Chem. Pharm. Bull. 45(10) 1711—1713 (1997)

Determination of the Stability Constants of Benzene and Alkylbenzenes with α-Cyclodextrin by Static Head-Space Gas Chromatography

Yoshihiro SAITO,*[,a] Kazunobu YOSHIHARA,[a] Izumi TANEMURA,[a] Haruhisa UEDA,[b] and Takatoshi SATO[a]

College of Pharmacy, Nihon University,[a] 7–7–1 Narashinodai, Funabashi-shi, Chiba 274, Japan, and Faculty of Pharmaceutical Science, Hoshi University,[b] 2–4–41 Ebara, Shinagawa-ku, Tokyo 142, Japan.

Received April 23, 1997; accepted June 16, 1997

The stability constants for the inclusion of benzene and alkylbenzenes with α-cyclodextrin (α-CyD) in aqueous solution have been determined using static head-space gas chromatography (SHSGC). The 1 : 1 and 2 : 1 (host : guest) stability constants obtained by this method were in reasonable agreement with the corresponding values in the literature. Therefore, it was concluded that the SHSGC method is useful for determining the stability constant of the volatile compound/CyD complex.

Key words benzene; alkylbenzene; α-cyclodextrin; stability constant; static head-space gas chromatography

The stability constant is of fundamental importance in understanding interactions in guest/cyclodextrin (CyD) systems. Several methods such as solubility measurement, NMR spectrometry and absorption spectroscopy have been employed to determine the stability constant for guest/CyD complexation.[1,2] However, these methods have both advantages and disadvantages. For instance, the solubility method has some essential problems in that a constant temperature cannot be maintained during the separation of the excess guest phase; furthermore, CyD molecules are adsorbed on the surface of the guest phase.[3] Also, the spectroscopic method is not applicable for complex formation involving only small spectral changes.[4] The static head-space gas chromatography (SHSGC) method has been used for determining the solubilization equilibrium constants of organic compounds between bulk and micelle phases.[5,6] The method has also been used for determining the stability constants for the CyD complex system.[7,8] However, to date there is no detailed report on the usefulness and accuracy of the method.

In the present study, we examined the applicability of the SHSGC method for determining the stability constants of benzene and alkylbenzenes with α-CyD. The reason for choosing these hydrocarbons is that they are known to complex with α-CyD and their concentrations in gas phase can be measured conveniently using gas chromatography.

Experimental

Materials The α-CyD used for the host was generously donated by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and was used after drying in a vacuum. The benzene and alkylbenzenes used for the guest were obtained from Waco Pure Chemical Industries, Ltd. (Osaka, Japan) and were used without further purification. Distilled water per injection JP (Japanese Phamacopoeia) was obtained from Ohtsuka Pharmacy Co., Ltd. (Tokyo).

Procedures Sample solutions (10 ml) containing various hydrocarbon concentrations together with a fixed α-CyD concentration (10 mM) in water were pipetted into 19.3 ml head-space vials and sealed using silicone septa and aluminum foil. The foil was placed between the silicone septum and the vial's contents to preclude absorption of hydrocarbons by the silicone septa. 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 had been established, 200 μl of hydrocarbon vapor from above the solution was drawn out from the vial using a gas-tight syringe. This sample was then analyzed with a gas chromatograph (GC, Shimadzu Co, GC-14B) equipped with a flame-ionization detector using a 1m × 3 mm i.d. glass column packed with PEG-20M. The injection and detection temperatures were maintained at 250 °C, and the column temperature was 110 °C. Nitrogen gas was used as the carrier gas, and the flow rate was kept at 30 ml/min. The area of each peak was measured by a Shimadzu Chromatopac C-R 6A integrator.

Results and Discussion

Theoretical Raoult's law and Henry's law are commonly used for ideal solution systems. Conventionally, Raoult's law is applied to a bulk solvent, and Henry's law to a solute, however, either can be used according to the standard state chosen.[9] In the real solutions, Raoult's law is defined by Eq. 1,

$$\frac{P_{\mathrm{B}}}{P_{\mathrm{B}}^{0}}=\gamma_{\mathrm{B}}^{\infty}X_{\mathrm{B}} \tag{1}$$

where P_{B} and P_{B}^{0} are the partial pressure of hydrocarbon (B) over the solution and the vapor pressure of B in the pure state, respectively. X_{B} is the molar fraction of B in the solution and $\gamma_{\mathrm{B}}^{\infty}$ is the limiting activity coefficient (extrapolated for the maximum dilution). The ratio of P_{B} and P_{B}^{0} is equal to that of the integrated GC peak areas ($A_{\mathrm{B}}/A_{\mathrm{B}}^{0}$), corresponding to the GC peaks obtained from the head-space of B in the pure state and of its solutions.

As for the benzene and alkylbenzene/CyD systems, the possible host : guest stoichiometric ratios are 1 : 1 and 2 : 1, as shown in Eq. 2.[10]

$$\mathrm{B}\underset{K_1}{\overset{\mathrm{CyD}}{\rightleftarrows}}\mathrm{B-CyD}\underset{K_2}{\overset{\mathrm{CyD}}{\rightleftarrows}}\mathrm{B-CyD_2} \tag{2}$$

Stability constants K_1 and K_2 for 1 : 1 and 2 : 1 complexes, respectively, are defined by Eqs. 3 and 4,

$$K_1=\frac{[\mathrm{B-CyD}]}{[\mathrm{B}][\mathrm{CyD}]} \tag{3}$$

$$K_2=\frac{[\mathrm{B-CyD_2}]}{[\mathrm{B-CyD}][\mathrm{CyD}]} \tag{4}$$

where [B] and [CyD] donate the concentrations of the free solutes, respectively. [B − CyD] and [B − CyD$_2$] don-

---

* To whom correspondence should be addressed.

ate the concentrations of the complexes. The mass balance of B and CyD in an aqueous solution is represented by Eqs. 5 and 6,

$$[\mathrm{B}]_t=[\mathrm{B}]+[\mathrm{B-CyD}]+[\mathrm{B-CyD_2}] \tag{5}$$

$$[\mathrm{CyD}]_t=[\mathrm{CyD}]+[\mathrm{B-CyD}]+2[\mathrm{B-CyD_2}] \tag{6}$$

where $[\mathrm{B}]_t$ and $[\mathrm{CyD}]_t$ are the total concentrations of CyD and B, respectively. Substituting Eqs. 3 and 4 into Eq. 5,

$$[\mathrm{B}]_t=[\mathrm{B}]+K_1[\mathrm{B}][\mathrm{CyD}]+K_1K_2[\mathrm{B}][\mathrm{CyD}]^2 \tag{7}$$

[CyD] can be deduced from Eq. 6 following the substitution of Eqs. 3 and 4, and rearrangement,

$$[\mathrm{CyD}]=\frac{-K_1[\mathrm{B}]-1+\sqrt{(K_1[\mathrm{B}]+1)^2+8K_1K_2[\mathrm{B}][\mathrm{CyD}]_t}}{4K_1K_2[\mathrm{B}]} \tag{8}$$

$[\mathrm{CyD}]_t$ and $[\mathrm{B}]_t$ are the known variables and [B] can be obtained experimentally from the calibration curve in the absence of CyD based on Eq. 1. Therefore, the stability constants K_1 and K_2 can be estimated by nonlinear least-squares program (MULTI) of [B] *versus* $[\mathrm{B}]_t$ using Eq.7.

Calibration Curve Figure 1 shows the plots based on Eq. 1 for the *p*-xylene aqueous solution as an example. The plot gave a straight line in accordance with Raoult's law. The same linear relationships have also been obtained for the other guests. These calibration curves were used to evaluate the [B] in the presence of α-CyD.

Stability Constant Figure 2 shows a typical plot of the [B] *vs.* $[\mathrm{B}]_t$ for *p*-xylene together with the calculated curve which gives the best fit of the experimental data. 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 stability constants determined by the SHSGC method are summarized together with values from the literature in Table 1. There are few reports concerning stability constants of benzene and alkylbenzenes with α-CyD, however, a report by Sanemasa and Akamine[10)] appeared using the volatilization rate technique which takes advantage of the volatility of the guest. The results of our study are in reasonable agreement with those of Sanemasa and Akamine. As for benzene and toluene systems, however, the precision of the stability constants obtained was not good. This may be because the injection of a given vapor sample into a gas chromatograph is difficult due to its high volatility.

The SHSGC method has already been used to determine the stability constant for the CyD complex system by Ito *et al.*[7)] and Hall *et al.*[8)] However, the usefulness and precision of this technique were not examined in detail in these papers. In this work, we showed that the SHSGC method can be used as an unique tool for determining the stability constant for guest/CyD complexation. The distinctive characteristics of this method are as follows:

1) It can be used to rapidly and accurately determine the stability constant for the volatile guest.
2) It is applicable even to guest which shows no change in the spectra.
3) The thermodynamic parameter for formation of complexes can be determined fairly accurately because the temperature of the sample solution is kept constant throughout the experimental process.

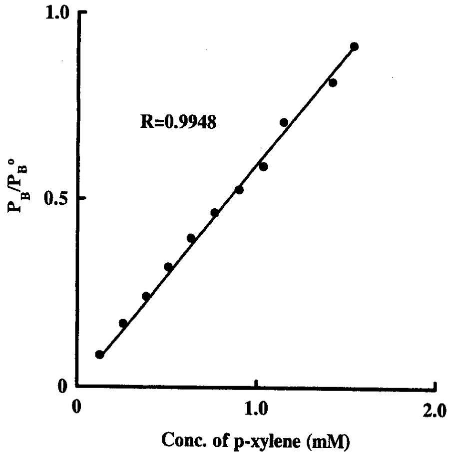

Fig. 1. Calibration Curve Based on Eq. 1 for *p*-Xylene Aqueous Solution at 25 °C

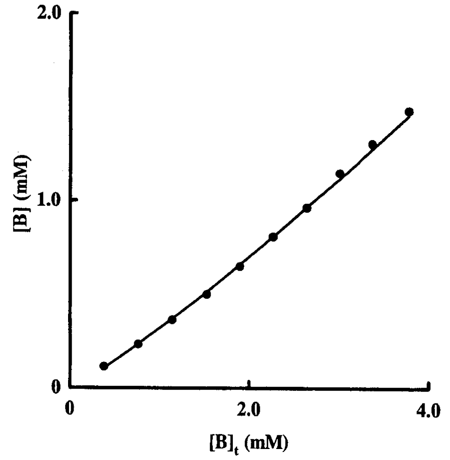

Fig. 2. Plots of [B] *vs.* $[\mathrm{B}]_t$ for *p*-Xylene Aqueous Solutions in the Presence of 10 mM α-CyD at 25 °C

●, observed; solid line, calculated curve from Eq. 7.

Table 1. Comparison of Stability Constants of Benzene and Alkylbenzenes with α-Cyclodextrin Complexes

Guest substance	K_1 (M^{-1})	K_2 (M^{-1})
Benzene	19±1 (17±2)	16±13 (17±3)
Toluene	36±4 (33±3)	41±18 (11±5)
o-Xylene	18±1 (22±8)	—
m-Xylene	60±5 (40±1)	39±13 (37±5)
p-Xylene	124±4 (72±7)	101±7 (140±40)

a) Literature values given in the parentheses are from ref. 10. *b)* Each value is the mean and S.D.

However, the SHSGC method has a disadvantage as do other methods, in that, as pointed out by Sanemasa and Akamine,[10)] its application is difficult for a low volatile guest. This problem can be solved, however, using the new competitive SHSGC method we have established. The value of this competitive technique will be dealt with in a following paper.

References

1) Szejtli J., Osa T., "Comprehensive Supramolecular Chemistry," Vol. 3 (Cyclodextrins), Pergamon, U.K., 1996, pp. 211—234.

2) Szejtli J., "Cyclodextrin Technology," Kluwer Academic Publishers, Netherlands, 1988, pp. 143—154.
3) Takuma T., Deguchi T., Sanemasa I., *Bull. Chem. Soc. Jpn.*, **64**, 480—484 (1991).
4) Suzuki M., Ueda S., Kusai A., *Chem. Pharm. Bull.*, **36**, 720—725 (1988).
5) Hayase K., Hayano S., *Bull. Chem. Soc. Jpn.*, **50**, 83—85 (1977).
6) Morgan M. E., Uchiyama H., Christian S. D., Tucker E. E., Scamehorn J. F., *Langmuir*, **10**, 2170—2176 (1994).
7) Ito K., Kikuchi K., Okazaki N., Kobayashi S., *Agric. Biol. Chem.*, **52**, 2763—2769 (1988).
8) Hall D., Bloor D., Tawarah K., Wyn-Jones E., *J. Chem. Soc., Faraday Trans., 1*, **82**, 2111—2121 (1986).
9) Behan J. M., Perring K. D., *Int. J. Cosmetic Sci.*, **9**, 261—268 (1987).
10) Sanemasa I., Akamine Y., *Bull. Chem. Soc. Jpn.*, **60**, 2059—2066 (1987).