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Interactions of oxyphenbutazone with different cyclodextrins in aqueous medium and in the solid state

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Abstract

The interactions between a nonsteroidal anti-inflammatory drugs, oxyphenbutazone (OPB), with two cyclodextrins, β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), have been studied in an aqueous medium and in the solid state. Differential scanning calorimetry, hot stage microscopy, thermogravimetric analysis and X-ray diffraction (XRD) powder have been the techniques used to characterize the interactions in the solid state. Although OPB forms inclusion compounds with β- and γ-CD in the aqueous medium, only the OPB/γ-CD inclusion compound was obtained in the solid state by the kneading method. The XRD powder used at different temperatures has proven be a useful tool in characterizing the behaviour of these binary systems.

Keywords: Oxyphenbutazone; β-Cyclodextrin; γ-Cyclodextrin; Differential scanning calorimetry; Hot stage microscopy; Thermogravimetry; X-ray diffraction powder

1. Introduction

To improve the bioavailability of poorly water soluble drugs when these are included in solid dosage forms of oral administration, it is possible to use different strategies of formulation such as formation of solid dispersions with hydrophilic carriers such as polyethyleneglycol 6000 [1], incorporation of biocompatible surfactants as sodium dodecyl sulphate or polysorbate 80 [2], or formation of inclusion compounds with cyclodextrins [3,4].

Cyclodextrins, are cyclic molecules constituted by 6, 7 or 8 glucose units forming the α-, β- and γ-CD, respectively. They are able to include in their apolar cavity some substances with an adequate size and molecular shape.

The molecules entrapped in the cyclodextrin cavity can modify some of their physico-chemical parameters such as solubility, melting temperature, decomposition process, etc. [5].

The nonsteroidal anti-inflammatory drugs (NSAD) constitute nowadays a therapeutic group, which are largely prescribed for patients with inflammatory conditions. They are used for chronic pain, rheumatoid arthritis and osteoarthritis. There are numerous papers about the formation of anti-inflammatory drug/cyclodextrin

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inclusion compounds as naproxen [6], indomethacin [7], etc. Some of these complexes are even on the market.

Oxyphenbutazone (OPB) is the active metabolite of phenylbutazone, a potent NSAD that has been shown to form inclusion complexes with different cyclodextrins in aqueous media [8] but there are no data about the isolation and characterization of these inclusion compounds in solid state.

Thermal behaviour of inclusion compounds can be drastically different which is shown by the corresponding physical mixtures. For this reason, the aim of this paper is to study the possible interactions between OPB and different cyclodextrins in the solid state by using analytical techniques (differential scanning calorimetry (DSC), hot stage microscopy (HSM), thermogravimetry and X-ray diffraction (XRD) powder), by which, we study the evolution of the samples during heating. Two natural cyclodextrins, β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) have been chosen in order to know the influence of the cyclodextrin apolar cavity size in the isolation of inclusion compounds in the solid state.

2. Experimental

2.1. Materials

OPB and β-CD were purchased from Sigma (St. Louis, MO).

γ-CD was a gift from Wacker-Química Ibérica (Barcelona, Spain).

2.2. Methods

2.2.1. Phase solubility study

A phase solubility study was performed according to the method reported by Higuchi and Connors [9]. An excess (5 mg) of OPB was weighed out into a series of test tubes. A constant volume of demineralized water or aqueous solutions of cyclodextrin containing increasing concentrations of β-CD and γ-CD (0.002–0.020 M) was added to each test tube. Test tubes were then closed and placed in an oscillating water bath and solutions were brought to solubility equilibrium at room temperature (22 °C), with constant shaking for 5 days. After attainment of equilibrium, the contents of the test tubes were filtered through Millipore cellulose filters (0.45 μm). The drug concentration in the filtered solutions was determined from the absorbance at 262 nm using a Beckman DU-7 spectrophotometer, and three replicates have been made for each assay. To nullify the absorbance due to the presence of cyclodextrins, the apparatus was calibrated with the corresponding blank for every assay.

2.2.2. Preparation of binary systems

Two types of binary systems were prepared: physical mixtures and kneaded systems. In both physical mixtures and kneaded systems, drug–cyclodextrin ratios were 1:1 and 1:2 mol/mol. Physical mixtures were prepared by homogeneus intermingling of previously sieved and weighed drug and cyclodextrin in a suitable container. Kneaded systems were prepared from physical mixtures by adding a small volume of distilled water. After moistening, the resultant systems were kneaded to produce a homogeneus dispersion. Once a homogeneus slurry was obtained, samples were dried at 40 °C for 24 h. All of the dried systems were crushed and sieved, and fractions smaller than 100 μm were collected for further study.

2.2.3. Hot stage microscopy

About 1 mg of sample was placed on a microscopic slide with cover and heated at a rate of 2 °C/min on a Kofler stage and samples were studied between 30 and 350 °C. Microscopic examinations were carried out by using a Thermogalen microscope fitted with the Kofler stage.

2.2.4. Differential scanning calorimetry

DSC curves of the pure materials and all binary systems were recorded on a Mettler TA 3000 differential scanning calorimeter (model DSC 20). About 5–10 mg of sample were placed in a pinholed aluminium sample pan with lid and heated in atmospheric air at a rate of 10 °C/min between 30 and 350 °C. The instrument was periodically calibrated with a standard sample of indium.

2.2.5. Thermogravimetric analysis

Thermogravimetric analyses were carried out using a Mettler TA 3000 thermogravimetric analyzer (TG-50). Samples (10 mg) were placed in a crucible of alumina and heated at a rate of 10 °C/min between 30 and 600 °C.

2.2.6. X-ray diffraction analysis

The X-ray measurement system consisted of an automatic powder diffractometer (Philips X'Pert MPD) with a Cu tube with $\lambda = 1.54056$ Å combined with a high temperature chamber (Anton-Paar HTK 10) with a Pt heating filament.

X-ray patterns were obtained at 25, 100, 150 and 200 °C with a heating rate of 10 °C/min. Before each measurement, the sample was maintained at the given temperature for 15 min.

X-ray patterns were obtained in the angular range of 4–40° 2θ, with 2 s fixed time for each 0.04° step (C.A.I., DRX, U.C.M.).

3. Results and discussion

3.1. Phase solubility study

The results obtained are graphed in the phase solubility diagram (Fig. 1), where can be observed that OPB solubility increases linearly in accord with the amount of cyclodextrin added to the aqueous medium. This indicates that OPB forms soluble inclusion compounds with β- and γ-CD. According with the phase solubility diagram classification of Higuchi and Connors, the diagram obtained is of A_L type [9]. Rajewski and Stella [10] have described that when there is a linear increase in guest molecule solubility with increasing cyclodextrin concentration, a cyclodextrin complex of the guest results from 1:1 mol/mol interaction. According with this theory, it is possible assume that a 1:1 mol/mol OPB/CD inclusion compound was formed. The apparent stability constant can be calculated from the slope and intercept of the linear portion of the phase solubility diagram, by using the equation [11]:

$$K_{1/1} = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})}$$

The stability constants obtained were 130 and 136/M for OPB/β-CD and OPB/γ-CD inclusion compounds, respectively.

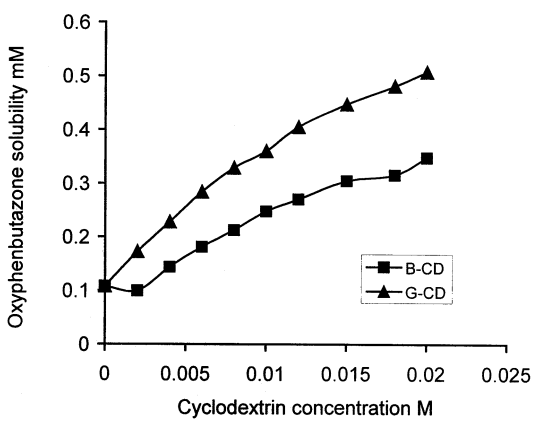

Fig. 1. OPB/cyclodextrin phase solubility diagram: (■) β-CD, (▲) γ-CD.

3.2. Hot stage microscopy

OPB sample was made up of small crystalline particles of irregular shape; they appeared coloured when they are observed with polarised light. During heating, melting occurred between 61.5 and 69.0 °C.

Under microscopic view, the β-CD sample was made up of dark, irregular-shaped and rough-surfaced particles, which became grey in polarised light. Upon heating only the melting of β-CD particles was detected (286–290 °C). The melting and decomposition of β-CD took place simultaneously, and was detected from the carbonisation of the melted particles.

The observation through the microscope of the γ-CD sample revealed that this substance was formed by irregular grey or black particles. With polarised light, coloured places in some particles can be detected; this indicates the crystalline nature of the sample. No changes were detected in the shape or appearance of the particles until the melting of the substance occurred (280–285 °C).

The samples of both OPB/β-CD (1:1 and 1:2 mol/mol) physical mixtures showed a similar thermal behaviour. Through the microscope, the particles of OPB from β-CD particles can be

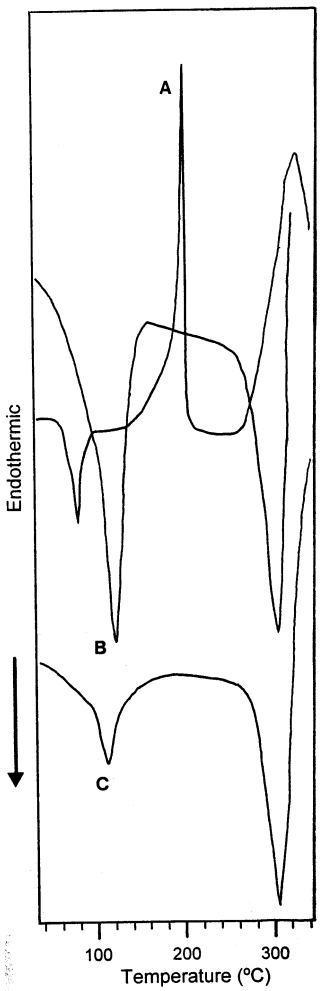

Fig. 2. DSC curves of OPB (A), β-CD (B) and γ-CD (C).

differentiated clearly due to their different morphologic appearance. In the range 61.5–69.0 °C the melting of OPB particles was produced. Afterwards, no interactions between melted OPB and β-CD particles were detected. Finally the fusion of the β-CD occurred in the 296–305 °C range.

HSM results from OPB/β-CD kneaded systems showed that samples were made up of mixed crystals formed by two different substances, which had their own thermal behaviour. When the temperature of the sample corresponded to that of the melting drug, partial fusion of mixed crystalline particles was produced. The rest of the sample remained unchanged until the β-CD melting temperature was reached. At that moment the fusion of the sample was completed.

At room temperature, in both OPB/γ-CD physical mixtures, the differentiation between particles of drug and cyclodextrin was possible because both components had a drastically different morphology. During the heating, the fusion of OPB particles was observed in the 61.5–69.0 °C range. As has already been explained in the behaviour of OPB/β-CD physical mixtures, interaction of γ-CD particles with melted drug was not detected either. From 165 °C the decomposition of melted OPB began and this process was simultaneous with the fusion of the γ-CD particles.

The behaviour of OPB/γ-CD kneaded systems observed in HSM was very different from their counterpart physical mixtures. The samples were made up of very small black particles which appeared grey under polarised light. During heating, no changes were detected until the melting of the systems was produced (295–300 °C).

These results could confirm that the OPB/γ-CD kneaded systems are inclusion compounds.

3.3. Differential scanning calorimetry

The DSC scan of pure OPB shows an endothermic peak corresponding to the fusion of the product. Later the base line is recovered and next an exothermic process attributable to the decomposition of the melted drug is detected (Fig. 2, graph A).

DSC curves of β- and γ-CD show similar designs. Both curves exhibit a first endothermic peak corresponding to dehydration process. The second endothermic peak indicates the fusion of the sample (Fig. 2, graphs B and C).

Thermal behaviour of all OPB/β-CD binary systems was similar, there is a little endothermic peak which indicates the fusion of the drug and next, the dehydration peak of β-CD in DSC curves (Fig. 3, graphs A–D) can be observed. In 150–200 °C range an exothermic peak corresponding to the OPB decomposition appears. At temperatures above 280 °C an endothermic peak, which indicates the fusion of β-CD, is detected in the four binary systems. The presence in both OPB/β-CD kneaded systems of the thermal phe-

nomena associated to pure OPB (melting endothermic peak and decomposition peak) reveals that no OPB/β-CD inclusion compound was formed.

DSC curves from both 1:1 and 1:2 OPB/γ-CD physical mixtures show all the characteristic thermal processes from the pure substances: melting process of OPB, dehydration process of γ-CD, decomposition of melted OPB and fusion of γ-CD (Fig. 4, graphs A and B). However, DSC curves from the corresponding kneaded systems indicate

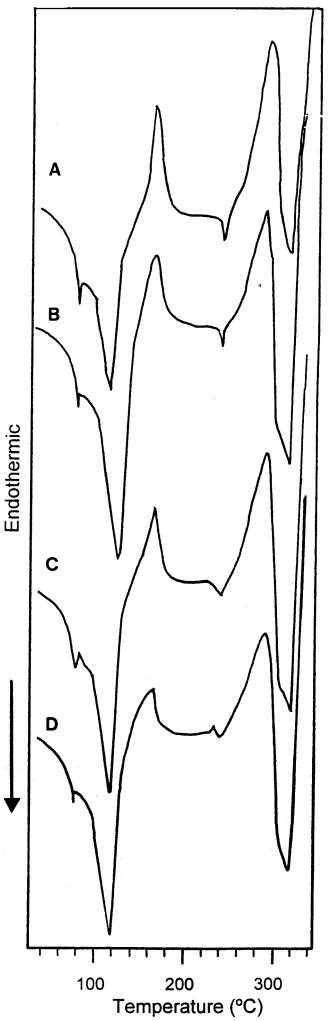

Fig. 3. DSC curves of OPB/β-CD binary systems: (A) 1:1 physical mixture, (B) 1:2 physical mixture, (C) 1:1 kneaded system, (D) 1:2 kneaded system.

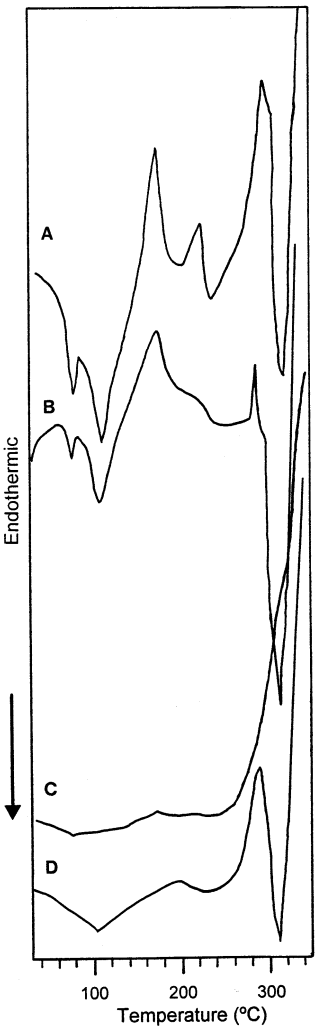

Fig. 4. DSC curves of OPB/γ-CD binary systems: (A) 1:1 physical mixture, (B) 1:2 physical mixture, (C) 1:1 kneaded system, (D) 1:2 kneaded system.

a very different thermal behaviour (Fig. 4, graphs C and D). The absence in DSC curves of drug melting peak in both kneaded systems could indicate that drug molecules were forming a true inclusion compound with γ-CD.

3.4. Thermogravimetric analysis

Thermogravimetric curve of OPB shows two steps of weight loss between 40 and 400 °C due to decomposition phenomenon. The first step of

weight loss, between 40 and 150 °C, occurred during the melting of the drug. For this reason when the melted sample was allowed to cool to room temperature, no recrystallization process was detected by thermomicroscopy. Thermogravimetric behaviour of both cyclodextrins was similar; the weight loss associated with the samples during heating happened in two steps: the first one corresponding to the dehydration process, later a second step of weight loss coinciding with the melting of the cyclodextrins (Table 1).

The data of OPB/β-CD binary systems obtained from the thermogravimetric study reveal a similar behaviour of both kneaded systems with respect to their corresponding physical mixtures (Table 2). All systems lost weight in two steps due to the dehydration process from β-CD and the decomposition from the melted OPB and the melted β-CD. The practically superimposable curves of kneaded systems with their counterpart physical mixtures, proves that OPB was not protected from decomposition phenomenon when the drug is in kneaded systems with β-CD, because in both binary systems OPB did not form an inclusion compound with β-CD.

To compare the weight loss shown by both OPB/γ-CD kneaded systems with their corresponding physical mixtures, different data in the decomposition process at low temperatures (40–150 °C) are seen (Table 3). The weight loss is lower in kneaded systems than in physical mixtures. This could indicate that the loss of the weight in the kneaded systems between 40 and 150 °C is due to the loss of hydration water, while the drug could remain unaltered in this temperature range.

Table 1
Thermogravimetric data from OPB, β-CD and γ-CD

Substance	Range T (°C)	Peak[a] (°C)	% Weight loss	Process
OPB	30–150	69.5	6.08	Decomposition
	250–400	270.0	51.74	Decomposition
β-CD	30–150	89.0	14.41	Dehydration
	250–400	315.0	73.44	Decomposition
γ-CD	40–160	82.5	9.52	Dehydration
	260–400	311.0	78.85	Decomposition

[a] The peak corresponds to the temperature at which the rate of weight loss was a maximum.

Table 2
Thermogravimetric data from OPB/β-CD binary systems

Substance	Range T (°C)	Peak[a] (°C)	% Weight loss	Process
PM[b] 1:1	40–150	86.0	12.18	A
	250–400	319.0	56.52	B
PM[b] 1:2	40–150	85.0	13.10	A
	250–400	317.0	66.39	B
KS[c] 1:1	40–150	86.0	12.61	A
	250–400	316.5	64.74	B
KS[c] 1:2	40–150	85.0	13.39	A
	250–400	316.5	66.85	B

A: decomposition of OPB and dehydration of β-CD.B: decomposition of both components.
[a] The peak corresponds to the temperature at which the rate of weight loss was a maximum.
[b] Physical mixtures.
[c] Kneaded systems.

Table 3
Thermogravimetric data from OPB/γ-CD binary systems

Substance	Range T (°C)	Peak[a] (°C)	% Weight loss	Process
PM[b] 1:1	40–150	71.0	8.90	A
	200–400	315.5	67.11	B
PM[b] 1:2	40–150	74.5	9.25	A
	200–400	314.5	67.10	B
KS[c] 1:1	40–150	48.0	6.89	C
	200–400	314.0	69.60	D
KS[c] 1:2	40–150	50.0	8.71	C
	200–400	313.5	76.27	D

A: decomposition of OPB and dehydration of γ-CD.B: decomposition of both components.C: dehydration of the system.D: decomposition of the system.

[a] The peak corresponds to the temperature at which the rate of weight loss was a maximum.

[b] Physical mixtures.

[c] Kneaded systems.

3.5. X-ray diffraction study

Fig. 5 (trace 1) shows the diffraction pattern of OPB at room temperature. The characteristic peaks of this substance appear at: 9.05°; 15.21°; 16.63°; 18.87°; 19.58°; 21.96°; 22.63°; 24.17° and 25.28° 2θ. Diffraction patterns obtained after heating of the sample at 100 °C (trace 2) and 150 °C (trace 3) do not show any diffraction peak, because at these temperatures the sample was completely melted, obviously a melted substance does not retain any diffraction peak characteristic of its crystalline structure.

Fig. 6 shows the evolution of β-CD diffraction pattern when the sample was heating. The diffraction pattern obtained at room temperature (trace 1) exhibits its different maximum diffraction peaks at 4.69°; 12.64°; 12.94°; 19.68° and 22.90° 2θ. When the sample was heated at 100 °C, a drastic change in its diffraction pattern was produced (trace 2). This modification is due to the fact that in the heating process, β-CD went through a dehydration process which implied a crystalline restructuring. If the sample heating continued until 150 °C (trace 3) there was a diffraction pattern practically identical to which was obtained at 100 °C. This indicates that at 100 °C the subsequent restructuring to the dehydration process was finished.

The diffraction pattern obtained from pure γ-CD at room temperature is shown in Fig. 7 (trace 1). The maximum diffraction peaks appear at: 5.12°; 6.19°; 12.33°; 13.95°; 15.41°; 16.40° and 18.79° 2θ. When this sample was heated to 100 °C, some differences in the diffraction pattern were produced (Fig. 7, trace 2). If the sample was heated to 150 °C, the change in the trace of diffraction pattern (Fig. 7, trace 3) continued. But when the product reached 200 °C, a diffraction pattern practically superimposable to which was obtained at 150 °C was recorded (Fig. 7, trace 4). These modifications can be attributed, as in the

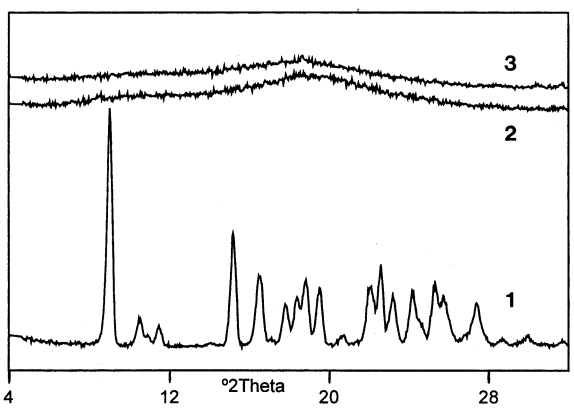

Fig. 5. XRDs patterns of OPB: (1) 25 °C, (2) 100 °C, (3) 150 °C.

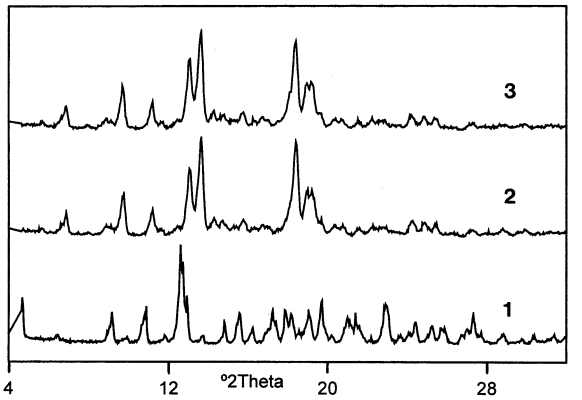

Fig. 6. XRDs patterns of β-CD: (1) 25 °C, (2) 100 °C, (3) 150 °C.

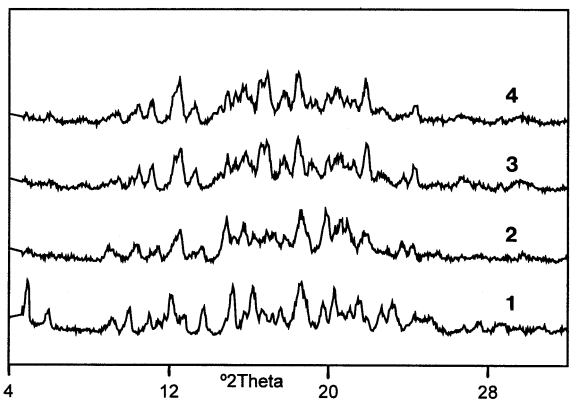

Fig. 7. XRDs patterns of γ-CD: (1) 25 °C, (2) 100 °C, (3) 150 °C, (4) 200 °C.

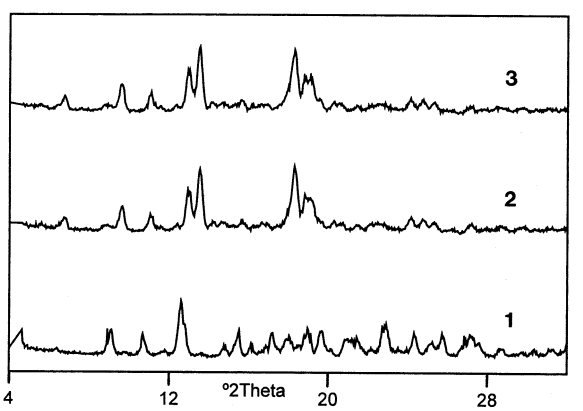

Fig. 8. XRD patterns of OPB/β-CD 1:2 physical mixture: (1) 25 °C, (2) 100 °C, (3) 150 °C.

case of β-CD, to the molecular restructuration after the water loss. The only difference was that in β-CD, the restructuration process was finished at 100 °C and in the case of γ-CD it was necessary to reach the temperature of 150 °C to complete the modification. These results are in agreement with the thermogravimetric data which indicate that the dehydration process of γ-CD continued until 150 °C.

Diffraction pattern of OPB/β-CD 1:2 physical mixture at room temperature (Fig. 8, trace 1) shows all of the principal diffraction peaks corresponding to β-CD. The only OPB diffraction peak detected is the shown at 9.05. When the sample was heated at 100 and 150 °C the OPB diffraction peaks disappeared, due to the fusion of the drug (Fig. 8, traces 2 and 3). In the same way, the diffraction patterns of OPB/β-CD 1:2 physical mixture at 100 and 150 °C exhibit the same trace that the corresponding to pure β-CD heated at 100 and 150 °C did.

Diffraction patterns of OPB/β-CD 1:2 kneaded systems are showed in Fig. 9. The trace obtained at room temperature (trace 1) exhibits the same principal peaks corresponding to its counterpart physical mixture, but the peaks are higher. This fact indicates an increase in the crystallinity of the sample attributable to a partial dissolution and subsequent recrystallization of the components during the kneading process. Neither the disappearance of any peak nor the appearance of new peaks is detected. The results obtained from XRD powder confirm the theory that OPB did not form an inclusion compound with β-CD in the kneaded system.

The evolution shown in the diffraction patterns of the OPB/β-CD 1:2 kneaded system during heating is similar to which happened in the corresponding physical mixture, as can be deduced from the observation of Fig. 9 (traces 2 and 3).

The evolution of OPB/γ-CD 1:2 physical mixture diffraction pattern happened during heating process is shown in Fig. 10. When the sample was analysed at room temperature, the diffraction pattern showed all the characteristic peaks corresponding to both components (Fig. 10, trace 1). When the sample was heated to 100 and 150 °C, the OPB particles melted and the diffraction

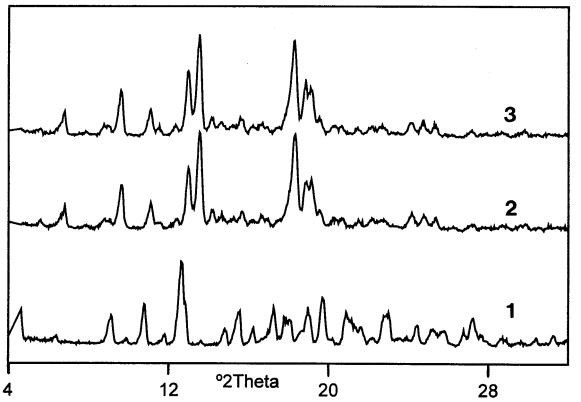

Fig. 9. XRD patterns of OPB/β-CD 1:2 kneaded system: (1) 25 °C, (2) 100 °C, (3) 150 °C.

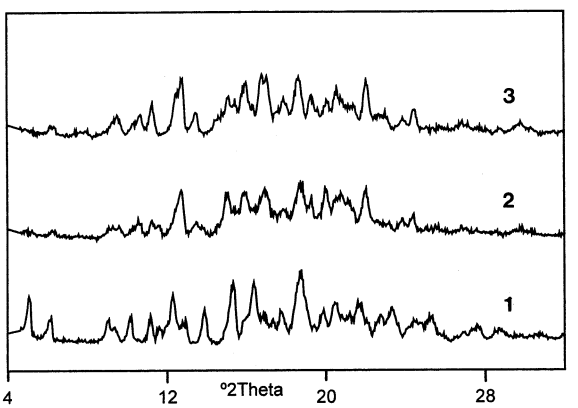

Fig. 10. XRD patterns of OPB/γ-CD 1:2 physical mixture: (1) 25 °C, (2) 100 °C, (3) 150 °C.

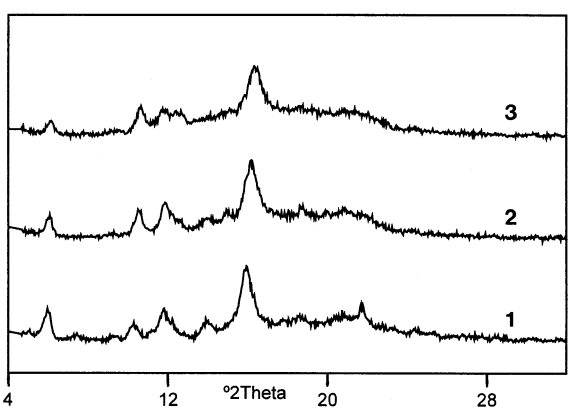

Fig. 11. XRD patterns of OPB/γ-CD 1:2 kneaded system: (1) 25 °C, (2) 100 °C, (3) 150 °C.

peaks corresponding to the drug disappeared. The evolution produced in diffraction peaks of γ-CD with the heating is in agreement with the modifications observed in the diffraction patterns of pure substance (Fig. 10, traces 2 and 3). This indicate that OPB melted was not able to interact with γ-CD. That is to say, the heating of the physical mixture did not produce the OPB/γ-CD inclusion compound.

The diffraction pattern of OPB/γ-CD 1:2 kneaded system recorded at room temperature is shown in Fig. 11 (trace 1). Its appearance indicates that the system was a crystalline substance and the principal diffraction peaks appeared at 6.03°; 10.24°; 11.76°; 13.86°; 15.99° and 21.71° 2θ. In comparing this diffraction pattern with this counterpart physical mixture, it can be deduced that in the kneading process an OPB/γ-CD inclusion compound was formed. When the sample was heated to 100 and 150 °C a displacement of the diffraction maxima to the right in the diffraction patterns (Fig. 11, traces 2 and 3) is observed. This can indicate that the heating went by a structural collapse, because the distances between planes were reduced. This theory is in agreement with the results obtained in thermogravimetric analysis. In the same way, a decrease of the height and an increase in the width of some peaks is detected, which could indicate a loss of crystallinity due to the heating process. It is significant that the evolution of the diffraction pattern of OPB/γ-CD 1:2 kneaded system during heating is very different from the behaviour shown by its counterpart physical mixture. This indicate that the OPB/γ-CD inclusion compound has a structure more thermostable than the pure components.

4. Conclusion

The results obtained allow to conclude that OPB forms inclusion compounds with β and γ-CD in aqueous media but, the kneading method has only allowed the isolation of the OPB/γ-CD inclusion compound in the solid state. In this study, XRD powder has been shown to be a very useful analytical tool, because on obtaining the

sample diffraction patterns at different temperatures, the remaining structure of OPB/γ-CD inclusion compound was demonstrated.

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