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CHARACTERIZATION, DISSOLUTION AND DIFFUSION PROPERTIES OF TOLBUTAMIDE-β-CYCLODEXTRIN COMPLEX SYSTEM

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ABSTRACT

The solubility of tolbutamide was increased about 2.5 fold due to complex formation with β-cyclodextrin (β-CD). Phase solubility studies showed that at high β-CD concentrations an insoluble microcrystalline complex was formed which had a stoichiometry of 1:2 (tolbutamide:β-CD); this

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was confirmed by chemical analysis. The endothermic peak for tolbutamide completely disappeared in differential scanning calorimetry studies and x-ray diffraction spectra indicated that the prepared complex was less crystalline than the parent drug. More evidence of the complex formation was obtained from infra-red studies. The dissolution of tolbutamide in pH 1.2 buffer was significantly enhanced by complexation with β-CD. after 20 minutes the % of drug released was 12 and 93 for the drug alone and the complex samples respectively. Increased solubility, decreased crystallinity, and improved wettability are responsible for the observed enhancement in dissolution rate. Diffusion studies across dimethyl polysiloxane revealed that the drug apparent permeability constant was concentration dependent and decreased in the presence of β-CD.

INTRODUCTION

Tolbutamide (1-butyl-3-(p-tolylsulfonyl) urea) belongs to the sulfonylurea class of oral hypoglycemic agents and has been popular in the management of certain cases of diabetes mellitus. The drug is poorly soluble and its dissolution rate is considered to be the rate-determining step

in its absorption from the gastrointestinal tract (1). The major route of degradation of tolbutamide is hydrolysis mainly to p-toluene sulfonamide (2). An investigation made on in-vitro dissolution rates of 18 commercially available brands of tolbutamide showed that marked variations in dissolution rates existed (3). Varley (4), has shown that minor changes in solid dosage form composition produces significant changes in serum tolbutamide and serum glucose levels. The modification of tolbutamide dissolution characteristics by its co-precipitation with PVP to obtain better bioavailability with less variation among subjects has been reported (5). The formation of solid dispersions with water soluble carriers such as urea and PEG-6000 is also effective in improving the dissolution characteristics of poorly soluble drugs like tolbutamide (6). Cyclodextrins and its congeners have been used to improve properties such as solubility, stability, palatability, and bioavailability of drugs. The dissolution characteristics of the sulfonylurea compound, acetohexamide were improved via inclusion complexation (7). The stability constants of several β-cyclodextrin-sulfonylurea complexes determined by a high performance liquid chromatography method were reported (8). The primary objective of the present study was to characterize the interaction of tolbutamide with

β-CD. The physicochemical properties of tolbutamide-β-CD system and the effect of β-CD on the dissolution properties of tolbutamide were investigated. Also, the diffusion properties of tolbutamide-β-CD preparations and the effect of β-CD on permeation on tolbutamide through a dimethyl polysiloxane membrane in an in-vitro diffusion model were studied.

MATERIALS AND METHODS

Materials

Tolbutamide and β-CD were purchased from Sigma Chemical Company. β-CD was recrystallized from water, dried and stored in a desiccator. All other reagents used were of analytical grade and deionized double distilled water was used in all studies.

Methods

Phase Solubility Studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors (9). Tolbutamide in amounts that exceeded its solubility (40 mg) were accurately weighed in each of 25 ml Erlenmeyer flasks to which were added 20 ml of water containing various concentrations of β-CD (0.001-0.024M). The securely capped flasks were shaken at 30 $\pm$ 0.5^{o}C for forty eight hours followed

by equilibration for six days. This amount of time was determined to be sufficient for equilibration. Following equilibration, the contents of the flask were filtered using 0.45 m HA-type millipore filter. The filtered solutions were appropriately diluted with methanol and analyzed using a Model 552 Perkin Elmer UV-vis spectrophotometer at 229 nm. It was confirmed that Beers law was obeyed and that no interference was observed from β-CD.

Preparation of Solid Complex

The solid complex was obtained by mixing appropriate amounts of β-CD and tolbutamide in distilled water. The amounts were calculated from the descending portion of the phase solubility diagram at the point (shown by an arrow in Fig. 1) where no solid drug existed and the solubility of β-CD was not exceeded. The mixture was shaken at 30 $\pm$ 0.5^{o}C for forty eight hours followed by equilibration for six days. The complex which precipitated as a microcrystalline powder was filtered and dried under vacuum at 40^{o}C overnight. The stoichiometry of the complex was determined by chemical analysis for all three batches prepared. Furthermore, a solubility study was performed on the prepared complex as described earlier under phase solubility studies.

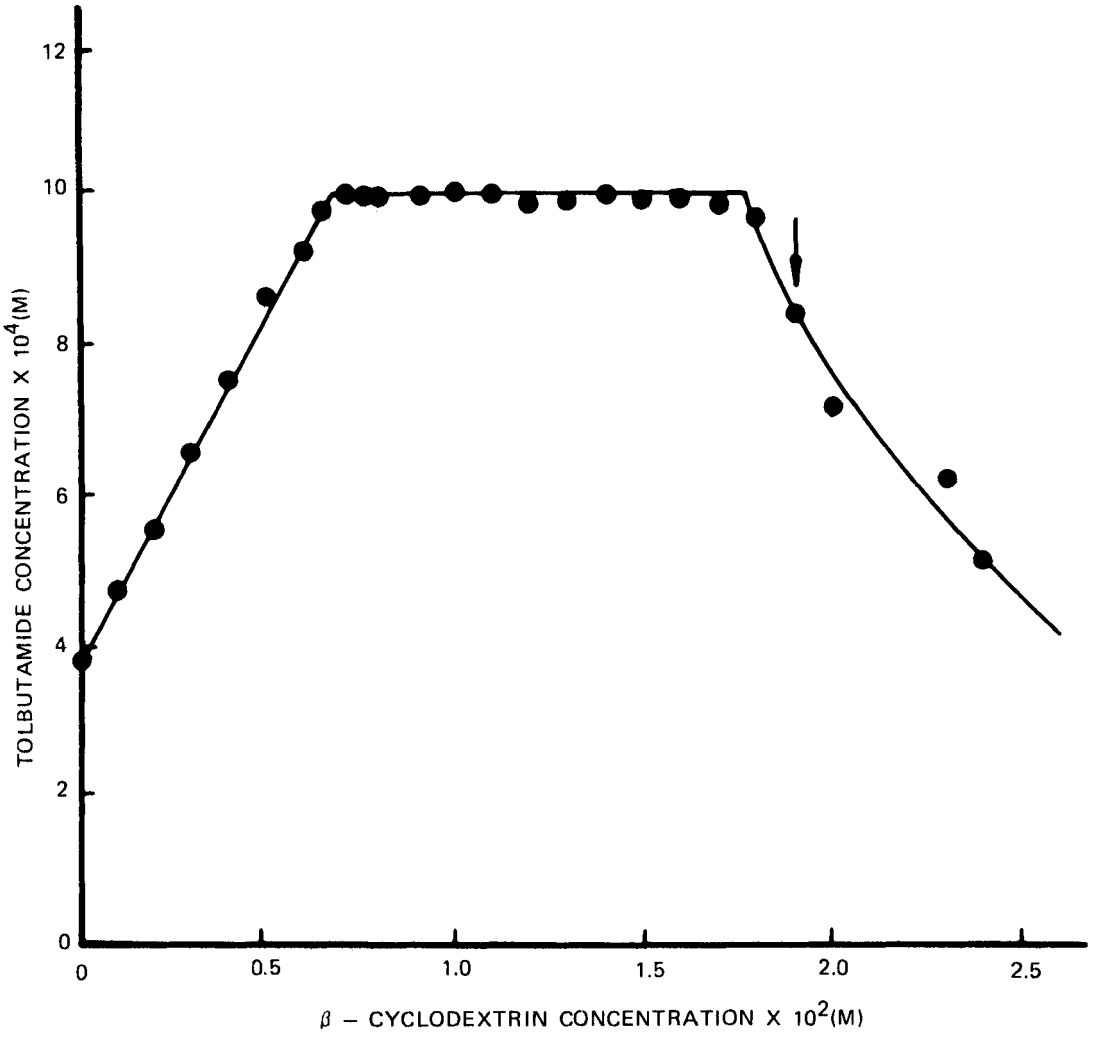

FIGURE 1.

Phase solubility diagram of tolbutamide-β-CD system in distilled water at 30 ± 0.5°C. The arrow indicates experimental conditions for the preparation of the solid complex.

Differential Scanning Calorimetry (DSC)

DSC scans were recorded on a Model 1B Perkin Elmer apparatus equipped with low temperature cell. Nitrogen was purged at a flow rate of 10 to 30 ml/minute. The instrument was set at a scan speed of 10°C/minute and a range of 8 millicalories/degree; 10 mg of tolbutamide or its equivalent were used for each scan.

X-Ray Diffraction Spectroscopy

X-Ray diffraction patterns were obtained using Norelco X-Ray diffractometer, Model 12045, Phillips Electronic Instrument with Ni filtered Cu-K radiation detector at a scanning rate of 1° 2θ/minute. The patterns for each samples were obtained at a 2θ range of 8°-40°.

Infra Red Spectroscopy (IR)

IR-spectra were obtained using a Model 257 Perkin Elmer IR spectrophotometer. The samples were prepared using the Nujol mull technique. The pellet compression technique was not used since β-CD is known to form complexes in solid state. (10). Each sample contained 10 mg of tolbutamide or its equivalent.

Dissolution Rate Studies

Dissolution studies were performed according to method II described in USP 20th edition. The USP

dissolution apparatus was a Hanson Model QC72RB (Hanson Research). Dissolution medium was 1/15 M pH 7.4 phosphate buffer or pH 1.2 hydrochloric acid/potassium chloride buffer. The stirring speed was 60 rpm and the temperature was maintained at 37 $\pm$ 0.5°C. Dissolution samples (passed through 22 and retained on 60 mesh USP standard) contained an equivalent of 100 mg of tolbutamide. A 1-2 ml sample was withdrawn at various time intervals using a filter pipette, diluted with the dissolution media and analyzed spectrophotometrically.

Diffusion Studies

In-vitro diffusion studies were performed using the system described by Mosher and Mikkelson (11). Glass diffusion cells were constructed from 25 ml Erlenmeyer flasks. The end of 1 cm side arm projection on each half cell had a ground glass opening finish with a circular opening in the middle. The surface area of the membrane effective for diffusion was 0.28 cm^2. The receptor half cell was positioned symmetrically with respect to the donor cell facing the membrane. Medical grade nonreinforced dimethyl polysiloxane sheeting (Dow Corning) was selected as a model membrane because it has permeability properties similar to that of biological membranes. The membrane was soaked overnight in distilled water

before each run. In order to investigate the effect of solution form on the diffusion of tolbutamide the following solutions were placed in the donor cell: a) saturated solution of tolbutamide in water; b) saturated solution of the prepared complex in water; c) saturated solution of tolbutamide in water containing 2% w/v β-CD; and d) suspension of tolbutamide in 0.1% methyl cellulose dispersion. Also the effect of β-CD on the diffusion of tolbutamide was investigated with the following solutions in the donor cell: a) saturated solution of tolbutamide in 1/15 M pH 5.4 phosphate buffer; and b) saturated solution of the drug in the same buffer but containing 0.5% w/v β-CD. The pH of the receptor compartment was adjusted to 7.4 using 1/15 M phosphate buffer in order to maintain sink conditions with respect to the permeable species. Both cells were capped with teflon lined stoppers in order to prevent loss of solvent by vaporization and the entire cell was placed in a water bath maintained at 37 $\pm$ 0.5°C. The solutions in both half cells were stirred vigorously with magnetic stir bars. Aliquot samples were withdrawn from the receptor compartment at various time intervals. A constant volume on the receptor side was maintained by replacing with fresh buffer solution

after each sampling time. The collected samples were analyzed spectrophotometrically.

RESULTS AND DISCUSSION

The phase solubility diagram obtained from tolbutamide and β-CD in distilled water at 30°C is as shown in Fig. 1. It can be classified as B_s-type phase solubility diagram (8). The increase in solubility observed in the initial ascending portion can be attributed to the interaction with β-CD and the formation of a complex which has higher solubility than the drug alone. When the solubility limit of the formed complex is exceeded, the ascending linear portion starts levelling off and further addition of β-CD result in the precipitation of a microcrystalline complex. The complex continues to form in the plateau region and precipitates from saturated solution as the concentration of β-CD is increased. The stoichiometry of the precipitating complex was calculated from the plateau region. It appeared that about 6.41×10^{-3} moles of tolbutamide reacted with 1.14×10^{-2} moles of β-CD, indicating a 1:2 (tolbutamide:β-CD) stoichiometric ratio. Stoichiometry obtained from chemical analysis was in fair agreement with that obtained from phase diagram. Assuming the stoichiometry of the precipitating complex to be 1:2 through-

out the descending portion of the curve the apparent formation constant $K_{1:2}$ can be calculated from the following equation (9):

$$K_{1:2} = \frac{S_B}{(S_X - S_B)(L_X - 2S_B)^2} \qquad (1)$$

where S_B is the molar solubility of the complex, S_X and L_X are the total molar concentrations of the substrate and the ligand respectively at any given point on the descending portion of the phase solubility diagram. S_B was estimated to be 2.3×10^{-4} M from the solubility study of the complex (fig. 2). Accordingly $K_{1:2}$ was calculated to be 1.15×10^{3} M^{-2}.

More evidence of the complex formation was obtained from the DSC thermograms (fig.3). The endotherm at 126°C equivalent to the melting point of tolbutamide completely disappeared in the inclusion complex samples.

X-Ray diffraction patterns of the powder samples revealed less crystallinity of complex as evidenced by fewer and broader peaks. The diffraction pattern of the physical mixture and the complex are shown in fig. 4. A peak shift from $2\theta = 18.3^{\circ}$ in β-CD to 18.0° in complex samples indicates higher d spacing in complex apparently due to enlarged cell dimension. This enlared cell dimension in β-CD cavity may be attributed to incorporation of tolbutamide in the β-CD cavity

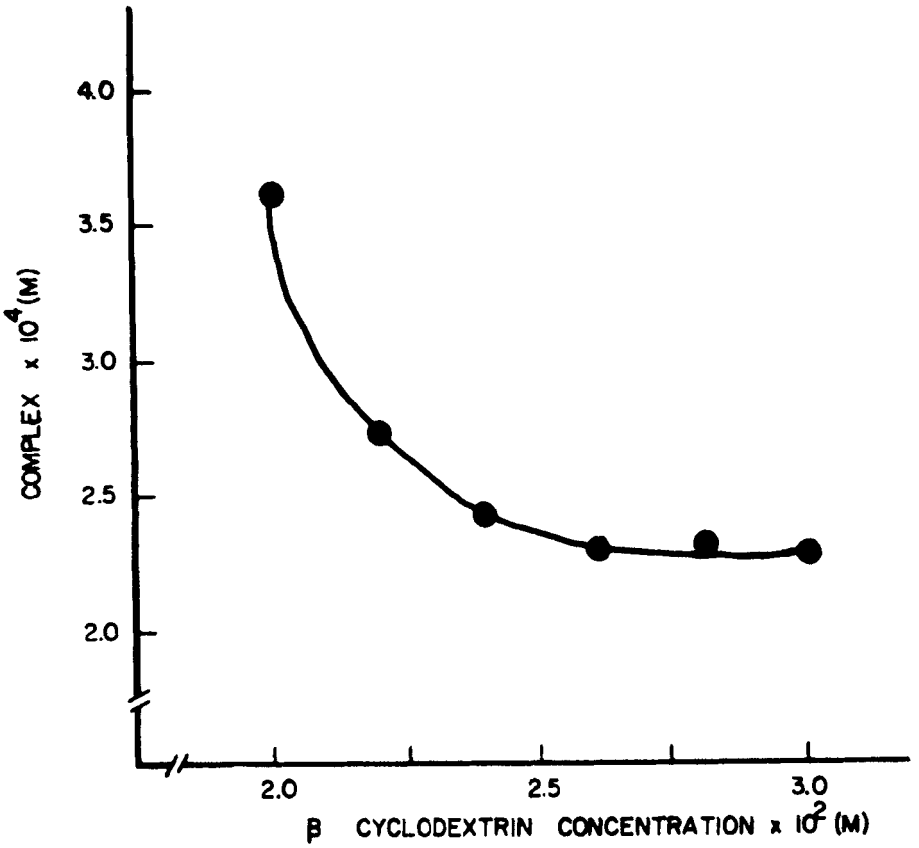

FIGURE 2.

Solubility of the inclusion complex of tolbutamide with β-CD as a function of β-CD concentration at 30°C.

indicating formation of an inclusion complex. Using the freeze drying preparation procedure, Kurozumi et al. (12) observed two broad peaks in diffraction patterns of inclusion compounds at an interplanar distance around 7.2-7.7° A and 4.8-5.4°A. The corres-

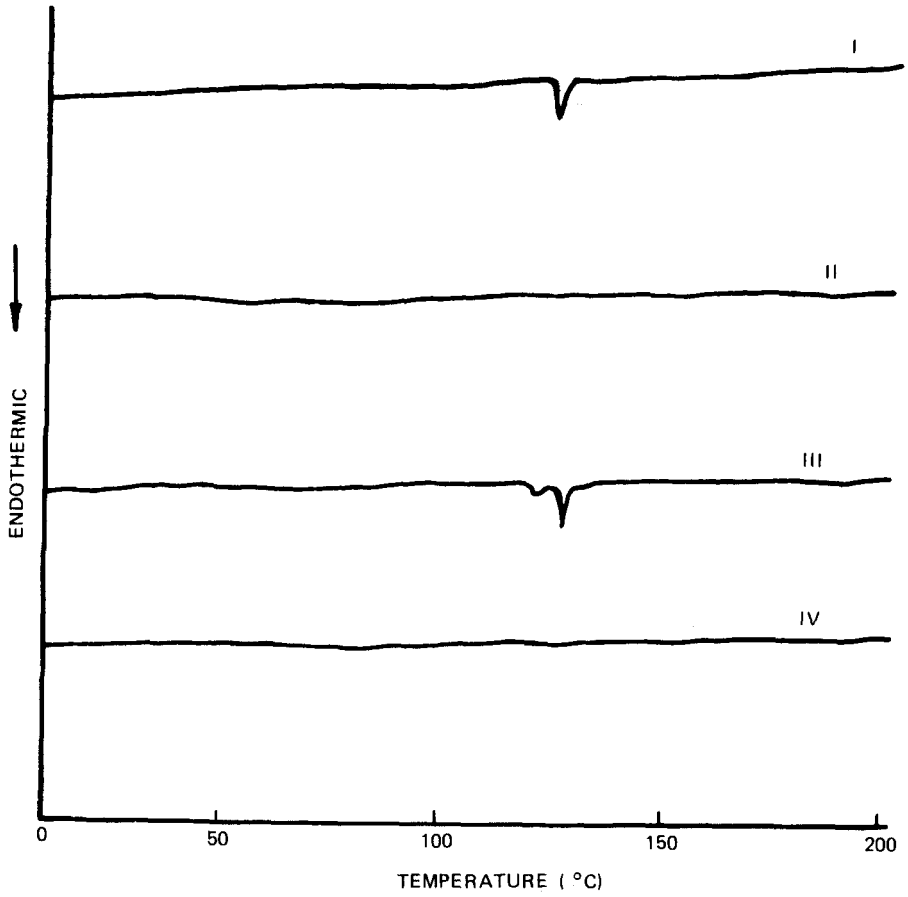

FIGURE 3.

Differential scanning calorimetry of: tolbutamide (I); β-CD (II); the physical mixture of tolbutamide and β-CD (1:2) (III); and the complex system of tolbutamide with β-CD (IV).

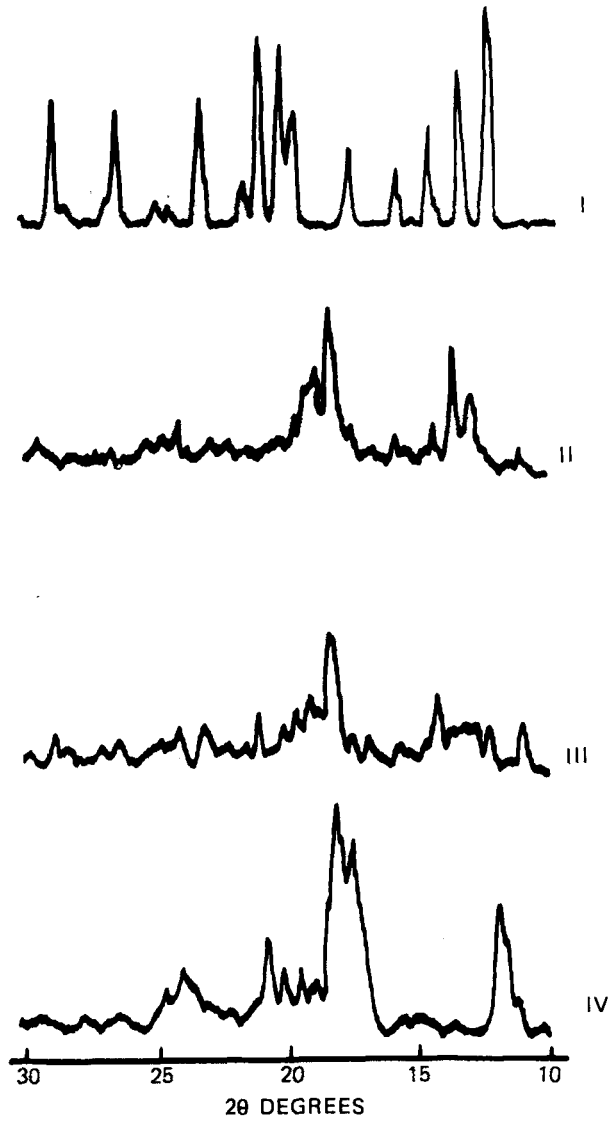

FIGURE 4.

Powder X-ray diffraction patterns of: tolbutamide (I); β-CD (II); the physical mixture of tolbutamide and β-CD (1:2) (III); and the complex of tolbutamide with β-CD (IV).

ponding values obtained in this work were 7.48^{O} A and 4.83-5.19^{O}A, are in good agreement.

The IR spectra shown in fig.5 show distinctive changes in the region of carbonyl absorption. The characteristic carbonyl stretching observed in both the drug and the physical mixture at 1700 cm^{-1} shifted to a higher wave number i.e. 1725 cm^{-1} with an increase in intensity and broadening of the band in the inclusion complex sample. A possible explanation of the shift to higher wave number may be the dissociation of the tolbutamide intermolecular hydrogen bonding existing between the C-O group of one molecule and N-H group of another molecule and the extablishing of weak forces in the complex system. Similar observation was reported in ibuprofen-β-CD complex system by Chow and Karara (13). The broadening band is probably due to the restriction of bending and stretching vibration within β-CD cavity (14).

Figures 6 and 7 show the dissolution profiles of tolbutamide, physical mixture, and the prepared complex at pH 1.2 and 7.4 respectively. There was significant enhancement in the dissolution rate of the complex compared to the free drug. After 20 minutes the percent of drug released was 12 and 93 for the powdered drug and the complex samples respec-

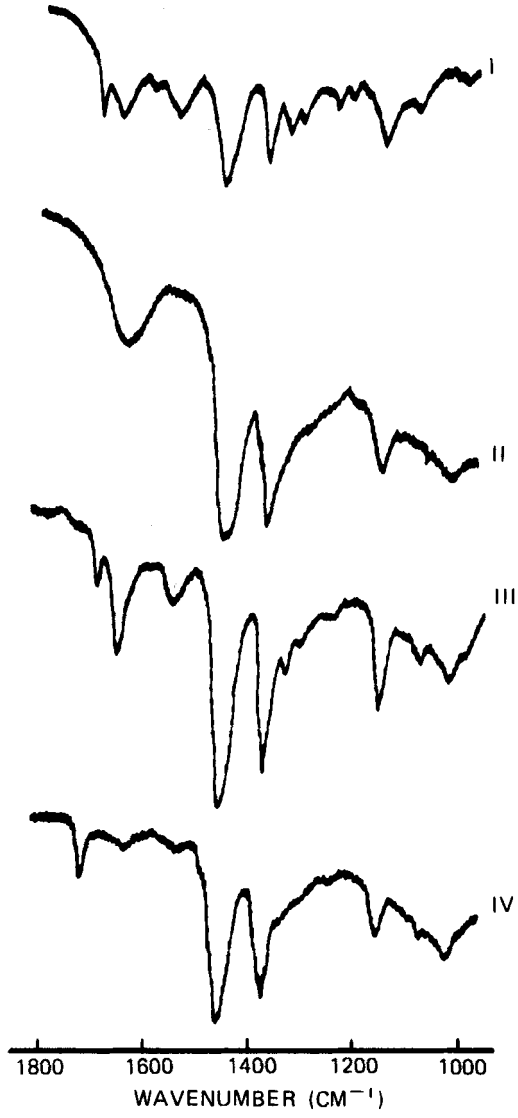

FIGURE 5.

IR spectra of: tolbutamide (I); β-CD (II); physical mixture of tolbutamide and β-CD (1:2) III; and the complex system of tolbutamide and β-CD (IV).

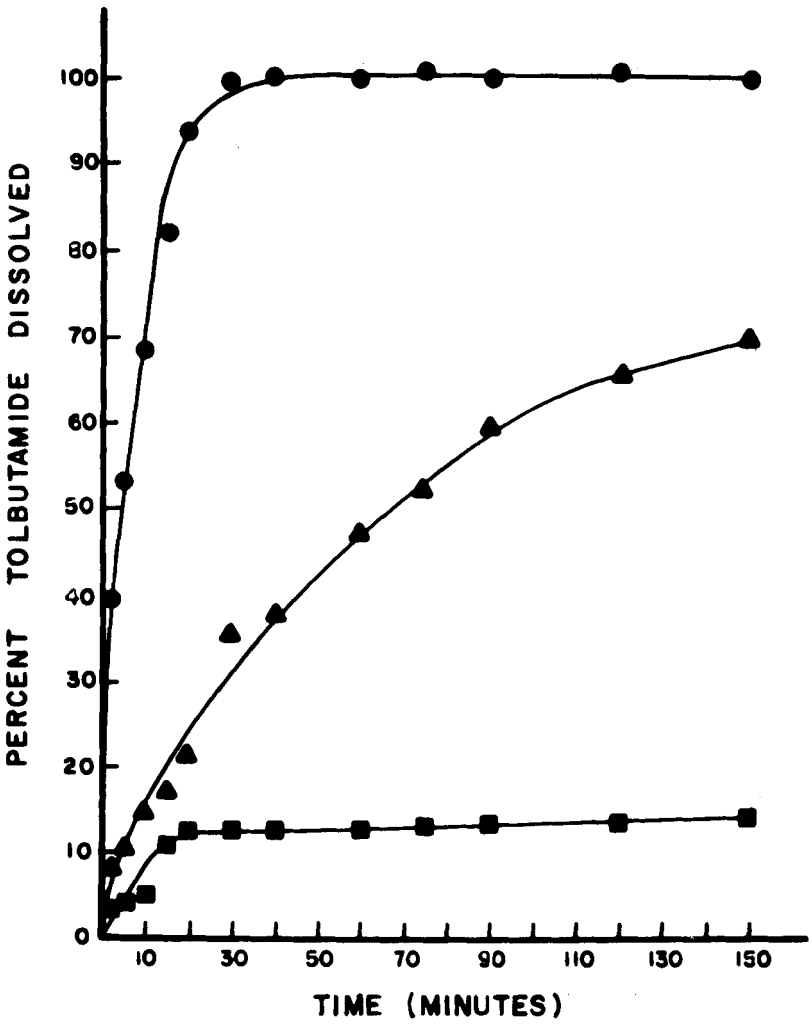

FIGURE 6.

Dissolution rate profiles of samples of ■ tolbutamide, ▲ physical mixture of tolbutamide and β-CD (1:2) and ● tolbutamide-β-CD complex in pH 1.2 buffer at 37°C.

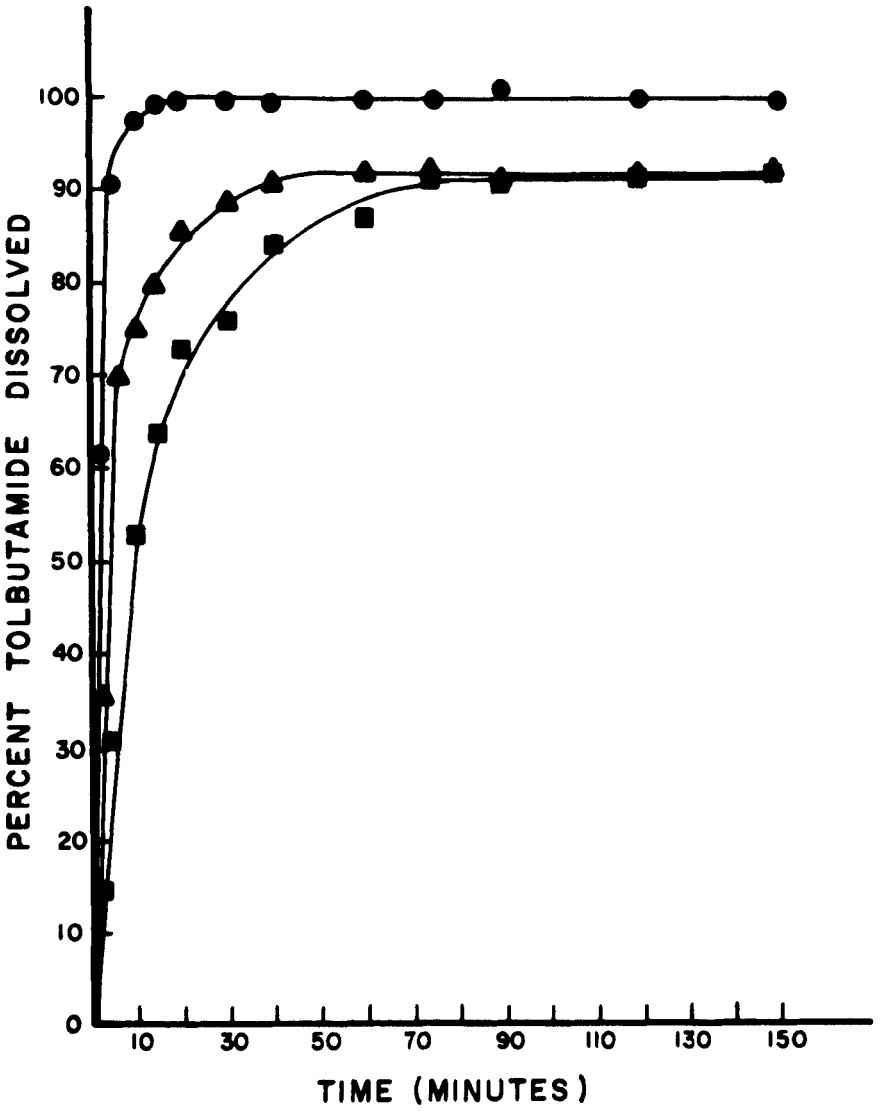

FIGURE 7.

Dissolution rate profiles of samples of ■ tolbutamide, ▲ physical mixture of tolbutamide and β-CD (1:2) and ● tolbutamide-β-CD complex in pH 7.4 buffer at 37°C.

tively (Fig. 6.). The amount of drug released at various time intervals in case of all dissolution samples at pH 7.4 was significant at 0.05 level of significance. It appears that the enhanced dissolution rate may be due to the increase in solubility, decrease in crystallinity, and enhanced wettability of the drug by inclusion complexation. Because of the large molecular weight of β-CD (approximately 1135) it is clear that the administration of β-CD complexed tolbutamide is not possible. However, incorporation of β-CD in solid tolbutamide formulations may result in a more uniform release of the drug. It should be kept in mind that fast dissolution of tolbutamide solid formulations is undesirable since that would result in a rapid fall in blood sugar levels. Also inclusion complexation may have some implications regarding the stability of the drug since, tolbutamide is known to decompose under various conditions (2,15).

The permeation behavior of tolbutamide from various solutions is shown in fig. 8. It is evident that the diffusion of tolbutamide from the suspension form was fastest in comparison to other solutions. When the suspension was placed on the donor side, the dissolved drug concentration in the donor solution remains essentially constant. Any loss of drug by

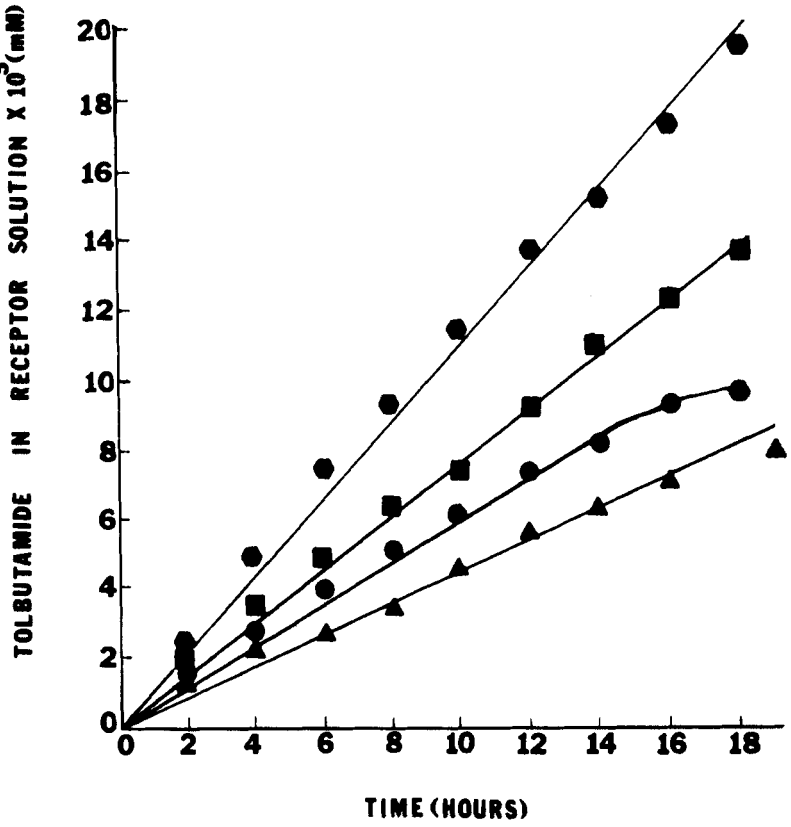

FIGURE 8.

Permeation profiles of tolbutamide under various conditions: ⬣ tolbutamide suspension in 0.1% methyl cellulose; ■ saturated solution of tolbutamide in 2% β-CD; ● saturated solution of tolbutamide in water; and ▲ saturated solution of the prepared complex in water.

permeation is replenished by the dissolution of the solid drug. Accordingly the release rate may be given by equation 2.

$$\frac{dC}{dt} = \frac{DAPC_o}{h} \qquad (2)$$

where D is the diffusion coefficient of the drug in the membrane, A is the surface area of the membrane, P is the partition coefficient of the drug between the membrane and the donor solution, and C_o is the solubility of the drug in the donor solution. Because of the constant release rate of the drug (zero order), the suspension showed a linear increase in drug concentration with time as shown in Fig. 8. The release rate of tolbutamide in 2% w/v β-CD was increased over a plain saturated solution mostly because more drug exist in the former solution. β-CD is not expected to permeate through the membrane because it is not soluble in it as shown previously by Nakano and his coworkers (16). Thus, it was reasonable to assume that β-CD does not partition into dimethyl polysiloxane membrane. Only the free (uncomplexed drug) species is capable of permeating across biological membranes as shown by Tokumura et al. (17). Even though the drug concentration at zero time on the donor side in case of saturated solution of complex was greater than plain

saturated solution of tolbutamide, the amount permeated was less in the former case. This is because the complex solution has less free drug which is the permeable species compared to the saturated solution of drug. The tolbutamide permeated in 2% w/v β-CD solution was greater than the saturated solution of the complex, even though the initial concentration in the donor compartment was almost the same, in both cases. In all permeation experiments the fraction of drug permeating to the receiver solution was negligible (<5%) so that the concentration gradient remained constant. The increased release rate of the suspension compared to that of the saturated solution of the drug was not expected on the basis of constant concentration gradient. This observation may be due to a change in the permeation properties of the membrane and/or aqueous diffusion layer resistance. Permeation behavior of tolbutamide from plain saturated solution in pH buffer 5.4 and a solution containing 0.5% β-CD is shown in fig. 9. The permeation rate was greater from the plain saturated solution compared to that containing 0.5% w/v β-CD. The concentration of the diffusing species was negligible since the receiver had a pH 7.4 buffer which would maintain the diffused molecule in the dissociated form. Because the concentration gradient was maintained throughout the permeation

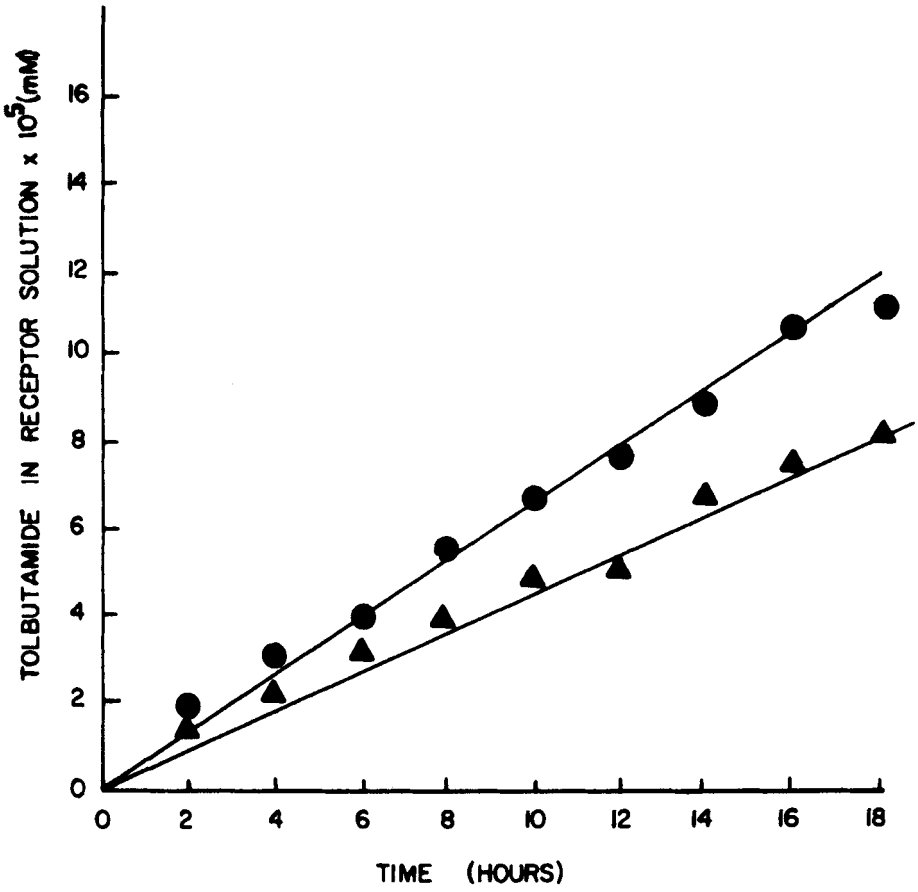

FIGURE 9.

Effect of β-CD on tolbutamide permeation. ●saturated solution of tolbutamide in pH 5.4 buffer and ▲saturated solution of tolbutamide in pH 5.4 buffer in the presence of 0.5% β-CD.

experiment, the following equation derived by Nakano and Patel (16) for a steady state diffusion may be used:

$$C_2 = \frac{K_P\ AC_o\ t}{V_2} \qquad (3)$$

where C_2 is the concentration of the diffusing species in the desorbing solution, V_2 is the volume of the desorbing solution, and K_P is the permeability constant which is the product of the diffusivity of the drug in the membrane and its membrane/water partition coefficient divided by the thickness of the membrane. The permeability constant were found to be 3.34 cm/hr for the plain saturated solution and 2.46 cm/hr for the solution containing 0.5% w/v β-CD. Statistical analysis using the students' "t" test on data shown in fig. 9 revealed no significant difference in the amount permeated at the end of two hours. However, the difference in the amount permeated at each time interval after two hours was statistically significant at 0.05 level of significance. It appears that the apparent permeability constant is concentration dependent and decreases in the presence of β-CD.

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