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Charge-transfer chromatographic study of the interaction of non-ionic surfactants with hydroxypropyl-β-cyclodextrin

Tibor Cserháti*, Esther Forgács

Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary

Abstract

The interaction of 38 ethoxylated and one non-ethoxylated non-ionic surfactants with hydroxypropyl-β-cyclodextrin (HPBCD) was studied by reversed-phase charge-transfer chromatography. The relative strength of interaction, the hydrophobicity and the specific hydrophobic surface area of the surfactants and the effect of methanol concentration on the strength of interaction were calculated. The presence of a phenyl group and the length of the alkyl chain in the hydrophobic moiety of the surfactants have the greatest impact on their hydrophobic character, and the role of the length of the polar ethylene oxide chain is negligible. Surfactants with a tributylphenol hydrophobic moiety did not form complexes with HPBCD, the cavity of HPBCD probably being too small for the insertion of the bulky tributylphenol group. Most surfactants formed complexes with HPBCD, but the strength of interaction varied considerably. Stepwise regression analysis indicated that both the hydrophobicity and the specific hydrophobic surface area of the surfactants significantly influenced the strength of interaction, demonstrating the importance of hydrophobic interactions in inclusion complex formation between non-ionic surfactants and HPBCD.

1. Introduction

Non-ionic surfactants show various types of biological activity. Polyethoxylated non-ionic surfactants with no similarities in the hydrophobic moiety are able to reverse multi-drug 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], and Polysorbate 80 and Polyoxyl 40 markedly influence the transport of drugs in monolayers of human intestinal epithelial (Caco-2) cells [4]. Nearly quantitative conversion of linoleic acid into its hydroperoxide was achieved in microemulsions containing non-ionic surfactants, water and an organic solvent [5]. Non-ionic surfactants enhanced the systemic absorption of α-melanocyte-stimulating hormone via the ocular route in rabbits [6]. Non-ionic surfactants derived from tris(hydroxymethyl)aminomethane performed well in the solubilization of subcellular proteins of rat hepatocytes and membrane antigens from tumour cells [7]. Triton X-100 stimulated the ATPase activity of P-glycoprotein at low concentration and inhibited it at higher concentrations [8]. Triton X-100 activated the lecithin:cholesterol acyltransferase enxyme [9].

Non-ionic surfactants also show toxic side-effects. Surfactants are cytotoxic, the cytotoxicity order being cationic > anionic = amphoteric > non-ionic. Triton X-100 had a ranking similar to

* Corresponding author.

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anionic surfactants [10]. Triton X-100 and Triton XR suppressed spore germination and germ tube growth of *Mucor mucedo* [11]. Polyethoxylated non-ionic surfactants inhibit the transport of 2,4-dinitrophenylglutathione from intact human erythrocytes. Surfactants possibly modify the arrangement of integral membrane proteins such as P glycoprotein and presumably the glutathione transporters [12]. Non-ionic surfactants inhibit the mineralization of phenanthrene in soil–water systems, probably by interacting with the membrane of soil microflora [13].

The biological activity of surfactants depends on the molecular structure. The toxicity of polyoxyethylene alkyl ethers decreased with increasing length of the alkyl chain and increased with increasing length of the polyoxyethylene head group [14]. The complex stability of 2-(1-naphthyl)acetic acid (NAA) with non-ionic surfactant micelles decreased with increasing logarithm of the length of the ethylene oxide chain for the Triton X series. The undissociated form of NAA formed more stable complexes [15].

Owing to their capacity to form inclusion complexes, cyclodextrins (CDs) are used in the stabilization and formulation of drugs, flavours and fragrances and also in agrochemistry [16]. Methylated CDs, but not CDs themselves, have surface activity [17]. Many surface-active agents can form inclusion complexes with CDs, resulting in striking changes in critical micelle concentration and surface tension [18,19]. The formation of inclusion complexes of some non-ionic surfactants with CDs decreases their phytotoxicity [20].

Charge-transfer reversed-phase chromatography has frequently been used to study various molecular interactions [21] such as the interaction of non-ionic surfactants with CDs [22] and with highly water-soluble CD derivatives [23].

The objectives of the work were the study the interaction of non-homologous series of non-ionic surfactants with a hydroxypropyl-β-CD derivative by charge-transfer chromatography and to find relationships between physico-chemical parameters, molecular structures and the relative strength of complex formation of surfactants.

2. Experimental

Charge-transfer chromatography was performed on Kieselgel 60 plates (Merck, Darmstadt, Germany) preimpregnated with *n*-hexane–paraffin oil (95:5, v/v). The structures of the non-ionic surfactants studied are given in Table 1. The surfactants were dissolved in methanol (20 mg/ml) and 4-μl volumes of solutions were spotted on the plates. The eluent was aqueous methanol with methanol concentrations between 50 and 80% (v/v) in steps of 5% (v/v). Hydroxypropyl-β-cyclodextrin (HPBCD) was purchased from Cyclolab Research and Development Laboratory (Budapest, Hungary) and was added to the eluent at concentrations of 0–37.5 mg/ml. After development the plates were dried at room temperature and the surfactants were detected with iodine vapour. Each determination was run in quadruplicate. The R_M value [$\log(1/R_F - 1)$], 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 HPBCD on the lipophilicity of surfactants and to take into consideration the effect of methanol concentration on inclusion complex formation, the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1C_1 + b_2C_2 + b_3C_1C_2 \quad (1)$$

where R_M is the R_M value for a surfactant determined at given methanol and HPBCD concentrations, R_{M0} is the R_M value extrapolated to zero methanol and HPBCD 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 the surfactant [24]), b_2 is the decrease in the R_M value caused by a 1 mg/ml change in the HPBCD concentration in the eluent (related to the relative strength of interaction), b_3 is the effect of methanol concentration on the complex formation and C_1 and C_2 are the concentrations of methanol and HPBCD, respectively. Eq. 1 was applied separately for each surfactant. When the relative standard deviation of parallel de-

Table 1
Structure of non-ionic surfactants: $Q–O(C_2H_4O)_{n_e}–H$

No.	Trade name	Q	n_e (average)
1	Tween 20	Sorbitan monolaurate	20
2	Tween 40	Sorbitan monopalmitate	20
3	Tween 60	Sorbitan monostearate	20
4	Tween 80	Sorbitan monooleate	20
5	Tween 61	Sorbitan monostearate	4
6	Tween 81	Sorbitan monooleate	5
7	Tween 65	Sorbitan tristearate	20
8	Tween 85	Sorbitan trioleate	20
9	Brij 30	Lauryl alcohol	4
10	Brij 35	Lauryl alcohol	23
11	Brij 56	Oleyl/cetylalcohol	10
12	Brij 76	Stearyl alcohol	10
13	Brij 78	Stearyl alcohol	20
14	Brij 96	Oleyl alcohol	10
15	Arkopal N50	Nonylphenol	5
16	Arkopal N60		6
17	Arkopal N80		8
18	Arkopal N90		9
19	Arkopal N100		10
20	Arkopal N110		11
21	Arkopal N150		15
22	Arkopal N230		23
23	Arkopal N300		30
24	Sapogenate T40	Tributylphenol	4
25	Sapogenate T60		6
26	Sapogenate T80		8
27	Sapogenate T100		10
28	Sapogenate T110		11
29	Sapogenate T130		13
30	Sapogenate T180		18
31	Sapogenate T300		30
32	Sapogenate T 500		50
33	Myrj 45	Stearic acid	8
34	Myrj 49		20
35	Myrj 51		30
36	Myrj 52		40
37	Myrj 53		50
38	Myrj 59		100
39	Span 80	Sorbitan monooleate	0

terminations was higher than 8%, the data were omitted from the calculations.

To test the validity of the hypothesis that with homologous series of solutes the slope and intercept values (b_1 and R_{M0} in Eq. 1) are strongly intercorrelated [25,26], the linear correlation was calculated between the two physico-chemical parameters:

$$R_{M0} = A + Bb_1 \quad (2)$$

To find the relationships between the physico-chemical parameters (lipophilicity and specific hydrophobic surface area) and the molecular substructures of surfactants, and to select the physico-chemical parameters and molecular substructures of the surfactants that significantly

Table 2
Parameters of multilinear correlations between R_M values of surfactants and concentrations of methanol (C_1) and hydroxypropyl-β-cyclodextrin (C_2) in the eluent: $R_M = R_{M0} + b_1C_1 + b_2C_2 + b_3C_1C_2$

Parameter	Surfactant[a]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
R_{M0}	3.89	5.02	4.89	5.01	5.74	5.63	5.63	5.94	4.52	4.76	4.91	5.94	5.46	4.63	4.22
$-b_1 \cdot 10^{-2}$	4.36	6.06	5.81	5.99	6.85	6.91	6.78	7.23	6.05	6.14	6.00	7.21	6.61	5.56	5.61
$s_{b_1} \cdot 10^{-2}$	0.62	0.35	0.48	0.44	0.91	0.45	0.48	0.37	0.38	0.47	0.40	0.60	0.46	0.47	0.51
$-b_2 \cdot 10^{-2}$	–	7.26	6.64	7.55	–	8.28	8.23	8.94	7.99	7.39	8.16	10.17	8.26	5.82	6.98
$s_{b_2} \cdot 10^{-2}$	–	0.98	1.33	1.21	–	1.24	1.32	1.04	1.06	1.31	1.10	1.68	1.28	1.32	1.41
$b_3 \cdot 10^{-3}$	–	10.71	9.86	11.67	–	12.62	12.54	13.59	12.06	11.24	11.91	14.85	11.86	8.68	10.12
$s_{b_3} \cdot 10^{-3}$	–	1.66	2.24	2.04	–	2.09	2.23	1.75	1.78	2.21	1.85	2.83	2.16	2.22	2.38
b_1 (%)	–	16.54	17.15	15.58	–	16.33	16.18	15.93	15.11	16.28	14.94	14.47	16.12	18.37	16.13
b_2 (%)	–	45.60	45.13	45.15	–	45.02	45.16	47.20	45.88	45.08	46.69	46.93	46.37	44.48	46.18
b_3 (%)	–	37.86	37.72	39.27	–	38.65	38.66	36.87	39.01	38.64	38.37	38.60	37.51	37.15	37.69
$F_{calc.}$	–	104.55	53.69	70.01	–	88.76	74.40	136.63	88.79	62.15	78.29	48.28	71.05	52.22	42.20
r^2	0.7923	0.9661	0.9361	0.9502	0.8360	0.9603	0.9530	0.9739	0.9603	0.9443	0.9553	0.9294	0.9509	0.9344	0.9201
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
R_{M0}	3.89	4.29	3.76	3.77	3.86	3.75	3.60	3.23	3.77	3.58	3.23	3.41	3.40	3.39	3.14
$-b_1 \cdot 10^{-2}$	5.14	5.78	5.03	5.06	5.15	4.98	4.68	3.98	5.01	4.69	4.22	4.41	4.39	3.36	3.99
$s_{b_1} \cdot 10^{-2}$	0.46	0.48	0.37	0.45	0.34	0.34	0.33	0.44	0.30	0.30	0.26	0.35	0.35	0.31	0.30
$-b_2 \cdot 10^{-2}$	6.86	8.03	6.68	6.96	7.45	7.44	6.27	5.37	–	–	–	–	–	–	–

	31	32	33	34	35	36	37	38	39						
$s_{b_2} \cdot 10^{-2}$	1.27	1.35	1.04	1.26	0.94	0.94	0.93	1.23	–	–	–	–	–	–	–
$b_3 \cdot 10^{-3}$	10.00	11.87	9.80	10.25	11.26	11.37	9.62	8.22	–	–	–	–	–	–	–
$s_{b_3} \cdot 10^{-3}$	2.14	2.27	1.75	2.12	1.58	1.58	1.56	2.08	–	–	–	–	–	–	–
b_1 (%)	15.18	14.59	15.19	14.72	13.97	13.52	14.83	14.75	–	–	–	–	–	–	–
b_2 (%)	46.58	46.60	46.44	47.70	50.99	46.46	45.70	45.77	–	–	–	–	–	–	–
b_3 (%)	38.24	38.81	38.37	37.58	35.04	40.02	39.47	39.48	–	–	–	–	–	–	–
$F_{calc.}$	43.28	48.44	61.86	42.23	79.35	74.16	70.24	28.55	–	–	–	–	–	–	–
r^2	0.9219	0.9296	0.9440	0.9592	0.9558	0.9529	0.9504	0.8862	0.9548	0.9491	0.9529	0.9240	0.9249	0.9350	0.9308
n	15	15	14	9	15	15	15	12	14						
R_{M0}	3.34	2.57	3.75	5.29	4.97	4.61	4.95	5.04	3.65						
$-b_1 \cdot 10^{-2}$	4.17	2.87	4.28	6.20	6.14	5.53	5.89	6.37	4.55						
$s_{b_1} \cdot 10^{-2}$	0.23	0.20	0.76	1.26	0.45	0.38	0.56	0.78	0.89						
$-b_2 \cdot 10^{-2}$	–	–	–	–	7.39	5.06	6.92	–	–						
$s_{b_2} \cdot 10^{-2}$	–	–	–	–	1.24	1.06	1.55	–	–						
$b_3 \cdot 10^{-3}$	–	–	–	–	11.21	8.20	10.47	–	–						
$s_{b_3} \cdot 10^{-3}$	–	–	–	–	2.09	1.80	2.61	–	–						
b_1 (%)	–	–	–	–	16.30	17.62	16.66	–	–						
b_2 (%)	–	–	–	–	45.15	44.83	45.01	–	–						
b_3 (%)	–	–	–	–	38.55	37.55	38.33	–	–						
$F_{calc.}$	–	–	–	–	69.38	98.37	41.22	–	–						
r^2	0.9789	0.9433	0.7264	0.7748	0.9498	0.9531	0.9183	0.8685	0.6838						

[a] Numbers 1–39 refer to surfactants in Table 1.

influence their complex-forming capacity, stepwise regression analysis was applied [27]. Stepwise regression analysis was applied four times:

(1) lipophilicity (R_{M0} in Eq. 1) being the dependent and the molecular substructures being the independent variables;

(2) specific hydrophobic surface area (b_1 in Eq. 1) being the dependent and the molecular substructures being the independent variables;

(3) relative strength of interaction (b_2 in Eq. 1) being the dependent and the R_{M0} and b_1 values being the independent variables; and

(4) relative strength of interaction (b_2 in Eq. 1) being the dependent and the R_{M0}, b_1 values and the molecular substructures being the independent variables.

In each instance the number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level.

3. Results and discussion

The simultaneous effects of methanol and HPBCD concentrations of the R_M values of surfactants 11 and 22 are shown in Figs. 1 and 2, respectively. The R_M values decrease in each instance with increase in methanol concentration, *i.e.*, these compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Eq. 1. An increase in HPBCD concentration also caused a decrease in R_M values, indicating complex (probably inclusion complex) formation. Interaction of the more hydrophilic HPBCD with the surfactant decreases the lipophilicity of the latter. This finding suggests that the biological properties (adsorption, uptake, half-life, etc.) of surfactant–HPBCD complexes may be different from that of uncomplexed surfactants, resulting in modified effectivity.

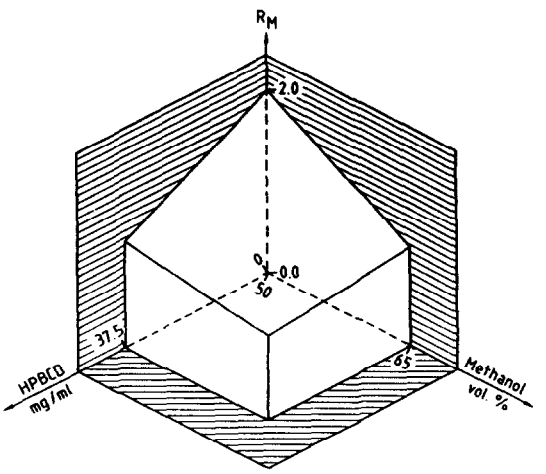

Fig. 1. Effects of methanol and hydroxypropyl-β-cyclodextrin (HPBCD) concentrations on the R_M value of surfactant 11 in Table 1.

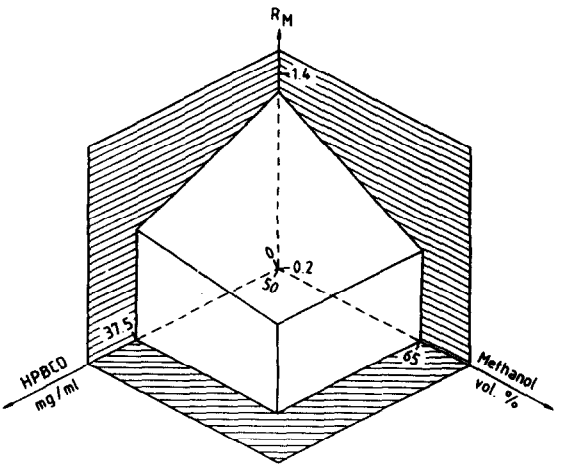

Fig. 2. Effects of methanol and hydroxypropyl-β-cyclodextrin (HPBCD) concentrations on the R_M value of surfactant 22 in Table 1.

The parameters of Eq. 1 are compiled in Table 2. Blank entries in Table 2 indicate that these independent variables did not significantly influence the R_M value of the surfactant. The equation fits the experimental data well, the significance levels in each instance being over 95% (see calculated F values). The ratios of variance explained were about 68–97% (see r^2 values). Most of the surfactants interact with HPBCD (the b_2 values differ significantly from zero), which means that in cosmetics and pesticide formulations containing both surfactants and HPBCD their possible interaction has to be taken into consideration. The parameters of Eq. 1 show large variations between the surfactants, demonstrating that the lipophilicity (R_{M0}),

specific hydrophobic surface area (b_1) and the capacity of surfactants to form inclusion complexes with HPBCD (b_2) differ considerably. This finding suggests also that the inclusion complex formation may influence differently the biological effects of individual surfactants. The complex-forming capacity of surfactants with HPBCD decreases considerably with increasing concentration of methanol in the eluent (see b_3 values). This result can be explained by the supposition that methanol also forms inclusion complexes with HPBCD. This complex is probably very weak; however, methanol is present at a higher concentration than the surfactant and the competition for the HPBCD cavity results in a decrease in the stability of surfactant–HPBCD complexes at higher methanol concentrations. The path coefficients (b_i values) indicate that changes in methanol concentration have the smallest and changes in HPBCD concentration the largest effect on the retention behaviour of surfactants.

A significant linear correlation was found between the intercept (lipophilicity) and slope (specific hydrophobic surface area) values of surfactants (Fig. 3). This finding indicates that from a chromatographic point of view these surfactants behave as a homologous series of compounds, although their hydrophobic moieties are considerably different. This surprising result

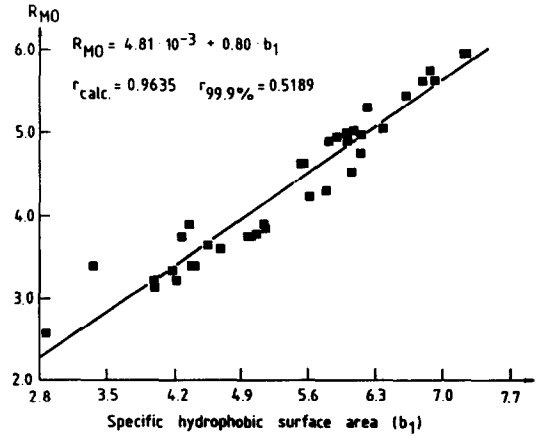

Fig. 3. Relationship between the lipophilicity (R_{MO}) and specific hydrophobic surface (b_1) of surfactants.

suggests that the hydrophilic ethylene oxide chains determine the retention behaviour of ethoxylated surfactants and the character of the hydrophobic moiety is of negligible importance. This can be explained by the assumption that the ethylene oxide chains point towards the polar mobile phase and the area of the hydrophobic surface of surfactants in contact with the non-polar support depends on the capacity of ethylene oxide chains to draw away the hydrophobic moiety from the non-polar support. This effect is really independent of the character of the hydrophobic moiety and depends only on the length of the ethylene oxide chain.

Each stepwise regression analysis found a significant relationship between the chromatographic parameter and molecular substructures (Table 3). The lipophilicity depends on the length of the alkyl chain and on the number of phenyl groups in the hydrophobic moiety of the surfactants (Eq. 3 in Table 3). These two hydrophobic substructures account for most of the change in lipophilicity (see r^2 values), the effect of the phenyl group being the stronger (see B values). The character of the relationship between the specific hydrophobic surface area and substructures is similar (Eq. 4), but the ratio of variance is markedly lower. This results suggests that other molecular characteristics not included in the calculation may also influence the specific hydrophobic surface area of surfactants. The fact that the hydrophobic molecular parameters account for most of the change in the complex-forming capacity of surfactants emphasizes the predominant role of hydrophobic forces in the inclusion complex formation (Eq. 5).

The best relationship describing the dependence of complex-forming capacity on the various parameters of surfactants includes both physico-chemical and structural characteristics (Eq. 6). The effects of the specific hydrophobic surface area and the number of phenyl groups have been discussed above. However, the significant role of the ester bonds need some explanation. It is well known that this substructure markedly modifies the spacial arrangement of the hydrophobic and hydrophilic moieties. This structural change may decrease the contact sur-

Table 3
Relationships between the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1), complex-forming capacity (b_2) and molecular substructures of surfactants[a,b]

$$R_{M0} = a + B_I x_I + B_{II} x_{II} \quad (3)$$

$$b_1 = a + B_I x_{II} \quad (4)$$

$$b_2 = a + B_I R_{M0} + B_{II} b_1 \quad (5)$$

$$b_2 = a + B_I b_1 + B_{II} x_{II} + B_{III} x_{III} \quad (6)$$

Parameter	Equation No.			
	3	4	5	6
n	39	39	24	24
a	4.48	6.02	−0.55	−3.51
B_I	$2.39 \cdot 10^{-2}$	−1.44	−2.08	1.74
s_{B_I}	$1.03 \cdot 10^{-2}$	0.26	0.67	0.20
B_{II}	−1.16	–	3.02	0.60
$s_{B_{II}}$	0.20	–	0.65	0.27
B_{III}	–	–	–	1.04
$s_{B_{III}}$	–	–	–	0.36
B_I (%)	28.36	–	39.75	63.17
B_{II} (%)	71.64	–	60.25	13.56
B_{III} (%)	–	–	–	23.27
$F_{calc.}$	36.98	–	28.30	30.18
r^2	0.6726	0.4627	0.7294	0.8191

[a] Results of stepwise regression analysis. Surfactants not interacting with hydroxypropyl-β-cyclodextrin were omitted from Eq. 5 and 6.
[b] x_I = Length of alkyl chain in the hydrophobic moiety of surfactants; x_{II} = number of phenyl groups in the hydrophobic moiety of surfactants; x_{III} = number of ether bonds in the hydrophobic moiety of surfactants.

face between the surfactants and the non-polar surface of the HPBCD cavity, resulting in a modified complex-forming capacity.

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