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SOLID LIPID NANOPARTICLES: A REVIEW

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ABSTRACT

Solid lipid nanoparticles (SLN) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research. 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. Hence solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence attracted wide attention of researchers. This review presents a broad treatment of solid lipid nanoparticles discussing their aims, production procedures, advantages, limitations and their possible remedies. Appropriate analytical techniques for the characterization of SLN like photon correlation spectroscopy, scanning electron microscopy, differential scanning calorimetry are highlighted. Aspects of SLN route of administration and the in vivo fate of the carriers are also discussed.

Key words: Colloidal drug carriers, Homogenization, TEM, PCS, Biodistribution targeting.

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]. Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system. SLN are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals[2,5,6].

In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles.

The reasons for the increasing interest in lipid based system are many – fold and include.

1. Lipids enhance oral bioavailability and reduce plasma profile variability.
2. Better characterization of lipoid excipients.
3. An improved ability to address the key issues of technology transfer and manufacture scale-up.

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Solid lipid nanoparticles are one of the novel potential colloidal carrier systems as alternative materials to polymers which is identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a solid lipid shown on Fig. 1. They have many advantages such as good biocompatibility, low toxicity and lipophilic drugs are better delivered by solid lipid nanoparticles and the system is physically stable.

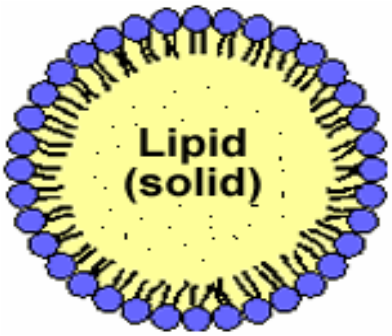

Fig. 1: Structure of solid lipid nanoparticle (SLN)

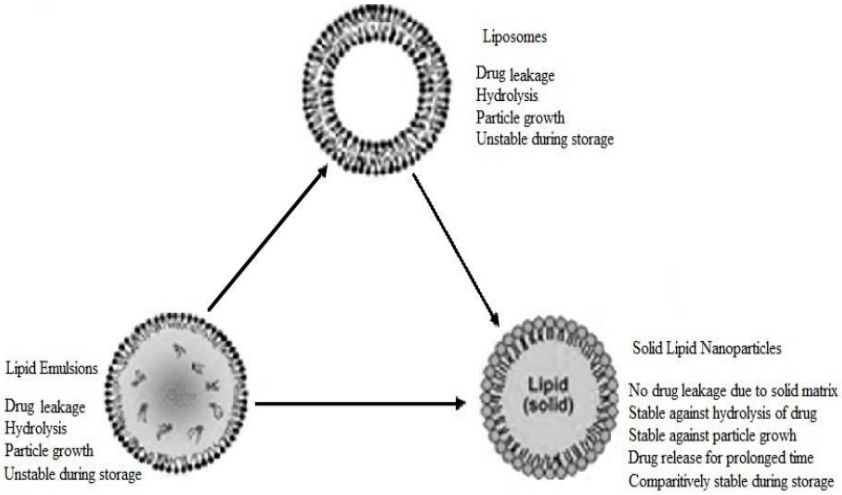

Fig. 2: A diagrammatic representation on SLN over emulsions and liposomes

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.

Advantages of SLN[1-4]

- Control and / or target drug release.
- Excellent biocompatibility[5].
- Improve stability of pharmaceuticals[4].
- 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 procedures.

Disadvantages of SLN[4,6]

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

Aims of solid lipid nanoparticles[6,9]

- Possibility of controlled drug release[5].
- Increased drug stability.
- High drug pay load[5].
- No bio-toxicity of the carrier.
- Avoidance of organic solvents.
- Incorporation of lipophilic and hydrophilic drugs.

Preparation of solid lipid nanoparticles[1- 4,6,43,52,56]

SLNs are prepared from lipid, emulsifier and water/solvent by using different methods and are discussed below.

Methods of preparation of solid lipid nanoparticles

1. High pressure homogenization
   A. Hot homogenization
   B. Cold homogenization
2. Ultrasonication/high speed homogenization
   A. Probe ultrasonication
   B. Bath ultrasonication
3. Solvent evaporation method
4. Solvent emulsification-diffusion method

5. Supercritical fluid method
6. Microemulsion based method
7. Spray drying method
8. Double emulsion method
9. Precipitation technique
10. Film-ultrasound dispersion

1. High pressure homogenization (HPH)

It is a reliable and powerful technique, which is used for the production of SLNs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 Km/h). Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated.

Two general approaches of HPH are hot homogenization and cold homogenization, work on the same concept of mixing the drug in bulk of lipid melt.

A. Hot homogenization: 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. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles.

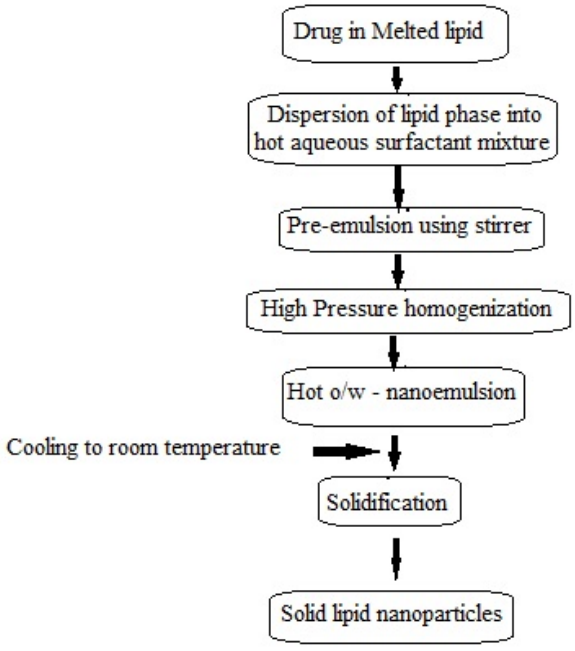

Fig. 3: Solid lipid nanoparticles preparation by hot homogenization process

B. Cold homogenization

Cold homogenization has been developed to overcome various problems associated with hot homogenization such as: Temperature-induced drug degradation, drug distribution into the aqueous phase during homogenization, Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or super cooled melts. In this technique the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or below room temperature, the gravitation force is strong enough to break the lipid microparticles directly to solid lipid nanoparticles.

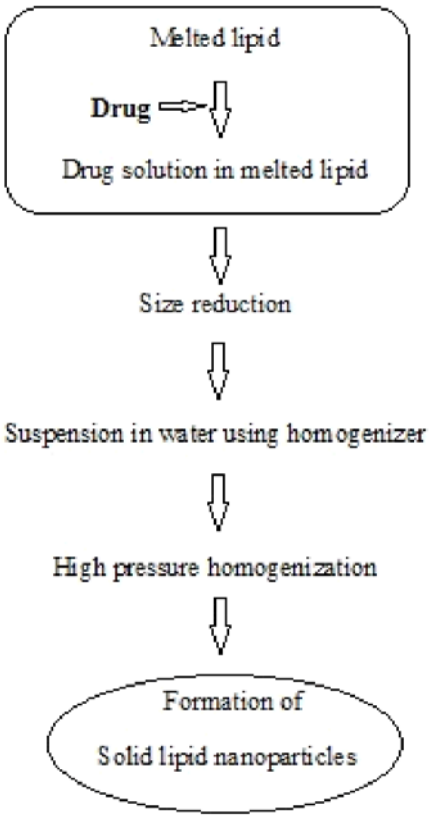

Fig. 4: Solid lipid nanoparticles preparation by cold homogenization process

Advantages

- Low capital cost.
- Demonstrated at lab scale.

Disadvantages

- Energy intensive process.
- Demonstrated at lab scale Biomolecule damage.
- Polydisperse distributions.
- Unproven scalability.

2. Ultrasonication/high speed homogenization

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.

Advantages

- Reduced shear stress.

Disadvantages

- Potential metal contamination.
- Physical instability like particle growth upon storage.

3. Solvent evaporation

SLNs can also prepared by solvent evaporation method. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar).

Advantages

- Scalable.
- Mature technology.
- Continuous process.
- Commercially demonstrated.

Disadvantages

- Extremely energy intensive process.
- Polydisperse distributions.
- Biomolecule damage.

4. Solvent emulsification-diffusion method

The particles with average diameters of 30-100 nm can be obtained by this technique. Voidance of heat during the preparation is the most important advantage of this technique.

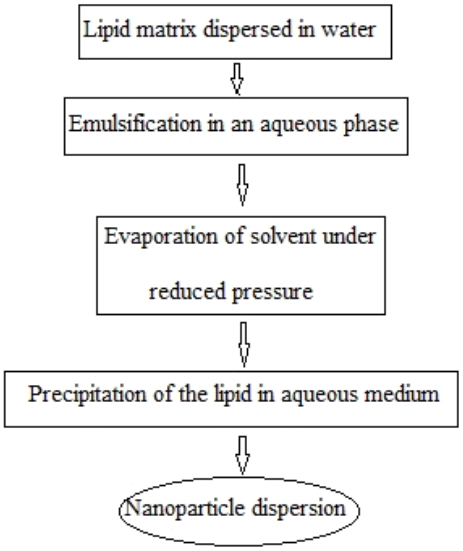

Fig. 5: Systematic representation for emulsification-diffusion method

5. Supercritical fluid method

This is an alternative method of preparing SLNs by particles from gas saturated solutions (PGSS).

Advantages

- 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 for this method.

6. Microemulsion based method

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 HPH based formulations.

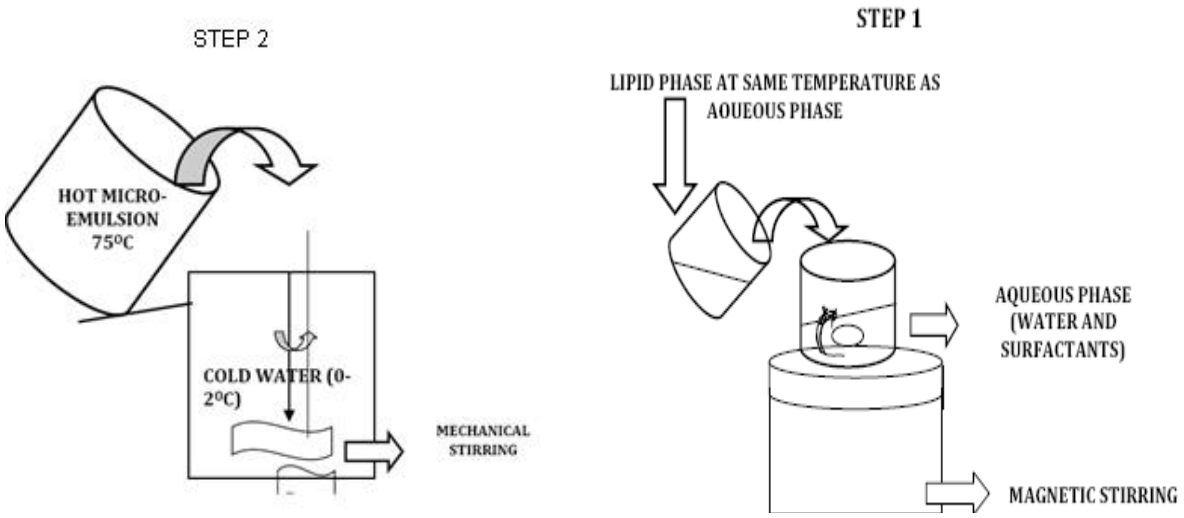

Fig. 6: Microemulsion method

Advantages

- Low mechanical energy input.
- Theoretical stability.

Disadvantages

- Extremely sensitive to change.
- Labor intensive formulation work.
- Low nanoparticle concentrations.

7. Spray drying method

It is an alternative technique to the lyophilization process. This recommends the use of lipid with melting point more than 70^{o}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.

8. Double emulsion method

Here the drug is encapsulated with a stabilizer to prevent the partitioning of drug in to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion.

9. Precipitation method

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

10. Film-ultrasound dispersion

The lipid and the drug were put into suitable organic solutions, after decompression, rotation and evaporation of the organic solutions, a lipid film is formed, then the aqueous solution which includes the emulsions was added. Using the ultrasound with the probe to diffuser at last, the SLN with the little and uniform particle size is formed.

Secondary Production Steps

Freeze drying

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required to achieve long term stability for a product containing hydrolysable drugs or a suitable product for per-oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions.

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.

Sterilization

Sterilization of the nanoparticles is desirable for parenteral administration and autoclaving which is applicable to formulations containing heat-resistant drugs. Effects of sterilization on particle size have been investigated and it was found to cause a distinct increase in particle size.

Spray drying

Spray drying might be an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a dry product. This method has been used scarcely for SLN formulation, although spray drying is cheaper as compared to lyophilization.

The lipids with melting points at temperature >70°C had been recommended for spray drying.

Influence of excipients[4,10,54]

Formulation variables in the product quality

Particle size

Alteration of the size significantly affects the physical stability, biofate of the lipid particles, and release rate of the loaded drug. Hence the size of the SLNs has to be controlled within reasonable range. Well formulated systems (liposomes, nanospheres and nanoparticles) should display a narrow particle size distribution in the submicron size range (as having size below 1μm), according to the definition of colloidal particles.

Influence of the ingredients on product quality

The particle size of lipid nanoparticles is affected by various parameters such as composition of the formulation (such as surfactant/ surfactant mixture, properties of the lipid and the drug incorporated), production methods and conditions (such as time, temperature, pressure, cycle number, equipment, sterilization and lyophilization). Large particle size is obtained at lower processing temperature. The hot homogenization technique gives a smaller particle size, generally below 500 nm, and a narrow particle size distribution as compared to cold homogenization. Mean particle size as well as polydispersity index (PI) values are reported to be reduced at increasing homogenization pressure up to 1500 bar and number of cycles (3-7 cycles).

Influence of the lipids

Using the hot homogenization, it has been found that the average particle size of SLN dispersions is increasing with higher melting lipids. However, other critical parameters for nanoparticle formation will be different for the different lipids. The examples include the velocity of lipid crystallization, the lipid hydrophilicity (influence on self-emulsifying properties and the shape of the lipid crystals (and therefore the surface area).

Further, increasing the lipid content over 5-10% resulted in larger particles (including microparticles) and broader particle size distribution in most cases.

Influence of the emulsifiers

The concentration of the surfactant/surfactant mixture strongly affects the particle size of the lipid nanoparticles. In general, smaller particle sizes were observed when a higher surfactant/lipid ratio was chosen. The decrease in surfactant concentration resulted in increase of particle size during storage.

Surfactants decrease the surface tension between the interface of the particles causing portioning of the particles and thereby increasing the surface area.

Drug incorporation models of SLN[6]

Factors affecting loading capacity of a drug in lipid are:

1. Solubility of drug in lipid melt.
2. Miscibility of drug melt and lipid melt.
3. Chemical and physical structure of solid matrix lipid.
4. Polymorphic state of lipid material.

Drug incorporation models are as follows

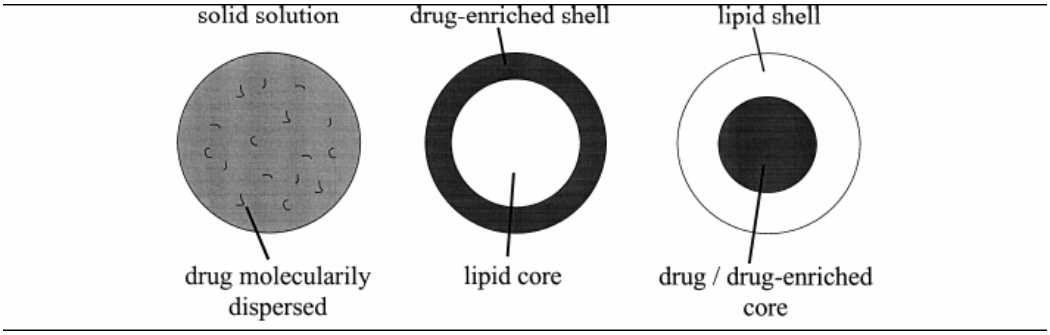

Fig. 7: Drug incorporation models

Solid solution model:

1. Drug is molecularly dispersed in lipid matrix when SLN is prepared by cold homogenization.
2. Drug-enriched shell model.
3. A solid lipid core forms upon recrystalization temperature of the lipid is reached.
4. Drug-enriched core model.
5. Cooling the nanoemulsion leads to a super saturation of the drug which is dissolved in the lipid melt leads to recrystalization of the lipid.

Fate of SLN after oral administration[4,9]

The oral route continues to be a challenge as well as the most attractive way to administer drugs because of its unquestionable commercial potential. Incorporation of drugs into lipid nanoparticles opens the perspective of enhanced and / or less variable bioavailability and prolonged plasma levels. While these systems may provide the greatest flexibility in the modulation of the drug release profile within GIT and provide protection against chemical degradation for labile drug molecules (Peptide drugs).

Drug incorporation and loading capacity[6,7]

The particle size, loading capacity and the size distribution of SLN's is found to vary with lipid (triglycerides, fatty acids, steroids, waxes etc), emulsifier (anionic, cationic, non - ionic) and the method of preparation etc.

Factors determining the loading capacity of the drug in the lipid are[4,6,7]

- Solubility of the melted lipid.
- Miscibility of the drug melt in the lipid melt.
- Chemical and physical structure of solid lipid matrix.
- Polymorphic state of lipid material.

The pre – requisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. Typically the solubility should be higher than required because, it decreases when cooling down the melt and might be even lower in the solid lipid. To enhance the solubility in the lipid melt one can add solubilizers. In addition, the presence of mono and diglycerides in the lipid used matrix material promotes drug solubilization. The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice lead drug expulsion.

Estimation of incorporated drug

Entrapment efficiency[1,6,14,28]

This is the prime importance in SLN, since it influences the release characteristics of drug molecule. The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the entrapped drug from the SLN formulation. This separation can be carried out using the techniques such as ultracentrifugation, centrifugation filtration and or gel permeation chromatography.

Centrifugation filtration[13]

Filters such as ultra free – mc or ultra sort – 10 are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining the amount of drug remaining in supernatant after centrifugation filtration/ultra-centrifugation of SLN suspension or alternatively by dissolution of the sediment in an appropriate solvent and subsequent analysis.

Principles of drug release[6,7]

The general drug principles of drug release from lipid nanoparticles are as follows:

- There is an inverse relationship between drug release and the partition co-efficient of the drug.
- Higher surface area due to smaller particle size in the nanometer size range gives higher drug release.
- Slow drug release can be achieved when drug is homogenously dispersed in the lipid matrix. It depends on the type and the drug entrapment model of SLN.
- Crystallinity behavior of the lipid and high mobility of the drug lead to fast drug release. There is an inverse relationship between crystallization degree and mobility of drug.

Factors contributing to a fast release are the large surface area, a high diffusion co - efficient due to small molecular size, low viscosity in the matrix and a short diffusion distance δ for the drug. The increase in the velocity with decreasing particle size was reported.

Storage stability of SLN[18,20]

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^{o}C - Most favorable storage temperature.

20^{o}C - Long term storage did not result in drug loaded SLN aggregation or loss of drug.

50^{o}C - A rapid growth of particle size was observed.

In vitro and *ex vivo* methods for the assessment of drug release from SLN[18,19,25]

A large number of drugs including very hydrophilic molecules have been postulated to be incorporated into SLN.

Various methods used to study the *in vitro* release of the drug are:

- Side by side diffusion cells with artificial or biological membrane[20].

- Dialysis bag diffusion technique[20] .
- Reverse dialysis bag technique[18].
- Agitation followed by ultracentrifugation or centrifugal ultra filtration[25].

In vitro drug release[10]

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.

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.

Ex vivo model for determining permeability across the gut[13,18]

Ahlin et al.[102] demonstrated the passage of enalaprilat SLN's across rat jejunum[13]. 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[18].

Analytical characterization of SLN

An adequate characterization of the SLN's is necessary for the control of the quality of the product. Several parameters have to be considered which have direct impact on the stability and release kinetics:

- Particle size and zeta potential.
- Degree of crystallinity and lipid modification.
- Co – existence of additional structures and dynamic phenomena.

Measurement of particle size and zeta potential[22,25]

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements 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. This method covers a size range from a few nanometers to about 3 microns. PCS is a good tool to characterize nanoparticles, but it is not able to detect larger micro particles. Electron Microscopy provides, in contrast to PCS and LD, direct information on the particle shape. The physical stability of optimized SLN dispersed is generally more than 12 months. ZP measurements allow predictions about the storage stability of colloidal dispersion.

Dynamic light scattering (DLS)[24,25]

DLS also known as PCS records the variation in the intensity of the scattered light on the microsecond time scale.

Static light scattering (SLS)/fraunhofer diffraction

SLS is an ensemble method in which the light scattered from a solution of particles is collected and fit into fundamental primary variable.

Acoustic methods

It measures the attenuation of the scattered sound waves as a means of determining size through the fitting of physically relevant equations.

Nuclear magnetic resonance (NMR)[28]

NMR can be used to determine both the size and qualitative nature of nanoparticles.

Electron microscopy[26,35]

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) are the direct method to measure nanoparticles, physical characterization of nanoparticles with the former method being used for morphological examination. TEM has a smaller size limit of detection.

Atomic force microscopy (AFM)

A probe tip with atomic scale sharpness is rastered across a sample to produce a topological map based on forces at play between the tip and the surface.

Powder X - ray diffraction and differential scanning calorimetry (DSC)[25,29]

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.

Sterilization of SLN[5,10]

For intravenous and ocular administration SLN must be sterile. The temperature reach during sterilization by autoclaving presumably causes a hot o/w micro emulsion to form in the autoclave, and probably alters the size of the hot nanoparticles. On subsequent slow cooling, the SLN reformed, but some nano-droplets may coalesce, producing larger SLN than the initial ones. SLN are washed before sterilization, amounts of surfactants and co surfactants present the hot systems are smaller, so that the nano-droplets may be not sufficiently stabilized.

Measurement of crystallinity and lipid modifications[4,29]

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.

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[27]. Their potential to characterize SLN dispersions has yet to be explored.

Co – existence of additional structures

The magnetic resonance techniques, nuclear magnetic resonance (NMR) and electron spin resonance (ESR) are powerful tools to investigate dynamic phenomena and the nano-compartments in the colloidal lipid dispersions. Dilution of the original SLN dispersion with water might cause the removal of the surfactant molecules from the particle surface and induce further changes such as crystallization changes of the lipid modification.

Parameter	Method of analysis
Molecular weight	Gel chromatography
Surface element analysis	X-ray photoelectron spectroscopy, Electrophoresis, Laser Doppler anemometry

Routes of administration and their biodistribution[2,3,7,52,101]

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.

Applications of SLN[4,51,101]

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[18-20,34]

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[34]

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[42]

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[47].

Solid lipid nanoparticles for targeted brain drug delivery[4]

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)[43].

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[4].

Solid lipid nanoparticles for parasitic diseases[4,51,85]

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[4]

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[50].

SLN applications for improved delivery of antiretroviral drugs to the brain[51]

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[51].

SLN applied to the treatment of malaria[51]

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)[52].

Targeted delivery of solid lipid nanoparticles for the treatment of lung diseases[4]

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[53].

Solid lipid nanoparticles in tuberculosis disease[4,51]

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[37]

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[66]

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[41]. 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[42]. 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[4]

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

SLN for potential agriculture applications[14]

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.

CONCLUSION

Solid lipid nanoparticles do not, as proposed, ‘‘combine the advantages of other colloidal drug carriers and avoid the disadvantages of them’’. 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). Sample dilution or water removal might significantly change the equilibria between the different colloidal species and the physical state of the lipid. 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. NMR, ESR and synchrotron irradiation will help the drug nanosuspensions coexist in the sample. Unfortunately, these aspects have not always been considered and the terminus 'drug incorporation' in the SLN literature is often misleading. 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.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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