REVIEW OF SOLID LIPID NANOPARTICLES

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***Abstract*: To improve the solubility and bioavailability solid lipid nanoparticles (SLNs) are the new approach in drug delivery system. Solid lipid nanoparticles (SLN) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research. . Solid lipid nanoparticles (SLN) are made of a solid lipid core with a monolayer phospholipid shell.Due to their unique size dependent properties, lipid nanoparticles offer possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could use for drug targeting. This review concentrated on advancement in lipid nanoparticles, method of preparation, secondary production steps, characterization, application and future of SLNs. This review presents a broad treatment of solid lipid nanoparticles discussing their advantages, limitations.**

***Keywords*: Solid lipid nanoparticles (SLNs), Colloidal drug carriers, Solid lipid**

INTRODUCTION

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as - emulsions, liposomes and polymeric micro – and nanoparticles[1]. The field of Novel Drug Delivery System is emerging at an exponential rate with the deep understanding gained in diversified fields of Biotechnology, Biomedical Engineering and Nanotechnology[2]. The overall goal of nanotechnology is the same as that of medicine: to diagnose as accurately and early as possible and to treat as effectively as possible without any side effects using controlled and targeted drug delivery approach[3]. Some of the important Drug Delivery System developed using Nanotechnology principles are- Nanoparticles, Solid Lipid Nanoparticles, Nanosuspension, Nanoemulsion, Nanocrystals[4]. SLNs are colloidal carrier system composed of a high melting point lipid as a solid core coated by aqueous surfactant and the drugs used are of BCS Class II and IV[5]. The term lipid in a broad sense includes triglycerides, partial glycerides, fatty acids, hard fats & waxes. A clear advantage of SLN is the fact that the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity[6]. SLNs are mainly prepared by high pressure homogenization or micro emulsification. SLNs prepared by any technique are in dispersion form which on long term storage results in instability mainly because of hydrolysis reactions so to increase their stability they can be converted into solid dry reconstituable powders through lyophilisation and a cheap and easy variant to lyophilisation is spray drying technique[7].

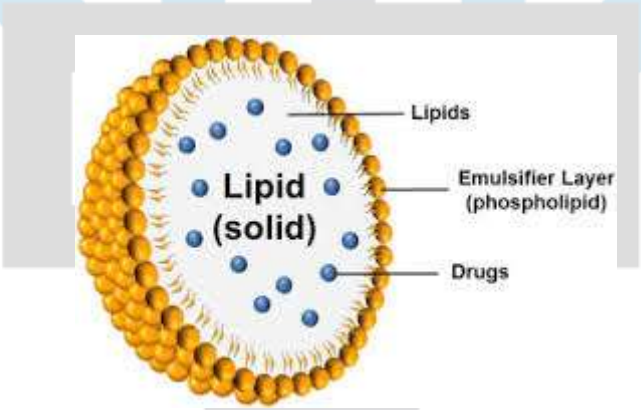


Fig. 1: Shows structure of Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid components which are in solid state at room temperature. The schematic representation of different particulate drug carriers such as emulsions and liposomes and their advantages are compared with SLNs in Fig. 2. SLNs combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes.

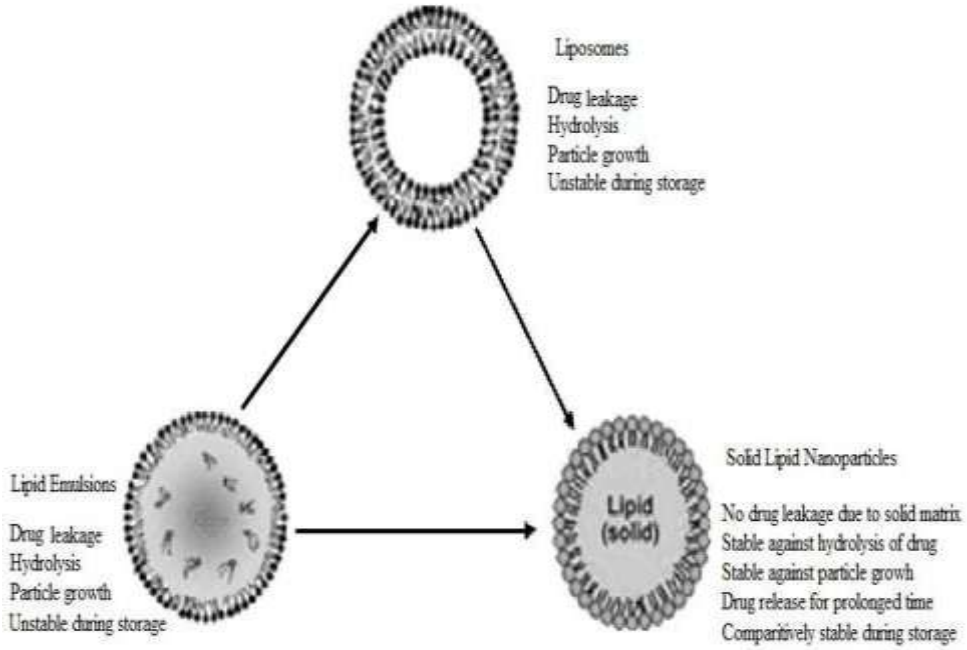


Fig. 2: Shows a diagrammatic representation on SLN over emulsions and liposome.

Advantages of SLN[1,9,10]

- Control and / or target drug release.
- Excellent biocompatibility[10].
- Improve stability of pharmaceuticals[9].
- High and enhanced drug content.
- Easy to scale up and sterilize.
- Better control over release kinetics of encapsulated compounds.
- Enhanced bioavailability of entrapped bioactive compounds.
- Chemical protection of labile incorporated compounds.
- Much easier to manufacture than biopolymeric nanoparticles.
- No special solvent required.
- Conventional emulsion manufacturing methods applicable.
- Raw materials essential the same as in emulsions.
- Very high long-term stability.
- Application versatility.
- Can be subjected to commercial sterilization procedure

Disadvantages of SLN[9,12]

- Particle growth.
- Unpredictable gelation tendency.
- Unexpected dynamics of polymeric transitions.

Routes of administration and their biodistribution[2,9,13,14,15]

The *in vivo* behavior of the SLN particles will mainly depend on the following points:

Administration route

Interactions of the SLN with the biological surroundings including: distribution processes (adsorption of biological material on the particle surface and desorption of SLN components into to biological surroundings) and enzymatic processes. Various administration routes are:

1. Parenteral administration

Peptide and proteins drugs are usually available for parenteral use in the market. Since their conventional oral administration is not possible due to enzymatic degradation in GI tract. Parenteral application of SLN reduces the possible side effects of drug incorporated with the increased bioavailability. These systems are very suitable for drug targeting.

2. Oral administration

Controlled release behavior of SLNs is reported to enable the bypass of gastric and intestinal degradation of the encapsulated drug, and their possible uptake and transport through the intestinal mucosa. However, the assessment of the stability of colloidal carriers in GI fluids is essential in order to predict their suitability for oral administration.

3. Rectal administration

When rapid pharmacological effect is required, in some circumstances, parenteral or rectal administration is preferred. This route is used for pediatric patients due to easy application.

4. Nasal administration

Nasal route is preferred due to its fast absorption and rapid onset of drug action also avoiding degradation of labile drugs in the GIT and insufficient transport across epithelial cell layers.

5. Respiratory delivery

Nebulisation of solid lipid particles carrying anti-tubercular drugs, anti-asthmatic drugs and anti- cancer was observed to be successful in improving drug bioavailability and reducing the dosing frequency for better management of pulmonary action.

6. Ocular administration

Biocompatibility and muco-adhesive properties of SLN improve their interaction with ocular mucosa and prolong corneal residence time of the drug, with the aim of ocular drug targeting.

7. Topical administration

SLN are very attractive colloidal carrier systems for skin applications due to their various desirable effects on skin besides the characteristics of a colloidal carrier system. They are well suited for use on damaged or inflamed skin because they are based on non-irritant and non-toxic lipids.

Aims of solid lipid nanoparticles[10]

It has been claimed that SLN combine the advantages and avoid the disadvantages of other colloidal carriers. Proposed advantages include-

- Possibility of controlled drug release and drug targeting.
- Increased drug stability
- High drug payload
- Incorporation of lipophilic and hydrophilic drugs
- No biotoxicity of the carrier
- Avoidance of organic solvents
- No problems with respect to large scale production and sterilization
- Increased Bioavailability of entrapped bioactive compounds
-

Table 1: Shows list of excipients used in sln preparation[10,16]

Lipids	Surfactants
Triglycerides	**Phospholipids**
Tricaprin	Soy lecithin (LipoidÒ S 75, LipoidÒ S 100)
Trilaurin	Egg lecithin (Lipoid E 80)
Trimyristin (Dynasan 114)	Phosphatidylcholine (Epikuron 170, Epikuron200)
Tripalmitin (Dynasan 116)	**Ethylene oxide/propylene oxide copolymers** Poloxamer 188
Tristearin (Dynasan 118) Hydrogenated coco-glycerides (SoftisanÒ142)	Poloxamer 182
	Poloxamer 407
Hard fat types	Poloxamine 908
WitepsolÒ W 35	**Sorbitan ethylene oxide/propylene oxide copolymers**
WitepsolÒ H 35	Polysorbate 20
WitepsolÒ H 45	Polysorbate 60
WitepsolÒ E 85	Polysorbate 80
Acyl glycerols	**Alkylaryl polyether alcohol polymers**
Glyceryl monostearate(ImwitorÒ900)	Tyloxapol
Glyceryl distearate(Precirol)	**Bile salts**
Glyceryl monooleate(Peceol)	Sodium cholate
Glyceryl behenate (CompritolÒ 888ATO)	Sodium glycocholate
Glyceryl palmitostearate (PrecirolÒ ATO 5)	Sodium taurocholate
Waxes	Sodium taurodeoxycholate
Cetyl palmitate Fatty Acids Stearic acid Palmitic acid Decanoic acid Behenic acid Acidan N12	**Alcohols** Ethanol ButanoL Butyric acid
	Dioctyl sodium sulfosuccinate Monooctylphosphoric acid sodium
Cycliccomplexes	
Cyclodextrin	
para-acyl-calix-arenes	

Solid lipid nanoparticles production procedure

The major problem for the SLNs to be introduced to the market is the use of excipients having no accepted status. For topical SLN, all excipients used in current topical cosmetic and dermal pharmaceutical products can be used. For oral administration of SLN, all excipients can be employed that are frequently used in traditional oral dosage forms such as tablets, pellets, and capsules. Even surfactants with cell membrane-damaging potential, e.g. SDS, can be used. SDS is contained in many oral products and accepted as an excipient by the regulatory authorities. In addition substances with accepted Generally Recognized As Safe (GRAS) status can be used. The situation is different for Parenteral administration as solid lipids have not yet been administered parenterally before-in contrast to liquid lipids (o/w emulsions for iv administration, prolonged release oil-based injectables for im administration). However, the glycerides used for SLN production are composed of compounds (glycerol, fatty acids) which are also present in emulsions for Parenteral nutrition[17].

The general excipients used in any SLN formulation are solid lipids, emulsifiers, co-emulsifiers and water. The term lipid is used here in a broader sense and includes triglycerides (e.g.tristearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate). All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently[10].

Influence of various excipients used on product quality Influence of the lipid

In hot homogenization it can be seen that average particle size of SLN dispersion is increasing with higher melting lipids and this is because of higher viscosity of dispersed phase. Some peculiar parameters are specific for every lipid like lipid crystallization, lipid hydrophilicity and shape of lipid crystals. Chemically most lipids are mixtures of various compounds so their composition can very from different suppliers and also from batch to batch but these small differences affect the quality of SLNs to a great extent (e.g. by changing the zeta potential, retarding crystallization processes etc.)Increasing the lipid content over 5%-10% result in larger particles and broader particle size distribution in most cases[10,18].

Influence of emulsifier

Choice of emulsifier has a great impact on quality of SLN. Reduction in surface tension and particle partitioning during homogenization is facilitated by increasing the emulsifier concentration. Reduction in particle size leads to increased surface area.

During SLN preparation the primary dispersion must contain excessive emulsifier to rapidly cover the new surfaces formed during High Pressure Homogenization; otherwise it will lead to agglomeration of uncovered new lipid surfaces. The time taken for

redistribution of emulsifier between new particle surfaces and micelles is different for different types of surfactants. It has been studied that Low Molecular Weight surfactants will take less time for redistribution and High Molecular Weight will take longer time for redistribution. The addition of some co-emulsifying agent like Sodium Glycocholate further decreases the particle size[10].

DRUG INCORPORATION MODELS

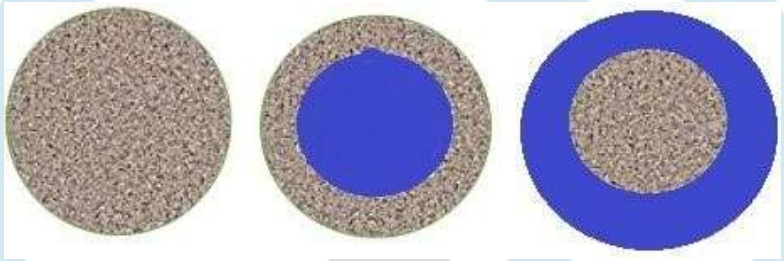
Solubility of drug and drug loading capacity are inversely proportional. Thus enhanced solubility results in reduced entrapment efficacy. To overcome this, Müller et al reported a cold homogenization technique which is performed at room temperature or below (0° C).[19]

Factors affecting loading capacity of a drug in lipid:[20]

- solubility of drug in lipid melt,
- miscibility of drug melt and lipid melt,
- chemical and physical structure of solid matrix lipid,
- polymorphic state of lipid material
- Solubility

There are three models of drug incorporation: [Figure2]

- Solid lipid solution
- Drug enriched shell
- Drug enriched core



Solid solution Drug enriched shell Drug enriched core

Fig. 3: Models of drug incorporation.

Solid solution

In the case of the solid solution model, the drug is molecularly dispersed in the lipid matrix when the particles are produced by the cold homogenization technique and using no surfactant or no drug-solubilizing surfactant. The drug has strongly pronounced interactions with the lipid.[21, 16]

Drug enriched shell

According to the drug-enriched shell model of drug incorporation, a solid lipid core forms when the recrystallization temperature of the lipid is reached. On reducing the temperature of the dispersion, the drug concentrates in the still liquid outer shell of the SLN.[16,22,18]

Drug enriched core

According to the drug-enriched core model of drug incorporation, cooling the nanoemulsion leads to a super saturation of the drug which is dissolved in the lipid melt at or close to its saturation solubility and the drug precipitates prior to lipid recrystallization. Further cooling finally leads to the recrystallization of the lipid surrounding the drug as a membrane.[19, 16]

ADVANCEMENT IN SLNS

As SLNs have several advantages of controlled and targeted drug delivery but have some limitations i.e.

- Limitation of drug load by the solubility of the drug in the solid lipid.
- Drug expulsion phenomenon when lipid crystallizes to the stable β-form.
- Particle concentration in the aqueous dispersions ranging from about 1% to a maximum of only 30%.

It was observed that drug was expelled out of SLN during storage due to highly ordered crystalline lipid matrix which was leaving very little space for drug molecules. To overcome these limitations of SLNs, Lipid Drug Conjugates (LDCs), Nanostructured Lipid Carriers NLCs) and Polymer lipid hybrid nanoparticles (PLNs) are introduced.[23]

Lipid drug conjugates (LDCs)

A major problem of SLNs is the low capacity to load hydrophilic drugs due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix.[24] LDCs are spherical in shape and lipid drug core are stabilized by a surfactant interfacial region. Fatty acids, acylglycerols, waxes and mixture of these are utilized as core lipids. Surface stabilizers includes bile salts, cholesterol, phospholipids, sphingomyelins. Use of ligands promote tissue targeting. LDC enables the incorporation of both hydrophilic (e.g., doxorubicin and tobramycin) and lipophilic (e.g., progesterone and cyclosporine A) drugs.[23]

Nanostructured lipid carriers (NLCs)

Nanostructured lipid carriers (NLCs), formulated with biocompatible solid and liquid lipids, are an improved generation of solid lipid nanoparticles (SLNs), providing a delivery system for various active drugs with controlled-release characteristic.[25,26] Addition of a liquid lipid to a solid lipid leads in a less ordered crystal lattice and increased imperfection that results in high drug entrapment and stability during storage. A comparison among Triptolide, TP-SLNs and TP-NLCs were made by *Cong Zhang et. al.* i.e. Fatty degeneration in the hepatocytes, dead cells in the macrophages manifested as a starry sky appearance in the spleen, and obvious kidney proximal tubular dilation were seen, which were also observed in male mice after oral administration of TP. However, in the TP-NLCs group at the same dose, no apparent changes were found[26]

Three models of NLCs were proposed: [Figure 3]

1. Imperfect type NLCs were prepared from a lipid mixture of spatially different lipids composed of different fatty acids. This provide a larger distances between the fatty acid chains of glycerides and general imperfection of the crystal lattice. This provide more space for guest molecule in molecular or amorphous form.
2. Amorphous type NLCs are prepared by using special lipids such as hydroxyl octacosanyl, hydroxyl stearate, isopropyl myristate, etc. that avoids the crystallization or transformation upon cooling.
3. Multiple type NLCs are analogous to w/o/w multiple emulsions since these are oil/ solid lipid/ water and prepared for the drugs those shows higher solubility in oils than in solid lipids. Such drugs are dissolved in oil and protected from degradation by the surrounding solid lipids.

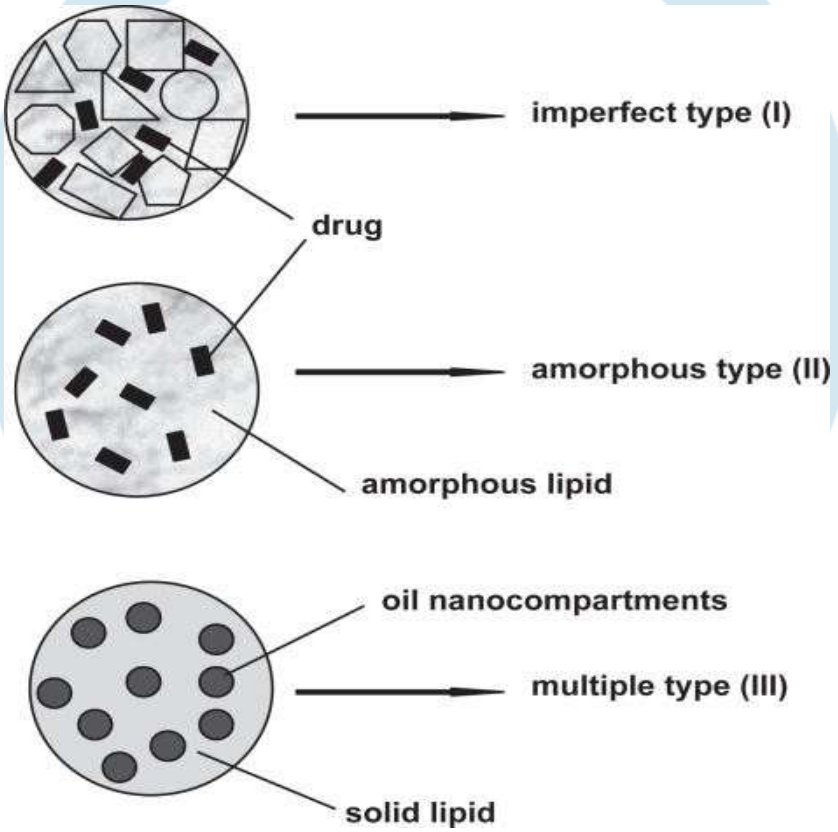


Fig. 4: Three models of NLCs

Polymer lipid hybrid nanoparticles (PLNs): [Figure 4] PLNs promising as drug delivery in treatment of cancer like breast cancer. PLNs composed of three components first is hydrophobic polymer core to encapsulate poorly water soluble drugs., second is hydrophilic polymeric shell to improve PLN stability and circulation half-life and third is lipid monolayer at core and shell interface that promote drug retention in polymer core. It has been shown in vitro that hybrid NPs possess the ability of carrying poorly water-soluble drugs with high encapsulation and loading yields, tunable and sustained drug release profiles, excellent serum stability, and differential targeting of cells.[27,28]

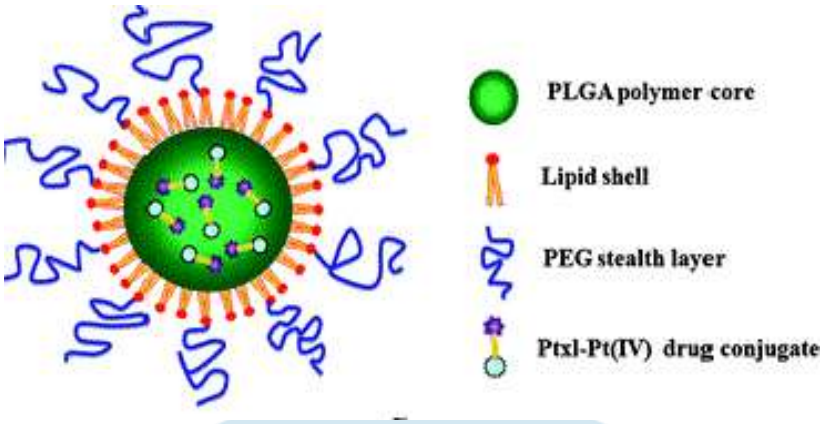


Fig. 5: Three models of NLCs.

SLNs PREPARATION TECHNIQUES (PRIMARY)

1. Emulsification solvent evaporation technique
2. Solvent emulsification-diffusion technique
3. Supercritical fluid technique
4. High pressure homogenization technique
5. Hot homogenization technique
6. Cold homogenization technique
7. Microemulsion based technique
8. Ultrasonication /high speed homogenization technique
9. Precipitation technique
10. Film-ultrasound dispersion technique
11. Double emulsion technique
12. Solvent Injection Technique
13. Membrane Contractor technique

Emulsification solvent evaporation technique

This technique is based on SLN dispersions by precipitation in oil/water emulsions. The lipophilic compound is dissolved in water immiscible organic solvent such as cyclohexane, which is emulsified in an aqueous phase. SLN dispersion is formed by precipitation of the lipid in the aqueous medium after evaporation of the solvent. The mean diameter of 25 nm with cholesterol acetate as model drug and lecithin/sodium glycocholate mixture as emulsifier has been reported for the prepared SLN. The reproducibility of the result was verified by Siekmann and Westesen, who produced the cholesterol acetate SLN with mean size of 29 nm.[29, 30]

Solvent emulsification-diffusion technique

SLNs can also be produced by solvent emulsification- diffusion techniques. The mean particle size depends upon lipid concentration in the organic phase and the emulsifier used. Particles with average diameters of 30- 100 nm can be obtained by this technique. Avoidance of heat during preparation is the most important advantage of this technique. In this method, the lipid matrix is dissolved in a water-immiscible organic solvent followed by emulsification in an aqueous phase. The solvent is evaporated under reduced pressure, resulting in a nanoparticulate dispersion formed by precipitation of the lipid in aqueous medium.[31]

Supercritical fluid technique

This is a novel technique which recently applied for the production of SLNs. A fluid is qualified as supercritical when its pressure and temperature exceed their respective critical value. Above the critical temperature, it is not possible to liquefy a gas by increasing the pressure. The supercritical fluid has unique thermo- physical properties. As the pressure is raised, the density of the gas increases without significant increase in viscosity while the ability of the fluid to dissolve compounds also increases. A gas may have little to no ability to dissolve a compound under ambient condition can completely dissolve the compound under high pressure in supercritical range. Therefore, its solvation power is altered by careful control of changes in temperature and pressure. Many gases like, CO_2, ammonia, ethane and CH_2FCF_3 were tried, but CO_2 is the best option for SCF technique because, it is generally regarded as safe, easily accessible critical point (31.5ºC,
75.8 bar), does not causes the oxidation of drug material, leaves no traces behind after the process, is inexpensive, non-inflammable, environmentally acceptable an easy to recycle or to dispose off. In the SCF phase or this technique generally use organic solvents (e.g. DMSO, DMFA) because they are fully miscible in SCF-CO_2. This technology comprises several processes for nanoparticles production such as Rapid Expansion of Supercritical Solution (RESS), Particles from Gas Saturated Solution (PGSS), Gas/Supercritical Anti- solvent (GAS/SAS), Aerosol Solvent Extraction Solvent (ASES), Solution Enhanced Dispersion by Supercritical fluid (SEDS), Supercritical Fluid Extraction of Emulsions (SFEE). Mainly SAS and PGSS were used for SLN preparation.[32]

High pressure homogenization technique

Hot homogenization technique

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A pre- emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high- shear mixing device (Ultra-Turrax). The quality of the final product is affected by the quality of pre-emulsion to a large extent and it is desirable to obtain droplets in the size range of a few micrometers. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures also accelerate the degradation rate of the drug and the carrier. The homogenization step can be repeated several times. It should always be kept in mind, that high pressure homogenization increases the temperature of the sample (approximately 10°C for 500 bar). In most cases, 3–5 homogenization cycles at 500– 1500 bar are sufficient. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to particle coalescence which occurs as a result high kinetic energy of the particles. The primary product is a nanoemulsion due to the liquid state of the lipid which on cooling at room temperature leads to solid particles. Due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a super cooled melt for several months.[8,7]

Cold homogenization technique

Cold homogenization method has been carried out to omit the following problems of the hot homogenization technique like temperature mediated drug and carrier degradation acceleration and consequently release of drug into the aqueous phase during homogenization. First stage in cold homogenization is the same with hot homogenization method but the next steps are different. The drug loaded lipid melt is cooled quickly by ice or liquid nitrogen for distribution of drug in the lipid matrix. The acquired particle sizes are in the range 50-
100 microns for this method. Disadvantages of cold homogenized samples are larger particle sizes and a broader size distribution. However, this method reduces the thermal exposure of the sample.[33]

Microemulsion based technique

This method is based on the dilution of microemulsions. As micro-emulsions are two-phase systems
composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-70°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2- 3°C) under stirring. SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step; achievable lipid contents are considerably lower compared with the High pressure homogenization based formulations.[34]

Ultrasonication /High speed homogenization technique

SLNs are also prepared by ultrasonication or high speed homogenization techniques. For smaller particle size combination of both ultrasonication and high speed homogenization is required. It reduces shear stress but has some disadvantages like potential metal contamination, physical instability like particle growth upon storage. In this probe sonicator or bath sonicator is used.[8]

Precipitation technique

The lipid is dissolved in an organic solvent (e.g., chloroform) and the solution is emulsified into an aqueous phase. After evaporation of the organic solvent, the lipid is precipitated, forming nanoparticles.[35]

Film-ultrasound dispersion technique

The lipid and drug are added to suitable organic solutions, and after decompression, rotation and evaporation of the organic solutions, a lipid film is formed. The aqueous solution containing emulsifier is then added to lipid film and, using probe sonication, SLNs are formed. Oleanolic acid SLNs have been produced using soybean phospholipid as a carrier using the film-ultrasound technique.[36]

Double emulsion technique

Double emulsion technique is used mainly for hydrophilic drugs. The drug was dissolved in aqueous medium and then was emulsified in melted lipid. This primary emulsion was stabilized by adding stabilizer (e.g. gelatine, poloxamer-407). Primary emulsion was dispersed in aqueous phase containing hydrophilic emulsifier (e.g. PVA). Thereafter, the double emulsion was stirred and was isolated by filtration .After evaporation of organic solvent by rotary, SLNs were recovered by centrifugation at 12000 ×g for 30 min at 4°C.

Solvent Injection Technique

It is a novel approach to prepare SLN, which has following advantages over other production methods like use of pharmacologically

acceptable organic solvent, easy handling and fast production process without technically sophisticated equipment. It is based on lipid precipitation from the dissolved lipid in solution. In this technique the solid lipid was dissolved in water-miscible solvent (e.g. ethanol, acetone, isopropanol) or a water miscible solvent mixture. Then this lipid solvent mixture was injected through an injection needle into stirred aqueous phase with or without surfactant. The resultant dispersion was then filtered with a filter paper in order to remove any excess lipid. The presence of emulsifier within the aqueous phase helps to produce lipid droplets at the site of injection and stabilize SLN until solvent diffusion was complete by reducing the surface tension between water and solvent.[8,37,38]

Table 2: Shows comparison of different formulation methods[8].

Formulation procedures	Advantages	Disadvantages
High pressure homogenisation	Low capital cost. Demonstrated at lab scale	Energy intensive process. Biomolecule damage. Polydisperse distributions. Unproven scalability.
Ultrasonication/ High speed homogenisation	Reduced shear stress	Potential metal contamination
Solvent Evaporation Method	Scalable. Continuous process. Commercially demonstrated	Extremely energy intensive process. Polydisperse distributions. Biomolecule damage.
Solvent Emulsification Diffusion Method	Voidance of heat during the production procedure.	
Super critical fluid method	Avoid the use of solvents. Particles are obtained as a dry powder, instead of suspensions. Mild pressure and temperature conditions. Carbon dioxide solution is the good choice as a solvent	Very expensive method
Micro emulsion based method	Low mechanical energy input. Theoretical stability.	Extremely sensitive to change. Labor intensive formulation work Low nanoparticle conc.
Membrane contractor method	Allow large scale production Stability demonstrated	

Membrane Contractor technique

It is a novel technique to prepare the SLN. In membrane contactor technique the liquid phase was pressed at a temperature above the melting point of the lipid through the membrane pore swallowing the formation of small droplets as indicated in Figure 2. The aqueous phase was stirred continuously and circulates tangentially inside the membrane module, and sweeps away the droplets being formed at the pore outlets. SLNs were formed by the cooling of the preparation at the room temperature. Here both the phases were placed in the thermostated bath to maintain the required temperature and nitrogen was used to create the pressure for the liquid phase. The influence of various process parameters (aqueous phase cross flow velocity, the lipid phase pressure, aqueous and lipid phase temperature, lipid phase amount and membrane pore size) were studied. The membrane contact or method is also used for the preparation of polymeric nanoparticles, by methods involving a polymerization of dispersed monomers (interfacial polymerization method) or a dispersion of preformed polymers (nanoprecipitation method). The advantages of this process of SLN preparation using a membrane contactor are shown to be its facility of use, the control of the SLN size by an appropriate choice of process parameters and it's scaling up ability.[32]

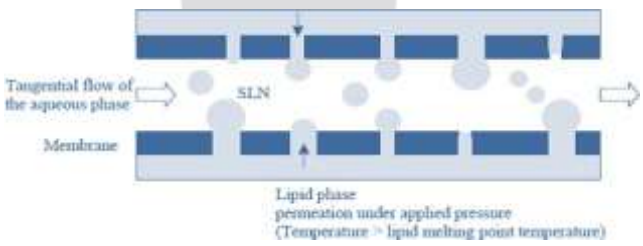


Fig. 6: Shows a Schematic diagram of Membrane Contractor for preparation of SLN

SECONDARY PRODUCTION STEPS

Sterilization

SLNs product should be sterilizes for parenteral application that can be achieved by autoclaving, filtration, gamma irradiation and aseptic production. Sterilization by autoclaving is very common and popular but the problem associated with this is its high

temperature and coalescence, as there is no applied shear. Increased temperature will result in melting of lipid particles and formation of o/w emulsion. Schwarz found that lecithin is a suitable surfactant for steam sterilization, because only a minor increase in particle size was observed.[10,5]

Lyophilisation

Lyophilisation gives long term stability for a product containing hydrolysable drugs or a suitable product for pre-oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions. In case of freeze drying of the product, all the lipid matrices used, form larger solid lipid nanoparticles with a wider size distribution due to presence of aggregates between the nanoparticles. The conditions of the freeze drying process and the removal of water promote the aggregation among SLNs. An adequate amount of cryoprotectant can protect the aggregation of solid lipid nanoparticles during the freeze drying process.[39]

Spray drying

It is an alternative and cheaper technique to the lyophilisation process. This recommends the use of lipid with melting point more than 70° C. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture. The addition of carbohydrates and low lipid content favour the preservation of the colloidal particle size in spray drying. The melting of the lipid can be minimized by using ethanol–water mixtures instead of pure water due to cooling leads to small and heterogeneous crystals, the lower inlet temperatures.[8,40]

STORAGE STABILITY OF SLN

The physical properties of SLN's during prolonged storage can be determined by monitoring changes in zeta potential, particle size, drug content, appearance and viscosity as the function of time. External parameters such as temperature and light appear to be of primary importance for long – term stability. The zeta potential should be in general, remain higher than -60mV for a dispersion to remain physically stable.

- 4°C - Most favorable storage temperature.
- 20°C - Long term storage did not result in drug loaded SLN aggregation or loss of drug.
- 50°C - A rapid growth of particle size was observed.

CHARECTERIZATION

Particle size and Zeta potential

The physical stability of SLNs depends on their particle size. Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for determination of particle size. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light, which is caused by particle movement. The particle size determination by photon correlation spectroscopy (PCS) detects size range of 3nm to 3µm and by laser diffraction in size range of 100 nm to 180 µm. Although PCS is a good tool to characterize nano-particles, but is capable for the detection of larger micro particles.[41] Zeta potential is an important product characteristic of SLNs since its high value is expected to lead to deaggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. It is usually measured by zetameter. Before measurement, SLN dispersions are diluted 50-fold with the original dispersion preparation medium for size determination and zeta potential measurement.[42]

Electron microscopy

Electron Microscopy methods such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to measure the overall shape and morphology of lipid nanoparticles. It permits the determination of particle size and distributions. SEM uses electrons transmitted from the surface of the sample while TEM uses electrons transmitted through the sample.[43,1]

Dynamic light scattering (DLS)

DLS also known as PCS records the variation in the intensity of the scattered light on the microsecond time scale. The variation results from interference of light scattered by individual particles under the influence of Brownian motion and quantified by completion of an auto correlation function. The advantage of the method are the lack of required calibration, sensitivity to submicrometer particles and speed of analysis.

Static light scattering (SLS)/Fraunhofer diffraction

In this method the pattern of light scattered from a solution of particles is collected and fit to fundamental electromagnetic equations in size is the primary variable. This method is fast but it requires advanced knowledge of particles optical qualities and more

cleanliness than DLS.

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

Atomic force microscopy (AFM)
In this technique, a probe tip with atomic scale sharpness is rastered across a sample to produce a topological map based on the forces at play between the tip and the surface. The probe can be dragged across the sample (contact mode), or allowed to hover just above (noncontact mode), with the exact nature of the particular force employed serving to distinguish among the sub techniques. That ultra-high resolution is obtainable with this approach, which along with the ability to map a sample according to properties in addition to size, e.g., colloidal attraction or resistance to deformation, makes AFM a valuable tool.[1]

Powder X - ray diffraction and Differential Scanning Calorimetry (DSC)
The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus the degree of crystallinity to be assessed. DSC can be used to determine the nature and the speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperature. Thermodynamic stability, lipid packing density and quantification are a serious challenge due to the increase, while drug incorporation rates decrease in the following order:
Super cooled melt < α-modification < β9-modification < β-modification.[44]
Due to the small size of the particles and the presence of emulsifiers, lipid crystallization modification changes might be highly retarded. Differential scanning calorimetry (DSC) and X- ray scattering are widely used to investigate the status of the lipid. Infrared and Raman spectroscopy are useful tools for investigating structural properties of lipids. Their potential to characterize SLN dispersions has yet to be explored.[45]

Acoustic methods
Acoustic spectroscopy, measures the attenuation of sound waves as a means of determining size through the fitting of physically relevant equations. In addition, the oscillating electric field generated by the movement of charged particles under the influence of acoustic energy can be detected to provide information on surface charge.[43]

***IN VITRO* DRUG RELEASE**

Dialysis tubing
In vitro drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in pre washed dialysis tubing which can be hermetically sealed. The dialysis sac then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for the drug content using a suitable analytical method.[43]

Reverse dialysis
In this technique a number of small dialysis sacs containing 1 mL of dissolution medium are placed in SLN dispersion. The SLN's are then displaced into the medium.[43]

Ex vivo model for determining permeability across the gut

Ahlin et al. demonstrated the passage of enalaprilat SLN's across rat jejunum. In short the rat jejunum (20 – 30 cm distal from the pyloric sphincter) was excised from the rats after sacrificing the animal used for the study. Qing Zhi Lu et al. excised 10 cm long segments of duodenum (1 cm distal to pyloric sphincter); jejunum (15 cm to pyloric sphincter), ileum (20 cm proximal to cecum) and colon (2 cm distal to cecum) were immediately cannulated and ligated on both sides used for their permeability studies. [46]

Applications of SLN[10,47,15]

There are several potential applications of SLNs some of which are given below:

SLN as potential new adjuvant for vaccines

Adjuvants are used in vaccination to enhance the immune response. The safer new subunit vaccines are less effective in immunization and therefore effective adjuvants are required. New developments in the adjuvant area are the emulsion systems. These are oil-in-water emulsions that degrade rapidly in the body. Being in the solid state, the lipid components of

SLNs will be degraded more slowly providing a longer lasting exposure to the immune system.

Solid lipid nanoparticles in cancer chemotherapy

From the last two decades several chemotherapeutic agents have been encapsulated in SLN and their *in-vitro* and *in-vivo* efficacy have been evaluated. Outcomes of these studies have been shown to improve the efficacy of chemotherapeutic drugs, simultaneously reduction in side effects associated with them. Improved stability of drugs, encapsulation of chemotherapeutic agents of diversified physicochemical properties, enhanced drug efficacy, improved pharmacokinetics and less *in-vitro* toxicity are the important features of SLN which make them a suitable carrier for delivering chemotherapeutic drugs. Several obstacles frequently encountered with anticancer compounds, such as normal tissue toxicity, poor specificity and stability and a high incidence of drug resistant tumor cells, are at least partially overcome by delivering them using SLN. The rapid removal of colloidal particles by the macrophages of the RES is a major obstacle to targeting tissues elsewhere in the body, such as bone marrow and solid tumors.

A) SLN as targeted carrier for anticancer drug to solid tumor[48-51]

SLN have been to be useful as drug carriers. Tamoxifen is an anticancer drug incorporated in SLN to prolong the release of drug after IV administration in breast cancer. Tumor targeting has been achieved with SLN loaded with drugs like methotrexate and camptothecin[51].

B) SLN in breast cancer and lymph node metastases[51]

Mitoxantrone SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of the drug.

Solid lipid nanoparticles for delivering peptides and proteins[52]

Solid lipid particulate systems such as solid lipid nanoparticles (SLN), lipid microparticles (LM) and lipospheres have been sought as alternative carriers for therapeutic peptides, proteins and antigens. The research work developed in the area confirms that under optimized conditions they can be produced to incorporate hydrophobic or hydrophilic proteins and seem to fulfill the requirements for an optimum particulate carrier system. Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral routes or by alternative routes such as oral, nasal and pulmonary. Formulation in SLN confers improved protein stability, avoids proteolytic degradation, as well as sustained release of the incorporated molecules. Important peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles and are currently under investigation. Several local or systemic therapeutic applications may be foreseen, such as immunisation with protein antigens, infectious disease treatment, chronic diseases and cancer therapy[53].

Solid lipid nanoparticles for targeted brain drug delivery[10]

The extremely small particle size of solid lipid nanoparticles, which are less than 50 nm, might be beneficial with respect to drug targeting. Small carrier size generally favors reduced uptake by the reticuloendothelial system. Drug targeting might also be possible by surface modification of solid lipid nanoparticles. SLNs can improve the ability of the drug to penetrate through the blood-brain barrier and is a promising drug targeting system for the treatment of central nervous system disorders. In a study to overcome the limited access of the drug 5-fluoro-2'-deoxyuridine (FUdR) to the brain, 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine (DO-FUdR) was synthesized and incorporated into solid lipid nanoparticles (DO- FUdR-SLN)[54].

The state of the art on surfactant coated poly (alkylcyanoacrylate) nanoparticles specifically designed for brain targeting is given by emphasizing the transfer of this technology to solid lipid matrices. The potential advantages of the use of solid lipid nanoparticles over polymeric nanoparticles are accounted on the bases of a lower cytotoxicity, higher drug loading capacity, and best production scalability. Solid lipid nanoparticles physicochemical characteristics are also particularly regarded in order to address the critical issues related to the development of suitable brain targeting formulations[10].

Solid lipid nanoparticles for parasitic diseases[10,47,57]

Parasitic diseases (like malaria, leishmaniasis, tryanosomiasis) are one of the major problems around the globe. Antiparasitic chemotherapy is the only choice of treatment for these parasitic infections, the reason for this is that these infections do not elicit pronounced immune response hence effective vaccination may not be possible. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent a second generation of colloidal carriers and have emerged as an effective alternative to liposomes mainly due to their better stability profile, ease of scalability and commercialization and relative cost efficacy. Moreover, SLN and NLC due to their particulate nature and inherent structure exhibit good potential in the treatment of parasitic infections. Recent reports including our investigation have validated their utility at least to some extent. However, the need of hour is to undertake extensive investigations on SLN and NLC matrices in order to extend their versatility with respect to encapsulation ability and target ability and to arrive at a versatile, effective and economical approach for the

delivery of anti-parasitic drugs.

Solid lipid nanoparticles for ultrasonic drug and gene delivery[10]

Drug delivery research employing micelles and nanoparticles has wide application in ultrasonic drug and gene delivery in recent years. Of particular interest is the use of these nanovehicles that deliver high concentrations of cytotoxic drugs to diseased tissues selectively, thus reducing the agent's side effects on the rest of the body. Ultrasound, traditionally used in diagnostic medicine, is finding a place in drug delivery in connection with these nanoparticles. In addition to their non-invasive nature and the fact that they can be focused on targeted tissues, acoustic waves have been credited with releasing pharmacological agents from nanocarriers, as well as rendering cell membranes more permeable. Ultrasonic drug delivery from micelles usually employs polyether block copolymers and has been found effective in vivo for treating tumors. Ultrasound releases drug from micelles, most probably via shear stress and shock waves from the collapse of cavitation bubbles. Liquid emulsions and solid nanoparticles are used with ultrasound to deliver genes *in vitro* and *in vivo*. The small packaging allows nanoparticles to extravasate into tumor tissues. Ultrasonic drug and gene delivery from nanocarriers has tremendous potential because of the wide variety of drugs and genes that could be delivered to targeted tissues by fairly non-invasive means[55].

SLN applications for improved delivery of antiretroviral drugs to the brain[47]

Human immunodeficiency virus (HIV) can gain access to the central nervous system during the early course of primary infection. Once in the brain compartment the virus actively replicates to form an independent viral reservoir, resulting in debilitating neurological complications, latent infection and drug resistance. Current antiretroviral drugs (ARVs) often fail to effectively reduce the HIV viral load in the brain. This, in part, is due to the poor transport of many ARVs, in particular protease inhibitors, across the blood- brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSBF). Studies have shown that nanocarriers including polymeric nanoparticles, liposomes, solid lipid nanoparticles (SLN) and micelles can increase the local drug concentration gradients, facilitate drug transport into the brain via endocytotic pathways and inhibit the ATP-binding cassette (ABC) transporters expressed at the barrier sites. By delivering ARVs with nanocarriers, significant increase in the drug bioavailability to the brain is expected to be achieved. Recent studies show that the specificity and efficiency of ARVs delivery can be further enhanced by using nanocarriers with specific brain targeting, cell penetrating ligands or ABC transporters inhibitors. Future research should focus on achieving brain delivery of ARVs in a safe, efficient, and yet cost-effective manner[47].

SLN applied to the treatment of malaria[47]

Despite the fact that we live in an era of advanced technology and innovation, infectious diseases, like malaria, continue to be one of the greatest health challenges worldwide. The main drawbacks of conventional malaria chemotherapy are the development of multiple drug resistance and the nonspecific targeting to intracellular parasites, resulting in high dose requirements and subsequent intolerable toxicity. Nanosized carriers have been receiving special attention with the aim of minimizing the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs. Several nanosized delivery systems have already proved their effectiveness in animal models for the treatment and prophylaxis of malaria. A number of strategies to deliver antimalarials using nanocarriers and the mechanisms that facilitate their targeting to Plasmodium spp-infected cells are discussed in this review. Taking into account the peculiarities of malaria parasites, the focus is placed particularly on lipid-based (e.g., liposomes, solid lipid nanoparticles and nano and microemulsions) and polymer-based nanocarriers (Nanocapsules and nanospheres)[47].

Targeted delivery of solid lipid nanoparticles for the treatment of lung diseases[10]

Targeted delivery of drug molecules to organs or special sites is one of the most challenging research areas in pharmaceutical sciences. By developing colloidal delivery systems such as liposomes, micelles and nanoparticles a new frontier was opened for improving drug delivery. Nanoparticles with their special characteristics such as small particle size, large surface area and the capability of changing their surface properties have numerous advantages compared with other delivery systems. Targeted nanoparticle delivery to the lungs is an emerging area of interest[56].

Solid lipid nanoparticles in tuberculosis disease[10,47]

SLN have longer stability and better encapsulation efficiency than liposomes and, as opposed to polymeric nanoparticles, the production process involves minimal amounts of organic solvents. SLN have been used to encapsulate Anti Tubercular Drugs (ATD) and were proved to be successful in experimental tuberculosis. Antitubercular drugs such as rifampicin, isoniazid, and pyrazinamide SLN systems were able to decrease the dosing frequency and to improve patient compliance. ATD were co-incorporated into SLN to evaluate the potential of these carriers in tuberculosis chemotherapy via the oral route. The finding of this study suggested that SLN have great potential in the delivery of ATD by reducing frequency of doses and improving patient compliance by better management of tuberculosis.

Transfection agent[57]

Cationic SLNs for gene transfer are formulated using the same cationic lipid as for liposomal transfection agents. The differences and similarities in the structure and performance between SLN and liposomes were investigated. PCS showed that the prepared SLNs were smaller in diameter than the corresponding liposomes while AFM supported the expected structural differences. DNA binding differed only marginally. Cationic lipid composition governs the in vitro transfection performance than the colloidal structure it is arranged in. Hence, cationic SLN extends the range of highly potent non-viral transfection agents by one with favorable and distinct technological properties. Combination of cationic SLN with the nuclear localization signal TAT2 increased transfection efficiency hundredfold.

SLN in cosmetic and dermatological preparations[58]

An area of big potential for SLN and with a short time-to market are topical products based on the SLN technology, that means pharmaceutical but also cosmetic formulations. SLN are considered as being the next generation of delivery system after liposomes[59]. Due to the lower risk of systemic side effects topical treatment of skin disease appears favourable, yet the stratum corneum counteracts the penetration of xenobiotics into viable skin. Particulate carrier systems may mean an option to improve dermal penetration. Since epidermal lipids are found in high amounts within the penetration barrier, lipid carriers attaching themselves to the skin surface and allowing lipid exchange between the outermost layers of the stratum corneum and the carrier appear promising. Besides liposomes, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been studied intensively[60]. Following the evaporation of water from the lipid nanodispersion applied to the skin surface, lipid particles form an adhesive layer occluding the skin surface. Then hydration of the stratum corneum may increase by which reducing corneocyte packing and widening of the inter-corneocytes gaps can facilitate drug penetration into deeper skin strata. Occlusive effects appear strongly related to particle size. Nanoparticles have turned out 15-fold more occlusive than microparticles, and particles smaller than 400 nm in a dispersion containing at least 35% lipid of high crystallinity has been most potent.

Solid lipid nanoparticles for lymphatic targeting[10]

The solid lipid nanoparticles (SLN) were developed and evaluated for the lymphatic uptake after intraduodenal administration to rats.

SLN for potential agriculture applications[61]

Essential oil extracted from *Artemesia arboreseens L* when incorporated into SLN, were able to reduce the rapid evaporation compared with emulsions and the systems have been used in agriculture as suitable carrier of safe pesticides.

MARKETED PRODUCTS OF SLNs

Lipid based drug delivery system have been used to improve the bioavailability of BCS class 2 drugs. Market survey data show that about 4% of commercial products of oral lipid based formulations are available in the US, UK and Japan market. Marketed products of SLNs are listed in Table no.3.

Table 3: List of Marketed Products.

Product Name	Main Active Ingredient	Producer/Distributors
Nano Lipid Restore	Coenzyme Q-10 and Omega unsaturated fatty acids.	Chemisches Laboratorium Dr. Kurt Richter, CLR Berlin
NLC Deep Effect	Coconut oil, tamanu tree extract	Beate Johnen
Intensive Serum Nanorepair Q-10	Q-10, Polypeptide, Mafane Extract (Antiwrinkle effect)	Dr. Rimpler Gmbh

FUTURE OF SLNs

SLNs can be developed as more effective drug delivery in future by taking consideration of industrial needs like simple technology, low cost, regulatory excipient status, tolerability, scale up, qualification and validation. Research must continue to develop a therapy through localized medical implants. Yih *et al.* developed a bio- micro electro mechanical micropumps for controlled release of drug for local action. Factors that should be taken in consideration in future research are efficacy, drug loading, targeting and toxicity. Studies are essential to evaluate the efficacy of implants over time when encapsulated and stored. Implantable devices or nanochips will provide improved therapeutics in disease management and potentially applied as gene therapy, antitumor, vaccines and in repairing damaged tissue, detecting mutated genes or detecting high hormone levels indicative of certain malignance. Further work needs to be carried out to understand the structure and dynamics of SLNs at the molecular level in *in-vitro* and *in-vivo* studies.

CONCLUSION

The present review has concentrated on newer approach of nano sized delivery carriers like solid lipid nanoparticle, nanostructured lipid carriers, lipid drug conjugates, Polymer lipid hybrid nanoparticles etc. The results cannot simply be regarded as nanoemulsions with a solid core. Clear advantages of SLN include the composition (physiological compounds), the rapid and effective production process including the possibility of large scale production, the avoidance of organic solvents and the possibility to produce carriers with higher encapsulation efficiency. Disadvantages include low drug-loading capacities, the presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals), the complexity of the physical state of the lipid (transformation between different modifications) and the possibility of super cooled melts which cause stability problems during storage or administration (gelation, particle size increase, drug expulsion). The appropriate characterization of the complex surfactant/lipid dispersions requires several analytical methods in addition to the determination of the particle size. Kinetic aspects to be taken into account. In summary, SLN are very complex systems with clear advantages and disadvantages to other colloidal carriers. Further work needs to be done to understand the structure and dynamics of SLN on molecular level *in vitro* and *in vivo* studies.

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