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Review Article

Excipients, drug release mechanism and physicochemical characterization methods of Solid lipid nanoparticles

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Abstract

From last thirty years, solid lipid nanoparticles (SLNs) gain much importance as drug delivery vehicle for enhanced delivery of the drugs, proteins, nutraceuticals and cosmetics. SLNs defined as a submicron size range nanoparticle with below 1000 nm and are mainly composed of lipids and surfactants, capable of incorporating both lipophilic and hydrophilic drugs. SLNs also used as controlled systems, targeted delivery and altered therapeutic efficacy purpose. A wide variety of methods such as double emulsion, solvent evaporation, ultra sonication, high-pressure homogenization and microemulsion used for SLNs production. This review provides the significance of SLNs in drug delivery with highlighting on selection of excipients, drug release mechanism, principles and limitations associated with their physicochemical and surface morphological characterization.

Keywords: Solid lipid nanoparticles, enhanced delivery, preparation, characterization, application.

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History:

Initially, particulate drug carriers investigated for many years include oil-in-water (O/W) emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules. The O/W emulsions have been introduced successfully to the clinic for parenteral nutrition in the 1950s. Based on these emulsions for parenteral nutrition, drug containing emulsion formulations have been developed, e.g., containing diazepam and etomidate. Trade products are Diazemuls, Diazepam-Lipuro and Etomidate-Lipuro. The only intention of these emulsions was to reduce drug side effects, e.g., pain of injection and inflammation at the injection site (e.g., diazepam)[1,2].

Despite the excellent tolerability of these O/W emulsions the number of products on the market is relatively low, indicating their limited success. One of the reasons preventing a broader introduction of emulsions for drug delivery is the physical instability which can be caused by the incorporated drug. In addition, the registered oils such as soybean oil, MCT and LCT and mixtures thereof show an insufficient solubility for drugs of possible interest to be incorporated into emulsions. Despite the fact that the emulsion is a very interesting delivery system, one has the impression that pharmaceutical companies are reluctant to pursue this delivery system further. A possible reason for this might be the necessity to search for new oils with improved solubility properties which would of course also require an expensive toxicity study[3].

Later, phospholipid vesicles rediscovered as `liposomes' in 1965 by Bangham found their way to the cosmetic market in 1986. It was the anti-aging product Capture (Dior) which smoothed the way for liposome-based pharmaceutical products. It is the first liposome product on the market. Finally, the first pharmaceutical products came to the market at the end of the 80s and beginning of the 90s, and include the synthetic lung surfactant Alveofact® (Dr Karl Thomae GmbH/Biberach in Germany) for pulmonary instillation, Epi-Pevaryl®, a topical product for anti-mycotic therapy (drug: econazole) and other products for intravenous injection (e.g. Ambisome® with amphotericin and cytotoxic-containing formulations like Doxil® and Daunosome®). However, the total number of products on the market is still limited. One of the reasons for this - apart from possible technological problems, is the non-availability of a `cheap' pharmaceutical liposome[3].

The number of products based on polymeric microparticles on the market is limited. After the introduction of the first wave of products (e.g. Enantone Depot®, Decapeptyl Depot®, Parlodel LA®, Parlodel LAR®), there was only a limited increase in the number of microparticulate products. The situation is even worse for polymeric nanoparticles, after more than 30 years of research; this delivery system

practically does not exist. An exception is the product Abdoscan produced by the company Nycomed, however, this is not a formulation for chronic treatment, it is a diagnostic agent[3].

There are quite a few well-known reasons for this, the cytotoxicity of polymers and the lack of a suitable large scale production method. Polymers accepted for use as implants are not necessarily also of good tolerability in the form of nanoparticles. In the nanometer size range and having a size of a few nanometers, the polymer can be internalized by cells (e.g., macrophages) and degradation inside the cell can lead to cytotoxic effects, e.g., as reported for polyester polymers[4,5].

There has been considerable interest in developing nanoparticles as effective drug delivery devices over the past few decades. Nanoparticles, generally, range from 10nm to 1000nm in diameter. Nanoparticles were first developed in 1970. They were actually devised as carriers for vaccines and anticancer drugs. The focus on developing means to target the tumors and also reduce the uptake of nanoparticles by the reticuloendothelial system was the first step in this direction[5].

Polymers from natural and synthetic sources have been used. Polymer based systems in the submicron range include water soluble polymer-drug conjugates, polymer nanocapusles and nanospheres. An advantage with these systems is the vast range of chemical modifications possible. The main problem, nevertheless, encountered with these systems is possible organic residues during the production process and the polymer cytotoxicity. Polymer hydrolysis during storage has to be taken into account and lyophilization is often required to prevent polymer degradation[6].

Table 1: Advantages and disadvantages of various colloidal drug carrier systems

Property	SLN	Polymer Nanoparticles	Liposomes	Lipid Emulsions
Systemic toxicity	Low	> or = to SLN	Low	Low
Cytotoxicity	Low	> = to SLN	Low	Low
Residues from organic solvents	No	Yes	May or may not	No
Large scale production	Yes	No	Yes	Yes
Sterilization by autoclaving	Yes	No	No	Yes
Sustained release	Yes	Yes	< or = to SLN	Yes
Avoidance of RES	Depend on size and coating	No	Yes	Yes

Since the beginning of the nineties attention from various research groups has focused on an alternative to polymeric nanoparticles, the solid lipid nanoparticles (SLN). The use of solid lipids as a matrix material for drug delivery is well-known from lipid pellets for oral drug delivery (e.g., Mucosolvan® retard capsules). Basically, lipids can be used which are well tolerated by the body (e.g., glycerides composed of fatty acids which are present in the emulsions for parenteral nutrition). Large scale production can be performed in a cost-effective and relatively simple way using high pressure homogenization leading to SLN[3,7].

A clear advantage of SLNs is the fact that the lipid matrix is made from physiological lipids which decrease the danger of acute and chronic toxicity. Also the possibility of control drug release and drug targeting with the higher drug pay load and feasibility for sterilization are some of the major advantages with these systems.

Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are emerging as alternative carriers to colloidal drug systems, for controlled systems and targeted delivery. These are in submicron size range (50-1000 nm) and are made of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs. SLNs combine the advantage of different colloidal carriers, for instance, like emulsions and liposomes, these are physiologically acceptable and like polymeric nanoparticles, controlled release of drug from lipid matrix can be anticipated[8,9].

SLNs are particles made from solid lipids (i.e., lipids solid at room temperature and also at body temperature) and stabilized by surfactant(s). By definition, the lipids can be highly purified triglycerides, complex glyceride mixtures or even waxes. Through the work of various research groups, the SLN carrier system has been characterized intensively[10-12].

The oral route is most predominant administered route of system for drug delivery. Despite the popularity and versatility of the oral route, significant problems remain. Not all drug molecules possess the physical, chemical or biological characteristics necessary for the successful therapy by oral route[13,14]. Problems such as poor solubility or chemical stability in the location of the gastrointestinal tract, poor permeability over the biological membranes or compassion to metabolism are well known to result in the refusal of potential drug candidates as oral applied products[15-20]. Lipid based drug delivery systems have been proposed as a means of by-passing some of more resistant chemical or physical barriers associated with poorly absorbed drugs[21-25]. Hence, various alternative drug delivery systems are developed to enhance the oral BA of these drugs. The delivery systems include; enhancement of solubility through solid dispersions, liquisolid compacts; avoid first-pass metabolism through buccal delivery or nasal route[0]; increase the stability and prolonged residence time

through floating systems, increase the mucoadhesive property; lipid based delivery systems for by passing metabolism with solid lipid nanoparticles, transfersomes, nanostructured lipid carriers and micronization for reducing particle size using nanosuspensions[26-50].

There are different techniques for the preparation of SLNs. Generally, the preparation of any nano carrier system requires a dispersed system as precursor, or else particles are produced through the use of a specific instrumentation[51]. This review mainly provides the insights onto the selection of excipients, drug loaded models and characterization methods used for the development of SLNs.

Factors to be considered in the formulation of SLN

Common ingredients used in the formulation of SLN are lipids (matrix materials), emulsifiers, co-emulsifiers and water. Charge modifiers, stealthing agents and homing devices are also used to meet the requirements of stability and targeting aspects. Various excipients used in the formulation of SLNs are listed in the Table 2.

Table 2: Excipients used in development of solid lipid nanoparticles

Lipid matrices (Solid lipid)	Beeswax Behenic acid Cetylpalmitate Cholesterol Glyceryl trilaurate (Dynasan 112) Glyceryl trimyristate (Dynasan 114) Glyceryl tripalmitate (Dynasan 116) Glyceryl tristearate (Dynasan 118) Glyceryl monostearate Glyceryl behenate (Compritol) Glyceryl monostearate (Imwitor 900) Hardened fat (Witepsol E 85) Monostearate monocitrate glycerol (Acidan N12) Softisan 142/Cetyl alcohol (75:25) Softisan 142 Solid paraffin Stearic acid Superpolysate Synrowax HRSC (mixture of glycerol tribehenate and calcium behenate) Witepsol E 85/Cetyl alcohol (75:25) Witepsol H5 WitepsolW 35
Emulsifiers	Phosphatidyl choline 95% (Epikuron 200) Soy lecithin (Lipoid S 75, Lipoid S 100) Egg lecithin (Lipoid E 80) Poloxamer 188 (Pluronic F 68) Poloxamer 407 Poloxamine 908 Polysorbate 80 Cremophor EL Solutol HS 15 Labrasol Vitamin E TPGS (D-alpha tocopheryl polyethylene glycol 1000 succinate) Vitamin E 6-100 (D-alpha tocopheryl acetate)
Co-emulsifiers	Tyloxopol Taurocholate sodium salt Taurodeoxycholicacid sodium salt Sodium dodecyl sulphate Sodium glycocholate Sodium oleate Cholesteryl hemisuccinate Butanol
Cryoprotectants	Trehalose, Glucose, Mannose, Maltose, Lactose, Sorbitol, Mannitol, Glycine, Polyvinyl pyrrolidone (PVP), Polyvinyl alcohol (PVA), Gelatin
Charge modifiers	Stearylamine Dicetylphosphate Dipalmitoyl phosphatidyl choline (DPPC). Dimyristoyl phophatidyl glycerol (DMPG).
Agents for improving circulation time	Polyethylene glycol Poloxamer

Selection of lipids

The rationale behind choosing lipid materials for developing oral pharmaceutical dosage forms had been reviewed recently. Lipid matrices used for the production of SLNs for i.v. administration should have the following appropriate properties[3, 52].

- ❖ They are capable of producing small size particles (in the nanometer size range) with a simultaneous low content of micro particles (>5µm).
- ❖ They possess sufficient loading capacity for lipophilic and possible also hydrophilic drugs.
- ❖ They should be stable in aqueous dispersions on long term storage, or alternatively they can be lyophilized or spray dried.
- ❖ They should not leave any toxic residues from the production process (e.g., solvents).
- ❖ They must be biodegradable.

Various lipids (matrix materials) used for the production of solid lipid nanoparticles are tristearin, tripalmitin or cetylpalmitate. Lipids of less ordered crystal lattices favour successful drug inclusion, as is observed in case of glyceryl monostearate[53] and glyceryl behenate SLN compared to SLN prepared using highly ordered crystal packing bees wax, cetylpalmitate. However, their long term stabilities were quite different. Within glycerides, the best physical stability was obtained for tripalmitate[54], followed by tribehenin and is due to the presence of 15% of monoglycerides in tribehenin which possess the surfactant properties[55]. On the other hand, glycery monostearate is extremely unstable and considerable particle growth takes place within a few days and is attributed to the presence of 50% of monoglycerides in glyceryl monostearate which are responsible for their physical destabilization[52].

Important point to be considered in the selection of drug carrier system is its loading capacity and also the intended use, for instance complex glycerides like hard fats are not suited for controlled release applications because these particles melt at body temperature[52]. Lipophilicity of the glyceride increases as the chain length of hydrocarbon increases. Therefore, lipophilic drugs are better soluble in lipid melts of longer fatty acid chain lengths[56].

Selection of emulsifier

Emulsifier should be non-toxic, compatible with other excipients, capable of producing desired size with minimum amount used and also provide adequate stability to the SLN by covering the surface of nanoparticles. From literature, it is evident that the type and amount of emulsifier, method of preparation, influence the size of the particles and also their stability. The amount of the emulsifier should be optimum to cover the surface of the nanoparticles. Lesser amounts of emulsifier result in particle aggregation and lead to increase in particle size. However, use of excess amount of emulsifier is avoided to prevent decrease in entrapment efficiency, burst release as observed in case of release studies of SLN and also toxic effects associated with surfactants[3]. The combined use of two or more emulsifying agents appears to produce mixed surfactant films at the interface.

Selection of co-emulsifier

Phospholipids used in the formulation of SLNs are neither soluble in continuous phase nor do they form highly dynamic micelles. The excess phospholipid molecules form small, predominantly unilamellar vesicles during homogenization process. Phospholipid molecules bound to vesicles, however, exhibit only a limited mobility. Therefore, they are not able to immediately cover the newly created interfaces during recrystallization. Due to the low mobility of the phospholipid molecules, sudden lack of emulsifier on the surface of the particle leads to particle aggregation and increase in the particle size of SLN. To avoid this, co-emulsifiers are employed. They stabilize the colloidally dispersed state of recrystallizing triglycerides. These water soluble emulsifiers are able to form micelles. Polymer molecules are able to diffuse to the particle surface in a much shorter time than do vesicles. However, it is not recommended to use rapid distributing surfactants like sodium lauryl sulphate due to their toxic effects[57,58].

Structure of solid lipid nanoparticles

SLNs consist of a core of solid lipid with the bioactives being a part of the lipid matrix (Figure 1). The particle is stabilized by a surfactant layer, which may consist of a single surfactant, but typically is composed of a mixture of surfactants. In general, the use of crystallized lipids instead of liquid lipids has been shown to increase control over release and stability of incorporated bioactive. This is because mobility of bioactives can be controlled by controlling the physical state of the lipid matrix[59].

PREPARATION METHODS OF SOLID LIPID NANOPARTICLES

Apart from the ingredients used for the preparation of SLNs, the method of preparation also greatly influences particle size, drug loading capacity, stability of the drug, etc. The techniques that could be employed for generating solid lipid nanoparticles are

1. High pressure homogenization
2. Hot homogenization[60]
3. Cold homogenization (for thermo labile drugs)
4. Microemulsion technique[61]
5. Solvent emulsification technique[62]
6. Solvent emulsifiation- diffusion technique[63, 64]
7. Solvent injection[65]
8. Double emulsion technique (for encapsulating hydrophilic drugs)[66]
9. Homogenization followed by Ultra sonication[67-73]
10. Membrane contactor as a new reported technique for SLN production[74]

DRUG INCORPORATION MODELS OF SLN

The prerequisite for a sufficient loading capacity is the high solubility of the drug in the lipid melt. Factors affecting loading capacity of a drug in lipid are[75]:

- ✓ Solubility of drug in molten lipid,
- ✓ Miscibility of drug melt and lipid melt,
- ✓ Chemical and physical structure of solid lipid matrix,
- ✓ Polymorphic state of lipid material.

There are basically three different models for the incorporation of active ingredients into SLN (Figure 1).

- ❖ Solid solution model (Homogeneous matrix model)
- ❖ Drug-enriched shell model
- ❖ Drug-enriched core model

Solid solution model

A homogeneous matrix with molecularly dispersed drug or drug being present in amorphous clusters is thought to be mainly obtained when applying the cold homogenization method and when incorporating very lipophilic drugs in SLN with the hot homogenization method. In the cold homogenization method, the bulk lipid contains the dissolved drug in molecularly dispersed form, mechanical breaking by high pressure homogenization leads to nanoparticles having the homogeneous matrix structure. The same will happen when the oil droplet produced by the hot homogenization method is being cooled, crystallize and no phase separation between lipid and drug occurs during this cooling process. This model is assumed to be valid for incorporation of, e.g., the drug prednisolone, which showed release from 1 day up to weeks[76,77].

Drug-enriched shell model

An outer shell enriched with active compound can be obtained when phase separation occurs during the cooling process from the liquid oil droplet to the formation of a solid lipid nanoparticle. The lipid can precipitate first forming a practically compound-free lipid core. At the same time, the concentration of active compound in the remaining liquid lipid increases continuously during the forming process of the lipid core. Finally, the compound-enriched shell crystallizes. This model is assumed, for example, for coenzyme Q10, the enrichment leads to a very fast release. A fast release can be highly desired when application of SLN to the skin should increase the drug penetration, especially when using the occlusive effect of SLN at the same time[78,79].

Drug-enriched core model

A core enriched with active compound can be formed when the opposite occurs, which means the active compound starts precipitating first and the shell will have distinctly less drug. This leads to a membrane controlled release governed by the Fick law of diffusion[80,81]. The three models presented each represent the ideal type. Of course, there can also be mixed types which can be considered as a fourth model. The structure of SLN obtained is a function of the formulation composition (lipid, active compound, and surfactant) and of the production conditions (hot vs. cold homogenization)[82].

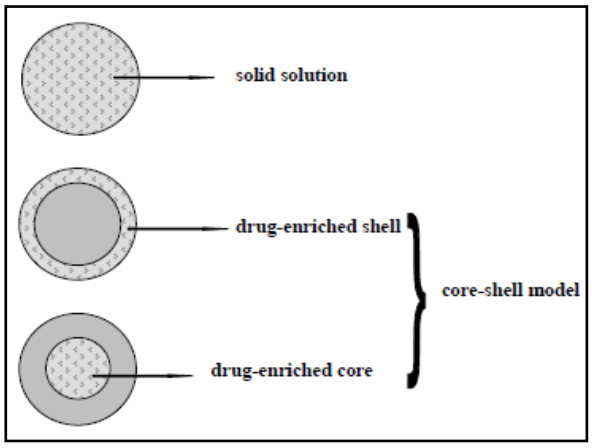

Figure 1: Models of drug incorporation into SLN

CHARACTERIZATION OF SOLID LIPID NANOPARTICLES

Several parameters which have to be considered in characterization are as follows:

Measurement Particle size and distribution

Size of nanoparticles can be determined by several methods such as photon-correlation spectrometry (PCS), Laser Diffraction (LD), Transmission Electron Microscopy (TEM), Scanning electron microscopy (SEM), SEM combined with energy-dispersive X-ray spectrometry and scanned probe microscopy. Among these methods, most widely used methods are PCS and electron microscopy (SEM, TEM) methods.

Photon Correlation Spectroscopy (PCS)

PCS method determines the hydrodynamic diameter of the nanoparticles. This technique is based on dynamic laser light scattering due to Brownian movement of particles in dispersion medium. PCS measures the fluctuation of the intensity of scattered light, which is caused by the particle movement. This method is suitable for the measurement of particles in the size range of few nanometers to 3 µm. Photon correlation spectroscopy (PCS) is also known as dynamic light scattering. The PCS device consists of a light source, a temperature-controlled sample cell, and a photomultiplier for detection of the scattered light[1,83, 84].

Laser Diffraction (LD)

This method is based on the dependency of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range. It is highly recommended to use PCS and LD simultaneously. It is noted, that both methods are not measuring particle sizes. Rather, they detect light scattering effects which are used to calculate particle sizes[85,86].

Measurement of shape and morphology

Transmission Electron Microscopy (TEM)

TEM determines the particle size with or without staining. TEM uses electrons transmitted through the specimen to determine the overall shape and morphology and both particle size as well as distribution. TEM allows visualization of nanoparticles after freeze fracturing and freeze substitution. Thus, it allows observation of their interior. Because this method is laborious and time-consuming, it is not useful for routine measurements[87,88].

Scanning Electron Microscopy (SEM)

SEM uses electrons transmitted from the specimen to determine the overall shape and morphology and both particle size as well as distribution. SEM has high resolution and the sample preparation is relatively easy. SEM imaging has no source–sample contacts and imaging is carried out in high vacuum and samples require pre-treatment[89].

Atomic Force Microscopy (AFM)

It is another advanced microscopic technique used for characterization of nanoparticles. This is a new tool to image the original unaltered shape and surface properties of the particles. In this technique, the force acting between the surface and probing tip results in a spatial resolution up to 0.01 nm for imaging. Sample preparation is simple, as no vacuum is needed during operation and that the sample does not need not be conductive. Hence, it allows the analysis of hydrated and solvent containing samples[90-93].

Measurement of zeta potential

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersions. In

general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot strictly be applied for systems which contain steric stabilizers, because the adsorption of steric stabilizers will decrease the zeta potential due to the shift in the shear plane of the particle[94-96].

Measurement of entrapment efficiency (EE %)

The entrapment efficiency of the system can be determined by measuring the concentration of free drug in the dispersion medium[97,98]. To separate dispersion medium, ultrafiltration can be employed using Centrisort separators. This consists of filter membrane (molecular weight cut-off 20,000 Daltons) at the base of the sample recovery chamber. The sample is placed in the outer chamber and sample recovery chamber is placed on top of the sample and subjected for centrifugation. The SLN along with the encapsulated drug remain in the outer chamber and aqueous phase moves into the sample recovery chamber through filter membrane. Analyzing drug concentration in aqueous phase gives entrapment efficiency.

Entrapment efficiency =

$$\frac{(\text{Wt. of the drug in system} - \text{Wt. of drug in aqueous phase}) * 100}{\text{Wt. of the drug in the system}}$$

Conclusion

A thorough understanding of the excipients profile, surfactant toxicity estimation, mechanism of drug loading and drug release from the nanoparticles, and detailed understanding of physicochemical characterization is necessary for the development and optimization of SLN as a potential drug delivery system. It is vital to understand the basic principles and the limitations of characterization techniques to use them effectively in characterizing SLN, both qualitatively and quantitatively.

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