"Development and optimization of luliconazole loaded nanostructure lipid carrier system for the treatment of fungal infection"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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Under the guidance of

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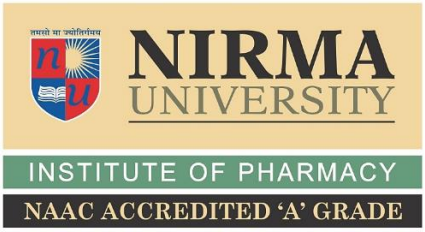

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May 2020

CERTIFICATE

This is to certify that the dissertation work entitled "Development and optimization of luliconazole loaded nanostructure lipid carrier system for the treatment of fungal infection" submitted by Mr. RAJ PACHANI with Reg.No. (18MPH111) in partial fulfillment for the award of Master of Pharmacy in "Name of a Department" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/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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CERTIFICATE OF ORIGINALITY OF WORK

This is to undertake that the dissertation work entitled "Development and optimization of luliconazole loaded nanostructure lipid carrier system for the treatment of fungal infection" Submitted by Raj Pachani (18mph111) in partial fulfillment for the award of Master of Pharmacy in "Name of M.Pharm. Programme" is a bonafide research work carried out by me at the "Name of Department", Institute of Pharmacy, Nirma University under the guidance of "Name of a Guide and Co-guide". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, this work is original and not reported anywhere as per best of my Knowledge.

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DECLARATION

I hereby declare that the dissertation entitled "Development and optimization of luliconazole loaded nanostructure lipid carrier system for the treatment of fungal infection" is based on the original work carried out by me under the guidance of Dr. Mayur Patel, Associate Professor, 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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ABSTRACT

This study aimed to formulate and evaluate luliconazole-loaded nanostructured lipid carrier for topical applications. This NLC prepared by a hot high shear homogenization method. Compritol 888 ATO as solid lipid, alpha-tocopherol as liquid lipid, tween80 use as a surfactant, and capriole 90 used as a co-surfactant. The particle size of luliconazole NLC between 49nm to 150nm it's optimum for topical nano-drug delivery. luliconazole NLC microscopy studies showed that all particles in a spherical shape. These results indicate that the studied luliconazole-NLC formulation with skin targeting may be a promising carrier for the topical delivery of luliconazole. PDI of luliconazole observed 0.351, so it is in good rang of PDI. Luliconazole NLC has excellent stability compared to the marketed formulation of luliconazole.

1.

INTRODUCTION

1.1 INTRODUCTION TO FUNGAL

A human frequently infected by bacteria, fungi, viruses, worms, prions, parasites and helminths. All of these fungal infections are most dangerous and a few years ago fungal infection was most feared. (1) Candida albicans is leading a cause of bloodstream infection in humans. (1) Mostly fungal disease found on various parts of skins like hair scalp and under the nails skin. The rate of fungal infection is continuously growing in developed and underdeveloped countries because one survey shows the 40 million peoples suffered by a fungal infection. Candida infection is life threating because it quickly penetrates deeper tissues, and it reached the bloodstream. Topical treatment for fungal infection has some benefits like, avoid first-pass metabolism, targeted drug delivery, highly patient compliance, increase the value of treatment, reduction of risk in systemic side effects. The effectiveness of topical drug delivery for fungal infection it depends on the penetration of drug by targeted tissue. Stratum corneum is the outmost film of the skin, so the drug is not easily penetrated this layer of skin so make a formulation like easily cross stratum corneum layer and give maximum drug concentration at targeted skin. For topical treatment come with the new formulation innovation like change in carrier system colloidal system, vesicular carriers and nanoparticles, so these types of carriers can cross the stratum corneum layer and give sustained-release. (2)

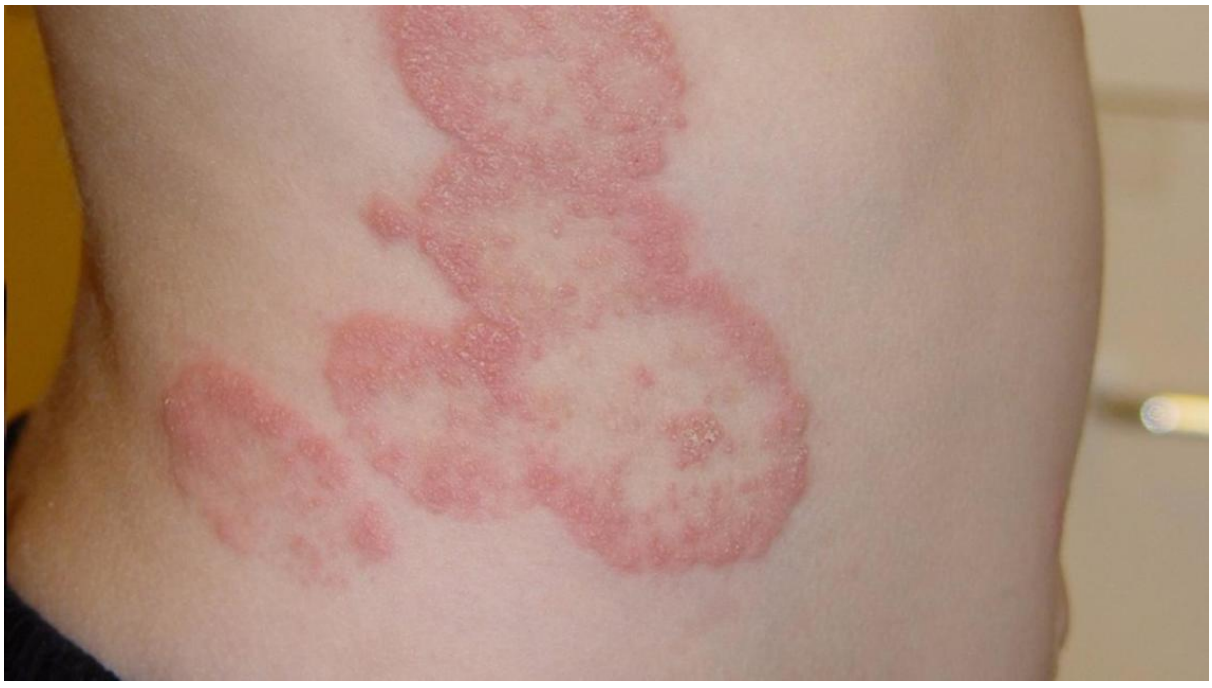

Figure 1.1: fungal infection on skin

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1.1.1 Pathophysiology

Only very few of fungal pathogenic was infected in healthy human cells, most of all harmless in case hosts have a weaker immune system, so is affected. In gastrointestinal tract epithelial surface prohibits microorganisms. In the respiratory tract, fungal spore and cell prevent to enter by mucociliary. One reason behind the fungal infection is a failure in the production of IL-10 because of weaker immune response genes. High dose of drugs in chemotherapy and corticosteroids they will damage mucosal barrier in results reduce the production of saliva, mucus and gastric acid, secretory Iga, as well as minimise peristalsis. Factors which promoting the fungal infection Depressed cellular immunity like corticosteroids, cyclosporin, anti-thymocyte globulin, cytotoxic drugs, Mucosal barrier injury, Poor hygiene, Usage of H2 receptor antagonists, and latest gastrointestinal operation. (1)

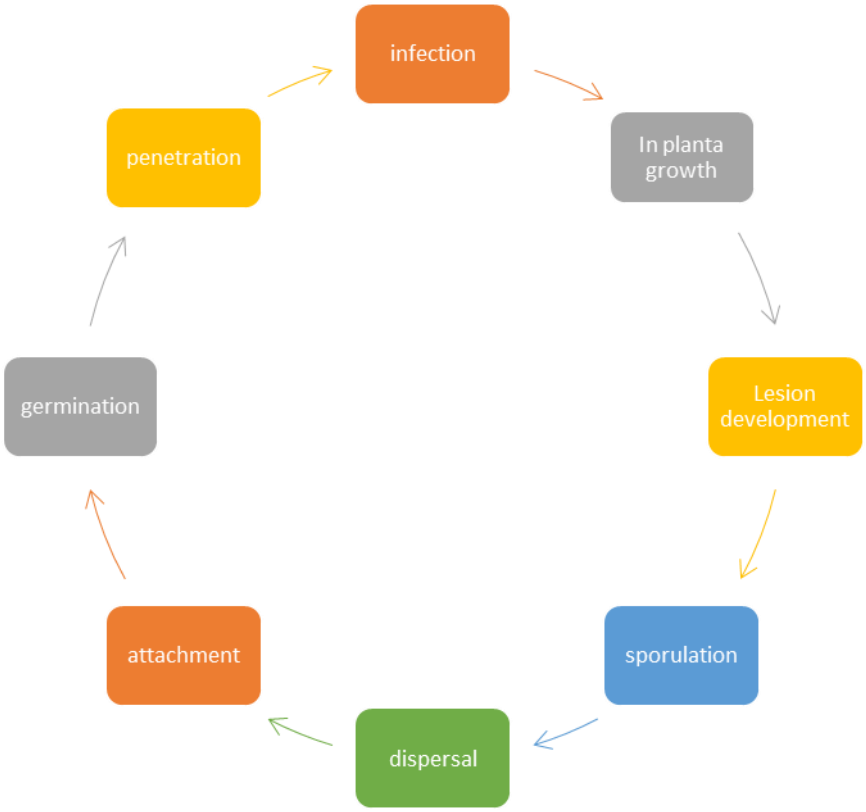

Figure 1.2: Pathophysiology of fungal infection

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1.1.2 Pathophysiology of antifungal agent

Azole is one of the most useful class for the treat fungal infection, mostly antifungal agents inhibit the cytochrome P 450 enzyme, which is necessary to synthesis of sterols. Ergosterol is the need for the fungal cell without ergosterol cells not able to live. (3) Allylamine work by

reticence squalene epoxidase which is an essential enzyme for the biosynthesis of ergosterol pathway so its actions as inhibit growing fungal cell. (2)

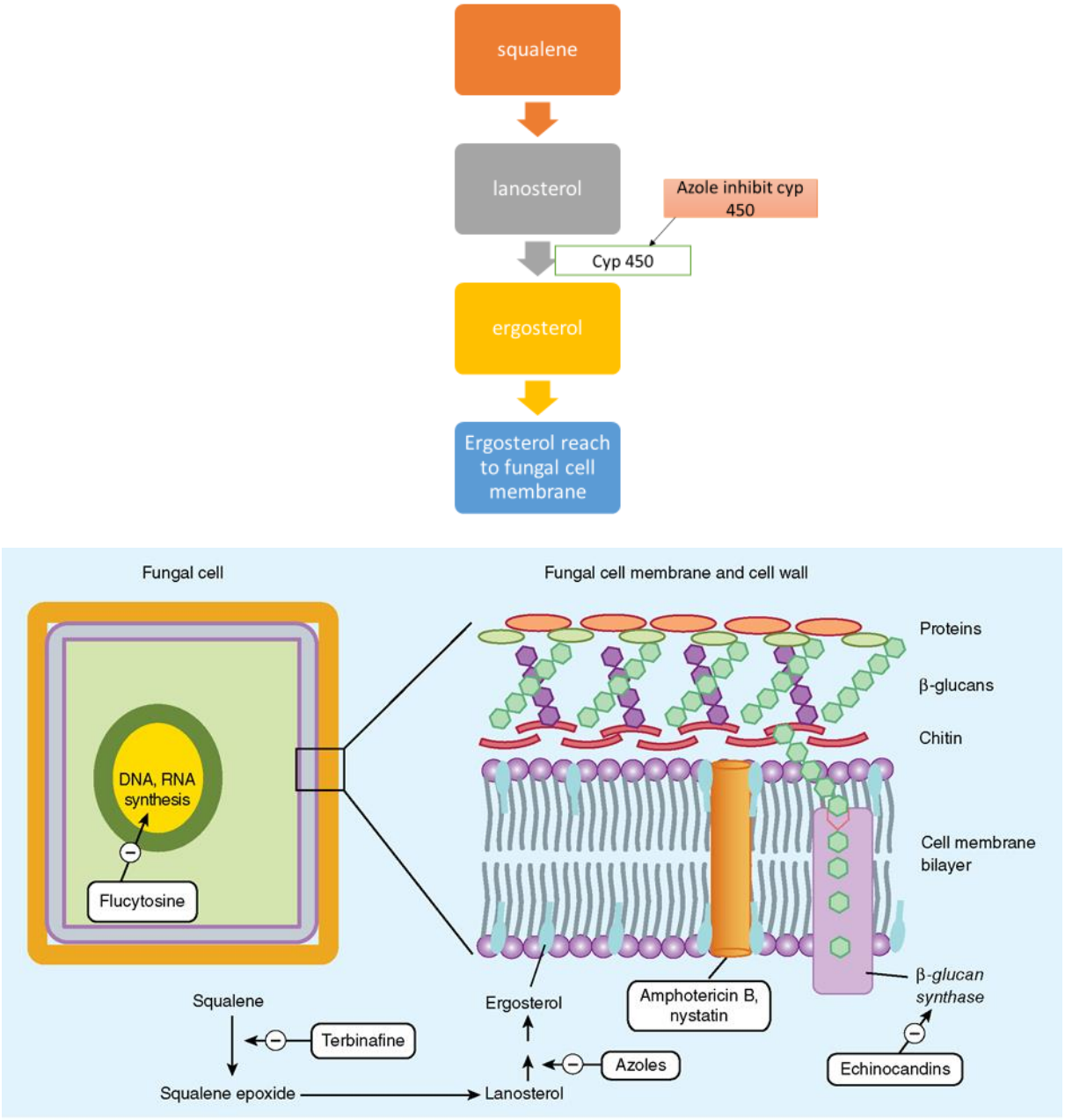

Figure 1.3: Pathophysiology of antifungal agent

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1.2 THE CONVENTIONAL DOSAGE FORMS

In conventional dosages forms like lotion, cream, gel, spray and ointment which use in the cure of fungal infection.

Azole class of antifungal work on the mechanism of stop the production of ergosterol and change the permeability of cell by bind with the phospholipid layer, which presents in the fungal cell membrane. Two three nitrogen presents in an azole circle and it is classifying by imidazole (e.g. clotrimazole, miconazole, ketoconazole) or triazole (e.g. fluconazole, itraconazole).

Polyene antifungal class drugs activity which binding irreversible to present ergosterol in the fungal cell membrane that is one reason behind polyene have extensive broad-spectrum antifungal activity compare to others classes. But this class has limited for only for topical use. This class have most common adverse allergic reaction to contact dermatitis.

Hydroxypyridone derivative like ciclopirox work as an antifungal, anti-inflammatory, and anti-bacterial properties. Enzymes are required in a cell for the mitochondrial electron transport system, and production of energy but ciclopirox inhibit these essential enzymes. It is most active against yeast and fungi, including dermatophytes. (2)

1.2.1 Drawbacks of Conventional Dosage Forms

Conventional dosage forms have some adverse action like erythema, burning, stinging, redness, and burning of the skin. The more problems in ocular fungal infection because conventional topical antifungal has low bioavailability, drug toxicity and very less ocular penetration. (4)

1.2.2 New drug delivery system in the healing of fungal infections

in the nanoparticle is a solid particle which sizes range in 10 to 1000 nm. Here drug was entrapped, dissolve, attached, or encapsulated to a nanoparticle model. It more categorized by fine particles and ultrafine particles, fine particle size between 2000 to 100 nanometre and an ultrafine particle size between 100 and 1 nanometres. Nano structured lipid carriers (NLCs) and solid lipid nanoparticle (SLNs) are types of nanoparticles which use for drug delivery to the skin layers. Solid lipid nanoparticle (SLNs) mostly in spherical shape and diameter of these spherical is 10 to 1000nm. In this system take only solid lipid so that lipophilic drugs can make easily soluble in this solid matrix. Using lipids in SLNs is a most favorable advantage because avoid organic solvents, and lipids also helps to penetrate skin layers. NLCS increase drug loading and give sustained drug release and good stability. Comparison

of SLNs and NLCs in fluconazole so NLCs of fluconazole give proper targeting skin and provide more drug retention on skin compare to SLNs of fluconazole. Here NLCs are considered better than SLNs. So here, the conclusion is a nanoparticulated drug-loaded system is more benefits compared to conventional dosages forms.

1.2.3 Classifications antifungal drugs (4)

Table1.1: Categorization of antifungal drugs

Sr no	Class	Subclass	Examples of drug
1	Antibiotics	Polyenes	Amphotericine B, hamycin, nystatin
		Echinocandine	Micafungine, anidulafungins
		Heterocyclic benzofuran	Griseofulvine
2	Antimetabolite	Flucytosin	5FC
3	Azole	Imidazoles	Clotrimazoles, econazoles, miconazoles, oxiconazoles, ketoconazoles
		Triazoles	Voriconazole, posaconazole, itraconazole, Fluconazole
4	Allylamine		Terbinafin

1.2.4 Marketed formulation (4)

Table1.2: Marketed formulation

Brand name	**Drug**	**Dosage form**	**Dose**
Natacyn (5%)	Natamycin	Suspension Ophthalmic	1 time/per day
Loceryl (5%)	Amorolfine	Nail lacquer	1 time/per day

Penlac (8%)	Ciclopiroxamine	Nail lacquer	1 time/per day
Onylac (8%)	Ciclopiroxamine	Nail lacquer	1 time/per day
Lotrimin (1%)	Clotrimazole	Cream	2 times/per day
Mycelex-G (1%)	Clotrimazole	Cream	2 times/per day
Gyne-Lotrimin (1%)	Clotrimazole	Cream	2 times/per day
Zocon (0.3%)	Fluconazole	Eye drop	3 times/per day
Syscan (0.3%)	Fluconazole	Eye drop	3 times/per day
Flucomet (0.7%)	Fluconazole	Eye drop	3 times/per day
Xolegel gel (2%)	Ketoconazole	Gel	1 time/per day
Lamisil (1%)	Terbinafine	Cream	2 times/per day
Ertaczo (2%)	Sertaconazole	Cream	2 times/per day
Manistat-ermD (2%)	Miconazole	Cream	Two times/per day
Micatin (2%)	Miconazole	Cream	Two times/per day
Spectazole (1%)	Econazole	Cream	Two times/per day
Econail (5%)	Econazole	Nail lacquer	One time/per day

Laprox (0.88%)	Ciclopiroxe	Cream	Two times/per day
Laprox (0.88%)	Ciclopiroxe	Gel	One time/per day

1.3 OVERVIEW OF LIPID NANOCARRIER

1.3.1 Solid lipid nanoparticle

In 1991 mostly, colloidal carrier system uses such as liposome and emulsion, so that's time solid lipid nanoparticle uses as an alternative of liposome and emulsion. These nanoparticulate systems have a size between 50 to 1000 nanometre. This drug-loaded particle is composed between melted lipid and it's dispersed in water surfactant liquid. Properties of SLNs is unique compare to liposomes and emulsion like high drug loading, large surface area, small size, and high stability so it gives good results in pharmaceutical. Nowadays, lipid drug delivery becomes quite interested in the pharmaceutical field because lipid helps to increase the bioavailability of a drug, targeted drug delivery, and smooth to the characterization of excipients in a lipid medium. (5)(6)

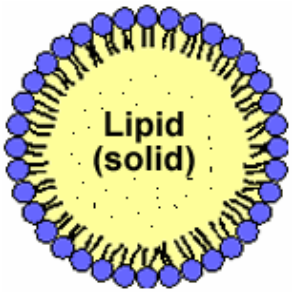


Figure 1.4: Structure of solid lipid nanoparticle (SLN)

(A. ABDUL SATHALI, P. EKAMBARAM 2011)

1.3.1.1 Model of SLNs (6)(5)

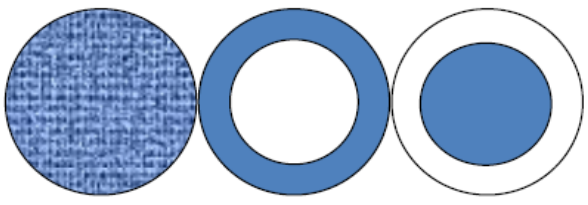


Figure 1.5: Model homogeneous matrix, Model drug-enrich to shell-type, Model drug-enrich to core type

(Neha Yadav, Sunil Khatak, 2013)

- Homogeneous matrix model.

  This model achieved by using cold homogenization technique and hot homogenization system. In cold homogenization procedure drug is uniquely separated in lipid medium using high-pressure homogenization because of mechanical force break particles and help to a homogenous matrix structure. In the same technique to the hot homogenization method here, oil droplets will be broken down and make a homogenous matrix model. Common examples of this model are a prednisolone drug in lipid medium and release of this prednisolone drug up to weeks.

- Drug-enriched to shell model.

  This model found throughout the phase separation process in SLNs like melted oil droplets to solid lipid nanoparticle. In this model, a blank lipid is found at the core and active drug-loaded lipid at outer layer of the shell. this model mostly uses for fast release of drug and applicable for a topical (skin disorder)

- Drug-enriched to core model.

  In this model, the API active lipid part founded at the core and less drug lipid part found at outer shell layer. This thing occurred when the drug-loaded part starts to precipitation first. This model follows the controlled release of drugs by fick law of diffusion.

Table1.3: List of List of excipients required in preparation of SLNs

Lipid	**Surfactant**
Triglycerides	**Phospholipids**
Trilaurin	Egg lecithin
Dynasan 114	Soy lecithin
Dynasan 116	Phosphatidylcholine
Dynasan 118	**Ethylene oxide**
Stearic acid	Poloxamine 908
Fatty Acids	Poloxamer 407
Palmitic acid	Poloxamer 182
Cyclodextrin	Poloxamer 188
Behenic acid	**Sorbitan ethylene oxide**
para-acyl-calix-arenes	Polysorbate 80
Cetyl palmitate	Polysorbate 60
Witepsolo E 85	Polysorbate 20
Witepsolo W 35	**Bile salts**
Witepsolo H 45	Sodium taurodeoxycholate
Witepsolo H 45	Sodium taurocholate
Tricaprin	Sodium glycocholate
Hydrogenated coco-glycerides	Sodium cholate
Glyceryl palmitostearate	**Alcohols**
Glyceryl behenate	ButanoL
Glyceryl monooleate	Ethanol
Glyceryl distearate	Butyric acid

1.3.1.3 Advantages of SLNs (5,6)

1. SLNs product easy to lyophilize because lyophilize product is more stable compare to other products
2. accessible to production in industry point of view
3. SLNs give excellent stability of products
4. compare to liposome easy to large scale production of SLNs
5. organic solvent replaces by lipid so less toxic
6. control release because of the encapsulated compound
7. used for topical and parenteral dosages forms

1.3.1.4 Disadvantages of SLNs (5,6)

1. partitioning effect observed during manufacturing time so not suitable for hydrophilic drug products
2. less drug loading capacity
3. during storage drug separate from lipid medium
4. a high amount of water in dispersion (75 to 100%)

1.3.2 INTRODUCTION OF NANO STRUCTURED LIPID CARRIER

The pharmaceutical company has a well-established process for manufacturing a nanostructured lipid carrier, but it's got problems in selection of lipids, surfactant and methods for preparation which lead to modified parameter like size, entrapment, drug loading, and shape also. In lipid formulation, NLCs have more demand to other dosage forms because it's use to raise the dispersible of API and bioavailability also because BCS class2 drugs follow low solubility nature. Nanostructured lipid carrier is a hybrid type of properties because it is a blend of liquid lipid and solid lipid. This binary mixture carrier is having a size between 10 to 500 nanometers. (7)

1.3.2.1 Types of NLCs

NLCs structure is nearby similar to SLNs, but based on drugs located in NLCs it's divided into three different types.

1. type1 NLCs: - imperfect crystal
2. type2 NLCs: - multiple type
3. type3 NLCs: - amorphous

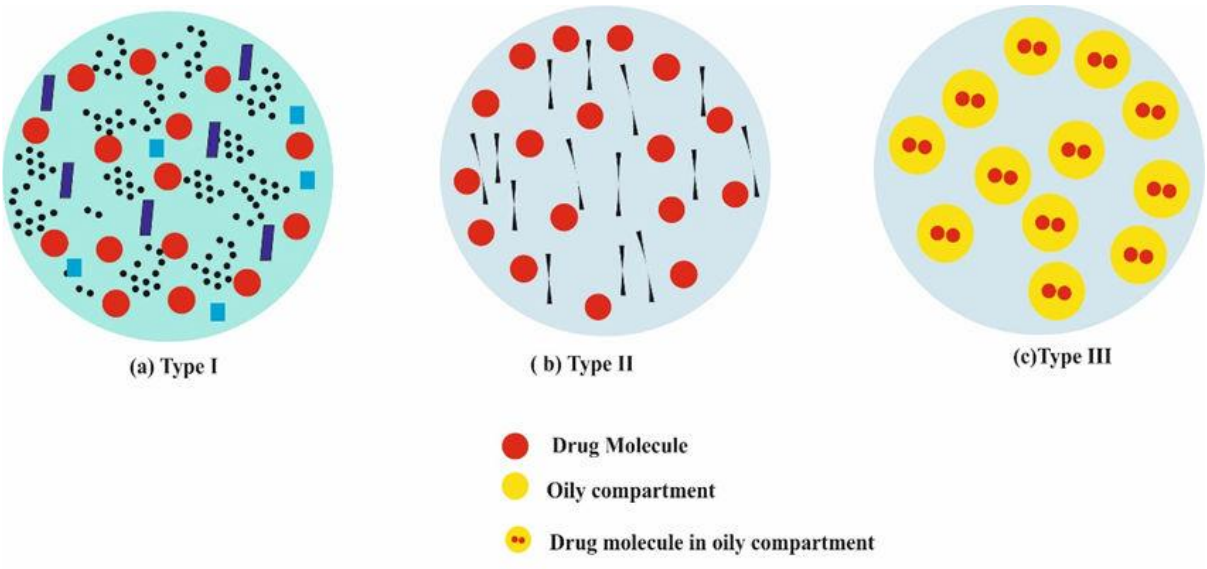


Figure 1.6: Types of NLCs

(Amit Sharma, Ashish Baldi, 2018)

- **type1: - imperfect crystal**

  In this type of NLCs solid matrix is found imperfectly structured. Here very badly matrix of solid structured if use different fatty acid like glycerides it helps to modify and improve structure. Mostly type 1 NLCs using lipids have properties like create crystal in matrix. They are adding less quantity of liquid lipid help to increase drug filling. (8)

- **type2: - multiple**

  In this type of NLCs formulation is oil in lipid in water (O/F/W), so it called multiple type of NLCs. This type of NLCs using throughout the cold time of hot homogenization system. The solubility of a drug is more in hot lipids compare to cooling lipids; it means solubility of drug less during the cooling phase after high shear homogenization. During storage drugs come out of lipid vesicle. Hence, it has very little stability, so add more amount of liquid lipid in formulation they help to prevent drugs comes out of nanoparticles. (7) (8)

- **type3: - amorphous**

  These types of NLCs obtained by specialized lipids like isopropyl myristate, hydroxyoctacosenyl hydroxystearate, and medium-chain triglycerides miglyol 812 add to solid lipid so not able to produce a crystalline state of solid and it remains an amorphous state. The amorphous phase prevents drug expulsion and increases drug loading. (7) (8) (9)

1.3.2.2 List of excipients required in production of NLCs (10)(11)

Table1.4: List of excipients required production in of NLCs

Lipids	Surfactant
Fatty acids	**Ionic surfactants**
Palmitic acid	Sodium dodecyl sulphure
Stearic acid	Sodium oleate,
Myristic acid	Sodium taurodeoxycholate
Dodecanoic acid	**Non-ionic surfactants**
Monoglycerides	Solutol HS15
Glyceryl monostearate	Poloxamer 407
Glyceryl behenate	Poloxamer 188
Diglycerides	Tyloxapol
Glyceryl dibehenate	Tween 20, 80,
Glyceryl palmitostearate	Span 20, 80, 85,
Triglycerides	**Amphoteric surfactants**
Caprate triglyceride,	Phospholipon 80 H
Caprylate triglyceride,	Phospholipon 90 H
Waxes	Hydrogenated egg phosphatidylcholine
Beeswax	Hydrogenated soy phosphatidylcholine
Carnauba	Egg phospholipid
Cetyl Palmitate,	**Co-surfactants**
Liquid lipids	Butanol
Isopropyl myristate	Butyric acid (10)(12)(6)
Squalene	
Hydroxyoctacosanyl hydroxystearate	
α-tocopherol	
capric triglycerides,	
caprylic	
Oleic acid	
Soya bean oil	
Cationic lipids	
Cetrimide tetradecy trimethyl ammonium bromide,	
Cetyl pyridinium chloride	

1.3.2.3 Advantages of NLCs (12)(13)

1. NLCs carriers give good efficient system because of the solid and liquid mixture in a matrix, so it is recognized safe and regulatory accepted
2. Increase the hydration of skin and elasticity also
3. The small size of NLCs particles easy to penetrate stratum corneum layer of skin.
4. Pharmaceutical and cosmetic industries approved all lipids which are used in an NLCs formulation.
5. Use for sustained/extended drug release
6. Increase skin occlusion effects
7. Particle size is controlled
8. Good entrapment for hydrophilic drug and lipophilic drug
9. Increased dispersion in an aqueous medium
10. Easy to prepare and scale-up for industries purposes
11. Better stability compared to other dosages forms

1.3.2.4 NLCs have some limitations like (12)(13)(10)

1. Sometimes surfactant gives irritation on the skin
2. Cytotoxic action because of matrix nature
3. Difficulties in selection of right lipid and surfactant during manufacturing

1.3.2.5 Formulation technique for SLNs and NLCs

1. High-pressure homogenization
    - *Hot homogenisation technique*
    - *Cold homogenisation technique*
2. Microemulsion
3. Solvent injection technique
4. Double emulsion method
5. ultrasonication technique

❖ **High-pressure homogenization**

High-pressure homogenization is the utmost mutual technique used for the making of SLNs, NLCs and parenteral emulsions. Here lipid goes for excessive pressure 101 to 2000 bar by using excessive shear pressure, so particles size occurs in a micrometre or nanometre range. This technique performed at less than room temperature (cold homogenization) or elevated temperature (hot homogenization). (11)(7)

Hot homogenization technique

In hot homogenization method, drug and molten lipid both constant stirrers using high shear devices. The aqueous solution also heats more than 2 to 3-degree Celsius in comparison to lipid after that aqueous solution and lipid drug mixture continues stirring using high shear devices when pre-emulsion is obtained after that it is homogenized by homogenizer after homogenization cool down to room temperature (7)(11)

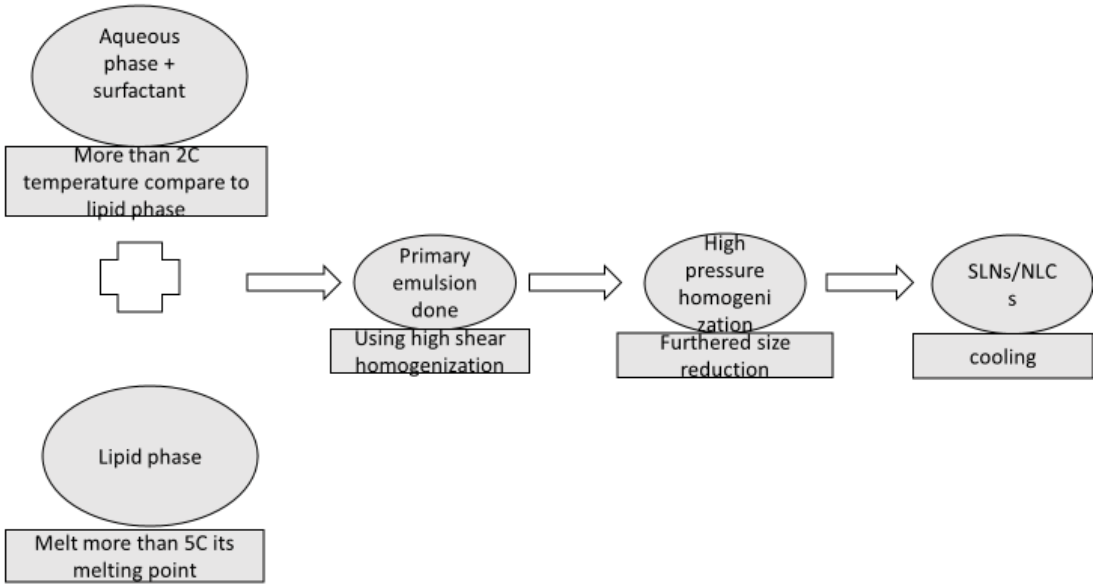


Figure 1.7: Schematic diagram of hot homogenization system

(Sarabjot kaur, Ujjwal Nautyal, 2015)

a. Cold homogenization technique

Hot homogenization techniques have some drawback, so it overcomes by using cold homogenization technique like API degradation at more temperature, loss of a drug during aqueous phase homogenization. The first step is common in both methods here melted drug lipid part is cooled down using liquid nitrogen or ice. (7)(8)

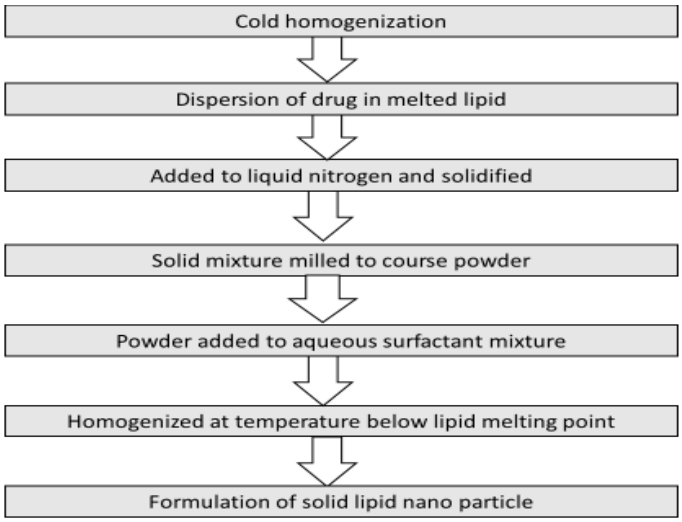


Figure 1.8: Schematic diagram of cold homogenization system

(Sarabjot kaur, Ujjwal Nautyal, 2015)

- Microemulsion

  In microemulsion technique, drug was dissolved in melted lipid, after that aqueous phase is heated the similar temperature as a lipidic medium when both reached the same temperature after that mixed using under mild stirring. During cooling of this solution, rapid crystallization of the oil drops. Microemulsion prepared in large quantities using temperature-maintained tank and then microemulsion transfer into cold water tank containers for precipitation. (11)(8)

- Solvent injection technique

  In this technique solvent selected like DMSO and ethanol because it rapidly distributes in water. The first step of this method is dissolving lipid insolvent after that this solvent fast added in an aqueous surfactant medium using an injection needle after migration lipid creates precipitation in surfactant medium. Here particle size was inversely proposal to a velocity of spreading process. Small particle size achieved when the higher velocity of system and large particle size achieved when low viscosity of system. The main advantages of this method are the fast production process, easy to Handel system, low shear stress and low temperature. in this system one disadvantages are using an organic solvent. (8)(9)

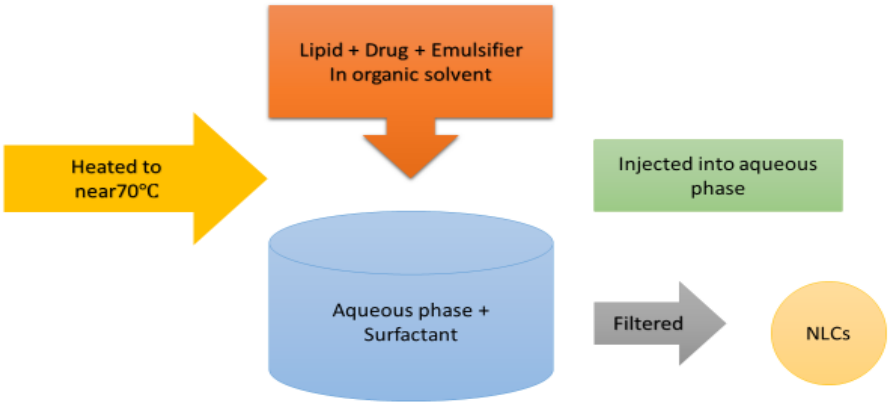


Figure 1.9: Schematic diagram of Solvent injection technique

(Sarabjot kaur, Ujjwal Nautyal, 2015)

❖ Double emulsion technique

This technique used for a hydrophilic drug because first dissolved hydrophilic drug in aqueous solution and emulsified in liquid lipid. Add hydrophilic emulsifier in an aqueous medium for stabilizing primary emulsion (8)

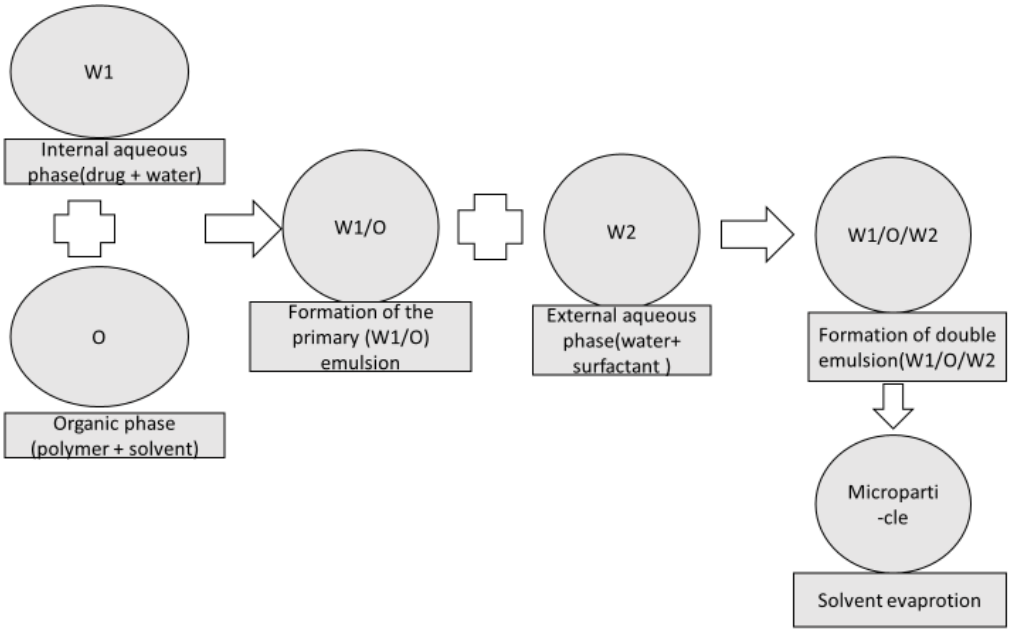


Figure 1.10: Schematic diagram of double emulsion technique

(Sarabjot kaur, Ujjwal Nautyal, 2015)

- Ultrasonication technique

In this method, ultrasonication prob is used for achieving the nanoparticle size of oil globules. A combination of solid lipid and liquid lipid melt first after that add drug in this mixture at constant stirring. Surfactant also heats more than 2 to 3 Celsius compare to the oil phase. under continues stirring add oil phase slowly into an aqueous phase. Both phases mixed adequately at 600 rpm for 10 minutes. This primary microemulsion goes for ultrasonication after ultrasonication gets oil globules in nanometres size (9)(11)

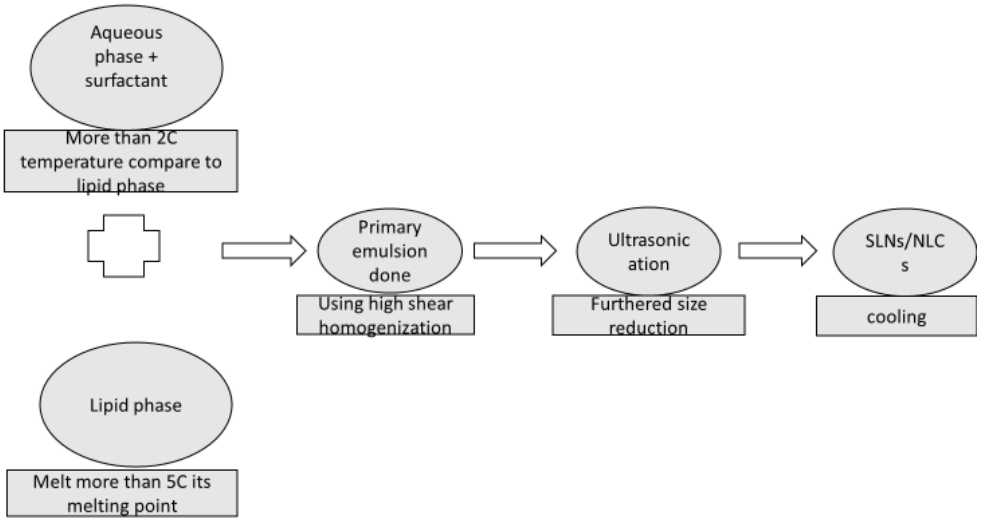


Figure 1.11: Schematic diagram of ultrasonication technique

(Sarabjot kaur, Ujjwal Nautyal, 2015)

1.3.2.6 Characterizations of SLNs and NLCs

- Particle size and zeta potential

Laser diffraction and photon co-relation spectroscopy are the greatest potent systems for the dimension of particle size. Light scatter because of particle moment in samples this scatter light measure by the PCS. So, PCS can easily detect the nanoparticle, but it's not useful for micro range particles. SLNS and NLCs optimized batch stable for more than one year, so the stability of nanoparticle determines by zeta potential of a sample. (5,6)

- Electron microscopy

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) both are uses for the find out morphological of nanoparticle. This both methods are direct to measure the morphology of particles. Rang of detection is a 50nm to 100100μm. (5,6)

- Powder X-ray emission technique and differential scanning calorimetry (DSC) technique

  Both techniques used for the measurement of crystallinity in sample and modification in lipids. The crystallinity rate determines with a comparison of bulk materials with dispersion. It gives details about the glass transits and the melting point of lipids. crystalline order of nanoparticle detects by the large angle of x-ray diffraction technique. (6) (5)

- Drug release

  The drug relief of the nanoparticle is checked by the dialysis method and Franz diffusion cell. Mostly NLCs give sustained release of a drug, so it provides prolong the half-life and inhibits enzyme attack in the body. Drug incorporated in lipid two types like more drug content outer side shell and more drug content core of the shell. The canter of shell drug gives a sustained release of a drug, and outer shell drug will give burst release actions. Drug release also depends on the oil percentage, types of selected surfactant, type of selected co-surfactant and manufacture temperature of NLC. (14)

- Rheology

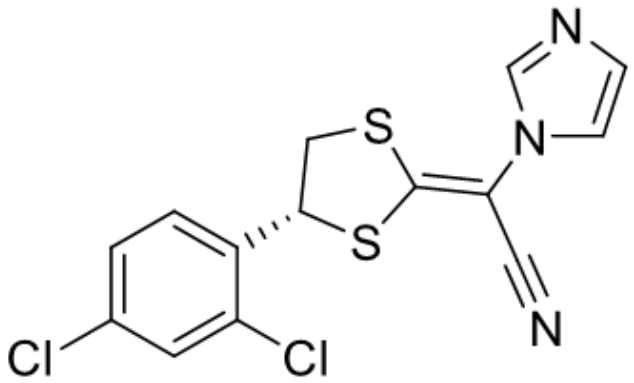
  Mostly viscosity of the prepared batch measured by using Brookfield Viscometer as per the sample set spindle number. Viscosity depends on the presence of lipids in the system if increase lipid content in batch so they follow non- Newtonian flow (6,14)(5)

- Polydispersity index

  Nanoparticle generally polydisperse in nature, so its measure by PDI of the nanoparticle. PDI value less than 0.3 is acceptable; the lower value of PDI indicates mono dispersion of nanoparticle. This PDI measured by the particle size analyser. (9)(7)

1.4 INTRODUCTION TO DRUG SUMMARY (15)

Table1.5: Drug Summary (luliconazole)

Drug name	Luliconazole
Structure	
IUPAC name	(2E)-2-[(4R)-4-(2,4-Dichlorophenyl)-1&3-dithiolan-2-ylidene]-2imidazol-1-ylacetonitril
Molecular formula	$C_{14}H_9Cl_2N_3S_2$
Molecular weight	355.28
Melting point	150 - 154 ℃
Solubility	Soluble in methanol and acetone, slightly soluble in ethanol, practically unsolvable in water.
BCS class	Class 2
Log p-value	4.27
Pharmacological category	Antifungal azoles class, cyp450 inhibitor during the synthesis of ergosterol
Protein Binding	>99%
Route of administration	Topical

1.5.1 INTRODUCTION TO EXCIPIENTS SUMMARY

Table1.6: Excipient Summary (Compritol ATO 888)

(Handbook of Pharmaceutical Excipients 814-819)

Excipients Name	Compritol ATO 888:
Non-Proprietary Name	Glyceryl Behenate USP, Glyceroli dibehenas EUR, Glyceryl dibehenate BP
Similar Name	23 dihydroxypropyl ester, Compritol 888 ATO 23dihydroxypropyl docosanat docosanic, glyceryl

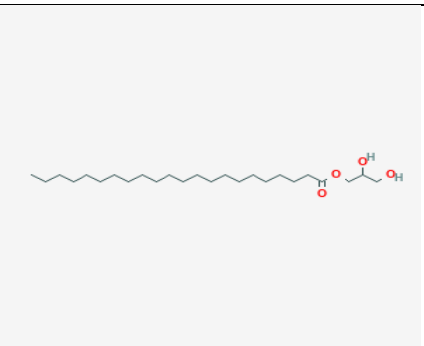
	behenate
Structure	
IUPAC name	2,3-di hydroxypropyl docosanoate
Description	Grey/white powder
HLB	2
Melting Point	68-77°C
Density.	0.94 g/cm3.
Acid amount (mg KOH/g)	4
Iodine amount (g I2/100g)	3
Solubility	Soluble in xylene, methylene chloride and chloroform. Insoluble in water, mineral oils, ethanol and ether.
Storage	Store below 35 °C

Table1.7: Excipient Profile (Alpha tocopherol)

(Handbook of Pharmaceutical Excipients, pg. 35 to 39)

Excipients name	Alpha Tocopherol
Non-proprietary Names	Tocopherol JP, Alpha tocopherol BP, RRR-a-Tocopherolum EUR, Vitamin E US
Synonyms	5,7,8-trimethyltocol; dl-a-tocophero; all-rac-

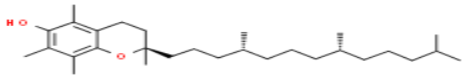
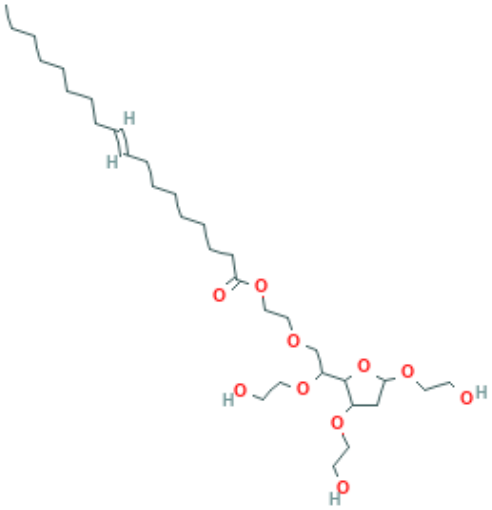
	atocopherol; synthetic alpha tocopherol
Structure	
IUPAC name	(2S)-2,5,7,8-tetra methyl-2-[(4S,8S)-4,8,12-trimethyltridecyl]-3,4-di hydro-2H-chromen-6-ol
Molecular Formula	$C_{29}H_{50}O_2$
Molecular Mass	430.7 g/mol
Functional Class	Antioxidant, vitamin E
Refractive index	1.50–1.508
Boiling point	235-240 °C
Density	0.947–0.954 g/cm3
Ignition point	340 °C
Solubility	Soluble in vegetable oil, acetone, ethanol, and ether. insoluble in water
Storage	Cool, dry place and protect from sunlit also. Store under inert gas and tight container also required.

Table1.8: Excipient Profile (Tween 80)

(Handbook of Pharmaceutical Excipient pg. 1536-1551)

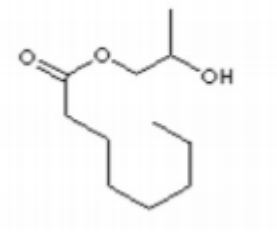
Excipients name	Tween80
Non-Proprietary Tags	Polysorbatum EUR, Polysorbate 80 BP, Polysorbate 80 USP
Similar name	Polysorbate 80

Structure	
IUPAC tag	Polyoxyethylene 20 sorbitan monooleate
Molecular Formula	C64H124O26
Molecular Weight	1310.304
Description	Better in test and yellowish thick oily liquid
Functional class	Emulsifier
Acid value (mg KOH/g)	2
Peroxide rate (mequi O/kg)	1-10
Hydroxyl rate (mg KOH/g)	65-80
Viscosity	400-430
Specific gravity	1.09
HLB	15
Flash time	150
Surface Tension	43
Solubility	Easily soluble in water and ethanol, insoluble in lipids.
Applications	emulsifying agents

Storage	Store in an amber colour bottle with nitrogen purging.

Table1.9: Excipient Profile (Capryol 90)

(Gattefosse Technical literature20,21)

Excipients name	Capryol 90
Structure	
Trade names	CAPRYOL 90, CAPRYOL PGMC, CAPRYOL PGMC ME 60
HLB	5
Boiling point	> 210°C
Flash point	> 140°C
Ignition temperature (auto ignition): > 250,00 °C	> 250°C
Uses	Use for SEDDS and SMEDDS formulations; solubilizer; co-surfactant in o/w emulsion system; topical dosage forms because also work as a permeation enhancer
Storage	Store in tightly packed containers because protect light and moisture again.
Solubity	Oils- soluble, ethanol-soluble, chloroform-soluble but insoluble in water.
Odour	Faint
Appearance	Oily liquid
Specific gravity	0.935 to 0.958

Value of acid	<= 1.00 mgKOH/g
Value OF saponification	270 to 285 mgKOH/g
Value of iodine	<= 1.0 gI2/100g
Value of peroxide	<= 6.0 meqO2/kg
Impurities of alkaline	<= 30 ppm NaOH

2. AIM AND OBJECTIVE

AIM AND OBJECTIVE

- The aim of this research work was development and optimization of luliconazole loaded nanostructured lipid carrier systems for the cure of fungal infection.
- Prepare novel formulation which gives a controlled release of luliconazole and increase permeation for topical application.
- To accomplish the anti-fungal action and overawed the drawbacks of conventional antifungal treatment.
- To inspect the result of altered formulation parameter on the characteristics of luliconazole loaded NLCs

3. LITERATURE REVIEW

3.1 Literature review on SLN and NLC benefits in the cure of fungal infection

Tais Gratieri et al. lipids nanoparticle similar to SLN and NLC studied for the cure of fungal infection, both systems give a sustained release of drug during treatment and increase skin moisture because of occlusion film on the skin. Moisture gives significant impacts on nail and skin permeation. It's complicated to penetrate drug molecules to nail plate because of its most resistance barrier. In recent times near 55 research articles reported about SLN and NLC for a fungal drug. These articles focused on nanoparticle ingredient's impact on in vivo antifungal studies, entrapment efficiency, drug release, and stability studies. Lipid nanoparticle like SLN and NLC shown good permeability compared to other formulation. Recently one research paper published they indicate voriconazole NLC directly applied to the nail plate. SLN/NLC gives better antifungal effect, and promoting cutaneous drug delivery evidence of this success is a high encapsulation of drug. (16)

Eliana B. Souto et al. As per the biopharmaceutical point, the skin is an exciting route for the treatment of skin disorders. Only small oil vesical can able to penetrate the stratum corneum layer of skin because of its most difficult and outer layer of skin. Large molecules like peptide and proteins are still challenging to cross the stratum corneum layer. The selection of lipid and surfactant plays an essential role in the characterization of SLN/NLC. all lipids are clinically non-toxic compare to organic solvent (17)

Buket Aksu et al. Forty million people faced fungal infection in this world. Topical therapy has some more advantages compared to other dosages forms like a drug at site infection, targeted drug delivery, and fewer side effects. Therefore, topical dosage forms select in the treatment of cutaneous diseases. Mostly conventional dosage form uses in the treatment of fungal infection, but these conventional dosage forms have some limitations this is overcome by SLN/NLC. Most effective factor in the topical dosage form is a characterization of SLN/NLC, types of formulation, selection of lipids and selection of surfactant because an only small particle can cross the stratum corneum layer of the skin (2)

3.2literature review on different formulation effect to the characterization of SLN/NLC

Malgorzata Sznitowska et al. Solid lipid microparticle obtained by the primary stage of emulsification during melted lipid mixture added in a surfactant solution. Ultrasound effect applied to this solid lipid microparticle to convert in a nanoparticle. With the help of the ultrasound effect get particle size below 1 Micrometre. Lipid, which uses during the experiments precirol ATO 5 and compritol 888 ATO, mixture with miglyol 812 also. When compriotol as a lipid and tween80 as emulsifier use, so get results 3 to 7 Micrometre and not extensive thermal sterilization is done. Solid lipid microparticle prepared by presirol and add tego care 450 as a surfactant, so get broad range in particle size 40 Micrometre average. Add one sonication step in compritol formulation batch, so get particle size in 200 to 500 nm, and it is optimum for nano-drug delivery. If tween 80 replace by tego care so get lipid nanoparticle size below 100 nm (18)

Chee Wun How et al. lipid nanoparticle gives more advantages as a drug carrier compares to other carrier systems. Liquid lipid gives more drug loading capacity compared to organic solvent materials. NLC80 and NLC20 prepared by using high-pressure homogenization techniques and it's stabilized by polysorbate80 and polysorbate20, respectively. Both batch of NLCs spherical this result observed by the help of Transmission electron microscopy (TEM). Photon correlation spectroscopy (PCS) results of average particle size of NLC20 and NLC80 were 261.63 ± 8.54 nm and 103.8 ± 0.1, respectively and zeta potential also observed by (PCS) were -31.57 ± 0.06 and -23.93 ± 0.75 mv, respectively. Those all results shown NLC80 more stable compare to NLC20. NLC80 melting point 5.72°C less compare to bulkiness lipid melting point (61.5 °C) while NLC20 shows two types of melting points at 54.81 and 59.12. So, based on these results, NLC80 gives better formulation compare to NLC20. The small particle size of NLCs increase drug loading capacity, provide more entrapment efficiency, and deliver good bioavailability of drugs so its promising drug delivery system. (19)

Julia C. Schwarz et al. Nanocarrier one of the most exciting drug delivery systems for dermal treatment. Nanostructured lipid carrier (NLC) and solid lipid nanoparticle prepared by using ultrasonic dispersion. This technique is rapid and convenient because nanostructured lipid carrier (NLC) was also developed by excessive pressure homogenization with the same

physicochemical properties. Results of in vitro skin experiments show good penetration properties and excellent skin permeation for flufenamic acid formulation. In semisolid NLC preparation addition of polymer must be required for rheological properties. These rheological properties are beneficial to dermal drug delivery and not affect the skin permeation property. If prepared nanostructured lipid carrier (NLC) by ultrasound method gives more stability over seven months, compared to high shear homogenization method. (18)

3.2 Literature review on antifungal loaded SLN/NLCs

E. B. Souto et al. Clinical use of ketoconazole gives some types of adverse reactions in healthy adults like pruritus, stinging, and irritation. The purpose of this paper presents information of ketoconazole in SLN and NLC and stability of drug-loaded nanoparticles. Lipid particle prepared by using solid lipid and liquid lipids were compritol 888 ATO and alpha-tocopherol, respectively. Ketoconazole loaded SLN suspension physically stable during storage but chemically not stable because of ketoconazole degradation under light exposure. Ketoconazole loaded NLC suspension show increase particle size during storage. Both SLN and NLC were physically stable in gel formation under a light protected package**.** (20)

Shaimaa El-Housiny et al. SLN is an essential formulation to treat topical disorders like a fungal infection. The purpose of this paper presents well known antifungal drug fluconazole loaded in SLN for treating Pityriasis Versicolor. compritol 888 ATO, precirol ATO5 used as a lipid and Cremophor RH40, Poloxamer 407 used as a surfactant during the preparation of SLN. In vitro release study and physiochemical properties of fluconazole loaded SLNs were observed. For rheological properties, SLN is incorporated in carbopol 934. Randomized controlled clinical trial performed on 30 diagnosed Pityriasis Versicolor patients and compared with marketed product Candistan1%cream. Take observation four weeks during clinical trials and compare results. Fluconazole SLN has a spherical shape so no aggregation between particles. The drug entrapment found between 55 to 83%. The zeta potential value found between 20 to 33 mV, so it indicates excellent stability of SLN. Compare to marketed product it’s give fourfold healing and super-fast therapeutic index in treatment of Pityriasis Versicolor. (21)

Prachi B. Shekhawat et al. Aim of this research paper was prepared clotrimazole loaded NLC and evaluation of this formulation. Clotrimazole SLN arranged by a hot excessive shear homogenization process. Oleic acid selected as liquid lipid, stearic acid as solid lipid, a blend of surfactants: poloxamer 188, Sodium lauryl sulfate, tween80, and lecithin used as a stabilizer. NLC characterized by laser diffraction technique all NLCs are spherical in shape and size between 220 to 300 nm. All formulation in amorphous phase this analyseds by DSC. In vitro penetration study performed using Franz diffusion cell clotrimazole gives good permeation. Compare to the marketed formulation, clotrimazole NLC increases skin target effect, so NLC of clotrimazole is a promising carrier for fungal infection treatment. (22)

Gajanan S Sanap et al. The study aimed was prepared and evaluate NLC loaded miconazole nitrate for the cure of fungal infection. Miconazole nitrate loaded NLC made by using hot high-pressure homogenization (HPH) technique. Miconazole nitrate NLC batch considered for entrapment efficiency, particle size, zeta potential, SEM, TEM, and DSC. This miconazole loaded NLC incorporated in carbopol gel for easy to apply, and it further evaluated for rheological properties, particle size, texture analysis, ex vivo permeation readings, in vitro drug release. Drug loaded NLC average particle size minus than 330 nm, and PDI was less than 0.350. After 3 months of storage semi-solid system showed the particle size minus than 250 nm and PDI less than 0.350. The marketed gel gives a rapid release of drugs with a lag time of 0.5 hours, and miconazole NLC provides a slow release of drug with lag time 1 hour. Drug release in high amount compared to marketed formulation of miconazole nitrate on abdominal skin of rats. Research results conclude it’s a successful formulation because of miconazole NLC hydrogel increase encapsulation efficiency, sustained release, good relief from fungal infection and excellent stability during storage. (23)

4.
MATERIALS AND METHODOLOGY

4.1 LIST OF MATERIAL

Luliconazole drug is gifted by intas pharmaceuticals limited, Ahmedabad. Compritol 888 ATO was obtained as a gift sample by Gattefosse India Pvt Ltd. Alpha tocopherol received as a gift sample from chemzone pharma limited, Ahmedabad. Tween 80 received from Sicso lab private limited. Capriole 90 obtained as a gift sample by Gattefosse India Pvt Ltd.

4.2 LIST OF EQUIPMENT

Table4.1: Types of equipment

Types of equipment	**Name of the company**
Centrifuge	Remi R24, India
UV visible apparatus	Shimadzu 1800uv., Japan
Optical microscope	Olympus iLED CX21., India
(TEM)Transmission Electron Microscope	TEM200kV, Technai20, Phillips., Holland
High-Speed Homogenize	IKA,T25, Digital Ultra Turrax

4.3 METHODOLOGY:

4.3.1 PREFORMULATION STUDY

Melting Point

Take the one end sealed capillary and filled the compound in this capillary. Take one beaker filled with the paraffin after beaker placed on the burner. Sampled filled capillary tie with thermometer with the help of thread after that thermometer placed in the beaker. Slowly heat the beaker with the help of burner. At one temperature solid sample start to melt. Noted down the range of melting point of a solid sample.

Table4.2: Melting point of drug

Reference melting point of drug	Observed melting point
148 to 156 °C	155 °C

UV spectra of drug

- **Drug calibration curve in methanol**

  10 mg drug dissolved in 100ml methanol solvent with the help of sonication. This solution called 100ppm stock solution of luliconazole in methanol. This stock solution was further diluted by methanol and prepared 3ppm, 5ppm, 7ppm, 9ppm, 11ppm, 13ppm and 15ppm. All different concentration solution analyses by UV spectroscopy. Lambda max of luliconazole was 295nm observed in UV spectroscopy, so it indicates a drug is in pure form

- **Drug calibration curve in chloroform: methanol**

  10 mg drug dissolved in a mixture of chloroform: methanol(2:1) 100 ml and prepared 100 ppm stock solution. This stock solution was further diluted by a combination of chloroform: methanol and prepared 88ppm, 8.5ppm, 9ppm, 9.5ppm, 10ppm, 10.5ppm. Those all solutions evaluated by UV spectroscopy at 295 lambda max.

4.3.2 SELECTION OF EXCIPIENTS

❖ Screening of solid lipid-based on solubility

Weighed all lipids 1 gm and heat until its melt after that add 2mg luliconazole drug in lipids. When 2mg drug was completely soluble after that again, add 2mg drug in lipids. At last final solid lipid selected, which gives more drug soluble.

❖ Screening of liquid lipid-based on solubility

As per solid lipid here weighed all 1gm liquid lipid and heat until its melt after that add 2mg luliconazole drug in lipid. When 2mg drug was completely soluble after that again, add 2mg drug in lipids. In the end, the final liquid lipid selected, which gives more drug soluble.

❖ The ratio of liquid lipid and solid lipid

Make a different ration of liquid lipid and solid lipid-like 50:50, 40:60, 30:70, 20:80, 10:90, 5:95. Mix proper ratio of liquid lipid and solid lipid with the help of vortex and

during vortex heat also provide by the hot plate. This process is done until 1 hour after that all sample store at 24 hours. Next day with the help of a filter paper check stain and no stain ratio. No stain ration selected for further experiments.

- **Screening of surfactant**

A less soluble drug in surfactant selected for further studies because surfactant is an outer layer of nano vesical. They prevent drug expulsion from vesical, so the drug remains in the lipid portion.

4.3.3 FORMULATION METHOD

- **Preparation of NLC**

Weighed accurately solid lipid and liquid lipid ratio and transfer it into the beaker. Heat until its melt with under constant stirring help of stirrer. After complete melt of lipid transfer accurate weighed drug in a beaker. After the complete soluble of the drug, add a surfactant in the lipid phase. Selected surfactant dissolves in distilled water and prepared aqueous phase. The temperature of the aqueous phase more compares to the lipid phase. When both reach at temperature after that lipid phase transfer into aqueous phase under high shear homogenization. Homogenizer helps to reduce the size of particle and convert it into a nanoparticle.

- **Preparation of gel**

Carbopol 937 used as a gelling agent. 1% w/v gelling agent adds into NLC batch dispersion under constant stirring until completed dissolve. After the completed dissolve of the gelling agent, NLC dispersion converted into gel. This gel further evaluated based on characterization

5. EXPERIMENTAL /PRACTICAL WORK

5.1PREFORMULATION

a. Melting point

- With the help of the capillary method to determine the melting point of luliconazole drug.

Table5.1: Melting point

Reference melting point of drug	Observed melting point
148 to 156 °C	155 °C

- The observed melting point was near to reference melting point

b. Drug calibration curve in methanol

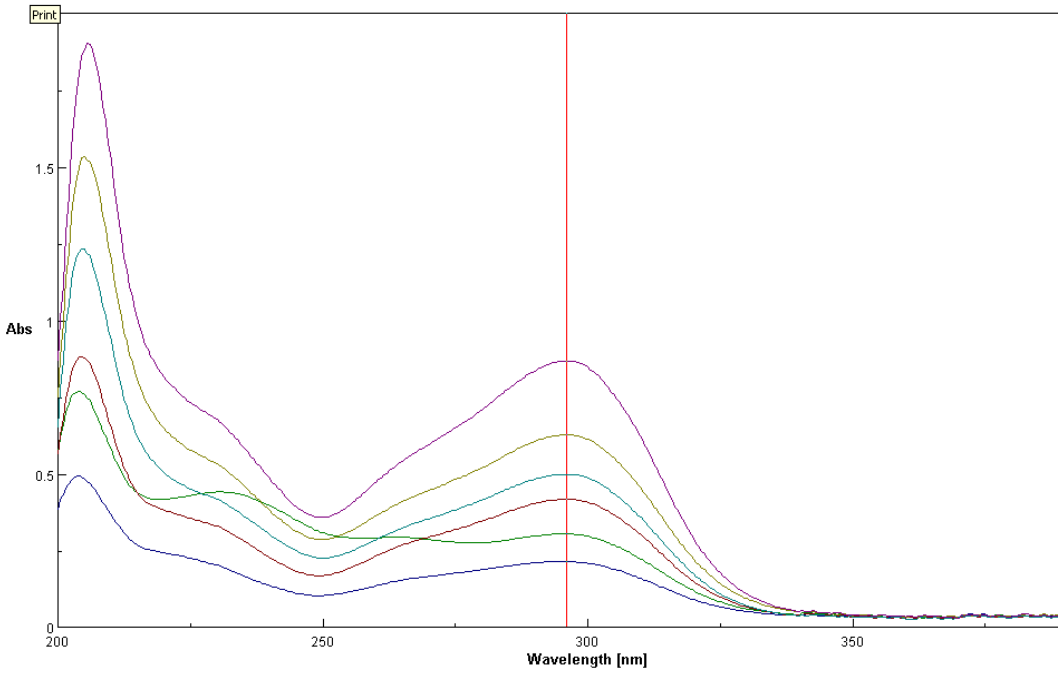


Figure 5.1: Overlay peak of UV absorption of luliconazole in methanol λmax=295 nm

Reading observed in UV spectroscopy

Table5.2: UV spectroscopy reading of luliconazole in methanol

Con	Abs1	abs2	abs3	Avg
0	0	0	0	0
3	0.20091	0.21766	0.215	0.21119
5	0.31937	0.30797	0.3113	0.31288
7	0.39552	0.42065	0.3973	0.40449
9	0.51052	0.50169	0.505	0.505737
11	0.60506	0.62995	0.609	0.61467
13	0.70106	0.74223	0.7108	0.71803
15	0.76216	0.87079	0.8151	0.816017

- **Calibration curve of the above reading**

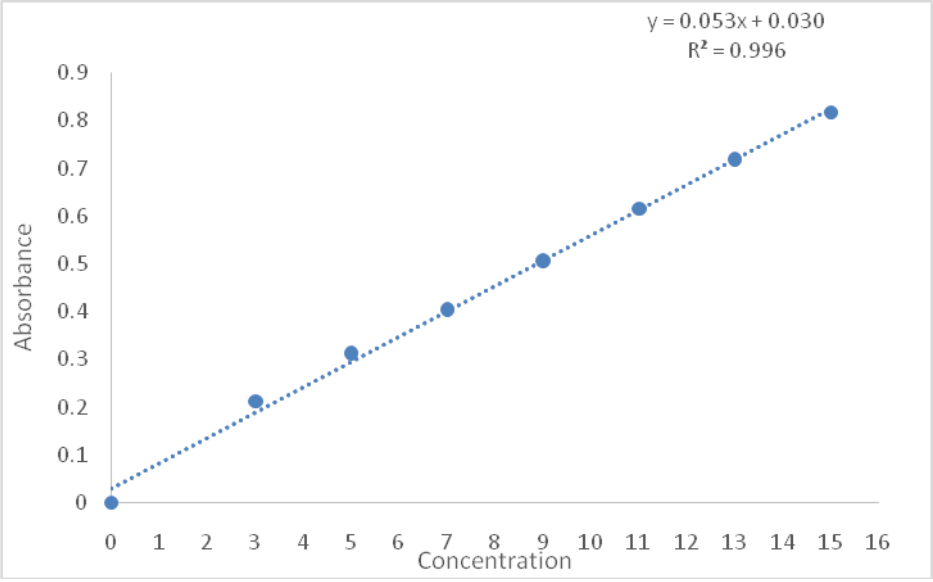


Figure 5.2: drug calibration curve in methanol

Regression Analysis

Table5.3: Regression analysis

Parameters for regression	Value
Slope	0.0506
Intercept	0.0562
Correlation coefficient	0.9996

c. Drug calibration curve in chloroform: methanol

- **Reading observed in UV spectroscopy**

Table5.4: UV spectroscopy reading of luliconazole in chloroform: methanol

Con	Abs1	Abs1	Abs1	Avg
0	0	0	0	0
8	0.379	0.375	0.378	0.377333
8.5	0.43555	0.45333	0.48744	0.458773
9	0.51355	0.52134	0.5316	0.522163
9.5	0.54462	0.53314	0.5469	0.541553
10	0.56849	0.5662	0.5765	0.570397
10.5	0.689	0.6599	0.697	0.681967

- **Calibration curve of the above reading**

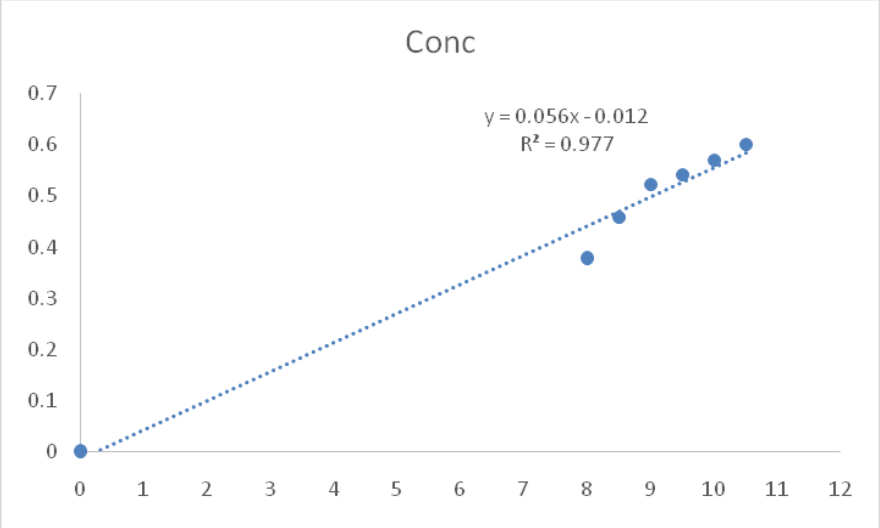


Figure 5.3: drug calibration curve in chloroform: methanol

Regression Analysis

Table5.5: Regression analysis

Parameters for regression	Value
Slope	0.1073
Intercept	0.467
Correlation coefficient	0.947

5.2 SELECTION OF EXCIPIENTS:

1. Solubility of drug in solid lipid

- Selected solid lipid gives more solubility of the drug compared to others lipids

Table5.6: Drug solubility in solid lipid

Solid lipids	Quantity of drug soluble (mg drug/ gm lipid)
Compritol 888 ATO	36
Glyceryl monostearate	2
Stearic acid	28
Precirol ATO 5	28

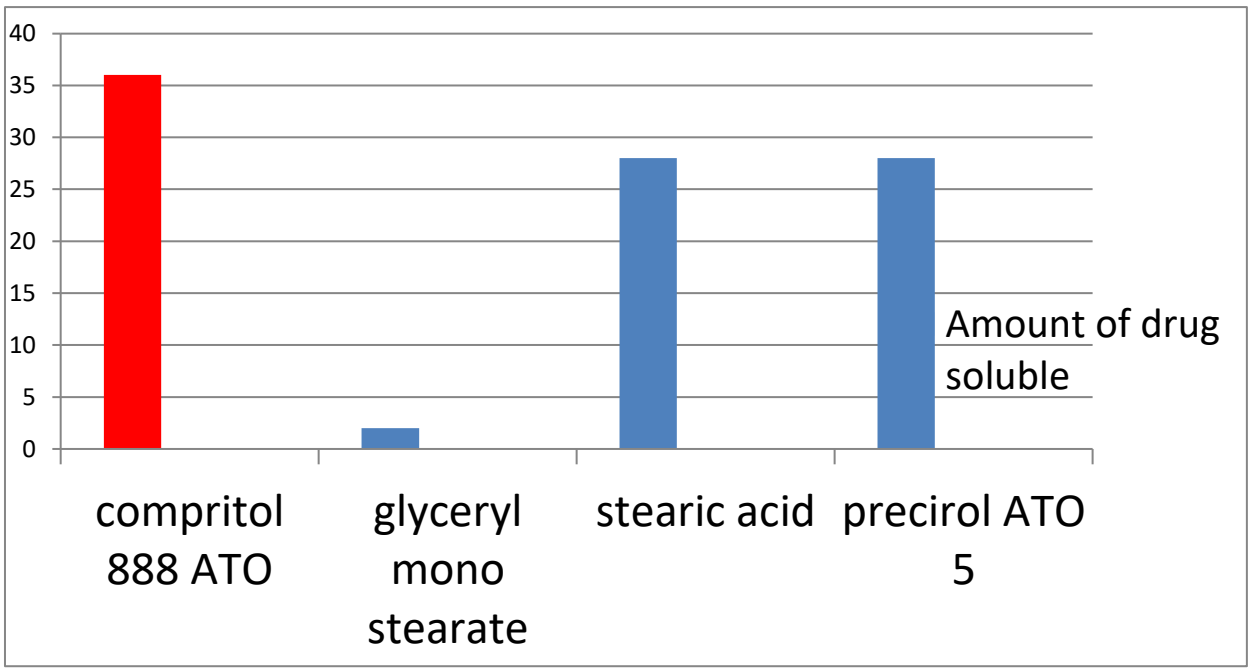


Figure 5.4: Drug solubility in solid lipid

- Compritol gives highest drug solubility compare to others lipids, so it's selected for future studies

2. Solubility of drug in liquid lipid

- Selected liquid lipid gives more solubility of the drug compared to others lipids

Table5.7: Drug solubility in liquid lipid

liquid lipids	Quantity of drug soluble (mg drug/ gm lipid)
Alpha-tocopherol	20
Corn oil	10
Olive oil	12
Isopropyl myristate	12

Isopropyl myristate (IPM)	16
Labarafill	4
Maisine	4
Sesame oil	4
Coconut oil	4
Oleic acid	4

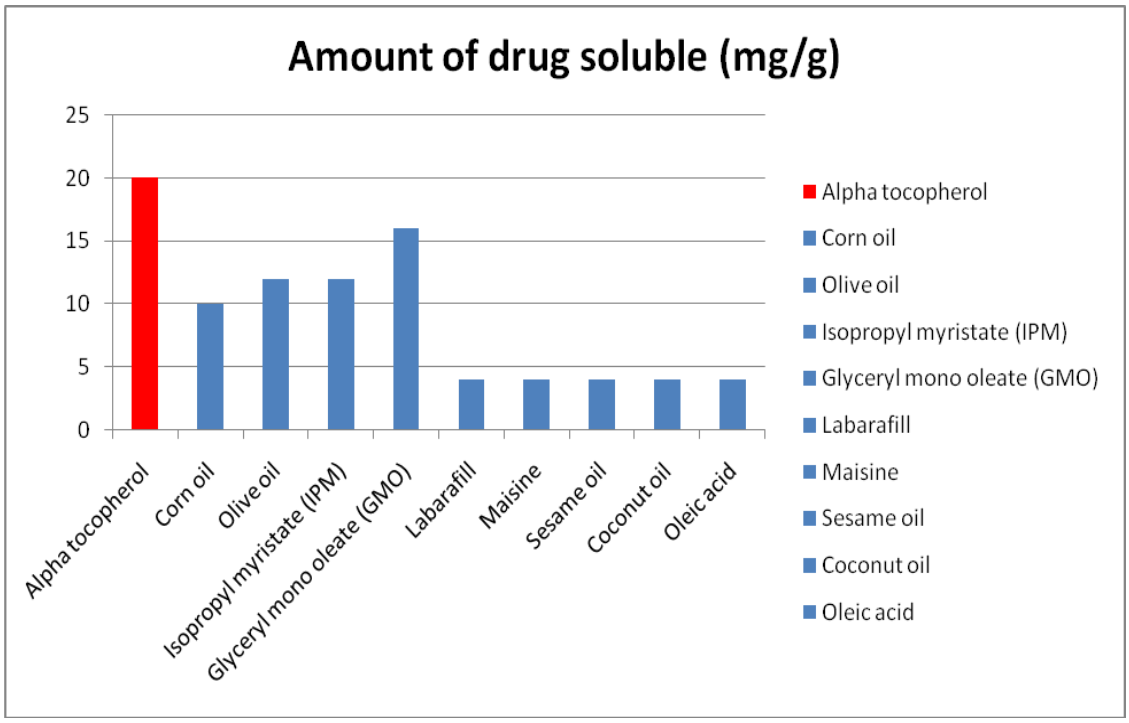


Figure 5.5: Drug solubility in liquid lipid

- Alpha-tocopherol gives highest drug solubility compare to others lipids, so it's selected for future studies

3. Selection ratio for liquid lipid: solid lipid

- Take the different amount of liquid lipid and solid lipid and mix it by using vortex after 24 hours check it gives stain or no stain. No stain ratio selected for further experiments.

Table5.8: Ratio for liquid lipid: solid lipid

liquid lipid: solid lipid ratio	Staining intensity
50:50	Intense
40:60	Intense
30:70	Intense
20:80	No stain
15:85	No stain
10:90	Intense
5:95	Intense

- 15:85 ratio of alpha-tocopherol: compritol 888 ATO: was selected for further study because it gives no stain

4. Screening different Type of Surfactant

- A less soluble drug in surfactant selected for further studies because surfactant is an outer layer of nano vesical. They prevent drug expulsion from vesical, so the drug remains in the lipid portion.

Table5.9: Drug solubility in surfactant

Types of surfactant	Quantity of drug soluble (mg/g)
Tween 20	4
Tween 80	2
Cremophor EL	4

Cremophor RH-40	4
Poloxamer P407	4
Poloxamer 188	2

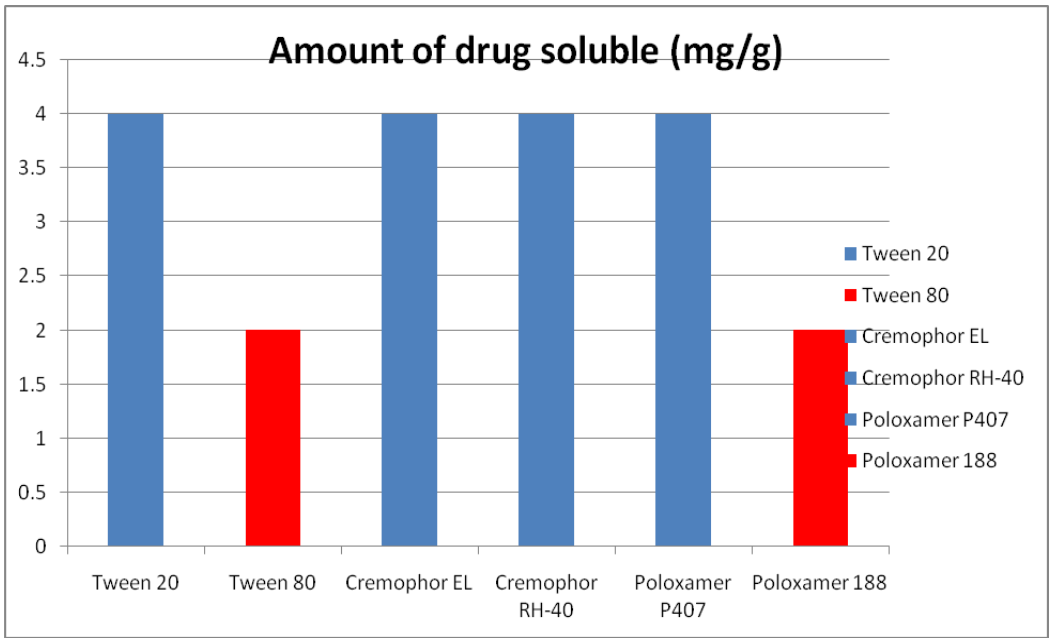


Figure 5.6: Drug solubility in surfactant

- Tween 80 selected as a surfactant for further trails because of it gives less soluble of a drug.

5.3 PRELIMINARY TRIALS

1. **Effect of surfactant on the formulation**

- Method of procedure: high shear homogenization for 13k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg.

Table5.10: Effect of surfactant on the formulation

Batch no	Con. of surfactant	Types of surfactant	Microscopic observation	Stability
1	1%	Poloxamer 188	Highly polydispersed particle and Drug crystals seen.	Unstable

2	1%	Tween80	Spherical monodispersed particle and no drug crystal seen	Stable

- Tween 80 was selected because it is a stable batch compared to poloxamer 188.

2. Surfactant concentration effect on formulation

- Method of procedure: high shear homogenization for 13k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg. Tween80 select as a surfactant with different concentration.
- At 13,000 rpm HSH speed and 10 minutes not able to reduce particle size.
- Particle size not in rang and PDI also inadequate for this batch.

3. Homogenization speed effect on formulation

- Method of procedure: high shear homogenization for 13k, 15k, 17k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg. 3%tween 80 as surfactant
- Here change in HSH speed also not gives good results for this formulation, PDI and Z-average even not in a proper range.

4. Co-surfactant effect on formulation

- Method of procedure: high shear homogenization for 13k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg. 3%tween 80 as a surfactant, add different types of co-surfactan
- Here capriol 90 and plurol oleique gives excellent results compared to other co-surfactant, so it is selected to further studies.

5. Co- surfactant continuous and varying concentration of surfactant

- Method of procedure: high shear homogenization for 13k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg. Tween 80 as a surfactant with varying concentration. Capriole 90 adds as co-surfactant.
- 3% and 5% of capriole 90 batches give good results compared to 1% batch of capriole 90.

6. Co- surfactant continuous and varying concentration of surfactant

- Method of procedure: high shear homogenization for 13k and 10 minutes. Liquid lipid: solid lipid ratio 15:85, 500mg total lipid mixture. Drug load 9mg. Tween 80 as a surfactant with varying concentration. Plurol oleique adds as co-surfactant.
- 3% and 5% of plurol oleique batches give good results compared to 1% batch of plurol oleique. 3% co-surfactant tween80 with capriol90 provides excellent results,

06. CONCLUSION

- NLC of luliconazole was successfully prepared and as per the reference optimized and evaluated with different parameter.
- The particle size of luliconazole NLC was 49.2nm observed, so it rang in good particle size.
- PDI of luliconazole observed 0.351, so it is in good rang of PDI.
- During microscopic observation of luliconazole, NLC was Spherical monodispersed particle and no drug crystal seen.
- Luliconazole NLC has excellent stability compared to the marketed formulation of luliconazole.

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