



Nanostructured Lipid Carriers: A Novel Drug Delivery System

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Abstract : Lipophilic drugs have low oral bioavailability. To enhance the oral bioavailability of the lipophilic drugs lipid matrices have been used for a long time. A third generation lipid matrix was introduced as Nanostructured lipid carrier(NLCs) which is a mixture of solid lipid mixed with some incompatible liquid lipids. NLC remains solid at room temperature or at body temperature. Due to its various advantages it also overcomes the problems of various lipid particulate carriers. It can be easily used as a carrier for drug which is given orally. Also used for a large scale production of drugs. It is a promising delivery which can be used in future for pharmaceutical market.

Keywords : Nanotechnology, targeted drug delivery, nanocarriers, NLCs.

Introduction

In the pharmaceutical industries everyday new drug molecule have been introduced, but only the production of new drug is not sufficient. The most common problem is low solubility which leads to low bioavailability. To overcome this problem a lipid based nanoparticle drug carrier was developed. This carrier system has no toxicity, greater drug loading capacity, drug targeting controlled release, physical and chemical stability.[1-3] Various lipid based nanoparticle carrier were developed as liposomes, Lipid emulsion(LE), Solid lipid nanoparticles(SLN) and Nanostructured lipid carrier(NLC). Eldem *et al.* [4]. SLN and NLC are two main types of lipid nanoparticles. These are used to deliver the drug via various routes like oral, parenteral, and topical with controlled release of drug delivery. SLN have some disadvantages as poor drug loading, high water content & drug expulsion during storage. To overcome these problems a new third generation drug carrier was introduced which is Nanostructured lipid carrier (NLC) [5].

NLC & Its Types

NLC is a mixture of solid lipids mixed with some incompatible liquid lipids. It remains solid at room temperature. It has various advantages like controlled release of drug from the carrier, biocompatible lipids, feasible to produce on large scale using the existing machinery, avoids first pass metabolism and drug protection from biochemical degradation. In NLC lipids can be used in higher ratio (up to 95%).

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NLC has been classified into three different classes based on the nano-structure, composition and ratios of solid and liquid lipids-

- a) Type I (The imperfect type)
- b) Type II (The multiple O/F/W type)
- c) Type III (The amorphous type)

Type I (The imperfect type) :

In this solid lipids and liquid lipids (oils) are blended. In liquid lipids drug solubility is higher than in solid lipids. 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[6]. As shown in the figure 1.

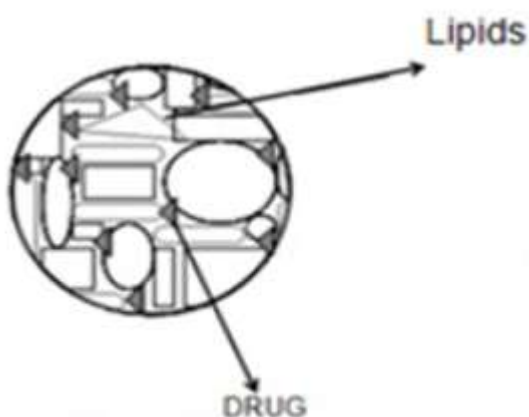


Figure 1: Type I

Type II (The multiple O/F/W type) :

In this multiple oil/fat/water, type II drug can be accommodated in the solid, but in the liquid lipids or oily parts at the increased solubility. The drug is dissolved in oily parts of the lipid matrix. These NLC was prepared by lipid-lipid precipitation. As shown in the figure 2.

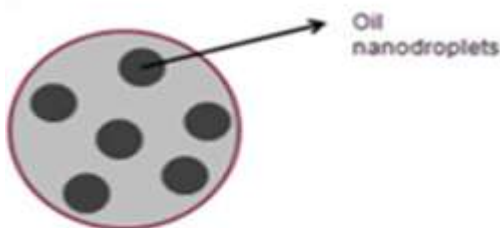


Figure 2: Type II

Type III (The amorphous type) :

This type of NLC is prepared by controlled mixture of special types of solid and liquid lipids (eg, Isopropylmyristate). In this lipids are mixed from being prevented from crystallizing. The lipid matrix is solid which is in amorphous state. The absence of crystallization avoids drug expulsion by crystallization. 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. Conjugation is performed by salt formation or covalent linkage[7]. As shown in the figure 3.

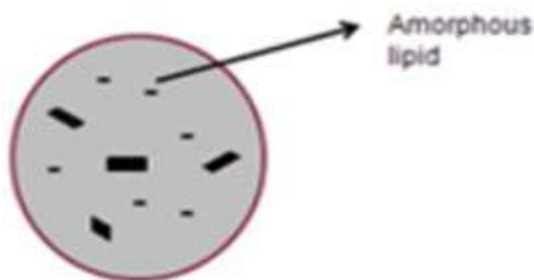


Figure 3: Type III

Composition of NLC

In the preparation of NLC various components are used as follows:

Solid lipids :

Compounds with high melting point i.e. higher than 40 °C are used as solid lipid. It should be biodegradable and safe [17]. Various solid lipids are used as Triglycerides- tricaprins, tristearin, trimyristin. Hard fat types- Glycerol monostearate, stearic acid, cetyl alcohol.

Liquid lipids :

The liquid lipids used are digestible oils from natural sources. It must have well tolerated GRAS status and also accepted for human use [17]. Natural and synthetic oils are used such as castor oil, mustard oil, codliver oil, medium chain triglycerides and oleic acid.

Aqueous surfactant :

Surfactants are also called emulsifying agents which lower the interfacial tension between two immiscible liquids or components. Hydrophilic surfactants are mostly used. Lipophilic or amphiphilic emulsifiers are used for the fabrication of NLCs [18]. Eg. Tween-80 and Pluronic F-68.

Co-emulsifier: Soya lecithin, phosphatidylcholine, egg lecithin.

Advantages of NLC's

- Better physical stability,
- Increased dispersability in an aqueous medium,
- Ease of preparation and scale-up,
- High entrapment of lipophilic drugs and hydrophilic drugs,
- Extended release of the drug,
- Controlled particle size
- Improve benefit/risk ratio,
- Small size of the lipid particles ensures close contact to the stratum corneum thus enhancing drug penetration into the mucosa or skin,
- Increased 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 [8].

Limitations of NLC's

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

- Irritative and sensitising action of some surfactants,
- Cytotoxic effects related to the nature of matrix and concentration,
- Lack of sufficient preclinical and clinical studies with these nanoparticles in case of bone repair, and
- Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better exploited [9].

Different Mechanism of NLC for Improved BioAvailability

Direct uptake :

This is done by GI tract i.e., by the intestinal lymphatic transport. As the drug is lipophilic in nature and a long chain of triglycerides is used, the NLC may stimulate the formation of chylomicron which follows the transcellular route of absorption. The compounds are transported with the help of triglycerides (TG) lipid core of the intestinal lipoproteins so for the formation of lipoproteins lipids are required. The hydrolysis of triglycerides starts in GIT by the lingual lipase and gastric lipase which form a crude triglyceride emulsion and then emptied in the duodenum. This crude emulsion stimulates the production of bile salt, biliary lipids and pancreatic juice. Monoglycerides (MG) and fatty acid (FA) are formed by the action of pancreatic lipase at the surface of emulsified TG.

By the enterocyte the long chain FA and MG are absorbed. The absorbed FA is re-esterified by monoacylglycerol pathway. After processing by number of organelles they are arranged to become the lipid core of chylomicron. These chylomicrons are stabilized by the addition of apolipoproteins and phospholipids [10]. The stabilized lipoprotein are then secreted into lamina propria and mesenteric lymphatic [11].

Muco-adhesion :

The formed nanoparticles adhere to the mucus by which they increase the residence time and due to which drug release from the carrier is increased [12].

Mixed micelle formation :

The lipids used in the formation of NLC are similar to dietary lipids which induce the bile secretion in the small intestine. The lipids on the action of enzymes undergo degradation to form the lipid digestion product and are mixed with bile to form the mixed micelle. The solubility of the drug increased by this phenomena and the transport of drug across the membrane promoted [13].

Increased permeability :

The surfactants change the intestinal permeability by various mechanisms. For eg. Poloxamer which deform the cell and tight junction of the intestinal epithelial cell promote the paracellular transport of NLC [14]. It also inhibits the P-glycoprotein efflux and increase the transport of NLC across the intestinal mucosa [15].

Inhibit drug degradation: In the GI environment some drugs are unstable. NLC helps to protect the drug by the lipids from chemical and enzymatic degradation, thereby delaying *in-vivo* metabolism [16].

Methods of Preparation

There are many techniques used for the preparation of NLCs. These techniques have been adopted from polymeric nanoparticles production procedure. The most commonly used technique is high pressure homogenization technique. The other techniques are emulsification method, solvent evaporation method, ultrasonication etc. The method for preparation is selected on the basis of which type of drug used, solubility of the drug, stability of the drug and its route of administration [17,19, 20].

1. High pressure homogenization :

High Pressure Homogenization Technique has been used as a reliable and powerful technique for the large-scale production of NLCs. It involves the melting of solid lipid materials first before mixing them with liquid lipid and drugs. After mixing, the molten liquid is scattered throughout the aqueous phase, which contains surfactants. The mixture is stirred to form the beginning of an emulsion. Lipid emulsions are pushed with high pressure (100-200bars) through a narrow gap of few micron ranges. So shear stress and cavitations 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. Homogenization may be performed either at elevated temperature (hot homogenization) or below room temperature (cold homogenization) [21].

a) Hot high pressure homogenization:

Hot homogenization is carried out at temperatures above the melting point of the lipid (100° C above the melting point of the lipid) and can therefore be regarded as the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by a high shear mixing device (Ultra-Turrax). The quality of the pre emulsion affects the quality of the final product to a large extent, and obtaining droplets in the size range of a few micrometers is desirable. In general, higher temperatures result in lower particle sizes because of the decreased viscosity of the inner phase [22]. This technique is illustrated in Figure 4. Hot homogenization has three basic problems. The first is temperature-dependent degradation of the drug, the second is the drug penetrates into the aqueous phase during homogenization and the third is complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts [23,24].

b) Cold high pressure homogenization :

Cold homogenization has been developed to overcome the problems of the hot homogenization technique such as, temperature mediated accelerated degradation of the drug payload, partitioning and hence loss of drug into the aqueous phase during homogenization. This method is suitable for heat-labile drugs or hydrophilic drugs. the cold homogenization is carried out with the solid lipid without melting as done in hot process. Drug along with lipid in solid state is milled to form microparticles, and further dispersed in a solution containing emulsifier. The pre-suspension formed is then subjected to high pressure homogenization at or below room temperature (25). In the cold HPH technique, lipid is melted above its melting point and drug is dissolved or dispersed in it. The system is cooled down by means of dry ice or liquid nitrogen. After solidification, the lipid mass is grounded using ball or mortar milling to yield lipid microparticles in a range between 50 and 100 µm. Then a microemulsion is formed by adding these microparticles into cold surfactant solution with stirring. This suspension is passed through a high pressure homogenizer at/or below room temperature and the microparticles are broken down to nanoparticles. This technique is illustrated in Figure 4.

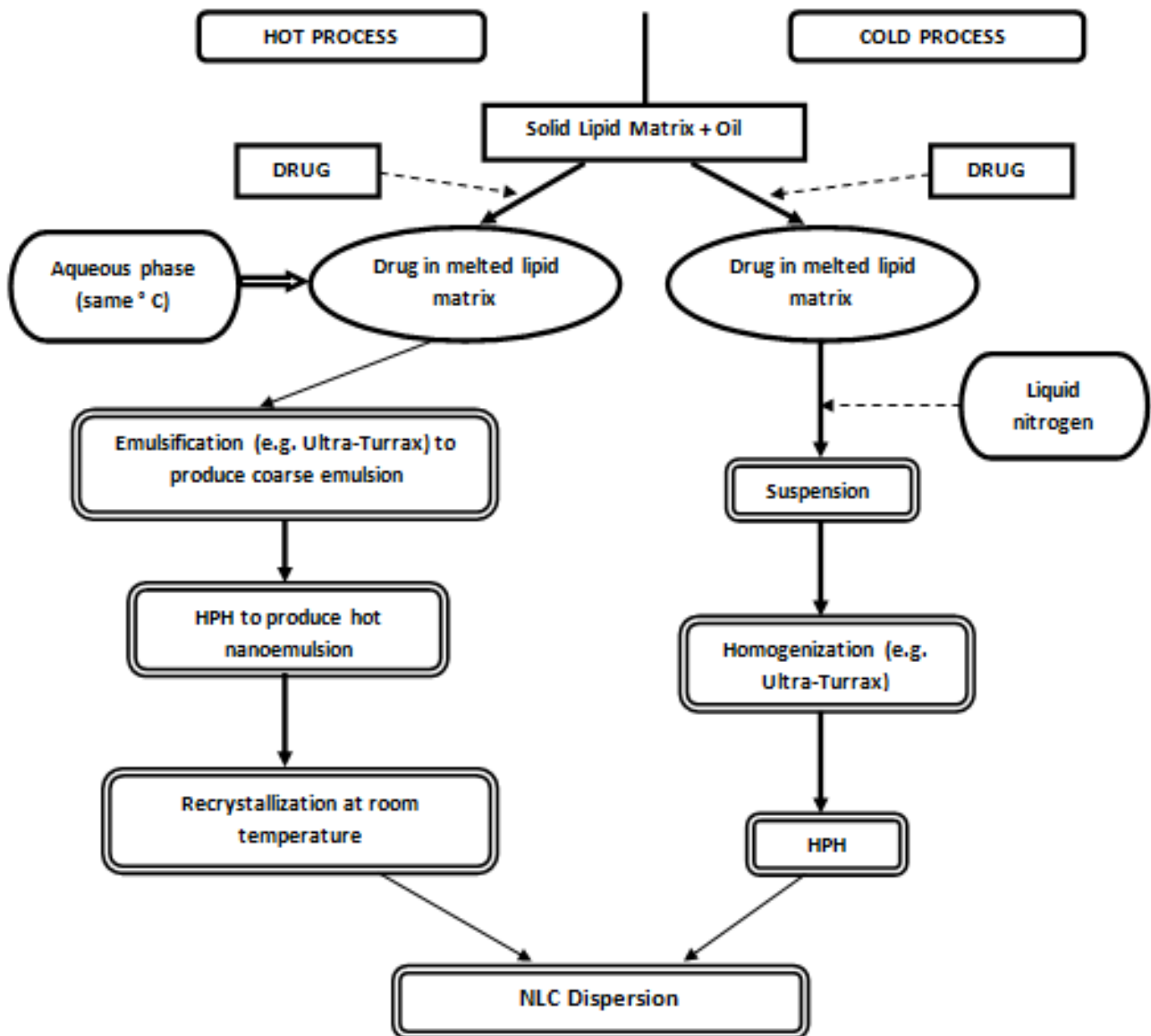


Figure 4: Schematic overview of the hot and cold homogenisation technique

2. Microemulsion technique :

The lipids (fatty acids or glycosides eg. lipid acid) are liquified and in this liquefied lipid the drug is dissolved. A mixture of water, surfactant and co—surfactant is heated at the same temperature as the lipid and added to the lipid melt under mild stirring. A clean microemulsion was obtained when the components were mixed in correct ratio. The formed microemulsion is the basis for the nanoparticle formation of a requisite size. This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water during a quantitative relation in the range 1:25-1:50. This dispersion in cold aqueous medium leads to rapid recrystallization of the oil droplets [26]. Surfactant and co-surfactant include lecithin, salt along with alcohol such as Butanol. The microemulsion was prepared in a large temperature-controlled tank and then pumped from this tank into a cold water tank for precipitation purpose [27]. As shown in the figure 5 [28].

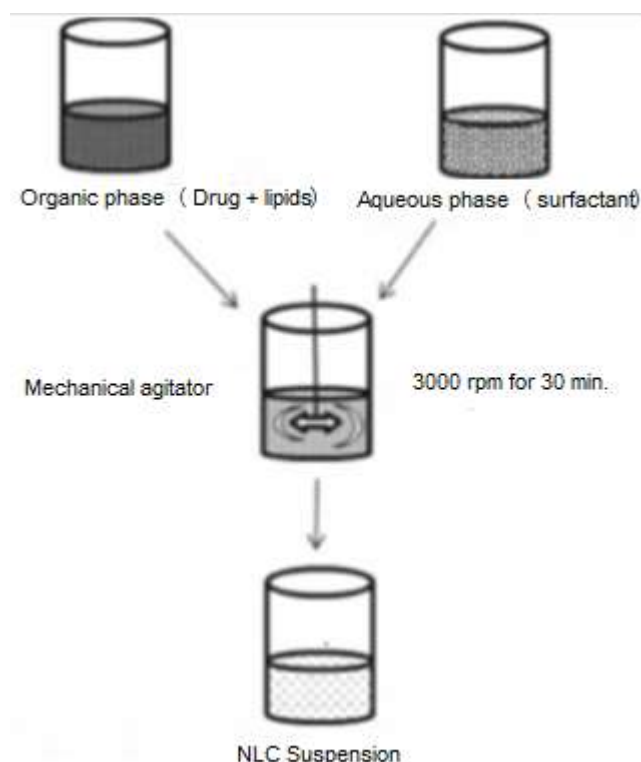


Fig. 5: Formation of NLC suspension

3. Solvent emulsification-evaporation technique :

This method is used for the production of the polymeric nanoparticles by solvent evaporation in o/w emulsion via precipitation [29,30]. In this technique the lipophilic material and hydrophobic drug were dissolved in a water immiscible organic solvent (eg. Cyclohexane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenization. The lipid precipitates upon evaporation of solvent and thus forming the nanoparticles. The big advantage of this technique is avoidance of any thermal stress, which makes it appropriate for incorporation of highly thermo labile drugs. A clean disadvantage is the use of organic solvent which may interact with drug molecule and limited the solubility of the lipid in the organic solvent [31].

4. Solvent emulsification-diffusion technique :

It consists of two different phases; organic phase and aqueous phase. In this technique organic solvent used (eg. Benzyl alcohol, ethyl acetate, methyl acetate, isopropyl acetate) must be partially miscible in water. Initially both the phases were mutually saturated in order to ensure the initial thermodynamic equilibrium of both the liquids. When heating is required for the solubilization of the liquid the saturation step was performed at elevated temperature. Then the drug and the lipid were dissolved in aqueous phase and the organic phase was emulsified with aqueous solution containing stabilizer using mechanical stirrer. After formation of this 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 to the continuous phase, forming the nanoparticles. In the whole process continuous stirring was maintained [32]. The obtained dispersion can be placed in vacuum desiccators for 24 hours to evaporate the residual organic solvent [33].

6. Melting dispersion method :

In this method, drug, solid lipid and organic solvents were melted together and the aqueous phase (water phase) is heated at the same temperature as organic phase separately. The organic phase is then added to the water phase with high speed stirring for few hours. The resulting mixture is cooled down to room temperature to obtain nanoparticles[34].

Parameters for Successful NLCs Formulation

The parameters for the successful formation of NLCs are shown in the figure 6.

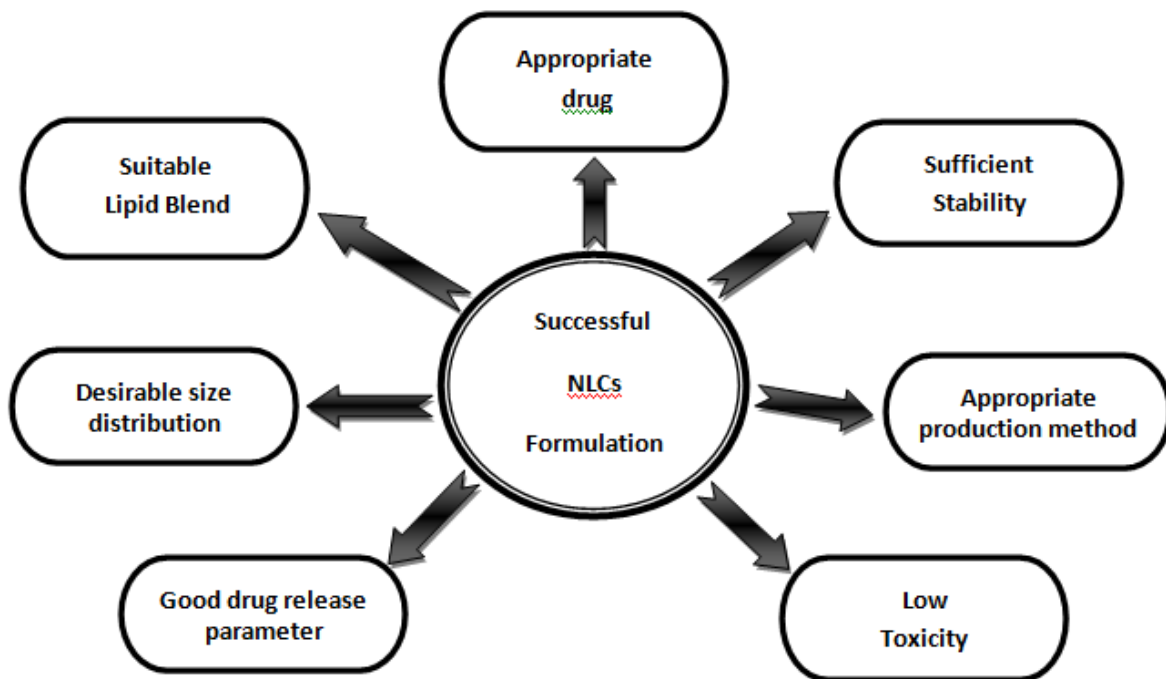


Figure 6: Parameters in producing a successful lipid nanoparticles formulation

Characterization of NLCs

Particle size and zeta potential :

Photon correlation Spectroscopy (PCS) is an established technique used for the measurement of the size and distribution Poly dispersity index (PDI) of NLCs [35].

Poly dispersity index: Determined by the equatio

$$PDI = [D(0.9) - D(0.1)] / D(0.5)$$

Where,

D (0.9) = PS immediately above 90% of sample

D (0.5) = PS immediately above 50% of sample

D (0.1) = PS immediately above 10% of sample

The PDI ranges from 0.0000 to 1.0000 i.e., monodisperse to very broad particle size distribution.

Entrapment efficiency (%) (E_e) and drug loading (DL) :

From the prepared NLCs formulation 1ml of the dispersion was dissolved in (1:1) mixture of 10ml of 7.4 PBS buffer and ethanol. Then this mixture was centrifuged at high rpm (10000-20000) for 40 min. at 25°C [36]. The rpm was selected on the bases of particle size. Lesser the particle size higher will be the rpm.

The entrapment efficiency (E_e) and drug loading (DL) were calculated as follows:

$$E_e = [(W_i - W_s) / W_i] \times 100\%$$

$$DL = [(W_i - W_s) / (W_i - W_s + W_L)] \times 100\%$$

Where,

W_i = weight of drug added initially

W_s = weight of drug in supernatant

W_L = weight of lipid mixture added

E_e can also be determined by suitable analysis of the obtained NLC and calculated as follows:

$$E = \frac{W_E}{W_I} \times 100\%$$

Where W_E = weight of the drug entrapped in NLC.

Shape and morphology :

The shape and the morphology of the NLC are determined by Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). These techniques can also be used for the determination of particle size and size distribution. SEM utilizes the electron transmission from the sample surface, whereas TEM utilizes the electron transmission through the sample. As compare to SEM, field emission scanning electron microscopy (FESEM) is used because it can detect the nanometer size range particle[37].

Applications of NLCs

There are various applications of NLCs in the pharmaceutical field some are as follows:

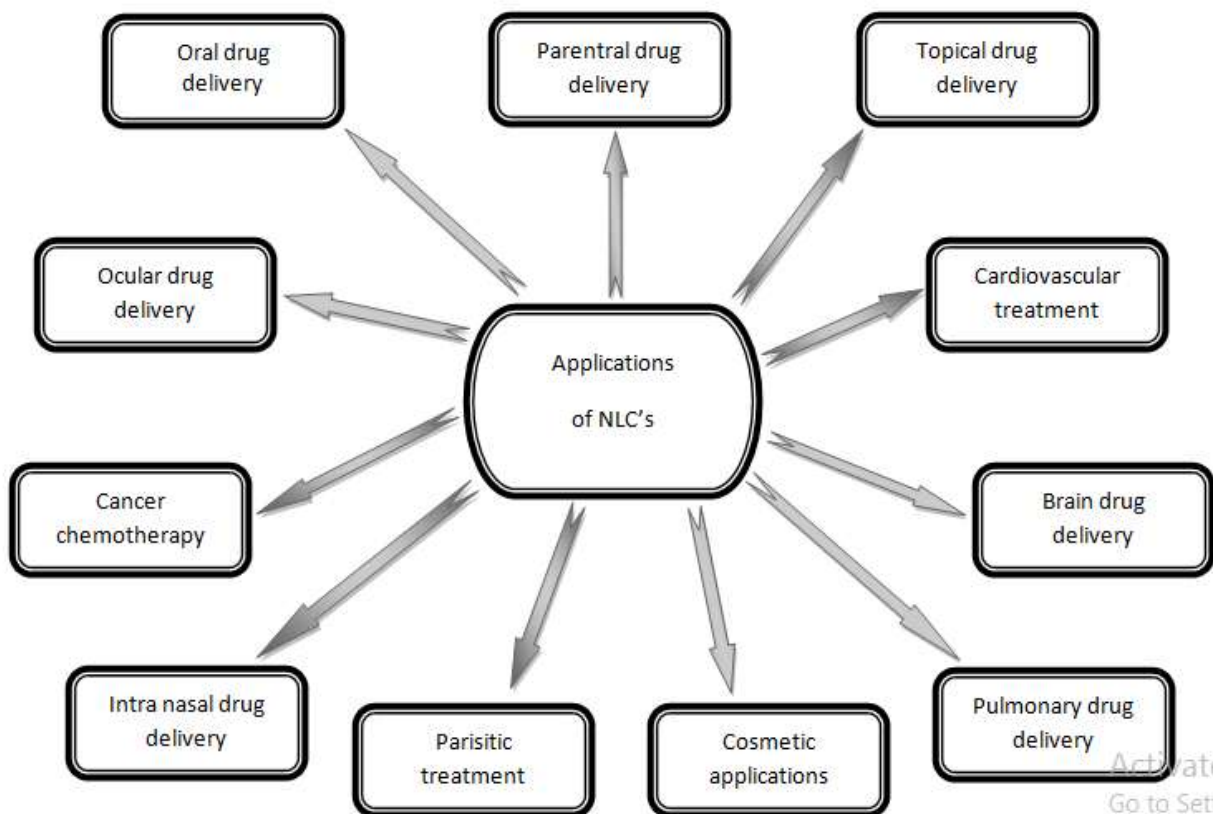


FIG.7: Applications of NLC's

NLCs for oral delivery :

Oral drug administration is most common and preferred route of drug administration due to good patient compliance and therapeutic success. But poor water solubility of drug is a limiting step for the absorption of them. Increased bioavailability and prolonged plasma level are described for oral administration of NLCs. The NLCs can protect the drug from harsh environment of GIT. To resolve the insolubility concern of lipophilic drugs NLCs are prepared by entrapment of lipophilic drugs. Repaglinide, an anti-diabetic agent with poor water solubility, has low oral bioavailability and a short half life [38]. It is suitable to load into NLCs for improving oral delivery. A good thing is that toxicity has not been observed in most cases [39].

NLCs for topical drug delivery :

Indian company V.B. Medicare made an invention in which they describe a method to prepare an NLC-based nanogel formulation, which focused at increasing the local bioavailability of drugs on the skin and enhance the dermal delivery of drugs. Eg. The anticancer agent 5-fluorouracil, which showed an improve permeation in vitro (Franz cell assay) and after application on skin [40].

NLCs for brain targeting :

Brain targeting not only increases the cerebrospinal fluid concentration of the drug but also reduces the dosing frequency and side effect. The major advantage of this route is the avoidance of first pass metabolism and rapid onset of action as compare to oral route [41]. The first invention was the treatment of Alzheimer's disease and the second one is the age-related disorder. A recent NLC formulation for the treatment of Alzheimer's disease and the Parkinson's disease was formulated which consist of glyceryl disterate and glyceryl behenate as solid lipid and glyceryl triacetate as liquid lipid at room temperature [40].

NLCs for ocular drug delivery :

Recent reports indicate that ocular bioavailability of lipophilic drugs could be increased by NLC formation (eg. Ibuprofen). The other benefits include protection of labile compounds and modulation of release behavior [42]. Lutein-loaded NLCs could protect the lutein in the presence of simulated gastric fluid and slowly release lutein in simulated intestinal fluid in an in-vitro study [43].

Cosmetic applications of NLC:

NLC can be used to formulate active compound in cosmetics e.g. prolonged release of perfumes. When using NLC it is even more flexible to incorporation of cosmetic compounds and modulation of release [44, 45]. A recent discovered feature is the sunscreen blocking effect of lipid nanoparticles. The other beneficial aspects of NLC in cosmetics are very broad as enhancing skin bioavailability of active compounds, controlled occlusion, UV protection, penetration enhancement and increase of physical and chemical stability [46].

Conclusion

It has been found that nano based delivery system has a lot of potential to increase the bioavailability of poorly soluble lipophilic drugs and also target the site of action. The smart NLC as the new generation have much more flexibility in drug loading, modulation of drug release and improved performance in producing final dosage form such tablets, cream, capsule, injectables. Permeation via the GI tract and BBB may be a future trend. NLC should be continued to extend their application to develop the alternative routes and to treat the other disease. For all the information gathered from the recent literature it can be concluded that NLC is an excellent drug carrier system for the treatment of disease.

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