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Solid Lipid Nanoparticles Preparation and Characterization

[1]N. AL-Haj and [2]A. Rasedee
[1]Laboratory Immunotherapeutic and Vaccine, Institute of Bioscience,
[2]Department of Pathology and Microbiology, Faculty of Veterinary Medicine,
Universiti Putra Malaysia, Serdang 43400, SDE, Malaysia

Abstract: The presents study aimed to prepare and characterize Solid Lipid Nanoparticles (SLNs) from palm oil materials. Hydrogenated palm oil and lecithin incorporated with surfactant were mixed and formed by High Pressure Homogenization (HPH) at elevated temperature. Appropriate analytical methods are needed for the characterization of SLN. The use of several analytical techniques is a necessity such as particle size which determined using Photon Correlation Spectroscopy (PCS). The change of particle charge was studied by Zeta Potential (ZP) measurements, while the melting and recrystallization behavior characterized by Differential Scanning Calorimetry (DSC). Data showed physical stability of the formulation. In conclusion, the SLN presented here are well suited for several applications including drug delivery.

Key words: Solid lipid nanaoparticles, transmission electron microscopy, high pressure homogenization, differential scanning calorimetry, photon correlation spectroscopy

INTRODUCTION

Solid Lipid Nanoparticles (SLN) (Mehnert and Mader, 2001; Muller *et al*., 2000) have been introduced to the literature as a carrier system for poorly water-soluble pharmaceutical drugs (Ugazio *et al*., 2002; Westesen *et al*., 1997) and cosmetic active ingredients (Jenning *et al*., 2000a; Lippacher *et al*., 2002, 2004; Wissing and Muller, 2003a, b). Advantages of SLN include a potentially wide application spectrum (dermal, oral, intravenous), the use of biodegradable physiological lipids or lipidic stabilisers which are Generally Recognized as Save (GRAS) or have a regulatory accepted status, the production without organic solvents and the possibility of scaling up to industrial production level (Wan *et al*., 2008; Gohla and Dingler, 2001; Liedtke *et al*., 2000). The predominant production technique till recently was the High Pressure Homogenization (HPH) method (Lander *et al*., 2000; Siekmann and Westesen, 1994). To avoid the organic solvents and the large amount of surfactants and other additions introduced, this method have some clear advantages for production of SLNs (AL-Haj *et al*., 2008; Kim *et al*., 2005).

MATERIALS AND METHODS

Lipid Matrices (LM) preparation: Present study was conducted in the Universiti Putra Malaysia November, 2007. Hydrogenated palm oil Softisan 154, solid lipid triglyceride mixture, was donated from Condea (Witten, Germany). Phospholipon 90 H is a completely hydrogenated lecithin from Phospholipid (Cologne, Germany). The lipid matrix composition was varied by incorporation of 0-50 % Phospholipon 90 H (w/w) within Softisan 154. Oleyl alcohol long-chain fatty alcohols and thimerosal used as a preservative were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The mixtures were stirred with a teflon coated magnet at 80°C until a transparent yellow solution was obtained. The solution was stirred at room temperature until solidification. Bidistilled water was used for all preparations and all chemicals were of pharmacopeial or reagent grade.

Solid Lipid Nanoparticles (SLN) preparation: Basic formulation of SLN contained 10% of Hydrogenated palm oil Softisan 154 and hydrogenated lecithin (lipid matrix), 1% Oleyl alcohol, 0.005% thimerosal and 89% bidistilled water (all w/w). All components were weighted into sealed containers and heated to 80°C. Thereafter a preemulsion was produced using an Ultra Turrax (Ika/Staufen, Germany) at 10,000 rpm for 10 min. The hot preemulsions were homogenised at a pressure of 1000 bar and a temperature of about 70°C with an EmulsiFlex^{w}-C5 (Avestin, Canada) high pressure homogeniser for 10 cycles. Subsequently, the dispersions were allowed to recrystallise at room temperature (Liedtke *et al*., 2000).

Corresponding Author: Dr. Rasedee Abdullah, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang 43400, SDE, Malaysia Tel: 603-89441582

Transmission Electron Microscopy (TEM): The morphology of the SLN was examined by TEM (Hitachi, Japan). The samples were stained with 2% (w/v) phosphotungstic acid for 30 sec and placed on copper grids with films for viewing.

Particle size measurement: The average diameter and Polydispersity Index (PI) of SLN were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3 (Malvern Instruments, Malvern, UK) at a fixed angle of 90 and at 25°C. The aqueous SLN dispersions were diluted with distilled water before analysis. Each value is the average of 3 measurements.

Zeta potential measurement: The particle charge was quantified as Zeta Potential (ZP) using a Zetasizer 4 at 25°C. Before measuring, each sample had to be diluted with demineralized particle free water to an adequate intensity. Each measurement was performed at least in triplicate. The pH values of the samples were always between 6.2±0.9.

Differential scanning calorimetry: Differential Scanning Calorimetry (DSC) was performed with a Mettler DSC 822e (Mettler Toledo, Greifensee, Switzerland). Samples containing ~10 mg nanoparticle dispersions (identical to 1-2 mg of solid lipid) were weighed accurately into standard aluminum pans using an empty pan as a reference. DSC scans were recorded at a heating and cooling rate of 5°C min^{-1}. The samples were heated from 25 to 85°C and cooled from 85 to 20°C under liquid nitrogen. Enthalpies were calculated using the Mettler Star software.

RESULTS AND DISCUSSION

Transmission electron microscopy: Solid Lipid Nanoparticles (SLN) are the new generation of nanoparticulate active-substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use (Wan *et al*., 2008). SLN are commonly produced by the hot homogenisation technique (AL-Haj *et al*., 2008). Alternatively a cold homogenisation process can be used to incorporate temperature sensitive drugs or to increase drug encapsulation. Nanoparticles were produced by either hot or cold homogenisation technique as described by Wissing and Muller (2002). Nanoparticles emulsified by a mixture of Lipid matrix and surfactant were produced at 1000 bar using the hot homogenisation technique. So, in practice, samples were taken after 10 homogenisation cycles lead to reproducible and satisfying results. The number of homogenisation cycles necessary to decrease the polydispersity and to get a small particle population was slightly different. The electron microscopy micrographs of SLN were shown in Fig. 1. The shape of SLN was spherical and particle size was approximately from 25 to 150 nm.

Average diameter and zeta potential: An adequate characterization of the solid lipid particles is a necessity for the control of the quality of the product. PCS the most powerful techniques for routine measurements of particle size. The average diameter of SLN measured by PCS Zetasizer 4 was 108.48 nm (n = 5) was showed in Table 1.

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion. In general, particle aggregation is less likely to

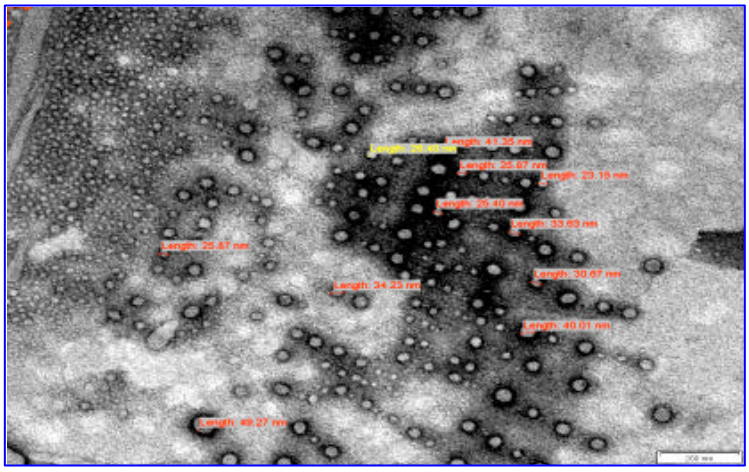

Fig. 1: TEM micrograph of SLN, bar = 200 nm

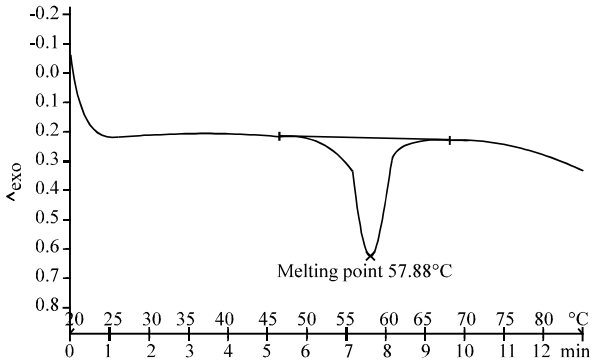

Fig. 2: Differential scanning calorimetry scans of SLN

Table 1: Particle size and zeta potential with polydispersity index (PI) of SLN

SLN: No.	Particle size (nm)	PI	Zeta potential (mV)
SLN 1	142.70	0.274	-14.4
SLN 2	107.30	0.219	-18.7
SLN 3	95.50	0.225	-14.8
SLN 4	66.20	0.182	-16.9
SLN 5	128.50	0.200	-12.2
Mean	108.48		-15.4

occur for charged particles (high zeta potential) due to electric repulsion (Müller *et al*., 2000). The zeta potential distribution of SLN was showed in Table 1. The mean zeta potential was -15.4 mV (n = 5). Therefore, this method had gained a relative good stability and dispersion quality.

Using DSC analysis, cooling scans are the most sensitive method to detect polymorphic forms. Freitas and Muller (1999) and Mühlen *et al*. (1998) used this method to investigate the different crystallization of SLN. The cooling curves obtained 1 day after production showed that the formulation recrystallized in different polymorphic forms. SLN cooling curve shows a main peak at 57.8°C which can be attributed to the beta modification (Fig. 2). But if the SLN cooling curve of the peak higher than 62°C suggested the presence of alpha modifications (Mühlen *et al*., 1998).

The melting points of colloidal systems were distinctly decreased by about 10-15°C. According to Wan *et al*. (2008) the melting point decrease of colloidal systems can be assigned to the colloidal dimensions of the particles in particular to their large surface to volume ratio and not to recrystallisation of the lipid matrices in a metastable polymorph possessing a lower melting point. Nevertheless, the melting point reduction of the different formulations has no apparent relation to the particle size data not show.

CONCLUSION

Solid Lipid Nanoparticles (SLN) as Drug carriers have unique characteristics that can enhance performance in a variety of dosage forms. We will focus on providing services for production, formulation and characterization of nanoparticles with clear advantages and disadvantages to other colloidal carriers.

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