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RESEARCH PAPER

Preparation and Characterization of Albendazole β-Cyclodextrin Complexes

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ABSTRACT

Albendazole (ABZ), mebendazole (MBZ), and ricobendazole (RBZ) are low-soluble anthelmintic benzimidazole carbamate drugs. To increase their aqueous solubility, three different types of β-cyclodextrins (CyDs): β-cyclodextrin (CD), hydroxypropyl-β-cyclodextrin (HPCD), and methyl-β-cyclodextrin (MCD) were used. Solubility depended on the type of CyDs. Increased solubility was obtained when the more substituted CyDs (HPCD or MCD) were used instead of nonsubstituted CD. Stability constants were calculated assuming a 1:1 stoichiometry. Calculated stability constant values depended on initial solubility of drug and pH of the medium. Solid ABZ complexes were prepared by coprecipitation and freeze-drying methods. These products were compared with physical mixtures of ABZ and CyDs. The characterization of these products was made by differential scanning calorimetry (DSC) and drug release studies. True inclusion complexes were obtained only by the freeze-drying method. Drug release studies showed that the freeze-dried inclusion complexes increased the solubility rate of ABZ, although a supersaturation effect was observed when drug release studies were performed in nonsink conditions. A bioavailability study on mice was done with a formulation of ABZ:HPCD complex and was compared to a conventional ABZ suspension. A significantly ($p < .05$) *shorter* T_{max} *of absorption was obtained by using the complex formulation. Greater and significant* ($p < .05$) *differences for AUC and* C_{max} *were observed.*

Key Words: *Albendazole; Bioavailability; Cyclodextrin; Solubility.*

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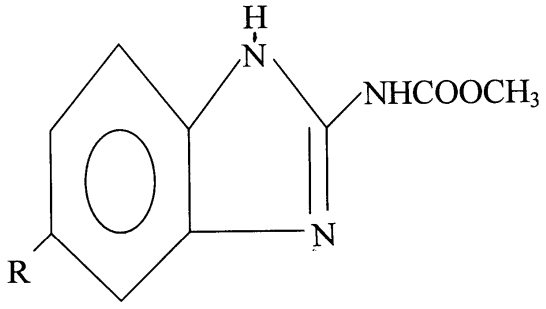
INTRODUCTION

Benzimidazole carbamates are anthelmintic drugs widely used in the treatment of intestinal and tissue nematode infections and, in higher doses, for therapy of echinococcosis. Among the different benzimidazole carbamates, we chose mebendazole (MBZ), albendazole (ABZ), and ricobendazole (RBZ). Figure 1 shows the chemical structures of these molecules. These drugs usually exhibit low water solubility, which reduces absorption by the oral tract (1).

In previous works, solid dispersion formulations were prepared with polyvinylpyrrolidone (2) and liquid formulations with Transcutol® (3) to improve the aqueous solubility and dissolution characteristics of ABZ. In this work, our aim was to study the effect of different cyclodextrins (CyDs) on ABZ solubility characteristics and their effect on bioavailability. To this end, we chose three different CyDs: β-cyclodextrin (CD), hydroxypropyl-β-cyclodextrin (HPCD), and methyl-β-cyclodextrin (MCD), which were mixed with ABZ in two media, distilled water or acid buffer. MBZ and RBZ are two other benzimidazole carbamate anthelmintic drugs less and more soluble, respectively, than ABZ; these were also solubilized with CyDs. The type of CyD with the best solubility results was used to prepare solid complexes of ABZ by means of coprecipitation and freeze-drying methods. The resultant products were characterized by differential scanning calorimetry (DSC) and dissolution release studies. A bioavailability study in mice was done with the best formulation to compare its characteristics with those of a reference suspension formulation.



MBZ R=-CO-C_6H_5
ABZ R=-S-CH_2-CH_2-CH_3
RBZ R=-SO-CH_2-CH_2-CH_3

Figure 1. Structures of different anthelmintic benzimidazole carbamates: mebendazole (MBZ), albendazole (ABZ), and ricobendazole (RBZ).

MATERIALS

Albendazole (Smithkline Beecham Pharmaceutical, UK), mebendazole (Sigma, St. Louis, MO), ricobendazole (Chemo Ibérica, Spain), β-cyclodextrin (Amaizo, USA), hydroxypropyl-β-cyclodextrin (Janssen, Belgium), methyl-β-cyclodextrin (Ringdex, France), Explotab (FMC, USA), and Avicel PH 101 (FMC) were used for the experiments.

METHODS

Solubility Studies

The solubility studies were carried out according to the method described by Higuchi and Connors (4). Different types and concentrations of CyDs were employed to prepare solutions in distilled water or in acid medium (ClH/ClK 0.2 M, pH 1.2), and an excess amount of the different drugs (MBZ, ABZ, or RBZ) was added. The tubes were closely fitted, sonicated for 15 min and then shaken at approximately 15 rpm and heated at 37°C for 1 week. Samples were made in triplicate. The suspensions were filtered through a 0.45-μm filter, properly diluted, and assayed for drug concentration by ultraviolet UV spectrophotometrical analysis at 284 nm for MBZ, 291 nm for ABZ, and 289 nm for RBZ. The stability constants were calculated from the solubility diagrams assuming a 1:1 stoichiometry.

Elaboration of Inclusion Complexes

Solid complexes were prepared using different ratios of ABZ and HPCD. The ratios were calculated on a molecular basis, and the following proportions were prepared: 1:1, 1:1.5, and 1:2. The complexes were made by the coprecipitation and freeze-drying methods.

Coprecipitation Method

For the coprecipitation, ABZ was dissolved in the minimum amount of ethanol, and HPCD was dissolved in distilled water. The mixture was stirred for 1 hr, and then it was slowly evaporated at room temperature. After 7 days, a crystalline powder was precipitated, recovered,

and sieved through a 0.247-mm mesh. The product was stored in a desiccator with silica gel for 3 days.

Freeze-Drying Method

For freeze-drying, ABZ was dissolved in acid medium (ClH/ClK 0.2 M, pH 1.2), and HPCD was dissolved in distilled water. The mixture of both solutions was stirred at room temperature for 24 hr and then freeze-dried for 2 days. The product was sieved through a 0.247-mm mesh. Complexes with MCD were also made in a 1:1 ratio in the same conditions as complexes formed with HPCD.

Elaboration of Physical Mixtures

Physical mixtures of ABZ and HPCD were prepared in the same molar ratios as inclusion complexes. To this end, the components were mixed with pestle and mortar, then the mixture was sieved through 0.247 mesh and stored in a desiccator with silica gel for 3 days.

In Vitro Characterization

Differential Scanning Calorimetry

Inclusion complexes, physical mixtures, free drug, and HPCD were subjected to DSC studies using DSC polymer equipment. The temperatures ranged between 35°C and 350°C, with a heat flow of 0.5 cal/°C min.

Dissolution Rate Studies

Dissolution rate profiles for the different products (ABZ as the raw material, physical mixtures, and complexes) were evaluated. To avoid floating of the products, the different samples were mixed with Explotab (5%), as the disintegrant agent, and Avicel PH 101, as diluent, and compacted at low tableting pressure. The disintegration time of the resultant products was always less than 5 min. The amount of ABZ for each experiment varied between 2 mg (sink conditions) and 50 mg (nonsink conditions). The dissolution studies were carried out in a USP 23 apparatus, method II. Dissolution media were 900 ml of distilled water or acid medium. The temperature of the study was 37°C, and the system was stirred at 100 rpm. Samples were taken at different time intervals: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min. They were then filtered and assayed by UV spectrophotometrical analysis at 291 nm. Experiments were made in triplicate.

In Vivo Characterization: Bioavailability Study

The bioavailability of ABZ:HPCD freeze-dried complex at a 1:1 ratio was compared with a conventional ABZ suspension. Both formulations were administered orally in a single dose of 20 mg/kg to Swiss CD-1 mice (weight 30 ± 3 g). After drug administration, groups of 6 mice were sacrificed, and blood samples were collected at different times (0.25, 0.5, 0.75, 1.5, 3, and 6 hr). Blood samples were heparinized and centrifuged individually after the extraction. Plasma samples were frozen until the high-performance liquid chromatography (HPLC) analysis, previously described (5).

The T_{max} and C_{max} were estimated as the mean values of time (T_{max}) taken to achieve maximum plasma concentrations (C_{max}) for each of the 6 mice used at each time point. The areas under the plasma concentration-time curve between 0 hr and 6 hr (AUC_{0-6}) were calculated by the trapezoidal method (6). Comparative statistical study among formulations was performed by a one-way analysis of variance (ANOVA) test.

RESULTS AND DISCUSSION

Figures 2, 3, and 4 show phase solubility diagrams for MBZ, ABZ, and RBZ, respectively, in distilled water. Figures 5, 6, and 7 show results in acid medium. The solubility of these drugs increases linearly with increasing concentration of CyDs. According to the Higuchi and Connors method (4), the phase solubility diagrams in our experimental conditions can be considered as A_L types. This behavior is characteristic of complexation with the stoichiometric ratio of drug:CyD of 1:1. The apparent stability constants *Kc* of the different anthelmintic drugs with the different CyDs in distilled water or in acid buffer medium were calculated from the slope and intercept S_0 of the initial straight-line portion of the diagram according to the following equation: $K = \text{Slope}/S_0 (1 - \text{slope})$.

Tables 1 and 2 show results of *Kc* (M^{-1}) and *r* values (in parentheses) for the different drug:CyD systems in distilled water and acid buffer medium, respectively. The stability constants correlate with the interaction forces between guest (drug) and CyD. In our systems, the stability constants usually have low values, which means a weak association-dissociation in both media tested. *Kc* values are characteristic of the pH in the liquid medium and also depend on the benzimidazole carbamate drug and type of CyD. *Kc* values between 200 and 5000 M^{-1}

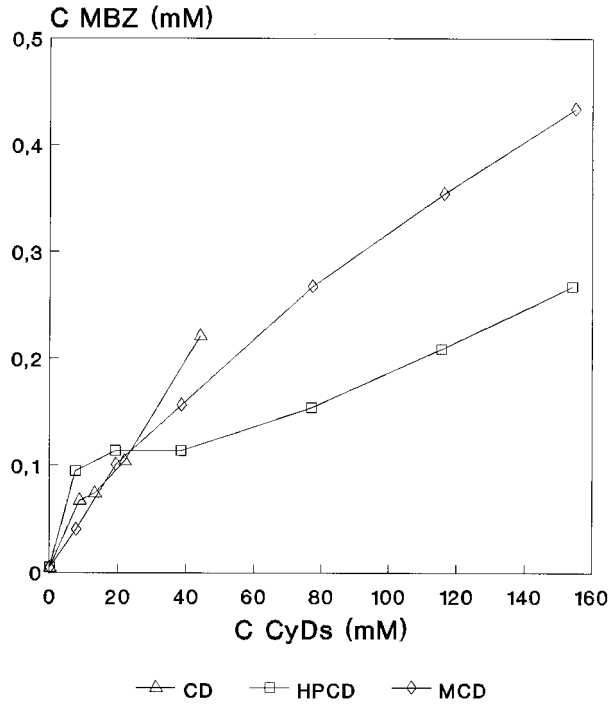


Figure 2. Phase solubility diagram of MBZ in distilled water in the presence of different CyDs.

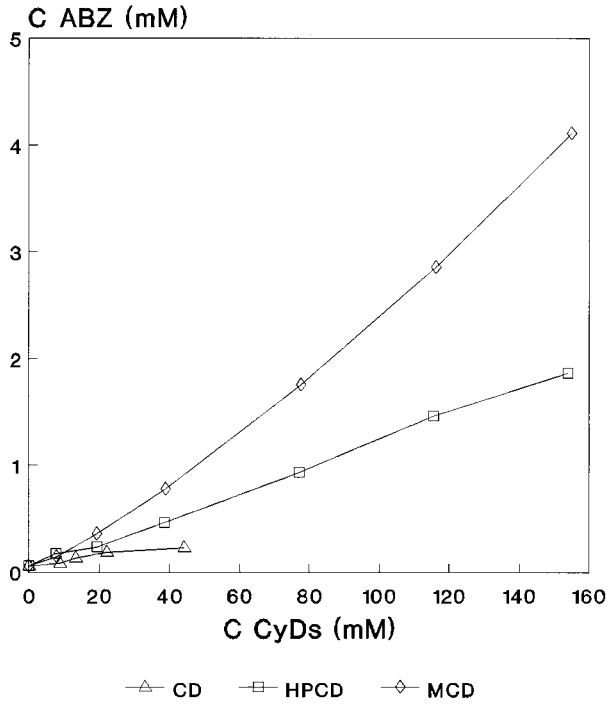


Figure 3. Phase solubility diagram of ABZ in distilled water in the presence of different CyDs.

are considered by various authors as the most suitable for the improvement of bioavailability of poorly soluble drugs (7). According to these criteria, the best results for the benzimidazole carbamate drugs are obtained with the less-soluble drugs (MBZ and ABZ). Clearly, drug solubility affects *Kc* values. When drug solubility increases, *Kc* values decrease. This effect can be due to the hydrophobicity of the drug and has been reported previously for some other molecules (8). If inclusion of CyDs depends on hydrophobicity of the drug, when hydrophobicity decreases (and water solubility increases), *Kc* must be lower.

Tables 1 and 2 and Figs. 2–7 show how drug solubility depends on the amount and type of CyD. The type of CyD that produces the highest solubilization effect depends also on the drug. For example, for the least-soluble drug (MBZ), the highest solubilization effect was achieved with CD. Nevertheless, for the more-soluble drugs (ABZ and RBZ), the best results were obtained with the most-soluble CyD (MCD).

At acid pH (1.2), higher solubility values were obtained due to the ionization effect of the three drugs, although with a lower stability constant than in distilled water. Benzimidazole carbamate drugs are basic drugs, so in acid medium they are in ionized form, which is most soluble, but as reported previously, ionized molecules produce weaker interactions with the hydrophobic cavities of CyDs than un-ionized ones (9,10). At acid pH, MBZ and RBZ increase their solubility by CyDs without regard to the type of CyD. For ABZ, the greatest solubility effect was obtained with MCD.

It can be concluded from this part of the work that, for MBZ, ABZ, and RBZ, drug solubility can be increased with CyDs. The best results depend on the drug, type of cyclodextrin, and pH of the medium.

Among the different CyDs studied, HPCD was chosen to prepare solid inclusion complexes because of its low toxicity and high solubilization effect in ABZ. Solid inclusion complexes were prepared by coprecipitation and freeze-drying techniques.

Figure 8 shows the DSC results for ABZ, HPCD, physical mixtures, coprecipitation, and freeze-drying systems at a ratio of 1:1. For the ABZ sample, a sharp endothermic peak is observed between 200.6°C and 200.9°C. The peak is not a clear single peak. In fact, it can be considered a double peak, which can be indicative of polymorphous or enantiomeric compounds. The ABZ polymorphous form has been reported by Zhu, Yang, and Botha (11). In the thermogram of HPCD, three endothermic peaks are observed. In the range of temperatures be-

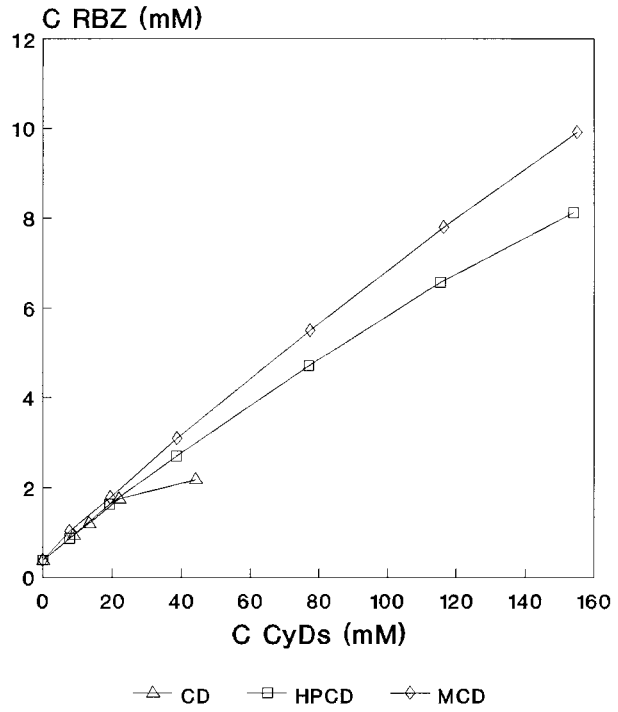


Figure 4. Phase solubility diagram of RBZ in distilled water in the presence of different CyDs.

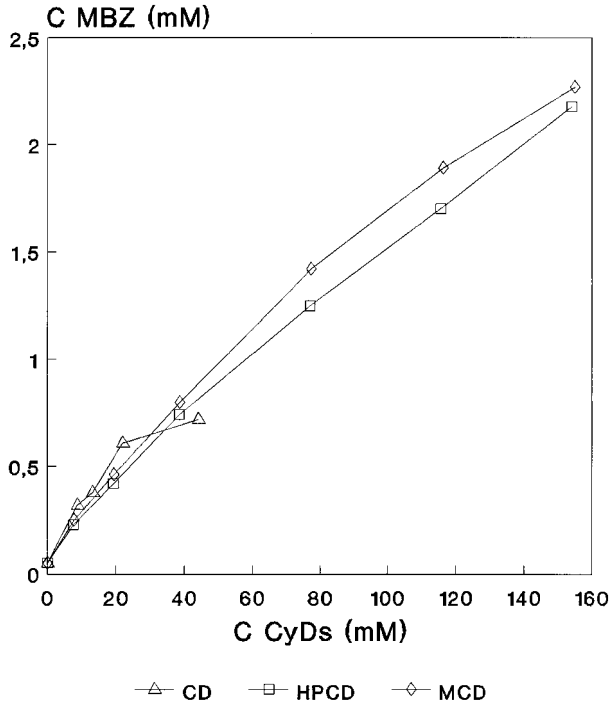


Figure 5. Phase solubility diagram of MBZ in acid buffer medium (ClK/ClH 0.2 M) in the presence of different CyDs.

tween 65°C and 75°C, a decomposition of approximately 1/3 of the weight of CyD is produced. In the range between 90°C and 100°C, loss of water is produced (the moisture content of the sample assayed by desiccation was between 3% and 4%). Near 350°C, the endothermic peak corresponding to HPCD fusion is observed. The physical mixture of ABZ and HPMC shows a behavior that can be considered a superposition of its two components. In the ABZ:HPCD systems obtained by the coprecipitation method, the DSC technique shows the endothermic peak corresponding to the melting of ABZ. Although this peak is partially reduced in size when compared to the ABZ as a raw material, the presence of this peak indicates that a true inclusion complex has not been achieved. Nevertheless, in the ABZ:HPCD systems obtained by the freeze-drying method, the endothermic peak corresponding to melting of ABZ is not present. A new peak appears at temperatures between 260°C and 270°C that can be attributed to melting of true inclusion complexes.

Figure 9 shows the dissolution rate profile of different ABZ samples in sink conditions. Solid systems, obtained by coprecipitation and freeze-drying methods, show faster and greater solubility than the reference ABZ and physical mixtures of ABZ:HPCD. The high ABZ dissolution obtained by the coprecipitation method is probably because most of the ABZ molecules are surrounded by HPCD molecules, which increase the hydrophilic characteristics of ABZ. Solid systems obtained by freeze-drying methods show the highest solubility rates at the beginning of the dissolution test (approximately 10 min). Later, a small decrease on solubility was observed. This effect is more important when the dissolution test is performed in nonsink conditions (see Fig. 10). For the solid systems obtained by coprecipitation and freeze-drying methods, an increase of solubility is achieved when the proportion ABZ:HPCD is increased to molar ratios 1:1.5 and 1:2 (results are not shown). Figure 9 shows there are few differences in dissolution between reference ABZ and the physical mixture of ABZ:HPCD. Perhaps, a slight enhancement of dissolution is achieved in the physical mixtures that can be due to the formation of a hydrodynamic layer of CyDs surrounding the ABZ particles in the acid medium. A similar effect was noticed by Ismail (12,13).

Figure 10 shows the dissolution rate profile of the different ABZ formulations in nonsink conditions. In clinical therapy, the doses of ABZ can be as high as 800 mg. With this amount of ABZ, perhaps sink conditions are not obtained in the digestive system. Dissolution tests in

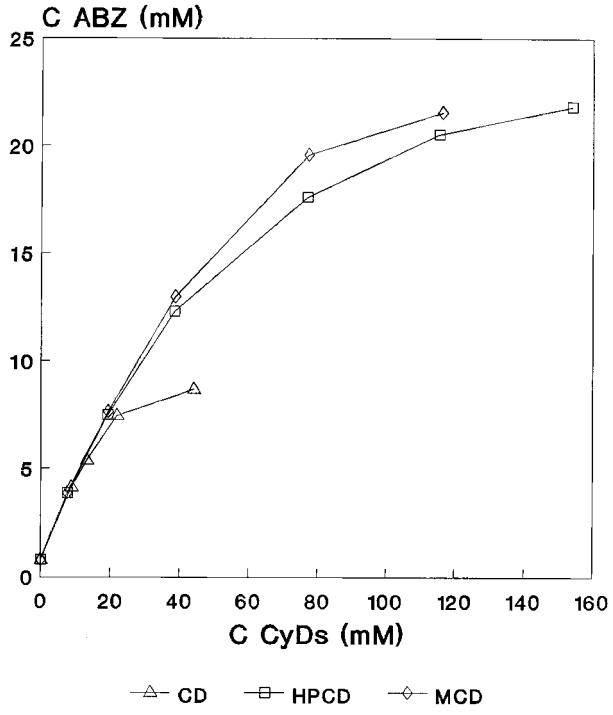


Figure 6. Phase solubility diagram of ABZ in acid buffer medium (ClK/ClH 0.2 M) in the presence of different CyDs.

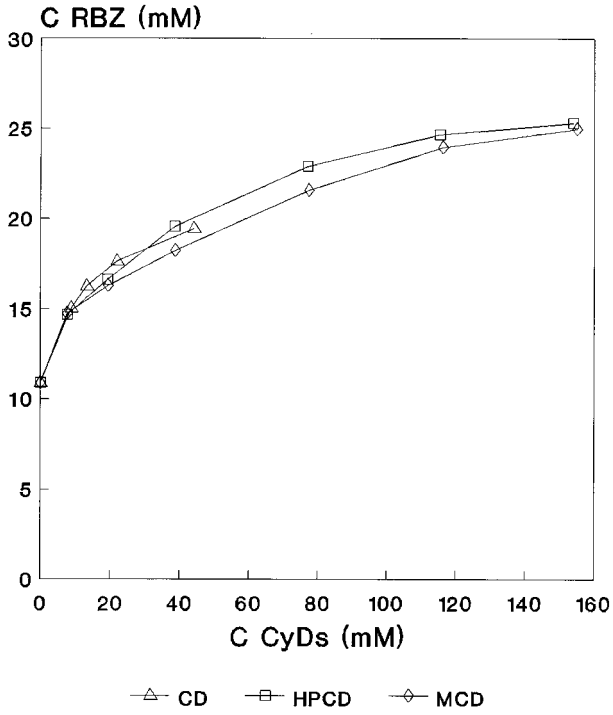


Figure 7. Phase solubility diagram of RBZ in acid buffer medium (ClK/ClH 0.2 M) in the presence of different CyDs.

acid buffer medium with 50 mg of ABZ samples were done to find the effect of being in nonsink conditions. These experimental conditions are probably more similar to the in vivo real situation than the experiments carried out with low doses of ABZ, which were used in order to be in sink conditions. In these dissolution conditions, the enhancement of solubility at some points of the dissolution test can be five times that of the reference. MCD and HPCD were employed to prepare inclusion complexes with ABZ. The effect on ABZ dissolution of these two different CyDs is very similar in acid buffer medium. The precipitation phenomenon observed and discussed in Fig. 9 is also observed in Fig. 10. This effect is even more evident in nonsink conditions than under sink conditions, probably because higher doses of ABZ are used; for this reason, ABZ precipitation is produced more markedly. This effect has been observed for piroxicam–polyethylene glycol 4000 in solid dispersions (14), nifedipine with polyethylene glycol 6000 and hydroxypropylmethyl cellulose (15), and different drug:CyD systems (16,17). This process has been described as a supersaturation phenomenon that can produce discrepancies between in vitro dissolution test results and in vivo bioavailability results. According to Pedersen (18), this effect can explain why, in some works, worse biological results than those expected are obtained.

A liquid formulation of ABZ with HPCD was chosen for bioavailability studies. The oral bioavailability of this new formulation was compared with an ABZ suspension. Both formulations were orally administered in mice. Plasma samples were taken at different times, and drug concentration was assayed by HPLC. Due to the fast

Table 1

Drug Solubility in Distilled Water

Drug/CyD	CD	HPCD	MCD
MBZ	1026	304.7	588.5
	(0.99)	(0.95)	(0.99)
ABZ	68.9	203.7	455.2
	(0.95)	(0.99)	(0.99)
RBZ	108.3	139.3	171
	(0.96)	(0.99)	(0.99)

Kc (M^{-1}) and *r* values (in parentheses) for the different drug:CyD systems.

Table 2

Drug Solubility in Acidic Medium

Drug/CyD	CD	HPCD	MCD
MBZ	289.6 (0.93)	270 (0.99)	288.2 (0.99)
ABZ	260.7 (0.91)	200.2 (0.95)	282.1 (0.96)
RBZ	19.5 (0.90)	8.69 (0.93)	8.3 (0.95)

Kc (M^{-1}) and *r* values (in parentheses) for the different drug:CyD systems.

ABZ metabolization, its main metabolite RBZ, which has also anthelmintic properties, was assayed. So, bioavailability characteristics of ABZ were studied depending on the RBZ concentrations obtained after ABZ administration. Mean plasma concentration time results are shown in Fig. 11. The following bioavailability parameters were determined: T_{max}, C_{max}, and $AUC_{0-6\ hr}$; they are shown in Table 3. Significant differences ($p < .05$) were obtained for the T_{max}, C_{max}, and $AUC_{0-6\ hr}$ values. When ABZ is administered as a liquid solution (HPCD complex), faster absorption is achieved than with the reference formulation of ABZ suspension. Similar results have been described previously using liquid solutions of ABZ solubilized by Transcutol (19) and with HPCD (20) and compared to suspensions.

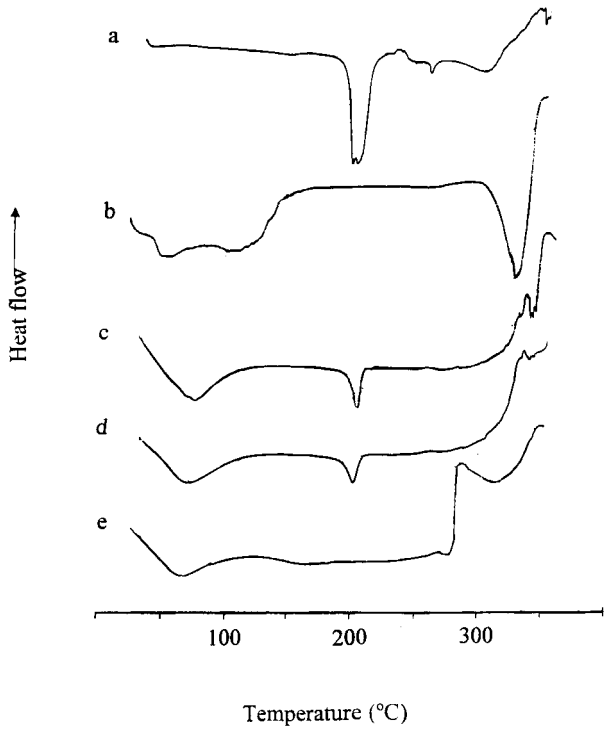


Figure 8. DSC thermograms of the following compounds: (a) albendazole; (b) HPCD; (c) physical mixture in a 1:1 ratio; (d) coprecipitation complex in a 1:1 ratio; and (e) freeze-dried complex in a 1:1 ratio.

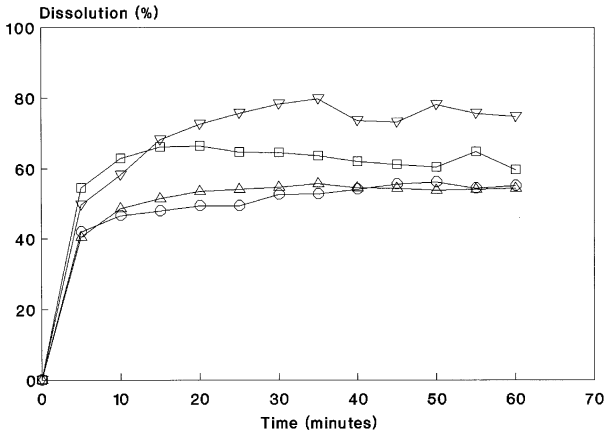


Figure 9. Dissolution rate profile in sink conditions in acid buffer medium for ○, ABZ; △, physical mixture in a 1:1 ratio; ▽, coprecipitation complex in a 1:1 proportion; and □, freeze-dried complex in a 1:1 ratio.

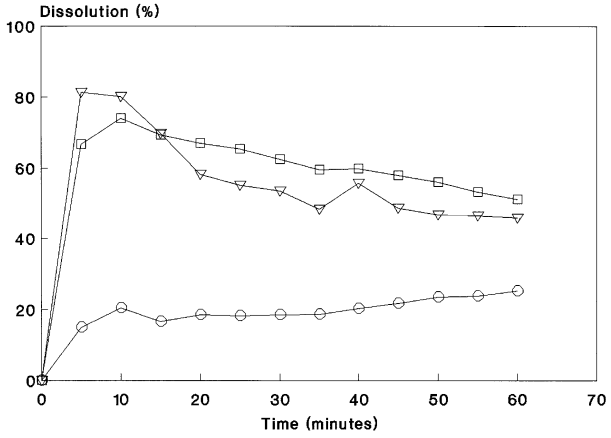


Figure 10. Dissolution rate profile in nonsink conditions in acid buffer medium for ○, ABZ; □, freeze-dried complex with HPCD in a 1:1 proportion; and ▽, freeze-dried complex with MCD in a 1:1 ratio.

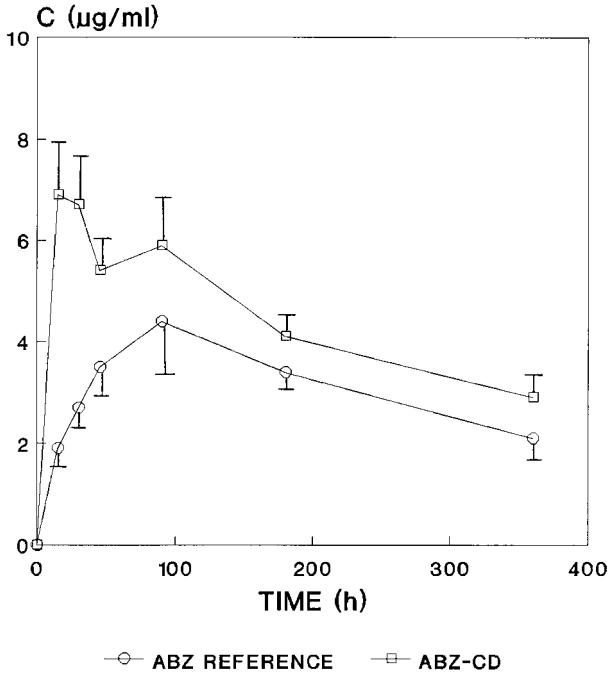


Figure 11. Mean plasma concentration-time profiles of RBZ after oral administration of two ABZ formulations equivalent to 50 mg ABZ/kg. Each point represents the average ± SE of six experiments. The freeze-dried complex of ABZ with HPCD (□) is in a 1:1 ratio.

CONCLUSIONS

Anthelmintic:CyD complexes can be obtained to increase the solubility of different benzimidazole carbamate drugs. To this end, HPCD and MCD are better options than CD. The freeze-drying method is useful for obtaining true inclusion complexes. Meanwhile, the coprecipitation method is also useful for obtaining solid products, although no true inclusion complexes were obtained. Products obtained by freeze-drying and coprecipitation methods had better solubility characteristics than ABZ as a raw material. The bioavailability results show that ABZ:HPCD complexes had faster absorption than a conventional ABZ suspension formulation. Furthermore, higher and significant ($p < .05$) values of AUC and C_{max} were obtained.

Table 3

Bioavailability Results

Formulation	T_{max} (hr)	C_{max} (µg/ml)	AUC_{0-6} (µg hr/ml)
ABZ:HPCD	0.33 (0.14)	7.6 (0.68)	26.17 (2.7)
ABZ reference	1.25 (0.43)	4.51 (0.6)	18.7 (2.1)

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