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*Research article*

# Impact of Parameter on Nanostructured Lipid Carrier Formulation and Approach of the Carrier for Cancer Treatment: a Brief Study

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**Abstract**

**Introduction.** For the last decade, nanotechnology has been studied extensively in the pharmaceutical field. Among all the nanotechnology formulation areas, nanostructured lipid carrier is enormously researched by formulation scientists as it is one of the focused areas of lipid carrier for the effective formulation.

**Materials and methods.** The nanostructured lipid carrier (NLC) consists of solid lipid, liquid lipid & surfactant for fabrication of formulation. Methods such as high energy methods, low energy methods and organic solvent-based methods are used for the preparation of NLC. As per literature study the High pressure homogenization is the most efficient method for fabrication of formulation.

**Results and discussion.** This carrier system has significant advantages such as high drug entrapment, improved bioavailability, stability during storage, and targeting the site with a better-controlled release making it a prominent area for the formulator to emphasize on it. Although many drugs are formulated with a nanostructured lipid carrier, it is a concern for researchers to find out the effectiveness of formulation by studying the process parameter and safety.

**Conclusion.** The present review was focused to study the impact of various parameters such as Lipid, surfactant, homogenization rate, preservative, Crystallinity, and surface charge on the formulation. The study also extended towards toxicity and biocompatibility, topical targeting & cancer treatment of the Nanostructured lipid carrier.

**Keywords:** nanostructured lipid carrier, toxicity & biocompatibility, surface charge, stability, topical targeting, cancer, brain tumor target

**Conflict of interest.** The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

**Contribution of the authors.** All authors participated in the discussion of the results and writing the text of the article.

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## ABBREVIATION

| | |
|---|---|
| NLC | Nanostructured lipid carrier |
| SL | Solid lipid |
| LL | Liquid lipid |
| BBB | Blood-Brain barrier |
| EE | Entrapment efficiency |
| PDI | Polydispersity Index |
| OA | Oleic acid |
| GMS | Glyceryl Monostearate |
| BHT | Butylated Hydroxytoluene |
| BHA | Butylated Hydroxyanisole |

## INTRODUCTION

Nanostructured Lipid Carriers (NLC) is a drug delivery system consisting of a mixture of solid along with liquid lipids, to form a lipid core matrix that is stabilized by using surfactants of particle size in the range of 10–1000 nm. [1, 2–92]. The use of liquid lipids in the matrix of solid lipids can make high imperfections to the crystal lattice and ultimately improve the capacity of drug loading and decrease drug expulsion while storing it [3]. The primary principle involved in the selection of lipid for the manufacture of Nanostructured lipid carriers is drug solubility study in different types of lipids, drug partitioning behavior in solid as well as liquid lipid & study of the compatibility of different lipid mixtures [4]. The rationale behind the NLC is oil (liquid lipid) should be integrated into the core of solid lipid which improves drug loading & imparts controlled release behavior as the drug dissolves in the oil and leads to encapsulating the drug in the solid core [87]. The poor bioavailability and low solubility of many drugs are challenging for formulators. Even though several carriers gradually developed nanostructured lipid carrier is one step ahead

among all carriers because of their numerous advantages in drug delivery. The lipid used in NLC formulation is biocompatible nature biodegradable with minimal toxicity [88]. The NLC system is composed of solid lipid & liquid lipid that make the carrier quite imperfect & hold more amounts of the drug. The characteristics such as low toxicity, controlled release, biocompatible, drug safety, high drug loading, and removal of organic solvent during production make the NLC as most acceptable lipid carrier and continuously gaining significance in drug delivery [89]

### *Structure of NLC*

The structure of NLC is categorized into 3 groups which are based on the drug molecule position to be found (figure 1, table 1) [5, 36, 37].

- NLC type I (imperfect crystal type).
- NLC type II (multiple types).
- NLC type III (amorphous type.

### *Component of NLC*

The different types of solid lipid, liquid lipid, and surfactant are used in formulation by studying their physical, and chemical properties and HLB value (table 2) [6, 7, 38, 42–45].

**Table 1.** **Type of NLC with characteristics [84]**

| NLC type | Name | Characteristics |
|---|---|---|
| I | Imperfect | The imperfect crystal structure terms as type I NLC. The solid matrix is arranged very bad way. The imperfection produced in NLC is due to the presence of various lipids in the formulation As the solid matrix is badly structured. The imperfection with numerous voids leads to more drug loading of a formulation. |
| II | Multiple | Type-II is called a multitype of NLC. The oil concentration is more in this type. The two lipid phases are separated during the process of crystallization. At a particular temperature, the miscibility problem can lead to the precipitation of small oily nanocompartment. When the drug solubility is less in lipid the more amount of liquid lipid addition is more advantageous to the solid matrix which prevents drug expulsion but liquid lipid exhibit high solubility |
| III | Amorphous | The type-III of NLC is amorphous. In this type, the NLC can be prepared by mixing lipids in a manner to avoid crystallization. Here the lipid matrix remains in an amorphous state. In this method chance of drug, expulsion is due to crystallization, which can be avoided by mixing solid lipids with other special type lipids such as isopropyl palmitate. |

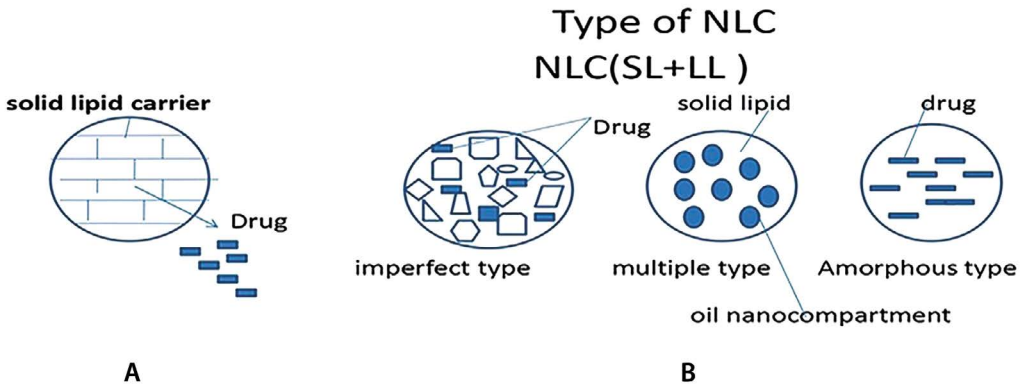


**Figure 1.** **Structural observation of different types of NLC [85]**

**Part (A) indicates drug expulsion easily from solid lipid carrier. Part (B) indicates it composed of solid lipid and liquid lipid and drug remain inside of carrier.**

**Abbreviations: SL – solid lipid; LL – Liquid lipid**

**Table 2.** **List of lipids & surfactants with their HLB value:**

| Solid lipid | HLB value | Liquid lipid | HLB value | Surfactant | HLB value |
|---|---|---|---|---|---|
| Monostearin | 3 | Oleic acid | 1 | Poloxamer 188, | 29 |
| Compritol-888 ATO | 5 | Linoleic acid | 8 | Sodium deoxycholate | 16–17 |
| Softisan145 | 10 | Migloyl 812 | 15.3 | Tween 80 | 15 |
| Precirol ATO 5 | 2 | Labrafac CC | 1 | Transcutol®P, | 4.2 |
| Dynasan114 | 3 | Soyabean oil | 7 | Span 40 | 6.7 |
| Imwitor 900 K | 3 | Labrafil M 1944CS | 4 | Gelucire 5./13 | 2 |
| Stearic acid | 15 | Isopropyl palmitate | 1.6 | Cremophor EL | 13 |
| Cetyl palmitate | 10 | Capryol 90 | 6 | Myverol 18-04 K | 3.8 |
| Gelucire® 44/14 | 11 | Capmul MCM | 5–6 | Brij® 78 | 15.31 |
| Gelucire® 43/01 | 1 | Isopropyl myristate | 2.8 | Kolliphor HS15 | 14–16 |

## MATERIALS AND METHODS

The NLC formulation is composed of solid lipid (SL), aqueous soluble surfactant, and liquid lipid (LL) as major components and used in proper ratio. The other excipients used are preservatives, antioxidants, and cryoprotectants to improve the stability of NLC. Usually the solid to liquid lipid ratio from 70:30 to 99.9:0.1 and surfactant from 0.5 to 5 % are used in the formulation [90]. The amorphous form of the solid matrix of NLC formed due to the presence of SL and LL. Fundamentally the presence of LL in NLC makes it differ from SLN (solid lipid nanocarrier). The contributory role of LL improves drug loading in NLC. Cryoprotectants are required when the lyophilization of NLC formulation is required and the agents include dextrose, sorbitol, PEG 4000, etc. [91]. To prevent oxidation of unsaturated fatty acid and chemical degradation of drug, antioxidants such as alpha-tocopherol, BHT, BHA are commonly used. The study also reveals that use of 5–10 % propylene glycol as a preservative; there is no significant change in zeta potential (ZP) and particle size compared with formulation without preservatives [92].

The various methods used to prepare NLC formulation are high-pressure homogenization, ultrasonication, microemulsion, solvent injection method, melts emulsification and solidification method, solvent emulsification/evaporation method, supercritical fluid method.

### *NLC Fabrication*

Two methods: a) high energy method; b) low energy methods and c) organic solvent-based method adopted to fabricate the NLC formulation. The first method conceptualized the high shear force produced from the equipment used in formulation preparation, pressure distortion, and mechanism for reducing particle size. However the second method i. e. low energy method used to reduce particle size does not depend on any specific amount of energy. Because of the easy scale-up & less time required for production HPH (high-pressure homogenization) method is more preferred compared to others [7]. The methods are presented in figure 2, table 3.

## RESULTS AND DISCUSSION

### *Concern for effective formulation of nlc:*

### *Size of particle & polydispersit*

The size of a particle and its distribution affect the characteristics such as solubility, biological performance, stability, and drug release of NLC. Generally, the particle diameter ranges from 10–1000 nm of NLC. Many formulation factors that affect the particle size are a type of lipid and its concentration, type of surfactant & its concentration, homogenization speed & cycle, process temperature, pressure, and lyophilization [8].

*Case study 1*. Jabber Emami et al, conducted a research study and found that oleic acid (OA) a solid lipid, and polaxomer (1 % as surfactant) have a contribution to the size of the particle. The presence of a higher concentration of OA (30 %) decreases viscosity inside the NLC as well as surface tension that leads to smaller particle size. The study was conducted for the formulation of NLC containing paclitaxel as a drug [9]. From the study polydispersity Index was found to decrease with increased OA content..It indicates that OA content influences nanoparticles with uniform particle distribution due to less size of the particle. A similar study was conducted by Hu et al using clobetasol as a drug, stearic acid, and various concentrations of OA used up to 30 % and found a decrease in particle size [10]. The above case study reveals that a higher concentration of oleic acid (as solid lipid) produce smaller particle due to lower surface tension & viscosity. The researcher's study

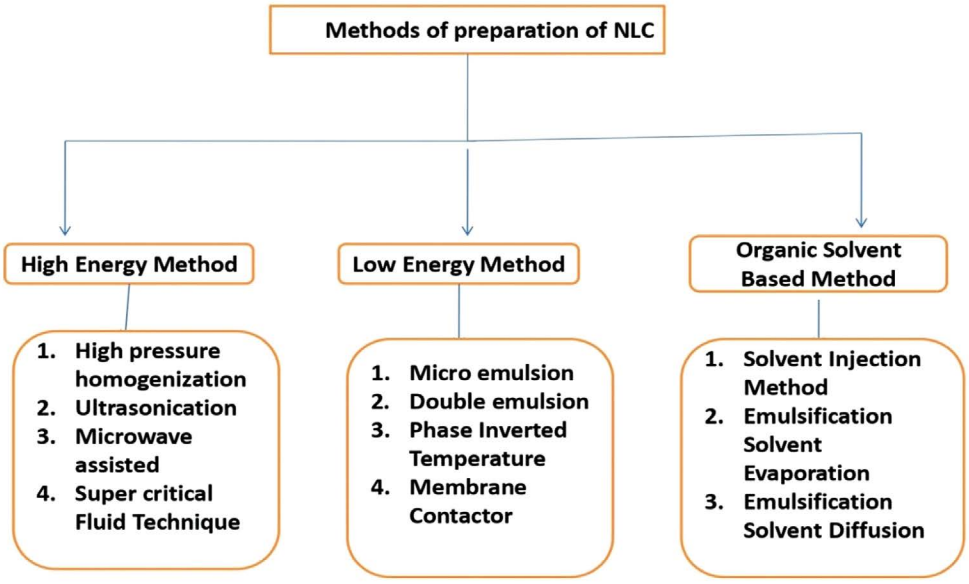


**Figure 2.** **Different methods of fabrication of NLC**

**Table 3.** **Various methods with process description for NLC fabrication**

| Name of methods | Description of process | References |
|---|---|---|
| High-pressure homogenization | Selected lipid mixture heat at temperature 50 °C above the M.P of SL (solid lipid) along with drug in a beaker. The surfactant solutions at the same temperature mix with the lipid-drug mixture and homogenized with high pressure. Then cool down & go for lyophilization to get NLC.<br>The high-pressure homogenization can be used as hot & cold HPH depending on the temperature environment used. | [76] |
| Ultrasonication | The lipids and drug mixture are prepared & mix with an aqueous surfactant at the same temperature using high shear pressure. The formed coarse emulsion was subjected to ultrasonication to make a nanosize emulsion & cool down to room temperature to get NLC | [77] |
| Supercritical fluid method | In this method melted solid lipid mix with carbon dioxide (SCF) with drug and surfactant. Then the preparation is atomized & sprayed in a specialized chamber followed by decompression & evaporation that form NLC | [78] |
| Microemulsion | This method involves a drug solution that has to be added to the lipid mixture along with an aqueous surfactant to produce an emulsion. It is also affected by hydrophilic & lipophilic phase ratios. The prepared emulsion is exposed to cold water with an agitation which gives a dispersion of NLC. The microemulsion method is suitable for a thermolabile drug | [78] |
| Double emulsion | This method involves the preparation of primary emulsion by mixing an aqueous solution of the drug with melted lipid and lipophilic surfactant. The primary emulsion is formed again mixed with an aqueous surfactant to form w/o/w emulsion. Then nanoparticles are purified by using the solvent evaporation method. The double emulsion method is suitable for formulation with low lipid content. | [79] |
| Membrane contractor | In this method melted lipid is mixed with drug and permeates through a ceramic membrane containing a specific size pore. Then aqueous surfactant solution passes tangentially to the membrane and removes the droplet at the outlet of the pores by using a brush. It cools at room temperature to get lipid nanoparticles, The pore size & and pressure applied at the lipid phase affect the size of the particle | [80] |
| Solvent Injection method | This method involves faster diffusion of aqueous solvent in a lipid matrix. The lipid matrix mixes with water-soluble solvent and is injected into the surfactant solution with continuous stirring. The aqueous solution will diffuse inside it. The precipitation of lipid nanoparticles was observed in an aqueous solvent. The major problem is solvent residue may remain in it | [81] |
| Solvent emulsification evaporation method | This method involves mixing lipid matrix containing drug with water-miscible organic solvent and it was dispersed in aqueous surfactant solution with continuous stirring then organic solvent evaporates from it leads to produce lipid nanoparticles | [82] |
| The solvent emulsification diffusion method | This method involves partly the use of water-miscible solvent to disperse lipid & drugs. The thermodynamic equilibrium is maintained with a proper ratio of water & organic solvent. Initially, O/W emulsion is formed by dispersing lipid & drug then diluted with water in a particular ratio so that solvent diffuses in a continuous phase to precipitate the nanoparticles | [83] |

report that the higher concentration of solid lipid (Oleic acid) i. e. up to 30 % concentration only shows a smaller particle size.

*Case study 2*. The particular research was conducted by X.Lin et al (2007) using medium-chain Triglyceride as a liquid lipid for NLC preparation. The research was targeted to know the effect of liquid oil on the particle size of NLC. In this work total lipid was constant but changes were made in the quantity of liquid lipid. The inference was found that initially increase in particle size with an increase in the amount of liquid oil and when oil proportion was above 20 % wt the particle size decreases. The case study reveals that when liquid lipid (oil) is above a specific weight in the formulation it leads to a decrease in particle size. Hence it can be concluded that the liquid lipid amount used in the formulation influences particle size [11].

*Case study 3*. A study by S.Anantachaisilp et al reveals that with increasing liquid lipid concentration from 0 to 10 %. The average particle size of the formulation decreases ( from 196 nm to 160 nm). The research data shows that with 0, 2.5, and 5 % concentration of liquid lipid there was a negligible difference in particle size & polydispersity index (PDI). However, at 7.5 and 10 %, there was a significant decrease in particle size and PDI [12]. The concluding remark of the case study was with increasing up to a certain concentration of liquid lipid the particle size decreased. Hence it can be concluded that different concentrations of liquid lipid have an impact on the size of particle & particle size distribution.

### *Impact of homogenization time & shear intensity*

*Case study 1*. A research study was conducted by Andree A. M. shimojo et al to know the impact of shear intensity and homogenization time on various characteristics of NLC formulation. Resveratol was used as a drug

candidate for the study. The research work used homogenization speeds 13 000, 19 000, and 24 000 as shear intensity (rpm) and time 2, 6, 10 as shear time (minutes). The report shows that a shear intensity of 24 000 rpm and shear time of 10 minutes resulting decreased particle size (142 nm) and PDI (0.353). The study also reveals the zeta potential of formulation (–11.67mV) and entrapment efficiency of 94.2 %. So it can be an inference that homogenization time and shear intensity have an impact on the characteristics of NLC formulation [13].

*Case study 2*. Effect of high shear homogenization (HSH) & Ultrasonication: A research study was conducted by V. trivedi et al (2021) to know the impact of HSH & ultrasonication by using Olanzapine as a drug candidate. It reveals that not only does HSH reduce the particle size but also there was an impact of ultrasonication on the reduction of particle size. The report shows that the range of particle size by HSH (148–191 nm) was reduced to a range of 118–164 nm with the use of ultrasonication for one minute. The study reveals that the contribution of sonication energy to nanostructured lipid carrier formulation leads to decrease particle size. Hence it can be the inference that the particle size of NLC formulation is affected by high shear homogenization & ultrasonication [14].

### *Effect of surfactant*

it was reported from many research works that surfactant reduces the size of the particle, maintains PDI, and enhances the stability of NLC formulation. Surfactant with a concentration of 1–5 % has an impact to reduce the particle size. The study also reveals that particle size reduction is directly proportional to the surfactant to total lipid ratio in the formulation [46].

*Case study 1*. An experimental work was carried out by Akramm Pezeshki et al for betacarotene NLC to know the effect of surfactant concentration on particle size, Zeta potential, and stability of the formulation. The study was conducted by using polaxomer 407 in different concentrations (2, 3, 4, 6 %). The report shows that the average particle size of the formulation was 88, 79, 94 and 115 nm concerning the above surfactant concentration respectively. from the experimental work, it was concluded that 3 % polaxomer concentration can effectively reduce particle size as compared with other concentrations. Hence surfactants have an impact on the particle size of NLC formulation. The zeta potential with optimized surfactant was 0.276 mV and after 60 days of storage and the mean particle size was 87 nm [15].

*Case study 2*. A research work was carried out by Ahmed R. Gardouh et al (2018) using Dermarol DCO®, Dermarol CCT® as liquid lipid, and Naterol GMS® as solid lipid along with using Tween 80 and span 20 as surfactants. The study aimed to determine the effect of surfactants on particle size. The surfactant concentration ratio of Tween 80 and span 20 was 2.5/1,5/1,10/1,15/1 w/w percentage. From the study, it was observed that the surfactant ratio of 5/1,2.5/1 was good to prepare NLC. With a surfactant ratio, of 2.5/1 the particle size ranges from 150 to 220 nm, and with a surfactant ratio of 5/1 particle size ranges from 157 to 324. Hence the study proved that the ratio of surfactant concentration has an impact on particle size [16].

*Case study 3.* F. Han et al (2008) reveal their research work that the effect of nonionic surfactants has more contribution as compared with other surfactants for the stability of NLC formulation. The work was conducted by using four types of surfactant (polaxomer 188, soya lecithin, sodium deoxycholate, tween 80). The study aimed to know the effect of surfactants on the characterization of NLC. The results indicate that the ionic surfactant (sodium deoxycholate) has a low emulsification ability but improves the ZP (zeta potential) of the formulation. But the surfactant such as non-ionic surfactant (polaxomer 188) shows additional stearic stability of formulation [47].

*Case study 4.* A study was conducted by Kovacevic et al. by using two surfactants ( Plurol Stearique® WL 1009 and Plantacare® 810), cetyl palmitate (SL), Miglyol 812 (LL) to prepare NLC 30 % w/w lipid stable up to 90 days. However, after increasing lipid concentration to 40 % w/w formulations. At a concentration of 1 %, both surfactants reduced the particle size to <200 nm, and the formulation containing particle size increased but was stabilized by Plurol Stearique® WL 1009. The surfactant Plantacare® 810 shows its stabilization effects even the lipid concentration at 50 % w/w but above it, the NLC shows a destabilization effect [49]. The study reveals that different types & concentrations of surfactants have an impact on the formulation.

### *The Crystallinity of NLC formulation*

It is important to understand the lipid phase behaviors when it was part of the formulation. Generally, a lipid with a temperature well above melting point can maintain some degree of ordering in the liquid phase termed the crystalline memory effect. However, high-temperature heating and subsequent cooling lead to destroying the memory and formation of different phases [17].

The composition & drop size of melted lipid is responsible for the crystalline behavior of particle of solid lipid. The process of nucleation depends on the process used, rate of cooling, and solidification of starting material (lipid) first from the mixture [18]. As the NLC contains both solid lipid & liquid lipid (oil) a specific amount of solid lipid may dissolve in liquid lipid with increased temperature. Usually, with high-pressure homogenization and process temperature more amount of solid lipid dissolve in oil. When the temperature of the mixture decreases leads to the initiation of a crystallization process that forms different crystalline forms (stable & metastable) which depends on lipid compatibility in a mixture [19]. Low energy polymorphs are formed due to the rapid cooling of liquid lipid. Upon slower cooling, the lipid molecules get sufficient time to organize into lamellae and formed

three-dimensional crystals. Many factors are responsible for the arrangement of molecules in a crystalline state such as temperature & cooling rate, the composition of lipid, and rate of agitation.

The most arguable parameter influencing lipid crystallization is subcooling (cooling of lipid below equilibrium point). As subcooling increases the rate of nucleation increases and decreases induction time for crystallization. The promotion of nucleation may be by agitation which produces mechanical disturbance (energy supplied to overcome the energy barrier) [20]. A higher agitation rate is responsible for small crystal formation and high crystallization rate however slow cooling rate and slow agitation produced mixed crystal that leads to an increased melting range. During the slow cooling stage, the lipid has more opportunity to form crystal lattice as the temperature remains more time in higher.

The very common method employed to investigate the polymorphic form and Crystallinity is DSC & X-ray diffraction. DSC can investigate the structure of NLC by mixing the behavior of SL (solid lipid) and LL (Liquid lipid). The process of fusion & breakdown of crystal lattice by cooling and heating give information about crystal ordering and polymorphism.XRD is also another technique to find out polymorphic changes. WAXS (wide-angle x-ray scattering and SAXS (small-angle x-ray scattering) were used to know the layer arrangement of crystal structure, and polymorphic form [21]. The storage time, amount of drug-loaded, and viscosity of formulation may significantly affect the Crystallinity of lipid-based nanoparticles. Using solid lipid (SL) with crystal lattice imperfection can enhance encapsulation efficiency and chemical stability more housing of drug in lipid matrix [22, 23]

## *Stability of NLC formulation*

Perkinetic flocculation may occur due to aggregation of a particle on long-term storage. During the storage period, all NLC preparation should maintain the properties of nanoparticles and also prevent the growth of microorganisms. The two methods are adopted: 1) freeze-drying of NLC preparation to remove water, and 2) use of preservatives. The freeze-dried product must maintain the therapeutics activity of the encapsulated drug. It should resuspend with water in less reconstitution time. However many studies revealed that there is an aggregation of a particle without the use of cryoprotectant during the freeze-drying process. The common cryoprotectants used in NLC formulation are microcelac, trehalose, dextrose, mannitol, sucrose, and Avicel RC597 [24].

Mostly for dermal products the preservative use to maintain stability (because preparations are fluid or semisolid). However, preservatives not only stabilize formulation but also it can destabilize. Hence its effect needs to be understood. The parameters are

- preservative ability to reduce zeta potential;
- hydrophobicity character of the particle surface;
- the affinity of preservative to the surface of the particle;
- stabilizer anchoring to particle surface (onto/into);
- interaction between preservative & stabilizer layer.

The study also revealed that the thermodynamic stability of lipid polymorphic form is affected by the melting point of the stabilizing agent. If the stabilizer has melting point greater than 50 °C it will maintain the lipid in low thermodynamic stability whereas the melting point less than 00C causes stable polymorphic transition [25]. The list of preservatives with their composition & effective concentration used in NLC formulation is presented in table 4 and figure 3 [26].

**Approaches for NLC stability.** Approaches such as lyophilization, the addition of non-ionic surfactant, spray drying, and the addition of hydrophilic substances (PEG 4000) make the formulation more stable. These are presented in table 5.

**Table 4. List of the preservatives [26]**

| Name | Effective range | Composition |
|---|---|---|
| Rokonsal®PB5 | 0.3–1.2 | Phenoxyethanol 71–73 %, methylparaben 14–15 %, ethylparaben 5.5–6.1 %, propylparaben 2.2–2.6 %, butylparaben 3.4–3.8 %, isobutylparaben 1.6–2 % |
| Euxyl® PE9010 | 1.0 | Phenoxyethanol 90 %, ethylhexylglycerin (1,2-propanediol, 3-(2-ethylhexyloxy) 10 % |
| Dermosoft® Octiol | 0.5–1.0 | Caprylyl glycol (octane-1,2-diol) |
| Phenonip® | 0.5–1.0 | Phenoxyethanol 70–75 %, methylparaben 14.5–16.5 %, ethylparaben 3.3–4.3 %, propylparaben 1.7–2.3 %, butylparaben 3.4–4.3 %, isobutylparaben 1.7–2.3 % |
| Hydrolite® 5 | 1.5–5.0 | Pentylene Glycol (1,3-pentanediol) |
| Euxyl® K702 | 0.2–1.0 | Phenoxyethanol 77 %, benzoic acid 13 %, dehydroacetic acid 6 %, polyaminopropylbiguanide 2 %, ethylhexylglycerol 1% , water 2 % |
| Ethanol 96% | >20 % v/v | Ethanol 96 % v/v |
| Propylene glycol | 10.0 | Propylene glycol, 1,2-propanediol |
| Euxyl® K700 | 0.5–1.5 | Phenoxyethanol 30 %, benzyl alcohol 30 %, potassium sorbate 15 %, tocopherol 15 %, water 10 % |

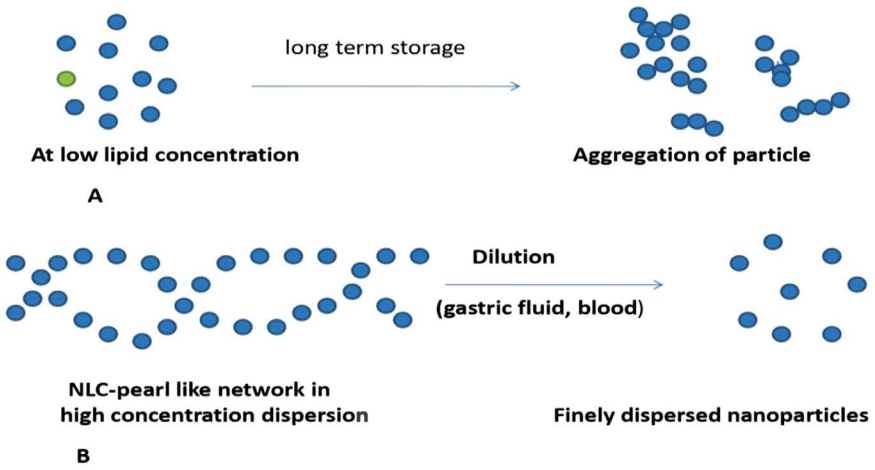


**Figure 3.** **Stabilization effect.**

**A – at low lipid concentration of formulation produces an aggregation of the particle on long-term storage; B – NLC pearl-like network converts to the finely dispersed particle on dilution with gastric fluid, and blood [24]**

**Table 5.** **Methods used for NLC stability**

| Approaches | Description | References |
|---|---|---|
| Lyophilization | It is the most efficient method for avoiding Ostwald ripening and avoid degradation of water-sensitive drugs but it is advisable to add cryoprotectant in the formulation during lyophilization | [71] |
| Spray drying | This method involves the spraying of preparation to get powder form which can be stored for a longer period for further use. Here to avoid lipid degradation, the melting point of lipid must be greater than the boiling point of sprayed liquid to be considered before spray. To decrease the M. P of the mixture, a water-alcohol system was used instead of using pure water in preparation | [72] |
| Addition of non-ionic surfactant (poloxamer) | Polaxomer of various grades are used as a stabilizing agent in nanoparticle formulation. The mechanical stability of nanoformulation is enhanced with the use of polaxomer 188 while 407 grade of polaxomer forms micelle with alcohol present in the formulation and makes it thermodynamic stable | [73, 74] |
| Addition of hydrophilic substance (PEG 4000) | The addition of substances like polyethylene glycol 4000 produces benefits like dispersibility, physical stability, lipid modification, and growth of NLC so that stability of NLC can be maintained | [74] |

## *Entrapment efficiency (EE)*

The characteristics of drug release affected by the drug entrapment efficiency. It can be defined as the ratio of the difference between the total amount of drug used in the formulation and unentrapped drug to the total amount of drug. The amount of solid lipid & liquid lipid in formulation affect the entrapment efficiency of the drug. Generally, the presence of a higher concentration of liquid lipid changes the nature of the crystal lattice and enhances the imperfection resulting in higher entrapment of the drug. The lipophilic nature of the drug also enhances the encapsulation efficiency due to the drug having more affinity to lipid [27]. The primary concern for improving EE depends on the solubility of the drug in lipid & the solubility of the drug in lipid must be more as compared with required because when melted lipid cools down it solidifies and there are chances of a decrease of solubility [48].

*Case study 1*: Jaber Emami et al conducted research work for NLC formulation with ART (Artemisinin) as a drug along with Compritol (solid lipid), and oleic acid (liquid lipid) & polaxomer (surfactant). By increasing the concentration of liquid lipid i.e oleic acid, the entrapment efficiency of formulation increased from 59 to 83 % indicating the effect of liquid lipid on EE [28]. Due to imperfection of NLC structure more space is created in the formulation as a result entrapment of drug is more [29].

## *Effect of Surface charge*

Particle aggregation or dispersion and stability of formulation affected by the surface charge of NLC. The various factors that affect the surface charge are ionic strength, ion types covering the aqueous phase & pH. Greater the electrostatic repulsion more the surface charge leads to less aggregation of particles. The zeta potential is the parameter used to measure the surface

charge. The stable NLC should have Zeta potential value ±30 mV. The amount of solid lipid and liquid lipid, type & concentration of surfactant as the formulation parameter affect the zeta potential value. The higher the liquid lipid concentrations in NLC formulation greater the net negative charges due to liquid lipid being mostly negative charge. If the formulation targeted the cancer cell, the NLC formulation must more cationic nature for electrostatic binding as cancer cells possess a high negative surface charge compared to a normal cell [30, 31]. Many factors contribute to their effect on zeta potential value is the size of the particle, nature of the particle & storage conditions. When the particle size changes it might affect the dispersion force and charge between the particles. Hence agglomeration tendency of particles affected resulting changes in the ZP (zeta potential) value [41].

## SAFETY AND BIOCOMPATIBILITY

The component used to prepare NLC are approved and used as pharmaceutical grade especially intended for topical use. A research study was conducted by D. Douroumis et al reveals that up to a dose of 1 mg/ml of lipid can be tolerated by cell line when drug-free NLC used. A report revealed by Almeida et al that by using cetyl trimethyl ammonium bromide (cationic surfactant) the lipid-based preparation showed less toxicity at a dose over 1 mg/ml. The study by R. H. Muller et al shows that using surfactants like polaxomer 188 and polysorbate 80 ensure biocompatibility & less toxicity of lipid formulation but it should be considered that even using a mixture of surfactant to enhance the stability of formulation may produce toxicity [32, 33].

*Case study 1*. M. L. Bondi et al conducted research work on the effect of NLC formulation composition on cytotoxicity. The formulation contains Compritol 888 ATO as solid lipid, Miglyol 812 as liquid lipid, and Epicuron 200 as surfactant along with simvastatin as API. The HuH6 & HuH7 were used as cell lines for the study. The cell exposure time was 72 h & the result suggests that no cytotoxicity during that period [34].

*Case study 2*. Group of the researcher (C. Vitorino et al) carried out a study to find out the effect of Nanostructured lipid carrier formulation on cell viability. The study includes Glyceryl tripalmitate as solid lipid, Miglyol 812 as liquid lipid, and Tween 80 as surfactant. The HaCaT was used as a cell line for study. The study was conducted for 72 h and the outcome of the study was 80 % cell viability [35].

## SKIN TARGETING

In NLC the particles are nanosize hence it has close contact with SC which leads to disturbing corneocyte arrangement resulting in more drug penetration into the deeper layer of skin. After applying NLC to the skin surface, the occlusion effect is produced due to the formation of a single layer of lipid film. The formations of the lipid layer prevent the loss of water from the skin surface and produced skin hydration. This skin hydration helps drug permeation into the deeper layer of skin [39, 40]. The process is depicted in figure 4.

## NLC APPROACH FOR CANCER THERAPY

Nanostructured lipid carrier which is an alternative to other colloidal carriers has enormous advantages such as high drug loading, stability of the formulation, controlled release & biocompatibility. Hence this carrier can be used for anticancer drug loading for better therapeutic effectiveness & targeting. The various types of the anti-cancer drug, formulation component, cell line use & outcome of research work were presented in table 4 below.

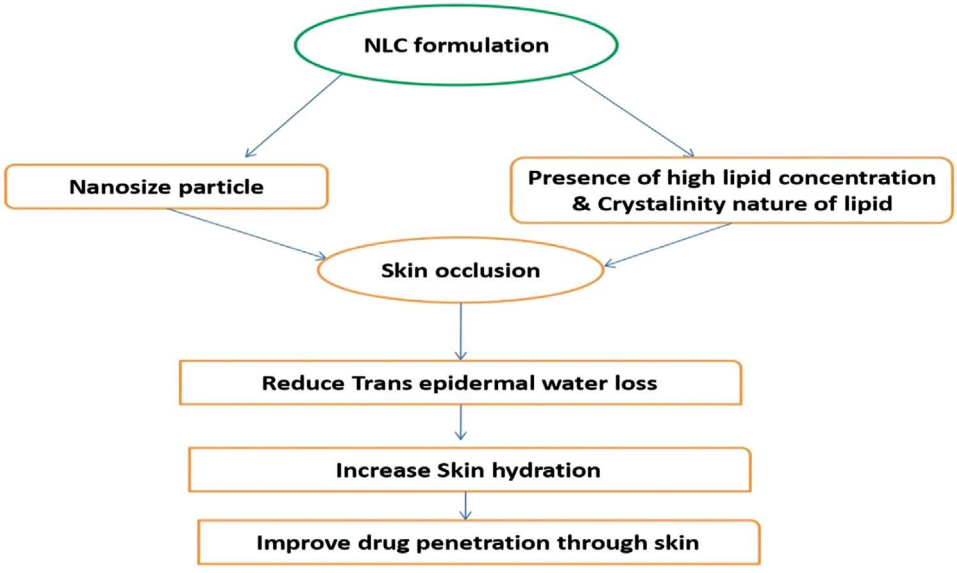


**Figure 4.** **Drug penetration through the skin by NLC formulation**

It has been reported that many limitations of an anti-cancer drug such as high toxicity to a normal cell, less specificity, stability problem, and variable pharmacokinetic profile make the drug limited use but NLC considers an effective carrier to address the problem. NLC offers more stability, high drug loading, and sustained release for better therapeutic effect. Due to advanced research hydrophilic drug (Decitabine) is now also formulated to NLC to improve drug action [65].

### *Drug action by passive target*

Passive target of the anti-cancer drug improved by changing the size of particle, shape, and surface character of NLC. Normally the pore size of a tumor's vessel range from 100 to 780 nm. Hence particle size plays a dominant role to enter into it [66].

*Case study 1*. The group of researchers Wang et al prepared NLC with paclitaxel and doxorubicin for lung cancer and find out an excellent result in terms of cytotoxicity on the H460 cell line. The particle size was 129.3 ± 4.2 nm and the formulation was effective as compared with pure drug [64].

*Case study 2*:. Group of researchers Z. P. Wand et al prepared an NLC formulation using berberine as an anti-cancer drug and evaluate its effectiveness on H22 tumor cells. The particle size of NLC was 138 nm which produces a better therapeutic response as compared with normal drug delivery [67].

### *Active tumor targeting*

This method involves the design of an active targeting ligand that improves more accumulation of drugs at the tumor site.

*Case study 1*. Group of researchers khajavinia et al conducted work by conjugating transferee with stearyl amine NLC with drug etoposide by using a K562 cell line and results show there was a 15-fold reduction in case of $IC_{50}$ compared with free drug [68].

*Case study 2*:. Varshosaz J et al conducted research work by conjugating retinoic acid with octadecylamine of NLC using 5-Fluorouracil as a drug. They found that prepared NLC shows higher efficacy in case of colorectal carcinoma (figure 5, table 6) [69].

## APPROACHES OF NLC FOR BRAIN TARGET

In general the BBB limit many drugs to delivery into brain tissue. So strategy must be considered that formulation should be penetrating the barrier. The remarkable qualities of NLC can target the brain which is discussed below [75].

A. **Nanosize particle.** As NLC possesses nanosize particles it can easily pass through the microvasculature of the brain.
B. **Lipid solubility.** In the present clinical research almost all drugs used are lipophilic. NLC is composed of solid and liquid lipid in which the drug is soluble. Due to the lipid solubility nature of the drug it can reach brain tissue by a lipid-mediated diffusion process.
C. **Protection & bioavailability of drug.** Nanostructured lipid carrier enhances bioavailability by improving drug encapsulation efficiency and protecting the drug from possible degradation. It prevents aggregation of particles and improves drug shelf life.
D. **The polymeric coating enhances stability & penetration.** The presence of RES (Reticuloenothelial system) is responsible for the rapid elimination of NLC from the body. This can be prevented by surface modification of particles with a hydrophilic polymer such as Poloxamer, PEG, etc. This surface modification enhances drug penetration through BBB.

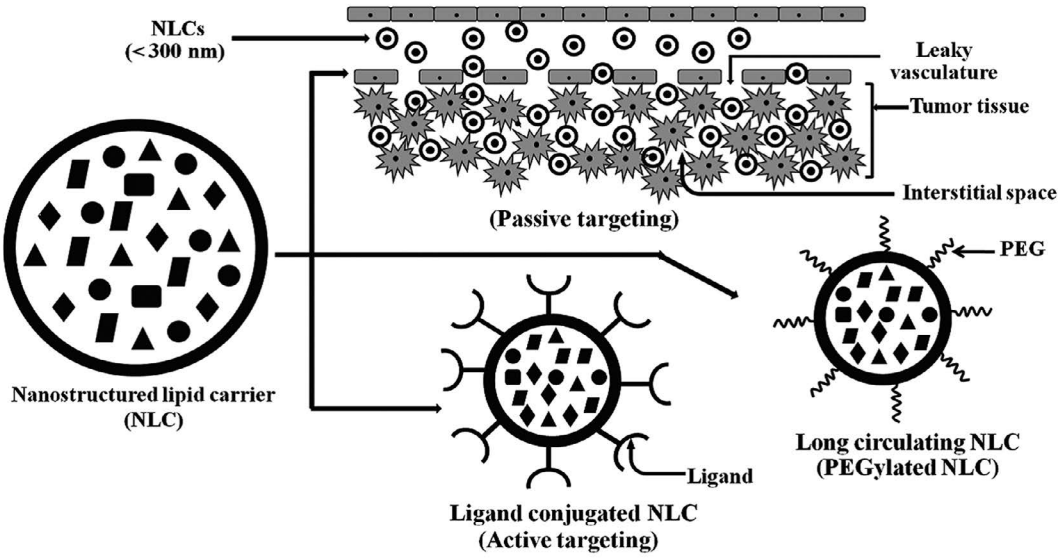


**Figure 5. Targeting of NLC for tumor [86] (figure used with permission from author)**

**Table 6.** **List of Anti-cancer drugs used for NLC formulation**

| Drug name | Cell line/Animal used | Component of formulation | Type of cancer | Outcomes | References |
|---|---|---|---|---|---|
| Imatinib | MCF-7 | Stearic acid, Sesame oil SLS, Tween 80 | Breast cancer | MTT cytotoxicity assay conducted using NLC loaded drug with blank. It was found that the NLC formulation shows more cytotoxicity toward cancerous cell | [50] |
| Miltefosine (HePC) | Michigan Cancer Foundation-7 (MCF-7) and squamous cell carcinoma-7 (SCC-7) /BALB/c mice | Stearic acid, Oleic acid, Tween 80 | Breast cancer | HePC-NLC shows enhanced pharmacokinetic character and reduced hemolytic potential compared with free drugs. | [51] |
| Docetaxel (DTX) | male Sprague-Dawley rats | Labrafil M 1944CS, Precirol ATO 5, DSPE-$PEG_{3K}$, (DSPE-$PEG_{2K}$, RIPL peptide | ovarian cancer | DTX-PEG-RIPL-NLCs suppressed tumors, evidenced by tumor volume change and histopathological examination. | [52] |
| Resveratrol (RSV) | MCF-7 | Stearic acid, Oleic acid, DPPH, polaxomer 188 | Breast cancer | In-vivo pharmacokinetic studies show a 9-fold increase in AUC values with RSV-FA-NLCs in comparison to free Resveratrol. | [53] |
| Etoposide (ETP) | SGC7901 cells | Glycerol monostearate, soybean phosphatidylcholine (SPC), oleic acid | Gastric cancer | The ETP-loaded NLC shows significant enhancement of in vitro cytotoxicity and in vivo antitumor effect against SGC7901 cells | [54] |
| Doxorubicin (DOX) and β-elemene (ELE) | A549 lung cancer cells, MRC-5 cells | Compritol® 888 ATO, Miglyol® 812, Lecithin | lung cancer | Cytotoxicity and synergistic effect observed in DOX/ELE Hyd NLC | [55] |
| Curcumin (CRN) | LNCaP cell lines | Oleic acid, stearic acid, Tween 80 | Prostate cancer | The CRN-NLC loaded formulation shows EE 92.9 % & shows substantial tumor volume suppression (40 %) | [56] |
| Paclitaxel (PTX) | H1299 (NCI-H1299) cells, S180 cells, and RAW264.7 cells | Compritol 888 ATO, GMS, Kolliphor ELP & HS15 | Breast cancer | RBC-loaded PTX-NLC enhanced the antitumor effect and extended the survival period significantly *in vivo* | [57] |
| Citral | MDA MB-231 cells | Palm oil, lipoid S-100, olive oil, Tween 80 | Breast cancer | By using MTT assay, NLC-Citral inhibited the proliferation of MDA MB-231 better compare with only citral | [58] |
| Tripterine | CACO-2 cell line/Male Sprague-Dawley rat | Precirol ATO-5, Polaxomer 188 and Labrafil M 1944 CS | Prostate cancer | NLC-loaded tripterine shows cell viability 4.7 times compare with the only tripterine solution and exhibit more permeability in a different area of the large intestine | [59] |
| Decitabine (DCB) | A549 Cell line/Male albino rat | Precirol ATO 5, Tween 80, polaxomer 188, Transcutol HP, Poloxamer 188 | Lung cancer | Affinity & cytotoxicity towards cancer cells improved and permeation improved 4-fold by NLC load with DCB | [60] |
| Isoliquiritigenin(ISL) | S180 and H22 cell lines/Female Kunming mice | GMS,polaxomer 188, Migloyl 812 | Liver cancer | Compare with ISL suspension the NLC-ISL shows more inhibition on cell line (H22 & S180) and NLC formulation exhibit 2.5 times more drug concentration in the mice model | [61] |
| 5-Fluorouracil (5-FU), Cisplatin (CDDP) | BGC823 human gastric cell line | GMS, Tween 80, soya lecithin, soya bean oil | Gastric cancer | The pro drug coated with stearic acid & Hyaluronic acid and cisplatin-loaded NLC shows an additive effect as compared with uncoated & free drug | [62] |
| Celecoxib (Cxb), Docetaxel (Doc) | A549 Cells/Nu mice | Compritol® ATO 888, sodium taurocholate, Migloyl 812 | Lung cancer | The aerosol form of Cxb with NLC shows a greater reduction in tumor volume | [63] |
| Paclitaxe l(PTX) & Doxorubicin (DOX) | NCL-H460 cells | Compritol® ATO 888, soybean phosphatidylcholine, oleic acid | Lung cancer | The cytotoxic effect by NLC loaded PTX and DOX enhanced 3 fold compared with NLC single drug & 9 times more in comparison with free drug | [64] |

*End of table 6*

| Drug name | Cell line/Animal used | Component of formulation | Type of cancer | Outcomes | References |
|---|---|---|---|---|---|
| Artemisinin | U-87MG brain cancer cell line | Oleic acid, Compritol®, Tween 80 | Brain tumor | The objective was transferrin-conjugated NLC to target brain tumors. The EE and particle sizes were 82.3 % & 145 ± 12.5 nm respectively. Transferring conjugated artemisinin NLC shows higher toxicity compare to free drugs towards cancer cells | [28] |
| Temozolorride | Porcine nasal mucosa, wistar rat | Gelucire, Vitamin E | Brain tumour | Prolong drug release & permeation enhanced by passing through the blood-brain barrier | [70] |

E. **Active & passive targeting.** for active targeting ligand should be used to bind with receptors expressed by the cancer cell. The active target enhances the high drug retention and reduced side effect. The passive target involves 'enhanced permeability. The abnormal cell (tumor) has high vascular permeability as compared with the normal cell. Due to this reason, nanocarriers easily penetrate. The effect was studied by Ming-Jan-Tsai et al using baicalein as a drug and found out 2–3 fold increase in drug amount in the targeted region.

F. **Release of the drug in a controlled manner.** As the active efflux system in BBB limit the therapeutic efficacy of the drug, NLC manages it by releasing the drug in a controlled manner which enhances the availability of the drug for a long period in the brain tissue. Hence the effectiveness of the drug increases.

## CONCLUSION

NLC, a smart generation of lipid carrier gaining more popularity day to day in research because of its numerous advantages over other carrier systems. The easy methods of fabrication, lipid biocompatibility, prolong drug action, high drug entrapment, and stability extends its scope to many pharmaceuticals & cosmetics. Due to its excellent skin targeting, it is one of the prominent research areas for an alternative route of administration. With the help of a nanostructured lipid carrier, the maximum amount of drug can be targeted for brain and cancer treatment. The above case study result shows the various parameters have an impact on formulation & it will provide a research idea to the reader for optimizing formulation. Therefore NLC can consider a "smart Nanolipid carrier" for effective treatment.

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