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Application of Freezing Point Depression to Drug Interaction Studies. I.[1] Interaction between Cyclodextrins and Alcohols

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The osmotic pressure of dilute aqueous solutions of a cyclodextrin and an alcohol were measured separately and in combination by the freezing point depression method with a commercially available osmometer. The results could be classified into two categories; in one, the osmotic pressure of combined solutes was equal to the sum of the individual values obtained separately. In the other case, such additivity was not maintained. In the latter case, it was assumed that the two substances form a complex. On the basis of this assumption, stability constants for inclusion complexes of cyclodextrin with a variety of alcohols were determined. The stability constants obtained here were in fair agreement with those obtained previously by other methods. Therefore, it was concluded that measurement of freezing point depression is a useful method for determination of the stability constants for complex formation in an aqueous solution. The advantages and disadvantages of this method are discussed.

Keywords——freezing point depression; osmotic property; osmotic pressure; stability constant; cyclodextrin; alchohol; inclusion complex

To determine the stability constants for complexes, solubility,[2] spectroscopic[3] and kinetic[4] methods are generally used. In addition, various methods such as potentiometry,[5] high performance liquid chromatography (HPLC)[6] and polarography[7] have been employed. These methods, however, have both advantages and disadvantages. For example, the spectroscopic method is not applicable to complex formation involving only small spectral changes.

We therefore examined the applicability of osmotic pressure measurement in terms of freezing point depression. The osmotic pressure of a solution containing both solute A and solute B was measured (π_{AB}), as were those of individual solutions containing only solute A and only solute B (π_A, π_B). There were two cases: in one case the π_{AB} value was equal to the sum of π_A and π_B values, and in the other case, the value was smaller. In the latter case, it could be assumed that the complex AB was formed in the aqueous solution, resulting in the decrease of osmotic pressure. On the basis of this assumption, we calculated the stability constant for the complex between A and B.

Some authors have suggested that complex formation between two components, or a self-associate formation, could be detected in terms of a lowering of vapor pressure.[8] However, they reported no data on the stability constants. Furthermore, vapor phase osmometry can not be applied to substances with low boiling point such as alcohols. In the present study, the applicability of the freezing point depression method to the inclusion of various alcohols with α- and β-cyclodextrins (α-, β-CD) was examined.

Experimental

Materials——α-CD and β-CD were from Nihon Shokuhinkako. Alcohols and other materials were of reagent grade.

Apparatus——An osmometer (Osmette Model 2007, Precision Systems, Inc.) was employed for determination of the osmotic concentration of aqueous solutions based on the freezing point depression. The apparatus was calibrated with standard solutions (100 and 500 mOsm/kg) supplied by the company.

Measurement of Osmotic Concentration——Solutions were made with distilled water. The final concentrations of α-CD, β-CD and alcohol were adjusted to 50, 10 and 50 mM, respectively. Osmolality was then measured with 2 ml of each solution. The reproducibility of the measurement of the freezing point was within ±0.001 °C, and 1 mOsm change in water causes a change of 0.00185 °C in freezing point (T_f). Consequently, the deviation of T_f for a 50 mM solution may be kept within 1% and that for a 10 mM solution within 5%.

Theoretical

A dilute solution exhibits a linear relationship between freezing point depression and molar concentration, that is, when ideal nonelectrolytes are dissolved in 1 kg of water, the osmotic concentration would be equal to the molality of the solution. Osmolality measured by the freezing point depression can then be defined as that mass of solute dissolved in 1 kg of water. In this study, however, the value of milliosmoles per 1 l of solution was used to present the osmotic concentration, for molarity can be taken as equivalent to molality in aqueous solutions more dilute than 0.1 molar.[9)]

Let us assume that a cyclodextrin (A) as a host and a guest compound (B) form a 1 : 1 complex (AB), whose stability constant (K) is given by Eq. 1,

$$A+B \rightleftharpoons AB$$

$$K=[AB]/[A]\times[B] \tag{1}$$

where [A] and [B] are the concentration of the free solutes and [AB] represents the concentration of the complex. Total molar concentration [M] and osmotic concentration $[\overline{M}]$ in the solution can be defined by Eqs.2 and 3, respectively.

$$[M]=[A]+[B]+2[AB]=[A_o]+[B_o] \tag{2}$$

where $[A_o]$ and $[B_o]$ are the total concentration of A and B

$$[\overline{M}]=[A]+[B]+[AB] \tag{3}$$

$$\Delta=[M]-[\overline{M}]=[AB] \tag{4}$$

Therefore, the difference (Δ) between [M] and $[\overline{M}]$ is equal to [AB] (Eq. 4). The concentrations of the free reactants [A] and [B] can be expressed as:

$$[A]=[A_o]-[AB] \tag{5}$$

$$[B]=[B_o]-[AB] \tag{6}$$

Substitution of the above equations into Eq. 1 gives:

$$K=\Delta/([A_o]-\Delta)\,([B_o]-\Delta) \tag{7}$$

where $[A_o]$, $[B_o]$ and Δ can be obtained experimentally. Therefore, the K value can be determined from the freezing point depression. Hereafter we call the determined (apparent) K "K_{osm}".

Results and Discussion

Additivity and Nonadditivity of Osmotic Concentration

The individual osmolality of α-CD, sucrose or *n*-butanol and that of α-CD/sucrose or α-CD/*n*-butanol in combination were measured (Table I). The osmolality of α-CD/sucrose

Table I. Osmolality of Aqueous Solutions of α-CD, Sucrose and *n*-Butanol Individually and α-CD/*n*-Butanol in Combination

System	Compound	Osmolality (mOsm/kg)
Individual	α-CD	50
	Sucrose	50
	n-Butanol	50
Mixture	α-CD + sucrose	100
	α-CD + *n*-butanol	69

Table II. Effect of Concentration on K_{osm} Values

Compound (mM)			Osmolality (mOsm/kg)	Stability constant K_{osm} (M^{-1})
n-Butanol	12.5		13	
	25		25	
	50		50	
	75		77	
	100		99	
α-CD	12.5		13	
	25		24	
	50		50	
	75		73	
	100		98	
n-Butanol	50 + α-CD	12.5	52	113
	50	25	55	108
	50	50	67	113
	50	75	84	102
	50	100	105	101
n-Butanol	12.5 + α-CD	12.5	20	115
	25	25	36	98
	50	50	67	113
	75	75	98	100
	100	100	124	109

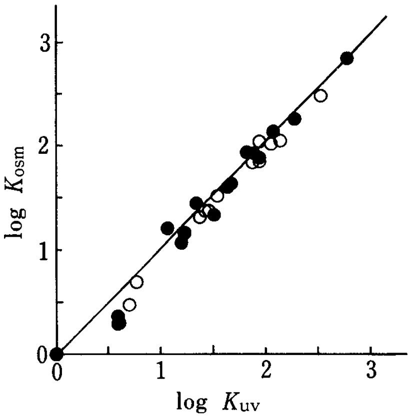

Fig. 1. Relationship between Stability Constants of Alcohol/CD Complexes Determined by Osmotic Method and UV Method

○, α-CD system; ●, β-CD system.

measured in combination was equal to the sum of their respective values measured separately. In contrast, that of α-CD/*n*-butanol measured in combination was smaller than the sum of their respective values measured separately. In the latter case, a complex was assumed to be formed between α-CD and *n*-butanol and behaves as if it were one molecule, resulting in a decline of apparent total number of molecules in the solution, because the osmolality of a dilute solution depends only on the number of dissolved molecules in solution. Based on this assumption, it is possible to obtain the stability constant from the degree of osmotic pressure depression (freezing point depression).

Effect of Concentration

The stability constant K_{osm} was measured with various concentrations of each solute according to Eq. 7. For an individual system, *n*-butanol or α-CD system, a linear relationship was holds between concentration and osmotic pressure, at least up to 100 mM. For the α-CD/*n*-butanol system, stability constants were measured in the concentration range from 12.5 mM to 100 mM (Table II). It is clear that the *K* values are independent of the concentration of α-CD and *n*-butanol and remain constant. In other words, it can be said that the stoichiometric ratio of the complex formed stayed constant in the concentration range studied. Similar results were obtained in other systems.

Determination of Stability Constant for Alcohol/Cyclodextrin Complex

It is known that cyclodextrin forms a 1 : 1 complex with various alcohols, but the complex formation does not result in significant spectral change. Matsui and Mochida

TABLE III. Comparison of Stability Constants of Various Alcohol and CD Complexes Determined by the Osmotic Method and the Spectroscopic Method

	Alcohol	α-CD		β-CD	
		K_{osm} (M^{-1})	K_{uv}[a] (M^{-1})	K_{osm} (M^{-1})	K_{uv}[a] (M^{-1})
1	Methanol	0	1	1	0
2	Ethanol	5	6	1	1
3	*n*-Propanol	20	23	5	4
4	2-Propanol	3	5	2	4
5	*n*-Butanol	110	89	15	17
6	2-Methyl-1-propanol	23	28	40	42
7	2-Butanol	23	26	14	16
8	2-Methyl-2-propanol	2	4	42	48
9	*n*-Pentanol	298	323	87	63
10	2-Methyl-1-butanol	102	110	131	120
11	3-Methyl-1-butanol	69	74	182	178
12	2,2-Dimethyl-1-propanol	—[b]	30	664	575
13	2-Pentanol	138	114	21	31
14	3-Pentanol	72	87	28	22
15	3-Methyl-2-butanol	—[b]	19	73	83
16	2-Methyl-2-butanol	32	34	88	81

a) Taken from Ref. 10. *b*) Could not be determined with accuracy due to the precipitation formed.

overcame this problem by using the so-called competitive indicator method[10]: an equilibrium is established between the host (CD) and an azo dye whose absorption spectrum differs significantly in its complexed and uncomplexed form. This equilibrum is then perturbed by the addition of a guest (alcohol), which competes with the azo dye for the host. By measuring the spectral change produced by this perturbation, the stability constant for the host–guest complex can be detected. On the other hand, Takagi and Maeda determined the K values from thermodynamic parameters.[11] The former method, however, needs the third compound, and the latter method involves fairly complicated procedures. In contrast, the osmotic method can be used to determine rapidly and directly the stability constant with a simple procedure when the complex is formed. Table III summarizes K_{osm} values obtained by the osmotic method for the complexation of α-CD and β-CD with various alcohols. For comparison, K_{uv} values obtained by Matsui and Mochida with the competitive indicator method are also listed in Table III.

A good correlation exists between K_{osm} and K_{uv} (Fig. 1). These results demonstrate that the osmotic method can give the K value as accurately as the other methods. It must be mentioned that an interaction with a relatively small K value can only be obtained by the osmotic method with fairly good accuracy, for Δ should be between 5 and 45 mOsm (considering the experimental deviation) when each solute concentration is 50 mM. The possible maximum variance of K obtained by this method depends on the instrumental error, and may be less than 20% when the K values are between 10 and 200 M^{-1} (Fig. 3). The slope of the straight line in Fig. 1 was a little greater than unity ($\log K_{osm} = 1.07 \log K_{uv} - 0.155$). The temperature difference might account for this: the K_{osm} values were determined at around 0°C (*i.e.* at freezing point). In contrast, the K_{uv} values were determined at room temperature. Therefore, K_{uv} values at 0°C (K_{uv}°) for a few pairs were estimated by extrapolating K_{uv} values determined at various temperatures to 0°C (Fig. 2). K_{uv}° values thus estimated came very close to the K_{osm} values (Table IV).

Table IV. K_{osm}, K°_{uv} and K_{uv} for Alcohol/α-CD Complexes

Compound	Stability constant (M^{-1})		
	K_{osm}	K°_{uv}	K_{uv}
α-CD + *n*-butanol	110	111	89
α-CD + 2-pentanol	138	137	114

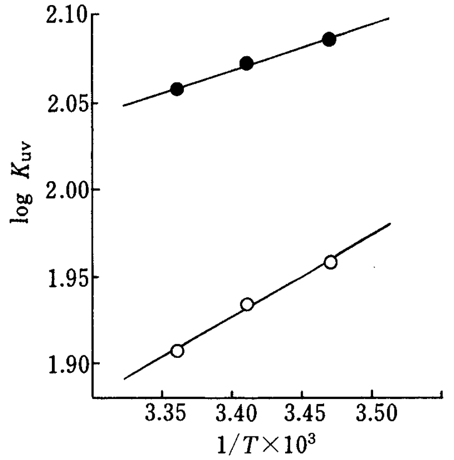

Fig. 2. Typical van't Hoff Plots for Stability Constants of Alcohol/CD Complexes

○, α-CD/*n*-butanol system; ●, α-CD/2-pentanol system.

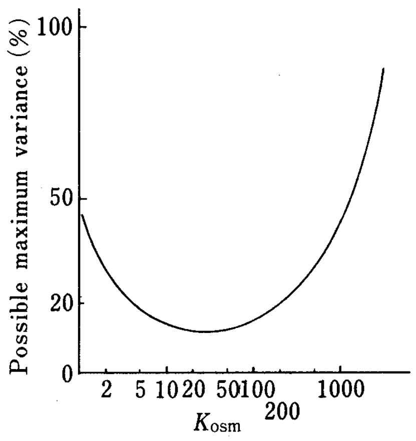

Fig. 3. The Variance (%) of K_{osm} Values Based on the Instrumental Error

Advantages and Disadvantages of the Osmotic Method

As mentioned above, the osmotic method has several advantages: the measurement can be conducted with a simple procedure within a short time and with good reproducibility. It can be applied to a combination for which quantitative measurements on complex formation by conventional methods, such as absorbance change, are impossible. On the other hand, it has some disadvantages: the measurement of K_{osm} values should be conducted only in aqueous systems, and the substrate must dissolve in water to a certain degree (>10 mM). The K_{osm} value should be relatively small (when each solute concentration is 50 mM, the range of K_{osm} is restricted to between 5 and 100 M^{-1}). In addition, it can be determined only at around 0°C, *i. e.* freezing point. Therefore, a thermodynamic parameter can not be determined by this method. In spite of these limitations, the osmotic method is concluded to be useful. It can give not only a rapid estimation of whether an interaction exists or not, but also the stability constant with fairly good accuracy.

References

1) A part of this work was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1985.
2) T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instr.*, **4**, 117 (1966).
3) H. A. Benesi and K. A. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
4) A. K. Colter, S. S. Wang, G. H. Megerle and P. S. Ossip, *J. Am. Chem. Soc.*, **86**, 1306 (1964).
5) T. Miyaji, Y. Kurono, K. Uekama and K. Ikeda, *Chem. Pharm. Bull.*, **24**, 1155 (1976).
6) K. Uekama, F. Hirayama, S. Nasu, N. Matsuo and T. Irie, *Chem. Pharm. Bull.*, **26**, 3477 (1978).
7) S. Yamaguchi and T. Tsukamoto, *Nippon Kagaku Kaishi*, **1978**, 1856.

8) H. N. Borazan and F. M. Goyan, *J. Pharm. Sci.*, **62**, 923 (1973); H. N. Borazan, *ibid.*, **64**, 770 (1976); F. Kopecky, M. Vojtekova and Bednarova-Hytterova, *Collect. Czech. Chem. Commun.*, **43**, 37 (1978); J.-Cl. Etter and Cl. Rey-Bellet, *Pharm. Acta Helv.*, **55**, 13 (1980).
9) A. Martin, J. Swarbrick and A. Cammarata, "Physical Pharmacy," 3rd ed., Lea and Febiger, Philadelphia, 1983, p. 146.
10) Y. Matsui and K. Mochida, *Bull. Chem. Soc. Jpn.*, **52**, 2808 (1979).
11) S. Takagi and M. Maeda, *J. Inclusion Phenom.*, **2**, 775 (1984).