ENHANCEMENT OF SOLUBILITY AND DISSOLUTION PROPERTY OF RAMIPRIL BY NANOCRYSTALLIZATION

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI-32

In partial fulfillment of the requirement

for the award of degree of

MASTER OF PHARMACY IN PHARMACEUTICS

Submitted By

(Reg. No: 261211301)

DEPARTMENT OF PHARMACEUTICS

COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE

MADURAI – 625 020

APRIL – 2014

CERTIFICATE

بسم الله الرحمن الرحيم
In the Name of Allāh, the Most Gracious, the Most Merciful

Prof. Dr. A. ABDUL HASAN SATHALI, M.Pharm., Ph.D,
Principal (i/c),
College of Pharmacy,
Madurai Medical College,
Madurai-625 020, (TN), India.
Email: drabdulhasan@rediffmail.com
Mob: 09443475400

Res: 19, Nallamani Nagar,
Lordu Nagar 12th Street,
K-Pudur,
Madurai – 625007. (TN), India.

CERTIFICATE

This is to certify that the dissertation entitled**, "Enhancement of Solubility and Dissolution Property of Ramipril by Nanocrystallization"** submitted by **Mr.P.Arjunkumar (M. Pharm II year)**, in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by him, under my guidance and supervision in the Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai-20 during the academic year 2013 – 2014.

This dissertation is forwarded to the Controller of Examinations, The Tamilnadu Dr. M.G.R. Medical University, Chennai-32.

Place : Madurai

Date :

(**Prof. Dr. A. ABDUL HASAN SATHALI)**

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

It is my pleasure to express my respectful regards and thanks to ***Dr. B. Santhakumar, M.Sc(F.Sc), M.D(F.M)., PGDMLE., Dip.N.B(F.M)*** *Dean, Madurai Medical College, Madurai for providing all kinds of supportive facilities required to carry out my project work.*

It is my immense pleasure and honour to express my deep sense of gratitude and heartfelt thanks to ***Prof. Dr. A. Abdul Hasan Sathali, M.Pharm., Ph.D.,*** *Principal (i/c)****,*** *College of pharmacy, Madurai Medical College, Madurai, for his excellence in guidance, contribution and encouragement which helped me in the successful completion of each and every stage of my project work.*

I express my heartiest thanks to ***Dr.Reddy's Laboratories, Hydrabad*** *for providing the drug Ramipril as gift sample and* ***Steril-gene Life science (P) Ltd, Pondicherry*** *(HPMC K15M) and* ***Shasun Pharmaceuticals, Pondicherry*** *(PVPK30) and* ***Nice chemicals, Cochin*** *(β-CD) and* ***Rankem fertilizers and chemicals (P) Ltd, New Delhi*** *(*Sodium Lauryl Sulphate) *for providing chemicals to carry out my project work.*

With immense pleasure I record here sincere and hearty thanks to ***teaching and non teaching staff*** *of Department of pharmaceutics for their support and valuable suggestions throughout my work.*

I also thank ***P.S.G. College of Pharmacy, Coimbatore, Karunya University, Coimbatore, and J.S.S College of Pharmacy, Ooty,*** *for their help in carrying out the evaluation (IR, DSC, Particle size, Zeta potential, SEM and X-ray diffraction) studies.*

I would like to give my sincere thanks to my classmates ***Mr. P. Kanniyappan., Mr. A. Manikkavasagan., Mrs. S. Ponnammal Asmi., Mr. C. Pravinkumar., Mr. J.Rajeshkumar., Mr. M. Ramanathan., Mr. Sankar Ganesh., Mr. S. Sudhakar*** *for their timely help and co-operation.*

I would like to thank my seniors and juniors for their moral support to carry out my project work.

I also extend my thanks to all the staff members and P.G. Students of Department of Pharmaceutical Chemistry and Pharmacognosy for their Co-operation.

I would like to express my heartfelt thanks to ***my parents, brother and sisters*** *for their moral support to successfully carryout my project work.*

I am extremely thankful to the staff of ***Laser Point*** *for their kind co-operation regarding printing and binding of this project work.*

Place : Madurai

Date :

(ARJUNKUMAR. P)

CONTENTS

CONTENTS

CHAPTER I

INTRODUCTION

CHAPTER-I

INTRODUCTION

Nanotechnology is derived from the Latin word “nano”, which means dwarf. One nanometer (nm) is equal to one-billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules. A human hair is approximately 80,000 nm wide, and a red blood cell is approximately 7000 nm wide. Atoms are smaller than 1 nm, whereas many molecules including some proteins range between 1 nm and larger.

The conceptual underpinnings of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman in his lecture, There’s plenty of room at the bottom. Feynman explored the possibility of manipulating material at the scale of individual atoms and molecules, imagining the whole of the Encyclopedia Britannica written on the head of a pin and foreseeing the increasing ability to examine and control matter at the nanoscale. The term nanotechnology was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to engineer materials precisely at the nanometer level. The primary driving force for miniaturization at that time came from the electronics industry, which aimed to develop tools to create smaller (and therefore faster and more complex) electronic devices on silicon chips.

In medicine and pharmaceutics, nanotechnology is used to improve human health at a molecular level. The novel and potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy. The advantages of nanotech drugs compared to conventional counterparts lie on the basis of particle size. Drugs/drug products with nano dimension can be used at a lower concentration and can lead to early onset of

bioactivity. Nano drug delivery systems (nanopharmaceutics) are, but not limited to, nanocapsules, nanospheres, nanosponges, nanoemulsions, solid lipid nanoparticles, nanovesicular systems (liposomes, niosomes), molecular systems (inclusion complexes) and nanocrystals.

Absorption of a drug is defined as the transition of a drug from the applied place to the blood and/or lymphatic circulation. The amount of absorbed active drug substance depends on physicochemical properties of the active drug substance, pharmaceutical dosage form and physiological characteristics. For complete absorption of the active drug substance from the gastrointestinal tract (GI), it must be completely dissolved **(Figure 1)**. However, most of the new chemical compounds developed as a drug have poor solubility in water. Water solubility of 40% of the active drug substances that are currently in use and 60% of the active drug substances that are at the investigation stage is very low. Low water solubility limits absorption and bioavailability of these drugs. For oral administration, conventional formulations of poorly water-soluble drugs are associated with erratic absorption in the GI tract and low/variable bioavailability.

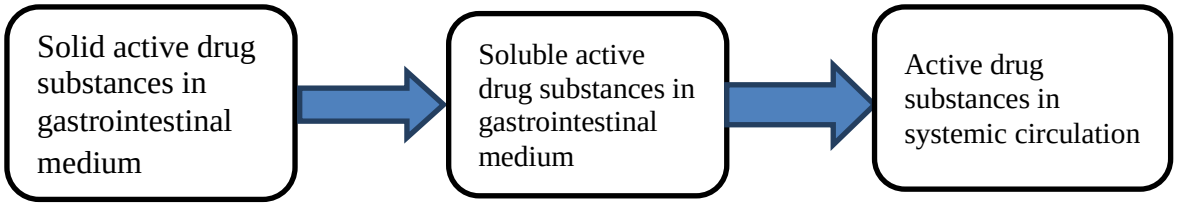


FIGURE 1: SCHEMATIC REPRESENTATION OF EVENTS THAT AN ACTIVE DRUG SUBSTANCE FACES IN THE GI MEDIUM

(Tugba Gulsun *et al*., 2010)

Thus, bioavailability of poorly water-soluble drugs will be affected positively when their dissolution rate is increased. These drugs show serious adverse clinical effects like non-steady absorption due to variability among patients and individual

patient dosing. Drugs which have high permeability but low solubility (Class II according to Biopharmaceutics Classification System) are not easily dissolved so they may not be absorbed from the GI tract sufficiently. Moreover, such drugs incorporated into conventional dosage forms are usually affected by the fasted or fed state of the patient. This situation eventually causes inappropriate dosing and low bioavailability.

Pharmaceutical approaches

For improving solubility of drugs, in order to enhance oral bioavailability. Solubilization of poorly water-soluble drugs increases dissolution rate and absorption leading to a significant improvement of drug bioavailability. Approaches to improve the solubility or to increase the available surface area for dissolution are classified as physical and chemical modifications.

- Physical modifications are decreasing particle size (micronization, nanosuspensions), formation of polymorphs/pseudopolymorphs (including solvates), complexation/solubilization (use of surfactants or cyclodextrines, addition of cosolvents) and preparation of drug dispersions in carriers (eutectic mixtures, non-molecular solid dispersions, solid solutions).
- Chemical modifications are synthesis of soluble prodrugs and salts.

Approaches such as adding a surfactant/cosolvent, complexation with cyclodextrins or/and preparing oil–in-water emulsions for intravenous applications have been developed for poorly water-soluble drugs, but these approaches have limited application since the active drug substance must have specific physicochemical properties (for example, cyclodextrins must have suitable molecular weight for optimal conjugation of the conical structure with the drug for such applications to be successful).

Solid dispersions are theoretically one of the appropriate methods for increasing dissolution rate, but molecules in the amorphous state are not thermodynamically stable; they can convert to the crystal form during storage. The use of surfactants or cosolvents sometimes leads to increased side effects and toxic reactions in the body. Potential disadvantages of salt forms include, high reactivity with atmospheric carbon dioxide and water resulting in precipitation of the poorly water-soluble drug. Polymorphs are different crystalline forms of a drug that may have different physicochemical properties and biological activities with respect to morphology, density, melting point, hardness, compression, solubility and bioavaliability. Therefore, the preparation of actual drug polymorphs is crucial during preformulation studies. An alternative drug delivery approach called micronization, has been developed to overcome poor solubility in water. Micronization of poorly soluble drugs, increases the dissolution rate of the drug due to the increase in surface area, but does not change the saturation solubility. In order to increase solubility and oral bioavailability, going down to the micron level may sometimes not be sufficient, so the next step, going down to the nano level, may be necessary.

Dissolution Rate vs. Particle Size

Intrinsic solubility can be explained as the number of moles of a substance per liter which dissolves in a particular solvent. Thus, dissolution is the process by which a solid substance dissolves. There are many factors that effect solubility; such as pH, cosolvent, surfactants, temperature and particle size. By changing these factors, solubility of active drug substances can be modified. Dissolution rate has been first described by Noyes and Whitney in 1897. In 1904, Nernst and Brunner explored the dissolution rate constant and diffusion coefficient of solutes. According to the Nernst Brunner equation, dissolution rate is proportional to the surface area of the active drug substance in contact with the dissolution medium.

$$Dw/dt = D/h \times S (Cs - Ct)$$

Where,

dw/dt: Dissolution rate (mg/s)

S: Effective surface area of the solid drug (cm2)

Cs-Ct: Concentration gradient (mg/mL)

Cs: Saturated concentration(mg/mL)

Ct: Concentration of the solute at time t (mg/mL)

h: Effective diffusion layer thickness (cm)

D: Diffusion coefficient (cm/s)

It can be deduced from the formula that changing particle size of the active drug substance, it is possible to change the specific surface area and the dissolution rate of the active drug substance in body fluids **(Figure 2)**. With this basic information in hand, it is clear that the dissolution rate and bioavailability of poorly water soluble drugs can be increased by decreasing the particle size of active drug substances. Additionally, particle size reduction results in a decrease in the diffusion layer thickness surrounding the drug particles and an increased concentration gradient between the surface of the drug particles(Tugba gulsun *et al*., 2009).

Total surface area 6cm^2 Total surface area 12cm^2 Total surface area 24cm^2

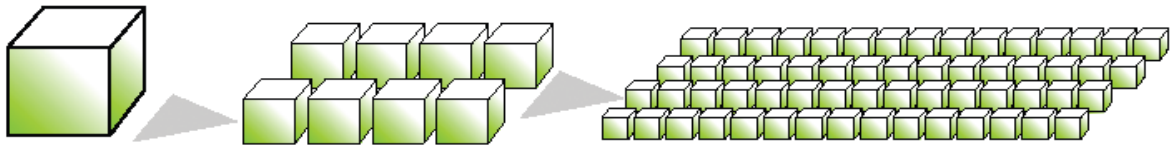


FIGURE 2: SURFACE AREA ENLARGEMENT BY PARTICLE SIZE REDUCTION NANONIZATION STRATEGIES FOR POORLY WATER SOLUBLE DRUGS (Huabing Chen *et al*., 2011)

Nanonization of hydrophobic drugs generally involves the production of drug nanocrystals through either chemical precipitation or disintegration. Alternatively, nanotechnology-based drug delivery systems such as nanoemulsions and polymeric micelles can be used. During the past decade, several drug nanoformulations have been clinically approved or are under clinical investigation. Major research efforts have been focused on the development of enabling nanoformulation technologies, new pharmaceutical materials and quality control to improve product properties while reducing production costs. New technological advances and unmet clinical needs provide the key driving force for the research and development of nanonization strategies.

a) Drug nanocrystals

Drug nanocrystals are nanoscopic crystals of the parent compound with dimensions less than 1 mm. According to the Noyes–Whitney equation , a decrease in particle size will lead to an increase in effective surface area in the diffusion layer, which, in turn, increases the drug dissolution rate. Drug nanocrystals are one of the most important strategies to enhance the oral bioavailability of hydrophobic drugs. Several following preparation methods for drug nanocrystals have been investigated they are,

- Nanoprecipitation,
- High-pressure homogenization and
- Media milling.

b) Nanoemulsions

Nanoemulsions are a nonequilibrium, heterogeneous system consisting of two immiscible liquids in which one liquid is dispersed as droplets in another liquid. Emulsions with nanoscopic droplet sizes (typically in the range of 20–200 nm) are

often referred to as submicron emulsions. Nanoemulsions are composed of oil droplets dispersed in an aqueous medium and stabilized by surfactant molecules **(Figure 3)**. Although nanoemulsions have a tendency for phase separation, kinetically stable nanoemulsions can be achieved with sufficient shelf stability and no apparent flocculation or coalescence. Advantages of nanoemulsions include increased drug loading and enhanced bioavailability. Commercial products that are nanoemulsions include Estrasorb1 and Flexogan1.

In a nanoemulsion, the oil droplets serve as the reservoir for hydrophobic drugs. The most widely used oil molecules include saturated and unsaturated fatty acids, fatty acid esters and soybean oils. Surfactant molecules play a key part in stabilizing the nanoemulsions. Nonionic or amphoteric surfactants such as poloxamer, lecithin and Tween 80 are commonly used. Combinations of various surfactants have also been used to control droplet size and improve the stability of nanoemulsions. The methods used for the production of nanoemulsions include

- High pressure homogenization,
- Microfluidization,
- Ultrasonication and
- Spontaneous emulsification.

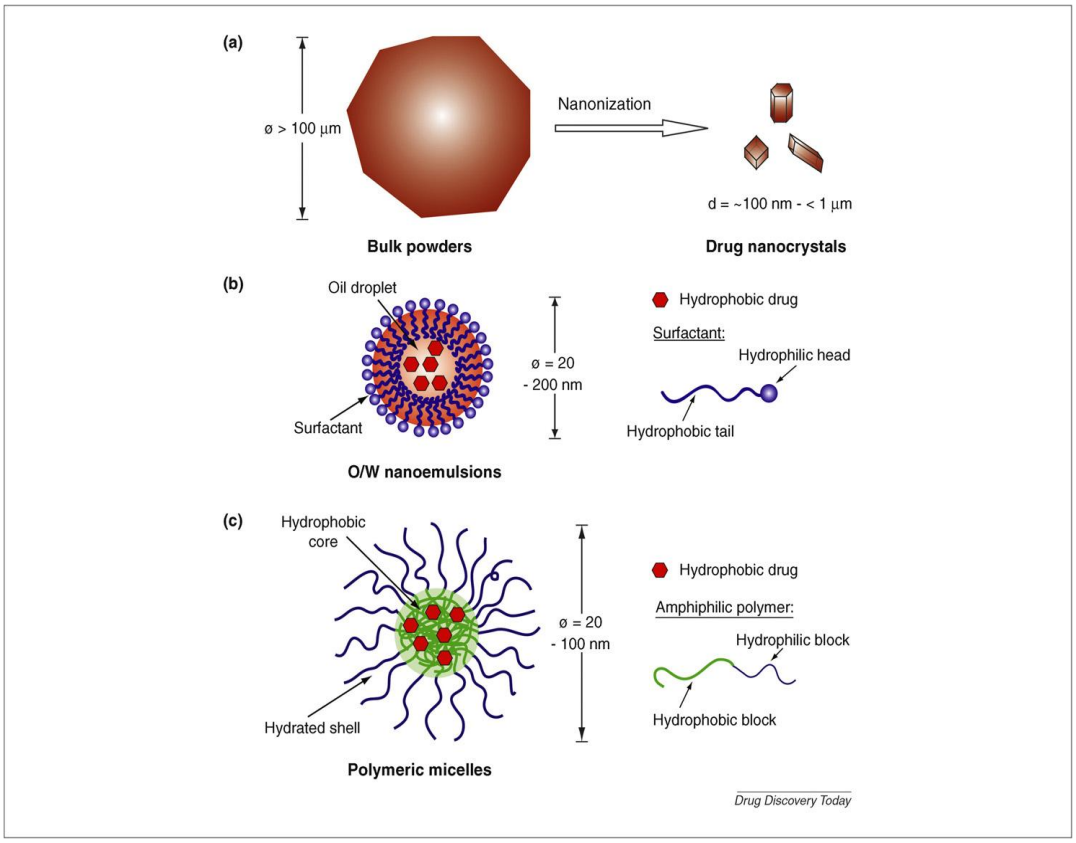


FIGURE 3: SCHEMATIC OF DIFFERENT NANONIZATION STRATEGIES TO INCREASE DRUG SOLUBILITY AND BIOAVAILABILITY

c) Polymeric micelles

Polymeric micelles have received considerable attention in the past two decades as a new multifunctional nanoplatform for the delivery of hydrophobic drugs. Polymeric micelles are nanosized (typically in the range of 20–100 nm) supramolecular constructs **(Figure 3)** formed from the self-assembly of amphiphilic block copolymers in aqueous environments. In water, the hydrophobic segment of the block copolymer self associates into a semisolid core, with the hydrophilic segment of the copolymer forming a coronal layer. The resulting core–shell architecture is important for drug delivery purposes; the hydrophobic core serves as a reservoir for water-insoluble drugs, and the outer shell protects the micelle from rapid clearance in circulation.

Poly(ethylene glycol) (PEG) is most commonly used for the hydrophilic segment. PEG molecules are biologically inert. Moreover, they are shown to prevent nonspecific protein adsorption to the micelle surface prolonging the blood circulation time of the micelles. Other polymers, such as poly(N-vinyl pyrrolidone) and poly(N-isopropyl acrylamide), are also used as hydrophilic blocks but with much less frequency. Compared with the hydrophilic blocks, the chemistry of core-forming hydrophobic polymers is much more diverse. Polyesters and poly(L-amino acids) are the most widely used polymers because of their biocompatibility and biodegradability. Examples include, but are not limited to, poly(lactic acid) (both L-isomer, or PLA and D,L-isomer, or PDLLA); poly(e caprolactone); poly(L-aspartic acid) (pAsp); and poly(L-glutamic acid). Recently, a new hydrotropic polymer design was reported to form polymeric micelles with high drug loading and excellent physical stability. This process involves the screening of hundreds of pharmaceutically safe molecules to identify candidate structures that enable heightened solubility for a chosen drug; then, the structural motif is incorporated as the hydrophobic segment to enhance its interactions with the drug. Using this approach, Park and co-workers have developed hydrotropic copolymers consisting of PEG and poly(4-(2-vinylbenzyloxy-N-picolylnicotinamide)) that provide efficient encapsulation of paclitaxel with high loading . This method might provide a universal strategy to produce tailor-made polymeric micelles that can achieve high drug loading and stable encapsulation of a wide variety of drugs. The methods used for the production of polymeric micelles include

- ✓ Dialysis,
- ✓ Solvent evaporation and
- ✓ Film sonication.

COMPARISON OF DIFFERENT NANOFORMULATION STRATEGIES

Currently, the formation of drug nanocrystals is the most established technique among the three strategies discussed in this review, with multiple clinically approved products. Large-scale production of drug nanocrystals is feasible with excellent reproducibility. This technique can formulate drugs with a wide range of solubility profiles, including drugs that are not soluble in either water or oils. The precipitation methods can also formulate drugs into amorphous or semicrystalline nanoparticles, whereas homogenization and media milling methods work better with drugs that have a high degree of crystallinity.

Drug nanocrystals also have fast dissolution rates, which make them an excellent choice for oral delivery. However, this strategy sometimes requires high-energy input, resulting in high production costs. Moreover, formulated nanocrystals often require surface stabilization. Owing to the fast dissolution kinetics nanocrystal formulations are not suitable for cytotoxic drugs with small therapeutic indices such as anticancer agents.

Nanoemulsions offer some crucial benefits over other nanonization techniques. High drug-loading content can be achieved easily using many clinically approved pharmaceutical ingredients (e.g. small molecular surfactants, lipids and oils). The production process is also inexpensive. Nanoemulsions are used for topical administration with several clinically approved products. Other routes of administration for drugs with large therapeutic indices are also used clinically. Drug nanoemulsions often suffer from poor stability, with the possibility of flocculation and coalescences upon storage. The lack of a controlled release mechanism is also a limitation for this nanoformulation technique to deliver cytotoxic agents.

Polymeric micelles have been explored extensively in the past decade because they can achieve improved blood stability and have excellent controlled release

properties. The higher hemostability of micelles allows prolonged circulation, enabling passive and active targeting to tumors for cancer treatment. In addition, a multifunctional design for polymeric micelles can also be achieved by incorporating imaging agents and therapeutic agents in the same micelle. Polymeric micelles are also suitable for intravenous administration to deliver a variety of cytotoxic drugs, a potential advantage over nanocrystals and nanoemulsions in cancer chemotherapy. The disadvantages of micelles include concerns over the safety of polymer carriers; only a few polymers, such as PLA, are clinically approved.

CHAPTER II

NANOCRYSTAL TECHNOLOGY – A REVIEW

CHAPTER-II

NANOCRYSTAL TECHNOLOGY – A REVIEW

Preparation of drug nanocrystals is basically a nanosizing method, which is utilized to enhance the oral bioavailability of poorly water-soluble drugs. Drug nanocrystals are nanoscopic crystals of the drug with dimensions less than 2000 nm as defined in the first patents in this field. Nanocrystal dispersions contain dispersion media (water, aqueous solutions or nonaqueous media), active drug substances and surface active agents or polymers required for stabilization. If necessary, other substances such as buffers, salts and sugars can be added.

There are many advantages of nanocrystal formulations designed for oral administration. They are as follows:

• Increased rate of absorption,

• Increased oral bioavailability,

• Rapid effect,

• Improved dose proportionality,

• Reduction in required dose,

• Applicability to all routes of administration in any dosage form. Contrary to micronized drugs, nanocrystals can be administered via several routes. Oral administration is possible in the form of tablets, capsules, sachets or powder; preferably in the form of a tablet. Nanosuspensions can also be administered via the intravenous route due to very small particle size, and in this way, bioavailability can reach 100 %,

• Reduction in fed/fasted variability,

• Rapid, simple and cheap formulation development.

• Possibility of high amounts (30-40 %) of drug loading,

• Increased reliability. Usually side effects are proportional to drug concentration, so decreasing the concentration of active drug substances leads to an increased reliability for patients.

• Sustained crystal structure. Nanocrystal technology leads to an increase in dissolution rate depending on the increase in surface area obtained by reduction of the particle size of the active drug substance down to the nano size range preserving the crystal morphology of the drug.

• Improved stability. They are stable systems because of the use of a stabilizer that prevents reaggregation of active drug substances during preparation. Suspension of drug nanocrystals in liquid can be stabilized by adding surface active substances or polymers,

• Applicability to all poorly soluble drugs because all these drugs could be directly disintegrated into nanometer-sized particles(Tugba Gulsun *et al*., 2010).

PROPERTIES OF NANOCRYSTALS

The main reasons for the increased dissolution velocity and thus increased bioavailability are:

Increase of dissolution velocity by surface area enlargement

The size reduction leads to an increased surface area and thus according to the Noyes-Whitney equation to an increased dissolution velocity. Therefore micronization is a suitable way to successfully enhance the bioavailability of drugs where the dissolution velocity is the rate limiting step. By moving from micronization further down to nanonization, the particle surface is further increased and thus the dissolution velocity increases too. In most cases, a low dissolution velocity is correlated with low saturation solubility **(Figure 4)**.

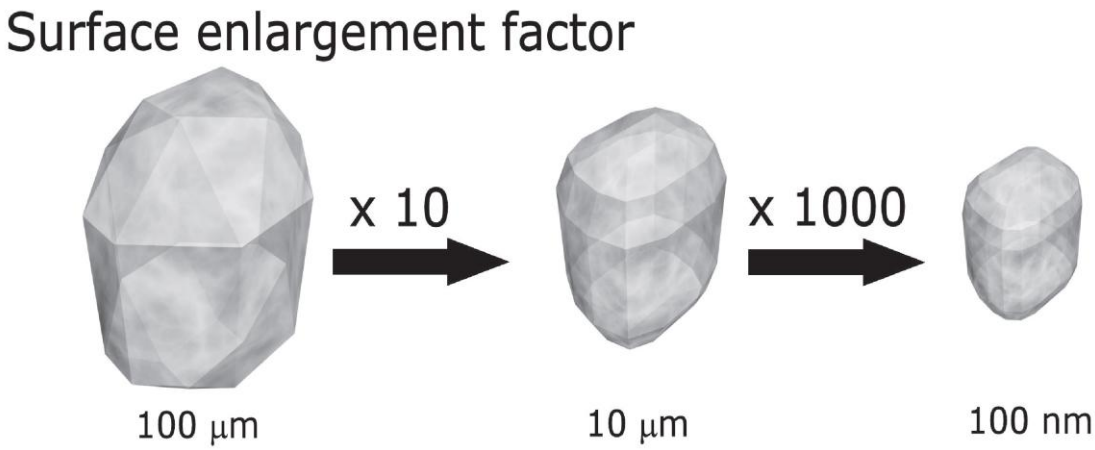


FIGURE 4: SURFACE ENLARGEMENT AND INCREASE IN NUMBER OF CRYSTALS BY PARTICLE SIZE DIMINUTION

(Rainer H Muller *et al*., 2008)

Increase in saturation solubility

The general textbook statement is that the saturation solubility C_s is a constant depending on the compound, the dissolution medium and the temperature. This is valid for powders of daily life with a size in the micrometer range or above. However, below a critical size of 1–2 μm, the saturation solubility is also a function of the particle size. It increases with decreasing particle size below 1000 nm.

Therefore, drug nanocrystals possess increased saturation solubility. This has two advantages:

1. According to Noyes and Whitney (1897), the dissolution velocity is further enhanced because dc/dt is proportional to the concentration gradient $(C_s-C_x)/h$ (C_s- saturation solubility, C_x - bulk concentration, h - diffusional distance).
2. Due to the increased saturation solubility the concentration gradient between gut lumen and blood is increased, consequently the absorption by passive diffusion **(Figure 5)**.

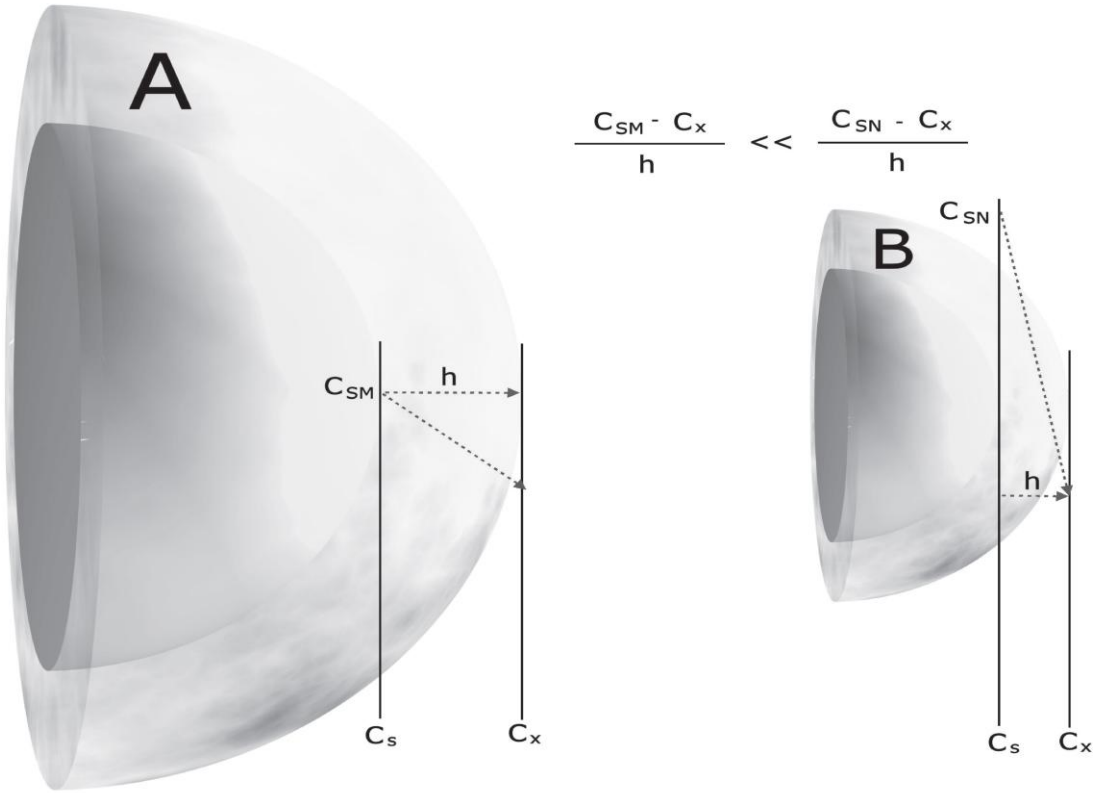


FIGURE 5:COMPARISON OF A MICROCRYSTAL (A) AND A NANOCRYSTAL (B) AND THEIR SURFACE CURVATURE AND CONCENTRATION GRADIENT OVER THE DIFFUSIONAL DISTANCE (h).

Abbreviations: C_s, drug-saturated water at surface (M, microcrystal; N, nanocrystal); Cx , bulk concentration at diffusional distance; h, diffusional distance. $dc / dt \sim (C_s - C_x) / h$ (Rainer Muller. H *et al*., 2008).

Advantages of an amorphous particle state:

It is well known that amorphous drugs possess a higher saturation solubility compared to crystalline drug material. Amorphous drug nanoparticles possess a higher saturation solubility compared to equally sized drug nanocrystals in the crystalline state. Therefore, to reach the highest saturation solubility increase, a combination of nanometer size and amorphous state is ideal.

Transferring all these facts to drug nanocrystals means that optimal drug nanoparticles with the highest increase in saturation solubility should have a size of

eg, 50 nm or 20–30 nm, and be amorphous. It can be concluded that the size matters regarding the increase in saturation solubility and consequently the increase in dissolution velocity caused by a higher C_s combine drug nanocrystals with traditional controlled release technology (eg, coated pellets) to avoid fast dissolution, excessively high plasma peaks and premature t_{max} and to reach prolonged blood levels.

To summarize, the optimal drug nanocrystal size and crystalline/amorphous state will depend on:

1. Required blood profile.
2. Administration route.
3. Stability of the amorphous state during shelf life of the product (Rainer Muller. H *et al*., 2008).

NANOCRYSTAL PREPARATION METHOD

Several preparation methods for drug nanocrystals have been investigated.

1. **Bottom-up technology**
   - Nanoprecipitation
2. **Top-down technology**
   - Homogenization
     - Ultrasonic homogenization.
     - High pressure homogenization.
   - Milling
3. **Top down and Bottom up technology**

Today, implemented preparation methods of nanocrystal formulations can be classified as “bottom- up”, “top-down”, “top down and bottom up”.

- "Bottom up" technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation.
- "Top-down" technology applies dispersing methods by using different types of milling and homogenization techniques. "Topdown" technology is more popular than "Bottom up" technology; it is known as "nanosizing". In other words, it is a process which breaks down large crystalline particles into small pieces.
- In "top down and bottom up" technology, both methods are utilized together. Spray drying is also a method for preparing drug nanocrystals, which is faster and more practical compared to the other methods (Tugba Gulsun *et al*., 2010).

1. Bottom up technology

- **Nanoprecipitation**

The nanoprecipitation method involves the formation of crystalline or semicrystalline drug nanoparticles by nucleation and the growth of drug crystals.

In a typical procedure, drug molecules are first dissolved in an appropriate organic solvent such as acetone, tetrahydrofuran or N-methyl-2-pyrrolidone at a supersaturation concentration to allow for the nucleation of drug seeds. Drug nanocrystals are then formed by adding the organic mixture to an antisolvent in the presence of stabilizers such as hydroxypropyl methylcellulose, polyvinylpyrrolidone, Tween 80, Poloxamer 188 or lecithin. The choice of solvents and stabilizers and the mixing process are key factors to control the size and stability of the drug nanocrystals. A combination of several stabilizers is often used for optimal effect.

The primary role of stabilizers is to inhibit excessive crystal growth or particle aggregation. The mixing step is crucial to produce a rapid and uniform supersaturated solution, which facilitates the formation of uniform and small drug nanoparticles. Other crucial factors include the drug concentration, volume ratio of antisolvent to solvent, temperature and viscosity (Huabing Chen *et al*., 2011).

Examples of products manufactured by the precipitation method are, Hydrosols and Nanomorph TM, which are developed by Sucker and Soliqs/Abbott respectively (Tugba Gulsun *et al*., 2010).

2. Top down technology

"Top down" technology can be applied by either homogenization or milling.

- **Homogenization**

i)Ultrasonic homogenization

ii)High pressure homogenization

i) Ultrasonic homogenization

One of the preparation methods of nanocrystals is homogenization by ultasonification. Ultrasonic probes are used to decrease the particle size in liquid or solid dispersed phase. Ultrasonic homogenization is quite effective for reducing the size of hard and soft particles. Homogenization by ultasonification is based on high frequency mechanical vibrations. Liquids are exposed to intense sound waves transmitted with ultrasonification. Ultrasonic probe provides controlled and reproducible ultrasonification. This is important for the quality of manufactured products and scale up studies (Tugba Gulsun *et al*., 2010).

ii) High-pressure homogenization

Another homogenization method is high pressure homogenization in which two types of homogenizers namely**, microfluidizers** and **piston gap homogenizers** are used.

a) Microfluidizer Technology

The microfluidizer is a jet stream homogenizer of two fluid streams collied frontally with high velocity (up to 1000m/sec) under pressures up to 4000 bar. There is a turbulent flow, high shear forces, particles collied leading to particle diminution to the nanometer range. The high pressure applied and the high streaming velocity of the lipid can also lead to cavitation additionally, contributing to size diminution. To preserve the particle size, stabilization with phospholipids or other surfactants and stabilizers is required. A major disadvantage of this process is the required production time. In many cases, 50 to 100 time- consuming passes are necessary for a sufficient particle size reduction. Skye Pharma Canada, Inc. (previously RTP, Inc.) applies this principle for its IDD-P™ technology to produce submicron particles of poorly soluble drugs.

b) Piston-gap homogenization in water (Dissocubes®)

Drug nanocrystals can also be produced by high-pressure homogenization using piston gap homogenizers. Depending on the homogenization temperature and the dispersion media, there is a difference between the Dissocubes® technology and the Nanopure® technology. Dispersion medium of the suspensions was water. A piston in a large bore cylinder creates pressure up to 2000 bar. The suspension is pressed through a very narrow ring gap. The gap width is typically in the range of 3-15 micrometer at pressures between 1500-150 bar. There is a high streaming velocity in the gap according to the Bernouli equation. Due to the reduction in diameter from

the large bore cylinder (e.g. 3 cm) to the homogenization gap, the dynamic pressure (streaming velocity) increases and simultaneously decreases the static pressure on the liquid. The liquid starts boiling, and gas bubbles occur which subsequently implode, when the suspension leaves the gap and is again under normal pressure (cavitation). Gas bubble formation and implosion lead to shock waves which cause particle diminution. The patent describes cavitation as the reason for the achieved size diminution (Suman Katteboinaa *et al*., 2009).

Nanopure® Technology

Another approach using the piston-gap homogenizer is the Nanopure® technology, owned and developed by Pharma- Sol GmbH in Berlin. The technology uses dispersion media with a low vapor pressure and optionally homogenization at low temperatures. The cavitation in the homogenization gap is very little or nonexistent. Even without cavitation, the size diminution was sufficient. The remaining shear forces, particle collisions and turbulences are sufficient to achieve nanoparticles. The optional low temperatures while homogenizing allow the processing of temperature labile drugs. It is possible to carry out the whole process in non-aqueous media to protect drugs from hydrolysis. Usage of oils, PEG or hot-melted polyethylene glycols can be directly filled into gelatin or HPMC capsules (Rainer Muller. H *et al*., 2008).

- **Milling**

In the milling method; pearl, bead or ball mills can be utilized to prepare a nanocrystal formulation. In this method, the active drug substance and the stabilizer are dispersed in the dispersion medium, and this mixture is then put into a grinder chamber. Balls are rotated at a very high speed and particle size of the drug gets smaller until nanocrystals are obtained.

Physicochemical characteristics of the nanocrystals depend on the number of milling balls, the amount of drug and stabilizer, milling time and speed, type of grinding chamber and temperature. In contrast with high pressure homogenization, it is a low energy technique. Grinder chambers are made from stainless steel, porcelain or hard material, and the balls are made from porcelain, glass, zirconium oxide, stainless steel, chromium, agate, or special polymer materials. The type of material that the balls are made of is very important since an interaction could take place between the material and the drug substance. In this regard, agate balls are frequently used in the pharmaceutical field as the possibility of such an interaction is minimized. Another important factor is the size of balls. When the balls with small diameter are used, grinding time is extended but smaller particles are obtained.

The mechanism of ball milling is that while grinding chamber is rotated, balls are rotated too, and at the end of this procedure, the particle size of the active drug substance is reduced by mechanical energy. Thus, particle size of active drug substances can be adjusted by changing the diameter and number of balls. Rotational speed of the grinding chamber is also very important. If the rotational speed is too low, balls cannot rotate effectively and grinding cannot be done efficiently. If rotational speed is very high, balls will remain at the edge of the grinding chamber due to centrifugal forces and grinding cannot bed one effectively. Moreover, for efficient grinding the volume of the balls should be 30 % to 50 % of the grinding chamber. The milling time depends on many factors such as the surfactant content, hardness of the drug, viscosity, temperature and energy input. The milling time can change from 30 minutes to hours or several days.

The main problem in this method is the contamination of the product as a result of erosion of balls or grinding chamber. Therefore, grinding time and the

material of balls/grinding chamber should be selected very carefully. This interaction can be reduced by using surface active agents or polymers (Tugba Gulsun *et al*., 2010).

Commercial products from this method include Rapamune1, Emend1, Tricore1, Megas ES1 and Invega1 (Huabing Chen *et al*., 2011).

3. Top down and bottom up technology

In "top down and bottom up" technology, both methods are used together. NanoEdge® is a product obtained by such a combination technology. As can be inferred, precipitation is followed by high pressure homogenization in this technology (Tugba Gulsun *et al*., 2010).

PROCESSING OF NANOSUSPENSION TO FORM NANOCRYSTALS (Sanjay Bansal *et al*., 2012).

The nanonization of drugs by various techniques generally results in a liquid product called nanosuspension. But these nanosuspensions are directly used as a final product only in some special cases e.g. as pediatric or geriatric dosage forms. In most of the cases, a dry dosage form (particularly for oral administration) is preferred, may be

a. for convenience,
b. to achieve a controlled drug delivery,
c. to prevent drug degradation,
d. to enable better drug targeting,
e. to increase the physical stability for long term storage and
f. to obtain a fine non-aggregated suspension in the gastro-intestinal tract after oral administration.

In such cases, the nanosuspension needs to be transformed into solid forms, which may be crystalline (Nanocrystals) or amorphous (Nanomorphs). Various techniques are used for this purpose like spray drying, freeze drying, pelletization or granulation.

1. Spray Drying

One of the preparation methods of nanocrystals is spray drying. This method is usually used for drying of solutions and suspensions. In a conical or cylindrical cyclone, solution droplets are sprayed from top to bottom, dried in the same direction by hot air and spherical particles are obtained. Spraying is made with an atomizer which rapidly rotates and provides scattering of the solution due to centrifugal effect. The solution, at a certain flow rate, is sent to the inner tube with a peristaltic pump, nitrogen or air at a constant pressure is sent to the outer tube. Spraying is provided by a nozzle. Droplets of solution become very small due to spraying; therefore, surface area of the drying matter increases leading to fast drying. Concentration, viscosity, temperature and spray rate of the solution can be adjusted and particle size, fluidity and drying speed can be optimized. The dissolution rate and bioavailability of several drugs, including hydrocortisone, COX-2 Inhibitor were improved utilizing this method.

2. Freeze drying

Another method for removing water from formulation is freeze drying. This however, is a complex and expensive process and the product obtained is highly sensitive to process parameters. This method is not suitable for industrial production.

A new technique based upon freeze drying was developed by de Waard. In this technique a mixture of the drug, solvent, and mannitol is cooled rapidly, resulting in separation of drug in a nanocrystal form encased by a matrix of mannitol.

This matrix increases the stability of the nanocrstallized drug without which the crystals may stick together and form one large crystal. De Waard also developed a spray-freeze-drying method which enables the process to be applied on an industrial scale. Another method developed by the same author was a spray-freeze-drying method which could make industrial application of this process simpler.

Lyophilization of drug nanoparticles produced in water-reduced media can be used to produce FDDS (Fast Dissolving Drug Delivery Systems). For parenteral application Nanopure can be lyophilized and reconstituted prior to injection with isotonic media (e.g. water with glycerol).

3. Pelletization

A number of pelletization techniques are known, but the most commonly used techniques are a) extrusion-spheronization and b) drug coating onto sugar spheres. The pelletization technique is selected on the basis of the required drug content, properties of the drug and the available equipment. A multi-particulate dosage form such as coated pellet system is obtained irrespective of the pelletization technique applied. These multi-particulate dosage forms show distinct advantages over single unit dosage forms such as faster and more predictable gastric emptying and more uniform drug distribution in GIT within different individuals.

a) Production of pellets containing drug nanocrystal-loaded matrix cores

The drug nanosuspension obtained by high-pressure homogenization is mixed with matrix material (fillers such as MCC, Lactose or Starch). Pellets are produced by extrusion-spheronization and can be subsequently coated with polymers to modify the drug release properties. Mucoadhesive budenoside nanocrystals were prepared using extrusion-spheronization. The obtained pellets were coated with Eudragit L 30 D- 55 to obtain enteric coating and delayed drug release . Another type of modified release

pellet formulations containing ibuprofen drug nanocrystals were produced by HPH. These ibuprofen containing pellets loaded with nanocrystals of the drug dissolved completely within 30 minutes from both formulations.

Spray coated pellets of hydrocortisone acetate were prepared (enteric coated) from mucoadhesive nanosuspension of this poorly soluble drug. The invitro dissolution tests showed an accelerated dissolution rate and an increased drug release for the pellets containing drug nanocrystals.

b) Production of pellets by Nanosuspension layering onto sugar cores:

The drug nanosuspension obtained by HPH is directly layered onto sugar beads and subsequently coated with polymers using the same equipment to modify the drug release properties.

SELECTION OF STABILIZERS FOR NANOCRYSTAL PREPARATION

Selection of stabilizers is very important in nanocrystal formulations because the type and concentration of stabilizer affect the final size of the particles, and also, stabilizers prevent nanocrystals from aggregating. Polymers or surface active agents exert their effect by covering the surface of drug nanocrystals and providing stabilization by creating a steric barrier. At the nano size, forces between particles due to dispersion or van der Waals forces come into play. Nanosized particles with a high surface area have high surface free energy (ΔG). Thus, particles tend to agglomerate in order to decrease the surface free energy leading to an increase in particle size and reduction in the surface area. Therefore, a stabilizer leads to a decrease in ΔG by decreasing the interfacial tension γs/l.

$$\Delta G = \Delta A \bullet \gamma s/l$$

γs/l: interfacial tension between the surface of solid and the surrounding liquid phase (joule/m2)

ΔG: change in surface free energy (joule)

ΔA: change in the surface area (m^2)

Jonghwii Lee and coworkers have reduced the particle size of seven different active drug substances by preparing nanocrystals. They investigated two different stabilizers (polyvinylpyrollidone and hydroxypropyl cellulose), their interaction with the active drug substances, and effects on surface energy and particle size. They found that interactions between stabilizers and active drug substances were complex and depended on many variables, such as presence of functional groups and surface energy. The concentration of the stabilizer is an important factor that affects the physical stability of the final product.

Hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127) are polymers suitable for use as stabilizers. The chains should be long enough to provide a steric layer, but not too big to slow down dissolution. Polysorbate 80 (nonionic), sodiumlaurylsulfate (SLS) and docusate sodium (DOSS) (both anionic) are some examples of suitable surfactant stabilizers for physical stability. Also, surfactants often help in the wetting, electrostatic stabilization and dispersion of the drug particles, which are usually very hydrophobic. HPMC E3, Povidone, DOSS, and SLS are some of the stabilizers that have been used in the nanocrystal formulations of drugs that are on the market today (Tugba Gulsun *et al*., 2010).

CHARACTERIZATION AND EVALUATION OF NANOCRYSTAL SUSPENSIONS(Lei Gao *et al*., 2008)

The essential characterization parameters for nanocrystal suspensions are as follows:

a) Size and size distribution

Size and size distribution are important characterizations of the nanosuspensions because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances. The mean particle size of nanosuspensionsis typically analyzed by photon correlation spectroscopy (PCS). Apart from the mean particle diameter, PCS can also yield the width of the particle size distribution, i.e. polydispersity index (PI). The PI value ranges from 0 (monodisperse particles) to 0.500 (broad distribution), and is an important index that governs the physical stability. For a long-term stability the PI should be as low as possible.

However, due to a narrow measuring range of PCS, approximately from 3 nm to 3 lm, it shows an incapability in the detection of the larger microparticles. Then the laser diffractometry (LD) is needed to investigate the content of particles in the micrometer range or aggregates of drug nanoparticles, especially for the nanosuspensions that are meant for parenteral and pulmonary delivery. The LD yields a volume distribution and possesses a measuring range of approximately 0.05–80 lm up to a maximum of 2000 lm, depending on the type of equipment employed. Typical characterization parameters of LD are diameters 50%, 90%, 99% represented by D50, D90 and D99 respectively (i.e. the D50 means that 50% of the volume of the particles is below the given size).

A Coulter counter analysis is essential for nanosuspensions to be administrated intravenously. Compared with a volume distribution of the LD analysis, the Coulter counter data give an absolute value, that is the absolute number of particles per volume unit for the different size classes. The size of the smallest blood capillary is about 5 lm, so even a small content of particles greater than 5 lm may cause capillary

blockade or emboli formation. So the content of microparticles in nanosuspensions should be controlled strictly by Coulter counter analysis.

b) Shape and morphous

Typically, the shape or the morphous of the nanocrystals can be determined using a transmission electron microscope (TEM) and/or a scanning electron microscope (SEM). A wet sample of suitable concentration is needed for the TEM analysis. When the original nanosuspensions are required to be processed into dried powder (e.g. by spray drying or lyophilization), a SEM analysis is essential to monitor changes of the particle size before and following the progress of the water removal. In general, agglomeration phenomenon may occur following water removal, leading to an increase of the particles' size which can be viewed through SEM. To minimize the extent of the increase of particle size, some excipients should be added as a protectant. For example, mannitol, generally used as a cryoprotectant in lyophilization, can recrystallize around nanocrystals during the water-removal operation, thus preventing particle interaction and agglomeration. An agglomeration within a certain extent is permitted when the particle size still in an accepted range, particularly the dried powder can be well redispersed into stable nanosuspensions. The shape of the drug crystals depends on their crystalline structure; different crystal shapes of different drugs were viewed under SEM.

c) Zeta potential

The measurement of the zeta potential allows the prediction about the storage stability of submicron colloidal dispersion. In general, particle aggregation is less likely to occur if particles possess enough zeta potential providing sufficient electric repulsion, or enough steric barrier providing sufficient steric repulsion between each other. According to the literature, a zeta potential of at least -30 mV for electrostatic

and -20 mV for sterically stabilized systems is desired to obtain a physically stable nanocrystal suspensions.

d) Crystalline state

The evaluation of crystalline state is necessary to understand the polymorphic changes that a drug might undergo when subjected to nanosizing. Differential scanning calorimetry(DSC) and X-ray diffraction can be used to evaluate the crystalline structure of the drug nanocrystals. It was reported that some drugs retained their crystalline state during homogenization, example danazol and nifedipine. However, for some drugs, for example azithromycin, the results of DSC and X-ray showed that amorphous state was generated during homogenization. Though it increases the saturation solubility, the amorphous state is a metastable state with a higher energy and leads to an instability during shelf time. Transforming the nanosuspension into stable dried solid powder by water removal can resolve this problem.

e) Saturation solubility and dissolution velocity

Determination of the saturation solubility and dissolution velocity is very important as it not only can help to assess the benefits compared to the microparticle formulation but also help to anticipate the in vivo performance (e.g. blood profiles, plasma peaks, and bioavailability). The nanosuspensions should be transferred into a dried powder before the investigation of the dissolution behavior. In the shakering experiments a different temperature can be used to determined the saturation solubility of a dried powder in a different artificial medium (i.e. physiologic saline, artificial gastric juice, or artificial intestinal juice). To determine the dissolution velocity,the methods described in the Pharmacopoeia can be used; also experiments in various physiological buffers should be performed.

f) Surface properties

The research on the surface parameters of nanosuspensions is very important, especially for the nanosuspensions to be administrated intravenously. The fate of the nanocrystals in vivo following injection, such as organ distribution, depends on its surface properties, such as surface hydrophobicity and interactions with plasma proteins. Therefore, some special techniques have to be used to evaluate the surface properties to give an idea of in vivo behavior. Hydrophobic interaction chromatography has been used to determine the surface hydrophobicity, and 2-D PAGE can be performed to measure the protein absorption after intravenous injection of nanosuspensions.

APPLICATIONS OF NANOCRYSTALS BY VARIOUS ROUTES OF ADMINISTRATION

a) Drug Nanocrystals for Oral administration

Enhancement in bioavailability of poorly soluble drugs after oral administration. Besides it has also been proved by various drug nanocrystal products placed in the market. A faster onset of action and decreased gastric irritancy has been reported when naproxen was formulated as nanosuspension. Due to fast dissolution of nanocrystals, the drug solubility is enhanced, making it bioequivalent in fed and fasting conditions. The bioadhesive nature of nanocrystals offers additional advantage of increased stay in the gastro-intestinal tract which enhances bioavailability. The nano size can be exploited for better drug targeting as reported for lymphatic drug uptake or for inflammatory tissues. Nanosuspensions can be formulated as more concentrated and less viscous. Patient has to take lesser dose of easily swallowed formulation (e.g. Megace ES). Nanoparticles provided sustained release of anti-

tubercular drugs – rifampin, isoniazid and pyrazinamide and considerably improved their efficacy after oral administration (Sanjay Bansal *et al*., 2012).

b) Parenteral Administration of Drug Nanocrystals

The parenteral application of poorly soluble drugs, particularly intravenous (IV) administration of practically insoluble compounds, using cosolvents, surfactants, liposomes, or cylcodextrines, is often associated with large injection volumes or toxic side effects. Carrier-free nanosuspensions enable potential higher loading capacity compared to other parenteral application systems. Using nanosuspensions, the application volume can be distinctly reduced compared to solutions. To fulfil the distinctly higher regulatory hurdles, the drug nanocrystals need to be produced in an aseptic process. Alternatively, nanosuspensions can be sterilized by autoclaving or alternatively by gamma irradiation as well as sterile filtration. When a drug is administered as a nanosuspension, the rapid dissolution of the nanocrystals will mimic the plasma concentration profile of a solution. Drug nanosuspensions can be formulated with accepted surfactants and polymeric stabilizers for IV injection. In contrast, solutions of poorly soluble drugs require the use of cosolvents and/or high surfactant contents (e.g., Chremophor EL in Taxol®), which can cause undesired side effects (Suman Katteboinaa *et al*., 2009).

c) Drug Nanocrystals for Pulmonary drug delivery

Poorly soluble drugs can be delivered directly to the lungs by nebulizing the aqueous nanosuspensions using mechanical or ultrasonic nebulizers. Using nanoparticles, drug is more evenly distributed in droplets. All aerosol droplets are likely to contain drug nanocrystals. Budenoside, poorly water soluble corticosteroid, has been successfully prepared as a nanosuspension for pulmonary delivery. It showed long term stability. No particle growth and aggregates formed over a period

of one year. In addition, Buparvaquone nanosuspension was formulated for an alternative treatment of lung infection (pneumonia) to deliver the drug at the site of lung infection using nebulization. Administration to infected guinea pigs of nebulized rifampin, isoniazid and pyrazinamide encapsulated in wheat germ agglutinin functionalized PLG nanoparticles was much more effective. Three doses administered fortnightly for 45 days were sufficient to produce a sterilizing effect in lungs and spleen. Drug nanocrystals showed an increased mucoadhesiveness leading to a prolonged residence time at the lung mucosa (Sanjay Bansal *et al*., 2012).

d) Drug Nanocrystals for Ophthalmic Drug Delivery

It could be shown that nanoparticles possess a prolonged retention time in the eye, most likely due to their adhesive properties. From this, poorly soluble drugs could be administered as a nanosuspension. The development of such colloidal delivery systems for ophthalmic use aims at dropable dosage forms with a high drug loading and a long-lasting drug action. The nanosuspensions were prepared by a modification of the quasi-emulsion solvent diffusion technique using variable formulation parameters (drug-to-polymer ratio, total drug and polymer amount, stirring speed). Nanosuspensions had mean sizes around 100 nm and a positive charge (zeta-potential of +40/+60 mV), this makes them suitable for ophthalmic applications. Stability tests (up to 24 months storage at 4 degrees C or at room temperature) or freeze-drying were carried out to optimize a suitable pharmaceutical preparation. In vitro dissolution tests indicated a controlled release profile of IBU from nanoparticles. In vivo efficacy was assessed on the rabbit eye after induction of an ocular trauma (paracentesis). An inhibition of the miotic response to the surgical trauma was achieved, comparable to a control aqueous eye-drop formulation, even though a lower concentration of free drug in the conjunctival sac was reached from the nanoparticle

system. Drug levels in the aqueous humour were also higher after application of the nanosuspensions; moreover, IBU-loaded nanosuspensions did not show toxicity on ocular tissues.

e) Drug Nanocrystals for Dermal Drug Delivery

Dermal nanosuspensions are mainly of interest if conventional formulation approaches fail. The use of drug nanocrystals leads to an increased concentration gradient between the formulation and the skin. The increased saturation solubility leads to supersaturated formulations, enhancing the drug absorption through the skin. This effect can further be enhanced by the use of positively charged polymers as stabilizers for the drug nanocrystals. The opposite charge leads to an increased affinity of the drug nanocrystals to the negatively charged stratum corneum (Suman Katteboinaa *et al*., 2009).

f) Drug Nanocrystals for Targeted drug delivery

Nanocrystals can have deep excess to the human body because of particle size and control of surface properties. So they can also be used for targeted drug delivery. Kayser developed a nanosuspention of aphidicolin to improve drug targeting against Leishmania-infected macrophages. He demonstrated that aphidicolin was highly active at a concentration in the microgram range. Similarly peptide dalargin was successfully targeted to the brain by employing surface modified poly(butyl) cyanoacrylate nanoparticles. Nanoparticles offer a promising new cancer treatment that may one day replace radiation and chemotherapy. Kangius RF therapy attaches microscopic nanoparticles to cancer cells and then cooks tumors inside the body with radio waves that heat only the nanoparticles and the adjacent cancerous cells. Muco-adhesive pellets or nanoparticles have been used as specific carrier systems for oral administration.

Bupravaquone nanosuspension was successfully used for targeting of *Cryptosporidium parvum*, (the organism responsible for cryptosporidium) by altering the mucoadhesive properties. Amphoterecin B as pulmonary nanosuspension was used to target conditions such as pulmonary asperigillosis (Sanjay Bansal *et al*., 2012).

PRODUCTS IN THE MARKET

All nanocrystals in the first four products were produced using the pearl mill technology by Elan Nanosystems. Prerequisite was the availability of production facilities at sufficiently large scale. In general candidates of first choice for Nanocrystal® technology are drugs with a relatively low dose.

a) Rapamune®

Rapamune® contains sirolimus (SRL, rapamycin) as the active drug, which is derived from S*treptomyces hygroscopicus* (actinomycetes) **(Figures 6)**. SRL is a macrocyclic immunosuppressive drug with a molecular weight of 914.2 and is related to tacrolimus which is produced from different species of *Streptomyces*.

SRL is being used mostly in a combination with cyclosporine or steroids to avoid organ rejection in patients after a kidney transplant. In cardiology SLR is used because of its antiproliferative effect to avoid a reoccurring constriction (restenosis) caused by a hyperplasia of the inner vascular after implantation of a stent into the vessels around the heart.

Rapamune was the first marketed product introduced in 2000 by Wyeth Pharmaceuticals. It is available in two formulations, as oral suspensions and as a tablet. The tablet has the advantage of being more user friendly. Comparing the oral bioavailability of solution and nanocrystal tablet, the bioavailability of the nanocrystals is 21% higher compared to the solution. The oral single dose of

Rapamune is 1 or 2 mg, the total tablet weight being approximately 365 mg for 1 mg formulation and approximately, 370 mg for the 2 mg formulation. This means it contains a very low percentage of its total weight as nanocrystals. An important point is that the drug nanocrystals are released from the tablet as ultra fine nanosuspension. In the event that crystal aggregation takes place to a pronounced extent, the dissolution velocity and subsequently the oral bioavailability of the BSC II drugs will be reduced. Therefore, there is an upper limit to load tablets with nanocrystals. In case the limit is exceeded and nanocrystals get in contact with each other within the excipient mixture of the tablet, the nanocrystals might fuse to larger crystals under the compression pressure during tablet production. For drugs with a low oral single dose such as sirolimus in Rapamune, incorporation into tablets causes few or no problems. A total nanoparticle load of less than 1% is well below the percentage being critical.

The main advantages of the nanocrystal technology in this product are the user friendliness and the higher bioavailability in comparison to the oral solution. As discussed previously, a smaller particle size leads to greater solubility and larger surface area, consequently increased dissolution velocity and thus greater bioavailability.

FIGURE 6: PACKAGING OF RAPAMUNE®

b) Emend®

The second product on the market was Emend®, introduced in 2001 by Merck (Winehouse Station, NJ) **(Figures 7)**. The drug is aprepitant, used for treatment of emesis (single dose is either 80 or 125 mg).

FIGURE 7: PACKAGING OF EMEND®

Aprepitant will only be absorbed in the upper gastrointestinal tract. Bearing this in mind nanoparticles proved to be ideal to ideally exploit this narrow absorption window. The large increase in surface area due to nanonization leads to rapid in vivo dissolution, fast absorption and increased bioavailability. The formulation of a tablet from micronized bulk powder made much higher doses necessary, leading to increased side effects (eg, other serotonin receptor induced effects like dizziness).

The drug nanocrystals are contained within a hard gelatin capsule as pellets. Aprepitant was formulated this way in order to make the drug easy to handle by healthcare providers and patients as capsules. In addition, the pellets can be administered via a stomach tube. Currently studies are being undertaken to evaluate the change in pharmacokinetics (if any) between the pellets and the capsules. One needs to bear in mind that a higher loading of the tablet with more than 125 mg drug in a 400 mg tablet is near the critical amount, which is about 30%. If the total drug

content exceeds 30%, the possibility of drug nanocrystals directly touching each other and fusing to larger crystals is enhanced. There are special methods and technologies required to produce high nanocrystal-loaded tablets. By applying this, up to 90% nanocrystal powder would be loaded into tablets.

This example of aprepitant demonstrates once again the importance of an increased bioavailability through nanonization. In case of a drug with a narrow absorption window which is also poorly soluble it is important to reduce the particle size to a size threshold that will make the drug bioavailable by enhancing the solubility.

c) Tricor®

Tricor® is being marketed by Abbott Laboratories and the active ingredient is fenofibrate, being available in 48 mg and 145 mg tablets **(Figures 8)**. Tricor is indicated as adjunctive therapy to diet in adult patients with primary hypercholesterolemia or mixed dyslipidemia to increase high-density lipoprotein cholesterol (HDL-C), reduce triglycerides (TG), reduce low-density lipoprotein cholesterol (LDL-C), reduce total cholesterol (Total-C), and reduce apolipoprotein B (Apo B).

Fenofibrate is a lipophilic compound and practically insoluble in water. Having no ionizable group, the solubility of fenofi brate was not infl uenced by changes in pH value through the application of food. The enhanced absorption of fenofibrate in fed patients can be explained with the availability of lipids and other surfactants (eg, cholesterol) in the food, thus solubilizing the fenofibrate. By nanonizing the drug, the solubility is enhanced, making it bioequivalent in fed and fasting conditions.

FIGURE 8: PACKAGING OF TRICOR®

Another product using nanocrystals to make an advanced fenofibrate is Triglide®, which is produced using the IDD-P®-technology (as discussed before) from Skye pharma and is marketed by Sciele Pharma Inc.

d) Megace ES®

In Megace ES® (ES stands for Enhanced Stability) (megestrol acetate) introduced by Par Pharmaceutical Companies, Inc., who licensed the Megace name from Bristol-Myers Squibb (New York, NY), the Nanocrystal technology leads to several advantages **(Figures 9)**.

Megestrol is mainly used to improve weight gain and appetite in patients undergoing chemotherapy or suffering from an HIV infection. It can also be used to treat psychologically induced anorexia. The precise mechanism of megestrol's antianorexic and anticachetic effects is unknown.

Megestrol is well absorbed in the gastrointestinal tract but absorption varies. A bioavailability study comparing the peak plasma concentration and extent of absorption of Megace ES and megestrol acetate oral suspension revealed that in unfed patients, the bioavailability of Megace ES is minimally reduced while there was a substantial food effect for megestrol acetate oral suspension.

FIGURE 9: PACKAGING OF MEGESTRAL

The improved rate of dissolution again resulting from the particle dimensions in the nanometer range leads to an enhanced bioavailability in people in fasted state.

The nanonized drug can be formulated in less volume, so the single dose the patient has to take (daily dose 625 mg of megestrol in 5 ml of fl uid) is reduced by the factor four compared to the oral solution available. This reduced volume and the improved bioavailability lead to a better patient compliance due to the possibility of flexible dosing in order to provide effective appetite stimulation and weight gain.

Another advantage is the reduced viscosity of the Megace ES formulation, which also leads to increased patient compliance. This viscosity reduction is a direct effect of the reduction of the particle size (no or little viscosity enhancement necessary to prevent sedimentation) (Rainer Muller. H *et al*., 2008).

CHAPTER III

LITERATURE REVIEW

CHAPTER-III

LITERATURE REVIEW

Chaudhar Bharat *et al*., 2013, enhanced the solubility and dissolution rate of Albendazole, a class II drug, by two different techniques, and compared them for improved drug delivery study. These techniques were inclusion complex of Albendazole with Hydroxypropyl-β cyclodextrin (HP- β-CD), and, converting drug into nanocrystal formulation by anti solvent precipitation techniques in the presence of Sodium Lauryl Sulfate (SLS) as stabilizers. The drug inclusion complex with HP-β-CD were prepared by Kneading method with different ratio of HP- β-CD. Finally, it was concluded that, Amongst these formulation techniques, Nanocrystalization techniques found to be more effective than inclusion complex with HP-β-CD

Abdul Hasan Sathali.A., and Gopinath.M., *et al*., 2013, developed Paliperidone nanocrytal in order to enhance the solubility and dissolution rate by decreasing the particle size of the drug and also sustained the drug release profile by using Eudragit L100 as polymer at different ratio. The Paliperidone nanocrystals were successfully prepared by nanoprecipitation method using different stabilizers (PVP K30, Poloxamer 1888, Poloxamer 407, combination of PVP K30 and Poloxamer 407, combination of PVP K30 and poloxamer 188). The formulations were evaluated for entrapment efficiency, morphology, size distribution, zeta potential, solubility studies and stability studies. The presence of stabilizers made the nanocrystal formulation more stable. The solubility studies and invitro dissolution studies suggested that the

nanocrystal formulations can improve the bioavailabilty of paliperidone when compared to pure drug. The X-ray powder diffraction (XRPD) confirmed that, there was no change in the crystalline state by this size reduction process. Stability studies were favourable for the development of this nanocrystal formulations.

Raghvendra., and Amlan Mishra., 2013, reviewed on nanocrystal technology in different fields, including medicine and pharmacy. Transferred material into nanodimension to develop a new innovative formulation for poorly soluble drugs. Nanocrystals have a wide variety of proven and potential applications. They have been used in the manufacture of filters that refine crude oil into diesel fuel, and, applied to flexible substrates to produce solar panels. Nanocrystals were emerged as key material due to their novel shape and size dependent chemical and physical properties.

Zuki Abu Bakar Zakaria *et al*., 2013, synthesized biobased calcium carbonate nanocrytals had demonstrated to be an effective carrier for delivery of anticancer drug Doxorubicin (DOX). These nanocrystls displayed high levels of selectivity and specificity in achieving effective cancer cell death without non-specific toxicity. The $CaCo_3$/DOX nanocrystals were relatively stable at neutral pH (7.4), resulting in slow release of drug, but, progressively dissociated in acidic pH (4.8) and faster release of DOX. They indicated that, $CaCo_3$/DOX nanocrystals were more sensitive and gave a greater reduction in breast cancer cell growth by controlled and targeted cancer therapy than free DOX.

Neusin Bolourchain *et al*., 2013, prepared Clarithromycin (CLA) nanoparticles from a ternary ground mixture in the presence of Sodium Lauryl sulfate(SLS) and

Polyvinyl pyrrolidone (PVP) as co-grinding water soluble compounds, in order to improve the drug dissolution rate. Different weight ratios of CLA:SLS:PVP were ground in a dry process by planetary ball mill using different grinding ball size. This formulation formed nanocrystals with enhanced solubility, after dispersing in water. X-ray diffraction, differential scanning calorimetry and Infrared spectrophotometry confirmed no chemical intraction and phase transition during the process. Accelerated stability studies confirmed that the co-ground mixture, remained unchanged in terms of dissolution rate, drug assay and particle size. The results revealed that the dissolution rate of ternary ground mixtures were much higher than that of the intact drug.

Koichi Baba *et al*., 2013, prepared steroid nanocrystals using the nano spray dryer B-90. The particle size was controlled by selecting the mesh aperture size. Submicron steroid particles in powder form were successfully obtained. These nanoparticles were confirmed to have a crystal structure using powder X-ray diffraction pattern analysis. Steroid nanocrystals were dispersed in aqueous solution and attractive as nanocrytals based eye drop solution for the treatment of Ophthalmic disorders in the near future.

Sanjay Bansal *et al*., 2012, studied a large number of drugs have been discovered, which have a better efficiency but their clinical application is restricted due to poor water solubility. Poor water solubility has become a leading challenge for the formulation of these compounds. Poor solubility is generally associated with poor bioavailability. Nanocrystal have the potential to overcome this issue. Drug nanocrystals are crystals with the size in the nanometer range (mean diameter < 1000nm).

Shailesh Soni *et al*., 2012, reviewed on nanosuspension contain submicron colloidal dispersion of pharmaceutical active ingredient particles in a liquid phase stabilized by surfactants. The poor water solubility of drug was major problem for drug formulation. The reduction of drug particles into the submicron range leads to a significant increase in the dissolution rate, bioavailability as well as improve stability. Nanosuspension consists of the pure poorly water soluble drug without any matrix material suspended in dispersion. Nanosuspension prepared by various methods such as media milling and high pressure homogenization have been used commercially, recently nanosuspensions were prepared by employing emulsion solvent diffusion method and microemulsion as templates.

Dianrui Zhang et al., 2012, magnified the clinical use of Amoitone B, by nanocrystal technology, in order to solve the poor solubility and dissolution rate problems. Optimized Amoitone B nanocrystal with small and uniform particle size were successfully prepared by microfluidization method. And investigated by morphology, size distribution, and zeta potential. The differential scanning calorimetry(DSC) study and X-ray diffraction (XRD) confirmed, there was no crystalline state changed in the size reduction process. Finally concluded that, developed Amoitone B nanocrystal not only increased saturation solubility, but also reduced its side effects, and expected to choice for intravenous delivery and further application to cancer therapy.

Dianrui Zhang *et al*., 2012, compared, different methods for preparation of stable riccardin D formulation by nano-technology. Nanocrystals were prepared in the evaporative precipitation into aqueous solution (EPAS) and the microfluidisation process. The characterizations of nanocrystals were compared by transmission

electron microscope, size distribution and zeta potential. Poloxamer 188, hydroxyl propyl methyl cellulose, PVP k30 were used as surfactant and polymers. The nanocrystals made in EPAS process were smaller, more uniform and had a narrow distribution than the microfluidisation nanocrystals. Differential scanning calorimetry and X-ray diffraction confirmed the crystalline states that were both reserved. The solubility were greatly improved by the two methods and the EPAS nanocrystals were more stable due to the smaller size. The stable nanocrystals were successfully achieved by the two methods.

Fude Cui *et al*., 2012, modified the nitrendipine nanocrystals surface with negative charge to improve bioavailability. The nanocrystals were prepared via precipitation high pressure homogenization method. The nanocrystal were dispersed into chitosan solution, the free chitosan was removed by centrifugation method to obtain the chitosan modified nanocrystal. The physical stability of the preparation was improved under ambient condition. During invitro drug release, nanocrystal showed slow release property. The surface modification by chitosan improved the bioavailability compared with initial nanocrystal. The intraction between the negatively charged nitrendipine nanocrystals and the positively charged chitosan, the particle size stability was increased.

Jiao Sun *et al*., 2012, prepared nanocrystals, without any surfactant or polymer using the solvent/nonsolvent method. The effect of size on their solubility, dissolution and oral bioavailability were investigated. A precipitation method was developed for producing suspension of naked coenzyme Q_{10} nanocrystal. The nanocrystal suspension was concentrated by ultrafiltration method. The particle effect on

dissolution was clearly influenced by the diffusion coefficients of the various dissolution media. For particle size 700nm nanocrystals, he AUC_{0-48} was 4.4 fold greater than that for the coarse suspension. But further the particle size was reduced to 80nm, the bioavailability was increased by 7.3 fold.

Maiti S *et al*., 2012, studied liquid-crystal and nanocrystal technology for solubilization of poorly water soluble drugs. Any material with a dimension of less than 1 micrometer ie 1000nm, should be referred to as a nanoparticles, not a nanocrystal. Crystalline nanoparticles are often provide single domain crystalline system that can be studied to provide information, that can be help to explain the behavior of macroscopic samples of similar material. Nanocrystal is also a registered trademark of Elan Pharma International Ltd(Ireland). That improves the bioavailability of drugs by nanoscale particles, that can be suspended in liquid, made into powder, pressed into tablets or encapsulated. As decreased size of the drug particles, may increase oral bioavailability of sparingly water soluble drugs. Drugs nanocrystals can be used for chemical stabilization of chemically labile drugs. The increased stability can be explained by a shield effect of the surfactant.

Filippos kesisoglou *et al*., 2012, studied, nanosuspension formulations of crystalline active pharmaceutical ingredients (API) with particle size in the submicron range, have been shown to increase oral bioavailability of compounds. Their ability to increase the oral bioavailability has been demonstrated in preclinical and clinical settings.

Siling Wang *et al*., 2012, revealed that, many drugs are abandoned early in discovery due to extremely low solubility in both aqueous and non-aqueous solvent. As

traditional formulation strategies fail to solve the intractable issue, there comes to drug nanocrystal regarded as universal, simple and industrially feasible. It includes buttom up method (Anti solvent precipitation, supercritical fluid process, spray drying) top down method(pearl milling, high pressure homogenization, combination technologies) and the latest second generation nanocrystals technologies, related physical instabilities(Sedimentation, agglomeration, crystal growth, and crystalline state conversion) and the methods to alleviate or tackle the stability problems are discussed. Oral and parenteral administration are two main applications of drug nanocrystals could enhance the intracellular uptake of macromolecules and protect them from denaturation.

Narendra Chary T *et al*., 2012, developed controlled release micropellets dosage form of Ramipril. The prototype formulation of micro pellets were prepared by using the fluid bed coater(FBC) with the air pressure 2.0 bar and spray rate 10-15ml/min. it is observed that at high pressure the pellets are breaking. Concerning results of prototype preparation of Ramipril the micro pellets were prepared by using HPMC Ethyl cellulose polymer as release retardant different concentration. Formulated micro pellets showed delayed invitro dissolution behavior, probably due to optimized concentration of polymer.

Yamini Pendyala., and Sudha Talasila., 2012, formulated and evaluated, mucoadhesive Ramipril microspheres for its potential use in the treatment of hypertension. Ramipril mucoadhesive microspheres four formulations were prepared by an emulsion solvent evaporation techniques. Among all the formulation F4 showed good dissolution profile with 81% of drug release in 2hours. The results showed a

sustained anti-hypertension effect over a longer period of time in case of mucoadhesive microspheres, compared to the powder. Finally concluded that, the prolonged gastrointestinal resisdence time and slow release of Ramipril resulting from the mucoadhesive microsphere.

Harish Chander *et al*., 2011., formulated and evaluated, fast dissolving tablets of Ramipril using direct compression technique (effervescent) with sodium bicarbonate, mannitol, polyvinylpyrrolidone, citric acid. Eight different formulation of Ramipril were prepared by using different ratio of NAHCO3: MANNITOL by direct compression method. The tablets were characterized by hardness, wetting time, weight variation, water Absorption Ratio, In Vitro Drug Release. The batches of all formulations, FDT4 batch with sodium bicarbonate:

mannitol (1:3) showed more release 97.56% than the other concentration and better results. Bioavailability of Ramipril can be increased by formulating it as a Fast Dissolving Tablet.

Phanchaxari M Dandagi *et al*., 2011, developed, griseofulvin formulation by nanocrystallization techniques for the enhancement of solubility and dissolution property of the drug. The drug Nanocrystals(NC) were prepared by emulsion solvent diffusion method. Two different solvents such as acetone and ethanol and two different stabilizers such as β-cyclodextrin and sodium lauryl sulphate(SLS) were evaluated in the process. Out of which formulation made of acetone were smaller size compared to ethanol. Formulation made by using acetone as solvent also possessed better dissolution velocity as compared to formulation prepared using ethanol as solvent.

Sinico C *et al*., 2011, developed piroxicam (PRX) orally disintegrating tablets (ODT) by using nanocrystal formulation in order to optimize dissolution properties of lipophilic, poorly soluble drug. Different nanocrystal formulations were prepared by using High pressure homogenization technique and Poloxamer 188 as stabilizer. Dissolution study of PRX ODT was compared to that of PRX coarse suspension ODT, PRX /Poloxamer 188 physical mixture and bulk PRX samples. Since the solubility of the different PRX polymorphic forms increased only slightly from bulk PRX (form I) to monohydrated, form II and form III. Finally concluded that the improvement in PRX dissolution rate was mainly caused by the increased surface to volume ratio due to the submicron dimension of the drug particles.

Yuan Gao *et al*., 2011, investigated the potential of oral and pulmonary baicalein nanocrystals to enhance the bioavailability. The baicalein nanaocrystal was prepared by anti solvent recrystallization techniques followed by high pressure homogenization. Invitro characterization was performed including particle size and distribution, zeta potential, dissolution, scanning electron microscopy, differential scanning after nanocrystal preparation. The mean relative bioavailability of oral baicalein nanocrystal was 1.67-fold that of oral baicalein crystal.

Mukesh S Patil *et al*., 2011, prepared Simvastatin nanoparticles by nanoprecipitation method using a partially water-miscible solvents and the mutual saturation of the aqueous and organic phases prior to form a nanosuspension in order to reduce the initial thermodynamic instability of the nanoparticles. Because of the self emulsifying properties of the methacrylic acid co-polymers, it was possible to prepare aqueous dispersion of colloidal size containing upto 30%w/v of Eudragit L100 using methanol

as a water miscible solvent with surfactant. Following oral administration in rats Simvastatin NP's provided significant increase in the bioavailability compared to a powder suspension formulation. Simvastatin NP's formulation was superior to marketed formulation with respect to invitro dissolution profile and in vivo hypolipidimic activity.

Basavaraj Nanjwade. K *et al.*, 2011, studied design and characterization of Lovastatin nanocrystals for solubility and dissolution enhancement. Some of the approaches were available for enhancing the dissolution of poorly soluble drugs. Nanonisation technique led to more soluble, more biologically available and safer dosage form of poorly soluble and bioavailable drugs. Lovastatin nanocrystals was formulated using simple precipitation method without using stabilizer or surfactant.The nanocrystals with less particle size was obtained with slight change in crystallinity. The nanocrystal formulated with acetone and methanol showed higher saturation solubility, less particle size, increased dissolution rate then the nanocrystal formulated with acetonitrile.

Suganeswari. M *et al.*, 2011, formulated the nanoparticles containing atorvastatin calcium and amlodipine besylate using nanoprecipitation technique with Pluronic F 68 and PLGA polymers. The prepared nanoparticles were characterized by particle size analysis, drug entrapment efficiency and *in vitro* drug release studies. The results concluded that the improvement in absorption rate, therapeutic efficacy and bioavailability of amlodipine and atorvastatin calcium.

Jingling Tang *et al.*, 2011, improved the oral bioavailabilty of genistein, by nanoparticles. Nanoparticles were prepared by the nanoprecipitation technique using

Eudragit E100 as carrier. The drug loaded nanoparticles were spherical on observation by transmission electric microscopy(TEM). Release of drug from the genistein nanoparticles was two times greater than that from the conventional capsules. These results suggested that a nanoparticle system was a potentially promising formulation for the efficient delivery of poorly water soluble drugs by oral administration.

Doaa Ahmed El-Setouhy *et al*., 2011, prepared itraconazole(ITZ) crystalline nanoparticles by using sonoprecipitation technique, in which both the solvent and antisolvent were organic in nature. The effect of stabilizer type(HPMC,HPC,Inutec SPI®, and Pluronic F127) drying method (oven and freeze drying) and matrix former used (Avicel PH101 and Aerosil® 200) on the dissolution performance as a key characteristic of nanocrystal was evaluated. Freeze dried ITZ nanocrystals containing Avicel PH101 showed better dissolution rate, when compared to other nanocrystals. Both inutec SPI® and Pluronic F127 were effective in preserving the rapid drug dissolution after 3 months of storage under different conditions.

Wei Wu *et al*., 2011, prepared silymarin glyceryl mono oleate/ poloxamer 407 liquid crystalline matrices (GMO/P407 LCM) to improve the oral bioavailability of silymarin. The silymarin GMO/P407 LCMs were prepared by a melting/ congealing method. The isotropic phenomenon observed under polarized light microscope confirmed the liquid crystalline structure at the junction of LCM and water. Most importantly a 3.5 fold increase in silymarin oral bioavailability as compared with Legalon® a commercial silymarin formulation.

Ming Thau sheu *et al*., 2011, formulated insitu formation of fenofibrate(FEB) nanocrystal from a self-microemulsifying drug delivery system to enhance oral

bioavailability. SMEDDS were formulated with Myritol and surfactant mixture(Smix) of D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) and either Tween 20 or Tween 80 at various oil/Smix ratios and water contents. The release rate from both groups obviously increased with increasing stirring rate. SMEDDS consisting of Myritol 318 and TPGS combined with Tween 80 at 4:1 was able to enhance the oral bioavailability of FFB. Tween 80 was better than Tween 20 in forming smaller particles, which enhanced the invivo absorption rate by faster the release rate and complete release were observed.

Peng Liu *et al.*, 2011, studied, nanosizing techniques were important tools for improving the bioavailability of water insoluble drugs. Here, a rapid wet milling method was employed to prepare nanosuspension. Photo correlation spectroscopy (PCS) results showed that the finest nanosuspension were obtained when 80wt% (to drug amount), pluronic F68 used as the stabilizer for indomethacin and 60wt% pluronic F127 for itrconazole. The morphology nanoparticles were observed by transmission electron microscopy (TEM), crystalline state of the drug before and after milling was confirmed using differential scanning calorimetry (DSC). The physical and chemical stabilities of the nanosuspension after storage for 2months at room temperature and 4^0C were investigated using PCS,TEM,and HPLC. There was, no obvious changes in particle size and morphology. And, there was no chemical degradation of the drug ingredients were seen.

Peng Quan *et al.*, 2011, developed solid formulation containing Nitrendipine nanocrystal for oral delivery. Nitrendipine nanocrystals were prepared by using a tandem precipitation homogenization process. The optimal process was follows firstly

nitrendipine/acetone solution(100mg/ml)was added to a polyvinyl alcohol solution (1mg/ml) at 10^{o}c, then the presuspension was homogenized for 20 cycles at 1000bar. The invitro dissolution rate of the nanocrystal was significantly increased and compared with the physical mixture and commercial tablets. The Cmax of the nanocrystal was approximately 15 folds and 10 folds greater than that of physical mixture and commercial tablet, respectively. And the $AUC_{0\rightarrow24}$ of the nanocrystals was approximately 41 folds and 10 folds greater than that of physical mixture and commercial tablet, respectively. The spray drying method was found to be suitable for conversion of the nanocrystal into solid form.

Huabing Chen *et al*., 2010, studied, poor water solubility of many drugs and drug candidates remains a major obstacle to their development and clinical application. Several nanonization techniques that seek to overcome these limitations for drug solubilization were presented. The nanoprecipitation method involves the formation of crystalline or semi crystalline drug nanoparticles by nucleation and the growth of drug crystal. Recent progress in the nanoprecitation techniques has centered on efforts to improve the production efficiency of high quality drug nanoparticles.

Suman katteboinaa *et al*., 2009, carried out the automation of the drug discovery process by technologies such as high throughout screening, combinatorial chemistry and computer aided drug design is leading to a vast number of drugs, candidates possessing a very good efficacy. Unfortunately, many of these drugs are exhibiting poor aqueous solubility. The use of drug nanocrystal is an universal formulation approach to increase the therapeutic performance of these drugs in any route of

administration. Drug nanocrystals were crystals with a size in the nanometer range, meaning that they are nanoparticles with a crystalline character.

Levent Oner *et al*., 2009, studied the major application areas of nanotechnology in pharmacy is nanoparticular drug delivery systems. Preparation of drug nanocrystals to improve the solubility of poorly water soluble drugs for oral delivery is also one of the important applications. Nanocrystal dispersion comprises water, active drug substance and a stabilizer. Different techniques can be used to prepare nanocrystal formulation of a drug powder such as homogenization, co-precipitation, spray drying and milling. Nanocrystals are physically stable due to the presence of stabilizers. The advantage of Nanocrystals formulations are enhanced oral bioavailability, improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration due to decreased particle size range. This technology will be substantially useful for the manufacture of poorly water soluble drug products for oral delivery.

N.Jawahar *et al*., 2009, prepared PLGA [Poly (DL-lactide/glycolide copolymer)] nanoparticals of carvedilol, to improved the bioavailability and prolonged the antihypertensive effect of the drug. Carvedilol encapsulated by nanoprecipitation method using PLGA and Pluronic F-68. They may have utility for site specific drug delivery since the small size of the particle and its biodistribution properties may allow their delivery to target sites.

Adlin Jino Nesalin. J *et al*., 2009, formulated Flutamide nanoparticles using chitosan polymer by ionic gelation technique. Nanoparticles of different core : coat ratio were formulated and analysed for total drug content, loading efficiency, particle size and *in*

vitro drug release studies. The drug loading capacity of nanoparticles containing drug : polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 63.3 ± 0.43, 66.3 ± 0.58, 68.1 ± 0.38, 75.2 ± 0.52, 71.0 ± 0.46. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation F4 registered highest entrapment of 75.2%. The particle size distribution was found to be 400nm by scanning electron microscopy (SEM) analysis. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core : coat ratio 1:4 was showed better sustained release for about 12hours as compared to other formulations.

Rainer Muller. H *et al*., 2008, developed nanocrystal technology, drug delivery and its clinical application. Transfer of material into the nanodimension changes their physical properties which were used in pharmaceutics to develop a new innovative formulation principle for poorly soluble drugs; the drug nanocrystals. The industrially relevant production technology, pearl milling and high pressure homogenization are reviewed . The physics behind the drug nanocrystals and changes of the physical properties are discussed. It is clearly ideally suited for drugs with solubility problems. Particle size diminution and the resulting increase in particle surface, curvature,saturation solubility and consequently the increased dissolution velocity are important factors.

Lei Gao *et al*., 2008, studied, formulation of poorly soluble drug was general intractable problem in pharmaceutical field, especially those compounds poorly soluble in both aqueous and organic media. It was difficult to resolve this problem using conventional formulation approaches, so many drugs were abandoned early in

discovery. Nanocrystals, a new carrier free colloidal drug delivery system with a particle size ranging from 100nm to 1000nm. They discussed the special features of drug nanocrystal with easily scaled up, which was the pre requisite to the development of a delivery system as a market product.

Rainer H Muller *et al*., 2007, investigated the feasibility of nanosuspension technology by high pressure homogenization to enhance the chemical stability of ascorbyl palmitate (AP), followed by lyophilization. Sodium dodecyl sulfate (SDS) and Tween 80 were chosen as emulsifying agents to stabilize the develop AP nanosuspension. After 3 months of storage at 3 different temperature (4^oC, 25^oC and 40^oC), the photon correlation spectroscopy (PCS) analysis of AP nanosuspension revealed that the mean particle size of these stabilized with SDS significantly increased compared to those stabilize with Tween 80. The percentage of AP remaining in nanosuspension stabilized with Tween 80 was higher than 90% after 3months storage at 4^oC, 25^oC and 40^oC. To increase the chemical stability of AP nanosuspension. A drug powder was prepared by lyophilization. The effect of the presence of cryoprotectant terhalose on the physical stability was evaluated at different concentrations. After redispersing the lyophilized product the mean size of AP nanosuspensions without terhalose was significantly higher compared with system with terhalose. It was found that the mean size of AP nanosuspensions stabilized with Tween 80 remained in the nanometer range and the amount of the active determined by HPLC, was more than 90% when stored at 3 different temperatures during 3 months. From the X-Ray diffracto grams, it was shown that AP remained in a crystalline state which is physiochemically and thermodynamically more stable than AP in an amorphous state.

Jonghwi Lee *et al*., 2006, enhanced the oral and parentral delivery of poorly water soluble pharmaceutical ingredients(API's), reports have been limited on the various drying procedures to convert a liquid nanocrystal dispersion into solid dosage forms. The solid dosage form should consist of nanocrystals that can be readily reconstitute into their original size upon dissolution in water. The freeze drying process of nanocrystal dispersion was examined at varying freezing rates. As freezing rate decrease, more particle-particle aggregation developed. A critical freezing rate, the dried nanocrystal can not be re-dispersed. Freeze drying at a freezing rate near the critical value produces dry powders of bimodal particle size distribution after re-dispersion. The study suggests that freezing rate was an important parameter in preparing solid dosage forms from nanocrystals dispersion.

Rainer H Muller *et al*., 2005, developed drug nanocrystal by bottom up technique (precipitation) was briefly described, main fours were given on particle diminution by high pressure homogenization. There was also a combination process of precipitation followed by a second high energy step eg homogenization. Finally suspension of drug nanocrystals in a liquid, were prepared and called as nanosuspension. The effect of production parameters (power density, number of homogenization cycles) on crystal size. As an important point the transfer of the liquid nanosuspension to patient convenient oral dosage forms such as tablets and/or capsules were described.

Amighi K *et al*., 2005, prepared nifedipine nanoparticles using high pressure homogenization. Nanoparticals were characterized in terms of size, morphology and redispersion characteristics following water removal. Saturation solubility and dissolution characteristics were investigated and compared to the un-milled

commercial NIF to verify the theoretical hypothesis on the benefit of increased surface area. Crystalline state evaluation before and following particle size reduction was also conducted through differential scanning calorimeter (DSC) and Powder X-Ray diffraction (PXRD). Through this study it has been shown that initial crystalline state is maintained following particle size reduction and that the dissolution characteristics of Nifedipine nanoparticles were significantly increased in regards to the commercial product. This approach should have a general applicability to many poorly water soluble drug entities.

Jonghwi Lee *et al*., 2005, reported the role of polymeric stabilizers for drug nanocrystal dispersions. Also successfully developed the wet comminution process for drug nanocrystals in the presence of polymeric stabilizers. The selection of proper stabilizer for the drug was chosen by studying the nature of interaction between polymeric stabilizers and drug by analyzing the steady state particle sizes of seven drugs obtain by wet comminution. The surface energy of drugs and polymers were measured by contact angle measurement. The stabilization ability of polymers and the subsequent steady state particle size of drug nanocrystal depend on various parameters including surface energy and specific interactions.

CHAPTER IV

AIM OF THE WORK

CHAPTER-IV

AIM OF THE WORK

During the last two decades, many modern technologies such as high throughput screening, combinatorial chemistry, and computer-aided drug design in the pharmaceutical research and development area is leading to a vast number of drug candidates possessing a very good efficacy. Unfortunately many of these drug candidates are exhibiting poor aqueous solubility. Although some approaches are available for enhancing the dissolution of poorly soluble drugs, but has certain drawbacks like low drug loading and large dose. However, a new solution to poorly water soluble drugs candidates are now available, that is, nanonisation, and it leads to much more soluble, more biologically available and safer dosage form of poorly soluble and poorly available drugs.

Ramipril is a prodrug, and is converted to the active metabolite Ramiprilat, by liver esterase enzyme. It is an angiotensin converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. Ramipril is chemically (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2yl]amino}propanoyl octahydrocyclopenta[b]pyrrole-2-carboxylic acid. Ramipril lowers the production of angiotensin II from angiotensin I by inhibiting angiotensin converting enzyme (ACE). Therefore, relaxing arterial muscles, while at the same time enlarging the arteries, allowing the heart to pump blood, more easily into large passageways.

The absolute oral bioavailability of Ramipril following oral administration is 28%-30%. It is soluble in methyl alcohol and sparingly soluble in water. (Harish Chander *et al*., 2011)

Drug nanocrystals are pure solid drug particles with a mean diameter below 1000nm. According to the Noyes-Whitey equation, a decrease in particle size will lead to an increase in effective surface area in the diffusion layer, which, in turn, increase the drug dissolution rate. Nanocrystal is also a registered trademark of Elan Pharma International Ltd (Ireland). Drug nanocrstals are one of the most important strategies to enhance the oral bioavailability of sparingly water soluble drugs. Drugs nanocrystals can be used for chemical stabilization of chemically labile drugs. The increased stability can be explained by a shield effect of surfactants and the drug protection by a monolayer made of degraded drug molecules which reduce the accessibility for destructive agents.

The present study is carried out to develop nanocrystal of Ramipril, in order to enhance solubility and bioavailability, by decreasing the particle size of the drug. Drugs nanocrystals are prepared by emulsion solvent diffusion method. Solubility and dissolution profile of obtained nanocrystals are compared with pure drug.

CHAPTER V

PLAN OF WORK

CHAPTER-V

PLAN OF WORK

1. STANDARD CURVES FOR RAMIPRIL

a) **Preparation of calibration medium**

b) **Estimation of absorption maximum (λmax)**

c) **Preparation of standard curves**

2. PREFORMULATION (COMPATABILITY) STUDIES

a) **Infrared (IR) spectroscopic studies**

3. FORMULATION OF RAMIPRIL NANOCRYSTALS

The nanocrystals are prepared by emulsion solvent diffusion method. The preparation process involves following steps.

a) **Formulation of Ramipril nanosuspensions**

b) **Lyophilization of nanosuspensions to obtain the nanocrystals**

4. CHARACTERIZATION OF RAMIPRIL NANOCRYSTALS

a) **Determination of drug content**

b) ***In vitro* dissolution studies**

c) **Determination of Particle size and Zeta potential**

d) ***Solubility studies***

5. SELECTION AND EVALUATION OF BEST FORMULATION

The best formulation is selected depending on the results obtained from particle size, *in-vitro* drug release studies and solubility studies

a) **Infrared (IR) spectroscopic studies**
b) **Scanning Electron Microscopy (SEM)**
c) **X-ray Powder Diffraction (XRPD) analysis.**

CHAPTER VI

MATERIALS AND EQUIPMENTS

CHAPTER-VI

MATERIALS AND EQUIPMENTS

MATERIALS USED:

MATERIALS NAME	SUPPLIERS
Ramipril	Gift samples from Dr.Reddy's Laboratories, Hydrabad.
Hydroxy Propyl Methyl Cellulose (HPMC) K15M	Gift samples from Steril-gene Life science (P) Ltd, Pondicherry.
Poly vinyl pyrrolidone (PVP K30)	Gift sample from Shasun Pharmaceuticals, Pondicherry.
β Cyclodextrin(BCD)	Nice chemicals, Cochin.
Sodium Lauryl Sulphate (SLS)	Rankem fertilizers and chemicals (P) Ltd, New Delhi.
Poly Ethylene Glycol (PEG) 6000	High Purity Laboratory Chemicals (P) Ltd, Mumbai.
Methanol	Universal Scientific Appliances, Madurai.
Potassium dihydrogen Phosphate	High Purity Laboratory Chemicals (P) Ltd, Mumbai.
Sodium Hydroxide	High Purity Laboratory Chemicals (P) Ltd, Mumbai.

EQUIPMENTS USED:

EQUIPMENTS NAME	MANUFACTURER'S
Electronic weighing balance	A & D Company, Japan.
UV-Visible spectrophotometer	Shimadzu Corporation, Japan.
Infrared spectroscopy	Spectrum RX-1, Perkin Elmer, German.
Homogenizer	M.S.E. Ltd., England
Refrigerator	Kelvinator, India.
Scanning electron microscope	Hitachi X650, Tokyo, Japan.
Particle size analyzer	Nano ZS 90, Malvern Instruments Ltd.,UK
Freeze dryer	Lyodel-Delvac Pumps Pvt. Ltd, USA.
Mechanical shaker	Secor, India.
Environmental chamber	Inlab equipments Pvt. Ltd., Madras.
Dissolution apparatus	Labindia – Disso 2000, India.
Ultra Sonicator	Vibronic's Ultrasonic processor, India.
X-ray diffractometer	Digaku XRD-462, Japan.

CHAPTER VII

DRUG PROFILE

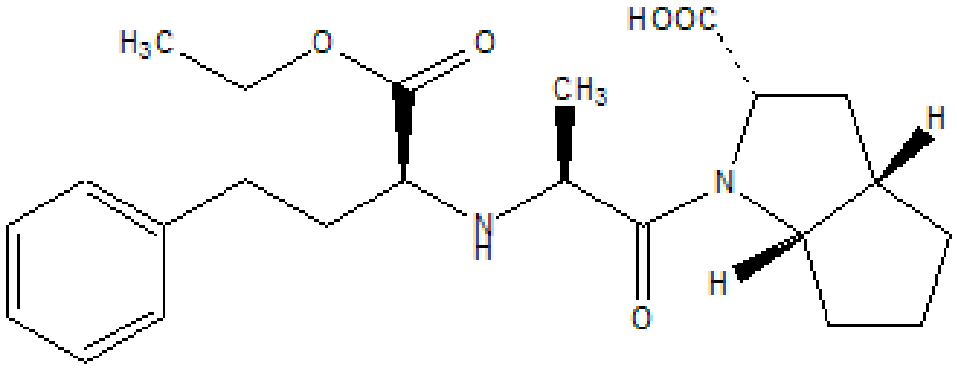
CHAPTER-VII

DRUG PROFILE

DRUG NAME : RAMIPRIL

SYNONYM : HOE-498; Ramiprilum

STRUCTURAL FORMULA :



CHEMICAL FORMULA : C_{23} H_{32} N_2O_5

CHEMICAL NAME:

(2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2yl]amino}propanoyl octahydrocyclopenta[b]pyrrole-2-carboxylic acid

DESCRIPTION:

Physical state	:	white crystalline powder.
Solubility	:	Ramipril is freely soluble in methanol and sparingly soluble in water
Molecular weight	:	416.5 g/mol
pKa	:	5.2
log P	:	1.47

Refractivity	:	111.19
Melting point	:	109° C
Polarizability	:	44.78

MECHANISM OF ACTION:

Ramipril is a prodrug, and is converted to the active metabolite Ramiprilat, by liver esterase enzyme. It is an angiotensin converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. Ramipril lowers the production of angiotensin II from angiotensin I by inhibiting angiotensin converting enzyme (ACE). Therefore, relaxing arterial muscles, while at the same time enlarging the arteries, allowing the heart to pump blood, more easily into large passageways. Ramipril also causes an increase in plasma rennin activity likely due to loss of feedback inhibition mediated by angiotensin II on the release of rennin and stimulation of releax mechanisms via baroreceptors.

PHARMACOKINETICS:

Absorption:

The absolute oral bioavailability of Ramipril following oral administration is 28%-30%. The extent of absorption is at least 50-60%. Food decreases the rate of absorption from the GI tract without affecting the extent of absorption, when oral administration is compare to intravenous administration.

Volume of distribution:

Mean volume of distribution at steady-state of Ramipril is approximately 90 liters.

Protein binding:

The plasma protein binding of racemic Paliperidone is 73%.

Metabolism:

Ramipril is metabolized in the liver to the active drug metabolite of Ramiprilat by hydrolysis via liver esterase enzymes. Other metabolites, diketopiperazine ester, the diketopiperazine acid, and the glucuronides of ramipril and ramiprilat, are inactive.

Route of elimination:

Ramipril is almost completely metabolised and the metabolites are excreted mainly via the kidneys (60%), and feces (40%).

INDICATIONS AND USAGE:

- Mild to moderate hypertension.
- Cardiac failure following myocardial infarction.
- To reduce proteinuria and the decline in glomerular filtration rate in patients with diabetic nephropathy and hypertension.
- To reduce the risk of myocardial infarction, stroke or cardiovascular death and to reduce the need for revascularization procedures in patients with an increased cardiovascular risk [such as manifest coronary heart disease (with or without a history of myocardial infarction), a history of stroke or a history of peripheral vascular disease]..
- To reduce the risk of myocardial infarction stroke or cardiovascular death in diabetic patients.

DOSING:

For hypertension: The dose range is 2.5 mg to 10 mg as a single daily dose. The antihypertensive effect is evident within one to two hours after intake of the medicine, peak effect occurs three to six hours after intake and has been shown to be maintained

for at least 24 hours at recommended doses. A maximum dose of 10 mg should not be exceeded.

For Post-Myocardial Infarction: The recommended dosage is 2.5 mg twice daily for two days. If well tolerated increase the dose to ramipril 5 mg twice daily. If patients are unable to tolerate 2.5 mg initially, 1.25 mg twice daily may be given initially and later increased to 2.5 mg twice daily.

For Non-diabetic and diabetic nephropathy: The recommended initial dose is 1.25 mg once daily. Depending on how the patient tolerates the medicine, the dose should be increased to doubled at intervals of 2 to 3 weeks. Maximum permitted daily dose is 10 mg.

To reduce the risk of myocardial infarction, stroke or cardiovascular death: The recommended initial dose is 2.5 mg once daily. Depending on the tolerability, the dose is gradually increased to doubling, after one week of treatment. Three weeks later, it should be doubled again to the usual maintenance dose of 10 mg once daily.

Dosage Adjustment in Renal Impairment: Drugs are not recommended for use in dialysis patients.

DOSAGE FORMS:

Capsule - Oral 1.25mg

Capsule – Oral 2.5mg

Capsule – Oral 5mg

Capsule – Oral 10mg

Capsule – Oral 15mg

Tablet – Oral 1.25mg

Tablet – Oral 2.5mg

Tablet – Oral 5mg

Tablet – Oral 10mg

Tablet – Oral 15mg

ADVERSE REACTIONS:

Haematological:

Less frequent: Decrease in white blood cell count, haemoglobin and haemocrit, bone marrow depression, anaemia, thrombocytopenia, agranulocytosis, haemolytic anaemia.

Cardiovascular:

Less frequent: Orthostatic effects including hypotension, myocardial infarction, cerebrovascular accident, palpitations and tachycardia.

Neurological:

More frequent: Dizziness, headache, fatigue.

Less frequent: Mood alterations, mental confusion, paraesthesia, vertigo, sleep disturbances.

Endocrine/Metabolic:

Less frequent: Hyperkalaemia, hyponatraemia, increases in blood urea, increases in serum creatinine.

Gastro-intestinal:

More frequent: Diarrhoea, nausea.

Less frequent: Abdominal pain, indigestion, dry mouth, pancreatitis, vomiting and taste disturbances.

Kidney/Genito-urinary:

Less frequent: Uraemia, oligouria, anuria, renal dysfunction, acute renal failure, impotence.

Liver/Hepatic:

Less frequent: Hepatitis (hepatocellular or cholestatic) jaundice, increase in liver enzymes, increase in serum bilirubin.

Musculoskeletal:

Less frequent: Asthenia.

Respiratory:

More frequent: Cough.

Less frequent: Bronchospasm, rhinitis, sinusitis.

Skin:

Less frequent: Rash, urticaria, diaphoresis, alopecia, pruritus psoriasis, severe skin disorders including pemphigus, toxic epidermal necrolysis, Stevens-Johnson Syndrome and erythema multiforme.

Others:

Less frequent: angioedema reactions like Angioedema of the face, which may be fatal, extremities, lips, tongue, glottis and/or larynx and intestinal angioedema. A symptom complex has been reported which may include fever, vasculitis, myalgia, arthritis/arthralgia, positive antinuclear antibodies (ANA), elevated erythrocyte sedimentation rate, eosinophilia and leucocytosis.

OVERDOSAGE:

While experience with Ramipril overdose is Severe hypotension, electrolyte disturbances and renal failure.

CONTRAINDICATIONS

Sensitivity to any of the components of ramipril, patients with a history of angioedema related to previous ACE-inhibitor therapy or angiotensin receptor blocker. Hereditary or idiopathic angioedema. Aortic stenosis. Hypertrophic obstructive cardiomyopathy. Severe renal function impairment (creatinine clearance below 30 mL/min). Renal artery stenosis in patients with a single kidney. Concomitant therapy with potassium sparing diuretics such as spironolactone, triamtrene, amiloride. Porphyria.

DRUG INTERACTIONS:

- Co-administration with Amiloride, increased risk of hyperkalemia.
- Co-administration with Azilsartan medoxomil, dual blockade of renin-angiotensin system. Increases risks of hypotension, hyperkalemia, renal impairment.
- Co-administration with Drospirenone, increased risk of hyperkalemia.
- Co-administration with Icatibant, may attenuate the antihypertensive effect of ACE inhibitors by pharmacodynamic antagonism. Monitor concomitant therapy closely.
- Concomitant therapy with Ramipril may increase the blood-glucose-lowering effect of insulin lispro and thus the chance of hypoglycemia should be monitored closely.
- Increases the serum levels of lithium, when administered with Ramipril.
- Co-administration with Spironolactone, potassium, Triamterene, increased risk of hyperkalemia.
- Tizanidine increases the risk of hypotension with Ramipril.
- Co-administration with Tobramycin, increased risk of nephrotoxicity.

BRAND NAME:

Cardiopril (Dr.Reddy's).,

Cardace (Sanofi Aventis).,

Hopace (Micro Cardicare).,

Ramace (Astra Zeneca).,

Ramipres (Cipla).,

Ramipro (Emcure).,

Ramiril (Micro labs).,

Ramistar (Lupin Pinnacle).,

Topril (Torrent Delta).,

(Drugbank.com., Clarke's Analysis of Drugs and Poisons 3rd Edition)

CHAPTER VIII

EXCIPIENTS PROFILE

CHAPTER-VIII

EXCIPIENTS PROFILE

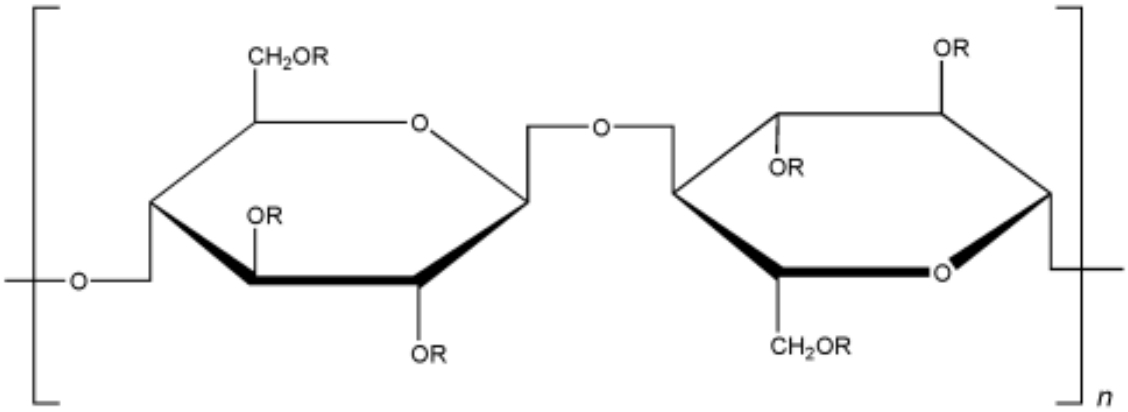
HYDROXY PROPYL METHYL CELLULOSE

SYNONYM:

- Hypromellose.
- Methocel

STRUCTURE:

(Hand book of Pharmaceutical Excipients. Pharmaceutical Press, London. 5th edtition)



where R is H, CH3, or CH3CH(OH)CH2

EMPIRICAL FORMULA:

It is a partly O-methylated and O-(2-hydroxypropylated) cellulose. It is available in several grades that vary in viscosity and extent of substitution.

MOLECULAR WEIGHT:

10 000–1 500 000 Dalton

DESCRIPTION:

- **Colour**: white or creamy-white fibrous or granular powder.
- **Odour**: odourless.
- **Taste**: Tasteless.

- **Solubility:** Soluble in cold water, forming a viscous colloidal solution; practically insoluble in hot water, chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents. Some grades are swellable in ethanol.
- **Melting point:** Browns at 190–2008°C; chars at 225–2308°C

METHOD OF MANUFACTURE:

A purified form of cellulose, obtained from cotton linters or wood pulp, is reacted with sodium hydroxide solution to produce a swollen alkali cellulose that is chemically more reactive thanuntreated cellulose. The alkali cellulose is then treated with chloromethane and propylene oxide to produce methyl hydroxypropyl ethers of cellulose. The fibrous reaction product is then purified and ground to a fine, uniform powder or granules. Hypromellose can then be exposed to anhydrous hydrogen chloride to induce depolymerization, thus producing low viscosity grades.

Typical viscosity values for 2 % (w/v) aqueous solutions of different viscosity grades of hpmc at 20°c:

Methocel K100 Premium LVEP : 100

Methocel K4M Premium : 4000

Methocel K15M Premium : 15000

Methocel K100M Premium : 100 000

Methocel E4M Premium : 4000

Methocel F50 Premium : 50

Methocel E10M Premium CR : 10 000

Methocel E3 Premium LV : 3

Methocel E5 Premium LV : 5

Methocel E6 Premium LV : 6

Methocel E15 Premium LV : 15

Methocel E50 Premium LV : 50

Metolose 60SH : 50, 4000, 10 000

Metolose 65SH : 50, 400, 1500, 4000

Metolose 90SH : 100, 400, 4000, 15 000

STORAGE CONDITION:

It should be stored in a well-closed container, in a cool, dry place.

HANDLING PRECAUTION:

- Hypromellose dust may be irritant to the eyes and eye protection is recommended.
- Excessive dust generation should be avoided to minimize the risks of explosion. Hypromellose is combustible.

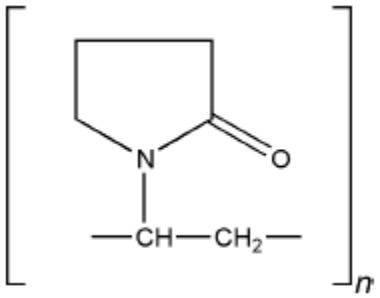
(Hand book of Pharmaceutical Excipients by Raymond C. Rowe et.al., 2009)

PVP K 30

SYNONYM:

- Kollidon
- Plasdone
- poly[1-(2-oxo-1-pyrrolidinyl)ethylene]
- Polyvidone;Polyvinylpyrrolidone
- PVP; 1-vinyl-2-pyrrolidinone polymer.

STRUCTURE:



CHEMICAL NAME:

1-Ethenyl-2-pyrrolidinone homopolymer

EMPIRICAL FORMULA:

$(C_6H_9NO)_n$ 2500–3 000 000

The USP 28 describes povidone as a synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in polymers of various molecular weights. It is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-value, in the range 10–120.

The K-value is calculated using Fikentscher's equation:

$$\text{Log } z = c \left(\frac{75k2}{1+1.5kc} \right)$$

where z is the relative viscosity of the solution of concentration c (in % w/v), and k is the K-value $\times 10^{-3}$

MOLECULAR WEIGHT: 50000

FUNCTIONAL CATEGORY:

Disintegrant; dissolution aid; suspending agent; tablet binder

DESCRIPTION:

Povidone occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder.

PROPERTIES:

Physical state	:	white powder
Solubility	:	freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil.

HANDLING PRECAUTIONS:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection is recommended

STABILITY AND STORAGE CONDITIONS:

Povidone may be stored under ordinary conditions without undergoing decomposition or degradation. However, since the powder is hygroscopic, it should be stored in an airtight container in a cool, dry place.

SAFETY:

Povidone is widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, povidone may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. Additionally it has no irritant effect on the skin and causes no sensitization.

(Handbook ofPharmaceutical excipients- 5th edition, 641-643).

BETACYCLODEXTRINS

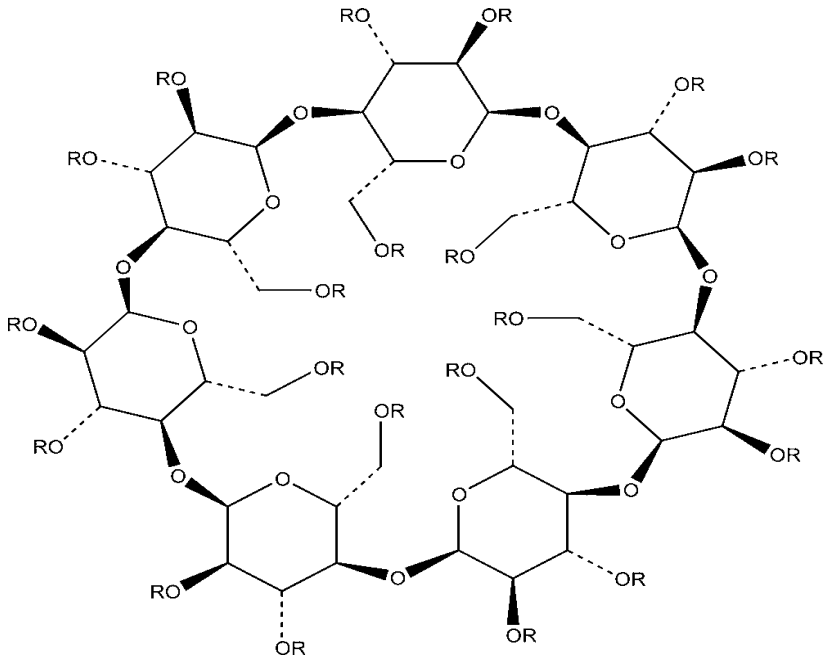
SYNONYMS:

- b-Cyclodextrin beta-cycloamylose
- Beta-dextrin
- Betadexum

EMPIRICAL FORMULA : $C_{42}H_70_{35}$

MOLECULAR WEIGHT : 1135

STRUCTURAL FORMULA:



FUNCTIONAL CATEGORY:

Solubilizing agent; stabilizing agent.

PHARMACOPEIAL SPECIFICATIONS:

SOLUBILITY :

Soluble 1 in 200 parts of propylene glycol, 1in50 of water at 208C, 1 in 20 at 508C; practically insoluble in acetone, ethanol (95%), and methylene chloride.

SPECIFIC ROTATION : D25= þ162.08;

SURFACE TENSION (AT 258C) n: 71 mN/m (71 dynes/cm);

STABILITY AND STORAGE CONDITIONS:

β-Cyclodextrin is stable in the solid state if protected from high humidity. β-Cyclodextrins should be stored in a tightly sealed container, in a cool, dry place.

METHOD OF MANUFACTURE:

Betacyclodextrin is produced by the action of the enzyme cyclodextrin glucosyltransferase upon starch or a starch hydrolysate. An organic solvent is used to direct the reaction that produces betacyclodextrin, and to prevent the growth of microorganisms during the enzymatic reaction. The insoluble complex of betacyclodextrin and organic solvent is separated from the non cyclic starch, and the organic solvent is removed in vacuum so that less than 1 ppm of solvent remains in the betacyclodextrin. The betacyclodextrin is then carbon treated and crystallized from water, dried, and collected.

SAFETY:

- nontoxic and nonirritant
- cyclodextrins are approved for use in food products and orally administered pharmaceuticals in a number of countries.
- Cyclodextrins are not irritant to the skin and eyes, or upon inhalation.
- There is also no evidence to suggest that cyclodextrins are mutagenic or teratogenic.

HANDLING PRECAUTIONS:

should be handled in a well-ventilated environment.

SODIUM LAURYL SULFATE

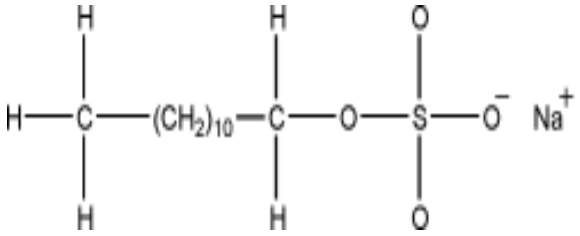
SYNONYMS:

- Dodecyl sodium sulfate.
- Elfan 240.
- Texapon K12P.
- Sodium dodecyl sulfate.
- Sodium monododecyl sulfate.

CHEMICAL NAME:

Sulfuric acid monododecyl ester sodium salt.

CHEMICAL STRUCTURE:



EMPIRICAL FORMULA: $C_{12}H_{25}NaO_4S$

MOLECULAR WEIGHT: 288.38

HLB VALUE: ≈ 40

FUNCTIONAL CATEGORY:

Anionic surfactant; detergent; emulsifying agent; skin penetrant; tablet and capsule lubricant; wetting agent.

APPLICATION IN PHARMACEUTICAL FORMULATION AND TECHNOLOGY:

Sodium lauryl sulfate is an anionic surfactant employed in a wide range of non parentral pharmaceutical formulations and cosmetics.

Use	Concentration (%)
Anionic emulsifiers, forms self emulsifying bases with fatty alcohols	0.5–2.5
Detergent in medicated shampoos	≈10
Skin cleanser in topical applications	1
Solubilizer in concentrations greater than critical micelle concentration	>0.0025
Tablet lubricant	1.0–2.0
Wetting agent in dentrifices	1.0–2.0

- It is a detergent and wetting agent effective in both alkaline and acidic conditions.
- In recent years it has found application in analytical electrophoretic techniques;SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresisis one of the more widely used techniques for the analysis of proteins.
- The sodium lauryl sulfate has been used to enhance the selectivity of micellar electrokinetic chromatography (MEKC).

DESCRIPTION:

SLS consists of white or cream to pale yellow- colored crystals, flakes, or powder having a smooth feel, a soapy. Bitter taste and a faint odour of fatty substances.

MELTING POINT:

204-207°c (for pure substance)

SOLUBILITY:

Freely soluble in water, giving an opalescent solution; practically insoluble in chloroform and ether.

STABILITY AND STORAGE CONDITION:

Sodium lauryl sulfate is stable under normal storage conditions. However, in solution, under extreme conditions i.e. PH 2.5 or below, it undergoes hydrolysis to lauryl alcohol and sodium bisulfate.

The bulk material should be stored in a well closed container away from strong oxidizing agents in a cool, dry place.

INCOMPATIBILITIES:

Sodium lauryl sulfate reacts with cationic surfactants, causing loss of activity even in concentrations too low to cause precipitation. Unlike soaps, it is compatible with dilute acids and calcium and magnesium ions. Solutions of sodium lauryl sulfate (PH 9.5-10.0) are mildly corrosive to mild steel, copper, brass, bronze and aluminium. Sodium lauryl sulfate is also in compatible with some alkaloidal salts and precipitates with lead and potassium.

HANDLING PRECAUTIONS:

- Inhalation and contact with the skin and eyes should be avoided; eye protection gloves, and other protective clothing, depending on the circumstances, are recommended.
- Adequate ventilation should be provided or a dust respirator should be worn. Prolonged or repeated exposure should be avoided.
- Sodium lauryl sulfate emits toxic fumes on combustion.

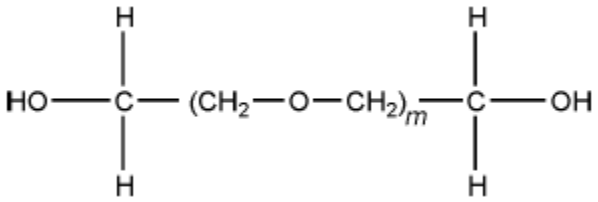
  (Hand book of Pharmaceutical excipients by Raymond C Rowe -5th edition, 1811- 1816).

PEG-6000

SYNONYM:

Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; PEG; Pluriol E; polyoxyethylene glycol.

STRUCTURE:



CHEMICAL NAME:

a-Hydro-o-hydroxypoly(oxy-1,2-ethanediyl)

EMPIRICAL FORMULA:

HOCH2(CH2OCH2)mCH2OH

where m represents the average number of oxyethylene groups. Alternatively, the general formula H(OCH2CH2)nOH may be used to represent polyethylene glycol, where n is a number m in the previous formula +1.

MOLECULAR WEIGHT : 6000

FUNCTIONAL CATEGORY:

Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.

DESCRIPTION:

The USPNF 23 describes polyethylene glycol as being an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and above are solids at ambient temperatures.

Solid grades (PEG>1000) are white or off-white in color, and range in consistency from pastes to waxy flakes. They have a faint, sweet odor. Grades of PEG 6000 and above are available as free-flowing milled powders.

PROPERTIES:

Physical state	:	white flakes
Solubility in water	:	soluble in water
Solvent solubility	:	soluble in methanol, ethanol(95%).

HANDLING PRECAUTION:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection is recommended.

STABILITY AND STORAGE CONDITIONS:

Polyethylene glycols are chemically stable in air and in solution, Polyethylene glycols should be stored in well-closed containers in a cool, dry place.

SAFETY:

Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials. Adverse reactions to polyethylene glycols have been reported, the greatest toxicity being with glycols of low molecular weight. However, the toxicity of glycols is relatively low.

CHAPTER IX

EXPERIMENTAL PROTOCOL

CHAPTER-IX

EXPERIMENTAL PROTOCOL

1. STANDARD CURVES FOR RAMIPRIL:

a) Preparation of calibration medium (Indian Pharmacopoeia 2010)**:**

Phosphate Buffer pH 6.8:

A known volume (50ml) of 0.2M potassium dihydrogen phosphate is placed in a 200ml volumetric flask. 22.4ml of 0.2M sodium hydroxide is added and makeup to the volume with distilled water.

• ***0.2 M potassium dihydrogen phosphate:***

A known quantity (27.218 g) of potassium dihydrogen phosphate is dissolved and diluted to 1000ml with water.

• ***0.2 M sodium hydroxide:***

A known quantity (8 g) of sodium hydroxide is dissolved and makeup to 1000ml with water.

b) Estimation of absorption maximum (λmax):

A known weight (10 mg) of drug (Ramipril) is dissolved in sufficient amount of methanol in 100ml volumetric flask and make upto 100ml with phosphate buffer pH (6.8) to prepare a primary stock solution (100µg/ml). The stock solution is further diluted using a phosphate buffer pH (6.8) solution to 10µg/ ml concentration. The resultant solution is scanned in the range of (200- 400nm) by UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) to get absorption maximum (λ max).

c) Preparation of standard curves:

From the above prepared stock solution, (5 to 25µg/ml) concentration solutions are prepared using the phosphate buffer pH (6.8) solution. The absorbance of these solutions are measured at λ max by UV- spectrophotometer (UV-1700 Shimadzu corporation, Japan). A standard curve is plotted using concentration on X-axis and the absorbance obtained on Y-axis. (Harish Chander., *et al,* 2011)

2. PREFORMULATION (COMPATABILITY) STUDIES:

preformulation studies are carried out by Infrared spectrophotometer in order to evaluate the drug and stabilizer interaction.

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectrum of the drug, stabilizers, and its physical mixtures are obtained by using IR spectrophotometer (Spectrum RX-1 Perkin Elmer, German). The pellets are prepared on KBr-press under hydraulic pressure of 150kg / cm^2; the spectra is scanned over the wave number range of 4000 to 400 cm^{-1} at the ambient temperature (Sinco.C *et al*., 2011).

3. FORMULATION OF RAMIPRIL NANOCRYSTALS:

The nanocrystals are prepared by emulsion solvent diffusion method. The preparation process involves following steps (Phanchaxari M Dandagi *et al*., 2011).

a) Formulation of Ramipril nanosuspensions;

b) Lyophilization of nanosuspensions to obtain the nanocrystals.

a) Formulation of Ramipril nanosuspensions:

i)Preparation of drug solution:

Accurately weighed sample (100 mg) of drug is added to methanol (10 ml) to prepare the drug solution.

ii) Addition of drug solution to aqueous solution containing stabilizers:

The above prepared drug solution is to be added into required quantity (10ml) of water containing stabilizers **(HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000)** with continuous stirring on homogenizer at 1000 rpm for 2 hours

iii) Removal of solvent:

The organic solvent is removed by continuous stirring for 3-4 hours at 500 rpm (Phanchaxari M Dandagi *et al*., 2011).

b) Lyophilization of nanosuspensions to obtain the nanocrystals:

Ramipril nanosuspensions are lyophilized by using freeze dryer (Lyodel-Delvac Pumps Pvt. Ltd, USA) to enhance the chemical stability of nanocrystals. The freshly prepared nanosuspensions are lyophilized with cryoprotective agent (mannitol). Briefly, Ramipril nanosuspensions are rapidly cooled down to -50^0C for 2 hours followed by primary drying at 1.03 mbar and secondary drying at 0.001 mbar (Rainer Muller. H *et al*., 2008).

4. CHARACTERIZATION OF RAMIPRIL NANOCRYSTALS:

All the formulations are evaluated for its particle size, zeta potential, drug content, and *in vitro* drug release studies

.

a) Determination of drug content:

Sample containing 10 mg equivalent of Ramipril nanocrystals are weighed and dissolved in methanol, and the volume is made upto 100ml with phosphate buffer pH(6.8). From the above solution 10 ml is pipetted out and made upto 100 ml with phosphate buffer pH(6.8). The absorbance of resulting solution is determined at λmax (277 nm) using UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) and the drug content is estimated (Phanchaxari M Dandagi *et al*., 2010).

b) *In vitro* dissolution studies:

USP dissolution apparatus Type II (paddle method) at rotation speed of 100 rpm is used for *in vitro* testing of drug dissolution of all nanocrystal formulations. For each batch, sample of 10 mg equivalent Ramipril nanocrystals containing in capsules are taken and subjected to dissolution studies with 900 ml of phosphate buffer pH(6.8) as dissolution medium. Bath temperature is maintained at 37±0.5°C throughout study.

A sample (5 ml) of the solution is withdrawn from the dissolution apparatus at predetermined time intravels of 5, 10, 20, 30, 40, 60, 90, and 120mins. The samples are replaced with fresh dissolution medium. Absorbance values of sample solutions are measured at λmax (277 nm) in UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan). The cumulative percentage drug release is calculated (Doaa Ahmed El-Setouhy *et al*., 2011)**.**

c) Determination of Particle size and Zeta potential:

The mean particle size (z-average), and zeta potential of Ramipril nanocrystal formulations are determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd., UK). The freeze dried powders are

redispersed with water to obtain a proper scattering intensity before measurement (Dianrui Zhang *et al*., 2012).

d) Solubility studies:

Solubility of Ramipril nanocrystal formulations are studied in different solvents such as distilled water and phosphate buffer pH(6.8). An excess amount of nanocrystal formulation is added in 10 ml of the pertinent solvents. The mixtures are stirred in a mechanical shaker for 24 hours. Visual inspection is carefully made to ensure there are excess Ramipril solids in the mixture, indicating saturation have been reached. The mixtures are then filtered and filtrates are diluted suitably to determine the solubility of Ramipril in each solvent (Abdul Hasan Sathali.A., and Gopinath.M., 2013).

5. SELECTION AND EVALUATION OF BEST FORMULATION:

The best formulation is selected depending on the results obtained from particle size, *in vitro* drug release studies and solubility studies.

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectrum analysis are carried out for the selected nanocrystal formulation to find out the interactions between the drug and excipients by using IR spectrophotometer (Spectrum RX-1 Perkin Elmer, German). The pellets are prepared on KBr-press under hydraulic pressure of 150kg / cm^2; the spectra is scanned over the wave number range of 4000 to 400 cm^{-1} at the ambient temperature (Sinco.C *et al*., 2011).

b) Morphological studies of nanocrystals by using Scanning Electron Microscopy (SEM):

Morphological evaluation of the selected Ramipril nanocrystal formulation is carried out in scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan). All samples are examined on a brass stub using carbon double-sided tape. Powder samples are glued and mounted on metal sample plates. The samples are gold coated (thickness ≈15–20 nm) with a sputter coater (Fison Instruments, UK) using an electrical potential of 2.0 kV at 25 mA for 10 min. An excitation voltage of 20 kV was used in the experiments (Yuan Gao *et al*., 2011).

c) X-ray Powder Diffraction (XRPD) analysis:

The crystalline state of the samples, including the drug and freeze-dried powders are studied in X-ray diffractometer (XRD-462, Digaku, Japan). XRPD is carried out in symmetrical reflection mode using Copper line as the source of radiation and the wavelength is set at 1.5405A°. Standard runs using a 40 kV and 30 mA in this process. Samples are performed with a scanning rate of 0.1000°/min and the scanning range of the 2θ from the initial angle 4° to the final angle 90° (Dianrui Zhang *et al*., 2011).

CHAPTER X

RESULTS AND DISCUSSION

TABLES & FIGURES

CHAPTER-X

RESULTS AND DISCUSSION

1. STANDARD CURVES FOR RAMIPRIL

a) Preparation of calibration medium

The calibration medium pH (6.8) were prepared by using phosphate buffer as per the I.P procedure.

b) Estimation of absorption maximum (λmax)

The λ max of ramipril was estimated by scanning the 10μg/ml concentration of the drug solution in buffer solution of pH (6.8). It showed the λ max of 277nm (Narendra Chary., *et al*., 2012) in buffer solution of pH (6.8). The results were shown in **(Figure 10a)**.

c) Preparation of standard curves

The standard curves of ramipril prepared by using phosphate buffer pH(6.8) were shown in **(Table 1)** and **(Figure 10b)**. The linear correlation coefficient was found to be 0.9995 for pH (6.8). ramipril obeys the Beer's law within the concentration range of 5 to 25 μg/ml.

2. PREFORMULATION (COMPATABILITY) STUDIES:

a) Infrared (IR) spectroscopic studies

Infrared (IR) spectroscopic studies were carried out to confirm the compatibility between drug and the stabilizers used for the preparation of nanocrystals. The IR studies were performed for pure drug, stabilizers and physical

mixture of drug with stabilizers. The spectra studied at 4000cm^{-1} to 400 cm^{-1} were shown in **(Table 2)** and **(Figure 11a-11m)**. The principal peaks for pure drug were observed at wave numbers 1373.36 cm^{-1}, 1228.70 cm^{-1}, 1186.26 cm^{-1}, 1063.78 cm^{-1}, 815.92 cm^{-1}, 753.23 cm^{-1}, 701.15 cm^{-1}. It was found from the spectra that there was no major shifting as well as any loss of functional peaks in the spectra of drug, stabilizers and physical mixture of drug with stabilizers. This clearly indicated that there was **no interaction** between the drug and the polymer and the drug was present in its unchanged form.

3. FORMULATION OF RAMIPRIL NANOCRYSTALS

The ramipril nanocrystals were prepared by emulsion solvent diffusion method (Phanchaxari M Dandagi *et al*., 2011). The principle of this method was based on the dissolution of the active drug substance in an organic solvent which was then added into a nonsolvent (miscible with the organic solvent). In the presence of, thereafter, the nanocrystals were precipitated. Basic advantage of the precipitation technique was that it was simple and had a low cost. Also, scale up was simple in this method (Phanchaxari M Dandagi *et al*., 2011).

Ramipril nanocrystals were prepared by following process:

a) Formulation of ramipril nanosuspensions;
b) Lyophilization of nanosuspensions to obtain the nanocrystals.

Various formulations of ramipril nanocrystals (F1 - F25) were prepared by using different stabilizers like HPMC K15M, PVP K30, β Cyclodextrin, SLS, and PEG 6000 at different concentrations (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) were selected shown in **(Table 3)**

4. CHARACTERIZATION OF RAMIPRIL NANOCRYSTALS

All the formulations were evaluated for its drug content, particle size, polydispersity index, and zeta potential, *in vitro* drug release studies and solubility studies.

a) Determination of drug content

The drug content of all nanocrystal formulations (F1 to F25) was in the range of 88.00% to 96.00%. The results were shown in **(Table 4)**. The results suggest that the process employed to prepare the nanocrystals shown uniform distribution of drug.

b) *In vitro* dissolution studies

The *invitro* dissolution studies of all formulations were compared with pure drug. The results of *in vitro* drug release studies from the ramipril nanocrystals were shown in the **(Table 5a-5e)** and in **(Figure 12a-12e)**. when compared the *In vitro* release profile of all the formulations are significantly greater than that pure drug ramipril.

Formulations (F1A – F1E) prepared using different concentration of stabilizer (HPMC K15M 0.1% to 0.5%) shown the percentage drug release of 53.39%, 61.10%, 67.97%, 71.12%, and 74.81% at 120 mins respectively.

Formulations (F2A – F2E) prepared using different concentration of stabilizer (PVP K30 0.1% to 0.5%) shown the percentage drug release of 73.29%, 74.04%, 75.68%, 75.67%, and 78.78% at 120 mins respectively.

Formulations (F3A – F3E) prepared using different concentration of stabilizer (β-cyclodextrin 0.1% to 0.5%) shown the percentage drug release of 75.69%, 77.98%, 81.12%, 81.12%, and 83.43% at 120 mins respectively.

Formulations (F4A – F4E) prepared using different concentration of stabilizer (SLS 0.1% to 0.5%) shown the percentage drug release of 60.46%, 72.49%, 78.69%, 81.02%, and 87.92% at 120 mins respectively.

Formulations (F5A – F5E) prepared using different concentration of stabilizer (PEG 6000 0.1% to 0.5%) shown the percentage drug release of 57.18%, 61.06%, 64.17%, 67.23%, and 71.76% at 120 mins respectively.

The percentage drug release of all the formulations were found to be in the following order

0.1% < 0.2% < 0.3% < 0.4% < 0.5%

The release rate of the drug from the nanocrystals were increased, on increasing the stabilizers concentration.

The increased percentage drug release of stabilizer (HPMC K15M) having formulation (F1A to F1E) indicates that, stabilizer HPMC K15M was used for the suspension's stabilization as this water soluble polymer offers adequate surface active properties and it indicates that they have increased the drug release (Amighi.K *et al*., 2005).

The increased percentage drug release of stabilizer (PVP K30) having formulation (F2A to F2E) indicates that, stabilizer PVP K30 was used as water soluble compound, in order to improve the drug dissolution rate (Noushin Bolourchian *et al*., 2013).

The increased percentage drug release of stabilizer (β-cyclodextrin) having formulation (F3A to F3E) indicates that, stabilizer β-cyclodextrin to form a network through intermolecular interaction, that colud protect and it can self-associate in aqueous solution to form nano-scale aggregates that have a minimum hydrodynamic

radius in order to improve the drug dissolution rate (Phanchaxari M Dandagi *et al*., 2011).

The increased percentage drug release of stabilizer (SLS) having formulation (F4A to F4E) indicates that, SLS was used as dispersion stabilizer, it prevents agglomeration of precipitated nanocrystal in the formulation by increasing the activation energy and reduce the surface tension existing between the drug particle and the solvent by providing wettability to the particls, in order to improve the drug dissolution rate (Phanchaxari M Dandagi *et al*., 2011).

The increased percentage drug release of stabilizer (PEG 6000) having formulation (F5A to F5E) indicates that, stabilizer PEG 6000 had long hydrophilic chain and it captured the water molecule through hydrogen bonding, which were formed between the hydroxyl group and ether bond of PEG and water molecule, in order to improve the drug dissolution rate (Peng Liu *et al*., 2011).

c) Determination of Particle size and Zeta potential:

i) Particle size:

Particle size, size distribution and zeta potential were important characterizations of the nanocrystals because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances (Dianrui Zhang *et al*., 2012).

The average diameters and polydispersity index of ramipril nanocrystals were listed in **(Table 7)** and **(Figure 13a and13b)**. In the present study the particle size of ramipril nanocrystals were ranged between 80.3nm to 300.6 nm.

ii) Zeta potential

The zeta potential of the nanocrystals were allowed predictions about the storage stability of colloidal dispersions. In general, the zeta potential value at least ±30 mV can ensure the physically stable nanocrystals when using ionic surfactants for electric repulsion; however, a zeta potential about ±20 mV can also signify long-term stability of the system when nonionic surfactants were applied for steric hindrance. In addition, the type of a key variable that had prominent effect on the mean zeta potential value (Dianrui Zhang *et al*., 2012).

The ramipril nanocrystals were characterized to evaluate the effect of stabilizers at different ratios and different on surface charge of nanocrystals. The results were showed in **(Table 8)** and **(Figure 14a and14b).**

Zeta potential values of the formulations code (F3C – F3E and F4D – F4E) prepared with different showed negative zeta potential (-19.7mV to -24.7mV) which indicated a stable preparation.

d) Solubility studies

Solubility studies of pure drug (RAMIPRIL) and selected formulation (F3C to F3E and F4D to F4E) were shown in **(Table 9)** and **(Figure 15a and 15b)**. Nanocrystal formulations (F3C to F3E and F4D to F4E) shown highest solubility in distilled water as compared with pure drug.

The solubility of formulations (F3C, F3D, F3E, F4D, F4E) and pure drug in phosphate buffer pH (6.8) were 1.177mg/10ml, 2.791mg/10ml, 5.680mg/10ml, 2.294mg/10ml, 5.182mg/10ml and 0.547mg/10ml respectively. Thus the solubility of RAMIPRIL in nanocrystal formulations (F3E and F4E) was increased approximately by ten folds when compared to pure drug.

Hence, the noticeable increased saturation solubility of ramipril in the formulation of nanocrystals was mainly attributed to the decreased particle size and increased surface area. The results can be explained by the Ostwald–Freundlich equation which demonstrates that the saturation solubility of the drug increases with reduction of particle size (Dianrui Zhang *et al*., 2012).

5. SELECTION AND EVALUATION OF BEST FORMULATION:

From the above results characterization, the following two formulations were selected as the best formulation showing,

For F3E

Particle size	: 80.3 nm.
In vitro drug release	: 85.74% in 2 hours
Solubility studies	: 3.565mg/10ml distilled water
	5.68mg/10ml phosphate buffer pH(6.8)

For F4E

Particle size	: 100.8 nm.
In vitro drug release	: 87.91% in 2 hours
Solubility studies	: 3.068mg/10ml distilled water
	5.182mg/10ml phosphate buffer pH(6.8)

a) Infrared (IR) spectroscopic studies:

Infrared (IR) spectroscopic studies were carried out for pure drug, stabilizers and selected nanocrystal formulations. The spectra studied at 4000cm^{-1} to 400 cm^{-1} were shown in **(Table 2)** and **(Figure 11a-11m)**. It was found from the spectra that there was no major shifting as well as any loss of functional peaks in the spectra of

drug, stabilizers and selected formulations **(F3E and F4E)**. This clearly indicated that there was **no interaction** between the drug and the polymer and the drug was present in its unchanged form.

b) Morphological studies of nanocrystals by using Scanning Electron Microscopy (SEM):

The morphology of selected nanocrystal formulations (F3E and F$E) was examined by Scanning Electron Microscopy (SEM). The SEM image of formulation was shown in **(Figure 16a and 16b)**. It was observed that the particle size of Ramipril nanocrystal formulations (F3E and F4E) was relatively small, uniform, crystal in shape and the mean size of particles were lower than 1 μm. This data was agreement with obtained data from Malvern zeta sizer analysis **(Rainer Muller .H *et al*., 2008)**.

c) X-ray Powder Diffraction (XRPD) analysis:

The XRPD patterns of pure drug (Ramipril) and formulations (F3E, F4E) were presented in **(Figure 17a, 17b and 17c)**.

The XRPD patterns of pure drug showed numerous sharp peaks (at 2θ 15.00°, 16.80°, 20.90°, 23.00°, 25.40° and 34.31°) which are the characteristic of a crystalline compound. Drug crystallinity peaks were also detectable in formulation as shown in **(Figure 17b and 17c)**.

This result confirmed that the characteristic peaks were still preserved indicating the crystalline state was not changed.

As we know, the amorphous form can generally enhance the dissolution rate and bioavailability of drugs due to its high-energy. According to that principle and with the XRPD analysis considered, the enhancement of dissolution rate of Ramipril

may be due to the reduction of particle size or the influence of stabilizers rather than the appearance of amorphous form. Moreover, compared with the amorphous form, the maintenance of crystalline state was beneficial to a long-term stability **(Dianrui Zhang *et al*., 2012)**.

TABLE 1: CALIBRATION OF RAMIPRIL USING PHOPSPHATE BUFFER pH (6.8)

S. NO	CONCENTRATION(µg/ml)	ABSORBANCE ± SD*
1	05	0.023 ± 0.0035
2	10	0.042 ± 0.0025
3	15	0.065 ± 0.0060
4	20	0.083 ± 0.0083
5	25	0.102 ± 0.0090

n=3* **γ = 0.99958**

TABLE 2: IR PEAKS OF DRUG, STABILIZERS, PHYSICAL MIXTURE OF DRUG WITH STABILIZERS AND FORMULATIONS

S. NO	DESCRIPTION	CHARACTERISTIC PEAKS (cm^{-1}) OBTAINED
1	RAMIPRIL	1373.36, 1228.70, 1063.78, 815.92, 753.23, 701.15
2	HPMC K15M	3642.69, 2934.79, 1650.16, 1459.20, 945.15
3	PVP K30	3748.78, 3398.69, 2956.01, 1667.52,1502.60
4	β-CYCLODEXTRIN	1490.06, 1457.27, 1363.72, 948.04, 651.00, 431.10
5	SLS	1654.01, 1470.77, 1248.95, 834.24, 634.60, 591.20
6	PEG 6000	2165.17, 1650.16, 1469.81, 1240.27, 961.55
7	RAMIPRIL + HPMC K15M	2935.76, 1743.71, 1187.23, 755.16, 355.88
8	RAMIPRIL + PVP K30	2867.28, 1654.01, 1442.80, 1187.23, 701.15
9	RAMIPRIL+ β-CD	2932.86, 1653.05, 1157.33, 1029.06, 702.11
10	RAMIPRIL+ SLS	2920.32, 1743.71, 1654.01, 1465.95, 1081.44
11	RAMIPRIL + PEG 6000	3281.02, 1349.25, 1280.78,845.81, 751.30
12	FORMULATION (F3E)	2932.86, 1653.05, 1157.33, 1029.06, 702.11
13	FORMULATION (F4E)	2920.32, 1743.71, 1654.01, 1465.95, 1081.44

TABLE 3: COMPOSITION OF RAMIPRIL NANOCRYSTALS

S.NO	FORMULATION CODE	SOLVENT/ORGANIC PHASE	DRUG CONCENTRATION(mg/10ml)	STABILIZERS CONCENTRATION(%)
1	F1A	METHANOL	100mg in 10ml	HPMC K15M(0.1%)
2	F1B	METHANOL	100mg in 10ml	HPMC K15M(0.2%)
3	F1C	METHANOL	100mg in 10ml	HPMC K15M(0.3%)
4	F1D	METHANOL	100mg in 10ml	HPMC K15M(0.4%)
5	F1E	METHANOL	100mg in 10ml	HPMC K15M(0.5%)
6	F2A	METHANOL	100mg in 10ml	PVP K30(0.1%)
7	F2B	METHANOL	100mg in 10ml	PVP K30(0.2%)
8	F2C	METHANOL	100mg in 10ml	PVP K30(0.3%)
9	F2D	METHANOL	100mg in 10ml	PVP K30(0.4%)
10	F2E	METHANOL	100mg in 10ml	PVP K30(0.5%)
11	F3A	METHANOL	100mg in 10ml	BCD(0.1%)
12	F3B	METHANOL	100mg in 10ml	BCD(0.2%)
13	F3C	METHANOL	100mg in 10ml	BCD(0.3%)
14	F3D	METHANOL	100mg in 10ml	BCD(0.4%)
15	F3E	METHANOL	100mg in 10ml	BCD(0.5%)
16	F4A	METHANOL	100mg in 10ml	SLS(0.1%)
17	F4B	METHANOL	100mg in 10ml	SLS(0.2%)
18	F4C	METHANOL	100mg in 10ml	SLS(0.3%)
19	F4D	METHANOL	100mg in 10ml	SLS(0.4%)
20	F4E	METHANOL	100mg in 10ml	SLS(0.5%)
21	F5A	METHANOL	100mg in 10ml	PEG6000(0.1%)
22	F5B	METHANOL	100mg in 10ml	PEG6000(0.2%)
23	F5C	METHANOL	100mg in 10ml	PEG6000(0.3%)
24	F5D	METHANOL	100mg in 10ml	PEG6000(0.4%)
25	F5E	METHANOL	100mg in 10ml	PEG6000(0.5%)

TABLE 4: DRUG CONTENT OF RAMIPRIL NANOCRYSTALS

S.No	STABILIZERS CONCENTRATION	FORMULATION CODE	AVG±SD
1	HPMC K15M(0.1%)	F1A	93.63±1.6226
2	HPMC K15M(0.2%)	FIB	89.63±1.9561
3	HPMC K15M(0.3%)	F1C	89.70±1.3856
4	HPMC K15M(0.4%)	F1D	88.00±0.0000
5	HPMC K15M(0.5%)	F1E	89.50±2.0621
6	PVP K30(0.1%)	F2A	92.86±1.8013
7	PVP K30(0.2%)	F2B	92.00±1.6055
8	PVP K30(0.3%)	F2C	90.33±1.0237
9	PVP K30(0.4%)	F2D	93.66±1.1547
10	PVP K30(0.5%)	F2E	93.00±1.4641
11	BCD(0.1%)	F3A	95.86±0.5011
12	BCD(0.2%)	F3B	95.33±1.5166
13	BCD(0.3%)	F3C	91.66±1.1547
14	BCD(0.4%)	F3D	95.33±1.5166
15	BCD(0.5%)	F3E	93.00±1.0000
16	SLS(0.1%)	F4A	90.66±1.5166
17	SLS(0.2%)	F4B	91.66±1.1547
18	SLS(0.3%)	F4C	94.33±1.1547
19	SLS(0.4%)	F4D	92.33±1.1547
20	SLS(0.5%)	F4E	96.00±1.7320
21	PEG6000(0.1%)	F5A	93.66±1.1547
22	PEG6000(0.2%)	F5B	95.33±0.5166
23	PEG6000(0.3%)	F5C	96.00±1.7320
24	PEG6000(0.4%)	F5D	95.33±1.5166
25	PEG6000(0.5%)	F5E	96.00±1.7320

n=3*

TABLE 5a: *IN VITRO* RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING HPMC K15M AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F1A±SD	F1B±SD	F1C±SD	F1D±SD	F1E±SD	
5	12.85±1.23	21.05±1.29	24.04±1.23	30.00±1.29	27.02±1.29	9.87±1.29
10	18.89±1.42	27.14±1.29	30.88±1.24	36.88±1.29	33.88±1.29	15.89±1.29
20	25.70±1.44	32.51±1.30	37.02±1.60	43.05±1.29	40.03±2.24	22.69±1.30
30	31.16±1.49	38.07±1.32	42.65±1.64	48.77±1.31	47.21±1.32	28.10±1.32
40	37.03±1.36	43.87±1.30	49.90±1.45	54.47±1.30	53.68±2.24	34.00±1.30
60	43.20±2.64	50.82±1.31	56.14±1.53	59.99±1.29	60.68±1.25	40.90±1.31
90	47.91±0.66	55.58±1.32	61.66±1.49	66.28±1.31	67.73±2.26	45.60±1.32
120	53.39±1.51	61.10±2.26	67.97±2.33	71.12±1.32	74.81±2.28	50.32±1.33

n=3*

TABLE 5b: *IN VITRO* RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING PVP K30 AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F2A±SD	F2B±SD	F2C±SD	F2D±SD	F2E±SD	
5	28.51±2.23	25.53±1.29	32.24±1.29	34.48±1.29	36.71±1.29	9.87±1.29
10	33.14±2.24	32.38±1.29	38.38±1.29	39.14±1.29	42.88±1.29	15.89±1.29
20	40.04±2.26	39.27±1.30	45.31±1.30	43.83±1.30	48.34±1.30	22.69±1.30
30	45.71±2.29	45.69±2.27	51.06±1.32	49.56±1.32	54.14±1.32	28.10±1.32
40	52.19±1.32	53.65±2.25	57.49±1.30	56.75±1.30	59.79±1.30	34.00±1.30
60	59.19±1.33	60.66±2.26	63.02±2.24	62.28±1.28	66.08±1.30	40.90±1.31
90	66.22±1.33	66.96±2.61	69.33±1.32	68.59±1.31	73.16±1.31	45.60±1.32
120	73.29±1.34	74.04±2.62	75.68±1.31	75.67±1.32	78.78±1.32	50.32±1.33

n=3*

TABLE 5c: *IN VITRO* RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING β-CYCLODEXTRIN AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F3A±SD	F3B±SD	F3C±SD	F3D±SD	F3E±SD	
5	32.24±1.29	34.48±1.29	38.20±1.29	40.44±1.29	42.68±1.29	9.87±1.29
10	38.38±1.29	41.38±1.29	45.13±1.29	47.38±1.29	49.63±1.29	15.89±1.29
20	45.31±1.30	47.57±1.29	52.09±1.30	54.35±1.30	56.61±1.30	22.69±1.30
30	51.06±1.32	53.36±1.31	57.94±1.32	60.24±1.32	62.53±1.32	28.10±1.32
40	58.23±2.24	59.02±1.30	64.31±1.30	66.58±1.30	68.85±1.30	34.00±1.30
60	63.77±1.32	63.82±1.31	70.62±1.31	72.91±1.31	75.20±1.31	40.90±1.31
90	70.09±2.26	70.88±1.32	75.49±1.32	77.78±1.32	80.08±1.32	45.60±1.32
120	75.69±1.33	77.98±1.32	81.12±1.31	83.43±1.31	85.74±1.31	50.32±1.33

n=3*

TABLE 5d: *IN VITRO* RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING SLS AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F4A±SD	F4B±SD	F4C±SD	F4D±SD	F4E±SD	
5	28.51±2.23	24.04±2.23	32.24±1.29	35.22±2.23	39.69±2.23	9.87±1.29
10	32.40±1.30	30.88±2.24	39.13±1.29	42.13±2.24	46.63±2.24	15.89±1.29
20	36.30±1.29	37.76±2.26	46.06±1.30	49.07±2.26	53.60±2.26	22.69±1.30
30	40.42±2.61	43.40±2.29	51.82±1.32	54.88±2.29	59.47±2.29	28.10±1.32
40	46.20±2.43	51.39±2.27	58.24±0.02	60.53±2.27	66.57±1.32	34.00±1.30
60	50.93±2.45	58.39±2.28	64.53±1.28	66.83±2.42	73.64±1.33	40.90±1.31
90	55.68±1.47	65.42±2.29	71.59±1.28	73.90±1.64	80.76±1.33	45.60±1.32
120	60.46±2.49	72.49±2.31	78.69±1.29	81.02±1.65	87.91±1.34	50.32±1.33

n=3*

TABLE 5e: *IN VITRO* RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING PEG 6000 AS STABILIZER

TIME IN Mins	FORMULATION CODE					PURE DRUG±SD
	F5A±SD	F5B±SD	F5C±SD	F5D±SD	F5E±SD	
5	15.09±2.23	19.56±2.23	22.54±1.29	24.78±1.29	27.02±1.29	9.87±1.29
10	21.88±2.24	26.38±2.24	28.63±2.24	31.63±1.29	33.13±2.24	15.89±1.29
20	26.48±2.26	31.75±1.31	35.50±2.25	37.77±2.24	37.79±2.25	22.69±1.30
30	31.95±2.29	37.30±1.33	41.11±2.28	43.41±2.28	43.43±2.28	28.10±1.32
40	37.81±2.27	43.11±1.32	46.89±2.26	49.16±2.26	50.68±2.44	34.00±1.30
60	44.73±2.28	48.56±1.61	53.85±2.28	55.40±1.61	57.67±1.46	40.90±1.31
90	50.94±1.47	54.05±2.28	59.37±1.34	61.67±1.46	64.69±1.48	45.60±1.32
120	57.18±1.65	61.06±2.29	64.17±1.34	67.23±1.65	71.76±1.50	50.32±1.33

n=3*

TABLE 6a: EFFECT OF PARTICLE SIZE ON *IN VITRO* RELEASE STUDIES OF RAMIPRIL NANOCRYSTALS CONTAINING β-CYCLODEXTRIN AND SLS AS STABILIZER

FORMULATION CODE	STABILIZERS CONCENTRATION	MEAN DIAMETER(nm)	% DRUG RELEASE
F3C	BCD(0.3%)	300.6	81.12
F3D	BCD(0.4%)	110.6	83.43
F3E	BCD(0.5%)	80.3	85.74
F4D	SLS(0.4%)	109.3	81.02
F4E	SLS(0.5%)	100.8	87.91

TABLE 7: EFFECT OF PARTICLE SIZE OF RAMIPRIL NANOCRYSTALS CONTAINING β-CYCLODEXTRIN AND SLS AS STABILIZER

FORMULATION CODE	STABILIZERS CONCENTRATION	MEAN DIAMETER(nm)	PDI
F3C	BCD(0.3%)	300.6	0.324
F3D	BCD(0.4%)	110.6	0.416
F3E	BCD(0.5%)	80.3	0.232
F4D	SLS(0.4%)	109.3	0.457
F4E	SLS(0.5%)	100.8	0.416

TABLE 8: ZETA POTENTIAL VALUES OF RAMIPRIL NANOCRYSTALS

FORMULATION CODE	STABILIZERS CONCENTRATION	ZETA POTENTIAL(mV)
F3C	BCD(0.3%)	-20.5
F3D	BCD(0.4%)	-22.3
F3E	BCD(0.5%)	-19.7
F4D	SLS(0.4%)	-24.7
F4E	SLS(0.5%)	-21.4

TABLE 9: COMPARISON OF SOLUBILITY OF SELECTED FORMULATION WITH PURE DRUG

S. NO	SOLVENT USED	SOLUBILITY IN EACH SOLVENT(mg/10ml)					
		PURE DRUG	F3C	F3D	F3E	F4D	F4E
1	DISTILLED WATER	0.174±0.02	0.879±0.05	2.238±0.09	3.565±0.09	1.077±0.05	3.068±0.09
2	PHOSPHATE BUFFER Ph(6.8)	0.547±0.05	1.177±0.05	2.791±0.09	5.68±0.14	2.294±0.09	5.182±0.14

n=3*

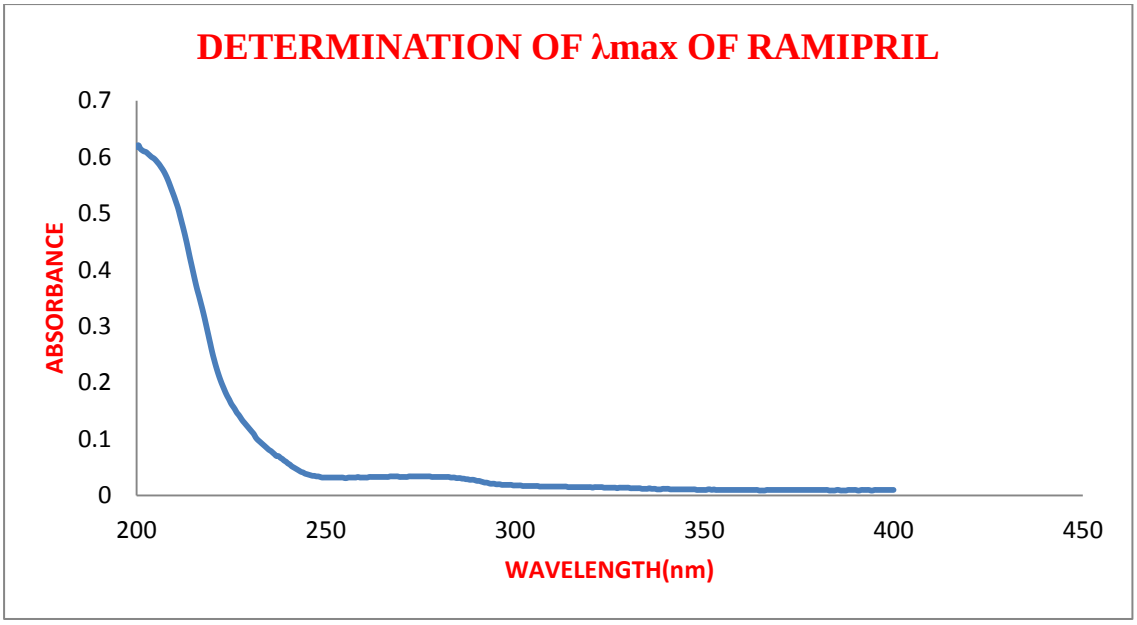


FIGURE 10a: λ MAX OF RAMIPRIL USING PHOSPHATE BUFFER pH (6.8)

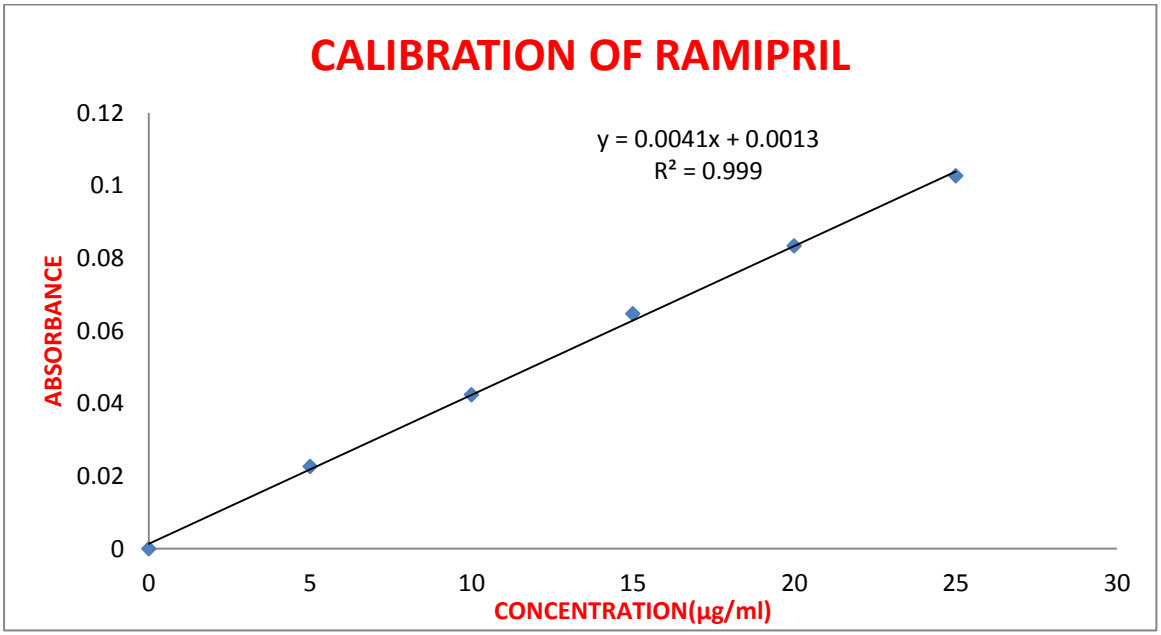


FIGURE 10b: CALIBRATION OF RAMIPRIL USING PHOSPHATE BUFFER pH (6.8)

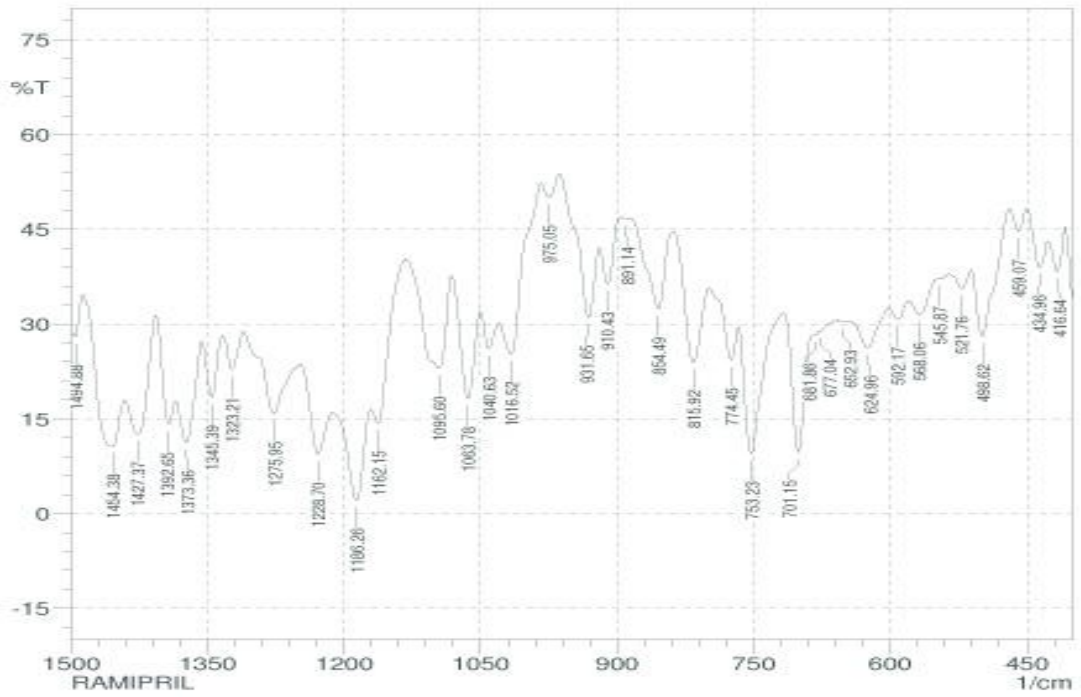


FIGURE 11a: IR SPECTRUM OF RAMIPRIL

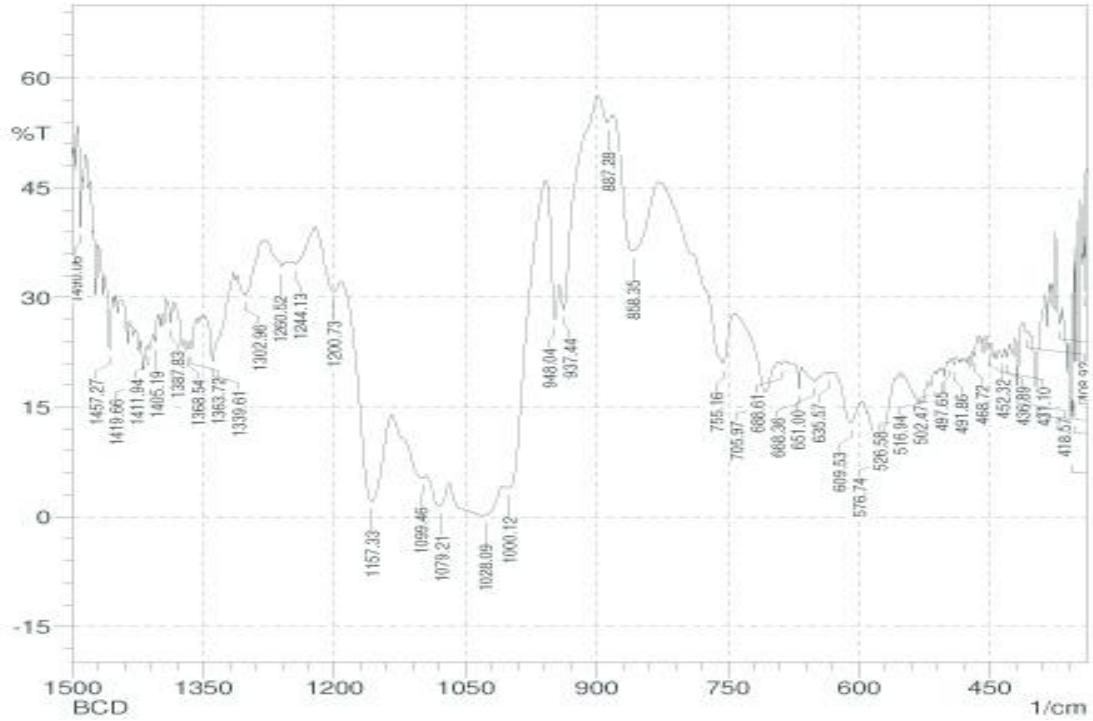


FIGURE 11b: IR SPECTRUM OF β-CYCLO DEXTRIN

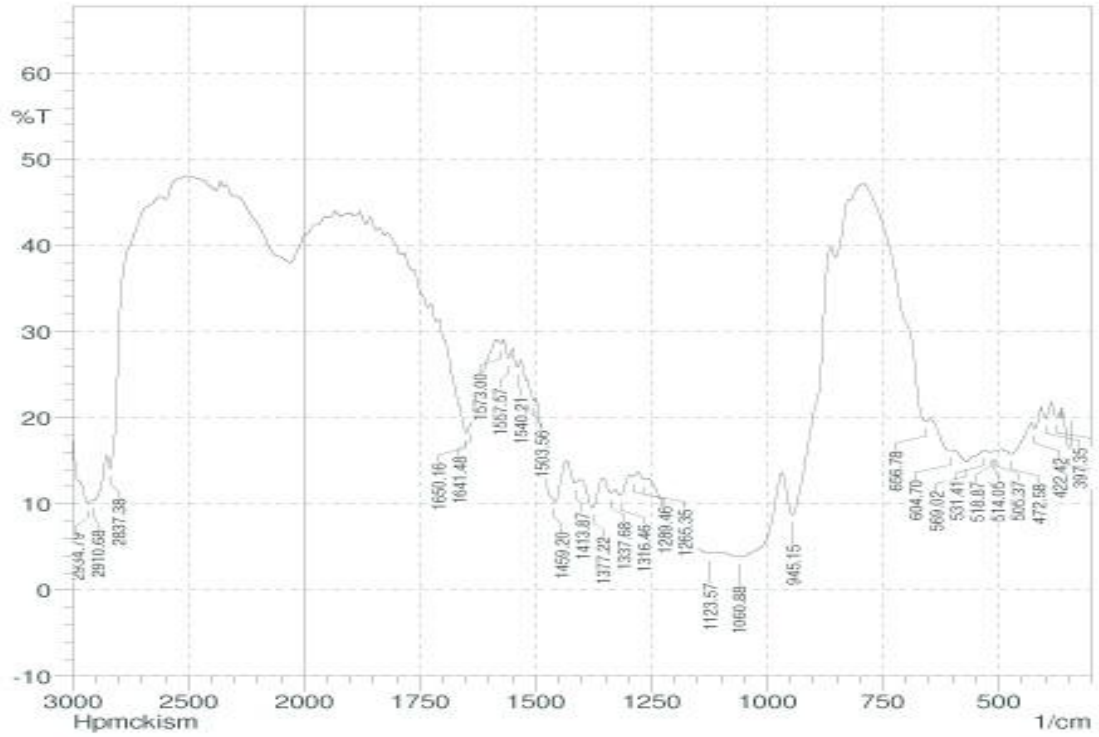


FIGURE 11c: IR SPECTRUM OF HPMC K15M

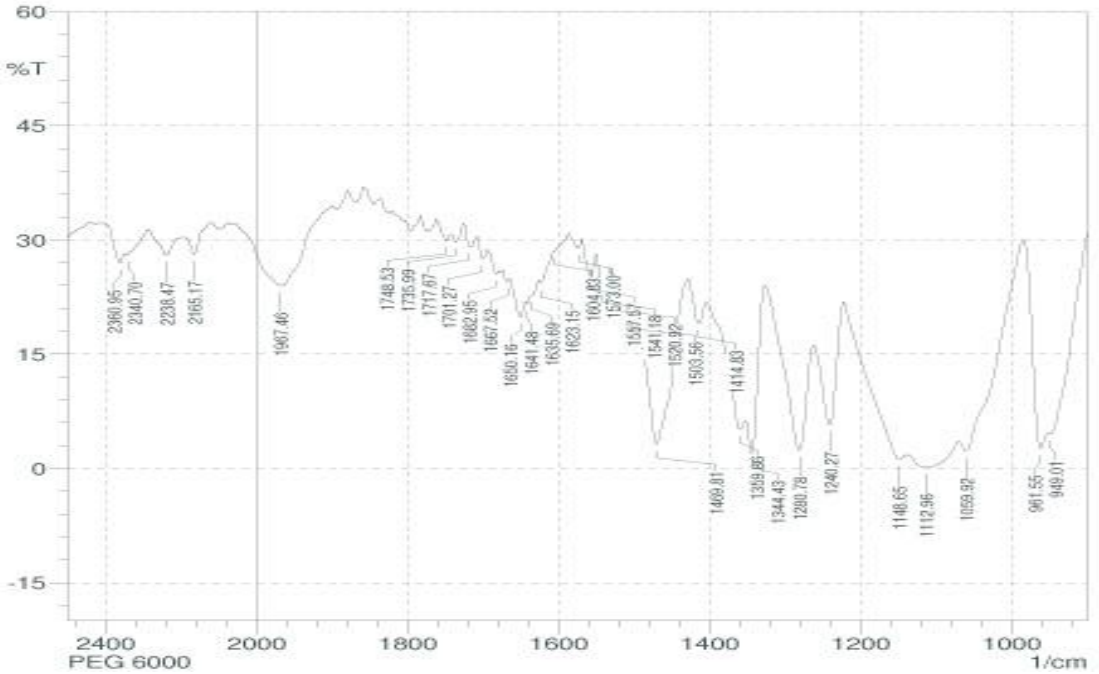


FIGURE 11d: IR SPECTRUM OF PEG 6000

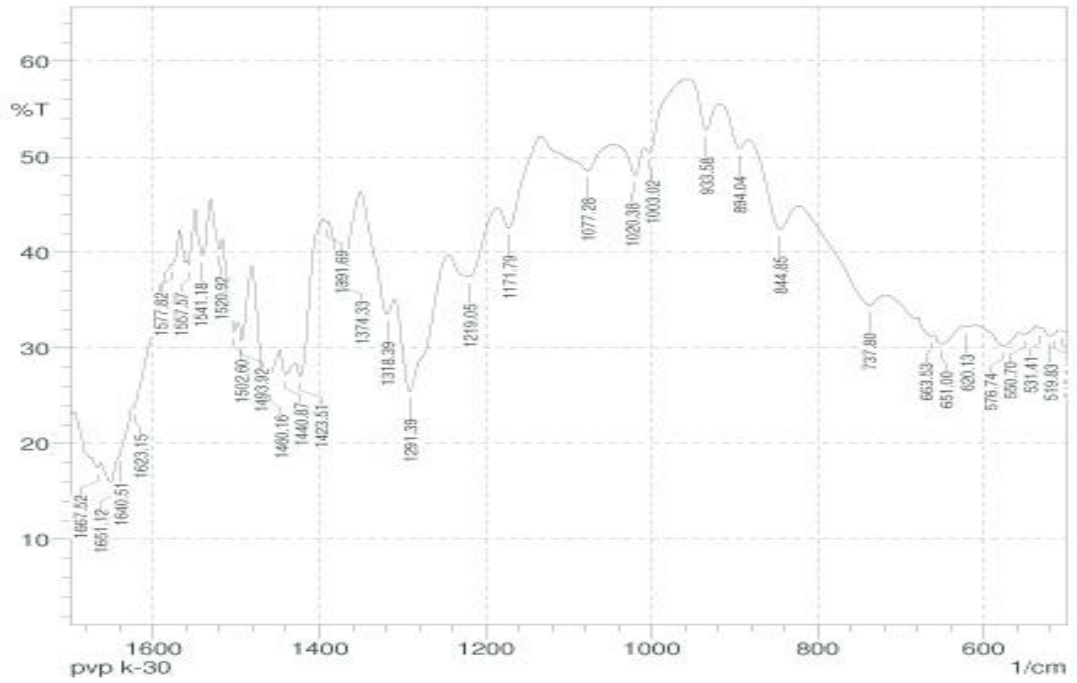


FIGURE 11e: IR SPECTRUM OF PVP K30

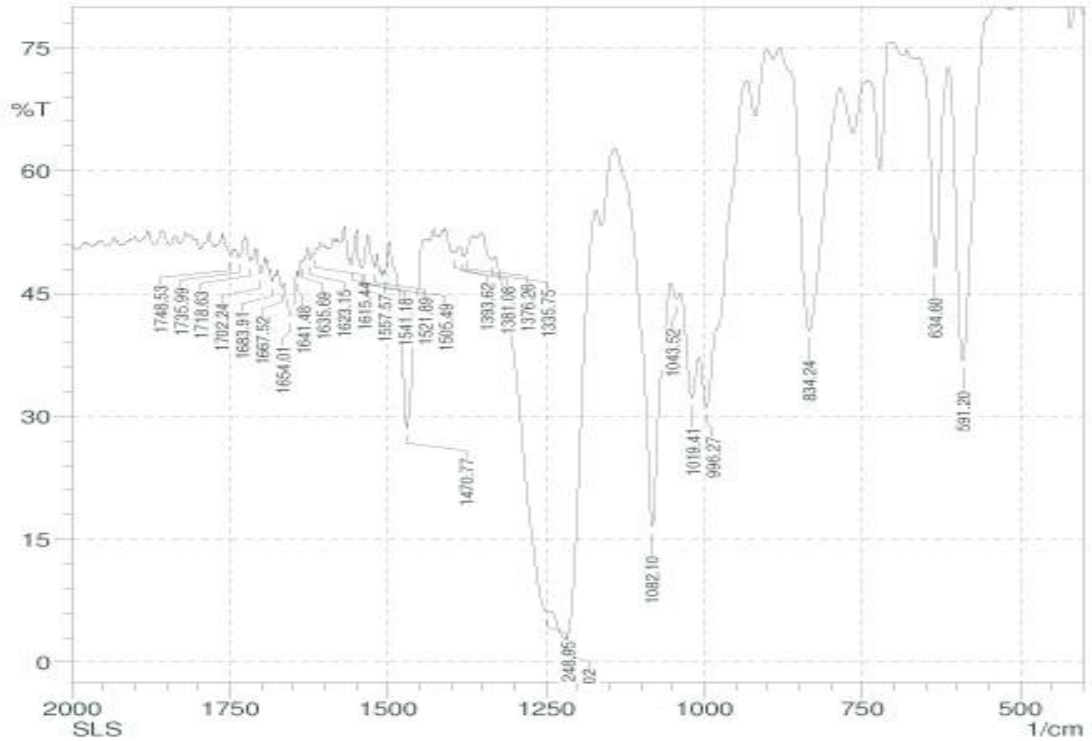


FIGURE 11f: IR SPECTRUM OF SLS

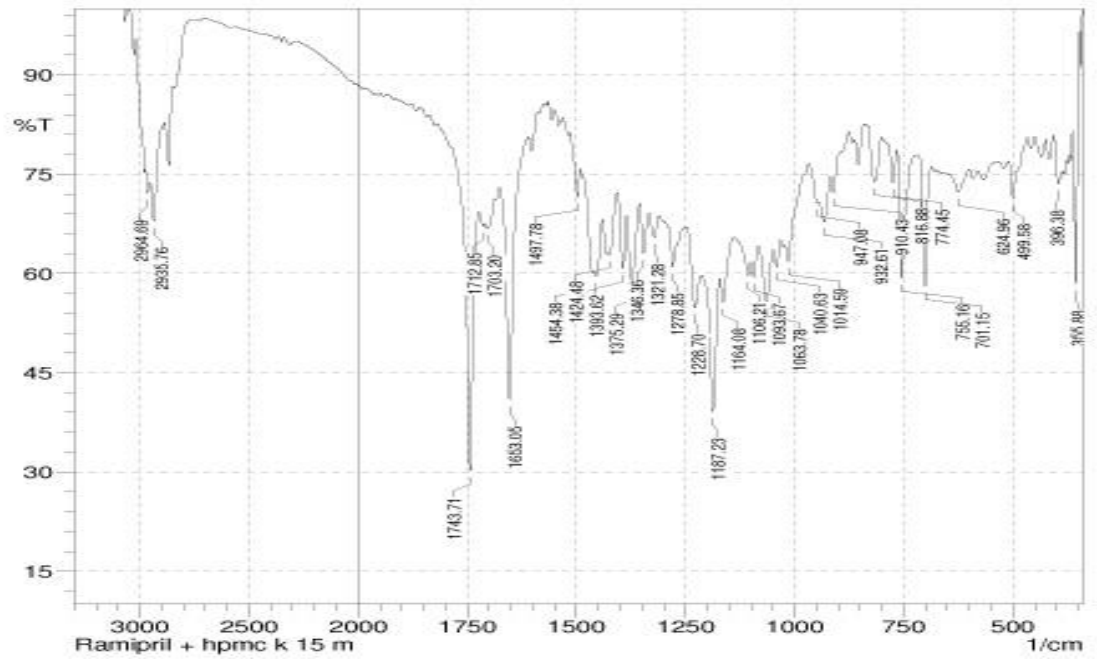


FIGURE 11g: IR SPECTRUM OF RAMIPRIL + HPMC K15M

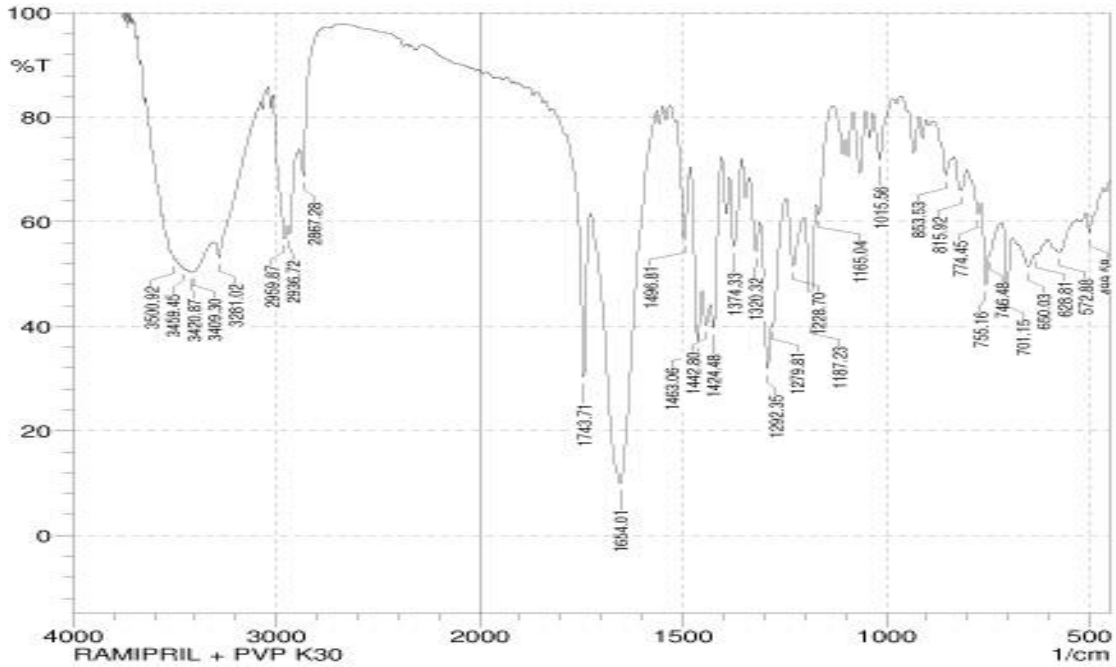


FIGURE 11h: IR SPECTRUM OF RAMIPRIL + PVP K30

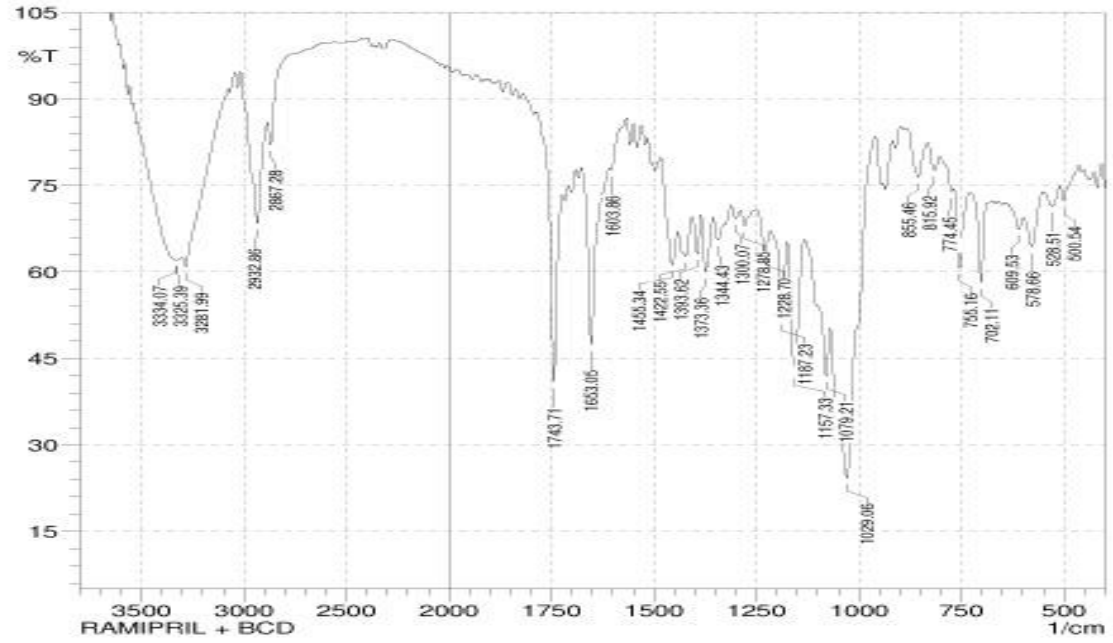


FIGURE 11i: IR SPECTRUM OF RAMIPRIL + β-CYCLO DEXTRIN

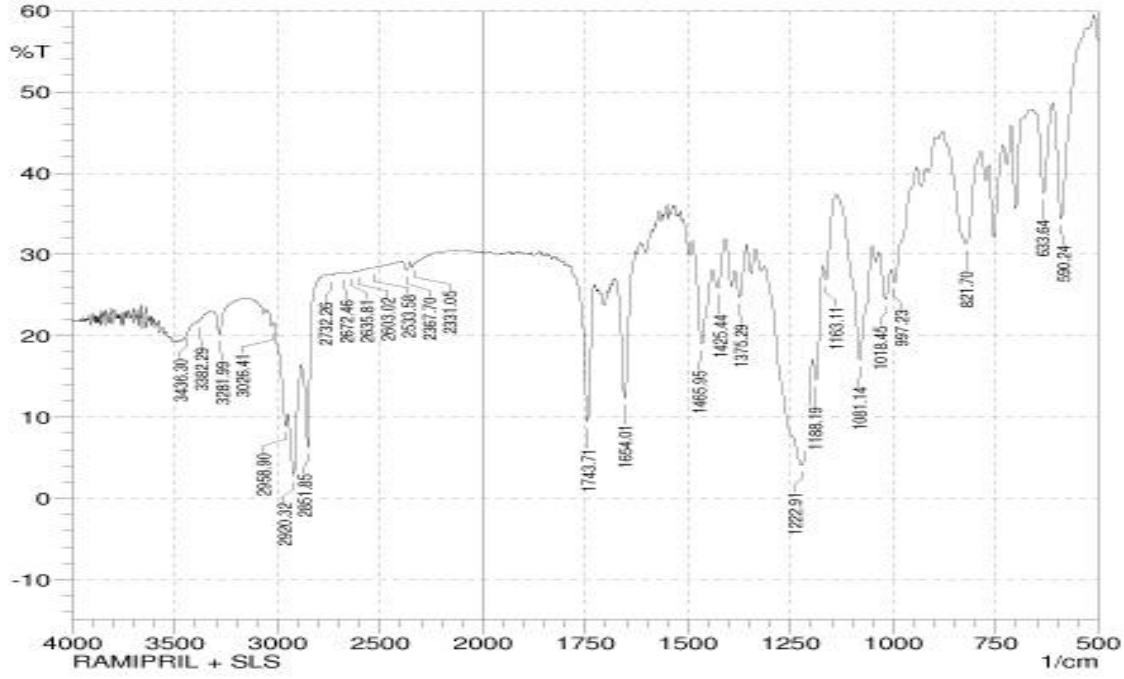


FIGURE 11j: IR SPECTRUM OF RAMIPRIL + SLS

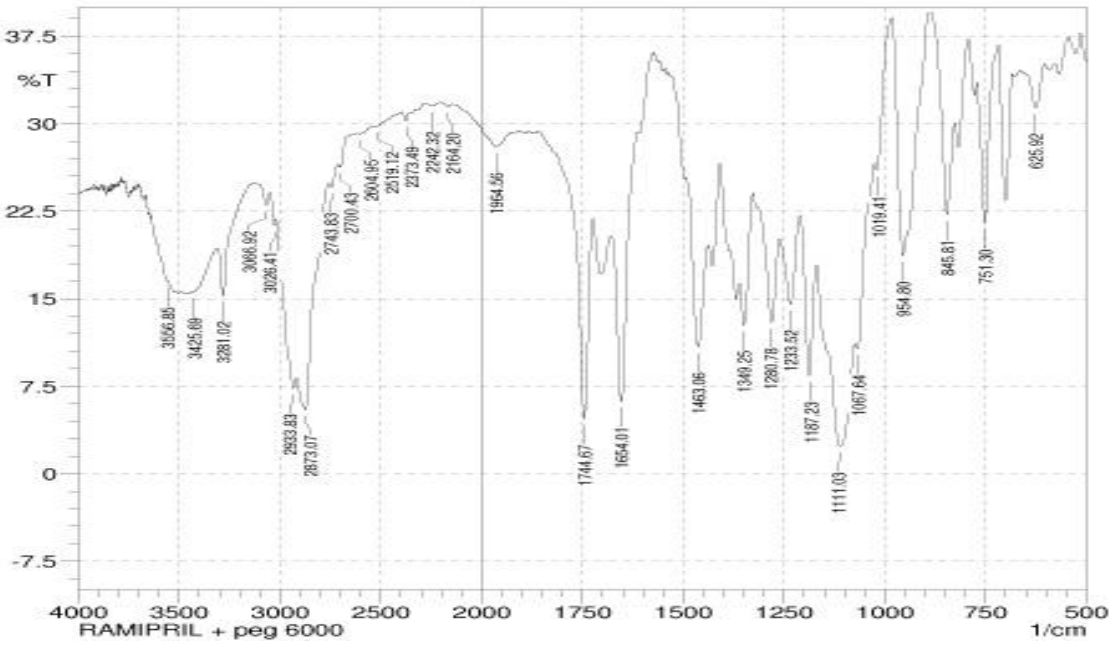


FIGURE 11k: IR SPECTRUM OF RAMIPRIL + PEG 6000

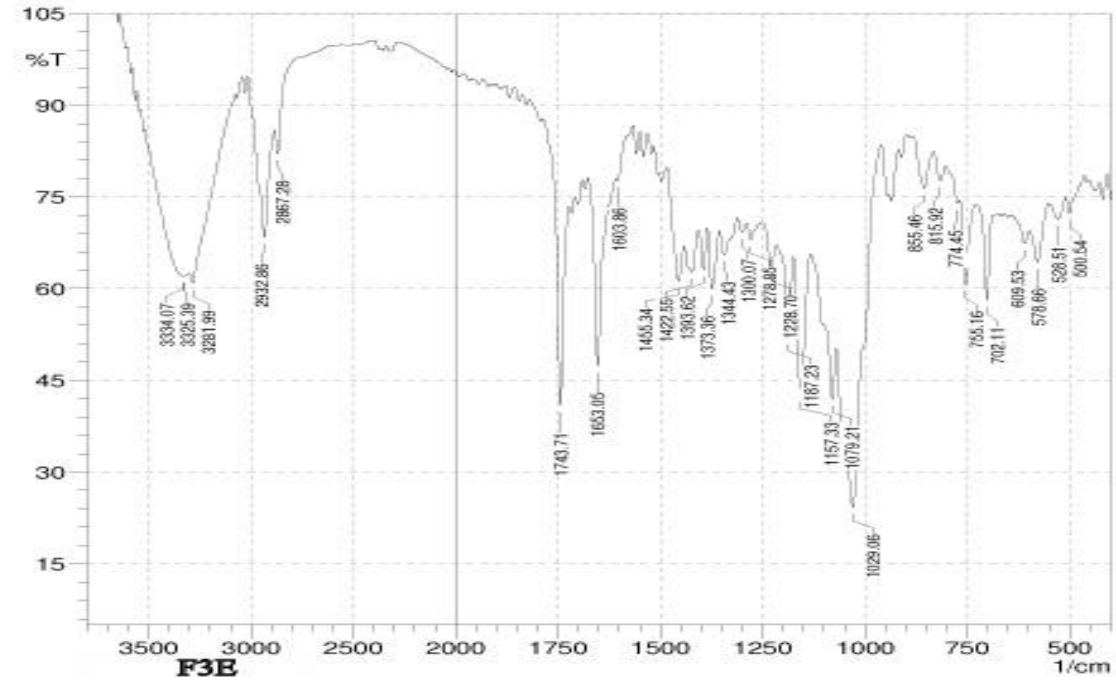


FIGURE 11l: IR SPECTRUM OF F3E

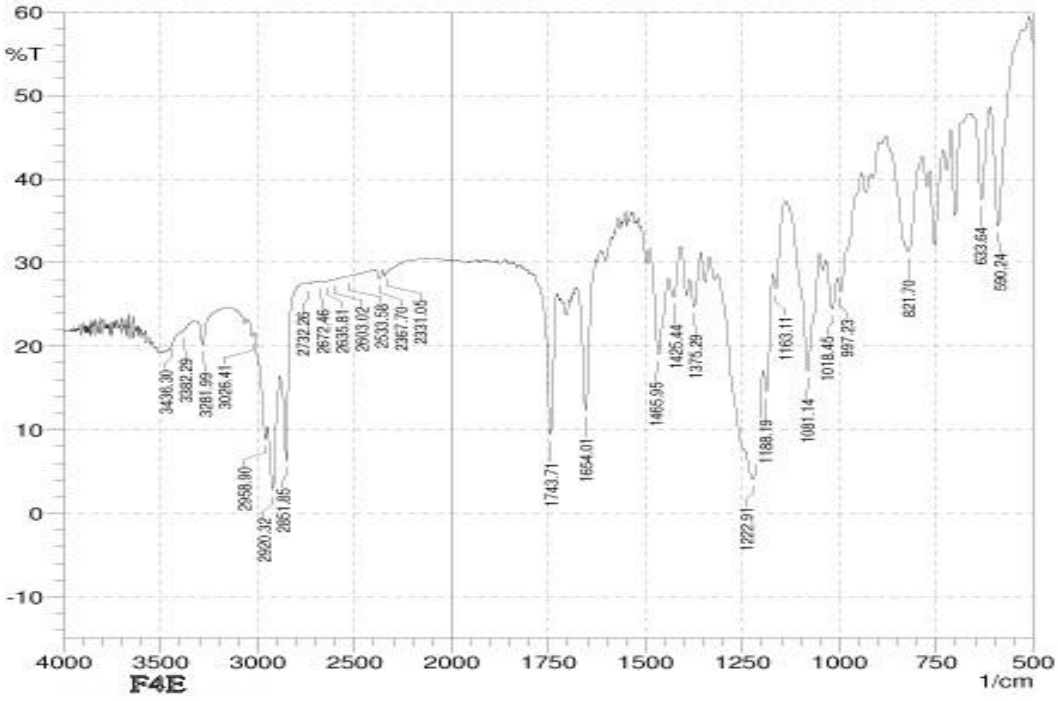


FIGURE 11m: IR SPECTRUM OF F4E

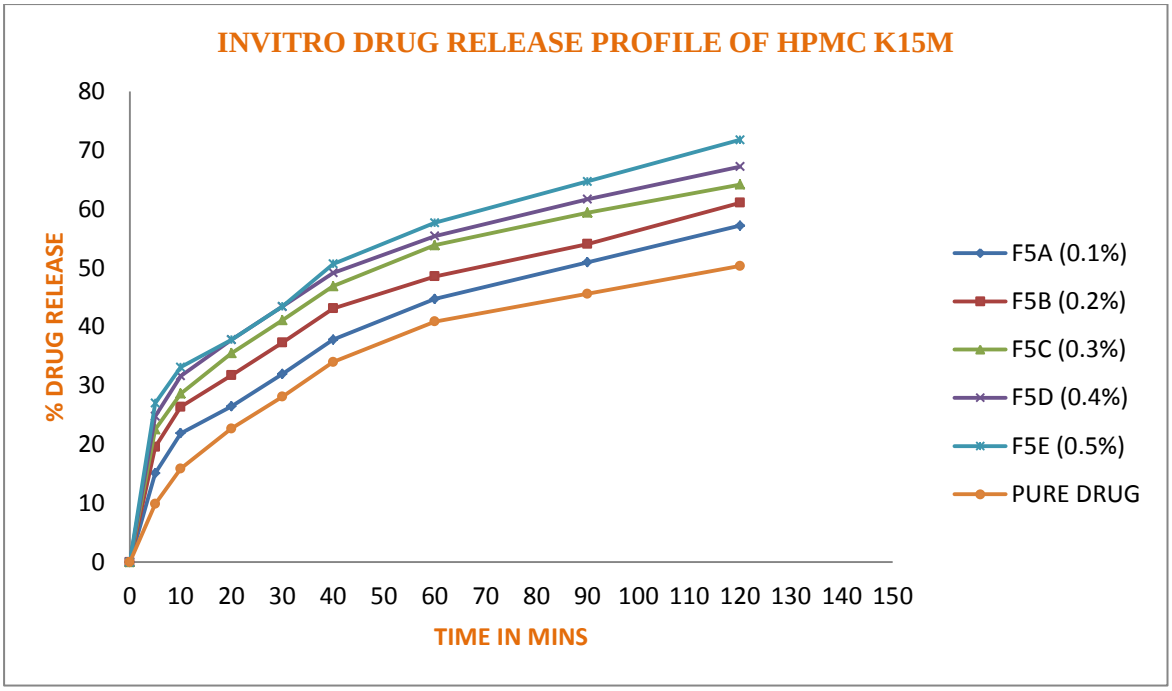


FIGURE 12a: COMPARISON OF *IN VITRO* DISSOLUTION RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING HPMC K15M AS STABILIZER

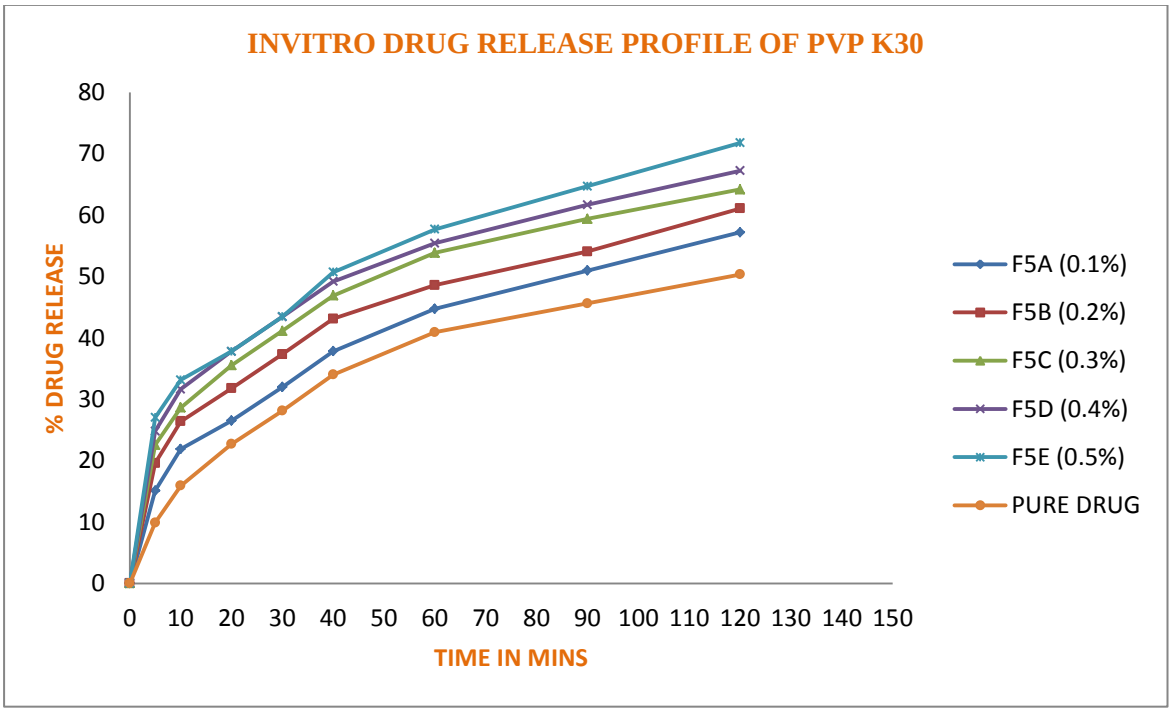


FIGURE 12b: COMPARISON OF *IN VITRO* DISSOLUTION RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING PVP K30 AS STABILIZER

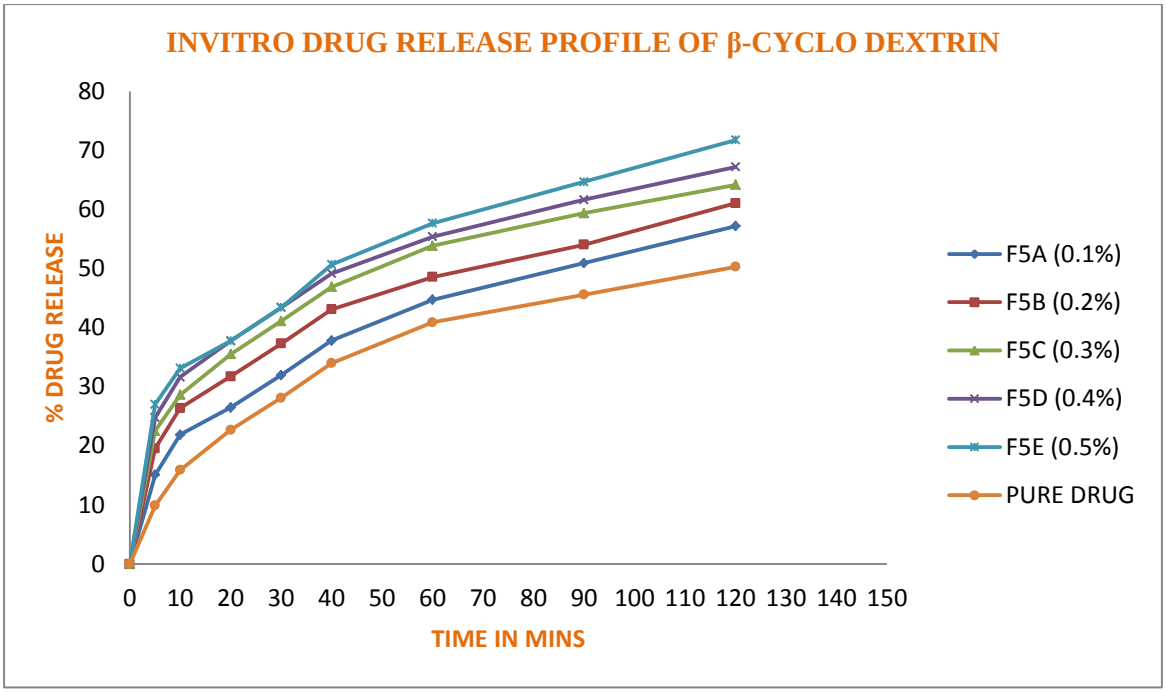


FIGURE 12c: COMPARISON OF *IN VITRO* DISSOLUTION RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING β-CYCLO DEXTRIN AS STABILIZER

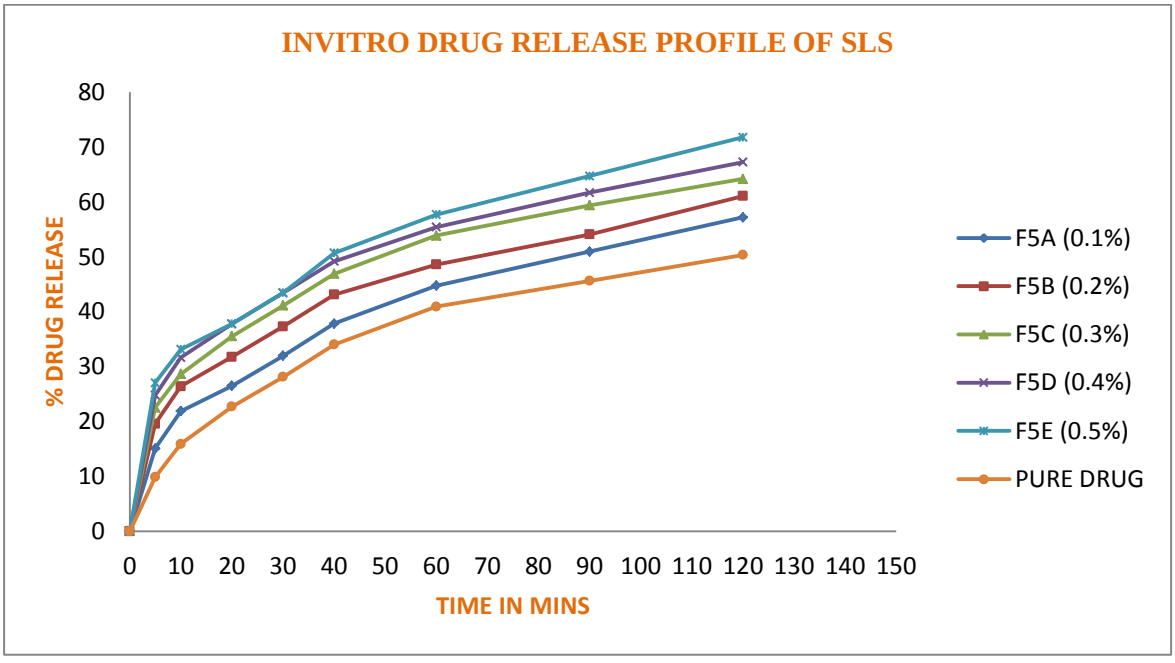


FIGURE 12d: COMPARISON OF *IN VITRO* DISSOLUTION RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING SLS AS STABILIZER

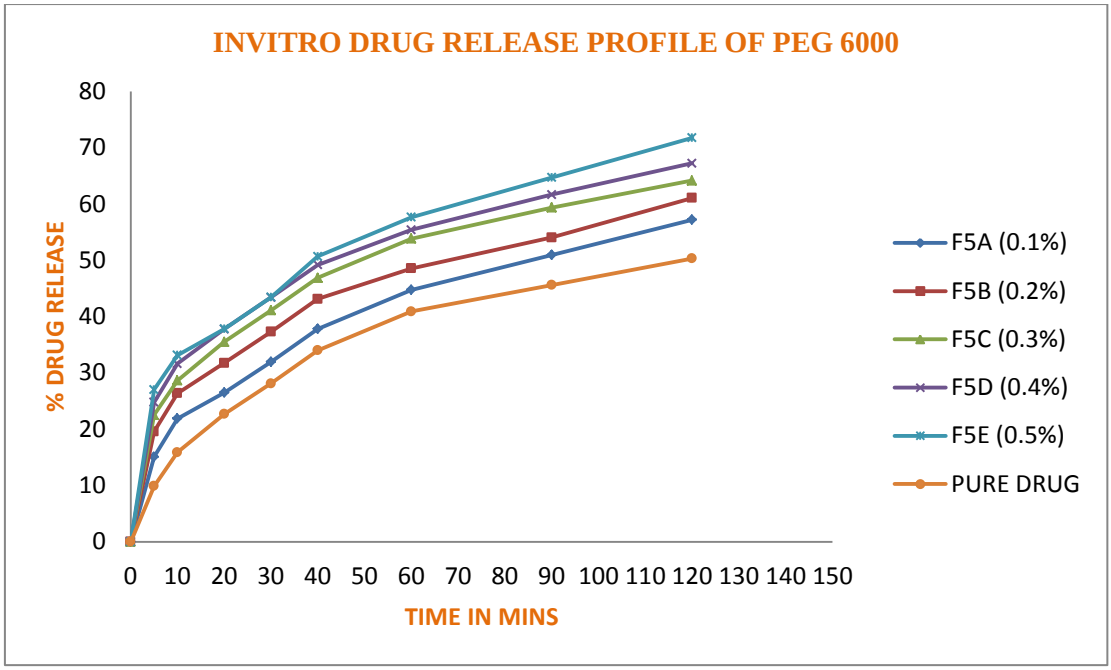
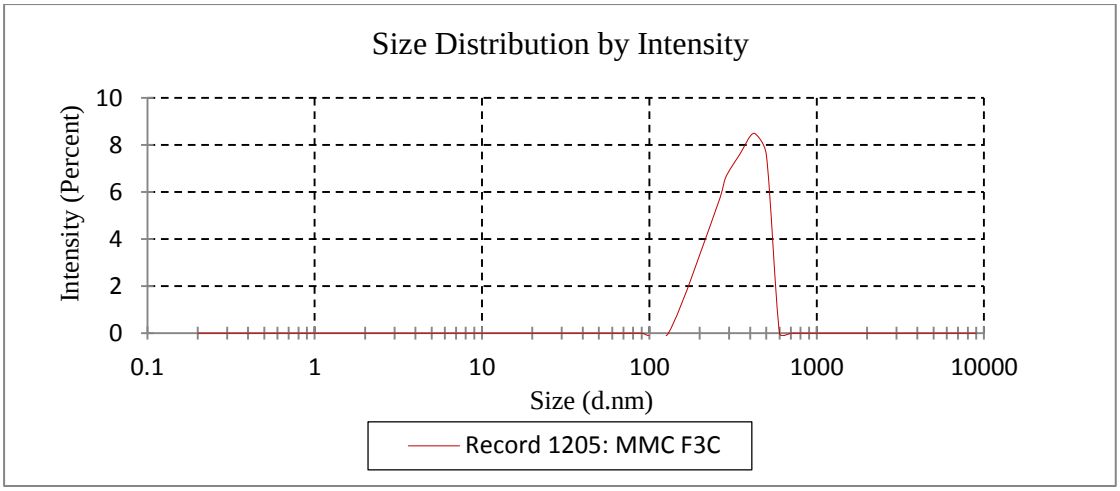
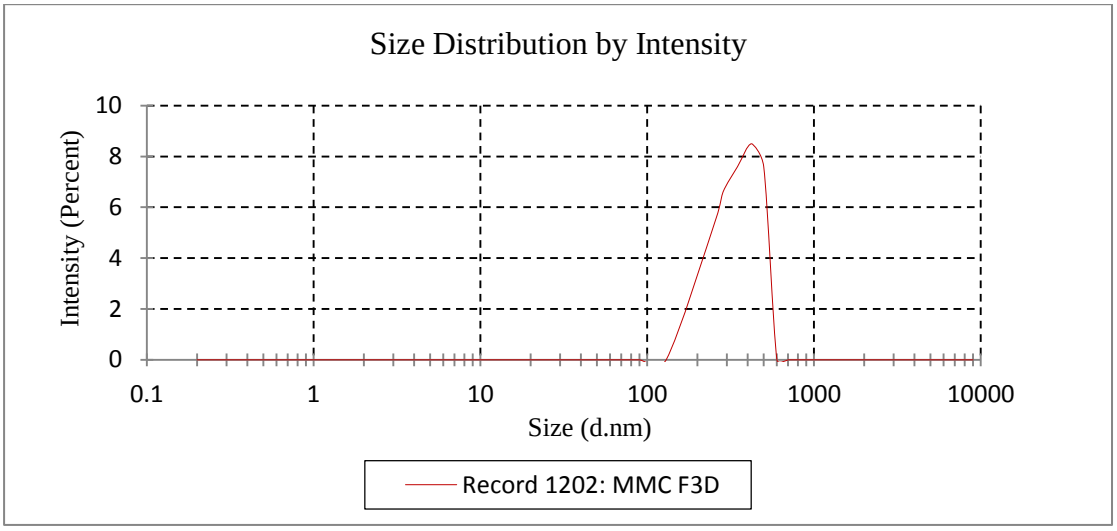


FIGURE 12e: COMPARISON OF *IN VITRO* DISSOLUTION RELEASE PROFILE OF RAMIPRIL NANOCRYSTALS CONTAINING PEG 6000 AS STABILIZER





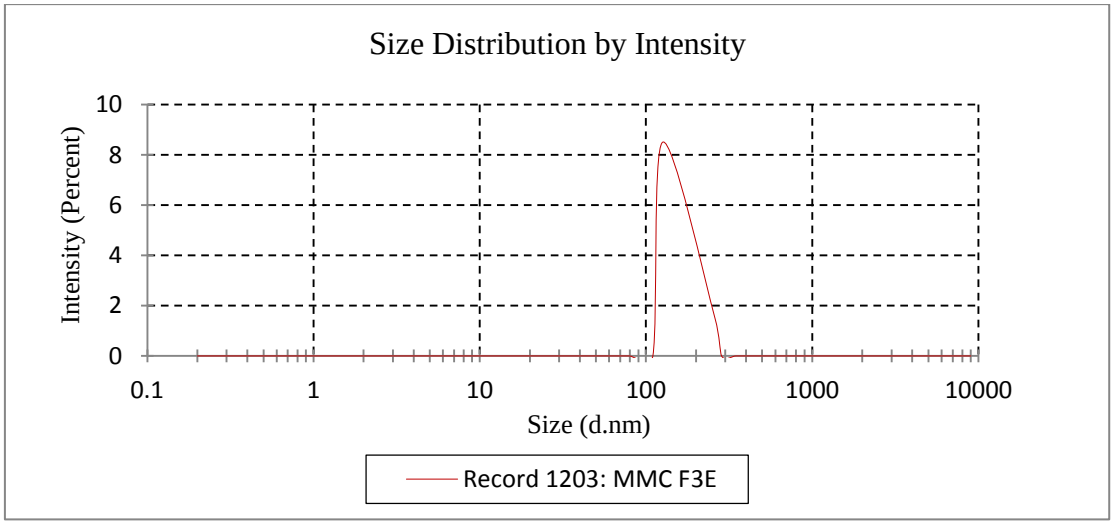
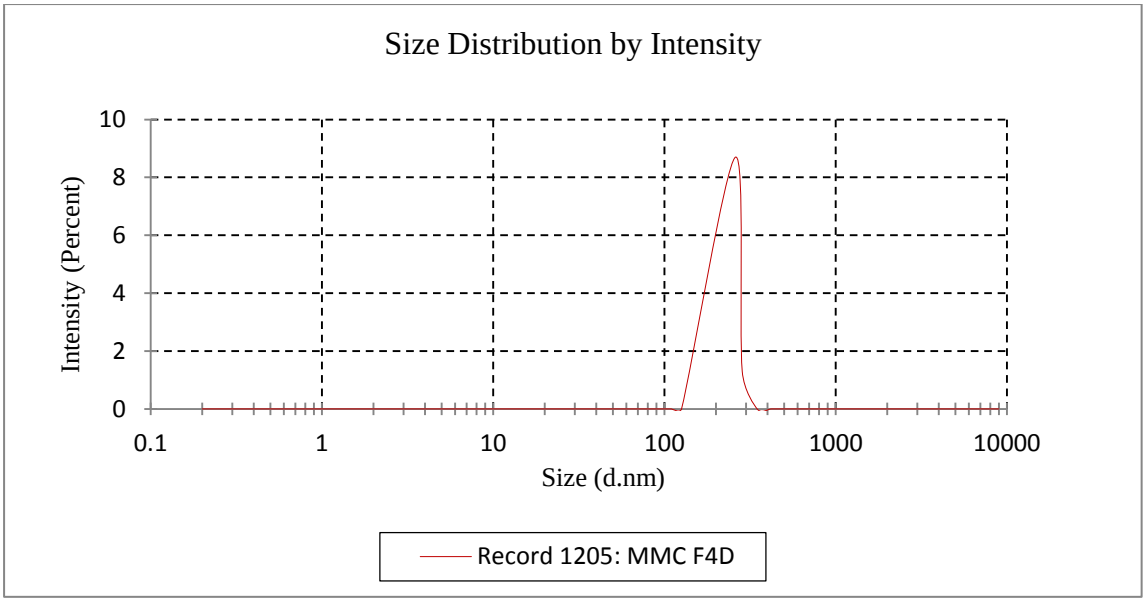


FIGURE 13a: PARTICLE SIZE DISTRIBUTION CURVE OF FORMULATIONS F3C, F3D AND F3E



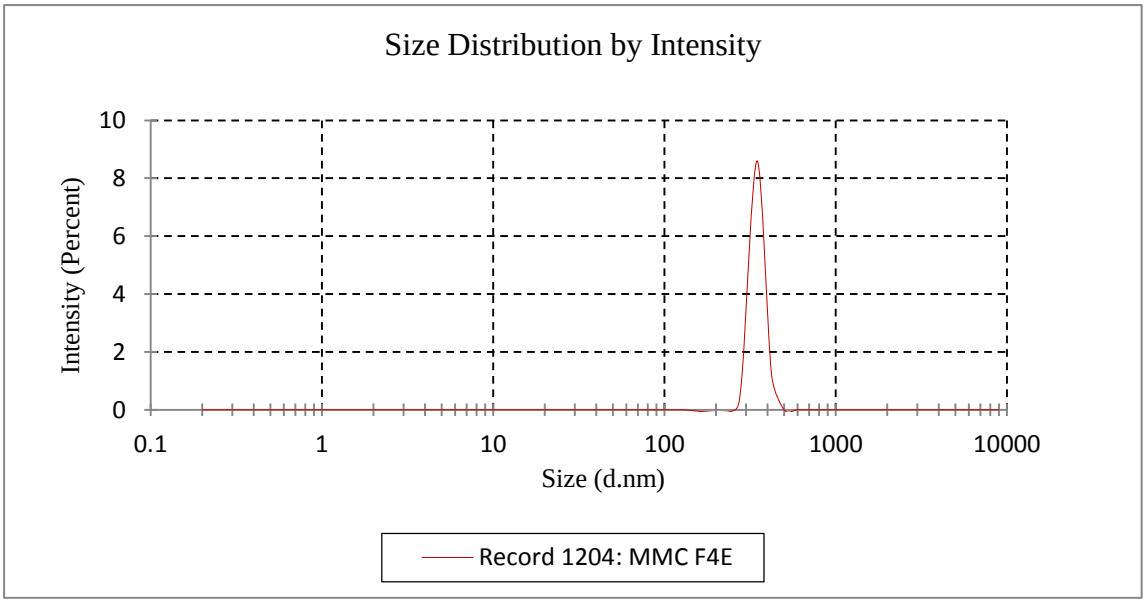
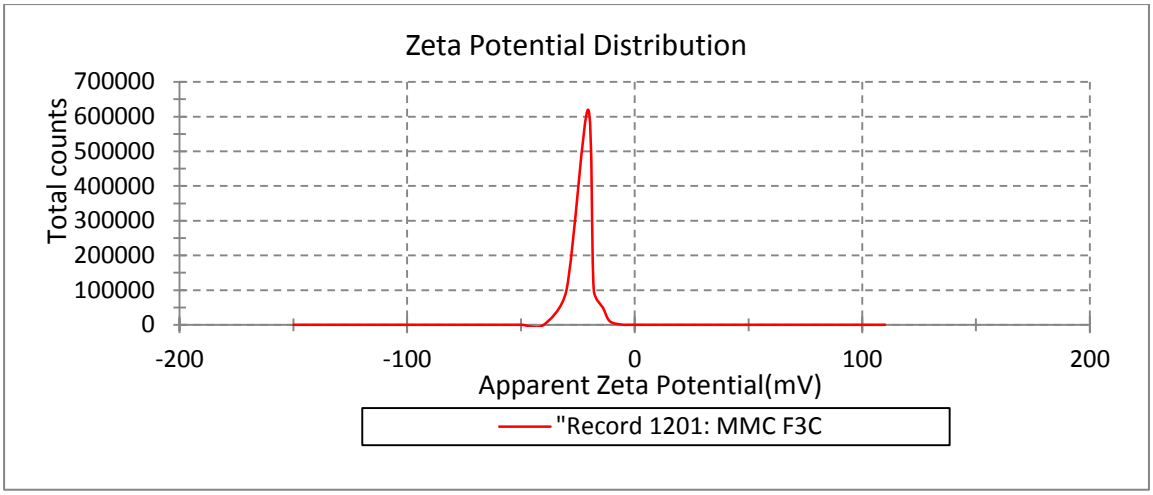
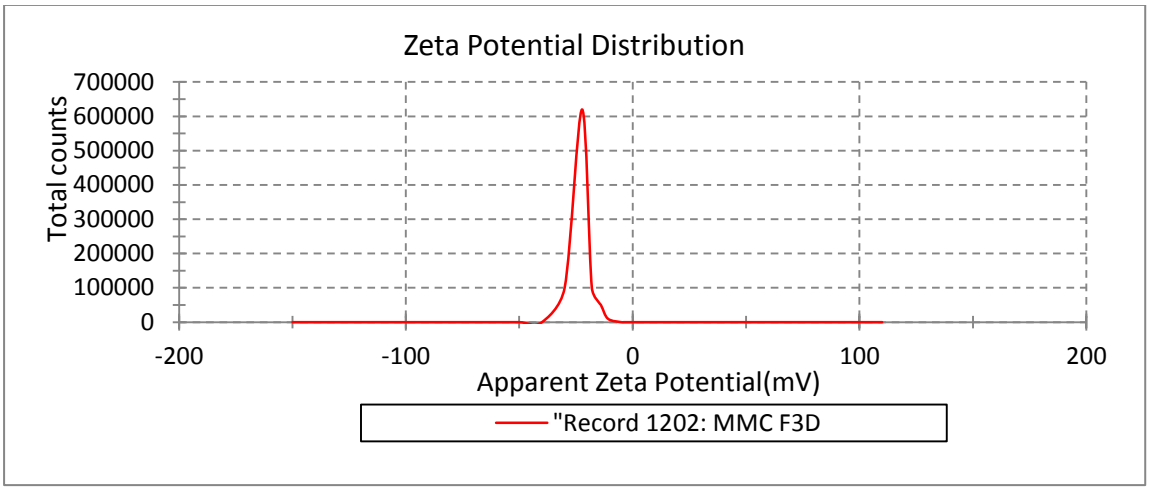


FIGURE 13b: PARTICLE SIZE DISTRIBUTION CURVE OF FORMULATIONS F4D AND F4E





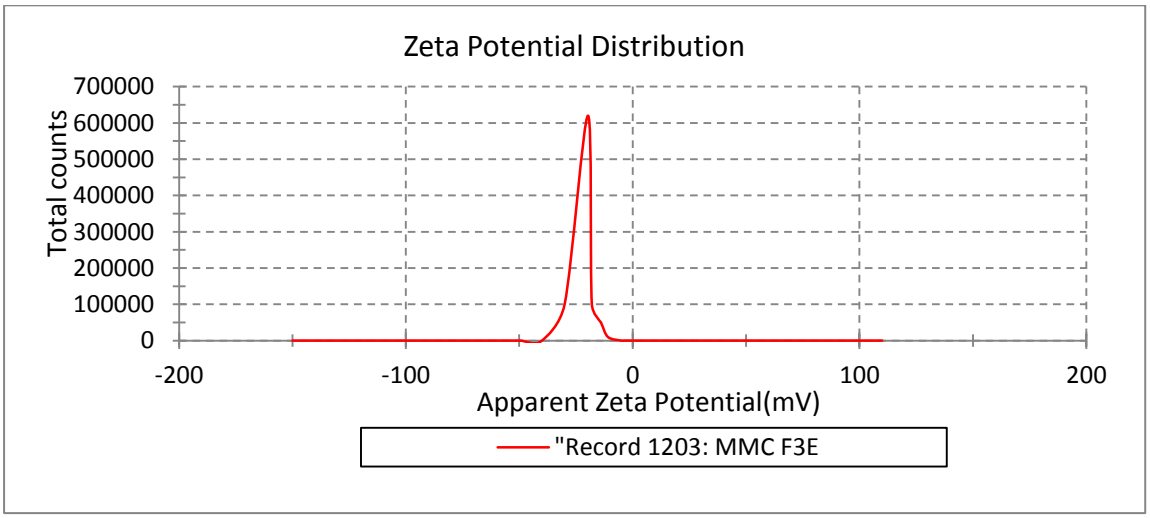
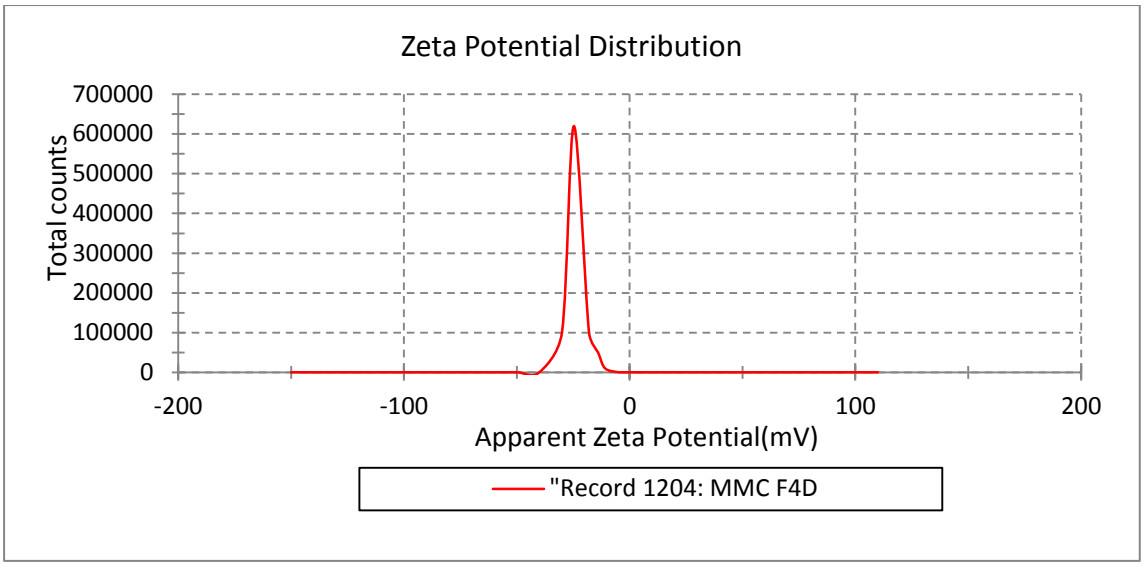


FIGURE14a: ZETA POTENTIAL CURVE OF FORMULATIONS F3C, F3D, AND F3E



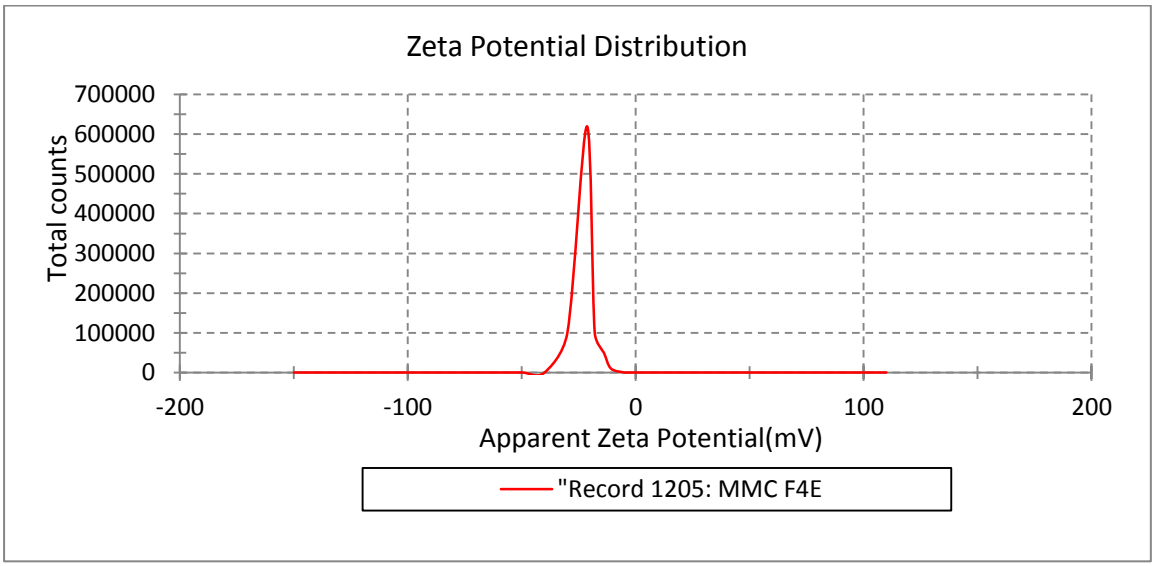


FIGURE14b: ZETA POTENTIAL CURVE OF FORMULATIONS F4D, AND F4E

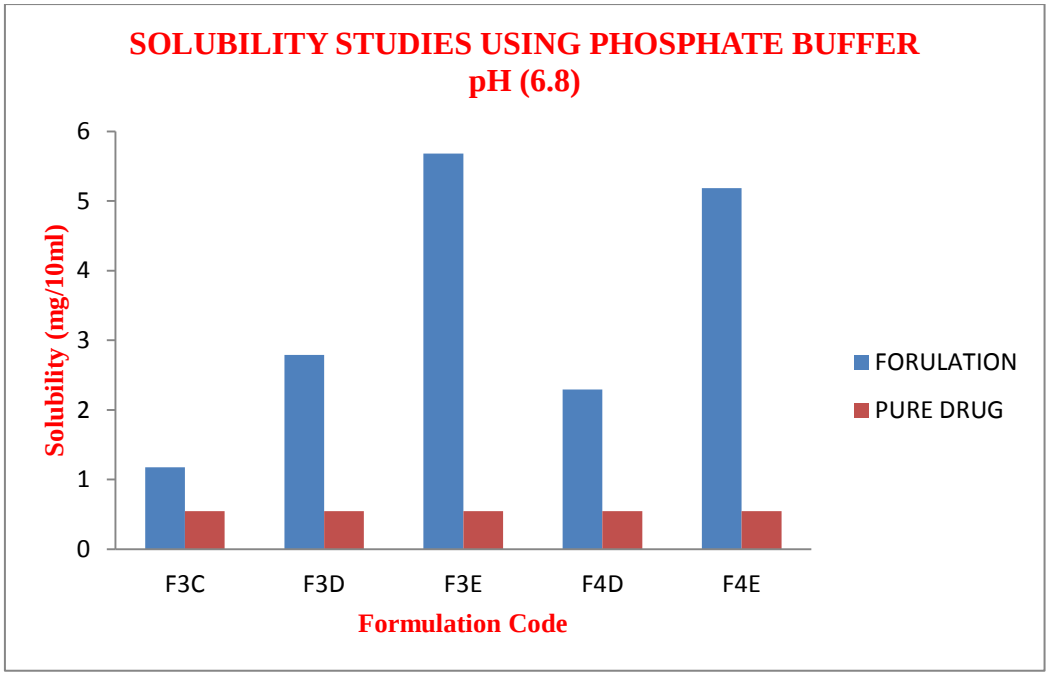


FIGURE 15a: COMPARISON OF SOLUBILITY OF SELECTED FORMULATIONS (F3C, F3D, F3E, F4D AND F4E) WITH PURE DRUG

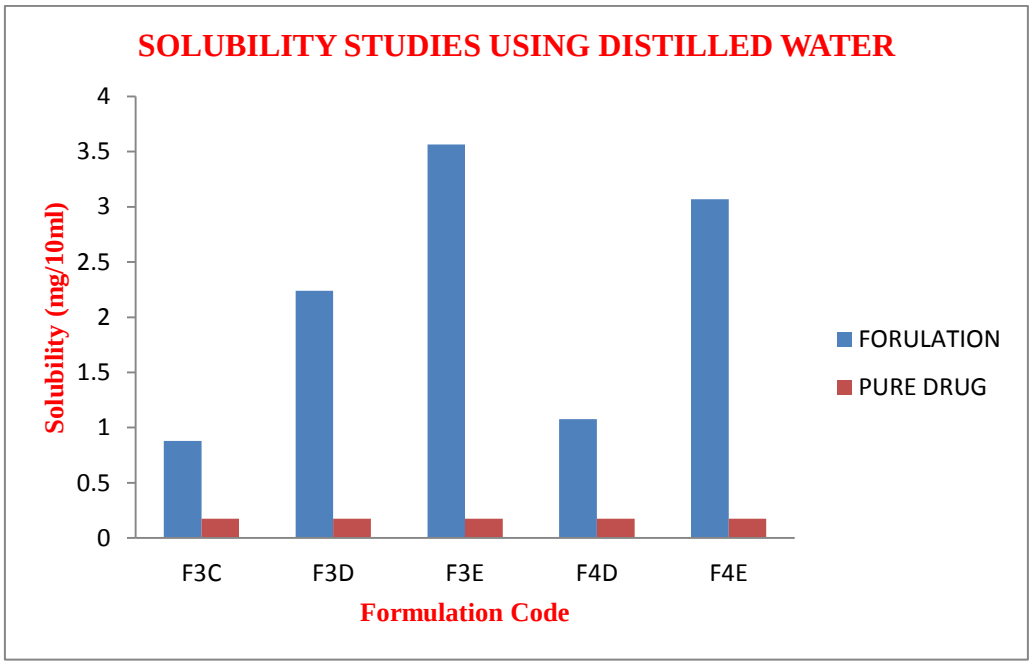


FIGURE 15b: COMPARISON OF SOLUBILITY OF SELECTED FORMULATIONS (F3C, F3D, F3E, F4D AND F4E) WITH PURE DRUG

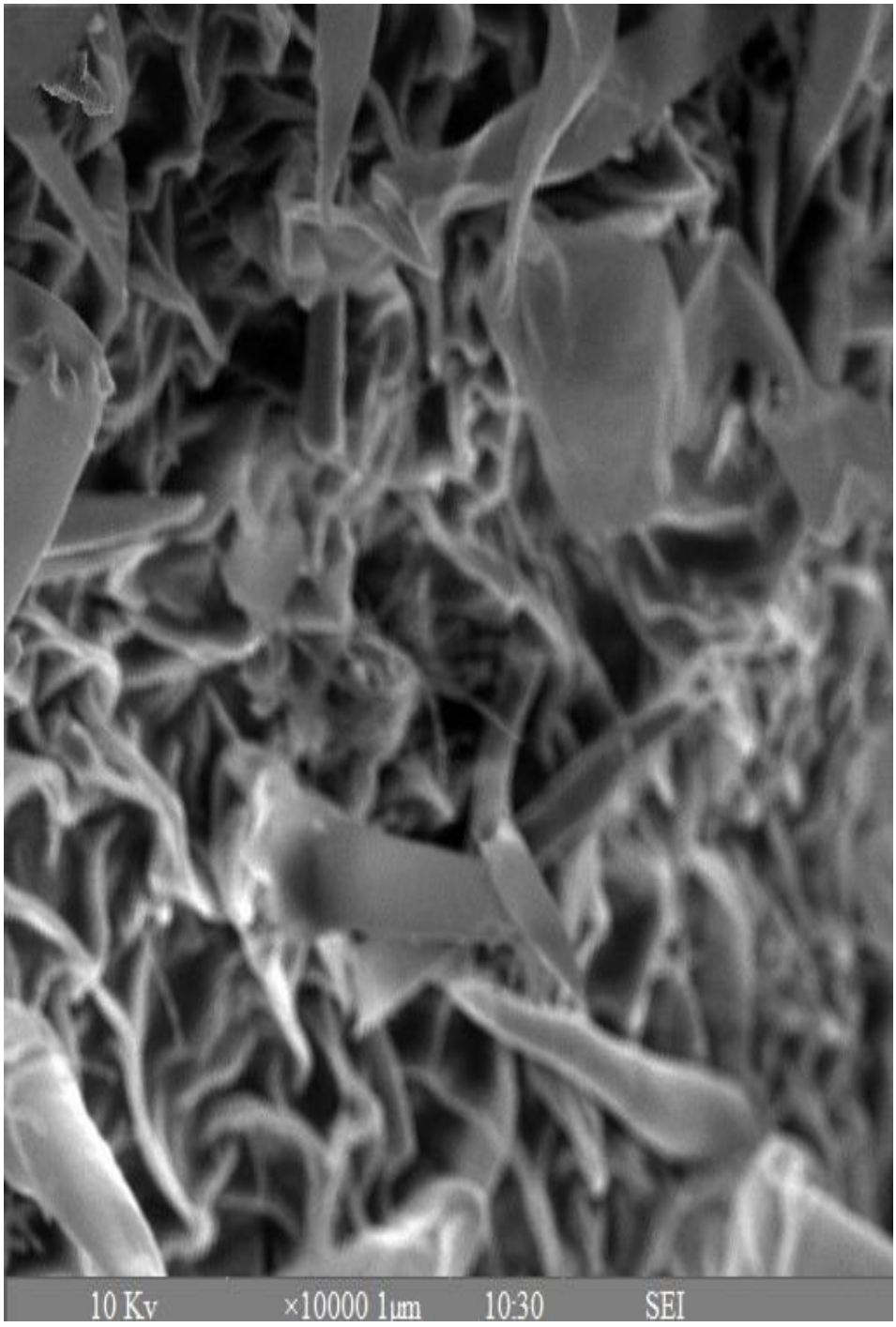


FIGURE 16a: SCANNING ELECTRON MICROSCOPY (SEM) IMAGE OF BEST FORMULATION (F3E)

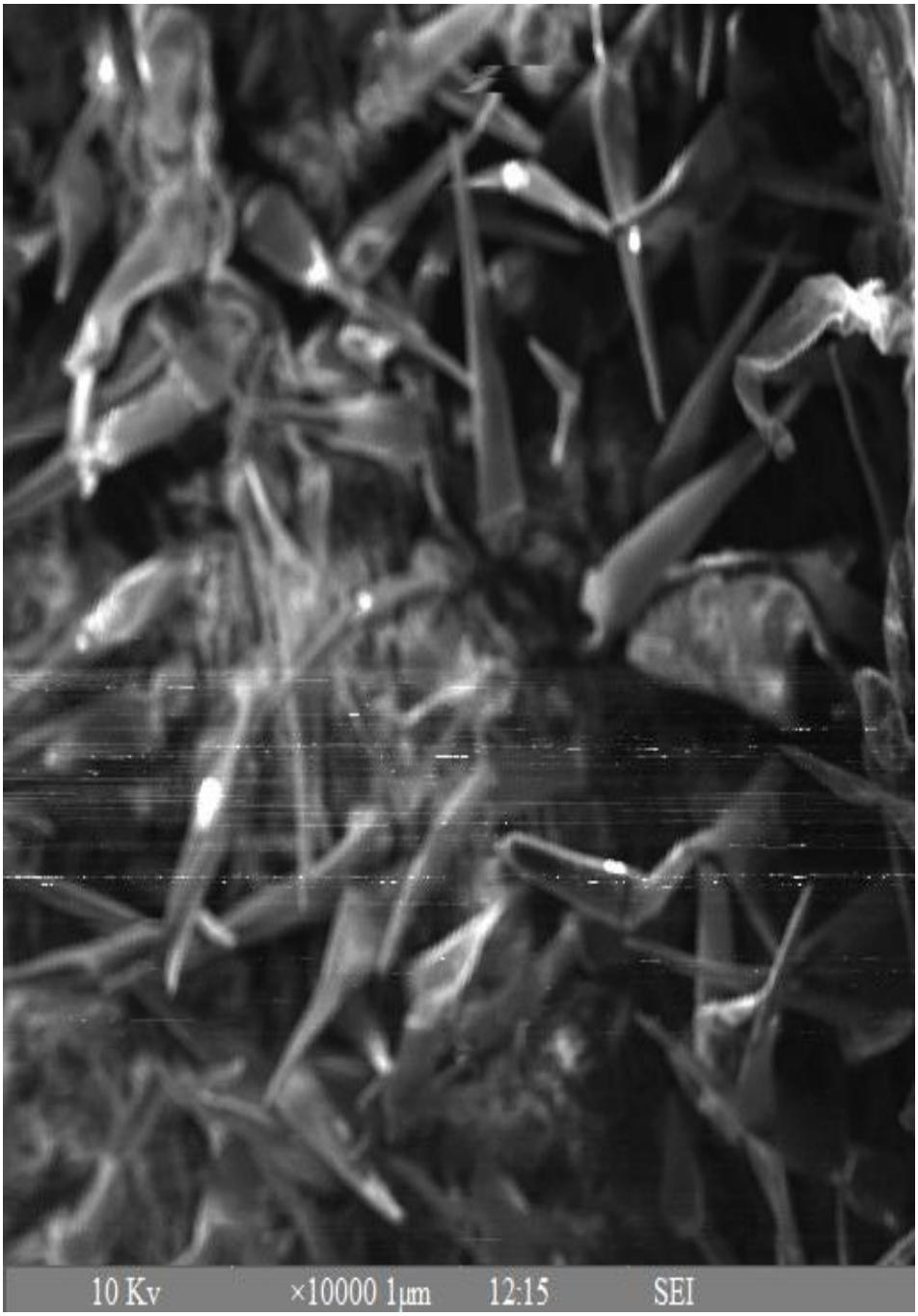


FIGURE 16b: SCANNING ELECTRON MICROSCOPY (SEM) IMAGE OF BEST FORMULATION (F4E)

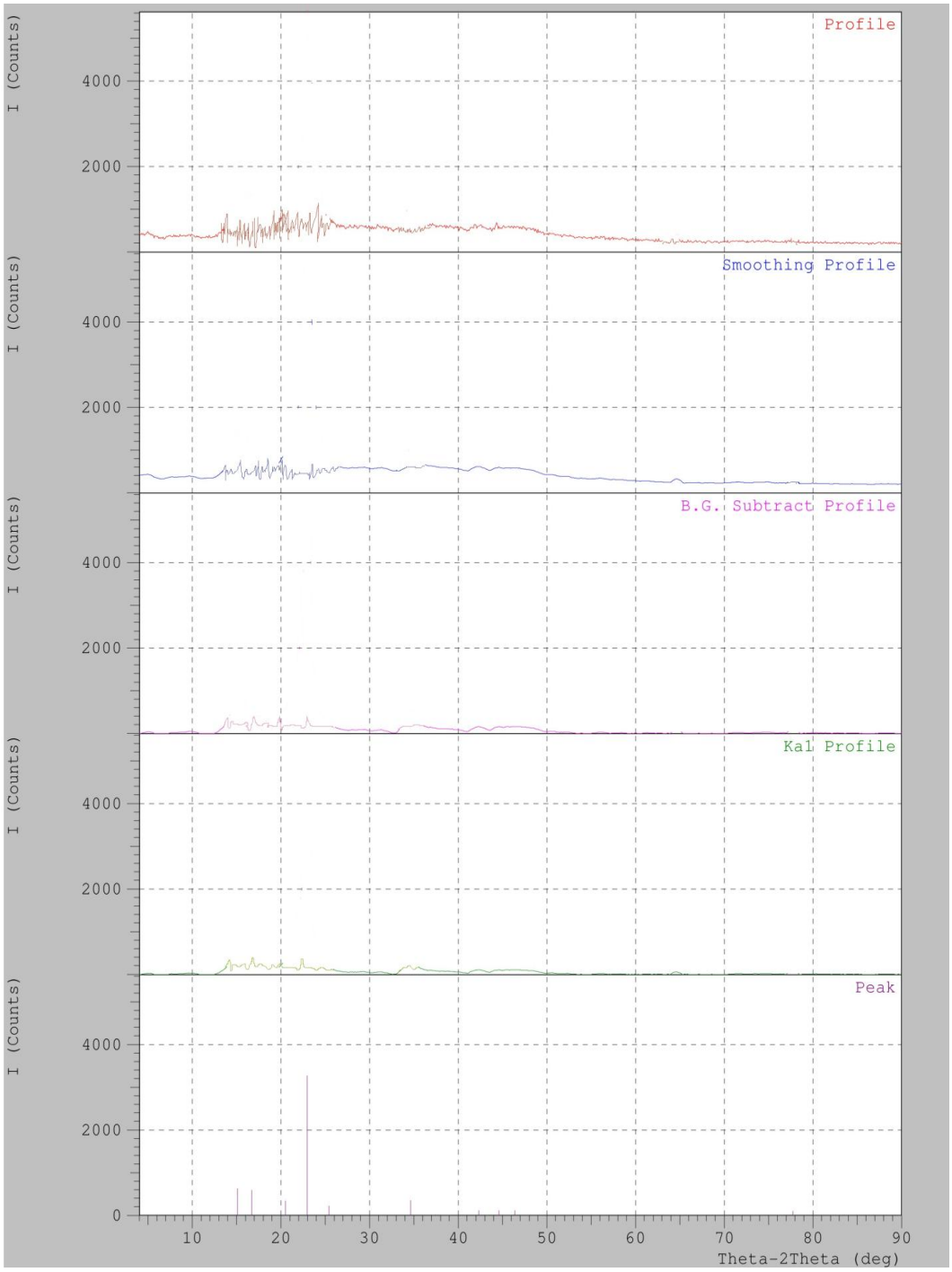


FIGURE 17a: X RAY DIFFRACTION PATTERN OF RAMIPRIL PURE DRUG

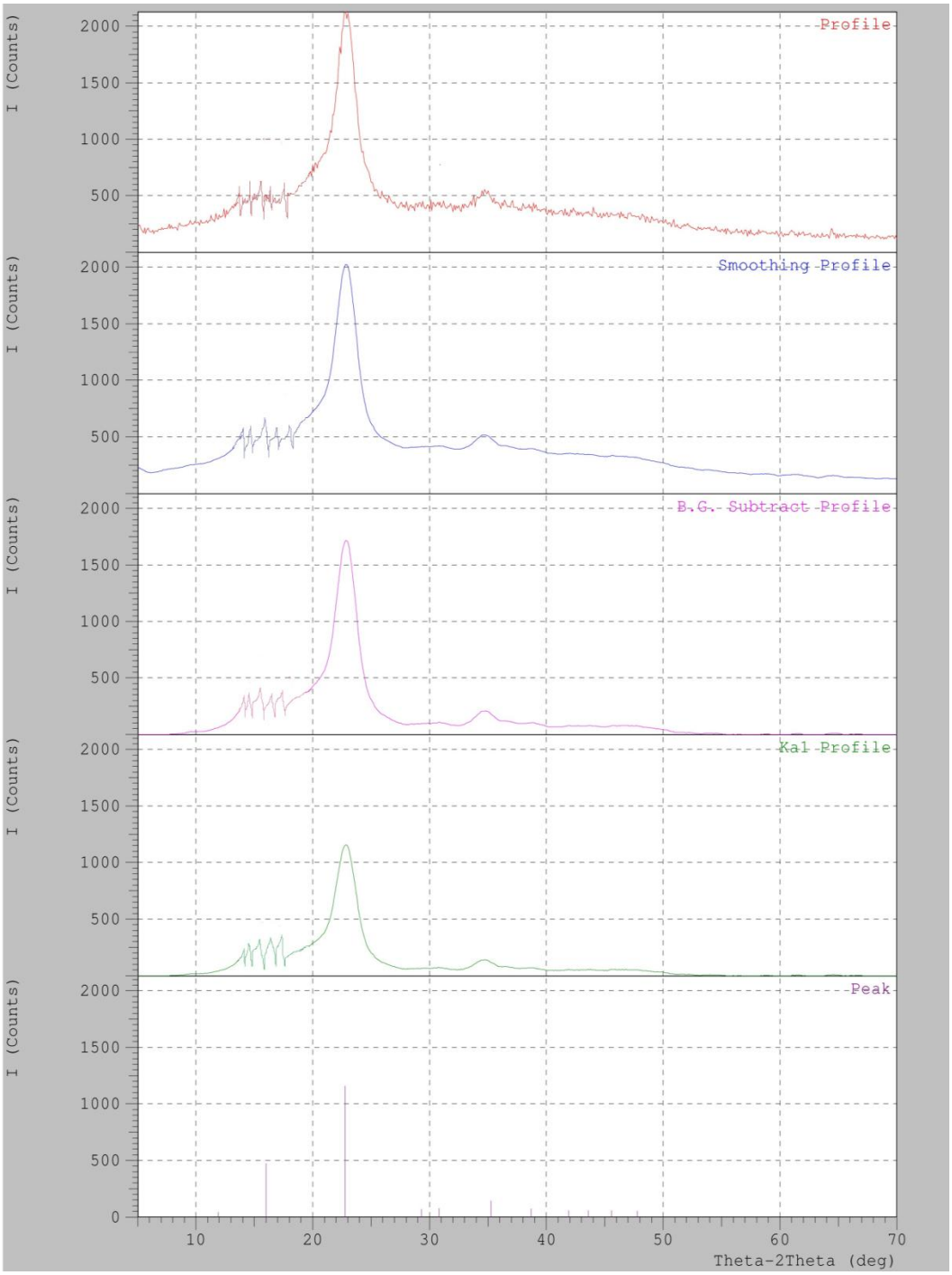


FIGURE 17b: X RAY DIFFRACTION PATTERN OF BEST FORMULATION (F3E)

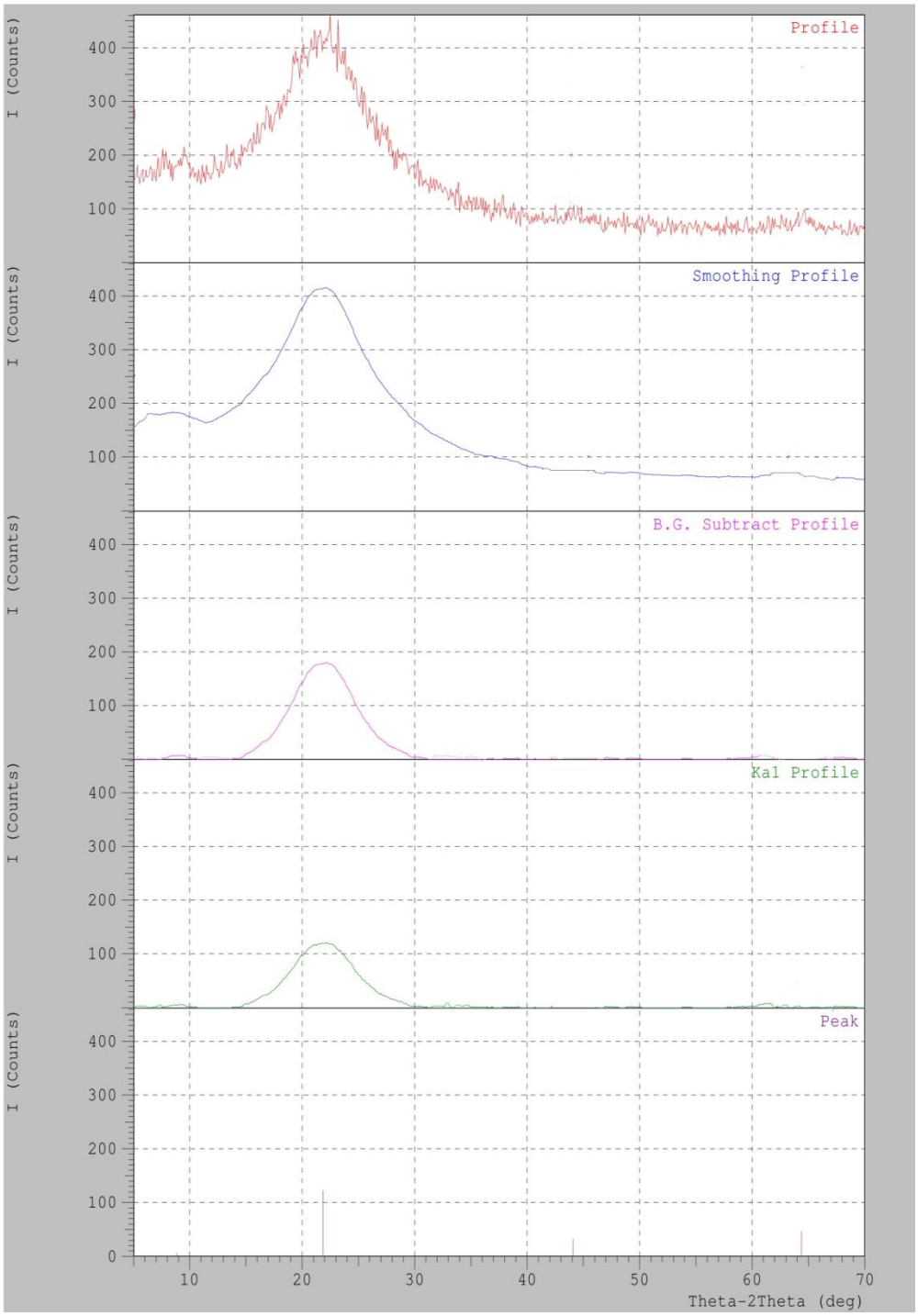


FIGURE 17c: X RAY DIFFRACTION PATTERN OF BEST FORMULATION (F4E)

CHAPTER XI

SUMMARY AND CONCLUSION

CHAPTER - XI

SUMMARY AND CONCLUSION

- In the present study an attempt has been made to develop nanocrystals of Ramipril in order to enhance solubility and dissolution rate by decreasing particle size of drug.
- The results of compatibility studies by Infrared spectroscopy showed no interaction between the drug and stabilizers.
- The Ramipril nanocrystals were successfully prepared by emulsion solvent diffusion method using different concentrations of stabilizers (HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000).
- The presence of stabilizers made the nanocrystal formulations more stable with increasing drug release.
- Particle size analyzer used to explore the particle size of Ramipril nanocrystals showed a suitable particle size in the range of 80.3nm to 300.6 nm.
- The polydispersity index of selected nanocrystal formulations(F3C, F3D, F3E, F4D, and F4E) was less than 0.5, which indicated a narrow size distribution of particles.
- Zeta potential value of Ramipril nanocrystals showed a negative surface charge (-19.7mV to -24.7mV). *In vitro* release study of all the formulations were showed a increased drug release with increase in concentration of different stabilizers (HPMC K15M, PVP K30, β-CYCLODEXTRIN, SODIUM LAURYL SULPHATE, and PEG 6000). Dissolution rate of all the formulations were improved when compared to pure drug.

- On the basis of drug release data F3C, F3D, F3E, F4D, and F4E showed a good release profile with more than 80% in 2 hours.
- The solubility of selected formulations (F3C, F3D, F3E, F4D, and F4E) in phosphate buffer pH(6.8) increased ten folds when compared to pure drug.
- SEM studies confirmed the morphology of the nanocrystal formulations. The crystalline state of the nanocrystal formulation was not altered according to the XRPD analysis.

CONCLUSION:

Hence, it was concluded that nanocrystallization was a good approach to enhance the dissolution property of Ramipril by emulsion solvent diffusion method. The solubility and *in vitro* dissolution studies suggested that the nanocrystal formulations can improve the bioavailability of the Ramipril by improving its solubility and dissolution rate when compared to pure drug. Thus nanocrystal drug delivery system can adopted to increase the solubility and dissolution rate of poorly soluble drug like Ramipril to enhance their bioavailability.

REFERENCES

REFERENCES

Abdul Hasan Sathali. A., Gopinath. M., 2013. Formulation and evaluation of Paliperidone Nanocrystals, *BioMedRx Vol1,* 1(5), 422-438.

Adlin Jino Nesalin. J., Gowthamrajan. K., Somashekhara. C. N., 2009. Formulation and evaluation of nanoparticles containing Flutamide, *Int. J. Chem Tech Res,* 1(4), 1331-1334.

Ahmed Elshafeey. H., Amany Kamel. O., Gehanne Awad. A.S., 2010. Ammonium methacrylate units polymer content and their effect on Acyclovir colloidal nanoparticles properties and bioavailability in human volunteers, *Colloids and Surfaces B: Biointerface,*.75, 398–404.

Amighi. K., Hecq. J., Deleers. M., Fanara. D., Vranckx., 2005. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of Nifedipine, *Int. J. Pharm,* 299, 167-177.

Annick Ludwig., Kathleen Dillen., Jo Vandervoort., Guy Van den Mooter., 2006. Evaluation of Ciprofloxacin-loaded Eudragit® RS100 or RL100/PLGA nanoparticles, *Int. J. Pharm,* 314, 72-82.

Basavaraj Nanjwade. K., Ganesh Derkar. K., Hiren Bechra. M., Veerendra Nanjwade. K., Manvi. F.V., 2011. Design and characterization of nanocrystals of Lovastatin for solubility and dissolution enhancement, *J. Nanomedic Nanotechnol,* 2(2), 1-7.

Bivash Mandal., Kenneth Alexander. S., Alan Riga. T., 2010. Sulfacetamide loaded Eudragit RL100 nanosuspension with potential for ocular delivery, *J. Pharm Pharmaceut Sci*,13(4), 510-523.

Chaudhari Bharat., Asija Rajesh., Asija Sangeeta., Patel Chirag.J., Patel Pinkesh., Patel Jaimin., 2013. Comparative study between Inclusion complex with hydroxypropyl-β-cyclodextrin and nanocrystal technology for enhancement of solubility and dissolution rate of poorly soluble drug Albendazole, *Journal of Drug Discovery and Therapeutics*, 1(1) 5-14.

Diane Burgess. J., Sudhir Verma., Rajeev Gokhale., 2009. A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions, *Int. J. Pharm*, 380, 216-222.

Dianrui Zhang., Guangpu Liu., Yang Jia., Dandan Zheng., Yue Liu., Cunxian Duan., Lejiao Jia., Qiang Zhang., Hongxiang Lou., 2012. Comparison of different methods for preparation of a stable riccardin D formulation via nano-technology, *Int. J. Pharm*, 422, 516-522.

Dianrui Zhang., Leilei Hao., Xiaoyong Wangb., Qingyan Xu., Siyang Song., Feihu Wang., Caiyun Li., Hejian Guoa., Yue Liu., Dandan Zhenga., Qiang Zhang., 2012. Studies on the preparation, characterization and pharmacokinetics of Amoitone B nanocrystals, *Int. J. Pharm*, 12583, 1-8.

Francesco Castelli., Chiara Messina., Maria Grazia Sarpietro., Rosario Pignatello., Giovanni Puglisi., 2003. Eudragit as controlled release system for anti-inflammatory drugs A comparison between DSC and dialysis experiments, *Thermochimica Acta*, 400, 227–234.

Fude Cui., Peng Quan., Kai Shi., Hongze Piao., Hongyu Piao., Na Liang., Dengning Xia., 2012. A novel surface modified Nitrendipine nanocrystals with enhancement of bioavailability and stability, *Int. J. Pharm*, 420, 366-371.

Hans de Waard., Henderik Frijlink. W., Woulter Hinrichs. L. J., 2011. Bottom-up preparation techniques for nanocrystals of liphophilic drugs, *Pharm. Res,* 28, 1220-1223.

Harish Chander., Sachin Kumar., and Bineeta Bhatt., 2011. Formulation and evaluation of fast dissolving tablet of Ramipril, *Pelagia Research Library,* 2 (6):153-160.

Hongwei Qiu., Victor Stepanov., Tsengming Chou., Ashok Surapaneni., Anthony R. Di Stasio., Woo Y. Lee., 2012. Single-step production and formulation of HMX nanocrystals, *J. Powder Tech,* 226, 235-238.

Huabing Chen., Chalermchai Khemtong., Xiangliang Yang., Xueling Chang., Jinming Gao., 2011. Nanonization strategies for poorly soluble drugs, *Drug Discovery Today,* 16, 354-360.

Jan Moschwitzer., Jan Salazar., Oliver Heinzerling., Rainer Muller. H., 2011. Process optimization of a novel production method for nanosuspensions using design of experiments (DoE), *Int. J. Pharm,* 420, 395-403.

Jawahar. N., Nagasamy Venkatesh. D., Sureshkumar. R., Senthil. V., Ganesh. G.N.K., Vinoth. P., Sumeet Sood., Samanta. M.K., 2009. Development and charecterization of PLGA-nanoparticles containing Carvedilol, *J. Pharm. Sci. & Res,* 1(3), 123-128.

Jens-Uwe Junghanns . A.H ., Rainer Müller. H., 2008. Nanocrystal technology, drug delivery and clinical applications, *International Journal of Nanomedicine,* 3(3), 295-309.

Jonghwi Lee., Ji-Yeun Choi., Ji Youn Yoo., Hae-Soo Kwak., Byeong Uk Nam., 2005. Role of polymeric stabilizers for drug nanocrystal dispersions, *J. Current Applied Physics*, 5, 472-474.

Jonghwi Lee., Yu Cheng., 2006. Critical freezing rate in freeze drying nanocrystal dispersions, *Journal of Controlled Release,* 111, 185–192.

Julijana Kristl., Andrej Dolenc., Sasa Baumgartner., Odon Planinsek., 2009. Advantages of Celecoxib nanosuspension formulation and transformation into tablets, *Int. J. Pharm,* 376, 204-212.

Jun Hu., Wai Kiong Ng., Yuancai Dong., Shoucang Shen., Reginald B.H. Tan., 2011. Continuous and scalable process for water-redispersible nanoformulation of poorly aqueous soluble Fenofibrate by antisolvent precipitation and spray-drying, *Int. J. Pharm,* 404, 198-204.

Koichi Baba., Kohji Nishida., 2013. Steroid Nanocrystals Prepared Using the Nano Spray Dryer B-90, *Pharmaceutics*, 5, 107-114.

Kristl. J., Baumgartner. S., Kocbek. P., 2006. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs, *Int. J. Pharm,* 312, 179-186.

Krutika Sawant., Chetan Detroja., Sandip Chavhan., 2011. Enhanced antihypertensive activity of Candesartan Cilexetil nanosuspension: Formulation, characterization and pharmacodynamic Study, *Sci. Pharm,* 79(3), 635-651.

Lei Gao., Dianrui Zhang., Minghui Chen., 2008. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system, *J. Nanopart. Res,* 10, 845-862.

Mohanraj. V.J., Chen. Y., 2006. Nanoparticles – Review, *Trop. J. Pharm.* Res, 5(1), 561-573.

Mukesh Patil. S., Kedar Bavaskar. R., Ghanashyam Girnar. A., Ashish Jain. S., Avinash Tekade. R., 2011. Preparation and optimization of Simvastatin nanoparticle for solubility enhancement and *in- vivo* study, *International Journal of Pharma Research and Development*, 2(12), 219-226.

Nanda Gopal Sahoo., Lin Li., Mitali Kakran., Zaher Judeh., 2012. Fabrication of Quercetin nanoparticles by anti-solvent precipitation method for enhanced dissolution, *J. Powder Tech*, 223, 59-64.

Narendra Chary.T., Sunitha kumarai.B., Vanamala Sudheer., Adarsh.D., Swathi.L., 2012. Studies on formulation development and *in-vitro* release kinetics of Ramipril Micropellets for controlled release**,** *International Research Journal of Pharmaceutical and Applied Sciences,* 2(4): 97-103.

Noushin Bolourchian., Malihe Shahbaziniaz., Seyed Mohsen Foroutan., 2013. Dissolution Rate Enhancement of Clarithromycin Using Ternary Ground Mixtures: Nanocrystal Formation, *Iranian Journal of Pharmaceutical Research*, 12(4):587-598.

Peng Liu., Xinyu Rong., Johanna Laru., Bert van Veen., Juha Kiesvaara., Jouni Hirvonen., Timo Laaksonen., Leena Peltonen., 2011. Nanosuspensions of poorly soluble drugs: Preparation and development by wet milling, *Int. J. Pharm*, 411, 215-222.

Phanchaxari Dandagi. M., Sumit Kaushik., Shaktish Telsang., 2010. Enhancement of solubility and dissolution property of Griseofulvin by nanocrystallization, *Int. J. Drug Dev. & Res*, 3(2), 180-191.

Plakkot. S., De Matas. M., York. P., Saunders., Sulaiman. B., 2011. Comminution of Ibuprofen to produce nano-particles for rapid dissolution, *Int. J. Pharm*, 415, 307-314.

Poovi. G., Dhanalakshmi. U.M., Narayanan. N., Neelakanta Reddy., 2011. Preparation and characterization of Repaglinide loaded Chitoson polymeric nanoparticles, *Res. J. Nanosci. Nanotechnol*, 1(1), 12-24.

Prasanthi. B., Basava Raju .D., Vijaya Ratna. J., 2012. Modulation of drug release kinetics of a highly water soluble drug from hydrophilic matrices, *Journal of Global Trends in Pharmaceutical Sciences*, 3(2), 698-707.

Raghvendra., Amlan Mishra., 2013. A Review on potential applications of nanocrystal technology, *Indian Journal Of Pharmaceutical Sciences and Research*, Vol 3, Issue 1, 9-13.

Rainer Muller.H., Cornelia Keck. M., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization, *Eur. J. Pharm. Biopharm*, 62, 3-16.

Rainer Muller. H., Veerawat Teeranachaideekul., Varaporn Junyaprasert. B., Eliana Souto. B., 2007. Development of Ascorbyl palmitate nanocrystals applying the nanosuspension technology, *Int. J. Pharm*, 354, 227-234.

Ravikumar. M.N.V., Mittal. G., Sahana. D.K., Bhardwaj. V., 2007. Estradiol loaded PLGA nanoparticles for oral administration: Effect of polymer molecular weight and copolymer composition on release behavior *in vitro* and *in vivo*, *Journal of Controlled Release*, 119, 77–85.

Raymond C. Rowe., Paul J. Sheskey., Sean C. Owen., 2006. Handbook of pharmaceutical excipients, *Pharmaceutical press, London. 5th edition*, 234-235.

Sahoo. S.K., Parveen. S and Panda. J.J., 2007. The present and future of nanotechnology in human health care, *Nanomedicine: Nanotechnology, Biology and Medicine*, 3, 20– 31.

Sanjay Bansal., Meena Bansal., Rachna Kumria., 2012. Nanocrystals: Current strategies and trends, *Int. J. Res. Pharm. and Biomed. Sci*, 3(1), 2229-3701.

Sanjay Jadhav. A., Shashikant Landge.B., Pramod Choudhari. M., Pavankumar Solanki. V., Saroj Bembalkar. R., Vijayavitthal Mathad. T., 2011. Stress degradation behavior of Paliperidone, an antipsychotic drug, and development of suitable stability-indicating RP-LC method, *Chromatography Research International*, 256812, 1-10.

Shailesh Soni., Tarun Patel., Bhaumik Thakar., Vikram pandya., Praful Bharadia., 2012. Nanosuspension: An approach to enhance solubility of drugs**,** *Journal of Pharmaceutics and Cosmetology*, 2(9), 49-63.

Sinico. C., Lai. F., Pini. E., Angioni. G., Manca. M.L., Perricci. J., Fadda. A. M., 2011. Nanocrystals as tool to improve Piroxicam dissolution rate in novel orally disintegrating tablets, *Eur. J. Pharm. Biopharm*, 79, 552-558.

Suganeswari. M., Anto Shering., Azhagesh raj., Bharathi. P., Sathish. B., 2011. Preparation, characterization and evaluation of nanoparticles containing hypolipidemic drug and antihypertensive drug, *Int. J. Pharm*, 2(3), 949-953.

Suman Katteboinaa., VSR Chandrasekar. P., Balaji. S., 2009. Drug nanocrystals: a novel formulation approach for poorly soluble drugs, *Int. J. Pharm Tech. Res*, 1(3), 682-694.

Suvakanta Dashl., Padala Narasimha Murthy., Lilakanta Nath., Prasanta Chowdhury., 2010. Kinetic modeling on drug release from controlled drug delivery systems, *Acta Poloniae Pharmaceutica-Drug Research,* 67(3), 217-223.

Tonglei Li., Qiang Zhang., Hua Zhang., Christin Hollis. P., 2011. Preparation and antitumor study of Camptothecin nanocrystals, *Int. J. Pharm,* 415, 293-300.

Tugba gulsun. R., Neslihan gursoy., Levent oner., 2009. Nanocrystal technology for oral delivery of poorly water-soluble drugs, *Fabad J. Pharm. Sci,* 34, 55–65.

Vishal Patel. R., Agarwal. Y. K., 2012. Nanosuspension: An approach to enhance solubility of drugs, *J. Adv. Pharm. Tech. Res,* 2(1), 81-87.

www.drugbank.com

www.drugs@fda.com

Yadav. A.V., Selvakumar Kalimuthu., 2009. Formulation and evaluation of Carvedilol loaded Eudragit e 100 Nanoparticles, *Int.J. PharmTech Res,* 1(4), 179-183.

YaminiPendyala., SudhaTalasila., 2012. Formulation and Evaluation of Chitosan Loaded Mucoadhesive Microspheres of Ramipril, *INTERNATIONAL JOURNAL OF PHARMACEUTICAL AND CHEMICAL SCIENCES,* 555-562

Yuan Gao., Jianjun Zhang., Huixia Lv., Kun Jiang., 2011. Enhanced bioavailability after oral and pulmonary administration of Baicalein nanocrystal, *Int. J. Pharm,* 420, 180-188.

Zuki Abu Bakar zakaria., Abdullahi Shafiu Kamba., Maznah Ismail., Tengku Azmi tengku Ibrahim., 2013. A pH-Sensitive, Biobased Calcium Carbonate Aragonite Nanocrystal as a Novel Anticancer Delivery System, *BioMed Research International,* Article ID 587451, 10 pages.