Micronization of *Magnolia* Bark Extract with Enhanced Dissolution Behavior by Rapid Expansion of Supercritical Solution

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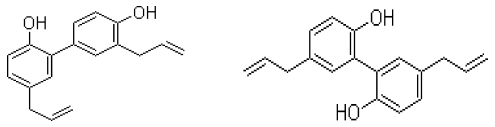
Received March 30, 2009; accepted October 24, 2009; published online November 10, 2009

A rapid expansion of supercritical solution (RESS) technology was presented for the micronization of Chinese medicinal material. *Magnolia* bark extract (MBE) obtained by supercritical carbon dioxide ($scCO_2$) extraction technology was chosen as the experimental material. RESS process produced 4.7 μm size MBE micropar ticles (size distribution, 0.2—24.1 μm), which was significantly smaller than the 55.3 μm size particles (size distribution, 8.3—102.4 μm) obtained from conventional mechanical milling. Dissolution rate study showed that drug dissolution was significantly enhanced by the RESS progress. At 90 min, the amount dissolved of mechanical milling MBE was 6.37 mg·l^{-1}, which was significantly lower than that of micronized MBE (14.77 mg·l^{-1}), according to the results of ANOVA ($p<0.01$). The effect of extraction temperature (30, 40, 50 °C), extraction pressure (200, 250, 300 bar) and nozzle size (50, 100, 200 μm) on the size distribution of microparticles was investigated. The characteristics of microparticles were also studied by differential scanning calorimetry (DSC), infrared spectroscopy (IR), scanning electron microscopy (SEM), and image analysis. This study demonstrates that RESS is applicable for preparing microparticles of MBE at low operating temperature; the process is simple without residual solvent.

Key words supercritical dioxide; micronization; rapid expansion of supercritical solution; magnolol; honokiol

Supercritical carbon dioxide ($scCO_2$) extraction technology is widely used in food preparation[1,2] and Chinese medicinal material extraction.[3,4] However, many Chinese medicinal material extracts have poor solubility or slow dissolution rate, which may limit absorption especially of drugs administered orally. Although various technologies can enhance the dissolution rate of poorly water-soluble drugs,[5—7] some disadvantages still remain, such as the need to use organic solvents, surfactants, or complex processes in their manufacture.[8,9] Mechanical methods include conventional methods for preparing Chinese medicinal materials particles. However the resultant particle size distribution is usually broad and does not reach to the micron or sub-micron level. Also, heat-sensitive materials degrade with milling. Furthermore, the extracts of Chinese medicinal material obtained by $scCO_2$ extraction technology can be sticky and butyraceous, and hard to pulverize by conventional mechanical disintegration. Rapid expansion of supercritical solution (RESS) technology is a new method to obtain microparticles in one step at low temperature without residual solvents and chemical reactivity for poor solubility drugs. The microparticles obtained by this method could greatly enhance drug solubility and bioavailability.[10,11] Although there have been many studies of RESS,[12,13] however, to date no study has reported preparation of Chinese medicinal material microparticles by this technology.

RESS technology was applied to prepare microparticles of Chinese medicinal mateatials in our work. *Magnolia* bark was chosen as the model herb. *Magnolia* bark is an aromatic herb; the bark from stems, branches, and roots is used as pharmaceutical item. It has broad biological activities[14—16]; At least 100 compounds have been identified in the bark including magnolol, honokiol, obovatol, 6′-*O*-methylhonokiol, magnaldehyde, magnolignan, and other water-insoluble active ingredients.[17,18] The known biological or pharmacological activity is associated with phenolic compounds such as magnolol and honokiol.[19—21] Magnolol and honokiol were used as the main index for product evaluation of *Magnolia* bark[22]; the structures are shown in Fig. 1. The aim of this study was to explore the possibility of applying the RESS method to micronize *Magnolia* bark extract (MBE) particles.



(A) (B)

Fig. 1. Structures of Honokiol (A, $C_{18}H_{18}O_2$) and Magnolol (B, $C_{18}H_{18}O_2$)

Experimental

Material *Magnolia* bark and β-cyclodextrin (β-CD) were provided by Department of Pharmacology, Zhujiang Hospital, Guangzhou, China. MBE (purchased from Guangzhou Institute of Pharmaceutical Industry, Guangzhou) obtained by $scCO_2$ extraction technology (extraction vessel I: pressure=250 bar, temperature=45 °C; extraction vessel II: pressure=60 bar, temperature=50 °C). The MBE was collected in extraction vessel II. MBE particles prepared by conventional mechanical milling were taken as the experiment material. The magnolol and honokiol content of MBE particles were 39.8% (w/w) and 40.6% (w/w), respectively. β-CD, magnolol and honokiol (pharmaceutical grade) were purchased from National Institute for Control of Pharmaceutical and Biological Products (China). Ethanol (analytical grade) was purchased from Tianjin Chemicals Company.

Apparatus The schematic diagram of the laboratory apparatus configured in RESS mode for this study is shown in Fig. 2. The schematic diagram was modified from Moribe *et al.*[12] The apparatus was mainly divided into two parts: an extraction chamber and an expansion chamber. The solvent CO_2 was introduced to the extraction vessel (internal volume: 1000 ml) in the extraction unit by a high-pressure syringe pump to a desired pressure (max. 500 bar). Two sintered plates were used on both the ends of extraction vessel to avoid any undissolved material carryover with the CO_2 flow. The extraction vessel containing solute was placed in a water bath to keep constant extraction temperature (±0.1 °C) by temperature controller. The pressure of extraction section was measured by online pressure transducer connected just before the valve and controlled through pump. After the extrac-

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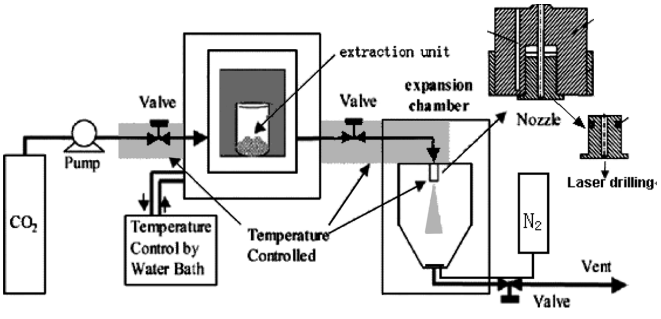


Fig. 2. Experimental Apparatus of Rapid Expansion of Supercritical Solutions (RESS)

Table 1. Factors and Levels for Orthogonal Test

Variable	Level		
	1	2	3
A. Extraction pressure (bar)	200	250	300
B. Nozzle size (μm)	50	100	200
C. Extraction temperature (°C)	30	40	50

Independent variables with three variation levels are listed. Extraction temperature varied at 30—50 °C, pressure 200—300 bar, and nozzle size 50—200 μm.

tion, definite amount of supercritical solution passed through the high-pressure stainless steel tubing and was expanded from a spray nozzle, which comprised a tungsten carbide stainless steel orifice in the precipitation unit. In the expansion chamber, rapid-phase change of sprayed supercritical solution across the nozzle into the atmospheric conditions induced high supersaturation of the solute and resulted in the formation of microparticles. The expansion chamber volume was 2 l and the spraying distance from the nozzle to a sintered plate placed on the bottom flange was 20 cm. Microparticles were precipitated on the sintered plate and dried by flowing nitrogen gas blown from the bottom of the expansion chamber.

Preparation of Micronized MBE Particles The extraction unit was loaded with 30.0 g of mixture of MBE particles and β-CD (1 : 2 w/w), which was placed in a water bath. Carbon dioxide was brought to the desired operating temperature and pressure conditions by controlling water bath temperature and the pump flow rate. The $scCO_2$ was then allowed to pass through the extraction unit to dissolve MBE and form a supercritical solution. Particles were formed by precipitation, due to the rapid reduction of solvent density during the expansion of supercritical solution across the nozzle to atmospheric conditions. Three nozzles of *L*/*D* ratio 20, 40, and 80 were used for expansion with a diameter of 200, 100 and 50 μm, respectively. Finally, microparticles were recovered on the sintered plate.

Optimization of RESS Process An orthogonal $L_9(3)^3$ test design was used to investigate the optimal preparation condition of micronized MBE particles with the particle size and the contents of magnolol and honokiol as indexes. Extraction temperature varied at 30—50 °C, pressure 200—300 bar, and nozzle size 50—200 μm. The influences of extraction temperature, pressure, and nozzle size on particle characteristics were studied. The experiments were conducted in triplicate under each processing condition. As seen in Table 1, nine tests were carried out at varied extraction temperature (30, 40, 50 °C), extraction pressure (200, 250, 300 bar), and nozzle size (50, 100, 200 μm). The range of each factor level was based on our previous results.

Characterization of Microparticles. Particle Morphology The microparticles were evaluated morphologically by scanning electron microscopy (SEM) (S-3000N, Japan). Pictures were taken at 20 kV and magnifications of 1500× and 2000×.

Particle Size Distribution Particle size of microparticle samples was determined by image analysis of photomicrographs (Q500MC, Germany). A certain number of particles were selected and analyzed by statistical software *SSPS 13.0*.

Differential Scanning Calorimetry A DSC system (NETZSCH DSC204, Germany) was used at scanning speed of 10 °C/min in the temperature range 30—250 °C to analyze 4.0 mg of powder samples in sealed aluminum pans.

Infrared Absorption Spectroscopy Studies of the IR spectra of the products were conducted with an IR spectrometer (FT-IR VECTOR-22 Bruker, Germany) using the KBr disc method (4000—450 cm^{-1}). The samples for analysis were mixed with desiccated KBr at about 1 : 100 (sample: KBr) ratio and pressed to form KBr pellets. The samples have been kept in a desiccator for 24 h before analyzing. For each sample, 16 scans were collected at a resolution of 4 cm^{-1} over the wave number region 4000—400 cm^{-1}.

Dissolution Studies Dissolution studies were performed following the ***Ch.P.***×C rotation-basket method in 900 ml of methanol–water (15 : 85) at 37 °C and 100 rpm. Accurately weighted samples containing the equivalent of 100 mg of the amount of magnolol and honokiol were spread in the dissolution medium. Then, 5 ml dissolution medium was piped at certain time (5, 10, 20, 30, 45, 60, 90 min) and passed through a 0.45 μm membrane filter. The withdrawn dissolution medium was replaced with equal quantities of fresh dissolution medium to maintain a constant volume. The concentrations of magnolol and honokiol in the withdrawn samples were determined by HPLC.

HPLC Conditions 1) The HPLC system consisted of a pump, a column (Hypersil BDS, 5 μm, 4.6×150 mm), an auto-injector and a UV-detector (Agilent 1100, U.S.A.). HPLC was performed at a flow-rate of 1.0 ml·min^{-1}, temperature 30 °C, using a mobile phase of methanol–water (78 : 22), and components were detected with a UV detector at 294 nm. The HPLC method was used for determining the contents of magnolol and honokiol in microparticles and MBE particles. Briefly, 20 mg of microparticles or MBE particles was weighed in a 50 ml volumetric flask, and reached the scale accurately with methanol. Then, 5 ml diluted solutions were absorbed and put into another 50 ml volumetric flask, and reached the scale accurately with methanol.[22)]

2) The HPLC system consisted of a pump, a column (Hypersil BDS, 5 μm, 4.6×150 mm), an auto-injector and a differential Refractive Index detector (Agilent 1100, U.S.A.). HPLC was performed at a flow-rate of 0.8 ml·min^{-1}, temperature 25 °C, using a mobile phase of methanol–water (15 : 85), and components were detected with a Refractive Index detector. The HPLC method was used for determining the β-CD in microparticles.[23)]

Results and Discussion

Preparation of Microparticles At the beginning of our work, microparticles in power form could not be obtained by RESS method. The particles collected on the sintered plate were viscous and sticky. This might be caused by the MBE, which contained some butyraceous and sticky ingredients. These ingredients were dissolved in the $scCO_2$ and precipitated in the expansion process. To obtain microparticles in powder form on one hand and further to increase its dissolution rate on the other hand, we need to search for a suitable adjuvant. It was found that addition of certain amount of β-CD to MBE (β-CD : MBE 2 : 1 w/w) resulted in microparticles in powder form. Furthermore, the β-CD was hardly soluble in $scCO_2$ fluid and would not precipitate combined with microparticles in the expansion chamber.

Optimization of Preparation Parameters of Microparticles Extraction pressure, extraction temperature, and nozzle size are generally considered the most important factors that affect particle size and yield (%) of the amount of magnolol and honokiol. Independent variables with three variation levels, *A* (extraction pressure 200, 250, 300 bar), *B* (nozzle size 50, 100, 200 μm), and *C* (extraction temperature 30, 40, 50 °C) are listed in Table 1. In the present study, all selected factors were examined by orthogonal $L_9(3)^3$ test design. The total evaluation index was used for statistical analysis. The analysis results of orthogonal test for microparticles, performed by statistical software *SPSS 13.0* with ANOVA method, are presented in Table 2. As seen in Table 2, the smallest mean size of microparticles sample was 4.7±2.1 μm and maximum yield of the amount of magnolol and honokiol was 90.3±1.0% (w/w). In view of orthogonal analysis, we

Table 2. Results of Orthogonal Experiment of Microparticles ($x\pm s$, $n=3$)

Number	*A*. Extraction pressure (bar)	*B*. Nozzle size (μm)	*C*. Extraction temperature (°C)	Mean size (μm)	Amount (%)
1	200	50	30	6.1±2.4	75.1±0.8
2	200	100	40	5.6±1.7	81.1±1.2
3	200	200	50	10.2±3.1	86.1±0.6
4	250	50	40	6.7±2.3	86.8±0.4
5	250	100	50	4.7±1.1	90.3±1.0
6	250	200	30	4.9±1.5	84.2±0.3
7	300	50	50	9.3±3.6	87.3±0.8
8	300	100	30	8.4±3.2	82.5±0.5
9	300	200	40	5.1±1.4	85.8±0.6
Mean size					
K1	21.90	22.10	19.40		
K2	16.30	18.70	17.40		
K3	22.80	20.20	24.20		
R	6.50	3.40	6.80		
Amount					
K1	242.30	249.20	241.80		
K2	261.30*	253.90	253.70		
K3	255.60*	256.10	263.70*		
R	19.00	6.90	21.90		

R refers to the result of extreme analysis; $*p<0.05$ *vs.* K1. The smallest mean size of microparticles sample was 4.7±2.1 μm and maximum yield of total-phenol amount was 90.3±1.0%. Factor influencing mean size and yield (%) of total-phenol amount are listed in decreasing order as follows: $C>A>B$, $C>A>B$, respectively. The smallest mean size of microparticles was obtained when extraction pressure, nozzle size, and extraction temperature were $A_2B_2C_2$ and the maximum yield of total-phenol amount was obtained when extraction pressure, nozzle size, and extraction temperature were $A_2B_3C_3$.

adopted statistical software to calculate the values of K and R. Factor that influenced the mean size and yield (%) of magnolol and honokiol are listed in decreasing order as follows: temperature>pressure>nozzle size, temperature>pressure>nozzle size, according to the R value. Thereofre the smallest mean size of microparticles was obtained when extraction pressure, nozzle size and extraction temperature were $A_2B_2C_2$ and the maximum yield of the amount of magnolol and honokiol was obtained when extraction pressure, nozzle size, and extraction temperature were $A_2B_3C_3$. According to the results of ANOVA (Table 3), we found a change in the factor levels for each factor had no statistically significant effect on particle size ($p>0.05$), whereas a change in pressure and temperature had a significant effect on yield of the amount of magnolol and honokiol ($p<0.05$). The biggest mean size was 10.2 μm and the smallest mean size was 4.7 μm with no statistically significant change among these 9 results. This may associate with agglomeration of particles occurring in expansion zone as the particles collide with each other and coagulate to form bigger particles in expansion process.[24] In view of yield, the cost of production, and time for industrialization, we set the optimum technology as follows: $A_2B_2C_3$ (250 bar, 100 μm, 50 °C). Through confirmatory test, we obtained MBE microparticles with mean size of 4.7 μm; yield of the amount of magnolol and honokiol was 91.2% (w/w).

Table 3. Results of ANOVA by *SPSS 13.0*

Dependent variable: mean size

Source	Type III sum of squares	df	Mean square	*F*	*p*
Temperature	8.142	2	4.071	0.548	0.646
Nozzle size	1.936	2	0.968	0.130	0.885
Pressure	8.269	2	4.134	0.556	0.643

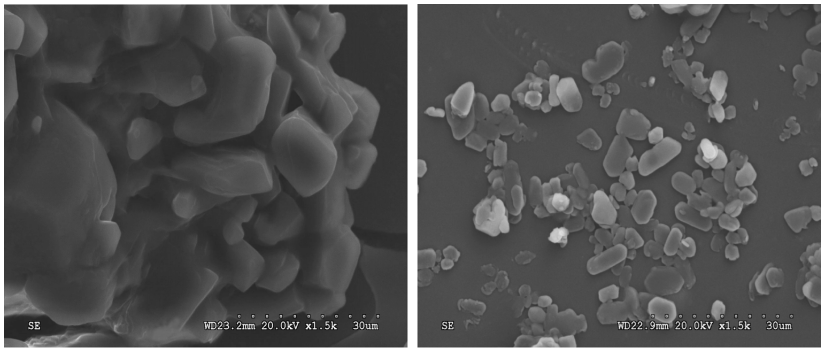
Dependent variable: together amounts

Source	Type III sum of squares	df	Mean square	*F*	*p*
Temperature	0.008	2	0.004	26.965	0.036*
Nozzle size	0.001	2	0.000	2.819	0.262
Pressure	0.006	2	0.003	21.309	0.045*

$*p<0.05$. A change in the factor levels for each factor (*A*, *B* or *C*) had no statistically significant effect on particle size ($p>0.05$), however, a change in factor *A* or *C* had significant effect on yield of total-phenol amount ($p>0.05$).

Characterization of Microparticles. Particle Morphology and Size Distribution Figure 3A shows that the MBE particles were irregular and gathered together. The microparticles obtained under the optimum preparation conditions were grayish, irregular schistous (Fig. 3B) and the average size was 4.7 μm, which was nearly 11 times smaller than that of previous MBE particles (55.3 μm). The particle size distribution of micronized MBE was 0.2—24.1 μm (Fig. 4), which is narrower than that of MBE particles, 8.3—102.4 μm. The SEM pictures showed all microparticles samples had similar morphologies and sizes (Fig. 5).

Result of Differential Scanning Calorimetry Analysis The obtained particles were analyzed by differential scanning calorimetry (DSC). The melting point of unprocessed MBE was 65.0 °C whereas RESS-processed MBE had a melting point 66.2 °C. As seen in Fig. 6, the peak of micronized particles was higher and narrower than that of MBE particles. This can be attributed to reduction of particle size and the amount of magnolol and honokiol elevation after RESS processing. The mean particle size reduced by 11 times and the amount of magnolol and honokiol elevated 10.8%. There was no peak corresponding to β-CD melting point in the curve d, suggesting the absence of β-CD in microparticles. Futher evidence of absence of β-CD in microparticles was the results of HPLC analysis. As seen in Fig. 7, only 0.11% (w/w) β-



A (×1. 5K, bar=30μm) **B** (×1. 5K, bar=30μm)

Fig. 3. SEM Pictures of MBE Particles (A, ×1.5K, Bar=30 μm) and Microparticles (B, ×1.5K, Bar=30 μm)

The MBE materials were irregular particles and gathered together. The mean size was 55.3 μm with distribution from 8.3—102.4 μm. The microparticles obtained under the optimum preparation conditions were grayish, irregular schistous and the mean size was 4.7 μm with distribution from 0.2—20.4 μm.

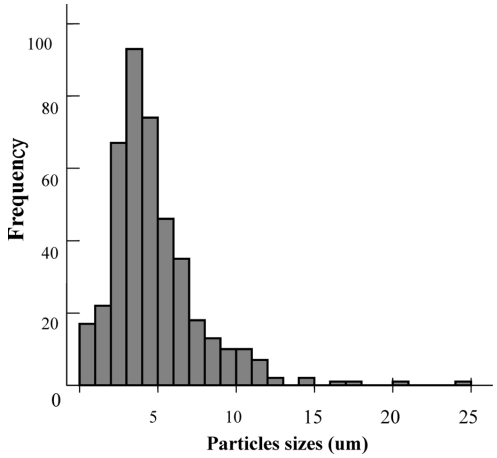


Fig. 4. Size Distribution of Microparticles from SEM Pictures (420 Particles Selected), RESS Treated Sample with the Preparation Condition of 250 bar and 50 °C, Nozzle Size=100 μm

The mean size of the microparticles was 4.7 μm with distribution from 0.2—20.4 μm.

CD was found in microparticles, which could be neglected. The MBE peak disappeared in curve e, but some (16.8%, w/w) magnolol and honokiol was detected by HPLC. These results demonstrate that magnolol and honokiol were entrapped in β-CD and the viscosity and sticky substances in MBE were also entrapped in β-CD, which supports the amount of magnolol and honokiol elevation in microparticles.

IR Spectroscopy Analysis[25] Figure 8 shows the IR spectra of MBE, β-CD, physical mixture, and product treated by RESS. Spectrum of the physical mixture can be considered as the result of addition of MBE and β-CD spectra. On the contrary, in the spectrum of the inclusion compound, shifts, disappearances, or attenuation of the characteristic MBE bands reveal a modification of the environment of MBE. For example, the C–OH bending band at 1217 cm^{-1} is no more detectable at this wavelength in the complex. The CH band at 1890 cm^{-1} belongs to the benzene derivatives of MBE and shifts to 1884 cm^{-1}. The CH aromatic deformation band at 907 cm^{-1} for MBE and the CH aliphatic deformation band at 1368 cm^{-1} for β-CD were also changed to 912 and 1372 cm^{-1}, respectively. These changes are probably related to the interaction and formation of intermolecular bonds between the guest (MBE) and the host (β-CD).

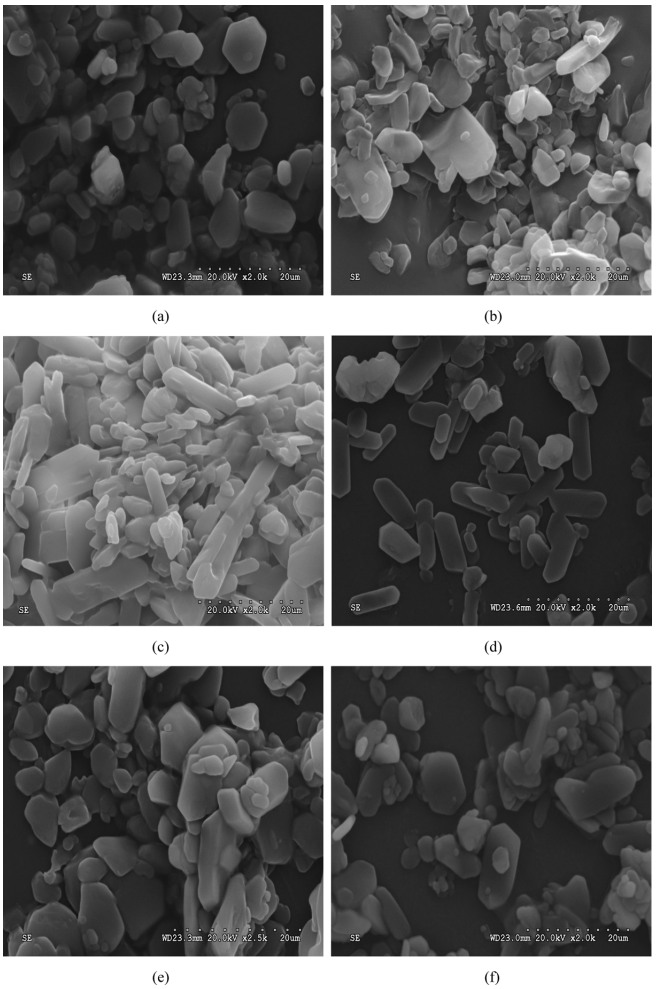


Fig. 5. SEM Micrograph of Microparticles Produced from RESS with Different Supercritical Conditions: (a) 40 °C/200 bar, (b) 40 °C /250 bar, (c) 30 °C/250 bar, (d) 50 °C/250 bar, (e) 30 °C/300 bar, and (f) 40 °C/300 bar, Bar Length=20 μm (×2K)

The microparticles produced from RESS with different supercritical conditions similar morphologies and sizes.

Dissolution Behavior Figure 9 shows the dissolution profiles for MBE particles and microparticles. The dissolution study was conducted with the amount of magnolol and

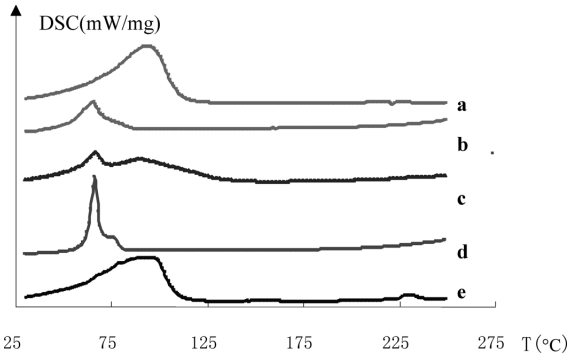


Fig. 6. DSC Curves of β-CD (a), MBE Particles (b), Physical Mixture of MBE Particles with β-CD (c), Microparticles (d), and Residual Material in Extraction Unit (e); RESS-Treated Sample with Preparation Condition of 250 bar/50 °C, Nozzle Size = 100 μm

The melting point of unprocessed MBE is 65.0 °C whereas RESS processed MBE had a melting point of 66.2 °C. The peak of micronized particles was higher and narrower than that of material particles. This can be attributed to the reduction of particle size and purity elevation after RESS-SC processing. There was no peak corresponding to β-CD melting point in curve d, suggesting absence of β-CD in microparticles. Further evidence of absence of β-CD in microparticles is the results of HPLC analysis. Only 0.11% (w/w) β-CD was found in microparticles, which could be neglected. The MBE peak disappeared in curve e, however, some (16.8%, w/w) magnolol and honokiol was detected by HPLC. These results demonstrate that magnolol and honokiol were entrapped in β-CD and the viscosity and sticky substances in MBE were also entrapped in β-CD, supporting the amount of magnolol and honokiol elevation in microparticles.

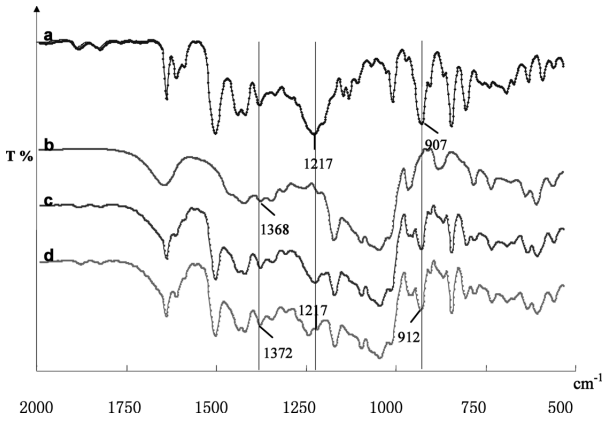


Fig. 8. IR Spectra of MBE (a), β-CD (b), Physical Mixture (c), and Product Treated by RESS (d)

The samples for analysis were mixed with desiccated KBr at about 1 : 100 (sample: KBr) ratio and pressed to form KBr pellets. For each sample, 16 scans were collected at a resolution of 4 cm^{-1} over the wave number region 4000—400 cm^{-1}. The C–OH bending band at 1217 cm^{-1} is no more detectable at this wavelength in the complex. The CH aromatic deformation band at 907 cm^{-1} for MBE and the CH aliphatic deformation band at 1368 cm^{-1} for β-CD were also changed to 912 and 1372 cm^{-1}, respectively. These changes are probably related to the interaction and formation of intermolecular bonds between the guest (MBE) and the host (β-CD).

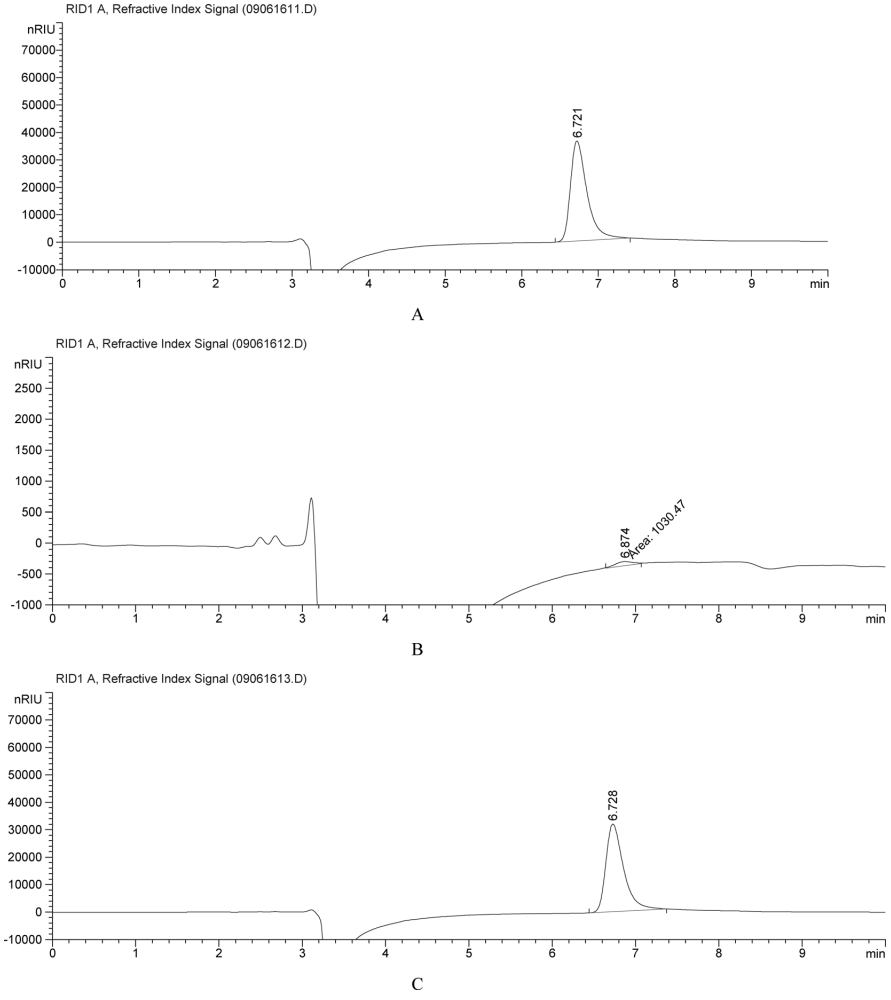


Fig. 7. Chromatograms of Reference Substances (A, β-CD) and Samples (B, Microparticles; C, Physical Mixture of β-CD with Microparticles)

Only 0.11% (w/w) β-CD was found in microparticles, which could be neglected.

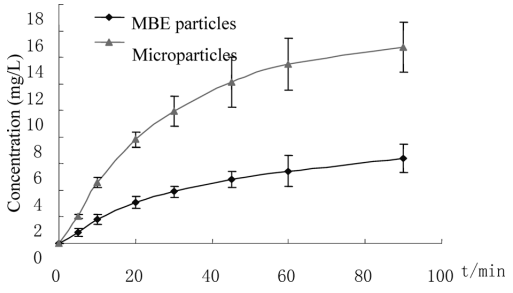


Fig. 9. Dissolution Curves for MBE Particles and Microparticles Prepared at 250 bar/50 °C, Nozzle Size=100 μm (n=3)

At 90 min, the amount dissolved of micronized MBE was 14.77 $\mu g \cdot ml^{-1}$, which is significantly higher than that of MBE particles (6.37 $\mu g \cdot ml^{-1}$).

honokiol as index for a time period of 90 min. The results suggest that drug dissolution was significantly enhanced by the RESS procedures. At 90 min, the amount dissolved of MBE particles was 6.37 $\mu g \cdot ml^{-1}$, which is significantly lower than that of microparticles (14.77 $\mu g \cdot ml^{-1}$), according to the results of ANOVA (F=73.59, p=0.001). Overall, the present study shows that micronized MBE has higher dissolution rate than MBE particles, possibly due to particle size reduction of microparticles. Furthermore, the amount of magnolol and honokiol elevation may act to enhance the dissolution rate; the amount of magnolol and honokiol in microparticles and MBE particles was 91.2% and 80.4%, respectively. Probably, it was not only the size of the particles that contributed to the dissolution but also the difference in weight, *i.e.* the MBE particles contained a product that was removed (or rather not extracted) in the RESS process. This could also contribute to a slower dissolution rate in the case of MBE particles.

Conclusion

The composition of MBE obtained by $scCO_2$ extraction technology contained butyraceous and sticky ingredients. It was hard to obtain microparticles in power form by conventional technology. RESS technique, however, was successfully applicable for microparticles production of MBE in this experiment. RESS could significantly micronize the particles size and purify the material in the same one-step process. During the RESS process, the viscosity and sticky substances in MBE were entrapped in β-CD, which is an important factor assisting to obtain microparticles in powder form. The present RESS technology provides an alternative and convenient method to increase MBE dissolution rate.

Acknowledgements The authors are grateful to Prof. K. Zhang for his assistance with DSC analysis and Dr. F. Ren for her help in particle size analysis. Financial support for Zhongyi Zhang was provided by National Nature Science Foundation of China.

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