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Preparation of solid lipid nanoparticles in co-flowing microchannels

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ABSTRACT

This work presents an effective and new method for producing solid lipid nanoparticles (SLNs) with small sizes (mean diameter less than 250 nm) and relatively narrow size distribution (polydispersity less than 0.26). The preparation process was conducted in a co-flowing microchannel assembled with inner and outer capillaries. A lipid-solvent phase was injected into the inner capillary, while an aqueous phase with surfactant was injected into the outer capillary. The solvent in the lipid-solvent phase diffused into the aqueous phase when the two phases meet in the outer capillary, resulting in the local supersaturation of lipid and finally the formation of SLNs. Softisan 100 (triglyceride mixture of fatty acids with chain lengths of C_{10} to C_{18}) was used as the test lipid and SLNs were prepared in the present microchannel system under various operation conditions. The mean diameter and the size distribution of the SLNs obtained were measured by dynamic light scattering (DLS) method and the particle morphology was examined by transmission electron microscopy (TEM). The results showed that the diameter of the SLNs decreased with the increases of the aqueous phase velocity and the lipid concentration, while increased slightly with the increases of the surfactant concentration and the lipid-solvent velocity under the test conditions. The corresponding mechanisms were also analyzed and discussed.

1. Introduction

Solid lipid nanoparticles (SLNs) have attracted increasing attention in recent years as an alternative drug delivery system to the traditional emulsions, liposomes and polymeric nanoparticles systems. This carrier system has advantages of excellent biocompatibility, low toxicity, physical stability and controlled release [1–4] and it is suitable for both lipophilic and hydrophilic drugs. SLNs delivery system has found applications in pharmaceutical and cosmetic areas, such as ocular delivery [5], oral administration [6–8], epidermal targeting [9–11], RNA and protein delivery [12,13] and sunscreen in cosmetic [14–16].

Numerous researches have investigated the production approaches of SLNs and several techniques have been developed successfully, such as high-pressure homogenization, microemulsion and solvent emulsification–diffusion [1–4,17–20]. High-pressure homogenization is one of the most widely used techniques, which includes hot homogenization and cold homogenization. Hot homogenization is carried out at temperatures above the lipid melting point. It can induce the degradation of temperature sensitive drugs or compounds such as proteins and vitamins. Cold homogenization overcomes this disadvantage. However, the SLNs obtained by the cold homogenization method always have much larger sizes and a broader size distribution. Microemulsion needs high concentrations of emulsifier and solvent emulsification–diffusion technique has the problem of solvent residue. In addition, all of these methods are performed in common equipments, and therefore, it is difficult to provide a uniform fluid field for mass transfer and control the forming process of SLNs.

Microchannel has been used successfully in producing nanoparticles or microspheres [21–25]. Kobayashil et al. reported a new method to prepare lipid microspheres using microchannel emulsification and solvent evaporation. Although the diameter was in micron-scale, the lipid microspheres obtained have narrow size distributions [21]. Takagi et al. prepared titania nanoparticles using a microreactor with axle dual pipes [26]. They found that monomodal particles with narrow sizes in the range of 40–150 nm could be produced using pipes with suitable diameters. Schubert and Müller-Goymann [27] proposed a novel method using solvent injection by a micro-sized needle for preparing SLNs. The mean size

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of SLNs by this approach was found in the range of 80–300 nm. Due to the stable fluid field, effective mass transfer efficiency and precise-controlled operation conditions in these microchannel systems, nanoparticles produced in microchannel also have narrow size distributions.

In this work, we present a method to produce SLNs in a co-flowing microchannel. The present microchannel system assembled with inner and outer capillaries. A lipid-solvent phase obtained by dissolving lipid in a water-miscible solvent is injected into the inner capillary, while an aqueous phase with surfactant is injected into the outer capillary at the same time. When these two fluids meet in the outer capillary, the solvent in the lipid phase diffuses rapidly into the aqueous phase, resulting in the local supersaturation of lipid and finally formation of SLNs. The effects of the aqueous phase flow rate, the lipid-solvent phase flow rate, the lipid concentration and the surfactant concentration on the size distribution and the morphology of SLNs will be investigated experimentally and the particle formation mechanisms in this microchannel system will also be analyzed.

2. Experimental

2.1. Materials

Softisan 100 from Sasol (China) Chemical Co. Ltd. (Nanjing, China) was used as lipid. Its melting point is about 33.5–35.5 °C [25]. Poloxamer 188 from BASF (China) Co. Ltd. (Shanghai, China) was employed as the surfactant. Other chemicals used were of analytical grade from local sources. All reagents were used as received.

2.2. SLNs preparation

Fig. 1 shows schematically the experimental system. It consists of a co-flowing microchannel, two precision syringe pumps (Model 11 plus, Harvard Apparatus Inc., Holliston, USA) for supplying the aqueous phase and the lipid-solvent phase, a digital inversion microscope (CFM-500E, Shanghai Changfang Optical Instrument Co. Ltd., Shanghai, China) equipped with video camera for capturing images of flow patterns in the microchannel and a stirred unit for collecting the SLNs dispersions. The inner and external diameters of the inner capillary are 110 and 490 μm, while these values of the outer capillary are 650 and 7000 μm, respectively. The length of the outer capillary is 618 mm and the length of the inner capillary inserted in the outer capillary is about 110 mm.

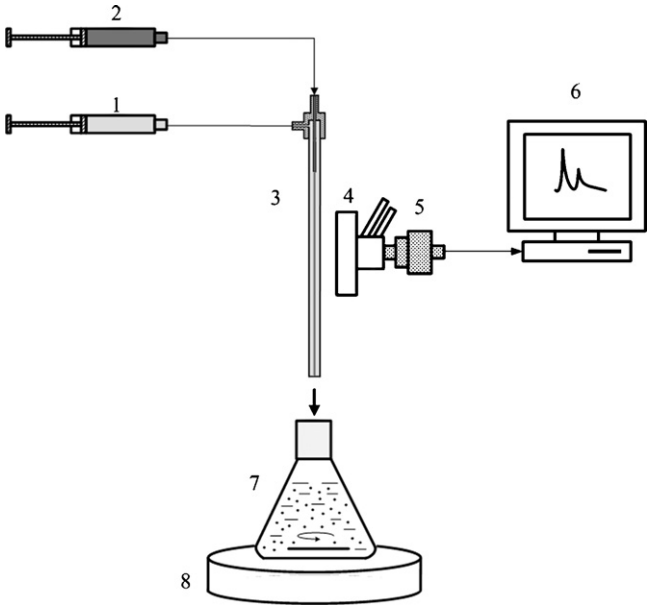

Fig. 1. The schematic diagram of experimental apparatus: (1) syringe for organic solution, (2) syringe for aqueous phase (3) microchannel, (4) digital inversion microscope, (5) video camera, (6) personal computer, (7) liquid container and (8) stirred collection unit.

The solution of Softisan 100 in acetone was pumped into the inner capillary, while at the same time the aqueous phase of poloxamer 188 was fed into the outer capillary. The flow rates of aqueous and lipid-solvent phases were both maintained at constant values in the whole preparation process. The images of flow field behaviors in microchannels were captured by a digital inversion microscope system and recorded in a personal computer. The effluent was collected in a conical flask containing 5 mL aqueous phase of poloxamer 188 and was stirred by a magnetic stir bar at low rotating speed (300 r/min) to prevent the aggregation of SLNs and keep the sample homogeneous. The sample was kept at room temperature for further analysis. The similar preparation process was performed under different conditions by varying the lipid-solvent phase velocity, the aqueous phase velocity, the surfactant concentration and the lipid concentration, respectively.

2.3. Properties of SLNs

Particle size distribution of the SLNs obtained was determined by dynamic light scattering (DLS) method using a 90 plus nanoparticle size distribution analyzer (Brookhaven Instruments Corporation, New York, USA) equipped with diode laser (658 nm) at scattering angles of 90° and the temperature was maintained at 25 °C. Morphology of the SLNs was examined using a PHILIPS CM 200 transmission electron microscopy after dropping the SLN dispersion sample on the holey copper net.

3. Results and discussion

3.1. Morphology characteristics of SLNs

The morphology characteristics of SLNs at the lipid phase velocity (U_L) of 0.05 m/s ($U_L = 4V_L/\pi D_{in}^2$, where V_L is the rate of lipid phase and D_{in} is the inner diameter of the inner capillary) and the aqueous phase velocity (U_A) of 0.15 m/s ($U_A = 4V_A/\pi D_{out}^2$, where V_A is the rate of aqueous phase and D_{out} is the inner diameter of the outer capillary), as an example, were analyzed by TEM. The lipid concentration used was 4 mg/mL and the surfactant concentration was 0.5% (w/w). Fig. 2 shows the TEM photograph obtained. The Reynolds numbers of the lipid solution flow Re_L ($=\rho_L U_L D_{in}/\mu_L$, where ρ_L and μ_L are the density and viscosity of the aqueous solution) and the aqueous phase Re_A ($=\rho_A U_A D_{out}/\mu_A$, where ρ_A and μ_A are the density and viscosity of the aqueous solution) were 12.9 and 67.3. Therefore, laminar flow was expected for both the lipid solution and aqueous phases. As can be seen, the SLNs obtained were near spherical in shape and approximately homogeneous in sizes. The particle diameters were in the range from 70 to 110 nm and the mean diameter was about 83 ± 6 nm.

3.2. Effect of aqueous phase velocity on the sizes of SLNs

The effect of the aqueous phase velocity on the properties of SLNs was examined. The SLNs preparation experiments were carried out at the aqueous phase velocities of 0.05, 0.1, 0.15, 0.2 and 0.25 m/s (the corresponding Re_A were 22.4, 44.8, 67.3, 89.7 and 112.1), respectively. The lipid-solvent phase flow rate was kept at 0.1 m/s ($Re_L = 24.3$) in all these runs. The lipid concentration was 4 mg/mL and the surfactant concentration was 0.5% (w/w).

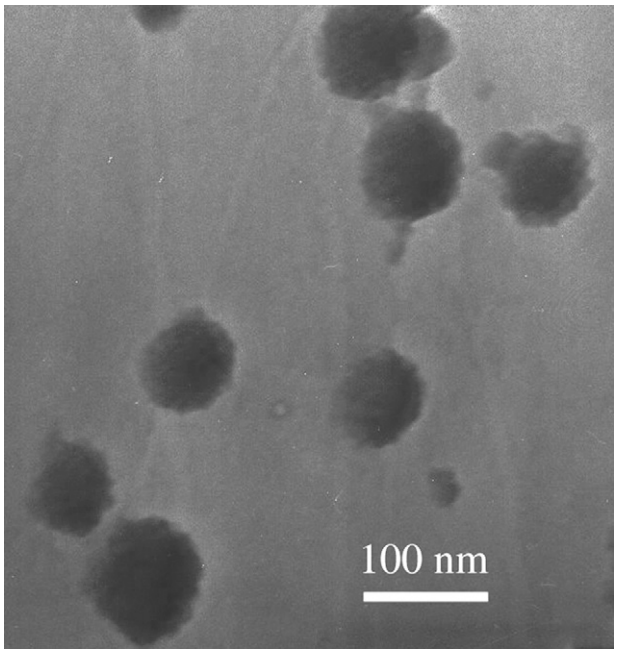

Fig. 2. Transmission electron microscopy microphotograph of the prepared SLNs.

Fig. 3 shows the size distribution of the SLNs produced at the aqueous phase velocity of 0.15 m/s. As can be seen, the particle sizes of these SLNs were about in the range from 80 to 320 nm. The mean diameter was 178 nm. Fig. 4 displays the mean diameters of the SLNs prepared at various aqueous phase velocities and the variation of the mean diameter with Re_A. It can be seen that the mean particle size decreased with the increase of aqueous phase velocity. Actually, separated flow of the inner and the outer fluids was formed at the beginning section where two liquids meet, as shown in Fig. 5. Along with the flow of these two fluids, the miscible solvent (acetone) in the lipid-solvent phase diffused into the aqueous phase, while at the same time the aqueous solvent (water) diffused into the lipid-solvent phase. Thus the spread of the inner fluid occurred at the downstream section along the outer capillary. For a given lipid-solvent phase velocity, the relative velocity of the inner and outer fluids was increased with the increase of the aqueous phase velocity. Therefore, the mass transfer rates of acetone molecules from the lipid-solvent phase into the aqueous phase were increased with the increase of the aqueous phase velocity or Re_A. The solvent in the lipid-solvent phase diffused more rapidly into the aqueous

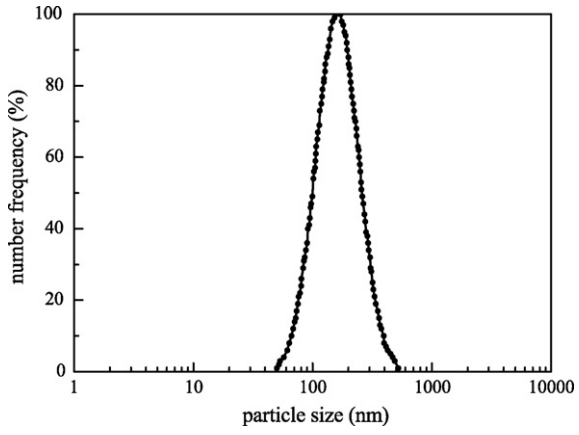

Fig. 3. Size distribution of SLNs measured by dynamic light scattering.

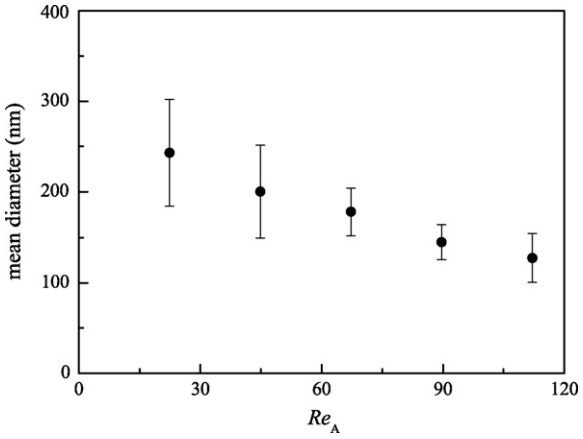

Fig. 4. Variation of the mean diameter of SLNs with Re_A.

solution at a high aqueous phase velocity than that at a low aqueous phase velocity, which accelerated the formation of smaller local super-saturation zones and consequently led to the smaller particle sizes.

3.3. Effect of lipid-solvent phase velocity on the size of SLNs

The influence of the lipid-solvent phase velocity on the properties of SLNs was also investigated experimentally. The lipid-solvent phase and the aqueous phase were the same as those used above in Section 3.2. The experiments were carried out at the lipid phase velocities of 0.01, 0.02, 0.05, 0.1, 0.2, 0.3 and 0.4 m/s (the corresponding Re_L were 2.6, 7.2, 12.4, 24.3, 48.6, 72.9 and 97.2, respectively), while the aqueous phase flow rate was maintained at 0.15 m/s ($Re_A = 67.3$). Fig. 6 shows the variation of the mean diameters at different lipid-solvent phase velocities with Re_L. It can be seen that the mean diameter increased slightly with the increase of Re_L. In fact, the averaged velocities of the lipid solution and the aqueous phase were different and varied from the meeting point along the downstream due to the variation of the flowing cross-areas. On one hand, for a given aqueous phase velocity and low lipid solution velocities (for example, $U_L < 0.2$ m/s or $Re_L < 48.6$ in this work) the increase of lipid velocity caused the decrease of relative velocity of the flow

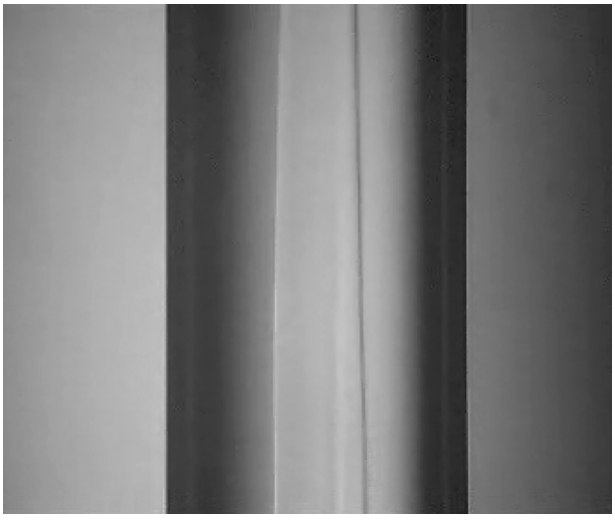

Fig. 5. Microscope image of the flow behaviors of the lipid-solvent and aqueous phases near the outlet of the inner capillary.

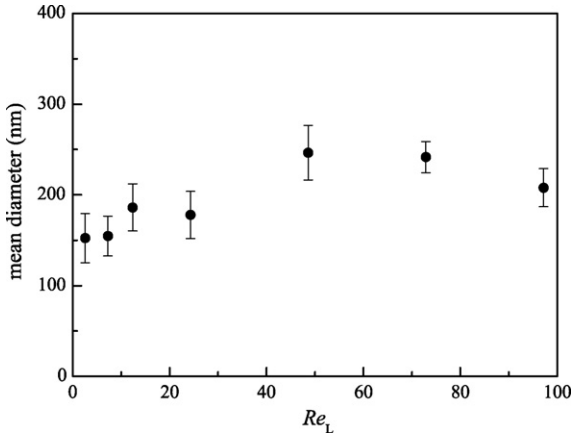

Fig. 6. Variation of the mean diameter of SLNs with ReL.

streams and induced the slight decrease of mass transfer rates of solvent. Thus, an increase of particle diameter was observed. However, when the lipid phase velocity or Re_L exceeded certain value (for example, U_L > 0.2–0.4 m/s or Re_L > 48.6–97.2), the increase of the relative velocity of the two streams with the increase of the lipid phase velocity or Re_L led to a slight decrease of the mean diameter. On the other hand, more amounts of the miscible solvent at a given moment needed to be transferred into the aqueous phase at a high lipid-solvent phase velocity compared with those at a low lipid-solvent phase velocity. This effect was dominated to the solvent transport compared with the positive contribution of the increase of velocity itself. Thus the complex trend of the diameter with Re_L was observed.

3.4. Effect of lipid concentration on the size of SLNs

Preparation of SLNs was also carried out using the aqueous solution with the surfactant concentration of 0.5% (w/w) at different lipid-solvent phases (2, 4 and 8 mg/mL). The aqueous phase velocity was maintained at 0.15 m/s (Re_L = 67.3) and the lipid phase velocity was kept at 0.05 m/s. The obtained values of the mean diameters were summarized in Table 1. It is observed that the mean diameters of SLNs were 208, 186 and 168 nm at the lipid concentrations of 2, 4 and 8 mg/mL, respectively, i.e., decreased slightly with the increase of the lipid concentration under the tested conditions. However, the diameter variation range was smaller compared with those obtained by changing velocities, indicating the weak effects of the lipid concentration on the particle diameter under the test conditions. The reason may be the fact that the increase of the lipid concentration could induce the easier formation of the smaller local lipid supersaturation zones and thus the decrease of the mean diameters of SLNs.

Table 1
The mean diameters of SLNs prepared under various lipid and surfactant concentrations (the aqueous and lipid-solvent phase velocities were 0.15 and 0.05 m/s)

Lipid concentration (mg/mL)	Surfactant concentration (%, w/w)	Mean diameter (nm)	Polydispersity
2	0.5	208 ± 23	0.168 ± 0.070
4	0.5	186 ± 22	0.216 ± 0.064
8	0.5	168 ± 21	0.251 ± 0.029
4	0.1	157 ± 26	0.210 ± 0.070
4	0.8	186 ± 55	0.212 ± 0.098
4	1.2	219 ± 41	0.131 ± 0.087

3.5. Effect of surfactant concentration on the size of SLNs

The experimental results of the mean diameters prepared using the aqueous fluid with surfactant concentrations of 0.1, 0.5, 0.8 and 1.2% were also summarized in Table 1. It was found that the particle sizes produced under different surfactant concentrations were changed in the range of 157–219 nm. Unlike those in the solvent injection method using a micro-sized needle by Schubert and Müller-Goymann [27], the mean diameter of SLNs prepared in the present capillary system increased slightly with the increase of poloxamer concentration. The possible reason is that the viscosity of the aqueous phase increased with the increase of the surfactant concentrations, which weakened the mutual diffusion of acetone and water. Thus smaller local lipid supersaturation zones were formed with the decrease of surfactant concentration, which resulted in the decrease of the diameters of SLNs.

4. Conclusions

SLNs can be successfully prepared in the present co-flowing microchannel. This is a simple and easy approach to produce SLNs with small diameters and slight narrow size distribution. The particle diameter was influenced by several factors in the present system, i.e., the velocities of the lipid-solvent and the aqueous phases, the lipid concentration and the surfactant concentration. It was found that the mean diameter of the SLNs decreased with the increases of the aqueous phase velocity and the concentration of Softisan 100, while increased slightly with the increases of the concentration of poloxamer 188 and the lipid-solvent velocity in most conditions. Among them, the velocity plays strong effects on the diameters of SLNs, while the concentrations of the lipid and surfactant have weak influences on the sizes of SLNs under the present test conditions. Therefore, by controlling these operation conditions SLNs with expected properties of small diameters and narrow size distribution can be produced by this method.

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