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International Journal of Pharmaceutics 308 (2006) 190–199
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Pharmaceutical Nanotechnology

Formation of phenytoin nanoparticles using rapid expansion of supercritical solution with solid cosolvent (RESS-SC) process

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Received 6 July 2005; received in revised form 22 September 2005; accepted 3 November 2005
Available online 13 December 2005

Abstract

Nanoparticles are of significant importance in drug delivery. Rapid expansion of supercritical solution (RESS) process can produce pure and high-quality drug particles. However, due to extremely low solubility of polar drugs in supercritical CO_2 (sc CO_2), RESS has limited commercial applicability. To overcome this major limitation, a modified process rapid expansion of supercritical solution with solid cosolvent (RESS-SC) is proposed which uses a solid cosolvent. Here, the new process is tested for phenytoin drug using menthol solid cosolvent. Phenytoin solubility in pure sc CO_2 is only 3 μmol/mol but when menthol solid cosolvent is used the solubility is enhanced to 1302 μmol/mol, at 196 bar and 45 °C. This 400-fold increase in the solubility can be attributed to the interaction between phenytoin and menthol.

Particle agglomeration in expansion zone is another major issue with conventional RESS process. In proposed RESS-SC process solid cosolvent hinders the particle growth resulting in the formation of small nanoparticles. For example, the average particle size of phenytoin in conventional RESS process is 200 nm whereas, with RESS-SC process, the average particle size is 120 nm, at 96 bar and 45 °C. Similarly at 196 bar and 45 °C, 105 nm average particles were obatined by RESS and 75 nm average particles were obtained in RESS-SC process. The particles obtained were characterized by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), dynamic light scattering (DLS) and differential scanning calorimetery (DSC) analyses. Phenytoin nanoparticle production rate in RESS-SC is about 400-fold more in comparision to that in RESS process.

Keywords: RESS; RESS-SC; Supercritical; Nanoparticle; Phenytoin

1. Introduction

The dissolution of poorly water-soluble drugs is a major concern for pharmaceutical industry, specially for the drugs whose dosage requirement is near their toxicity limits. The particle size reduction is one of the methods which can achieve desired bioavailability of poorly soluble drugs, as the dissolution rate can be enhanced by reducing the particle size (Unno et al., 1984; Yakou et al., 1984). Mechanical methods have been used for particle size reduction but broad size distribution and difficulty in commuting are some of the problems associated with these methods. Also, heat-sensitive materials can degrade by milling. To overcome these disadvantages, new methods have been devised including supercritical fluid (SCF)-based particle size reduction methods (Tom and Debenedetti, 1991). These can be divided into two major processes: rapid expansion of supercritical solution (RESS) for CO_2-soluble drugs and supercritical antisolvent (SAS) process for CO_2-insoluble drugs.

In RESS process, the desired solute is solubilized in SCF and then resulting solution is expanded through a nozzle to cause a sudden decrease in the solubility and hence, particle formation (Tom et al., 1994; Turk et al., 2002). Homogenous nucleation in RESS is caused by supersaturation and several mathematical models have been presented to explain this process theoretically (Kwauk and Debenedetti, 1993; Shaub et al., 1995; Helfgen et al., 2003). In SAS process, the desired solute is dissolved in an organic solvent and then injected inside SCF media causing small particle formation by volumetric expansion and removal of solvent (Luna-Barcenas et al., 1995; Werling et al., 2000; Elvassore et al., 2001; Reverchon et al., 2001). RESS is simpler and less expensive when compared to SAS process. But the solubility of most polar drugs is almost negligible in supercritical CO_2 (sc CO_2) which makes RESS process unviable for practical application. Due to this reason, other less benign

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doi:10.1016/j.ijpharm.2005.11.005

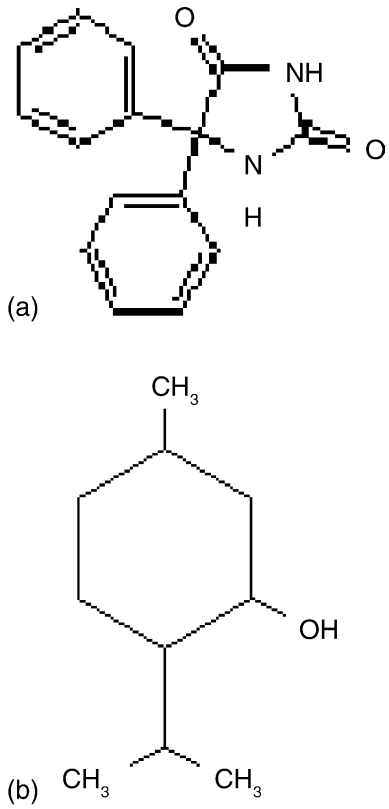


Fig. 1. Chemical structure of phenytoin (5,5-diphenyl-2,4-imidazolidinedione) (a) and menthol (b).

SCFs been used to produce polar particles using RESS method (Reverchon et al., 1995). Also, organic compounds tend to agglomerate due to their adhesive nature, resulting in agglomeration which generally produces bigger particles. Both challenges are addressed in this work by improving the drug solubility in sc CO_2 and also producing sub-100 nm particles by reducing the particle growth. Here, the new concept is tested for phenytoin drug.

Phenytoin (5,5-diphenyl-2,4-imidazolidinedione; Fig. 1) is widely used as anticonvulsant and antiepileptic drug. As phenytoin is a blocker for inactivated sodium channels, it is also used as antiarrhythmic drug for treatment of heart rhythm disturbances (Dylag et al., 2004). The side effects of phenytoin include nausea, insomnia and other central nervous system disorders (Page et al., 2002; Reynolds, 1982). Phenytoin is a highly crystalline compound having high melting point of 295–298 °C due to the strong intermolecular hydrogen bonding. Phenytoin solubility in water is as low as 80 μmol/l (Stella et al., 1999). For better bioavailability, low melting prodrugs have been proposed which later on convert to phenytoin (Stella et al., 1999). Also, some excipients have been added to phenytoin to obtain better dissolution (Hashim and El-Din, 1989). For example, β-cyclodextrin–phenytoin complexation has been used for enhancing phenytoin bioavailability (Tsuruoka et al., 1981). Most common route of phenytoin exposure is oral, though parenteral mode is used intravenously in *status epilepticus*.

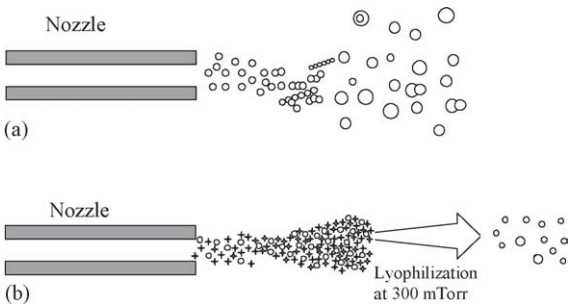


Fig. 2. Schematic of RESS (a) and RESS-SC (b) process.

Due to the high polarity it is difficult to solubilize phenytoin in sc CO_2. At 196 bar and 45 °C, phenytoin solubility in sc CO_2 is only 3 μmol/mol. With this low solubility RESS is not economically viable for industrial production. Earlier, micrometer-sized phenytoin particles were formed by supercritical-assisted atomization process after dissolving in methyl alcohol (Reverchon, 2003). To overcome the limitation of low solubility, this work proposes the addition of a solid cosolvent to enhance the phenytoin solubility in sc CO_2.

Though the mathematical modeling of RESS predicts particles of size less than ∼20 nm at the tip of the nozzle, particles experimentally obtained are in the range of 200–1000 nm (Helfgen et al., 2003). In conventional RESS process, each particle is surrounded by same kind of particles in the expansion zone which results in larger particles due to coagulation (Fig. 2a). So far various solvents and techniques have been used for phenytoin crystals modifications (Nokhodchi et al., 2003). A new method, rapid expansion of supercritical solution with solid cosolvent (RESS-SC), has been proposed which overcomes this particle growth in expansion zone resulting in smaller nanoparticles. In RESS-SC, phenytoin particles are surrounded by a solid cosolvent, avoiding surface to surface interaction to other phenytoin particles, hence hindering the particle growth. RESS-SC concept is shown in Fig. 2b. The cosolvent is simply removed by sublimation using a lyphilizer, which is carried out after the particle recovery from the expansion chamber.

1.1. Choice of cosolvent

The choice of cosolvent is very important as it needs to provide polar interaction to enhance solubility in CO_2. Polar cosolvents including acetone, ethanol have been tried so far which are liquid at operating and exit conditions and can cause particle dissolution (Dobbs et al., 1987; Dobbs and Johnston, 1987; Liu et al., 2000; Jin et al., 2004). In this work, solid cosolvent is proposed which should have following properties:

- sufficiently high vapor pressure for easy removal by sublimation;
- solid at nozzle exit conditions (typically −5 to 25 °C, observed experimentally);
- appreciable solubility in sc CO_2;
- non-reactive with desired solute or sc CO_2;

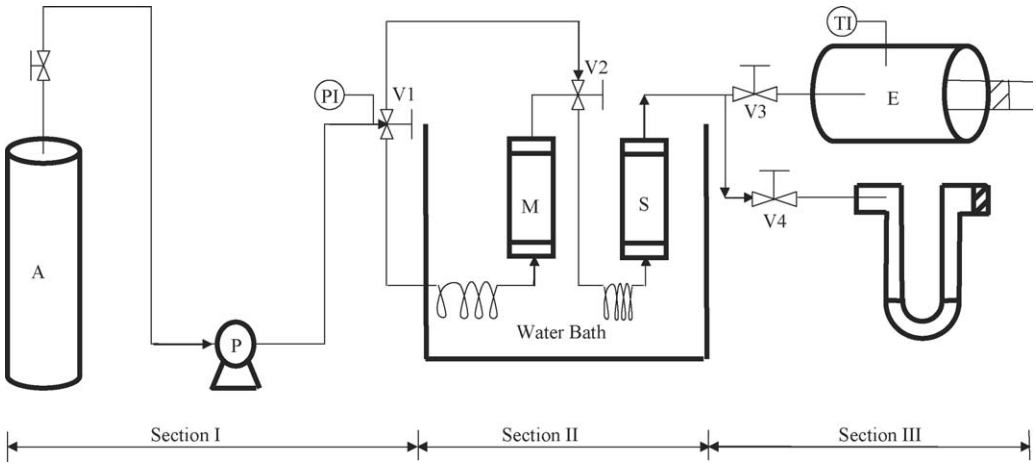


Fig. 3. Schematic of RESS-SC experimental apparatus.

- non-flammable and non-toxic;
- inexpensive.

Menthol is one such compound which meets all these requirements (Fig. 1b). Its melting point is 34–36 °C with high vapor pressure. Also, menthol has comparatively high solubility in sc CO_2 (Sovova and Jez, 1994; Thakur and Gupta, 2005). Menthol is already widely used in food and pharmaceutical industry.

In this work, menthol solubility is measured at 45 °C by gravimetric analysis whereas phenytoin solubility is measured by UV analysis at 264 nm. Size and morphology of obtained particles were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), differential scanning calorimetery (DSC), dynamic light scattering (DLS) and Fourier-transform infrared spectroscopy (FTIR).

2. Experimental

2.1. Materials

CO_2 (99.99% pure) from Air Gas, menthol (99% pure) with melting point of 34–36 °C from Fisher Scientific, phenytoin (>98% pure) from Aldrich were used as received. ACS grade (200 proof) ethanol was purchased from Pharmco products.

2.2. Apparatus

The schematic of RESS-SC process is shown in Fig. 3. The apparatus is mainly divided into three parts: a pre-extraction chamber (Section 1), an extraction chamber (Section 2) and an expansion chamber (Section 3). In Section 1, P is a high-pressure syringe pump for pressurizing CO_2 at desired pressure using CO_2 from cylinder A. Two vessels, M and S, were used as extraction column for menthol and phenytoin, respectively, in Section 2. Glass wool was used on both the ends of vessels M and S to avoid any undissolved material carry over with the CO_2 flow. Both vessels containing solute and cosolvent were kept in a water bath to keep constant extraction temperature (±0.1 °C) by temperature controller. The pressure of extraction section was measured using an online Heise ST-2H pressure transducer connected just before valve V1. Section 3 is either an expansion chamber for RESS-SC experiment or a U-tube for solubility experiments and is kept at atmospheric pressure and ambient conditions. Valve V1 connects Section 1 with Section 2 whereas valve V3 connects Sections 2 and 3. Valves V1 and V2 are three-way valves for CO_2 bypass connection to vessel S to perform conventional RESS experiments for comparison. Glass wool was used at the end of the expansion chamber outlet or at the end of the second leg of the U-tube to entrap the particles. Temperature in the expansion chamber was recorded to be less than 5 °C using a thermocouple.

2.3. Solubility measurement

A syringe pump was filled with CO_2 from tank A and set at a desired pressure. Vessels, M and S, having 7 ml capacity each, were filled with menthol and phenytoin powders. These vessels act as extraction/solubilization columns. After filling, the extraction columns were connected as per Fig. 3. Both columns were kept in a water bath at a desired temperature in such a way that the inlet was at the bottom while the outlet was at the top for proper distribution of SCF. CO_2 was fed to the first extraction column and was allowed to stabilize for 30–45 min by closing valve V2 at a desired pressure. After stabilizing the first column, menthol-enriched CO_2 was supplied to the second extraction column containing phenytoin using valve V2. Again, the entire system was equilibrated for 60–75 min before expanding phenytoin solution in the U-tube. The U-tube was kept in an ice bath with glass wool at the other end to trap the formed particles. For pure phenytoin solubility experiments, only one column was used filled with phenytoin and system was equilibrated for 110–120 min. For measuring phenytoin solubility, powder obtained in the U-tube was dissolved in ethyl alcohol and was analyzed by UV spectrophotometer (Spectronic Genesys 2) set at 264 nm.

For pure menthol solubility at 45 °C extraction column was filled with menthol powder and sc CO_2 was expanded in U-tube after 120 min of stabilization (to reach equilibrium). Gravimetric analysis method was used for menthol solubility.

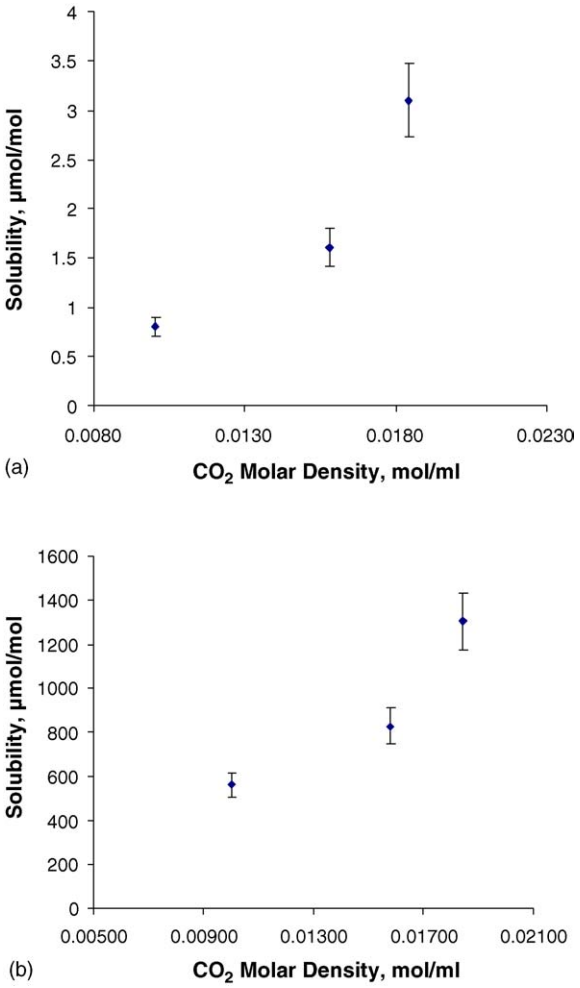


Fig. 4. Phenytoin solubility in sc CO_2: (a) without menthol and (b) with menthol.

2.4. Particle formation by RESS-SC

For RESS-SC, the expansion chamber was used for the expansion of menthol/phenytoin/CO_2 solution from a desired pressure to atmospheric pressure. A tube nozzle (PEEK nozzle from Upchurch) with a fixed diameter of 64 μm was used for expansion. Particles were collected in the expansion chamber. Before analyzing the particles for size, crystallinity or morphology, the particles were subjected to 300 mTorr (absolute) vacuum for 24 h to remove all the menthol by sublimation. No change in weight of particles was observed by applying vacuum for additional 3 h. Also, the lyophilized powder did not give any mint smell. These were the sufficient tests for ensuring menthol absence from lyophilized phenytoin particles. After menthol removal, the particles were analyzed by SEM, dynamic light scattering, differential scanning calorimeter and powder X-ray diffraction methods along with FTIR. RESS experiments were also conducted for phenytoin prior to RESS-SC experiments for comparison.

2.5. SEM analysis

The particle size and morphology analysis was carried out using SEM (Zeiss, model DSM940). For analysis, the particles were attached to the carbon tape on the top of SEM aluminum stubs and were coated with gold using a sputter coater (Electron Microscopy Sciences, model 550X) for two runs of 1 min each. In order to have a proper representation of the particles collected in the expansion chamber SEM micrographs of different regions were obtained. In menthol/phenytoin system, wherein menthol was the cosolvent, processed powder was first kept in a high vacuum of 300 mTorr (absolute) to remove all the menthol from the particle mixture. All the SEM analyses were done after 60–70 h of experiments at an accelerating voltage of 10 kV.

2.6. DSC analysis

Thermal analysis was carried out using DSC (TA instruments, model DSC Q100) for processed and unprocessed phenytoin particles. Analysis was performed for 1.5 mg phenytoin sample at a temperature heating rate of 5 °C/min and a temperature range of 30–300 °C.

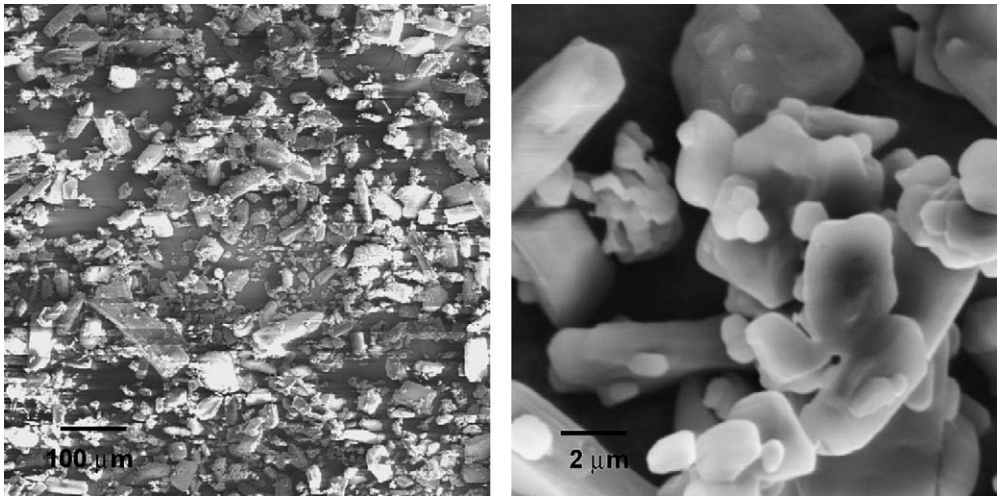


Fig. 5. Original phenytoin particles.

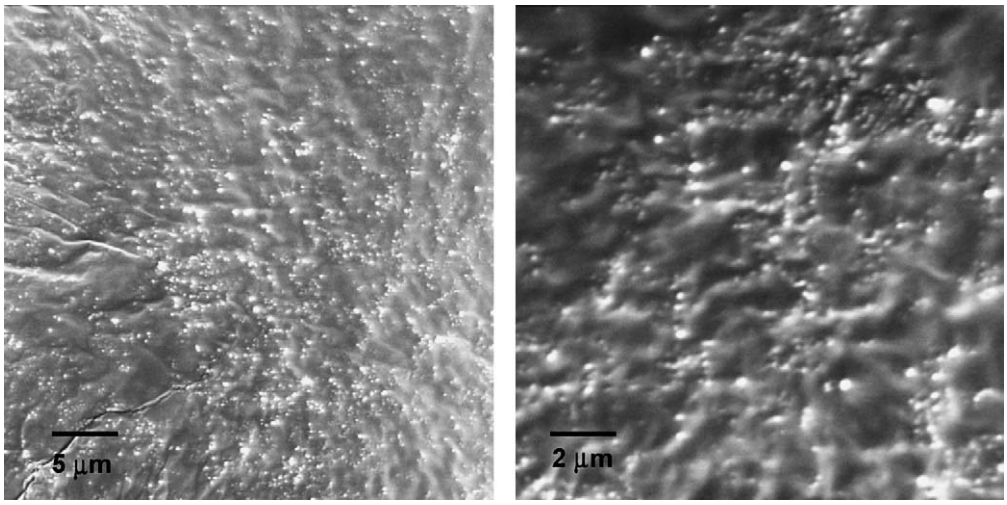


Fig. 6. Phenytoin particles obtained from RESS at 96 bar and 45 °C.

2.7. X-ray diffraction

Phenytoin particle crystallinity was analyzed using Rigaku X-ray diffractometer which was equipped with Cu $K\alpha_1$ radiation source and a Miniflex gonoiometer. The powder was filled to same depth inside the sample holder by leveling with spatula and scanning rate (2° min^{-1}) was same for all XRD analysis.

2.8. FTIR analysis

Chemical analysis of unprocessed and RESS-SC processed phenytoin particles were performed by FTIR spectroscopy using Nicolet instrument. The spectra were collected in transmission mode at room temperature in 4000–400 cm^{-1} range at a resolution of 2 cm^{-1}.

2.9. DLS analysis

Nanosuspension in water was made for phenytoin particles from RESS-SC process. The water was pre-saturated with phenytoin to avoid dissolution of the nanoparticles. The suspension was analyzed in DLS (PSS NICOMP model 380) for measuring hydrodynamic radius of phenytoin particles. Measurements were made using laser light of 638 nm wavelength with a 90° scattering angle at room temperature.

3. Results and discussion

3.1. Solubility enhancement

Table 1 summarizes the pure menthol solubility at 45 °C in sc CO_2. Menthol solubility is as high as 0.147 mol/mol at 196 bar and 45 °C. On the contrary, phenytoin has extremely low solubility in sc CO_2 as shown in Table 2. Due to high crystallinity and molecular polarity, phenytoin has a limited solubility in sc CO_2, for example, only 3 μmol/mol at 196 bar and 45 °C.

Menthol-saturated sc CO_2 can solubilize higher amount of phenytoin, at the given pressure and temperature as shown in Table 3. It is quite evident from Tables 2 and 3

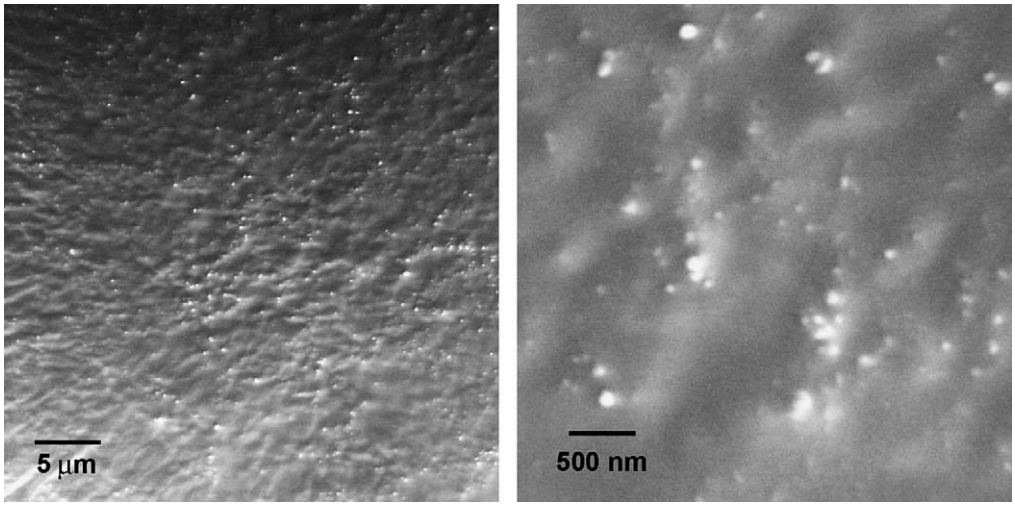


Fig. 7. Phenytoin particles obtained from RESS at 196 bar and 45 °C.

Table 1
Solubility of pure menthol in sc CO_2

Pressure (bar)	Temperature (°C)	CO_2 density Span and Wagner (1996) (mol/ml)	Solubility (mmol/mol)	Standard deviation (mmol/mol)
96	45	0.01004	43	5
129	45	0.01582	84	9
163	45	0.01746	121	7
196	45	0.01844	147	10

Table 2
Solubility of phenytoin in pure sc CO_2

Pressure (bar)	Temperature (°C)	CO_2 density (mol/ml)	Solubility (μmol/mol)	Standard deviation (μmol/mol)
96	45	0.01004	0.8	0.1
129	45	0.01582	1.6	0.3
196	45	0.01844	3	0.4

Table 3
Solubility of phenytoin in CO_2 with menthol solid cosolvent

Pressure (bar)	Temperature (°C)	CO_2 density (mol/ml)	Solubility (μmol/mol)	Standard deviation (μmol/mol)	Enahncement factor[a]
96	45	0.01004	561	45	701
129	45	0.01582	829	90	518
196	45	0.01844	1302	125	434

[a] Ratio of phenytoin solubility in CO_2 with menthol to that without menthol.

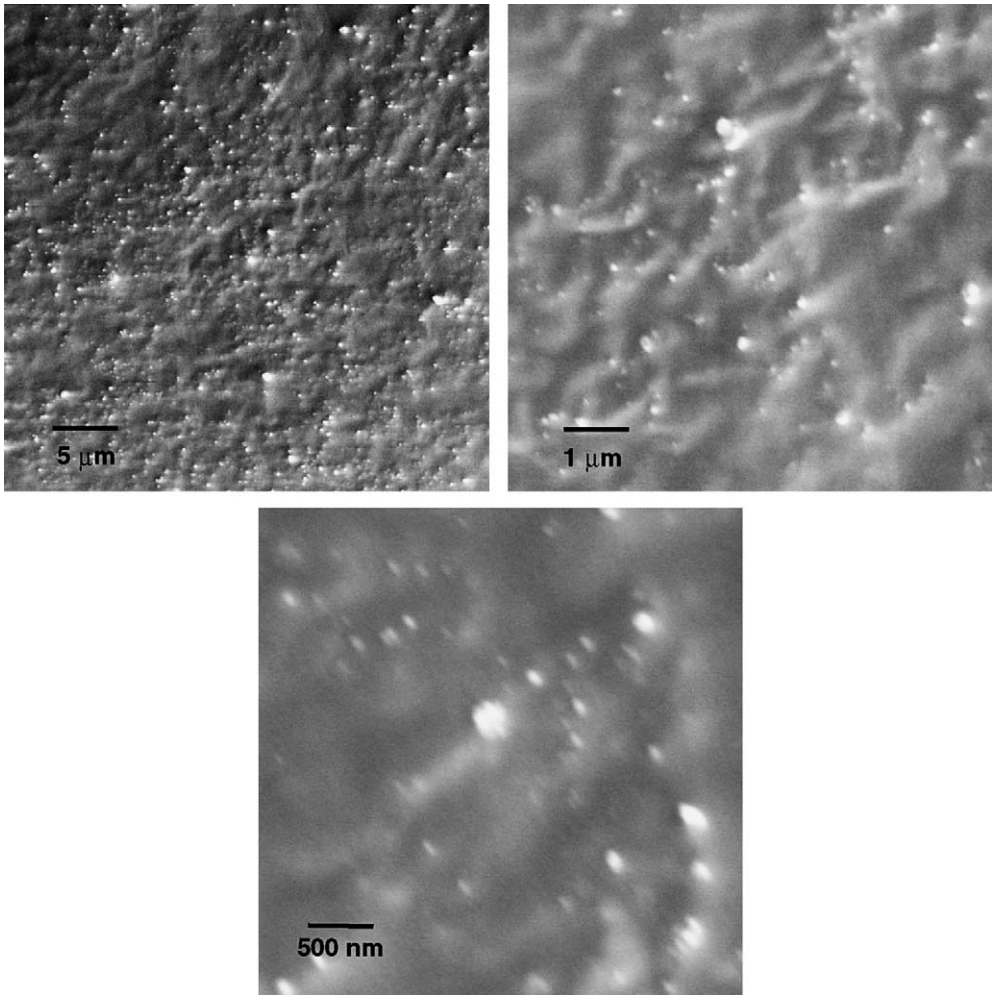


Fig. 8. Phenytoin particles obtained from RESS-SC at 96 bar and 45 °C.

that phenytoin solubility is enhanced as high as 400-fold using menthol cosolvent. The enhancement can be attributed to the polar interaction sites provide by menthol phenytoin solubilization. Similar results were obtained earlier with griseofulvin and 2-aminobenzoic acid solubility with menthol cosolvent (Thakur and Gupta, 2005, submitted for publication). Maximum solubility of 1302 μmol/mol was measured at 196 bar and 45 °C. Though griseofulvin is hydrophobic drug still drug solubility increases with polar cosolvents (Gioannis et al., 2004). Fig. 4 shows the variation of phenytoin solubility with and without menthol with increasing density. Phenytoin solubility increases with increase in density, which is typical for solubility in supercritical fluids.

3.2. Phenytoin nanoparticles

After addressing the solubility issue, the next goal was to form phenytoin nanoparticles by rapid expansion. The original unprocessed particles were rectangular-shaped with average length of 4 μm long and width of 3 μm (Fig. 5). Fig. 6 shows particles from RESS at 96 bar and 45 °C. The average size of phenytoin particles was 200 nm. There is not only a change in the particles morphology from rectangular to spherical but also the particle size after processing with sc CO_2. Experiments were also performed at a higher pressure of 196 bar to analyze pressure effect on particle size. The average particle size reduced to 105 nm at the higher pressure (Fig. 7). Due to lower solubility, exiting sc CO_2 has lower concentration of phenytoin which helps in obtaining smaller nanoparticles, as opposed to microparticles for other more CO_2-soluble drugs.

After processing with menthol-enriched sc CO_2, phenytoin concentration increases but still the particles obtained are in nanometer range. Fig. 8 shows SEM micrograph for RESS-SC processed phenytoin particles at 96 bar with average size of 120 nm. Here, again the particles are in spherical shape. As phenytoin is surrounded by menthol solid cosolvent, even higher phenytoin concentration in RESS-SC process produces nanometer particles. This verifies the scheme proposed for RESS-SC (Fig. 2b). Like RESS process, the particle size reduces to 75 nm at higher pressure of 196 bar in RESS-SC process (Fig. 9). This decrease in particle size is due to higher supersaturation value at higher pressure, while keeping same exit

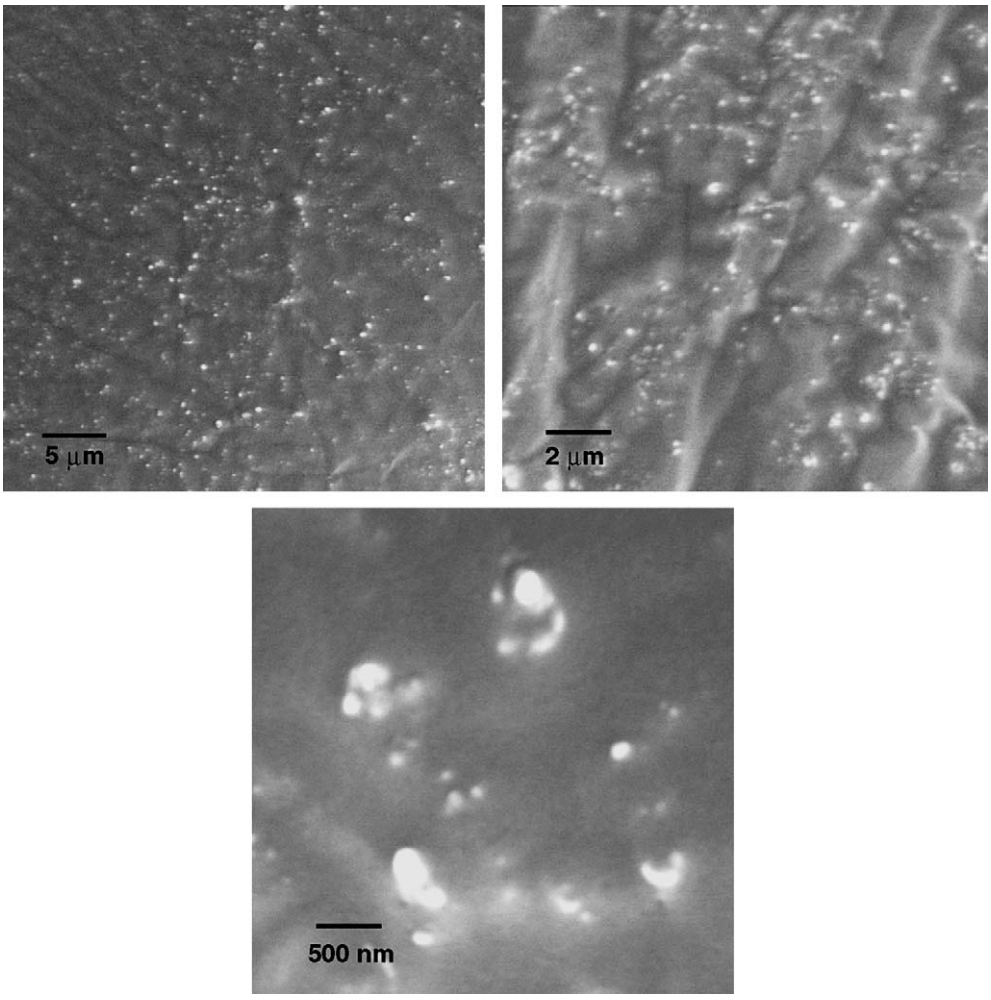


Fig. 9. Phenytoin particles obtained from RESS-SC at 196 bar and 45 °C.

condition. However, the particle size reduction is not that significant considering 20% standard deviation in size measurement. Several researchers observed a similar behavior of pressure effect on particle size for their RESS experiments (Thakur and Gupta, submitted for publication; Charoenchaitrakool et al., 2000).

All RESS-SC processed SEM micrographs presented here are for menthol-free phenytoin particles. These were obtained after menthol cosolvent is removed by sublimation. Digital pictures of vial containing menthol and phenytoin after lyophilization are taken with a CCD camera (Sony model DFW-V500) having close focus lens (maximum magnification 10×). Fig. 10a shows the overall picture of vial containing menthol fibers at rim and drug particle at bottom of the vial. Parafilm was used at the top of the vial to trap menthol which can be seen in Fig. 10b. Fig. 10c and d shows close-up pictures of rim and base of the vial. The purpose of Fig. 10 is to show physical difference between drug particles and menthol particles. Earlier, precipitation by compressed antisolvent (PCA) and gas antisolvent (GAS) process had been used, but micrometer-sized rod-like phenytoin particles were obtained in both methods using two different solvents (Fig. 11) (Muhrer et al., submitted for publication).

RESS-SC processing does not affect the chemical structure of the drug based on FTIR analysis. Fig. 12 shows the FTIR spectra of RESS-SC processed and unprocessed phenytoin particles. In the spectra, –NH strech can be seen at 3280 cm^{-1} frequency whereas –C=O strech is evident at 1780 cm^{-1}. Both the spectra overlap each other, though for RESS-SC processed particles slight bump at 3500 cm^{-1} can be seen which may be because of the ambient moisture attracted to the large surface area present on the phenytoin nanoparticle surface.

DSC was performed for thermal analysis of RESS-SC processed and unprocessed phenytoin particles. Here, DSC analysis was performed for qualitative explanation of the crystallinity change and not so much for quantitative analysis. The melting point of unprocessed phenytoin is 296.78 °C whereas after processing it is 296.62 °C. There is no significant change in melting point which suggests that particle crystal form does not change after RESS-SC. Fig. 13 shows heat flow with temperature plot of unprocessed and RESS-SC processed phenytoin particles.

To further investigate particles crystallinity XRD analysis was performed. Fig. 14 shows the XRD intensity variation with 2θ for unprocessed and processed phenytoin particles. Though all the peaks are overlapping, RESS-SC processed phenytoin particles have lower intensity values (Fig. 14b). Though care has been taken to analyze same sample mass for XRD, due to lower bulk density of processed particles there might be some difference in the mass used. This difference in sample mass can be the cause of lower intensity of processed particles. XRD of pure menthol particles is shown elsewhere (Thakur and Gupta, 2005).

According to SEM analysis, the average size of phenytoin particles was 75 nm from RESS-SC at 196 bar and

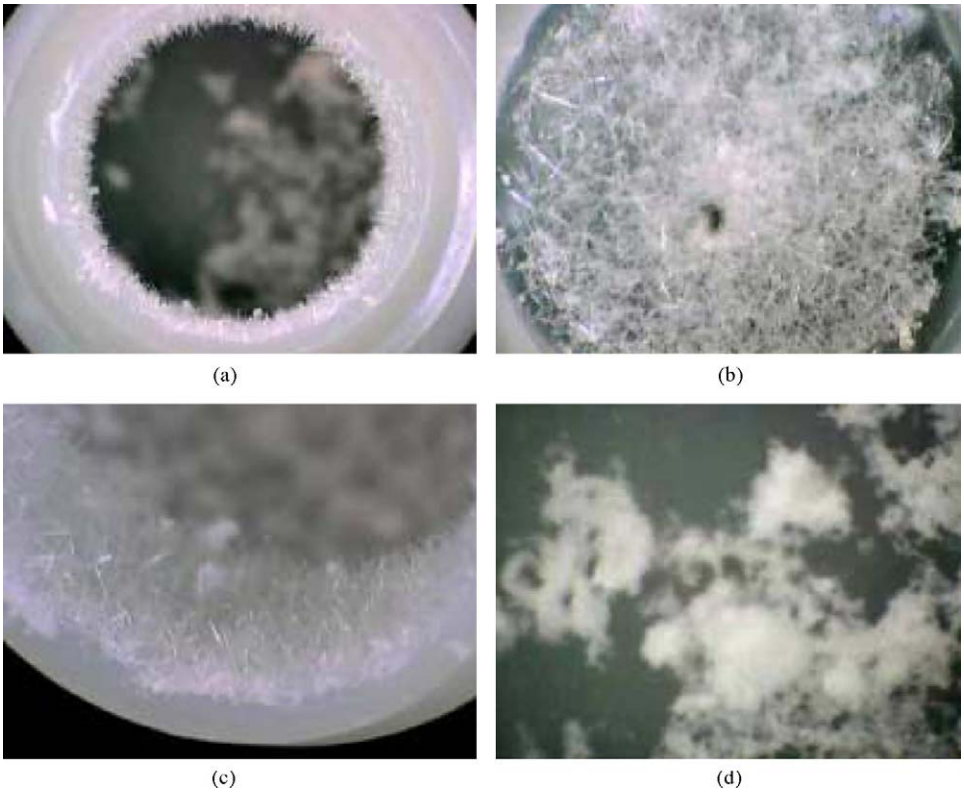
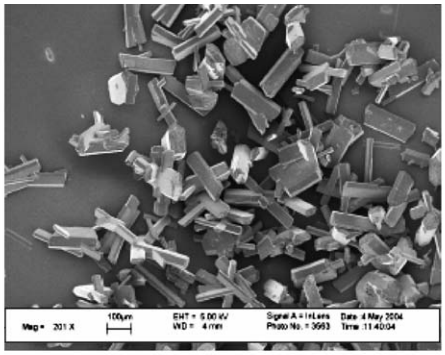
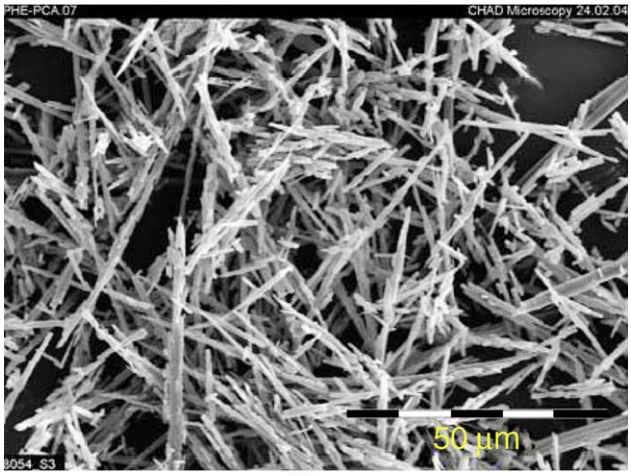


Fig. 10. Optical pictures of vial containing menthol fibers on rim (a and c) and bottle cap (b) and phenytoin particles at bottom of vial (d) after partial lyophilization.



(a)



(b)

Fig. 11. (a) GAS recrystallization and (b) PCA precipitation of phenytoin particles from acetone (Muhrer et al., submitted for publication; Charoenchaitrakool et al., 2000).

45 °C. These particles were also subjected to DLS analysis to obtain hydrodynamic size, which gave a number average diameter of 57.4 nm for phenytoin nanosupension. Fig. 15 shows the DLS number average distribution for the nanosuspension. Such a small hydrodynamic size makes these phenytoin particles ideal candidates for injectable drug delivery.

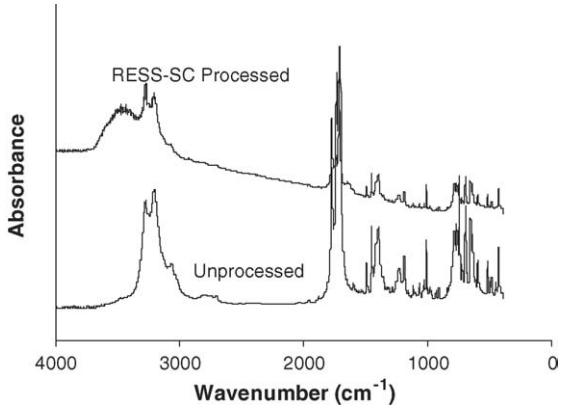


Fig. 12. FTIR analysis of unprocessed and RESS-SC processed phenytoin particles.

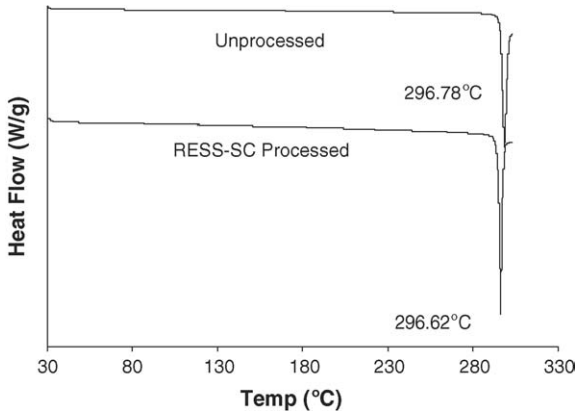
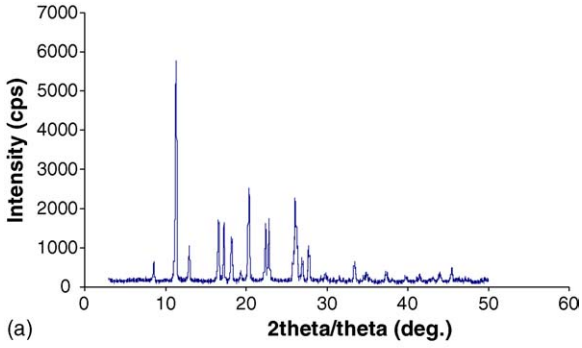


Fig. 13. DSC thermograph of unprocessed and RESS-SC processed phenytoin particles.



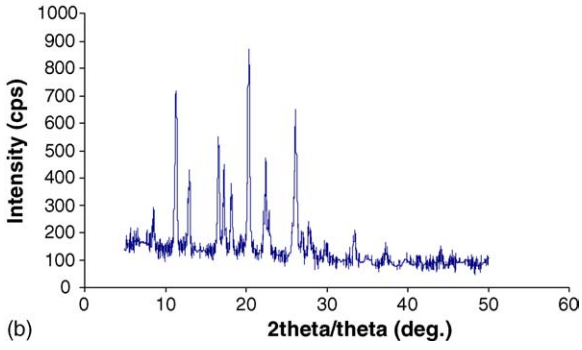


Fig. 14. XRD analysis of: (a) unprocessed and (b) RESS-SC processed phenytoin particles.

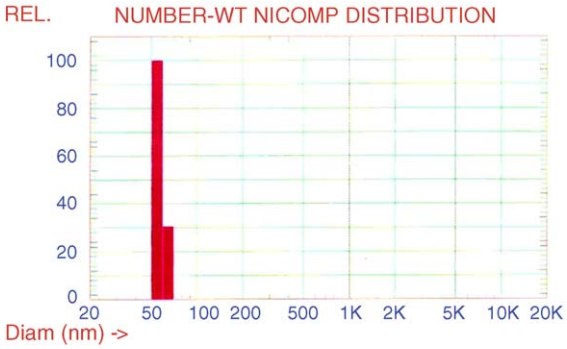


Fig. 15. DLS analysis of phenytoin nanosuspension.

4. Conclusion

The two major issues of the RESS process: (a) low solubility in sc CO_2 and (b) formation of nanoparticles are addressed in this work for phenytoin drug using menthol solid cosolvent. Menthol cosolvent not only enhances the phenytoin solubility in sc CO_2 but also form particles as small as 75 nm. At 196 bar and 45 °C, solubility of phenytoin is only 3 μmol/mol, which is enhanced to 1302 μmol/mol by using menthol cosolvent. Due to the enhancement, phenytoin nanoparticle production rate in RESS-SC is about 400-fold more in comparision to that in RESS process. FTIR, XRD, DLS and DSC analyses are used to characterize the obtained phenytoin particles.

Acknowledgements

We thank Dr. Michael Miller for his help in SEM analysis. Authors also like to thank Dr. T. Albrecht-Schmitt for XRD, Dr. M. Byrne and Mr. Asa Vaughn for DSC analysis and Ms. Haley Brooks for experimental assistance.

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