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ORIGINAL ARTICLE

Physicochemical characterization of drug-cyclodextrin complexes prepared by supercritical carbon dioxide and by conventional techniques

Ali Al-Marzouqi · Baboucarr Jobe · Giovanna Corti · Marzia Cirri · Paola Mura

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Abstract The objective of this study was to investigate the effectiveness of supercritical carbon dioxide (SC CO_2) technique for preparing solid complexes between β-cyclodextrin and three local anesthetic agents (benzocaine, bupivacaine, and mepivacaine) by comparing it to more traditional methods such as kneading, co-evaporation, co-grinding, and sealed-heating. Effects of variation of experimental conditions, i.e. temperature, pressure and exposure time, on the products prepared by SC CO_2 method were also examined. The products obtained were characterized by powder X-ray diffractometry and Fourier transform infrared spectroscopy, and tested for dissolution properties. Results suggested the possibility of complex formation between β-cyclodextrin and the three anesthetic agents, and indicated that it was influenced by the preparation technique. The co-grinding method was the only one resulting in completely amorphous products for all three drugs. Almost amorphous products, with only limited residual crystallinity, were obtained by co-evaporation and kneading techniques, while SC CO_2 and sealed-heating methods gave rise to more crystalline systems. As for the SC CO_2 method, temperature (for benzocaine and bupivacaine) or exposure time (for mepivacaine) had significant effects on the solid-state properties of the final products. Dissolution studies indicated that all the examined methods were more effective than the simple physical mixing in improving drug dissolution properties, but the different rank orders observed for the different drugs suggested that there is no general rule for the selection of the most effective preparation method, which depends on the type of drug-Cyd system considered. Nevertheless, in all cases, products obtained by the SC CO_2 method showed satisfactory dissolution properties.

Keywords Supercritical carbon dioxide mepivacaine · Cyclodextrin · Inclusion complex · Benzocaine · Bupivacaine · Mepivacaine

A. Al-Marzouqi (✉) · B. Jobe
Department of Chemical & Petroleum Engineering, U.A.E. University, Al-AinP.O. Box 17555, UAE
e-mail: hassana@uaeu.ac.ae

G. Corti · M. Cirri · P. Mura
Dipartimento di Scienze Farmaceutiche, Universita di Firenze, Polo Scientifico di Sesto Fiorentino, 50019 Sesto Fiorentino, Firenze, Italy

Introduction

Local anesthetics are a class of drugs able to induce pain relief by causing physicochemical disturbance of the neuron myelin sheath and thus inhibiting the opening and closing of sodium ion channels in neural membranes [1]. Despite their short half-lives, anesthetic drugs are widely used for regional anesthesia during surgery as well as for regional control of acute and chronic pain [2]. However, anesthetic drugs with prolonged actions are preferred in order to reduce the toxicity and dosage frequency of such drugs.

Among the various approaches that have been used to improve the performance and prolong the action of anesthetic drugs, complexation with cyclodextrins is one of the most interesting and promising ones. Cyclodextrins are cyclic oligomers of glucose which, because of the particular arrangement of their glucosidic units, have cone-like structures, whose exterior

surface has hydrophilic properties, whereas the interior is hydrophobic in nature. The particular feature of cyclodextrin molecules allows the formation of non-covalent inclusion complexes with various drugs of appropriate size and polarity leading to changes in their physicochemical and biopharmaceutical properties, improving their solubility, dissolution rate, chemical stability and bioavailability and reducing side effects and toxicity [3–10]. Moreover, cyclodextrins can act like a sort of reservoir, allowing a controlled release of the drug included in their cavity, thus prolonging its duration of action [11–12]. Finally, inclusion complexation may suppress unpleasant odors or tastes associated with the drug, reduce its volatility and avoid incompatibility problems [13–14].

Different techniques (i.e. kneading, co-evaporation, co-grinding, sealed-heating, spray-drying, freeze-drying) have been used for the preparation of solid inclusion complexes between cyclodextrins and various drugs [15–18]. The most proper method for a given drug-cyclodextrin system must be carefully selected, considering a series of factors including yield, simplicity, rapidity, ease of scaling up, low cost, and the performance of the obtained product [19]. In fact, it has been proven that the preparation technique can clearly affect the properties of the final solid systems [20–23]. The use of supercritical fluid carbon dioxide (SC CO_2) [24] has been recently proposed for the preparation of inclusion complexes between some anti-inflammatory or antifungal drugs and different cyclodextrins [25–30]. The main advantages of such an approach are the good solvent properties of supercritical CO_2, which avoids the use of water or organic solvents (and thus the need to resolve toxic solvent residual), and the lack of toxicity of CO_2, which returns to the gaseous state after decompression.

Benzocaine, bupivacaine, and mepivacaine are local anesthetic agents whose poor water solubility restrict their use in parenteral administration, and limit their application to topical formulations for treating pain [31–32]. The properties of these drugs could be enhanced by complexation with cyclodextrins, which would both improve drug solubility and act as a reservoir, thus prolonging their duration of action. At present, only the freeze-drying method has been used to examine the ability of some anesthetic drugs to form complexes with β-cyclodextrin and its derivatives [33–34]. Therefore, the aim of the current study was to investigate the actual effectiveness of SC CO_2 method for obtaining solid inclusion complexes between β-cyclodextrin, and the above anesthetic agents, by comparing it to traditional techniques, such as kneading, co-evaporation, high-energy co-grinding and sealed-heating. These traditional techniques were mainly selected on the basis of their low cost, practicality, and simplicity. The products were characterized by X-ray diffractometry and Fourier transform infrared spectroscopy, and tested for dissolution properties. For the complexes prepared by the SC CO_2 method, the effects of variations of experimental conditions (temperature, pressure and exposure time) were also investigated.

Materials and methods

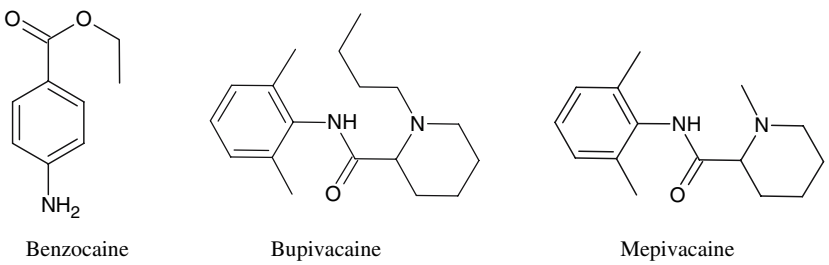
Materials

Benzocaine (BZC), bupivacaine hydrochloride (BPVH), mepivacaine hydrochloride (MPVH) and β-cyclodextrin (β-Cyd) (Fig. 1) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and solvents were of analytical grade.

Preparation of drug:Cyd solid systems

Physical mixtures at 1:2 drug:Cyd mol/mol ratio were prepared by gently blending the previously weighed and sieved drug and β-cyclodextrin powders, in a mortar with a spatula.

Kneaded products were obtained by adding a small volume of a water-ethanol (50/50 v/v) solution to the drug-β-Cyd physical mixture and kneading thoroughly with a pestle to obtain a homogeneous slurry, continuing until the solvent was completely removed. The sample was kept in a desiccator overnight to remove traces of solvent.



Benzocaine Bupivacaine Mepivacaine

Fig. 1 Chemical structure of local anesthetics benzocaine, bupivacaine and mepivacaine

Co-evaporated products were prepared by dissolving known amounts of β-Cyd and drug (to obtain the desired molar ratio) in bi-distilled water and ethanol, respectively, and then mixing the two solutions. The solvents were then removed using a rotary evaporator at 75 °C and 210 rpm. The sample was kept in a desiccator overnight to remove traces of solvents.

Co-ground products were obtained by co-grinding the drug-β-Cyd physical mixtures in a high-energy vibrational mill (Retsch, GmbH, Düsseldorf, Germany) at 24 Hz for 60 min.

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

Preparation of drug:Cyd solid systems by supercritical carbon dioxide method

The supercritical fluid experimental apparatus consisted of a 260 ml syringe pump and 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 [29, 35]. Temperature and pressure within the vessels were measured and could be independently adjusted. Preparation of inclusion complexes by the SC CO_2 technology started by filling the 10 ml cell with the drug-Cyd physical mixture. The system was then pressurized and heated up to the desired pressure and temperature and left in a static mode for 3 h. At the end of the process, the pressure in the cell was dropped to atmospheric pressure within 15 min. The contents of the cell were ground and homogenized in a mortar.

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectra of individual drugs, β-Cyd, and selected drug-Cyd binary systems were obtained as Nujol dispersion using a Perkin-Elmer Mod. 1600 FTIR spectrophotometer in the 4,000–600 cm^{-1} wave number range.

Powder X-ray diffractometry (PXRD)

The PXRD patterns of individual drugs, β-Cyd, and the different drug-Cyd combinations were recorded 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. 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.

Dissolution studies

Dissolution studies were performed in water at 37 ± 0.5 °C according to the dispersed amount method, by adding a suitable amount of drug or drug-equivalent to 250 ml of water, in a 400 ml beaker. A 19 mm diameter glass three-blade propeller was immersed at 25 mm from the bottom and rotated at 100 rpm. Suitable aliquots were withdrawn with a 0.45 μm filter-syringe at specified times and spectrometrically assayed for drug concentration (UV/VIS 1601 Shimadzu). The same volume of fresh medium was added and the correction for the cumulative dilution was calculated. Each test was repeated three times (C.V. <5%). Dissolution Efficiency (D.E.) was calculated from the area under the dissolution curve at time t [36].

Results

Characterization of drug-Cyd solid binary systems

FTIR and PXRD analyses were used to investigate the effectiveness of the examined methods for the formation of solid complexes between the selected anesthetic drugs and β-Cyd. For the SC CO_2 method, the influence of different exposure time, pressure and temperature, on the complex formation were also investigated. Previous studies about preparation of itraconazole complex with β-Cyd by the SC CO_2 method, showed that an increase of the exposure time had a positive influence on the inclusion formation, ensuring a better inclusion yield [29–30]. Therefore, a 3 h exposure time was used in most of the runs in this investigation. However, to evaluate the effect of this parameter, some runs were conducted with 15 min exposure time. Moreover, the same study indicated that higher pressures allowed higher inclusion yields for the itraconazole system. Therefore, most of the runs in this investigation were performed at 45 MPa, while a few runs were carried out at 10 MPa. Finally, also an increase in temperature should give rise to an improved interaction between the components, and thus, to investigate the role of this factor, experiments were performed at 50, 75 and 100 °C.

Infrared spectroscopy

Infrared spectra of pure drug, pure β-Cyd, and drug-β-Cyd (1:2 mol–mol) products obtained by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating, and SC CO_2 method are presented in Fig. 2 A, B, and C for BZC, BPVH and MPVH, respectively. The FTIR spectra of all physical mixtures can be considered as the result of the sum of those of the pure components, indicating the absence of interactions between each examined drug and β-Cyd. In all cases, spectra of physical mixtures maintained for 3 h in an oven at 100 °C were essentially identical to those of the corresponding original products, indicating that only an increase in temperature is not enough to promote drug-Cyd interaction. On the contrary, the FTIR spectra of products obtained by SC CO_2, sealed-heating, kneading, co-evaporation, and co-grinding were different from that of the corresponding physical mixture, indicating some drug-Cyd interactions. Comparing the FTIR spectra of the binary products with those of pure drug and Cyd, different degrees of modification, such as shift of peaks, or their attenuation up to almost complete disappearance, were observed depending on the preparation method, indicative of different degrees of inclusion formation and/or sample amorphization.

With regard to the influence of the experimental conditions used in the SC CO_2 method on the characteristics of the products obtained, in the case of BZC (Fig. 2A), a decrease of pressure (from 45 to 10 MPa) and a shortening of exposure time (from 3 h to 15 min) only gave rise to slight spectral differences. In particular, the band of the carbonyl stretching vibration at 1,686 cm^{-1}, typical of pure drug, was only maintained in the product treated at 10 MPa for 15 min, and that of aromatic NH_2 in plane bending vibration at 1,636 cm^{-1} appeared at 1,637 cm^{-1} for the sample treated at 10 MPa for 15 min, while it shifted at 1,642 cm^{-1} for the product obtained at 45 MPa and 3 h exposure time. These results seem to indicate a minor influence of the pressure and exposure times used in the SC CO_2 method in promoting interactions between BZC and β-Cyd.

These findings were essentially confirmed in the case of products with BPVH (Fig. 2B). In fact, some appreciable spectral differences were observed by increasing the temperature from 50 to 75 up to 100 °C, by keeping the pressure (45 MPa) and the exposure time (3 h) constant in the samples obtained by the SC CO_2 method. On the contrary, no important modifications of the FTIR spectra were observed by keeping the temperature constant at 100 °C and reducing the pressure to 10 MPa and the exposure time to 15 min. Products obtained by sealed-heating, kneading, and co-grinding methods resulted in similar FTIR spectra to those of the products obtained by SC CO_2 at 75 °C while the FTIR spectrum of the co-evaporated product was somewhat similar to those of the products prepared with SC CO_2 at 100 °C.

Some different results were instead obtained for the binary systems with MPVH (Fig. 2C). In fact in this case no appreciable differences were observed in FTIR spectra of the products obtained by the SC CO_2 method when increasing the temperature from 75 to 100 °C and the pressure from 10 to 45 MPa, and keeping the exposure time constant at 15 min. On the contrary, products exposed to SC CO_2 for 3 h, at any of the tested temperatures and pressures, resulted in some spectral differences. Therefore, in this case exposure time seems to affect the characteristics of the products treated with SC CO_2 more than temperature variations.

The spectra of sealed-heated, kneaded and co-ground products were similar to those of the samples exposed to SC CO_2 for 3 h (at any of the tested temperatures and pressures), while some differences were observed in the pattern of the co-evaporated system, where the characteristic carbonyl stretching band of the drug at 1,675 cm^{-1} was shifted to 1,666 cm^{-1}.

Powder x-ray diffraction (PXRD) analysis

Figure 3 A, B, and C show, respectively, the PXRD of pure BZC, BPVH and MPVH, together with those of pure β-Cyd, and their corresponding 1:2 mol–mol systems obtained by the different preparation methods.

The diffraction patterns of all pure drugs and β-Cyd displayed several sharp peaks, indicative of their crystalline nature. Some slight crystallinity loss can be noted in all untreated physical mixtures (25 °C) and in the corresponding mixtures heated for 3 h at 100 °C, but they were substantially the sum of the spectra of the respective pure components. Interestingly, in the case of BZC, some new peaks were observed in the samples prepared by SC CO_2 as indicated by arrows in Fig. 3A. In detail, the spectral pattern of the sample treated with SC CO_2 at 45 MPa and 50 °C for 3 h exhibited 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 are attributed to the formation of new bonds induced by the 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 very few low-intensity peaks and one new

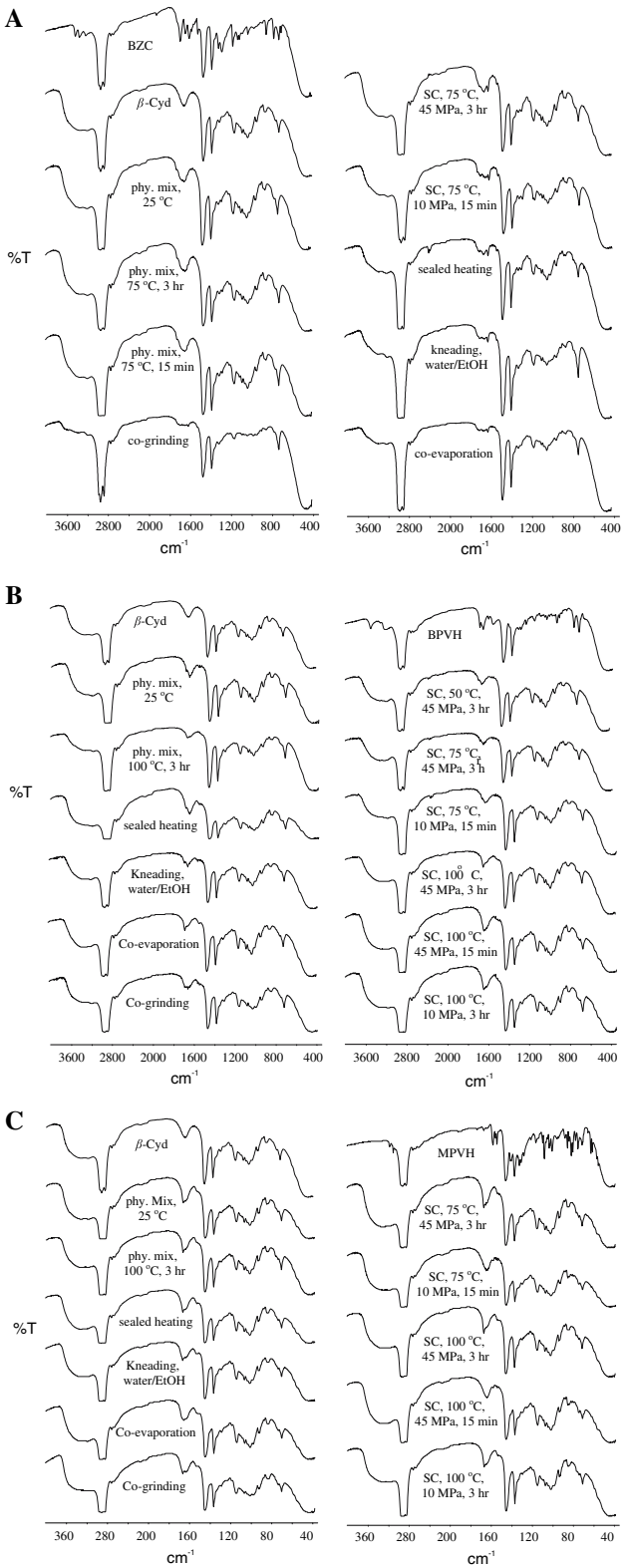


Fig. 2 FTIR spectra of pure drug, pure β-Cyd, and drug-β-Cyd (1:2 mol–mol) systems prepared by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating, and SC CO_2 at different temperatures, pressures, and exposure times.(**A**) benzocaine (BZC) system, (**B**) bupivacaine (BPVH) system, (**C**) mepivacaine (MPVH) system

peak obtained at a 2θ value of 26.8, suggesting partial drug amorphization and/or complexation in this product. The pattern of the product treated with SC CO_2 at 75 °C and 10 MPa for 15 min was similar to that of the sample exposed at the same temperature but higher pressure (45 MPa) and longer exposure time (3 h), except that only three new peaks, instead of four, were observed, lacking the additional new peak at 13.5 2θ. These results are in agreement with the results obtained by FTIR analysis, suggesting a poor influence of higher pressures or longer exposure times in facilitating interactions between BZC and β-Cyd using the SC CO_2 method.

The sealed-heated product also 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, indicating that the two products have similar solid state structural characteristics. A pattern with a very few low-intensity peaks was obtained for the kneaded and co-evaporated samples, suggesting partial drug amorphization and/or complexation in these products.

A diffuse pattern was obtained for the co-ground product, indicating full drug amorphization as a consequence of the intimate dispersion of BZC in the carrier induced by the mechanical treatment, and/or of the inclusion of the drug in the Cyd cavity.

As for the BPVH samples (Fig. 3B), products treated with SC CO_2 displayed crystalline patterns, with some drug crystallinity loss. Higher crystallinity losses were observed for the products exposed to 75 and 100 °C at higher pressure (45 MPa) and longer exposure time (3 h), as compared to the sample treated at the same pressure and exposure time but at lower temperature (50 °C). No additional new peaks were observed in any of the BPVH-β-Cyd samples treated with SC CO_2, contrary to what was observed in the case of the corresponding BZC-β-Cyd products.

The BPVH-β-Cyd product obtained by sealed-heating also displayed a crystalline pattern with most characteristic peaks at lower intensities except the peak at a 2θ value of 21, which had a higher intensity than that in the pure drug or Cyd. The products prepared by kneading and co-evaporation methods resulted in similar diffraction patterns, showing high crystallinity losses, like in the case of samples exposed to SC CO_2 for 3 h at 45 MPa and 75 or 100 °C. An almost completely diffuse pattern was obtained for the BPVH-β-Cyd product obtained by co-grinding, indicating complete drug amorphization and/or complexation.

The MPVH-β-Cyd samples obtained by the SC CO_2 method also displayed crystalline patterns with some

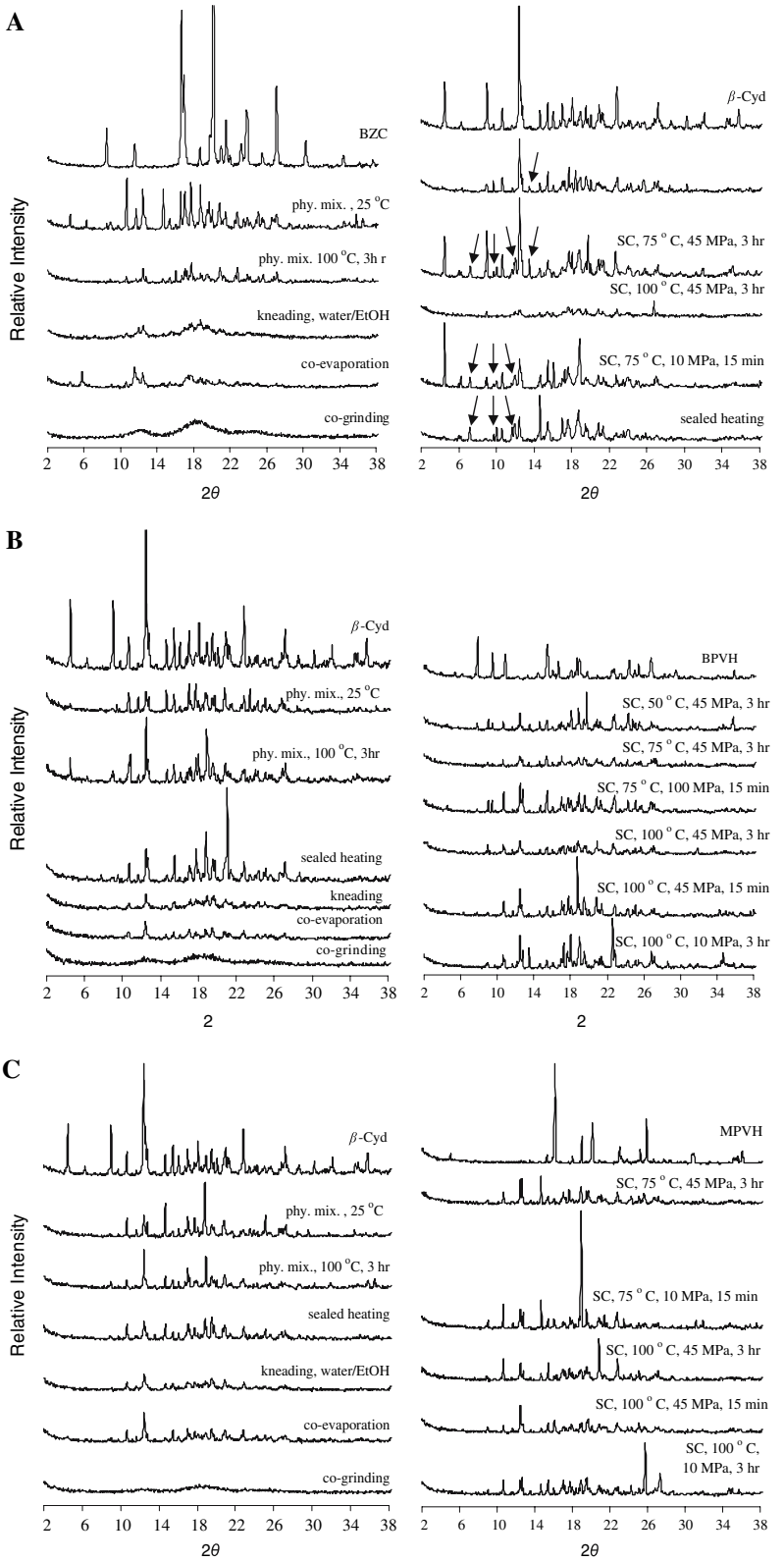


Fig. 3 PXRD patterns of pure drug, pure β-Cyd, and drug-β-Cyd (1:2 mol–mol) complexes prepared by physical mixing, kneading, co-evaporation, co-grinding, sealed-heating, and SC CO_2 at different temperatures, pressures, and exposure times. (**A**) benzocaine (BZC) system, (**B**) bupivacaine (BPVH) system, (**C**) mepivacaine (MPVH) system

drug crystallinity loss (Fig. 3C). In particular, in the sample treated at 75 °C and 10 MPa for 15 min, some characteristic peaks of the drug disappeared or their intensities were decreased (i.e. peaks at 2θ values of 16, 20 and 26) while the intensity of the peak at 2θ value of 19 was increased. Samples exposed to SC CO_2 under

other temperature, pressure or exposure time conditions all resulted in a decrease in the intensity of the drug peaks, and did not display an increase in the intensity of any peaks. Moreover, like the BPVH-β-Cyd samples, the MPVH-β-Cyd systems prepared by the SC CO_2 method did not result in any new peaks, contrary to what was observed for BZC-β-Cyd products.

The MPVH-β-Cyd product obtained by sealed-heating displayed a crystalline pattern similar to that of the product treated with SC CO_2 at 75 °C and 45 MPa for 3 h. As mentioned above, similar results were obtained for the corresponding BZC-β-Cyd samples treated with these two methods, showing the similarities between the characteristics of the products obtained by sealed-heating method and SC CO_2 method at 75 °C and 45 MPa for 3 h. However, the BPVH-β-Cyd systems did not show such a similarity between the two methods.

On the other hand, the results obtained for BZC-β-Cyd, BPVH-β-Cyd and MPVH-β-Cyd products prepared by kneading and co-evaporation methods resulted in similar diffraction patterns, showing high crystallinity losses. Nevertheless, in all cases the highest crystallinity loss for the three binary systems was observed for the products obtained by the co-grinding method, which showed an almost completely diffuse pattern, indicative of complete drug amorphization and/or inclusion of the drug in the Cyd cavity.

Dissolution studies

The mean dissolution curves of BPVH from the binary systems with β-Cyd prepared by the different techniques are shown in Fig. 4, while the main dissolution parameters are collected in Table 1. It is evident that the technique used for obtaining BPVH-β-Cyd solid systems influenced the dissolution properties of the final product, and that all the examined preparation methods were more effective than the simple physical mixing. The rank order of the dissolution rates was COE > GR > SC CO_2 > KN > S.H. > P.M.

Analogous results were obtained for the various BZC-β-Cyd binary systems, but a different rank order in the dissolution performance was found, i.e. S.H. > SC $CO_2 \approx$ GR > COE > KN > PM, as it can be seen in Fig. 5, where the Dissolution Efficiency values at 60 min obtained for the two series of binary systems with the two drugs are compared. In particular, it can be observed that sealed-heating was the least effective technique in the case of BPVH-β-Cyd products, whereas it was the best one for the BZC-β-Cyd binary systems; on the contrary, co-evaporation was the most successful

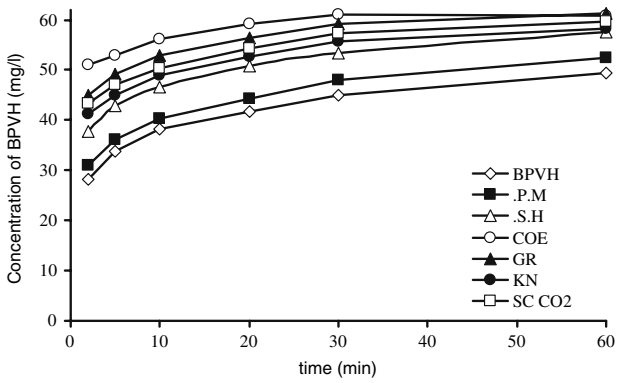


Fig. 4 Mean dissolution curves of bupivacaine (BPVH) from the 1:2 mol–mol binary systems with β-Cyd obtained by the different preparation methods. Key: (◇) BPVH alone; (■) P.M.; (△) S.H.; (□) SC CO_2 (50 °C, 45 MPa, 3 h); (▲) GR; (○) COE; (●) KN

preparation method in the case of BPVH, while it was almost the worst in the case of BZC. On the other hand, with both drugs, products obtained by the SC CO_2 method showed satisfactory dissolution properties, similar to those of the corresponding ones obtained by co-grinding, and only slightly lower than the corresponding most effective system, i.e. the co-evaporated product for BPVH or the sealed-heated one for BZC. Similar results were also found in the case of MPVH-β-Cyd systems; however, due to the greater dissolution properties of these products, it was not possible to perform dissolution tests under the same experimental conditions used for BZC and BPVH binary systems, and therefore, the results were not directly comparable.

Conclusions

Solid systems of BZC, BPVH and MPVH with β-Cyd in the 1:2 mol:mol ratio were prepared by SC CO_2 method and compared to products obtained using dif-

Table 1 Percent dissolved (P.D.) and dissolution efficiency (D.E.) at 30 min and relative dissolution rate (Rdr) of BPVH from the 1:2 mol–mol binary systems with β-Cyd prepared by the different methods

Sample	P.D.30	D.E.30	Rdr 2 min
BPVH	74.8	63.0	–
BPVH-β-Cyd P.M.	80.1	67.1	1.1
BPVH-β-Cyd KN	92.7	80.9	1.4
BPVH-β-Cyd COE	100	92.1	1.8
BPVH-β-Cyd GR	98.7	87.0	1.6
BPVH-β-Cyd SC CO_2*	95.4	83.5	1.5
BPVH-β-Cyd S.H.	88.8	77.1	1.3

* (50 °C, 45 MPa, 3 h)

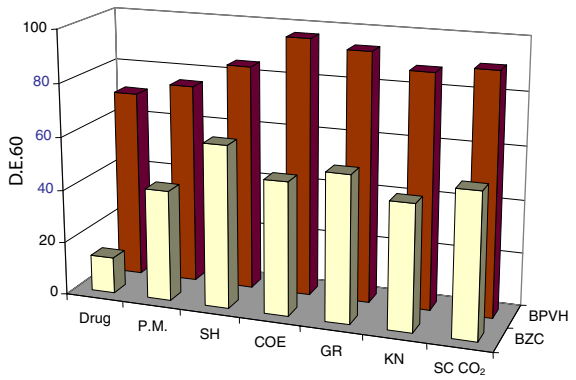


Fig. 5 Dissolution efficiency at 60 min (D.E.60) of bupivacaine (BPVH) and benzocaine (BZC) from their 1:2 mol–mol binary systems with β-Cyd prepared by different methods

ferent techniques such as physical mixing, kneading, co-evaporation, high-energy co-grinding, and sealed-heating. A similar PXRD pattern was observed for the BZC-β-Cyd, BPVH-β-Cyd, and MPVH-β-Cyd systems obtained by co-grinding, suggesting that this technique leads to complete amorphization and/or complexation of the drugs with β-Cyd. The other methods instead led to crystalline or partially amorphous products depending on both the type of drug and the preparation method. The different degrees of modification observed in the spectral analyses of products prepared by diverse methods suggest the possibility of drug-Cyd interactions of different strengths, which may give rise to different degrees of inclusion formation and/or amorphization of the sample.

Dissolution studies indicated that all the examined methods were more effective than the simple physical mixing in improving the drug dissolution performance of the final product. However, the different rank orders observed for the different drugs suggested that there is no general rule for the selection of the most effective preparation method, which depends on the type of drug-Cyd system considered. Nevertheless, in all cases, products obtained by the SC CO_2 method showed satisfactory dissolution properties. Therefore, supercritical fluid technology proved to be a novel and useful complexation method of anesthetic drugs into β-Cyd. Moreover, products obtained using SC CO_2 should provide minimal side effects in humans, compared to those obtained by techniques requiring the use of organic solvents, since this method has no toxic solvent residuals. However, also with this technique, the most effective conditions to obtain the best result with the shortest exposure time and the most appropriate temperature and pressure values should be carefully investigated.

References

1. Covino, B., Vassallo, H.: Local Anesthetics. Grune and Stratton, New York (1976)
2. Benumof, J.: Clinical Procedures in Anesthesia and Intensive Care. JB Lippincott, Philadelphia (1992)
3. Szejtli, J.: Cyclodextrins in Pharmacy, Dordrecht. Kluwer, The Netherlands (1994)
4. Duchene, D., Wouessidjewe, D.: Pharmaceutical uses of cyclodextrins and derivatives. Drug Dev. Ind. Pharm. **16**, 2487–2499 (1990)
5. Lee, B., Lee, J.: Enhancement of solubility and dissolution rate of poorly water-soluble naproxen by complexation with 2-hydroxypropyl-β-cyclodextrin. Arch. Pharm. Res. **18**, 22–26 (1995)
6. Dhanaraju, M., Kumaran, K., Baskaran, T., Moorthy, M.: Enhancement of bioavailability of griseofulvin by its complexation with beta-cyclodextrin. Drug Dev. Ind. Pharm. **24**(6), 583–587 (1998)
7. Dollo, G., Thompson, D., Le Corre, P., Chevanne, F., Le Verge, R.: Inclusion complexation of amide-typed local anesthetics with β-cyclodextrin and its derivatives. III. Biopharmaceutics of bupivacaine-SBE7-βCD complex following percutaneous sciatic nerve administration in rabbits. Int. J. Pharm. **164**(1–2), 11–19 (1998)
8. Greczy, J., Bruhwyler, J., Scuvee-Moreau, J., Seutin, V., Masset, H., Van Heugen J., Dresse, A., Lejeune, C., Decamp, E., Szente, L., Szejtli, J., Liegeois, J.: The inclusion of fluoxetine into gamma-cyclodextrin increases its bioavailability: behavioural, electrophysiological and pharmacokinetic studies. Psychopharmacology **151**(4), 328–334 (2000)
9. Nagarsenker, M., Meshram, R., Ramprakash, G.: Solid dispersion of hydroxypropyl beta-cyclodextrin and ketorolac: Enhancement of in-vitro dissolution rates, improvement in anti-inflammatory activity and reduction in ulcerogenicity in rats. J. Pharm. Pharmacol. **52**, 949–956 (2000)
10. Kang, J., Kumar, V., Yang, D., Chowdhury, P., Hohl, R.: Cyclodextrin complexation: influence on solubility, stability and cytotoxicity of camptothecin, an antineoplastic agent. Eur. J. Pharm. Sci. **15**, 163–170 (2002)
11. Stanley, T.: New routes of administration and new delivery systems of anesthetics. Anaesthesiolog **38**, 665–668 (1988)
12. Szejtli, J.: Cyclodextrins and their Inclusion Complexes. Akademiai Kiado, Budapest (1982)
13. Jones, S. Grant, D., Hadgraft, J., Parr, G.: Cyclodextrins in the pharmaceutical sciences. Part I: Preparation, structure and properties of cyclodextrins and cyclodextrin inclusion compounds. Acta Pharm. Technol. **30**, 213–223 (1984)
14. Irie, T., Uekama K.: Protection against the photosensitized skin irritancy of chlorpromazine by cyclodextrin complexation. J. Pharmacobiol. Dyn. **8**(9), 788–791 (1985)
15. Mura, P., Faucci, M., Bettinetti, G.: The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-β-cyclodextrin. Eur. J. Pharm. Sci. **13**, 187–194 (2001)
16. Cirri, M., Rangoni, C., Maestrelli, F., Corti, G., Mura, P.: Development of fast-dissolving tablets of flurbiprofen-cyclodextrin complexes. Drug Dev. Ind. Pharm. **31**(7), 697–707 (2005)
17. Mura, P., Furlanetto, S., Cirri, M., Maestrelli, F., Corti, G., pinzauti, S.: Interaction of naproxen with ionic cyclodextrins in aqueous solution and in the solid state. J. Pharm. Biomed. Anal. **37**, 987–994 (2005)
18. Veiga, M., Merino, M., Cirri, M., Maestrelli, F., Mura, P.: Comparative study on triclosan interactions in solution and

in the solid state with natural and chemically modified cyclodextrins. J. Incl. Phenom. **53**(1), 77–83 (2005)
19. Hirayama, F., Uekama, K.: Cyclodextrins and their Industrial Uses. Editions de Santé, Paris (1987) pp. 133
20. Blanco, J., Vila-Jato, J.L., Otero, F., Anguiano, S.: Influence of the method of preparation on inclusion complexes of naproxen with different cyclodextrins. Drug Dev. Ind. Pharm. **17**, 943–957 (1991)
21. Mura, P., Adragna E., Rabasco, A., Moyano, J., Perez-Martinez, J., Arias, M., Gines, J.: Effects of the host cavity size and the preparation method on the physicochemical properties of ibuproxam-cyclodextrin systems. Drug Dev. Ind. Pharm. **25**, 279–287 (1999a)
22. Mura, P., Faucci M.T., Manderioli A., Bramanti G.: Influence of the preparation method on the physicochemical properties of binary systems of econazole with cyclodextrins. Int. J. Pharm. **193**, 85–95 (1999b)
23. Mura, P., Faucci, M.T., Parrini, P.L., Furlanetto, S., Pinzauti, S.: Influence of the preparation method on the physicochemical properties of ketoprofen-cyclodextrin binary systems. Int. J. Pharm. **179**, 117–128 (1999c)
24. Kiran, E., Brennecke J.: Supercritical Fluid Engineering Science, ACS Symposium Series 514, American Chemical Society, Washington DC (1993)
25. Van Hees, T., Piel, G., Evrard, B., Otte, X., Thunus, L., Delattre, L.: Application of supercritical carbon dioxide for the preparation of a piroxicam-β-cyclodextrin inclusion compound. Pharm. Res. **16**, 1864–1870 (1999)
26. Junco, S. Casimiro T., Ribeiro, N., da Ponte, M.N., Cabral Marques, H.: A comparative study of naproxen-beta cyclodextrin complexes prepared by conventional methods and using supercritical carbon dioxide. J. Incl. Phenom. **44**(1–4), 117–121 (2002)
27. Charoenchaitrakool, M., Dehghani, F., Foster, R.F.: Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl-β-cyclodextrin. Int. J. Pharm. **239**(1–2), 103–112 (2002)
28. Lai, S., Locci E., Piras, A., Porcedda, S., Lai, A., Marongiu B.: Imazalil-cyclomaltoheptaose (β-cyclodextrin) inclusion complex: Preparation by supercritical carbon dioxide and ^{13}C CPMAS and ^{1}H NMR characterization. Carbohydr. Res. **338**(21), 2227–2232 (2003)
29. Al-Marzouqi A., Shehatta, I., Jobe, B., Dowaidar, A.: Phase solubility and inclusion complex of itraconazole with β-cyclodextrin using supercritical carbon dioxide. J. Pharm. Sci. **95**, 292–304 (2006)
30. Shehatta, I., Al-Marzouqi, A., Jobe, B., Dowaidar, A.: Enhancement of aqueous solubility of itraconazole by complexation with cyclodextrins using supercritical carbon dioxide. Can. J. Chem. **83**(10), 1833–1838 (2005)
31. de Jong, R.H.: Local Anestethics. Springfield, Illinois (1994)
32. Strichartz, G.R., Sanchez, V., Arthue, R., Chafetz, R., Martin, D.: Fundamental properties of local anesthetics. II. Measured octanol:buffer partition coefficients and pK(a) values of clinically used drugs. Anest. Analg. **71**(2), 158–170 (1990)
33. Dollo, G., LeCorre, P., Chevanne, Le Verge, R.: Inclusion complexation of amide-typed local anaesthetics with ß-cyclodextrin and its derivatives. I. Physicochemical characterization. Int. J. Pharm. **131**(2), 219–228 (1996)
34. Pinto, L., Fraceto L., Santana, M., Pertinhez, T., Oyama Junior, S., de Paula, E.: Physico-chemical characterization of benzocaine-β-cyclodextrin inclusion complexes. J. Pharmaceut. Biomed. **39**(5), 956–963 (2005)
35. Hassan, A., Tang, Y., Ayres, J.: Itraconazole formation using supercritical carbon dioxide. Drug Dev. Ind. Pharm. **30**(10), 1029–1035 (2004)
36. Khan, K.A.: The concept of dissolution efficiency. J. Pharm. Pharmacol. **27**(1), 48–49 (1975)