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Studies on crystallinity state of puerarin loaded solid lipid nanoparticles prepared by double emulsion method

Zhen Li · Li Yu · Liqiang Zheng · Fei Geng

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Abstract In this work, solid lipid nanoparticles (SLN) have been prepared from water-in-oil-in-water double emulsion, using monocaprate as solid lipid, sorbitan monooleate (Span 80) and polyoxyethylene sorbitan monolaurate (Tween 20) as emulsifier, and puerarin as target drug. The morphology of SLN with drug loaded or not was investigated by the transmission electron microscope (TEM). The crystal order and structure of particles were studied by differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD), respectively. The results indicate that the diameters of SLN with puerarin inside are larger than those without drugs. The analysis of WAXD and DSC shows that the state of crystallinity SLN prepared by double emulsion method was worse than that of SLN prepared by microemulsion. And also the drug-loaded SLN presents a less ordered crystallinity than the drug-free SLN. But both the drug-free and drug-loaded SLN exist in an amorphous state. The reasons of the phenomenon have been discussed.

Keywords Double emulsion · Puerarin · Solid lipid nanoparticles

Introduction

Solid lipid nanoparticles (SLN) consist of solid lipids in nanosized range dispersed in aqueous medium have attracted increasing attention in recent years as an alternative drug delivery system to the traditional emulsions, liposomes and polymeric nanoparticles systems [1, 2]. However, there were some problems for SLN which is prepared by traditional way: microemulsion method. On one hand, microemulsion method using O/W microemulsion which leads to limited capacity of some water-soluble drugs, because during the process of emulsification, the water-soluble drugs tend to escape from oil phase to aqueous phase. On the other hand, as a drug carrier with solid lipid, it is easy to lead to drug expulsion [3]. SLN prepared by double emulsion method (W/O/W), can improve the ability of encapsulation of water-soluble drug such as protein [4, 5]. Because of the complex formulation of surfactants doped with lipid, the crystallinity decreases, and theoretically it can be loaded with more drugs.

Puerarin (structure shown in Fig. 1), is one of the major isoflavonoid compounds isolated from the root of the wild leguminous creeper, *Pueraria lobata* (Wild) Ohwi which has been used in traditional Chinese medicine for centuries [6]. It exhibits a great variety of biological and pharmacological activities. Previous studies showed that puerarin may be used clinically for improvement of blood circulation, prevention of cardiovascular diseases such as antihypertension, anti-arteriosclerosis, and dilating coronary arteries, decreasing myocardial oxygen consumption and improving microcirculation in both animals and humans suffering from cardiovascular disease [7–9].

In the present work, double emulsion technique was employed as the method to prepare SLN. Puerarin was chosen to add into these nanoparticles. To investigate the effect of drug on the morphology of SLN, nanoparticles loaded with or without puerarin. The crystalline states of these two kinds of SLN were studied by DSC and WAXD. In addition to this, the crystallization states of SLN prepared by the method of double emulsion and microemulsion were also investigated by DSC.

Z. Li · L. Yu (✉) · L. Zheng · F. Geng
Key Laboratory of Colloid and Interface Chemistry, Shandong University, Ministry of Education, Jinan 250100, China
e-mail: ylmlt@sdu.edu.cn

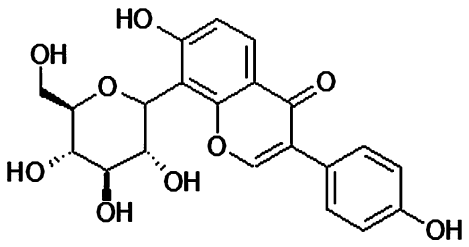

Fig. 1 Structure of puerarin

Experimental

Materials

Monocaprate (1-monocaprate glycerol, MC, purity >95%) was obtained from Danisco Cultor (Denmark). Tween 20 (polyoxyethylene sorbitan monolaurate, analytical reagent) was purchased from Beijing Medicinal Co. (China) and Span 80 (sorbitan monooleate, analytical reagent) from Tianjin Shentai Medicinal Co. (China). Puerarin was provided by Unilever Co., Ltd. (China). All materials were used directly without further purification. Water used was doubly distilled and deionized.

A double emulsion is usually prepared in two main modes: one-step emulsification and two-step emulsification [10–13].

In this paper, the two-step emulsification procedure was chosen to prepare SLN. Briefly, 0.023 g of puerarin and 0.593 g of MC were accurately weighed and mixed in sealed glass bottle. The mixture was stirred and heated up to 60 °C which was above the melting point of the lipid in order to form the primary microemulsion (W/O). Then 0.593 g of Span 80 and 0.213 g of deionized water were added into the bottle stirring for 40 min at the same temperature, and a clear oil-in-water microemulsion was obtained. After that, the primary emulsion was dispersed in the outer aqueous phase, containing 0.024 g of Tween 20 at 60 °C. The mixture was gently magnetically stirred for 30 min. A clear W/O/W double emulsion was obtained. The SLN dispersions were obtained by dispersing the warm (60 °C) W/O/W microemulsion into cold distilled water (2–3 °C) under mild mechanical mixing at a ratio of 1:20 (microemulsion: water, v/v) for 30 min. Then the SLN dispersions were dialyzed twice with water using a membrane with cut off 10,000–12,000 Da in an ultrasonic cleaning tank in order to eliminate a large proportion of the surfactant molecules to obtain the emulsion.

Preparation of SLN with the microemulsion method

In this paper, the preparations of SLN with microemulsion method were carried out according to the previous reports [14–17]. Briefly, to form the microemulsion (O/W), 0.300 g MC was weighed in sealed glass bottles stirring to heat up to 60 °C. Then the solution was dispersed into 1.200 g distilled water containing 0.500 g Tween 20 and mixed under magnetic stirring for several minutes at the same temperature to form the microemulsion. The SLN dispersions were obtained under the same condition with the double emulsion method.

Preparation of freeze-dried SLN

Freeze-dried SLN were obtained by freezing SLN suspensions in an aqueous solution at −25 °C overnight, and then the samples were transferred to a freeze-dryer (FD-1000, Tokyo Rikakikai Co., Japan) at −50 °C for 72 h. Thus SLN powders were obtained.

Characterization

Transmission electron microscopy

The particle sizes of the SLN were determined by transmission electron microscopy (TEM,JEM-100 CX II, JEOL Ltd.). Transmission electron microscopy was performed using a negative-staining method. A drop of the SLN dispersion was spread on a 200-mesh copper grid coating and the excess droplets were removed with filter paper. After 5 min, a drop of 2 mass% uranyl acetate in ethanol was placed onto the copper. The grid was dried at room temperature and then observed by TEM. The images were taken with $\lambda = 0.037$ Å at an acceleration voltage of 100 kV.

Differential scanning calorimetry (DSC)

The state of crystallinity of the lipid nanoparticles was determined with a differential scanning calorimeter (DSC, Perkin-Elmer, America). For DSC measurements, standard aluminum pans with accurately weighed 2–5 mg samples were tightly sealed. Baselines were determined using an empty pan, and all the thermograms were baseline-corrected. Samples were heated at the scanning rate of 5 °C/min in the temperature range from 0 to 100 °C. Transition temperatures were determined from the endothermic peak minima while transition enthalpies were obtained by integration of the endothermic transitions using linear baselines.

Wide angle X-ray diffraction (WAXD)

Crystalline structures of the particles were investigated using a WAXD (D8 Advance, Bruker Axs, Germany).

Diffractograms were performed from the initial angle $2\theta = 10°$ to the final angle $2\theta = 60°$. A Cu Kα radiation source was used, $\lambda = 1.5418$ nm, and the scanning rate was 0.3 S/step.

Results and discussion

Particle morphology

Figure 2 shows the TEM images of SLN without or with puerarin loaded. It can be seen from Fig 2 that both SLN with and without drug loaded are in the similar morphology. Both of them are spherical in shape and even in the distribution of particle diameter on the whole. There is no obvious aggregation. It indicates that the system made from double emulsion is monodispersed. According to these images, the diameters of SLN without and with puerarin loaded are about 80–90 nm and 130–150 nm, respectively. In previous literature, the incorporation of drug into nanoparticles always leaded to increase of their size compared with the blank nanoparticles [18, 19]. Based on the above results, the addition of puerarin enable to increase the size of nanoparticles which shows the drug indeed was loaded on SLN.

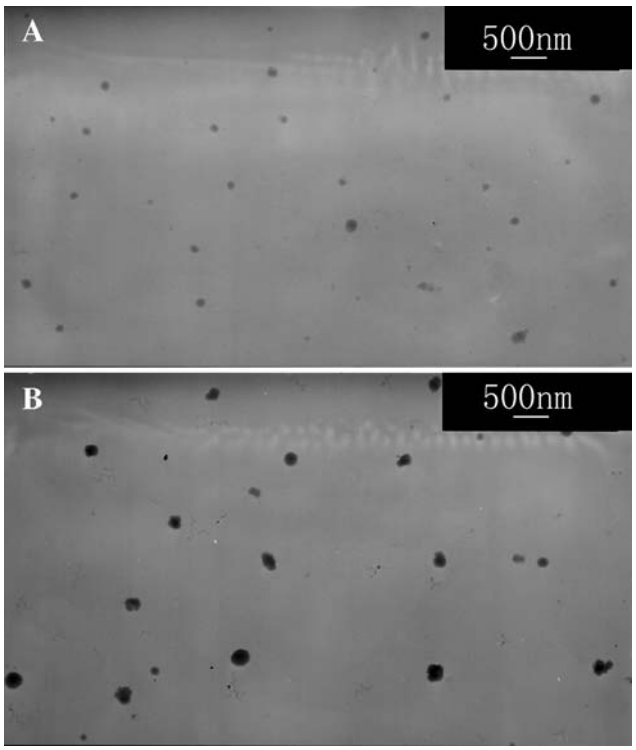

Fig. 2 TEM images of SLN **a** SLN without puerarin loaded, **b** SLN with puerarin loaded

Differential scanning calorimetry

Differential scanning calorimetry was extensively used to investigate the crystallization behavior of crystalline materials [20–22]. And thermoanalytical studies were used to study the SLN in this work. During the experiment, in order to investigate the different effect on the state of crystallization behavior of SLN prepared by double emulsion method and microemulsion method, the freeze-dried of SLN was used for DSC experiment. And also the SLN with or without puerarin were studied.

Figure 3 presents the DSC thermograms of the SLN prepared by double emulsion, microemulsion and pure MC. The pure MC exhibits a maximum peak at 50.4 °C. SLN exhibits a maximum peak at 36.8 °C by microemulsion method, while shows a weaker peak at about 24.3 °C. When the pure MC was turned into SLN, the melting point was depressed whether the SLN prepared by double emulsion method or microemulsion method. The onset temperatures of MC-SLN prepared by microemulsion method and SLN of double emulsion method are, respectively, 44.9 °C, 27.9 °C, 34.3 °C. The decrease of maximum and the onset temperature can be attributed to the small size effect and the interactions of Mc with emulsifier, which were explained by the Thomson equation [23]. The melting enthalpies for MC-SLN prepared by microemulsion method and double emulsion method are 376.80, 101.61, and 70.72 J/g, respectively. The SD value is 1% for the enthalpy. It can be seen from Fig. 3, compared to the DSC thermograms of the pure lipid, there is a sharp decline in enthalpy from the pure lipid to the nanoparticles. For less ordered crystals or amorphous solids, the melting of the substance requires much less energy than crystalline substances which need to overcome lattice forces [24].

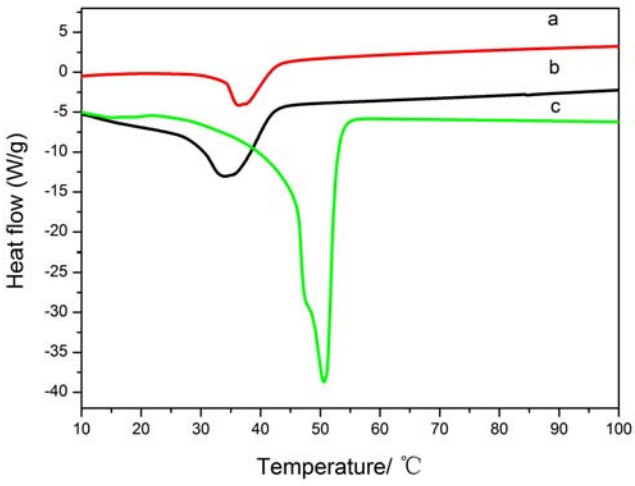

Fig. 3 DSC curves **a** SLN prepared by double emulsion method; **b** SLN prepared by microemulsion method; **c** MC

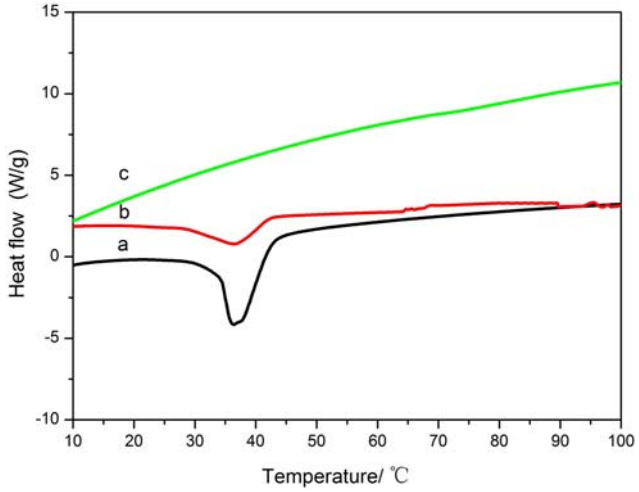

Fig. 4 DSC curves **a** drug-free SLN, **b** drug-loaded SLN, **c** puerarin

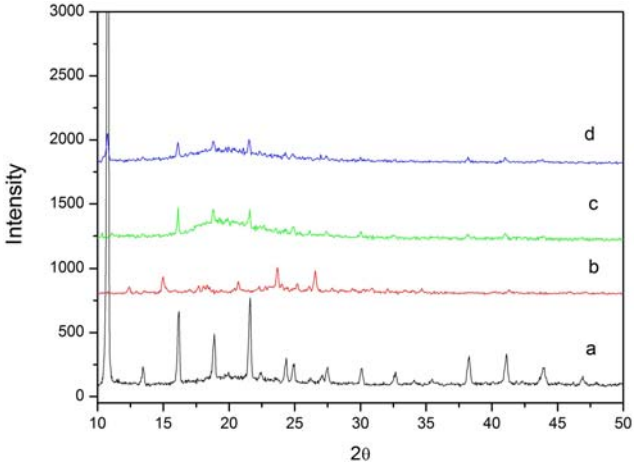

Fig. 5 WAXD pattern **a** MC, **b** Puerarin, **c** SLN prepared by microemulsion method, **d** SLN prepared by double emulsion method

Therefore, the lower melting enthalpy values suggest lower ordered lattice arrangements and vice versa. As a result, it can be concluded that the lipid within the nanoparticles should be in a less ordered arrangement compared to the pure MC. In addition, the SLN of double emulsion method is less ordered crystal according to the DSC analysis, because SLN of double emulsion is prepared by two different kinds of surfactants while the other is prepared by only one kind. Further more, a less ordered crystal lipid matrix is favorable for encapsulating more drug molecules.

In order to investigate the effect of the drug loaded on SLN, the DSC curves of puerarin, SLN with and without drug (Fig. 4) were determined. After computation, the melting enthalpy after adding puerarin is 52.39 J/g which is lower than that of SLN without loaded drugs (70.72 J/g). It manifests that when the drugs are added into SLN, the lipid crystals in an orderly situation have been further disrupted which can reduce the crystallization property of nanoparticles. Therefore, the crystallinity of the lipid gradually declines from MC to SLN loaded with drugs.

Wide angle X-ray diffraction

The inner structures of the SLN were studied by X-ray diffraction [25, 26]. The lipid crystalline structure, which is related to the chemical nature of the lipid, is a key factor that can determine whether the liquid oil is expelled or firmly incorporated into a carrier system [3]. It is proposed that the structure of less ordered arrangement in the nanoparticles would be beneficial to increase the drug payload [24]. In order to investigate the changes of the microstructure in the lipid crystallization process, X-ray diffraction experiments were performed. Diffractograms of the lyophilized SLN as well as the raw materials (pure MC and puerarin) are displayed in Fig. 5. The diffraction curve of pure MC is different from that of SLN and puerarin. The diffraction patterns of pure MC show remarkable differences from those of SLN loaded or unloaded drug. The diffractograms of MC exhibit sharp peaks at 2θ scattered angles 10.7°, 16.2°, 18.8° and 21.5°, indicating the crystalline nature of MC. Compared with the pure lipid MC, the peak intensities of the SLN without drugs are weaker, which indicates that the crystallinity state in these particles is worse than that of pure MC. It can be seen that the addition of the puerarin has not changed the position of the signals of MC but weaken their intensity. This difference can be ascribed to the existence of puerarin that has destroyed the crystallize process of monocaprate.

Conclusion

Double emulsion method was employed to prepare the SLN. The experimental results reveal that drug-loaded SLN are similar to the drug-free SLN in shape, but the particle size is larger than that of the drug-free SLN. DSC and WAXD experiments show that the particles made by double emulsion method are less ordered or amorphous, which indicates that the SLN prepared by double emulsion method may have a good drug-loaded capacity. After adding drugs, the lipid presents a worse state of crystallinity because of the destruction of the crystallization of solid lipid.

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