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Process parameters and morphology in puerarin, phospholipids and their complex microparticles generation by supercritical antisolvent precipitation

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ABSTRACT

The aim of the study was to develop and evaluate a new method for the production of puerarin phospholipids complex (PPC) microparticles. The advanced particle formation method, solution enhanced dispersion by supercritical fluids (SEDS), was used for the preparation of puerarin (Pur), phospholipids (PC) and their complex particles for the first time. Evaluation of the processing variables on PPC particle characteristics was also conducted. The processing variables included temperature, pressure, solution concentration, the flow rate of supercritical carbon dioxide (SC-CO_2) and the relative flow rate of drug solution to CO_2. The morphology, particle size and size distribution of the particles were determined. Meanwhile Pur and phospholipids were separately prepared by gas antisolvent precipitation (GAS) method and solid characterization of particles by the two supercritical methods was also compared. Pur formed by GAS was more orderly, purer crystal, whereas amorphous Pur particles between 0.5 and 1 μm were formed by SEDS. The complex was successfully obtained by SEDS exhibiting amorphous, partially agglomerated spheres comprised of particles sized only about 1 μm. SEDS method may be useful for the processing of other pharmaceutical preparations besides phospholipids complex particles. Furthermore adopting a GAS process to recrystallize pharmaceuticals will provide a highly versatile methodology to generate new polymorphs of drugs in addition to conventional techniques.

1. Introduction

A successful formulation design of particulate products depends upon the physico-chemical properties of the constituent powders. Bioavailability, powder flow, compression characteristics and physical stability are affected by physical properties of the drug substances and particulate formulation additives. Therefore, the control of physical properties of pharmaceutical materials and the development of robust production processes are vital (Rehman et al., 2004). Improving the dissolution properties of a poorly soluble active substance is also a major concern of the pharmaceutical industry. It implies increasing dissolution rates and improving permeation through biological membranes. In fact, it has been shown that, for most poorly soluble compounds that are orally administered, the bio-absorption process is rate-limited by dissolution in gastrointestinal fluids; in the case of oral administration, the effective bioavailability of compounds is also limited by solubility issues. Many parameters related to solid morphology influence the dissolution rate of a compound, among which the particle size, the crystal habit and crystal pattern have a key-role (Perrut et al., 2005a). Many technological methods, such as micronization, formation of solvates, adsorbates, phospholipids complexes, microspheres, or more often, solid dispersions have been reported. They all aim at enhancing the dissolution characteristics of slightly or poorly water-soluble drugs or changing particle morphology. In spite of the tremendous potential of phospholipids complex systems for improving drug bioavailability, the methods traditionally used to prepare these systems are always time-consuming and have problems of grinding or difficult removal of the solvent. To overcome these problems, a technology using supercritical fluids (SCF) has been proposed to prepare solvent free solid particles or composite systems with reduced dimensions and different morphologies without the need of a grinding procedure (Moneghini et al., 2001).

Supercritical particle generation process is a new and efficient route for improving the bioavailability of pharmaceutically active compounds. SCF methods promise the control of particle size (PS) and size distribution (PSD) in the micrometric or nanometric ranges. These expectations derive from a continuous, adjustable

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solvent power and selectivity obtained at varying process parameters such as pressures and temperatures, etc. Diffusivity of SC-CO_2 is about two orders of magnitude greater than that of liquids. So SCF-based processes can quickly transfer mass and offer performance unattained using conventional solvents (Porta and Reverchon, 2005b). Rapid expansion of supercritical solutions (RESS), supercritical anti-solvent (SAS) and particles from gas saturated solutions (PGSS) are three families of SCF processes. Supercritical processes give micro- or nanoparticles with narrow size distribution, and also can be used to achieve microencapsulation, surface coating of an active substance particle with a polymer, or co-crystallization, co-precipitation with excipients such as poly(vinyl pyrrolidone) (PVP), poly(ethylene glycol) (PEG), ethyl cellulose (EC), poly(lactic acid) (PLA), Gelucire and other polymers, or host molecules like cyclodextrins (Kim et al., 1996; Falk and Randolph, 1998; Ghaderi et al., 1999; Elvassore et al., 2001b; Corrigan and Crean, 2002; Sethia and Squillante, 2002; Snavely et al., 2002; Wang et al., 2002; Meziani et al., 2004; Pathak et al., 2004; Reverchon and Marco, 2004; Rodier et al., 2005; Duarte et al., 2006). In addition, several other advantages can be noted depending on the chosen process configuration: high purity of products, control of crystal polymorphism, possibility of processing thermolabile molecules, single-step process, easy downstream processing, and environmentally friendly technology (Fages et al., 2004). As most pharmaceutical materials are insoluble or sparingly soluble in SCF, the SAS processes including SEDS and GAS methods are of greater importance. In GAS method, a solution of the compound in an organic solvent is in contact with a supercritical solvent that causes solid precipitation by anti-solvent effect. The organic solvent is eventually entrained by the supercritical fluid and either neat particles of a unique compound, or microspheres of an ingredient embedded in an excipient may be generated. SEDS technique is developed by York and Hanna to overcome the constraints of RESS and GAS processes to produce controlled particulate materials with defined morphology. This process features a highly turbulent flow of solvent and CO_2, leading to a very fast mixing or dispersion. Thus, mass transfer is not limited by molecular diffusion or convective phenomena. By using this technique, it is possible to control the size, shape and morphology of the material of interest (Rehman et al., 2001). In supercritical methods, CO_2 is the most widely used solvent. Its innocuity and "green" characteristics make it the best candidate for the pharmaceutical industry. Particularly, the critical temperature of CO_2 is very near to room temperature, which favors the processing of thermolabile compounds (Porta and Reverchon, 2005a).

Besides pure drug particles, these different supercritical fluid processes are being developed to design composite particles with several purposes. They include the preparation of sustained-release drugs by incorporating the active compound in a slow-dissolving (bio-degradable or bio-erodable) matrix, the stabilization of fragile molecules (mainly bio-molecules) in the solid form, and bioavailability enhancement of poorly soluble compounds by incorporating the active substances in a fast dissolving hydrophilic excipient. For the last purpose, most work has been focused on the preparation of particles consisting of an active drug substance with PVP or cyclodextrins (Perrut et al., 2005b). The preparation of drug phospholipids complex by supercritical fluid precipitation has not been reported ever. Till now, there is only one publication related with SCF-produced phospholipids. It reports the formation of nanoparticles of cyclosporine-A stabilized by phospholipids (Young et al., 2003). Their method uses SC-CO_2 as a solvent and it is rapidly expanded in the presence of water to form a suspension of nanoparticles. While, our work uses SC-CO_2 as an antisolvent and is able to produce a dry powder with a one-step process. Our product is not an aqueous suspension which may be dried in subsequent steps. And most importantly, the nanoparticles suspension stabilized by phospholipids cannot be defined as a phospholipids complex. Moreover, the effect of the SEDS process parameters on the particle size, size distribution and morphology of the produced phospholipids complex has never been studied either.

Incorporation of drugs into phospholipids has frequently been reported to increase the dissolution of poorly soluble drugs, often leading to improved drug bioavailability (Gabetta et al., 1987; Wu et al., 1998). Such dosage forms are referred to as phospholipids complex, defined as the complex of one or more active ingredients in phospholipids carrier at solid state prepared in suitable conditions. Phospholipids complex formation technique has been widely accepted as a method for improving bioavailability of dissolution-dependent poorly soluble drugs. Both the type of phospholipids and the method of phospholipids/drug combination manufacture have great impact on the characteristics of phospholipids complex formed. Several techniques used for the preparation of phospholipids complex before include the most widely used coprecipitation in solvents, spray or freeze drying and grinding. Yet they are time and energy consuming (Rodier et al., 2005). Apart from the spray-drying which is a single-step process that converts a liquid feed into a dried powder, all other techniques require multi-stages. In this study, SC-CO_2 in SCF method is suggested as a new complex formation medium for the preparation of phospholipids complex due to its properties of improved mass transfer and increased solvating power. What is more, SCF technology is an environmentally friendly technique that allows more precise control of particle size and morphology while minimizing organic solvent use. In addition to micronization, the pharmaceutical application of the SCF process enables the modification of the solid-state properties of drug or its composite particles. The diversity of experimental parameters can vary the conditions for nucleation and crystal growth steps in a wide range. Hence, particles having different internal crystalline structures and external characteristics can be precipitated.

Therefore, the purpose of this study is to demonstrate the feasibility of formulating puerarin and its phospholipids complex particles for the first time using SEDS and GAS processes. Pur is one of the major effective constituents of *Pueraria lobata (wild.) ohwi* with poor solubility. The potential of this micronization technology was also explored by studying its influence on the morphology, particle size and size distribution. To achieve this purpose, the solutions were dispersed and atomized by the SC-CO_2. The organic solvent, which is soluble in the supercritical CO_2, was extracted and solid microparticles were formed. The PSD and morphology have been shown to be affected by process variables such as flow rate, solute concentration, temperature, pressure and the relative flow rates of drug solution to CO_2. So their relationship was studied. Plain drug and plain phospholipids particles were prepared besides producing drug-phospholipids complex particles by SEDS. Research has been done to optimize the process conditions to make the particles sizing as small as possible and the morphology more regular. In this investigation, attempts have also been made using a GAS technique to prepare particles of Pur and phospholipids and comparing particles prepared by two SCF methods for the first time as well.

2. Materials and methods

2.1. Chemicals

Absolute ethanol (Analytical grade, purity 99.7%) was bought from Guangzhou Chemical Reagent Two Factory (Guangzhou, China). Puerarin was obtained from Beijing Union Pharmaceutical Factory (Beijing, China). Soya phospholipids PC70 were purchased from Degussa Texturant System Deutschland GmbH&Co. KG (Ausschlager Elbdeich, Hamberg). Liquid CO_2 (Instrument grade, purity

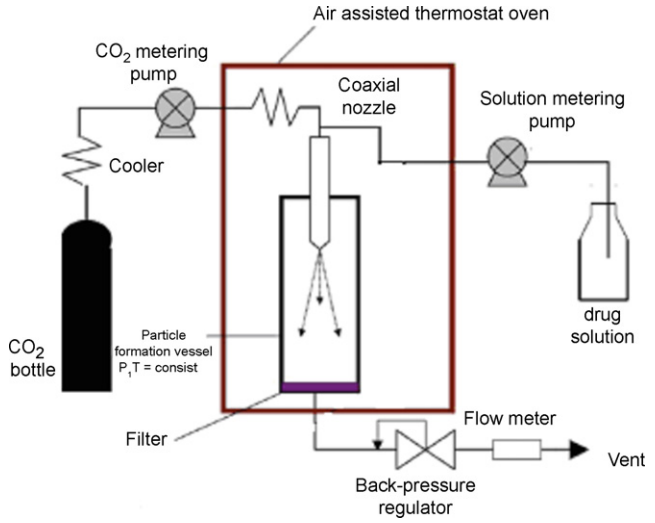

Fig. 1. Schematic diagram of the experimental apparatus for the SEDS process adapted from Rehman et al. (2004).

99.5%) was purchased from Zhonghong Industrial Gas (Shenzhen) Ltd. (Shenzhen, China).

2.2. Apparatus for SCF process

A schematic diagram of the SEDS apparatus is shown in Fig. 1. In the semi-continuous SEDS experiment, CO_2 was stored in a liquid CO_2 storage tank and firstly cooled by Caron water-cooled constant temperature bath (Model 2050W, Caron Products and Services, Inc., Marietta, OH) to prevent cavitations before being delivered. Then the CO_2 passed through a heat exchanger to ensure that it was supercritical before entering the 0.1 mm nozzle. The nozzle consisted of two concentric tubes and a small premixing chamber. The apparatus for preparation of puerarin, phospholipids or their complex powder were composed of two pumps, one for delivering liquid CO_2, and the other for drug solution. Both of them were controlled by Isco Series D pump power controller (Teledyne Isco, Inc., Lincoln, NE). The CO_2 and the organic solutions were delivered to the stainless steel 250 ml CL-1078 high pressure particle formation vessel (Thar Designs Inc., Pittsburgh, PA) through this small nozzle at the desired operating temperature and pressure in co-current mode. The CO_2 flow rate was shown on the syringe pump controller. The temperature of the high-pressure vessel was controlled using Athena 2000-B heaters (Model 2000-B, Athena Controls, Inc., Plymouth Meeting, PA) attached to the sides of the vessel.

The equipment for GAS is almost the same except that one pump for delivering drug solution was closed and the drug solution was put into the particle formation vessel beforehand. Details of the experiment procedure were given below.

2.3. Preparation of solutions

Preparation of puerarin or phospholipids solution: A stock solution of puerarin was prepared by weighing out an accurate amount of 10.0 g puerarin and dissolving it into 100 ml absolute ethanol. A stock solution of phospholipids was prepared by weighing out an accurate amount of 3.6 g phospholipids PC70 and dissolving it into 10 ml absolute ethanol by stirring.

Preparation of puerarin and phospholipids mixture solution: A mixture of solutes in 100 ml absolute ethanol was prepared at a puerarin concentration of 100 mg/ml and phospholipids PC70 of 120 mg/ml.

2.4. SCF processes

Preparation of puerarin or phospholipids by GAS: In the batch GAS step configuration, the precipitation unit attached to Isco syringe pump was initially loaded with a given amount of puerarin or phospholipids solution. Then the supercritical antisolvent was added until the final pressure was reached. The vessel was filled with SC-CO_2 at the desired pressure (10 MPa) and temperature (38 °C) and left for 3 h without any agitation. A pure constant carbon dioxide flow was then maintained in order to completely remove the residual solvent. After this washing step which lasted for approximately 90 min, the autoclave was depressurized for 30 min at the experimental temperature. At the end, solid product was scraped out with a spatula from the filter located at the bottom of the vessel and subjected to handling and storage conditions identical to the unprocessed materials.

Preparation of puerarin, phospholipids or their complex by SEDS: In semi-continuous operation, the liquid solution and the supercritical antisolvent were continuously added into the precipitation unit in co-current mode. This was slightly more complicated than the batch mode, because the role of the liquid-injection device became crucial. In addition, the ratio between the solution and the antisolvent flow rates could be the key parameter for the evolution of the precipitation process (Elvassore et al., 2001a). The organic liquid solution was sprayed through a nozzle into high-pressure cylinder. The high velocity of the SC-CO_2 stream thoroughly mixed and dispersed the solvent stream and extracted the solvent, leaving dry powder in the vessel. The concentration of drug and/or carrier in the droplets increased, leading to rapid particle formation. A range of operating temperature (30–40 °C), pressures (8–12 MPa) was applied to produce complex powder. The flow rate of CO_2 was 45 ml/min at all combinations of pressure and temperature, except at 10 MPa and 35 °C, where the flow rates were studied varying between 25 and 65 ml/min. Proportion of flow rate of solution to supercritical CO_2 was from 1% to 5%. At the end of each experiment the microparticles were flushed with CO_2 at the flow rate of 25 ml/min for 90 min or more to remove any residual solvent to avoid the re-condensation of the liquid inside the chamber. The vessel was then slowly depressurized for 60 min and the powder was collected. The CO_2 gas was vented through the back pressure regulator outlet to the atmosphere. Pure phospholipids or drug was processed using SEDS besides PPC. The constant mass ratio of drug to phospholipids of 1:1.2 used for preparing the particles was optimized by conventional solvent preparation method.

2.5. Particle size and morphology

Scanning electron microscopy (SEM): The particle morphology of the processed and unprocessed materials was observed with a Jeol JSM-6460LV electron scanning microscope (Japan Electron Optics Ltd., Tokyo, Japan). Powder samples were manually dispersed on an aluminum stub with a thin self-adhered carbon film. The samples were coated with a thin layer of gold using an ion sputter under 0.5 mbar argon atmosphere (at room temperature for 90 s, at an accelerating voltage of 20 kV, working distance of 15 mm, and at 1000, 3000 and 5000 magnification).

Particle size distribution: PSD was measured by laser diffraction using Master Size 2000 laser particle analyzer (Malvern Instruments Ltd., Malvern, UK). The instrument covers a particle size range between 0.020 and 2000.0 μm. The method used for analysis was dry method.

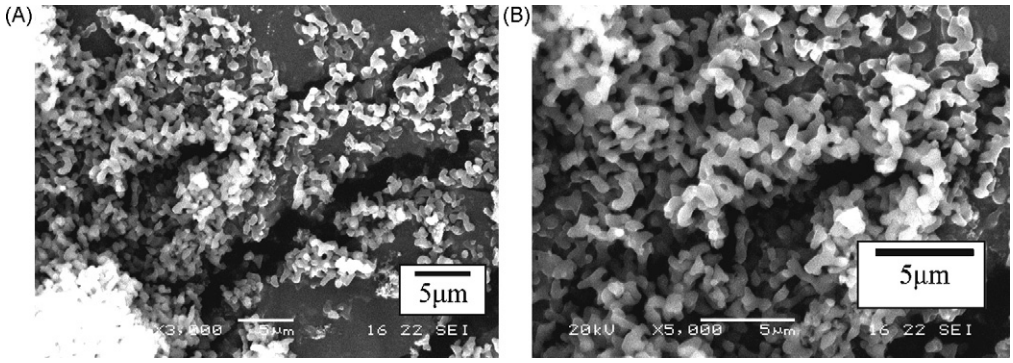

Fig. 2. SEM pictures of Pur precipitated by SEDS: (A) ×3000 and (B) ×5000.

3. Results and discussion

3.1. Puerarin and phospholipids particles by GAS

By GAS method, precipitation of puerarin or phospholipids could be obtained. The GAS processed puerarin was white fluffy powder all collected at the vessel bottom with the production yield of about 90%. The obtained phospholipids were light yellow loose solid which was a little viscous with the production yield of about 65%. Attempts were also made to prepare their complex (PPC) by varying the experimental conditions, such as increasing the solution concentration, augmenting pressure or temperature, prolonging the precipitation time, etc. Yet, results were not ideal with only precipitation of puerarin or a very low production yield of the complex. This was probably due to the increased solubility of phospholipids in SC-CO_2 under these conditions when ethanol co-existed in the system. Then the above reason resulted in complete extraction of both the solvent and the phospholipids. This was confirmed by the presence of phospholipids in ethanol, which appeared yellow in the solvent collected under the back pressure regulator. Therefore, we only prepared the complex of puerarin and phospholipids by SEDS and focused on the effect of experimental conditions onto the size and morphology of the complex particles in order to optimize the conditions for preparing PPC by SCF method.

The Pur particles collected under the same conditions with phospholipids were formed as pure crystals that we will discuss below, sizing 20.40 μm on average with the size distribution between 2.89 and 37.09 μm.

3.2. Puerarin and phospholipids particles prepared by SEDS

By SEDS, at the conditions of 35 °C, 10 MPa, CO_2 flow rate 45 ml/min, flow rate proportion of CO_2 to solution 1% and puerarin concentration 100 mg/ml, Pur particles sizing 6.47 μm (see Fig. 2) and phospholipids particles sizing 4.92 μm (see Fig. 3B) were obtained. Compared with the commercial phospholipids of 4.67 μm (Fig. 3A), SEDS-phospholipids showed no advantage over the particle size. The original phospholipids were compact and continuous film-like particles with ruffles on the surfaces. While after SEDS, its morphology changed to films with more smooth surfaces. The original phospholipid was known to be amorphous (this part of work would be published elsewhere). After processed with SEDS, the amorphous characteristics of PC did not change although the surface morphology was a little different. The particle size of commercial Pur was 25.68 μm, while the particle size of Pur prepared by either SCF method was decreased remarkably. Especially by the manipulation of process conditions in SEDS method, the particle size of Pur was reduced fourfold which was more favorable to its oral absorption. During the GAS process, the liquid solution was expanded less rapidly than the SEDS process. In both processes, the solute in solution was depleted owing to the formation of nuclei and to the crystal growth. A rapid increase in the supersaturation generally led to a high nucleation rate, which then resulted in numerous small crystals or particles. So, the GAS process was supposed to imply higher growth rates and thus to give larger crystals (Taki et al., 2001). Although the reduction of particle size by GAS was not as distinctive as SEDS, the transformation of Pur particle morphology by GAS was remarkable. The unprocessed Pur was composed of variously sized cascading flakes (Fig. 4) whilst after processed with GAS, the particles transformed into a totally different crystal form. Overall observation of SEM photomicrographs of the two crystal forms indicated that the GAS processed crystals showed more ordered appearances with clean surfaces and they were directionally arranged in prisms (Fig. 5). The phenomenon was consistent with the literature (Sang et al., 2003). This means that the GAS process provides suitable environment for the solid

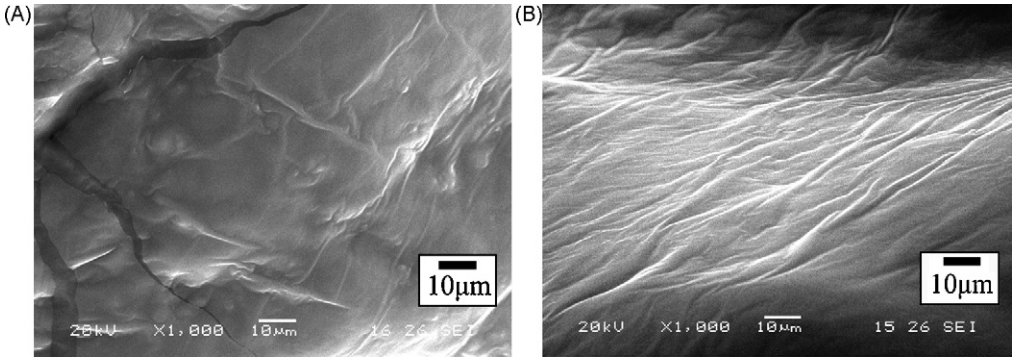

Fig. 3. SEM pictures of phospholipids: (A) commercial PC and (B) SEDS-PC.

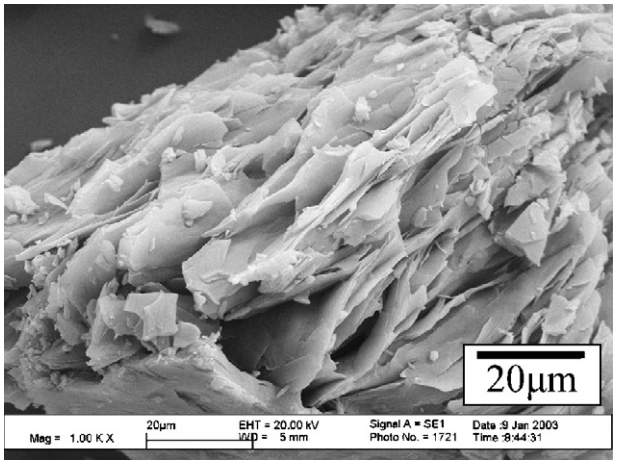

Fig. 4. SEM picture of commercial Pur.

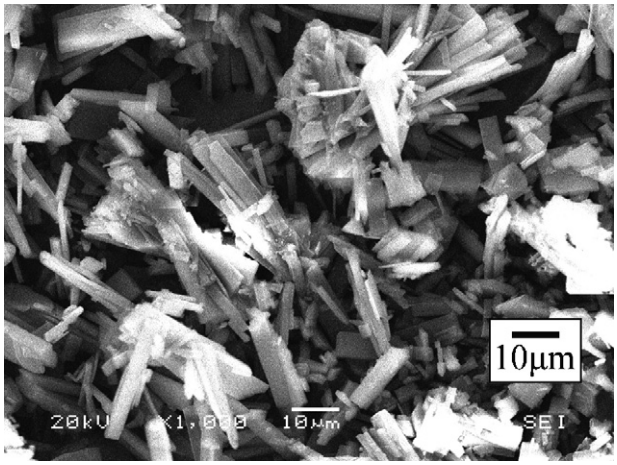

Fig. 5. SEM picture of Pur precipitated by GAS.

growth of a single crystal by minimizing the conditions for growth-related imperfections and solvent occlusion into the crystal faces. These results showed that the GAS process may provide the possibility to control and improve the morphology of drug particles and to provide another approach for compound recrystallization. Pur by SEDS showed no crystal structure. In SEM picture (Fig. 2) the particles were comprised of microparticles, and the size of single ones if not congregated was even smaller than 0.5 μm. Large aggregates were formed by coalescence of particles due to both the impacts undergone by the particles during the precipitation and the interactions between the solvent and the solute as pointed out by Badens et al. (2001). When the solution was sprayed into the precipitator, the liquid drops swelled out causing precipitation of the solid. EtOH solubilized in supercritical CO_2 still partly interacted with the solute. And all the above produced the coalescence of microparticles. That's also our main task in the following research to make the aggregation as less as possible. The Pur sample by SEDS was very light, voluminous powder displaying a radically altered gross morphology. This was consistent with the conclusion deduced by Sethia and Squillante (2002) that the morphology of particles would be changed by SCF process by formation of a light voluminous powder made of little particles. By forming small particles with larger surface areas, the dissolution rate of the drug would be further increased.

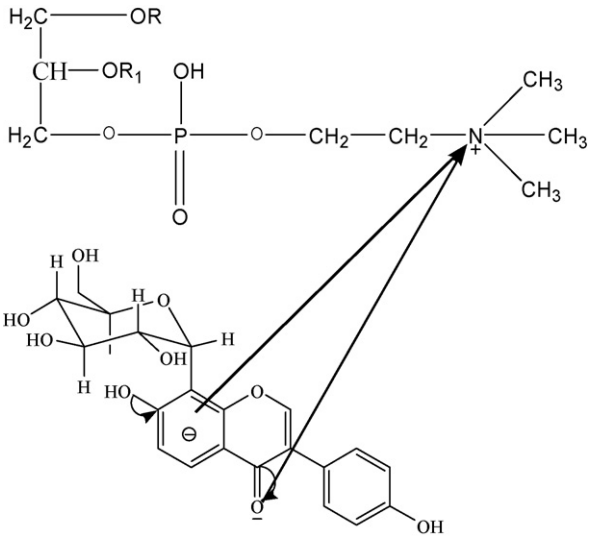

Fig. 6. Schematic diagram of spatial arrangement and action position of PPC.

3.3. Puerarin, phospholipids and their complex particles by SEDS

The formation mechanism of PPC was confirmed by nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy (IR) showing that there was a profound interaction between Pur and the polar end of PC. In the molecule structure of Pur, owing to the electron donating effect of the benzene ring, a relatively negative charged center existed that served as a likely site of complexation; on the other hand, the carboxyl oxygen at position 4 was nucleophilic or basic because of the withdrawal of electrons by the oxygen. As observed in phospholipids molecule, the nitrogen at the polar end presumably could become strongly electrophilic or acidic. The complexation could thus occur as a result of charge-transfer interaction between the nucleophilic Pur and the electrophilic nitrogen of phospholipids, but not simply a physical mixture. The proportion of Pur complexed with PC was 1:1.2. Fig. 6 showed the action position between Pur and PC and their spatial arrangement. As an index for evaluating the formation of PPC, "proportion of complexed", which means the amount of the complexed Pur relative to the used Pur, was used besides other evaluating indexes, such as yield, PS and PSD.

According to literature and initial studies, SEDS processing parameters such as concentration of puerarin, flow rate ratio of drug to CO_2 solution, CO_2 flow rate, temperature and pressure inside the particle-formation vessel that were employed during the microparticle preparation in this study were summarized in Table 1. The limits of the experimental field were defined as follows:

- Precipitation pressure range: from 8 to 12 MPa.
- Range of precipitation temperature: from 30 to 40 °C.
- CO_2 flow rate: from 25 to 65 ml/min.
- Proportion of flow rate of solution to SC-CO_2: from 1 to 5%.
- Pur concentration range in EtOH: from 60 to 150 mg/ml.

To probe possible mechanisms which may control particle characteristics, a systematic exploration was conducted to study the effects of the process parameters on the morphology, yield, particle size and size distribution. The reproducibility of the experimental results had been duly checked. A good reproducibility of the obtained particle size and size distribution was observed. Fig. 7 showed the particle size distribution curve at the best set of conditions. The trends in particle size and shape resulting

Table 1
Working conditions, yield, proportion of puerarin complexed, mean diameter, span and PSD for PPC precipitation from EtOH solution by SEDS

Sample no.	Pressure (MPa)	Temperature (°C)	Conc[a] (mg/ml)	Flow rate[b] (ml/min)	R[c] (%)	Yield (%)	Proportion of complexed (%)	Mean diameter (μm)	Span	PSD
1	10	35	100	45	5	45.0	72.2	2.55	2.15	0.43–5.90
2	10	35	100	45	3	43.4	42.3	4.03	2.36	0.97–10.46
3	10	30	100	45	1	50.0	61.3	4.37	1.72	1.53–9.03
4	10	40	100	45	1	82.5	100.0	6.82	1.42	3.37–13.02
5	8	35	100	45	1	70.9	44.2	4.31	1.54	1.91–8.54
6	12	35	100	45	1	91.2	87.1	7.14	1.24	3.83–12.69
7	10	35	100	65	1	90.8	86.0	6.01	1.46	2.816–11.58
8	10	35	100	25	1	92.1	100.0	7.29	1.28	3.94–13.26
9	10	35	150	45	1	76.4	100.0	6.78	1.36	3.34–12.59
10	10	35	100	45	1	93.3	97.8	5.93	1.29	2.80–10.43
11	10	35	80	45	1	87.6	91.5	6.63	1.39	3.04–12.24
12	10	35	60	45	1	86.8	100.0	7.34	1.27	3.91–13.24
13[d]	10	35	100	45	1	95.1	–	6.47	1.21	3.31–11.10
14[e]	10	35	0	45	1	64.0	–	4.92	1.21	2.71–8.64

[a] Pur concentration in EtOH.
[b] Flow rate of the SC-CO_2.
[c] Proportion of flow rate of solution to CO_2.
[d] Concentration of phospholipids is 0 mg/ml.
[e] Concentration of phospholipids is 120 mg/ml.

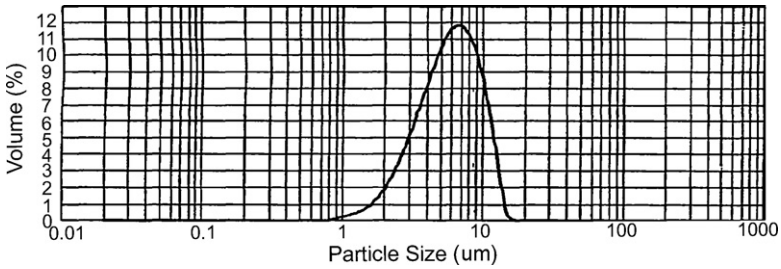

Fig. 7. Particle size distribution of PPC particles prepared by SEDS.

from manipulation of these variables were discussed accordingly.

3.3.1. Effect of relative flow rates of drug solution to CO_2

Several experiments were conducted in the flow rate proportion range of 1–5%, with the other operating conditions maintaining at pressure of 10 MPa, temperature of 35 °C, CO_2 flow rate of 45 ml/min and puerarin concentration of 100 mg/ml in ethyl alcohol. When the flow rate proportions of solution to CO_2 were 3% and 5%, the shape of particles were similar to the phospholipids which were fusing in the surface as shown in Fig. 8A. From the SEM pictures of 1% proportion (Fig. 8B), it was clear that the micronized material was comprised of nearly spherical particles sizing only about 1 μm constituting aggregates. It was found that by solubility measurement, ethanol may perceptibly improve the solubility of phospholipids in SC-CO_2 and the higher the percentage of the cosolvent, the better the solubility (Badens et al., 2001). When the proportion increased, the fraction of solvent in the system also increased and so was the solubility of phospholipids in SC-CO_2. The higher solubility of phospholipids in SC-CO_2 led to the less contact chance between phospholipids and Pur. Thus the proportion of complexed and production yield of PPC decreased. This also resulted in the precipitation of phospholipids in the surface of puerarin with similar morphology as phospholipids. In addition, Charoenchaitrakool et al. (2002) reported that the contact time between SC-CO_2 and the physical mixture of drug and carrier in the static mode had a positive influence on the complex formation. In this study, the fact that the contact time between Pur and phospholipids was shortened when the flow rate proportion was increased, and the increased solubility of phospholipids in SC-

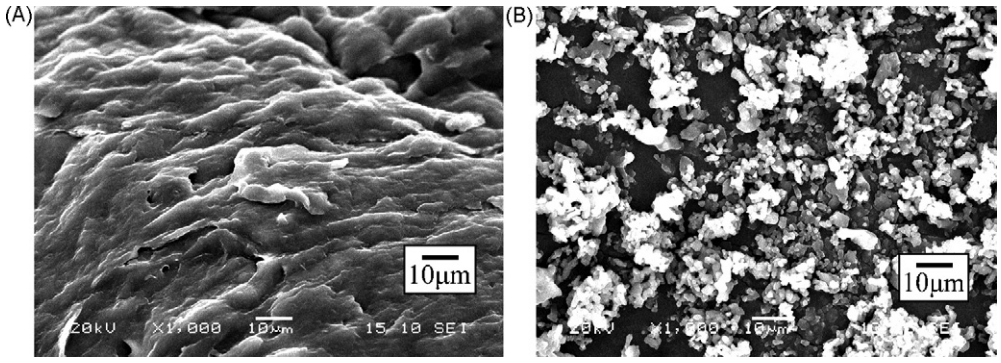

Fig. 8. SEM photographs of PPC particles prepared at different flow rate proportions of solution to CO_2: (A) 3% and (B) 1%.

CO_2 all led to the decreased proportion of complexed and yield for PPC.

The influence of the flow rate ratio also showed that the particle size and size distribution decreased with the augment of the flow rate proportions of solution to CO_2. This result was generally attributed to a faster atomization of the liquid phase. This resulted in the formation of smaller droplets and accelerated mass transfer. The increase of the CO_2 transfer rate induced higher supersaturation in the liquid phase which was more favorable to the formation of nuclei than to the crystal growth (Badens et al., 2001).

3.3.2. Effect of precipitation temperature

Experiments were performed at fixed conditions as mentioned above while varying the temperatures only. It was reported the melting point of phospholipids was 60 °C and it would degrade at about 100 °C (Chen, 2002). Since the phospholipids had a low melting point, the experiments were started at lower precipitation temperatures of 30–40 °C to avoid problems of particles sintering or phospholipids degradation. With the precipitator temperature set at 30 °C, PPC particles formed large agglomerates fusing together like the ones reported in Fig. 9A. When the precipitator temperature was increased to 35 °C and 40 °C, similar partly connected, nearly spherical particles were produced (Fig. 9B–D), just bigger at 40 °C. This was because the CO_2 in the precipitation vessel was not in the supercritical state when the temperature was low. The lower penetration and solubility of the medium resulted in the large amount of precipitation of PPC particles coalescing together as a result of the more slowly extraction of ethanol. At a higher temperature of 40 °C, the particles connected only partly because of the faster coagulation rates from thermal energy and faster diffusion in the medium. Increasing temperature would increase the solubility of the pharmaceutical in the organic solvent, hence moving the position of the saturation and critical supersaturation lines upwards in addition to changing their shape. The magnitude of the generated supersaturation was decreased as the profile moved closer to the saturation line. This was followed by a gradual decline-depletion in the supersaturation as the nuclei grew, i.e., a high growth rate followed (Bakhbakhi et al., 2006). So the particle size became bigger at higher temperatures.

It was also observed that higher production yield and complexed proportion of PPC were obtained at higher temperatures. CO_2 and ethanol mass transfer rates were expected to be increased by small increases of temperature (Randolph et al., 1993). The increases in mass transfer rates would tend to increase PPC proportion of complexed and production yield.

3.3.3. Effect of precipitation pressure

To investigate the effect of antisolvent pressure on the particle sizes resulted from the SEDS process, several experiments were conducted in which other parameters were held constant while the pressure was varied. Experiments were carried out at pressures of 8, 10 and 12 MPa, 35 °C, CO_2 flow rate of 45 ml/min, solution flow rate to CO_2 of 1% and puerarin concentration of 100 mg/ml in ethyl alcohol. The higher the pressure, the higher the production yields, the proportion of complexed, and the larger the particle size. The pressure appeared to have little effect on the particle shape of PPC, which exhibited as more or less agglomerated spherical particles (Fig. 10).

The results showed that particle size was increased at higher pressures. It was known that the pressure was the most relevant parameter in controlling particle size during the batch antisolvent process of GAS, but it was not the case for the semi-continuous process such as SEDS (Badens et al., 2001). E. Badens et al. made a research on variations of E_v vs. pressure and temperature for ethanol–CO_2 mixtures. E_v, the volumetric expansion of the liquid phase, was defined as: $E_v = (V - V^0)/V^0 \times 100$, where V^0 is the initial volume of ethanol and V is the liquid phase volume after the dissolution of CO_2 at temperature T and pressure P. The results showed that the variation of E_V was slow at low pressures, but the variation was tremendously accelerated at high pressures and the asymptotic value of the pressure at 308 K was estimated as 7.5 MPa. They also concluded that within the range of the study and for a given tem-

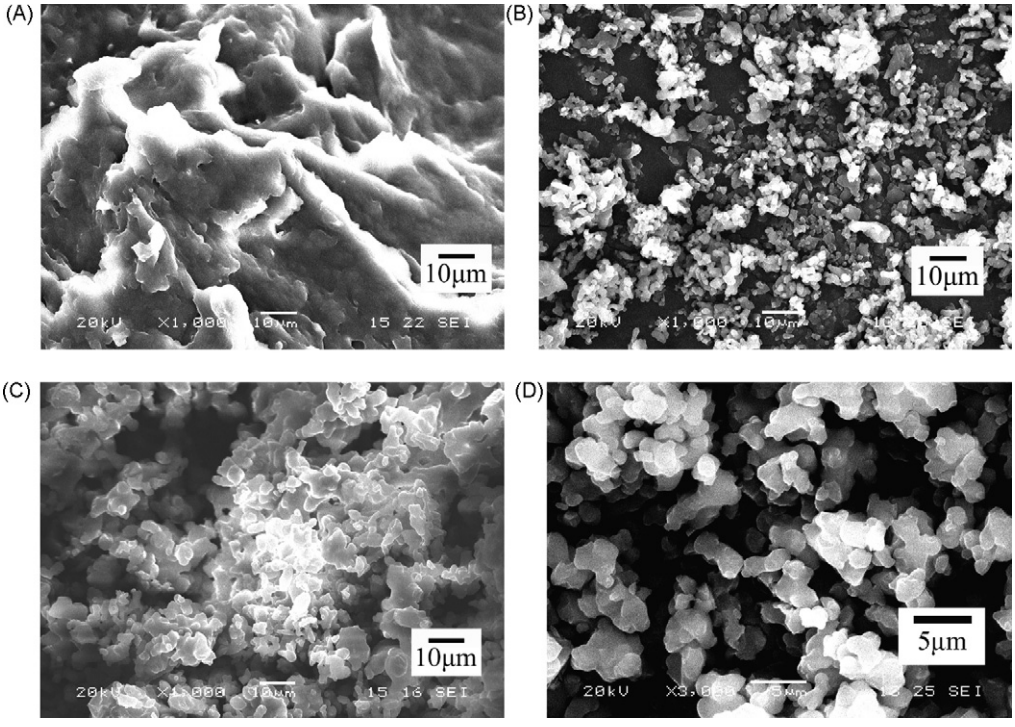

Fig. 9. SEM photographs of PPC particles prepared at different temperatures: (A) 30 °C, (B) 35 °C, (C) and (D) 40 °C.

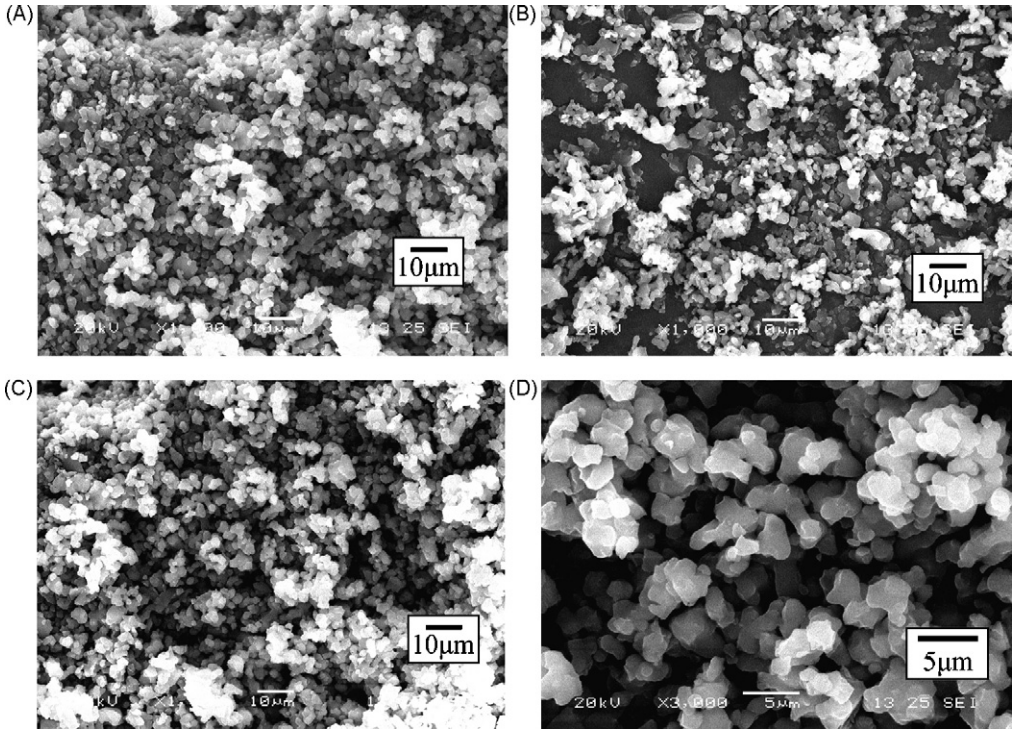

Fig. 10. SEM photographs of PPC particles prepared at different pressures: (A) 8 MPa, (B) 10 MPa, (C) and (D) 12 MPa.

perature, the transition pressure was not perceptibly influenced by variations of the relative amounts of CO_2, ethanol and phospholipids. In our experiment, at 8 MPa the average particle size was 4.31 μm, while at 12 MPa the average particle size was 7.14 μm. Fig. 10A and C showed the nearly spherical shapes of the particles obtained at 8 and 12 MPa. This trend of increased particle size with increasing antisolvent pressure was opposite with that seen by Dixon and Bodmeier (1993). Our explanation for the opposite trend observed in this study was the same with the literature reported by Randolph et al. (1993). The higher ethanol and CO_2 mass transport rates expected at lower pressures caused more rapid swelling of the initial droplets. Rapid swelling in turn resulted in a high

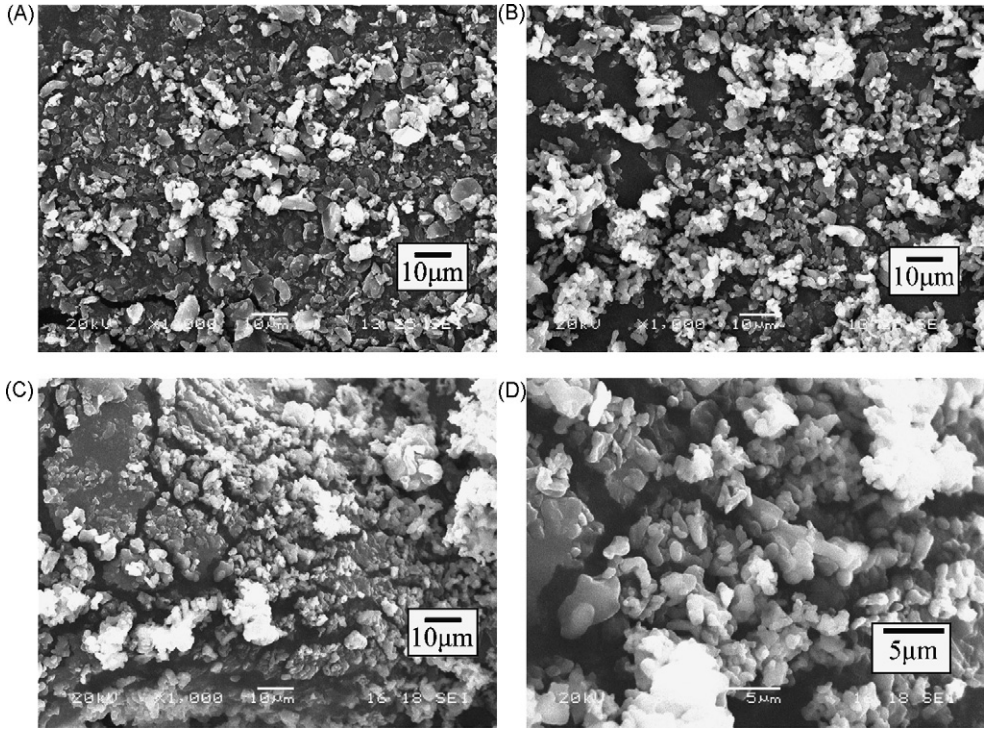

Fig. 11. SEM photographs of PPC particles prepared at different CO_2 flow rates: (A) 25 ml/min, (B) 45 ml/min, (C) and (D) 65 ml/min.

degree of supersaturation, and subsequently high nucleation rates yielded smaller particles. Besides this reason, this was also probably due to the increased solubility of Pur and phospholipids in CO_2 at higher pressures as reported by Badens et al. (2001) and Wang et al. (2006), thus resulting in a decreased nuclei density and larger particles.

3.3.4. Effect of CO_2 flow rate

The flow rate of CO_2 was varied at 25.0, 45.0 and 65.0 ml/min. Similar particle morphology at different flow rates was observed (Fig. 11), with a decrease of the particle size from 7.29 μm at 25 ml/min to 6.01 μm at 65 ml/min. Previous work on the fluid dynamics of precipitation in SCF had shown that large flow velocities could increase particle size because of reduced residence time in the nozzle and increased variability of solvent concentration in the flow (Rehman et al., 2001). The different result in our study indicated that high turbulence of SC-CO_2 should generally result in more efficient mixing and lead to the production of small particles. However it seemed that the shorter contact time did have an effect on the PPC formation. Compared with the 100.0% at lower CO_2 flow rate, the proportion of complexed at the highest flow rate of 65 ml/min decreased to 86.0%. To achieve higher complexing percentage and smaller particle size, the medium CO_2 flow rate should be appropriate.

3.3.5. Effect of solution concentration

In all experiments, the mass proportion of Pur to phospholipids was always held at 1:1.2. Then we varied the concentration of the Pur–EtOH solution from 60 to 150 mg/ml. Different Pur concentrations produced particles with remarkably distinct morphology. More diluted solution produced the solid product appearing agglomerated in large blocks (Fig. 12A). It was evident that the swollen structure was formed by micronic particles fused together. The formation of macroparticles could be due to the spray of the solution into the precipitator. The liquid drops swelled out causing precipitation of the solid. Then, ethanol diffused into the CO_2 bulk phase leaving the solid particles tightly tied together. At the concentration of 80 mg/ml (Fig. 12B) there were flakes structures besides particles. And when the concentration increased to 100 mg/ml (Fig. 12C), the particle morphology was partially connected particles. Then the particles prepared at 150 mg/ml (Fig. 12D) appeared massive block besides irregular microparticles. A possible explanation was that diluted solution produced lower supersaturation. At the lower supersaturation the nucleation rate decreased and particle growth would dominate over nucleation. Once the nuclei were formed, they could grow into a larger size such as lamellar structures or aggregated in blocks. The size of droplets in SEDS was governed by a number of parameters such as the internal diameter of the capillary nozzle, the density and the flow rate of the liquid solution, etc. The viscosity of the phospholipids solution was also an important factor. Increasing the phospholipids concentration would result in higher viscosities. Thus it had a stabilizing effect on the jet and bigger droplets were yielded. High phospholipids concentrations may even reduce the atomization forces to such a low level that it was not sufficient to break up the jet into droplets (Taki et al., 2001). In that condition, the precipitation kinetic was much faster than the droplet formation kinetic and fibers were formed rather than microparticles.

An increase in Pur concentration firstly gave a reduction in particle size, then an increase in particle size at Pur concentrations above 100 mg/ml. This could be explained by a combined effect of two factors in terms of nucleation and growth of particles. First, primary particle size was decreased with an increase of the solute concentration (supersaturation). And second, particle agglomerated at high Pur concentrations. The higher the nuclei concentration, the stronger the interaction between the clusters formed in the liquid drop. In this way, particles agglomerated in large blocks. It was commonly admitted that increasing the solute concentration would result in larger particles and enlarged PSD. However, our results showed that, the particle size

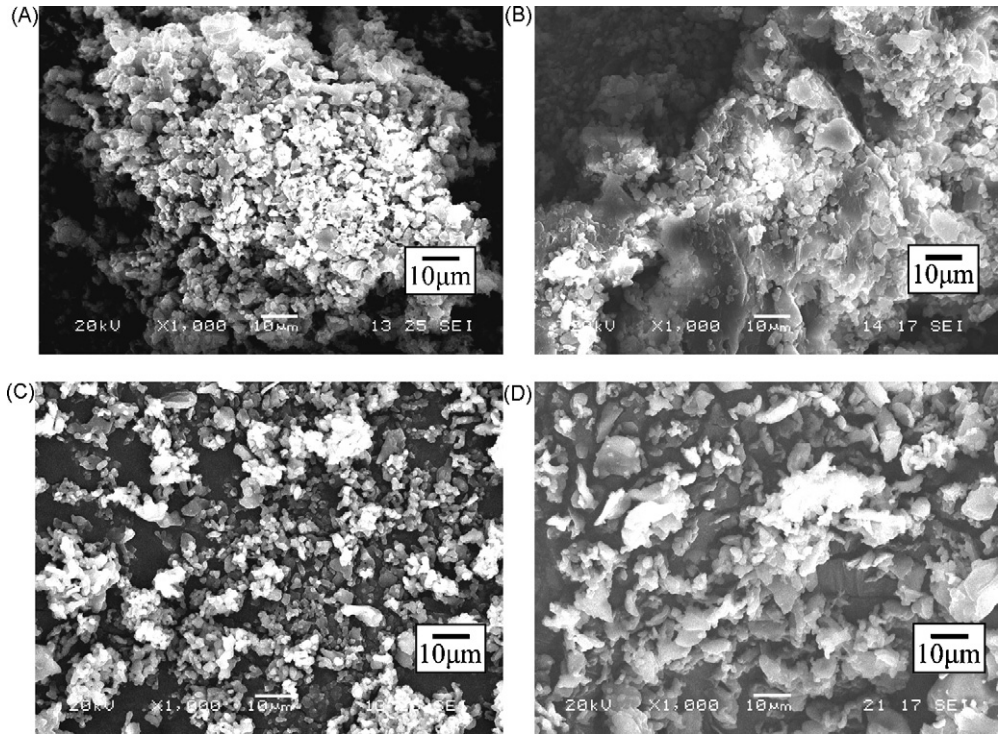

Fig. 12. SEM photographs of PPC particles prepared at different Pur concentrations: (A) 60 mg/ml, (B) 80 mg/ml, (C) 100 mg/ml, and (D) 150 mg/ml.

decreased at first, then increased with the enhancement of the Pur concentration, whereas no change for the proportion of complexed and production yield. The phospholipids concentration acted upon the droplet size in two opposite ways. On the one hand, increasing the phospholipids concentration resulted in a higher viscosity of the liquid solution and thus the bigger droplets. On the other hand, because phospholipids were surfactants, increasing its concentration in the liquid phase yielded a decrease of the interfacial tension and thus stabilized smaller objects. The fact that there were not only phospholipids but also Pur in our study made the situation more complicated. Thus the exact explanation for the particle size changing trend could not be elucidated clearly.

To sum up, in the study of SEDS process, the production yield, proportion of complexed, and especially the particle size and morphology were determined and evaluated to optimize the conditions for preparing the PPC. The optimum conditions were 35 °C, 10 MPa, CO_2 flow rate 45 ml/min, flow rate proportion of CO_2 to solution 1% and puerarin concentration 100 mg/ml. Because under these conditions, nearly spherical just partly connected micron-sized (2.80–10.43 μm) particles with the average size of 5.93 μm were obtained. Although the size was somewhat smaller compared with the size of particles (6.32 μm) prepared by conventional solvent evaporation method, we noticed that in SEM pictures the PPC particles were comprised of microparticles whose size was only about 1 μm. So here the size of 5.93 μm should be the magnitude of aggregates size. The actual PPC particles prepared by SEDS should be much smaller than those from the conventional method, thus facilitating its dissolution rate and further enhancing the bioavailability of Pur which should be confirmed in future research.

4. Conclusions

In this study, the feasibility of preparing a phospholipids complex using SEDS was investigated for the first time. Compared with GAS method, the SEDS was more effective in the reduction of puerarin particle size to submicron range. The SEDS process for the preparation of PPC had been optimized at the pressure of 10 MPa, temperature of 35 °C, flow rate of CO_2 45 ml/min, flow rate ratio of drug to CO_2 solution of 1%, and Pur concentration of 100 mg/ml. Agglomerates of 5.93 μm and well-formed primary particles of only about 1 μm were obtained under these optimized conditions. The concentration of solution, flow rate of CO_2 and relative flow rate of solution to CO_2 were the major parameters controlling particle size and morphology. The results of this study demonstrated that a supercritical process could be an efficient method for phospholipids complex formation. Since SEDS gave micronized drug or drug phospholipids coprecipitates with much smaller size in powdered form in one step, it showed many advantages over conventional methods for pure drug and drug phospholipids complex preparation. Besides phospholipids complex, the SEDS process may provide other different interesting applications in pharmaceutical technology.

The Pur and phospholipids particles were also prepared successfully by GAS. The GAS processed Pur crystals showed more ordered appearances with cleaner surfaces in prisms. It had been proved that adopting a GAS process to recrystallize pharmaceutical compounds would provide highly versatile methodology to generate new polymorphs of drugs in addition to conventional crystallization techniques. The obtained experimental results motivated further work and provided directions for future experimental and theoretical investigation about the potential of the GAS recrystallization technology.

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