Drug Nano-Particles Formation by Supercritical Rapid Expansion Method; Operational Condition Effects Investigation

Zabihi, Fatemeh*+

Department of Chemical Engineering, Faculty of Engineering, Ayatollah Amoli Branch, Islamic Azad University, Amol, I.R. IRAN

Akbarnejad, Mohammad Mahdi

Research Institute of Petroleum Industry, Tehran (RIPI), I.R. IRAN

Vaziri Yazdi, Ali

Department of Chemical Engineering, Faculty of Engineering, Science & Research Branch, Islamic Azad University, Tehran, I.R. IRAN

Arjomand, Mahdi

Department of Chemical Engineering, Faculty of Engineering, Tehran South Branch, Islamic Azad University, Tehran, I.R. IRAN

Safekordi, Ali Akbar

Faculty of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, I.R. IRAN

ABSTRACT: *Dissolution pressure and nozzle temperature effects on particle size and distribution were investigated for RESS (Rapid Expansion of Supercritical Solution) process. Supercritical CO_2 was used as solvent and Ibuprofen was applied as the model component in all runs. The resulting Ibuprofen nano-particles (about 50 nm in optimized runs) were analyzed by SEM and laser diffraction particle size analyzer systems. Results show that in low supercritical pressure ranges, depending on the solvent and solid component properties (Lower than 105 bar for Ibuprofen-CO_2 system), nozzle temperature should be as low as possible (80-90˚C for Ibuprofen-CO_2 system). In the other hand in high supercritical pressure ranges (above 105 bar), high nozzle temperatures work better. The border line of these two areas depends on the solvent phase behavior. Rapid Expansion of Supercritical Solution into a liquid solvent (RESOLV) was also studied with and without the presence of surfactant and compared with RESS process by measuring of formed particles size, size distribution and dissolution rate. Results show that the RESS process generally creates better conditions for achieving fine and uniform organic powders (with mean particles size of 40-180 nm), in contrast to the RESOLV method (minimum particles size of 80-400 nm).*

KEY WORDS: *Supercritical, Rapid expansion, Dissolution rate, Mean particle size, Size distribution.*

* *To whom correspondence should be addressed.*

+ E-mail: elyze_125@hotmail.com

1021-9986/11/1/7 9/$/2.90

INTRODUCTION

Size distribution is a crucial parameter in mass transfer of organic and inorganic active substances such as pharmaceuticals. Many physical properties of powdered solids can be fine tuned by minor changes in the mean particle size [1-3]. For the solid pharmaceuticals, bioavailability (absorbed percentage of initial dosage of drug) is often limited by low water solubility. Particle size reduction provides a larger surface area and improves the rate of water dissolution [4-6]. In addition to the higher dissolution rate, smaller diameter particles correspond to a better activity, easier absorption, longer circulating capacity in the blood, stability against degradation and reduction of undesirable side effects [6, 7]. Various methods including; crashing, grinding, milling, spray drying, freeze-drying, re-crystallization of solute particles using liquid anti-solvents, surfactant-aid dispersion, use of organic solvents, emulsions and microemulsions and solid dispersion technology, are used to convert a solid bulk material into a fine powder [8-10]. However, some of these techniques introduce various problems or limitations such as broad distribution in particle size, excessive solvent use and disposal, thermal and/or chemical degradation of biological substances and contamination with unwanted residues [10, 11]. Rapid expansion of supercritical solution (RESS method) offers considerable promise as a means for the production of films, crystalline or amorphous powders with narrow and controllable particle size distribution. Actually the mild operational conditions and high efficiency has rendered the RESS method very attractive to process the thermally sensitive pharmaceuticals [17, 18]. During this process, the substance of interest is dissolved in a supercritical fluid, resulting in a solute laden supercritical phase which is then subjected to rapid expansion by passing through a nozzle at sonic speed into a chamber in ambient condition (spray receiver). So, the solute precipitates because of the reduction of the density and, as a result solvent power of supercritical fluid [12-14]. A modified form of RESS process (Rapid Expansion into a Liquid Solvent / RESOLV) has been investigated recently. It is claimed that due to higher mass transfer resistance in the liquid phase spray receiver in RESOLV, aggregation and agglomeration is much more limited as compared to RESS and thus a lower mean particle size is achieved [15, 16].

The combination of high super saturation ratios and a rapid propagating mechanical perturbation are the distinguishing characteristic of RESS and RESOLV processes [16, 17]. Precipitation through evaporation of liquid solvent eliminates all the advantages of supercritical RESS expansion. Therefore thermodynamic conditions after and before expanding, should be controlled in order to avoid any solvent liquefaction. Precipitation path consists of three sections: (1) Pre-expansion zone (from immediately after the solubilization cell to the nozzle entrance), (2) Nozzle path and (3) Receiving environment (free jet zone). As soon as entering into the receiving environment, the supercritical solution separates into a low density gas and a solid precipitate, as the density and solvent power are reduced due to lower pressure in sections (1) and (2) [17]. Temperature limitation in the first section is very important, because in low temperatures, solvent liquefaction occurs and solute particles precipitate from liquid phase. On the other hand, in very high temperatures, the solute component may be degraded [18, 19]. Therefore, the pre-expansion zone should be set at a high enough temperature to compensate the temperature drop brought about due to pressure drop, to avoid precipitation of solute in the pre-expansion zone. However, the residence time is very low in short nozzle (section 2) nozzle temperature is one of the most important operational conditions, because of its effect on supercritical solution density during nozzle path. Nozzle temperature should be kept high enough to prevent precipitating in the nozzle path [20, 21]. By using a nozzle with low L/D (Length: Diameter) ratio, nozzle temperature can be controlled easily and the fluid flow can be considered steady state and one-dimensional [26-28].

At the nozzle exit (between 2 & 3 sections) solvent power drops suddenly because of pressure reduction and the nuclei appear. The driving force for particle formation is the Supersaturation Ratio (S) [22-24].

$$S = \frac{y_E(T_E, P_E)}{y^*(T, P)} \rangle 1 \quad (1)$$

Nucleation in RESS process is generally controlled by two consecutive mechanisms:

1-Nucleus formation

2- Growth

Particle formation rate direct depends on the S factor. By increasing the particle formation rate, smaller and

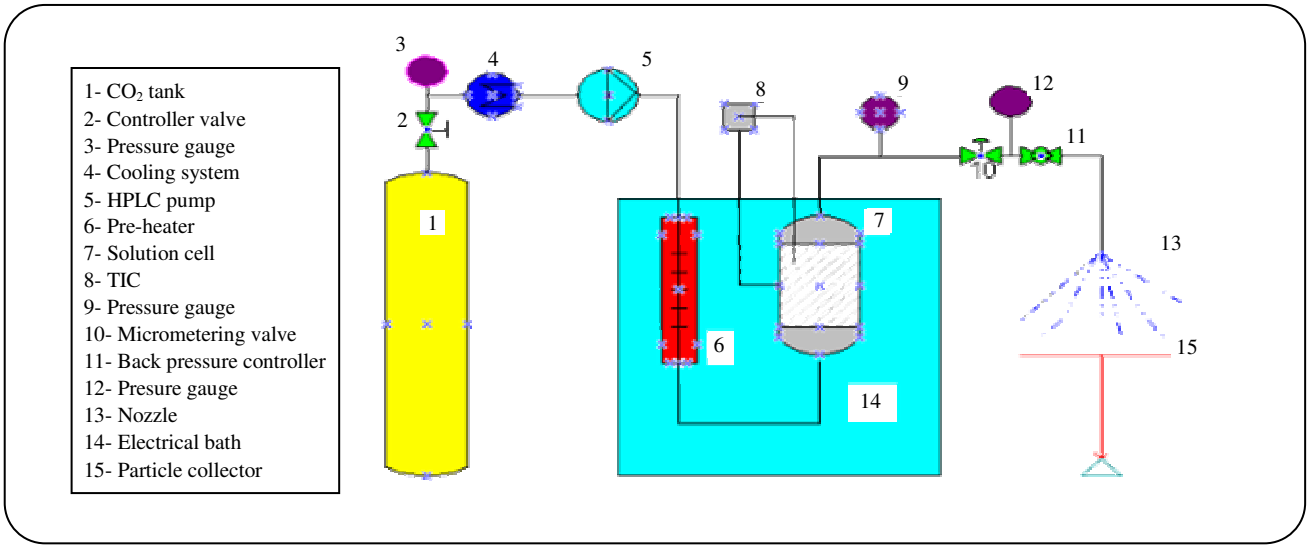

Fig. 1: RESS Apparatus.

more uniform nuclei are formed [25]. High S values always lead to high precipitation rates. So, numerous nuclei appear without having enough time to grow up. In the constant pre-expansion condition, $y_E(T_E , P_E)$ value remains constant. Therefore the S factor is only a function of the after-expansion condition and the $y^*(T , P)$ value. Spraying fluid density and consequently the $y^*(T , P)$ value are impacted by nozzle temperature.

A rough estimate for the choice of the process can be given based on the solubility of the solid component in the supercritical fluid. In addition size and morphology of RESS processed particles are clearly affected by supercritical solution concentration, just before expansion [29-31]. High concentration of expanding solution causes aggregation of nuclei because of the high cohesions potential. In low pressures and low concentrations, solution concentration is not high enough to bring about cohesion and coagulation. On the other hand, in higher pressures, because of higher solution concentration, the nuclei coagulate and large clusters are formed.

Therefore the dissolution pressure and nozzle temperature are selected as essential parameters in the RESS process and their effect on the resulting physical properties of Ibuprofen particles, is investigated in this work. We also compared the effect of spray receiving environment by running the RESS and RESOLVE processes under the same conditions.

Carbon dioxide was chosen as our supercritical fluid which is commonly used especially for heat-sensitive materials. It is non-toxic and chemically inert with the relatively low supercritical pressure and temperature [5, 17, 32].

The required dosages of Ibuprofen can be lowered by improving its effectiveness by increasing the dissolution rate in the biological environment [10, 12, 33]. Mean particles size effect on the Ibuprofen bioavailability was investigated using the dissolution test. Experiments were designed based on middle pressure ranges (80-130 bars) which makes the process more feasible and attractive from practical and economical aspects.

EXPERIMENTAL SECTION

Materials

Ibuprofen (Sina Daru , 99.99% purity) was used as received and CO_2 (Roham Gaz, 99.95%) was also used as solvent. Ethanol (99.8%, Sigma Aldrich) and Acetone (99.9%, Sigma Aldrich) were used in analytical grade form.

Apparatus

The experimental apparatus is shown in Fig.1. It is similar to the regular form of RESS system which is made of two parts: the solution cell and the expansion or crystallization part. The solvent (CO_2) flows through a 0.2µm filter to a cooling system (F38-Me, Julabo) from the reservoir to liquefy the gas solvent. Then liquid solvent is compressed to the desired pressure by means of an HPLC pump (LC 6A, Shimadzu) and after passing through a pre-heater coil, enters in to the solution cell

(extraction part, 16 cm height and 7mm I.D., S.S.) which is loaded with solid sample. Both coil and cell are located in an air bath, where temperature is kept constant at 35 ˚C (±1ºC) by a TIC. Immediately after the extraction cell, the supercritical solution passes through a micro-metering valve (1″/4 , S.S, Swagelok) to set the flow rate, a back pressure valve (KPR Series, Swagelok) and an expansion device (a stainless steel capillary, 5 mm length and 0.05mm I.D.). Passing through the expansion device, causes the pressure reduction and the solute precipitate from the solution in the form of tiny solid particles. The produced particles are collected on a glass slide directly. All connections and tubes are stainless steel and wrapped by wire heaters equipped with a TIC, to keep temperatures constant. The pressures in the CO_2 reservoir, extraction cell and immediately after the flow controller valve and before the expansion device are also measured and controlled by pressure gauges in ±0.1 bar accuracy (Ashcroft, f5503).

Spraying flow rate is constant and the same in all runs. Nozzle tip is kept vertical, relative to collection surface at the distance of 0.5 cm. In each run, sample collection starts after 4 min to ensure condition consistency.

Analysis

Morphology and the mean particle size were determined using SEM (Hitachi, S-570) images. Size distribution curves were obtained by Particle Size Analyzer system (CiLAS, 1068-Liquid) in order to emphasize the SEM results. Effect of particles size and morphology of the model component on dissolution rate was also determined by dissolution rate tester (Erveka DT70) equipped with a UV spectrometer system (Shimadzu, UV-1700). The dissolution tester, works based on the drug concentration measurement, in a phosphate buffer solution (PH=6.1), in equal time intervals.

Ibuprofen Solubility in Supercritical CO_2

Solid component solubility in supercritical solvent should be determined before starting the precipitation tests. We obtained the Ibuprofen saturated mole fraction in supercritical CO_2 in twelve different pressures (80-130 bars) at 35˚C by a dynamic method. We also obtained saturated solubility values at 40˚C and 45˚C in triplicate. All the solubility values were compared with the reference data to ensure the reproducibility and precision of our method. Our results are in good agreement (max. 7% deviation) with the reference data [34].

RESULTS AND DISCUSSION

Data was obtained in 25 runs. Six different supercritical solution pressures (80, 90, 100, 110, 120 and 125 bars) and in each pressure, five different nozzle temperatures (80, 85, 90, 95 & 100°C) were set as experimental variables. Except for the above mentioned variables, all other parameters were kept constant during all runs. Experimental conditions were selected based on Ibuprofen physical properties and CO_2 phase behavior and critical point (31.5°C & 72.5 bars). In 120 and 125 bar experiments series, particles were too aggregated to determine their exact morphology, size and distribution. All other products were analyzed by SEM and particle size analyzer systems and their mean particles size (d.a.) and Particle Size Distribution (PSD) were calculated by Eqs. (2) and (3) [35].

$$\text{d.a.} = \frac{\sum \text{nd}}{\sum \text{n}} \tag{2}$$

$$\text{PSD} = \frac{\sum \text{n}\left|\text{d} - \text{d}_\text{a}\right|}{\sum \text{n}} \tag{3}$$

Our results show a novel trend of particle size and distribution based on nozzle temperature and supercritical solution pressure before expanding. In supercritical systems, there is always a threshold supercritical solution pressure. For the supercritical solution low pressure ranges relative to this threshold pressure, before the expansion process, precipitated particles size will increase with increasing nozzle temperature. On the other hand, in upper supercritical solution pressures, low nozzle temperatures, cause solvent liquefaction and large nucleus formation. However in higher nozzle temperatures, even though the solvent doesn't liquefy but initial nucleus stick to each other and large clots are formed. This threshold limit depends on the solvent and solid character and their solubility behavior, that for Ibuprofen-CO_2 system is about 105-110 bar (See Figs. 2 and 3).

Fig. 2 shows that in high supercritical pressures (above 105-110 bar), large nucleus appear initially, even in high nozzle temperature. Because of high supercritical solution concentration, initial nucleus immediately aggregate, due to frequent nuclei collisions. But in low pressures, because of lower concentrations, the nuclei are

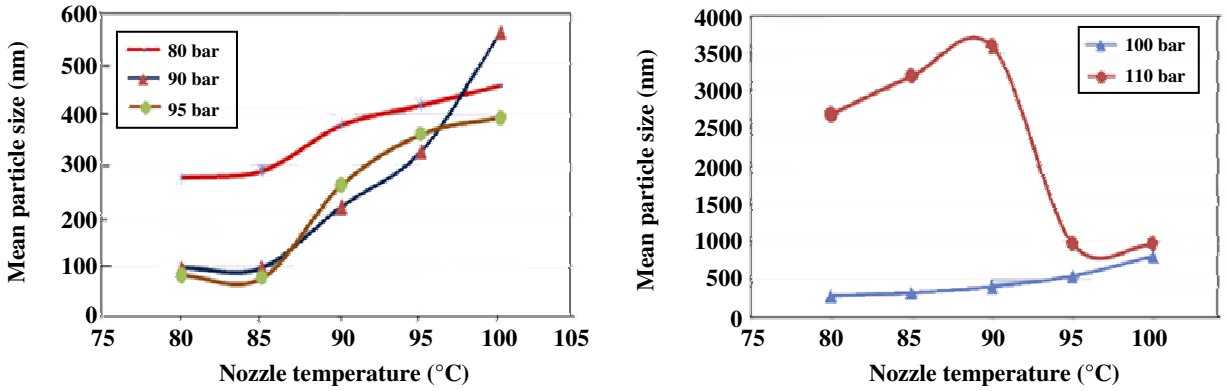

Fig. 2: Mean particle size changes with nozzle temperature.

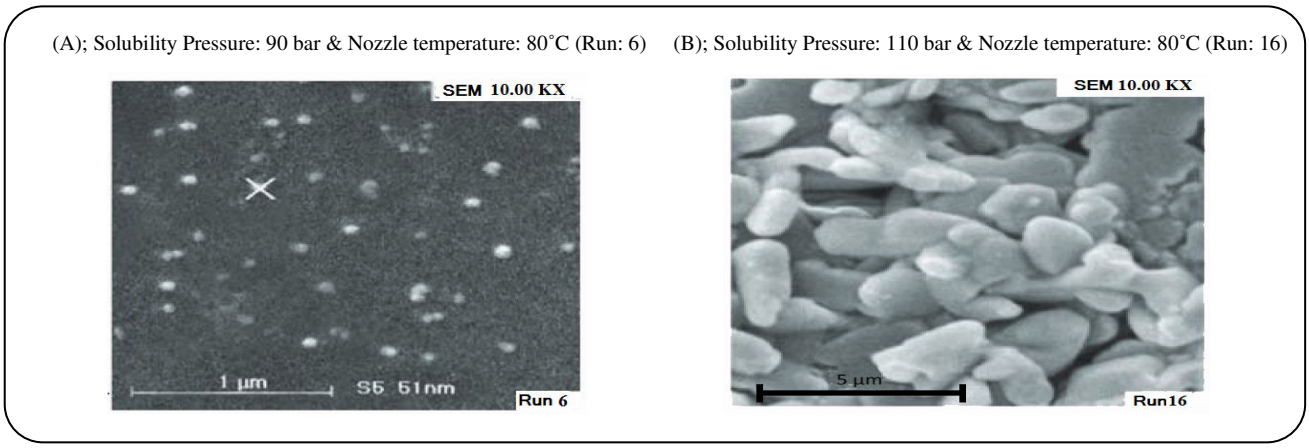

Fig. 3: SEM images of the particle, formed in (A); run 6 and (B); run 16.

small and collide less frequently [36, 37]. Fig. 3 shows the SEM images of particles collected from runs 6 & 16 which are also shown in Fig. 2.

In low nozzle temperatures, low values of $y^*(T, P)$ increase the S factor (see Eq. (1)) and small and uniform nuclei (in 50 nm range) are formed. This claim has been clearly proved in our experiments, by comparing the mean particle sizes and size distributions of particles produced in different nozzle temperatures in 80 and 90 bars.

The isenthalpic assumption of fluid motion in nozzle is valid for our experiments, and the thermodynamic conditions (T and P values) at the nozzle entrance should be selected in such a way to prevent the formation of two phases during expansion [38-40]. In high pressure ranges (~above 100 bar), lower nozzle temperatures drops expanding path into the 2-phase area. Precipitation from liquid phase causes large crystals formation that are likely to agglomerate in the liquid phase. Whereas, in lower pressure range (80-100 bar), even 340 K for nozzle does not result in liquid phase formation. Results have also shown that in 100 and 110 bars, against 80 and 90 bar, particles become larger with high crystalline character, when nozzle temperature is adjusted at 80-90 °C (See Fig. 3). However in more than 100 bar, even in 90-100°C, large and wide distribution particles were formed, because of high supercritical solution concentration and numerous nucleus collisions that leads to large clusters formation. Meanwhile high nozzle temperatures causes S values reducing that leads to large initial particle formation as occurred in our (19) and (20) runs (110 bar, 95 and 100°C).

Effects of Expansion Receiving Environment

The optimum run (run 6), was repeated four times by RESOLV method. Once the supercritical solution was sprayed into water medium and three times into solutions of surfactants (Poly ethylene glycol solution; 10%, 25% and 50% v/v respectively). Analysis showed that,

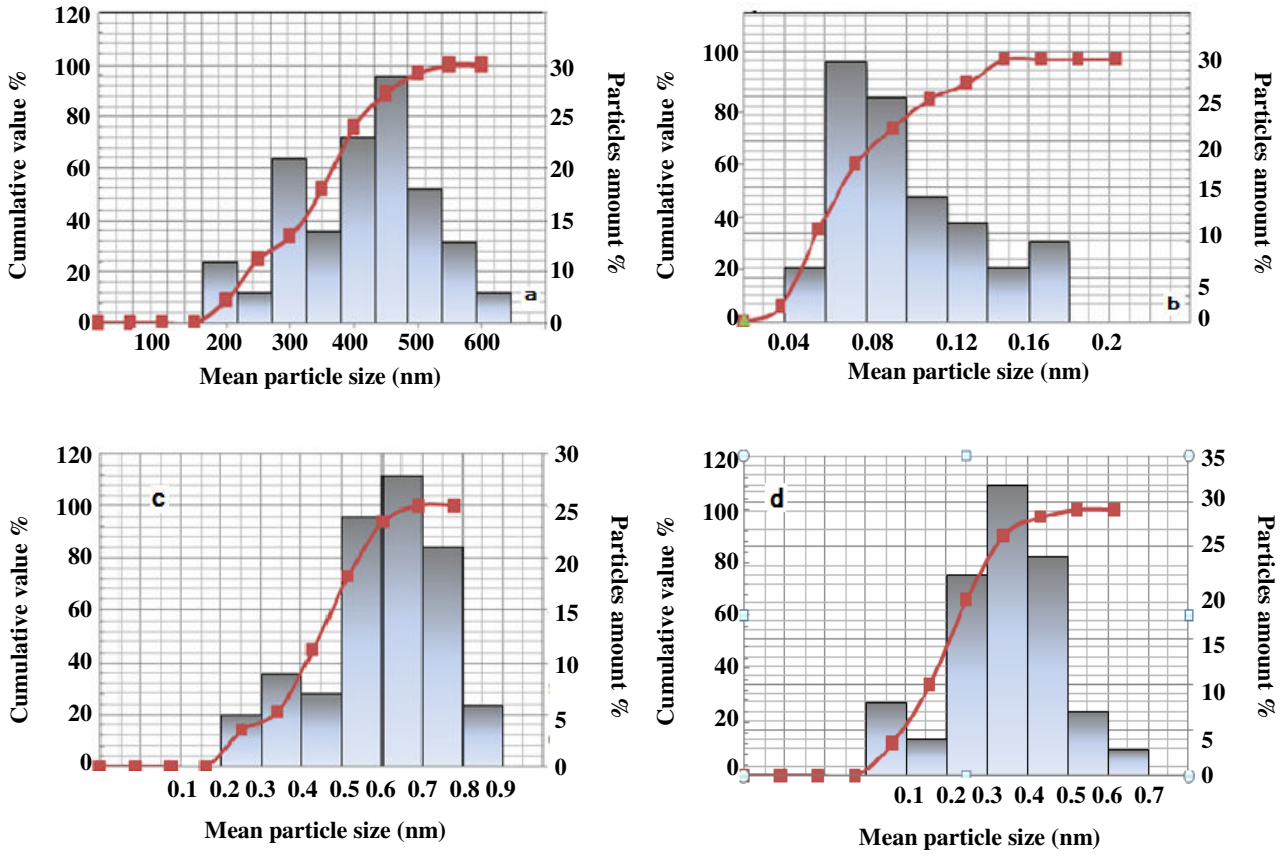

Fig. 4: PSA curves of Ibuprofen particles (a) Original sample (b) Processed sample in run 6 (c) Processed sample by run 6 condition expanded into pure water (d) Processed sample by run 6 expanded into water +polyethylene glycol (25%)

volume percent of surfactant in the receiving solution has no significant impact on particles quality. Achieved particles were compared by PSD and dissolution time analysis (See Fig. 4).

Expansion into the pure water results in a water solution that can be directly formulated as a consumer product. However the intensified nano-particle surface behavior in water results in appearance of large aggregates. Using of a surfactant within the receiving liquid can solve this problem [41, 42]. Fig. 4 shows the PSD curves of the original particles, RESOLV & RESS products. Based on the literatures [references needed], dry expansion leads to smaller and more uniform particles formation relative to liquid receiving expansion, however a highly dense receiving environment prevents nucleus growth. Because of liquid low temperature, density of free jet gas increases. So, large initial nucleuses form as a result of low S factor. Low diffusion factor prevents nucleuses growth but particles aggregate rapidly because of their surface properties in the nano scale. By adding an adequate surfactant this problem can be solved to some extent.

Dissolution Studies

Dissolution rate of precipitated particles obtained from runs 6 and 16 and original component were compared. The concentrations of Ibuprofen in aqueous environments were measured spectrophotometrically by measuring the absorbance at λ=221nm, in 1, 2, 5, 10 and then every 10 minutes to 120 minutes after starting, respectively. The dissolution profiles are shown in Fig. 5.

Fig. 5 shows that the dissolution rate of large crystals obtained from run (16) (solubility Pressure: 110 bar, nozzle temperature: 80°C) didn't improve in contrast to the original sample, but particles obtained from run 6 (solubility Pressure: 90 bar, nozzle temperature:80°C) exhibit considerably higher dissolution rate. This enhancement is based on reducing surface area and structure crystalline

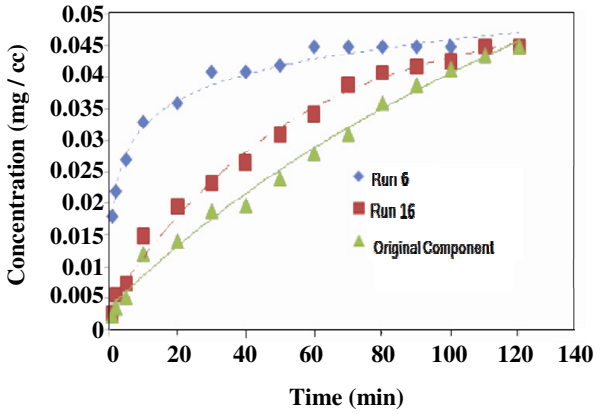

Fig. 5: Dissolution profiles of products achieved from runs 6 (Solubility Pressure: 90 bar & Nozzle temperature: 80˚C), 16 (Solubility Pressure: 110 bar & Nozzle temperature: 80˚C) and the original sample.

form. Destruction of crystalline character is an important advantage of RESS process which is not observed in other micronization methods.

CONCLUSION

A new parameter (Nozzle Temperature) effect on RESS processed ibuprofen particles has been investigated at the present work. Results showed that in low solubility pressures (< $2P_c$) fine and uniform particles form, especially in low nozzle temperatures. In high pressures (above 110 bar), low nozzle temperatures lead the precipitation process to face with 2-phase condition. Of course, in this area, high nozzle temperatures (above 90°C), increase S factor and small initial nucleuses form those easily stick to each other. Also in RESOLV process, particles are formed in an instable suspension form. At the presence of surfactants, a more uniform suspension is conformed, but a separation stage should be considered to extract the surfactant from the main component which needs the extra separation and purification process.

Symbols

d	Diameter of an species of particles
d.a.	Mean particles size
n	Number of particles of the species d
P	Absolute pressure
PSD	Mean particles distribution
S	Super saturation ratio
T	Absolute temperature
$y_E (T_E,P_E)$	Saturated mole fraction in pre-expansion condition
$y^* (T,P)$	Mole fraction in free jet condition

Received : May 20, 2009 ; Accepted : May 21, 2010

REFERENCES

[1] Ya Ping Sun, Radhakishan Guduru, Feng Lin, A Static Methods Coupled with Gravimetric Analysis for the Determination of Solubility of Solids in Supercritical Carbon Dioxide, *Ind. Eng. Chem. Res.*, **39**, p. 4663 (2000).

[2] True l. Rogers, Keith P. Johnston and Robert O. Williams, Solution-Based Particle Formation of Pharmaceutical Powders by Supercritical or Compressed Fluid CO_2 and Cryogenic Spray-Freezing Technologies, *Drug Development and Industry Pharmacy*, **27**(10), p. 1003 (2001).

[3] Rahoma S. Mohamed, Pablo G. Debenetti, Robert K. Prud'home , Effects of Process Conditions on Crystals Obtained from Supercritical Mixtures, *AIChE*, **35**(2) (1989) , p. 325 (2002).

[4] Sundeep Sethia, Eemilio Squinlante, Physicochemical Characterization of Solid Dispersions of Carbamazepine Formulated by Supercritical Carbon Dioxide and Conventional Solid Evaporation Method, *Journal of pharmaceutical Science*, **91**(9), p. 1948 (2002).

[5] Ranjit Thakur and Ram B. Gupta, Supercritical CO_2 Based Silica Coating of Gold Nano-particles Using Water-in-Oil Microemulsions, *Ind. Eng. Chem. Res.*, **44**, p. 7380 (2005).

[6] Estella J, Echeverria JC, Laguna M, et al., Effect of Supercritical Dying Conditions in Ethanol on the Structural and Textural Properties of Silica Areoles, *J. Porous Materials*, **15**, p. 705 (2008).

[7] Chen Y, Koberstein JT., Fabrication of Block Copolymer Monolayers by Adsorption from Supercritical Fluids: A Versatile Concept for Modification and Functionalization of Polymer Surfaces, *Langmuir*, **24**, p. 10488 (2008).

[8] Neil Foster, Fariba Dehghani, Kiang M. Charoenchiakool and Barry Warwick, Application of Dense gas Techniques for the Production of Fine Particles, *AAPS Pharm. Sci.*, **5**(2), p. 112 (2003)

[9] Pablo J. Debenetti, Homogenous Nucleation in Supercritical Fluid, *AIChE*, **36**(9), p. 1289 (1990).

[10] Ashish K.Lele, Annette D. Shine, Morphology of Precipitated from a Supercritical Solvent, *Ind. Eng. Chem. Res.*, **33**, p. 1476 (1994).

[11] Jung J. Perrut, Particle Using by Supercritical Fluid, *J. of Supercritical Fluids*, **20**, p. 179 (2001).

[12] Hirunsit P., Huang Z., Srinophakon T., Charoenchaitrakool M., Kawi S., Particle Formation of Ibuprofen- Supercritical CO_2 System from Rapid Expansion of Supercritical Solution, Powder Technology, **154**, p. 83 (2005).

[13] Debenetti P.G., Tom J.W., Kwauk X., Yeo S.-D., Rapid Expansion of Supercritical Solutions (RESS): Fundamentals and Applications, *Fluid Phase Equilibria*, **82**, p. 311 (1993).

[14] Victor Stepanov, LevN. Krasnoperov, Inga B. Elkina, Xuyean Zhang, Propellants, Production of Nanocrystalline RDX by Rapid Expansion of Supercritical Solutions, *Explosives, Pyrotechnics*, **30**(3), p. 178 (2005).

[15] Dean W. Matson, John L. Folton, Robert C.Peterson and Richard D. Smith, The Prepration of Polycarbosylane Power and Fibers during Rapid Expansion of Supercritical Fluid, *Material Letters*, **4**(10), p. 429 (1986).

[16] Kosal E., Lee C.H., Holder G.D., Modeling Solubility of Biological Compounds in Supercritical Fluids , *J. of Supercritical Fluids*, **5**, p. 169 (1992).

[17] Coimbra P., Duarte C.M.M., Sousa H.C., Solubility of Flurbiprofen in CO_2 and CO_2 + Methanol, *Fluid Phase Equilibria*, **239**, p. 188 (2006).

[18] Charoenchaitracool M., Dehghani F., Foster N.R., Application of Dense Gas Techniques for the Production of Fine Particles, *Ind. Eng. Chem. Res.*, **39**, p. 4794 (2000).

[19] Jingdai Wang, Jizhung Chen, Yongrong Yang, Supercritical Technology in Brazil: System Investigated, *J. of Supercritical Fluids*, **33**, p. 159 (2005).

[20] Helfgen B., Hils P., Holzknech Ch., Turk M., Schaber K., Time-Resolved Aerosol Collector for CCSEM/EDX Single-Particle Analysis, *Aerosol Science*, **32**, p. 295 (2001).

[21] Eun-Seok Song, Keun Hyung Lee, Youn-Woo Lee, "CFD Simulations of the Supercritical Fluid Process", 1st International Symposium on Applications of Supercritical Fluids in Green Chemistry and Material Science, Beijing, China, 2007.03.03

[22] Wlliams J.R., Clifford A.A., Bartle K.D., Kee T.P., The Production of Fine Particles of Metal Complexes using Supercritical Fluids, *Power Technology*, **96**, p. 158 (1989).

[23] Defne Kyrak, Ugur Akman, Oner Hortacsu, The Solubilities of Xanthone and Xanthene in Supercritical Carbon Dioxide: Structure Effect, *J. of supercritical Fluids*, **26**, p. 17 (2003).

[24] Corazza M.L., Cardozo Filho L., Dariva C., Modeling and Simulation of Rapid Expansion of Supercritical Solutions, *Braz. J. Chem. Eng.*, **23**(3), p. 214 (2006).

[25] Jaques Fages, Hubert Luchard, Jean-Jaques Letorneau, Martial Sauceau, Particle Generation for Pharmaceutical Applications Using Supercritical Fluid Technology, *Powder Technol.*, **141**, p. 219 (2004).

[26] Nuray Yildiz, Sebnem Tuna, Onur Duker, Alya Calimli, Particle Size Design of Digitoxin in Supercritical fluids, *J. of Supercritical Fluids*, **41**, p. 440 (2007).

[27] Markus Weber, Lynn M. Russell, Pablo G. Debenetti, Hydrodynamics Modeling and Analysis of Rapid Expansion Systems of Supercritical Solutions (RESS), *J. of Supercritical Fluids*, **23**, p. 65 (2002).

[28] Alessi P., Cortesi A., Kikic I., Foster N.R., Colombo I., Supercritical-Fluid Processing Technique for Nanoscale Polymer Particles, *Ind. Eng. Chem. Res*, **35**, p. 4718 (1996).

[29] Jong-Hyun Kim, Thomas E. Paxton and David L. Tomasko, Rapid Expansion from Supercritical to Aqueous Solution to Produce Submicron Suspensions of Water-Insoluble *Drugs, Biotechnol Prog.,* **12**, p. 650 (1996).

[30] Jouyban A., Rehman M., Shekounov B.Y., Chan H.K., Study of Interaction between Ibuprofen and Nicotinamide Using Diggerential Scanning Calorimetry, Spectroscopy, and microscopy and formulation of a Fast-Acting and Possibly Better Ibuprofen Suspension for Osteoarthritis Patients, *J. Pharm. Sci*., **91**(5), p. 1287 (2002).

[31] Nagi A. Alhaj, Mariana N. Shamsudin, Hana F. Zamri, Rasedee Abdullah, Extraction of Essential Oil from Nigella Sativa Using Supercritical Carbon Dioxide: Study of Antibacterial Activity, *American*, *J. Pharmacology and Toxicology*, **3**(4), p. 225 (2008).

[32] Gina R. Shaub, Joan F.Brennecke and Mark J. McCready, Supercritical Fluid Extraction of Palm Oil Components, *J. of Supercritical Fluids*, **8**, p. 318 (1995).

[33] Cherniyak Y., Henon F., Herris R.B., Gould R.D., Metal Nanoparticles Prepared in Supercritical Carbon Dioxide, *Ind. Eng. Chem. Res*., **40**(26), p. 6118 (2001).

[34] Alessi P., Cortesi A., Kikic I., Foster N.R., Colombo I., Particle Production of Steroid Drugs Using Supercritical Fluid Processing, *Ind. Eng. Chem. Res.*, **35**, p. 4718 (1996).

[35] Mahajani S. M., Sharma M. M., Sridhar T., Direct Hydration of Propylene in Liquid Phase and Under Supercritical Conditions in the Presence of Solid Acid as Catalyst, *Chem. Eng. Sci*., **57**, p. 4877 (2002).

[36] Michael Turk, Ralph Lietzow, Particle Production by Supercritical Antisolvent Processing, *Techniques AAPS Pharm. Sci. Tech.*, **5**, p. 103 (2004).

[37] Petra Sencar-Bozic, Stane Srcic, Zeljko Knez, Janez Kerk, Improvement of Nifedipine Dissolution Characteristics Using Supercritical CO_2 , *International Journal of Pharmaceutical*, **148**, p. 123 (1997).

[38] Anderian Tandya, Fariba Dehghani, Neil R Foster, Nanomaterial and Supercritical Fluids, *J. of Supercritical Fluids*, **37**, p. 272 (2006).

[39] Pankaj Pathak, Mohammed J. Meziani, Tarang Desai, Ya-ping Sun, Formation and Stabilization of Ibuprofen Nanoparticles in Supercritical Fluid Processing, *J. of Supercritical Fluids*, **37**, p. 279 (2006).

[40] Sang-Do Yeo, Erogan Kiran, Formation of Polymer Particles with Supercritical Fluids: A Review, *J. Supercritical Fluids*, **34**, p. 287 (2005).

[41] Ajay Kumar Gupta, Mona Gupta, Synthesis and Surface Engineering of Iron Oxide Nanoparticles for Biomedical Applications, *Biomaterials*, **26**, p. 3995 (2005).

[42] Gupta AK, Gupta M., Synthesis and Surface Engineering of Iron Oxide Nanoparticles for Biomedical Applications, *Biomaterials*, **18**, p. 3995 (2005).