International Journal of Pharmaceutics 376 (2009) 116–122

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

# Micronization of silybin by the emulsion solvent diffusion method

Zhi-Bing Zhang[a], Zhi-Gang Shen[a,*], Jie-Xin Wang[a], Hai-Xia Zhang[a], Hong Zhao[a], Jian-Feng Chen[b,**], Jimmy Yun[c]

[a] Sin-China Nano Technology Center, Key Lab for Nanomaterials, Ministry of Education, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] Research Center of the Ministry of Education for High Gravity Engineering and Technology, Beijing University of Chemical Technology, Beijing 100029, PR China
[c] Nanomaterials Technology Pte. Ltd., 28 Ayer Rajah Crescent #03-03, Singapore 139959, Singapore

ARTICLE INFO

*Article history:*
Received 3 March 2009
Received in revised form 22 April 2009
Accepted 23 April 2009
Available online 3 May 2009

*Keywords:*
Silybin
Emulsion solvent diffusion
Poorly water-soluble drug
Amphiphilic compound
Morphology

ABSTRACT

Micronized silybin particles were successfully prepared by emulsion solvent diffusion method. Uniform spherical and rod-shaped particles with a mean size of 2.48 and 0.89 μm could be obtained using sodium dodecyl sulfate (SDS) concentration of 0.1 wt% at 30 and 15 °C, respectively. The characterization of silybin particles by SEM and particle size distribution (PSD) indicated that with the increase of temperature from 15 to 30 °C, the as-prepared particles became bigger and had a tendency to turn into spherical shapes; with the increase of SDS concentration from 0.02 to 0.1 wt%, the span of PSD became narrower while the mean particle size kept almost unchanged. XRD patterns and FT-IR spectra showed that the spherical and rod-shaped silybin particles possessed decreased crystallinity; however, the chemical structure and components were similar to those of the commercial silybin powder. Dissolution tests demonstrated that both of the spherical and rod-shaped silybin particles exhibited significantly enhanced dissolution rate when compared to the commercial silybin powder.



## 1. Introduction

Silybin is a main biologically active component in silymarin, which is an antihepatotoxic polyphenolic substance isolated from the milk thistle plant named silybum marianum, and it has been widely used as a therapeutic agent for a variety of acute and chronic liver diseases (Flora et al., 1998; Kvasnicka et al., 2003). However, the therapeutic effects of silybin are discounted by its extremely poor aqueous solubility, which results in poor oral absorption and bioavailability (Pepping, 1999; Wachter and Zaeske, 2000). To solve this problem, several approaches such as formation of silybin–phospholipid complex (Xiao et al., 2006), silymarin solid dispersions (Sun et al., 2008), silymarin encapsulated liposomes (El-Samaligy et al., 2006), silymarin self-microemulsifying drug delivery system (Wu et al., 2006) and so on, have been employed to improve the dissolution rate of silybin or silymarin thus enhancing its bioavailability.

In fact, for biopharmaceutic class II drugs, the bio-absorption process is rate-limited by dissolution in gastrointestinal fluids. According to the Noyes–Whitney equation, the dissolution rate of poorly water-soluble drugs could be increased by reducing the particle size to the micro- or nano-scale thus increasing the interfacial surface area (Müller and Peters, 1998; Douroumis and Fahr, 2006). The conventional approaches to produce untrafine drug particles can be divided into top-down and bottom-up techniques (Keck and Müller, 2006; Rabinow, 2004). In the case of top-down techniques which include jet-milling, pear/ball milling and high-pressure homogenizing, the bulk drugs are comminuted into micro- or nano-sized range by the use of mechanical force (Rasenack et al., 2004). However, these techniques need high energy input and exhibit some disadvantages in practice such as contamination of drugs, variation of crystal structures, uncontrolled particle morphology, and broad particle size distribution (Keck and Müller, 2006; Kharb et al., 2006). In the last decade, bottom-up techniques that rely on dissolving the drug in a solvent and precipitating it by the addition of a non-solvent, like supercritical fluid (SCF) technique and liquid precipitation, have been widely investigated to obtain ultrafine drug particles, such as cephradine (Zhong et al., 2005), cefuroxime axetil (Zhang et al., 2006), danazol (Rogers et al., 2002; Zhao et al., 2007), ibuprofen (Rasenack et al., 2004), etc. Silybin particles with a mean size of about 10 μm could be prepared by SCF technique (Wei, 2008). However, this SCF technique needs enormous production costs, and is difficult to control and scale-up.

Emulsion solvent diffusion (ESD) method, proposed by Kawashima et al. (1989a,b) and developed from the spherical crystallization technique (Kawashima et al., 1982), is an effective way to prepare drug-loaded polymeric micro/nanoparticles for mask-

* Corresponding author. Tel.: +86 10 64447274; fax: +86 10 64423474.
** Corresponding author. Tel.: +86 10 64446466; fax: +86 10 64434784.
*E-mail addresses:* shenzg@mail.buct.edu.cn (Z.-G. Shen), chenjf@mail.buct.edu.cn (J.-F. Chen).

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doi:10.1016/j.ijpharm.2009.04.028

ing taste, controlled release, drug targeting, etc. (Gao et al., 2006; You et al., 2006; Tsujimoto et al., 2007). In the usual applications of ESD method, drug and polymer are dissolved in a suitable solvent with or without a bridging liquid. The solution is then added into an aqueous medium (as a poor solution) under stirring and the emulsion droplets are immediately formed in the external poor solution. As the ESD proceeds, the solvent diffuses out of the droplets and water diffuses into the droplets. Therefore, the drug and polymer are co-precipitated, leading to the solidification of emulsion droplets. Using ESD method, silybin polymeric particles with a mean size of about 800 μm could be produced (Hu et al., 2006). However, so far, very little attention has been paid to the micronization of drugs through ESD method (She et al., 2007; Makhlof et al., 2008), and no mechanism has been developed to explain why ESD method can be applied to produce ultrafine drug particles.

Based on the fact that silybin emulsion could be formed through the mixing of drug solvent and aqueous solution, the aim of this study was to prepare ultrafine silybin particles utilizing ESD method. In this study, no bridging liquid was introduced and the as-prepared silybin particles exhibited a significantly enhanced dissolution rate. In addition, the possible particle formation mechanism was given. The experimental parameters were also investigated, and the resulting silybin particles were characterized in detail.

## 2. Materials and methods

### 2.1. Materials

Silybin (purity: 98.6%) was supplied by Panjin Huacheng Pharmaceutical Co., Ltd. (Liaoning, China). Tween-80 and acetone were of analytical grade and obtained from Chemical Reagent Company (Beijing, China). SDS was purchased from Biodee Biotechnology Co., Ltd. (Beijing, China). Methanol (HPLC grade) was provided by FisherChemical (New Jersey, USA). Deionized water was purified by Hitech-K Flow Water Purification System (Hitech instruments Co., Ltd., Shanghai, China).

### 2.2. Methods

#### 2.2.1. Preparation of silybin particles

Silybin was dissolved in acetone, and the solution was filtered through a 0.45 μm nylon membrane to remove the solid impurities. Afterwards, 10 ml of the drug solution (20 mg/ml) was poured into 100 ml deionized water containing 0.01–0.10 wt% SDS under magnetic agitation (1000 rpm). Immediately after pouring the solution into water, the system turned opalescent. The mixture was stirred for 1 min and then stored for about 6–10 min till the translucent emulsion droplets turned into opaque particles. The solidified particles were recovered by filtration and washed with deionized water. The resultant filter paste was dried in an oven at 60 °C for 12 h. All experiments were carried out in triplicate.

#### 2.2.2. Scanning electron microscopy (SEM) and particle size distribution (PSD)

The morphology of the silybin samples was examined using a model JSM-6360LV scanning electron microscopy (SEM) system (JEOL, Japan). The dry powder or a glass slide with sample was fixed on an aluminium stub using double-sided adhesive tape and sputter coated with gold. The particle size and distribution were determined using Image-Pro Plus software (release 5.0, MediaCybernetics, USA) via the obtained SEM photographs. The width of rod-shaped particle or the diameter of spherical particle was prescribed as the specific particle size. At least 500 particles were measured. Gauss fitting curves were also constructed to give an apparent illustration for PSD comparison.

#### 2.2.3. Drug content analyses

HPLC (Waters Corporation, Miford, MA, USA) was used to determine silybin contents of the as-prepared samples. The stationary phase, $C_{18}$ column (150 mm × 4.6 mm, 5 μm particle size), was kept at 25 °C. The mobile phase was a mixture of methanol–water–glacial acetic acid (48:52:1). The flow rate was 1.0 ml/min. Effluent was monitored at 287 nm, and as silybin is an isomeric compound, two peaks can be detected at about 12 and 13 min. Silybin concentrations were calculated according to the calibration curves which were constructed by plotting peak areas of silybin versus silybin concentrations and were linear over the concentration range of 0.3–30 μg/ml ($R^2$ = 0.9999). The data reported was the average of three measurements.

#### 2.2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded with a Nicolet model 8700 spectrometer (Nicolet thermo electron instrument corporation, USA) in the range of 400–4000 $cm^{-1}$ using a resolution of 2 $cm^{-1}$ and 32 scans. Samples were diluted with 1% of KBr mixing powder and pressed to obtain self-supporting disks.

#### 2.2.5. X-ray diffraction studies (XRD)

X-ray diffraction analysis was performed using XRD-6000 diffractometer (Shimadzu Inc., Japan) to detect any changes in the physical characteristics and crystallinity. The measuring unit consisted of a rotating anode in transmission technique with a specification that Cu Kα1 radiation was generated at 30 mA and 40 kV. Sample powder was grounded and placed in an aluminium sample holder. The scanning speed was 5°/min from 5° to 50° with a step size of 0.05°.

#### 2.2.6. Specific surface area

The specific surface area was measured using $N_2$ adsorption method. In this method, calculation was implemented by Surface Area Analyzer ASAP 2010-M (Micromeritics Instrument Corporation, USA) based on the BET equation. Before measuring, sample powder was degassed for at least 4 h.

#### 2.2.7. Physical stability studies

Dry powder of each silybin sample was sealed in polyethylene bag and re-dispersed uniformly in deionized water (1 mg/ml), respectively. All resulted test samples were stored under room conditions (temperature: 15–25 °C, relative humidity: 30–60%), suspended samples were collected at different times for SEM characterization, while powder samples were collected for SEM and XRD analysis.

#### 2.2.8. Dissolution testing

Dissolution testing for drug powder was carried out using a dissolution apparatus (D-800LS, Tianjin, China) following the USP Apparatus II (paddle) method. Paddle speed and bath temperature were set at 100 rpm and 37.0 ± 0.5 °C, respectively. A 0.5 wt% Tween-80 aqueous solution was employed as the dissolution medium, and the dissolution rate tests were performed under sink conditions. Each drug powder (30 mg) was placed into a vessel containing 900 ml dissolution medium. The samples (1 ml) were withdrawn at specific time intervals and immediately filtered through a 0.45 μm syringe filter. Then the filtrate was injected onto a HPLC column and detected at a wavelength of 287 nm. Each powder sample was characterized in triplicate.

## 3. Results and discussion

### 3.1. The possible particle formation mechanism

In previous reports on the applications of the ESD method, the mechanism for emulsion droplet formation has not been given. In this paper, a postulation on the particle formation mechanism was made. Silybin molecule contains hydrophilic parts (hydroxyl groups and hydroxymethyl group) and hydrophobic parts (oxobenzopyranyl group and methoxyphenyl group), and can be generally regarded as an amphiphilic drug. After silybin is dissolved in a good solvent and subsequently poured into the aqueous solution, the hydrophilic parts of silybin tend to align with the aqueous phase, whereas the hydrophobic parts are repelled from the aqueous phase. Accordingly, the silybin molecules, which exist in the contact surface of water-miscible solvent and water, spontaneously assemble into bilayers (Antonietti and Förster, 2003; Seo et al., 2006). As a result of the tendency to minimize the surface energy, these bilayers close up into small vesicles in which the good solvent and silybin molecules are enriched. Hence, small emulsion droplets are formed. Once the droplets are deposited on glass slide by solvent evaporation, uniform nanospheres with a mean size of ~240 nm appear (as shown in Fig. 1). As the solvent system is miscible, the good solvent and water counter-diffuse out of and into the droplets, respectively (Ré and Biscans, 1999). Meanwhile, these small droplets can coalesce into big droplets due to the collision and interfacial tension variation (Toyota et al., 2006; Kawashima et al., 1995). With the progression of solvent diffusion, silybin in the droplets becomes supersaturated, leading to the crystallization of silybin. Therefore, the droplets are gradually solidified to form uniform particles.

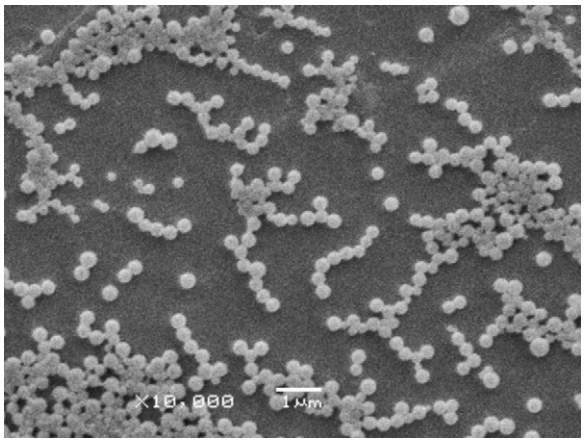


**Fig. 1.** SEM image of droplets deposited on glass slide by solvent evaporation.

### 3.2. Effect of temperature

SEM images of the commercial silybin powder and the particles obtained at different temperatures were shown in Fig. 2. The commercial silybin powder showed irregular-shaped particles with a wide particle size distribution from 1 to 40 μm. Comparatively, the particles prepared by ESD method at 15, 23 and 30 °C appeared to be uniform short rods, irregular spheres and monodispersed spheres respectively and the mean particle sizes obtained at different temperatures are 0.89, 1.74 and 2.48 μm, respectively. This suggests that the particle size and morphology were strongly dependent on the processing temperature. There are several reasons which

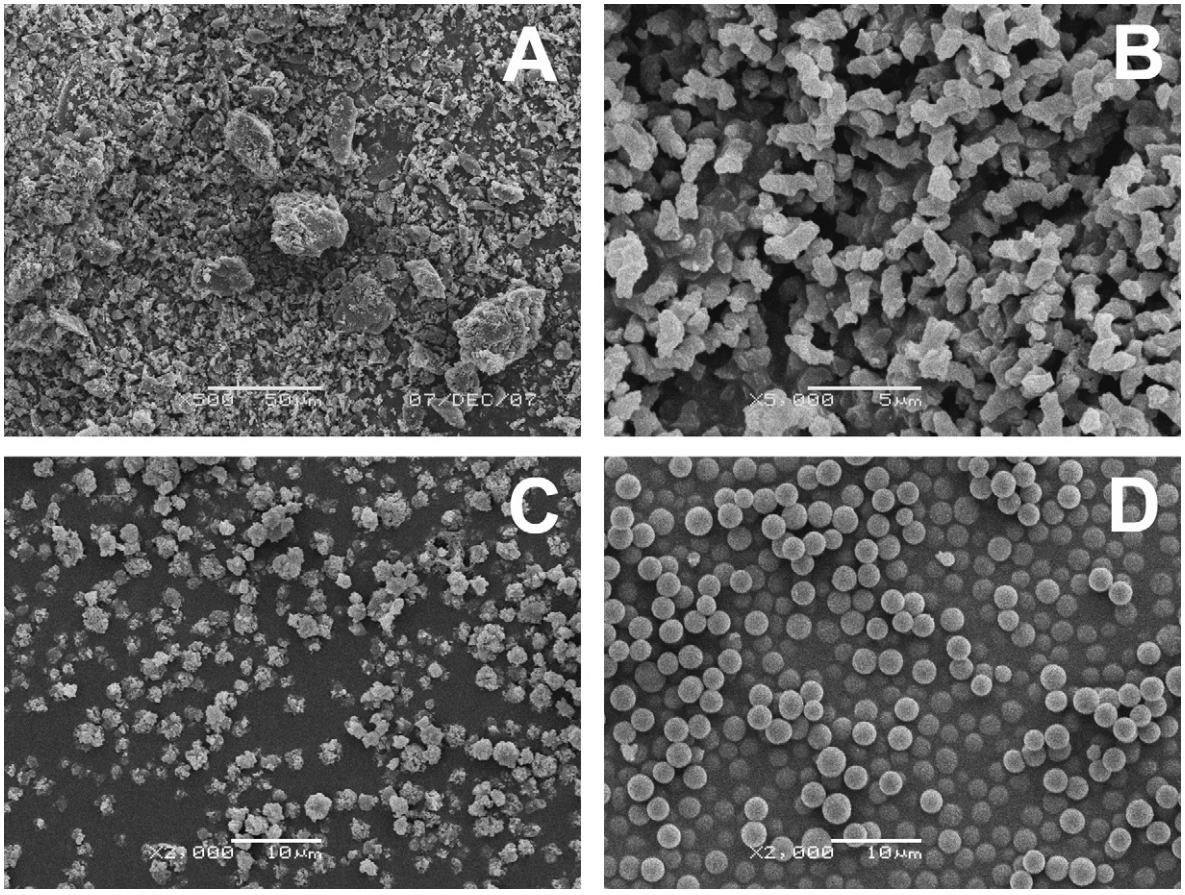


**Fig. 2.** SEM images of (A) commercial silybin powder and particles prepared at different temperatures (SDS concentration: 0.10 wt%): (B) 15 °C; (C) 23 °C; (D) 30 °C.

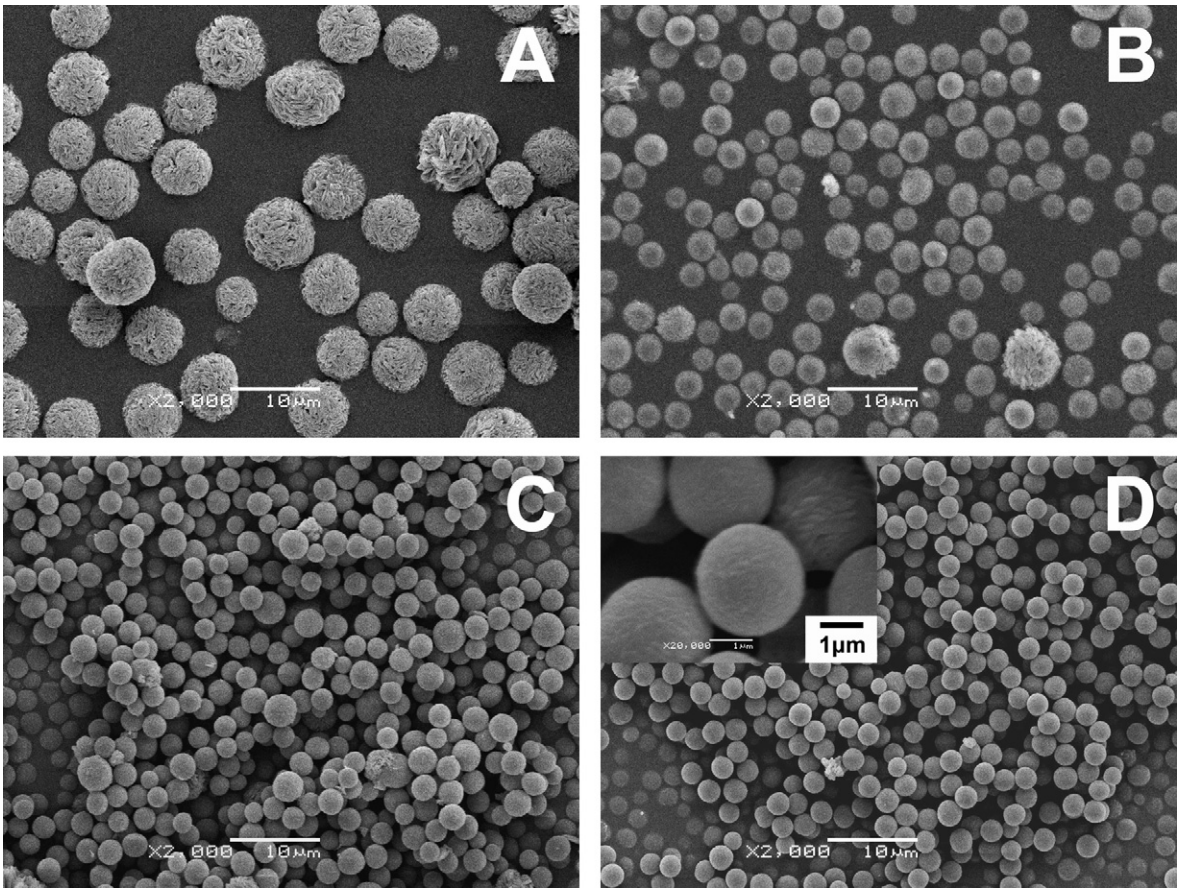


**Fig. 3.** SEM images of silybin spherical particles prepared at 30 °C under different SDS aqueous solutions: (A) 0.00 wt%; (B) 0.01 wt%; (C) 0.02 wt%; (D) 0.10 wt%.

might be responsible for this. Firstly, the interfacial tension of the droplets increased due to the decreasing density of SDS adsorbed on the droplet surface with the increase of temperature. This could cause the droplets to coalesce into bigger ones. Secondly, the kinetic energy of the droplets was increased with the increase of temperature, resulting in higher opportunity and intensity of the mutual collision, which promoted the coalescence of the droplets. Thirdly, because of the temperature dependence of diffusion, the droplets could be consolidated in a non-spherical shape at lower temperature (Cui et al., 2003). Consequently, rod-shaped silybin particles were formed owing to the low diffusion rate. In addition, it was worth noting that when the temperature was below 5 °C, it took much longer time (>60 min) for the semi-transparent mixed solution to become opaque. However, the resultant particles were almost the same with those obtained at 15 °C.

### 3.3. Effect of SDS concentration

Different SDS concentrations were investigated to understand the effects of SDS on particle size and morphology, and SEM images of resultant particles were shown in Fig. 3. Without the addition of SDS, relatively big spherical particles with a mean size of 6.68 μm and coarse surface were obtained. In contrast, smaller spherical particles with smooth surface could be achieved with the use of SDS, and the mean sizes of particles obtained under three SDS concentrations were 2.97 μm (0.01 wt%), 2.49 μm (0.02 wt%) and 2.48 μm (0.10 wt%), respectively, indicating that the mean particle size decreased slightly with the increase of SDS concentration. It is worth noting that when SDS concentration was changed from 0.02 to 0.10 wt%, the span of PSD became obviously narrower although the mean size of particles changed slightly as shown in Fig. 4. Such a result may be ascribed to the following two reasons. On one hand, with the increase of SDS concentration, the interfacial tension of the droplets decreased and the diffusion property was modified. As a result, the smaller particles with smooth surface were formed. However, when the SDS concentration increased over a certain level, the emulsion droplets formed at the initial stage could no longer adsorb more SDS molecules; meanwhile, the interfacial tension kept increasing with the decrease of solvent concentration in the droplets. So the coalescence of droplets occurred in

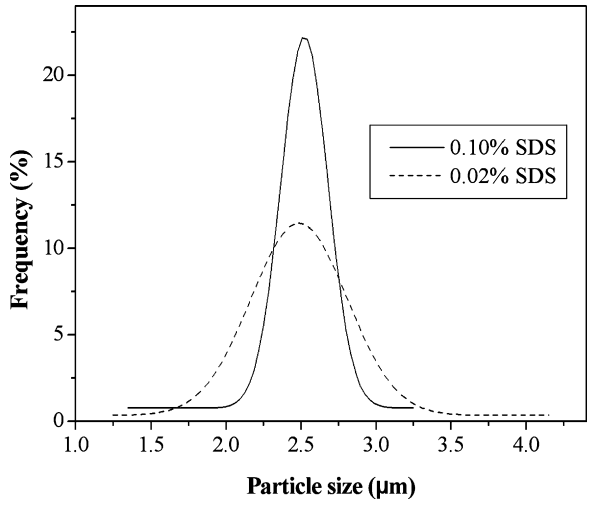


**Fig. 4.** PSD of silybin spheres precipitated at 30 °C under SDS concentration of 0.02 and 0.10% respectively.

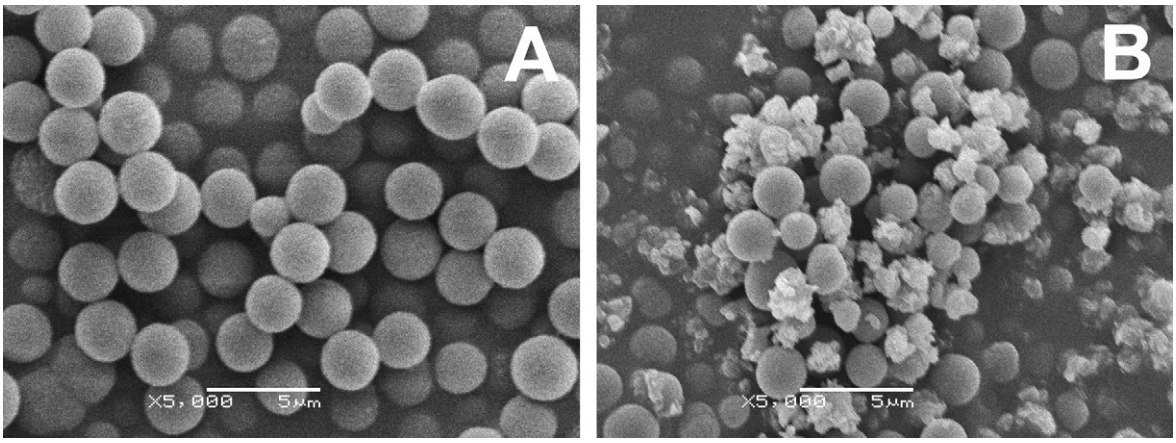


**Fig. 5.** SEM images of silybin spherical particles prepared from 0.10 wt% SDS aqueous solution at 30 °C with stirring for (A) 1 min and (B) 7 min.

almost the same way under different SDS concentrations, causing nearly the same mean size of the resultant particles. On the other hand, the interfacial tension of aqueous solution decreased with the increase of SDS concentration, which might make the newly formed emulsion droplets distribute and subsequently collide more evenly, leading to the formation of more uniform particles with much narrower PSD.

### 3.4. Effect of stirring time

Fig. 5 illustrates the effect of stirring time on silybin particle morphology. Uniform spherical particles could be obtained by stirring the mixed solution for 1 min and subsequently storing at 30 °C for 6 min. However, some irregular particles could be observed when the mixed solution was stirred continuously for 7 min. During the ESD process, prolonged stirring could aggravate the collision of uncompletely solidified particles, and hence some particles might disrupt into pieces. In addition, it was found that once the uniform spherical particles have been formed, no particles could be deformed by restarting the intense stirring. This also suggests that the completely solidified particles were stable and hard to be broken up.

### 3.5. The drug content analyses

Since the uniform particles were produced from SDS aqueous solution, some SDS might remain in the silybin particles. Therefore, purity of the drug was examined using HPLC. The results showed that drug contents for both spherical and rod-shaped particles reached more than 99% (99.3 ± 0.41 and 99.5 ± 0.35%), which proved that most of the SDS had been removed by washing the filter paste several times with deionized water.

### 3.6. FT-IR spectroscopy

FT-IR spectra of the commercial silybin powder, spherical and rod-shaped silybin in the range of 500–4000 $cm^{-1}$ were compared carefully and it could be seen that the spectrum of spherical or rod-shaped drug particles showed no obvious difference from that of the commercial silybin in the whole area of the silybin absorption bands. So it could be concluded that the addition of SDS and the employment of ESD process did not change the chemical composition of silybin.

### 3.7. X-ray diffraction studies

XRD study was performed to determine the physical state of the commercial silybin powder, spherical and rod-shaped silybin. The corresponding patterns were displayed in Fig. 6. The crystallinity peaks were found in the diffraction patterns of all three samples, demonstrating that the samples were crystalline. Obviously, all three samples had almost the same peak positions, clearly indicating that the as-prepared silybin powders had the same crystalline structure as that of commercial silybin. In addition, it could also be seen that the rod-shaped particles had the lowest peak intensities, suggesting the lowest crystallinity and the smallest size which was consistent with the SEM results. It is believed that poorly water-soluble pharmaceuticals with lower crystallinity and smaller size usually exhibit higher dissolution rate and bioavailability (Sarkari et al., 2002; Zhong et al., 2005). Accordingly, the decrease in crystallinity and the size reduction of as-prepared silybin particles are expected to improve its dissolution rate and bioavailability.

### 3.8. Physical stability studies

Most drugs exhibit structural polymorphism, which can affect the bioavailability and mechanical properties of drug particles (Singhal and Curatolo, 2004). It is necessary to investigate the stability of particles in the solid-state forms or in suspensions. Stability studies indicated that the dry powder of commercial silybin, rod-shaped and spherical particles could be stably held in polyethylene bags under room conditions for more than 3 months without any changes. Moreover, when uniformly re-dispersed in deionized water without any surfactant, all the samples manifested no morphology change.

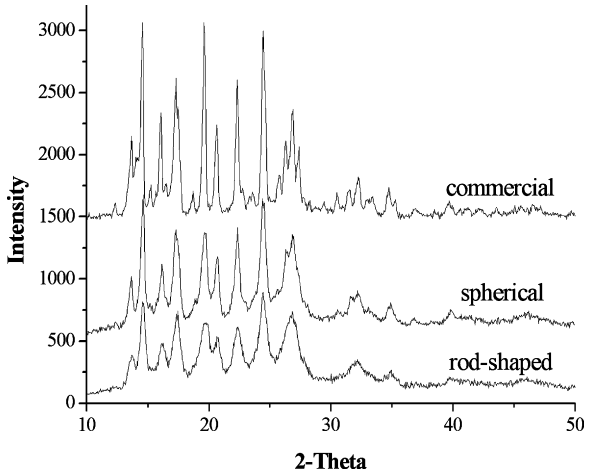


**Fig. 6.** XRD patterns of commercial silybin powder, spherical and rod-shaped silybin particles.

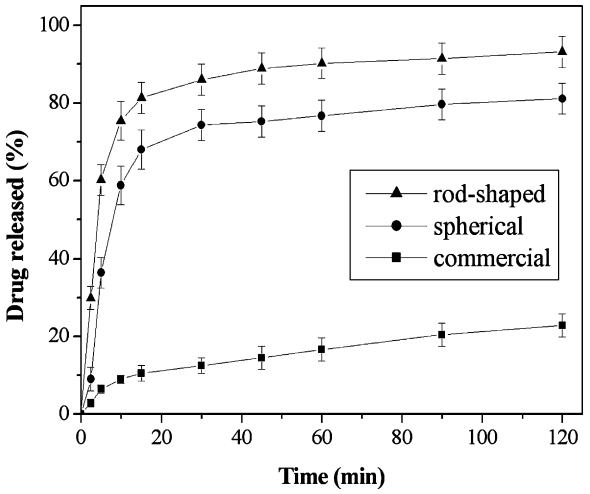


**Fig. 7.** Dissolution profiles of commercial silybin powder, spherical and rod-shaped silybin particles.

### 3.9. Dissolution test

The in vitro release profiles of the commercial silybin powder, spherical and rod-shaped silybin particles were shown in Fig. 7. The dissolved amount of drug of the spherical and rod-shaped particles increased to 74.3 and 86.0% after 30 min, while only 11.5% of the commercial silybin was dissolved. After 120 min, about 81.1% of the spherical silybin and 93.1% of the rod-shaped silybin was dissolved, respectively, while only 22.8% of the commercial drug was dissolved. The increase of the dissolution rates of the products is mainly attributed to the much better uniformity, the decreased crystallinity, the reduction of the particle size, the increase of BET surface area (increased from 2.87 $m^2/g$ for the commercial silybin powder to 3.09 and 6.77 $m^2/g$ for the spherical and rod-shaped particles) and the improvement of particle dispersion. The increase of the dissolution rate of rod-shaped particles, compared with that of the spherical ones, was mainly resulted from the decreased crystallinity and the increased BET surface area. Therefore, ESD method is an effective way for decreasing the particle size to enhance the dissolution rate of amphiphilic and poorly water-soluble drugs.

## 4. Conclusions

In this study, ESD method was employed to prepare uniform micronized silybin particles. In this process, the particle size and morphology could be well controlled by temperature and SDS concentration. With the increase of temperature from 15 to 30 °C, the morphology of the prepared silybin particles gradually transformed from rod-shaped to spherical while the mean particle size increased from 0.89 to 2.48 μm. Moreover, the mean particle size decreased and the PSD became narrower with the increase of SDS concentration. Compared to the commercial silybin powder, the as-prepared silybin particles possessed decreased crystallinity and showed very similar chemical composition. More importantly, the dissolution of the spherical and rod-shaped silybin particles was markedly improved when compared to the commercial silybin powder. Therefore, ESD method offers a potentially feasible way to prepare micronized drug particles with controlled size and morphology.

## Acknowledgements

This work was financially supported by National Natural Science Foundation of China (Nos. 20806004 and 20821004) and the Talent Training Program of Beijing City (2007B022).

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