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NANOSTRUCTURED LIPID CARRIERS: NOVEL DRUG DELIVERY SYSTEM

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Abstract: To overcome the limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals**.** Nanostructured lipid carriers (NLC) are interesting delivery systems for enhancing the penetration of an active substance through the skin after topical administration. A new generation of nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructured has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. A lipid carrier has bright future, because of their intrinsic property to improve the bioavailability of lipophilic drugs with low aqueous solubility. SLN and NLC offer an economical and patient-friendly device for administration of drugs by topical routes. The present review gives insights on the definitions and characterization of NLC as colloidal carriers including the production techniques, stability techniques and suitable formulations. This review paper also highlights mechanism of skin penetration and the importance of NLC in pharmaceutical applications.

Keywords: NLC (Nanostructured lipid carrier), colloidal carriers, lipophilic pharmaceuticals

1. INTRODUCTION:

1.1 Lipid Based Drug Delivery System:[1, 2, 3, 4, 5, 6, 7, 8]

There is always a growing need for novel drug delivery systems to deal with the vast majority of the new chemical drug entities that have poor solubility or permeability and to improve the delivery of existing drugs. Polymeric drug delivery systems have been widely developed and provide an attractive alternative for progressive and long term delivery of therapeutic agents as well as their wealth of possible chemical modification. Nevertheless, the number of products based on polymeric micro and nanoparticles in the market is still limited because of the toxicity of polymers and of solvent residues used in their production, high cost of biodegradable polymers, potentially toxic/allergic end products of biodegradable polymers and the lack of suitable large-scale production methods.In order to overcome these problems, a great deal of interest has focused on lipid based carriers such as lipid emulsions, liposome's or lipid nanoparticles. Lipid-based delivery systems (LBDDS) are an accepted approach and an emerging field for drug delivery and have attracted different research groups because of their inherited properties, their biocompatibility and biodegradability of physiologically tolerated lipids, physiochemical diversity, lower toxicity, high incorporation efficiency for lipophilic drugs, protection of drugs from degradation, improved bioavailability, and controlled-release characteristics, challenges in stability and manufacturing at the commercial scale and suitability for drug delivery at different sites of administration. LBDDS formulations can be tailored to meet a wide range of product requirements dictated by disease indication, route of administration and considerations of cost, product stability, toxicity, and efficacy.The use of LBDDS for the topical treatment of skin diseases is very attractive, since epidermal lipids are found in high amounts within the penetration barrier thus decreasing their systemic absorption by localization in skin layers. Besides liposomes, solid lipid nanoparticles and nanostructured lipid carriers showed potentials in dermal targeting as well as cosmetic products by overcoming the stability problems of liposome's. Moreover, transdermal drug application has gained increasing importance for systemic treatment, e.g. with drugs subject to extensive first-pass elimination such as glyceryl trinitrate or estrogens as well as for the sustained suppression of chronic pain. Extensive research has been conducted on the use of Nano emulsions, micro emulsions, liposome's and most importantly lipid nanoparticles for the transdermal delivery of various drug groups.

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1.2 Lipid Nanoparticles as Drug Delivery Systems: [9]

Lipid nanoparticles gain more interest than micro particles; they combine both technology of lipid

sciences and Nano sciences. Generally, the size is considered as a major issue for pharmaceutical applications since it greatly influences the *in vitro* and *invivo* studies. The term nanoparticles refers to Nano spheres or Nano capsules that are, respectively, matricial having a homogeneous structure in the whole particle, or vesicular exhibiting a typical core-shell structure varying in size from 10 to 1000nm. They have liquid core or solid core. To overcome the limitations of polymeric Nano-particles lipids are put forward as an alternative carrier, particularly for lipophilic pharmaceuticals.

1.3 There are two types of lipid Nano-particles :[10, 11, 12, 13, 14]

a) Solid Lipid Nano-particles (SLN)

b) Nano-structured Lipid Carriers NLC).

SLNs and NLCs as an alternative colloidal carriers to traditional carriers (emulsion, liposome's and polymeric Nano-particles) are developed in the last decade.SLN are produced by replacing the liquid lipid (oil) of an o/w emulsion by solid lipid being solid at both room and body temperature.SLN are composed of 0.1%(w/w) to 30%(w/w) solid lipid dispersed in an aqueous medium and stabilized with 0.5%(w/w) to 5%(w/w) surfactant. The mean particle size of SLN is ranging from about 40 to 1000nm.In the second generation of the lipid Nano particle technology i.e. NLC, the particles are produced using blends of solid lipids and liquid lipids (oils).The ratio of solid lipid to liquid lipid is 70:30 to 99.9:0.1. In NLC overall solid content could increase up to 95%.The particle diameter of NLC ranging 10-1000nm.To overcome the limitations of SLN, the second generation of nanoparticles NLCs are developed. IN NLCs the solid and liquid lipids are mixed in such a way that particles solidifies upon cooling but does not recrystallize, remaining in amorphous state. Due to this accommodation of drug in the particle for a longer time occur and that can leads to increase the drug loading capacity of the system. SLN and NLC are novel systems composed of physiological and biodegradable lipid materials suitable for topical, dermal and trans-dermal application that show low toxicity. The Nano size ensures a close contact to the stratum corneum and can enhance the amount of drug penetrated into the skin. An increased skin hydration effect is observed due to the occlusive properties of lipid Nano particles. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. A controlled release from NLCs possible due to their solid lipid matrix. When drug is supply over prolong period of time this become an important tool, to reduce the systemic absorption, and when drug produces irritation in high concentration.

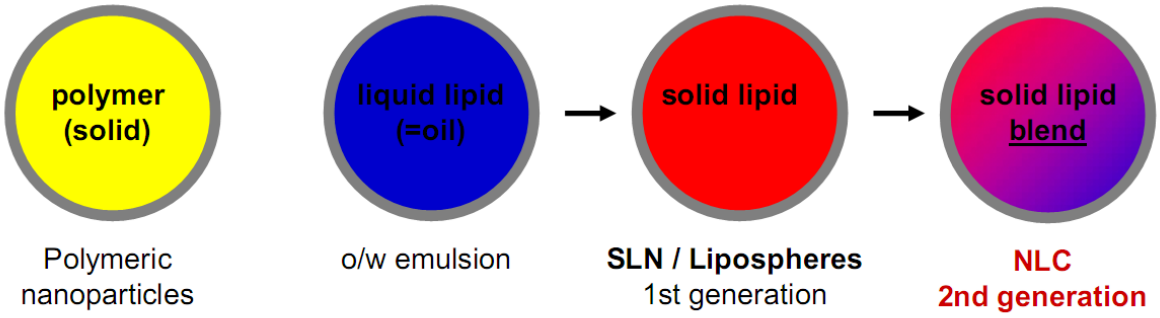

Fig.1: Traditional Carriers and Lipid Nanoparticles [10]

2. Why Lipid Nanoparticles? [15]

- Better control over release kinetics of encapsulated compound
  - Engineering via size and lipid composition.
  - Melting can serve as trigger.
- Enhanced bioavailability of entrapped bioactive.
- Chemical protection of labile incorporated compounds.
- Much easier to manufacture than bio polymeric nanoparticles.
- No special solvents required.
- Wider range of base materials (lipids).

- Conventional emulsion manufacturing methods applicable.
- Raw materials essential the same as in emulsions.
- Very high long-term stability.
- Application versatility:

a. Can be subjected to commercial sterilization procedures.

b. Can be freeze-dried to produce powdered formulation.

3. Compositionof Nanostructured Lipid Carrier (NLC: (13, 16, 17, and 18)

Lipid nanoparticles with solid particle matrix are derived from o/w emulsions by simply replacing the liquid lipid (oil) by a solid lipid, i.e. being solid at body temperature. The first generation of solid lipid nanoparticles (SLN) was developed at the beginning of the nineties. They were produced from a solid lipid only. In the second generation technology of the nanostructured lipid carriers (NLC), the particles are produced by using a blend of a solid lipid with a liquid lipid, this blend also being solid at body temperature.

In the NLC very different lipids were blended to form the matrix that means solid lipids and liquid lipids. Due to their difference in structure they cannot fit together very well to form a perfect crystal, the matrix contains a lot of imperfection to accommodate drug in molecular form and amorphous clusters.

In the second generation of the lipid nanoparticle technology, the particles are prepared using blends of solid lipids and liquid lipids (oils). To obtain the blends from the particles matrix, solid lipid are mixed with liquid lipids (oils), preferably in a ratio of 70:30upto a ratio of 99.9:0.1.Due to the oil in these mixtures a melting point depression compared to the pure solid lipid is observed, but the blends obtain are also solid at body temperature. This second generation of nanoparticles is called nanostructured lipid carriers. The overall solid content of NLC increased up to 95%.These second generation of submicron particles can be loaded with cosmetics and pharmaceutical actives as well.

3.1 Ingredients used in the formulation of NLC :(13)

The essential ingredients for NLCs include lipids, emulsifiers and water.

Emulsifiers

The emulsifiers have been used to stabilize the lipid dispersions. Most of the investigations employ hydrophilic emulsifiers such as Pluronic F68 (poloxamer 188), polysorbates (Tween), polyvinyl alcohol, and sodium deoxycholate. Lipophilic or amphiphilic emulsifiers such as Span 80 and lecithin are employed for fabrication of NLCs if necessary. It has been found that the combination of emulsifiers can prevent particle aggregation more efficiently. Polyethylene glycol (PEG), sometimes added in NLCs, resides on the Nano particulate shell to prevent uptake by the reticuloendothelial system (RES) and to prolong the circulation time of drugs. Table 1 summarizes the detailed information pertaining to the materials used for NLCs. Another prerequisite for NLCs' stability is the ability for preservation. The preservatives can impair the physical stability of lipid dispersions. Obeidat *et al*. demonstrate that Hydrolite® 5 is proved suitable for the preservation of coenzyme Q10- loaded NLCs.

Table 1: The excipients for composing nanostructured lipid carriers (NLCs)

Ingredient	Material
Solid Lipid	Tristearin, stearic acid, cetylpalmitate, cholesterol, Precirol® ATO 5, Compritol® 888 ATO, Dynasan®116, Dynasan® 118, Softisan® 154, Cutina® CP, Imwitor® 900 P, Geleol®, Gelot® 64, Emulcire® 61
Liquid Lipid	Medium chain triglycerides, paraffin oil, 2-octyl dodecanol, oleic acid, squalene, isopropyl myristate,vitamin E, Miglyol® 812,Transcutol® HP, LabrafilLipofile® WL 1349, Labrafac® PG, Lauroglycol® FCC, Capryol® 90
Hydrophilic Emulsifier	Pluronic® F68 (poloxamer 188), Pluronic® F127 (poloxamer 407), Tween 20,Tween 40,Tween 80,polyvinyl alcohol, Solutol® HS15, trehalose, sodium deoxycholate, sodium glycocholate, sodium oleate, polyglycerol methyl glucose distearate
Lipophilic Emulsifier	Myverol® 18-04K, Span 20, Span 40, Span 60
Amphiphilic Emulsifier	Egg lecithin, soya lecithin, phosphatidylcholines, phosphatidylethanolamines, Gelucire® 50/13

Lipids

Both solid and liquid lipids are included in NLCs for constructing the inner cores. The solid lipids commonly used for NLCs include glycerylbehenate (Compritol® 888 ATO), glycerylpalmitostearate (Precirol® ATO 5), fatty acids (e.g. stearic acid), triglycerides (e.g.

tristearin), steroids (e.g. cholesterol), and waxes (e.g. cetylpalmitate). These lipids are in a solid state at room temperature. They would melt at higher temperatures (e.g. > 80°C) during the preparation process. Liquid oils typically used for NLCs consist of digestible oils from natural sources. The medium chain triglycerides, such as Miglyol® 812, are often utilized as the constituents of liquid lipids because of their similar structures to Compritol®. Other oily components such as paraffin oil, 2- octyldodecanol, propylene glycol dicaprylocaprate (Labrafac®), isopropyl myristate and squalene are included as well. Alternatively, the fatty acids, such as oleic acid, linoleic acid, and decanoic acid, are included in NLCs for their value as having oily components and as being penetration enhancers of topical delivery. In general, these lipids are already approved by European and American regulatory authorities for clinical applications and for their "generally recognized as safe" (GRAS) status. There is a need for novel and biocompatible oils that are cost effective, non-irritating, and capable of being sterilized before application. Vitamin E (α-tocopherol) and other tocols have been investigated as materials for nanoemulsions. Tocols can serve as a choice of oils for NLCs because of their stability, ease of production on a large scale, and good solubility in lipophilic drugs. NLCs produced using natural oils from plants are also currently popular. Averina*et al*. have used Siberian pine seed oil and fish oil from Baikal Lake as the liquid oils since they show acceptable physical and chemical stability to NLCs.

3.2 Types of NLC :[6, 10, 13, 19]

The three types of NLC can be summarized

1. The imperfect type
2. The amorphous type
3. The multiple types

A potential problem in SLN is the formation of a perfect crystal, which can be compared to a dense 'brick wall'. Using different molecules, i.e. different 'stones' to build the matrix or 'wall' leaves enough imperfections to accommodate the drug . Drug load in SLN is limited due to the formation of the lipid crystal. Drug expulsion is caused by an ongoing crystallization process towards a perfect crystal. Thus, by avoiding crystallization, one can avoid these obstacles which is realised in the NLC type 2. The lipid matrix is solid but not crystalline it is in an amorphous state. This can be achieved by mixing special lipids, e.g. hydroxyl octacosanyl hydroxyl stearate with isopropylmyristate. The solid character of the particles was proven by NMR measurements and the lack of crystallinity by DSC analysis10. The third type of NLC is a multiple system, being comparable to w/o/w emulsions. In this case it is an oil-in-solid lipid-in-water dispersion. The solid lipid matrix contains tiny liquid oil Nano compartments. This NLC type uses the fact that for a number of drugs, the solubility in oils is higher than their solubility in solid lipids.

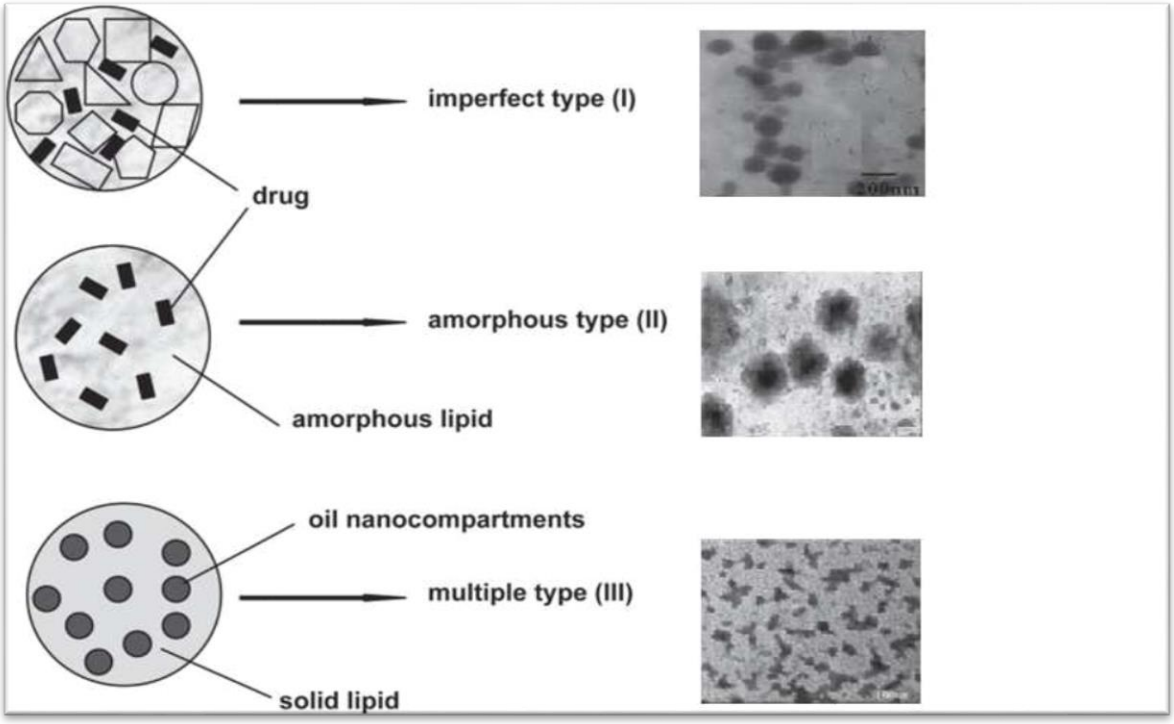

Fig.2: Types of NLC: (I) imperfect type,(II) amorphous type and (III) multiple type[10]

4.

5. **Advantages of NLC :**[10, 13]

- Better physical stability,
- Ease of preparation and scale-up,
- Increased dispersability in an aqueous medium,
- High entrapment of lipophilic drugs and hydrophilic drugs,
- Controlled particle size,
- An advanced and efficient carrier system in particular for substances,
- Increase of skin occlusion,
- Extended release of the drug,
- One of the carriers of choice for topically applied drugs because their lipid components have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations,
- Small size of the lipid particles ensures close contact to the stratum corneum thus enhancing drug penetration into the mucosa or skin,
- Improve benefit/risk ratio,
- Increase of skin hydration and elasticity and
- These carriers are highly efficient systems due to their solid lipid matrices, which are also generally recognized as safe or have a regulatory accepted status.

6. **Limitation of NLC:**[10, 13]

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

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

7. Preparation Procedures Of Nanostructured Lipid Carriers (NLC):[10, 13, 15, 16]

There many methods for the preparation of lipid nanoparticulate DDS. The method used is dictated by the type of drug especially its solubility and stability, the lipid matrix, route of administration, etc.

6.1 High Pressure Homogenization Technique

HPH has been used as a reliable and powerful technique for the large-scale production of NLCs, lipid drug conjugate, SLNs, and parenteral emulsions. In High Pressure Homogenization technique lipid are pushed with high pressure (100-200bars) through a narrow gap of few micron ranges. So shear stress and cavitations are the forces which cause the disruption of particle to submicron range. Normally the lipid contents are in the range of 5-10%. In contrast to other preparation technique High Pressure Homogenization does not show scaling up problem. Basically there are two approaches for production by high pressure homogenization, hot and cold homogenization techniques. For both the techniques drug is dissolved in the lipid being melted at approximately 5- 10^{o} C above the melting point.

6.2 Hot Homogenization Technique

In this technique the drug along with melted lipid is dispersed under constant stirring by a high shear device in the aqueous surfactant solution of same temperature. The pre-emulsion obtained is homogenized by using a piston gap homogenizer and the obtained nanoemulsion is cooled down to room temperature where the lipid recrystallises and leads to formation of nanoparticles.

6.3 Cold homogenization technique

Cold homogenization is carried out with the solid lipid containing drug. Cold homogenization has been developed to overcome the problems of the hot homogenization technique such as, temperature mediated accelerated degradation of the drug payload, partitioning and hence loss of drug into the aqueous phase during homogenization. The first step of both the cold and hot homogenization methods is the same. In the subsequent step, the melt containing drug is cooled rapidly using ice or liquid nitrogen for distribution of drug in the lipid matrix. Cold homogenization minimizes the thermal exposure of the sample.

6.4 Micro emulsion technique

The lipids (fatty acids or glycosides e.g. stearic acid) are melted and drug is incorporated in molten lipid. A mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipids and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratios for micro emulsion formation. Thus the micro emulsion is the basis for the formation of nanoparticles of a requisite size. This micro emulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot micro emulsion with water in a ratio in the range 1:25 – 1:50. This dispersion in cold aqueous medium leads to rapid recrystallization of the oil droplets.

6.5 Solvent emulsification-evaporation technique

In solvent emulsification-evaporation technique, the hydrophobic drug and lipophilic material were

dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenizer. To improve the efficiency of fine emulsification, the coarse emulsion was immediately passed through the micro fluidizer. Thereafter, the organic solvent was evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates of SLNs. Here the mean particle size depends on the concentration of lipid in organic phase. Very small particle size could be obtained with low lipid load (5%) related to organic solvent. The big advantage of this method is the avoidance of any thermal stress, which makes it appropriate for the incorporation of highly thermo labile drugs. A clear disadvantage is the use of organic solvent which may interact with drug molecules and limited the solubility of the lipid in the organic solvent.

6.6 Solvent emulsification-diffusion technique

In solvent emulsification-diffusion technique, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, methyl acetate) must be partially miscible with water and this technique can be carried out either in aqueous phase or in oil. Initially, both the solvent and water were mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquid**.** When heating is required to solubilize the lipid, the saturation step was performed at that temperature. Then the lipid and drug were dissolved in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous Solution containing stabilizer (dispersed phase) using mechanical stirrer. After the formation of o/w emulsion, water (dilution medium) in typical ratio ranges from 1:5 to 1:10, were added to the system in order to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles. Here the both the phase were maintain at same elevated temperature and the diffusion step was performed either at room temperature or at the temperature under which the lipid was dissolved. Throughout the process constant stirring was maintained. Finally, the diffused solvent was eliminated by vacuum distillation or lyophilization.

6.7 Phase inversion temperature (PIT) method

Phase inversion of O/W to W/O emulsions and vice versa induced by temperature change is a long known method to produce micro emulsions stabilized with non-ionic surfactants. The technique is based on the change in the properties of polyoxyethylated surfactants at different temperatures. The hydrophilic-lipophillic balance (HLB) value of surfactants defined by Griffin is valid at 25°C. At this temperature the hydrophilic parts of the SAC molecules are hydrated to a certain extent. An increase in the temperature causes dehydration of the ethoxy groups. As a result, the lipophilicity of the molecules of the SAC rises with corresponding decrease in HLB value. At a certain point the affinity of the SAC to the aqueous and lipid phase is equal - this temperature is defined as the phase inversion temperature. This particulate state is characterized by very low surface tension and presence of complex structures in the system. If the temperature is further increased the SAC's affinity to the lipid phase becomes higher enough to stabilize emulsions of w/o type.

6.8 Melting dispersion method

In melting method, drug and solid lipid are melted in an organic solvent regarded as oil phase, and simultaneously water phase is also heated to the same temperature as oil phase. Subsequently, the oil phase is added to a small volume of water phase and the resulting emulsion is stirred at high speed for few hours. Finally, it is cooled down to room temperature to yield nanoparticles.

6.9 High Shear Homogenization or Ultra sonication Technique

Ultra sonication based on the mechanism of cavitation .In first step, the drug was added to previously melt solid lipid. In second step, the heated aqueous phase (heated to same temperature) was added to the melted lipid and emulsified by probe sonication or by using high speed stirrer or aqueous phase added to lipid phase drop by drop followed by magnetic stirring. The obtained pre-emulsion was ultrasonicated using probe sonicator with water bath (at 0^{o}C). In order to prevent recrystallization during the process, the production temperature kept at least 5^{o}C above the lipid melting point. The obtained product was filtered through a 0.45µm membrane in order to remove impurities carried in during ultra-sonication.

6.10 Solvent injection (or solvent displacement) technique

Technique in which a solvent that distributes very rapidly in water (DMSO, ethanol) is used. First the lipid is dissolved in the solvent and then it is quickly injected into an aqueous solution of surfactants through an injection needle. The solvent migrates rapidly in the water and lipid particles precipitate in the aqueous solution. Particle size depends on the velocity of distribution processes. Higher velocity results in smaller particles. The more lipophilic solvents give larger particles which may become an issue. The method offers

advantages such as low temperatures, low shear stress, easy handling and fast production process without technically sophisticated equipment (e.g. high-pressure homogenizer). However, the main disadvantage is the use of organic solvents.

6.11 Double emulsion technique

In double emulsion technique the drug (mainly hydrophilic drugs) is dissolved in aqueous solution, and further emulsified in melted lipid. The primary emulsion is stabilized by adding stabilizer that is dispersed in aqueous phase containing hydrophilic emulsifier, which is followed by stirring and filtration. Double emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be modified in order to sterically stabilize them by means of the incorporation of lipid-PEG derivatives.

8. Physicochemical Characterization of NLC :[11, 13]

The physicochemical characterization for NLCs is essential to confirm quality control and stability. Both physical and chemical properties can be determined for NLCs. Microscopic and macroscopic techniques are used in development of colloidal system84. Various techniques like particle size analysis, zeta-potential, transmission electron microscopy, differential scanning calorimetry (DSC), X- Ray scattering, polarized light microscopy, laser diffraction (LD), field-flow fractionation (FFF) were performed to investigate the structure, mobility and molecular environment of the compounds. These techniques also reveal the physical and chemical stability of formulation, surface charge tends to determine the particles will flocculate or not.

7.1 Particle Size

The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size and as particle size decreases, surface area characteristics increases as a function of total volume, photon correlation spectroscopy (PCS) based on laser light diffraction provides an appropriate method for investigation and can be applied for particles ranging below 200 nm and up to 1μm 86. For particles below 200nm Rayleigh's theory holds that the scattering intensity to be proportional to the sixth potency of the particle diameter. Both, Fraunhofer's and Rayleigh's theories, are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption and the size of the particles as well as the refractive indices of both the particles and the dispersion medium.

7.2 Zeta potential (ZP)

Zeta potential is the electric potential of a particle in a suspension. It is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions. In suspensions the surfaces of particles develop a charge due to ionization of surface groups or adsorption of ions. This charge depends on both the surface chemistry of the particles and the media around these particles. The surface charge generates a potential around the particle, which is at the highest near the surface and decays with distance into the medium. The zeta potential can be measured by determining the velocity of the particles in an electrical field (electrophoresis measurement).

7.3 Scanning electron microscopy (SEM)

This technique can be used to investigate the shape of the particles prepared and to assess the particle size of these particles. Aqueous NLC dispersions can be applied and spread on a sample holder (thin carbon film). The samples will be placed inside of the vacuum column of the microscope and the air was pumped out of the chamber. An electron gun placed at the top of the column emits a beam of high energy primary electrons. The beam of the electrons passes through the lenses which concentrates the electrons to a fine spot and scan across the specimen row by row. As the focused electron beam hits a spot on the sample, secondary electrons are emitted by the specimen through ionization. A detector counts these secondary electrons. The electrons are collected by a laterally placed collector and these signals are sent to an amplifier.

7.4 Differential scanning calorimetry (DSC)

DSC is usually used to get information about both the physical and the energetic properties of a compound or formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature. DSC and powder is performed for the determination of the degree of crystallinity of the particle dispersion. The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion.

7.5 Nuclear magnetic resonance (NMR)

NMR can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle. Nuclear Magnetic Resonance (NMR). The

mobility of the solid and liquid lipids is related to the width at half amplitude of the signals. Broad signals and small amplitudes are characteristics of molecules with restricted mobility and strong interactions. The higher line width of NLCs compared to the physical mixture of the materials added in NLCs indicates the interaction of liquid oil with the solid lipid. Immobilization of the nanoparticles of NLCs is stronger compared to SLNs with totally crystallized cores.

7.6 Atomic Force Microscopy (AFM)

AFM is optimal for measuring morphological and surface features that are extremely small. AFM does not use photons or electrons but a very small sharp tipped probe located at the free end of a cantilever driven by interatomic repulsive or attractive forces between the tip and surface of the specimen. Although electron microscopy is still frequently used, the AFM technique offers substantial benefits: real quantitative data acquisition in three dimensions, minimal sample preparation times, flexibility in ambient operating conditions, and effective magnifications at the nano levels.

7.7 X-ray Scattering

With X-ray scattering experiments characteristic interferences the generated from an ordered microstructure. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacing'd'. According to Bragg's equation 'd' can be calculated $d= n/\lambda\ 2 \sin \theta$ Where, λ is the wavelength of the X- ray being used, n is an integer and nominates the order of the interference and θ is the angle under which the interference occurs.

7.8 Transmission Electron Microscopy

It is a technique where colloidal samples could be visualized at high resolution. Sufficient contrast can be given to a thin film of the frozen sample by use of osmium tetra- oxide. This allows the sample to be viewed directly in the TEM (at temperature -196^{o} C).The adjustment of the temperature to -196^{o} C leads to a very poor pressure, so that the examination of the sample is possible by preservation of microstructure despite the high vacuum.

7.9 Drug Release

The controlled or sustained release of the drugs from NLCs can result in the prolonged half-life and retarded enzymatic attack in systematic circulation. The drug release behavior from NLCs is dependent upon the production temperature, emulsifier composition, and oil percentage incorporated in the lipid matrix. The drug amount in the outer shell of the nanoparticles and on the particulate surface is released in a burst manner, while the drug incorporated into the particulate core is released in a prolonged way. Sustained release of the drugs can be explained considering both drug partitioning between the lipid matrix and water, as well as the barrier function of the interfacial membrane. The dialysis method and the utilization of the Franz cell are the modes for measuring in vitro drug release from nanoparticles. The interpretation of in vitro drug release profiles should consider the specific environment in the in vivo status. Enzymatic degradation of lipid nanoparticles may be influenced to a relevant extent by the composition of the particles.

8. Topical benefit of NLC :[11, 15]

8.1 Increase of skin occlusion:

The lipid film formation on the top of the skin and the subsequent occlusion effect was reported for lipid nanoparticles. By using very small lipid particles, which are produced from highly crystalline and low melting point lipids, the highest occlusion will be reached. Particles smaller than 400 nm containing at least 35% lipid of high cystallinity have been most effective. Souto *et al*. found a higher occlusive factor for SLN in comparison to NLC of the same lipid content. Comparing NLC with different oil content showed that an increase in oil content leads to a decrease of the occlusive factor.

8.2 Increase of skin hydration and elasticity:

The reduction of Trans epidermal water loss (TEWL) caused by occlusion leads to an increase in skin hydration after dermal application of SLN, NLC or formulations containing them. An *in vivo* study showed that the SLN containing o/w cream increased the skin hydration significantly more than the conventional o/w cream. In this study the skin hydration effect after repetitive application of an o/w cream containing SLN and a conventional o/w cream was investigated for 28 days26. A significant higher increase in skin hydration was found by Müller *et al*. for an NLC-containing cream compared to conventional cream.

8.3 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 SLN or NLC leads to a reduction of corneocytes packing and an increase in the size of the corneocytesgaps. This will facilitate the percutanious absorption and drug penetration to the deeper skin layers.

8.4 Improve benefit/risk ratio:

Skin atrophy and systemic side effect occurred after applying conventional prednicarbate cream could be avoided when this drug was formulated as lipid nanoparticle. Predinicarbate uptake was enhanced and it was accumulated in the epidermis with a low concentration in the dermis. Tretinoin loaded-SLN formulation was studied by Shah *et al* concerning skin irritation. One of the major disadvantages associated with the topical application of tretinoin is the local skin irritation such as erythema; peeling and burning as well as increased sensitivity to sunlight. In the *in vitro* permeation studies through rat skin they found that SLN based tretinoin gel hasa permeation profile comparable to that of the market tretinoin cream. But on the other hand, Draize patch test showed that SLN based tretinoin gel resulted in remarkably less erythremic episodes compared to the currently marketed tretinoin cream and hence, a better benefit/risk ratio is expected for the formulations containing tretinoin-loaded SLN. Conclusively,applying SLN or NLC can enhance skin penetration of incorporated actives, promote theepidermal targeting and minimize the systemic side effects and therefore, the benefit/riskratio is improved.

8.5 Enhancement of chemical stability of chemically labile compounds:

Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives, e.g. coenzyme Q 10, ascorbyl palmitate, and retinol (vitamin A).

8.6 Enhancement of UV blocking activity:

Some side effects of organic UV blockers were reported due to the penetration of these compoundsinto the skin causing skin irritation and allergicreaction. This penetration can be reduced by incorporating these compounds in lipid nanoparticles. It was found that incorporating benzophenone in SLN not only improves the UV blocking activity evaluated using *in vitro* photoprotection assay but also reduces the absorption of the benzophenone into the skin in comparison to a conventional nanoemulsion. Improving the UV blocking activity allows the reduction of the concentration of the UV blocker while maintaining the protective level of the conventional formulation.

9. CONCLUSION

Lipid carriers have bright future, because of their intrinsic property to improve the bioavailability of lipophilic drugs with low aqueous solubility. SLN and NLC offers an economical and patient friendly device for administration of drugs by topical routes.Technologically, the straight forward production of nanocarriers avoiding organic solvents should be a relevant criterion for industrial scale-up (e.g. Solid Lipid Nanoparticles and Nanostructured Lipid Carries) conditioning the becoming of topical formulations for the 21st century's dermatological practices.

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