DESIGN DEVELOPMENT AND EVALUATION OF CEFIXIME SOLID LIPID NANOPARTICLES FOR ENHANCEMENT OF ORAL BIOAVAILABILITY

A Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

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DECLARATION

I hereby declare that the dissertation work entitled **" DESIGN DEVELOPMENT AND EVALUATION OF CEFIXIME SOLID LIPID NANOPARTICLES FOR ENHANCEMENT OF ORAL BIOAVAILABILITY"** submitted to the Tamil Nadu Dr. M.G.R. Medical university, Chennai, in partial fulfillment for the degree of **Master of Pharmacy** in **Pharmaceutics** was carried out, under the guidance of **DR. K. SELVARAJU, M. Pharm., Ph.D.,** at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020.

This research work either in part or full does not constitute any of other thesis / dissertation.

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EVALUATION CERTIFICATE

This is to certify that the research work entitled **"DESIGN DEVELOPMENT AND EVALUATION OF CEFIXIME SOLID LIPID NANOPARTICLES FOR ENHANCEMENT OF ORAL BIOAVAILABILITY"** submitted by **Mr. BASKAR B (Reg No. 261810901)** to the Tamil Nadu Dr. M.G.R. Medical university, Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** at the Department of Pharmaceutics, is a bonafide work carried out by the candidate at KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2019-2020 and the same was evaluated.

Examination Center: K.M.C.H College of Pharmacy, Coimbatore

Date:

Internal Examiner **External Examiner**

Convener of Examination

Dedicated to Almighty, My Family, Teachers & Friends

ACKNOWLEDGEMENT

This thesis becomes a reality with the kind support and help of many individuals. First of all I would like to extend my sincere thanks to God Almighty for the wisdom he bestowed upon me, the strength, peace of my mind and good health in order to finish this research.

I would like to express my whole hearted gratitude to my father **Mr. Boobal R** my mother **Mrs. Amutha B** and my brothers **Mohan raja B, Gokul Raj B**, for their unconditional love, affection, guidance, encouragement and motivation. They remembered me in their prayers and supported me emotionally and financially thus enabled me to concentrate fully on my studies without any worries.

I acknowledge my sincere thanks to my guide **DR. K. Selvaraju, M. Pharm., Ph.D.,** Department of Pharmaceutics. KMCH college of Pharmacy, for his constant insight, guidance, kindness, persistent encouragement and sharing his knowledge and expertise that helped me to learn and complete my work.

It is my pleasure to express my deep sense of thanks to **DR. K.S.G. Arul kumaran, M. Pharm., Ph.D., Professor, HOD**, Department of Pharmaceutics.

I owe a deep sense of obligation to **DR. A. Rajasekaran, M. Pharm., Ph.D., Principal,** KMCH College of Pharmacy for his suggestions, encouragement and support.

I take this opportunity to convey my sincere thanks to our most beloved Managing Trustee, Hon'ble **Dr. Nalla G. Palaniswami** and respected Trustee Madam **Dr. Thavamani D. Palaniswami**, Kovai Medical Center Research and Educational Trust, Coimbatore for all the facilities provided for me at the institution.

I put forth my unlimited gratitude to my all other teaching staffs for their immense support, timely help and valuable suggestion.

I extent my heartfelt gratitude to **Mr. C. Sankar, M. Pharm., Ph.D.,** for their timely suggestions, guidance help and support throughout my work, which helped me to finish my work successfully.

I owe my debt of gratitude to our esteemed staffs **Miss. Gayathri M. Pharm, Mr. S. Muthukumar M.Pharm, Mr. N. Tamilselvan, M.Pharm, Ph.D.** for their invaluable support and encouragement in completing my project successfully.

I am thankful to lab technicians especially **Mrs. Akila, Mrs. Selvi, Mrs. V. Sridevi** for helping me during my project work.

I wish to thank **Mrs. Jeeva, Sudha, Dhana** & Chemical store in-charge, computer lab technicians, and all those who have co-operated with me during my project work.

I profusely acknowledge all my seniors **Nijanthan, Suresh kumar, Raj kumar, Kanupriya, Treesa, and Shyamala** for the advice, affection and encouragement throughout this journey.

I am indebted to my friends and batch mates **Karthikaa, Raj kannan, Aravindhan, Siva kumar, Santhuru, Dharsan, Rajesh, Palanichami, Balamurugan, Nithish, Sridevi, Pavithra, Maharaja, Sundharajan, Sumitha, Anitta, Anu, Anjalai, Jinu, Haritha, Malathi, Jayanthi, Nadhiya, Nivetha and Satheesh** for the dedication, love and support they gave me during the hard times. Their selfless support and motivation throughout the research is beyond words.

I take this opportunity to extend my indebtedness, gratitude to all my juniors for their memorable company and involvement – especially to **Sujithra**, **Hamesh, Arul, Hari, Salabha and Muneesh** and so on…

Last but not least, I would like to thank each and everyone who are all part of this successful completion of my thesis.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
AUC	Area under the Curve
C_{max},	Maximum Concentration
O/W	Oil in Water
CFX	Cefixime Trihydrate
DSC	Differential Scanning Calorimetry
EE	Entrapment Efficiency
FTIR	Fourier Transform Infra Red
DNA	Deoxy Ribonucleic Acid
MRT	Mean Residence Time
IDSA	Infectious Diseases Society of America
MSSA	Methicillin Sensitive Staphylococcus Aureus
gm	Gram
hr	Hour
HPH	High Pressure Homogenization
m^2/g	Meter square per gram
mg	Milligram
min	Minute
nm	Nanometre
NPS	Nanoparticles
PBS	Phosphate buffer saline
PCS	Photon Correlation Spectroscopy
GMS	glycerylmonostearate
PI	polydispersity indexs
SEM	Scanning Electron Microscopy
SLN	Solid Lipid Nanoparticles
$T_{1/2}$	Half life
TEM	Transmission Electron Microscopy

UV	Ultra Violet
ZP	Zeta potential
λ max	Lambda max (maximum absorption)
µg	Microgram
µl	Microliter
PXRD	Powder X-ray diffractometry

1. INTRODUCTION

An antibiotic is a substance which is active against bacteria and fight against bacterial infections. Antibiotic medications are widely used in the treatment and prevention of such infections They may either kill or inhibit the growth of bacteria.[1,2]

A Cephalosporin's are a group of β-lactam antibiotics which is discovered by Edward Abraham and his fellow workers in Oxford produced by *Cephalosporium acremonium*, a mould nurtured from a Sardinian sewage out fall by Giuseppe Brotzu in 1948 [3].Cephalosporin's are a class of antibiotics routinely used for a variety of infections, many of which are recommended first line therapies in North American Infectious Diseases society guidelines such as the Infectious Diseases Society of America (IDSA).

In general, there are six different generations of cephalosporins, and drugs in each generation are used in different indications. While the core structure is the same for ß-lactams, changes in position 7 of the β-lactam ring are what differentiate the spectrum of activity for each of the cephalosporin generations [4].

First generation cephalosporins include cefazolin and cephalexin, and these agents are known for their coverage of methicillin-sensitive *Staphylococcus aureus* (MSSA) and streptococci with some Gram-negative bacilli coverage. First generation cephalosporins are commonly prescribed for surgical site infection (SSI) prophylaxis for almost all surgeries, either as monotherapy or as combination therapy [5].

Cefazolin is also used as prophylaxis with the insertion of a cardiac device and as prophylaxis for endometritis. This generation is used as first line therapy for Group A streptococcus (GAS) pharyngitis, outpatient treatment for mild diabetic foot infections (DFIs), mild-to-moderate intra-abdominal infections (IAIs), cholecystitis, combat wounds, and prosthetic joint infections (PJIs)[6-8]. Additionally, first generation cephalosporins can be used as alternative therapy for uncomplicated cystitis and alternative prophylaxis against infective endocarditis[9].Cefazolin can also be used as definitive therapy for vertebral osteomyelitis, infections from endoscopic urologic procedures with mucosal trauma, necrotizing fasciitis, pyomyositis, SSIs, and in antibiotic locks. More recently, cefazolin, a first-generation cephalosporin, has been examined as a first line agent for treating MSSA infections, including bacteremia and endocarditis[10-12].

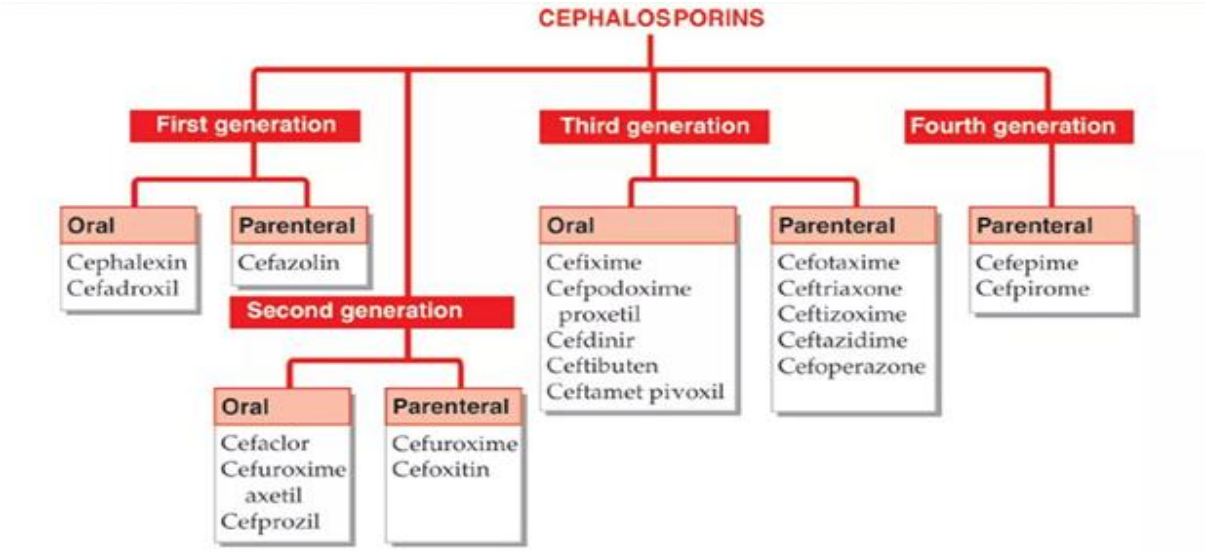


Fig no 1: Classification of Cephalosporins

Second generation cephalosporins are broken upinto two groups which is true second generation cephalosporins and the cephamycins. The true second generation cephalosporins include cefuroxime and cefprozil, whereascephamycins include cefoxitin, cefotetan, and cefmetazole. This class has good coverage against enteric Gram-negative bacilli, *Haemophilu sinfluenzae*, and *Neisseria* spp., with most second generation cephalosporins displaying moderate coverage against streptococcus and staphylococcus. Cefoxitin has moderate coverage of both Gram-positive and Gram-negative anaerobes. Because of the anaerobic coverage, cefoxitin is used prophylactically in multiple surgeries, including cardiac, biliary, appendectomy, small intestine, colorectal, head and neck, hysterectomy, and urologic [5]. Cefoxitin can also be prescribed for use in treating pelvic inflammatory disease (PID), moderate severity DFIs, human and animal bites, early localized or early disseminated Lyme or Lyme-induced arthritis, and mild-to-moderate severity IAIs.

Third generation cephalosporins are the most prescribed cephalosporins and are the first generation to be considered an extended-spectrum cephalosporin. This class includes ceftriaxone, cefotaxime, ceftazidime, ceftazidime/avibactam, cefdinir, cefpodoxime, and cefixime. They are more stable to common β-lactamases produced by Gram-negative bacilli, which offers good coverage against enteric Gram-negative bacilli. However, third generation cephalosporins are hydrolyzed by broad-spectrum β-lactamases, such as extended-spectrum β-lactamases (ESBLs), AmpC-producing organisms, and carbapenemases among others [13].

Additionally, this class has good coverage against *Streptococcus* spp., moderate coverage against MSSA, and ceftazidime has in vitro activity against *Pseudomonas aeruginosa*. Due to its broad-spectrum of activity, third generation cephalosporins are preferred for a large variety of indications. They are recommended for use as first line prophylaxis against inpatient spontaneous bacterial peritonitis (SBP), biliary or colorectal or liver transplant SSIs, and infections post-urologic procedures, and are recommended as alternative prophylactic agents for neutropenic infections and infective endocarditis. This class is heavily used as first line therapy for most infections, including SBP, sexually transmitted infections (gonorrhea, chlamydia, PID, epididymitis, proctitis), moderate-to-severe DFIs, outpatient treatment of CAP, inpatient, non-intensive care unit (ICU) treatment of CAP, outpatient empiric treatment for suspected infection in Human Immunodeficiency Virus (HIV) patients, inpatient empiric treatment for suspected infection in HIV patients, pyelonephritis, necrotizing fasciitis, glanders, SSIs, human and animal bites, Lyme disease with CNS involvement and with or without parenchymal involvement, mild-to-severe IAIs, healthcare-associated (HCA) complicated IAIs, encephalitis, PJIs, HCA meningitis/ventriculitis, and community-acquired meningitis.

Ceftazidime is the only cephalosporin with a Food and Drug Administration (FDA)-approved indication for the inpatient treatment of febrile neutropenia, however, its use is not recommended due to a lack of reliable activity against Gram-negative bacilli and streptococcus **[14, 19]**.

Third generation cephalosporins can also be used as alternative treatment options against a variety of infections, including syphilis, gonorrhea, acute bacterial rhinosinusitis, endometritis, GAS pharyngitis, uncomplicated cystitis, infectious diarrhea in Acquired Immune Deficiency Syndrome (AIDS) patients, *Vibrio cholera*, *Yersinia enterocolitica*, combat wounds, PJIs, and community-acquired meningitis **[15,16]**.

Cefepime is a fourth-generation cephalosporin that is active against MSSA, Streptococcus spp., *P. aeruginosa*, and enteric Gram-negative bacilli. Cefepime is commonly used as first line therapy for empiric febrile neutropenia, HAP/VAP, severe DFIs, *P. aeruginosa* isolated in CAP, severe intra-abdominal infections, cholecystitis, cholangitis, HCA biliary infections, PJIs, and HCA meningitis/ventriculitis**[17]**. It can also be used as alternative therapy for community-acquired meningitis and definitive therapy for vertebral osteomyelitis and also used in culture-negative infective endocarditis.

The fifth generation cephalosporins are otherwise known as anti-methicillin-resistant *S.aureus* (MRSA) cephalosporins, which includes ceftaroline and ceftibiprole. These agents offer good coverage against Gram-positive cocci (e.g., MSSA, MRSA, and *Streptococcus* spp.) and enteric Gram-negative rods, with the exception of extended-spectrum beta-lactamase producers, *Acineto bacterbaumanii*, and *Stenotrophomonas maltophilia* . Ceftaroline is available for use in the US and is recommended as a first line agent for treating SSIs. Recent data from the capture trial also suggest that ceftaroline is suitable for treating infective endocarditis. Ceftibiprole provides additional antimicrobial activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa* and used as a therapy in the treatment of nosocomial infections and CAP caused by MRSA **[18].**

The newer cephalosporins, ceftolozane/tazobactam, has not categorized into an existing generation due to its unique spectrum of activity. This agent provides good coverage against enteric gram-negative bacilli, *P. aeruginosa*, and *Streptococcus* spp. It is currently FDA-approved for treating complicated IAIs and pyelonephritis. Ceftolozane/tazobactam also has results pending for treatment of HAP **[19].**

1.1 TARGETED DRUG DELIVERY SYSTEM:

Targeted drug delivery system is the most challenging research areas in pharmaceutical sciences. By producing colloidal delivery systems like liposome, micelles and nanoparticles, new challenges have opened for improving drug delivery [20]. Nanoparticles are the nano sized solid colloidal particles which usually ranges from 10 to 1000 nm (1.0μm), in which active drug or biologically active material are dissolved, entrapped, and/or to which the active principle is adsorbed or attached[21]. In the last decade, lipids are one of the carriers that have suitable property for the delivery of drugs with poor water solubility. If any therapeutic agent is added into the lipid, therapeutic usefulness of drugs will be maximized. Nowadays, Lipid-based nano carriers are an acceptable approach and have gained significance in the current era because of their various prominent properties, such as low toxicity, improved bioavailability, high biocompatibility, high drug-loading efficiency, and high protection from degradation in the gastro intestinal tract. Various lipids used for the preparation of lipid nano carriers are those which are biodegradable and showing biocompatibility in physiological media or biological fluid **[22].**To overcome the limitations of polymeric nanoparticles, lipids are incorporated as a carrier, especially for lipophilic pharmaceuticals. These lipid nanoparticles are known as solid lipid nanoparticles (SLNs), which are attracting the wide attention of

formulators worldwide [23]. It is the new generation of submicron-sized lipid emulsions in which the liquid lipid has been replaced by a solid lipid. SLN gives properties like small size, large surface area, high drug loading capacity, interaction of phases at the interfaces to improve the performance of pharmaceuticals, nutraceuticals and other materials [24].Various Drug Delivery System developed using Nanotechnology principles including Nanoparticles, Solid Lipid Nanoparticles, Nanosuspension, Nanoemulsion,Nanocrystals[23].

1.1.1 Nanoparticles [25-31]:

Nanoparticles are solid polymeric, submicronic colloidal system size ranging from 5-300nm which consist of macromolecular substances that vary in size 10nm to 1000nm. The drug is dissolved, entrapped, adsorbed, attached or encapsulated into the nanoparticle matrix.

Depending upon the method of preparation, nanoparticle, nanosphere or nanocapsule can be obtained with different properties.

Nanosphere are matrix system in which drug is physically and uniformly dispersed throughout, then particles prepared by using different polymers such as polyalkyl cyanoacrylate & poly lactides or they can be solid lipid nanosphere prepared by using lipids like dipalmitoyl – phosphatidyl choline.

Nanocapsule are ultrafine vesicular system with a diameter less than 1 µm in which the drug is confined to a cavity surrounded by a unique polymer membrane and having aqueous or oily core containing drug substances.

Nanoparticles holds much interest, because in this range materials can have different and enhanced properties compared with the same materials of a larger size due to the following two major principle factors. The increased surfaces are of quantum effect. These factors which can enhances the properties such as reactivity, strength, electrical characteristics & in vivo behaviour hence a much greater surface area per unit mass compared with the larger particles leading to greater reactivity.

The advantages of using nanoparticles loaded with drugs, because of their small size can penetrate through small capillaries and are taken up by cells and allow the drug release at right rate and dose at specific sites in the body for a certain time to release the accurate delivery, which enhances the therapeutic effect and reduces the toxicity and side effects. The use of biodegradable materials for nanoparticles preparation shows sustained release within the target site over a period of days or even weeks.

1.2 Types of NPS as carrier for drug & diagnostic agents

- Polymeric NPS
- Nanosuspensions and nanocrystals
- Polymeric micelles
- Ceramic NPS
- Liposome's
- Fullerenes and dendrimers
- SLN (Solid lipid nanoparticles)
- Magnetic nanoparticles
- Nanoshells coated with gold
- Nanomers and carbon nanotubes

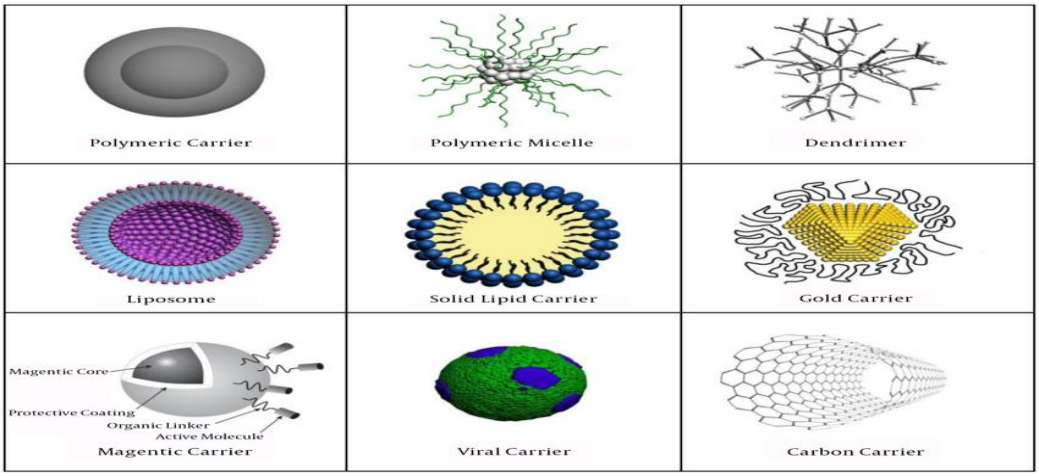


Fig No 2: Types of Nanoparticles

1.3 SOLID LIPID NANOPARTICLES:

Solid lipid nanoparticles were discovered by Gasco and Muller in 1991 it represents an alternative carrier system to tradition colloidal carriers. Lipids are commonly used as an alternative carrier for polymeric nanoparticles, especially for lipophilic pharmaceuticals and lipid nanoparticles which is known as solid lipid nanoparticles (SLNs). The system consists of spherical solid lipid particles in the ranges of nanometer it is dispersed in water or in aqueous surfactant solution. It is identical for oil-in-water type emulsion for parenteral nutrition but the liquid lipid of the emulsion has been substituted by a solid lipid, which yields a Solid Lipid Nanoparticles. SLNs are colloidal particles derived from oil-in-water emulsions by replacing liquid lipids with a lipid matrix that is solid at body temperature and stabilized by the use of surfactants. SLN are prepared by a combination of lipids, fatty alcohol, wax, triglycerides and

surfactants [32].Solid lipid nanoparticles are one of the novel potential colloidal carrier systems which is used as substitute materials to polymers which is indistinguishable to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion which is replaced by a solid lipid. They have advantages such as good biocompatibility, low toxicity and the system is physically stable. Solid lipid nanoparticles may be a promising sustained– release and drug targeting system for lipophilic drugs.

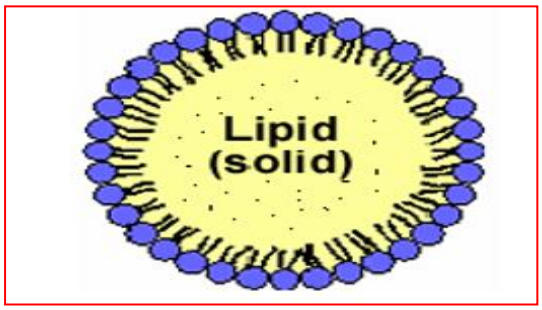


Fig No 3: Structure of SLN

Solid lipid nanoparticles (SLNs) are considered to be the most efficient lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid mechanisms which are in solid state at room temperature.

1.3.1 Advantages of solid lipid nanoparticles [32]

The advantages of SLNs including the following such as:

- SLNs can be enhancing the bioavailability of entrapped bioactive.
- Better control over release kinetics of encapsulated compound.
- Drug stability of SLNs for three years has been developed. This is of more importance
- Compared to the other colloidal carrier systems.
- It is easy scale up and excellent biocompatibility.
- Enhanced bioavailability of entrapped bioactive compounds.
- Controlled and targeted release of the incorporated drug can be achieved.
- Increased scope of drug targeting can be achieved by coating with or attaching ligand to SLNs.
- Excellent reproducibility with use of different methods as the preparation procedure.

- SLNs particularly those in the range of 120 -200 nm are not taken up readily by the cells present in the RES and thus bypass liver and spleen filtration.
- The feasibility of incorporating both hydrophilic and hydrophobic drugs.
- The carrier lipids are biodegradable and safe.
- Avoidance of organic solvents.
- Feasible for large scale production and sterilization.
- In SLNs the lipid matrix is made from physiological lipid which decreases the danger of acute and chronic toxicity.

Table No: 1 Different material used for the preparation of SLNs

Lipids	Surfactants and Co surfactants
Triglycerols Tricaprin Trilaurin TrimyristinTripalmitin, Tristearin	**Phospholipids** Phosphatidylcholine Soy lecithin Egg lecithin
Acylglycerols Glycerol behenate Glycerol monostearate Glycerol palmitostearate	**Ethylene oxide/propylene oxide copolymers** Poloxamer 188 Poloxamer 182 Poloxamer 407 Poloxamer 908
Fatty acids Decanoic acid Behenic acid Stearic acid Palmitic acid	**Sarbitan ethylene oxide/propylene oxide copolymers** Polysorbate 20 Polysorbate 60 Polysorbate 80
Waxes Cetylpalmitate	**Alkylaryl polyether alcohol polymers** Tyloxapol
Cyclic complexes Cyclodextrin	**Bile salts:** Sodium cholate Sodium taurodeoxycholate Taurocholic acid sodium salt Sodium taurocholate Sodium glycocholate
Hard fat types Witepsol W 35 Witepsol H 35	**Alcohols** Ethanol Butanol

1.4 PREPARATION OF SOLID LIPID NANOPARTICLES: [25, 29, 30, 31]

The performance of SLNs greatly depends on the method of preparation which in turn influences the particle size, drug loading capacity, drug release, drug stability etc. Different approaches exist for the production of finely dispersed lipid nanoparticle dispersions.

Methods of Preparation [33-35]

- High pressure homogenization

a) Hot homogenization.

b) Cold homogenization.

- Ultrasonication/high speed homogenization

a) Probe ultrasonication.

b) Bath ultrasonication.

- Solvent evaporation method.
- Solvent emulsification-evaporation method.
- Supercritical fluid method.
- Micro emulsion based method.
- Spray drying method.
- Double emulsion method.
- Precipitation technique. .

1.4.1 HIGH PRESSURE HOMOGENIZATION (HPH)

It is a dependable and powerful technique, which is used for the production of SLNs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap. The fluid which accelerates on a very short distance to very high velocity (over 1000 km/h). Very high shear stress and cavitations forces interrupt the particles down to the

submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated. Two general approaches of HPH are hot homogenization and cold homogenization; work on the same concept of mixing the drug in bulk of lipid melt.

HOT HOMOGENIZATION:

Hot homogenization is carried out at temperatures above the melting point of the lipid and can consequently be regarded as the homogenization of an emulsion. A preemulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles frequently results in an increase of the particle size due to high kinetic energy of the particles.

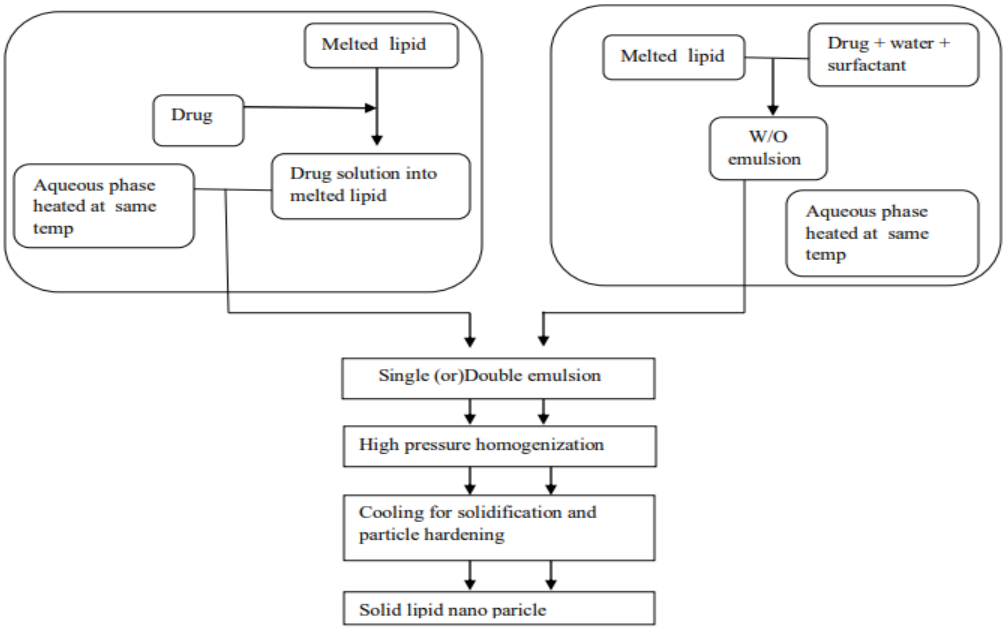


Fig no 4: Solid lipid nanoparticles prepared by hot homogenization process

COLD HOMOGENIZATION:

Cold homogenization has been developed to beat various problems associated with hot homogenization such as: Temperature-induced drug degradation, drug distribution into the aqueous phase during homogenization, Complexity of the crystallization step of the

nanoemulsion leading to several modifications and/or super cooled melts. In this technique the drug containing lipid melt is cooled, the solid lipid ground to lipid micro particles and these lipid micro particles are dispersed in a cold surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or less than room temperature, the cavitations force is strong enough to break the lipid micro particles directly to solid lipid nanoparticles.

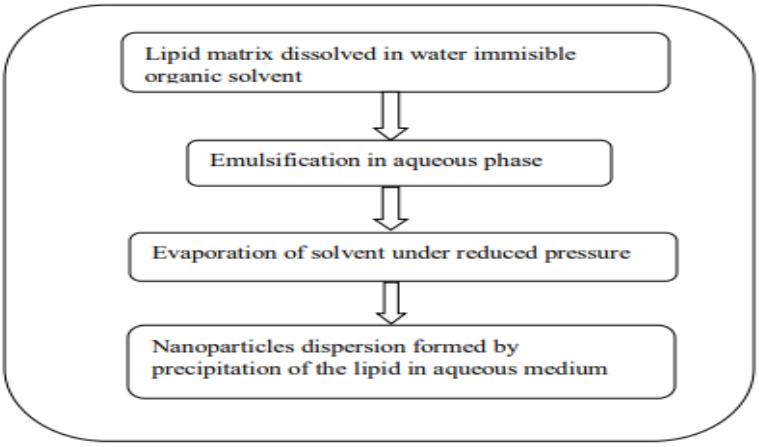


Fig No 5: Solid lipid nanoparticles prepared by cold homogenization process

1.4.2 ULTRASONICATION/HIGH SPEED HOMOGENIZATION:

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

1.4.3 SOLVENT EVAPORATION:

SLNs are also prepared by solvent evaporation method. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was separate from the emulsion by evaporation under reduced pressure.

1.4.4 SOLVENT EMULSIFICATION-DIFFUSION METHOD:

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

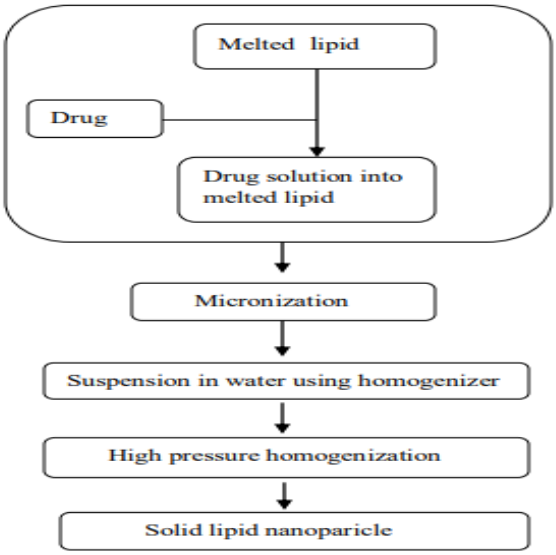


Fig No 6: Systematic representation for emulsification-diffusion method

1.4.5 SUPERCRITICAL FLUID METHOD:

This is a relatively new technique for SLN production and has the advantage of solvent-less processing. There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method.

1.4.6 MICRO EMULSION BASED METHOD:

This method is based on the intensity of micro emulsions. As micro emulsions are two phase systems composed of an inner and outer phase (e.g. o/w micro emulsions). They are made by stirring an optically transparent mixture at 65-70°C which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot micro emulsion is diffused in cold water (2-3°C) under stirring. SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and

prevent aggregation. Due to the dilution step; achievable lipid contents are considerably lower compared with the HPH based formulations.

1.4.7 SPRAY DRYING METHOD:

It is an alternative technique to the lyophilisation process. This recommends the use of lipid with melting point more than 70^0 C. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture.

1.4.8 DOUBLE EMULSION METHOD:

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

1.4.9 PRECIPITATION METHOD:

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

1.5 SECONDARY PRODUCTION STEPS [36, 37]:

1.5.1 Freeze drying:

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required to achieve long term stability for a Product containing hydrolysable drugs or a suitable product for per-oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions.

In case of freeze drying of the product, all the lipid matrices used, form larger solid lipid nanoparticles with a wider size distribution due to presence of aggregates between the nanoparticles. The conditions of the freeze drying process and the removal of water promote the aggregation among SLNs. An adequate amount of cryoprotectant can protect the aggregation of Solid lipid nanoparticles during the freeze drying process.

1.5.2 Sterilization:

Sterilization of the nanoparticles is desirable for parenteral administration and autoclaving which is applicable to formulations containing heat-resistant drugs. Effects of sterilization on particle size have been investigated and it was found to cause a distinct increase in particle size.

1.5.3 Spray drying:

Spray drying might be an alternative procedure to lyophilisation in order to transform an aqueous SLN dispersion into a dry product. This method has been used scarcely for SLN formulation, although spray drying is cheaper as compared to lyophilisation. The lipids with melting points at temperature >70°C had been recommended for spray drying.

1.6 DRUG INCORPORATION MODELS AND TYPES OF SLNs [38]:

Factors affecting loading capacity of a drug in lipid are

1. Chemical and physical structure of solid matrix lipid

2. Polymorphic state of lipid material

3. Solubility of drug in lipid melt

4. Miscibility of drug melt and lipid melt

Drug incorporation models are as follows:

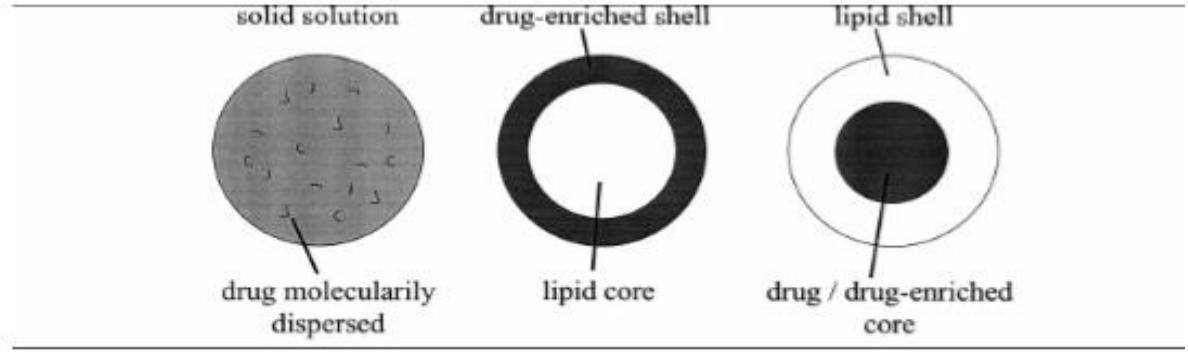


Fig no 7: Drug incorporation models

Solid solution model:

Drug is molecularly dispersed in lipid matrix when SLN is prepared by cold homogenization

Drug-enriched shell model:

A solid lipid core forms upon recrystalization temperature of the lipid is reached.

Drug-enriched core model:

Cooling the nanoemulsion leads to a super saturation of the drug which is dissolved in the lipid melt leads to re crystallization of the lipid.

1.7 POSSIBLE PROBLEMS IN SLN PREPARATION AND SLN PERFORMANCE[33, 31, 38]:

1.7.1. High pressure-induced drug degradation:

It has been shown to decrease the molecular weight of polymers. High stress has been assumed to be the major cause and evidence of free radical formation was reported. But it's not a serious problem for majority of the drugs.

1.7.2. Lipid crystallization and drug incorporation:

The following three key aspects should be considered:

A) Super cooled melts:

The main reason for the formation of super cooled melts is the size dependence of crystallization processes. The tendency of the formation of super cooled melts increases with decreasing droplet size. It is therefore necessary to proof the solid state of the lipid by appropriate analytical techniques (NMR, X-ray or DSC).

B) Lipid modification:

The crystallized lipid may be present in several modifications of the crystal lattice. During storage, rearrangement of crystal might occur in favour of thermodynamically stable configurations and resulted in expulsion of drug. The utilization of higher drug-loading capacity in unstable configurations prevents lipid modification during storage.

C) Particle shape:

The shape of lipid nanoparticles may significantly differ from a sphere, lipids prefer to crystallize in the platelet form. This can be overcome by increasing the surfactant concentration.

1.7.3. Co existence of several colloidal species:

Unstable drugs will hydrolyze rapidly in contact with water and the distribution equilibrium of the drug between the different environments will be distorted. This can be overcome by increasing the matrix viscosity.

1.8. EVALUATION PARAMETERS FOR SLNs: [39-41]

In order to expand a drug product of high quality, a precise physicochemical characterization of the SLNs is necessary. Characterization of solid lipid nanoparticles is a grave challenge due to the small size of the particles and complexity of the system.

1.8.1 Particle size Measurement:

Many techniques are available for particle size analysis and zeta potential like scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunnelling microscopy (STM) and photon correlation spectroscopy (PCS). To determine the particle size the best suitable methods are Photon correlation spectroscopy (PCS) and laser diffraction (LD). PCS is also known as dynamic light scattering which measures the fluctuation of the intensity of the scattered light, which is caused by particle movement.

1.8.2 Measurement of Zeta potential:

Zeta potential measurement can be carried out using zeta potential analyzer or zeta meter. Zeta potential provides information about the magnitude of the electrostatic repulsion or attraction between particles in the aqueous suspension of SLN. Zeta potential can serve as an important parameter in the predictions for long term stability of the formulations. High values of zeta potential (e.g., more than +30mV or less than-30mV) can stabilize the colloidal suspension by electric repulsion, Electric repulsion generally results in less contact between the particles and less aggregation. For example colloidal systems that containing stearic

stabilizers which express good and long term stability when the zeta potential is as low as around 0mV.

1.8.3 Electron Microscopy:

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide way to directly observe nanoparticles. SEM is however better for morphological examination. TEM has a small size limit of detection. Transition electron microscopy and light microscopy both are based on same principle but one difference is that in light microscopy light is used instead of electron.

1.8.4 Differential Scanning Calorimetry (DSC):

Differential Scanning Calorimetry (DSC) which is used to measures differences in the amount of heat required to increase the temperature of a sample compared to a reference. Differences in heat flow may be positive or negative and are presented as function of the temperature. During phase transition there were differences in the samples when compared to the reference. The rate of crystallinity is estimated using DSC by comparing the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion.

1.8.5 Entrapment efficiency:

By measuring the concentration of free drug in the dispersion medium the entrapment efficiency of the drug is determined. Ultracentrifugation carried out using the Centrisart, that consist of filter membrane (molecular weight cut-off 20,000Da) at the base of the sample recovery chamber. The SLNs along with encapsulated drug remain in the outer chamber and aqueous phase moves into the sample recovery chamber. The HPLC or UV spectrophotometer method was used to measure the amount of the drug present in the aqueous phase.

1.8.6 Stability Studies:

Drug loaded SLNs are stored at 25°C for 6 months and average size and entrapment efficiency are determined.

1.8.7 Effect of sterilization:

To observe the effect of sterilization on particle size, zeta potential and entrapment efficiency, blank and drug dispersions are autoclaved at 121°C for 20 minutes.

1.8.8 Powder X-ray diffractometry (PXRD):

PXRD studies are performed in order to identify the crystallinity behaviour of the SLN.

1.8.9 *In-vitro* drug release:

Dialysis tubing:

The SLN dispersion is located in prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for drug content using a suitable analytical method.

Reverse dialysis:

In this technique a number of small dialysis sacs containing 1 ml of dissolution medium are placed in SLN dispersion. The SLNs are then displaced into the dissolution medium. The direct dilution of the SLNs is possible with this method.

Franz diffusion cell:

The solid lipid nanoparticle diffusion is; placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The dispersion is then dialyzed against a appropriate dissolution medium at room temperature; the samples are withdrawn from the dissolution medium at suitable intervals and analyzed for drug content. The maintenance of sink condition is essential.

1.9 PHARMACOKINETIC ANALYSIS:

Serum concentration versus time data for drug in individual rats is analyzed by non compartmental estimations. Relative bioavailability, C_{max}, T_{max}, AUC and MRT can be estimated.

1.10 APPLICATION OF SOLID LIPID NANOPARTICLES:[42-50]

Oral SLNs in anti tubercular chemotherapy:

Anti tubercular drugs such as rifampicin, isonizide, pyrazinamide-loaded SLN systems, were able to decrease the dosing frequency and improve patient compliance. By using the emulsion solvent diffusion technique this anti tubercular drug loaded solid lipid nanoparticles are prepared.

SLNs for topical use:

SLNs used for topical application for various drug such as anticancer, vitamin-A, isotretinoin, flurbiprofen .Using glyceryl behenate, vitamins A-loaded nanoparticles can be prepared. This method is useful for the improvement of penetration with sustained release. The isotretinoin-loaded lipid nanoparticles were formulated for topical delivery of drug.

SLNs as Cosmeceuticals:

The SLNs have been applied in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. SLN and NLCs have proved to be controlled release innovative occlusive topicals. Better localization has been achieved for vitamin A in upper layers of skin with glyceryl behenate SLNs compared to conventional formulations

Stealth Nanoparticles:

These provide a novel and unique drug-delivery system they evade quick clearance by the immune system. Such nanoparticles can target specific cells. Stealth SLNs have been successfully tested in animal models with marker molecules and drugs. Antibody labeled stealth Lipo bodies have shown increased delivery to the target tissue in accessible sites

SLN as a carrier for vaccines:

Adjuvants are used to improve immune response during vaccination. The safer novel subunit vaccines are less efficient in immunization, and for this reason, efficient adjuvant are needed. Emulsion systems of SLNs have been recently employed to use the adjuvant. This is O/W emulsions that are degraded in the body after administration

For Parenteral Application:

SLN are very suitable for systemic delivery because they consist of physiologically well-tolerated ingredients and they have good storage capabilities after lyophilization and/or sterilization.

A targeted carrier for solid tumors:

SLNs have been reported to be useful as drug carriers to treat neoplasm.

SLNs for potential agriculture application

Essential oil extracted from Artemisia arboreseens L when incorporated in SLN, were able to reduce the rapid evaporation compared with emulsions and the systems have been used in agriculture as a suitable carrier of ecologically safe pesticides.

SLNs as gene vector carrier

SLN can be used in the gene vector formulation. There are several recent reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and other nucleic acids. The gene transfer was optimized by incorporation of a diametric HIV-1 HAT peptide (TAT 2) into SLN gene vector.

SLN applied to the treatment of malaria:

Despite the fact that we live in an era of advanced technology and innovation, infectious diseases, like malaria, continue to be one of the greatest health challenges worldwide. The drawbacks of conventional malaria chemotherapy are to develop multiple drug resistance and the nonspecific targeting to intracellular parasites, resulting in increased dose requirements and subsequent in tolerable toxicity.

2. REVIEW OF LITERATURE

Waghware BG *et al.*, (2018) [51] developed a novel technique, solid lipid nanoparticles Olmesartan Medoxomil, antihypertensive drug used as model drug to improve its aqueous solubility, dissolution rate & ultimately bioavailability by preparing solid lipid nanoparticles using solvent emulsification-evaporation method. The prepared solid lipid nanoparticles were characterized by SEM, FTIR, DSC and XRD. The solubility profile was compared with pure Olmesartan Medoxomiland found that more than three-fold increase in solubility of OlmesartanMedoxomil SLNs. *In vitro* release of OLMSLNs formulation was shown to be improved as compared to pure drug.

Zeynep Kipriy *et al.*, (2017) [52] carried out preparation and evaluation of carvedilol-loaded solid lipid nanoparticles for targeted drug delivery. The findings of the present study show that carvedilol loaded solid lipid nanoparticles were prepared successfully by hot homogenization and ultrasonication process. The molecular state of carvedilol changed from the crystalline state to the amorphous state following incorporation into solid lipid nanoparticles. The developed formulation is stable and safe, and represents a promising system for the sustained and controlled delivery of carvedilol to target cells, tissues and organs.

Mangal B. Gahandule *et al.*, (2016) [53] developed a simple, accurate and precise area under curve (AUC) spectrophotometric method for estimation of cefixime trihydrate and validation according to ICH Q2 (R1) guideline. The area selected for estimation of cefixime trihydrate was between 262.80 to 303.60 nm. The method represented correlation coefficient (R2=0.999) at concentration rang 5-25 µg/ml. The Satisfactory value of percent relative standard deviation for the intraday and inter-day precision indicates that method was precise. The recovery of the cefixime trihydrate was found upto 100.12 %. The developed methods can be successfully applied in routine work for the estimation of cefixime trihydrate in its pharmaceutical dosage form.

Venkateswara Reddy *et al.*, (2015) [54] formulated floating tablets containing Cefixime offers a suitable and practical approach in serving desired objective of retaining the drug in the stomach to increase the its bioavailability. The tablets were prepared by direct compression method and total of 12 formulations are developed employing HPMC K100M and HPMC K15M as polymers for sustaining the drug release and sodium bicarbonate as the gas generating agent. Various polymers have been selected and subjected to IR-spectroscopic studies. The powder blends of all the formulations have shown good flow properties. Other parameters such as hardness, friability, drug content uniformity, Floating lag time and in-vitro dissolution studies were performed and the results were satisfactory.

Muder al hayder *et al.*, (2015) [55] formulated a cefixime dispersible tablet and evaluate the flow ability, wet ability, disintegration time and in vitro dissolution with impact to a marketed cefixime tablet. It is evaluated based on disintegration time using a direct compression technique except formula (F7) which was prepared by wet granulation. Different super disintegrants such as croscarmellose sodium (CCS) and crospovidone (CP) were used and evaluated for disintegration time. Seven formulations were prepared and evaluated for flow ability, hardness, wetting time, disintegration time and in vitro drug release. The best formulation of super disintegrants was CP at a concentration of 10% (Formula F5) as it gave a rapid disintegration time (25 s) and less wetting time (20 s) compared to the other formulae. It was found that the dispersible tablets of cefixime proved to show a better release profile in all aspects as compared to the marketed formulation (Zimaks®). Using different super disintegrants or methods of compression have significant effects on the hardness and wetting time of cefixime tablets.

Satish M Havnoor *et al.*, (2014) [56] carried out preparation, characterization and *in vivo* evaluation of Isradipine loaded nanoparticles for hypertension. Reported that improving the oral bioavailability of isradipine by loaded solid lipid nanoparticles using triglycerides, monoglyceride and poloxamer 188 as surfactant. The drug release to be 99% within 12 hours mechanism followed by diffusion and erosion.

Preeti *et al.*, (2013) [57] investigated the effect of hydrophilic polymers PVP and HPMC on cefixime (CFX) complexation with β-cyclodextrin (β-CD) to study the solubility and dissolution rate of Cefixime. The binary (CFX-β-CD) and terenary mixtures (CFX – β-CD-PVP, CFX –β-CD-HPMC) were prepared by physical mixing. The increase in dissolution rate was found to be higher for the ternary systems than respective binary compositions. CFX-β-CD-HPMC ternary system was found to exhibit faster dissolution profile as compared to other systems.

Surender *et al.*, (2013) [58] developed solid lipid nanoparticles of cefixime by solvent evaporation method using compritrol ATO 888 as the lipid core. The drug release in the first 10 h was about 95.37% of the total drug. The relative bioavailability of cefixime-SLNs was found to be 1.87. These results showed that cefixime absorption is enhanced by using solid lipid nanoparticles.

A.R. Gardouh *et al.*, (2013) [59] studied a study on solid lipid nanoparticles of glyceryl mono stearate containing dibenzoyl peroxide, Erythromycin base and Triamcinolone acetonide as model drugs. High shear hot homogenization method was used for the preparation of solid lipid nanoparticles loaded with three model lipophilic drugs. By using different standard physical and imaging methods, the prepared solid lipid nanoparticles were evaluated. Infrared spectroscopy and thermal procedures were used for studying the stability of prepared formulae. When compared with the pure drugs and commercially available formulation, model drugs showed faster release patterns significantly ($p<0.05$). Finally, in this study they concluded that high encapsulation efficiency was observed with solid lipid nanoparticles with small particle size and relatively high loading capacity for Erythromycin base, Dibenzoyl peroxide and Triamcinolone acetonide as model drugs.

Praveen Kumar Gaur *et al.*, (2013) [60] prepared Diclofenac sodium loaded solid lipid nanoparticles using guggul lipid as major lipid component. Melt-emulsion sonication/low temperature solidification methods were used for the preparation of SLNs and characterized for in vitro drug release, physical parameters & accelerated stability studies and formulated into gel. The highest in vitro drug release was given by GMS nanoparticle 1 and stearic acid nanoparticle 1. When compared to commercial emulgel in receptor fluid, guggul lipid nanoparticle gel 3 exhibited 104.68 times higher drug content. It also showed higher Cmax which is almost 8-12

times greater than commercial Emulgel at 4 hours. Finally, in this study, they concluded that SLN with guggul lipid showed good physical properties with acceptable stability and also a promising permeation profile.

Rakesh Kumar Sharma *et al.*, (2013) [61] studied on the solid lipid nanoparticles as carrier of Metformin for transdermal delivery. Evaluation was done for particle size, surface morphology and in vitro- in vivo release studies. Evaluation for patches was done by ex-vivo skin permeation studies. Solvent diffusion technique was used for the preparation of Metformin solid lipid nanoparticles using polymethacrylic acid as polymer, propylene glycol as solvent and Soya lecithin as lipid base. Permeation of high cumulative amount of drug was observed with ex-vivo permeation studies. Finally, the authors concluded that for diabetes patients, transdermal delivery of Metformin solid lipid nanoparticles is pain less, safe and cost effective drug delivery system.

Shagufta Khan *et al.*, (2012) [62] developed Dithranol loaded solid lipid nanoparticles. Dithranol – a poorly soluble drug was encapsulated in SLNs by revision 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 well suitable for several applications including drug delivery.

Priyanka K, *et al*., (2012) [63] studied 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.

Panakanti Pavan Kumar *et al.*, (2012) [64] conducted a study on the formulation of Atorvastatin (ATR) loaded solid lipid nanoparticles by hot homogenization followed by ultra sonication technique and optimization of formulation and process parameters to formulate preferred SLN dispersion. In this study, the effects of composition of lipid materials, zeta potential, surfactant mixture & sonication time on particle size, *invitro* drug release behavior and drug entrapment efficiency were investigated. Transmission Electron Microscopy (TEM) was used to determine the shape and surface morphology which showed fairly spherical shape of nanoparticles. When compared to the dispersion of pure drug, the ATR SLN formulation had controlled drug release over a period of 24 hrs was demonstrated by the in-vitro drug release study.

Shivashankar *et al.*, (2012) [65] formulated and evaluated pH-sensitive controlled release cefixime microspheres based on cross linked chitosan and acryl amide-grafted-poly ethylene glycol prepared by precipitation and crosslinking methods. The 80% drug was released at 11.5 h for C-grafted copolymer 50 (hydrolyzed) in pH 7.4 media compared to 38% drug released in pH 2. It is evident from this study that chitosan microspheres could be further developed to serve as an effective biodegradable carrier for controlled release of cefixime.

Subhra Prakash Bhattacharyya *et al.*, (2012) [66] conducted a study on Flurbiprofen loaded solid lipid nanoparticles, formulation and optimization by using response surface methodology. Flurbiprofen is a non- steroidal anti inflammatory drug which is poorly water soluble. Modified solvent injection method was used for the preparation of Flurbiprofen solid lipid nanoparticles dispersions by using different ratio of tripalmitin and stearic acid a lipid and pluronic-F-68 in different amounts as emulsifier. To systematically optimize the drug entrapment efficiency, particle size and drug release, a central composite design for 2 factors at 3 levels each was employed. This study concluded that the effect of the 2 factors on different response variables helped in identifying the optimum formulation with excellent dissolution profile and stability.

Ekambaram P *et al.*, (2011) [67] studied on the formulation and evaluation of solid lipid nanoparticles of Ramipril. In this study, in order to increase the Ramipril bioavailability and also to overcome the side effects by using the lipids like glyceryl mono oleate and glyceryl mono stearate along with the stabilizers like poloxamer 188, tween 80 and span 20, solid lipid nanoparticles of Ramipril were prepared. Evaluation for the prepared formulation was done in the aspects of drug content, entrapment efficiency, particle size analysis, in-vitro drug release, stability, scanning electron spectroscopy and Fourier transform- infrared studies. When compared to the other formulations with different lipids and surfactants a formulation that contains glyceryl mono oleate which was stabilized with span 20 showed smaller particle size, narrow particle size distribution and prolonged drug release.

Suchetha *et al.*, (2011) [68] studied on solubility enhancement of cefixime by preparing solid dispersions using natural polymer i.e., guar gum by various techniques such as physical mixing, kneading and solvent evaporation methods using different drug polymer ratio. The results indicated that the solubility and dissolution of cefixime solid dispersions were improved compared to pure drug.

Wenzhong Zhou *et al*., (2011) [69] studied formulation of ofloxacin-loaded SLN and determined the effect of fatty acids on the characteristics and pharmacokinetics of SLN. The results showed that the encapsulation efficiency and loading capacity of SLN varied with fatty acids in the order of stearic acid> palmitic acid> tetradecanoic acid. Pharmacokinetic study revealed that the fatty acids increase the bioavailability of SLN. These results suggest that enrofloxacin-loaded SLN are promising formulations for sustained release while fatty acids had significant influences on the characteristics and performance of the SLN.

Silva A.C. et al., (2011) [70] 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.

Remya KS *et al.*, (2010) [71] performed the formulation development, evaluation and comparative study of the effects of super disintegrants in Cefixime 50 mg oral disintegrating tablets. The super disintegrants were sodium starch glycolate and cross carmellose sodium. The formulated tablets were evaluated for various tableting properties, like hardness, thickness, friability, weight variation, and disintegration time and dissolution rate. Comparative evaluation of the above-mentioned parameters established the superiority of the tablets formulated with cross carmellose sodium to those formulated with sodium starch glycolate.

3. AIM AND OBJECTIVES

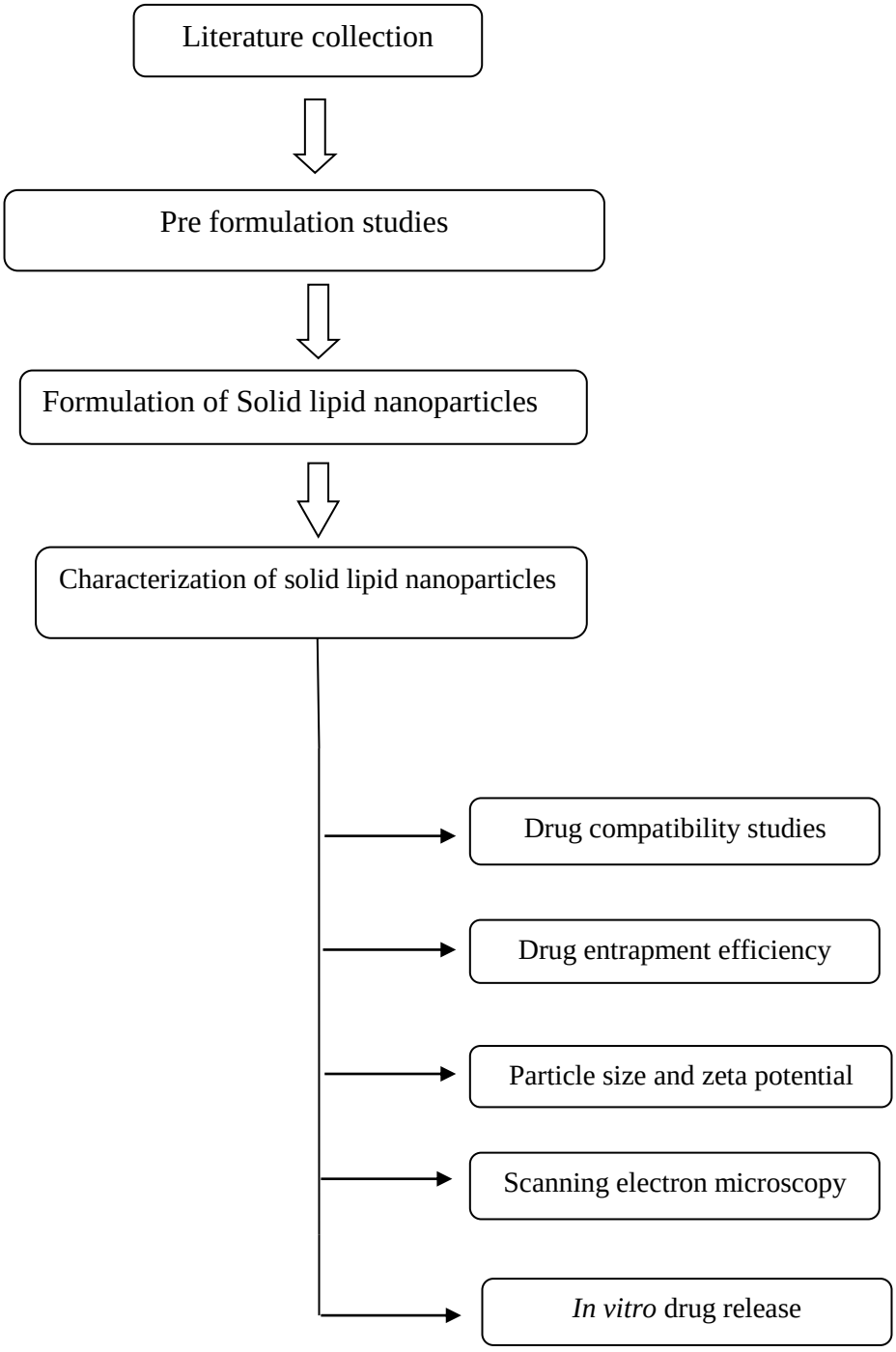
AIM

The aim of the present study is to design develop and evaluate cefixime Solid Lipid Nanoparticles for enhancement of oral bioavailability.

OBJECTIVES

- To perform pre-formulation studies
- To formulate SLN by using homogenization followed by ultra sonication technique
- To characterize drug-loaded SLNs using various techniques.
- To evaluate particle size and zeta potential of the formulation.
- To perform *in–vitro* drug release compliance with the established criteria

4. PLAN OF WORK



5. DRUG PROFILE

5.1 CEPHALOSPORIN: [72-78]

These are the group of semi-synthetic antibiotics derived from cephalosporin- C obtained from a fungus cephalosporium. They are chemically related to penicillin. The nucleus consists of β-lactum ring fused to a dihydrothiazine ring (7-amino- cephalosporinic acids). By addition of different side chain at position 7 of β-lactum ring (altering spectrum activity) and position of 3 of dihydrothiazine ring (affecting pharmacokinetics) a large number of semi-synthetic compounds have been produced. These have been conventionally divided into 4-generation.

Drug: Cefixime trihydrate.

Generic Name: Cefixime trihydrate.

Chemical Name: 7-[2-(2-aminothiazol-4-yl)-2 (carboxymethoxy-imino) acetamido] - 3-vinyl-3 cephem-4-carboxalic acid trihydrate.

Molecular Formula: $C_{16}H_{15}N_5O_7S_2$ $3H_2O$

Molecular Weight: 507.5 g/mol

Description: A white to light yellow crystalline powder.

Category: Antibiotic

Physico-chemical properties:

Melting Point: 218º to 225ºC.

5.2 STRUCTURE:

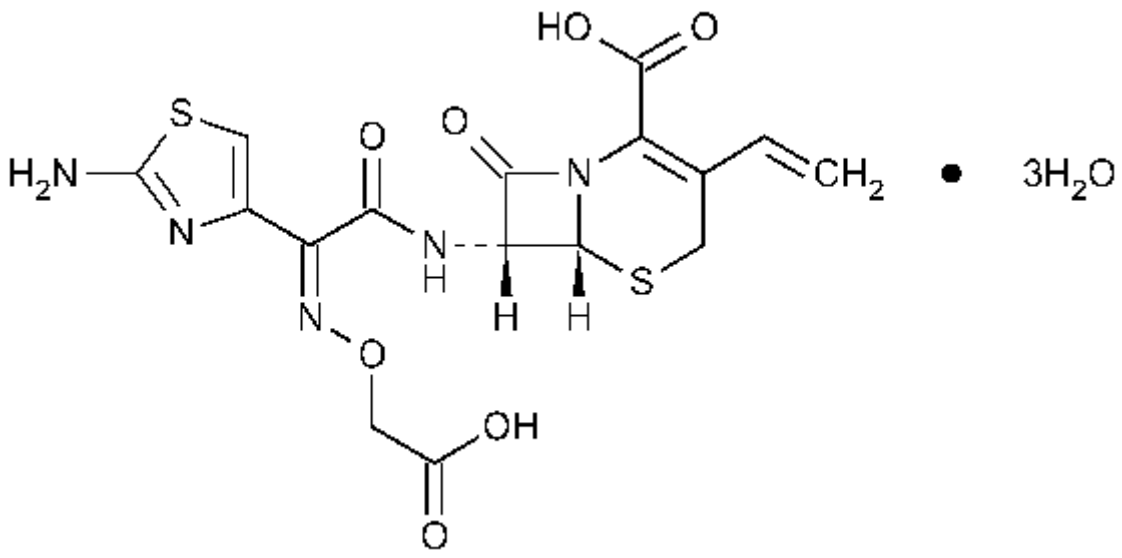


Fig no 8: Molecular structure of Cefixime trihydrate

Solubility: Practically insoluble in water and ether, slightly soluble in alcohol and acetone, freely soluble in methanol, sparingly soluble in dehydrated alcohol.

Dissociation constant: pKa=3.73

Storage: Store in air tight container.

Standards: Cefixime contain the equivalent of not less than 950 µg and not more than 1030 µg of cefixime ($C_{16}H_{15}N_5O_7S_2$) per mg calculated on anhydrous basis.

5.3 MECHANISM OF ACTION (BACTERICIDAL):

Cefixime inhibit the third and final stage of cell wall formation by preferentially binding to one or more penicillin binding proteins (PBPs) that are located in the cytoplasmic membrane beneath the cell wall of susceptible bacteria.

Its affinity for penicillin binding protein explains the rapid Lytic action of cefixime relative to that of other oral active cephalosporin.

5.4 PHARMACOKINETICS PROFILE:

Absorption:

Only 40 to 50% of an oral dose of cefixime is slowly and incompletely absorbed from gastrointestinal tract whether taken before or after meals. However, time of maximal absorption is increased approximately 50 minutes when administered with food. Peak plasma concentration is achieved in between 3 to 4 hours and is about 2µcg/ml when given in a single dose of 200 mg in adult. In children some pharmacokinetic responses have seen with 8 mg/Kg dose.

Distribution:

Cefixime is widely distributed in body tissue and fluids. It achieves high concentration in tonsils.Maxillary sinuses, bile and bile duct. About 65-70% of Cefixime is bound to plasma protein in healthy subjects.

Elimination:

No biologically active metabolites of cefixime have been identified in plasma or urine. Approximately 50% of the absorbed dose is excreted unchanged in the urine in 24 hours. More than 10% of an administered dose is excreted via bile; the serum half-life of cefixime in healthy subject is 3 to 4 hours.

Pharmacokinetic Data:

Route of administration ..... oral

Percent bioavailability (oral) ..... 47±15

Volume of distribution (liter/Kg)..... 0.30±0.03

Plasma protein bound (%) ..... 67±1

Peak time (hrs) (t_{max}) .... 3-4[a]

Peak plasma concentration (C_{max})..... 1.7 to 2.9µg/ml

Metabolized (%)..... None

Urinary excretion (%)..... 47±& and 10% of dose excreted in bile

Half life (hrs) ..... 3±0.4 increase in renal disease

Clearance ($mlmin^{-1}$ Kg^{-1})..... 1.2±0.2 decrease in renal disease

[a] – response in 200 mg single oral dose administration.

5.5 INDICATION:

Cefixime is indicated in the treatment of the following infection when caused by susceptible strains of the designated microorganisms. This is only oral cephalosporin that is recommended by the Centre of Disease Control and Prevention (CDC) for treatment of gonococci infections.

- Urinary tract infection caused by Escherichia coli.
- Otitis media caused by hemophilus influenza,
- Pharyngitis and tonsillitis caused by Staphylococcus pyrogens.
- Uncompleted gonorrhea caused by Nisseria

5.6 CONTRAINDICATION:

Cefixime is contraindicated in patients with known allergy in the cephalosporin group of antibiotics. Antibiotics including cefixime should be administered cautiously to any patients who had demonstrated some form of allergy.

5.7 DRUG INTERACTION: No significant drug interactions have been seen.

5.8 ADVERSE REACTION: Following adverse reaction have been reported:

- Gastrointestinal: Diarrhea, loose motions, abdominal pain, dyspepsia, nausea, vomiting.

- Hypersensitivity reactions: Skin rashes, urticaria, and drug fever.
- Central nervous system: Headache and dizziness.
- Others: Genital pruritis, vaginitis.

5.9 PHARMACOLOGICAL PROFILE:

Adult Dose: The recommended dose of cefixime is 200-400mg daily as a single dose or in two-divided doses. For treatment of cervical/ urethral gonococci infection, a single dose of 400 mg is recommended.

Children: 8 mg/kg/ day. It may be administered in a single dose or may be given in two-divided doses in 4 mg/Kg every 12 hours.

6. EXCIPIENTS PROFILE

6.1 GLYCERYL MONO STEARATE (GMS) [79]:

STRUCTURE:

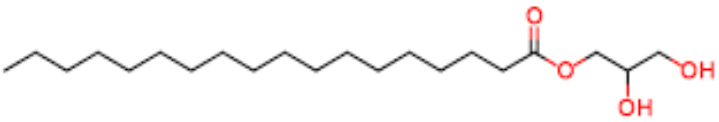


Fig 9: Chemical structure of glyceryl mono stearate

Synonyms	**:**	Aldo MS; Arlaacel129; Atoms 150; Cithrol GMS N/E; Estol 1473; Glycerol Monostearate; Glycerol stearate; GMS; Protachem GMS-450; Rita-GMS; Simulsol 165.
Chemical name	**:**	Octadecanoic acid monoester with 1, 2, 3-propanetriol.
Molecular formula	**:**	$C_{21}H_{42}O_4$.
Molecular weight	**:**	358.57 (for pure material).
Boiling Point	**:**	$\approx 240^0C$
Melting point	**:**	$55\text{-}60^0C$
Description	**:**	White or cream colored waxy solid in the form of beads, flakes or Powders. It is waxy to touch and has a slight fatty odor and taste.
Solubility	**:**	Soluble in hot ethanol (95%), ether, chloroform, hot acetone, mineral Oil and fixed oils. Practically insoluble in water, but readily dispersible in hot water with the aid of an anionic or cationic agent.

Uses **:** GMS is a food additive used as a thickening, emulsifying, anticaking, and preservative agent; an emulsifying agent for oils, waxes, and solvents; a protective coating for hygroscopic powders; a solidifier and control release agent in pharmaceuticals; and a resin lubricant. It is also used in cosmetics and hair-care products.

GMS is largely used in baking preparations to add "body" to the food. It is somewhat responsible for giving ice cream and whipped cream their smooth texture. It is sometimes used as an antistaling agent in bread.

6.2 TWEEN 80:

STRUCTURE:

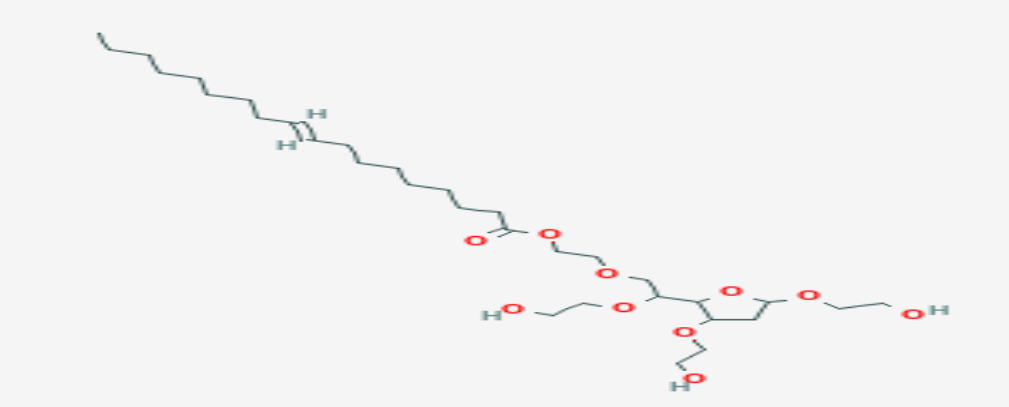


Fig No.10: Chemical structure of Tween 80

Synonym	**:**	Polysarbate 80, PEG (80) sorbitan monooleate, polyoxyethylene, sorbitanmonooleate
Chemical Name	**:**	Sorbitan mono-9-octadecanoate poly (oxy-1, 2-ethanediyl)
Molecular Formula	**:**	$C_{64}H_{124}O_{26}$
Molecular Weight	**:**	1310 g/mol
Boiling point	**:**	> 100°C
Viscosity	**:**	300–500 centistokes (@25°C)
Density	**:**	1.06–1.09 g/mL, oily liquid.

Description : Polysorbate, a substance formulated by the reaction of sorbitan. Fatty acid ester. It is a hydrophilic on ionic surfactant.

Solubility : It is very soluble in water. Also soluble in organic solvents like Ethanol, cotton seed oil, corn oil, ethyl acetate, methanol, toluene.

Uses : Polysorbate 80 is also used as a surfactant in soaps and cosmetics (including eye drops), or a solubilizer such as in a mouthwash. A solubilizing agent acts as a surfactant and increases the solubility of one agent in a further. A substance that would not usually dissolve in a particular solution is able to dissolve with the use of a solubilizing agent.[80]

6.3 POLOXAMER 188

STRUCTURE:

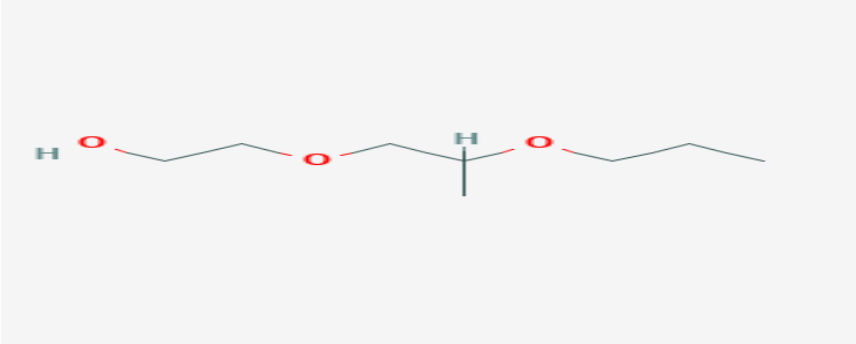


Fig No.11: Chemical structure of poloxamer 188

Synonym : Lutrol F 68, Pluronic F 68

Chemical name : Polyethylene-Polypropylene Glycol

Molecular formula : $C_8H_{18}O_3$

Molecular weight : 162.23 g/mol

Boiling Point : 260°C

Melting point : 52-57°C

Description : White to off white granules

Solubility : Soluble in water, ethanol; partially soluble in toluene. Insoluble in kerosene and ethylene glycol.

Uses : Poloxamers are polymers used for drug delivery as formulation excipients. They are used in pharmaceutical formulations as surfactants, emulsifying agents, solubilizing agent, dispersing agents, and as in vivo absorbance enhancers. They are also used in topical dosage forms and rectal suppositories.

6.4 SPAN 20

STRUCTURE:

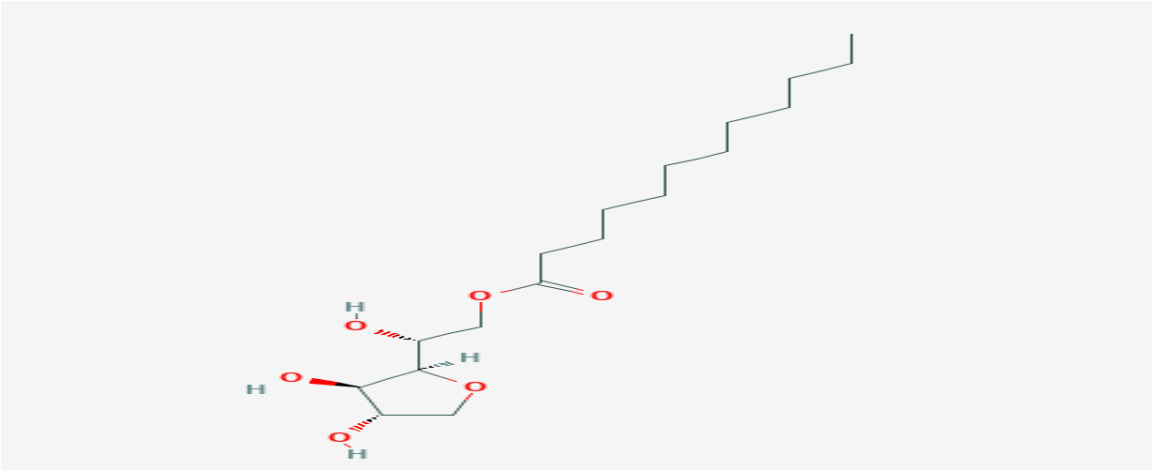


Fig No.12: Chemical structure of span 20

Synonyms : Arlace 20, Armotan ML Glycomul, Sorbitan laurate, Sorbitan mono laurate

Chemical name : Sorbitan mono dodecanoate

Empirical formula : $C_{18}H_{34}O_6$

Molecular weight : 346 g/mol

Description : Yellow viscous liquid

Boling Point	**:**	516.1℃ at 760 mm/Hg
Solubility	**:**	Sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water although insoluble, they are generally dispersible
Applications	**:**	Emulsifying agent, nonionic surfactant, solubilizing agent wetting, dispersing and suspending agent. Widely used in cosmetics, food products and as an emulsifying agent in emulsions, creams and ointments.

7. MATERIALS AND METHODS

Table No 2: LIST OF MATERIALS USED

SL NO.	NAME OF DRUGS	MANUFACTURER
1	Cefixime trihydrate	Pharmafabrikon
2	Glyceryl monostearate	mohini organics pvt.ltd.
3	Span 20	mohini organics pvt.ltd.
4.	poloxamer 188	Sigma-Aldrich
5	Tween 80	Gemco Scientific Company
6	Ethanol	Ponmani & Co.

Table No 3: LIST OF INSTRUMENTS USED

S. No	INSTRUMENTS	MODEL	MANUFACTURER
1.	Analytical weighing balance	AX200	Shimadzu
2.	Magnetic stirrer	MLH	Remi
3.	High Speed Homogenizer	Unidrive-X1000	CAT Scientific
4.	Probe Sonicator	VT- PROBE 250	V- Tech
5.	UV Spectrophotometer	UV-1700	Pharmaspec,Shimadzu
6.	FTIR spectrophotometer	4100	JASCO
7.	pH meter	Cyber scan	Eutech Instrument. Singapore
8.	Zeta sizer	Malvern Zetasizer (MAL 1021384)	Malvern Instruments Ltd
9.	Cooling Centrifuge	C-30BL	Remi
10.	Bath sonicator	2200MH	LIFE CARE
11.	Glass wares	borosilicate	Mumbai, India

7. METHODOLOGY

7.1 STANDARDIZATION OF CEFIXIME TRIHYDRATE:

7.1.1 Preparation of phosphate buffer (pH 7.2)

About 2.38g of disodium hydrogen phosphate, 0.19g of potassium dihydrogen phosphate and 8.0 g of sodium chloride were dissolved in water to produce 1000ml; the pH was adjusted into pH 7.2.

7.1.2 Preparation of standard stock solution:

Standard stock solution of cefixime was prepared by dissolving 10mg of drug in 10 ml of Phosphate buffered saline.

7.1.3 Preparation of working stock solution:

From the stock solution 1ml was taken 10ml standard volumetric flask and was made up with phosphate buffered saline. Further it was diluted with phosphate buffered saline pH 7.2 to reach concentration range of 0-15 µg.

7.2 Determinations of λmax & preparation of calibration curve:

The 10 µg/ml solution of cefixime trihydrate was scanned at 200-400 nm in UV spectrophotometer to find out the maximum absorbance (λ max) of cefixime trihydrate. The standard stock solution of drug was prepared by dissolving 100 mg ofcefixime trihydrate in 5 ml methanol and diluted with phosphate buffer solution pH 7.2 up to 100 ml. From the above stock solution, different concentrations (0, 3, 6, 9, 12 and 15µg/ml) were prepared using phosphate buffer pH 7.2 solutions and absorbance was measured in UV- spectrophotometer for the plotting of standard calibration curve. The obtained standard curve was used for the estimation of entrapment efficiency and percentage drug release.

7.3 COMPATIBILITY STUDIES FOR DRUG AND EXCIPIENTS:

Compatibility studies were performed for the verification of interactions present between the drug and excipients. It gives information needed for the selection of excipients with the drug for formulation of solid lipid nanoparticles. Infrared spectrophotometry technique is used to check the compatibility studies between lipids (GMS) drugs (cefixime trihydrate) and surfactant (span 20, Tween 80, poloxamer 188).

7.4 FOURIER TRANSFORMS INFRARED SPECTROSCOPIC STUDIES (FT-IR):

FT-IR studies were performed for the verification of interactions between cefixime trihydrate, lipids surfactants and its physical mixture by KBr pellet technique using FT-IR spectrophotometer (Shimadzu, Japan). The IR spectrum of the physical mixture was compared with the spectrum of pure cefixime trihydrate materials and excipients for the assessment of compatibility. The scanning range is 450-4000 cm^{-1} and the resolution is 4 cm^{-1}[81-84].

7.5 PREPARATION OF CEFIXIME TRIHYDRATE LOADED SOLID LIPID NANOPARTICLES:

Cefixime trihydrate loaded SLN were prepared by hot homogenization followed by the ultrasonication method. Cefixime trihydrate and GMS were dissolved in a mixture of methanol and chloroform (1:1). Organic solvents were completely removed using a rotary flash evaporator. The embedded lipid layer was melted by heating to 5°C above the melting point of the lipid. An aqueous phase was prepared by dissolving the stabilizers (tween 80 or poloxamer 188 or span 20) in distilled water (sufficient to produce 30 ml) and heating to the same temperature of the oil phase. The hot aqueous phase was added to the oil phase and homogenization was performed (at 2500 rpm and 70°C) using a mechanical stirrer for 30 minutes. The coarse oil in water emulsion so obtained was sonicated using probe soincator for 25 minutes. Cefixime trihydrate loaded SLN was finally obtained by allowing the hot nanoemulsion to cool to room temperature, and was stored at 4°C in the refrigerator. **(85)**

Table No 4: Formulation development of solid lipid nanoparticle

Formulation code	Drug (mg)	Lipid % (GMS)	Surfactant %	
F1	200	6	Tween 80	1
F2	200	6		1.5
F3	200	6		2
F4	200	6	Poloxamer 188	1
F5	200	6		1.5
F6	200	6		2
F7	200	6	Span 20	1
F8	200	6		1.5
F9	200	6		2

7.6 CHARACTERIZATION OF CEFIXIME TRIHYDRATE LOADED SOLID LIPID NANOPARTICLE:

The formulated cefixime trihydrate loaded solid lipid nanoparticles were characterized for their, entrapment efficiency, particle size, polydispersity index, zeta potential, *in vitro* drug release, kinetic modeling and SEM.

7.6.1 Determination of Physicochemical properties:

The physicochemical properties such as color, odor and stability of formulated cefixime trihydrate loaded SLN were evaluated after dispersion of cefixime trihydrate loaded SLN using centrifugation at 2000 rpm for 30 min.

7.6.2 Entrapment efficiency:

The entrapment efficiency of SLN dispersion was determined by the centrifugation method. SLN dispersion (containing an equivalent to 5 mg of drug) was centrifuged at 20000 rpm for one hour in a refrigerated centrifuge to collect the supernatant liquid. The collected

liquid was filtered to measure the free drug concentration after suitable dilution with a fresh phosphate buffer saline pH 7.2. The absorbance was measured at 288 nm in a UV spectrophotometer [86] to calculate the entrapment efficiency using the following formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount taken - free drug}}{\text{Amount taken}} \times 100$$

7.6.3 Particle size and zeta potential:

Particle size and zeta potential of cefixime loaded SLN was measured using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments) at 25 ° C. Prior to measurements all samples were diluted using ultra purified water to yield a suitable scattering intensity.

7.6.4 *In-Vitro* Drug Release Study:

The *invitro* drug release study of the tablets Was Performed Using USP Type II apparatus paddle at 37°C±0.5°C using phosphate saline buffer pH 7.2 (900 ml) as a dissolution medium and 50 rpm. At the predetermined time intervals, 10 ml samples were withdrawn and replaced with fresh dissolution media. With drawn samples diluted, and assayed at 288nm using a Shimadzu UV- spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a calibration curve.

7.6.5 Kinetic modeling:

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots. The exact mechanism by which the SLN formulation follows determined by korse-meyerpeppas model (log drug release vs. log time) [87].

Table No: 5 Diffusion exponent values indicating drug release mechanism

Release exponent (n)	Drug transport mechanism	Rate as a function of Time
0.5	Fickian diffusion	$t^{-0.5}$
0.5 < n <1	Anomalos transport	t^{n-1}
1	Case II transport	Zero order release
>1	Super case II transport	t^{n-1}

7.6.6 Scanning electron microscopy:

Average particle size and surface morphology of the best formulation was evaluated using scanning electron microscopy. The sample was spread on an aluminum stub and allowed to dry at room temperature. The dried sample was sputter coated with gold for 40 seconds using Hitachi Ion-Sputter E-1010. The images were captured with the Hitachi S-3400 Scanning Electron Microscope.

8. RESULTS AND DISCUSSION

8.1 STANDARDIZATION OF CEFIXIME LOADED SLNs:

Determination of λ max & preparation of calibration curve:

The absorbance maximum (λ max) of cefixime was found to be 288 nm. The standard calibration curve of cefixime is shown in Figure 13. The standard calibration curve was shows good linearity with the r^2 value 0.998.

Table No 6: Standard calibration curve of cefixime

Concentration (mcg/ml)	Absorbance (nm)
0	0
3	0.142
6	0.272
9	0.424
12	0.567
15	0.689

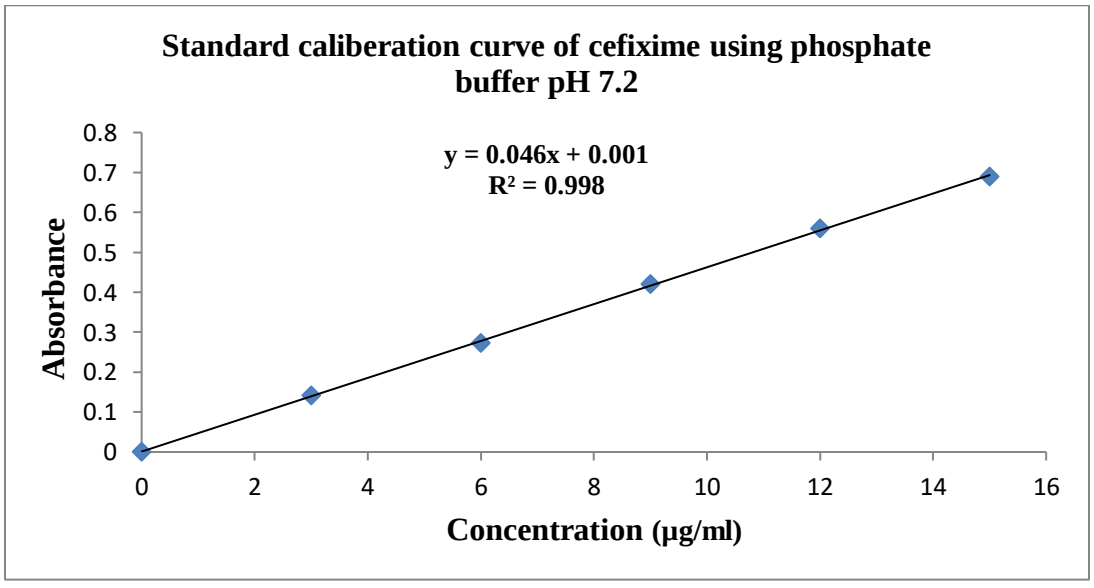


Fig No 13: Standard calibration curve of cefixime

8.2 COMPATIBILITY STUDIES OF DRUG AND EXCIPIENTS:

Fourier Transform Infrared Spectroscopic studies (FT-IR)

The IR Spectra of drug, lipid excipients are shown in Figure 14, 15. The spectrum was studied at 4000 cm^{-1} – 400 cm^{-1}. The spectra and major peaks of individual drug, lipid and their mixtures are given in the Figure 14, 15. From the spectra it was clear that there was no interaction between the selected lipids, drug and mixtures. Hence the selected lipid was found to be compatible in entrapping the selected drug with carriers without any mutual interactions.

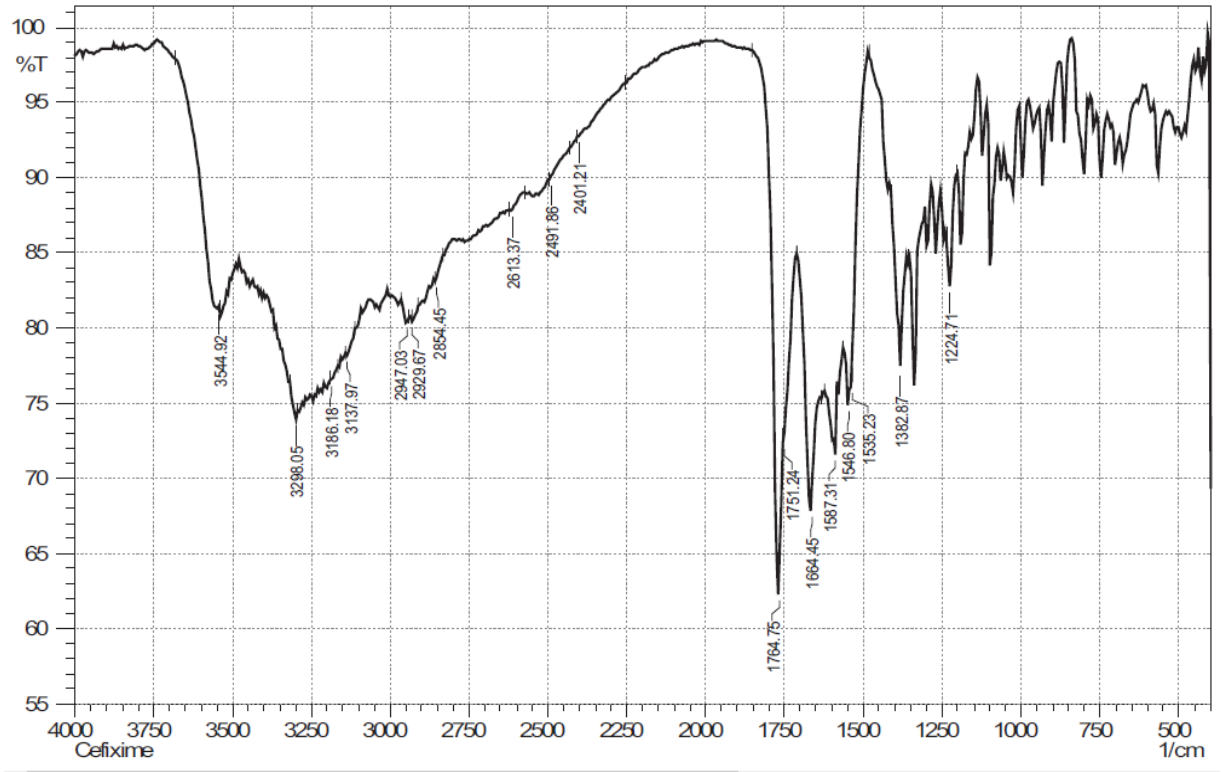


Fig No 14: FT-IR spectra cefixime

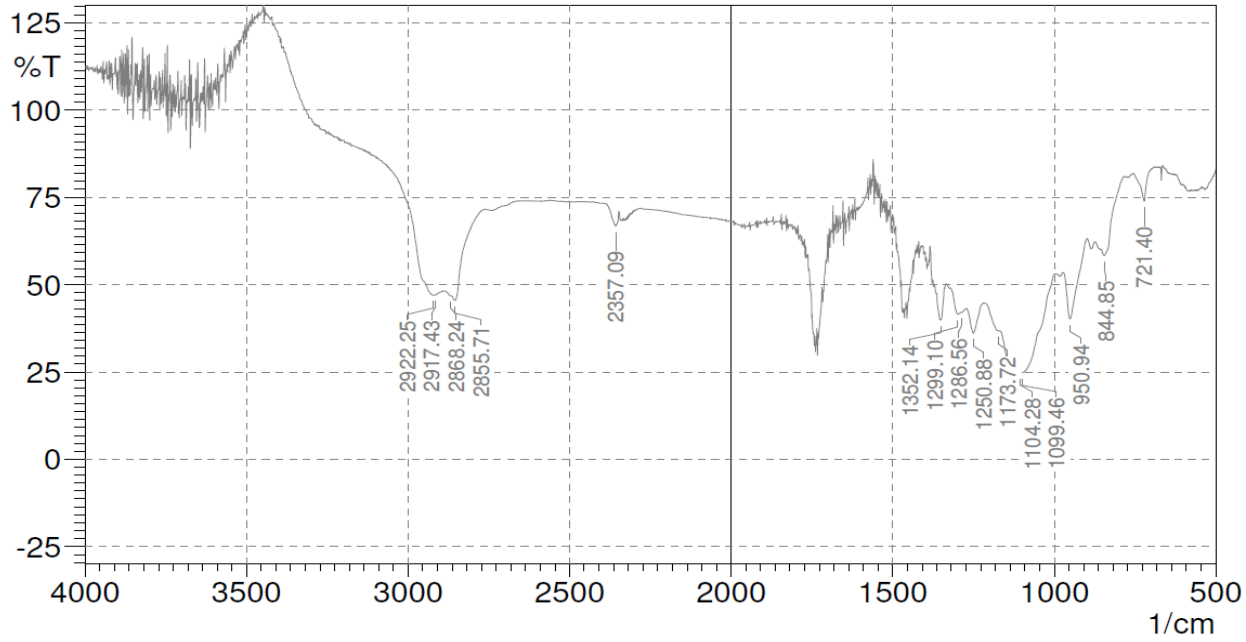


Fig No 15: FT-IR spectra Cefixime + Excipients

8.3 PREPARATION OF CEFIXIME LOADED SLNs:

Homogenization followed by ultrasonication is a reliable, simple and reproducible method for preparing cefixime SLN. The prepared SLN dispersion was found to be uniform and homogenous in appearance.

8.4 CHARACTERIZATIONOF CEFIXIME LOADED SOLID LIPID NANOPARTICLES:

8.4.1 Determination of physicochemical properties:

The formulated Cefixime loaded SLN dispersion was shows milky white in appearance, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation at 2000 rpm for 30 min.

8.4.2 Determination of entrapment efficiency

The results of entrapment efficiency of formulated SLNs are shown in the Table 7.The entrapment efficiency of the formulations F1-F3 (Tween 80) at different ratios (1%, 1.5%,and 2%) shows 73.83±0.83,77.83±0.35,80.6±0.70.The entrapment efficiency of the formulation F4-F6 (Poloxamer 188) at different ratios (1%, 1.5%, and 2%) shows 81.3±0.74,83.5±0.45 and 84.8±0.51. The entrapment efficiency of the formulation F7-F9 (Span 20) at different ratios (1%, 1.5%, and 2%) shows 69.3±0.60, 73.2±0.4 and 75.1±0.2.

From the above results it shows that the entrapment efficiency of the formulations increases with increase of surfactant concentration. This was because that when the lipid concentration increases there would be more lipid to entrap the drug molecules. Among the various surfactants used poloxamer 188 shows highest drug entrapment.

Table No 7: Entrapment efficiency of cefixime trihydrate loaded SLN

S. No	Formulation code	Entrapment efficiency (%)	
1	F1	Tween 80	73.83±0.83
2	F2		77.83±0.35
3	F3		80.6±0.70
4	F4	Poloxamer 188	81.3±0.74
5	F5		83.5±0.45
6	F6		84.8±0.51
7	F7	Span 20	69.3±0.60
8	F8		73.2±0.4
9	F9		75.1±0.2

8.4.3 Effect of surfactants on the particle size of Cefixime SLN:

The particle size of the prepared drug loaded SLNs are shown in Table 8.

SLN dispersion prepared using poloxamer 188 2.0% as stabilizer (F4, F5 and F6) showed lower particle size than the other surfactants irrespective of the lipids studied. This result could be explained due to the higher molecular weight of poloxamer 188 and higher HLB value of poloxamer 188 when compared to tween 80 and span 20.The particle size of various SLN, stabilized with different surfactants, increased in the order of Poloxamer 188 > Tween 80 > Span 20.

Table No 8: Particle size, PDI, Zeta potential of cefixime loaded SLNs

S. No	Formulation code	Surfactant	Mean diameter	PDI	Zeta potential
1	F1	Tween 80	381.1	0.056	-14.2
2	F2		151.5	0.647	-19.1
3	F3		69.51	1.000	-12.3
4	F4	Poloxamer 188	33.58	0.354	-1.10
5	F5		22.03	0.281	-1.20
6	F6		5.229	0.273	-3.65
7	F7	Span 20	421	0.071	-17.3
8	F8		908.8	0.054	-14.267
9	F9		478.0	0.315	-11.07

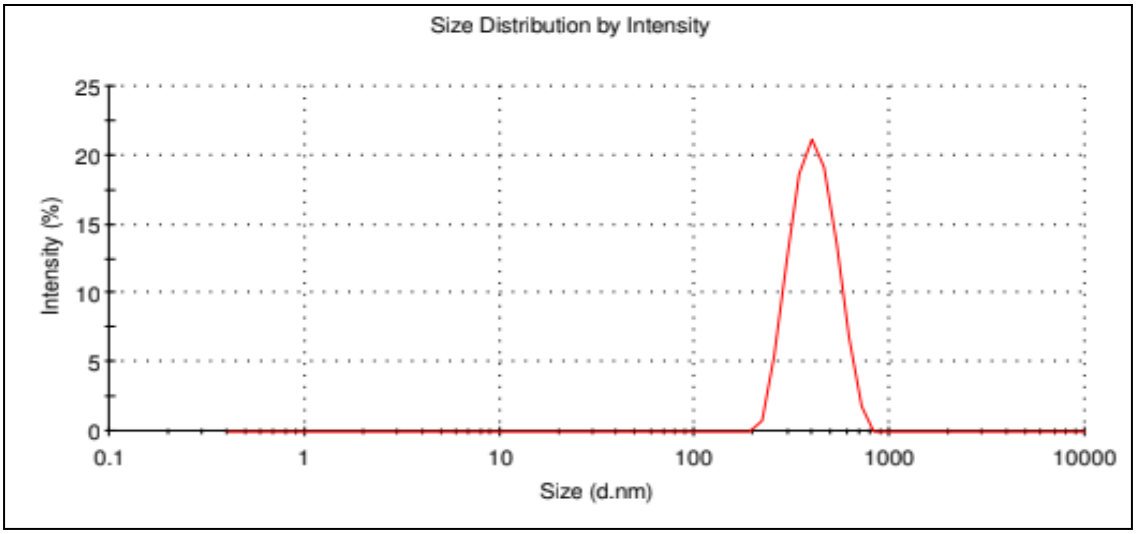


Fig No: 16 Particle size of F1 containing 1% Tween 80

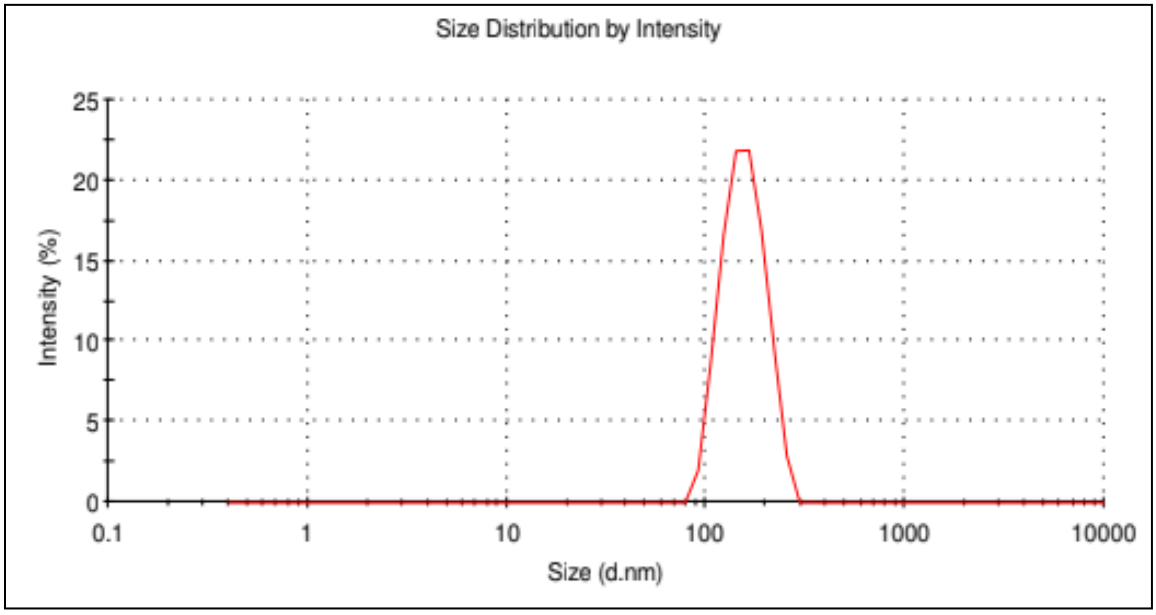


Fig No: 17 Particle size of F2 containing 1.5% Tween 80

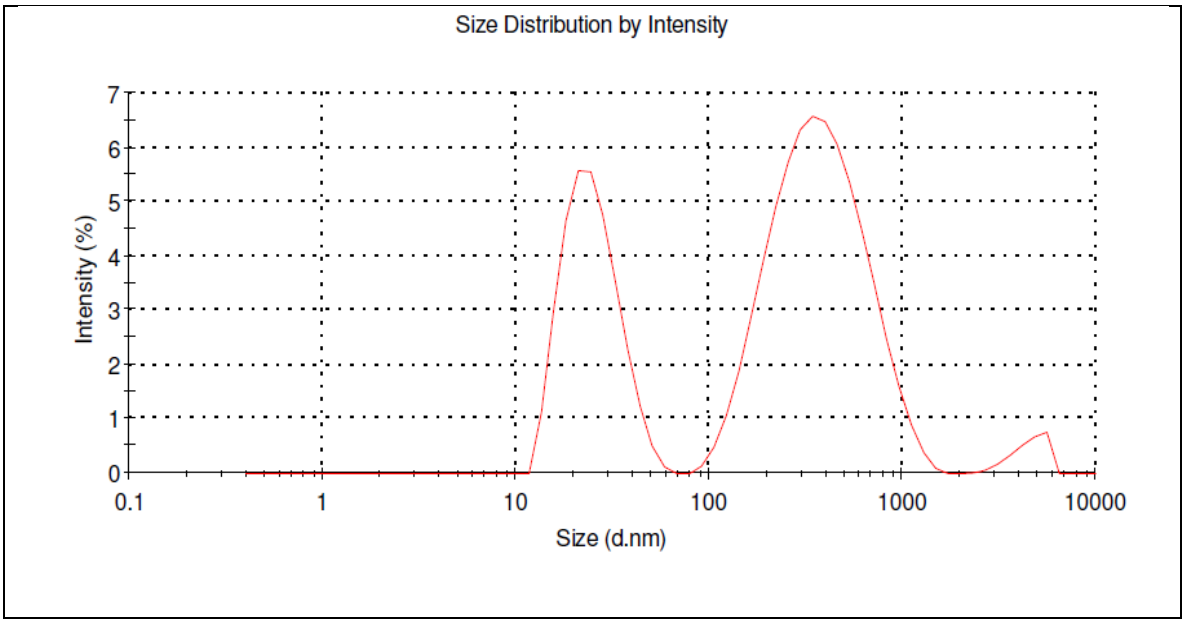


Fig No: 18 Particle size of F3 containing 2% Tween 80

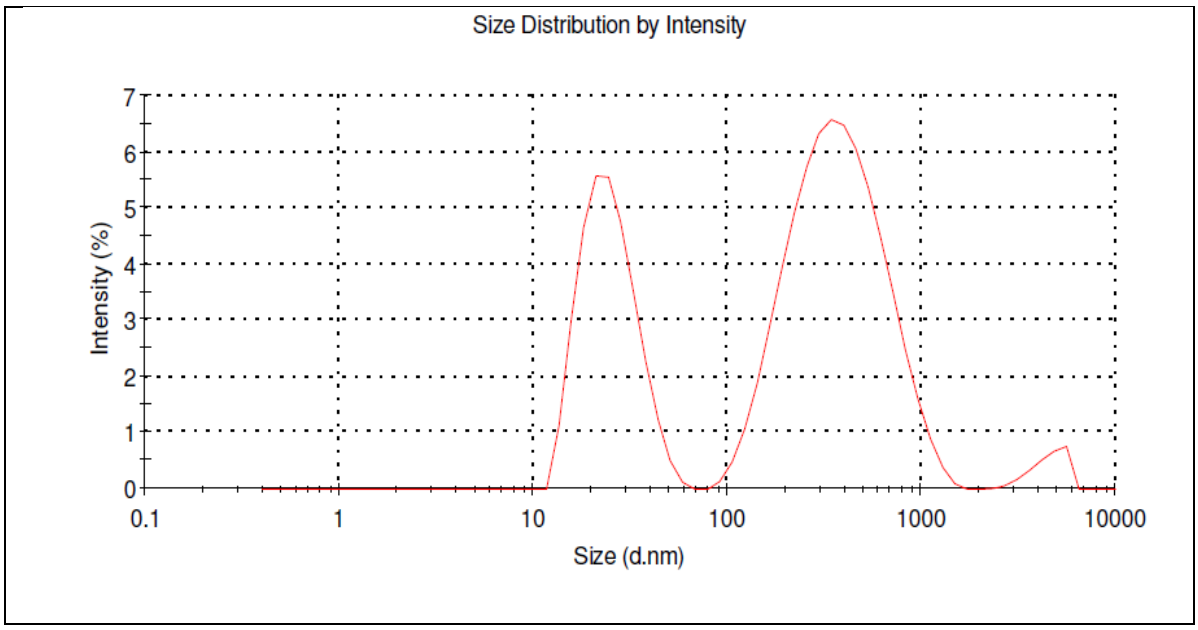


Fig No: 19 Particle size of F4 containing 1% Poloxamer188

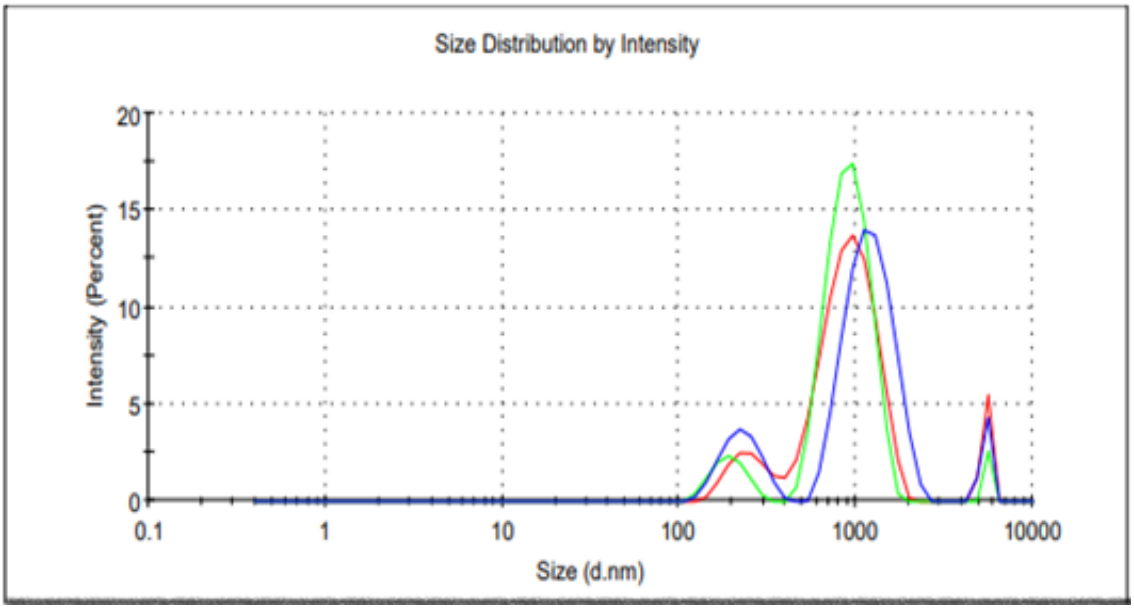


Fig No: 20 Particle size of F5 containing 1.5% Poloxamer188

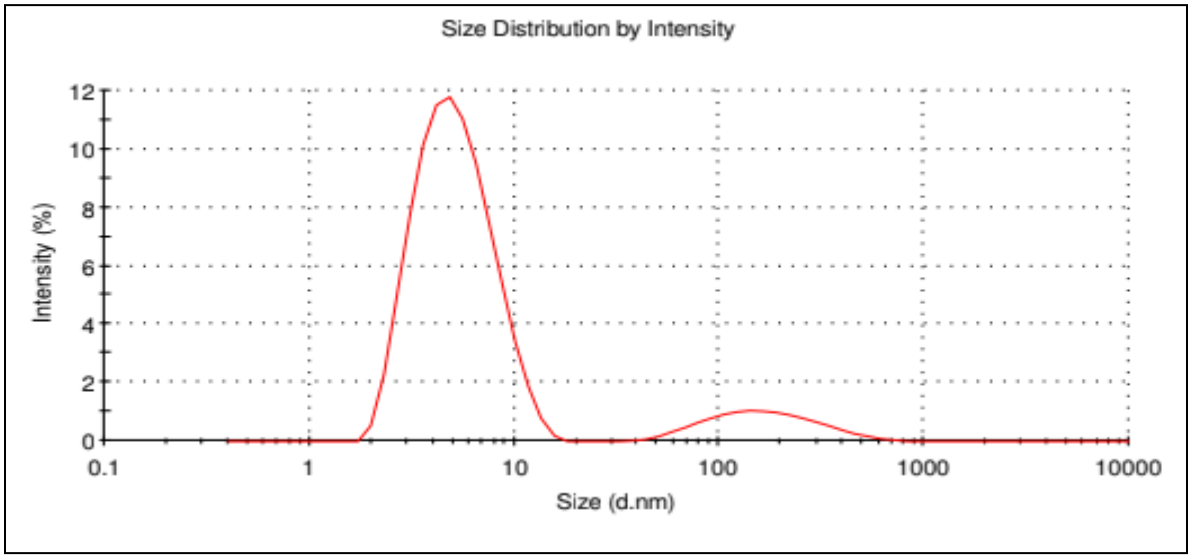


Fig No: 21 Particle size of F6 containing 2% Poloxamer188

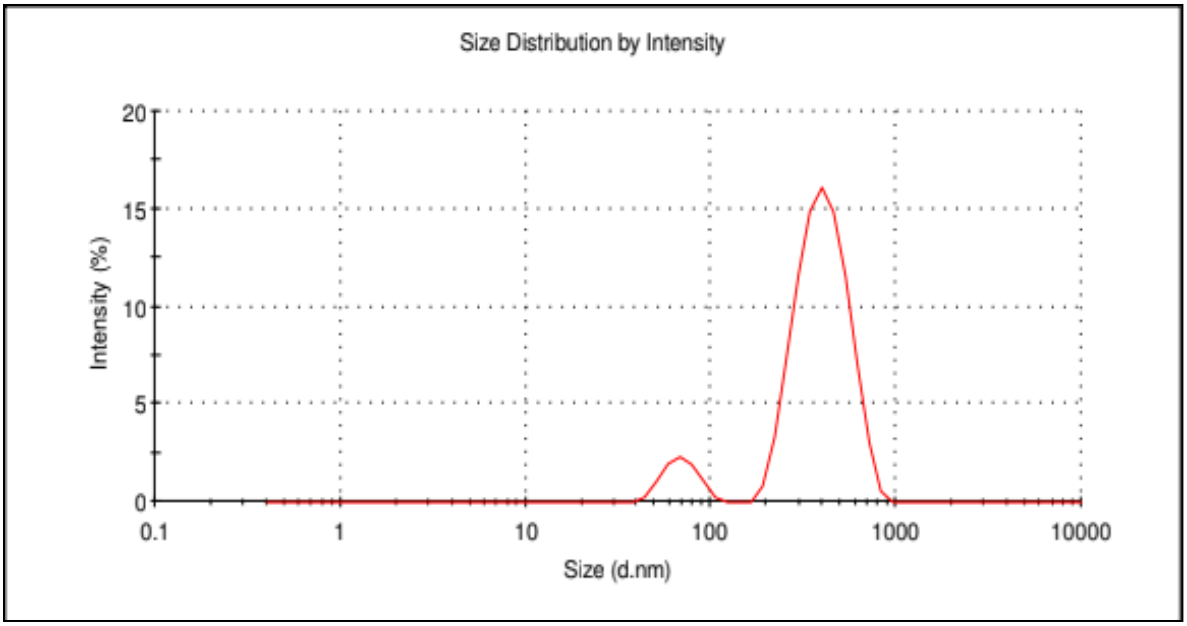


Fig No: 22 Particle size of F7 containing 1% Span 20

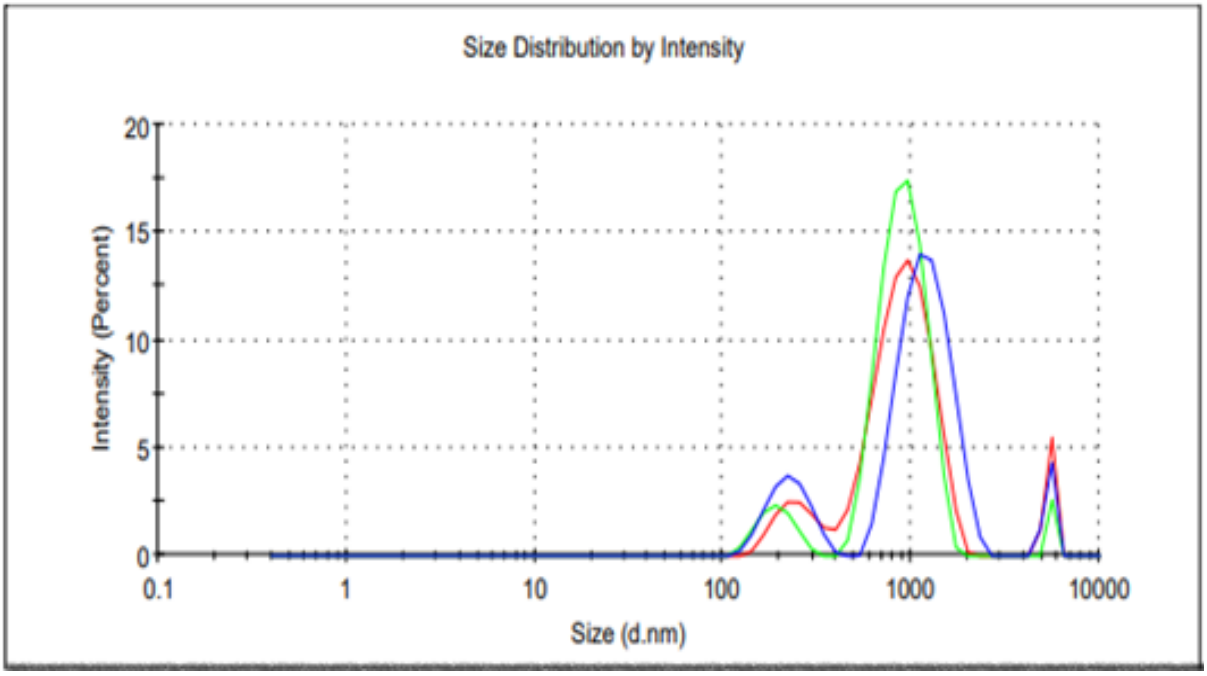


Fig No: 23 Particle size of F8 containing 1.5% Span 20

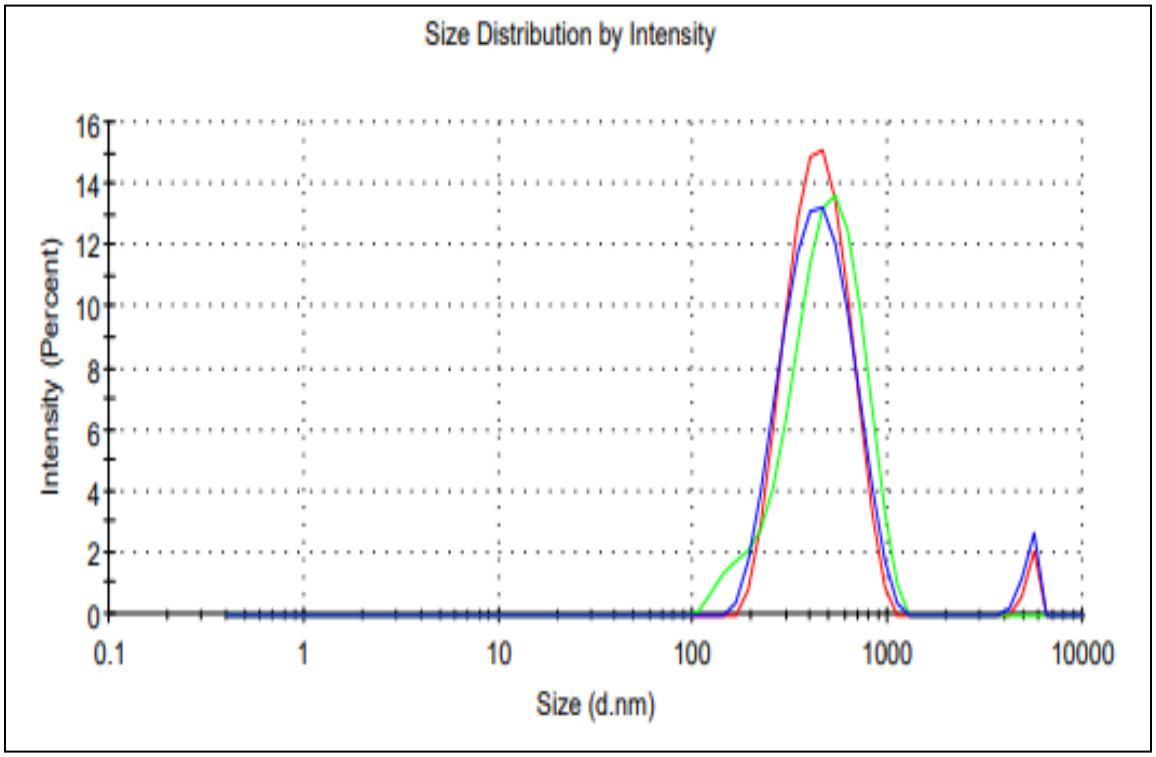


Fig No: 24 Particle size of F9 containing 2% Span 20

8.5 POLY DISPERSITY INDEX (PDI):

The PDI of the formulations are shown in Table 8 and was smaller than 0.5, which indicates a relative homogenous dispersion. Polydispersity index indicates particle size distribution, which ranges from 0 to 1. Theoretically, mono disperse populations indicates PI = 0. However, PI < 0.2 was considered as narrow distribution and those greater than 0.5 indicate high homogenicity. PDI of F6 is 0.273

8.6 ZETA POTENTIAL:

The zeta potential of the formulations is shown in Table 8. Zeta potential of formulations shows negative zeta potential respectively.

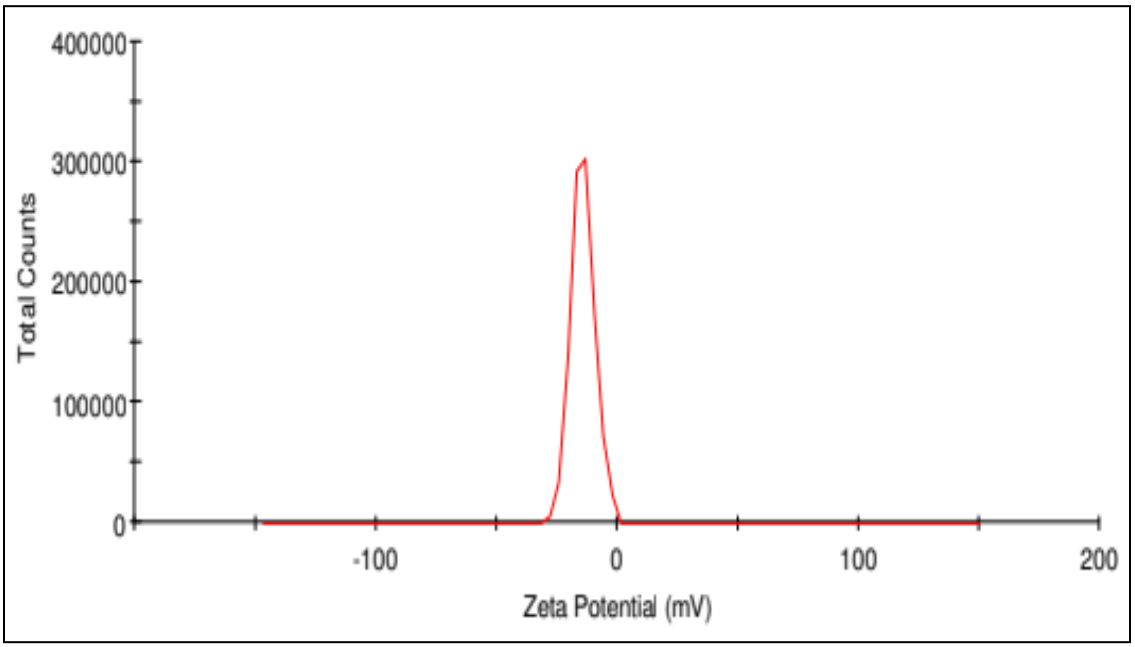


Fig No: 25 Zeta potential of F1 containing 1% Tween 80

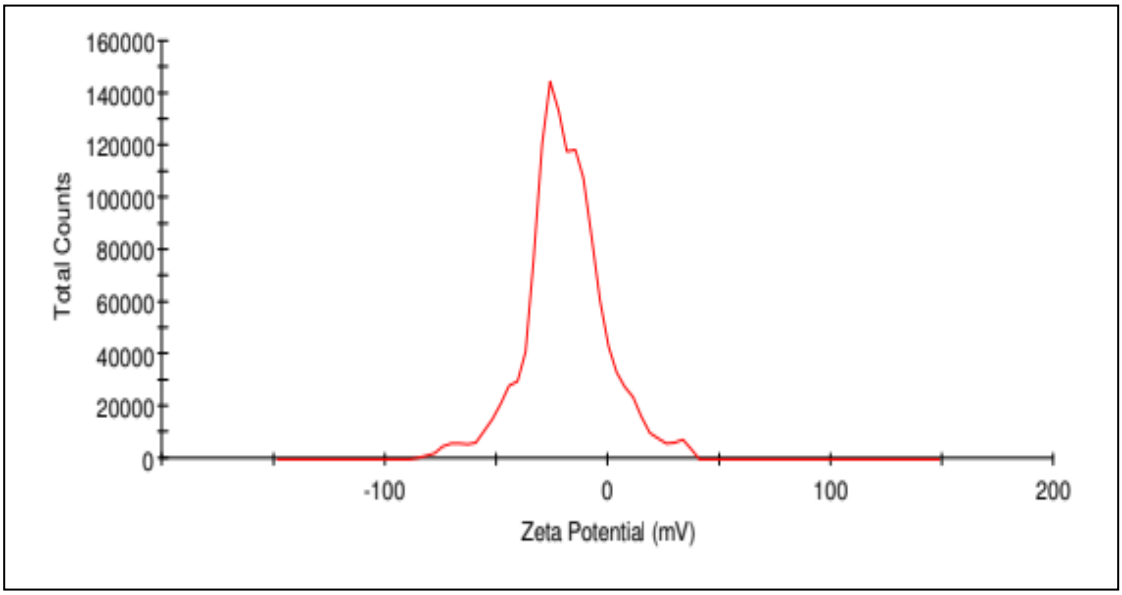


Fig No: 26 Zeta potential of F2 containing 1.5% Tween 80

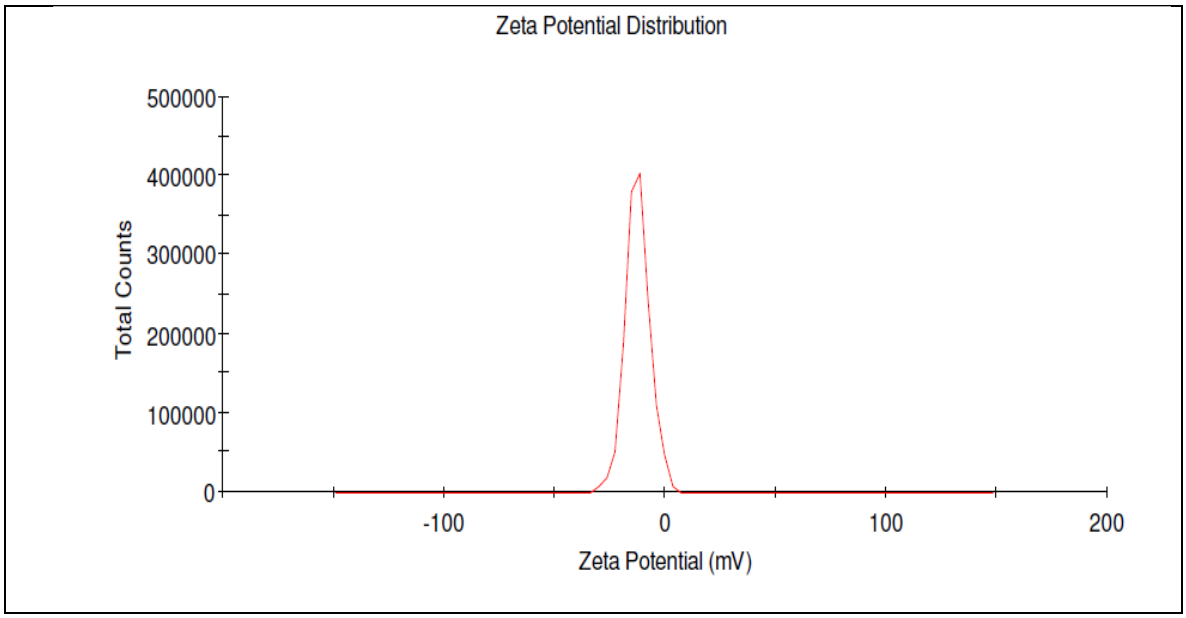


Fig No: 27 Zeta potential of F3 containing 2% Tween 80

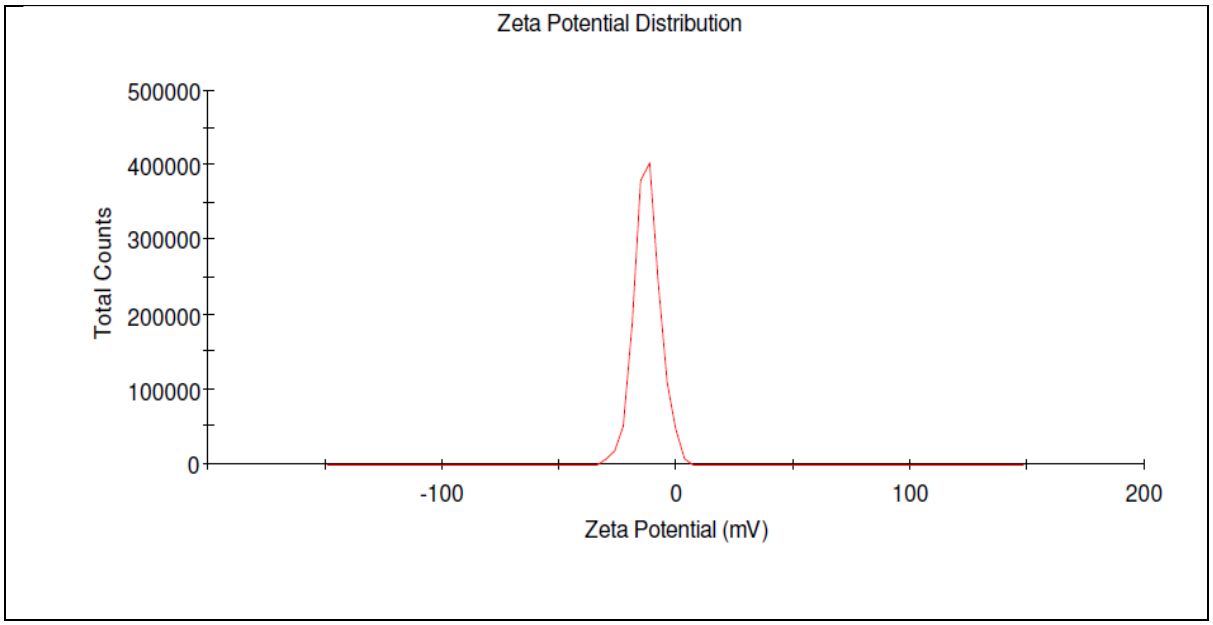


Fig No: 28 Zeta potential of F4 containing 1% Poloxamer 188

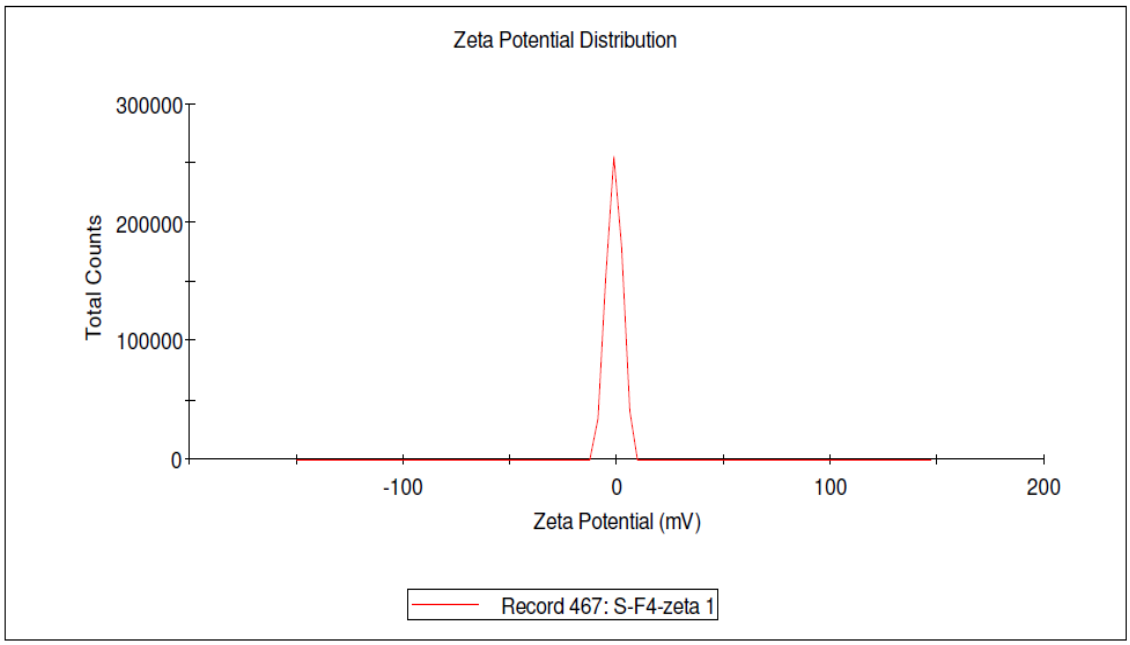


Fig No: 29 Zeta potential of F5 containing 1.5% Poloxamer 188

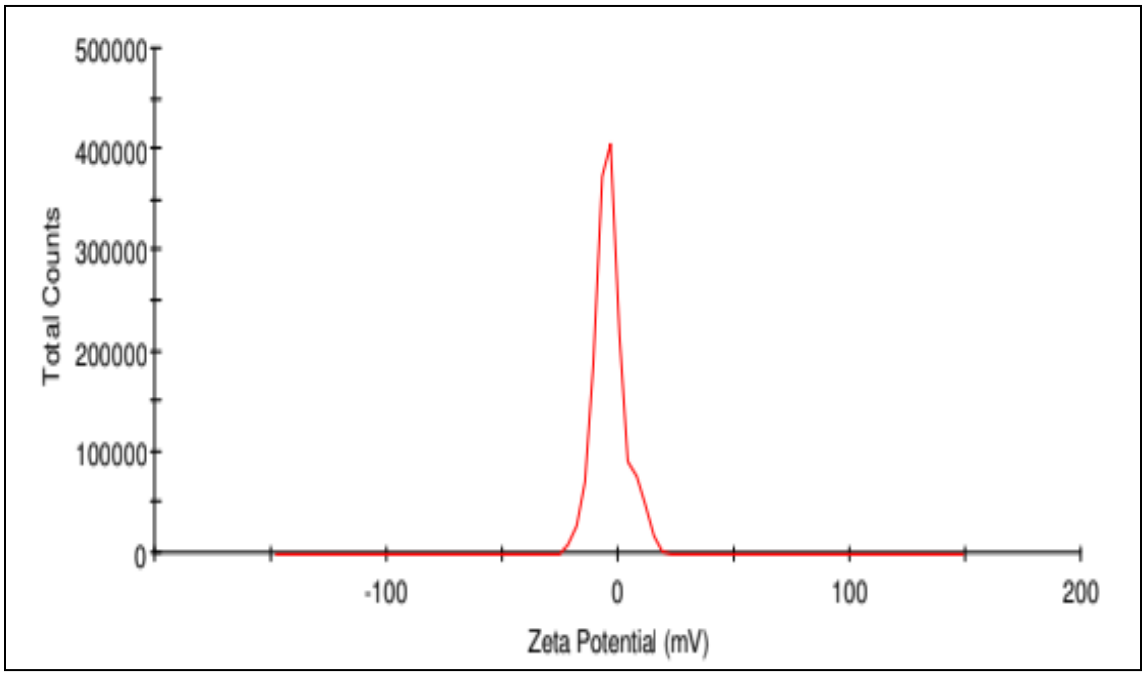


Fig No: 30 Zeta potential of F6 containing 2% Poloxamer 188

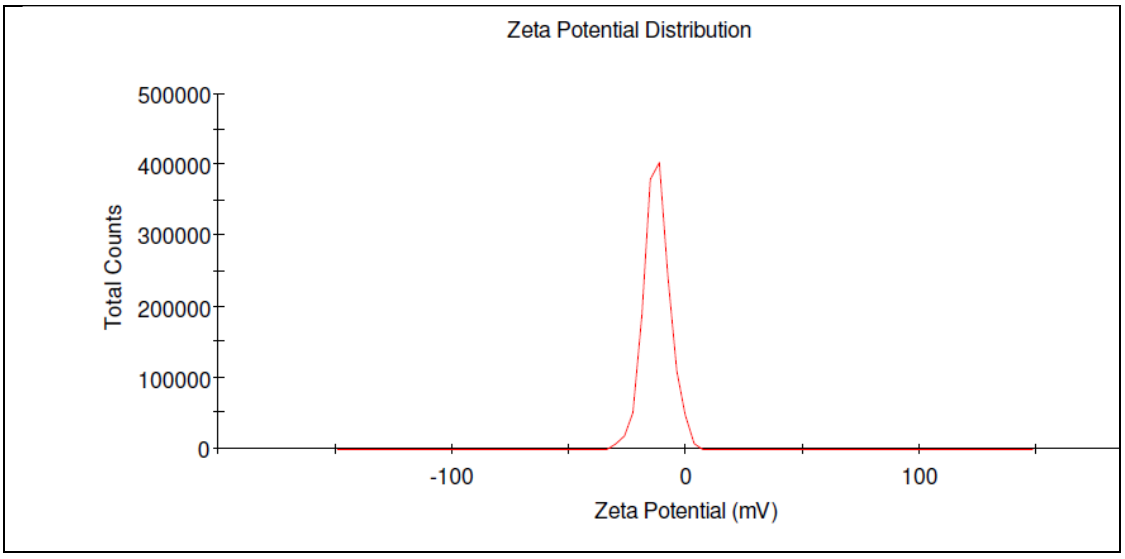


Fig No: 31 Zeta potential of F7 containing 1% Span 20

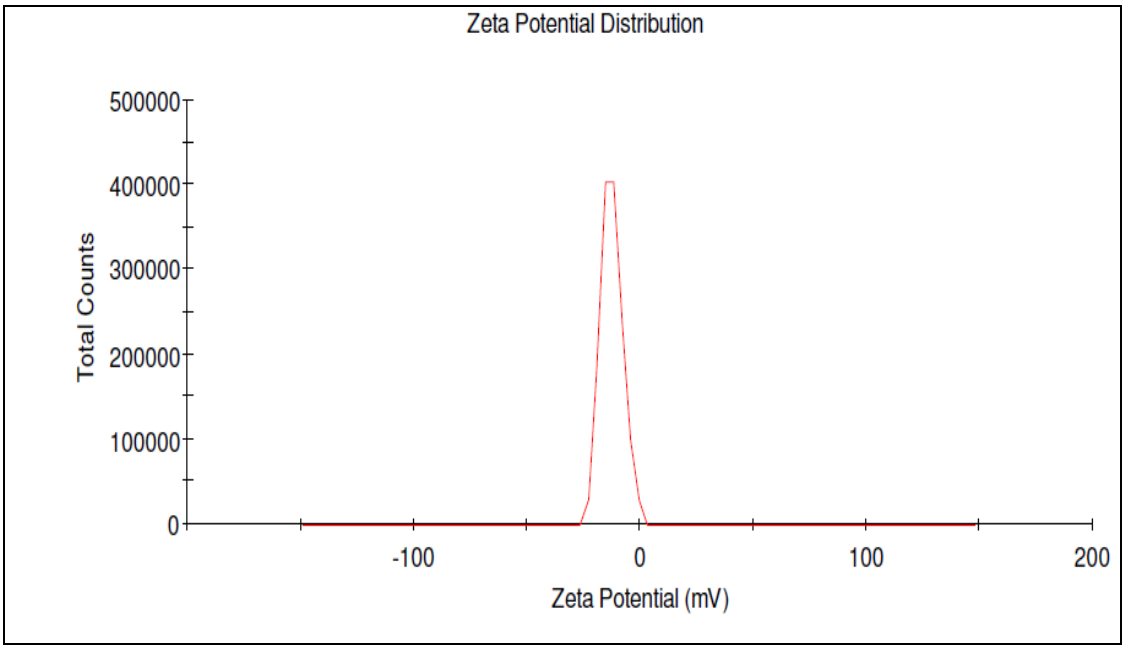


Fig No: 32 Zeta potential of F8 containing 1.5% Span 20

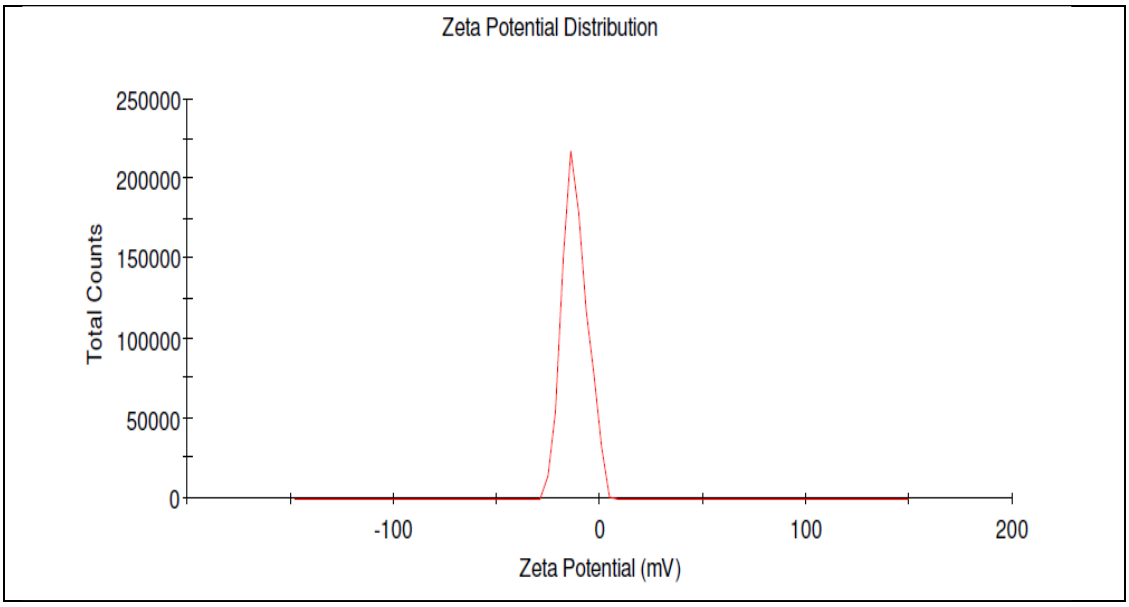


Fig No: 33 Zeta potential of F9 containing 2% Span 20

8.7 EVALUATION OF CEFIXIME LOADED SOLID LIPID NANOPARTICLE:

In vitro DRUG RELEASE:

From the results mentioned in Table 9, it was seen that F6 showed drug release of 94.71±0.53% for Cefixime SLN at the end of 5 hrs with better bio availability.

Table no 9: *in vitro* drug release of cefixime loaded SLN

TIME (MINS)	Tween 80			Poloxamer 188			Span 20		
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
15	6.77±0.21	7.67±0.21	8.12±0.85	9.78±0.63	13.85±0.85	16.71±0.63	5.56±0.21	6.92±0.42	8.28±0.21
30	18.97±0.42	17.63±0.63	18.68±0.42	20.79±0.43	25.01±0.84	28.03±0.42	14.46±0.43	14.76±0.85	20.04±0.21
60	23.98±0.21	24.88±.64	25.04±0.43	29.71±0.21	38.02±0.42	41.19±0.64	21.12±0.40	23.67±0.21	29.11±0.22
120	39.84±1.2	37.44±2.1	39.69±0.21	43.92±1.4	55.1±0.63	51.81±0.62	35.61±0.43	36.37±0.64	43.92±1.49
180	49.71±0.21	56.78±0.43	57.39±0.43	59.82±0.43	71.18±1.1	70.29±0.63	44.57±0.64	42.32±0.43	53.80±0.43
240	58.40±1.7	65.94±0.44	70.31±1.92	75.01±0.42	83.38±0.2	85.65±0.41	53.69±0.21	58.29±0.53	65.96±0.42
300	63.19±0.2	75.11±0.25	80.40±0.64	83.1±0.01	89.73±0.42	94.71±0.53	60.88±0.42	67.07±0.21	70.47±1.92

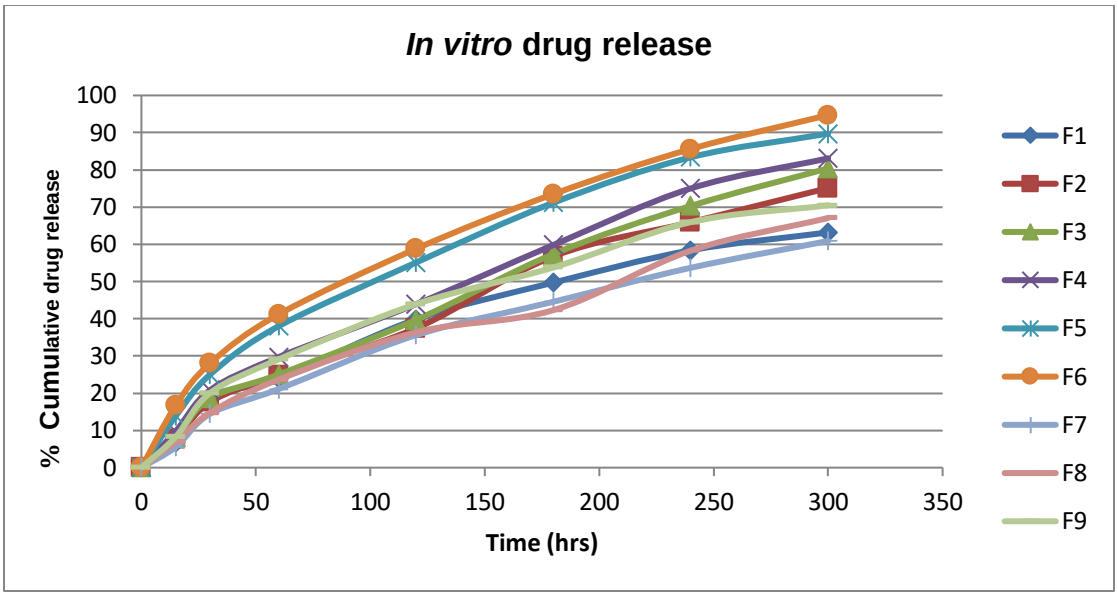


Fig no: 34 *in vitro* drug release of cefixime loaded SLN

8.8 SCANNING ELECTRON MICROSCOPY:

The morphology of cefixime loaded SLN dispersion was examined by scanning electron microscope. The best formulation, F6 (formulation containing 2% poloxamer) was chosen for SEM studies. The SEM photograph was shown in the Figure 30. It revealed that the SLN dispersion shows the particle size was found to be 500 nm in size smooth surface as shown in Figure 35.

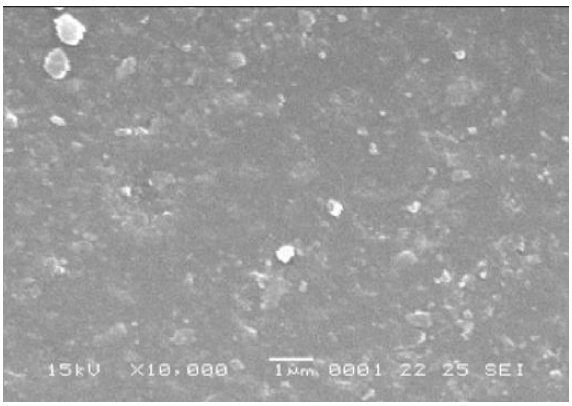


Fig No: 35 Image of cefixime loaded SLN by Scanning Electron Microscopy

8.9 RELEASE KINETICS:

The kinetics and mechanism of drug release were studied by release kinetics, the n, k and r^2 values are indicated in the Table 10. A formulation shows first-order release which had higher linearity than the zero-order or Higuchi model.

The exact mechanism of the release kinetics was determined by First order model. Results indicated that the SLN formulations followed Non fikian model of release kinetics.

Table no 10: Release kinetics of formulated SLN (F6)

Code	Zero order		First order		Higuchi Model		Korsmeyarpeppas	
	k	r^2	k(hr^{-1})	r^2	k(hr^{-1})	r^2	n	r^2
F6	1.878	0.8685	0.039	0.9977	11.948	0.9451	0.633	0.9867

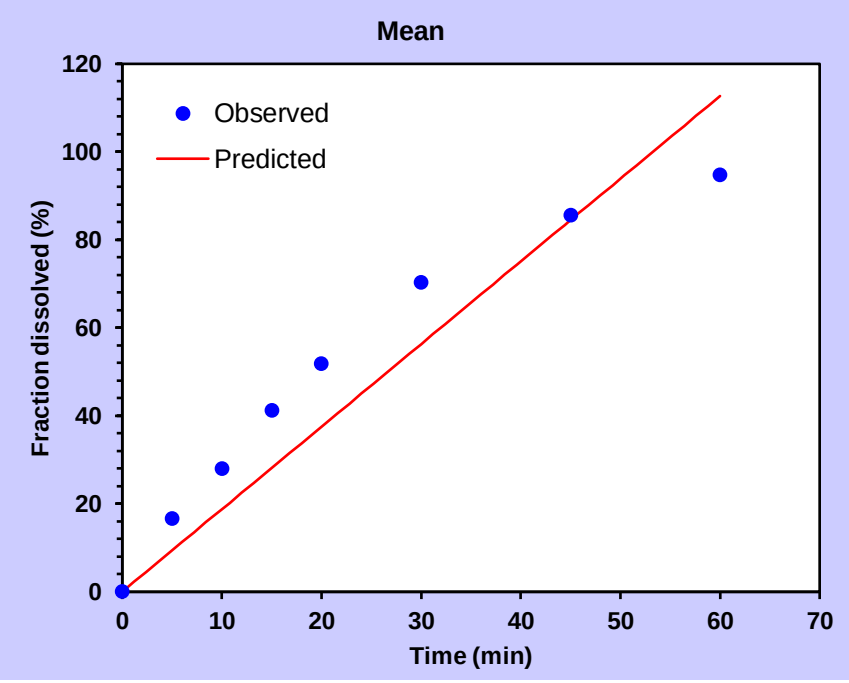


Fig No 36: Zero order drug release

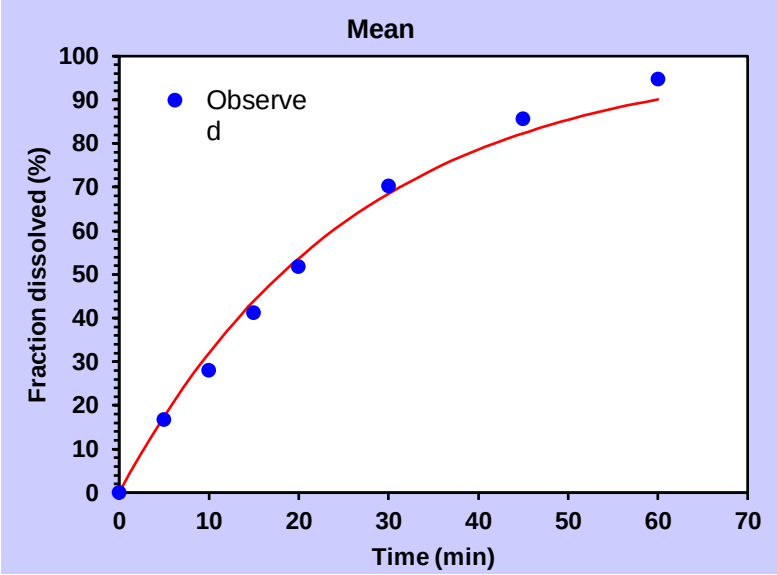


Fig No 37: First order drug release

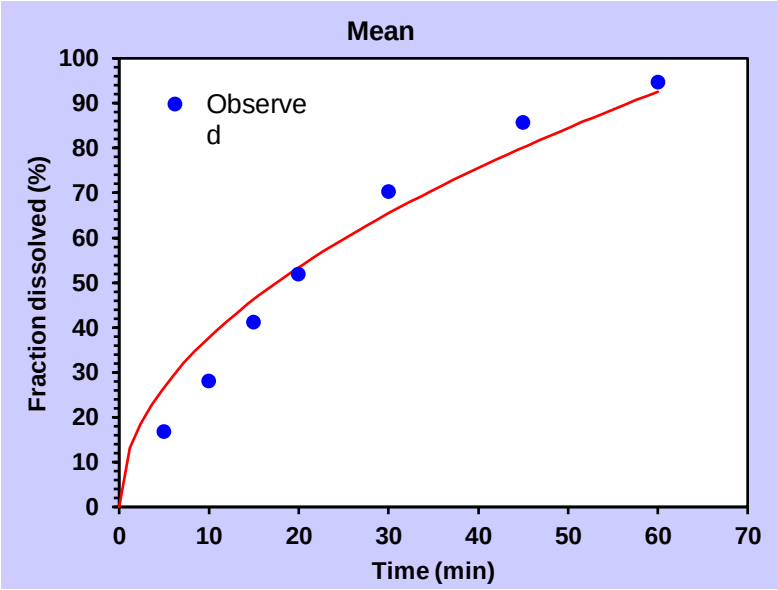


Fig No 38: Higguchi model drug release

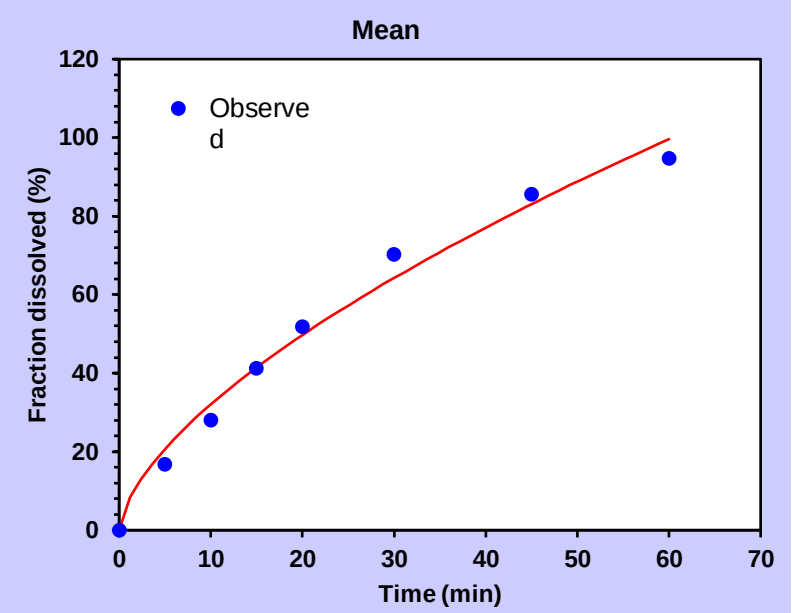


Fig No: 39 Korsmeyar peppasmodel of drug release

9. SUMMARY

The present research study was to estimate the effect of lipid on the preparation of cefixime-loaded SLN to improve its better bioavailability

- FT-IR study was confirms that there was no interaction between the selected lipid, drug, surfactants and mixtures. SLNs were prepared by hot homogenization followed by ultrasonication using lipid GMS and surfactant Tween 80, Polaxamer 188, and Span 20.

- Result suggests that the hot homogenization and ultrasonication method was a feasible method for preparing cefixime- loaded Soild Lipid Nanoparticles.

- SLN formulations were estimated for its particle size, morphology, entrapment efficiency, release studies, FTIR, *invitro* kinetics, scanning electron microscope and polydispersity index.

- From the particle size studies, it was observed that increase in surfactant concentration from 1 to 2 % decreases the mean particle size of the formulation.

- SLN dispersion prepared using poloxamer 188 (2.0%) as stabilizer (F4, F5 and F6) showed lower particle size than the other surfactants irrespective of the lipids studied.ss

- The release of cefixime trihydrate from the SLN formulation follows first order model of release kinetics.

- The entrapment efficiency was found to increase with increase in surfactant concentration and with increase in lipid concentration.

- The particle size of formulations with best entrapment was found out in the range of 5.229 nm.

- The poly dispersity index was within 0.273 indicating a uniform size distribution.

- The zeta potential of the formulation with best entrapment was in the range of -3.65 mV.
- The best formulation was selected according to entrapment, particle size & *invitro* drug release.
- The selected formulations were lyophilized.
- The *invitro* drug release shows a biphasic release pattern.
- The release kinetics shows that the formulations were diffusion controlled. Korsmeyer peppas n values were shows more than 0.6 indicating non fickian diffusion.
- SEM studies revealed that the SLN formulation are in the nanometric range and are spherical in shape with smooth surface.

10. CONCLUSION

In our study poorly bio available drug cefixime was successfully incorporated into solid lipid nanoparticles by hot homogenization and ultrasound dispersion method.It was concluded that optimum formulation was exhibits uniform dispersity index, nano particle size,better zeta potential,high percentage of entrapment efficiency, and expected drug release pattern. The poloxamer 188 had significant influence on the drug release of cefixime. So that SLNs offer a promising delivery system for the enhancement of the bioavailability of poorly soluble drug cefixime.

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ABSTRACT

Solid Lipid Nanoparticles (SLNs) of Cefixime were prepared by hot homogenization and ultrasound dispersion method using glyceryl monostearate (GMS) as the lipid. Particle size and zeta potential of Cefixime loaded SLN was measured using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments) SLN dispersion prepared using Poloxamer 188 2.0% as stabilizer (F4, F5 and F6) showed lower particle size than the other surfactants. The entrapment efficiency (EE) of SLN dispersion was determined by the centrifugation method. Among the various surfactants used Poloxamer 188 shows highest drug entrapment. The *in-vitro* drug release study was determined using USP Type II apparatus paddle in phosphate saline buffer pH7.2. The drug release of F6 formulation at the end of 5 hrs was about 94.71±0.53%. The morphology of Cefixime loaded SLN dispersion was examined by scanning electron microscope. The above observation shows that Cefixime absorption is enhanced by using solid lipid nanoparticles formulations.

Keywords: SLNs, Hot homogenisation, Scanning electron microscope, Zeta potential.