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RESEARCH ARTICLE

Characterization of Inclusion Complexes between Miconazole and Different Cyclodextrin Derivatives

Hajnal Kelemen[1], Gabriel Hancu[1*], Serban Andrei Gâz-Florea[2], Eniko Nemes-Nagy[3], Lajos Attila Papp[1], Eleonora Mircia[4]

1. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
2. Department of Organic Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
3. Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
4. Department of Pharmaceutical Industry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

Objective: Miconazole, an imidazole antifungal derivative, is a very hydrophobic compound, a major drawback in obtaining topical pharmaceutical formulations with optimal bioavailability. Cyclodextrins (CDs) may increase local drug delivery by enhancing the drug release and/or permeation. The aim of the study is the characterization of inclusion complexes between miconazole and different CD derivatives. **Methods**: Several CD derivatives were tested in the experiments. The binary systems between miconazole and different CDs were prepared in 1:1 molar ratios by physical-mixture and kneading methods. Differential scanning calorimetry (DSC) and Fourier transformed-infrared spectroscopy (FT-IR) methods were used to characterize solid state interactions between miconazole and CDs in their binary systems. **Results**: The FT-IR analysis suggests the formation of a new solid phase, indicating a molecular interaction between the components. The DSC analysis sustains the hypothesis of formation of partial inclusion complexes between miconazole nitrate and CD. **Conclusion**: The thermic behaviour of the complexes depends both on the preparation method and the composition of the products.

Keywords: miconazole, cyclodextrins, complexation, differential scanning calorimetry, Fourier transform-infrared spectroscopy

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Introduction

Miconazole nitrate, (1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]imidazole) (MNZ) is a first generation imidazole antifungal substance.

The chemical structure of miconazole nitrate is presented in Figure 1.

MNZ is a white or almost white powder, very slightly soluble in water; slightly soluble in alcohol; sparingly soluble in methanol. MNZ is an imidazole antifungal which inhibits 14α-demethylase (a cytochrome P450 enzyme), thus blocking the demethylation of lanosterol to ergosterol, the principarl sterol of fungal membranes; this inhibition disrupts membrane structure and function and, thereby, inhibits fungal cell development. It has a wide activity spectrum icluding *Candida spp*., *Epidermophyton spp*., *Malassezia spp., Microsporum spp* or *Trichophyton spp*. It also posses activity against some Gram-positive bacteria including staphylococci and streptococci [1].

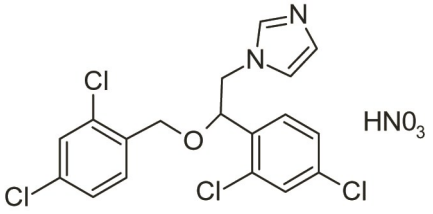

Fig. 1.Chemical structure of miconazole nitrate

MNZ is used in the treatment of superficial candidiasis, and of the skin infections like dermatophytosis and pityriasis versicolor. MNZ is used mainly as a topical drug for cutaneous mycoses; being applied usually as cream, lotion, or powder in the treatment of several fungal infections of the skin [2].

MNZ may be given orally as a oral gel, chewing gum and bioadhesive bucal tablets for the treatment of oropharyngeal and intestinal candidiasis; however after oral use, nausea and vomiting have been reported. Oral administration of MNZ leads to erratic and unpredictable bioavailability because of its poor dissolution and absorption in the gastrointestinal tract [3].

Due to the presence of an imidazole cycle, MNZ is a basic compound (pKa 6.77), and due to the presence of the aromatic rings it is a liphophilic substance (log P 5.86); MNZ has a water solubility 0,7μg/mL at 25°C [4].

Solubility is one of the most important parameter of a pharmaceutical substance; an appropriate pharmacological response is strongly related to the concentration of drugs in the systemic circulation. To overcome this problem, various formulation strategies can be applied to increase the dissolution characteristics such as solid dispersion, micronization, salt formation, liquid-solid techniques or cyclodextrin complexation [5].

Cyclodextrins (CDs) are cyclic oligosaccharides obtained from starch by enzymatic hydrolysis. Native CDs are built from 6, 7 or 8 units of D-glucopyranose linked by oxygen bridges and are called α, β and γ-CD. CDs have a hydrophilic external surface and a hydrophobic internal

* Correspondence to: Gabriel Hancu
E-mail: gabriel.hancu@umftgm.ro

cavity in which they can incorporate different guest molecules by hydrophobic interactions. A number of chemically modified CDs have been prepared to increase the inclusion capacity and to modify physico-chemical properties of the native CDs. The use of CDs in the pharmaceutical field is most often due to their ability to form inclusion complexes with various active substances. The chemical structure of the three native CDs is presented in Figure 2 [6].

Inclusion complexes are complexes that comprise two or more molecules in which one of the molecules, the "host"; includes partially or totally, the other molecule, the "guest", generally in stoichiometric proportions. The bonds between CD and the the "guest" molecules are of weak energy (hydrogen bonds or Van der Walls forces), therefore this phenomenon is in balance and the dissociation is reversible. The bond may then be stabilized by secondary valency bonds, or by dipolar bonds between the support molecules and the included ones, if an energy gain is obtained. Therefore, the geometric factor, size and symmetry of the molecules involved play an essential role in their formation [6].

CD complexation may improve some physicochemical properties of drugs, such as stability and water solubility and consequently also their bioavailability. Due to these particular properties, CDs can encapsulate a variety of hydrophobic molecules inside their cavity through non-covalent interactions. Because of the different size of the CD cavity, the different types of CDs allow the complexation of well-defined chemical structures [7-10].

Thermoanalytical techniques (differential scanning calorimetry, thermogravimetry) are frequently used in the investigation of the thermal properties of CDs and their inclusion complexes [11].

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference material is measured as a function of temperature, as the two specimens are subject to identical temperature regimes in a heated or cooled environment at a controlled rate. DSC is a frequently used thermal analytical technique because of its ability to provide detailed information about both the physical and energetic properties of a substance [12].

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, as well as the shifting of the melting peak of the guest. The melting enthalpy is an indication of the amount of guest not involved in the interaction with the CD [13-17].

In previously published articles, inclusion complexes of azole derivatives with CDs in aqueous solution and in the solid phase were studied by solubility methods, spectroscopy (UV, IR), thermal analysis, and X-ray diffractometry, and their modes of interaction were assessed [18].

An NMR spectroscopic study was published in order to evaluate the complexation of fluconazole with β-CD, the results confirmed the formation of an inclusion complex in aqueous solution [19-20]. Complexes of fluconazole and bifonazole, miconazole, voriconazole prepared with different CDs by various methods such as kneading, co-evaporation, physical mixing and spray-dried were characterized by Fourier transform infrared spectroscopy (FT-IR) and DSC studies; the effect of complexation on the dissolution rate of MNZ was studied [21-23].

The purpose of this study is to evaluate the possibility of interaction of antifungal drug MNZ through complexation with different types of CD. The binary systems between MNZ and different CDs were prepared in one molar ratios 1:1, using several methods (mixing, kneading). The resulted complexes were characterized by means of thermal analyses by DSC and FT-IR.

Materials and Methods

Materials

Miconazole nitrate of pharmaceutical grade was acquired from Gedeon Richter (TîrguMureș, Romania); while the CDs were purchased from Cyclolab Ltd. (Budapest, Hungary): native α-CD, β-CD, γ-CD and derivatized randomlymethylated-β-CD (RAMEB) and hydroxypropyl–β-CD (HP-β-CD) were used. All the chemical reagents were of analytical grade.

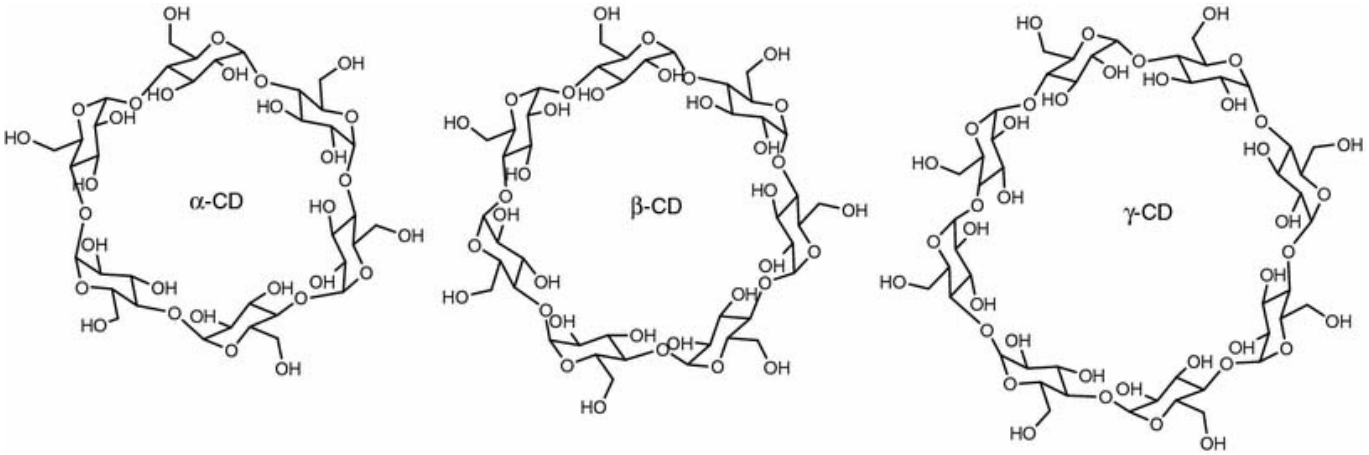

Fig. 2. Chemical structure of native CDs

Preparation of the binary systems

Physical mixtures (PM): the components were mixed in a mortar and sieved through a 100 μm sieve.

Kneaded products (KP): physical mixtures of MNZ and different CDs were mixed with the same quantity of a 50% ethanolic solution. The obtained mixture 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 μm sieve.

These methods are simple and provide a high yiel; the molecular ratio of the products were 1:1.

Differential scanning calorimetry (DSC)

The temperature and enthalpy measurements were performed using a Mettler Toledo DSC 823e Thermal Analysis system (Schwerzenbach, Switzerland). Approximately 1-2 mg of the active material or binary systems were examined in aluminum pans between 25 - 400°C in a nitrogen atmosphere (flow rate of 50 mL/min.). The heating rate was 10°C/min.

Fourier-transformed - infrared spectroscopy (FT-IR)

FT-IR analysis provides 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. The IR spectra of MNZ, CDs derivatives and their binary systems were recorded using a FT-IR 470 Plus, (AbleJasco, Japan) spectrometer. The resolution was 4 cm^{-1}, the wave number range was 2000-400 cm^{-1} and the scan number was 64. The samples were included in KBr pellets. Analyses were performedat room temperature.

Results and Discussion

Differential scanning calorimetry (DSC)

Differences in the thermal behaviour of MNZ, CDs, and the corresponding inclusion complexes were evident. As shown in Figure 3, MNZ exhibits a characteristic endothermic fusion peak at 187.71°C corresponding to the MNZ melting point. Furthermore, α-CD, β-CD, γ-CD, HP-β-CD and RAMEB showed broad endothermic events in the temperature range from 30 - 95°C, which are related to the loss of adsorbed water, and small endo- or exothermic effects at 210 – 325°C due to thermal degradation. DSC thermograms of the physical mixture of MNZ and α-CD, β-CD, γ-CD, HP- β-CD and RAMEB show the existence of the endothermic peak of MNZ indicating interactions between the CDs and MNZ. The MNZ peak area in the physical mixture with different CDs decreased, indicating a more intense interaction of MNZ with CDs. This endothermic peak is still present in kneading products complexes which show an endothermic peak at 131.21 – 133.35°C, and also a new endothermic peak at the range of 140° - 160 C was observed, which shows a possible complex formation.

The analysis of binary systems MNZ-RAMEB and MNZ-HP-β-CD showed the modification of the MNZ endothermic peak, indicating a more intense interaction of MNZ with the CDs. The MNZ peak in the kneading products decreased between 187.7 - 181.9°C indicating a more intense interaction of MNZ with the CDs. The absence of the characteristic peak is a strong evidence of MNZ inclusion into the CD cavity. This can be explained by the amorphization, molecular encapsulation of the drug into the CD cavity, or both.

Fourier-transformed - infrared spectroscopy (FT-IR)

FT-IR spectroscopy was used in order to investigate the changes upon host - guest interaction between MNZ and CDs. FT-IR is useful to identify the vibrational mode of the drug and suggesting the interactions between molecules in solid state.

The FT-IR spectra of MNZ presented in Figure 4 reveal numerous absorption bands in the fingerprint region.

The bands at 1589 and 1561 cm^{-1} in the experimental spectrum are assigned to the C–C stretching vibration of the two dichlorobenzene groups. The band at 1510 cm^{-1}corresponds to C–C stretching vibration of the imidazole group as well as the CH bending of the imidazole group, the C6 of the aliphatic part of MNZ. The band at 1468cm^{-1}corresponds to the CH bending of the two dichlorobenzene groups and to the CH bending of the C6 and C17.

The changes observed in the FTIR spectra of the various samples, such as shift of peaks or their reduction in intensity up to almost complete disappearance, depends on the preparation method, suggesting different degrees of interaction and amorphization in different products.

In the spectrum of β-CD there is a wide absorption band in the 1200-1000 cm^{-1} area, attributed to the glucopyranosic ring. Another broad and strong absorption band in the 3000 cm^{-1} domain is attributed to -OH stretching. For the binary systems, the 1600-600 cm^{-1} domain was chosen to highlight the modification of spectra due to complexation, CDs mask the characteristic peaks of groups that are included in their cavity.

The absorption spectrum of the complex of MNZ with β-CD shows that the absorption bands of primary and secondary -OH groups flanking the narrower and wider rims of CD do not appear or shift in the complex. This may indicate that the free deformation of these groups is somewhat hindered in the complex. The absorption band around 3280 cm^{-1} is, according to literature data, the range of water vibration vibrations. The fact that this band appears only in the case of pure CD indirectly suggests that the molecule entering the cavity during the complex formation displaces the previously contained water molecules in the cavity Figure 5.

Generally, all comlexes showed reduced intensity by comparison to pure MNZ; these changes in the FT-IR spectra might be related to possible drug - CD interactions.

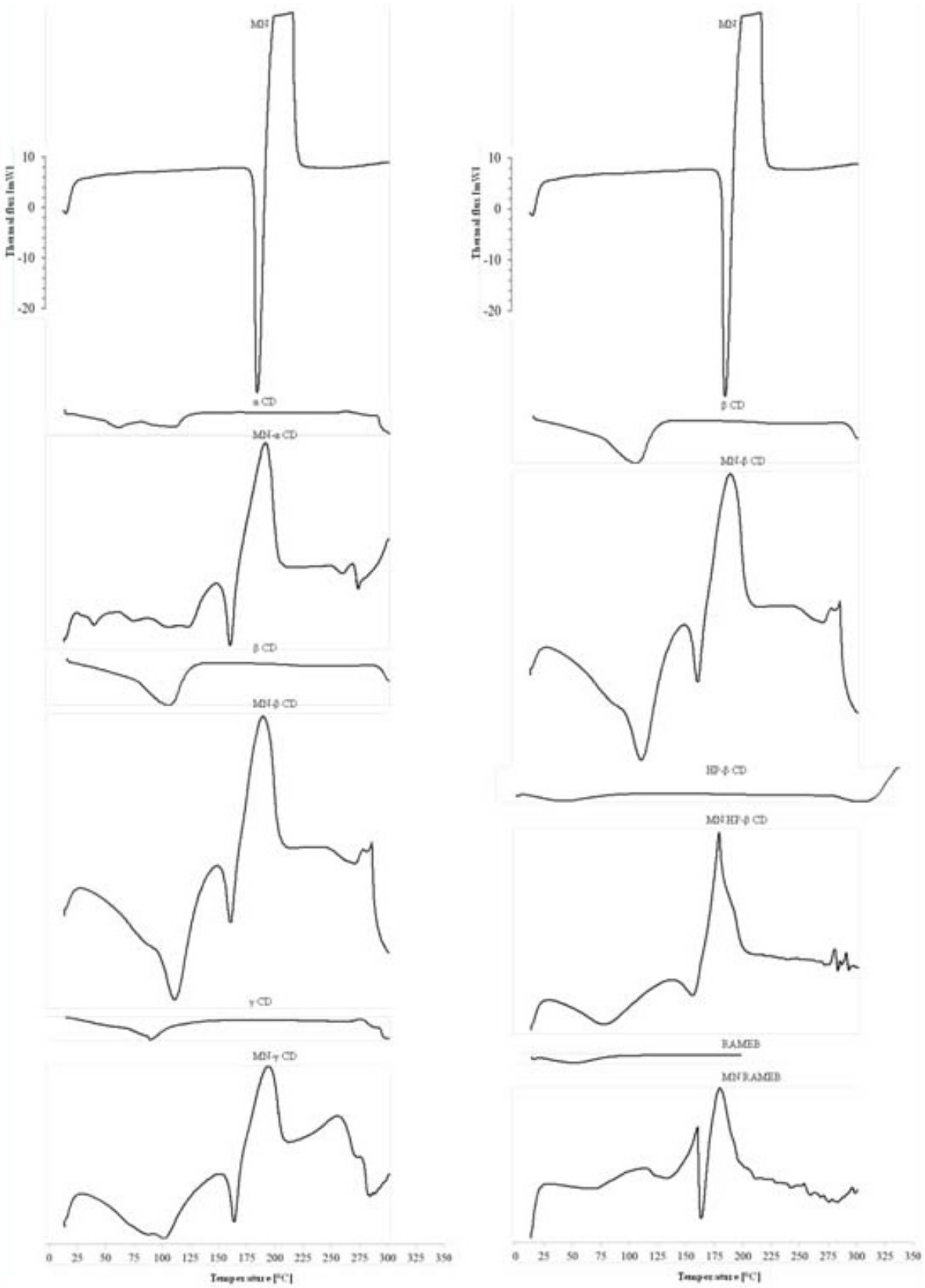

Fig. 3. DSC thermograms of miconazole nitrate (MN), CDs, and their complexes

The disappearance of characteristic frequencies of imidazole ring from the absorption spectra of the complexes in range of 1000-650 cm^{-1} indicates that it fits the cavity of the HP-β-CD. Together with the imidazole ring the aliphatic carbon atom bonded to N of the imidazole was also encapsulated (Figure 6).

Evidence of complex formation was obtained also by FT-IR spectroscopic measurements of the bands corre-

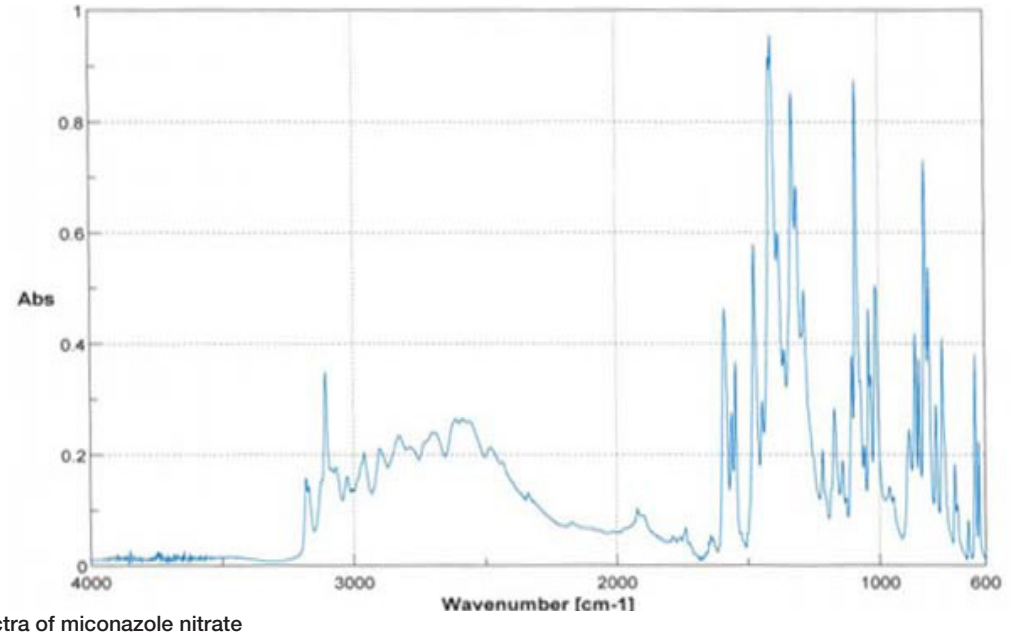

Fig. 4. FT-IR spectra of miconazole nitrate

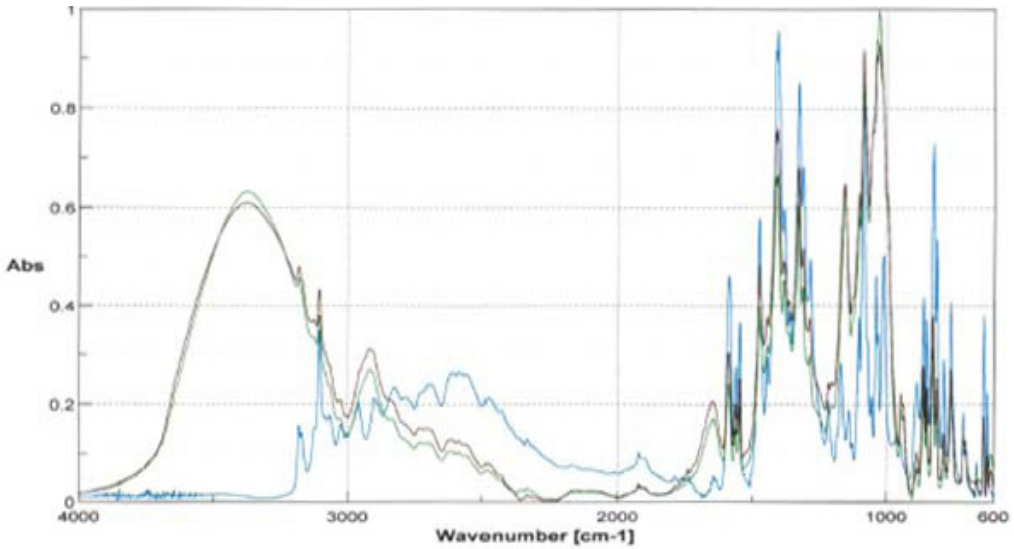

Fig. 5. FT-IR spectra of MNZ-β-CD (MNZ is marked with blue, KP complex marked with green and the PM complex marked with red)

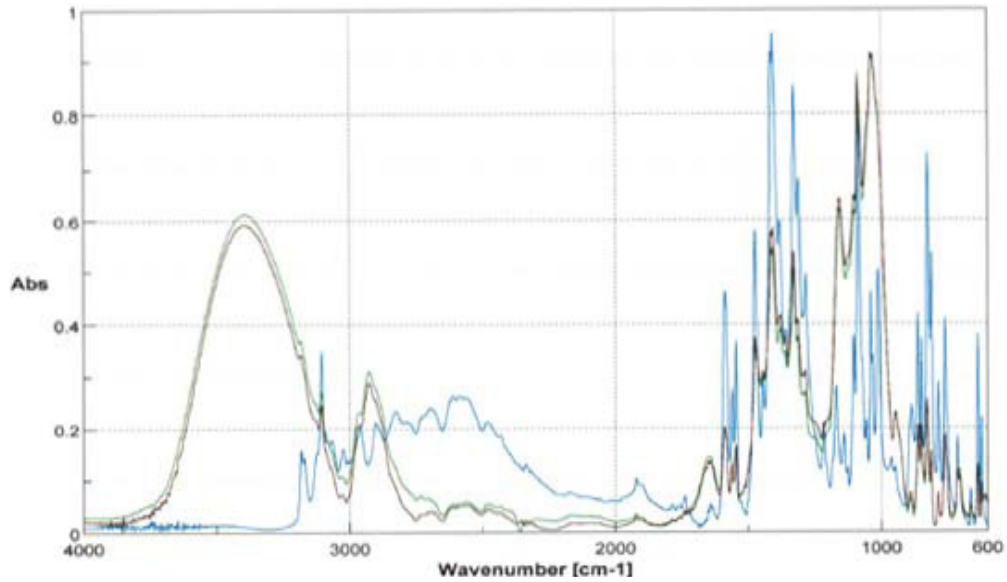

Fig. 6. FT-IR spectra of MNZ complexes with HP-β-CD (MNZ is marked with blue, KP complex marked with green and the PM complex marked with red)

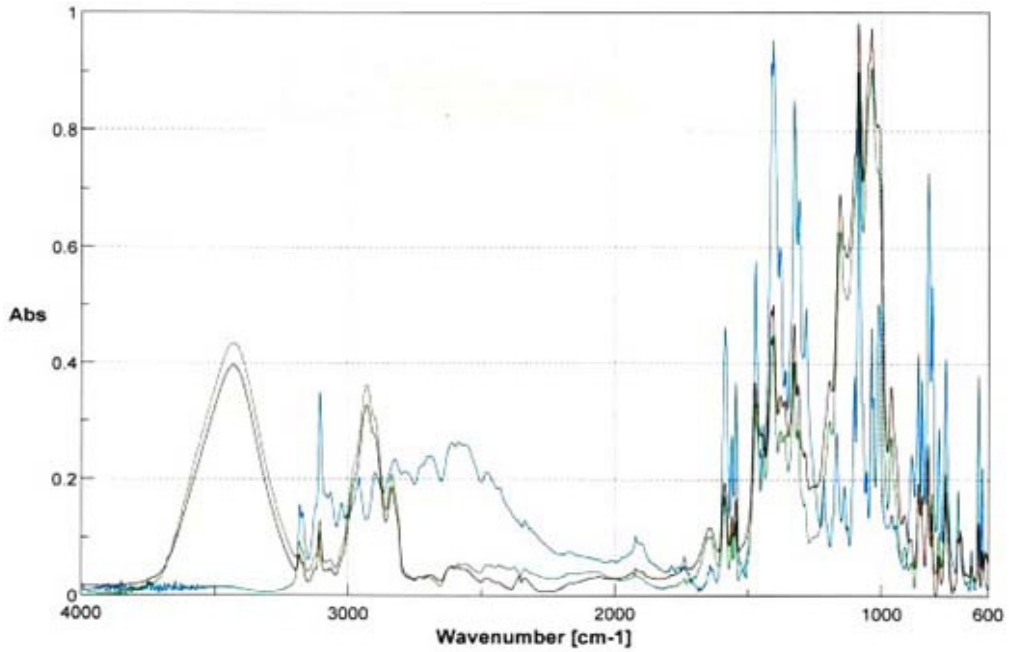

Fig. 7. FT-IR spectra of MN complexes with RAMEB (MN is marked with blue, KP complex marked with green and the PM complex marked with red)

sponding to the functional groups of MNZ involved in the complexation.

Changes in IR such as the disappearance or intensity of characteristic bands, the emergence of new bands, demonstrate the interaction between miconazole and CDs.

From the spectra it appears that the strongest interaction is with RAMEB (Figure 7) followed by HP-β-CD.

Conclusions

The thermoanalytical analysis data sustains the hypothesis of partial inclusion complex formation between MNZ and CDs. The thermic behaviour depends both on the preparation method and the composition of the product. The DSC analysis point at the formation of a new solid phase due to a molecular interaction between MNZ and CD, suggested by the shifting of the melting peak of the guest and the reduction of the melting enthalpies values, observed in the thermograms of the binary systems.

It was revealed that the properties of the products of CDs with MNZ are influenced by nature of the CD. The FT-IR analysis suggest the formation of a new solid phase, indicating a molecular interaction between the components; changes observed in the FT–IR spectra, such as shift of characteristic absorption bands of MNZ, disappearance or reduction in intensity and appearance of new bands might be related to possible drug - CD interactions or amorphization of the complexation product.

Conflict of interest

None to declare.

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