Solubilization of Synthetic Perfumes by Nonionic Surfactants and by Sulfoalkyl Ether *β*-CDs

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Abstract

Head-space gas chromatography has been used to study the solubilization of synthetic perfumes by nonionic surfactants and by sulfoalkyl ether *β*-cyclodextrins. This study shows that the addition of nonionic surfactants reduces the vapor pressure of these perfumes due to reduction of the free perfume concentration in water caused by partition of the solute between surfactant micelles and water. The relationship between solubilization capacity of nonionic surfactants and characteristics of both the surfactant and the synthetic perfumes was evaluated. Similar vapor pressure reduction also observed in perfumes in sulfoalkyl ether *β*-cyclodextrins. The stability of the inclusion complexes depends on the structures of both perfume molecules and substitution group on sulfoalkyl ether *β*-cyclodextrins. The results also indicated that in some cases there may be more than one perfume molecule included in a *β*-CD molecule while there is no indication of complexes formed between more than one *β*-CD molecule and a single perfume molecule.

Introduction

Cyclodextrins are known by their ability to include a wide range of guest molecules to form noncovalently bonded inclusion complexes. The stability of these inclusion complexes in solution is characterized by the stability constant or dissociation constant. The stability constant can be defined as the equilibrium constant of the formation of the inclusion complex in the form of

$$K = \frac{[\text{Complex}]}{[\text{CD}] \cdot [\text{Guest}]}, \quad (1)$$

where [Complex], [CD] and [Guest] represent the concentration of cyclodextrin complex, empty cyclodextrin and free (un-included) guest molecules, respectively.

In many industrial applications of cyclodextrins such as cosmetic or pharmaceutical formulation, the final effects of the addition of cyclodextrin depend on the stability of the inclusion complexes as well as the ability to release active ingredients at desired conditions. It is believed that the inclusion complexes with stability constants around 2000 M^{-1} are preferred for practical applications [1].

Several experimental methods have been developed to study cyclodextrin inclusion complexes in solution including maximum solubility method, conductometry, microcalorimetry, potentiometry, circular dichroism, NMR, fluorescence, UV/Visible spectrometry, HPLC and head-space gas chromatography. Solubility method is the simplest and most used method for slightly water soluble guest molecules

while UV/Visible spectroscopy [2, 3] and HPLC [4] are also widely used. Head-space GC has been used in study of solute-surfactant association equilibria of volatile organic solutes [5, 6] and it can be used for study of volatile organic inclusion complexes with cyclodextrins. However, there are not many publications on head-space GC in this area [7].

Head-space gas chromatography is a technique to determine physical properties of condensed phase by analyzing gas phase, into which some part of the components of the condensed phase are partitioned with the establishment of equilibrium [8]. If volatile components are present in an equilibrium in solution, the analysis of equilibrium vapor permits the determination of equilibrium constants in solution. Association constants of volatile organic compounds can be obtained by head-space gas chromatography [9, 10].

When a complex is in solution or liquid form, head-space GC can give direct measurements of volatility reduction by inclusion complexation between cyclodextrin and volatile organic substances. A head-space GC experiment has shown that the addition of cyclodextrin to an aqueous solution of aroma components has depressed the vapor pressure of these aroma components and increased the retention time of aroma from evaporation. Because the vanishing rate of aroma components is proportional to the concentration of free aroma components in solution, head-space gas chromatography can provide direct measurement of the free aroma component.

Synthetic perfumes are widely used in various commercial products. If solubility of the perfumes in the medium is insufficient to provide desired odor intensity, a solubilizer must be used to increase the solubilization of the perfume. Nonionic surfactants usually serve as such a solubilizer. Eth-

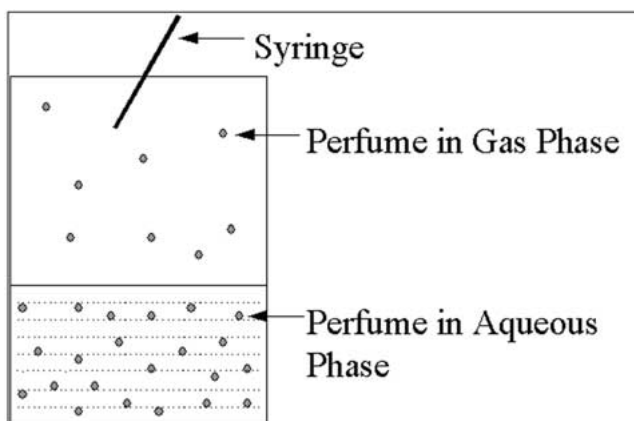* Present address BJ Services, U.S.A., 16610 Aldine-Westfield Road, Houston, TX 77032, U.S.A.

Figure 1. Schematic diagram of head-space GC technique.

oxylated fatty alcohols based on C_{12} to C_{18} alcohols and seven to nine ethoxyl units have proved very useful [11]. Solubilization of synthetic perfumes by anionic and/or nonionic surfactant systems has been systematically studied by Abe's research group [12–14]. The solubilization behavior of synthetic perfumes has been characterized by their hydrophobic property as well as the synergistic effect of anionic/nonionic surfactant mixtures.

Cyclodextrins can be excellent solubilizers for various flavor components to improve their solubility, their stability to heat, to oxygen and to light, and reduce their volatility. However, systematic information on cyclodextrin inclusion complexation of synthetic perfumes have not been reported [15]. The effectiveness of cyclodextrin complexation to a targeted ingredient depends on the association constant of the CD inclusion complex and competition from other ingredients in the formulation. This work studied the inclusion complexation between sulfoalkyl ether β-cyclodextrins and synthetic perfumes and the association constants of the inclusion complexes are strongly influenced by the structure of the perfumes and of the CD derivatives.

Head-space GC for inclusion complexation study

The head-space GC technique is based on the equilibrium distribution of the substance between the coexisting phases. For many dilute liquid-gas systems a direct proportionality between the concentration of a given component in the equilibrium phases can be established.

$$C^g = KC^1.$$

Here K is distribution coefficient, and C^g and C^1 represent the concentrations of a given substance in gas phase and liquid phase, respectively.

One of the techniques to measure K is gas chromatography. The head-space GC analysis used in present study is schematically illustrated in Figure 1.

The investigated solution with known concentration of the synthetic perfumes C_1^0 is placed in a fixed volume container. The container is kept at constant temperature to establish the liquid-gas phase equilibrium. The quantitative

representation of head-space GC is accomplished by standard calibration of peak area of GC measurement against C_1^0. However, due the analysis of a large number of samples over a wide range of concentrations in current study, the calculation of sample concentration in the liquid phase was done according to a calibration. This method has been used almost exclusively in solubilization study of volatile substance by surfactant micelle solutions and by cyclodextrin solutions. The absolute concentration of synthetic perfumes in vapor phase can be estimated, provided that a relationship between peak area of GC measurement and concentration of the perfumes has been established [16]. In the presence of surfactant micelles or cyclodextrins, the vapor pressure of the perfumes was decreased and the peak area vs. perfume concentration is interpreted in terms of the fraction of perfume bound to the micellar aggregates or inclusion complexed by cyclodextrins.

The computation of the fraction of free perfume and bound perfume can be illustrated by Figure 2. The horizontal separation between two curves at any ordinate represents A and B. B corresponds to the perfume fraction solubilizing in the micellar phase or included by cyclodextrin cavity, while A is the fraction remaining in the aqueous phase as free perfume. By knowing the fractions of perfume in both aqueous phase and the bonded phase, a distribution constant can be calculated.

Assuming that a partition-like interaction is responsible for the perfume distribution between surfactant micellar pseudophase and water phase, the overall amount of perfume in solution is divided into perfume incorporated into micellar phase and associated with water phase. The water and micellar pseudophases are interspersed but remain chemically distinct, with characteristic properties. The concentration based partitioning of perfume between these two pseudophases can be defined as

$$\frac{C_{\text{Tot.}}}{C_w} = \frac{C_w + C_{\text{mic.}}}{C_w} = 1 + \frac{C_{\text{mic.}}}{C_w}. \qquad (2)$$

where $C_{\text{tot.}}$ is the total concentration of perfume in solution, C_w and $C_{\text{mic.}}$ are the concentration of perfume in "water" pseudophase and in micellar pseudophase, respectively. The partition coefficient is defined as

$$K_{mw} = \frac{\left(\dfrac{\text{Perfume Concentration in Micellar Phase}}{\text{Concentration of Surfactant in Micellar Phase}} \right)}{\text{Perfume Concentration in Water Phase}}. \qquad (3)$$

Then Equation (2) can be modified to

$$\frac{C_{\text{tot.}}}{C_w} = 1 + K_{mw} \cdot [\text{Surf.}], \qquad (4)$$

where [Surf.] is total concentration of surfactant and when total concentrations are well above the CMC, [Surf.] $\gg$ CMC. The total concentration of perfume is known and the free perfume in water phase can be measured by head-space GC experimentally. The partition coefficient, K_{mw}, then can be estimated from the slope of the plot of $C_{\text{tot.}}/C_w$ vs. [Surf.].
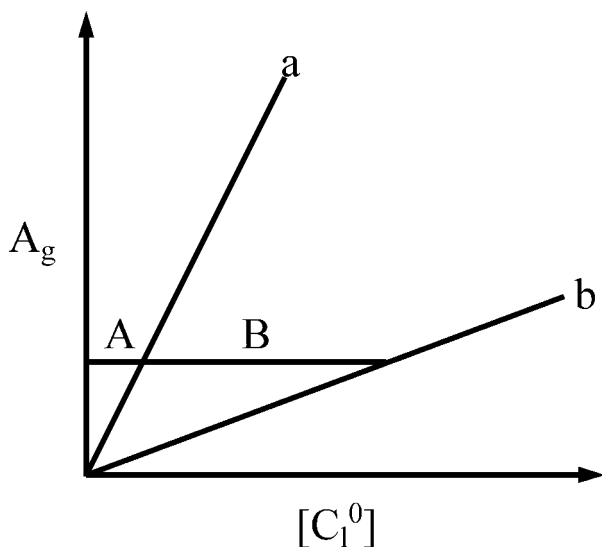
Figure 2. Peak area plot for a perfume in solution in the absence of surfactant or cyclodextrin (a) and in the presence surfactant or cyclodextrin (b).

In the case of cyclodextrin inclusion complexation, the association constant, K, of the inclusion complex can be represented by the following derivations, assuming 1:1 complexation:

$$[CD]_t = [CD]_f + [CD\text{-}S]$$

$$[S]_t = [S]_f + [CD\text{-}S]$$

$$K = \frac{[CD\text{-}S]}{[CD]_f \cdot [S]_f} \tag{5}$$

$$[CD]_t = \frac{1}{K}\left(\frac{[S]_t}{[S]_f} - 1\right) + ([S]_t - [S]_f), \tag{6}$$

where $[CD]_t$, $[CD]_f$ and $[CD\text{-}S]$ are the total cyclodextrin concentration, free cyclodextrin concentration and cyclodextrin inclusion complex concentration, respectively. $[S]_t$ and $[S]_f$ are the total perfume concentration and free perfume concentration in the aqueous phase. By utilizing nonlinear least square regression, the association constant can be computed.

Experimental

All studies were performed using a HP5890 gas chromatograph equipped with a flame ionization detector. The analyses were done on a 10 m × 0.53 mm id capillary column with a stationary phase of polydimethylsiloxane. Helium was used as carrier gas at various flow rates depending on the particular perfume analyzed. A HP 2310 integrator was used to record the signals. Carrier gas flow rate and experimental temperature range were adjusted to meet the most appropriated experimental condition for each perfume.

Sulfoalkyl ether β-cyclodextrins were synthesized and characterized as described previously [20]. Sulfoethyl ether β-CD has an average degree of substitution of 3.36 and contain 1.19 water per CD; sulfopropyl ether β-CD has an average degree of substitution of 1.6 and with 4.62 water per CD and the average degree of substitution of sulfobutyl ether β-CD is 2.46 with 4.53 water per CD. The average molecular weights for sulfoethyl, sulfopropyl and sulfobutyl ether β-CDs are 1593.2, 1448.6 and 1605.2, respectively. Benzyl alcohol (>99%), benzyl acetate (>99%), phenethyl alcohol (>99%), phenethyl acetate (>99%) and ethyl phenylacetate (>99%) were purchased from Aldrich and used as received. Polyethylene glycol mono-p-nonylphenyl ether with 10, 15 and 20 oxylethylate units and $C_{16}POE_{20}$ were used as received from TCI, Japan. All the solutions were prepared with deionized water. A sample solution (10 mL) was placed in an EPA certified 40 ml sample vial, sealed airtight with a silicon septum, and placed in a water bath at 20 °C for 24 hours to achieve equilibrium before injection. A gas-tight syringe was used for the sample injection and all samples were injected manually. A 20 μl sample was injected into gas chromatograph each time. Table 1 illustrates all the structures and physical constants of synthetic perfumes and nonionic surfactants.

Results and discussion

All data on nonionic surfactant micellar solutions were analyzed according to Equation (3) to calculate the partition coefficient of perfumes by plotting total perfume concentration over free perfume concentration as a function of total surfactant concentrations. A clear linear relationship was obtained in each system. The linear curve gave an intercept of 1 within the experimental error, which supports the partition-like interaction model. From the slope of each straight line, the value of the partition coefficient of each synthetic perfume between water pseudophase and surfactant micellar pseudophase can be calculated (Table 2).

The calculated K_{mw} indicates that in each surfactant solution the partition coefficients are in the order of benzyl alcohol < phenethyl alcohol < benzyl acetate < ethyl phenylacetate < phenethyl acetate. For a homologous series of synthetic perfumes, the perfume with higher hydrophobicity has higher partition coefficient in surfactant micelles. Generally speaking, the alcohol is more hydrophilic or less hydrophobic than acetate while molecules with longer hydrocarbon chains are more hydrophobic than those with short hydrocarbon chains. Benzyl alcohol is the most polar perfume and has the highest water solubility in the absence of surfactant which means it has the lowest hydrophobicity. Thus it has the lowest partition coefficient in each surfactant solution. On the other hand, phenethyl acetate has the lowest water solubility or highest hydrophobicity and the highest partition coefficient.

For the apolar esters, such as benzyl acetate, ethyl phenylacetate and phenethyl acetate, the partition coefficients in different surfactant micelles are approximately the

Table 1. Structure, molecular weight and abbreviation of nonionic surfactants and synthetic perfumes

Structure	Name	MW	Abbreviation
$C_9H_{19}C_6H_4(OCH_2CH_2)_{10}OH$	Polyoxyethylenated p-nonylphenol ($n = 10$)	660.9	APE ($n = 10$)
$C_9H_{19}C_6H_4(OCH_2CH_2)_{15}OH$	Polyoxyethylenated p-nonylphenol ($n = 15$)	881.1	APE ($n = 15$)
$C_9H_{19}C_6H_4(OCH_2CH_2)_{20}OH$	Polyoxyethylenated p-nonylphenol ($n = 20$)	1101.4	APE ($n = 20$)
$C_{16}H_{33}(OCH_2CH_2)_{20}OH$	Polyoxyethylenated straight-chain alcohol	1123.5	AE ($n = 20$)
$C_6H_5CH_2OH$	Benzyl alcohol	108.14	BA
$C_6H_5CH_2CH_2OH$	Phenethyl alcohol	122.17	BAC
$C_6H_5CH_2OCOCH_3$	Benzyl acetate	150.18	PEA
$C_6H_5CH_2CH_2OCOCH_3$	Phenethyl acetate	164.20	PEC
$C_6H_5CH_2COOCH_2CH_3$	Ethyl phenylacetate	164.20	EPC

Table 2. K_{mw} of synthetic perfumes in nonionic surfactants

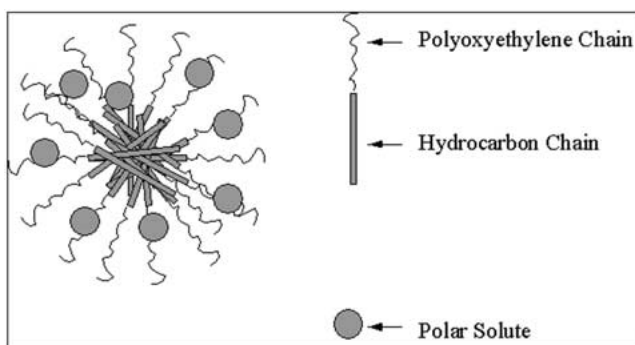|	APE ($n = 10$) M^{-1}	APE ($n = 15$) M^{-1}	APE ($n = 20$) M^{-1}	AE ($n = 20$) M^{-1}
Benzyl alcohol	8.0 ± 0.8	14.6 ± 0.9	16.3 ± 0.5	19.3 ± 0.6
Benzyl acetate	29.4 ± 1.0	33.5 ± 1.9	30.2 ± 3.4	35.6 ± 1.4
Phenethyl alcohol	11.4 ± 0.5	14.3 ± 0.8	22.2 ± 1.3	23.1 ± 1.4
Phenethyl acetate	64.7 ± 1.1	57.8 ± 1.7	63.0 ± 3.0	57.5 ± 3.8
Ethyl phenylacetate	55.7 ± 2.3	51.9 ± 1.1	51.2 ± 1.7	47.0 ± 3.5

Figure 3. Schematic diagram of polar solute solubilization in nonionic surfactant.

same within experimental error. This suggests that each surfactant has similar solubilization capacity to these esters. This fits well with a solubilization model illustrated in Figure 3. For a surfactant containing polyoxyethylene chain as its head group, the micelles could consist of two parts, an inner core of hydrocarbon tails and an outer shell of hydrated polyoxyethylene. Polar organic solutes such as benzyl alcohol or phenol, which have strong affinity to the polyoxyethylene group, may be incorporated at least partly into the outer shell. This type of solubilization is classified as inclusion into the polyoxyethylene exterior of micelles rather than as adsorption on the surface of micelles [17].

Apolar solutes such as esters are mainly solubilized inside the micellar core or deeply within the palisade layer. The incorporation site of a solubilized solute depends on its relative hydrophobic and hydrophilic tendencies. Because of the hydrocarbon tails of the nonionic surfactants used, the solubilization capacities of these surfactants should be in the same range for an apolar solute. Also, partition coefficients of apolar esters in these surfactants are in the same range. It was reported that solubilization of benzene by nonionic surfactants is only slightly decreased when the EO number in the nonionic surfactants varies from 9 to 40. Particularly, at relatively low surfactant concentration there is no significant difference in the benzene solubilization in various nonionic surfactants with different EO numbers [10]. Our results are in agreement with the above observation and the solubilization of apolar solutes being independent of the EO unit number of the nonionic surfactants.

For benzyl alcohol or phenethyl alcohol in the above surfactant solutions, the partition coefficients increase in the order of APE ($n = 10$) < APE ($n = 15$) < APE ($n = 20$) < $C_{16}POE_{20}$. This is in agreement with the prediction from the above solubilization model. Polar solutes such as benzyl alcohol are solubilized within the palisade layer of EO units surrounding the hydrophobic core. The solubilization of polar solutes increases with the EO unit number in the nonionic surfactants. The partition coefficient for each synthetic perfume is plotted against the surfactant type (Figure 4).

Head-space GC measurement of the same synthetic perfumes in sulfoalkyl ether β-CDs was conducted. By assuming 1:1 inclusion complexation, the stability constant, K, can be estimated from the parameter P_1 that is obtained from nonlinear least square regression in each plot of synthetic perfumes in sulfoalkyl ether β-CDs by $P_1 = 1/K$. Table 3 lists all the calculated Ks.

The stability constant of inclusion complexes formed between synthetic perfumes and each sulfoalkyl ether β-CD is in the order of benzyl alcohol < phenethyl alcohol < benzyl acetate < ethyl phenylacetate < phenethyl acetate,

Table 3. Stability constants of inclusion complexes between synthetic perfumes and sulfoalkyl ether β-CDs

	Sulfoethyl ether β-CD (M^{-1})	Sulfopropyl ether β-CD (M^{-1})	Sulfobutyl ether β-CD (M^{-1})
Benzyl alcohol	13	12	12
Benzyl acetate	92	158	124
Phenethyl alcohol	24	43	37
Phenethyl acetate	106	397	262
Ethyl phenylacetate	195	206	238
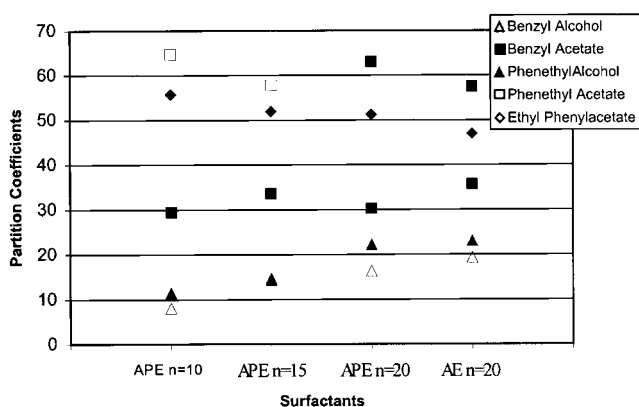
Figure 4. Partition coefficients of synthetic perfumes in nonionic surfactants.

Figure 5. Benzyl alcohol in surfactants and sulfoalkyl β-CDs.

Figure 6. Benzyl acetate in surfactants and sulfoalkyl β-CDs.

except for the sulfoethyl ether β-CD. This order is similar to that of partition coefficients in nonionic surfactants.

The binding between cyclodextrins and guest perfumes depends on both hydrophobicity of the guest molecule and the geometric accommodation of the guest molecule into the CD cavity. When the hydrophobicity of various guest molecules differs greatly and their sizes and geometric structures are similar, the stability constants of the inclusion complexes are mainly determined by the hydrophobicity of the guest molecules, such as is the case with benzyl alcohol compared to benzyl acetate and phenethyl alcohol compared to phenethyl acetate. The more hydrophobic the guest molecule, the more stable the inclusion complex becomes. When the geometric structure and size of the guest molecules have significant differences and their hydrophobicity is similar, the geometric fit of the guest molecule into the CD cavity becomes the main factor in determining the stability of the inclusion complexes. Among the three esters, benzyl acetate forms the least stable inclusion complex and phenethyacetate forms the most stable complex with each of the sulfoalkyl ether β-CDs, except with sulfoethyl ether β-CD.

The trend is not as obvious as that for different perfumes in the same sulfoalkyl ether β-CD. Most of the perfumes form the more stable inclusion complex with sulfopropyl β-CD, followed by that with sulfobutyl ether β-CD and with sulfoethyl ether β-CD. A low average substitution degree of sulfopropyl ether β-CD could be the reason. It was suggested that the high substitution degree of β-CD derivatives
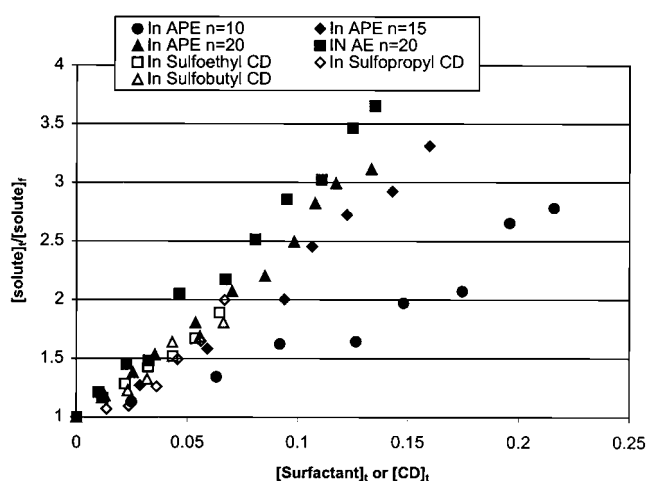
may block the accessibility of the guest molecule to the CD cavity [19]. Another factor influencing stability constant is the size of the substituent group. A bulky substituent group limits the accessibility to CD cavity. Combination of several factors such as hydrophobicity of the substituent group, average substitution degree, geometric feature of the substituent group, and positional distribution of the substitution must be considered [20].
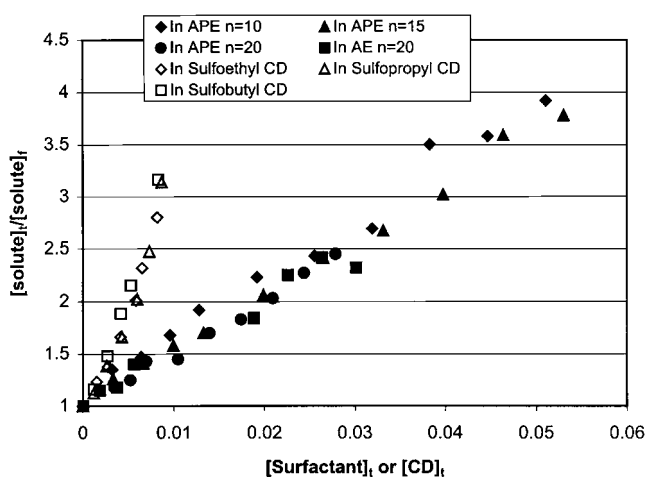

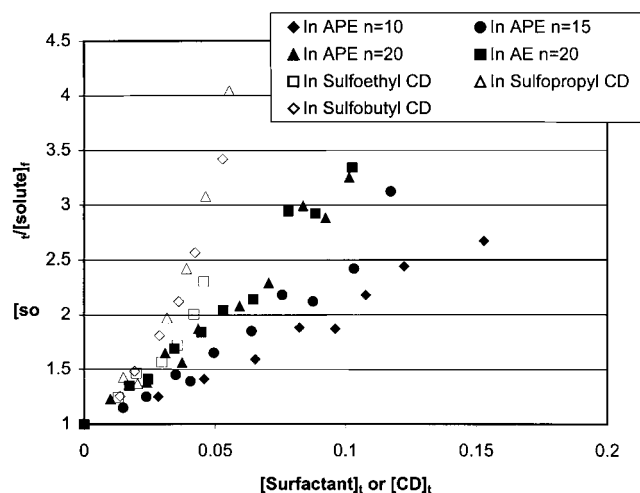*Figure 7.* Ethyl phenylacetate in surfactants and sulfoalkyl β-CDs.

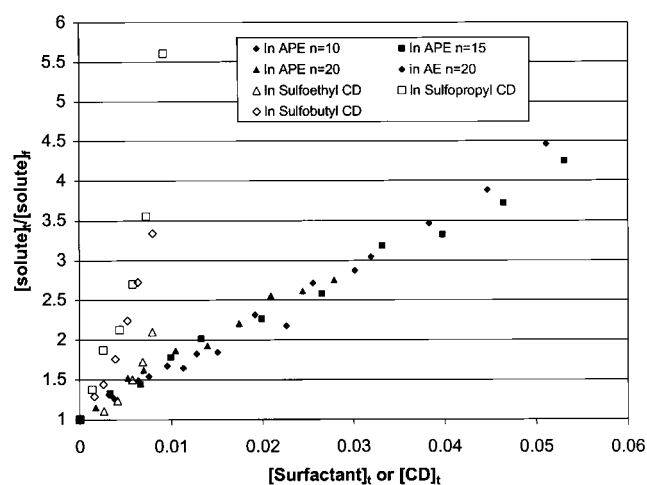*Figure 8.* Phenethyl alcohol in surfactants and sulfoalkyl β-CDs.

Figure 9. Phenethyl acetate in surfactants and sulfoalkyl β-CDs.

β-CDs that can be added to the liquid phase is less than 0.5; otherwise, the gas phase has too little perfume to be detected by head space GC. Unfortunately, it is not possible to have a clear indication to predict the complexation stoichiometry through Job plot analysis.

Conclusion

Head-space gas chromatography has been used to study the solubilization of synthetic perfumes by nonionic surfactants and by sulfoalkyl ether β-cyclodextrins. This study has shown that both nonionic surfactants and cyclodextrin derivatives reduce the vapor pressure of these perfumes. The solubilization capacity of nonionic surfactants depends on properties of both the surfactant and synthetic perfumes. Sulfoalkyl ether β-CDs can form stable inclusion complexes with a wide range of guest molecules. The sulfoalkyl ether β-CDs synthesized in this work have the combined characteristics of low average substitution degree and high water solubility, which will confer great advantages in their possible industrial applications as compared with other β-CD derivatives.

Head-space GC is an effective way to characterize the inclusion complexes formed between sulfoalkyl ether β-CDs and synthetic perfumes. The stability of the inclusion complexes depends on both hydrophobicity of the guest molecules and the size of the substituent on β-CD and degree of the substitution, while the degree of substitution is especially important. The results from the head-space GC on solubilization of synthetic perfumes in nonionic surfactants support the partition-like model.

Average substitution degree is of particular importance in this case. When the guest molecule is small enough, such as benzyl alcohol, there is no preference among all three sulfoalkyl ether β-CD derivatives. Binding constants of the inclusion complexes between benzyl alcohol and sulfoalkyl ether β-CDs are almost the same. As the guest's molecular size becomes large, a more stable inclusion complex was formed as the β-CD derivatives have a low average substitution degree.

Figures 5 to 9 summarized the concentrations of the free synthetic perfumes in the presence of nonionic surfactants or sulfoalkyl ether β-CDs. One observation is that there is an upward deviation from a linear relationship based on Equation (7) for perfume complexation with CD derivatives. This is particularly apparent for the phenethyl alcohol and phenethyl acetate while it is slightly deviated in the case of benzyl alcohol, benzyl acetate and ethylphenyl acetate. However, there is no evidence to indicate the downward deviation from the 1:1 inclusion assumption. The deviation may suggest the ratio of CD molecule to perfume molecule in their complexation. Job plot analysis could be an effective way to confirm the complex stoichiometry. However, due to the limitation of the head space GC method, the maximum molar fraction of

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