Original Research Article

Inclusion complexes of Fluconazole with methylated-beta-cyclodextrin: influence on physicochemical properties of the guest molecule

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Abstract:

The increase in the incidence of fungal infections and fungal resistance has prompted the research for better therapeutic strategies such as the optimization and improvement of existing antifungal drugs. Fluconazole, one of the most used and well-tolerated antifungal drugs has a series of drawbacks hampering the obtention of an optimal therapeutic response. Complexation with cyclodextrins represents a very-well known approach for improvement of pharmaceutical profile of drugs. The present study aims to obtain and to evaluate the solid inclusion complexes of fluconazole with methylated beta-cyclodextrin, as preliminary experiments in formulation of improved pharmaceutical product of fluconazole. The differential scanning calorimetry, Fourier transform infrared spectroscopy, and X-ray powder diffractometry were used to characterize the binary systems between fluconazole and methylated-beta-cyclodextrin. The encapsulation of fluconazole into methylated-beta-cyclodextrin cavity resulted in modifications of the physicochemical properties of the antifungal agent. The formation of the fluconazole-cyclodextrin inclusion complex was confirmed by thermal and spectral methods. Based on the experimental data, a real interaction in solid state between the antifungal agent and cyclodextrin was proved, leading us to propose the kneading product in 1:1 molar ratio for future development of improved pharmaceutical formulation containing fluconazole.

Keywords: fluconazole; methylated beta-cyclodextrin; inclusion complex; thermal analysis; spectroscopic analysis.

Citation:

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1. Introduction

Nowadays, the increase in fungal resistance has prompted the research for better therapeutic strategies, such as the optimization and improvement of existing antifungal drugs [1]. In particular the appearance of *Candida* resistant strains is due to prescription without confirmation of the etiological agent, use for prophylactic purposes, long duration of treatment and reduced penetration of the antifungal agent into the cell, due to the intervention of efflux pumps and the formation of biofilms [2].

Fluconazole (FLU) (Figure 1) is a bis-triazole fungistatic derivative mainly used to treat oropharyngeal, esophageal candidiasis and cryptococcal meningitis [3,4]. Fluconazole has better safety profile compared to other triazole derivatives, being prescribed as a first line in infections with *Candida* [5]. It is worth mentioning that, although the spectrum of FLU covers a large number of *Candida* spp., the *Candida glabrata* presents a dose-dependent susceptibility, and *Candida krusei* is completely resistant [6]. Fluconazole hampers the conversion of lanosterol to ergosterol, by inhibiting the fungal cytochrome P450 dependent enzyme lanosterol 14α-demethylase, which leads to the reduction of ergosterol in the plasma membrane, and an accumulation of potentially toxic sterols. Recent findings suggest an additional mechanism of action based on generation of ROS and associated membrane damage [7]. Although FLU is well tolerated, irritation of gastro-intestinal tract and serious hepatotoxicity have been reported [8]. FLU is included in the WHO Model List of Essential Medicines-23rd List (2023), as an antifungal medicine, as capsules (50 mg), injections (2 mg/mL in vial), oral liquid (50 mg/5 mL), and powder for oral liquid (50 mg/mL) [9].

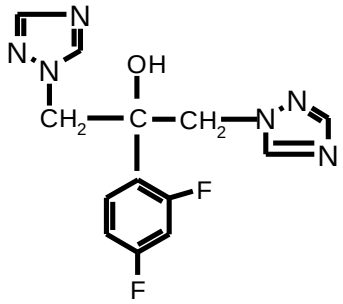


Figure 1. Structural formula of FLU [1].

Fluconazole, 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl) propan-2-ol has low water-solubility, instability, poor dissolution, and hygroscopicity, limiting its pharmaceutical formulations, and its dissolution rate. The Biopharmaceutics Classification System (BCS) classifies FLU as a BSC class II drug (low solubility, high permeability) [10]. Its low hydrophilicity decreases effectiveness when administered orally [11]. In particular, eye drop formulations have to overcome specific drawbacks, related to the low ocular bioavailability due to poor water solubility and eye clearance mechanism; also, the emerge of resistant species demands higher concentrations than usual to be administrated [12].

The dramatic increase in the fungal resistance to drugs, and in the invasive fungal infections, led to a high necessity for development of new delivery systems that can increase the drug availability and avoid the mechanism of fungal resistance [5].

In this context, different strategies have been used to optimize the dissolution rate of FLU, such as obtention of solid dispersion with inert hydrophilic polymers, complexation with sulfonatocalix-4-naphtalene, the preparation of microemulsion with diethylene glycol monomethyl ether, nanosizing, and crystalline structure modulation [13]. However, serious limitations were reported in relation to this conventional methods, involving physical instabilities of solid dispersions, problems of grinding or difficulties in removing the toxic organic solvent [14].

Cyclodextrins (CDs) have attracted a large pharmaceutical interest, some of them being already used as pharmaceutical excipients. Cyclodextrins are able to form inclusion complexes with a large variety of drugs, which may lead to improvement in physicochemical properties of drug molecule, its stability, dissolution properties, its biological properties and bioavailability, and to decrease toxicity [15].

Many researches aimed to improve some unfavorable properties of FLU, using complexation with CDs. Yurtdaş et al. prepared inclusion complexes of FLU with beta-CD, using different preparation techniques, in order to improve the physicochemical and biopharmaceutical properties of antifungal agent. The freeze-drying, spray-drying, co-evaporation techniques led to the formation of inclusion complex, which was not achieved using the kneading method. Moreover, the release of FLU from hard cellulose capsules prepared with beta-CD complexes was higher as compared to commercial capsules, and it was attributed to high energetic amorphous state and inclusion complex formation [14].

Li and co-workers prepared inclusion complexes of FLU with beta-CD and hydroxypropyl-beta-CD respectively, by co-precipitation method, aiming to gain structural insight of inclusion complexes. A 1:1 stoichiometry of complexation was achieved and a 1-D chain structure was proposed, with one triazolyl ring of FLUC being inserted into the narrower end of the CD cavity, and the 2,4-difluorophenyl ring into the wider end [16].

Kelemen et al. investigated the effect of native cyclodextrins, alfa-, beta-, and gamma-CD, and of two derivatives, hydroxypropyl-beta-CD and random methylated beta-cyclodextrin (RAMEB), on dissolution properties of FLU using the physical and the kneading methods. The results pointed out that RAMEB was the most suitable CD for complexation [17].

Oral mucoadhesive nanogels based on carbopol 940 and gelatin, loaded with FLU-thiolated beta-CD complex for improved adhesion were prepared, aiming to address the problem of short residence time and poor dissolution of antifungal agent. Thiolated beta-CD was chosen, due to its property to enhance solubility and permeability, along with its enhanced mucoadhesive properties. The authors found an 18-fold improved mucoadhesion on the oral mucous membrane of the goat when compared to simple nanogels, and enhanced permeation and a first-order concentration-dependent drug release, in *in vitro* permeation study [11].

A ternary complex between FLU, polyvinylpyrolidone, and hydroxypropyl-beta-CD was obtained, using the kneading technique, with the purpose to improve the solubility of FLU. The phase solubility study confirmed a stoichiometry of 1:1, and a linear increase of solubility of FLU with an increase in CD concentration. The *in vitro* release studies correlated with thermal and spectroscopic analysis of the ternary complex, led the authors to propose the complex as a suitable carrier for oral delivery [18].

This study aims to prepare and investigate the binary systems between FLU and RAMEB, a chemically modified CD derivative frequently used for improving drug solubility.

2. Materials and Methods

2.1. Chemicals and Reagents

Fluconazole (M: 306.277 g/mol; water solubility: 1 mg/L) was received from Gedeon Richter (Târgu Mureș, Romania), methylated-beta-cyclodextrin (RAMEB) (CY-2004.1; M: 1303 g/mol) was received from Cyclolab R&D Ltd., Budapest, Hungary; ethanol (Chimopar, Bucharest, Romania). All the reagents were of analytical grade and distilled water was used in experiments.

Differential scanning calorimetry measurements were assessed using a Mettler Toledo STAR Thermal Analysis System, DSC 821 (Mettler Inc., Schwerzenbach, Switzerland). Approximately 2-3 mg of active material or product was examined, in the following parameters: heating rate: -5 °C/min, Ar flow rate 10 L/h, the temperature range of 25-300 °C.

The X-ray spectra were recorded using a Rigaku ULTIMA IV Diffractometer, radiation CuKα with 2θ angles between 5° and 40°, functioning at 40 kV and 40 mA.

Fourier transform infrared spectra were recorded with a Shimadzu Prestige-21 spectrometer in the range 400-4000 cm^{-1} with a resolution of 4 cm^{-1} using the KBr pellet method.

2.2. Sample Preparation

Binary systems between FLU and RAMEB were obtained using the physical mixture method and the kneading method, in three molecular ratios (drug:CD): 2:1, 1:1 and 1:2. After preparation, the samples were stored in glass vials.

2.3. Methodological Protocols:

For the preparation of binary mixtures, two simple and feasible laboratory methods were used.

Preparation of physical mixtures (PM)

Exactly weighted amounts of FLU and RAMEB were mixed in a ceramic mortar with pestle until a homogeneous mixture was obtained.

Preparation of kneaded products (KP)

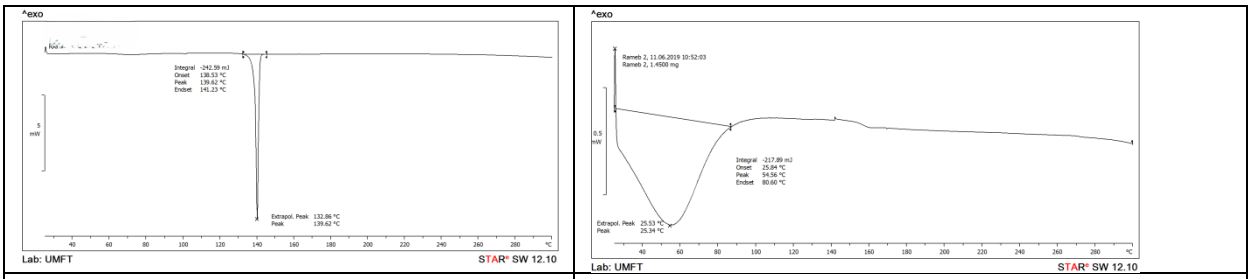
Exactly weighted amounts of FLU and RAMEB were homogenized in a ceramic mortar with a pestle, and a 50% (w/w) aqueous ethanol solution was added, corresponding to the molar ratio. The kneading process was continued until the bulk of solvent evaporated. The resulting paste-like product was dried in the oven at 105 °C, until constant mass, and then was pulverized.

3. Results

In order to determine the phenomenon of molecular encapsulation between the active pharmaceutical ingredient and RAMEB, the data of pure ingredients were compared to those obtained for physical mixtures and kneaded products.

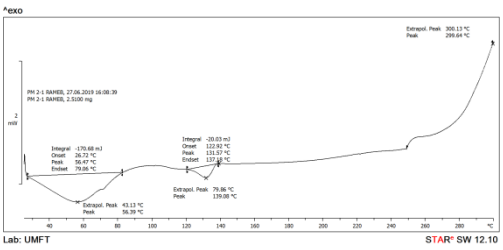
3.1. Differential scanning calorimetry

Figures 2-4 present the DSC thermograms for FLU (Fig. 2 (a), RAMEB (Fig. 2 (b), their physical mixtures in the molar ration of 2:1, 1:1, and 1:2 (Fig.3 (a) - (c)), and their kneaded products in the molar ratio of 2:1, 1:1 and 1:2 (Fig. 4(a) - (c)).

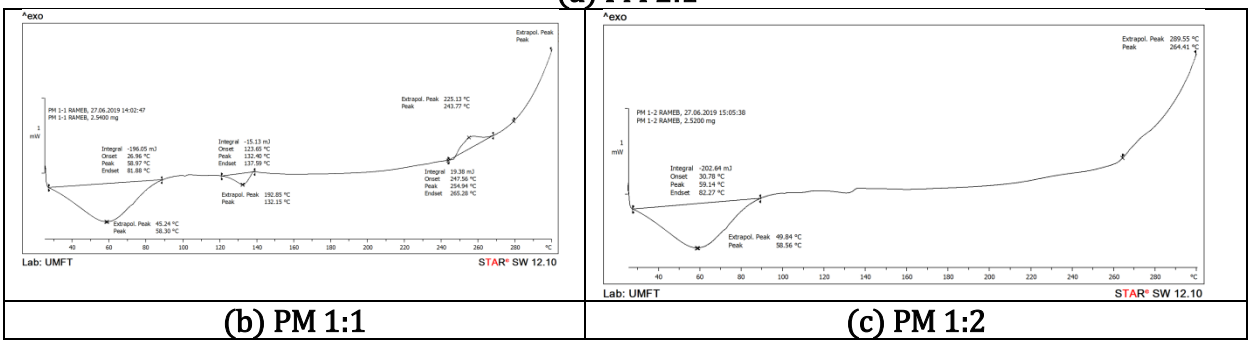


(a) FLU (b) RAMEB

Figure 2. The thermogram of FLU (a) and of RAMEB (b).

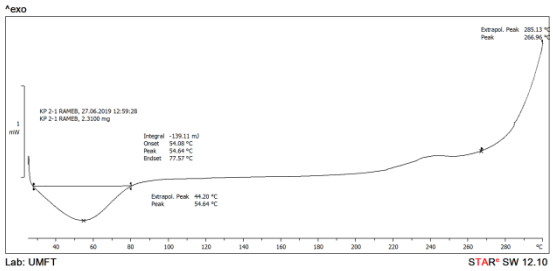


(a) PM 2:1

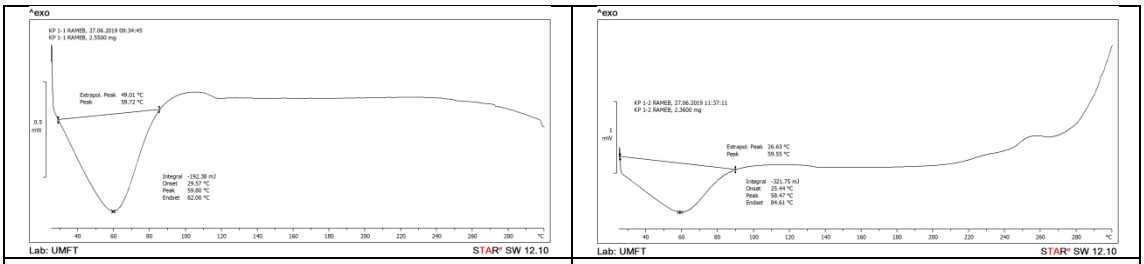


(b) PM 1:1 (c) PM 1:2

Figure 3. Thermograms of FLU:RAMEB physical mixtures.



(a) KP 2:1



(b) KP 1:1 (c) KP 1:2

Figure 4. Thermograms of FLU:RAMEB kneaded products.

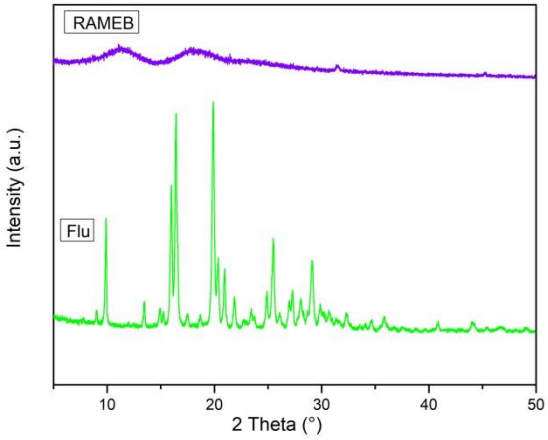
The DSC data for FLU, RAMEB and their inclusion complexes are presented in Table 1.

Table 1. The obtained DSC data for FLU and the FLU : RAMEB physical mixtures and kneaded products.

	T onset (°C)	T peak (°C)	T offset (°C)
FLU	**138.53**	**139.62**	**141.23**
PM 2:1	122.92	131.57	137.18
PM 1:1	123.65	132.40	137.59
PM 1:2	-	-	-
KP 2:1	-	-	-
KP 1:1	-	-	-
KP 1:2	-	-	-

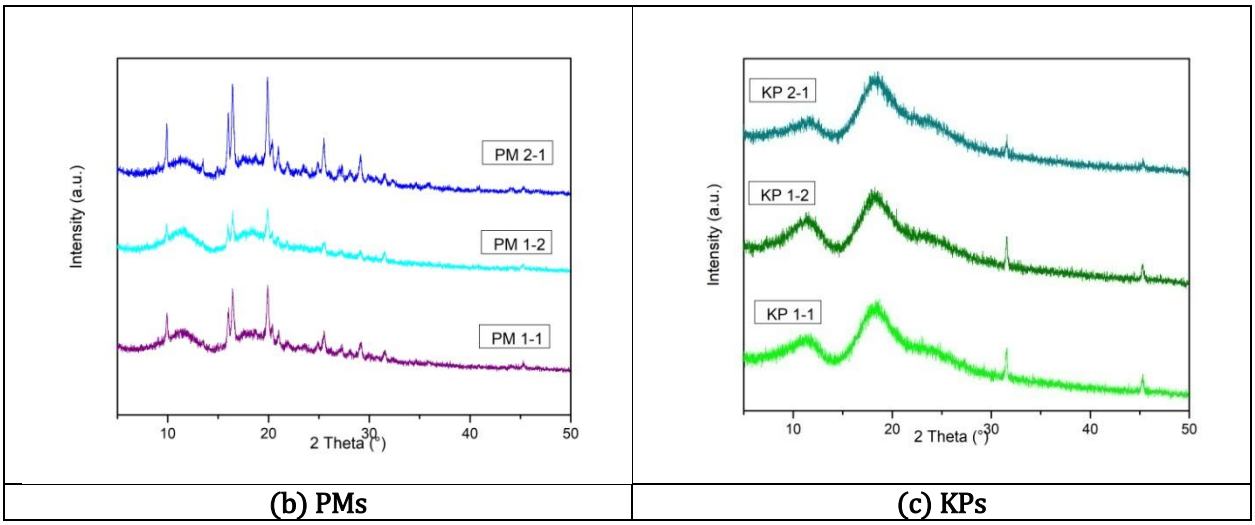
3.2. Powder X-ray diffractometry

Figure 5 presents the diffraction patterns of FLU, RAMEB, and Figure 6 presents the diffractograms of their binary systems obtained by physical mixture method and by kneaded method.



(a)

Figure 5. PXRD spectra of FLU and RAMEB

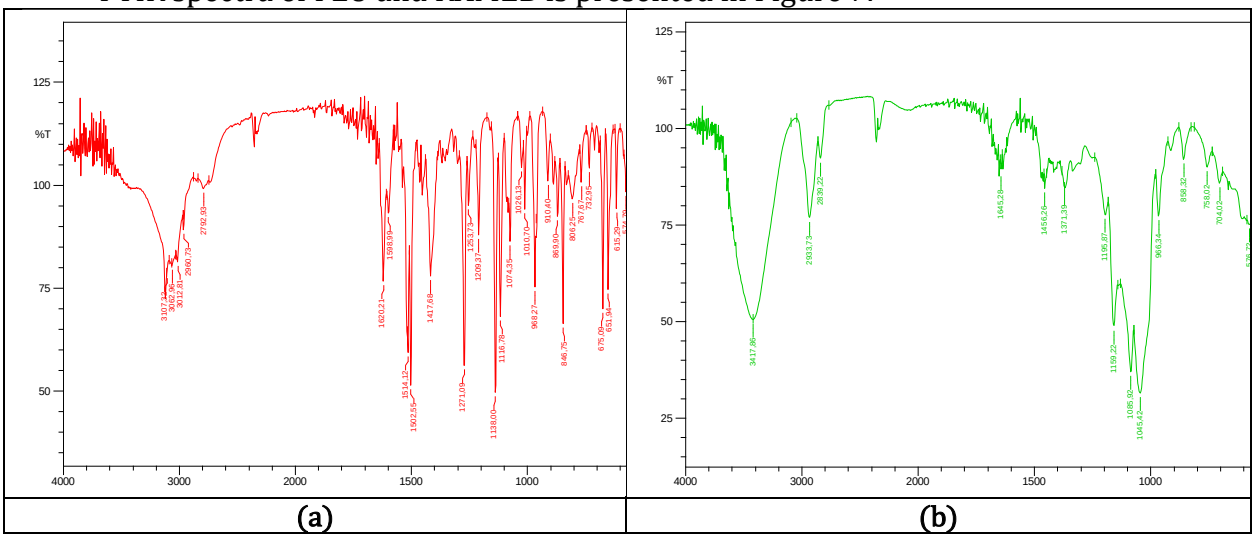


(b) PMs (c) KPs

Figure 6. PXRD spectra of FLU and RAMEB (a) and their PM (b) and KP (b)

3.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of FLU and RAMEB is presented in Figure 7.



(a) (b)

Figure 7. FTIR spectrum of FLU (a) and RAMEB (b)

FTIR spectra of physical mixture products are presented in Figure 8 (a-c).

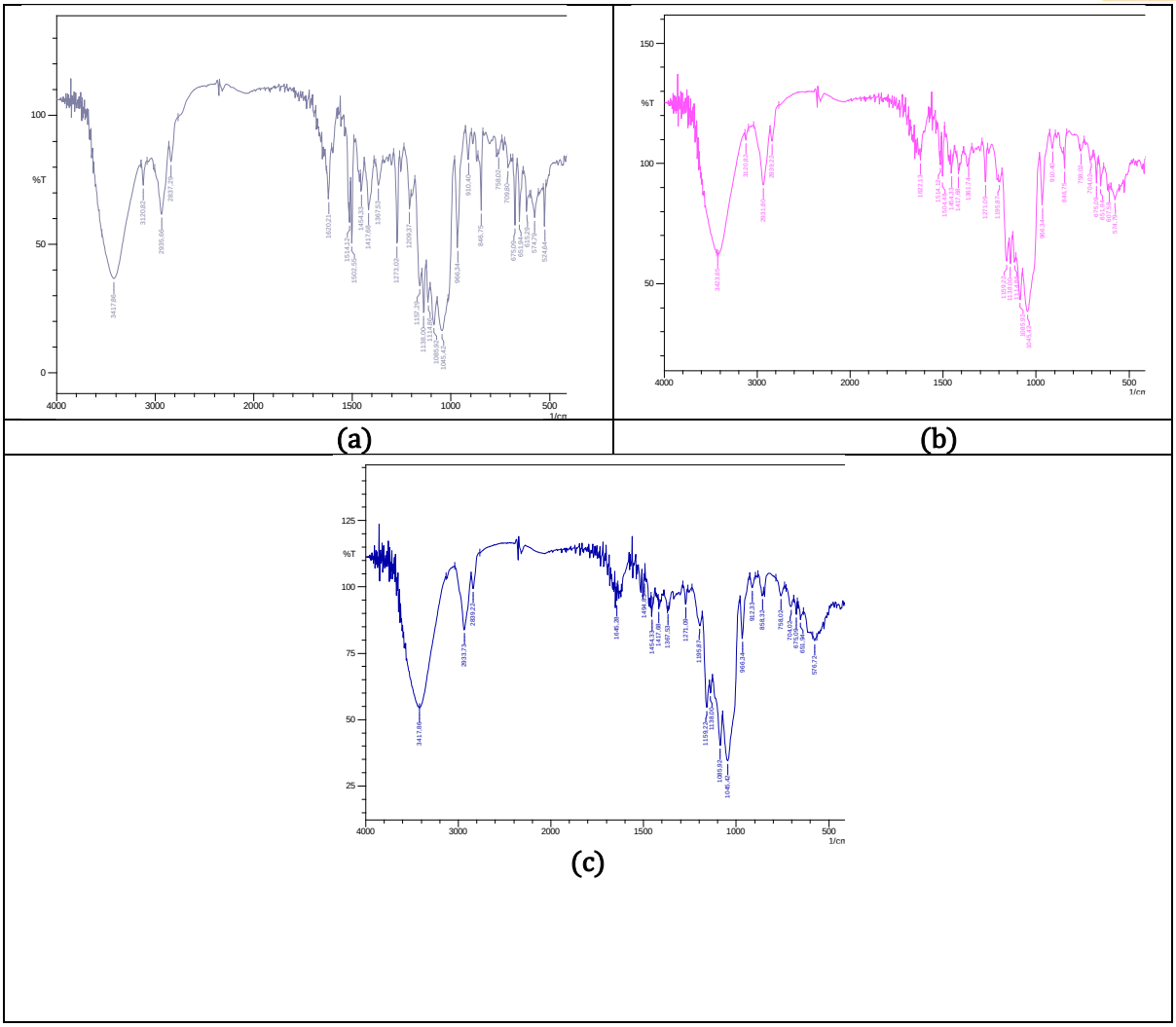
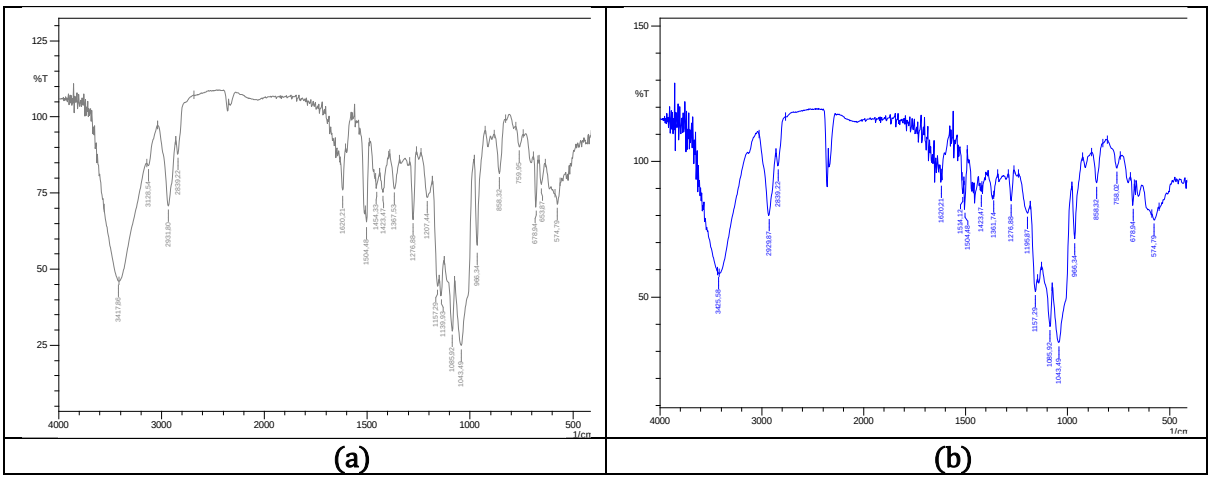


Figure 8. The obtained spectra for PMs between FLU and RAMEB (a) PM 2:1; (b) PM 1:1; (c) PM 1:2.

FTIR spectra of kneaded products are presented in Figure 9 (a-c).



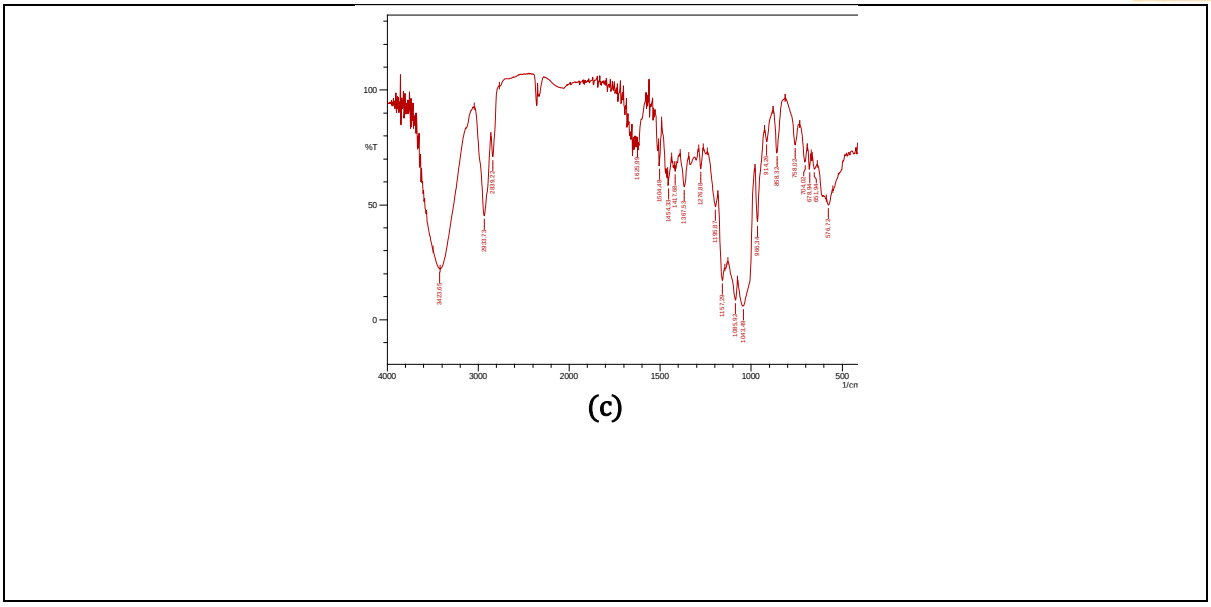


Figure 9. The obtained spectra for KPs between FLU and RAMEB (a) KP 2:1; (b) KP 1:1; (c) KP 1:2.

4. Discussion

The analytical characterization of an inclusion complex involves several techniques, in order to provide suitable and reliable information about the new formed compound, evidencing their advantages and limits.

4.1. Differential scanning calorimetry

The DSC analysis is one of the most frequently used analyses to characterize the inclusion complex between a host molecule and the cyclodextrin [19]. The disappearance of the characteristic peak of the guest from the binary system thermogram is considered strong evidence of molecular encapsulation of the guest into the CD cavity and/or the formation of an amorphous solid dispersion [17].

The DSC pattern for FLU (Figure 2a) showed a sharp endothermic peak due to the melting of the compound at 139.62 °C, which is within the reported melting range [2, 13, 14, 17,20]. The melting endotherm reflects the crystalline nature of FLU. The DSC curve of RAMEB (Figure 2b) exhibited a single large endothermic peak between 25.84 °C and 80.60 °C, which corresponds to a dehydration process, in accordance with the results determined by Kelemen et al. [17].

The PM products in 2:1 and 1:1 molar ratio showed endothermic peaks, with reduced intensities, at 131.57 °C, and at 132.40 °C, evidencing the presence of free FLU (Figures 3a-b). In the case of PM 2:1, we noticed a notable decrease in the crystallinity of FLU (Figure 3c). A reduction of the peak area, and the shift of the peak' temperature to smaller values may be related to possible drug-CD interaction or loss of drug crystallinity [19].

The characteristic peak of FLU was not observed in the DSC profiles of all the KPs, attributable to an encapsulation process and/ or amorphization induced by heating (Figures 4a-c).

4.2. Powder X-ray diffractometry

The PXRD analysis is widely used to ascertain the results obtained from DSC analysis; the technique also allows to acquire information about the intensity of drug-CD solid state interaction [19]. A reduction in peak's intensity, peaks shifting and/or disappearance may be attributable to a change from the crystalline state to amorphous state [5].

The diffractogram of FLU (Figure 5a) showed several distinct intense diffraction peaks, highlighting its crystalline structure. The peaks were identified at angles (2θ): 9.9°, 16.01°, 16.4°, 19.9 and 25.5° in good agreement with other studies [5, 13, 14, 16]. RAMEB showed a PXRD spectrum characteristic for amorphous substance (Figure 5a).

The systematic comparison of the PXRD patterns of the single components, their physical mixture, and the kneaded products (putative inclusion complexes) allows revealing changes of the solid-state properties [19]. In case of PM products, the spectra were not the simple sum of those of pure components, suggesting the formation of a new solid state (Figure 6a). The PXRD spectra of KPs revealed a marked reduction in the peak's intensities, and the apparition of two new peaks, at approximately 2θ angle of 33° and 45° (Figure 6b).

The appearance in the KPs spectra of new diffraction peaks, together with the evident reduction of their intensities, is indicative of new solid-state formation. The results are in good agreement with those obtained by DSC analysis, eliminating the assumption of heat induced interaction between the components, as well as the hypothesis of masking of the main representative peaks of the drug by the amorphous RAMEB, considering the higher content in weight of CD.

4.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR technique was performed to determine the vibrational changes in host-guest interaction between the active pharmaceutical ingredient and the CD, which are being disturbed throughout the molecular encapsulation process [11]. Bands shifting, reduction of their intensities, and disappearance of some bands may indicate the molecular interactions between the components, in complex formation process [5]. Upon complexation, the characteristic peaks of groups that are included in the CD cavity are masked [17].

The FTIR spectrum of FLU (Figure 7a) is characterized by the presence of some vibrational bands in the fingerprint region, in good agreement to those mentioned by other researchers [6, 11, 14, 21]:

- At 3600-2500 cm^{-1} O-H stretching vibrations from intramolecular and intermolecular hydrogen bonds;
- At 1620 and 1514 cm^{-1} absorption bands due to C=C stretch from the aromatic ring;
- At 1502 and 1417 cm^{-1} bands due to the triazole ring stretch;
- At 1271 cm^{-1} for C-F stretching vibration;
- At 1138 cm^{-1} for triazole ring breathing in plane;
- At 1026 cm^{-1} for C-H aromatic ring vibration;
- At 960 cm^{-1} and 846 cm^{-1} for C-H triazole ring

The RAMEB absorption spectrum (Figure 7b) is characterized by the presence of a wide absorption band in the region 1200 – 1000 cm^{-1}, attributed to the glucopyranosic ring, and determined by the stretching vibration of the C-O moiety from alcohols. The FTIR spectrum also presents a broad absorption band, between 3700 and 3025 cm^{-1}, that can be

assigned to the stretching vibrations of the OH groups, with the peak located at 3417.86 cm^{-1}.

For the binary systems, the 1600-600 cm^{-1} domain was chosen to highlight the modification of spectra due to molecular interactions between the components.

In case of binary systems, the FTIR spectra showed clear modifications as compared to the drug. There were detectable reductions of several peak's intensities, and bands shifting, suggesting host-guest interactions due to complexation. The spectra of PM products (Figures 8a-c) showed fewer modifications as compared to the KPs (Figures 9a-c). For PMs, the host guest interactions were more visible as the quantity of RAMEB increased, but in case of KPs, the molar ratio had no influence on the modifications visible on the spectra, with few exceptions (the band at 1620 cm^{-1} was detectable in both KP 2:1 and KP 1:1; the band at 1514 cm^{-1} was present in KP 1: 1, and the band at 1417 cm^{-1} was present only in the case of KP 1:2).

It is worth mentioning the shift of the peak characteristic of RAMEB from 3417.86 cm^{-1} to 3423.65 cm^{-1} in case of PM 1:1, to 3425.58 cm^{-1} in case of KP 1:1, and to 3423.65 cm^{-1} in case of KP 1:2, which supports the interaction between the components.

5. Conclusions

In this study, the formation of inclusion complex between the antifungal drug fluconazole and RAMEB was investigated in solid state, using thermal and spectroscopic analysis. Differences in the thermal and spectroscopic behaviours of raw materials and the corresponding binary products were evident, suggesting a real interaction in molecular state between the components. Based on these results, and correlating the data obtained from the three analyses, the kneading product in 1:1 molar ratio is the proposed product for future development of improved pharmaceutical formulation containing fluconazole.

Author Contributions:

Conceptualization, [R.P.; C.T.]; Methodology, [R.R., R.L., C.T.]; Formal Analysis, [S.B., R.R., R.L]; Investigation, [R.P., I.O.A., D.C.]; Resources, [C.D., C.T.]; Writing—Original Draft Preparation, [R.P., I.O.A., S.B., D.C.]; Writing—Review & Editing, [C.D., C.T.]; Visualization, [R.P., I.O.A., C.D., D.C., R.R., R.L, S.B., C.T.]; Supervision, [C.T.]; Project Administration, [C.T.].

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest.

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