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The Interaction of Zipeprol with β-Cyclodextrin

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Abstract □ The characteristics of zipeprol-β-cyclodextrin system were studied by circular dichroism, competitive UV method and dialysis method. In this experiment, binding constants by competitive UV method, circular dichroism and dialysis method were 155 M^{-1}, 187 M^{-1}(± 5%)and 315 M^{-1}, respectively. It shows that zipeprol forms 1:1 complex with β-cyclodextrin by circular dichroism and 1:2 by dialysis method. pH profile shows that binding force seems to be a hydrophobic interaction. It is suggested that benzene ring be accomodated in the cavity of β-cyclodextrin.

Key words □ β-cyclodextrin, zipeprol, circular dichroism, dialysis method, competitive UV method, inclusion complex.

Zipeprol,[4-(2-methoxy-2-phenylethyl)-α-(methoxy phenylmethyl)-1-1-piperazineethanol], antitussive, is considerably bitter. Therefore, it is necessary to mitigate the bitterness of this compound in aqueous dosage form. In previous report, it was attempted to mitigate the bitterness by means of complexation with β-cyclodextrin[1]. Bitterness test was carried out using caffeine as reference standard.[2]

Cyclodextrin is cyclic oligomers containing six or more D-glucose units linked 1-4 and is doughnut-shaped and includes a variety of drugs with holes of doughnut. The internal diameter of these cyclodextrins is 5-6 Å for the α(glucose unit: 6), 7-8 Å for the β(glucose unit: 7) and 9-10 Å for the γ entities (glucose unit: 8).

Inclusion complexes of β-cyclodextrin (abbreviated to CDx) with various drugs have been extensively applied in pharmaceutical field, e.g. the enhancement of solubility[3,7,9], stability[3], dissolution rate[8], bioavailability[10] of insoluble drugs, the masking effect of smell, taste, volatilability of drugs and the retardation of the cleavage of drugs.

It was reported that CDx forms complexes with a variety of drugs, e.g. barbiturates[13,14], amines[5], alcohols[6], hydrocortisone[7], prostaglandins[8,9], anti-inflammatory fanamates[15], sulfonyl urea[16], 2-substituted naphthalenes[17], non-aromatic ring[18], phenol derivatives[19], azo dyes[20], carboxylic acid[19,22], benzene derivatives[21].

The binding state of the host and guest molecules has been studied by circular dichroism (CD)[14,15,18], IR, UV, X-ray techniques, NMR, potentiometric titration, competitive UV method[6,16,23] and dialysis method. It has been reported that the forces holding together these complexes seem to be Van der waals, hydrogen bonding as well as hydrophobic bonding.[9,15,18,20,22]

It was reported that aliphatic groups[8,9], amine group[15] and benzene group[21] were accomodated in the cavity of CDx. Zipeprol seems to be a good guest molecule because it contains hydrophobic groups such as benzene group, aliphatic group, amine group. Interaction of CDx with zipeprol in aqueous solution was examined by circular dichroism(CD), dialysis method and competitive UV method in this experiment. Stoichiometry and binding constant were determined and effect of pH on this interaction was investigated to gain insight into mechanism and geometry of the inclusion process.

EXPERIMENTAL METHODS

Materials and apparatus

β-cyclodextrin(CDx), zipeprol(ZP) and methyl orange(MO) were obtained from Pacific pharmaceutical Co., LTD., Yang Ji pharmaceutical Co., LTD., and Shio Yo pure chemicals Co., LTD., respectively. All materials were used without further purification. All solutions were prepared in double distilled water. UV scanning, UV absorbances, CD spectra and CD data treatment were recorded by a Pye Unicam SP1750, a LKB, a JASCO Model J-20C spectropolarimeter and MULTI computer, respectively.

Methods

CD method: The solutions containing CDx (0.5×10^{-2} M) and ZP ($0.5\text{-}2.0 \times 10^{-2}$ M) were equilibrated at 25 °C during 24-30 hr. Conformational changes of complexes were detected by CD mea-

surement. In order to adjust the pH of the solutions, HCl solutions (pH 1.2-6) and phosphate buffers(pH 6-8) were used in this experiment. The reason to use HCl solution is that citrate buffer and acetate buffer have CD spectra at 220-250 nm.

Competitive UV method: MO(2.0×10^{-5} M) and CDx($1.3\text{-}7.8 \times 10^{-3}$ M) in H_2SO_4-Na_2SO_4 buffer solutions (pH 3) were equilibrated at 25 °C and absorbances were read at 508 nm. Binding constant (K_f) of MO, 358 M^{-1}, is determined by Hildebrand-Benesi plot.[8,12] MO (2.0×10^{-1} M), CDx ($1.3\text{-}7.8 \times 10^{-3}$ M) and ZP ($3.9\text{-}6.5 \times 10^{-3}$ M) in H_2SO_4-Na_2SO_4 buffer (pH 3) were equilibrated at 25 °C and then UV absorbances were read at 508 nm in 1-cm quartz cell.

Dialysis method: First, the following experiment was carried out to verify the impermeability of CDx. CDx solution was added to one chamber and distilled water to the other in dialysis cell. For equilibrium, dialysis cell was shaken at 25 °C during 12-15hr. The solution of later chamber was pippetted and added to concentrated HCl solution. Qualitative analysis was carried using Betrand's method[23]. It must be sure that analytical results are negative. Equilibrium dialysis was performed in cells with two chambers separated by semi-permeable membranes. The solution (7 m*l*) containing ZP ($0.5\text{-}4.0 \times 10^{-2}$ M) and CDx(2.0×10^{-2} M) was added to one chamber and distilled water (7 m*l*) to the other. For equilibrium, dialysis cell was shaken at 25 °C during 12-13 hr. Samples were taken from water compartment and UV absorbances were read at 508 nm.

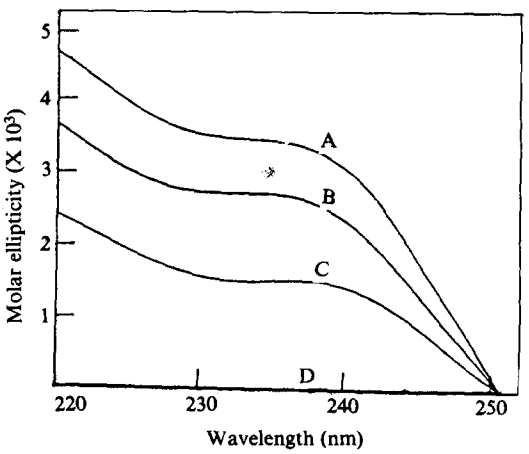

Fig. 1. Circular dichroism of ZP-CDx system at 25° (in H_2O).
Key: A, ZP (1.0×10^{-2}M) + CDx (1.5×10^{-2}M);
B, ZP (1.0×10^{-2}M)+CDx (1.0×10^{-2}M);
C, ZP (1.0×10^{-2}M)+CDx (0.5×10^{-2}M);
D, base line or only ZP or only CDx.

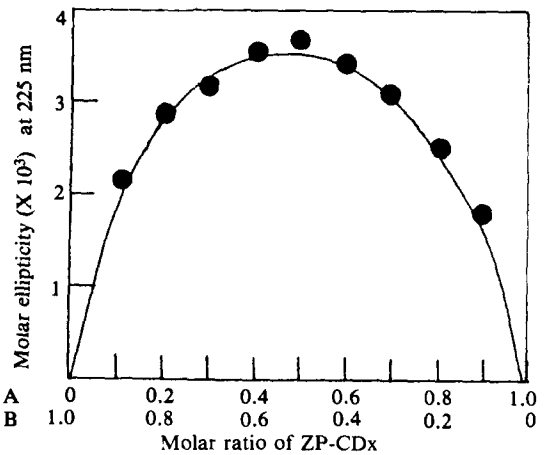

Fig. 2. Continuous variation plots for zipeprol-CDx system at 25°C.
Key: A,CDx (2.0×10^{-2} M);B, ZP(2.0×10^{-2}M).

RESULTS AND DISCUSSION

Fig. 1 shows that the CD spectra of ZP in the presence of CDx has positive peak at 220-250 nm. Since CDx and ZP have no CD spectrum at 220-250 nm, observed ellipticity at 220-250 nm is due to the complex of ZP with CDx. Therefore, ZP has shown to generate extrinsic cotton effects on binding to CDx.

To determine the stoichiometric ratio, the induced optical activity was quantitatively measured. Fig. 2 shows the continuous variation plots of the ellipticity change at 225 nm for ZP-CDx system. When the composition ratio of ZP-CDx was 1:1, molar ellipticity was maximum value. Therefore, ZP forms 1:1 complexes with CDx.

Consequently,[13] A + B = C

If A and B denote the initial concentration of CDx and ZP, respectively, and if C denotes the equilibrium concentration of the complex, then K, binding constant, is given by

$$K = \frac{C}{(A-C)\cdot(B-C)} \qquad \text{Eq. (1)}$$

Upon rearrangement

$$C = \frac{1+K(A+B) \pm [1+2K(A+B)}{2K}$$

$$\frac{\pm K^2(A+B)^2 - 4K^2AB]^{1/2}}{} \qquad \text{Eq. (2)}$$

In dilute solutions, the observed ellipticity is proportional to the concentration of the complex at any fixed wavelength. Then, ZP and CDx have no CD curve.

$$C = E_{obs}/P \qquad \text{Eq. (3)}$$

where E_{obs} is the observed ellipticity and P is the proportionality constant for a given pathlength of cell at the particular wavelength of measurement. And CDx concentration (0.5×10^{-3} M) was constant and intrinsic cotton effects of CDx are not observed above 220 nm.[22]
Consequently,

$$E_{obs} = P \cdot \frac{1+K(0.005+B) - [1+K(0.01+2B)}{2K} + \frac{K^2(0.005+B)^2 - 0.02K^2B]^{1/2}}{} \qquad \text{Eq. (4)}$$

Observed ellipticity values at 225, 228, 230, 232 nm were read and applied to non-linear regression method[12] using a damping gause-newton method with aid of a digital computer(MULTI computer). As shown in Fig. 3, curve equation is $Y^2 - (X + 0.01)Y + 0.005 = 0$ and K was found to be 187 M^{-1} ($\pm$ 5%).

Fig. 4 shows the effect of pH on the binding constant. As shown in Fig. 4, the binding constant increases slightly with increasing pH. The binding force seems to be a hydrophobic bonding. It is suggested that benzene group of ZP should be accomodated in the cavity of CDx.[21]

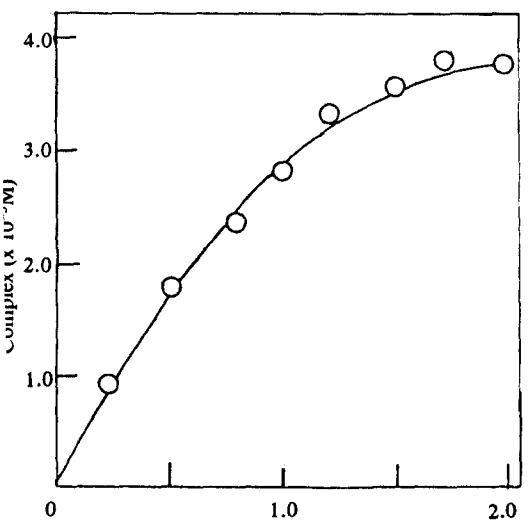

Fig. 3. Computer data: Plot of ZP-CDx complex concentration against CDx concentration (in H_2O).

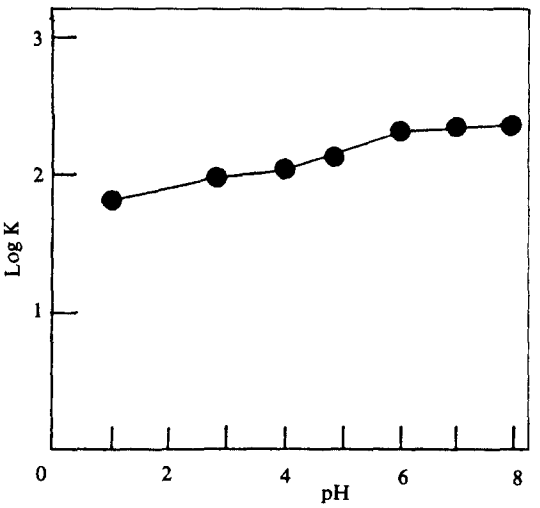

Fig. 4. The pH profile for binding constant of ZP-CDx system at 25°C.

It was reported that azo dyes such as MO form 1:1 complexes with CDx and CDx includes MO at the benzene-sulfate sides.[20] When MO and CDx form a complex in acidic solution, the complexed form of MO absorbs light much less intensely than the free form. If a third solution, capable of forming a complex, is added into the solution, some of the complexed MO will be competitively displaced, with a corresponding increase in the absorption intensity.[16]

When ZP was added in the complex of MO with CDx as shown in Fig. 5, there was a corresponding increase in the absorption intensity. Also, as given in Fig. 5, UV spectra reveals isobestic points at approximately 410, 590 nm. Therefore, MO and ZP competitively form the complexes with CDx.

Binding constant(K_D) of ZP is treated as follows[20]; the induction process is ommited and only important equations are written.
Let D, L and I represent ZP, CDx and MO, respectively.

$$I + L = IL \qquad K_I = (IL)/(I) \cdot (L) \qquad \text{Eq. (5)}$$

$$D + L = DL \qquad K_D = (DL)/(D) \cdot (L) \qquad \text{Eq. (6)}$$

Defining the indicator ratio

$$Q = (I)/(IL) = (E - E_{IL}) / (E_I - E) \qquad \text{Eq. (7)}$$

where E_I and E_{IL} are the molar absorptivities of free and complexed MO, respectively, and E is the ap-

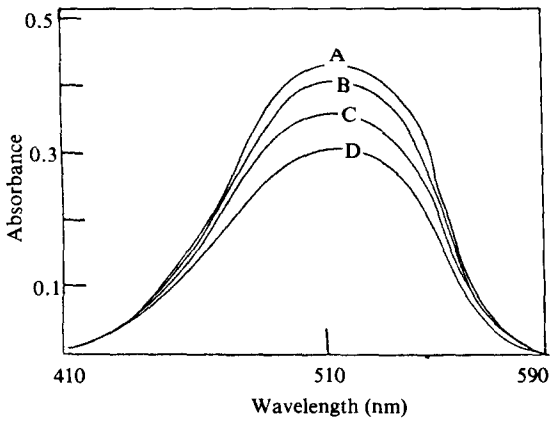

Fig. 5. UV spectra of MO-ZP-CDx system in H_2SO_4-Na_2SO_4 buffer (pH 3).
Key: A, MO (2.0×10^{-5}M);
B, MO (2.0×10^{-5}M) + CDx (2.6×10^{-3} M) + ZP (3.9×10^{-3}M);
C, MO (2.0×10^{-5}M) + CDx (2.6×10^{-3} M) + ZP (2.6×10^{-3}M);
D, MO (2.0×10^{-5}M) + CDx (2.6×10^{-3} M).

parent molar absorptivity in any solvent containing ZP and MO. If the total concentration of MO (2.0×10^{-5} M) is constant in all solutions, the absorptivities can be replaced by absorbances.
The quantity, P, is defined as follows:

$$P = L_t - \frac{1}{Q \cdot K_I} - \frac{I_t}{Q+1} \qquad \text{Eq.(8)}$$

Therefore, Eq.(8) may be written

$$P = (D_t \cdot K_D)/(Q \cdot K_I + D)$$
$$D_t/P = (K_I/K_D) \cdot Q + 1 \qquad \text{Eq. (9)}$$

K_I, 358 M^{-1}, was determined by the Hildebrand-Benesi plot and K_D would be determined by ploting D_t/P against Q where P was obtained using Eq.(8). As shown in Fig. 6 and Eq.(9), K_D, 155 M^{-1}, was determined by the slope.

In dialysis method, CDx wasn't permeable. As shown in Fig. 7, binding constant(K) and binding sites(v) were determined by the Scatchard plot which is as follows;

$$r/D = -rK + vK \qquad \text{Eq. (10)}$$

Ploting r/D against r, K = -slope, 315M^{-1} and v = X-intercept, 0.42, namely 1:2 complex (1 ZP: 2 CDx), where r is mole of ZP bound per moles of

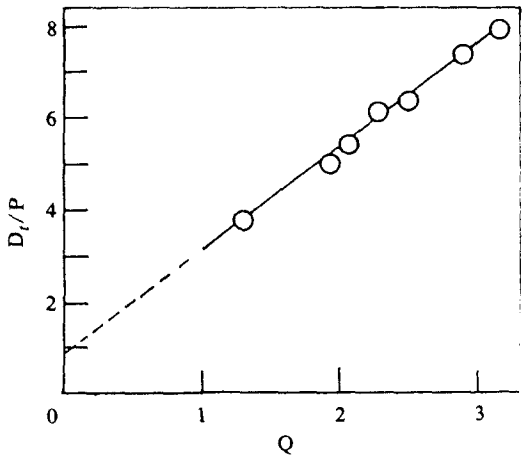

Fig. 6. Plot of D_t/P against Q for ZP-CDx system.

CDx and D is the concentration of free ZP.

Binding constant and stoichiometry are given in Table I. In the results, it is suggested as follows; ZP has two benzene groups. Its is nearly symmetric and it is not observed CD spectrum at 220-250 nm. If only one benzene group of ZP is accomodated in the cavity of CDx, it is suggested that ZP-CDx complex be asymmetric. Therefore, it has CD curve at 220-250 nm. If two benzene rings of ZP are accomodated in the cavity of CDx, it is suggested that two CDx molecules symmetrically exist out of ZP molecule. Therefore, it has no CD curve at 220-250 nm. Consequently, 1:1 stoichiometry was observed in CD method. This fact is also supported by competitive UV method. After one benzene group is accomodated in the cavity of CDx in diluted solution

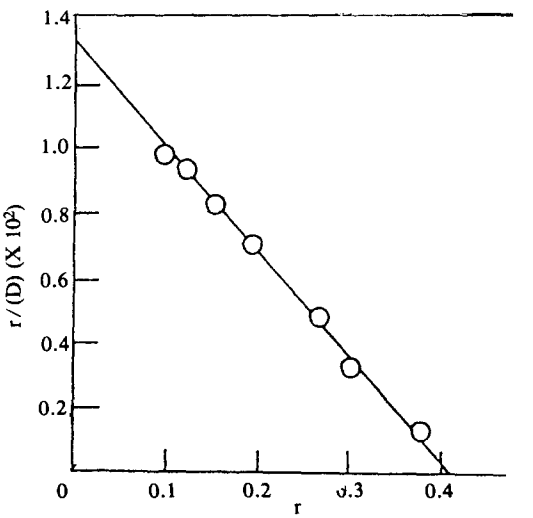

Fig. 7. Scatchard plot for ZP-CDx system.

Table 1. Binding constant and stoichiometry

	binding constant	stoichiometry
CD	187 M^{-1}(±5%)	1 : 1
dialysis	315 M^{-1}	1 : 2
competitive UV	155 M^{-1}	1 : 1

is somewhat difficult that the other benzene ring accomodated in the cavity of CDx because of eric hindrance. Thus, spectroscopic method is on- applicable to the diluted solution.

However, total interactions among molecules inuding symmetric complex can be detected by the uilibrium dialysis. Present results indicate that vo benzene rings of one ZP molecule are accomoated in the cavity of two CDx molecules in concenated solution. This means that apparent binding nstant measured by dialysis is larger than that by ectroscopic method.

Although three different methods have been us-, these techniques have yielded somewhat limited formation on the nature involved in the forma- n of the complex. Therefore, in the study of Dx-ZP interaction, it is reasonable that various ethods are used to cover the deficiency of the inrmation given by only one method.

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