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Evaluation of supercritical fluid technology as preparative technique of benzocaine–cyclodextrin complexes—Comparison with conventional methods

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Abstract

The objective of this study was to investigate the effect of the preparation method on the physico-chemical properties of complexes prepared between β-cyclodextrin (β-Cyd) and benzocaine (BZC). In particular, the effectiveness of a new technique based on supercritical carbon dioxide (SC CO_2) for preparing solid drug–cyclodextrin complexes was investigated and compared to other more conventional methods such as kneading (KN), co-evaporation (COE), co-grinding (GR) and sealed-heating (S.H.). Effects of temperature, pressure and exposure time on the properties of complexes prepared by SC CO_2 technology were also studied. The different systems were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), powder X-ray diffractometry (PXRD) and dissolution test according to the dispersed amount method. The co-grinding (GR) method resulted in amorphous products while other methods led to crystalline or partially amorphous products depending on both the method and its experimental conditions. SC CO_2 method revealed to be an effective technique for preparing solid systems between β-cyclodextrin and benzocaine, avoiding the use of organic solvents (and problems of their complete removal) and allowing an easy scale-up of the process. As for the influence of the experimental conditions in promoting the solid-state drug–carrier interaction when using the SC CO_2 method, temperature seemed to play the major role, whereas pressure and exposure times had more limited effects. Dissolution tests confirmed a limited but favourable effect in increasing the exposure time, while indicated a possible interaction effect between temperature and pressure in influencing the dissolution performance of the final product. The best product obtained by the SC CO_2 method showed dissolution properties similar to those of the co-ground product and only slightly lower than the system obtained by sealed-heating, which was the most effective technique.

Keywords: Supercritical fluid carbon dioxide; Benzocaine; β-Cyclodextrin; Characterization; Dissolution

1. Introduction

In recent years, supercritical fluid technology has given new important directions in both research and application fields. This is mainly due to the peculiar properties of supercritical fluids (SCF), which merge the properties of gases and liquids and can be changed from gas-like to liquid-like values by small changes in the pressure and/or temperature. Because of these special characteristics, supercritical fluids have found several useful applications in chemical reactions, environmental remediation, polymer processing, extraction, separation or impregnation procedures, food and pharmaceutical processing [1–5]. As for food and pharmaceutical applications, carbon dioxide is the preferred supercritical fluid (SC CO_2), since it is non-toxic, non-flammable, chemically stable, inexpensive, environmentally acceptable and easily separated from the mixture, and, in addition, it has a relatively low critical temperature and a moderate critical pressure.

Among its possible uses in the pharmaceutical field, the SC CO_2 technology has been recently applied to the preparation of inclusion complexes of some anti-inflammatory and antifungal drugs with different cyclodextrins [6–11]. Cyclodextrins are cyclic oligosaccharides whose cone-shaped cavity allows formation of non-covalent inclusion complexes with appropriately

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sized host molecules, thus modifying their physico-chemical and biological properties [12]. Cyclodextrin complexation has been successfully utilized to improve dissolution and consequently therapeutic effectiveness and bioavailability of several drug molecules [12,13], including local anesthetics [14–16]. Furthermore, cyclodextrins can act as drug delivery systems that would allow a controlled release of the drug leading to a longer duration of action [17,18].

Several techniques have been proposed for complex preparation, such as kneading (KN), high-energy co-grinding, co-evaporation (COE), freeze-drying and spray-drying. The choice of the most suitable method for a given drug–cyclodextrin system should be carefully evaluated, taking into account several factors such as good yield, simplicity, rapidity, ease of scaling up, low cost and the performance of the obtained product [19]. In fact, it has been demonstrated that the preparation method can significantly influence the characteristics of the end products [20–22].

Benzocaine (BZC) is a local anesthetic agent of ester-type, widely used in topical, dermal and mucous formulations; however, its poor aqueous solubility (about 5 mM) limits its parenteral administration [23,24]. Furthermore, its anesthetic action is characterized by a rapid but short effect, compared with the potential duration of pain [25]. The preparation of benzocaine–β-cyclodextrin (β-Cyd) inclusion complexes by freeze drying, in order to improve drug solubility, stability and bioavailability, has been recently examined [16].

Taking into account all these considerations, the aim of the present paper was to investigate the effectiveness of the SC CO_2 technique for preparation of an inclusion compound of benzocaine with β-cyclodextrin in comparison with some common traditional methods (high-energy ball-milling, kneading, sealed-heating (S.H.) and co-evaporation). The influence of variations of the experimental conditions used in the SC CO_2 method (temperature, pressure and time of exposure) on the inclusion complex formation has also been evaluated. The physico-chemical properties of the solid complexes prepared by different methods were determined by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy and powder X-ray diffractometry (PXRD) and compared with those of the corresponding physical mixtures (P.M.). The dissolution properties of the various binary systems were evaluated according to the dispersed amount method.

2. Experimental methods

2.1. Materials

Benzocaine and β-cyclodextrin were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents and solvents were of analytical grade.

2.2. Preparation of physical mixtures

The 1:1 or 1:2 mol/mol drug–Cyd physical mixtures were prepared by homogeneous mixing of previously weighed powders in a mortar with a spatula for 15 min.

2.3. Preparation of drug–cyclodextrin solid complexes

2.3.1. Supercritical carbon dioxide method (SC CO_2)

The supercritical fluid experimental apparatus was composed of a 260-ml capacity syringe pump, a controller system (ISCO 260D), and an ISCO series 2000 SCF Extraction system (SFX 220) consisting of a dual-chamber extraction module with two 10-ml stainless steel vessels as described previously [4,11]. Temperature and pressure within the vessels were measured and could be independently adjusted. Inclusion experiments with SC CO_2 started by filling the 10-ml cell with a physical mixture of drug–Cyd at the desired drug to Cyd molar ratio. The system was then pressurized and heated to the desired pressure and temperature and left for 3 h in a static mode. At the end of the process, the pressure in the cell was dropped to atmospheric pressure within 15 min. The powder obtained was ground and homogenized in a mortar.

2.3.2. Kneading (KN)

Kneaded products were prepared by adding a small volume of ethanol or water–ethanol (50/50, v/v) to a known amount of the physical mixture consisting of BZC and β-Cyd at the desired molar ratio. The resultant mixture was kneaded thoroughly with a pestle to obtain a homogeneous slurry and continued until the solvent was completely removed. The sample was kept in a desiccator overnight to remove traces of solvent.

2.3.3. Co-evaporation (COE)

Co-evaporated products were obtained by dissolving known amounts of β-Cyd in bidistilled water at 25 °C and drug (giving the desired drug: β-Cyd molar ratio) in ethanol at the same temperature. The solutions were added together after the powders were completely dissolved. The solvent was then removed using a rotary evaporator at 75 °C and the sample was kept in a desiccator overnight to remove traces of solvents.

2.3.4. Co-grinding (GR)

Co-ground products were prepared by ball-milling the drug–β-Cyd physical mixture at the desired molar ratio in a high-energy vibrational micro-mill (Retsch, GmbH, Düsseldorf, Germany) at a frequency of 24 Hz for 60 min.

2.3.5. Sealed-heating (S.H.)

Sealed-heated products were prepared by placing a known amount of the drug–β-Cyd physical mixture at the desired molar ratio in a glass container, adding 10 μl bidistilled water and then sealing the glass container using a flame. The sample was kept for 3 h in an oven at 75 °C, after which the sample was removed and kept in a desiccator overnight to remove traces of water.

2.4. Differential scanning calorimetry (DSC)

Thermal analyses of the individual components or BZC–β-Cyd combinations were performed using a differential scanning calorimeter (Mettlet TA4000) equipped with a DSC 25 cell. Weighed samples (5–10 mg, Mettler M3 Microbalance) were

scanned in Al pans pierced with a perforated lid at 10 °C/min from 30 to 130 °C under static air, unless mentioned otherwise.

2.5. Fourier transform infrared spectroscopy (FTIR)

FT-IR spectra (Perkin-Elmer Mod. 1600) of individual BZC, β-Cyd and BZC–β-Cyd binary systems were obtained as Nujol dispersion in the 4000–600 cm^{-1} region.

2.6. Powder X-ray diffractometry (PXRD)

The powder X-ray diffraction patterns of individual BZC, β-Cyd and drug–cyclodextrin combinations were determined using the X-ray diffractometer (Bruker D8-advance®), with Cu Kα radiation, voltage 40 kV, current 40 mA and 2θ over a 2–70° range at a scan rate of 1° min^{-1}. The Sol-X® solid-state Si (Li) detector was used. C/Ni Goebel-Spiegel mirrors in the incident beam were used as monochromator; 1.0 mm divergence, 0.2 scatter and 0.1 for the receiving slits were used.

2.7. Dissolution studies

Dissolution rates of BZC, both alone and from the different drug-carrier binary systems, were determined in water at 37 ± 0.5 °C according to the dispersed amount method, by adding 500 mg of drug or drug-equivalent to 250 ml of water, in a 400 ml beaker. A glass three-blade propeller (19 mm diameter) was immersed in the beaker 25 mm from the bottom and rotated (f = 100 min^{-1}). Suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 μm) at the specified times and the drug concentration was spectrometrically assayed (UV/VIS 1601 Shimadzu). The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated. Each test was repeated three times (coefficient of variation < 5%). Dissolution was characterized through the percent of drug dissolved after 10 min, as index of the rate of dissolution, and the dissolution efficiency (D.E.), as index of the totality of the process. Dissolution efficiency was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time [26].

3. Results and discussion

Different analytical techniques, such as DSC, FTIR and PXRD, were used to characterize and compare the physicochemical properties of the solid complexes prepared between BZC and β-Cyd, in order to investigate and compare the potential and effectiveness of the different preparation methods. For the SC CO_2 method, the influence of varying experimental conditions such as temperature (50, 75 or 100 °C), pressure (10 or 45 MPa), exposure time (3 h or 15 min) and drug–Cyd mol/mol ratio (1:2, 1:1) on the complex formation was also investigated. Similar studies with an antifungal drug (itraconazole) showed that the time of exposure had a positive influence on the inclusion formation, since increasing the exposure time ensured a better inclusion yield [10,11]. Therefore, 3-h exposure time was used in most of the runs in this investigation while a few runs were conducted with 15-min exposure time. Moreover, higher pressures resulted in higher inclusion yields for the itraconazole system [10,11]. Therefore, most of the runs in this investigation were performed at 45 MPa while a few runs were carried out at 10 MPa. A molar ratio of 1:2 (BZC–β-Cyd) was used throughout this investigation. However, in order to evaluate the effect of the drug:Cyd molar ratio, a few runs were conducted with a 1:1 molar ratio.

3.1. DSC analysis

DSC curves for pure components and their 1:1 and 1:2 drug:Cyd mol/mol products obtained by SC CO_2 method at different temperature (50, 75 and 100 °C), exposure time (15 min or 3 h) and pressure (10 and 45 MPa) conditions are shown in Fig. 1. DSC curves of the physical mixtures as such or after exposure to the same temperatures and for the same exposure times as the products obtained by SC CO_2 were also recorded, in order to separately investigate the simple effect of the sample thermal treatment on its thermal behaviour. In fact, previous studies showed that a 3-h exposure of β-Cyd alone at high temperature (130 °C) or to SC CO_2 at 130 °C and 45 MPa resulted in some different thermal changes [11].

The thermal curve of pure BZC showed a typical sharp melting endotherm at about 94 °C indicative of its anhydrous and crystalline state, while pure β-Cyd exhibited a broad endothermal effect corresponding to its dehydration. The DSC curve for the untreated physical mixture consisted of the superimposition of the thermal profiles of drug and Cyd with no significant changes in the drug melting peak parameters (except for a 4 °C shift to lower temperature), suggesting no drug–Cyd interactions. The thermal profile of the physical mixtures exposed at 50 and 75 °C for 3 h did not show significant differences from that of the untreated physical mixture, while the drug peak in the physical mixture exposed to 100 °C was reduced in size and slightly shifted to a lower temperature (88 °C).

As it can be seen in Fig. 1, temperature had a significant effect on the thermal behaviour of the products obtained by the SC CO_2 method. DSC curve of the BZC–β-Cyd sample treated with SC CO_2 at 50 °C, 45 MPa and 3-h exposure time was similar to that of the physical mixture exposed to 100 °C, presenting a reduced-size drug melting peak at 88 °C followed by the endothermal effect corresponding to the β-Cyd dehydration. The decrease in intensity of the BZC endothermic peak observed in such samples suggests possible drug–Cyd interactions and/or loss of drug crystallinity. When the temperature in the SC CO_2 method was increased to 75 °C, the drug peak disappeared and instead a weak and broad endothermal effect extending from 65 to 90 °C was observed. A similar DSC curve was also obtained for the product treated with SC CO_2 at the higher temperature of 100 °C. On the contrary, such results were not observed in the corresponding physical mixtures exposed at the same temperatures for the same times. Therefore, these findings proved that the changes in the thermal behaviour observed for the samples treated with SC CO_2 at 75 and 100 °C cannot be due to the simple thermal treat-

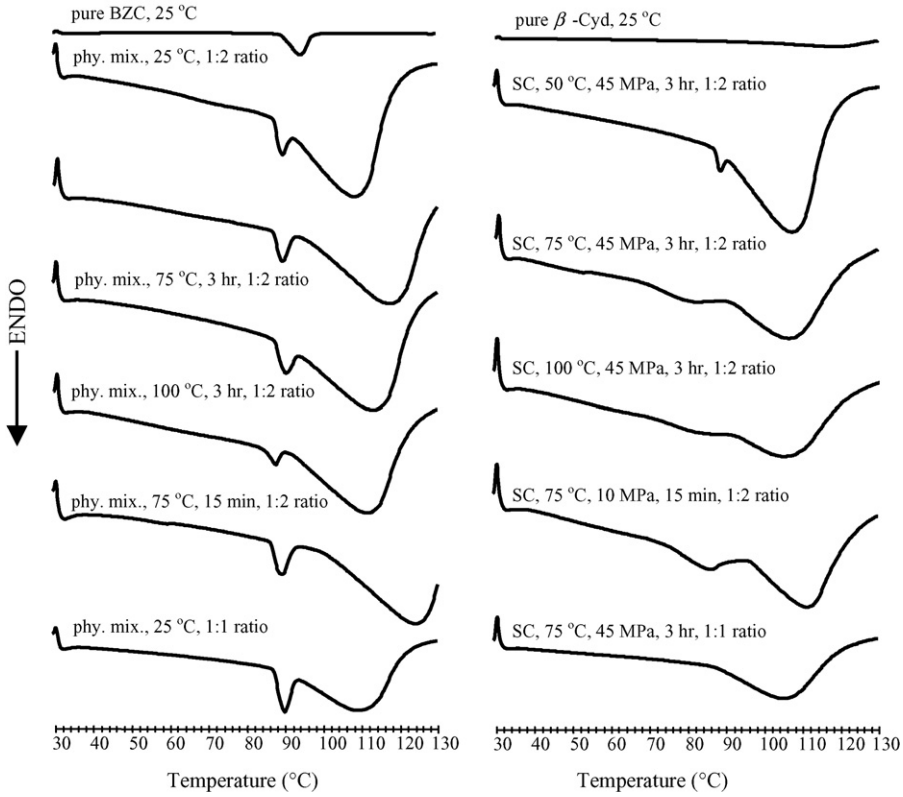

Fig. 1. DSC curves of pure benzocaine (BZC), pure β-Cyd and BZC/β-Cyd (1:1 and 1:2 mol–mol) binary systems prepared by the SC CO_2 method at different temperatures, pressures and exposure times and corresponding physical mixtures.

ment sustained, but, on the contrary, they have to be attributed to the formation of an inclusion complex and/or to the sample amorphization resulting from a combined effect of both temperature and SC CO_2. Moreover, these results show that increasing the SC CO_2 temperature enhances the effectiveness in inclusion complex formation and/or sample amorphization.

Since almost complete disappearance of the BZC peak was observed for the product treated with SC CO_2 at 75 °C, 45 MPa and 3 h exposure time, another experiment was conducted to see if similar results can be obtained at lower pressure (10 MPa) and shorter exposure time (15 min). As displayed in Fig. 1, the thermal profile of the physical mixture exposed to 75 °C for 15 min was, as expected, the sum of the thermal profiles of drug and Cyd, indicating no interactions between the components. On the contrary, DSC curve for the product treated with SC CO_2 at 75 °C and 10 MPa for 15 min was substantially similar to that obtained for the sample exposed to SC CO_2 at the same temperature but higher pressure (45 MPa) and longer exposure time (3 h), except that the broad endothermic effect replacing the drug peak was slightly more marked and well defined, indicative of some residual crystallinity of uncomplexed drug. This result suggests that temperature is a critical factor to promote interactions between BZC and β-Cyd using SC CO_2 method, whereas there is no need to use high pressure or long exposure times. Finally, Fig. 1 also compares DSC curves for 1:1 mol/mol BZC–β-Cyd products prepared by physical mixing at room temperature (25 °C) and SC CO_2 method upon 3 h exposure at 75 °C and 45 MPa. As can be seen, the results obtained for the systems at 1:1 molar ratio are similar to those observed for the corresponding samples at the 1:2 ratio, showing the presence of the unchanged BZC melting peak in the physical mixture and the total disappearance of the same peak in the SC CO_2 product. This finding seems to suggest a complete drug–β-Cyd interaction also for the sample in the 1:1 mol/mol ratio prepared by the SC CO_2 method upon the above conditions (3 h at 75 °C and 45 MPa).

Fig. 2 shows the DSC curves for BZC–β-Cyd 1:2 mol/mol products obtained by conventional methods such as kneading (with ethanol or water–ethanol 50/50, v/v), co-evaporation, co-grinding and sealed-heating. The DSC curve for the product obtained by kneading with ethanol was similar to that of the physical mixture exposed at 100 °C for 3 h, showing a reduction in the intensity of the drug fusion peak, with a concomitant shift to lower temperature (88 °C), indicative of some drug–Cyd interaction. A different thermal behaviour was instead observed for the sample prepared by kneading with water–ethanol (50/50, v/v), which lacked the drug melting peak, and only showed a very broad endothermal dehydration band, thus suggesting complete drug amorphization, and/or inclusion complexation. The DSC curve for the product obtained by sealed-heating was very similar to that of the product treated with SC CO_2 at 75 °C, 45 MPa and 3 h exposure time. This may be attributed to the similar conditions used for preparing the two samples. In fact, in the sealed-heating method, the sample, added of 10 μl bidistilled water, was kept at 75 °C for 3 h in a sealed glass container. An analogous thermal profile was detected also for the product obtained by the co-evaporation method, where only a further

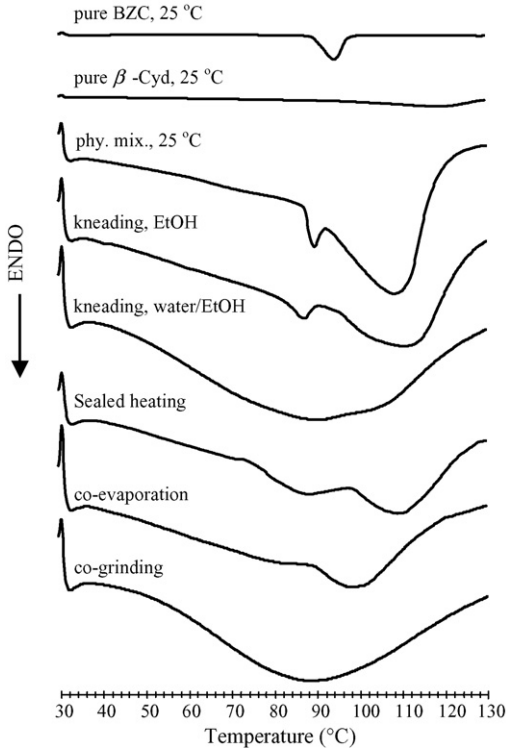

Fig. 2. DSC curves of pure benzocaine (BZC), pure β-Cyd and BZC/β-CD(1:2 mol–mol) binary systems prepared by kneading, co-evaporation, co-grinding and sealed-heating.

weakening of the broad endotherm replacing the drug peak and a concomitant shift to lower temperature of the β-Cyd dehydration band was observed. Finally, the product obtained by co-grinding method displayed only a very broad endothermal effect, similar to that observed for the sample prepared by kneading with water–ethanol (50/50, v/v).

3.2. Fourier transform infrared spectroscopy

FTIR spectra of pure components, and BZC–β-Cyd (1:2 mol/mol) products obtained by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating and SC CO_2 methods are presented in Fig. 3. The FTIR spectrum of BZC–β-Cyd physical mixture freshly prepared at 25 °C can be considered as the result of the sum of BZC and β-Cyd spectra, thus confirming the absence of solid-state interactions between the components, as indicated by DSC results. No modifications were observed after 3 h exposure of the physical mixture at 75 °C, thus confirming that the simple thermal treatment, at least in such a temperature range, did not promote any drug–Cyd interaction. On the contrary, the FTIR spectra of BZC–β-Cyd products obtained by SC CO_2, sealed-heating, kneading, co-evaporation and co-grinding methods showed some differences with respect to those of the physical mixtures, revealing a modification of the environment of BZC and thus indicating some drug–Cyd interactions, in accordance with the results obtained by DSC analysis. The changes observed in the FTIR spectra of the various samples, such as shift of peaks, or their reduction in intensity up to almost complete disappearance, depended on their preparation method, suggesting different

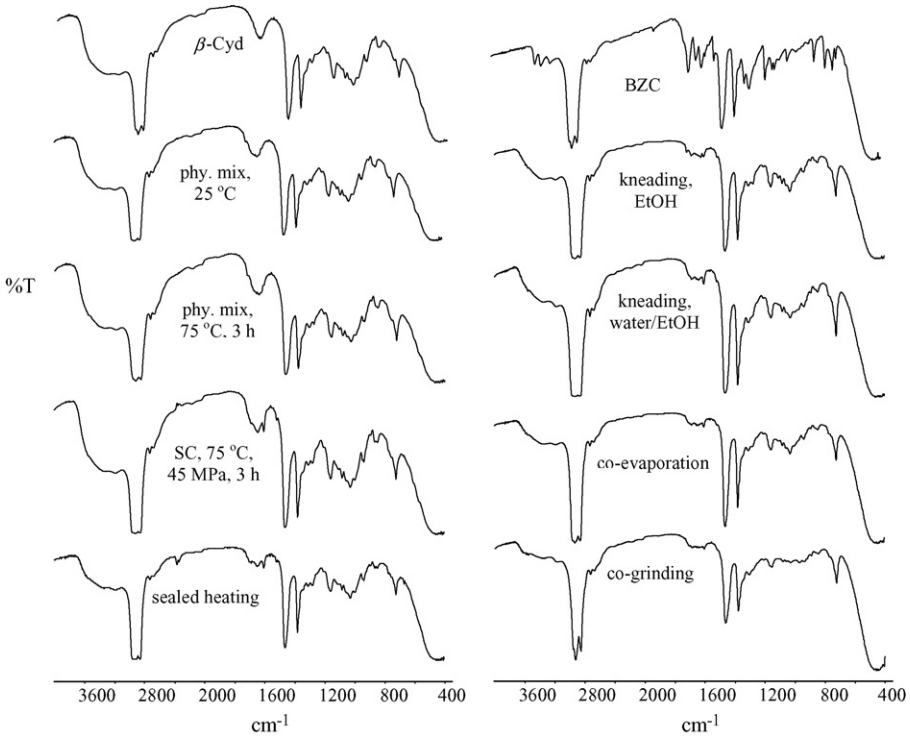

Fig. 3. FTIR spectra of pure benzocaine (BZC), pure β-Cyd and BZC/β-Cyd (1:2 mol–mol) binary systems prepared by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating and SC CO_2 at different temperatures, pressures and exposure times.

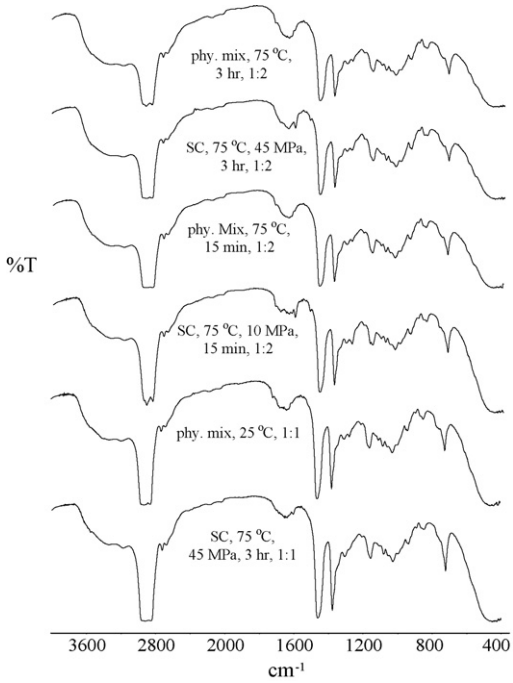

Fig. 4. FTIR spectra of benzocaine/β-Cyd (1:1 and 1:2 mol–mol) binary systems prepared by physical mixing and SC CO_2 at different temperatures, pressures and exposure times.

degrees of interaction and/or amorphization in the different products.

The effect of varying pressure (45 or 10 MPa) and exposure time (15 min or 3 h) on the products obtained by SC CO_2 method is illustrated in Fig. 4. As it can be seen, only slight differences can be detected between the FTIR spectrum of the product treated with SC CO_2 at 75 °C and 10 MPa for 15 min and that of the sample exposed at the same temperature but at higher pressure (45 MPa) and longer exposure time (3 h). In particular, the band at 1686 cm^{-1}, characteristic of pure drug, was only maintained in the product treated with SC CO_2 at 10 MPa for15 min, and that at 1636 cm^{-1} appeared at 1637 cm^{-1} for the sample treated at 10 MPa for 15 min, while it shifted to 1642 cm^{-1} for the product obtained at 45 MPa and 3 h exposure time. Therefore, in agreement with the findings of DSC analysis, these results suggest that there is no need to use high pressure or long exposure times to facilitate interactions between BZC and β-Cyd using SC CO_2 method. As for the effect of the drug–Cyd molar ratio, the FTIR spectra of BZC–β-Cyd products at 1:1 mol/mol ratio prepared by physical mixing at 25 °C and by the SC CO_2 method at 75 °C, 45 MPa and 3 h exposure time were substantially similar to those of the corresponding products at 1:2 molar ratio (shown in Fig. 3), confirming DSC results.

3.3. Powder X-ray diffractometry (PXRD)

Powder X-ray diffractometry was used to investigate in more depth the differences in the solid-state between BZC–β-Cyd products prepared by the different methods. Fig. 5 shows the X-ray powder diffraction patterns of pure BZC, pure β-Cyd and their 1:2 mol–mol systems obtained by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating and SC CO_2 method. The diffraction pattern of both BZC and β-Cyd displayed several sharp peaks, indicative of their crystalline nature. A crystalline pattern, given by the sum of the spectra of pure components, was obtained for the untreated physical mixture. Some crystallinity loss was observed in physical mixtures heated for 3 h at 50 and 75 °C, and it became clearly more intense for the physical mixtures exposed at 100 °C for 3 h. The characteristic peaks of BZC and β-Cyd, even though markedly reduced in intensity, were still detectable in the product obtained by kneading with ethanol, indicating that the drug maintained a residual crystallinity in this product and only partially interacted with β-Cyd. On the contrary, a diffuse pattern, with a very few low-intensity peaks, was obtained for the sample prepared by kneading with water–ethanol (50/50, v/v), suggesting an

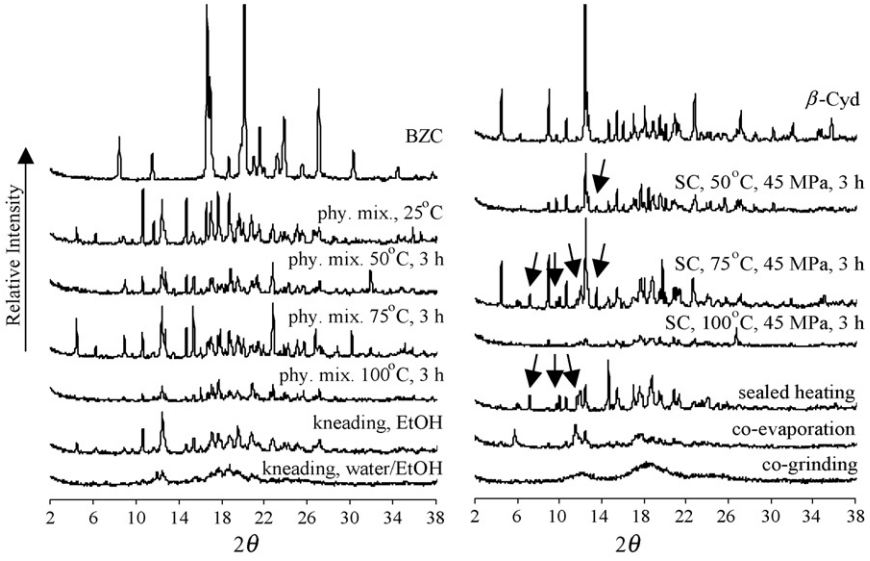

Fig. 5. PXRD patterns of pure benzocaine (BZC), pure β-CD and BZC/β-Cyd (1:2 mol–mol) binary systems prepared by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating and SC CO_2 at different temperatures, pressures and exposure times.

almost complete drug amorphization and/or complexation. Interestingly, the presence of new diffraction peaks was detected in samples prepared by SC CO_2 and sealed-heating methods, as indicated by arrows on Fig. 5. In particular, the sample treated with SC CO_2 at 45 MPa and 50 °C for 3 h showed a new peak at a 2θ value of 13.5, while the product exposed to 75 °C and the same pressure and exposure time resulted in four new peaks (at 2θ values of 7.2, 10, 12 and 13.5). These new peaks were attributed to the formation of a new solid phase induced by complexation of BZC with β-Cyd. The sample treated with SC CO_2 at 100 °C and 45 MPa for 3 h showed a diffuse pattern with a very few low-intensity peaks, suggesting drug amorphization and/or complexation. The sealed-heated product resulted in a crystalline pattern with three new peaks observed at the same values of 2θ as in the sample treated with SC CO_2 at 75 °C, thus confirming the analogies between these products observed in DSC and FTIR studies. The PXRD spectrum of the co-evaporated product exhibited some residual crystallinity peaks emerging from a substantially amorphous pattern, indicative of nearly total amorphization and/or complexation of the sample. A completely diffuse pattern was instead obtained for the co-ground product, suggestive of total drug amorphization as a consequence of the homogeneous dispersion of BZC in the carrier and/or inclusion in the carrier cavity induced by co-grinding.

The PXRD pattern of the product obtained by SC CO_2 method at shorter exposure time (15 min) and lower pressure (10 MPa) at 75 °C (Fig. 6) was similar to that obtained for the sample exposed to SC CO_2 at the same temperature but higher pressure (45 MPa) and longer exposure time (3 h) (shown in Fig. 5), exhibiting the presence of three additional new peaks at the same 2θ values of 7.2, 10 and 12. The only difference was the absence of the fourth new peak observed at the 2θ value of 13.5 in the product treated with SC CO_2 at 75 °C and 45 MPa for 3 h. These results are in agreement with the results obtained by DSC and FTIR analysis, suggesting that lower pressure and shorter exposure time can be adequate to induce interactions between BZC and β-Cyd using SC CO_2 method. Fig. 6 also compares PXRD patterns of BZC–β-Cyd products at 1:1 mol/mol ratio prepared by physical mixing at 25 °C and by SC CO_2 method at 75 °C, 45 MPa and 3 h exposure time. Interestingly, the sample obtained by the SC CO_2 method only showed some crystallinity decrease with respect to the corresponding equimolar physical mixture, whereas no new peaks were detected, contrary to the corresponding SC CO_2 product at 1:2 drug–Cyd molar ratio. These results seem to indicate a better drug–Cyd solid-state interaction in the sample with higher Cyd amount.

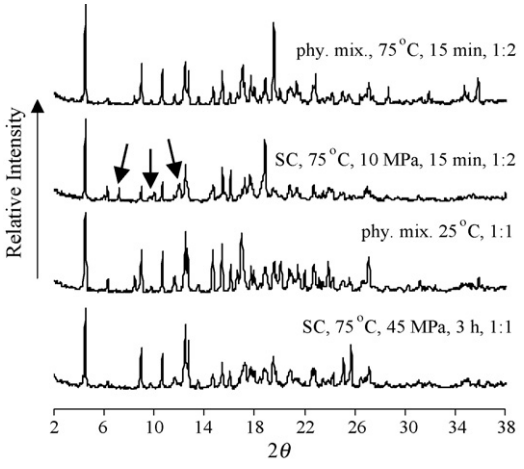

Fig. 6. PXRD patterns of benzocaine/β-Cyd (1:1 and 1:2 mol–mol) binary systems prepared by physical mixing and SC CO_2 at different temperatures, pressures and exposure times.

3.4. Dissolution studies

The mean dissolution curves of BZC from the binary systems with β-Cyd obtained by different preparation methods examined are presented in Fig. 7A. The relative dissolution rates of the different systems, calculated by dividing the amount of drug dissolved at 2 min by that obtained with the pure drug after the same time, give a comprehensive picture of the performance of each technique tested (Fig. 7B). The results in terms of dissolution efficiency [26] and percent of active ingredient dissolved are collected in Table 1.

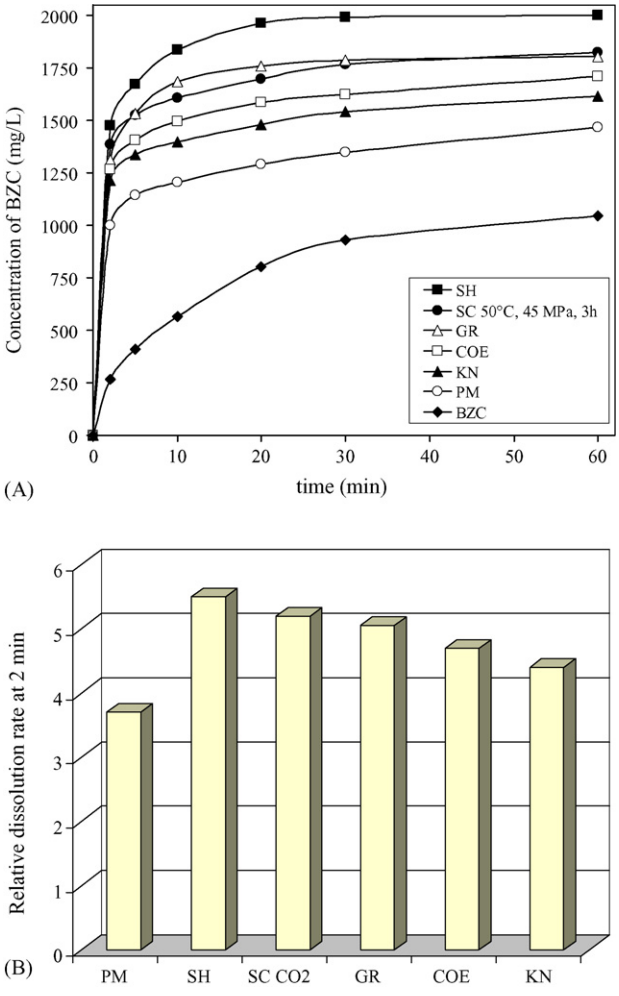

Fig. 7. (A) Mean dissolution curves of benzocaine (BZC) from the 1:2 mol–mol binary systems with β-Cyd obtained with the different preparation methods; (B) relative dissolution rates of the different systems at 2 min with respect to the pure drug.

Table 1
Percent dissolved at 30 min (P.D.30) and dissolution efficiency at 60 min (D.E.60) for pure benzocaine (BZC) and the 1:2 mol–mol binary systems with β-Cyd obtained by different methods

Sample	P.D.30	D.E.60
BZC	16.0	13.6
P.M.	45.0	40.8
KN	51.4	47.1
COE	54.2	50.0
GR	59.5	54.5
SC CO_2 (50 °C, 45 MPa, 3 h)	59.0	54.0
S.H.	65.5	61.1

Table 2
Percent dissolved at 30 min (P.D.30) and dissolution efficiency at 60 min (D.E.60) for benzocaine (BZC) from the 1:2 mol–mol binary systems with β-Cyd obtained by the SC CO_2 method under different experimental conditions

Experimental conditions				
Temperature (°C)	Pressure (MPa)	Exposure time (min)	P.D.30	D.E.60
50	45	180	59.0	54.0
75	45	15	39.1	38.0
75	10	180	49.3	45.9
100	10	180	53.0	51.6
100	45	15	40.5	39.6
100	45	180	47.3	45.6

The technique used for obtaining BZC–β-Cyd solid systems clearly affected the dissolution performance of the final product, and the rank order of the dissolution rates was S.H. > SC CO_2 ≈ GR > COE > KN > P.M. The increased dissolution rate of the simple physical mixture in comparison with drug alone can be attributed to the improved powder wettability, as well as to the formation of readily soluble complexes in the dissolution medium [27]. The best product obtained by the SC CO_2 method showed dissolution properties similar to those of the co-ground product and only slightly lower than the system obtained by sealed-heating, which was the most effective technique. The effects of varying temperature, pressure and exposure time conditions when using the SC CO_2 technique on the dissolution properties of the final product are summarized in Table 2. Dissolution tests showed a limited but favourable effect in increasing the exposure time, thus substantially confirming the results obtained in solid-state studies. However, contrary to the results observed in solid-state studies, a possible interaction effect between temperature and pressure in influencing the dissolution performance of the final product has been found. In fact, interestingly, the best results were obtained for products treated for longer exposure time (3 h), by using high temperature (100 °C) and low pressure (10 MPa) values or low temperature (50 °C) and high pressure (45 MPa) values.

4. Conclusions

BZC–β-Cyd systems were prepared by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating and SC CO_2 method and characterized by DSC, FTIR and PXRD analysis and dissolution studies. It was shown that the properties of the solid systems are influenced by the preparation method. BZC–β-Cyd products prepared by sealed-heating and SC CO_2 (at 75 °C, 45 MPa and 3 h exposure time) resulted in similar DSC curves, FTIR spectra and PXRD patterns, indicating that the two products have similar solid-state properties. An amorphous PXRD pattern was observed for the BZC–β-Cyd product obtained by co-grinding, suggesting that this method leads to complete drug amorphization and/or complexation. The other examined methods led to crystalline or partial amorphous products depending on the method, indicating the possibility of drug–Cyd interactions of different strengths, which may lead to different degrees of inclusion formation and/or amorphization of the sample.

The new supercritical fluid technology proved to be a useful and effective method for preparing solid BZC–β-Cyd complexes with few processing steps. In comparison with the other examined methods, the SC CO_2 has some important technical advantages. In particular, with respect to co-evaporation and kneading methods, the SC CO_2 method avoids the use of organic solvents, and therefore the need for removing toxic solvent residual, whereas, in comparison with the sealed-heating method, the SC CO_2 one presents an easier scale-up. Effectiveness of the SC CO_2 method in terms of dissolution performance (referring to the product obtained under the best experimental conditions) is comparable to that of the high-energy co-grinding method, and only slightly less than the sealed-heating-method, that gave the best results. Efficacy, safety and pharmacokinetics of the new inclusion complexes obtained by different methods will be further investigated by both in vitro and in vivo experiments. The SC CO_2 method will also be tested for other Cyds as well as for other drugs, in order to better investigate the potential of this technology and optimize the experimental conditions.

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