A New Competitive Method Using Static Head-Space Gas Chromatography for the Determination of Cyclodextrin Complex Stability Constants

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The stability constants for the inclusion of alkanediol compounds with α-cyclodextrin (α-CyD) in aqueous solution have been determined by a competitive method using static head-space gas chromatography (SHSGC). The stability constants obtained by this method were in agreement with those obtained by the calorimetry method. The competitive method is applicable to the low volatile guest–CyD systems. Therefore, it was concluded that the competitive SHSGC measurement is another useful means of determining the stability constants of complex formation in an aqueous solution.

Key words alkanediol; α-cyclodextrin; stability constant; static head-space gas chromatography; competitive method

Various methods such as solubility measurement, absorption spectroscopy, and NMR spectrometry have been employed to obtain the stability constant for the cyclodextrin (CyD) complex.[1,2] These methods, however, have both advantages and disadvantages. For instance, the absorption spectroscopic technique is not applicable to complexes which do not exhibit a useful spectral change upon complexation.[3] To overcome this disadvantage indirect methods such as competitive spectrophotometry and spectrofluorometry have been developed.[4] We recently used static head-space gas chromatography (SHSGC) for determining the stability constants of benzene and alkylbenzenes–α-CyD complexes.[5] The SHSGC method is easy, reliable and accurate, but as true of other methods has a disadvantage in that its application to a low volatile guest is difficult.

In this paper, we report a new competitive method using SHSGC to determine the stability constants in low volatile guest–CyD systems. This technique is applied to the study of complexation between alkanediols and α-CyD. The reason for choosing these alkanediols is that they are compounds with very low volatility and are known to form complexes with α-CyD.[6]

Experimental

Materials The α-CyD used for the host was kindly donated by Nihon Shokuhin Kako Co., Ltd. and was used after drying in a vacuum. The α,β- and α,ω-alkanediols were obtained from Aldrich Chemical Co. and 1-hexanol was obtained from Wako Pure Chemical Industries, Ltd.; these were used without further purification. Distilled water (pH 6.3) per injection JP (Japanese Phamacopoeia) was obtained from Ohtsuka Pharmacy Co., Ltd.

Procedures The SHSGC method used was the same as described previously.[5] Briefly, sample solutions (10 ml) containing various alkanediol concentrations in the presence of a fixed α-CyD and 1-hexanol concentration were pipetted into 19.3 ml head-space vials and sealed using silicone septa and aluminum foil. The vials were thermostated at 25±0.1 °C and shaken for 24 h prior to analysis. After the equilibrium was established, 200 μl of 1-hexanol vapor from the top of the solution was drawn out of the vial by a gas-tight syringe. The sample was then analyzed with a gas chromatograph (GC, Shimadzu Co, GC-14B), equipped with a flame-ionization detector. A glass column 1 m × 3 mm i.d. with PEG-20M was used. The temperature of the detection was kept at 250 °C and the column temperature was 110 °C. Nitrogen was used as the carrier gas (flow rate, 30 ml/min). The peak area of 1-hexanol was measured by a Shimadzu Chromatopac C-RA integrator.

Results and Discussion

Theoretical Figure 1 shows the proposed mechanism of the competitive SHSGC method. The volatile guest (A) is expelled from the CyD cavity by the addition of the low volatile guest (B), resulting in an increment of the A concentration in the vapor phase and the free A concentration in liquid phase. In equilibrium, the two guests share the CyDs corresponding to their stability constants based on the assumption that no ternary complexes are formed, but only two binary complexes which do not interfere with each other.[2] If we assume that the equilibrium between the guests and CyD involves 1 : 1 complexes then, in principle, we can evaluate the stability constant for the low volatile guest–CyD complex from the competitive SHSGC data. In calculations, we are applying the equations proposed by Connors to determine the stability constant by competitive spectrophotometry.[7,8] Stability constants K_A and K_B for 1 : 1 complexes are defined by Eqs. 1 and 2,

$$K_A=\frac{[\text{A-CyD}]}{[\text{A}][\text{CyD}]} \tag{1}$$

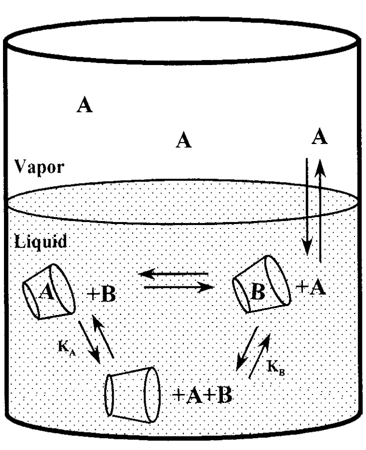

Fig. 1. Schematic Illustration of Competitive Model by Static Head-Space Gas Chromatography

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$$K_B=\frac{[\text{B-CyD}]}{[\text{B}][\text{CyD}]} \tag{2}$$

where [A], [B] and [CyD] denote the concentration of the free solutes, respectively. [A-CyD] and [B-CyD] denote the concentration of the complexes. The mass balance of A, B and CyD in aqueous solutions is represented by Eqs. 3—5:

$$[\text{A}]_t=[\text{A}]+[\text{A-CyD}] \tag{3}$$

$$[\text{B}]_t=[\text{B}]+[\text{B-CyD}] \tag{4}$$

$$[\text{CyD}]_t=[\text{CyD}]+[\text{A-CyD}]+[\text{B-CyD}] \tag{5}$$

where $[\text{A}]_t$, $[\text{B}]_t$ and $[\text{CyD}]_t$ are the total concentration of A, B and CyD, respectively. By substituting Eq. 1 into Eq. 3,

$$[\text{A-CyD}]=\frac{K_A\cdot[\text{CyD}]\cdot[\text{A}]_t}{1+K_A[\text{CyD}]} \tag{6}$$

The ratio $P=[\text{A}]/([\text{A}]_t-[\text{A}])$ is substituted into the definition of [CyD] using Eq. 1,

$$[\text{CyD}]=\frac{1}{K_AP} \tag{7}$$

which is used in Eq. 6 to give:

$$[\text{A-CyD}]=\frac{[\text{A}]_t}{P+1} \tag{8}$$

By substituting Eqs. 7 and 8 into Eq. 5, [B-CyD] can be rewritten as shown in Eq. 9:

$$[\text{B-CyD}]=[\text{CyD}]_t-\frac{1}{K_AP}-\frac{[\text{A}]_t}{P+1} \tag{9}$$

[B-CyD] can also be written by combining Eqs. 2, 4 and 7 as follows:

$$[\text{B-CyD}]=\frac{K_B\cdot[\text{CyD}]\cdot[\text{B}]_t}{1+K_B[\text{CyD}]}=\frac{K_B[\text{B}]_t}{K_AP+K_B} \tag{10}$$

Rearranging Eq.10 gives:

$$[\text{B}]_t=[\text{B-CyD}]\left(\frac{K_AP}{K_B}+1\right) \tag{11}$$

[A] and K_A in Eq. 11 are unknown for the determination of K_B. However, [A] can be obtained from the calibration curve in the absence of CyD based on Raoult's law as described in a previous paper.[5]

In the real solutions, Raoult's law is defined by Eq. 12:

$$\frac{P_A}{P_A^0}=\gamma_A^\infty X_A \tag{12}$$

where P_A and P_A^0 are the partial pressure of the volatile guest (A) over the solution and the vapor pressure of A in the pure state, respectively. X_A is the molar fraction of A in the solution and γ_A^∞ is the limiting activity coefficient. The ratio of P_A and P_A^0 is equal to that of the integrated GC peak areas (A_A/A_A^0), corresponding to the GC peaks from the head-space of A in the solution and of A in the pure state. K_A can also be determined in a separate experiment by means of direct SHSGC.[5] Therefore, the K_B for the low volatile guest can be estimated by nonlinear least-squares program (MULTI[9]) of $[\text{B}]_t$ *versus* [A] according to Eq. 11. Also, the stoichiometry of the low volatile guest–CyD complex is estimated by a continuous variation method as a function of [B-CyD] and molar fraction of B.

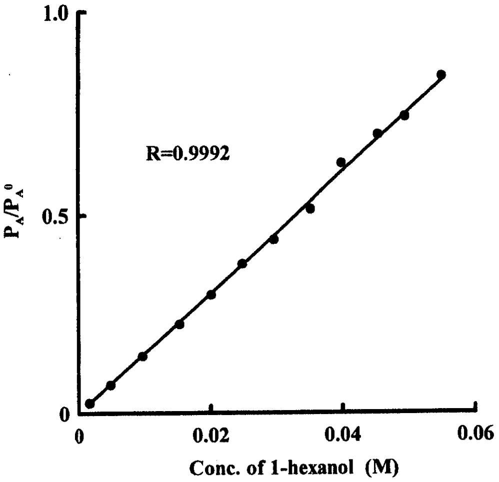

Fig. 2. Calibration Curve Based on Raoult's Law for 1-Hexanol Aqueous Solution

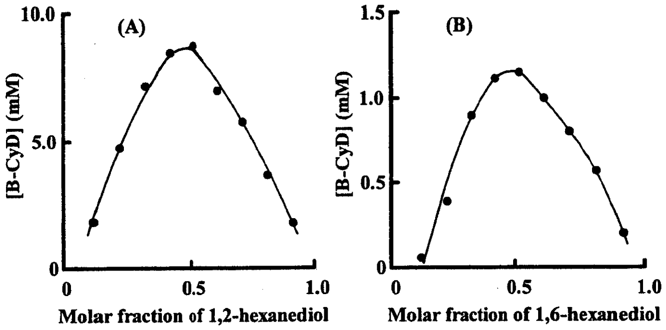

Fig. 3. Continuous Variation Plots for the 1,2-Hexanediol–α-CyD System (A) and 1,6-Hexanediol–α-CyD System (B)

Stoichiometry of Alkanediols In the present work, 1-hexanol was used as the volatile guest (A). Figure 2 shows the plot based on Raoult's law for the 1-hexanol aqueous solution; the plot gave a straight line in accordance with this law. These calibration curves were used to evaluate [A] in the presence of α-CyD and alkanediols. The continuous variation method has usually been applied to binary systems, however, it can also be applied to three-component systems.[10] The 1-hexanol was fixed at 10 mM and the total concentration of α-CyD and alkanediol (B) was kept constant at 40 mM for 1,2-hexanediol and 15 mM for 1,6-hexanediol, respectively. Under these conditions, the concentrations of the alkandiol–α-CyD complex ([B-CyD]) estimated from Eq. 9 were plotted as a function of the alkanediol molar fraction. K_A for the 1-hexanol–α-CyD complex in Eq. 9 used a value (701 M^{-1}) obtained by the direct SHSGC method.[5] Figure 3 shows the continuous variation plots for the 1,2-hexanediol–α-CyD and 1,6-hexanediol–α-CyD systems. Both plots gave the maximum values at hexanediols molar fraction of 0.5 indicating a 1 : 1 stoichiometry of the complexes. The same results were also obtained for the other alkanediols.

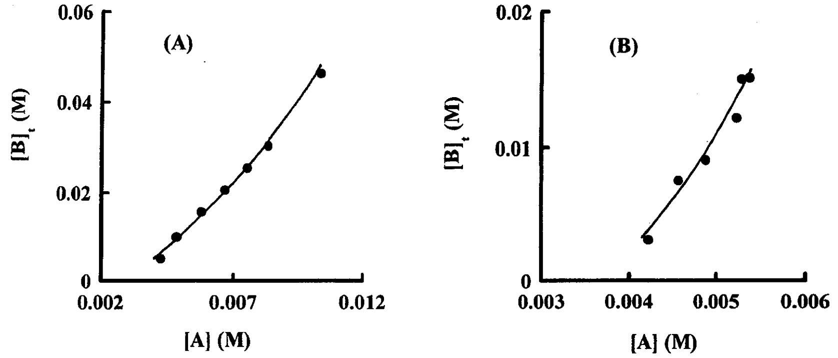

Fig. 4. Plots of $[B]_t$ *vs.* [A] for the 1,2-Hexanediol–α-CyD System (A) and 1,6-Hexanediol–α-CyD System (B)

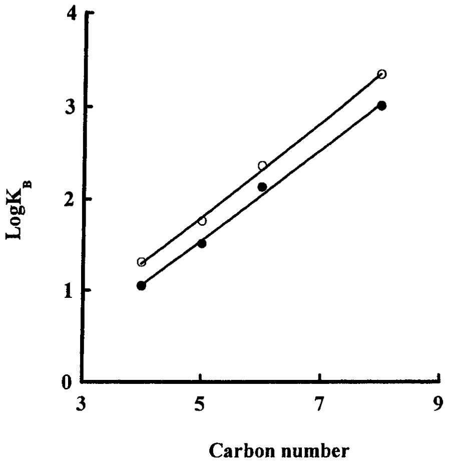

Fig. 5. Effects of Carbon Number on Stability Constants of Alkanediols–α-CyD Complexes

○, α,β-alkanediol; ●, α,ω-alkanediol.

Table 1. Comparison of Stability Constants of Alkanediols with α-Cyclodextrin Complexes

Alkanediol	This work	Calorimetry[a]
	K_B (M^{-1})	K_B (M^{-1})
1,2-Butanediol	20± 1	12.8± 0.4
1,4-Butanediol	11± 1	8 ± 1
1,2-Pentanediol	57± 3	78 ± 5
1,5-Pentanediol	32± 2	31 ± 1
1,2-Hexanediol	227± 5	185 ±43
1,6-Hexanediol	133± 5	94 ± 7
1,2-Octanediol	2113±129	—
1,8-Octanediol	999± 63	—

Each value is the mean and S.D. *a*) Literature values are from ref. 6.

Stability Constant Figure 4 shows the plots according to Eq. 11 for the 1,2-hexanediol–α-CyD and 1,6-hexanediol–α-CyD systems together with the calculated curve which gave the best fit of the experimental data. The concentrations of α-CyD were fixed at 30 mM for 1,2-hexanediol and at 15 mM for 1,6-hexanediol and the concentrations of 1-hexanol were kept at 24 mM for 1,2-hexanediol and at 15 mM for 1,6-hexanediol, respectively. The concentration ranges of alkanediols were (5—46) mM for 1,2-hexanediol and (3—15) mM for 1,6-hexanediol, respectively. Since the plots satisfied Eq. 11, the K_B values can be calculated. The similar nonlinear regressions were also obtained for the other alkanediols examined in this study. The precision of the stability constant is commonly derived from a single experiment consisting of a set of data points fitted to a model function by least-squares analysis.[1] The K_B values with S.D. for the alkanediol–α-CyD complexes were estimated as shown in Table 1. There was reasonable agreement between our results and those obtained by Andini *et al.*[6] in a micro calorimetric study. The values of K_B were plotted as $\log K_B$ against the total number of carbon atoms of the alkanediols in Fig. 5. The plots formed approximately straight lines, increasing monotonously with carbon atoms. Accordingly, this supports the idea that the cavity of α-CyD accommodates the hydrophobic moiety of alkanediols. It was also shown that the affinity of the α,β-alkanediols toward α-CyD is higher than that of α,ω-alkanediol.

In conclusion, the competitive SHSGC method compensates for the disadvantage of the direct SHSGC method, in that the former is applicable for determining the stability constant of low volatile guest–CyD systems. This method can determine the stability constant as rapidly and accurately as the direct SHSGC method. Since both direct and competitive SHSGC methods are applicable to guests which show no change in the spectra, it is expected that determination of the stability constants for almost all guest–CyD complexes will be feasible using these techniques.

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