



Release control of fragrances by complexation with β -cyclodextrin and its derivatives

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Abstract

The release control of fragrances, benzyl acetate (BA), citral (CR), linalool (LL), citronellol (CL) and linalyl acetate (LA), was conducted using β -cyclodextrin (β -CyD), 2-hydroxypropyl- β -CyD (HP- β -CyD) and 2,6-di-*O*-methyl β -CyD (DM- β -CyD). The release rate of the fragrances from 30% ethanol/water solution was significantly suppressed by the complexation with these CyDs, and the suppressing effect increased in the order of β -CyD < HP- β -CyD < DM- β -CyD. The concentration-dependent change of the release rate was quantitatively analyzed to obtain the stability constant (K_c) of the fragrance-CyD complexes. These K_c values were in good agreement with those determined by the solubility method. The results suggest that the release of fragrances can be prolonged by the complexation with β -CyDs and their effects can be controlled by choosing appropriate CyD derivatives with higher K_c values and by setting proper concentrations of the host molecules.

Keywords Cyclodextrin · Inclusion complex · Fragrances · Release control · Stability constant

Introduction

Olfaction has two important roles in the lives of all animals, i.e. fundamental and psychological roles [1, 2]. The former is to help protect from enemies, including foods, by detecting danger signs such as unpleasant smell. The latter has become more important for human beings, invigorating the spirit and mind in everyday life through a contact with pleasant fragrances or sometimes damping the spirit by unpleasant smell. Recently, fragrances of natural and synthetic oils have received attention in medical fields, because of their pharmacological and psychological effects [3–7]. For example, flavors of lavender and roses have a sedative effect while those of lemongrass and peppermint have a stimulant effect. A number of essential oils have been used for the treatment of stress, anxiety, epilepsy and pain etc. However, most of flavor components are highly volatile and quickly

disappear from atmosphere. Therefore, it is very important to control the release rate of fragrances for enhancement of their effects. Encapsulation is one of the efficient methods for sustained releases of fragrances, among which are the encapsulations into micro- and macro-pores of inorganic and organic materials, nanofibers, nanofilms and cyclodextrins [8–13].

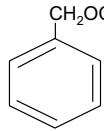
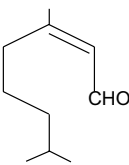
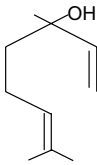
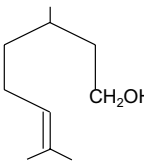
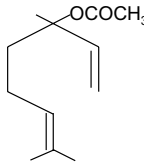
In this study, we conducted the release control of several fragrances utilizing the complexation with parent β -cyclodextrin (β -CyD), 2-hydroxypropyl- β -CyD (HP- β -CyD) and 2,6-di-*O*-methyl- β -CyD (DM- β -CyD). In this study, benzyl acetate (BA), citral (CR) and linalool (LL) were mainly employed for the release control, in addition to citronellol (CL) and linalyl acetate (LA). CR and LA were used as fragrance models with a stimulant activity, while BA, CL and LL were those with a sedative activity. Further, concentration-dependent decreases of the release rate were quantitatively analyzed to obtain the stability constant of the complexes. Table 1 shows chemical structures of the fragrances used, together with the solubility in water and the partition coefficient in *n*-octanol/water determined in this study.

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Table 1 Chemical structures and some physicochemical properties of fragrances used in this study

Fragrance	BA	CR	LL	CL	LA
Structure					
Partition coefficient (PC) ^a	2.4	2.6	2.1	2.8	1.9
Solubility in water (g/dL) ^b	0.15	0.03	0.15	0.02	0.05

^aLog (PC), *n*-octanol/H₂O at 25 °C^bAt 25 °C

Experimental

Materials

LA and CL were donated by Shiseido Ltd. (Tokyo, Japan). BA, LL and CR were purchased from Kanto Chemicals (Tokyo, Japan). β -CyD, HP- β -CyD and DM- β -CyD were supplied from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Other chemicals and solvents used were of analytical grade, and deionized double-distilled water was used throughout the study.

Measurements of solubility and partition coefficient

An excess amount of fragrances was added in water and the mixture was shaken at 25 °C for 1 week. After the equilibrium was attained, the mixture was centrifuged. In the case of BA, an aliquot of the supernatant was appropriately diluted with water and concentrations of the fragrance in the solutions were spectrophotometrically measured at 256 nm. In the case of CL, CR, LA and LL, the supernatants were filtered through a 0.2 μ m filter, the filtrates were diluted with water and then analyzed for CL, LA and LL at 215 nm and for CR at 260 nm, using a Hitachi U-3200 spectrometer (Tokyo, Japan).

Oil/water partition coefficients of the fragrances were measured using *n*-octanol. The mixture of *n*-octanol and water (1:30 v/v) containing each fragrance was shaken for 5 h. After the mixture was centrifuged, the water phase was analyzed for fragrances using a Hitachi L-6000 high-performance liquid chromatograph (Tokyo, Japan) at the following conditions: a Shodex ODS packed column (5 μ m, 4 mm \times 150 mm, Tokyo, Japan), a mobile phase of 80% methanol/water, a flow rate of 1.0 mL/min, and the

detection at 215 nm for CL, LA and LL, 256 nm for BA and 260 nm for CR.

Interaction studies

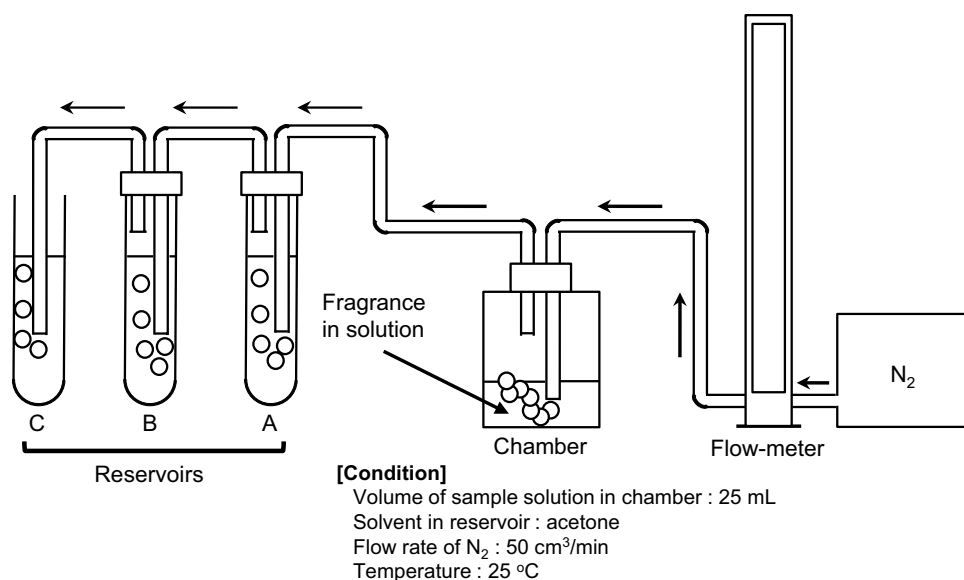
The interaction of BA, CR and LL with β -CyDs was studied by the solubility method [9] and circular dichroism (CD) spectroscopy. The solubility studies were conducted according to the method of Higuchi and Connors [14] under the conditions same as those described in the measurements of solubility, except of the solvent and the shaking period. As a solvent, 30% ethanol/water solution was used, because this mixed solution was used in the release experiments described below. Concentrations of fragrances were measured spectrophotometrically, as described above.

CD spectra were recorded on a JASCO J-60 spectropolarimeter at concentrations of 1.0×10^{-3} and 1.0×10^{-2} M for fragrances and β -CyDs, respectively, in 30% ethanol/water solution at room temperature.

Release studies

The release rates of fragrances were monitored by the strip-trap method [15], using the home-made apparatus shown in Fig. 1. Fragrances dissolved in 30% ethanol/water were put in the chamber into which nitrogen gas was bubbled at a constant flow rate. Evaporated fragrances were trapped in acetone in three reservoirs A–C. The volume of 30% ethanol/water in the chamber: 25 mL, the volume of acetone in each reservoir: 20 mL, the flow rate of N₂: 50 cm³/min, and temperature: 25 °C. Fragrances in acetone in the reservoirs were analyzed by gas-chromatography under the following conditions: a Shimadzu GC-7A gas-chromatograph (Kyoto, Japan), a Shimadzu Hi Cap CBP1-S25-050 column, detection: FID, carrier gas: N₂, pressure of the carrier gas: 2.0 kgf

Fig. 1 Apparatus used for fragrance-release study



cm², air pressure: 0.5 kg/cm², rate of temperature increase: 4 °C/min, column temperature: 70–220 °C, detection temperature: 250 °C.

Figure 2 shows concentration–time profiles of BA, LL and CR in 30% ethanol/water in the chamber and in acetone of three reservoirs. The concentrations of the fragrances in the chamber decreased with time, with concomitant increase in the reservoir A. The fragrances were negligibly detected in the reservoirs B and C. These results indicated that the released fragrances were completely trapped in the first reservoir A, confirming no leaks of the fragrances from the apparatus and no degradations of the fragrances under the experimental conditions.

Results and discussion

Release of fragrances in the absence of CyDs

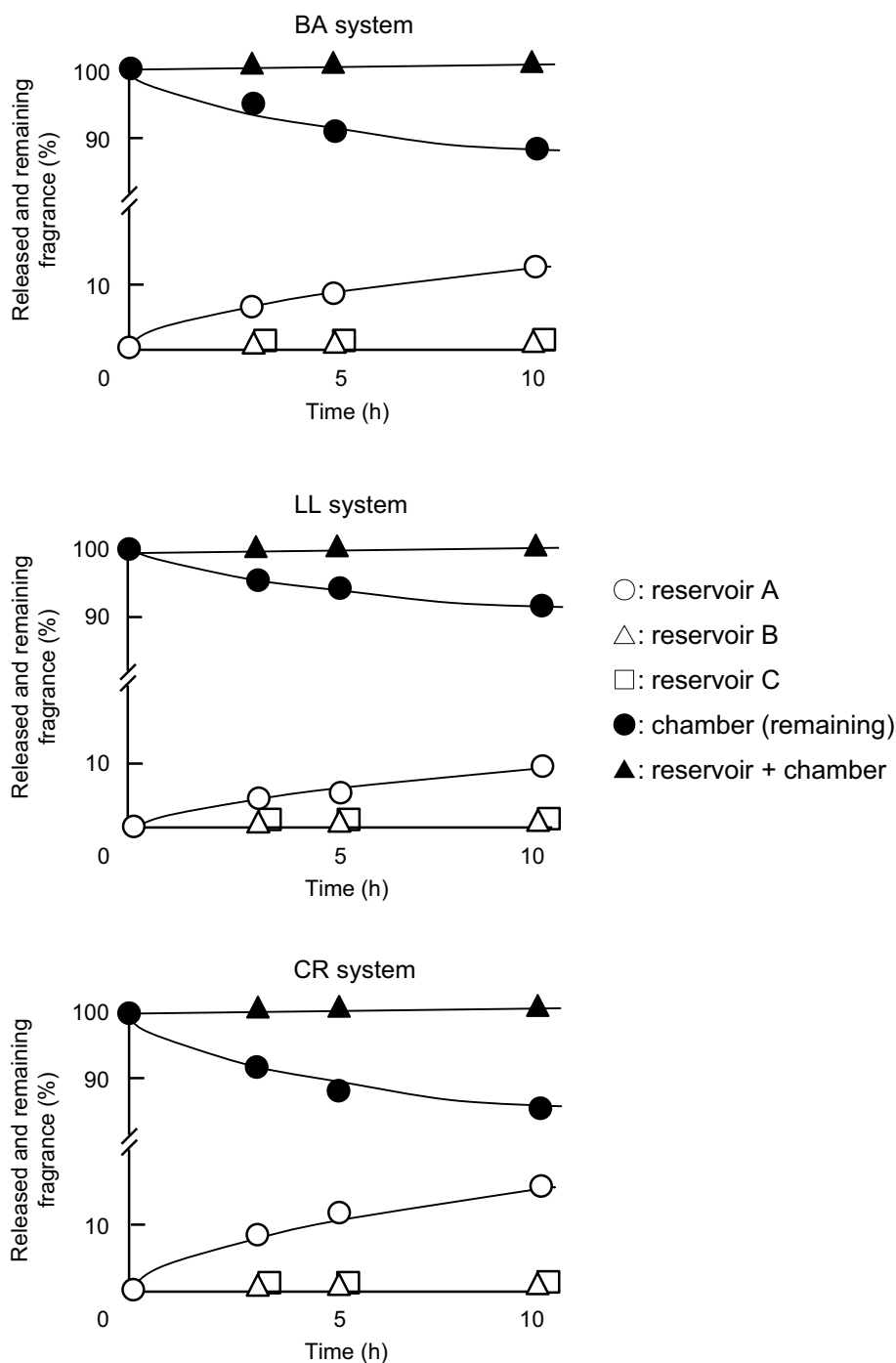
Figure 3 shows the released% of BA, LL and CR after 5 h as a function of ethanol concentration in the chamber. The released amounts of fragrances were almost constant below 30% ethanol concentrations, above which they decreased, indicating a high affinity of the fragrances to hydrophobic solvents. The solvents of higher ethanol concentrations are suitable for accurate monitoring of the release rate of fragrances, while the solubility of parent β -CyD is limited and the competitive inclusion occurs in such solvents. Therefore, 30% ethanol/water solution was used as a medium of the donor chamber, which gave the constant released amount at least up to 5 h. Figure 4 shows the release rates of BA, LL and CR from 30% ethanol/water solution, as a function of fragrance concentration. As described later, the release amount–time profiles were linear under the experimental

conditions, the release rate of fragrances was analyzed according zero-order kinetics. As shown in Fig. 4, the release rate increased with increasing the concentration of fragrances, although it was not a first order to the concentration probably due to self-aggregation of fragrances in the releasing medium at high concentrations. Figure 5 shows the relationship between the release rate and partition coefficient of the fragrances, where the release rates were those in the fragrance concentration of 1.0×10^{-3} M and the partition coefficients were those of *n*-octanol/water (Table 1). The release rate of fragrances was linearly increased with their partition coefficient, indicating a linear free energy (ΔG) relationship between the two parameters [16, 17], i.e. $\ln(\text{release rate}) = 0.77 \ln(\text{PC}) - 7.9$ (correlation coefficient = 0.96). The increase in the hydrophobicity of fragrances facilitates their escaping rate from water, a hydrophilic environment.

Release of fragrances in the presence of β -CyD, HP- β -CyD or DM- β -CyD

Figure 6 shows the comparison of the release rates of BA, CR, CL, LA and LL (1.0×10^{-3} M) fragrances in the presence of β -CyDs (1.0×10^{-2} M) from 30% ethanol/water solution. The release rate of fragrances was suppressed by the addition of β -CyDs and the suppressing effect was in the order of DM- β -CyD > HP- β -CyD > β -CyD. Since the relatively large suppression of β -CyDs was observed in fragrances of BA, CR and LL, the concentration-dependent decrease in the rate was in detail investigated. Figure 7 shows the release-time profiles of BA, as an example, in the presence of different concentrations of HP- β -CyD. It was apparent that the release amount of BA linearly increased with time both in the absent and presence of HP- β -CyD, indicating a zero-order release under

Fig. 2 Trapped fragrances in reservoir (A–C) and remaining fragrances in 30% (v/v) ethanol solution, measured by strip-trap method at 25 °C. BA: 8.4×10^{-3} M, CR: 1.0×10^{-3} M, LL: 8.4×10^{-3} M



the experimental conditions. The zero-order release rate constants were determined from the slope of the plots. Figure 8 shows the effect of β -CyD, HP- β -CyD and DM- β -CyD on the release rate constant of BA, CR and LL. The release rates were decreased with increasing CyD concentrations, showing a feature of the saturated kinetics. These profiles were analyzed according to Scheme 1, where the fragrance forms the 1:1 complex with CyDs in the liquid phase of the chamber and the fragrance in the free form takes part in the release. Therefore,

the ratio (k/k_0) of the release rate constants in the absence (k_0) and in the presence of β -CyDs is expressed as Eq. (1) in which $(Fra)_f$ and $(Fra-CyD)$ stand for the concentration of fragrances in a free form and that of the complex, respectively. The 1:1 stability constant (K_c) of the complexes is defined as Eq. (2) in which $(CyD)_f$ stands for the concentration of β -CyDs in a free form. The total concentrations of fragrances $(Fra)_t$ and β -CyDs $(CyD)_t$ were expressed in Eqs. (3) and (4). Equation (5) is obtained by substituting Eqs. (2), (3) and (4) into Eq. (1).

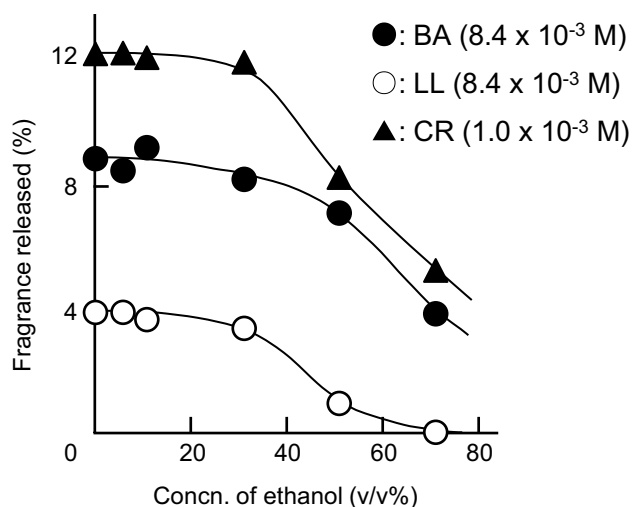


Fig. 3 Released and adsorbed amounts of fragrance after 5 h, as a function of ethanol concentration in chamber, measured by strip-trap method at 25 °C

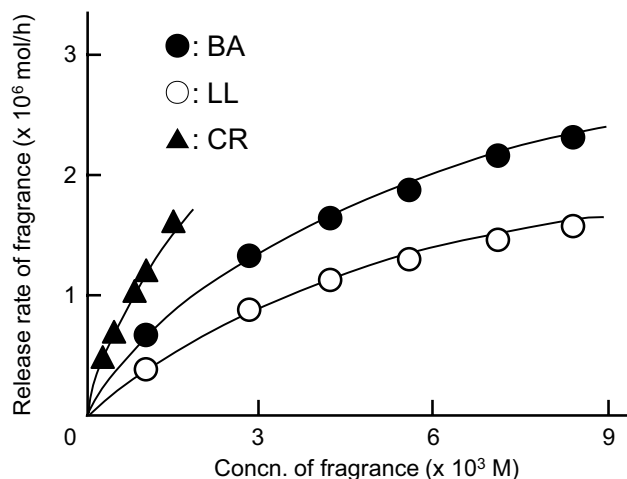


Fig. 4 Release rate of fragrance from 30% ethanol solution to gas-phase, measured by strip-trap method at 25 °C

$$r = k/k_0 = (\text{Fra})_t / [(\text{Fra})_f + (\text{Fra} - \text{CyD})] \quad (1)$$

$$Kc = (\text{Fra} - \text{CyD}) / [(\text{Fra})_f \times (\text{CyD})_f] \quad (2)$$

$$(\text{Fra})_t = (\text{Fra})_f + (\text{Fra} - \text{CyD}) \quad (3)$$

$$(\text{CyD})_t = (\text{CyD})_f + (\text{Fra} - \text{CyD}) \quad (4)$$

$$(\text{CyD})_t = (1 - r) \left[(\text{Fra})_t + \frac{1}{(r \times Kc)} \right] \quad (5)$$

The release rate constants (k) of Fig. 8 were converted to the relative rate constants (k/k_0), and the (k/k_0) versus

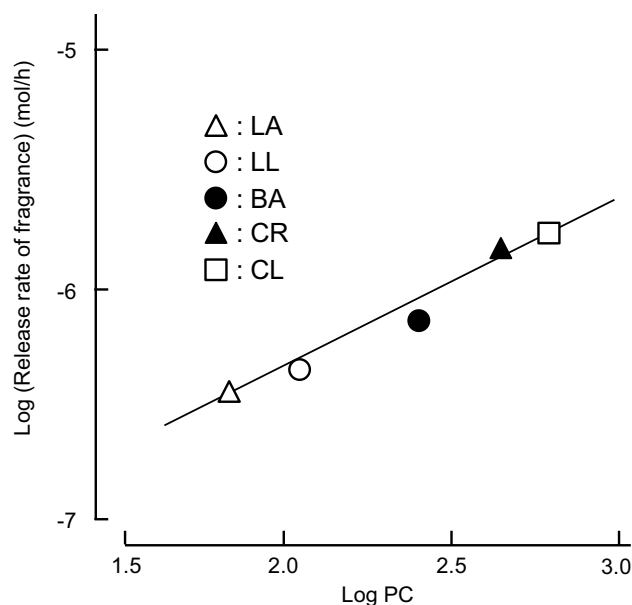


Fig. 5 Relationship between release rates of fragrances and their partition coefficients (PC, *n*-octanol/water)

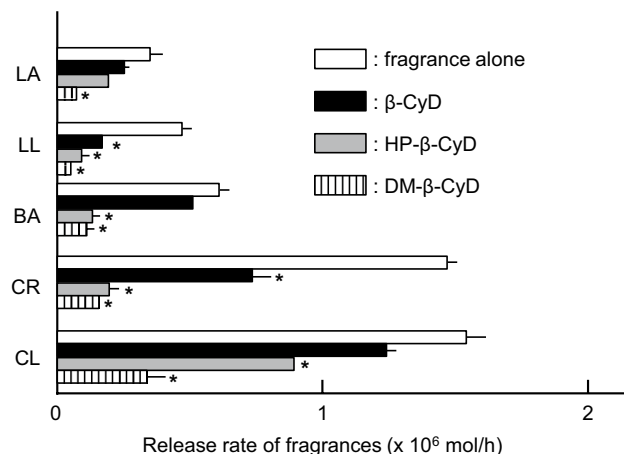


Fig. 6 Effects of CyDs (1×10^{-2} M) on release rates of fragrances (1×10^{-3} M) from 30% ethanol solution, measured by strip-trap method at 25 °C. Each value represents the mean \pm SE of three experiments. * $p < 0.05$ versus fragrance alone

($\text{CyD})_t$ profiles were analyzed by Eq. (5) using a non-linear least-squared method [18] to obtain the Kc values. The results were shown in Table 2. The fragrances, LL and CR, having the non-cyclic chemical structure had a similar affinity to the cavity of β -CyD, HP- β -CyD and DM- β -CyD. The Kc values of LL and CR were larger than that of BA, a cyclic fragrance. It was apparent that the inclusion ability to the fragrances increased in the order of β -CyD < HP- β -CyD < DM- β -CyD, which was consistent with the suppressing effect to the release rate.

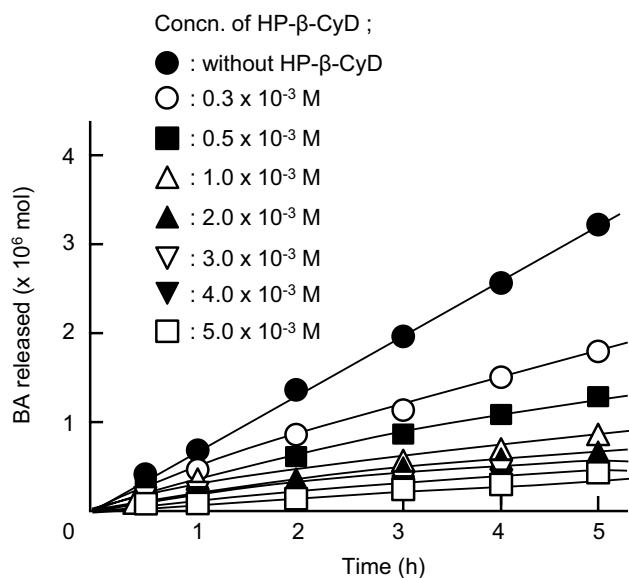
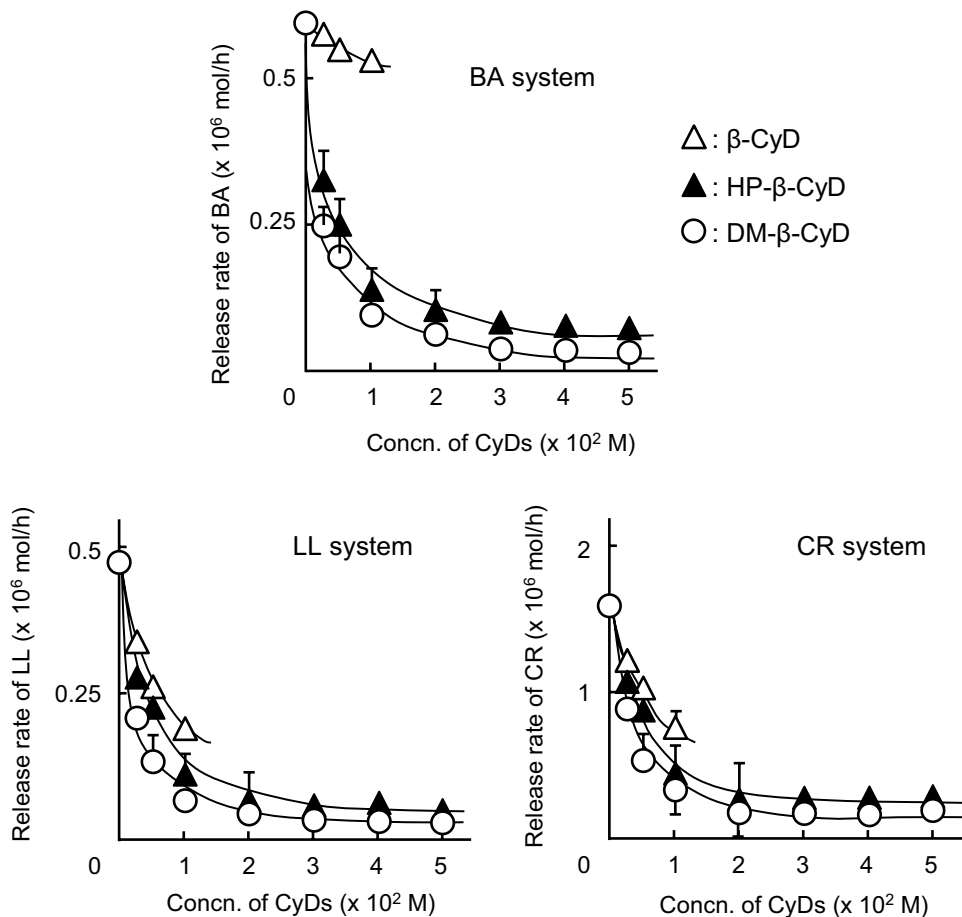


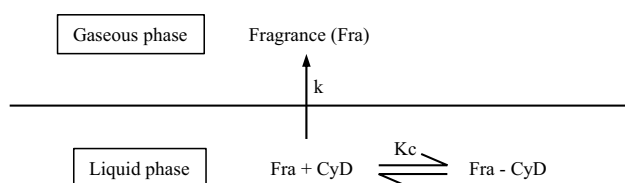
Fig. 7 Effects of HP- β -CyD on release of BA (1×10^{-3} M) from 30% ethanol solution, measured by strip-trap method at 25 °C

Fig. 8 Effects of β -CyDs on release rates of fragrances (1×10^{-3} M) from 30% ethanol solution, measured by strip-trap method at 25 °C. Each value represents the mean \pm SE of three experiments



Interaction of fragrances with β -CyDs

The interaction of BA and CR with β -CyDs was confirmed by CD spectroscopy and the solubility method. Figure 9 shows CD spectra of the fragrances in the presence of β -CyD, HP- β -CyD and DM- β -CyD in 30% ethanol/water solution. The optical activity was induced at around 250 nm with a negative sign. Because intrinsic Cotton effects of these β -CyDs are observed only below 220 nm and the fragrances have no asymmetric carbons in a molecule, these CD bands are attributable to the induced optical activity of the fragrances [19, 20], indicating that the fragrances are embedded in the asymmetric locus of the β -CyD cavities. The magnitude of the induced CD was DM- β -CyD > HP- β -CyD > β -CyD, which was in accordance with that of the stability constants of the complexes. Figure 10 shows the phase solubility diagrams of BA, LL and CR with β -CyD, HP- β -CyD and DM- β -CyD in 30% ethanol/water solution. The solubility of the fragrances increased linearly with CyD concentrations, showing the A_L type diagram according to the classification of Higuchi and Connors [14], suggesting the formation of 1:1 (host:guest) inclusion complexes under the experimental conditions [20]. The K_c values of the complexes were calculated using Eq. (6) [14] in which



Scheme 1 Proposed scheme for release of fragrances from solution of CyD complex

the slope and intercept stand for those of linear lines of the diagrams. Unfortunately, the accurate stability constant of BA- β -CyD complex could not be determined because of small changes in the release rate and the solubility by the addition of β -CyD (Figs. 8, 10).

$$Kc = \text{Slope} / [\text{intercept} (1 - \text{slope})] \quad (6)$$

The Kc values determined by the solubility method were similar to those determined by the kinetical method, although the former values were slightly lower than those of the latter, which may be due to the different experimental conditions such as concentrations of the fragrances.

The Kc values were in the order of DM- β -CyD complex > HP- β -CyD complex > β -CyD complex. This order may change depending on ethanol concentrations, because the solvent forms the ternary fragrance/ethanol/CyD complexes and/or works as a competitive agent to the fragrance/CyD complexes. The stability constant of BA/HP- β -CyD complex in the absence of solvent in aqueous solution is reported to be 310 M^{-1} [13]. This value decreased to about 200 M^{-1} (see Table 2) in 30%v/w ethanol solution that corresponds to 5.2 M calculated using the density of ethanol = 0.8. It is apparent that ethanol definitely hinders the access of fragrances to CyD cavities, although it is difficult to discriminate between the ternary complexation [21, 22] and the competitive inclusion. Therefore, the retarding effect of CyDs on the release rate of fragrances may be greater at lower ethanol concentrations, but we employed 30% ethanol solution as a release medium, because of the reasons described above and the quick dissolution of fragrances with low aqueous solubility in the medium. Further studies should be done to elucidate the detailed inhibition mechanism of ethanol in the complexation.

Table 2 Stability constants (Kc , M^{-1}) of Fra-CyD complexes in 30% ethanol solution at 25 °C

System	Method					
	Release rate			Solubility		
	BA	LL	CR	BA	LL	CR
β -CyD	–	120	130	–	86	83
HP- β -CyD	180	230	190	120	180	190
DM- β -CyD	270	290	310	220	250	260

Fig. 9 CD Spectra of fragrances ($1 \times 10^{-3} \text{ M}$) in the presence of CyDs ($1 \times 10^{-3} \text{ M}$) in 30% ethanol solution

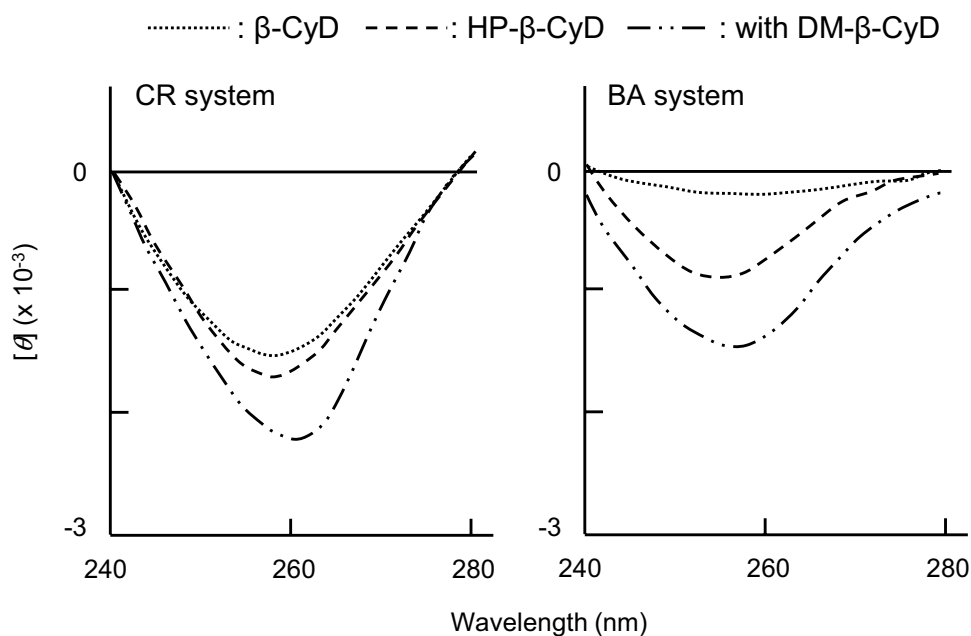
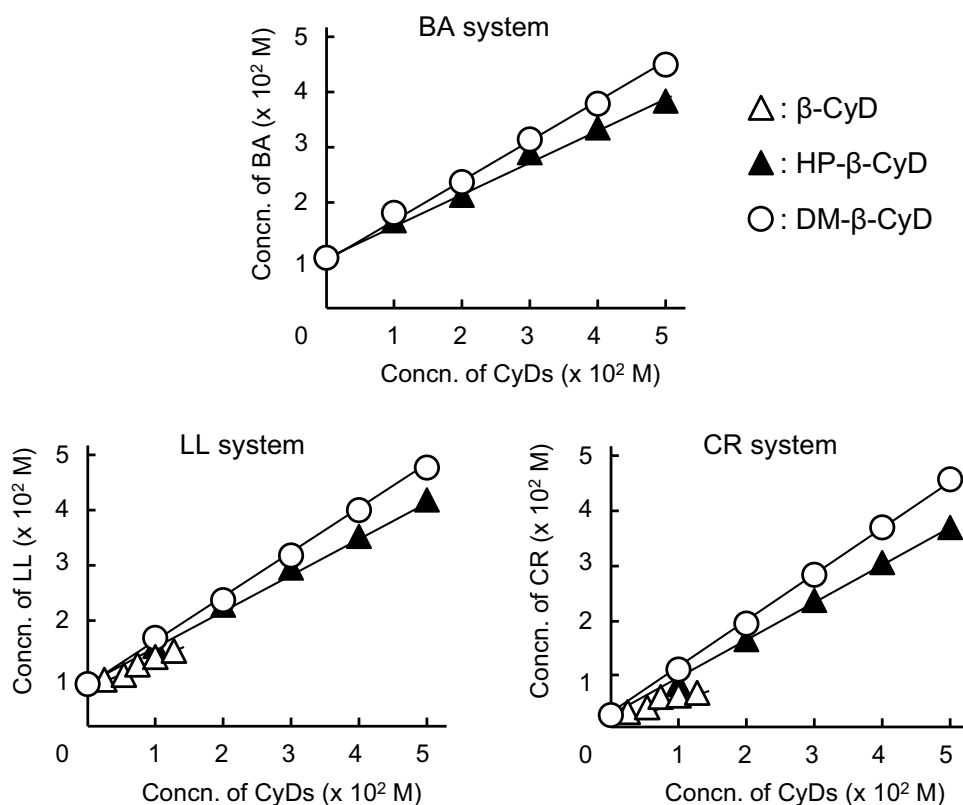


Fig. 10 Phase solubility diagrams of fragrance-CyD systems in 30% ethanol solution at 25 °C



In conclusion, the release of fragrances can be prolonged by the complexation with β -CyDs and their effects can be controlled by choosing appropriate CyD derivatives with higher K_c values and by setting appropriate concentrations of the host molecules.

References

- Mitsui, T. (ed.) *New Cosmetic Science*. Elsevier, Amsterdam (1997)
- Adorjan, B., Buchbauer, G.: Biological properties of essential oils: an updated review. *Flavour Fragr. J.* **25**, 407–426 (2010)
- Haehner, A., Maass, H., Croy, I., Hummel, T.: Influence of room fragrance on attention, anxiety and mood. *Flavour Fragr. J.* **32**, 24–28 (2017)
- Dobetsberger, C., Buchbauer, G.: Actions of essential oils on the central nervous system: an updated review. *Flavour Fragr. J.* **26**, 300–316 (2011)
- Sousa, O.V., Silverio, M.S., Del-Vechio-Vieira, G., Matheus, F.C., Yamamoto, C.H., Alves, M.S.: Antinociceptive and anti-inflammatory effects on the essential oil from *Eremanthus erythropappus* leaves. *J. Pharm. Pharmacol.* **60**, 771–777 (2008)
- Kuroda, K., Inoue, N., Ito, Y., Kubota, K., Sugimoto, A., Kakuda, T., Fushiki, T.: Sedative effects of the jasmine tea odor and (R)-(-)-linalool, one of its major odor components, on autonomic nerve activity and mood states. *Eur. J. Appl. Physiol.* **95**, 107–114 (2005)
- Nomura, T., Kurihara, K.: Liposomes as a model for olfactory cells: changes in membrane potential in response to various odorants. *Biochemistry* **26**, 6135–6140 (1987)
- Greenblatt, H.C., Dombroski, M., Klishevich, W., Kirkpatrick, J., Bajwa, I., Garrison, W., Redding, B.K.: Encapsulation and controlled release of flavours and fragrances. In: Karsa, D.R., Stephenson, R.A. (eds.) *Encapsulation and Controlled Release*, pp. 148–162. Woodhead Publishing Limited, Cambridge (1993)
- Zhang, J., Peppard, T.L., Reineccius, G.A.: Preparation and characterization of nanoemulsions stabilized by food biopolymers using microfluidization. *Flavor Fragr. J.* **30**, 288–294 (2015)
- Kfoury, M., Pipkin, J.D., Antle, V., Fourmentin, S.: Captisol[®]: and efficient carrier and solubilizing agent for essential oils and their components. *Flavour Fragr. J.* **32**, 340–346 (2017)
- Helena, M., Marques, C.: A review on cyclodextrin encapsulation of essential oils and volatiles. *Flavour Fragr. J.* **25**, 313–326 (2010)
- Loftsson, T.: Cyclodextrins in skin delivery. *Cosmet. Toilet.* **115**, 59–66 (2000)
- Tanaka, M., Matsuda, H., Sumiyoshi, H., Arima, H., Hirayama, F., Uekama, K., Tsuchiya, S.: 2-Hydroxypropylated cyclodextrins as a sustained-release carrier for fragrance materials. *Chem. Pharm. Bull.* **44**, 416–420 (1996)
- Higuchi, T., Connors, K.A.: Phase-solubility techniques. *Adv. Anal. Chem. Instrum.* **4**, 117–210 (1965)
- Nunez, A.J., Maarse, H.: Headspace methods for volatile components of grapefruit juice. *Chromatographia* **21**, 44–48 (1986)
- Wells, P.R.: *Linear Free Energy Relationships*. Academic Press, New York (1968)
- Davis, S.S., Higuchi, T., Rytting, J.H.: Determination of thermodynamics of the methylene group in solutions of molecules. *J. Pharm. Pharmacol.* **24**, 30P–46P (1972)

18. Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T.: A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharm. Dyn.* **4**, 879–885 (1981)
19. Kodaka, M.: Sign of circular dichroism induced β -cyclodextrin. *J. Phys. Chem.* **95**, 2110–2112 (1991)
20. Uekama, K., Hirayama, F., Irie, T.: Cyclodextrin drug carrier systems. *Chem. Rev.* **98**, 2045–2076 (1998)
21. Hamai, S.: 1:1:1 inclusion compounds of β -cyclodextrin with fluorene and alcohols or nitriles in aqueous solution. *Bull. Chem. Soc. Jpn.* **62**, 2763–2767 (1989)
22. Liao, Y., Bohne, C.: Alcohols effect on equilibrium constants and dissociation dynamics of xanthone-cyclodextrin complexes. *J. Phys. Chem.* **100**, 734–743 (1996)