July 1998 Notes Chem. Pharm. Bull. 46(7) 1777—1179 (1998) 1177

Simultaneous Determination of the Stability Constants for Fragrance Materials with 2-Hydroxypropyl-β-Cyclodextrin by Static Head-Space Gas Chromatography

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Received January 13, 1998; accepted March 18, 1998

The simultaneous determination of the stability constants for the inclusion of fragrance materials with 2-hydroxypropyl-β-cyclodextrin (2HP-β-CyD) was investigated in a mixed fragrance materials system using static head-space gas chromatography (SHSGC). The 1 : 1 stability constants obtained by this method are in reasonable agreement with the corresponding values in the literature. The time required for determination of the stability constant is markedly reduced by this method.

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

We have recently proposed two new methods using static head-space gas chromatography (SHSGC) for determination of the stability constant of cyclodextrin (CyD) complexes called the "direct SHSGC method"[1)] and the "indirect (competitive) SHSGC method."[2)] These methods have been successfully applied to the determination of the stability constants for benzene derivatives and alkanediols–α-cyclodextrin complexes. Since the stability constants of volatile and non-volatile guests can be determined by a combination of these methods, the determination of stability constants for almost all guest–CyD complexes is feasible using these techniques.

Some investigators have tried to solubilize fragrance materials into an aqueous media using CyDs, as well as surfactants, to form a clear aqueous solution.[3,4)] Hence, understanding how CyDs interact with fragrance materials in aqueous solution is essential for industrial applications of CyDs, especially in the cosmetic field.[5)]

In this paper, we investigated the simultaneous determination of the stability constants for fragrance materials–2-hydroxypropyl-β-cyclodextrin (2HP-β-CyD) complexes in a mixed fragrance materials system using an extension of the direct SHSGC method.

Experimental

Materials The 2HP-β-CyD (average substitution degree, 4.4) used for the host was donated by Nihon Shokuhin Kako Co., (Tokyo, Japan) and was used after vacuum drying. Fragrance materials used for the guest, (−)-α-pinene, (−)-β-pinene, myrcene, and (+)-limonene were obtained from Aldrich Chemical Company, Inc. (Wisconsin, U.S.A.) and were used without purification. These fragrance materials are the main constituents in orange oil. The reason for choosing these fragrance materials was that we could compare the reliability of the estimated stability constants obtained in this study with the values obtained from a solubility method using the SHSGC technique recently established by us.[6)] The molecular weights of these fragrance materials are the same (136.24). Their chemical structures, inorganic/organic (I/O) balances, and vapor pressures[7)] are shown in Table 1. The I/O balance is the ratio between the inorganic values of the functional groups (I) and organic values of the functional groups (O) of organic molecules, which is applied to define the hydrophilicity of the fragrance materials.[8)] The I/O values were calculated by summation of I and O values described by Fujita[9)] for all the groups in a molecule. The I/O value increases with an increase in their hydrophilicity. Distilled water (pH 6.3) per injection JP (Japanese Pharmacopoeia) was obtained from Ohtsuka Pharmacy Co. Ltd. (Tokyo Japan).

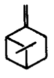
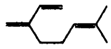
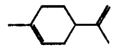
Sample Preparation Various amounts of an equimolar mixture of fragrance materials were added to an aqueous solution (10 g) with and without 38 mmol kg^{-1} 2HP-β-CyD weighted into 19.5 ml head-space vials. These sample solutions were sealed using silicone septa and aluminum foil. The vials were then thermostated at 25±0.1 °C and shaken for 24 h prior to analysis. After the equilibrium was established, 200 μl of the fragrance material vapor from above the solution was determined by GC.

GC Analysis The analytical conditions for GC were the same as described previously[1)] except for the following conditions; the column temperature was 90 °C and the flow rate of nitrogen gas used as the carrier gas was 20 ml/min.

Results and Discussion

Theoretical In equilibrium, the four fragrance materials share the 2HP-β-CyD corresponding to their stability constants based on the assumption that no complexes are formed, except four binary complexes which do not interfere with one another. If each fragrance material in the vapor is separated and determined by GC, in principle, we can evaluate the stability constants for the four fragrance materials (F)–2HP-β-CyD complexes by this method as reliably as the direct SHSGC method.[1)] If we assume that the equilibrium between a fragrance component (F_i) and 2HP-β-CyD involves a 1 : 1 complex as

Table 1. Properties of the Fragrance Materials

Fragrance materials	Structure formula	I/O	Vapor pressure[a)] (Pa, 25 °C)
(−)-α-Pinene		0.11	586.6
(−)-β-Pinene		0.11	386.4
Myrcene		0.033	220.0
(+)-Limonene		0.074	186.6

a) Data taken from ref. 7.

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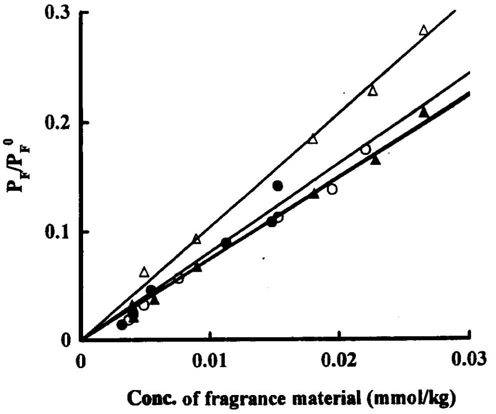

Fig. 1. Calibration Curves Based on Henry's Law for Mixed Fragrance Materials in Aqueous Solution

△, (+)-limonene (r=0.9965); ●, (−)-α-pinene (r=0.9732); ▲, myrcene (r=0.9955); ○, (−)-β-pinene (r=0.9931).

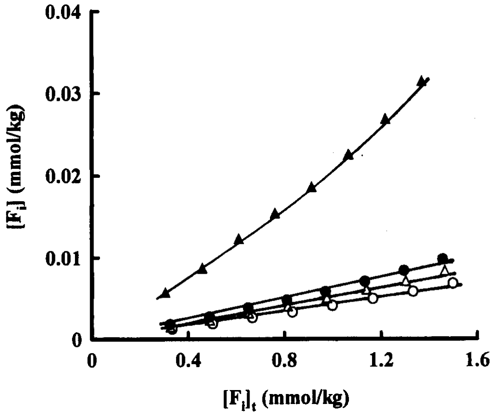

Fig. 2. Plots of $[F_i]$ *vs.* $[F_i]_t$ for Fragrance Material Solutions in the Presence of 38 mmol kg^{-1} 2HP-β-CyD

▲, myrcene; ●, (−)-α-pinene; △, (+)-limonene; ○, (−)-β-pinene.

shown in Eq. 1,

$$[F_i]+[CyD] \overset{K}{\leftrightarrows} [F_i-CyD] \tag{1}$$

Stability constant (K) for a 1 : 1 complex is defined by Eq. 2,

$$K=\frac{[F_i-CyD]}{[F_i][CyD]} \tag{2}$$

where $[F_i]$ and $[CyD]$ denote the concentrations of the free solutes, respectively. $[F_i-CyD]$ denotes the complex concentration. The mass balance of F_i in aqueous solution is represented by Eq. 3,

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

where $[F_i]_t$ is the total concentration of F_i. Substituting Eq. 2 into Eq. 3,

$$[F_i]=\frac{[F_i]_t}{1+K[CyD]} \tag{4}$$

$[F_i]_t$ is known and $[F_i]$ can be obtained experimentally from the calibration curve (Henry's law) in the absence of 2HP-β-CyD as described previously.[1] [CyD] can also be determined from the mass balance of CyD by Eq. 5,

$$[CyD]=[CyD]_t-[F-CyD]_t \tag{5}$$

where $[CyD]_t$ is the total concentration of CyD and is known. $[F-CyD]_t$ is the total concentrations of complex for four fragrance materials and is also calculated from the sum of each complex obtained from Eq. 3. Therefore, the stability constant for each fragrance material can be estimated by a nonlinear least-squares program (MULTI[10]) of $[F_i]$ *versus* $[F_i]_t$ using Eq. 4.

Calibration Curve Figure 1 shows the calibration curves for each fragrance material in the absence of 2HP-β-CyD. P_F and P_F^0 are the vapor pressure of F over the solution and that of F in the pure state, respectively. We were able to make a quantitative separation of fragrance material vapor above the solution containing the mixed fragrance materials by GC. The plots gave straight lines in accordance with Henry's law for each fragrance material. These calibration curves were used to evaluate $[F_i]$ in the presence of 2HP-β-CyD.

Stability Constant Figure 2 shows the plots according to Eq. 4 for each fragrance material together with the calculated curves which give the best fit of the experimental data. The theoretical values agreed well with the experimental values (data points) for each fragrance material. The results in Fig. 2 also suggest that the stoichiometry of complexes between fragrance materials and 2HP-β-CyD is 1 : 1. The apparent stability constants determined by this method are summarized together with S.D. and are compared with the literature values in Table 2. The S.D. values were derived from a single experiment consisting of a set of data points fitted to a model function by least-squares analysis.[5] The K values determined in this study are in reasonable agreement with those obtained from the solubility method using the SHSGC technique (solubility SHSGC method).[6] We also investigated the relationship between the I/O values and K values for each fragrance material, but no close correlation could be seen. To our knowledge, there is no paper dealing with the simultaneous determination of a stability constant in a mixed guest system. The time required for the determination of the stability constants can be markedly reduced by this method. To put it in concrete terms, the experimental time required for the determination of stability constants for the four fragrance materials by this method can be shortened to about 1/4 that required with the conventional direct SHSGC method which individually determines the stability constants. In addition, this method possesses the same advantage of the direct SHSGC method which is characterized by simplicity and accuracy. However, this method may not be applied to a system in which an interaction among guests can occur; that is, the

Table 2. Comparison of the Stability Constants for Fragrance Materials/2HP-β-CyD Complexes

Fragrance materials	This work ($\times 10^{-3}$ kg/mol)	Solubility SHSGC method[a] ($\times 10^{-3}$ kg/mol)
(−)-α-Pinene	4.75±0.04	5.78±0.14
(−)-β-Pinene	7.07±0.07	7.36±0.16
Myrcene	1.36±0.01	1.24±0.04
(+)-Limonene	5.63±0.06	4.73±0.07

a) Literature values are from ref. 6. *b*) Data are the mean±S.D.

application of this method may be limited to mixed guest systems in which the physicochemical properties of the guests are similar to one another. However, to clarify this point, further studies using a combination of various guests are needed.

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