"A COMBINED APPROACH OF NANOPARTICLES WITH IONTOPHOROSIS USING MACROLIDE ANTIBIOTIC FOR THE TREATMENT OF EYE DISEASES"

A Thesis Submitted to

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IN

PHARMACEUTICAL TECHNOLOGY &

BIOPHARMACEUTICS

BY

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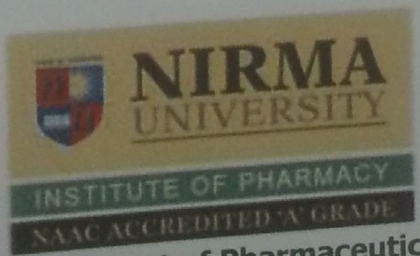

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CERTIFICATE

This is to certify that the dissertation work entitled "A combined approach of Nanoparticles with Iontophorosis using macrolide antibiotic for the treatment of eye diseases" submitted by Mr. Harshit Parikh with Regn. No. (14MPH107) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "A combined approach of Nanoparticles with Iontophorosis using macrolide antibiotic for the treatment of eye diseases", is based on the original work carried out by me under the guidance of Dr. Jigar N. Shah, Assistant professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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LIST OF ABBREVIATIONS

CLA	Clarithromycin
SLNs	Solid Lipid Nanoparticles
mg	Milligram
%EE	Entrapment Efficiency
%DL	Drug loading
Ps	Particle size
TEM	Transmission Electron Microscopy
HLB	Hydrophilic-Lipiphilic Balance
GRAS	Generally Regarded As Safe
nm	Nanometer
HPH	High pressure homogenization
µm	Micrometer
ppm	Parts Per Million

Abstract

A Combined approach of nanoparticles with iontophorosis using macrolide antibiotic for the treatment of eye diseases

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The aim of the research was to formulate Solid Lipid nanoparticles of clarithromycin for the treatment of endophthalmitis and to enhance the permeation using a novel non-invasive technique 'Ionotophorosis'. Solid lipid nanoparticles were prepared by nano-precipitation technique followed by probe sonication. For the selection of lipid and surfactant, solubility studies were done. From the solubility study, Stearic acid, Tween 80 and Transcutol P were selected. Preliminary trials were then carried out by varying the concentration of lipid, Surfactant and co-surfactant ratio and Surfactant concentration. From the preliminary trails, fraction factorial design was used for the screening. From the fraction factorial design, two significant factors were found i.e Drug-lipid ratio and Sonication time. For further optimization 3^2 full factorial design was applied. The dependent variable selected were particle size, % entrapment efficiency and % drug loading. Design space was obtained and the formulation was optimized. In-vitro diffusion studies using by multi diffusion cells was performed in phosphate buffer 7.4. To enhance the permeation of drug, iontophorosis technique was used. Ex-vivo study was performed on the goat eye and the permeation was checked. The iontophorosis technique was found to be very useful as systemic toxicity can be avoided due to enhanced permeation. So, with combination of the nanoparticles and iontophorosis, controlled release formulation along with increased permeation can be formulated.

1. INTRODUCTION

1.1 ANATOMY OF EYE:[1][2][3]

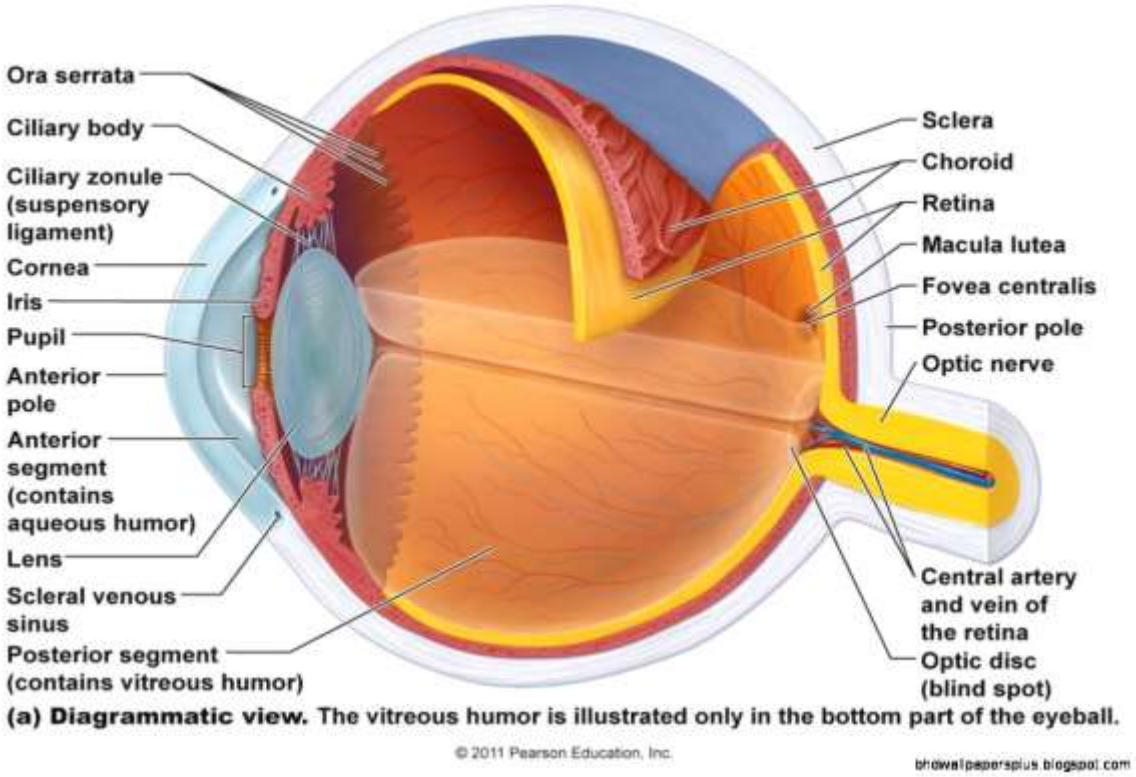


Figure 1.1 Anatomy of eye

Eye is considered as one of the most unique and complicated organ of the body. Many ophthalmic formulations are developed for treating the eye but they have certain limitations as protective mechanisms are present such as rapid drainage of foreign substances, barriers present in eye, blinking of eye which removes the drug. Thus to deliver drug knowledge of anatomy and physiology of eye is very important.

The diameter of an adult eye is 2.5cm. Eye is made up of two segments: Anterior segment and Posterior segment. The anterior segment consist of the iris, ciliary body, pupil and aqueous humor. The posterior segment consist of sclera, lens, choroid, retina and vitreous humor.

1.1.1 Cornea

The cornea is an outer transparent coat that covers the iris. It directs the light onto the retina as it is dome shaped. It can be divided into five parts: the epithelium, bowman's membrane, the lamellar stroma, the endothelium and descemet's membrane. The main pathways for drugs to cross the corneal epithlium are paracellular and transcellular route. Lipophilic drugs penetrate through transcellular pathway whereas hydrophilic drugs penetrate through paracellular pathway. Cornea has the most nerves than any part of the body.

1.1.2 Sclera

It is the white colour portion of eye. It is made up of large collage fibres and fibroblasts. Sclera gives shape to the eyeball and protects the inner part by making the eyeball more rigid. It is divided into three layers: episclera, the sclera and melanocytic layer.

1.1.3 Conjunctiva

The conjunctiva forms and maintains the percomeal tear film and protects the eye. It starts from cornea to the visible part of sclera upto the inside of the eyelid. It is made up of epithelium substantia propria and episclera. The tiny blood vessels present in the eye nourishes the conjunctiva.

1.1.4 Retina

It covers the 2/3 rd region of the posterior eyeball. It is a thin, transparent and reddish colour due to presence of blood vessels. It is the only part of eye where blood vessels can be seen. Retina captures light ray with the help of photoreceptors and convert them into electrical impulses. These impulses are sent to by brain via optic nerve to get the images. There are two types of photoreceptors cones and rods. The cones are present in macula and are responsible for the vision.

1.1.5 Aqueous humor

It is present in the anterior part of the eye. It is a fluid present between the cornea and iris. Aqueous humor is produced by ciliary body and drains into the extraocular venous system. It helps in maintaining the intraocular pressure and supplies nutrients to the lens and cornea.

1.1.6 Vitreous Humor

It is present in the posterior region of eye. It is a transparent gell like mass located behind the lens. It protects the lens by acting as the suspension and maintains the shape of the posterior segment of the eye.

1.1.7 Iris

It is the coloured part of the eye. It is located between cornea and the lens and is attached to the ciliary body. The main function of iris is to regulate the amount of light entering the eye through pupil. The center of iris is called pupil. Circular muscles are present in the iris which is responsible for dilation and constriction of the pupil.

1.1.8 Choroid

It is present in the internal surface of the sclera. It has many blood vessels which provides nutrition to the posterior part of the eye. A brown colour pigment melanin present in the choroid absorbs stray light and prevents reflection and scattering of light within the eye.

1.2 Barriers present in ocular drug delivery:[1]

1. **Lacrimal fluid – eye barrier:** Drug absorption from the lacrimal fluid to eye is prevented by corneal barrier formed by maturation of epithelial cells. The epithelial cells form tight junction that prevents the drug permeation. So the lipophilic drugs have more permeability than the hydrophilic drugs. The main route of drug entrance

from the lacrimal fluid to the aqueous humor is transcorneal permeation inspite of the tightness of the corneal epithelial layer.

2. **Blood – ocular barriers**: It is divided into two barriers : blood – aqueous barrier and blood – retinal barrier. The blood – aqueous barrier is present in anterior part of the eye. It is made up of endothelial cells. This barriers prevents the permeation of plasma albumin and hydrophilic drugs from plasma to the aqueous humor. The blood – retinal barrier is made up of retinal pigment epithelium. Drugs can easily enter chrodial extravascular space but it is difficult to enter retina due to retinal pigment epithelium.

3**. Drug loss from ocular surface**: Due to the flow of lacrimal fluid, the instilled drug is removed from the surface of the eye. The extra volume of the instilled fluid enters the vasolacrimal duct. Drugs can also be removed due to systemic absorption instead of ocular absorption. Systemic absorption takes place from conjuctival sac or after the flow of solution to nasal cavity. Drugs having low molecular weight is absorbed in systemic circulation very fast. Ocular bioavailability of only 10 % is obtained as most of the drugs are cleared by the systemic absorption.

1.3 Common disorders related to eye

Table 1.1 Disorders of eye

Cataract	
Conjunctival and sclera disorders	
Viral Conjunctivitis	It is a communicable infection caused by adenovirus
Allergic Conjunctivitis	It is generally caused by allergens
Pinguecula and Pterygium	They are the growth of conjunctiva which can result in irritation
Trachoma	It is caused by Chlamydia trachomatis
Episcleritis	It is an inflammation of the episcleral tissue

Scleritis	It is an inflammation of sclera and deep episclera which may lead to loss of vision
Corneal	
Corneal ulcer	It is an inflammation by bacteria, fungi, virus in the corneal epithelial
Keratitis	It non-ulcerative inflammation caused by infection
Keratoconus	It is bulging of the cornea which leads to loss of visual acuity
Keratomalacia	It is caused due to nutritional deficiency
Glaucoma	
Eyelid and tearing disorders	
Blepharitis	It is an inflammation of edges of eyelid with swelling or redness
Canaliculitis	It is an inflammation of the inner corner of the eyelid
Dacryocystitis	It ia an infection of lacrimal sac
Trichiasis	It is misalignment of eyelashes and rubs against the eyeball
Dacryostenosis	It is narrowing of the nasolacrimal duct
Eye socket disorder	
Infection of the orbit	It is the infectio of tissue around the eyes or within the eye socket
Inflammation of orbit	It is due to systemic imflammation
Tumors of the orbit	It occurs behind in the tissue behind the eye
Cavernous sinus thrombosis	It is a rare disorder with clotting of blood in the cavernous sinus

1.2 INTRODUCTION TO ENDOPHTHALMITIS[4][5]

Endophthalmitis is an intraocular inflammatory infection of anterior and posterior region of eye. It is the infection generally caused due to organisms that enter the eye through blood stream, intravenous drugs, surgery in eye or other parts of eye or sepsis. Bacterias, fungi or parasites are the organisms that cause endophthalmitis. It can lead to permanent loss of vision.

Figure 1.2 Endophthalmitis disorder in patient

1.2.1 Classification of Endophthalmitis[4]

Endophthalmitis is divided into two classes: Infectious Endophthalmitis and Non-infectious Endophthalmitis. Infectious Endophthalmitis can further be classified into Endogenous infection and exogenous infection. Endogenous infections are due to entering of micro-organism to the eye in the bloodstream whereas exogenous infections are entering of micro-organism from the external environment i.e. due to trauma or ocular surgery.

Table 1.2 Classification of Endophthalmitis

	Exogenous	Endogenous
Infectious Endophthalmitis	Acute onset post-operative • Cataract surgery • Glaucoma filtering surgery	
	Delayed onset post-operative	
	Conjuctival filtering bleb-associated	
	Post-traumatic	
Non-infectious Endophthalmitis	Sympathetic ophthalmia	
	Sterile uveitis	
	Phacoanaphylactic Endophthalmitis	

1.2.2 Causes of Endophthalmitis

Endophthalmitis is caused by bacteria, fungi or parasites by affecting retina, lens, uveal track, aqueous humor or vitreous humor. Both gram positive and gram negative bacterias are responsible for endophthalmitis but most of the cases occur due to gram positive bacteria. Gram positive bacteria accounts for 70% of the cases which include Streptococcus species such as S aureus. Gram negative bacteria accounts for only 5.9% of the cases which include Klebsiella pneumonia, Pseudomonas aeruginosa, E.coli, Serratia Marcescens etc. Causative organisms of fungal infection include aspergillus, candida, Pseudallecheria boydii, Histoplasma, Coccidoides, Blastomyces, Cryptococcus, Sporothrix. In post-operative endophthalmitis the main source of infections are conjunctiva and eyelid. Other sources include lacrimal system, contaminated surgical instruments or irrigating fluids.

1.2.3 Signs and symptoms[5]

- Loss of vision or decrease in sight
- Conjuctival hyperaemia
- Hypopyon
- Lid swelling
- Corneal edema
- Loss of red reflex
- Vitritis
- Anterior chamber inflammation
- Enlarging capsular plaque

1.2.4 Diagnosis of Endophthalmitis[6]

The diagnosis is based on the symptoms, an examination of the eye, cultures, and sometimes antibody or DNA testing. Cultures may be taken from the aqueous humor and the vitreous humor and plated on sheep blood agar, chocolate agar, thioglycolate broth, and Sabrouraud's dextrose media and smears are treated with Giemsa and Gram staining to determine which organism is responsible and which drugs are most active against them.

1.2.5 Drugs used in endophthalmitis

Mainly intravitreal injections of antibiotics are given to the patient having endophthalmitis. Other routed of administration include subconjuctival, topical and systemic route

Table 1.3 Drugs used in endophthalmitis

Route of administration	Drugs
Intravitreal route	Vancomycin Amikacin
Subconjuctival	Ceftazidime

	Vancomycin Dexamethasone
Topical	Vancomycin Amikacin
Systemic	Prednisone Ceftazidime Amikacin

1.2.6 Treatment of endophthalmitis[7]

Treatment of endophthalmitis depends on the severity and extent of inflammation. Primary objectives of the treatment incluse control of infection, restoration of vision, manage complication. Secondary objectives include manage globe shape, debulking and improve blood retinal barrier.

Process of treatment

I. Medical
   a) Anti-microbial therapy:
      - Intravitreal
      - Topical
      - Peribulbar
      - Systemic
   b) Anti-inflammatory therapy
      - Intravitreal
      - Topical
      - Systemic
   c) Supportive therapy
      - Anti glaucoma
      - Corneal

II. Surgical
   a) Vitrectomy

1.3 OCULAR DRUG DELIVERY SYSTEM[3] [2]

1.3.1 Routes of ocular delivery

Depending on the types of target tissue it is divided into three routes: Topical, intraocular and systemic route.

1) **Topical route**: It includes eye drops, suspensions, in-situ gel, contact lens liposomes, micro and nanoparticles. It is one of the common route of administration due to its convenience and safety. The disadvantage of this route is its short contact time with the eye so prolonged release formulation such gels ocular inserts etc. need to be prepared. Other disadvantages include low bioavailability, high dosing frequency. The drug after administration is rapidly eliminated from from precorneal region due to nasolacrimal duct and dilution by tear fluid.
2) **Intraocular route**: Intraocular routes are given for increasing the intraocular concentration along with decreased systemic effects. Most of the intraocular injection should maintain prolong release as they are eliminated from the anterior region due to the flow of aqueous humor or from the posterior region through retina. This route has low patient compliance as multiple injection have to be taken which causes inconvenience and pain and may led to other complication such as retinal detachment, cataract and endophthalmitis.
3) **Systemic route**: This route has poor access to the eye due to blood-ocular barrier. This barrier is further divided into blood-aqueous barrier and blood-retinal barrier. Blood-aqueous barrier prevents the blood to enter anterior chamber whereas the blood-retinal barrier prevents the drug from entering vitrous body. Drugs having active transport or are lipophilic can easily cross the blood-retinal barrier. For the systemic delivery high dose is required due to less blood flow in the posterior region.

1.3.2 Approaches of ocular delivery

1.3.2.1 Conventional approach:

Conventional dosage forms include eye drops, suspensions, ointments, gels. They account for almost 90% of all the ophthalmic formulation due its patient compliance, ease of administration, low cost. They have many disadvantages such as low residence time, low bioavailability, elimination from nasolacrimal drainage.

1.3.2.2 Colloidal drug delivery system:

1) **Liposomes:** They are the vesicular systems composed of phospholipids bilayers. They can encapsulate both lipophilic and hydrophilic drugs. They are reservoir type of system so they useful for posterior region of eye. Advantages of liposomes are that they increase the half-life and decrease the intraocular side effects of the drugs. Disadvantages of liposomes include chemical instability, degration of liposomes on storage. Liposomes having negative or no charge have fast precorneal clearance whereas positively charged liposomes have prolonged retention time due to adherence to the epithelial layer which is negatively charged.

2) **Niosomes:** They are vesicles of non-ionic surfactant. They are used to deliver hydrophobic or amphiphilic drugs. Advantages of niosomes are they chemically stable, are non toxic and do not require any special procedure for preparation. There is an increase in ocular bioavailability of niosomes as compared to solution as surfactant act as penetration enhancers.

3) **Microemulsion:** They are the dispersion prepared by homogenising oil phase and aqueous phase. They contain surfactants which act as permeation enhancer. They also increase the bioavailability of drugs. They help to increase solubility of lipophilic and hydrophilic drugs and decrease the systemic side effects. Microemulsion decrease the drainage from the cornea due to adsorption of the droplets.

4) **Nanoparticles:** They are the polymeric particles ranging in the size of 1 to 500 nm. They act by dissolving, entrapping or encapsulating the drug with the polymer or lipid. They are divided in two categories: nanospheres and nanocapsules. Nanospheres are the solid monolithic spheres made of solid polymeric network. Nanocapsules are the polymeric membrane having cavity in the center. Nanoparticles decrease the nasolacrimal drainage and increase the ocular residence time. Different types of polymers are to prepare nanoparticles which can give different release of the drug.

1.3.2.3 Ocular inserts:

They are the solid dosage form divided in three types: Laciserts, SODI, minidisc. Ocusert is a membrane-controlled reservoir system which allow the drug to diffuse from the reservoir at a slow determined rate for a long period of time.

Laciserts are the rod shaped device made of HPC without any preservative. It was developed by Merck, Sharp and Dohme in 1981.

Sodi is an oval shaped wafer used by cosmonauts. It is a thin sterile film made of arcylamide,, ethylacrylate, etc. the film is kept in cul-de-sac and gets wetted by tear and acquires the shape of the globe. Film turns into viscous mass and then it becomes solution.

Minidiscs are contoured dick with convex front and concave back which is in contact with eyeball. It has the diameter of4-5mm. It is made up of silicon. Minidisc can be hydrophilic and hydrophobic to give sustained release.

1.4 INTRODUCTION TO SOLID LIPID NANOPARTICLES

1.4.1 Introduction[8][9]

Lipid nanoparticles are important because of their nano-scale properties to give therapeutic efficacy to desired site of action. They are generally used to reduce the slow and incomplete dissolution of BCS class II and class IV drugs. SLNs are the colloidal carrier system varying in size from 50 to 1000nm. They are generally composed of biodegradable lipids dispersed in aqueous surfactant solution. The main reason for preparing SLNs is to give sustained or controlled release of the drug. They can be developed for various routes of administration such as ocular, oral, pulmonary, parenteral, dermal etc. and can be characterised for in vitro and in vivo studies.

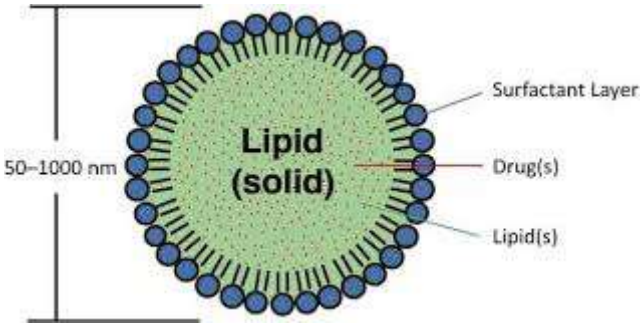


Figure 1.3 solid lipid nanoparticle

ADVANTAGES

- Both hydrophobic and hydrophilic drugs can be incorporated.
- They improve bioavailability of poorly water soluble drugs
- Low toxicity as biodegradable lipids are used
- Site specific drug delivery is possible
- Scaling up is possible as they have excellent reproducibility.
- They give controlled release and drug targetting for several days.
- Avoidance of organic solvent

DISADVANTAGES

- Drug loading capacity may be poor
- Drug expulsion due to crystallinity of lipid

1.4.2 Excipients used for SLNs formulation

Table 1.4 excipients used in SLNs formulation

Lipids	
Compritol 888 ATO	Lauric acid
Precirol ATO 5	Tristrarin
Stearic acid	Glyceryl monostrarate
Gelicire 44/14	Palmitic acid
Stearyl amine	Octadecyl amine
Surfactants and co-surfactants	
Tween 20,60,80	Cremophore EL
Poloxamer 188,407	Transcutol P
Egg lecithin	Sodium taurochoate
Soya lecithin	

Mostly solid lipids, surfactants and water are used for preparation of SLNs. Lipids such as fatty acids, Triglycerides, waxes etc. are generally used. Particle size of the SLNs increases as the amount of lipid increases. Surfactants having HLB values in the range of 6 to 18 are used for the preparation of SLNs. Concentration of surfactant is very important for the smaller particle size and for prevention of agglomeration. Combination of two surfactants can also be used for the stability of SLNs. All the excipients used should be GRAS(Generally Regarded As Safe) listed.

1.4.3 Method of preparation of SLNs[10][11]

Different methods for preparation of SLNs include

1) High Pressure Homogenization
    a) Hot homogenization
    b) Cold homogenization
2) High speed homogenization along with Probe sonication
3) Microemulsion followed by probe sonicator
4) Nano-precipitation technique
5) Solvent diffusion
6) Solvent Evaporation
7) Supercritical fluid method

(1) High pressure homogenization

This method is widely used for the preparation of SLNs due to its large scale production. HPH uses pressure between 100 – 2000 bars to push the liquid the through small gap of few micrometers. Due to very high force and short distance to travel for fluid, it break the particle to submicron size. Two methods are used for proper homoginization.

(a) Hot homogenization

(b) Cold homogenization

(a) Hot homogenization

In this method the lipid is melted 5 to 10°C above the melting point. Drug is solubilised in the lipid. The aqueous phase is also heated at the same temperature and both are homogenized to form a pre – emulsion. Then the pre – emulsion is subjected to HPH at room temperature to obtain SLNs.

Lower particle size is achieved at higher temperature due to decreased viscosity of the internal phase. The number of cycles and pressure used in the HPH has to be optimized. By increasing the pressure and number of cycles may result in an increased particle size.

(b) Cold homogenization

In this, the lipid is melted and drug is solubilized in it. The mixture is rapidly cooled by liquid nitrogen or dry ice. Then the lipid is milled by mortar pastel to obtain micron size particles. Both lipid and the aqueous phase are heated at the same temperature and they are homogenised to form the pre-emulsion. Afterwards it is subjected to HPH at room temperature to form SLN. This method is mainly used for hydrophilic drugs.

(2) High speed homogenization followed probe sonication.

This method is widely used at lab scale as the equipments are easily available. Some of the problems associated with are broad PDI, contamination due to metal probe, growth of particle on storage. In this method the lipid is melted above the melting point of lipid and the drug is solubilised in it. The aqueous phase is also heated at the same temperature. The aqueous phase containing surfactant is added drop-wise to the lipid phase and homogenized at high speed. It is then subjected to probe sonication to obtain SLNs.

(3) Microemulsion method

It is a two phase system having internal phase and external phase. Both the phases are stirred at 65 – 70 ° C which has lipid, emulsifier and water. The heated microemulsion is then dispersed in cold water with continuous stirring. The SLNs formed are washed with distilled water and filtered.

(4) Nano-precipitation method

In this method the lipid is dissolved in the water miscible solvent. Drug is also dissolved in the solvent. The aqueous phase contains emulsifier. The solvent phase

is added to the aqueous phase drop-wise with continuous stirring. SLN will be precipitated when the solvent gets evaporated. A disadvantage of this method is that organic solvents are needed for the use.

(5) Solvent evaporation

In solvent evaporation, solid lipid and drug are dissolved in water immiscible organic solvent. The organic solvent is then emulsified in an aqueous phase using homogenizer. Afterwards the emulsion is stirred at room temperature to evaporate the organic solvent. As the solvent evaporates lipid will precipitate and SLNs will be formed.

(6) Solvent diffusion method.

In this method, water immiscible solvent is used. Firstly the equilibrium is formed by saturating solvent and water. The drug and lipid are dissolved in the saturated liquid. They are then emulsified with aqueous phase. Then the diffusion of solvent to the continuous phase takes place to form the SLNs. The advantage of this method is that it is easy to implement and scale up, high reproductibility, narrow size distribution

(7) Super critical Fluid Technology.

This is a novel method for the production of SLNs. This method include different process such as rapid expansion of supercritical solution, particles from gas saturated solution (PGSS), aerosol solvent extraction solvent (ASES), supercritical fluid extraction of emulsions (SFEE). The advantage of this technique is that the particles are obtained as dry powder. Different gases like CO_2, ethane, ammonia, $CHClF_2$ etc are used. CO_2 is mainly used among those as it is inexpensive, non-inflammable and acceptable to environment. Solubility enhancers such as ethanol are used to increase the solubility of less soluble solvent in the SCF phase.

1.4.4 Characterization of SLNs

Characterization of SLN is very important for the quality. Different parameters to be evaluated for SLNs are particle size, zeta potential, Differential scanning calorimetry (DSC), Atomic force microscopy, X ray diffraction, FTIR, Transmission electron microscopy , Scanning electron microscopy.

(1) Particle size

This is one of the most important parameter of SLNs. It is used to determine physical stability. Different methods to determine the particle size includes photon correlation microscopy, laser diffraction. PCS is used for measuring the fluctuation of the intensity of the scattered light caused by particle movement. Particle size measured by this is in the range 3 nm to 3 µ m. The laser diffraction depends on the diffraction angle on the particle size. More intense scattering of the smaller particle is caused at high angles as compared to larger ones. Factors such as nature of lipid, lipid concentration, type and concentration of surfactant are important for the particle size.

(2) Zeta potential

Zeta potential measures stability of colloidal systems. It indicates extent of particle – particle repulsion forces to avoid agglomeration and aggregation. High zeta potential shows higher stability irrespective of positive or negative value. The value of zeta potential mainly depends on the nature of surfactants.

(3) Differential scanning calorimetry

It is a thermal analytical method used for the determination of melting point, transition temperature of solid lipid by comparing the sample and reference. In SLNs melting and recrystallisation studies are done. Crystallinity of lipid is one of the important properties as it can affect drug incorporation and release rates.

(4) Drug entrapment and loading capacity

Entrapment efficiency is based on the separation of lipids from the aqueous by ultracentrifugation, gel filtration or by dialysis. The amount of drug in aqueous phase is analysed by UV Spectrometry or HPLC.

The entrapment efficiency and drug loading area calculated by the formula

$$\text{Entrapment efficiency} = \frac{\text{Initial drug weight - free drug weight}}{\text{Initial drug weight}} \times 100$$

$$\text{Drug loading} = \frac{\text{Initial weight - free drug weight}}{\text{weight of lipid}} \times 100$$

(5) In vitro drug release

In vitro drug release can be performed by three methods :

(a) Franz diffusion cell

(b) Dialysis bag

(c) Reverse dialysis method

(a) In franz diffusion cell the donor compartment is filled with the SLNs and the receptor compartment is filled with suitable buffer. Both are separated by the semi – permeable membrane. At certain time point aliquots of sample are withdrawn and analyzed by UV Spectroscopy or HPLC. Equal amount of buffer is added after withdrawing to maintain the sink condition.

(b) Dialysis bag method

In this the SLN formulation is filled in the bag (dialysis membrane) and sealed at both the ends. The bag is then kept in the beaker containing buffer, which acts as the receptor compartment. At the specified time interval samples are collected and analyzed and replaced with same volume of buffer to maintain the sink condition.

(c) Reverse dialysis method

In this method, different bags of the suitable buffer of 1 ml are made and dipped in the receptor compartment Slns formulation. One bag of buffer is opened at specific time interval and analyzed. At the other time point another bag is analyzed.

(6) Electron microscopy

(a) Transmission electron microscopy:

It is used for analyzing morphology of the particle. Electrons that are transmitted through the sample are analyzed and an image is obtained of the sample which shows the interaction of the electrons.

(b) Atomic force microscopy

It gives a three dimensional image of the particles. It measures the force acting between the probe tip and the surface of the particle and spatial resolution upto 0.01 nm can be achieved.

(c) X ray diffraction

It is used to know the crystalline structure of the SLNs formulation. It determines the diffraction pattern of the crystalline ingredient. Polymorphic change in the ingredient can be known.

1.5 Introduction to drug and Excipients

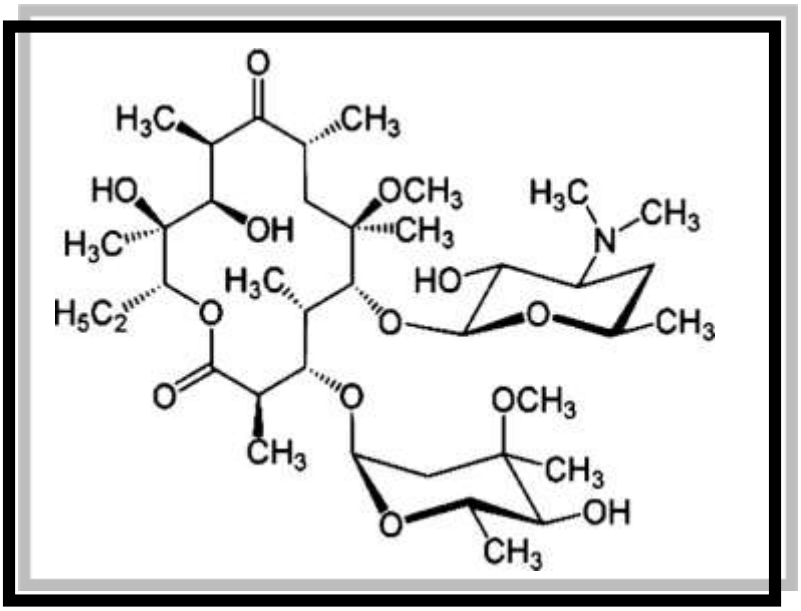
1.5.1 Clarithromycin

Name: Clarithrmycin

Molecular formula: $C_{38}H_{69}NO_{13}$

Molecular weight: 747.95 g/mol

Structural formula:



IUPAC name: ((3R,4S,5S,6R,7R,9R,11R,12R,133S,14R)-6-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2R,4R,5S,6S0-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione.

Category: Macrolide antibiotic

Appearance: white to off-white, crystalline powder

Solubility: Soluble in acetone, slightly soluble in methanol and accetonitrile, practically in soluble in water

Content: NLT 96% and NMT 102%

Melting point: 215-217^{o}C

Log P: 3.16

PKa: 8.99

Half life: 3.3 to 4.9 hours

Bioavailability: 52 to 55%

Protein binding: 42 to 72%

Volume of distribution: 191 to 306 liters

Active metabolite: 14-hydroxyclarithromycin

Excretion: Urine- 18.4%; faeces- 4.4%

Mechanism of action: It acts by inhibiting bacterial protein synthsis of 50S ribosomal subunit thereby inhibiting translation of peptides

Side effects: stomach pain, indigestion gas, vomiting, rashes and itching, headace

1.5.2 Stearic acid:

Nonproprietary Names:

BP: Stearic Acid

JP: Stearic Acid

PhEur: Stearic Acid

USP-NF: Stearic Acid

Synonyms:

Acidum stearicum; cetylacetic acid; Crodacid; Cristal G; Cristal S; Dervacid; E570; Edenor; Emersol; Extra AS; Extra P; Extra S; Extra ST; 1-heptadecanecarboxylic acid; Hystrene; Industrene; Kortacid 1895; Pearl Steric; Pristerene; stereophanic acid; Tegostearic.

Chemical Name and CAS Registry Number:

Octadecanoic acid

Functional Category:

Emulsifying agent; solubilizing agent; tablet and capsule lubricant.

Molecular weight: 284.47

Empirical Formula: $C_{18}H_{36}O_2$

Structural Formula:

Description: Stearic acid is a hard, white or faintly yellow-colored, glossy, crystalline solid or a white or yellowish white powder. It has a slight odor (with an odor threshold of 20 ppm) and taste suggesting tallow.

Physical Properties:

Boiling point: 383 ^oC

Flash point: 113^oC (closed cup)

Melting point: 69–70^oC

Moisture content Contains practically no water.

Partition coefficient Log (oil : water) = 8.2

Refractive index: 1.43

Solubility: Freely soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.

Applications: It is widely used in oral and topical formulation. In oral formulation it is mainly used as tablet and capsule lubricant. It also acts as binder and for tablet coating. It is used as tablet coating to give sustained release. In topical formulation, it is used as emulsifying and solubilising agent. Stearic acid is used as the hardening agent in glycerine suppositories. Stearic acid is also widely used in cosmetics and food products.

1.5.3 TWEEN 80

Nonproprietary Names:

BP: Polysorbate 80,

PhEur: Polysorbate 80,

USP-NF: Polysorbate 80

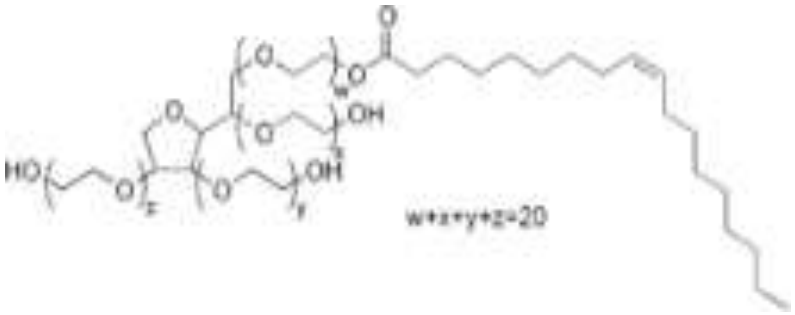
Synonyms: Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O- 20K; Montanox 80; polyoxyethylene 20 oleate; polysorbatum 80; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2-ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Polysorbate 80.

Chemical Name and CAS Registry Number: Polyoxyethylene 20 sorbitan monooleate [9005-65-6]].

Functional Category: Dispersing agent; emulsifying agent; nonionic surfactant; solubilising agent; suspending agent; wetting agent.

Empirical Formula: $C_{64}H_{124}O_{26}$

Structural Formula:



Molecular Weight: 1128

Description: Tween 80 has a characteristic odor and a warm, somewhat bitter taste. Its color and physical form at 250 ºC is Yellow oily liquid.

Physical Properties:

HLB Value: 15

Specific Gravity: 1.08 at 250C

Viscosity (dynamic): 425 mPa S at 250C;

Solubility: Soluble in water, ethanol & insoluble in mineral oil, vegetable oil with water.

Application: Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its

anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound.

1.5.4 Transcutol P

IUPAC name: 2-(2-Ethoxyethoxy)ethanol

Trade names: Carbitol, Carbitol cellosolve, Transcutol, Dioxitol, Poly-solv DE, and Dowanol DE.

CAS register number: 111-90-0

Chemical formula: $C_6H_{14}O_3$

Molecular weight: 134.18 g/mol

Boiling point: 196°C to 202°C

Uses

- It is used as a solvent for dyes, nitrocellulose and resins.
- It is used in non-aqueous stains for wood, for setting the twist and conditioning yarns and cloth, in textile printing, textile soaps, lacquers, cosmetics and quick-drying varnishes and enamels.
- It is also used in brake fluid diluent and in organic synthesis.
- It is used to determine saponification values of oils and as neutral solvent for mineral oil-soap and mineral oil-sulphated oil mixtures.

Aim & Objective

Formulation of ocular diseases are one of the most challenging task for the formulation scientists as eye is the most sensitive part of the body. Any toxic material present in it may damage eye or lead to loss of vision. Eye disorders include cataract, uveitis, endophthalmitis, diabetic retinopathy, etc. Endophthalmitis is an infection of eye which is caused by bacteria, fungi or viruses which enter eye through exogenous or endogenous route. Different routes of administration include topical, intra-vitreal and systemic route. Intra-vitreal route is the most favourable route but it has many limitations as multiple injections have to be taken which may damage the eye. Disadvantage of conventional dosage form is rapid pre-corneal drainage of drug and low bioavailability of drug.

The aim to the project was to formulate and optimise Solid lipid nanoparticles for ocular delivery and increase permeation using iontophorosis. Lipids used in solid lipid nanoparticles are bio-degradable and non-toxic in nature. Lipids also give sustained release as they degrade slowly. Iontophorosis was used to avoid taking multiple injection by intra-vitreal route. Iontophorosis can increase permeation by crossing the barriers present in the eye by application of small amount of current.

Objectives:

1) Formulation, development and optimization of Solid lipid nanoparticles.
2) To study the combined effect of SLNs with iontophorosis to increase the permeation

3. Literature Review

3.1 For SLNs

Article name	Information	Reference
Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: Effects of process variables.[12]	Solid lipid nanoparticles (SLNs) of a hydrophobic drug, tretinoin, by emulsification–ultrasonication method were prepared. Variables such as homoginization time, sonication time, Surfactant concentration, lipid concentration, surfactant type, lipid type were studied. Almost all the process variables had noticeable impact on formulation.	S. Das et al.
Influence of different surfactants on the technological properties and in vivo ocular tolerability of lipid nanoparticles[13]	Surfactants are necessary for the preparation of the nanoparticles. As the surfactant concentration increases the particle size decreases so optimum concentration of surfactant is necessary. So, the different surfactants were studied for ocular tolerability.	A. Leonardi et al.
Formulation Optimization of Erythromycin Solid Lipid Nanocarrier Using Response Surface Methodology[14]	The effect of two variables, including lipid concentration ($X1$) and ratio of surfactant : cosurfactant ($X2$) with their interactions, were studied. These results showed that the	Anil Kumar Sahu et al.

	SLNs obtained in this study could potentially be used as a carrier with an initial dose and prolonged release.	
Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system.[15]	The SLNs were prepared by o/w microemulsion technique. The prepared SLNs were in the colloidal size range with good particle size distribution, PI, high entrapment efficiency, and had good stability on storage.	Mohd. Abul Kalam et al.
Development of a Novel Method for Fabrication of Solid Lipid Nanoparticles: using High Shear Homogenization and Ultrasonication[16]	The objective of the present investigation was to develop SLNs of capsaicin by a novel method using modified high shear homogenization and ultrasonication technique. The study confirmed that the method developed was simple and effective to formulate SLNs of poorly soluble drugs without organic solvents.	Sharma A et. al.
Indomethacin-Loaded Solid Lipid Nanoparticles for Ocular delivery: Development, Characterization, and In Vitro Evaluation[17]	The indomethacin SLNs developed could significantly improved the chemical stability and in vitro corneal permeability. Thus increasing the ocular bioavailabity	HIPPALGAONKAR et al.
Diclofenac sodium-loaded solid lipid nanoparticles prepared	They prepared solid lipid nanoparticles by solvent evaporation method. Ethanol	

by emulsion/solvent evaporation method[18]	was used as the solvent. GMS and phospholipid were taken as lipids and 1% PVA and 1% PEG 400 as solvent. They were successfully able to incorporate the drug in SLNs.	Dongfei Liu et al

3.2 For Clarithromycin

Article name	Information	Reference
Review of Macrolides (Azithromycin, Clarithromycin), Ketolids (Telithromycin) and Glycylcyclines (Tigecycline)[19]	The advanced macrolides have several distinct advantages over erythromycin, including improved oral bioavailability, longer half-life, higher tissue concentrations, enhanced antimicrobial activity	Zuckerman et al
Clarithromycin: review of a new macrolide antibiotic with improved microbiologic spectrum and favorable pharmacokinetic and adverse effect profiles.	Comparision was made between clarithromycin and erythromycin in terms of in vitro activity, pharmacokinetics, pharmacodynamics, clinical efficacy, and toxicity. clarithromycin appeared to expand the traditional spectrum of macrolide antibiotics	Sturgill MG et al
Formulation and evaluation of clarithromycin poorly soluble drug as microemulsion[20]	They prepared o/w microemulsion and increased the bioavailability by increasing the solubility of the drug. They selected ethyl oleate and olive oil as the oil phase, DGMO-C and	Rahul suthar et al.

	HCO-40 as surfactant. From the particle size, zeta potential and drug release the successufully increased the bioavailability of clarithromycin	

3.3 For Nanoparticles and iontophorosis

Article name	Information	Reference
In Vivo Transscleral Iontophoresis of Amikacin to Rabbit Eyes[21]	Enhancement was observed in iontophoretic delivery to the rabbit eye. as the current is increased from 0- to 4-mA, the amount of drug delivered systemically and to the individual tissues increases.	D. L. VOLLMER et al
Nanoparticulate Transscleral Ocular Drug Delivery [22]	Nanoparticulate along with the iontophorosis gives sustained release as well as non-invasive method to deliver drug to the posterior segment	Jigar N Shah et al
Charged nanoparticles delivery to the eye using hydrogel iontophorosis[23]	They studied the permeation of charged fluorescent nanoparticles into rabbit eye by iontophorosis. They compared the permeation of negative and positively charged nanoparticles. On applying the current of 1.5mA negatively charged nanoparticles showed good penetration in anterior part whereas positively charged	Esther Eljarrat-Binstock et al.

	nanoparticles showed better permeation in the posterior region	

3.4 Patents

Title	Patent no. & year	Innovators
Clarithromycin formulations having improved bioavailability[24]	US 10/509,704, 2005	Ashok Rampal, Rajeev S Raghuvanshi, Manoj Kumar Paruthi
Transscleral delivery[25]	US 13/279,179, 2012	Eugene R. Cooper, David M. Kleinman, Thierry Nivaggioli, Philippe JM Dor,Sreenivasu Mudumba
Solid lipid nanoparticles entrapping hydrophilic/ amphiphilic drug and a process for preparing the same	PCT/IN2012/000154, 2012	Indu Pal Kaur, Rouit Bhandari
Ocular iontophoresis device	US 11/297,942, 2012	Eyegate Pharma

4. EXPERIMENTAL WORK

4.1 Materials used

Table 4.1 List of material used

Materials	Company
Clarithromycin (CLA)	Century Pharmaceuticals Ltd., Vadodara
Stearic acid	Central Drug House, New Delhi
Tween 80	Central Drug House, New Delhi
Transcutol P	Gattefosse India pvt. Ltd.
Ethanol	
Glyceryl monostearate	Central Drug House, New Delhi
Compritol 888ATO	Gattefosse India pvt. Ltd.
Gelucire 43/01	Gattefosse India pvt. Ltd.
Precirol ATO5	Gattefosse India pvt. Ltd.
Lauric acid	Central Drug House, New Delhi
Glyceryl tristearate	Gattefosse India pvt. Ltd.
Tween 20	Central Drug House, New Delhi
Span 80	Central Drug House, New Delhi
Span 20	Central Drug House, New Delhi
Span 60	Central Drug House, New Delhi
PEG 400	Central Drug House, New Delhi
Propylene Glycol	Central Drug House, New Delhi
Stearyl amine	Hi-Media, Mumbai
Potassium dihydrogen ortho phosphate	Central Drug House, New Delhi
Sodium hydroxide	Central Drug House, New Delhi
Sodium lauryl Sulphate	Central Drug House, New Delhi
Distilled water	Freshly prepared in Lab

4.2 LIST OF EQUIPMENT USED

Table 4.2 List of equipment used

Equipment	Company name
Propeller stirrer	Remi motors, mumbai
Magnetic stirrer with hot plate	EIE Instruments Pvt. Ltd. India
Digital balance	Citiweigh-tejas exports, India
Hot air oven	EIE Instruments Pvt. Ltd. India
Bath sonicator	EIE Instruments Pvt. Ltd. India
Probe sonicator	Brookfield Engineering Laboratories,
Vortex mixer	Remi motors Ltd.
Melting point apparatus	
pH meter	Analab scientific pvt. Ltd.
UV visible spectrophotometer	Shimadzu UV 1800 corporation, Japan
Ultra-Centrifugation	Remi motors, Mumbai
Multi-diffusion cell	Orchid scientific labs
Particle size analyzer	Malvern Zetasizer
TEM	Tecnai

4.3 IDENTIFICATION OF CLA

4.3.1 MELTING POINT DETERMINATION

Melting point of drug is the temperature at which the solid state of drug gets converted to liquid state at atmospheric pressure to check the impurity present as compared with the reported value. Digital melting point apparatus was used to check the melting point.

Table 4.3 Melting point determination

Reported value	Observed Value
215-217^{o}C	214-216^{o}C

Conclusion:

The observed value of melting point was found to be in the range which shows that the drug was pure as compared to that of standard CLA powder.

4.3.2 IR SPECTRA OF CLA:

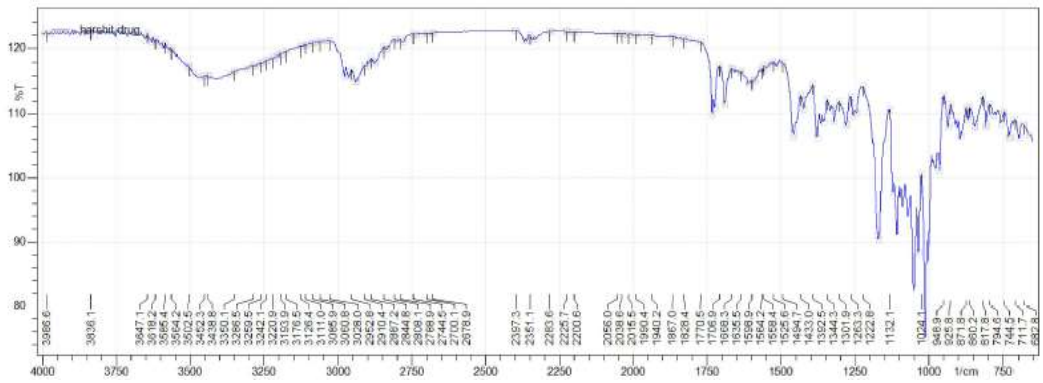


Figure 4.1 Test IR spectra of CLA

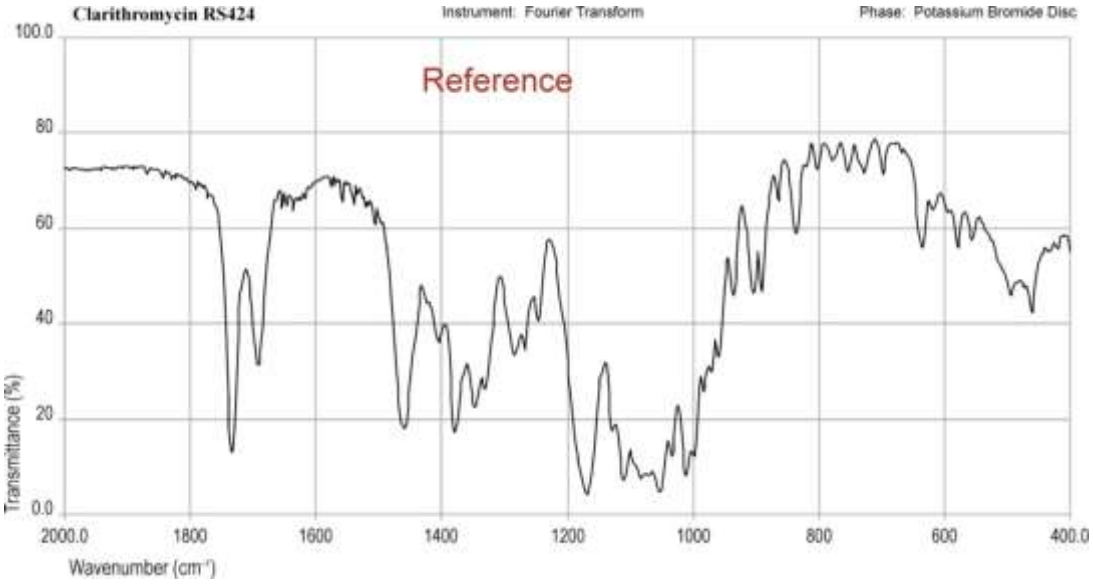


Figure 4.2 Reference IR spectra of CLA

Table 4.4 Comparison of Characteristic and observed Peaks

Functional group	Characteristic peak (cm^{-1})	Observed peak (cm^{-1})
Hydrogen bond between O-H	3470-3570 cm^{-1}	3547
Alkane stretching peaks	2780-3000 cm^{-1}	2843
C=O, lactone	1734-1745 cm^{-1}	1750
C=O, ketone	1690-1680 cm-1	1686
N-CH_3	1425- 1470cm-1	1435
CH_2	1200-1390 cm-1	1280
C-O-C streching	1052 cm-1, 1110 cm-1, 1174 cm-1	1110

Conclusion:

It was concluded that the observed frequency match with the standard frequency for the presence of functional group. So the drug was in the pure state.

4.3.3 UV ABSORPTION SPECTRA OF CLA:

PROCEDURE:

Absorption spectra of CLA was measured by dissolving 100 mg of drug in small quantity of methanol. The volume was made upto 100 ml to make 1000 ppm solution. Absorbance of the solution was measured by UV spectrophotometer. Solution showed peak at 207 nm.

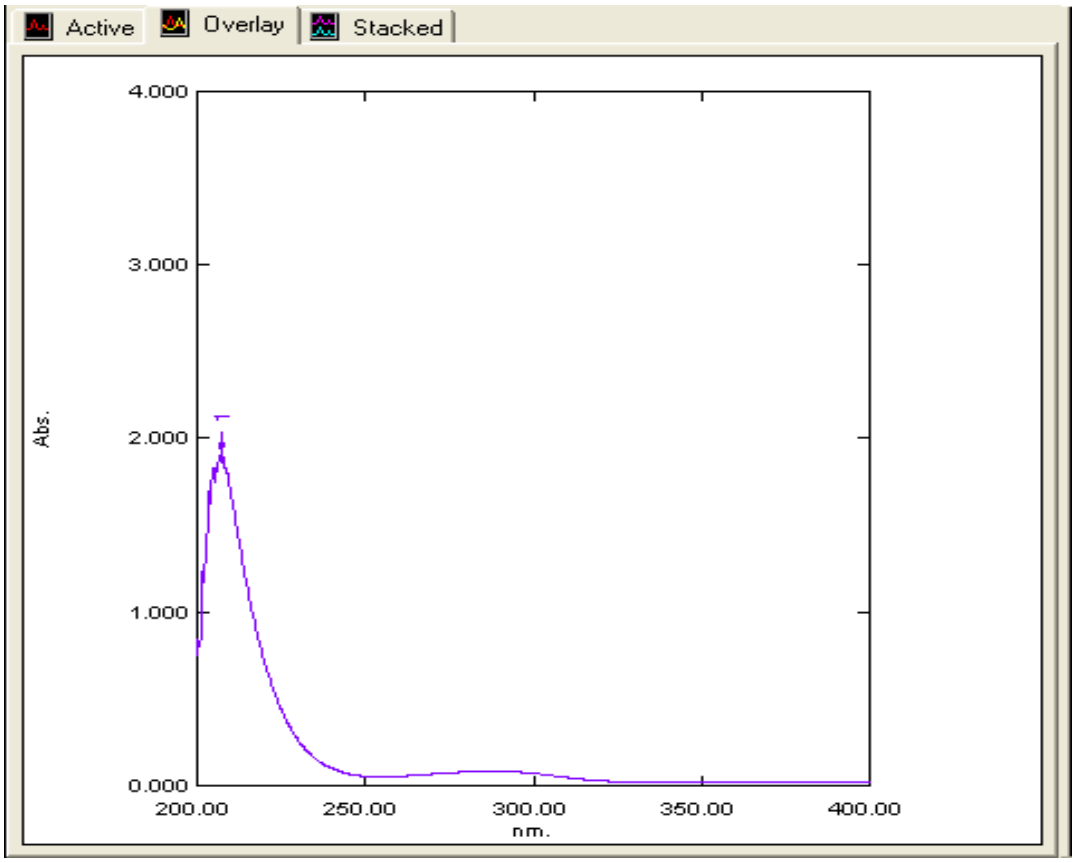


Figure 4.3 UV absorbance spectra of CLA

Conclusion:

Solution shows absorbance maxima at 207 nm which indicate that CLA was in pure form.

4.4STANDARD CALIBRATION CURVE OF CLA

4.4.1 STANDARD CALIBRATION CURVE OF CLA IN METHANOL

Procedure

Preparation of stock solution:

100mg CLA was accurately weighed and dissolved in 50ml methanol. Volume was made up to 100ml using methanol to make final concentration of 1000 ppm solution of CLA

Preparation of dilutions from stock solution

From the stock solution 2 ml, 3 ml, 4 ml, 5 ml and 6 ml solution were pipetted out in 10ml volumetric flask and volume was made up 10 ml with methanol. Absorbance was measured of all the solution at 207 nm absorption maxima by taking methanol as the blank. Measurement was done in triplicate. Standard curve was plotted of absorbance v/s concentration.

Table 4.5 Standard curve of CLA in methanol

Sr. No.	Concentration (ppm)	Absorbance (nm)			Average absorbance (nm)
		1	2	3	
1	0	0	0	0	0
2	200	0.262	0.261	0.262	0.261
3	300	0.379	0.380	0.379	0.379
4	400	0.491	0.491	0.490	0.490
5	500	0.621	0.620	0.620	0.620
6	600	0.713	0.714	0.713	0.713

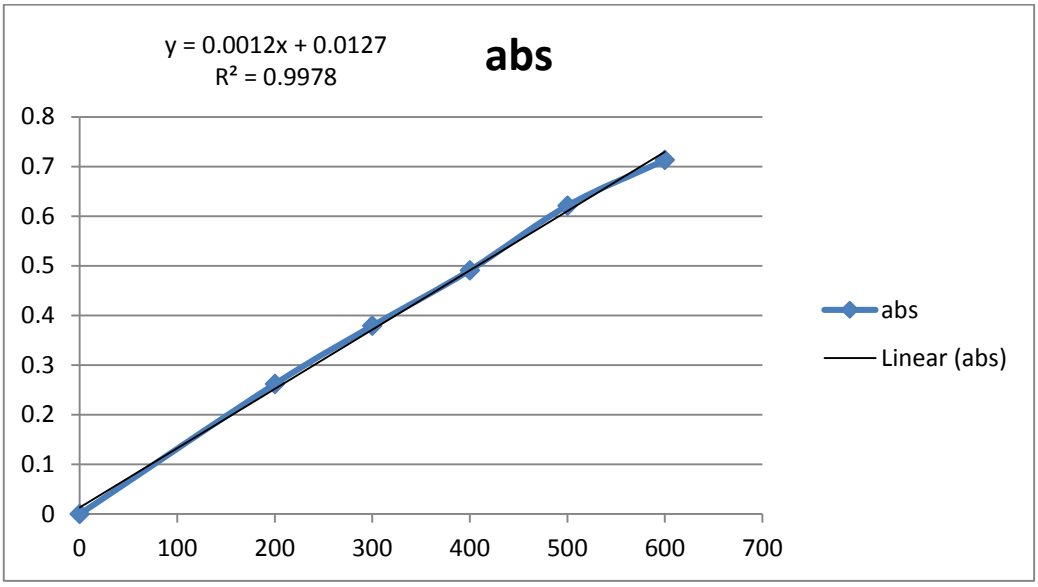


Figure 4.4 Standard curve of CLA in methanol

Table 4.6 Regression analysis of CLA in methanol

Regression parameter	Value
Correlation coefficient	0.997
Slope	0.001
Intercept	0.012

Conclusion:

The regression analysis data of CLA standard curve in methanol shows linear relationship with Correlation coefficient of 0.997. It showed that Beer-Lambert's law was obeyed for the drug concentration range in 200 to 600 ppm.

4.4.2 STANDARD CALIBRATION CURVE OF CLA IN PHOSPHATE BUFFER 7.4

Procedure:

Preparation of stock solution

100mg accurately weighed CLA was dissolved in Phosphate buffer 7.4 using 1% SLS and volume was made up to 100ml with phosphate buffer form 1000ppm solution.

Preparation of dilutions from stock solution

From the stock solution 1ml, 1.5 ml, 2 ml, 2.5 ml, and 3 ml solution were pipetted out and volume was made up to 10ml using Phosphate buffer 7.4 to get a concentration ranging from 100 ppm to 300 ppm. Measurement was done in triplicate. Standard curve was plotted of absorbance v/s concentration.

Table 4.7 Standard calibration curve of CLA in Phosphate buffer 7.4

Sr. No.	Concentration (ppm)	Absorbance (nm)			Average absorbance (nm)
		1	2	3	
1	0	0	0	0	0
2	100	0.277	0.277	0.278	0.277
3	150	0.383	0.382	0.384	0.383
4	200	0.479	0.480	0.480	0.479
5	250	0.561	0.561	0.562	0.561
6	300	0.654	0.653	0.653	0.653

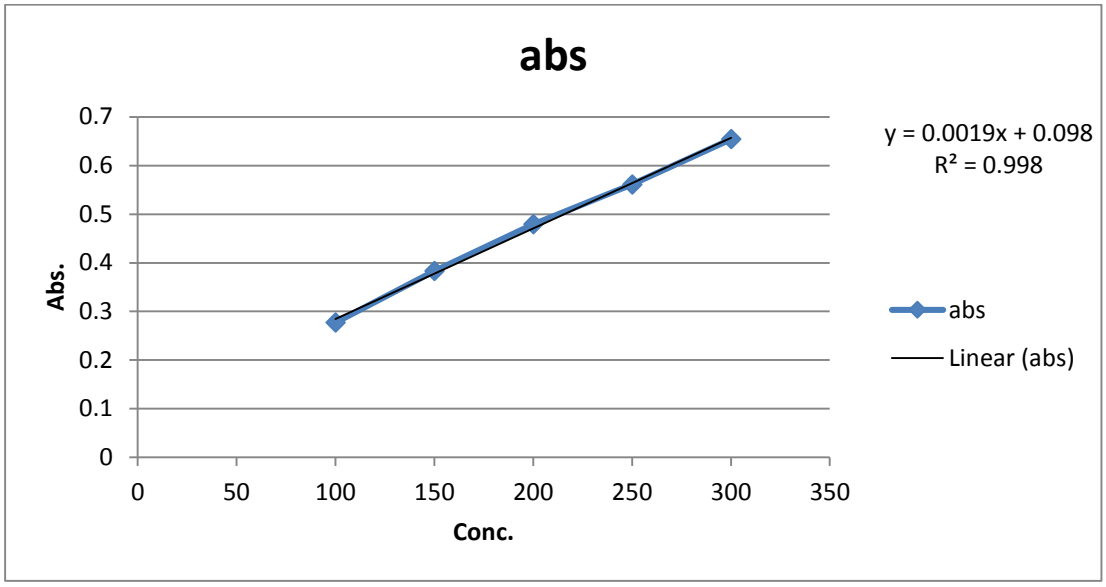


Figure 4.5 Standard calibration curve of CLA in PBS 7.4

Table 4.8 Regression analysis of CLA in Phosphate buffer 7.4

Regression parameter	Value
Correlation coefficient	0.998
Slope	0.001
Intercept	0.098

Conclusion:

The regression analysis data of CLA standard curve in Phosphate buffer 7.4 shows linear relationship with Correlation coefficient of 0.998. It showed that Beer-Lambert's law was obeyed for the drug concentration range in 100 to 300 ppm.

4.5 DRUG EXCIPIENT COMPATIBILITY STUDY:

4.5.1 FT-IR:

Drug excipient compatibility was studied by FT-IR spectrophotometer. IR spectra of drug sample, lipid, Surfactants and their combination were studied.

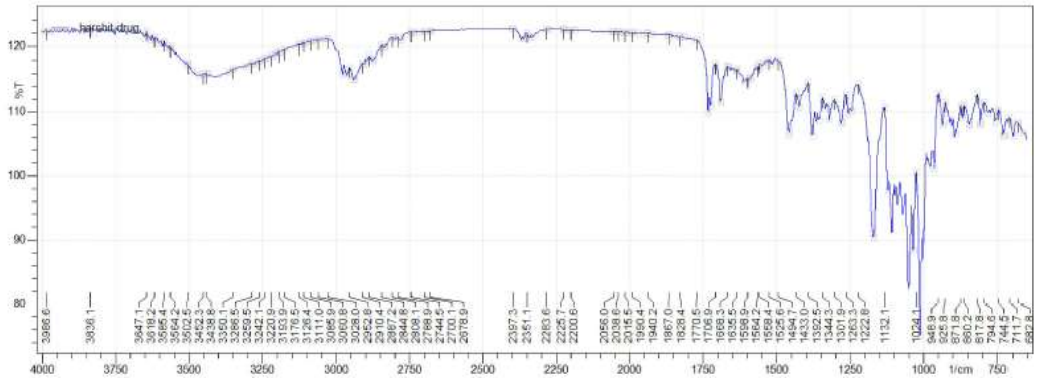


Figure 4.6 Test IR spectra of CLA

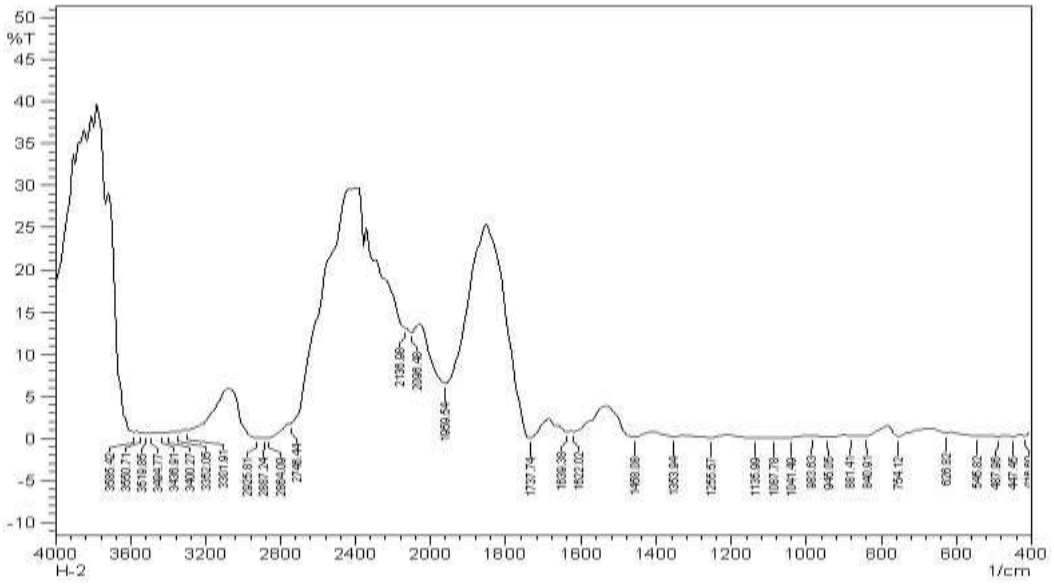


Figure 4.7 IR spectra of Tween 80

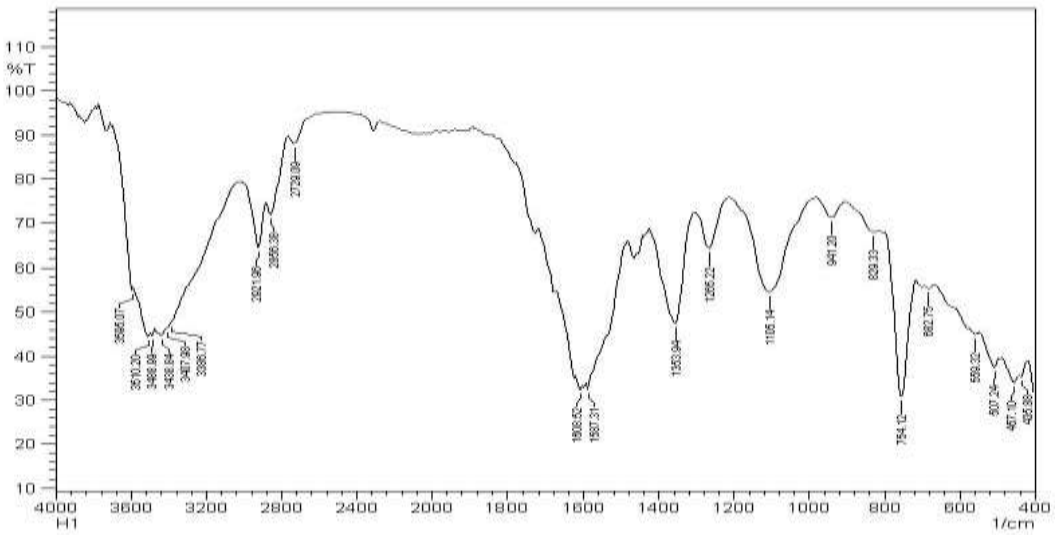


Figure 4.8 IR spectra of Transcutol P

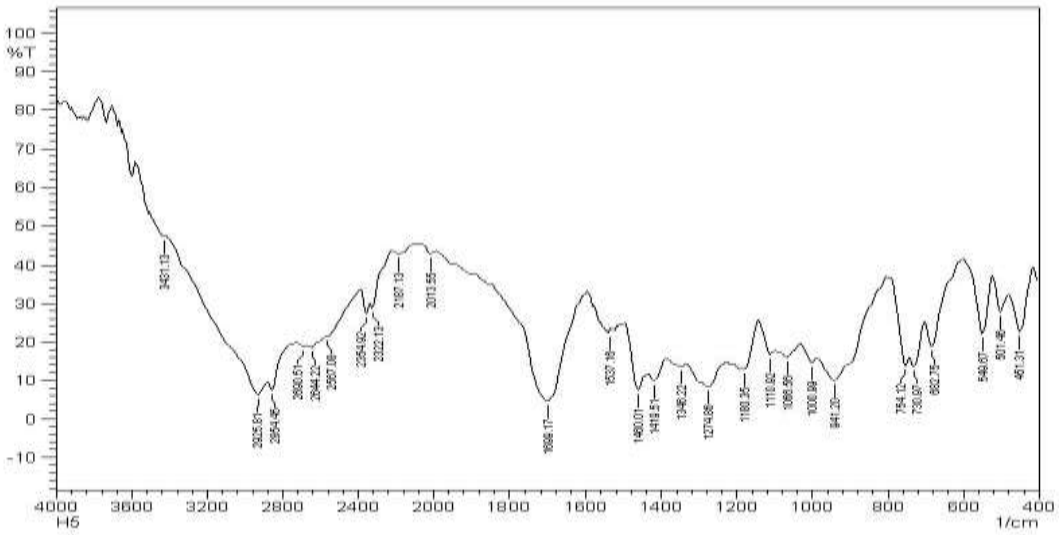


Figure 4.9 IR spectra of CLA + stearic acid

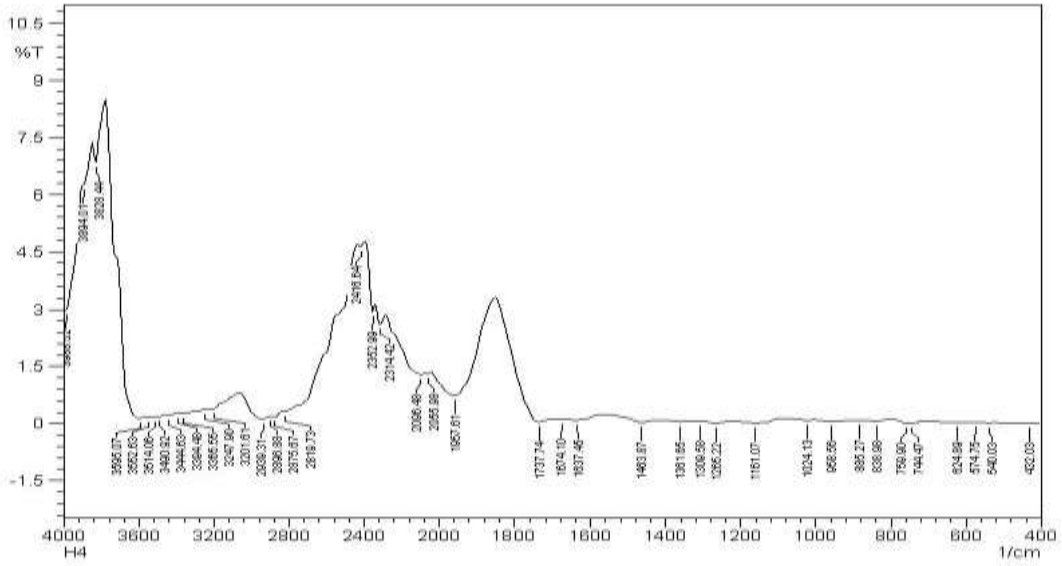


Figure 4.10 IR spectra of CLA + Tween 80

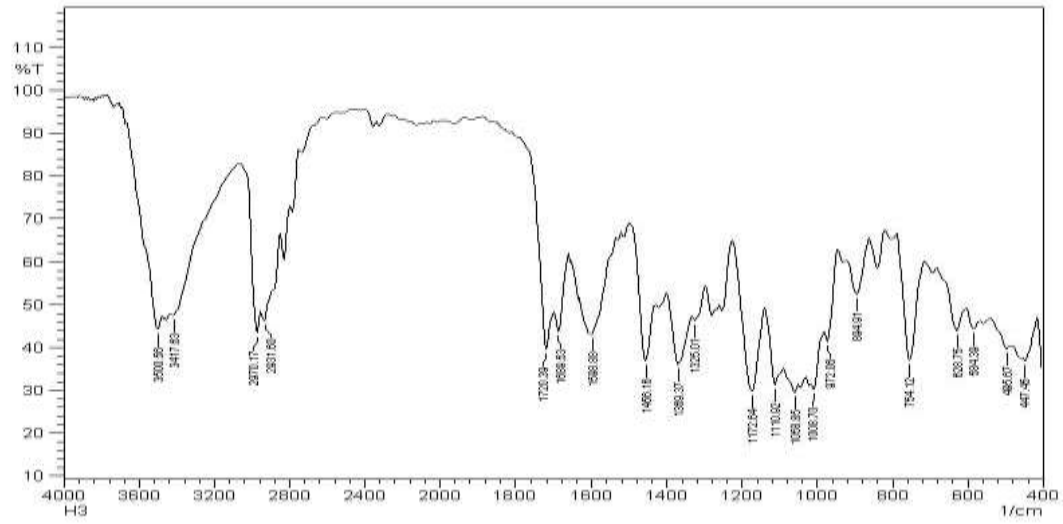


Figure 4.11 IR spectra of CLA + Transcutol P

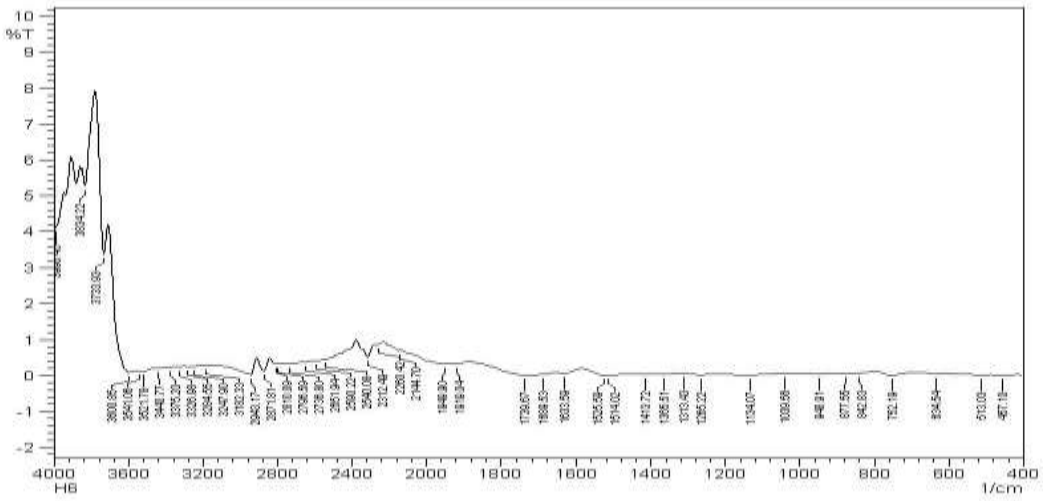


Figure 4.12 IR spectra of CLA + stearic acid + tween 80 + Transcutol P

Conclusion:

IR spectra of CLA, lipids, surfactants and their combination showed peak at specific frequency based on the functional group present. So it can be concluded that there was no interaction between drug and excipients.

4.6 SOLUBILITY STUDIES:

The solubility of CLA in various solid lipids and surfactants were studied and screened in which CLA showed maximum solubility in lipid and minimum solubility in surfactant were selected for formulation.

4.6.1 Solubility of drug in lipid

Solubility of drug in lipid is necessary for the preparation of SLNs. Lipid in which drug is most soluble has to be selected because if the drug is not soluble in the lipid then it may lead to decrease in encapsulation efficiency and drug loading. The drug may not bind to the lipid and may remain as free drug thereby not giving sustained release as desired.

4.6.1.1 Procedure of solubility study drug in Surfactants

1. 10 mg of lipid was taken in the vial and melted above its melting point in the water bath
2. 10 mg of drug was added to the melted lipid and vortexed
3. Another 10 mg of lipid was added if the drug was not soluble and added continuously until the drug was soluble

Table 4.9 Solubility of CLA in different lipids

Lipid	Amt req to dissolve the drug(mg)
Stearic acid	125
GMS	1050
compritol 888 ATO	750
Gelucire 43/01	1150
Softemul	1430
monegly T18	2170
Geleol	1560
precirol AT05	2350

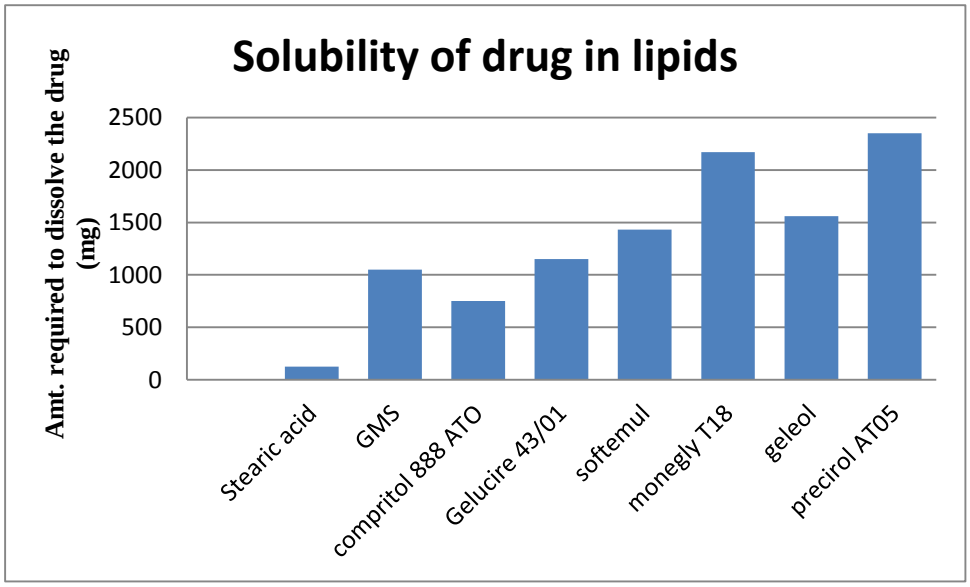


Figure 4.13 Solubility of CLA in different lipids

Conclusion: From the above data it was concluded that least amount of Stearic acid was required to solubilize CLA when compared with other lipids. Also, stearic acid acts as charge inducer as it has anionic charge.

4.6.2 Solubility of drug in Surfactants

Solubility of drugs in surfactants in important because the prepared formulation may leach out of the solid lipid and dissolve in the surfactant or the drug present on the surface of the lipid may dissolve the drug in surfactants. So the surfactant should be selected in such a way that the drug should be least soluble in the surfactants.

4.6.2.1 Procedure of solubility study drug in Surfactants

1

- 10 mg of surfactant was taken in the vial and heated above its melting point in the water bath

2

- 10 mg of drug was added to the melted lipid and vortexed

3

- Another 10 mg of surfactant was added if the drug was not soluble and added continuously untill the drug was soluble

Table 4.10 Solubility of CLA in different surfactants

Surfactants	Amt req to dissolve the drug (mg)
Tween 20	1650
Tween 80	2260
Span 80	330
Stearyl amine	1010
Propelene glycol	830
PEG-400	1260
Transcutol P	230

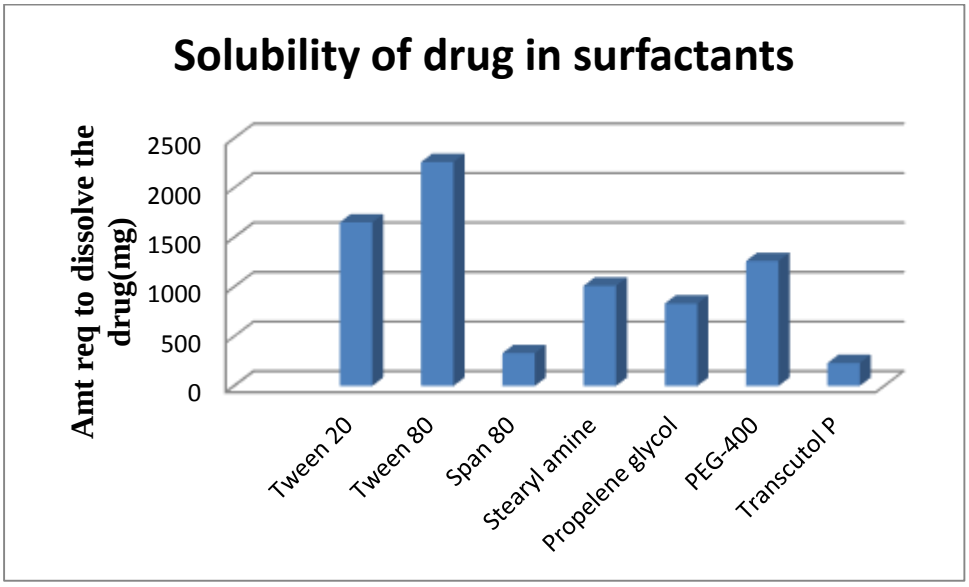


Figure 4.14 solubility of CLA in different surfactants

Conclusion: From the above study it was concluded that the drug was least soluble in tween 80. So it was selected as surfactant. Transcutol P was selected as co-surfactant.

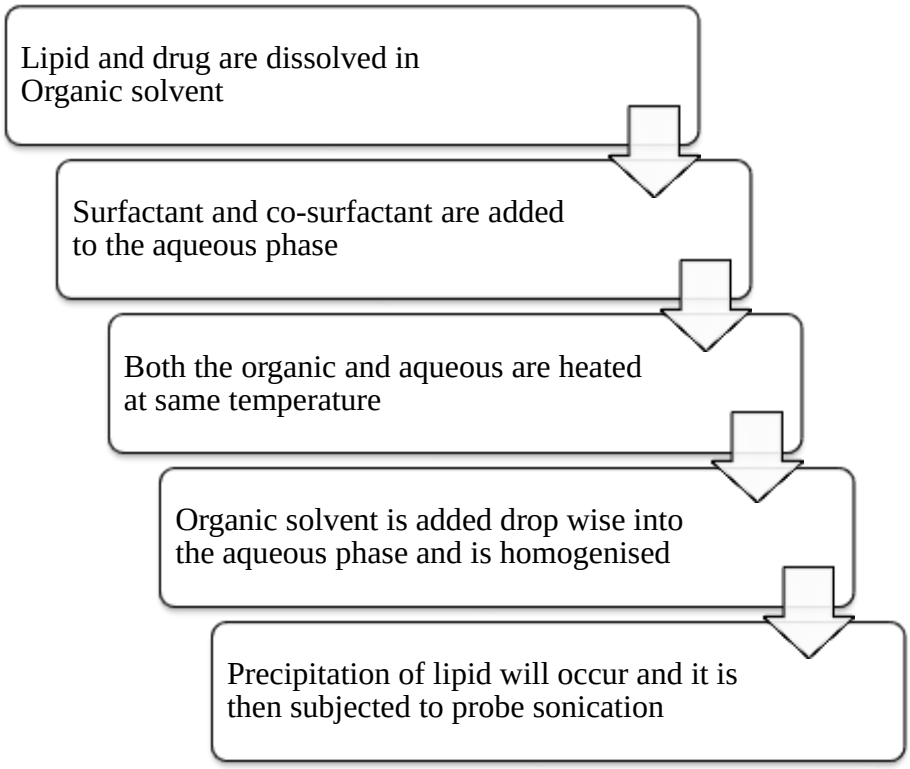
4.7 PRELIMINARY TRIALS:

4.7.1 Final selection of Excipients for preliminary trials

Table 4.11 Final selected excipients

Stearic acid	Lipid
Tween 80	Surfactant
Transcutol P	Co-Surfactant
Ethanol	Organic solvent

4.7.2 Method of preparation of SLNs:



4.7.3 Evaluation of SLNs

1) Method for finding Entrapment efficiency and drug loading:

- 2 ml of the formulation was filled in the micro-centrifuge tube.
- It was centrifuged at 15000 rpm for 30 minutes to separate the supernatant layer and the pellet
- The supernatant layer was discarded
- The pellet was dissolved with methanol and the volume was made up to 10 ml with methanol
- The contents were measured by UV spectrophotometer to obtain the absorbance
- The concentration of drug was found out and Entrapment efficiency and Drug loading were calculated.

$$\text{Entrapment efficiency} = \frac{\text{Initial drug weight - free drug weight}}{\text{Initial drug weight}} \times 100$$

$$\text{Drug loading} = \frac{\text{Initial weight - free drug weight}}{\text{weight of lipid}} \times 100$$

2) Particle size and zeta-potential: Particle size and zeta-potential were measured by Malvern zetasizer

3) In-vitro drug release: It was performed in franz- diffusion cell.

- Phosphate buffer 7.4 was filled in the receptor compartment
- 1% SLS was added to the buffer as the drug was not soluble in the buffer
- SLNs formulation was filled in the donor compartment.
- The donor and the receptor compartment are separated by the semi-permeable membrane which was previously dipped overnight in PBS 7.4
- At every hour samples were withdrawn and equal amount of fresh buffer was added to maintain the sink condition
- Samples were analyzed by UV spectrophotometer.

After the selection of stearic acid as lipid, Tween 80 as surfactant and Transcutol P as co-surfactant batches were taken for the selection of Surfactant : co-surfactant ratio.

Different batches were taken for 1:9, 3:7, 5:5, 7:3, 4:6, 2:8, and 9:1 ratio of surfactant and co-surfactant

Based on the solubility study of drug in lipid, different amount of lipid selected were 150 mg, 200 mg and 250 mg.

Concentration of surfactants selected were 1%, 1.5% and 2%

Homogenization speed was kept constant at 5000 rpm

Probe Sonication time was selected as 5 mins.

4.7.2 Trials for 3:7 surfactant ratio

Different batches were taken for 3:7 surfactant ratio. Different amount of lipid taken was 150mg, 200mg and 250mg. Different concentration of surfactant and co-surfactant taken was 1%, 1.5% and 2%.

Table 4.12 Trials for 3:7 surfactant ratio

BATCH NO.	STEARIC ACID(mg)	TRANSCUTOL P (ml)	TWEEN 80 (ml)	WATER (ml)	ETHANOL (ml)
F1	150	0.14	0.06	20	5
F2	200	0.21	0.09	20	5
F3	250	0.28	0.12	20	5
F4	150	0.14	0.06	20	5
F5	200	0.21	0.09	20	5
F6	250	0.28	0.12	20	5
F7	150	0.14	0.06	20	5
F8	200	0.21	0.09	20	5
F9	250	0.28	0.12	20	5

Evaluation for 3:7 surfactant ratio

Table 4.13 Results of %EE, %DL and PS

% Entrapment efficiency	% Drug loading	Particle Size
85.9	28.63	555.8
77.8	19.45	
60.8	12.16	
78.5	26.16	
45.2	11.3	671.6
60.8	12.16	
91.6	21.97	
89.6	19.58	
97.9	19.48	643.9

Conclusion:

From the above results it was found that entrapment efficiency and drug loading was more in the batches having 150 mg of lipid. Particle size was obtained in the range of 500 to 700nm.

4.7.3 Trials for 5:5 surfactant ratio

Table 4.14 Trials for 5:5 surfactant ratio

BATCH NO.	STEARIC ACID(mg)	TRANSCUTOL P (ml)	TWEEN 80 (ml)	WATER (ml)	ETHANOL (ml)
F10	150	0.1	0.1	20	5
F11	200	0.15	0.15	20	5
F12	250	0.2	0.2	20	5
F13	150	0.1	0.1	20	5
F14	200	0.15	0.15	20	5
F15	250	0.2	0.2	20	5
F16	150	0.1	0.1	20	5
F17	200	0.15	0.15	20	5
F18	250	0.2	0.2	20	5

Evaluation for 5:5 surfactant ratio

Table 4.15 Results of %EE, %DL and PS of 5:5 surfactant ratio

% Entrapment efficiency	% Drug loading	Particle size
89.3	29.76	559
91.2	22.8	
81.5	16.3	526
98.9	32.96	
97.9	24.47	
92.7	20.68	
94.3	31.43	
96.5	27.57	
98.4	21.44	598.4

Conclusion:

From the above results it was found that % EE of was more when 200 mg of lipid was used. The Drug loading was more when 150 mg of lipid was used. Particle size was found to be in the range of 500 to 600 nm.

4.7.4 Trials for 4:6 surfactant ratio

Table 4.16 Trials for 6:4 surfactant ratio

BATCH NO.	STEARIC ACID(mg)	TRANSCUTOL P (ml)	TWEEN 80 (ml)	WATER (ml)	ETHANOL (ml)
F19	150	0.12	0.08	20	5
F20	200	0.18	0.12	20	5
F21	250	0.24	0.16	20	5
F22	150	0.12	0.08	20	5
F23	200	0.18	0.12	20	5
F24	250	0.24	0.16	20	5
F25	150	0.12	0.08	20	5
F26	200	0.18	0.12	20	5
F27	250	0.24	0.16	20	5

Evaluation for 4:6 surfactant ratio

Table 4.17 Results of %EE, %DL and PS of 5:5 surfactant ratio

% Entrapment efficiency	% Drug loading	Particle size(nm)
84.8	28.28	671.6
82.2	27.4	
78.6	26.2	
87.1	21.77	
87.6	21.9	
82.8	20.7	643.9
77.1	15.42	
86.6	17.32	
81.4	16.28	754.9

Conclusion:

It was concluded that %EE and %DL of 150 mg lipd was found to be high. The particle size was in the range of 600 to 800 nm. It was also found that 250 mg of lipid did not give good %EE and %DL so it was not used for further trials

4.7.5 Trials for 2:8 surfactant ratio

Table 4.16 Trials for 8:2 surfactant ratio

BATCH NO.	STEARIC ACID(MG)	TRANSCUTOL P (ML)	TWEEN 80 (ML)	WATER	ETHANOL
F28	150	0.16	0.04	20	5
F29	150	0.24	0.06	20	5
F30	150	0.32	0.08	20	5
F31	200	0.16	0.04	20	5
F32	200	0.24	0.06	20	5
F33	200	0.32	0.08	20	5

Evaluation for 2:8 surfactant ratio

Table 4.19 **Results of %EE, %DL and PS of 4:6 surfactant ratio**

% Entrapment efficiency	% Drug loading	Particle size
61.8	17.8	1038
76.4	16.6	2095
52.1	16.1	
81.5	13.2	
75.7	11.7	
84.6	15.2	2353

Conclusion:

It was found the 2:8 surfactant ratio was not found to be stable as the particle size was more and %EE and %DL were less.

4.8 Screening Design:

Screening designs are used to screen the main significant effects from the many potential factors which can affect the formulation. There are two types of screening design: Fractional factorial design and Plackett Burman design.

Fractional factorial design is used when the number of factors are five or more. Total number of runs can be calculated by 2^{n-1} where, n= no. of factors

Fractional factorial screening design was applied for screening of the factors. Total five factors were selected which may have significant effect on SLNs formulation. So, the total number of runs was 2^{5-1}=16.

The factors which were selected for screening are:

Process Variables:

1) Homogenization speed
2) Sonication time

Formulation variables:

1) Drug-lipid ratio
2) Surfactant concentration
3) Surfactant ratio

Table 4.20 coded and values of Independent variables

Independent Variable	Levels	
	+1	-1
A: Homogenization speed (rpm)	12000	6000
B: Sonication time (mins)	10	5
C: Drug-lipid ratio (mg)	1:4	1:3
D: Surfactant ratio	7:3	5:5
E: Surfactant concentration(%)	3%	1%

Table 4.21 Dependent variable Fractional factorial design

Dependent Variables	R1: Particle size R2: % Entrapment Efficiency R3: % Drug loading

Table 4.22 list of coded value of Fractional factorial design

Batch no.	Coded value				
	A	B	C	D	E
1	1	-1	1	-1	1
2	-1	1	1	-1	1
3	1	1	1	1	1
4	-1	1	-1	-1	-1
5	-1	-1	1	1	1
6	1	-1	-1	-1	-1
7	-1	1	1	1	-1
8	1	-1	-1	1	1
9	1	1	-1	-1	1
10	-1	-1	-1	1	-1
11	-1	-1	-1	-1	1
12	1	-1	1	1	-1
13	-1	-1	1	-1	-1
14	1	1	1	-1	-1
15	-1	1	-1	1	1
16	1	1	-1	1	-1

Table 4.23 list of actual values

Batch no.	Actual values				
	A	B	C	D	E
1	12000	5	200	5:5	3
2	6000	10	200	5:5	3
3	12000	10	200	3:7	3
4	6000	10	150	5:5	1
5	6000	5	200	3:7	3
6	12000	5	150	5:5	1
7	6000	10	200	3:7	1
8	12000	5	150	3:7	3
9	12000	10	150	5:5	3
10	6000	5	150	3:7	1
11	6000	5	150	5:5	3
12	12000	5	200	3:7	1
13	6000	5	200	5:5	1
14	12000	10	200	5:5	1
15	6000	10	150	3:7	3
16	12000	10	150	3:7	1

Table 4.24 Values of dependent variables

Batch no.	Dependent variable		
	Particle size	**% EE**	**%DL**
1	115	84.9	21.22
2	357.4	69.9	17.47
3	333.6	72.4	18.1
4	276.3	79.1	26.36
5	112.8	85.8	21.45
6	123.6	92.4	30.8
7	369.3	72.5	18.12
8	112.6	87.9	29.3
9	413	49.9	15.96
10	134.1	87.3	26.86
11	119.2	83.7	27.9
12	129.7	85.7	21.42
13	124.2	84.5	21.12
14	396.8	75.2	18.85
15	284.4	77.1	25.7
16	396.8	74.9	24.96

Evaluation of batches:

A) Effect on particle size

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.264E+005	5	45283.77	28.48	< 0.0001	significant
A-Homoginiz	*2926.81*	*1*	*2926.81*	*1.84*	*0.2047*	
B-Sonication	*2.217E+005*	*1*	*2.217E+005*	*139.43*	*< 0.0001*	
C-Drug-lipid	*699.60*	*1*	*699.60*	*0.44*	*0.5221*	
D-Surfactant	*39.69*	*1*	*39.69*	*0.025*	*0.8776*	
E-Surfactant	*1053.00*	*1*	*1053.00*	*0.66*	*0.4347*	
Residual	15900.25	10	1590.02			
Cor Total	2.423E+005	15				

Figure 4.15 Anova of effect of particle size of fractional factorial design

Conclusion: The model was found to be significant and Sonication time had significant effect as the f- value was less than 0.05.

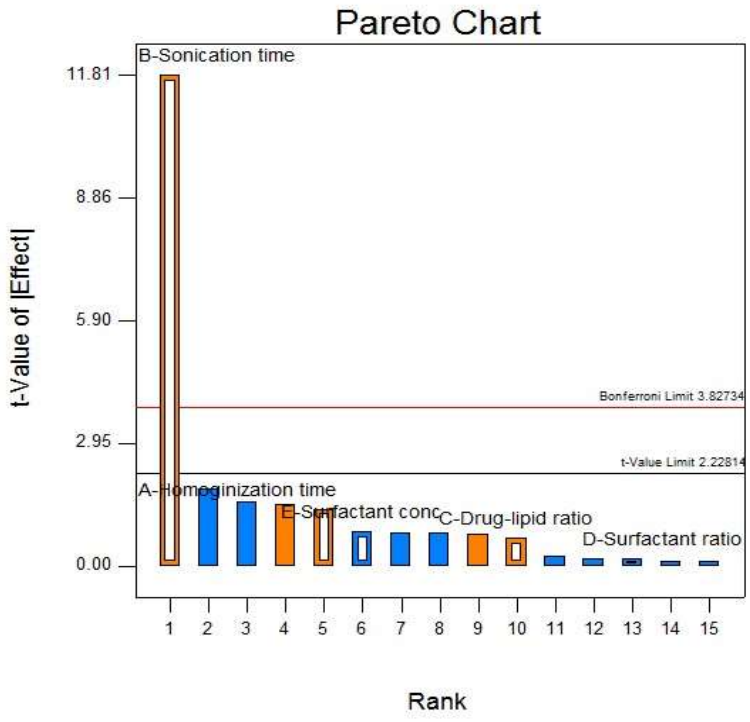


Figure 4.16 pareto chart of effect on particle size

Conclusion:
It was concluded from the pareto chart that t-value of Sonication time was above the limit so it had significant impact on the formulation.

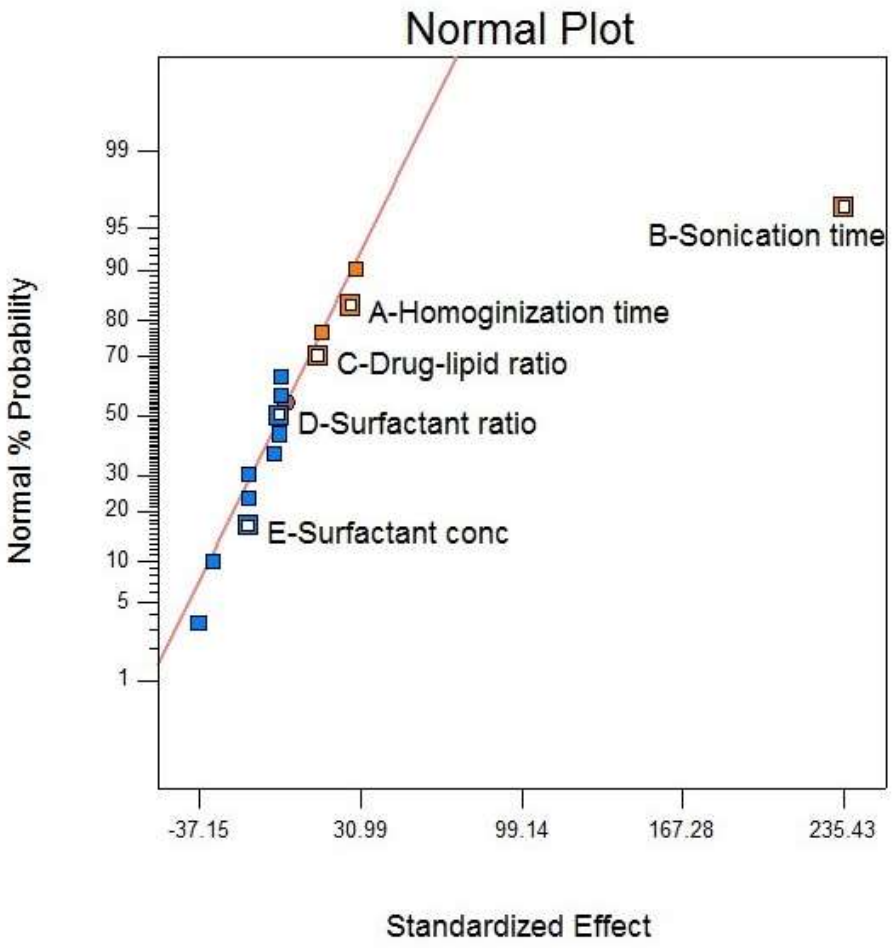


Figure 4.17 Normal plot of effect on particle size

Conclusion:

From the normal plot of effect on particle size it was concluded that Sonication time had most deviation from the center line

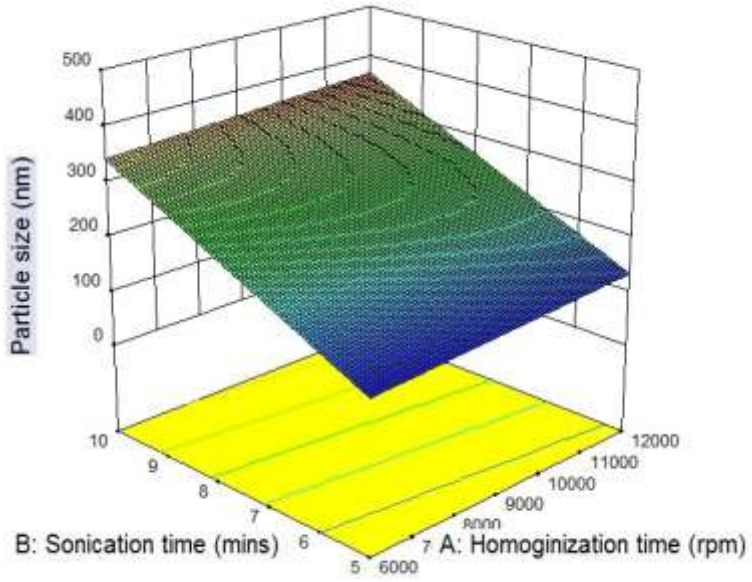


Figure 4.18 3D surface plot of effect on particle size of fractional factorial design

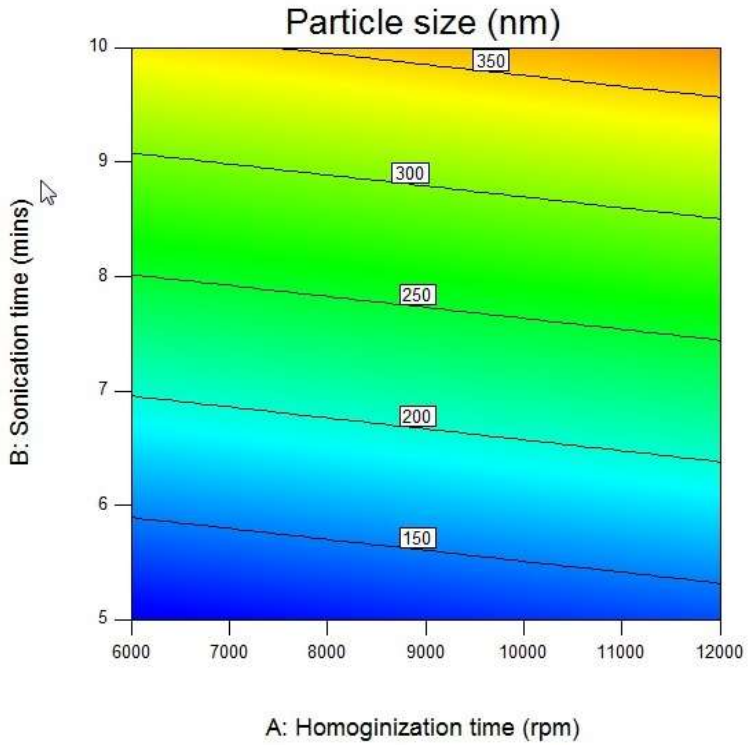


Figure 4.19 contour plot of effect on particle size of fractional factorial design

Conclusion:

From contour plot and 3D response surface plot it can found that Sonication time has significant effect on the formulation whereas homogenization time did not have significant effect

b) Effect of Entrapment efficienc

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1122.78	5	224.56	4.06	0.0284	significant
A-Homoginiz	*21.62*	*1*	*21.62*	*0.39*	*0.5459*	
B-Sonication	*948.64*	*1*	*948.64*	*17.14*	*0.0020*	
C-Drug-lipid	*0.022*	*1*	*0.022*	*4.066E-004*	*0.9843*	
D-Surfactant	*42.25*	*1*	*42.25*	*0.76*	*0.4027*	
E-Surfactant	*110.25*	*1*	*110.25*	*1.99*	*0.1884*	
Residual	553.37	10	55.34			
Cor Total	1676.15	15				

Figure 4.20 Anova of %EE of fractional factorial design

Conclusion:

From the Anova table it was the model was found to be significant. The f- value of the factor sonication time was less than 0.05 so it had significant effect on the formulation.

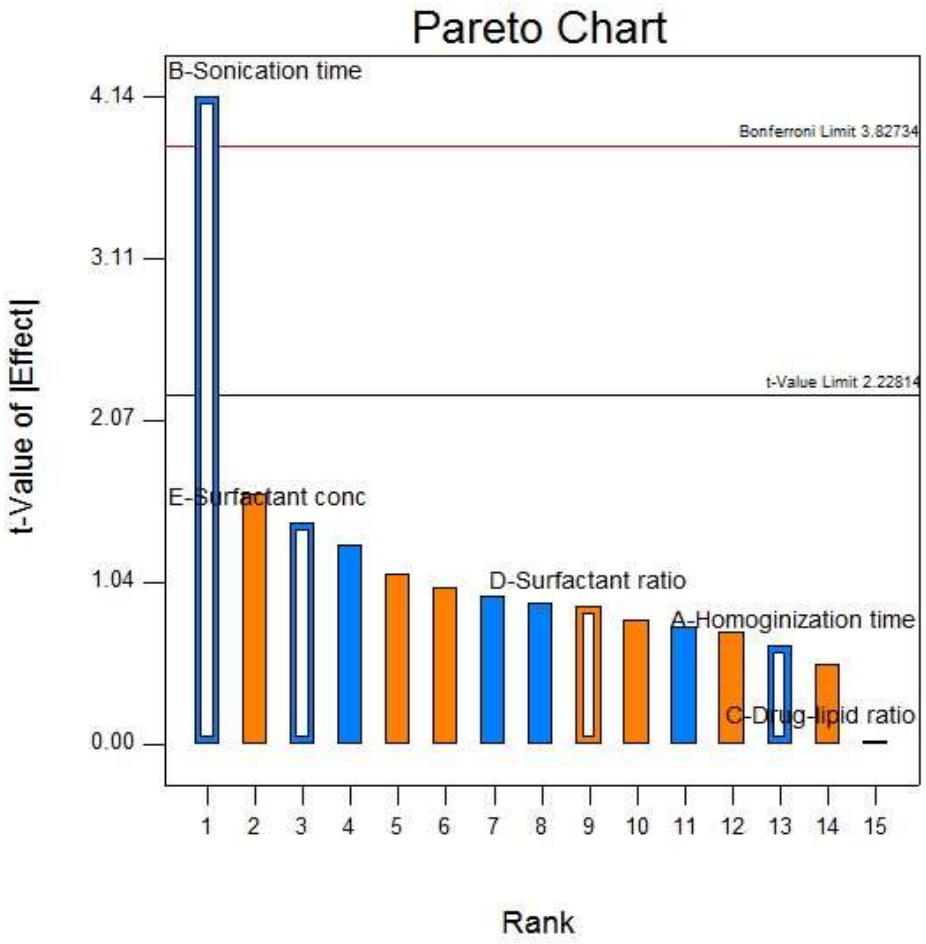


Figure 4.21 Pareto chart of %EE

Conclusion:

From the pareto chart of effect on entrapment efficiency it was concluded that the Sonication time had significant impact on the entrapment efficiency.

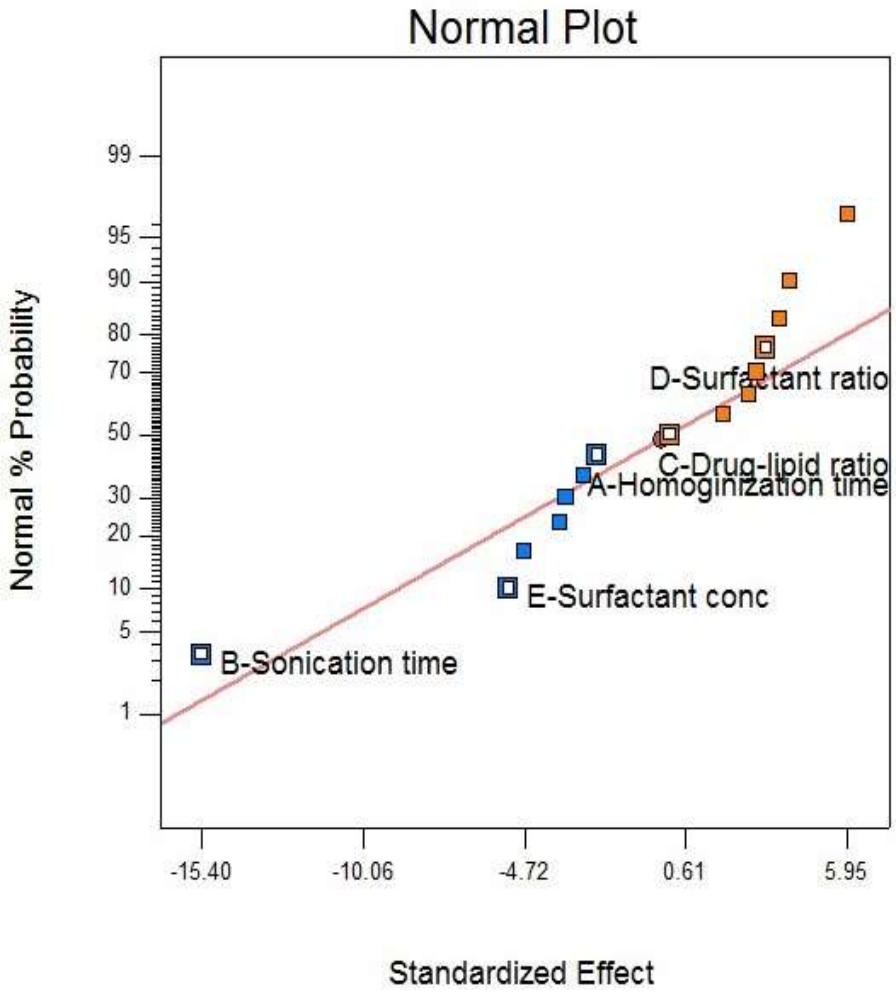


Figure 4.22 Normal plot of %EE

Conclusion:

From the normal plot it was found that Sonication time had effect on entrapment efficiency as it deviates from the line.

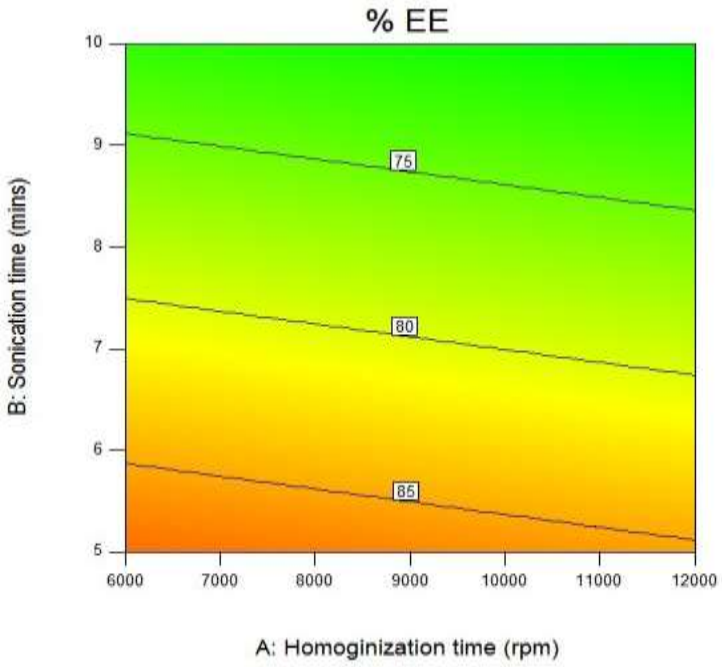


Figure 4.23 Contour plot of %EE of fraction factorial design

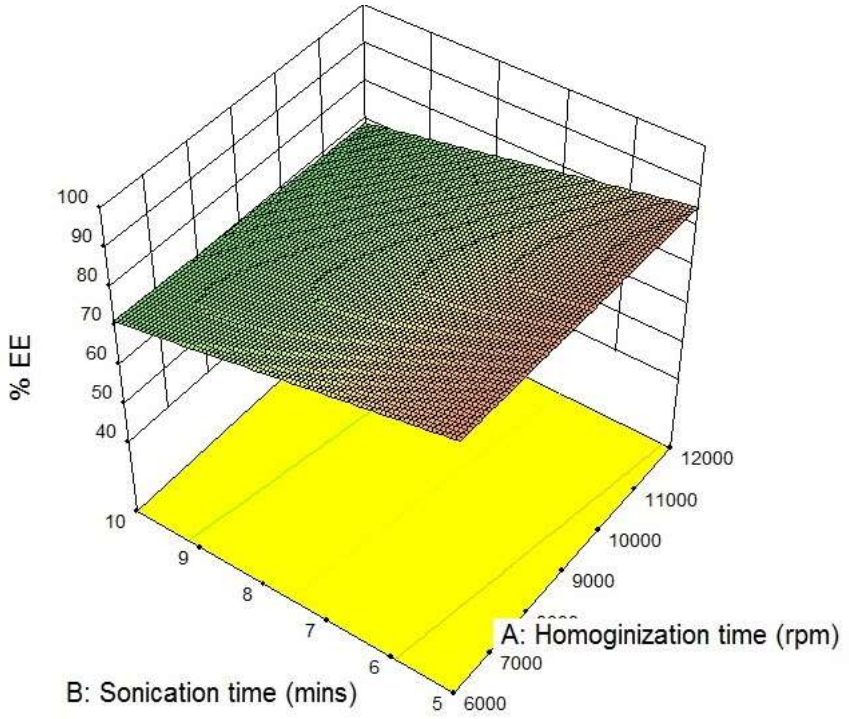


Figure 4.24 3D surface plot of %EE of fractional factorial design

Conclusion: From the contour plot and 3D surface response plot it was found that homogenization time did not have any significant effect on entrapment efficiency whereas Sonication time had impact on it.

c) Effect on Drug loading

Response 3 %DL

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	243.14	5	48.63	6.47	0.0062	significant
A-Homoginiz	*1.19*	*1*	*1.19*	*0.16*	*0.6986*	
B-Sonication	*74.65*	*1*	*74.65*	*9.94*	*0.0103*	
C-Drug-lipid	*156.75*	*1*	*156.75*	*20.87*	*0.0010*	
D-Surfactant	*2.43*	*1*	*2.43*	*0.32*	*0.5824*	
E-Surfactant	*8.12*	*1*	*8.12*	*1.08*	*0.3229*	
Residual	75.12	10	7.51			
Cor Total	318.26	15				

Figure 4.25 Anova of %DL of fractional factorial design

Conclusion:

From the Anova table it was found that model was significant. F- value of Sonication time and drug – lipid ratio were less than 0.05 so it they had significant impact on the drug loading.

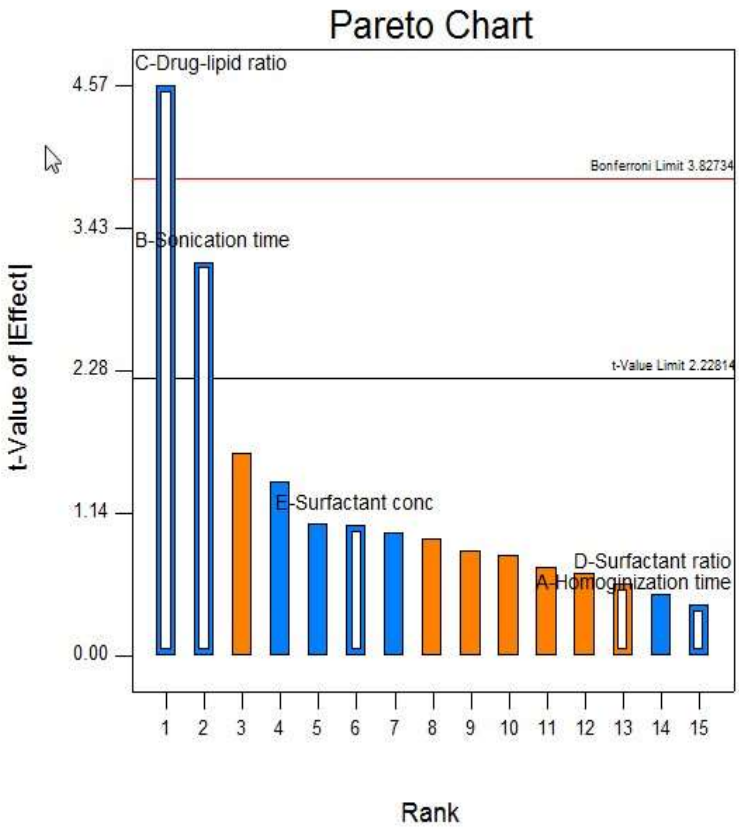


Figure 4.26 Pareto chart of %DL

Conclusion: From the Pareto chart it was concluded that Drug – lipid ratio and Sonication time had significant effect on the drug loading as the t-value was above the limit.

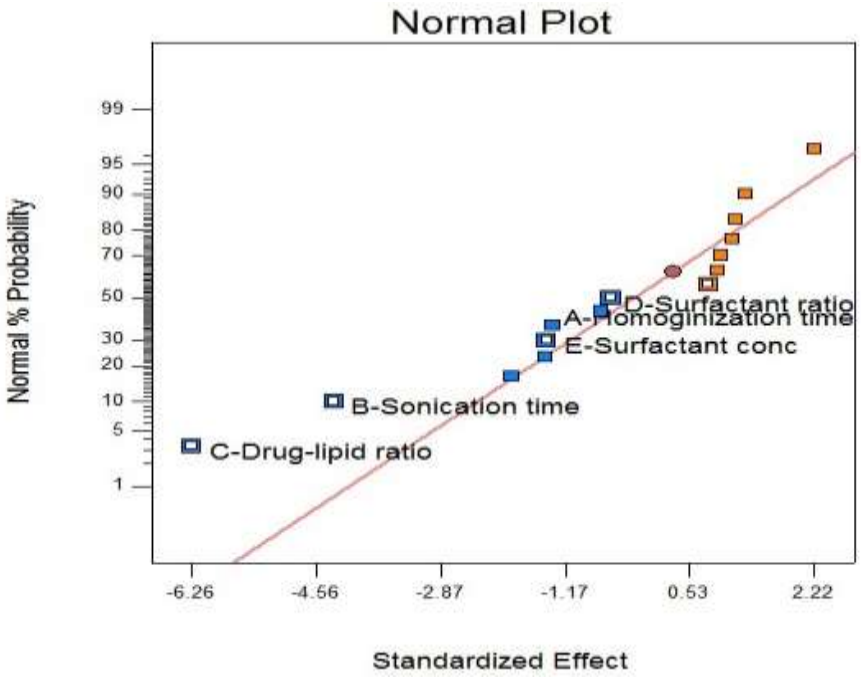


Figure 4.27 Normal plot of %DL

Conclusion:

From the Normal plot of drug loading it was found that drug – lipid ratio and Sonication time deviate from the line so they had significant effect on the drug loading.

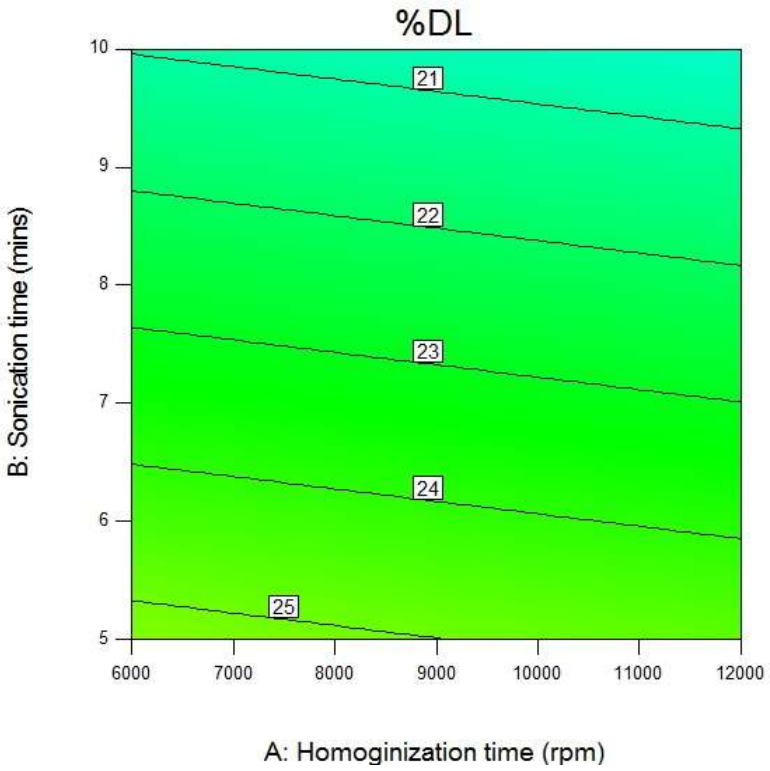


Figure 4.28 Contour plot of %DL of fractional factorial design

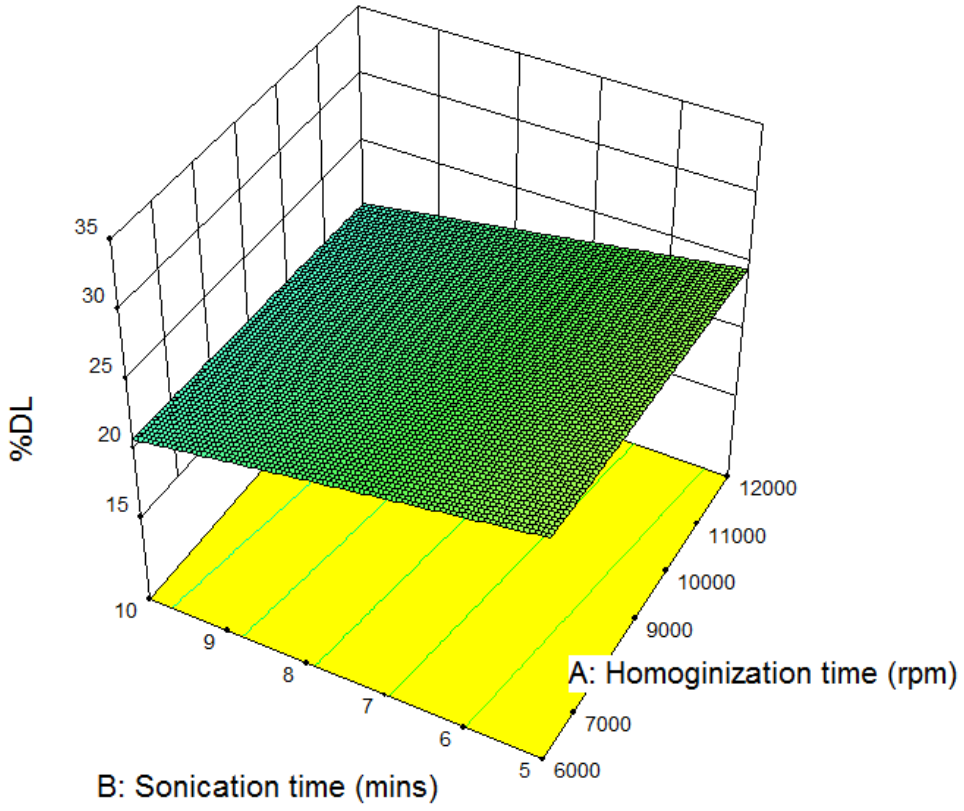


Figure 4.29 3D surface plot of %DL of fractional factorial design

Conclusion:

From the overall fractional factorial design it was found that two factors were significant i.e. Sonication time and Drug-lipid ratio. So for the further optimization the above two factors were used and 3^2 full factorial.

4.9 Optimization design

A 3^2 full factorial design was used to study the effect of independent variables on the dependent variables. Two factors which were found to be significant from the screening design were applied and 3 levels were selected. So total 9 runs were performed.

Homogenization speed, surfactant ratio and surfactant concentration were kept constant at 9000 rpm, 5:5 and 3%

Table 4.25 coded values and actual of Independent variables of full factorial design

Independent Variable	Levels		
	+1	0	-1
A: drug-lipid ratio (mg)	175	150	125
B: Sonication time (mins)	8	6	4

Table 4.26 Dependent variables of full factorial design

Dependent Variables	R1: Particle size
	R2: % Entrapment Efficiency
	R3: % Drug loading

Table 4.27 coded and actual value

Batch no.	Coded value		Actual value	
	A	B		
1	0	+1	150	8
2	-1	+1	125	8
3	+1	+1	175	8
4	+1	0	175	6
5	0	-1	150	4
6	-1	0	125	6
7	-1	-1	125	4
8	+1	-1	175	4
9	0	0	150	6

Table 4.28 Values of dependent variables

Batch no.	Coded value		Particle size Nm	% entrapment efficiency	% drug loading
	A	B			
1	0	+1	339.2	70.7	23.56
2	-1	+1	404.1	74.9	29.96
3	+1	+1	315.9	65.7	18.77
4	+1	0	162.5	79.4	22.68
5	0	-1	236.4	80.4	26.86
6	-1	0	172.9	76.9	30.76
7	-1	-1	273.6	79.6	31.84
8	+1	-1	245.2	81.9	23.4
9	0	0	154.1	87.2	29.06

Evaluation of the optimization design

a) Effect of particle size

ANOVA for Response Surface Quadratic model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	58488.85	5	11697.77	44.03	0.0052	significant
A-drug-lipid r	*2688.17*	*1*	*2688.17*	*10.12*	*0.0501*	
B-sonication	*15402.67*	*1*	*15402.67*	*57.97*	*0.0047*	
AB	*894.01*	*1*	*894.01*	*3.37*	*0.1639*	
A^2	*732.17*	*1*	*732.17*	*2.76*	*0.1955*	
B^2	*38771.84*	*1*	*38771.84*	*145.94*	*0.0012*	
Residual	797.03	3	265.68			
Cor Total	59285.89	8				

Figure 4.30 Anova of PS of full factorial design

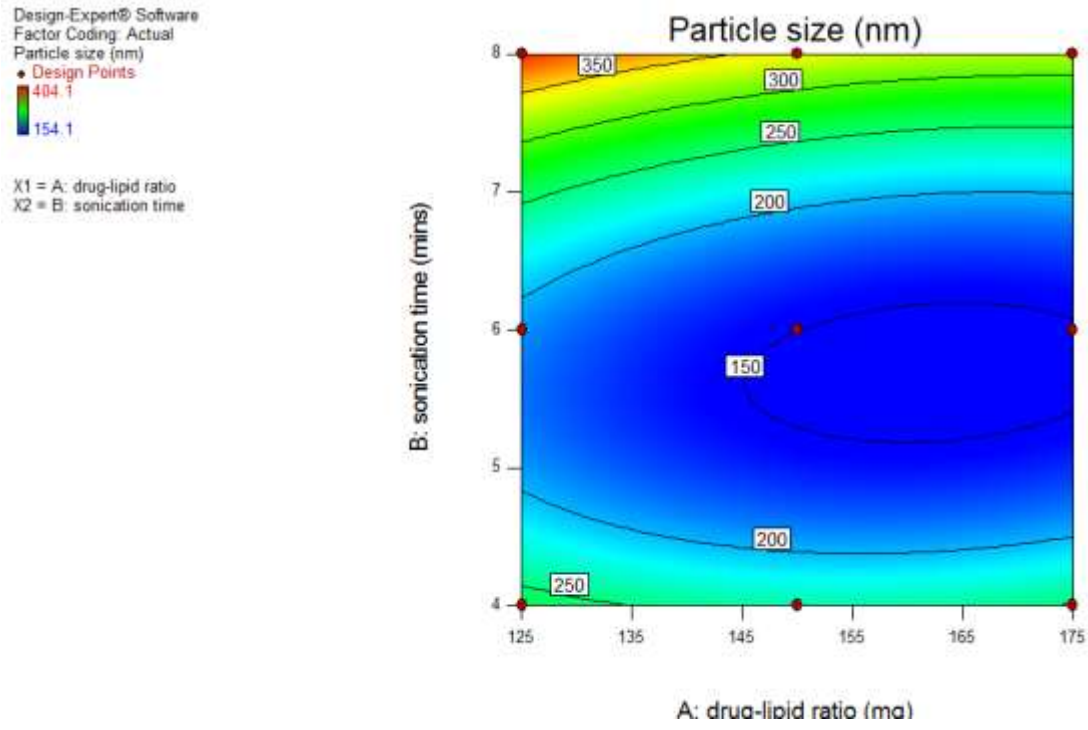


Figure 4.31 contour plot of effect on particle size of full factorial design

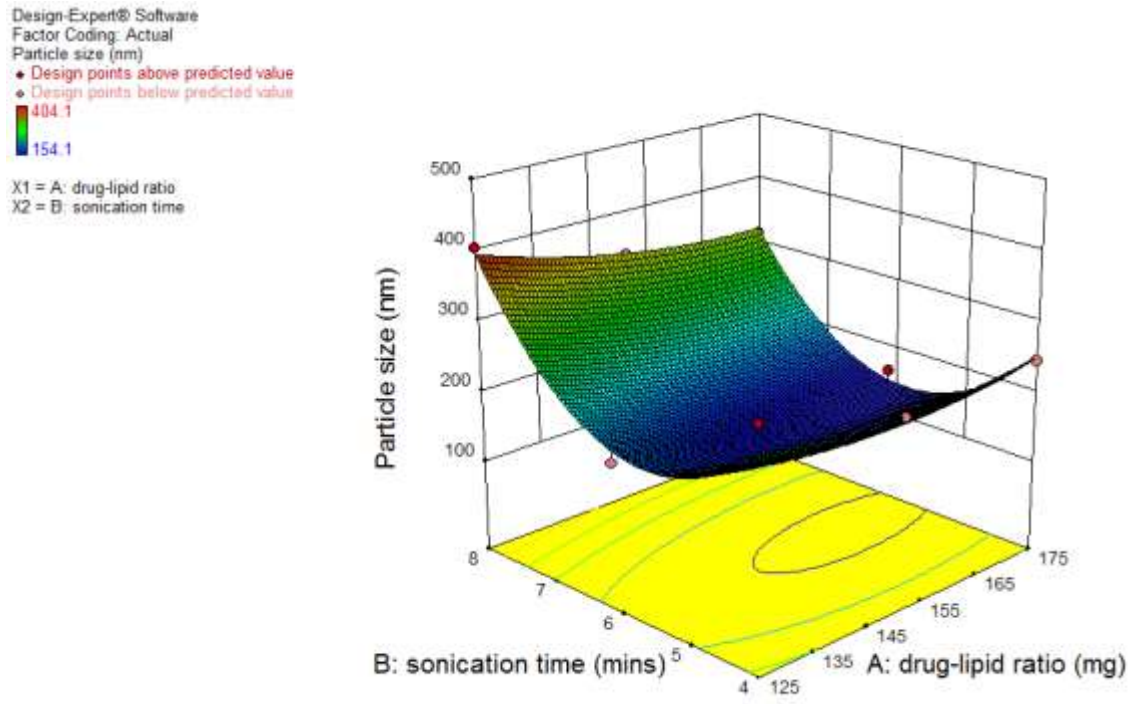


Figure 4.32 3D surface plot of PS of full factorial design

Polynomial Equation:

Particle size= +150.41- 21.17*A+ 50.67*B- 14.95*AB+ 19.13*A^2+ 139.23*B^2

Interpretation:

From the contour plot and 3D surface plot it can be seen that the most of the particle size are in smaller range. From the Anova table it was seen that Sonication time had significant effect on the particle size. From the polynomial equation it was found that drug-lipid ratio had negative effect on particle size whereas Sonication time had positive effect on particle size.

B) Effect of entrapment efficiency:

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	274.22	5	54.84	3.41	0.1705	not significant
A-drug-lipid r	*3.23*	*1*	*3.23*	*0.20*	*0.6844*	
B-sonication	*156.06*	*1*	*156.06*	*9.72*	*0.0526*	
AB	*33.06*	*1*	*33.06*	*2.06*	*0.2469*	
A^2	*18.40*	*1*	*18.40*	*1.15*	*0.3629*	
B^2	*63.47*	*1*	*63.47*	*3.95*	*0.1410*	
Residual	48.19	3	16.06			
Cor Total	322.41	8				

Figure 4.33 Anova of %EE of full factorial design

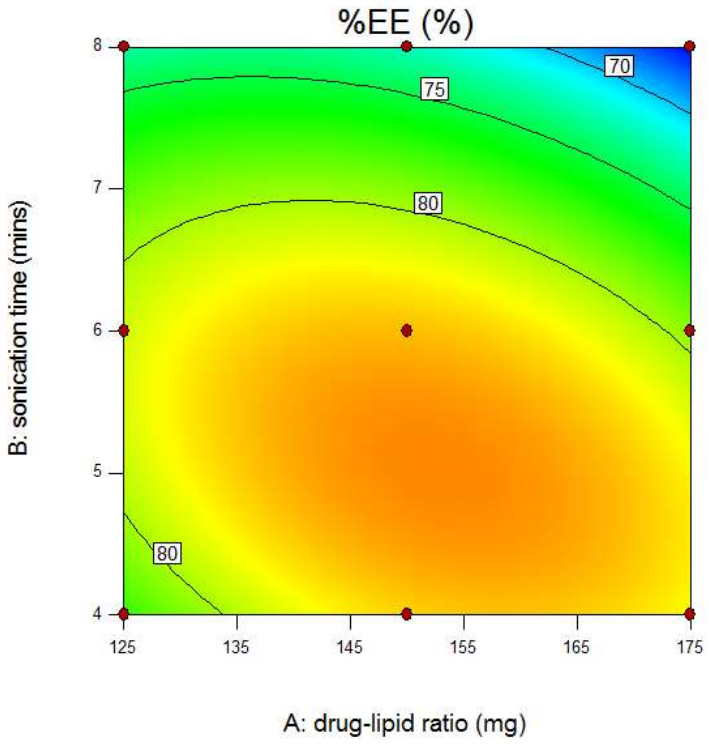


Figure 4.34 contour plot of effect on %EE of full factorial design

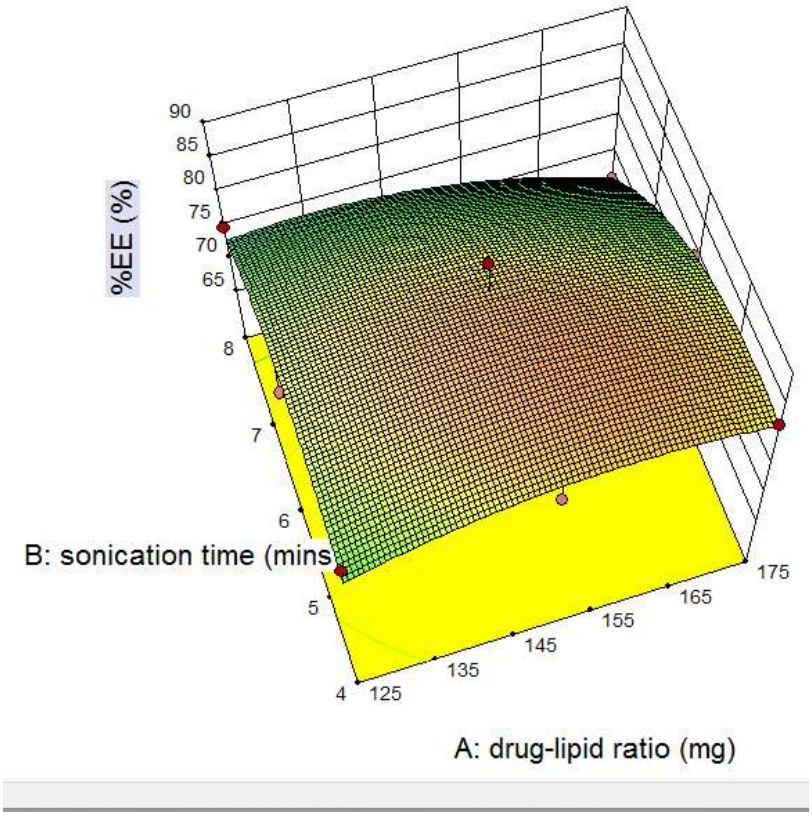


Figure 4.35 3D surface plot of %EE of full factorial design

Polynomial Equation of % Entrapment efficiency:

$\%EE = +83.19 - 0.73*A - 5.10*B - 2.87*AB - 3.03*A^2 - 5.63*B^2$

Interpretation:

From the contour plot and 3D surface response it was interpreted that Entrapment efficiency was more when the Sonication time was less and drug-lipid ratio was near the center point. From the polynomial equation it was observed that drug lipid ratio and Sonication time both had negative effects

C) Effect on % Drug loading

ANOVA for Response Surface Linear model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	143.94	2	71.97	31.68	0.0006	significant
A-drug-lipid r	*127.91*	*1*	*127.91*	*56.31*	*0.0003*	
B-sonication	*16.03*	*1*	*16.03*	*7.06*	*0.0377*	
Residual	13.63	6	2.27			
Cor Total	157.57	8				

Figure 4.36 Anova of %DL of full factorial design

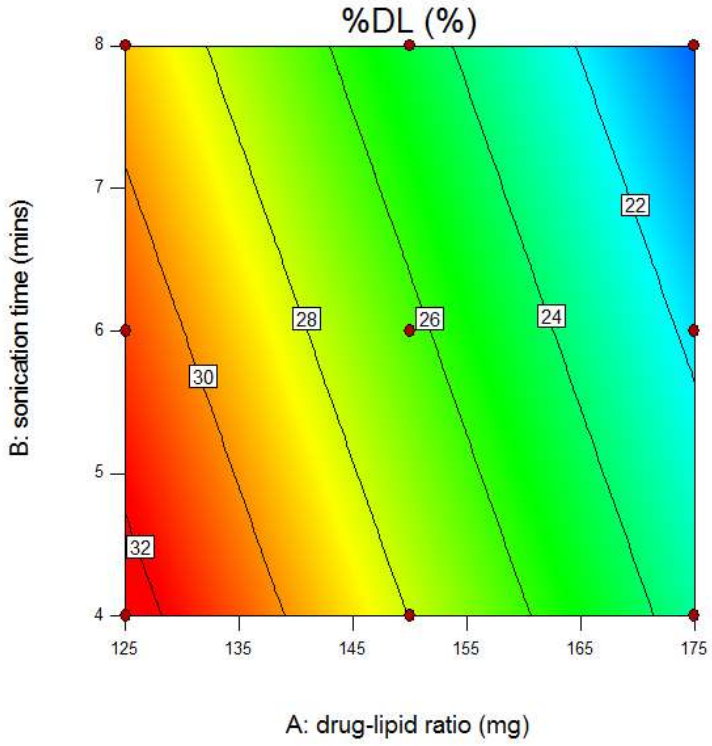


Figure 4.37 contour plot of effect on %DL of full factorial design

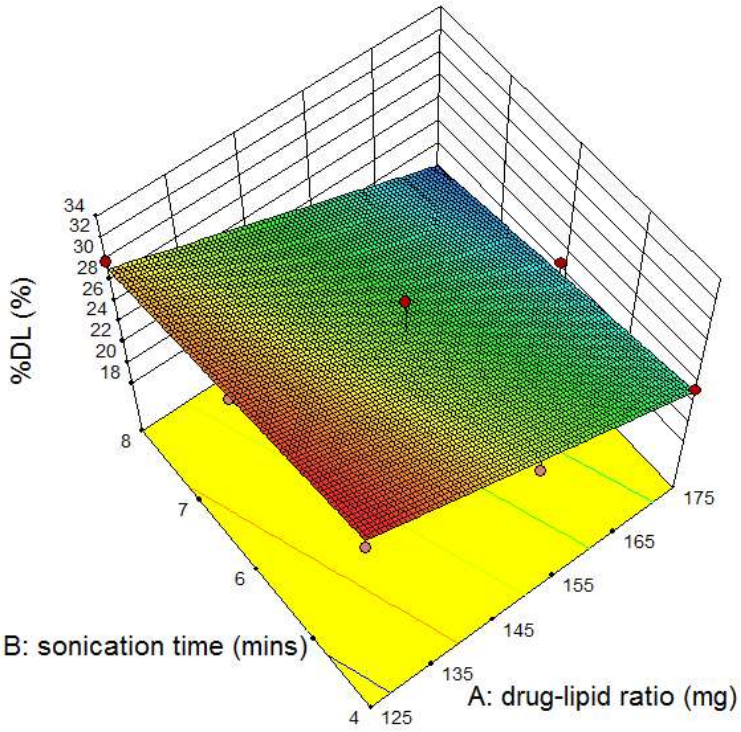


Figure 4.38 3D surface plot of %DL of full factorial design

Polynomial equation:

% drug loading= +26.32- 4.62*A- 1.63*B

Interpretation:

From the contour plot and 3D surface response plot it was observed that drug loading increases as the drug-lipid ratio and Sonication time decreases. From the Anova table both factors were found to be significant as the f- value was less than 0.05. From the polynomial equation it was interpreted that both factor had negative effect on the entrapment efficiency.

In-vitro diffusion:

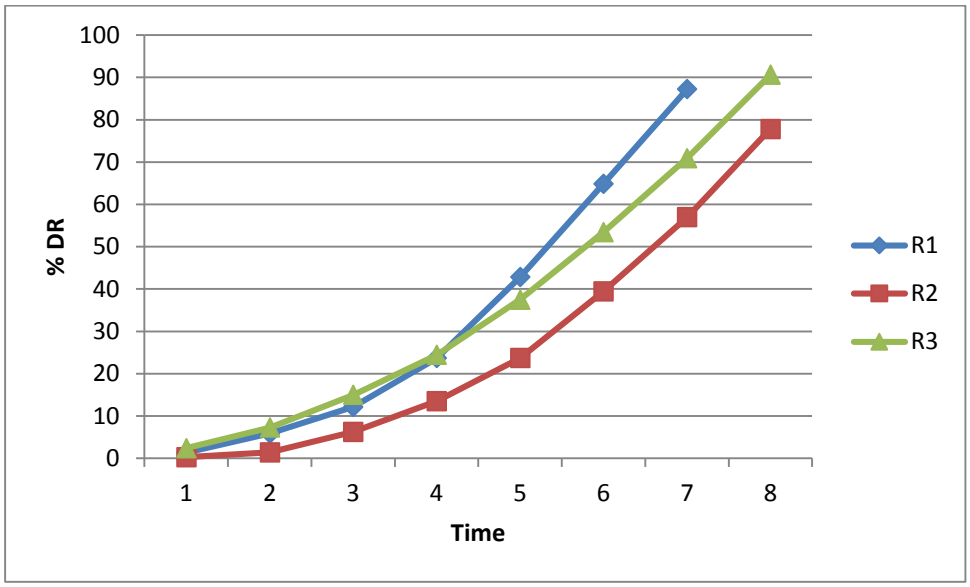


Figure In-vitro release of R1,R2 and R3

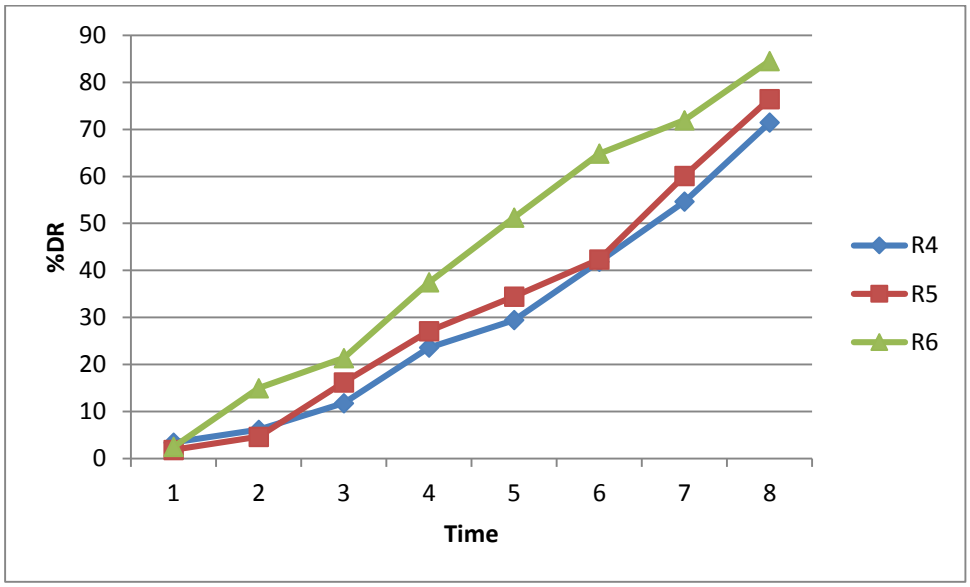


Figure In-vitro release of R4,R5 and R6

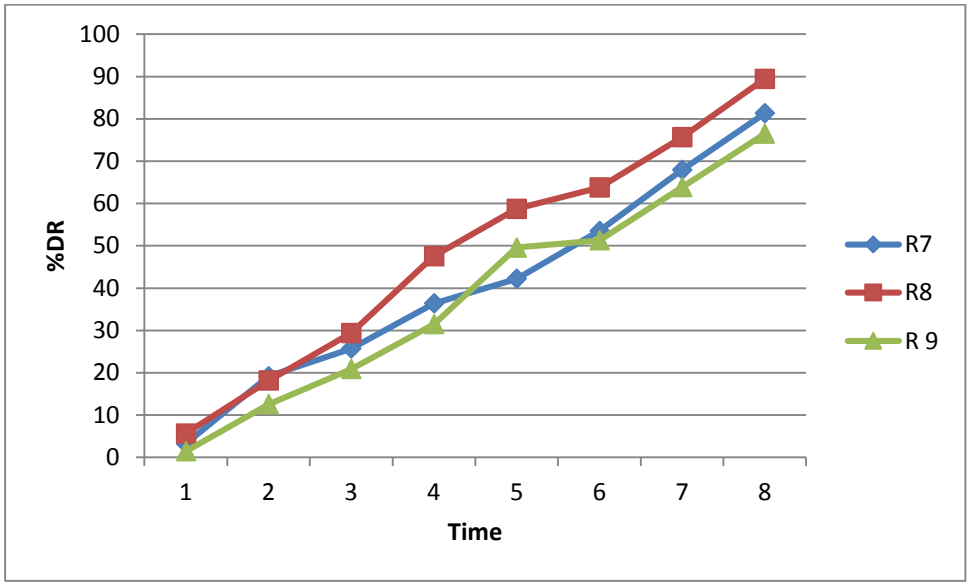


Figure In-vitro release of R7,R8 and R9

Overlay plot:

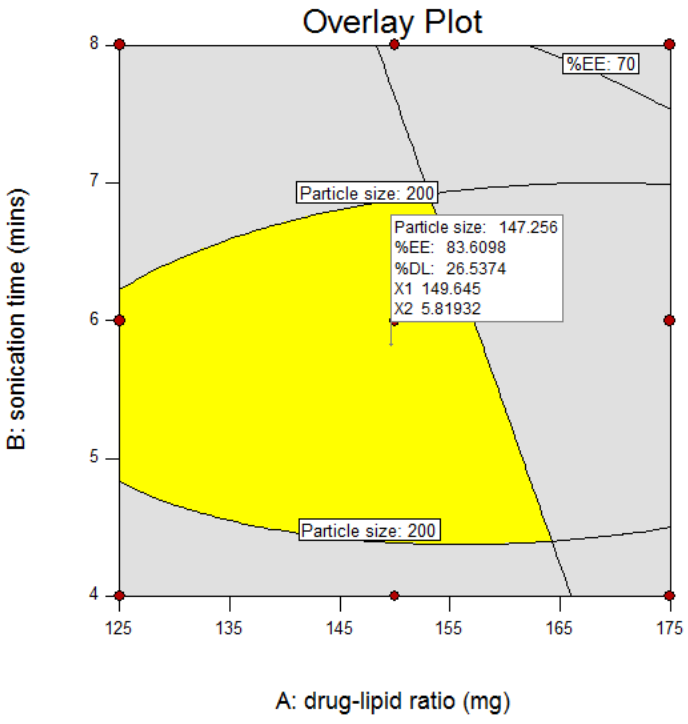


Figure 4.39 Design space of full factorial design

Design space was obtained by selecting the required range for each dependent variable to get the optimized batch. Optimized batch was selected by adding flags to the design space the point of design which showed the best response was selected as the optimized batch and it was further evaluated.

Formulation of optimized batch (S1):

Table 4.29 formulation of optimize batch

Ingredients	Quantity
Stearic acid	149.64 mg
Tween 80	0.3 ml
Transcutol P	0.3 ml
Water	20 ml
Ethanol	5 ml

The prepared batch was homogenized at 9000 rpm and sonicated for 5.8 mins.

Evaluation of optimized batch:

Table 4.30 evaluation of optimized batch

Parameter	Predicted value	Observed value
Entrapment efficiency	83.60	81.3
Drug loading	26.53	27.1
Particle size	147.256	157.2
Zeta potential	-	-17.23
In-vitro drug release	-	89.42

Conclusion:

It was concluded that observed value of optimized batch was almost similar to the predicted value.

TEM Images of optimized batch

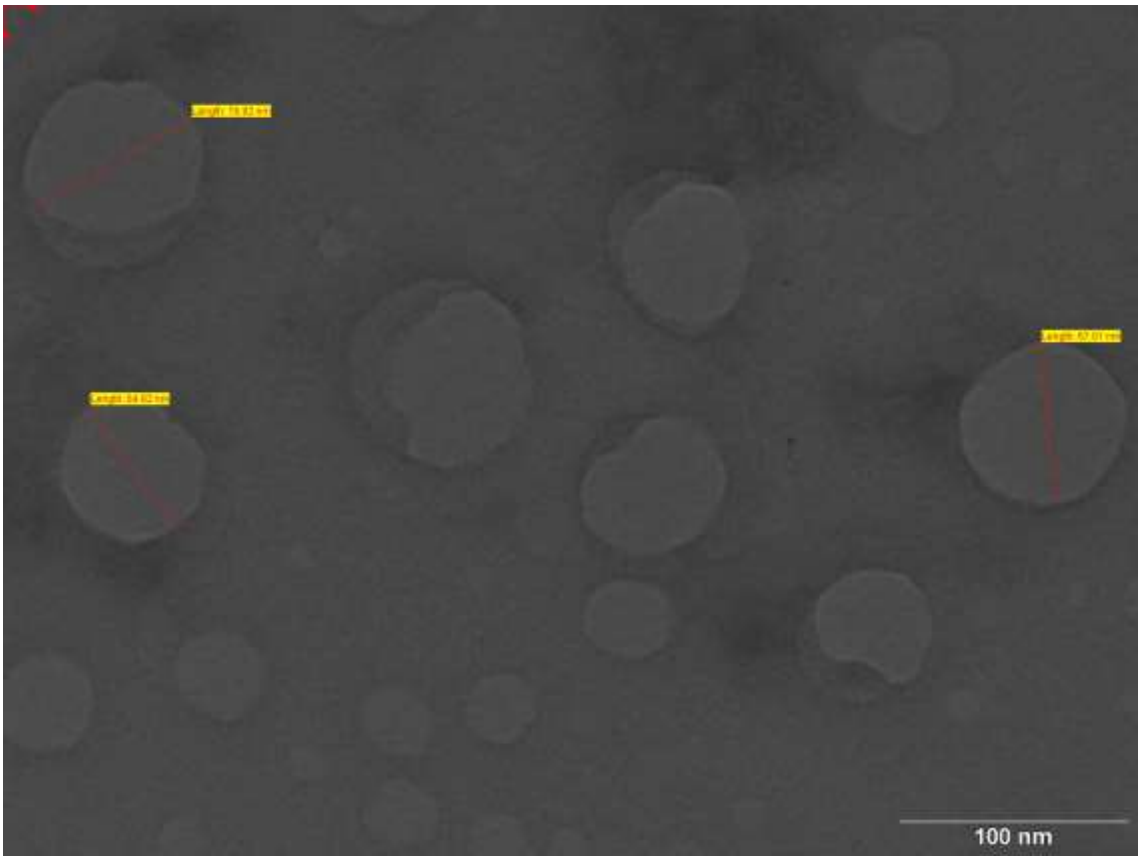
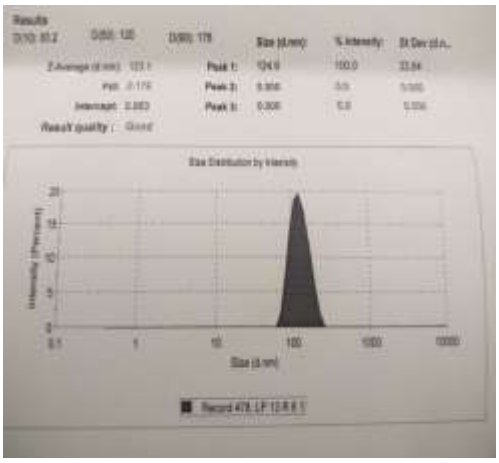
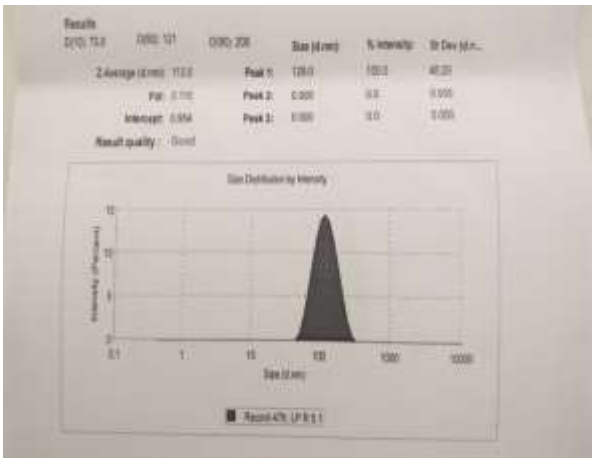


Figure TEM image

Particle Size Images:





4.10 Iontophrosis:

- Electrodes were prepared by dipping the wires i.e. anode and cathode in 0.1N HCl for 24 hrs by applying 12V current.
- Diffusion study was performed by keeping Cathode in the receptor compartment whereas anode was kept in the donor compartment.
- %DR was found out

Conclusion:

After applying iontophorosis it was found that the drug permeation had increased considerably.

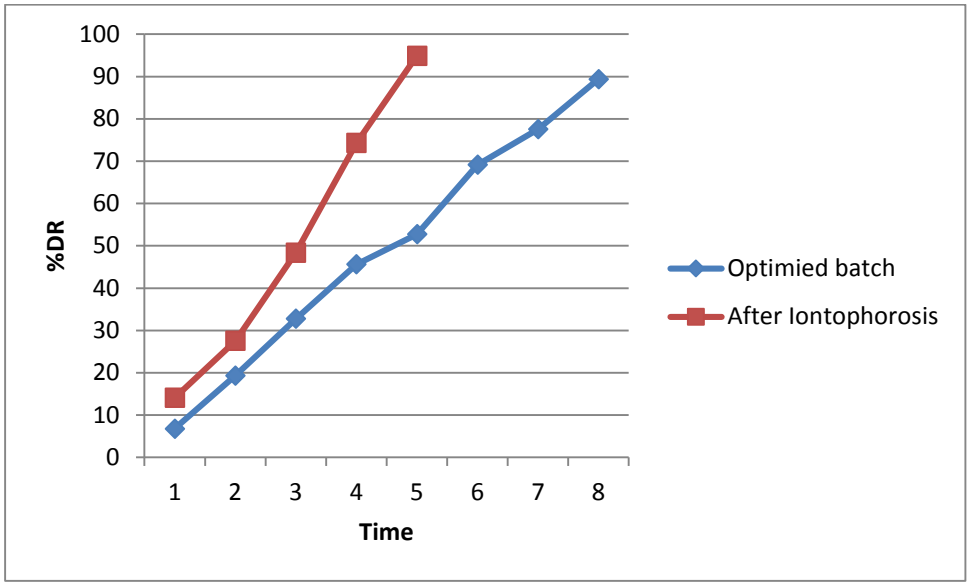


Figure comparison of %DR of optimised and iontophorosis of optimised batch

Conclusion:

From the graph of drug release it can be found that the drug release had increased after using iontophorosis technique.

4. Summary

1. Solid lipid nanoparticles were prepared by nano-precipitation technique for ocular delivery. Iontophorosis was used to enhance the permeation of the drug.
2. Solubility of drug in different lipid and drug was studied and based on that Stearic acid, Tween 80 and Transcutol P were selected as lipid, surfactant and co-surfactant.
3. Different amount of lipid, surfactant ratio, surfactant concentration were tried in the preliminary trials.
4. Then screening design was applied to screen significant factors. The design applied was fractional factorial design and five factors were selected. From that Sonication time and drug-lipid Ratio were found to be significantly affecting the formulation.
5. Then full factorial design was used for the optimization of the batch. Significant factor obtained from the screening design were used. 3^2 full factorial design was used. Final batch was optimized from the design space obtained.
6. Evaluation parameters such as particle size, Entrapment efficiency, Drug loading, zeta-potential and in-vitro drug release were evaluated.
7. Iontophorosis was used on the optimized formulation to enhance the permeation of drugs.
8. So, it can be concluded that Solid lipid nanoparticles along with iontophorosis technique can increase the permeation of the drugs and intra-vitreal injections can be avoided.

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