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Solubility Method Using Static Head-Space Gas Chromatography for Determination of the Stability Constants of Fragrance Materials with 2-Hydroxypropyl-β-cyclodextrin

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The stability constants for the inclusion of fragrance materials with 2-hydroxypropyl-β-cyclodextrin (2HP-β-CyD) in aqueous solution have been determined at 25 °C by a solubility method. The solubility of fragrance in 2HP-β-CyD solution was determined from the concentration of fragrance in vapor which was in equilibrium with that in the solution using static head-space gas chromatography (SHSGC). The stability constants of (+)-limonene and linalool obtained by this method were in rough accord with the reported values. Because of its advantages of simplicity, less amount of guest used and the ease of temperature control, it was concluded that the SHSGC method is another useful technique for determining the solubility and stability constant of a volatile guest/CyD system in aqueous medium.

Key words fragrance material; 2-hydroxypropyl-β-cyclodextrin; static head-space gas chromatography; solubility; stability constant

The solubility method comprising a phase solubility diagram has frequently been used to study the interaction between a guest and a cyclodextrin (CyD), because this technique offers information on the stability constant and complex stoichiometry.[1,2] The solubility method makes use of an increase in the guest solubility in water as a result of complex formation by a CyD. The experiment requires determination of the apparent solubility of the guest as a function of the total concentration of the CyD. We recently used static head-space gas chromatography (SHSGC) to determine the stability constant of CyD complexes, and reported that such determination for almost all guest/CyD complexes is feasible using direct and indirect (competitive) SHSGC methods.[3,4] These SHSGC methods are simple and precise; an important characteristic is that the temperature of the sample solution is kept constant throughout the experimental process.

In this paper, we report on a solubility method using this technique to determine the stability constants in fragrance materials/2-hydroxypropyl-β-cyclodextrin(2HP-β-CyD) systems.

Experimental

Materials The 2HP-β-CyD (average substitution degree: 4.4) used for the host was generously donated by Nihon Shokuhin Kako Co., (Tokyo, Japan) and was used after vacuum drying. (−)-α-pinene, (−)-β-pinene, myrcene, (+)-limonene and linalool were used for the guests. These were obtained from Aldrich Chemical Company Inc. (WI, U.S.A.) and were used without further purification. These fragrance materials are the main constituents in orange oil. Distilled water (pH 6.3) per injection JP (Japanese Pharmacopoeia) was obtained from Ohtsuka Pharmacy Co., Ltd. (Tokyo).

Procedures The SHSGC technique used was described previously.[3,4] Briefly, sample solutions (10 ml) containing various concentrations of fragrance materials and 2HP-β-CyD in water were pipetted into 19.5 ml head-space vials and sealed using silicone septa and aluminum foil. The vials were then thermostatted at 25±0.1 °C and shaken for 24 h prior to analysis. After equilibrium was established, 200 μl of fragrance 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 similar to those previously described.[3,4]

Results and Discussion

Activity (a_F) of the fragrance material (F) in the solution is defined by Eq. 1,[5]

$$a_F = \frac{A_F}{A_F^0} \tag{1}$$

where A_F and A_F^0 are the integrated GC peak areas obtained from the head-space of the fragrance material in the solution and its pure state, respectively. The partial vapor pressure of the fragrance material (P_F) is then obtained by multiplying the activity calculated in Eq. 1 with the vapor pressure of the pure fragrance (P_F^0) at 25 °C.

$$P_F = a_F \cdot P_F^0 \tag{2}$$

Figure 1 shows the experimental curves of the relative peak area (A_F/A_F^0) as a function of linalool fed in the presence and absence of 2HP-β-CyD as an example. When the activity of linalool in solution reached about 1, the relative peak area did not change any further despite the increasing amounts of linalool fed into the system. This phenomenon has been used to determine the solubility of a substance with significant volatility, that is, the point of attaining a plateau on the curve can be looked upon as the solubility of solute in solution.[6] In general, it is thought that the amount of substance in the vapor over the solution will be extremely small. However, this amount should not be overlooked for high volatile substances such as the fragrance materials used in this study, because the actual quantity in the solution after equilibrium differs widely from the quantity added initially. We calculated the number of moles of fragrance material in the vapor from the partial vapor pressure of the fragrance calculated in Eq. 2 and the ideal gas equation, and then determined the actual fragrance concentration in the solution by subtracting the number of moles in the vapor from the total amount of fragrance added to the

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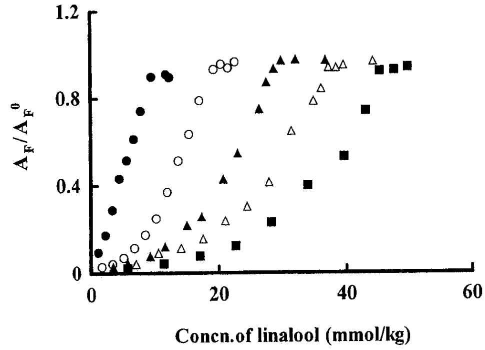


Fig. 1. Relative Peak Area as a Function of the Amount of Linalool Fed

2HP-β-CyD concentration (mmol/kg): ●, 0; ○, 10; ▲, 20; △, 30; ■, 40.

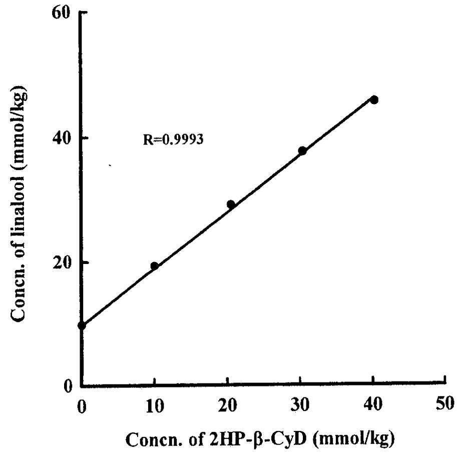


Fig. 2. Phase Solubility Diagram of Linalool as a Function of 2HP-β-CyD Concentrations

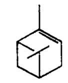
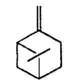
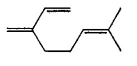
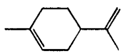
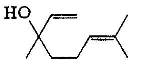
vial.$^{5)}$ These corrected fragrance concentrations in the solution are used as the values for the x axis in Fig. 1.

The phase solubility diagram of linalool as a function of the 2HP-β-CyD concentration is given in Fig. 2. The solubility of linalool increased linearly as the concentration of 2HP-β-CyD increased, resulting in an A_L type phase solubility curve according to Higuchi and Connors.$^{7)}$ An apparent stability constant (K, $\mathrm{kg\,mol^{-1}}$) was then calculated in terms of Eq. 3 using the slope and intercept of the linear line of the phase solubility diagram assuming that a 1 : 1 complex is formed.

$$K=\frac{\text{slope}}{\text{intercept}(1-\text{slope})} \tag{3}$$

Similar linear regressions were also obtained for the other fragrance materials/2HP-β-CyD systems examined in this study and their K values were calculated by the same method. The apparent stability constants determined by this method are summarized together with values from the literature in Table 1. The results for (+)-limonene and linalool are in accord with the literature values, however, the K value of (−)-α-pinene does not agree with that in the literature.$^{8)}$ Due to a lack of stability constant data available in the literature, it is difficult at present to judge which is more reliable. However, we obtained a K value of $5.39\times10^3\,\mathrm{kg\,mol^{-1}}$ for the (−)-α-pinene/2HP-β-CyD system using a direct SHSGC method. This compares closely to the K value of $5.78\times10^3\,\mathrm{kg\,mol^{-1}}$ for the (−)-α-pinene/2HP-β-CyD system obtained by the present solubility method.

Table 1. Comparison of Stability Constants of Fragrance Materials with 2HP-β-CyD Complexes

Fragrance material	Structure formula	This work$^{a)}$ ($\times10^{-3}$ kg/mol)	Solubility method ($\times10^{-3}$l/mol)
(−)-α-Pinene		5.78 ± 0.14	$0.578^{8)}$
(−)-β-Pinene		7.36 ± 0.16	—
Myrcene		1.24 ± 0.04	—
(+)-Limonene		4.73 ± 0.07	$3.350^{9)}$
Linalool		0.94 ± 0.11	$1.610^{9)}$

a) Mean ± S.D., $n=4$.

Conclusions

We showed in this work that the SHSGC technique can be used as a unique tool for determining the solubility and stability constants of fragrance materials/CyD systems in aqueous medium. The distinctive characteristics of this technique are as follows.

1) It can be used to rapidly and simply determine the solubility of the volatile guest.

2) The temperature of sample solution is kept constant throughout the experimental process.

3) The amount of guest required can be appreciably lower than in conventional techniques.

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