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Characterization and Stability of Nanostructured Lipid Carriers as Drug Delivery System

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Abstract: Recently more focus has been put to the development of innovative drug-delivery systems that includes polymer nanoparticles, emulsions and liposomes and solid lipid nanoparticles (SLNs). The SLNs have been proposed to be an alternative colloidal drug delivery system. The aim of this study was preparation and characterization of solid lipid nanoparticle (SLN) using varieties of emulsifier for encapsulation of the drug with poor water solubility. In these study four types of solid lipid nanoparticles were prepared based on different compositions of palm oil (S154) and lecithin (Lipoid 100) using the high pressure homogenization method. The SLN formulation had the following (palm oil+lecithin) compositions: SLN-01 (90+10%, respectively), SLN-02 (80+20%, respectively), SLN-03 (70+30%, respectively) and SLN-04 (60+40%, respectively). The SLNs were characterized and the optimum stability factors for one year storage determined. The parameters used to characterize the SLNs were particle size and polydispersity index (particle sizer), zeta potential (zetasizer), crystallinity (differential scanning calorimetry and wide angle X-ray diffraction), ultrastructure (transmission electron microscopy). Varying the palm oil and lecithin compositions resulted in SLNs of variable sizes and zeta potentials. The particle sizes of SLN-01, SLN-02, SLN-03 and SLN-04 were 298.40±11.80, 255.40±3.20, 145.00±3.39 and 273.00±86.50 nm, respectively, while the zeta potentials were -19.44±60.00, -19.50±1.80, -17.83±10.00 and -13.33±2.30 mV, respectively. Thermoanalysis and X-ray diffraction analysis showed that the SLNs had lower crystallinity than bulk lipid. The SLNs were generally round and uniform in shape under transmission electron microscopy. The SLN dimensional data suggested they had high quality physicochemical characteristics, which are conducive for the loading of poor water solubility drugs.

Key words: Colloidal system, drug delivery, characterizations, solid lipid nanoparticles

INTRODUCTION

Colloidal drug delivery system has improved the characterization of diagnosis and treatment (Abbasalipourkabir *et al.*, 2010; Saraf *et al.*, 2011b). Some lipid based system such as liposomes, noisomes, ethosomes and transfersomes were used (Elsayed *et al.*, 2006). Polymeric nanoparticulate systems have been developed to improve the drug loading and the physicochemical stability of other colloidal carriers (Saraf *et al.*, 2011a). Recently solid lipid nanoparticles (SLN) as colloidal particulate drug delivery system for poorly water-soluble pharmaceutical drugs (Ugazio *et al.*, 2002) and cosmetic active ingredients (Jenning *et al.*, 2000) have received much attention from drug development researchers (Fontana *et al.*, 2005). The solid lipid nanoparticles contain a lipid matrix composed of physiologically tolerable lipids that is not toxic (Abbasalipourkabir *et al.*, 2011a). However, Biodegradable

nanoparticles improve the therapeutic significance of diverse drugs and biological agents (Shenoy and Amiji, 2005). The use of SLNs has several advantages including avoids organic solvent for production of SLN, capacity of large scale production, widespread application, high bioavailability, provides protection of drug from degradation agent like water and light and improved controlled-drug release (Yuan *et al.*, 2007). In recent years some investigators have reported a number of preparations techniques, physical and chemical characterization (Wissing and Muller, 2002) and long term storage stability (Freitas and Muller, 1999). Until now, much research was conducted to develop nanoparticles, drug delivery (Oppenheim, 1981). Several stabilizers such as phospholipids, poloxamer, bile salts, polysorbates and other ionic and nonionic surfactants have been used in preparation of SLN (Friedrich and Muller-Goymann, 2003). The physical stability and administration route are affected by the type of emulsifier used in formulation (Abbasalipourkabir *et al.*, 2011c).

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The common techniques for production of SLN include High Pressure Homogenization (HPH), high shear homogenization combined with ultrasound, solvent emulsification/evaporation and microemulsion techniques (Hou *et al.*, 2003). Among these techniques, HPH is most suitable for the production of SLNs because it can be handled and scaling-upped easily. Using the HPH technique, drug incorporation is through dissolving or dispersing the drug in melted lipid (Abbasalipourkabir *et al.*, 2011b). The drug can either be located in the matrix or linked to the particle surface (He *et al.*, 2007).

The objective of this study was to produce SLN using hydrogenated palm oil and lecithin as surfactant. The production of the nanoparticle is by the HPH technique. The SLNs were physically, structurally and biologically characterized.

MATERIALS AND METHODS

Hydrogenated palm oil (Softisan 154 or S154) was a gift from Condea (Witten, Germany). It contains triglyceride mixture of natural, hydrogenated, even chained and unbranched fatty acids with a melting point o f about 55-60°C. Hydrogenated soybean lecithin (Lipoid S100-3, containing 90% phosphatidylcholine, including 12-16% palmitic acid, 83-88% stearic acid, oleic acid and isomers, and linoleic acid] was a gift from Lipoid (Ludwigshafen, Germany). Thimerosal and Sorbitol were purchased from Sigma. Oleyl alcohol, also called octadecenol, or cis-9-octadecen-1-ol, is a fatty alcohol and non-ionic surfactant or emulsifier is usually used for cosmetic product and carrier for medications was purchased from Fluka and water was used in bidistilled quality.

Preparation of solid lipid nanoparticles: Solid lipid nanoparticles were prepared according to the HPH technique (Schubert and Muller-Goymann, 2005). In the present study palm oil (S154) as solid lipid, soy lecithin (S100) as emulsifier and oleyl alcohol as co-surfactant were used. In order to determine the effect of soy lecithin on the characteristics of SLN, four types of solid lipid nanoparticles were prepared based on different compositions of palm oil (S154) and lecithin (Lipoid 100) using the high pressure homogenization method. The SLN formulation had the following (palm oil+lecithin) compositions: SLN-01 (90+10%, respectively), SLN-02 (80+20%, respectively), SLN-03 (70+30%, respectively) and SLN-04 (60+40%, respectively). Each batch was weighed, mixed and ground in a ceramic crucible and then heated up to 65-70°C until a clear yellowish solution was obtained. A solution consisting of 1 mL oleyl alcohol, 0.005 g Thimerosal, 4.75 g Sorbitol and 89.25 mL bidistilled water was added to each of the lipid matrices. The mixtures were stirred on a magnetic stirrer using a Teflon coated magnet, for 30 min at room temperature. The SLNs, according to composition, in form of emulsion were produced using an Ultra Turrax®(Ika, Staufen Germany) at 13000 rpm for 10 min and EmulsiFlex® -C50 (CSA10, Avestin, Ottawa, Canada) high pressure homogenizer at temperature of approximately 60°C. The homogenization pressure used 1000 bar for 20 cycles. The SLN formulations were allowed to solidify at room temperature.

Physical characterization of solid lipid nanoparticles: Mean particle size (diameter, nm+S.D.) and size distribution (polydispersity index, PI) of solid lipid nanoparticles were determined using the high performance particle sizer (HPP5001, Malvern Inst. UK.). Measurement was at 25°C performed in triplicates. Prior to measurement, each sample was dispersed in filtrated bidistilled water by sonic water bath. The zeta potential (electrophoretical movement) of the nanoparticle formulations was then measured by Zeta potential analyzer (Zeta sizer; ZEN-2600; Malvern, UK.), in triplicates.

Morphology and crystallinity of solid lipid nanoparticles: The morphology of SLN was viewed using a transmission electron microscopy (Hitachi H-7100, Japan). After dispersion with water, the samples were negatively stained with 1.5% (w/v) phosphotungstic acid for viewing.

The bulk lipid and SLN formulation were measured for melting point using differential scanning calorimetry (DSC). The DSC analysis was performed using the Mettler DSC 822e (Mettler Toledo, Switzerland). Thermograms were recorded in the temperature range of 20-120°C with heating rate of 5°C min^{-1}. An empty aluminum sample pan was used as reference. Wide angle X-ray Diffraction (WXRD) was used to determine the crystal characteristics of the SLN preparation. The WXRD analysis experiment was conducted using the X-ray machine (Philips, X' Germany) installed on PW3050/60 X-ray tube with copper anode.

Statistical analysis: All the data were subjected to one way Analysis of Variance (ANOVA) followed by Post Hoc multiple comparison and Duncan test after verification of the normal distribution of the data.

RESULTS AND DISCUSSION

Physical analyses: The particle sizes, particle size distribution, specific surface area and zeta potential of

Table 1: Effect of different amount of lecithin on SLN characteristics

Formulations	Particle size (nm)	PI	A_{spec} (m² g⁻¹)	Zeta potential (mV)
SLN-01	298.40±11.80[b]	0.77±0.33	10.06[b]±0.40	-19.44±60.00
SLN-02	255.40±3.20[b]	0.80±0.29	11.74[b]±0.14	-19.50±1.800
SLN-03	145.00±3.39[a]	0.15±0.03	20.69[a]±0.50	-17.83±10.00
SLN-04	273.00±86.50[b]	0.95±0.10	11.79[b]±3.91	-13.33±2.30

All values are represented as Means±SD, (n = 5), [a,b]Values in each column with different superscripts are significantly different (p<0.05) ,The amount of 1% (w/w) oleyl alcohol was fixed, Assessment was 3 days after preparation

Table 2: Effect of storage time on SLN-03 characteristics

Storage time (month)	Particle size (nm)	PI	A_{spec} (m² g⁻¹)
0	145.00±3.39[a]	0.15±0.03	20.69±0.50
6	177.92±1.30[b]	0.15±0.08	16.86±0.12
9	202.20±5.03[c]	0.26±0.05	14.84±0.37
12	219.80±4.08[d]	0.27±0.13	13.65±0.26

All values are represented as Means±SD, (n = 5) [a,b,c,d]Values in each column with different superscripts are significantly different (p<0.05),The amount of 1% (w/w) oleyl alcohol was fixed, Assessment was 3 days after preparation

Table 3: Effect of different homogenization pressures on SLN-03 characteristics

Pressure (bar)	Particle size (nm)	PI	A_{spec} (m² g⁻¹)
500	233.40±8.05[b]	0.23±0.31	12.86±0.4[b]
1000	145.00±3.39[a]	0.15±0.03	20.69±0.50[a]
1500	236.20±7.43[b]	0.25±0.21	12.70±0.39[b]

All values are represented as Means±SD, (n = 5) [a,b]Values in each column with different superscripts are significantly different (p<0.05),The amount of 1% (w/w) oleyl alcohol was fixed, Assessment was 3 days after preparation

different SLN formulations are presented in Table 1. The results revealed that differences in particle size between the SLN formulations are significant (p<0.05) and the optimum concentration for lecithin is 30% of lipid matrix to achieve the smallest particle size. The critical amount for lecithin (30%) in SLN is necessary in order to reduce particle size from 298.4±11.80-145±3.39 nm and Polydispersity Index (PI) from 0.95±0.10-0.19±0.05. Lecithin provides interface between oil and water (Schubert and Muller-Goymann, 2005). As lecithin concentration increased, particles size decreased. This is probably due to the presence of lecithin with multilayer structure on the particle surfaces (Westesen and Siekmann, 1997).

In the present study, during high pressure homogenization the temperature was kept lower than the melting point of lipid matrix. Therefore, the high viscosity of lipid matrix caused effective reduction in particle size (Mehnert and Mader, 2001). Appropriate control of particle size is necessary to obtain maximum physical stability of dispersions (Attama and Muller-Goymann, 2007).

Zeta potential is an important factor to the determination of stability of colloidal formulation (Mountasser *et al.*, 2002). In formulations where the zeta potential is high, aggregation of particles is unlikely to occur, because there is electrical repulsion (Mehnert and Mader, 2001). The surface of SLNs prepared in this study) at a fixed nonionic emulsifier concentration of 1% (w/w), carried a negative charge ranging from -13.33±2.30-19.44±6 mV. The negatively surface charge indicates that anionic surfactant chains on the surface are exposed (Wong *et al.*, 2006). Negative surface charge reduces the adherence of nanoparticles to interstitial tissue and cell surfaces; therefore distant distribution of the particles is promoted (Ikomi *et al.*, 1999). By the results obtained from the SLN formulations, SLN-03 was chosen for subsequent experiments.

Measurement of particle size of SLN-03 formulation stored at room temperature fresh and after storage time of 6, 9 and 12 month (Table 2) showed significant (p<0.05) increase in the particles although still within the nanometer range. The rate of increase in particle size was

ranged from 22 to 51% with storage. Lecithin at low concentrations strongly binds to the particle surface and becomes immobilized. Thus agglomeration and particle growth does not occur due to limited mobility of lecithin (Schubert and Muller-Goymann, 2005).

Different homogenization pressures ranging from 500 to 1000 bar for preparation of the SLN-03 were carried out. The results of SLN-03 characterization are shown in Table 3. Homogenization provides cavitations forces, which break down the particles to the smaller sizes (Schwarz and Mehnert, 1997). Although, increments of homogenization pressure from 500 to 1000 bars significantly (p<0.05) decreased mean particle size and increased specific surface area, further increase to 1500 bars had significantly (p<0.05) increased particle size and decreases specific surface area. It is postulated that at high homogenization pressure, the particles may have coalesced as a result of the high kinetic energy of the particles, which impaired the homogenization process. Increase in the kinetic energy to particle collision and consequently coagulation may have occurred. The high particle collisions also distort the surfactant film coating the particle surface and enhance particle aggregation (Siekmann and Westesen, 1994).

The surface characteristics can be improved using some materials. Rad (2010) reported the developed drug-loaded nanocapsules by interfacial deposition of polymer by means of sunflower seed oil. In this study the effect of three nonionic surfactants; oleyl alcohol, tween 80 and poloxamer 188 at concentration of 1% (w/w) on the characteristics of SLN-03 formulation were assessed. The result of the study is shown in Table 4. The particle size of all formulations of SLN-03 was in the nanometer range, and the difference between the particle sizes and specific surface areas were comparable. The type of surfactants
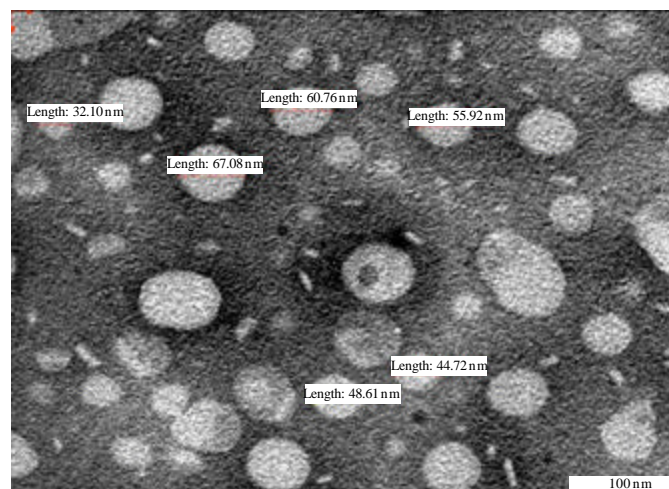
Fig. 1: SLN-03 at 7 days after preparation. The particles were negatively stained with aqueous solution of phosphotungstic acid (PTA) 10 min prior to imaging. Particles exhibited spherical and uniform shapes

Table 4: Effect of different surfactants on SLN-03 characteristics

Emulsifiers	Particle size (nm)	PI	A_{spec} (m^2 g^{-1})	Zeta potential (mV)
Oleyl alcohol	145.00±3.39[a]	0.15±0.03	20.69±0.50[a]	-17.83±1.0
Tween 80	211.13±66.03[a]	0.71±0.36	15.94±6.44[a]	-1.33±0.5
Poloxamer 188	497.83±91.14[b]	0.22±0.15	6.26±1.55[b]	-12.50±1.0

All values are represented as Means±SD, (n = 5) [a,b]Values in each column with different superscripts are significantly different (p<0.05),The amount of 1% (w/w) oleyl alcohol was fixed, Assessment was 3 days after preparation

and their concentrations are important for the quality of SLN products (Muller *et al.*, 1995). The composition of surfactants not only affects particle size, but also improves the absorption of the incorporated drugs in the intestine. It is possible that the surfactants contribute to the increase in permeability of the particle at the intestinal membrane and/or it could have improved the affinity between the lipid particles and the intestinal membrane (Song *et al.*, 2005). As another examples, Lalanne *et al.* (2007) and Poovi *et al.* (2011) developed a particulate delivery system in order to improve drug bioavailability.

In the present study SLN formulation with oleyl alcohol gained the smallest size. The effect of oleyl alcohol and tween 80 in the reduction of particle size may be attributed to the better location of these surfactants on the surface of lipid. The strong interface between oleyl alcohol and the lipidic core results in immobilization of the lipid. With poloxamer (sterically stabilizing polymer), there could be a partial collapse of polymer adsorption layer leading to particle aggregation and enlargement of the particle size (Venkateswarlu and Manjunath, 2004).

Transmission Electron Microscopy (TEM): The TEM image of SLN-03 after 7 days preparation is shown in Fig. 1. The particles were nonporous, spherical and uniform in shape. The ranges of size measured by TEM are smaller than those determined by the particle sizer (HPP5001, Malvern Inst. UK). This discrepancy is probably due to the difference in the measurement conditions. After 9 month storage at room temperature, there was no significant change in the particles shape (data not shown).

Differential scanning calorimetry (DSC): Untreated S154 showed a sharp and single endothermic peak upon heating at 58.88°C and SLN-03 showed a broader single endothermic heating peak at 57.88°C (Fig. 2). The Differential Scanning Calorimeter (DSC) is a useful instrument for the study the melting point and grade of crystalline materials like SLN (Attama and Muller-Goymann, 2007). In this study melting point of SLN was lower than untreated S154. When the melting point of triglycerides in the SLN was depressed in comparison to the bulk lipid, it can be suggested that triglycerides in SLN might be in the β form (Bunjes *et al.*, 2003). The decreased melting point may be due to small particle size (nanometer range), their high specific area and the presence of surfactant and Kelvin effect (Kristl *et al.*, 2008).

Wide angle X-ray diffraction analysis (WAXD): Wide angle X-ray diffraction (WAXD) pattern of untreated bulk lipid (S154) and SLN-03 dispersion is shown in Fig. 3. The
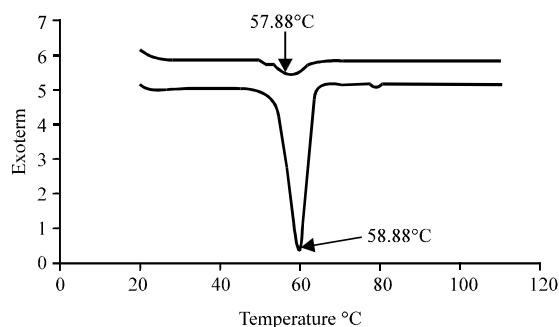
Fig. 2: DSC Thermogram of Bulk Lipid (S154) and SLN. The thermogram was recorded within 7 days after preparation (Scan rate: $5°C\,min^{-1}$)
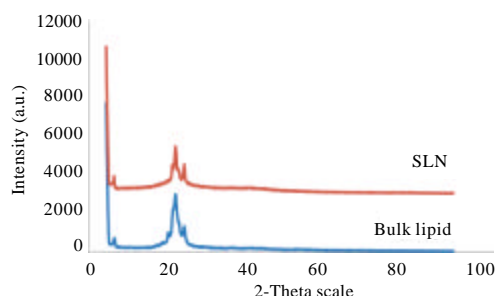

Fig. 3: Wide Angle X-ray Diffraction pattern of untreated S154 (bulk lipid) and SLN-03 at 7 days after preparation

degree of crystallinity is compared on the basis of peak intensity (Venkateswarlu and Manjunath, 2004). The result showed that crystallinity degree was reduced when S154 is converted to SLN. The reduced crystallinity of SLN may be related to the incorporation of lecithin in bulk lipid (S154). It was shown that lipid within nanoparticles should be in a less ordered arrangement compared to the bulk materials (Hou *et al.*, 2003). Thus X-ray diffraction data obtained in this study were in good agreement with the results established by DSC thermogram.

CONCLUSION

In this study we incorporated Lipoid S100 (soy lecithin) into the palm oil matrix to produce SLNs. A crucial concentration of 30% lecithin was necessary decrease the particle size. Also of the three stabilizers studied, the oleyl alcohol with concentration of 1% was able to keep the SLN in smaller size with high surface area. Another advantage of oleyl alcohol is that poor water-solubility drug like tamoxifen that can be dissolved in oleyl alcohol phase. Homogenization of SLN at 1000 bar

for 20 cycles was found to be the optimum condition in the preparation the SLN or small mean diameter.

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