RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Parameter Optimization of Solid Lipid Nanoparticles Formulation

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Abstract

Solid lipid nanoparticles (SLN) are colloidal lipid carriers, which used for improving the bioavailability of lipophilic and hydrophilic drugs. SLN are composed of biodegradable lipids such as hard fats, highly purified triglycerides, monoglycerides, complex glyceride mixtures or waxes which are solid at room temperature. In the present study, different formulation variables, including the type and quantity of lipid and surfactant were optimized for the preparation of SLN. SLN were prepared by a modified micro emulsion-based method and their particle size was evaluated. Two types of surfactants Tween 80 and Poloxamer 188 were used. Also, the monoglyceride (Glyceryl monostearate) and wax (Cetyl palmitate) were used as lipid. The optimum SLN formulae were at lipid concentration 5%. The optimum surfactant concentration was 6%. SLN with a surfactant Tween 80 were smaller in size than Poloxamer 188 SLN. Glyceryl monostearate formed a smaller size SLN than Cetyl palmitate. Increasing Glyceryl monostearate concentration in the lipid ratio resulted in the formation of small size SLN.

1. Introduction

Solid lipid nanoparticles (SLN) are colloidal lipid carriers, which are composed of biodegradable lipids such as hard fats, highly purified triglycerides, monoglycerides, complex glyceride mixtures or waxes which are solid at room temperature. Unlike liposomes and emulsions, the particle-matrix of SLN is composed of solid lipids (Mulla, Hiremath et al. 2012).

There are many advantages of SLN such as the possibility of controlled drug release and targeting, the ability of incorporation of lipophilic and hydrophilic drugs, good biocompatibility and low biotoxicity, avoiding the use of organic solvents in the production cycle and easy large scale production. Oral administration of SLN could increase the lymphatic transport of drugs, which decreased first-pass hepatic metabolism and increased oral bioavailability of the drug

(Mulla, Hiremath et al. 2012, Ebrahimi, Javadzadeh et al. 2015). Various methods were available for the production of SLN such as hot and cold high-pressure homogenization, high shear homogenization and ultrasonication, solvent emulsification/evaporation process, and micro emulsion-based technique. The principle of this latter method was to disperse a warm oil-in-water microemulsion into a large amount of cold water forming SLN. So, this method was easily processed at the laboratory scale as a microemulsion, the key intermediate product of this preparation, would be formed spontaneously (Boonme, Souto et al. 2013). But the removal process of excess water was difficult so that some modifications applied to the method helped to decrease the dispersion ratio to 1:2 which improved the stability of the prepared SLNs (Aboutaleb, Noori et al. 2012). This study aimed to optimize different formulation variables, including the type and quantity of surfactant for the preparation of SLNs. Also, study the effect of using lipids with different molecular structures and different concentrations on the particle size of SLN.

2. Experimental
2.1. Materials

Cetyl palmitate was kindly provided by Gattefosee, Saint-Priest, France. Glyceryl monostearate was purchased from Alpha Chemika, Mumbai, India. Poloxamer 188 was purchased from Caisson laboratories, Utah, United States. Tween 80 was purchased from Elnasr pharmaceutical chemicals, Al Qalyubia, Egypt.

Distilled water and freshly prepared reverse osmosis (RO) water were used in all experiments. All chemicals were used directly as received without further purification.

2.2. Methods
2.2.1. Preparation of solid lipid nanoparticles

SLN were prepared by a modified micro emulsion-based technique. The lipids (cetyl palmitate/Glyceryl monostearate) and the emulsifiers (Tween 80/Poloxamer 188) were molten and mixed using magnetic stirring at 80°C then, up to 30 ml of RO water at the same temperature was added.

This coarse pre-emulsion was treated with high shear homogenization using an Ultra-Turrax homogenizer (Ultra- Turrax T – 25, IKA, Germany) at 15,000 rpm for 15 minutes, with 30 seconds intervals every two minutes; the resulting microemulsion was immediately dispersed in cold distilled water while stirring (2°C to 3°C) with a ratio 1:2 for distilled water and pre-emulsion respectively. The principle of this latter method is to add a warm oil-in-water microemulsion into a large amount of cold water which leads to precipitation of the lipid phase forming fine particles called SLN [2, 3]. Two different surfactants (Tween 80 and Poloxamer 188) were evaluated at three different concentrations (2%, 4% and 6% w/w) and 1% as a co-surfactant. Two types of lipids (cetyl palmitate and glyceryl monostearate (GMS)) were used at three different concentrations (1%, 5% and 10% w/w) as shown in Table 1.

2.2.2. Particle size analysis

Particle size was measured using dynamic laser light scattering apparatus at 25ºC (Mastersizer 2000 vers. 5.54, hydro 2000 S, Malvern Instruments Ltd., Malvern, Worcs, UK). Prior to the measurements, all samples were diluted using ultra-purified water to yield a suitable scattering intensity. DLS data were analyzed at 25ºC using the general-purpose mode.

3. Results and Discussion
3.1. Effect of surfactant type and concentration on the particle size of SLN

The use of nonionic surfactants increased SLN stability through their steric properties (Ebrahimi, Javadzadeh et al. 2015). As shown in **Error! Reference source not found.**, low molecular weight surfactants gave smaller size SLN due to their ability to cover interfacial spaces more quickly, where Tween 80 (molecular weight 1310 Dalton's) formed smaller SLN than Poloxamer 188 (Avg. molecular weight 8400 Dalton's). This property was observed in a previous study, where Tween 80and phosphatidylcholine based SLN formed a smaller particle size than polyvinyl alcohol (PVA) and Polyvinyl pyrrolidone (PVP) based SLN (Ebrahimi, Javadzadeh et al. 2015).

Table 1. Composition of various SLN at different lipid concentration 1, 5 and 10% using different surfactant concentration and different lipid ratio

SLN Formula code			Cetyl palmitate in lipid ratio	Glyceryl monostearte in lipid ratio	Tween 80 (w/w %)	Poloxamer 188 (w/w %)
Prepared at lipid concentration 1%	Prepared at lipid concentration 5%	Prepared at lipid concentration 10%				
FA11	FB11	FC11	1	0	2	1
FA12	FB12	FC12	1	0	4	1
FA13	FB13	FC13	1	0	6	1
FA14	FB14	FC14	1	0	1	2
FA15	FB15	FC15	1	0	1	4
FA16	FB16	FC16	1	0	1	6
FA21	FB21	FC21	0	1	2	1
FA22	FB22	FC22	0	1	4	1
FA23	FB23	FC23	0	1	6	1
FA24	FB24	FC24	0	1	1	2
FA25	FB25	FC25	0	1	1	4
FA26	FB26	FC26	0	1	1	6
FA31	FB31	FC31	1	1	2	1
FA32	FB32	FC32	1	1	4	1
FA33	FB33	FC33	1	1	6	1
FA34	FB34	FC34	1	1	1	2
FA35	FB35	FC35	1	1	1	4
FA36	FB36	FC36	1	1	1	6
FA41	FB41	FC41	1	2	2	1
FA42	FB42	FC42	1	2	4	1
FA43	FB43	FC43	1	2	6	1
FA44	FB44	FC44	1	2	1	2
FA45	FB45	FC45	1	2	1	4

FA46	FB46	FC46	1	2	1	6
FA51	FB51	FC51	2	1	2	1
FA52	FB52	FC52	2	1	4	1
FA53	FB53	FC53	2	1	6	1
FA54	FB54	FC54	2	1	1	2
FA55	FB55	FC55	2	1	1	4
FA56	FB56	FC56	2	1	1	6
FA61	FB61	FC61	1	3	2	1
FA62	FB62	FC62	1	3	4	1
FA63	FB63	FC63	1	3	6	1
FA64	FB64	FC64	1	3	1	2
FA65	FB65	FC65	1	3	1	4
FA66	FB66	FC66	1	3	1	6
FA71	FB71	FC71	3	1	2	1
FA72	FB72	FC72	3	1	4	1
FA73	FB73	FC73	3	1	6	1
FA74	FB74	FC74	3	1	1	2
FA75	FB75	FC75	3	1	1	4
FA76	FB76	FC76	3	1	1	6

Tween 80 had a long hydrophobic tail which might initiate crystallization of the lipid matrix of nanoparticles due to its affinity to the lipid, a small portion of the surfactant molecules localized in the lipid matrix not only attached to the surface and thus promoted lipid recrystallization. So, surfactant was able to stabilize the formed droplets when they left the homogenization gap to avoid coalescence. Similar results had been obtained for SLN containing Cetyl palmitate as solid lipid and Plurol Stearique (PS), Plantacare 810 (PL) as emulsifiers (Kovacevic, Savic et al. 2011). It seemed that surfactant molecular weight and structure played an important role in the particle size of SLN.

Decrease of surfactant concentration led to an increase in the particle size because the surfactant was insufficient to cover the interface and made particles aggregation. Also, increased the surfactant concentration led to a decrease in the particle size due to the reduction in interfacial tension between the aqueous and lipid phases and form a steric barrier on the particle surface, thereby protecting the particles from coagulation(Reddy and Murthy 2005). **Error! Reference source not found.** showed the obtained results. At high surfactant concentrations, bridging between the nanoparticles might be formed and might cause toxicity (Pooja, Tunki et al. 2016). Therefore, two different surfactants (Tween 80 and Poloxamer 188) were evaluated at three different concentrations (2, 4 and 6% w/v). The optimum concentration of Poloxamer 188 was 1% of the

total formula and up to 10% of total surfactant
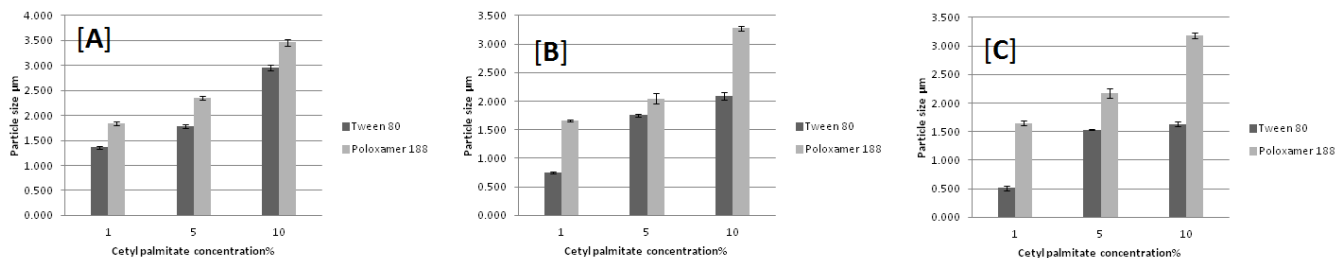
Fig. 1: Effect of surfactant type on the particle size of SLN containing Cetyl palmitate as a lipid: (A), surfactant concentration 2%; (B), surfactant concentration 4%; (C), surfactant concentration 6% (mean±SE, N=3)
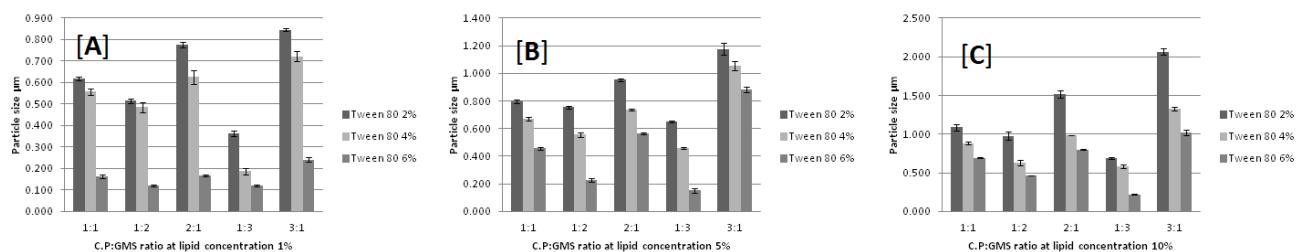
Fig. 2: Effect of Tween 80 concentration on the particle size of SLN: (A), lipid concentration 1%; (B), lipid concentration 5%; (c), lipid concentration 10% (mean±SE, N=3)
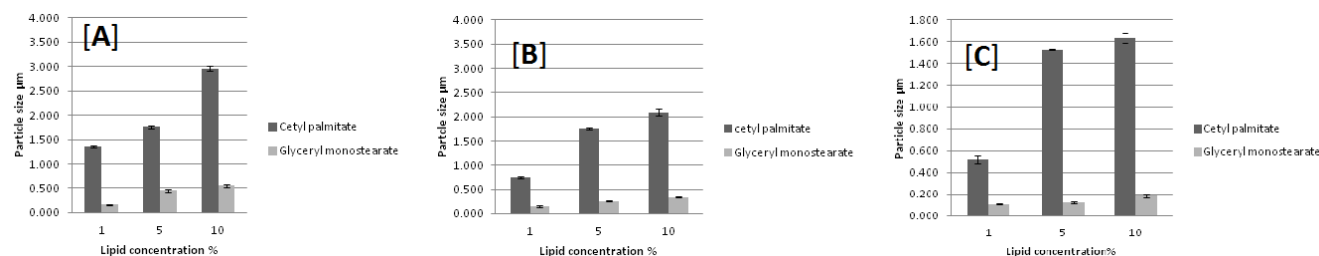
Fig. 3: Effect of lipid type and concentration on the particle size of SLN using Tween 80 as a surfactant: (A), surfactant concentration 2%; (B), surfactant concentration 4%; (C), surfactant concentration 6% (mean±SE, N=3)
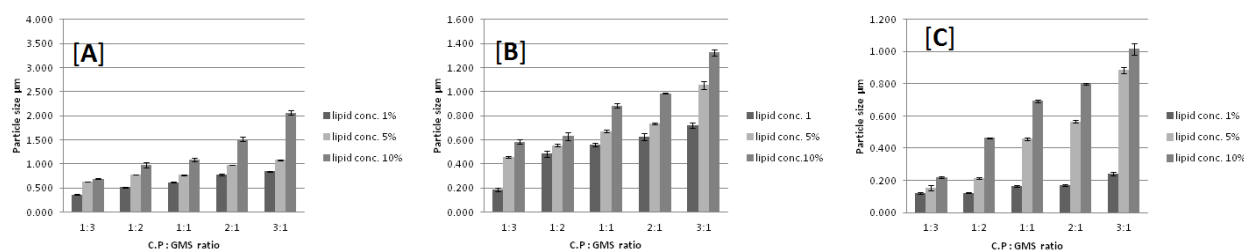
Fig. 4: Effect of increasing Cetyl palmitate concentration in lipid ratio on the particle size of SLN using Tween 80 as a surfactant: (A), surfactant concentration 2%; (B), surfactant concentration 4%; (C), surfactant concentration 6% (mean±SE, N=3)

(Kumar, Chandrasekar et al. 2007, Aboutaleb, Noori et al. 2012). High Poloxamer concentrations (more than 1.5%) would make a decrease in entrapment efficiency and caused toxic effects (Kumar, Chandrasekar et al. 2007). The increase in Poloxamer concentration might also increase the particle size due to the extensive dehydration of propylene oxide and ethylene oxide blocks within the Poloxamer molecule during emulsification and hot homogenization conditions, leading to reduction of steric repulsion activity (Reddy and Murthy 2005). Tween 80 at concentration 6% was optimum to give stable SLN. This result was agreed with that obtained by Aboutaleb et al. on Cetyl palmitate SLN using Tween 80 and Poloxamer 188 as surfactants for improving the antimycobacterial activity of rifampin (Aboutaleb, Noori et al. 2012).

3.2. Effect of lipid type and concentration on the particle size of SLN

The size of SLN was affected by the number of carbon atoms in the fatty acid chain of the lipid (Baek, Shin et al. 2012, Boonme, Souto et al. 2013). Glyceryl monostearate had a smaller number of carbon atoms on its fatty acid chain than Cetyl palmitate resulting in smaller size SLN (**Error! Reference source not found.**). Also, increasing the carbon chain length of fatty acid enhanced the entrapment efficiency and drug loading of SLN, because the longer chain fatty acids were more hydrophobic and would increase the accommodation of lipophilic drugs (Baek, Shin et al. 2012).

Glyceryl monostearate is a monoglyceride and it was reported that emulsifying monoglycerides might lead to a different content of water in the SLN lipid matrix which decreased the stability of SLN (Radomska-Soukharev 2007). Stability of SLN prepared with wax (Cetyl palmitate) was greater than glyceride SLN (Glyceryl monostearate) (Kumar, Chandrasekar et al. 2007).

Increase of the lipid content led to an increase in the particle size of SLN due to increase of viscosity of organic phase that resulted in slower diffusion of the organic phase in the continuous phase (Kommavarapu, Maruthapillai et al. 2015). The lipid content increased the packing density of lipid molecules in a given space which reduced the drug release(Narala and Veerabrahma 2013). Employing more than 5% of lipid led to increasing the size of SLN in micrometer range. These results were in

agreement with previous studies (Aboutaleb, Noori et al. 2012, Mulla, Hiremath et al. 2012). Low lipid concentration (1 and 2%) led to drug deposition indicating the poor encapsulation efficiency of the drug (Kumar, Chandrasekar et al. 2007).

According to the previous results, increasing Cetyl palmitate concentration in the lipid ratio resulted in a formation of large size SLN (**Error! Reference source not found.**) and increasing Glyceryl mono-stearate concentration in the lipid ratio resulted in the formation of small size SLN.

Conclusion

The particle size of SLN depended on the type and the concentration of the surfactant and lipid. Tween 80 gave smaller particle size SLN than Poloxamer188. Increasing the surfactant concentration led to a decrease in the particle size of SLN. Tween 80 at concentration 6% was optimum to give small size SLN and the optimum concentration of Poloxamer 188 was 1% of the total formula.

Employing 5% of lipid was optimum and Glyceryl monostearate gave smaller size SLN than Cetyl palmitate but Cetyl palmitate enhanced the stability of SLN.

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