Review Article

Title:

A focus on Fabrication, Characterization, Stability, Skin Targeting, Patent, Safety and Toxicity of Nanostructured Lipid Carrier

Abstract:

Background: The advanced development of lipid nanocarrier contributes a lot to the domain of therapeutic effectiveness of the drug. However the parameter such as drug loading, drug release, stability, and targeting influence much more towards the limitation of many lipid nanocarriers. The Nanostructured lipid carrier, the second generation of lipid carrier has more promising advantages over others and have tremendous targeting ability to skin for drug administration

Objective: The present review paper focus to understand the different fabrication technique, impact of lipid and surfactant on formulation effectiveness, characterization of formulation, and Crystalinity concept of lipid which have an impact on stability & drug loading. Focus on a parameter such as Transepidermal water loss , skin occlusion, and hydration which determine the ability of the carrier to target the skin. Hence the effectiveness of the drug improved. This review also focused on patents based on Nanostructured lipid carriers.

Method of preparation: many methods have been adopted to prepare Nanostructured lipid carriers and among all High-pressure homogenization method is considered as best one.

Conclusion: Because of numerous advantages of this carrier system such as biocompatibility of lipid, high drug encapsulation, stability over others, it is considered as a major focused area for researchers. The new domain of Nanostructured lipid carrier is transdermal drug administration by targeting the skin, hence more research is focused on topical preparation. However, toxicity must have to be studied in humans. So by considering all factors one can rename it as " smart nano lipid carrier".

Keywords: Nanostructured Lipid carrier, skin occlusion, stability, skin targeting, fabrication, safety and Toxicity, patent, Transepidermal water loss

1. Introduction:

In the last decade the nanoparticulate carrier imparting a promising drug delivery system for drugs. Among the nanoparticulate carrier, lipid nanoparticles carrier is the emerging carrier for recent development. As many drugs are structurally designed and well-formulated but their toxicity, low bioavailability, stability make them limited for use. Hence by choosing the route of administration along with lipid nanocarrier removes the boundary of limitation. The various lipid carriers used in the formulation are liposome, niosome, Solid Lipid Nanoparticles (SLN), Nanostructured Lipid Carriers (NLC). Among these, the NLC is now a promising carrier for researchers as it provides more advantages over other lipid carriers for drug delivery. The solid lipid nanoparticles which contain only solid lipid produce more limitations to formulation such as poor drug loading capacity(which is attributed due to lipid crystalline nature), the expulsion of drug content (because of perfect crystalline lattice formation), and stability concern of formulation over long storage [1,2]. However, NLCs are second-generation lipid carriers that consist of solid lipid and liquid lipid enhancing drug entrapment capacity and preventing leakage of the drug during storage [3,4]. Hence the current study is concerned with how NLC is a promising delivery system through the skin by studying the important parameter such as skin barrier & permeability, skin hydration & occlusion, TPEL(Trans Epidermal Water Loss), skin targeting, and stability aspect of the formulation[5,6,7]. Skin's enormous surface area makes drug ease administration and acts as a barrier for drug molecules having a molecular weight greater than 500 Da[8]. The top layer of skin called the epidermis act as a barrier that limits many drugs from their effectiveness. Hence NLC is the approach equipped with nanotechnology and lipid carriers that can make effectiveness through the skin.

NLC has particle diameter ranges from 10-1000 nm consisting of solid lipid & liquid lipid which are biocompatible. The presence of different fatty acid carbon chain in liquid lipids make NLC with a less organized crystalline structure. Hence improving loading capacity for drug accommodation. The presence of Liquid lipids is an excellent solubilizer of drugs than Solid Lipid (SLS). The NLC produces low cytotoxicity/systemic toxicity as it is composed of physiological and biodegradable lipids. The nanosizes of lipid particles enhance drug penetration through the stratum corneum. The controlled release from this carrier is also possible due to the solid lipid matrix [9,10]

Table 1:Type of NLC model along with characteristics:

NLC type	Characteristics	References
Imperfect crystal types	-Nanoemulsion is formed by blending SL(solid lipid) & LL (liquid lipid) followed by cooling and highly disorder matrix formed due to crystallization process - characterized by low liquid lipid -Disordered matrix contain more space due to gap between fatty acid chain which will accompany more drug - NLC matrix does not form a high order structure due to different chain lengths of fatty acid & other glycerol	[11]
Multiple carrier types	- It is Oil/fat/water system - In the solid matrix, the oil compartment distributed - High drug solubility in nanosized lipid oil compartment - A high concentration of liquid lipid is used as a drug that has poor solubility in solid lipid - Drug entrapment is more - Prolong release is achieved because of being surrounded by a solid lipid matrix - Drug leakage minimized	[12],[13]
Amorphous types	- Mixing of special lipid to form amorphous state (e.g - hydroxyoctacosanyl hydroxyl stearate or isopropyl myristate) - Drug leakage minimized due to crystallization of lipid matrix	[14]

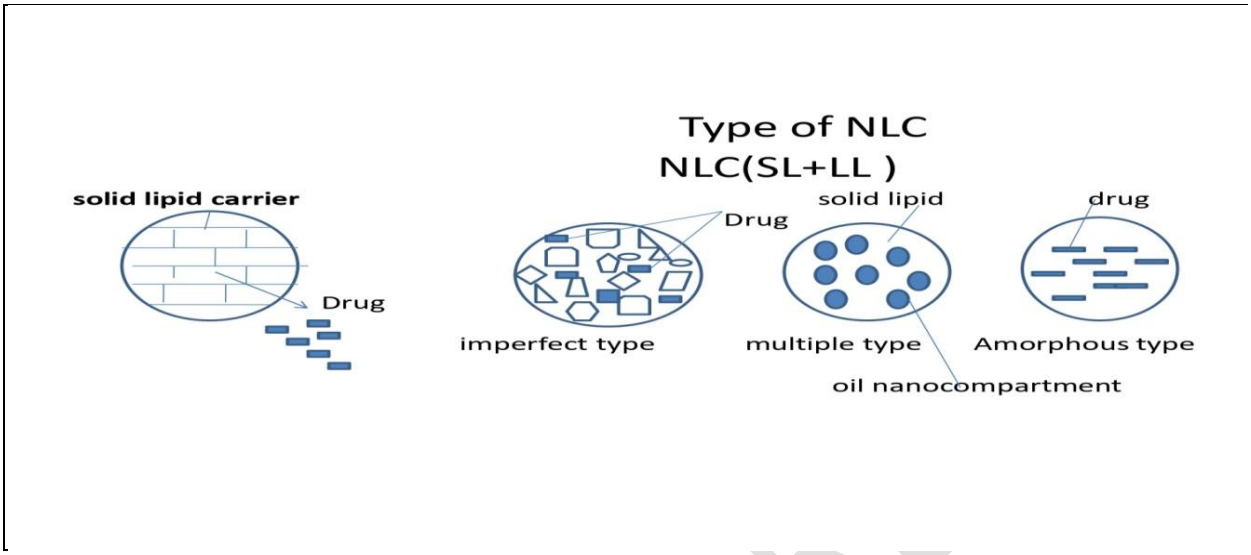

Figure 1: Different types of NLC [15]

2. NLC fabrication:

2.1 *Ingredients used for NLC*: The Nanostructured lipid carrier contains major component that are solid lipid, Liquid lipid, surfactants, and water. Normally surfactants are dispersed in water and they add to the lipid mixture followed by homogenization. The ratio of SL and LL is from 70:30 to 99.9:0.1. The concentration of surfactant varies from 0.5-5% [16]

Table-2: List of the ingredients used in NLC:[17,18,19,20,21,22]

Component	**Trade name**	**Chemical name**	**Melting point**
Solid Lipids	Compritol®888 ATO	Glyceryl behenate	69°C -74°C
	Precirol ATO®5	Glyceryl palmitostearate	50°C -60°C
	Crodamol™CP,Precifac ATO, Cutina CP®	Cetyl palmitate	47°C -54°C
	Glycerol tripalmitate	Tripalmitin	44.7°C - 67.4°C
	Octadecanoic acid	Stearic acid	68°C-70°C
	Glycerin Monostearate	Glyceryl monostearate	57°C -65°C
	Softisan 142	Hydrogenated coco-glycerides	42°C-44°C
	Dynasan 114	Glyceryl trimyristate	55°C -58°C
	Softemul 165	Glycerol stearate& PEG 100 stearate	50°C -60°C
	Dynasan®116	triacylglycerol of palmitic acid	62ºC - 64ºC
	Elfacos® C 26	Hydroxyoctacosanyl Hydoxystearate	80ºC
	Imwitor 900®	Mono diglyceride	54°C - 64°C.
	Syncrowax ERLC	Ethylene glycol ester	60°C - 68°C
Liquid Lipids	Myverol 18-99K	Monoacylglycerols	-
	Gelucire®44/14	Lauroyl Polyoxylglycerides	-
	Epikuron™200	Soy lecithin	-

	Miglyol®812, labrafac, Softisan^{R}378	Caprylic/Capric triglycerides(C8/C10)	
	Oleic Acid	Oleic acid (9Z)-Octadecenoic acid	
		Linoleic acid	
	Caproyl 90	Propylene glycol monocaprylate	-
	Capmul^{R} MCM	Glyceryl Caprylate/Caprate	-
Surfactants			HLB value
	Tween®20	Polyoxyethylene sorbitan laurate	16.7
	Tween®80	Polyoxyethylene sorbitan oleate	15
	Lutrol®F68	Poloxamers 188	29
	Lutrol®F127	Polaxomer 407	21.5
	Cremophor EL	polyoxyl castor oil	12-14
	Solutol®HS15, Kolliphor®HS 15	Macrogol-15-hydroxy stearate	15
	Phospholipon^{R} 80/H	Phosphatidylcholine	9
	Epikuron^{TM}200	Soybean lecithin	7-10
	Cremophor^{R} RH 40	PEG-40 Hydrogenated castor oil	14-16
	Labrasol^{R}	Caprylcaproyl macrogol glycerides	8-12
	Gelucire® 44/14	Lauroyl polyoxyl-32 glycerides	14
	Span 20	Sorbitan monolaurate	8.6
	Sodium oleate	Sodium oleate	18.0
	Polyvinyl alcohol	Polyvinyl alcohol	15-19

2.2 *Lipids and surfactants as component of NLC***:**

To formulate the NLC lipid is the primary component of the formulation. It influences a parameter such as drug encapsulation, stability, and prolonged action. The lipids used in the carrier are biodegradable and non-toxic, and physiologically acceptable. Even though many lipids are available and they have GRAS (Generally Recognized As safe) status, the choice of suitable lipid for NLC is of more concern. The characteristics such as solubility of the drug in lipid and partition coefficients are extremely vital for the selection of lipid. Most of the study reveals that the solubility of the drug in lipid influences the drug loading/encapsulation efficiency [23]. The research also reveals that drug loading, charge, and size of the particle are also affected by the degree of crystallization of lipid [24]. The melting point of lipid also has a vital role as the higher melting point of lipid leads to an increase in the viscosity of the dispersed phase which increases particle size. The other characteristics such as lipid hydrophilicity & crystal shape also influence the NLC quality. The increase in lipid amount 5-10 percent leads to an increase in particle size[25]. Hence it is highly concerning to select the suitable lipid for NLC.

The crystallization, stability& toxicity of NLC are affected by surfactant type and concentration [26]. The choice of surfactant is also based on the route by which the drug is administered, the effect on particle size, and HLB value. Due to crystallization during NLC formulation particle surface area increases that leading to the whole system being unstable. Therefore the selection of surfactant becomes necessary to make the formulation stable. Another important parameter of surfactant is rHLB (required HLB) value for lipid can be calculated by dispersing in a mixture of surfactant with different HLB values followed by high-pressure homogenization to find out least particle size[27,28]

Other excipients as a component of NLC: There are other categories of excipients such as counter ion (ionic polymer and organic salts) used to minimize the problem associated with water-soluble drug encapsulation. The surface modifiers were also used to reduce the NLC formulation from phagocytic uptake by macrophages. Various polymers like polyethylene glycol can be used to coat the lipid particle so that drug residence time can be increased in the systemic circulation. This surface modification can also improve the physical stability and drug targeting [23,29].

2.3 NLC method of fabrication: The method is categorized into 3 groups namely [30]

i) High energy method

ii) Low energy method

iii) Organic solvent-based method

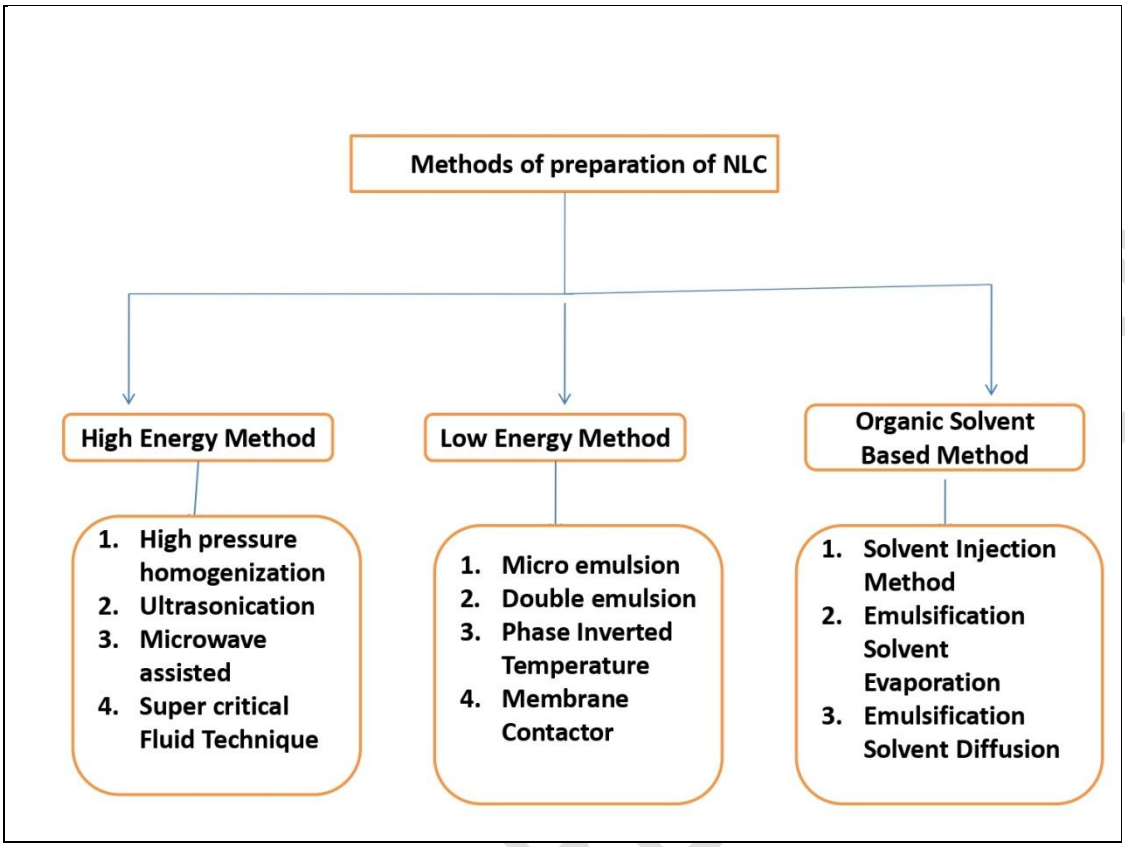

Figure 2: different methods used for the preparation of NLC:

Methods of NLC preparation:

High energy method based on the requirement of equipment that can produce high shear force, distortion of pressure, or the mechanism involved in particle size reduction. The low energy method does not require any specific amount of energy for the reduction of particle size. However, the solvent-based method involves the requirement of organic solvent on a mechanistic basis to the system for the reduction of particle size [30]. Among all the methods high-pressure homogenization method is the most accepted & well-reported method for the research work because of its less production time & easy scale-up process. This method is again categorized into two parts - the hot method and the cold method.

In the hot method, initially solid lipid has to be melted above 5-10^{o}c of its melting point, then liquid lipid has to be added to it & mixed for a few minutes to ensure proper mixing of it. Then surfactant solution heat at the same temperature as lipid mixture. At the same temperature, surfactant solution was added to lipid mixture followed by homogenization under high pressure (500-800 bar) to form nanoemulsion. Subsequently, the mixture allows cooling below room temperature to give NLC[31,21,32]. In the cold homogenization method, the melted hot mixture of lipid & drug is allowed to cool by using nitrogen of ice. Then the mass is ground into fine particles. The obtained macroparticle has to be dispersed in a cold aqueous solution containing surfactant/stabilizer followed by homogenization with high pressure. To get particles with average size & good polydisperse index it is necessary to use high pressure with more number cycles as compared with the hot homogenization method [33,34,35].

Ultrasonication: The cavitation mechanism used for the ultrasonication method. Initially the solid lipid and liquid lipid melted at a temperature higher than the melting point of solid lipid. The drug substance needs to add the lipid mixture. The next step involves preparing the aqueous phase of surfactant heated at the same temperature and adding drop by drop to the lipid mixture with constant stirring. The obtained emulsion is sonicated by using a probe sonicator.

Microemulsion method: Lipid mixture containing drug mixed with surfactant and co-surfactant with proper ratio to form a microemulsion. The prepared microemulsion (hot) is diluted with cold water so that breaking of microemulsion takes place which forms nanoemulsion.

Phase inversion method: This method involves the use of heating as well as the cooling cycles for the formulation component. During this cycle the temperature used in increasing order of $4^{o}C$/min from $25^{o}C$ to $80^{o}C$ and bring back the temperature to $60^{o}C$. Due to this Thermal treatment inversion of emulsion occurs.

Solvent emulsification/Evaporation methods: This method involves the dissolution of solid lipid and liquid lipid with drugs in an organic solvent (water - immiscible). Then it is emulsified in an aqueous phase using high shear homogenization. Instead of using temperature, low pressure (40-50 bar) is used to evaporates the organic solvent. This method is suitable for the thermo labile drug as the method use low - pressure technique. However presence of residual solvent (as heat avoided) makes this method limited for use.

Membrane contractor method: The lipid mixture is placed in a pressure vessel above its MP(melting point). Under applied pressure, the lipids allow passing through the pores of the ceramic membrane to produce tiny droplets. With constant stirring, the aqueous phase allows to flow tangentially inside the ceramic membrane and remove droplets formed at the outlet. Then bring the preparation to room temperature so that lipid particle formed.

3. Various Methods of fabrication , procedure involved along with advantages and disadvantages of methods:

Table 3: Different methods & procedure for fabrication of NLC:

Methods of fabrication	Procedure involved	Advantages of method	Disadvantages of method	References
Hot high-pressure homogenization	Drug lipid mixture emulsified in a hot aqueous solution containing surfactant at same temperature followed by homogenization with high pressure then cool to room temp to form NLC	Simple & cost effective method	Not suitable for thermo labile drug	[36],[37]
Cold high-pressure homogenization	The melted drug–lipid mixture solidified using liquid nitrogen or ice and milled to get microparticles. Then it dispersed in cold aqueous surfactant & homogenized at high pressure below room temp. It requires more pressure (500-1200 bar) compare with HPH	Suitable for thermo labile drug &large scale production	The presence of macroparticle affect dispersion quality	[37],[38]
Ultrasonication	Methods involve direct mixing of melted lipid phase with heated with aqueous surfactant solution using ultrasonication. Probe Sonication is more useful to obtain the narrow distribution of NLC.	Simple & feasible for production as significant available of ultrasonicator	Large polydispersity & moderate product stability	[39]
Microemulsion	The lipid–drug mixture is dispersed in the hot aqueous solution of surfactant at the same temperature to form a microemulsion. Then hot micro emulsion is poured into cold water to form nanoemulsion which will produce NLC upon recrystallization.	Scale-up process easy	Dilution of the particle due to high volume of water, a high concentration of surfactant used	[40],[41]
Phase inversion technique	Under this method mixture of lipid, drug, surfactant, and water is formed by stirring & exposed to heat & cold cycle (3 cycles). Then dilute with cold water to induce shock which will produce NLC by phase inversion.	Suitable for thermo sensitive drugs, avoid using organic solvents	The process is complex & require more time	[42]
Membrane contractor	This method involves the passing of melted lipid over the membrane to produce tiny lipid particles & at the same time aqueous phase is circulated in the membrane to remove lipid droplets from the pore. Then cool at room temperature.	Simple methodology	It may not be more effective as particles may stick to the membrane	[43],[44]
Solvent diffusion method	In this method, an organic solvent such as benzyl alcohol is used to dissolve lipid. To maintain the thermodynamic equilibrium organic solvent is saturated with water. The o/w emulsion is diffused into the water with continuous stirring to produce solidification of the dispersed phase.	Water miscible solvent used	Use of organic solvent	[4],[7]
Solvent emulsification evaporation	The lipid dissolves in a water-immiscible solvent like cyclohexane. After that it is emulsified in an aqueous surfactant solution with continuous stirring .then lipid is precipitating on the removal of organic solvent	Suitable to thermo sensitive drug	Use of organic solvent Ultrasonication required	[45]
Solvent Injection method	Dissolve lipid in water-miscible solvent & quickly inject the preparation into an aqueous solution containing surfactant through the needle.	Easy process	Use of organic solvent	[46],[47]

4. Different parameters of NLC formulation , its description and test methods :

Table 4: Characterization of NLC:

Parameter	Description	Test method	References
Morphology (particle size & distribution)	- Stability of NLC mostly affected by Particle size & distribution -Particle with smaller size & limited distribution tend to reduce aggregation &improve physical stability - Increase amount of liquid lipid may increase particle size - Low concentration of surfactant produce larger NLC particle compare with the high surfactant-to-lipid ratio	-Transmission Electron microscopy (TEM) - Scanning electron microscopy (SEM) -Dynamic light scattering (DLS)	[31],[50],[51]
Zeta potential	-This parameter analyze the NLC repulsion of particle & measure the long term stability -Greater surface charge increases electrostatic repulsion & decrease aggregation between particle -The stable Nanostructured Lipid Carrier should have a minimum ZP of ±30Mv -Formulation parameters like liquid lipid & SL concentration and surfactant nature have a significant impact on NLC surface charge -Higher LL to SL ratio, the impact is less as LL mostly negative charge	- Dynamic light scattering (DLS)	[52],[17]
Crystalinity	-Lipid crystal lattice structure affect encapsulation efficiency and drug release rate from NLC -Amount of drug-loaded, viscosity of preparation, and storage time have an impact on the Crystalinity of NLC -More the crystal lattice imperfection, more the encapsulation of drug due to entrapment & housing of drug-enhanced	Differential scanning Calorimetry (DSC) X-ray Diffraction (XRD)	[53],[54]
Drug load & Encapsulation efficiency	-The nature and amount of drugs have a significant impact on entrapment efficiency - There is inverse relationship was observed between the amount of drug-loaded and entrapment efficiency - Lipophilic drug uniformly solubilized in LL/SL mixture and entrapped for a long period	Ultracentrifugation & spectroscopic analysis	[55],[56]
In vitro drug release	- Factors such as liquid lipid quantity, type of solid lipid, the surfactant used, the quantity of drug and location in NLC, pH of medium affect the drug release - Release of drug from NLC controlled by diffusion of drug or erosion of matrix which depends on drug entrapped in NLC core, in the matrix or the shell. - Due to more surface area & shorter diffusion path, small particle size results in faster drug release compare with larger particle	Dialysis bag method, Franz diffusion cell	[57]

4.1: Parameter of NLC formulation:

Morphology: The effectiveness of NLC formulation depend on particle size and shape. The study shows that particle of NLC formulation ranges from 10-1000nm. However depending on site- specific action, the range may vary or be specific (50-300 nm for chemotherapeutic agent). The physical stability of NLC formulation depends on particle size & its distribution throughout the formulation. The parameter like entrapment efficiency, cellular uptake, the potential for a target is affected by the shape of particles present in the formulation. The analysis technique such as TEM (Transmission Electron Microscopy) and SEM (scanning Electron Microscopy) is necessary to find out the shape of the particle. Generally, NLC formulation shows the spherical particle with low surface area[31].

Surface charge: The surface charge of particles affected by the concentration of lipid mixture and surface active agent. The term 'Zeta potential ' is used to measure the surface charge. The determination of zeta potential (ZP) of a particle based on electrophoretic mobility. Higher the zeta potential value (> +30 mV) lesser the particle aggregation. Generally, the dispersion should have ZP either more than +30mV or less than -30mV[52].

Entrapment Efficiency(EE): Entrapment efficiency is the percentage amount drug entrapped in particle and determine the efficiency of the formulation. The lipophilic drug entrapped more as compared with hydrophilic one as the drug easily solubilized in lipid. The release rate of the drug in NLC formulation is significantly affected by EE as a high entrapment value changes the concentration gradient [55]. The formula for EE is as below

$$EE=[(WI-WS)/WI] \times 100$$

Where WI : Initial amount of drug added to the formulation
WS: Amount of drug present in the supernatant
WL: Total weight of lipid in formulation

In vitro drug diffusion study: A Dialysis bag is used for in-vitro drug diffusion study. Initially the dialysis membrane activated by soaking with distilled water overnight for better results. The prepared NLC formulation is required to be put inside the bag and both ends to be sealed . The total experiment need to conduct under sink condition. At specific time intervals samples are withdrawn and replaced with fresh media. The sample is analyzed with UV-visible spectrophotometer[57].

5. Screening methods for solid lipid & Liquid lipid:

Liquid lipid: Liquid lipid can be selected depending on the solubility of the drug in it. The excess amount of drug added to 2 ml of various liquid lipid in a small vial. Then the vial stoppered tightly & continued stirring with the help of a mechanical shaker at 25^{o}C for 24-48 hours followed by centrifugation for 30 minutes at 37^{o}C. The collected supernatant was suitably diluted & analyzed with a UV-Vis spectrophotometer [48]

Solid lipid: one of the methods used to select solid lipid-based on the solubility of the drug in it. This can be performed by incremental addition of the drug to solid lipid at above its melting point until the excess of drug fails to dissolve in it. Usually, solid lipid has to be melted above 5-10^{o}C of its melting point. Then depending on the solubility of drug amount in various solid lipids it can be chosen for NLC formulation [49]

6. Lipid matrix crystalline behavior:

The crystalline behavior of the lipid matrix is to be studied as it is fundamental to optimize the formulation. The melting point depression (temperature much below its melting point) of the liquid mixture is responsible for the crystallization of lipid. The crystallization of lipid occurs only when the lipid blend of NLC cooled below its CTT (critical crystalline temperature). The crystallization of the internal structure of lipid determines the shape of the particle, amount of drug incorporation, and stability of the formulation. The characterization of Crystalinity of NLC study utmost importance as encapsulated drug undergoes polymorphic changes leads to leakage of the drug, impact on release rate and encapsulation efficiency [2,58]. There are structural changes of lipid during heating and cooling of the mixture, which lead to different polymorphic formations [59]. Therefore control of transition of the polymorphic form allows the metastable crystalline form to entrap more drugs [60] and stable polymorphic forms of nanoparticles are formed.

Depending on the cooling rate of NLC preparation and solidification of starting material, the nucleation process starts from the inner layer of lipid [61,62]. That is why depending on the preparation process and composition, the internal structure of lipid particle has various conformations like gel, liquid crystal, etc.

The study also reported melting point of the stabilizing agent can affect the lipid polymorphic form of thermodynamic stability. The melting point of stabilizer greater than 50^0c maintains the lipid in low thermodynamic stability as compared with lipid having melting point $<0^o$c (which favors stable polymorphic transition)[63]. Two possible ways that the crystallization process modulation is mediated by lipid having low molecular weight. In the first way interaction between molecules of low molecular weight lipid with triglyceride molecules. On the other hand, heterogeneous nucleation process induction leads to organized of minor lipids into the micellar structure.

DSC (Differential scanning calorimetry) and X-ray diffraction are the two possible methods that investigate the crystalline status.DSC gives information about the change in physical & chemical properties as a function of temperature due to heat loss or gain. This information tells about the status of lipid, crystallization, and melting of solid lipid used in NLC [23].DSC is used to analyze the crystalline nature of lipid in a pure state and after processing (freeze-dried powder). The solid lipid & liquid lipid compatibility identified by DSC and help to analyze the polymorphic transition. The degree of Crystalinity or RI (recrystallization index) can be measured by DSC data

$$RI\ (\%) = \frac{\Delta H \text{ of NLC}}{\Delta H \text{ of bulk lipid x concentration of lipid}} \times 100$$

Where,

ΔH of NLC = melting enthalpy of 1g NLC preparation

ΔH bulk = melting enthalpy of 1g bulk lipid

ΔH is given in j/g & concentration given in percentage

XRD is the technique that helps to determine crystal structure & various polymorphic forms and reveals compound polymorphic structural changes. In different ways, lipids may aggregate to give polymorphic forms like micelles, laminar phases. Wide range X-ray scattering (WAXS) and small-angle X-ray scattering (SAXS) give information on layer arrangement, polymorphic behavior, crystal structure. The length of long & short spacing of lattice and drug localization in it can also be studied by X-ray diffraction [64,65].

7. NLC stability concern:

The long-term storage of NLC may lead to aggregation due to perikinetic-flocculation (flocculation due to Brownian motion of colloidal particles). A pearl-like network arrangement observed with NLC of highly concentrated dispersion leads to prevent collision and as a result, perikinetic flocculation can be avoided. This pearl-like network converts to fine particles once it is in contact with gastric fluid on administration [57]

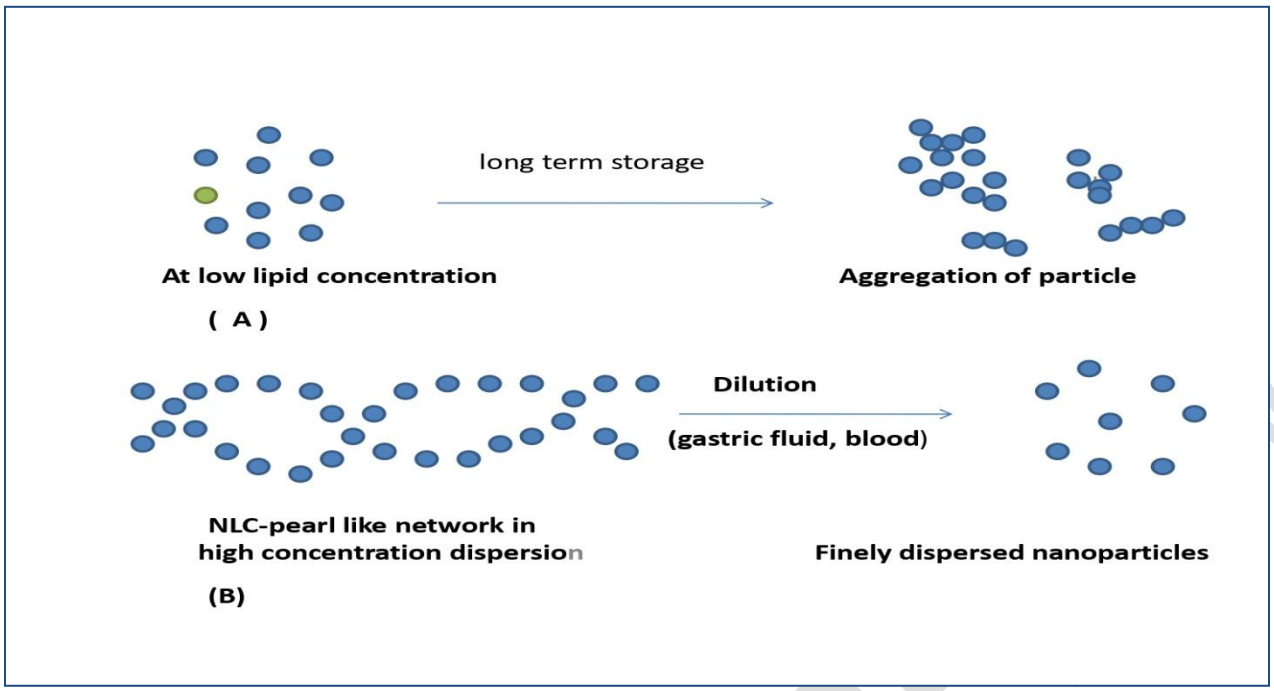

Figure 3: stabilization effect A) particles collide to form an aggregate in low lipid concentration dispersion B) In high concentration NLC dispersion, pearl-like network dispersed into fine particles upon dilution

As NLC formulation possesses less water in comparison with solid lipid nanoparticles, then care must be considered to avoid bacterial growth and changes in initial particle size. There are two possible ways to preserve the stability of NLC. One is to remove water content by freeze-drying (by converting nanoparticles liquid dispersion to solid). The second possible way is to add a preservative to NLC preparation [66,67]. Generally, the freeze-dried nanoparticles should maintain stability by preventing changes in particle diameter, reducing reconstitution time, and maintaining the appearance while maintaining drug activity [68]. As the freeze-drying process leads to aggregation of particles it is necessary to add cryoprotectant. A group of researchers (Beloqui *et al*) conducted the study to know the effect of cryoprotectant on NLC formulation by taking different concentrations. of trehalose, sucrose, sorbitol (5,10,15% W/V) respectively [69,70,71].The study concluded that trehalose is effective to prevent the aggregation of particles. Another study was conducted by Varshosaz *et al* using microcelac, Avicel PH 102, Avicel RC 591, Mannitol, and sucrose at different concentrations and found that Avicel RC 591 at 1% concentration exhibit effective agent to prevent the increase of particle size [66]. So it needs to be attention for the formulator that the lyophilization process only does not improve stability but required adding cryoprotectant for it. Another way to prevent the instability of NLC is the use of preservatives. Obeidat et al conduct a study by using eleven different preservatives and study their influence on particle size, ZP (zeta potential) & other physical stability of NLC formulation loaded with Q10. They collected the sample at 3,6,12 month intervals (sample store at room temp.) and the result found that seven preservatives out of 11 show efficacy for the stability of NLC formulation (Hydrolite 5 was the best effective preservative)[67]**.**

In topical NLC preparation preservative is added to maintain physical stability but preservative also causes destabilization of NLC. So preservatives are categorized into various types based on their impact on NLC preparation.

Table -5:Different preservatives & their impact on NLC stability

Example of preservative	**Impact on stabilization of NLC**
Ethanol	Preservative causing major stability problem
Caprylyl glycol	Minor stability issue by preservative
Pentylene Glycol (pentylene + propylene glycol)	Preservative with stabilizing effect
Propylene glycol	Have no impact on the stability

A multifactorial phenomenon is related to physical stability or the effect of destabilization. Examples of such factors are the nature of particle stabilizer, the affinity between particle surface and preservative, a preservative with stabilizer layer interaction, anchoring of stabilizer onto/into the surface, preservative ability to reduce zeta potential, surface hydrophobicity of particle[67].

8. **Skin as targeting organ for NLC:**

8.1. *Skin barrier***:** Human body covers the skin as the largest organ having a surface area of approximately 2 sq.m. It serves as a permeability barrier against the transdermal absorption of many biological agents [72]. Skin acts as a major factor to determine drug delivery aspect such as permeation & drug absorption through the dermis. The skin is composed of mainly three layers such as epidermis, dermis, and lower layer of adipose tissue. The stratum corneum (SC), the outermost layer of skin is the rate-limiting barrier for the movement of various chemical substances. The coenocytes of the stratum corneum embedded in a lipid matrix have a significant role in the permeability of substance. Lipids that are present in SC are ceramide, phospholipids, sterol ester, cholesterol-3 sulfate & free fatty acid. The stratum corneum also contains sebaceous lipid (composed of triglyceride, wax ester & squalene). This organized structure of lipid is completely related to barrier properties of skin [73]. The factors which are responsible to target skin for NLC are skin permeation, skin hydration & elasticity, skin occlusion, Transepidermal water loss

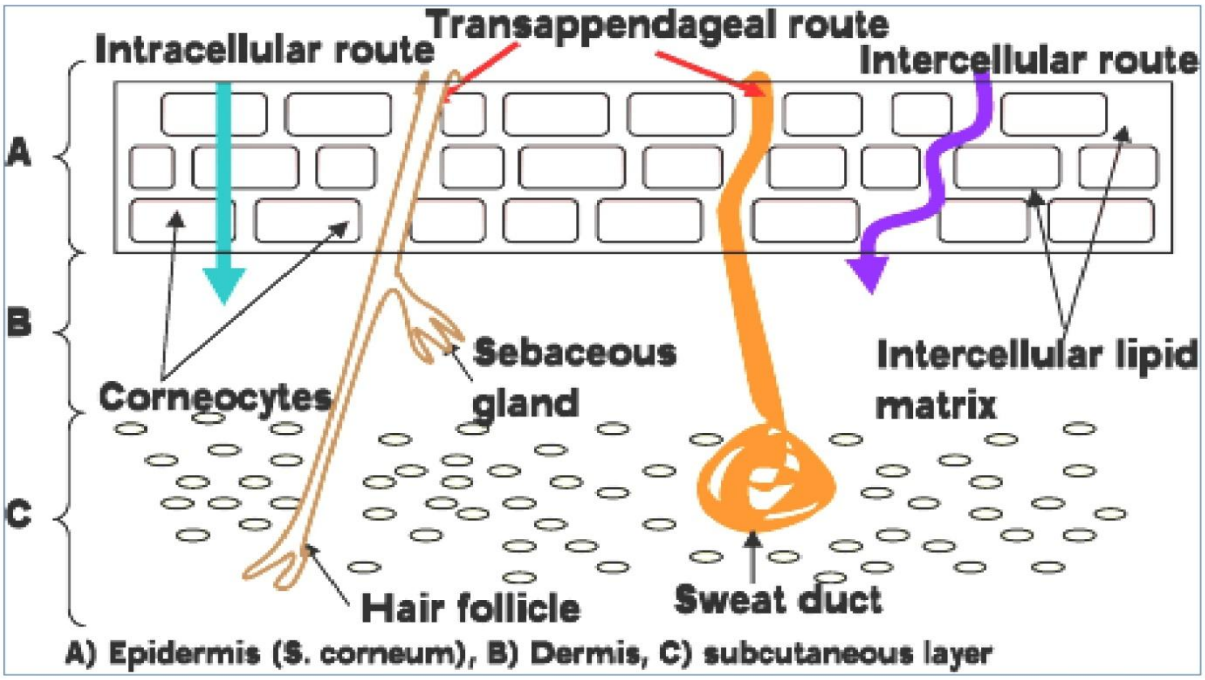

Figure -4: Drug penetration through the different route of the skin

8.2. Skin permeation: The Transepidermal pathway contains intercellular and transcellular routes as micro pathways for the transport of drugs. As two pathways involved in the transport of drugs through intercellular lipid, more research work focused on to understanding the organization of the structure and composition in the SC. The following mechanism involves drug penetration through the skin

i) Lipid present in formulation mediating increased transdermal drug delivery through the appendegeal route.

ii) By skin fluidizing property lipid acts as a penetration enhancer.

iii) Direct skin-carrier drug exchange through 'collision complex transfer'.

Among the appendageal route, the hair follicle is the most penetration pathway for NLC. As NLC contains more lipid as a component it may exchange with skin lipid and facilitate drug penetration. However other factors responsible for drug permeation are particle size, aggregation form, the solubility of a particle in skin lipid, particle surface charge, and capacity to form a film over skin[7].

8.3. Skin hydration: The hydration state of the stratum corneum normally ranges from 10-20%.The content of lipid and water has a significant influence on the skin frictional resistance. The presence of biocompatible lipid in NLC produces occlusive action which enhances skin hydration. Due to skin hydration, corneocyte packing is loosened and an expanded gap leads to more drug penetration [74]. As a particle of less size in NLC, the capillary channel of nanometer pores will be very smaller. Hence decrease the hydrodynamic evaporation of water [75].When the concentration of lipid is more in formulation leads to more occlusion resulting in increased hydration. Corneometer is the instrument used to measure skin hydration. This instrument measures the conductance of the dielectric medium. The dielectric properties changes as the skin hydration level increases.

8.4. Transepidermal water loss (TEWL): It is a good indicator to know the impaired barrier function of SC. It is the passive evaporation of water to the environment through the skin due to vapor pressure gradient. The increase in TEWL indicates disruption of SC and depletion of intercellular fluid [6]. When NLC is used in topical the TEWL is lesser due to skin occlusion resulting in skin hydration. The nanosizes of the particle of NLC have more surface area and improve the particle contact with the stratum corneum. The lipid particle forms a thin film over the skin and reduces the evaporation of water. The other factor responsible for TEWL is the size of the particle, amount of lipid, and presence of emollients in the formulation [76,77]

8.5. Skin occlusion: occlusion involves hydration skin due reducing of TEWL.The presence of lipid in formulation produces film over skin leads to occlusion effect. With lipid formulation like NLC 'controlled occlusion effect' can be achieved by i) at a given lipid concentration the occlusion effect can be increased by reducing the particle size or ii) at a given particle size by increasing the lipid concentration [76]. The characteristics of lipid such as low melting point & high Crystalinity can attribute more occlusion effect

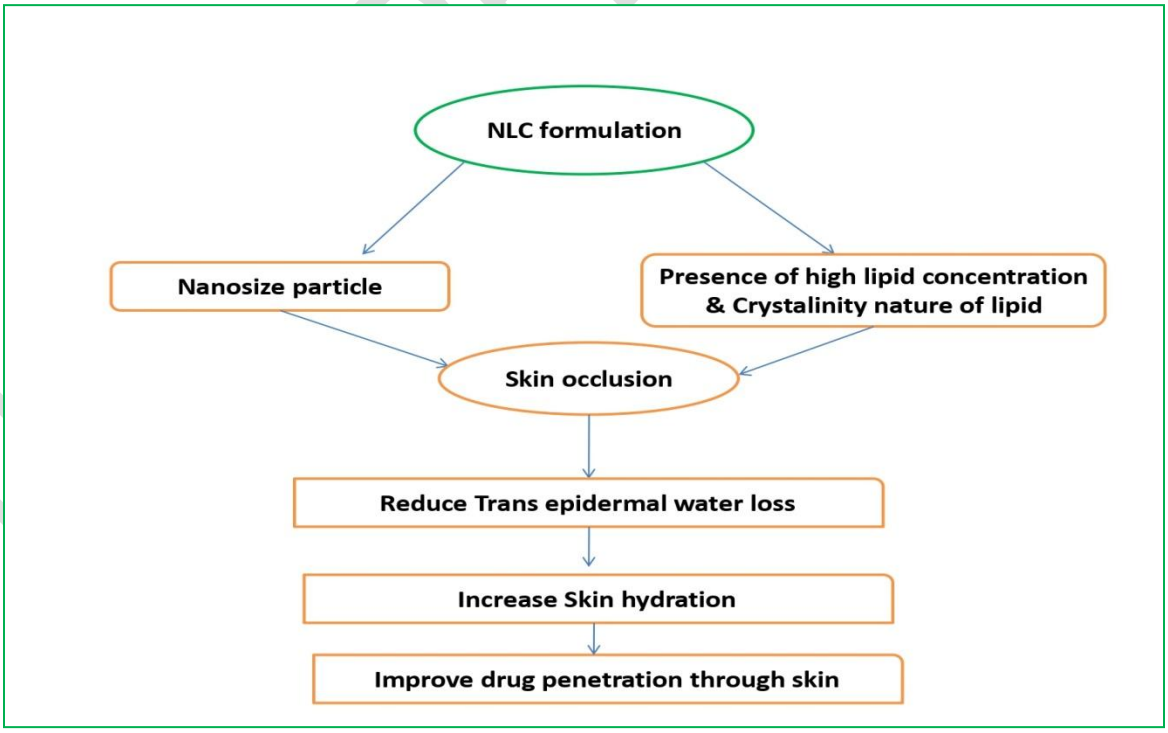

Figure -5: Skin as a target organ for NLC

9. List of the drugs used to prepare NLC topical formulation along with their research outcomes:

Table 6: Literature survey on the drug used in NLC topical formulation for targeting skin:

Name of drug	Category	Dosage form	Method used	Composition of formulation(solid lipid, liquid lipid, surfactant)	The particle size & Zeta potential	Encapsulation efficiency	Research output	References
Aceclofenac	Non-steroidal Anti-inflammatory drug for rheumatoid arthritis	Gel	Ultrasonication/high-speed homogenization	Stearic acid, oleic acid, Tween 80	< 500 nm	75-85%	Optimized formulation converted into topical gel &shows the sustained release profile	[78]
Betamethasone	Glucocorticoids for treatment of atopic dermitis	Ointment	Melt emulsification method	Precirol ATO 5,oleicacid,Tween 80,span 80	169.1 nm, −23.4 mV	85%	-Topical ointment NLC formulation shows high skin retention (35.43 µg/g) and low penetration (0.87 µg/ml) -More advantage for skin retention as it was better for drug release	[79]
Clindamycin	Antifungal for treatment of Acne	Gel	High-pressure homogenization	Stearic acid, oleic acid, Pluronic F- 68	258.83 nm,-19.0 mV	-	-Topical gel formulation shows stability with short term stability study -No change of pH,viscosity& consistency	[80]
clotrimazole	Antifungal drug for skin occlusion effect	semisolid	Hot high-pressure homogenization	Dynasan116,tyloxapol, Migloyl 812	<1 µm	>50%	- Research carried out for both SLN & NLC for topical delivery -particle diameter same after 3 months for SLN & NLC -NLC shows a faster release profile than SLN	[81]
Dexamethasone	Corticosteroid for allergic reaction and skin inflammation	hydrogel	Ultrasonic homogenization	Compritol ATO888, Mygliol 812, Tween 80 and Span 80	224.4 nm	-	-Research shows hydrogel containing NLC was 7.3 times higher than dexamethasone ointment -Skin deposition of hydrogel was 3.8 times more than solution	[82]
Diclofenac	Non-steroidal Anti-inflamatory drug for pain and inflammatory	Gel	Hot high-pressure homogenization	GMS, lanolin PEG-75, Phospholipon® 90G,precirol ATO 5, Tween 80	<126 nm	78.26%	-Research reported that high drug loading achieved by smaller particle size which improved drug penetration & in vivo efficacy improved	[83]

	condition							
Etoricoxib	COX-2 inhibitor for treatment of inflammation & allied condition	Gel	Melt emulsification & low-temperature solidification method	Stearic acid, oleic acid, Tween 80	244 nm,-11.9 mV	69-76%	-Invitro drug release pattern experience brust effect & prolong release -Zeta potential value predict good stability	[84]
Flurbiprofen	Platelet aggregation inhibitor for treatment of gout, rheumatoid arthritis	Hydrogel	Hot high-pressure homogenization	Dynasan114,Epikuron 200,cpatex 355,polysorbate 80	150-300 nm,-21.7 mV	>90%	-formulation done for SLN & NLC -NLC shows faster release compared with SLN -NLC shows sustained release over 24 hr.	[85]
Ibuprofen	NSAID for treatment of osteoarthritis and other musculoskeletal diseases	Gel	Hot high-pressure homogenization	Witepsol E85, Miglyol 812, Lutrol F68	106 nm,-18.4 mV	98.51%.	NLC gel is of great potential to increase drug permeation through the skin and enhance the efficacy	[86]
ketoprofen	NSAID for treatment of musculoskeletal disorders	Gel	Melt emulsification& low-temperature solidification method	Compritol 888 ATO, Labrafac Lipophile, LutrolF68	298 nm	77%.	-Drug-cyclodextrin complex loaded to NLC -NLC hydrogel exhibit better permeation than plain drug-loaded NLC	[87]
Lansoprazole	PPI (proton pump Inhibitor), objective to protect drug form gastric degradation	Hydrogel	Ultrasonication	GMS, Stearyl amine, Pluronic F65	90-210 nm,-61.9 to +3.2 mV	-	NLC hydrogel showed that drug elimination significantly reduced and prolong the mean residence time	[88]
Lidocaine	Local anesthesia	Gel	Ultrasound dispersion method.	Compritol 888 ATO, Precirol ATO 5, Miglyol 810	72.1 nm	95.9%,	-Formulation prepared with three carriers (NE, SLN, NLC) - NLC gel resulted in a six-fold increase in the duration of anesthesia compared with a market gel product	[89]
Methotrexate	Used for Rheumatoid arthritis	Hydrogel	Hot micro-emulsion method	Phospholipon S 100, Gelucire® 50/13, Transcutol®P	181.5 ± 11.5 nm, −16.58 ± 1.8 mV	-	-NLC gel formulation along with chemical enhancer (CE) can improve therapeutic efficacy compared with only NLC gel (without CE)	[90]
Minodoxil	used in case of Alopecia	Gel	Melt dispersion	Tristearin, Oleic acid, Tween 80, soya	280 nm, 42.40 mV	86.09%	A biphasic release pattern was observed in NLC gel and provided	[91]

			Ultrasonication	lecithin, Pluronic F-68			a fast release initially for skin saturation followed by a slow-release profile to maintain the skin concentration.	
Nebivolol	Used for hypertension (Selective β1-blocker)	Gel	High-pressure homogenization	Glyceryl monostearate, oleic acid, Span 80, Cremophor EL	228 nm, -29mV	95%	-Increase encapsulation efficiency along with stability and sustainable transdermal effect has been observed.	[92]
pioglitazone	Antihypertensive	Gel	High-pressure homogenization	Apifil, labrasol, Carbopol,Tween 80	81.33 to 181.87 nm,-27.5 mV	63.46–87.56%,	- The pharmacokinetic study showed 2.17 times enhanced bioavailability in comparison to oral tablet	[93]
Quercetin and resveratrol	Anti-cancer	Gel	Melt emulsification and ultrasonication	Precirol ATO 5, Compritol ATO 888, Labrasol, Labrafil M2125CS, Labrafil M1944CS, Captex GTO	191 nm ± 5.20 ,−10.00 mV ± 0.30 a	92.85 ± 0.25%	-NLC gel formulation evaluated for permeability study - The enhanced drug deposition in the epidermal layer was observed through dermatokinetic and CLSM studies.	[94]
Sildenafil	Phosphodiesterase type 5 inhibitor, used for erectile dysfunction	Gel	Modified high-shear homogenization technique	Cetyl palmitate, Glycerol monolinoleate, Cremophor® RH 40, Span 85	<1µm	97.5%,	- Formulation was prepared with both SLN & NLC - Improved SC transdermal permeation & prolonged action	[95]
Tadalafil	Phosphodiesterase-5 inhibitor, used for erectile dysfunction	Gel	Hot-melted ultrasonic method	Glyceryl monostearate, Oleic acid, Tween 80	< 0.5 µm.	89.6%,	-The Tadalafil-loaded NLC dispersion with skin permeation enhancers (ethanol and limonene)exhibited the highest flux - Tadalafil-loaded NLC gel with selected permeation enhancers showed tolerance against toxicity in HaCaT cells.	[96]

10. List of Different patents based on Nanostructured lipid carrier :

Table 7: List of patents for Nanostructured lipid carrier formulation:

Patent number	Patent publication date	Title of the patent	Description	Applicant of patent	References
RO135202	30.09.2021	Process for dual encapsulation of two categories of bioactive plant-based principles in the same nanostructure distributio n system	The invention comprises a dual nanocarrier .The NLC formulation contains licorice extract & wild yam extract which provide sustained release and enhance antioxidant & anti-inflammatory effect.	Ac helcors.r.l.	[97]
EP3876913	15.09.2021	Artificial tears	It involves the preparation of NLC consisting of solid lipid outer shell & liquid lipid as the liquid core. It used for dry eye disorder	Waterford institute of tech	[98]
AU2021104317	19.08.2021	An Artemether-Loaded Nanostructured Lipid Carrier (NLC) Nanogel Composition and A Method for Formulation of the Artemether-Loaded (NLC) Nanogel	The research involves the preparation of lyophilized NLC formulation and covert into a gel for the treatment of malaria.	Nnamani, Petra Obioma	[99]
AU2021104270	19.08.2021	Desvenlafaxine succinate loaded Nanostructured lipid ca rrier (NLC) for brain targeting via nasal route	The NLC preparation improved the bioavailability of the lipophilic drugs by crossing the Blood - Brain Barrier through the nasal route and producing an antidepressant effect	Fatma, Bushra Kumar, Vikram Kushwaha, Swatantra K S Mantry, Shubhrajit Mohanto, Sourav Srivastava, Dipti Tiwari, Pallavi	[100]
CN113041234	29.06.2021	Cannabidiol lipid nanoparticles, freeze-dried powder, and preparation method	Encapsulation of Cannabidiol in NLC for improvement of bioavailability & sustained release	Shanghai normal university, East china university of science and technology	[101]
AU2021102817	17.06.2021	Novel formulations of 5-fluorouracil against diabetic retinopathy and process thereof.	Present research involved for preparation of 5-Fluorocil loaded NLC with enhanced drug absorption and molecular targeting for diabetic retinopathy	Lovely Professional University	[102]
CN112641727	13.04.2021	Antioxidant water-in-oil-in-water type micro-nano multiple emulsion as well as preparation method and application thereof	Modification of lipoic acid into water- soluble derivative and incorporated in NLC formulation. The obtained preparation has better antioxidant activity & improve bioavailability	Beijing Technology and business university	[103]
US20210085618	25.03.2021	Ocular drug delivery	An Ocular delivery system was prepared where the outer shell is solid lipid and liquid lipid in the core. Therapeutic agent present in core used for an eye disorder	Waterford Institute Of Technology	[104]
IN202141009486	12.03.2021	Ant psoriatic effects of clobetasol loaded nano structured lipid carriers on imiquimod induced psoriasis	Preparation of novel NLC loaded with clobetasol for Psoriasis treatment	Mr. Ramesh reddy kudamala, prof.venkata satyanarayana suggala,	[105]

				prof.jayasankar reddy veeram, Dr. sucharitha palagati	
MYPI 2019005424	19.03.2021	Nanostructured lipid carrier co mposition and method for enhanced trans-epidermal absorption of Ficus deltoidea extract	The NLC preparation contain Ficus-detoides extract improve epidermal absorption so that antioxidant & anti-inflammatory action improved	UniversitiTeknologi Malaysia	[106]
KR1020200117 345	14.10.2020	Nanostructured lipid carrier co ntaining econazole and film-forming topical pharmaceutical composition containing same	Topical film-forming NLC formulation containing econazole for long term anti-bacterial effect	The Industry Amp; Academic Cooperation InChungnam National University (IAC)	[107]
KR1020200051 997	14.05.2020	Cosmetic composition for delaying skin aging containing active ingredient stabilized with nanostructure lipid carrier	NLC preparation contains retinol, tocopherol and glutathione as core active ingredient for the treatment of skin wrinkle with enhanced stability	Coreana Cosmetics Co., Ltd.	[108]
IN2018110212 13	13.12.2019	Novel nanostructured lipid carrier-based ophthalmic controlled release formulation for treatment in fungal keratitis	The poor solubility & low permeability nature of the drug is used to formulate NLC for the treatment of fungal keratitis. The research aim was to improve retention time & increase solubility in intraocular tissue.	Manish Kumar Ajay Pathania Vipin Saini A. Pandurangan Shailendra Bhatt PrernaSarup	[109]
MYPI 2018300001	22.07.2019	A Nanostructured solid lipid carrier encapsulates bromelain extract	The objective of the invention was to prepare Bromelain extract loaded NLC for better penetration and stabilize the physicochemical properties of extract	Universiti Teknologi Malaysia	[110]
CN109602706	12.04.2019	Ferulic acid nanostructured lipid carrier and preparation method thereof	The objective was to prepare Ferulic Acid (FA) loaded NLc with a lipid concentration 2.7 % . The hot melt emulsification method used to prepare FA-loaded NLC for better entrapment & stability	Shaanxi University of Chinese medicine	[111]
AU2018285694	20.12.2018	Nanostructured lipid carriers and stable emulsions and uses thereof	To prepare NLC containing lipid phase along with other oil core which delivers the active ingredient to cell for generating of immune response like the vaccine	Infectious Disease Research Institute	[112]
CN108853054	23.11.2018	Cyclic peptide modified gambogic acid nanostructured lipid carrier and preparation method thereof	The prepared NLC formulation with modified gambogic acid is able to target tumor & strong penetration effect in tumor tissue.	Tianjin University of traditional Chinese medicine	[113]
CN107115531	01.09.2017	Nanostructured lipid carrier modified by glycolipid polymer as well as preparation method and application of Nanostructured lipid carrier	The method involves for encapsulation of hydrophobic drug A-317491 in NLC by using modified glycolipid polymer. The concentration of drug has improved in ectopic endometrium tissue.	Zhejiang University	[114]
US15163724	26.01.2017	Topical nano drug formulation	The prepared NLC gel contains spironolactone as a biomolecule for acne vulgaris disorder. The	Hamidreza Kelidari Majid Saeedi	[115]

			formulation shows improved skin penetration and drug release.		
CN106176677	07.12.2016	N-acetyl-L-cysteine modified curcumin nanostructured lipid carrier used for oral administration	Work- based on the preparation of NLC load with curcumin. The formulation contains N-acetyl-L-cysteine modified accelerator which improves the solubility of curcumin in water & improves oral bioavailability.	China Pharmaceutical University	[116]
WO2016065444	06.05.2016	Method for producing nanostructured lipid carriers on triblock copolymers, nanostructured lipid carriers thereby produced and uses thereof.	The Present invention related to producing NLC with triblock copolymer (one liquid lipid & two solid lipids) using hot homogenization as the method. This cosmetic preparation is used for moisture retention in the hair fiber.	UniversidadeEstadual De Campinas - Unicamp [BR]/[BR]	[117]
IN276/MUM/2014.	11.09.2015	Idebenone lipid nanocarrier composition for the treatment of neurodegenerative disorders	The nanoprecipitation technique is used for formulating NLC by the solvent evaporation method. The preparation containing Idebenone as biomolecule for treatment of Alzheimer's disease	Sachin Subhash Salunkhe	[118]
CN104367549	25.02.2015	Psoralen-doxorubicin-loaded composite nanostructured lipid carrier preparation and preparation method thereof	The investigation includes Psoralen-doxorubicin as two active biomolecules used to prepare NLC. The formulation contains 40-120 part of SL & 10-30 part of LLThe preparation used for multi - drug resistance of leukemia cells.	Liaoning University	[119]

11. Safety and toxicity**:** A group of researchers (C Vario *et al*) conducted experimental work for the safety of NLC formulation in topical route. They used Compritol ATO, Migloyl 182 as lipid, and Tween 80 and polaxomer 188 as a surfactant for formulation. The prepared formulation was applied to the skin of the rat. It was observed that formulation remains 24 hr. in application site & no systemic absorption. Hence indicate the safety of formulation. Rahman et al carried-out research work using Zerumbone loaded NLC to know the toxicity of formulation. The oral route used for the experimental work uses mice as the animal. The formulation was composed of palm oil, Lipoid S 100, thimeosal, olive oil. The histopathological study report that the formulation does not have a toxicity effect on the kidney, liver & lungs. Bruge et al conducted research work to know the effect of various lipid carriers of NLC formulation on cytotoxicity in human dermal fibroblast using Precirol ATO 5,compritol 888 ATO, GMS, Dynasan 118,migloyl 812,softisan 100, and polaxomer 188 as ingredients for formulation. From the study, they found Compritol 888 ATO was the safest lipid as it has a neutral cytotoxic effect. V.R Salvi and P.Pawar with their research study found that because of biocompatible lipid, nonionic & biocompatible surfactant of NLC formulation without the use of organic solvent, lipid nanoparticles are non-toxic & relatively safe for ocular drug delivery [120]
12. **Conclusion**: NLC, a new generation of lipid carrier gaining more popularity as it has numerous advantages over others. The vigorous institutional research also progresses remarkably owing to its stability &effectiveness. The biocompatibility of lipid, high drug loading, prolonged-release, and non-use of organic solvent made the NLC more numerous areas for researchers. Among all the routes of administration skin targeting of NLC is the new domain for cosmetic research as well as topical formulation due to its occlusion and skin hydration effect. From the various methods of preparation HPH (High-pressure Homogenization) is considered as the most used method because of its scalability. The factor considered is its toxicity in humans to be evaluated. As day by day NLC formulation occupies more places in the market, we can predict its prospectiveness with more advancement in near future. Therefore by considering the above NLC can be termed as 'smart nano lipid carrier'.

List of Abbreviations:

NLC: Nanostructured Lipid Carrier
SL: solid lipid
LL: Liquid Lipid
ZP: Zeta potential
EE: Entrapment Efficiency
TEWL: Trans Epidermal water Loss
WAXS: Wide Angle X-ray scattering
SAXS: Small Angle X-ray Scattering (SAXS)
CCT: Critical Crystalline Temperature
PPI: Proton pump Inhibitor
NSAID : Non-steroidal anti-inflammatory drug

Ethics approval and consent to participate: Not applicable

Human and animal rights**:** Not applicable

Consent for publication : Not applicable

Availability of data and material: The data and material that support the finding of this manuscript are available on request**.**

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