J Incl Phenom Macrocycl Chem (2007) 57:197–201
DOI 10.1007/s10847-006-9196-9

ORIGINAL ARTICLE

Mebendazole complexes with various cyclodextrins: preparation and physicochemical characterization

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Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 18 January 2007

Abstract Mebendazole is an antihelmintic drug, active against many intestinal parasites. Its systemic efficacy is limited by its poor water solubility. The use of natural or derivatized cyclodextrins permeated to multiply notably its apparent solubility, especially with permethyl β-cyclodextrine (PM β-CD) (multiplied by 4700). The inclusion complex formation between mebendazole and this methylated β-cyclodextrin, was characterized by mass spectrometry, powder X-ray diffractometry and Fourier transform infrared spectroscopy: mebendazole seemed to be included in permethyl β-cyclodextrin by its aromatic rings. To prepare inclusion complex of mebendazole and PMβ-CD by solvent evaporation, acetone may be used and the ratio using lower amount of cyclodextrin (MBZ:CD, 1:2) should be used.

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Keywords Mebendazole · Cyclodextrins · Inclusion complex · Physicochemical characterization

Introduction

Mebendazole (MBZ), a benzimidazole carbamate derivative, is an anthelmintic, active drug against intestinal nematode infection and hydatid disease when administered in high doses (2.4 g/day, for 1–6 months) [1].

Nevertheless, its low biodisponibility (5–10%) is due to its poor water solubility which limits therapeutic potentialities for systemic use against *Ecchinococcus granulosus*, for instance. Enhancing its solubility could be an alternative to the use of high doses, generally associated with adverse side effects (gastro-intestinal disturbances, alopecia, reversible bone marrow depression...). Cyclodextrins (CDs), a group of cyclic oligosaccharides, able to form inclusion complexes, are generally efficient vectors contributing to solubilization and stabilization of host drugs. In oral preparations, co-administration of CDs has been reported to increase drug absorption and efficiency of poorly water-soluble drugs like tolbutamide or phenytoin [2, 3].

In this work, we have investigated the complexation of MBZ with CDs. Solubility studies of α-CD, β-CD, γ-CD, HPα-CD, HPβ-CD, HPγ-CD and PMβ-CD/MBZ complexes were carried out according to the method of Higuchi and Connors [4]. All complexes were prepared by solvent evaporation and in case of complexation with PMβ-CD, the best solubilizing agent, the solvent influence was studied: acetonic or methanolic solution of MBZ added to PMβ-CD solution, with or without 6% (v/v) formic acid were tested.

This latter complex was characterized by Mass Spectrometry (MS), X-Ray Diffractometry (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

Experimental

Materials

MBZ (Fig. 1) ($C_{16}H_{13}N_3O_3$; MM: 295.3 g mol^{-1}; melting point 290°C) was obtained from Janssen Cilag (France). α-, β-, γ-cyclodextrins and hydroxypropyl α-, β-, γ-cyclodextrins (HPα-, HPβ-, HPγ-CD) were obtained from Roquette and Wacker. Permethyl β-cyclodextrin (PMβ-CD) was provided by Orsan. All other chemicals and reagents were USP-NF quality.

MBZ quantification by HPLC

Mebendazole (MBZ) concentration was evaluated by an HPLC method with a Jasco AS950 pump, equipped with a Cromasil® C18 (18 cm) reverse phase column and a Merck multichannel photodetector L3000. Mobile phase consisted in a mixture of acetonitrile and sodium acetate buffer 0.25 N (1/1, v/v), pH 5.7. Flow rate was fixed to 1 ml/min, injection volume to 20 µl and detection wavelength to 289 nm. In that case, retention time was comprised between 5 and 6 min.

Phase solubility studies

Phase solubility studies were performed according to the method of Higuchi and Connors [4]. Increasing concentration of CDs water solutions were prepared, the highest concentration of natural CDs corresponding to their saturated concentration. An excess amount of MBZ (2 mg/ml) was added to these solutions. After 1 week equilibrium, in a steam room at 37°C and centrifugation, supernatant was filtered on 0.22 µm membrane filter and assayed for drug content by HPLC method at 289 nm.

Preparation of PMβ-CD inclusion complexes (IC) and physical mixtures

Inclusion complexes between MBZ and PMβ-CD were prepared by solvent evaporation.

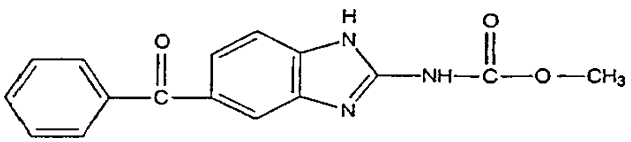

Fig. 1 MBZ molecular structure

PMβ-CDs was dissolved in 5 ml water. To this solution, acetonic or methanolic solution of MBZ was added, with or without 6% (v/v) formic acid, in a 1/1, 1/2 or 2/1 molar ratio (MBZ over cyclodextrin). The whole solutions were stirred for 2 weeks in a hood at 37°C ± 2°C.

The corresponding physical mixture was obtained by thoroughly mixing the MBZ with PMβ-CD.

Mass spectrometry (MS)

Mass spectrometry analysis was performed on a Hewlett Packard S973 mass spectrometry.

Samples were introduced by a direct insertion probe heated with an increasing temperature (2 min at 30°C, rise in temperature until 450°C at a 10°C/min rate and 2 min stabilization at 450°C). Analysis was achieved by electronic impact (70 eV). Two types of diagrams were analyzed: total ionic flow (expressed in abundance according to the time) and mass spectrum at accurate time during temperature increase.

Powder X-ray diffractometry

X-Ray diffraction spectra were obtained with a Siemens d5005 diffractometer equipped with a Nickel-filtered copper Kα radiation. Thirty milligram-samples filtered through a 180 mesh membrane was placed on the center of a quartz plate and analysis was performed at 45 kV and 40 mA; with a 0.02° 2θ step size, a 1°/min counting rate and 5–25° 2θ analysis range.

Fourier transform infrared spectroscopy (FT-IR)

The Fourier Transform Infrared Spectra were obtained from a Perkin Elmer IR spectrometer. Samples were prepared by the potassium bromide disk method and scanned for absorbance from 3500 to 1000 cm^{-1}.

Results and discussion

Phase solubility studies

Solubility diagrams of MBZ with pristine CDs (Fig. 2), HPβ- and HPγ-CD (Fig. 3) resulted in an A_L type Higuchi phase solubility diagram. Thus, complexes formed with these CDs were of the first order with regards to the host molecule [4] whereas HPα-CD (Fig. 3) and PMβ-CD (Fig. 4) resulted in a A_p type Higuchi phase solubility diagram leading to formation of a higher order than one in the host molecule, indicating the formation of 1/1 and 1/2 stoichiometric ratio of MBZ/CD.

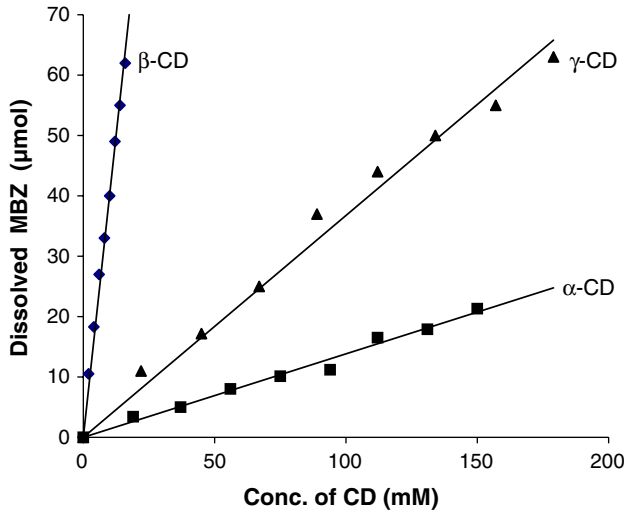

Fig. 2 Higuchi phase solubility diagrams of MBZ in presence of α, β and γ-CD

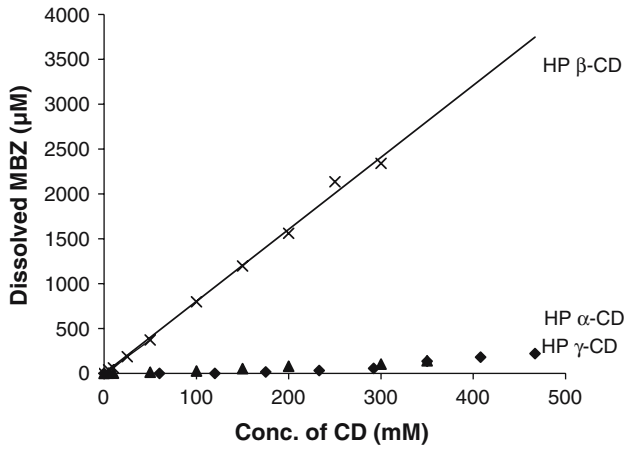

Fig. 3 Higuchi phase solubility diagram of MBZ in presence of HPα-, HPβ- and HPγ-CD

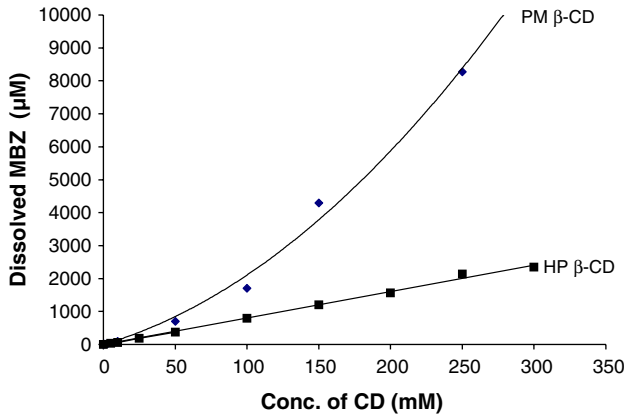

Fig. 4 Higuchi phase solubility diagrams of MBZ in presence of HPβ-CD and PMβ-CD

Apparent solubilities were 35- and 4700-fold increased in presence of β-CD (the best solubilizer among pristine CDs) and PMβ-CD (the best solubilizer among derivatized CDs), respectively. However, these apparent solubilities were limited to the maximum solubilization ability of these CDs: 16 and 250 mmol/l in case of β-CD and PMβ-CD, respectively. The largest increase of MBZ solubility was obtained by using PM β-CD, consequently, preparation of inclusion complex was rather done with this derivatized cyclodextrin.

Mass spectrometry analysis

All mass spectra confirmed the presence of MBZ specific fragments at m/z = 295, 218 and 186, at its volatization time of 4.5 min [5]. MBZ remained stable during the complex formation (Fig. 5). Spectrum of MBZ and PMβ-CD physical mixture was a simple addition of their two individual spectra: there was no interaction between MBZ and PMβ-CD in physical mixture. In the mass spectra of inclusion complex, a new peak appeared about 6 min indicating complex formation. MBZ residual peak was smaller in spectra of 1/2 MBZ/CD ratio complex indicating a less important interaction between MBZ and PMβ-CD.

X-ray diffractometry studies

Further evidence of complex formation was obtained by X-ray powder diffraction (Fig. 6). An amorphous pattern lacking crystalline peaks was observed for PMβ-CD. Nevertheless, MBZ diffractogram was well crystallized. Physical mixture diffractogram was the superimposition of each pure component's diffractograms. Complex prepared with methanol or acetone

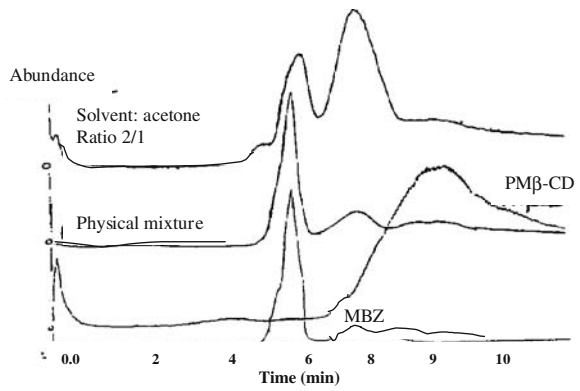

Fig. 5 Mass spectra of MBZ, PMβ-CD, physical mixture, inclusion complex prepared with acetone in 2/1 molar ratio

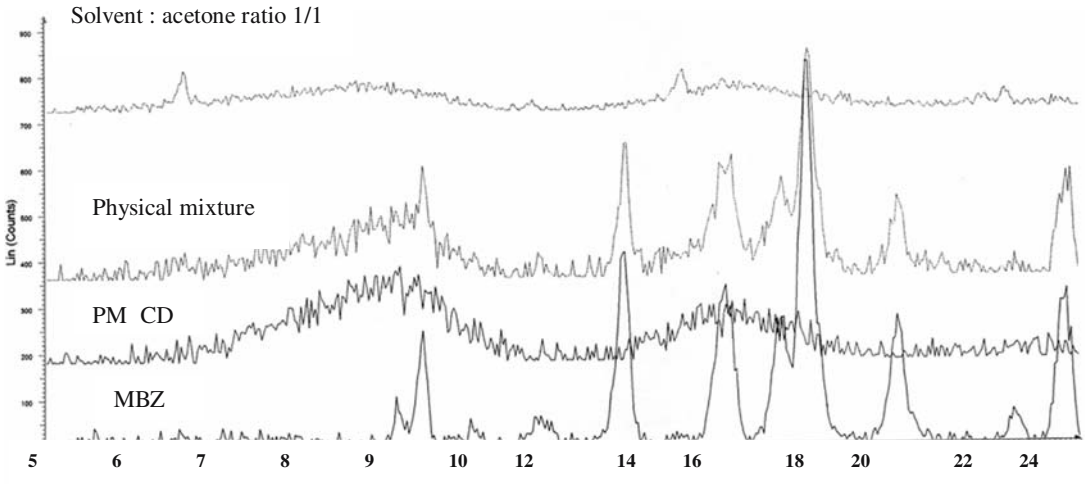

Fig. 6 X-Ray diffractograms of MBZ, PMβ-CD, physical mixture, and inclusion complex prepared with acetone in 1/1 molar ratio

were amorphous which emphasized its inclusion in PMβ-CD. However, diffractograms of complex prepared with formic acid presented peaks at different positions from those from MBZ or PMβ-CD (not shown), which indicate the formation of another crystalline state.

Fourier transform infrared spectroscopy studies

FTIR spectrum of MBZ (Fig. 7), presented main specific peaks, characteristic from C form [6]: free N–H stretching frequency at 3412 cm^{-1}; carbonyl stretching frequency at 1720 cm^{-1}. In complex spectra, most of these peaks disappeared and modifications in benzimidazole stretching could be observed.

FT-IR spectra of physical mixture did not demonstrate any significant difference from the spectra of each pure component. Any significant difference had been found between spectra of complex prepared in different molar ratios. However, MBZ residual peaks originating from complex prepared with acetone, were less distinct than the ones prepared with methanol. Those originating from complex prepared with formic acid disappeared. These observations were in adequation with MBZ polymorphs crystallization ability in well-chosen solvents: form C was recrystallized by de Villiers et al. (2005) in methanol, form B in chloroform and form A in glacial acetic acid. Moreover, form C should be preferred as its solubility is sufficient to ensure optimal bioavailability and it is less

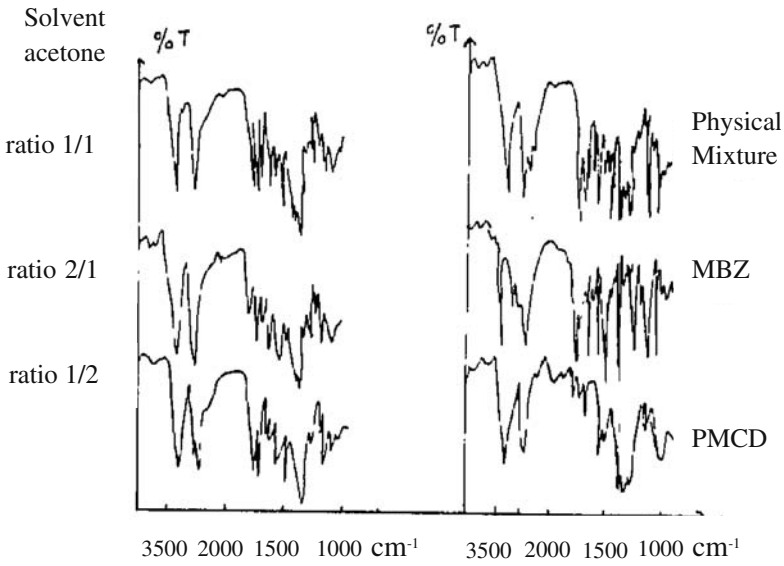

Fig. 7 FTIR spectra of MBZ, PMβ-CD, physical mixture and inclusion complex prepared with acetone in 1/1, 1/2, and 2/1 molar ratios

toxic than form B whereas form A has no anthelmintic activity [7, 8].

Conclusion

MBZ was well-included in PMβ-CD, the CD which had the best solubilization ability. Formic acid should be avoided in the inclusion complex preparation between MBZ and PMβ-CD, as in that case, another crystalline state was formed, probably polymorph A which has no anthelmintic activity.

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