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A Novel Method to Obtain a β-Cyclodextrin Inclusion Compound by Solid State Reaction: the Ketoprofen Case Revisited

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Abstract. The ketoprofen/beta-cyclodextrin (β-CD) inclusion compounds may be conveniently obtained through a solid state reaction at room temperature in the presence of saturated water vapour. Relative to the corresponding complexes produced with the usual coprecipitation method, the new compounds have a higher ketoprofen content and/or different properties. In particular, the formation reaction is not accompanied by a loss of hydration water, which is released in distinct stages upon heating. The thermodynamics of the dehydration process is discussed.

Key words: β-Cyclodextrin, ketoprofen, hydration.

1. Introduction

Inclusion compounds of cyclodextrins and organic molecules are usually obtained by coprecipitation [1], or kneading [2]. To achieve complexation in a relatively short time, the use of a solvent, or both a solvent and a mechanical action, is apparently needed. It may be desirable to obtain these complexes in even simpler ways but, in order to do so, we have to achieve a deeper understanding of the inclusion chemistry and driving forces involved. A few years ago a new method for preparing cyclodextrin inclusion compounds was proposed, based upon heating mixtures of drugs and α or β – cyclodextrin (or a derivative of β – cyclodextrin) in a sealed container [3–6]. Several parameters, such as heating temperature and time, drug vapour pressure, the CD's initial water content and crystallinity and mixing molar ratio, were found to affect the formation of the inclusion compound. However, no attempt was made to obtain inclusion compounds at room temperature. Moreover, little attention was paid to the role of hydration water, since no discussion was given of the water content and dehydration behaviour of complexes prepared by different routes.

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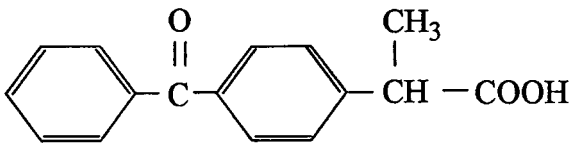
In a previous work [7], analysis of complexes between ketoprofen [2-(3-benzoylphenyl)propionic acid]



and beta-cyclodextrin (β-CD) prepared by coprecipitation has shown that the inclusion reaction apparently leads to removal of just one of the 11-water molecules of β-CD, and to a new arrangement of the remaining ones. The question naturally arises as to whether this phenomenon occurs also when the reaction takes place in water vapour, rather than in solution.

This work has two purposes:

(1) to verify that in mechanical mixtures of ketoprofen and β-CD complexation does occur by a solid – state reaction, in reasonable times, at room temperature (rt) and in a vapour – saturated atmosphere;
(2) to compare the properties of the complexes obtained by coprecipitation and by solid – state reaction in wet air.

We have characterised the solid phases by X-ray powder diffraction and thermal analysis. In particular, we simultaneously recorded the DSC (Differential Scanning Calorimetry) and TGA (Thermogravimetric Analysis) responses with a single sample in order to establish quantitative relationships between changes of enthalpy and the thermal dehydration process.

2. Experimental

2.1. SAMPLE PREPARATION

Ketoprofen (Carlo Erba, Italy) and β-CD-11H_2O (Roquette, France) were used as starting materials. The inclusion compounds were prepared by two different methods:

1. *Coprecipitation*: the reagents were suspended in water and three different starting compositions were studied with ketoprofen/β-CD molar ratios of 1 : 2, 1 : 1, and 2 : 1. The suspensions were stirred for one week, filtered and then stored at 100% relative humidity (RH) for four months, or more, always at rt. These samples will be indicated as C12, C11, and C21.
2. *Reaction in vapour*: physical mixtures of the solid reagents were left at rt (21–25 °C) and 100% RH for five months, or more. The mechanical mixing of powders was achieved, in minutes or hours, by stirring (manually, with a spatula) and/or shaking (with a shaker). Comparison of samples prepared with different mixing procedures did not reveal appreciable differences. Eight different samples were produced, with ketoprofen/β-CD molar ratios of 1 : 3,

1 : 2, 1 : 1, 2 : 1, 3 : 1, 4 : 1, 6 : 1, 7 : 1. They will be indicated as S13, S12, ..., S71. We noted that at rt and with a RH between 50% and 60% (standard storage conditions for hydrated β-CD) no reaction occurs in the mixtures over one year.

Pure β-CD received both treatments: the water-extracted sample will be indicated by Cβ-CD and the vapour – equilibrated one by Sβ-CD.

2.2. APPARATUS AND PROCEDURES

Thermal measurements were performed with samples of about 10 mg, using the simultaneous TGA/DSC apparatus STA 625 by Polymer Laboratories (UK). Measurements were carried out in the temperature range 18 °C–140 °C under a flow of N_2 (3 L/h) bubbled through water at rt. The scanning rate was 0.5 °C/min and, when possible, each run was replicated several times. Uncertainties of the numerical results, when determined through more than three measurements, are in terms of the *standard deviation* (rather than standard error of the mean).

X-ray diffraction patterns were obtained with a powder X-ray diffractometer (Philips PW1710) using the CuKα radiation selected by a monochromator made with a bent graphite crystal.

3. Results and Discussion

3.1. XRD MEASUREMENTS

XRD patterns of physical mixtures immediately after preparation ("time-zero") are compared in Figure 1 with the patterns of pure ketoprofen and β-CD. Ketoprofen reflections are barely visible in the 1 : 2 mixture, where the ketoprofen is less than 10% in weight, and become more evident as the ketoprofen fraction increases. The traces of the mixtures appear to be superpositions of the patterns of the constituents, as expected if no reaction had taken place.

The effect of complexation upon the diffraction pattern can be seen in Figures 2–4 where the "time-zero" pattern is compared with the one after storage in a wet atmosphere and, when available, with the coprecipitated sample of the same composition. For the 1 : 2 composition (Figure 2), the vapour equilibrated sample (b) has a pattern with additional reflections, relative to the zero-time pattern (a). Furthermore, the common reflections are broader in the equilibrated sample than in the zero-time sample. Related to this broadening, is the fact that spectrum (a) has a maximum height 2.5 times larger than (b). The pattern of the coprecipitated sample (c) shares many of the features of the spectrum of the vapour equilibrated sample. However the two samples have substantially different reflections in the 9°–15° interval. In both the coprecipitated and vapour equilibrated 1 : 2 samples there is no free ketoprofen; therefore, the two complexes have the same average composition but they are significantly different in structure.

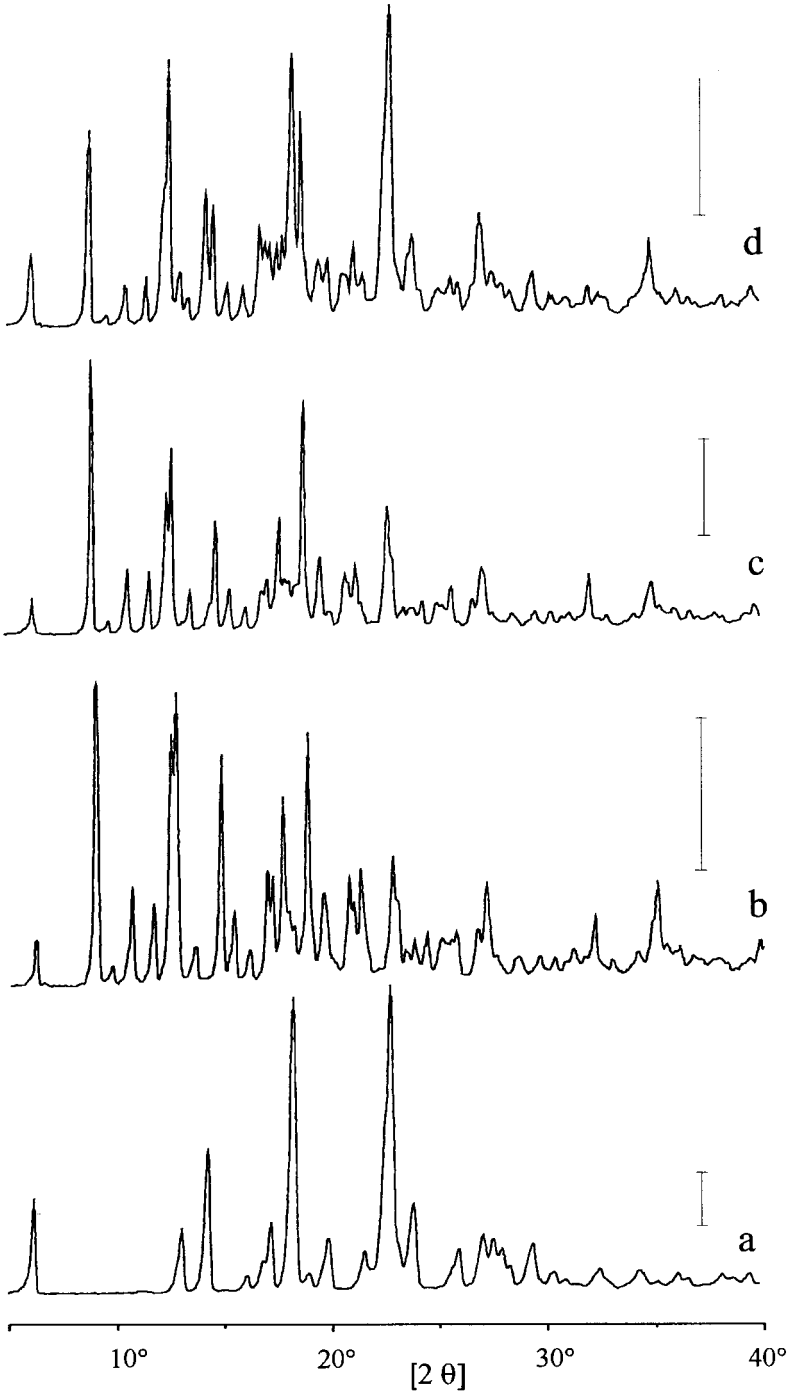


Figure 1. XRD of ketoprofen (a), β-CD (b), and two mechanical mixtures at "time-zero" with compositions 1 : 2 (c) and 4 : 1 (d). In order to have the same maximum height in all patterns, the spectra are represented with different vertical scales, indicated by the vertical bars corresponding to 1000 counts.

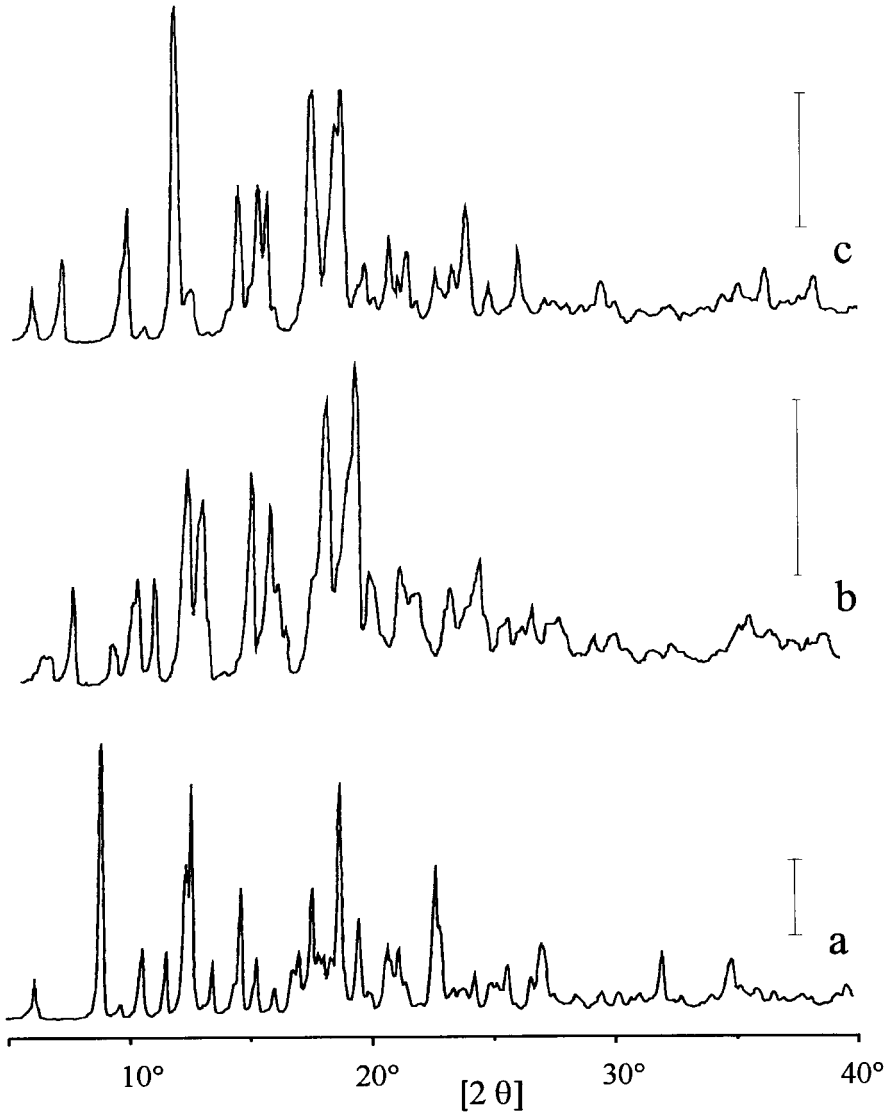


Figure 2. Comparison of XRD patterns for a mechanical 1 : 2 mixture at "time-zero" (a), for an equilibrated S12 sample (b) and a coprecipitated C12 sample (c).

In the 2 : 1 composition (Figure 3), again the reacted samples give patterns (b, c) broader than the "time-zero" sample (a). Patterns (b) and (c) are now very similar, and resemble the pattern of the 1 : 2 coprecipitated sample (Figure 2c). Some of the differences in relative intensities between patterns (b) and (c) are related to the different contents of free ketoprofen, which is higher for the coprecipitated sample. Note, in particular, that the dominant reflection of ketoprofen at 22.5° gives in (c) a

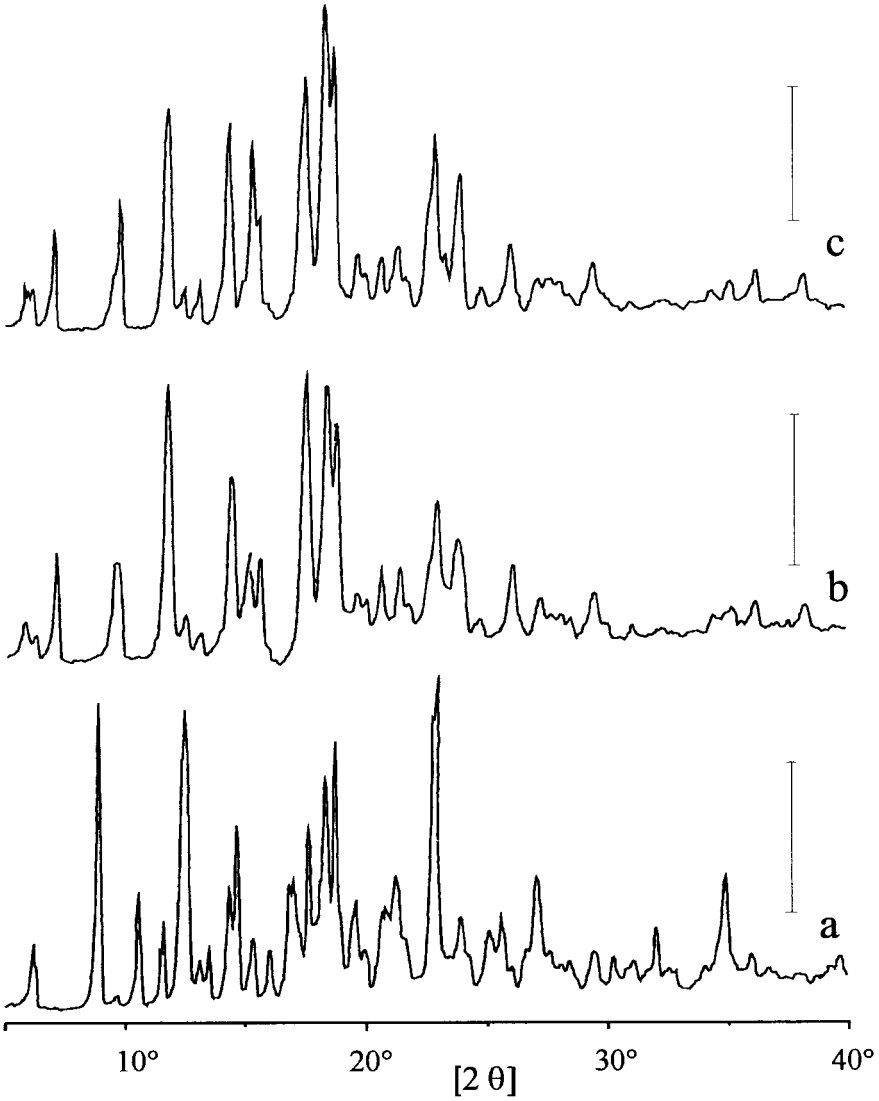


Figure 3. Comparison of XRD patterns for a mechanical 2 : 1 mixture at "time-zero" (a), for an equilibrated S21 sample (b) and a coprecipitated C21 sample (c).

peak higher than in (b). The most significant differences between patterns (b) and (c) are now seen near $2\vartheta \approx 15°$.

Figure 4 shows the "time-zero" (a) and vapour equilibrated (b) patterns of the 4 : 1 composition, for which no coprecipitated sample has been prepared. The vertical scales are the same, and the dominant features of both patterns are the reflections of free ketoprofen. The reaction causes the near extinction (or shifting) of all β-CD reflections. If the contribution of the free ketoprofen is discounted,

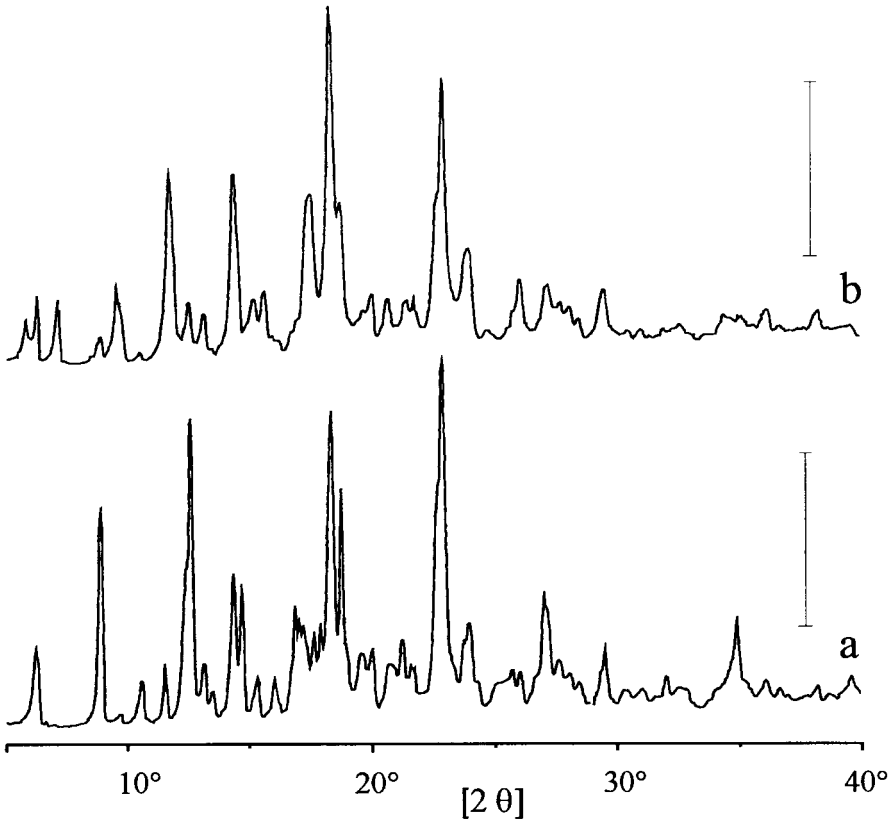


Figure 4. Comparison of XRD patterns for a mechanical 4 : 1 mixture at "time-zero" (a), and the equilibrated S41 sample (b). In this Figure, the vertical scales (1000 counts bar) are the same.

the (b) pattern is similar, both for peak positions and intensities, to that of the 2 : 1 vapour equilibrated sample (See Figure 3b).

3.2. KETOPROFEN/β-CD RATIO OF COMPLEXES

The DSC trace of pure ketoprofen shows only the melting peak near 94 °C which yields a heat of fusion of 114.2 ± 1.1 J/g. As described previously [7], the amount of free ketoprofen in the mixtures has been evaluated from the area of this melting peak; since the total amount of ketoprofen in each sample is known, the fraction of the reacted ketoprofen is thus obtained. The composition of the sample will be written as:

$$n(\beta\text{-CD} \cdot x\text{H}_2\text{O} \cdot y\text{ketoprofen}) + z\text{ketoprofen}$$

where $(ny + z)/n$ is the ketoprofen/β-CD molar ratio of the original mixture, z are the moles of free ketoprofen, and the water content of the complex, x, is obtained from the final mass of the sample (see, however, the procedure described in [7]).

For all compositions, the y vs. time plots show that a few weeks of storage in a wet atmosphere are sufficient to complete the reaction, and attain a stable value of y. Note that the equilibrium value of y depends upon the starting composition, and that it achieves a nearly constant value of 1.15 ± 0.03 for a ketoprofen/β-CD molar ratio $\geq 3:1$. This can be clearly seen in Figure 5, where the results obtained with the coprecipitated samples are also reported for comparison. These data confirm that, for ketoprofen/β-CD ratios of $1:1$ and $2:1$, the y value of coprecipitated samples is near 0.58, as found before [7]. In the S12 and C12 samples all the ketoprofen has reacted ($y = 0.5$). The same is true for the S13 sample, where $y = 0.33$.

It is customary to describe complexation in terms of a mixture of simple stoichiometric species: the y value of 0.58 observed in the coprecipitated samples would be obtained if 75% of the ketoprofen molecules are bonded to two β-CDs while the remaining 25% are bonded one-to-one. Similarly, the y value of 1.15, characteristic of the ketoprofen-rich mixtures, would imply that 85% of β-CD molecules are bonded to one ketoprofen molecule, while 15% are bonded to two. However, we will argue that this description is somehow misleading, and of little use in understanding the microscopic interactions which lead to the complex.

A qualitative explanation of the difference in complexation between corresponding coprecipitated and vapour equilibrated samples is as follows. In coprecipitated samples, complexation takes place in an aqueous solution, with a ketoprofen concentration which is usually limited by the small solubility of this compound, which is smaller than the solubility of β-CD. Therefore, as long as the solution is saturated, complexation takes place with an effective ratio of the two reagents which is determined by their solubility, rather than by their overall amounts. On the other hand, complexation with a low ketoprofen/β-CD ratio is a very slow process, and the slightly soluble complex precipitates long before having had the possibility of reaching the equilibrium stoichiometry.

It seems that vapour equilibration is more efficient than coprecipitation in binding ketoprofen to β-CD. Apparently, β-CD forms with ketoprofen a range of complexes, and the statistical distribution among these is preparation and concentration-dependent. For example, for the $2:1$ composition, only about one quarter of ketoprofen reacts in solution (C21 sample), while almost one half of ketoprofen reacts in the solid state (S21 sample) (see Figure 5). However, in the S21 sample, there is plenty of excess ketoprofen to reach the empirical complexation limit of $y = 1.15$; the fact that this does not happen means that the microscopic interactions are too subtle to be described in terms of formation energies of a few well defined "inclusion" species. Also the complexation limit of the ketoprofen-rich mixtures, achieved already with the $3:1$ composition, may point to a complex crystallo-

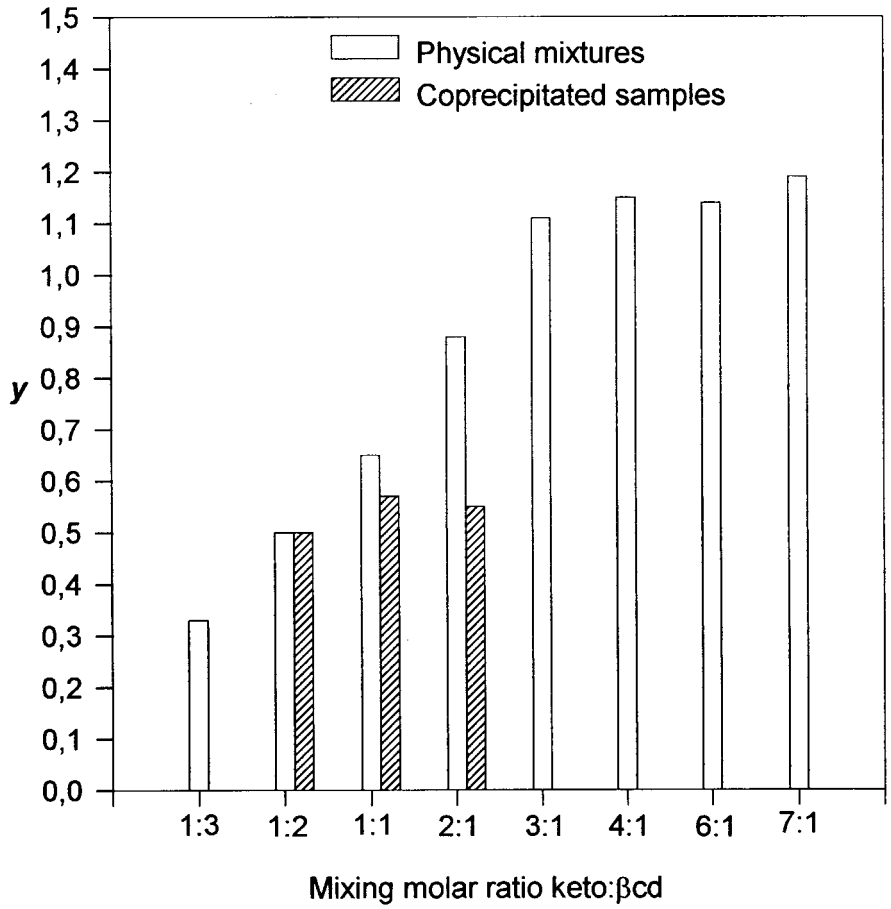


Figure 5. Ketoprofen/β-CD equilibrium complexation ratio for vapour-stabilised and coprecipitated samples of different initial compositions.

graphic structure, similar but, according to X-ray data, not identical to that achieved by coprecipitation.

3.3. β-CD WATER CONTENT

Samples of Cβ-CD and Sβ-CD display very similar TGA/DSC traces. A TGA/DSC curve of a Sβ-CD sample is reported, as an example, in Figure 6. The small weight increase at the beginning of the scan is believed to be related to the run starting a few degrees below rt (the storage temperature) in order to record the beginning of dehydration. Water loss occurs in a single stage in the 26–90 °C interval and corresponds to 11.9 ± 0.2 moles of water per mole of β-CD.

Evaluation of the enthalpy change associated with dehydration is somewhat ambiguous because the DSC trace displays a rather asymmetric peak, where the

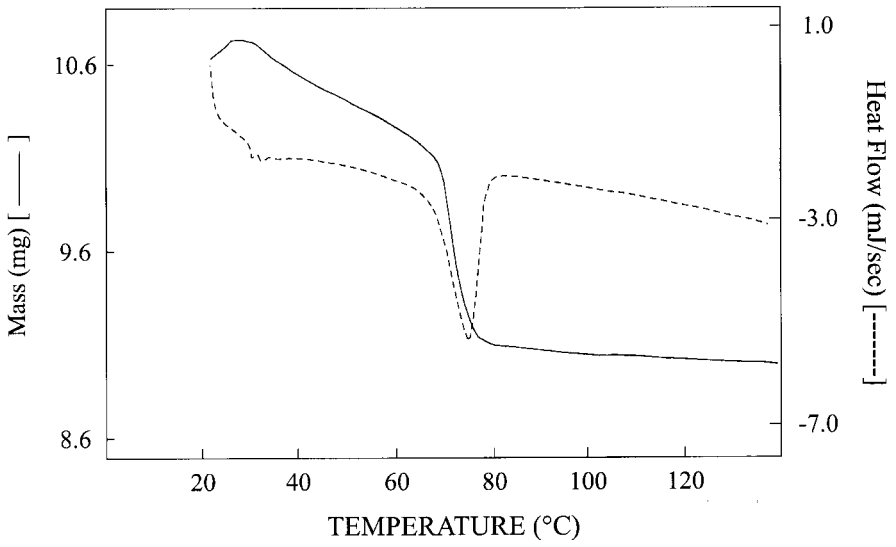


Figure 6. Simultaneous TGA/DSC scan of the Sβ-CD sample.

baseline cannot be accurately identified. However, by consistently using the same peak – closing criterion, our enthalpy data can be reproduced within ~1.5%: over a set of 10 fully hydrated samples we measured a dehydration enthalpy of 43.8 ± 0.7 kJ/mole H_2O.

3.4. WATER CONTENT OF COPRECIPITATED SAMPLES

The TGA/DSC traces of coprecipitated samples are qualitatively similar to those of vapour equilibrated samples (see Figure 7). There are three distinct stages of mass loss, with corresponding DSC peaks; the fourth DSC peak at 94 °C is due to ketoprofen melting, and it is absent in the C12 sample. Since the DSC peaks occur in well defined temperature intervals, we have associated with the enthalpy intake given by the DSC peak areas (ΔH_1, ΔH_2, ΔH_3) the amounts of water (n_1, n_2 and n_3) released in these temperature intervals. Outside these intervals, dehydration continues with a low rate, and there is water loss, but not obvious heat intake, from 70°C to 140°C. There is a major difference relative to pure β-CD, where a single dehydration stage is present. The pertinent parameters are the moles of water lost per mole of β-CD at various stages (n_i) and the corresponding dehydration enthalpies (ΔH_i); they are summarised in Table 1.

Since a substantial amount of water is released between the stages, the overall water content, n_{tot}, is always higher than $n_1 + n_2 + n_3$. The mean values of n_{tot} are somewhat different among the samples of different composition (overall deviation of n_{tot} = 0.4), but rather close to the common mean value of 10.8 reported before [7], which should be compared with $n_{\text{tot}} = 11.9 \pm 0.2$ of pure β-CD samples.

Table I. Number of H_2O moles per mole of β-CD released at various stages and specific dehydration enthalpies of different complexes

Sample	n_{tot}	n_1	n_2	n_3	ΔH_1 J/mole H_2O	ΔH_2 J/mole H_2O	ΔH_3 J/mole H_2O
C12	10.44 ± 0.95	3.74 ± 0.20	3.74 ± 0.09	0.97 ± 0.13	43790 ± 3470	45800 ± 1000	26300 ± 1500
C11	11.31 ± 0.09	4.08 ± 0.17	4.00 ± 0.11	0.38 ± 0.08	48050 ± 760	48040 ± 460	18400 ± 4600
C21	10.74 ± 0.25	3.51 ± 0.29	3.84 ± 0.12	0.43 ± 0.11	48500 ± 3660	48540 ± 680	16100 ± 3100
S12	11.77 ± 0.17	3.25 ± 0.31	3.28 ± 0.07	3.05 ± 0.17	40410 ± 600	39600 ± 6500	40500 ± 3400
S11	11.78 ± 0.04	4.05 ± 0.06	4.63 ± 0.01	0.86 ± 0.02	44290 ± 460	44000 ± 2100	17300 ± 600
S21	11.92 ± 0.30	3.85 ± 0.37	4.65 ± 0.07	0.89 ± 0.08	44370 ± 30	44700 ± 1600	15900 ± 2600

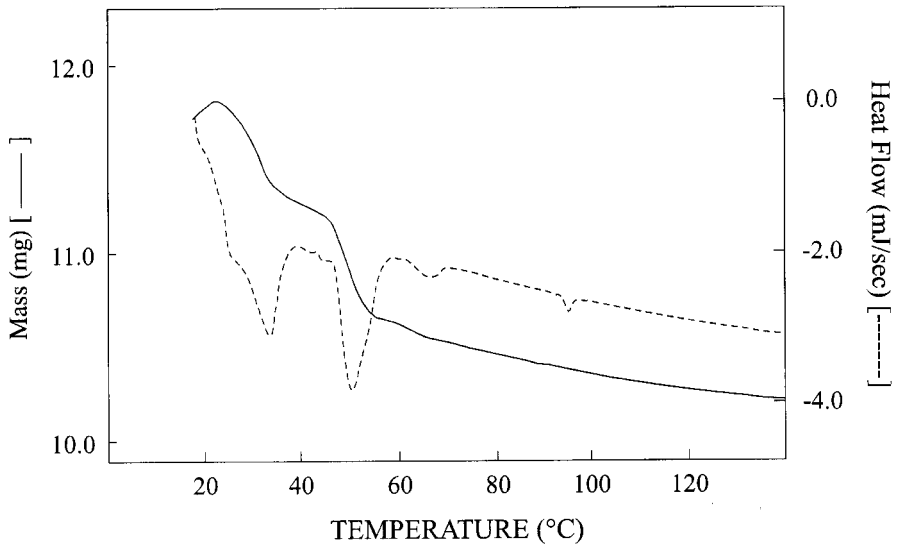


Figure 7. Simultaneous TGA/DSC scan of the S11 sample.

Relative to the other two samples, the C12 composition shows dehydration enthalpies significantly smaller for stages 1 and 2 and higher for stage 3. However, also for this sample, stage 3 has the lowest dehydration enthalpy per mole of water, contrary to the expectation that the more strongly bonded water is released last when heating. This is believed to be related to modifications of the β-CD structure occurring during dehydration [8] and/or with the so called entropy – enthalpy compensation effect [9, 10].

3.5. WATER CONTENT OF EQUILIBRATED SAMPLES

The TGA/DSC curve of a vapour equilibrated sample is shown in Figure 7. A significant difference with respect to the coprecipitated samples is the total water content, which has an overall average $n_{tot} = 11.8 \pm 0.1$, i.e. identical, within the experimental error, with that of pure β-CD. Therefore, the "rule" found in our previous work [7], for which every reacted ketoprofen molecule replaces a single water molecule in β-CD, applies to the coprecipitated samples but not to the vapour stabilised ones. As it is very unlikely that a keto molecule can enter the β-CD cavity without changing its water content, the invariance of the total amount of water indicates the following:

(a) redistribution between in-cavity and out-of-cavity water occurs as a consequence of guest inclusion;
(b) such a redistribution depends upon the complexation route, which in turn affects the host/guest ratio of the inclusion compound.

The dehydration enthalpies of the first two stages, ΔH_1 and ΔH_2, are systematically lower in the vapour stabilised samples, relative to those of the corresponding coprecipitated samples. For the S12 sample, ΔH_3 is unexpectedly high, and about equal to the dehydration enthalpies per mole of water of the first two stages. Note also, from Table 1, that n_3 for the S12 and C12 samples is significantly higher than in all other samples. Qualitatively, these observations mean that the complexes formed in the presence of excess β-CD are appreciably different, from the thermodynamic point of view, relative to those obtained in keto-rich mixtures. One view of the entropic – enthalpic compensation is that there is competition between the two terms in determining the equilibrium water content. As temperature increases and part of the water is released, the entropy of the remaining water, which moves over all available positions, increases and it becomes easier to overcome the enthalpic barrier of dehydration. If this mechanism is at work, it is not surprising that compensation is stronger ($\Delta H_1 - \Delta H_3 \approx 18$ kJ/mole H_2O) in C12, where $n_3 \approx 1.0$, than in S12 ($\Delta H_1 - \Delta H_3 \approx 0$ kJ/mole H_2O), where $n_3 \approx 3.0$.

4. Concluding Remarks

Inclusion complexes between ketoprofen and β-CD have been obtained by equilibrating physical mixtures of the solid components in a water vapour saturated atmosphere. As the equilibrated complexes consistently have the same water content of the equilibrated β-CD, we may be tempted to say that water vapour acts as a catalyser. However, the starting product (as received β-CD) has a *lower* water content than the equilibrated products (ca 11 mol H_2O/mol β-CD vs. 11.9 mol H_2O/mol β-CD). Thus, water vapour enters the reaction and must be considered a *reagent* rather than a catalyser. Probably, the water vapour acts as a ketoprofen carrier. How this happens and why the same amount of water enters the β-CD with or without carried ketoprofen is not clear at present. Both questions deserve further studies. We stress that no reaction occurs, over one year, in mixtures at rt and relative humidity between 50% and 60%, i.e., when no water intake occurs. Therefore, we may call the proposed preparation route "vapour promoted solid state reaction".

Both the amount of ketoprofen reacting with a β-CD molecule and the complexation rate increase on increasing the guest/host ratio. Typical reaction times are only a few weeks, and the method is more efficient than coprecipitation, besides being more suited for industrial production. With three or more moles of ketoprofen per β-CD mole, we have a new inclusion compound, not obtained through coprecipitation, with a guest/host ratio of about 1.15, which appears to be indefinitely stable at rt and RH $\sim$50%.

We have pointed to similarities and differences among the complexes studied in this work. It is clear that the preparation route plays a major role and, for ketoprofen – rich samples, two well defined and distinct complexation limits are obtained by coprecipitation (y = 0.58) and solid state reaction (y = 1.15). The

differences between the 1 : 2 samples prepared by the two methods are more subtle, but noticeable.

A key point of this work is the analysis of the dehydration process in pure and complexed β-CD. Water vapour not only acts as a catalyst in the solid state reaction, but water redistribution, rather than removal, is a major consequence of complexation. This holds true also for the coprecipitated samples, where about one water molecule is lost per ketoprofen molecule. Both types of complexes display a similar multistage process of thermal dehydration, with the enthalpy of dehydration of the highest temperature stage which is usually substantially smaller than at the lower temperatures. This phenomenon is believed to be related to the entropy-enthalpy compensation effect [8–10].

References

1. D. Amdidouche, H. Darrouzet, D. Duchene and M. C. Poelman: *Int. J. Pharm.* **54**, 175 (1989).
2. S. Y. Lin and Y. H. Kao, *Int. J. Pharm.* **69**, 211 (1991).
3. Y. Nakai, K. Yamamoto, K. Terada, and D. Watanabe: *Chem. Pharm. Bull.* **35**, 4609 (1987).
4. Y. Nakai, K. Yamamoto, K. Terada, T. Oguchi, H. Saito, and D. Watanabe, *Chem. Pharm. Bull.* **37**, 1055 (1989).
5. Y. Nakai, K. Yamamoto, T. Oguchi, E. Yonemochi, and T. Hanawa: *Chem. Pharm. Bull.* **38**, 1345 (1990).
6. Y. Nakai, K. Yamamoto, T. Oguchi, E. Yonemochi, and T. Hanawa, *Chem. Pharm. Bull.* **39**, 1532 (1991).
7. A. Marini, V. Berbenni, G. Bruni, P. Mustarelli, F. Giordano, and M. Villa, *J. Incl. Phenom.* **22**, 221 (1995).
8. A. Marini, V. Berbenni, G. Bruni, V. Massarotti, P. Mustarelli and M. Villa, *J. Chem. Phys.* **103**, 7532 (1995).
9. B. Zhang and R. Breslow, *J. Am. Chem. Soc.* **115**, 9353 (1993).
10. Y. Inoue, Y. Liu, L. Tong, B. Shen, and D. Jin, *J. Am. Chem. Soc.* **115**, 10637 (1993).