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BIFONAZOLE-β-CYCLODEXTRIN INCLUSION COMPLEXES
Thermal analysis and X-ray powder diffraction study

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Abstract

The complexation of bifonazole, an antimycotic hydrophobic imidazole derivative, with β-cyclodextrin (β-CD) was investigated in solid phase, using the following complementary techniques: differential scanning calorimetry (DSC), thermogravimetric analysis (TG) and X-ray powder diffractometry. The β-CD-bifonazole samples were prepared in both aqueous medium by coprecipitation and in solid state by kneading method and the β-CD-bifonazole binary diagrams were drawn. The experimental results demonstrate the formation of two binary compounds, β-CD-bifonazole and $(\beta\text{-CD})_x$-bifonazole (x=2 or 4). The first compound may be an inclusion compound and the second a crystallized compound, in which the bifonazole is not necessarily included in the cyclodextrin internal cavity.

Keywords: β-cyclodextrin, bifonazole, coprecipitation, inclusion complexes, kneading method, thermal analysis, X-ray powder diffraction

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides constituted of α-(1,4)-linked D-glucopyranose units. β-CDs (Fig. 1) are composed of 7 glucopyranose units. The CD molecule features a cylinder-shaped, macro ring structure with a large internal axial cavity: the outer surface is hydrophilic, but the internal cavity is apolar [1, 2]. CDs, or so-called host molecules, have been recognized as useful pharmaceutical excipients, as they are able to interact with appropriately sized molecules to result in the formation of host-guest inclusion complexes. These non-covalent complexes offer a variety of physico-chemical advantages over the unmanipulated drugs including the possibility for increased water solubility, solution stability, dissolution rate and bioavailabil-

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ity [3, 4]. Several methods have been employed to obtain the cyclodextrin complexes, such as kneading, coprecipitation, freeze drying, spray drying, cogrinding and sealed heating [5].

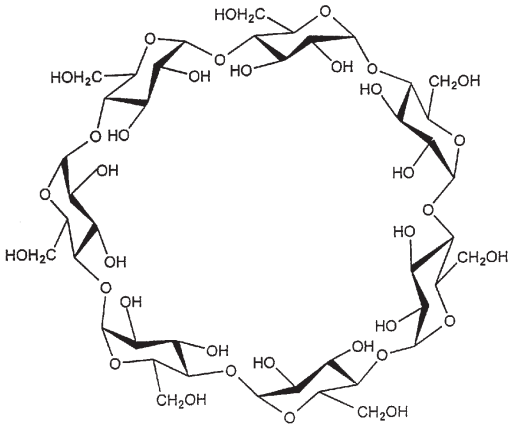

Fig. 1 β-cyclodextrin chemical structure

Imidazole derivatives, such as bifonazole (Fig. 2), are used for the treatment of onychomycosis. These hydrophobic compounds have a weak penetration into hydrophilic human nail matrices. Their inclusion in the apolar cyclodextrin cavity could improve this penetration considering the hydrophilic character of the cyclodextrin cavity rim.

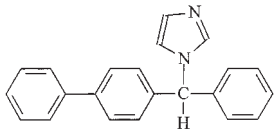

Fig. 2 Bifonazole chemical structure

Thermal analysis and X-ray powder diffraction analysis of pharmaceuticals are generally used as screening methods for the purity determination and the characterization of excipients and active compounds. For the study of cyclodextrins, DSC is often used to determine the existence of an inclusion complex: the existence of a melting endotherm of the crystallized compound to be included meaning that the complex is not formed, the lack of this melting endotherm means the formation of a complex. It is likely that the endothermic peaks shown on DSC curves is due to invariant equilibria of binary systems. Thus, the aim of this study was to draw the phase diagrams of the β-CD-bifonazole system in order to show the existence of intermediate compounds. The composition of the compounds, the limits of the different phase domains and the invariant equilibria which occur could be drawn in relation to mole fraction and temperature. Samples obtained by

both preparation methods, kneading and coprecipitation, were analyzed and the results were compared.

Materials and methods

Solvents and samples

Bifonazole was obtained from Lipha (Lyon, France). β-cyclodextrin was provided by the Roquette Laboratories (Lestrem, France). Acetone (Prolabo, Paris, France) was used without further purification. Distilled water was applied.

Apparatus

X-ray diffraction patterns were obtained with CGR and INEL CPS 120 high resolution powder diffractometers by using the wavelength of CuK_{α_1} radiation (λ=1.5406 Å).

DSC measurements were carried out by using a heat flux TA 2000 apparatus. The instrument was flushed with nitrogen. Experiments were performed with a heating rate of 10°C min^{-1} over the temperature range of 20–300°C, on 5 to 10 mg samples which were transferred into weighed open aluminium crucibles. In this way, water and volatile compounds escape during the heating process. In order to allow the accurate evaluation of endothermic effects in a β-CD-bifonazole system, a thermal cycle was set up [6, 7]. The samples were first heated to 135°C and kept at this temperature for 5 min, then allowed to cool to 100 and finally rescanned up to 300°C. Thus, the invariance peak observed in the β-CD-bifonazole binary systems was isolated from the large endothermic peak corresponding to the loss of water molecules which represented a component of the β-cyclodextrin molecules.

TG analyses were performed on a DuPont 951 Thermogravimetric Analyzer. The instrument was flushed with nitrogen.

Preparation of solid inclusion complexes

Kneading method

The method we used was based on that of Furata *et al.* [8]. Various quantities of β-CD were mixed with 10 mg of bifonazole. Distilled water was added to have a final mole ratio water: β-CD equal to 35. Then, the mixture was gathered in stainless steel crucibles which contained two stainless steel balls. The crucibles were placed on the axis of a tridimensional movement MM10 microstirrer (LaboModerne, Paris, France) and mixed continuously for an hour at a rotating speed of 60 rpm. Then, the samples were dried and stored at room temperature. The whole range of compositions was investigated by steps of mole fractions x=0.05.

Coprecipitation

Solutions of β-CD (4% w/w) prepared with distilled water were heated to 65°C. 10 mg of bifonazole were dissolved in 25 mL acetone and added to the β-CD aqueous

solutions. The final solutions were then mixed continuously with an Ika magnetic stirrer (Prolabo, Paris, France) and heated to 65°C. The organic solvent was allowed to evaporate and the mixtures were cooled to 5°C. The crystals were separated by filtration through 0.2 μm Sartorius cellulose acetate membrane filters (Goettingen, Germany). Then, the samples were dried and stored at room temperature [9]. The whole range of compositions was investigated by steps of mole fractions x=0.05.

Results and discussion

X-ray powder diffraction study

Twenty-one samples were analyzed.

Firstly, the pure compounds, bifonazole and β-cyclodextrin, were analyzed with a CGR powder diffractometer. Then, all the patterns obtained for the other compositions were compared with the pure compounds. This procedure has been carried out with samples prepared by both kneading and coprecipitation methods.

By comparing the X-ray diffraction patterns, the different phases present at room temperature could be identified for each composition. Two new compounds, named X and Y, were observed on the patterns obtained for samples prepared by coprecipitation. Only one was observed with the kneading method.

Figure 3 summarizes the results obtained from both sample series. The ranges of composition where X, Y, β-CD and bifonazole are present, are shown.

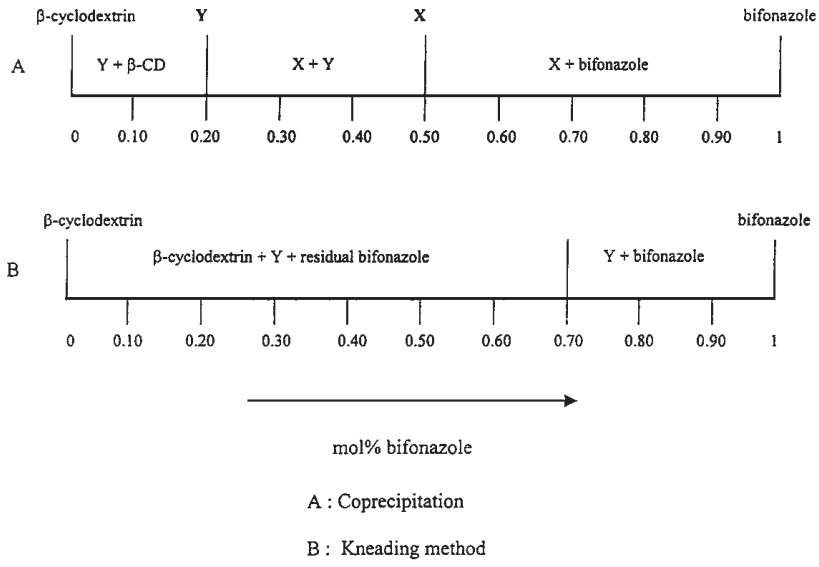

Fig. 3 Schematic representation showing the ranges of composition in which X, Y, β-CD and bifonazole exist for samples prepared by coprecipitation (A) and kneading method (B)

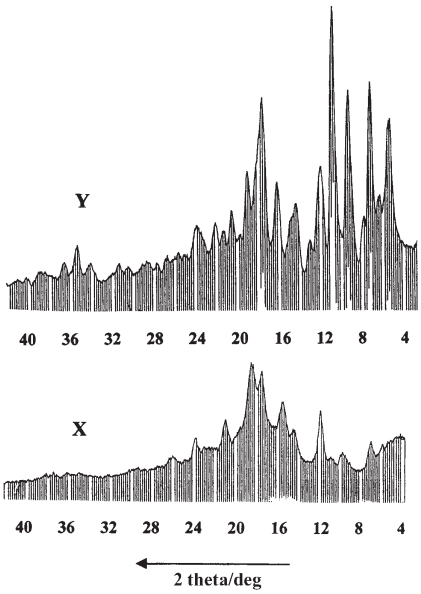

Fig. 4 X-ray diffraction patterns (CGR diffractometer) of Y and X compounds obtained by the coprecipitation method

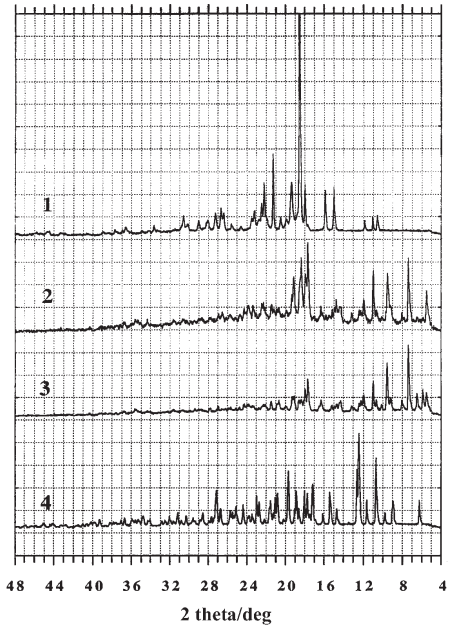

Fig. 5 X-ray diffraction patterns obtained from INEL diffractometer: 1 – pure bifonazole; 2 – $X_{\text{bifonazole}}$=0.2; 3 – $X_{\text{bifonazole}}$=0.5; 4 – pure β-cyclodextrin

Coprecipitation

For $0 \leq x \leq 0.20$ two phases were observed: Y and β-CD. $x=0.20$ corresponds to pure Y. For $0.20 < x \leq 0.50$ two phases were observed, Y and X. $x=0.50$ corresponds to the new compound X. For $x \geq 0.50$, both X and bifonazole phases were observed.

Figure 4 shows the X-ray diffraction patterns obtained for both compounds X ($x_{bifonazole}=0.50$) and Y ($x_{bifonazole}=0.20$) with the CGR diffractometer. Analyses were then carried out with a high resolution INEL diffractometer. In Fig. 5, pure bifonazole and β-cyclodextrin are compared to preparations obtained by coprecipitation method for $x_{bifonazole}=0.20$ and $x_{bifonazole}=0.50$. These analyses confirm that neither bifonazole nor β-cyclodextrin were present in these samples. Only mixtures of X and Y compounds could be identified.

Tables 1 and 2 show the lists of the interreticular distances of X and Y compounds obtained from CGR and INEL diffractometers.

Table 1 Interreticular distances of X compound obtained from both CGR and INEL diffractometers

CGR			INEL	
2 theta/°	d_{hkl}/Å	Intensity	2 theta/°	d_{hkl}/Å
			5.54	15.94
7.0	12.62	M	7.20	12.27
9.8	9.02	M	10.20	8.66
11.8	7.49	S	11.90	7.43
14.4	6.15	w	14.80	5.98
15.5	5.71	M		
17.5	5.06	M	17.65	5.02
			18.35	4.83
18.4	4.82	S	18.50	4.79
			19.35	4.58
21.0	4.23	M		
24.0	3.70	w		

Kneading method

Some peak intensity changes, observed between untreated and kneaded bifonazole X-ray patterns, show that the kneading treatment induces a decrease in the crystallization degree of the compounds. Nevertheless, the samples prepared by the kneading method only showed the formation of the Y compound. A high proportion of this compound was obtained for the compositions equal to 0.50 and 0.70. Furthermore, the X-ray patterns show that free bifonazole (non bonded with β-CD molecules) exists in the whole range of compositions. Thus, it can be assumed that equilibria were not completely reached by this preparation method: the complexation was only partial.

Table 2 Interreticular distances of Y compound

CGR			INEL	
2 theta/°	d_{hkl}/Å	Intensity	2 theta/°	d_{hkl}/Å
5.6	15.77	M	5.90	14.97
6.2	14.24	w	6.48	13.63
7.2	12.27	S	7.35	12.02
7.8	11.32	vw	8.03	11.00
			9.15	9.66
9.3	9.50	S	9.53	9.27
			10.10	8.75
			10.70	8.26
10.8	8.18	S	10.95	8.07
12.0	7.37	M	12.20	7.25
			12.40	7.13
13.0	6.80	vw	13.20	6.70
14.5	6.10	M	14.30	6.19
15.0	5.90	M	14.90	5.94
			15.20	5.82
16.2	5.47	M	16.28	5.44
17.6	5.03	S	17.65	5.02
			17.95	4.94
19.0	4.67	M	19.10	4.64
			19.30	4.59
19.8	4.48	vw	20.00	4.44
20.6	4.31	w	20.65	4.30
21.3	4.17	w	21.50	4.13
22.0	4.04	w	22.15	4.01
23.8	3.74	M	24.20	3.67

Conclusions

The X-ray diffraction patterns of samples prepared by coprecipitation showed two binary compounds, called X and Y. The pure intermediate compounds were obtained for mole fractions x=0.50 and x=0.20. The kneading method was less successful. No X compound was found in these conditions. Only the Y compound formed in the whole range of composition. In the β-CD rich part, residual bifonazole was observed. But for $x_{bifonazole}$>0.70, β-CD was no more observed (Fig. 3).

At this stage of the study, the powder diffraction patterns offered no information on bifonazole inclusion in the cyclodextrin cavity. Nevertheless, considering the bi-

fonazole size and the internal diameter of the β-CD cavity, the X binary compound may be a 1:1 (β-CD:bifonazole) inclusion compound, while it is unlikely that the Y stoichiometry, 4:1 (β-CD:bifonazole) corresponds to that of an inclusion compound. In the latter case, bifonazole and β-CD probably form a crystallized intermediate compound, in which the bifonazole molecules are not necessarily included in the internal cyclodextrin cavity.

β-*CD – bifonazole phase diagram*

Pure bifonazole

The DSC curve of pure bifonazole showed a sharp endothermic peak at T_f=148.7°C, corresponding to the bifonazole melting point. The melting enthalpy, ΔH_f, is equal to 110 J g^{-1}. The decomposition starts above 225°C. When liquid was quenched from 175°C, glass formed and after a second heating, crystallization took place between 90–135°C. This result is confirmed by adding the values of the enthalpy changes associated with the exothermic and endothermic effects: $\Delta H_{exo}+\Delta H_{endo}\approx 0$ (Fig. 6).

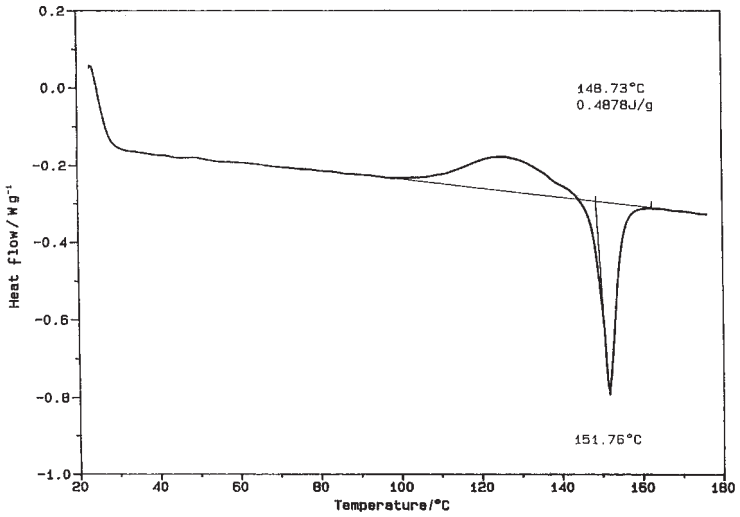

Fig. 6 DSC curve of bifonazole after quenching of liquid from 175°C

Pure β-cyclodextrin

The DSC and TG curves of pure β-CD are shown in Fig. 7. A first endothermic effect occurs between 20 and 150°C and corresponds to the dehydration of β-CD. The water loss is confirmed by the thermogravimetric curve, indicating that β-CD contains 12 moles of water which are lost up to 150°C. A second thermal effect, at 212°C, represents a processus corresponding probably to a molecular reorganization of the β-CD. No mass loss is observed at this temperature. The last endothermic peak at 288 is fol-

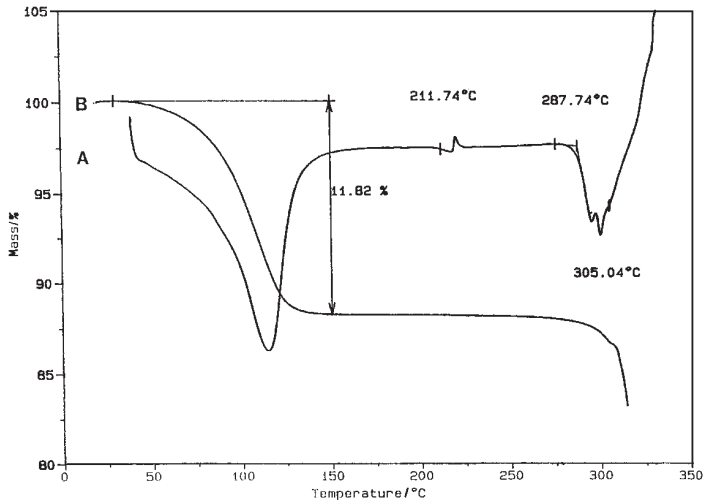

Fig. 7 DSC (A) and TG (B) curves of pure β-CD

lowed by an exothermic peak at 305°C. These two peaks are associated with the decomposition of β-CD [1].

β-CD – bifonazole system

The β-CD-bifonazole samples prepared by both coprecipitation and kneading methods were submitted to the thermal cycle described in the experimental section. This treatment was used to separate the endothermic peaks associated with the dehydration and the invariant equilibrium. A perfect base line could be obtained which made it possible to integrate the invariant peak. Thus, all the water was lost before the invariant equilibrium started. The phase equilibria which appeared at temperatures higher than of the β-CD dehydration were studied. 21 samples were analyzed, with bifonazole mole fractions from 0 to 1, by steps of 0.05.

Analysis of samples prepared by coprecipitation

Phase diagram

Analyses were carried out over the whole range of compositions between β-CD and bifonazole. The results are shown in Fig. 8.

At the β-CD end (x= 0.05 to 0.15) of the diagram, there is no visible endotherm before the β-CD decomposition temperature, except for the large one corresponding to the loss of water.

At the bifonazole-rich end, a double endothermic peak occurs for x=0.95 which is characteristic of an invariant equilibrium followed by a liquidus monovariant. On this DSC curve, the invariant temperature is 140.5 and the liquidus temperature is 148.7°C.

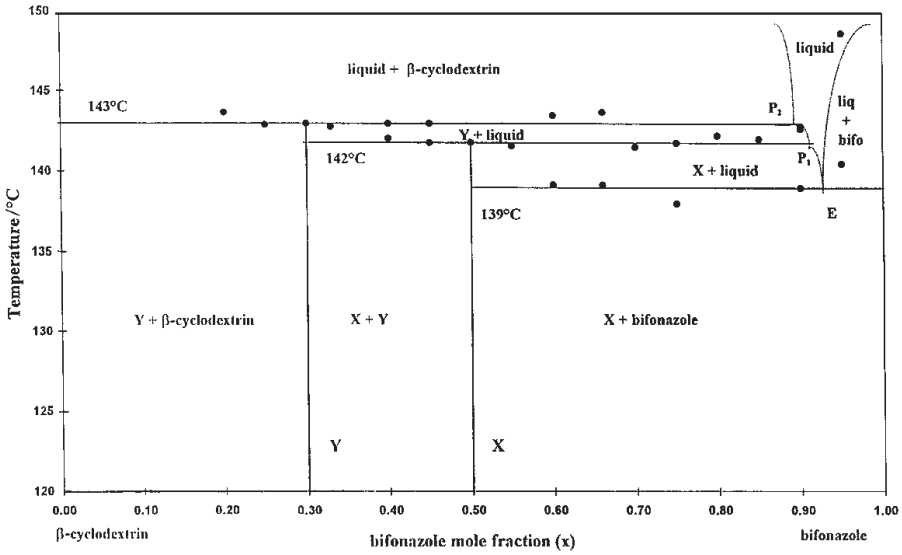

Fig. 8 β-CD-bifonazole binary phase diagram drawn from samples prepared by coprecipitation

The interpretation of the DSC curves obtained for all the other compositions is rather difficult. All are associated with eutectic and peritectic equilibria related to the existence of either X or Y compounds, at temperatures between 139 and 143°C.

The peaks do not appear to be single peaks. For some compositions, it is possible to define two onset temperatures because of the two slope peaks. In the composition range from x=0.25 to x=0.35, the onset temperature is about 143°C. For x=0.40 and x=0.45, two onset temperatures can be determined at 142 and 143°C.

On the bifonazole-rich end of the diagram, the first onset temperature is 139°C. It is attributed to an eutectic equilibrium.

Then, some peaks present a second slope whose onset is either 142 or 143°C from x=0.50 to x=0.65, and 142°C all the way from x=0.70 to 0.90. The differences between these temperature values are weak, but their distribution over the composition range is consistent with the existence of two invariant equilibria due to the incongruent melting of compounds X and Y.

Thus, it can be assessed that at 139°C an eutectic equilibrium occurs which corresponds to the reaction:

$$X_{solid} + \text{bifonazole}_{solid} \leftrightarrows \text{eutectic liquid E}$$

The onset observed at 142°C is attributed to a peritectic equilibrium:

$$X_{solid} \leftrightarrows Y_{solid} + \text{peritectic liquid } P_1$$

The onset observed at 143°C is attributed to a second peritectic equilibrium:

$$Y_{solid} \leftrightarrows \beta\text{-}CD_{solid} + \text{peritectic liquid } P_2$$

The composition of the eutectic liquid is $x_{bifonazole} \approx 0.93$, and the compositions of the peritectic liquids P_1 and P_2 are situated in the interval 0.90–0.93.

Tammann diagram

As the three temperatures given above were close, measurement of the enthalpies of the three invariant equilibria was not possible. Thus, a Tammann diagram was plotted in which, for each composition, the whole thermal effect was taken into account (Fig. 9).

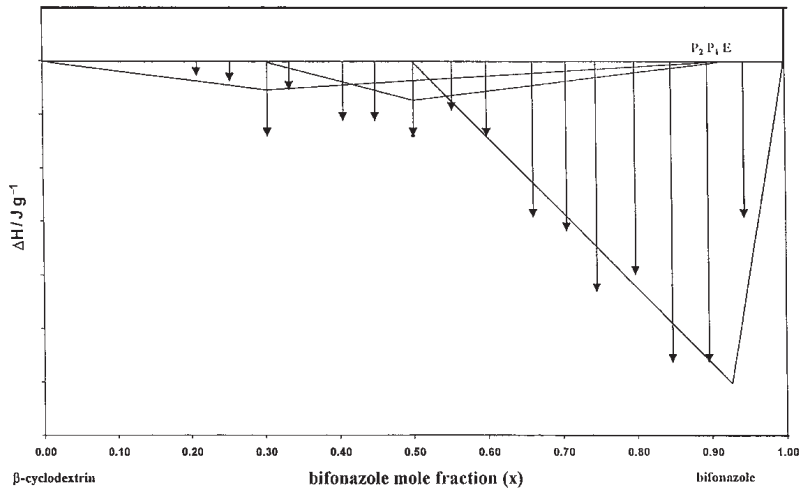

Fig. 9 Tammann diagrams drawn from samples prepared by coprecipitation

However, the different slopes which are shown in this diagram suggest that each ΔH value results in the superposition of two or three thermic effects. From these values, three Tammann diagrams were reconstituted for the three invariant equilibria.

In the bifonazole rich part of the diagram, the major thermal effect seems to be that of the eutectic equilibrium which occurs at 139°C. It can be assessed from the Tammann diagram that the mole fraction of the eutectic is $0.90<x<0.95$ and the composition of the X compound is $x=0.50$.

In the β-CD-rich part of the diagram, the thermic effects are very weak. In the middle part of the diagram, between $x=0.20$ and $x=0.50$, the two peritectic equilibria are superposed and the Tammann diagram suggests that the Y compound occurs at $x \approx 0.3$ (β-CD:bifonazole=2:1).

X-ray diffraction analysis of quenched samples

Further study was carried out to determine, by means of X-ray diffraction analysis, the composition at temperatures higher than those of eutectic and peritectic invariants of samples prepared by the coprecipitation method.

First, pure β-CD was quenched from 180 and 260°C and analysed by powder X-ray diffraction method with CGR diffractometer. Figure 10 shows the diffraction patterns. In both experiments (Fig. 10, B and C) β-CD has lost water and room temperature pattern (A) is not observed. Furthermore, the B and C patterns are not similar, which must be due to the structure modification at 212°C observed on the DSC curve of Fig. 7.

In the coprecipitation method, three samples corresponding to bifonazole mole fraction $0.3 \leq x \leq 0.5$ in which both X and Y compounds were present, were quenched from 130, 160 and 220°C. Powder X-ray diffraction analyses were performed just after quenching. The diffraction patterns were compared to those obtained at room temperature. After quenching from 130°C, the pattern was not modified: both diffraction peaks of X and Y were present. After quenching from 160 and 220°C, i.e. higher than the eutectic and peritectic invariants, only two wide peaks were observed for θ=8.85°

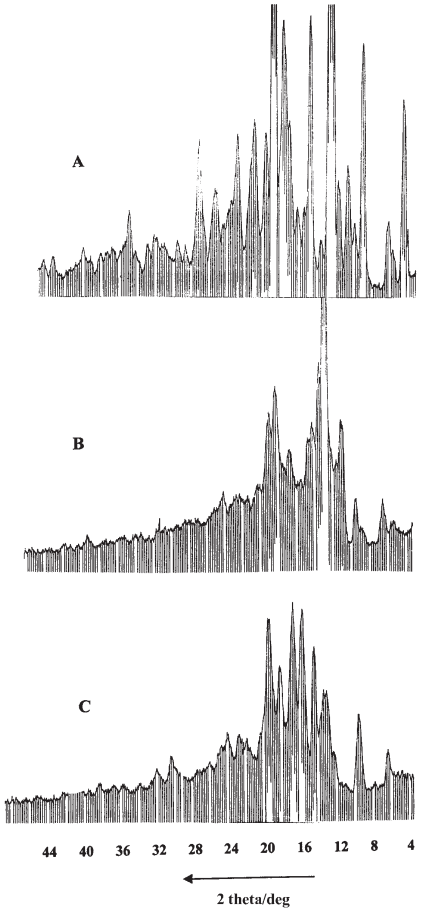

Fig. 10 X-ray diffraction patterns (CGR diffractometer of quenched β-cyclodextrin): A – room temperature; B – quenched from 180°C; C – quenched from 260°C

and θ=5.85°: the sample seems to be amorphous. The temperatures which were chosen for these two quenching correspond to the β-CD – liquid domain of the phase diagram (Fig. 8). The liquid phase is bifonazole-rich and it has been shown that bifonazole became amorphous by cooling. But no peak was present which belong to the quenched β-CD (as observed in Fig. 10).

It can be assumed that the incongruent melting of X and Y compounds is followed by a modification of the β-CD and that the resulting product becomes amorphous when quenched. These results involve some limits to the phase diagram representation since above the peritectic invariants a modified form of β-CD is present.

Analysis of samples prepared by kneading method

Analyses were carried out over the whole range of compositions between β-CD and bifonazole. The results are shown in the diagram of Fig. 11.

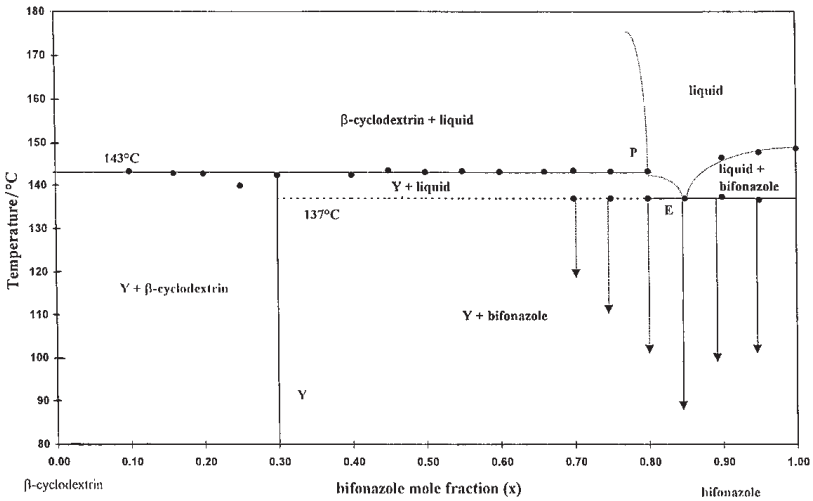

Fig. 11 β-CD-bifonazole binary phase diagram drawn from samples prepared by kneading method and partial Tammann diagram

For 0.70≤*x*≤0.95, an eutectic invariant is shown at 137°C which is associated with a three-phase equilibrium involving solid bifonazole, eutectic liquid and a second solid phase. It can be assessed that this second phase is the Y compound observed on X-ray diffraction patterns. Moreover, the temperature of this invariant equilibrium (137°C) is lower than that of the eutectic invariant observed with coprecipitation samples between solid X and solid bifonazole (139°C).

Then, the eutectic equilibrium is:

$$Y_{solid} + \text{bifonazole}_{solid} \leftrightarrows \text{eutectic liquid E} \quad (T \approx 137°C)$$

The onset at T=137°C is no longer observed for x<0.70.

For x=0.95 and x=0.90 a double endothermic effect occurs and a typical liquidus is observed, following the eutectic invariant, at 147.8 and 146.5°C.

As the onset temperature of invariant peaks slightly increases up to 143°C, for $x \leq 0.80$, it is likely that a second invariant takes place, starting from this mole fraction.

This invariant is a peritectic invariant resulting in the three-phase equilibrium:

$$Y_{solid} \leftrightarrows \beta\text{-}CD_{solid} + \text{peritectic liquid P} \quad (T=143°C)$$

where the composition of the peritectic liquid is $x_{bifonazole} \approx 0.80$.

X-ray diffraction patterns showed that bifonazole was present in the whole range of compositions, which suggests that equilibrium was not obtained when samples were prepared by the kneading method. This must be the reason why a coherent Tammann diagram could not be plotted with DSC results. Thus, only the start of the Tammann diagram in the bifonazole-rich part of the diagram was drawn, where only one invariant equilibrium occurs. This made it possible to obtain the eutectic composition: $x \approx 0.85$.

Conclusions

When studying the complexation of cyclodextrin inclusion compounds by differential scanning calorimetry, the melting enthalpy of the compound to be included is usually measured. Then, as $\Delta H_f=0$, the guest molecules are thought to be included, since there is no longer any melting point associated with the presence of a crystallized compound, before the β-CD decomposition.

It appeared that systems guest molecule-β-CD obtained from preparation methods such as kneading or coprecipitation were binary systems to which the Gibbs phase rule might be applied and that the measured enthalpy did not correspond to the melting of a pure compound but to an invariant equilibrium involving three-phases. Thus, the existence of a binary compound is consistent with a non null enthalpy associated with the peritectic decomposition of the compound occurring at a lower temperature than the decomposition temperature of pure β-CD.

The β-CD-bifonazole phase diagram, plotted for samples prepared by the coprecipitation method, shows three invariant equilibria at 139, 142 and 143°C. All these temperatures are lower than the melting temperature of pure bifonazole, which is consistent with three-phase equilibria. The first is an eutectic equilibrium between a new binary compound 1:1 (β-CD:bifonazole) and bifonazole. The second is a peritectic equilibrium associated with the incongruent melting of the 1:1 compound. The third is a second peritectic equilibrium due to the incongruent melting of a second binary compound which forms at $x_{bifonazole} \approx 0.3$ and which may be a 2:1 compound (β-CD:bifonazole). The approximate compositions of the two new compounds are determined from the Tammann diagram results.

X-ray powder diffraction patterns confirmed the existence of the two new binary compounds, though the compositions of Y compound given by the X-ray diffraction

were found to be 4:1 rather than 2:1 as concluded from the DSC analysis. The X-ray diffraction study also made it possible to identify the two-phase fields of the diagram.

The phase diagram plotted for samples prepared by kneading method, shows only two invariant equilibria. The first one, at T=137°C is an eutectic equilibrium between a binary compound which is identified from X-ray patterns as the x≈0.3 compound. The second one, at T=143°C, corresponds to the peritectic decomposition of this compound. Both DSC and X-ray diffractometry indicate that the 1:1 compound is not present.

Although it can be assessed from X-ray diffraction patterns and from DSC that two binary compounds were formed for x≈0.3 and x≈0.5 respectively, none of these two analysis techniques makes it possible to determine whether these compounds are inclusion complexes or binary compounds without any inclusion.

Nevertheless, it is likely that the 1:1 compound is an inclusion complex whereas the 2:1 compound is a binary compound where β-CD-bifonazole inclusion complex and β-CD molecule without an inclusion can exist. Crystal structure and NMR analyses are being carried out to test these hypotheses.

References

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