Drug Development and Industrial Pharmacy, 25(1), 111–115 (1999)

COMMUNICATION

Solubility, ^{1}H-NMR, and Molecular Mechanics of Mebendazole with Different Cyclodextrins

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ABSTRACT

The solubility behavior and binding constants (K_{ass}) of mebendazole with α-, β-, γ-, and hydroxypropyl-β-cyclodextrins (HP-β-CD) have been investigated in simulated intestinal juice by the Higuchi and Connors method. A_L diagrams have been obtained. The equilibrium has also been studied in simulated gastric fluid with HP-β-CD. The phase solubility, ^{1}H-NMR, and molecular mechanics studies revealed the formation of a 1:1 complex.

INTRODUCTION

Mebendazole is an important drug against hydatid disease; however, it presents low solubility in aqueous solutions and, consequently, poor absorption from the intestinal tract (1). To increase the drug's solubility, many new formulations have been proposed (2,3) but unfortunately without much success.

Cyclodextrins increase the solubility and dissolution rate of poorly soluble drugs, and as drug carriers, they control the drug release at the gastrointestinal level (4). It was suggested that the optimum margin of the K_{ass} to get good bioavailability should be between 200 and 5000 M^{-1} (5).

We propose to improve the mebendazole solubility by the formation of inclusion compounds with cyclodextrins in simulated intestinal juice. In addition, we propose to determine if the inclusion constants are in accord with Szejtli parameters (5) and to know the probable geometry of complexes.

MATERIALS AND METHODS

Dimethyl sulfoxide (DMSO)-d_6 (99.9 atom %D) was purchased from Aldrich. NaCl and NaOH were supplied by Mallinckrodt. Mebendazole, sodium citrate, and NaH_2PO_4 were purchased from Sigma. α, γ, and hydro-

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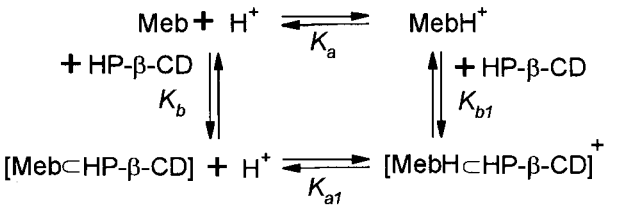
xypropyl-β-cyclodextrins (HP-β-CDs) were donated by Cerestar. β-CD was provided by Arancia.

Phase Solubility and Dissolution Rate Studies

Solubility measurements were performed according to the Higuchi and Connors method (6). The used cyclodextrin concentrations were 0, 10^{-4}, 5×10^{-4}, 10^{-3}, 4×10^{-3}, 8×10^{-3}, and 10^{-2} M. The solution medium was 0.1 M phosphate buffer aqueous solution, pH 7.5, ionic strength 0.1 M of NaCl at 25°C. The samples were analyzed by UV/vis electronic spectroscopy (diode array HP8452A Hewlett Packard spectrophotometer with a Peltier HP 8909OA system). The same procedure was accomplished with HP-β-CD in 0.1 M citrate buffer aqueous solution, pH 1.8, ionic strength 0.1 M of NaCl.

Scheme I shows the possible equilibria at the simulated gastric fluid without enzymes:



From scheme I, the authors proposed the equation

$$[\text{Meb}]_{\text{total}} = [\text{Meb}]_{\text{free}} + \frac{[\text{Meb}]_{\text{free}}[\text{H}^+]}{K_a} + \frac{\left(K_b + \frac{K_{b1}[\text{H}^+]}{K_a}\right)[\text{Meb}]_{\text{free}}[\text{CD}]_{\text{total}}}{1 + [\text{Meb}]_{\text{free}}\left(K_b + \frac{K_{b1}[\text{H}^+]}{K_a}\right)} \quad (1)$$

A simple equilibrium 1:1 was found in simulated intestinal fluid, and K_{ass} were obtained from the Higuchi and Connors model.

The dissolution behavior of mebendazole singly and with HP-β-CD in simulated intestinal fluid without enzymes was directly determined. Mebendazole 1 mg was placed into 2.5 ml of the different essayed solutions at 25°C and at 500 rpm. The spectroscopic measurements were accomplished each 90 sec for 225 minutes.

The authors found that experimental data fit the equation

$$[\text{Meb}] = \frac{k_{dis}}{k_{pp}}(1 - e^{-k_{pp}t}) + \frac{k'_{dis}}{k'_{pp}}(1 - e^{-k'_{pp}t}) \quad (2)$$

where, k_{dis} and k'_{dis} are the dissolution rate constants, k_{pp} and k'_{pp} are the precipitation rate constants, and t is time.

NMR and Molecular Mechanics Studies

^{1}H-NMR studies have been carried out in DMSO-d_6 solutions, because of the lower mebendazole solubility in aqueous solutions. NOESY spectra were collected in a 300-MHz Varian Unity Plus System at 25°C.

Structures of host and guest molecules either isolated or in the form of inclusion complexes were optimized with Hyperchem (Hypercube Inc.) by using molecular mechanics with the MM2 forcefield and the conjugate gradient optimizer. The convergence criterion was 0.1 kcal/mol Å in energy gradient.

RESULTS AND DISCUSSION

Solubility and Dissolution Rate Studies

The phase solubility diagrams for the complex formation among mebendazole and various cyclodextrins in simulated intestinal fluid at 25°C are presented in Fig. 1(a). In all cases, the solubility of mebendazole increased linearly as a function of [CD] with the slopes less than unity; thus, the solubility diagram can be classified as A_L type according to Higuchi and Connor's terminology (6). This indicates the formation of the 1:1 inclusion complexes in each case.

The studied cyclodextrins are adequate candidates for our purposes in accord with the Szejtli parameters (5), to exception of α-CD. Binding constants are 150 ± 30 (r^2 = 0.968), α-CD; 1500 ± 100 (r^2 = 0.977), β-CD; 780 ± 10 (r^2 = 0.998), γ-CD; and 1800 ± 300 (r^2 = 0.995), HP-β-CD. These results show that HP-β-CD presents the best behavior in this respect.

K_{ass} depends on the degree of interaction between mebendazole and the cyclodextrin cavity, and it is related to their size relationship. The β-CD and its derivatives provide an ideal space for the adequate inner fitting of the mebendazole. HP-β-CD is a special case because the hydroxypropyl groups have free movement and change the size cavity and depth of this cyclodextrin.

At the sight of the results, the studies of solubility and binding constants of the mebendazole with HP-β-CD in simulated gastric fluid have been made. In this medium, the 98% is mebendazole H^+ and the 1:1 binding constant (K_{b1}) is 350 ± 30 M^{-1}. The mebendazole pK_a value decreases from 3.5 to 2.8 due to the cyclodextrin. These data indicate that the complexes with mebendazole are more stable than these with mebendazole H^+. Thus, we suppose that the equilibria in the stomach probably do

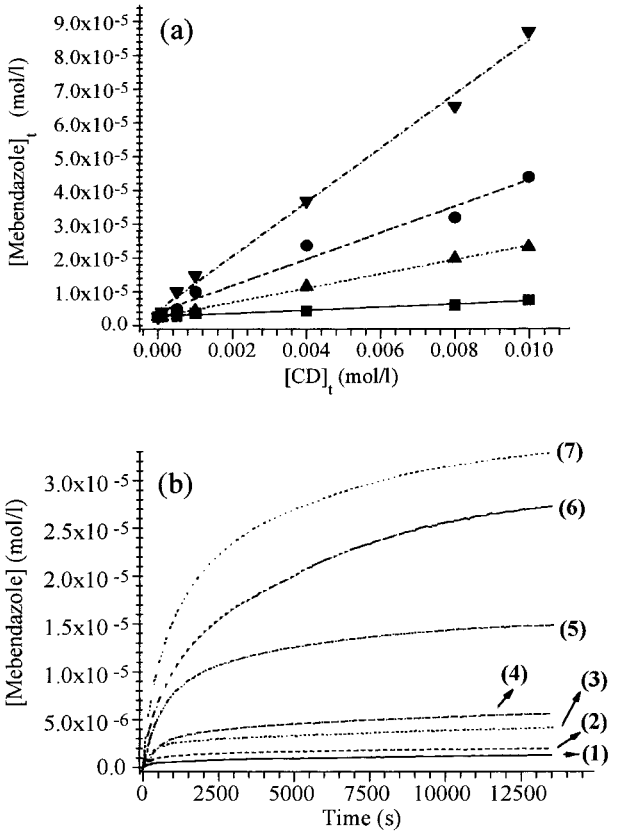


Figure 1. (a) Phase solubility diagram of mebendazole and several cyclodextrins in simulated intestinal juice at 25°C: ■, α-CD; ●, β-CD; ▲, γ-CD; ▼, HP-β-CD. (b) Dissolution profiles of mebendazole in simulated intestinal juice with different Hp-β-CD concentration at 25°C: (1), 0 M; (2), 10^{-4} M; (3), 5×10^{-4} M; (4), 10^{-3} M; (5), 4×10^{-3} M; (6), 8×10^{-3} M; (7), 10^{-2} M.

not significantly influence the posterior equilibrium in the intestinal tract.

The dissolution experiment [Fig. 1(b)] shows the dissolution profiles of mebendazole in simulated intestinal fluid singly and with different [HP-β-CD], because this cycloamilose presents the highest binding constant. A linear tendency is observed for the rate constants in function of cyclodextrin concentration. The linear fit of these results are $k_{dis} = 2.88 \times 10^{-9} + 2.75 \times 10^{-6}$ [HP-β-CD] and $k'_{dis} = 3.27 \times 10^{-11} + 4.03 \times 10^{-7}$ [HP-β-CD].

^{1}H-NMR Analyses and Molecular Mechanics

To know the geometry of the inclusion complexes, NOESY studies were carried out. Figure 2 shows the NOESY spectrum of mebendazole with γ-CD in DMSO-d_6 at 25°C. The cross-peaks indicate that the —OH2 and —OH3 give NOE with aminic protons of the mebendazole and —OH6 and —H6 interact with the methyl group of the guest. Similar behavior has been observed with β-CD. We conclude that one guest molecule is introduced within γ-CD and β-CD through their wide part and the imidazolic group is fitted in the inner, practically in its totality.

NOESY spectrum of mebendazole with α-CD shows interaction between phenyl group of the guest and —OH2 and —OH3 of this cycloamilose. We establish that this phenyl group is introduced within α-CD through its wide part.

Taking into account the experimental results, the authors made molecular mechanics to support and visualize the possible structures of the complexes (7).

All possible orientations of the guest molecule with respect to the host were considered. We optimized the geometry for several configurations of the mebendazole with each cyclodextrin. In the case of the α-CD, the minimal energy was found when the benzyl group of the mebendazole is partially included within the cavity by the wide part. We suppose that the small observed binding constant is due to the weak interaction because of the little contact surface between the aromatic ring of the guest and the cyclodextrin.

The inclusion compounds with the other three cycloamiloses present a similar geometry among them. In the three cases, the carbamate group of the guest enter into the cavity by the wide part. However, the inclusion depth is different. In the case of HP-β-CD, the contact surface is higher than in the other cases. We consider that the larger binding constant is due to this. Differences in the β- and γ-CDs binding constants (K_b γ-CD $<$ K_b β-CD) may be attributed to the weaker interaction in the γ-CD inclusion compound because of the larger distance between the guest and the host within the cavity. In Fig. 2, the inclusion complex structure of mebendazole is observed with γ-CD, obtained from ^{1}H-NMR and molecular mechanics.

The molecular mechanics results are in accord with the possible geometry obtained from NMR and with the binding constant magnitude.

CONCLUSIONS

The cyclodextrins increase the mebendazole solubility by the formation of inclusion complexes in simulated intestinal fluid. HP-β-CD shows the best behavior. Unionized mebendazole is less included than mebendazole H^+.

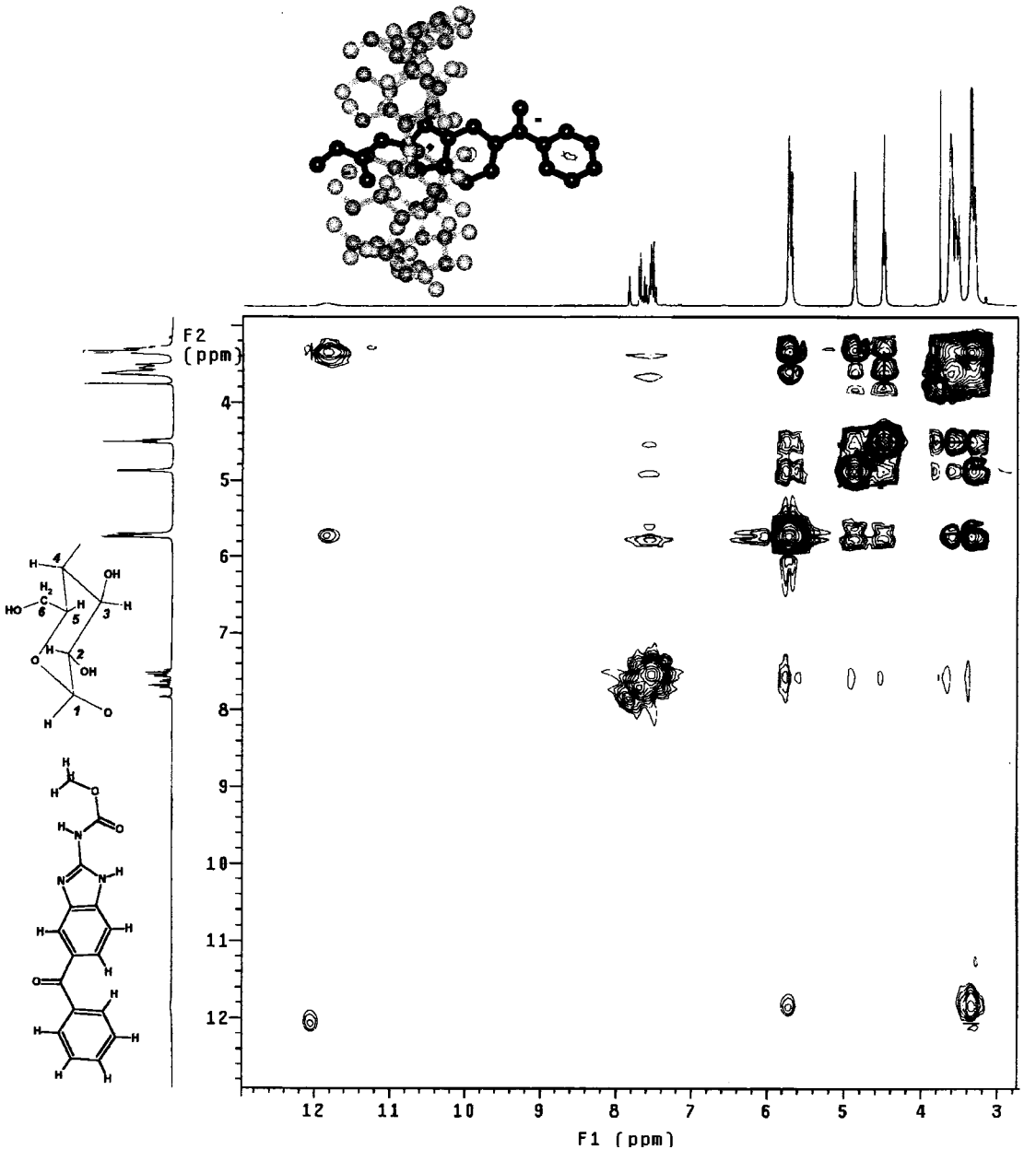


Figure 2. NOESY spectrum for mebendazole with γ-cyclodextrin in DMSO-d_6. ''A'' corresponding to H6, H3, and H5 of the cycloamilose. The structure at the top shows the inclusion compound geometry of mebendazole with γ-CD obtained from ^{1}H-NMR and molecular mechanics.

Dissolution studies indicate that the dissolution rate increased with the [HP-β-CD]. From NMR and molecular mechanics studies, the most probable geometry may be proposed. They are in agreement with the binding constant values.

ACKNOWLEDGMENTS

We thank Cerestar, Arancia, and Janssen laboratory for the donation of the cyclodextrins and mebendazole. We also thank DGAPA for financial support. We are very grateful to Joseph Doshner for his helpful suggestion and to Nuria Esturau for the technical assistance.

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