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PREPARATION & OPTIMIZATION OF ANTIFUNGAL NOVEL TOPICAL DRUG DELIVERY SYSTEM

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Abstract: The present study is to optimize and characterize a Nanostructured Lipid Carrier (NLC) loaded transdermal drug delivery system loaded by antifungal drug. The goal of this research is to optimize the NLCs and to study its potential for the topical delivery of Luliconazole by prolonging the drug release through the NLC transdermal gel. Preformultion studies for the drug and excipients studies are carried out by IR. Pre-optimization studies were carried out for the selection of suitable formulation variables from lipids, surfactant and process variables from ultrasonication time. From the selected variables, 15 NLC formulation were designed by using Solvent diffusion technique and optimized based on the impact of independent variable on dependent variables like Particle Size and percentage Entrapment Efficiency (% EE) by applying Box- Behnken design utilizing multiple linear regression method (Design expert software). The selected optimized NLC formulation was fabricated into transdermal gel. It was evaluated for viscosity, Drug content, pH, Spread ability and In vitro diffusion studies.

INTRODUCTION: In the present situation, conventional medication is investigating a novel drug delivery system due to the defective factors like less solubility, reduced absorption, more fluctuation plasma drug concentration and variation in concentration of drug in plasma and also due to food – drug interaction, patient related factors like improper prescription, no patient compliance, more toxicity, which playing a vital role in loss of desirable in-vivo pharmacological effect. These consequences lead to failed conventional delivery system which paved way for novel drug delivery system like Nanostructured Lipid carrier (NLC)**.** The use of lipid as a delivery or carrier system for less water soluble, lipophilic medicines (BCS Class II pharmaceuticals like Luliconazole) results in a possible improvement in solubility and bioavailability of drugs in the aforesaid class. In this era, lipid nanoparticles like NLC are the potential carrier than existing lipid particulate systems like Liposome and Polymeric Nanoparticles. NLC ensures good stability both physically and chemically, excellent drug loading capacity, good release controlling carriers and it can use in various administrative routes like oral, ocular, parenteral, dermal, rectal and pulmonary.

NLCs shows the following unique properties such as

- Good physiochemical diversity compared to all other lipid carrier system
- Good biocompatibility when compared to Solid Lipid Nanoparticles (SLN)
- High potential ability to increase the bioavailability of poorly aqueous soluble drugs
- Good target ability by selective lymphatic uptake mechanism

• Feasibility of scaling-up to large scale.

Nano structured lipid carrier: Nanostructured lipid carriers (NLCs) are the novel drug carrier and these are the generation next to solid lipid nanoparticles (SLNs). NLC carrier contains two mixtures of solid lipid connected with the spatially arranged liquid lipid phase. Due to this spatial alternative arrangement of solid lipid and liquid lipid the drug loading efficiency has enhanced, and also drug load will overcome the crystallinity character of lipid matrix which has high ability to convert solid lipid to crystals. In NLC the particle will be in amorphous form due to presence of both solid and liquid lipid in the form of chain or in blocks, which prevents the expulsion of drug in recrystallization state during storage. NLC acts as a good alternative carrier for conventional systems such as solutions, suspensions and ointments. NLC is a micellar colloidal drug carrier nanoparticle dispersions consist of particles in the range of 10–500nm in diameter. To obtain a good NLC matrix the solid lipids and liquid lipids are mixed together. Due to presence of oil in NLC there will be a melting point depression will be there when compared to SLN. Solid lipid and liquid lipid ratio in NLC can be extended upto 95%. NLC are not spherical in shape as other lipid carrier. They have liquid lipid droplets embedded in solid lipid particles which bind together with the help of surfactatnt. Usually they are seen in blocks or in chain form as shown in Figure 1.1

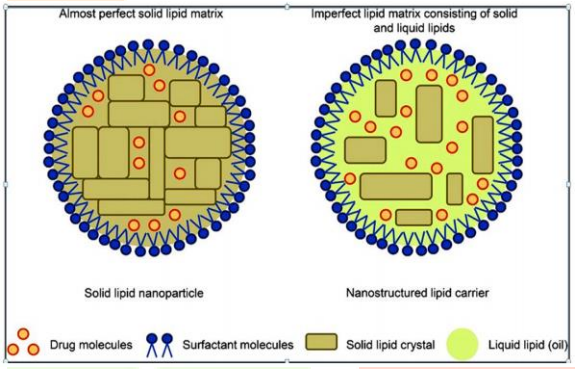


Figure 1.1: Difference between the structure of SLN and NLC

Advantages of NLC:

- Excellent loading efficiency, good permeability and high stability.
- It protects the sensitive drug molecules from degradation like stomach acid degradation, enzymatic degradation.
- Due to slower degradation rate in in-vivo condition it provides desires and better controlled drug release.
- It provides superior protection to the loaded or encapsulated drugs.
- It shows excellent tolerability and acceptability due to its structural changes by solid lipid and liquid lipid modification.
- For its highly lipophilic in nature NLCs has been used to administer lipophilic drugs.
- It has more drug loading capacity, due to its structural mixture of solid and liquid lipids. So that they can't fit together very well as crystal form, (i.e.) it has more flaws in its molecular structure.
- Compared to polymeric and other nanoparticle, NLC has lower toxicity because of the absence of the solvents in the production process
- It use very low cost of excipients so that NLC formulation is cost effective and can ease to scale up the batch to manufacturing scale.
- It shows excellent tolerability in different biological environment condition like change in pH and temperature condition.

Disadvantage of NLC:

- Phase separation of lipid and aqueous phase takes place due to precipitation of tiny oily nano compartments in the liquid lipid phase.
- Risk of gelation, particle growth due to improper storage condition (10).

A Reverse Micellar method to form nanocarrier is subjected for the encapsulation of hydrophobic drugs in lipid nanoparticle, and to enhance its solubility. Surfactants in non-polar medium forms Reverse Micelles (RM) which acts as multimolecular entities to form nanoparticle.

NLCs have high ability to encapsulate hydrophobic molecules in an oily core of lipid nano droplets. Both hydrophobic and hydrophilic drugs can be encapsulated to form a stable lipid core. Therapeutic action of the drug molecule is effected by both the drugs and lipid carrier system. Lipid carriers are novel drug delivery system with high therapeutic efficacy, good stability, minimum toxicity and side effects with good patient compliance. Lipid carrier colloidal systems, due to their high stability, were used for drug targeting by lymphatic uptake mechanism and also for topical application. NLC shows small lipid platelet structure due to more oil content between solid lipids. However, both SLN and NLC possess numerous features which are advantageous for topical route of application. Different types of NLC are shown in Figure 1.2.

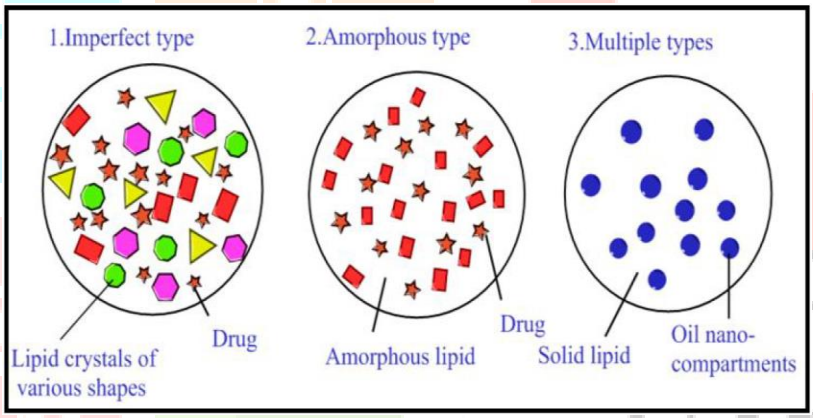


Figure 1.2: Different types of NLC: 1- Highly imperfect matrix; 2-Non-crystalline amorphous NLC (versus SLN with high crystallinity); 3-Multiple O/F/W type

Nanostructured Lipid Carriers Classification:

NLC - Imperfect type: Imperfect type of NLC was produced by blending both liquid lipid and solid lipid to enhance the capacity of drug loading efficiency. On fabrication of NLC, the liquid lipids nanoparticles were cooled to produce a crystalline molten state at room temperature condition.

NLC - Amorphous type: Amorphous type NLCs were formulated to reduce the expulsion of drug by blending different variety of lipids like isopropyl myristate and Hydroxyloctacosanyl hydroxy stearate. The lipid matrix will be in amorphous state.

NLC - Multiple type: Multiple type of NLC was produced by using more quantity of oil in solid lipid. It will enhance the solubilization of certain poorly soluble drugs. If drug is not soluble in lipid it will results in precipitate formation.

Materials used in Nanostructured lipid carriers

The materials used for preparation of NLCs are solid lipid, liquid lipid and surfactants. Initially they have to meet the compatibility between the mixtures for a good formulation. The solid lipid used in NLCs should contain melting point more than 40ºC. It should be biodegradable in nature and must have the stability on body temperature condition. Some of the materials used in NLC formulation are tabulated in Table 1.1.

Table 1.1: Materials used in NLC formulation

Solid Lipid	Liquid Lipid	Surfactant
Bees Wax	Compryol 90	Tween 20 Span
Compritol	Cetiol V	20,40,60 Sodium glycolate
Witepsol	Softigen	Polyvinyl alcohol
Cholesterol	Oleic acid	Tween 80,60,20
Carnauba Wax	Miglyol 181	Miranol ultra 32
Imwitor	Captex 225	Soya lecithin
Dynasan	Isopropyl myristate	Poloxamer 407,188 Soya &
	Liquid Paraffin	Egg lecithin,
		Phosphatidyl
		cholines

Mechanism of drug release in NLC: The medication or drug entrapped in NLC bypasses the hepatic metabolism and target the desired site of action. The drug encapsulated lipid molecule achieves the specific site of action by binding with the predetermined receptors and enters the channel reasonable for its activity. NLC get through the external layer of cell reasonable for its activity. NLC get through the external layer of cell membrane and they were consumed by the biological fluids of the cell. Due to interaction of biological fluids the lipid layer become feeble and nourishes the drug release in specific site of action in a controlled manner. The mechanism of NLC drug release is shown in Figure 1.3.

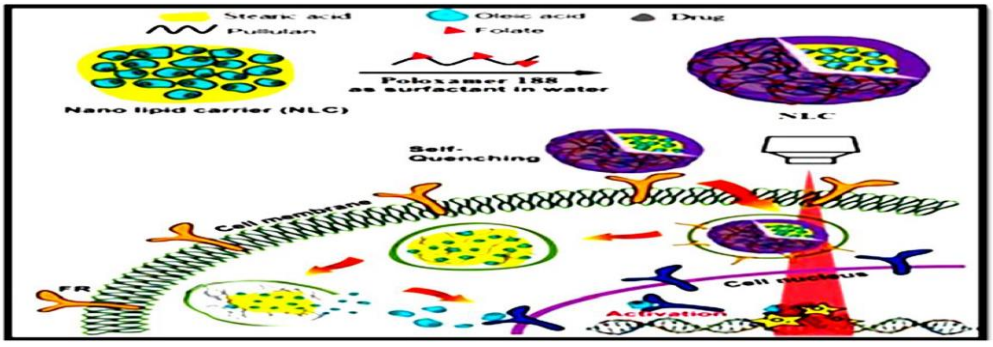


Figure 1.3: Mechanism of drug release from NLC

Method of preparation of NLC:

1. Hot homogenization method: This method is carried out by melting the lipid above its melting point. Then weighed quantity of drug is dispersed in the above melt. A pre-emulsion is formed. An aqueous phase containing surfactant was heated at same temperature and kept under homogenization by using high speed homogenizer. To this aqueous phase add lipid phase with high homogenization. Temperature above the melting point of lipid was

maintained throughout the process. There will be a reduction in particle size due to the maintenance of constant temperature and homogenization speed. The main disadvantage is when there is an increase in high temperature there is degradation in drug characteristics. After completion of homogenization process the formulation is cooled to room temperature, which leads to solidification of the particles.

2. Cold homogenization method: The drug is dispersed in a melted lipid and then it is cooled to room temperature, and then the cooled solid lipid ground to microparticles. Then the cold surfactant solution is prepared. To this cold surfactant solution the lipid microparticle solution is dispersed to get a pre suspension. Then the pre suspension is kept under homogenization at room temperature. The homogenization force and gravitation force exhibited in particles helps to break the lipid microparticles into NLC. In this process drug degradation will not occur when compared to hot homogenization technique.

3. Solvent evaporation technique: A weighed quantity of lipid is dissolved in an organic solvent like ethanol, methanol and cyclohexane. Prepared organic solvent is then emulsified with aqueous phase containing emulsifier or surfactant. The Final solution is kept in high- pressure homogenization for 10-15 min. Organic solvent is then removed from the emulsion mixture by evaporating the mixture under reduced pressure of about 40–60 mbar. NLC dispersion is formed after evaporation of organic solvent. NLC carrier is formed due to the precipitation of the lipid in the aqueous medium. The particle size diameter of about 25 nm can be achieved by this method.

4. Solvent emulsification-diffusion method: In this technique, the weighed quantity of lipid material is dispersed directly in organic phase. Emulsification process is done by dispersing the lipid material in aqueous phase containing surfactant, under high pressure by using homogenizer. Precipitation of the lipid in aqueous media is then obtained by the formation of Nanoparticles dispersion. By this technique 30- 100 nm particle size diameter can be obtained.

5. Micro emulsion based method: This method is based on micro emulsions dilution process. It is made by continuous stirring of optically transparent mixture at 65-70°C, which is normally made out of a fatty acid with low melting point containing surfactant, co-surfactant and water. The above hot microemulsion is dispersed into ice water under continuous mixing by magnetic stirrer. The proportion of hot microemulsion and cold water should be in the range of 1:50 and 1:25.

6. Double emulsion method: In this process, lipids get melted and the hydrophilic drugs that already dissolved in aqueous solution containing emulsifier or stabilizer is dispersed to get a primary emulsion. In the next step this primary emulsion is again dispersed into the water containing hydrophilic emulsifier under continuous stirring to get a double emulsion. The surfaces of lipid Nanoparticles are coated with lipid-PEG derivative in order to get a stabilized nanoemulsion. A major disadvantage of this technique is the formation of large number of microparticles.

7. Precipitation method: Glycerides are dissolved in an organic solvent such as chloroform and emulsified in an aqueous phase in this technique. The lipid precipitates to form NLC once the organic solvents have evaporated.

8. High speed homogenization followed by ultra-sonication method: Required quantities of drug, phospholipids are dissolved in suitable solvent like acetone and methanol solution containing desired quantity of fatty acids at 70°C under homogenization of 15000 rpm for 10 min to get a primary emulsion. This primary emulsion is further ultrasonicated for 15 minutes to get an O/W type of emulsion and also ultrasonication is done to avoid the crystallization of lipids. The finally obtained primary emulsion is then subsequently cooled down to room temperature with continuous stirring. The obtained NLC dispersions is lyophilized to get an amorphous NLC powders and stored in a desiccator for further studies.

Table 1.2: Methods for preparation of NLC and their importance during formulation

Sr. No	Methods for preparation of NLC	Importance
1	Hot Homogenization Method	➢ No use of organic solvents and feasibility to large scale production. ➢ The drug can be degraded due to application of high temperature. ➢ Due to elevated kinetic energy of the particles at homogenization speed it will leads to enhancement of particle size. Homogenous particle size
2	Cold Homogenization Method	➢ Highly thermolabile substance can be encapsulated. ➢ Drug degradation due to high temperature, distribution of drug into the aqueous phase during hot homogenization are overcome by this method
3	Micro emulsion technique	➢ The micro droplets already exist in the microemulsion, so no need of mechanical energy is required to achieve nanoparticles ➢ Cost effective technique
4	Solvent evaporation method	➢ 25 nm diameter of NLCs can obtained by this technique ➢ No sophisticated equipment needed
5	Solvent emulsification-diffusion method	➢ 30 - 100 nm diameter of NLCs can obtained by this technique ➢ Suitable for thermolabile drugs
6	Double emulsion method	➢ This technique is used mainly for hydrophilic drugs ➢ Main disadvantage is formation of more microparticle
7	Precipitation technique	Simplified and reproducible method for formulation of NLC
8	High-speed homogenization followed by ultra-sonication metho	It is a low temperature solidification method, it leads to good feasibility and cost effect NLC formulation

Mechanism of NLC in enhancing bioavailability: The behavior of NLCs in body to enhance bioavailability is discussed in the following Table 1.3

Table 1.3: Mechanism of NLC in enhancing bioavailability

Mechanism of NLC	Function of NLC in enhancing bioavailability
Direct uptake	In GIT NLC is transport by intestinal lymphatic system. NLC has long chain triglycerides which stimulate the production of chylomicron, which absorbed efficiently through transcellular route. NLC are highly lipophilic compounds transported by intestinal lymphatic system that avoids first pass hepatic metabolism, which leads to enhancement of bioavailability.
Mucoadhesion	NLC adhere to the mucus membrane , so that there will be increasing in residence time of carrier in mucus leads to controlled release of drug , which further leads to enhancement of bioavailability
Mixed micelle formation	NLC lipid is similar to dietary lipids, which induces bile secretion. This lipid on enzyme degradation will form byproduct, which blended with bile to form a mixed micelle. This mixed micelle will enhance the solubility and permeation of drugs across the membrane, which leads to increase in bioavailability.
Increased permeability	By adding surfactant like Poloxamer there will be increase in permeability lead to enhancement of bioavailability.
Inhibits drug degradation	NLC inhibits drug degradation by protecting the drug from enzymatic and chemical degradation, which leads to increase in bioavailability.
NLC through skin	It bypasses the first pass metabolism and more permeation will leads to enhancement of bioavailability of drugs.

Application of Nanostructured Lipid Carriers:

1. It is, applied as topical formulation, as sustained oral dosage forms preparation, Inhalation type of drug delivery system, parenteral formulation, drug targeting, and potential way to treat cancer.

2. Treatments for skin diseases are done such as skin dermatitis, psoriasis and inflammations.

3. The carrier with high biocompatibility is used in cosmetic industries, pharmaceutical industries and biochemical industries.

4. Recently proteins, peptides, all types of hydrophilic and lipophilic drugs can be entrapped within this NLC carrier.

NLCs as topical drug delivery systems:

Topical drug application has been introduced since long time to achieve several purposes on different levels (skin surface, epidermis, dermis and hypodermis). However, several problems have been reported with the conventional topical preparations e.g. low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation. A lot of research groups paid attention to the topical application of the SLN and NLC. Many features, which these carrier systems exhibit for dermal application of cosmetics and pharmaceutics, have been pointed out. SLN and NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis.

Increase of skin occlusion

Following the evaporation of water from the lipid nanodispersion applied to the skin surface, lipid particles form an adhesive layer occluding the skin surface. Then hydration of the stratum corneum may increase which by reducing corneocyte packing and widening of the inter-corneocytes gaps can facilitate drug penetration into deeper skin strata. Occlusive effects appear strongly related to particle size. Nanoparticles have turned out 15-fold more occlusive than microparticles, and particles smaller than 400 nm in a dispersion containing at least 35% lipid of high crystallinity have been most potent.

Increase of skin hydration and elasticity: The reduction of transepidermal water loss (TEWL) caused by occlusion leads to an increase in skin hydration after dermal application of NLC or formulations containing them.

Skin as route of administration: Skin is the largest organ of the body, making up 16% of body weight, with a surface area of 1.8 m2. It has several functions, the most important being to form a physical barrier to the environment, allowing and limiting the inward and outward passage of water, electrolytes and various substances while providing protection against microorganisms, ultraviolet radiation, toxic agents and mechanical insults. There are three structural layers to the skin: the epidermis, the dermis and subcutis. Hair, nails, sebaceous, sweat and apocrine glands are regarded as derivatives of skin. Skin is a dynamic organ in a constant state of change, as cells of the outer layers are continuously shed and replaced by inner cells moving up to the surface. Although structurally consistent throughout the body, skin varies in thickness according to anatomical site and age of the individual.

SKIN ANATOMY

The epidermis is the outer layer, serving as the physical and chemical barrier between the interior body and exterior environment; the dermis is the deeper layer providing the structural support of the skin, below which is a loose connective tissue layer, the subcutis or hypodermis which is an important depot of fat.

1. Epidermis:

The epidermis is stratified squamous epithelium. The main cells of the epidermis are the keratinocytes, which synthesise the protein keratin. Protein bridges called desmosomes connect the keratinocytes, which are in a constant state of transition from the deeper layers to the superficial. The epidermis varies in thickness from 0.05 mm on the eyelids to 0.8±1.5 mm on the soles of the feet and palms of the hand. Moving from the lower layers upwards to the surface, the four layers of the epidermis are:

• Stratum Basale (basal or germinativum cell layer)

• Stratum Spinosum (spinous or prickle cell layer)

• Stratum Granulosum (granular cell layer)

• Stratum Corneum (horny layer).

Stratum Basale: The innermost layer of the epidermis which lies adjacent to the dermis comprises mainly dividing and non-dividing keratinocytes, which are attached to the basement membrane by hemidesmosomes.

Stratum Spinosum: As basal cells reproduce and mature, they move towards the outer layer of skin, initially forming the stratum spinosum. Intercellular bridges, the desmosomes, which appear as `prickles' at a microscopic level, connect the cells. They play a significant role in immune reactions of the skin, acting as antigen-presenting cells.

Stratum Granulosum: Continuing their transition to the surface the cells continue to flatten, lose their nuclei and their cytoplasm appears granular at this level.

Stratum Corneum: The final outcome of keratinocyte maturation is found in the stratum corneum, which is made up of layers of hexagonal-shaped, non-viable cornified cells known as corneocytes. In most areas of the skin, there are 10±30 layers of stacked corneocytes with the palms and soles having the most. Each corneocyte is surrounded by a protein envelope and is filled with water-retaining keratin proteins. The cellular shape and orientation of the keratin proteins add strength to the stratum corneum. Surrounding the cells in the extracellular space are stacked layers of lipid bilayers. The resulting structure provides the natural physical and water-retaining barrier of the skin.

2. Dermoepidermal junction/basement membrane: This is a complex structure composed of two layers. Abnormalities here result in the expression of rare skin diseases such as bullous pemphigoid and epidermolysis bullosa. The structure is highly irregular, with dermal papillae from the papillary dermis projecting perpendicular to the skin surface.

3. Dermis: The dermis varies in thickness, ranging from 0.6 mm on the eyelids to 3 mm on the back, palms and soles. It is found below the epidermis and is composed of a tough, supportive cell matrix. Two layers comprise the dermis: a thin papillary layer and a thicker reticular layer. The papillary dermis lies below and connects with the epidermis. It contains thin loosely arranged collagen fibres. Thicker bundles of collagen run parallel to the skin surface in the deeper reticular layer, which extends from the base of the papillary layer to the subcutis tissue. The dermis is made up of fibroblasts, which produce collagen, elastin and structural proteoglycans, together with immunocompetent mast cells and macrophages. Collagen fibres make up 70% of the dermis, giving it strength and toughness. Elastin maintains normal elasticity and flexibility while proteoglycans provide viscosity and hydration. Embedded within the fibrous tissue of the dermis are the dermal vasculature, lymphatics, nervous cells and fibres, sweat glands, hair roots and small quantities of striated muscle.

4 Subcutis: This is made up of loose connective tissue and fat, which can be up to 3 cm thick on the abdomen.

5 Blood and lymphatic vessels: The dermis receives a rich blood supply. A superficial artery plexus is formed at the papillary and reticular dermal boundary by branches of the subcutis artery. Branches from this plexus form capillary loops in the papillae of the dermis, each with a single loop of capillary vessels, one arterial and one venous. The veins

drain into mid-dermal and subcutaneous venous networks. Dilatation or constriction of these capillary loops plays a direct role in thermoregulation of the skin.

6 Nerve supply: The skin has a rich innervation with the hands, face and genitalia having the highest density of nerves. All cutaneous nerves have their cell bodies in the dorsal root ganglia and both myelinated and non-myelinated fibres are found. Free sensory nerve endings lie in the dermis where they detect pain, itch and temperature.

Derivative structures of the skin

1 Hair: Hair can be found in varying densities of growth over the entire surface of the body, exceptions being on the palms, soles and glans penis. Follicles are most dense on the scalp and face and are derived from the epidermis and the dermis. Each hair follicle is lined by germinative cells, which produce keratin and melanocytes, which synthesise pigment. The hair shaft consists of an outer cuticle, a cortex of keratinocytes and an inner medulla. The root sheath, which surrounds the hair bulb, is composed of an outer and inner layer.

2 Nails: Nails consist of a dense plate of hardened keratin between 0.3 and 0.5 mm thick. Fingernails function to protect the tip of the fingers and to aid grasping. The nail is made up of a nail bed, nail matrix and a nail plate. The nail matrix is composed of dividing keratinocytes, which mature and keratinise into the nail plate. Underneath the nail plate lies the nail bed.

3 Sebaceous glands: These glands are derived from epidermal cells and are closely associated with hair follicles especially those of the scalp, face, chest and back; they are not found in hairless areas. They are small in children, enlarging and becoming active at puberty, being sensitive to androgens. They produce an oily sebum by holocrine secretion in which the cells break down and release their lipid cytoplasm. The full function of sebum is unknown at present but it does play a role in the following:

• Maintaining the epidermal permeability barrier, structure and differentiation

• Skin-specific hormonal signalling

• Transporting antioxidants to the skin surface

• Protection from UV radiation.

4 Sweat glands: There are thought to be over 2.5 million on the skin surface and they are present over the majority of the body. They are located within the dermis and are composed of coiled tubes, which secrete a watery substance. They are classified into two different types: eccrine and apocrine.

• Eccrine glands are found all over the skin especially on the palms, soles, axillae and forehead. They are under psychological and thermal control. Sympathetic (cholinergic) nerve fibres innervate eccrine glands. The watery fluid they secrete contains chloride, lactic acid, fatty acids, urea, glycoproteins and mucopolysaccharides.

• Apocrine glands are larger, the ducts of which empty out into the hair follicles. They are present in the axillae, anogenital region and areolae and are under thermal control. They become active at puberty, producing an odourless proteinrich secretion which when acted upon by skin bacteria gives out a characteristic odour. These glands are under the control of sympathetic (adrenergic) nerve fibres.

Topical treatment of skin disease

Topical treatment of skin diseases is very attractive, since systemic load of active pharmaceutical ingredients (API) and thus also systemic side effects are reduced as compared to parenteral or oral drug administration. Hence application of drugs (preparations of API) to the skin surface was and still is not only used for skin disease but also for local antirheumatic therapy to control gastrointestinal side effect. Moreover, drug application to the skin surface avoids the major fluctuations of plasma levels typical for repeated administration of rapidly eliminated drugs while it also allows to circumvent the first passage of API through liver after intestinal absorption. Thus transdermal drug application has gained still increasing importance for systemic treatment, e.g. with drugs subject to extensive first-pass elimination such as glyceryl trinitrate or estrogens as well as for the sustained suppression of chronic pain. The main penetration barrier protecting humans both from excessive water loss and harm due to toxic agents and microorganisms from the environment consist of the horny layer formed by corneocytes due to apoptosis of keratinocytes during the passage of these cells from the basal layer of the epidermis to the surface. Below the dead horny layer there is the viable epidermis (50–100 μm) followed by the dermis (1–2 mm). Micrometer wide hair follicles reaching from the dermis or even the subcutis to the skin surface and passing through the epidermis are the most important appendages of human skin. Small particles can make close contact with superficial junctions of corneocyte clusters and furrows between corneocyte islands may favour accumulation for several hours allowing for sustained drug release. While this holds also true with liposomes, they are different in so far as they collapse which is considered as compromising percutaneous penetration. SLN and NLC should stay as they are for longer and remain at the application site because of a pronounced adhesive effect. Indeed, adhesion increases with decreasing particle size. Moreover, the drug is dissolved or finely dispersed within the lipid matrix of the carrier or adheres to the carrier surface both facilitating contact to the outermost skin layers. Moreover, a lipid film found covering the skin surface may facilitate dermal absorption because of an additional occlusive effect. Luliconazole is a newly added imidazole antifungal agent most commonly used against infections caused by Candida and Trichophyton spp. Luliconazole exerts antifungal action by inhibiting 14-alpha-lanosterol demethylase in the fungal cell wall. 14-alpha lanosterol demethylase is a membrane protein of CYP51 class in the cytochrome P450 superfamily of enzymes involved in ergosterol biosynthesis. Ergosterol is responsible for maintaining integrity of fungal cell membrane. Commercially, luliconazole is available as 1%w/w topical cream. The clinical efficacy of luliconazole is hindered by low aqueous solubility and hence, this in turn reduces the dermal bioavailability. In comparison to other azoles, luliconazole exhibits 1 to 4 fold lower MIC against Trichophyton species and is active against strains which are resistant to fluconazole. Various strategies have been explored to improve the topical delivery of anti- fungals. Use of permeation enhancers has several side effects like systemic toxicity. Many researchers have worked on nanoparticulate systems such as nanosponges and microemulsion based gel.

Lipid Nanoparticle for oral delivery of Raloxifen was investigated by **Punna Rao Ravi** et al., **(2014).** Punna Rao Ravi et al., designed Box–Behnken design to optimize the manufacturing conditions of formulation of lipid nanoparticle. Lipid nanoparticle was prepared by micro-emulsion technique. The following variables are used for optimization like type of surfactant (polysorbate 80), concentration of surfactant (1% and 5% w/v), temperature of surfactant solution (25 and 75 ºC), volume of external phase (10 and 30 ml), speed of homogenization (7500 rpm and 12500 rpm), time of homogenization (2 and 16 min), amount of lipid (0.5 and 1.5 g), time of ultrasonication (5 and 15 min), ultra-sonication amplitude (70% and 100%), ultrasonication pulse (continuous and pulse mode) and temperature during homogenization (60 and 75 ºC).

DRUG AND EXCIPIENT PROFILES

Table 1.4: Drug Profile of Luliconazole

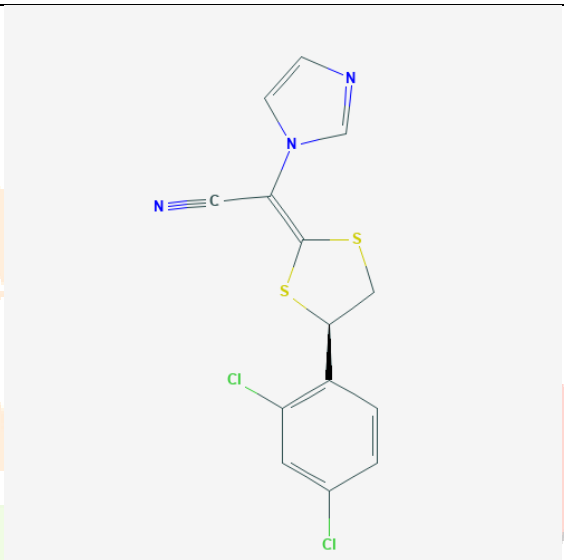
Luliconazole	
IUPAC Name	(2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile
Description	Off-white to yellowish crystalline powder
Molecular formula	C14H9Cl2N3S2
Molecular weight	354.3g/mol.
Melting point	152 °C
Chemical Structure	
Solubility	Methanol, Acetone
Category	Luliconazole is an antifungal that belongs to the azole class.
Log P	4
Bioavailability	1%
Half life	27.5 h
Dissociation constant (Pka)	6.34
Mechanism of action	Luzu (luliconazole) Cream 1% is an antifungal that belongs to the azole class. Luliconazole appears to inhibit ergosterol synthesis by inhibiting the enzyme lanosterol demethylase.
Adverse effect	Difficult to breathing, swelling of face, lips, tongue, or throat. Severe burning, redness, swelling, or stinging after applying the medicine.

Table 1.5: Campritol ATO 888

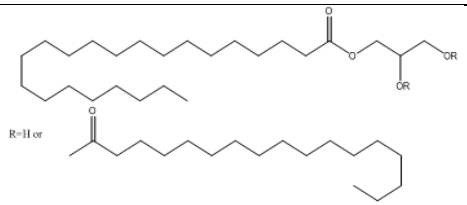
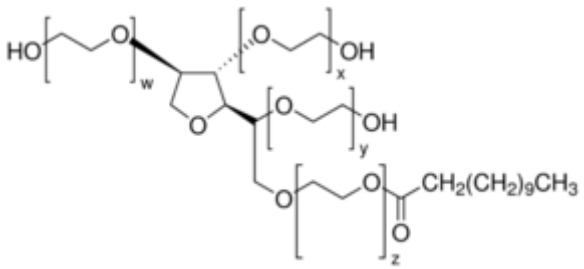
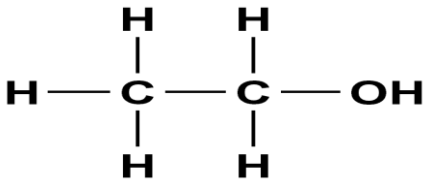
Campritol 888	
IUPAC Name	2, 3-dihydroxypropyl docosanoate
Synonym	Glyceryl behenate
Molecular wt.	414.671g/mol
Molecular Formula	$C_{25}H_{50}O_4$
Structure	
Physical and chemical properties	Appearance : Fine powder Color: Gardner Scale Odor: Faint Melting point : 65-77 °C HLB: 2 Solubility**:** Chloroform and Dichloromethane Insoluble : Ethanol, Hexane, Mineral Oil and Water
Use	Surfactant, emulsifying agent and viscosity-inducing agent in emulsions or creams.

Table 1.6: Profile of Tween 20

Tween 20	
Description	Tween 20 is a polyoxyethylene sorbitol ester that belongs to the polysorbate family. It is a nonionic detergent having a molecular weight of 1,225 daltons, assuming 20 ethylene oxide units, 1 sorbitol, and 1 lauric acid as the primary fatty acid.
Chemical name	Tween 20
Molecular Formula	C26H50O10
Molar mass	1,227.54 g/mol
Structure	
Other names	Polysorbate 20.

Physical and chemical properties	Form: Viscous Liquid Color: Yellow To Yellow-Green Freezing point: 110 °C Boling point:> 100°c Density: 1.1 g/ml Vapor pressure: < 1.4 hPa Storage temp.: Store at RT. PH: 6-8 HLB : 16.7 Solubility: Soluble in water, ethanol, methanol, ethyl acetate and dioxane
Uses	TWEEN 20 is a nonionic detergent widely used in biochemical applications. It has been used as an emulsifying agent for the preparation of stable oil-in-water emulsions. TWEEN 20 has been used in pre-extraction of membranes to remove peripheral proteins (used at 2% for extraction of membrane-bound proteins).

Table 1.7: Profile of Ethanol

Ethanol	
Nonproprietary Names	**BP:** Ethanol (96%) **JP:** Ethanol **PhEur:** Ethanol (96 per cent) **USP:** Alcohol
Chemical Name	Ethanol
Synonym	Ethanolum (96 per centum); ethyl alcohol; ethyl hydroxide; grainalcohol; methyl carbinol.
Empirical Formula	C_2H_6O
Molecular Weight	46.07
Structure	
Physical and Chemical Properties	**Boiling point**-78.15^{0}C **Flammability-** It is readily flammable, burning with a blue, smokeless flame. **Flash point**-148^{0}C (closed cup) **Solubility-** Alcohol is miscible with chloroform, ether, glycerin, and water (with rise of temperature and contraction of volume). **Specific gravity-** 0.8119–0.8139 at 20^{0}C

Functional Category	Antimicrobial preservative; disinfectant; skin penetrant; solvent.
Description	Alcohol is a clear, colorless, mobile, and volatile liquid with a slight, characteristic odor and burning taste.
Uses	Emulsifying agent, Solubilizing agent, Wetting agent
Applications	
Ethanol and aqueous ethanol solutions of various concentrations are widely used in pharmaceuticalformulations and cosmetic. Although ethanol is primarily used as a solvent, it is also employed as a disinfectant andin solutions as an antimicrobial preservative. Topical ethanolsolutions are used in the development of transdermal drug deliverysystems as penetration enhancers. Ethanol has also been usedin the development of transdermal preparations as a co-surfactant.	

Table 1.8 Profile of Capryol 90

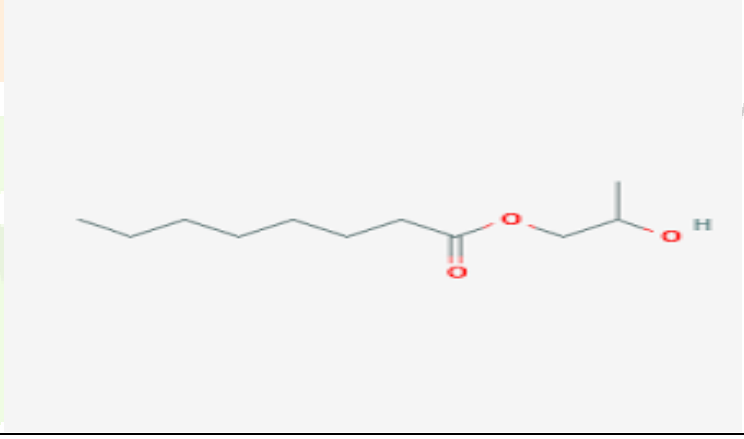
Capryol 90	
IUPAC Name	2-hydroxypropyl octanoate
Molecular Formula	C11H22O3
Structure	
Physical and Chemical Properties	**Molecular Weight: 202.29** **Density**: 0.46 g/mL **Molar mass**: 202.29g/mol
Description	Capryol 90 (propylene glycol monocaprylate) is a nonionic water-insoluble surfactant used as cosurfactant in oral lipid-based formulation. Cosurfactant and solubilizer in topical dosage forms.
Applications	
Is a colourless, viscous, colorless liquid. It is miscible with water alcohol, and many solvents. This versatile PO (propylene oxide) derivative has wide range of applications including industrial solvents, paint and coating solvents, polyester and alkyd resins, antifreeze coolants, heat transfer fluids, deicing fluids, plasticizers, detergents and surfactants, and bactericide.	

Table 1.9 Profile of Oleic acid

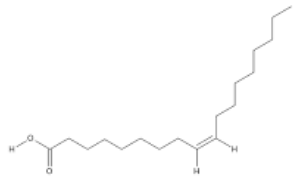
Oleic Acid	
IUPAC Name	(Z)-octadec-9-enoic acid
Molecular Formula	C18H34O2
Structure	
Physical and Chemical Properties	**Boiling point:** 286°C at 13.3 kPa (100 mmHg) (decomposition at 80–100°C) **Density:** 0.895 g/cm^3 **Melting point** 13–14°C **Solubility:** Miscible with benzene, chloroform, ethanol (95%), ether, hexane, and fixed and volatile oils; practically insoluble in water. **Viscosity:** 26 mPas (26 cP) at 25°C
Description	A yellowish to pale brown, oily liquid with a characteristic lard-like odor and taste. Oleic acid consists chiefly of (Z)-9-octadecenoic acid together with varying amounts of saturated and other unsaturated acids. It may contain a suitable antioxidant.
Applications	
It is included in the FDA Inactive Ingredients Database (inhalation and nasal aerosols, tablets, topical and transdermal preparations). It is included in nonparenteral medicines (metered dose inhalers; oral capsules; oral prolonged release granules; topical creams and gels) licensed in the UK.	

Table 2.0 Profile of Acetone

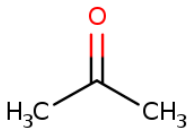
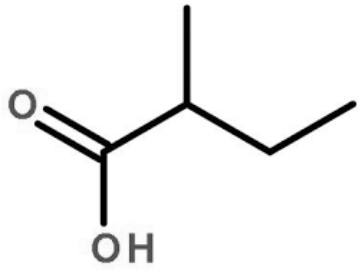
Acetone	
IUPAC Name	Propan-2-one
Molecular formula	C_3H_6O
Structure	
Physical and Chemical Properties	Appearance: Colourless liquid Odor: Pungent, irritating, floral, cucumber like Melting point : −94.7 °C (−138.5 °F; 178.5 K) Boiling point : 56.05 °C (132.89 °F; 329.20 K) Solubility in water: Miscible Solubility: Miscible in benzene, diethyl ether, methanol, chloroform, ethanol. Density: 0.7845 g/cm3 (25 °C) log P: −0.16
Description	Acetone is a colorless, flammable liquid that evaporates easily. It is an organic compound because carbon atoms are present in acetone's chemical formula, which is (CH3)2O. It consists of three carbon atoms, six hydrogen atoms, and one oxygen atom.
Used	It is widely used because it can easily mix with water and evaporates quickly in the air. Acetone is widely used in the textile industry for degreasing wool and degumming silk.

Table 2.1 Profile of Carbopol

Carbopol 934 or Carbomer	
Name	Carbomer 934 (NF); Polyacrylic acid; Carbopol 934 (TN)
Molecular formula	(C3H4O2)n
Structure	
Physical and Chemical Properties	**pH:** 2.5–4.0 for a 0.2% w/v aqueous dispersion; **Melting point:** Decomposition occurs within 30 minutes at 260oC **Solubility:** Swellable in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are threedimensionally crosslinked microgels.
Description	Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powders with a characteristic slight odor.

Used	Carbomers are used extensively in nonparenteral products, particularly topical liquid and semisolid preparations

Preformulation Studies:

Prior to the development of dosage forms, it is essential that certain fundamental, physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. These properties could affect drug performance and development of an efficacious, stable and safe dosage form

Identification and Characterization:

Organoleptic Evaluation:

Both drugs were evaluated for colour, odour and texture.

Melting Point Range:

The melting point of compound is the temperature at which it changes from a solid to liquid. This is a physical property often used to identify compounds. Melting point of the Luliconazole was determined by Thiel's tube method. The melting range was recorded from first start to completely melt of sample.

Ultra-Violet Absorption Maxima:

A standard solution (1µg/ml) of Luliconazole was prepared in methanol and scanned by UV-VIS Double beam spectrophotometer between 200 to 400nm.

Solubility Studies:

Solubility is defined as the concentration of the solute in a solution when equilibrium exists between the pure solute phase and the solution phase. Solubility is determined by exposing an excess of drug powder to the solvent and assaying after equilibrium has been established.

The solubility of Luliconazole was studied in various solvents. Excess amounts of drugs was added to different solvents (10ml each) and agitated for 24 h using rotary shaker at room temperature. The contents were filtered through filter paper (whatmann filter paper No. 44) to obtain clear solutions, and the concentration of drug was estimated spectrophotometrically.

Quantitative Estimation of Drug:

Preparation of calibration curve of Luliconazole in methanol

Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol to give a solution of 1000 µg/ml concentration. From this solution 10 ml was taken and diluted to 100 ml using methanol to get a stock solution of 100µg/ml. From this stock solution various aliquots ranging from 4.20 µg/ml were prepared. The absorbance of these solutions was measured spectrophotometrically against reference blank.

Formulation Development (Broad Range Optimization)

A) Solid Lipid: Solid lipids used in this work are well tolerated, of GRAS status, accepted for human use and they are also in vivo biodegradable. Selection of solid lipid was based on the solubility and partitioning behavior of both drugs.

Solubility of drug Luliconazole in various solid lipids were observed when it gives a visibly clear solution in lipid, when seen under normal light with naked eye. The lipids used for this study were Glyceryl behenate (Compritol 888 ATO) and Cetyl Alcohol. This is performed by dissolving increasing amounts of drug in various melted solid lipids and determining the maximum amount of the active that could be dissolved in each lipid. After dissolution, the lipid/drug mixtures were cooled down to room temperature for solidification. The solid mixtures are visually observed for the presence or absence of crystalline active (when this ingredient is a solid substance at room temperature).

B) Liquid Lipid and Surfactant:

The oils used in this work are well tolerated and accepted for human use. Different liquid lipids and surafacatant were selected on the basis of solubility of drugs in liquid lipids/surfactact. The lipids used for this study were oleic acid, capryiol 90. Oils (10 gm) oils, surfactants is Tween 20 and tween 80 in 100ml-capacity stopper glass vials and shaken on a shaker for 24 hours at ambient temperature. Suspension was centrifuged at 4000 rpm for 10 min and the concentration of Luliconazole in the supernatant was determined by UV spectrophotometer after appropriate dilution with methanol at λmax about 299 nm.

C) Screening of Excipients:

After analysing the solubility of Luliconazole in suitable solvent methanol, ethanol, water and all the excipients, oils and surfactants were selected. Excipients were selected and solubility study was done for Luliconazole within all the excipients. These excipients were selected on the basis of maximum solubility of Luliconazole in different oils and surfactants

D) Compatibility Study:

The main objective of this study is drug/excipients compatibility consideration and practical studies are to define, as quickly as possible, real and possible interactions between Drug and excipient. In the solid dosage form the drug is in intimate contact with one or more excipient; the latter could affect stability of drug. Chemical interaction between the drug, lipid and surfactants were studied by FT-IR technique. The IR spectrum of the physical mixture was compared with those of pure drug and lipid and peak matching was done to detect any appearance or disappearance of peaks.

The compatibility study was carried out at 55°C for 14 days with moisture and without Moisture in sealed glass container of individual drug and Drug: Excipient (1:1). Individual IR graph were taken before placing the ingredient and drug into the glass vials and these vials were kept for 14 days for 55°C in duration of 14 days all the vials were observed for any colour change, gas formation caking and liquefaction and lastly after 14 days its IR was studied.

Table 2.3: Drug: excipients ratio for compatibility study at 55°C ± 2°C / 75% RH ± 5

Drug + Excipient	Ratio	Temperature	With moisture	Without moisture
Luliconazole	1	55°C	14 days	14 days
Luliconazole + Capryol 90	1:1	55°C	14 days	14 days

Formulations and Development:

Preparation of trial batches using different combination of Lipids and surfactants:-

The preparation of trial batches using different oils, surfactants for checked morphology. For these trial batches we was selected the excipient for optimization of nano structured lipid carrier.

Table 2.4: Preparation of trial batches using different combination of oils and surfactants.

Trial Batch	Solid Lipid	Liquid Lipid	Surafcatnt Concentraion
F1	Compritol ATO	Capryol 90	1%
F2	Compritol ATO	Capryol 90	4%

Formulation Optimization:

Optimization of the selected three parameters was carried out by using Box-Behnken design of response surface methodology with support of design Expert software version 13. Box-Behnken statistical design that is an independent, rotatable or nearly rotatable, quadratic design having the treatment combinations at the midpoints of the edges of the process space and at the center. The two-level, three-factor Box-Behnkan design was selected for study. Drug lipid ratio, surfactant concentration, were identified as key process parameters influencing entrapment efficiency. These are considered as three independent variables and tested at two different level for the formulation of NLC. This design of experiments has total 15 runs including five center points. An advantage of the Box-Behnken design is that it does not contain combination for which all factors are simultaneously at their highest or lowest level. So, these designs are useful in avoiding experiments performed under extreme conditions, for which unsatisfactory result in avoiding experiments performed under extreme conditions, for which unsatisfactory result are often obtained. Conclusions made with respect to the following second-order polynomial equation with owing constraint of the magnitude of coefficients and mathematical signs. $Y = B0 + B1A + B2B + B3C + B11A^2 + B22B^2 + B33C^2 + B_{12}$ AB + B_{13} AC + B_{23} BC. Where Y was predicted response (s); B_0 was an intercept, B_1,B_2,B_3 were linear coefficients; B_{11},B_{22}, and B_{33} were squared coefficients and quadratic term; B_{12},B_{13}, and B_{33} were interaction coefficients; and A, B, and C were independent variables. Positive sign before a factor in polynomial equations represents that the response increases with the factor. On the other hand, a negative sign means the response and factors have reciprocal relation.

Optimization of Liquid to total lipid ratio %w/w/ Lipid Phase Concentration %w/v/Surfactant Concentration %w/v: BBD was used in the study for optimization of NLCs. A three level design was employed for exploring reponses which were constructed with design expert software (version 9). Independent variables included Liquid to total lipid ration (X1), Total lipid phase concentration (X2) and surfactant concentration (X3).The dependent variables include particle size (y1) and entrapment efficiency (y2). The levels for independent variables were selected on the basis of pre-formulation studies.

Table 2.5: Independent variables and their selected levels for formulation of Nano Structured Lipid Carrier.

Variables	Independent Variables	Levels		Dependent Variables
		-1	+1	
1	A) Liquid to total lipid Ratio (% w/w)	10	40	Particle Size, Entrapment efficiency.
2	B) Lipid Phase Conc. (% w/v)	0.1	1	
3	C) Surfactant conc. (%w/v)	1	4	

Table 2.6: Box-Behnken experimental design for three variables.

Run	Variable A	Variable B	Variables C
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	0	-1	-1
10	0	+1	-1
11	0	-1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0

Selection of formulation Technique:

Nanostructured lipid carriers (NLCs) can be prepared by several methods like hot high pressure homogenization, Solvent diffusion method, microemulsion technique, high shear homogenization with ultrasonication and solvent emulsification evaporation technique. Out of which solvent diffusion techniques was tried. Formulation parameters were taken as given in some literatures so as to evaluate the feasibility of technique.

Solvent Diffusion Method followed by sonication:

After selection and optimization offormulation , Luliconazole loaded NLCs were formulated via solvent diffusion technique. The formulation table is given Table. Drug loaded NLCs were formulated via Solvent Diffusion Technique technique. Compritol® ATO 888 and Capryol 90 were mixed and melted at 70ºC to obtain a clear lipid phase. Drug was then added to melted lipid mixture to obtain a clear solution and were then completely dissolved into a mixture of acetone (6 ml) and ethanol (6 ml). Meanwhile, an aqueous surfactant solution with tween 20 was prepared and heated at the same temperature. The hot melted lipid solution was then dispersed drop wise via syringe in the hot surfactant

phase with continuous stirring on stirrer at 1000rpm for the specified time interval i.e. 10 minutes. The obtained emulsion was then sonicated, using a probe sonicator for 10 min at 40% amplitude. NLCs dispersion so obtained was cool and stored.

Table 2.7 Optimized NLCs Formulation Table

Formulation No.	Std	Run	Factor 1 A: Liquid to total lipid ratio %w/w	Factor 2 B: Lipid Phase Concentration %w/v	Factor 3 C: Surfactant Concentration %w/v
F1	2	1	40	0.1	2.5
F2	3	2	10	1	2.5
F3	11	3	25	0.1	4
F4	8	4	40	0.55	4
F5	12	5	25	1	4
F6	13	6	25	0.55	2.5
F7	6	7	40	0.55	1
F8	5	8	10	0.55	1
F9	1	9	10	0.1	2.5
F10	14	10	25	0.55	2.5
F11	10	11	25	1	1
F12	4	12	40	1	2.5
F13	7	13	10	0.55	4
F14	15	14	25	0.55	2.5
F15	9	15	25	0.1	1

Table 2.8: Preparation of Nano structured lipid carrier.

Sr. no.	Liquid Lipid (mg)	Solid Lipid (mg)	Total Lipid (mg)	Surfactact (%)	Drug (mg)	Response 1 Particle size (nm)	Response 2 Entrapment efficiency (%)
1	40	60	100	2.5	10	205.6	96.6
2	100	900	1000	2.5	100	678.3	80.2
3	25	75	100	4	10	120.4	86.3
4	220	330	550	4	55	416.5	95.2
5	400	600	1000	4	100	632.5	87.5
6	137.5	412.5	550	2.5	55	425.9	88.6
7	220	330	550	1	55	536.7	96.2
8	55	495	550	1	55	694.4	79.5

9	10	90	100	2.5	10	290.4	78.6
10	137.5	412.5	550	2.5	55	428.3	89.3
11	250	750	1000	1	100	695.6	89.7
12	400	600	1000	2.5	100	724.3	95.4
13	55	495	550	4	55	418.8	79.5
14	137.5	412.5	550	2.5	55	430.2	88.7
15	25	75	100	1	10	225.6	88.2

Validation of optimized formulation

Table 2.9: Optimized Batch Preparation of Nano Structured Lipid Carrier.

Factor 1 A: Liquid to total lipid ratio %w/w	Factor 2 B: Lipid Phase Concentration %w/v	Factor 3 C: Surfactant Concentration %w/v	Drug (%)	Particle size (nm)	Entrapment efficiency (%)
0.927	0.304	0.331	10	482.135	84.389

Characterization of drug loaded NLCs:

Measurement of Particle Size:

The Particle size of NLC system was measured by Horiba Particle Size Analyzer. For the determination of particle size, the diluted sample was transferred into a disposable plastic cuvette. The cuvette was then manually shaken for about 5–10 sec and then kept on the sample holder. Average particle size and polydispersity index were measured by particle size analyzer at a 90° angle at 25 °C. All measurements were carried out in triplicate.

Zeta potential measurement

The surface charge on the nanoparticles was quantified by measuring the zeta potential using Zetasizer. Prior to analysis, NLC suspensions were diluted with double distilled water (1:100) to obtain uniform dispersions. All reported values are the mean of three separate measurements.

Drug Entrapment Efficiency (DEE)

A volume of 5.0 ml of each drug-loaded sample was centrifuged with centrifuge tube (10000 MWCO) in centrifuge at 5000 rpm for 30 minutes at 25ºC to separate the lipid and aqueous phase. The supernatant was then diluted with methanol, filtered through 40µm filter paper and the absorbance of the sample was noted by the UV-VIS spectrophotometer. The entrapment efficiency of NLC was calculated as follows:

% Drug Entrapment Efficiency = Total amount of drug - Amount of drug in supernatant X 100 / Total amount of drug.

In- vitro drug diffusion study

The luliconazole formulation (2.0 ml) was enclosed in a dialysis bag (cellulose membrane, mw cutoff 12,400) and bag is placed in 40 ml release medium in beaker which was maintained at 37°C and at 100 rpm. Phosphate buffer (PBS, PH 7.4) was used as the release medium and analysed spectrophotometrically at 299 nm. After sampling, 0.5 ml of fresh medium was added in the release medium to maintain the sink condition

Preparation of drug loaded NLC based gel:

NLC-based gel was prepared according to the formula (Table 5.8). Carbopol was firstly dispersed in purified water and subsequently, LZ-NLC dispersion, propylene glycol was incorporated into the blank gel using a high speed stirrer at 1000 rpm for 5 min. The NLC-gel was immediately neutralized with triethanolamine until pH 5.0–6.0. The formed gel was left equilibrating for 24 h at room temperature (25 ± 1 ◦C). Meanwhile, LZ PBS (10.0 mg/ml) was used to prepare the LZ-carbopol-gel.(40)

Table3.0 Composition of LZ NLC gel formulation (%m/m)

Formulation Composition	LZ NLC Gel (g)
LZ NLC	50
Carbopol 940	0.75
Propylene glycol	7.5
Triethavolamine	2.5
Water	100

Characterization of Drug loaded NLC based topical gel: The basic physicochemical parameters were studied for the developed formulation.

A. Physical Appearance:

The prepared gel formulation was inspected visually for their color, clarity, homogeneity and appearance.

B. Formulation pH:

The pH values of 1% aqueous solution of the prepared gel was measured by a pH meter. 1 gm of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of formulation was done in triplicate and average values are calculated.

C. Viscosity:

To measure the viscosity, all the formulated gels were taken in beakers and placed beneath the spindle RV-7 and the spindle was rotated at 10 rpm in Brook Field Viscometer.

D. Spreadibility:

The Spreadibility of the gel was evaluated by using slides method. 1gm of gel was kept between the two slides. The preweighted plate was kept above the gel and more weights were added on the plate until the gel stop spreading. Final

cumulative weight and the total time taken by the gel to spread was measured and noted. Then total weight applied and mass of the gel were compared by the time.

Spreadibility= Mass x Length/ Time

E. Drug Content:

To determine the drug content of gel, 10 mg of drug loaded NLC based gel was weighed and mixed to the phosphate buffer saline (PBS pH 7.4) and the concentration of LZ was observed spectrophotometrically. The NLC gel base containing the identical amount of ingredients without drug was used as a blank.

F. In vitro diffusion study:

The experiment was conducted in Franz diffusion cell of 40ml capacity. Pretreated egg membrane was fixed in between the donor and receptor cells. The receiver contains phosphate buffer pH 6.8 and methanol [1:1] solution. LZ NLCs gel (1gm) and LZ Carbopol gel (1 gm) were applied on the egg membrane and donor compartment was clamped. Water circulation was maintained at 37±1oc with 20 rpm on the magnetic stirrer.1ml sample was withdrawn every 1 hr with the syringe and replace with fresh PBS solution to maintain the sink condition. Samples were analyzed by UV spectroscopy at λmax 299 nm. The study was conducted for 12 hrs.

Result:

Preformulation:

Organoleptic Evaluation

Table 3.1 Results of Organoleptic evaluation of Luliconazole

Evaluation Parameters	Observed Results	Specification
Colour	Off-white to yellowish crystalline powder	Off-white to yellowish crystalline powder
Odor	Odorless	Odorless
Texture	Solid Crystalline	Solid Crystalline

Melting Point

Table 3.2 Melting Point of Luliconazole

Parameter	Drug	Observed Value	Specification
Melting Point	Luliconazole	152 °C	152 °C-154^{0}C

Ultra-Violet Absorpotion Maxima (λmax):

The λmax of Luliconazole was found to be 299nm in methanol as solvent system as shown in fig. 6.1

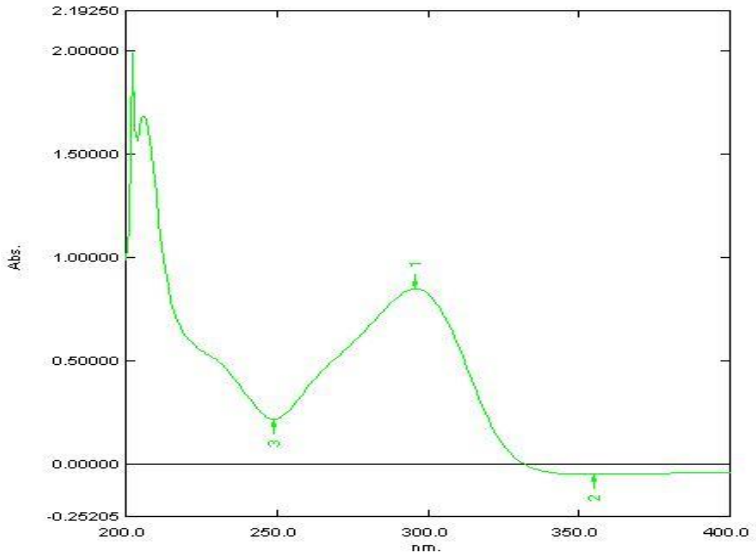


Figure: Ultraviolet Absorption Maxima of Luliconazole.

Solubility Studies:

Table 3.3 Solubility Studies of Luliconazole

S. No.	Solvents	Solubility (mg/ml)	Solubility of Luliconazole
1.	Distilled Water	0.28 mg/ml	Practically Insoluble
2.	Methanol	31 mg/ml	35 mg/ml
3.	Ethanol	10.12 mg/ml	10

Quantitative Estimation of Drug (Preparation of Calibration Curve):

Standard Calibration curve of Luliconazole

Table 3.4 Standard Curve of Luliconazole at 299 nm

Conc µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance 4	Absorbance 5	Absorbance 6	Average	SD
4	0.168	0.17	0.167	0.165	0.172	0.171	0.169	0.002
8	0.328	0.329	0.328	0.286	0.287	0.285	0.307	0.021
12	0.473	0.471	0.471	0.450	0.449	0.451	0.461	0.011
16	0.619	0.621	0.624	0.649	0.648	0.648	0.635	0.014
20	0.786	0.786	0.784	0.848	0.849	0.847	0.817	0.031

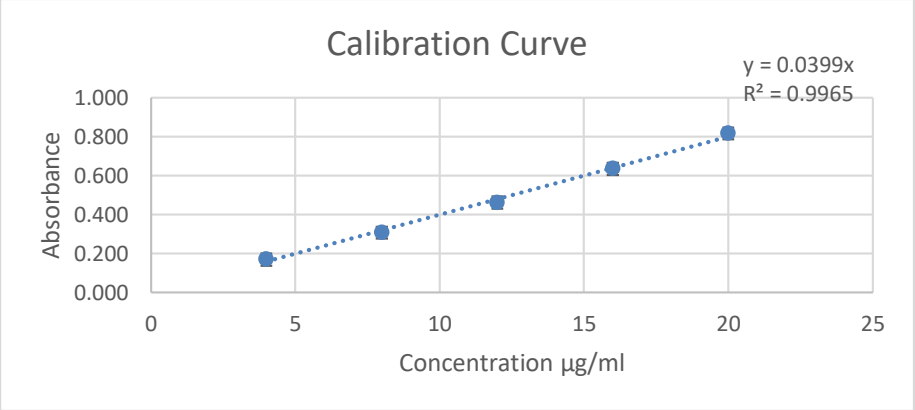


Figure: Standard curve of Luliconazole in methanol 299 nm

IR Spectroscopy:

IR Spectra of Luliconazole:

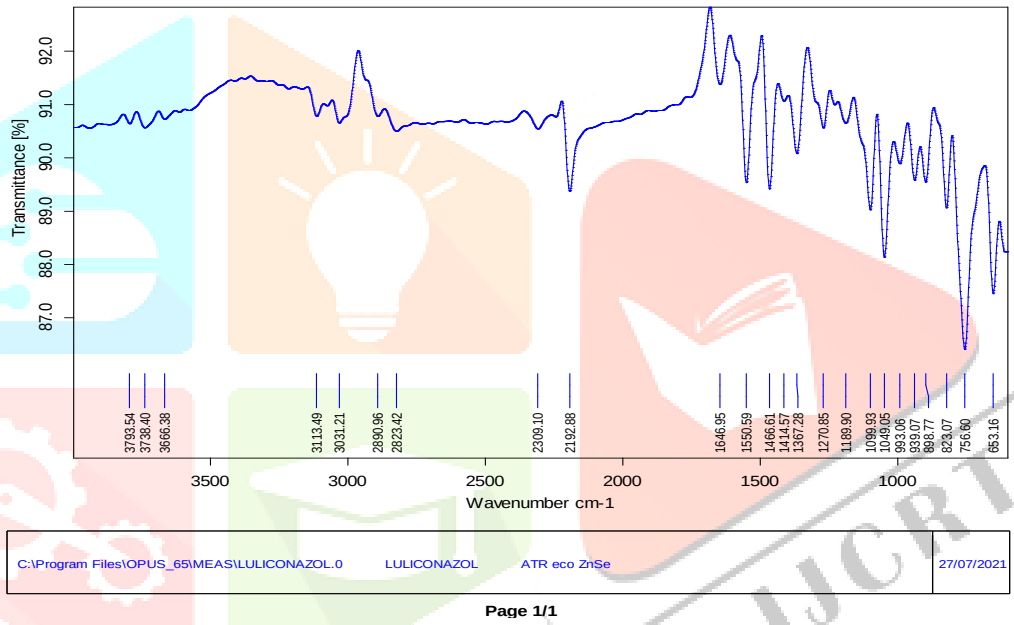


Figure: Infrared Spectra of Luliconazole

Table 3.5 Wavenumber (cm-1) representing functional groups present in IR spectra of Luliconazole

S. No.	Wavenumber (cm-1)	Functional Group
1.	**3031.21**	aromatic C-H stretching
2.	**2990.96**	aliphatic C-H stretching
3.	**2823.42**	S-H stretching
4	**2192.88**	C≡N stretching
5.	**1550.99**	C=C alkene stretching
6.	**1646.96**	C=N stretching
7.	**1466.61**	aromatic C=C stretching
8.	**756.60**	C-Cl bond stretching

Conclusion: The IR spectrum of the pure Luliconazole sample was recorded by FT-IR spectrometer as shown which was compared with standard functional group frequencies of Luliconazole. The major peaks observed and corresponding functional groups are given.

Identification and characterization of Lipids:

I) Compritol 888 ATO

a) Organoleptic characteristics

The organoleptic characteristics of compritol 888 ATO such as colour, odour were studied. Colour of compritol 888 ATO was found white and it was odourless in nature.

Table 3.5: Organoleptic properties of compritol 888 ATO

Organoleptic properties	Standard	Observation
Colour	White	White
Odour	Faint odour	Faint odour
Taste	Tasteless	Tasteless

b) Melting Point

Melting point of drug Compritol 888 ATO was determined by capillary method. The temperature at which drug goes in the liquid state was consider as a melting point of Compritol 888 ATO. Pactrically it was found that drug gets melts at 74-76°C. Reported melting point of the drug Compritol 888 ATO is 65-77°C.

c) FT-IR spectra

A small amount of drug in the form of power was placed on selenium bromide crystal and FT-IR spectrum were run. The FT-IR spectra of Compritol 888 ATO are given below.

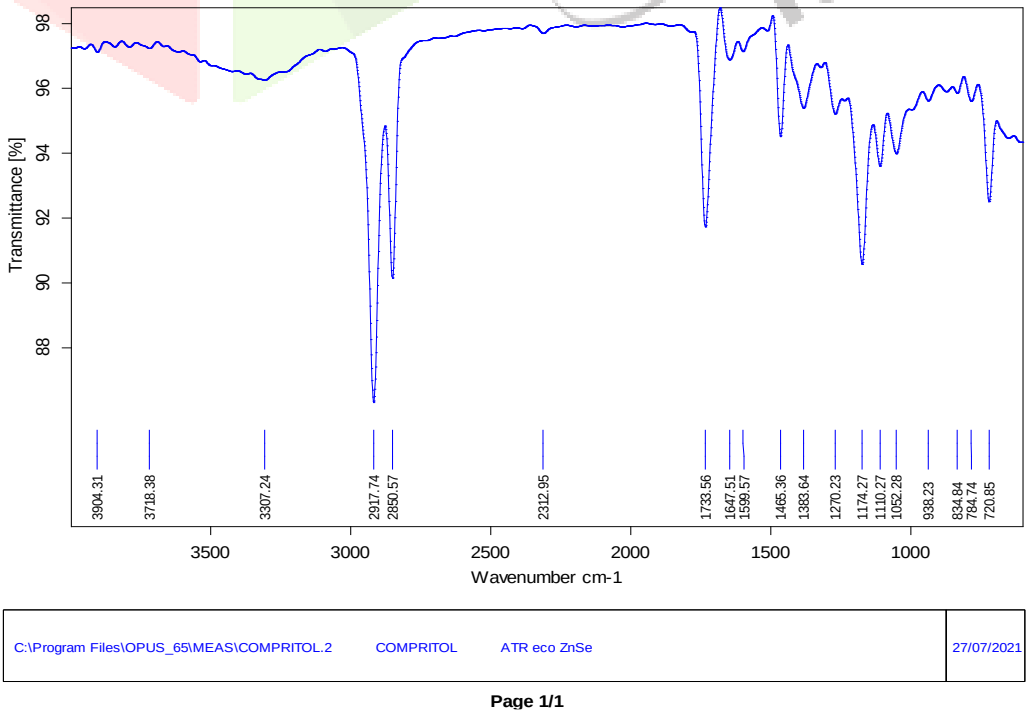


Figure: FT-IR spectra of Compritol 888 ATO.

II) Capryol 90

a) Organoleptic characteristics

The organoleptic characteristics of capryol 90 such as colour, odour were studied. Colour of capryol 90 was found clear yellowish and it was odourless in nature.

Table 3.6: Organoleptic properties of compritol 888 ATO

Organoleptic properties	Standard	Observation
Colour	Clear yellowish	Clear yellowish
Odour	Odouless	Odouless
Taste	Tasteless	Tasteless

IR Spectra of Capryol 90:

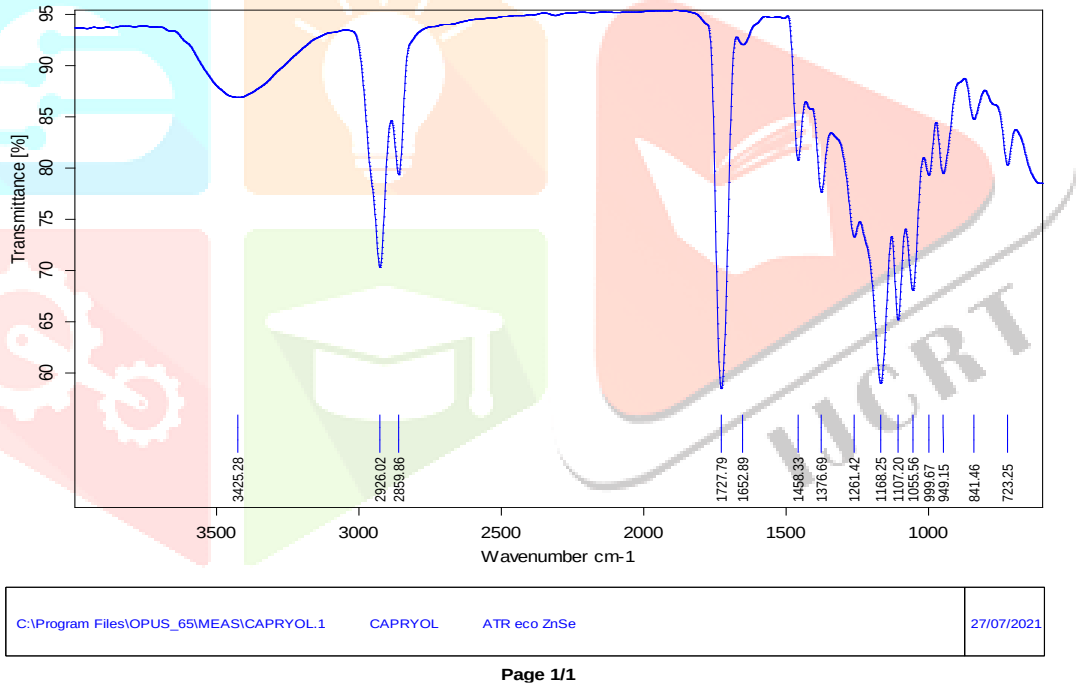


Figure: Infrared Spectra of Capryol 90

Table 3.7 Wavenumber (cm-1) representing functional groups present in IR spectra of Capryol 90

Sr. No.	Wavenumber (cm-1)	Functional Group
1.	3425.29	(O-H)
2.	2926.02	(C-H) aliphatic
3.	1727.79	(C=O)
4.	1168.25	(C-O)

Drug Excipient Compatibility Studies:

IR spectra of Luliconazole and Capryol 90:

In the formulation of NLCs liquid lipid plays important role which dissolve the drug and control the release. Hence the compatibility with the liquid lipid studied with FT-IR.

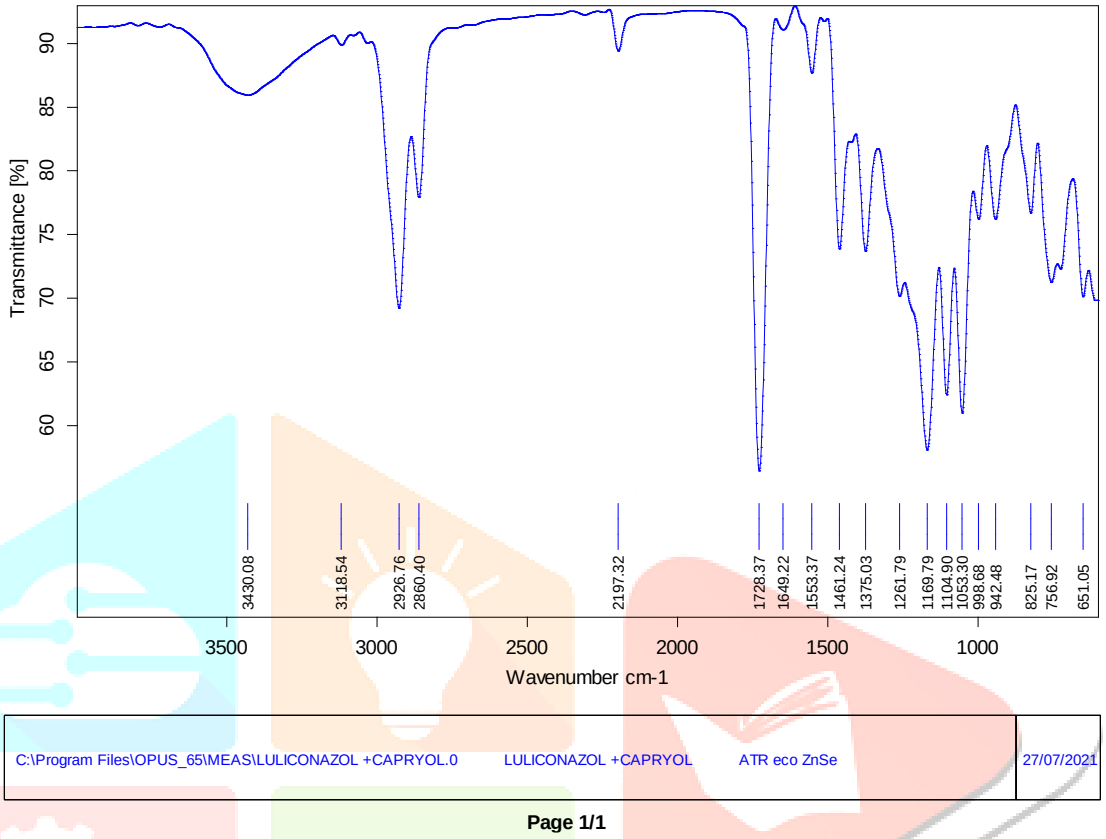


Figure: Infrared Spectra of Luliconazol and Capryol 90

It was found that selected liquid lipid was compatible with Luliconazole. There were no colour changes, caking or liquefaction found in the sample of Luliconazole and Capryol 90. Also no chemical degradation seen in the mixture as the FT-IR of mixtures was same before compatibility study and after compatibility study.

Formulation development

Selection of Lipids (Solid Lipid and Liquid Lipid):

Solid Lipid Solubility

Luliconazole was found to be very much soluble in Compritol 888 ATO without any presence of solid crystalline structure as compared to cetyl alcohol.

Liquid Lipid

Capryol 90 was selected as liquid lipid on the basis of drug's solubility in liquid lipid as shown in Table 6.7.

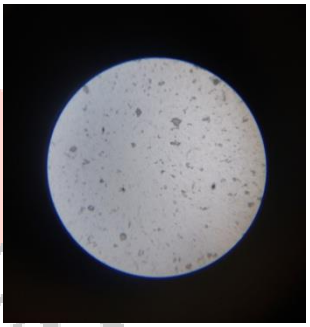
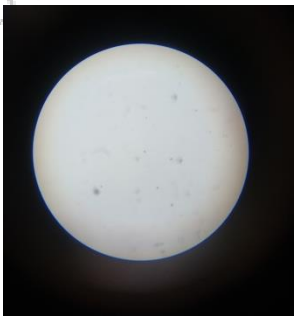
Table 3.8 : Selection of Liquid Lipids for LZNLC on the basis of solubility

Sr. No.	Liquid Lipid	Solubility of LZ (mg/ml)
1.	**Capryol 90**	**31.4 mg/ml**
2.	Olic Acid	27.6 mg/ml

All observation are carried out in triplicate.

Evaluation of Trial batch:

Physicochemical property of trial batch

Trial Batch	Solid Lipid	Liquid Lipid	Surfactant Concentration	Entrapment Efficiency	Globule Size
F1	Compritol ATO	Capryol 90	1%	75%	
F2	Compritol ATO	Capryol 90	4%	82%	

Significance

In trial batch result of drug Entrapment efficiency is good but the result of globule size of pre-emulsion was obtained 3.6 µm & 2.9 µm. It was observed that drug: lipid ratio has positive effect on globule size. With increasing surfactant conc, it shows reduced interfacial tension between lipid and aqueous phase thereby resulting in lower size.

Formulation Optimization

Table 3.9: Entrapment efficiency and particle size of different batches prepared as per DOE.

Sr. no.	Liquid Lipid (mg)	Solid Lipid (mg)	Total Lipid (mg)	Surfactact (%)	Drug (mg)	Response 1 Particle size (nm)	Response 2 Entrapment efficiency (%)
1	40	60	100	2.5	10	205.6	96.6
2	100	900	1000	2.5	100	678.3	80.2
3	25	75	100	4	10	120.4	86.3
4	220	330	550	4	55	416.5	95.2
5	400	600	1000	4	100	632.5	87.5
6	137.5	412.5	550	2.5	55	425.9	88.6
7	220	330	550	1	55	536.7	96.2
8	55	495	550	1	55	694.4	79.5
9	10	90	100	2.5	10	290.4	78.6
10	137.5	412.5	550	2.5	55	428.3	89.3
11	250	750	1000	1	100	695.6	89.7
12	400	600	1000	2.5	100	724.3	95.4
13	55	495	550	4	55	418.8	79.5
14	137.5	412.5	550	2.5	55	430.2	88.7
15	25	75	100	1	10	225.6	88.2

Statistical Analysis:

The model were evaluated in terms of stastistically significant coefficient, standardized main effects (SEM) and R^2 values varies feasibility and gried searches were conducted to find the composition of optimized formulation and varies 3D response surface graphs were drawn by using design expert softwere 13. By intensive gride searches performed whole experimental region, optimum cheked point were selected to validate the choosen experimental domain.

Table 4.0: Particle size ANOVA for Response surface 2F1 model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.199E+05	6	69977.37	4.91	0.0215	Significant
A-liquid to total lipid ratio	58670.25	1	58670.25	4.11	0.0771	
B- lipid phase	32385.13	1	32385.13	2.27	0.1703	
C-surfactant	1.951E+05	1	1.951E+05	13.68	0.0061	
AB	4726.56	1	4726.56	0.3314	0.5807	
AC	1.205E+05	1	1.205E+05	8.45	0.0197	
BC	8510.06	1	8510.06	0.5967	0.4621	
Residual	1.141E+05	8	14262.33			Not significant
Lack of fit	72028.16	6	12004.69	0.5707	0.7484	

Table 4.1: Entrapment efficiency ANOVA for Response surface 2F1 model.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	375.96	6	62.66	2.79	0.0903
A- liquid to total lipid ratio	153.13	1	153.13	6.83	0.0310
B-lipid phase	63.28	1	63.28	2.82	0.1315
C-surfactant	0.1013	1	0.1013	0.0045	0.9481
AB	17.64	1	17.64	0.7866	0.4010
AC	8.41	1	8.41	0.3750	0.5573
BC	133.40	1	133.40	5.95	0.0406
Residual	179.40	8	22.42	-	-
Lack of Fit	128.11	6	21.35	0.8326	0.6358

The analysis of variance is essential to test significance and adequacy of the model. It subdivide the total variation of the result in to sources of variation, the model and experimental error, shows whether the variation from the model insignificant when compared to the variation due to residual error Fisher's F- test value, which is the ratio between the mean square of the model and the residual error, performs this comparisons. A model, were significant, with very

small p- values (p<0.05). The other term coefficients were not significant (p>0.05). The "Lack of Fit Test" compares the residual error to the pure error from replicated design points. The lack of fit F-value of is not significant as the p-value is >0.05. The non-significance lack of fit showed that the model was valied for the present work.

Table 4.2: Statistical summary

Response	Model	Adjusted R^2	Predicted R^2	Sequential p- value
Particale size	2F1	0.6261	0.2482	0.0877
Entrapment efficiency	2F1	0.4347	0.2484	0.1464

Influence of variables

1) Particle Size

Particle size is the important parameter of NLC formulation. It is mainly depends on the added concentration of liquid to total lipid ratio, surfactant, lipid ratio. All of these describe with the response surface plots as shown in following figure.

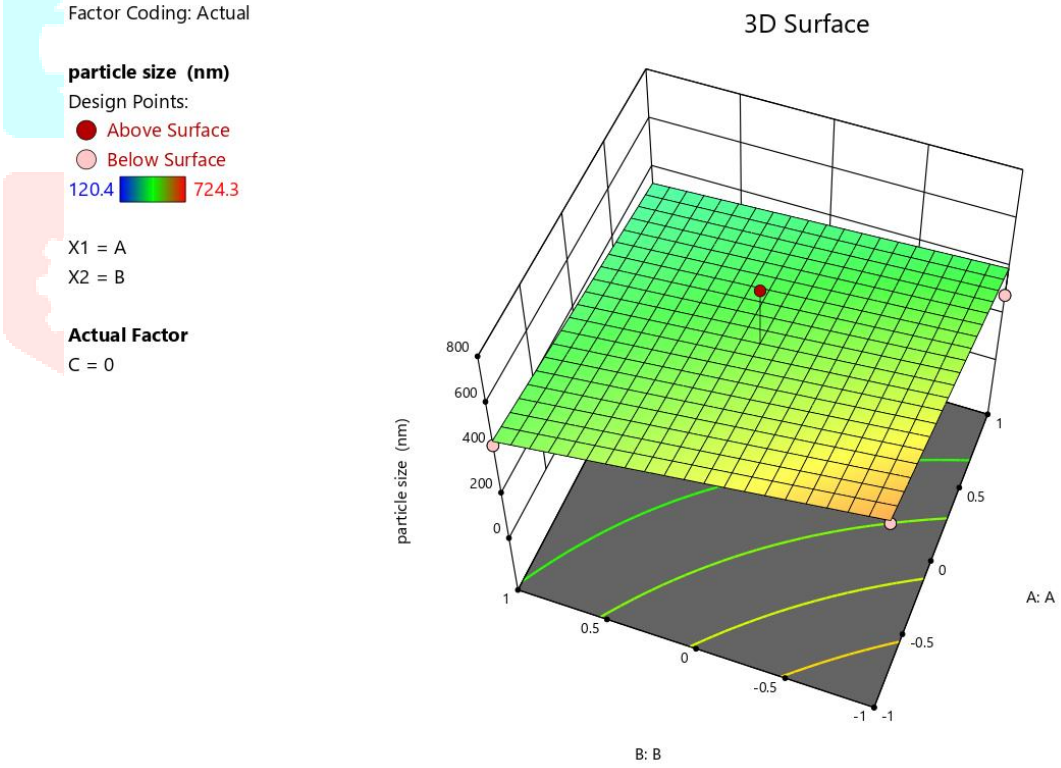


Figure: 3D response surface curve of liquid to total lipid ratio and lipid phase concentration on particle size.

Significance: Fig Indicates that with increase in liquid to total lipid as well as increase in particle size of NLC formulation and lipid phase concentration increases as well as decrease in particle size of NLC.

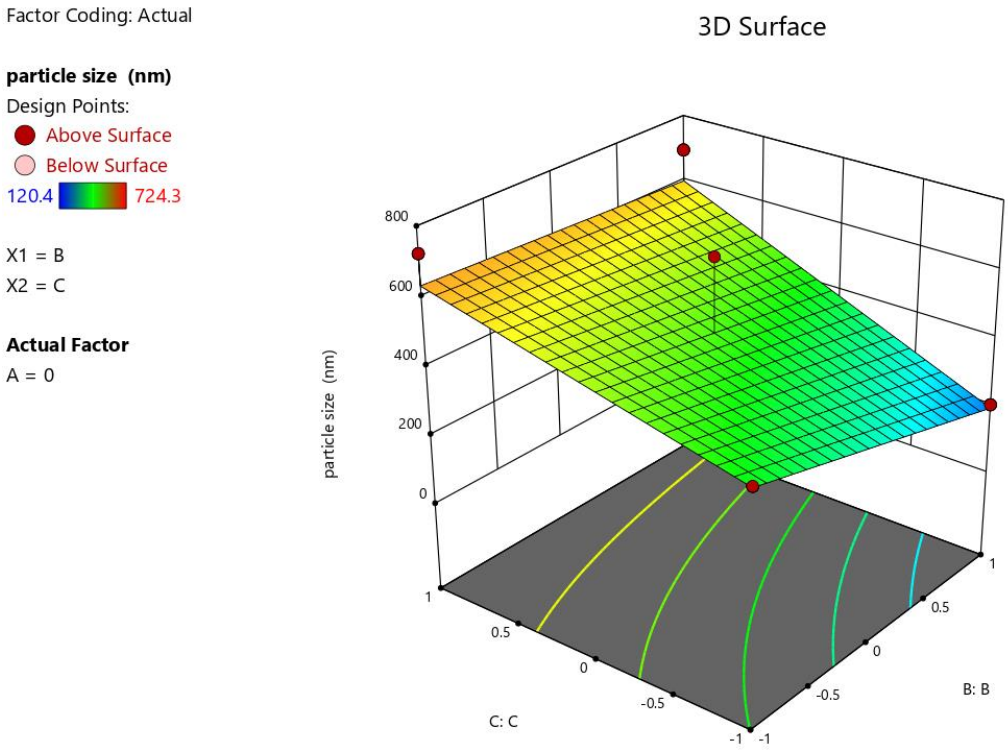


Figure: 3D response surface curve of liquid to total ratio and surfactant conc on particle size

Significance

Fig no. 8.7. Indicates that with increase in liquid to total ratio as well as increase in particle size of NLC formation and increase in surfactant conc as well as increase particle size.

2) Entrapment Efficiency

Entrapment efficiency is the important parameter of NLC formulation. It is mainly depends on the added concentration of liquid to total lipid ratio, lipid phase conc and surfactant conc. All of these describe with the response surface plots as shown in following figure.

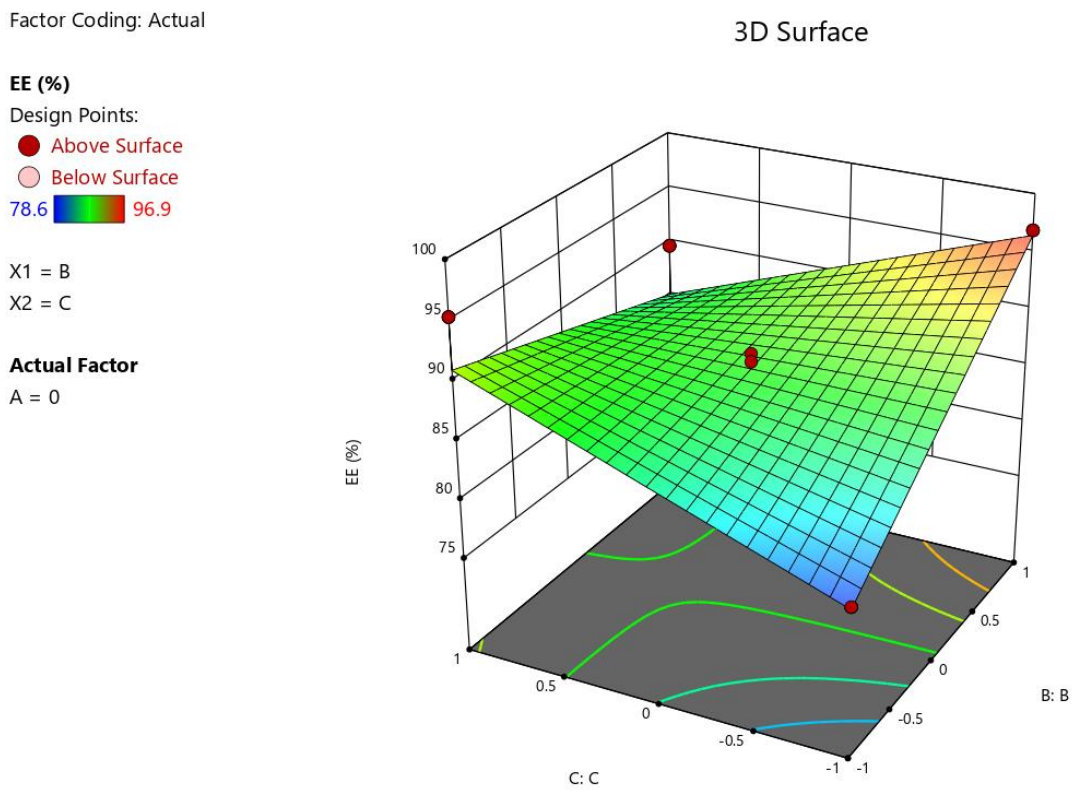


Figure: 3D response surface curve of surfactant con and lipid phase con on entrapment efficiency.

Significance:

Fig no.8.11. Indicates that with increase in surfactant con. does not showed much effect on entrapment efficiency. As well as increase in lipid phase con. thane entrapment efficiency increases of NLC formulation.

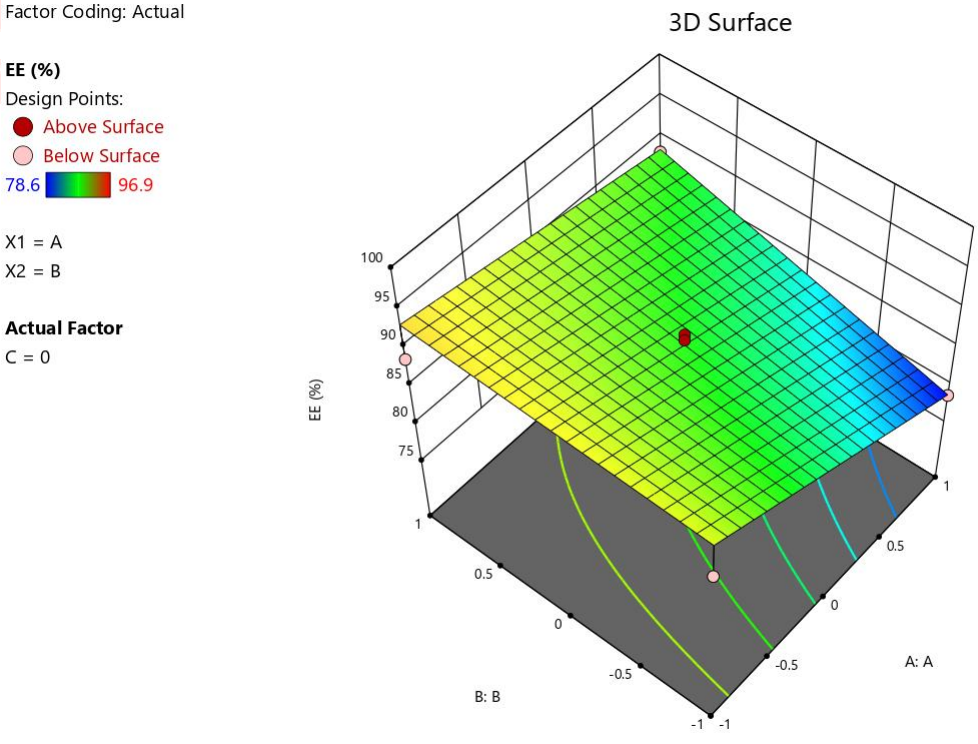


Figure: 3D response surface curve of liquid to total lipid ratio and lipid phase con on Entrapment efficiency.

Significance:

Fig no.8.9. Indicates that with increase in liquid to total lipid ratio as well as decrease in entrapment efficiency of NLC formulation and increase in lipid phase con as well as increase in entrapment efficiency.

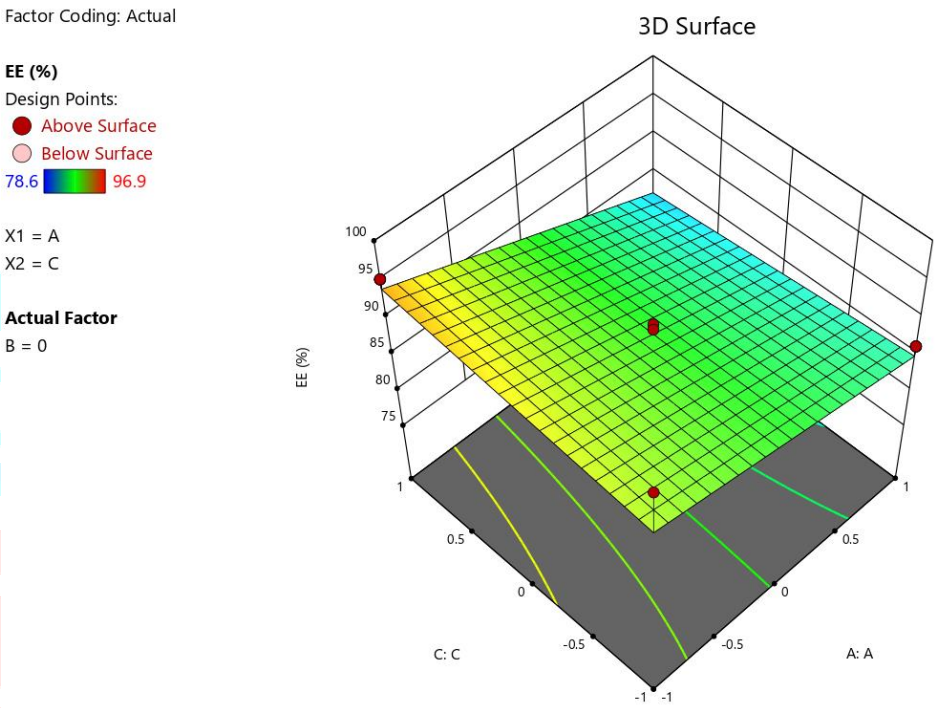


Figure: 3D response surface curve of liquid to total ratio and surfactant conc on entrapment efficiency

Significance:

Fig no. 8.10 Indicates that with increase surfactant con as well as does not showed much effect on entrapment efficiency as well as increase in liquid to total lipid ratio decreases the entrapment efficiency of NLC formulation.

Prediction of optimized formulation

a) Final equation in term of Coaded Factor

Particle size =

461.57-85.64A– 63.63B + 156.16C– 34.38AB – 173.55AC + 46.13BC

Entrapment efficiency =

87.99– 4.38 A + 2.81 B – 0.1125C+ 2.10 AB - 1.45AC – 5.78 BC

b) Final equation in terms of actual factor

Particle size

85.64 liquid to total lipid ratio – 63.63 lipid phase con + 156.16 surfactant con – 34.38 liquid to total lipid ratio ± lipid phase con – 173.55 liquid to total lipid ratio ± surfactant con + 46.13 lipid phase con ± surfactant con.

Entrapment efficiency

4.38 liquid to total lipid ratio + 2.81 lipid phase con – 0.1125 surfactant con + 2.10 liquid to total lipid ratio ± lipid phase con - 1.45liquid to total lipid ratio ± surfactant con – 5.78 liquid to total lipid ratio ± surfactant con.

Optimized Formula

From the set variables, the software calculated and predicts the formula which achieved the set target as near as possible. Optimize formulation formula was showed in the following table.

Table 4.3: Formula for optimized batch of NLC as per DOE.

Factor 1 A: Liquid to total lipid ratio %w/w	Factor 2 B: Lipid Phase Concentration %w/v	Factor 3 C: Surfactant Concentration %w/v
0.927	0.304	0.331

Table 4.4: Physicochemical properties of optimized batch

Entrapment Efficiency (%)	Particle size	Zeta potential (mV)	Polydispersity Index
93.27	306.8 nm	-16.8	0.202

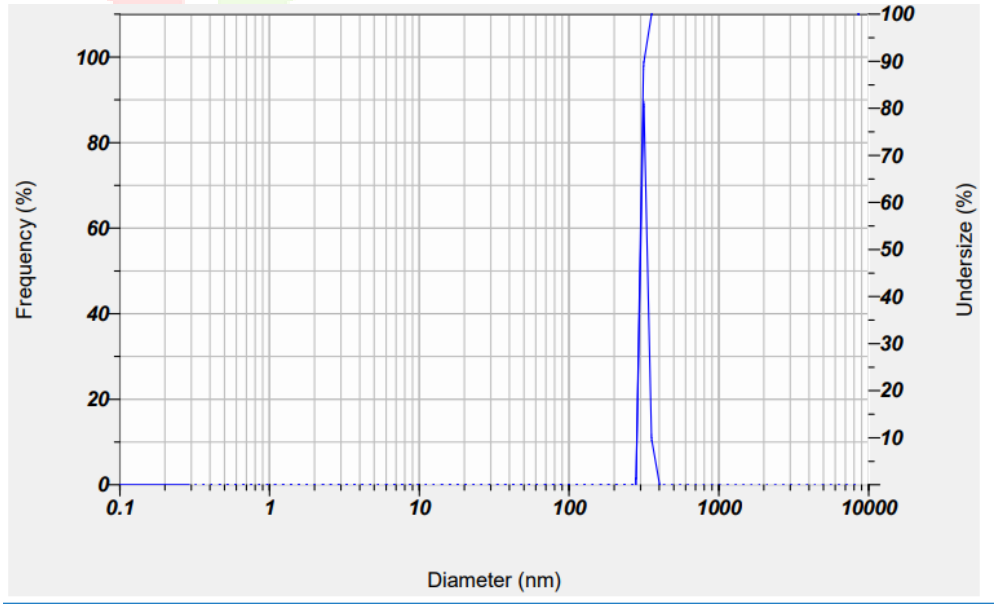


Fig: Particle Size Analysis

The percent entrapment efficiency was found to be 93.27. The zeta potential that is repulsion and attraction charge between particles was -16.8 mV. Which indicate good physical stability. Polydispersity index was found to be 0.202 which is acceptable rang, lower the PDI which indicate the decrease in aggregation of particle and increase the long term stability.

In-Vitro drug release

Percentage of drug release from Luliconazole NLC was studied by dialysis method.

Table 4.5: In Vitro drug release study.

Time (min)	Plain drug	Optimized batch
30	08.42	04.23
60	17.20	11.36
90	24.62	17.25
120	32.51	24.56
150	41.25	31.27
180	48.65	38.94
210	57.35	46.25
240	65.89	51.27
270	78.14	55.68
300	84.65	61.28
330	97.50	69.35

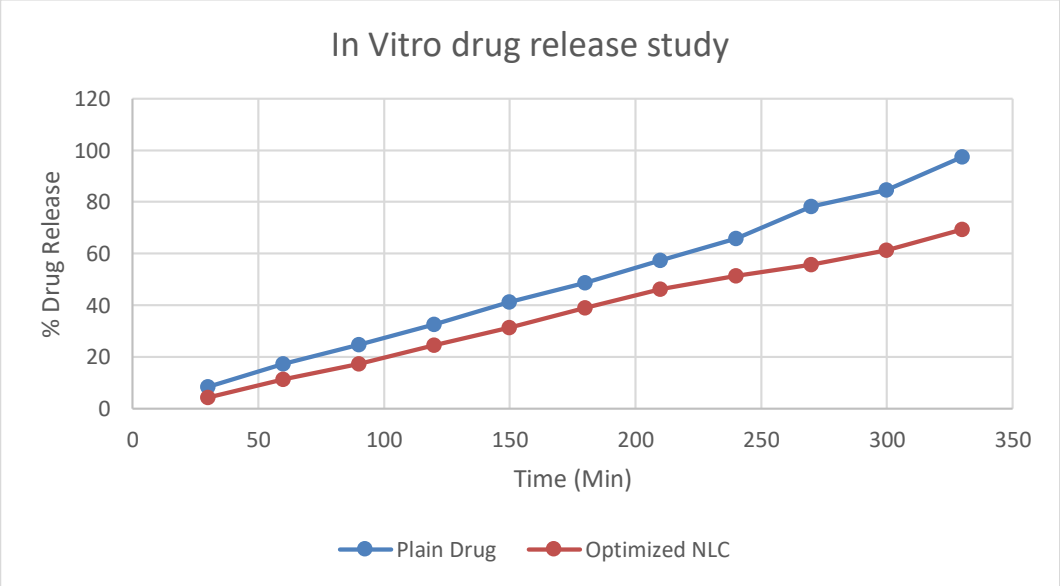


Fig : % Drug Release Study

From the above result it was concluded that the release of plain drug was shown 97.50 % in 330 min. The drug release of optimized batch was found to be 69.35 % in 330 min.

Preparation of Drug Loaded NLC Gel:

After optimization of the NLCs, LZ NLC was used for the formulation of gel.

Charachterization of Drug Loaded NLC Topical Gel:

Physical Appearance

Gel formulation was transparent, clear and homogeneous in texture.

pH

pH ranges between 7.1 – 7.4, which could easily be tolerated on skin without irritation.

Viscosity

LZ NLCs shows optimum viscosity i.e. 56000-63000 cps.

Spreadability:

LZ-NLCG is quite easy spreadable on application and shows the value of 216.85 g.cm/min.

Drug Content

Percent drug content of LZ NLC was found to be 98.76 %.

The basic physicochemical parameters were studied for the developed NLC based gel formulation as complies in Table:

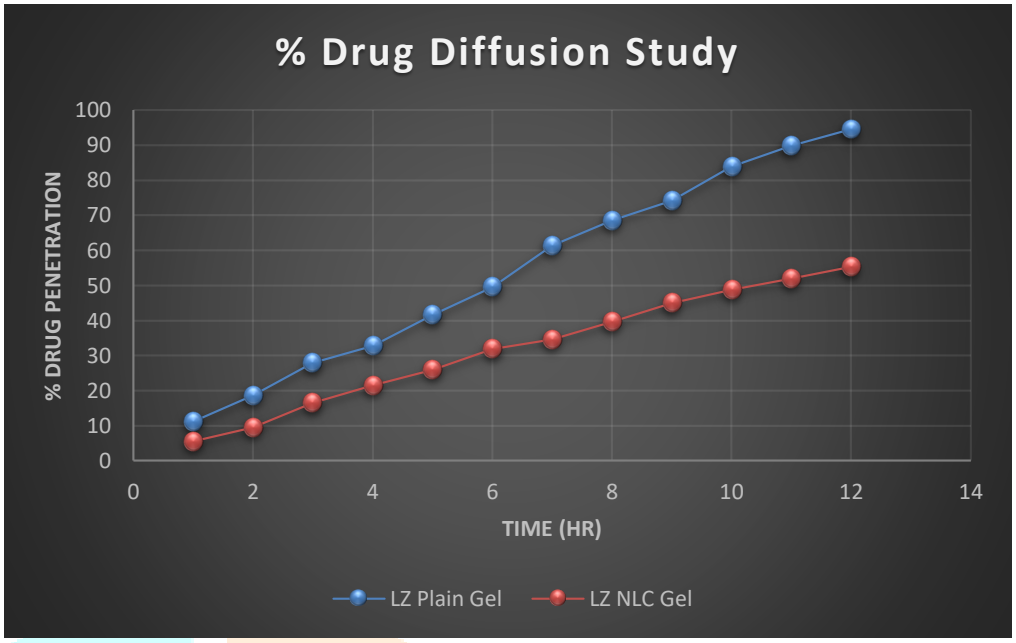
Table 4.6: Results of pH, Viscosity, Spreadability and Drug Content

Sr No	Formulation	pH	Viscosity (cps)	Spreadability (g.cm/min)	Drug Content %
1	LZ NLC Gel	7.2	56000-63000	216.85	98.76

In vitro drug diffusion study:

From the diffusion study of the LZ carbolpol gel and LZ NLC gel it was observed that the rate of diffusion of the LZ is more in LZ Carbopol gel while the LZ NLC gel diffuses slowly in controlled manner. And releases the drug in predetermined rate and no burst occur in the NLC gel formulation which directly reduces the skin irritation of the drug. There was an increase in the percentage of drug release in LZ carbopol gel as compared to the LZ NLCs Gel. Which explodite our hypothesis to get the optimum release of the luliconazole.

Fig : % Drug Diffusion of LZ Carbopol Gel and LZ NLC Gel



Characterization of Drug Loaded NLCs :

Measurement of Particle size, Polydispersity Index (PDI) and Zeta Potential: LZ-loaded NLC showed optimum particle size. This might be due to optimum concentration of combination of surfactant and lipid which decrease the particle size because of its synergism. It is observed that particle size decreases with increase in surfactant concentration, Smaller size helps in targeting and increases penetration of drug through biological membranes. The foremost criterion for nanoparticle-loaded topical drug delivery system is its permeation through the skin; especially in the case of NLC, particle size should be small enough to permeate the skin. Therefore, entrapment efficiency can be overruled or is considered less significant than particle size for the development of NLC formulation. The zeta potential values for all formulations were in the range of −16.3 to −17.5 mV. A high zeta potential will confer storage stability, i.e., the dispersion will resist aggregation. It is investigated that zeta potential increases first and then decreases. This may be credited to the surfactant (Tween 20). Ionic emulsifier leads to the highest zeta potential because after ionization in water, it gets adsorbed on particle/water interface and forms electric double layer. Polydispersity index is the measure of particle size distribution of nanoparticles. Samples with broad size distribution have polydispersity values less than 0.5 which clearly indicates a uniform dispersion. The values of polydispersity index (PDI) lies in the range of 0.224 to 0.462. Formulation revealed the smallest value of PDI viz. 0.224. It is observed that value of PDI increases with increase in solid lipid.

Drug Entrapment Efficiency: Entrapment efficiency of LZ-loaded NLC was found to be in the range of 79.46 % w/w to 99.99 % w/w. It is investigated that as the concentration of liquid lipid (Capryol) increases, entrapment also increases. Encapsulation efficiency is always correlated with the crystallinity degree of lipid nanoparticles. The more capryol in the mixture, the higher the encapsulation efficiency. Incorporation of capryol increases the amorphous proportion in the solid lipid matrix, and as a result decreases the overall particle crystallinity, thereby improving the encapsulation efficiency.

In vitro release study of drug loaded NLCs LZ-loaded NLC displayed optimum in vitro drug release up to 24 h. The in vitro release of the drug from the NLC dispersion was found to be biphasic, with the initial burst effect followed by gradual release of the drug.

The initial burst release might be due to either the presence of unentrapped drug in the NLC dispersion or due to localization of liquid lipid in outer shell which contains lipophilic drug in dissolved form and leads to initial burst release at initial stage. Release may occur by matrix erosion or diffusion. Initial burst release provides the drug for immediate therapeutic action and improves the drug penetration while sustained release deliver the drug over a prolonged period of time and maintain therapeutic drug concentration at the site of action. This clearly indicates that sustained release could be obtained by using this formula with single dosing frequency.

Preparation of Drug Loaded NLC based Gel: After optimization of NLCs, LZ NLCs were used for the formulation of gel. Characterization of Drug Loaded NLC Topical gel: Formulations were transparent, clear, and homogeneous in texture. pH ranges between 7.1–7.4 range for formulations, which could easily be tolerated on skin without irritation. LZ NLCs has optimum viscosity. LZ NLCG is quite easy spreadable on application. The diffusion study revealed that the prepared NLCs diffuses drug in a controlled manner and no burst release of the drug observed through NLC gel formulation.

Conclusion:

The present investigation was carried out to develop and optimized a novel dosage form 'Nanostructured lipid carriers' (NLCs) loaded Gel of Luliconazole.

From the study conducted, the following conclusion was drawn:

1. A successful attempt was made to perform preformulation studies. All the experiments were performed and all the values complied as per the literature.

2. All the IR studies displayed appropriate peaks and graphs as given in literature. Thus, revealing no interaction between drugs and excipients.

3. Formulation development was successfully accomplished involving broad range and narrow range optimization.

4. Solid lipid and liquid lipid were aptly selected on the basis of solubility and the ratio of SL to LL was selected on the basis particle size and entrapment efficiency. Foremost criterion for the selection of any parameter was resultant NLC particle size which should be lowest so as to increase permeation into skin.

5. Solevent Diffusion Technique was profitably tried for the formulation of NLCs.

6. Mixture of surfactants and ratio was significantly optimized on the basis of lowest particle size and highest entrapment efficiency.

7. Process parameters viz. stirring speed, stirring time and homogenization cycles were taken from literature on the basis of particle size.

8. After optimization of all parameters, NLC were successfully formulated and characterized for particle size, zeta potential, polydispersity index, drug entrapment efficiency and in vitro drug release.

9. On the basis of results of characterization, optimized formulation was selected and loaded into gel and further characterized for gel evaluation parameters like pH, viscosity, spreadability, drug content and diffusion study.

The present study has proved that poor water soluble drug Luliconazole could be significantly formulated in NLC loaded gels. Inflammation could effortlessly be cured by drug loaded NLCs. Lipids utilized in NLC could give emollient effect while gel will results in skin hydration. In this fashion, drugs can successfully be incorporated into NLCs and subsequently loaded into gel. The formulation can be significantly employed with sustained release to match the physiological and clinical requirement. The potential of NLC as carriers for topical administration was confirmed by the results obtained, demonstrating drug penetration. Lulicoanazole loaded NLC have shown excellent characterization parameters with regard to particle size, PI, zeta potential, and in vitro release profiles. Even, the phenomenon by which these mechanisms occur are not completely recognized, but active pharmaceutical ingredient embedded nanoparticles extends the anti-inflammatory effect presenting prolonged release. This research discloses sufficient facts that targeting and prolonged release effect can be achieved with immense prospective in dermal delivery.

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