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Interaction of diclofenac sodium with β- and hydroxypropyl-β-cyclodextrin in solution

B. Pose-Vilarnovo, L. Santana-Penín, M. Echezarreta-López, M.B. Pérez-Marcos, J.L. Vila-Jato and J.J. Torres-Labandeira*

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain
**Correspondence*

The formation of inclusion compounds of diclofenac sodium with β- and hydroxypropyl-β-cyclodextrins was studied in solution by phase solubility techniques and by ^{1}H-NMR spectroscopy. Phase solubility diagrams revealed the formation of a 1/1 complex with both cyclodextrin derivatives in simulated gastric juice pH 1.2, with a similar stability constant, i.e. 100.6 M^{-1} for β-cyclodextrin and 115.8 M^{-1} for hydroxypropyl-β-cyclodextrin. The continuous variation method was utilized to elucidate the stoichiometry, determine the formation of complex with 1/1 and 1/2 drug/cyclodextrin ratio in deuterated water. Solid inclusion complexes were obtained by freeze-drying. X-ray diffractometry and differential scanning calorimetry showed differences between the complexes and their corresponding physical mixture and individual components. The solubility of diclofenac sodium increased significantly in the presence of cyclodextrins. Enhancement was better from the β-cyclodextrin systems.

La formation de complexes d'inclusion entre la β- et l'hydroxypropyle-β-cyclodextrine a été étudiée en solution par la méthode du diagramme de solubilité de phases et par spectroscopie RMN du proton. Les diagrammes de solubilité de phases révèlent la formation d'un complexe 1/1 avec les deux cyclodextrines en milieu gastrique artificiel de pH 1,2, avec des constantes de stabilité très semblables: 100,6 M^{-1} pour la β-cyclodextrine et 115,8 M^{-1} pour l'hydroxypropyl-β-cyclodextrine. La méthode par variation continue, utilisée pour élucider la stoechiométrie du complexe, a permis de mettre en évidence la formation d'un complexe 1/1 et 1/2 entre le principe actif et la cyclodextrine dans l'eau deutérée. Des complexes d'inclusion solides ont été obtenus par lyophilisation. La diffractométrie de rayons X et l'analyse enthalpique différentielle ont révélé des différences entre les complexes et les mélanges physiques correspondants ainsi que les composés pris séparément. La solubilité du diclofénac de sodium est significativement augmentée en présence de cyclodextrine. L'augmentation de solubilité est la plus forte pour les systèmes à base de β-cyclodextrine.

Keywords: Diclofenac sodium — β-cyclodextrin — Hydroxypropyl-β-cyclodextrin — Nuclear magnetic resonance — X-ray diffractometry — Differential scanning calorimetry — Dissolution properties.

Mots clefs: Diclofénac de sodium — β-cyclodextrine — Hydroxypropyl-β-cyclodextrine — Résonance magnétique nucléaire — Diffractométrie de rayons X — Analyse enthalpique différentielle — Dissolution.

Diclofenac sodium is a widely used non-steroidal anti-inflammatory and analgesic drug. It has limited water solubility, especially in gastric juice (about 15 µg/ml), and it is unstable in aqueous solution. This limited solubility in an acidic medium engenders problems in its oral bioavailability and it is a drawback in terms of its formulation in controlled release devices.

Cyclodextrins form inclusion complexes with a variety of guest drugs, increasing their solubility and dissolution rate [1, 2], bioavailability [3, 4] and stability [5, 6]. The ability of diclofenac sodium to form inclusion complexes with same cyclodextrin derivatives has been studied. The complexation was found to improve the solubility [7-10], stability [8] and *in vitro* corneal permeability [11] of the drug.

The aim of this study was to improve the solubility of diclofenac sodium in artificial gastric juice pH 1.2 by its complexation with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. The molecular association between diclofenac sodium and both cyclodextrins and the stoichiometry of the complex formed were examined by ^{1}H-NMR studies. Freeze-drying was employed for the preparation of diclofenac sodium/cyclodextrin solid inclusion compounds. X-ray diffractometry and differential scanning calorimetry were used to characterize the systems prepared. The influence of complexation on drug dissolution behaviour was also analysed.

I. EXPERIMENTAL

1. Materials

Diclofenac sodium (2-[(2,6-dichlophenyl) amino] benzeneacetic acid monosodium salt) was purchased from Sigma Chemical Co. (St. Louis, MO, United States), β-cyclodextrin and hydroxypropyl-β-cyclodextrin were generously supplied by Roquette-Laisa España (Spain) and Janssen Pharmaceutical (Belgium), respectively. All other reagents were of analytical grade.

2. Phase solubility diagrams

Solubility diagrams were obtained according to Higuchi and Connors [12] in gastric juice of pH 1.2. The apparent stability constant of the diclofenac/β-cyclodextrin and diclofenac/hydroxypropyl-β-cyclodextrin complexes, assuming 1/1 stoichiometry, were calculated from the slope of the initial straight portion of the solubility diagram.

3. ^{1}H-NMR

Nuclear magnetic resonance spectra were recorded on a Brucker WN 300 spectrometer (Brucker Anal.) at 25°C. Samples were dissolved in deuterated water. The internal reference was a peak due to small amounts of DHO and H_2O present as impurities (assigned a value of $\delta = 4.6$ ppm).

4. Preparation of physical mixtures

The physical mixtures of an appropiate amount of diclofenac/β-cyclodextrin and diclofenac/hydroxypropyl-β-cyclodextrin in the 1/1 molar ratios were obtained by pulverizing and subsequent mixing in a Turbula T2C mixer (5 min at 30 r/min).

5. Preparation of the inclusion complexes

The solid inclusion complexes of diclofenac with β-cyclodextrin and hydroxypropyl-β-cyclodextrin (1/1 mol/mol) were prepared using the freeze-drying method. Both components were dissolved in 0.2 N aqueous ammonium hydroxide. The solution was filtered (0.45 µm) and frozen by immersion in liquid nitrogen. Freeze-drying was completed in 48 h in a Lyph-lock 6 apparatus (Labconco).

6. Characterization of the solid state inclusion complexes

6.1 Thermal analysis

Differential scanning calorimetry was performed on a Shimadzu DSC-50 system with a DSC equipped with a computerized data station TA-5 WS/PC. General conditions: scanning rate 10°C/min^{-1}, scanning temperature range 50 to 250°C.

6.2 X-ray

X-ray powder diffraction patterns were recorded on a Philips X-ray diffractometer (PW-1710 BASED) using Cu-K_α radiation.

7. Dissolution studies

In vitro dissolution studies of pure drug, physical mixtures and the inclusion complexes were carried out by placing the corresponding amount of product in a hard-shell colourless gelatin capsule in simulated gastric fluid (USP XXIII). The capsule was placed in a stainless steel cylinder to avoid its flotation. Powdered samples containing 50 mg of diclofenac or its equivalent in complexed or physically mixed form in the gelatin capsule were placed in 900 ml of the dissolution medium in a beaker at 37°C for 180 min and shaken at 500 r/min. At predetermined time intervals, samples were taken for spectrophotometric determination of diclofenac concentration ($\lambda = 276$ nm, $E_{1\%,\,1\,cm} = 283.85$) following filtration. All samples were analysed in triplicate. Dissolution efficiencies after 180 min (DE_{180}) were calculated according to Khan [13]. The effects of drug formulation on dissolution efficiency at each pH were investigated by one-way analysis of variance with the Student-Newman-Keuls test for multiple comparisons.

II. RESULTS AND DISCUSSION

1. Phase solubility diagrams

Complex formation of diclofenac with β-cyclodextrin and hydroxypropyl-β-cyclodextrin were studied by a solubility method. *Figure 1* shows the equilibrium phase solubility diagrams obtained for the diclofenac/β-cyclodextrin and diclofenac/hydroxypropyl-β-cyclodextrin in artificial gastric juice without enzymes (USP 23). Both diagrams can be classified as the A_L type according to Higuchi and Connors [12]. This indicates that, within the cyclodextrin concentration range tested, a soluble complex is formed. On the other hand, because both straight lines have a slope less than unity, it was assumed that the increase in solubility was due to the formation of a 1/1 mol/mol complex. The apparent stability constant, K, was calculated according to *equation 1*:

$$K = \text{Slope}/S_0\,(1 - \text{slope}) \qquad \textbf{Eq. 1}$$

where S_0 is the solubility of diclofenac in the absence of cyclodextrins. The obtained values were 100.6 M^{-1} for β-cyclodextrin and 115.8 M^{-1} for hydroxypropyl-β-cyclodextrin, which indicates a similar interaction between the drug and both cyclodextrin derivatives in the conditions used in the study.

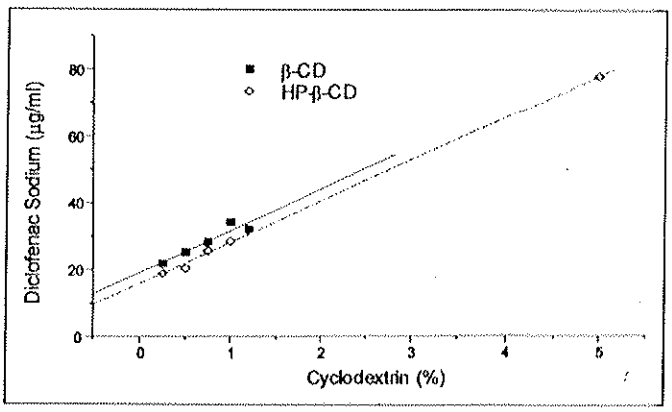

Figure 1 - Solubility diagrams. Plot of diclofenac concentration versus cyclodextrin concentration.

2. ^{1}H-NMR studies

The spectrum for diclofenac, diclofenac/β-cyclodextrin and diclofenac/hydroxypropyl-β-cyclodextrin in a 1/1 molar ration are shown in *figure 2*. The assignments for the different protons were made based on chemical shifts [14]. Information about the interaction of diclofenac with cyclodextrins from nuclear magnetic resonance (NMR) is primarily inferred from the changes in chemical shifts and line shape. Under the present conditions, only the shifts of the signals were observed. No new peaks are present in the spectra of diclofenac/cyclodextrin mixtures. This observation implies that complexation is a dynamic process, the included drug is in a fast exchange (relative to the nuclear magnetic resonance timescale) between the free and bound states [15].

The insertion of a guest molecule into the cyclodextrin cavity is clearly reflected by changes in ^{1}H-NMR chemical shift values [16]. The effects of β-cyclodextrin and hydroxypropyl-β-cyclodextrin at different concentrations on the ^{1}H-NMR spectrum of diclofenac are shown in *figures 3 and 4*, respectively. Only protons H3' and H5' shifted significantly downfield with increasing cyclodextrin concentrations. Chemical shifts are

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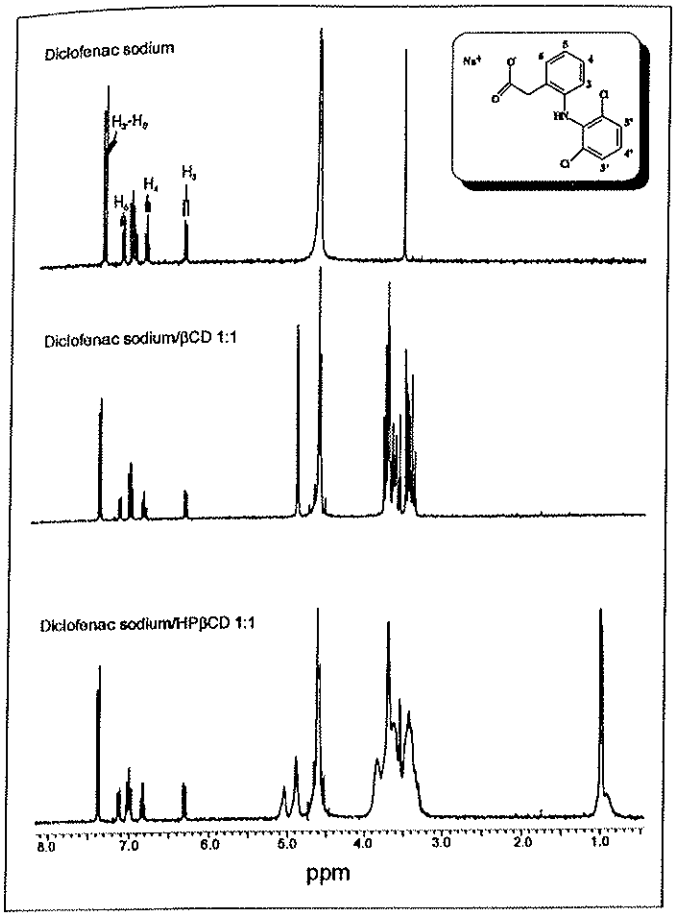

Figure 2 - Nuclear magnetic resonance spectra of the products indicated.

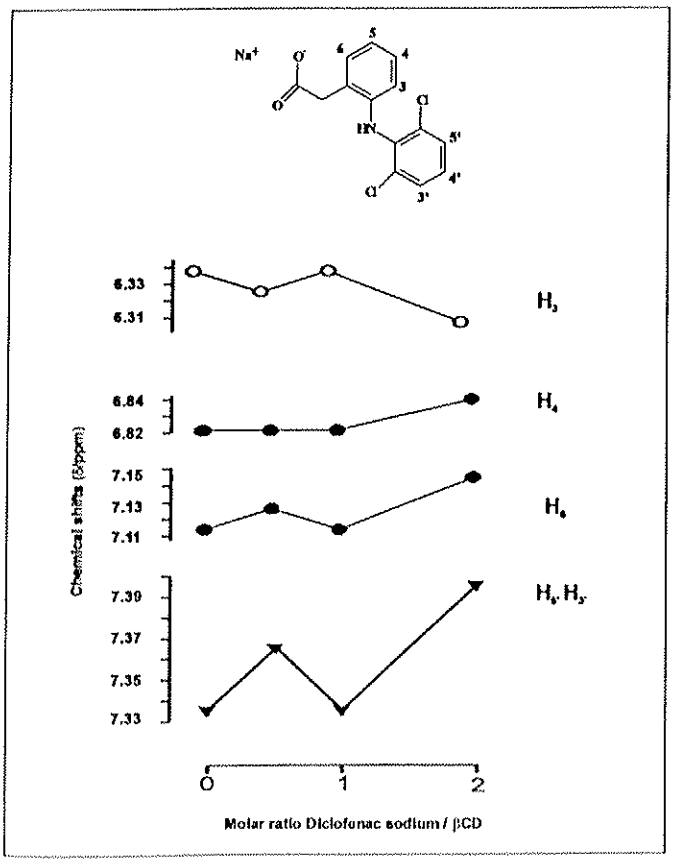

Figure 3 - Chemical shifts of protons of diclofenac in solutions varying in β-cyclodextrin concentration.

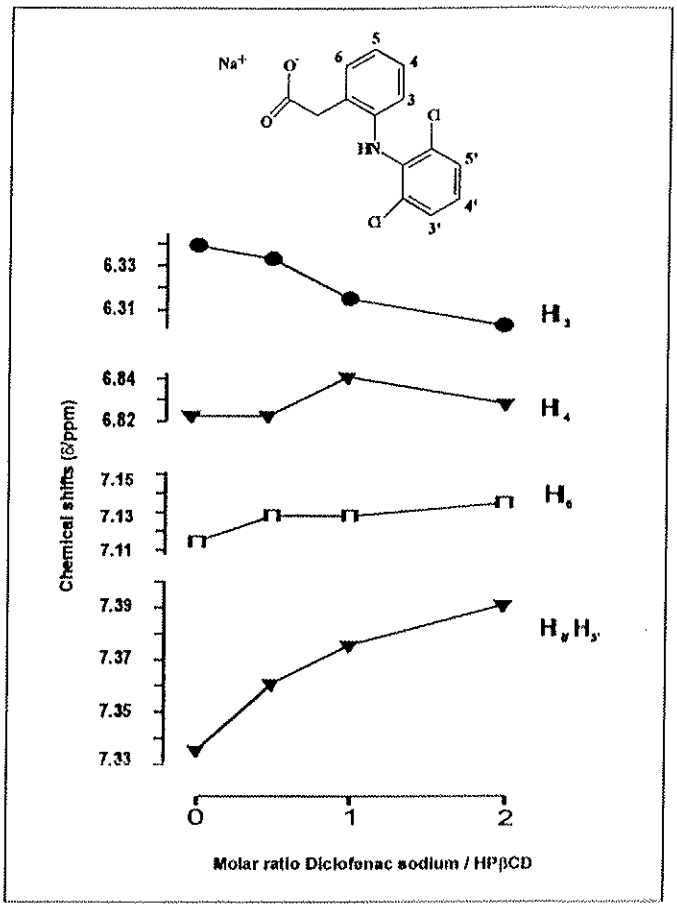

Figure 4 - Chemical shifts of protons of diclofenac in solutions varying in hydroxypropyl-β-cyclodextrin concentration.

quite important even in a 1/1 molar ratio. This suggests that this part interacts with the cyclodextrin cavity and is encapsulated in the molecule. On the other hand, proton H3 shows upfield shifts. This effect can be attributed to the interaction of that part of the molecule with the edge of the cyclodextrin cavity establishing hydrogen bonds with the hydroxyl groups, and justified the biggest changes found with the cyclodextrin derivative. H4 and H6 do not show changes downfield up to a molar ratio of 1/2.

The angled conformation of the two aromatic groups of diclofenac have been described with a torsional angle of 69 degrees [17], and therefore the existence of a complex with the whole molecule in the cyclodextrin cavity is impossible. The conformation carried out using the «minimize utility» of CS Chem3D Pro (MOPAC-MNDO) gives a structure of diclofenac sodium as shown in *figure 5*, with an angle C1'-N-C2 of 128∫. This conformation may allows the formation of 1/1 drug/cyclodextrin complex in which the 2,6-dichlophenyl group is inside the cavity, or a 1/2 with both rings complexed (*figure 5*).

In order to confirm the above NMR results, continuous variation plots [18] were constructed from proton shift data. The total concentration of cyclodextrin and diclofenac in the experiments was kept constant (10.5 mM with β-cyclodextrin and 9.0 mM with hydroxypropyl-β-cyclodextrin), the ratio r (r=[diclofenac]/([diclofenac]+[cyclodextrin])) being varied from 0.2 to 0.8. Plots of the observed $\Delta\delta \cdot [\text{diclofenac sodium}]_t$

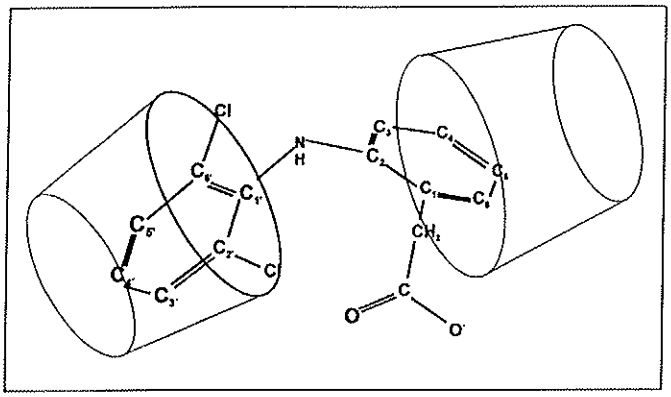

Figure 5 - Structure of diclofenac /β-cyclodextrin inclusion complexes in aqueous solution concluded from ^{1}H-NMR studies.

as a function of r leads to the Job plots presented in *figures 6 and 7* for β-cyclodextrin and hydroxypropyl-β-cyclodextrin, respectively.

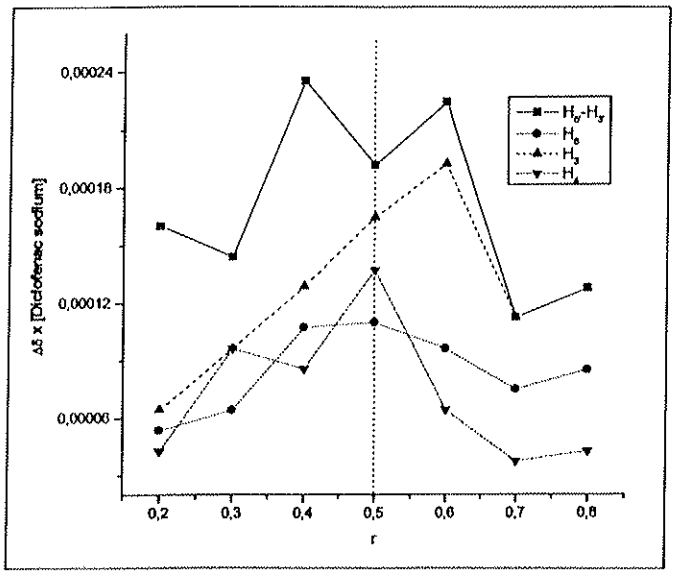

Figure 6 - Continuous variation plot for protons of diclofenac in the presence of different relative concentration of β-cyclodextrin.

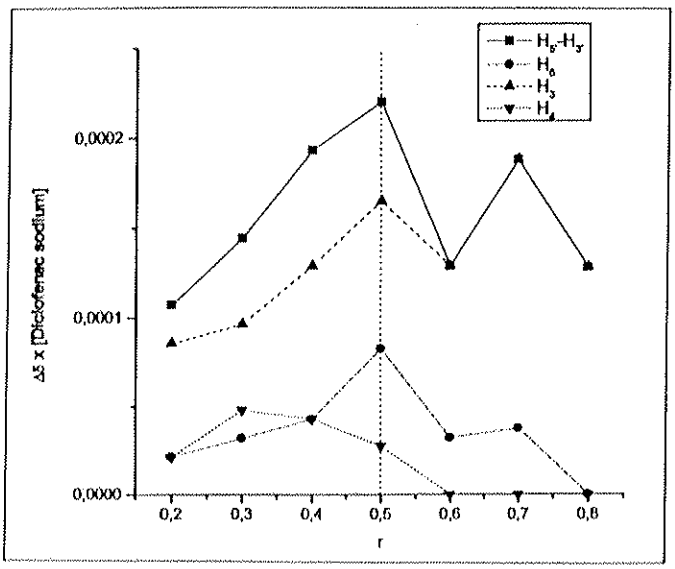

Figure 7 - Continuous variation plot for protons of Diclofenac in the presence of different relative concentration of hydroxypropyl-β-cyclodextrin.

The protons used in the analysis where H3, H3'–H5', H6 and H4. The Job plots indicate that complex stoichiometry is predominantly 1/1; however, slight skewing of the curves for some of the protons to the left of r = 0.5 suggests the presence of 1/2 complexes (r 0.4 to 0.5). Similar results were described by other authors in diclofenac/β-cyclodextrin inclusion complex [19].

3. Preparation and characterization of solid inclusion complexes

Solid inclusion complexes diclofenac/cyclodextrin molar ratio of 1/1 were prepared by the freeze-drying method.

Figure 8 shows the powder X-ray diffraction pattern of the complexes prepared in comparison with that of a physical mixture at the same molar ratio. The diffraction patterns of the physical mixtures were found to be simple superposition of those of the drug and cyclodextrins, while the complex was apparently different. The inclusion complexes are markedly less crystalline than either the physical mixture or the individual components, corresponding to a new solid phase. This is more evident in the hydroxypropyl-β-cyclodextrin system, because of the amorphous characteristics of the hydroxypropyl derivative.

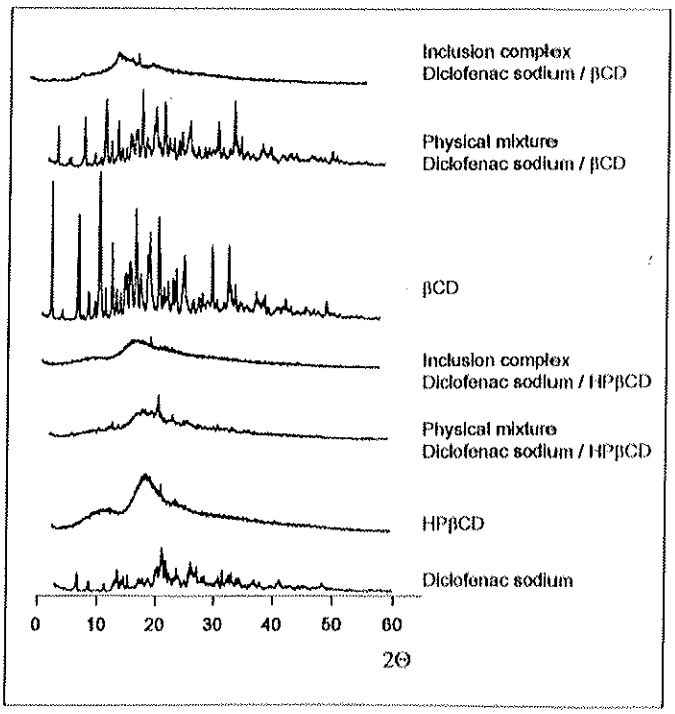

Figure 8 - X-ray diffractograms corresponding to the indicated products.

Figure 9 illustrates the differential scanning calorimetry thermograms of the preparations. The drug does not have any significant peak in the temperature range analysed because the melting and decomposition point is 283 to 285°C. The peaks corresponding to the evaporation of water appeared in the range of 50 to 150°C. Those peaks decrease and even disappear in the case of inclusion complexes [20].

These results indicate that inclusion of the drug within the cyclodextrin cavity can be achieved by a freeze-drying process.

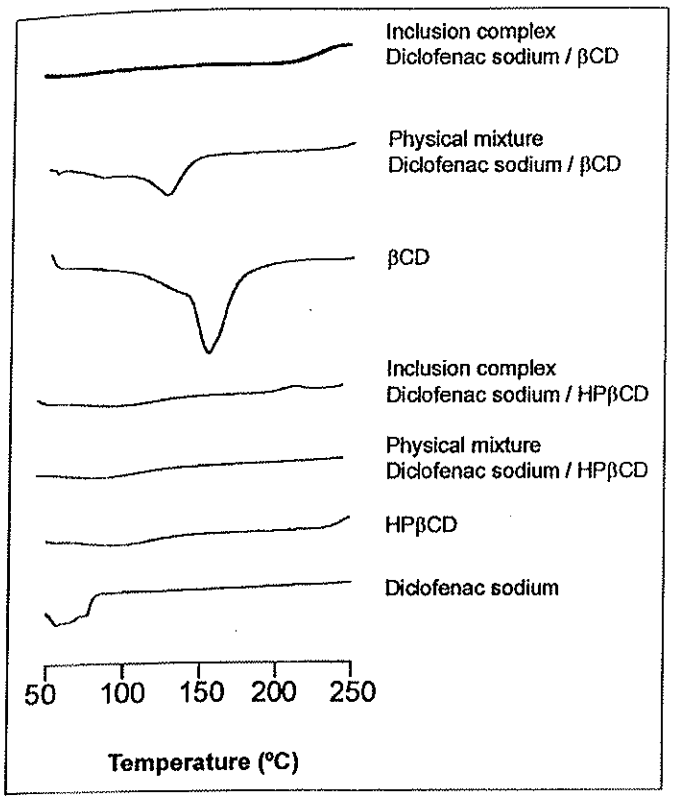

Figure 9 - Differential scanning calorimetry curves corresponding to the indicated products.

4. Effects of complexation on the dissolution behaviour of the drug

Figures 10 and 11 show the dissolution profiles of diclofenac, physical mixture and inclusion complexes in artificial gastric juice.

One-way analysis of variance indicates that the factor

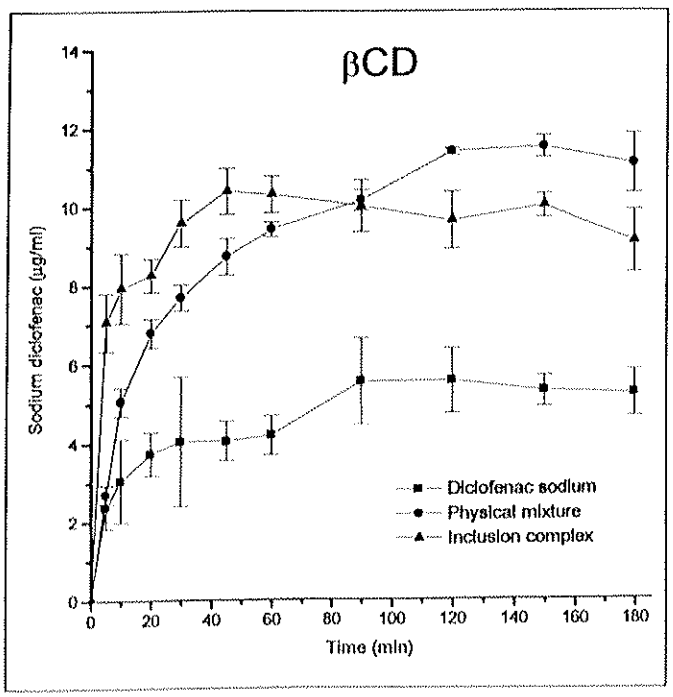

Figure 10 - Dissolution profiles of diclofenac and its β-cyclodextrin systems in artificial gastric juice without enzymes.

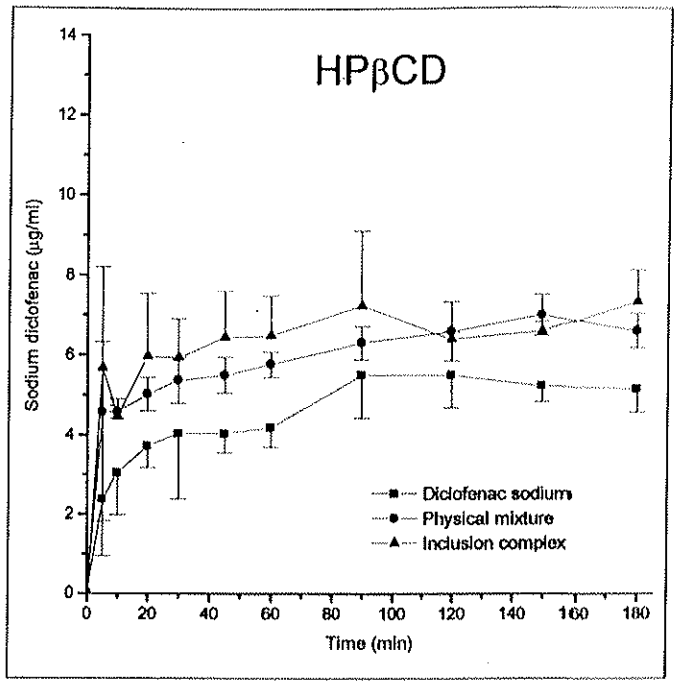

Figure 11 - Dissolution profiles of diclofenac and its hydroxypropyl-β-cyclodextrin systems in artificial gastric juice without enzymes.

formulation has a significant effect on 0 to 180 min dissolution efficiency ($F_{4,10} = 81.9, \alpha < 0.01$). The Student-Newman-Keuls test for pairwise multiple comparison grouped the preparations in the following order (from lowest to highest DE_{180}):

diclofenac<PM-HPβCD<FD-HPβCD<PM-BCD<FD-BCD

where PM-HPβCD: physical mixture diclofenac/hydroxypropyl-β-cyclodextrin; PM-BCD: physical mixture diclofenac/β-cyclodextrin; FD-HPβCD: freeze-dried diclofenac/hydroxypropyl-β-cyclodextrin complex; FD-BCD: freeze-dried diclofenac/β-cyclodextrin complex

Therefore, the presence of cyclodextrin in the system increases the dissolution properties of the drug. This effect is higher with β-cyclodextrin despite the fact that the stability constant calculated from the solubility diagram is slightly smaller for the natural cyclodextrin. However, similar results were found with a physical mixture and the inclusion complex.

In conclusion, it can be stated that diclofenac can form inclusion complexes with β- and hydroxypropyl-β-cyclodextrin. Solubility and NMR study evidence that the stoichiometry of those complexes in solution is 1/1 mol/mol. Solid inclusion complexes were prepared by a freeze-drying method. Differential scanning calorimetry and X-ray diffraction showed the interaction of both cyclodextrins with diclofenac and confirm the formation of a new specie. The presence of cyclodextrins, either as a physical mixture or an inclusion complex, improves the dissolution of diclofenac in artificial gastric juice. This effect has been found higher when the systems were prepared with β-cyclodextrin.

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