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Complex Formation of Some Nonionic Surfactants with Hydroxypropyl-β-cyclodextrin and with Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin*

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Abstract. The interaction of six nonionic surfactants α-[4-(1,1,3,3-tetra-methylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) with hydroxypropyl-β-cyclodextrin (HPβCD) and dimethyl-β-cyclodextrin (DIMEB) was studied by reversed-phase thin-layer chromatography in the presence and absence of sodium chloride. Each surfactant formed complexes with both cyclodextrin derivatives; however, the strength of interaction varied considerably. DIMEB formed more stable inclusion complexes with the surfactants than did HPβCD. A longer ethyleneoxide chain decreased the strength of interaction, whereas sodium chloride exerted a negligible impact. Principal component analysis indicated that both the hydrophobicity and the specific hydrophobic surface area of the surfactant influenced the complex formation indicating the hydrophobic character of the interaction.

Key words: Nonionic surfactants, hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin, hydrophobic interaction.

1. Introduction

Nonionic surfactants display a range of biological activities. Polyethoxylated nonionic surfactants with no similarities in the hydrophobic moiety are able to reverse multidrug resistance in a human leukaemic cell line [1] and nonylphenyl nonylethoxylate breaks down the polymer aggregates of scleroglucan [2]. Tween-80 enhances the intestinal absorption of the anthelminthic drug albendazole in rat gut [3], whereas polysorbate-80 and polyoxyl-40 markedly influence the transport of drugs in monolayers of human intestinal epithelial (Caco-2) cells [4].

The biological activity of surfactants depends on the molecular structure. The toxicity of polyoxyethylene alkyl ethers decreased on increasing the length of the alkyl chain, and increased on increasing the length of the polyoxyethylene headgroup [5]. The complex stability of 2-(1-naphthyl)acetic acid with nonionic surfactant micelles decreased with the logarithm of the length of the ethyleneoxide chain for the Triton X series. The nondissociated form of NAA formed more stable complexes [6].

Due to their capacity to form inclusion complexes cyclodextrins (CDs) are used in the stabilization and formulation of drugs, flavors, and fragrances, and also in agrochemistry [7]. Methylated CDs, but not CDs themselves, have surface activity

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[8]. Many surface active agents can form inclusion complexes with CDs, resulting in striking changes in the critical micelle concentration and surface tension [9, 10]. The formation of inclusion complexes of some nonionic surfactants with CDs lessens their phytotoxicity [11].

Reversed-phase thin-layer chromatography has been frequently used to study various molecular interactions [12] such as the interaction of nonionic surfactants with CDs [13] and with highly water-soluble CD derivatives [14].

The objectives of the work were the study of the interaction of nonionic surfactants with a hydroxypropyl- and dimethyl-β-CD by charge-transfer chromatography and to find relationships between physicochemical parameters, molecular structures and the relative strength of complex formation with surfactants.

2. Materials and Methods

Polygram UV_{254} plates (Macherey-Nagel, Dürren, Germany) were preimpregnated with n-hexane : paraffin oil 95 : 5 v/v. The nonionic surfactants α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) contained on average 5 (Compound **I**), 7.5 (**II**), 10.5 (**III**), 11.5 (**IV**), 16 (**V**) and 30 (**VI**) ethyleneoxide groups per molecule. They were the gift of Union Carbide Austria, the purity of compounds **I–IV** and compounds **V–VI** was 95 and 70%, respectively. The surfactants were separately dissolved in methanol to give a concentration of 5 mg/mL, and 4 μL of solutions were spotted on the plates. The eluent was aqueous methanol with methanol concentrations between 30–57.5 vol. % in steps of 2.5 vol. %. Hydroxypropyl-β-cyclodextrin (HPBCD) and heptakis(2,6-di-*O*-methyl-β-cylcodextrin (DIMEB) were purchased from CYCLOLAB, Research and Development Laboratory (Budapest, Hungary), and were added to the eluent in concentrations of 0–15 mg/mL. Each experiment was also run in the presence of 0.15 M NaCl. The use of sodium chloride as an eluent additive was motivated by the fact that salts can modify the strength of interaction between cyclodextrins and various guest molecules [15, 16]. After development the surfactants were detected by UV absorption spectra. Each determination was run in quadruplicate. The R_M value, given by $\log(1/R_f - 1)$, which characterizes the molecular lipophilicity in reversed-phase thin-layer chromatography was calculated for each surfactant and eluent.

To separate the effects of methanol and CD derivatives on the lipophilicity of surfactants the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 \tag{1}$$

where R_M is the R_M value for a surfactant determined at given methanol and CD concentrations; R_{M0} is the R_M value extrapolated to zero methanol and CD concentrations; b_1 is the decrease in the R_M value caused by a 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of surfactant [17]); b_2 is the decrease in the R_M value caused by a 1 mg/mL

TABLE I. Parameters of multilinear correlations between the R_M values of surfactants and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in salt-free eluent ($n = 18$).

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$$

Parameter	Surfactant					
	I	II	III	IV	V	VI
R_{M0}	2.89	2.71	2.82	2.81	2.80	1.87
$-b_1 \cdot 10^{-2}$	3.39	3.04	2.98	3.15	2.89	2.02
$s_{b1} \cdot 10^{-3}$	4.96	4.57	5.12	4.98	3.50	2.60
$-b_2 \cdot 10^{-2}$	4.50	4.53	5.17	4.74	3.73	1.49
$s_{b2} \cdot 10^{-3}$	6.81	6.29	7.04	6.84	4.81	3.57
b_1 %	50.86	48.02	44.24	47.76	51.58	65.07
b_2 %	49.14	51.98	55.76	52.24	48.42	34.93
$F_{calc.}$	28.79	30.65	28.89	28.11	40.90	30.16
r^2	0.7825	0.7930	0.78310	0.7785	0.8364	0.7904

concentration change of CDs in the eluent (related to the relative strength of interaction); C_1 and C_2 are the concentrations of methanol and CDs, respectively. Equation 1 was applied separately for each surfactant and eluent system.

Principal component analysis (PCA) was used to assess the similarities and dissimilarities between the complex forming capacity of HPβCD and DIMEB and the hydrophobic molecular parameters of the surfactants [18]. The complex forming capacity of surfactants with HPβCD and DIMEB in salt-free and salt-containing environments, the lipophilicity and specific hydrophobic surface area of surfactants in salt-free and salt-containing environments as well as the average number of ethyleneoxide groups per molecule were the variables and the surfactants were the observables. The ratio of variance explained was set to 99%. The two dimensional nonlinear maps of principal component loadings and variables were also calculated [19].

3. Results and Discussion

The R_M value of each surfactant decreased in each instance with an increase in the methanol concentration, i.e., these compounds do not show any anomalous retention behavior in this concentration range that would invalidate the evaluation using Equation 1. An increase in CD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic CDs with the surfactant reduces the lipophilicity of the latter.

The parameters of Equation 1 are compiled in Tables I–IV. The equation fits the experimental data well, the significance levels in each instance being over 99.9% (see calculated F values) proving the excellent applicability of the equation. The ratios of variance explained were about 69–91% (see r^2 values). The parameters of

TABLE II. Parameters of multilinear correlations between the R_M values of surfactants and the concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in salt-containing eluent ($n = 16$).

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$$

Parameter	Surfactant					
	I	II	III	IV	V	VI
R_{M0}	2.93	3.33	3.01	3.11	2.85	1.96
$-b_1 \cdot 10^{-2}$	3.51	4.24	3.39	3.67	2.93	2.12
$s_{b1} \cdot 10^{-3}$	3.33	4.65	3.89	4.58	5.22	3.27
$-b_2 \cdot 10^{-2}$	3.68	5.08	4.64	5.14	4.97	2.35
$s_{b2} \cdot 10^{-3}$	3.84	5.36	4.48	5.29	6.02	3.77
b_1 %	52.38	49.02	45.74	45.16	40.48	50.96
b_2 %	47.62	50.98	54.26	54.84	59.52	49.04
$F_{calc.}$	69.05	58.49	62.87	54.56	35.84	27.28
r^2	0.9080	0.8931	0.8998	0.8863	0.8366	0.7958

TABLE III. Parameters of multilinear correlations between the R_M values of surfactants and the concentrations of methanol (C_1) and dimethyl-β-cyclodextrin (C_2) in salt-free eluent ($n = 18$; N.S. = not significant).

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$$

Parameter	Surfactant					
	I	II	III	IV	V	VI
R_{M0}	1.15	2.09	1.30	2.11	1.29	1.75
$-b_1 \cdot 10^{-2}$	N.S.	1.97	N.S.	1.91	N.S.	1.92
$s_{b1} \cdot 10^{-3}$	–	8.38	–	8.87	–	4.41
$-b_2 \cdot 10^{-2}$	7.61	8.95	8.15	8.70	7.05	4.51
$s_{b2} \cdot 10^{-3}$	11.23	11.52	11.24	12.19	11.41	6.06
b_1 %	–	23.26	–	23.19	–	36.96
b_2 %	–	76.74	–	76.81	–	63.04
$F_{calc.}$	45.89	33.41	52.54	28.23	38.16	27.64
r^2	0.7297	0.8068	0.7555	0.7792	0.6918	0.7755

Equation 1 show high variations between the surfactants proving that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of surfactants to form inclusion complexes with CD derivatives (b_2) differ considerably. This finding probably indicates that the inclusion complex formation may influence differently not only the chromatographic retention parameters of surfactants but also their biological effects outlined above.

DIMEB forms stronger complexes with the surfactants than does HPβCD. This result can be explained by the following assumptions:

TABLE IV. Parameters of multilinear correlations between the R_M values of surfactants and the concentrations of methanol (C_1) and dimethyl-β-cyclodextrin (C_2) in salt-containing eluent ($n = 16$; N.S. = not significant).

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2$$

Parameter	Surfactant					
	I	II	III	IV	V	VI
R_{M0}	2.23	2.65	2.42	2.63	1.40	1.87
$-b_1 \cdot 10^{-2}$	2.26	3.01	2.36	2.82	N.S.	2.02
$s_{b1} \cdot 10^{-3}$	9.78	11.71	10.53	10.12	–	5.32
$-b_2 \cdot 10^{-2}$	8.72	9.52	9.52	9.34	7.60	4.57
$s_{b2} \cdot 10^{-3}$	11.28	13.51	12.15	11.68	12.49	6.14
b_1 %	22.99	26.74	22.27	25.83	–	33.78
b_2 %	77.01	73.26	77.73	74.17	–	66.22
$F_{calc.}$	31.16	25.25	32.20	32.71	37.03	27.77
r^2	0.8166	0.7829	0.8214	0.8237	0.7117	0.7987

a. the dimensions of the DIMEB and HPβCD cavities are different and surfactants fit better into the DIMEB cavity;

b. the bulkier substituents on the outer surface of HPβCD decrease the availability of the cavity for the surfactants.

Sodium chloride has a lower impact on the strength of interaction than does the type of CD. This finding indirectly suggests that the CD-surfactant interaction is of a hydrophobic character which is less dependent on the environmental salt concentration than the various hydrophilic forces. The insignificant influence of methanol on the retention of some surfactants in the presence of DIMEB can be explained by the fact that DIMEB forms very strong complexes with the surfactants and this process overshadows the relatively weak effect of methanol.

The results of principal component analysis (PCA) are compiled in Table V. The first principal component explains the overwhelming majority of the variance. This finding indicates that the characteristics of the nine variables can be described by only one background variable. Unfortunately, PCA does not define this background variable as a concrete physicochemical entity, it only indicates its mathematical possibility. As the hydrophobic parameters and relative strength of interaction (probably depending on hydrophobic forces) have positive loadings and the number of hydrophilic ethyleneoxide groups per molecule has negative loadings it is reasonable to assume that the first PC is the overall indicator of the hydrophobicity of surfactants.

The distribution of various parameters on the two dimensional nonlinear map of PC loadings entirely supports our previous conclusions (Figure 1). The hydrophobic parameters form a well-defined cluster whereas the number of ethyleneoxide groups per molecule is separated. This finding proves again that hydrophobic molecular

TABLE V. Similarities and dissimilarities between the physicochemical parameters and complex forming capacity of surfactants. Results of principal component analysis.

PC component	Eigenvalues	Variance explained %	Total variance explained %
1	8.11	90.13	90.13
2	0.45	4.99	95.12

	Parameters	Principal component loadings No of PC component 1	2
A	Hydrophobicity in salt-free environment	0.94	−0.21
B	Specific hydrophobic surface area in salt-free environment	0.93	−0.34
C	Hydrophobicity in the presence of 0.15 m NaCl	0.98	0.14
D	Specific hydrophobic surface area in the presence of 0.15 M NaCl	0.92	0.15
E	relative strength of interaction with HPβCD in salt-free environment	0.97	−0.08
F	relative strength of interaction with HPβCD in the presence of 0.15 M NaCl	0.86	0.40
G	relative strength of interaction with DIMEB in salt-free environment	0.98	0.17
H	relative strength of interaction with DIMEB in the presence of 0.15 M NaCl	0.99	0.03
I	number of ethyleneoxide groups per molecule	−0.97	0.23

parameters influence the inclusion complex formation of surfactants both with HPβCD and DIMEB and the impact of sodium chloride on the interaction is of secondary importance.

The surfactant with the longest hydrophilic ethyleneoxide chain separates well from the others on the two dimensional nonlinear map of PC variables (Figure 2). This finding indicates that the length of the ethyleneoxide chain may have a marked influence not only on the lipophilicity but also on the formation of inclusion complexes. This somewhat surprising result can be explained by the supposition that the long hydrophilic chain increases the solubility of the surfactant in the aqueous environment around the CD molecules weakening the possible hydrophobic interactions between the bulky substituted benzene ring of surfactants and the apolar inner wall of the cyclodextrin cavity.

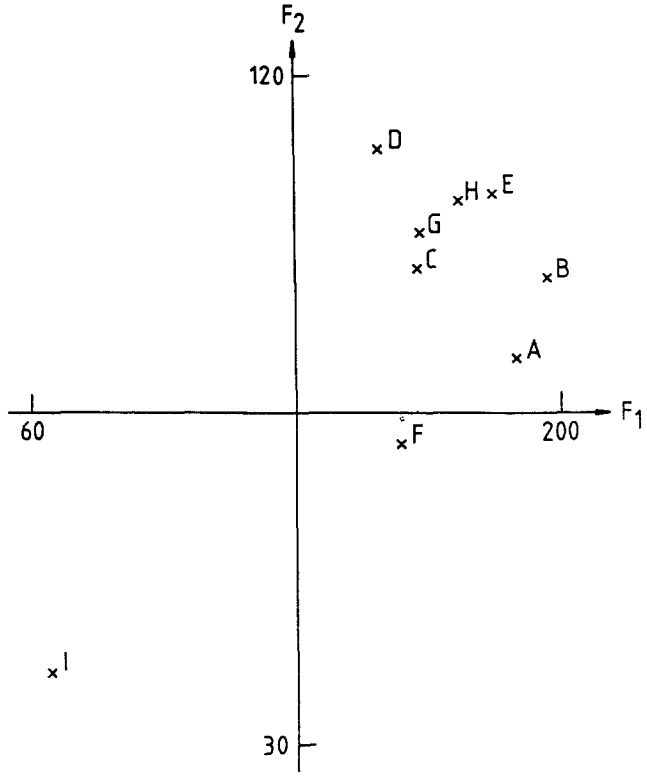

Fig. 1. Two-dimensional nonlinear map of PC loadings. Number of iterations: 148; maximum error: 1.12×10^{-2}. For symbols see Table V.

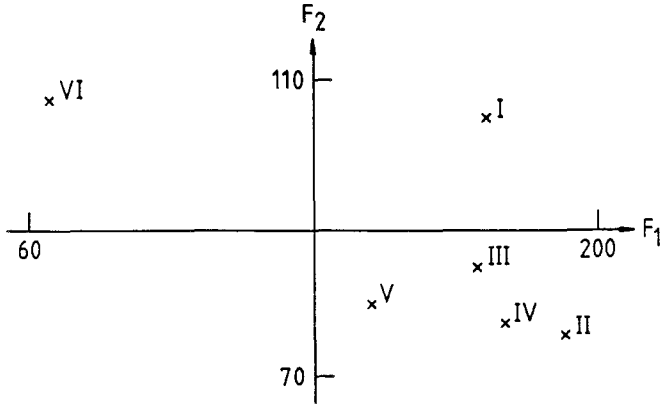

Fig. 2. Two-dimensional nonlinear map of PC variables. Number of iterations: 108; maximum error: 3.39×10^{-3}. Numbers refer to surfactants in Materials and Methods section.

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