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Nanostructure Lipid Carrier (NLC) - An Overview

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ABSTRACT

Having developed exponentially in nanotechnology, the target was on a therapeutic undertaking, especially for objective drug therapy. In 1980, K. Eric Drexler developed and popularized the Nanotechnology concept. The nanocarriers had a revolutionary approach developed. During the last few decades, lipid nanoparticles (LNPs) have brought special interest. Two major types of lipid-based nanoparticles are solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs). SLNs have been developed to overcome the inconvenience of other colloidal carriers, such as emulsions, liposomes and polymeric nanoparticles because they have advantages such as good release profile and objective drug delivery with excellent physical strength. This paper reviews the types of NLCs, skin penetration mechanism, stability related issues along with their manufacturing techniques, characterization and applications toward objective drug supply.

1. INTRODUCTION

Newer technologies have emerged to encounter the problems associated with pharmaceuticals of low water solubility and insufficient bioavailability. Hence, there is an expanding need to fabricate a pharmaceutical carrier which can circumvent these matters. The newly developed system should be free of toxicity, should possess high drug loading capability, possible surface modification and controlled release specifications, along with an assurance of safety to the drug from possible physical and chemical degradation. The developed carrier should possess feasible production and low cost attributes also [1-3].

Colloidal systems investigated hitherto have their own drawbacks. Inadequacies often met with the colloidal systems such as liposomes, micro & nanoemulsions, nanocapsules, nanosponges & polymeric nanoparticles are the quick dilapidation by the stomach pH or by the enzymes of the intestines, constrained physical and chemical steadiness throughout storage [4-6], large scale production, quick release of drug from carrier system, steadiness hitches, scums of the organic solvents used in preparation, toxic polymers used [7-8] and many to point. These constraints restrict the usage of colloidal carrier as a pharmaceutical carrier system.

Solid lipid nanoparticles represent themselves as an alternate carrier system to emulsions, liposomes and polymeric nanoparticles as they are being formulated from solid lipids only. Drug leaching from the crystal lattice is the major drawback from the solid lipids that restricts there use [9]. Nanolipid carriers have emerged to overwhelm the drawbacks associated with solid lipid nanoparticles. High drug payload, less drug expulsion during storage, stable solid lipid matrix by the amalgamation a liquid lipid with solid lipid and a less tendency of unpredictable gelation are the features which distinct them from SLN's [2, 10-14]. Further, controlled drug delivery and increased chemical stability with possibility on large scale production enables NLC's to accepted gleefully as an efficient pharmaceutical carrier [2, 12, 15-17].

2. Nanostructured lipid carrier NLC as compare to solid lipid nanoparticle SLN:

Nanostructured lipid carrier (NLC), the second generation innovative lipid nanoparticle that acts as a bioactive carrier system, has been developed to overcome some potential limitations of the solid lipid nanoparticle (SLN). The review of Menhert and Mader highlight these aspects:

- Pay-load for number of drugs is too low.
- Drug expulsion during storage.
- High water content of SLN dispersion.

To overcome drug expulsion during storage, use of lipid blends which do not form a highly ordered crystalline arrangement is needed. The matrix of NLCs is composed of mixture of spatially different lipid molecules, normally mixture of solid and liquid lipid, which makes more imperfection in the matrix to accommodate more drug molecules than SLN. Despite the presence of liquid lipid, NLC matrix is solid at room/body temperature. NLCs adopt mixtures of a solid lipid and liquid lipid and remain in the solid state by controlling the content of liquid lipid. NLCs can more strongly immobilize drugs and prevent the particle from coalescing by virtue of the solid matrix compared to emulsions [18, 19] due to the lower risk of systemic side effects. In addition, the expulsion of drug entrapped in NLC during storage is minimized or avoided. NLC is an alternative carrier to other drug carrier systems such as liposomes and polymeric nanoparticles because it has combined the advantages of other colloidal carriers and avoided their disadvantages. These includes high amounts of drug payload, increasing drug stability, the possibility to control drug release and targeting, and avoidance of organic solvents [20].

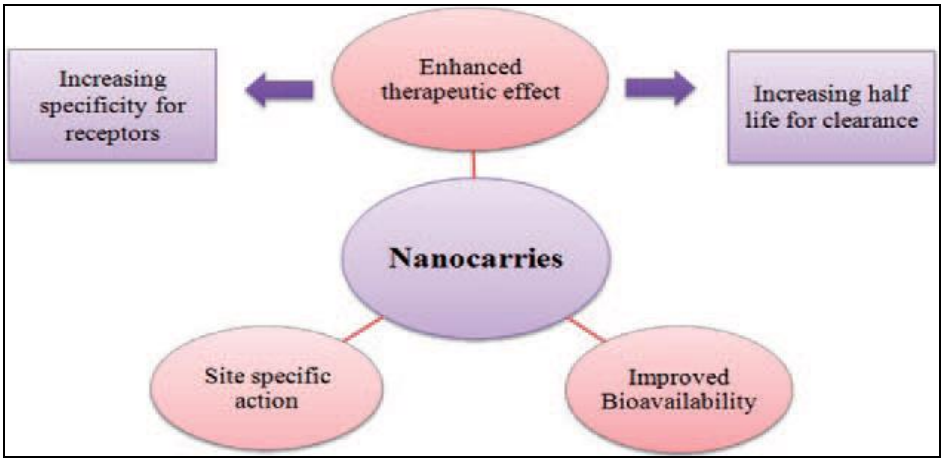
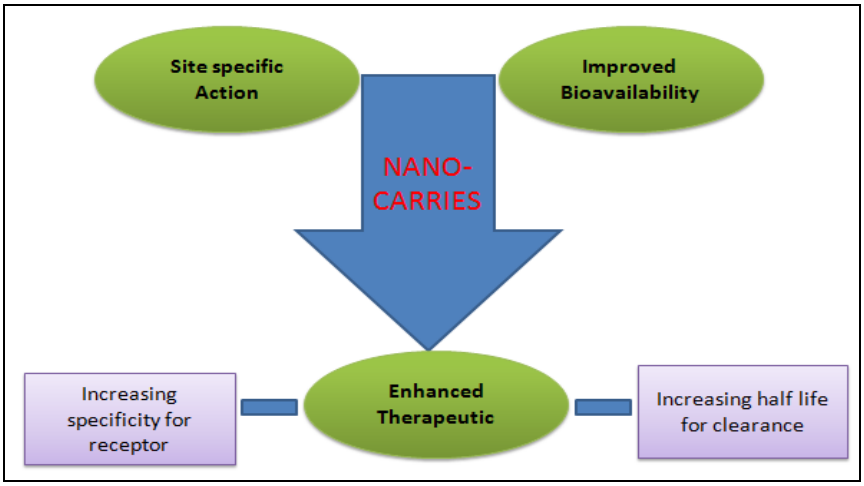


Figure No. 1: Important aspects of nanocarriers



3. ADVANTAGES OF NLCs:

- Better physical stability,
- Ease of preparation and scale-up,
- Increased dispersibility in an aqueous medium,
- High entrapment of lipophilic drugs and hydrophilic drugs,
- Controlled particle size,
- An advanced and efficient carrier system in particular for lipophilic substances,
- Increase of skin occlusion,
- Extended release of the drug,
- One of the carriers of choice for topically applied drugs because their lipid components have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations,
- Small size of the lipid particles ensures close contact to
- the stratum corneum thus enhancing drug penetration into the mucosa or skin,
- Improve benefit/risk ratio,
- Increase of skin hydration and elasticity and [21],

- These carriers are highly efficient systems due to their solid lipid matrices, which are also generally recognized as safe or have a regulatory accepted status [22].

4. LIMITATIONS OF NLC's

Despite the great potential of NLCs in targeted delivery, they face certain limitations like:

- Cytotoxic effects related to the nature of matrix and concentration,
- Irritative and sensitising action of some surfactants,
- Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better exploited, and
- Lack of sufficient preclinical and clinical studies with these nanoparticles in case of bone repair [23].

5. TYPES OF NLC: Different methods have been proposed for creating NLCs:

I. Imperfect Type NLC (Imperfectly Structured Solid Matrix): Spatially different lipids are mixed, and thus imperfections in the crystal order of lipid nanoparticles are provided. Large distances between fatty acid chains in the matrix structure of lipid nanoparticles can be increased by using glycerides composed of very different fatty acids. Therefore, the matrix contains imperfections to accommodate the drug in amorphous clusters. Mixing small amounts of chemically very different liquid lipids (oils) with solid lipids in order to achieve the highest incompatibility leads the highest drug payload.

II. Amorphous Type (Structureless Solid Amorphous Matrix): This kind of NLC can be achieved by mixing solid lipids with special lipids, e.g., hydroxy octacosanol hydroxy stearate, isopropyl myristate or medium chain triglycerides such as Miglyol® 812. Therefore, drug expulsion caused by the process of crystallization to β forms during storage is prevented by the special structure of the lipid matrix since NLC are solids in an amorphous but not crystalline state [24].

III. Multiple Type (Multiple Oil in Fat in Water (O/F/W) Carrier): The solubility of the drug in the lipophilic phase decreases during the cooling process after homogenization and the crystallization process during storage. Continuously reducing drug solubility leads to drug expulsion from the lipid nanoparticles especially when the drug concentration in the

formulation is too high. Solubility of many drugs in a liquid lipid is higher than in a solid lipid. When lipids lack appropriate drug solubilities, addition of a higher amount of liquid lipid to the lipophilic phase displays the advantages of the solid matrix which prevented drug leakage while the liquid regions (oily nano compartments) show comparatively high solubility for lipophilic drugs [25].

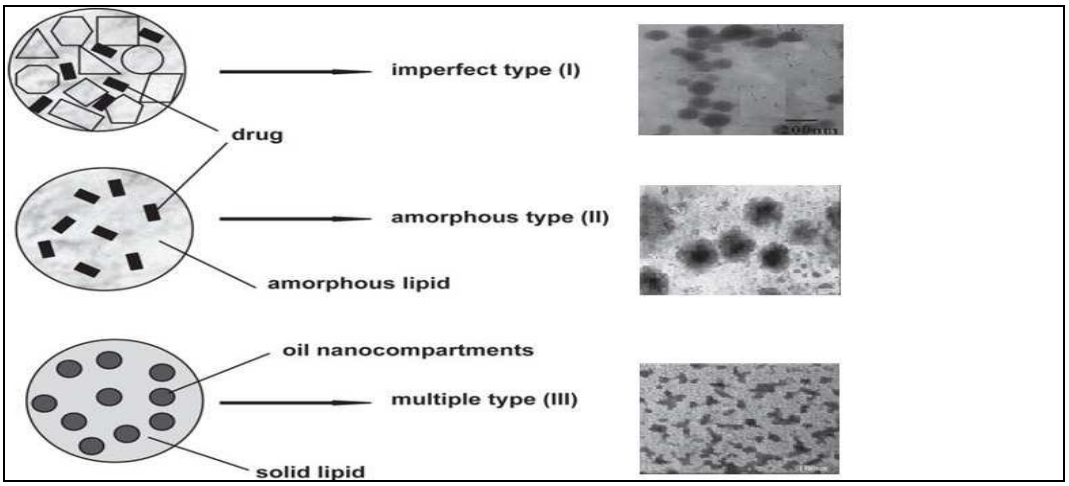


Figure No. 2: Types of NLC: (I) imperfect type, (II) amorphous type and (III) multiple type

6. EXCIPIENTS USED IN NANOSTRUCTURED LIPID CARRIERS:

6.1 Lipids:

Both solid and liquid-lipids are included in NLCs for constructing the inner cores. The solid lipids commonly used for NLCs include glyceryl behenate (Com- pritol® 888 ATO), glyceryl palmitostearate (Precirol® ATO 5), fatty acids (e.g. stearic acid), triglycerides (e.g. tristearin), steroids (e.g. cholesterol), and waxes (e.g. cetyl palmitate). These lipids are in a solid state at room temperature. They would melt at higher temperatures (e.g. > 80°C) during the preparation process. Liquid oils typically used for NLCs consist of digestible oils from natural sources. The medium chain triglycerides, such as Miglyol® 812, are often utilized as the constituents of liquid lipids because of their similar structures to Compritol® [26]. Other oily components such as paraffin oil, 2-octyl dodecanol, propylene glycol dicaprylocaprate (Labrafac®), isopropyl myristate and squalene are included as well. Alternatively, the fatty acids, such as oleic acid, linoleic acid, and decanoic acid, are included in NLCs for their value as having oily components and as being penetration enhancers of topical delivery. In general, these lipids are already approved by European and American regulatory authorities

for clinical applications and for their "generally recognized as safe" (GRAS) status. There is a need for novel and biocompatible oils that are cost effective, non-irritating, and capable of being sterilized before application. Vitamin E (α-tocopherol) and other tocols have been evaluated as materials for nanoemulsions [27]. Tocols can serve as a choice of oils for NLCs because of their stability, ease of production on a large scale, and good solubility in lipophilic drugs. NLCs produced using natural oils from plants are also currently popular. Averina et al. [28, 18] have used Siberian pine seed oil and fish oil from Baikal Lake as the liquid oils since they show acceptable physical and chemical stability to NLCs.

6.2 Emulsifiers:

The emulsifiers have been used to stabilize the lipid dispersions. Most of the investigations employ hydrophilic emulsifiers such as Pluronic F68 (poloxamer 188), polysorbates (Tween), polyvinyl alcohol, and sodium deoxycholate [18, 29 30]. Lipophilic or amphiphilic emulsifiers such as Span 80 and lecithin are employed for fabrication of NLCs if necessary. It has been found that the combination of emulsifiers can prevent particle aggregation more efficiently [31]. Polyethylene glycol (PEG), sometimes added in NLCs, resides on the nanoparticulate shell to prevent uptake by the reticuloendothelial system (RES) and to prolong the circulation time of drugs. Table 1 summarizes the detailed information pertaining to the materials used for NLCs. Another prerequisite for NLCs' stability is the ability for preservation. The preservatives can impair the physical stability of lipid dispersions. Obeidat et al.[32] demonstrate that Hydrolite® 5 is proved suitable for the preservation of coenzyme Q10- loaded NLCs.

Table No. 1: The excipients for composing nanostructured lipid carriers (NLCs)

S. No.	Ingredient	Material
1.	Solid lipids	Tristearin, stearic acid, cetyl palmitate, cholesterol, Precirol® ATO 5, Compritol® 888 ATO, Dynasan®116, Dynasan® 118, Softisan® 154, Cutina® CP, Imwitor® 900 P, Geleol®, Gelot® 64, Emulcire® 61
2.	Liquid lipids	Medium chain triglycerides, paraffin oil, 2-octyl dodecanol, oleic acid, squalene, isopropyl myristate, vitamin E, Miglyol® 812, Transcutol® HP, Labrafil Lipofile® WL 1349, Labrafac® PG, Lauroglycol® FCC, Capryol® 90
3.	Hydrophilic emulsifier	Pluronic® F68 (poloxamer 188), Pluronic® F127 (poloxamer 407), Tween 20, Tween 40, Tween 80, polyvinyl alcohol, Solutol® HS15, trehalose, sodium deoxycholate, sodium glycocholate, sodium oleate, polyglycerol methyl glucose distearate
4.	Lipophilic emulsifiers	Myverol® 18-04K, Span 20, Span 40, Span 60
5.	Amphiphilic emulsifiers	Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire® 50/13

7. Techniques of Production:

7.1 HPH:

HPH has been used as a reliable and powerful technique for the large-scale production of NLCs, lipid drug conjugate, SLNs, and parenteral emulsions. The lipid is pushed with high pressure (100 – 2000 bars) through a very high shear stress, resulting in disruption of particles down to the sub micrometer or nanometer range. Generally, the lipid contents are in the range of 5 – 10%. In contrast to other preparation technique, high pressure homogenisation does not show scaling up problem. Homogenisation may be performed either at elevated temperature (hot homogenisation) or below room temperature (cold homogenisation) [33].

7.2 Hot Homogenisation 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 homogenised by using a piston gap homogeniser and the obtained nanoemulsion is cooled down to room temperature where the lipid recrystallises and leads to formation of nanoparticles [34].

7.3 Cold Homogenisation Technique:

Cold homogenisation is carried out with the solid lipid containing drug. Cold homogenisation has been developed to overcome the problems of the hot homogenisation technique such as, temperature mediated accelerated degradation of the drug payload, partitioning and hence loss of drug into the aqueous phase during homogenisation. The first step of both the cold and hot homogenisation methods is the same. In the subsequent step, the melt containing drug is cooled rapidly using ice or liquid nitrogen for distribution of drug in the lipid matrix as shown in the Figure 3. Cold homogenisation minimizes the thermal exposure of the sample [35].

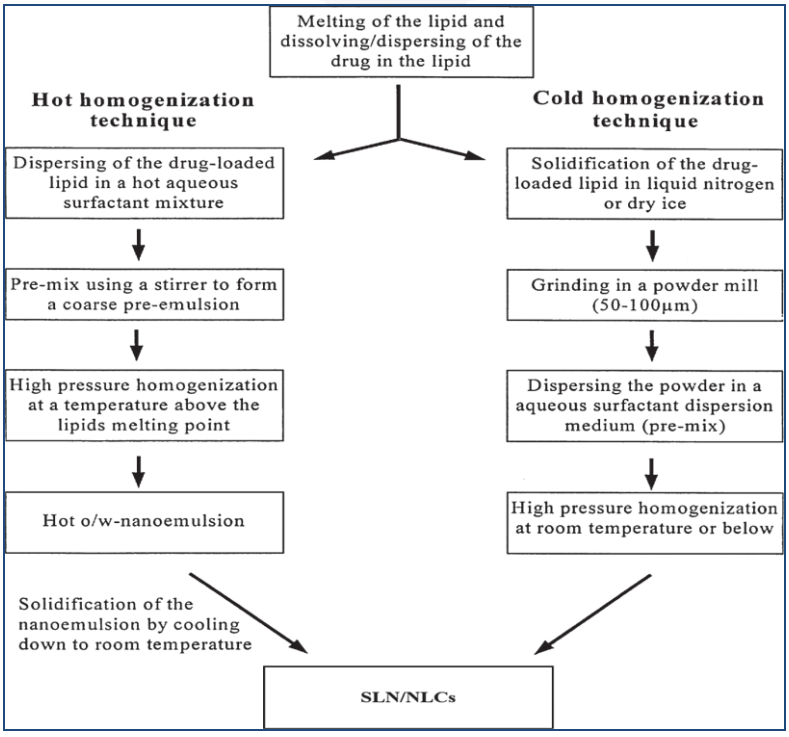


Figure No. 3: Schematic overview (valid only for lipophilic drug or protein) of the hot and cold homogenisation technique [2]

7.4 Microemulsion Technique:

The lipids (fatty acids or glycosides eg. lipid acid) are liquified and drug is integrated in liquified lipid. A mixture of water, co-surfactant(s) and also the surface-active agent is heated to a similar temperature because the lipids are added beneath gentle stirring to the lipid soften. A clear, thermodynamically stable system is created once the compounds are mixed within the correct ratios for microemulsion formation. therefore the microemulsion is the basis for the formation of nanoparticles of a requisite size. This microemulsion is then spread in a very cold liquid medium below gentle mechanical mixing of hot microemulsion with water during a quantitative relation in the range 1:25 – 1:50. This dispersion in cold liquid medium ends up in fast re-crystallisation of the oil droplets [36].

Surfactants and co-surfactants include lecithin; biliar salts along with alcohols such as butanol. Excipients such as butanol are less commonly used due to their regulatory aspects. The microemulsion is formulated in a large, temperature-controlled tank and then pumped from this tank into a cold water tank for the precipitation step [37].

7.5 Solvent- Diffusion Method:

It consists of two different phases; organic phase consists of the LM, lipophilic surfactant and drug which are dissolved in organic solvents at elevated temperature. The resultant organic solution was quickly dispersed into the aqueous surfactant solution at room temperature (25 °C) under high mechanical agitation for a period of time to obtain NLC. The obtained dispersion can be placed in vacuum desiccators for 24h to evaporate the residual organic solvent [38].

7.6 Spray Drying:

This system is a substitute to lyophilization method that leads to the production of pharmaceutical product from aqueous SLN dispersion. Spray drying is cost effective method rather than lyophilization, but is not used for the production of lipids commonly. Because the high temperatures and shear forces used in this way, leads to particle aggregation. According to earlier studies, lipids with melting point greater than 70 °C are suitable for spray drying [39].

7.7 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 produce nanoparticles [40].

7.8 Solvent Injection:

The common principle of the solvent injection method is similar to the solvent diffusion method. In case of solvent injection method, lipids are dissolved in a water-miscible solvent (e.g. acetone, isopropanol and methanol) or water-miscible solvent mixture and quickly injected into an aqueous solution of surfactants through an injection needle [41]. The favour of this method are the easy handling and fast production process without technically sophisticated equipment (e.g. high-pressure homogeniser). However, the main disadvantage is the use of organic solvents [10].

7.9 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 stabilised by adding stabiliser 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 could be modified in order to sterically stabilise them by means of the incorporation of lipid-PEG derivatives [42].

7.10 Supercritical Fluid Extraction of Emulsions (SFEE):

SFEE is a relatively novel approach for SLN preparation. This method uses a supercritical fluid such as carbon dioxide for solvent extraction from o/w emulsions. Carbon dioxide is a good option, but it should be noted that it can’t dissolve many of the drugs. Therefore supercritical anti-solvent precipitation (SAS) can be an alternative method to SFEE [43-44].

7.11 Ultrasonication or High Speed Homogenization:

One of the methods for the production of LNPs is ultrasonication or high-shear homogenization. The aqueous phase containing a large amount of surfactant, and lipid phase is dispersed in this phase. The high amount of surfactant will be considered as a disadvantage. Another disadvantage of this method is that it doesn't produce a narrow particle size distribution, thus leading to instability during storage. This technique uses from the simple instruments that can be found in every lab unlike hot and cold homogenization [45-46].

8. STRATEGIES EMPLOYED FOR OVERCOMING THE ISSUES RELATED TO STABILITY OF NLCS SPRAY DRYING:

It is an alternative and cheaper technique to the lyophilization process. This recommends the use of lipid with melting point more than 70 ºC. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture. The addition of carbohydrates and low lipid content favour the preservation of the colloidal particle size in spray drying. The melting of the lipid can be minimized by using ethanol–water mixtures alternative of pure water due to cooling leads to small and heterogeneous crystals, the lower inlet temperatures.

8.1 Lyophilisation

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been right to achieve long term stability for a product containing hydrolysable drugs or a suitable product for per –oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions. However, when SLN are lyophilised without cryoprotectant, the final product commonly results in the aggregation of particles. Some of the most widely used cryoprotectants are trehalose, sorbitol, glucose, sucrose, mannose and maltose. Schwarz and Mehnert reported trehalose as the most effective cryoprotectant in preventing particle growth [47].

8.2 Stabilizing Agent:

a. Poloxamers

Poloxamer 188 used in a formulation that was developed and then in human plasma and whole blood showed an increased whole blood permeability of networks and it was also realized that the increased fibrin permeability was due to fibrin fibres arrangement. The alterations of fibrin are the main reason to increase the mechanical stability contributing to antithrombotic and rheological effects [48-49]. The increase in stability of the gel formulation using Poloxamer with organic solvents such as ethanol, propylene glycol, glycerol and PEG 400. Poloxamer 407 in the presence of these organic solvents, self assembles into two liquid crystal structures namely micellar cubic and hexagonal structures that are thermodynamically stable. Poloxamer 407 in combination with a liposome showed an increase in stability of liposome formulation by increasing half life, preventing aggregation and fusion of phosphatidylcholine multilamellar vesicles [50]. The low stability of poloxamer hydrogel in an aqueous solution lead to the combination development of poloxamer 407 with acrylate and thiol groups of 17.5 wt % at body temperature. It was observed with an immediate crosslinking formed between acrylate and thiol that modified poloxamer 407 property, giving rise to a remarkable increase in stability of drugs about four times and for its potential application in controlled drug release [51].

b. Polyethylene Glycol:

In general, surface modification of colloidal particles by coating with a hydrophilic substance like polyethylene glycol (PEG) reported to bring following benefits:

• Providing good physical stability and dispersability of colloids,

• Improving presence of colloids in blood circulation for systemic use

• Increasing stability of colloids in body fluids such as gastrointestinal (GI) fluids,

• Acceleration of colloid transport across the epithelium,

• Modulation of interaction of colloids with mucosa for specific delivery requirements and drug targeting,

• Increasing biocompatibility and decreasing thrombogenicity of drug carriers [40]

9. CHARACTERIZATION OF NLC's:

9.1 Particle Size Analysis:

Photon correction spectroscopy (PCS): photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern, UK). The measurements were obtained in triplicates (n = 3) and standard deviations calculated at a fixed angle of 173° and at 25°C. The aqueous NLC were diluted with distilled water prior to analysis to prevent back-scattering effect. (PCS) based on laser light diffraction provides an appropriate method for investigation and can be applied for particles ranging below 200 nm and up to 1μm 86. For particles below 200nm Rayleigh's theory holds that the scattering intensity to be proportional to the sixth potency of the particle diameter. Both, Fraunhofer's and Rayleigh's theories, are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption and the size of the particles as well as there fractive indices of both the particles and the dispersion medium[52,53].

9.2 pH Analysis:

The determination of the pH of a formulation intended for cutaneous application is extremely important, since it must be compatible with the pH of the application site. The natural pH of the skin comes from the secretions of sweat and sebaceous glands, and lactic acid production, which leads to the formation of a protective film over the entire skin surface, designated hydrolipidic film. The skin normally has an average pH of 5.5, although this may vary slightly depending on the area of the body [54]. The evaluation of the pH was performed in all prepared HGs on days 7 and 30 after storage at different temperatures. For this, a glass pH electrode (Basic 20; Crison Instruments, Barcelona, Spain) was directly dipped in each semisolid formulation. All analyses were performed in triplicate (means ± SD)[55].

9.3 Atomic Force Microscopy (AFM):

To study morphological changes and also the particle size of NLC's before and after lyophillization AFM micrographs were taken. AFM observation were performed by a nanosurf mobile S, Atomic Force Microscopy (nanosurfe AG, Liestal, Switzerland). The images were obtained by measurement of interaction forces between the tip and sample surface. The experiments were done in air at room temperature (25 ºC) operating in non-contact mode. droplets of suspension were placed on a small mica disk . The measurements

were performed in different sample locations. the amplitude AFM images were taken before and after freeze drying NLC's in optimized condition of freeze drying, i.e., freezing temperature of -70 ºC applied at a time period of 24 h, and sublimation time of 48 h. image data were analysed with Easyscan 2 software[56].

9.4 Zeta Potential Measurement:

Laser doppler electrophoresis technique was applied to measure particle electrostatic charge. The analysis was done with Zetasizer Nano ZS (Malvern, UK) and the results were expressed as zeta potential (ZP). The measurements were performed in triplicates at pH of 7.26 ± 0.13 to mimic physiological pH [57].

9.5 Differential Scanning Calorimetry (DSC): Differential scanning calorimetry is used to determine the speciation of crystallinity and polymorphism of bulk materials, drugs, and drug nanoparticles by measurement of glass and melting point temperatures at their respective enthalpies. [57] Differential scanning calorimeter (822e, Mettler Toledo, Greifensee, Switzerland). Approximately 10 mg of bulk lipid, drug and lyophilized NLC were placed in pinhole bottom sealed aluminum pans with lids and heated. An empty aluminum pan was used as the reference. Differential scanning calorimetric curves were recorded across a temperature range of 20°C–80°C, with a constant linear heating rate of 5°C per minute in pure ultrahigh dry nitrogen. The analysis was repeated three times and values are expressed as the mean of three determinations. Finally, the enthalpies were calculated using the Mettler Star software [58, 59].

9.6 High-Performance Liquid Chromatographic (HPLC) Analysis: The HPLC system included a Hitachi L-2130 pump, a Hitachi L-2200 sample processor, and a Hitachi L-2400 UV detector. A 25-cm-long, 4-mm inner diameter stainless steel C18 column (Merck, Darmstadt, Germany) was used. The mobile phases consisted of methanol: water (80:20) for calcipotriol and acetonitrile: water (15:85) at pH 2.7 adjusted with phosphoric acid for methotrexate. The flow rate was 1 ml/min. The UV detector was set to wavelengths of 265 and 303 nm for calcipotriol and methotrexate, respectively [60].

9.7 Rheological Study: The rheological properties of the prepared lipid nanoparticles were measured using Brookfield's viscometer (Brookfield LV-DV II+, USA) [61]. The sample (20 g) was placed in a beaker and allowed to equilibrate for 5 min. The measurements were carried at ambient temperature using the suitable spindle. The spindle speed rate was

increased in ascending order from 1 to 100 rpm and then in descending order speed setting from 100 to 1 rpm with each kept constant for 10 seconds before a measurement was made [62, 63].

9.8 Transmission Electron Microscopy (TEM): A drop of diluted NLC dispersions was placed onto the surface of a copper grid coated with carbon. Upon drying, the grids with mesh size of 300 were stained with 2% phosphotungstic acid, (PTA) (w/v) for 120 s and dried at room temperature. The NLC samples were placed onto sample holders and probed with transmission electron microscopy (TEM) (Hitachi H-7100, Japan) [64].

9.9 Scanning Electron Microscopy (SEM): The morphological characteristic of NLC was determined by a scanning electron microscope (JEOL-JSM-6360, Japan). One drop of sample was placed on a slide and excess water was left to dry at room temperature. The slide was attached to the specimen holder using double coated adhesive tape and gold coating under vacuum using a sputter coater (Model JFC-1100, JEOL, Japan) for 10 minutes, and then investigated at 20kV [65].

9.10 Wide-angle X-ray Diffraction (XRD): The geometric scattering of radiation from crystal planes within nanoparticle dispersion can be determined by wide angle X-ray diffraction to assess the degree of crystallinity. An X-ray diffractometer (Philips, Hamburg, Germany), equipped with a copper anode (γ = 1.5406 Å) for radiation was used to detect the crystallinity of the lyophilized NLC. Powdered samples of lipid, drug , and lyophilized NLC about 10 mm in length were placed on the top of X-ray plates, exposed to a voltage of 45 kV and a 40 mA current at room temperature, with a scanning speed of 5° per minute and a scanning range of 2θ. The X-ray diffractogram patterns were recorded over the range of 20°–80° [66, 67].

9.11 Confocal Laser Scanning Microscopy (CLSM): to investigate the structure of NLC's a drop of glycerol was applied upon samples and examined using confocal FV-1000 station installed on a inverted microscope IX-81(Olympus, Tokyo, Japan). The emitted fluorescence was detected through spectral detection channel [68].

9.12 Ultra Filtration: colloidal dispersion can pass through an ordinary filter paper, because the pore size of filter paper is large. If this filter paper is impregnated with colloidion, the pore size reduces. Such modification filter papers are called ultra filters. The colloidal dispersion is filtered through ultrafilter to remove all electrolytes. Colloidal particles are

retained on the filter paper as slime. These are collected an dispersed in a pure dispersion medium to get a sol. Ultra filtration proceeds very slowly , so pressure or suction is applied to increase the rate of filtration [69].

9.13 Drug Entrapment Efficiency: A volume of 2.0 ml of each drug-loaded sample was centrifuged (Microfuge, Remi motors, Mumbai) at 12500 rpm for 45 minutes to separate the lipid and aqueous phase. The supernatant was then diluted with methanol, filtered through 40µm filter paper (Hi-media, Mumbai) and the drug content was determined by the UV-VIS spectrophotometer (UV1800, Shimadzu, Japan) at 273 nm. The entrapment efficacy of NLC was calculated as follows:

$$EE = Wa - Ws / Wa \times 100$$

$$DL = Wa - Ws / Wa - Ws + Wl \times 100$$

Where EE is entrapment efficiency, DL is Drug loading,

Wa stands for the mass of aceclofenac added to the formulation, and

Ws is the analyzed weight of the drug in supernatant and W is the weight of lipid added [70, 71].

9.14 Nuclear Magnetic Resonance (NMR): NMR can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle. Nuclear Magnetic Resonance (NMR). The mobility of the solid and liquid lipids is related to the width at half amplitude of the signals [72]. Broad signals and small amplitudes are characteristics of molecules with restricted mobility and strong interactions. The higher line width of NLCs compared to the physical mixture of the materials added in NLCs indicates the interaction of liquid oil with the solid lipid. Immobilization of the nanoparticles of NLCs is stronger compared to SLNs with totally crystallized cores consider the specific environment in the in vivo status. Enzymatic degradation of lipid nanoparticles may be influenced to a relevant extent by the composition of the particles.

9.15 Drug Release: The controlled or sustained release of the drugs from NLCs can result in the prolonged half-life and retarded enzymatic attack in systematic circulation. The drug

release behavior from NLCs is dependent upon the production temperature, emulsifier composition, and oil percentage incorporated in the lipid matrix [73]. The drug amount in the outer shell of the nanoparticles and on the particulate surface is released in a burst manner, while the drug incorporated into the particulate core is released in a prolonged way. Sustained release of the drugs can be explained considering both drug partitioning between the lipid matrix and water, as well as the barrier function of the interfacial membrane [74, 75]. The dialysis method and the utilization of the Franz cell are the modes for measuring in vitro drug release from nanoparticles. The interpretation of in vitro drug release profiles should consider the specific environment in the in vivo status. Enzymatic degradation of lipid nanoparticles may be influenced to a relevant extent by the composition of the particles.

10. FACTORS AFFECTING DRUG RELEASE:

The release study must be performed to compare the capacity of different samples to retain the drug incorporated for a longer time and release it slowly from the lipid matrix of the nanoparticles. Many factors that could affect the release profile of the drug from the NLC system. The effect of the particle size, the lipid matrix, the surfactant, the drug concentration in the lipid matrix and the drug type can be studied [40].

10.1 Particle Size: The particle size of a colloidal system (e.g. NLC) is a crucial factor for the release of the material(s) incorporated inside the particles.

10.2 Lipid Matrix: Different lipid matrices lead to different release profiles. The lipids have different crystals order and crystallization modification, different melting points and different hydrophilic lipophilic balance (HLB) values, e.g. Apifil HLB = 9.4, Compritol 888 HLB = 2. This makes the affinity of the drug to be entrapped within the lipid matrix different from one lipid to another.

10.3 Surfactant: Surfactants as they are used to stabilize the particles in the dispersion media (or emulsify the oil in water) may affect the structure of the lipid nanoparticles. This happens because of the interaction between the emulsifying agent molecules and the lipid molecules. Moreover, the ability of the surfactant to stabilize the oil droplets (in the lipid melted state during homogenization) and form smaller NLCs gives the surfactant also a role through the size of the formed lipid particles. The physicochemical properties of the NLCs are essentially influenced by the type of surfactant used.

10.4 Drug Loading: Drug loading might affect the release profile. It depends on the affinity of the drug to mix with the lipid and be enclosed in the matrix.

10.5 Drug Type: The drug type affects the release profile because with the different compositions of drugs there are different affinities to the lipid matrix. Nanostructured lipid carriers have unique characteristics that can enhance the performance of a variety of incorporated drug forms [40].

11. MECHANISM OF SKIN PENETRATION OF NLCs:

Nanosized particles can make close contact with superficial junctions of SC and furrows between corneocyte islands, allowing superficial spreading of the active agents. Following the evaporation of water from the nanosystems applied to the skin surface, particles form an adhesive layer occluding the skin. Hydration of SC thus increases to reduce corneocyte packing and widen inter-corneocyte gaps. Hydration also influences partitioning of the drug into SC [76]. Intact nanoparticles sized above 100 nm are not considered to permeate the SC because of their dimensions and rigidity [77]. Although the particles do not penetrate across SC, uptake ofthe components is to be expected. Since epidermal lipids are rich in SC, lipid nanoparticles attaching to the skin surface would allow lipid exchange between SC and the nanocarriers [78]. Lipid nanoparticles have the potential to deliver drugs via the follicles [79]. Furthermore, each follicle is associated with sebaceous glands, which release sebum, creating an environment enriched in lipids [80]. This environment is beneficial for trapping of lipid nanoparticles. Sebum is a mixture of triglycerides, squalene and waxes. Some glyceride lipids present in NLCs may accelerate the entrance into the follicles/sebaceous glands. The possible mechanisms involved in skin permeation enhancement by NLCs are depicted in [81].

12. APPLICATIONS OF NLCs:

12.1 Oral Drug Delivery:

Oral drug administration is common and preferred route due to good patient compliance, non-invasiveness and therapeutic success, but poorly water-solubility of drugs is limiting step for the absorption of them. Thus an approach is needed to improve the bioavailability of drugs. Lipid-based delivery systems in the recent decades have shown many advances for this purpose. These systems include a wide range of formulations such as self-nanoemulsifying drug delivery system (SNEDDS), self-microemulsifying drug delivery system (SMEDDS),

nanoemulsions, SLNs and NLCs. Since in these systems, drug is dissolved in the lipid thus makes the potential for improving the bioavailability of poorly soluble drugs in water, especially lipophilic drugs. In fact, these systems can increase dissolution of drug, residence time and lymphatic uptake. A good thing is that toxicity has not been observed in most cases [82-85].

12.2 Topical Delivery:

Topical route has been greatly exploited for the drug delivery to dermal areas employing lipid based nanoparticles. In recent years, many studies and experiments have been performed on topical application of NLCs for their unique properties [86-90]. NLCs can enhance the apparent solubility of entrapped drugs, which can form high concentration gradient on skin to facilitate drug permeation. The nano-sized particles tightly adhere to the skin surface and release the drugs in a more controlled manner [81]. Therefore NLCs are used for topical application of various categories of drugs for improvement of penetration along with sustained release. Another benefit of NLCs for topical delivery of active compounds is the short time required to market these products.

Experimental studies have confirmed the significant improvement in therapeutic response and reduction in local side effects of acitretin NLCs loaded gel indicating its effectiveness in the topical treatment of psoriasis. The prepared NLCs were spherical in shape as shown in Figure 7A and the release study showed biphasic drug release pattern with an initial sustained release phase for up to 10 h followed by a steady drug release phase. Figure 7B indicates the release profile of acitretin suspension and acitretin NLC in phosphate buffer pH 7.4 containing 3% w/v sodium lauryl sulphate [92].

Idebenone loaded nanostructured lipid carriers (I-NLCs) were prepared for topical delivery of antioxidant idebenone and evaluation of its sun protection efficacy. Sun protection factor (SPF) value for I-NLCs was found to be 23 which represents that lipid nanocarriers (LNC) have standards of blocking of 94 – 96% of Ultraviolet-B rays [93]. The potential of NLCs loaded with lipophilic calcipotriol and hydrophilic methotrexate for topical therapy of psoriasis was investigated. The study confirmed that NLC systems are a promising carrier for the topical delivery of anti-psoriatic drugs as revealed by enhanced skin permeation, negligible skin irritation and the compatibility of the two drugs [94]. In another study anti-fungal drug ketoconazole loaded NLCs were physically more stable as compared to SLN as

the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure [95].

12.3 Pulmonary Drug Delivery:

Inhalation drug delivery represents a potential delivery route for the treatment of several pulmonary disorders. NLCs have greater stability against the shear forces generated during nebulisation compared to polymeric nanoparticles, liposomes and emulsions. NLCs are comprised of an inner oil core surrounded by an outer solid shell and hence allow the high payload of a lipophilic drug [6]. NLCs in pulmonary disorders seems to be promising strategy since lung epithelium can be directly reached resulting in faster onset of action, desired dose and dosing frequency can be reduced as compared to other administered routes like oral and undesirable side effects of drugs can be avoided. Bioadhesive properties of NLCs are due to their small particle size as well lipophilic character lead to longer residence time in lungs [96-97].

12.4 Ocular Delivery:

The characteristic features of SLNs and NLCs for ocular application are the improved local tolerance and less astringent regulatory requirements due to the use of physiologically acceptable lipids. The other benefits include the ability to entrap lipophilic drugs, protection of labile compounds, and modulation of release behaviour [98]. SLNs have been used for ocular drug delivery in the last decades. Recently, further investigations employing NLCs as ocular delivery systems have become known in Cyclosporine loaded NLCs the mucoadhesive properties of the thiolated non-ionic surfactant Cysteine polyethylene glycol stearate (Cys-PEG-SA) and NLC modified by this thiolated agent were evaluated. Cys-PEG-SA and its resultant NLC provided a promising system with prolonged residence time [99]. Lutein-loaded NLCs could protect the entrapped lutein in the presence of simulated gastric fluid and slowly released lutein in simulated intestinal fluid in an in-vitro study [100]. Triamcinolone acetonide (TA)- loaded NLCs increased ocular absorption and enhanced prolonged drug residence time in the ocular surface and conjunctival sac, by sustained drug release from the delivery system, it also reduced precorneal drug loss [101].

12.5 Drug Delivery to Brain:

Brain targeting not only increases the cerebrospinal fluid concentration of the drug but also reduces the frequency of dosing and side effects. The major advantages of this administration route are avoidance of first pass metabolism and rapid onset of action as compared to oral administration. LNC (e.g. NLC) of this generation are considered to be one of the major strategies for drug delivery without any modification to the drug molecule because of their rapid uptake by the brain, bioacceptability and biodegradability. Further, the feasibility in scale-up and absence of burst effect make them more promising carriers for drug delivery. In addition, NLC further enhanced the intranasal drug delivery of duloxetine in the brain for the treatment of major depressive disorder. Bromocriptine (BC) a dopamine receptor agonist has been also incorporated in NLCs for controlled delivery of drug to provide long-lasting therapeutic effects possibly extending BC half-life in vivo for the treatment of Parkinson ' s disease [102-103].

12.6 Cardiovascular Treatment:

Lipid nanoparticles as a carrier system has superiorities mainly prolonged circulation time and increased area under the curve (AUC) with manageable burst effect. NLCs would provide highly desirable physic-chemical characteristics as a delivery vehicle for lipophilic drugs. Drug loading and stability were improved. Tanshinone (TA) loaded NLCs the in-vitro incubation tests confirmed that TA-NLC could bind to apoA-I specifically. Macrophage studies demonstrated that TA-NLC incubated with native HDL could turn endogenous by association to apo-lipoproteins, which cannot trigger immunological responses and could escape from recognition by macrophages [104]. Nifedipine loaded NLCs Nanoparticle suspensions were formulated with negatively charged phospholipid, dipalmitoyl phosphatidylglycerol in preventing coagulation to improve solubility and hence bioavailability of drug [105]. In Lovastatin loaded NLCs, NLCs were developed to promote oral absorption of lovastatin. More than 70% lovastatin was entrapped in the NLCs. The in-vitro release kinetics demonstrated that lovastatin release could be reduced by up to 60% with lipid nanoparticles containing Myverol as the lipophilic emulsifier. NLCs showing the slowest delivery. The oral lovastatin bioavailability was enhanced from 4% to 24% and 13% when the drug was administered from NLCs containing Myverol and SPC as surfactants respectively [106].

12.7 Parasitic Treatment:

Novel colloidal delivery systems have gained considerable interest for antiparasitic agents with focus on 3 major parasitic diseases viz. malaria, leishmaniasis and trypanosomiasis. Lipid Nanoparticles combine advantages of traditional colloidal drug carrier systems like liposomes, polymeric nanoparticles and emulsions but at the same time avoid or minimize the drawbacks associated with them. The delivery system should be designed in such a way that physicochemical properties and pharmacokinetic properties are modulated of the antiparasitic agents in order to improve biospecificity (targetability) rather than bioavailability with minimization in the adverse effects associated with it. SLNs and NLCs have ability to deliver hydrophobic and hydrophilic drug with more physical and biocompatibility Dihydroartemisnin (Anti-malarial) loaded NLCs The drug release behaviour from the NLC exhibited a biphasic pattern with burst release at the initial stage and sustained release subsequently [107].

12.8 Intranasal Drug Delivery:

The use of nanocarriers provides suitable way for the nasal delivery of antigenic molecules. These represent the key factors in the optimal processing and presentation of the antigen. Nasal administration is the promising alternative non invasive route of drug administration due to fast absorption and rapid onset of action, avoiding degradation of labile drugs (peptides and proteins) in the GI tract and insufficient transport across epithelial cell layers. The development of a stable nanostructured lipid carrier (NLC) system as a carrier for curcumin (CRM) biodistribution studies showed higher drug concentration in brain after intranasal administration of NLCs than PDS. The results of the study also suggest that CRM-NLC is a promising drug delivery system for brain cancer therapy [108]. In addition, NLC further enhanced the intranasal drug delivery of duloxetine in the brain for the treatment of major depressive disorder. Nanostructured Lipid Carriers (NLCs) of Asenapine maleate to improve the bioavailability and enhance the uptake of ASN to the brain [109].

12.9 Parenteral Delivery:

The nano-drug delivery systems such as nanomicelles, nanoemulsions and nanoparticles has displayed a great potential in improved parenteral delivery of the hydrophobic agents since last two decades. NLC has been considered as an alternative to liposomes and emulsions due to improved properties such as ease in manufacturing, high drug loading, increased flexibility

in modulating drug release profile, and along with these, their aqueous nature and biocompatibility of the excipients has enabled ability and easy abolishment.

Another reported example is NLCs of artemether (Nanoject) that offers significant improvement in the anti-malarial activity and duration of action as compared to the conventional injectable formulation. Nanoject can be considered as a viable alternative to the current injectable intramuscular (IM) formulation [110-111].

Bufadienolides a class C-24 steroid also proved to be effective in terms of enhanced haemolytic activity and cytotoxicity with reduced side effects when incorporated in NLCs [112].

12.10 Gene Transfer:

LNPs penetrate to biological membranes effectively through receptor-mediated pathway because lipids are the most important components of cell membranes. Thus enhance the uptake of genetic compounds [113-114]. The delivery of some bioactive to particular sites in the body and their release behavior is directly dependent to particle size [115]. The achievement of gene therapy (with DNA and RNA transfer) depends on the new bioactive delivery techniques. While 1980; more than 400 clinical studies in gene therapy have been reported. Delivery vectors are used in gene transfer due to restricted ability of naked DNA transfer to cells owing to propensity to enzymatic degradation [116-120]. Cationic SLNs are interesting and proper nonviral gene delivery vector for systemic delivery. SLNs directly bond with DNA and can be used for gene transfection. Genospheres (such as cationic SLNs) have large potential for targeted gene delivery. Genospheres generally carry materials such as plasmid DNA, DNA and other nucleic acids. Three issues are important about them: composition of cationic SLN, their ability to condense DNA and transferring of nucleic acid to cells [121, 115]. NLCs can be effectively used as novel nonviral gene transfer vector that offers a promising approach for gene therapy [122].

12.11 In Food Industry:

Because of its good stability and high loading capacity, the NLCs are widely applied in the pharmaceutical field. It was seldom reported that the NLC was applied as a nutritional supplement carrier in food industry for the capsule and beverage preparations. However, there are certain difficulties related to the raw material supply, availability and environmental

factors due to which there is still a great risk for food industry to invest in this area. Coenzyme Q10-loaded NLCs for food application were developed to enhance the physicochemical stability and bioavailability [123].

12.12 Chemotherapy:

Recent studies have shown that NLCs not only enhanced the efficacy and stability but also reduced side effects of many cytotoxic drugs. Diff erent nanosystems have been developed with anti-cancer drugs, for example, the albumin – paclitaxel nanoparticles were approved in early 2005 in the chemotherapy for metastatic breast cancer; etoposide NLCs were found to be cytotoxic against human epithelial-like lung carcinoma cells; stabilisation and prolonged release of topotecan NLCs in treatment of refractory ovarian and small-cell lung cancer. Advantages of incorporating anti-cancer drugs in NLCs include high drug loading efficiency; prolonged release profile; increased chemical stabilisation; increased cytotoxicity. As these NLCs avoid some potential problems associated with SLN, such as drug leakage during storage and decreased loading capacity. They act by prolonging the exposure of tumour cells to anti-tumour drug and enhancing permeability and retention effect to further increase the therapeutic effect [124].

It has also been reported that hyaluronic acid coated NLC could prolong the circulation time of paclitaxel (PTX) in blood and increase the accumulation of PTX in the tumour. The results of this experiment indicated that HA-NLCs (hyaluronic acid coated, paclitaxel-loaded, nanostructured lipid carriers) showed higher anti-tumour efficacy and fewer side effects than Taxol® in B16-bearing Kunming mice. The overall targeting efficiency of HA-NLC in the tumour was 14.46%, approximately 1.4 times that of Taxol® [125].

12.13 Cosmetic Applications:

LNPs such as SLNs and NLCs are one of the excellent vehicles for cosmetic and dermatological application. They have some characteristics which make them talented carriers for cosmetic applications for instance protection of sensitive compounds against chemical degradation and enhancement the water content of the skin. The use of LNPs as carriers for sunscreens, anti-acne and anti-ageing actives has been investigated. In fact, due to the high control behaviors of LNPs on skin penetration of active substances, they have UV-blocking and skin hydration behavior. In cosmetic products, reduction of the desire to scratch

and skin damage is important. Since these formulations bear a resemblance to skin structure, there is no disruption and toxic effect when used topically [126].

13. CONCLUSION

Lipid nanoparticles such as SLN, NLC, LNC and so on were always potential carrier systems with strong therapeutic applications. The purpose of this work was to highlight the role that NLCs play as a novel drug delivery system for different drug categories. A large range of structural models for SLNs and NLCs have been demonstrated based on the structure and arrangement of lipids and drugs in the particles. NLC's extremely unordered lipid matrix structured, enhanced drug encapsulation and stability, and good release profile also made them popular in the field of nano-pharmaceutical research and other applications. The aim has been to developed therapeutic nanotechnology undertaking, particularly for targetted drug therapy. As the new generation, the smart NLCs offer far more flexibility in drug loading, release modulation and improved performance in producing final dosage forms such as creams, tablets, capsules and injectables. Extending their applications should continue the effort to develop alternative routes and treat other diseases with NLCs. Permeation via the gastrointestinal tract and BBB could be a trend of the future. Another consideration for future development is the combination of the two therapeutically active agents to be included in a single nanosystem.

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