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Review Article

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Nano structured lipid carrier based drug delivery system

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ABSTRACT

The primary objective of writing this article is to put the emphasis on the importance of nano structured based drug delivery systems. Although drugs as such can be delivered in the body using different routes but most of the routes esp. have its own limitations like poor solubility, absorption, first pass metabolism and poor bioavailability, hence need for this nano structured based drug delivery systems was developed. This technique not only will help us in overcoming the above mentioned drawbacks but also this helps us in reducing the dose, systemic side effects and in addition to these, this also helps us in delivering the drugs to the site of action. These nano based systems can be used to deliver variety of drugs through different routes of administration including oral, topical, transdermal, ocular and parenteral. This review outlines the process involved in the preparation of NLC's, its characterization and evaluation and its pharmaceutical applications.

Keywords: Nano- structured lipid carriers, solvent evaporation, melt emulsion, pharma fields.

INTRODUCTION

In the present era not too many new chemical entities are coming in market primarily due to the fact that either they have poor solubility or incomplete absorption. Various methodologies have been explored to overcome this issue but none of them possess all the prerequisites. Hence this Nanostructured lipid carriers (NLCs) is being explored present a relatively new type of colloidal drug delivery system that consists of solid lipid and liquid lipid, and offers the advantage of improved drug loading capacity and release properties . nanostructured lipid carriers (NLCs) are systems that have been successfully used for topical , dermal , Transdermal administration . these systems consist of aqueous dispersions of solid nanoparticles, composed of a mixture of solid and liquid lipids, and stabilized by one or two surfactants[1]. NLCs are efficient systems to improve skin hydration, due to their physiological lipid composition and occlusive effect properties. Typically, NLC dispersions present a low viscosity, which is not advantageous for topical application, because it decreases the time of permeance at the application site. To avoid this, NLCs can be incorporated into traditional semisolid systems (eg, hydrogels [HGs]), increasing the consistency of final formulations and also the long-term stability of the incorporated nanoparticles.(, NLCs have the usual particle diameter ranging 10–1000 nm. NLCs drug delivery system have many advantages like high biocompatibility, controlled drug release, high bioavailability, and the possibility of large industrial scale production. Drug delivery system based on NLCs also have no problems with different routes of administration, such as oral, intravenous, pulmonary and transdermal administration[2-7]. However, the various kinds of lipid NLC components results in the imperfections type structures, amorphous state type or multiple type to adjust more drug

and decrease the drug leakage during storage[8]. Poorly water soluble drugs loaded by lipid formulations have been studied for oral route and have reported to enhance the oral bioavailability by numerous research teams[9,10] but there are very less reports for oral administration on NLC system) Solid lipid nanoparticles (SLN) combining the advantages of colloidal carriers, had attracted increased attention as a drug delivery system when it was introduced in 1991. To overcome these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals. These lipid nanoparticles are known as solid lipid nanoparticles (SLNs) and Nanostructured lipid carriers, which are attracting wide attention of formulators world-wide.

SLNs and NLCs are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsion, liposomes and polymeric nanoparticles). They are new a generation of submicron-sized lipid emulsion where the lipid (oil) has been substituted by a solid lipid. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials.[11]

ADVANTAGES OF NLC's

• Better physical stability,
• Ease of preparation and scale-up,
• Increased dispersability in an aqueous medium,
• High entrapment of lipophilic drugs and hydrophilicdrugs,
• Controlled particle size,
• An advanced and efficient carrier system in particular for 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
• 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 [12].

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,
• Iritative 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 [13].

2.TYPES OF NLC:

It is well known from the study of suppositories that highly ordered crystalline lipid matrices will lead to drug expulsion .Lipid nanoparticles and microparticles made from blends of solid lipids can experience this, especially when nanoparticles are prepared from highly purified lipids ,for example ,tristearin. The formation of highly ordered modifications , particularly during storage the little space for drug molecules ,and the expulsion of drugs leads to drug crystals in suspensions and solid dosage forms[14] .To avoid this problem ,the particles should have a controlled nanostructure that offers enough space to accommodate the drug. Different approaches were taken for an optimized nanostructure of NLCs.

2.1 Type I

Solid lipids and liquid lipids (oils) are blended .The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered , imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs . In general ,drug solubility is higher in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils).During the production process ,the liquid lipid particles(nanoemulsions) are cooled from the molten state to room temperature to crystallize and form

solid particles. At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase which leads to phase separation, that means precipitation of tiny oily nanocompartments[15]

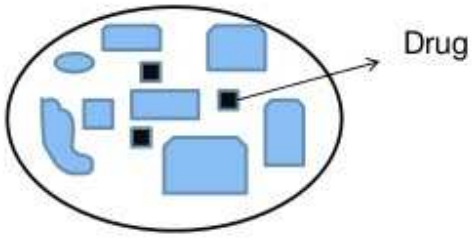

FIG 1 Type I NLC's

2.2 Type II : In this multiple oil/fat/water ,type II drug can be accommodated in the solid,but at increased solubility in the oily parts of the lipid matrix. at high oil concentrations a miscibility gap of two lipids occurs during the cooling phase, leads to phase separation , that means precipitation of tiny oily nanocompartments

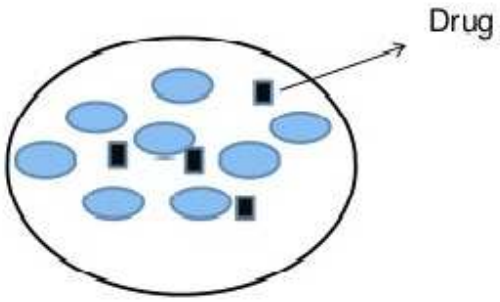

FIG.2 type II NLC's

2.3 Type III: In this lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in an amorphous state. The absence of crystallization avoids drug expulsion by crystallization. Lipid particles are preferentially suited to incorporate lipophillic drugs; hydrophilic drugs can only be incorporated at a low percentage (however, this is still sufficient for highly potent peptides and proteins). In a further variation of the lipid matrix, water-soluble drugs were conjugated with a lipid, thus forming a water-insoluble lipidic conjugate. The lipid conjugate powder was melted and processed in the same way as the other types to yield a lipid drug conjugate (LDC) nanoparticle. Depending on the conjugate ,this lipidic conjugate has a drug loading of up to 30–50% for water-soluble drugs. Conjugation is performed by salt formation or covalent linkage [16]

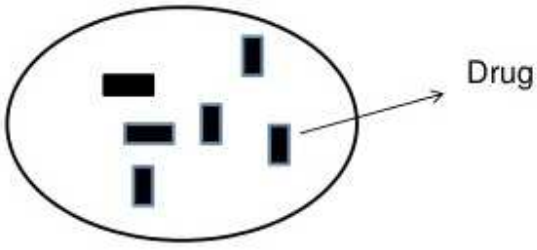

FIG.3 Type III NLC's

3. EXCIPIENTS USED IN NANO STRUCTURED LIPID CARRIERS:

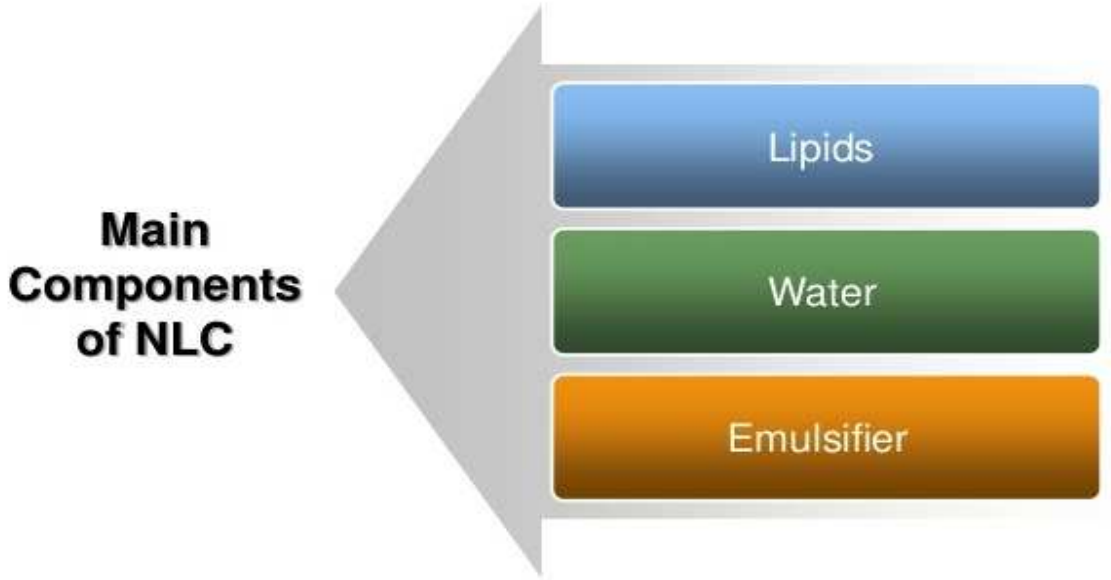

FIG.4 components of NLC's

3.1 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® [17]. Other oily components such as paraffin oil, 2octyl 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 costeffective, non-irritating, and capable of being sterilized before application. Vitamin E (α-tocopherol) and other. tocols have been investigated as materials for nanoemulsions [18]. 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. [19,20] 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. [Refer table no.1]

3.2 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 [21-23]. 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 [24]. Polyethylene glycol (PEG), sometimes added in NLCs, resides on the nanoparticulate shell to prevent uptake by the reticulo endothelial system (RES) and to prolong the circulation time of drugs. Another prerequisite for NLCs' stability is the ability for preservation. The preservatives can impair the physical stability of lipid dispersions.[25] demonstrate that Hydrolite® 5 is proved suitable for the preservation of coenzyme Q10 loaded NLCs. [Refer table no.1]

3.3 UV blockers: uv blockers helps to protect skin from ultraviolet radiation of the sun and it lowers the risk of skin cancer, some sunscreen products contains either an organic chemical compound that absorbs uv light . Examples: Avobenzone:- absorb UV-A radiation(maximum absorption at 357 nm)

3.4 Aqueous medium: water used in all experiments was purified by reverse osmosis.

Table no.1 lipids and emulsifiers

Ingredients	Materials
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
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
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
Lipophilic emulsifier	Myverol® 18-04K, Span 20, Span 40, Span 60
Amphiphilic emulsifier	Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire® 50/13

4. METHODS EMPLOYED IN FABRICATION OF NLC's

There are several methods for the preparation of lipid nanoparticulate DDS. in this type of DDS the drug especially depends on solubility and stability, the lipid matrix, route of administration, etc.

4.1 High pressure homogenization :

High Pressure Homogenization Technique has been used as a reliable and powerful technique for the large-scale production of NLCs, lipid drug conjugate, SLNs, and parenteral emulsions. In High Pressure Homogenization technique lipid are pushed with high pressure (100-200bars) through a narrow gap of few micron ranges. So shear stress and cavitation are the forces which cause the disruption of particle to submicron range. Normally the lipid contents are in the range of 5-10%. In contrast to other preparation technique High Pressure Homogenization does not show scaling up problem .Basically there are two approaches for production by high pressure homogenization, hot and cold homogenization techniques [26]. For both the techniques drug is dissolved in the lipid being melted at approximately 510º C above the melting point.

FIG.5 High Pressure Homogenizer

a) Hot High pressure homogenization: in this process the lipid and drug are melted(10^0 C above the melting point of the lipid) are combined with an aqueous surfactant solution at the same temperature . a hot pre-emulsuion is formed using high shear device (e.g.Ultra-Turrax).then hot pre-emulsion is processed in a temperature controlled high pressure homogenization at 500 bar using piston gap homogenizer.the obtained nanoemulsion recrystallizes upon cooling down at room temperature leads to formation of NLC's[27]

b) Cold high pressure homogenization: this method is suitable for heat-labile drugs or hydrophilic drugs. The lipid and drug are melted together and rapidly cooled under liquid nitrogen forming solid lipid micro particles, a pre-suspension is formed by homogenization of the particles in a cold surfactant solution. The pre-suspension is then further homogenized in a high pressure homogenization at or below room temperature at predetermined homogenization condition to produce NLC . In this both high pressure homogenization techniques are suitable for processing lipid concentrations of upto 40% and generally they yield very narrow particle size distributions. Cold homogenisation minimises the thermal exposure of the sample [28].

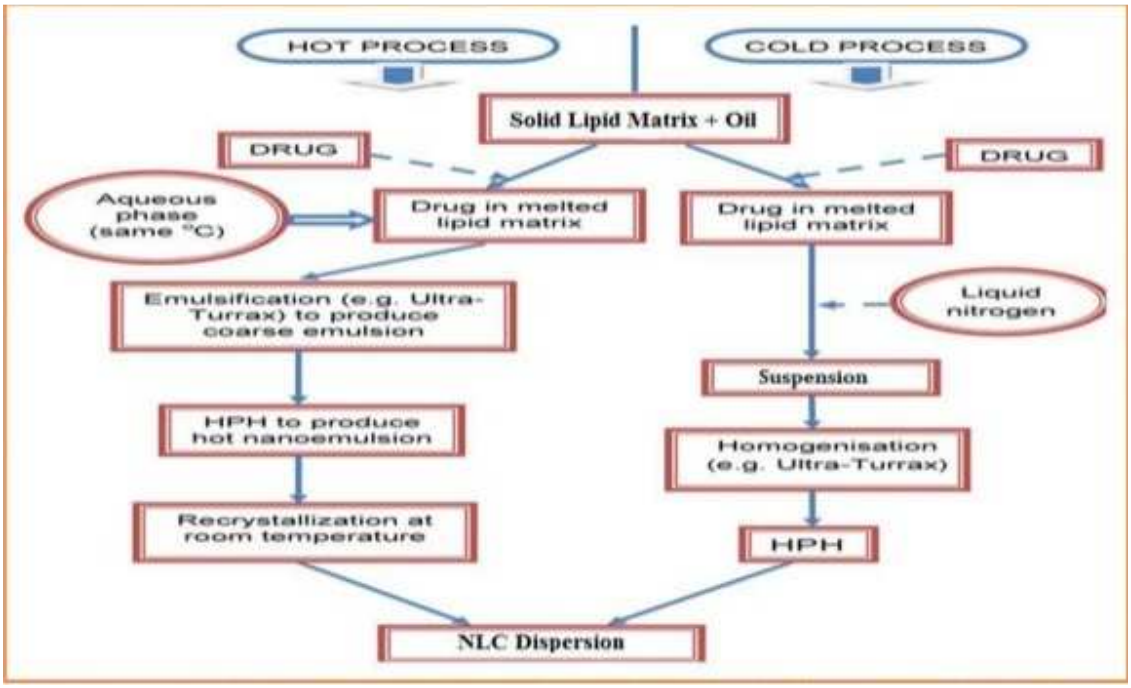

FIG.6 Flow chart of Hot HPH and Cold HPH

4.2 Microemulsion technique: The lipids (fatty acids or glycosides eg. lipid acid) are liquified and drug is incorporated 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 beneath 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 recrystallisation of the oil droplets. [29].

4.3 Solvent emulsification-evaporation technique: In solvent emulsification-evaporation technique, the hydrophobic drug and lipophilic material were dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenizer. To improve the efficiency of fine emulsification, the coarse emulsion was immediately passed through the microfluidizer. Thereafter, the organic solvent was evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates of SLNs. Here the mean particle size depends on the concentration of lipid in organic phase. Very small particle size could be obtained with low lipid load (5%)

related to organic solvent. The big advantage of this method is the avoidance of any 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 limited the solubility of the lipid in the organic solvent [30].

4.4 Solvent emulsification-diffusion technique: In solvent emulsification-diffusion technique, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, 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 in order to ensure the initial thermodynamic equilibrium of both liquid. When heating is required to solubilize the lipid, the saturation step was performed at that temperature. Then the lipid and drug were dissolved in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous solution containing stabilizer (dispersed phase) using mechanical stirrer. After the formation of o/w emulsion, water (dilution medium) in typical ratio ranges from 1:5 to 1:10, were added to the system in order to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles. Here the both the phase were maintain at same elevated temperature and the diffusion step was performed either at room temperature or at the temperature under which the lipid was dissolved. Throughout the process constant stirring was maintained. Finally, the diffused solvent was eliminated by vacuum distillation or lyophilization [31].

4.5 Phase inversion temperature (PIT) method: Phase inversion of O/W to W/O emulsions and vice versa induced by temperature change is a long known method to produce microemulsions stabilized with non-ionic surfactants . The technique is based on the change in the properties of polyoxyethylated surfactants at different temperatures. The hydrophilliclipophillic balance (HLB) value of surfactants defined by Griffin is valid at 25°C. At this temperature the hydrophilic parts of the SAC molecules are hydrated to a certain extent. An increase in the temperature causes dehydration of the ethoxy groups. As a result, the lipophilicity of the molecules of the SAC rises with corresponding decrease in HLB value. At a certain point the affinity of the SAC to the aqueous and lipid phase is equal - this temperature is defined as the phase inversion temperature. This particulate state is characterized by very low surface tension and presence of complex structures in the system. If the temperature is further increased the SAC's affinity to the lipid phase becomes higher enough to stabilize emulsions of w/o type [32].

4.6 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 [33].

4.7 High Shear Homogenization or Ultrasonication Technique: Ultrasonication based on the mechanism of cavitation. In first step, the drug was added to previously melt solid lipid. In second step, the heated aqueous phase (heated to same temperature) was 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 was ultrasonicated using probe sonicator with water bath (at 0ºC). In order to prevent recrystalization during the process, the production temperature kept at least 5ºC above the lipid melting point. The obtained product was filtered through a 0.45µm membrane in order to remove impurities carried in during ultrasonication [34].

4.8 Solvent injection (or solvent displacement) technique: Technique in which a solvent that distributes very rapidly in water (DMSO, ethanol) is used [35]. 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 lipid particles precipitate in the aqueous solution.As shown in Figure 6 schematic overview of Solvent injection method. Particle size depends on the velocity of distribution processes. Higher velocity results in smaller particles. The more lipophilic solvents give larger particles which may become an issue. The method offers advantages such as low temperatures, low shear stress, 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.

4.9 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 [36].

4.10 Strategies employed for overcoming the issues related to stability of NLCs

Spray drying: Spray drying It is an alternative and cheaper technique to the lyophilization process. This recommends the use of lipid with melting point more than 70ºC. 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 favor the preservation of the colloidal particle size in spray drying. The melting of the lipid can be minimized by using ethanol–water mixtures instead of pure water due to cooling leads to small and heterogeneous crystals, the lower inlet temperatures [32].

Lyophilisation: Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required 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 [37].

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 observed 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 [38,39]. 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[40]. 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 [41].

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[32]

5. CHARACTERIZATION OF NLC's

5.1 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 bidistilled 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 tobe 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[42,43]

5.2 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[44].

5.3 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) (AL-Haj et al., 2008[45].

5.4 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 [46].

5.5 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 (25^0 C) 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 -70^0 C applied at a time period of 24 h, and sublimation time of 48 h. image data were analysed with Easyscan 2 software[47].

5.6 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 [48].

5.7 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.[49] differential scanning calorimeter (822e, Mettler Toledo, Greifensee, Switzerland). Approximately 10 mg of bulk lipid, drug and lyophilized NLC were placed in pin-hole 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[50,51].

5.8 Wide-angle X-ray diffraction (XRD): The geometric scattering of radiation from crystal planes within a nanoparticle dispersion can be determined by wideangle 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°[52,53]

5.9 Rheological study: The rheological properties of the prepared lipid nanoparticles were measured using Brookfield's viscometer (Brookfield LV-DV II+, USA).[54] 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[55,56]

5.10 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 ×100
DL =Wa – Ws/ Wa − Ws + Wl ×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*l* is the weight of lipid added [57,58].

5.11 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 a 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 [59].

5.12 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].

5.13 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.[61] 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)[62].

5.14 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 [63]. 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

5.15 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 [64]. 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 [65,66]. 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.

5.16 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[32]

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.

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.

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. Depending on the HLB of the surfactant and the molecular weight of the surfactant molecules, the affinity of the surfactant to the lipid differs. Having the surfactant molecules embedded in the lipid matrix might dramatically affect the crystallization of the lipid, and leave spaces in the lipid lattice. These spaces will give rise to higher loading capacity of drug, incorporation in imperfections inside the particle matrix and eventually a slower release profile. 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.

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.

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[32]

6. APPLICATIONS OF NLC's

There are various applications of NLCs in the pharmaceutical field some are as follow-

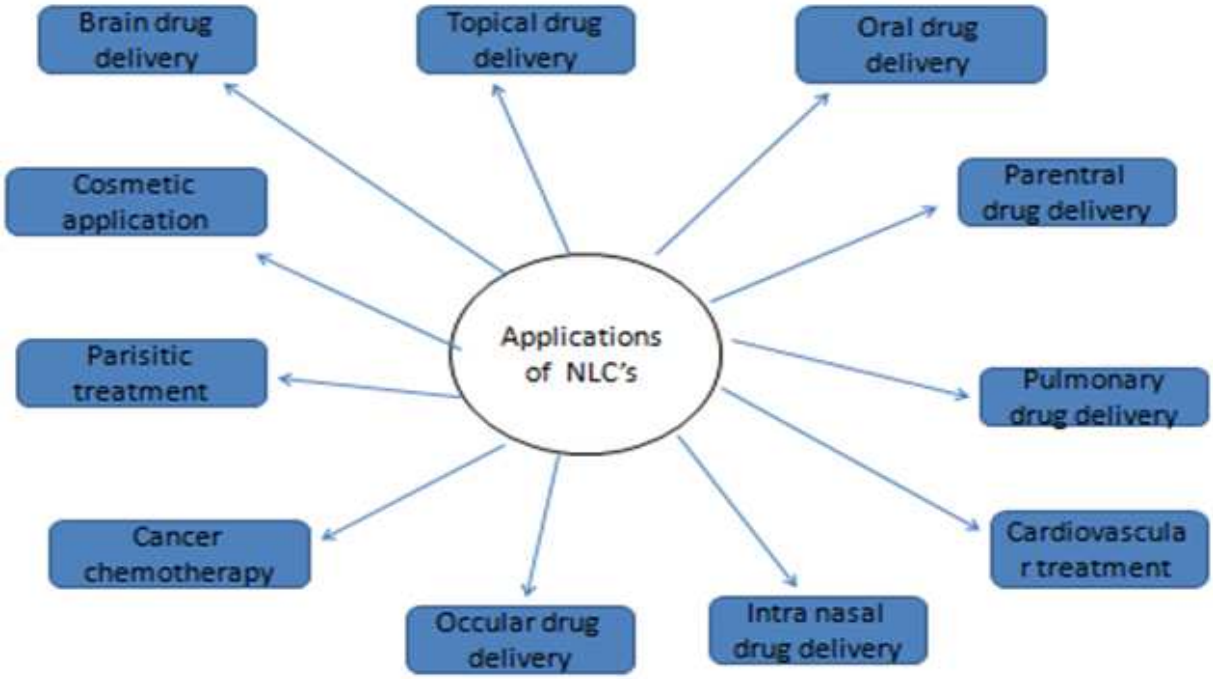

FIG.7 applications of NLC's

6.1 Oral drug delivery

Interest in NLCs for oral administration of drugs has been increasing in recent years. Increased bioavailability and prolonged plasma levels are described for peroral administration of NLCs. The lipid nanocarriers can protect the drugs from the harsh environment of the gastrointestinal tract. The lipophilic drugs can be entrapped by NLCs to resolve insolubility concerns. Repaglinide, an anti-diabetic agent with poor water solubility, has low oral bioavailability and a short halflife [67]. It is suitable to load into NLCs for improving oral delivery. Date et al. [68] prepare repaglinide NLCs with Gelucire 50/13 as an amphiphilic lipid excipient. Gelucire 50/13(stearoyl macrogolglycerides) has been previously used for the preparation of solid dispersions for improving the aqueous solubility of lipophilic drugs [69]. DSC studies indicate that Gelucire 50/13 interacts with Precirol® and that this interaction suppresses polymorphic transitions of both components. The NLCs exhibit a significantly greater decrease of the blood glucose level (about 2-fold) in rats compared to marketed repaglinide tablets The chemotherapeutic agent etoposide is used as a model drug. Etoposide is a poorly water-soluble drug and a substrate of Pglycoprotein with a considerable intra- and interpatient variation of oral bioavailability. PEG or distearoylphosphatidylethanolamine- PEG (DSPEPEG) is added into NLCs as a stabilizer to increase circulation time. The absorption of etoposide in the intestine is evaluated by an in vitro diffusion chamber. The formulations with smaller size are easier to penetrate across the intestine wall. A pharmacokinetic study is conducted in rats. After oral administration at a drug dose of 180 mg/kg, the relative bioavailability etoposide from standard NLCs, PEG-containing NLCs, and DSPE-PEG-containing NLCs is enhanced 1.8-, 3.0- and 3.5-fold, respectively, compared with control dispersion. DSPE-PEG-containing NLCs display the highest cytotoxicity against lung carcinoma cells among all carriers tested.

6.2 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. Nanostructured Lipid Carriers (NLCs) of Asenapine maleate to improve the bioavailability and enhance the uptake of ASN to the brain[70]. In Bromocriptine loaded NLCs the In-vivo results showed bromocriptine NLCs have rapid onset of action and longer duration and higher brain levels as compared to that of solution, entrapment efficiency was also increased [71].

6.3 Topical drug delivery: Tacrolimus – loaded NLCs were successful prepared. The penetration rate of these NLCs through the skin of a hairless mouse was greater than that of Prototopic®. In vitro penetration tests revealed that the tacrolimus-loaded NLCs have a penetration rate that is 1.64 times that of the commercial tacrolimus ointment, Protopic®[72].An increase of skin penetration was reported forcoenzyme Q 10 (Q10)-loaded SLN compared toQ10 in liquid paraffin and isopropanol. Thecumulative amounts of Q10 were determined performing a tape stripping test. After five stripsthe cumulative amount of Q10 was 1%, 28%and 53% of the applied amount from the liquidparaffin , the isopropanol and the SLN formulation, respectively. Similar results were achieved by another study for Q10loaded NLC.

6.4 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 nebulization 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 drug8. NLCs in pulmonary disorders seems to be promising strategy (discussed in table 2) 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 smallparticle size as well lipophilic character lead to longer residence time in lungs [73,74].

6.5 Cancer Chemotherapy: In supplement, the function of NLC in cancer chemotherapy is presented and hotspots in research are emphasized. It is foreseen that, in the beside future, nanostructured lipid carriers will be further advanced to consign cytotoxic anticancer compounds in a more efficient, exact and protected manner. ZER into NLC did not compromise the anti-proliferative effect of ZER. Both ZER and ZER-NLC significantly induced apoptosis via the intrinsic pathway in time-dependent manner. The proposed mechanism of apoptosis of cancer cells

induced by ZER and ZER-NLC is via activation of caspase-9 and caspase-3, inhibition of anti-apoptotic protein, and stimulation of proapoptotic protein expressions. Loading of ZER into NLC will increase the bioavailability of the insoluble ZER in the treatment of cancers [75].g l-arginine lauril ester (AL) into nanostructure lipid carriers (NLCs) and then coating with bovine serum albumin(BSA),pH-sensitive membranolytic and lysosomolytic nanocarriers (BSAAL-NLCs) were developed to improve the anti-cancer effect y render more nanocarriers lysosomolytic capability with lower cytotoxicity, as well as improved therapeutic index of loaded active agents[76].

6.6 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 (targetablity) 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 [77].

6.7 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[78]. 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 [79]. 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[80].Triamcinoloe 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 [81].

6.8 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 noninvasive 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 [82].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.

6.9 Parentral drug 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 alongwith these, their aqueous nature and biocompatibility of the excipients has enabled intravenous delivery of the drug with passive targetingability 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 asa viable alternative to the current injectable intramuscular(IM) formulation [83,84].Bufadienolides a class C-24 steroid also proved to be effectivein terms of enhanced haemolytic activity and cytotoxicity with reduced side effects when incorporated in NLCs[85]. Nanostructured lipid carriers (NLCs) were prepared and optimized for the intravenous delivery of β Elemene (β-E) β-E-NLCs showed a significantly higher bioavailability and anti-tumor efficacy than Elemene injection. β-E-NLCs described in this study are well-suited for the intravenous delivery of β-E[86].

6.10 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. Tashinone (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 [87].Nifedipine loaded NLCs Nanoparticle suspensions were formulated with negatively charged phospholipid, dipalmitoyl phosphatidylglycerol in preventing coagulation to improve solubility and hence bioavailability of drug [88]. 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 [89].

6.11 Cosmetic Applications of NLC : Lipid nanoparticles—SLN and NLC—can be used to formulate active compounds in cosmetics, e.g. prolonged release of perfumes. Incorporation of cosmetic compounds and modulation of release is even more flexible when using NLC. In addition, the release of insect repellents has been described [90-93]. A feature of general interest is the stabilisation of chemically labile compounds. The solid matrix of the lipid nanoparticle protects them against chemical degradation, e.g. Retinol [94-96] and coenzyme Q10. A recently discovered feature is the sunscreen blocking effect of lipid nanoparticles. Similar to particles such as titanium dioxide the crystalline lipid particles scatter UV light, thus protecting against UV irradiation. In addition, it was found that incorporation of sunscreens leads to a synergistic UV blocking effect of the particulate blocker lipid nanoparticle and the molecular blocker. In vitro, crystalline lipid nanoparticles with the same sunscreen concentration exhibited twice the UV protection effect compared with an O/W emulsion loaded with the sunscreen.

CONCLUSION

It has been found that nano based drug delivery systems has lot of potential to enhance the bioavailability of poorly soluble drugs and also target the site of action. These gives additional edge to deliver some potent drugs like in various cancer. The present study states that the NLC's are the drug delivery system that reach the targeted site in the body, at the right time, at right concentration. The aim has been to develop therapeutic nanotechnology undertaking, particularly for targeted drug therapy The NLCs as the new generation offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms such as creams, tablets, capsules and injectables. The effort to develop alternative routes and to treat other diseases with NLCs should be continued to extend their applications. As these delivery systems can be easily scaled up for large scale manufacturing, hence has a lot of potential in the forth coming years in the pharmaceutical field.

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