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Diclofenac/β-Cyclodextrin Binary Systems: A Study in Solution and in the Solid State

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Abstract

This study was carried out with the aim to optimize the dissolution properties of diclofenac (DIC) – a non-steroidal anti-inflammatory drug sparingly soluble in water – through association with β-cyclodextrin (βCD). The effect of βCD on the aqueous solubility of DIC was evaluated by the phase solubility method. The amount of DIC dissolved increased linearly with the addition of βCD according to an Al type plot and without precipitation of the complex. The apparent stability constant of the complex, calculated supposing a 1:1 stoichiometry, was 295 M^{-1}; this value was confirmed by circular dichroism analysis. DIC/βCD interactions were also studied in water by ^{1}H and ^{13}C NMR spectroscopy. Equimolar DIC/βCD solid systems were prepared by physical-mixing, kneading, co-evaporation and freeze-drying, and their properties in the solid state studied by Differential Scanning Calorimetry, X-ray powder diffractometry and Fourier-Transform Infrared analysis. For sake of comparison, the mixture of DIC and βCD separately lyophilized was investigated too. The results demonstrated that the freeze-dried product had the highest degree of amorphization and they were in agreement with the existence of an inclusion complex in the solid state. The dissolution profiles of the drug from each solid system were affected by its physico-chemical properties, the freeze-dried being the most rapidly dissolving form.

Introduction

The gastrotoxicity of non-steroidal anti-inflammatory drugs (NSAIDs) has been ascribed to both the inhibition of protective prostaglandin synthesis (cyclooxygenase-1 inhibition) and a local detrimental effect on the mucus gel layer of the gastrointestinal tract [1]. It has been demonstrated that oral formulations of NSAIDs containing cyclodextrins can provide a better gastric tolerability and a faster onset of pharmacological activity than uncomplexed drugs [2–5]. This effect has been attributed to the increase of both drug aqueous solubility and dissolution rate exerted by cyclodextrins through drug complexation. Furthermore, the preparation method of NSAIDs/cyclodextrin binary systems plays an important role in determining their physico-chemical properties, and in turn affects the dissolution performance [6–7].

Diclofenac (DIC) is a lipophilic NSAID sparingly soluble in water (its solubility is 1 μg/ml), with a strong analgesic and gastrolesive component. Although some studies on the interactions between DIC and different cyclodextrins have been reported [8–9], the influence of the preparation procedure on both overall physico-chemical properties of the complex and its pharmacological activity has not been investigated in depth.

In a more general attempt to optimize the pharmacological profile of DIC, in this work our aim was to investigate the effectiveness of β-cyclodextrin (βCD) containing systems for improving the dissolution profile of DIC. The interactions between DIC and βCD were studied in solution by the phase solubility method, circular dichroism, ^{1}H and ^{13}C NMR spectroscopy. DIC/βCD solid systems in equimolar ratio were prepared by different methods (physical mixing, kneading, co-evaporation, freeze-drying) and fully characterized by thermal analysis (DSC), Fourier Transform Infrared analysis (FTIR), X-ray powder diffractometry, and scanning electron microscopy (SEM). A physical mixture of DIC and βCD separately freeze-dried was also considered for comparison purpose. The influence of the physico-chemical properties of the solid systems on dissolution profiles has been highlighted and discussed.

Materials and methods

Chemicals

Diclofenac acid (DIC) and β-cyclodextrin (βCD) were kindly supplied by ICS (Milano, Italy) and SPAD (Cas-

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sano Spinola, AL, Italy), respectively. All chemicals used throughout the study were of analytical reagent grade.

Solubility studies

Solubility studies were performed according to Higuchi and Connors [10]. An excess amount of DIC (50 mg) was added to 25 ml of water or βCD aqueous solutions (from 1.0×10^{-3} to 12.5×10^{-3} M) and shaken in a sealed glass container at 25 °C until equilibrium (achieved after 7 days). An aliquot was withdrawn, filtered (filter HA-0.45 μm, Millipore) and analyzed for DIC content by spectrophotometry at the wavelength of 275 nm (PU 8700 Philips spectrophotometer). Assuming the formation of a complex with a 1:1 stoichiometric ratio, the apparent stability constant ($K_{1:1}$) was calculated from the linear graph obtained by plotting the molar concentration of DIC in the solution versus each βCD molar concentration according to the equation:

$$K_{1:1} = \text{slope}/[\text{intercept}(1\text{-slope})].$$

Each experiment was performed in triplicate; the coefficient of variation associated to each measurement was never greater than 3%.

Spectroscopic studies

Spectra were collected on solutions containing DIC (5.0×10^{-5} M) and βCD (from 1.0×10^{-3} to 1.0×10^{-2} M) in 0.1 M phosphate buffer pH 7.0 in the range 240–340 nm by a Jasco J710 spectropolarimeter. The instrument was calibrated by using (+)-10-canphosulphonic acid. A quantitative analysis of the ellipticity variation as a function of βCD concentration was performed according to the modified Scott's equation [11]:

$$\frac{[G] \cdot [CD] \cdot d}{\Delta\psi} = \frac{1}{\Delta\theta} \cdot [CD] + \frac{1}{K_{1:1}\Delta\theta},$$

where [G] is the total molar concentration of the guest, [CD] is the molar concentration of uncomplexed βCD (which can be considered equivalent to total CD concentration), $\Delta\psi$ is the difference between experimental ellipticities of the guest in the absence and presence of cyclodextrins at a definite wavelength, $\Delta\theta$ is the difference in the molar ellipticity coefficient between included and free guest, $K_{1:1}$ is the apparent stability constant and d is the path-length of the cell. For DIC/βCD system, ψ and θ of DIC are zero.

Each experiment was performed in triplicate; the coefficient of variation associated to each measurement was never greater than 3%.

NMR studies

^{1}H and ^{13}C NMR spectra were collected on a Bruker AMX500 Spectrometer at 20 ± 0.1 °C. Tetramethylsilane was used as an external reference and no correction was made for susceptibility of the capillary. Solutions containing a constant DIC concentration (2×10^{-2} M) and increasing βCD amounts (from 0 to 8×10^{-2} M) were made up in D_2O by adding 0.1 M NaOD to dissolve DIC.

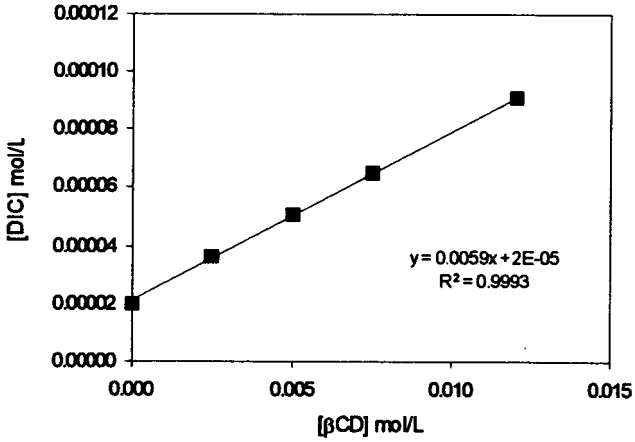

Figure 1. Phase solubility plot of diclofenac/βCD system in water.

Preparation of DIC/βCD solid systems

DIC and βCD were sieved and the corresponding 75–150 μm granulometric fraction collected. The stoichiometric ratio employed to prepare solid systems was always 1/1 (mol/mol).

For the preparation of physical mixture (PM), DIC and βCD powders were blended in an agate mortar until a homogeneous mixture was obtained. The kneaded product (KN) was prepared by wetting DIC and βCD powders in a mortar with a low amount of an ethanol/water solution (1:1 v/v). The mixture was ground thoroughly to obtain a paste and dried under vacuum at 40 °C up to constant weight. The other solid systems were prepared from solutions obtained dissolving 1 g of physical mixture in 1 L of a 0.2 g/L ammonium hydroxide aqueous solution. The co-evaporated product (CE) was obtained by evaporating the solution under vacuum at 40 °C and drying the residue up to constant weight. The freeze-dried product (FD) was prepared by freeze-drying the DIC/βCD aqueous ammonia solution in a Modulyo Edwards apparatus. A physical mixture of DIC and βCD separately freeze-dried (MSFD) was prepared too. Neither residual ammonia (Nessler's test) nor decomposition product of DIC was detected in any DIC/βCD mixture (TLC analysis in chloroform:acetone 4:1 v/v on silica precoated plates).

Differential scanning calorimetry

DSC measurements were carried out on a Mettler DSC 30 apparatus equipped with a TC II probe. Samples ranging from 10 to 15 mg were put in pierced aluminum pans and scanned at a rate of 5 °C/min. Dry nitrogen was used as purge gas.

X-Ray analysis

X-Ray powder diffraction patterns were collected on a Philips PW 3710 diffractometer in the 2–40° 2θ range. Kα radiation of Cu was generated at 40 kV and 30 mA.

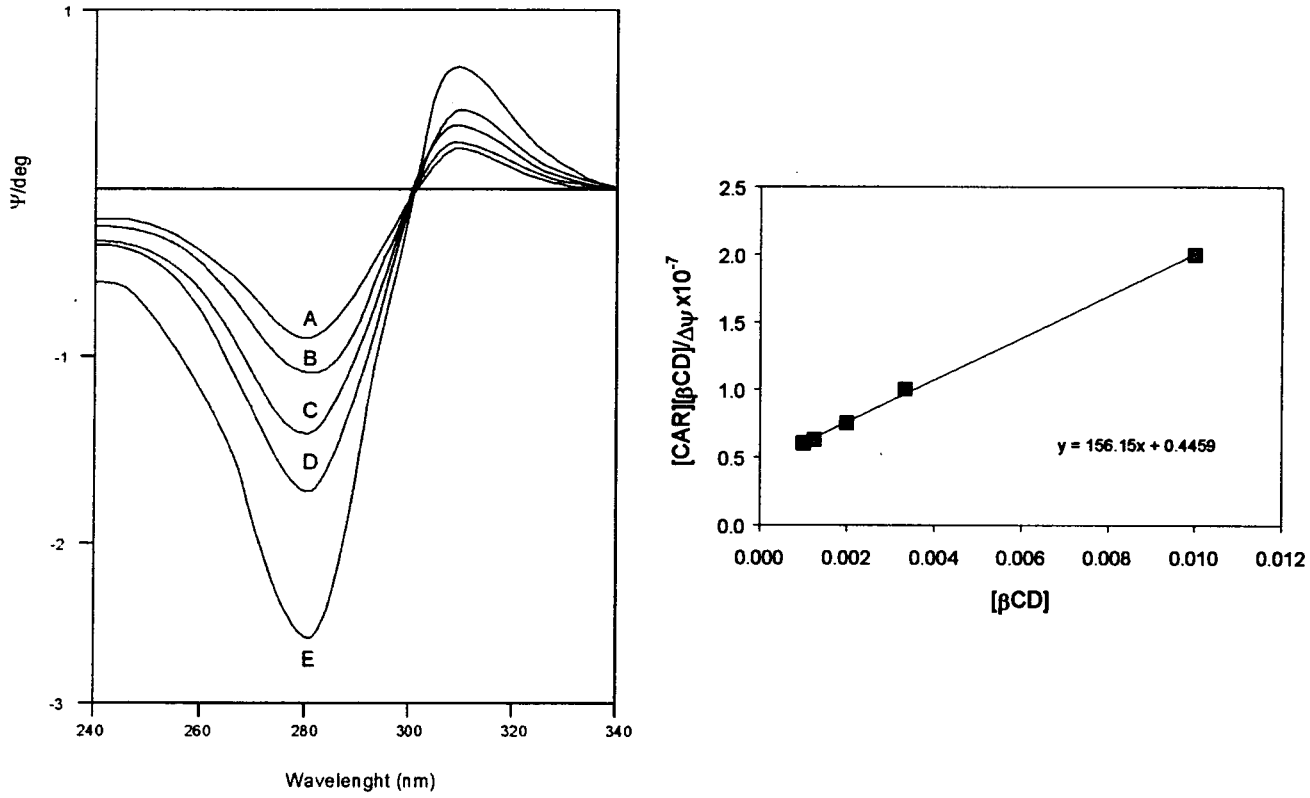

Figure 2. Left: circular dichroism spectra of diclofenac (5.0×10^{-5} M) in the presence of 1.0×10^{-3} M (A), 2.5×10^{-3} M (B), 5.0×10^{-3} M (C), 7.5×10^{-3} M (D), 1.0×10^{-2} M (E). Spectra were taken in phosphate buffer. Right: Scott's plot for diclofenac/βCD system. Ellipticity values were taken at 282 nm.

Microscopy

The morphology of physical mixture and freeze-dried products was checked by Scanning Electron Microscopy (SEM) (S-2300 Hitachi). The sample was placed on a metal stub and coated with gold under vacuum.

Infrared spectroscopy

FTIR spectra (KBr disk) were obtained on a Bruker IFS-48 apparatus applying Fourier transformation of 32 scans.

Dissolution studies

Dissolution profiles of DIC and DIC/βCD solid systems were evaluated according to the USP 24 method. Ten mg of DIC or equivalent amounts of DIC/βCD systems were added to 1 L of water at 37.0 ± 0.1 °C in a Sotax ATII apparatus. Suitable aliquots were removed at scheduled times, filtered and spectrophotometrically analyzed for DIC content at 275 nm.

Each experiment was performed in triplicate; the coefficient of variation associated to each measurement was never greater than 3%.

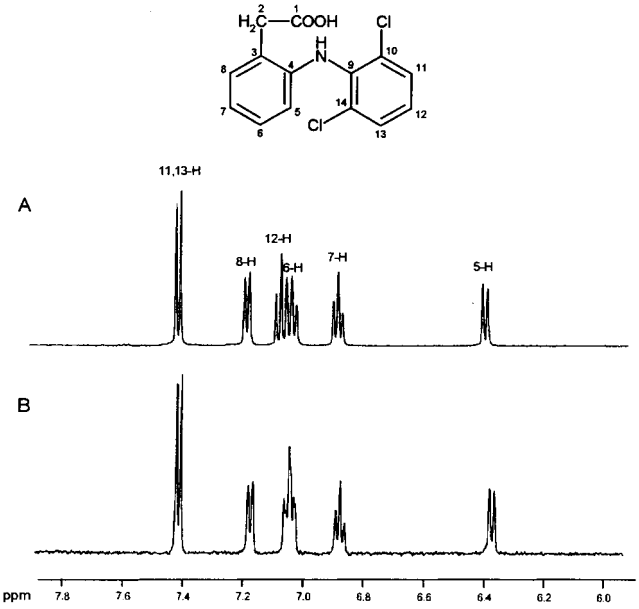

Figure 3. Effect of βCD on ^{1}H NMR spectrum of diclofenac in D_2O/NaOD: (A) diclofenac and (B) diclofenac/βCD (1:1 molar ratio). Diclofenac concentration was 0.02 M.

Results and discussion

The equilibrium phase solubility diagram of DIC/βCD system is reported in Figure 1. The increase in βCD molar concentration resulted in a linear increase of DIC solubility, giving an AL type trend [10]. The apparent stability constant was 295 M^{-1} as calculated from the regression equation reported in Figure 1, and assuming the formation of a complex with a 1:1 stoichiometry.

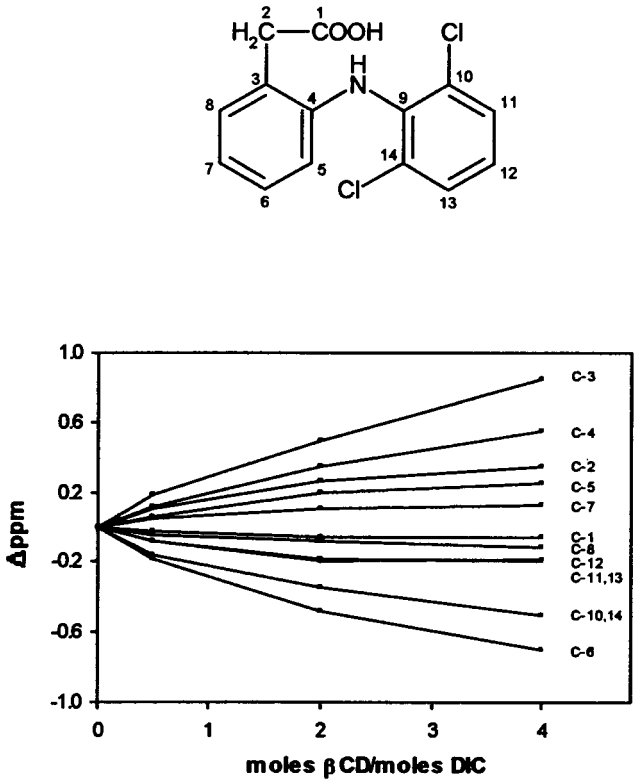

Figure 4. Diclofenac ^{13}C chemical shifts *versus* βCD concentration reported according to mole ratio method. Diclofenac was dissolved in D_2O containing a minimum amount of NaOD (diclofenac concentration was 0.02 M).

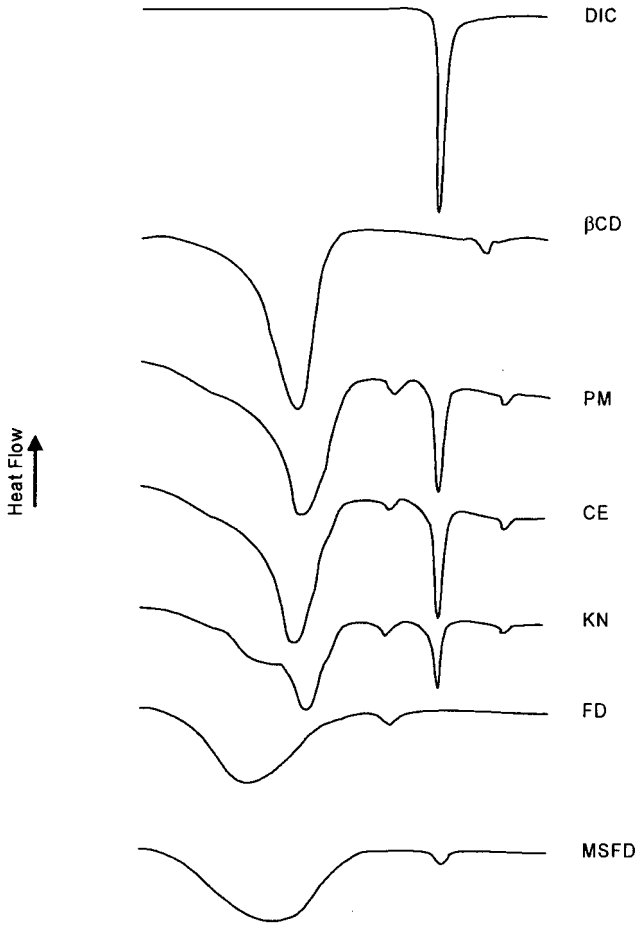

Figure 5. DSC thermograms of diclofenac (DIC), β-cyclodextrin (βCD) and diclofenac/βCD systems. Key: physical mixture (PM); co-evaporated (CE); kneaded (KN); freeze-dried (FD); mixture of diclofenac and βCD separately freeze-dried (MSFD).

Figure 2 shows the effect of different amounts of βCD on the dichroism spectrum of DIC. It is well known that complexes formed between chiral non-absorbing cyclodextrins and non-chiral light-absorbing guests, such as DIC, can induce Cotton effects on the dichroic spectrum. These effects can be referred mainly to the optical activity of the guest molecule induced by inclusion of its cromophore portion into the chiral cavity and partly to conformational changes of cyclodextrins [12]. The spectra of DIC-containing solutions were recorded in the region corresponding to its long-wavelength isotropic absorption bands. The curve of DIC in water containing βCD showed both a negative band at 281 nm and a small positive band at 307 nm. Increasing the amount of βCD strongly increased the ellipticity of both peaks. The variation of the molar ellipticity of the negative peak as a function of βCD concentration was analyzed quantitatively applying Scott's equation (Figure 2). The linearity of the plot was consistent with the hypothesis of a complexation reaction with a 1:1 stoichiometry. The apparent stability constant, calculated according to the equation reported in the experimental section, was 351 M^{-1}, in good agreement with the value determined by the phase solubility method.

Complementary information on the complex was gained by studying DIC/βCD interaction by NMR. Figure 3 shows the effect of βCD on the ^1H NMR spectrum of DIC. The absence of new peaks indicates that complexation is a dynamic process involving fast exchange (relative to the nuclear magnetic resonance timescale) between complexed and free state of the drug. In the presence of βCD, 11,13-H and 12-H protons were slightly shifted upfield giving chemical shifts of about 0.03 ppm, whereas 5-H and 6-H gave downfield shifts, suggesting that both phenyl rings of DIC are involved in complex formation within cyclodextrin cavity. Similar 1H NMR shifts have been reported in the literature [7, 13–14]. However, the multiplicity of ^1H signals often makes very difficult and sometimes impossible a quantitative evaluation of chemical shift changes. Thus, ^{13}C NMR spectroscopy can provide supplementary information on the environment of individual carbons and intermolecular interactions. In Figure 4, the ^{13}C chemical shift values are plotted as a function of host/guest ratio according to the mole ratio method [15]. The negative sign of Δ ppm indicates an upfield shift whereas the positive sign a downfield shift. The C-1 carboxylate anion of DIC did not show any significant shift, indicating that this function is not directly involved in the inclusion within cyclodextrin cavity. In the presence of βCD, the aliphatic C-2 showed a very pronounced downfield shift with respect to C-3 and C-4. A significant upfield shift was shown by C-10 and C-14, namely by carbons bearing Cl atoms, although the more pronounced effect was evident on C-6. These results suggest that the complexation of DIC within βCD cavity

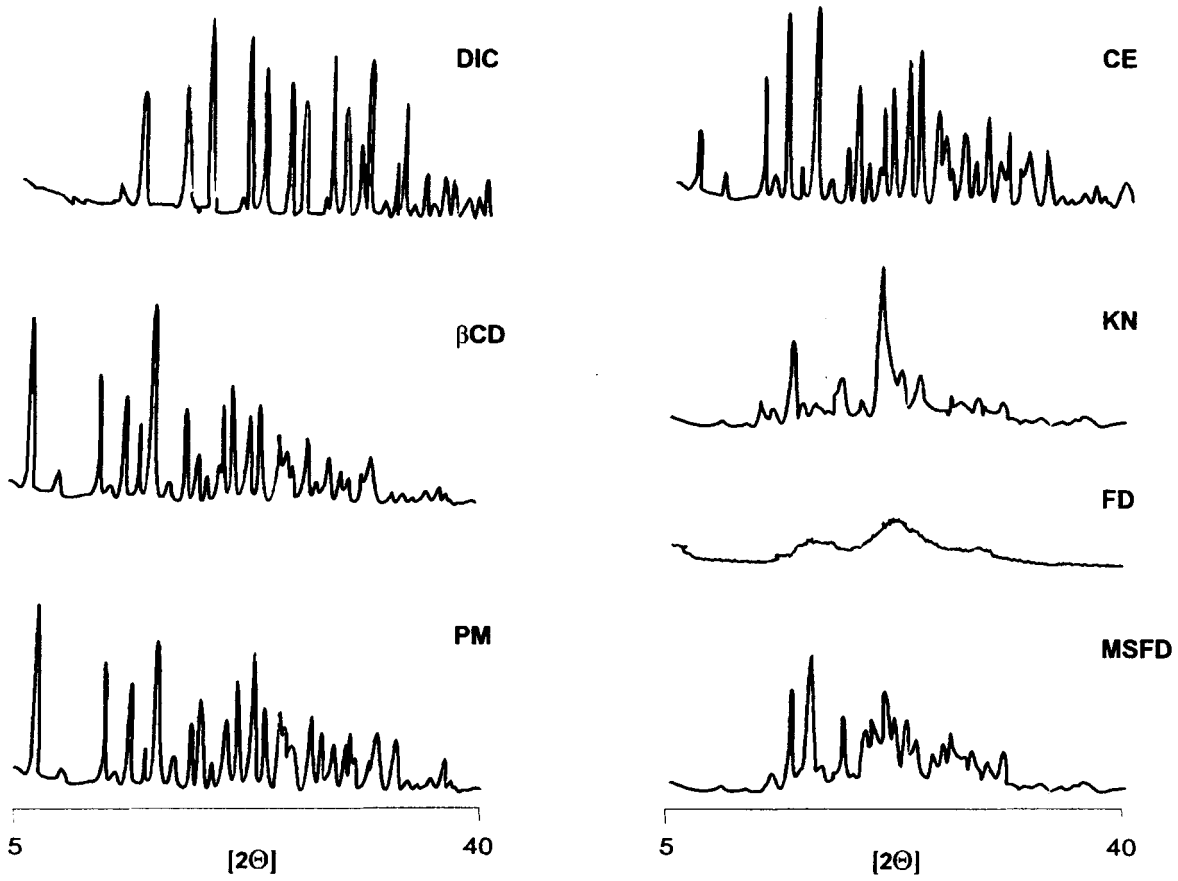

Figure 6. X-ray powder diffraction patterns of diclofenac (DIC), β-cyclodextrin (βCD) and diclofenac/βCD systems. Key: physical mixture (PM); co-evaporated (CE); kneaded (KN); freeze-dried (FD); mixture of DIC and βCD separately freeze-dried (MSFD).

involves both the aromatic rings of DIC and confirms the hypothesis that multiple equilibria exist in solution [14].

The studies in solution indicate that DIC forms a complex with βCD having an apparent stability constant falling in the range 200–5000 M^{-1}, considered as adequate to achieve an inclusion complex in the solid state [16]. Thus, we prepared drug/βCD solid systems by different techniques and characterized their physico-chemical properties. DIC/βCD solid systems were prepared, besides physical mixing, by kneading, co-evaporation and freeze-drying, i.e. the techniques reported to be effective to give drug/βCD complexes in the solid state [17].

The thermal profiles of DIC, βCD and their mixtures are reported in Figure 5. As can be seen, DIC showed a fusion endothermic peak at 178 °C (ΔH = 196 J/g) whereas βCD shows the typical dehydration peak at about 100 °C. The thermograms of the physical mixture, coevaporated and kneaded products showed the melting peak of DIC and the dehydration peak of βCD although the enthalpy value of DIC peak is lower than pure crystalline DIC. On the contrary, the melting peak of DIC disappeared in the freeze-dried product and the dehydration peak of βCD was broader and shifted to a lower temperature. These results indicated a partial loss of crystallinity of DIC in the binary systems – a typical consequence of the modification of pre-existing hydrogen bonds of crystalline drug and the occurrence of interactions with βCD. In order to elucidate whether the complete amorphization of FD could be attributed to lyophilization process, freeze-dried DIC and a physical mixture of DIC and βCD separately freeze-dried was also investigated. Freeze-dried DIC exhibited an endotherm at the same temperature of crystalline DIC although the enthalpy of fusion was slightly decreased (ΔH = 142 J/g) (data not shown). The thermogram of DIC and βCD separately freeze-dried (MSFD) showed a small endotherm at DIC melting temperature together with a broadening of βCD dehydration peak. These results indicate that the solid state properties of DIC are not substantially affected by freeze-drying procedure. Similar results have been observed for ibuprofen and ketoprofen [18].

The X-ray powder diffraction patterns of DIC, βCD and their mixtures (Figure 6) showed that DIC maintained its crystallinity in the physical mixture and coevaporated product. On the other hand, the kneaded product displayed an appreciable loss of crystallinity probably due to the fact that the interactions between drug molecules in the original crystalline structure are prevented upon cyclodextrin addition. The freeze-dried system was completely amorphous, as already suggested by calorimetric data. The pattern of the physical mixture of DIC and βCD separately freeze-dried clearly indicated a residual crystallinity of the binary system. Therefore, the freeze-drying procedure in itself contributes to a loss of crystallinity of the drug but not to such an extent that a complete amorphization occurs.

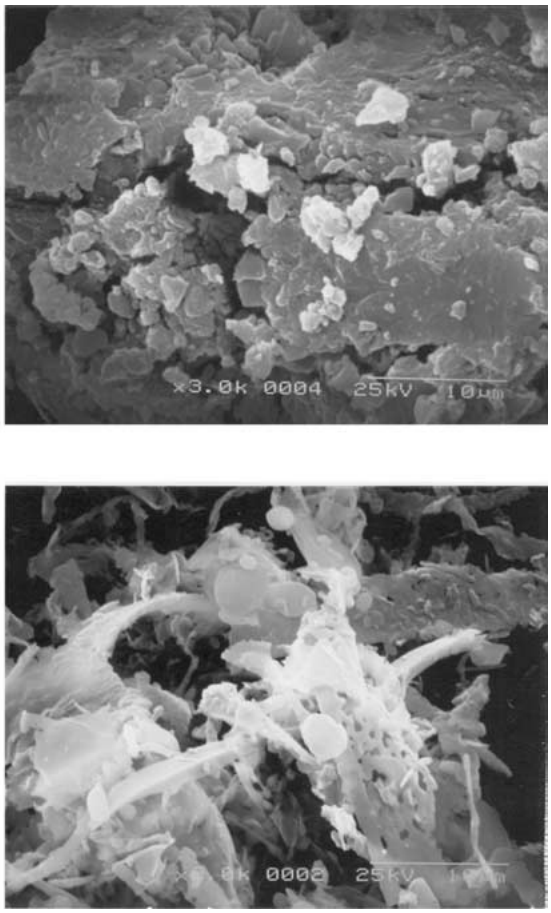

Figure 7. SEM micrographs of diclofenac/βCD powders: (A) physical mixture and (B) freeze-dried product.

The scanning electron micrographs taken on DIC/βCD systems prepared by physical mixing and freeze-drying (Figure 7) further showed that DIC/βCD physical mixture is characterized by a crystalline structure, which is lost in the freeze-dried sample. Freeze-drying process resulted also in the formation of a powder with a quite porous structure.

The FT-IR spectra collected on DIC, βCD, physical mixture, freeze-dried product and the mixture of DIC and βCD separately freeze-dried are reported in Figure 8. The spectra of coevaporated and kneaded products were superimposed to physical mixture (data not shown). The characteristic CO stretching band of DIC at 1694 cm^{-1} was evident in the physical mixture, coevaporated and kneaded products, whereas disappeared in the freeze-dried sample. This suggested that in the case of freeze-dried product a modification of the electronic environment of the CO group occurred, probably due to the achievement of a monomolecular dispersion of DIC within βCD. This hypothesis was supported by the fact that the spectrum of the mixture of DIC and βCD separately freeze-dried was superimposed to that of the physical mixture. Thus, we could infer that the interaction between DIC and βCD freeze-dried product involved CO group and was different with respect to the other solid systems, probably due to the occurrence of an inclusion complexation rather than a mere amorphization of the powder. On the basis of these results and the fact that freeze-drying can

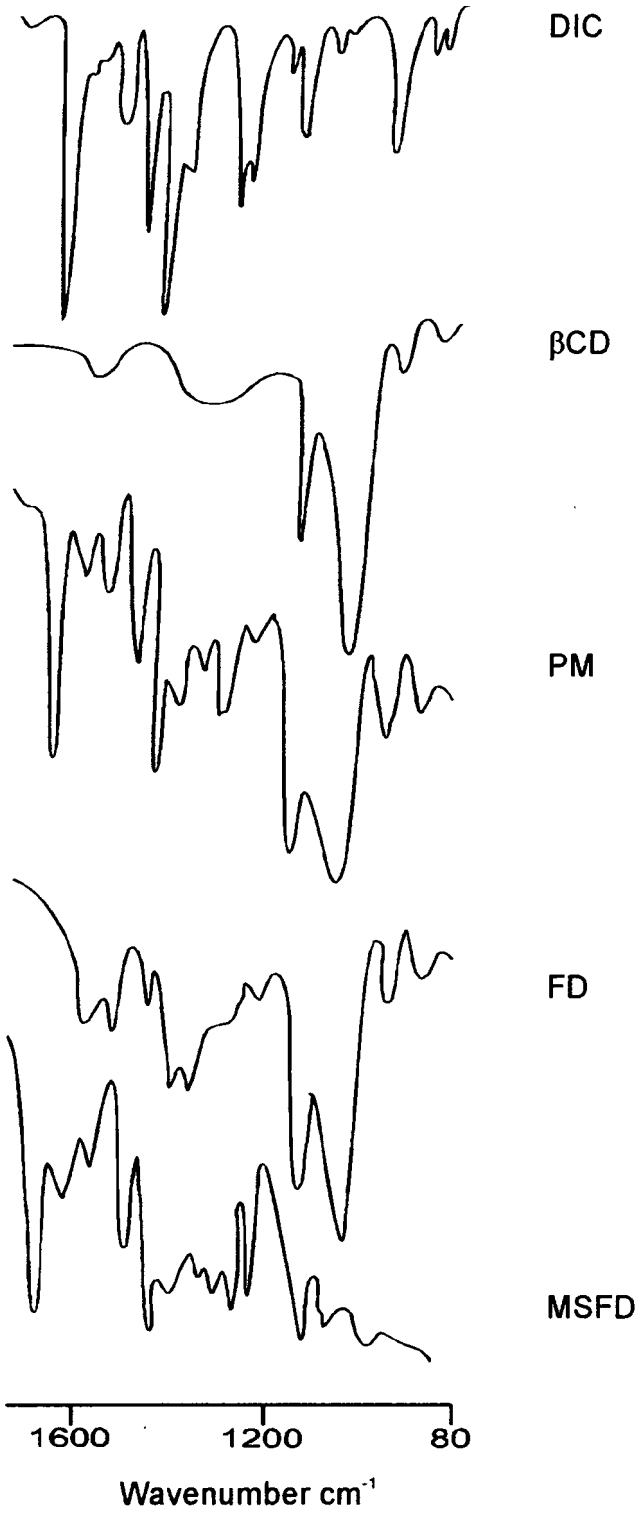

Figure 8. FTIR spectra of diclofenac (DIC); β-cyclodextrin (βCD) and diclofenac/βCD systems. Key: physical mixture (PM); freeze-dried (FD); mixture of DIC and βCD separately freeze-dried (MSFD).

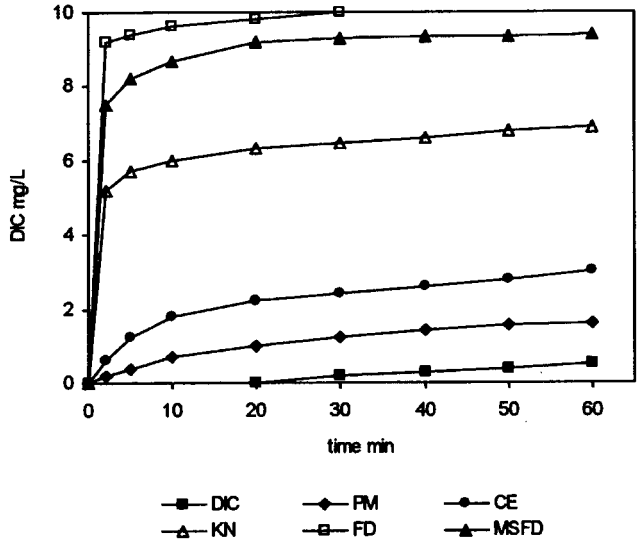

Figure 9. Dissolution profiles of diclofenac and diclofenac/β-cyclodextrin systems in water at 37 °C. Key: diclofenac (DIC); physical mixture (PM); co-evaporated (CE); kneaded (KN) freeze-dried (FD); mixture of DIC and βCD separately freeze-dried (MSFD).

allow to save in the solid state the inclusion complex existing in solution, the hypothesis that in the case of freeze-dried product an inclusion complex is formed can be reasonably formulated.

The dissolution profiles of DIC and different DIC/βCD solid systems are reported in Figure 9. At each time, DIC amounts dissolved from all the cyclodextrins-containing systems were higher than form crystalline drug. As expected, the highest dissolution performance at each time point and a quite stable supersaturation state was obtained in the case of the freeze-dried product. The dissolution curve of DIC and βCD separately freeze-dried was intermediate between the freeze-dried and co-evaporated products. Thus, the increase in DIC dissolved from all CD-containing systems was ascribed to both the capability of CD to form a soluble complex in solution and their different amorphization degree.

In conclusion, βCD can be suggested as complexing and solubilizing agent for DIC. The freeze-drying procedure allows the preparation of a rapidly dissolving form of DIC, which could be proposed as new formulation to optimize its pharmacological profile.

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