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# Nanostructure Lipid Carrier (NLC): the new generation of lipid nanoparticles

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**ABSTRACT**

Nanotechnology having developed exponentially, the aim has been on therapeutic undertaking, particularly for targetted drug therapy. In 1980 K. Eric Drexler developed and popularized the concept of nanotechnology. The nanocarriers has became a revolutionary approach. Nanocarriers are at forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicines and research. Nanostructure lipid carriers have attracted expanding scientific and commercial vigilance in the last couple of years as alternate carriers for the pharmaceutical consignment. A new generation of nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructured has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. The present review gives insights on the definitions and characterization of NLC as colloidal carriers including the production techniques, stability techniques and suitable formulations. This review paper also highlights mechanism of skin penetration and the importance of NLC in pharmaceutical applications.

**Keywords**: nanotechnology, targetted drug delivery, nanocarriers, NLCs.

## Introduction

Since the beginning of 20th century, nanotechnology growing interest from the pharmaceutical technology research groups worldwide. It has practically made its influence in all technical fields. Industry estimates suggest that approximately 40% of lipophilic drug candidates fail due to solubility and formulation stability issues, which has been solved by various novel and advanced lipophilic drug delivery technologies [1]. The lipids employed to prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) so, that drugs can be delivered at the required site of action with controlled release with low acute and chronic toxicity [2]. Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology.To overcome the drawbacks associated to the traditional colloidal systems such as emulsions, liposomes and polymeric nanoparticles, various nanotechnology platforms like Nanostructured Lipid Carrier, fullerenes, nanotubes, quantum dots, nanopores, dendrimers, liposomes, magnetic nanoprobes and radio controlled nanoparticles are being developed.

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## NLC as compare to 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 apects:

- 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.NLC has attracted increasing scientific and commercial attention during the last few years [3,4] 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 [5].

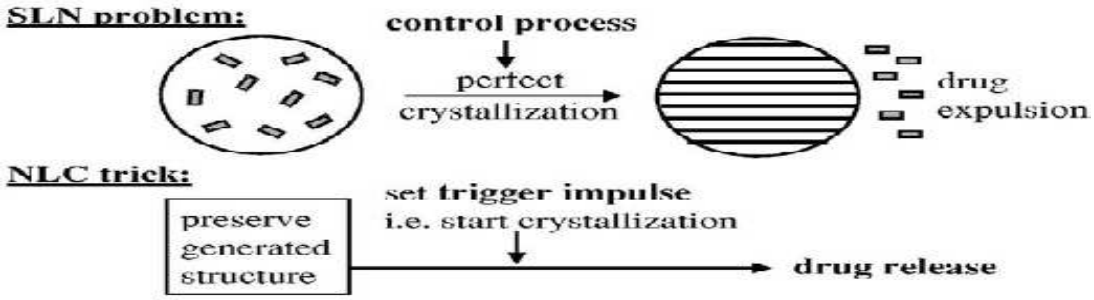



**Figure1: Triggered release of drug from NLC the transform form of SLN**

NLCs are composed of biocompatible solid lipid matrices and liquid lipid which have different chemical structure from the solid lipid [6]. Besides, NLCs have the usual particle diameter ranging 10–1000 nm.Nanostructured lipid carriers (NLC) are the second generation SLN composed of solid lipid matrix which are incorporated with liquid lipids [7]. Among the nanostructured lipid carriers that contain solid lipids together with liquid oils are, Miglyol®, α-tocopherol, etc [8]. The presence of liquid lipids with different fatty acid C-chains produces NLC with less organized crystalline structure and therefore provides better loading capacity for drug accommodation [9]. Liquid lipids are better solubilizers of drugs than solid lipids.These carriers are composed of physiological and biodegradable lipids exhibiting low systemic toxicity and low cytotoxicity[10]. Most of the used lipids have an approved status or are excipients used in commercially available pharmaceutical preparations. The small size of the lipid particles ensures close contact to stratum corneum and can increase the amount of drug penetrating into mucosa or skin. Due to their solid lipid matrix, a controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over prolonged period of time, to reduce systemic absorption, and when drug produces irritation in high concentrations [11,12,13]. NLC have been shown to exhibit a controlled release behavior for various active ingredients such as ascorbyl palmitate, clotrimazole,ketoconazole, and other antifungal agents.

**Advantages of NLCs**

- 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 forsubstances,
- 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 [14].

**Limitation of NLCs**

Despite the great potential of NLCs in targeted delivery, theyface 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 [15].

**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 (Figure1. upper). 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, eg, hydroxy octa cosanylhydroxystearate, isopropylmyristate 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 [Figure 1.middle] [16].

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 nanocompartments) show comparatively high solubility for lipophilic drugs [Figure 1. Lower] [17].

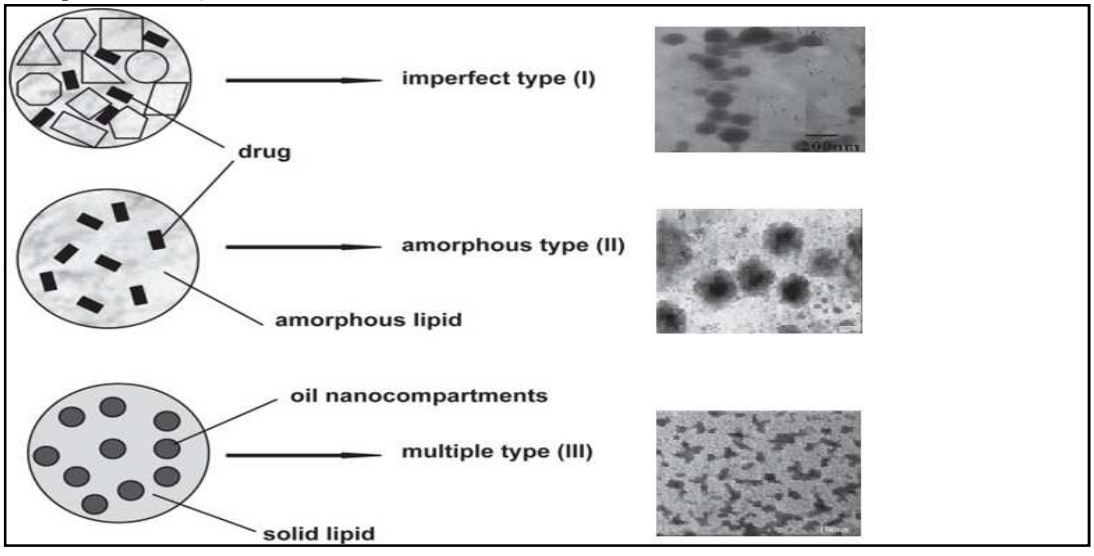



**Figure: 2Types of NLC: (I) imperfect type, (II) amorphous type and (III) multiple type**

**Ingredients used in the formulation of Nano structured lipid carrier drug delivery systems**

The essential ingredients for NLCs include lipids, water, and emulsifiers.

**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[18-20]. Lipophilic or amphiphilic emulsifiers such as Span 80 and lecithin are employed for fabrication ofNLCs if necessary. It has been found that the combination of emulsifiers can prevent particle aggregation more efficiently[21]. 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*.[22] demonstrate that Hydrolite® 5 is proved suitable for the preservation of coenzyme Q10-loaded NLCs

**Table 1: The excipients for composing nanostructured lipid carriers (NLCs)**

| Ingredient | Material |
|---|---|
| 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 emulsifiers | Myverol® 18-04K, Span 20, Span 40, Span 60 |
| Amphiphilic emulsifiers | Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire® 50/13 |

**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® [23]. 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 investigated as materials for nanoemulsions [24]. 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.* [25,26] 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.

**Preparation procedures of nanostructured lipid carriers (NLCs)**

There many methods for the preparation of lipid nanoparticulate DDS. The method used is dictated by the type of drug especially its solubility and stability, the lipid matrix, route of administration, etc.

**High Pressure Homogenization Technique**

HPH 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 [27]. For both the techniques drug is dissolved in the lipid being melted at approximately 5-10º C above the melting point.

**Hot Homogenization Technique**

In this technique the drug along with melted lipid is dispersedunder 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 [28].

**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 2. Cold homogenisation minimises the thermal exposure of the sample [29].

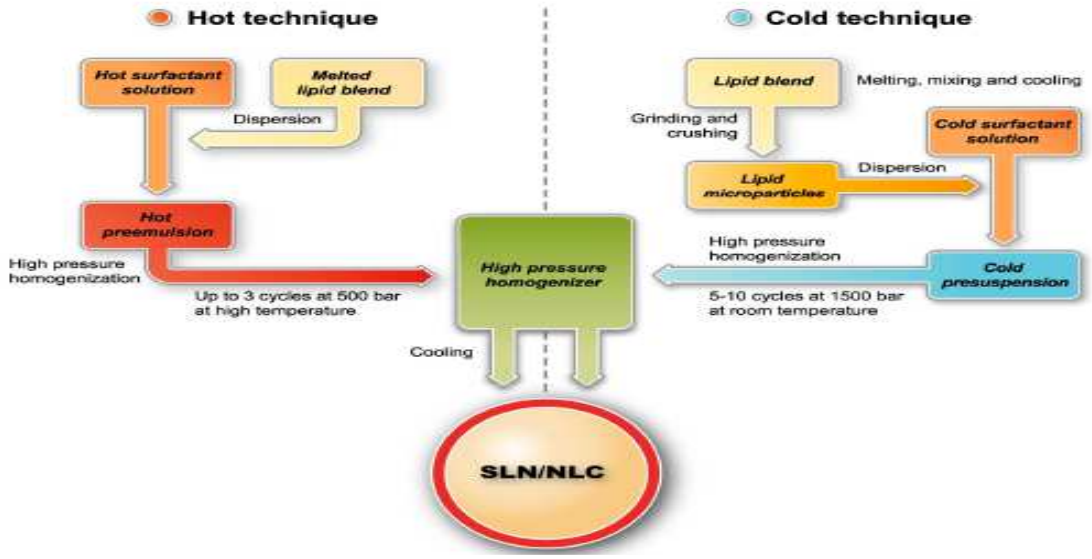



**Figure 3: Schematic overview of the hot and cold homogenisation technique**

**Microemulsion technique**

The lipids (fatty acids or glycosides eg. Stearic acid) are melted and drug is incorporated in molten lipid.A mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipids and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratios for microemulsion formation. Thus the microemulsion is the basis for the formation of nanoparticles of a requisite size.This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water in a ratio in the range 1:25 – 1:50. This dispersion in cold aqueous medium leads to rapid recrystallisation of the oil droplets. [30].

**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 [31].

**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[32].

**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 [94]. The technique is based on the change in the properties of polyoxyethylated surfactants at different temperatures. The hydrophillic-lipophillic 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 lowsurface 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.

**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].

**High Shear Homogenization or Ultrasonication Technique**

Ultrasonication based on the mechanism ofcavitation. 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 bydrop followed by magnetic stirring. The obtained pre-emulsion was ultrasonicated usingprobe 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 impuritiescarried in during ultrasonication [34].

**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 smallerparticles. 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.

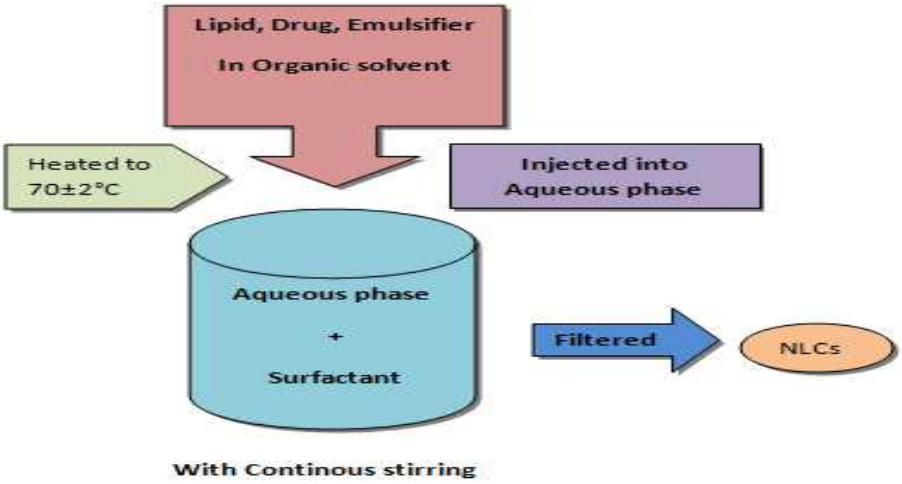



**Figure 4: Schematic representation of solvent injection method**

**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].Various factors require for successful formulation as shown inFigure 5 Parameters in producing a successful lipid nanoparticle formulation

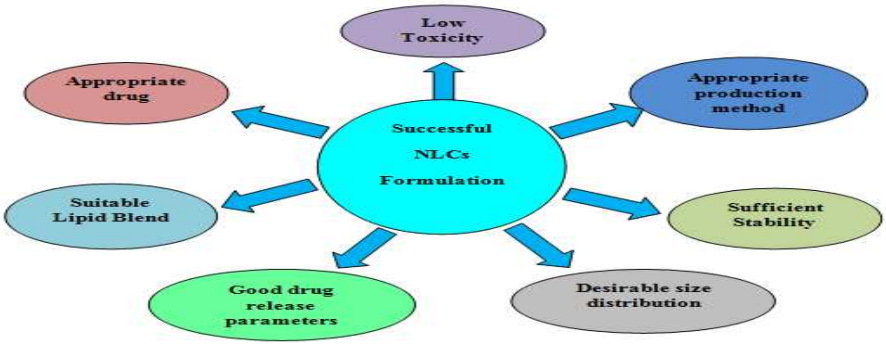



**Figure 5: Parameters in producing a successful lipid nanoparticle formulation**

**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.

**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 that 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].
- There was also 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**

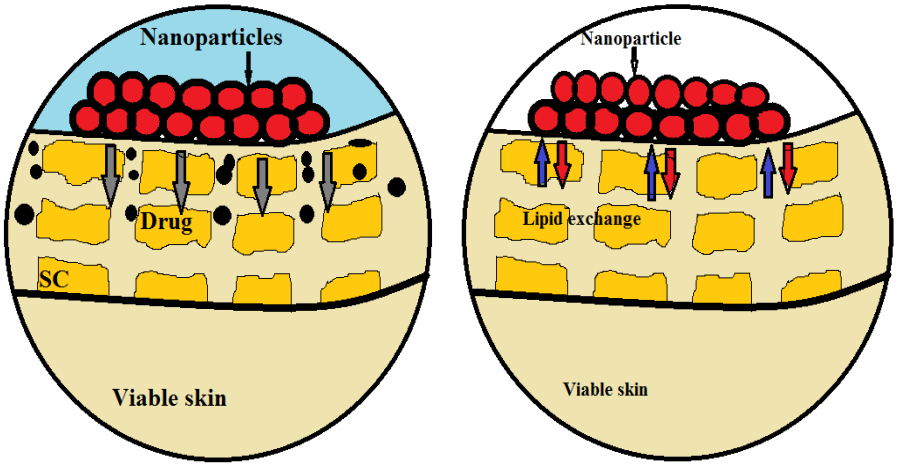
**I**In general, surface modification of colloidal particles by coating with a hydrophilic substance like polyethylene glycol(PEG) reported to bring following benefits:

- 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.

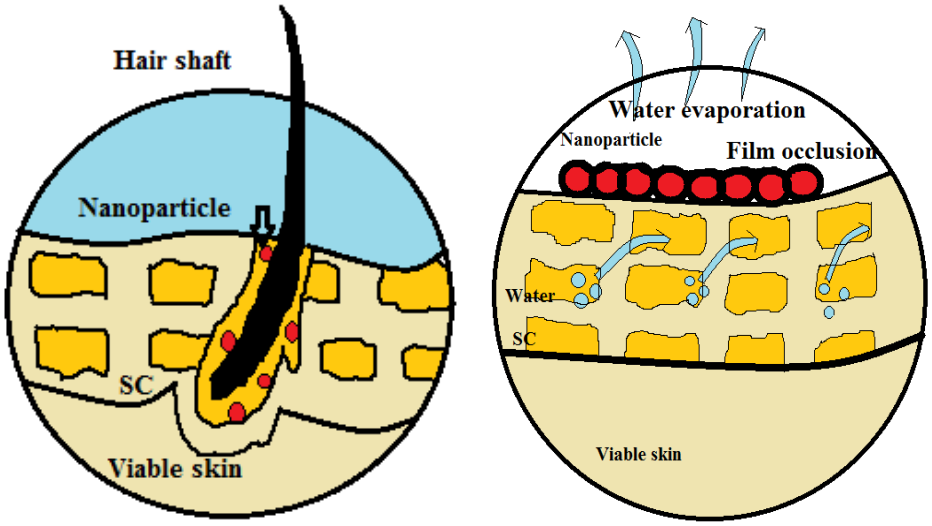
**Mechanism of skin penetration of NLCs**

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 theskin. Hydration of SC thus increases to reduce corneocyte packing and widen inter-corneocyte gaps. Hydration also influences partitioning of the drug into SC[42]. Intact nanoparticles sized above 100 nm are not

considered to permeate the SC because of their dimensions and rigidity [43].Although the particles do not penetrate across SC, uptake ofthe components is to be expected. Since epidermal lipids arerich in SC, lipid nanoparticles attaching to the skin surface would allow lipid exchange between SC and the nanocarriers[44]. Lipid nanoparticles have the potential to deliver drugsvia the follicles [45]. Furthermore, each follicle is associatedwith sebaceous glands, which release sebum, creating anenvironment enriched in lipids [46]. This environment isbeneficial for trapping of lipid nanoparticles. Sebum is amixture of triglycerides, squalene and waxes. Some glyceridelipids present in NLCs may accelerate the entrance intothe follicles/sebaceous glands. The possible mechanismsinvolved in skin permeation enhancement by NLCs are depictedin Fig. [6].




**Close contact to skin surface.skin hydration by particle occlusion**




**Lipid exchange between SC and NLCsEntrance into follicles and sebaceous glands**

**Figure 6: Possible mechanisms for skin permeation enhancement of drugs or active ingredients from Nanostructure lipid carriers (NLCs)**

**Applications of NLCs**

**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 [47]. It is suitable to load into NLCs for improving oral delivery. Date et al. [48] 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 [49]. 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 P-glycoprotein with a considerable intra- and inter-patient variation of oral bioavailability. PEG or distearoylphosphatidylethanolamine- PEG (DSPE-PEG) 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.

**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[50]. 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 [51].

**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®[52].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 determinedperforming 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 SLNformulation, respectively. Similar results wereachieved by another study for Q10-loaded NLC.

**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 longerresidence time in lungs[53,54].

**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 [55].g l-arginine lauril ester (AL) into nanostructure lipid carriers (NLCs) and then coating with bovine serum albumin(BSA),pH-sensitive membranolytic and lysosomolyticnanocarriers (BSA-AL-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[56].

**Parasitic treatment**

Novel colloidal delivery systems have gained considerable interest for anti-parasitic 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 physico-chemical properties and pharmacokinetic properties are modulated of the anti-parasitic agents (formulated as NLCs shown in table 5) 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 [57].

**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[58]. SLNs have been used for ocular drug delivery in the last decades. Recently, further investigations employing NLCs as ocular delivery systems have become knownIn 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 [59]. 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[60].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 [61].

**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 [62].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[50].

**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 [63,64].Bufadienolides a class C-24 steroid also proved to be effectivein terms of enhanced haemolytic activity and cytotoxicitywith reduced side effects when incorporated in NLCs[65]. 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[66].

**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 [67].Nifedipine loaded NLCs Nanoparticle suspensions were formulated with negatively charged phospholipid, dipalmitoyl phosphatidylglycerol in preventing coagulation to improve solubility and hence bioavailability of drug [68]. 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 [69].

**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 [70,71]. 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[72] 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.

**Physicochemical characterization ofNLCs**

The physicochemical characterization for NLCs is essentialto confirm quality control and stability. Both physicaland chemical properties can be determined for NLCs.Microscopic and Macroscopic techniques are used in development of colloidal system84. Various techniques like particle size analysis, zeta-potential, transmission electron microscopy, differential scanning calorimetry (DSC), X- Ray scattering, polarized light microscopy, laser diffraction (LD), field-flow fractionation (FFF) were performed to investigate the structure, mobility and molecular environment of the compounds. These techniques also reveal the physical and chemical stability of formulation, surface chargetend to determine the particles will flocculate or not.

**Particle Size**

The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size andas particle size decreases, surface area characteristics increases as a function of total volume, photon correlation spectroscopy (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, theabsorption and the size of the particles as well as therefractive indices of both the particles and the dispersion medium.

**Zeta potential (ZP)**

Zeta potential is the electric potential of a particle in a suspension. It is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions. In suspensions the surfaces of particles develop a charge due to ionization of surface groups or adsorption of ions. This charge depends on both the surface chemistry of the particles and the media around these particles. The surface charge generates a potential around the particle, which is at the highest near the surface and decays with distance into the medium. The zeta potential can be measured by determining the velocity of the particles in an electrical field (electrophoresis measurement).

**Scanning electron microscopy (SEM)**

This technique can be used to investigate the shape of the particles prepared and to assess the particle size of these particles. Aqueous NLC dispersions can be applied and spread on a sample holder (thin carbon film). The samples will be placed inside of the vacuum column of the microscope and the air was pumped out of the chamber. An electron gun placed at the top of the column emits a beam of high energy primary electrons. The beam of the electrons passes through the lenses which concentrates the electrons to a fine spot and scan across the specimen row by row. As the focused electron beam hits a spot on the sample, secondary electrons are emitted by the specimen through ionization. A detector counts these secondary electrons. The electrons are collected by a laterally placed collector and these signals are sent to an amplifier.

**Differential scanning calorimetry (DSC)**

DSC is usually used to get information about both the physical and the energetic properties of a compound or formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature. DSC and powder is performed for the determination of the degree of crystallinity of the particle dispersion. The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion.

**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 [73]. 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.

**Atomic Force Microscopy (AFM)**

AFM is optimal for measuring morphological and surface features that are extremely small. AFM does not use photonsor electrons but a very small sharp-tipped probe located at the free end of a cantilever driven by interatomic repulsive or attractive forces between the tip and surface of the specimen[74]. Although electron microscopy is still frequently used, the AFM technique offers substantial benefits: real quantitativedata acquisition in three dimensions, minimal sample preparation times, flexibility in ambient operating conditions, and effective magnifications at the
nano levels [75].

**X-ray Scattering**

With X-ray scattering experiments characteristic interferences the generated from an ordered microstucture. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacing'd'.According to Bragg's equation 'd' can be calculated

$$d= n/\lambda\ 2 \sin \theta$$

Where, $\lambda$ is the wavelength of the X- ray being used, n is an integer and nominates the order of the interference and $\theta$ is the angle under which the interference occurs.

**Transmission Electron Microscopy**

It is a technique where colloidal samples could be visualized at high resolution. Sufficient contrast can be given to a thin film of the frozen sample by use of osmium tetra- oxide. This allows the sample to be viewed directly in the TEM (at temperature -196º

C).The adjustment of the temperature to -196º C leads to a very poor pressure, so that the examination of the sample is possible by preservation of microstructure despite the high vacuum.

**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 [76]. 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 [77,78]. 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.

**Factors affecting the 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.

**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.

**Conclusion**

In the20th century, Paul Ehrlich envisioned his magic bullet concept; the idea that drugs reach the right site in the body, at the right time, at right concentration. The aim has been to developed therapeutic nanotechnology undertaking, particularly for targetted drug therapy The smart 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. Permeation via the gastrointestinal tract and BBB may be a future trend. The combination of two therapeutically active agents to be included in a single nanosystem is another consideration for future development.

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