MODIFICATION OF THE MORPHOLOGY OF CAFFEINE AND CIMETIDINE USING SUPERCRITICAL CO_2

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Abstract: Supercritical fluid technique (SCF), in this case using supercritical CO_2 as a medium, offers promising approach of morphology changes of materials and moreover reduction of particle size at moderate operating conditions. Thus SCF could be suitable for treatment of many thermally unstable compounds, for example the pharmaceuticals [1]. In this study a commercially supplied device Spe-ed SFE-4 (Applied Separations, USA) has been used for size reduction of selected biologically active substances cimetidine and caffeine. Procedures were carried out by techniques of rapid expansion of supercritical mixtures (RESS) or using supercritical CO_2 as an anti-solvent (GAS). Submicron particles and nanoparticles of caffeine were prepared using RESS procedure; moreover the increasing of temperature caused the reduction of caffeine nanoparticles size and had an impact on the shape of the resulting particles. Cimetidine submicron particles were obtained using GAS method. In this case higher pressure leads to the smaller particles which have enormous tendency to agglomerate; the influence of higher temperature could be summarized as positive. It is evident that the use of supercritical CO_2 could lead to interesting morphology modification, as well as to reduction of the particles size. This has a positive impact on improvements in the terms of bioavailability of drugs.

Keywords: Supercritical fluid, submicron particles, micronization, cimetidine, caffeine

Introduction

Supercritical fluids (SCFs) combine properties of gases and liquids such as low viscosity and high density; therefore they are suitable for preparation of nanoparticles. Moreover, supercritical fluids (SCFs) enable reduction of particle size at moderate operating conditions; thereby SCFs would be suitable for many thermally unstable compounds; as e.g. pharmaceuticals. CO_2 is the most used SCF because of low critical points and non-toxic properties [1-4].

Rapid expansion of supercritical solutions (RESS) is a process consisted of the saturation of the supercritical fluid with solid substrate followed by the depressurization of the solution through a heated nozzle into a low pressure chamber. It produces a rapid nucleation of the substrate in the form of very small particles that are collected from the gaseous steam [1-4].

Supercritical CO_2 (sc-CO_2) can be used in addition to the direct method (RESS) as so called gas anti-solvent (GAS); in the case of GAS procedure solid material is first dissolved in a suitable organic solvent then the sc-CO_2 is loaded to the vessel (three-component system). Pressing of sc-CO_2 into the solution with the solute causes a volume expansion of the liquid phase; resulting decrease of density decreases solvent power of the organic solvent and then causes the precipitation of the solute out of solution. Particle size and morphology could be influenced in the both cases (the RESS and GAS methods) by using different temperature, pressure, but also by design and temperature of output nozzles, duration of static or dynamic mode (i.e. the length of the sample remaining in the autoclave) and moreover by the concentration of sample inside of the autoclave [1, 5].

This paper introduces results from: i) preparation of nano/microparticles of caffeine using RESS method observing changes in caffeine size distribution depending on temperature and ii) preparation of nano/microparticles of cimetidine using GAS procedure observing an influence of temperature and in particular pressure on cimetidine particle size. Commercial system Spe-ed SFE-4 (Applied Separations Inc., USA), which is mainly designed for extraction of organic compounds from solid samples, was used (Fig. 1).

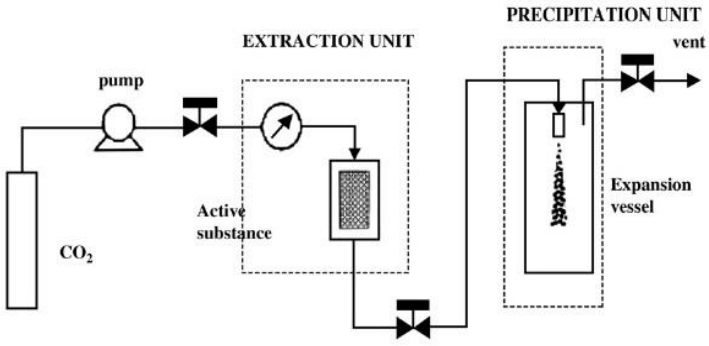

Figure 1: Schema of SFE apparatus, RESS method [2].

Experimental

Caffeine [6] (i.e.: 1,3,7-trimethylxanthine) and cimetidine [7] (i.e.: 1-cyano-2-methyl-3-(2-(((5-methyl-4-imidazolyl)methyl)thio)ethyl)guanidine) were used as the test substances, CO_2 (SIAD, purity 4.8) was employed as a solvent (RESS) and anti-solvent (GAS) supercritical medium and methanol and dichloromethane as auxiliary solvents were used as well. All the named chemicals were supplied by Sigma Aldrich.

Nanoparticles and microparticles of caffeine (0.2 g sample weight) were prepared by RESS method using the temperatures of 45, 50, 80 and 100 °C. Pressure was set to a constant value of 150 bar. Cimetidine (0.1 g) was dissolved in 800 µl of methanol and the influence of temperature was followed at 40, 50 and 70 °C, as well as the influence of pressure, at 150, 200 and 250 bar. After the process, cimetidine remained in autoclave. It was washed out with dichloromethane and dried at ambient temperature. The temperature of the output nozzles for all processes was set to a constant value of 110° C.

To determine the particle shape and morphology scanning electron microscopy (SEM) was used (Quanta FEG 450), X-ray power diffraction (XRD) analysis was used (Rigaku UltimaIV) for measurement of crystallinity and particle size distribution was determined by Zetasizer Nano ZS – Malvern for caffeine and Nanopartica SZ, Horiba for cimetidine particles.

Results

Scanning electron microscopy has showed that original caffeine particles before using sc-CO_2 formed crystals of approximate size of 10 to 30 micrometres (Fig. 2a), while particles prepared by RESS at temperatures 50 °C (Fig. 2b) formed particles and nanoparticles, which agglomerated into larger units. Caffeine nanoparticles prepared by RESS at temperatures of 80 °C (Fig. 2c) and 100 °C (Fig. 2d) also agglomerated into larger units; however the shape and morphology were absolutely different, formed needle-like nanoparticles.

Similarly, scanning electron microscopy has confirmed the influence of the temperature and moreover of the pressure to the size and morphology of cimetidine particles. As it is shown in the Figure 3, where the original sample of cimetidine (a) shows the particles size of units to tens of micrometres, with increasing temperature and pressure (b) 50 °C, 150 bar and (c) 40 °C, 200 bar there are evident only small changes while the highest temperature as well as pressure (d) 50 °C, 250 bar caused the obvious difference in shape and size of particles together with evidence to create agglomerates.

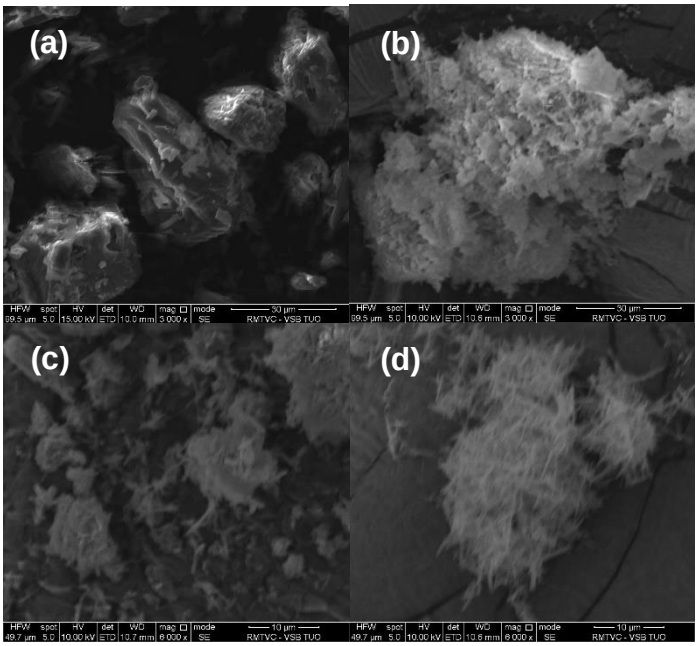

Figure 2: Comparison of SEM images of caffeine particles of unprocessed, original sample (a), after SCF procedure, using 50 °C (b), after SCF procedure, using 80 °C (c) and after SCF procedure, using 100 °C (d). Notice: the scale of a, b- images is around 98 μm, the scale of c, d- images is around 47 μm

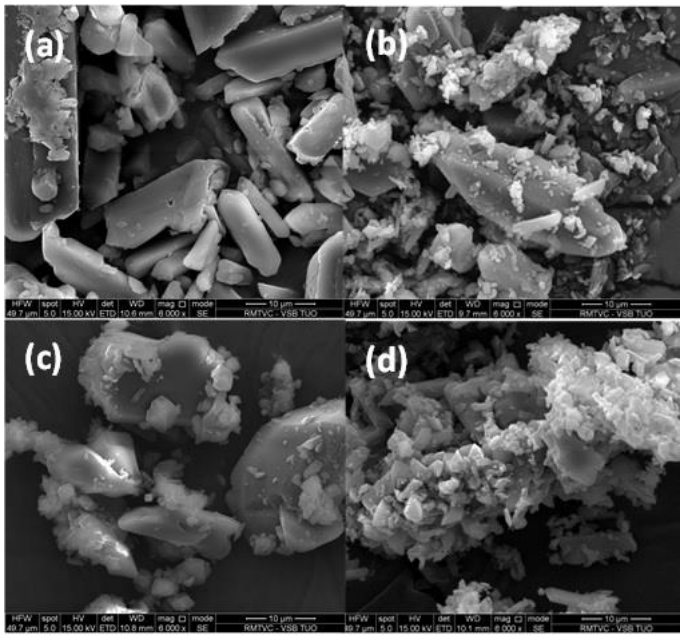

Figure 3: Scanning electron microscopy images of original sample of cimetidine (a), using sc-CO_2, 50 °C, 150 bar (b), using sc-CO_2, 40 °C, 200 bar (c) and using sc-CO_2, 50 °C, 250 bar (d), the scale of images is around 47 μm.

X-ray powder diffraction has determined the crystallinity of the material. A comparison of the caffeine samples is shown on the XRD pattern of caffeine structure (Fig. 4, left). The position of the diffraction lines of original sample and the prepared samples are unchanged. It is apparent that the intensity of diffractions of the sample prepared by RESS at 45 °C (Fig. 4, left - b) opposite of original sample (Fig. 4, left - a) decreased. More significant decrease in intensity is observed in the case of the sample prepared by RESS at 100 °C (obvious in Fig.4, left - c), the scale is about one order of magnitude lower). This reduction in intensity occurs due to the lowering of crystallinity.

In the case of cimetidine samples (Fig. 4, right), it is obvious that positions of typical diffraction lines of samples prepared under SCF influence (Fig. 4, right, b, c, d) remain unchanged, small changes in ratios of typical diffractions intensities opposite of original cimetidine sample (Fig. 4, right - a) were observed rather due to reduce of crystallinity or insufficient sample volume than changes in the structure of cimetidine.

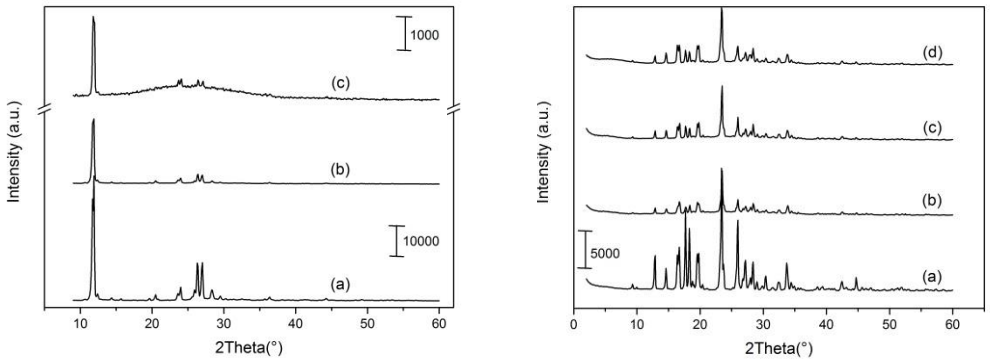

Figure 4: X-ray powder diffraction measurement of samples. Left (a) original caffeine particles; (b) caffeine particles prepared by RESS using 45 °C, 150 bar and (c) 100 °C, 150 bar. Right (a) original cimetidine particles; (b) cimetidine particles prepared by GAS using 50 °C, 150 bar; (c) 40 °C, 200 bar and (d) 50 °C, 250 bar.

Particle size distribution was measured for both type of samples, caffeine and cimetidine, by different devices: by Zetasizer Nano ZS – Malvern and Nanopartica SZ, Horiba, respectively. The sizes of the prepared caffeine particles are in the range from 50 to 1500 nm compared to original particles with approximate size from 10 to 30 micrometres. The graph of particle size distribution (Fig. 5) shows that at the temperature of 100 °C the volume distribution shifted to lower size, this was confirmed by the measuring of SEM as well.

The values of particle size distribution of cimetidine samples show that opposite of the original sample when the particle size distributions was around 26.7 μm the range of samples prepared by using of sc-CO_2 was from 3.1 μm to 21.9 μm; so this technique can achieve a substantial reduction in values of particle size distribution.

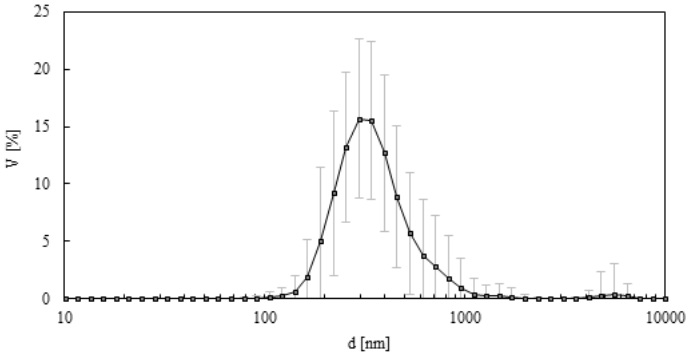

Figure 5: The volumetric particle size distribution of caffeine prepared by RESS method at 100 °C measured by Zetasizer Nano ZS – Malvern.

Conclusions

The preparation and consequent characterization of nano/microparticles of caffeine and cimetidine using sc-CO_2 as solvent or anti-solvent confirmed influence of the temperature and the pressure, eventually their combinations, on the size of newly formed nano/microparticles. In addition, the changes in these parameters influenced evidently shape and morphology of the formed particles, which could have an importance for improving properties as better solubility and bioavailability. It can be also concluded that commercial devices Spe-ed SFE-4 could be used for micronization of selected organic substances, and further testing is required to optimize processes leading to the smallest particles in a narrow range of particle size distribution.

Acknowledgement

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