Stability constants for 1:1 complexes formed between 2-hydroxypropyl-β-cyclo-dextrin with an average substitution degree of 4.4 and benzene and alkylbenzenes as guests by modified static head-space gas chromatography method

KATSUMASA MISAWA[1], YOSHIHIRO SAITO[1,*], KANAME HASHIZAKI[1], HIROYUKI TAGUCHI[1], NAOTAKE OGAWA[1] and HARUHISA UEDA[2]

[1]*College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan;* [2]*Faculty of Pharmaceutical Science, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*

Abstract

Modified static head-space gas chromatography method (M-SHSGC method) was used to determine the stability constants of benzene and alkylbenzenes with 2-hydroxypropyl-β-cyclodextrin inclusion complexes. The 1:1 stability constants were in agreement with those obtained by conventional SHSGC method. The M-SHSGC method precludes the necessity of the calibration curve by the use of Henry's law constant of guest. Consequently, it is more advantageous than conventional SHSGC method because the time required to determine the stability constant is markedly reduced.

Introduction

The stability constant is of fundamental importance in understanding interactions in guest/cyclodextrin (CD) systems. It has been determined using physical methods including calorimetry [1], adsorption spectroscopy [2], fluorescence spectroscopy [3], and NMR [4]. Each method has its advantages and disadvantages as well as limitations. We previously presented static head-space gas chromatography (SHSGC) method for determining the stability constant of an inclusion complex of a volatile guest with CD [5, 6]. This method was successfully applied for the determination of the stability constant for the CD complex system. SHSGC method is based on the principle that only the free guest is in an equilibrium between gas and liquid phases, whereas the complexed component is non-volatile. Therefore, the guest concentration in the head-space corresponds to that of the free guest in the liquid phase. In this manner, the free concentration of guest in the liquid phase can be directly estimated. However, conventional SHSGC method requires a calibration curve to evaluate the concentration of free guest in the presence of CD. Here, we report on a modification of SHSGC method (M-SHSGC method) for determining the stability constants of benzene and alkylbenzenes with 2-hydroxypropyl-β-cyclodextrin (2HP-β-CD) inclusion complexes. In this study, we used Henry's law constant for the guest

instead of a calibration curve to evaluate the concentration of free guest. This resulted in a reduction in the time required to determine the stability constant.

Experimental

Materials

The 2HP-β-CD (average substitution degree: 4.4) used for the host was from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and was used after drying in a vacuum. Analytical grade reagent benzene and alkylbenzenes used for the guest were purchased from Waco Pure Chemical Industries, Ltd. (Osaka, Japan) and used without further purification. Water for injection (Japanese Phamacopoeia) was obtained from Ohtsuka Pharmacy Co., Ltd. (Tokyo, Japan).

Procedures

The SHSGC technique used was the same as previously described [5]. Briefly, sample solutions (15 ml) containing various concentrations of 2HP-β-CD together with a fixed concentration of aromatic hydrocarbon in water or various concentrations of aromatic hydrocarbon together with a fixed concentration of 2HP-β-CD in water were pipetted into 32.6-ml head-space vials and sealed using silicone septa and aluminum foil. The vials were then thermostated at 25 ± 0.1 °C and shaken for

*Author for correspondence. E-mail: ysaito@pha.nihon-u.ac.jp

24 h prior to analysis. This time period was sufficient for attaining equilibrium. After equilibrium was established, aromatic hydrocarbon vapor above the solution was withdrawn using a gas-tight syringe. The gas 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 for GC were as follows:

The column temperature was 90 °C for benzene and toluene, and 100 °C for xylenes. Nitrogen was used as the carrier gas at a pressure of 30 kPa for benzene and toluene and 40 kPa for xylenes. The injection and detection temperature was 250 °C.

Theoretical

Number of moles of complex

Figure 1 shows the mechanism employed in M-SHSGC method. Two equilibria exist for the aromatic hydrocarbon (H)/2HP-β-CD system in the head-space of the vial: (1) Each aromatic hydrocarbon in 2HP-β-CD is in equilibrium with each free aromatic hydrocarbon in the liquid phase, and (2) each free aromatic hydrocarbon in the liquid phase is in equilibrium with each monomeric hydrocarbon in the gas phase. The relationships between these equilibria are the underlying principles of M-SHSGC method. The number of moles of aromatic hydrocarbon in 2HP-β-CD in the liquid phase, n_{comp}^H, is represented by Equation (1) which was derived from a mass balance:

$$n_{comp}^H = n_t^H - n_g^H - n_l^H \qquad (1)$$

Here, n_t^H is the total number of moles of aromatic hydrocarbon added to the vial. n_g^H and n_l^H are the number of moles of aromatic hydrocarbon in the gas and liquid phases, respectively. n_g^H is determined using the ideal gas equation:

$$n_g^H = \frac{P^H V_g}{RT} \qquad (2)$$

Here, P^H is the partial pressure (Pa) of aromatic hydrocarbon above the solution, V_g is the gas volume (m^3), R is gas constant (Pa m^3 K^{-1} mol^{-1}) and T is the experimental temperature (K). n_l^H can also obtained from Equation (4) through Henry's law (Equation (3)):

$$P^H = K_H^H C_l^H \qquad (3)$$

$$n_l^H = \frac{P^H V_l}{K_H^H} \qquad (4)$$

Here, K_H^H is the Henry's law constant (Pa m^3 mol^{-1}) for the aromatic hydrocarbon. The Henry's law constant for many organic compounds can be obtained from the literature [7, 8]. C_l^H is the concentration of free aromatic hydrocarbon (mol m^{-3}) in the liquid phase and V_l is the volume of the liquid phase (m^3). By substituting Equations (2) and (4) into Equation (1) and rearranging, the following relationship is derived:

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

All parameters except P^H in Equation (5) are known for the determination of n_{comp}^H. The peak area obtained by GC of the aromatic hydrocarbon in the sample taken from the head-space is proportional to the concentration of the aromatic hydrocarbon in the gas phase (its vapor pressure). As a result, the equation $A^H/A_0^H = P^H/P_0^H$ holds true, where A^H and A_0^H are the integrated GC peak areas obtained from the head-space of the aromatic hydrocarbon in solution and in its pure state, respectively, and P_0^H is the vapor pressure of the aromatic hydrocarbon in its pure state. Therefore, the number of moles of aromatic hydrocarbon in 2HP-β-CD can be determined from Equation (5) by measuring the ratio of the GC peak areas (A^H/A_0^H). The vapor pressures [9] and
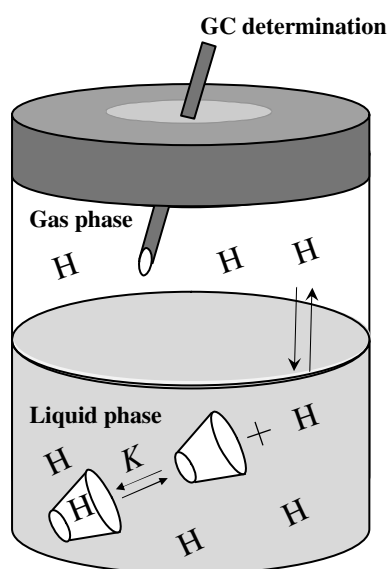

Figure 1. Schematic illustration of the M-SHSGC method.

Table 1. Vapor pressures and Henry's law constants for benzene and alkylbenzenes at 25 °C

|	Vapour Pressure [a] (Pa)	Henry's law constant [b] (Pa·m^3 mol^{-1})
Benzene	12573.9	554
Toluene	3764.5	632
o-Xylene	876.2	500
m-Xylene	1116.8	721
p-Xylene	1176.1	710

[a] Literature values are from ref. [9].
[b] Literature values are from ref. [7].

Henry's law constants [7] of the aromatic hydrocarbon used in this study are shown in Table 1.

Determination of the stability constant

If we assume that the equilibrium between the aromatic hydrocarbon (H) and 2HP-β-CD (CD) involves a 1:1 complex as shown in Equation (6),

$$H + CD \xleftrightarrow{K} H\!-\!CD \qquad (6)$$
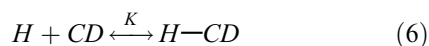
then the stability constant (K) is defined by Equation (7):

$$K = \frac{[H\!-\!CD]}{[H][CD]} \qquad (7)$$

Here, $[H]$ and $[CD]$ denote the free concentrations of aromatic hydrocarbon and 2HP-β-CD (mol dm^{-3}), respectively, and $[H\!-\!CD]$ denotes the complex concentration (mol dm^{-3}). The mass balance equations for this system are:

$$[CD_t] = [CD] + [H\!-\!CD] \qquad (8)$$

$$[H_t] = [H] + [H\!-\!CD] \qquad (9)$$

Here, $[CD_t]$ and $[H_t]$ are the total concentrations of 2HP-β-CD and aromatic hydrocarbon (mol dm^{-3}), respectively. Combining Equation (7) with Equation (8) gives:

$$\frac{[CD_t]}{[H\!-\!CD]} = \frac{1}{K[H]} + 1 \qquad (10)$$

$[CD_t]$ is known and $[H\!-\!CD]$ can be experimentally obtained from Equation (5). $[H]$ can also be determined from the mass balance of aromatic hydrocarbon using Equation (9).

Therefore, the stability constants (K) can be calculated from the slope ($1/K$) of a linear fit using Equation (10).

Results and discussion

Stability constants of aromatic hydrocarbon/2HP-β-CD complexes

Figures 2 and 3 show the plots of $[CD_t]/[H\!-\!CD]$ vs. $1/[H]$ for each aromatic hydrocarbon/2HP-β-CD system
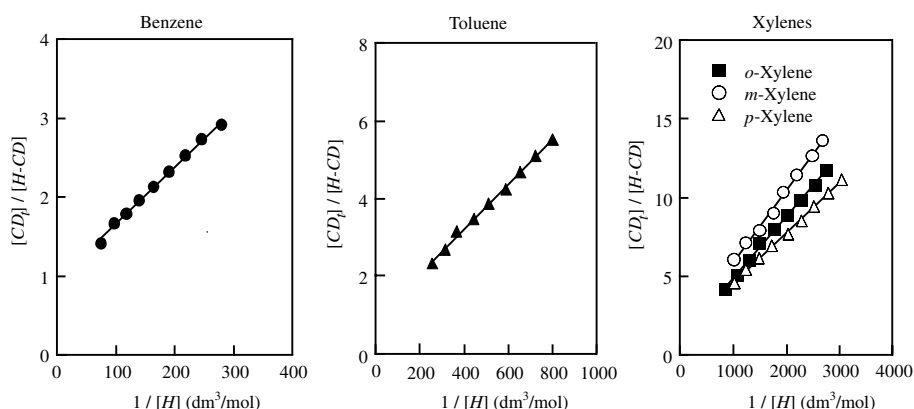

Figure 2. Plots of $[CD_t]/[H\!-\!CD]$ vs. $1/[H]$ for aromatic hydrocarbon/2HP-β-CD systems at a fixed guest concentration. Guest concentrations are 20 mmol dm^{-3} for benzene, 6 mmol dm^{-3} for toluene, 2 mmol dm^{-3} for *o*-xylene, and 1.6 mmol dm^{-3} for *m*-xylene and *p*-xylene.
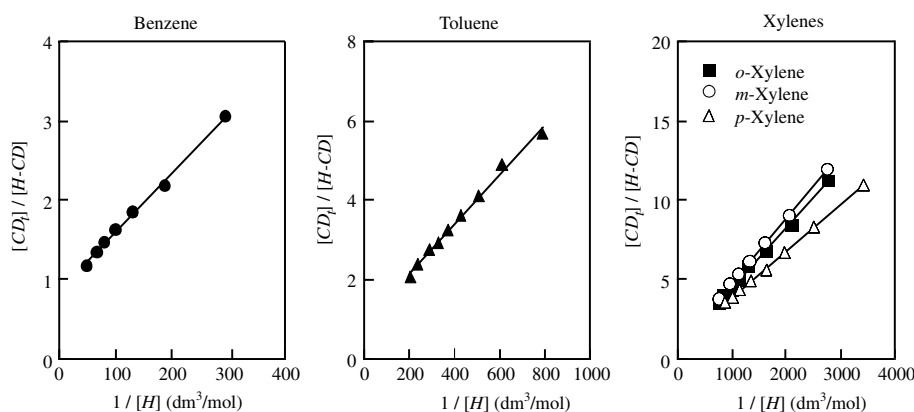

Figure 3. Plots of $[CD_t]/[H\!-\!CD]$ vs. $1/[H]$ for aromatic hydrocarbon/2HP-β-CD systems at a fixed 2HP-β-CD concentration. 2HP-β-CD concentration is 10 mmol dm^{-3}.

at fixed aromatic hydrocarbon and CD concentrations, respectively. The plots yielded straight lines according to Equation (10) for each aromatic hydrocarbon. The slopes of these lines were used to estimate the stability constants for each aromatic hydrocarbon/2HP-β-CD system by M-SHSGC method, and are summarized with the values obtained by conventional SHSGC method in Table 2. The K-values determined by M-SHSGC method are in agreement with the values obtained by conventional SHSGC method. In general, the stability constant can be determined using the difference in averaged physical properties of guest (e.g. the difference in fluorescence intensity and the difference in chemical shift seen using NMR) when adding CD at a fixed guest concentration [3, 4]. On the other hand, M-SHSGC method can be applied for a fixed guest and CD concentration as shown in Figures 2 and 3. When the guest or CD is limiting, M-SHSGC method is useful because the limiting species can be conserved by choosing the experimental conditions.

Stoichiometry of aromatic hydrocarbon/2hp-β-cd complexes

We assumed that the stoichiometry of the aromatic hydrocarbon/2HP-β-CD was 1:1 because a good linear fit according to Equation (10) was obtained as shown in Figures 2 and 3. However, we further confirmed this using the continuous variation method. This method is usually used to determine the stoichiometry of the CD complex [10]. In continuous variation plots, the difference in a physical parameter in relation to the complex concentration is plotted as a function of the mole fraction of guest. For our continuous variation plots produced using M-SHSGC method, however, the complex concentrations obtained from Equation (5) were used. This is a unique feature of M-SHSGC method. Figure 4 shows continuous variation plots for the aromatic hydrocarbon/2HP-β-CD systems. The total concentration of aromatic hydrocarbon and 2HP-β-CD was kept constant at 1.5 mmol dm^{-3}. The plots gave maximum values at a mole fraction of 0.5 and had symmetrical shapes, suggesting that 1:1 complexes formed.
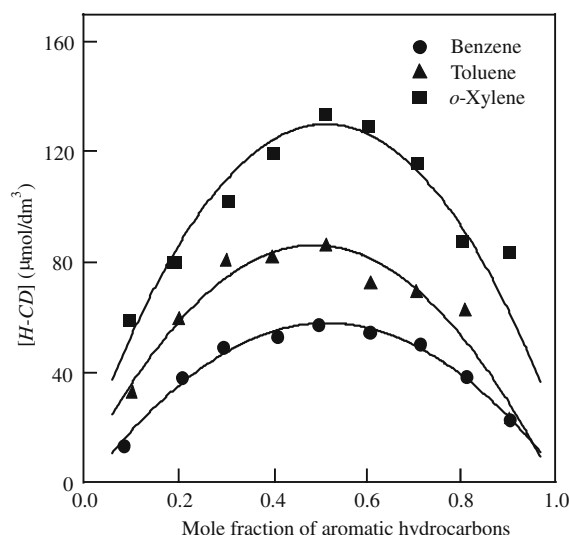

Figure 4. Continuous variation plots for aromatic hydrocarbon/2HP-β-CD systems. Total concentration of guest and 2HP-β-CD is 1.5 mmol dm^{-3}.

Conclusions

The stability constants of aromatic hydrocarbon/2HP-β-CD systems were determined using Henry's law constants for the aromatic hydrocarbon. M-SHSGC method can be used to accurately determine the stability constants for aromatic hydrocarbon /2HP-β-CD systems. This method is advantageous compared to conventional SHSGC method because the time required to determine the stability constant can be markedly shortened by up to a factor of 1/2 of that required by conventional SHSGC method.

Acknowledgements

Table 2. Comparison of the stability constants of benzene and alkylbenzenes with 2HP-β-CD complexes

	M-SHSGC (a fixed guest concn.) (dm^3 mol^{-1})	M-SHSGC (a fixed CD concn.) (dm^3 mol^{-1})	SHSGC (kg mol^{-1})
Benzene	139	135	142
Toluene	182	159	156
o-Xylene	263	263	289
m-Xylene	222	244	238
p-Xylene	323	345	376

References

1. M. Bastos, L.-E. Briggner, I. Shehatta, and I. Wadso: *J. Chem. Thermodyn.* **22**, 1181 (1990).
2. S. Hamai: *Bull. Chem. Soc. Jpn.* **73**, 861 (2000).
3. B.D. Wagner and S.J. Fitzpatrick: *J. Incl. Phenom.* **38**, 467 (2000).
4. L. Caron, S. Tilloy, E. Monflier, J.-M. Wieruszeski, G. Lippens, D. Landy, S. Fourmentin, and G. Surpateanu: *J. Incl. Phenom.* **38**, 361 (2000).
5. Y. Saito, K. Yoshihara, I. Tanemura, H. Ueda, and T. Sato: *Chem. Pharm. Bull.* **45**, 1711 (1997).
6. Y. Saito, I. Tanemura, H. Ueda, and T. Sato: *Chem. Pharm. Bull.* **46**, 1177 (1998).
7. D. Mackay and W.Y. Shiu: *J. Phys. Chem. Ref. Data* **10**, 1175 (1981).
8. J. Hine and P.K. Mookerjee: *J. Org. Chem.* **40**, 292 (1975).
9. C.L. Yaws: Handbook of Vapor Pressure, Gulf, Houston (1994).
10. K. Hirose: *J. Incl. Phenom.* **39**, 193 (2001).