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Review Article

Solid Lipid Nanoparticles: Methods of Preparation

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ABSTRACT

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. The SLN combine the advantages (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) of other traditional colloidal systems. This review describes the different ways of SLN production such as high pressure homogenization, ultrasonication, solvent emulsification/ evaporation, microemulsion, spray drying and double emulsion method.

INTRODUCTION

Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules [1]. Looking for drug carrier formulations increasing the bioavailability and consisting of well tolerated excipients, the Solid Lipid Nanoparticles (SLN) are alternative drug carrier systems [2]. In contrast to emulsions and liposomes, the particle matrix of SLN is composed of solid lipids. In the past few years, lipid matrices became extremely popular in controlling release of drugs [3]. General features of SLN are their composition of physiological compounds, possible routes of administration by i.v., oral and topical, the relatively low costs of excipients. The other advantage is easy large scale production [4].

Nanosized drug delivery systems have been developed to overcome one or several of the following problems: (i) low and highly variable drug concentrations after per oral administration due to poor absorption, rapid metabolism and elimination (ii) poor drug solubility which excludes i.v. injection of an aqueous drug solution (iii) drug distribution to other tissues combined with high toxicity (e.g. cancer drugs).

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Several systems, including micelles, liposomes, polymer nanoparticles, nanoemulsions and nanocapsules have been developed. During the last few years, solid lipid nanodispersions (SLN) have attracted increased attention.

In the past, solid lipids have been mainly used for rectal and dermal applications. In the beginning of the 80s, Speiser and coworkers developed solid lipid microparticles (by spray drying) [5] and "Nanopellets for peroral administration"[6]. These Nanopellets were produced by dispersion of melted lipids with high speed mixers or ultrasound. The manufacturing process was unable to reduce all particles to the submicron size. A considerable amount of microparticles was present in the samples. This might not be a serious problem for peroral administration, but it excludes an intravenous injection. "Lipospheres", described by Domb, are close related systems.[7-9] They are also produced by means of high shear mixing or ultrasound and also often contain considerable amounts of microparticles.

The quality of the SLN has been significant improved by the use of high pressure homogenization (HPH) in the early 90s [10-12]. Higher shear forces and a better distribution of the energy force more effective particle disruption, compared with high shear mixing and ultrasound. Dispersions obtained by this HPH are called Solid Lipid Nanoparticles (SLN™). Most

SLN dispersions produced by high pressure homogenization (HPH) are characterized by an average particle size below 500 nm and a low microparticle content. Other production procedures are based on the use of organic solvents HPH/solvent evaporation[13] or on dilution of microemulsions[14-15].

METHODS OF PREPARATION

1. High pressure homogenization
   a. *Hot homogenization technique*
   b. *Cold Homogenization technique*
2. Ultrasonication or high speed homogenization
3. Micro emulsion based SLN preparations
4. Double emulsion method
5. Solvent emulsification/evaporation
6. Spray drying method
7. SLN preparation by using supercritical fluid

1. High pressure homogenization

a. Hot homogenization technique

The hot homogenization is carried out at temperatures 5 – 10 ^{0} c above the melting point of lipid. Therefore, it is in fact the homogenization of an emulsion. A preemulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device (Ultraturrax). The quality of the preemulsion is very important for the final product quality. In general, higher temperatures result in lower particle sizes due to the decrease of the viscosity of the inner phase. The obtained pre-emulsion was then subjected to HPH. Five homogenization cycles at 500 bar. The produced hot O/W nanoemulsion is cooled down to room temperature, the lipid recrystallizes and leads to solid lipid nanoparticles[16].

Kovacevic A *et al.,* were prepared solid lipid nanoparticle by using hot homogenization technique. They used two polyhydroxy surfactants Plurol®Stearique WL1009 – (PS) and Plantacare® 810 – (PL). Analysis was performed by photon correlation spectroscopy (PCS), laser diffraction (LD), zeta potential measurements and differential scanning calorimetry (DSC) The particle size of solid lipid nanoparticle was found 200 nm by both of surfactant and All dispersions with both surfactants were physically stable for 3 months at room temperature[17].

Alex MR *et al.,* had prepared solid lipid nanoparticle of poor orally available lopinavir with glyceryl behenate. SLN with mean particle size of 230 nm (polydispersity index, PDI < 0.27) and surface electrical charge of approx. −27 mV, were produced by hot homogenization process. From the intestinal lymphatic transport study it became evident that SLN increased the cumulative percentage dose of lopinavir secreted into the lymph, which was 4.91-fold higher when compared with a conventional drug solution in methyl cellulose 0.5% (w/v) as suspending agent[18].

Silva AC *et al.,* were encapsulated risperidone in solid lipid nanoparticles. The hot high pressure homogenization (HPH) and the ultrasound (US) technique were used as production methods for SLN. All the studies on the SLN formulations were done in parallel, in order to compare the results and conclude about the advantages and limitations of both techniques. The particle sizes were in the nanometer range for all prepared SLN formulations and the zeta potential absolute values were high, predicting good long-term stability[19].

Helgason T *et al.,* had shown effect of surfactant surface coverage on formation and stability of Tween 20 stabilized tripalmitin solid lipid nanoparticles. Emulsion droplets after homogenization had a mean particle diameter of 134.1 ± 2.0 nm and a polydispersity index of 0.08 ± 0.01. Upon addition of 1–5% w/w Tween 20, SLN dispersions became increasingly stable. At low added Tween 20 concentration (<1% w/w) the SLN formed gels but only increased slightly at higher surfactant concentrations (>1% w/w)[20].

b. Cold Homogenization technique

The primary step for preparing solid lipid nanopartical by cold homogenization method is similar to the hot homogenization .The active compound is dissolved or dispersed in melted solid lipid. The active containing lipid melt is cooled down. After solidification the mass is crushed and ground to obtain lipid microparticles. The lipid microparticles are then dispersed in a cold surfactant solution yielding a cold pre-suspension of micronized lipid particles. This suspension is passed through a high pressure homogenizer at room temperature applying typically 5–10 cycles at 1500 bar [21,22]].

2. Ultrasonication

The solid lipid was heated 5–10 ◦C above its melting point, and then added to a mixture of surfactants and water, previously heated at the same temperature. A pre-emulsion was obtained under stirring with an Ultra-Turrax T25 (Janke &

Kunkel GmbH, Germany), at 8000rpm for 5 min. A sonication probe (6mm diameter) was placed in this pre-emulsion, by means of an Ultrasonic processor VCX130 (Sonics, Switzerland). A power output with amplitude of 70% was applied for 20 min, which lead to droplet breakage by acoustic cavitation, and subsequent formation of nanoparticles. The o/w nanoemulsion formed was transferred to glass vials and immediately cooled down to room temperature to generate SLN. For drug-loaded SLN, the drug was added to the solid lipid before melting and sonication [23].

Castelli F *et al.,* were prepared Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) of indomethacin by ultrasonication method. They were studied organization and distributions of the different ingredients originating each type of nanoparticle system were studied by differential scanning calorimetry (DSC) technique. Furthermore, mean particle size and percentage of drug encapsulation were also determined [24].

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3. SLN preparations by dilution of microemulsions or liquid crystalline phases

SLN preparation techniques which are based on the dilution of microemulsions have been developed by Gasco and coworkers. microemulsions as two systems composed of an inner and outer phase (e.g. O/W-microemulsions). They are made by stirring an optical transparent mixture at 65-70°C, typically composed of a low melting lipid fatty acid (e.g. stearic acid), emulsifier (e.g. polysorbate 20, polysorbate 60, soy phosphatidylcholin, taurodeoxycholic acid sodium salt), co-emulsifiers (e.g. Butanol, Na-monooctylphosphate), and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. Typical volume ratios of the hot microemulsion to the cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion [25].

According to the literature, the droplet structure is already contained in the microemulsion, and therefore, no energy is required to achieve submicron particle sizes. The temperature gradient and the pH-value determine the product quality in addition to the composition of the microemulsion. High temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step, lipid contents which are achievable are considerably lower, compared with the HPH based formulations. Another disadvantage includes the use of organic solvents.

a similar approach to produce SLN. A hot liquid crystalline phase (instead of a microemulsion) is diluted in cold water to yield a solid lipid nanodispersion.34 This approach avoids the use of high pressure homogenization and organic solvents, and therefore represents an interesting opportunity [16].

Ali H *et al.,* prepared and characterized lipid nanoparticles by microemulsion technique that combined simvastatin and tocotrienol rich fraction (TRF) as potential anticancer therapy. The entrapment of simvastatin in the oily nanocompartments, which were formed by TRF inclusion into the solid matrix of the nanoparticles, was verified by its high entrapment efficiency and the absence of endothermic or crystalline peaks when blends were analyzed by DSC and PXRD, respectively. They reported that no any significant change in particle size (~100 nm) was observed after storage for six months [26].

Souza LG *et al.,* A microemulsion technique was employed to prepare SLNs and NLCs and produced homogeneous, small size, negatively charged lipid nanoparticles with high entrapment efficiency and satisfactory drug loading. However, low recovery of topotecan was observed when the microemulsion temperature was high and in order to obtain high quality nanoparticles, and precise control of the microemulsion temperature is critical. Nanoencapsulation sustained topotecan release and improved its chemical stability and cytotoxicity. Surprisingly, there were no significant differences between the NLCs and SLNs, and both are potential carriers for topotecan delivery [27].

Miglietta A *et al.,* were prepared solid lipid nanoparticle of doxorubicin and paclitaxel by dispersing warm microemulsions in cold water. SLN were investigated on two cell-lines, human promyelocytic leukemia (HL60) and human breast carcinoma (MCF-7). Cellular uptake of SLN was determined by incorporating 6-coumarin as fluorescent marker. The cytotoxicity of doxorubicin incorporated in SLN was higher compared to the conventional doxorubicin solution, even at the lower concentrations. Paclitaxel in SLN was about 100-fold more effective than free paclitaxel on MCF-7 cells, while on HL60 cells a lower sensitivity was achieved with paclitaxel in SLN [28].

Kuo YC *et al.,* prepared Cationic solid lipid nanoparticles (CSLNs) with entrapped saquinavir (SQV) by microemulsion method. CSLNs were stabilized by polysorbate 80, and the lipid phase contained cationic stearylamine (SA) and dioctadecyldimethyl ammonium bromide (DODAB) and nonionic Compritol 888 ATO (CA) and cacao butter (CB). results indicated that a mixture of SA andDODAB and a mixture of CA and CBwere beneficial to the entrapment efficiency of SQV [29].

4. Double emulsion method

To our knowledge, this technique has so far not been applied to the formation of lipid nanoparticles. Therefore, at first a number of preliminary experiments were conducted in order to select the most appropriate conditions for nanoparticle formation. One hundred microliter of milli-Q water (inner aqueous phase) were added to a 1 ml dichloromethane solution containing 100 mg of tripalmitin and different amounts of lecithin (oily phase). This mixture was dispersed with an ultrasonic probe (Branson Sonifier 250) for 15 s at 20 W leading to a W/O emulsion. A double emulsion W/O/W was formed after addition of different volumes of a 2% Poloxamer solution (outer aqueous phase) to the previous W/O emulsion followed by sonication for 1 min at 20 W. This double emulsion was then diluted to 10 ml with a 1% Poloxamer solution. The solvent was evaporated for 6 h under stirring. The influence of variables such as volume of the outer aqueous phase and lecithin concentration on the particle size distribution of nanoparticle suspensions was investigated [30].

Xie S *et al.,* were prepared solid lipid nanoparticle by double emulsion technique. They studied the effect of PLGA composition on the emulsifying activity was studied with PLGA of different lactic/glycolic acid ratios (90/10, 75/25, 50/50). The results demonstrated that the glycolic acid monomer ratio significantly affected the emulsifying activity of PLGA. Increasing the glycolic acid monomer ratio from 10% to 50% decreased the minimum PLGA content needed to produce stable w/o emulsions. With same PLGA contents, increase of the glycolic acid monomer ratio increased the stable time of the w/o emulsion, yielded smaller and narrower-distributed SLN, and enhanced the encapsulation efficiency and loading capacity [31].

5. solvent emulsification evaporation

The solvent emulsification/evaporation processes adapts techniques which have been previously used for the production of polymeric micro- and nanoparticles. The solid lipid is dissolved in a water-immiscible organic solvent (e.g. cyclohexane, or chloroform) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. Westesen prepared nanoparticles of tripalmitate by dissolving the triglyceride in chloroform. This solution was emulsified into an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure. The mean particle size ranges from approximately 30 to lOOnm depending on the lecithin/co-surfactant blend. Particles with very small diameters (30 nm) were obtained by using bile salts as co-surfactants. Comparable small particle size distributions were not achievable by melt emulsification of similar composition. The mean particle size depends on the concentration of the lipid in the organic phase. Very small particles could only be obtained with low fat loads (5 w%) related to the organic solvent. With increasing lipid content, the efficacy of the homogenization declines due to the higher viscosity of the dispersed phase [22].

Trotta M *et al.,* were prepared solid lipid nanoparticle by solvent emulsification evaporation technique. To increase the lipid load the process was conducted at 47±2 ◦C and in order to reach submicron size a high-shear homogenizer was used. Particle size of the solid lipid nanoparticles (SLN) was affected by using different emulsifiers and different lipid loads. By using lecithin and taurodeoxycholic acid sodium salt, on increasing the GMS percentage from 2.5

to 10% an increase of the mean diameter from 205 to 695 nm and from 320 to 368 nm was observed for the SLN prepared using benzyl alcohol and butyl lactate, respectively [32].

Rahman Z *et al.,* were prepared by solvent evaporation method and characterized by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRD), fourier infrared spectroscopy (FTIR), near infrared spectroscopy (NIR) and NIR-chemical imaging (NIR-CI). The objective of this investigation is to evaluate compositional variations and their interaction of the solid lipid nanoparticle (SLN) formulation of risperidone using response surface methodology of design of experiment (DOE) and subsequently, characterize the SLN by non-destructive methods of analysis [33].

Shah AK *et al.,* were developed solid lipid nanoparticles (SLN) of tretinoin (TRE) with the help of facile and simple emulsification-solvent diffusion (ESD) technique and to evaluate the viability of an SLN based gel in improving topical delivery of TRE. The developed SLN were characterized for particle size, polydispersity index, entrapment efficiency of TRE and morphology. Studies were carried out to evaluate the ability of SLN in improving the photostability of TRE as compared to TRE in methanol [34].

6. Spray drying method

It's an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a drug product. It's a cheaper method than lyophilization. This method cause particle aggregation due to high temperature, shear forces and partial melting of the particle. Freitas and Mullera recommends the use of lipid with melting point >70 0 for spray drying. The best result was obtained with SLN concentration of 1% in a solution of trehalose in water or 20%trehalose in ethanol-water mixtures (10/90 v/v) [35].

7. SLN preparation by using supercritical fluid

This is a relatively new technique for SLN production and has the advantage of solvent-less processing. There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) was the good choice as a solvent for this method [36].

REFERENCES

[1] Manmode AS, Sakarkar DM, Mahajan NM. Nanoparticles-tremendous therapeutic potential: a review. Int J Pharm Tech Res. 2009; 1(4):1020-27.

[2] K. Peters, R.H. Muller. Nanosuspensions for the oral administration of poorly soluble drugs, European Symposium on formulation of poorly available Drugs for Oral Administration, APGI, Paris, 1996:330–33.

[3] R.H. Műller, K. Mäder, S. Gohla. Solid Lipid Nanoparticles (SLN) for controlled drug delivery - a review of the state of the art, Eur. J. Pharm. Biopharm. 2000; (50): 161–77.

[4] R.H. Műller, K.Mehnert. Solid Lipid Nanoparticles (SLN)—an alternative colloidal carrier system for controlled drug delivery, Eur. J. Pharm. Biopharm. 41 (1995) 62–69.

[5] Eldem T, Speiser P and Hincal. A Optimization of spray-dried and congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. Pharm Res. 1991; (8):47-54

[6] Speiser P. Lipidnanopellets als Tragersystem fur Arzneimittel zur peroralen Anwendung. 1990. European Patent EP 0167825.

[7] Domb AJ. Lipospheres for controlled delivery of substances. 1993. United States Patent No. 5188837

[8] Domb AJ. Long acting injectable oxytetracycline-liposphere formulation. Int J Pharm.1995; (124):271-8.

[9] Domb AJ. Liposphere parenteral delivery system. Proc Intl Symp Control Rel Bioact Mater. 1993; (20):346-7.

[10] Siekmann B and Westesen K. Submicron-sized parenetral carrier systems based on solid lipids, Pharm. Pharmacol Lett. 1992 (1):123-26.

[11] Miiller RH, Lucks JS. Arzneistofftrager aus festen Lipidteilchen, Feste Lipidnanospharen (SLN). 1996. European Patent No. 0605497.

[12] Miiller RH, Mehnert W, Lucks JS, Schwarz C, zur Miihlen A, Weyhers H, Freitas C and Riihl D. Solid Lipid Nanoparticles (SLN) -An Alternative Colloidal Carrier System for Controolled Drug Delivery. Eur J Pharm Biopharm. 1995; (41):62-9.

[13] Sjostrom B and Bergenstahl B. Preparation of submicron drug particles in lecithinstabilized o /w emulsions. I. Model studies of the precipitation of cholesteryl acetate. Int J Pharm. 1992; (88):53-62.
[14] Cavalli R, Caputo O and Gasco MR. Solid lipospheres of doxorubicin and idarubicin. Int J Pharm. 1993; (89):R9-R12.
[15] Gasco MR. Method for producing solid lipid microspheres having a narrow size distribution. 1993. United States Patent No. 5250236.
[16] Torchilin VP. Nanoparticle as a drug carriers. Imperial College Press. 2006; 187-93.
[17] Kovacevic A, Savic S, Vuleta G, Muller RH, Keck CM. Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): Effects on size, physical stability and particle matrix structure. Int J Pharm. 2011; (406):173-72.
[18] Alex MR, Chacko AJ, Jose S, Souto EB. Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. Eur J Pharm Sci. 2011; (42):11-8
[19] Silva AC, González E, García ML, Egea MA, Fonseca J, Silva R, *et al*. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): High pressure homogenization versus ultrasound. Colloids and Surfaces B: Biointerfaces. 2011; (86):158-65.
[20] Helgason T, Awad TS, Kristbergsson K, McClements DJ, Weiss J. Effect of surfactant surface coverage on formation of solid lipid nanoparticles. Journal of Colloid and Interface Science. 2009; (324):75-81.
[21] Pardeike J, Hommoss A, Muller RA. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. . Int J Pharm. 2009; (366):170-84.
[22] Mehnert W, Mader K. Solid lipid nanoparticles production, characterization and applications. Adv Drug Del Rev. 2001;(47):165–96
[23] Puglia C, Blasi P, RizzaL, Schoubben A, Bonina F, Rissi M. Lipid nanoparticles for prolonged delivery: An in vitro and in vivo investigation. Int J Pharm. 2008; (357):295-304.
[24] Castelli F, Puglia C, Sarpietro MG. Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry. Int J Pharm. 2005; (304):231-38.
[25] Luisa Rizza b, Francesco Bonina, Gasco MR. Solid lipid nanospheres from warm micro-emulsions. Pharma Technol Eur. 1997:52-58.
[26] Ali H, Shirode AB, Sylvester PW, Nazzal S. Preparation, characterization, and anticancer effects of simvastatin–tocotrienol lipid nanoparticles. Int J Pharm. 2010; (389):323-31.
[27] Souza LG, Silva EJ, Martins ALL, Mota MF, Braga RC, Lima EM, *et al*. Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. Eur J Pharm Sci. Bio. 2011; (79):189-96.
[28] Miglietta A, Cavalli R, Bocca C, Gabriel L, Gasco MR. Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. 2000; (210):61-7.
[29] Kuo Y, Chen H. Entrapment and release of saquinavir using novel cationic solid lipid nanoparticles. Int J Pharm. 2099; (365)206-13.
[30] Fuentes MG, Torres D, Alonso MJ. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloids and Surfaces B: Biointerfaces. 2002; (27):159-68.
[31] Xie S, Wang S, Zhu L, Wang F, Zhou W. The effect of glycolic acid monomer ratio on the emulsifying activity of PLGA in preparation of protein-loaded SLN. 2009; (74):358-61.
[32] Trotta M, Debernardi F, Caputo O. Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. Int J Pharm. 2003; (57):153-60.
[33] Rahman Z, Zidan AS, Khan MA. Non-destructive methods of characterization of risperidone solid lipid nanoparticles. Eur J Pharm Sci. Bio. 2010; (76):127-37.
[34] Shah AK, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery. Int J Pharm. 2007; (345):163-71.
[35] Freitas C, Mullera RH. Spray-drying of Solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm 1998; (46):145-51.
[36] Cavalli R, Marengo E, Rodriguez L, Gasco MR. Effects of some experimental factors on the production process of solid lipid nanoparticles. Eur J Pharm Biopharm 1996; (43):110-5.