Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 281–289

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejpb

Reseach paper

Study of the solid state of carbamazepine after processing with gas anti-solvent technique

M. Moneghini[a,*], I. Kikic[b], D. Voinovich[a], B. Perissutti[a], P. Alessi[b], A. Cortesi[b], F. Princivalle[c], D. Solinas[b]

[a]*Department of Pharmaceutical Sciences, University of Trieste, Italy*
[b]*Department of Chemical, Environmental and Raw Materials Engineering, University of Trieste, Trieste, Italy*
[c]*Department of Earth Sciences, University of Trieste, Trieste, Italy*

Received 13 November 2002; accepted in revised form 22 April 2003

Abstract

The purpose of this study was to investigate the influence of supercritical CO_2 processing on the physico-chemical properties of carbamazepine, a poorly soluble drug. The gas anti-solvent (GAS) technique was used to precipitate the drug from three different solvents (acetone, ethylacetate and dichloromethane) to study how they would affect the final product. The samples were analysed before and after treatment by scanning electron microscopy analysis and laser granulometry for possible changes in the habitus of the crystals. In addition, the solid state of the samples was studied by means of X-ray powder diffraction, differential scanning calorimetry, diffuse reflectance Fourier-transform infrared spectroscopy and hot stage microscopy. Finally, the in vitro dissolution tests were carried out. The solid state analysis of both samples untreated and treated with CO_2, showed that the applied method caused a transition from the starting form III to the form I as well as determined a dramatic change of crystal morphology, resulting in needle-shaped crystals, regardless of the chosen solvent. In order to identify which process was responsible for the above results, carbamazepine was further precipitated from the same three solvents by traditional evaporation method (RV-samples). On the basis of this cross-testing, the solvents were found to be responsible for the reorganisation into a different polymorphic form, and the potential of the GAS process to produce micronic needle shaped particles, with an enhanced dissolution rate compared to the RV-carbamazepine, was ascertained.

Keywords: Supercritical CO_2; Gas anti-solvent; Carbamazepine; Polymorphism

1. Introduction

In the 1970s and 1980s, supercritical fluid technology was mainly focused on extraction and separation. However in the last 10 years, interest in supercritical fluid processing has also arisen in other areas. In particular, supercritical carbon dioxide is known to be a quasi-ideal solvent medium for preparation of pharmaceutical formulations such as microparticle formation, impregnation with supercritical solutions and microencapsulation, with the additional advantage of being able to remove completely the residual solvent in the processed carrier/drug formulation.

The particle formation processes involving supercritical fluid are based on the rapid expansion of supercritical solution (RESS) and gas anti-solvent (GAS) crystallisation. The latter is mostly used for recrystallisation of powders, the most important limitation of RESS development being the excessively low solubility of substances in supercritical fluids. The GAS method originates from the knowledge that the absorption of a gas in a liquid causes the liquid to expand. In particular, when a solution is expanded sufficiently by a gas, the liquid phase is no longer a good solvent for the solute (drug, polymer or both) and nucleation takes place. Advantages include higher solute throughput and flexibility of solvent choice [1–5].

The objective of this study was to investigate the applicability of the GAS method to the formation of carbamazepine (CBZ) particles and to control their characteristics by determining the influence of the process and the solvents on solid-state properties of drug particles. CBZ, a well-known anticonvulsivant drug, characterised by a slow and irregular gastro-intestinal absorption due to its

---

[*] Corresponding author. Department of Pharmaceutical Sciences, University of Trieste, Piazza le Europa 1, 34127 Trieste, Italy. Tel.: +39-40-558-3105; fax: +39-40-52-572.

E-mail address: moneghin@univ.trieste.it (M. Moneghini).

0939-6411/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0939-6411(03)00092-4

low water solubility [6], has been extensively investigated in terms of solid-state studies [7–11]. Previous data have shown that CBZ exists as several polymorphic and pseudo-polymorphic forms [12–14].

With regards to supercritical fluid processing, Bettini et al. [15] investigated whether mixtures of CBZ polymorphs could be processed in dynamic and static condition in supercritical CO_2 to select the pure stable form III, and found that the conversion in the stable crystalline phase could be promoted by treating the mixture at 55°C and 350 bar. Edwards et al. [16] processed all anhydrous CBZ polymorphs by solution enhanced dispersion with supercritical fluids (SEDS™), showing that polymorph generation is influenced by crystallisation kinetics, solvent effects and temperature. In our previous work, we improved the dissolution properties of carbamazepine, applying GAS technique for the preparation of solid dispersions with PEG 4000, maintaining the original polymorphic form of the drug unaltered [17]. More recently, Sethia and Squillante [18] compared the physico-chemical properties and dissolution performances of CBZ in solid dispersions, formulated by supercritical carbon dioxide and conventional solvent evaporation method, using PEG 8000, Gelucire 44/14 and vitamin E TPGS NF (d-α-tocopheryl PEG1000 Succinate) as carriers. The authors found that CBZ after CO_2 treatment converted into form I, whilst the conventional solvent evaporation method gave a CBZ sample consisting of a mixture of form III (starting) and form I.

In this work, we investigated the influence of GAS method on the physico-chemical properties of pure CBZ, while considering the effects of using different solvents. After selection of the appropriate solvents on the basis of their CO_2-volumetric expansions, the precipitation of the drug was carried out by GAS technique and conventional evaporation method (RV) from acetone, ethylacetate and dichloromethane. Then, the characterisation of drug solid state encompassed the use of X-ray powder diffraction (XRD), differential scanning calorimetry (DSC), hot stage microscopy (HSM) and diffuse reflectance Fourier-transform infrared spectroscopy (DRIFT) analyses. The shape and size of drug were determined by scanning electron microscopy analysis (SEM) and image analyses. Finally, the in vitro dissolution tests were performed.

2. Materials and methods

2.1. Materials

Carbamazepine USP-grade was provided by Sigma-Aldrich Chemicals (Steinheim, Germany, Germany) and was used as received. All the solvents (acetone, dichloromethane, ethylacetate) were of analytical grade and were provided by Carlo Erba (Milan, Italy). CO_2 (purity 99.9%) was supplied by SIAD (Trieste, Italy).

2.2. Solubility studies

The solubility of carbamazepine in supercritical CO_2 was determined using the dynamic method at the temperature of 40°C and at three different pressures: 100, 130 and 160 bar. The detailed experimental equipment and method have been previously reported by MacNaughton et al. [19]. The determination of solubility was based on the mass of CBZ solubilised and the corresponding volume of CO_2. The gas volume was measured with a precision of ± 0.01 l and an accuracy of $\pm 0.4\%$ whereas the mass of solute was determined with a precision of ± 0.2 mg and an accuracy of $\pm 0.2\%$. The system pressure was measured with an accuracy of $\pm 0.1\%$ and the temperature with a precision of ± 0.1 K.

2.3. Volumetric expansion

The apparatus used in this study and the applied method are very similar to that reported in literature [19].

The volumetric expansion can be calculated using the following equation:

$$\Delta V\% = 100\,\frac{V(p,\,T) - V_0(p_0,\,T)}{V_0(p_0,\,T)} \tag{1}$$

where $V(p, T)$ was the volume in the liquid phase added to the supercritical CO_2 at a constant temperature T, with increasing p; V_0 was the initial volume at ambient pressure p_0.

2.4. Preparation of the samples

The detailed experimental equipment has been already described by Kikic et al. [20]. A precipitation vessel with a capacity of 50 ml was loaded with a 7 ml solution of pure drug (with a concentration ranging about 2% w/w) in acetone, or dichloromethane, or ethylacetate. The supercritical CO_2 was added from the bottom of the chamber and when the liquid phase expanded, the formed particles were retained in the vessel by a suitable filter (0.2 μm pore size). During the particle formation, the temperature was fixed at 40°C and the pressure was 63 bar for acetone and dichloromethane, and 53 bar for ethylacetate.

For comparison purposes, samples of CBZ from the same lot were recrystallised following a procedure used in a previous study [21]: an appropriate amount of CBZ (2% w/w solution) was added to the appropriate solvent in a round bottom flask under stirring. The solvent was then removed under reduced pressure in a rotary evaporator (Buchi R-114, Flawill, Switzerland) at 40 ± 1°C. Then, the samples were kept for 5 days in a desiccator under vacuum at room temperature before the characterisation.

2.5. Characterisation of the samples

2.5.1. Particle size and shape analysis

Particle size analysis and dynamic shape characterisation of the CO_2-treated samples and starting CBZ were determined using a Galai Mod.CIS-100 Particle Size and Shape Analyzer (Tecno Galenica s.r.l.- Cernusco S/N, Milan, Italy) using the software: Galai WCIS-100 (version 1.36) and Galai WSHAPE (version 1.37), respectively. Small amounts of each sample were uniformly dispersed using a powder-dispersor Galai PD-10 on top of a microscopy glass slide. For each powder batch, three microscopy glass slides were prepared, examining at least 20,000 particles per sample. The size of each sample was determined as specific length, specific width, while the shape was expressed as aspect ratio and shape factor. For each particle 36 values of Feret's diameter were determined. The area of the particle is calculated multiplying the area occupied by a single pixel by the number of pixels corresponding to the particle (1 pixel corresponds to 0.9 μm). The perimeter is the number of pixels of the external particle contour. The aspect ratio (AR) is a shape parameter giving the squareness of an object: a value approaching 0 indicates a line, while a value of 1 is typical of a square or a circle. It is computed as follows:

$$\mathrm{AR} = \frac{d_{\min}}{d_{\max}} \qquad (2)$$

where $d_{\min}$ indicates the minimum Feret diameter and $d_{\max}$ indicates the maximum Feret diameter.

The shape factor (SF) is defined as the smoothness of the particle, if the value is close to 0 the particle is elongated and/or irregular, if it is close to 1, then it is smooth and rounded. In particular SF results from:

$$\mathrm{SF} = \frac{4\pi A}{p^2} \qquad (3)$$

where A and p are the area and the perimeter of the particle, respectively.

2.6. Scanning electron microscopy analyses (SEM)

The shape and surface characteristics of the samples were observed by SEM. Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milan, Italy) and examined using a scanning electron microscope (model 500, Philips, Eindhoven, The Netherlands) at 10 KV accelerating voltage using the secondary electron technique.

2.7. Hot-stage microscopy (HSM)

Physical changes in the samples on heating were monitored performing hot stage microscopy studies. A hot plate (FP 52 Mettler, Greifensee, Switzerland), connected to a temperature controller (FP 5 Mettler) was used. A little amount of each sample was placed on a glass slide with a cover glass and heated at 10°C/min in the temperature range of 30–200°C. The changes in the samples were observed via an optical microscope (Reichert Biovar, Wien, Austria) (magnification 10 ×).

2.8. Differential scanning calorimetry (DSC)

Calorimetric analyses were performed with a DSC mod. TA 4000 (Mettler), equipped with a measuring cell DSC 20. The calibration of the instrument was performed with indium, zinc and lead for the temperature, and with indium for the measurement of the enthalpy. Samples, containing about 2 mg of CBZ were placed in pierced aluminium pans and heated at the scanning rates of 10 and 40°C per min from 30 to 210°C, under air atmosphere.

2.9. X-ray powder diffraction studies (XRD)

Samples were studied by means of XRD technique using a STOE D500 (Siemens, Munich, Germany) diffractometer with Cu Kα radiation, monochromatised by a secondary flat graphite crystal. The scanning angle ranges from 3 to 40° of 2θ, steps were of 0.04° of 2θ, and the counting time was of 2 s/step. The current used was 20 mA and the voltage 40 kV.

2.10. DRIFT spectroscopy

Fourier transform-infrared spectra were obtained on a FT-IR spectrometer (FT-IR 200 Jasco, Tokyo, Japan) using the diffuse reflectance method (DRIFT). The samples were ground gently with anhydrous KBr, to avoid polymorphic transition possibly induced by extended grinding. The scanning range was 400–4000 cm^{-1} and the resolution was 2 cm^{-1}.

2.11. Determination of drug dissolution

The dissolution tests were performed according to the rotating paddle method (USP 24). A dissolution apparatus (model DT-1, Erweka, Heusenstamm, Germany) was employed with a stirring rate of 100 rpm and was maintained at 37 ± 0.1°C. The dissolution medium was 900 ml of freshly demineralised water. An accurately weighed sample of CBZ as a powder before and after CO_2 and RV treatment, corresponding to a suitable amount (18 mg) of CBZ for sink conditions ($C \ll C_s$) was dispersed in 900 ml of dissolution medium. The aqueous solution was filtered and continuously pumped to a flow cell in a spectrophotometer (model 552, Perkin-Elmer, Norwalk, USA) and absorbance values were recorded at the maximum wavelength of the drug (285 nm).

Finally, the analysis of variance (one-way ANOVA test) was carried out on dissolution data, comparing the mean value of each sample and its relative standard deviation.

3. Results and discussion

The solubility results are the average of at least three separate measurements. The maximum deviation between the measurements was ±3% giving a good indication of the expected accuracy of the results. Since the overall error (i.e. pressure, temperature, mass and volume) related to the individual equipment errors is significantly lower, the deviation is due to random experimental errors associated with the difficulties of working with high-pressure supercritical fluids and with the problems connected to the continuous flow technique (such as partial clogging of the regulating valve inducing possible fluctuations of flow rate).

The solubility of CBZ in CO_2, expressed as molar fractions, at 100 bar is in the order of 10^{-6}, the limit of the experimental apparatus (gravimetric determination). Its solubility at 130 and 160 bar is 2.63 and 3.93 10^{-5}, respectively. These low values for CO_2 solubility highlight the necessity of using GAS technology to enhance drug precipitation.

As for the volumetric expansion of the solvents at 40°C, according to Kordikowski et al. [22], dichloromethane, acetone and ethylacetate presented the highest expansion capacity at the tested temperature, and were therefore chosen as solvents for precipitation experiments.

The precipitation of CBZ from acetone and ethylacetate, gave a very light, voluminous powder, as typical of CO_2-precipitated samples [17,18,23,24], whilst the gross morphology of sample treated with dichloromethane was less voluminous and characterised by the presence of long needles.

The results of laser granulometer studies, expressed as values of specific length, specific width, shape factor and aspect ratio for each sample, are reported in Table 1. These results highlight that the processing of carbamazepine with GAS technique changed both the size and shape of drug crystals dramatically. To go into more details, a remarkable reduction of the crystal dimensions with a restricted particle size distribution was obtained with all three cosolvents, ethyl acetate seemingly being the most efficient vehicle to micronise this drug. The values of shape factor and aspect ratio were quite constant for all three CO_2-treated samples, pointing to elongated crystals. On the other hand, starting CBZ consisted of homogeneous particles in terms of size, with a regular shape, presumably similar to parallelepiped, and with a practically smooth surface. This data was confirmed by SEM analysis, where prismatic crystals having rounded edges and smooth surface (Fig. 1a) could be observed. Conversely, the appearance of the sample treated with supercritical CO_2 was very different, consisting predominantly of needle-shaped crystals, with size dependent on the solvent used (Figs. 1b–d). Moreover, the appearance of the crystals of CO_2-samples was identical to the polarised light images of the trigonal form recorded by Rustichelli et al. [14] and to the SEM images reported by Roberts et al. [25]. It must be noted that in the case of the acetone-sample, the needles are arranged in the fashion of a paint brush. Comparing these photomicrographs with the samples recrystallised by removal of the solvents in a rotary evaporator (RV-samples) revealed a great difference in shape: acetone-RV (Fig. 2a) and dichloromethane-RV samples (Fig. 2b) looked similar to large and thin foils with irregular edges, whilst ethylacetate-RV (Fig. 2c) was arranged in sponge-like irregular particles.

The examination by optical microscopy (HSM) of pure CBZ revealed, in agreement with previous observation, the presence of prismatic agglomerates. Starting from 160°C, the formation of needle-shape crystals could be noticed, followed by their melting between 190 and 192°C.

In the sample treated with acetone, the presence of long needle-shape particles was initially predominant, and few fragments were observed. During heating, the small particles disappeared, turning into needles at temperatures higher than 165°C. Finally, the fusion of the crystals occurred at 189–192°C. A very similar situation was observed analysing the sample precipitated from ethylacetate. On the other hand, the carbamazepine previously dissolved in dichloromethane, consisted of little needle-shaped particles with some prismatic crystals. When increasing the temperature, some needles started to spread out from the edges of the prisms, and the complete transformation into needles was ended at 170°C. Again, the crystals melted in the 189–192°C range, according to the endothermal event subsequently registered by DSC.

The DSC traces of pure and treated drug, performed at the scanning rate of 10°C/min, only showed a melting endotherm at about 192°C with a heat of fusion of 103.5 J/g (data not shown). Yet, In order to understand whether a polymorphic transition happened during the process, the same analyses were performed using a different scanning rate, as previously suggested by several authors [12,14,15,17,18]. In fact, the DSC traces, as well as the melting point values, depend on the analytical scanning rate, and very different DSC curves can be observed owing to

Table 1
Results of image analysis of the starting CBZ and CO_2-processed samples (mean ± SD, $n = 20000$)

	CBZ-USP	Acetone	Dichloromethane	Ethylacetate
Specific length (μm)	365.70 ± 15.49	161.86 ± 15.35	173.36 ± 15.88	97.46 ± 9.73
Specific width (μm)	237.09 ± 10.85	40.35 ± 3.78	38.65 ± 3.57	37.27 ± 3.24
Shape factor	0.87 ± 0.36	0.64 ± 0.45	0.62 ± 0.47	0.68 ± 0.39
Aspect ratio	0.74 ± 0.31	0.56 ± 0.44	0.52 ± 0.47	0.59 ± 0.41

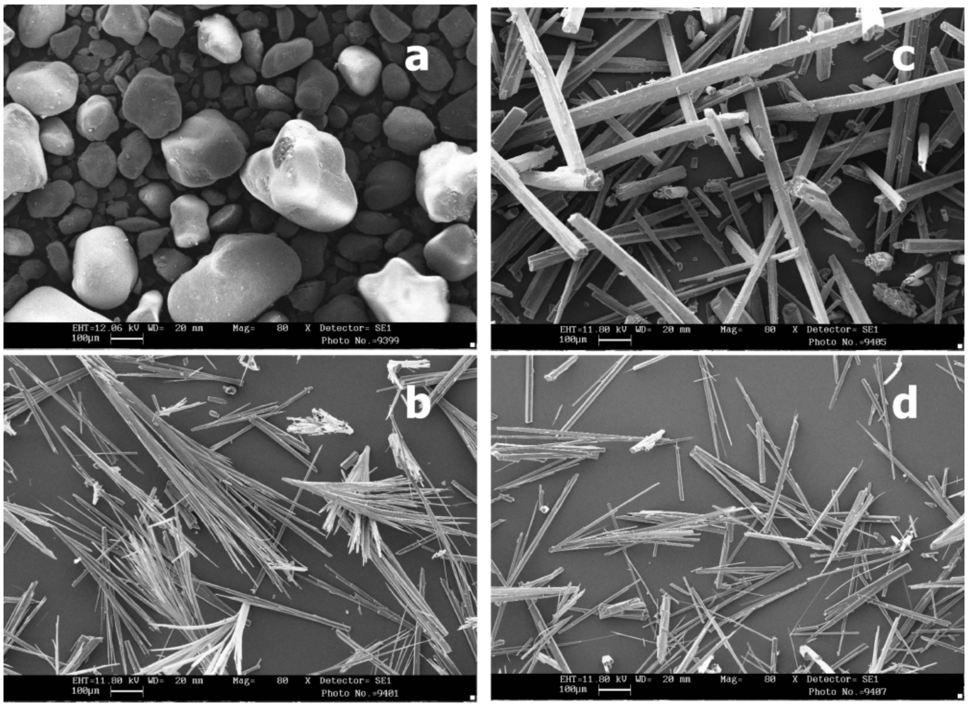

Fig. 1. SEM pictures of: (a) starting CBZ-USP; (b) CBZ-CO_2 processed with acetone; (c) CBZ-CO_2 processed with dichloromethane; and (d) CBZ-CO_2 processed with ethylacetate.

differences in the rate of transformation of CBZ form III → I form: a low scan rate causes the solid-solid transition into form I at around 155°C, therefore the DSC curves only show the corresponding peak of fusion at about 190°C. Hence, the DSC analyses of treated and starting CBZ were repeated performing the analysis at 40°C min^{-1}. The corresponding DSC traces are reported in Fig. 3. In this case, two endotherms of fusion are present: the first peak corresponded to the melting of the form III (m.p. about 175°C), followed by crystallisation and melting of form I

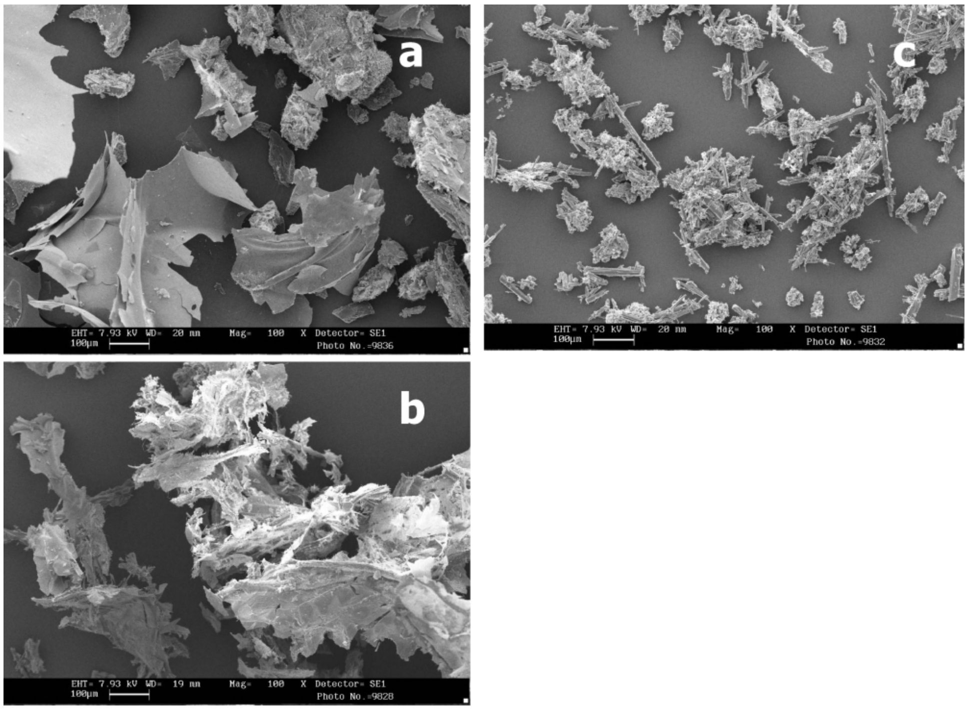

Fig. 2. SEM pictures of: (a) CBZ-RV recrystallised from acetone; (b) CBZ-RV recrystallised from dichloromethane; and (c) CBZ-RV recrystallised from ethylacetate.

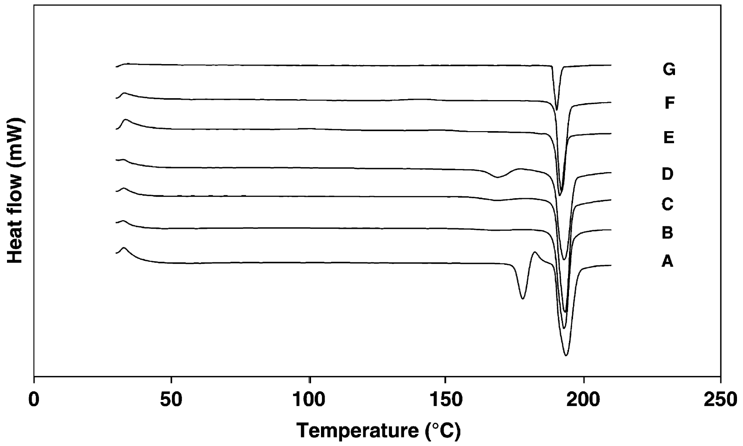

Fig. 3. DSC traces: (A) starting CBZ-USP; (B) CBZ-CO_2 processed with acetone; (C) CBZ-CO_2 processed with ethylacetate; (D) CBZ-CO_2 processed with dichloromethane; (E) CBZ-RV recrystallised from acetone; (F) CBZ-RV recrystallised from ethylacetate; and (G) CBZ-RV recrystallised from dichloromethane.

(m.p. about 190°C). The DSC curve of the pure drug (Fig. 3A) showed the following thermal events: an endothermic peak occurred at low temperature (177.2°C) corresponding to the melting of the monoclinic form III ($\Delta H = 25.2$ J/g), which subsequently recrystallised into the trigonal form I. Finally, the trigonal form melted at 192.3°C, as shown by the sharp endotherm at this temperature. These findings were in good agreement with the results reported by Bettini et al. [15].

The three samples treated with supercritical CO_2 attested a remarkable reduction of the endothermal event corresponding to the melting of the monoclinic polymorph (III). Further, no evidence of recrystallisation into the trigonal form could be found. This fact seems to attest a sensible decrease of the percentage of the form III in the treated samples. According to the previous findings observed by HSM, very similar trends were exhibited from the samples precipitated with acetone and ethylacetate (Figs. 3B,C). In fact, in both DSC traces, the fusion at 168.4°C of the form III occurred with a very scarce enthalpy (about 5 J/g). On the other hand, in the DSC curve of CBZ treated with dichloromethane (Fig. 3D), this peak was more intense (12.8 J/g). It must be also pointed out that in all treated samples the maximum of the peak, corresponding to the melting transition of the monoclinic form, shifted to lower temperatures (from 177 to 168°C). The thermal analysis of

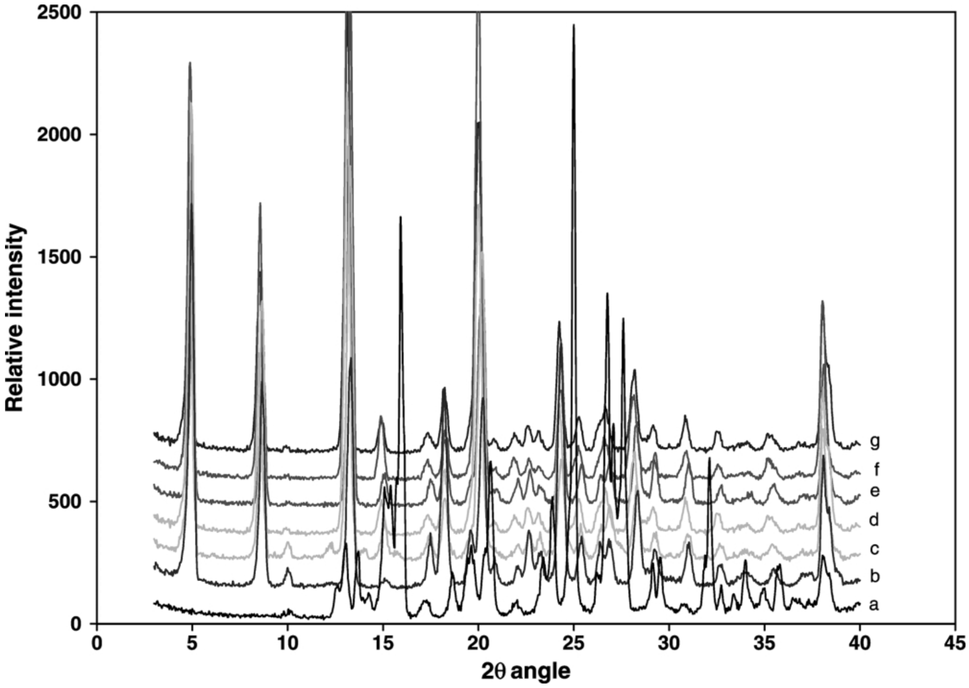

Fig. 4. XRD patterns of: (a) starting CBZ-USP; (b) CBZ-RV recrystallised from dichloromethane; (c) CBZ-RV recrystallised from ethylacetate; (d) CBZ-RV recrystallised from acetone; (e) CBZ-CO_2 processed with ethylacetate; (f) CBZ-CO_2 processed with dichloromethane; and (g) CBZ-CO_2 processed with acetone.

Table 2
Main differences in DRIFT spectra of starting (USP) and treated samples of carbamazepine

Absorbance position, cm^{-1}	USP	CO_2-acetone	CO_2-CH_2Cl_2	CO_2-ethylacetate	RV-acetone	RV-CH_2Cl_2	CO_2-ethylacetate
3600–3341	3466.4	3484.6	3485.1	3484.4	3483.9	3482.8	3484.3
3251–3076	3195.7[a] 3166.1[a]	3156.1	3155.8	3156.3	3156.3	3156.1	3157.2
1717–1568	1676.2	1690.1	1689.8	1691.0	1989.8	1689.6	1690.6
1318–1058	– –	1270.8 1130.6	1271.2 1131.6	1270.8 1131.6	1271.2 1131.1	1271.2 1131.8	1271.3 1130.8
1058–933	984	954.6	953.2	953.6	954.2	954.6	953.8

[a] These bands were reported as typical of form III by Lowes et al. [9].

the three RV-samples (Figs. 3E–G) indicated a complete transformation of the starting CBZ from the form III to the polymorph I, as attested by the absence of the first peak ranging at 170°C and the presence of a sharp endotherm at 191°C. Moreover a slight amorphisation of the sample recrystallised from dichloromethane (Fig. 3G) was noticed.

The XRD patterns of the treated and untreated samples are depicted in Fig. 4. Commercial USP-CBZ (Fig. 4A) was identical to that of β CBZ USP reference standard and to that reported by the International Centre for Diffraction Data: the most providing identification is the absence of peaks from 2 to 10° 2θ, as also reported by several authors [9,12,14]. Further, the diffractogram presented high intensity peaks at 15.9, 24.9, 26.7, 27.02, 27.5 and 32° 2θ. The XRD patterns of the sample CO_2-treated with acetone (Fig. 4G) could be clearly distinguished from the starting one. Two high intensity peaks were recorded at values lower than 10° 2θ (4.88 and 8.5° 2θ) and the intensity of the signals detected at 19.9, 24.22, 38° 2θ increased remarkably. These results were in agreement with those published for the trigonal polymorph by several authors, e.g. Edwards et al. [16]. This behaviour was quite consistent in the other CO_2-treated samples in terms of 2θ values (Figs. 4E,F). However, sensible changes in the intensity of the signals could be found between the samples. As suggested by several authors and in particular by Kala et al. [26] and Suryanarayanan [10], the amount of form I inside the sample has been calculated on the basis of the intensity of the two major peaks exhibited at values less than 10° 2θ. The acetone CO_2-treated sample was the richest in terms of trigonal I form, followed by ethylacetate CBZ and dichloromethane CBZ. The XRD patterns of the RV-treated samples indicate the complete reorganisation into the I-form (Figs. 4B–D). All these findings were in complete agreement with the results of previous thermal analyses.

The main results of the DRIFT analysis on the starting CBZ and treated samples are reported in Table 2. Clearly, the starting CBZ presented the typical bands of the form III,

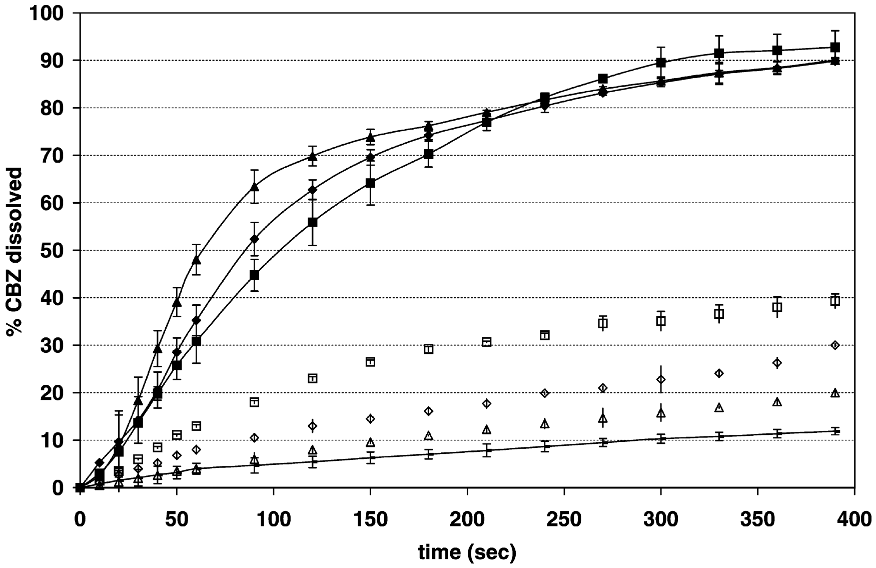

Fig. 5. In vitro dissolution profiles of starting CBZ-USP (−); CBZ-RV recrystallised from ethylacetate (△); CBZ-RV recrystallised from dichloromethane (◇); CBZ-RV recrystallised from acetone (□); CBZ-CO_2 processed with dichloromethane (■); CBZ-CO_2 processed with acetone (◆); and CBZ-CO_2 processed with ethylacetate (▲).

in substantial agreement to those reported by Krahn and Mielk [12], whilst the main bands of CO_2- and RV-treated samples were attributable to the trigonal form [12].

Even though previous physico-chemical characterisations had attested the almost complete transformation from polymorph III (USP 24 RS) to form I after GAS-processing with the tested solvents, the aim was to test the dissolution performance of the CO_2-treated samples compared to the RV-samples. The dissolution profiles are depicted in Fig. 5. Noticeably, a remarkable increase of the CBZ dissolution rate was achieved by its processing with supercritical CO_2 as compared to the RV-samples. In fact the time necessary for the dissolving of 90% of the drug ($t_{90\%}$) was about 6.5 min, whereas the RV-samples showed a $t_{90\%}$ of about 1h. These great differences between the dissolution rates were attributable to changes in crystal size and morphology. It is plausible that the better crystal shape of all the CO_2-treated CBZ (needles) favoured the dissolution process, as previously reported [17,27]. Furthermore, the RV-samples seemed to have larger dimensions compared to those treated with supercritical CO_2 (as apparent from the observation of the RV-crystals with the optical microscopy and SEM). It must be also pointed out that no significant differences ($P = 0.01$) were found between the dissolution performances of the samples obtained with different solvents in the same process. Conversely, a significant difference ($P = 0.01$) was found between the dissolution profiles of the CO_2 samples and the RV-samples.

4. Conclusions

The above reported experimental evidence shows that treating with supercritical CO_2 solution of commercial CBZ in acetone, ethylacetate or dichloromethane, at the performed operating conditions, yields to a sample mostly made up of form I. Since this transition into the trigonal form also occurred by simple recrystallisation in vacuum evaporator, it is reasonable to infer that this transformation is due to the solvents rather than to the treatment with supercritical CO_2. The photomicrographs of the samples treated and untreated with CO_2 illustrated that the GAS method changed the appearance of the crystals between the samples. In fact, while the untreated CBZ was mainly characterised by prisms, crystals with predominantly needle-like structures were present in CO_2-treated CBZ and large thin foils or sponge-like structures were observed after RV-treatment. Further, the CO_2-samples were arranged in particles with reduced dimensions and a satisfactory homogeneous distribution, thus confirming that GAS technique is a viable means to produce micronic particles with restricted particle size distribution.

Acknowledgements

The authors wish to thank the Italian Ministry of University and Scientific Research (MIUR) and Regione Friuli Venezia Giulia for financial support and Dr Formichi and Tecno Galenica s.r.l. for laser diffractometer analyses.

References

[1] P.M. Gallagher, M.P. Coffey, V.J. Krukonis, N. Klasutis, Gas antisolvent recrystallization: a new process to recrystallize compounds in supercritical fluids, Am. Chem. Soc. Symp. Ser. 406 (1989) 334–354.

[2] B. Subramaniam, R.A. Rajewski, K. Snavely, Pharmaceutical processing with supercritical carbon dioxide, J. Pharm. Sci. 86 (1997) 885–890.

[3] E. Reverchon, Supercritical Antisolvent Precipitation: its application to microparticle generation and products fractionation, Proceedings of the 5th Meeting on Supercritical Fluids, Tome 1; M. Perrut, P. Subra (Eds), ISBN 2-905-267-28-3, March 23–25, Institut National Polyclinique de Lorraine, Nice, France, 1998, 221–236.

[4] E. Reverchon, Supercritical anti-solvent precipitation of micro- and nano-particles, J. Supercrit. Fl. 15 (1999) 1–21.

[5] J. Jung, M. Perrut, Particle design using supercritical fluids: literature and patent survey, J. Supercrit. Fl. 20 (2001) 179–219.

[6] R.H. Levy, W.H. Pitlick, A.S. Troupin, J.R. Green, J.M. Neal, Pharmacokinetics of carbamazepine in normal man, Clin. Pharmacol. Ther. 17 (1975) 657–658.

[7] H. Pöhlmann, C. Gulde, R. Jahn, S. Pfeifer, Polymorphie, Teilchengröβe und Blutspiegelwerte von Carbamazepin, Pharmazie 30 (1975) 709–711.

[8] R. Céolin, S. Toscani, M.-F. Gardette, V.N. Agafonov, A.V. Dzyabchenko, B. Bachet, X-ray characterisation of the triclinic polymorph of carbamazepine, J. Pharm. Sci. 86 (1997) 1062–1065.

[9] M.M.J. Lowes, M.R. Caira, A.P. Lötter, J.G. Van Der Watt, Physicochemical properties and X-ray structural studies of the trigonal polymorph of carbamazepine, J. Pharm. Sci. 76 (1987) 744–752.

[10] R. Suryanarayanan, Determination of the relative amounts of anhydrous carbamazepine (C15H12N20) and carbamazepine dihydrate (C15H12N2O.2H2O) in a mixture by powder X-ray diffractometry, Pharm. Res. 6 (1989) 1017–1024.

[11] L. Borka, A. Lönmo, R. Winsnes, Semiquantitative IR spectroscopy on the crystal polymorphs of carbamazepine- a special case in the Ph. Eur. and USP, Pharm. Acta Helv. 67 (1992) 231–233.

[12] F.U. Krahn, J.B. Mielck, Relations between several polymorphic forms and the dihydrate of carbamazepine, Pharm. Acta Helv. 62 (1987) 248–254.

[13] Y. Kobayashi, S. Ito, S. Itai, K. Yamamoto, Physicochemical properties and bioavailability of carbamazepine polymorphs and dihydrate, Int. J. Pharm. 193 (2000) 137–146.

[14] C. Rustichelli, G. Gamberini, V. Ferioli, M.C. Gamberini, R. Ficarra, S. Tommasini, Solid-state of polymorphic drug: carbamazepine, J. Pharm. Biomed. Anal. 23 (2000) 41–54.

[15] R. Bettini, L. Bonassi, V. Castoro, A. Rossi, L. Zema, A. Gazzaniga, F. Giordano, Solubility and conversion of carbamazepine polymorphs in supercritical carbon dioxide, Eur. J. Pharm. Sci. 13 (2001) 281–286.

[16] A.D. Edwards, B.Y. Shekunov, A. Kordikowski, R.T. Forbes, P. York, Crystallization of pure anhydrous polymorphs of carbamazepine by solution enhanced dispersion with supercritical fluids (SEDS™), J. Pharm. Sci. 90 (2001) 1115–1124.

[17] M. Moneghini, I. Kikic, D. Voinovich, B. Perissutti, J. Filipovic-Grcic, Processing of carbamazepine-PEG 4000 solid dispersions with supercritical carbon dioxide: preparation, characterisation, and in vitro dissolution, Int. J. Pharm. 222 (2001) 129–138.

[18] A.D. Sethia, E. Squillante, Physicochemical characterization of solid dispersions of carbamazepine formulated by supercritical carbon dioxide and conventional solvent evaporation method, J. Pharm. Sci. 91 (2002) 1949–1957.

[19] S.J. MacNaughton, I. Kikic, N.R. Foster, P. Alessi, A. Cortesi, I. Colombo, The solubility of antiinflammatory drugs in supercritical carbon dioxide, J. Chem. Eng. Data 41 (1996) 1083–1086.

[20] I. Kikic, M. Moneghini, D. Voinovich, B. Perissutti, Effect of GAS technique on the solid state of carbamazepine, 2nd International Meeting on High Pressure Chemical Engineering. 7–9 March 2001 Hamburg, Germany.

[21] M. Moneghini, D. Voinovich, B. Perissutti, F. Princivalle, Action of carriers on carbamazepine dissolution, Pharm. Dev. Technol. 7 (2002) 289–296.

[22] A. Kordikowski, A.P. Schenk, R.M. Van Nielen, C.J. Peters, Volume expansions and vapor-liquid equilibria of binary mixtures of a variety of polar solvents and certain near-critical solvents, J. Supercrit. Fl. 8 (1995) 205–216.

[23] E. Reverchon, G. Della Porta, R. Taddeo, P. Pallado, A. Stassi, Solubility and micronization of griseofulvin in supercritical CHF_3, Ind. Eng. Chem. Res. 34 (1995) 4087–4093.

[24] M. Sarkary, I. Darrat, B.L. Knutson, Generation of microparticles using CO_2 and CO_2-philic antisolvents, AIChE J. 46 (2000) 1850–1859.

[25] R.J. Roberts, R.S. Payne, R.C. Rowe, Mechanical properties for polymorphs of sulphathiazole and carbamazepine, Eur. J. Pharm. Sci. 9 (2000) 277–283.

[26] H. Kala, U. Haak, F. Fahr, P. Pollandt, Zum kristallografischen verhalten von carbamazepin-zubereitungen unter preβdruck, Pharmazie 41 (1986) 61–62.

[27] P. Giunchedi, U. Conte, A. La Manna, A swellable polymer as carbamazepine dissolution rate enhancer, Boll. Chim. Farm. 129 (1990) 17–20.