Complexation of Various Fragrance Materials with 2-Hydroxypropyl- β -cyclodextrin

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2-Hydroxypropyl- β -cyclodextrin (2HP- β -CD) is more soluble in water than α , β , or γ cyclodextrin. Although water-insoluble substances are dissolved by surfactants, the surfactants induced a number of problems. Therefore, we have studied a method for dissolving water-insoluble perfumes using 2HP- β -CD, without the need of a surfactant.

First, complexes of various perfumes with 2HP- β -CD were prepared, and the solubilized amount of the perfumes was determined by high-performance liquid chromatography (HPLC). As a result, water-insoluble perfumes were dissolved in water by 2HP- β -CD, even though a surfactant was not used.

The use of proton nuclear magnetic resonance ($^1\text{H-NMR}$) confirmed that these dissolution phenomena were based on the inclusion mechanism.

Next, factors which caused differences in the inclusion among perfumes were discussed. Among the perfumes with a hydroxide (OH) group and an aromatic ester group and perfumes of low molecular weight (150–160), some perfumes were highly soluble. Obtaining the stability constant (K_e') through Higuchi's formula confirmed that the solubilization of perfumes was dependent on the concentration. It was proven that the solubilized amount of perfumes is in close relation with the addition of 2HP- β -CD, in other words, the apparent stability constant.

Keywords 2-hydroxypropyl- β -cyclodextrin; 2HP- β -CD; fragrance material; cosmetic; $^1\text{H-NMR}$; solubility; solubilized amount; substitution degree; stability constant

Surfactants in cosmetic preparations have been regarded as important raw materials for use in emulsion, dispersion, and solubilization techniques for many years. However, studies on surfactants used as a solubilizing agent (such as polyoxy ethylene octyl dodecyl ether and polyoxy ethylene hydrogenated castor oil) reveal different problems. These problems involve high sensitivity to human skin,^{1,2)} poor appearance due to cloudiness or turbidity caused when surfactants dissolve water-insoluble substances, and an uncomfortable feeling due to a foam residue like that of soap. To eliminate these problems, the authors tried to use cyclodextrin derivatives to solubilize any water-insoluble substances.

It is well known that cyclodextrin, in which 6–8 glucose molecules are circularly connected by an α -1,4 glycoside bond, is capable of including water-insoluble substances.³⁾ However, β -cyclodextrin ($\text{C}_6\text{H}_{10}\text{O}_5$)₇ scarcely dissolves in water, so it is difficult to use β -cyclodextrin instead of a surfactant. Cyclodextrin derivatives have been intensely studied in recent years. Among them the authors selected 2-hydroxypropyl- β -cyclodextrin (2HP- β -CD), because it is easily soluble in water, inexpensive, convenient to use in manufacturing, and safe. The authors conducted an experiment to dissolve water-insoluble substances in water using 2HP- β -CD. They selected perfumes as the water-insoluble substances. Most perfumes have low molecular weights. Since some perfumes have a functional group, they are essential raw materials in cosmetic components and are suitable hosts. By producing complexes of different perfumes with 2HP- β -CD, the authors obtained solubilized amounts of each and studied the difference between the amount of perfume included. As a result, some interesting facts were observed.

Experimental

Materials Host experiments were performed using 2HP- β -CD made by Nihon Shokuhin Kako Ltd. Eight typical fragrance materials were used as the guest material.

We calculated the degree of substitution using the average number of 2-hydroxypropyl groups per molecule of 2HP- β -CD estimated by proton nuclear magnetic resonance ($^1\text{H-NMR}$). We allowed them to stand for 12 h at 25 °C. Table I shows the list of guest materials.

Specimen Preparation Method The discrete fragrance material used as a guest was directly and gradually added to an aqueous solution in which 2HP- β -CD was dissolved. The mixture was stirred at 25 °C for 12 h with a magnetic stirrer. Later, the concentration of the guest in the filtrate was determined.

Figure 1 shows the flow chart for this preparation method.

Determination of the Degree of Substitution The degree of substitution was calculated from the $^1\text{H-NMR}$ integration ratio of anomeric protons and methyl protons at the terminal of the hydroxypropyl. However, the degree of substitution was expressed as the number of hydroxypropyl group per mol of cyclodextrin.

Solubility Studies Solubility measurements were carried out according to Higuchi and Connors.⁴⁾ Excess amounts of fragrance materials were added to aqueous solutions containing 2HP- β -CD (degree of substitution (DS)=4.3) at various concentrations, and the mixtures were equilibrated by shaking at 25 °C for about 2 weeks. Thereafter, the mixtures were filtered and after appropriate dilution with methanol, the concentration of the fragrance materials in the filtrate was measured by high-performance liquid chromatography (HPLC).

Determination Method The concentration of fragrance materials in the solution was determined by HPLC with the calibration curve method.

The HPLC equipment (Nihon Bunko 860-CO) used and the conditions are as follows: Column; CAPCELLPAK C₁₈ SG 4.6 $\times$ 250 mm (Shiseido), pump flow rate; 1 ml/min, quantity of the solution; 4.0 μl .

Table II gives the solvent and λ_{max} for each fragrance material in the HPLC.

Fig. 1. Flow Chart

Add 2HP- β -CD to water, mix together and slowly add the fragrance to the mixture, stir using a stirrer and filter. Use the filtrate as the inspection body.

TABLE I. List of Guests Used

Fragrance materials	Structural formula	Molecular weight
Linalool		154.25
Citronellol		156.27
Citral		152.24
Limonene		136.24
Methyl ionone	α Type	206.33
Benzyl acetate		150.17
Linalyl acetate		196.29
Methyl dihydro-jasmonate		226.32

Calculation of the Stability Constant By preparing a solubility phase plot of the concentration of fragrance material *versus* the 2HP- β -CD according to the method of Higuchi *et al.*,⁴⁾ the authors used the least square method for linear regression and calculated the apparent stability constant (Ke') using the following formula.

$$Ke' = \frac{S_i - S_0}{[S_i - (S_i - S_0)][L_i - (S_i - S_0)]}$$

where S_i and L_i are the guest and CD addition concentrations, respectively. S_0 is the solubility of the guest alone.

NMR Spectra NMR spectra were taken on a JEOL-GX-400 spectrometer (^1H , 400 MHz). Samples (10 mg) were dissolved in 10 mg of the solvent.

Results and Discussion

Solubilized Amount Using HPLC, we determined the solubilized amount for each of the eight types of fragrance materials in water in the absence of 2HP- β -CD. Next, we similarly determined the solubilized amount for each type of fragrance material in a solution containing 5% 2HP- β -CD. We then determined the solubilized amount for each type of the fragrance materials from the difference between the solubilities in solutions of 2HP- β -CD and the solubilities in water alone. Thereafter, we calculated the ratio of the guest to the host. Table III shows these results.

TABLE II. The Solvents and Ultraviolet Spectrum (UV) λ_{max} for Each Fragrance Material in the HPLC

Fragrance material	λ_{max} (nm)	Solvent water : methanol
Linalool	215	3 : 7
Citronellol	215	3 : 7
Citral	280	3 : 7
Limonene	215	1 : 9
Methyl ionone	260	1 : 9
Benzyl acetate	256	2 : 8
Linalyl acetate	215	1 : 9
Methyl dihydro-jasmonate	215	3 : 7

TABLE III. The Solubilized Amount of the Fragrance Materials

Fragrance material	Solubility (%) 25 °C		Molar ratio guest/host
	Water	2HP- β -CD 5% soln.	
Linalool	0.15	0.58	0.82
Citronellol	0.02	0.47	0.83
Citral	0.03	0.41	0.71
Limonene	0.003	0.08	0.17
Methyl ionone	0.002	0.25	0.38
Benzyl acetate	0.15	0.63	0.94
Linalyl acetate	0.05	0.30	0.37
Methyl dihydro-jasmonate	0.04	0.42	0.49

TABLE IV. The Relationship between Substitution Degree and Solubilized Amount

Degree of substitution	Benzyl acetate (%)		Linalool (%)	
	2HP- β -CD 5% soln.	2HP- β -CD 10% soln.	2HP- β -CD 5% soln.	2HP- β -CD 10% soln.
3.6	0.55	1.16	0.56	1.08
4.6	0.60	1.11	0.59	1.17
5.5	0.55	1.04	0.56	1.09
8.1	0.52	0.97	0.48	0.97

Substitution Degree and Solubilization of 2HP- β -CD

The solubilization was measured using 2HP- β -CD with a substitution degree of 4.3. However, when producing 2HP- β -CD, scattering occurs in the degree of the substitution. Therefore, we conducted an experiment to examine the change in the solubilization concerning the four lots of the actually produced samples. We selected benzyl acetate and linalool with a relatively large solubilization, and we examined the relationship between substitution degree and solubilization (Table IV).

This result shows that in the substitution degree range of 3.5–8.1 (Nihon Shokuhin Kako), as the substitution degree increases, the solubilization tends to decrease, though only slightly, and there is a peak at 3.5–4.6.

Confirmation of Clathration by ^1H -NMR By using 2HP- β -CD, it was possible to solubilize a considerable amount of the fragrance materials without using a surfactant. We examined them with ^1H -NMR in order to determine whether or not this solubilization phenomenon was based on clathration. In our experiment, we used benzyl acetate as a kind of fragrance material. We prepared the sample to

Fig. 2. The Shift ^1H -NMR Spectra of β -CD and 2HP- β -CD by Solubilizing Fragrance Material

(1) Spectra obtained when 10 mM of benzyl acetate was added to 10 mM of β -CD. (2) Spectra of β -CD. (3) Spectra obtained when 10 mM of benzyl acetate was added to 10 mM of 2HP- β -CD. (4) Spectra of 2HP- β -CD.

Fig. 3. Solubility Phase

Linalool is used to compose the solubility phase.

determine whether equal moles of the host and the guest were contained in each milliliter of heavy water. First, as a model we used β -CD which is known to produce a clathration phenomenon, mixed it with benzyl acetate, and compared its spectrum and that of the β -CD not mixed with a guest. As a result, we confirmed that $\text{C}_2\text{-H}$, $\text{C}_3\text{-H}$ and $\text{C}_6\text{-H}$ shifted to a high magnetic field compared with the other protons.⁵⁻⁷⁾ Next, we conducted an experiment using 2HP- β -CD in the same way. Also in 2HP- β -CD, $\text{C}_2\text{-H}$, $\text{C}_3\text{-H}$ and $\text{C}_6\text{-H}$ shifted to a high magnetic field due to the guest mixture as in the case of the β -CD (Fig. 2). These results indicate that the solubilization of the fragrance materials used in this experiment is based on clathration.

Stability Constant The stability constant is considered to be an important parameter for predicting the clathration phenomenon. According to the usual method^{3,4)} we obtained the apparent stability constant for each fragrance material.

Figure 3 shows the phase solubility diagram of linalool in water with 2HP- β -CD (DS=4.3). The solubility of

TABLE V. Solubilized Amounts and the Apparent Stability Constant

Fragrance material	Apparent stability constant (M^{-1}) $K'_{1:1}$	Solubility 2HP- β -CD 5% soln./water
Linalool	1610	+
Citronellol	3740	+++
Citral	1560	++
Limonene	3350	+++
Methyl ionone	29500	++++
Benzyl acetate	306	+
Linalyl acetate	137	+
Methyl dihydro-jasmonate	712	+

+, The value obtained by dividing the solubility of the perfume in the presence of 2HP- β -CD 5% by the solubility of the perfume dissolved in water is small. ++, The value of the ratio is slightly high. +++, The value of the ratio is quite high. ++++, The value of the ratio is very high.

linalool increased with CD concentration, showing an A_L type solubility diagram according to Higuchi and Connors.⁴⁾ Assuming that a 1:1 complex is initially formed, the apparent stability constant (K_e') of the complex was calculated in terms of Eq. 1,⁴⁾ using the slope and intercept of the straight line of the diagram.

$$K_e' = \frac{\text{slope}}{\text{intercept} (1 - \text{slope})} \quad (1)$$

Also, apparent stability constants of the complexes of other fragrance materials with 2HP- β -CD could be calculated by the same method, because of their similar behavior in aqueous solution containing 2HP- β -CD. As Table V demonstrates, we found a relatively high correlation between the solubilized amount of the fragrance materials in water and 5% 2HP- β -CD aq.

A stability constant is one of the parameter which explains the degree of the contribution of 2HP- β -CD added to raise the solubility of a guest.

For instance, although the stability constant of methyl ionone is very high, methyl ionone hardly dissolves in water, so that the calculated value of the solubility of a guest in a 2HP- β -CD 5% solution and the solubility of a guest in water are very high.

Thus, the comparison between the value of the stability constant and the value obtained by dividing the solubility of the guest in the presence of 2HP- β -CD by the value of the guest dissolved in water shows a considerable correlation.

Although we have omitted details because of space limitations, we also found a considerable correlation in the fragrance material molecular weight, the polarity value, and the amount solubilized.

Conclusions

By using 2HP- β -CD, it was possible to solubilize a considerable amount of fragrance materials without using a surface active agent. We examined them with ^1H -NMR in order to determine whether or not this solubilization phenomenon was based on inclusion. The results of the ^1H -NMR study indicate that the solubilization of the fragrance materials used in this experiment is dependant on formation of an inclusion complex.

The relationship between solubility of guests and degree of substitution on 2HP- β -CD shows that in the substitution degree range of 3.5—8.1, as the substitution degree increases, the solubilized amount tends to decrease, though only slightly, and there is a peak at 3.5—4.6.

From the measured solubilities of the fragrance materials, it has been found that a fragrance material of lower molecular weight or higher polarity tends to be more soluble in water and that solubility correlated fairly well with the apparent stability constant.

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