[Chem. Pharm. Bull. 24(6)1155—1159(1976)]

UDC 547.854.5.03.08: 547.458.6.08

Simultaneous Determination of Complexation Equilibrium Constants for Conjugated Guest Species by Extended Potentiometric Titration Method: On Barbiturate-β-Cyclodextrin System[1,2]

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(Received July 18, 1975)

The equilibrium constants of barbiturate–β-cyclodextrin complexations in aqueous media were determined by potentiometric titration method. The stability constants for complexes of unionized and ionized barbiturates with β-cyclodextrin and also the dissociation constants of complexed barbiturates can be determined simultaneously by simple procedure with good reproducibility. The results were fairly agreement with those obtained by ultraviolet, circular dichroism, and solubility method. This procedure is applicable even when the complexation accompanies no significant spectral change. The stoichiometric treatment presented was found to be pertinent also to other complexation and surfactant solubilization systems of weakly acidic or basic guest molecules.

In the preceding studies the inclusion of various barbituric acid derivatives within cyclodextrin was investigated by means of ultraviolet (UV), circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra. As has been reported various methods (UV, CD, and solubility studies) are applicable for the determination of the equilibrium constants of the inclusion complexation between β-cyclodextrin and various guest molecules.[4,5] Donbrow and others used potentiometric titration to determine the equilibrium constant of the solubilization of weak organic acid in nonionic micelle.[6] In the present study the potentiometric titration method was extended to more complicated system and proved to be applicable for β-cyclodextrin–barbiturate system, where both of unionized and ionized barbiturate molecule form 1:1 complexes. The results obtained were in fair agreement with those obtained by UV, CD, and solubility studies and the reproducibility and accuracy are not less than the spectral methods. The advantage of this method is that two equilibrium constants, those for unionized and ionized substrates, can be obtained by simple procedure even when significant spectral change does not occur by complexation. The stoichiometric treatment on the present system was proved to be pertinent also to other complexation and surfactant solubilization systems for weakly acidic or basic guest molecules, which will be reported later. The titration was carried out at various temperature and the thermodynamic parameters were calculated.

Experimental

Materials——Sodium phenobarbiturate, calcium cyclobarbiturate, sodium pentobarbiturate, and sodium amobarbiturate were commercially obtained and recrystallized from ethanol. Sodium salts of mepho-

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[1] A part of this study was presented at the 94 th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.

[2] This report constitutes Part II of the studies entitled "Inclusion Complexation of Barbiturates with β-Cyclodextrin in Aqueous Solution." Part I: M. Otagiri, T. Miyaji, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1146 (1976).

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[4] *a*) A.L. Thakkar and P.V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971); *b*) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *ibid.*, **61**, 1841 (1972).

[5] K. Koizumi, K. Mitsui, and K. Higuchi, *Yakugaku Zasshi*, **94**, 1515 (1974).

[6] *a*) M. Donbrow and C.T. Rhodes, *J. Chem. Soc.*, 6166 (1966); *b*) M. Donbrow, P. Molyneux, and C.T. Rhodes, *ibid.*, A561 (1967).

barbituric acid and methalbituric acid were prepared by the usual method from commercially available free barbituric acids. β-Cyclodextrin was favored by Teijin Ltd.

Methods——The titration of aqueous sodium barbiturate (30 ml of 2×10^{-3} M) containing various concentration of β-cyclodextrin (2×10^{-3} M to 1.6×10^{-2} M) with HCl (about 2×10^{-2} M) was carried out in a thermostatted beaker under the stream of nitogen gas using pH-stat, Toa Denpa HS-2A type.

The determination of equilibrium constants by UV and CD methods were performed in the same manner as previously descrived.[2]

The procedure of the solubility study was same to that of Higuchi and Lach[7] and 1×10^{-1} M phosphate buffer of pH 5.0 was used as the solvent. The concentration of barbiturate was determined by UV spectrophotometry at pH 10.0.

Theory

Unionized barbiturates have been known to form inclusion complex with β-cyclodextrin in the molar ratio of 1: 1[4b] and the ionized monoanion of barbiturate also forms complex in the same molar ratio as was verified in the preceding study.[2] Therefore the possible complexation process may be represented as Chart 1. Values K_a and $K_{a'}$ are the first step

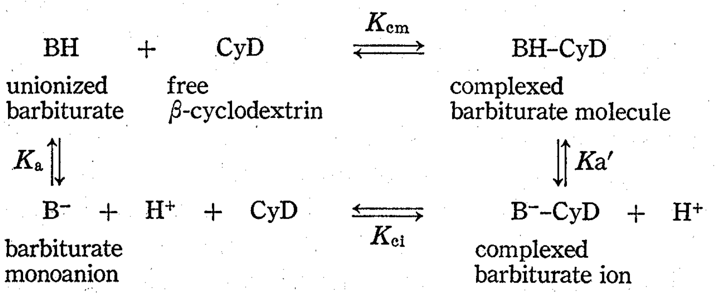

Chart 1. Equilibrium Scheme for the Complex Formation of Barbiturate with β-Cyclodextrin

dissociation constants of non-complexed and complexed barbituric acid respectively as follows:

$$K_a = \frac{[B^-][H^+]}{[BH]} \tag{1}$$

$$K_a' = \frac{[B^-\text{–}CyD][H^+]}{[BH\text{–}CyD]} \tag{2}$$

It is assumed that the secondary dissociaton can be disregarded in this experimental conditions. Values K_{cm} and K_{ci} are the dissociation constants of the inclusion complexes of unionized and ionized barbituric acid, respectively, as represented by the following equations.

$$K_{cm} = \frac{[BH][CyD]}{[BH\text{–}CyD]} \tag{3}$$

$$K_{ci} = \frac{[B^-][CyD]}{[B^-\text{–}CyD]} \tag{4}$$

Meanwhile, the equilibrium constants in Chart 1 hold the following relation.

$$K_a \times K_{cm} = K_a' \times K_{ci} \tag{5}$$

At the half neutralization point there is relationship as following equation.

$$[B^-] + [B^-\text{–}CyD] = [BH] + [BH\text{–}CyD] \tag{6}$$

From equation (1)—(6) the hydrogen ion concentration at the half-neutralization, $[H^+]_h$, can be expressed as follow:

$$[H^+]_h = K_a' + \frac{K_a'(K_{ci}-K_{cm})}{K_{cm}+[CyD]} \tag{7}$$

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[7] T. Higuchi and J.L. Lach, *J. Am. Pharm. Ass. Sci. Ed.*, 43, 349 (1954).

Equation 7 is hyperbolic with respect to $[\mathrm{H^+}]_h$ and [CyD] and finally can be reduced to $[\mathrm{H^+}]_h = K_{a'}$ when [CyD]=excess. Since equation 7 is held in any concentrations of β-cyclodextrin, the following relation can be expressed between two arbitrarily selected CyD concentrations, which are designated as $[\mathrm{CyD}]_1$ and $[\mathrm{CyD}]_2$, respectively:

$$\frac{\Delta[\mathrm{CyD}]}{\Delta[\mathrm{H^+}]_h} = \frac{K_{cm}+[\mathrm{CyD}]_1}{K_a'(K_{cm}-K_{ci})}[\mathrm{CyD}]_2 + \frac{K_{cm}[K_{cm}+[\mathrm{CyD}]_1]}{K_a'(K_{cm}-K_{ci})} \qquad (8)$$

where $\Delta[\mathrm{H^+}]_h$ is the difference of $[\mathrm{H^+}]_h$'s at the two [CyD]'s and $\Delta[\mathrm{CyD}] = [\mathrm{CyD}]_1 - [\mathrm{CyD}]_2$. When $[\mathrm{CyD}]_1$ is held constant and $[\mathrm{CyD}]_2$ is varied, linear relation should be obtained between $\Delta[\mathrm{CyD}]/\Delta[\mathrm{H^+}]_h$ and $[\mathrm{CyD}]_2$.

Referring to equation 8, K_{cm} is determined from the linear relationship by (Intercept)/(Slope) and K_a' can be calculated by the following equation.

$$K_a' = \frac{K_{cm}+[\mathrm{CyD}]_1}{(\mathrm{Intercept})} + K_a \qquad (9)$$

Value K_a is obtainable by the usual titration method in the absence of β-cyclodextrin and K_{ci} can be determined by equation 5 substituting the obtained equilibrium constants.

Results and Discussion

Equilibrium Constants Determined by Potentiometric Titration

Figure 1 shows the titration curves of sodium phenobarbiturate with HCl in the absence and presence of β-cyclodextrin. The curve shifts to alkaline side as the concentration of β-cyclodextrin increases.

In equation 7 [CyD] can be assumed to be equal to the added concentration of β-cyclodextrin, $[\mathrm{CyD}]_T$, when the β-cyclodextrin concentration is largely in excess. Figure 2 shows the typical plots of experimentally obtained $[\mathrm{H^+}]_h$ against $[\mathrm{CyD}]_T$ in the titration of sodium phenobarbiturate.

Figure 3 shows the plots according to equation 8, assuming that $[\mathrm{CyD}]_2$ equals to $[\mathrm{CyD}]_T$. The linear plot obtained verifies Chart 1 and the stoichiometric treatment described above.

The equilibrium constants of various barbiturate–β-cyclodextrin systems determined by the potentiometric titration are shown in

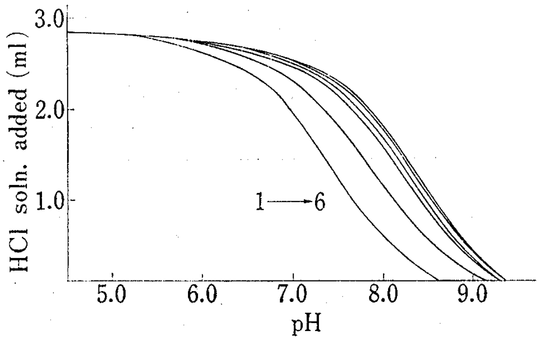

Fig. 1. Titration Record of Sodium Phenobarbiturate (2×10^{-3} M, 30 ml) with Hydrochloric Acid (2.14×10^{-2} M) at 25°

1; no β-cyclodextrin, 2—6; concentration of β-cyclodextrin: 2×10^{-3}, 5×10^{-3}, 8×10^{-3}, 1.0×10^{-2} and 1.6×10^{-2} M

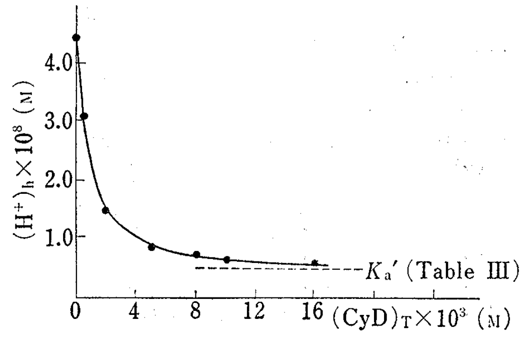

Fig. 2. Plots of $(\mathrm{H^+})_h$ *versus* Total CyD Concentration for Phenobarbiturate–CyD System at 25°

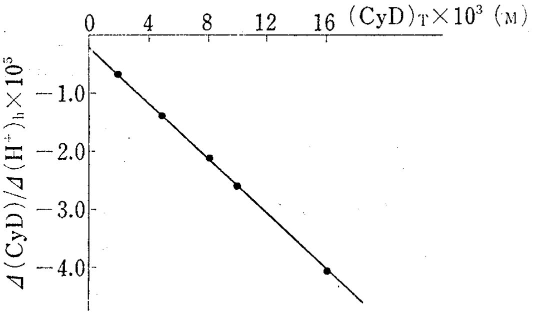

Fig. 3. Plots according to Equation 3 for Phenobarbituric Acid–CyD System, $(\mathrm{CyD})_1=0$

Table I. Equilibrium Constants of Various Barbiturate–CyD Systems at 25° determined by Potentiometric Method

Compound	$K_a \times 10^8$ (M)	$K_a' \times 10^9$ (M)	$K_{cm} \times 10^4$ (M)	$K_{ci} \times 10^3$ (M)
Phenobarbital	4.46	5.20	5.92	5.21
Mephobarbital	1.55	3.52	6.15	2.71
Amobarbital	1.02	5.37	10.0	1.89
Cyclobarbital	2.75	3.24	8.64	7.25
Pentobarbital	0.89	2.23	9.26	3.70
Metharbital	0.51	2.00	26.0	6.67
Barbital	1.15	6.76	61.0	10.3

Table I. All K_a values are larger than K_a' values, indicating that protolytic dissociation of barbituric acid is suppressed by the inclusion. The ratio of $K_a/K_{a'}$, the magnitude of the suppression, is larger when 5-substituents include phenyl or cyclohexenyl ring. Also the longer the substituent chain the stronger the inclusion, which is in accordance with the previous results.

Comparison with Other Methods

Table II shows the stability constants of the inclusion which were determined by the titration and the other methods. Values $1/K_{cm}$ and $1/K_{ci}$ are equal to K_f and K_i in the previous paper.[2] As is seen the potentiometric titration method gives values fairly consistent with those by the other methods. The advantage of this method is that all equilibrium constants, K_a, $K_{a'}$, K_{cm} and K_{ci} are simultaneously determined by the analysis of a series of titration curves.

Table II. Stability Constants (M^{-1}) of Barbiturate–CyD Complexes determined by Various Method at 25°

Compound	Potentiometry		UV		CD		Solubility
	$1/K_{cm}$	$1/K_{ci}$	$1/K_{cm}$	$1/K_{ci}$	$1/K_{cm}$	$1/K_{ci}$	$1/K_{cm}$
Phenobarbital	1690	190	1650	160	2940	390	3300
Mephobarbital	1630	370	1500	130	1460	320	1800
Pentobarbital	1080	270	1040	390	1050	380	810
Cyclobarbital	1160	140	1400	200	—	—	1110
Amobarbital	1000	530	1190	410	—	—	1290
Barbital	160	100	150	:	—	—	210
Metharbital	390	150	190	100	—	—	120

—; not determined
: ; unobtainable because of small spectral change

Table III. Equilibrium Constants of Barbiturate–CyD Systems at Various Temperature

Compound	Temp. (°K)	$K_a \times 10^8$ (M)	$K_a' \times 10^9$ (M)	$K_{cm} \times 10^4$ (M)	$K_{ci} \times 10^3$ (M)
Phenobarbital	283	3.24	2.01	2.39	3.85
	293	4.46	5.20	5.92	5.21
	308	5.25	6.07	13.0	11.2
	323	7.08	13.1	22.1	15.2
Mephobarbital	298	1.55	3.52	6.15	2.71
	308	2.14	3.40	11.3	7.11
	323	2.95	4.40	19.1	12.8

TABLE IV. Thermodynamic Parameters for the Complex Formation of Barbiturate with CyD

Compound	Equilibrium constant	ΔH (kcal/mole)	ΔS (cal/mole deg)
Phenobarbital	K_a	3.51	−21.9
	K_a'	8.10	−11.0
	$1/K_{cm}$	−10.3	−20.1
	$1/K_{ci}$	−6.60	−12.1
Mephobarbital	K_a	4.11	−19.3
	K_a'	1.50	−33.0
	$1/K_{cm}$	−8.10	−12.7
	$1/K_{ci}$	−9.30	−28.0

Temperature Dependency of Inclusion

On phenobarbiturate and mephobarbiturate, the stability constants were determined at various temperature and the results are summarized in Table III. Table IV shows thermodynamic parameters on the complexation which are fairly in agreement with those obtained from UV spectral change in the previous study. The discussion on the thermodynamic data were made previously. Both of unionized and ionized barbiturate are complexed with negative ΔH and the inclusion is unfavorable with respect to ΔS. The numerical correlation on ΔH and ΔS as follows, which is supposed from the equilibrium scheme, are held within experimental error.

$$(\Delta H \text{ for } K_a) - (\Delta H \text{ for } 1/K_{cm}) = (\Delta H \text{ for } K_a') - (\Delta H \text{ for } 1/K_{ci}) \qquad (10)$$

$$(\Delta S \text{ for } K_a) - (\Delta S \text{ for } 1/K_{cm}) = (\Delta S \text{ for } K_a') - (\Delta S \text{ for } 1/K_{ci}) \qquad (11)$$