



ELSEVIER

Journal of Chromatography A, 722 (1996) 33–40

JOURNAL OF
CHROMATOGRAPHY A

Reversed-phase chromatographic study of the interaction of non-ionic surfactants with sodium dodecyl sulfate

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Abstract

The interaction of 18 non-ionic surfactants (9 nonylphenylethylene oxide oligomers, containing in average 5, 6, 8, 9, 10, 11, 15, 23 and 30 ethylene oxide groups per molecule, and 9 tributylphenylethylene oxide oligomers, containing in average 4, 6, 8, 10, 11, 13, 18, 30 and 50 ethylene oxide groups per molecule) with the anionic surfactant sodiumdodecylsulfate (SDS) was studied by reversed-phase chromatography, and the relative strength of interaction was calculated separately for each non-ionic surfactant–SDS pair. Stepwise regression analysis was used for the determination of molecular substructures and physicochemical parameters accounting for the interaction. The retention of non-ionic surfactants decreased in the presence of SDS indicating the formation of SDS–non-ionic surfactant complexes. In each instance the SDS–surfactant complex was less hydrophobic than the surfactant itself. The relative strength of interaction increased with increasing specific hydrophobic surface area of surfactants and decreased with increasing number of polar ethylene oxide groups per molecule. This finding indicates that hydrophobic forces are involved in the complex formation and the alkyl chain of SDS is bound to the hydrophobic moiety of surfactants. The relative strength of interaction significantly decreased with the increasing concentration of methanol in the solution, indicating the hydrophobic character of the interaction.

1. Introduction

Surfactants are extensively used in pharmaceutical [1] and agrochemical formulations [2], in cosmetics etc. which considerably improve the application parameters [3,4] and biological efficiency of various drugs [5] and pesticides [6]. Surfactants exert beneficial effects in biotechnology, i.e. they reduce the aflatoxin production by the fungus *Aspergillus flavus* by 96 to 99% at 1% (wt/vol) [7] and induce hydrogen production in Cyanobacteria [8]. Surfactants increase the decomposition of pollutants in soil [9], but on the

other hand they are environmental pollutants. Surfactants may also have adverse biological effects, for example they show marked eye irritation potential [10,11].

Non-ionic and ionic surfactants are frequently used together in a wide variety of formulations, they can interact with each other [12–15] and with the active ingredient or ingredients [16]. Thus, it has been established that the polar head group of SDS is located in the ethylene oxide region of a non-ionic surfactant [17]. The characteristics of micelles formed of ionic and non-ionic surfactants shows non-ideal behavior [18]. It was established that hydrophilic forces are involved in the binding of surfactants to each other [19].

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Reversed-phase thin-layer chromatography has been previously used to study the interaction on non-ionic surfactants with amino acids [20,21], and with various cyclodextrin derivatives [22,23]. These studies used similar methodology, however, the interacting molecular species were entirely different. The objectives of this work were to study the interaction of some non-ionic surfactants with sodium dodecylsulfate (SDS) by means of reversed-phase thin-layer chromatography, to determine the influence of sodium chloride on the interaction and to elucidate the role of molecular parameters in the complex formation. The use of sodium chloride additive was motivated by the fact that characteristics of SDS are different in the presence of salt [24].

2. Experimental

The chemical structures of non-ionic surfactants are listed in Table 1. DC-Fertigplatten KIESELGEL 60 (Merck, Darmstadt, Germany)

Table 1
Chemical structures of non-ionic surfactants.

No.	Common name	General structure $Q-O(C_2H_4O)_{n_c}-H$	
		Q	n_c (average)
1	Arkopal N50	Nonylphenol	5
2	Arkopal N60		6
3	Arkopal N80		8
4	Arkopal N90		9
5	Arkopal N100		10
6	Arkopal N110		11
7	Arkopal N150		15
8	Arkopal N230		23
9	Arkopal N300		30
10	Sapogenate T40	Tributylphenol	4
11	Sapogenate T60		6
12	Sapogenate T80		8
13	Sapogenate T100		10
14	Sapogenate T110		11
15	Sapogenate T130		13
16	Sapogenate T180		18
17	Sapogenate T300		30
18	Sapogenate T500		50

were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v). The non-ionic surfactants were separately dissolved in methanol to give a concentration of 5 mg/ml and 2 μ l of solution was spotted on to the plates. As the object was to study the complex formation between the non-ionic surfactants and SDS and not the study of the effect of SDS on the separation of surfactants, the surfactants were separately spotted on the plates. Methanol–water mixtures were used as eluents with the methanol concentration varying between 45–65 vol.% in steps of 5 vol.%. SDS was dissolved in the eluent in the concentration range of 0–70 mM. As the salts may modify the capacity of non-ionic surfactants to interact with SDS each experiment was run in salt free eluents and in eluents containing 0.5 M NaCl (end concentration). Development was performed in sandwich chambers (22 \times 22 \times 3 cm) at room temperature, and the running distance was ca. 15 cm. The chambers were not presaturated. After development the plates were dried at room temperature and the spots were detected by iodine vapours. 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 system.

To separate the effects of methanol and SDS on the lipophilicity of surfactants and to take into consideration the possible effect of methanol concentration on the strength of interaction the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 + b_3 \cdot C_1 \cdot C_2 \quad (1)$$

where $R_M = R_M$ value for a surfactant determined at given methanol and SDS concentrations; $R_{M0} = R_M$ value extrapolated to zero methanol and SDS concentrations; $b_1 =$ decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of surfactants) [25]; $b_2 =$ decrease in the R_M value caused by 1 mg/ml concentration change of SDS in the eluent (related to the relative strength of interaction); $b_3 =$ common effect of methanol and

SDS concentrations on the R_M , and C_1 and $C_2 =$ concentrations of methanol and SDS, respectively. Eq. 1 was applied separately for each drug. When the coefficient of variation of the parallel determinations was higher than 6%, the data were omitted from the calculations.

Stepwise regression analysis was applied [26] to find the physicochemical parameters of non-ionic surfactants which significantly influence their capacity to interact with SDS. The relative strength of interaction (b_2) was the dependent variable whereas the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of Eq. 1, the number of ethylene oxide groups per molecule and the presence of nonylphenyl and tributylphenyl moiety in the surfactant molecule, were the independent variables, respectively. According to the additivity rule the hydrophobicity of non-ionic surfactants has to depend linearly on the number of ethylene oxide groups per molecules. In this instances the use of both hydrophobicity and the number of ethylene oxide group in the calculation is redundant. However, the ethylene oxide groups are more or less in folded state in solution depending on the length of the ethylene oxide chain. This folding results in non-linear dependence of the hydrophobicity of surfactants on the number of ethylene oxide groups, therefore the inclusion of both

physicochemical parameters in the calculation is justified. Calculation was separately carried out for salt-free and salt-containing systems. The number of accepted independent variables was not limited and the acceptance limit was set to the 99% significance level. In the common multivariate regression analysis the presence of independent variables exerting no significant influence on the change of dependent variable considerably decreases the significance level of the equation. Stepwise regression analysis selects the independent variables which significantly influence the dependent variable. This selection procedure increases the reliability of the calculation.

To elucidate the effect of salt on the stability of the non-ionic surfactants–SDS complexes linear correlations were calculated between the b_2 and b_3 values of Eq. 1 determined in salt-free and in salt containing systems.

3. Results and discussion

The simultaneous effect of methanol and SDS concentrations on the R_M values of surfactants 6 and 9 are shown in Figs. 1 and 2, respectively. The R_M values decrease in each instance with increase in methanol concentration, i.e. these

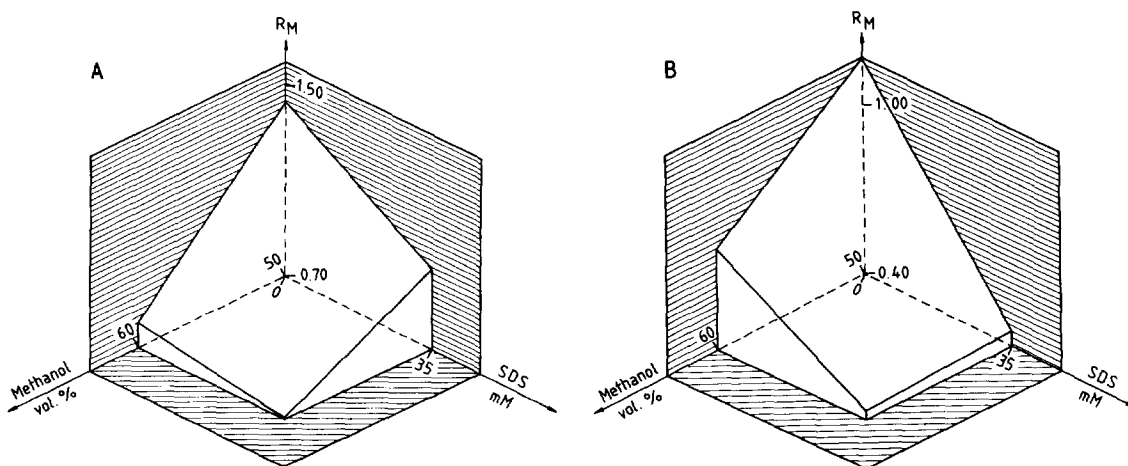


Fig. 1. Effects of methanol and sodium dodecylsulfate (SDS) concentrations on the R_M value of surfactant 6 in Table 1. A = salt-free eluents; B = eluents containing 0.5 M NaCl (end concentration).

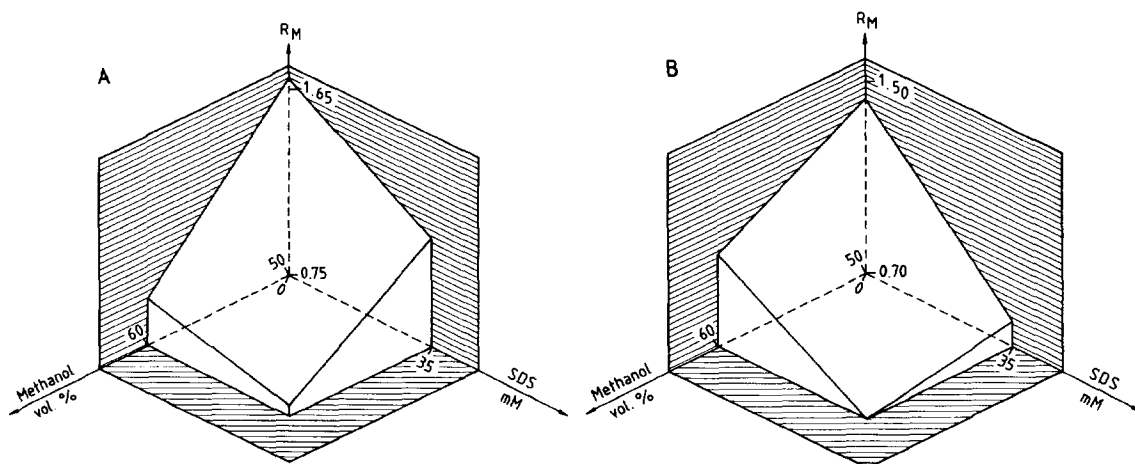


Fig. 2. Effects of methanol and sodium dodecylsulfate (SDS) concentrations on the R_M value of surfactant 9 in Table 1. A = salt-free eluents; B = eluents containing 0.5 M NaCl (end concentration).

compounds do not show any anomalous retention behaviour in this concentration range that would invalidate the evaluation using Eq. 1. An increase in the SDS concentration also caused a decrease in R_M values, indicating complex formation. Interaction of the more hydrophilic SDS with the non-ionic surfactants decreases the lipophilicity of the latter.

The parameters of Eq. 1 calculated for salt-free eluents are compiled in Table 2. The equation fits the experimental data well, the significance level in each instance being over 95% (see calculated F values). The ratios of variance explained were about 65–93% (see r^2 values). Each surfactant interacts with SDS (the b_2 values differ significantly from zero). The parameters of Eq. 1 differ considerably, demonstrating that the lipophilicity (R_{M0}), specific hydrophobic surface area (b_1) and the capacity of non-ionic surfactants to form complexes with SDS (b_2) differ significantly. The differences between the interactive capacity of nonylphenyl and tributylphenyl derivatives with the same number of ethylene oxide groups are fairly low. This finding indicates that both hydrophobic moieties have approximately the same possibility to bind to the apolar alkyl chain of SDS. The interactive capacity of surfactants with SDS decreases with increasing concentration of methanol in the eluent (see b_3

values) suggesting the hydrophobic character of the interaction. The path coefficients (b_1 % values) indicate that the changes in methanol concentration have the smallest and changes in SDS concentration the largest effect on the retention behaviour of surfactants.

Stepwise regression analysis found a significant relationship between the complex forming capacity (b_2) and the specific hydrophobic surface area of surfactants (b_1) and the number of ethylene oxide groups per molecule (n_e):

$$b_2 = 1.22 + (0.72 \pm 0.08) \cdot b_1 - (3.69 \pm 0.67) \cdot 10^{-2} \cdot n_e \quad (2)$$

$$r^2 = 0.9298 \quad F = 99.28$$

The results of Eq. 2 prove that the interaction of SDS is stronger with non-ionic surfactants having a shorter polar ethylene oxide chain and a larger specific hydrophobic surface area. The path coefficients indicated that the effect of specific hydrophobic surface area is dominant in the complex formation (61.34%). It can be assumed that the apolar alkyl chain of SDS and the hydrophobic moiety of the surfactants are bound by hydrophobic forces. The ethylene oxide groups being weak Lewis acids repulse the acidic sulfate head of SDS decreasing in this manner the stability of the complex.

Table 2

Parameters of multilinear correlations between R_M values of non-ionic surfactants and concentrations of methanol (C_1) and sodium dodecylsulfate (C_2) in the eluent (salt free systems).

Parameter	Surfactant number								
	1	2	3	4	5	6	7	8	9
R_{M0}	6.09	4.64	4.60	5.06	5.03	4.58	4.99	4.87	4.87
$-b_1 \cdot 10^{-2}$	8.63	6.34	6.26	6.86	6.81	6.29	6.73	6.36	6.54
$s_{b1} \cdot 10^{-2}$	1.08	0.52	0.61	0.72	0.67	0.74	0.66	0.82	0.96
$-b_2 \cdot 10^{-2}$	7.83	5.94	5.63	5.78	5.66	5.41	5.36	4.69	4.56
$s_{b2} \cdot 10^{-2}$	1.32	0.64	0.75	0.88	0.83	0.91	0.81	1.00	1.18
$b_3 \cdot 10^{-4}$	12.22	9.28	8.86	8.94	8.82	8.56	8.27	7.03	6.88
$s_{b3} \cdot 10^{-4}$	2.33	1.13	1.33	1.56	1.46	1.61	1.43	1.77	2.08
$b_1\%$	13.47	13.09	13.51	14.40	14.51	12.77	15.11	16.32	17.05
$b_2\%$	47.41	47.60	47.21	47.10	46.89	47.49	46.75	46.66	46.14
$b_3\%$	39.12	39.31	39.28	38.50	38.60	39.47	38.14	37.02	36.81
r^2	0.8571	0.9328	0.9028	0.8969	0.9053	0.8614	0.9093	0.8679	0.8333
F	29.98	69.45	46.42	43.50	47.81	31.08	50.13	32.84	24.99
	10	11	12	13	14	15	16	17	18
R_{M0}	5.52	4.91	5.25	5.10	3.86	4.75	4.33	3.57	4.54
$-b_1 \cdot 10^{-2}$	7.54	6.62	7.22	6.88	5.05	6.37	5.62	4.18	5.94
$s_{b1} \cdot 10^{-2}$	0.75	0.75	0.79	0.82	0.54	0.60	0.69	1.07	0.88
$-b_2 \cdot 10^{-2}$	6.30	5.40	6.15	5.58	4.57	5.50	5.08	3.09	3.91
$s_{b2} \cdot 10^{-2}$	0.92	0.92	0.97	1.01	0.66	0.74	0.85	1.31	1.08
$b_3 \cdot 10^{-4}$	9.58	8.09	9.60	8.53	7.05	8.61	8.02	4.51	5.65
$s_{b3} \cdot 10^{-4}$	1.62	1.63	1.72	1.78	1.17	1.31	1.49	2.31	1.90
$b_1\%$	14.58	14.98	14.21	14.93	13.55	14.04	13.45	16.42	18.15
$b_2\%$	47.38	47.47	46.98	47.05	47.61	47.01	47.19	47.20	46.41
$b_3\%$	38.04	37.55	38.81	38.02	38.84	38.95	39.36	36.38	35.44
r^2	0.9133	0.8950	0.8844	0.8755	0.8958	0.9102	0.8547	0.6465	0.8481
F	52.66	42.62	38.24	35.15	42.97	50.66	29.41	9.15	27.92

Numbers refer to non-ionic surfactants in Table 1. $n = 19$; s = standard deviation. $R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 + b_3 \cdot C_1 \cdot C_2$.

The parameters of Eq. 1 calculated for eluents with 0.5 M NaCl end concentration are compiled in Table 3. The results are similar to those compiled in Table 2: the equation fits the experimental data well, the significance level in each instance being over 99.9% (see calculated F values). The ratios of variance explained were about 91–97% (see r^2 values). Each surfactant interacts with SDS, however, the relative strength of interaction is markedly higher than in salt-free eluents. This finding can be tentatively explained by the following theoretical considerations:

(a) salt exerts a salting-out effect on both types

of surfactants facilitating the occurrence of hydrophobic forces between the apolar molecular substructures.

(b) salt probably is in a highly dissociated form in the eluents. The free ions surround the acidic sulfate group in SDS decreasing in this manner the repulsive forces.

Methanol concentration exerts a similar effect on the strength of interaction as in salt-free eluent indicating also in these instances the involvement of hydrophobic forces in the interaction.

The results of stepwise regression analysis support our previous conclusions:

Table 3

Parameters of multilinear correlations between R_M values of non-ionic surfactants and concentrations of methanol (C_1) and sodium dodecylsulfate (C_2) in the eluent (0.25 M NaCl end concentration in the eluents)

Parameter	Surfactant number								
	1	2	3	4	5	6	7	8	9
R_{M0}	4.45	4.59	3.85	4.12	4.57	3.67	4.15	3.03	3.19
$-b_1 \cdot 10^{-2}$	5.76	6.12	4.79	5.09	5.85	4.75	5.29	3.23	3.52
$s_{b1} \cdot 10^{-2}$	1.04	0.83	0.67	0.85	0.68	0.46	0.52	0.59	0.62
$-b_2 \cdot 10^{-2}$	16.22	15.65	11.12	12.13	13.87	8.65	10.88	4.94	5.31
$s_{b2} \cdot 10^{-2}$	2.83	2.24	1.81	2.33	1.87	1.25	1.40	1.61	1.68
$b_3 \cdot 10^{-4}$	2.53	2.42	1.66	1.83	2.13	1.26	1.65	0.66	0.71
$s_{b3} \cdot 10^{-4}$	5.18	4.12	3.33	4.28	3.42	2.29	2.57	2.95	3.09
$b_1\%$	6.65	7.30	8.10	7.88	7.85	10.20	8.98	12.29	12.44
$b_2\%$	50.61	50.41	50.79	50.69	50.29	50.17	49.94	50.81	50.76
$b_3\%$	42.74	42.29	41.11	41.43	41.86	39.63	41.08	36.90	36.80
r^2	0.9457	0.9652	0.9675	0.9518	0.9720	0.9807	0.9727	0.9453	0.9488
F	69.71	111.07	119.01	79.04	138.86	203.68	179.03	69.17	74.15
	10	11	12	13	14	15	16	17	18
R_{M0}	4.76	3.66	3.32	3.95	4.66	3.81	4.67	3.75	3.95
$-b_1 \cdot 10^{-2}$	6.03	4.28	3.82	4.75	6.21	4.44	6.15	4.50	4.98
$s_{b1} \cdot 10^{-2}$	1.14	1.37	0.98	0.82	0.68	0.91	0.80	0.62	0.72
$-b_2 \cdot 10^{-2}$	17.17	14.88	10.74	13.25	13.99	11.20	13.09	7.23	5.46
$s_{b2} \cdot 10^{-2}$	3.09	3.75	2.65	2.24	1.86	2.46	2.18	1.67	1.96
$b_3 \cdot 10^{-4}$	2.67	2.25	1.55	2.00	2.17	1.65	2.05	1.05	0.82
$s_{b3} \cdot 10^{-4}$	5.68	6.82	4.86	4.11	3.41	4.51	4.00	3.07	3.59
$b_1\%$	6.59	5.52	6.88	6.81	8.18	7.55	8.60	11.42	15.69
$b_2\%$	50.69	51.89	52.17	51.30	49.83	51.39	49.48	49.53	46.49
$b_3\%$	42.72	42.59	40.95	41.89	41.99	41.06	41.92	39.05	37.82
r^2	0.9431	0.9158	0.9400	0.9614	0.9705	0.9465	0.9539	0.9570	0.9300
F	66.25	43.49	62.67	99.70	131.54	70.78	82.79	88.37	53.15

Numbers refer to non-ionic surfactants in Table 1. $n = 16$, s = standard deviation. $R_M = R_{M0} + b_1 \cdot C_1 + b_2 \cdot C_2 + b_3 \cdot C_1 \cdot C_2$.

$$b_2 = -1.30 + (3.89 \pm 0.65) \cdot R_{M0} - (0.20 \pm 0.03) \cdot 10^{-2} \cdot n_c \quad (3)$$

$$r^2 = 0.8930 \quad F = 62.62$$

The overall molecular hydrophobicity increases and the number of ethylene oxide groups per molecule decreases the stability of the surfactants complexes. In the presence of salt the hydrophobicity and not the specific hydrophobic surface area influences significantly the complex formation. We assume that the folding of the polar ethylene oxide chains of the surfactant molecules is different in the presence of salt

resulting in modified capacity to interact with SDS. Unfortunately, we have no experimental prove to support this hypothesis. It cannot be excluded that the stoichiometry of SDS–non-ionic surfactant complexes is different in salt-free and in salt-containing eluents. As reversed-phase chromatography is not suitable for the determination of the stoichiometry of complexes, the determination can be carried out only by other physicochemical methods.

Significant linear correlations were found between the relative strength of interactions (b_2) and the effects of methanol concentration on the strength of interaction (b_3) determined in salt-

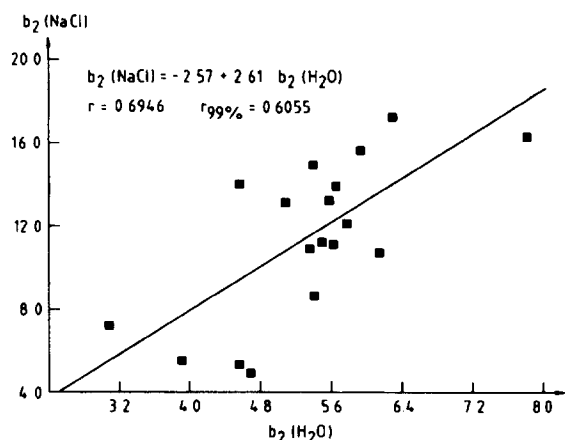


Fig. 3. Relationship between the relative strength of interaction determined in salt-free ($b_2(\text{H}_2\text{O})$) and in salt-containing eluents ($b_2(\text{NaCl})$).

free and in salt-containing eluents (Figs. 3 and 4). The data suggest that the interaction is considerably stronger in the presence of salt, and effect of methanol is similar in both eluent systems. Although the relationships plotted in Figs. 3 and 4 are statistically significant (F_{calc} values are 14.92 and 13.58 for Figs. 3 and 4, respectively the tabulated F value corresponding to 99% significance level being 8.68) the ratio of variance explained are fairly low (48.25% and

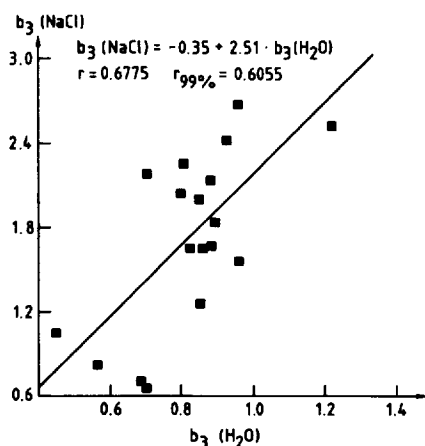


Fig. 4. Relationship between the effect of methanol concentration on the relative strength of interaction determined in salt-free [$b_3(\text{H}_2\text{O})$] and in salt-containing eluents [$b_3(\text{NaCl})$].

45.90% for Figs. 3 and 4, respectively). This finding indicates that these data are not interchangeable and they have to be used separately in future structure–activity relationship calculations.

It can be concluded from the data that non-ionic surfactants readily interact with SDS forming complexes of undefined stoichiometry, and the complex formation involves hydrophobic forces.

Acknowledgement

This work was supported by a grant for Cooperation in Science and Technology with Central and Eastern European Countries: “Enhanced removal and prevention of environmental pollution by attachment and immobilization of bacteria at surfaces”.

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