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Inclusion Complexation of Cyclodextrin with Prothionamide in Aqueous Solution

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The inclusion of β-cyclodextrin (β-CyD) with prothionamide in aqueous phase was investigated by circular dichroism(CD), ultraviolet (UV) absorption, and solubility technique. The results suggested that a region of drug chromophore was located within the asymmetric center of β-cyclodextrin.

Solubility and spectral changes were quantitatively treated to obtain stoichiometric ratio, which was found to be 1 : 1, and formation constants which were determined by solubility, CD, and UV method were 257, 367, and 389 M^{-1}, respectively.

Also, the formation constant of the inclusion complex was determined by CD method at various pH. The result was that K_c depended upon the pH of medium, and this fact also supported that thioamide moiety was accomodated in the cavity of β-cyclodextrin.

Inclusion complexes of β-cyclodextrin (β-CyD) with various drugs have been extensively applied in pharmaceutical field to enhance the stability[1] of the unstable drugs and the solubility[2], dissolution rate[3], membrane permeability[4], and bioavailability[5] of slightly soluble drugs.

In preceding papers, complexation of β-CyD with various drugs, such as N-phenylanthranilic acids[6-8], phenothiazines[9], barbiturates[10-12], cinnamates[13,14], prostaglandins[15-17], and sulfonamides[18,19] in aqueous solution have been reported. It has then been shown that the forces holding together these complexes seem to be hydrophobic interaction, van der Waals force, and hydrogen bonding and that the magnitude of these forces depends upon the spatial relationship between host and guest molecules.

After the inclusion of optically inactive compounds within a cavity of β-CyD has been recently known to generate extrinsic Cotton effects[20], it has been demonstrated

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that various compounds show the induced circular dichroism(CD) by the formation of inclusion complexes with β-CyD and that CD method is useful to examine the mode of interaction of cyclodextrins with drug molecules[5-9,11,13,19-21].

It was previolusy reported that the sulfur containing guest molecules, such as phenothiazines[9], thiobarbiturates[11], sulfamethizole, and sulfathiazole[19], were very favorably included within β-CyD cavity. For the elucidation of inclusion mechanism, prothionamide is a useful guest molecule because it contains a sulfur atom and has both the hydrophobic and hydrophilic group in itself. Also, the effects of its inclusion are subtly reflected in various spectra.

In the present study, the interaction of β-CyD with prothionamide in aqueous solution was examined by circular dichroism(CD), ultraviolet(UV) spectra, and solubility method. Induced CD, UV absorption change, and solubilizing effect were quantitatively investigated. Stoichiometric relationship and formation constants for complex formation were reported. Also, the effect of pH on this interaction was investigated to gain further insight into mechanism and geometry of the inclusion process. From these observations the mode of inclusion will be discussed.

Experimental

Materials—Prothionamide was favored by Dong-A Pharm. Co., Ltd. and recrystallized from EtOH-water. β-Cyclodextrin (β-CyD) was obtained commercially from Tokyo Kasei Kogyo Co., Ltd. and recrystallized from water and dried with P_2O_5 in vacua. Its specific rotatory power in water was $[\alpha]_D^{25} = 162.0 \pm 0.5°$. Distilled water and deionized water were used. All other materials and solvents were of reagent grade purity.

CD and UV Absorption Studies—β-CyD equivalent to 1.0×10^{-2}M was added into the experimental bottle containing 25 m*l* of prothionamide solution(2.0×10^{-4}M) and was then shaken for 50−70 hrs at 25°C in the temperature-controlled shaking bath (Grant Instruments Ltd.) until equilibrium was attained. After equilibration, the CD and UV spectra were recorded by a Jasco 20 C automatic recording spectropolarimeter and a Unicam SP-1750 ultraviolet spectrophotometer, respectively.

The molar ellipticity, $[\theta]^\lambda$ and the molar absorptivity, ε^λ of guest molecule (prothionamide) in the presence of β-CyD were respectively calculated from the following equation [1] and equation [2] at the maximum wavelength of spectra on the basis of total drug concentration.

$$[\theta]^\lambda = \frac{10^2 \cdot \theta}{C \cdot l} \quad \cdots\cdots [1]$$

(unit: deg. cm^2. $dmole^{-1}$)

$[\theta]^\lambda$: the observed ellipticity at wavelength (deg.)

C : the concentration of drug (moles/liter)

l : pathlength (cm)

$$\varepsilon^{\lambda} = \frac{A}{C \cdot l} \quad \cdots\cdots [2]$$

(unit: $cm^2 \cdot mole^{-1}$)

A : the observed absorbance

The optical anisotropy factors (g values) which were proportional to the magnitude of the induced Cotton effects were calculated from the equation [3] at the maximum wavelength of CD spectrum.

$$g = \frac{[\theta]}{3300 \cdot \varepsilon} \quad \cdots\cdots [3]$$

The effect of β-CyD (1.0, 2.5, and 10.0×10^{-3}M) on UV absorption spectrum of prothionamide(constantly 1.0×10^{-4}M) was measured.

Also, the induced CD spectra for prothionamide-β-CyD complex system were investigated. In this experiment, prothionamide concentration was constantly 3.0×10^{-4} M and β-CyD concentration added was varied from 1.0 to 10.0×10^{-3} M.

Stoichiometric Relationship—The ratio of complex formation was determined by the continuous variation plot of the molar ellipticity changes. Both the concentrations of prothionamide and β-CyD solution were 1.0×10^{-3}M and the composition ratios of the samples were made variable. After shaking for 60 hrs, the ellipticities of the various solutions were quantitatively measured at 320 nm of the induced CD bands.

Determination of Formation Constant—The formation constant, K_c, of the prothionamide-β-CyD complex was determined by solubility, CD, and UV method on the basis of 1 : 1 stoichiometry.

Solubility method: The solubility study was carried out according to Higuchi and Lach (25). Excess amount of prothionamide (50 mg) was added buffer or aqueous β-cyclodextrin solution (varied from 0.1 to 1.8×10^{-2}M) and was shaken at 25°C±0.5° for 50–70 hrs in a temperature controlled shaking bath until the system attained equlibrium. After equilibration, the solution was filtrated through Cotton filter. The sample solution was then suitably diluted with distilled water and the concentration of prothionamide in the filtrate was determined by UV spectrophotometry at 289 nm. The presence of β-CyD in the experimental condition did not interfere on the spectrophotometrical assay.

The formation constant for the complex was calculated from the phase diagram obtained according to the method of Higuchi and Lach by equation [4].

$$K_c = \frac{\text{slope}}{S_0 \cdot (1 - \text{slope})} \quad \cdots\cdots [4]$$

S_0 : Solubility of prothionamide in water (intercept)

Circular Dichroism method: The formation constant, K_c, of prothionamide-β-CyD complex was determined spectropolarimetrically. The ellipticities of prothionamide (constantly 3.0×10^{-4}M) in the presence of β-cyclodextrin (varied from 1.0 to 10.0×10^{-3}M) were measured at 305nm of the induced CD bands.

The K_c was determined according to the equation [6] derived from the conventional Scott's equation [5] (26)

$$\frac{a \cdot b}{\theta} = \frac{1}{K_c \cdot \varepsilon_c} + \frac{b}{\varepsilon_c} \quad \cdots\cdots [5]$$

$$K_c = \frac{\text{slope}}{\text{intercept}} \quad \cdots\cdots [6]$$

where a is the total concentration of prothionamide, b is the total concentration of β-cyclodextrin, ε_c is the difference of the molar absorptivities for free and complexed prothionamide, and θ is the change in the ellipticity of the prothionamide by the additon of β-CyD.

Ultraviolet absorption method: The formation constant, K_c was also determined spectrophotometrically. UV absorption changes of prothionamide (constantly 3.0×10^{-4}M) in the presence of β-CyD(varied from 1.0 to 10.0×10^{-3}M) were quantitatively measured at the maximum wavelength(292 nm) due to the complex formation.

The K_c was determined according to Scott's equation [7] in the same way as CD method;

$$\frac{a \cdot b}{d} = \frac{1}{K_c \cdot \varepsilon_c} + \frac{b}{\varepsilon_c} \quad \cdots\cdots [7]$$

where d is the change in the absorbance of prothionamide by the addition of β-CyD.

pH Profile for Formation Constant—For the pH profile experiment, K. Ikeda *et al.* (11) used the solutions adjusted to appropriate value by the addition of 0.1 M NaOH or 0.1 M HCl to phosphate buffer (pH 7.0), but these solutions didn't maintain the buffer system. And so, this experiment used HCl buffer(pH 2), acetate buffer (pH 3–5), and phosphate buffer(pH 6–8). These buffer solutions didn't interfere the complexation and CD study for the complex. The CD method was used for the determination of formation constant at various pH's.

Results and Discussion

Induced CD and UV Absorption Changes—Since β-CyD has a large asymmetric cavity, various compounds have been shown to generate the extrinsic Cotton efffects by the formation of inclusion complexes. The induced CD of the complexes is generally characterized by their sign, magnitude, and wavelength of the location. According to the symmetry rule of Schellman(27), the sign of the induced Cotton effect depends upon the spatial relationship between the asymmetric center and the perturbed chromophore, whereas the magnitude of the optical activities seems to depend upon the rigidity of the complex formed.

Figure 1 Shows the CD and UV spectrum of prothionamide following the binding to β-CyD. The prothionamide absorption band at 289 nm is assigned to the resonance between pyridine ring and thiocarbonylamide group. Since β-CyD has neither CD nor absorption band at longer wavelength than 220 nm, this CD band above 220 nm can be attributed to the induced optical activity of prothionamide by the formation of inclusion complex with β-CyD. Prothionamide-β-CyD complex system showed two positive ellipticities; the strong peak at 205 nm and the very weak peak at 243 nm,

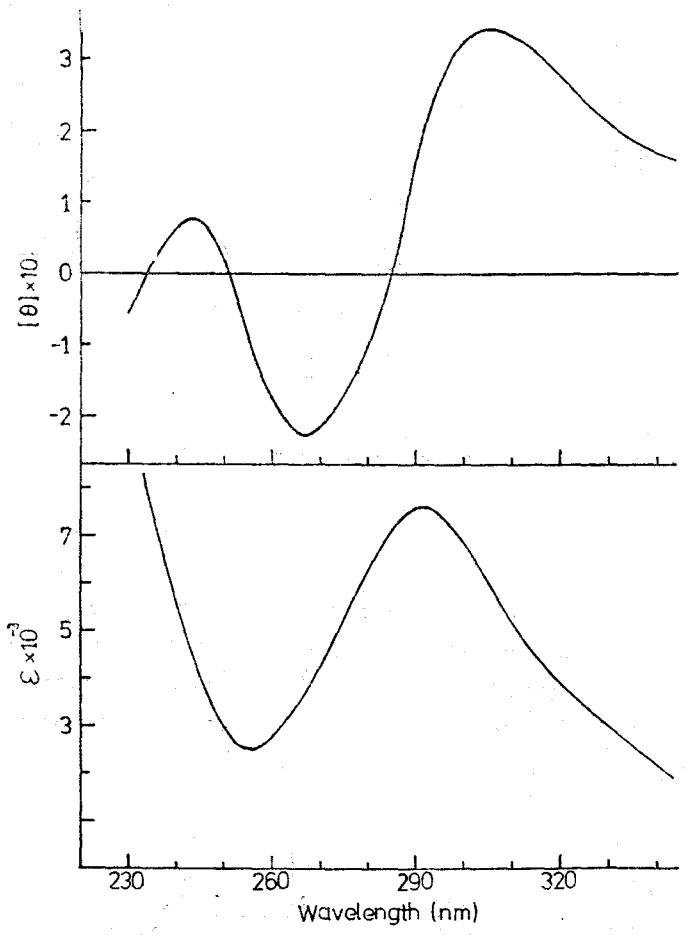

Figure 1—Circular dichroism(upper) and ultraviolet(lower) absorption spectra of prothionamide-β-cyclodextrin system. Concentration; prothionamide, 2.0×10^{-4} M; β-cyclodextrin, 1.0×10^{-2} M.

and one negative ellipticity; the relatively strong peak at 268 nm. The fact that prothionamide generates the induced Cotton effect by complexation with β-CyD may indicate that the asymmetric center of β-CyD is located in a region of drug chromophore which makes positive and negative contributions to Cotton effect.

Table 1 summarizes spectral characteristics such as λmax, [θ], ε, and g value obtained from prothionamide bound to β-CyD, where the facts mentioned above are quantitatively shown. As shown in Table 1, optical anisotropy factors(g values), which were proportional to the induced Cotton effects, were determined. The g value at 263 nm was the largest of the three peaks and g at 305nm was a little lower than at 268nm. And g at 243 nm was the smallest. From these results it was assumed that a region of chromophore participated in the inclusion.

Figure 2 shows the effect of β-CyD on the UV absorption spectrum of prothionamide where absorption maximum(λ max) and intensity changed concomitantly by the

Table I —Induced CD and UV Absorption Bands by the Binding of Prothionamide to β-Cyclodextrin*

	λmax, nm	243	268	305
CD	Molar Ellipticity, [θ] (deg. cm dmole)	0.7×10^3	-2.3×10^3	3.4×10^3
	Optical Anisotropy Factor, g	0.46×10^{-4}	1.81×10^{-4}	1.70×10^{-4}
	λ max, nm	292		
UV	Apparent molar Absorption Coefficient, ε	0.76×10^4		

* : Concentration of prothionamide and β-CyD were 2.0×10^{-4}M and 1.0×10^{-2}M, respectively, in 0.1M sodium phosphate buffer(pH 7.0).

increasing amount of β-CyD with the isosbestic points at 250 and 300 nm. By the binding to β-CyD, the UV peak was generally shifted to longer wavelength(bathochromic shift) with the concomitant decrease in the molar absorptivity(hypochromic shift). Similar UV spectral changes were observed when the drugs were dissolved in less polar solvents such as ethanol-buffer and dioxane-buffer mixtures(2). These results apparently indicated that the drug chromophore was located within the hydrophobic cavity of β-CyD.

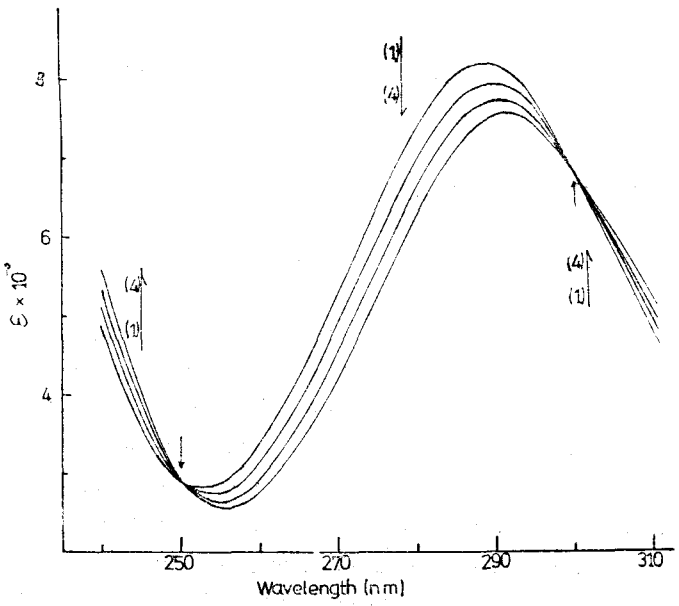

Figure 2—Effect of β-cyclodextrin on UV absorption spectrum of prothionamide in 0.1M phosphate buffer(pH 7.0). Concentration of prothionamide: constantly 1.0×10^{-4}M, concentration of β-CyD added:(1) 0, (2) 1.0×10^{-3}M, (3) 2.5×10^{-3}M, (4) 10.0×10^{-3}M.

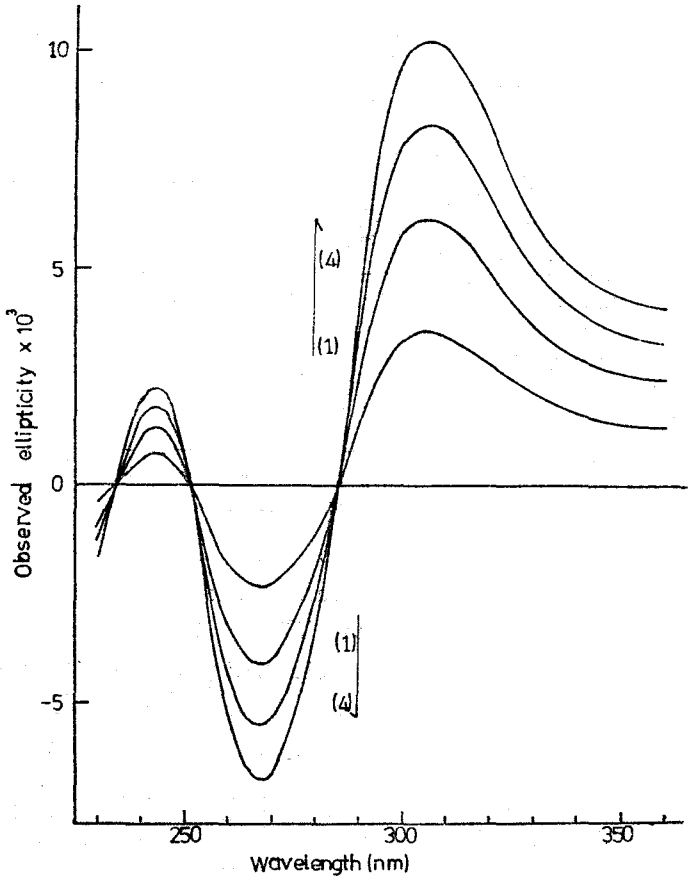

Figure 3—Induced CD curves for prothionamide-β-CyD complex. Concentration of prothionamide: constantly 3.0×10^{-4}M, concentration of β-CyD added: (1) 1.0×10^{-3}M, (2) 2.5×10^{-3}M, (3) 5.0×10^{-3}M, (4) 10.0×10^{-3}M.

Also, Figure 3 shows the induced CD curves for prothionamide-β-CyD complex where CD spectrum changed concomitantly by the addition of β-CyD. This indicated that the addition of β-CyD increased the complexed amount of drug and that the circular dichroism(CD) method could be quantitatively used.

Stoichiometric Relationship—Figure 4 shows a continuous variation diagram of the observed ellipticity changes for prothionamide-β-CyD system at 320 nm by CD method. When the composition ratio of prothionamide and β-CyD was 1 : 1, the observed ellipticity was the largest value. Also, both sides of plot were nearly symmetrical. This indicated the 1 : 1 complex formation with the presence of isosbestic points as shown Figure 2.

Formation Constants of Inclusion Complex—Induced optical activity, UV absorption, and solubility change due to the complex formation between β-CyD and prothionamide were quantitatively treated to obtain formation constant, K_c. Figure 5 shows the solubility of prothionamide as a function of β-CyD concentration in buffer. The plateau was not observed in this plot until β-CyD concentration attained to its

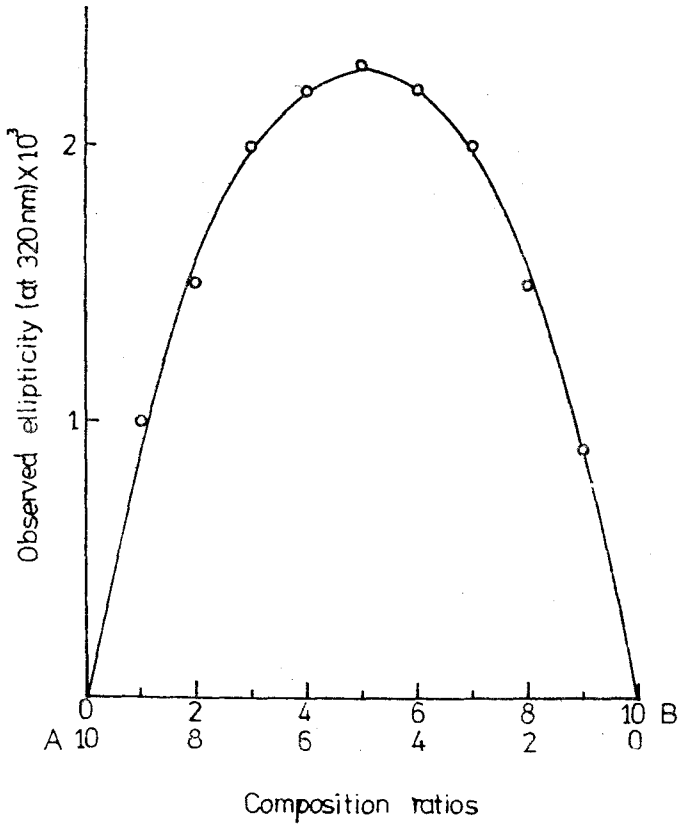

Figure 4—Continuous variation plots for prothionamide-β-cyclodextrin system in 0.1 M sodium phosphate buffer(pH 7.0) at 25°C. A, prothionamide, 1.0×10^{-3} M; B, β-CyD, 1.0×10^{-3}M.

limiting solubility. But the solubilizing effect of β-CyD on prothionamide was large. Formation constant was then calculated on the basis of 1 : 1 from the slope and intercept of the solubility diagram. Figure 6 shows Scott's plot of ellipticity change for prothionamide-β-CyD system. Also, Figure 7 shows Scott's plot of UV absorption change for complex system. UV absorption change was very small and had large error range, but application of UV absorption change to Scott's equation showed linear plots, indicating 1 : 1 stoichiometry. Formation constants of inclusion complex were calculated from the slope and intercept of the Scott's plots. Table Ⅱ summarizes K_c values obtained by solubility method fairly agreed with those by spectroscopic methods.

Table Ⅱ—Formation Constants of β-Cyclodextrin-Prothionamide Complex by Various Methods

Method	Formation Constant, $K_C(M^{-1})$
Solubility Method	257
CD Method	367
UV Absorption Method	389

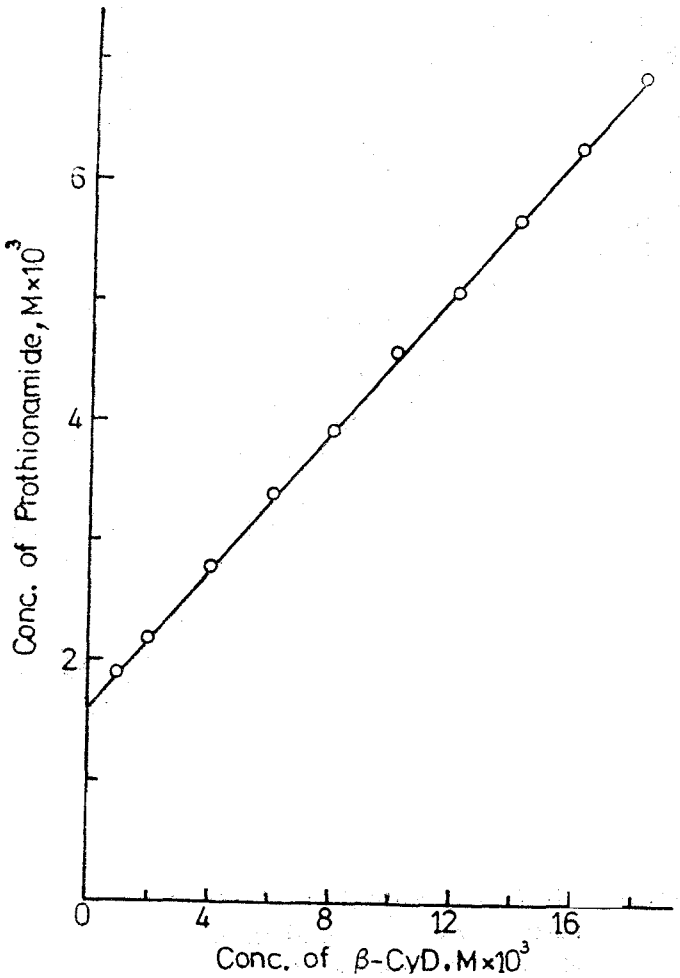

Figure 5—Solubility of prothinamide as a function of β-cyclodextrin concentration in 0.1M sodium phosphate buffer (pH 7.0) at 25°C.

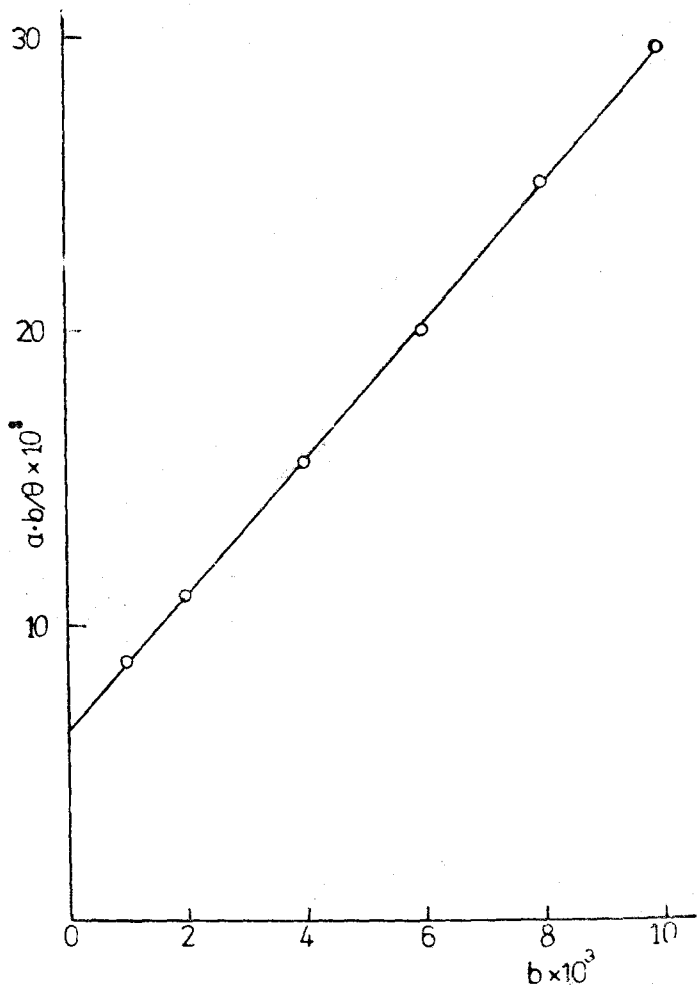

Figure 6—Scott's plot for interaction between prothionamide and β-cyclodextrin in 0.1M sodium phosphate buffer(pH 7.0) at 25°C by CD Method. a, conc. of prothionamide, 3.0×10^{-4}M; b, conc. of β-CyD; θ, observed ellipticity.

pH Profile for Formation Constant—Figure 8 shows the pH dependency of the stability constant for prothionamide-β-CyD complex system, where typical sigmoid curves were obtained with an inflection point around pH 3.5. The higher was pH, the larger was K_c in pH range from 2 to 8. This also supported that thioamide moiety was accommodated in the cavity of β-CyD.

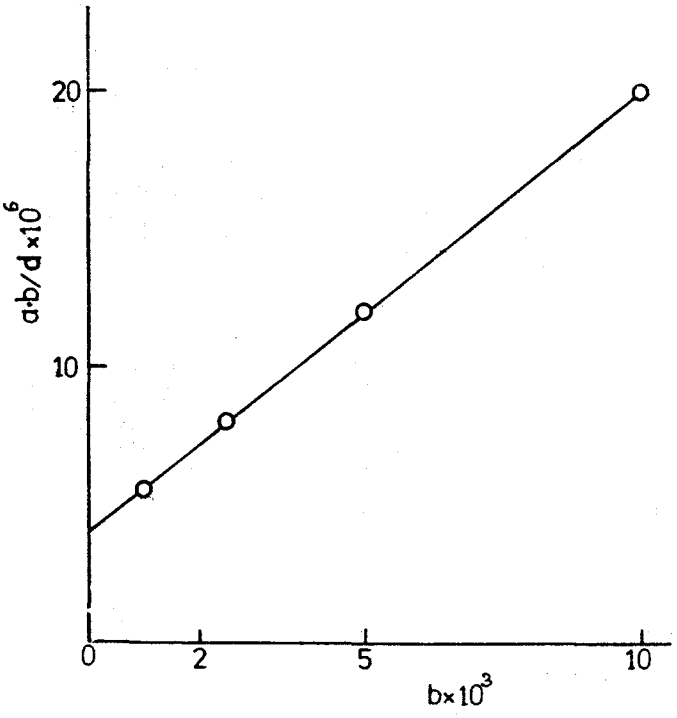

Figure 7—Scott's plot for interaction between prothionamide and β-cyclodextrin in 0.1M sodium phosphate buffer(pH 7.0) at 25°C by UV absorption method. a: conc. of prothionamide, 3.0×10^{-4}M; b: conc. of β-CyD; d: change in absorbance of prothionamide on adding β-CyD.

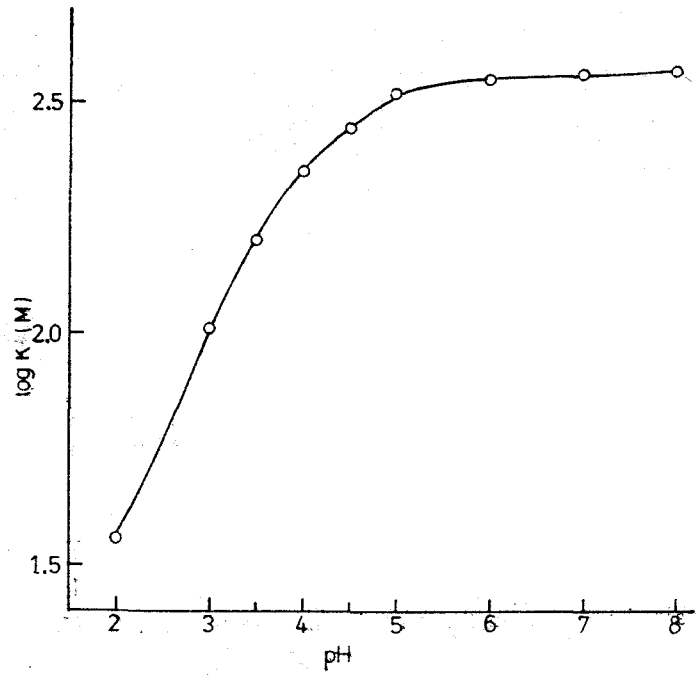

Figure 8—The pH profile for formation constant of prothionamide β-cyclodextrin complex at 25°C.

Conclusion

1. The results indicated that the chromophore of prothionamide was located within the hydrophobic cavity of β-cyclodextrin due to the inclusion complexation.

2. The isosbestic points and the continuous variation plots showed 1 : 1 complex formation.

3. The formation constants of β-CyD-prothionamide complex by solubility, CD, and UV method were 257, 367, 389M^{-1}, respectively,

4. The formation constant of β-CyD-prothionamide depended upon the pH of medium, and this fact also supported that thioamide moiety was accommodated in the cavity of β-CyD.

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