DEVELOPMENT OF MUCOADHESIVE TABLETS OF CEFUROXIME AXETIL LOADED IN SOLID LIPID NANOPARTICLES

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: 261211308)

DEPARTMENT OF PHARMACEUTICS

COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE

MADURAI – 625020

APRIL – 2014

CERTIFICATES

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

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CERTIFICATE

This is to certify that the dissertation entitled**, "Development of Mucoadhesive Tablets of Cefuroxime axetil loaded in Solid Lipid Nanoparticles"** submitted by **Mr. Sankar Ganesh** (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)

(For IAEC / CPCSEA usage)

Proposal number	: G.Sankar/M.Pharm/IAEC/126 /KMCP/ 261211308/2013-14
Date first received	:01.10.2013
Date received after modification (if any)	: NA
Date received after second modification (if any)	: NA
Approval date	: 10.10.2013
Expiry date	: 28.02.2014
Name of IAEC / CPCSEA chairperson	: N.CHIDAMBARANATHAN

Date:10.10.2013

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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(i/c).,*** *Principal, 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 ***Sterilgene, Pondicherry*** *for providing the drug* ***Cefuroxime axetil*** *as gift sample and* ***Orchid Pharma, Chennai*** *for providing Compritol ATO 888 and* ***Madras Pharma, Chennai*** *for providing Poloxamer and* ***Central Drug House (P) Ltd, New Delhi*** *for providing chemicals to carry out my project work.*

With immense pleasure I record here my 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, Particle size, Zeta potential, SEM and X-ray diffraction) studies.*

I would like to give my sincere thanks to my classmates ***Mr. P.Arjunkumar., Mr. P. Kanniyappan., Mr. A. Manikkavasagan., Mrs. S. Ponnammal Asmi., Mr. C. Pravinkumar., Mr. J.Rajeshkumar., Mr. M. Ramanathan., 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 :

(SANKAR GANESH)

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CHAPTER 1

INTRODUCTION

CHAPTER – 1

INTRODUCTION

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale.

One nanometer (nm) is equal to one-billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules. (Sahoo S. K et al, 2007)

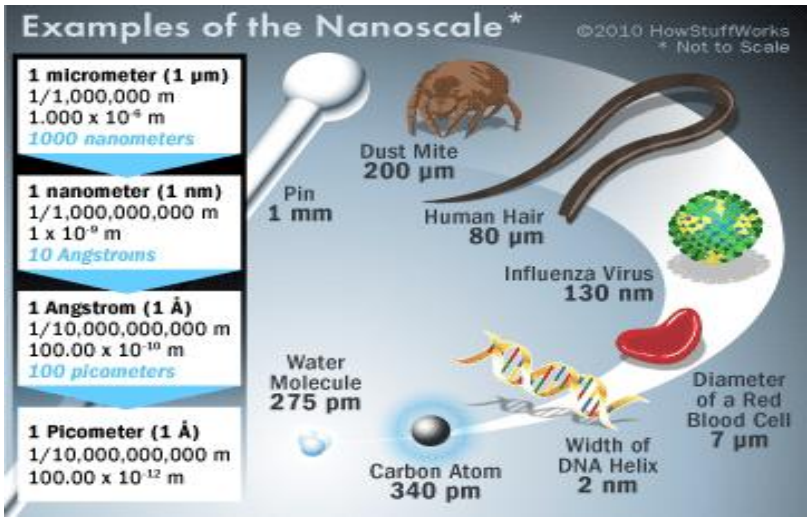


Figure 1

Nanotechnology in drug delivery and its significance

In pharmaceutical industries, nanotechnology can address issues such as extending product life, or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance.

Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to improve drug efficacy and reduces drug toxicity.

Nanotechnology offers a solution for using the numerous chemical entities for treating brain disorders that are not clinically useful, because of the presence of the blood-brain barrier.

The increased vascular permeability coupled with an impaired lymphatic drainage in tumors allows an enhanced permeability and retention effect of the nanosystems in the tumors or inflamed tissues.

The tendency of nanosystems to specifically localize in the reticulo-endothelial system (RES) also presents an excellent opportunity for passive targeting of drugs to the macrophages present in the liver and spleen.

(Sanjeeb k. Sahoo et al, 2003)

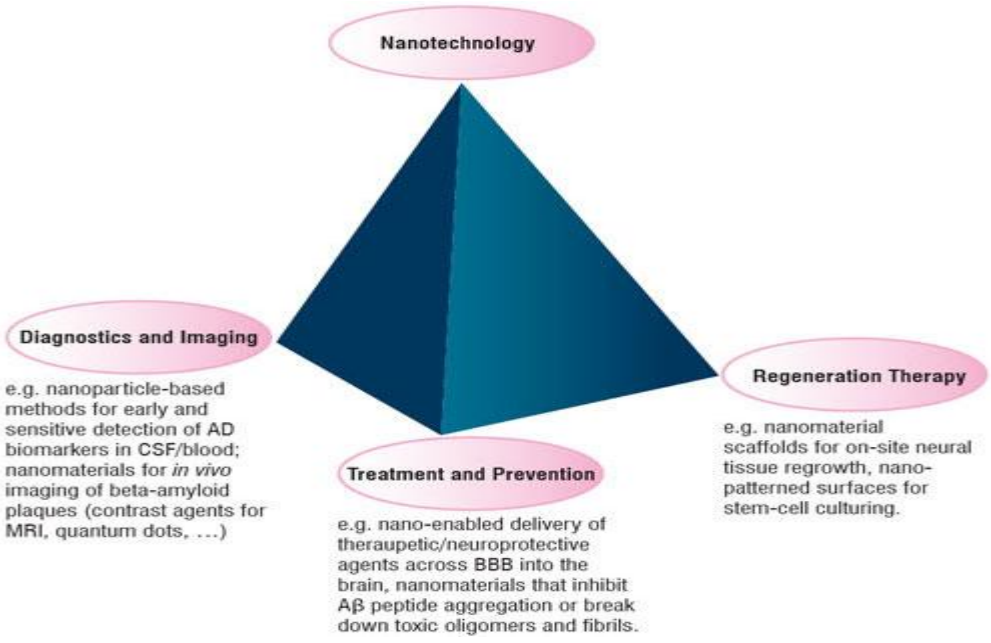


Figure 2

NANOANTIBIOTICS

Nanomaterials, which either show antimicrobial activity by themselves or elevate the effectiveness and safety of antibiotics administration, are called "nanoantibiotics".

Antimicrobial nanoparticles (NPs) tackle multiple biological pathways found in broad species of microbes and many concurrent mutations would have to occur in order to develop resistance against NPs' antimicrobial activities. Unlike many antimicrobial agents currently being used, antimicrobial NPs may not pose direct and acute adverse effects, although there is potential toxicity upon long-term exposure.

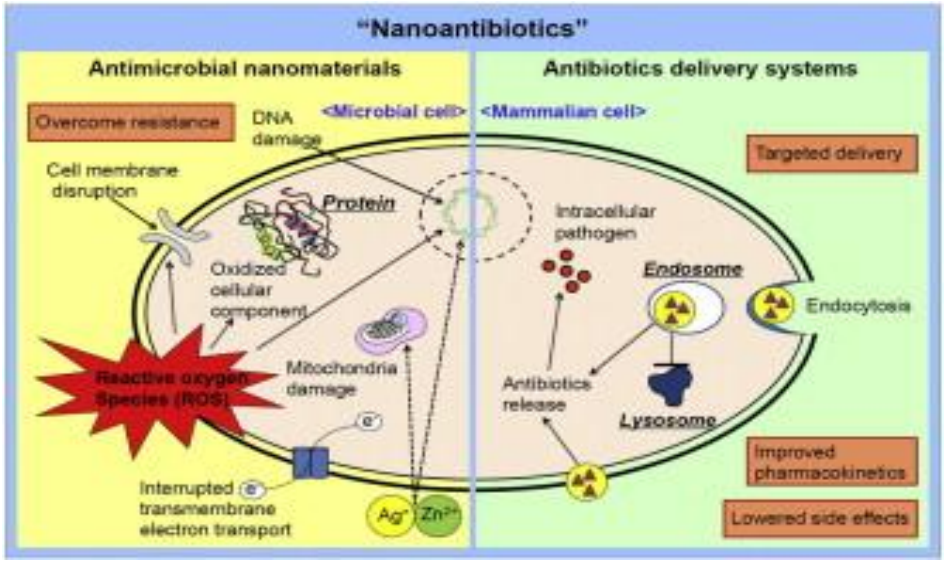


Figure 3

Preparation of antimicrobial NPs could be cost-effective, compared with antibiotics synthesis, and they are quite stable enough for long-term storage with a prolonged shelf-life. In addition, some NPs can withstand harsh conditions, such as high temperature sterilization, under which conventional antibiotics are inactivated.

(Young Jik Kwon et al, 2011)

Advantages of Nanoantibiotics

Antibiotics delivery using nano materials offer multiple advantages,

- Overcoming resistance
- Improved solubility
- Sustained and controlled release
- Improved patient-compliance

- Minimized side effects
- Enhanced cellular internalization
- Controllable and relatively uniform distribution in the target tissue.

Antimicrobial Nanometals

Antibacterial nanometals consist of metals and metal oxides, naturally occurring antibacterial substances, carbon-based nanomaterials, and surfactant-based nano-emulsions. High surface area to volume ratios and unique chemico-physical properties of various nanomaterials are believed to contribute to effective antimicrobial activities.

Antimicrobial mechanisms of nanomaterials include,

- Photocatalytic production of reactive oxygen species (ROS) that damage cellular and viral components
- Compromising the bacterial cell wall/membrane
- Interruption of energy transduction
- Inhibition of enzyme activity and DNA synthesis

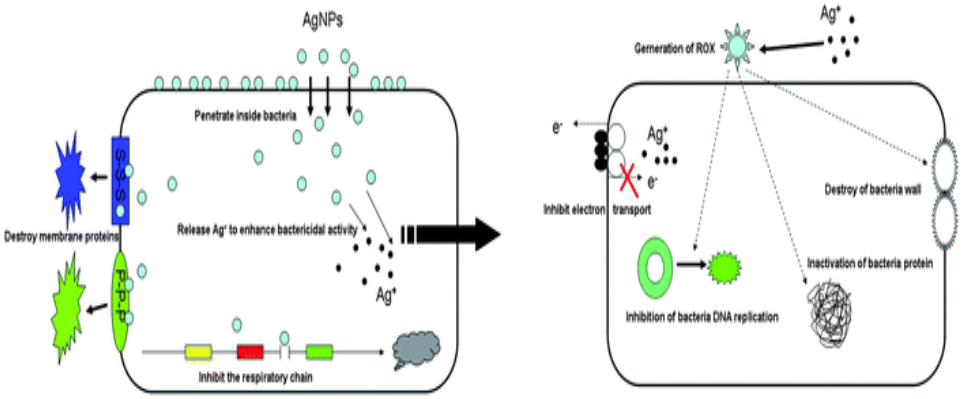


Figure 4

Some of the various types of antimicrobial nanometals being used are,

- Silver Nanoparticles (Ag NPs)
- Zinc oxide Nanoparticles (ZnO NPs)

- Titanium dioxide Nanoparticles (TiO_2 NPs)
- Gold Nanoparticles (Au NPs)
- Aluminium and Copper Nanoparticles (Al & Cu NPs)
- Nitric oxide (NO) releasing Nanoparticles
- Carbon Nanotubes (CNTs)

(Jagat R Kanwar et al, 2010)

Nanoparticle carriers

Novel nanomaterials, NPs in particular have improved solubility of poorly water-soluble drugs, prolonged drug half-life and systemic circulation time, and sustained and stimuli-responsive drug release, which eventually lowers administration frequency and dose. Moreover, minimized systemic side effects via targeted delivery of antimicrobial drugs as well as combined, synergistic, and resistance-overcoming effects via co-delivery of multiple antimicrobial drugs can be achieved using NP carriers.

Some of the nanocarriers used are,

- Liposomes
- Polymeric nanoparticles (NPs)
- Solid Lipid Nanoparticles (SLNs)
- Dendrimers

The use of nanotechnology in immunization, design and delivery of antimicrobial drugs, and diagnosis and control of cross-infections, particularly in overcoming antibiotics-resistant pathogens, serve as a promising alternative to the current antibiotics-based approaches.

(Young Jik Kwon et al)

SOLID LIPID NANOPARTICLES

Solid lipid nanoparticles (SLNs) are first introduced in 1991, which are the forefront of rapidly developing field of nanotechnology with several potential applications in drug delivery and research. SLNs are sub micron colloidal carrier ranging from 50-1000nm, which are composed of physiological lipid disperse in water or aquoeus surfactant solutions.

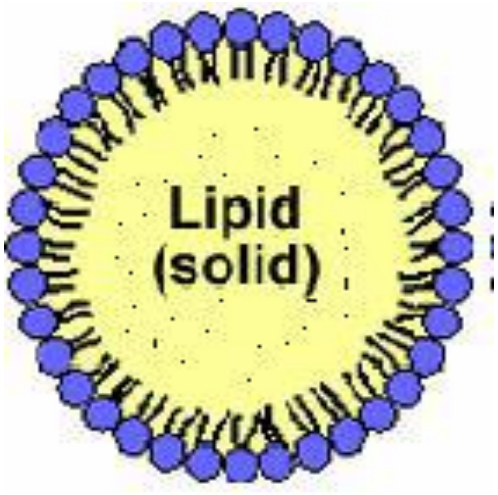


Figure 5

SLNs posses a solid lipid core matrix that can solubilise lipophilic molecules and the lipid core is stabilized by surfactants. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and the stability in nanometer size. Many biocompatible or biodegradable lipids, which are solid at room temperature can be obtained in high purity are generally recognized as safe (GRAS) and are inexpensive. Some of commonly used solid lipids include triglycerides, carnauba wax, beeswax, cetyl alcohol, emulsifying wax, cholesterol and cholestryl butyrate. Nano- and micro-particles made of these lipids and suspended in water offer an option for formulating both BCS Class II and IV drugs as well as biologics

that may overcome the issues of shelf life stability, cost and toxicity associated with the use of organic solvents. (Abdul Hasan Sathali A et al, 2012)

Advantages of SLNs

- Smaller size and relative narrow size distribution
- Controlled and sustained release of active drug
- The incorporated drug is protected from onslaughts of biochemical degradation.
- Can be lyophilized
- Relatively cheap and stable
- Use of physiological lipids
- Avoidance of organic solvents
- Improved bioavailability
- Chemical protection of labile incorporated compounds
- Very high long term stability
- Application versatility

Disadvantages of SLNs:

- Particle size increase
- Polymorphic transitions
- Gelation tendency

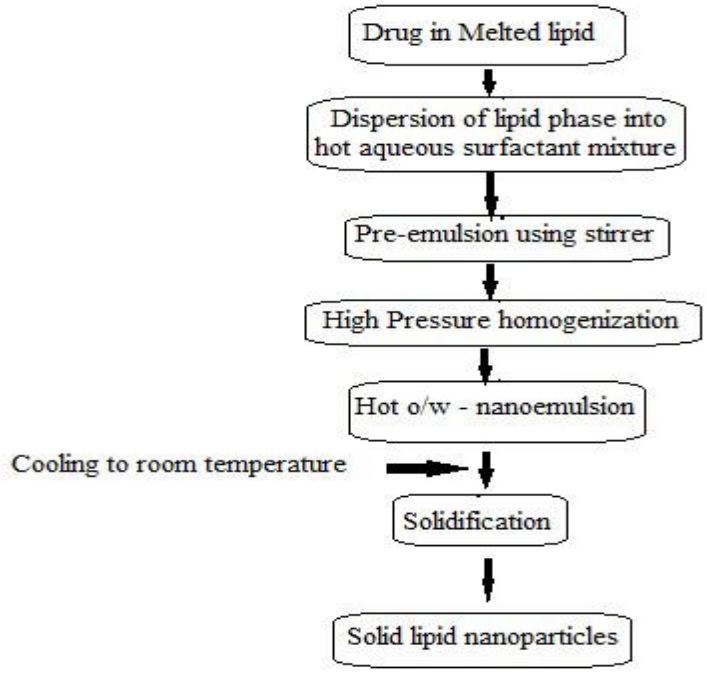
Methods of Preparation of SLNs:

High Pressure Homogenization:

It is a reliable and powerful technique, which pushes a liquid with high pressure (100-2000bar) through a narrow gap.

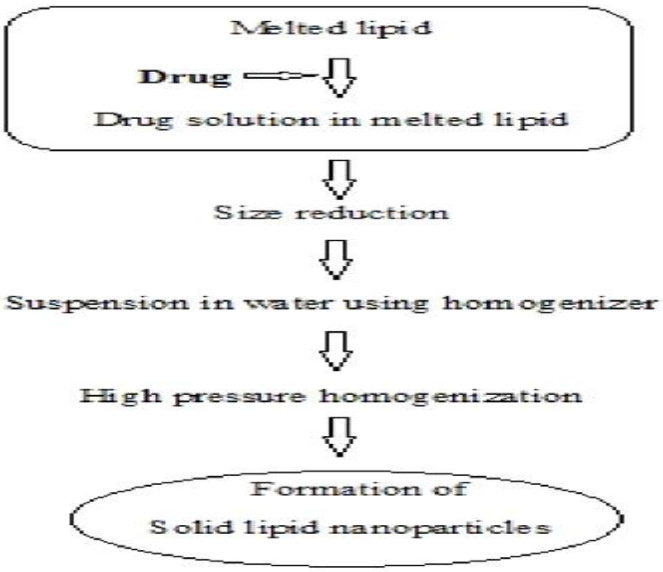
- **Hot homogenization:**

  It is carried out at temperature above the melting point of lipid.



- **Cold homogenization:**

It has been developed to overcome various problems associated with hot homogenization such as temperature induced drug degradation, drug distribution into the aquous phase during homogenization, complexity of crystallization step of nanoemulsion leading to several modification and/super cooled melts.

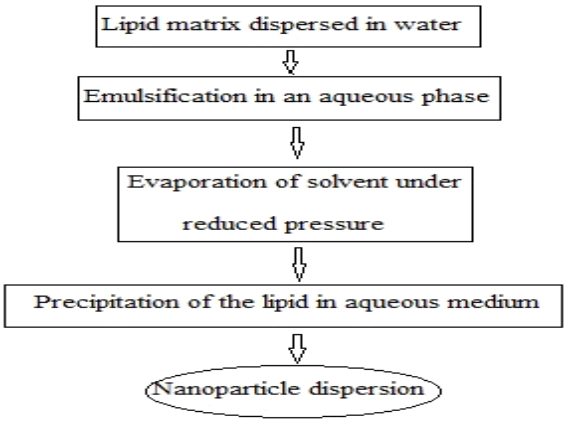


Ultrasonication/ High speed homogenization:

SLNs are also prepared by ultrasonication/high speed homogenization techniques. For smaller particle size combination of both ultrasonication and high speed homogenization is required.

Solvent Emulsification diffusion method:

The particles with average diameters of 30-100nm can be obtained by this technique. Avoidance of heat during the preparation is the most advantage of this technique.



Supercritical fluid method:

This is an alternative method of preparing SLNs by particles from gas saturated solutions (PGSS).

Spray drying method:

It is an alternative process to lyophilization. It recommends the use of lipid with melting point more than 70ºC. Best results were obtained with SLN of 1% in a solution of trehalose in water or 20% in ethanol water mixure.

Double emulsion method:

In this method drug is encapsulated with a stabilizer to prevent the partitioning of drug in to external water phase during solvent evaporation in external water phase of w/o/w double emulsion.

Precipitation method:

The glycerides are dissolved in an organic solvent and the solution will be emulsified in an aquous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

Film ultrasound dispersion:

The lipid and drug were put into suitable organic solution, after decompression, rotation and evaporartion of the organic solutions, a lipid film is formed, then the aquoux solution which includes the emulsion was added.

Secondary Production Step:

Freeze drying:

Lyophiilization is a promising way to increase chemical and physical stability over extended periods of time. Transformation in to the solid state would prevent the Oswald ripening and avoid hydrolytic reactions.

Spray drying:

Spray drying might be an alternative procedure to lyophilisation in order transforms an aqueous SLNs dispersion in to dry product. The lipids with melting point at temperature greater than 70°C had been recommended for spray drying.

(Shagufta Khan et al, 2012)

Drug release from SLN:

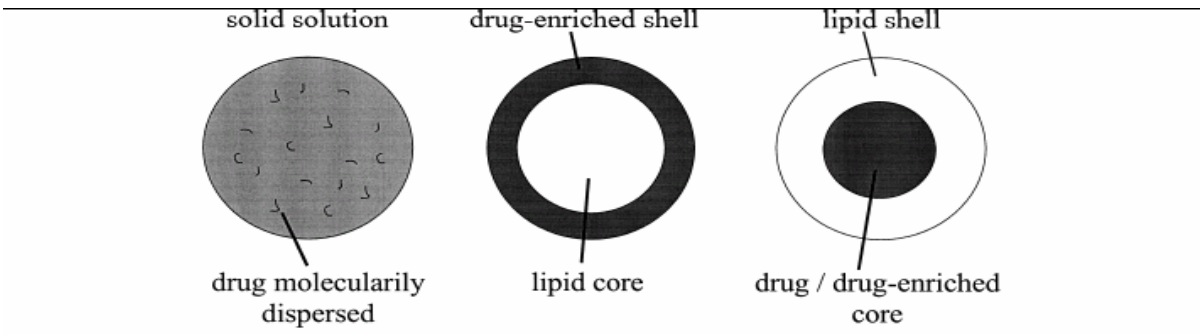


Figure 6

Homogenous matrix model:

In this with many drugs being present in amorphous clusters or molecularly dispersed is mainly obtained when incorporating highly lipophillic drugs in to SLN using hot homogenization method or by avoiding drug solubilising surfactants.

Drug enriched shell with lipid core:

The drug enriched shell with lipid core model will be obtained when performing the production. During production, the drug is partition to the water phase and upon cooling the lipid precipitate first forming a practically drug free lipid core due to phase separation. At the same time the drug re-partitions in to the remaining liquid lipid phase and drug concentration in the outer shell increasing gradually. Finally drug enriched shell crystallizes. The amount of drug partitioning will increase with increase of aquous solubility of drug.

Drug enriched core with lipid shell:

A drug enriched core obtained when dissolving drug in the lipid melts at or dose to its saturation solubility. In here, cooling of the formed nanoemulsion will lead to supersaturation of drug in melted lipid and on further cooling will lead to precipitation of lipid surrounding the drug enriched core as a membrane, due to increased diffusional distance and hindering effect of surrounding solid lipid shell, the carrier system shows sustained release profile. (Meghana S. Kamble et al, 2012)

Factors determining the loading capacity of the drug in the lipid:

- Solubility of the melted lipid.
- Miscibility of the drug melt in the lipid melt.
- Chemical and physical structure of solid lipid matrix.
- Polymorphic state of lipid material.

The pre-requisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. Typically the solubility should be higher than required because, it decreases when cooling down the melt and might be even lower in the solid lipid. To enhance the solubility in the lipid melt one can add solubilizers. In addition, the presence of mono and di-glycerides in the lipid used matrix material promotes drug solubilization. The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice lead drug expulsion. (Abdul Hasan Sathali A et al, 2012)

Routes of Administration:

- Oral administration
- Parentral administration
- Rectal administration
- Nasal administration
- Respiratory administration
- Ocular administration
- Topical administration

(Shah Chandni V et al, 2011)

Applications of SLNs:

- SLNs for parasitic diseases
- SLNs as potential new adjuvant for vaccines
- SLNs in cancer chemotherapy
    i. SLNs as targeted carrier for anticancer drug to solid tumor
    ii. SLN in breast cancer and lymph node metastases

- SLNs for malarial disease
- SLNs in treatment of tuberculosis
- SLNs for brain targeted drug delivery
- SLNs for improved delivery of antiretroviral drugs to the brain
- SLNs for lung targeted drug delivery
- SLNs for lymph targeting
- SLNs for ultrasonic drug and gene delivery
- SLNs for delivery of peptides and proteins
- SLNs in cosmetical and dermatological preparations

(Krishna Sailaja A et al, 2011)

ORAL DRUG DELIVERY SYSTEM

Oral drug delivery is most widely utilized route of administration among all the routes that have been explored. An ideal drug delivery system should deliver an appropriate amount of drug to the desired site with a desired rate to optimize the drug therapy. Due to the considerable therapeutic advantages over conventional oral drug delivery dosage forms, various oral controlled release dosage forms have been developed.

Controlled Drug Delivery System:

The term controlled release on the other hand, has a meaning that goes beyond the scope of sustained drug action. It also implies a predictability and reproducibility in the drug**.**

Advantages of controlled drug delivery system

- Decreased incidence and/or intensity of adverse effects and toxicity
- Better drug utilization
- Controlled rate and site of release
- More uniform blood concentration
- Improved patient compliance
- Reduced dosing frequency
- prolonged therapeutic effect
- A greater selectivity of pharmacological activity.

Gastroretentive Drug Delivery System:

Dosage forms that can be retained in the stomach are called gastroretentive drug delivery system (GRDDS). These are the systems which can remain in gastric

region for several hours and significantly prolongs the gastric residence time of drug. After oral administration, such a delivery system would be retained in stomach**.**

Types of Gastroretentive Delivery Systems:

- Bioadhesive system
- Floating system
- Unfolding system
- Size increasing system
- Density controlled system

(Aashima Hooda et al, 2012)

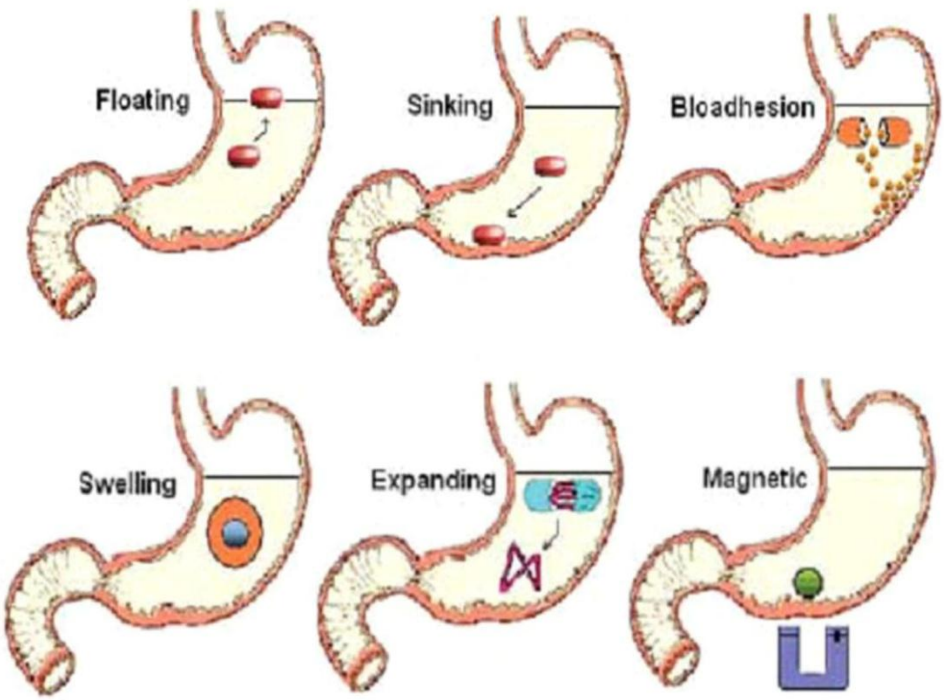


Figure 7

MUCOADHESIVE DRUG DELIVERY SYSTEM

A bioadhesive can be defined as a substance with the ability to interact with biological materials and is capable of being retained there. It involves the use of bioadhesive polymers which are usually macromolecular, hydrophilic gelling

substances with numerous hydrogen bond forming groups, such as carboxyl, hydroxyl, amide and sulfate groups (e.g., crosslinked polyacrylic acids, sodium carboxymethyl cellulose, sodium alginate and carrageenan) that can adhere to the epithelial surface of the GIT.

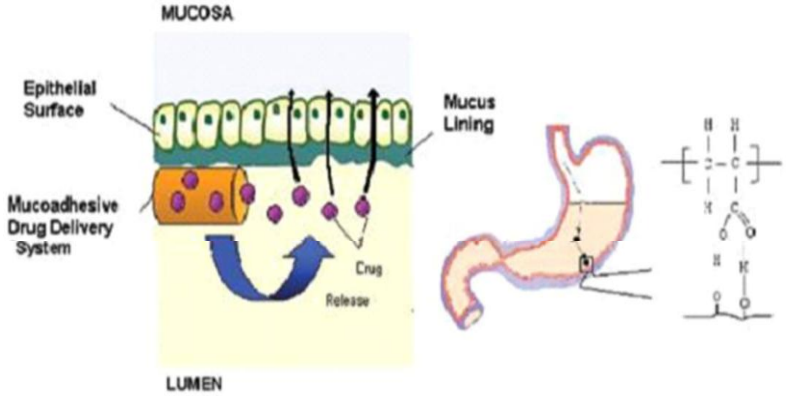


Figure 8

(Aashima Hooda et al, 2012)

Theories of Mucoadhesion

Several bioadhesion theories have been discussed.

1) Electronic theory

It defined as the electron transfer from contact of an adhesive polymer with a glycoprotein network; they form an electrical interface at adhesive polymer and glycoprotein network. Adhesion can produce by attractive forces across the double layer.

2) Absorption theory

Absorption theory is defined as the cause after initial contact between two surfaces that is material surface because a force formed between two surfaces, the force is two types of chemical bond that is,

i. Primary chemical bond of covalent bond: they are high strength so they cause permanent bonds.

ii. Secondary chemical bond has types of force of attraction like electrostatic force, Vander Waals forces, hydrogen and hydrophobic bonds.

3) Wetting theory

They are only beneficial for liquid bioadhesive systems, analyses adhesive and contact behaviour means they have ability of a liquid or a paste to spread over a biological system.

The equation is

$$Wa = Ya + Yb - Yab$$

Where, Wa = work of adhesion = energy/cm 2

a and b - biological membrane

Work of cohesion equation is

$$Wc = 2YA - (Y\ A + Y\ AB)$$

$$Wc = 2Y\ A \text{ or } Y\ B$$

Bioadhesive material B spreading on a biological substrate A so spreading coefficient that is,

$$SB/A = YA - (YB + YAB)$$

SB/A should be positive for a bioadhesive material to adhere to a biological membrane.

4) Diffusion Theory:

This theory provides the information that the polymer chains and the mucus mix to a sufficient depth to form a semi permanent adhesive bond. The polymer

chains penetrate the mucus depends on the diffusion coefficient and the time of contact.

5) Fracture Theory:

This theory related for difficulty of separation of two surfaces after adhesion,

The equation,

G = (E e/L) 1/2

E = Young's formula of elasticity

e = Fracture energy

L= Critical crack length

(Chein Y. W. - Novel drug Delivery System)

Gastric emptying

The process of gastric emptying occurs during both fasted state and fed state however, the pattern of motility differs markedly in these two states. In the fasted state, it is characterized by an inter digestive series of electrical events, which propagate both through stomach as well as small intestine every 2-3 hours. This activity is called as inter digestive myo electric complex, and is often divided into four consecutive phases.

- **Phase I**

  It is a quiet period lasting from 30-60 min, with rare contractions.

- **Phase II**

  It is a period of similar duration consisting of intermittent action potentials gradually increases an intensity and frequency as phase progresses.

- **Phase III**

  It is a short period of intense, large regular contractions lasting from 10-20 min. As it serves to sweep undigested materials out of stomach and down in small intestine. It is termed as 'housekeeper waves'. As the phase III of one cycle reaches the distal part of small intestine, the phase III of next cycle begins in duodenum.

- **Phase IV**

  It is a brief transitional phase that occurs between phase III and phase I of two consecutive cycles. In the fed state, the gastric emptying rate is slowed since the onset of IMC is delayed.

Types of Mucoadhesive Dosage Forms:

- Tablets
- Gels & ointments
- Films
- Patches

Factors Affecting Mucoadhesion:

- Molecular weight
- Cross linking & Swelling
- Hydrophilicity
- Concentration of active polymer
- pH
- Spatial conformation

CHAPTER - 2

LITERATURE REVIEW

CHAPTER – 2
LITERATURE REVIEW

Hassan M. Ghonaim et al., 2013, designed and characterized glyceryl monosterate solid lipid nanoparticles[SLN]. Glyceryl monosterate SLN containing dibenzoyl peroxide, erythromycin base and triamicinolone acetonide as model drugs were prepared by hot homogenization method. The prepared SLNs were characterized by different physical and imaging methods. Thus SLNs with and smooth surface particle size having high encapsulation could be obtained by hot homogenization technique.

Jaspreet Randhawa et al., 2013, has done a review on high melting lipid based approach for drug delivery. Poor solubility of newly developed drug molecules is the main problem in recent drug discovery research, so novel drug delivery approaches are being used to deliver these molecular entities for pharmacological action. Colloidal carriers have been used to administer poorly soluble drug, but solid lipid nanoparticles are found to be most reliable carriers for this type of drugs due to its advantage over carriers. SLNs have the potential to solve the drug delivery problems with safe excipients used in its formulation. In this review all the aspects of SLNs production, stability, characterization, differentiation based on route, preservation and storage have been discussed.

Pinitphon Prombutara et al., 2012, formulated nisin loaded solid lipid nanoparticles [SLN] for sustained anti microbial activity. Here, nisin – a natural antimicrobial agent used as a preservative in food was encapsulated in imvitor 900 based SLN by high pressure homogenization. Nisin loaded SLNs had particle size of 159 ± 6.4 to 167 ± 8.6 nm and had a zeta potential of - 28.3 ± 0.15 to - 29.2 ±

0.12 mV and entrapment efficiency of 69.2 ± 0.04 to 73.6 ± 0.04 % . Finally it was concluded that nisin from SLNs showed better antimicrobial action for 20 to 15 compared to free nisin.

Yitao Wang et al., 2012, has developed emodin loaded solid lipid nanoparticles. The objective of the present study was to prepare and evaluate emodin loaded SLNs and evaluate their anti tumor activity in vitro. Poloxamer 188 and tween 80 were used as surfactants. The prepared SLNs were characterized for their particle size, drug entrapment efficiency, zeta potential, stability and in vitro drug release studies. MTT assay showed emodin- SLN could enhance in vitro cytotoxicity against human breast cancer cell line MCF-7 and MDA-MDB-231 cells flow cytometric analysis showed more significant cell cycle arrest effect in MCF-7. In vitro drug release showed 72 hour drug release from SLN, exhibiting a sustained action.

Yaping Li PhD et al., 2012, has developed solid lipid nanoparticles loaded with candesartan cilexetil to enhance oral bioavailability. Candesartan – a poorly aqueous soluble, very low orally absorbing drug was encapsulated in SLN by film homogenization technique. The prepared SLNs were characterized for their particle size, entrapment efficiency (91.33%) the pharmacokinetic results indicated improved oral bioavailability of candesartan over 12- fold in SLNs.

De Pintu Kumar et al., 2012, has done project on formulation and evaluation of solid lipid nanoparticles of poorly water soluble model drug ibuprofen. Ibuprofen was encapsulated in SLN by hot homogenization method to enhance solubility and dissolution rate. Stearic acid used as lipid matrix and phospholipon 80 H was used as surfactant and tween 80 as stabilizer. Prepared SLNs were characterized for size

distribution, entrapment efficiency, drug release and stability. In vitro drug release studies showed higher release through dialysis membrane than pure drug. Hence SLNs prove to be a more efficient carrier for ibuprofen.

Kesavan Bhaskar Reddy et al., 2012, has done formulation and in vitro assessment of itraconazole loaded solid lipid nanoparticles for topical delivery. Itraconazole – a poorly water soluble drug was encapsulated in SLNs by hot homogenization method, using dynasan 118, phospatidylcholine and polysorbate 80 at varied concentrations. The formulated itraconazole-SLNs were evaluated for their particle size, zeta potential, entrapment efficiency, DSC, FTIR, P-XRD studies, stability, in vitro and in vivo permeation studies. The optimized formulation showed drug release of 83.4% and drug permeation of 1173µg/cm^2 after 24 hours. Results showed that incorporation of drug into SLNs showed better drug release.

Yamasai Madhusudan Rao et al., 2012, formulated atorvastatin loaded solid lipid nanoparticles by hot homogenization followed by ultrasonication technique. The mean particle size. Poly dispersity index, zeta potential and entrapment efficiency were found to be 50 ± 6.2 nm, 0.08 ± 0.011, 10.40 ± 4.68 mV and 88.7 ± 6.08% respectively. In vitro drug release showed controlled release over a period of 24 hours, comparing to pure drug. Stability studies showed that there was no physical instability over a period of 3 months.

Priyanka K, Abdul Hasan Sathali. A, 2012, developed preparation and evaluation of montelukast sodium loaded solid lipid nanoparticle. Montelukast – a poor orally available, high presystemically metabolized drug was chosen to formulate SLN by hot homogenization followed by ultrasonication technique.

Compritol ATO 888, stearic acid, and glyceryl monosterate were used as lipid matrix and polyvinyl alcohol as surfactant. The formulated SLNs were characterized for their drug content, entrapment efficiency, in vitro drug release, particle size analysis, scanning electron microscopy, FTIR, DSC and stability studies. Entrapment efficiency was found to be 42% to 92%, in vitro drug release studies showed cumulative drug release of 59% containing stearic acid and lowest of 28% containing compritol after 12 studies. From all these studies SLNs of compritol ATO 888 showed best lipid formulation.

Kaushik. M et al., 2012, formulated and evaluated solid lipid nanoparticles of aceclofenac. Aceclofenac – a poorly soluble drug wass encapsulated using glyceryl behenate as lipid carrier and poloxamer 188 as surfactant by solvent injection method. The mean particle size measured by laser diffraction was 226.9 nm and surface morphology was determined by scanning electron microscopy. The entrapment efficiency was found to be 90% and in vitro drug release was found to be 90.22%.

Shagufta Khan et al., 2012, developed dithranol loaded solid lipid nanoparticles. Dithranol – a poorly soluble drug was encapsulated in SLNs by adaptation of lipid dispersions method. Appropriate analytical methods were needed for characterization of SLNs such as particle size, percentage entrapment, percentage drug loading and percentage yield. Morphology of SLNs were characterized with scanning and transmission electron microscopy. In vitro drug release studies were carried out using HIMEDIA dialysis bag. In conclusion, SLNs presented were wll suited for several applications including drug delivery.

Lakshmi Sirisha Kotikalapudi et al., 2012, formulated and evaluated domperidone solid lipid nanoparticles. SLNs were prepared by hot homogenization followed by ultrasonication technique. The prepared SLNs were characterized for particle size, polydispersity index, zeta potential, entrapment efficiency and in vitro drug release. The mean particle size, poly dispersity index, zeta potential and entrapment efficiency of optimized formulation were found to be 56 nm, 0.154, 34 mV, 98.5%. P-XRD and DSC studies showed that drug was in amorphous state. In vitro drug release studies showed controlled drug release for a period of 48 hours. Thus, fairly spherical shaped, stable and controlled release domperidone-SLNs could be prepared by hot homogenization followed by ultrasonication method.

Rassoul Dinarvand et al., 2012, improved antimicrobial activity if rifampin using soid lipid nanoparticles. Rifampin loaded SLNs were prepared by modified micro emulsion method. The prepared SLNs were characterized for their particle size, zeta potential, encapsulation efficiency, morphology and antibacterial activity against mycobacterium fortuitum. The resulting SLNs were spherical with diameter about 100 nm, with low negative zeta potential and encapsulation efficiency of 82%, with sustained release for 72 hours. The minimum inhibitory concentration of rifampin -SLNs were eight times less than free rifampin. Thus it was concluded that SLNs show a promising vehicle for enhanced antimicrobial effect.

Subhra Prakash Bhattacharya et al., 2012, developed flurbiprofen loaded solid lipid nanoparticles. Flurbiprofen – a poorly water soluble drug was encapsulated in SLNs by modified solvent injection method, using different ratios of stearic acid

and tripalmitin as lipid matrix and pluronic F-68 as emulsifier. The main aim of the project was to optimize the prepared SLNs by response surface methodology. A central composite design for 2 factors at 3 levels each was employed to systemically optimize particle size, drug entrapment efficiency and drug release in 1 hour. The effect of 2 factors on various response variables helped in finding optimum formulation with excellent distribution profile and stability.

Abdul Hasan Sathali A et al., 2012, has done a review on Solid Lipid Nanoparticles. In this review, a broad treatment of SLNs discussing their aims, production procedures advantages, limitations and their possible remedies. Due to their unique size dependent properties, lipid nanoparticles offer possibility to develop new therapeutics. Solid Lipid Nanoparticles, the forefront of rapidly developing field of nanotechnology has several potential applications in drug delivery and research. Appropriate analytical techniques for characterization of SLN like photon correlation spectroscopy, scanning electron microscopy, differential scanning colorimetry were highlighted.

Shailesh S. Chalikwar *et al*., 2012, formulated nimodipine loaded SLN a highly lipophilic anti-hypertensive drug. SLN prepared with palmitic acid, poloxamer 188 and soya lecithin as lipid, surfactant and co-surfactant by high pressure homogenisation. The pharmacokinetic study of optimized SLNs conducted in male albino wistar rat shows 2.08-fold increased in relative bioavailability than that of NMD solution, when administrated orally. SLN were a promising drug delivery for transporting the lipophilic drugs to the intestinal lymphatic region resulted in increased oral bioavailability of drug and reduction in dosing frequency.

Ramteke K.H et al., 2012, has done a review on solid lipid nanoparticles (SLNs). SLNs are the colloidal drug carrier system, suitable for intravenous administration, consisting of spherical solid lipid particles in nanometer size ranges. Different production methods production methods which are suitable for large scale production and the applications are also discussed here. Characterization using photon correlation spectroscopy, scanning electron microscopy, differential scanning calorimetry is also described. Thus the importance of SLNs, if appropriately investigated, may solve many complex diseases.

Shaguft Khan et al., 2012, has done a review on solid lipid nanoparticles. SLNs, due to their unique size dependent properties, offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers, offer a new prototype in drug delivery that could be used for secondary and teritiary levels of drug targeting. Different production methods which are suitable for large scale production and applications of solid lipid nanoparticles are described. Appropriate analytical techniques for characterization of SLN like photon correlation spectroscopy, Scanning electron microscopy and differential scanning calorimetry are discussed. Hence SLNs hold great promise for reaching the goal of controlled and site specific drug delivery.

E. B. Souto et al ., 2011, developed lopinavir loaded solid lipid nanoparticles for intestinal targeting. The poor orally available lopinavir was encapsulated in glyceryl behenate based SLNs by hot homogenization followed by ultrasonication method. SLNs were characterized using differential scanning colorimetry, wide angle x-ray scattering and atomic force microscopy for their solid characteristics and homogenous distribution. From intestinal lymphatic transport study, SLNs

increased cumulative percentage dose of lopinavir, which was 4.91 fold higher than drug suspended in methyl cellulose (0.5%) as suspending agent. The AUC of lopinavir-SLN was 2.91 fold higher than lopinavir in methyl cellulose. Accelerated stability studies showed that there was no significant change in mean particle size and Polydispersity index after storage at 25 ± 2ºC / 60 ± 5% RH. Shelf life of optimized formulation was found to be 21.46 months.

Silva A.C. *et al*., 2011, prepared risperidone- loaded solid lipid nanoparticle for oral administration by hot high pressure homogenisation and ultrasound. Prepared SLN showed the particle size in nanometer range, predicted good long term stability. Commercial oral formulations (suspension and tablets) of risperidone maximum concentration of 4mg since required a frequent dose administration. Concluded that two lipids compritol ATO 888 and Imwitor 900K suitable for RISP-loaded SLN. For a drug ≥4% present as insoluble drug carrier was observed. Imwitor 900K was selected for production of 3%(w/w) RISP-loaded SLN and the lipid tested for oral delivery.

Wen Zhong Zhou et al., 2011, prepared and evaluated ofloxaxin loaded palmitic acid solid lipid nanoparticles. SLNs were prepared by hot homogenization and ultra sound method. SLNs were characterized for their particle size, encapsulation efficiency, Polydispersity index, loading capacity and zeta potential, which were found to be 156.33 ± 7.51 nm, 4.40 ± 0.16%, 0.26 ± 0.04, 4.40 ± 0.16%, -22.7 ± 1.40 mV respectively. Pharmacokinetic results demonstrated that SLNs increase bioavailability by 2.27 fold and extended mean residence time of drug from 10.50 hours to 43.44 hours. The overall results indicate SLNs to be promising drug delivery to enhance pharmacological action of ofloxaxin.

Min – Shing Chen *et al.*, 2011, suggested the delivery system of solid lipid nanoparticles could enhanced its oral bioavailability. Monostearin and Soya lecithin were used as lipid and emulsifiers. The bioavailability of puerarin formulated as phosphor lipid complex was 1.46- fold higher than that with puerarin suspended in water. Puerarin was incorporated into SLN, the relative bioavailability of puerarin was 310% and indicated that incorporated into SLNs enhanced the absorption of puerarin after oral administration. The decreased excretion in feces and increased excretion of puerarin in urine suggested the improved absorption after entrapped into nanoparticles.

Maria Antonietta Casadei et al., 2011, designed the system SLN dextran hydrogel containing ketconazole for topical delivery. Ketaconazole – a broad spectrum anti fungal agent suffers from poor water solubility and chemical degradation, which was overcome by incorporating the drug into SLNs for topical delivery. All SLN formulations had good entrapment properties and were able to protect drug from UV degradation. Antifungal efficacy was tested against Candida albicans, whereas skin tolerability was tested on rabbits.

Marreto R.N. *et al.*, 2011, developed SLN and NLC with high drug load of topotecan. SLNs were prepared by microemulsion technique using Stearic acid and oleic acid as solid and liquid lipids, soya lecithin sodium, taurodeoxycholate as emulsifiers. Homogenous, small sized, negatively charged lipid nanoparticle with high entrapment efficiency and drug load was obtained. SLNs showed slower degradation in vivo provided better control of drug release and protected encapsulated drug. SLN and NLC showed no difference with respect to all

parameters of mean particle size, polydispersity index, zeta potential, entrapment efficiency and drug loading.

Young Jik Kwon et al., 2011, has done review on 'Nanoantibiotics'. The main drawbacks for conventional antimicrobial agents were the development of multiple drug resistance and adverse side effects. Drug resistance enforces high dose administration of antibiotics, often generating intolerable toxicity, development of new antibiotics and requests for significant economic, labour and time investments. Several classes of pathogenic microorganisms developed resistance against several antibiotics, which could be overcome by antimicrobial nanoparticles and nano sized carriers. Thus, this review had summarized emerging efforts in combacting against infectious disease, particularly using antimicrobial nanoparticles and antibiotics delivery systems as new tools to tackle the current challenges in treating infectious disease.

Vandana B Patravale et al., 2011, has done a review on overcoming poor oral bioavailability using nanoparticles formulations. Oral delivery of drugs with poor aqueous solubility and poor enzymatic and/or metabolic stability was very challenging. However, the advent of nanotechnology has revolutionized the field of oral drug delivery. In this review, an overview of various nano architectures such as nanosuspensions, lipid and polymeric nanoparticles, inorganic nano structures had been discussed and their advantages and challenges associated with their delivery were also discussed.

Lireni C Humtsoe et al., 2011, has done a review on Brain delivery by solid lipid nanoparticles for CNS drugs. Brain has been the most delicate organ in the body and drugs accessing to brain has been severely limited by some factors such as

blood brain barrier, P-gp efflux mechanisms. This review highlights about the advantages of SLN over the other colloidal carriers as well as the advantages of nanoparticles for brain targeting, some proposed mechanisms to cross blood brain barrier, incorporation models and release of drugs form SLN.

Jithan Aukunuru et al., 2010 designed systemic delivery of diclofenac sodium after topical application of gels incorporated with drug loaded solid lipid nanoparticles. Diclofenac sodium-SLNs were prepared by hot homogenization followed by sonication technique and the prepared SLNs were incorporated in freshly prepared carbopol gel. The gels enriched with SLNs sustained the drug release for 24 hours both in vitro and in vivo. Results suggested enhancement in systemic delivery of diclofenac sodium with gels incorporating SLNs.

Arvind k Bansal et al., 2010, has done a review on self emulsifying drug delivery system to improve bioavailability. Through this delivery system, followed by their oral administration, they rapidly dispense in gastro intestinal fluids, gidding micro/ nano emulsified drug can easily be absorbed through lymphatic pathways by passing hepatic first pass metabolisms owing to their smaller particle size. The different types of self emulsifying formulations, their formulation, characterization, biopharmaceutical aspects, advantages and recent development are discussed. Finally self emulsifying drug delivery systems show a promise for better drug delivery of poorly bioavailable drugs.

A Malzert Freon et al., 2010, has done a research on influence of a solubility enhancer on formulation of lipidic nanoparticles with improved drug loading rates. Here a poorly water and lipid soluble drug is encapsulated in lipidic nano formulation without using organic solvents, by adding a solubility enhancer such

as Labrasol, through low energy phase inversion temperature method. Labrasol does not prevent phase inversion and it takes part in the micro emulsion structuring, probably bicontionous type. From results of partial least squaring pseudo ternary liagzoms, the nanoparticles present a core shell structure, were labrsol is all encapsulated and contributes to formation of oily liquid core of nanoparticle. So highly drug loaded lipidic nanocarriers were developed without using the silightest organic solvent trace and making it easy. Possible dose adjustment could also be achieved.

Hoo – Kyun Choi et al., 2009, formulated solid lipid nanoparticles loaded with doxorubicin. SLNs were prepared by solvent emulsification – diffusion method. The mean particle size, entrapment efficiency, drug loading were found to be 199 nm, 67.5 ± 2.4% and 2.8 ± 0.1% respectively. In conclution, SLNs with small particle size, high entrapment efficiency and relative high drug loading for doxorubicin could be obtained by this method.

Anil K. Sharma et al., 2009, developed solid lipid nanoparticles of lamivudine for brain targeting. Lamivudine, the most widely used drug for treatment of AIDS, was incorporated into SLNs by emulsion solvent diffusion technique. The optimum rotation speed for better drug entrapment and percentage yield was in the range of 1000 to 1250 rpm. The in vitro drug release from optimized formulation was found to be 40% - 50% in PBS and SGF for 10 hours. After 24 hours more than 65% of drug was released from all formulations in both mediums, meeting the requirement for drug delivery for prolonged period of time.

Andrew Laxley et al., 2009, has done a review on solid dipid nanoparticles. Here poorly water soluble drugs such as class II and III BCS drugs, which have poor

bioavailability are formulated in to a drug delivery vehicle that specifically targets tissue or cells to maximize therapeutic index. A common formulation approach with such compounds is focus on creating and stabilizing very small particles of the drug in an attempt to increase the surface area available for dissolution in vivo, and hence the rate of dissolution and consequently plasma or tissue levels of drug.

Seitaro Kamiya *et al*., 2008, designed the nifedipine lipid nanoparticle and investigated the prepared formulation without using any organic solvents. A mean particle size of approximately 50nm could be prepared without organic solvents by a combination of roll milling and high pressure homogenisation. The X-ray diffraction peak of sample presented identical position and showed no peak shift was induced by interaction with lipid. The particle size of suspension was maintained for long time by adding gelatin powder to the NI-lipid suspension. The mean particle size of 55nm was retained as nanoparticle.

Linden H et al., 2007, has given a conference report about poor solubility issues. The major challenges in oral delivery of new drugs such as absorbtion, sufficient and reproducible bioavailability are discussed. During discovery of new technologies, tremendous knowledge has been accumulated on biological factors like transporters metabolizing enzymes and efflux systems as well. Research tools and technologies have been and are will be developed to assess the impact of these factors on drug absorption for new chemical entities. The impact of compounds with poor solubility on analytical evaluation, prediction of oral absorption, substance selection, material and formulation stratagies and development are discussed here.

Antonio J Almeida et al., 2007, has done a review on solid lipid nanoparticles as a delivery system for peptides and proteins. Solid lipid particulate systems have been sought as alternative carriers for therapeutic peptides, proteins and antigens. The research work developed in area confirms that under optimized conditions they can be produced to incorporate hydrophobic or hydrophilic proteins. Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed on to SLNs and further administerd by parentral routs or by alternative routs such as nasal and pulmonary. Formulation of SLN confers improved protein stability avoids proteolytic degradation as well as sustained release of incorporated molecules. So far SLNs prove to be a promising tool for administering protein molecules.

Xiangliang yang *et al.*, 2005, investigated the anti-inflammantory activity and hepatotoxicity of triptolide loaded SLN. The anti-inflammantory activities of triptolide – SLN were stronger than the free triptolide. Oral observation occurred and nanoparticles were likely to cross the gastro-intestinal barrier to deliver their drug content in the blood, lymph or target organ. Lymphatic up take by the M cells of the teyer's batches appears to be a major sight of translocation of solid particulates. It depended on particle characteristics such as size or surface properties. It was concluded that solid lipid nanoparticle delivery system enhanced triptolide absorption, increased its bioavailability and obtained sustained, controlled effects.

Sanjeeb K Sahoo et al., 2003, has done a review on nanotech approaches to drug delivery and imaging. Nanotechnology is expected to create innovations and play a critical role in various biomedical applications not only in drug delivery by also

in molecular imaging, biomarkers and bio sensors. Target specific drug therapy and methods for early diagnosis of pathologies are the priority research areas where nanotechnology would play a vital role. In this review, various nanotechnology based drug delivery and imaging approaches and their economic impact on pharmaceutical and biomedical industries are discussed.

Sven Gohla et al., 2000, has done a review on solid lipid nanoparticles for controlled drug delivery. Solid lipid nanoparticles were first introduced in 1991 as an alternative carrier system to traditional colloidal carriers. This review have presented the state of art regarding production techniques for SLN, drug incorporation, loading capacity and drug release, especially focusing on drug release mechanisms. Relevant issues for the introduction of SLN to the pharmaceutical market, such as status of excipients, toxicity, tolerability aspects and sterilization and long term stability inducing industrial large scale production were also discussed. The potential of SLN to be exploited for the different administration routes were highlighted.

Sven H. Gohla et al., 2000, formulated vitamin A loaded solid lipid nanoparticles. Vitamin A loaded SLNs were incorporated in hydrogel and o/w cream and tested with respect to their influence on drug penetration into porcine skin. Because of polymorphic transition of lipid carriers with subsequent drug expulsion following the application to skin, the drug localizing action appears to be limited for 6 to 24 hours. Best results were obtained with retinol-SLN incorporated in o/w cream retarding drug expulsion.

Gujjar Chaitanya Yogananda et al., 2013, developed muoadhesive formulation of Quetiapine fumarate using non-gelling polymer. Mucoadhesive tablets were

prepared using non-gelling polymers such as lambda carrageenan and effective thickner propylene glycol alginate, by dry granulation techniques. Dissolution profiles were compared with marketed preparations, in which formulation with propylene glycol alginate had comparable dissolution profile to that of marketed formulation.

Rao B Umamaheswara et al., 2012, designed and characterized sustained release mucoadhesive tablet o glipizide. Here, glipizide a short biological half life drug was formulated in to mucoadhesive tablets using different combination of polymers such as HPMC K4 M, HPMC K100 M, Carbopol 71G by wet granulation method. Preformulation studies and post compression evaluation were carried out for the formulations. Hence mucoadhesive tablets of glipizide show a promising improvement for diabetic administration.

Vitaliy V Khutoryanskiy et al., 2012, has formulated chitosan based mucoadhesive tablets for oral delivery of ibuprofen. Chitosan and its half acetylated derivative have been compared as excipients. Powder formulation for tableting was prepared by either co-spray drying or by physical co-grinding. Polymer-drug interactions and degree of drug crystallinity were assessed by infrared spectroscopy and differential scanning calorimetry. Mucoadhesive property of prepared tablets was analyzed by their detachment from pig gastric mucosa over a range of pH. Increased polymer-drug interactions were seen for spray dried particles. Higher drug loading was observed for chitosan based microparticles than half acetylated samples. Swelling and drug release was observed with half acetylated chitosan tablets. These results indicate a potential

sustained drug delivery for oth chitosan and its half acetylated derivative as mucoadhesive tablet excipients.

Goswami Dhruba Sankar et al., 2011, has formulated and evaluated mucoadhesive tablets of famotidine by wet granulation method. Since the drug has a short halft life, it was formulated in to mucoadhesive tablets using natural and synthetic polymers. Evaluations were done for the fabricated tablets. In vitro drug release studies showed formulation containing HPMC K4 M and tragacanth having better muco adhesive property. Thus the present investigation showed the combination of HPMC K4 M and traganch as hydrophilic polymers for preparation of famotidine mucoadhesive tablets.

Inderbir Singh et al., 2011, has formulated and evaluated muucoadhesive matrix tablets of Taro gum by direct compression method. The prepared tablets were evaluated for bioadhesive strength and invitro dissolution parameter. The mucoadhesive detachment force was found to increase with taro gum concentration increase. Invitro drug release follows first order kinetics and shows best linearity with higuchi mode. PVP K 30 has indirect effect on all the factors byincreasing tenstile strength and making the tablet firm and intact.

Remeth Dias et al., 2010, studied in vitro absorbtion of mucoadhesive tablets of acyclovir. In here the absorbtion of acyclovir was improved using permeation enchancer such as sodium lauryl sulphate. From the perfusion studies of intestinal model, the permeability of mucoadhesive tablets were found to increase with increase in sodium lauryl sulphate (4%) comparing to marketed formulations. Thus mucoadhesive tablets with permeation enhancers shows promising developments in increasing bioavailability of drugs.

Akant Priyo Singla et al., 2010, characterized mucoadhesive tablets of ciprofloxacin, by wet granulation technique. Combination of hydrophilic and hydrophobic polymers was used and further evaluation studies were performed for the mucoadhesive tablets. In vitro release studies showed formulation containing HPMC and tragacanth had better mucoadhesive property. Since fluroquinolones appear to have effect in patients not responding to trimethoprim and sulfamethaxazole. Mucoadhesive tablets of ciprofloxacin prove to be potential in many disease conditions.

Mahesh D Chavanpatil et al., 2006, has formulated novel sustained release, swellable and bioadhesive gastro rententive drug delivery system for ofloxacin using polymers like psyllium husk, HPMC K100 M by wet granulation method. Evaluation studies were conducted and invitro release studies followed Higuchi kinetics and drug release mechanism was found to be of anomalous or non-fickian type. The bio adhesive property of developed formulation was found to be significant in combination as compared to HPMC K100 M and psyllium husk alone. The evaluation studies were compared with marketed formulation.

CHAPTER -3

AIM OF THE WORK

CHAPTER - 3

AIM OF WORK

Drug resistance enforces high dose administration of antibiotics, often generating intolerable toxicity, development of new antibiotics, and requests for significant economic, labor, and time investments. The main drawbacks for conventional antimicrobial agents are the development of multiple drug resistance and adverse side effects. Several classes of antimicrobial nanoparticles (NPs) and nanosized carriers for antibiotics delivery have proven their effectiveness for treating infectious diseases, including antibiotics resistant ones, in vitro as well as in animal models.

Cefuroxime axetil is a second generation cephalosporin antibiotic generally used for lower and upper respiratory tract infections, genitor-urinary tract infections, skin and soft tissue infections. It is a prodrug that gets converted into cefuroxime after oral absorption. The main site of absorption of cefuroxime axetil is in the stomach. The marketed preparation has very poor oral bioavailability (25% - 30%) and is variable with presence or absence of food. The main side effects of cefuroxime axetil are gastrointestinal disturbances including nausea, vomiting, diarrhoea.

The main aim of this study is to formulate and evaluate mucoadhesive tablets of cefuroxime axetil loaded Solid lipid nanoparticles, for reducing the drug resistance, improving the bioavailability, dose reduction, controlled release of drug and also to target the drug at its specific site of absorption (Stomach). Solid lipid nanoparticles are formulated using various lipids [compritol ATO 888, glyceryl mono stearate (GMS), glyceryl mono oleate(GMO), stearic acid, palmitic acid] at different concentrations.

Pluronic F68 is used as surfactant/stabilizer and soya lecithin is used as co surfactant to increase the solubility of drug in lipid. The best formulation is selected and lyophilized to dry powder form. Mucoadhesive tablets are prepared from the lyophilized SLN using suitable mucoadhesive polymers (carbopol, HPMC K15) by direct compression method. Further characterization for the finished formulation is carried out.

CHAPTER - 4

PLAN OF WORK

CHAPTER - 4

PLAN OF WORK

1. **STANDARDIZATION OF CEFUROXIME AXETIL:**
   - **a.** Preparation of 0.07N HCl buffer
   - **b.** Determination of λ_{max} & preparation of calibration curve

2. **COMPATIBILITY STUDIES OF DRUG AND EXCIPIENTS:**
   - **a.** Fourier Transform Infrared spectroscopic studies (FT-IR)

3. **FORMULATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:**

   Solid lipid nanoparticles of cefuroxime axetil are prepared by using various lipids at different concentrations by hot homogenization followed with ultrasonication technique.

4. **CHARACTERIZATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:**
   - **a.** Determination of drug content
   - **b.** Determination of drug entrapment efficiency
   - **c.** Particle size & zeta potential
   - **d.** *In vitro* release studies
   - **e.** Kinetics of drug release

5. SELECTION AND EVALUATION OF BEST FORMULATION:

a. Solubility studies

b. Microbiological assay

c. Lyophilization of best formulation

d. X- ray diffraction studies

e. Morphology of SLN by scanning electron microscopy (SEM) technique

f. Statistical analysis

6. COMPRESSION OF LYOPHILIZED SLN LOADED WITH CEFUROXIME AXETIL INTO MUCOADHESIVE TABLETS:

Mucoadhesive tablets are compressed, using suitable mucoadhesive polymers, by direct compression method.

7. CHARACTERIZATION OF MUCOADHESIVE TABLETS OF LYOPHILIZED SLN LOADED WITH CEFUROXIME AXETIL:

a. Precompression evaluation of powder blend:

i. Estimation of drug content of lyophilized SLN

ii. Angle of repose

iii. Bulk density

iv. Tapped density

v. Carr's index

vi. Hausner's ratio

b. Post compression evaluation studies:

i. Drug content of fabricated mucoadhesive tablets

ii. Thickness & diameter

iii. Hardness

iv. Weight variation

v. Friability test

vi. Fourier Transform Infrared spectroscopic studies (FT-IR)

vii. Determination of swelling index

viii. *Invitro* release studies

ix. *Invitro* release kinetics

x. *In vitro* mucoadhesive strength determination

xi. Determination of *in vitro* residence time

xii. *Ex vivo* stomach permeability studies

xiii. *In vivo* gastroretentive time in rabbit stomach

CHAPTER - 5

MATERIALS AND EQUIPMENTS

CHAPTER - 5

MATERIALS

S.No.	INGREDIENTS	SUPPLIERS
1	Cefuroxime Axetil	Gift sample obtained from Steril - gene Life Sciences Pvt Ltd.
2	Compritol ATO 888	Orchid Pharma, Chennai.
3	Glyceryl monosterate	Central Drug House (P) Ltd.
4	Glyceryl monooleate	Otto Chemicals, Mumbai.
5	Palmitic acid	Central Drug House (P) Ltd., New Delhi.
6	Stearic acid	Central Drug House (P) Ltd., New Delhi.
7	Pluronic F68	Gift sample obtained from Madras Pharmaceuticals.
8	Soyalecithin	Otto Kemi.
9	Methanol	Universal Scientific Suppliers, Madurai.
10	Chloroform	Spectrum Reagents and Chemicals, Cochin.
11	Carbopol 934	Gift sample obtained from Madras Pharmaceuticals.
12	HPMC K15M	Gift sample obtained from Madras Pharmaceuticals.
13	Magnesium Stearate	Nice Chemicals (P) Ltd., Kerala.
14	Talc	Nice Chemicals (P) Ltd., Kerala.
15	Conc. Hydrochloric acid	Spectrum Reagents and Chemicals, Cochin.

EQUIPMENTS

S.No.	EQUIPMENTS	DISTRIBUTORS
1.	Rotary Flash Evaporator	Super fit rotary flash evaporator
2.	Ultra Sonicator	Vibronic's Ultrasonic processor
3.	Centrifugator	Eppendorf Centrifuge 5417 R
4.	Mechanical stirrer	Scientific industries
5.	Electronic Balance	A&D Company, Japan
6.	Magnetic Stirrer	MC Dalal & co
7.	Single Punch Tablet Compression Machine	Cadmach Machinery Co. Pvt., Ahmadabad.
8.	Disintegration Apparatus	Rolex, India.
9.	Digital Tablet Dissolution Test Apparatus	Disso 2000, Lab India, Mumbai.
10.	Friability Test Apparatus	Indian Equipment Corporation, Mumbai.
11.	Tablets Hardness Tester(Monsanto)	Praveen Enterprises, Bangalore.
12.	Vernier Caliper	Linker, Mumbai.
13.	UV Visible Spectrophotometer	UV Pharma Spec 1700, Shimadzu
14.	Stability chamber	Inlab equipments.
15.	Rotary shaker	Secor, India.
16.	Scanning electron microscope	Hitachi X650, Tokyo, Japan
17.	Particle size analyser	Malvern Instrument, U.K.
18.	FT-IR	Shimadzu, Japan.
19.	Differential Scanning Calorimetry	DSC Q 200, Mumbai.

CHAPTER - 6

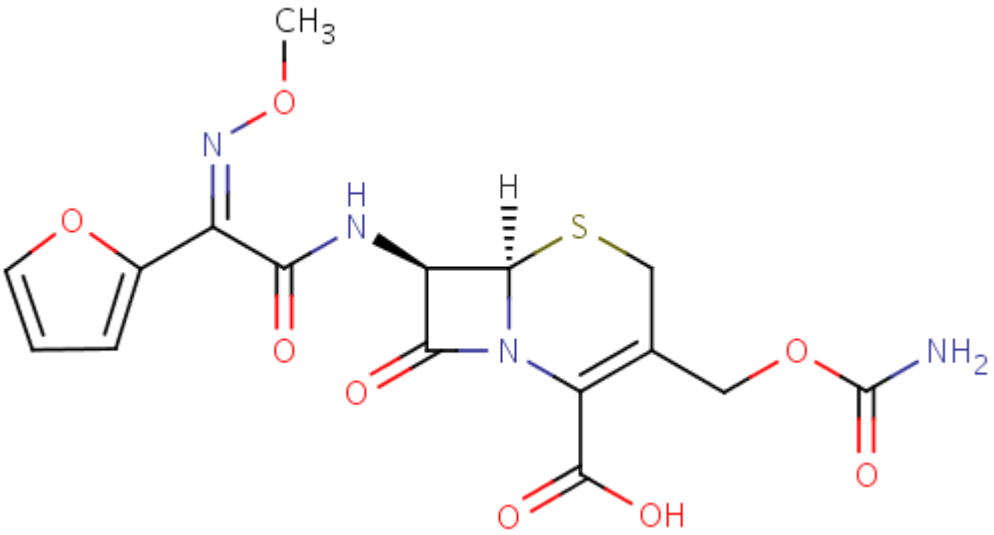
DRUG PROFILE

CHAPTER - 6
DRUG PROFILE

Cefuroxime axetil

Cefuroxime axetil is a β lactam antibiotic belonging to 2nd generation cephalosporin antibiotic and is active against β lactamase producing strains.

STRUCTURAL FORMULA:



EMPIRICAL FORMULA:

$C_{16}H_{16}N_4O_8S$

CHEMICAL NAME:

(6R,7R)-3-[(carbamoyloxy)methyl]-7-[(2Z)-2-(furan-2-yl)-2 (methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

DESCRIPTION:

Nature : White powder.

Solubility : Freely soluble in, methanol and acetone, and slightly soluble in water and dehydrated alcohol.

Melting point : 135.5°C

Molecular weight : 424.4

MECHANISM OF ACTION:

Cefuroxime axetil is a 2nd generation cephalosporin antibiotic which acts by binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall; it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefuroxime interferes with an autolysin inhibitor

PHARMACOKINETICS:

Absorption:

Following oral administration of cefuroxime axetil, the drug is absorbed from the GI tract as the 1-(acetyloxy)ethyl ester and rapidly hydrolyzed to cefuroxime.

Oral bioavailability is 37-52 %

Distribution:

50 % bound to plasma proteins

Metabolism:

No metabolism after hydrolysis from cefuroxime axetil to cefuroxime.

Elimination:

Mean plasma half-life is 1.2-1.6 hours

Excreted unchanged principally in urine.

Therapeutic indicatons:

For many bacterial infections such as bronchitis, sinusitis, tonsillitis, ear infections, skin infections, gonorrhea, and urinary tract infections.

Dose

500-1000 mg per day

Side effects:

Gastro intestinal disturbances such as, vomiting, diarrhoea, nausea.

Drug interactions:

Probenacid reduces the renal clearance of cefuroxime.

Diuretics increases possible risk of nephrotoxicity.

Precautions:

Should not be given to patients with ahistory of GI disease especially colitis.

Contra-indications:

Known hypersensitivity to cefuoxime or other cephalosporins.

Brand names:

- Ceftin
- Cefurax
- Elobact
- Kefurox
- Oraxim
- Sharox
- Supacef
- Zinacef
- Zinnat

CHAPTER - 7

EXCIPIENTS PROFILE

CHAPTER - 7

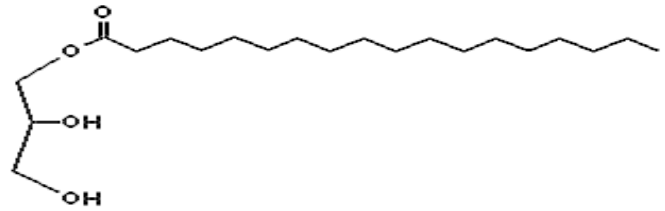
EXCIPIENTS PROFILE

GLYCERYL MONOSTEARATE

Synonym:

Glyceryl stearate, Monostearin

Structure:



Chemical name:

3-Stearoyloxy-1,2-propanediol; Glyceryl stearate; Alpha-Monostearin; Monostearin; Octadecanoic acid, 2,3-dihydroxypropyl ester; Glycerin 1-monostearate; Glycerin 1-stearate; Glycerol alpha-monostearate; Glyceryl 1-monostearate; Stearic acid alpha-monoglyceride; Stearic acid 1-monoglyceride; 1-Glyceryl stearate; 1-Monostearin; 1-Monostearoylglycerol; 1,2,3-Propanetriol 1-octadecanoyl ester.

Empirical formula:

$CH_3 (CH_2)_{16}COOCH_2CHOHCH_2OH$

Molecular weight:

358.56

Functional category:

Emulsifying agent

Description:

White or cream colored waxy solid.

Properties:

Physical state	:	white powder
Melting point	:	63 - 68 ^{o}C
Boiling point	:	> 100 ^{o}C
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0

Stability and storage conditions:

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

Safety:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

Handling precautions:

Keep away from heat and ignition.

Regulatory status:

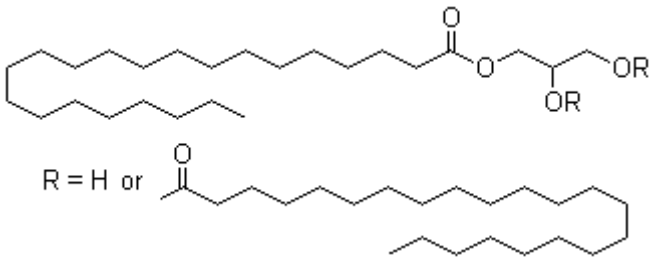
Induced in the FDA inactive ingredients and recognized by GRAS status. **(**Handbook of Pharmaceuticals Excipients, 2009, 831-824)

GLYCERYL BEHENATE

Synonyms:

Compritol 888 ATO; 2,3-dihydroxypropyl docosanoate; docosanoic acid, glyceryl monobehenate, 1,2,3-Propanetriol docosanoate.

Structure:



Empirical formula:

$C_3H_8O_3 \cdot x(C_{22}H_{44}O_2)$

Molecular weight:

414.66

Functional category:

Coating agent

Tablet binder

Tablet and capsule lubricant

Description:

Fine white powder or hard waxy mass with a faint odor.

Properties:

Physical state : Fine white powder

Melting point : 65–77ºC

Boiling point : 306 ºC

Solubility : Soluble, when heated, in chloroform and dichloromethane.

Practically insoluble in ethanol(95%), hexane, mineral oil and water.

HLB value : 12

Stability and storage conditions:

It should be stored in a tight container, at a temperature less than 358C.

Safety:

It is generally regarded as a relatively nonirritant and nontoxic material.

Handling precautions:

It emits acrid smoke and irritating fumes when heated to decomposition.

Regulatory status:

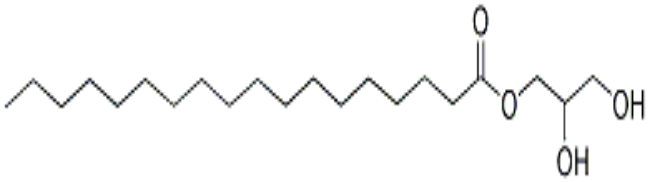
Included in the FDA Inactive Ingredients Guide(capsules and tablets). (www.sciencelab.com, www.parchem.com, Handbook of Pharmaceuticals Excipients, 2009, 819-824.)

GLYCERYL MONOOLEATE

Synonym:

Glyceryl monooleate, monoolein

Structure:



Chemical name:

3-Stearoyloxy-1,2-propanediol; Glyceryl stearate; Alpha-Monostearin; Monostearin; Octadecanoic acid, 2,3-dihydroxypropyl ester; Glycerin 1-monostearate; Glycerin 1-stearate; Glycerol alpha-monostearate; Glyceryl 1-monostearate; Stearic acid alpha-monoglyceride; Stearic acid 1-monoglyceride; 1-Glyceryl stearate; 1-Monostearin; 1-Monostearoylglycerol; 1,2,3-Propanetriol 1-octadecanoyl ester

Empirical formula:

$CH_3 (CH_2)_{16}COOCH_2CHOHCH_2OH$

Molecular weight:

358.56

Functional category:

Emulsifying agent

Description:

White or cream colored waxy solid.

Properties:

Physical state	:	soft solid waxy
Melting point	:	40 ^{o}C
Boiling point	:	> 100 ^{o}C
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0

Stability and storage conditions:

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

Safety:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

Handling precautions:

Keep away from heat and sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. **(**www.sciencelab.com, www.parchem.com,)

STEARIC ACID

Synonyms:

Cetylacetic acid; stereophonic acid; Tegostearic.

Structure:

Chemical name:

Octadecanoic acid

Empirical formula:

$C_{18}H_{36}O_2$

Molecular weight:

284.47

Functional category:

Emulsifying agent

Solubilizing agent

Tablet and capsule lubricant

Description:

It is a hard, white or faintly yellow-colored, crystalline solid or a white or yellowish white powder.

Properties:

Physical state : Crystalline solid/white or yellowish powder.

Melting point : 554°C

Boiling point	:	383°C
Solubility	:	Freely soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane and propylene glycol; practically insoluble in water.
HLB value	:	15

Stability and storage conditions:

It is a stable material; an antioxidant may also be added to it. The bulk material should be stored in a well-closed container in a cool and dry place.

Safety:

It is generally regarded as a nontoxic and nonirritant material. However, consumption of excessive amounts may be harmful.

Handling precautions:

Stearic acid dust may be irritant to skin, eyes, and mucous membranes. Eye protection, gloves, and a dust respirator are recommended. Stearic acid is combustible.

Regulatory status:

Included in the FDA Inactive Ingredients Guide (sublingual tablets; oral capsules,

solutions, suspensions, and tablets; topical and vaginal preparations).

PALMITIC ACID

Synonyms:

Acidum palmiticum; cetylic acid; n-hexadecoic acid; hexadecylic acid;

Structure:

Chemical name:

Hexadecanoic acid

Empirical formula:

C16H32O2

Molecular weight:

256.42

Functional category:

Emulsifying agent

Skin penetrant

Tablet and capsule lubricant

Description:

Palmitic acid occurs as white crystalline scales with a slight characteristic odor and taste.

Properties:

Physical state	:	White crystalline scales
Melting point	:	64 °C
Boiling point	:	352 °C
Solubility	:	Soluble in ethanol (95%); practically insoluble in water.
HLB value	:	15

Stability and storage conditions:

The bulk material should be stored in a well-closed container in a cool and dry place.

Safety:

Palmitic acid is used in oral and topical pharmaceutical formulations and is generally regarded as nontoxic and nonirritant at the levels employed as an excipient. However, palmitic acid is reported to be an eye and skin irritant at high levels and is poisonous by intravenous administration.

Handling precautions:

Observe normal precautions appropriate to the circumstances and quantity of material handled. When palmitic acid is heated to decomposition, carbon dioxide and carbon monoxide are formed.

Regulatory status:

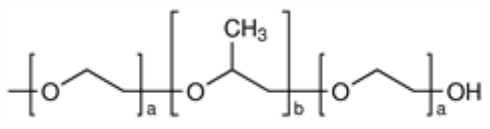
GRAS listed. Included in the FDA Inactive Ingredients Database (oral tablets). Included in nonparenteral medicines licensed in the UK.

POLOXAMER 188

Synonym:

Lutrol F 68, Pluronic F 68

Structure:



Chemical name:

Polyethylene-Polypropylene Glycol

Empirical formula:

$HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$

Molecular weight:

8400.00

Functional category:

- Emulsifying agent
- Sensitize drug resistant cancers to chemotherapy

Description:

White to off white granules

Properties:

Physical state	:	white powder
Solubility in water	:	soluble in water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	29.0

B**iological effects of poloxamer:**

Originally thought to be inert carrier molecules work led by Kabanov has recently shown that some of these polymers have a very real effect on biological systems independently of the drug they are transporting. The poloxamers have been shown to incorporate into cellular membranes affecting the microviscosity of the membranes.

Stability and storage conditions:

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

Safety:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

Handling precautions:

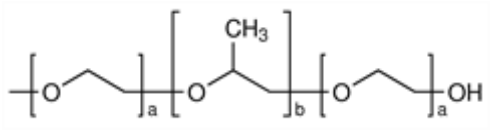
Keep away from heat and sources of ignition.

SOYA LECITHIN

Synonym:

Lecithin, soy lecithin

Structure:



Chemical name:

Polyethylene-Polypropylene Glycol

Empirical formula:

$HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$

Molecular weight:

8400.00

Functional category:

- Emulsifying agent
- Sensitize drug resistant cancers to chemotherapy

Description:

Light brown to brown liquid

Properties:

Physical state	:	Brown liquid
Solubility in water	:	soluble in water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	15.0

Stability and storage conditions:

It is stable if stored at the temperature of 2-8ºC. Product looses its potency/performance above 45ºC. No hazardous polymerization occurs.

Safety:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

Handling precautions:

Keep away from heat and light.

CHAPTER - 8

EXPERIMENTAL PROTOCOL

CHAPTER - 8

EXPERIMENTAL PROTOCOL

1) STANDARDIZATION OF CEFUROXIME AXETIL: (Indian Pharmacopoeia 2010)

a. Preparation of 0.07N HCl buffer:

Measure 6.93 ml of hydrochloric acid and gradually dissolve it in specified amount of distilled water with continuous stirring and make this solution up to 1000 ml using distilled water to prepare 0.07N HCl buffer solution.

b. Determination of λ_{max} & preparation of calibration curve:

The standard stock solution of Cefuroxime axetil is prepared by dissolving 100 mg of drug in 5 ml methanol and diluted with 0.07N HCl buffer solution up to 100 ml. From the above stock solution, drug having different concentrations of 5, 10, 15, 20 and 25μg /ml is prepared using 0.07N HCl buffer solution with appropriate dilution.

The 10 ug/ml solution is scanned in UV spectrophotometer to find the λ max and the absorbance of the samples is measured at λ_{max} (281nm). A graph is plotted by taking concentration in X-axis and absorbance in Y-axis to obtain the standard curve.

The standard curve prepared is used to estimate drug content, entrapment efficiency and percentage drug release.

2) COMPATIBILITY STUDIES FOR DRUG AND EXCIPIENTS:

Compatibility studies are carried out to confirm there are no interactions existing between the drug and excipients. It gives information needed for selection of excipients with the drug for the formulation of

nanosuspension. Infrared spectrophotometry technique is used to check the compatibility studies between lipids (compritol ATO 888, glyceryl monostearate, glyceryl monooleate, stearic acid & palmitic acid) and drug.

a. Fourier Transform Infrared Spectroscopic studies (FT-IR):

IR studies are carried out to find whether there are interactions between pure drug, lipids, surfactants and its physical mixture by KBr pellet technique using FTIR spectrophotometer (shimadzu, RX 1, Japan). The IR spectrum of the physical mixture is then compared with the spectrum of pure drug (cefuroxime axetil) to assess the compatibility of the excipients and drug. The scanning range is 450-4000 cm^{-1} and the resolution is $4cm^{-1}$.

(De Pintu Kumar et al., 2012)

3) FORMULATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:

Cefuroxime axetil loaded solid lipid nanoparticle is prepared by hot homogenization method followed by ultrasonication using different lipids at different concentrations [1%, 2%, 4%, 6%, 8% and 10%].

In hot homogenization method, the solid lipid and soya lecithin are dissolved in a mixture of chloroform and methanol in ratio of 1:1, to which drug is added. Now this mixture is taken in a rotary flash evaporator and all the organic solvents are completely removed. The resulting residue is melted approximately 5 – 10°C above the melting point of the lipid. A 2% aqueous surfactant solution of 40ml is prepared and is heated to the same temperature of the lipid phase. Now the hot aqueous surfactant solution is added to the lipid phase. Homogenization is carried out at 2000 rpm by using mechanical

stirrer for 1 hr. Temperature is maintained 5 – 10°C above the melting point of the lipid to prevent lipid recrystalization.

After homogenization is finished, the obtained coarse emulsion is allowed to cool to room temperature, while stirring at 400 rpm for 30 minutes. The dispersion is then ultrasonicated using a probe sonicator processor for 10 minutes. **(**Priyanka & Abdul Hasan Sathali .A et al., 2012)

4) CHARACTERIZATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:

The formulated cefuroxime axetil loaded solid lipid nanoparticles are characterized for their drug content, entrapment efficiency, particle size, polydispersity index, zeta potential, *in vitro* drug release and kinetics of drug release.

a. Determination of Physicochemical properties:

The formulated SLNs are to characterize for their physicochemical properties such as color, odor and stability after centrifugation over 2000 rpm for 30 minutes.

b. Determination of drug content:

The total drug content of the SLN formulations is determined by spectrophotometric analysis. One milligram equivalent of cefuroxime axetil loaded SLN is dissolved in (1 ml) of methanol and the volume is made up to 100 ml by using 0.07N HCl buffer solution to make 10 µg/ ml concentration. The absorbance is measured at 281 nm (λ max) using UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan). From the absorbance, drug content is calculated.

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

c. Determination of drug entrapment efficiency:

The entrapment efficiency (EE) is the ratio of amount of drug incorporated into the SLNs to the total drug content. The entrapment efficiency of cefuroxime axetil loaded SLNs is directly determined by the centrifugation method. 1ml of SLN is taken in a centrifuge tube and the nanoparticles are separated in a high speed cooling centrifuge (Eppendorf Centrifuge 5417 R, Germany) at 14,000 rpm for 90 min at 4°C. Then the supernatant liquid is made up to desired volume with 0.07N HCl buffer solution to measure the absorbance of free drug at 281nm by using UV Spectrophotometer, (Shimadzu UV-1700 pharma spec, Japan) to estimate the unentrapped drug for the calculation of % EE.

The percentage entrapment efficiency (%EE) is calculated by following formula:

$$\% EE = \frac{\text{Total amount of drug taken - Unentrapped drug}}{\text{Total amount of drug taken}} \times 100$$

(Yitao Wang et al., 2012, Nisha & Abdul Hasan Sathali A *et al*., 2013)

d. Particle size and zeta potential:

Particle size and zeta potential of drug loaded SLN dispersion with best entrapment efficiency is done by photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments, UK) at 25°C. Prior to measurements all samples are diluted using ultra purified water to yield a suitable scattering intensity.

(E. B. Souto et al., 2011, Yamasai Madhusudan Rao et al., 2012)

e. *In vitro* release studies:

In vitro drug release study of cefuroxime axetil from SLN formulations is determined by using dialysis bag diffusion method using 0.07N HCl buffer solution as dissolution medium.

The dialysis bags are soaked in distilled water for 24 hrs before use. SLN equivalent to 1 mg of cefuroxime axetil is placed inside the dialysis bag and sealed at both ends with threads. The dialysis bag is immersed in receptor compartment containing 100 ml of 0.07N HCl buffer solution in 250 ml beaker maintained at 37°C ± 1°C and magnetically stirred at 100 rpm. Samples are withdrawn at predetermined time intervals of 30 min for first 2 hrs and every 60 min for 10 hrs. Sink condition is maintained by replacing with fresh buffer solution after each sample withdrawal. The content of cefuroxime axetil in the samples is determined by using UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan) at 281 nm.

(Kaushik M *et al*., 2012, Priyanka K, Abdul Hasan Sathali A *et al*., 2012, Lakshmi Sirisha Kotikalapud et al., 2012)

f. Kinetics of drug release:

In order to understand the release kinetics of a drug, the results of *in vitro* drug release studies of nanoparticles were fit to various kinetic equations such as zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), and Higuchi's model (cumulative % drug release vs. square root of time). Values of r^2 and k were calculated for the linear curve obtained by regression analysis of the above plots. The exact mechanism of drug release was determined by the Korsemeyer–Peppas model (log drug

release vs. log time). (R. N. Marreto et al., 2011, Kesavan Bhaskar Reddy et al., 2011)

5) SELECTION AND EVALUATION OF BEST FORMULATION:

The best formulation selection is based on the results obtained from particle size, zeta potential, entrapment efficiency, *in vitro* release studies and kinetics of drug release.

a. Solubility studies:

The solubility of the pure drug is compared with the solubility of best formulation. An approximately(10 mg) equivalent of pure drug and cefuroxime axetil loaded solid lipid nanoparticles are introduced in 25 ml stoppered standard conical flask containing 10 ml distilled water. The sealed flask is agitated on a rotary shaker for 24 hr. An aliquot is withdrawn and filtered and the filtrate is suitably diluted and analysed in UV spectrophotometer (Shimadzu UV-1700 pharma spec, Japan).

(Nisha. N & Abdul Hasan Sathali A *et al*., 2013)

b. Microbiological assay:

Microbiological activity of drug loaded SLNs is evaluated by determination of bacteria colony forming units after incubation of 1% suspension of S. aureus bacteria in MRS medium at 30^0 for 24hrs with drug loaded SLNs.

c. Lyophilization of the best formulation:

The nanoparticles are lyophilized using a programmable freeze-dryer (Shin PVTFD10R, Shinil Lab, Korea). Cryoprotectant is added to the SLN dispersion before freezing. Slow freezing is carried out on the shelves in the freeze dryer (shelf temp.−40º C). The samples are lyophilized for 24 h from

−40º C to 25º C at an increasing rate of 5ºC/h. Lyophilized products are reconstituted by sonication.

d. X- ray diffraction studies:

PXRD studies are performed in order to indentify the crystallinity behavior of the SLN. (Yaping Li, PhD et al., 2011, Lakshmi Sirisha Kotikalapudi et al., 2012)

e. Morphology of SLN by scanning electron microscopy (SEM) technique:

Scanning electron microscopy is an excellent tool for physical observation of morphological features of particle both initially and degradation process. It is helpful to examine particle shape and surface characteristics such as surface area and bulk density. The formulations are poured in a circular aluminum stubs using double adhesive tape, and coated with gold in HUS – 5GB vaccum evaporator and observed in Hitachi S – 3000N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X. (Hassan M. Ghonaim et al., 2013, Jithan Aukunuru et al., 2010)

f. Statistical analysis

Statistical analysis for the determination of differences in permeability profiles of cefuroxime axetil loaded SLNs and cefuroxime axetil pure drug solution was assessed by the use of Student's t-test (Graph pad Instat Version 3.0 software). Statistical probability (p) values less than 0.05 were considered significantly different (R. N. Marreto *et al*., 2011)

6) COMPRESSION OF LYOPHILIZED SLN LOADED WITH CEFUROXIME AXETIL INTO MUCOADHESIVE TABLETS:

Controlled release mucoadhesive matrix tablets of lyophilized SLN loaded with cefuroxime axetil are formulated by direct compression technology. Lyophilized drug loaded SLN and the polymers (carbopol 934 & HPMC K15M) in the ratio of 1:1.5 and the other excipients are screened through 40 mesh sieve. All materials are accurately weighed and mixed intimately for 15 minutes. The directly compressible mixture are compressed using single stroke tablet punching machine fitted with 12 mm flat faced punch. Before compression, the surface of die and punch are lubricated with magnesium stearate. (Vitaliy V. Khutoryanskiy et al., 2012, Yadav V.D. 2013 et al., 2011)

7) CHARACTERIZATION OF MUCOADHESIVE TABLETS OF LYOPHILIZED SLN LOADED WITH CEFUROXIME AXETIL:

a. Precompression evaluation of powder blend:

i. Estimation of drug content of lyophilized SLN:

Approximately weighed quantity of 100 mg equivalent of cefuroxime axetil is taken and transferred into a 100 ml volumetric flask. It is dissolved in methanol and made up to the volume with 0.07N HCl buffer. Subsequently the solution in volumetric flask is filtered and suitable dilutions are made and analyzed at λ_{max} using UV-Visible spectrophotometer (Shimadzu UV-1700, pharma spec, Japan). The drug content of each sample is estimated from standard curve of cefuroxime axetil using 0.07N HCl buffer.

(A. S. Gudigennavar *et.al.*, 2013)

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

ii. Angle of Repose:

Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane. In this method, the powder is allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose is then calculated by measuring the height and radius of the heap of granules formed. (Aulton M.E., 2002 and Satyabrata Bhanja *et.al.*, 2013)

$$\text{Tan } \theta = h/r$$

$$\theta = \tan^{-1} (h/r)$$

Where,

θ = Angle of repose

h = Height of the heap

r = radius of the heap

The relationship between the angle of repose and powder flow is given as,

Angle of repose	Powder flow
$<25^0$	Excellent
$25\text{-}30^0$	Good
$30\text{-}40^0$	Passable
$>40^0$	Very poor

iii. Bulk density (gm /ml) :

Bulk density is the ratio between given mass of powder and its bulk volume. Bulk density measurements are carried by placing fixed weight of powder in graduated cylinder and volume occupied is measured and initial

bulk density (gm/ml) is calculated. It is expressed in gm/ml. Bulk density is calculated by using following formula, (Rao G. Umamaheshwara *et al.*, 2012)

$$\text{Bulk Density} = \frac{\text{Weight of the powder}}{\text{Bulk volume of powder}} = \frac{W}{V}$$

iv. Tapped density (gm/ml):

A known quantity of sample is transferred to a graduated cylinder and placed on tapped density apparatus and operated for a fixed number of taps (100). It is the ratio of weight of sample to tapped volume.

(Rao G. Umamaheshwara *et.al.*, 2012)

$$\text{Tapped Density} = \frac{\text{Weight of the powder}}{\text{Tapped volume of powder}} = \frac{W}{V_f}$$

v. Carr's index:

It indicates powder flow properties. It is expressed in percentage and is give

$$\mathbf{I = \frac{D_t - D_b}{D_t} \times 100}$$

Where, Dt is the tapped density of the powder

D_b is the bulk density of the powder.(Vinod Kombath Ravindran et al., 2012)

% Compressibility	Flow ability
5 – 12	Excellent
12 – 16	Good
18 – 21	Fair Passable
23 – 35	Poor
33 – 38	Very Poor
< 40	Very Very Poor

vi. Hausner ratio:

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

$$\text{Hausner ratio} = \frac{D_t}{D_b}$$

Where,

D_t is the tapped density.

D_b is the bulk density.

Lower Hausner ratio (<1.25) indicates better flow properties than higher ones (>1.25). (Vinod Kombath Ravindran et al., 2012)

b. Post compression evaluation studies:

i. Drug content of fabricated mucoadhesive tablets:

Approximately weighed quantity of 100 mg equivalent of cefuroxime axetil is taken and transferred into a 100 ml volumetric flask. It is dissolved in methanol and made up to the volume with 0.1N Hydrochloric acid. Subsequently the solution in volumetric flask is filtered and suitable dilutions are made and analyzed at λ_{max} using UV-Visible spectrophotometer (Shimadzu UV-1700, pharma spec, Japan). The drug content of each sample is estimated from standard curve of Clozapine using 0.07N HCl buffer.

(A. S. Gudigennavar *et.al.,* 2013)

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

ii.Thickness & Diameter:

Three tablets are randomly selected from each formulation and thickness and diameter are measured individually by vernier caliper. It is

expressed in millimeter (mm) and average is calculated.

(D. Krishnarajan *et.al.,* 2013)

iii. Hardness:

Tablet requires a certain amount of hardness and resistance to friability to withstand mechanical shakes of handling in manufacture, packing and shipping. The hardness of the tablets is determined using Monsanto hardness tester. It is expressed in Kg/cm^2. Three tablets are randomly selected from each formulation and hardness of the tablets is determined. The results are expressed in average value. (Akant Priya Singla *et. al.,* 2010)

iv. Weight variation:

Twenty tablets are randomly selected from each formulation and average is determined. Then individual tablet is weighed and individual is compared with average weight. The tablet passes the IP test if not more than 2 tablets are outside the percentage limits and if no tablet differs by more than 2 times the percentage limit. (Indian Pharmacopoeia 1996, Page no: 736)

Average weight	**Maximum % difference allowed**
130 mg or less	± 10%
130 mg to 324 mg	± 7.5%
More than 324 mg	± 5%

v. Friability test:

The friability of tablets is determined using **Roche Friabilator**. Twenty tablets are selected from each batch. The tablets are initially weighed (initial weight) and transferred into Friabilator. The Friabilator is rotated at 25 rpm for

4 minutes, after which the tablets are removed. Loose dust is removed from the tablets as before and the tablets are weighed again (final weight).

The percentage friability is then calculated by,

$$F = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

% Friability of tablets less than 1% is considered acceptable.

(Akant Priya Singla *et. al.,* 2010, Goswami Dhruba Sankar et al., 2011)

vi. Fourier Transform Infrared spectroscopic studies (FT-IR):

Infrared spectrometry of the SLN formulation is carried out to find out the interactions between the drug and excipients used.

vii. Determination of swelling index:

Swelling of tablet due to the excipients particles involves the absorption of a liquid resulting in an increase in weight and volume. Liquid uptake by the particle may be due to saturation of capillary spaces within the particles or hydration of macromolecule. The liquid enters the particles through pores and bind to large molecule, breaking the hydrogen bond and resulting in the swelling of particle. The extent of swelling can be measured in terms of % weight gain by the tablet. In each formulation batch one tablet is weighed and placed in a Petri plate containing 25ml of 0.07N HCl buffer. After an hour interval of time the tablet is removed from petri plate, and excess of buffer is removed by using filter paper. The same procedure is repeated up to 12 hours. (Vitaliy V. Khutoryanskiy *et.al.,* 2012). Swelling index is calculated by using the following formula.

$$\text{Swelling index} = (W_2 - W_1)/W_1 \times 100$$

Where,

W_1- Initial weight of the tablet,

W_2 – hydrated weight of the tablet.

viii. *In vitro* release studies:

In vitro release studies are performed by using USP type II Paddle dissolution apparatus. 900 ml of freshly prepared 0.07N HCl buffer is used as dissolution medium. Temperature is maintained at 37° C ± 1° C. Samples (5ml) are withdrawn at regular intervals of 30 minutes and the same volume of fresh dissolution medium is replaced after every withdrawal. The withdrawn samples are analyzed by UV- visible spectrophotometer at λ_{max}. The studies are done in triplicate. **(Akant Priya Singla *et. al.*, 2010).**

ix. *In vitro* release kinetics:

In controlled or sustained release formulations the three most important rate controlling mechanisms are,

- Diffusion
- Swelling and
- Erosion

The In vitro release profiles obtained from the mucoadhesive tablets are fitted to zero order, first order, Higuchi, Hixson Crowell, Korsemeyer & Peppas model kinetics, to find out the mechanism of drug release.

Release Kinetics Model	Equation
Zero Order	$Q_t = Q_0 + K_0 t$
First Order	$\text{In } Q_t = \text{In } Q_0 + K_0 t$
Hixson-Crowell	$Q_0^{1/3} – Qt^{1/3} + K t$
Higuchi	$Q = KH. t^{1/2}$
Korsmeyer – Peppas	$M_t / M_0 = a.tn$

Fitness of release profiles to linear equations is assessed by comparing the coefficients of determination (r) values. For cylinder type of systems,

n< 0.45	:	Classical Fickian diffusion
n=0.45 to 0.89	:	Anomalous Non Fickian transport i.e. coupled diffusion in the hydrated matrix and polymer relaxation (Indicators of both phenomenon)
n=0.89	:	Case II relaxational release transport - Zero order release(Polymer relaxation or swelling controlled systems)
n> 0.8	:	Super Case II transport.

(Inderbir Singh *et. al.*, 2011)

x. *In vitro* mucoadhesive strength determination:

Bioadhesive strength of the mucoadhesive tablets is measured on modified physical balance. A modified physical balance is used for determining the *ex vivo* mucoadhesive strength of prepared mucoadhesive tablets. Fresh sheep stomach mucosa is obtained from a local slaughterhouse. Sheep stomach mucosa is tied to the glass petri dish, which is filled with 0.07N HCl buffer so that it just touched the mucosal surface. The tablet is placed on the stomach mucosa. The preload of 5 gm is placed on the tablet and the balance is kept in this position for 5 minutes. Then weight of 5 gm is removed from the right hand pan, which is loaded along with the tablet over the mucosa. Then water is added slowly to the right hand pan until the tablet is detached from the mucosal surface. (A. S. Gudigennavar *et.al.*, 2013)

Mucoadhesive strength = Weight of the water to detach the tablet from the mucosal surface (gm)

$$\text{Force of Adhesion (N)} = \frac{\text{Mucoahesive strength (gm) x 9.81 kg (1N)}}{1000}$$

$$\text{Bond strength } (N/m^2) = \frac{\text{Force of adhesion (N)}}{\text{Surface area of the tablet } (m^2)}$$

xi. Determination of i*n vitro* residence time:

In vitro residence time for tablets is determined using USP disintegration apparatus. The disintegration medium composes of 800 ml of 0.07N HCl buffer and temperature is maintained at 37°C ± 2˚C. A segment of sheep stomach mucosa about 3 cm in length is glutted to glass slide and mucoadhesive tablet is placed on to the wet sheep stomach mucosa. The glass slide vertically attached to disintegration apparatus is completely immersed in 0.07N HCl buffer. The time taken for the tablet to detach from sheep gastric mucosa is recorded as the mucoadhesion time.

(Rao G. Umamaheswara *et al.*, 2012)

xii. *Ex vivo* stomach permeability studies:

This study is performed after approval by the Institutional Animal Ethical Committee using male Wistar rat. Rat stomach mucosa is used to determine the drug permeation profile. Rats fasting for 18 – 20 hours are anaesthetized by some ether sprinkled to a piece of cotton wool in a glass container equipped with a lid. After making a midline incision in the abdomen, stomach is separated and is washed with 0.07N HCl buffer to remove any remaining gastric contents. The separated stomach tissue is incised to suitable size similar to the size used for Franz Diffusion cell. A modified Franz Diffusion cell is used for permeability studies, it consist of one donor compartment and a receptor compartment. The receptor compartment is filled with 54 ml of pH 1.2 phosphate buffer simulating the blood circulation and the donor compartment is filled with 5 ml of 0.07N HCl buffer simulating gastric

content. Temperature is maintained at $37^0 \pm 1^0$C. The separated stomach epithelium was mounted between the two chambers and stomach epithelium was allowed to stabilize. After stabilization of stomach epithelium, the mucoadhesive tablet is adhered on stomach epithelium. This system was placed on a thermostatic cum magnetic stirrer to generate stirring in the receptor compartment. Periodically, samples are withdrawn and same volume fresh medium is replaced. The aliquots are analyzed spectrophotometrically at 281nm. (A. S. Gudigennavar et al., 2013)

xiii. *In vivo* gastroretentive time in rabbit stomach:

The clearance has been obtained from the institutional ethical board (Institutional Animal Ethical Committee, Madurai Medical College, Madurai) for performing *in vivo* x-ray studies in rabbits. It is carried out to evaluate the mucoadhesive property of the formulated (best formulation) mucoadhesive tablets. For this study, mucoadhesive tablets containing barium sulphate (as X-ray opaque material) is used (instead of cefuroxime axetil). The tablet is administered orally to rabbit along with 30 ml of 5% dextrose solution by using stomach tube (No.12 French catheter) and 20ml syringes. X-ray photographs are taken at different time intervals (0, 2, 4, 6, 8 and 10 hr) and observed for the position of the tablet

(Aashima Hooda et al., 2012, A. S. Gudigennavar et al., 2013)

CHAPTER - 9

RESULTS AND DISCUSSION

TABLES & FIGURES

CHAPTER - 9

RESULTS AND DISCUSSION

1) STANDARDIZATION OF CEFUROXIME AXETIL:

a. Preparation of 0.07N HCl buffer:

The calibration medium of 0.07N hydrochloric acid buffer was prepared as per Indian Pharmacopoeia., 2010.

b. Determination of λ_{max} & Preparation of calibration curve:

The absorption maximum (λ_{max}) of cefuroxime axetil was estimated by using UV spectrophotometer. It was done by scanning the drug solution (10µg/ml) in between 200-400 nm region. The obtained spectrum showed that the absorption maximum (λ_{max}) at 281 nm. The absorbance spectrum was shown in **figure-1A.**

The standard calibration curve of cefuroxime axetil was prepared by using 0.07N HCl buffer. The absorbance was measured at λ_{max} of 281 nm. Good linearity was observed with the plot. The 'r^2' value was found to be 0.99982 as shown in **table-1**, which was very nearer to '1' and hence obeyed "Beer-Lambert" law within the concentration range of 5-25 µg/ml. The calibration plot of cefuroxime axetil was shown in **figure-1B.**

2) COMPATIBILITY STUDIES OF DRUG AND EXCIPIENTS:

a. Fourier Transform Infrared Spectroscopic studies (FT-IR):

The IR Spectra of pure drug, lipidic excipients were shown in the **figure-3A-3J**. The spectrum was studied at 4000 cm^{-1} – 400 cm^{1}. The spectrum of pure drug shows crystalline nature with sharp bands (shown below in table) indicating crystallinity.

IR interpretation of cefuroxime axetil

S.NO	FUNCTIONAL GROUPS	OBTAINED WAVE NUMBER cm^{-1}
1	C-N Stretching	1329
2	C-S Stretching	755
3	C=O in β-lactam	1733
4	C=N Stretching	1527
5	C-O Stretching in Ester	1249
6	C=O Stretching in Ester	1757
7	C=C Stretching (Aromatic)	1558
8	OCH_3 group	1680

In the physical mixture of all the formulations as shown in **figure 3K-**3**O**, the peaks of cefuroxime axetil shown above was retained indicating that there were no interaction between drug and excipients.

3) FORMULATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:

The composition of the formulation was shown in **table-2**. All the formulations were spontaneously formed when the aqueous phase containing surfactant was added drop wise to the stirred melted lipid along with co-surfactant at the same temperature with or without cefuroxime axetil, resulting in a colloidal suspension. The prepared SLN dispersion was found to be uniform and homogenous in appearance.

4) CHARACTERIZATION OF CEFUROXIME AXETIL LOADED SOLID LIPID NANOPARTICLES:

a. Determination of Physicochemical properties:

The SLN dispersion was milky white in appearance, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 minutes).

b. Determination of drug content:

The percentage drug content for all the formulations (SLN1-SLN30) were shown in the **table-3.** The drug content was found in the range of 98.48% - 96.97%, indicating uniform distribution of drug in formulations.

c. Determination of drug entrapment efficiency:

The results of EE were shown in the **tables 3A–3E** and **figure-2**. The EE of the formulations SLN1-SLN6 (glyceryl behenate (compritol ATO 888) at different ratios (1%, 2%, 4%, 6%, 8% & 10%) showed 49.88 ± 0.88% to 68.09 ± 0.68%; the formulation SLN7-SLN12 (glyceryl monostearate at different ratios (1%, 2%, 4%, 6%, 8% & 10%) showed 45.16 ± 1.52 to 62.88 ± 0.67%; the formulation SLN13-SLN18 (glyceryl monooleate at different ratios (1%, 2%, 4%, 6%, 8% & 10%) showed 27.52 ± 0.94% to 45.07 ± 1.22%; the formulation SLN19-SLN24 stearic acid at different ratios (1%, 2%, 4%, 6%, 8% & 10%) showed 22.79 ± 2.02% to 37.34 ± 1.25%; the formulation SLN25-SLN30 (palmitic acid at different ratios (1%, 2%, 4%, 6%, 8% & 10%) showed 18.28 ± 2.00% to 33.91 ± 1.86%. The influence of surfactant and lipid concentrations was discussed below.

From the above results it showed that the EE of the formulations increase with increase of lipid concentration. This was because that when the lipid concentration increases there would be more lipid to entrap the drug molecules. Among the various

lipids used compritol showed highest drug entrapment, because of the presence of long chain fatty alcohols. Because of the long chain fatty alcohols, the lipid could accommodate more drug molecules in it comparing to the other lipids. The order of EE of the lipids was given as,

Compritol>Glyceryl monostearate>Glyceryl monoleate>Stearic acid>Palmitic acid

The effect of surfactant on the EE was also studied. Generally when the particle size of the formulation was reduced cohesive forces exists between them, which would lead to particle aggregation. Therefore in order to overcome this hurdle the use of surfactant was applied. Pluronic F68 was selected as the outer phase stabilizer, which get coated on the outer surface of the nano particles, thereby preventing their aggregation (Abdul Hasan Sathali A et al., 2012). A 2% concentration of the surfactant was applied for an effective stabilization, because upto certain extend the effect of the surfactant increases and beyond that there were no effective results (Lakshmi Sirisha Kotikalapudi et al,. 2012).

When the drug was not evenly distributed in the lipid phase, the proper entrapment of the drug molecules in the lipid matrix may not be achieved, so the use of a co-surfactant was needed. Here soya lecithin was used as co-surfactant/solubilizer in the formulations, which solubilizes/disperse the drug in the lipid matrix evenly.

(Lakshmi Sirisha Kotikalapudi et al,. 2012)

d. Particle size & Zeta potential:

Nanoparticles were characterized by mean particle diameter and their distribution. The particle size of the formulations with best EE was shown in **table-5** and their distribution curves were shown in **figure 10A-10E**.

Formulation SLN6 prepared using lipid-compritol ATO 888 containing showed mean particle size of 512.6nm.

Formulation SLN12 prepared using lipid-glyceryl monostearate showed mean particle size of 148.nm.

Formulation SLN18 prepared using lipid-glyceryl monooleate showed mean particle size of 104.3nm.

Formulation SLN24 prepared using lipid-stearic acid showed mean particle size of 319.5nm.

Formulation SLN30 prepared using lipid-Palmitic acid showed mean particle size of 467.2nm.

Surfactant plays an important role in particle size of the formulations. Surfactant was used for stearic stabilization of the formulated nano particles, preventing them from aggregating to form micro particles. To produce an optimum particle size for the formulations, pluronic F68 at 2% concentration was used which was kept constant for all the formulations.

The various lipids and their various concentrations also contribute to the particle size. Here the melting point of the lipids plays an important role. The higher the melting point of the lipid the higher would be its particle size, lower the melting point lower would be its particle size(Maria Antonietta Casadei et al,. 2011). The order of melting point of the lipids used were given as,

Glyceryl monooleate<Glyceryl monostearate<Stearic acid<palmitic acid<Compritol

The concentrations of the lipids also influence the particle size. An increase in particle size was observed when the concentrations of lipids were increased. This was because when the concentrations of the lipids were increased the amount of surfactant

used could not completely emulsify the lipids and also the surfactant could not give enough stearic stabilization. But since the particle size of the highest concentrations of the different lipids were not more than 515 nm, the formulations with best EE ie, highest lipid concentrations were selected.

Polydispersity index (PDI):

The PDI for the formulations as shown in **table-5** and **figure 11A-11E** is smaller than 0.5, which indicates a relative homogenous dispersion. Polydispersity index indicates particle size distribution, which ranges from 0 to 1. Theoretically, monodisperse populations indicates PI = 0. However, PI < 0.2 was considered as narrow distribution and those greater than 0.5 indicate high homogenicity (Krutika Sawant et., 2013).

The zeta potential of the formulations with best EE was evaluated, which was shown in **table-6** and **figure 11A-11E**. Zeta potential of formulations SLN6, SLN12, SLN18, SLN24 & SLN30 showed negative zeta potential of-9.42mV, -22.9mV, -16.5, -10.5 & -15.9 resectively.

Zeta potential of about -25mV allows an ideal stabilization of nanoparticles because the repulsive forces prevent aggregation upon ageing. All nanoparticles showed a high negative residual charge due to chemical nature of the lipid matrix (stearic acid/oleic acid/behenic acid) and surfactant used (Krutica Sawant et al., 2013)

e. *In vitro* release studies:

The *invitro* drug release of the formulations showed a biphasic release pattern ie, both first and zero order drug release as shown in **table 7A-7E** and **figure 4A-4E.**

Burst effect

From the obtained drug release data, a burst release was seen in all the formulations. Higher lipid ratios led to lower burst release in the first two hours

(23.19±1.21, 28.35±0.73, 29.79±1.53, 33.40±0.86, 36.17±0.60) showing lower release from the lipid due to higher EE and lower lipid concentrations led to higher burst release (33.25±1.71, 40.17±0.49, 43.63±2.19, 46.76±1.94, 50.05±1.48) showing higher release due to lower EE.

This might be due to the presence of unentraped drug on the outer surface of the nanoparticles. This shows that the formulated SLNs were in "Drug Enriched Shell Model". The faster release was due to the presence of larger surface area, as the particles were in nano size. The burst release decreases with an increase in lipid concentration, because there would be more amount of lipid to entrap the drug molecules.

The burst release might be also useful for producing immediate action.

Sustained effect

Followed by the burst release for 2 hours, SLN formulations showed sustained effect for 12 hours.

The release for SLN1-6 (compritol ATO 888) was 68.80±1.55 – 53.31±1.13

The release for SLN7-12 (glyceryl monostearate) was 71.83±1.08 – 57.28±1.02

The release for SLN13-18 (glyceryl monooleate) was 87.37±1.33 – 69.90±1.67

The release for SLN19 - 24 (stearic acid) was 92.48±2.21 –77.53±1.53

The release for SLN25 - 30 (palmitic acid) was 94.01±1.41 – 80.90±0.91

Among the various lipids used, compritol ATO 888 showed more sustained release than the glyceryl monooleate and glyceryl monostearate due to its longer carbon chain length than the other two lipids. Moreover GMO and GMS, were lipids with lower melting point when compared to compritol ATO 888, can produce a controlled release from SLN. This is due to the presence of solid solution throughout the particle combined with the slow diffusion of drug from the lipid matrix.

The order of drug release from the three lipids as follows:

Glyceryl monooleate>Glyceryl monostearate>Stearic acid>palmitic acid>Compritol.

From the results it was concluded that higher lipid concentration and longer carbon chain length of fatty acids sustained the drug release from SLNs.

f. Kinetics of drug release:

The data obtained from the drug release studies were plotted in various kinetic models such as,

- Cumulative percentage drug release Vs time (zero order rate kinetics)
- Log cumulative percentage drug remaining Vs time (first order rate kinetics)
- Cumulative percentage drug release Vs square root of time (Higuchi classical diffusion model)
- Cube root of percentage drug remaining Vs time (Hixon Crowell erosion equation).
- Log cumulative percentage drug release Vs log time (Korsmeyer Peppas exponential equation)

The r^2 values and k values were shown in **table 8A-8E** and **figures 5A-9E**

Among the models tested, the drug release profile of all formulations were best fitted with first order with r^2 values ranging from 0.973-0.988 and Higuchi model with r^2 values ranging from 0.979 - 0.992. From the results higuchi release kinetics showed purely diffusion controlled.

The 'n' values obtained from Hixon Crowell were within 0.4-0.8 which indicated that the drug release mechanism followed Non-Fickian diffusion (Abdul Hasan Sathali .A and Priyanka .K., 2012)

5) SELECTION AND EVALUATION OF BEST FORMULATION

Based on entrapment efficiency and *in vitro* release

Based on the entrapment efficiency, the formulations containing compritol ATO 888 as lipid showed higher entrapment. This might be due to longer chain length of the lipid. Due to high entrapment, drug release from SLNs was sustained. So according to this release profile formulation SLN6 (compritol ATO 888-10%) was selected as one of the best formulations.

Based on particle size

The optimized formulations SLN12 & SLN18 (GMS-10% & GMO-10%) with lowest particle size was selected for further evaluation studies.

Selected best Formulation code	Entrapment efficiency (%) ± SD	Drug release (%) ± SD	Particle size (nm)
SLN6	68.09 ± 0.68	53.31 ± 1.13	512.6
SLN12	62.88 ± 0.67	57.28 ± 1.02	148.0
SLN18	45.07 ± 1.22	69.90 ± 1.69	104.3

a. Solubility studies:

Solubility results were showed in **table-9** and **figure-12**. The solubility of cefuroxime axetil in distilled water was found to be 0.241 mg/ml at room temperature which increased significantly to 0.871 ± 0.002 mg/ml (SLN18-104.3nm), 0.730 ± 0.003 mg/ml (SLN12- 148 nm), 0.508 ± 0.004 mg/ml (SLN6- 512.6 nm) after formulating as solid lipid nanoparticle. This was indicating that drastic increase in

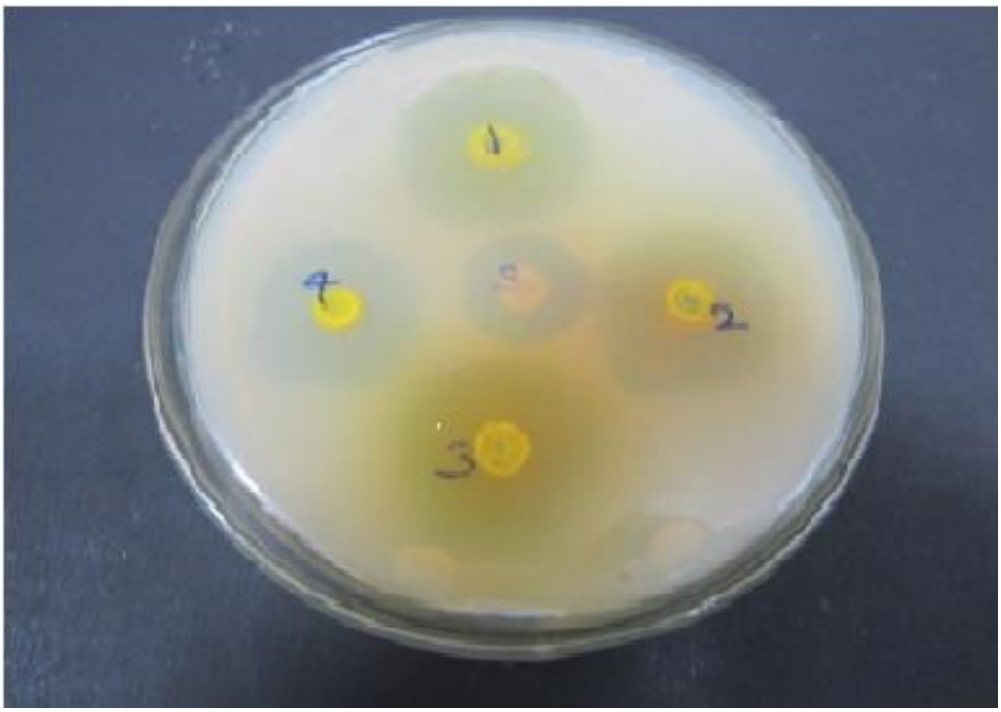
surface area, resulting from particle size reduction, greatly enhanced the solubility of the drug (Abdul Hasan Sathali .A and Nisha .N,. 2012)**.**

b. Microbiological assay:

The microbiological action of the formulated SLNs was shown below.

S. No.	Composition	Zone of Inhibition
1	Pure drug - **1**	16
2	GMO - **2**	19
3	GMS - **3**	21
4	Compritol - **4**	17
5	Standard - **S**	16

The zone of inhibition of SLNs was found to be much higher than that of pure drug (cefuroxime axetil) and standard () in a 24 hours study. This was achieved because of their nano size (Compritol-512.6nm, GMS-148nm, and GMO-104.3nm). And also the SLNs showed continuous antimicrobial action throughout 24 hours, due to their controlled action.



c. Lyophilization of best formulation:

Dry amorphous formulations (F1, F2 & F3) were obtained after lyophilization process.

d. X-ray diffraction studies:

The x-ray diffraction studies showed amorphous state of drug in formulations as shown in **figure-22A, 22B, 22C & 22D.**

e. Morphology of SLN by Scanning electron microscopy (SEM) technique:

From the obtained SEM images, the SLNs showed a spherical shape and also an almost smooth appearance as shown in the **figure-21, 22 & 23**

6) COMPRESSION OF LYOPILIZED SLN LOADED WITH CEFUROXIME AXETIL INTO MUCOADHESIVE TABLETS:

The individually weighed powder blends of lyophilized SLN along with other excipients mentioned in **table-10** were compressed in to tablets in a single punch tablet compressing machine. Each tablet contains 125mg eqiuivalent of cefuroxime axetil lyophilized SLN. Carbapol 934 and HPMC K15M were used as mucoadhesive polymers, magnesium stearate and talc as lubricants. The prepared mucoadhesive tablets were white in colour and round in shape**.**

(Margret Chandira et al., 2009**)**

7. CHARACTERIZATION OF MUCOADHESIVE TABLETS OF LYOPHILIZED SLN LOADED WITH CEFUROXIME AXETIL:

a. Precompression evaluation of powder blend:

i. Estimation of drug content:

The drug content of the formulations F1, F2 & F3 were found to be 90.93%, 97.73% & 94.33%, showing an uniform distribution of drug.

ii. Angle of Repose

Angle of repose for the formulations F1, F2 & F3 were 28.66°, 29.28° & 27.39° and the powder blends of all formulations shows good flow properties. The results of angle of repose of all formulations were shown in **table-11 & figure-13.**

iii. Bulk density (gm /ml)

The bulk density for the formulations F1, F2 & F3 were 0.544g/cm^3, 0.555 g/cm^3 & 0.554 g/cm^3. The results indicated that the powder blends of all twenty formulations have good flow properties. The results were summarized in **table -11 & figure-14**.

iv. Tapped density (gm/ml)

Tapped density for the formulations F1, F2 & F3 were 0.664g/cm^3, 0.676 g/cm^3 & 0.624 g/cm^3, indicating the presence of smaller particles occupying the voids between the the bigger particles. The results of all the formulations were summarized in **table-11 & figure-16.**

v. Carr's index:

The carr's index of the formulations F1, F2 & F3 were found to be 18.06%, 17.89% & 11.16% which indicated that the powder blend was fairly passable. The results of all the formulations were summarized in **table-11.**

vi. Hausner's ratio:

The Hausner ratio of the formulations F1, F2 & F3 were found to be 1.21, 1.21 & 1.12. Since a very low Hausner ratio (<1.25) was obtained the formulations showed better property. The results of all the formulations were summarized in **table-11.**

b. Post compression evaluation studies:

i. Drug content of fabricated mucoadhesive tablets:

The drug content of the formulations F1, F2 & F3 as given in **table-12** were 119.68mg (95.75%), 121.45mg (97.16%) & 120.75mg (96.60%) which shows an uniform drug content in the formulations and also it complies with USP limit (not less than 90% & not more than 110%)

ii. Thickness & Diameter:

The thickness for the formulations F1, F2 & F3 was 6mm, 5mm & 6mm. The results were summarized in **Table-12**. The diameter of all the formulations was 12mm. the results indicated an uniform particle size distribution and no deformities.

iii. Hardness:

The hardness for the formulations F1, F2 & F3 was 7kg/cm^3, 6 kg/cm^3 & 7kg/cm^3. The results indicated that the tablets of all formulations have good hardness, which in turn protects them from mechanical damage. The results were summarized in **Table-12.**

iv. Weight variation:

The weight of all the formulations ranges from 799.5mg ± 40, 800.2mg ± 40 & 800.7mg ± 40 and were tabulated in **Table-12**. The formulations F1, F2 & F3 tablets passed weight variation test and the weight variation was within the standard pharmacopoeial limits of ± 5% of the weight. The results indicated that all tablets of each formulation were of uniform weight.

v. Friability test:

Friability of the formulations F1, F2 & F3 was 0.43%, 0.33 % & 0.28%. The results indicated that the friability for tablets of all formulations were below 1% (I.P. limit 1%) and hence exhibit good mechanical resistance. The results were shown in **Table-12.**

vi. Fourier Transform Infrared spectroscopic studies (FT-IR):

From the obtained FT-IR interaction studies as shown in **figures 3P-3R,** the peaks obtained from pure cefuroxime axetil (1329cm^{-1}, 755 cm^{-1}, 1733 cm^{-1} & 1529 cm^{-1}) were also found in the final formulations as well, which indicated that there were no interactions between drug and excipients.

vii. Determination of swelling index:

The swelling index of the formulations F1, F2 & F3 at the 12^{th} hour as given in **table-14** and **figure-17** was 215%, 194.23% & 212.51% which shows an optimum swelling efficiency due to the presence of hydrophilic polymers (HPMC K15M & Carbopol). **(**Margret Chandira et al., 2009**)**

viii. *Invitro* release studies:

The invitro drug release studies of formulations F1, F2 & F3 as give in **table-15** and **figure-18** was 67.19%, 65.70% & 68.41%. The controlled release rate was achieved because of the hydrophilic polymers, (HPMC K15M & Carbopol) which on hydration swells in an controlled manner resulting in controlled release of drug.

ix. ***Invitro* release kinetics:**

The r^2 value of higuchi kinetics for formulations F1, F2 & F3 as given in **table-16** amd **figure 19A-19E,** was 0.968, 0.975 & 0.964 which were all less than 1 indicating pure diffusion process. The n vlue of korsmeyer peppas kinetics were less than o.4 indicagin g that the releade follows fickian diffusion ie., drug release by diffusion. (Inderbir Singh et al., 2011)

***x. In vitro* mucoadhesive strength determination:**

The invitro mucoadhesive strength of formulations F1, F2 & F3 as given in **table-13** and **figure-16** was 37.01gm, 34.63gm & 37.86gm This bioadhesive strength was acheived by the formation of secondary bioadhesion bonds with mucin and interpenetration of the polymer chains in the interfacial region, by the hydrophilic pllymers. **(**Margret Chandira et al., 2009**)**

xi. Determination of *in vitro* residence time :

The in vitro gastro residence time for formulation F1, F2 & F3 was 10hrs 28mins, 10hrs 38mins & 10hrs 18mins as given in **table-13.** This indicates that the formulation adheres to the gastric mucosa long enough to deliver the drug efficiently for more than 10hours.

xii. *Ex vivo* stomach permeability studies:

From permeation studies, the formulation F1, F2 &F3 as given in **table-17** & **figure-20** showed drug permeation of 90.49%. From this study it was noted that the formulation containing lyophilized SLN showed more permeation than that of pure drug and mucoadhesive tablets containing plain drug. This might be due to the presence of nano particle size.

xiii. *In vivo* gastroretentive time in rabbit stomach:

From the **figure 23A-23F,** it was clearly shown that even at the 8^{th} hour the tablet still adheres to the mucosal membrane. This was due to the presence of mucoadhesive polymers (HPMC K15M & carbopol 934).

TABLE-1 CALIBRATION OF CEFUROXIME AXETIL USING BUFFER 0.07N HCl

S. No	CONCENTRATION (µg/ml)	ABSORBANCE
1	5	0.180 ± 0.002
2	10	0.353 ± 0.006
3	15	0.535 ± 0.008
4	20	0.707 ± 0.005
5	25	0.887 ± 0.002

r = 0.99982

TABLE-4A ENTRAPMENT EFFICIENCY OF SLN USING GLYCERYL BEHENATE (COMPRITOL) AS LIPID

S. No	LIPID %	ENTRAMENT EFFICIENCY % ± SD
1	1	49.88 ± 0.88
2	2	53.87 ± 0.36
3	4	57.62 ± 0.71
4	6	61.77 ± 1.09
5	8	66.25 ± 1.04
6	10	68.09 ± 0.68

n=3*

TABLE-4B ENTRAPMENT EFFICIENCY OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

S. No	**LIPID %**	**ENTRAMENT EFFICIENCY % ± SD**
1	1	45.16 ± 1.52
2	2	44.3 ± 2.34
3	4	50.42 ± 1.62
4	6	54.18 ± 1.42
5	8	56.78 ± 2.33
6	10	62.88 ± 0.67

TABLE-4C ENTRAPMENT EFFICIENCY OF SLN USING GLYCERYL MONOOLEATE AS LIPID

S. No	**LIPID %**	**ENTRAMENT EFFICIENCY % ± SD**
1	1	27.52 ± 0.94
2	2	29.42 ± 0.81
3	4	32.29 ± 1.16
4	6	37.68 ± 0.18
5	8	40.85 ± 1.32
6	10	45.07 ± 1.22

n=3*

TABLE-4D ENTRAPMENT EFFICIENCY OF SLN USING STEARIC ACID AS LIPID

S. No	**LIPID %**	**ENTRAMENT EFFICIENCY % ± SD**
1	1	22.79 ± 2.02
2	2	27.04 ± 2.31
3	4	30.57 ± 0.39
4	6	33.54 ± 1.17
5	8	35.25 ± 1.56
6	10	37.34 ± 1.25

TABLE-4E ENTRAPMENT EFFICIENCY OF SLN USING PALMITIC ACID AS LIPID

S. No	**LIPID %**	**ENTRAMENT EFFICIENCY % ± SD**
1	1	18.28 ± 2.00
2	2	21.57 ± 1.10
3	4	24.74 ± 1.09
4	6	27.41 ± 2.11
5	8	30.55 ± 0.68
6	10	33.91 ± 1.86

n=3*

TABLE-3 DRUG CONTENT

S. No	CODE	DRUG CONTENT (mg) ± SD
1	SLN 1	122.76 ± 0.71
2	SLN 2	122.27 ± 1.23
3	SLN 3	123.22 ± 0.71
4	SLN 4	121.92 ± 0.89
5	SLN 5	121.09 ± 1.87
6	SLN 6	121.92 ± 0.73
7	SLN 7	120.98 ± 1.42
8	SLN 8	115.07 ± 5.79
9	SLN 9	118.73 ± 4.95
10	SLN 10	118.38 ± 5.03
11	SLN 11	114.60 ± 4.42
12	SLN 12	121.09 ± 2.32
13	SLN 13	122.16 ± 1.06
14	SLN 14	121.92 ± 0.89
15	SLN 15	122.86 ± 0.93
16	SLN 16	122.51 ± 1.41
17	SLN 17	123.33 ± 1.07
18	SLN 18	120.15 ± 0.74
19	SLN 19	121.45 ± 1.27
20	SLN 20	121.18 ± 2.59
21	SLN 21	121.69 ± 1.08
22	SLN 22	122.51 ± 1.27
23	SLN 23	121.69 ± 1.81
24	SLN 24	122.36 ± 1.51
25	SLN 25	121.68 ± 2.40
26	SLN 26	121.80 ± 2.21
27	SLN 27	122.39 ± 1.59
28	SLN 28	122.27 ± 1.23
29	SLN 29	121.21 ± 1.59
30	SLN 30	121.21 ± 2.13

n=3*

TABLE-5 PARTICLE SIZE OF FORMULATIONS WITH BEST ENTRAPMENT EFFICIENCY

CODE	LIPID (%)	MEAN DIAMETER (nm)	PDI
SLN 6	10	512.6	0.534
SLN 12	10	148.0	0.230
SLN 18	10	104.3	0.252
SLN 24	10	319.5	0.421
SLN 30	10	467.2	0.395

TABLE-6 ZETA POTENTIAL OF FORMULATIONS WITH BEST ENTRAPMENT EFFICIENCY

S. No	CODE	ZETA POTENTIAL (mV)
1	SLN 6	-9.42
2	SLN 12	-22.9
3	SLN 18	-16.5
4	SLN 24	-10.2
5	SLN 30	-15.9

TABLE-9 COMPARISON OF SOLUBILITY AMOUNG BEST FORMULATIONS

TIME IN HOURS	SOLUBILITY (mg/ml) ± SD			
	PURE DRUG	**SLN 6**	**SLN 12**	**SLN 18**
24 hours	0.241 ± **0.003**	0.508 ± **0.004**	0.730 ± **0.003**	0.871 ± **0.002**

n=3*

Table-10 FORMULA

INGREEDIENTS	F1 (SLN6-Compritol)	F2 (SLN12-GMS)	F3 (SLN18-GMO)
Lyophilized cefuroxime SLNs	468 mg	468	468
Carbopol 934	100 mg	100 mg	100 mg
HPMC K15M	200 mg	200 mg	200 mg
Talc	16 mg	16 mg	16 mg
Magnesium stearate	16 mg	16 mg	16 mg

Table-11 PRECOMPRESSIONAL EVALUATION OF POWER BLEND

CODE	ANGLE OF REPOSE Ɵ ±SD*	BULK DENSITY (g/ml)±SD*	TAPPED DENSITY (g/ml)±SD*	DRUG CONTENT %	CARR'S INDEX %	HAUSNER RATIO
F1	28.66 ± 0.88	0.544 ± 0.00	0.664 ± 0.003	90.93	18.06 ± 0.42	1.21 ± 0.005
F2	29.28 ± 1.32	0.555 ± 0.00	0.676 ± 0.006	97.73	17.89 ± 0.83	1.21 ± 0.005
F3	27.39 ± 1.41	0.554 ± 0.04	0.624 ± 0.005	94.33	11.16 ± 0.08	1.12 ± 0.005

n=3*

Table-12 POST COMPRESSION EVALUATION OF MUCO ADHESIVE TABLETS CONTAINING LYOPHILIZED CEFUROXIME AXETIL SLN

CODE	HARDNESS (KG/CM^2)	THICKNESS (MM)	DIAMETER (MM)	% FRIABILITY (%)	AVERAGE WEIGHT (mg) [±5 %(±40mg)]	DRUG CONTENT (mg)
F1	7	6	12	0.43	799.5	119.68
F2	6	5	12	0.33	800.2	121.45
F3	7	6	12	0.28	800.7	120.75

Table-13 POST COMPRESSION EVALUATION OF MUCO ADHESIVE TABLETS CONTAINING LYOPHILIZED CEFUROXIME AXETIL SLN

CODE	MUCOADHESIVE STRENGTH (gm)±SD*	FORCE OF ADHESION (N)±SD*	BOND STRENGTH (N/m^2) ±SD*	IN VITRO RESIDENCE TIME (Hrs)
F1	37.01 ± 1.09	0.362 ± 0.011	0.724 ± 0.014	10hrs 28mins
F2	34.63 ± 1.11	0.339 ± 0.011	0.678 ± 0.021	10hrs 38mins
F3	37.86 ± 0.88	0.371 ± 0.008	0.742 ± 0.011	10hrs 18mins

n=3*

Table-14 SWELLING INDEX OF MUCOADHESIVE TABLETS

TIME (HOURS)	F1 (SLN6-COMPRITOL)	F2 (SLN12-GMS)	F3 (SLN18-GMO)
1	23.28 ± 0.43	11.18 ± 0.11	25.77 ± 0.58
2	49.83 ± 0.76	36.51 ± 0.42	41.70 ± 0.66
3	66.29 ± 0.53	52.73 ± 0.35	58.99 ± 0.49
4	85.61 ± 0.23	69.20 ± 0.22	76.53 ± 0.72
5	101.39 ± 0.11	84.80 ± 0.26	93.33 ± 0.64
6	120.73 ± 0.37	102.00 ± 0.39	110.50 ± 0.53
7	136.21 ± 0.15	117.30 ± 0.47	127.79 ± 0.61
8	150.06 ± 0.67	133.84 ± 0.31	144.09 ± 0.43
9	165.89 ± 0.62	149.43 ± 0.10	163.62 ± 0.63
10	181.47 ± 0.59	163.66 ± 0.29	179.54 ± 0.88
11	197.00 ± 0.88	178.38 ± 0.31	196.46 ± 0.75
12	215.00 ± 0.47	194.23 ± 0.16	212.51 ± 0.70

n=3*

TABLE-16 INVITRO RELEASE KINETICS

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F 1	0.869	4.147	0.954	-0.032	16.87	0.968	0.963	0.309	0.934	-0.095
F 2	0.889	4.147	0.961	-0.031	16.75	0.975	0.962	0.330	0.944	-0.093
F 3	0.861	4.167	0.951	-0.033	0.964	17.01	0.964	0.300	0.929	-0.096

Table-15 CUMULATIVE % DRUG RELEASE OF MUCOADHESIVE TABLETS

TIME (HOURS)	F1 (SLN6-COMPRITOL)	F2 (SLN12-GMS)	F3 (SLN18-GMO)
0.5	26.34 ± 0.51	24.23 ± 0.40	27.29 ± 0.20
1.0	29.05 ± 0.42	26.81 ± 0.42	30.00 ± 0.31
1.5	31.90 ± 0.42	29.46 ± 0.31	33.26 ± 0.47
2.0	34.14 ± 0.23	31.56 ± 0.81	35.50 ± 0.31
3.0	37.81 ± 0.31	35.46 ± 0.05	39.09 ± 0.53
4.0	41.67 ± 0.23	39.71 ± 0.20	42.96 ±0.20
5.0	45.27 ± 0.11	43.71 ± 0.11	46.42 ± 0.35
6.0	48.12 ± 0.11	45.750 ± 0.31	48.73 ± 1.50
7.0	51.18 ± 0.31	49.14 ± 0.71	52.33 ± 0.40
8.0	55.11 ±0.31	53.08 ± 0.71	56.13 ± 0.31
9.0	58.91 ± 0.31	56.84 ± 0.05	60.00 ± 0.42
10.0	61.63 ± 0.51	59.66 ± 0.61	62.65 ± 0.82
11.0	65.09 ± 0.23	63.46 ± 0.11	66.17 ± 0.40
12.0	67.19 ± 0.73	65.70 ± 0.42	68.41 ± 0.61

n=3*

TABLE-17 *EX VIVO* DRUG PERMEATION STUDIES OF CEFUROXIME AXETIL ACROSS RAT STOMACH EPITHELIUM

TIME (HOURS)	PURE DRUG ±SD	MUCOADHESIVE TABLET LOADED WITH FREE DRUG ±SD	MUCOADHESIVE TABLET LOADED WITH LYOPHILIZED SLN (±SD)
0	0	0	0
0.5	6.13±0.12	6.31±0.54	23.09±0.63
1	10.20±0.22	8.55±0.23	26.49±0.45
1.5	15.63±0.76	9.57±0.51	29.88±0.21
2	19.02±0.55	12.22±0.62	34.63±0.87
3	23.09±0.54	14.87±0.89	43.45±0.68
4	27.17±0.11	16.49±0.23	47.53±0.59
5	28.52±0.88	18.53±0.64	52.99±0.75
6	35.99±0.23	20.16±0.43	58.42±0.48
7	38.70±0.55	21.794±0.55	63.77±0.48
8	40.74±0.34	23.62±0.32	69.40±0.49
9	43.45±0.19	26.27±0.23	75.47±0.30
10	47.53±0.45	29.93±0.95	81.53±0.52
11	50.92±0.57	33.80±0.39	86.58±0.75
12	53.44±0.34	37.02±0.13	90.49±0.45

n=3*

TABLE-2 FORMULA

S.NO	CODE	COMPOSITION					
		LIPID	PERCENTAGE (%)	SURFACTANT	% W/V	CO- SURFACTANT	% W/V
1	SLN 1	Compritol ATO 888	1	Poloxamer 188	2	Soya lecithin	2
2	SLN 2	Compritol ATO 888	2	Poloxamer 188	2	Soya lecithin	2
3	SLN 3	Compritol ATO 888	4	Poloxamer 188	2	Soya lecithin	2
4	SLN 4	Compritol ATO 888	6	Poloxamer 188	2	Soya lecithin	2
5	SLN 5	Compritol ATO 888	8	Poloxamer 188	2	Soya lecithin	2
6	SLN 6	Compritol ATO 888	10	Poloxamer 188	2	Soya lecithin	2
7	SLN 7	Glyceryl monostearate	1	Poloxamer 188	2	Soya lecithin	2
8	SLN 8	Glyceryl monostearate	2	Poloxamer 188	2	Soya lecithin	2
9	SLN 9	Glyceryl monostearate	4	Poloxamer 188	2	Soya lecithin	2
10	SLN 10	Glyceryl monostearate	6	Poloxamer 188	2	Soya lecithin	2
11	SLN 11	Glyceryl monostearate	8	Poloxamer 188	2	Soya lecithin	2
12	SLN 12	Glyceryl monostearate	10	Poloxamer 188	2	Soya lecithin	2
13	SLN 13	Glyceryl monooleate	1	Poloxamer 188	2	Soya lecithin	2
14	SLN 14	Glyceryl monooleate	2	Poloxamer 188	2	Soya lecithin	2
15	SLN 15	Glyceryl monooleate	4	Poloxamer 188	2	Soya lecithin	2

S.NO	CODE	COMPOSITION					
		LIPID	PERCENTAGE (%)	SURFACTANT	% W/V	CO- SURFACTANT	% W/V
16	SLN 16	Glyceryl monooleate	6	Poloxamer 188	2	Soya lecithin	2
17	SLN 17	Glyceryl monooleate	8	Poloxamer 188	2	Soya lecithin	2
18	SLN 18	Glyceryl monooleate	10	Poloxamer 188	2	Soya lecithin	2
19	SLN 19	Stearic acid	1	Poloxamer 188	2	Soya lecithin	2
20	SLN 20	Stearic acid	2	Poloxamer 188	2	Soya lecithin	2
21	SLN 21	Stearic acid	4	Poloxamer 188	2	Soya lecithin	2
22	SLN 22	Stearic acid	6	Poloxamer 188	2	Soya lecithin	2
23	SLN 23	Stearic acid	8	Poloxamer 188	2	Soya lecithin	2
24	SLN 24	Stearic acid	10	Poloxamer 188	2	Soya lecithin	2
25	SLN 25	Palmitic acid	1	Poloxamer 188	2	Soya lecithin	2
26	SLN 26	Palmitic acid	2	Poloxamer 188	2	Soya lecithin	2
27	SLN 27	Palmitic acid	4	Poloxamer 188	2	Soya lecithin	2
28	SLN 28	Palmitic acid	6	Poloxamer 188	2	Soya lecithin	2
29	SLN 29	Palmitic acid	8	Poloxamer 188	2	Soya lecithin	2
30	SLN 30	Palmitic acid	10	Poloxamer 188	2	Soya lecithin	2

TABLE-7A COMPARISON OF CUMULATIVE % DRUG RELEASE OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL BEHENATE (COMPRITOL) AS LIPID

TIME(HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD					
	SLN 1 COMPRITOL 1%	SLN 2 COMPRITOL 2%	SLN 3 COMPRITOL 4%	SLN 4 COMPRITOL 6%	SLN 5 COMPRITOL 8%	SLN 6 COMPRITOL 10%
0.5	8.87 ± 1.65	6.98 ± 0.86	11.60 ± 1.01	7.36 ± 1.29	4.44 ± 0.71	2.55 ± 1.23
1.0	18.44 ± 1.57	16.43 ± 1.00	20.26 ± 1.03	15.49 ± 1.15	13.19 ± 0.59	11.00 ± 1.32
1.5	26.56 ± 2.02	23.97 ± 1.16	27.83 ± 1.25	21.79 ± 1.15	19.28 ± 0.60	17.44 ± 1.15
2.0	33.25 ± 1.71	30.54 ± 1.16	33.31 ± 1.21	27.39 ± 1.16	24.76 ± 0.58	23.19 ± 1.21
3.0	38.78 ± 1.95	36.04 ± 1.31	36.66 ± 1.06	31.82 ± 1.04	29.07 ± 0.61	27.30 ± 1.11
4.0	43.04 ± 1.81	40.27 ± 1.35	39.76 ± 1.39	35.16 ± 1.20	32.38 ± 0.59	30.68 ± 1.20
5.0	46.38 ± 1.99	43.68 ± 1.21	42.70 ± 1.40	37.96 ± 1.20	35.06 ± 0.62	32.97 ± 1.06
6.0	49.67 ± 1.83	47.04 ± 1.21	45.66 ± 1.42	40.69 ± 1.35	37.95 ± 0.63	35.84 ± 1.07
7.0	53.16 ± 1.70	50.32 ± 0.94	48.55 ± 1.27	43.63 ± 1.37	40.68 ± 0.76	38.74 ± 1.08
8.0	56.32 ± 1.71	53.45 ± 0.81	51.09 ± 1.25	46.22 ± 1.23	43.52 ± 0.64	41.66 ± 1.09
9.0	59.31 ± 1.73	56.79 ± 1.01	53.75 ± 1.29	49.02 ± 1.11	46.49 ± 0.65	44.60 ± 1.10
10.0	62.32 ± 1.76	59.97 ± 0.97	56.42 ± 1.15	51.75 ± 1.12	49.38 ± 0.55	47.39 ± 0.98
11.0	65.36 ± 1.81	63.08 ± 0.98	59.22 ± 1.16	54.40 ± 0.98	52.11 ± 0.55	50.29 ± 1.12
12.0	68.80 ± 1.55	66.51 ± 1.27	62.03 ± 1.17	57.18 ± 0.99	55.06 ± 0.78	53.31 ± 1.13

n=3*

TABLE-7BCOMPARISON OF CUMULATIVE % DRUG RELEASE OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL MONOSTEARATE AS LIPID

TIME(HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD					
	SLN 7 GMS 1%	SLN 8 GMS 2%	SLN 9 GMS 4%	SLN 10 GMS 6%	SLN 11 GMS 8%	SLN 12 GMS 10%
0.5	16.78 ± 0.71	14.90 ± 0.32	12.54 ± 0.58	10.94 ± 0.43	9.15 ± 1.27	6.51 ± 1.29
1.0	25.23 ± 0.87	24.36 ± 0.32	21.97 ± 0.75	19.59 ± 0.65	17.59 ± 1.35	15.96 ± 0.57
1.5	32.85 ± 0.32	32.16 ± 0.57	29.18 ± 0.66	26.50 ± 0.82	24.76 ± 1.48	23.30 ± 0.73
2.0	40.17 ± 0.49	38.72 ± 0.99	34.86 ± 0.93	31.67 ± 0.92	30.20 ± 1.18	28.35 ± 0.73
3.0	45.76 ± 0.33	43.16 ± 1.72	38.23 ± 0.84	35.39 ± 1.20	33.81 ± 1.04	32.03 ± 1.02
4.0	49.33 ± 0.45	46.23 ± 2.16	41.15 ± 0.85	38.38 ± 1.33	36.88 ± 0.91	34.90 ± 1.22
5.0	52.07 ± 0.45	48.95 ± 2.18	43.91 ± 0.69	41.30 ± 1.35	39.50 ± 1.29	37.69 ± 1.07
6.0	54.75 ± 0.51	51.97 ± 2.20	46.60 ± 0.70	44.06 ± 1.40	42.34 ± 1.47	40.51 ± 1.1
7.0	57.35 ± 0.61	54.92 ± 2.22	49.41 ± 0.80	47.04 ± 1.41	45.11 ± 1.32	43.26 ± 1.30
8.0	60.35 ± 0.60	57.71 ± 2.10	52.24 ± 0.92	49.85 ± 1.27	47.90 ± 1.25	46.13 ± 1.15
9.0	63.09 ± 0.63	60.71 ± 2.27	55.10 ± 0.98	52.59 ± 1.28	50.43 ± 1.57	48.93 ± 1.07
10.0	65.95 ± 0.47	63.55 ± 2.14	57.98 ± 1.06	55.35 ± 1.29	53.08 ± 1.74	51.75 ± 1.01
11.0	68.92 ± 1.07	66.31 ± 2.02	61.07 ± 1.32	58.13 ± 1.03	55.84 ± 1.85	54.60 ± 0.93
12.0	71.83 ± 1.08	69.28 ± 1.69	64.28 ± 1.52	59.74 ± 1.12	58.72 ± 1.72	57.28 ± 1.02

n=3*

TABLE-7C COMPARISON OF CUMULATIVE % DRUG RELEASE OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL MONOOLEATE AS LIPID

TIME(HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD					
	SLN 13 GMO 1%	SLN 14 GMO 2%	SLN 15 GMO 4%	SLN 16 GMO 6%	SLN 17 GMO 8%	SLN 18 GMO 10%
0.5	19.33 ± 1.72	16.97 ± 1.13	16.97 ± 1.13	16.97 ± 1.13	7.17 ± .099	3.59 ± 1.17
1.0	28.10 ± 1.85	26.38 ± 1.00	26.38 ± 1.00	26.38 ± 1.00	16.57 ± 1.07	12.77 ± 1.30
1.5	36.30 ± 1.87	34.84 ± 1.01	34.84 ± 1.01	34.84 ± 1.01	25.13 ± 0.99	21.38 ± 1.32
2.0	43.63 ± 2.19	41.88 ± 0.19	41.88 ± 0.19	41.88 ± 0.19	33.11 ± 0.61	29.79 ± 1.53
3.0	50.47 ± 2.04	48.61 ± 0.41	48.61 ± 0.41	48.61 ± 0.41	40.03 ± 0.74	36.59 ± 1.54
4.0	57.09 ± 1.68	54.83 ± 0.26	54.83 ± 0.26	54.83 ± 0.26	45.23 ± 1.18	41.28 ± 1.67
5.0	62.35 ± 1.27	60.08 ± 0.46	60.08 ± 0.46	60.08 ± 0.46	48.97 ± 1.32	44.80 ± 1.69
6.0	66.44 ± 1.45	63.95 ± 1.02	63.95 ± 1.02	63.95 ± 1.02	52.65 ± 1.33	48.62 ± 1.70
7.0	70.00 ± 1.42	67.49 ± 1.31	67.49 ± 1.31	67.49 ± 1.31	55.97 ± 1.21	52.01 ± 1.84
8.0	73.59 ± 1.53	70.96 ± 1.04	70.96 ± 1.04	70.96 ± 1.04	59.72 ± 1.21	55.81 ± 1.74
9.0	77.11 ± 1.55	74.45 ± 0.77	74.45 ± 0.77	74.45 ± 0.77	63.40 ± 1.23	58.88 ± 1.75
10.0	80.38 ± 1.56	78.07 ± 0.92	78.07 ± 0.92	78.07 ± 0.92	67.10 ± 1.24	62.55 ± 1.77
11.0	83.95 ± 1.57	81.63 ± 0.92	81.63 ± 0.92	81.63 ± 0.92	70.37 ± 1.39	65.97 ± 1.51
12.0	87.37 ± 1.33	85.21 ± 0.93	85.21 ± 0.93	85.21 ± 0.93	74.04 ± 1.42	69.90 ± 1.69

n=3*

TABLE-7D COMPARISON OF CUMULATIVE % DRUG RELEASE OF CEFUROXIME AXETIL LOADED SLN USING STEARIC ACID AS LIPID

TIME(HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD					
	SLN 19 STEARIC ACID 1%	SLN 20 STEARIC ACID 2%	SLN 21 STEARIC ACID 4%	SLN 22 STEARIC ACID 6%	SLN 23 STEARIC ACID 8%	SLN 24 STEARIC ACID 10%
0.5	21.78 ± 1.69	17.35 ± 2.15	13.20 ± 0.71	9.62 ± 1.23	8.68 ± 1.39	7.83 ± 0.71
1.0	30.66 ± 2.00	26.27 ± 2.06	22.17 ± 0.87	19.29 ± 1.15	18.34 ± 1.15	16.63 ± 0.59
1.5	38.91 ± 2.04	34.57 ± 1.93	30.52 ± 0.66	28.47 ± 1.09	27.22 ± 1.15	52.21 ± 0.57
2.0	46.76 ± 1.94	43.04 ± 1.83	38.85 ± 0.73	35.65 ± 1.21	34.20 ± 0.94	33.40 ± 0.86
3.0	54.78 ± 1.92	50.93 ± 1.96	46.32 ± 0.74	43.09 ± 1.18	41.15 ± 0.32	40.91 ± 0.88
4.0	61.55 ± 1.93	56.34 ± 1.83	52.26 ± 0.78	48.90 ± 1.33	46.85 ± 0.21	46.13 ± 1.41
5.0	65.55 ± 1.95	60.95 ± 2.15	56.83 ± 0.47	54.19 ± 1.24	51.18 ± 0.54	50.93 ± 1.10
6.0	69.40 ± 1.97	64.85 ± 1.86	60.88 ± 0.76	56.89 ± 1.35	54.42 ± 0.27	54.26 ± 0.96
7.0	73.75 ± 2.14	69.15 ± 2.08	64.86 ± 0.76	60.94 ± 1.27	59.10 ± 0.75	57.52 ± 1.44
8.0	77.85 ± 1.72	73.03 ± 2.21	68.60 ± 0.77	64.54 ± 1.66	63.16 ± 1.55	60.43 ± 1.61
9.0	81.23 ± 1.47	77.12 ± 1.77	73.22 ± 0.71	69.59 ± 1.35	68.29 ± 1.36	66.11 ± 1.20
10.0	84.54 ± 1.49	80.58 ± 1.48	77.03 ± 0.97	73.65 ± 1.36	71.96 ± 0.98	70.42 ± 0.95
11.0	88.92 ± 1.77	84.17 ± 2.06	80.49 ± 0.65	77.37 ± 1.28	75.66 ± 1.23	73.72 ± 1.59
12.0	92.48 ± 2.21	87.50 ± 1.93	83.79 ± 0.90	80.17 ± 1.17	78.45 ± 0.81	77.53 ± 1.53

n=3*

TABLE-7E COMPARISON OF CUMULATIVE % DRUG RELEASE OF CEFUROXIME AXETIL LOADED SLN USING PALMITIC ACID AS LIPID

TIME(HRS)	CUMULATIVE PERCENTAGE DRUG RELEASE ± SD					
	SLN 25 PALMITIC ACID 1%	SLN 26 PALMITIC ACID 2%	SLN 27 PALMITIC ACID 4%	SLN 28 PALMITIC ACID 6%	SLN 29 PALMITIC ACID 8%	SLN 30 PALMITIC ACID 10%
0.5	25.55 ± 0.99	24.23 ± 0.58	22.35 ± 0.56	19.05 ± 1.60	16.31 ± 1.81	13.01 ± 0.48
1.0	34.76 ± 0.85	33.62 ± 0.49	31.15 ± 0.71	27.53 ± 1.94	24.96 ± 1.81	21.44 ± 0.65
1.5	42.93 ± 1.16	42.06 ± 0.59	39.28 ± 0.57	35.72 ± 1.76	33.50 ± 1.50	29.00 ± 0.43
2.0	50.05 ± 1.48	48.50 ± 0.76	45.51 ± 0.72	42.96 ± 0.58	40.53 ± 0.86	36.17 ± 0.60
3.0	56.66 ± 1.06	55.20 ± 0.87	51.80 ± 0.88	35.72 ± 2.21	46.39 ± 1.75	42.56 ± 0.50
4.0	61.55 ± 0.91	60.83 ± 0.78	58.34 ± 0.88	53.27 ± 2.23	52.69 ± 2.41	49.20 ± 1.13
5.0	65.73 ± 1.19	64.06 ± 0.75	60.98 ± 0.60	58.41 ± 2.38	58.01 ± 2.22	54.48 ± 1.04
6.0	70.13 ± 0.93	68.44 ± 0.63	66.28 ± 0.75	65.19 ± 2.12	63.09 ± 1.55	58.97 ± 0.78
7.0	74.38 ± 1.21	73.15 ± 1.06	71.90 ± 0.63	69.30 ± 1.99	66.90 ± 1.25	62.27 ± 0.69
8.0	79.61 ± 1.22	78.74 ± 0.97	76.92 ± 0.76	71.66 ± 2.01	69.23 ± 1.26	65.59 ± 0.90
9.0	83.75 ± 1.23	82.12 ± 0.65	79.72 ± 0.80	75.44 ± 2.21	72.43 ± 1.15	69.61 ± 0.87
10.0	87.55 ± 1.25	86.38 ± 0.82	83.30 ± 0.81	79.07 ± 1.80	75.94 ± 0.24	73.66 ± 0.65
11.0	90.72 ± 0.97	89.63 ± 0.67	86.99 ± 0.65	82.64 ± 2.09	80.13 ± 1.07	76.98 ± 0.72
12.0	94.01 ± 1.41	92.44 ± 0.95	89.59 ± 0.51	86.04 ± 1.68	84.27 ± 1.02	80.90 ± 0.91

n=3*

TABLE-8A RELEASE KINETICS OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL BEHENATE (COMPRITOL) AS LIPID

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
SLN 1	0.891	4.917	0.973	-0.038	0.979	19.82	0.947	0.566	0.947	-0.110
SLN 2	0.905	4.852	0.971	-0.035	0.982	19.74	0.940	0.615	0.954	-0.106
SLN 3	0.866	4.174	0.944	-0.029	0.975	16.33	0.957	0.465	0.922	-0.089
SLN 4	0.897	4.084	0.956	-0.027	0.981	16.45	0.949	0.564	0.939	-0.083
SLN 5	0.912	4.050	0.962	-0.026	0.981	16.56	0.917	0.662	0.948	-0.081
SLN 6	0.918	4.006	0.964	-0.025	0.980	16.56	0.879	0.771	0.951	-0.079

TABLE-8B RELEASE KINETICS OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL MONOSTEARATE AS LIPID

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
SLN 7	0.840	4.699	0.944	-0.038	0.970	18.13	0.970	0.419	0.916	-0.110
SLN 8	0.847	4.553	0.944	-0.036	0.971	17.60	0.961	0.430	0.918	-0.104
SLN 9	0.862	4.238	0.943	-0.031	0.972	16.46	0.956	0.447	0.921	-0.092
SLN 10	0.878	4.140	0.949	-0.029	0.979	16.26	0.960	0.476	0.929	-0.088
SLN 11	0.880	4.049	0.947	-0.027	0.975	16.06	0.949	0.509	0.928	-0.084
SLN 12	0.890	4.056	0.951	-0.027	0.973	16.30	0.922	0.574	0.934	-0.083

TABLE-8C RELEASE KINETICS OF CEFUROXIME AXETIL LOADED SLN USING GLYCERYL MONOOLEATE AS LIPID

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
SLN 13	0.879	6.016	0.986	-0.065	0.988	23.63	0.989	0.455	0.968	-0.168
SLN 14	0.885	5.917	0.984	-0.064	0.987	23.38	0.983	0.477	0.966	-0.159
SLN 15	0.879	5.776	0.979	-0.055	0.979	23.06	0.966	0.515	0.957	-0.148
SLN 16	0.909	5.690	0.984	-0.048	0.987	23.16	0.966	0.596	0.968	-0.137
SLN 17	0.907	5.501	0.979	-0.044	0.982	22.55	0.942	0.647	0.962	-0.127
SLN 18	0.912	5.332	0.977	-0.040	0.980	22.19	0.900	0.783	0.961	-0.118

TABLE-8D RELEASE KINETICS OF CEFUROXIME AXETIL LOADED SLN USING STEARIC ACID AS LIPID

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
SLN 19	0.874	6.264	0.978	-0.079	0.988	24.43	0.990	0.437	0.972	-0.191
SLN 20	0.888	6.130	0.986	-0.066	0.988	24.45	0.983	0.484	0.971	-0.170
SLN 21	0.904	6.071	0.988	-0.059	0.987	24.29	0.977	0.544	0.974	-0.158
SLN 22	0.911	5.940	0.986	-0.053	0.987	24.18	0.961	0.603	0.972	-0.147
SLN 23	0.920	5.871	0.988	-0.051	0.989	23.97	0.959	0.622	0.975	-0.143
SLN 24	0.917	5.787	0.984	-0.049	0.985	23.74	0.954	0.648	0.971	-0.138

TABLE-8E RELEASE KINETICS OF CEFUROXIME AXETIL LOADED SLN USING PALMITIC ACID AS LIPID

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
SLN 25	0.868	6.189	0.972	-0.085	0.992	23.65	0.993	0.393	0.973	-0.199
SLN 26	0.873	6.150	0.978	-0.080	0.991	23.60	0.992	0.403	0.973	-0.191
SLN 27	0.884	6.094	0.986	-0.072	0.993	23.61	0.994	0.423	0.974	-0.179
SLN 28	0.892	5.949	0.987	-0.062	0.992	23.35	0.991	0.455	0.971	-0.163
SLN 29	0.893	5.870	0.983	-0.058	0.989	23.28	0.985	0.487	0.967	-0.154
SLN 30	0.912	5.853	0.988	-0.053	0.992	23.58	0.945	0.536	0.974	-0.147

FIGURE-1A DETERMINATION OF λ_{MAX} OF CEFUROXIME AXETIL

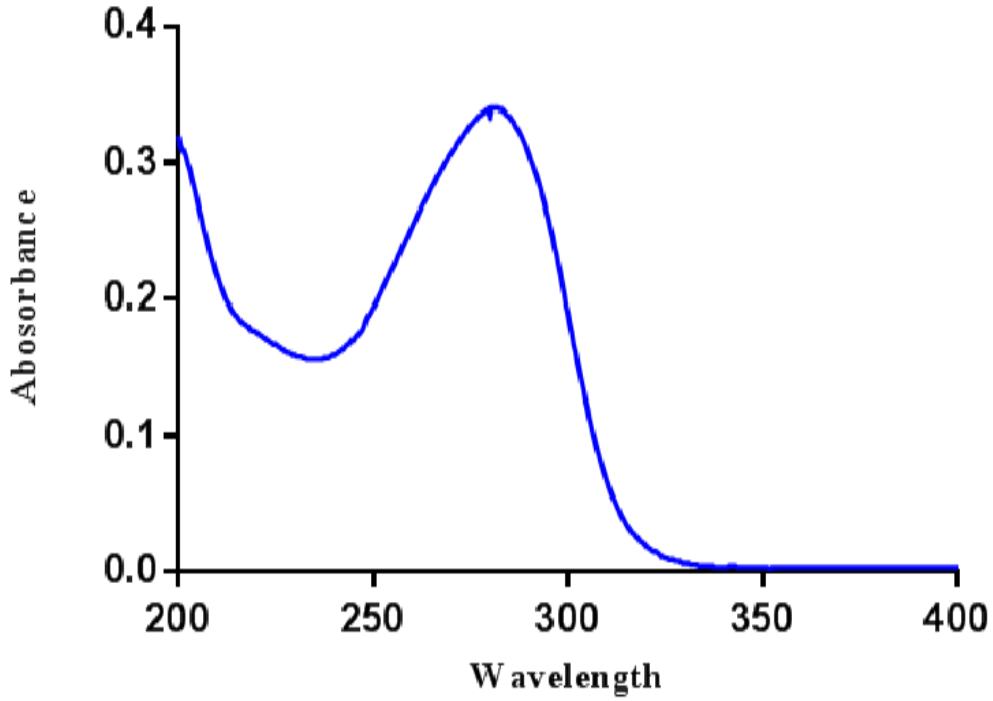


FIGURE-1B CALIBRATIONOF CEFUROXIME AXETIL

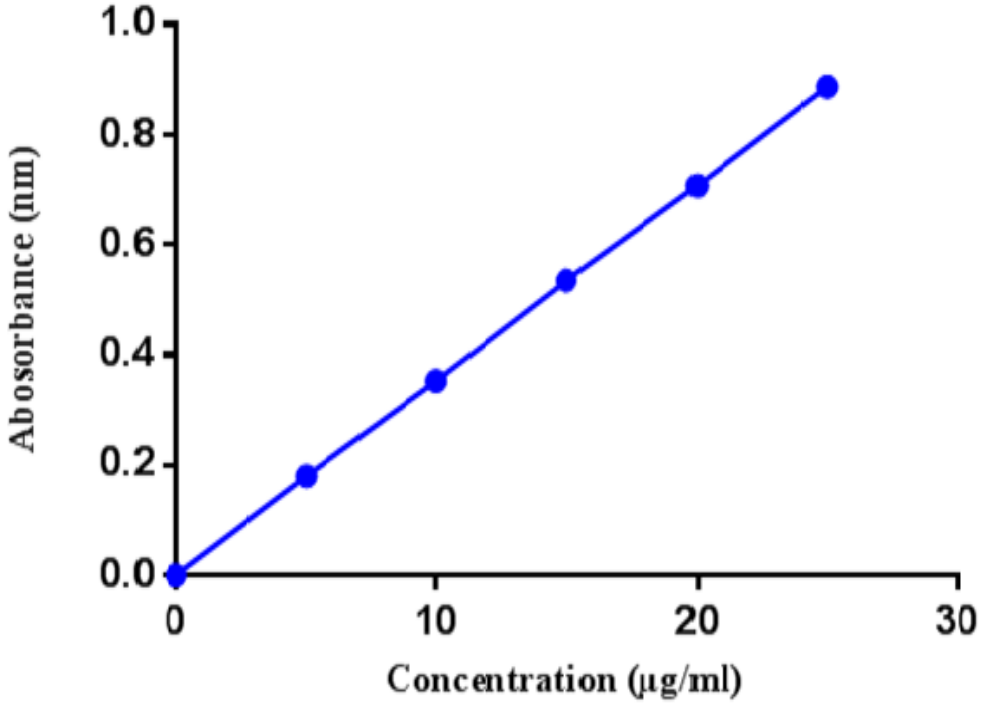


FIGURE-2 ENTRAPMENT EFFICIENCY OF CEFUROXIME AXETIL LOADED SLN USING DIFFERENT LIPIDS AT DIFFERENT CONCENTRATIONS

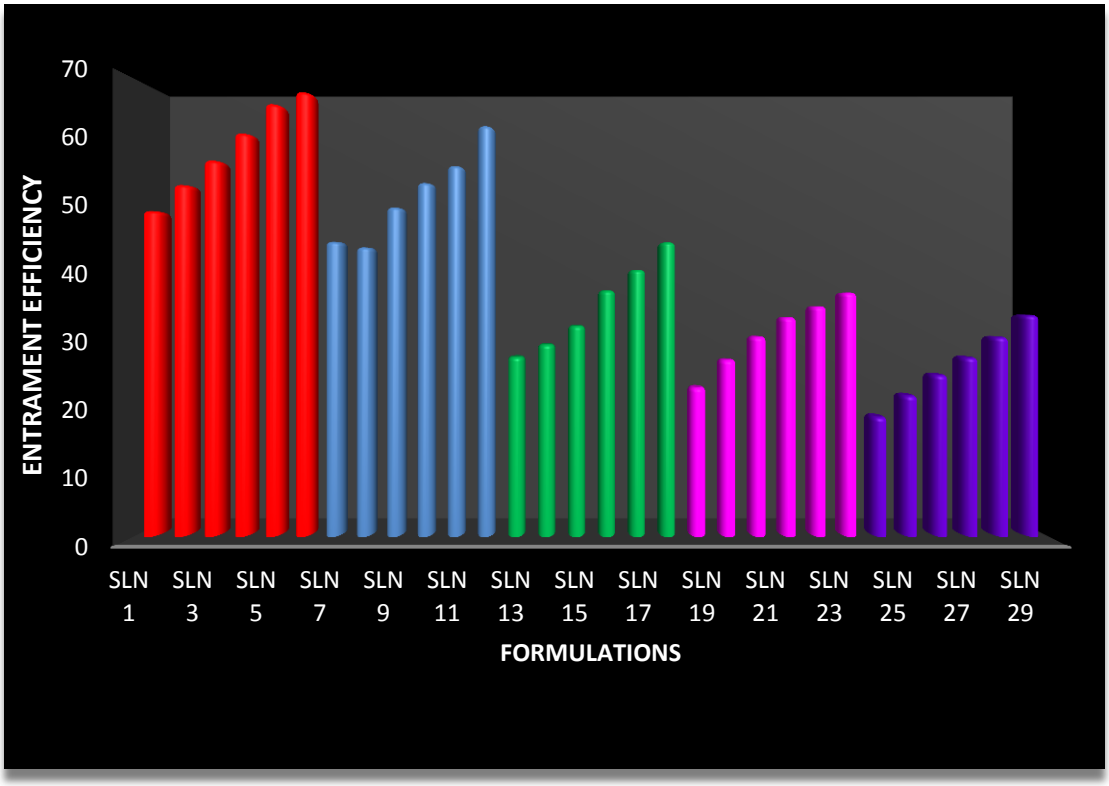


FIGURE-3A FT-IR SPECTRA OF CEFUROXIME AXETIL

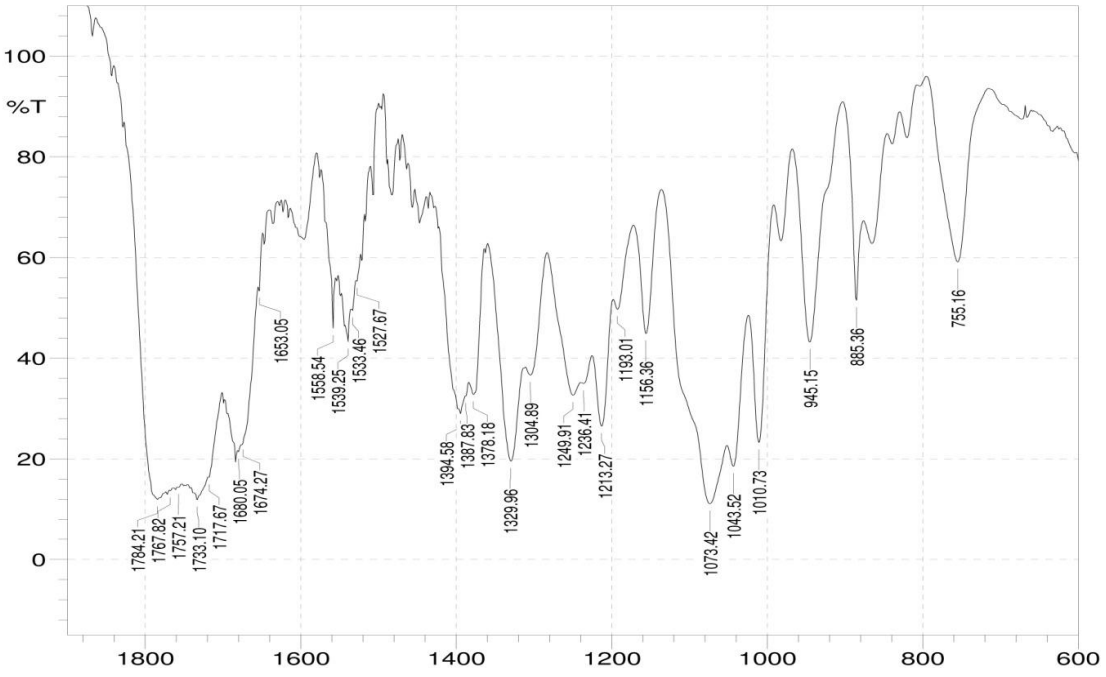


FIGURE-3B FT-IR SPECTRA OF GLYCERYL BEHENATE (COMPRITOL ATO 888)

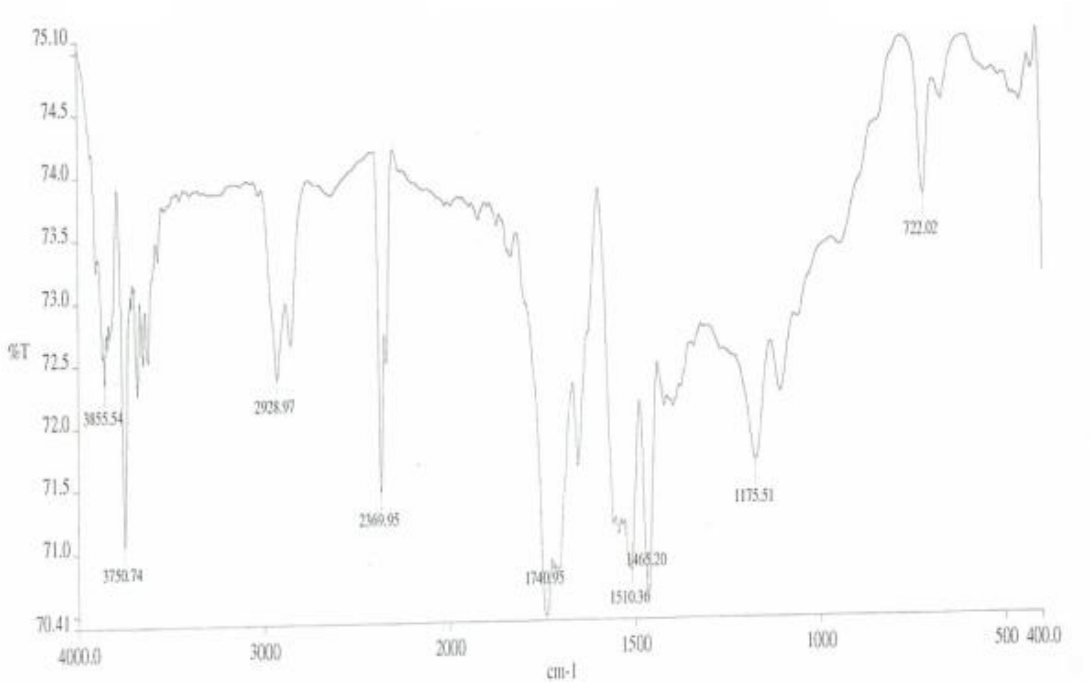


FIGURE-3C FT-IR SPECTRA OF GLYCERYL MONOSTEARATE

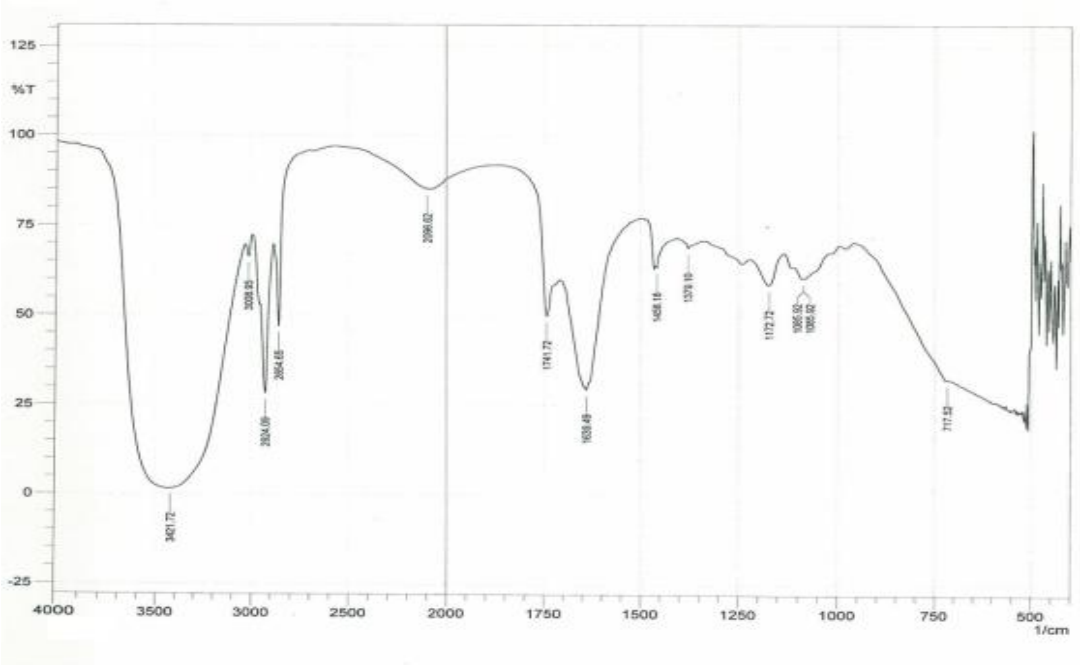


FIGURE-3D FT-IR SPECTRA OF GLYCERYL MONOOLEATE

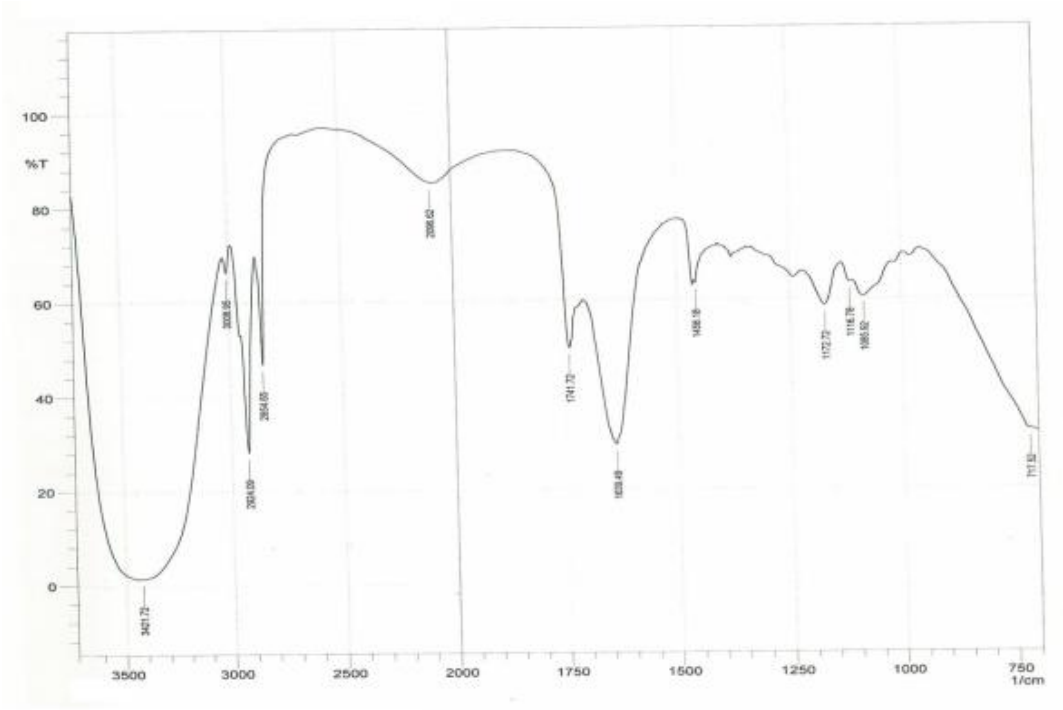


FIGURE-3E FT-IR SPECTRA OF STEARIC ACID

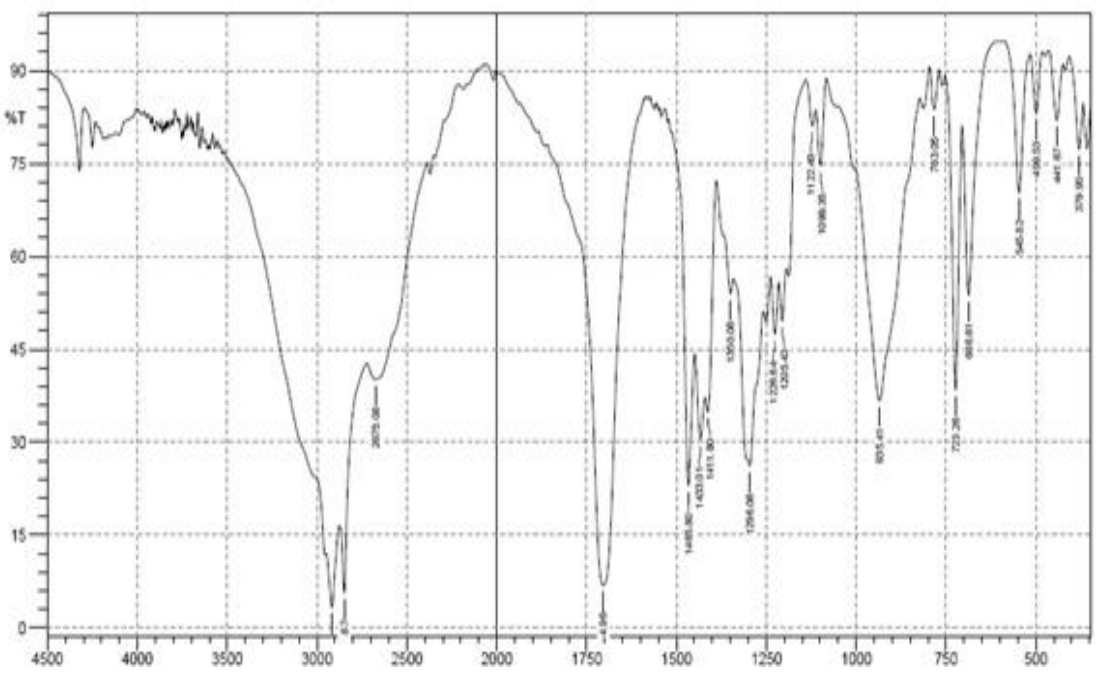


FIGURE-3F FT-IR SPECTRA OF PALMITIC ACID

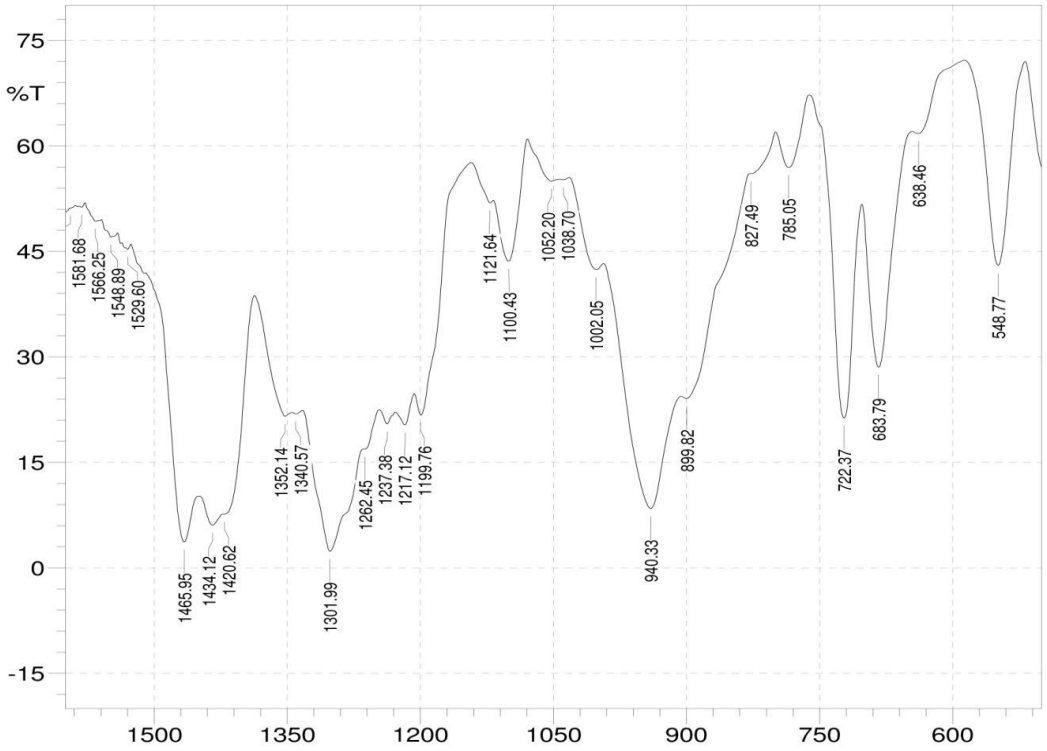


FIGURE-3G FT-IR SPECTRA OF POLOXAMER 188

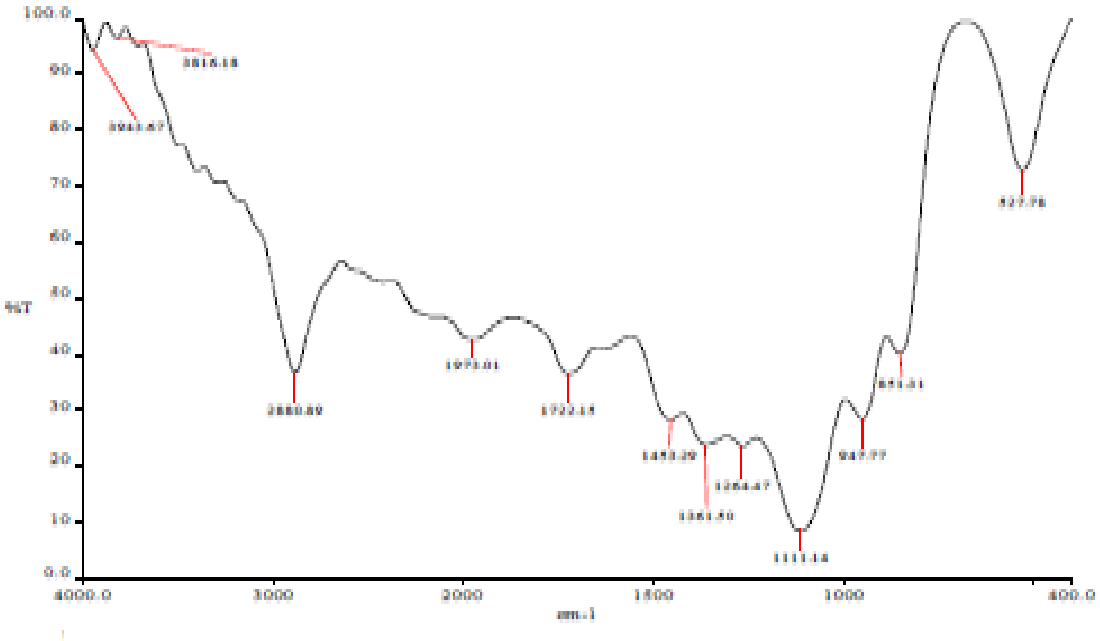


FIGURE-3H FT-IR SPECTRA OF SOYA LECITHIN

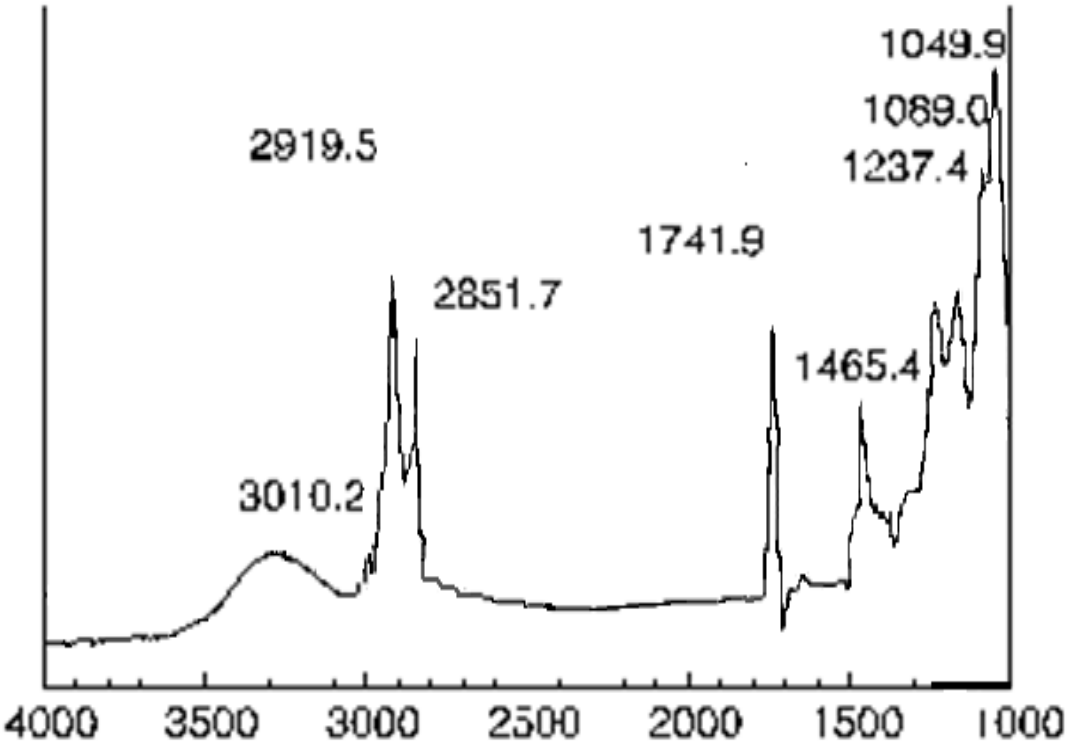


FIGURE-3I FT-IR SPECTRA OF CARBOPOL 934

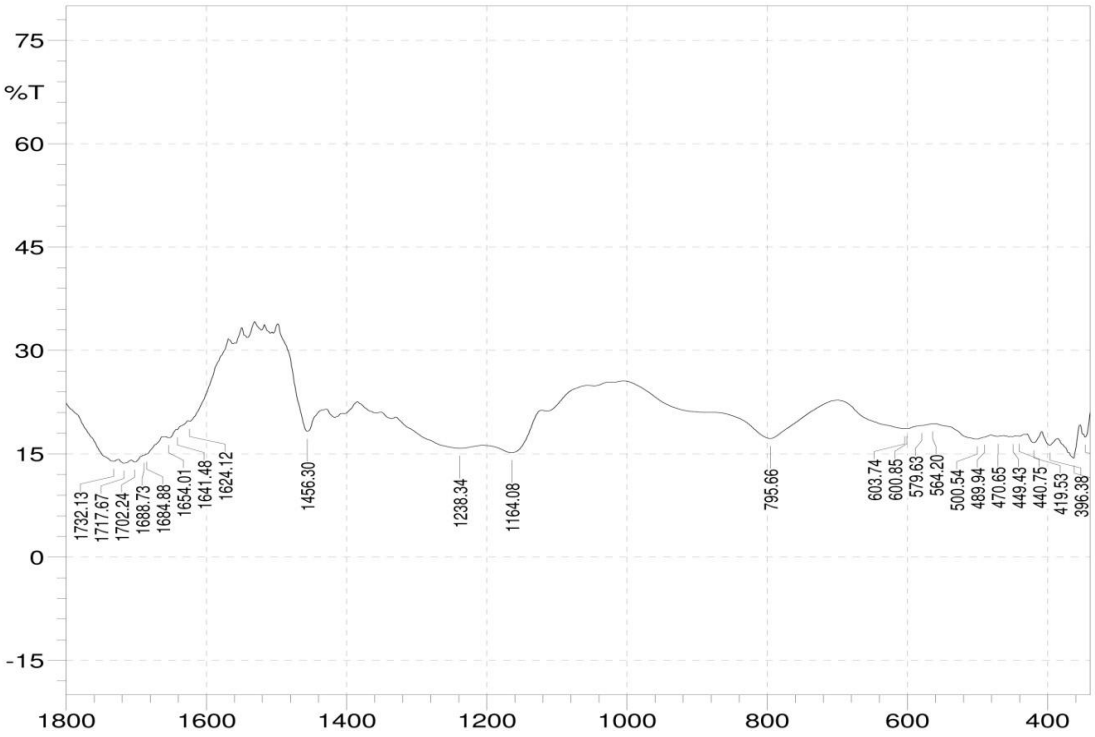


FIGURE-3J FT-IR SPECTRA OF HPMC K15M

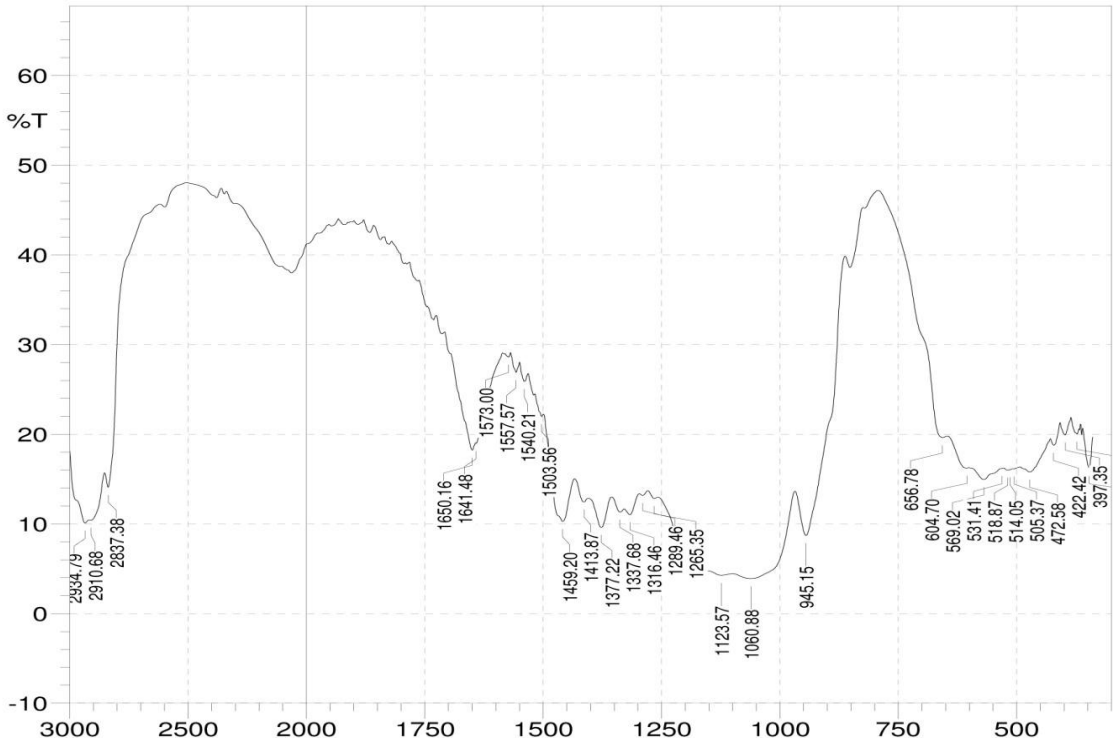


FIGURE-3K FT-IR SPECTRA OF PHYSICAL MIXTURE (CEFUROXIME AXETIL + COMPRITOL + HPMC K15M + CARBOPOL 934)

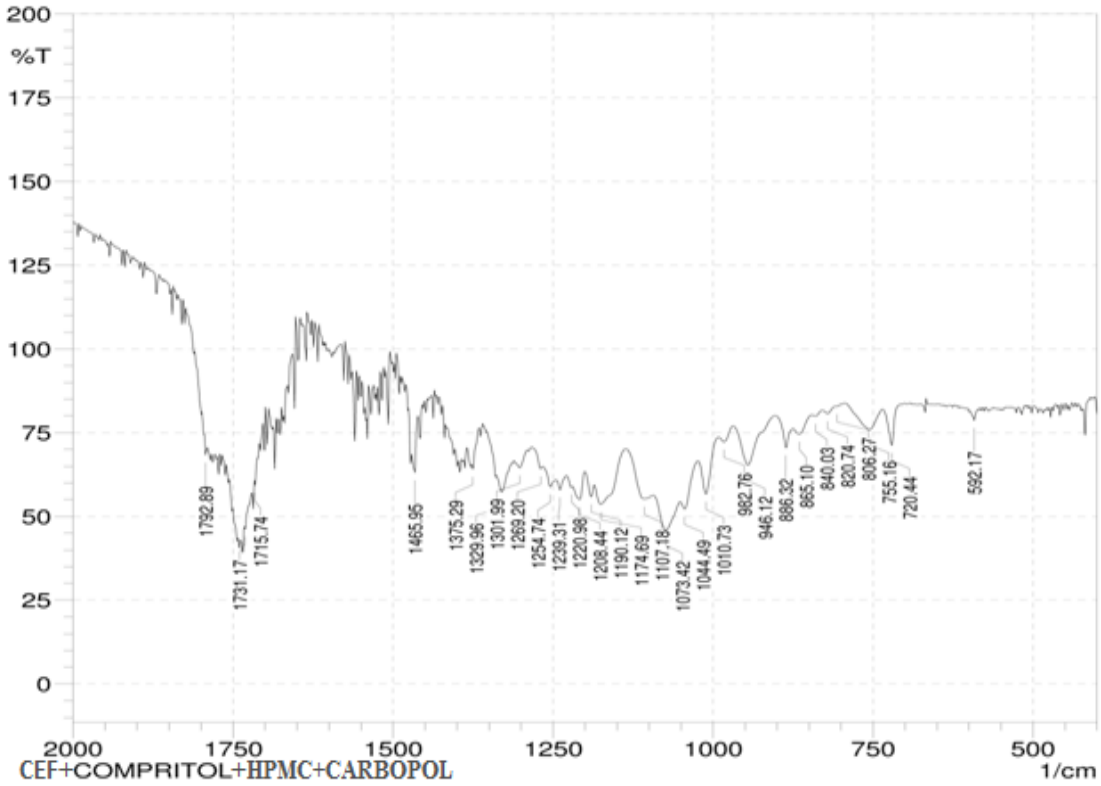


FIGURE-3L FT-IR SPECTRA OF PHYSICAL MIXTURE (CEFUROXIME AXETIL + GMS + HPMC K15M + CARBOPOL 934)

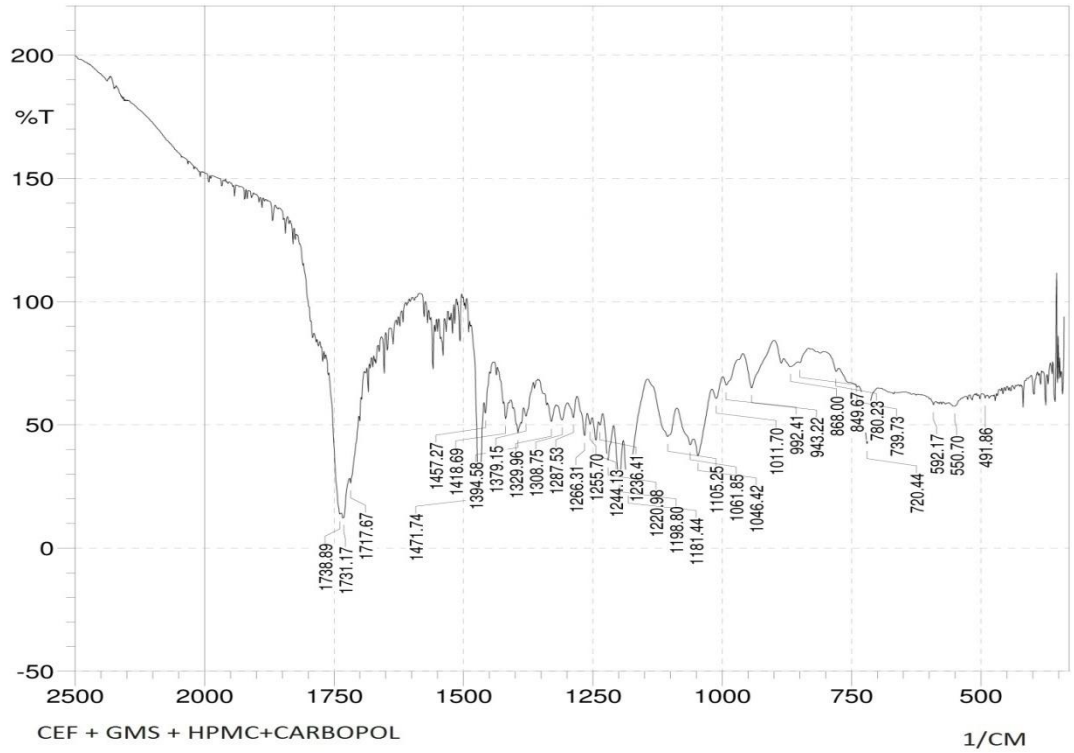


FIGURE-3M FT-IR SPECTRA OF PHYSICAL MIXTURE (CEFUROXIME AXETIL + GMO + HPMC K15M + CARBOPOL 934)

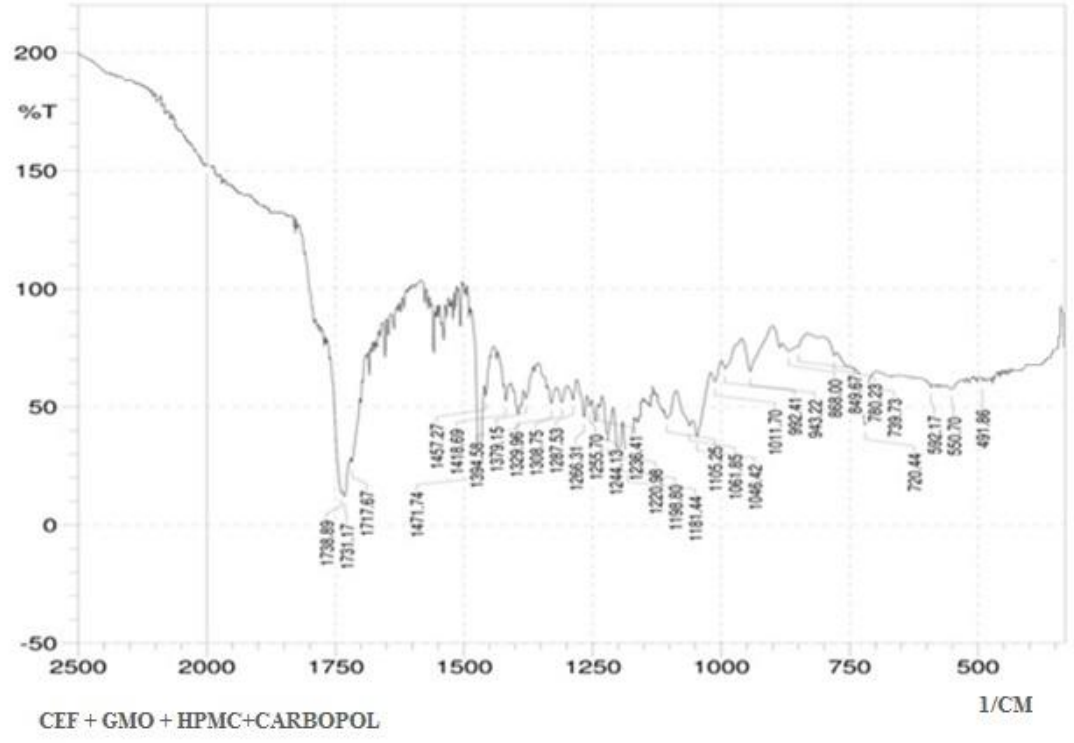


FIGURE-3N FT-IR SPECTRA OF PHYSICAL MIXTURE (CEFUROXIME AXETIL + STEARIC ACID + HPMC K15M + CARBOPOL 934)

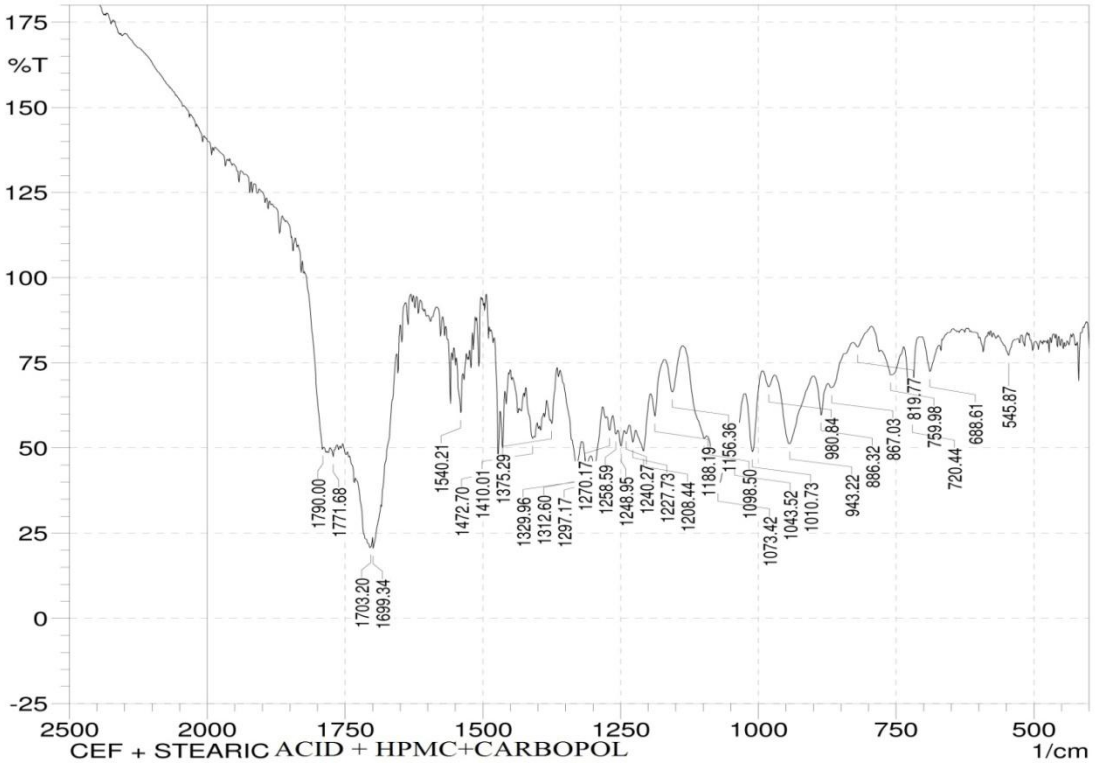


FIGURE-3O FT-IR SPECTRA OF PHYSICAL MIXTURE (CEFUROXIME AXETIL + PALMITIC ACID + HPMC K15M + CARBOPOL 934)

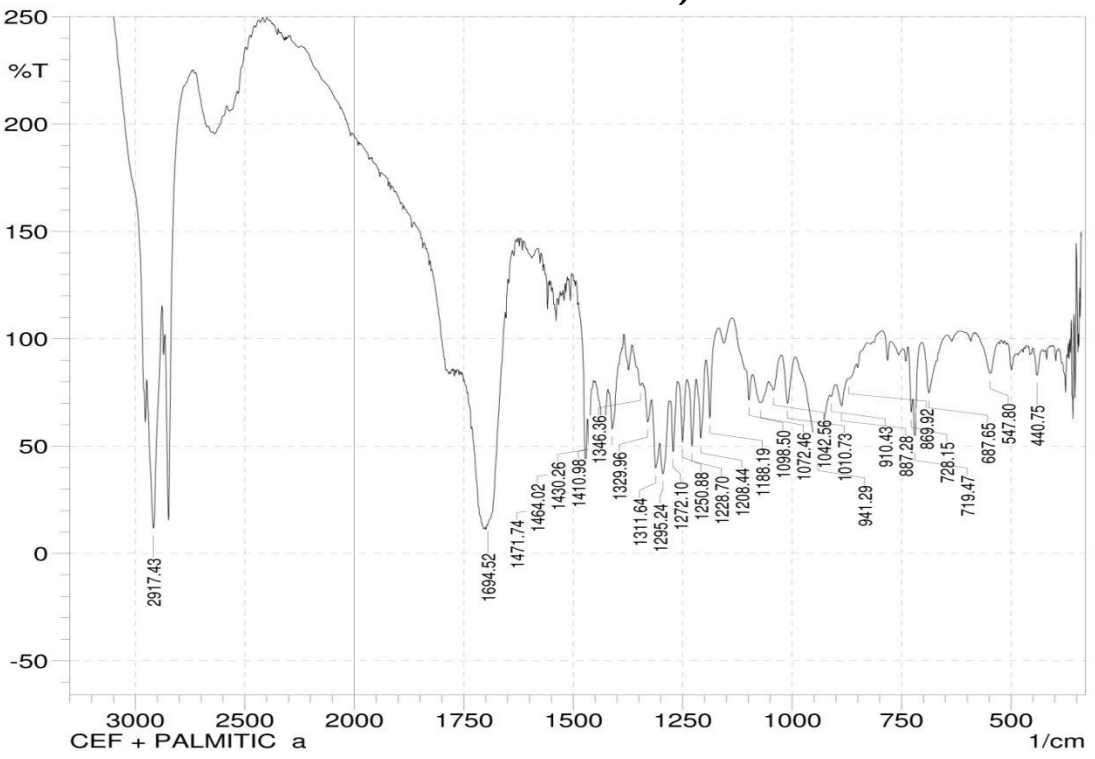


FIGURE-3P FT-IR SPECTRA OF FINAL FORMULATION-F1 (CEFUROXIME AXETIL + COMPRITOL + HPMC K15M + CARBOPOL 934)

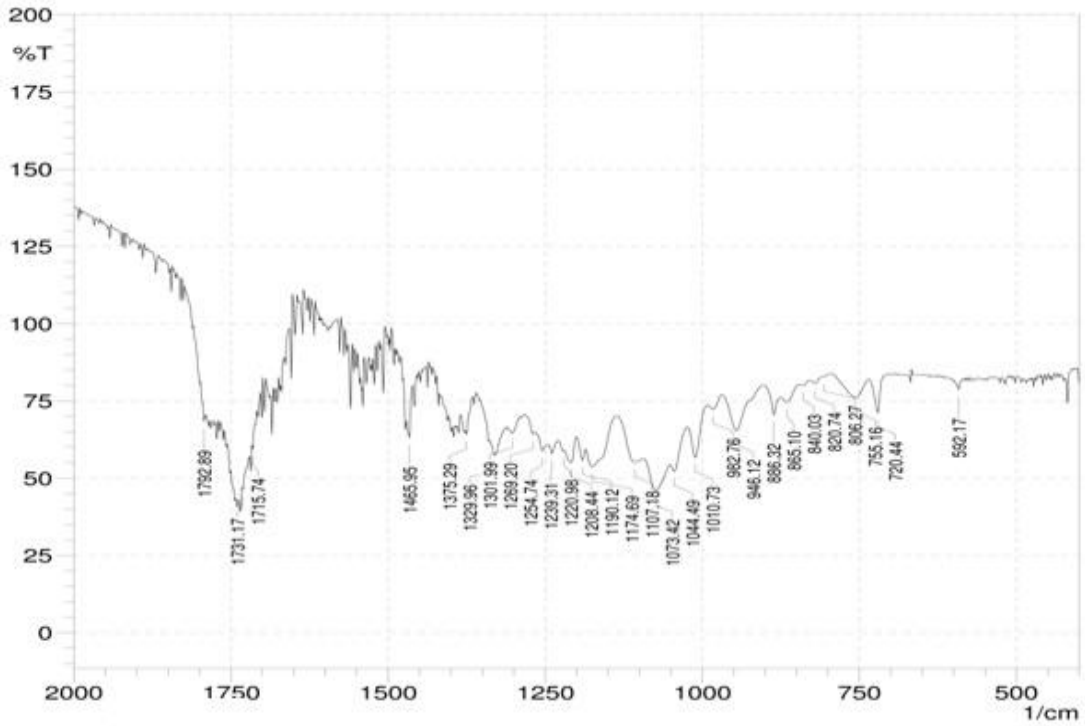


FIGURE-3Q FT-IR SPECTRA OF FINAL FORMULATION-F2 (CEFUROXIME AXETIL + GMS + HPMC K15M + CARBOPOL 934)

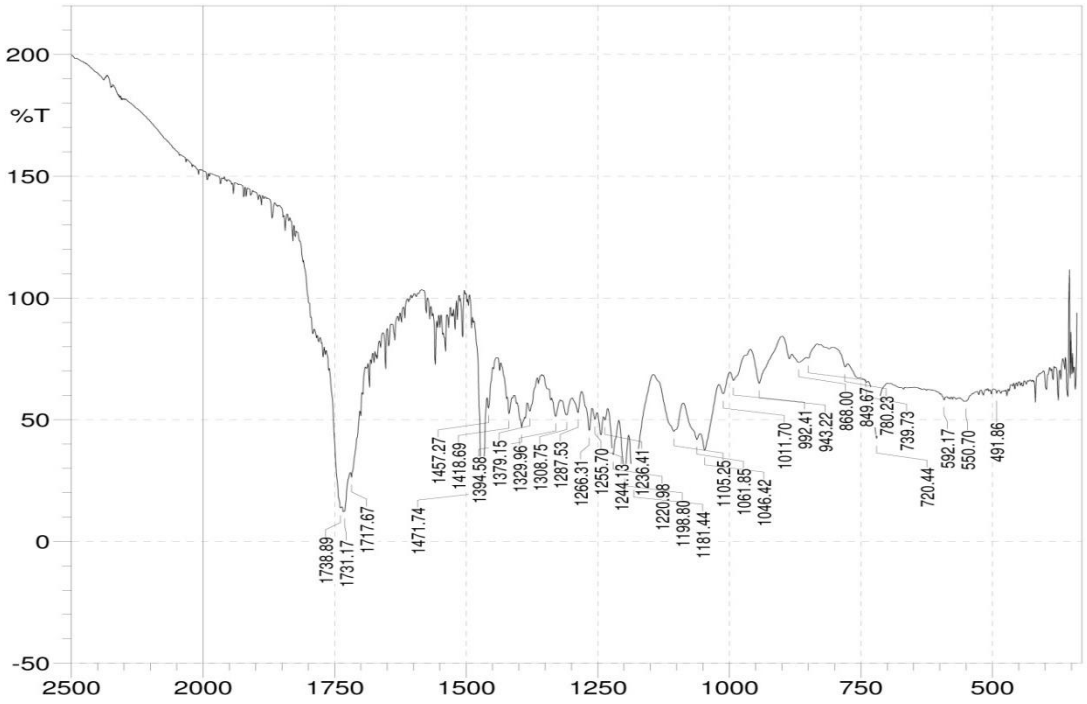


FIGURE-3R FT-IR SPECTRA OF FINAL FORMULATION-F3 (CEFUROXIME AXETIL + GMO + HPMC K15M + CARBOPOL 934)

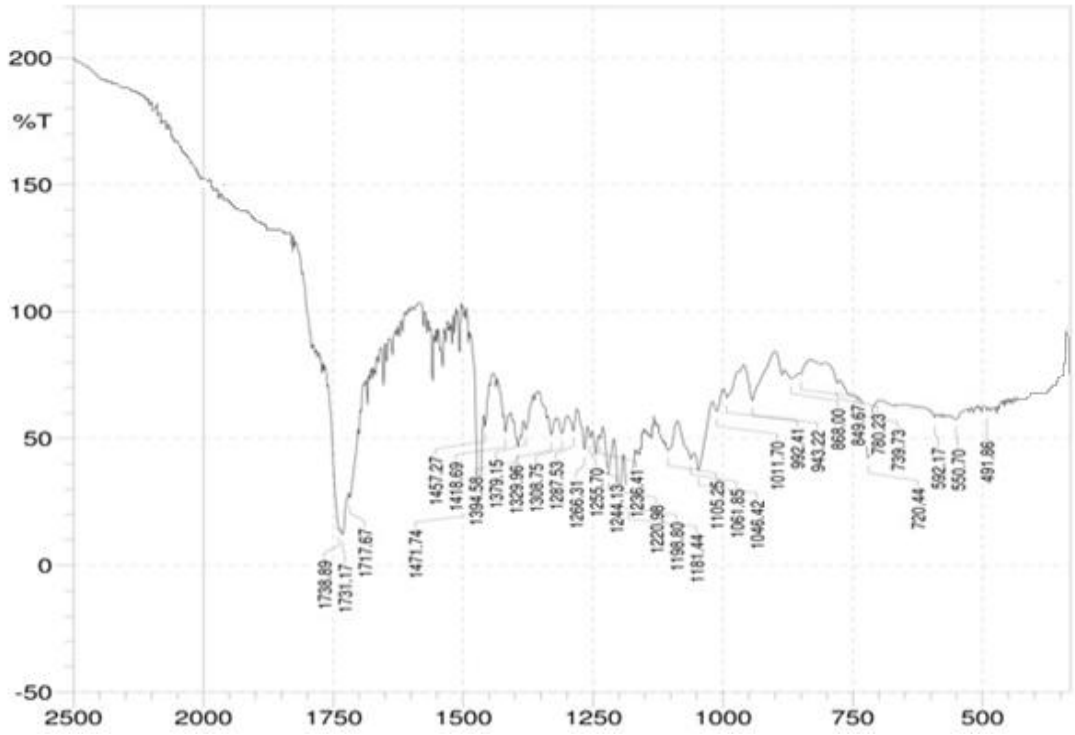


FIGURE-4A INVITRO DRUG RELEASE STUDIES OF SLN LOADED WITH CEFUROXIME AXETIL USING COMPRITOL AS LIPID

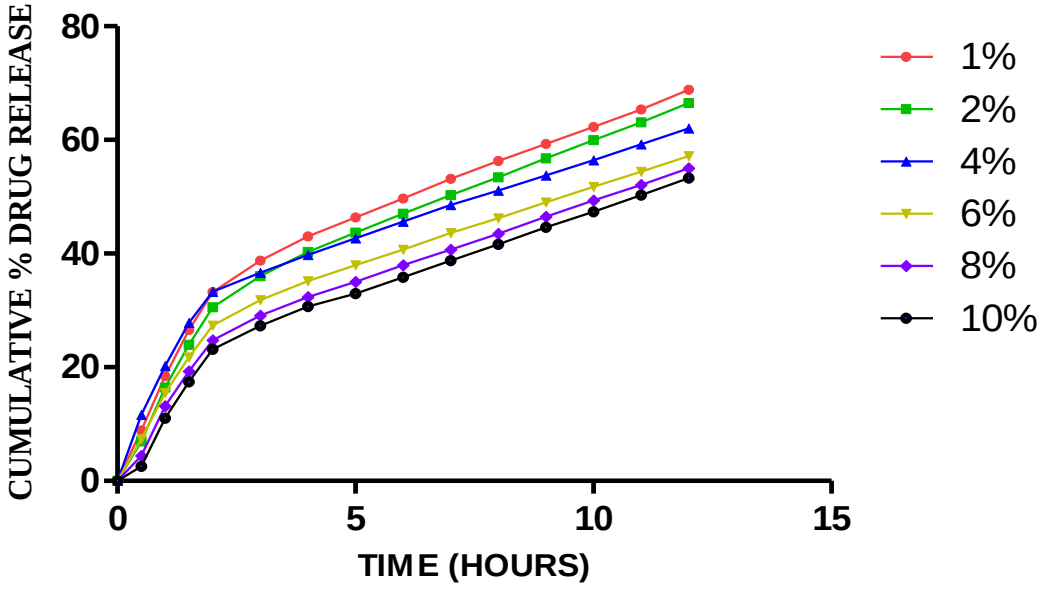


FIGURE-4B INVITRO DRUG RELEASE STUDIES OF SLN LOADED WITH CEFUROXIME AXETIL USING GLYCERYL MONOSTERATE AS LIPID

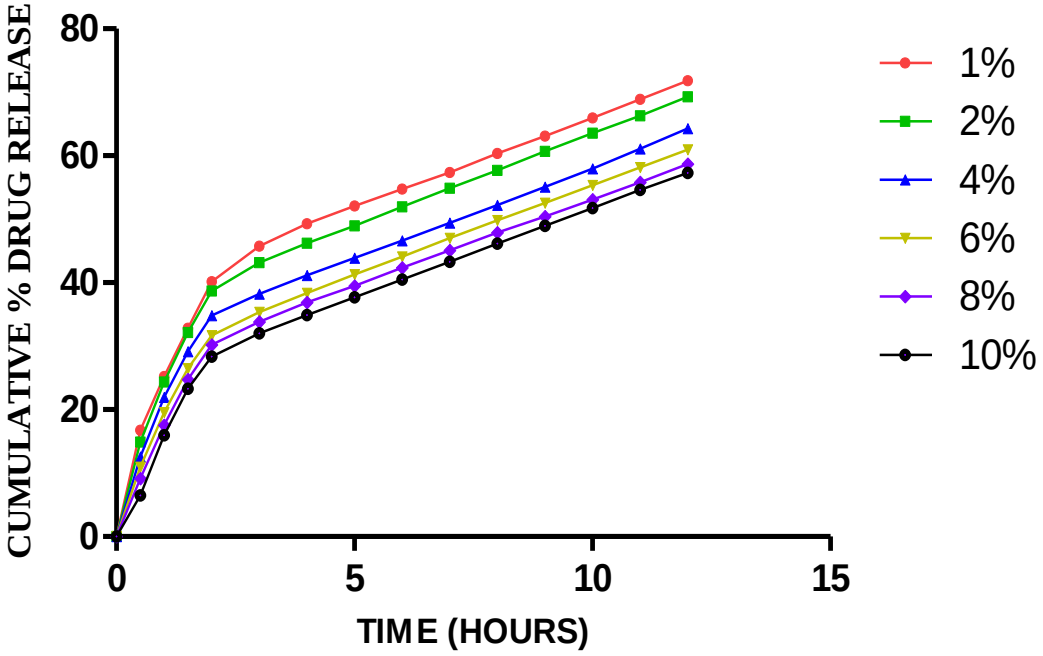


FIGURE-4C INVITRO DRUG RELEASE STUDIES OF SLN LOADED WITH CEFUROXIME AXETIL USING GLYCERYL MONOSTERATE AS LIPID

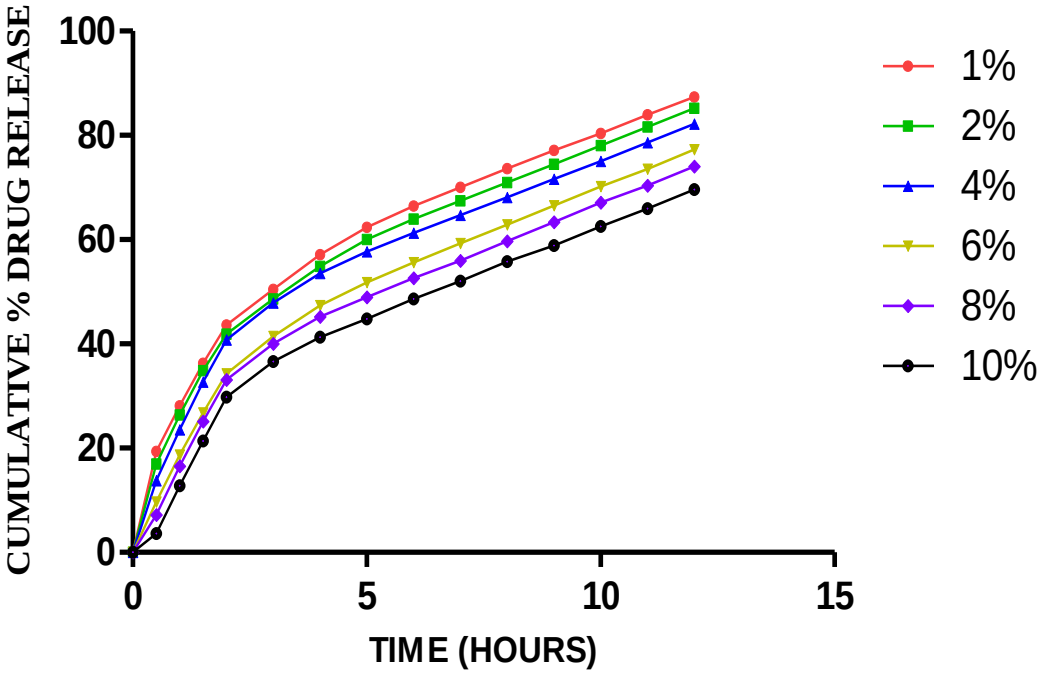


FIGURE-4D INVITRO DRUG RELEASE STUDIES OF SLN LOADED WITH CEFUROXIME AXETIL USING STEARIC ACID AS LIPID

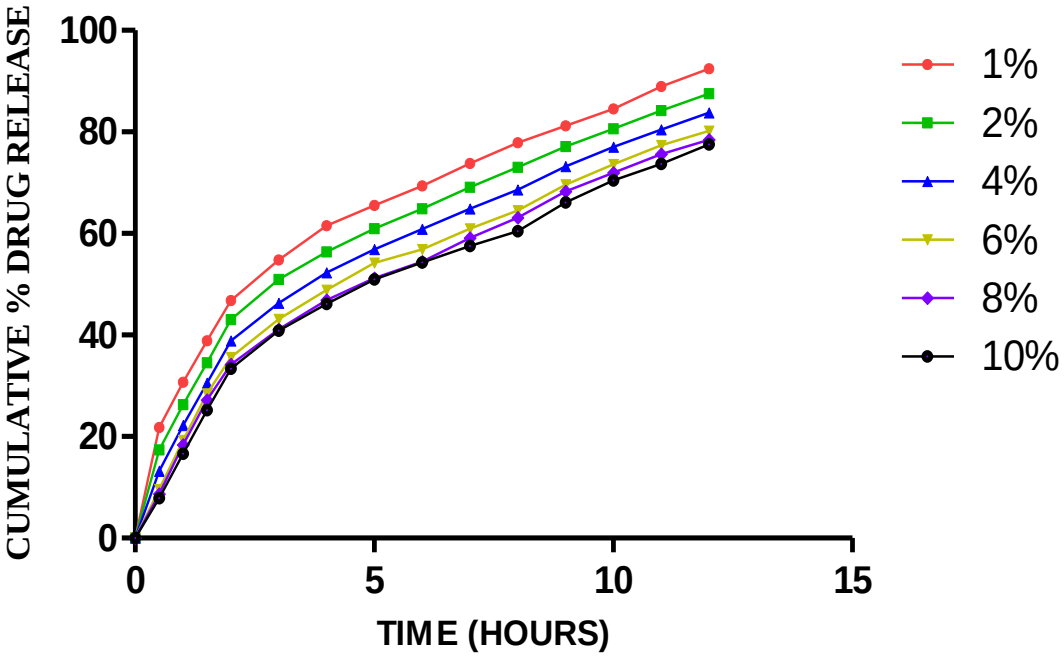
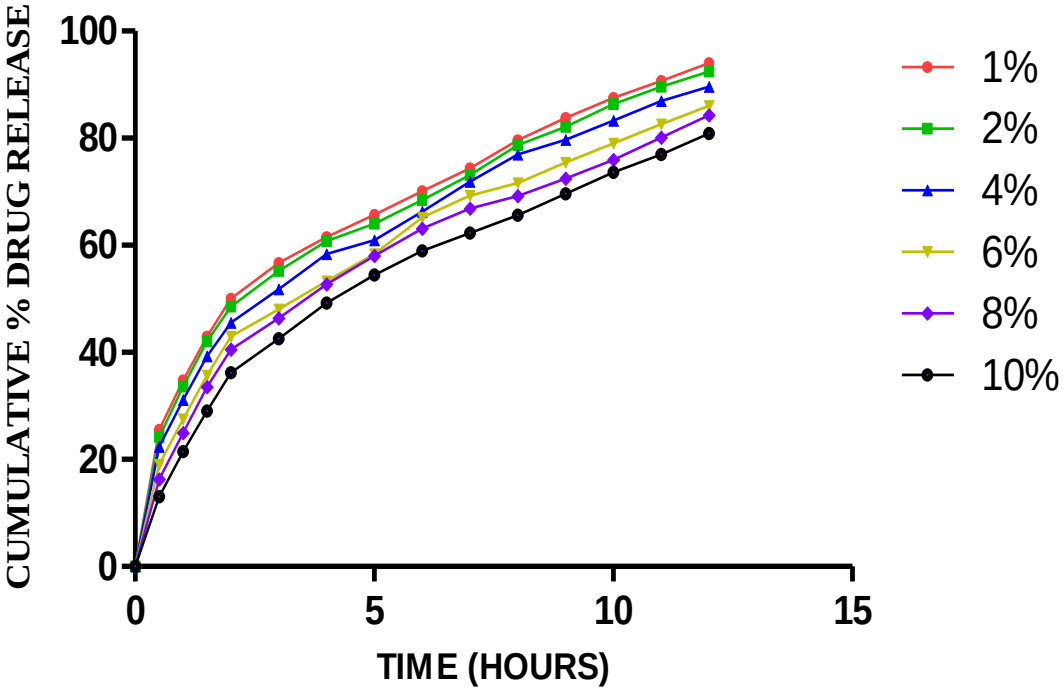


FIGURE-4E INVITRO DRUG RELEASE STUDIES OF SLN LOADED WITH CEFUROXIME AXETIL USING PALMITIC ACID AS LIPID



RELEASE KINETICS

FIGURE-5A FIRST ORDER KINETICS OF SLN USING COMPRITOL AS LIPID

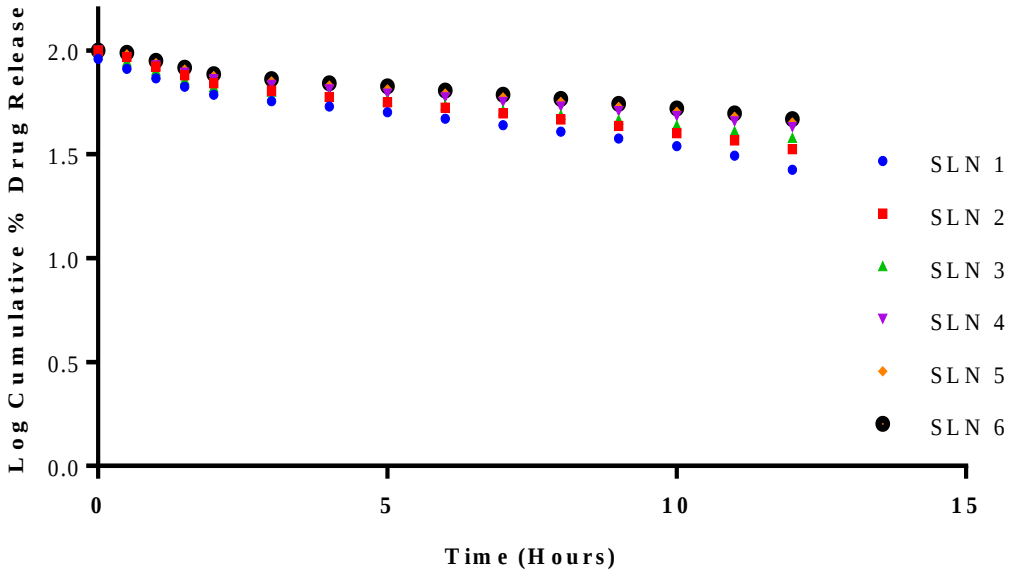


FIGURE-5B FIRST ORDER KINETICS OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

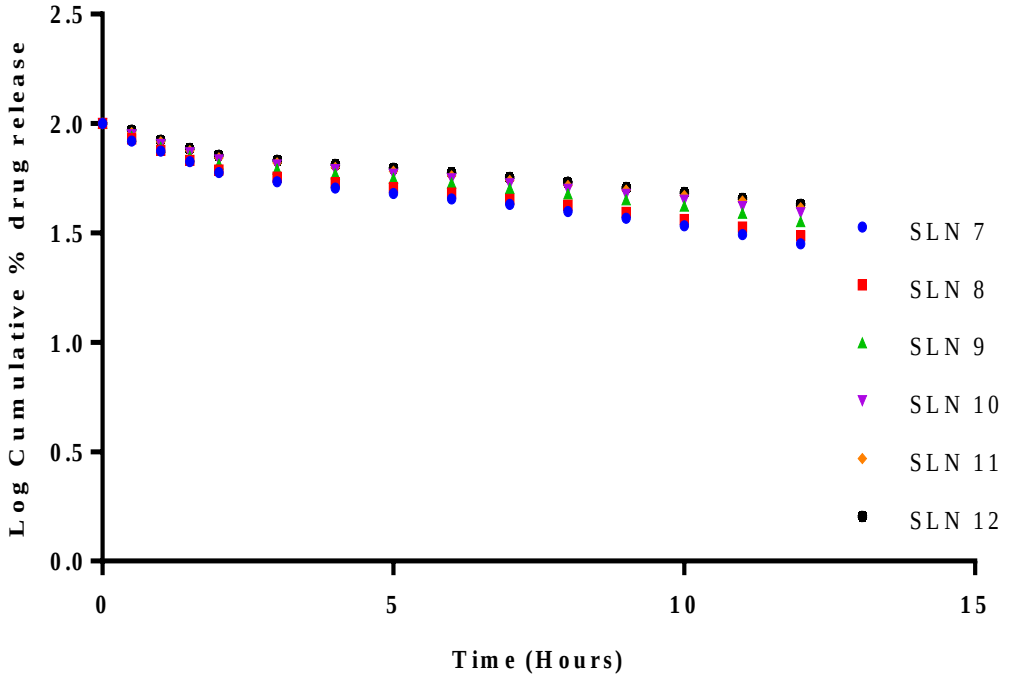


FIGURE-5C FIRST ORDER KINETICS OF SLN USING GLYCERYL MONOOLEATE AS LIPID

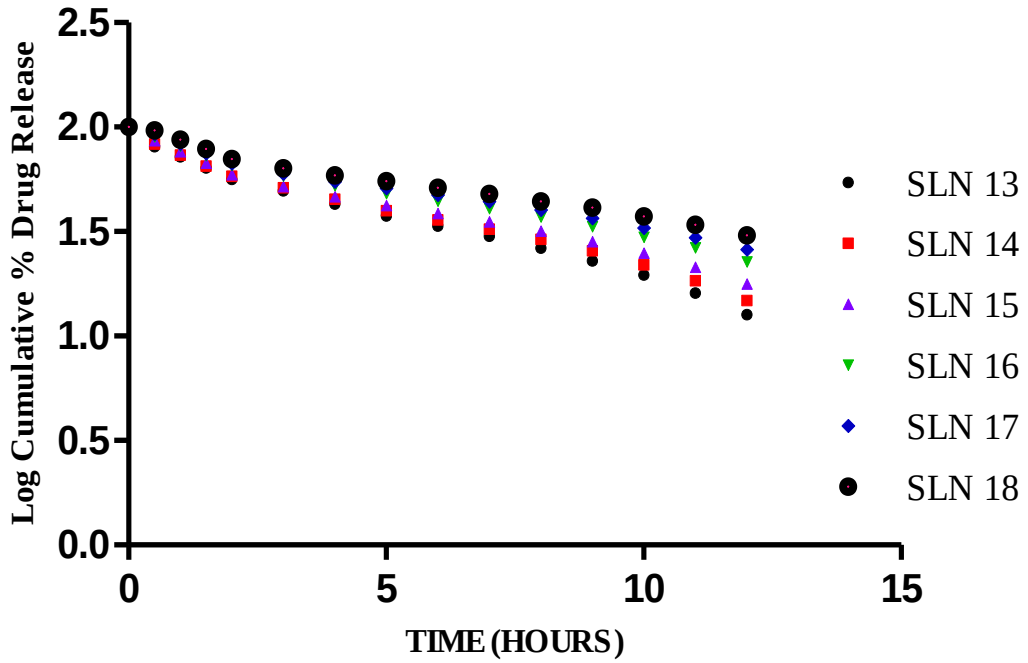


FIGURE-5D FIRST ORDER KINETICS OF SLN USING STEARIC ACID AS LIPID

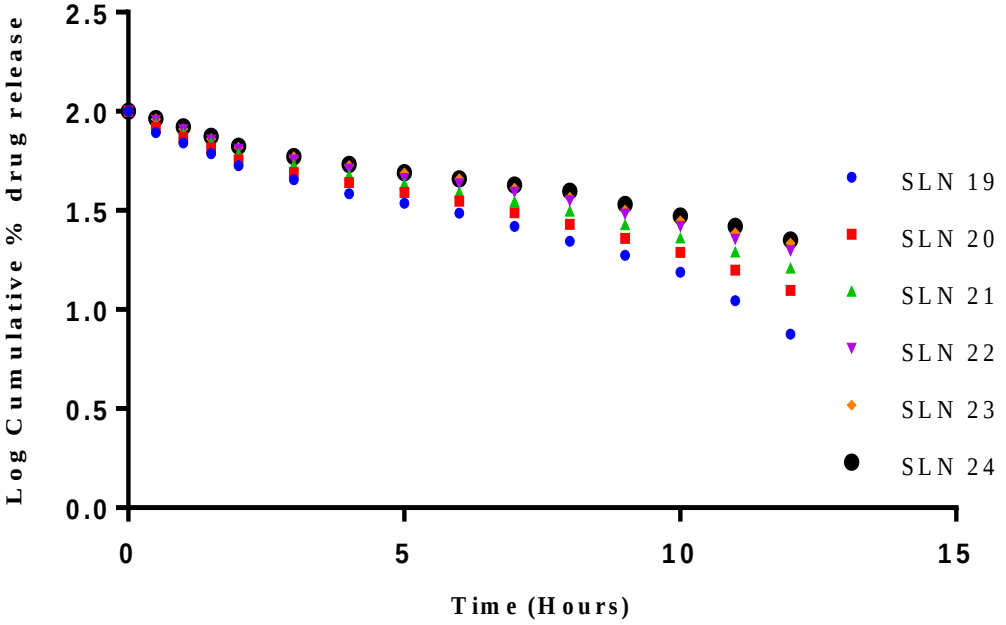


FIGURE-5E FIRST ORDER KINETICS OF SLN USING PALMITIC ACID AS LIPID

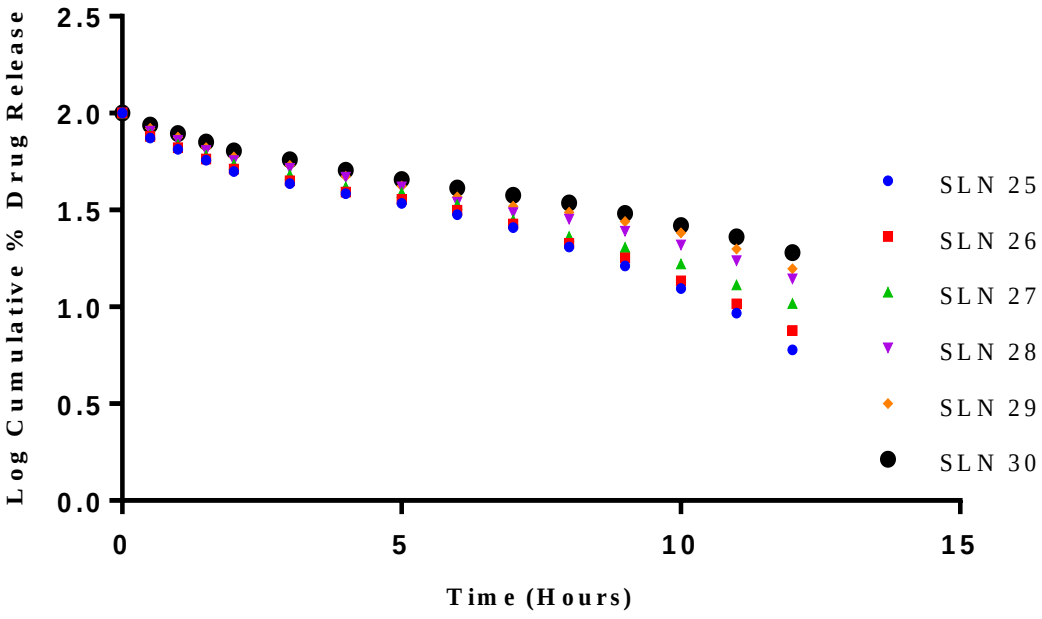


FIGURE-6A ZERO ORDER KINETICS OF SLN USING COMPRITOL AS LIPID

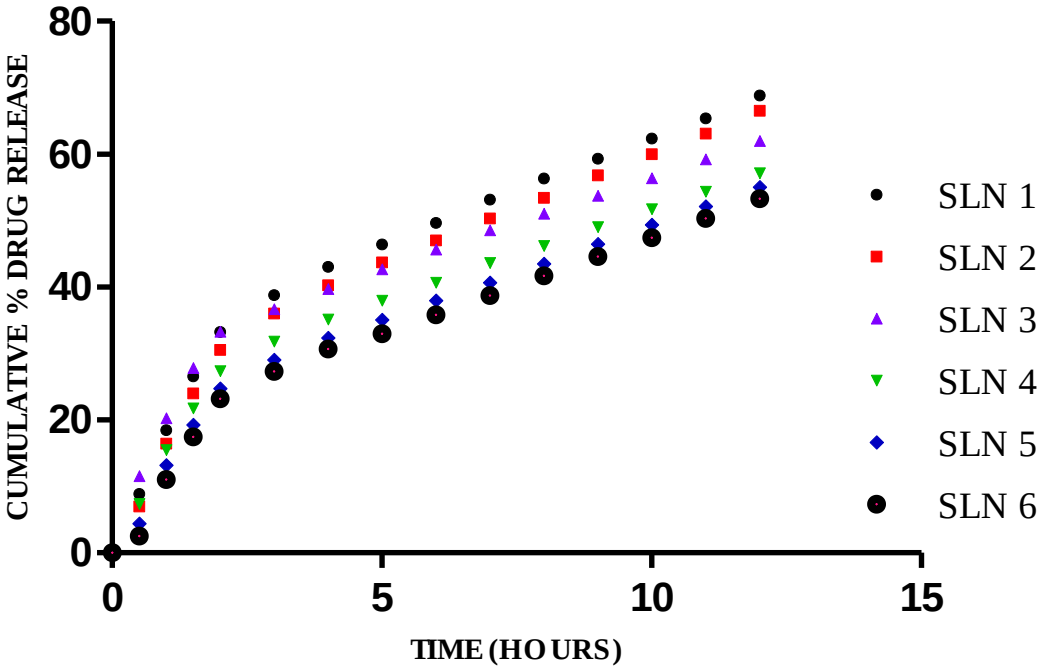


FIGURE-6B ZERO ORDER KINETICS OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

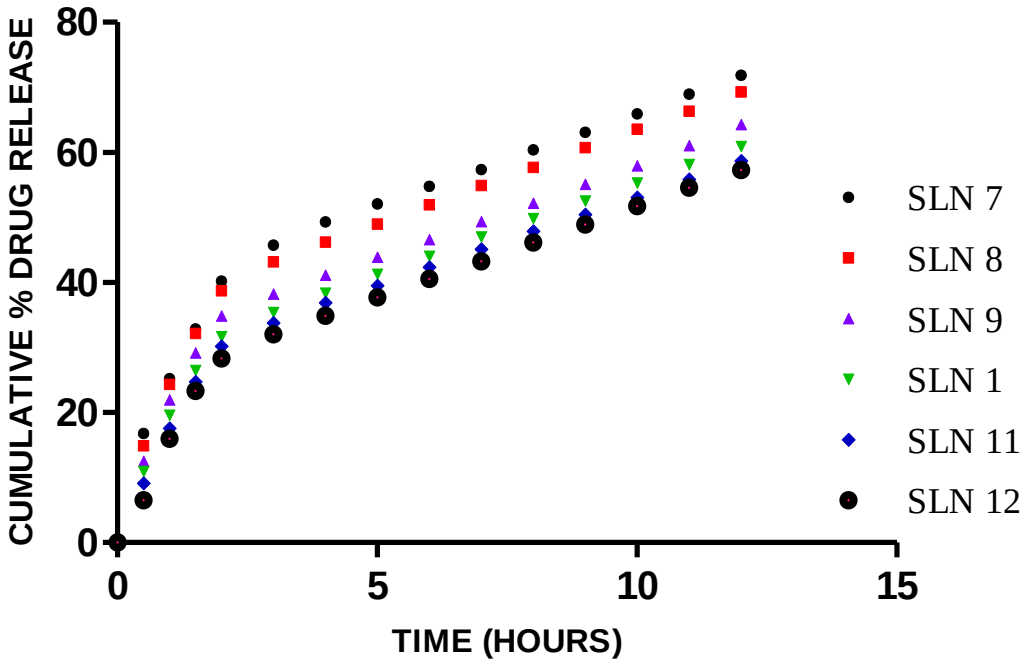


FIGURE-6C ZERO ORDER KINETICS OF SLN USING GLYCERYL MONOOLEATE AS LIPID

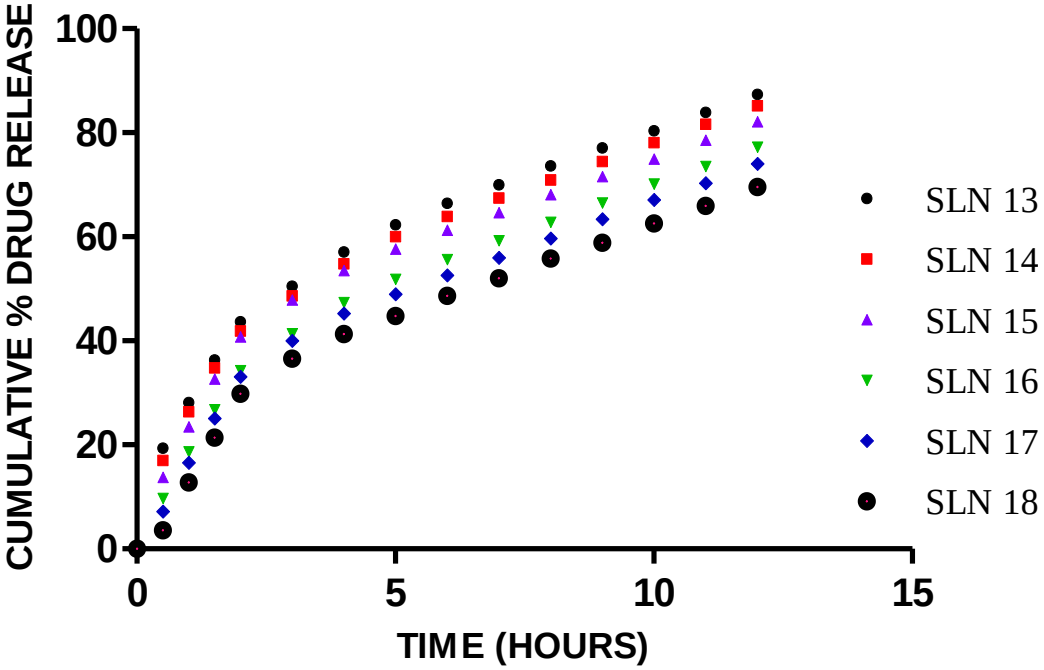


FIGURE-6D ZERO ORDER KINETICS OF SLN USING STEARIC ACID AS LIPID

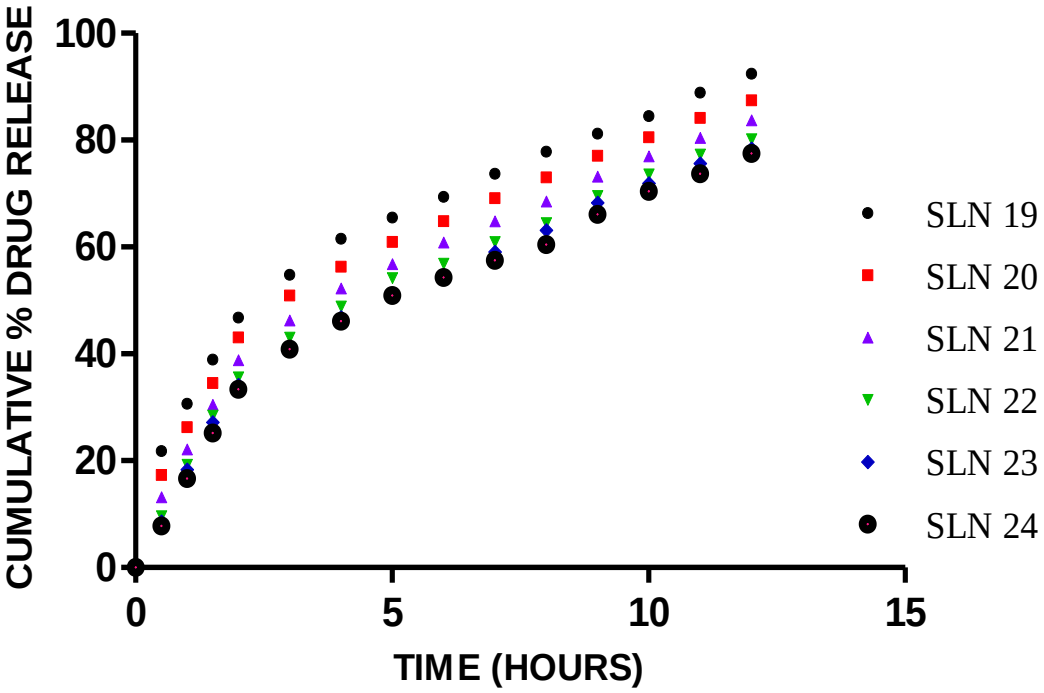


FIGURE-6E ZERO ORDER KINETICS OF SLN USING PALMITIC ACID AS LIPID

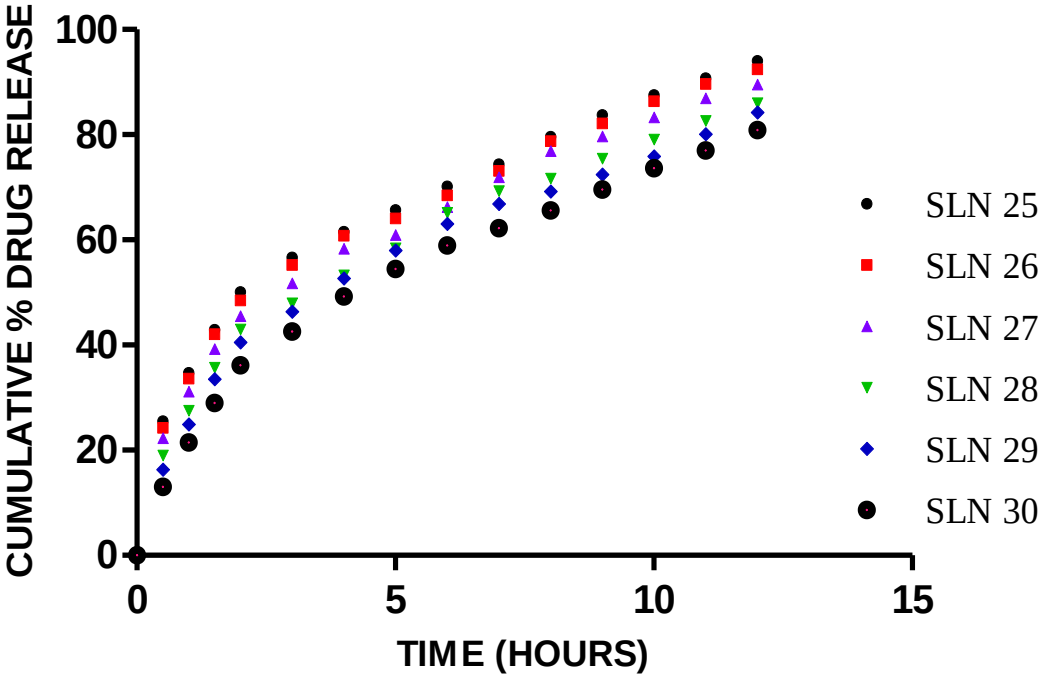


FIGURE-7A HIGUCHI MODEL RELEASE KINETICS OF SLN USING COMPRITOL AS LIPID

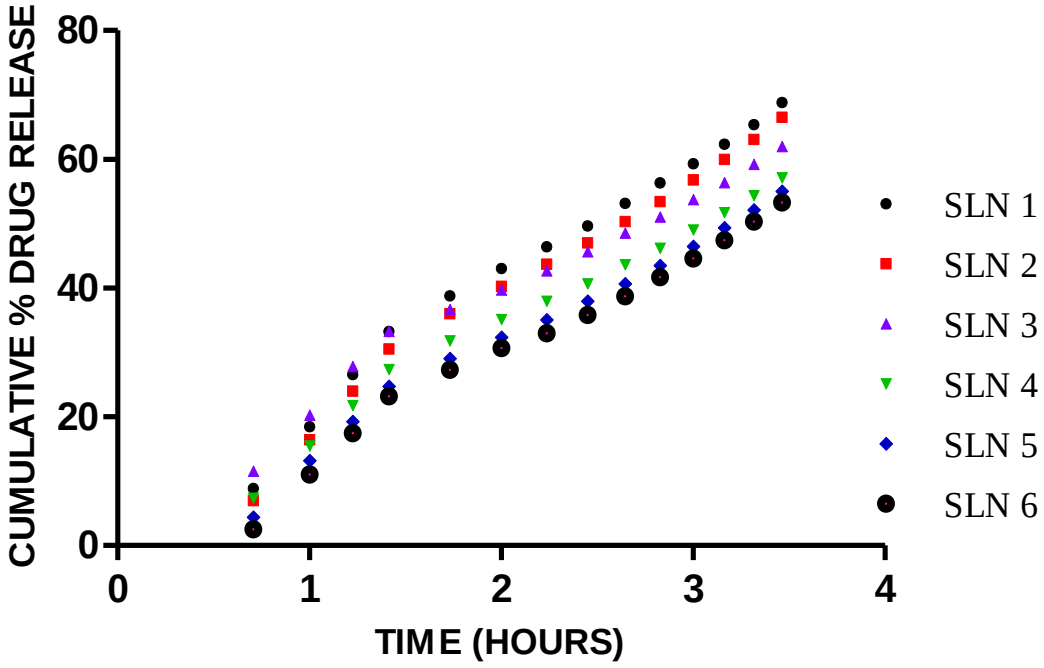


FIGURE-7B HIGUCHI MODEL RELEASE KINETICS OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

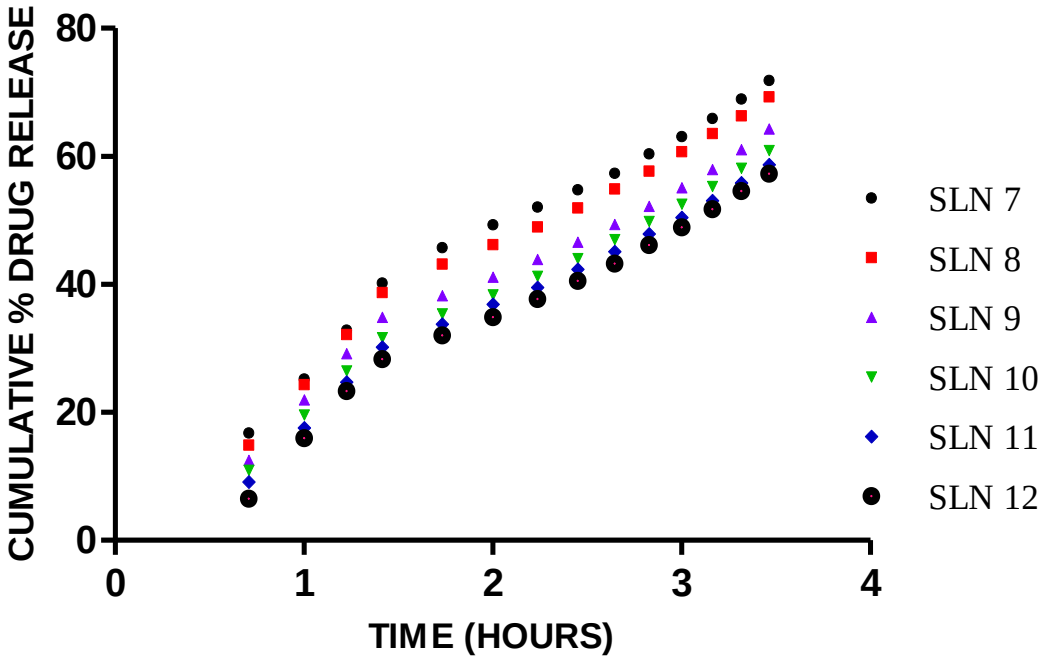


FIGURE-7C HIGUCHI MODEL RELEASE KINETICS OF SLN USING GLYCERYL MONOOLEATE AS LIPID

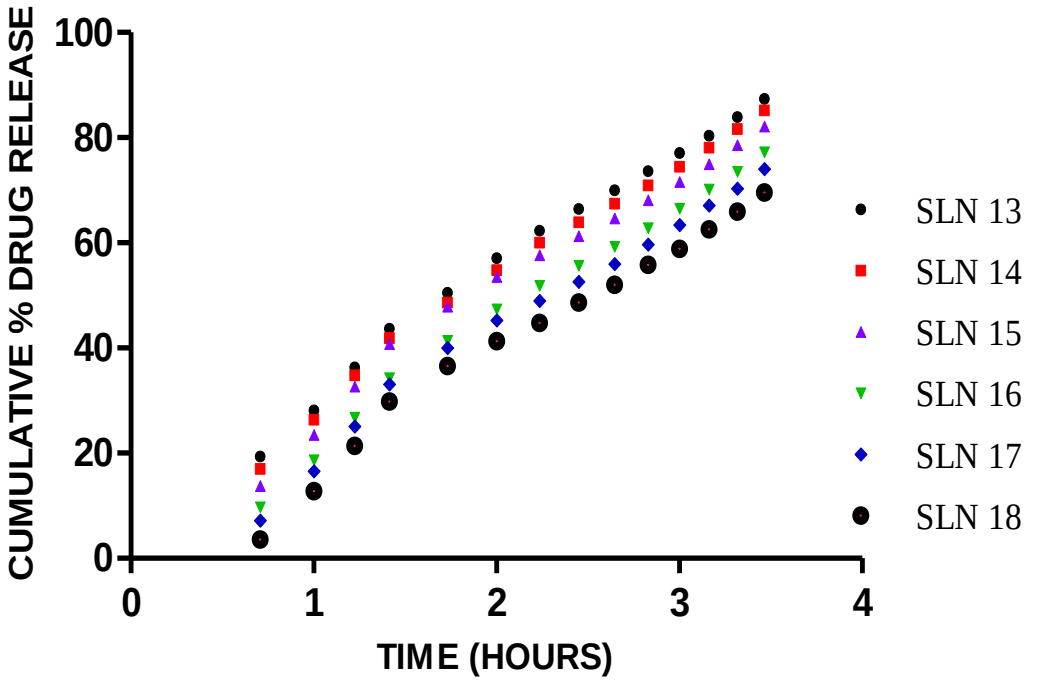


FIGURE-7D HIGUCHI MODEL RELEASE KINETICS OF SLN USING STEARIC ACID AS LIPID

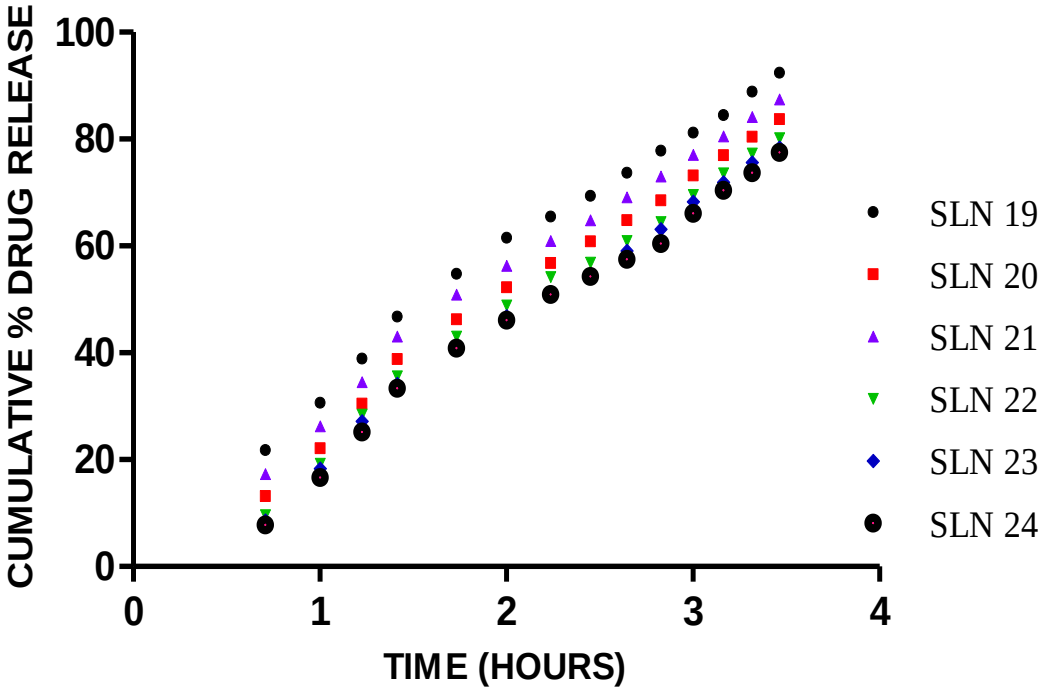


FIGURE-7E HIGUCHI MODEL RELEASE KINETICS OF SLN USING PALMITIC ACID AS LIPID

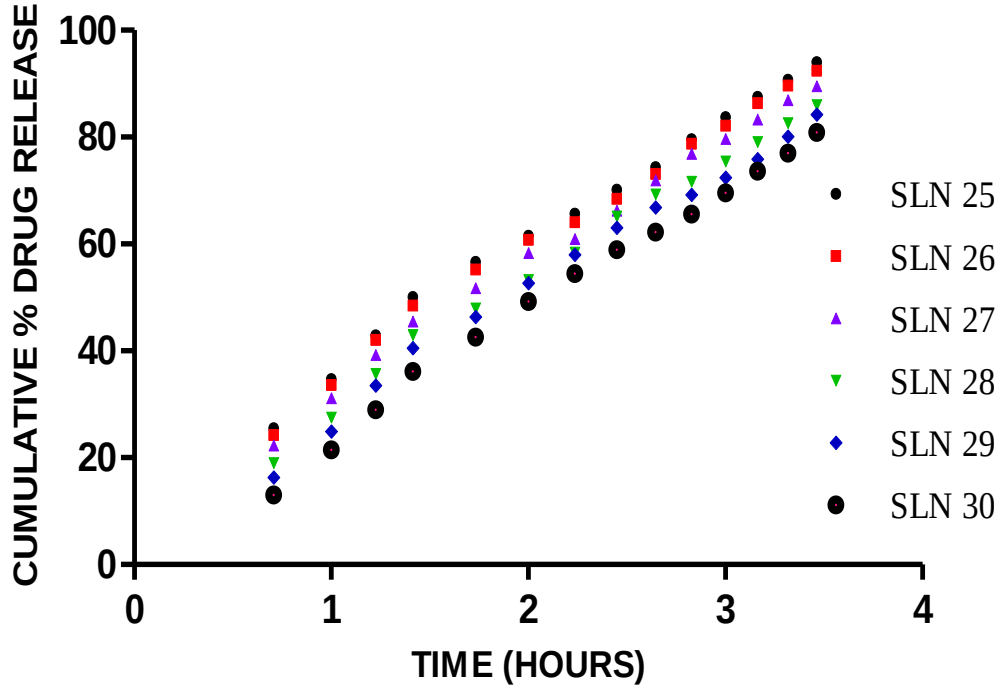


FIGURE-8A KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SLN USING COMPRITOL AS LIPID

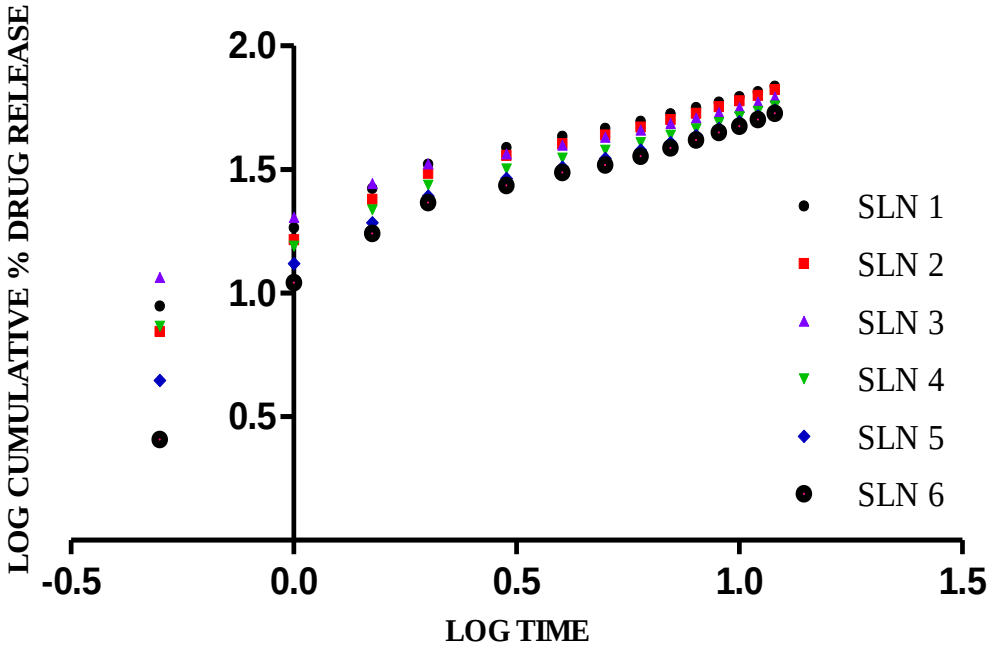


FIGURE-8B KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

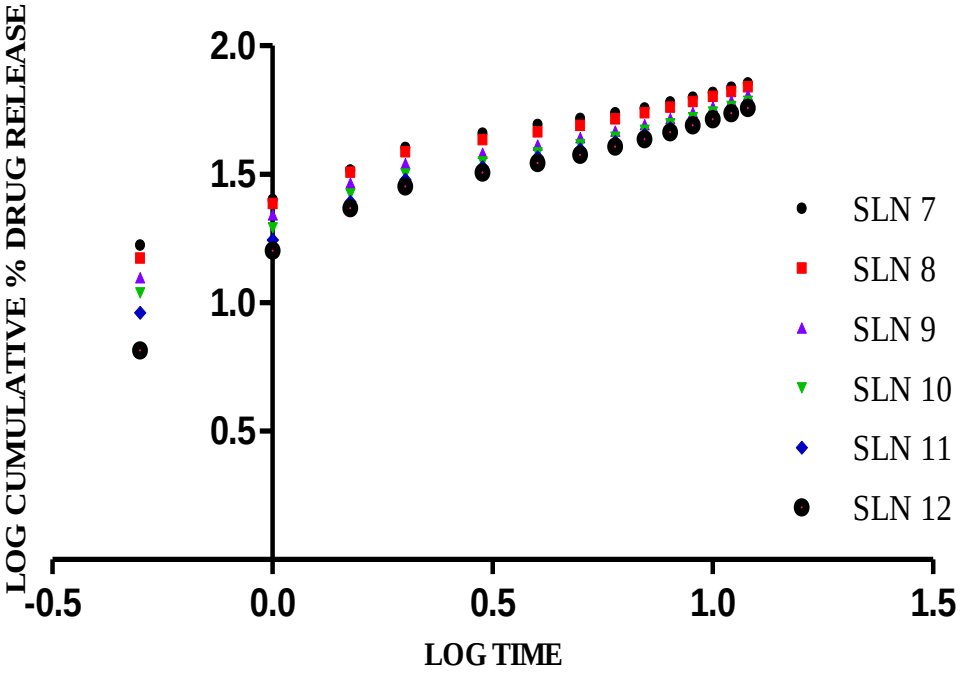


FIGURE-8C KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SLN USING GLYCERYL MONOOLEATE AS LIPID

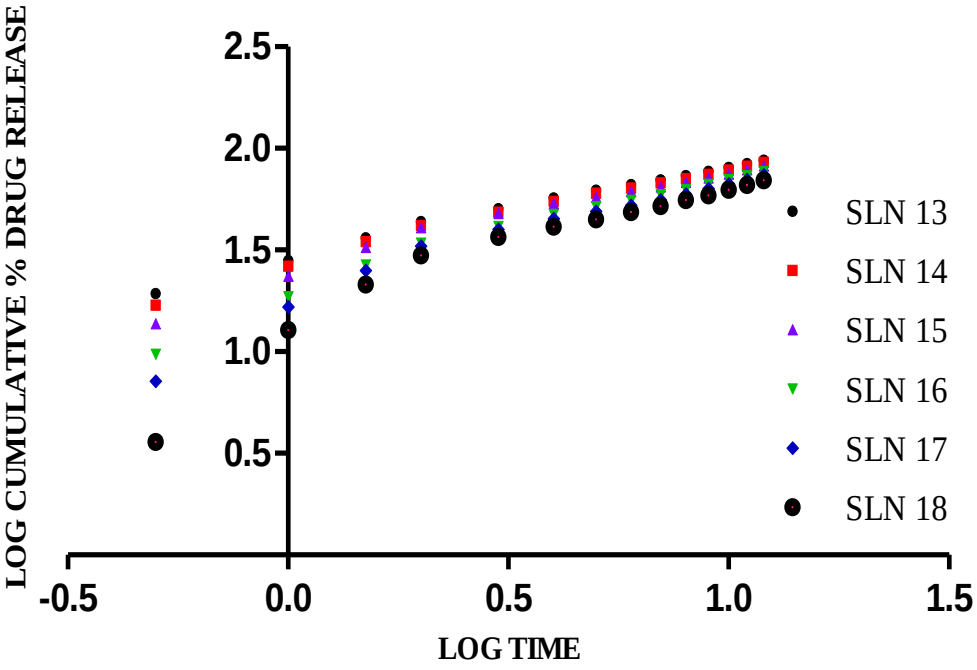


FIGURE-8D KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SLN USING STEARIC ACID AS LIPID

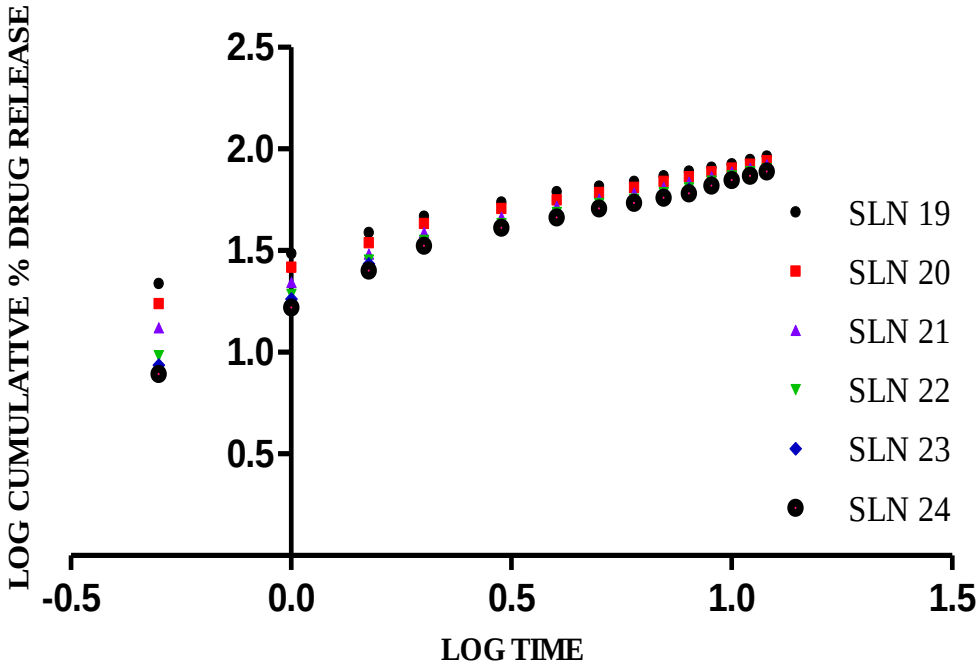


FIGURE-8E KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SLN USING PALMITIC ACID AS LIPID

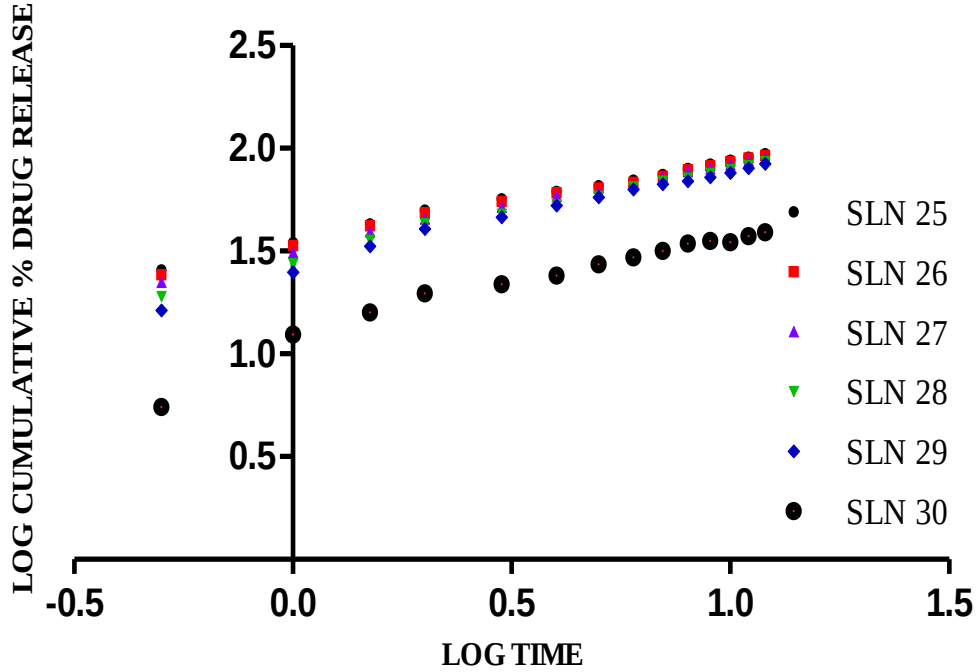


FIGURE-9A HIXSON CROWELL MODEL KINETIC RELEASE OF SLN USING COMPRITOL AS LIPID

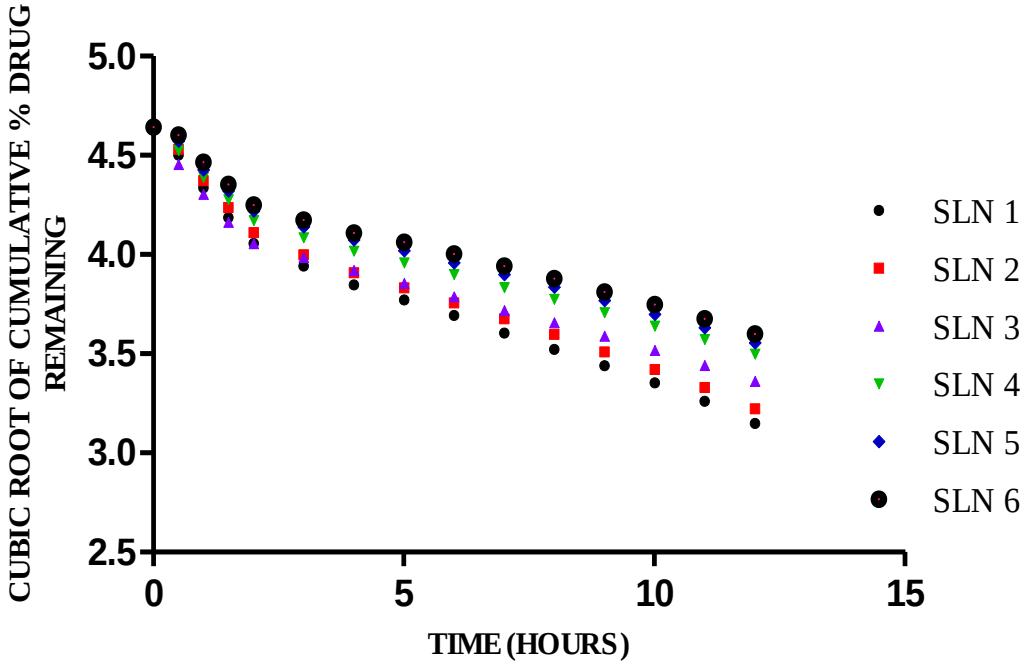


FIGURE-9B HIXSON CROWELL MODEL KINETIC RELEASE OF SLN USING GLYCERYL MONOSTEARATE AS LIPID

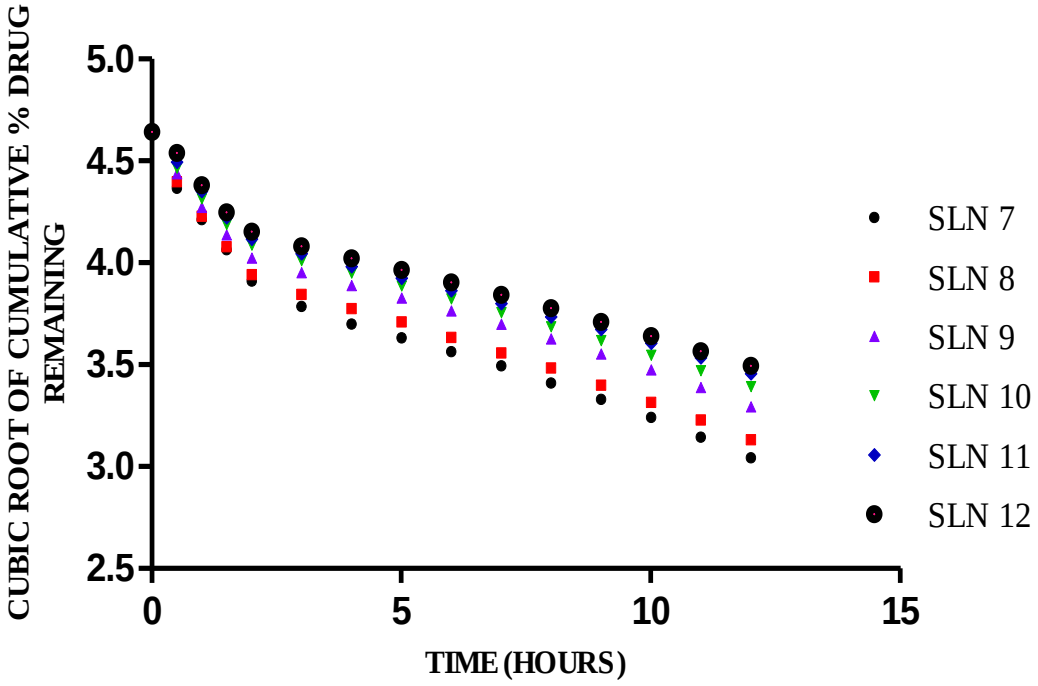


FIGURE-9C HIXSON CROWELL MODEL KINETIC RELEASE OF SLN USING GLYCERYL MONOOLEATE AS LIPID

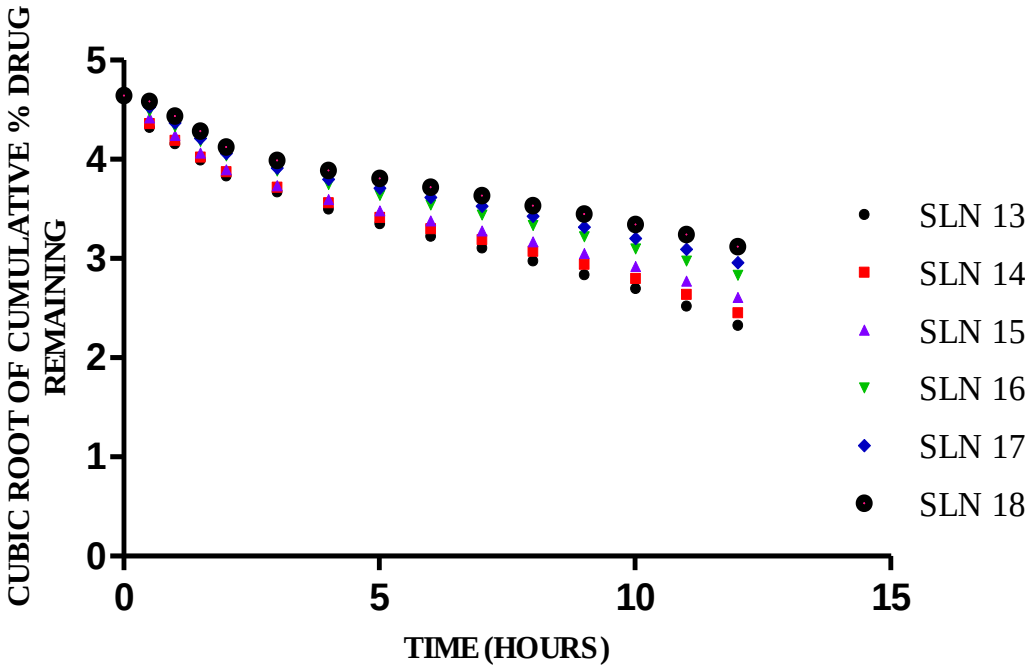


FIGURE-9D HIXSON CROWELL MODEL KINETIC RELEASE OF SLN USING STEARIC ACID AS LIPID

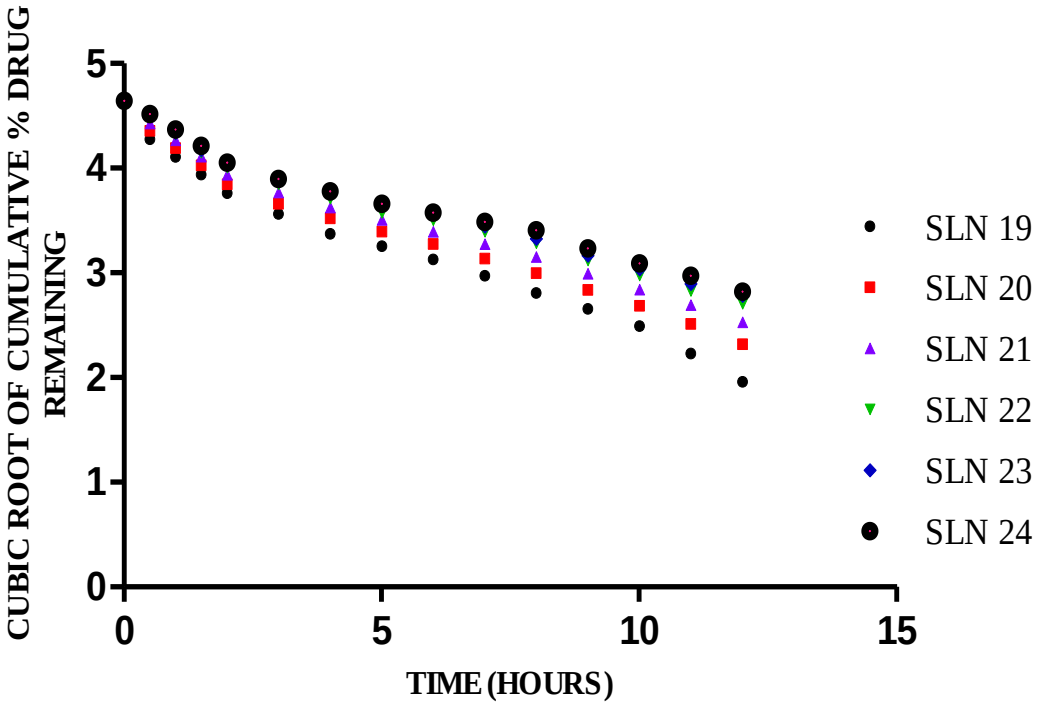


FIGURE-9E HIXSON CROWELL MODEL KINETIC RELEASE OF SLN USING PALMITIC ACID AS LIPID

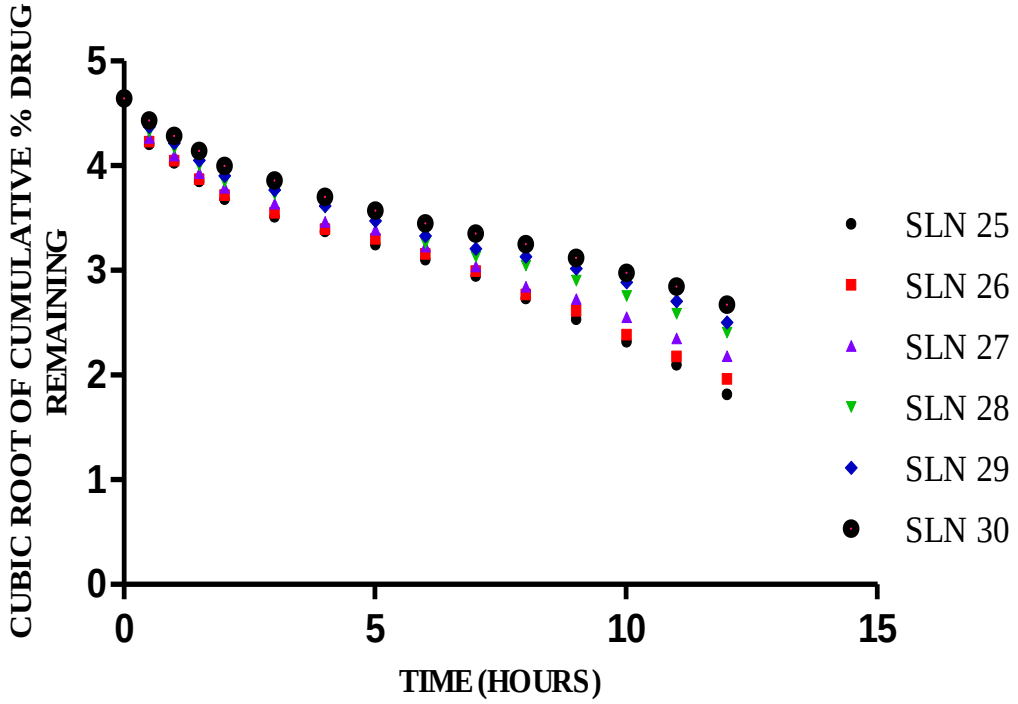


FIGURE-10A PARTICLE SIZE DISTRIBUTION CURVE OF SLN6 (COMPRITOL ATO 888-10%)

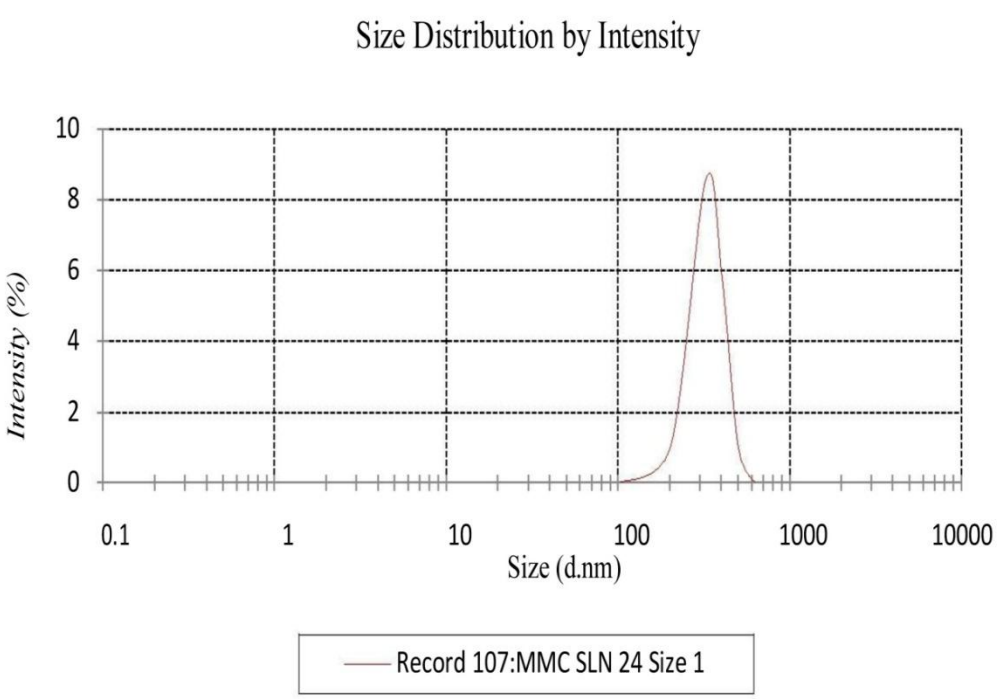


FIGURE-10B PARTICLE SIZE DISTRIBUTION CURVE OF SLN12 (GLYCERYL MONOSTEARATE-10%)

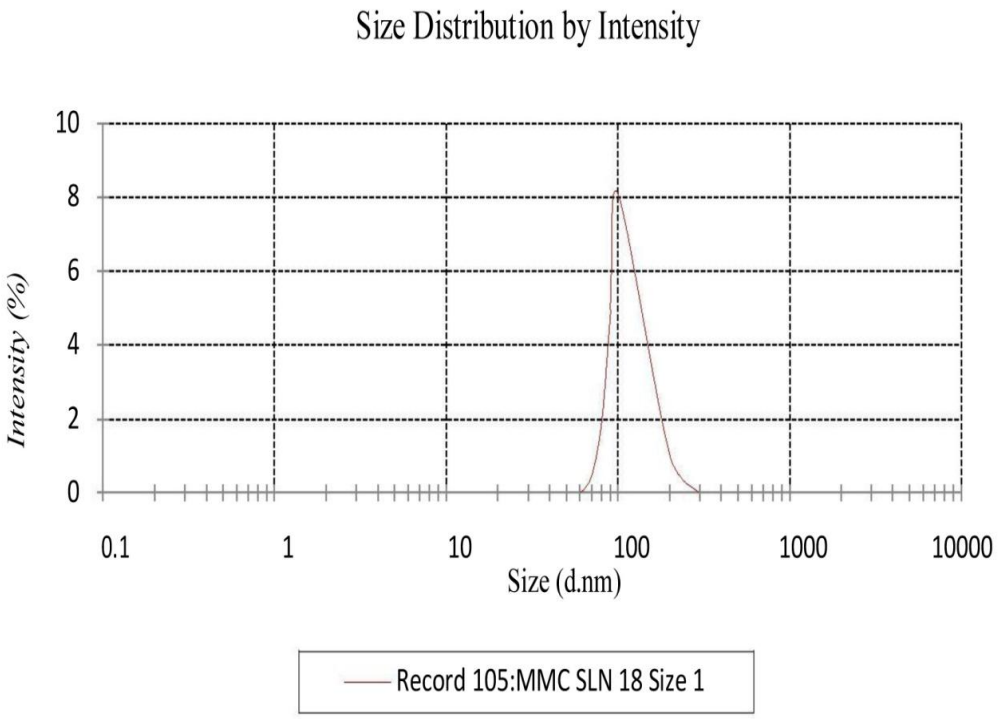


FIGURE-10C PARTICLE SIZE DISTRIBUTION CURVE OF SLN18 (GLYCERYL MONOOLEATE-10%)

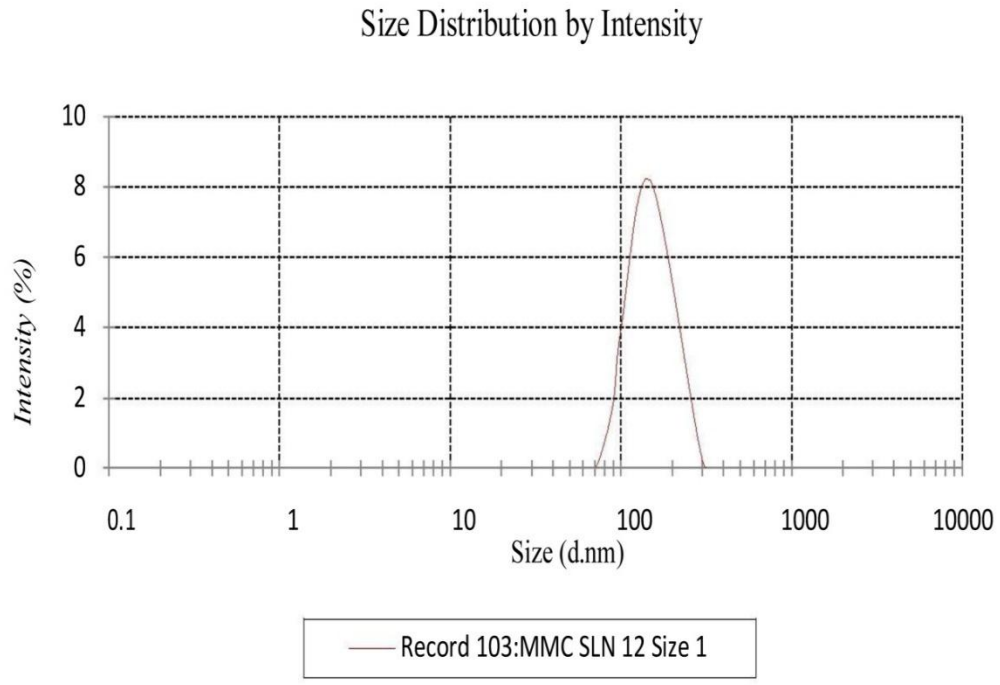


FIGURE-10D PARTICLE SIZE DISTRIBUTION CURVE OF SLN24 (STEARIC ACID-10%)

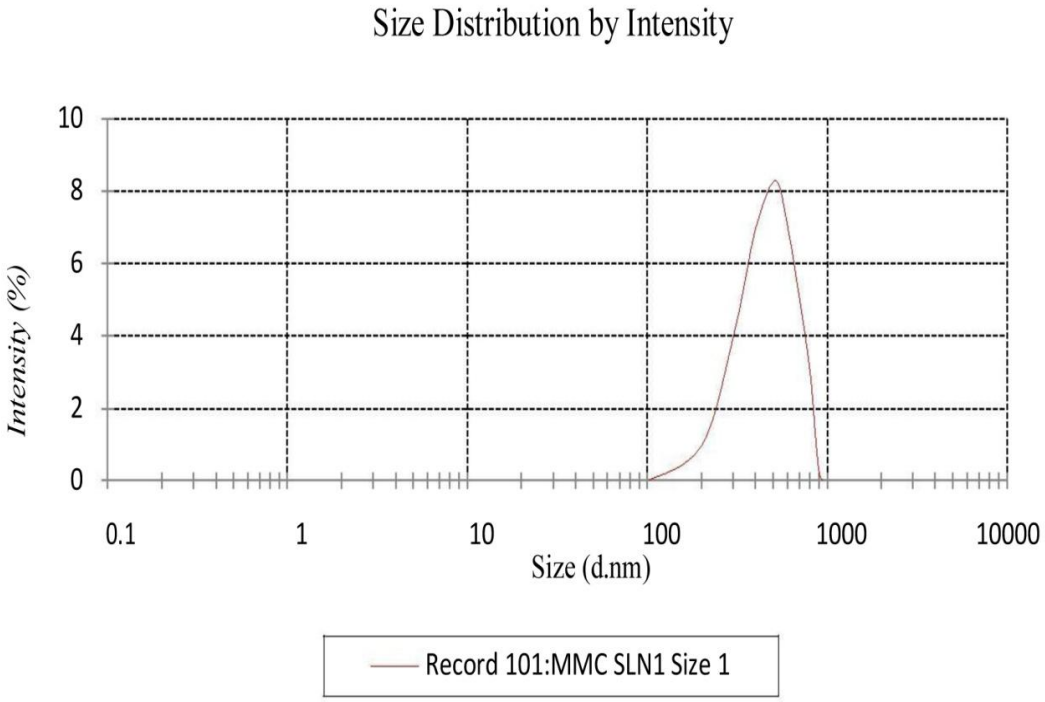


FIGURE-10E PARTICLE SIZE DISTRIBUTION CURVE OF SLN30 (PALMITIC ACID-10%)

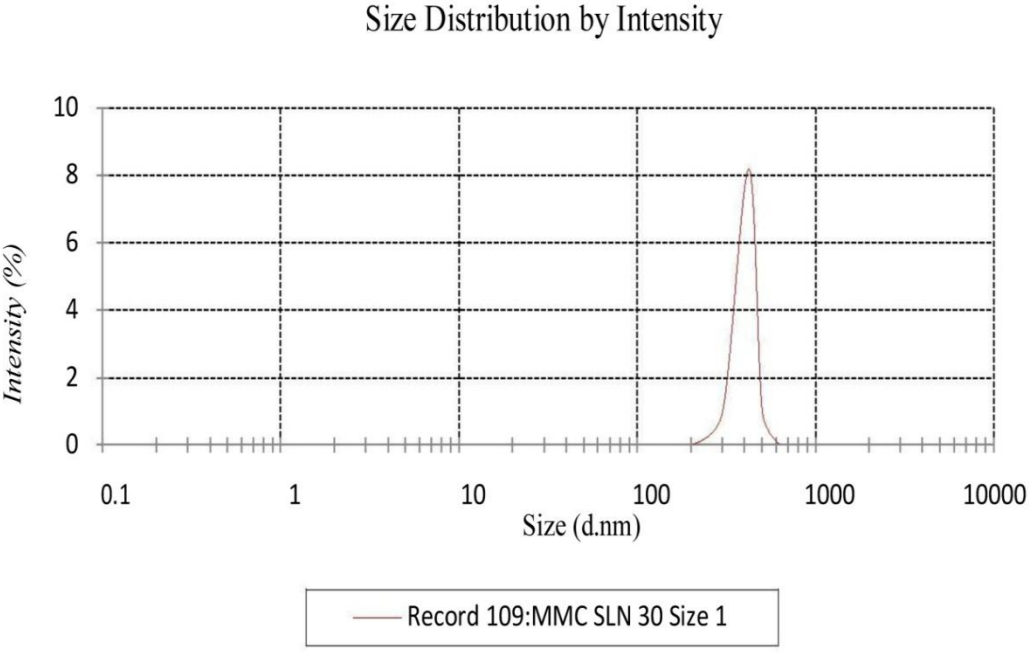


FIGURE-11A ZETA POTENTIAL CURVE OF SLN6 (COMPRITOL ATO 888-10%)

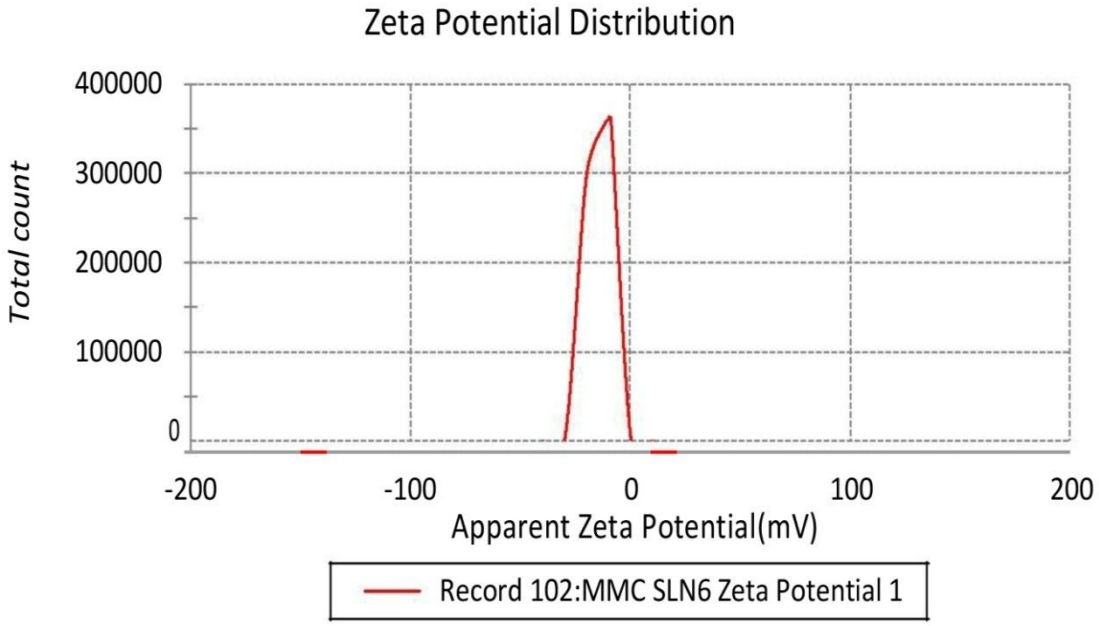


FIGURE-11B ZETA POTENTIAL CURVE OF SLN12 (GLYCERYL MONOSTEARATE-10%)

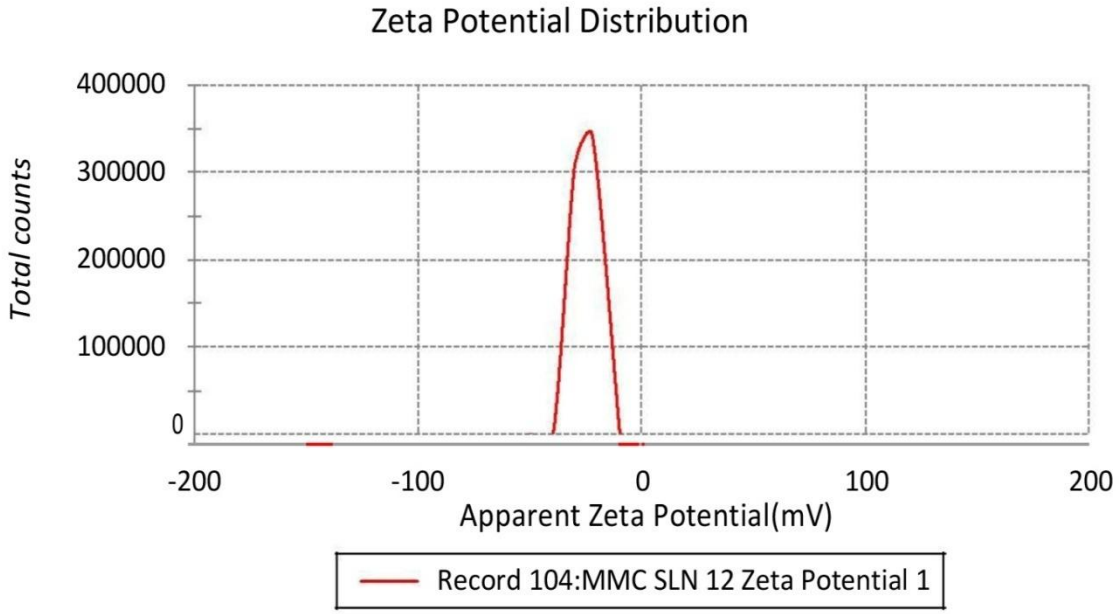


FIGURE-11C ZETA POTENTIAL CURVE OF SLN18 (GLYCERYL MONOOLEATE-10%)

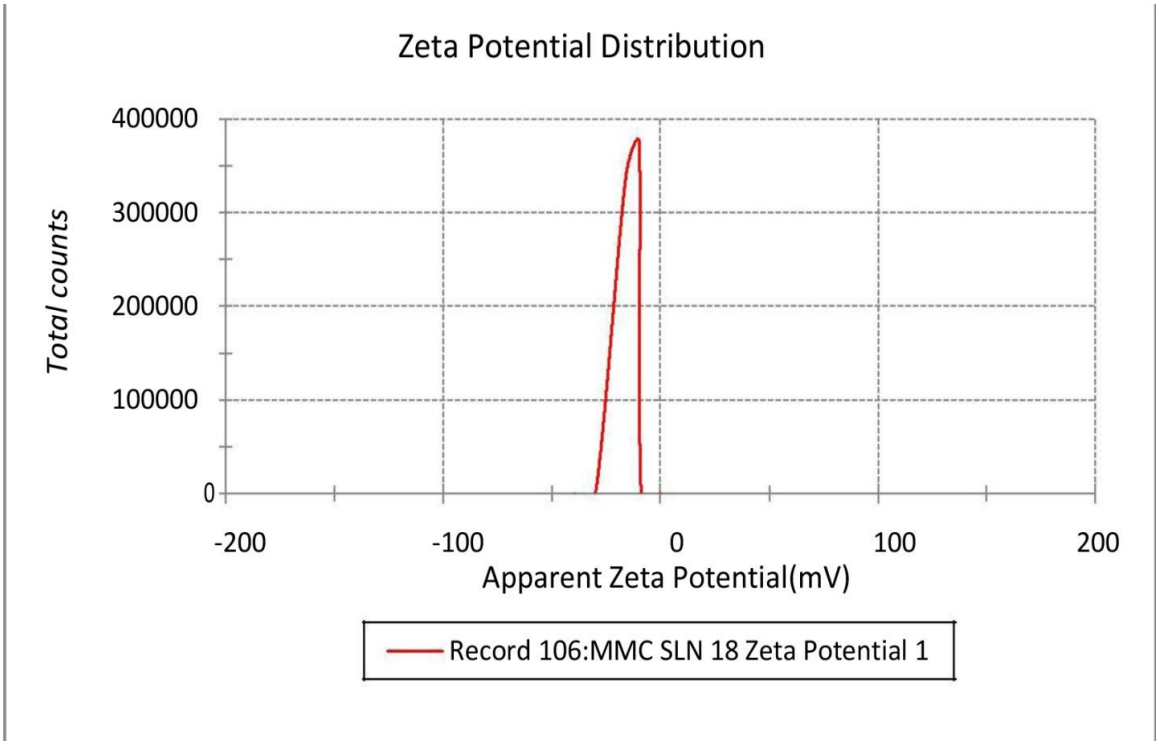


FIGURE-11D ZETA POTENTIAL CURVE OF SLN24 (STEARIC ACID-10%)

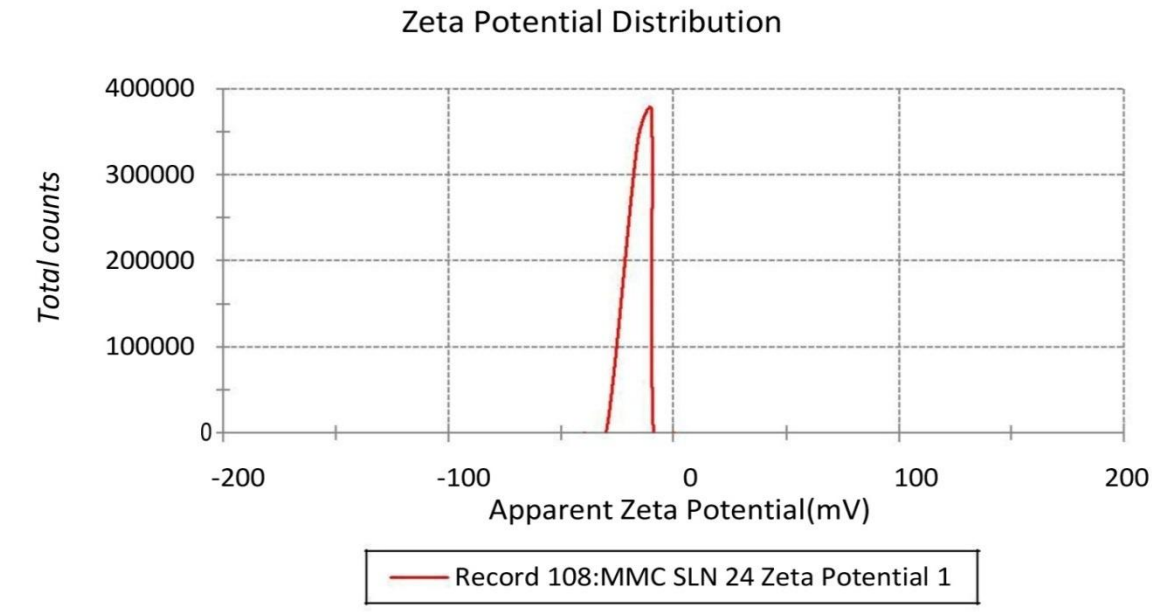


FIGURE-11E ZETA POTENTIAL CURVE OF SLN30 (PALMITIC ACID-10%)

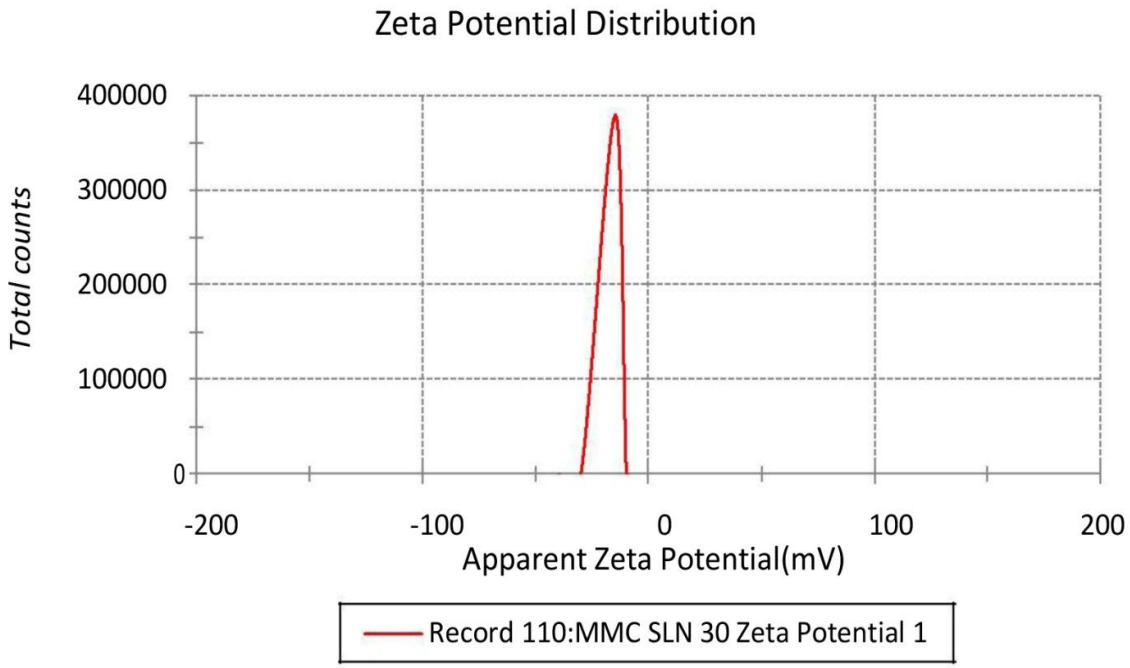


FIGURE-12 SOLUBILITY STUDIES

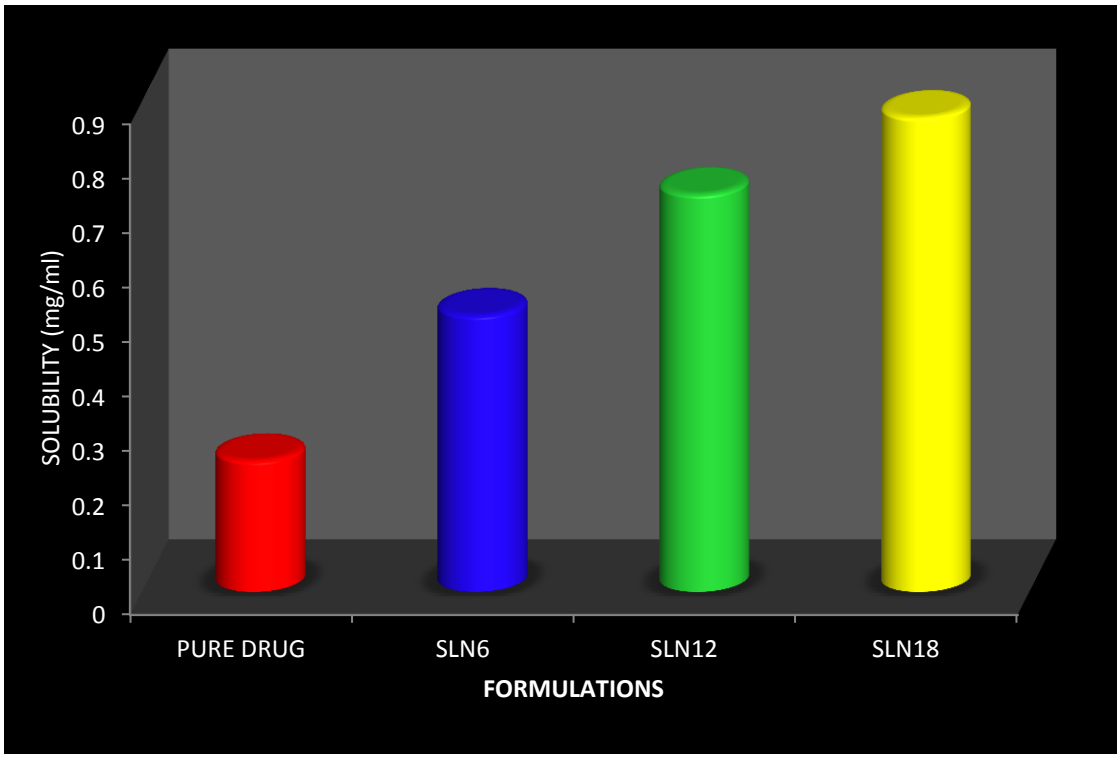


FIGURE-13 ANGLE OF REPOSE

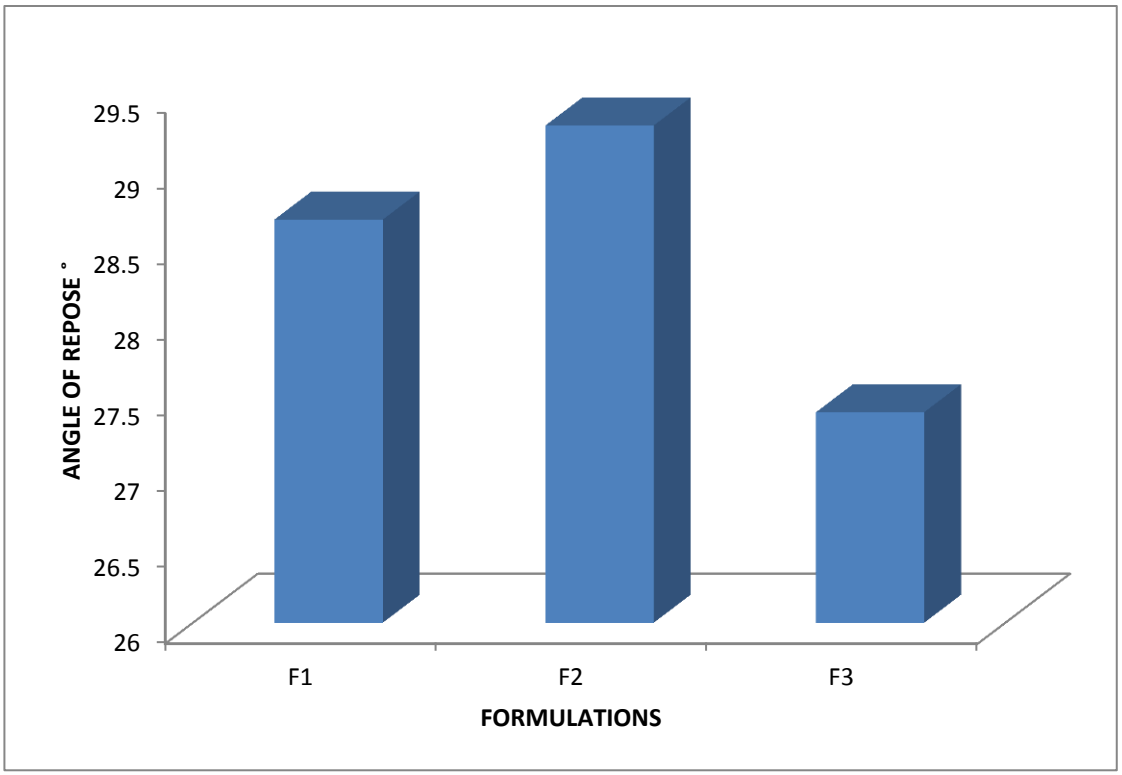


FIGURE-14 BULK DENSITY

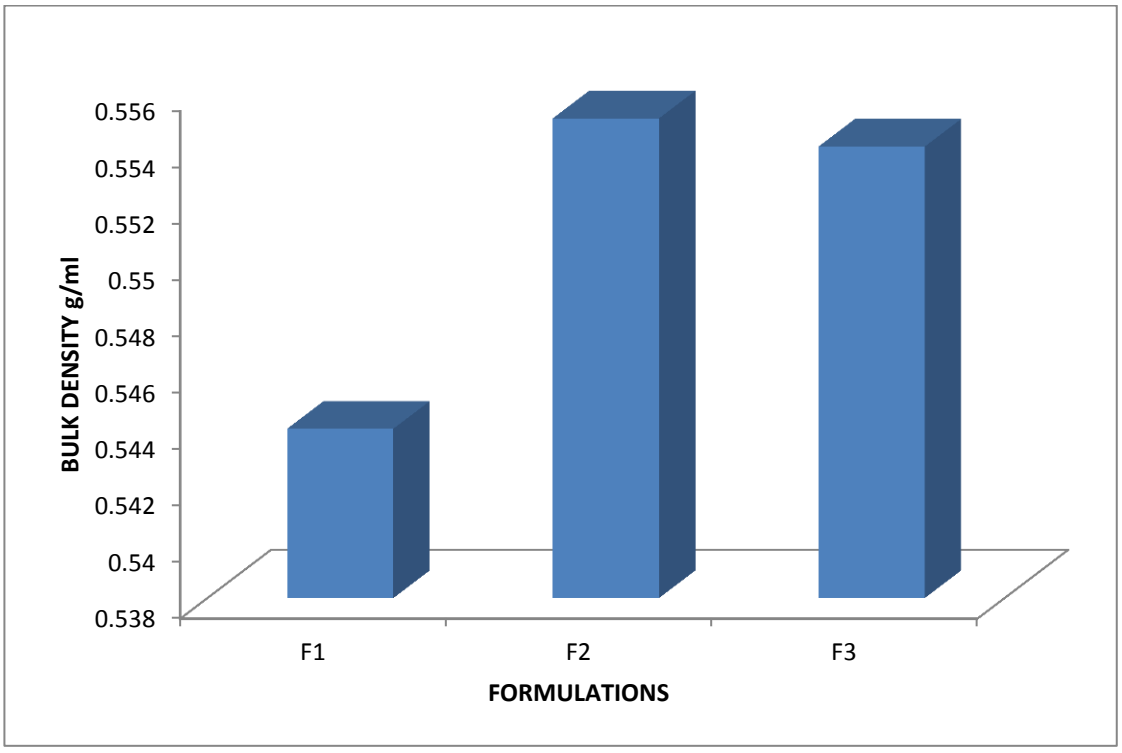


FIGURE-15 TAPPED DENSITY

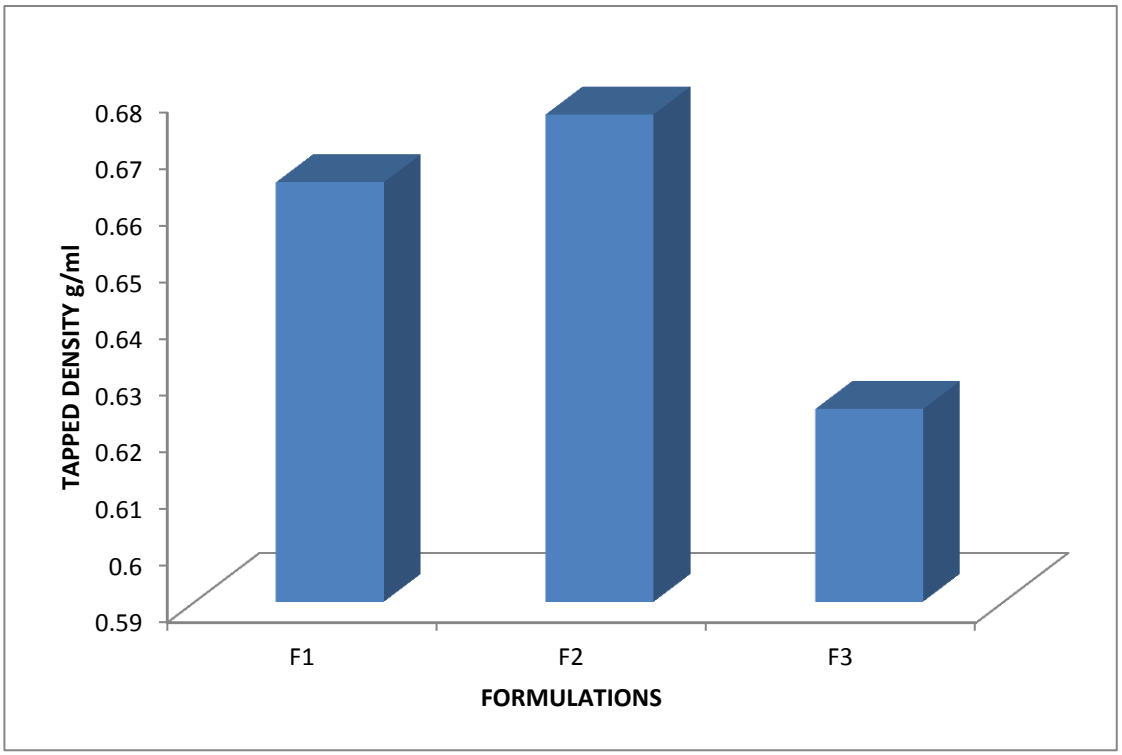


FIGURE-16 MUCOADHESIVE STRENGTH

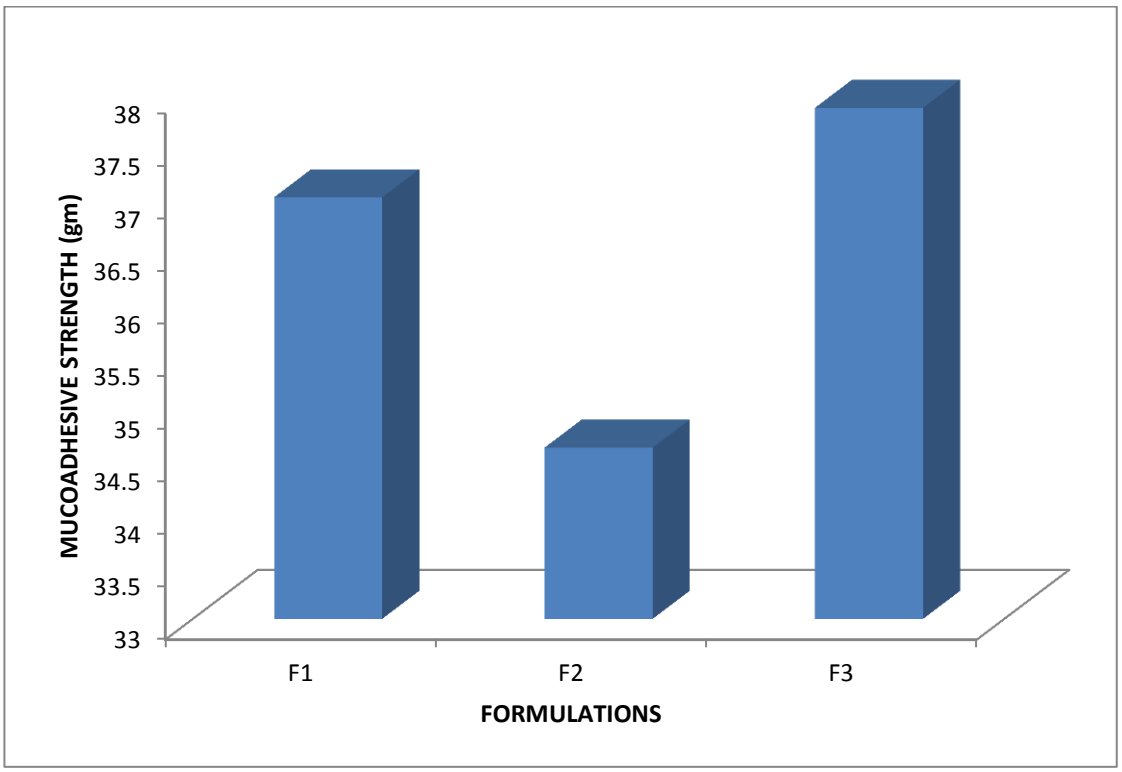


FIGURE-17 SWELLING INDEX

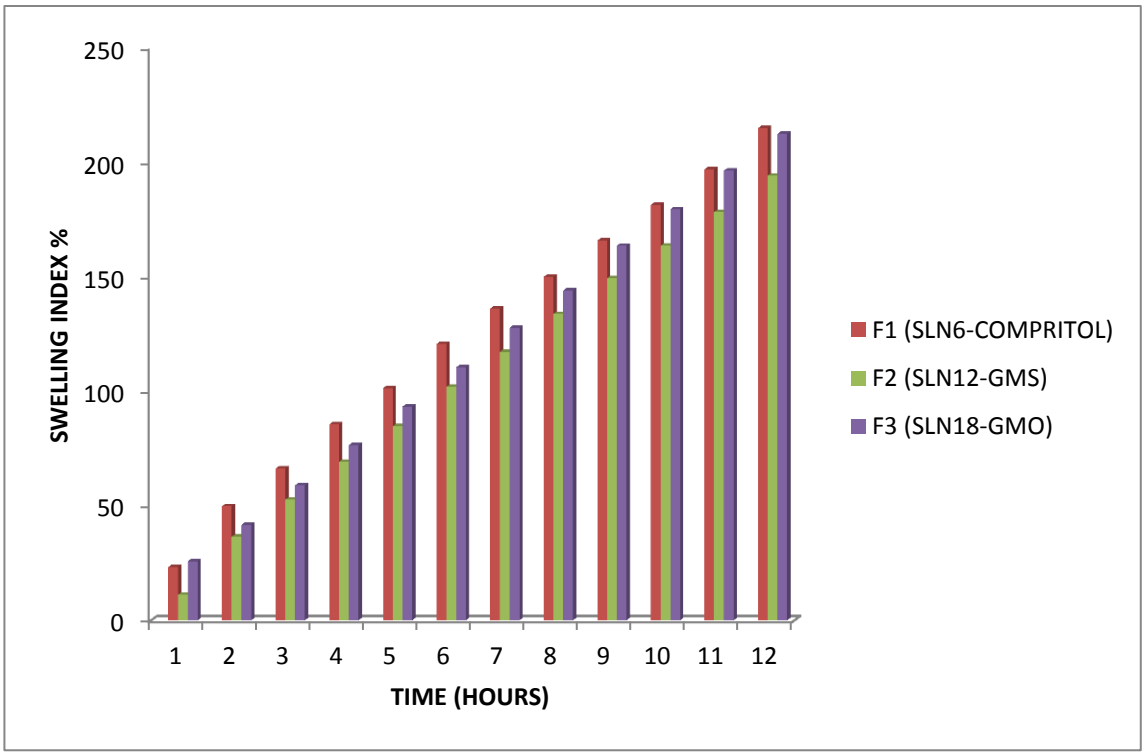


FIGURE-18 INVITRO DRUG RELEASE OF MUCOADHESIVE TABLETS

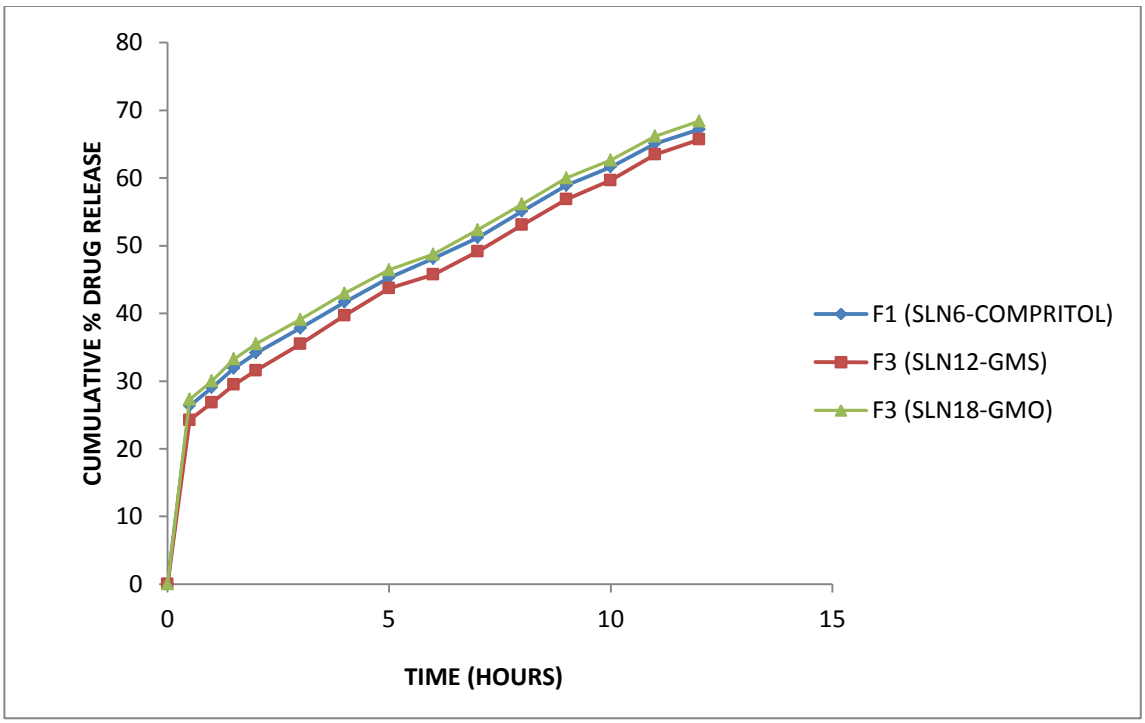


FIGURE-19A ZERO ORDER INVITRO RELEASE KINETICS

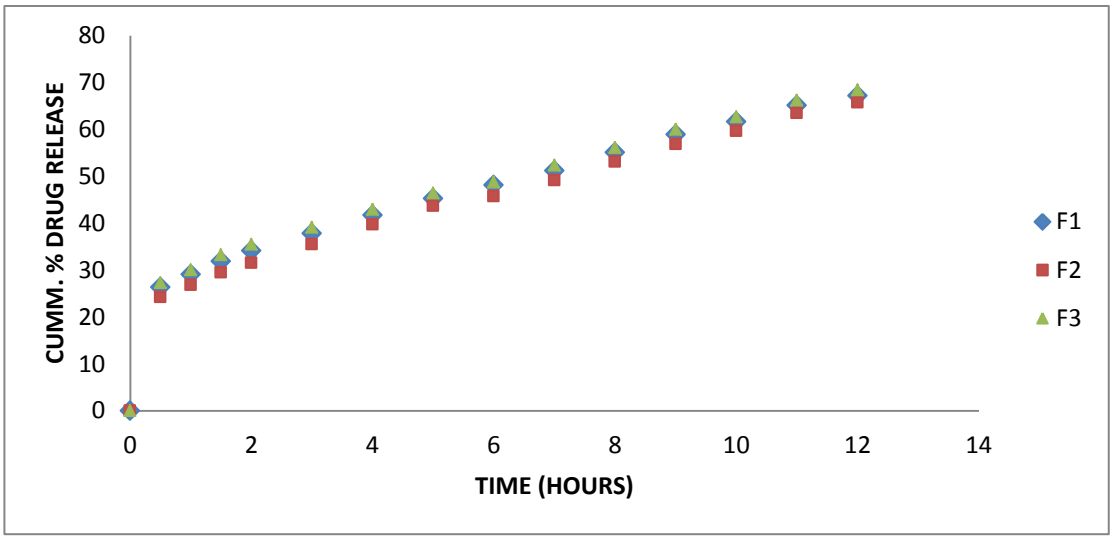


FIGURE-19B FIRST ORDER INVITRO RELEASE KINETICS

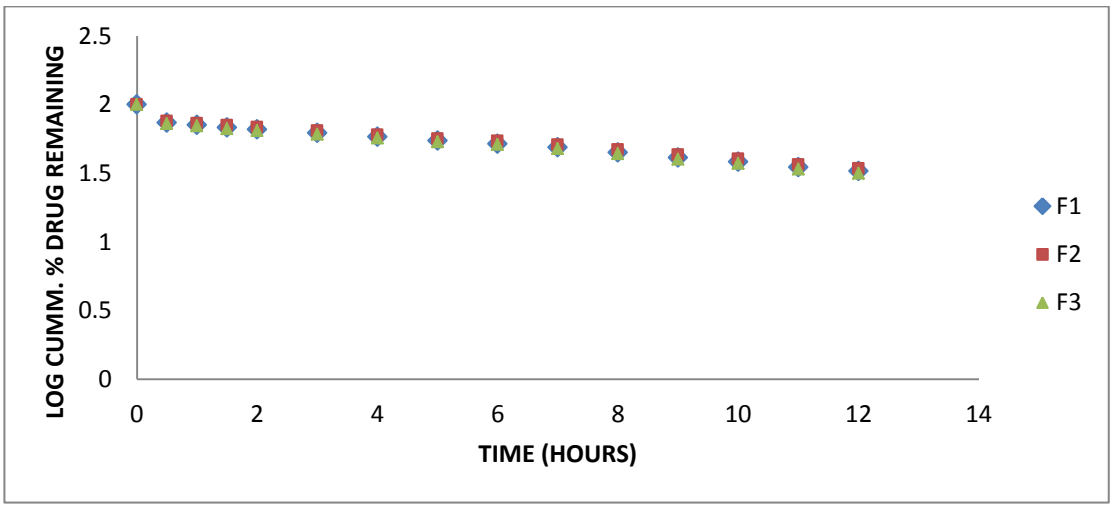


FIGURE-19C HIXON CROWELL INVITRO RELEASE KINETICS

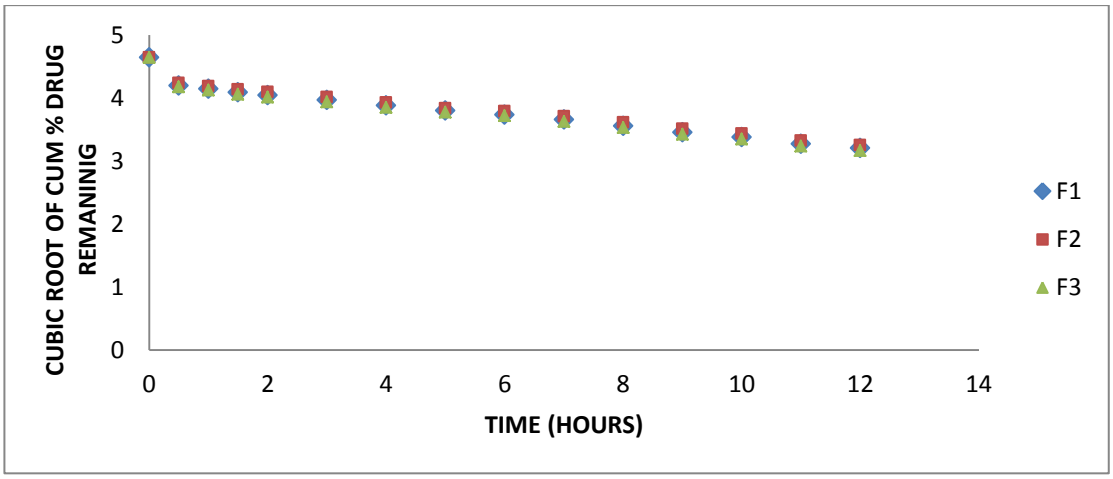


FIGURE-19D KORSMEYER PEPPAS INVITRO RELEASE KINETICS

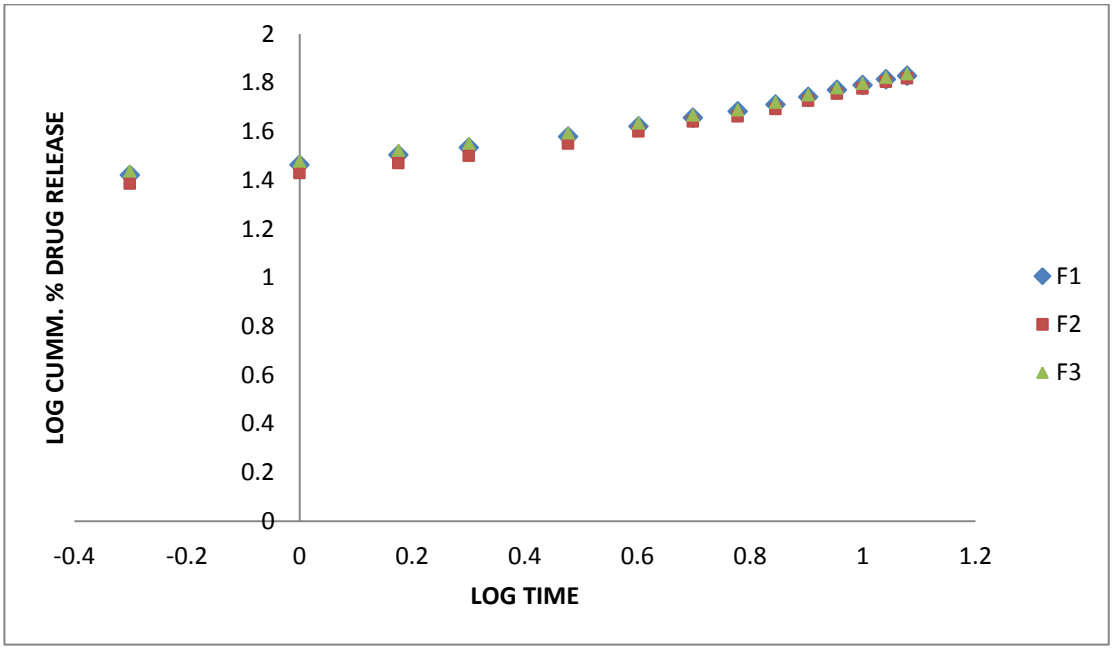


FIGURE-19E HIGUCHI INVITRO RELEASE KINETICS

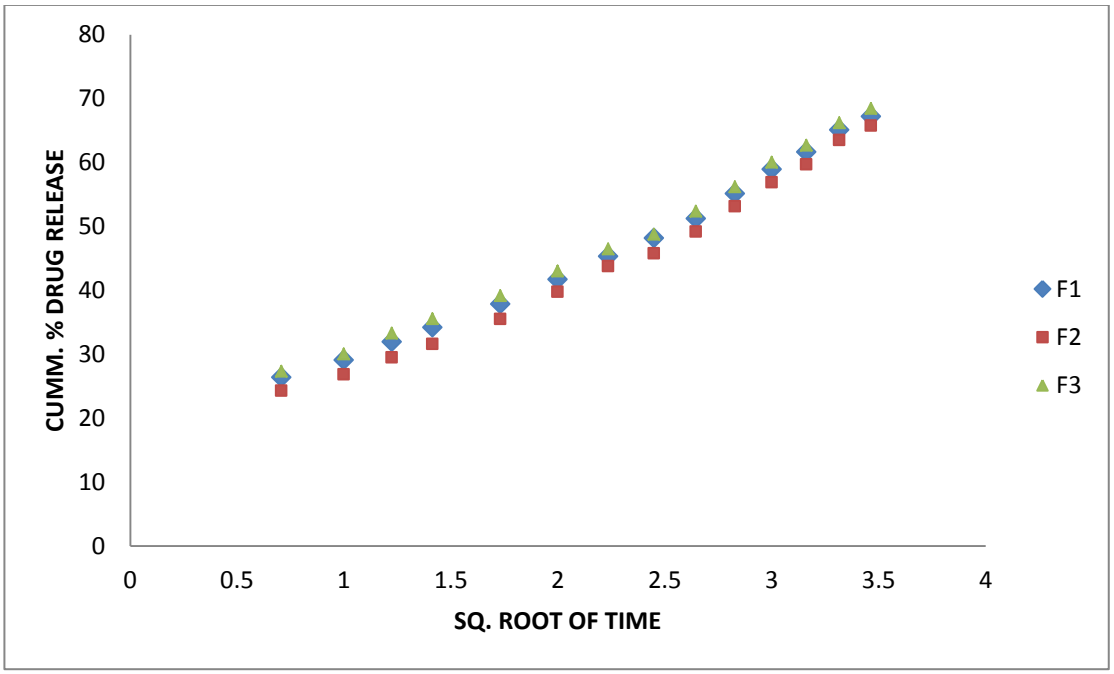


FIGURE-20 *EXVIVO* DRUG PERMEATION STUDIES THROUGH RAT STOMACH

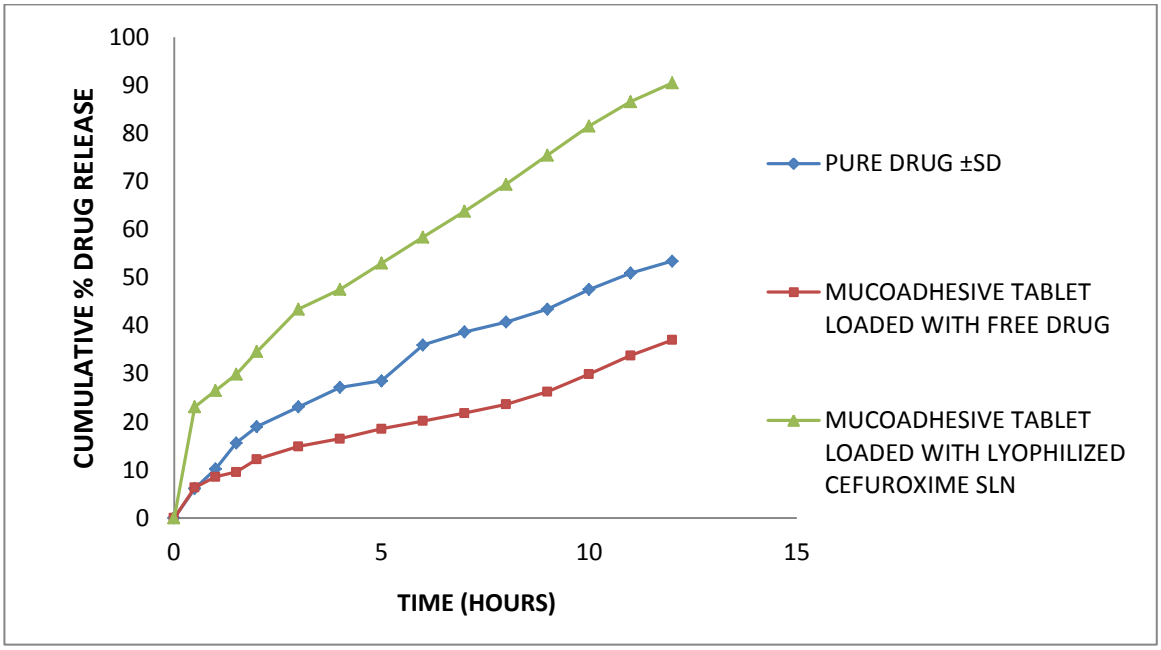


FIGURE-21A SEM IMAGE OF SLN6 (COMPRITOL)

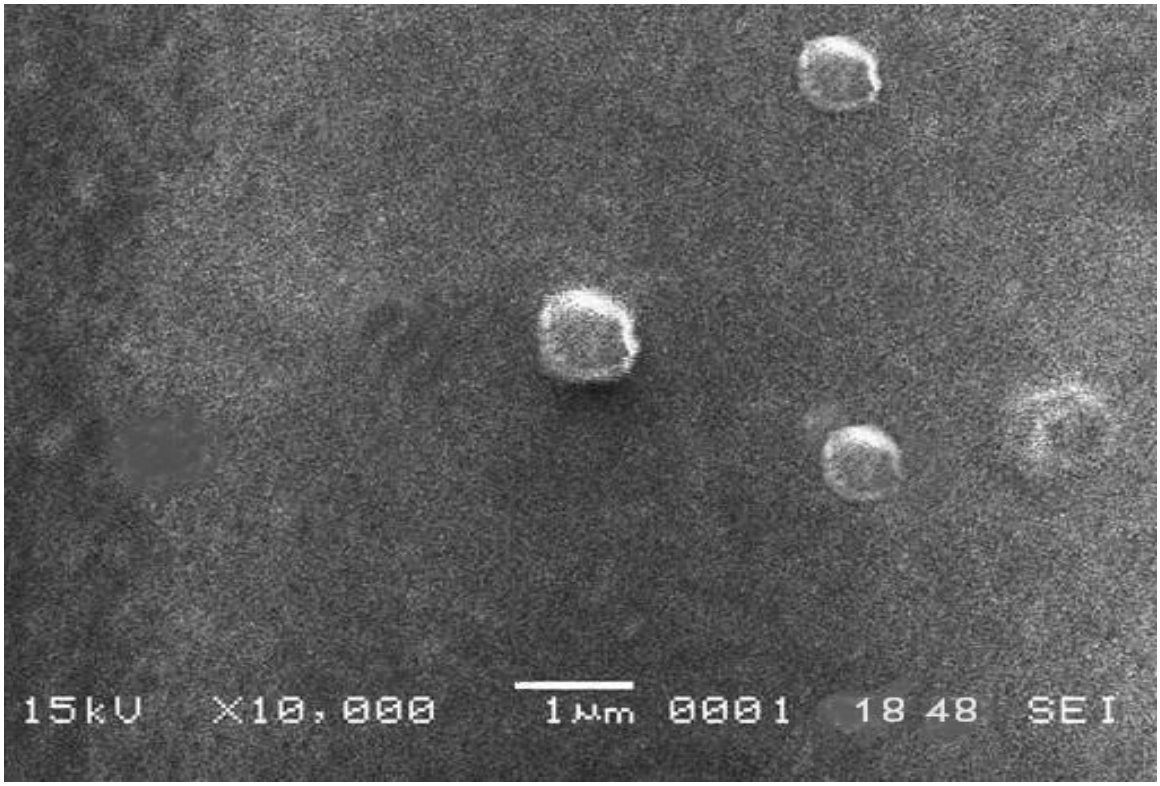


FIGURE-21B SEM IMAGE OF SLN12 (GMS)

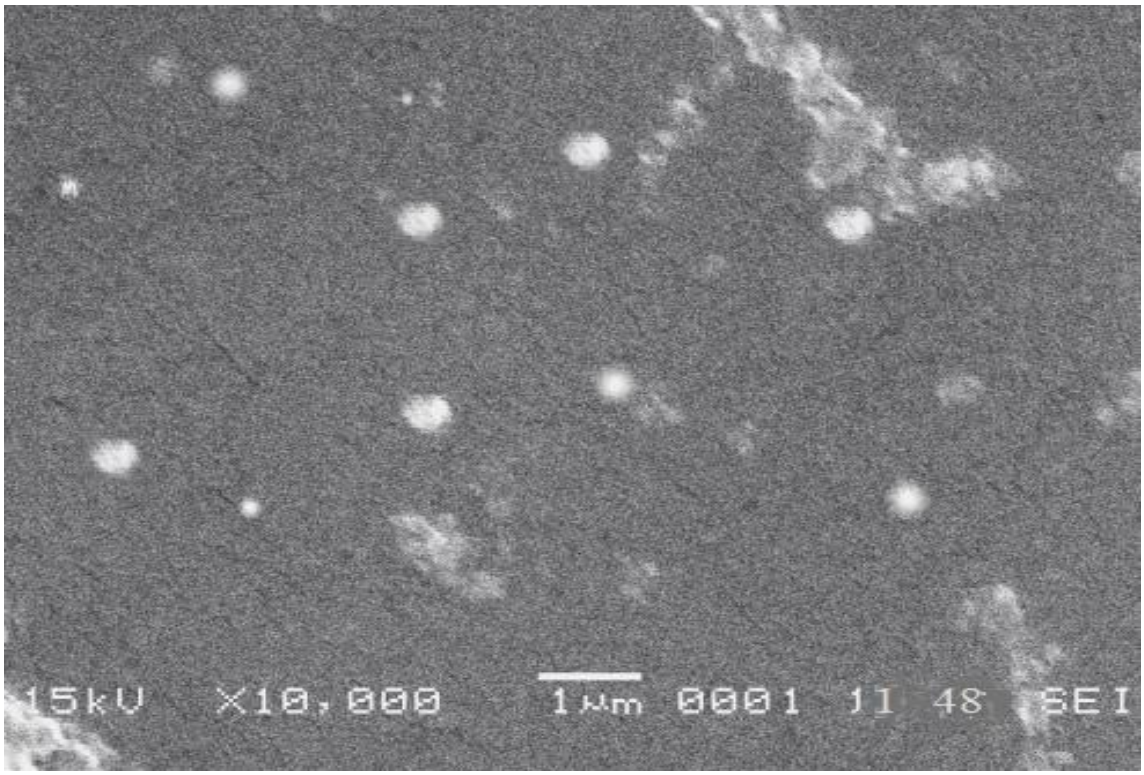


FIGURE-21C SEM IMAGE OF SLN18 (GMO)

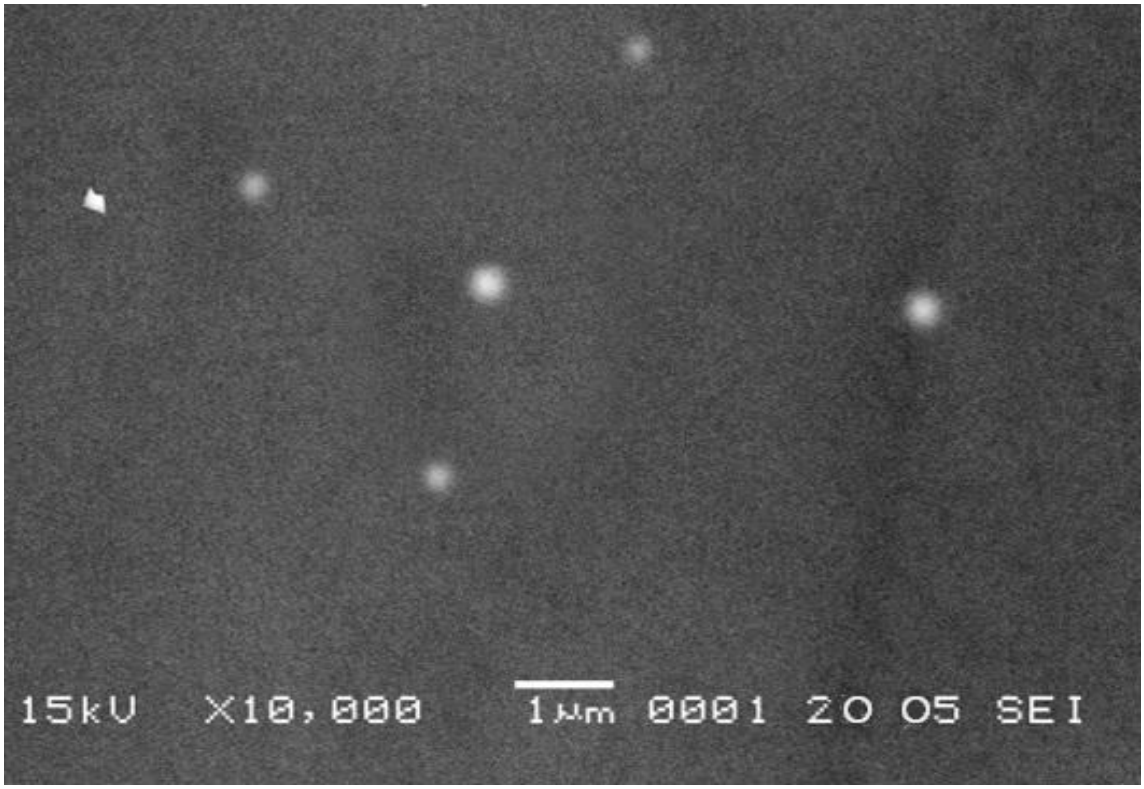


FIGURE-22A X-RAY DIFFRACTION OF CEFUROXIME AXETIL

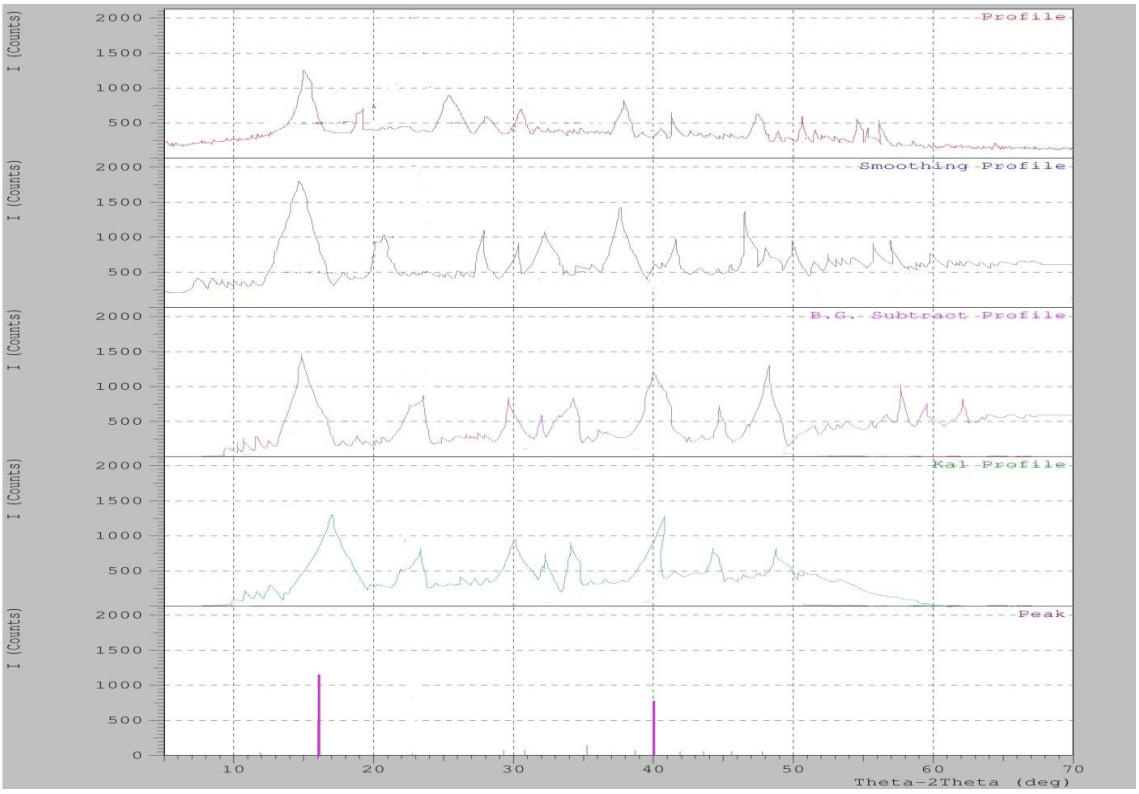


FIGURE-22B X-RAY DIFFRACTION OF LYOPHILIZED SLN6

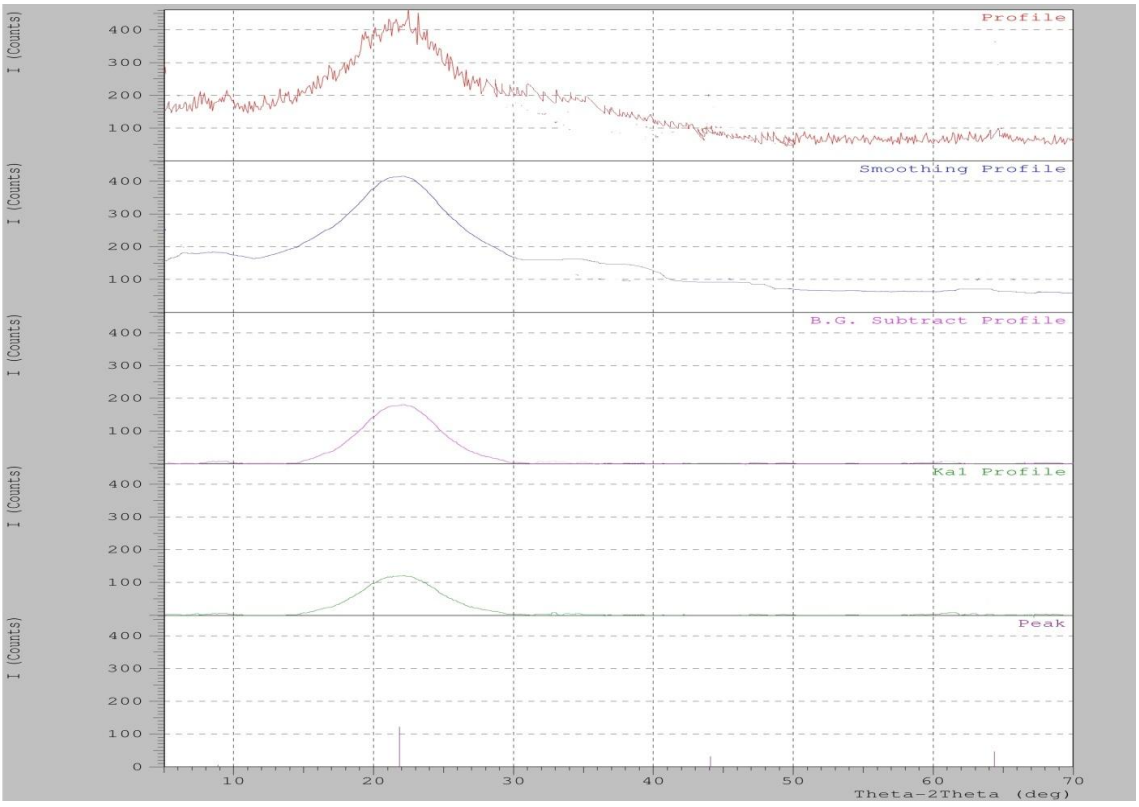


FIGURE-22C X-RAY DIFFRACTION OF LYOPHILIZED SLN12

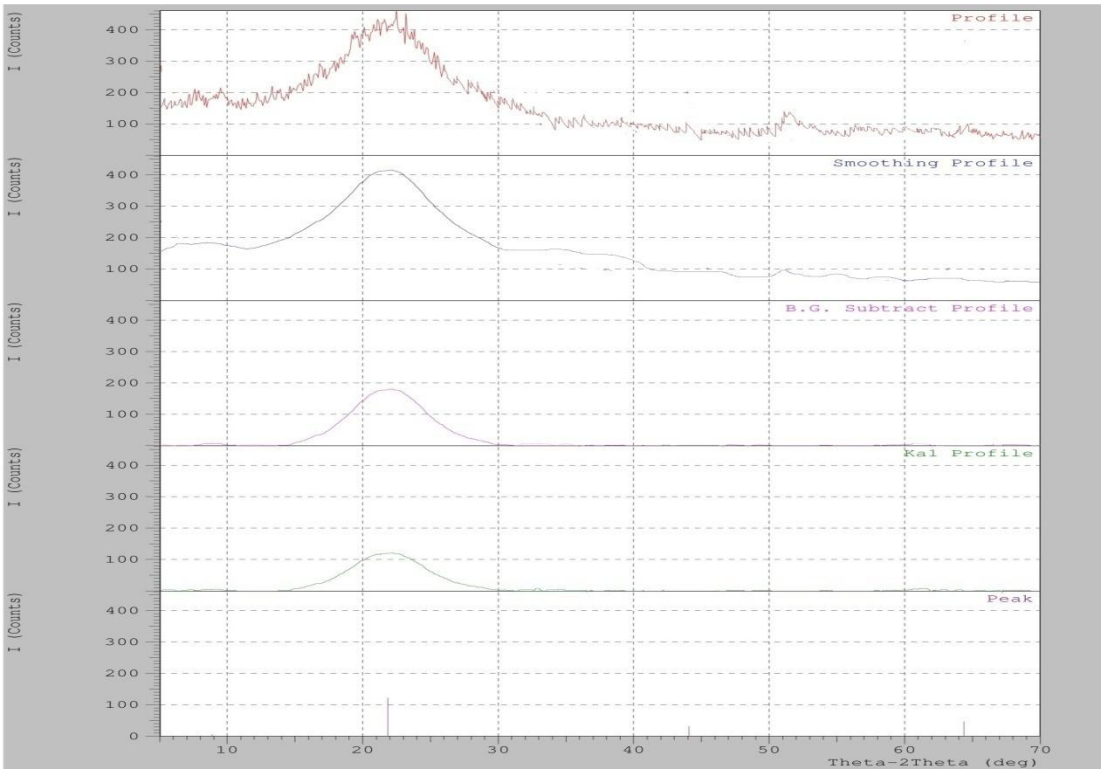


FIGURE-22D X-RAY DIFFRACTION OF LYOPHILIZED SLN18

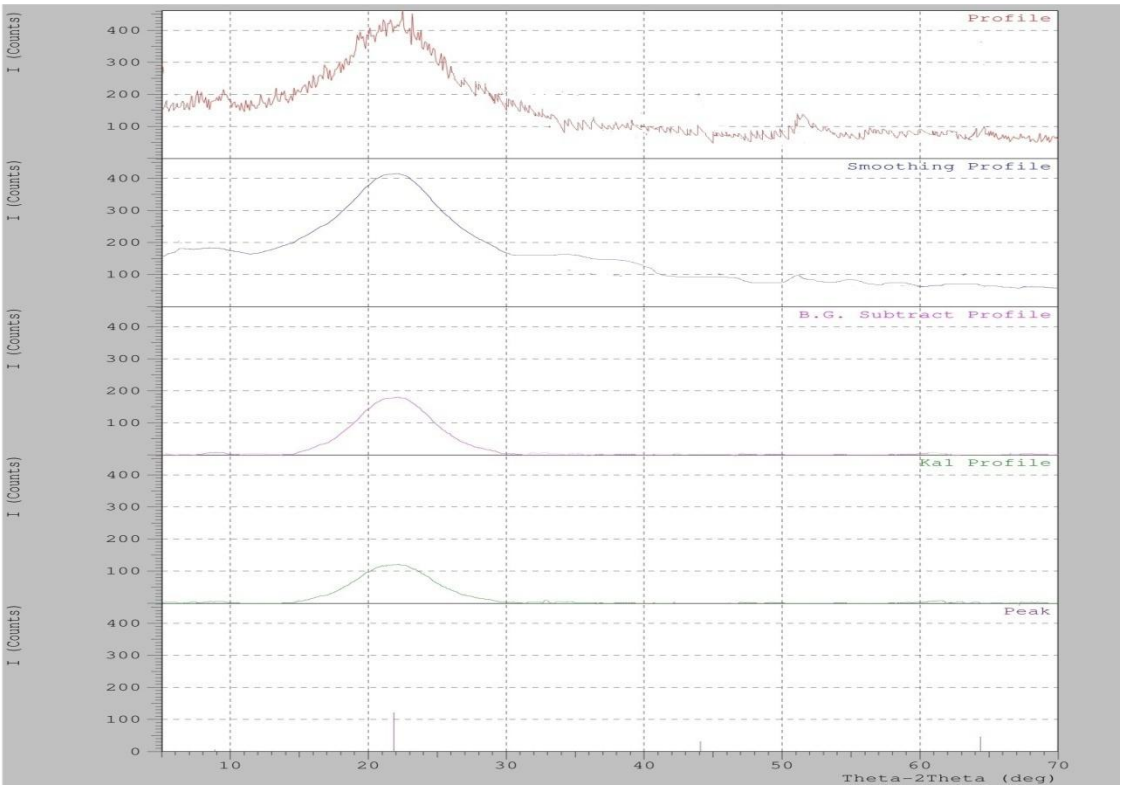


FIGURE-23A INVIVO STUDIES IN RABBIT STOMACH (CONTROL)

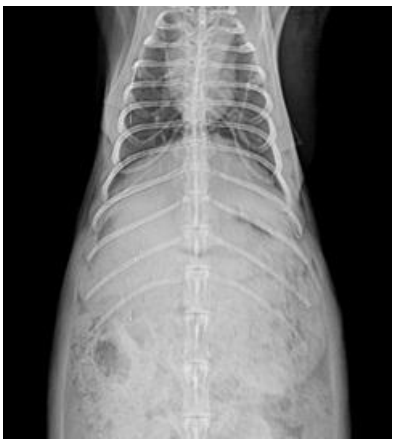


FIGURE-23B INVIVO STUDIES IN RABBIT STOMACH (0 HOUR)

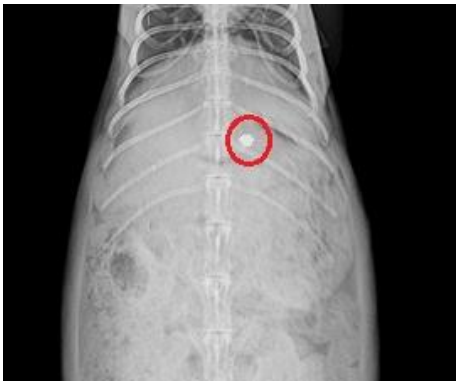


FIGURE-23C INVIVO STUDIES IN RABBIT STOMACH (2 HOUR)

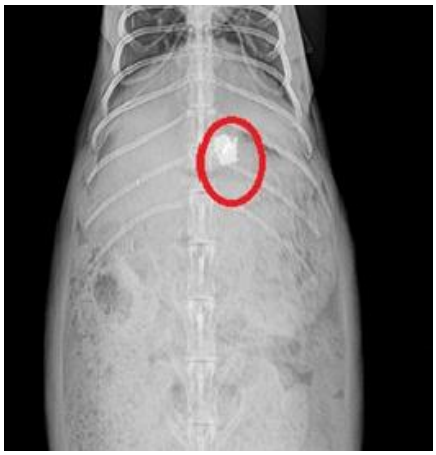


FIGURE-23D INVIVO STUDIES IN RABBIT STOMACH (4 HOUR)

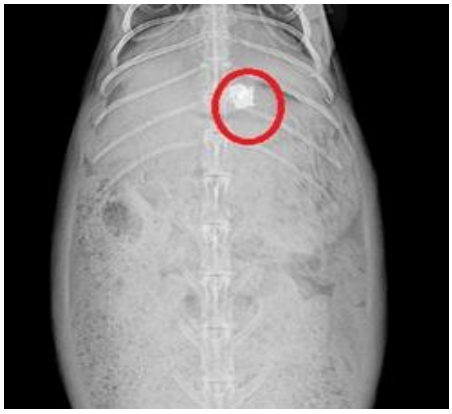


FIGURE-23E INVIVO STUDIES IN RABBIT STOMACH (6 HOUR)

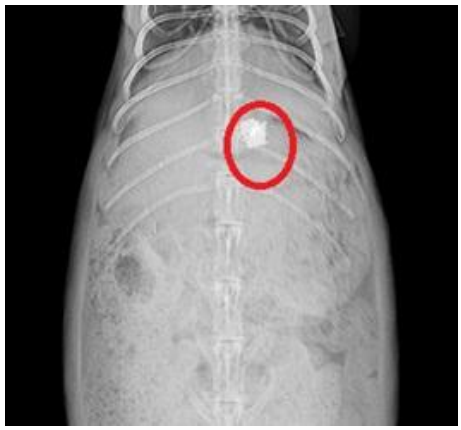
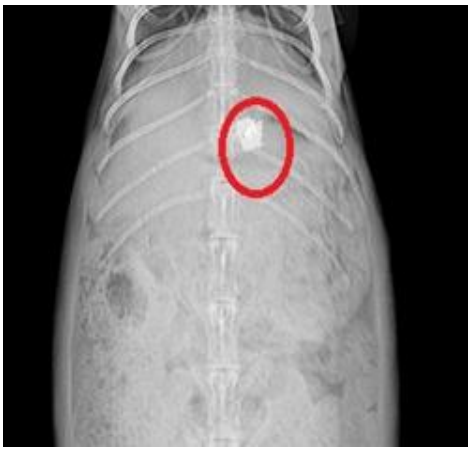


FIGURE-23F INVIVO STUDIES IN RABBIT STOMACH (8 HOUR)



CHAPTER - 10

SUMMARY AND CONCLUSION

CHAPTER - 10

SUMMARY & CONCLUSION

- The main purpose of this investigation was to develop mucoadhesive tablets of cefuroxime axetil loaded in solid lipid nanoparticles (SLNs) to improve cefuroxime axetil bioavilability.
- From the FT-IR studies there were no interactions between the drug and excipients was seen.
- SLNs were prepared by hot homogenization followed by ultrasonication using various lipids such as glyceryl behenate (compritol ATO 888), glyceryl monostearate, glyceryl monooleate, stearic acid & palmitic acid and surfactants pluoric F68 & soyalecithin.
- Entrapment efficiency of the formulations increase with increase in lipid percentage with an optimum surfactant concentration.
- The particle size of formulations with best entrapment was found out in the range of 104.3nm-512.6nm.
- The polydispersity index was within 0.55 indicating an uniform size distribution.
- The zeta potential of the formulation with best entrapment was in the range of -9.42—22.9mV.
- The invitro drug release showed a biphasic release pattern ie., burst release followed by sustained effect in 12hours.
- The release kinetics showed that the formulations were diffusion controlled. Korsmeyer peppas n values were more than 0.4 indicating, non fickian diffusion.

- The best formulation was selected according to entrapment, particle size & invitro drug release.
- Solubility studies were done for the best formulation which showed a higher solubility for formulation with lowest particle size.
- The selected formulations were lyophilized.
- The x-ray diffraction studies showed an amorphous form of the drug.
- SEM images showed that the formulation were spherical in shape and had an almoat smooth appearance.
- The selected lyophilized formulations were then compressed into mucoadhesive tablets using suitable polymers (HPMC K15M & carbopol 934) by direct compression method.
- The mucoadhesive approach was applied to target the drug to the upper part of gastro intestinal tract, since cefuroxime axetil absorption happens only at 1.2pH.
- The precompression studies showed good flowability of the powder blend.
- The post compression evaluation studies were evaluated for the fabricated tablets and were within the limits.
- Since hydrophilic polymers were used good swelling of the tablets were observed.
- Since carbopol is a very good mucoadhesive polymer optimum mucoadhesion was observed during mucoadhesive strength studies.
- The invitro drug release of the formulations showed an optimum drug release due to the presence of swellable hydrophilic polymers for 12hours in a controlled manner.

- The invitro release kinetics showed that the formulation follow diffusion controlled drug release. The n value of formulations in korsmeyer peppas were less than 0.4 indicating fickian diffusion.
- The exvivo studies showed an increase in drug permeation in formulation with lyophilized SLN loaded with drug due to the nano size of the SLN formulation in it.
- From the invivo x-ray studies done in rabbit, it was shown that the mucoadhesive formulation was retained for 8hours.

From these studies it was concluded that mucoadhesive tablets of cefuroxime axetil loaded in SLNs prove to be a successful gastroretentive oral delivery of the poor bioavailable drug. Since the drug was incorporated into lipids and the particle size was reduced to nano, enhanced permeation was observed and solubility was also increased due to increased surface area. The presence of muco adhesive polymer results in adhesion of tablet to the upper gastric mucosa resulting in targeted drug delivery. The overall results indicate the success of developing mucoadhesive tablets of cefuroxime axetil loaded in SLNs for stomach targeting.

ANNEXTURE

ANNEXURE

Investigator declaration

- I certify that I have determined that the research proposal herein is not unnecessarily duplicate of previously reported research.
- I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedures.
- For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
- I will obtain approval from the IAEC / CPCSEA before initiating any significant changes in this study.
- Certified that performance of experiment will be initiated only up on review and approval of scientific intent by appropriate expert body (institutional scientific advisory committee / funding agency / other body (to be named)
- Institutional biosafety committee (IBC) certification of review and concurrence will be taken (required for studies utilizing DNA agents of human pathogens)
- I shall maintain all the records as per format (Form D)

Signature

(G.Sankar)

Name of Investigator

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.