Solubility and Micronization of Griseofulvin in Supercritical CO_2 with Cosolvent Acetone

Guoqin Hu*, Hongyan Chen, Jianguo Cai, Xiu Deng

Chemical Engineering Research Center, East China University of Science and Technology, Shanghai 200237, China

Abstract Griseofulvin (GF) is an antifungal drug whose pharmaceutical activity can be improved by particle size reduction. In this study the rapid expansion of supercritical solution (RESS) was employed to micronize GF. Carbon dioxide (CO_2) with cosolvent acetone was chosen as a supercritical mixed solvent. The solubility of GF in supercritical CO_2 with cosolvent acetone was measured using a dynamic apparatus at pressures between 120 and 320bar, temperatures at 313,323 and 333K and cosolvent concentration at 1.5, 3.0, 4.5 and 6.0 mol%. The effect of the pre-expansion pressure, the pre-expansion temperature, diameter of the nozzle, concentration of cosolvent and expansion chamber temperature on the precipitated particles was investigated. The results showed that the mean particle size of griseofulvin precipitated by RESS was less than 1.2 μm. An increase in pre-expansion pressure, pre-expansion temperature, concentration of cosolvent resulted in decrease in particle size with the range of operating condition studied. With the decrease of nozzle diameter and pre-expansion temperature the particle size reduces. The crystallinity and melting point of the original material and the processed particle by RESS were tested by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). No evident modification in the crystal habit was founded at the experimental conditions tested. The morphology of particles precipitated was analyzed by scanning electron microscopy (SEM).

Keywords griseofulvin, micronization, RESS process, cosolvent

1. INTRODUCTION

Many drugs exhibit poor solubility in water, and absorption rate in the gastrointestinal tract are very low because of its big size. In the pharmaceutical industry, micronization techniques are used to improve dissolution rates of drugs into the biological environment. Several conventional techniques have been utilized for particle size reduction. These include mechanical comminution (crushing, grinding, and milling), spray-drying, freeze-drying, and recrystallization of the solute particles from solutions using liquid antisolvents. The disadvantages of using these conventional techniques are thermal and chemical degradation of products, large amounts of solvent used and associated disposal problems, broad particle size distributions, and solvent residues [1].

A novel technique, the rapid expansion of supercritical solutions (RESS), was recently developed for micronization of particles. In the RESS process, solutes are dissolved in a supercritical fluid, resulting in a solute-laden supercritical phase. By reduction of the pressure

*Corresponding author. Tel: (021) 64253329; E-mail: hu-guoqin@sohu.com.

by an expansion device, fine particles with a narrow size distribution can be obtained. One advantage of micronization by the RESS process is the ability to produce solvent-free product. Low critical temperature solvents such as CO_2 (T_C = 304.25K) can be used for the precipitation of thermally labile materials without the risk of degradation. The application of RESS can also be extended to the processing of shock and chemically sensitive substances, for the formation of thin films and fibers, for microencapsulation, and for mixing of powders [2].

Griseofulvin (GF) is a pharmaceutical with a powerful antifungal action. However, it gives rise to problems of bioavailability and toxicity. Indeed, the percentage of GF that is absorbed (bioavailability) compared to the initial dose, is low. Moreover, its toxicity threshold is close to the actual therapeutic dosage. Therefore, it should be safer and better to use GF if it were possible to reduce the dose through an increase in its bioavailability. Bioavailability should be improved by a further comminution with regard to the particle size usually used [3].

Reverchon[4] used CHF_3 as supercritical solvent to micronize GF. However, Its cost was very high. Chen Hongyan [5] used CO_2 as supercritical solvent, but the solubility of GF in supercritical CO_2 was very low. In this study the RESS was employed to micronize GF. The supercritical fluid was carbon dioxide with cosolvent acetone. The cosolvent acetone is to increase the solubility of GF in supercritical CO_2.

2. MATERIAL, EXPERIMENTAL APPARATUS, and METHODS

2.1 Materials

Griseofulvin (purity 99.2%) was supplied by Shanghai Chinese Pharmaceutical Factory. It consisted of particles ranging from about 2.0 to 18μm.

CO_2 (purity 99.8%) was purchased from Shanghai Nanhui Chemical Company and acetone (analytic purity >99.5%) from Shanghai Feida Company.

2.2 Experimental Apparatus

The experimental apparatus used for RESS is shown in figure 1. Liquid CO_2 from cylinder was fed to a compressor and delivered through a preheating coil, which was controlled by a water bath. The supercritical CO_2 at the desired dissolving pressure and temperature then flows through the dissolver where the griseofulvin, blended with cotton wool to prevent solute caking, was charged. The cosolvent was added to dissolver by plunger pump which can control the flow rate of the cosolvent. The filter paper was installed in the outlet of the dissolver to prevent physical entrainment of solute. The stream flowing from the dissolver entered into the injector formed by a thermostated tube (L = 200mm, D =3mm, stainless steel) that contains an interchangeable laser-drilled nozzle. Then the solute - laden CO_2 was jetted to the expansion chamber (L=300mm, D=100mm) which temperature was controlled by a thermostated water bath, causing the dissolved solute to precipitate out from the fluid phase. The precipitated particles were collected by glass slides. In this study nozzles with diameters of 20,30,40,50,60 μm and the length of 350 μm was used. Temperatures in the pre-expansion and nozzle part were controlled through wire heater connected to a controller.

The solubility of griseofulvin in supercritical CO_2 with cosolvent acetone was measured. The apparatus was similar to the apparatus of RESS. The expansion chamber of RESS experiment was replaced by two U-shaped glass tubes and a cooled bath. The expanded solution flowed through two U-shaped glass tubes packed with wool and immersed in a cooled bath. The solubility was determined by weighting the collected solute with an analytical balance and the total volume of

gas measured with the wet test meter at ambient conditions.

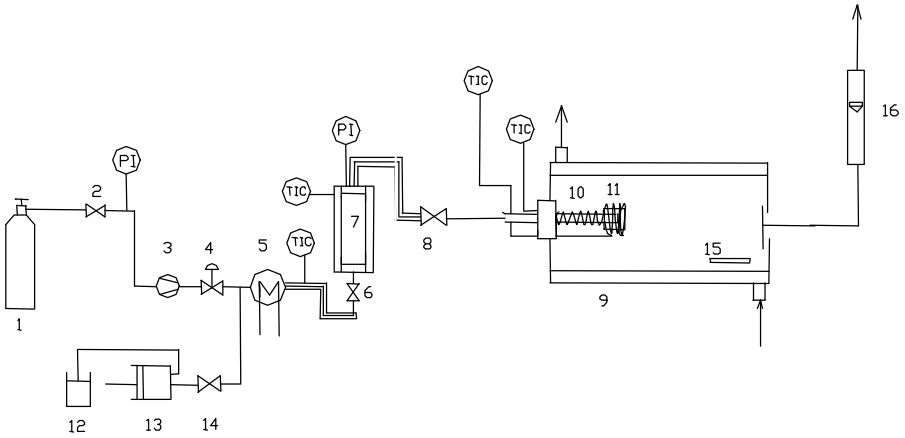


Figure 1 Schematic diagram of the apparatus

1 CO_2 cylinder; 2, 6, 8,14 valve; 3 compressor; 4 regulator; 5 heater;
7 extractor; 9 expansion chamber; 10 pre-heater; 11 heater of nozzle;
12 feed tank of cosolvent; 13 plunger pump; 15 glass slide; 16 rotameter;

2.3 Particle Characterization

The crystallinity and melting point of the original material and precipitates were tested by X-ray diffraction (XRD, Japan, Kugaku, D/MAX-rB) and differential scanning calorimetry (DSC, Universal V2.3C TA Instrument). The morphology of particles precipitated by RESS was analyzed by scanning electron microscope (SEM, British, Cambridge, S-250MK3). Particle size distribution (PSD) was subsequently determined by counting at least 150 particles from the optical microscope images.

3. RESULTS AND DISCUSSION

3.1 Solubility Experiments

The solubility in supercritical solvent is a key factor to perform RESS process. Because of the solubility of griseofulvin in supercritical CO_2 is very low we try to modify the polar of CO_2 by adding to cosolvent to improve the solubility of griseofulvin in supercritical fluid. We used acetone, methanol, ethanol and ethyl acetate as cosolvents to study the solubility of griseofulvin. The cosolvent acetone and ethyl acetate can increase the solubility of griseofulvin in supercritical fluid. But because at present acetone has been used as extractive solvent in production of GF and has lower boiling point we chose acetone as cosolvent. We emphatically investigate the effect of acetone as cosolvent on the solubility of griseofulvin in supercritical CO_2.

The isothermal solubilities of GF correlated by modified Peng-Robinson equation, at temperatures 313, 323 and 333K and pressures range from 120 to 320bar, in binary mixture of 3.0mol% acetone-CO_2 was shown in figure 2. The phenomenon of retrograde vaporization is also observed in the ternary system as a consequence of the influence of density and vapor pressure on the solute solubility. Meanwhile crossover point $((\partial y/\partial T)_P = 0)$ was observed. The crossover pressure is 170bar or so. The correlation of the parameter of GF was shown in table 1.The results of correlation is in agreement with literature.

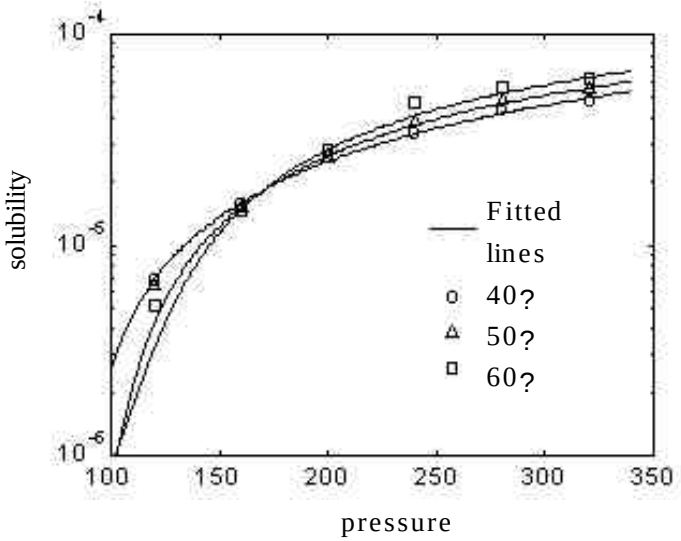


Figure 2 correlations of solubilities in CO_2-acetone mixture using modified PR equation

Table 1 Correlation parameter of GF using MPR equation

temperature (K)	a_2 (Pa m^6/mol^2)	b_2 ($m^3/mol*10^6$)	%AARD
313	9.01	107.96	7.0
323	8.85	115.10	4.6
333	7.74	113.71	16.9

3.2 RESS Experiments

The following tables showed the experimental series performed to analyze the effects on crystal characteristics of variations in the following selected process parameters: (1)pre-expansion pressure, (2) different nozzle size , (3) concentration of cosolvent, (4) pre-expansion temperature, (5) expansion chamber temperature .

3.2.1 Pre-expansion Pressure

Table 2 Pre-expansion pressure and results

P (MPa)	Diameter of GF(μ m)
20	1.01
24	0.99
28	0.98
32	0.97

When the parameter of pre-expansion pressure was studied, the other parameters such as pre-expansion temperature, nozzle temperature, diameter of nozzle, expansion chamber temperature were maintained constant. From the results of table 2 the pre-expansion pressure had slight effect on the precipitates. An increase in pre-expansion pressure resulted in a slight decrease in particle size. This may be due to the fact that when the pressure was raised, the solubility of GF

in supercritical CO_2 with cosolvent acetone was increased. And then the supersaturation of GF in the same pre-expansion condition was enhanced.

3.2.2 Nozzle Size

Table 3 Size of nozzle and results

Size of nozzle(μ m)	Diameter of GF(μ m)
20	0.88
30	0.90
40	0.92
50	1.03
60	1.10

The effect of nozzle size on precipitates was investigated for sizes from 20 to 60 when other parameters were changeless. The average diameters of the particles precipitated by RESS were 1 μ m or so. An increase of the nozzle diameter caused the increase in particle size. Following the literature [6] after supercritical solution jets from exit of nozzle, the supersaturation of the solute increases promptly, the generation and growth of crystal nuclei can be finished between exit of nozzle and Mach disk. At the same condition of supersaturation the smaller the nozzle diameter is, the faster the jetting velocity is, the shorter the growth of crystal nuclei. Therefore an increase of the nozzle diameter caused the increase in particle size. This result showed agreement with regard to literature[7].

3.2.3 Concentration of Cosolvent

Table 4 Concentration of cosolvent and results

Concentration of acetone (mol %)	Diameter of GF(μ m)
0.50	1.03
1.00	0.98
1.50	0.90
2.00	0.84

The influence of this parameter can be evaluated from the results in table 4. The concentration of cosolvent acetone increased , the average size decreased from 1.03μ m to 0.84μ m. Following the literature a decrease of the solubility of solute in supercritical fluid induces a marked increase of particle sizes due to lower supersaturation and to the decrease of nucleation rate [8,9]. Because of the increase of concentration of cosolvent acetone causing the increase of the solubility of GF in supercritical mixed fluid, and as a consequence, the average size decreased from 1.03μ m to 0.84 μ m.

3.2.4 Pre-expansion Temperature

When the effect of the pre-expansion temperature on precipitates was studied, the other parameters were kept constant. The results showed that an increase in pre-expansion temperature resulted in an increase in particle size. This may be due to the fact that as the pre-expansion temperature increased, the supersaturation and nucleation rate of GF in supercritical solution decreased and the particle size became bigger. The similar result was founded by Charoenchaitrakool [10] as to benzoic acid in supercritical CO_2.

Table 5 pre-expansion temperature and result

Pre-expansion temperature(K)	Mean diameter of precipitate (μ m)
333	0.97
353	0.99
373	1.01
393	1.08
413	1.10

3.2.5 expansion chamber temperature

Table 6 Expansion chamber temperature and result

Expansion chamber temperature (K)	Mean diameter of precipitate (μ m)
283	1.10
293	1.03
303	0.99
313	0.98
323	0.96

When the effect of the expansion chamber temperature on precipitates was studied, the other parameters were kept constant. The results showed that an increase in expansion chamber temperature resulted in a decrease in particle size. This may be due to the fact that as the expansion chamber temperature increased, the supersaturation and nucleation rate of GF in supercritical solution enlarged and the particle size became smaller.

3.2.6 Particles Characterization

The following process parameters of particles characterized are extraction temperature 333K, Pre-expansion pressure 240bar, pre-temperature 383K, diameter of nozzle 50μ m, expansion chamber temperature 303K and 3.0 mol% acetone concentration.

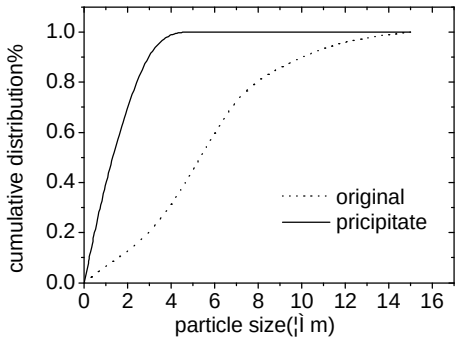
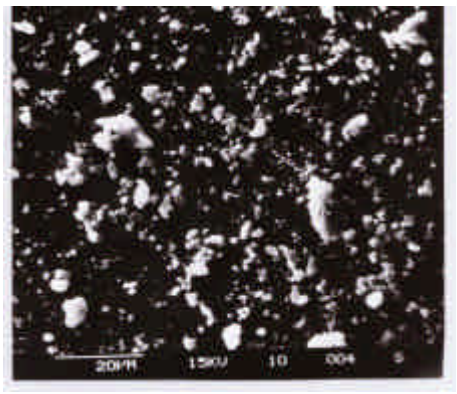
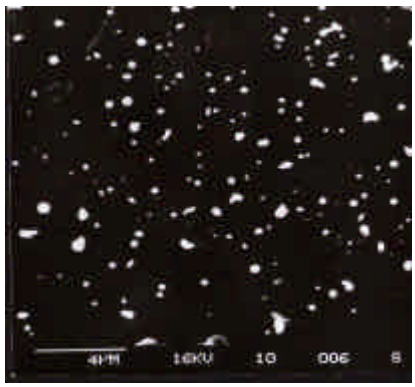


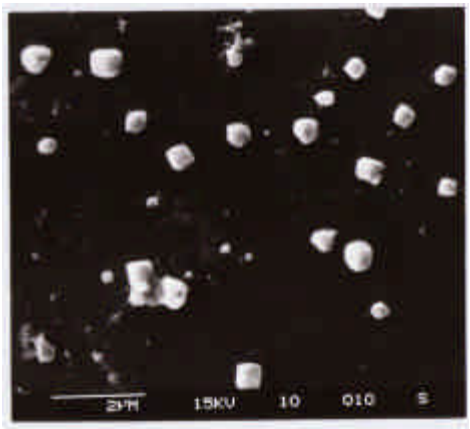
Figure 3 cumulative distribution profile of GF



(a)

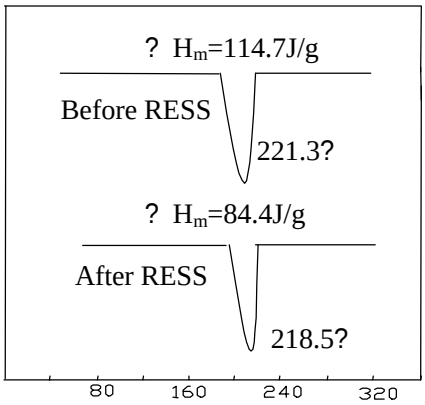


(b)



(c)

Figure 4 SEM of GF (a) original; (b) (c) microparticles by RESS;



Temperature (?)

Figure 5 DSC results of griseofulvin before and after RESS processing.

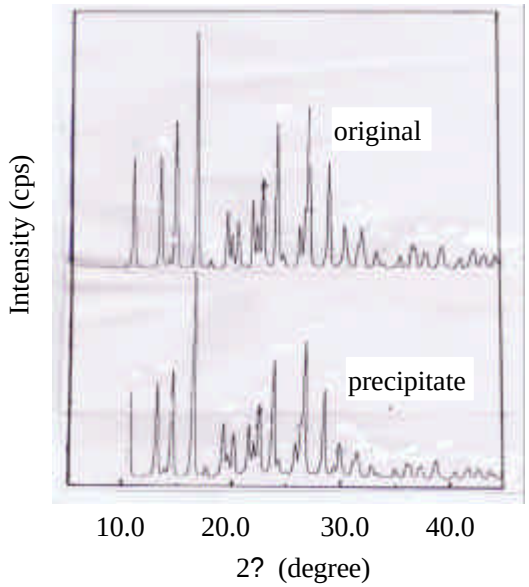


Figure 6 XRD patterns of GF before and after RESS processing.

The cumulative distribution of GF before and after RESS was shown in figures 3.The SEM images of particles before and after RESS was shown in figures 4. Figure 3 showed that the average size of the particles micronized by RESS was smaller than that of the original GF. Meantime, the cumulative distribution of the micronized GF was narrower than that of the original. In this study we only founded quasispherical particles. The particles obtained in this study were equivalent to that in Reverchon RESS experiment.

As is illustrated in figure 5 and 6, similar melting points and X-ray diffraction patterns were observed for the original material and the precipitates obtained by RESS. On the basis of the melting point and XRD investigation the processes griseofulvin is similar to the 0riginals.

However , the heat of melting (? H_m) obtained from DSC analysis and the intensity of the XRD peaks of the processed griseofulvin were slightly lower compared with the original material. These results indicated a slight reduction in the degree of crystallinity of griseofulvin after processing with RESS.

4. CONCLUSIONS

Micronization of GF was successfully performed by RESS using supercritical CO_2 with cosolvent acetone. Increasing the pre-expansion temperature enlarged the particle size. Raising pre-expansion pressure, expansion chamber temperature and concentration of cosolvent degreased the particle size. The smaller the diameter of nozzle the smaller the particle preciptated by RESS is. The precipitates were analyzed for crystallinity using XRD and DSC. Only slight modification in the crystal habit was found in the experimental conditions.

REFERENCE

1 Subramaniam, B., Rajewski, R. A., Snavely, K., J. Pharm. Sci. 1997, 86 (8), 885.

2 Tom, J.W., Debenedetti, P. G., J. Aerosol Sci. 1991, 22(5), 555.

3 Shargel, L., Yu, A. B.C., Applied Biopharmaceutics and Pharmacokinetics ,

Prentice Hall: New York, 1980.
4 Reverchon, E., Porta, G.D., Taddeo, R., Ind. Eng. Chem.Res. 1995,24,4087.
5 CHEN Hongyan, dissertation of Ph. D. East China University of Science and Technology 1999.
6 Debenedetti. P. G., AIChE. J.1990, 36, 1289.
7 Peterson, R. C., Matson D. W., Polymer Engineering and Science, 1987,27 (22), 1693
8 Tom, J.W., Debenedetti, P. G., Jerome, R. J. Supercrit. Fluids. 1994, 7, 9.
9 Debenedetti, P. G., Int. Symp. Supercrit. Fluids (Strasbourg), 1994, 3, 213.
10 Charoenchaitrakool, M., Dehghani, F., and Foster, N. R., Micronization by Rapid Expansion of Supercritical Solutions to Enhance the Dissolution Rates of Poorly Water-Soluble. Ind. Eng. Chem. Res. 2000, 39: 4794—4802.