Biolog. J. Armenia, *Special issue: Cyclodextrins*, 2001

INTERACTION OF IBUPROXAM WITH γ-CYCLODEXTRIN IN SOLUTION AND IN SOLID STATE

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Interaction between Ibuproxam (Ib) and γ-cyclodextrin (γ-CD) was studied in liquid and solid states. In solution, complexation was evaluated by solubility studies. Also, we studied the effect of temperature on the complexation, to propose a model of the complexation mechanism in solution. The results suggested that complexation is a spontaneous and exothermic process. In the solid state, binary systems were prepared by kneading, heating in a sealed container, and spray-drying methods. The products obtained were studied using Differential Scanning Calorimetry (DSC), Hot Stage Microscopy (HSM), Fourier Transformed Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and compared by their dissolution rate performance. SEM, DSC and HSM indicated that Ib is partially found as crystalline non-complexed form in the kneaded sample, while in the sealed-heated and spray-dried products Ib is totally dispersed in the carrier. Finally, this inclusion process in the γ-CD significantly increases the dissolution rate of Ib, especially in the spray-dried system, where the amorphous nature of this one plays a significant role.

Изучена реакция между ибупроксамом (Ib) и γ-циклодекстрином (γ-ЦД) в жидком и твердом состоянии. В растворе комплексообразование оценивалось по результатам растворимости. Изучали также влияние температуры на комплексообразование, чтобы предложить модель механизма комплексообразования в растворе. Результаты показали, что комплексообразование - спонтанный и экзотермический процесс. В твердом состоянии бинарные системы изготовлялись смешиванием, нагреванием в изолированном контейнере, а также методом распылительной сушки Полученные продукты изучались дифференциальной сканирующей колориметрией (ДСК), жарофазной микроскопией (ЖФМ), трансформационной инфракрасной спектроскопией Фурие (ТИКФ), сканнирующей электронной микроскопией (СЭМ) и сравнивались по их скорости растворимости. Результаты СЭМ, ДСК и ЖФМ показали, что Ib частично обнаруживается в виде кристаллической некомплексной формы в смешанном образце, тогда как в герметически нагретых и в препаратах, высушенных распылительной сушке Ib полностью диспергирован в носителе. В конечном счете, этот процесс включения в γ-ЦД значительно увеличивает скорость растворимости Ib, особенно при распылительной сушке, где его аморфная природа играет важную роль.

Ուսումնասիվել է իբուպրոքսամի (ԻԲ) և γ-ցիկլոդեքստրինի (γ-ՑԴ) միջև ռեակցիան հեղուկ և պինդ վիճակում: Լուծույթում համալիրագոյացումը գնահատվել է ըստ լուծելիության: Ուսումնասիրվել է նաև ջերմաստիճանի ազդեցությունը համալիրագոյացման վրա, առաջարկելու համար լուծույթում համալիրագոյացման մեխանիզմի մոդել: Արդյունքները ցույց են տվել, որ համալիրագոյացումը ինքնածին և էկզոթերմիկ պրոցես է: Պինդ վիճակում բինար սիստեմները պատրաստվել են խառնելով, տաքացնելով մեկուսացված կոնտեյների մեջ, ինչպես նաև փոշիացման-չորացման մեթոդներով: Ստացված նյութերը ուսումնասիրվել են դիֆերենցիալ սկանող կոլորիմետրիայի (ԴՍԿ), շոգե-ֆազային միկրոսկոպիայի (ՇՖՄ), Ֆուրիեի

տրանսֆորմացիոն ինֆրակարմիր սպեկտրոսկոպիայի (ՖՏԻԿ), սկանող էլեկտրոնային միկրոսկոպիայի (ՍԷՄ) մեթոդներով և համեմատվել են ըստ նրանց լուծելիության արագությունների: ՍԷՄ, ԴՍԿ և ՇՖՄ արդյունքները ցույց են տվել, որ ԻԲ-ն մասնակիորեն գտնվում է բյուրեղային ոչ համալիր ձևով խառնված նմուշում, այն դեպքում որ հերմետիկորեն տաքացված և փոշիացմամբ չորացված պատրաստուկներում ԻԲ-ն ամբողջապես ցրված է կրիչի մեջ: Վերջապես γ-ՑԴ -ում ներառման պրոցեսը բարձրացնում է ԻԲ-ի լուծելիության արագությունը, հատկապես փոշիացնող չորացնող համակարգում, որում նրա ամորֆ բնույթը մեծ դեր է խաղում:

Introduction

Cyclodextrins (CDs) are well-known complexing agents widely used in the pharmaceutical field [1]. The CD complexation has been very employed to improve the stability, dissolution rate and bioavailability in oral, rectal, dermal and parenteral administration [2, 3].

Ibuproxam (Ib) is a non-steroid anti-inflammatory agent with good analgesic and antipyretic properties [4]. It is characterised by a poor aqueous solubility, which limits its dissolution rate and, consequently, its bioavailability.

Earlier investigations showed that Ib complexation with γ-CD increases its solubility [5-7]. However, some aspects, such as thermodynamics of the complexation process and the isolation of solid complexes have not been treated extensively. For this reason, the primary objective of the present study was to investigate the potentiality of interaction of Ib with γ-CD with the aim to reach solid inclusion complexes able to yield further improvement of drug dissolution characteristics. Moreover, the thermodynamic parameters of the complexation were determined, in order to explain the mechanism of this one at the liquid phase.

Materials and Methods

Ib was supplied by Manetti and Roberts (I-Firenze) and γ-CD by Cyclolab (H-Budapest).

Study of the complexation in aqueous solution.

The complexation of Ib with γ-CD in aqueous solution at different temperatures (25, 37 and 50 °C) has been studied using the solubility method described by Higuchi and Connors [8]. 50 mg of Ib were added to 10 ml solutions containing various concentrations of γ-CD (10- 100 mM). The Erlenmeyer flasks were sealed and stirred for one week, until the solubility equilibrium was reached. Then, their content was filtered through 0.22 μm cellulose nitrate membrane filters. The filtrates, properly diluted with distilled water, were analysed spectrophotometrically at 262.5 nm. The apparent 1:1 stability constant (Kc) was calculated from the slope of the phase solubility diagram following the Higuchi and Connors equation.

Preparation of the solid complexes.

The samples, according to the solubility curve results, were prepared in 1:1 molar ratio by the following methods:

Kneading: γ-CD and Ib were kneaded in a mortar with the aid of few drops of ethanol, until a homogenous paste was obtained. The process continued for 45 min and the final paste was dried in an oven at 35 °C for 24 hours.

Spray-drying: The required amounts of Ib and γ-CD were dissolved, respectively, in 400 ml of 96 % ethanol and 400 ml of purified water. Both solutions were mixed by sonication (20 min), to produce a clear solution, which was then spray-dried (Büchi 190M miniSpray-Dryer, Switzerland). The drying conditions were: flow rate: 400 ml· h^{-1}; inlet temperature: 115 °C; outlet temperature: 70 °C; air flow rate: 400 Nl· h^{-1}.

Heating in a Sealed Container: 400 mg of a 1:1 mol:mol Ib-γ-CD physical mixture was sealed in a 2 ml glass ampoule containing 125 µL of distilled water. The ampoule was vigorously shaken and then heated at 100 °C for 2 h. The final product was dried under the same conditions that the kneaded system.

Physical Mixture: drug and cyclodextrin in equimolar proportion and previously sieved, were mixed during 15 min in a flask under manual agitation.

All the obtained products were finally ground and sieved (50- 200 µm).

Study of the complexation in solid state.

DSC measurements were carried out using a DSC equipment (Mettler TA4000) equipped with a DSC 25 cell. Samples of 5-8 mg were put into aluminium crucibles, which firm was pierced to allow the leaving of gases evolved during the heating process. The conditions were: static air atmosphere, temperature range from 30 to 320 °C and 10 °C· min^{-1} heating rate.

Different observations were made during heating using a HSM (Mettler model FP82HT) attached to an Olympus BH-2 microscope. Approximately 0.1 mg of samples was placed on glass slides with coverglass and heated at 5 °C· min^{-1}.

The infrared spectra of the different samples were obtained by means of a Bomem M-120 IR equipment. The samples were mixed with KBr and compressed as disks. The selected wavenumber ranged between 600 and 4000 cm^{-1}, being the speetra resolution of 4 cm^{-1} and 20 the number of scans.

The microscopic features of the single raw materials were compared with those of the products obtained by kneading, spray-drying and sealed heating by examination under the SEM (Philips XL30). Samples were previously coated with Au, in order to make them conductor.

Dissolution rate studies.

The dissolution rate studies were performed according to the USP 23 paddle method. The samples, equivalent to 40 mg of Ib, were previously placed into hard gelatine capsules. Dissolution medium was 1000 mL of distilled water. The stirring speed was 50 rpm and the temperature 37 ± 0.5 °C. Aliquots of 3 ml were withdrawn at settled time intervals using a filter syringe, and analysed spectrophotometrically at 262.5 nm. All tests were performed in triplicate.

Results and Discussion

Complexation in aqueous solution.

Figure 1 shows the obtained phase solubility diagrams. The solubility of Ib increased in a linear fashion as function of CD concentration, being classified as A_L Higuchi type curves. In this system a water soluble complex may exist in the solution since no precipitation was observed even at concentration of CD as high as 100 mM.

The variation of stability constant as a function of temperature was used to determine the thermodynamic parameters for Ib inclusion complex with γ-CD. The stability constants calculated at different temperatures and the thermodynamic parameters for the complexes are shown in Table 1. For 1:1 complex formation, the equilibrium constant Kc, was determined according to $Kc = m / S_0 (1-m)$, where m is the slope of solubility diagram shown in Figure 1, and S_0 is the intrinsic solubility of Ib. As shown in Figure 1, temperature significantly affected the interaction. So, the Kc values decrease with increase in temperature. For calculate the thermodynamic parameters of the inclusion phenomenon, we have previously calculated the association constants at three different temperatures.

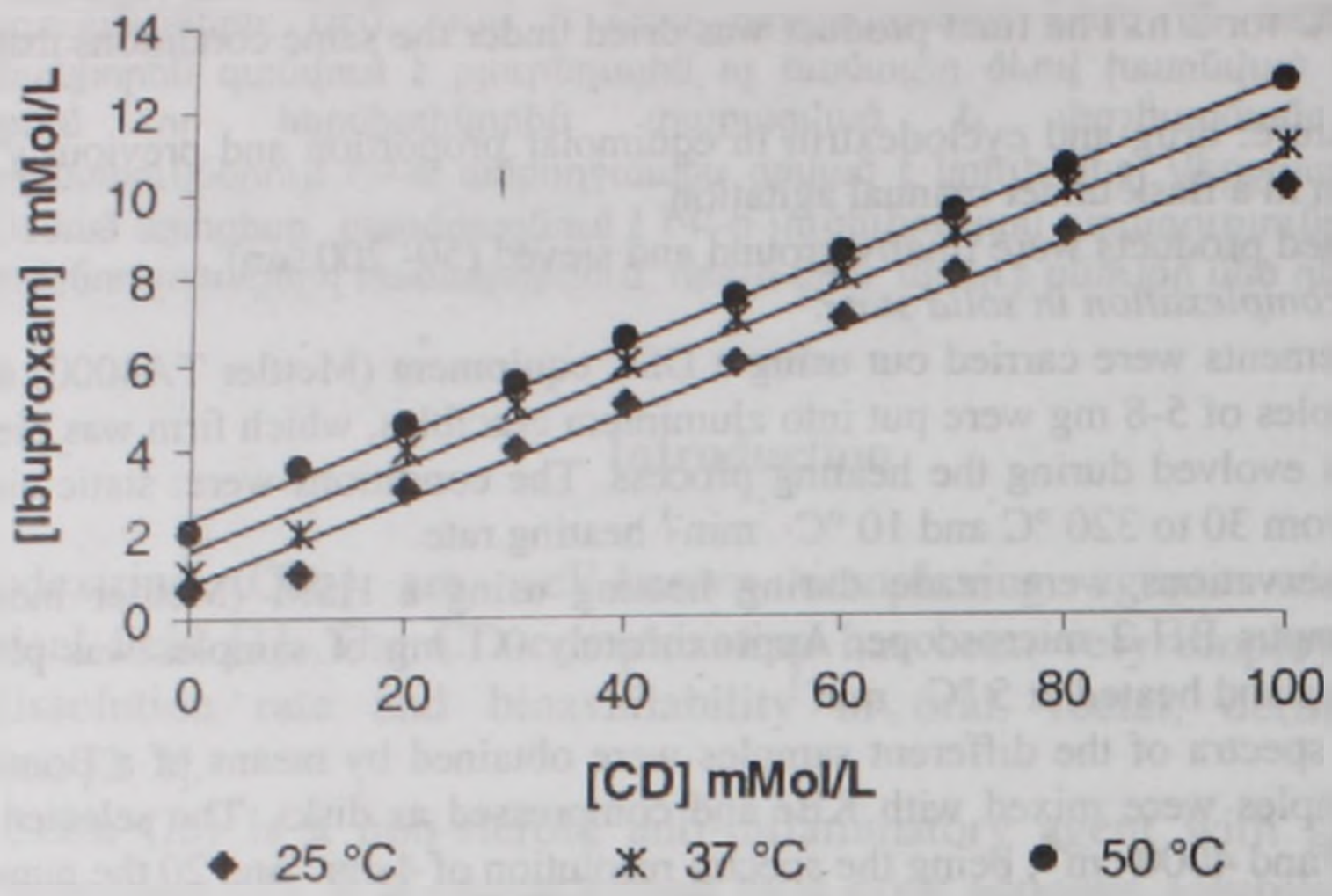


Figure 1. Phase-solubility diagram of Ib-γ-CD system at 25, 37 and 50°C. The calculation of the thermodynamic parameters was carried out using the following equations:

$$\ln \frac{K_2}{K_1} = \frac{\Delta H}{R}\left[\frac{T_2 - T_1}{T_2 \cdot T_1}\right] \qquad \text{Eq. (1)}$$

$$\Delta Gi = -RTi \ln Ki \qquad \text{Eq. (2)}$$

$$\Delta Si = \frac{\Delta Hi - \Delta Gi}{Ti} \qquad \text{Eq. (3)}$$

Where K_1 and K_2 are the stability constants at 25 at 37 °C respectively, R is the gas constant (8.314 J· mol^{-1}· K^{-1}), being T_1 and T_2 the temperatures in Kelvin degrees.

Table 1. Thermodynamic parameters calculated from solubility studies at different temperatures.

Temperature (K)	Slope	*R*	K_c (M^{-1})	ΔG (KJ· mol^{-1})	ΔH (KJ· mol^{-1})	ΔS (J· mol^{-1}· K^{-1})
298	0.09979	0.9915	142.0	-12.278	-34.25	-73.73
310	0.10303	0.9898	73.6	-11.079	-34.25	-74.74
323	0.10248	0.9982	48.7	-10.434	-34.25	-73.73

The large negative magnitude of enthalpies (ΔH) obtained for all the interactions, suggesting that strong intermolecular forces are involved in the complex formation, as hydrogen bonding, which energy is between 12 and 20 KJ· mol^{-1} [9].

It is worth noting that the complexes gave negative entropy changes (ΔS), which were unfavourable for the complex formation. These results may indicate a more ordered state upon complexation. The overall entropy change is the sum of two opposite effects. First, a negative contribution, due to the reduction of traslational and rotational degrees of freedom of the host and guest molecules by complexation. Also, a positive effect, related with the solvent ordering, which is due to the displacement of the highly ordered water molecules of the cavity of the CD and those surrounding the hydrophobic portions of the guest molecule

when it interact with the inner of the CD. In our case, the first factor seems to be the prevalent.

On the other hand, negative free energy values (ΔG) were obtained for all the temperatures, indicating that the inclusion process is spontaneous. This is due to the negative ΔH change that compensates the unfavourable ΔS, indicating also the exothermic nature of the complexation process under study. This contribution of ΔH to the ΔG value confirm that this type of interaction is enthalpy driven.

The Kc values decrease whit increase in temperature, as found for a large variety of drugs [10], can be interpreted as a result of displacement of cavity water molecules by means of the entering guest, enabling them to form full hydrogen bonds with adjacent water molecules.

Complexation in solid state.

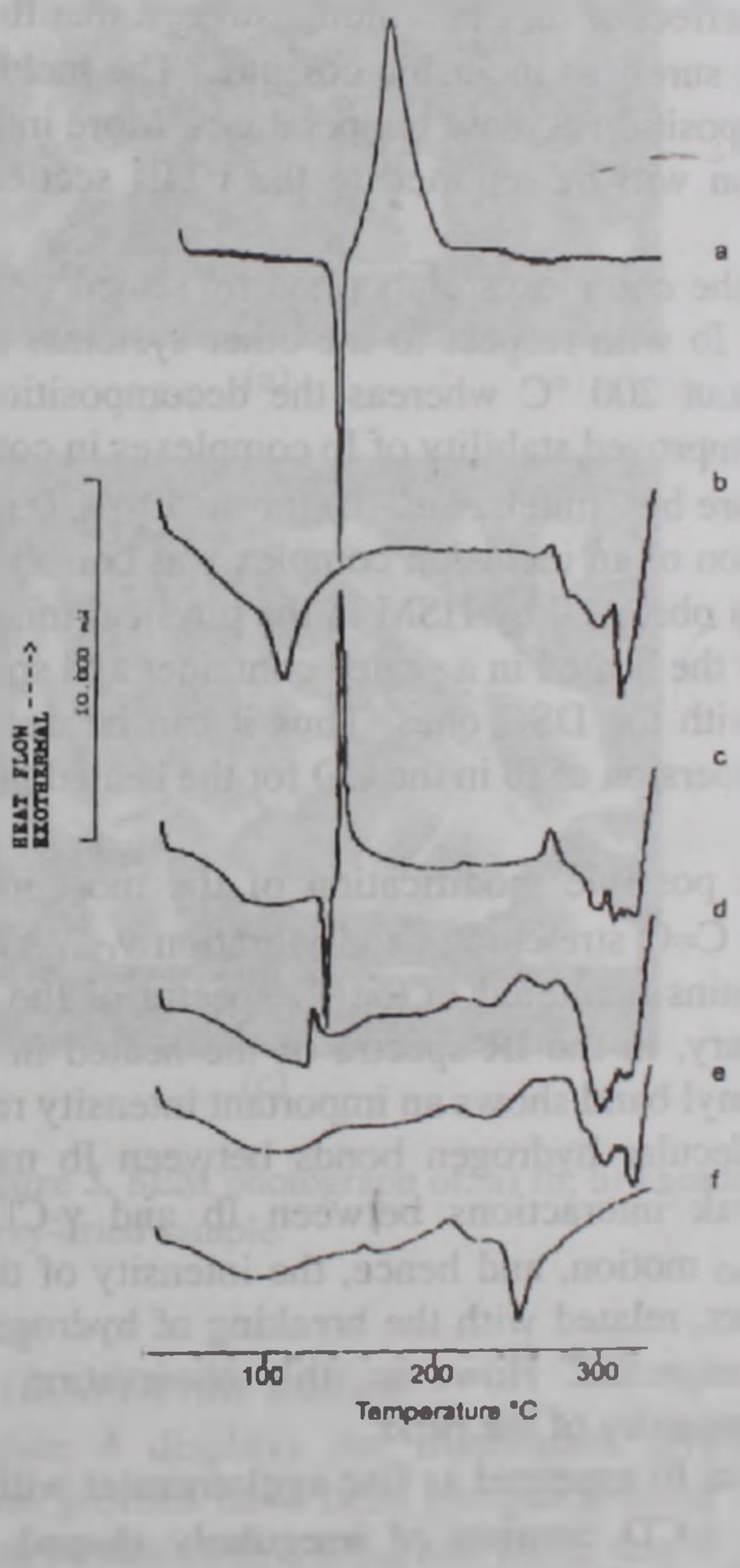


Figure 2. DSC curves of: a) Ib; b) γ-CD; c) physical mixture; d) kneaded; e) heated in a sealed container and f) spray-dried.

The DSC traces of the systems under study are represented in Figure 2. The DSC curve of Ib (Figure 2a) is characterised by the presence of typical endo- and exo-thermal peaks (endo: Tonset = 126.1 (± 0.6)°C, Tpeak = 130.2 (± 0.7)°C, ΔHf = 129 (± 9) J· g^{-1} and exo: Tonset = 147.2 (± 1.8)°C, Tpeak = 165.3 (± 0.6)°C, ΔHf = 573 (± 63) J· g^{-1}) corresponding to the melting and oxidative degradation of the drug, respectively. These results are in accordance with the literature data [6]. On the other hand, the γ-CD (Figure 2b) shows a broad endothermic effect around 100 °C related to the CD dehydration.

The thermogram corresponding to the physical mixture (Figure 2c) displays the endo-exothermic effect of Ib (endo: Tonset = 122.9 (± 0.7)°C, Tpeak = 131.5 (± 0.5)°C, ΔHf = 115 (± 10) J· g^{-1} and exo: Tonset = 138.3 (± 1.9)°C, Tpeak = 142.6 (± 0.6)°C, ΔHf = 290 (± 30) J· g^{-1}). It is important to note that the exothermic effect shows a significant difference in the temperature and shape of the peak, which may be attributed to

the degradation of the drug itself. If we take into account that this is an oxidation process, in the pure drug, when melts, the contact with the oxygen is limited to the outer layer in the crucible. The rest of the drug is protected against oxygen by its own liquid, which delays the oxidation process. On the contrary, in the physical mixture, the drug, in a relatively low w/w percentage is molten beneath the carrier particles, allowing its simultaneous contact with the atmospheric oxygen.

The thermogram corresponding to the kneaded system (Figure 2d) reveals that the endo-exothermic effect of Ib does not disappear (endo: Tonset = not determinable, Tpeak = 120.4 (± 0.7)°C, ΔHf = not determinable and exo: Tonset = 122.9 (± 1.4)°C, Tpeak = 124.9 (± 0.8)°C, ΔHf = 28 (± 4) J· g^{-1}). In this product, a clear size reduction and broadening of both peaks is observed, with a concomitant shift to lower temperatures. This result may be interpreted in terms of incomplete inclusion formation between Ib and γ-CD [13-14].

The sealed heating (Figure 2e) and spray-drying (Figure 2f) treatments leaded to the total disappearance of the endo-exothermic effect of Ib. This finding suggest that Ib exists in a molecularly dispersed state into the γ-CD, surely as inclusion complex. The included drug should not suffer neither melting not decomposition at these temperatures. More information about the nature of the drug-CD interaction will be reported in the FTIR section of this paper.

The thermograms also revealed that the complexes elaborated by sealed heating and spray drying have an improved stability of Ib with respect to the other systems. Thus, the complexes start their decomposition at about 200 °C whereas the decomposition of the commercial Ib arises at about 165 °C. This improved stability of Ib complexes in comparison with pure Ib appears described in the literature by Zmitek *et al.* (6) for the Ib-γ-CD complex.

The feasibility of the possible formation of an inclusion complex was corroborated by HSM. The melting process of Ib was clearly observed by HSM in the physical mixture and, partially, in the kneaded product, but not for the heated in a sealed container and spray-dried samples. These results are in accordance with the DSC ones. Thus it can be deduced the reality of the formation, at least, of a true dispersion of Ib in the CD for the heated in a sealed container and spray-dried products.

The IR studies were focused on the possible modification of the most interesting absorption band of Ib, corresponding to the C=O stretching band vibration $\nu_{C=O}$, situated at 1634 cm^{-1} in the pure drug. This band remains unaltered in the IR spectra of the physical mixture and kneaded system. On the contrary, in the IR spectra of the heated in a sealed container and spray-dried systems, the carbonyl band shows an important intensity reduction. It is related with the breaking of intermolecular hydrogen bonds between Ib molecules, accompanied by the establishment of weak interactions between Ib and γ-CD in the complexed state [13], which reduce the $\nu_{C=O}$ motion, and hence, the intensity of this band. Also, a possible shift to higher wavenumbers, related with the breaking of hydrogen bonds between drug molecules [14] would be expected. However, this observation was not possible, due to the strong reduction of the intensity of the band.

The SEM analysis revealed as the commercial Ib appeared as fine agglomerates with smooth surfaces (Figure 3a). On the other hand, γ-CD consists of irregularly shaped crystals.

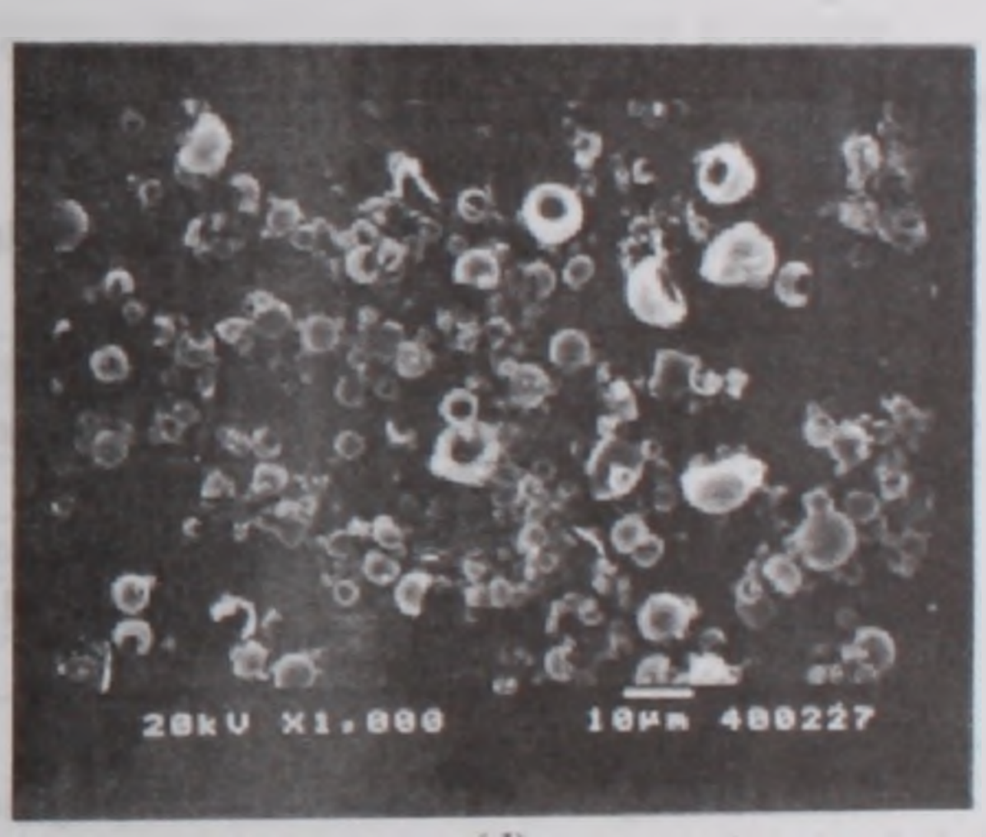
The kneaded mixture photograph (Figure 3b) is characterised by the influence of the preparing technique in the sample morphology. Thus, mixed crystals constitute the kneaded product, which present adhered crystals of original Ib. This observation is in agreement with the results obtained from the former analytical techniques, that reveal the kneaded system does not constitutes a true inclusion complex. Figure 3c shows the heated in a sealed

container product, where the original morphology of both Ib and γ-CD has disappeared, being impossible the differentiation of the components. The spray-drying technique, in contrast, yields products of amorphous appearance (Figure 3d), with the presence of spherical homogeneous particles, which display a quite significant reduction in the particle size. These particles are distinguished by the absence of agglomeration. These observation help to contemplate the presence of a single component in the preparations obtained by sealed heating and spray-drying methods.

(a)

(b)

(c)



(d)

Figure 3. SEM photograph of: a) Ib; b) kneaded sample; c) heated in a sealed container sample and d) spray-dried sample.

Dissolution rate studies.

Figure 4 displays the dissolution profiles of Ib and the Ib-γ-CD systems. The dissolution profiles have been compared using the dissolution efficiency parameter [15] at 15, 30 and 60 min (DE_{15}, DE_{30} and DE_{60}, respectively) (Table 2).

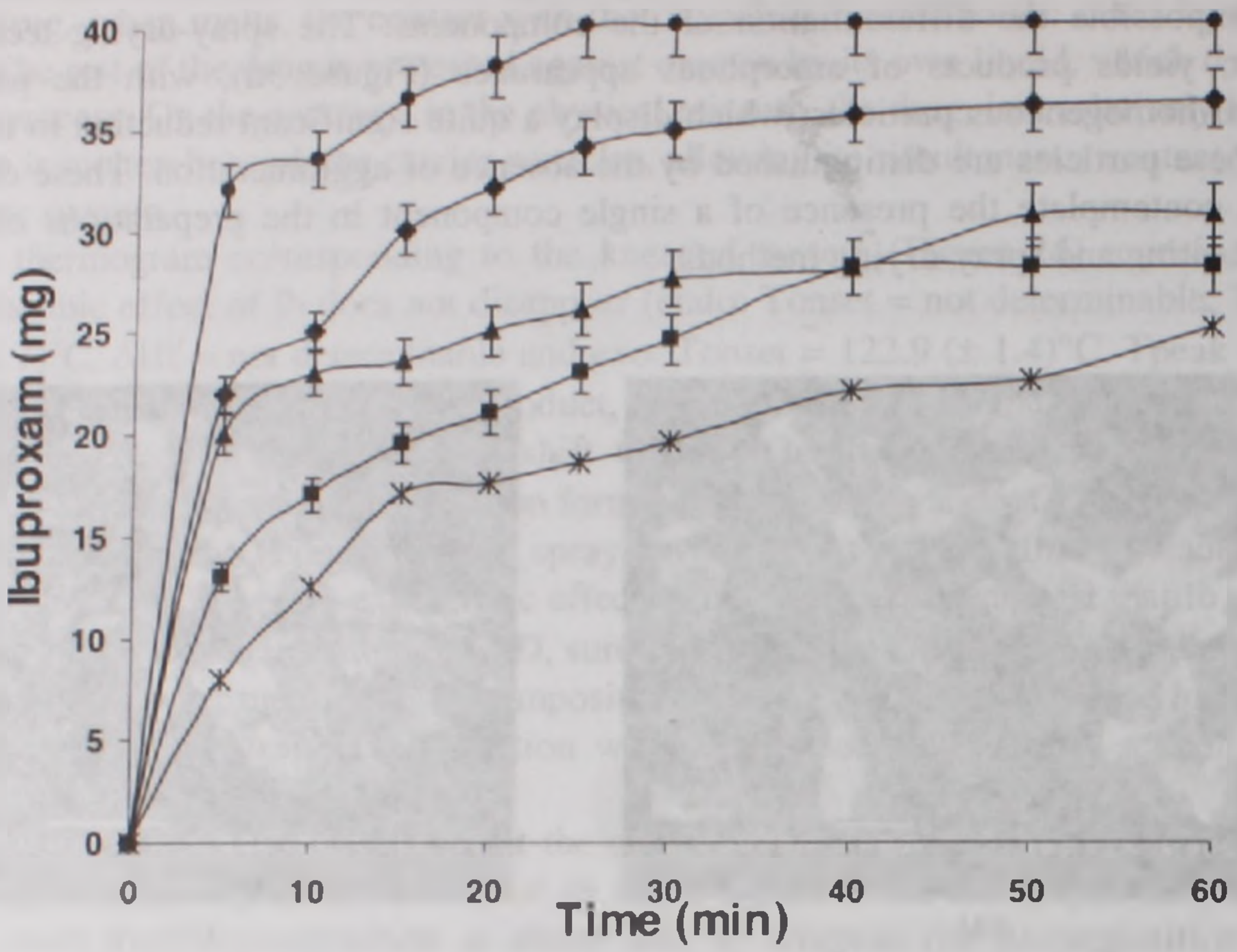


Figure 4. Dissolution curves of Ib and Ib-γ-CD binary systems: ✱: Ib; ■: physical mixture; ▲: kneaded; ●: heated in a sealed container and ●: spray-dried.

Table 2. Dissolution efficiency values at 15, 30 and 60 min for the Ib and Ib-γ-CD binary systems.

	Elaboration Method	DE15	DE30	DE60
Binary Systems	Physical Mixture	0.331	0.441	0.563
	Kneaded	0.456	0.547	0.638
	Heated in a sealed container	0.510	0.668	0.780
	Spray-Dried	0.698	0.833	0.917
Ibuproxam		0.242	0.347	0.451

It is clearly observed that the physical mixture and kneaded sample improve only slightly the dissolution rate of Ib. The low dissolution rate from the kneaded system may be attributed to the fact that the isolated product has a low percentage of complexed drug.

For the heated in a sealed container and spray-dried samples, we can appreciate a notable increase in the release rate of the drug. These results are due to the major interaction between drug and CD in these systems in contrast with the physical mixture and kneaded sample, which is translated in a more effective inclusion complexation of Ib into the CD cavity. In the spray-dried sample, the special increase in the dissolution rate may be also explained by the higher specific surface of the product produced from the molecular dispersion and, also, by the amorphous structure of the product.

Acknowledgements. The authors wish to thank Dr. Mª del Carmen Ortiz Mellet (Department of Organic Chemistry, Faculty of Chemistry, University of Seville) for her kind help in carrying out and interpreting the IR spectra.

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