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NANOSTRUCTURED LIPID CARRIERS (NLC) FOR TOPICAL DELIVERY OF ANTIFUNGAL DRUGS: RECENT ADVANCEMENT FABRICATION AND CHARACTERIZATION METHOD

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ABSTRACT

Fungal infections are common amongst global populations. The major problem is drug targeted delivery because the therapeutic dose does not reach the targeted sites. Nanotechnologies have proven their efficacy in many diseases. In this review work, we have highlighted the gross structure of the skin and various methods for the preparation of nanostructured lipid carrier's which can play in the development of future research related to fungal infections. Nanocarrier systems are the potential for immediate drug release triggered by typical formulations, thus

reducing the risk of some allergic/toxic reactions. Furthermore, the need of the hour is to design to improve the penetration power of antifungal drugs, resulting in more effective and well-organized treatment in fungal skin infections. When more commercial preparations become usable, these carrier systems can prove their effectiveness over traditional drug delivery systems.

Keywords: Fungal infection, lipid nanoparticles, nanostructured lipid carriers, and topical route

INTRODUCTION

Human skin is the outer visible portion of the body shown in **(Figure 1)**. Bristly and glabrous skins are the two types of skin that exist. Skins foresee a fundamental opposition effort in verifying the body against microbes and excessive water adversity because it interacts with the earth. Its various limits include security, temperature law, sensation, supplement vitamin-D amalgamation, and the security of supplement vitamin-B folates. Wounds will attempt to heal by confining scar tissue. Individual skin pigmentation differs from mass to mass, and skin type can range from dry to smooth **[1, 2]**.

MATERIAL AND METHODS

Epidermis

The epidermis is the main barrier to external environments, preventing bacteria from penetrating the skin and causing infection **[3]**. Epidermis cellular portion is made up of:

Keratinocytes: These are found on the basal layer of skin's which behave as a wall barrier against various environmental elements.

Melanocytes: Melanin-producing cells present in the stratum basal layer of the skin and the uvea of the eye.

Langerhans cells: Langerhans cells act as antigen-displaying cells.

Merkel cells: Merkel cells are oval receptor cells that are involved in touch, shape separation, and texture **[4]**.

The epidermis layer of skin consists of the following four sub-layers:

Stratum corneum: It is made up of keratinized cells that cover the superficial surface of the skin.

Stratum lucidum: It has a transparent appearance when examined under a microscope. It has a transparent look because it is made up of a clear layer of dead skin cells.

Stratum granulosum: The granular layer, also known as the stratum granulose, is made up of moved Keratinocytes.

Stratum spinosum: It is available in between the stratum granulosum and stratum basal. Keratinization starts in the stratum spinosum **[5]**.

Dermis

Sweat glands, oil organs, nerve finishing, hair follicles, blood, and lymph vessels are all found under the epidermis layer of skin, which is dense, fibrous, and elastic, imparting flexibility and strength to the skin muscles. It assists in the skin's maintenance and repair **[6]**.

Hypodermis

It is the lowermost layer of skin free from connective tissue, flexible filaments, and cells (e.g., fibroblasts, macrophages, and adipocytes). It acts as an insulator as well as a vitality hold **[6]**. New enhancements are being developed to ensure the most efficient delivery of active drugs molecule for the healing of skin diseases **[7]**. New treatments are being developed regularly, but they are insufficient for medications to work properly. The treatments may appear to be successful in principle, but the findings of an in-vitro study on routine measurements may vary from those of an in-vivo study. Different methods are used to improve the efficacy of each drug such as enhancing the body's solvency or absorption at the treatment location **[8]**.

TOPICAL ANTIFUNGAL

The epidermis, dermis, and hypodermis are the three main layers of the human skin, which are effective and well-organized membranes. The deepest layer of the stratum corneum is dead and keratinized cells make up the epidermis. It's a great way to prevent drugs from getting into the system, the exterior of body **[9]**. According to a study, nearly 40 million people in developing countries have been exposed to illnesses that are more likely to spread. Because of the trade-off with invulnerable function, parasitic contaminants may become a real and fast problem **[10]**. One of the most common causes of tinea and onychomycosis is dermatophytes. Candidiasis is also one of the most common superficial cutaneous parasitic infections **[4]**. When the immune system is weakened, candida can attack other body tissues/organs as well as the blood, resulting in life-threatening foundational candidiasis **[11, 12]**.

Topical treatment of fungal infections

The stratum corneum is the best for testing dermal delivery and new methods have been explored to increase its permeability. Microemulsions, vesicular transporters with ethosomes, liposomes, and niosomes are examples of colloidal drug carriers. The new carrier dermal administration of antifungal by dermal targeting includes both lipid and polymeric particulate carrier systems **[13, 14]**. There have been a few advantages in the treatment of fungal diseases, such as focusing

on the infectious site and reducing the risk of unwanted adverse reactions, increasing the treatment's viability, and ensuring more patient compliance. A variety of newly topical antifungal drug compounds have been used to treat several dermatological skin infections. Polyenes, azoles, and allylamine/benzyl amines are the most common types of topical antifungals. Cicloprox is a topically applied antifungal agent. These medications currently exist in convectional dosage forms like creams, gels, salves, and showers **[15]**. The list of most common skin diseases, as well as their antifungal operators and treatments, are shown in **(Table 1)**.

Effective topical antifungal treatment is dependent on drug penetration power through the targeted tissue. As a result, the most effective drug concentration levels in the blood should be successfully achieved through the skin. The drug substances should pass via the stratum corneum, to reach the high amount of drug concentration deep down the skin layer, particularly the viable epidermis, when antifungals are applied topically. Antifungal agents can be delivered into the skin more effectively using carriers such as colloidal carrier systems, vesicular systems, and nanoparticles. After dermal administration, antifungal compounds should reach an effective therapeutic drug concentration level in the viable epidermis **[16]**.

NANOSTRUCTURED CARRIER

Nano lipid carriers systems are the second generation of ingenious lipid nanoparticles are a potential bioactive carrier system, **(Figure 2)** depicts the potential limitations of the solid lipid nanoparticles (SLN) given in topical delivery of drugs **[17]**. The major drawback of the solid lipid nanocarriers (SLN) devices is their existence in solid form at room temperature. These biocompatible solid lipids and liquid lipid frameworks, solid lipid framework has a different structure entity than liquid lipid and are used to make nano lipid carriers **[18]**. Nano lipid carriers also have a standard molecule diameter of 10–400 nm. This carrier is a type of solid lipid network (SLN) that is fused with liquid lipids **[19]**. Miglyol, α-tocopherol, and other nanostructured based lipid carriers that encloses lipids in solid-state and oils phase **[20]**. Based upon the nanostructure, synthesis, and proportions ratio of solid and liquid lipids, they can be classified into three distinct types shown in **(Figure 3)**.

Imperfect type

This is made by combining synthetically different solid and liquid lipids, resulting in imperfections and increased drug loading. As

a result, the matrix has defects that force the drug to form amorphous clusters **[21]**.

Amorphous type

The amorphous form is made using a special type of lipid (for example, isopropyl myristate) to produce amorphous nano lipid carriers. Solid lipids are combined with a special liquid lipid such as molecules of hydroxyocta cosanylhydroxystearate, isopropyl myristate, or medium-chain triglycerides like Miglyol 812 to produce this form of Nano lipid carrier **[22]**.

Multiple type

Oil nano compartments are encapsulated with solid lipid in various forms. The drug substance is dissolved in oil phase compartments. The lipid–lipid ppt technique was used to make it. When lipids need proper drug solubility, expansion of a larger volume of liquid lipid to the lipophilic phase indicates the need for the solid matrix, which prevents the leakage of the drug molecule, while oily nano compartments show better solubility for the lipophilic nature of drugs **[22]**.

COMPOSITION OF NANOSTRUCTURED LIPID CARRIER

The key ingredients of a nanostructured lipid carrier are lipids, water, and an emulsifier **[12]**.

Emulsifiers

Lipid dispersions can be stabilized by the use of emulsifiers. Among the findings from the literature available, it has been concluded that hydrophilic emulsifiers are used for stabilizing lipid dispersions, for example, Pluronic F68, tweens (20, 80), PVA, and sodium deoxycholate **[23, 26]**. Along with these emulsifiers, lipophilic emulsifiers for example lecithin and Span 80 were commonly used in designing nano lipid carriers. A mixture of emulsifiers is used to avoid particle aggregation and improve efficacy **[27]**. Polyethylene glycol (PEG) is added to Nano lipid carriers, it remains on the nanoparticulate shell, preventing reticuloendothelial system absorption and improving systemic bioavailability.

Lipid Excipients

The internal core of nano lipid carriers is made up of both the solid and liquid nature of lipids. Glyceryl behenate, glyceryl palmitostearate, unsaturated fats, triglycerides, steroids and waxes cetyl palmitate are several of the strong lipids commonly used for nano lipid carriers. At room temperature, all the above lipids exist in solid-state. During the preparation process, they will melt at a higher temperature that is more than 80°C during the readiness process **[28]**.

In European and American regulatory authorities bodies have already approved these lipids as clinically safe. There is a high requirement for novel and biocompatible oils that are economic, non-toxic, and sterilizable before use. Nano lipid carriers made from natural plant oils are also prevalent right now **[29, 30]**.

PREPARATION METHOD FOR NANOSTRUCTURED LIPID CARRIERS

High-pressure homogenization

This method is most reliable and efficient for large-scale processing of nano lipid carriers, lipid–drug conjugates, parenteral, and SLNs. High pressure (100–200 bars) is used to force the lipid through a small gap of some few micron ranges in the high-pressure homogenization technique. Shear stress and cavitations force are responsible for particle disruption in the submicron scale. In most of the case, lipid content is present between the ranges of 5-10%. In general, there are two techniques are used for the preparation of NLCs **[31]**. The drug substance is solubilized in lipid that is being melted around 5–10°C above the freezing point in both techniques.

Hot homogenization

According to this technique, the drug substance is mixed with previously melted lipid and immersed in an aqueous surfactant solution at the same temperature with the help of high shear force. Then obtained pre-emulsion phase is homogenized through a piston gap homogenizer, and finally, the resulting nanoemulsion is cooled at room temperature and developed the nanoparticles after recrystallizes the lipid **[32]**. Graphical representation of hot homogenization technique shown in the **(Figure 4)**.

Cold homogenization technique

This method the solid lipid contains drug molecule. Cold homogenization was created solely to defeat the homogenization associated issues, such as degradation of the drug substance, partitioning and drug loss into the aqueous system of homogenization process as shown **(Figure 4)**. The drug is cooled through liquid nitrogen gas or dry ice for drug delivery to the lipid matrix. Thermal exposure of the drug is minimized with cold homogenization **[33]**.

Microemulsion technique

The lipids are dissolved, and the drug molecule is mixed with melted lipid. Water, Smix (combination of surfactant and co-surfactants) are heated at the previous temperature of the lipids and then add in previously molten lipid with constant vortexing. The hot microemulsion is dispersed in a cold aqueous phase system with constant stirring among the water in a

1:25–1:50 ratio. The oil droplets are easily recystallized after being dispersed in a cold aqueous phase **[34]**.

Solvent emulsification-evaporation technique

In this method the hydrophobic drug and lipophilic material were dissolved dichloromethane, cyclohexane, toluene, and chloroform all the solvents are immiscible with aqueous phase then emulsified in an aqueous phase system with the help of high-speed homogenizer **[35]**. By moving the coarse-emulsion with the help of mechanical vortexing, then the performance of fine emulsification can be increased. The method's key benefit is that it avoids thermal stress, which makes it superlative incorporation of extremely thermolabile drugs. The specific drawback of organic solvent it can be interfere with drug substance and reduce the solubility of lipid **[36]**.

Solvent emulsification-diffusion technique

In solvent emulsification-diffusion method, the partially water miscible solvents are used some example of partially miscible solvent like butyl lactate, ethyl acetate, IPA, benzyl alcohol and methyl acetate, and this technique may be carried out in either an aqueous or in oil phase. To ensure primarily thermodynamic equilibrium of solvent and water and both phases were equally saturated. When heating is heating necessarily requires for solubilizing the lipid, the saturation step was carried out at that temperature. Following the development of the oil/water type of emulsion, water (continous phase) was applied to the system in a typical ratio ranging from 1:5 to 1:10 to enable the solvent diffusion into the continuous phase, resulting lipid aggregation. Finally, vacuum distillation or lyophilization is used to remove the diffused solvent **[37]**.

Phase inversion temperature method

Temperature-induced phase inversion between the O/W to W/O types of emulsions and vice versa is a eminent process for the development of microemulsions, it stabilized using nonionic surfactants **[8]**. At 25°C, the surfactant value on the HLB scale (hydrophilic lipophilic balance) is valid. The presence of compound structures in the system and a very low surface tension characterize this particulate state. If the temperature is raised even higher, the surface active compounds preference for the lipid phase increases to the point that w/o emulsions can be stabilized.

Melting dispersion method

The drug molecule and solid lipid are melted with organic solvent, which is referred to as the oil phase, while the aqueous phase and oil

phase are heated at 70-80°C. The oil phase is mixed with a small quantity of aqueous phase, and stirred using vortex shaker for a few hours. Finally, produced nanoparticles cooled at room temperature **[38]**.

Ultrasonication or high shear homogenization technique

The cavitation mechanism underpins ultrasonication. The drug is first mixed with melted lipid. In second stage heated the aqueous phase as the same temperature then add melted lipid and emulsified using probe sonication or a high-speed magnetic stirrer, or titrate the liquid lipid phase with drop by drop aqueous phase by using magnetic stirring. The obtained pre-emulsion is ultrasonicated with the help of water bath and probe–sonicator (at 0°C). To eliminate the impurities introduced during ultrasonication, the obtained product is filtered using a 0.45µm membrane filter **[39]**.

Solvent injection (solvent displacement) technique

The solvent like (dimethyl sulfoxide and ethanol) distributes rapidly in water is used in this technique **[40]**. Lipid is first dissolved in the solvent then immediately injected into an aqueous phase surfactant solution using injectable needle. Precipitate the lipid particles into aqueous solution as the solvent migrates rapidly through the water. Particles with a higher velocity are smaller. Larger particles are generated by more lipophilic solvents, which can become a problem.

Low temperatures, easy handling, low shear stress, and a quick production process are all advantages of this system, which requires no technically sophisticated equipment example high-pressure homogenizer. The use of organic solvents, on the other hand, is the main drawback.

Double emulsion technique

In this technique mostly hydrophilic drugs is dissolved in aqueous solution and then emulsified with melted lipid. The primary emulsion is stabilized by using stabilizer in an aqueous phase containing a hydrophilic emulsifier, accompanied by mechanical stirring and filtration. The use of a double-emulsion technique eliminates the need of melted lipid in order to make peptide-loaded lipid nanoparticles and the surface of nanoparticles can be adjusted to make them sterically stable by adding lipid-PEG derivatives **[9]**. Various parameters required for an effective lipid nanoparticle formulation as shown in **(Figure 5)**.

CHARACTERIZATION METHODS

The physicochemical characterization of Nano lipid carriers is necessary to confirm the quality and safety. To investigate the structure of nano lipid carrier, versatility, and

atomic state of the mixes, various techniques were used, including particle size analysis, differential scanning calorimetry (DSC), zeta potential (ZP), transmission electron microscopy (TEM), X-Ray dissipating, laser diffraction, enraptured light microscopy, and field-flow fractionation. These procedures reveal the formulation's physical and chemical stability the surface charge, in general, determines whether the particles can flocculate or not **[8]**.

Particle size

The physical strength of vesicle dispersion is completely dependent on particle size. Decreases the particle size then increases the particle surface area. Photon Correlation Spectroscopy (PCS) is working on the principle of laser light diffraction and examine particle's diameters ranging from 200 nm to 1 μm. Rayleigh's theorem states that the scattering rate of particles smaller than 200 nm corresponds to the sixth power of the particle diameter **[8]**.

Zeta potential

It is the electric potential of a particle in the form of a suspension formulation. It is a very helpful parameter used to demonstrate the physical stability of colloidal dispersions. The surface charge creates a potential about the particle that is strongest due to near the surface and diminishes on the separation of particle from the medium. It is used for measuring particle speed in an electrical field.

This method is used to determine the molecular size of particles. On a sample holder, aqueous Nano lipid carrier dispersions can be added and dispersed. The samples are placed in the magnifying lens vacuum section, and the air in the chamber is sucked out of the cavity. Light emission is produced by an electron gun located at the top of the column. The emitted the electrons by the light and pass through the lens, and scans the specimen row by row. The electrons are then collected, and then signals are sent to an amplifier and then recorded **[8]**.

Differential scanning calorimetry

The differential scanning calorimetry method is most widely used to obtain information about the compound or formulation of physical and energetic properties. It calculates the amount of heat lost or gained as a function of temperature due to physical or chemical changes in the drug sample. The degree of crystallinity of powdered particle scattering is calculated by using DSC. The crystallinity rate of powdered is measured using DSC and comparing the mass material's liquefying enthalpy/g to the scattering's softening enthalpy/g **[8]**.

Atomic force microscopy

This technique is appropriate for monitoring extremely tiny morphological features of drug formulation. Instead of using photons/electrons, it uses a small sharp-tipped probe at the terminal end (free) of a cantilever that is guided by the interatomic forces between the tip and the specimen's surface **[44]**. While electron microscopy is commonly used, the Atomic force microscopy technique has many advantages, including real-time quantitative data achievement in three dimensions, quick preparation, versatile conditions, and efficient magnifications at the nanoscale **[45]**.

In-vitro drug release profile

To estimate the rate of drug release profile from the formulation, in-vitro drug release employs commonly accepted Franz diffusion cells. It entails the application of a drug product to a membrane that separates donor and recipient chambers (synthetic membrane, excised animal skin, or excised human skin) is commonly used to investigate the drug release profile **[46]**. In-vivo, the receiver chamber maintains the sink conditions. The release rate profile of drug delivery obtained using this study is assuming thought to be comparable with in-vivo conditions **[47]**. This parameter has been widely used in drugs to demonstrate the drug release profile of the formulations and better understand cutaneous drug transport mechanisms **[48, 49]**.

Controlled drug release from nano lipid carriers will result in a longer half-life and retarded the slower enzymatic attack in systemic flow. Drug release profile patterns are affected by various factors including the temperature at which nano lipid carriers are manufactured are dependent upon the composition of emulsifier, and oil percentage incorporated in lipid matrix **[50]**. The amount of drug in outer shells of nanoparticles and released the surface in a flash manner, while the drug substance is incorporated in core matrix and released prolong period.

The drug release profile study must be carried out to compare the ability of various formulations to hold the drug sample integrated for a prolonged period and slowly release the drug from the lipid matrix nanoparticles.

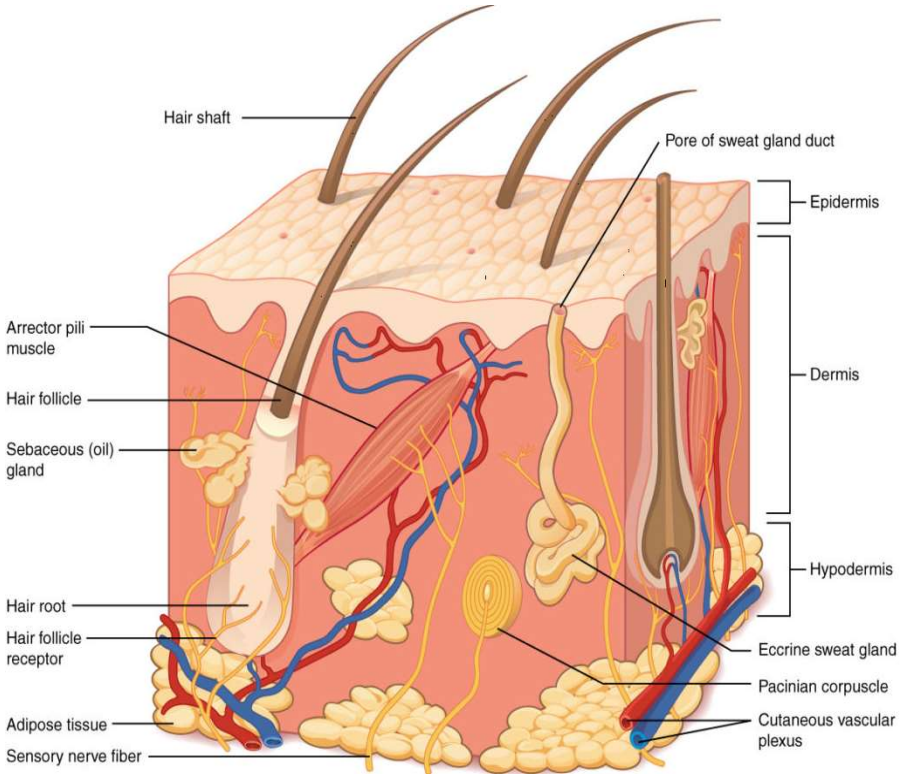

Figure 1: Structure of the Skin

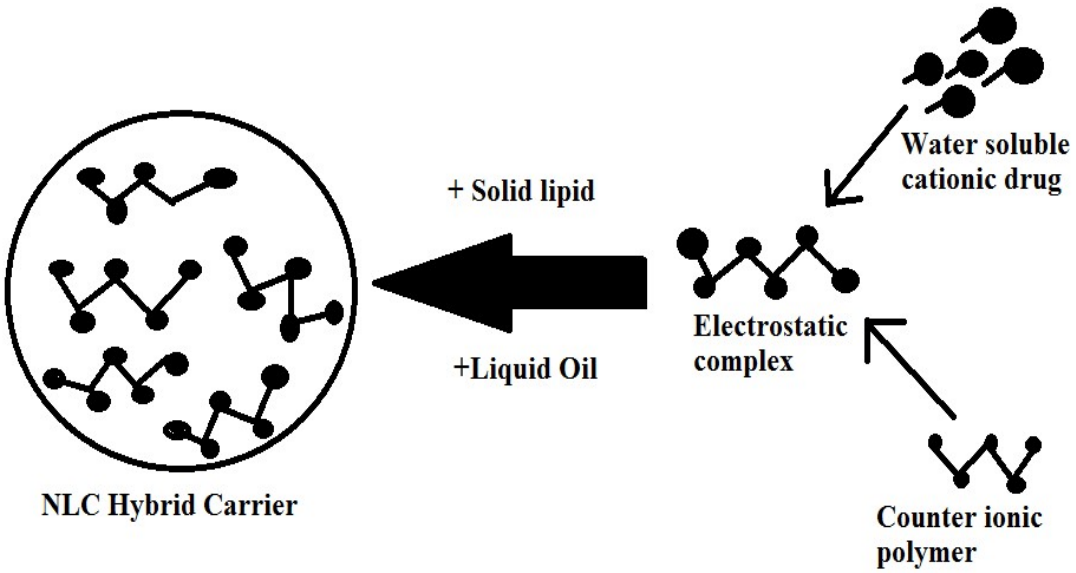

Figure 2: Nanostructured lipid carrier structure

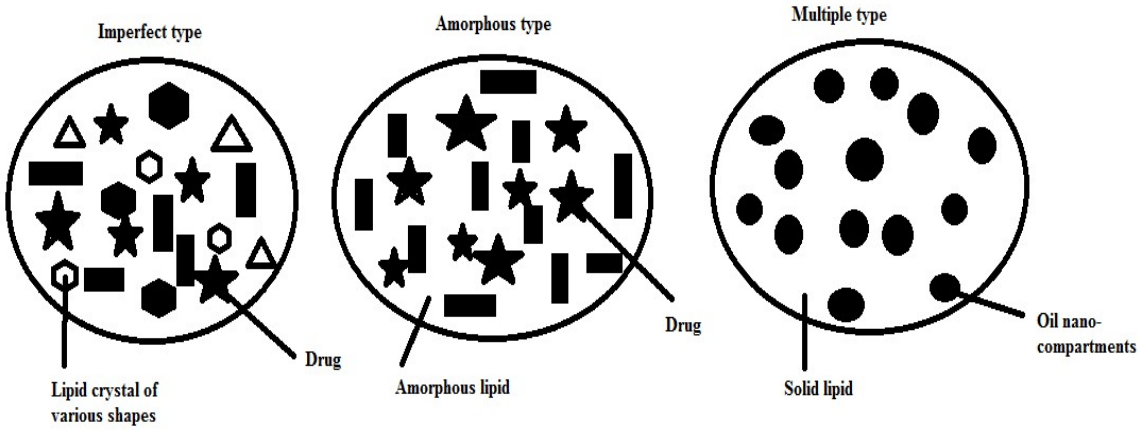

Figure 3: (A) Imperfect type, (B) Amorphous type, and (C) Multiple type [21].

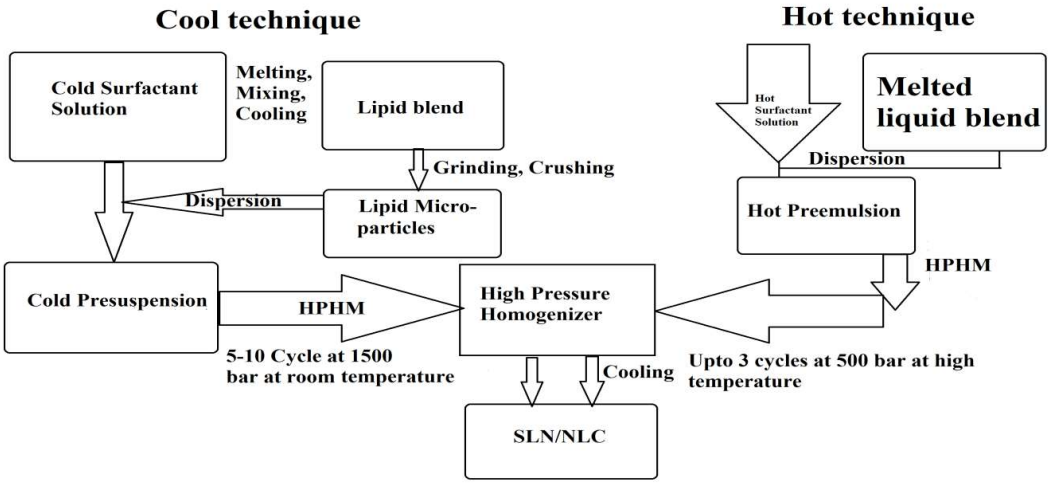

Figure 4: Schematic representation of Cool and Hot homogenization technique of NLC (Nanostructured lipid carrier) [8].

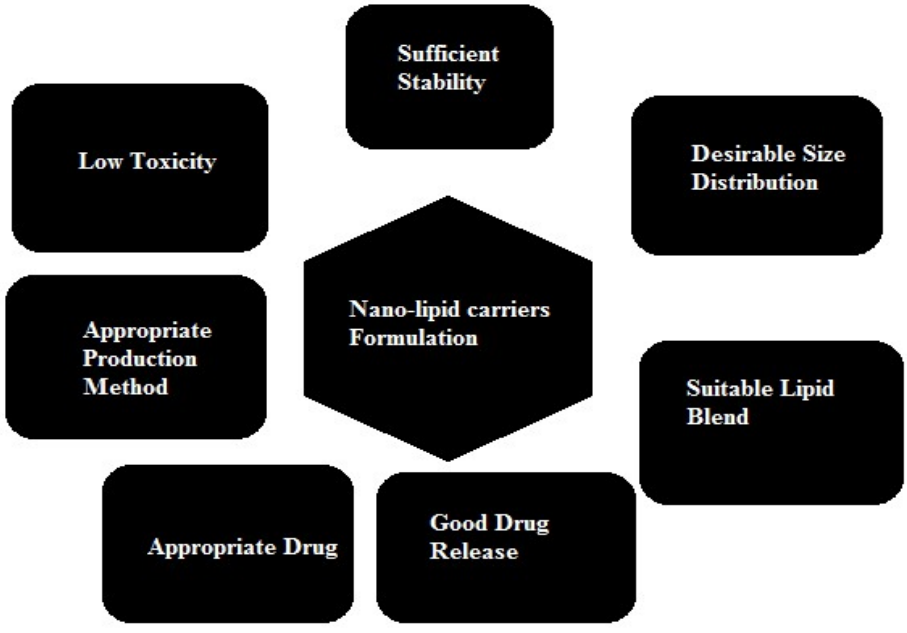

Figure 5: Representing some parameters for producing a successful nanostructured lipid carrier formulation

Table 1: The most common skin diseases, antifungal agents, affected site and treatments [48]

Sr. No.	Antifungal drugs	Lipid	Mode of action (MOA)	Method of preparation (MOP)	Cause
1	Oxiconazole	Carbopol	It act to destabilize the fungal cytochrome P450 51 enzyme	Ultrasonication method	Lysis of the fungal cell membrane
2	Miconazole nitrate	Dynasan 116	To inhibit the CYP2C9	High pressure homogenization method	Cutaneous
3	Amphotericin-B	Lutrol-F 127, phospholipon 90 H	Am-B selectivity bind ergosterol that causes leakage of fungal cells	High pressure homogenization method	Systemic Fungal Infection
4	Terbinafine	Glyceryl monostearate, glyceryl behenate, glyceryl palmitostearate	Inhibits ergosterol synthesis by inhibiting the fungal squalene	Micro-emulsification technique	Tinea pedis
5	Voriconazole	Compritol 888 ATO	It bind and inhibits the ergosterol synthesis by inhibiting the CYP450-dependent 14-Alpha sterol demethylase enzyme	High pressure homogenization method	Urogenital tract infections
6	Terbinafine hydrochloride	Compritol 888 ATO	It inhibits the fungal and cell wall synthesis	High pressure homogenization method	Mycoses
7	Bifonazole	Dynasan 114	Inhibiting the production of ergosterol	Ultrasonication method	Superficial fungal infections
8	Clotrimazole	Tyloxapol	Inhibit the growth of individual entity of candida or fungal cell by changing the permeability of the fungal cell wall	Hot homogenization technique	Rashes

CONCLUSIONS

Nanostructured lipid carriers have a significantly higher drug-loading capability than other typical delivery systems, such as creams, ointments, and gels, which have historically been used to treat fungal skin infections though deep down-seated. Nanocarrier systems are the potential for immediate drug release triggered by typical formulations, thus reducing the risk of some allergic/toxic reactions. Furthermore, the need of the hour is to design to improve the penetration power of antifungal drugs, resulting in more effective and well-organized treatment in fungal skin infections. When more commercial preparations become usable, these carrier systems can prove their effectiveness over traditional drug delivery systems.

LIST OF ABBREVIATIONS

NLC: Nano Lipid Carrier; SLN: Solid Lipid Nanoparticles; AFM: Atomic Force Microscopy; DSC: Differential Scanning Calorimetry; PCS: Photon Correlation Spectroscopy; ZP: Zeta Potential; PEG: Poly Ethylene Glycol; MOP:

Method of Preparation; MOA: Mode of Action; HLB: Hydrophilic Lipophilic Balance; O/W: Oil in Water; W/O: Water in Oil; TEM: Transmission Electron Microscopy.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE- Not applicable

COMPETING INTERESTS- The author has declared that no conflicts of interest exist.

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AUTHOR'S CONTRIBUTION- In the present review, MK analyzed the data related to fungal disease and various treatments approaches and were the most important contribution in making the manuscript. AK, NS, KA and PP contributed the various nanotechnologies for treatment approaches. SK, LS, RKP and NS elaborated on the skin disease part in the manuscript. All authors read and approved the final manuscript.

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