

SOLID-STATE CHARACTERIZATION OF BIFONAZOLE- β -CYCLODEXTRIN BINARY SYSTEMS. NOTE I

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Abstract

Antimycotic bifonazole (BIF) is a very hydrophobic compound, which is a major drawback for obtaining topical pharmaceutical formulations with optimal bioavailability and for the penetration into hydrophilic human nail matrices. Cyclodextrins may increase local drug delivery by enhancing the drug release and/or permeation. The binary systems between bifonazole and β -cyclodextrin were prepared in two (BIF: β -CD) molar ratios - by physical-mixture and kneaded method. Hot-stage microscopy (HSM), differential scanning calorimetry (DSC), X-ray diffractometry and Fourier-transformed infrared spectroscopy (FT-IR) were used to characterize solid state interactions between bifonazole and β -cyclodextrin (β -CD) in their binary systems. The X-ray diffractograms, as well as the FT-IR analysis suggest the formation of a new solid phase, indicating a molecular interaction between the components. The HSM analysis and the DSC analysis sustain the hypothesis of formation of partial inclusion complexes between BIF and β -CD.

Rezumat

Bifonazolul (BIF) este un antimicotic puternic lipofil, hidrofobicitatea sa accentuată fiind un dezavantaj major pentru obținerea formelor farmaceutice de uz local cu o bună biodisponibilitate și pentru penetrarea în matricea unghială hidrofilă. Ciclodextrinele pot crește disponibilitatea locală prin îmbunătățirea eliberării substanței medicamentoase și /sau penetrabilitatea acesteia. Sistemele binare formate din bifonazol și β -ciclodextrină au fost preparate în două rapoarte molare (BIF- β -CD), utilizând metoda amestecului fizic și metoda malaxării. Pentru caracterizarea interacțiunii în stare solidă a sistemelor binare dintre bifonazol și β -ciclodextrină au fost utilizate analiza termomicroscopică, calorimetria cu scanare diferențială, difracția razelor X și spectroscopia în IR. Rezultatele difracției razelor X și analiza spectrală în IR indică posibilitatea formării unei faze solide noi, prin stabilirea unei interacțiuni moleculare între componentele sistemului binar. Analiza

termomicroscopică și calorimetria cu scanare diferențială susțin ipoteza formării unui complex de incluziune parțial între bifonazol și β -ciclodextrină.

Keywords: bifonazole, β -cyclodextrin, inclusion complex.

Introduction

Mycotic infections represent a continuous challenge due to the rise in resistance to classic antimycotic drugs and to the development of opportunistic fungal infections in patients receiving long-time and /or broad spectrum antibiotic therapy, as well as in patients with impaired immunity system [10].

Bifonazole (BIF) is an imidazole derivative (Figure 1), with antimycotic activity, used for the treatment of dermal- and onychomycosis. BIF is a very hydrophobic compound, practically insoluble in water, which is a major drawback for obtaining topical pharmaceutical formulations with optimal bioavailability, which commonly consists of aqueous systems [6], and for the penetration into hydrophilic human nail matrices [3].

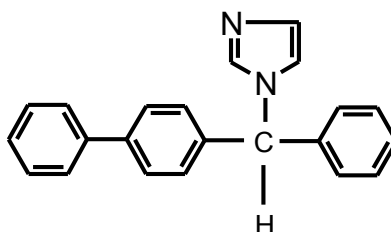


Figure 1

Chemical structure of bifonazole

The complexation of drugs with cyclodextrins, considered pharmaceutical excipients, is a very studied and useful approach for improving definite characteristics of pharmaceutical interest [5]. Cyclodextrins can accommodate molecules inside their non-polar cavity to form inclusion complexes [2], thus changing the physicochemical and biopharmaceutical properties of drugs [5]. Cyclodextrins may increase local dermal drug delivery by enhancing the drug release and/or permeation [3, 11]. Beta-cyclodextrin (β -CD) is a natural cyclodextrin, composed of 7 α -(1,4)-linked D-glucopyranose units, mentioned in the European Pharmacopoeia [14].

Hot-stage microscopy (HSM), differential scanning calorimetry (DSC) X-ray diffractometry and Fourier-transformed infrared spectroscopy (FT-IR) were used to characterize solid state interaction between BIF and β -CD

in their binary systems. The binary systems were prepared in two (BIF- β -CD) molar ratios by physical-mixture and kneaded method.

An indication of the host-guest molecular interaction, provided by DSC analysis, is the disappearance of the melting endotherm of the guest in the DSC thermogram of the binary system [12], as well as the shifting of the melting peak of the guest [1, 4]. The melting enthalpy is an indication of the amount of guest not involved in the interaction with the cyclodextrin [12]. The X-ray diffractometry and FT-IR analysis provide the detection of inclusion complexation in terms that the diffraction and IR spectra patterns of the complex must be clearly distinct from that resulting by the superimposition of individual diffraction and IR spectra patterns [8].

Materials and Methods

Bifonazole (1-[(R,S)-biphenyl-4-yl]phenylmethyl]-1-H-imidazole) was kindly provided by Gedeon Richter S.A. (Târgu Mureș, România). β -cyclodextrin was purchased from Cyclolab R&D (Budapest, Hungary). Other chemical reagents were of analytical grade purity requested by the European Pharmacopoeia 7th ed [14] and by the Romanian Pharmacopoeia 10th ed. [15].

Preparation of the binary systems

Physical mixtures (PM): the components were mixed in a mortar and sieved through a 100 μ m sieve. Kneaded products (KP): physical mixtures of BIF and β -CD were mixed with the same quantity of a 50 % ethanolic solution. The obtained paste was kneaded until the bulk of the solvent had evaporated. After drying at room temperature and then in the oven at 105°C, the KP were pulverized and sieved through a 100 μ m sieve. These methods are simple and provide a high yield. The molecular ratio of the products were 2:1, 1:1 BIF: β -CD. The products were stored in ambient circumstances [7, 9, 13].

Thermal analysis

The temperature and enthalpy measurements were performed with a Mettler Toledo STAR^c Thermal Analysis system, version 6.0 (Schwerzenbach, Switzerland). Approximately 2-5 mg of active material or binary systems were examined between 25-300 °C in argon flow (10 L/h). The heating rate was 5°C/min.

The relative degree of crystallinity (RDC) of BIF in the PM and KP samples, as a percentage of the BIF mass fraction, was calculated according to the relation [1, 4]:

$$\text{BIF}_{\text{RDC}} = 100 \cdot \frac{\Delta H_{\text{binary system}}}{\Delta H_{\text{uncomplexed BIF}} \cdot c}$$

where BIF_{RDC} is the uncomplexed guest %; $\Delta H_{\text{binary system}}$ is the normalized integral value for the product; $\Delta H_{\text{uncomplexed BIF}}$ is the normalized integral value for the active ingredient; and c is the percentage of active ingredient in the product.

HSM analysis was performed with a hot-stage thermomicroscope LEICA MZ6, (LEICA Microsystems, Swiss).

X-ray powder diffractometry

The X-ray spectra were recorded with a DRON UM-1 diffractometer (Russia) with $\text{CuK}_{\alpha 1}$ radiation ($\lambda = 1.54051 \text{ \AA}$) over the 2θ within 2-32 degrees. The measurement conditions were as follows: target, Cu; filter, Ni; 35 kV acceleration potential, 20 mA current, 1 s time constant, angular range of $2^\circ < 2\theta < 32^\circ$, and angular step of 0.030° .

Fourier-transformed infrared spectroscopy

The IR spectra of BIF, β -CD and their binary systems were recorded using a FT-IR 670 Plus, Able Jasco (Japan) spectrometer. The resolution was 4 cm^{-1} , the wave number range was $2000\text{--}400 \text{ cm}^{-1}$ and the scan number was 64. The samples were included in KBr pellet. Analyses were performed at room temperature.

Results and Discussion

Differential scanning calorimetry

The DSC thermogram of β -CD is presented in Figure 2. The thermogram of the host molecule shows a broad endothermic effect between $40\text{--}110^\circ\text{C}$, corresponding to the dehydration process. The melting temperatures, the specific melting enthalpies and the RDC values are presented in Table I.

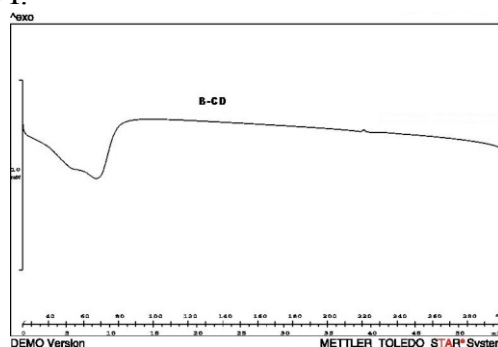


Figure 2

The DSC thermogram of β -CD

Very fine shifts of the melting temperatures of the binary systems to smaller values, and decreases of the melting enthalpies with decreasing the amount of BIF may be observed. These phenomena may be an index of the dispersion of the crystals of guest molecule in the matrix of β -CD, and the formation of highly energetic crystals of BIF [5]. BIF preserves a high relative degree of crystallinity in the PM and KP.

Table I
Results of DSC analysis of BIF and of binary systems with β -CD and the RDC of BIF in the binary systems

Sample	T_{peak} (°C)	ΔH (Jg ⁻¹)	BIF _{RDC} (%)
BIF	150.89	-118.2	100%
PM 2:1	150.78	-34.17	81
PM 1:1	150.43	-22.18	87
KP 2:1	150.38	-34.97	84
KP 1:1	150.47	-21.9	86

Hot-stage microscopy

Using the HSM analysis, the crystalline states of BIF and β -CD at the temperature of 25°C and 145°C were compared with the crystalline states of the binary products.

Figure 3 (A-D) reveals the results of HSM analysis. The melting of BIF takes place at 145°C, while β -CD does not exhibit any modification of its crystalline structure. The melting of β -CD takes place at 200°C.

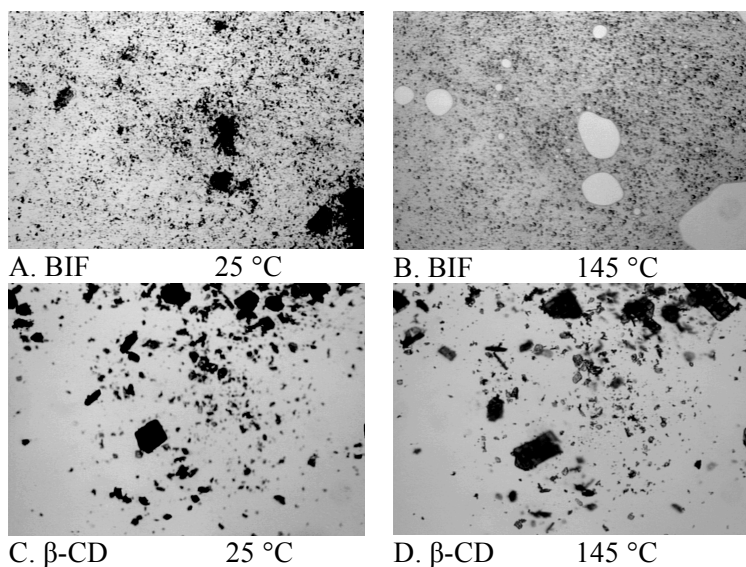


Figure 3 (A-D)
HSM photomicrographs of BIF and β -CD

The results of HSM analysis for the physical mixture products are presented in Figure 4 (A-D). In all cases, the presence of some free uncomplexed BIF crystals with melting temperature of 145°C, is visible.

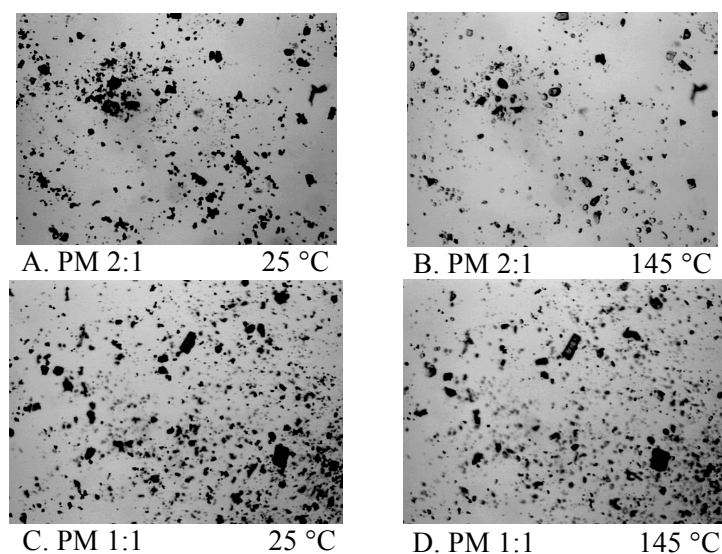


Figure 4 (A-D)

HSM photomicrographs of crystals of PM products

As the Figure 5 (A-D) reveals, one may observe also the presence of some free crystals of BIF, which melt at 145°C.

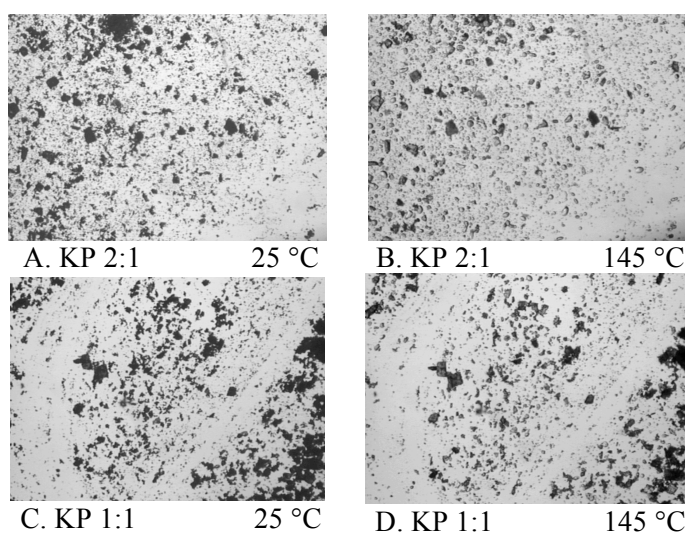


Figure 5 (A-D)

HSM photomicrographs of crystals of KP products

X-ray analysis

The diffractograms of BIF and β -CD, presented in Figure 6 (A-B), reveal characteristic peaks, arguing the crystalline nature of the substances. The most intense peaks in BIF diffractogram correspond to the following values of the 2θ angle: 15.9682, 18.5322, 19.3698, 21.3330.

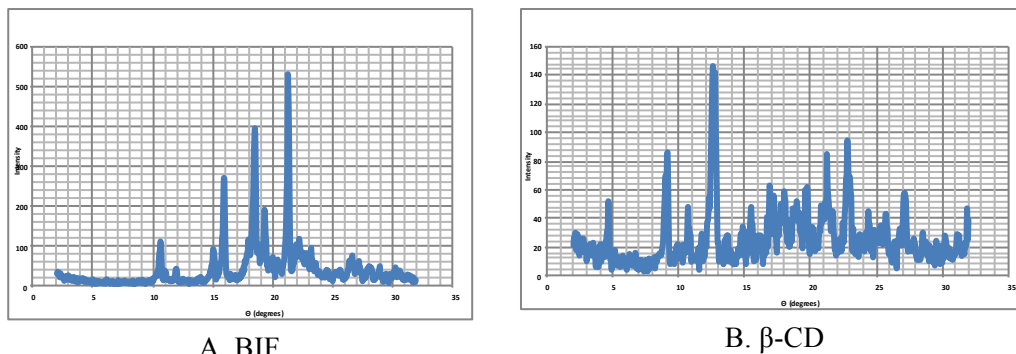


Figure 6 (A-B)
X-ray diffractograms of BIF and β -CD

The diffractograms of PM products, presented in Figure 7 (A-B), and the results of X-ray analysis emphasize the cancellation or the reduction of the intensities of some characteristic peaks of bifonazole.

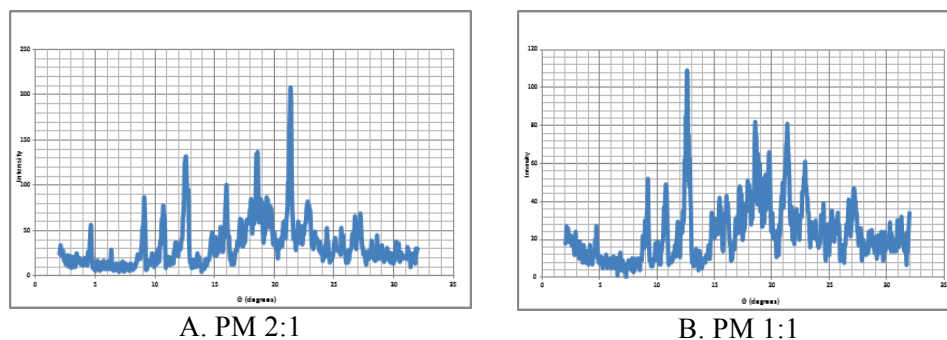


Figure 7 (A-B)
X-ray diffractograms of physical mixtures

The diffractograms of the KP, presented in Figure 8 (A-B) and the results of X-ray analysis reveal a less pronounced intensity of some characteristic peaks of BIF compared with pure BIF and with PMs, and a decrease of intensities with decreasing the amount of BIF in the binary system.

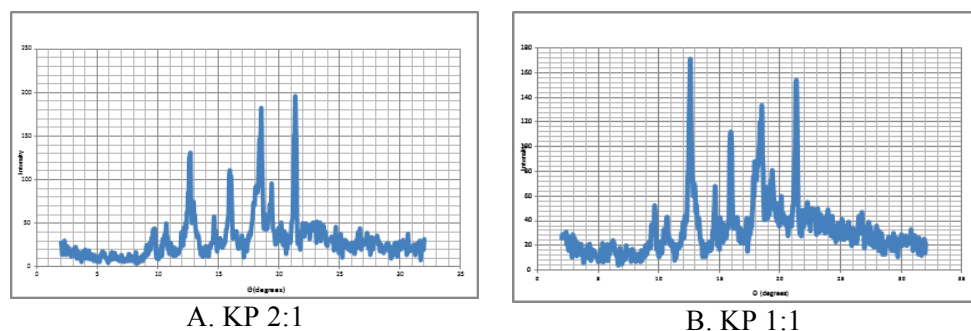


Figure 8 (A-B)
X-ray diffractograms of KPs

The changes of peak intensities and the disappearance of some peaks of BIF, observed in the diffractograms of all binary systems, support the hypothesis that complexation with β -CD induce a decrease of the crystallization degree of the guest-molecule.

Using the Bragg equation, the intensities and the relative intensities of the main peaks for BIF, β -CD and their binary systems were calculated. The results are presented in Table II.

Table II

The intensities and the calculated relative intensities of the main peaks

d (Å)		9.50	4.87	4.15	3.53	2.79	2.41	2.32	2.12	1.99
BIF	Intensity			112		272	397	192	527	
	I/I ₀ (%)			21		52	75	36	100	
β -CD	Intensity	52	86		146				85	94
	I/I ₀ (%)	36	59		100				58	64
PM 2:1	Intensity	56	87	78	132	100	136	87	208	82
	I/I ₀ (%)	27	42	38	63	48	65	42	100	39
PM 1:1	Intensity	27	52	49	109	43	75	54	81	61
	I/I ₀ (%)	25	48	45	100	39	67	50	74	56
KP 2:1	Intensity	14	27	50	131	111	182	96	196	49
	I/I ₀ (%)	7	14	26	67	57	93	49	100	25
KP 1:1	Intensity	12	37	43	171	112	133	81	154	49
	I/I ₀ (%)	7	21	25	100	65	78	48	90	29

The results obtained by X-ray analysis support the DSC results, indicating the preservation of a high BIF relative degree of crystallinity in the PM and KP.

FT-IR spectroscopy

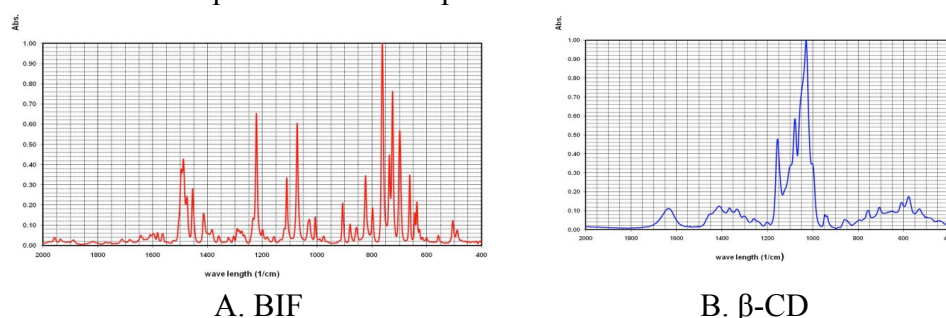
The FT-IR spectra of BIF presented in Figure 9A, reveal numerous absorption bands in the fingerprint region. The most intense peaks and the corresponding absorbances from the BIF spectrum are presented in Table III.

Table III

The values of the most intense peaks and the corresponding absorbances in the BIF spectrum

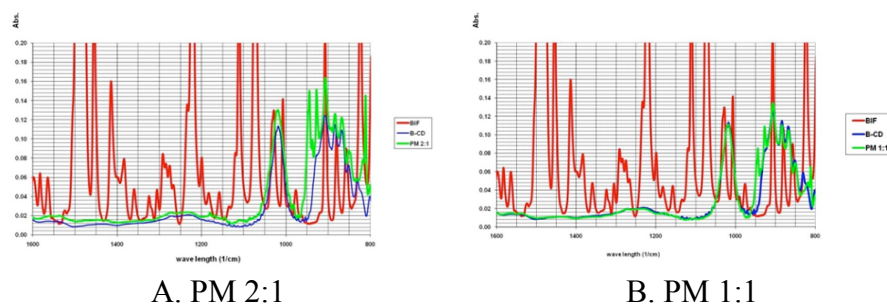
Wave length (cm ⁻¹)	Absorbance
762	0.632
725	0.482
1221	0.401
1073	0.383

In the spectrum of β -CD (Figure 9B) there is a wide absorption band in the 1200-1000 cm⁻¹ area, attributed to the glucopyranosic ring. For the binary systems, the 1600-800 cm⁻¹ domain was chosen to highlight the modification of spectra due to complexation.

**Figure 9 (A-B)**

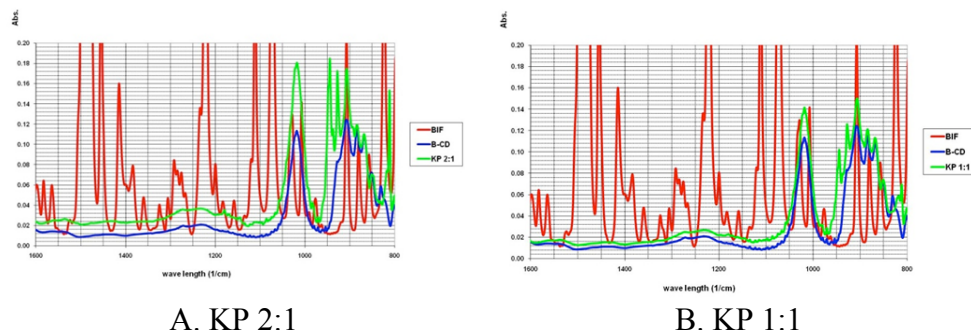
FT-IR spectra of bifonazole and β -cyclodextrin

The Figure 10 (A-B) represent the FT-IR spectra of PM products, where two major phenomenon were highlighted: the appearance of some new absorption bands and the shift of other absorption bands in the 1100-800 cm⁻¹ region, and the disappearance of the characteristic absorption bands of BIF between 1600-1100 cm⁻¹.

**Figure 10 (A-B)**

FT-IR spectra of the PM products

Similar observations could be highlighted by the IR spectra of KPs, presented in Figure 11. In the 1100-800 cm^{-1} area, the shifting of some absorption bands, as well as the appearance of some new absorption bands, were observed. In the 1600-1000 cm^{-1} region the disappearance of the BIF characteristic absorption bands is revealed.



A. KP 2:1

B. KP 1:1

Figure 11 (A-B)

The FT-IR spectra of the kneaded products

Conclusions

The decreasing of intensities of the peaks, and/or the lack of some BIF characteristic peaks in the binary systems, observed in the X-ray diffractograms, as well as the missing and/or the shifting of some characteristic absorption bands of bifonazole, and the appearance of some new absorption bands, highlighted by the FT-IR analysis of BIF, suggest the formation of a new solid phase, indicating a molecular interaction between the components.

The HSM analysis and the DSC analysis sustain the hypothesis of formation of partial inclusion complexes between BIF and β -CD.

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