Laxmidhar sahoo /J. Pharm. Sci. & Res. Vol. 12(4), 2020, 475-487

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Nanostructured Lipid Carrier (NLC) -A Promising Drug Delivery for Transdermal Application

Laxmidhar sahoo

Assistant professor,Roland instituite of Pharmaceutical Sciences,khodasingi,BerhampurOdisha,760010,india

Abstract:

Skin application of pharmaceutical products is one of the methods used for drug administration. The problem of limited drug penetration via topical application makes searching for safe drug carriers that will provide an expected therapeutic effect of utmost importance. Research into safe drug carriers began with liposome structures, paving the way for work with nanocarriers, which currently play a large role as drug vehicles. Nanostructured lipid carriers (NLC) consist of blended solid and liquid lipids (oils) dispersed in an aqueous solution containing a surfactant. These carriers have many advantages: good biocompatibility, low cytotoxicity, high drug Content,they enhance a drug's stability and have many possibilities of application (oral, intravenous, pulmonary, ocular, dermal). The present study emphasize to evoke an interest in the current art of NLC by discussing the various parameter like need of NLC,types of lipid, drug used in various NLC formulation,characterization,skin occlusion, stability factor. This analysis indicates the possibility of using NLC for dermal and transdermal drug application.

Key word:NLC,characterization ,lipids,stability,high speed homogenizer

INTRODUCTION :

In the last decade, drug delivery research is clearly moving from the micro to the nanometer scale. In simple terms, a drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of this substance in the body. The process of drug delivery includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action.[1]. The oral route is the most important and conventional method of drug administration. Unfortunately, oral drug delivery systems have many significant limitations, such as drug degradation in the gastrointestinal track (by enzymes, pH), pre-systemic metabolism or toxic side effects. One of the methods which could overcome problems associated with the oral route is transdermal drug delivery (TDD) [2-3].

Lipid-based formulations as effective dermal vehicles for drugs :

Nanotechnology is a modern and rapidly evolving trend in dermal and transdermal drug delivery which includes several forms of nanocarriers such as liposomes, nanoemulsions, nanocrystals, polymeric nanoparticles, lipid nanocarriers and dendrimers. Lipid nanocarriers show essential advantages over conventional drug forms and they are formulated with biodegradable, non-toxic and non-irritant lipids. The small size (from 40 to 800 nm) of lipid nanocarriers allows to adhere them to the lipid film of SC and to increase the number of drug molecules that penetrate into deeper layers of the skin. Moreover, they demonstrate the occlusion effect, which results in increased skin hydration, thereby improving the absorption of the drug [4-5].

Nano structured lipid Carriert (NLC) as New era for Topical Drug delivery :

Topical drug application has been introduced since long time to achieve several purposes on different levels (skin surface, epidermis, dermis and hypodermis). However, several problems have been reported with the conventional topical preparations e.g. low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation. A lot of research groups paid attention to the topical application of the SLN and NLC. Many features,

which these carrier systems exhibit for dermal application of cosmetics and pharmaceutics, have been pointed out. SLN and NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis.[74]

FACTORS RESPONSIBLE FOR PENETRATION OF NLC FORMULATION THROUGH SKIN :

Many factors are responsible for drug penetration when topical application of NLC, such as concentration & type of lipids and surfactants, NLC production method ,drug localization in the NLC .The mechanism of drug release from an NLC consists of diffusion and lipid particle degradation in the body [10]. Drug release is affected by alteration from a highly disorganized lipid structure to one with more organized stable modifications. It is ideal to achieve controlled release from an NLC that is triggered by an impulse after application. Impulses for designing skin preparations with NLC can be temperature increase or water evaporation leading to changes in the lipid structure and,in consequence, to drug release. A drug release profile might be modified according to composition of the lipid matrix, surfactant concentration and production parameters.

Advantages of Nanostructured lipid carrier [7,8]

- Feasibilities of carrying both lipophilic and hydrophilic drugs
- Water based technology (avoid organic solvents).
- Control and targeted drug release.
- Improve stability of pharmaceuticals.
- High and enhanced drug content (compared to other carriers).
- Most lipids being biodegradable,
- More affordable (less expensive than polymeric/surfactant based carriers).
- Easier to validate and gain regulatory approval.

Limitation of Nanostuctured lipid carrier

- Cytotoxic effects related to the nature of matrix and concentration
- Irritative and sensitising action of some surfactants,
- Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better exploited.
- Pay-load for a number of drugs too low

DRUG ENCAPSULATION IN NLCS :[68]

There are three ways of incorporating or encapsulating drug. within the lipid nanoparticles or NLCs.

1. Homogenous matrix of solid solution: In this method of encapsulation, the drug is homogenously dispersed into the lipid matrix of the particles and the drug release occurs by diffusion process.
2. Drug-enriched shell; In this method, the drug is concentrated on to the outer most layer or shell of the lipid nanoparticles. This type of nanoparticles exhibit burst release of the drug due to precipitation and solubilisation mechanism.
3. Drug-enriched core: In this method, prolonged release is observed due to the saturation solubility of drug in the lipid.

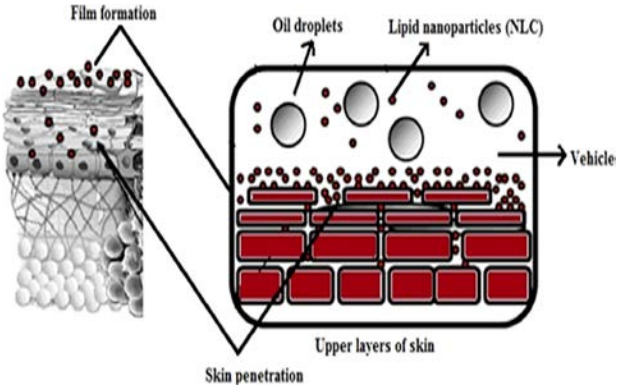
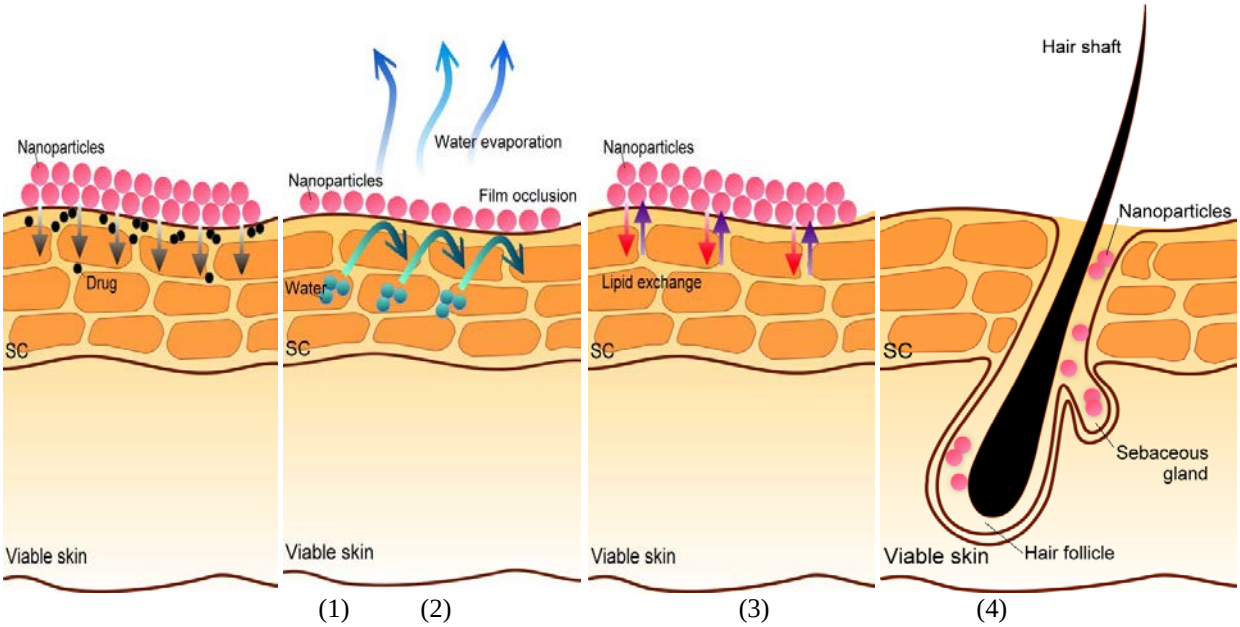


Figure:1 Mechanism of drug penetration into skin

ENHANCEMENT OF SKIN PERMEATION AND DRUG TARGETING :

The stratum corneum in healthy skin has typically a water content of 20% and provides relatively an effective barrier against percutaneous absorption of exogenous substances skin hydration after applying NLC leads to a reduction of corneocytes packing and an increase in the size of the corneocytes gaps. This will facilitate the percutaneous absorption and drug penetration to the deeper skin layers.[11]



(1) (2) (3) (4)

1. Close contact to skin surface 2. Skin hydration by particle occlusion
3. Lipid exchange between SC and NLCs 4. Entrance into follicles and sebaceous glands [111].

Figure-2

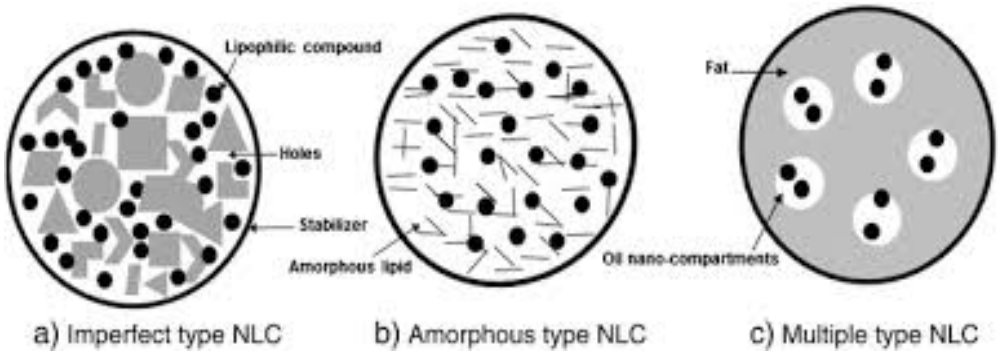


Figure-3

TYPES OF NLC:[9]

Type I (highly imperfect matrix)

Type II multiple types

Type III amorphous type

Type I (highly imperfect matrix) :

In Type I NLC, low conc of liquid lipid (oil) is used compared to solid lipid. Oil and solid lipids are blended to o/w nano-emulsion and when cooled from molten state to room temperature, forms solid particle, due to crystallization process, leads to highly disordered, imperfect lipid matrix offering space for drug molecules and amorphous structure of drug.

Type II multiple types :

In Type II NLC, there is a high oil concentration. During crystallization process, phase separation of the two lipids occurs. At certain temperature, they have miscibility gap leading to precipitation of tiny oily nano-compartment. When lipids lack drug solubilities, the addition of higher amount of liquid lipid to the lipophilic phase display the advantages of the solid matrix which prevented drug leakages while liquid lipid shows high solubility for lipophilic drug.

Type III amorphous type :

In the amorphous type of Nano structured lipid carrier , by controlled mixture of lipids, particles were created which were solid, not crystalline but in an amorphous state. This amorphous state needs to be preserved

COMPOSITION OF NLC:

In general terms, the ingredients for the preparation of NLC include a solid lipid, a liquid lipid , a surfactant and water/solvent, in addition to the active molecules to be incorporated. other agents like counter-ions and surface modifiers also incorporated. It is intended the use of starting materials classified as Generally Recognized as Safe-GRAS[12]

Excipients used in preparation of NLC (Topical) :[13,14,15,16,64,65] [Table-1)

Solid lipid	**HLB value**	**Melting point**	
Stearic acid	15	68-70°C	
Beeswax	12	62-64°C	
Dynasan 116	6-10	62-64°C	
Dynasan 114	-	55-58°C	
Gelucire 44/14	14	42-44°C	
GMS	3.8	55-60°C	
Apifil	9.4	59-70°C	
Compritol 888 ATO	> 2.0	69-74°C	
Monecol PC		45°C	
Softemul 165	>11	54°C	
Dynasan 118		71°C	
Liquid lipid			**Boiling point**
Seasame oil	7-5	−5°C	230-233°C
Ethyl oleate	10.7-14	−32°C	216-218°C
Capmul MC	3-4	25-30°C	145-149°C
Cetiol V	5-6	18.2°C	494-495°C
Miglyol 812	15	<0°C	240-270°C
Oleic acid	17	13-14°C	194-195°C
Transcutol	4.2	76°C	197-205°C
EMULSIFIER			
Pluronic F-127	22	52-57°C	
Tween 20	16.7	21°C	
Span 20	8.6	>200°C	
Cremophor RH40	13	16-26°C	
Solutol HS	14-16	30°C	
Labrasol	14	44-48°C	
Span 80	4.3	45.2°C	
Polaxamer 188	>24	52-57°C	
Gelucire 44/14	>10	44°C	

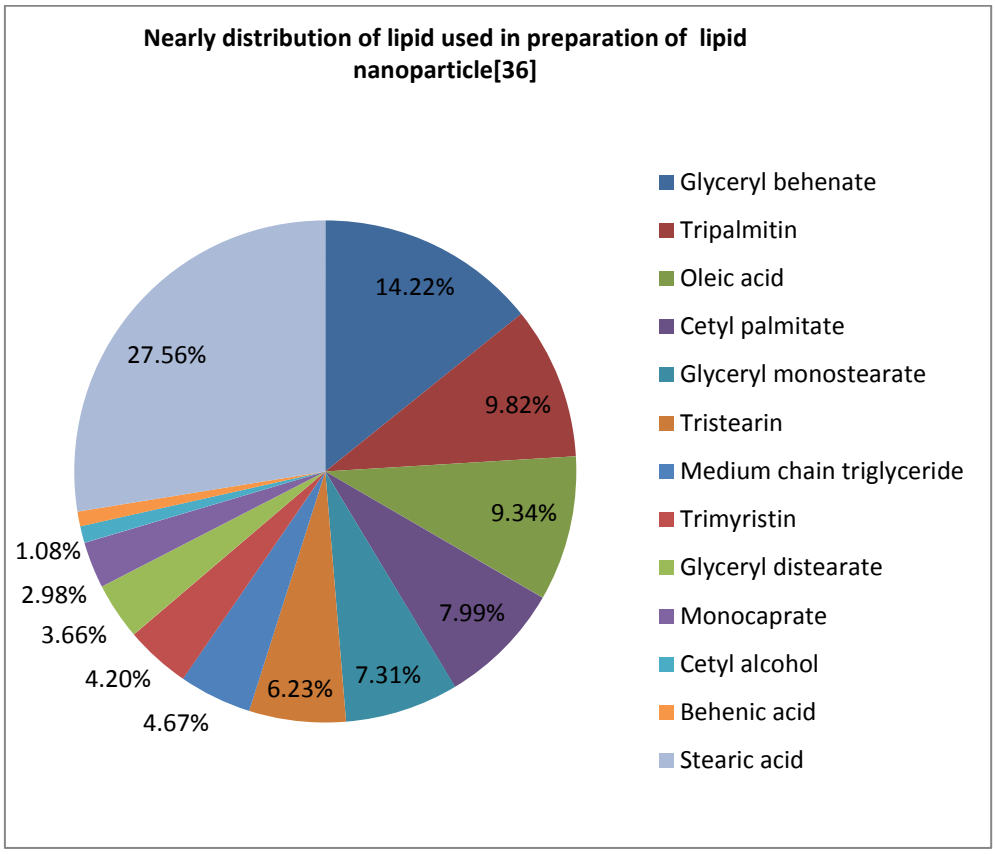


Figure-4

LIPID:

The primary component of nanostructure lipid carriers that govern drug loading capacity, prolong action and stability of the formulations is lipid. Solid lipids like fatty acids, triglyceride, diglyceride, monoglyceride, steroids and waxes have been used for formulating NLC. Physiologically acceptable, biodegradable, non-toxic and generally-recognized-as-safe (GRAS) status lipids are preferred for preparation of lipid nanoparticles . Choice of suitable lipids is essential preceding their utilization in preparation of nanoparticulate carriers. The type and structure of lipid affects various characteristics of nanocarriers. Practically, solubility or evident partition coefficient of bioactives in the lipid has been suggested as the best fitting criteria for choosing a suitable lipid. The solubility of the drug molecules in lipid is interpretative as it affects drug loading and encapsulation efficiency [17]

Screening of solid and liquid lipid :

Liquid lipid : The solubility of drug in various liquid lipids and surfactants was determined by adding excess amounts of drug in 3-5 ml of oils in small vials. The vials were tightly stoppered and were continuously stirred to reach equilibrium for 72 h at 25 ^{0}C in a mechanical shaker. After that, the mixtures were centrifuged using High Speed Centrifuge at 5000 rpm for 30 min at 37^{0}C.The supernatant was separated, dissolved in methanol and solubility was quantified by UV Spectrophotometer [83]

Solid lipid : The solubility determination of drug in various solid lipids was performed by adding drug in increments of 1 mg until it failed to dissolve further in the molten solid lipids (which were heated at 5 ^{0}C above their melting point). The amount of solid lipids required to solubilize drug was calculated. [84]

Surfactants :

The type and concentrations of surfactant exert influence on quality and efficacy of NLC. It has been found that toxicity, physical stability and crystallinity of NLC are greatly influenced by choice of surfactant [18]. Surfactant systems also have an impact on extent of drug dissolution and drug permeability. Surfactants are chosen based on of route of administration, hydrophilic-lipophilic balance (HLB) value, effect on particle size and lipid modification. Surface active agents (emulsifiers) are adsorbed on the interface where they reduce the tension between lipid and aqueous phases because of their amphipathic nature[19]

Required HLB (rHLB) plays an important role while selecting suitable type and amount of surfactant for NLC formulation.16 rHLB of lipids and lipid matrix is determined to calculate the amount of emulsifiers to be added in formulation. The rHLB value for lipid is the HLB value of emulsifier which is necessary for appropriate emulsification i.e. reduction of interfacial tension between oil and water phase. This also assists in achieving a stable nano system and small particle size of NLCs[20,21]

Other ingredients :

Organic salts and ionic polymers may be employed as counter-ions in formulation of nano structure carriers to overcome the challenge of encapsulating water soluble drug molecules. Surface-modifiers are another category of excipients used in formulation of NLC to minimize their phagocytic uptake by the macrophages in reticuloendothelial system (RES). Lipid particles are

coated with hydrophilic polymers like PEG, poloxamines or poloxamers to increase the residence time of drug molecules in systemic circulation. Surface modification may offer other advantages like enhanced physical stability and biocompatibility, drug targeting, increased transport across epithelium.[22]

METHODS EMPLOYED IN FABRICATION OF NLC:[32]

1. High-pressure homogenization Technique:
   a. Hot homogenization
   b. Cold homogenization
2. Ultrasonication/high-speed homogenization Technique
3. Solvent Emulsification Evaporation Technique
4. Solvent emulsification-diffusion Technique
5. Supercritical fluid Technique,
6. Microemulsion based Technique
7. Spray drying Technique
8. Double emulsion Technique
9. Precipitation technique
10. Solvent injection technique
11. Membrane contractor technique
12. Film-ultrasound dispersion

High-pressure homogenization technique *:*

High pressure used in homogenization technique makes it possible to avoid use of organic solvents in preparations and render them eco friendly. Additionally high-pressure homogenization is easy to scale up and an attractive technique being used in the manufacturing of pharmaceuticals and cosmetics for topical application. Hot homogenisation is performed at elevated temperature and cold homogenization is done below room temperature. Active ingredient is dissolved or dispersed in the molten lipid before to the high pressure homogenization, in both approaches. High pressure (100–2000 bar) moves the fluid in the narrow gap in homogenizer[33]

Hot homogenization :

In this approach homogenization is conducted at elevated temperature. The solid lipids are melted at a temperature above 5-10°C above their melting point. A dispersion is obtained by adding liquid lipid and drug to be encapsulated. The mixture is dispersed in aqueous solution of surfactant (s) heated to same temperature by high shear mixing device and leads to formation of pre emulsion. The pre-emulsion is introduced in high pressure homogenizer at controlled temperature. Generally 3 to 5 cycles at 500-1500 bar are sufficient for homogenization. The lipid recrystallizes and causes formation of nanoparticles as nanoemulsion is gradually cooled down. Employment of high temperature during the process may lead to degradation of heat sensitive ingredients. Another problem which may arise is reduction in emulsifying capacity of surfactants due to high temperature as surfactants have cloud point lower than 85°C. This may induce instability to nanocarriers [34,35]

Cold homogenisation :

In this technique lipid melt containing active agent is rapidly cooled to being solidify using liquid nitrogen or dry ice, then milled and ground before being dispersed in cold surfactant phase and subsequently homogenized at room temperature. Pressure used in cold process is higher i.e. 5-10 cycles of 1500 bar. This approach minimizes the thermal exposure of drug and well suited for thermolabile drugs. Improved drug entrapment efficiency and uniform distribution of drug within the lipid are other benefits of the method. However it results in nanoparticles of more variable sizes [37,38]

Solvent-emulsification evaporation method :

In this method, the lipids (solid lipid + liquid lipid) along with drug are dissolved in a water immiscible organic solvent (cyclohexane, chloroform)[39]. The obtained mixture is dispersed into aqueous solution of emulsifiers producing an o/w emulsion. Evaporation under reduced pressure is employed to remove solvent from the emulsion. Evaporation leads to the dispersion of nanoparticles in the aqueous phase (by lipid precipitation in the aqueous medium). This method avoids any thermal stress, but usage of organic solvent is a disadvantage. Particle size can vary from 30-100 nm according to the solid lipid and surfactant [38]

Solvent-emulsification diffusion method :

In this technique, solvent and water are mutually saturated to maintain initial thermodynamic equilibrium. Afterwards, the lipids and drug is dissolved in the water-saturated solvent. Solvent containing drug and lipids are emulsified in a solvent-saturated aqueous emulsifier solution by a homogenizer to form an o/w emulsion. The lipid nanoparticles precipitate after dilution with excess water (ratio: 1:5–1:10) due to diffusion of the organic solvent from the emulsion droplets to the continuous phase. The solvent can be removed by ultrafiltration or lyophilisation. Solvent diffusion is more innovative and most of the solvent employed show a better safety profile compared to volatile solvents [38].

Microemulsion Method :

Utilizing a microemulsion approach, the lipid carrier is warmed and softened, and after that drugs, emulsifier assistant emulsifier, and deionized water are added to yield a blend with a straightforward appearance and a thermodynamic strength like that of oil-in-water (O/W)-type microemulsion. The microemulsion is immediately dispersed in ice water (4 0C), shaping an NLC dispersion system. The sizes of the nanoparticles and particles from microemulsion and weakening are amazingly near the temperature distinction between the cold water and the microemulsion, which is a key factor in planning little molecule size NLCs. Quick cooling and solidification can keep the collection of a few particles. The benefits of this strategy incorporate its low drug substance and effortlessness, while the weaknesses are the plenitude of assistant emulsifier and emulsifier required.[40]

Double emulsion technique :

This method is mainly used for the production of lipid nanoparticles loaded with hydrophilic drugs. This technique overcomes the problem of escapism of water soluble moiety in aqueous phase from oily phase as investigated in microemulsion method.[41] In this method, drug is firstly dissolved in aqueous solvent (inner aqueous phase) and then is dispersed in lipid phase (Molten solid

lipid + liquid lipid+ lipophilic surfactant+ lipophilic active moiety) to produce primary emulsion (w/o). Both lipid and the aqueous phase are maintained at same temperature. Stabilizer prevents loss of drug to the external phase during solvent evaporation. Afterwards, primary emulsion is dispersed into a large volume of surfactant aqueous solution followed by sonication to form a double emulsion (w/o/w). The lipid nanoparticles are then purified by ultrafiltration or solvent evaporation[42]

Nanoprecipitation Method :

In this approach, the polymer and drug are dissolved in an organic solvent to form an organic phase. The organic phase is then added slowly to the aqueous phase to form an emulsion and then the organic solvents are removed. The basic principle of this method is to deposit a polymer from a lipophilic solution, which is a mixture of polar organic solvent and water [69]

Solvent Injection technique :

It is a viable new technique to manufacture lipid nanoparticles. In this technique, lipids are solubilized in water-miscible solvent (e.g., acetone, methanol, ethanol, isopropyl alcohol) or water-soluble solvent mixture and then rapidly injected into aqueous surfactant solution under continuous stirring. Resultant dispersion is filtered in order to eliminate excess lipid.[43] The technique relies on rapid diffusion of the solvent over the solvent lipid interfaced with the aqueous phase.[44] The particle size of nanocarriers depends on diffusion rate of the organic solvent through the lipid-solvent interface. This method offers the advantage of easy handling, efficiency, versatility, no employment of technical equipment (e.g., high-pressure homogenizer) and use of approved organic solvents.[43]

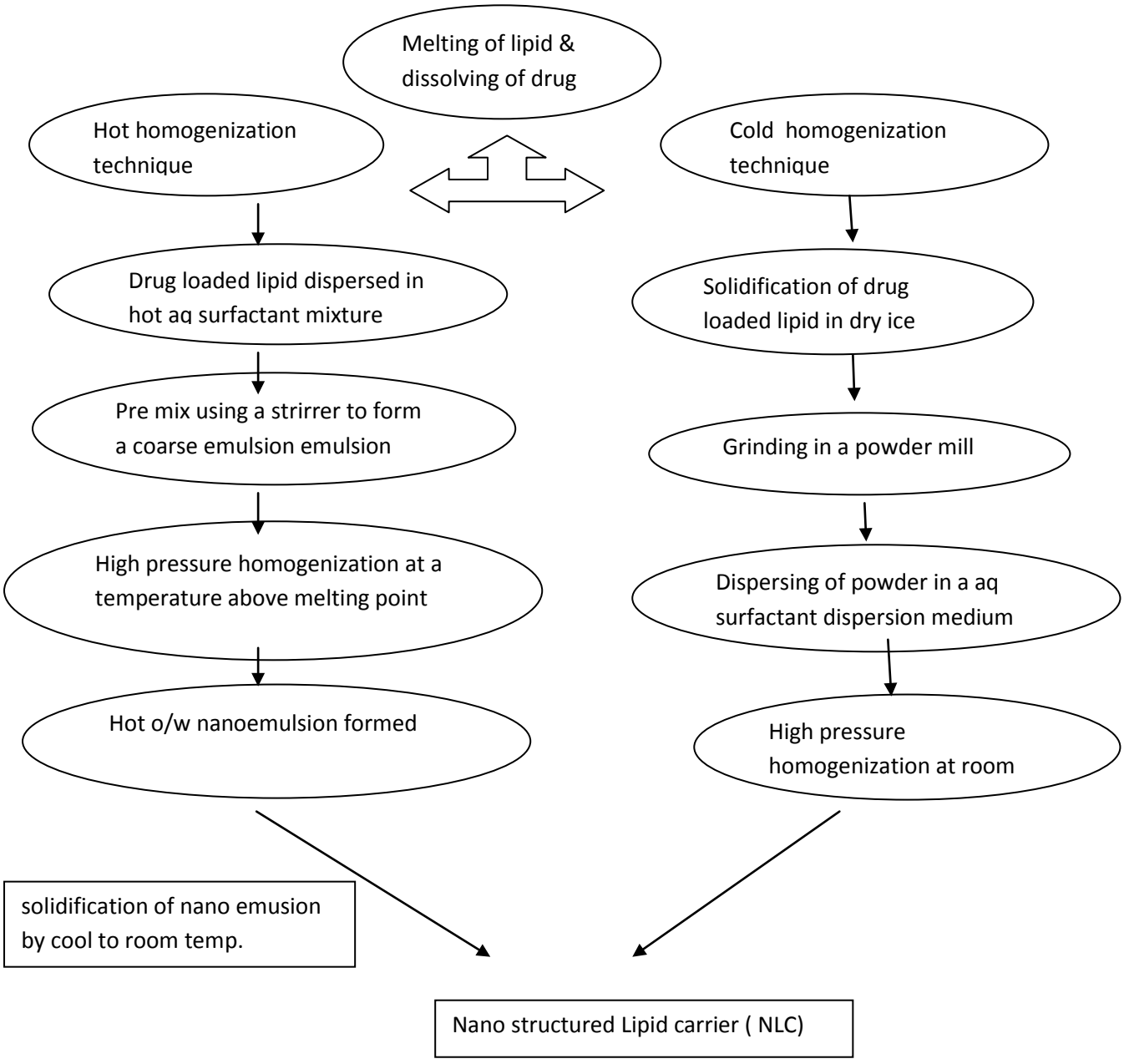


Figure-5 Hot homogenization & cold homogenization technique:

Membrane contactor technique :

Membrane contactor is used to identify membrane systems that are employed to "Keep in contact" two phases. The lipid phase, at a temperature above its melting point is placed in a pressurized vessel. It is allowed to permeate through ceramic membrane pores under applied pressure to form small droplets. The aqueous phase, under continuous stirring, flow tangentially inside the membrane module, and brush away the droplets formed at the pore outlets. Cooling of the preparation to room temperature leads to the formation of lipid particles. Temperature of aqueous and lipid phase, aqueous phase tangential-flow velocity and pressure of lipid phase and membrane pore size are the process parameters affecting size of lipid nanocarriers. The benefits of this new process of membrane emulsification are commercial scalability and control on particle size by fitting optimized parameters.[45]

LIST OF SOME DRUG USED IN NLC PREPARATION AND THEIR ENTRAPMENT EFFICENCY/PARTICLE SIZE (TABLE-2)

DRUG	SOLID LIPID	LIQUID LIPID	SURFACTANT	METHOD	ENTRAPMENT EFFICIENCY/PARTICLE SIZE	REFERENCE
Amoitone B	Polyethylene glycol stearate GMS	Caprylic/capric triglyceride	Pluronic F68, Soya lecithin	Emulsion evaporation, low temperature solidification	68.17 ±0.94%	47
Aceclofenac	Stearic acid	Oleic acid	Phospholipon 90 H	High Speed Homogenization method or ultrasonication	75-85 %	25
artemether	Gelucire1 43/01 pellets , Compritol 888	Transcutol P	Phospholipon 85G	hot homogenization/ultrasonication methods.	61%	70
betamethasone	Precirol ATO-5	OA(oleic acid)	Tween 80	melt emulsification method	85%	46
Brimonidine base	GMS	Castor oil	Poloxamer188	HPH	10.51%	48
CoenzymeQ10	Hard Stearin	GTCC	Alkyl polyglycoside	HPH	99.58 ± 0.0061%	49
Celastrol	Precirol ATO-5	Labrasol	Lecithin, TPGS, Poloxamer188	Solvent diffusion	88.6 ± 0.37%	50
Curcumin	CP	Miglyol812	Solutol HS15, Soya lecithin	Film-ultrasonic emulsion evaporation	96.7 ±0.146%	51
Celecoxib	Kollicream, CP	Miglyol812ic	Solutol HS15, Soya lecithin	low temperature solidification	103.5 ±32.6 nm	52
clobetasol	Monostearin	caprylic/capric triglycerides	sodium dodecyl sulfate	Solvent diffusion method	69.61±2.54%	99
Clindamycin phosphate	Stearic acid	O.A	Pluronic F-68	HPH	38-67%	29
Docetaxel	Stearic acid, Glycerin monostearate	Olive oil, MCT, OA	Pluronic F68, Cremophor EL	HPH	60.5 ± 5.0%	53
Dexamethasone	glycerol trilaurate	Tristearin, Chain Triglycerides Miglyol812	Phospholipids	Solvent diffusion	86.7 ±3.9%	54
diclofenac	GMS,precirol ATO 5	Lanolin PEG-75	Phospholipon 90 G	Hot homoginization	78.26%	85
Etoricoxib	Stearic acid	oleic acid	tween 80	melt-emulsification and low temperature solidification	69-76 %	24
Fenofibrate	Compritol888, ATO	M1944CS, Labrafil	Soya lecithin, Polysorbate80	HPH	99%	55
flubiprofen	Trimyristin Dynasan 144	Captex 355 EP/NF	Soy phosphatidyl choline,tween 80	hot homogenization	>90 %	23
Gentiopicroside	Glycerin monostearate	OA	Polysorbate80, Poloxamer188	Solvent diffusion	38.19 ±1.61%	56
Ibuprofen	Witepsol E85	Miglyol 812	Lutrol F68	hot high-pressure homogenization	98.51%	26
ketorolac	Compritol 888 ATO	Miglyol 812	LutrolF68	tirring/ultrasonication method	92.3%	27
Lornoxicam	Compritol888ATO Lanette O	OA	Pluronic F68	HPH	97.89 ±0.25%	57
Lovastatin	Cholesteryl oleate, cholesterol	Trioleate	Soybean lecithin	Solvent diffusion	96.2 ±1.3%	58
Lercanidipine HCl	GMS	Linseed oil, Labrafil	Polysorbate80	Ultrasonication and emulsion evaporation	214.97 nm	60
lansoprazole	GMS	stearylamine	Pluronic F68	Probe sonication	90−210 nm	28
Lidocaine	Compritol 888 ATO, Precirol ATO 5	Miglyol 810	Tween 80,	ultrasounddispersion method	95.9%	81
Minoxidil	Soya lecithin	OA	Polysorbate80	Ultrasonication and emulsion evaporation	86.09%	59
Methotrexate	Stearic acid Gelucire®50/13	Transcutol	Phospolipon S 100	Hot microemulsion method by using high shear homogenizer	181.5 ± 11.5 nm	97
Nebivolol	Glyceryl monostearate	OA	Span 80	HPH	95%	67
Paclitaxel	Cholesterol	OA	Poloxamer188, Polysorbate80	Solvent diffusion	72 ± 11.6%±	61
Pioglitazone	apifil	labrasol	Tween-80	HPH	>70%	82

DRUG	SOLID LIPID	LIQUID LIPID	SURFACTANT	METHOD	ENTRAPMENT EFFICIENCY/PARTICLE SIZE	REFERENCE
Quercetin	Imwitor 900 K	MCT(medium chain triglyceride)	Tween80, Span20, Soybean lecithin	HPH	91%	62
sildenafil citrate	Cetyl palmitate	Glycerol momolinoleate	Span 85	high-shear homogenization	97.5%,	66
simvastatin	compritol	softigen	Polaxmer 188	High speed homogenizer	88.40 ± 2.4	30
thymoquinone	Lipoid S100	oil	Thimerosal, Polysorbate80	HPH	75 ± 2.4 nm	63
tadalafil	Dynasan Stearic acid	Miglyol 812,OA	Tween 80,	High speed homogenizer & probe sonication	89.6%	31
terbinafine	GMS	labrasol	Pluronic F-127	HPH	80.24 ±4.56	83

CHARACTERIZATION OF LIPID NANOPARTICLES:

Measurement of Particle Size :

Particle size might be determined by dynamic light scattering (DLS), optical single particle sizing (OSPS), laser diffraction (LD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM) and freeze fracture electron microscopy (FFEM) [71,72]. Photon correlation spectroscopy (alias dynamic light scattering or quasi-elastic light scattering) is based on the measurement of the fluctuations in scattered light arising from Brownian motion[76]. It provides the average particle size (z-average) and polydispersity of the system as a measure of the particle size distribution. It characterizes particles of few nanometers to about 3 microns. Laser diffractometer (LD) can characterize a wide range from the nanometer to the micrometer range particles.

Measurement of Zeta Potential :

Almost all particles in contact with a liquid acquire an electric charge on their surface. The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions close to the surface. Thus an electrical double layer exists around each particle. The liquid layer surrounding the particle has an inner region called the Stern layer, where the ions are strongly bound and an outer and diffuse region, where the ions are less firmly attached. Within the diffuse layer there is an imaginary boundary inside which the ions and particles form a stable entity. When a particle moves, ions within the boundary move with it, but any ions beyond the boundary do not move with the particle. This boundary is called the surface of hydrodynamic shear or slipping plane. Zeta potential (ζ) is the potential that exists at this boundary and it is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions[73] . Generally the zeta potential of dispersion should be either less than -30 mV or greater than +30 mV for electrostatic stabilization of NLC [75]. Zeta potential estimation can be done by Laser Doppler electrophoresis, using a Malvern ZetaSizer Nano ZS. By applying an electric field across the sample, particles with a zeta potential will migrate toward the electrode of opposite charge with a velocity relative to the magnitude of the zeta potential. The velocity is measured utilizing the technique of Laser Doppler anemometry, also known as Laser Doppler velocimetry[77]

Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively. Since most cellular membranes are negatively charged, zeta potential can affect a nanoparticle's tendency to permeate membranes, with cationic particles generally displaying more toxicity associated with cell wall disruption. Positive charge possesses a membrane destabilizing and concomitantly destructive effect resulting from an interaction of positive charge and negative charge of membrane. Thus, positively charged lipids are not approved by Food and Drug Administration (FDA) for clinical use[78]. Unlike particle size or molecular weight, ZP is not only dependent on the particles but also on their environment such as pH, ionic strength and also the types of ions present [88].

Drug entrapment efficiency (EE) and drug loading (DL):

Entrapment efficiency (EE) is a critical factor which needs to be optimized during formulation design as it has an impact on the drug release and also on the cost-effectiveness of the formulation. It is the percentage amount of drug that gets entrapped in the nanoparticle and reflects the efficiency of the NLC formulation. For lipophilic drugs, the entrapment is high as the drug gets homogenously solubilized within the lipid. Further, the entrapment is increased due to the formation of a rigid solid lipid particle after cooling, which keeps the drug entrapped within the lipid system. Lipids with imperfections in the crystal structure show higher EE. In NLC, the presence of liquid lipids increases imperfections in the crystal structure, thereby further increasing the EE.[98]

The obtained dispersion of NLc was centrifused at high rpm (10000-20000) for a period of 10-15 minutes.The rpm was selected based on the particle size hence lesser the particle size,higher the rpm required to centrifuge. The supernatant was analyzed by HPLC or UV visible spectrophotometer to determine the amount of drug in it.the Drug entrapment efficiency (EE) and drug loading (DL) is calculated as follows [79]. In NLC, the solid lipid matrix encloses tiny oil section in which drug solubility is considerably higher which increases their total drug loading capacity. Therefore the liquid lipids present in NLCs affect their entrapment efficiency to a great extent

by creating imperfections in a highly ordered crystal matrix and consequently providing sufficient space for a large amount of drug to lodge successfully [96]

$EE= [(W_I-W_S)/W_I] X100$

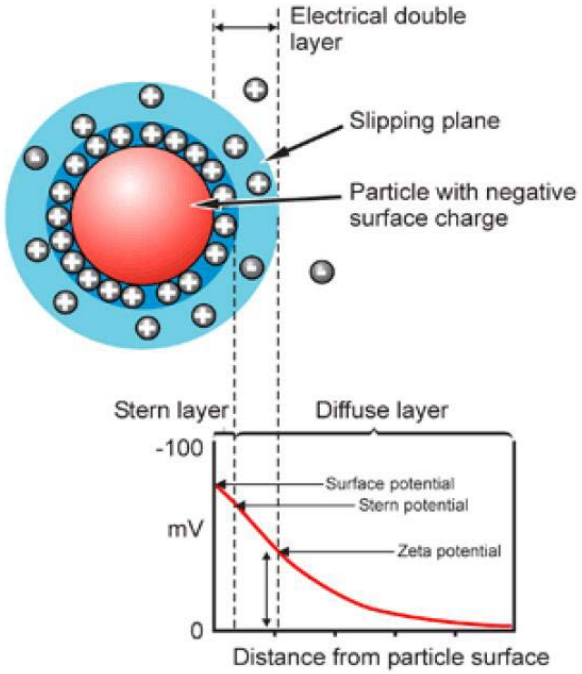
$DL = [(W_I-W_S)/ W_I-W_S +W_L]X100$

Where

W_I = Weight of drug added initially

W_S = weight of drug in supernatant

W_L=Weight of lipid mixture added



[Graphical presentation of Zeta potential of nano particle in solution][73]

Figure-6

Polydisperse index (PDI):

polydispersity is a measure of particle homogeneity and it varies from 0 to 1. The nearer the value of polydispersity to zero, the higher is the homology between the particle [80]. PCS can also measure the polydispersity represented as the polydispersity index (PI) provided by the particle size distribution of the sample. Low PI (0.1–0.25) indicates narrow size distribution whereas PI above 0.5 indicates a broad size distribution hence more polydispersity [86]. PCS shows that NLC being non-spherical particles differ from a nanoemulsion with respect to Brownian motion. This asymmetry of particles leads to higher PI in NLC as compared to nanoemulsions [87].

Particle morphology :

Morphology refers to the external characteristics such as shape and surface structure of particles. Anisometric particles have high surface area, in addition to short diffusion pathways as compared to spherical particles[86]. Due to the higher surface area, non-spherical particles require higher amounts of surfactants for stabilization. Further, particle morphology significantly influences drug loading, encapsulation efficiency, drug release characteristics, pharmacokinetics, biodistribution and targeted delivery of NLC. It also plays an important role in cellular uptake, receptor binding and interaction with cells [89,90]. PCS and DLS analyze the particle size assuming that the particles are spherical in shape. However, NLC are not spherical solid lipid particles embedded with oil droplets, rather they are solid platelets with the oil present between the solid platelet and the surfactant layer [91].further difficulties in analyzing particle size might arise if the sample contains populations of different size ranges or exhibit flocculation. Hence, additional analytical methods are required which can provide direct information regarding these characteristics of the NLC. Electron microscopy techniques like scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) are extremely useful in determining the particle size, state of aggregation, morphology, surface topography and even the internal structure of the NLC.

SEM gives information about the surface characteristics and threedimensional morphology of the particle. For SEM, the nanodispersion is usually converted into a powder, mostly by freeze drying. These dried particles are then sputtered with a conductor such as gold providing a coating on the surface before measurement under high vacuum. The resolving power of SEM is 3–4 nm which is quite low as compared to TEM. It also does not provide any information about the internal structure of the particles [92,93]

In vitro drug diffusion studies :

In vitro drug release studies of NLC can be performed using dialysis bag technique. The activation of dialysis membrane was carried out. The experiments were carried out under sink conditions. 10 mg of NLC formulation can loaded into a cellulose membrane dialysis bag (molecular weight cut-off 12,400, Sigma–Aldrich Co., St. Louis, MO, USA), immersed in 200 mL of pH 7.4 phosphate buffer containing 0.5-1% surfactant solution magnetically stirred at 32 ^{0}C at pH 7.4. Samples should collect at predetermined intervals from the receiver solution, replaced with equal volumes of fresh solvent, and spectrometrically determined drug concentration. [94,95]

APPROACHES EMPLOYED FOR OVERCOMING THE ISSUES RELATED TO STABILITY OF NLCS :

Lyophilisation :

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required to achieve long term stability for a product containing hydrolysable drugs or a suitable product for per –oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions.However, when SLN are lyophilised without cryoprotectant, the final product commonly results in the aggregation of particles. Some of the most widely used cryoprotectants are trehalose, sorbitol, glucose, sucrose, mannose and maltose. Schwarz and Mehnert reported trehalose as the most effective cryoprotectant in preventing particle growth [100]

Stabilizing agent used for NLC stability as follows:

a)Poloxamers :

Poloxamer 188 used in a formulation that was developed and then in human plasma and whole blood showed that showed an increased whole blood permeability of networks and it was also observed that the increased fibrin permeability was due to fibrin fibres arrangement. The alterations of fibrin are the main reason to increase the mechanical stability contributing to antithrombotic and rheological effects [101,102].There was also increase in stability of the gel formulation using Poloxamer with organic solvents such as ethanol, propylene glycol, glycerol and PEG 400. Poloxamer 407 in the presence of these organic solvents, self assembles into two liquid crystal structures namely micellar cubic and hexagonal structures that are thermodynamically stable.

Poloxamer 407 in combination with a liposome showed an increase in stability of liposome formulation by increasing half life, preventing aggregation and fusion of phosphatidylcholine multilamellar vesicles[103].

The low stability of poloxamer hydrogel in an aqueous solution lead to the combination development of poloxamer 407 with acrylate and thiol groups of 17.5 wt % at body temperature. It was observed with an immediate crosslinking formed between acrylate and thiol that modified poloxamer 407 property, giving rise to a remarkable increase in stability of drugs about four times and for its potential application in controlled drug release [104].

b) **Polyethylene glycol**

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

- Providing good physical stability and dispersability of colloids
- Improving presence of colloids in blood circulation for systemic use
- Increasing stability of colloids in body fluids such as gastrointestinal (GI) fluids,
- Acceleration of colloid transport across the epithelium,
- Modulation of interaction of colloids with mucosa for specific delivery requirements and drug targeting,
- Increasing biocompatibility and decreasing thrombogenicity of drug carriers. [105]

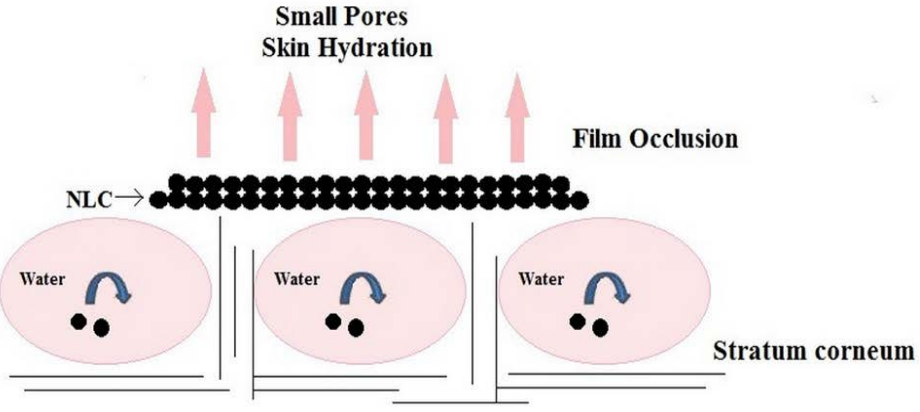
SKIN OCCLUSION :

In general, lipid nanocarriers have peculiar epidermal occlusive characteristics which by inhibiting water evaporation could enhance bioactive penetration into the stratum corneum. Nanoparticles were found to be 15 times more occlusive than microparticles. Müller et al mentioned NLC as "invisible, penetration enhancing occlusive plastic foil''.[106].Scope of NLC occlusive feature depends on the following factors:

- **Particle size**: Small particle size of the nanocarriers diminishes water evaporation from the skin. Occlusion factor of lipid micro particles of >1 µm diameter was found to be 10%, whereas lipid nanoparticles of 200 nm size showed 50% occlusion.[107]
- **Crystallinity and concentration of lipids**: High concentration of lipid (50%-60%) present in NLC formulation act as occlusive agent and is responsible for retaining the moisture in the stratum corneum [108]. Formulation having particles size less than 400 nm and at least 35% lipid of high crystallinity was found to be most effective. An increase in oil content in NLC formula causes a decrement in occlusive factor.[109]. Supercooled melts (noncrystalline nanoparticles) have no occlusive properties [110]. An enhanced skin hydration effect showed up due to the occlusive nature of NLCs.[107]

CONCLUSION :

The Nanostructured Lipid carrier(NLC) have always been potential carrier systems with good therapeutic applications. The purpose of this work was to highlight the role of NLCs as a novel drug delivery system for various categories of drugs. They are the new generation, smart, flexible systems offering for enhanced drug loading, modulation of release and improved performance in producing final dosage forms. They are easy to scale up and can be modulated to achieve the desired particle size and release profile, improved drug loading and higher stability of the therapeutics.



[Occlusion effect of NLC]
FIGURE-7

REFERENCES:

1. Attama AA, Momoh MA, Builders PF (2012) Lipid Nanoparticulate Drug Delivery Systems : A Revolution in Dosage Form Design and Development, *Recent Advances in Novel Drug Carrier Systems*, doi.org/10.5772/50486
2. Alkilani AZ, McCrudden MTC, Donnelly RF. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. *Pharmaceutics* 2015;7:438–470.
3. Bolognia JL, Jorizzo JL, Schaffer JV. Dermatology. 3 rd. ed. St. Louis: Elsevier Health Sciences; 2012 [chapter 124]
4. Montenegro L, Lai F, Offera A, Sarpietro MG, Micicche L, Maccioni AM, *et al*. From nanoemulsions to nanostructured lipid carriers: a relevant development in dermal delivery of drugs and cosmetics. *J Drug Deliv Sci Tech* 2016;32:100–112.
5. Wissing AS, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). *Int J Pharm* 2003;254:65-68.
6. Soma D, Attari Z, Reddy MS, Damodaram A, Koteshwara KBG. Solid lipid nanoparticles of irbesartan: preparation, characterization, optimization and pharmacokinetic studies. *Braz J Pharm Sci* 2017;53(1):1-10.
7. Kiran Kumari*, Anupam Kr. Sachan and Saurabh Singh, nanostructured lipid carriers, methods of preparation and applications**,** *ejpmr, 2019,6(7), 250-257*
8. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. *Adv Pharm Bull* 2015;5(3):305- 13. doi: 10.15171/apb.2015.043
9. Dhruv K. Purohit, Tanaji D. Nandgude, Sushilkumar S. Poddar : Nano-lipid Carriers for Topical Application: Current Scenario: *Asian Journal of Pharmaceutics* • Jan-Mar 2016 (Suppl) • 9 (5) | S1
10. Beloqui AB, Solinis MA, Rodriguez-Gascón A, Almeida AJ, Preat V. Nanostructured lipid carriers: promising drug delivery systems for future clinics. *Nanomed – Nanotechnology* 2016;12:143–161.
11. Hajare AA, Mali SS, Ahir AA, Thorat JD, Salunkhe SS, Nadaf SJ, *et al*. Lipid nanoparticles: A modern formulation approach in topical drug delivery systems. *J Adv Drug Deliv* 2014;1:30-7
12. J. Pardeike, A. Hommoss, R.H. Müller, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products, *Int. J. Pharm*. 366 (2009) 170–184. doi:10.1016/j.ijpharm.2008.10.003.
13. Dale M. In: Sweetman SC, editor. The Complete Drug Reference. 36th ed. London, UK: *Pharmaceutical Press*; 2009. p. 3694.
14. Chen ML. Lipid excipients and delivery systems for pharmaceutical development: A regulatory perspective. *Adv Drug Deliv Rev* 2008;60:768-77.
15. Shinde G, Rajesh KS, Prajapati N, Murthy RS. Formulation, development and characterization of nanostructured lipid carrier (NLC) loaded gel for psoriasis. *Sch Res Lib Pharm Lett* 2013;5:13-25.
16. Cirri M, Bragagni M, Mennini N, Mura P. Development of a new delivery system consisting in “drug-in cyclodextrin-in nanostructured lipid carriers” for ketoprofen topical delivery. *Eur J Pharm Biopharm* 2012;80:46-53.
17. Shah R, Eldridge D, Palombo E, Harding I. *Lipid Nanoparticles: Production, Characterization and Stability*. UK: Springer; 2015
18. Karn-Orachai K, Smith SM, Phunpee S, Treethong A, Puttipipatkhachorn S, Pratontep S, et al. The effect of surfactant composition on the chemical and structural properties of nanostructured lipid carriers. *J Microencapsul* 2014;31(6):609-18. doi: 10.3109/02652048.2014.911374
19. Jaiswal P, Gidwani B, Vyas A. Nanostructured lipid carriers and their current application in targeted drug delivery. *Artif Cells Nanomed Biotechnol* 2016;44(1):27-40. doi: 10.3109/21691401.2014.909822
20. Keck CM, Baisaeng N, Durand P, Prost M, Meinke MC, Muller RH. Oil-enriched, ultra-small nanostructured lipid carriers (usNLC): a novel delivery system based on flip-flop structure. *Int J Pharm* 2014;477(1-2):227-35. doi: 10.1016/j. ijpharm.2014.10.029
21. Affandi MMM, Julianto T, Majeed A. Development and stability evaluation of astaxanthin nanoemulsion. *Asian J Pharm Clin Res* 2011;4:Suppl 1:142-8.
22. Uner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *Int J Nanomedicine* 2007;2(3):289-300.
23. Kesavan Bhaskar et al , Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, in vitro, ex vivo and in vivo studies, Lipids Health Dis. 2009; 8: 6
24. Sachan Anupam Kr, Gupta Ankita and Arora Mona. Formulation & characterization of nanostructured lipid carrier (nlc) based gel for topical delivery of etoricoxib. *Journal of Drug Delivery & Therapeutics*. 2016; 6(2):4-13
25. Naglakshmi Sethuraman, Shanmuganathan S, Sandhya K, Anbarasan B. Design, Development and Characterization of Nano Structured Lipid Carrier for Topical Delivery of Aceclofenac**.** *Indian Journal of Pharmaceutical Education and Research* 2018 (52) - 4
26. Blanka Sütő,Szilvia Berkó,Gábor Kozma, Ákos Kukovecz. Development of ibuprofen-loaded nanostructured lipid carrier-based gels: characterization and investigation of in vitro and in vivo penetration through the skin. *International Journal of Nanomedicine* 2016:11 1201–1212
27. Carmelo Puglia, Rosanna Filosa, Antonella Peduto, Paolo de Caprariis, Luisa Rizza, Francesco Bonina. Evaluation of Alternative Strategies to Optimize Ketorolac Transdermal Delivery. *AAPS PharmSciTech* 2006; 7 (3)
28. Lin WJ , Duh YS .Nanostructured lipid carriers for transdermal delivery of acid labile lansoprazole. *Eur J Pharm Biopharm*. 2016 Nov;108:297-303
29. Ishrat et al , formulation and evaluation of nano particulate drug delivery system, *International Journal of Pharmacy Research and Technology* / Issue (2) 2018
30. Brito Rajet al. Formulation, in-vitro and in-vivo pharmacokinetic evaluation of simvastatin nanostructured lipid carrier loaded transdermal drug delivery system, *Future Journal of Pharmaceutical Sciences* (2019) 5:9
31. Baek, Jong-Suep, Pham, Cuong Viet, Myung, Chang-Seon and Cho, Cheong-Weon .Tadalafil-loaded nanostructured lipid carriers using permeation enhancers.*International journal of pharmaceutics*. 2015,495. 2, 701-709
32. Patel, *et al*.: Lipid Nanoparticles for Skin Penetration: *International Journal of Pharmaceutical Investigation***,** Vol 9, Issue 4, Oct-Dec, 2019
33. Leonida MD, Kumar I. Bionanomaterials for Skin Regeneration. Switzerland: *Springer International Publishing*; 2016. p. 55-6.
34. Jain P, Rahi P, Pandey V, Asati S, Soni V. Nanostructure lipid carriers: a modish contrivance to overcome the ultraviolet effects. *Egypt J Basic Appl Sci* 2017;4(2):89-100. doi: 10.1016/j.ejbas.2017.02.001
35. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: structure preparation and application. *Adv Pharm Bull* 2015;5(3):305- 13. doi: 10.15171/apb.2015.043
36. A. Gordillo-Galeano, C. Elizabeth Mora-Huertas, Solid lipid nanoparticles and nanostructured lipid carriers: A review emphasizing on particle structure and drug release, *European Journal of Pharmaceutics and Biopharmaceutics* (2018),
37. Hernández-Sánchez H, Gutiérrez-López GF. *Food Nanoscience and Nanotechnology*. New York: Springer; 2015. p. 124-5.
38. Wong HL, Li Y, Bendayan R, Rauth MA, Wu XY. Solid lipid nanoparticles for anti-tumor drug delivery. In: Amiji MM, ed. *Nanotechnology for Cancer Therapy*. *Boca* Raton: CRC press, Taylor & Francis Group; 2007.
39. Iqbal MA, Md S, Sahni JK, Baboota S, Dang S, Ali J. Nanostructured lipid carriers system: recent advances in drug delivery. *J Drug Target* 2012;20(10):813-30. doi: 10.3109/1061186x.2012.716845
40. Shao Z, Shao J, Tan B, Guan S, Liu Z, Zhao Z, He F, Zhao J. Targeted lung cancer therapy: Preparation and optimization of transferrin-decorated nanostructured lipid carriers as novel nanomedicine for co-delivery of anticancer drugs and DNA. *Int J Nanomed*, 2015; 10: 1223-33.
41. Shi L, Li Z, Yu L, Jia H, Zheng L. Effects of surfactants and lipids on the preparation of solid lipid nanoparticles using double emulsion method. *J Dispers Sci Technol* 2011;32(2):254-9. doi: 10.1080/01932691003659130
42. Uner M. Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems. *Pharmazie* 2006;61(5):375- 86.

43. Schubert MA, Müller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles-- evaluation of the method and process parameters. *Eur J Pharm Biopharm* 2003;55(1):125-31. doi: 10.1016/s0939- 6411(02)00130-3
44. Anuradha K, Senthil Kumar M. Development of Lacidipine loaded nanostructured lipid carriers (NLCs) for bioavailability enhancement. *Int J Pharm Med Res* 2014;2(2):50-7
45. Charcosset C, El-Harati A, Fessi H. Preparation of solid lipid nanoparticles using a membrane contactor. *J Control Release* 2005;108(1):112-20. doi: 10.1016/j.jconrel.2005.07.023
46. *Xin Kong, Yuan Zhao, Peng Quan, Liang Fang*, Development of a topical ointment of betamethasone dipropionate loaded nanostructured lipid carrier**,** asian journal of pharmaceutical s c i e n c e s 11 (2 0 1 6) 248–254
47 Luan, J.; Zhang, D.; Hao, L.; Qi, L.; Liu, X.; Guo, H.; Li, C.; Guo, Y.; Li, T.; Zhang, Q.; et al. Preparation, characterization and pharmacokinetics of Amoitone B-loaded long circulating nanostructured lipid carriers. *Colloids Surf. B Biointerfaces* **2014**, 114, 255–260.
48. El-Salamouni, N.S.; Farid, R.M.; El-Kamel, A.H.; El-Gamal, S.S. Effect of sterilization on the physical stability of brimonidine-loaded solid lipid nanoparticles and nanostructured lipid carriers. *Int. J. Pharm.* **2015**, 496, 976–983.
49. Piao, H.; Ouyang, M.; Xia, D.; Quan, P.; Xiao, W.; Song, Y.; Cui, F. In vitro-in vivo study of CoQ10-loaded lipid nanoparticles in comparison with nanocrystals. *Int. J. Pharm.* **2011**, 419, 255–259.
50. Zhou, L.; Chen, Y.; Zhang, Z.; He, J.; Du, M.;Wu, Q. Preparation of tripterine nanostructured lipid carriers and their absorption in rat intestine. *Die Pharm.* **2012**, 67, 304–310.
51. Hong,W.; Chen, D.W.; Zhao, X.L.; Qiao, M.X.; Hu, H.Y. Preparation and study in vitro of long-circulating nanoliposomes of curcumin. *China J. Chin. Mater. Med*. **2008**, 33, 889–892.
52. Patlolla, R.R.; Chougule, M.; Patel, A.R.; Jackson, T.; Tata, P.N.; Singh, M. Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. *J. Control. Release* **2010**, 144, 233–241.
53. Choi, K.O.; Choe, J.; Suh, S.; Ko, S. Positively Charged Nanostructured Lipid Carriers and Their Effect on the Dissolution of Poorly Soluble Drugs. *Molecules* **2016**, 21, 672.
54. Zhao, C.; Fan, T.; Yang, Y.; Wu, M.; Li, L.; Zhou, Z.; Jian, Y.; Zhang, Q.; Huang, Y. Preparation, macrophages targeting delivery and anti-inflammatory study of pentapeptide grafted nanostructured lipid carriers. *Int. J. Pharm.* **2013**, 450, 11–20.
55. Tran, T.H.; Ramasamy, T.; Truong, D.H.; Choi, H.G.; Yong, C.S.; Kim, J.O. Preparation and characterization of fenofibrate-loaded nanostructured lipid carriers for oral bioavailability enhancement. *AAPS PharmSciTech* **2014**, 15, 1509–1515.
56. Zhang, K.; Lv, S.; Li, X.; Feng, Y.; Li, X.; Liu, L.; Li, S.; Li, Y. Preparation, characterization, and in vivo pharmacokinetics of nanostructured lipid carriers loaded with oleanolic acid and gentiopicrin. *Int. J. Nanomed*. **2013**, 8, 3227–3239.
57. Yang, X.; Zhao, L.; Almasy, L.; Garamus, V.M.; Zou, A.;Willumeit, R.; Fan, S. Preparation and characterization of 4-dedimethylamino sancycline (CMT-3) loaded nanostructured lipid carrier (CMT-3/NLC) formulations. *Int. J. Pharm. **2013***, 450, 225–234.
58. Gu, X.; Zhang, W.; Liu, J.; Shaw, J.P.; Shen, Y.; Xu, Y.; Lu, H.; Wu, Z. Preparation and characterization of a lovastatin-loaded protein-free nanostructured lipid carrier resembling high-density lipoprotein and evaluation of its targeting to foam cells. *AAPS PharmSciTech* **2011**, 12, 1200–1208.
59. Uprit, S.; Kumar Sahu, R.; Roy, A.; Pare, A. Preparation and characterization of minoxidil loaded nanostructured lipid carrier gel for effective treatment of alopecia. *Saudi Pharm. J. SPJ* **2013**, 21, 379–385.
60. Ranpise, N.S.; Korabu, S.S.; Ghodake, V.N. Second generation lipid nanoparticles (NLC) as an oral drug carrier for delivery of lercanidipine hydrochloride. *Colloids Surf. B Biointerfaces* **2014**, 116, 81–87.
61. Emami, J.; Rezazadeh, M.; Varshosaz, J.; Tabbakhian, M.; Aslani, A. Formulation of LDL Targeted Nanostructured Lipid Carriers Loaded with Paclitaxel: A Detailed Study of Preparation, Freeze Drying Condition, and In Vitro Cytotoxicity. *J. Nanomater*. **2012**, 2012, 3.
62. Beloqui, A.; Solinis, M.A.; des Rieux, A.; Preat, V.; Rodriguez-Gascon, A. Dextran-protamine coated nanostructured lipid carriers as mucus-penetrating nanoparticles for lipophilic drugs. *Int. J. Pharm*. **2014**, 468, 105–111.
63. Abdelwahab, S.I.; Sheikh, B.Y.; Taha, M.M.; How, C.W.; Abdullah, R.; Yagoub, U.; El-Sunousi, R.; Eid, E.E. Thymoquinone-loaded nanostructured lipid carriers: Preparation, gastroprotection, in vitro toxicity, and pharmacokinetic properties after extravascular administration. *Int. J. Nanomed*. **2013**, 8, 2163–2172.
64. M. SHAH*et al, Solid Lipid Nanoparticles of a Water Soluble Drug Ciprofloxacin Hydrochloride, *Indian Journal of Pharmaceutical Sciences* September - October 2012
65. M. Shah and Y. Agrawa, High throughput screening: an in silico solubility parameter approach for lipids and solvents in SLN preparations, *Pharmaceutical Development and Technology*, 2013; 18(3): 582–590
66. Elnaggar YS, El-Massik MA, Abdallah OY. Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles. *Int J Nanomedicine* 2011;6:3195–3205.
67. Ghazy E, Abdulrasool AA, Al-Tamimi JJ, Ayash N. Nebivolol hydrochloride loaded nanostructured lipid carriers as transdermal delivery system: Part 2: Hydrogel preparation, evaluation and permeation study. *TJMS* 2016;3(2):1-16.
68. P. Jaiswal et al., Nanostructured lipid carriers and their current application in targeted drug delivery, Artificial Cells, *Nanomedicine, and Biotechnology*, 2016; 44: 27–40
69. Qianwen Li et al, A Review of the Structure, Preparation, and Application of NLCs, PNPs, and PLNs**,** *Nanomaterials* **2017**, 7, 122
70. P.O. Nnamani et al., Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application, *International Journal of Pharmaceutics* 477 (2014) 208–217
71. Mehnert W, Mäder K (2001) Solid lipid nanoparticles: Production, characterization and applications. *Adv. Drug Deliv. Rev.* **47**, 165–196.
72 Parhi R, Suresh P (2012) Preparation and characterization of solid lipid nanoparticles-a review. *Curr. Drug Discov. Technol.* **9**, 2–16.
73. Zetasizer Nano Series User Manual. MAN 0317 Issue 1.1.
74. Muller R H, Radtke M, Wissing S A. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, *Adv Drug Deliv Rev*, 2002; 54(1):131-55
75. Thatipamula R, Palem C, Gannu R, Mudragada S, Yamsani M. Formulation and in vitro characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. *Daru* 2011;19(1):23-32.
76. Loo C, Basri M, Ismail R, Lau H, Tejo B, Kanthimathi M, et al. Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion. *Int J Nanomedicine* 2013;8:13-22. doi: 10.2147/ijn.s35648
77. Biopharmaceutical research and development service, Contract analytical services, Zeta potential. Available from: https://www.coriolis-pharma.com/contract-analytical-services/zeta-potential/. Accessed February 17, 2019.
78. Honary S, Zahir F (2013) Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2). *Trop. J. Pharm. Res.* **12**, 265–273.
79. Natrajanet al,Nano structured lipid carrier : A promising drug delivery system,*Global Journal Of Nanomedicine*,2017,1(5)
80. S. Uprit et al, Preparation and characterization of minoxidil loaded nanostructured lipid carrier gel for effective treatment of alopecia, *Saudi Pharmaceutical Journal* (2013) 21, 379–385
81. Pathak and Nagarsenker, Formulation and Evaluation of Lidocaine Lipid Nanosystemsfor Dermal Delivery, *AAPS PharmSciTech*, Vol. 10, No. 3, September 2009
82. Sohrab Alam, Mohammed Aslam et al, Nanostructured lipid carriers of pioglitazone for transdermalapplication: from experimental design to bioactivity detail, *Drug Deliv, Early Online*: 1–9
83. Bharti Gaba, Mohammad Fazil, Saba Khan, Asgar Ali, Sanjula Baboota, Javed Ali, Nanostructured lipid carrier system for topical

delivery of terbinafine hydrochloride, *Bulletin of Faculty of Pharmacy*, Cairo University (2015) 53, 147–159
84. Shete H, Patravale VS. Long chain lipid based tamoxifen NLC. Part I: Preformulation, formulation development and physicochemical characterization. *Int J Pharm* 2013;454(1):573–83.
85. Chien Ngoc Nguyen et al, Nanostructured lipid carriers to enhance transdermal deliveryand efficacy of diclofenac, *Drug Deliv. and Transl*. Res. DOI 10.1007/s13346-017-0415-2
86. F. Tamjidi, M. Shahedi, J. Varshosaz, Nanostructured lipid carriers (NLC): a potential delivery system for bioactive food molecules, *Innov. Food Sci. Emerg. Tech*. 19 (2013) 29–43.
87. K. Jores, W. Mehnert, M. Drechsler, H. Bunjes, Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy, *J. Control. Release* 95 (2) (2004) 217–227
88. R. Xu, Progress in nanoparticles characterization: sizing and zeta potential measurement, *Particuology* 6 (2) (2008) 112–115.
89. S.M. Moghimi, A.C. Hunter, Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective, *Annu. Rev. Pharmacol. Toxicol*. 52 (1) (2012) 481–503.
90 N.P. Truong, M.R. Whittaker, C.W. Mak, The importance of nanoparticle shape in cancer drug delivery, Expert Opin. *Drug Deliv*. 12 (1) (2015) 129–142
91. K. Jores, A. Haberland, S. Wartewig, K. Mader, Solid lipid nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and Raman spectroscopy, *Pharm. Res*. 22 (11) (2005) 1887–1897
92. V. Teeranachaideekul, E.B. Souto, Cetyl palmitate-based NLC for topical delivery of coenzyme Q10 – development, physicochemical characterization and in vitro release studies, *Eur. J. Pharm. Biopharm*. 67 (1) (2007) 141–148.
93 S. Das, W.K. Ng, R.B.H. Tan, Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? Eur. *J. Pharm. Sci*. 47 (1) (2012) 139–151.
94. Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC*). Eur J Pharm Biopharm* 2008;70:633–40.
95.. Cirri M, Bragagni M, Menni N, Mura P. Development of a new delivery system consisting drug- in cyclodextrin- in nanostructured lipid carriers; for ketoprofen topical delivery. *Eur J Phram Biopharm* 2011;32(4):21–32
96. Muller R, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – A review of the state of the art. *Eur J Pharm Biopharm* 2000;50:161–77.
97. Garg, N. K., Singh, B., Tyagi, R. K., Sharma, G. and Katare, O. P. (2016). Effective transdermal delivery of methotrexate through nanostructured lipid carriers in an experimentally induced arthritis model. *Colloids Surfaces B Biointerfaces* 147, 17–24.
98. C.L. Fang, S.A. AlSuwayeh, Nanostructured lipid carriers (NLCs) for drug delivery and targeting, *Recent. Pat. Nanotechnol*. 7 (1) (2013) 41–55.
99. Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characteristics of monostearin nanostructured lipid carriers. *Int J Pharm* 2006;314:83-9.
100. Trotta M, Debernardi F, Caputo O., Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. *Int J Pharm*. 2003;257: 153– 160.
101. Molpeceres, J., Guzman, M., Bustamante, P., Rosario, M.D., *Int. J. Pharm*. 1996; 130(1); 75- 81.
102. Yong, C.S., Yu-Kyoung, O., Hyun, S.J., Jong- Dal, R.J., Ho-Dong, K., Chong-Kook, K., Han-Gon, C., *Eur. J. Pharm. Sci*. 2004; 23(4–5);347–353.
103. Nogueiras-Nieto, L., Sobarzo-Sánchez, E., Gomez-Amoza, J.L., Otero-Espinar, F.J., *Eur. J.Pharm. Biopharm*. 2012; 80; 585–595.
104. Goldi, K., Huang, J., Chatlapalli, R., Krishnendu, G., Arwinder, N., *AAPS PharmSci*. 2011; 12(4); 1-10.
105. Reithmeier, H., Herrmann, J., & Göpferich, A. (2001). *Lipid microparticles as a parenteral controlled release device for peptides. Journal of Controlled Release, 73(2-3), 339–350*
106. Müller RH, Shegokar R, Keck CM. 20 years of lipid nanoparticles (SLN and NLC): present state of development and industrial applications. *Curr Drug Discov Technol* 2011;8(3):207-27. doi: 10.2174/157016311796799062
107. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev* 2002;54 Suppl 1:S131-55. doi: 10.1016/s0169- 409x(02)00118-7
108. Loo C, Basri M, Ismail R, Lau H, Tejo B, Kanthimathi M, et al. Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion. *Int J Nanomedicine* 2013;8:13-22. doi: 10.2147/ijn.s35648
109. Wissing S, Muller R. The influence of the crystallinity of lipid nanoparticles on their occlusive properties. *Int J Pharm* 2002;242(1-2):377-9. doi: 10.1016/s0378-5173(02)00220-x
110. Beck R, Guterres S, Pohlmann A. *Nanocosmetics and Nanomedicines: New Approaches for Skin Care*. Berlin: Springer; 2014
111. Fang CL, Al-Suwayeh SA, Fang JY. Nanostructured lipid carriers (NLCs) for drug delivery and targeting. *Recent Pat Nanotechnol* 2013;7(1):41-55. doi: 10.2174/1872210511307010041