A Modified Method Using Static Head-Space Gas Chromatography for Determining the Stability Constants of 1-Alkanol/α-Cyclodextrin Complexation

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A modification of the conventional static head-space gas chromatography method (SHSGC method) to determine stability constants for 1-alkanol/α-CD inclusion complexes was investigated. The 1 : 1 stability constants determined by this modified SHSGC method are in reasonable agreement with the corresponding values reported previously. The modified SHSGC method precludes the necessity of the calibration curve by the use of Henry's law constant of guest. Consequently, the modified SHSGC method is more advantageous than the conventional SHSGC method because the experimental time required for determination of the stability constant is markedly reduced.

Key words 1-alkanol; α-cyclodextrin; stability constant; static head-space gas chromatography; modification

The stability constant is of fundamental importance in understanding interactions in guest/cyclodextrin (CD) systems. The stability constant has been determined using spectroscopic methods such as absorption spectroscopy and fluorescence spectroscopy.[1)] It is difficult, however, to determine directly the stability constant by these spectroscopic methods if the guest has no chromophoric groups. Alkanols other than aromatic alkanol are the best known example of such a case. We previously proposed new methods using static head-space gas chromatography (SHSGC) for determining the stability constant of the CD complex, called the "direct SHSGC method,[2)]" the "indirect (competitive) SHSGC method,[3)]" the "solubility SHSGC method,[4)]" and the "simultaneous SHSGC method.[5,6)]" These methods have been successfully applied to the determination of the stability constants for the CD complexes including the alkanol/α-CD system. However, the conventional SHSGC methods require the calibration curve to evaluate the concentration of free guest in the presence of CD. In this study, the use of Henry's law constant of guest instead of using the calibration curve was adopted for evaluating the concentration of free guest. This must bring the shortening of experimental time required for the determination of the stability constant.

Herein, we report a modification of the direct SHSGC method (modified SHSGC method) for determination of the stability constants of 1-alkanol/α-CD inclusion complexes.

Experimental

Materials The α-CD used for the host was from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and was used after drying in a vacuum. 1-Alkanols used for the guest of reagent grade were purchased from Tokyo Kasei Kogyo Co., Ltd. and used without further purification. Water for injection (Japanese Phamacopoeia) was obtained from Ohtsuka Pharmacy Co., Ltd. (Tokyo).

Procedures The SHSGC technique used was the same as described previously.[2)] Briefly, sample solutions (15 ml) containing various concentrations of α-CD and a fixed concentration of 1-alkanol in water were pipetted into 32.6 ml head-space vials, and sealed using silicone septa and aluminum foils. The vials were then thermostated at 25±0.1 °C and shaken for 24 h prior to analysis. This time period was found to be sufficient for attaining equilibrium. After equilibrium was established, 1-alkanol vapor from above the solution was drawn out from the vial using a gas-tight syringe. This sample was then analyzed by gas chromatography (GC, Shimadzu Co., GC-14B), with a flame-ionization detector using a 1 m×3 mm i.d. glass column packed with PEG-20M. The analytical conditions of GC were as follows:

The column temperature: 80 °C for 1-butanol; 100 °C for 1-pentanol and 1-hexanol; and 105 °C for 1-heptanol and 1-octanol. The pressure of nitrogen gas used as the carrier gas: 40 kPa for 1-butanol, 1-pentanol, and 1-hexanol; and 60 kPa for 1-heptanol and 1-octanol. The injection and detection temperature: 250 °C.

Theoretical

Number of Moles of Complex The total number of moles of 1-alkanol (n_t^A) added to the vial in the presence of α-CD is represented by Eq. 1 from the mass balance.

$$n_t^A = n_g^A + n_l^A + n_{comp}^A \quad (1)$$

where n_g^A and n_l^A are the number of moles of 1-alkanol in the gas and aqueous phases, respectively. n_{comp}^A is the number of moles of 1-alkanol which has formed the complex in the aqueous phase. The n_g^A is determined by using the ideal gas equation.

$$n_g^A = \frac{P^A V_g}{RT} \quad (2)$$

where P^A is the partial pressure (Pa) of 1-alkanol over solution, V_g is the volume of gas phase (m^3) in vial, R is gas constant ($Pa\,m^3\,K^{-1}\,mol^{-1}$) and T is experimental temperature (K). The n_l^A is also obtained from Eq. 4 by the use of Henry's law (Eq. 3).

$$P^A = K_H^A C_l^A \quad (3)$$

$$n_l^A = C_l^A V_l = \frac{P^A V_l}{K_H^A} \quad (4)$$

where K_H^A is Henry's law constant ($Pa\,m^3\,mol^{-1}$) of 1-alkanol. We can easily obtain the K_H^A values for many organic compounds from the literatures.[7,8)] The C_l^A is the concentration of free 1-alkanol ($mol\,dm^{-3}$) in aqueous phase and V_l is the volume of aqueous phase (m^3) in vial. By substituting Eqs. 2 and 4 into Eq. 1 and rearranging,

$$n_{comp}^A = n_t^A - P^A\left(\frac{V_g}{RT} + \frac{V_l}{K_H^A}\right) \quad (5)$$

For determination of the n_{comp}^A, all parameters except P^A in Eq. 5 are known. Fortunately, P^A can be obtained by multiplying the activity (a) calculated in Eq. 6 with the vapor pressure of the pure 1-alkanol (P_0^A) as shown in Eq. 7.[4)]

$$a = \frac{A^A}{A_0^A} \quad (6)$$

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Table 1. Vapor Pressures and Henry's Law Constants of 1-Alkanols

1-Alkanols	Vapor pressure[a] (Pa)	Henry's law constant ($Pa \cdot m^3/mol$)
1-Butanol	903.9	0.83[b]
1-Pentanol	325.6	1.26[b]
1-Hexanol	122.1	1.72[c]
1-Heptanol	28.4	1.89[b]
1-Octanol	10.4	2.50[c]

a) Literature values are from ref. 9. *b*) Literature values are from ref. 10. *c*) Literature values are from ref. 11.

$$P^{A}=aP_0^{A} \tag{7}$$

A^{A} and A_0^{A} are the integrated GC peak areas obtained from the head-space of the 1-alkanol in the solution and its pure state, respectively. Therefore, the number of moles of complex can be determined from Eq. 5 by measuring the ratio of the GC peak area (A^{A}/A_0^{A}). Vapor pressures[9] and Henry's law constants[10,11] of used 1-alkanols are shown in Table 1.

Determination of Stability Constant If we assume that the equilibrium between 1-alkanol (A) and α-CD involves a 1 : 1 complex as shown in Eq. 8,

$$A+CD \overset{K}{\longleftrightarrow} A-CD \tag{8}$$

Stability constant (K) for 1 : 1 complex is defined by Eq. 9.

$$K=\frac{[A-CD]}{[A][CD]} \tag{9}$$

where $[A]$ and $[CD]$ denote the free concentrations of 1-alkanol and α-CD ($mol\,dm^{-3}$), respectively. $[A-CD]$ denotes the complex concentration ($mol\,dm^{-3}$). The mass balance of α-CD in aqueous solution is represented by Eq. 10,

$$[CD_t]=[CD]+[A-CD] \tag{10}$$

where $[CD_t]$ is the total concentration of α-CD ($mol\,dm^{-3}$). Combining Eq. 9 with Eq. 10 gives,

$$[A-CD](1+K[A])=K[A][CD_t] \tag{11}$$

Eq. 11 may be transformed into,

$$\frac{[CD_t]}{[A-CD]}=\frac{1}{K[A]}+1 \tag{12}$$

$[CD_t]$ is known and $[A-CD]$ can be experimentally obtained from Eq. 5. $[A]$ can also be determined from the mass balance of 1-alkanol by Eq. 13.

$$[A]=[A_t]-[A-CD] \tag{13}$$

where $[A_t]$ is the total concentration of 1-alkanol ($mol\,dm^{-3}$) and is known. Therefore, the stability constants (K) can be calculated from the slope ($1/K$) of the linear fit using Eq. 12.

Results and Discussion

Stability Constants of 1-Alkanol/α-CD Complexes Figure 1 shows the plots of $[CD_t]/[A-CD]$ *vs.* $1/[A]$ for each 1-alkanol/α-CD system. The plots gave good straight lines according to Eq. 12 for each 1-alkanol. The regression equations for each 1-alkanol are shown in Table 2. The slopes of these straight lines were used to estimate the stability constants for each 1-alkanol/α-CD system. The stability constants determined by this modified SHSGC method are summarized together with values from the literature as shown in Table 3. The K values determined in this study are in reasonable agreement with previously reported values[12,13] except for 1-octanol. Due to a lack of stability constant data for 1-octanol available in the literature, it is difficult at present to judge which is the more reliable. However, in comparison with the K values of other 1-alkanols it appears that the value obtained by spectrophotometry is too large.

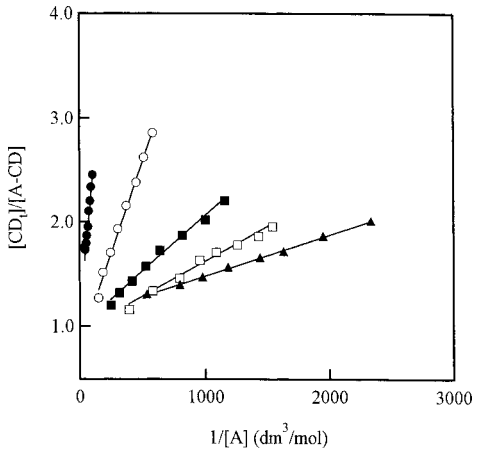

Fig. 1. Plots of $[CD_t]/[A-CD]$ *vs.* $1/[A]$ for 1-Alkanol/α-CD Systems
●, 1-butanol; ○, 1-pentanol; ■, 1-hexanol; □, 1-heptanol; ▲, 1-octanol.

Table 2. Characteristic Parameters Based on Eq. 12 for 1-Alkanol/α-CD Systems

1-Alkanols	Slope	Intercept	R^2
1-Butanol	1.35×10^{-2}	0.907	0.990
1-Pentanol	3.50×10^{-3}	0.826	0.995
1-Hexanol	1.07×10^{-3}	0.982	0.992
1-Heptanol	6.70×10^{-4}	0.933	0.985
1-Octanol	3.95×10^{-4}	1.084	0.999

Table 3. Comparison of the Stability Constants for 1-Alkanol/α-CD Complexes

1-Alkanols	This work (dm^3/mol)	Spectrophotometry[a] (dm^3/mol)	Surface tension[b] (kg/mol)
1-Butanol	74	89	93
1-Pentanol	286	324	378
1-Hexanol	935	891	698
1-Heptanol	1493	2291	1270
1-Octanol	2532	6310	3316

a) Literature values are from ref. 12. *b*) Literature values are from ref. 13.

Stoichiometry of 1-Alkanol/α-CD Complexes It is assumed that the stoichiometry of 1-alkanol/α-CD is 1 : 1, because the good linear relation according to Eq. 12 as shown in Fig. 1 was valid. However, we further confirmed it by the continuous variation method. The continuous variation method has usually been applied to determine the stoichiometry of the CD complex.[14,15] In the continuous variation plots, the difference in a physical parameter (*e.g.*, the difference in fluorescence intensity and the difference in chemical shift by NMR) related to the concentrations of complex is generally plotted as a function of mole fraction of guest. In our continuous variation plots, however, the concentrations of complex obtained from Eq. 5 themselves can be used. This is one of the unique features of this modified SHSGC method. Figure 2 shows the continuous variation plots for the 1-pentanol/α-CD and 1-heptanol/α-CD systems. The total concentration of 1-alkanol and α-CD was kept constant at $10\,mmol\,dm^{-3}$. Both plots gave maximum values at a mole fraction of 0.5 and highly symmetrical shapes, suggesting that 1 : 1 complexes are formed. The same results were also obtained for the other 1-alkanols.

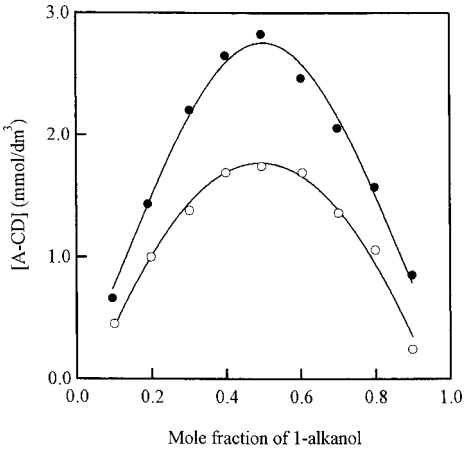

Fig. 2. Continuous Variation Plots for 1-Pentanol/α-CD System (○) and 1-Heptanol/α-CD System (●)

Conclusions

A study was made of the determination of the stability constants of 1-alkanol/α-CD systems by the use of Henry's law constants of 1-alkanols. In this study, we showed that the modified SHSGC method can determine the stability constants for 1-alkanol/α-CD systems accurately. The modified SHSGC method is more advantageous than the conventional SHSGC method because the experimental time required for the determination of the stability constant can be markedly shortened.

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