# A Comprehensive Investigation Of Nanostructured Lipid Carrier: A Promising Drug Delivery System For Future Clinics.

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**Abstract:** Lipid nanocarriers are being developed as substitutes for emulsions, liposomes, and polymeric nanoparticles. Furthermore, second-generation lipid carriers known as nanostructured lipid nanocarriers which are used in a variety of therapeutic approaches were created to address issues with solid lipid nanoparticles. Though their appropriateness for hydrophilic medicines is now well established, NLCs were originally primarily explored for the delivery of lipophilic medications. Lipids' development as a promising medication delivery system is due to their biocompatible characteristics. It was discovered to possess higher qualities in comparison to other lipid compositions. The NLC's architecture, preparation techniques, characterisation, and stability are all covered in this article. Because NLCs are compatible, non-immunogenic, and biologically non-toxic, they will be extensively studied lipid nanocarrier systems. Many NLC manufacturing processes are covered, along with ways to increase stability by turning NLCs into powder or utilising hydrophobic -ion pairing. In order to support the continued use of NLCs in the future, this study attempts to give an overview of the state of the art at the moment regarding NLCs as well as their contemporary methodologies and therapeutic uses. This research also serves as a review of current NLC-based patents. Lastly, the existing challenges and future progress are explained.

**Index Term:** Lipids, Nanostructured Lipid Carrier, HPH, Patents, etc.

1. **Introduction:** Drug-loaded nanocarriers have drawn a lot of attention as potential drug delivery systems during the past few decades as a variety of drug delivery technologies have arisen. Different kinds of nanostructures allowed for the targeted distribution of medications by shielding the encapsulated medications from physiological conditions they would face on their way to the intended locations. Additionally, drug release might be regulated with reduced variation in drug plasma levels due to nanoconstructs [1]. In order to distribute both hydrophilic and hydrophobic medications and enhance their pharmacokinetics and pharmacological characteristics, nanocarriers based on biocompatible polymers, lipids, and oils have gained prominence. The benefit of lipid nanocarriers made of synthetic or natural lipids is that they can regulate drug release while still being biocompatible and biodegradable [2].

Solid lipid nanoparticles (SLNs), the first generation of lipid-based nanoparticles, were first introduced as a viable alternative to conventional lipid carriers like emulsions, liposomes, and polymeric nanoparticles in the early 1990s. This was because of their low toxicity, ability to be produced on a large scale, and accessibility of their excipients. Furthermore, labile medications are shielded by the use of solid lipids (SL), and SLNs do not always require the presence of

non-aqueous solvents. However, the solid lipid matrix of SLNs causes a nearly perfect crystal lattice to develop, which leaves limited space for the incorporation of drugs. This reduces the loading capacity and increases the risk of drug leakage during storage. Researchers focused their attention on a created formulation known as Nanostructured Lipid Carriers (NLCs) in order to solve the previously noted drawbacks of lower drug encapsulation and storage instabilities of SLNs [3].

Muller et al. suggested creating NLCs, or second-generation lipid nanoparticles, by incorporating liquid lipid (LL) into the matrix. NLCs consist of a mixture of spatially distinct lipid molecules, meaning that a liquid lipid and a solid lipid are combined. Liquid lipid is added to increase drug loading capacity, decrease particle size, and lessen the chance of gelation and
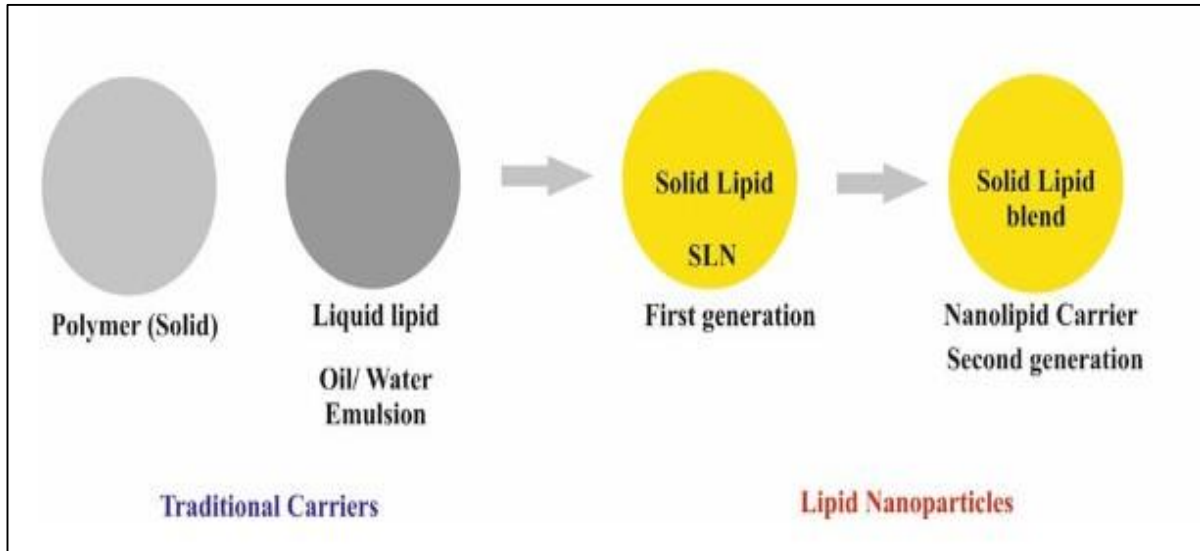


drug leakage during storage by causing a distortion in the production of ideal lipid crystals (Figure 1) [4].

**Figure 1: Nanostructured Lipid Carrier**

Nanotechnology and medicine share the same ultimate objectives precise and timely diagnosis, as well as efficient and side effect free treatment. Because of their greater surface area to volume ratio, nanocarriers exhibit enhanced therapeutic drug pharmacokinetics and biodistribution, which reduces toxicity by preferentially accumulating at the target location [5]. Lipid nanoformulations are used to create therapeutic dispersions that are relatively soluble. They may modify the production of solubilized phases from which drug absorption occurs readily and lessen the distinctive limits of sluggish and poor dissolving of fairly water-soluble medicines, such as Biopharmaceutics Classification System (BCS) class II.
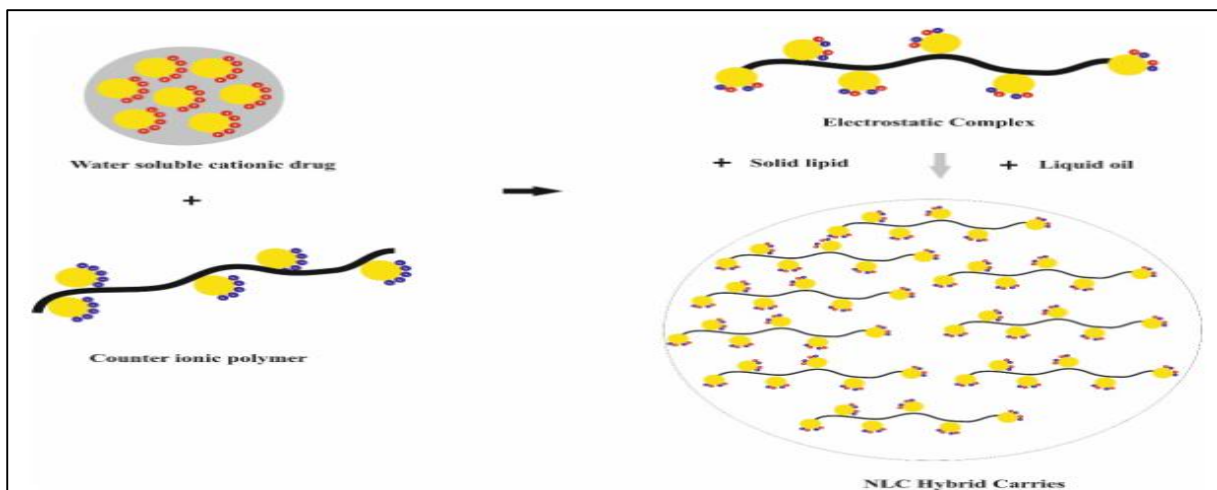


**Figure 2:Mixture of solid- lipid with liquid-lipid for nano lipid carrier hybrid.**

NLCs consist of a binary mixture of liquid lipid (oil) and solid lipid (solid) as a hybrid carrier, with an average size of 10-500 nm. As seen in Figures 2, the mixture of NLCs is composed of a long chain of liquid and lipid (oil) with a ratio of 99.9: 0.1 and a short chain of solid and lipid with a ratio of 70:30 [6].

2. **Lipid Nanocarrier:** Different types of lipid carriers can be distinguished based on their physicochemical properties and production technique. As seen in the following figure, these consist of liposomes, niosomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) as shown in Figure 3.

Lipid nanocarriers of the first generation are called SLNs. Depending on the drug's thermal stability, these are designed to synthesise drugs in solid lipids, ideally using a cold or hot homogenization process. As was previously said, NLCs provide better drug loading, faster release rates, and storage stability because they use a blend of liquid and solid lipid in their formulations, which helps them overcome the drawbacks of SLNs [7]. The different features of SLN and NLCs are listed in Table 1. The integrated medication may be released from the lipid matrix if a polymorphic transition to low energy modification occurs during storage. The likelihood of using SLN as a delivery system for several commercial goods is higher. Nevertheless, this technique has certain drawbacks as well:

1) The majority of medications have too little payload;
2) Medications expel during storage; and
3) The nanolipid dispersions have a high water content [8].
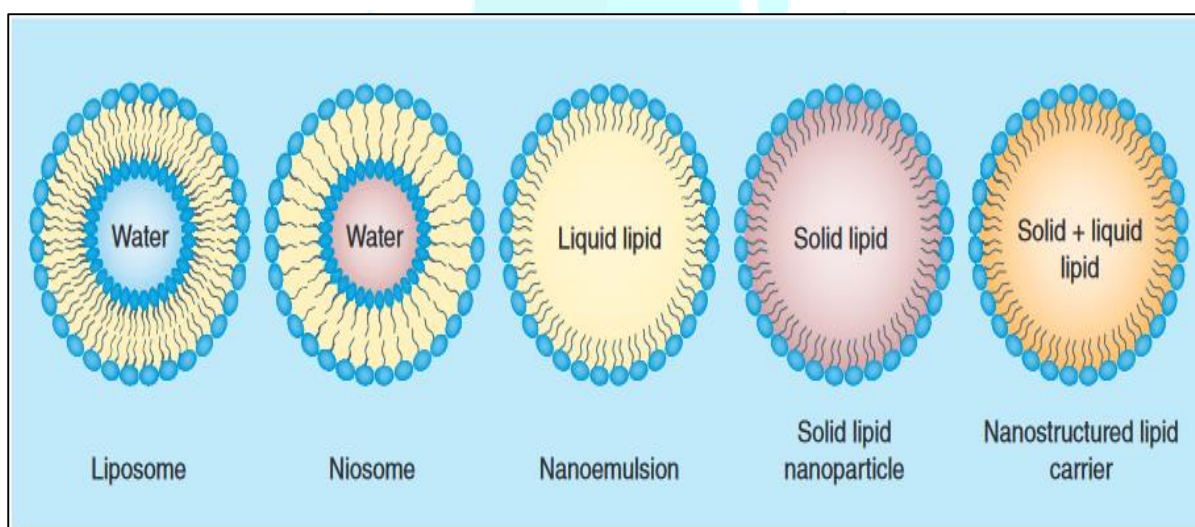


**Figure 3: Various lipid-based systems explored for drug delivery applications.**

Lipid blends that do not form a highly structured crystalline arrangement are required to prevent medication ejection during storage. In order to hold more drug molecules than SLN, the matrix of NLCs is made up of a mixture of spatially distinct lipid molecules, typically a mixture of liquid and solid lipid. This results in more imperfections in the matrix. At room temperature or body temperature, the NLC matrix is solid even though there is liquid lipid present. By adjusting the amount of liquid lipid present, NLCs can absorb combinations of solid and liquid lipids and stay in the solid form. Compared to emulsions, NLCs have a stronger ability to immobilise medicines and prevent the particle from aggregating due to their solid matrix. Like SLNs, NLCs are similarly characterised by low toxicity, biodegradation, drug protection, gradual release, and the avoidance of organic solvents during manufacturing [9]. A list of the different characteristics of SLNs and NLCs may be found in Table 1.

**Table 1: Comparison between properties of SLN and NLCs**

| Properties | SLNs (Developed since early 1990s) | NLCs (Developed since early 2000s) |
|---|---|---|
| 1)Drug encapsulation efficiency | Lesser drug encapsulation efficiency due to formation of highly ordered crystalline arrangement. | Higher drug encapsulation efficiency as blend of solid lipids and liquid lipids form disordered structure providing higher space for drug loading Also due to high solubility of drug in liquid lipids. |
| 2)Shelf life storage | Drug expulsion takes place during storage due to polymorphic transition of high-energy modification formed during fabrication to low-energy modification during storage. | No polymorphic transition takes place and drug expulsion is prevented. |
| 3)Release rate | Slower release as mobility of drug in crystalline form is less. | Faster release as mobility of drug in crystalline form is high. |

3. **Structural type of NLCs:** Although NLCs and SLNs have somewhat similar structures, NLCs have three extremely distinct traits. Varied kinds of NLCs are produced, depending on the lipid blend composition and the varied production methods. The fundamental idea is to give the lipid matrix a specific nanostructure in order to improve the payload for active compounds and decrease compound ejection during storage. The three categories of NLCs can be summed up as follows (Figure 4):

    a. The imperfect type

    b. The amorphous type

    c. The multiple types [10].
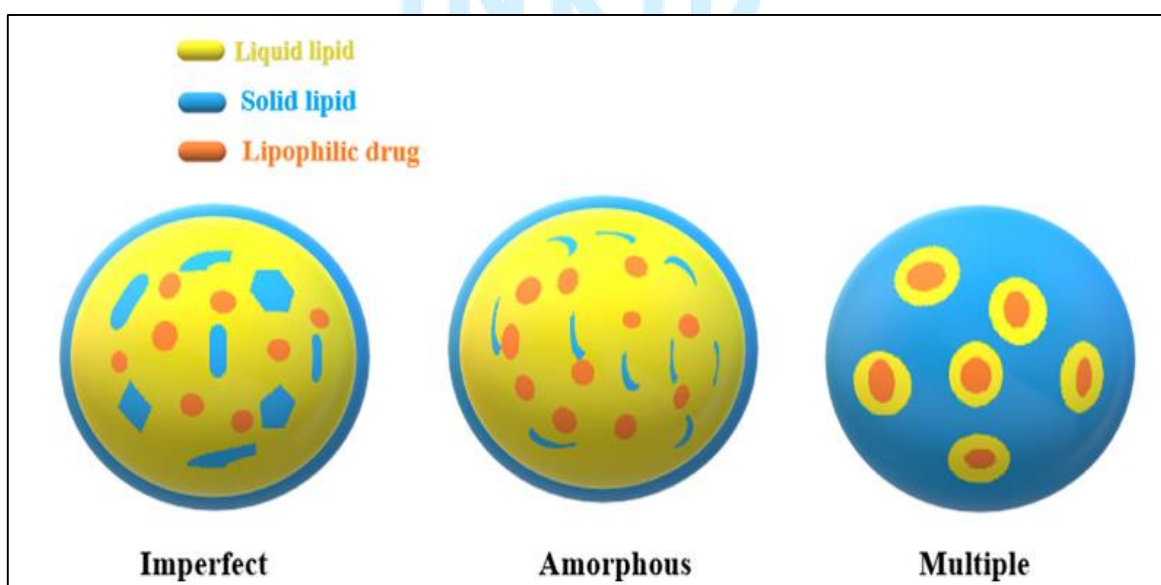


**Figure 4: Types of NLCs**

The specification of particular type of NLCs has beed described in Table 2.

**Table 2:Features of types of NLCs**

| Sr.No. | NLC type | Nature of matrix | Comments |
|---|---|---|---|
| 1 | Imperfect | Imperfectly structured solid matrix | Spatially different lipids are mixed creating imperfections in the crystal order of lipid nanoparticles; Show high-drug pay load. |
| 2 | Amorphous | Structure less solid amorphous matrix | Formed by mixing solid lipids with special lipids like hydroxyoctacosenyl hydroxystearate, isopropyl myristate or medium chain triglycerides such as miglyol 812 thus preventing drug repulsion, moderate drug payload. |
| 3 | Multiple | Multiple oil in fat in water | Solubility of drug in lipophilic phase decreases during the cooling process after homogenisation and crystallisation process during storage. |

4. **Drug encapsulation in NLCs:** Drug incorporation or encapsulation into lipid nanoparticles, or NLCs, can be done in three different ways. Drug-enriched shell, drug-enriched core, and homogenous matrix of solid solution are exactly what they are.

   a) The drug is uniformly distributed within the lipid matrix of the particles in the homogeneous matrix of solid solution method of encapsulation. The drug is released through the process of diffusion.

   b) The drug is concentrated on the outermost layer, or shell, of the lipid nanoparticles in the drug-enriched shell approach. Due to a precipitation and solubilization mechanism, these particular nanoparticles show burst release of the medication.

   c) Drug-enriched core: The drug's saturation solubility in the lipid is the reason behind this method's delayed release [11].

5. **Componant and Formulation attributes:** Similar to lipid nanoemulsions, which are primarily of the oil in water (O/W) type, lipids, surfactants, and water make up the main constituents of NLC. But at room temperature, a solid lipid matrix is created when a portion of the oil is swapped out for a solid lipid. The ideal ratio for blending solid lipids and oils is between 70:30 and 99.9:0.1. A larger percentage of oils can be employed in several emulsion NLC. Surfactant solutions ranging from 0.5% to 5% aid in stabilising the system.Lipid concentrations that are generally regarded as safe (GRAS) or unlikely to have a substantial harmful effect should be employed [12].Table 3 contains a list of common lipids, oils, and surfactants utilised in NLC formulation.

5.1.**Role of surfactant and lipid in formulation development:** The effectiveness and quality of lipid nanoparticles and nanolipid carriers are significantly influenced by the characteristics and concentrations of surfactant. Due to their amphiphilic character, these surface active agents are preferentially positioned at interfacial regions where they reduce the interfacial tension between lipid and aqueous phases. Low emulsification efficiency ionic surfactant sodium deoxycholate can be used to boost the charge of the nanoparticles, which is linked to an increase in electrostatic repulsion and an improvement in the colloidal system's physical stability. An further steric stabilisation effect provided by non-ionic emulsifiers, particularly Poloxamer 188, prevents the tiny particles in the colloidal system from aggregating [13].

**Table 3:Various componant of NLCs**

| Sr. No. | Componant of NLCs | Specification | Examples |
|---|---|---|---|
| 1) | Lipids | 1)Fatty acids | Dodecanoic acid, Myristic acid, Palmitic acid and Stearic acid. |
| | | 2)Monoglycerides | Glyceryl monostearate, and Glyceryl behenate. |
| | | 3)Diglycerides | Glyceryl palmitostearate and Glyceryl dibehenate. |
| | | 4)Triglycerides | Caprylate triglyceride, Caprate triglyceride, Glyceryl and tribehenate/Tribehenin. |
| | | 5)Waxes | Cetyl Palmitate, Carnauba, and wax Beeswax. |
| | | 6)Liquid lipids | Soya bean oil, Oleic acid, Medium chain triglycerides (MCT)/caprylic- and capric triglycerides, α-tocopherol/Vitamin E, Squalene Hydroxyoctacosanyl hydroxystearate and Isopropyl myristate. |
| | | 7)Cationic lipids | Cetyl pyridinium chloride (hexadecyl pyridinium chloride, CPC), Cetrimide(tetradecyl trimethyl ammonium bromide, CTAB. |
| 2) | Surfactants | 1)Ionic surfactants | Sodium taurodeoxycholate, Sodium oleate, Sodium dodecyl sulphure |
| | | 2)Non-ionic surfactants | Span 20, 80, 85, Tween 20, 80, Tyloxapol, Poloxamer 188 Poloxamer 407, Solutol HS15 |
| | | 3)Amphoteric surfactants | Egg phospholipid Soy, Hydrogenated soy phosphatidylcholine, Hydrogenated egg phosphatidylcholine. |
| | | 4)Co-surfactants | Butanol, Butyric acid |

**5.2. Surface modifiers :**
- Dipalmitoyl-phosphatidyl-ethanolamine conjugated withpolyethylene glycol 2000 (DPPE-PEG2000).
- Distearoyl-phosphatidyl-ethanolamine-N-poly (ethylene glycol)2000 (DSPE-PEG2000).
- Stearic acid-PEG 2000 (SA-PEG2000).
- α-methoxy-PEG 2000-carboxylic acid-α-lipoamino acids(mPEG2000-C-LAA18).α-methoxy-PEG 5000-carboxylic acid-α-lipoamino acids(mPEG5000-C-LAA18)
- Ionic polymers: Dextran sulphate sodium salt [6].

**5.3. Excipients for NLCs:** Solid lipids such as glyceryl behenate (Compritol 888 ATO), glyceryl palmitostearate (Precirol ATO 5), fatty acids, steroids, and waxes are frequently utilised in

NLC formulations. It is solid for these lipids at room temperature.During the preparation, they melt at higher temperatures (such as > 80ºC). Digestable oils derived from natural sources make up the liquid oils commonly used for NLCs [14]. Table 4 displays the excipients used in the creation of NLCs.

**Table 4:Excipients of NLCs**

| Ingredient Material | Material |
|---|---|
| Solid lipids | Gelot 64, Emulcire 61, Tristearin, stearic acid, Softisan 154, Cutina CP, Imwitor 900 P, Geleol, cetyl palmitate |
| Liquid lipids | Lauroglycol FCC, Capryol 90, Medium chain triglycerides, paraffin oil, 2-octyl dodecanol, Miglyol 812, Transcutol HP, Labrafil Lipofile WL 1349, Labrafac PG |
| Hydrophilic emulsifiers | Polyvinyl alcohol, Solutol HS15, polyglycerol methyl glucose distearate Pluronic F68 (poloxamer 188), Pluronic F127, Tween 20, Tween 40, Tween 80 |
| Lipophilic emulsifiers | Span 40, Span 60, Myverol 18-04K, Span 20 |
| Amphiphilic emulsifiers | Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire 50/13 |

6. **Fabrication methods of NLCs:** The preparation techniques of lipid nanoparticles, like SLN and NLC, are quite similar. The formulation's use of liquid lipids or lack thereof makes all the difference. NLCs can be made using a variety of techniques, depending on the energy input, including solvents and high- and low-energy (Figure.5) [15].

   6.1. **High-Energy Method:** To create NLCs, this approach employs hot and cold high-pressure homogenization strategies. The high-shear/high-speed homogenization method is another approach that fits into this group**.**

      6.1.1. **Hot High-Pressure Homogenization Method:** Here, the drug(s) and liquid lipids are added after the solid lipids are melted by heating them to a temperature that is typically 5 to 10 °C over the melting point. The resulting dispersion is then added to a hot surfactant solution in water. After the mixture is homogenised under high pressure (100–2000 bar), a hot oil in water primary emulsion forms. This emulsion cools and solidifies into the shape of NLCs. As lipid viscosity progressively decreases, higher temperatures aid in the reduction of particle size. The emulsion is often ultrasonically treated to achieve a narrow particle size distribution [15–16].

      6.1.2. **Cold High-Pressure Homogenization Method:** In the cold, high-pressure homogenization process, liquid or solid lipids and/or drug(s) are melted and then solidified using dry ice or liquid nitrogen. After milling and sorting the mixture into a cold surfactant solution, a presuspension is created. This is subsequently homogenised at high pressure (5–10 cycles, 1500 bar pressure), which produces NLCs. The use of few hazardous solvents, a simple scaling-up procedure, and speedy NLC formulation are some benefits of both of these approaches [15, 17].

**6.1.3. High Shear/High-Pressure Homogenization Method:** Lipids, both liquid and solid, are melted at a temperature 5–10 °C above their melting temperatures before being combined with the medication. A surfactant solution is added to this and heated simultaneously to an analogous temperature. After cooling and ultrasonication, this mixture is homogenised at a greater shear pressure to produce a low-particle-sized hot oil in water nano-emulsion that settles into a homogenous NLC formulation [15,18].

**6.1.4. Melt Emulsifcation Homogenization Method:** Using this technique, the medication is combined with liquid and solid lipids and mixed into an aqueous surfactant solution. The mixture is then exposed to probe sonication. To produce NLCs, the mixture is then cooled [15].

## 6.2. Low-Energy Method

**6.2.1. Microemulsion Method:** Using this technique, which is a less complicated way of creating NLCs, drug is added to melted lipid and combined with a mildly heated liquid lipid. In order to create a microemulsion, the melted lipid mix is then combined with the aqueous emulsifier solution and lipid blend while being continuously stirred. Within 20–50 times the volume of the microemulsion, the microemulsion is quickly dispersed in ice-cold water (2-4 °C), causing microemulsion globules to precipitate and form NLCs. Homogeneous preparation and the production of smaller particles are supported by ice-cold water without grouping. Lyophilization could be used to counteract dilution caused by high water volume. A large volume of emulsifiers and coemulsifers is needed for this straightforward procedure [15, 19].

**6.2.2. Double Emulsion Method:** The hydrophilic drug-containing aqueous phase is dissolved into the organic phase, which consists of melting solid and liquid lipids, creating the main water in oil emulsion. A w/o/w double emulsion is created when this primary emulsion is once more disseminated into the aqueous phase, enclosing the hydrophilic drug in the inner watery continuous phase [15,20].

**6.2.3. Membrane Contractor Method:** Using this technique, the melted lipid is forced through the membrane's pores at a pressure that raises the system's temperature above the lipids' melting temperatures. The aqueous surfactant, flowing immediately below the membrane, disperses the lipid globules emerging from the pores under turbulence. NLCs form after cooling to ambient temperature. Particle size is determined by a number of factors, including temperature, pressure in the lipid phase, membrane pore size that may be blocked, and the complex and instrument intensive nature of the process [15, 19].

**6.2.4. Coacervation Method:** This technique eliminates the need for hazardous solvents for developing thermosensitive lipids as NLCs. Here, lipidic blend in an acidic environment (coacervation solution) is combined with an amphiphilic emulsifier to create NLCs [15].
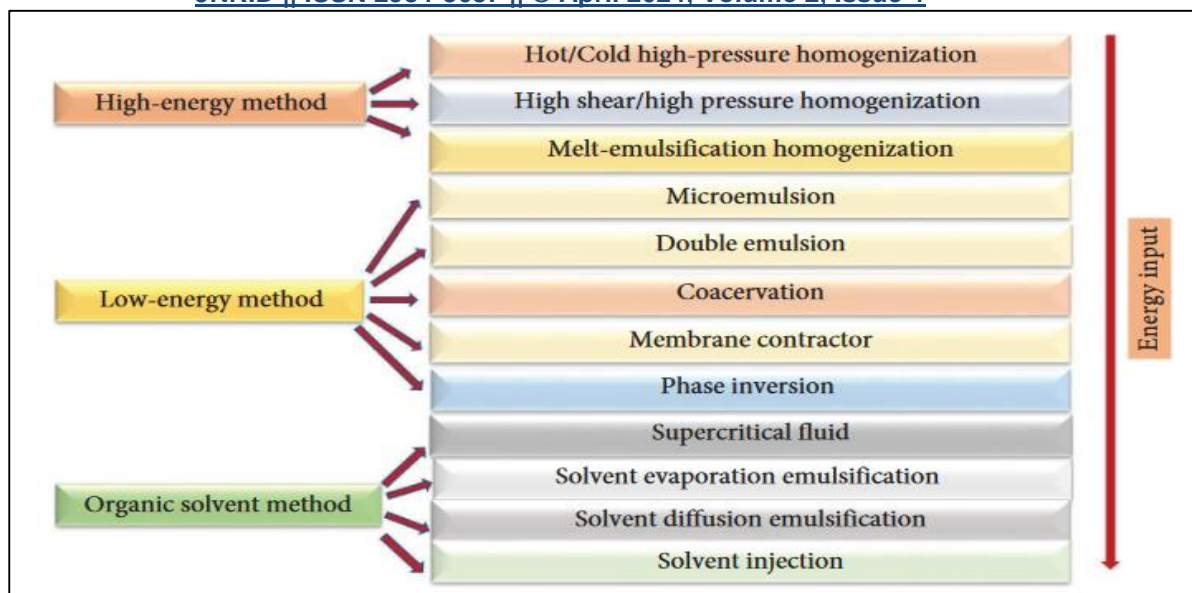
**Figure 5:Fabrication methods of NLC**

### 6.3.Organic Solvent Employed Method

**6.3.1. Solvent Evaporation Emulsifcation Method:** This process includes dissolving drugs and lipids in water-immiscible organic solvents, such as dichloromethane (DCM), cyclohexane, dimethyl sulfoxide (DMSO), and chloroform. After that, the mixture is agitated in an emulsifying aqueous phase and subjected to further sonication or homo-mogenization to produce a homogenous NLC formulation with a consistent particle size and distribution as shown in Figure.6 [2,15]. One clear drawback is the usage of organic solvents.

**6.3.2. Solvent Difusion Emulsifcation Method:** To dissolve the lipids and medication, water-mixable organic solvents such as methanol, ethanol, acetone, benzyl alcohol, and ethyl formate are used. To separate the mixture into a separate lipid phase, the mixture is sonicated at a high temperature. This lipid phase is then continuously stirred while being combined with an aqueous surfactant solution that is kept at a temperature comparable to the lipid phase. To obtain nanosized lipid carriers, the dispersion was agitated at room temperature to cool and evaporate the organic solvent [15, 22].
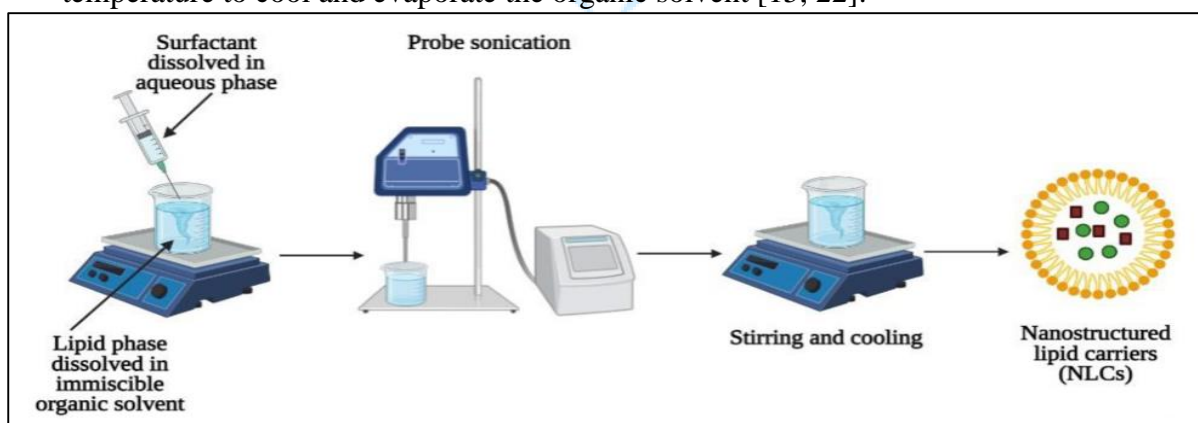


**Figure 6:Emulsification solvent evaporation technique**

**6.3.3. Solvent Injection Method:** When water-miscible organic solvents are used, the procedure is fairly similar to the solvent difusion method. The globules are injected out of the needle, whereas the lipid is injected into the aqueous surfactant solution. To facilitate the rapid solubilization of the lipid, a stirrer was used to maintain turbulence in the surfactant solution. The resulting emulsion is filtered to remove unnecessary fat. The size and size distribution are proportionally affected by the choice of surfactant and solvent concentrations. The formulator can employ basic approaches without the need for complex apparatus, which makes the method itself distinctive [15, 23].

**6.3.4. Supercritical Fluid Method:** The emulsifier is used to solubilize the medicine and lipids in an organic solvent, creating an organic solution. An oil-in-water emulsion is produced by high-pressure homogenization after this is distributed into the watery phase. In order to guaranteetotal solvent removal, lipid nanoparticles are prepared by infusing the o/w emulsion from the top of an extraction column and concurrently adding a supercritical fluid, usually carbon dioxide, at a constant flow rate [15,24].

## 6.4.Additional techniques in NLCs fabrication

**6.4.1. Hydrophobic ion pairing:** Drugs that are hydrophilic barely dissolve in the lipidic phase, which limits the effectiveness of their encapsulation into lipid-based nanoparticles (LBNs). But it can be added by making medications more lipophilic using methods like hydrophobic ion paring, and then it can be encapsulated in LBNs for increased solubility. It has been discovered that combining these ionised medications with additional hydrophobic compounds that have oppositely charged groups can enhance their incorporation into lipophilic bends (LBNs) (Figure.7). Because of the ionic interaction between counterions and charged drug groups, a "new" complex drug with a greater partition coefficient in the non-watery phase is formed. Thus, the complex medication may be loaded into the lipidic carrier with a longer retention period if its lipophilicity was increased.This leads to an enhancement in permeability across the membrane, which improves bioavailability generally. Anionic or cationic counterions can be found in the list of counterions paired with pharmaceuticals; they usually contain one or more charged groups. While quaternary amines and alkylamines are frequently used as cationic counterions, ionic surfactants, fatty acids (oleic acid, stearic acid, deoxycholic acid, and their salts), sulphates, phospholipids (dimyristoyl phosphatidyl glycerol), and anionic polymers (dextran sulphate) have been widely used as cationic counterions [25–26].

**6.4.2. Surface modification**
   a) **Surface charge modification:** It is significant to remember that one of the main elements affecting the colloidal dispersion's physical stability and cellular absorption capability is the surface charge of the NPs. Inevitably, nano-dispersion like NLCs is not an exception. Destabilised ingredients such as Ostwald ripening, coalescence, flocculation, coagulation, creaming, and sedimentation can lead to aggregation over the storage term. To maintain a high surface charge and guarantee the electrostatic repulsion between particles, charge agents could be used with NLCs. Additionally, a longer retention time on cells was made possible by the propensity of positively charged NPs to stick to the negatively charged cell membrane (Figure.7 ). A few often used charge modifiers are N, N-di-(beta stearoyl), dicetyl phosphate, cetylpyridinium chloride, and s stearyl amine [25].
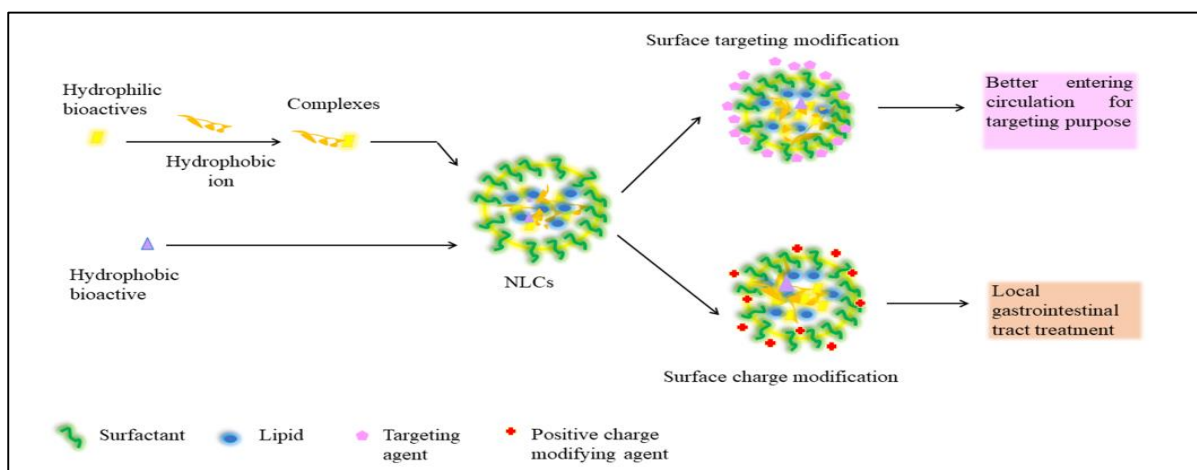


**Figure 7:Additional techniques for NLCs fabrication.**

b) **Surface modification for targeting delivery:** By controlling the abrupt burst release triggered by lipid-based nanostructures, NLCs can be further coated to further leverage their durability throughout gastrointestinal processing. Additionally, the surface modification of NLCs by polymers can be applied to target specific areas, improve mucoadhesion, and extend blood circulation (Figure.7). The combination of these two properties allows for the modification of polymer molecular weight and structure, which in turn allows for the customisation of drug delivery systems to achieve specific therapeutic goals. This fabrication process makes use of a variety of synthetic and natural polymers. Particularly, polymers that are frequently used include polyesters, co-polymers, polysaccharides, alginate, dextran, polyethylene glycol (PEG), and chitosan; their derivatives are also used for coating and stabilising materials when combined with NLCs [25].

7. **Transformation of NLC into powder form:** The incorporation of various colloidal structures such as micelles, mixed micelles, liposomes, and nano-emulsions in the aqueous dispersion of NLCs affects the stability of entire systems. Some transformations may occur within the system during storage to gain higher thermodynamic stability; however, this can result in several relevant stability issues such as increasing particle size, then leading to a wider size distribution range, caused by Oswald ripening or coalescence, gelation of dispersion caused by the lipid bridge between the particles, and drug expulsion out of the lipid matrix caused primarily by lipid recrystallization. The parameters PS, PDI, ZP, or DSC are commonly used to determine the stability of dispersion systems such as NLCs [25].

As previously documented, the long-term stability of NLCs is on the verge of aggregation, similar to that of SLNs. It can be linked to a variety of material qualities or preparation variables. In terms of lipid concentration, highly concentrated occupied lipid dispersion was shown to be more stable than low concentration, with no change in particle size following dilution. This was described as follows: free movement of particles in a dispersion with a low concentration of lipid drives aggregation via collisions and peri-kinetic flocculation, whereas lipid dispersion in a more dense occupation creates pearl chain-like structures, which minimise collisions [25,27].

Pre-solidifying temperature is another aspect that may be crucial to stability. After dispersion, the temperature must be lowered to solidify the solid lipid component, generating the microstructure of NLCs. The study of rambutan kernel NLCs discovered that pre-solidifying the dispersion at 5 C gave greater stability compared to pre-solidifying it at 25 C [28]. This might be at a lower temperature of 5 C; the higher the degree of supercooling, the faster the rate of nucleation and crystal formation. As a result, even if the temperature returns to normal temperature, only the surrounding crystals will disintegrate, while the nuclei inside will remain stable after 28 days [25,28].

8. **Physiochemical Characterisation of NLCs:** NLC physicochemical characterisation is critical for confirming quality control and stability. NLCs have physical and chemical properties that may be determined. Particle size and zeta potential are the most commonly used metrics for defining NLCs. Furthermore, differential-scanning calorimetry (DSC), X-ray, nuclear magnetic resonance (NMR), and Raman spectroscopy are used to characterise the lipid nanoparticles. The encapsulation efficiency and drug-release behaviour of the medication when it is incorporated into NLCs give essential information for judging the practicality of NLCs as drug delivery devices [29].

   8.1. **Particle Size:** The most powerful technologies for routine particle size assessment are photon correlation spectroscopy (PCS) and laser diffraction. PCS is often referred to as dynamic light scattering. It measures the variability in the intensity of scattered light caused by particle mobility. This approach spans a specific size range of a few nm to 3 m. Laser diffraction can detect the greater size. This conclusion is based on the diffraction angle's dependence on particle radius. The types and ratios of lipid and emulsifier utilised

in NLCs have a significant impact on particle size. More emulsifiers always promote more thorough emulsification and a more stiff structure, allowing the size to be reduced[29-30].

**8.2. Zeta Potential:** Surface charge measurements are used to evaluate the dispersion and aggregation processes that affect particle stability in applications. Because of electrostatic repulsion, particle aggregation or fusion is less likely to occur for charged particles. Because of binding to the paracellular portion of the BBB, which is rich in anionic sites, a positively charged surface of NLCs is effective for penetrating the blood brain barrier (BBB) [31]. The determination of zeta potential is useful for formulation design in order to check if the cationic surface is attained. A negative charge on the particle surface is sometimes required to stabilise nanoparticulate systems during storage [29].

**8.3. Electron Microscopy:** The particle radius and size distribution of NLCs can also be evaluated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Furthermore, electron microscopy is useful in studying the form and morphology of the particles. SEM uses electrons that are transmitted from the surface of the sample, whereas TEM uses electrons that are communicated through the specimen. The SEM has a high resolution and is simple to prepare samples for. TEM enables for the visualisation of nanoparticles following freeze-drying or freeze-thawing [29].

**8.4. Atomic Force Microscopy (AFM):** AFM is ideal for measuring extremely minute morphological and surface characteristics. AFM does not employ photons or electrons, but rather a very small sharp-tipped probe situated at the free end of a cantilever and powered by interatomic repulsive or attraction interactions between the tip and the specimen's surface [29]. Although electron microscopy is still widely employed, the AFM technique provides significant advantages, including real-time quantitative data gathering in three dimensions, short sample preparation periods, flexibility in operating conditions, and effective magnifications at the nano level [32].

**8.5. Surface Tension:** The surface tension of water at 20°C is 72.8 dynes/cm. The addition of lipids and emulsifiers can dramatically reduce surface tension to a lower value. Because of the emulsification process of the entire system, the surface tension falls as emulsifier concentration increases. The surface tension of lipid nanoparticles is frequently evaluated using the Wilhemy plate method. Another way for determining surface tension in nanoparticulate systems is to measure the contact angle [29,33].

**8.6. Differential Scanning Calorimetry (DSC):** DSC provides information about the melting and recrystallization behaviour of solid lipids derived from SLNs and NLCs. DSC is based on the notion that different lipid modifications have different melting points and enthalpies. The degree of crystallinity of NLCs is estimated by dividing the enthalpy of NLCs by the enthalpy of bulk lipids, which is computed on the basis of total weight collected. The degree of crystallinity of nanoparticles diminishes as the liquid lipid ratio in the particles increases. This finding indicates that liquid oil is the primary factor lowering crystallinity and enhancing the less ordered structure of NLCs.The NLCs with a smaller size, a higher surface area, and a greater number of emulsifiers experience a decrease in enthalpy and a fall in melting point of the lipids. The loading of liquid oil disrupts the crystal arrangement, resulting in additional space for medication molecules. DSC profiles are useful in predicting preferential drug dissolution in solid or liquid lipids [29,34].

**8.7. X-ray Diffraction:** Both DSC and X-ray diffraction are commonly employed to examine lipid status. Polyphorphism has been observed in lipid molecules with a lengthy hydrocarbon chain. Wide-angle X-ray diffraction can reveal the crystalline order of NLCs. To confirm DSC results, the polymorphism status of the nanoparticles identified by X-ray can be used. The length of the lipid lattice's long and short spacing can be measured using X-ray scattering [29,35].

**8.8. Parelectric Spectroscopy**: Parelectric spectroscopy is based on the frequency dependence of dipole density and mobility when subjected to an electromagnetic field shift. This method is used to identify the structure and dynamics of SLNs and NLCs. Parelectric

spectroscopy has been shown to be a versatile instrument since it provides insight into the experimental details and function of open ended coaxial probes used when measuring liquid dispersions and even when analysing living material for medical diagnostic purposes [29,36].

**8.9. Nuclear Magnetic Resonance (NMR) Spectroscopy:** Proton NMR spectroscopy is used to explore the mobility of materials in the inner core of NLCs. The mobility of solid and liquid lipids is proportional to the signal width at half amplitude. The properties of molecules with restricted mobility and strong interactions include broad signals and tiny amplitudes. The interaction of liquid oil with solid lipid is indicated by the greater line width of NLCs compared to the physical combination of the materials put in NLCs. The immobilisation of NLC nanoparticles is stronger than that of SLNs with completely crystallised cores [29,37].

**8.10. Raman Spectroscopy:** Raman spectroscopy detects vibrations in molecules after they have been excited by a powerful laser beam. Water produces only broad peaks at 3500 cm -1. The bands reflecting the order of lipid chains are of importance in the context of oil inclusion in a crystalline lattice. The symmetric stretching modes of the methylene groups at 2840 cm-1 and the sharp band of the asymmetric stretching mode at 2880 cm-1 are both signs of a high degree of conformational organisation of hydrocarbon chains in NLCs [29,38].

**8.11. Molecular environment:** The lipophilic fluorescent dye Nile red can be utilised as a fluorometric spectroscopic marker. The solvatochromism of Nile red elucidates the molecular environment or polarity of NLCs. Nile red is a lipophilic benzophenoxazone that fluoresces strongly in organic solvents and lipid environments. Nile red has a maximal emission wavelength near 600 nm, which corresponds to its high lipophilicity. Nile red emission spectra can shift to shorter wavelengths as ambient polarity decreases. When Nile red is moved into a more polar environment, such as an aqueous phase or a nanoparticulate shell, the emission maximum shifts to a longer wavelength and the fluorescence intensity decreases. In NLCs, Nile red is preferentially located in the fluid lipid phase [29,39].

**8.12. Drug Encapsulation Efficiency:** Determining drug-loading efficiency is critical for NLCs since it influences release characteristics.Lipophilic medication molecules may diffuse uniformly in the lipid matrix or enrich the core or particle shell. Hydrophilic medicines are often loaded in the aqueous and interfacial phases. A significant solubility of the medication in the lipids is required to achieve high loading capacity. Because solubility diminishes when the melt cools and may even be lower in solid lipids, it should be more than required. The percentage of medicines encapsulated in NLCs is determined by separating the interior and exterior phases. Different procedures, such as ultrafiltration, ultracentrifugation, Sephadex gel filtration, and dialysis, are routinely employed to separate the dispersions. In comparison to SLNs, the incorporation of liquid oil to solid lipid in NLCs causes significant crystal order disruption. The resulting matrix shows significant lattice imperfection and provides greater area for the medicines. As a result, drug entrapment efficiency and loading capacity are improved [12,29].

**8.13. Drug Release:** Controlled or sustained drug release from NLCs can result in a prolonged half-life and delayed enzymatic attack in systemic circulation. The drug release behaviour of NLCs is affected by the manufacturing temperature, emulsifier composition, and oil content in the lipid matrix. The amount of drug on the outer shell of the nanoparticles and on the particulate surface is released in a burst manner, whilst the amount of drug incorporated into the particulate core is released over time. Sustained drug release can be described by taking into account both drug partitioning between the lipid matrix and water, as well as the barrier function of the interfacial membrane.The dialysis method and the use of the Franz cell are the modes for assessing in vitro drug release from nanoparticles. The interpretation of in vitro drug release profiles should take into account the particular environment in the in vivo situation. The composition of lipid nanoparticles may influence enzyme degradation to some extent [12,29,40].

9. **Stability of nanostructured lipid carriers:** NLCs may contain additional colloidal structures that contribute to their stability, such as micelles, mixed micelles, liposomes, and nanoemulsions. There are also some significant storage stability concerns, such as particle size augmentation, dispersion gelation, and drug ejection from the lipid matrix. Gelation occurs as a result of the creation of a network and lipid bridges between the particles. Particle size (Photon correlation spectroscopy, PCS; Laser diffraction, LD), zeta potential (ZP), and thermal analysis (Differential scanning calorimetry, DSC) are commonly used to assess the physical stability of these dispersions. Several studies [8,41] found that SLN dispersion was physically stable for more than a year.

Long-term storage of lipid dispersions causes aggregation and shell development, as seen with SLNs. In the case of extremely concentrated NLC dispersions, the particles form a 'pearl-like network', resulting in collision and perikinetic flocculation. The network is disrupted after NLC injection and dilution with gastrointestinal fluid, releasing single, non-aggregated particles. Lipid particle dispersions with low lipid content (below 30%, outside patent coverage) and with 35% lipid were created at the same surfactant concentration. The low particle dispersion aggregated following storage, but the gel-like NLC dispersion remained stable and, after dilution, single particles with no size increase were produced [8]. Freely diffusible nanoparticles in low concentration dispersions can collide and aggregate (upper), however in highly concentrated dispersions the particles are trapped in a network, where subsequent dilution with water releases non-aggregated definite nanoparticles (as illustrated in Figure 9).
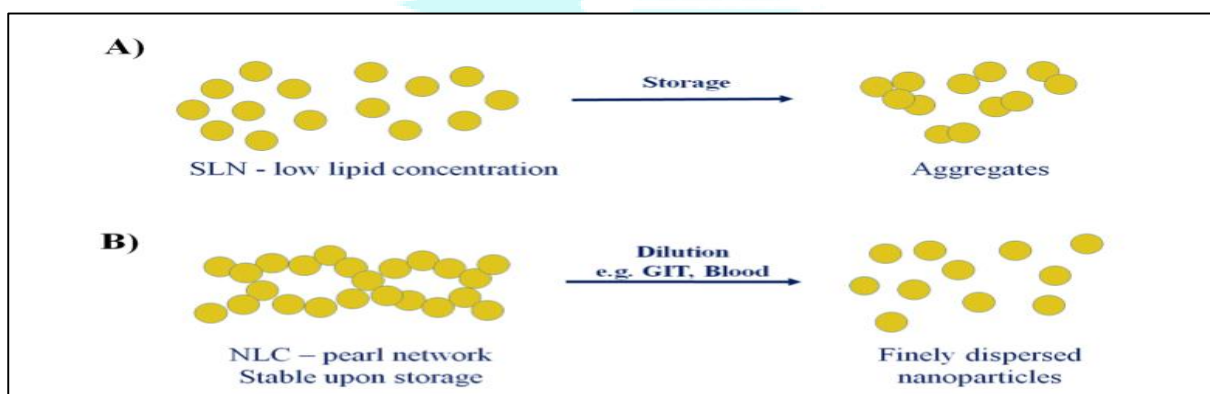


**Figure 9:Stabilization effect: A) in low lipid concentration dispersions such as SLN, B) pearl network formation inhighly concentrated NLC dispersions.**

### 9.1. Strategies employed for overcoming the issues related to stability of NLCs :

**9.1.1. Polyethylene glycol:** In general, surface modification of colloidal particles with a hydrophilic material such as polyethylene glycol (PEG) has been shown to provide the following benefits**:**

- Improving colloids' physical stability and dispersability,
- Increasing colloids' presence in blood circulation for systemic application,
- Increasing colloidal stability in body fluids such as GI fluids,
- Accelerating colloid transport across the epithelium,
- Modulating colloidal interaction with mucosa for specific delivery requirements and drug targeting,
- Increasing drug carrier biocompatibility and lowering thrombogenicity, and

**9.1.2. Spray drying:** In addition to optimal storage conditions, SLNs/NLCs dispersions can be spray dried to boost stability. However, for spray drying, the lipid matrix's melting point should be more than 70°C.

**9.1.3. Lyophilisation:** Lyophilisation is another effective method of increasing stability.When SLN is lyophilised without a cryoprotectant, the end product frequently results in particle aggregation. Trehalose, sorbitol, glucose, sucrose, mannose, and maltose are some of the most commonly utilised cryoprotectants.

Trehalose was identified by Schwarz and Mehnert as the most effective cryoprotectant in inhibiting particle development [8].

10. **Drug release:** The release of drugs from a matrix is determined by the rate of degradation and diffusion in the case of NLCs. It is widely recognised in the literature that precise and regulated release is required beyond diffusion and degradation. When a particle is administered the release, an impulse should be used to trigger the particle[6,42].

Because of their disordered and unorganised lipid structure, the drugs will have to be confined in NLCs. The structure of the lipid can be modified using various methods and procedures, which leads to the conversion of the structure of the lipid molecule and hence the initiation of continuing drug release, as shown in Figure.  It was discovered that this procedure is critical when NLCs are included into cream for use on the skin as well as for the treatment of various dermatological diseases such as psoriasis and ezema. These types of NLCs are beneficial and have extremely advantageous features when used by rubbing; this method enhances the temperature and water evaporation from the formulation; cyclosporine-lipid particles are being developed to treat psoriasis based on this method [6,43].
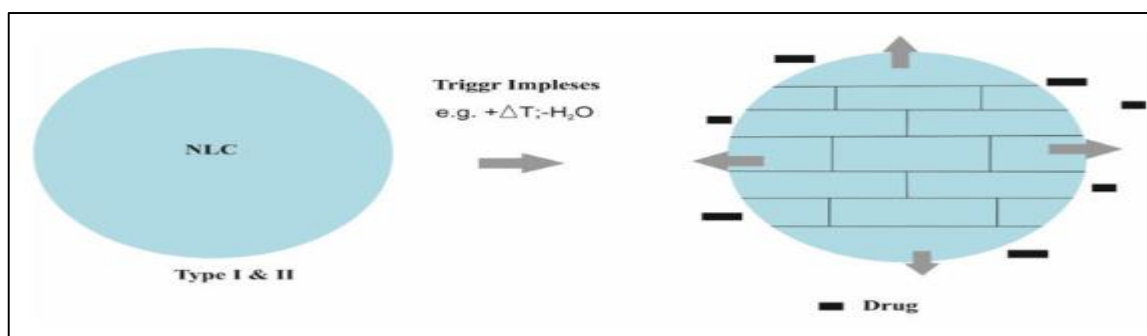


**Figure 10: The drug release from NLC by initiating the alteration from a extremely disordered lipid structure to more ordered stable modifications.**

11. **Recent Applications of Nanostructured Lipid Carriers**: Several NLC formulations are now marketed for medicinal and cosmetic uses, most likely due to their well-established biocompatibility due to the usage of lipids. The development and characterisation of NLCs to investigate treatments for various diseases and disorders is a continuous process. Teixeira et al. summarised the therapeutic applications of lipid nanoparticles in the treatment of several illnesses. The report summarises that lipid nanoparticles are ideal for the integration of medications from BSC classes II and IV that would otherwise cause problems with bioavailability due to their physicochemical features. In addition, patents involving the formulation of BCS class II and IV medicines as lipid nanoparticles are summarised [15,44]. Some of the recent investigations are discussed here in Table .5 considering various routes of administration and physiological complications.

   11.1. **Therapeutic applications**

   11.1.1. **Oral drug delivery:** The oral route of delivery of drugs is the most convenient route of drug administration. The bioavailability (BA) of poorly water soluble drugs is reduced by GI barriers and biochemical processes such as efflux by enterocyte transporters, hydrophilic environment, gut wall metabolism by enzymes and resident microflora, and hepatic first pass metabolism, which can be overcome by formulating drugs in NLCs [15,45].

   11.1.2. **Transdermal drugs delivery:** Some orally delivered medicines degrade owing to stomach acid, first pass hepatic metabolism, and intestinal metabolism. These factors are to blame for medication loss and decreased bioavailability, which necessitates frequent administration of dose form and subject to a variety of side effects. Although transdermal drug administration is an appealing alternative, it does have some drawbacks. Transdermal medication distribution is influenced by the physicochemical features of the drug molecules. To diffuse through subcutaneous tissue, the drug must have an optimal partition coefficient, as well

as a low molecular weight and melting point.Drug delivery by chemical mediators such as permeation enhancers and physical means such as iontophoresis and electroporation can have a deleterious impact on skin tissue integrity and function. Lipid nanocarriers outperform traditional transdermal drug delivery methods. Lipids are non-irritant, biocompatible, and can be formulated in nano size ranges. The application of NLC formulation to the stratum corneum ensures tight contact between the lipid structures of NLC and the skin, resulting in excellent drug penetration. It also moisturises the skin by producing an occlusive layer and decreasing transepithelial water loss [15,46].

**11.1.3. Ocular drug delivery:** Topical drug delivery to the anterior part of the eyes is challenging due to precorneal drug loss, nasolachrimal drainage, conjunctival drug absorption, and the principal barrier, the cornea, which hinders the passage of most medications. The presence of tight blood-ocular barriers prevents medication access into the posterior ocular segment.For posterior segment medication delivery, intravitreal and subconjuctival injections are used, however these might cause tissue damage and decrease patient compliance. Lipid nanoparticles were discovered to improve medication transcorneal permeability, resulting in effective ocular drug delivery [15,47].

Polymeric systems are the most commonly employed carriers, however because of the possibility of local toxicity and foreign body interactions, NLCs were created with biocompatible and non-toxic lipids in mind [15,48]. Lipid nanoparticles have previously been investigated for the ocular delivery of a wide range of therapeutic compounds, including anti-inflammatory medications (corticosteroids and nonsteroidal anti-inflammatory drugs), antibacterials, antifungals, antivirals, anti-glaucoma agents, and gene delivery. The use of positively charged lipids and coating with positively charged polymer is advantageous in ocular medication administration because it allows carriers to be retained on the precorneal surface due to binding with negatively charged mucin. Recently, the NLCs are surface modified or coated with the positively charged molecules and mucoadhesive components to improve corneal residence of carriers which possibly leads to enhanced transcorneal permeation [15]**.**

**11.1.4. Drug delivery to central nervous system:** Drug distribution to the central nervous system is a difficult undertaking due to the presence of the Blood Brain Barrier (BBB), which hinders drug passage due to tight endothelial junctions of capillaries. Only highly lipophilic medications can get through the barrier, and important nutrients are transported by an active transport system. The mechanism of BBB disruption for medication administration is intrusive and unpleasant. Non-invasive techniques such as intranasal (IN) drug administration, nanoparticles, prodrugs, and the use of chimeric peptides (vector mediated) can all be used to replace it.Polymeric nanoparticles and liposomes have demonstrated increased medication biodistribution and bioavailability. Surface modification of nanocarriers has been found to improve carrier uptake across the BBB. NLC have shown inhibition of p-gp efflux pump thereby achieved higher drug concentration in brain with potential of circulating for longer period of time [15,49].

**11.1.5. Intranasal route:** Oral and intravenous medication administration for CNS disorders necessitates repetitive dosage, resulting in increased and prolonged drug exposure to systemic circulation, causing organ toxicities and related side effects. To avoid these complications, olfactory and trigeminal nerve pathways are used for direct drug administration to the brain via intranasal routes [15,50].

**11.1.6. Intravenous route:** When treating CNS illnesses, oral medication delivery causes physiological difficulties. As a result, parenteral NLC therapy has been devised to overcome side effects and organ damage while targeting the brain specifically [15].

**11.1.7. Pulmonary drug delivery:** Drugs are given in the lungs in appropriate formulations to treat local ailments, and therefore for systemic drug delivery due to the increased surface area for drug absorption. For deep lung distribution in the alveolar region, the mean aerodynamic diameter of particles must be between 1 and 3 m for systemic medication delivery via pulmonary route. If local delivery is anticipated, particle characteristics must be tweaked or adjusted to ensure greater drug disposal in the infected/targeted area. Aerosolized lipid nanocarriers are used in pulmonary applications. These have been shown to be successful due to their lower toxicological profiles, biodegradability and biocompatibility, and ability to release medication over extended periods of time [15,51].

**11.1.8. Chemotherapy:** Recent research has revealed that NLCs not only improve the efficacy and stability of several cytotoxic medicines, but also lessen their negative effects. For example, albumin-paclitaxel nanoparticles were approved in early 2005 in the chemotherapy for metastatic breast cancer; etoposide NLCs were discovered to be cytotoxic against human epithelial-like lung carcinoma cells; and stabilisation and prolonged release of topotecan NLCs in the treatment of refractory ovarian and small-cell lung cancer. The benefits of including anti-cancer medications in NLCs include higher drug loading efficiency, a longer release profile, increased chemical stability, and increased cytotoxicity. Because these NLCs avoid some of the possible issues associated with SLN, such as drug loss during storage and limited loading capacity, they are a better alternative. They work by extending the time that tumour cells are exposed to anti-tumor drugs and increasing permeability and retention to boost the therapeutic effect [8,52].

## 11.2. Miscellaneous

**11.2.1. Cosmetics and cosmeceuticals:** Because lipids are responsible for skin hydration and moisturization, a lipid nanocarrier system is a viable option for cosmetics. The incorporation and concentration of propylene glycol and lecithin on skin hydration, surface occlusion, and transepithelial water loss were studied and found to have favourable effects. The researchers created argan oil-based NLCs and put them into hydrogel for dermal treatment, which enhanced skin moisture. Argan oil NLCs can be combined with other APIs to provide synergistic effects in transdermal applications [15].

**11.2.2. Food technology:** The food and cosmetics industries are gradually replacing synthetic excipients with natural ones. The antimicrobial ingredient menthol, which has low stability and insolubility, was synthesised as NLC, which demonstrated increased antibacterial action against gramme positive bacteria [103]. Lycopene, an antioxidant, was added for food fortification in the form of NLCs to improve its solubility and stability [15].

**Table 5:Application of NLCs**

| Drug | Lipid Componant | Fabrication Method | Particle Size | Research Highlights | Ref |
|---|---|---|---|---|---|
| Vinpocetine | Monostearin (I) Miglyol 812 Lecithin | HPH | 107-132 | Relative bioavailability of NLC formulation was 32% compared with suspension | [53] |
| Deoxyarbutin | Cetyl palmitate MCT Poloxamer 188 and Polyethylene glycol 400 | High shear homogenisation and ultrasonication | 500 | NLC showed highest permeation into skin as compared to nanoemulsion and conventional emulsion. | [54] |
| Lercanidipine Hydrochloride | Labrafil 2130M Linseed Oil Tween 80 and poloxamer 188 | Solvent evaporation | ~214.4 | NLCs released drug in a controlled manner for a prolonged period of time as compared to plain drug. | [55] |
| Celecoxib | Compritol Miglyol 812 Sodium taurocholate | HPH | 217 ± 20 | Controlled release of drug 888 ATO Cytotoxicity of NLC formulation due to prolonged release and cell internalization of nanoparticle | [56] |
| Ibuprofen | Compritol 888 ATO, Gelucire 44/14, Miglyol 812, Stearylamine, Transcutol P, Cremphor EL | Hot emulsion - ultrasonication | | Apparent permeability coefficients were 1.28 and 1.36 times more than that of the control preparation. Prolonged precorneal retention time with steraylamine Controlled-release property | [57] |
| Doxorubicin | Precirol ATO 5, Squalene Soybean phosphatidylcholine | High shear homogenization and ultrasonication | 110 ± 20 | Higher bioavailabilty and lower clearance Significantly decreased exposure of healthy tissues to cytotoxic effects enhanced anti tumor activity | [58] |

12. **Recent Patents on Nanostructured Lipid Carriers :** Minimal regulatory obstacles and the use of non-toxic, biodegradable, and biocompatible excipients like as lipids and emulsifiers are major factors for NLC's rising popularity and global success. All of the components employed are either generally regarded as safe by regulatory agencies or have been approved for the encapsulation of active substances in pharmaceutical and food applications. In either scenario, using all materials within an acceptable and allowed range is critical. Most are derived from natural sources, such as fatty acids and glycerol. These are well tolerated and have been shown

to reduce cytotoxic or adverse drugresponses. Lipid nanostructure carriers have been used to examine numerous medicinal substances throughout the last few years[59]. Table 6 gives a summary of patents in Nanostructured Lipid Carriers.

### Table 6:Patents on NLCs

| Patent Number | Patent Name | Applicant | Publication Date | [Ref] |
|---|---|---|---|---|
| US2022005441 6A | Nanostructured lipid carriers and stable emulsions and uses thereof | Infectious Disease Research Institute, Seattle, WA (US) | 24.02.2022 | [60] |
| EP3638207 | Nanostructured lipid carriers and stable emulsions and uses thereof | Infectious Disease Res Inst | 22.04.2020 | [61] |
| IN20181102121 3 | Novel nanostructured lipid carrier- based ophthalmic controlled release formulation for treatment in fungal keratitis | Manish Kumar Ajay Pathania Vipin Saini A. Pandurangan Shailendra Bhatt Prerna Sarup | 13.12.2019 | [62] |
| MYPI 2018300001 | A Nanostructured solid lipid carrier encapsulates bromelain extract | Universiti Teknologi Malaysia | 22.07.2019 | [62] |
| CN110013471 | Nanostructured lipid carrier (NLC) for collaborative treatment of glioma as well as preparation method and application of NLC | Stomatology/Affil iated Stomatology Hospital of Guangzhou Medical University | 16.07.2019 | [63] |
| CN108904450 | Polymer thermosensitive liposome loaded with yeast glucan and carnosic acid | Guangzhou Jiayuan Pharmaceutical Technology Co., Ltd. | 30.11.2018 | [62] |

13. **Safety/toxicity concern:** The biocompatibility of SLNs and NLCs is critical in every formulation development study. The encapsulation of the medications in SLN and NLC, as well as their targeting of specific cells, are predicted to improve selectivity and hence reduce toxicity issues.This is accomplished by either improving nanoparticle-cell interaction (e.g., targeting moieties and a positive charge surface) or by bypassing drug efflux transporters (e.g., p-glycoprotein). However, the lipidic content of both SLN and NLC should not affect cell viability, however surfactants employed in formulations may be harmful. The half maximal inhibitory concentration value is the most efficient technique to compare cell viability. IC50, on the other hand, is not tested in every toxicity research. In the literature, cell sensitivity to different lipidicformulations is usually expressed as cell viability and it shows that cells rarely toleratehigher concentrations of lipid carriers. Interestingly, few studies suggest that cancer celllines may be more sensitive to SLNs and NLCs than non-cancer cell lines; however, morestudies are needed to conclude [64].

14. **Conclusion:** Nanoparticulate delivery technologies have been widely used in the biomedical industry throughout the previous decade. Among the different types of nanocarriers, NLC have shown considerable promise in the efficient delivery of medicines via pulmonary, topical, intranasal, ophthalmic, and oral routes. Because of their tiny size and ability to cross the BBB even without surface functionalization, they are an attractive choice for therapeutic administration across the brain. Furthermore, the excipients utilised in the manufacture of NLC are biocompatible, biodegradable, non-irritating, and the majority of them have GRAS approval. They are simple to scale up and can be modified to achieve the desired particle size and release profile, as well as increased drug loading and therapeutic stability. Despite the introduction of a number of cosmetic dermal NLC products to the market, no NLC formulations for therapeutic or diagnostic use have achieved regulatory approval or even reached the clinical study stage. NLC have been actively researched at the academic level in recent years for their potential as delivery methods targeting multiple organs. However, the toxicity of nanoparticle buildup in non-target organs/tissues such as the liver, heart, spleen, and lungs must be considered. As a result, in order to properly explore the potential of NLC as a delivery method, it must be thoroughly researched at both the preclinical and clinical levels.

15. **Approaches to clinical trials:** Although NLCs have significant potential as drug delivery vehicles, preclinical and clinical research is currently lacking. As a result, there is a need to broaden the scope of their applications to encompass clinical trials in accordance with applicable ethical laws. This could be related to a lack of critical analysis of NLCs' safety profile as medication transporters. However, cutaneous and oral applications were the most common. Finally, the potential properties of NLCs might be pursued further with additional research on their absorption, distribution, metabolism, and excretion. Methods for scaling up production and applying them in clinical trials in the near future should also be clinically studied.

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