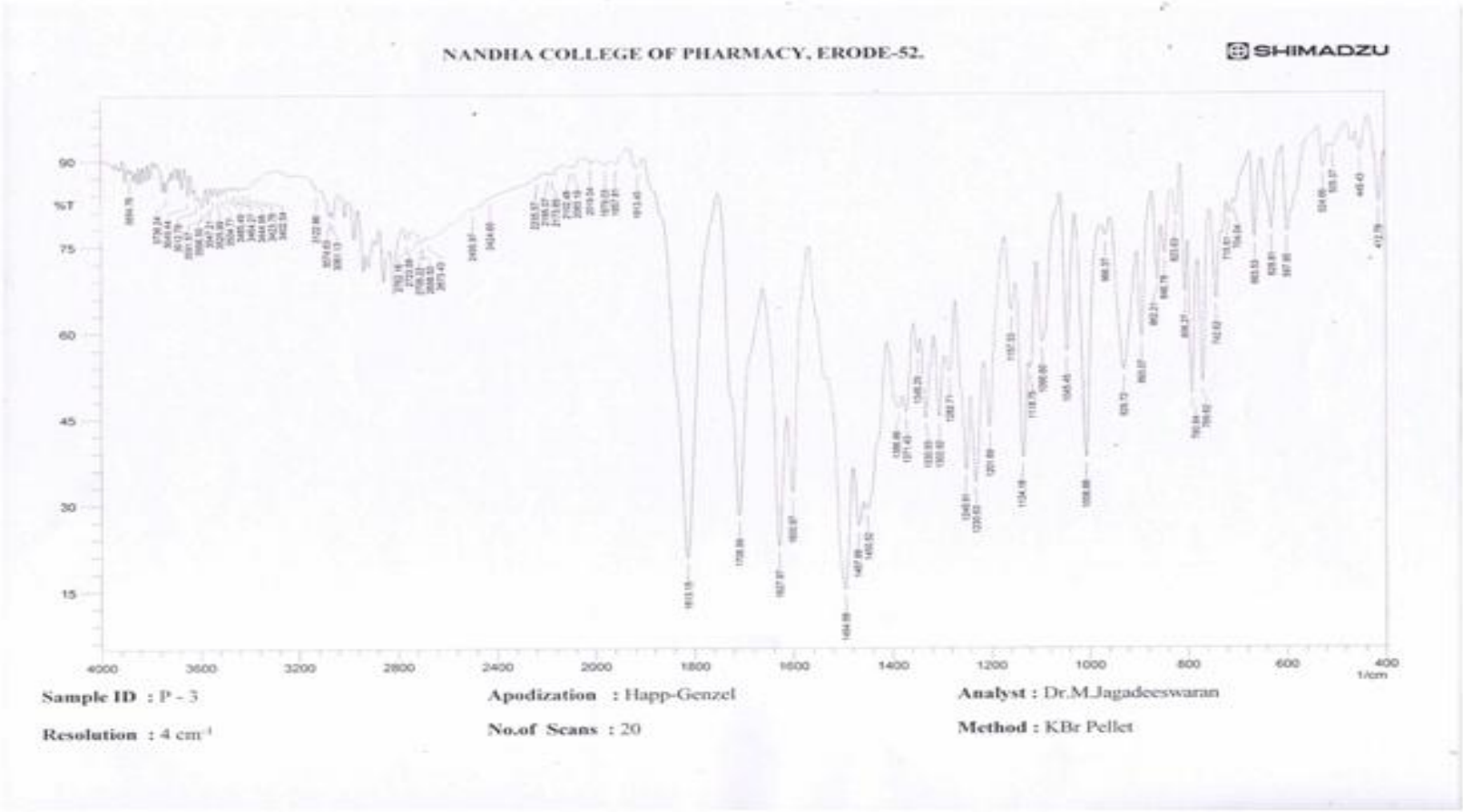



**Fig. 10: FT-IR spectra of P-III**

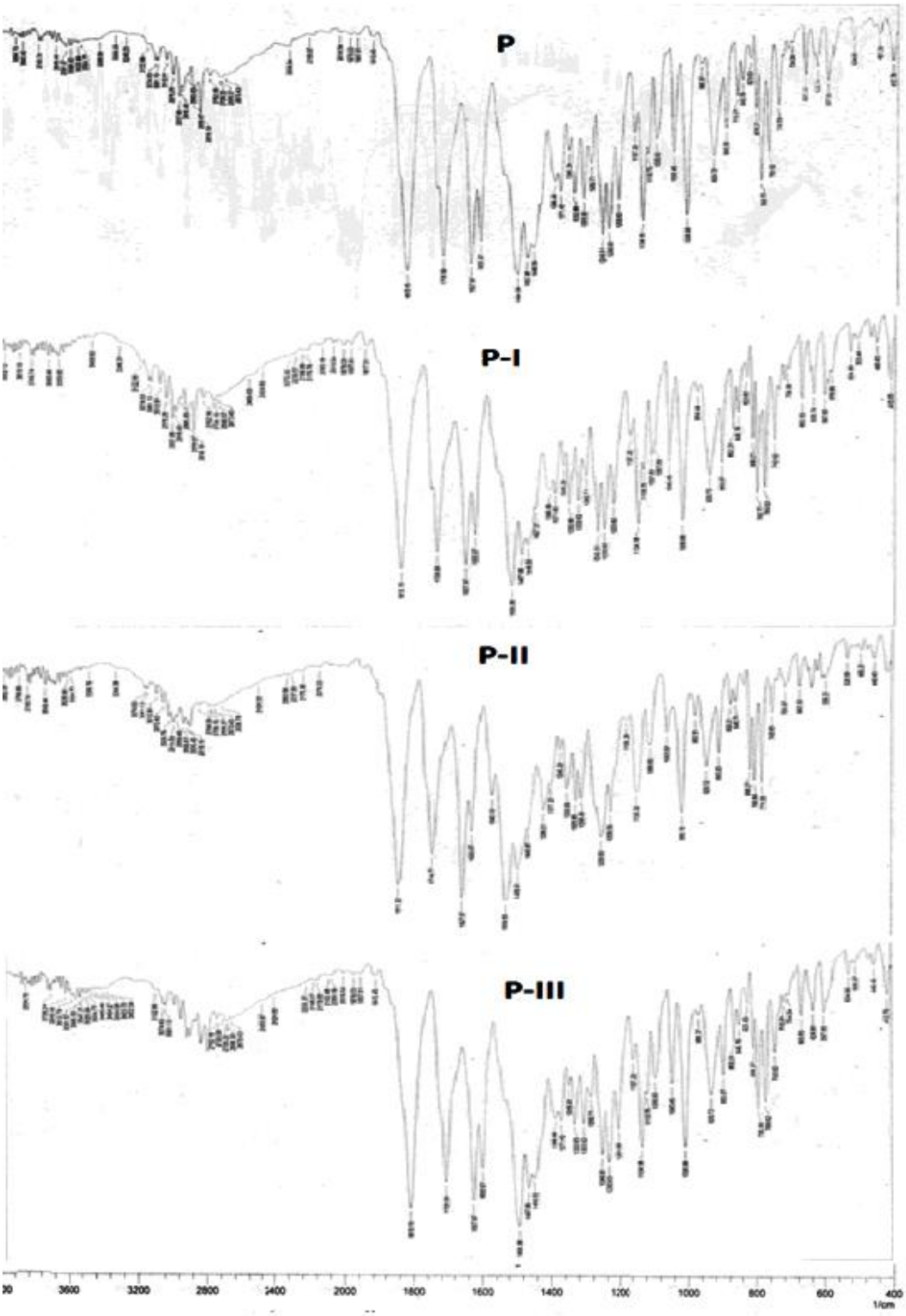



**Fig. 11: Superimposed IR spectrum of P, P-I, P-II and P-III**

### 6.1.1.5 Differential Scanning Calorimetry (DSC) [132]

The DSC study was carried out to study compatability or any other interaction of drug and solvent after the formation of crystal. The DSC thermograms were showed in Fig. 12 for pure drug of prulifloxacin and in Fig. 13-16 for prepared crystals. The DSC curves of all prepared crystal form showed endothermic peaks at 224.01°C (103.5 J/g), 226.43°C (216.6 J/g), 222.33°C (90.34 J/g) and 224.91°C (108.7 J/g) for pure form of prulifloxacin (P), crystals of P-I, P-II and P-III respectively. The exothermic peaks were obtained at 268.37°C, 266.78°C, 267.74°C, and 265.33°C for P-0, P-I, P-II and P-III respectively. From endothermic peaks obtained in the DSC curves of crystalline forms of prulifloxacin, P-III was having similar melting point of pure drug, where as P-II was showing fusion point at 222.33ºC indicates that it may be meta stable polymorph. Higher melting point (from endothermic peak) of the P-I form of crystal indicated that it may be more stable polymorph than other forms.

**Table 8: DSC data of prulifloxacin (P) and its crystals**

| S.No | Crystal Form | Solvent used | DSC data | |
|---|---|---|---|---|
| | | | Peak fusion point (°C) | Heat of diffusion (J/g) |
| 1. | Prulifloxacin (P) | Pure drug | 224.01 | 103.5 |
| 2. | P-I | Acetonitrile | 226.44 | 216.6 |
| 3. | P-II | Acetone | 222.33 | 90.34 |
| 4. | P-III | Dichloromethane | 224.91 | 108.7 |

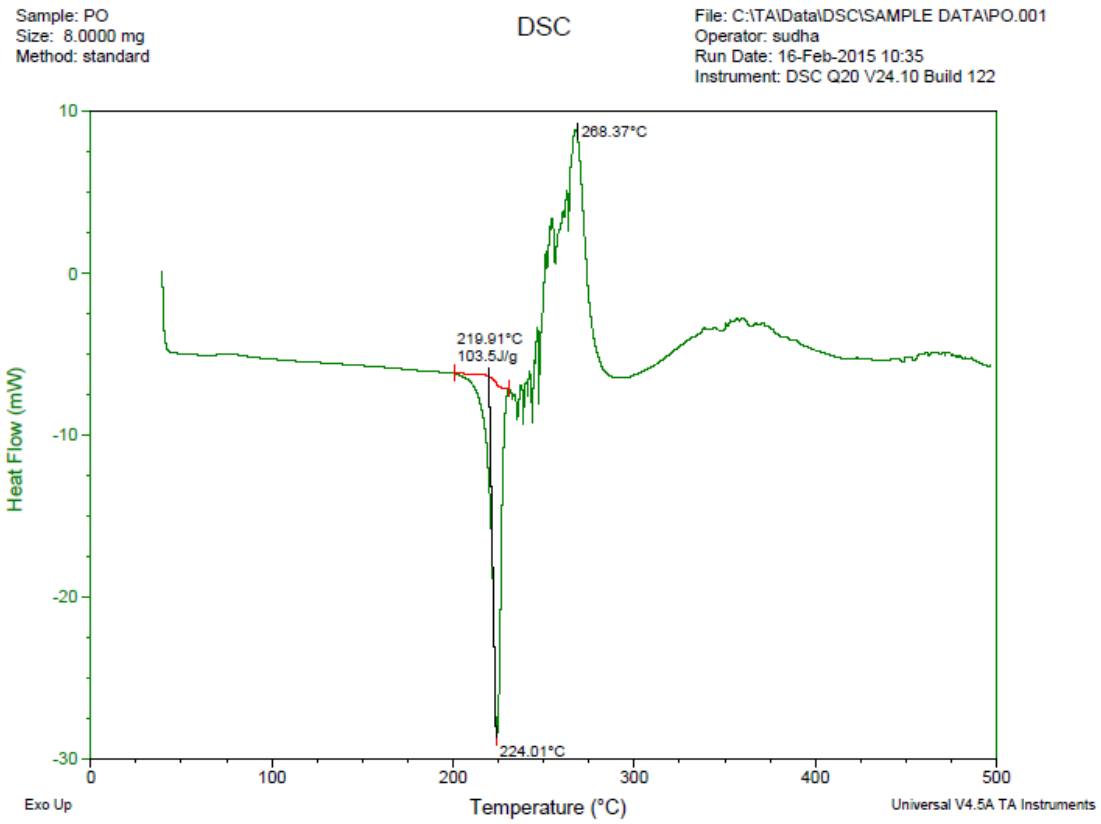



**Fig. 12: DSC thermogram of prulifloxacin (P)**

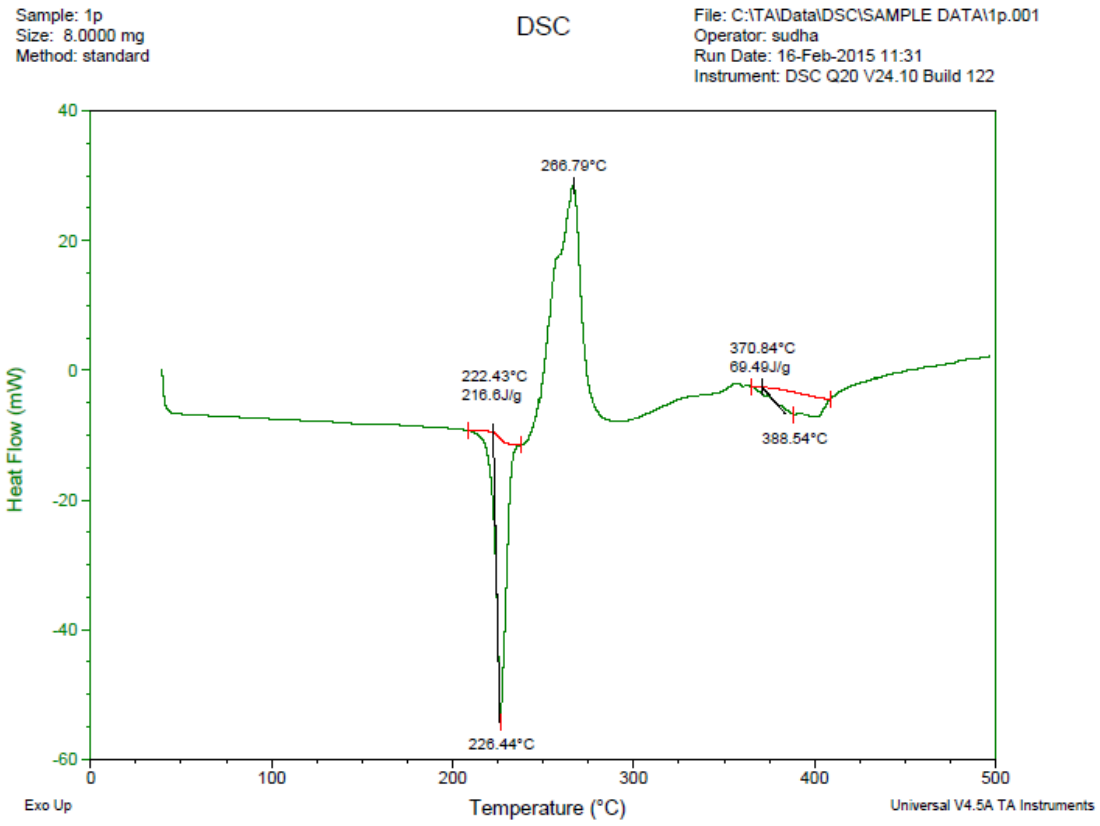



**Fig.13: DSC thermogram of P-I**

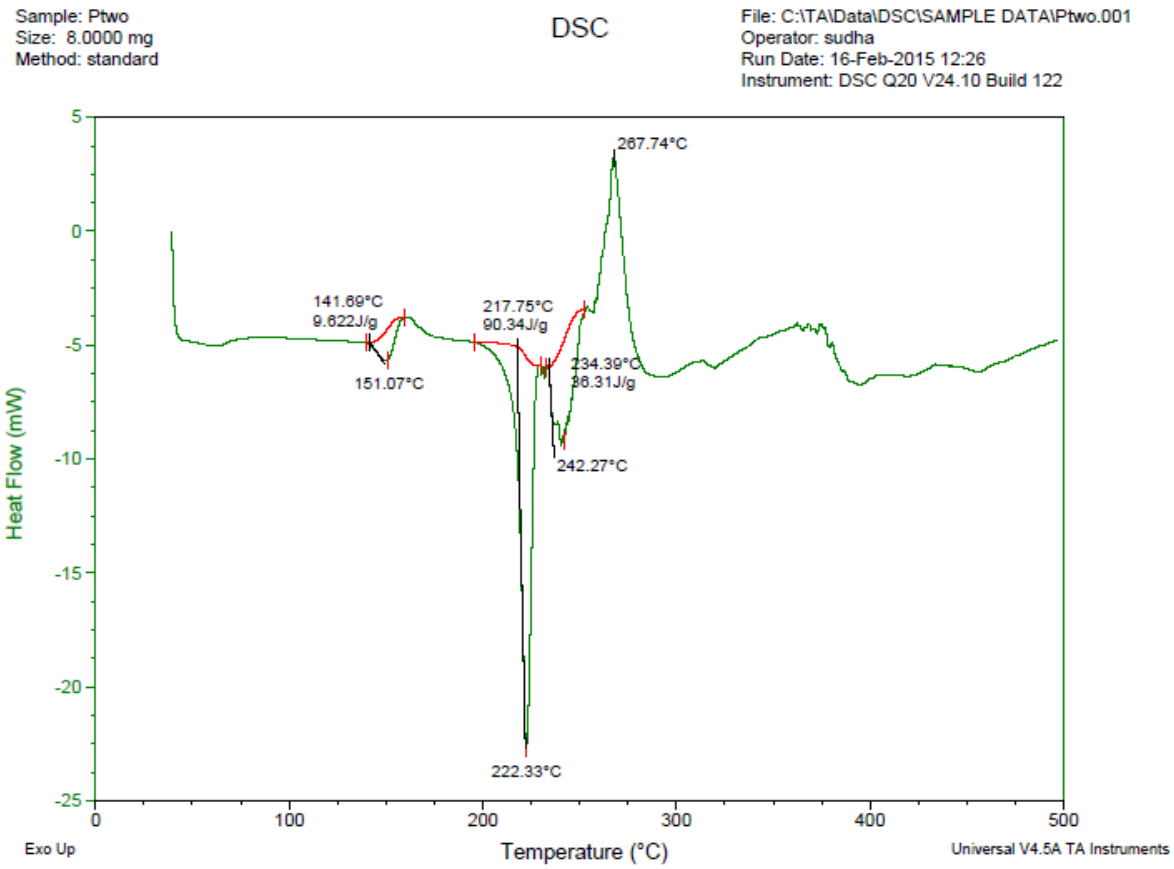



**Fig. 14: DSC thermogram of P-II**

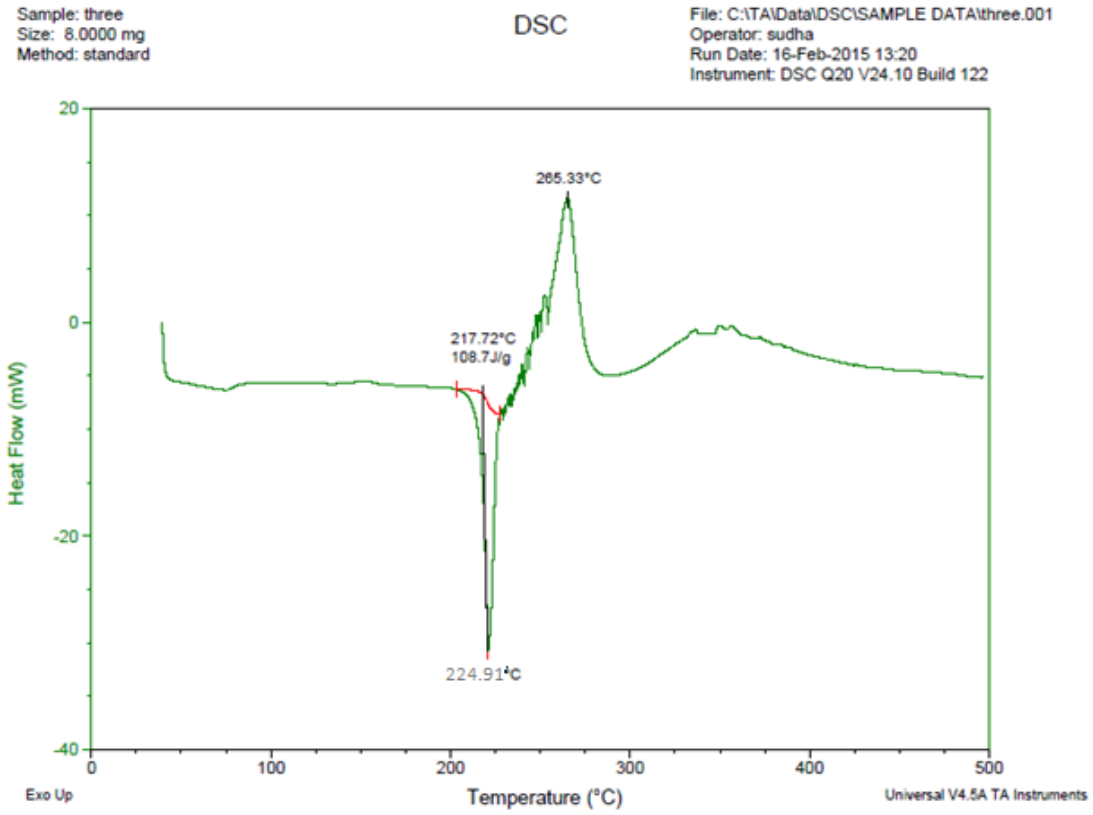



**Fig. 15: DSC thermogram of P-III**

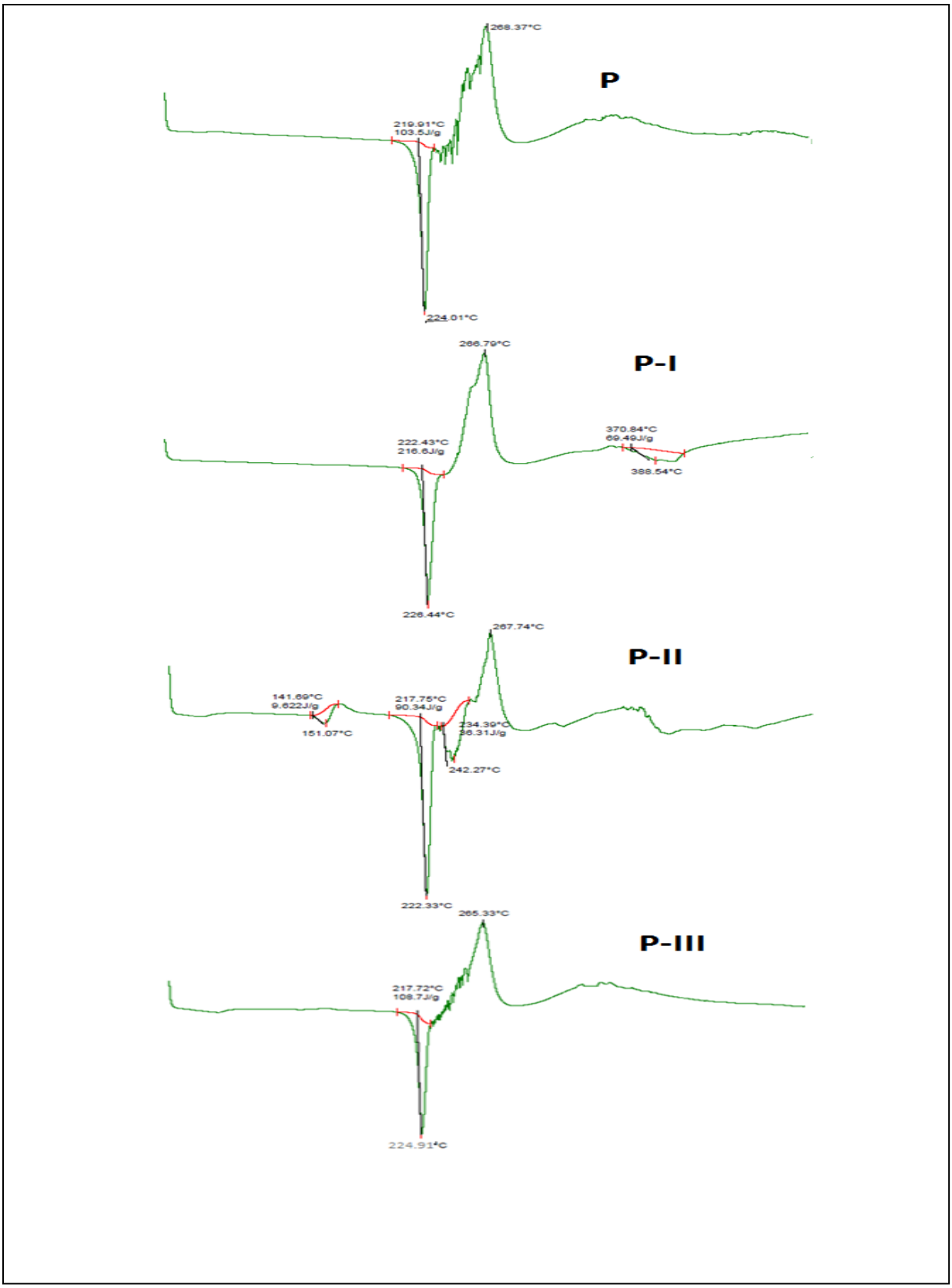



**Fig. 16: Superimposed DSC Spectra of P, P-I, P-II and P-III**

### 6.1.1.6 Powder X-Ray Diffraction (PXRD) [133]

Considering powder X-ray diffraction to be the ideal technique for characterizing polymorphs, all the crystal forms were subjected for PXRD studies. This XRD spectrum (Fig.17-21) showed different distinction in the position of the peak, clearly indicating different crystal lattice and data were tabulated in Table 9. The presence of new peaks at 11.90°, 31.00° and 51.01 °2 theta in P-I and also appearance of high intensity peak at 17.73 º2 theta made it different from the commercial sample. High intensity line at 23.69º 2 theta was present in pure form but, less intensity peaks only appeared in all the prepared crystal forms. P-II intense peak at 13.89º, 17.48º, 23.46 º2 theta and these peaks were absent in remaining crystals forms. Peaks obtained at 11.60, 20.78 and 27.76 º2 theta in pure form and 11.70, 20.68 and 27.60 º2 theta in P-III form showed approximately similar intensity. Based on these report, P-III form may have similar PXRD pattern with that of pure form of drug. Besides the difference in the position of 2θ values, the peak intensity counts were also different in all the forms. All these three forms showed well resolved diffraction patterns with various characteristic peaks, hence it was confirmed that the polymorph of prulifloxacin P-I, P-II and P-III were properly obtained. Appearance and disappearance of some peaks on the XRD spectrum represented the complete formation of crystals of P-I, P-II and P-III with different crystal lattice which was successively achieved by cooling crystallization method.

**Table 9: PXRD data for prulifloxacin (P), P-I, P-II and P-III**

| P-0 | | P-I | | P-II | | P-III | |
|---|---|---|---|---|---|---|---|
| **2θ** | **I%** | **2θ** | **I%** | **2θ** | **I%** | **2θ** | **I%** |
| **11.60** | **221** | **11.90** | **303** | **11.20** | 40 | **11.70** | **193** |
| 12.20 | 613 | 12.40 | 301 | 11.50 | 84 | 12.11 | 460 |
| 13.80 | 68 | 14.11 | 163 | **13.89** | **450** | 14.06 | 216 |
| 15.29 | 160 | 15.18 | 52 | 15.10 | 57 | 16.02 | 338 |
| 18.66 | 198 | **17.73** | **1379** | **17.48** | **381** | 18.57 | 80 |
| **20.78** | **176** | 20.90 | 100 | 20.50 | 45 | **20.68** | **238** |
| 22.46 | 44 | 22.90 | 273 | 22.78 | 179 | 22.90 | 415 |
| **23.69** | **1221** | **23.72** | **579** | **23.46** | **327** | **23.50** | **682** |
| **27.76** | **212** | 27.72 | 71 | 27.30 | 73 | **27.60** | **117** |
| 30.63 | 171 | **31.00** | **43** | 30.33 | 59 | 31.20 | 25 |
| 33.96 | 173 | 33.89 | 127 | 33.00 | 26 | 33.77 | 112 |
| 37.80 | 73 | 37.82 | 114 | 37.56 | 38 | 38.92 | 25 |
| 39.18 | 45 | 40.43 | 47 | 38.00 | 18 | 40.21 | 44 |
| 43.70 | 44 | 44.82 | 43 | 43.40 | 23 | 43.61 | 44 |
| 45.07 | 55 | 45.78 | 82 | 45.78 | 24 | 45.61 | 36 |
| 49.86 | 40 | **51.01** | **104** | 49.03 | 18 | 49.75 | 31 |
| 55.42 | 40 | 55.32 | 46 | 54.73 | 19 | 50.95 | 25 |
| 58.42 | 59 | 58.46 | 66 | 58.19 | 23 | 58.24 | 41 |
| 77.50 | 64 | 77.44 | 55 | 77.38 | 119 | 77.44 | 94 |

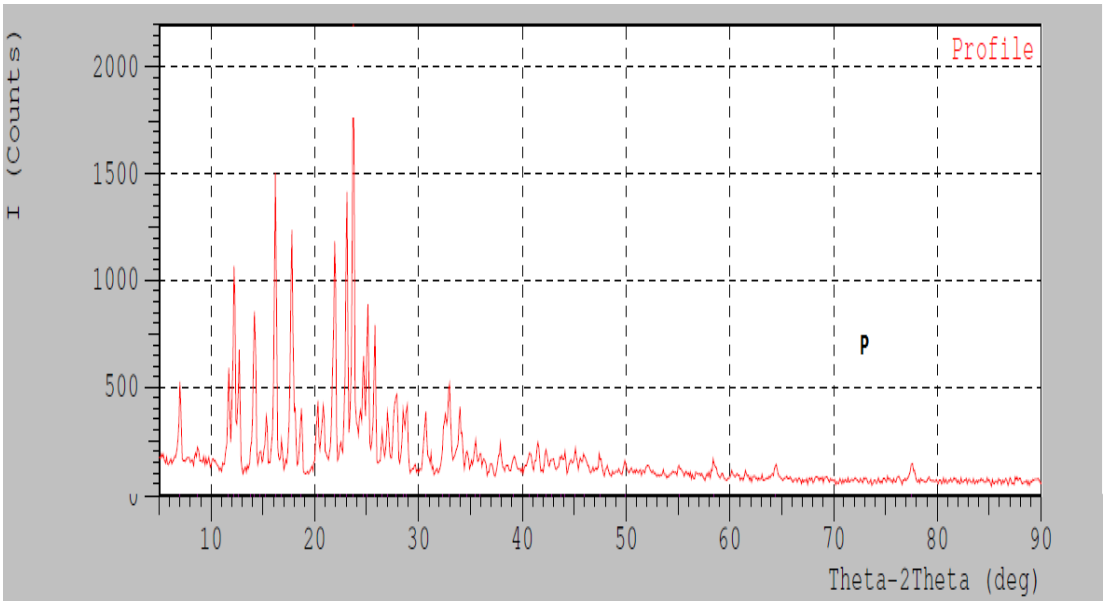



**Fig. 17: PXRD spectra of prulifloxacin (P)**

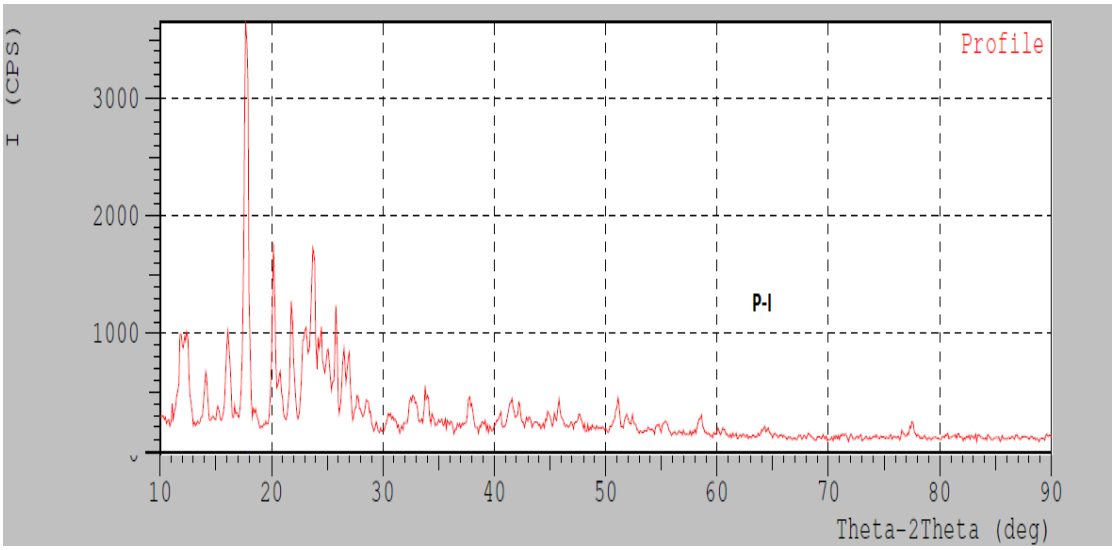



**Fig. 18: PXRD spectra of P-I**

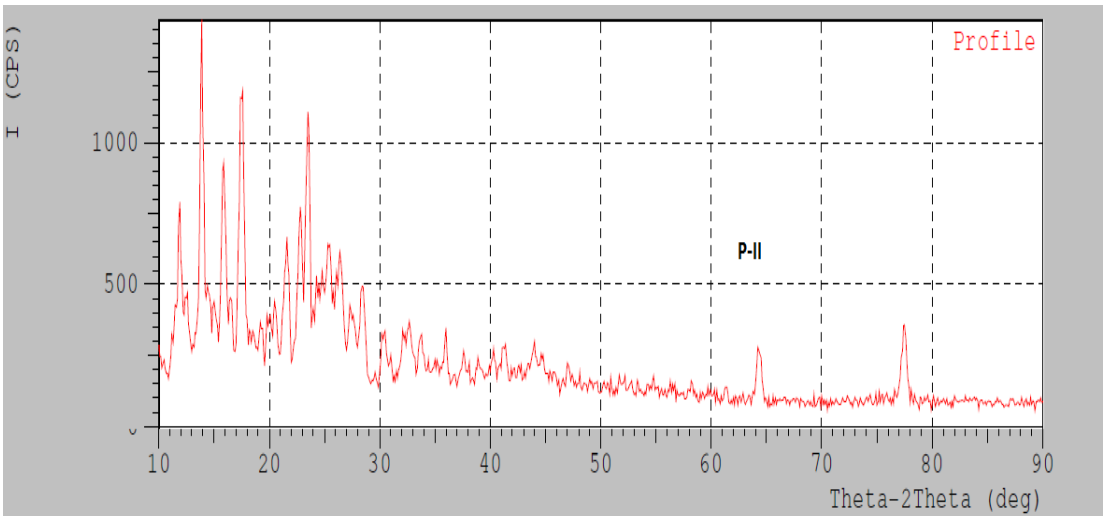



**Fig. 19: PXRD spectra of P-II**

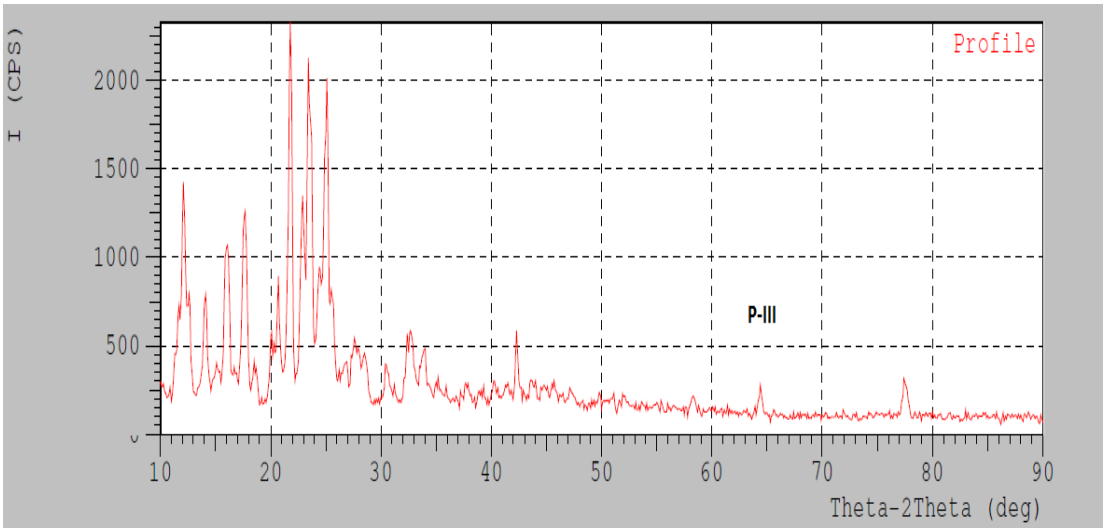



**Fig. 20: PXRD spectra of P-III**

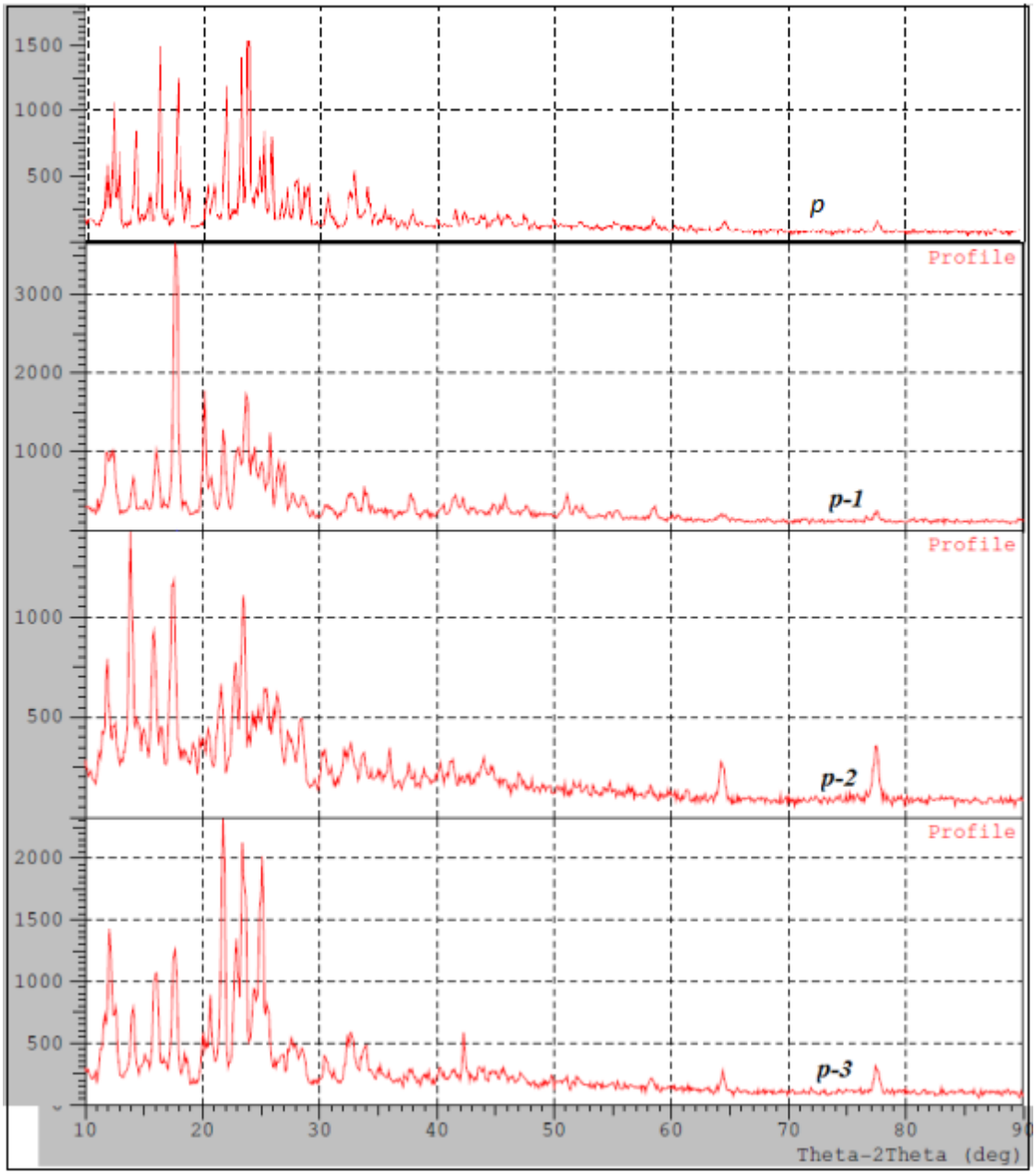



**Fig. 21: Superimposed PXRD Spectra of P, P-I, P-II and P-III**

### 6.1.2 *IN-VITRO* DISSOLUTION AND SOLUBILITY STUDIES

#### 6.1.2.1 Determination of absorption maxima for prulifloxacin

A fourth generation fluoroquinolone, prulifloxacin was selected as target drug for this present work due to its poor aqueous solubility and poor drug absorption and bioavailability. Characterization of its pure drug was done by measuring its melting point and maximum absorbance (λ max) of drug in UV-Visible spectroscopy. The λ max of prulifloxacin was analysed by suitably diluting the drug in phosphate buffer pH 6.8 and it observed at **272 nm** (Fig.22).

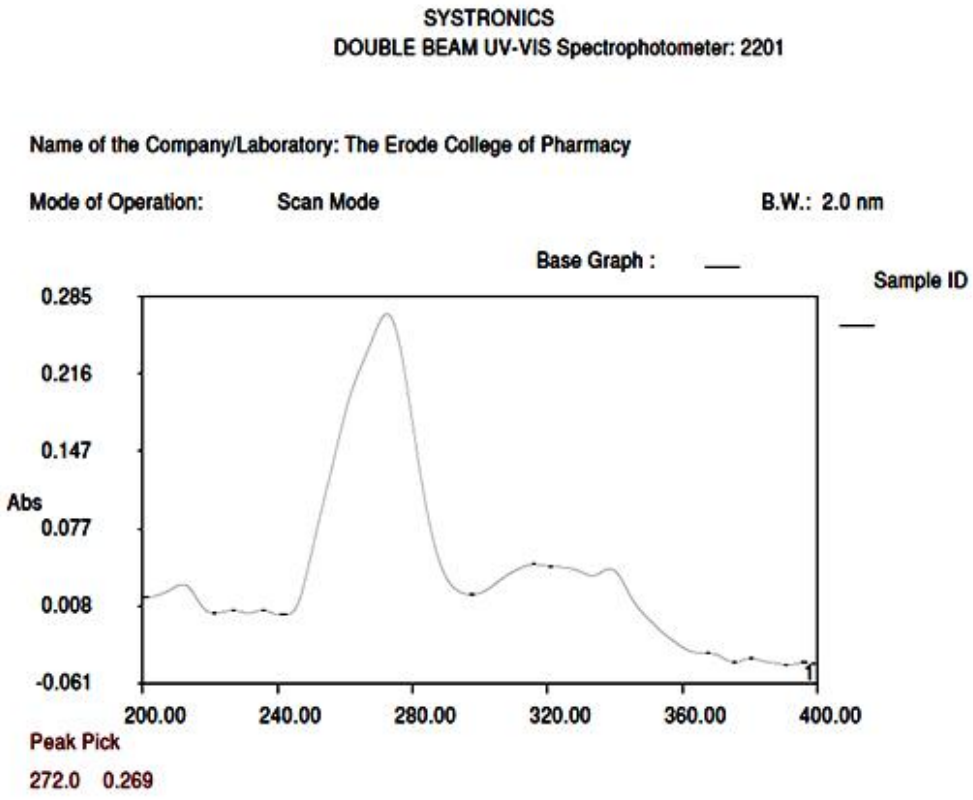



**Fig. 22: Determination of λ $_{max}$ graph for prulifloxacin**

### 6.1.2.2 Preparation of standard curve

A standard calibration curve (Fig.23) of prulifloxacin was constructed using same solvent and it obeyed Beers –Lambert's law in the concentration range of 5 – 50 μg/ml with good linearity. The results were tabulated in Table 10. The above curve showed the regression equation of y = 0.0201x + 0.0094 with correlation coefficient ($R^2$ = 0.9989) as nearer to one which indicated good linearity.

**Table 10: Data for standard curve of prulifloxacin in pH 6.8**

| S.No. | Concentration (mcg/ml) | Absorbance (at 272 nm)* |
|---|---|---|
| 1 | 5 | 0.104 |
| 2 | 10 | 0.212 |
| 3 | 15 | 0.319 |
| 4 | 20 | 0.410 |
| 5 | 25 | 0.518 |
| 6 | 30 | 0.609 |
| 7 | 35 | 0.713 |
| 8 | 40 | 0.832 |
| 9 | 45 | 0.915 |
| 10 | 50 | 0.989 |

(*n=3)

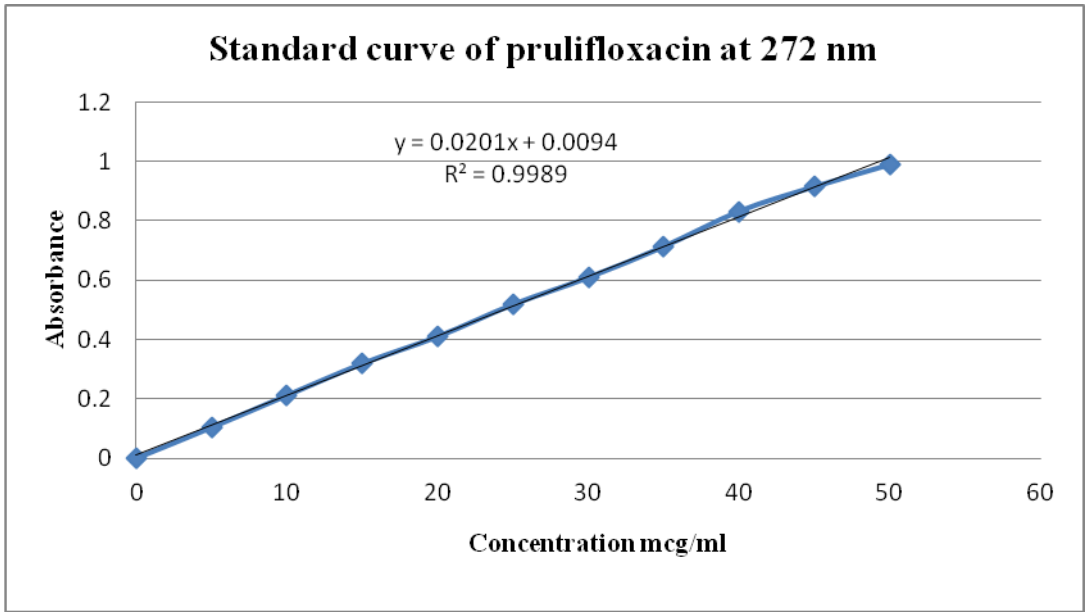



**Fig. 23: Standard curve for prulifloxacin**

### 6.1.2.3 Solubility assessment

Solubility of pure drug was found to be 0.45 mg/ml. Improvement in solubility was observed with crystal form of P-II, where as P-I and P-III form showed least solubility than P-II. Solubility of crystal forms follows in the order of P-II>P-I>P-III and the results for solubility measurement were tabulated in Table 11.

**Table 11: Solubility studies of crystals and pure drug**

| S.No. | Crystal Forms | Solubility (mg/ml) |
|---|---|---|
| 1 | Pure | 0.45 |
| 2 | P-I | 0.65 |
| 3 | P-II | 0.85 |
| 4 | P-III | 0.50 |

### 6.1.2.4 *In-vitro* dissolution studies[125,134]

The dissolution profile of all the three crystalline forms and pure form were studied in pH 6.8 buffer solution. Comparative *in-vitro* drug release graph obtained from dissolution studies were showed in Fig.24 and their results were presented in Table 12 . The result has shown that a marked difference in their dissolution behavior of the crystal form compared to that of pure drug. After 240 min, the cumulative percentage drug release of each form was calculated by the use of calibration graph obtained at **272 nm**. Increasing order of drug release was as followed P<P3<P1<P2, the values of % drug release were 51.06%, 58.40%, 72.80%, and 89.40% respectively. Due to more solubility of P-II form showed highest percentage drug release. From solubility and dissolution studies, crystal form (P-II) obtained from acetone showed highest cumulative percentage drug release when compared to others.

**Table 12: *In-vitro* Dissolution rate profile of crystal forms of prulifloxacin**

| Time ( Min) | Percentage drug release (Mean ± SD*) | | | |
|---|---|---|---|---|
| | **Prulifloxacin (P)** | **P-I** | **P-II** | **P -III** |
| 15 | 5.20±0.21 | 19.0±0.16 | 33.80±0.14 | 8.40±0.15 |
| 30 | 11.20±0.26 | 32.0±0.32 | 52.0±0.22 | 17.80±0.21 |
| 45 | 25.60±0.16 | 39.60±0.31 | 60.80±0.32 | 23.60±0.12 |
| 60 | 33.0±0.11 | 52.20±0.18 | 69.0±0.17 | 29.40±0.14 |
| 120 | 47.0±0.28 | 66.60±0.15 | 82.80±0.28 | 47.0±0.22 |
| 240 | 51.06±0.21 | 72.80±0.11 | 89.40±0.23 | 58.40±0.44 |
| * All values represent mean ( n = 4) ± SD | | | | |

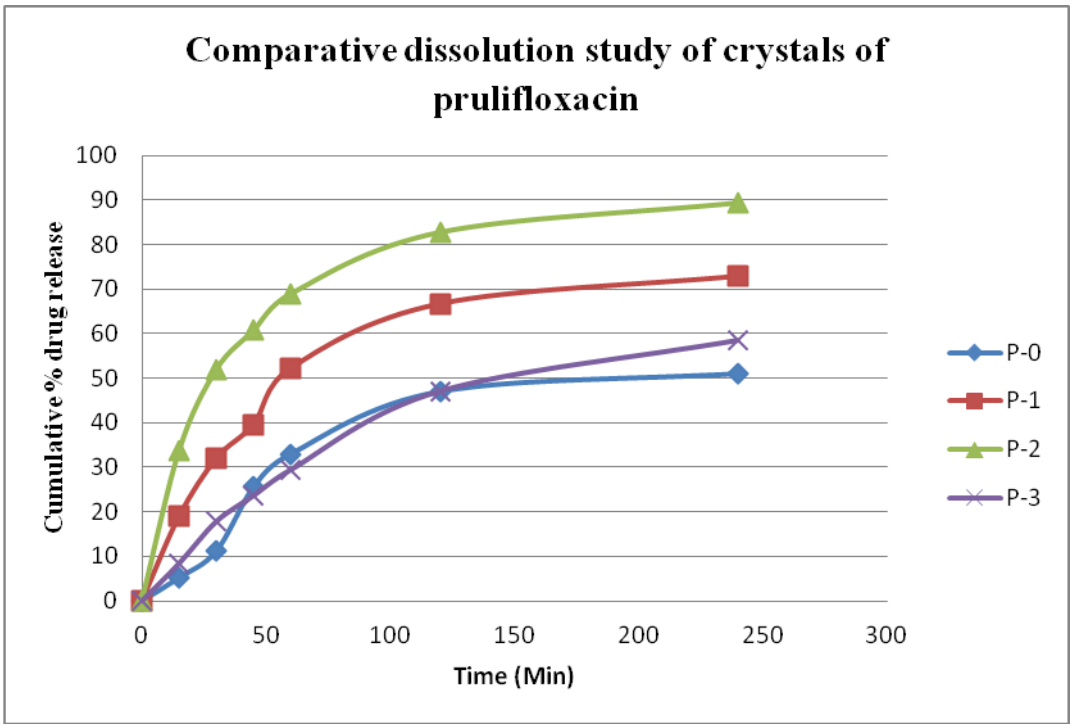



**Fig. 24: *In-vitro* Dissolution rate for crystal forms of prulifloxacin**

### 6.1.2.5 Drug content

The drug content was found to be good and uniform among the different batches of crystals prepared and ranged from 98.07 to 99.76 %. The results obtained from drug content determination were represented in Table 13.

**Table 13: Percentage drug content for prepared crystal forms**

| S.No. | Crystal Forms | Drug content (%)* |
|---|---|---|
| 1 | P-I | 98.07±2.21 |
| 2 | P-II | 99.76±1.24 |
| 3 | P-III | 99.15±1.41 |

(n=3)

### 6.1.2.6 Stability studies [75]

All the crystals were screened for accelerated stability studies and did not show any physical changes during the study period. The drug content were observed (n=3) for all the crystals (Table 14) which were quite stable at accelerated storage conditions. The stability of each crystalline form was proved by determining the percentage content under the above said accelerated storage condition. Values nearly 100 % indicated that all the polymorphic forms were stable without any alteration on the physical characters.

**Table 14: Stability study data for crystal forms of prulifloxacin**

| Crystal form | Drug content *(%) | | |
|---|---|---|---|
| | After 30 days | After 60 days | After 90 days |
| P-I | 99.35±0.2291 | 98.94±0.4007 | 98.69±0.2651 |
| P-II | 99.42±0.1553 | 98.86±0.1652 | 98.38±0.1997 |
| P-III | 99.52±0.2502 | 98.88±0.4804 | 98.63±0.2022 |

(*n = 3)

### 6.1.3 PHARMACOKINETIC STUDY IN RAT [135]

The pharmacokinetic parameter like maximum plasma concentration ($C_{max}$) and time ($T_{max}$) were visually inspected from the pharmacokinetic profiles. From the *in-vitro* dissolution profile of the pure and all three crystalline forms of prulifloxacin, the P-II form showed highest solubility and percentage drug release which was co-related with *in-vivo* oral bioavailability studies using animal model.

The mean plasma concentration versus time profiles and other pharmacokinetic parameters of pure drug and crystal form P-II after oral administration to Sparque-Dawley rats (male) were determined. For the determination of the plasma drug concentration, a sensitive HPLC method was developed and partially validated by linearity, precision and specificity studies. A chromatographic peak was observed at a retention time of 7.0 ± 0.2 min (Fig. 25) using the mixture of phosphate buffer pH 3.2 & acetonitrile in the ratio of 60:40 v/v using UV absorbance detector at 285 **nm**.

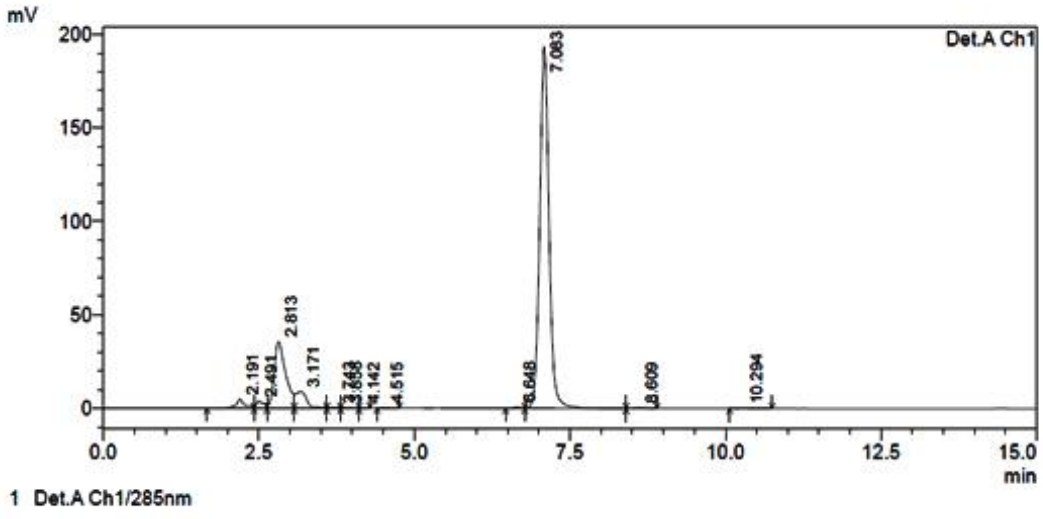



**Fig. 25: HPLC chromatogram for prulifloxacin**

The metabolite of prulifloxacin (ulifloxacin) obeyed linearity in the concentration range from 0.25 µg/ml to 3 µg/ml. The plasma drug concentration of prulifloxacin at predetermined time intervals was calculated from the linearity graph. The drug concentration in rat plasma was determined by extrapolating the linearity graph with peak area of each plasma sample which was collected from Sparque-Dawley rat at above said predetermined time interval (0, 0.5, 1, 2, 4, 8, 12 and 24 hrs). The plasma drug concentration-time curve of prulifloxacin pure drug and crystal form

P-II were presented in Fig. 26-28 and other pharmacokinetic parameters (Table 15) were calculated by non compartmental model using PK solution 2.0 software.

**Table 15: Pharmacokinetic parameters of prulifloxacin (pure & P-II)**

| S.No. | PK parameters | Prulifloxacin(P) | Crystal (P-II) |
|---|---|---|---|
| 1 | $C_{max}$ | 1.54±1.1 µg/ml | 1.88±1.2 µg/ml |
| 2 | $T_{max}$ | 1 hr | 1 hr |
| 3 | Half life ($T_{1/2}$) | 9.62±1.8 hr | 9.28±1.2 hr |
| 4 | Elimination rate constant | 0.072±0.16 $hr^{-1}$ | 0.074 ±0.26 $hr^{-1}$ |
| 5 | AUC $_{(0-t)}$ | 13.30±3.2 µg.hr/ml | 14.43±3.8 µg.hr/ml |
| 6 | AUC $_{(\alpha)}$ | 13.58 ±3.7µg.hr/ml | 14.70±3.6 µg.hr/ml |

All the values were expressed as mean ± S.D. (n=6)

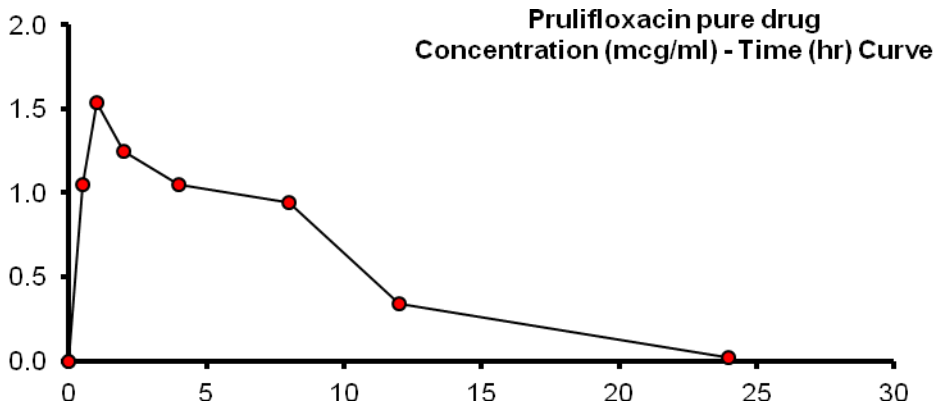



**Fig. 26: Mean plasma concentration Vs time curve of prulifloxacin (P)**

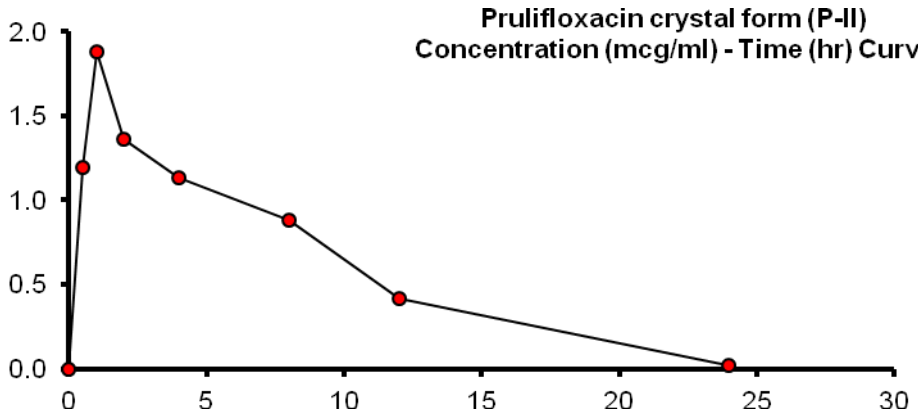



**Fig. 27: Mean plasma concentration Vs time curve of P-II**

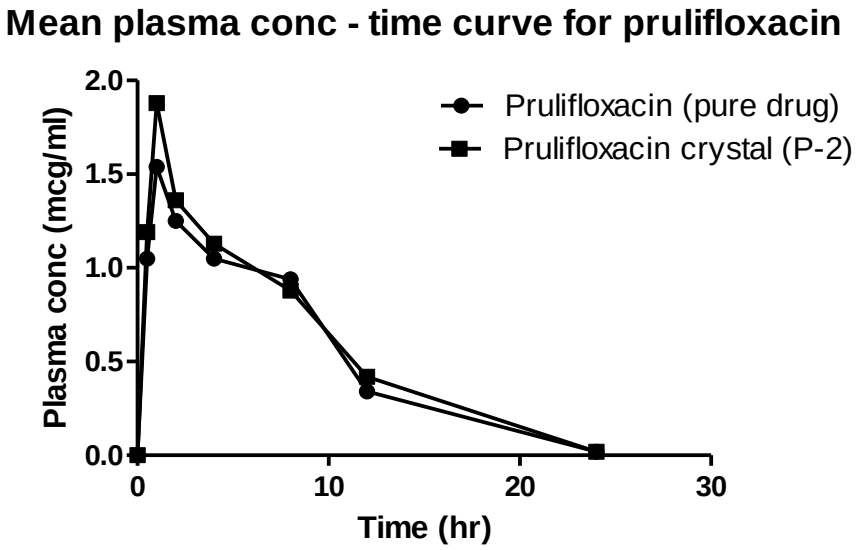



**Fig. 28: Plasma conc-time curve after oral administration of prulifloxacin (P) and P-II crystal form**

Significant differences were observed among these two forms, peak plasma concentration of crystalline form was higher ($C_{max}$ 1.88 µg/ml) when compared to pure drug with approximately similar $T_{max}$ of 1 hr. Comparison of AUC $_{(0-\infty)}$ of pure drug with crystal P-II showed that higher serum level of prulifloxacin was achieved in the group of animal which was administered with P-II crystal form which indicated

about the higher bioavailability than pure form. The apparently longer half life ($T_{1/2}$) observed for pure form (9.62 hr) after oral administration indicated that it had prolonged absorption due to low aqueous solubility when compared to crystalline form. The above observation from pharmacokinetic studies was consistent with the result of *in-vitro* dissolution studies. The dissolution property (highest percentage drug release for P-II form) of prulifloxacin crystal should have a considerable impact on their blood plasma concentration level.

## 6.2 GEMIFLOXACIN MESYLATE

### 6.2.1 PREPARATION AND CHARACTERIZATION OF CRYSTAL FORMS

#### 6.2.1.1 Preparation of crystal forms from different solvents[8]

Gemifloxacin was procured as raw material from Yarrow chem. Products, Mumbai and it was used as such for the preparation of crystal forms. Gemifloxacin crystal forms were tried to prepare from different solvents viz: dimethyl formamide, methanol, ethanol, acetonitrile, chloroform, ethyl acetate, acetone, isopropanol, dichloromethane, n-hexane, benzene, toluene, dimethyl sulfoxide and cyclohexane. But the pure drug of Gemifloxacin had shown appreciable solubility only in isopropanol (G-I), chloroform (G-II), dichloromethane (G-III) and benzene (G-IV), these solvents only gave different types of crystals.

#### 6.2.1.2 Characterization of crystal forms

Melting points of all the developed crystal forms of G-I, G-II, G-III and G-IV were found 206ºC, 208ºC, 209ºC and 214ºC respectively. It was found that the melting point of G-I was lower than the pure drug of gemifloxacin (G) 212ºC, hence it may be the meta stable polymorph. The melting point of G-IV was slightly higher than that of pure form. G-II and G-III were similar in their melting point values, 208ºC and 209ºC respectively. The percentage yield of prepared crystal forms such as G-I, G-II, G-III and G-IV were 83.33%, 70.00%, 66.66% and 73.33% respectively (Table 16).

**Table 16: Crystal yield and melting point of crystal forms of gemifloxacin mesylate**

| S.No. | Crystal Forms | Crystal yield (%) | Melting point (°C) |
|---|---|---|---|
| 1 | Gemifloxacin (G) | -- | 212 |
| 2 | G-I | 83.33 | 206 |
| 3 | G-II | 70.00 | 208 |
| 4 | G-III | 66.66 | 209 |
| 5 | G-IV | 73.33 | 214 |

### 6.2.1.3 Scanning Electron Microscopy (SEM) [136]

SEM analysis of pure drug and newly developed crystal forms represented the various shapes of each form with different size and results were shown in Table 17. G-I had thick rod shaped crystals and G-II showed crystals of rectangular shape with smaller size. Plate like crystals and bricks like crystals were obtained for crystal forms of G-III & G-IV. The regularly shaped particles of each forms indicated about the formation of different crystal form. The SEM images for all the obtained crystal forms were shown below in Fig. 29-33.

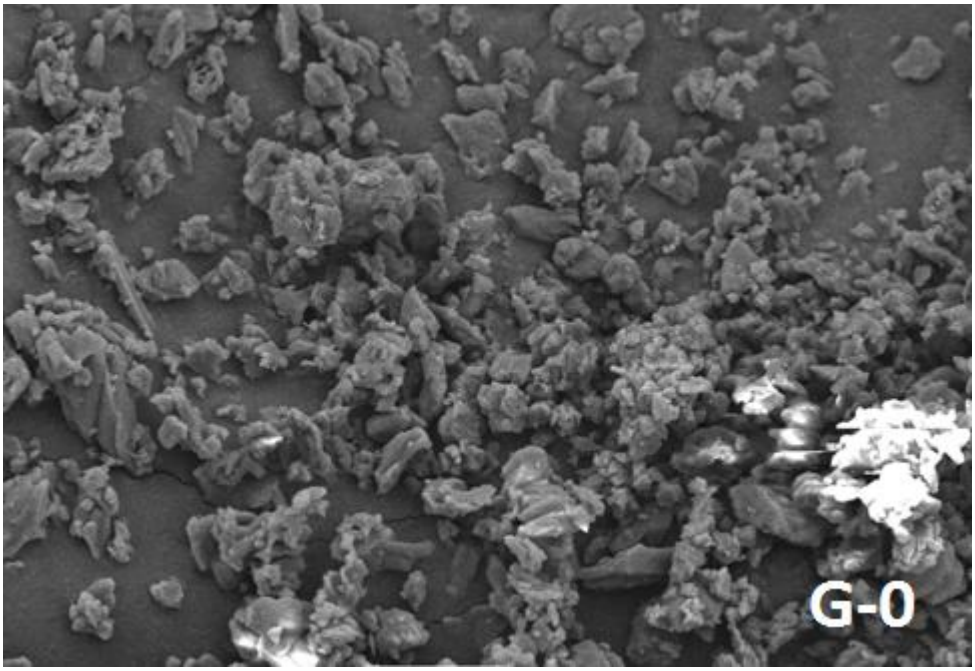



**Fig.29: SEM photograph of gemifloxacin (G)**

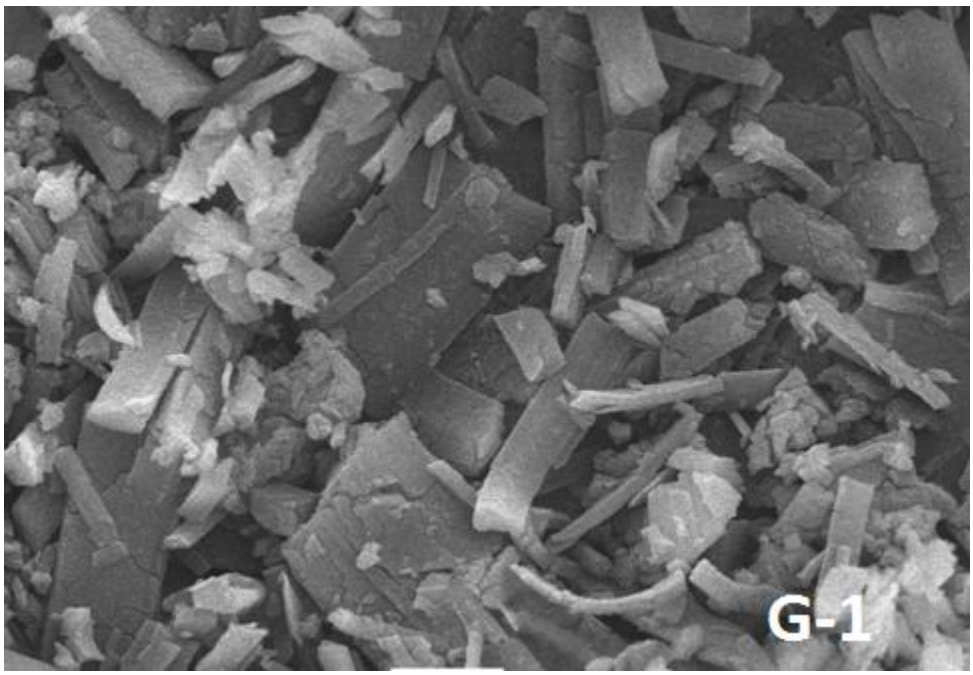



**Fig. 30: SEM photograph of G-I**

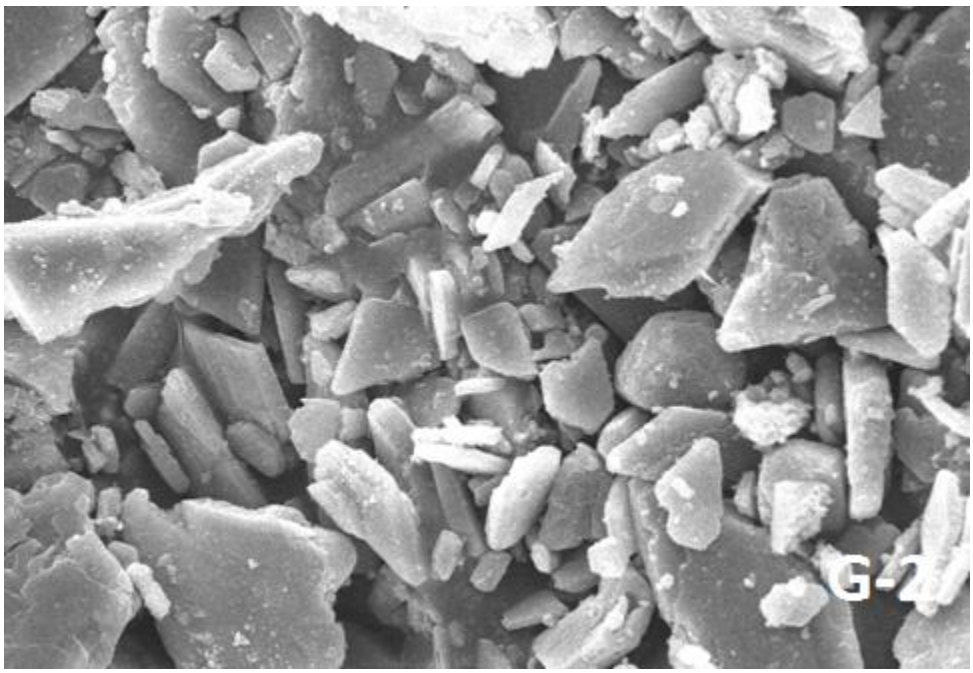



**Fig.31: SEM photograph G-II**

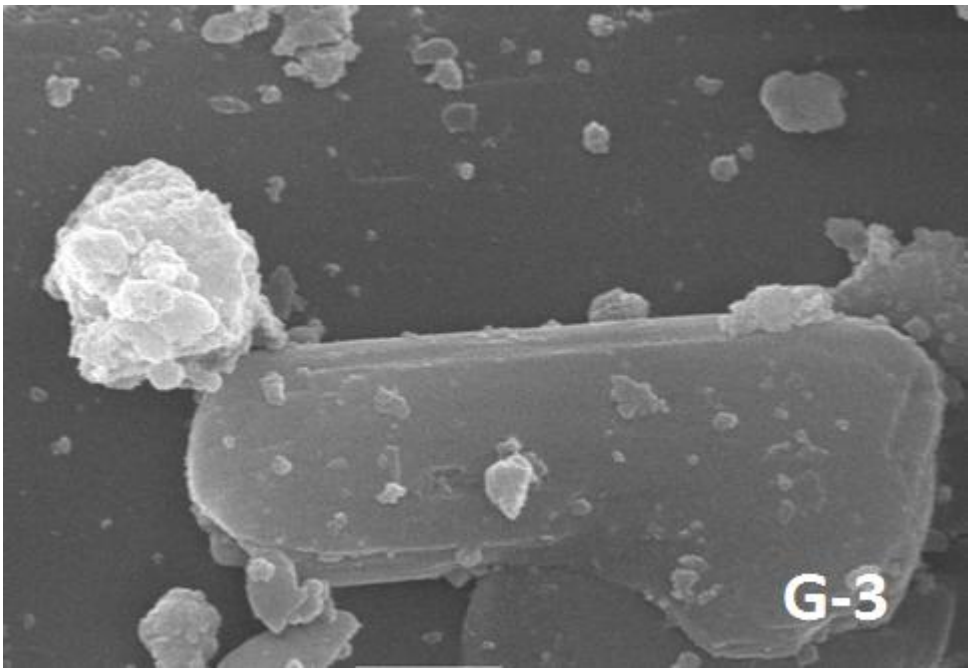



**Fig.32: SEM photograph G-III**

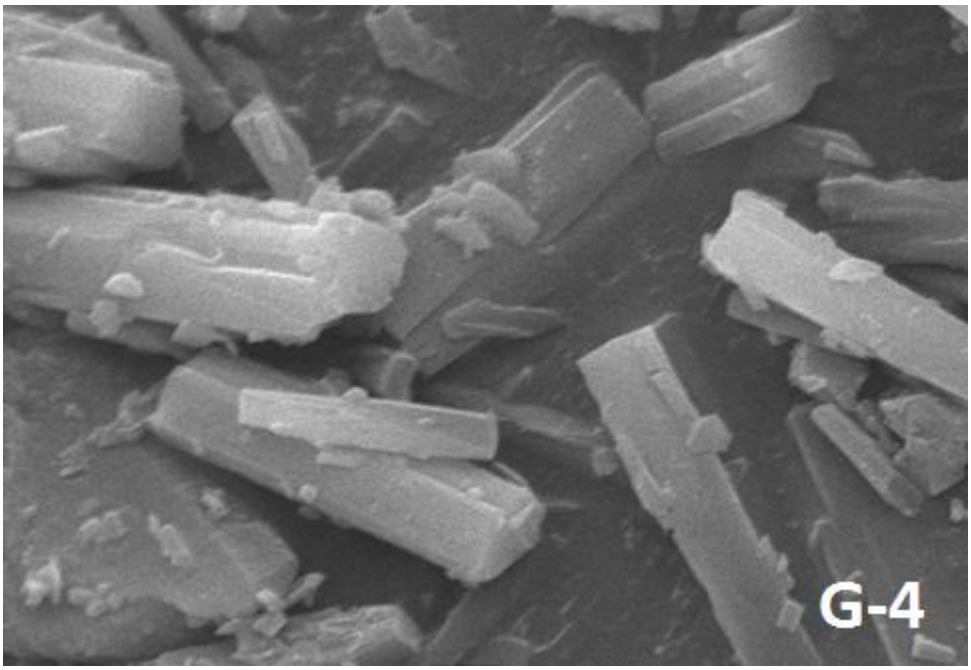



**Fig.33: SEM photograph of G-IV**

**Table 17: SEM analysis of crystal forms**

| S.No. | Crystal Forms | Shape |
|---|---|---|
| 1 | G-I | Thick rod shape |
| 2 | G-II | Rectangular |
| 3 | G-III | Plate like |
| 4 | G-IV | Bricks like |

### 6.2.1.4 FT-IR Spectroscopy [137]

The results of IR spectrum of pure drug and obtained crystal forms revealed that there was no major alteration in the characteristic peaks of functional groups which indicated that there was no interaction between the drug and solvents during the crystallization process. FT-IR spectrum for pure form & crystalline forms of gemifloxacin mesylate was showed in Fig. 34-38 and comparison of their absorption bands were tabulated in Table 18. The frequency range for C-H stretching appeared between 2700-3300 $cm^{-1}$, but the peaks of gemifloxacin (G), G-I, G-II, G-III, G-IV showed at 2702$cm^{-1}$, 2706$cm^{-1}$, 2704$cm^{-1}$, 2704$cm^{-1}$, 2704$cm^{-1}$. The absorption band of N-H bending ranged between1500-1700 $cm^{-1}$. Peaks at 1329 $cm^{-1}$, 1510 $cm^{-1}$, 3254 $cm^{-1}$ appeared in G-I form where as it was shifted to 1400 $cm^{-1}$, 1504 $cm^{-1}$, 3213 $cm^{-1}$ respectively in G-II form. Peaks at 1400 $cm^{-1}$, 1631 $cm^{-1}$, 30219 $cm^{-1}$ appeared in G-III where as it was shifted to 1365 $cm^{-1}$, 1631 $cm^{-1}$, 3228 $cm^{-1}$ respectively in G-IV.

This shifting could not alter the functional group and had not shown the difference in their chemical nature and pharmacological action. Hence, they may be considered as chemically identical.

**Table 18: Interpretations of FT-IR peaks for gemifloxacin mesylate and its crystal forms**

| S.N. | Sample | C-H Stretching ($cm^{-1}$) | C-F Stretching ($cm^{-1}$) | N-H Bending ($cm^{-1}$) | 0-H Stretching ($cm^{-1}$) | C-C Stretching ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | Frequency range for functional groups | 3300-2700 | 1400-1000 | 1700-1500 | 3700-3000 | 1700-1600 |
| 2 | Gemifloxacin mesylate (G) | 2702 | 1365 | 1631 | 3228 | 1631 |
| 3 | G-I | 2706 | 1329 | 1510 | 3254 | 1631 |
| 4 | G-II | 2704 | 1400 | 1504 | 3213 | 1631 |
| 5 | G-III | 2704 | 1400 | 1631 | 3219 | 1631 |
| 6 | G-IV | 2702 | 1365 | 1631 | 3228 | 1631 |

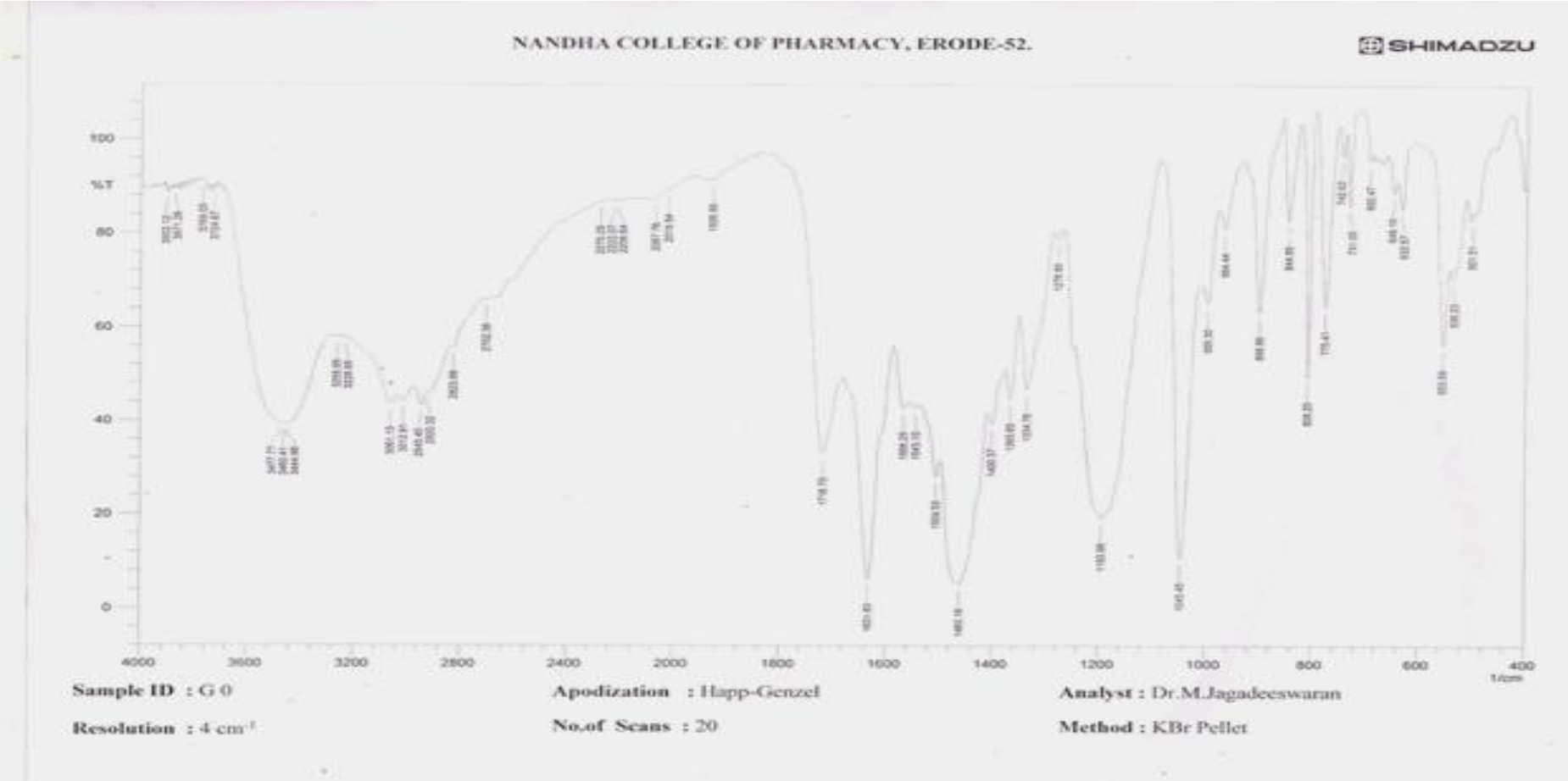



**Fig. 34: FT-IR spectra of gemifloxacin mesylate (G)**

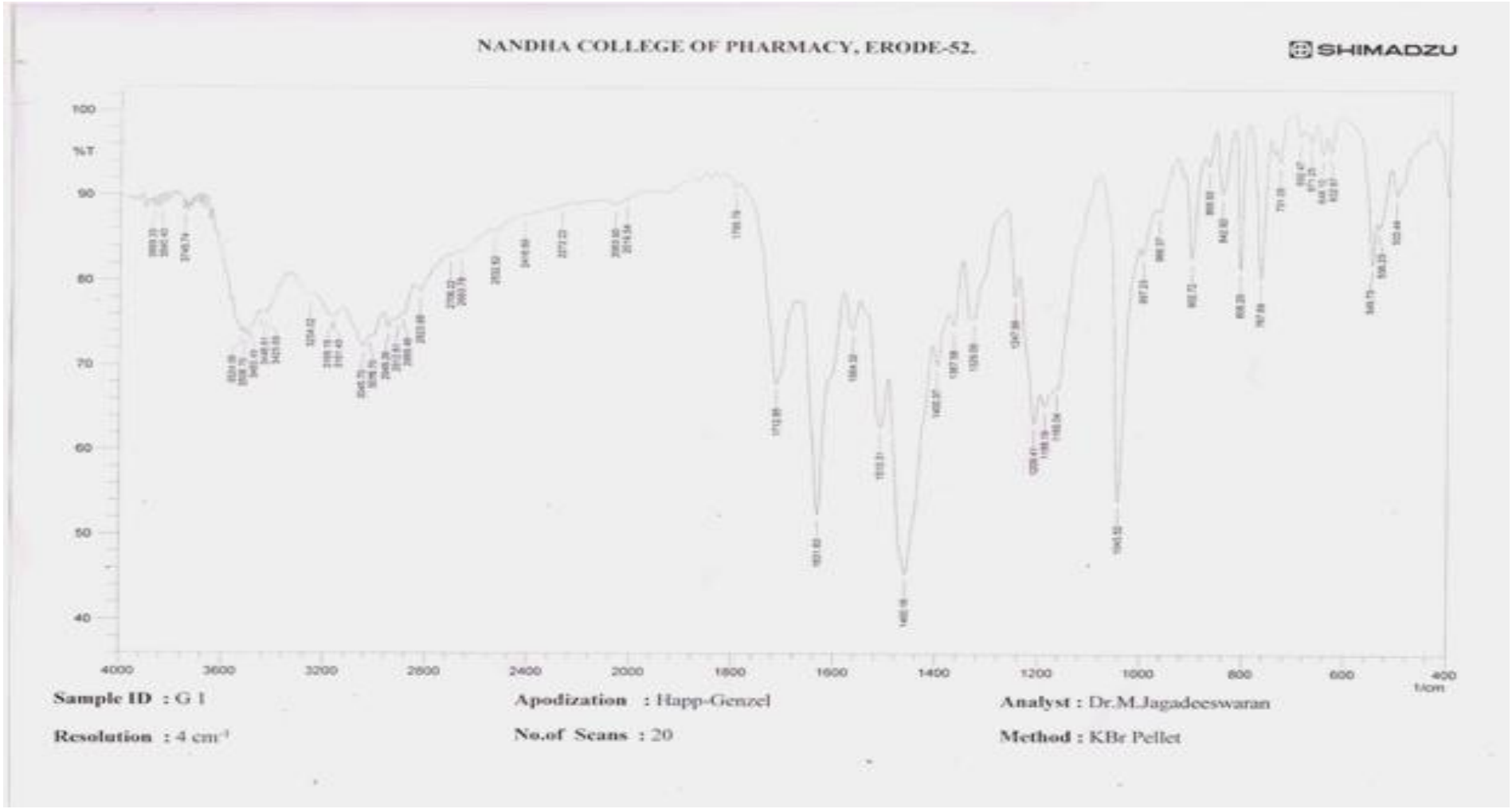



**Fig.35: FT-IR spectra of G-I**

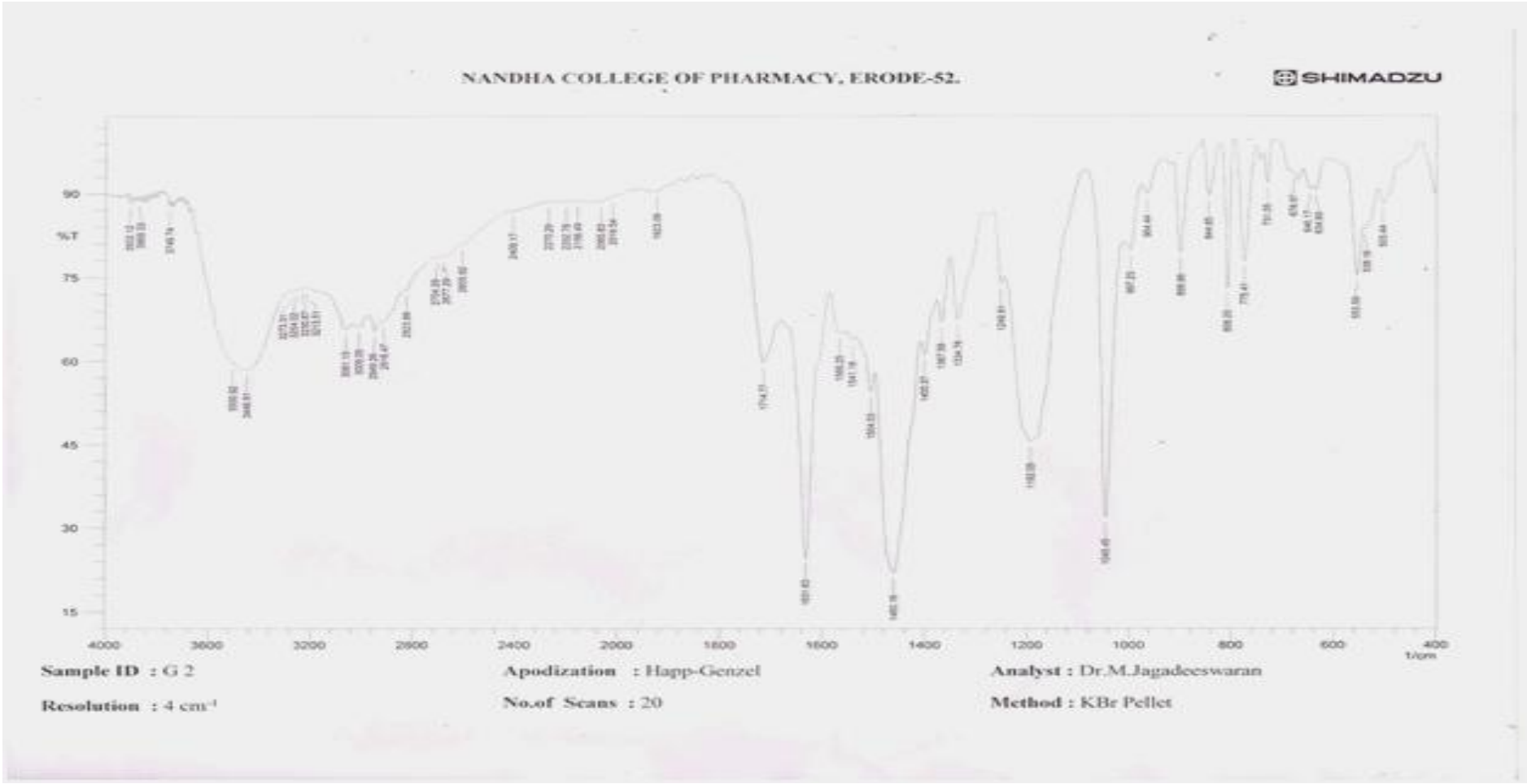



**Fig. 36: FT-IR spectra of G-II**

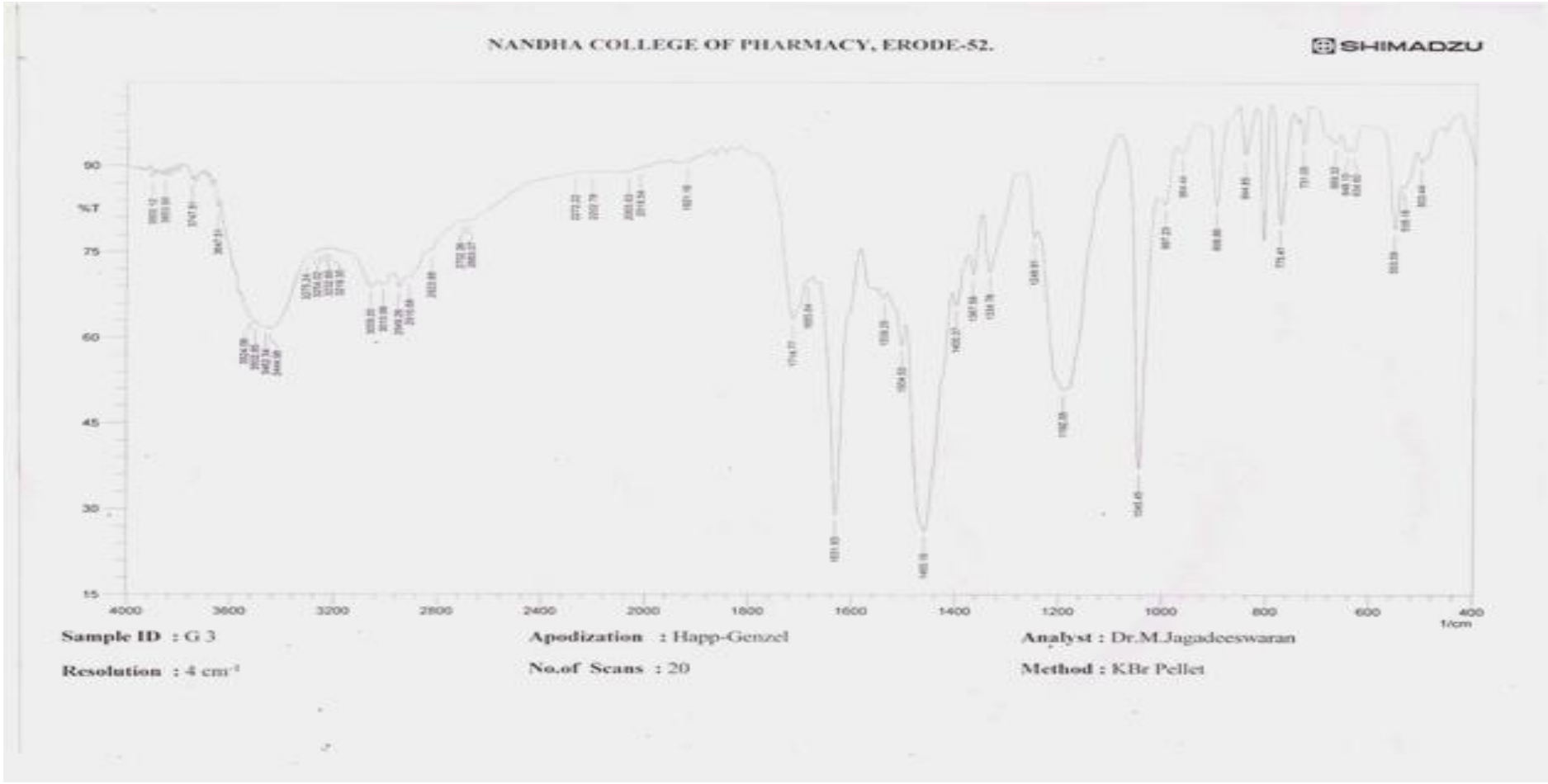



**Fig. 37: FT-IR spectra of G-III**

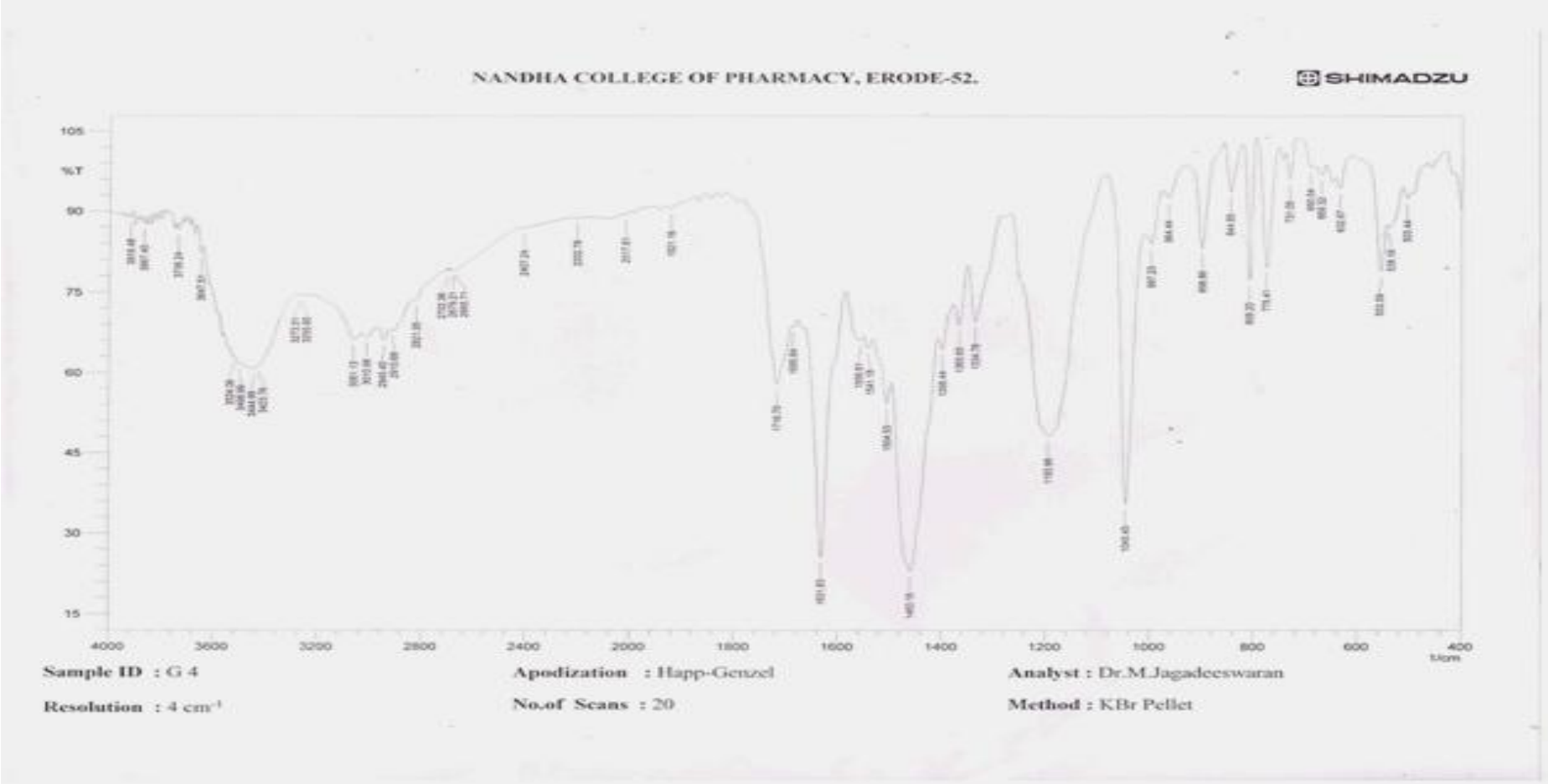



**Fig. 38: FT-IR spectra G-IV**

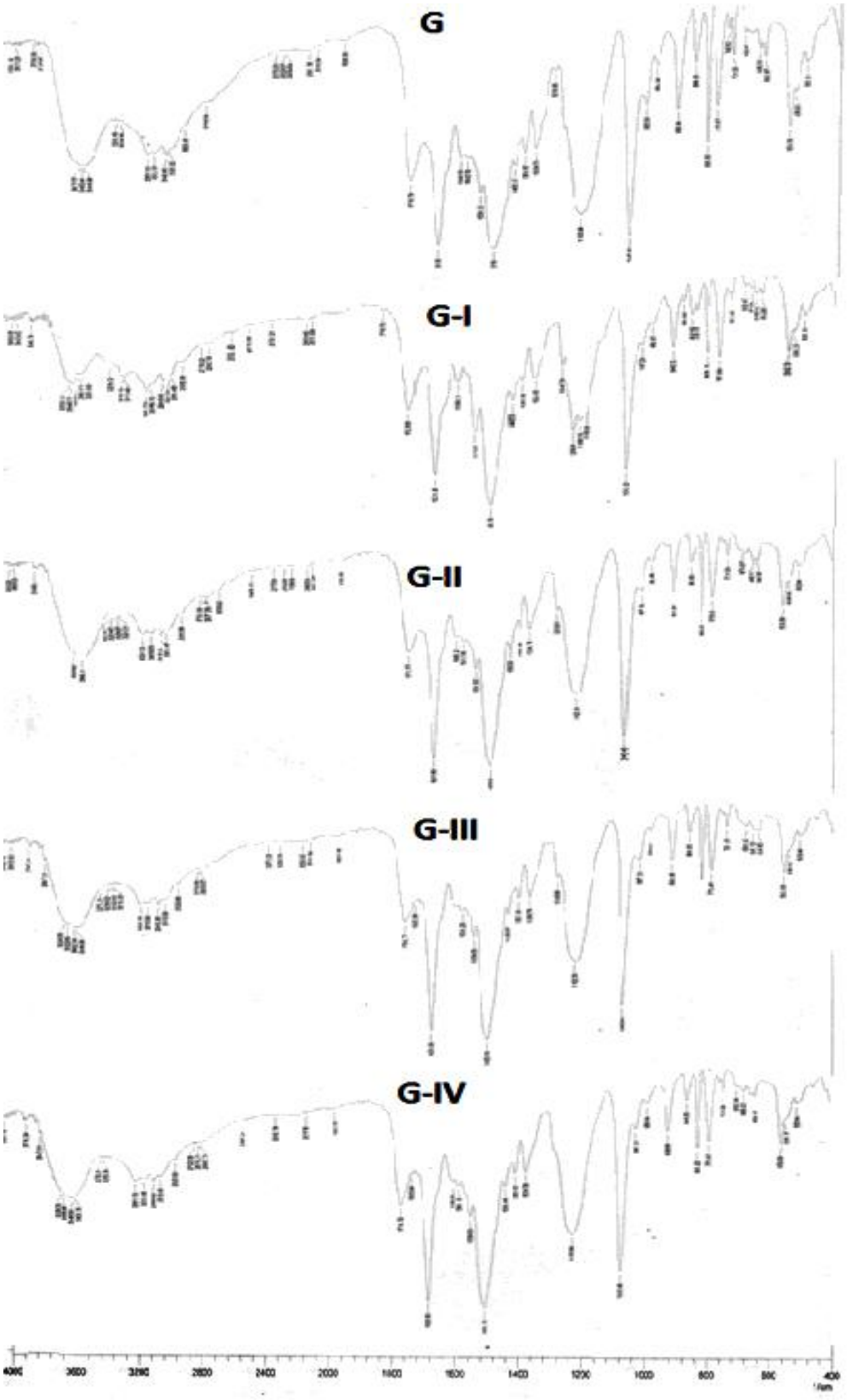



**Fig. 39: Superimposed IR spectrum of G, G-I, G-II, G-III and G-IV**

### 6.2.1.5 Differential Scanning Calorimetry (DSC) [138]

The DSC thermograms of pure drug of gemifloxacin mesylate and its crystalline forms showed endothermic peaks at 212.8°C, 206.6°C, 208.2°C, 209.8°C and 214.2°C which described about their different melting points (Table 19). Among all these crystals, G-I had the lowest melting point which indicated that it may be meta stable polymorph. Hence, meta stable polymorphic form of G-I crystal may had more solubility and better percentage drug release than other forms. When compared with pure drug (m.p 212.8 ºC) G-IV had slightly higher melting point where as G-II had melting point similar to that of G-III. DSC thermograms of pure gemifloxacin mesylate and its crystalline forms along with their superimposed thermogram were shown in Fig. no. 40-45.

**Table 19: DSC & TGA data of polymorphs and gemifloxacin mesylate**

| S.No | Crystal Form | Solvent used | DSC & TGA data | |
|---|---|---|---|---|
| | | | Peak fusion point (ºC) | Inflection point (ºC) |
| 1. | Gemifloxacin (G) | Pure drug | 212.8 | 208.8 |
| 2. | G-I | Isopropanol | 206.6 | 204.1 |
| 3. | G-II | Chloroform | 208.2 | 208.7 |
| 4. | G-III | Dichloromethane | 209.8 | 206.8 |
| 5. | G-IV | Benzene | 214.2 | 206.5 |

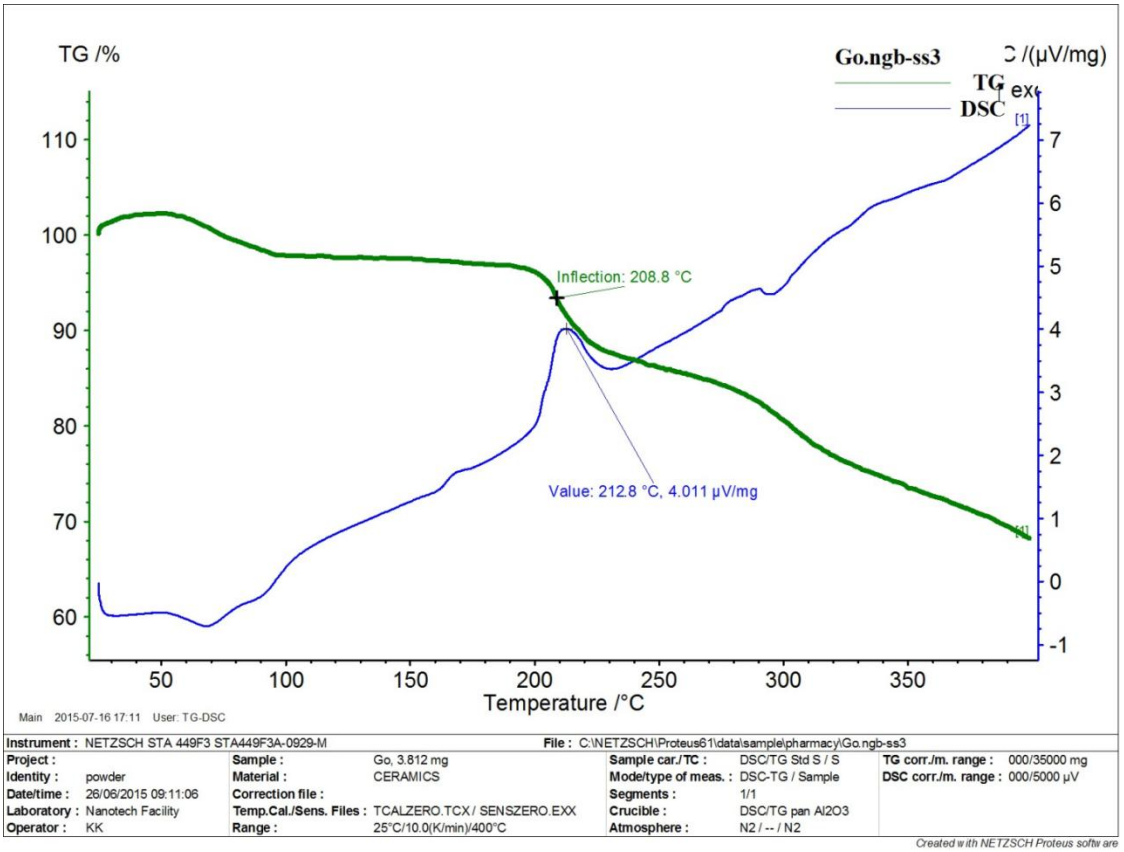



**Fig.40: DSC & TG thermogram of gemifloxacin mesylate (G)**

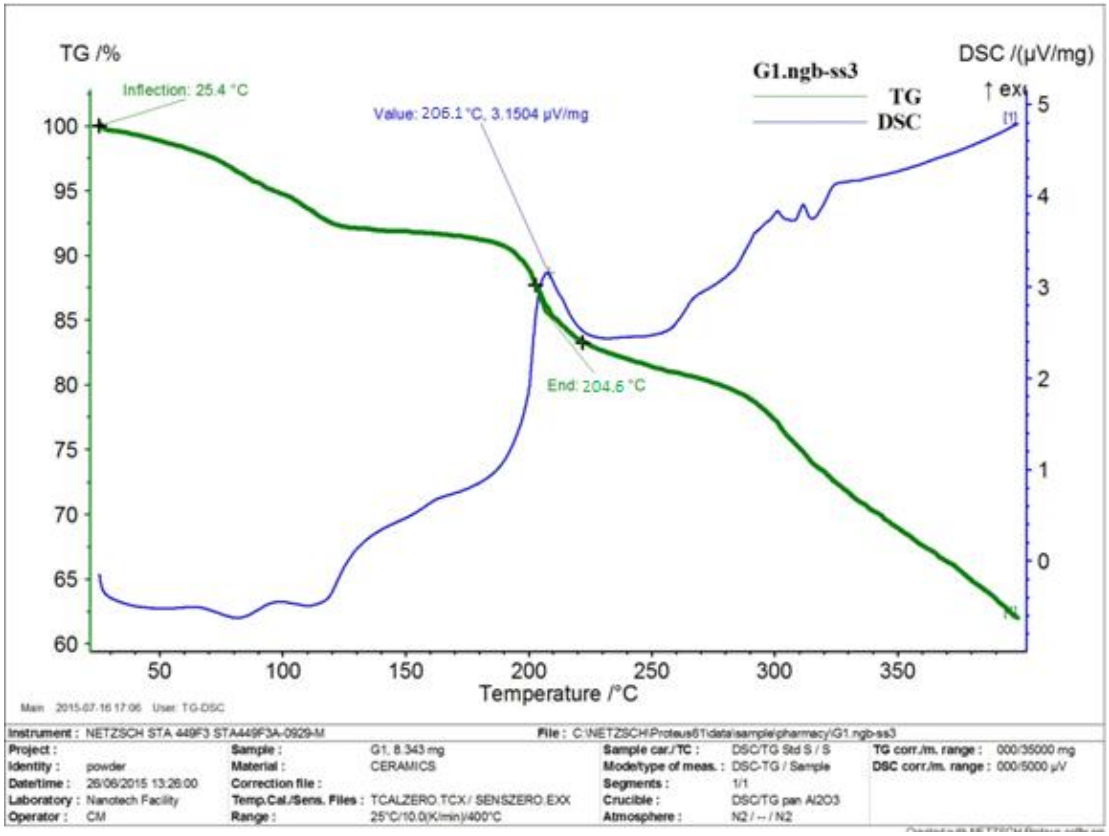



**Fig. 41: DSC & TG thermogram of G-I**

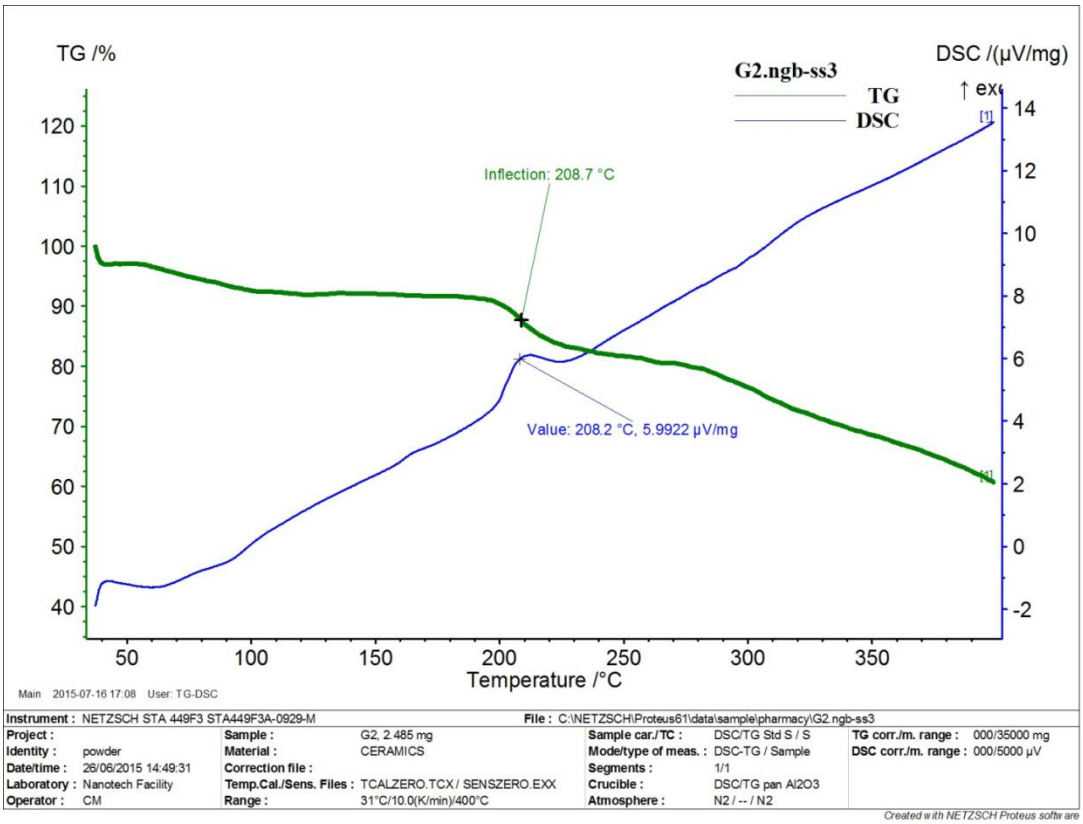



**Fig. 42: DSC & TG thermogram of G-II**

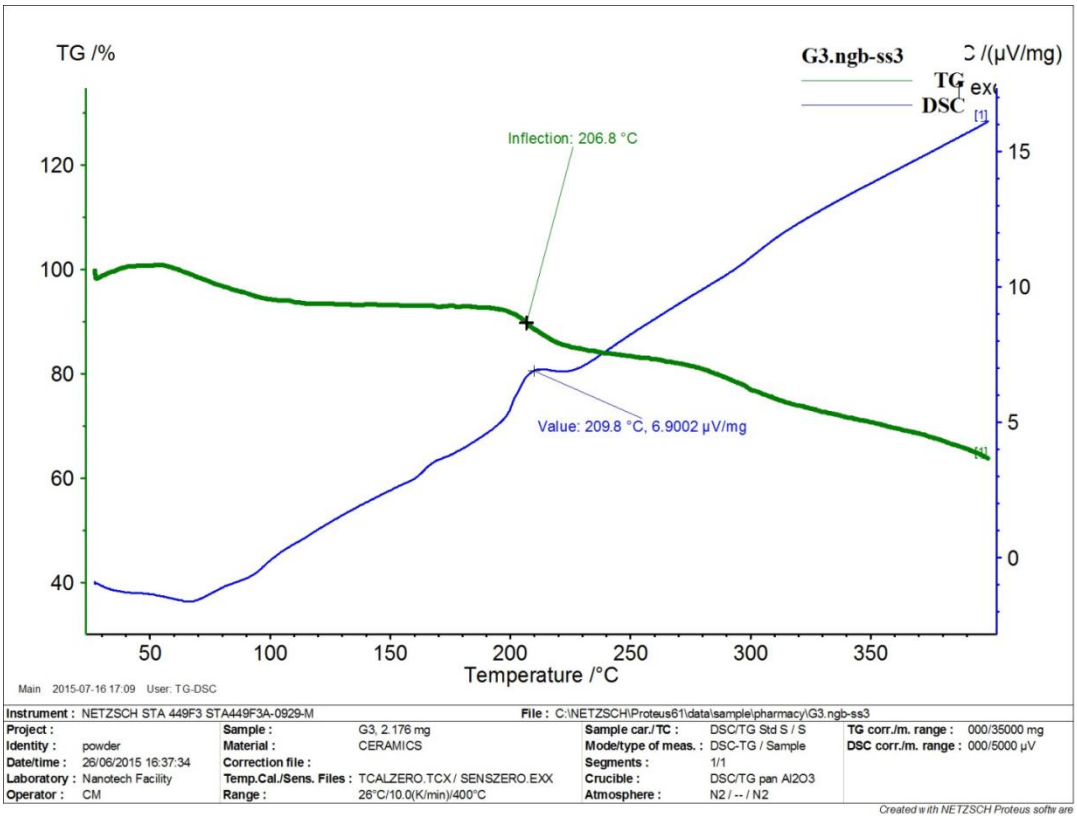



**Fig.43: DSC & TG thermogram of G-III**

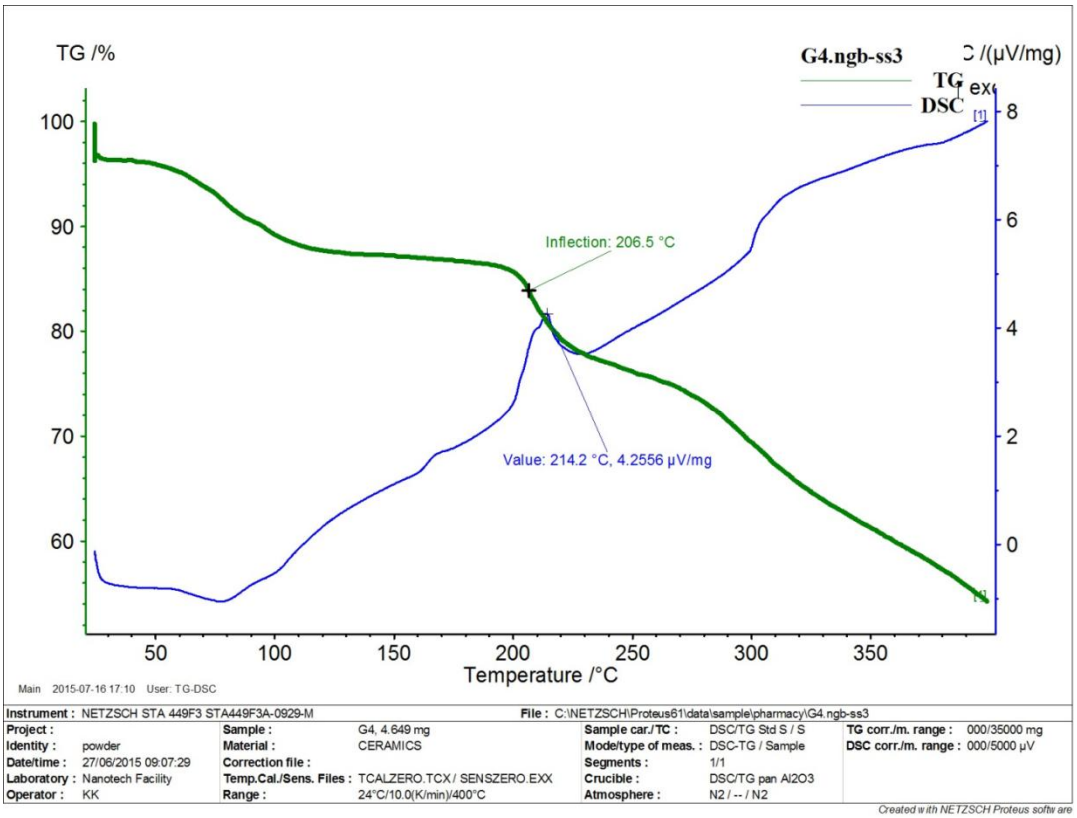



**Fig. 44: DSC & TG thermogram of G-IV**

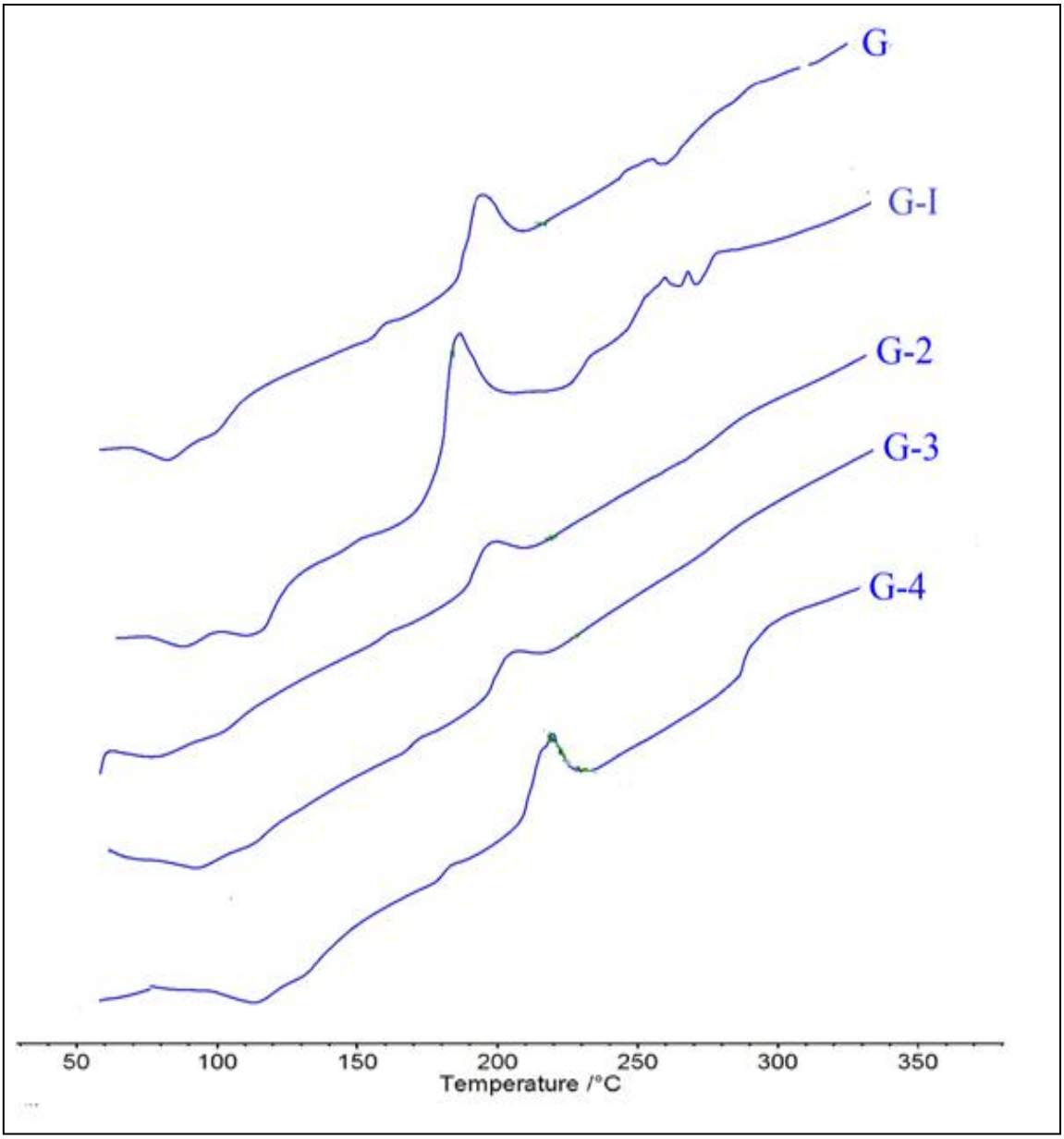



**Fig. 45: Superimposed DSC thermogram of G, G-I, G-II, G-III and G-IV**

### 6.2.1.6 Powder X-Ray Diffraction (PXRD) [139]

The difference in the position of 2θ values of all the prepared crystal forms of gemifloxacin mesylate were confirmed by well resolved diffraction patterns with their corresponding peaks. All the intensity lines (relative intensity) observed in the powder pattern of the crystal forms G-I to G-IV was not observed in pure drug. The high intensity line (186) for pure drug G at 16.3 º2 theta was not seen in G-I (128), G-II (33), G-III (68) and G-IV (67). The highest intense peak (359) was only observed in pure drug at 20.75 º2 theta than crystal forms. G-I form showed characteristic intense line at 12.10, 14.60, 16.30, 25.25 and 26.25 º2 theta which were absent in G-II and G-IV. G-III intense peaks were observed at 25.25 and 25.95 º2 theta which were present in G and G-IV and above were absent in G-II. G-I crystal form intense peak (128) was observed at 17.9 º2 theta, which was not obtained in remaining crystals and pure gemifloxacin mesylate. For G-III form high intensity peak (116) was observed at 12 º2theta, the peak intensity was 49, 66, 23, 22 for G, G-I, G-II and G-IV respectively.

Appearance and disappearance of some peaks on the PXRD spectrum of pure drug and its crystal forms (Fig. 46-50) were clearly indicating about the formation of different crystal lattice for all the crystal forms. The superimposed PXRD spectrum of G, G-I, G-II, G-III and G-IV was represented in Fig. 51. Based on the inference, it was concluded that the synthesized crystals existed in four different polymorphic forms.

**Table 20: PXRD data for gemifloxacin mesylate G, G-I, G-II, G-III and G-IV**

| G-0 | | G-I | | G-II | | G-III | | G-IV | |
|---|---|---|---|---|---|---|---|---|---|
| **2θ** | **I%** | **2θ** | **I%** | **2θ** | **I%** | **2θ** | **I%** | **2θ** | **I%** |
| 10 | 45 | 10 | 19 | 10 | 4 | 10 | 28 | 10 | 22 |
| 10.05 | 23 | 10.05 | 22 | 10.05 | 10 | 10.05 | 20 | 10.05 | 15 |
| 10.1 | 20 | 10.1 | 21 | 10.1 | 17 | 10.1 | 20 | 10.1 | 20 |
| 10.85 | 54 | 10.85 | 29 | 10.85 | 16 | 10.85 | 18 | 10.85 | 29 |
| 11 | 70 | 11 | 35 | 11 | 12 | 11 | 31 | 11 | 21 |
| 11.3 | 48 | 11.3 | 32 | 11.3 | 19 | 11.3 | 26 | 11.3 | 26 |
| 11.95 | 28 | 11.95 | 62 | 11.95 | 21 | 11.95 | 73 | 11.95 | 30 |
| **12** | **49** | **12** | **66** | **12** | **23** | **12** | **116** | **12** | **22** |
| 12.55 | 113 | **12.10** | 107 | 12.15 | 23 | 12.10 | 153 | 12.10 | 29 |
| 12.9 | 36 | 12.9 | 35 | 12.9 | 24 | 12.9 | 35 | 12.9 | 25 |
| 13.4 | 19 | 13.4 | 25 | 13.4 | 13 | 13.4 | 15 | 13.4 | 22 |
| 14 | 71 | 14 | 74 | 14 | 22 | 14 | 31 | 14 | 51 |
| 14.5 | 57 | 14.5 | 81 | 14.5 | 22 | 14.5 | 85 | 14.5 | 51 |
| 14.90 | 138 | **14.60** | 117 | 14.60 | 29 | 14.60 | 119 | 14.60 | 39 |
| 15 | 160 | 15 | 69 | 15 | 24 | 15 | 49 | 15 | 47 |
| **16.3** | **186** | **16.3** | **128** | **16.3** | **33** | **16.3** | **68** | **16.3** | **67** |
| 17.05 | 72 | 17.05 | 69 | 17.05 | 15 | 17.05 | 64 | 17.05 | 30 |
| 17.9 | 80 | **17.9** | **128** | 17.9 | 20 | 17.9 | 98 | 17.9 | 56 |
| 18.3 | 107 | 18.3 | 65 | 18.3 | 23 | 18.3 | 56 | 18.3 | 45 |
| 19.1 | 122 | 19.1 | 99 | 19.1 | 19 | 19.1 | 75 | 19.1 | 53 |
| 20 | 59 | 20 | 67 | 20 | 26 | 20 | 47 | 20 | 39 |
| **20.75** | **359** | 20.75 | 72 | 20.75 | 24 | 20.45 | 113 | 20.75 | 48 |
| 21.1 | 67 | 21.1 | 59 | 21.1 | 15 | 21.1 | 39 | 21.1 | 42 |
| 22 | 113 | 22 | 98 | 22 | 24 | 22 | 64 | 22 | 63 |
| 25.90 | 371 | **25.25** | 154 | 25.25 | 24 | **25.25** | 182 | 25.80 | 177 |
| 26.0 | 304 | **26.25** | 345 | 26.25 | 41 | **25.95** | 206 | 26.20 | 158 |

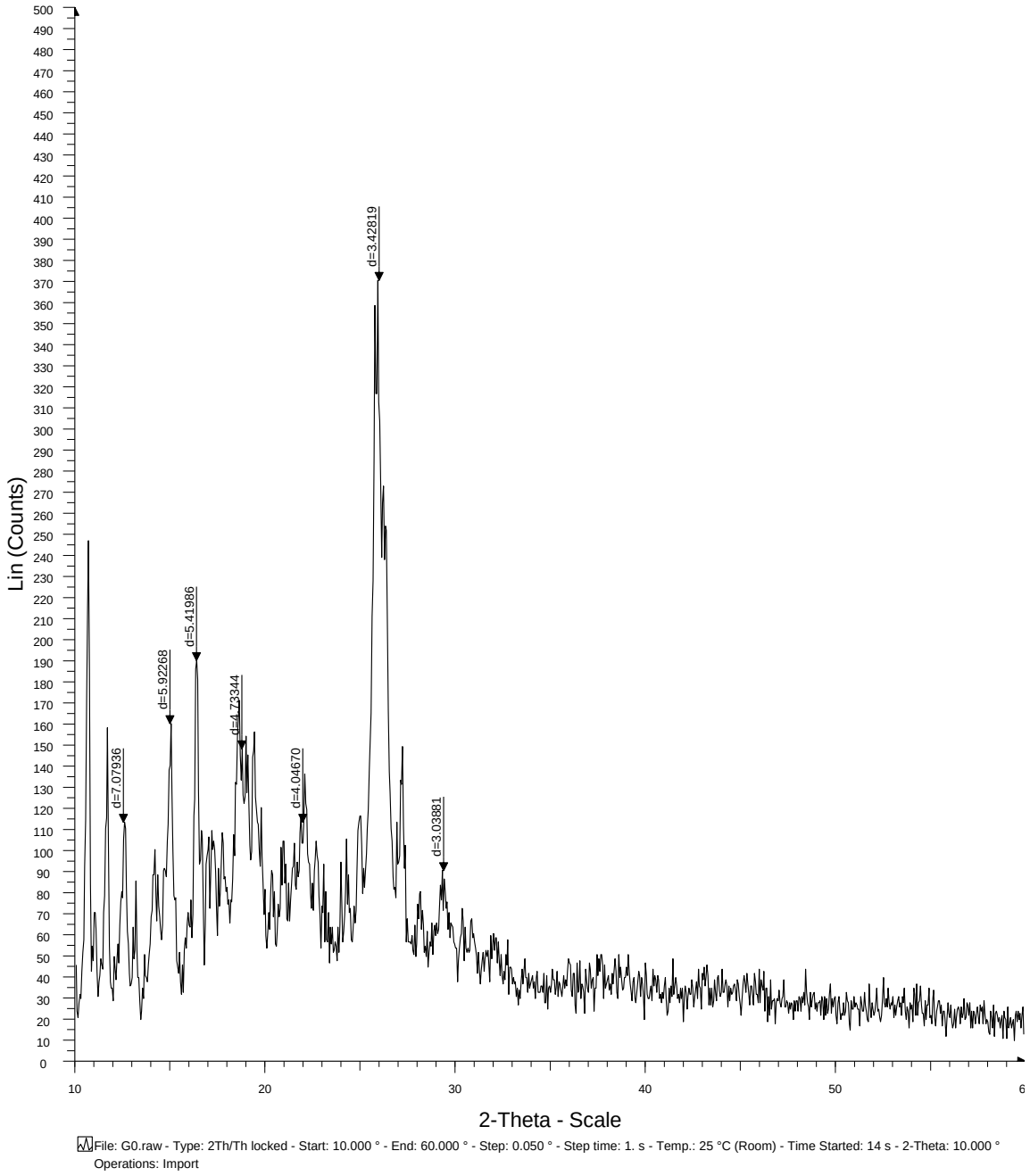



**Fig. 46: PXRD spectrum of gemifloxacin mesylate (G)**

EX/2204/2

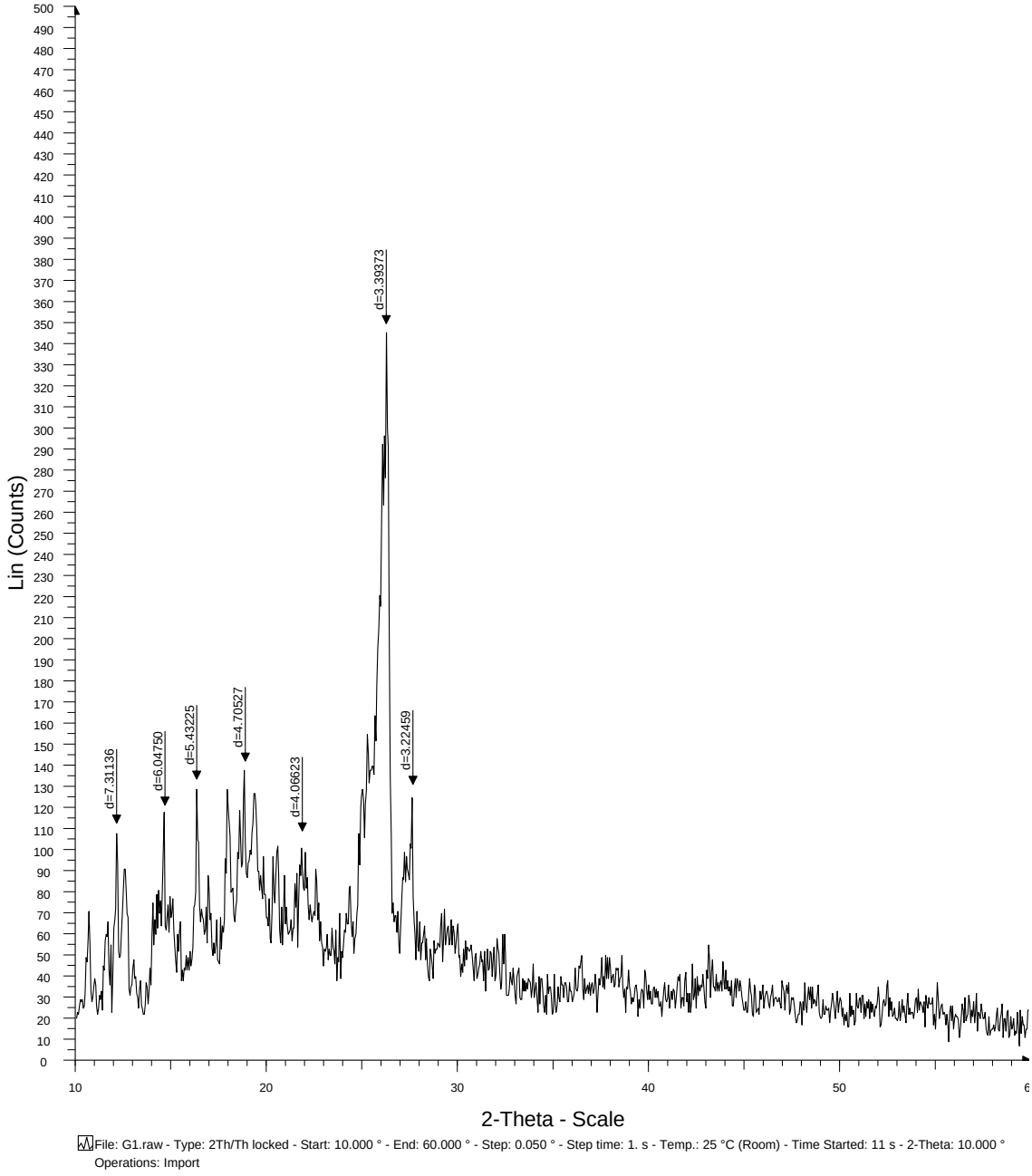



**Fig. 47: PXRD spectrum of G-I**

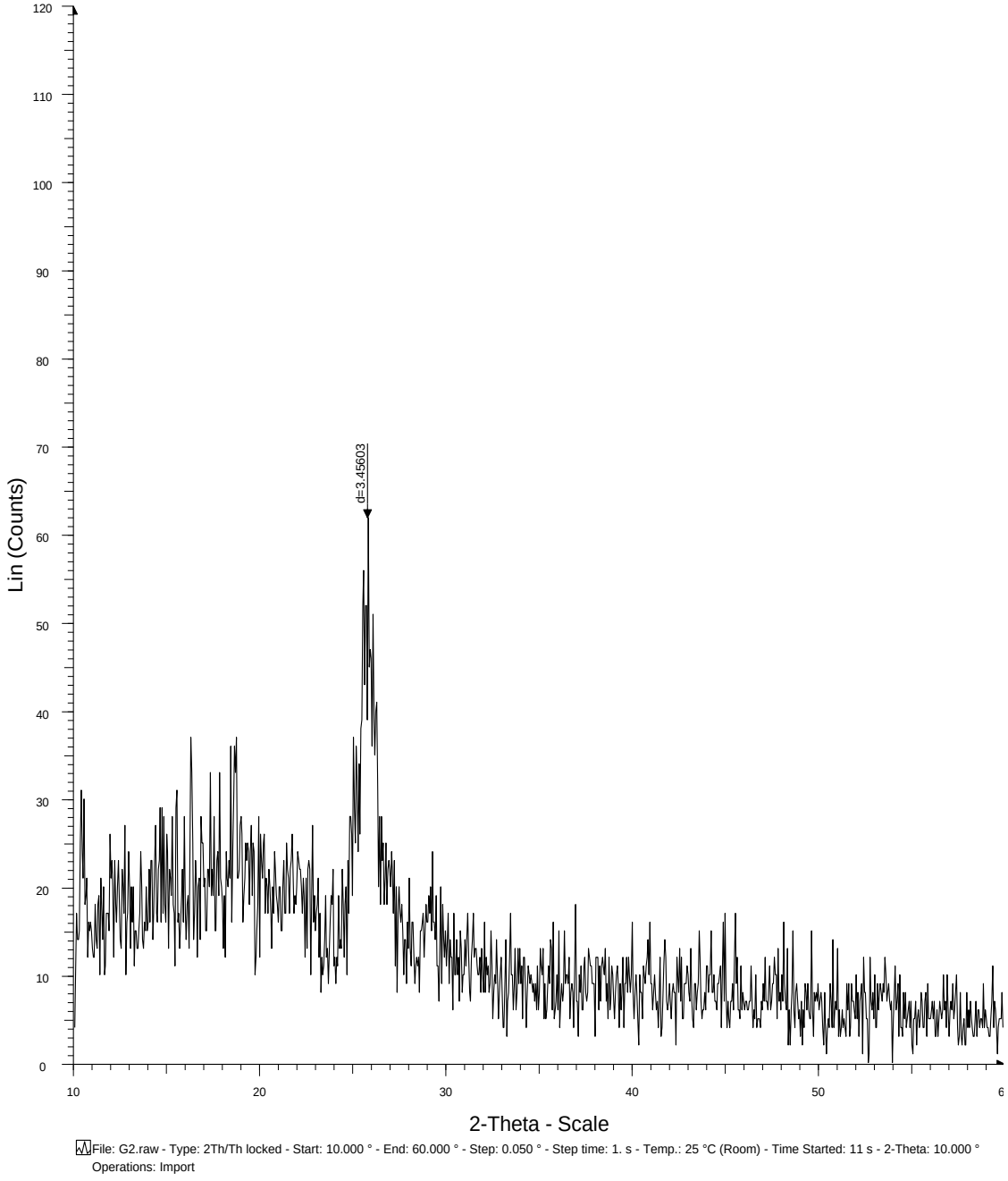



**Fig. 48: PXRD spectrum of G-II**

EX/2204/4

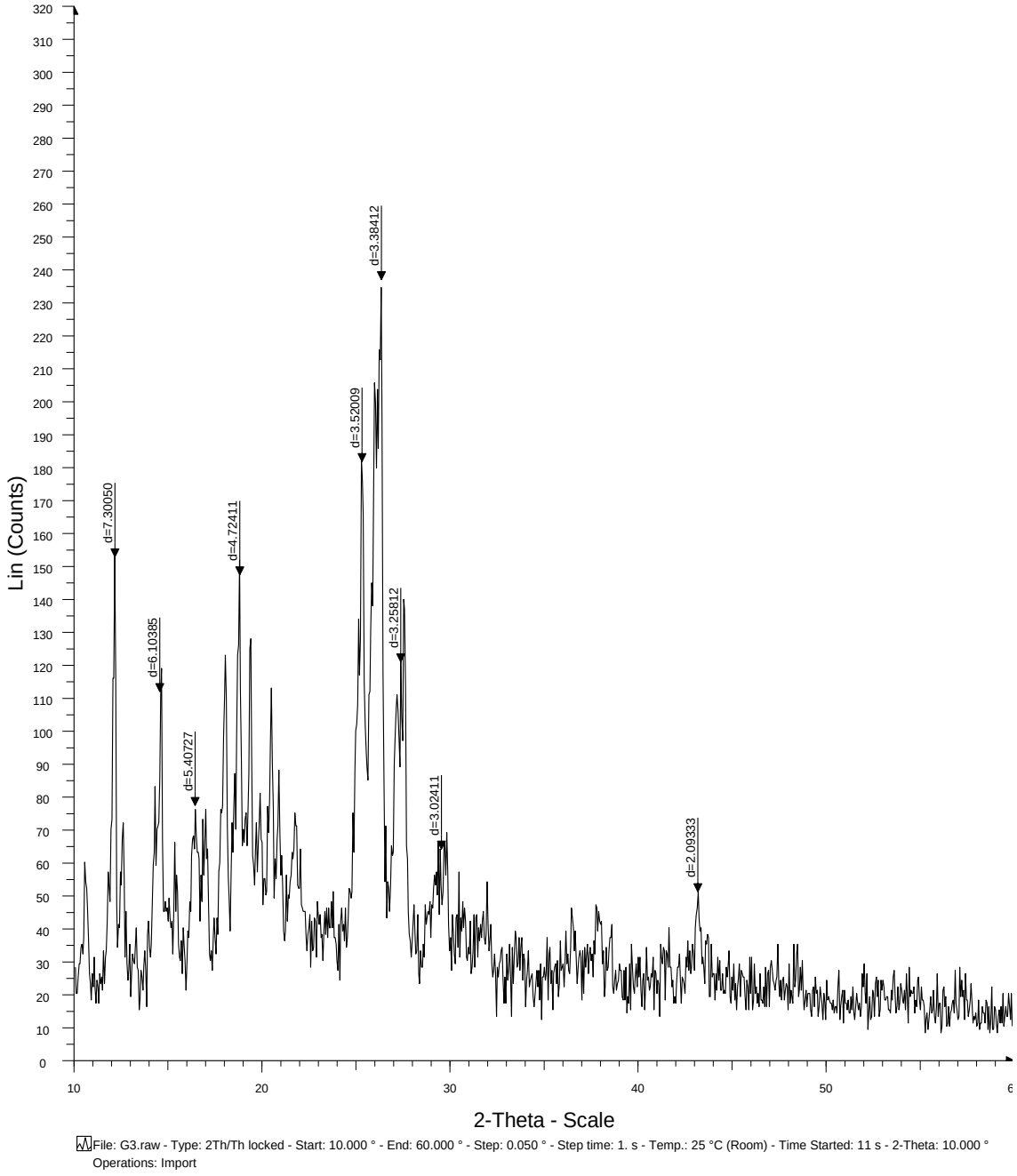



**Fig. 49: PXRD spectrum of G-III**

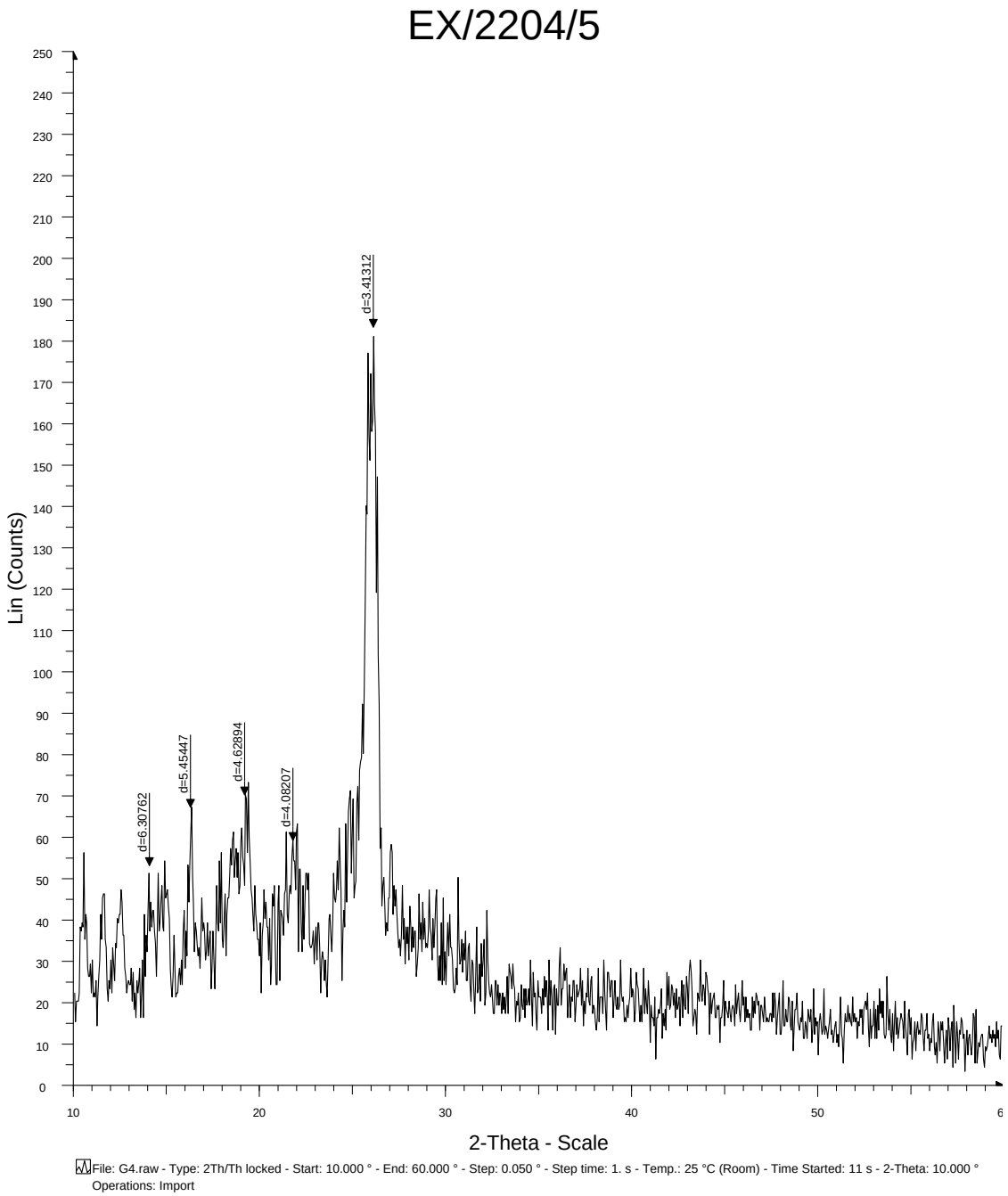



**Fig. 50: PXRD spectrum of G-IV**

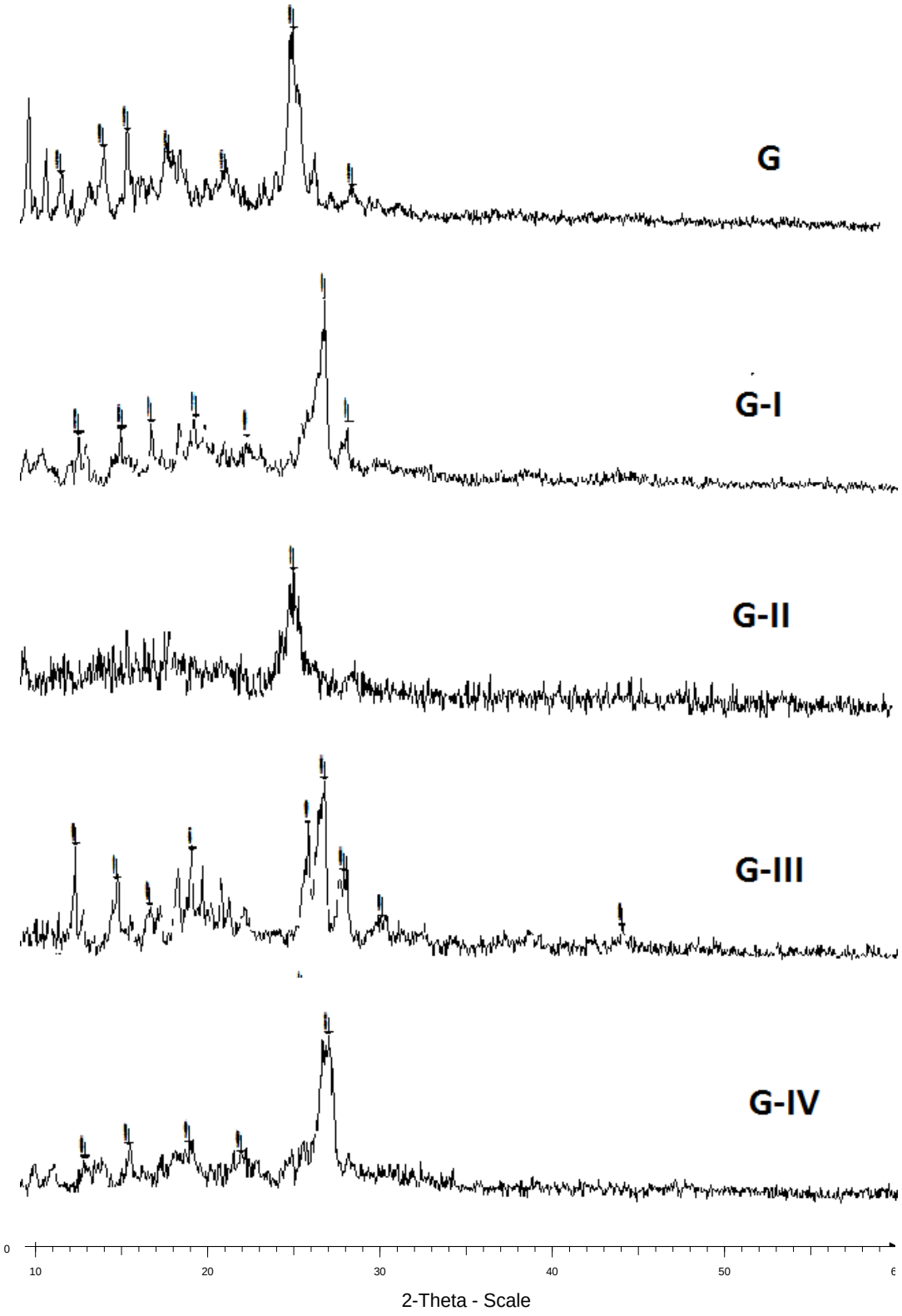



**Fig. 51: Superimposed PXRD spectra of G, G-I, G-II, G-III and G-IV**

### 6.2.2 *IN- VITRO* DISSOLUTION AND SOLUBILITY STUDIES

#### 6.2.2.1 Determination of absorption maxima for gemifloxacin mesylate

A fourth generation fluoroquinolone, gemifloxacin mesylate was also selected as another target candidate for the present work. Physical state of selected active constituent is potential importance in preformulation studies and for getting better bioavailability and stability of active drug. At least, it should be possible to synthesize different crystal forms of selected pharmaceutical entity with attendant variations in physicochemical and biological activity. Characterization of its pure drug was done by measuring its melting point and maximum absorbance (λ max) of drug in UV-Visible spectroscopy. The λ max of gemifloxacin mesylate was measured by suitably diluting the drug in phosphate buffer pH 7.0 and it was observed at **271 nm** (Fig. 52).

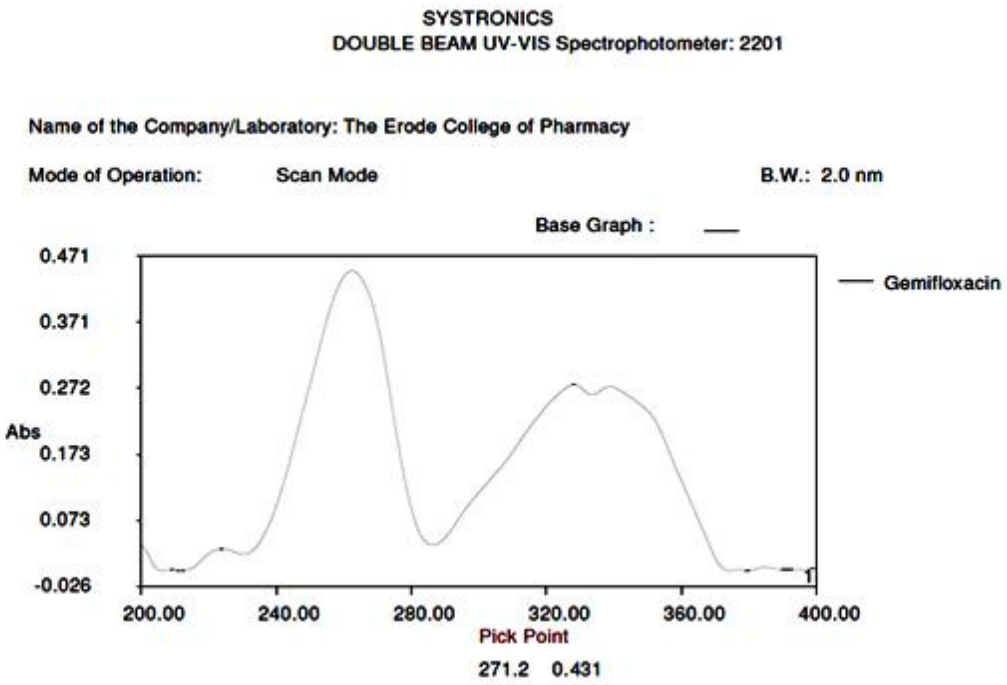



**Fig. 52: Determination of $\lambda_{max}$ graph of gemifloxacin mesylate**

### 6.2.2.2 Preparation standard curve

A standard calibration curve (Fig. 53) of prulifloxacin was constructed using phosphate buffer pH 7.0 and it obeyed Beers –Lambert's law in the concentration range of 1 - 10 μg/ml with good linearity. The results were tabulated in table 21. The above curve showed the regression equation of y = 0.0684x + 0.0185 with correlation coefficient ($R^2$ = 0.9981) as nearer to one which indicated good linearity.

**Table 21: Standard curve of gemifloxacin mesylate in phosphate buffer pH 7.0**

| S.No. | Concentration (mcg/ml) | Absorbance at (271nm)* |
|---|---|---|
| 1 | 1 | 0.087 |
| 2 | 2 | 0.165 |
| 3 | 3 | 0.240 |
| 4 | 4 | 0.294 |
| 5 | 5 | 0.363 |
| 6 | 6 | 0.431 |
| 7 | 7 | 0.494 |
| 8 | 8 | 0.554 |
| 9 | 9 | 0.628 |
| 10 | 10 | 0.710 |

(*n=3)

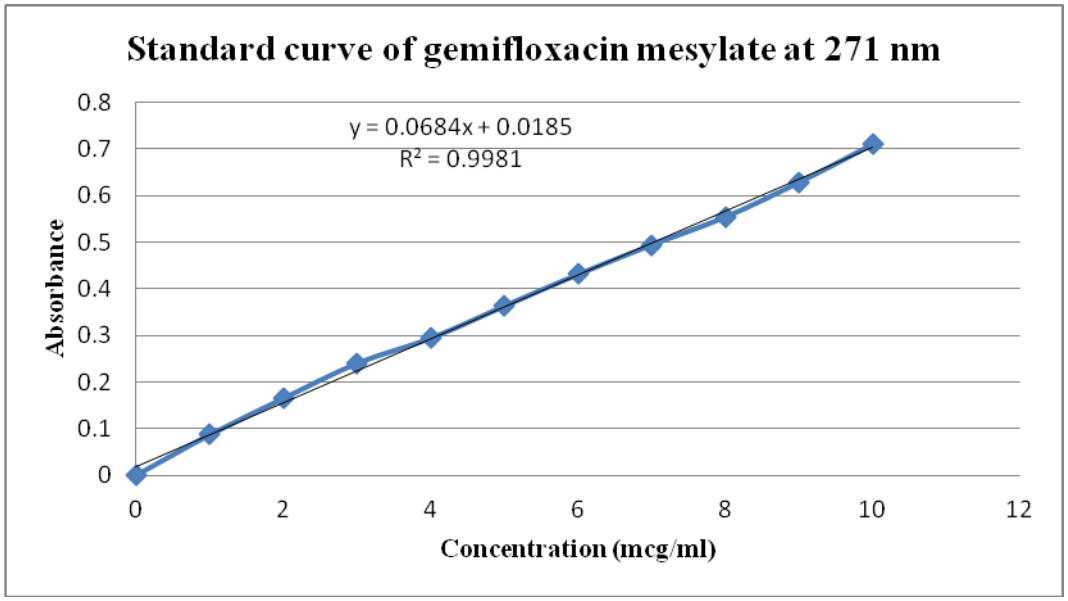



**Fig. 53: Standard curve of gemifloxacin mesylate**

### 6.2.2.3 Solubility assessment

Solubility of pure drug was found to be 0.79 mg/ml. Improvement in solubility was observed with crystal form of G-I than other crystal forms. Solubility of crystal forms follows in the order of G-I>G-II>G>G-III>G-IV. The result for solubility measurement for pure drug of gemifloxacin mesylate and its crystal forms were tabulated in Table 22.

**Table 22: Solubility studies of crystals and pure drug**

| S.No. | Crystal Forms | Solubility (mg/ml) |
|---|---|---|
| 1 | Pure | 0.79 |
| 2 | G-I | 0.90 |
| 3 | G-II | 0.81 |
| 4 | G-III | 0.76 |
| 5 | G-IV | 0.72 |

### 6.2.2.4 *In-vitro* Dissolution studies [96]

The *in-vitro* dissolution study of pure and crystalline forms were performed using phosphate buffer (pH 7.0) and the cumulative percentage drug release of each crystals was calculated by the use of standard calibration graph obtained at **271nm** and results were represented in Table 23. Increasing order of drug release followed as G-I, G-II, pure, G-IV and G-III, the value of percentage drug release were as 95.85%, 88.40%, 84.78%, 75.0% and 72.6% respectively. Due to more solubility of G-I form; it showed highest percentage drug release. Hence, from the solubility and dissolution studies, crystal (G-I) obtained from isopropanol was having highest cumulative percentage drug release, when compared to other forms. The comparative *in-vitro* drug release graph for obtained crystals of gemifloxacin mesylate with crystal form was plotted and showed in Fig. 54.

**Table 23: *In-vitro* Dissolution rate profile of crystal forms of gemifloxacin mesylate**

| Time ( Min) | Percentage drug release (Mean ± SD*) | | | | |
|---|---|---|---|---|---|
| | G-0 | G-I | G-II | G-III | G-IV |
| 15 | 3.8±0.30 | 9.9±0.23 | 7.9±0.28 | 7.3±0.45 | 8.2±0.16 |
| 30 | 9.5±0.74 | 15.8±0.52 | 10.9±0.61 | 10.3±0.85 | 11.4±0.42 |
| 45 | 15.3±0.11 | 27.0±0.31 | 15.3±0.42 | 13.4±0.38 | 20.5±0.41 |
| 60 | 29.7±0.23 | 44.1±0.43 | 34.2±0.54 | 32.4±0.15 | 33.0±0.14 |
| 90 | 38.7±0.46 | 72.42±0.32 | 49.3±0.54 | 42.3±0.35 | 45.5±0.67 |
| 120 | 54.1±0.74 | 88.02±0.37 | 79.83±0.30 | 60.12±0.26 | 62.01±0.95 |
| 240 | 84.78±0.38 | 95.85±0.53 | 88.4±0.36 | 75.0±0.75 | 72.6±0.48 |
| All values represents means (n = 4) ± S.D | | | | | |

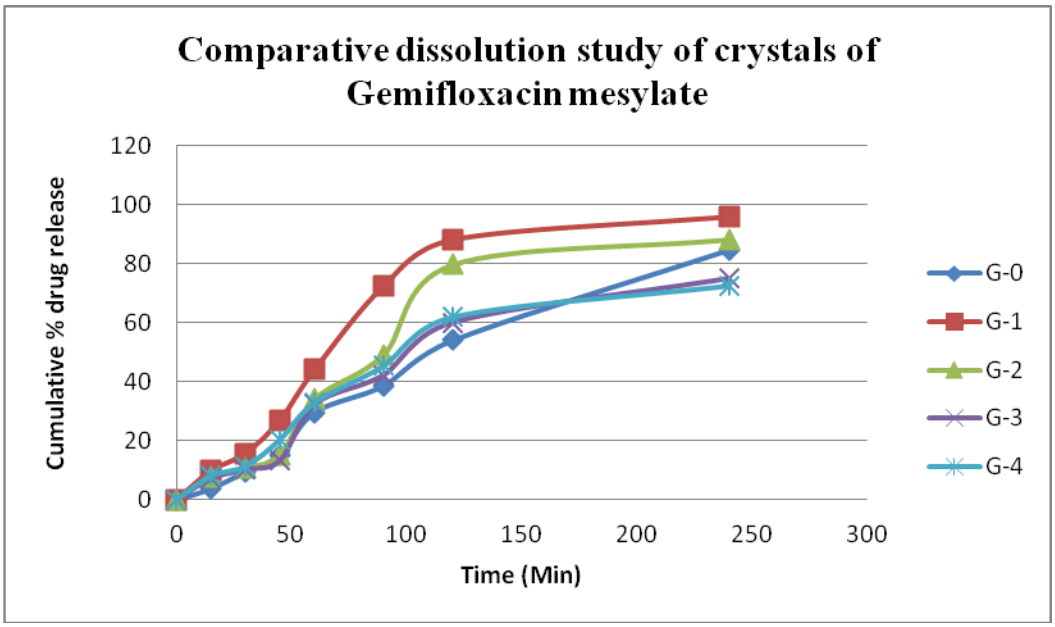



**Fig. 54: *In-vitro* dissolution rate for crystal forms of gemifloxacin mesylate pure & crystal forms**

### 6.2.2.5 Drug content

The drug content was determined for all the prepared crystal forms of gemifloxacin mesylate and the results were uniform and nearer to 100%. The data were tabulated in Table 24.

**Table 24: Percentage drug content for prepared crystal forms**

| S.No. | Crystal Forms | Drug content (%)* |
|---|---|---|
| 1 | G-I | 99.15±1.45 |
| 2 | G-II | 98.88±1.37 |
| 3 | G-III | 98.54±2.63 |
| 4 | G-IV | 98.49±1.88 |

(*n=3)

### 6.2.2.6 Stability studies[75]

All the crystals were stored in stability chamber for accelerated stability studies and did not show any physical changes during the study period. The drug content were determined at end of the study (n=3) for all the prepared crystals and they were quite stable at accelerated storage conditions. Values nearly 100 % indicated that all the polymorphic forms were stable without any alteration on the physical characters results which were tabulated in Table 25

**Table 25: Stability study data for crystal forms of gemifloxacin mesylate**

| Crystal form | Drug content *(%) | | |
|---|---|---|---|
| | After 30 days | After 60 days | After 90 days |
| G-I | 98.35±0.2531 | 98.24±0.2397 | 97.19±0.6318 |
| G-II | 98.12±0.3413 | 97.51±0.2632 | 97.08±0.4267 |
| G-III | 98.24±0.2202 | 98.04±0.5432 | 97.37±0.5987 |
| G-IV | 98.88±0.7865 | 98.12±0.5809 | 97.43±0.3963 |

(*n = 3)

### 6.2.3 PHARMACOKINETIC STUDY IN RATS [140]

The pharmacokinetic parameters observed in the male Sprague-Dawley rat following oral administration of amorphous and crystalline form of gemifloxacin mesylate demonstrated the relationship between the *in-vivo* oral absorption and *in-vitro* drug release.

The mean plasma concentration versus time profiles and other pharmacokinetic parameters of amorphous and crystal form of gemifloxacin mesylate after oral administration to Sparque-Dawley rats (male) were determined using PK solution 2.0 software. The plasma drug concentration was estimated by partially validated sensitive RP-HPLC method using UV absorbance detector at 265 **nm**. A chromatographic peak was observed at a retention time of 5.0 ± 0.2 min (Fig. 55) using the mixture of phosphate buffer pH 6.8 & acetonitrile in the ratio of 80:20 v/v. The drug gemifloxacin obeyed linearity in the concentration range from 0.25 µg/ml to 5 µg/ml. The plasma drug concentration of gemifloxacin at predetermined time intervals was calculated from the linearity graph.

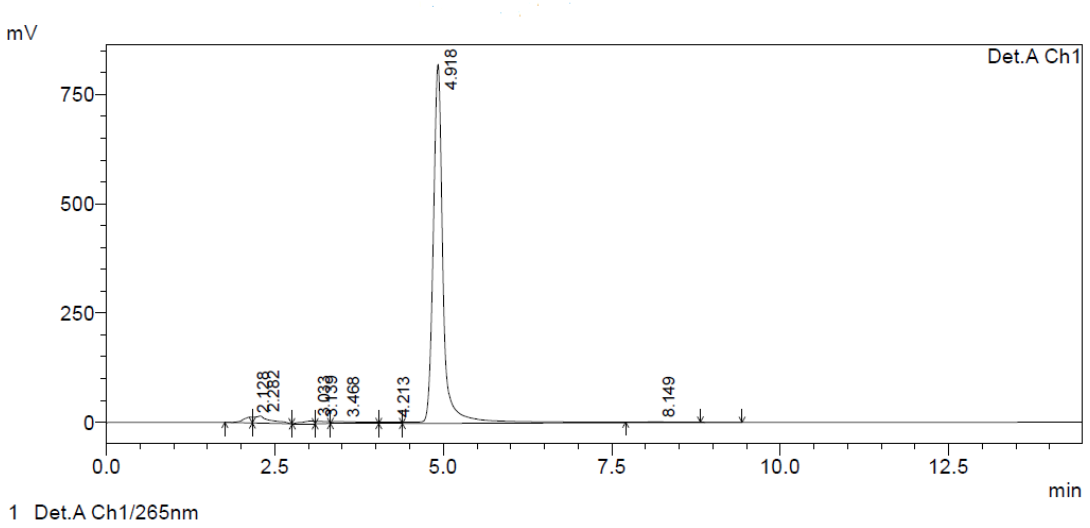



**Fig. 55: HPLC chromatogram for gemifloxacin mesylate**

The drug concentration of each plasma samples were determined by extrapolating the linearity graph with peak area at above said predetermined time interval (0, 0.5, 1, 2, 4, 8, 12 and 24 hrs). The plasma drug concentration-time curve of gemifloxacin pure drug and optimized crystal form were plotted and presented in Fig. 56-58 and other pharmacokinetic parameters (Table 20) were calculated by non compartmental model using PK solution 2.0 software.

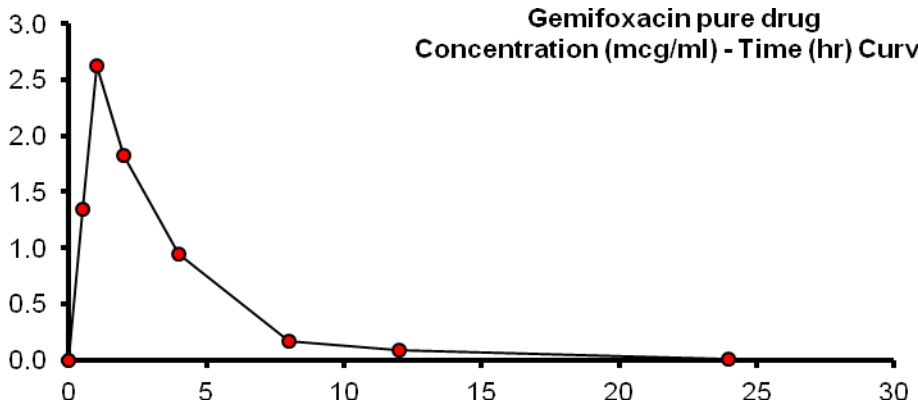



**Fig. 56: Mean plasma concentration Vs time curve of gemifloxacin mesylate (G)**

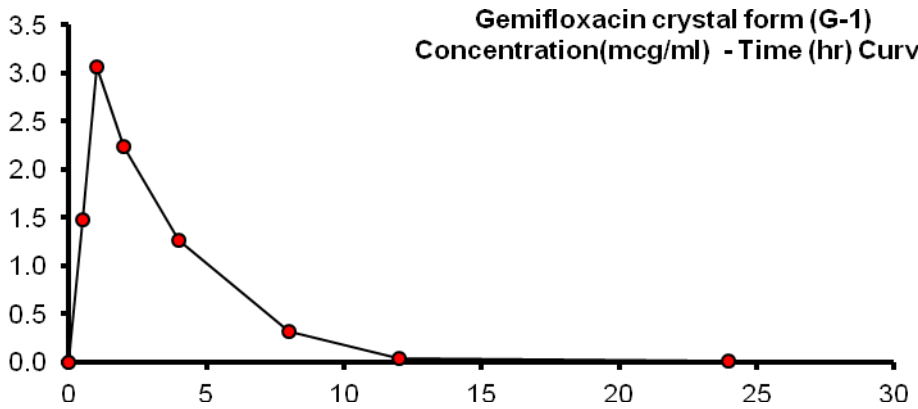



**Fig. 57: Mean plasma concentration Vs time curve of (G-I)**

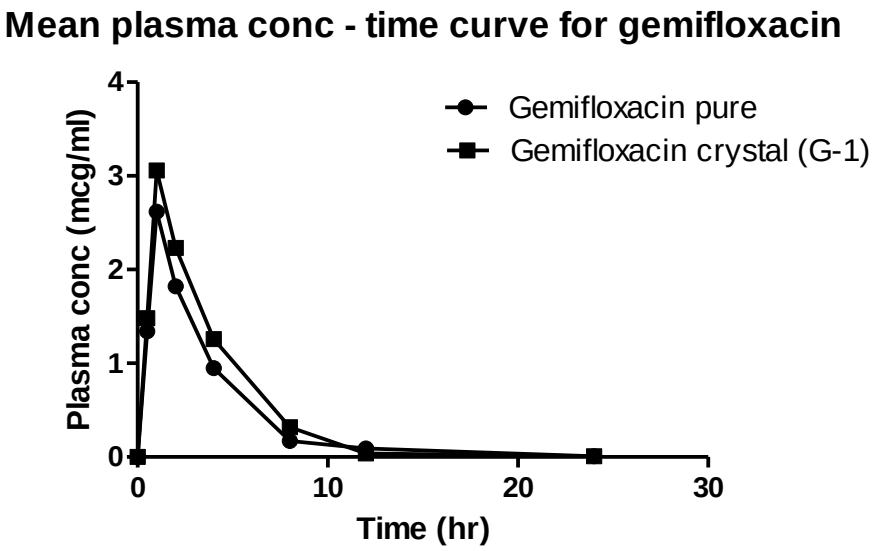



**Fig. 58: Plasma conc-time curve after oral administration of gemifloxacin mesylate G and G-I crystal**

**Table 26: Pharmacokinetic parameters of gemifloxacin (Pure & G-I)**

| S.No. | PK parameters | Gemifloxacin(Pure) | Crystal (G-I) |
|---|---|---|---|
| 1 | $C_{max}$ | 2.62±1.06 µg/ml | 3.06±1.02 µg/ml |
| 2 | $T_{max}$ | 1 hr | 1 hr |
| 3 | $T_{1/2}$ | 5.29±1.3 hr | 4.78±1.9 hr |
| 4 | E. Rate constant | 0.1310±0.13 $hr^{-1}$ | 0.1448±0.17 $hr^{-1}$ |
| 5 | $AUC_{(0-t)}$ | 9.675±2.15 µghr/ml | 11.82±2.26 µghr/ml |
| 6 | $AUC_{(\alpha)}$ | 9.75 ±2.86µghr/ml | 11.88±2.25 µghr/ml |

*All values were expressed as mean ± S.D. (n=6).

The maximum plasma concentration for gemifloxacin mesylate polymorphs (G-I) was observed as high ($C_{max}$ of 3.06 μg /ml) than the amorphous form with approximately similar $T_{max}$ value of 1 hr. The animals administered with optimized crystal form of gemifloxacin mesylate (G-I) showed higher value of $AUC_{(0-\infty)}$ of 11.88 $\mu g.hr.ml^{-1}$ with apparently shorter half life of 4.78 hr which proved that it had more aqueous solubility and rapid absorption from oral route. Hence, the experimental findings of pharmacokinetic studies correlated more closely with *in-vitro* percentage drug release. Hence, the pharmacokinetic study supports rapid and higher oral absorption in addition with higher bioavailability G-I crystalline form when compared with pure form.

# 7. SUMMARY AND CONCLUSION

Prulifloxacin and Gemifloxacin are fourth generation Fluoroquinolones; they are broad-spectrum antibiotics which are active against both gram positive and gram-negative bacteria. They are mainly prescribed for the bacterial infections of complicated lower urinary tract infection, acute respiratory tract infection, simple cystitis, skin and soft tissue infection, E.N.T infection, bone and joint infection in children and adults and also community acquired pneumonia.

The existence of various solid-state forms of active ingredients can drastically alter the physico-chemical properties of a pharmaceutical product. This may affect its effectiveness, stability, bioavailability, therapeutic efficacy and suitability of a particular formulation. Therefore, the development of suitable solid form is critical for the success of drug product. Solids may be prepared in a particular crystal form through various crystallization techniques. These solid forms are differing from each other with respect to many properties such as internal crystal lattice, crystal shape, solubility, dissolution rate, flow properties and compaction behavior.

Particularly for drugs that are poorly water soluble, polymorphism in formulation plays a vital role since it could significantly influence the dissolution rate and solubility required to achieve adequate bioavailability. The present work was focused with the aim of preparation of various crystals of prulifloxacin and gemifloxacin mesylate using the solvents of varying polarity and by change of phase. The main objectives of this study was also achieved by conducting the experiments to characterize the newly developed crystals by Scanning Electron Microscopy, FT-IR

spectroscopy, Differential Scanning Calorimetry, Powder X-ray Diffraction, *in-vitro* dissolution study and *in-vivo* pharmacokinetic study.

**Prulifloxacin**

The different crystal forms of prulifloxacin (P-I, P-II, P-III) were prepared by cooling crystallization method using acetonitrile, acetone and dichloromethane respectively. SEM photographs showed a distinct difference in the morphology of newly developed polymorphs such as plate like crystal for P-I, rod or needle shaped for P-II and prismatic for P-III which believed the formulation of different polymorphs with their corresponding crystal morphology.

The FT-IR spectra of pure drug and crystalline forms of selected drug were almost identical and the main absorption bands were appeared in the entire spectrum which indicated that there was no alternation in the functional group. On the basis of FT-IR spectroscopy the crystal forms of prulifloxacin could be categorized in to three different crystal forms.

From DSC analysis peak fusion point were observed at 224.01ºC, 226.44ºC , 222.33ºC and 224.91 ºC for pure form, P-I, P-II and P-III. The DSC thermogram analysis revealed that the crystal obtained from acetonitrile showed endothermic peak at 226.43º C corresponding to its melting point indicated its more stability than others. Shift of the endothermic peak towards lower temperature in P-II indicated lower melting point, hence it may be the formation of meta stable polymorph.

Powder X-ray diffraction pattern of all crystal forms showed that P-I and P-II were not identical with any of the other spectra indicating that they are two different polymorphs. PXRD spectra of P-III have similar intense peaks as that of pure form.

From the results of above study, three different categories of polymorphs were identified.

Solubility of all the crystals was determined by determining the concentration of the drug after exposing the crystals to phosphate buffer for 24 hours. The maximum solubility was found for P-II form while the others have least solubility. P-II form showed highest percentage drug release in its dissolution profile when compared to pure form and other crystals. Finally it can be concluded that the three different polymorphs of prulifloxacin were identified based on SEM, FT-IR, DSC and PXRD studies. These polymorphs have shown difference in their solubility and dissolution profiles.

Samples stored at accelerated stability condition had shown no significant changes in their drug content, demonstrating their good stability. The result of dissolution study of P-II crystal form showed impact on the pharmacokinetic studies after oral administration to Sparque-Dawley rat. The highest $C_{max}$ (1.88 μg/ml) and high area under the curve vale ($AUC_{\infty}$ 14.70 μg/ml) supported the better oral absorption and greater bioavailability of P-II crystal form of prulifloxacin than the pure drug. Hence, the above process increased the confident to develop the further formulation.

**Gemifloxacin mesylate**

The crystal forms of gemifloxacin mesylate were prepared by solvent evaporation method using isopropanol, chloroform, dichloromethane and benzene to get the fine crystals of G-I, G-II, G-III and G-IV form respectively. The SEM images of developed crystals showed different shapes and sizes due to the nature of various solvents used in their preparation. Thick rod shaped for G-I, rectangular for G-II, plate like for G-III and bricks shape for G-IV demonstrated the formation of different crystals.

FT-IR spectroscopy of prepared crystal had not shown any major alternation in comparison with pure form which indicated that these crystal forms were different in their crystal habbit, but identical chemically. From the FT-IR spectrum of crystals of gemifloxacin mesylate could be categorized in to four crystal forms.

DSC thermograms of the above crystalline form showed changes in melting point due to alternation in the internal molecular arrangement and other intermolecular interaction. From DSC analysis peak fusion points were observed at 206.6 ºC, 208.2 ºC, 209.8 ºC, 214.2 ºC for G-I to G-IV respectively. Lowest melting point of G-I described that it may be meta stable polymorph.

The differences in the line intensities of few peaks on the XRPD spectrum of prepared crystal forms and pure form indicated the existence of different forms of crystal. Powder X-ray diffraction of all crystal forms shown spectacular results. Comparison of the spectra shown that G-I, G-II, G-III and G-IV have distinct crystal structure. From the results, all prepared crystal forms were identified as four different polymorphs.

The observation from the dissolution rate profiles and solubility measurement of all the four crystalline forms explained that the G-I crystal had highest solubility and percentage drug release. Maximum solubility was found in G-I. Rest of the other crystal forms offered more or less the same solubility. The drug content observed for all the developed crystals between 97% - 98% indicated good stability after storage at accelerated stability condition.

Finally it can be concluded that the four different polymorphs of gemifloxacin mesylate were identified based on SEM, FT-IR, DSC and PXRD studies.

The mean peak plasma concentration of G-I form ($C_{max}$ 3.06 µg/ml) was more than the pure form which was indicative of the rate and extent of oral absorption. The absorption rate of G-I forms showed greatly up at 1 hr with highest $AUC_{\infty}$ value (11.88µg/ml) which expressed the higher bioavailability than pure form. This improved pharmacological response provided the potential to develop the newer dosage form of gemifloxacin mesylate in future.

# BIBLIOGRAPHY


1. John FB. Polymorphism – A critical consideration in Pharmaceutical Development, Manufacturing and Stability**. J Validation Technol.** 2008; 14(4): 15-23.

2. Andre S Raw, M.scott Furness, Devindar S Gill, Richard C Adams, Franko Holcombe, Lawrence X Yu. Regularity considerations of Pharmaceutical Solid Polymorphism in abbreviated new drug application (ANDAs). **Adv Drug Deliv Rev**. 2004; 56:397-414.

3. Sudha R, Vippagunta, Harry G Brittain, David JW Grant. Crystalline solids. **Adv Drug Deliv Rev.** 2001; 48:3-26.

4. Thenge RR, **Ganesan V,** Tekade NP. Studies on crystal forms of lomifloxacin hydrochloride: Preparation, characterization and dissolution profile**. Int J Res Pharm Sci.** 2013; 4(1):12-17.

5. Sevukarajan M, Tamizhvanan K, Sodanapalli R, Satheesh babu JM, Naveen kumar B, Sreekanth Reddy B, Sethu Krishna J, Vivekananda U. Sarada K, Hyndavi N, Hima bindu R and Roopa Lahari K. Crystal engineering technique-An emerging approach to modify physicochemical properties of active pharmaceutical ingredient. **Int J Chem Pharm Sci.** 2012; 3(1):15-29.

6. John Haleblian, Walter Macrone. Pharmaceutical applications of polymorphism. **J Pharm Sci**. 1969; 58(8): 911-929.

7. Rolf Hilfiker, Fritz Blatter, Markus V Raumer. Polymorphism in the Pharmaceutical Industry. Wiley Online Library 2006.

8. Harry G Brittan. Polymorphism in pharmaceutical solids. 2nd Ed. New York, USA: Informa Health care; 2009.

9. Pangarkar PA, Tayde AM, Uttarwar SG, Wanare RS. Drug Polymorphism: An overview. **Int J Pharm Technol.** 2013; 5(1): 2374-2402.

10. Subramanyam CVS. Principles and Practices of Pharmaceutical Engineering. Delhi: Vallabh Prakshan; 2002.

11. Kaisar Raza, Pramod Kumar, Shanti Ratan, Ruchi Malik, Shweta Arora. Polymorphism: The phenomenon affecting the performance of drugs. **SOJ Pharm Pharm Sci.** 2014; 1(2): 1-10.

12. Rahul Purohit, Venugopalan P. Polymorphism: An overview**. J Sci Edu Resonance.** 2009; 882-893.

13. Eun Hee Lee. A practical guide to Pharmaceutical polymorph screening and selection. **Asian J Pharm Sci.** 2014; 9: 163-175.

14. Sawant KD, Naidu AV. Preparation, characterisation of hydrates and anhydrous forms of anti-migraine drug – frovatriptan succinate. **Res J Pharm Sci.** 2013; 2(5): 1-10.

15. Gandhi Saurabh, Chandrul Kaughal. Pharmaceutical solid polymorphism in abbreviated new drug application (ANDA) – A regularity perspective**. J Chem Pharm Res.** 2011; 3(3): 6-17.

16. Veerendra K, nanjwade N, Rajesh G, Aswini N, Manvi FV, Basavaraj K, Nanjwade. Crystal structures of prulifloxacin and its solvates. **Adv Chem Lett.** 2013; 1: 1-8.

17. Edina Vrenic. Recent advances in the identifications and prediction of polymorphs. **Bosn J Basic Med Sci. III**. 2003; 3: 32-36.

18. Madhu Pudipeddi, Abu TM Serajuddin. Trends in solubility of polymorphs**. J Pharm Sci**. 2005; 94(5): 929-939.

19. Dharmendra Singhal, William Curatolo. Drug polymorphism and dosage form design: a practical perspective. **Adv Drug Deliv Rev.** 2004; 56: 335-347.

20. Daniel A Srider, William Addicks, Walter Evens. Polymorphism in generic drug product development. **Adv Drug Deliv Rev.** 2004; 56: 391-395.

21. Cesur S, Gokbel S. Crystallization of mefenamic acid and polymorphs. **Cryst Res Technol.** 2008; 43(7): 720-728.

22. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. Part II. 4th Ed. New delhi: CBS publishers and distributors; 2007.

23. Gurdeep R Chatwal. Instrumental methods of chemical analysis. 5th Ed. Mumbai: Himalaya publishing house; 2003.

24. Jagmohan. Organic ananlytical chemistry. 1st Ed. Delhi: Narosa publishing house; 2003.

25. David G Watson. Pharmaceutical analysis, 2nd Ed. China: Elsevier churchill living stone; 2007.

26. Kenneth A Connors. A text book of pharmaceutical analysis. 3rd Ed. A wiley-Insterscience publication.

27. Opticalmicroscopy.[Internet][updateon5.11.2015]
Available from: http://en.wikipedia.org/wiki/optical_ microscope

28. GeochemicalInstrmentationandAnalysis-Scanning Electron Microscopy. [Internet][updateon04.06.2015]
Availablefrom:http://serc.carleton.edu/research_education/geochemsheets/techniques/SEM.html

29. ScanningElectronMicroscopy.[Internet][updatedon16.12.2015]
Available from: http://en.wikipedia.org/wiki/scanning_electron_microscope.

30. Panagopoulou-Kaplani, Malamataris S. Preparation and characterization of a new insoluble polymorphic form of glibenclamide. **Int J Pharm**. 2000; 195: 239-246.

31. Giron D. Investigations of polymorphism and pseudo polymorphism in pharmaceuticals by combined thermo analytical techniques. **J Therm Anal Calorim**. 2001; 64: 37-60.

32. Guang Wei Lu, Michael Hawley, Mak Smith, Brenda M. Geiger, William pfund. Characterization of a novel polymorphic form of celecoxib. **J Pharm Sci.** 2006; 95(2): 305-317.

33. Ramesh Panchagnula, Prakash Sundaramurthy, Omathanu Pillai, Shrutidevi Agarwal Yaswanth Ashokraj. Solid-state characterization of mefenamic acid**. J Pharm Sci.** 2004; 93(4): 1019-1029.

34. Schinzer WC, Bergren MS, Aldrich DS, Chao RS, Dunn MJ, Jaganathan A, Madden LM. Characterization and interconversion of polymorphs of premafloxacin, a new quinolone antibiotic. **J Pharm Sci.** 1997; 86(12): 1426-1431.

35. Lee M Katrincic, Yan T Sun, Robert A Carlton, Ann M Diederich, Ronald L Mueller, Frederick G Vogt. Characterization, selection and development of an orally dosed drug polymorph from an enantiotropically related system. **Int J Pharm**. 2009; 366: 1-13.

36. Antibiotics[Internet][updatedon21.10.2015]
Available from: https://en.wikipedia.org/wiki/Antibiotics

37. Hooper DC. Mode of action of fluoroquinolones. Drugs. 1999; 58(2): 6-10.

38. Antibiotics classification & mechanism.[Internet] [updated 2.12.2015]
Availablefrom:http://upload.orthobullents.com/topic/9059/images/ antibioticsmechanismofaction.jpg.

39. Fluoroquinolone. [Internet] [review on Jan-2015]
Availablefrom:http://msdmanuals.com/professional/infectious-disease/bacteria-andbacterial-drugs/fluoroquinolones.

40. Fluoroquinolone. General pharmacology.[Internet] [update on 16.04.2015]
Available from:http://www.urology-textbook.com/fluoroquinolones.html.

41. Conduct of Pharmacokinetic Studies in Animals. [Internet] [update on 3.1992]
Availablefrom:http://ec.ewropa.eu/health/files/eudralex/vol7/a/7ae3aen.pdf.

42. Conduct of pharmacokinetic studies in target animal species. [Internet] [update on 08.09.2000]
Availablefrom:http://www.ema.europa.eu/docs/enGB/documentlibrary/Scientificguideline/2009/10/WC5000+pdf

43. Metabolism and Pharmacokinetic studies. [Internet] [update on 22.10.2015]
Availablefrom:http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM07871.pdf.

44. Clinical Pharmacokinetic studies of pharmaceuticals.[Internet] [update on 24.10.2015
Available from: http//www.nihs.go.jp/phar/pdf/C1PkEng011122.pdf.

45. Chandrani Gunaratna, Meloney Cregor, Candice Kissinger. Pharmacokinetic and pharmacodynamic studies in rats using a new method of automated blood sampling. **Curr Separation.** 2000; 18(4): 153-157.

46. Ali Aghazadeh-Habashi, Saeed Sattari France M.Pasutto, Fakhreddin Jamali. Single dose pharmacokinetics and bioavailability of glucosamine in the rat**. J Pharm Sci.** 2002; 181: 181-184.

47. Donglu Zhang, Gang Luo, Xinxin ding, Chuang Lu. Pre clinical experimental models of drug metabolism and disposition in drug discovery and development. **Acta Pharm Sinica B.** 2012; 2(6): 549-561.

48. Guidance for industry: Bioequivalence studies with pharmacokinetic end points for drugs submitted order an ANDA.[Internet] [update on 08.07.2015].
Availablefrom:http://www.fda.gov/Drugs/GuidancecomplianceRegulatory Information/Guidance/default.html

49. Brahmankar DM, Sunil B Jaiswal. Biopharmaceutics and pharmacokinetics. New Delhi: Vallabh prakashan: 2005.

50. Ibrahim HG, Pisano F, Bruno A. Polymorphism of phenylbutazone: Properties and compressional behavior of crystals. **J Pharm Sci.** 1977; 66(5): 669-673.

51. Walkling WD, Almond H, Paragamian V, Batuyios NH, Meschino JA, Appino JB. Difenoxin hydrochloride polymorphism**. Int J Pharm.** 1979; 4: 39-46.

52. Lagas M, Lerk CF. The polymorphism of sulphathiazole. **Int J Pharm**. 1981; 8: 11-24.

53. Laszlo Borka, Eli Shefter. The polymorphism of triamcinolone diacetate. **Int J Pharm.** 1983; 16: 93-96.

54. Siegfried Lindenbaum, Elisabeth S Rattie, Gary E Zuber, Mary E Miller, Louis J Ravin. Polymorphism of auranofin. **Int J Pharm.** 1985; 26: 123-132.

55. Satoshi Kitamura, Luh-Chian Chang, Keith Guillory J. Polymorphism of mefloquione hydrochloride. **Int J Pharm.** 1994; 101: 127-144.

56. Martinez-Oharriz. Polymorphism of disflunisal: Isolation and solid-state characteristics of a new crystal form. **J Pharm Sci.** 1994; 83(2): 174-177.

57. Hiroaki Kitaoka, Chisa Wada, Reimei Moroi, Hideo Hakusui. Effect of dehydration on the formation of leveofloxacin pseudopolymorphs. **Chem Pharm Bull.** 1995; 43(4): 649-653.

58. Yuji Chikaraishi, Makoto Otsuka, Yoshihisa Matsuda. Preparation of amorphous and polymorph piretanide and their physicochemical properties and solubilites. **Chem Pharm Bull.** 1996; 44(8): 1614-1617.

59. Renli Teng, Dennis Girard, Thomas D Gootz, George Foulds, Theodore E Liston. Pharmacokinetics of trovafloxacin, a new quinolone, in rats, dogs and monkeys**. Am Soc Microbiol.** 1996; 40(3): 561-566.

60. Kristl A, Srcic S, Vrecer F, Sustar B, Vojnovic D. Polymorphism and pseudopolymorphism: influencing the dissolution properties of the guanine derivate acyclovir. **Int J Pharm.** 1996; 139: 231-235.

61. Kakkar AP, Manmohan Singh, Mendiratta. Isolation and characterization of ciprofloxacin-HCL crystals. **Drug Dev Ind Pharm.** 1997; 23(11): 1063-1067.

62. Mohamed A Hassan, Mutaz Sheikh salem, Mohammed S, Sueliman, Naji M.Najib. Characterization of famotidine polymorphic forms. **Int J Pharm.** 1997; 149: 227-232.

63. Dilraj Singh, Peter V, Marshali, Len Shields, Peter York. Solid-state characterization of chlordiazepoxide polymorphs. **J Pharm Sci**. 1998; 87(5): 655-662.

64. Llacer JM, Gallardo V, Parera M, Ruiz MA. Formation of ondansetron polymorphs. **Int J Pharm**. 1999; 177: 221- 229.

65. Jan-Olav Henck, Maria Kuhnert-Brandstatter. Demonstration of the terms enantiotropy and monotropy in polymorphism research exemplified by flurbiprofen. **J Pharm Sci**. 1999; 88(1): 103-108.

66. Zornoza A, Deno, Martin C, Goni M, Martinez Oharriz MC, Velaz I. Evidence for polymorphism in glisentide. **Int J Pharm**. 1999; 186: 199-204.

67. Rustichelli C, Gamberini G, Ferioli V, Gamberini MC, Ficarra R, Tommasini S. Solid state study of polymorphic drugs: carbamazepine**. J Pharm Biomed Anal.** 2000; 23: 41-54.

68. Vrecer F, Vrbine M, Meden A. Characterization of piroxicam crystal modification. **Int J Pharm.** 2003; 256: 3-15.

69. Rahul V Manek, William M Kolling. Influence of moisture on the crystal forms of niclosamide obtained from acetone and ethyl acetate. **AAPS Pharm Sci Tech.** 2004; 5(1): 1-8.

70. Elquidra R, Unlu N, Capan Y, Sahin G, Dalkara T, Hincal AA. Effect of polymorphism on *in-vitro, in-vivo* properties of carbamazepine conventional tablets. **J Drug Del Sci Tech**. 2004; 14(2): 147-153.

71. Tofilo Mazon Cardoso, Patrik Oening Rodrigues, Hellen Karine Stulzer, Marcos Antonio Segatto Silva. Physical-Chemical Characterization and polymorphism determination of two nimodipine samples deriving from distinct laboratories. **Drug Dev Ind Pharm.** 2005; 31: 631-637.

72. Barbas R, Prohens R, Cristina puigjaner. A new polymorph of norfloxacin complete characterization and relative stability of its trimorphic system. **J Therm Anal Calorim.** 2007; 89(3): 687-692.

73. Mange Ramyadev, Anwar R Shaikh, **Ganesan V**, Rajani Giridhar, Renu Chandha. Studies on the crystal forms of pefloxacin: preparation, charactgerization and dissolution profile. **J Pharm Sci.** 2008; 97(7): 2637-2648.

74. Antonio Llinas, Jonathan C, Burley, Timothy J Prior, Robert C Glen, Jonathan M Goodman. Concomitant hydrate polymorphism in the precipitation of sparfloxacin from aqueous solution**. Cryst Growth Des.** 2008; 8(1): 114-118.

75. Nighute AB, Blise SB. Preparation and evaluation of microcrystals of cefuroxime axetil. **Int J Pharm Tech Res.** 2009; 1(3): 424-430.

76. Mahalaxmi R, Ravi Kumar, Shivanand Pandey, Arun Shirwaikar, Annie Shirwaikar. Effect of recrystallisation on size, shape, polymorph and dissolution of carbamazepine. **Int J Pharm Tech Res.** 2009; 1(3): 725-732.

77. Maria Klimakow, Klaus Rademann, Franziska Emmerling. Toward novel pseudo-polymorphism of nifedipine: Elucidation of a slow crystallization process. **Cryst Growth Des.** 2010; 10: 2693-2698.

78. Alok Tripathi, Joshi BS, Joshi. Polymorphism in rabeprazole sodium**. Rasayan J Chem.** 2010; 3(4): 736-744.

79. Renu Chanda, Poonam Arora, Rupinder Kaur, Anupam Saini, Jain DVS. Crystal modification of 5-flurouracil preparation and characterization. **J Pharm Educ Res.** 2010; 1(2): 77-82.

80. Modha NB, Chotai NP, Patel VA, Patel BG. Preparation, characterization and evaluation of fluconazole polymorphs**. Int J Res Pharm Biomed sci.** 2010; 1(2): 124-127.

81. Mohd R Abu Bakar, Zoltan K Nagy, Chris D Rielly, Sandy E Dann. Investigation of the riddle of sulfathiazole polymorphism. **Int J Pharm.** 2011; 414: 86-103.

82. Pandu Ranga Rao V. Preparation of stable new polymorphic form of atorvastatin calcium. Scholars Research Library, **Der Pharm Lett.** 2011; 3(5): 48-53.

83. Wei Da, Yuefang Zhou, Yafei Gong, Chunshun Zhao. Investigation of physico-chemical properties and *in-vitro, in-vivo* evaluation of agomelatine polymorphs. **Asian J Pharm Sci**. 2013; 8: 181-190.

84. Anchal Kulshrestha, Dhaval Joshipura. Polymorphism in fexofenadine hydrochloride. **Der Pharma Chemica.** 2013; 5(1): 279-283.

85. Marco Farias and Renato Carneiro. Simulataneous quantification of three polymorphic forms of carbamazepine in the presence of excipients using raman spectroscopy. **Molecules.** 2014; 19: 14128-14138.

86. Aline Quelian Penna Garbuio, Tassia Hanashiro, Blanca Elena Ortega Markman, Fernado Luiz Affonoso Fonseca, Fabio Ferreira Perazzo, Paulo Cesar Pires Rosa. Evaluation and study of mebendazole polymorphs present in raw materials and tablets available in the Brazilian Pharmaceutical market. **J Appl Pharm Sci.** 2014; 4(11): 001-107.

87. Natalia L Calvo, Teodoro S Kaufman, Ruben M Maggio. A PCA-based chemometrics-assisted ATR-FTIR approach for the classification of polymorphs of cimetodine: Application to physical mixtures and tablets. **J Pharm Biomed Anal.** 2015; 107: 419-425.

88. Rajamma AJ, Sateesha SB, Narode MK, Prashanth VRSS and Karthick AM. Preparation and crystallographic analysis of gliclazide polymorphs. **Indian J Pharm Sci**. 2015; 24: 34-40.

89. Prulifloxacin [Internet] modified 2015 sep 11.
Available from: http://en.wikipedia.org/wiki/prulifloxacin

90. Shadong Panxin Chemical Co. Ltd [Internet] 2015.
Available from: www.foreign-trade.com/showcase.cfm?c=cathy1108&p=123842.

91. Prulifloxacin|CAS 123447-621|Santa Cruz Biotech [Internet] 2015.
Available from: www.scbt.com/datasheet-204868-prulifloxacin.html.

92. Gemifloxacin mesylate [Internet] modified 11.9.2015.
Available from: http://en.wikipedia.org/wiki/gemifloxacin

93. Gemifloxacin mesylate|CAS 210353-53-0|Santa Cruz Biotech [Internet] 2015.
Available from: www.scbt.com/datasheet-211557-gemifloxacin-mesylate.html.

94. Lookchem, Look for chemical all over the world [Internet] 2015.
Available form: www.lookchem.com/cas-175463-14-6.html.

95. Indian Pharmacopoeia. Vol I. Ghaziabad: The Indian Pharmacopoeia Commission; 2014.

96. **Ganesan V,** Punitha S. Sperical crystals of moxifloxacin: To improve solubility, dissolution rate and micromeritics properties. **Asian J Pharm Res Dev.** 2013: 1(6): 41-51.

97. Venkadari Rammohan Gupta, Srinivas Mutalik, Madhobhai M Patel, Girish K Jani. Spherical crystals of celecoxib to improve solubility, dissolution rate and micromeritics properties. **Acta Pharm.** 2007; 57: 173-184.

98. Ferdinando Giordano, Andrea Gazzaniga, Jose Ramon Moyano, Paolo Ventura, Margherita Zanol, Tiziana Peveri, Lorenza Carima. Crystal forms of piroxicam pivalate: preparation and characterization of two polymorphs. **J Pharm Sci.** 1998; 87:333-337.

99. Palash Sanphul, Bipul Sarma, Ashwini Nangia. Phase transformation in conformational polymorph**. J Pharm Sci**. 2011; 100(6): 2287-2299.

100. William L Rocco, Jon R Swanson. Win 63843 polymorphs: prediction of enantiotropy. **Int J Pharm**. 1995; 117: 231-236.

101. Selma Sehic, Gabriele Betz, Sejerzada Hadzidedic, Silvia Kocova El-Arini, Hans Leuemberger. Investigation of intrinsic dissolution behavior of different carbamazepine samples. **Int J Pharm.** 2010; 386: 77-90.

102. Elisabetta Maccaroni, Enrica Alberti, Luciana Malpezzi, Norberto Masciocchi, Chiara Valdiskovic. Polymorphism of linezolid: A combined single-crystal, powder diffraction and NMR study. **Int J Pharm.** 2008; 351: 144-151.

103. Marion WY Wong, Alan G Mitchell. Physicochemical characterization of a phase change produced during the wet granulation of chlorpromazine hydrochloride and its effect on tableting. **Int J Pharm.** 1992; 88: 261-273.

104. Wang Y, Wenslow RM, Mc Cauley JA, Crocker LS. Polymorphic behavior of an NK1 receptor anatagonist. **Int J Pharm.** 2002; 243: 147-159.

105. Christopher Buxton P, Ian R, Lynch and Johan M. Roe. Solid-state forms of paroxetine hydrochloride. **Int J Pharm**.1988; 42: 135-143.

106. Renu Chandha, Poonam Arora, Rupinder Kaur, Anupam Saini, Madan Lal Singla, Dharamvir singh Jain. Characterization of solvatomorphs of methotrexate using thermoanalytical and other techniques. **Acta Pharm.** 2009; 59: 245-247.

107. Xiaohong Pan, Thomas Jullian, Larry Augsburger. Increasing the dissolution rate of a low-solubility drug through a crystalline-amorphous transition: A case study with indomethicin. **Drug Dev Ind Pharm.** 2008; 34: 221-231.

108. Gul Majid Khan, Zhu Jiabi. Preparation, characterization and evaluation of physicochemical properties of different crystalline forms of ibuprofen. **Drug Dev Ind. Pharm.** 1998; 24(4): 463-471.

109. Yadav AV, Dabka AP, Shete AS. Crystal engineering to improve physicochemical properties of mefloquine hydrochloride. **Drug Dev Ind Pharm.** 2010; 36(9): 1036-1045.

110. Hiroyuki Imaizumi, Naoki Nambu, Tsueji Nagai. Stability and several physical properties of amorphous and crystalline forms of indomethacin. **Chem Pharm Bull.** 1980; 28(9): 2565-2569.

111. Srinivas Mutalik, Parambil Anju, Krishnan Manoj, Achutha Nayak Usha. Enchancement of dissolution rate and bio-availability of aceclofenac: A chitosan-based solvent change approach. **Int J Pharm.** 2008; 350: 279-290.

112. Dengning Xia, Fude Cui, Hongze Piao, Dongmei un, Hongyu Piao, Yanboo Jiang, Mei Onyang, Peng Quan. Effect of crystal size on the *in-vitro* dissolution and oral absorption of nitrendipine in rats. **Pharm Res.** 2010; 27: 1965-1976.

113. Ravisankar P, Devala Rao G, Devadasu Ch, Sudhakar Saibabu G, Srinivasa Babu P. A validated RP-HPLC method for the assay of prulifloxacin in marketed drug product using levofloxacin as an internal standard. **Int J Chem Sci.** 11(1): 95-105.

114. Bruno C Hancock, Glenn T Carlson, Dauda D Ladipo, Beth A Langdon, Matthew P Mullarney. Comparison of the mechanical properties of the crystalline and amorphous forms of a drug substance. **Int J Pharm.** 2002; 241: 73-85.

115. Hadi A Garekani, James L Ford, Michael H Rubinstein, Ali R Rajabi-Siahboomi. Formation and compression characteristics of prismatic polyhedral and thin plate-like crystals of paracetamol**. Int J Pharm.** 1999; 187: 77-89.

116. Liang Fang, Sachihiko Numajiri, Daisuke Kobayashi, Hideoueda, Koji Nakayama, Hiroshi Miyamae, Yasunori Morimoto. Physicochemical and crystallographic characterization of mefenamic acid complex with alkanolamines. **J Pharm Sci.** 2004; 93(1): 144-154.

117. Yukiko Umeda, Toshiro Fukami, Takayuki Furuishi, Toyofumi Suzuki, Katsuhisa Tanjoh and Kazuo Tomono. Characterization of multicomponent crystal formed between indomethacin and lidocaine. **Drug Dev Ind Pharm.** 2009; 35(7): 843-851.

118. Gary Nichols, Christopher S Frampton. Physicochemical characterization of the orthorhombic polymorph of paracetamol crystrallized from solution. **J Pharm Sci.** 1998; 87(6): 684-693.

119. Mino R Caira, Giampiero Bettinetti, Milena Sorrenti, Laura Catenacci. Relationships between structural and thermal properties of anhydrous and solvated crystalline forms of brodimoprim. **J Pharm Sci.** 2007; 96(5): 996-1007.

120. Agatonovic-Kustrin S, Wu V, Rades T, Saville D, Tuker I G. Powder diffractometric assay of two polymorphic forms of ranitidine hydrochloride. **Int J Pharm.** 1999; 184: 107-114.

121. Jarring K, Larsson T, Stenslanol B, Ymen I. Thermodynamic stability and crystal structures for polymorphs and solvates of formoterol fumarate. **J Pharm Sci.** 2006; 95(5): 1144-1161.

122. Veerendra K Nanjwade, Manvi FV, Shamrez Ali M, Basavaraj K, Nanjwade. Development and evaluation of prulifloxacin tablet. **Int J Drug Formul Res.** 2011; 2(6): 302-314.

123. Lim Yee Lyn, Huan Wen Sze, Adhiyaman Rajendran, Gorajana Adinarayana, Kamal Dua, Sanjay Garg. Crystal modification and dissolution rate of piroxicam. **Acta Pharm.** 2011; 61: 391-402.

124. Vinod Labhasetwar, Deshmukh SV, Dorle AK. Studies on some crystalline forms of ibuprofen. **Drug Dev Ind Pharm.**1993; 19(6): 631-641.

125. **Vellaichamy Ganesan**, Raju Thange, Naresh Vinjamuri, Srinivasarao Prathipati. Crystal forms of lomifloxacin: Preparation, characterization and dissolution profile. **Indonesian J Pharm.** 2013: 24(4): 238-244.

126. Sabiruddin Mirza, Inna Miroshnyk, Jyrki Heinamaki, Osmo Antikainen, Jukka Rantanen, Pia Vuorela, Heikki Vuorela, Jouko Yliruusi. Crystal morphology engineering of pharmaceutical solids: tabletting performance enhancement. **AAPS Pharm Sci Tech.** 2009; 10(1): 113-119.

127. Gee T, Andrews JM, Ashby JP, Marshall G Wise R. Pharmacokinetics and tissue penetration of gemifloxacin following a single oral dose. **J Antimicrob Chemother.** 2001; 47: 431-434.

128. Ann Alen, Elizabeth Bygate, Stuart Oliver, Martin Johnson, Christopher ward, Ae-Jin Cheon, Youn Sung Choo, In-chull Kim. Pharmacokinetics and tolerability of gemifloxacin after administration of single oral doses to healthy volunteers. **Antimicrob Agent Chemother.** 2000; 44(6): 1604-1608.

129. Yunoos Mohammad, Pragati Kumar, Azmath Hussain, Harish. Development and validation of RP-HPLC method for the estimation of gemifloxacin mesylate in bulk and pharmaceutical dosage forms. **E-J Chem.** 2010; 7(4): 1621-1627.

130. Puneet Sharma, Zoran D Zujovic, Graham A Bowmaker, William A Denny, Sanjay Garg. Evaluation of a crystalline nanosuspension: Polymorphism, process induced transformation and *in-vivo* studies. **Int J Pharm.** 2011; 408: 138-151.

131. Van Tonder EC, Caira MR, Botha SA, Lotter AP. Polymorphism of doxylamine succinate and X-ray structural study of Form-I and of doxylamine succinate 0.5 succinic acid. **Int J Pharm.** 1990; 63: 35-42.

132. Dominic P, Gerald S Brenner, James M Stevenson, Siegfried Lindenbaum, Alan W Doughlas, S David Klein, James A McCauley. High resolution spectroscopic evidence and solution calorimetry studies on the polymorphs of enalapril maleate. **Int J Pharm.** 1986; 28: 183-191.

133. Getsoian A, Lodaya RM, Blackburn AC. One-solvent polymorph screen of carbamazepine. **Int J Pharm.** 2008; 348: 3-9.

134. Ranjit Dash, Ajith Kumar Acharya, Sanysi Swan, Mayank Barg, Hemant Kumar Choudhary, Khageswar Meher. Formation and evaluation of spherical crystal of etoricoxib. **Int J Pharm Biol Arch**. 2011; 2(4): 1123-1129.

135. Pandit JK, Gupta SK, Gode KD, Mishra B. Effect of crystal form on the oral absorption of phenylbutazone. **Int J Pharm.** 1984; 21: 129-132.

136. Walter Cabri, Pado Ghetti, Marco Alpegiani, Giovanni Pozzi, Angel Justo-Erbez, Jose Ignacio Perz-Martinez, Rosario Villalon-Rubio, Carmen Monedero-Perales M, Angel Munoz-Ruiz. Cefdinir: A comparative study of anhydrous vs monohydrate form micro structure and tabletting behavior**. European J Pharm Bio Pharm.** 2006; 64: 212-221.

137. Praveen Chaudhari, Pravin Uttekar, Nishant Waria, Amit Ajab. Study of different crystal habits formed by recrystallisation process and study effect of variables. **Res J Pharm Tech**. 2008; 1(4): 381-385.

138. Takeshi Uchida, Etsuo Yonemochi, Toshio Oguchi, Katsuhide Terada, Keiji Yamamoto, Yoshinobu Nakai. Polymorphism of tegafur: Physico-chemical properties of four polymorphs**. Chem Pharm Bull.** 1993; 41(9): 1632-1635.

139. Christian Nather, Inke Jeb. Investigations on the polymorphism and pseudopolymorphism of clobetasone butyrate. **Collect Czech Chem Commun.** 2011; 76(5): 553-566.

140. Ensio Laine, Paavo Kahela, Riitta Rajala. Terttu Heikkila, Kirsti Saarnivaara, Ilse Piippo. Crystal forms and bioavailability of erythromycin. **Int J Pharm.** 1987; 38: 33-38.