Inhibitory Effect of 2-Hydroxypropyl-β-cyclodextrin on the Foaming Generated by the Phosphodiester Compound of Vitamin C and E, EPC-K1

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2-Hydroxypropyl-β-cyclodextrin (HP-β-CyD) was examined for potential use as an inhibitor of foaming generated by L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6-yl-hydrogen phosphate] potassium salt (EPC-K1). Ultraviolet (UV), circular dichroism (CD) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopic studies suggested the formation of inclusion complexes of EPC-K1 with HP-β-CyD in aqueous solution. HP-β-CyD inhibited the foaming generated by EPC-K1 in aqueous solution in a concentration dependent manner. The inhibitory effect of HP-β-CyD on the foaming was consistent with a restoring effect on the lowered surface tension of aqueous solutions containing EPC-K1. In addition, there is a negative correlation between the free EPC-K1 concentration calculated from the stability constants of the 1 : 1 and 1 : 2 EPC-K1/HP-β-CyD complexes and the surface tension of aqueous solutions containing EPC-K1 and HP-β-CyD. Therefore, the inhibitory effect of HP-β-CyD on the foaming generated by EPC-K1 could be attributable to the abatement in the surface activity of EPC-K1 by inclusion complexation with HP-β-CyD. These data suggest that HP-β-CyD is useful in topical liquid preparations such as lotions of EPC-K1 used in pharmaceutics and cosmetics.

Key words 2-hydroxypropyl-β-cyclodextrin; EPC-K1; foaming; surface tension; inclusion complex

L-Ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6-yl-hydrogen phosphate] potassium salt (EPC-K1), is a chimera compound of *dl*-α-tocopherol and L-ascorbic acid, showing humectant and antioxidant activities.[1—3] EPC-K1 is a surface active agent due to the presence of both hydrophilic and hydrophobic moieties in the molecule and thus apt to generate foaming in aqueous solution. This physicochemical property of EPC-K1 has up to the present restricted the range of its pharmaceutical and cosmetical applications.

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of covalently linked glucopyranose rings that provide for drug solubilization through the formation of dynamic inclusion complexes.[4] Natural CyDs, especially β-CyD, the glucose heptamer, however, are poorly soluble in water. To improve solubility, various chemical modifications including alkylation and hydroxyalkylation of the hydroxyl group of β-CyD have been conducted.[5,6] Of the modified CyDs evaluated, 2-hydroxypropyl-β-CyD (HP-β-CyD) has been most widely used due to its higher aqueous solubility, superior solubilizing activity and lack of local and systemic toxicities.[7—9]

In this study, the complex formation of EPC-K1 with HP-β-CyD in aqueous solution was investigated by ultraviolet (UV), circular dichroism (CD), and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopic methods. In addition, the effect of HP-β-CyD on the foaming generated by EPC-K1 in aqueous solution was investigated. Furthermore, the mechanism of the inhibitory effect of HP-β-CyD on the foaming is discussed from the viewpoint of the relationship between the complexation and the surface tension of aqueous solutions containing EPC-K1 and HP-β-CyD.

Experimental

Materials EPC-K1 was purchased from Senju Pharmaceutical Co., Inc. (Osaka, Japan). HP-β-CyD was donated by Nihon Shokuhin Kako, Co., Inc. (Shizuoka, Japan); the average substitution degree of 2-hydroxypropyl groups per β-CyD molecule was determined to be 4.3 by means of ^{1}H-NMR.[10] All other chemicals and solvents were of analytical reagent grade and double-distilled water was used throughout the study.

UV and CD Spectroscopies UV and CD spectra were recorded at 25 °C with a Jasco Ubest-55 spectrophotometer and a Jasco J-720 recording polarimeter (Tokyo, Japan), respectively. The concentrations of EPC-K1 and HP-β-CyD were 10.0 and 0—0.5 mM in water, respectively.

^{1}H-NMR Spectroscopy ^{1}H-NMR spectra were recorded at 25 °C on a JEOL JNM-EX400 spectrometer (Tokyo, Japan) operating at 400 MHz. The concentrations of EPC-K1 and HP-β-CyD were 10.0 and 0—50.0 mM in deuterium oxide (D_2O), respectively. ^{1}H-Chemical shifts were measured using acetone as an external reference with an accuracy of ±0.001 ppm.

Foaming Tests Aqueous solutions (50 ml) containing EPC-K1 at the indicated concentration in the absence and presence of HP-β-CyD were added to test tubes (head space volume, 65 ml) and then shaken for 20 min at 300 times/min using an Iuchi shaker NM-1 (Tokyo, Japan) at 25 °C. The time required for complete elimination of the foam was noted.

Surface Tensions The surface tension of EPC-K1 solutions in the absence and presence of HP-β-CyD was measured by using a Wilhelmy type surface tensiometer. In brief, the tip of a glass plate was slung vertically on a surface of the solution (50 ml) and the tensile strength was measured using a Shimadzu surface tensiometer ST-1 (Tokyo, Japan) at 25 °C.

Results and Discussion

Inclusion Complexation of EPC-K1 with HP-β-CyD The inclusion complexation of EPC-K1 with HP-β-CyD was investigated by UV, CD and ^{1}H-NMR spectroscopies. As shown in Fig. 1, EPC-K1 had a UV absorption at 258 nm and a shoulder at about 237 nm and gave two negative and one positive Cotton effects at about 220, 280 and 245 nm, respectively, due to asymmetric carbons in this molecule. HP-β-CyD significantly increased the intensity of the UV absorption at 258 nm and decreased that at 237 nm. By the addition of HP-β-CyD, the intensity of the negative peak of the CD

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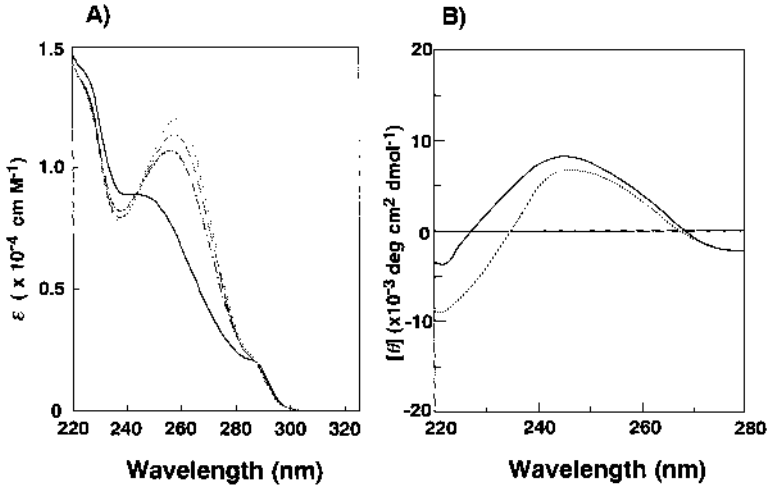

Fig. 1. UV (A) and CD (B) Spectra of EPC-K1 (0.1 mM) in the Absence and Presence of HP-β-CyD (0.1—0.5 mM) in Water at 25 °C

Concentration of HP-β-CyD: ——, 0 mM; –·–·–, 0.1 mM; – – –, 0.3 mM; ------, 0.5 mM.

band at about 220 nm increased and that of 280 nm changed only slightly, whereas that of the positive peak at about 245 nm decreased significantly. These spectral changes clearly indicated that EPC-K1 interacts with HP-β-CyD in aqueous solution.

^{1}H-NMR spectroscopic studies were carried out in detail to gain insight into the interaction of EPC-K1 with HP-β-CyD. Figure 2 shows the ^{1}H-NMR spectra of EPC-K1 (10 mM) in the absence and presence of HP-β-CyD (10 mM) in D_2O solution. The H4, H5 and H6 proton signals of EPC-K1 appear at about 4.2, 3.7 and 3.3 ppm, respectively, and the H16—H18 and H32—H35 methyl signals are observed at about 1.8—2.0 and 0.6 ppm, respectively (see Fig. 2 for the proton numbering). Unfortunately, the assignment of the other protons of EPC-K1 was difficult because of the overlap of the signals. With the addition of HP-β-CyD, the signals of the H4 and the methyl protons shifted downfield (Fig. 2C), whereas those of the H5 and H6 protons overlapped with the HP-β-CyD signals (Fig. 2B). It is of interest to note that the proton signal of EPC-K1 split into several peaks and the intensity of the newly emerged peaks increased at the expense of the original peak intensity, as HP-β-CyD concentration increased. Such spectral changes in the H4 and H32—H35 proton signals are shown in Figs. 3A and 3B, respectively. The intensity of the broad signal of the H4 proton at 4.26 ppm decreased with the concomitant increase of a new signal at 4.35 ppm, as HP-β-CyD concentration increased. Similarly, the intensity of the broad signal of the H32—H35 methyl protons decreased proportionately to the increase of a new doublet signal at about 0.72 ppm and broad signals at 0.64 and 0.68 ppm. The sum of the areas of the new and original peaks was constant. Figures 3C and 3D show changes in the area of the original signal of the H4 and the methyl protons, *i.e.*, the change in $RI_{4.2}$=(area of the 4.20—4.32 signal)/(area of the 4.20—4.40 ppm signals) for the H4 proton and $RI_{0.6}$=(area of the 0.55—0.62 ppm signal)/(area of the 0.55—0.75 ppm signals) for the methyl protons, as a function of the HP-β-CyD/EPC-K1 molar ratio. Both RI values decreased with increasing HP-β-CyD concentration and reached zero at higher concentrations of the host, although the saturation point was different for the $RI_{0.6}$ and $RI_{4.2}$ values. In general, CyD complexes give an averaged NMR signal of free and complexed species when they are in a fast equilibrium with free components. However, there are two possibilities for the signal separation: 1) Chiral discrimina-

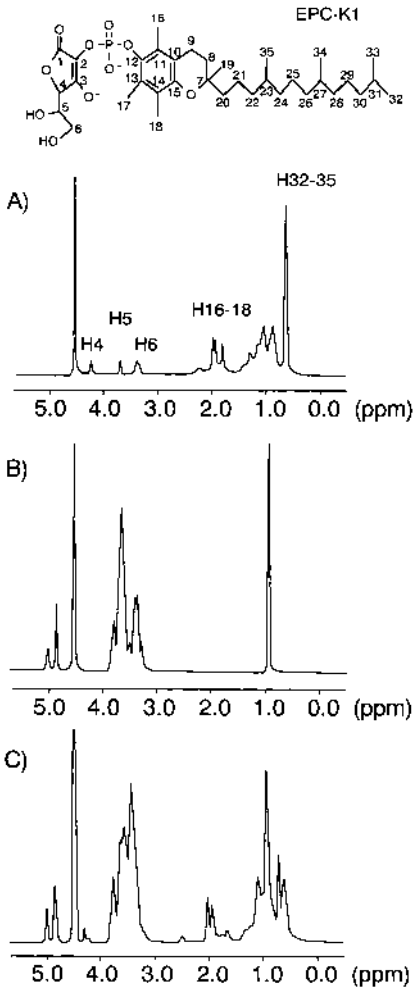

Fig. 2. ^{1}H-NMR Spectra of EPC-K1 (10 mM) in the Absence and Presence of HP-β-CyD (10 mM) in D_2O at 25 °C

(A) EPC-K1 alone, (B) HP-β-CyD alone, (C) EPC-K1 with HP-β-CyD.

tion through CyD inclusion giving signals of each enantiomer and 2) Slow exchange of a guest between the free and complexed states giving signals for both species. The second mechanism seems to be operative in the EPC-K1/HP-β-CyD system, because chiral discrimination should give a 1 : 1 peak arising from each enantiomer, but in the present case the original signals completely disappeared. Therefore, we conclude that EPC-K1 forms a stable inclusion complex with HP-β-CyD, the exchange rate of which is sufficiently slow to be observed on the ^{1}H-NMR time scale.

The signal separation due to the slow exchange rate has been reported for guest molecules such as α,ω-alkanedicarboxylate and substituted viologens having hydrophobic long alkyl chains.[11—14] The change of RI values was quantitatively analyzed to obtain stability constants for the EPC-K1/HP-β-CyD complex. We assumed that two HP-β-CyD molecules are involved in the inclusion of the vitamin E moiety of the EPC-K1 molecule, *i.e.*, 1 : 2 (guest : host) complexation, because of the following reasons: 1) As described above, the saturation point for the RI values is different for the H4 and the methyl protons (H32—H35, Figs. 3C, 3D), suggesting higher-order complexation, 2) vitamin E forms a 1 : 2 complex with 2,6-di-*O*-methyl-β-CyD, as reported previously,[15] 3) the interaction with the highly hydrophilic vitamin C moiety is very weak,[16] and 4) Corey–Pauling–Koltun molecular model constructions indicated that two HP-β-CyD molecules are enough to include the vitamin E moiety. Furthermore, we assumed that the first binding site is the benzopyran and its neighboring methylene moieties and the second one is the remaining alkyl moiety, because of the following reasons: 1) The $RI_{4.2}$ value for the H4 proton in the neighborhood of the vitamin C moiety was saturated at lower concentration of the host, compared with that of the $RI_{0.6}$ value of the H32—H35 methyl groups, and 2) the four methyl groups at 0.60 ppm gave two or three new signals at about 0.64 ppm, 0.68 ppm (shoulder) and 0.72 ppm (doublet) due to the addition of HP-β-CyD, two methyl groups of which shifted at lower HP-β-CyD concentrations followed by the remaining methyl groups at higher HP-β-CyD concentrations. The consecutive 1 : 2 inclusion complexation of EPC-K1 and HP-β-CyD can be described by Eq. 1:

$$[\text{EPC-K1}]_f+[\text{CyD}]_f \overset{K_{1:1}}{\leftrightarrows} [\text{EPC-K1}\cdot\text{CyD}]+[\text{CyD}]_f \overset{K_{1:2}}{\leftrightarrows} [\text{EPC-K1}\cdot 2\text{CyD}] \quad (1)$$

where $[\text{EPC-K1}]_f$=concentration of free EPC-K1, $[\text{CyD}]_f$= concentration of free HP-β-CyD, and $[\text{EPC-K1}\cdot\text{CyD}]$ and $[\text{EPC-K1}\cdot 2\text{CyD}]$=concentrations of 1 : 1 and 1 : 2 complexes, respectively. The 1 : 1 and 1 : 2 stability constants ($K_{1:1}$ and $K_{1:2}$) and the mass balance are described by Eqs. 2—5:

$$K_{1:1}=[\text{EPC-K1}\cdot\text{CyD}]/([\text{EPC-K1}]_f[\text{CyD}]_f) \quad (2)$$

$$K_{1:2}=[\text{EPC-K1}\cdot 2\text{CyD}]/([\text{EPC-K1}\cdot\text{CyD}][\text{CyD}]_f) \quad (3)$$

$$[\text{EPC-K1}]_t=[\text{EPC-K1}]_f+[\text{EPC-K1}\cdot\text{CyD}]+[\text{EPC-K1}\cdot 2\text{CyD}] \quad (4)$$

$$[\text{CyD}]_t=[\text{CyD}]_f+[\text{EPC-K1}\cdot\text{CyD}]+2[\text{EPC-K1}\cdot 2\text{CyD}] \quad (5)$$

where $[\text{EPC-K1}]_t$ and $[\text{CyD}]_t$ are total concentrations of EPC-K1 and HP-β-CyD, respectively. As described above, the RI value expressed a fraction of free guest molecule. Therefore, the concentration of the 1 : 1 and 1 : 2 complexes

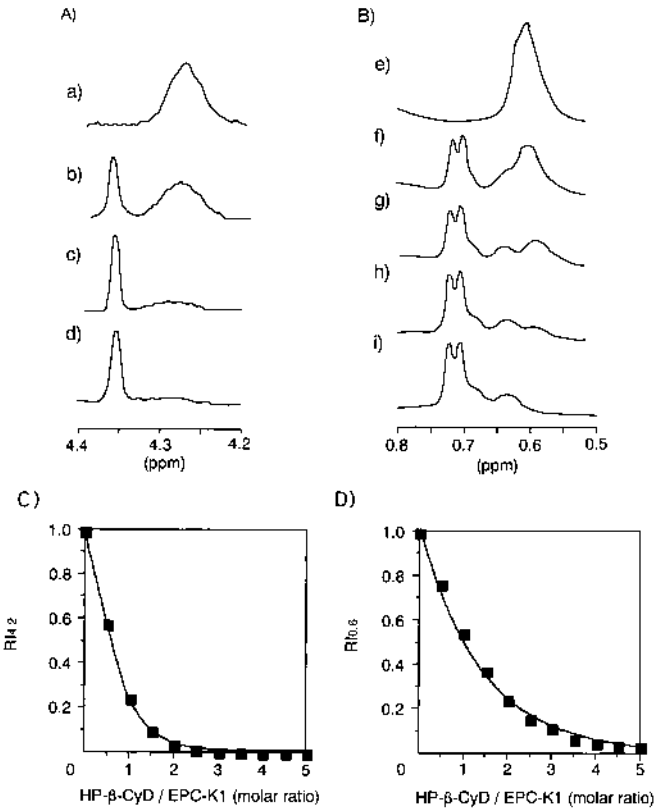

Fig. 3. Effect of HP-β-CyD Concentration on ^{1}H-NMR Spectra of the H4 Proton (A) and the H32—H35 Methyl Protons (B) in D_2O at 25 °C and Change of the $RI_{4.20}$ (C) and the $RI_{0.6}$ Values (D) as a Function of Host/guest Molar Ratio

The concentration of EPC-K1 is 10 mM and that of HP-β-CyD is described below: (a), (e), 0 mM; (b), 5 mM; (c), (f), 10 mM; (d), (g), 20 mM; (h), 30 mM; (i), 40 mM.

can be described by Eq. 6, in terms of the $RI_{4.2}$ value, by assuming that the chemical shift of the H4 was changed by the 1 : 1 complexation, but not by the 1 : 2 complexation, because the H4 proton of the vitamin C moiety is far from the terminal alkyl chain of the 1 : 2 binding site.

$$[\text{EPC-K1}]_t(1-RI_{4.2})=[\text{EPC-K1}\cdot\text{CyD}]+[\text{EPC-K1}\cdot 2\text{CyD}] \quad (6)$$

On the other hand, the concentration of the complexes can be described by Eq. 7, in terms of the $RI_{0.6}$ value of the four methyl groups, where two methyl groups are involved in the 1 : 1 complexation and four methyl groups in the 1 : 2 complexation. Thus, each coefficient (4, 2, 4) in Eq. 7 represents the number of methyl groups involved in the complexation, respectively.

$$4[\text{EPC-K1}]_t(1-RI_{0.6})+2[\text{EPC-K1}\cdot\text{CyD}]+4[\text{EPC-K1}\cdot 2\text{CyD}] \quad (7)$$

Eqs. 8 and 9 for $K_{1:1}$ and $K_{1:2}$ were derived from Eqs. 1—7.

$$K_{1:1}(\text{M}^{-1})=\{2(1-RI_{4.2})-(1-RI_{0.6})\}\times 1000/\{[\text{CyD}]_t-2(1-RI_{0.6})[\text{EPC-K1}]_t\}RI_{4.2} \quad (8)$$

$$K_{1:2}(\text{M}^{-1})=\{2(1-RI_{0.6})-(1-RI_{4.2})\}\times 1000/\{2(1-RI_{4.2})-(1-RI_{0.6})\{[\text{CyD}]_t-2(1-RI_{0.6})[\text{EPC-K1}]_t\} \quad (9)$$

The 1 : 1 and 1 : 2 stability constants were obtained by measuring changes in the $RI_{0.6}$ and $RI_{4.2}$ values as a function of HP-β-CyD concentration and analyzed using by Eqs. 8 and 9. The results were as follows: $K_{1:1}=2.08\times 10^3\ \text{M}^{-1}$ and

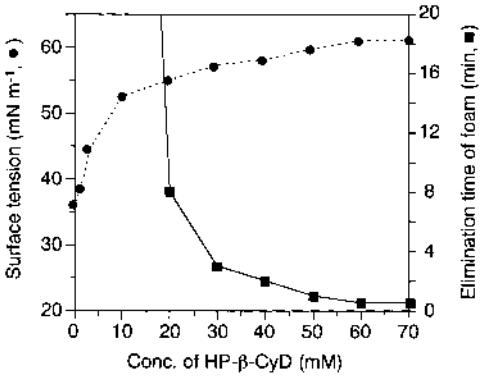

Fig. 4. Effect of HP-β-CyD Concentration on the Foaming (■) and the Surface Tension (●) in the EPC-K1 Solution

The EPC-K1 (0.2 mM) solution in the absence and presence of HP-β-CyD shaken for 20 min at 25 °C, the time by which the foam was eliminated was noted. The surface tension was measured by the Wilhelmy method at 25 °C.

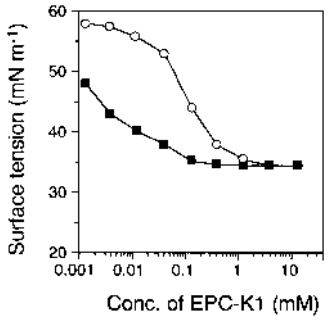

Fig. 5. Effect of EPC-K1 Concentration on the Surface Tension of EPC-K1 Solutions in the Absence (○) and Presence (■) of HP-β-CyD

The concentration of HP-β-CyD added was 3.6 mM. The surface tension was measured by the Wilhelmy method at 25 °C.

$K_{1:2}=4.85\times10^2\,\mathrm{M}^{-1}$ at a molar ratio of $[\text{EPC-K1}]_t/[\text{CyD}]_t=0.5$, $K_{1:1}=2.40\times10^3\,\mathrm{M}^{-1}$ and $K_{1:2}=2.5\times10^2\,\mathrm{M}^{-1}$ at 1.0, $2.15\times10^3\,\mathrm{M}^{-1}$ and $2.34\times10^2\,\mathrm{M}^{-1}$ at 1.5, ... and $2.21\times10^3\,\mathrm{M}^{-1}$ and $4.46\times10^2\,\mathrm{M}^{-1}$ at 5.0. Here the larger deviation of the $K_{1:2}$ value may be due to the broad ^{1}H-NMR signals. The averaged $K_{1:1}$ and $K_{1:2}$ values are 2.25×10^3 and $3.47\times10^2\,\mathrm{M}^{-1}$, respectively. Previous papers reported that the slow exchange of CyD complexes into free components may occur when they have a stability constant higher than $10^4\,\mathrm{M}^{-1}$.[12—14] The EPC-K1/HP-β-CyD complex had a 1 : 1 stability constant lower than $10^4\,\mathrm{M}^{-1}$. However, EPC-K1 and HP-β-CyD formed 1 : 1 and 1 : 2 complexes and these EPC-K1/HP-β-CyD complexes have relatively larger stability constants under this experimental condition. These results suggest that two HP-β-CyD molecules are cooperatively involved in the inclusion, forming a stable 1 : 2 complex with a slow exchange rate.

Inhibitory Effect of HP-β-CyD on the Foaming A foam was generated by shaking EPC-K1 solutions, and then the time required to eliminate the foam was measured. The addition of HP-β-CyD into EPC-K1 solutions markedly accelerated the elimination rate of foams in a concentration dependent manner (Fig. 4). HP-β-CyD also inhibited the generation of foams (data not shown). The surface tension of EPC-K1 solutions was increased by the addition of HP-β-CyD in a concentration dependent manner (Fig. 4). The restoring effect of HP-β-CyD on the surface tension was observed at higher concentrations of EPC-K1, as shown in Fig. 5, although the effect was diminished when the concentration of EPC-K1 was higher than that of HP-β-CyD, probably because of an increase in the fraction of the free guest molecule. In addition, the critical micelle concentration (CMC) values of EPC-K1 in the absence and presence of HP-β-CyD were *ca.* 0.4 and 4 mM under these experimental conditions, respectively. The increase in CMC values caused by the presence of HP-β-CyD indicates that the complexation of EPC-K1 with HP-β-CyD decreases the micellar formation ability of EPC-K1, although the difference in the free energy values for the CyD complexation and micelle formation of EPC-K1 is unknown. Therefore, the inhibitory effect of HP-β-CyD on the foaming may be correlated with an increasing effect on the surface tension.

Anti-foams are classified into foam breakers and foam inhibitors.[17,18] The foam breakers act upon the foams already formed by spreading on the surface of the foam membrane, whereas the foam inhibitors act to inhibit the foaming by driving out the foaming agent from the surface of the foam membrane. Under the present experimental conditions, HP-β-CyD was added to EPC-K1 solution before shaking, thus it is likely that HP-β-CyD predominantly plays a role as a foam inhibitor.

Most foam inhibitors such as higher alcohols, higher fatty acids and silicone oils are surfactants which possess lower hydrophile-lipophile balance (HLB) values.[19] The inhibitory effects of such foam inhibitors could be attributed to the competitive effect regarding an adsorption of the foaming agent to a water/air surface. However, the inhibitory mechanism of HP-β-CyD seems to be different from those of the previously mentioned foam inhibitors, because HP-β-CyD is significantly hydrophilic. In addition, the surface tension values of EPC-K1 solutions containing HP-β-CyD augmented to a maximum value (60 mN m^{-1}) that corresponds to that of the solution containing HP-β-CyD alone, as HP-β-CyD concentration increased, *i.e.*, the surface tension of the solution containing 0.2 mM of EPC-K1 and 70 mM of HP-β-CyD was 60 mN m^{-1} and that containing 70 mM of HP-β-CyD alone was 60 mN m^{-1} (see Fig. 4). These results suggest that the surface activity of EPC-K1/HP-β-CyD solutions originates with the free guest molecule. Thus, we examined the relationship between the concentration of free EPC-K1 and surface tension (Fig. 6). Here, the concentration of free EPC-K1 was calculated as follows: firstly, free HP-β-CyD concentration was calculated by substituting the data of Fig. 4 and the $K_{1:1}$ and $K_{1:2}$ values in Eqs. 10 and 11 derived from Eqs. 1—4.

$$K_{1:1}\cdot K_{1:2}[\text{CyD}]_f^3+\{K_{1:1}\cdot K_{1:2}(2[\text{EPC-K1}]_t\cdot[\text{CyD}]_t+K_{1:1}])\}[\text{CyD}]_f^2 +(1+K_{1:1}[\text{EPC-K1}]_t-K_{1:1}[\text{CyD}]_t)[\text{CyD}]_f-[\text{CyD}]_t \quad (10)$$

$$[\text{EPC-K1}]_f=(2[\text{EPC-K1}]_t-[\text{CyD}]_t+[\text{CyD}]_f)/(2+K_{1:1}[\text{CyD}]_f) \quad (11)$$

As shown in Fig. 6, a good linear negative correlation ($r=-0.994$) was obtained between the free EPC-K1 concentration and the surface tension of aqueous solutions containing EPC-K1 and HP-β-CyD. These results suggest that HP-β-CyD inhibited the foaming of EPC-K1 through forming an inclusion complexation with the guest rather than a competitive effect on the adsorption of the guest to the air/water surface.

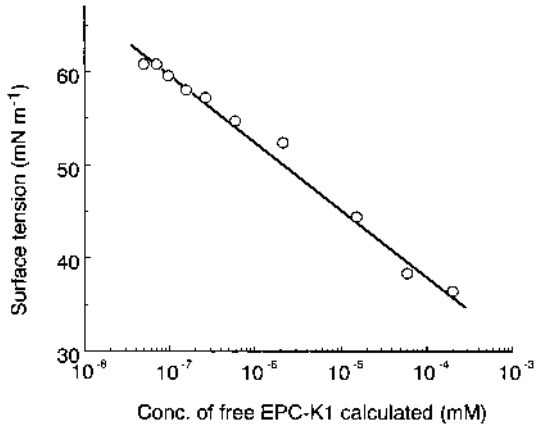

Fig. 6. Relationship between the Concentration of Free EPC-K1 and the Surface Tension of an Aqueous Solution Containing EPC-K1 and HP-β-CyD

The concentration of free EPC-K1 was calculated from the $K_{1:1}$ and $K_{1:2}$ stability constants determined by the NMR spectroscopic method described in the text.

In conclusion, our results indicate that HP-β-CyD inhibits the foaming generated by shaking an EPC-K1 solution by reversing the decrease in surface tension of an EPC-K1 solution *via* an inclusion complexation. The data suggest that HP-β-CyD is useful in preparing topical liquid preparations such as lotions of EPC-K1 used in pharmaceutics and cosmetics.

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