Review Article

Recent Advances in the Development of the Nanostructured Lipid Carriers for the Topical Fungal Infections

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Abstract

Topical fungal infections are one of the often faced diseases worldwide. The first choice for the treatment of fungal infection is topical therapy due to its advantages such as decreasing the risk of systemic side effects and targeting the drug at the site of fungal infection. The treatment of the fungal infection depends on the penetration of the drug molecules through the outermost layer of the skin (stratum corneum) at an effective concentration. The disadvantages of topical treatment are its lack of drug adherence at the site of application and bigger particle size of drug molecules. Nanostructured lipid carriers are the advanced form of lipid nanoparticles and carry the nano range of the drug molecules, which helps to achieve localized and slow release. The topical route is generally preferred due to the possible side effects of oral medication. Advances in the field of formulation may soon render outdated conventional products such as creams, ointments, and gels. Several carrier systems loaded with antifungal drugs have shown promising results in the treatment of skin fungal infections.

Keywords: *Fungal infection, lipid nanoparticles, nanostructured lipid carriers, topical*

Introduction

The human skin is the outer covering of the body [Figure 1]. There are two general sorts of skin, bristly and glabrous skin (smooth). Because it interfaces with the earth, skins expect a fundamental opposition work in verifying the body against pathogens and unnecessary water adversity. Its diverse limits are assurance, temperature rule, sensation, amalgamation of supplement D, and the security of supplement B folates. Truly hurt skin will endeavor to repair by confining scar tissue. In individuals, skin pigmentation varies among masses, and skin type can go from dry to smooth.[1]

Epidermis

The epidermis is the primary boundary for external condition that keeps pathogens away from going into the skin and causing infection.[3] The cellular component of epidermis consists of:

Keratinocytes: These are available on the basal layer of the skin and act as an obstruction from various ecological components.

Melanocytes: Melanin-creating cells that are situated in the stratum basal of skin and uvea of the eye.

Langerhans cells: They fill in as antigen-displaying cells.

Merkel cells: These are the oval receptor cells that are connected with the feeling of touch, separation of shape, and texture.[3,4]

The epidermis consists of the following four sub-layers:

Stratum corneum: It includes keratinized cells that cover the uppermost layer of the skin.

Stratum lucidum: It is also named as translucent appearance under a microscope. As it gives a translucent appearance which is a clear layer of dead skin cells.

Stratum granulosum: It is also known as the granular layer as it comprises moved keratinocytes.

Stratum spinosum: It is available in between the stratum granulosum and stratum basal. Keratinization starts in the stratum spinosum.[3-6]

Dermis

The dermis is present beneath the epidermis layer and is thick, fibrous, and elastic, imparting flexibility and strength to the skin,[5] and comprises sweat glands, oil organs, nerve

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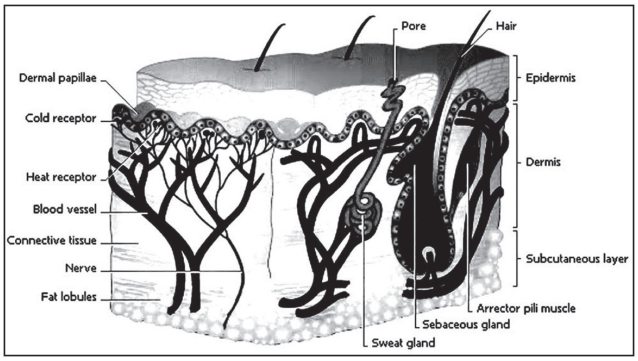

Figure 1: Structure of the skin[2]

finishing, hair follicles, blood, and lymph vessels. It helps in the maintenance and repairing of the skin.[6]

Hypodermis

The hypodermis is the lowermost layer of the skin comprising free connective tissue, flexible filaments, and cells (e.g., fibroblasts, macrophages, and adipocytes). It works as a vitality hold and insulator.[6]

New enhancements are being produced for the best possible conveyance of the drugs for the treatment of skin diseases.[7] New therapies is growing on daily basis but they are not sufficient for medications to work appropriately. The treatments may appeared effective hypothetically, but the in-vitro study on routine measurements may show the different results of *in-vivo* study. There are different techniques are used to increase the effectiveness of every medication used in these treatments like increasing the solvency in the body or by ingestion at the site.[8]

Topical Antifungal

Human skin is an efficient, organized membrane, and it has three primary layers, which are called epidermis, dermis, and hypodermis. Stratum corneum, the farthest layer of the epidermis is formed by dead and keratinized cells, and it is an excellent barrier to the entrance of drug through the skin.[9] A study shows the result that near about 40 million persons have experienced spreadable diseases more likely in immature countries. The infestation of parasitic contaminants can be real and quick due to trading off with invulnerable function.[10] Dermatophytes are one among the most incessant reasons for tinea and onychomycosis. Candidiasis is additionlly among the most accross the broad shallow cutaneous parasitic infection.[4] Indeed, candida can attack further tissues just as the blood, which prompts life-threatening foundational candidiasis, when the insusceptible framework is weakened.[11,12]

Topical treatment of fungal infections

The best test for dermal delivery is stratum corneum, and so as to improve its permeability, new approaches have been investigated. Colloidal drug carriers, such as micro emulsions, vesicular transporters with ethosomes, liposomes and niosomes. Both lipid and polymeric particulate carrier systems are among that new carrier dermal administration of antifungals by dermal targeting.[13-15]

For the treatment of fungal infections, there have been a few superiorities, including targeting on the site of disease, a reduction of the risk of body reactions, enhancement of the viability of treatment, and high patient compliance. Different kinds of topical antifungal compounds have been used in the treatment of a variety of dermatological skin infections. Topical antifungals are mainly categorized into polyenes, azoles, and allyl amine/benzylamines. Ciclopirox is an antifungal agent that is used topically. Currently, these drugs are commercially available in conventional dosage forms, for example, creams, gels, salves, and showers. The common skin diseases with their viable antifungal operators and treatment are shown in Table 1.

The efficiency of the topical antifungal treatment relies on the penetration of drugs through the targeted tissue. Consequently, the effective drug concentration levels should be accomplished in the skin. In the topical administration of antifungals, the drug substances should pass the stratum corneum, which is the peripheral layer of the skin, to reach lower layers of the skin, especially into the viable epidermis. Delivery of antifungal agents into the skin can be increased with the carriers including colloidal systems, vesicular carrier, and nanoparticles. Antifungal drugs should achieve effective therapeutic level in viable epidermis after dermal administration.[16]

Nanostructured Lipid Carrier

Nanostructured lipid carrier (Nano lipid carriers), the second-generation of inventive lipid nanoparticles that will go about as a bioactive carrier system, [Figure 2] has been created to control some potential restrictions of the solid lipid nanoparticle (SLN).[17] It stays solid at room temperature.

Nano lipid carriers are made of biocompatible solid lipid frameworks and liquid lipid, which has a distinctive synthetic structure from that of the solid lipid.[18] In addition, Nano lipid carriers have the standard molecule diameter of 10–1000 nm. Nanostructured lipid carriers (Nano lipid carriers) are the second-generation SLN made of solid lipid network, which are fused with liquid lipids.[19] Among the nanostructured lipid carriers that contain solid lipids together with liquid oils are Miglyol, α-tocopherol, and so on.[20]

Nano lipid carriers can be arranged into three unique types depending on the nanostructure [Figure 3], synthesis, and proportions of solid and liquid lipids.

Imperfect type

This is produced by blending synthetically different solid and liquid lipids, which gives the imperfections and enhances the drug loading. Subsequently, the matrix contains defects to oblige the drug in amorphous clusters.[21]

Multiple type

The multiple type contains oil nanocompartments that are encapsulated with solid lipid. The drug is dissolved in the oil

Table 1: Common skin diseases, antifungal agents, affected site, and treatment

S. no.	Antifungal drugs	Lipid	Mode of action (MOA)	Method of preparation (MOP)	Cause	Reference
1	Miconazole nitrate	Dynasan 116	To inhibit the CYP2C9	High pressure homogenizer method (HPHM)	Cutaneous mycoses	[40]
2	Terbinafine hydrochloride	Compritol 888 ATO	It inhibits the fungal and cell wall synthesis	HPHM	Mycoses	[41]
3	Clotrimazole	Tyloxapol	Inhibit the growth of individual candida or fungal cells by altering the permeability of the fungal cell wall	Hot homogenization technique	Rashes	[1]
4	Oxiconazole	Carbopol	It acts to destabilize the fungal cytochrome P450 51 enzyme	Ultrasonication method	Lysis of the fungal cell membrane	[42]
5	Voriconazole	Compritol 888 ATO	It binds and inhibits ergosterol synthesis by inhibiting CYP450-dependent 14-alpha sterol demethylase	High-pressure homogenization	Urogenital tract infections	[46]
6	Amphotericin B	Lutrol-F 127, phospholipon 90H	AmB selectivity bind ergosterol that causes leakage of fungal cells	High-pressure homogenization	Systemic fungal infections	[47]
7	Bifonazole	Dynasan 114	Inhibiting the production of ergosterol	Ultrasonication	Superficial fungal infections	[48]
8	Terbinafine	Glyceryl monostearate, glyceryl behenate, glyceryl palmitostearate	Inhibits ergosterol synthesis by inhibiting the fungal squalene	Microemulsion technique	Tinea pedis	[49]

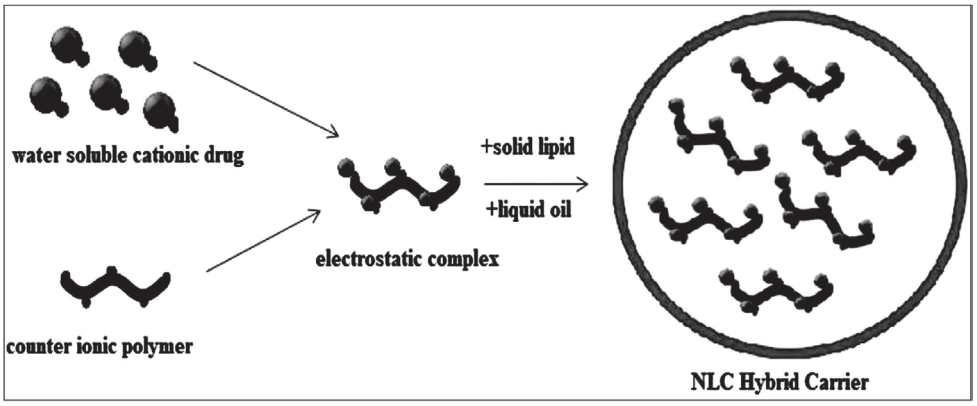

Figure 2: Structure of nanostructured lipid carrier

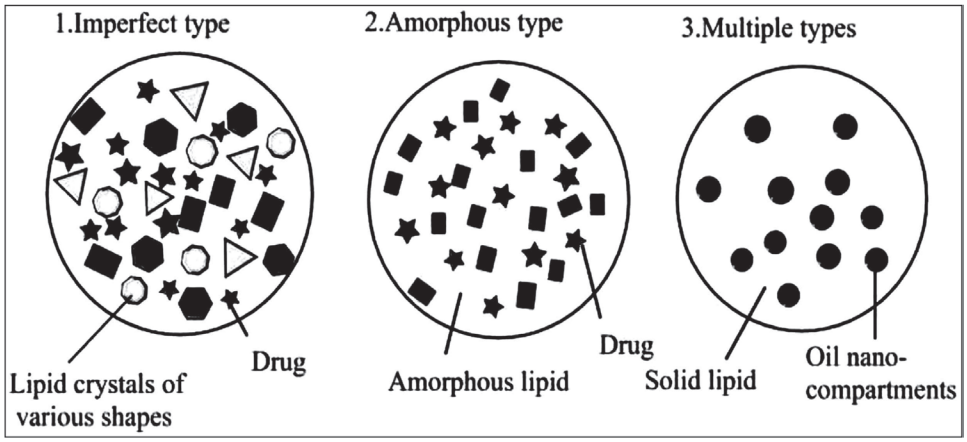

Figure 3: (A) Imperfect type, (B) amorphous type, and (C) multiple type[21]

compartments. It was prepared by lipid–lipid precipitation method. At the point when lipids need proper drug solubility, expansion of a higher amount of liquid lipid to the lipophilic phase shows the benefits of the solid matrix, which prevents the drug leakage, whereas the oily nanocompartments show relatively high dissolvability for lipophilic drugs.[22]

Amorphous type

The amorphous type is prepared by the special type of lipids (e.g., isopropyl myristate) with the end goal that the Nano lipid carriers obtained are in amorphous state. This type of Nano lipid carriers is obtained by the mixing of solid lipids with special liquid lipids (e.g., hydroxyocta cosanylhydroxystearate, isopropyl myristate or medium chain triglycerides, such as Miglyol 812).[22]

Composition of Nanostructured Lipid Carriers

Lipids, water, and emulsifier are considered as the main ingredients of nanostructured lipid carrier.[12]

Emulsifiers

Lipid dispersions can be stabilized by the use of emulsifiers. Among the findings from literature available, it has been concluded that hydrophilic emulsifiers are used for stabilizing lipid dispersions, for example, Pluronic F68 (Poloxamer 188), polysorbates (Tween), polyvinyl alcohol, and sodium deoxycholate.[23-26] Beside these emulsifiers, for formulating the Nano lipid carriers we generally used lipophilic or amphiphilic emulsifiers such as Span 80 and lecithin. To prevent particle aggregation, a combination of emulsifiers are used for better efficacy.[27] On incorporation of polyethylene glycol (PEG), in Nano lipid carriers, it stays on the nanoparticulate shell to prevent uptake by the reticuloendothelial system and to enhance systemic bioavailability.

Lipids

Nano lipid carriers are composed of both solid and liquid lipids for developing the internal core. The solid lipids generally used for Nano lipid carriers include glyceryl behenate (Compritol 888 ATO), glyceryl palmitostearate (Precirol ATO 5), unsaturated fats (e.g., stearic corrosive), triglycerides (e.g., tristearin), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate). These all lipids are in solid state at environmental temperature. They would dissolve at higher temperatures (e.g., >80°C) amid the readiness process.[28]

In general, these lipids are already approved by the European and American regulatory authorities for clinical applications and for their "generally recognized as safe" status. There is a need for novel and biocompatible oils that are cost-effective, nonirritating, and capable of being sterilized before application. Nano lipid carriers produced using natural oils from plants are also currently popular.[29,30]

Preparation of Nanostructured Lipid Carriers

High-pressure homogenization technique

High-pressure homogenization technique is one of the most reliable and powerful techniques for the large-scale production of Nano lipid carriers, lipid–drug conjugate, SLNs, and parenteral emulsions. In high-pressure homogenization technique, high pressure is introduced to push the lipid (100–200 bars) through a narrow gap of few micron ranges. So, shear stress and cavitation are the forces that cause the disruption of particle to submicron range. Normally, the lipid contents are in the range of 5%–10%. Basically, there are two approaches for production by high-pressure homogenization, hot and cold homogenization techniques.[31] For both the techniques, the drug is dissolved in the lipid that is being melted at approximately 5°C–10°C above the melting point.

Hot homogenization technique

In this technique, the drug along with melted lipid is dispersed under constant stirring by a high shear device in the aqueous surfactant solution of same temperature. The pre-emulsion obtained is homogenized by using a piston gap homogenizer

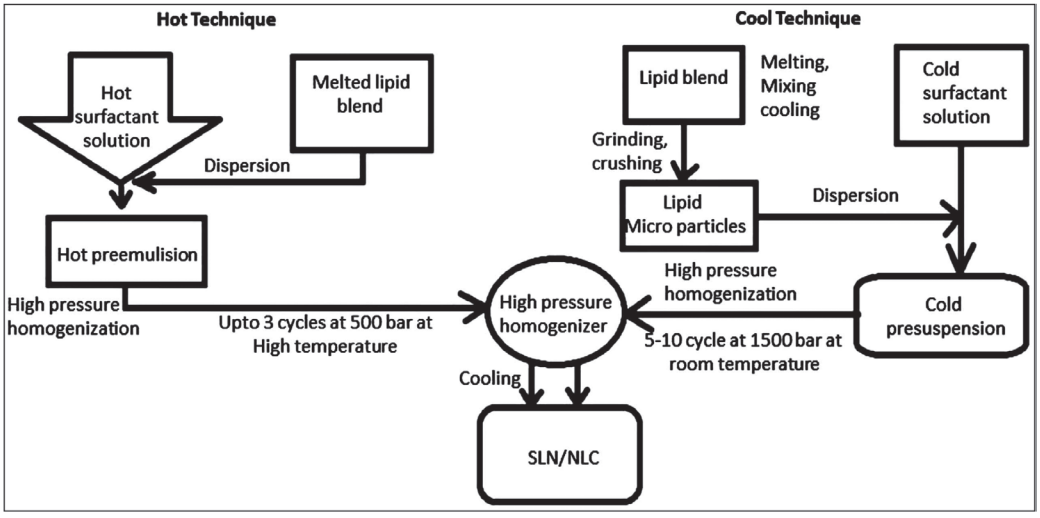

Figure 4: Schematic overview of the hot and cold homogenization technique of NLC (nanostructured lipid carrier)[8]

and the obtained nanoemulsion is cooled down to room temperature where the lipid recrystallizes and leads to formation of nanoparticles[32] [Figure 4].

Cold homogenization technique

Cold homogenization technique is carried out with the solid lipid that contains drug. Cold homogenization has been developed just to minimize the problems of the hot homogenization technique, such as temperature-mediated accelerated degradation of the drug payload, partitioning, and hence loss of drug into the aqueous phase during homogenization. In the proceeding step the drug is cooled rapidly using liquid nitrogen or dry ice for drug distribution in lipid matrix as shown in Figure 4. Cold homogenization minimizes the thermal exposure of the sample.[33]

Microemulsion technique

The lipids (fatty acids or glycosides, e.g., stearic acid) are melted, and the drug is incorporated in the molten lipid. A mixture of water, cosurfactant(s), and the surfactant is heated to the same temperature as the lipids and added under mild stirring to the molten lipid. This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water in a ratio in the range 1:25–1:50. This dispersion in cold aqueous medium leads to rapid recrystallization of the oil droplets.[34]

Solvent emulsification–evaporation technique

In this technique, the hydrophobic drug and lipophilic material were dissolved in a water immiscible organic solvent (e.g., cyclohexane, dichloromethane, toluene, and chloroform), which is then emulsified in an aqueous phase using high-speed homogenizer. The efficiency of fine emulsification can be improved by passing the coarse emulsion through the microfluidizer. The main advantage of the method is to avoid thermal stress, which makes it appropriate for the incorporation of highly thermolabile drugs. A clear disadvantage is the use of organic solvent, which may interact with drug molecules and limit the solubility of the lipid in the organic solvent.[35]

Solvent emulsification–diffusion technique

In solvent emulsification–diffusion technique, the solvent used (e.g., benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, and methyl acetate) must be partially miscible with water, and this technique can be carried out either in aqueous phase or in oil. Initially, both the solvent and water were mutually saturated to ensure the initial thermodynamic equilibrium of both liquids. When heating is required to solubilize the lipid, the saturation step was performed at that temperature. After the formation of oil/water emulsion, water (dilution medium), in typical ratio ranging from 1:5 to 1:10, was added to the system to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles. Finally, the diffused solvent was eliminated by vacuum distillation or lyophilization.[36]

Phase inversion temperature method

Phase inversion of oil/water to water/oil emulsions and vice versa induced by temperature change is a long-known method to produce microemulsions stabilized with nonionic surfactants.[8] The value of surfactant on HLB (hydrophilliclipophillic balance) is valid at 25°C.This particulate state is characterized by a very low surface tension and the presence of complex structures in the system. If the temperature is further increased, the surface active compounds affinity to the lipid phase becomes higher enough to stabilize emulsions of water/oil type.

Melting dispersion method

In melting method, drug and solid lipid are melted in an organic solvent regarded as oil phase, and simultaneously water phase is also heated to the same temperature as oil phase. Subsequently, the oil phase is added to a small volume of water phase, and the resulting emulsion is stirred at high speed for few hours. Finally, it is cooled down to room temperature to yield nanoparticles.[37]

High shear homogenization or ultrasonication technique

Ultrasonication is based on the mechanism of cavitation. In first step, the drug is added to previously melted solid lipid. In second step, the heated aqueous phase (heated to same temperature) is added to the melted lipid and emulsified by probe sonication or by using high-speed stirrer or aqueous phase added to lipid phase drop by drop, followed by magnetic stirring. The obtained pre-emulsion is ultrasonicated dousing probe–sonicator with water bath (at 0°C). The obtained product is filtered through a 0.45-µm membrane to remove impurities carried in during ultrasonication.[38]

Solvent injection (or solvent displacement) technique

Technique in which a solvent that distributes very rapidly in water (dimethyl sulfoxide and ethanol) is used.[39] First, the lipid is dissolved in the solvent and then it is quickly injected into an aqueous solution of surfactants through an injection needle. The solvent migrates rapidly in the water and the lipid particles precipitate in the aqueous solution. Higher velocity results in smaller particles. The more lipophilic solvents give larger particles, which may become an issue.

This method offers advantages such as low temperatures, low shear stress, easy handling, and fast production process without technically sophisticated equipment (e.g., high-pressure homogenizer). However, the main disadvantage is the use of organic solvents.

Double-emulsion technique

In double-emulsion technique, the drug (mainly hydrophilic drugs) is dissolved in aqueous solution, and further emulsified in melted lipid. The primary emulsion is stabilized by adding stabilizer that is dispersed in aqueous phase containing hydrophilic emulsifier, which is followed by stirring and filtration. Double-emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles, and the surface of the nanoparticles can

be modified to sterically stabilize them by means of the incorporation of lipid-PEG derivatives.[9] Various parameters required for a successful lipid nanoparticle formulation as shown in Figure 5.

Physicochemical Characterization of Nano Lipid Carrier

The physicochemical portrayal of Nano lipid carriers is essential to confirm the quality control and security. Different strategies such as particle size analysis, zeta potential (ZP), transmission electron microscopy, differential scanning calorimetry (DSC), X-Ray dissipating, enraptured light microscopy, laser diffraction, and field-flow fractionation were performed to investigate the structure, versatility, and atomic condition of the mixes. These procedures uncover the physical and chemical stability of the formulation, the surface charge will, in general, decide whether the particles will flocculate or not.[8]

Particle size

The particle size is critical parameter in producing control and quality because the physical strength of vesicle dispersion relies upon particle size, as particle size decreases than the surface area increases. PCS (Photon Correlation Spectroscopy) is based upon the laser light diffraction, which provide appropriate method for examination and can be applied for particles ranging from 200 nm–1 μm. For particles below 200 nm, Rayleigh's hypothesis holds the scattering intensity to be corresponding to the sixth power of the particle diameter.[8]

Zeta potential

ZP is the electric potential of the particle in a suspension. It is a parameter, which is helpful for the assessment of the physical stability of colloidal dispersions. The surface charge produces a potential around the particle, which is at the highest near the surface and decays with separation into the medium. The ZP can be estimated by determining the speed of the particles in an electrical field (electrophoresis measurement).

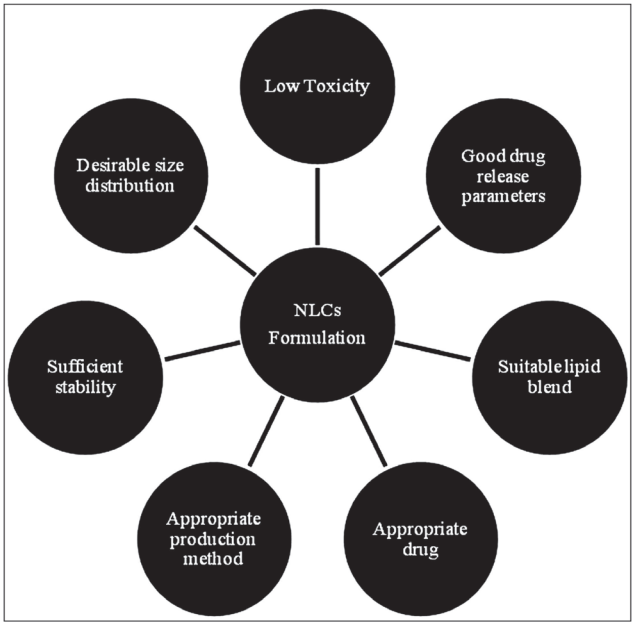

Figure 5: Parameters in producing a successful nanostructured lipid carrier formulation

This method can be used to investigate the shape of the particles prepared and to assess the molecular size of these particles. Aqueous Nano lipid carrier dispersions can be applied and spread on a sample holder (thin carbon film). The samples are put within the vacuum segment of the magnifying lens, and the air is siphoned out of the chamber. An electron gun placed at the top of the column discharges a light emission. The light emission electrons pass through the lens, which concentrates the electrons to a fine spot and scans across the specimen row by row. Then the electrons are gathered and these signs are sent to amplifier.[8]

Differential scanning calorimetry

DSC is generally used to get information about both the physical and the energetic properties of a compound or formulation. It measures the heat loss or gain because of physical or chemical changes within the sample as a function of the temperature. DSC of powder is performed for the determination of the degree of crystallinity of the particle scattering. The rate of crystallinity using DSC is evaluated by examination of the liquefying enthalpy/g of the mass material with the softening enthalpy/g of the scattering.[8]

Atomic force microscopy

Atomic force microscopy (AFM) is optimal for measuring morphological and surface features that are extremely small. AFM does not use photons or electrons but a very small sharp-tipped probe located at the free end of a cantilever driven by interatomic repulsive or attractive forces between the tip and the surface of the specimen.[43] Although electron microscopy is still frequently used, the AFM technique offers substantial benefits: real quantitative data acquisition in three dimensions, minimal sample preparation times, flexibility in ambient operating conditions, and effective magnifications at the nano levels.[44]

In vitro drug release

In-vitro release uses widely accepted Franz diffusion cells to estimate the rate of drug release from drug products. It involves the application of a drug product onto a membrane (synthetic membrane, excised animal skin, or excised human skin) that separates the donor and receiver chambers. The receiver chamber simulates sink conditions *in vivo*. The rate of delivery obtained from these studies is assumed to be similar to the *in vivo* situation. The method has been widely used in the discovery research for screening formulations and understanding the mechanism of cutaneous drug transport.

The controlled release of the drugs from Nano lipid carriers can result in the prolonged half-life and retarded enzymatic attack in systematic flow. The drug release behavior from Nano lipid carriers is dependent on the production temperature,

emulsifier composition, and oil percentage incorporated in the lipid matrix.[45] The drug amount in the outer shell of the nanoparticles and on the particulate surface is released in a burst manner, whereas the drug incorporated into the particulate core is released in a prolonged way.

The release study must be performed to compare the capacity of different samples to retain the drug incorporated for a longer time and to release it slowly from the lipid matrix of the nanoparticles.

Conclusion

Nanostructured lipid carriers have greater drug-loading capacity and huge advantage over the other conventional delivery systems, including creams, ointments, and gels, which are traditionally being used for the treatment of skin fungal infections, even if they are deep seated. Carrier systems have the ability to overcome the immediate drug release caused by these conventional formulations, hence they avoid the possibility of induction of allergic reactions. Moreover, they are especially customized to enhance the penetration of the antifungal agents, leading to more effective treatment of skin fungal infections, especially the deeper ones. These carrier systems are expected to slowly replace the conventional systems as more commercial preparations become available.

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Conflicts of interest

There are no conflicts of interest.

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