Journal of Inclusion Phenomena and Macrocyclic Chemistry **43:** 251–257, 2002.

Investigation on the Interaction of Bendazac with β-, Hydroxypropyl-β-, and γ-Cyclodextrins

BRUNELLA CAPPELLO*, CLELIA DI MAIO and MARIA IERVOLINO
Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131 Naples, Italy

(Received: 6 December 2001; in final form: 11 March 2002)

Key words: aqueous solubility, Bendazac, β-cyclodextrin, ^{13}C-NMR, dissolution rate, DSC, FT-IR, γ-cyclodextrin, hydroxypropyl-β-cyclodextrin, solid binary systems

Abstract

The interactions of Bendazac, a topical non-steroidal anti-inflammatory drug, with β-cyclodextrin, hydroxypropyl-β-cyclodextrin and γ-cyclodextrin were investigated to evaluate possibilities to improve the drug's poor water solubility and eventually to enhance the topical delivery of Bendazac. Phase solubility studies demonstrated the ability of the selected cyclodextrins to complex with Bendazac and increase drug solubility. The amount of solubilized Bendazac increased linearly with the addition of each cyclodextrin according to A_L type plots. ^{13}C-NMR studies showed that the Bendazac A-ring was included in the cavity of the three cyclodextrins. The γ-cyclodextrin was also able to include the B-ring of Bendazac, forming a complex where one drug molecule fitted into two cyclodextrin molecules. Equimolar solid systems of the drug with each cyclodextrin carrier were prepared using various techniques (physical mixing, spray-drying and freeze-drying). The results of differential scanning calorimetry and Fourier transform infrared analysis, performed on the solid systems, demonstrated that freeze-dried and spray-dried products had a high degree of amorphization and agreed with the hypothesis of the existence of drug–cyclodextrin interaction in the solid state. The cyclodextrins tested were able to improve the dissolution of Bendazac. The dissolution profile of the drug was also affected by the physico-chemical properties of each solid system, the freeze-dried products being the most rapidly dissolving forms.

Introduction

Bendazac ((1-benzyl-1H-indazol-3-yloxy)acetic acid) is a topical non-steroidal anti-inflammatory drug used for the treatment of inflammatory skin disorders evolving towards necrosis [1]. In ophthalmology, Bendazac (BEN) is employed in the management of cataract, due to its ability to prevent lens protein denaturation and opacification [2]. The interest in the co-formulation of BEN and cyclodextrins (CDs) lies in the possibility to favourably modify the drug's poor water solubility and permeability through biological membranes (eye cornea and skin).

CDs are cyclic oligosaccharides capable of forming water-soluble inclusion complexes with many compounds by taking up the molecule in part or in its entirety into their relatively hydrophobic cavity [3–4]. They have been employed in pharmaceutical formulations to improve the aqueous solubility, the stability and the bioavailability of a series of proximate principles [5–7]. During recent years CDs have also been suggested to act as penetration enhancers for topical drug delivery [8–10]. This effect has been related to the capability of CDs to increase the aqueous solubility of lipophilic water-insoluble drugs, without affecting the drug's intrinsic ability to permeate biological membranes [10]. Actually, CDs do enhance drug permeability without causing physico-chemical changes within the biological barriers, since hydrophilic CDs and their drug complexes are only able to penetrate into biological membranes with considerable difficulty [8–11]. In general, it is thought that the CDs enhance transdermal or transcorneal drug delivery by increasing drug availability at the barrier surface, where drug molecules partition from the CD cavity into the lipophilic barrier [10].

The effectiveness of CDs to improve the physico-chemical properties of drugs depends on their capacity to form an inclusion complex and, hence, on the type and degree of drug-CD interactions. These, in turn, are governed by the structural features of the specific drug and the CD.

In this work the interactions of BEN with different CDs, in both solution and solid states, were investigated with an ultimate goal to evaluate possibilities to improve the rather unfavourable physico-chemical properties of BEN with regard to optimal topical formulations. The following CDs were selected: β-cyclodextrin (β-CD), because its unique cavity size is suitable for complexation of most drug molecules; hydroxypropyl-β-cyclodextrin (HP-β-CD), due to the higher water solubility with respect to the native β-CD; γ-cyclodextrin (γ-CD), chosen for evaluating the effect of a larger cavity than β-CD's on BEN complexation. These CDs

* Author for correspondence.

were also selected because they are considered safe upon dermal and ocular applications [12–14].

Solution studies by the phase solubility method and NMR spectroscopy were performed to investigate the ability of the CDs to complex with and dissolve BEN. Solid systems of BEN and each CD were prepared by physical mixing, spray-drying and freeze-drying, as these methods are known to yield products with distinctive properties in the solid state [15, 16]. All solid systems were characterized by differential scanning calorimetry (DSC) and Fourier transform infrared analysis (FT-IR). Dissolution studies on the binary systems were also performed to evaluate the influence of the physicochemical properties of the solid systems on the dissolution rate of BEN.

Experimental

Chemicals

Bendazac was kindly supplied by Aziende Chimiche Riunite Angelini Francesco (Rome, Italy); β-cyclodextrin (Kleptose®) and hydroxypropyl-β-cyclodextrin (MS = 0.40) by Roquette Frères (Lestrem, France), whereas γ-cyclodextrin was a commercial sample from Cyclolab (Budapest, Hungary). All substances were used without further purification. All chemicals were of analytical reagent grade. Double distilled water was used throughout the study.

Solution studies

Phase solubility studies

Solubility studies were performed in unbuffered water, according to the Higuchi and Connors method [17]. An excess amount of BEN (10 mg) was added to 10 mL of water or CD aqueous solutions (from 1×10^{-3} to 1.2×10^{-2} M of β-CD; from 1×10^{-3} to 5×10^{-2}M of HP-β-CD and γ-CD) in screw-capped glass vials; these were mechanically shaken (SS40-D Grant shaking bath) at 25 °C until equilibrium was achieved (5 days). Aliquots were withdrawn, filtered (filter HA-0.45 μm, Millipore) and spectrophotometrically analysed for BEN content (Shimadzu UV-1204 spectrophotometer) at 307 nm. The presence of CDs did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate; the coefficient of variation associated with each measurement was never greater than 3%.

^{13}C-NMR studies

^{13}C-NMR spectra were recorded on a Bruker WM-250 spectrometer. Solutions containing a constant concentration of BEN (2×10^{-2} M) and variable concentrations of each CD (from 0 to 8×10^{-2} M) were prepared in a 0.1 N solution of NaOD in D_2O to ensure drug solubilization. Spinning tubes of 4 mm i.d. containing 0.5 mL of solution were employed. Tetramethylsilane was used as external reference and no correction was made for the susceptibility of the capillary. Chemical shifts were calibrated with an accuracy of 0.01 ppm.

Preparation of solid binary systems

BEN and CDs were sieved (IG3/WET/MS, Giuliani, Torino) and the corresponding 75–150 μm granulometric fractions collected. The BEN/CD solid systems were all prepared in 1:1 (mol/mol) stoichiometric ratio.

For the preparation of the physical mixtures (PMs), BEN and each CD were blended in a mortar until a homogeneous mixture was obtained. For the preparation of the freeze-dried (FDs) and the spray-dried (SDs) products, 1 g of each PM was dissolved in 600 mL of water containing 0.06 g/L ammonium hydroxide to ensure drug solubilization. After 24 hours of agitation at room temperature, the solutions were frozen at −70 °C and freeze-dried in a Modulyo Edwards apparatus, obtaining the corresponding FD products. The same solutions were atomised in a Büchi 190 Mini Spray Dryer to prepare the SD products. The spray-drying system was equipped with a 0.5 mm nozzle and atomisation was performed under the following conditions: N_2 flow rate, around 700 L·h^{-1}, inlet temperature, 145 °C, outlet temperature, 73 °C. No residual ammonia (Nessler's test) was detected in any of the SDs and FDs.

Solid state studies

Differential scanning calorimetry

DSC measurements were carried out using a Mettler DSC 30 apparatus equipped with a TC II probe. Samples were weighed (10–15 mg) (Mettler M3 microbalance) in Al pans pierced with a perforated lid, and scanned at 10 °C/min in the 30-250 °C temperature range. Dry nitrogen was used as purge gas.

X-ray analysis

X-ray powder diffraction analysis was performed by a Philips PW3710 diffractometer in the 3-40° 2θ range (scan rate 1° min^{-1}). Kα radiation of Cu was generated at 40 kV and 20 mA.

Infrared spectroscopy

FT-IR spectra (KBr disk) were obtained on a Bruker IFS-48 apparatus applying Fourier transformation of 8 scans.

Dissolution studies

The dissolution studies of BEN and the different BEN/CD solid systems were performed according to the USP 24 method (Apparatus 2). 200 mg of BEN or equivalent amounts of BEN/CD blends were added to 200 mL of water (non-sink conditions) at 37.0 ± 0.1 °C in a Sotax AT7 apparatus at a rotation speed of 100 rpm. Suitable aliquots were removed at different time intervals, filtered and spectrophotometrically analysed for BEN content, as for solubility studies. A correction was calculated for the sampling. Each test was performed in triplicate; the coefficient of variation associated with each measurement was never greater than 3%.

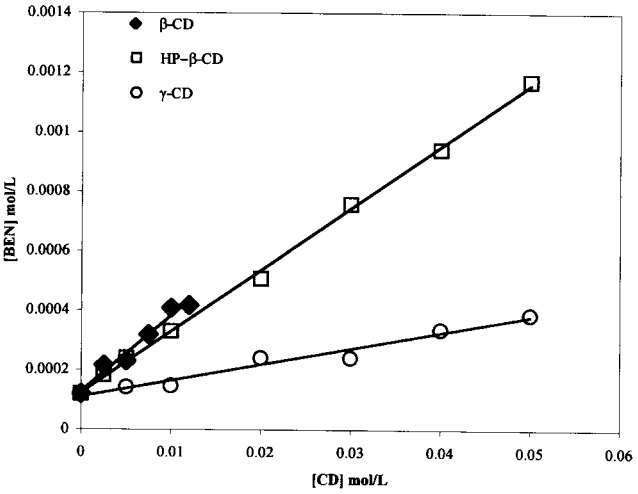


Figure 1. Phase solubility diagrams of BEN at increasing amounts of the different CDs (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

Table 1. Stability constants of BEN/CD complexes (± indicates the S.D. of the respective values)

CD	$K_{1:1}$ (M^{-1})
β-CD	201 ± 5
HP-β-CD	172 ± 4
γ-CD	49 ± 1

Results and discussion

Solution studies

The equilibrium phase solubility plots for the various BEN/CD systems are reported in Figure 1. Drug solubility increased proportionally as the CD molar concentration increased. At the highest concentration tested (5×10^{-2} M), HP-β-CD and γ-CD increased BEN solubility about 10- and 3-fold, respectively. With β-CD a three fold enhancement in drug solubility was obtained at the concentration of 1.2×10^{-2} M, which indeed was the highest concentration tested due to the limited β-CD aqueous solubility. According to the Higuchi and Connors classification [17], the diagrams obtained were of A_L type, since they were characterized by a straight line pattern. This type of diagram indicates the formation of a soluble complex, thereby increasing the total amount of drug in solution. The assumption of a 1:1 drug/CD stoichiometry is usually made for A_L type diagrams in the absence of additional information. However, as pointed out by Higuchi and Connors [17], an A_L type diagram does not necessarily mean that only a 1:1 complex is formed.

Assuming a 1:1 stoichiometry, the apparent stability constants ($K_{1:1}$) of the complexes were calculated from the slope of the phase solubility diagrams and the drug solubility in water [17]. The $K_{1:1}$ values obtained are reported in Table 1. The stability constant of the complexes of BEN with β-CD and with HP-β-CD were similar, indicating that the hydroxypropyl substituent groups did not much affect the affinity of β-CD for BEN. Additionally, the $K_{1:1}$ value of the BEN/γ-CD complex is lower than those of the complexes with the β-CDs. However, it is important to remark that ^{13}C-NMR studies (see below) demonstrated a 1:2 stoichiometry for the BEN/γ-CD complex. Therefore, the calculated $K_{1:1}$ value has poor significance in the case of this complex.

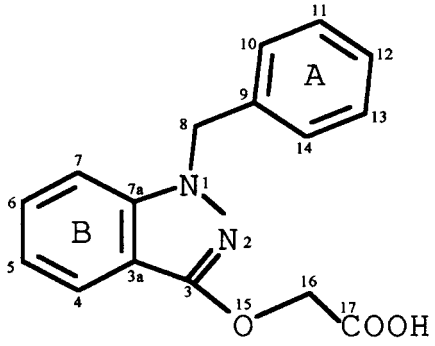
^{13}C-NMR studies were performed to investigate both the nature of the interactions between BEN and the CDs and the stoichiometry of the complexes in solution. The chemical shift changes of BEN carbons, due to the interaction with each CD, as a function of the CD concentration were examined. NMR data analysis was performed according to the mole ratio method [18], which provides useful information on the portions of a drug molecule involved in the interaction with CDs. As a matter of fact, the transfer of a guest molecule from the free state to the CD cavity in solution has been reported to cause an upfield shift of the ^{13}C-NMR signals of the lead carbons included and a downfield shift of the ^{13}C-NMR signals of the carbons externally close to the wider rim of the hollow CD cone [19, 20]. The chemical shift changes measured were also analysed according to Job's method of continuous variations [21], which is known to provide indications on the stoichiometry of the complexes.

Figure 2(A–C) shows the chemical shift changes in the BEN carbon atoms induced by the CDs and analysed according to the mole ratio method. The profiles of the carbons showing the highest variations in chemical shift are only reported; the negative sign of Δ ppm (i.e., the difference in BEN chemical shifts in the presence and absence of CD) refers to an upfield shift, whereas the positive sign indicates a downfield shift. In the presence of β-CD (Figure 2A) the upfield shifts of the C10-14, C11-13 and C12 carbon atoms suggest that the BEN phenyl group, indicated as the A-ring, was included within the CD cavity. The downfield shifts of the C8 and C9 carbon atoms confirm this hypothesis.

The same portions of drug molecule were involved in the interaction with HP-β-CD, since similar patterns were observed (Figure 2B).

In contrast to the β-CDs tested, in the presence of γ-CD (Figure 2C) not only were the carbons of the A-ring of BEN (C10-14, C11-13 and C12) shifted upfield but also those of the condensed phenyl group indicated as the B-ring (C3a, C4, C5, C7 and C7a). This indicates the interaction of both the phenyl rings with the hollow γ-CD cone, but does not provide definite information on the drug/CD ratio. It is worthy of note that the signal displacements observed in the presence of γ-CD were smaller than those observed with the other CDs, indicating the weaker interaction between the drug and this CD, probably due to its larger cavity size compared to the β-CD.

Figure 3(A–C) shows Job's plots obtained for the interaction between BEN and the CDs tested. They indicate a 1:1 stoichiometric ratio with β-CD and HP-β-CD (Figures 3A and 3B respectively) because these complexes display the greatest displacement at $r = 0.5$ (r is the molar fraction of CD in the global amount of CD and BEN. It is reported in



Structure of Bendazac
(Arbitrary numbers were assigned to the atoms in the structure for ease in presentation of data)

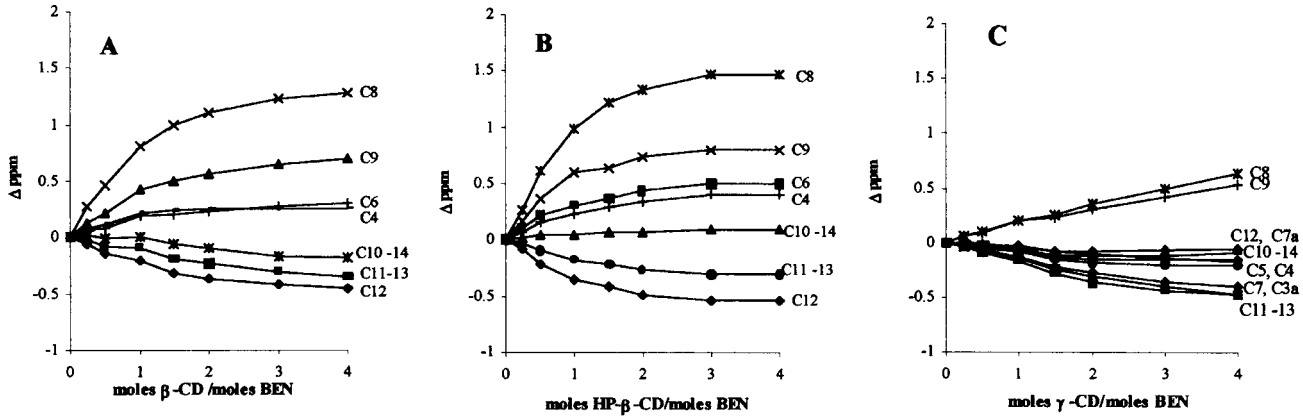


Figure 2. Cyclodextrin-induced ^{13}C-chemical shifts of BEN plotted as a function of the molar ratio of each CD to BEN: β-CD (A), HP-β-CD (B), and γ-CD (C).

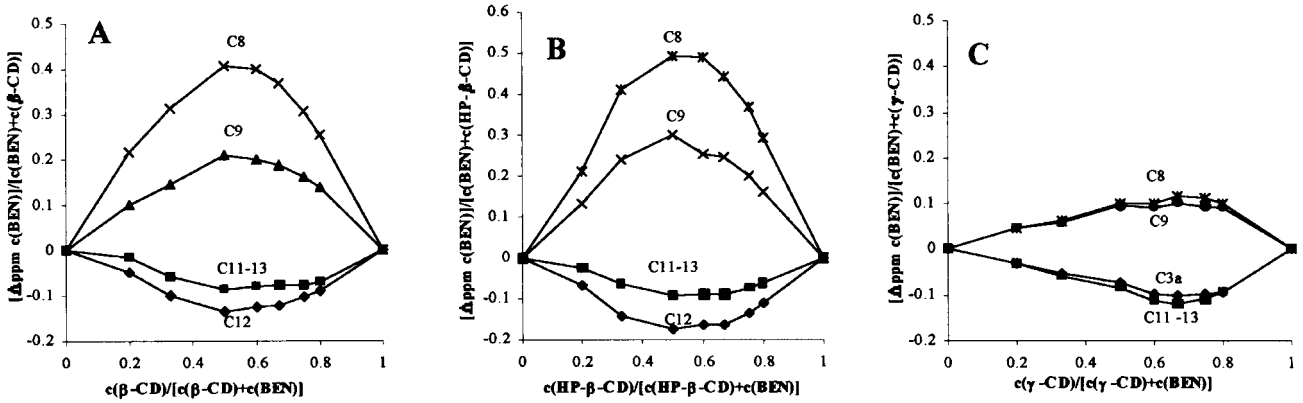


Figure 3. Continuous variation plots of cyclodextrin-induced ^{13}C-chemical shifts of BEN: β-CD (A), HP-β-CD (B), and γ-CD (C).

the figures as the value of the abscissa, and varies from 0 to 1). The plots obtained in the presence of γ-CD (Figure 3C) show curves having an asymmetrical pattern, with $r = 0.66$, suggesting that BEN can form a complex with two γ-CD molecules.

The results of Job's plots and those of the mole ratio method indicate that the A-ring and the B-ring of BEN fitted into two different γ-CD molecules.

Solid state studies

The DSC curves of the pure components and of the various drug-CD equimolar solid systems are shown in Figure 4. Both β-CD and γ-CD exhibited a broad endothermic effect corresponding to their dehydration. The broader endotherm was associated with water loss from amorphous HP-β-CD. The thermal curve of BEN was indicative of its crystalline anhydrous state and showed an endothermic peak at 160 °C, corresponding to the melting point of the drug.

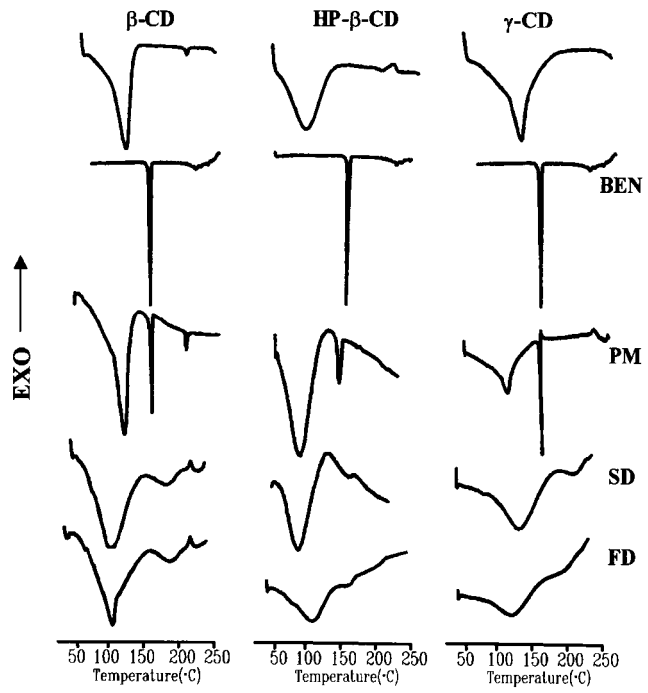


Figure 4. DSC thermograms of pure β-CD, HP-β-CD, γ-CD, BEN and of BEN/CD equimolar systems: physical mixture (PM), spray-dried product (SD), freeze-dried product (FD).

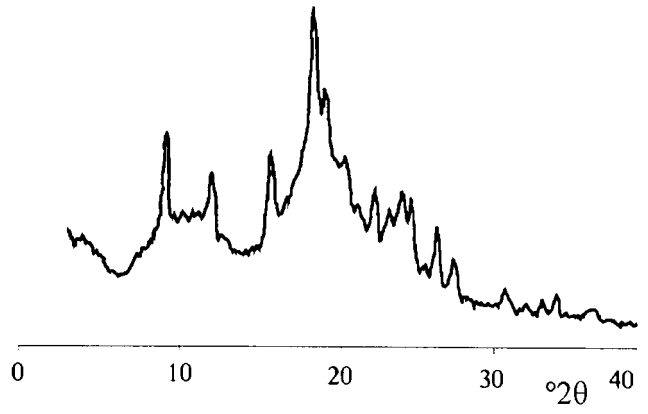


Figure 5. X-ray powder diffraction pattern of the BEN/HP-β-CD physical mixture.

The PMs of BEN with β-CD and γ-CD were subjected to the same transitions of both the drug and the CD and at the same temperatures, while, in the thermal curve of the PM with HP-β-CD, the melting peak of BEN had a reduced intensity and was shifted to a lower temperature than what is typical for the drug. This modification of the DSC melting peak depends on an interaction between BEN and HP-β-CD [22] and may indicate partial amorphization of the drug. However, X-ray diffraction analysis on the PM sample BEN/HP-β-CD (Figure 5) showed that the drug retained its crystalline form at room temperature. Therefore, the observed thermal behaviour is due to a drug-carrier interaction induced by the thermal energy supplied to the sample in the DSC scan [23] and does not depend on drug amorphization.

In all the SDs and the FDs, the dehydration peak of the CDs broadened and the drug's endothermal effect disappeared, indicating total drug amorphization. This phenomenon, though not unequivocally attributable to inclusion complex formation is, however, undoubtedly indicative of a stronger interaction in the SD and FD products between BEN and the CDs [16].

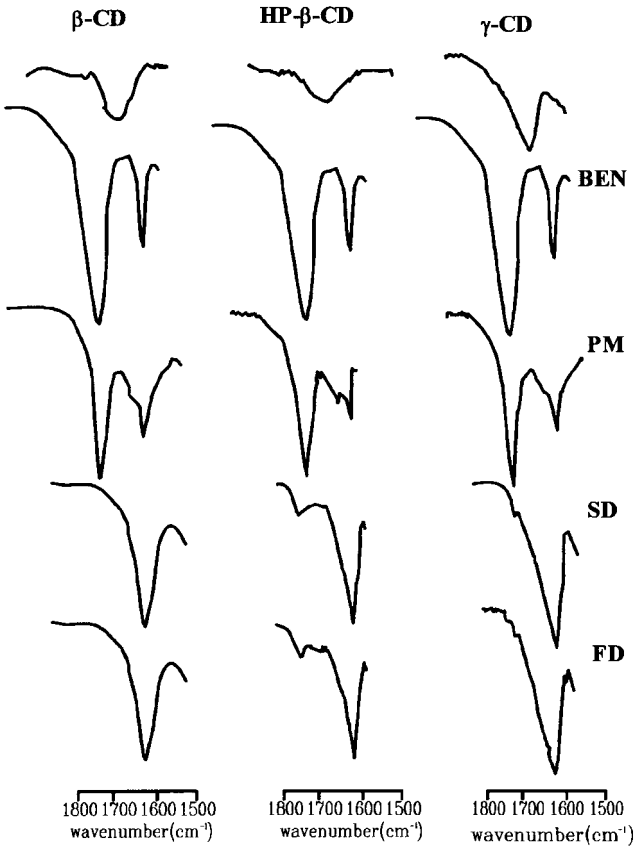


Figure 6. FTIR spectra of pure β-CD, HP-β-CD, γ-CD, BEN and of BEN/CD equimolar systems: physical mixture (PM), spray-dried product (SD), freeze-dried product (FD).

The carbonyl stretching region of the infrared spectra of BEN and its different equimolar systems with CDs are presented in Figure 6. The IR spectrum of BEN gave a sharp peak at 1720 cm^{-1}, corresponding to the carboxyl carbonyl stretching band of BEN. This band is of diagnostic value to elucidate drug-CD interactions.

The carbonyl band appeared unchanged in all PMs. On the other hand, in the SD and FD products this band was affected by the nature of the CD. In the IR spectra of SD and FD products with HP-β-CD, the carbonyl band, at 1720 cm^{-1}, appeared noticeably reduced and was shifted to a higher wavenumber (1740 cm^{-1}). This behaviour can be attributed to the breakdown of the intermolecular hydrogen bonds probably associated with the crystalline BEN molecule, and the formation of a monomeric dispersion of the drug as a consequence of the interaction with the CD. Such an interaction could result in the inclusion of the drug monomer in the CD cavity or in the formation of hydrogen bonding of the monomeric drug with the CD [24]. In the SDs and FDs with β-CD and γ-CD the carbonyl band of BEN disappeared. This phenomenon has been attributed to the restriction of the C=O into the CD cavity, and is probably a consequence of inclusion complex formation [25, 26].

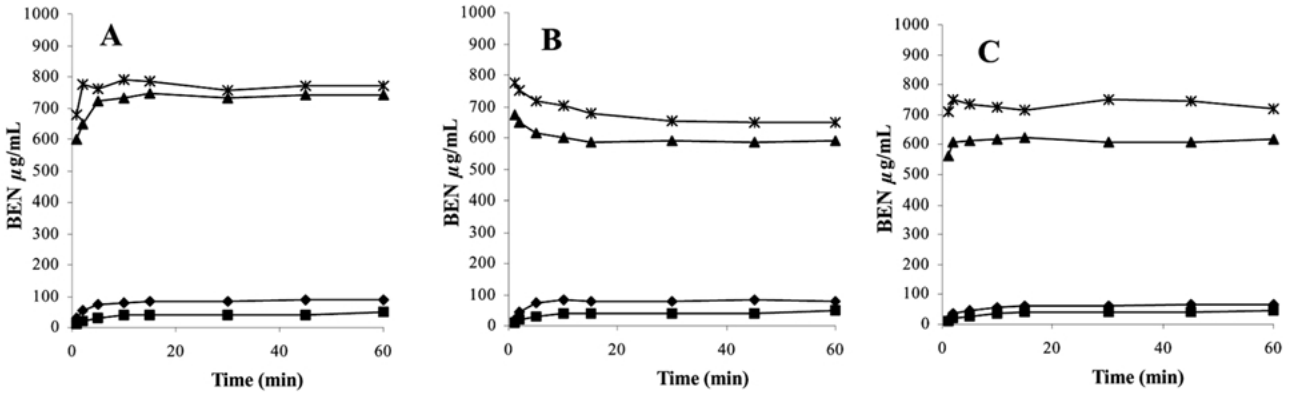


Figure 7. Dissolution curves of BEN (■) and of BEN/β-CD (A), BEN/HP-β-CD (B) and BEN/γ-CD (C) equimolar systems: physical mixture (♦), spray-dried product (▲), freeze-dried product (∗) (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

As to solid state interactions, in contrast to what emerged from the in-solution interactions, no significant differences were found if the macrocycle was changed (β-CD or γ-CD), whereas the presence of hydroxypropyl substituents on the β-CD molecule played a role in the way the carrier interacted with the drug in the solid state.

Dissolution studies

The mean dissolution curves of BEN and the various solid systems with CDs are reported in Figure 7.

From the PMs only a slight increase in drug dissolution rate was observed with respect to pure BEN. In contrast, the SDs and FDs provided dissolution rates considerably higher than the pure drug. It is evident that SD and FD products containing both β-CD and γ-CD gave quite stable supersaturated states (Figure 7A and 7C, respectively). The same samples with HP-β-CD showed a high initial dissolution rate followed by a slight decrease in the amount of drug dissolved (Figure 7B). This indicates that a less stable supersaturation state is achieved with HP-β-CD.

Table 2 shows the relative dissolution rates of the PMs, SDs and FDs with each carrier, calculated as the ratio of the amount of drug dissolved at 2 min to that obtained with the pure drug. The PM with γ-CD provided the lowest dissolution rate increase. The highest relative increases in dissolution rate were achieved with the FDs of the three CDs tested, which gave similar values. The SD products provided relative dissolution rates slightly lower than the FD products. The very good performance of SDs and FDs can be explained by the closer contact between drug and carrier, the decrease in drug crystallinity, as well as by a phenomenon of drug-CD interaction occurring in the solid state.

Table 2. Relative dissolution rate at t = 2 min of BEN from physical mixture (PM), spray-dried (SD) and freeze-dried (FD) samples with β-CD, HP-β-CD, or γ-CD

	β-CD	HP-β-CD	γ-CD
PM	2.4	2.1	1.6
SD	29.5	29.5	27.7
FD	35.2	34.1	34.1

Conclusions

A significant enhancement of BEN solubility has been achieved by associating the drug with CDs, particularly HP-β-CD. This CD is able to form with BEN a complex almost as stable as β-CD, but does not suffer the solubility limitations of the parent CD, and displays higher solubilizing ability than γ-CD. Complexation of BEN with HP-β-CD as well as with β-CD occurs at a 1:1 stoichiometric ratio, whereas one BEN molecule forms a complex with two γ-CD molecules.

The existence of drug-CD interaction has also been evidenced in the solid state in spray-dried and freeze-dried samples.

The results of dissolution studies show that spray-drying and freeze-drying processes are very effective in enhancing the effect of the CDs on the dissolution rate of BEN.

The improvement of BEN solubility obtained with the selected CDs promises an enhanced drug permeability through the corneal and skin barriers.

Acknowledgements

This work was kindly supported by a grant from Regione Campania (P.O.P. Misura 5.4.2 – 1999).

References

1. J.E.F. Reynolds: *Martindale, The Extra Pharmacopoeia, 31st ed.*, p. 24, Royal Pharmaceutical Society, London (1996).
2. J.A. Balfour and S.P. Clissold: *Drugs* **3**9, 575 (1990).
3. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988).
4. D. Duchêne: *New Trends in Cyclodextrins and Derivatives*, Edition de Santé (1991).
5. T. Loftsson and M.E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
6. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
7. R.A. Rajewski and V.J. Stella: *J. Pharm. Sci.* **85**, 1142 (1996).

8. T. Loftsson and T. Jarvinen: *Adv. Drug Deliv. Rev.* **36**, 59 (1999).
9. H. Matsuda and H. Arima: *Adv. Drug Deliv. Rev.* **36**, 81 (1999).
10. T. Loftsson and M.Màsson: *Int. J. Pharm.* **225**, 15 (2001).
11. V.J. Stella, V.M. Rao, E.A. Zannou, and V. Zia: *Adv. Drug Deliv. Rev.* **36**, 3 (1999).
12. U. Vollmer, B.W. Muller, J. Peeters, J. Mesens, B. Wilffert,and T. Peters: *J. Pharm. Pharmacol.* **46**, 19 (1994).
13. M. Vitòria, L.B. Bentley, R.F. Vianna, S. Wilson, and J.H. Collett: *J. Pharm. Pharmacol.* **49**, 397 (1997).
14. P. Saarinen-Savolainen, T. Jarvinen, K. Araki-Sasaki, H. Watanabe, and A. Urtti: *Pharm. Res.***15**, 1275 (1998).
15. J. Blanco, J.L. Vila-Jato, and F.A.S. Otero: *Drug Dev. Ind. Pharm.* **17**, 943 (1991).
16. P. Mura, M.T. Faucci, P.L. Parrini, S. Furlanetto, and S. Pinzauti: *Int. J. Pharm.* **179**, 117 (1999).
17. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
18. K.A. Connors: *Binding Constants. The Measurement of Molecular Complex Stability*, John Wiley, New York (1987).
19. Y. Inoue: *Annual Reports on NMR Spectroscopy* **27**, 59 (1993).
20. E. Redenti, M. Pasini, P. Ventura, A. Spisni, M. Vikman, and J. Szejtli: *J. Incl. Phenom.* **15**, 281 (1993).
21. M. Suzuki and Y. Sasaki: *Chem. Pharm. Bull.* **32**, 832 (1984).
22. K.H. Kim, M.J. Frank, and N.L. Henderson: *J. Pharm. Sci.* **74**, 283 (1985).
23. P. Mura, G.P. Bettinetti, A. Manderioli, M.T. Faucci, G. Bramanti, and M. Sorrenti: *Int. J. Pharm.* **166**, 189 (1998).
24. Y. Nakai, K. Yamamoto, K. Terada, and K. Akimoto: *Chem. Pharm. Bull.* **32**, 685 (1984).
25. H. Hatanaka, F. Komada, Y. Mishima, and K. Okumura: *J. Pharm. Sci.* **82**, 1054 (1993).
26. D.D. Chow and A.H. Karara: *Int. J. Pharm.* **28**, 95 (1986).