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Mixtures of nonionic and anionic surfactants: interactions with low-molecular-mass homopeptides[☆]

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Abstract

The interaction between low molecular-mass homopeptides and mixtures of nonionic and anionic surfactants has been assessed by using reversed-phase thin-layer chromatography. The relative strength of interaction for mixtures of sodium dodecylsulfate and tridecylalcohol diglycolate (GNX) at the molar ratios of 8:2, 6:4, 4:6 and 2:8 has been calculated and its relationship with the physicochemical parameters (number of amino acid units, hydrophobicity, side chain bulkiness, electronic characteristics) of peptides has been computed by stepwise regression analysis. Each peptide interacted with each surfactant mixture the strength of interaction markedly depending on both the character of the peptide and the composition of the surfactant mixture. The hydrophobicity and electronic properties of the amino acid units exerted the highest influence on the strength of interaction at the highest concentration of the nonionic surfactant (GNX) whereas the number of amino acid units in the peptide molecule and the bulkiness of the amino acid side chain governed the strength of interaction at the lowest concentration of GNX. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In analytical chemistry, particularly in micellar electrokinetic chromatography (MEKC) [1] two phase separations and LC using micellar mobile phases [2,3], partition between the surfactant micelles and analytes to be separated is made use of.

With charged (cationic [4] or anionic [5]) surfactants at submicellar concentrations can act as counter ions changing both the charge and polarity of entities separated.

It has been established the surfactants readily bind to proteins [6,7] modifying thereby structure [8,9], physico-chemical properties, and enzymatic activity [10,11]. To the best of our knowledge the molecular basis of protein-surfactant interaction has not been studied in detail and the interactive forces involving in the binding have not been elucidated.

Various chromatographic methods have been extensively used for the study of molecular interactions [12]. The advantages of these techniques are the low

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quantity of compounds needed for the investigation and the low requirement for purity (impurities are separated during the chromatographic process). Although thin-layer chromatography (TLC) is a very simple and today rarely used method [13] its application is highly recommended when a large number of retention data are needed (i.e. for preliminary screening tests, or for the prediction of HPLC behaviour of a considerable number of analytes [14,15]). TLC as a rapid and versatile method has also been employed for the measurement of molecular interactions [16,17]. TLC techniques allow the simultaneous measurement of more than one interaction, however, the stoichiometry of complexes and the absolute value of the energy of interaction cannot be determined.

In our previous communication we have proven that both anionic and nonionic surfactants readily bind to low-molecular-mass homopeptides, and the relative strength of interaction depended on the number of amino acid units in the peptide, side chain bulk and electronic properties and hydrophobicity of the amino acids [18].

As our previous results proved that the strength of interaction between surfactants and low-molecular-mass peptides shows considerable differences we considered worth while to broaden the spectrum of surfactant mixtures used.

The objectives of the study were the determination of the interaction of surfactant mixtures with homologous series of peptides, the elucidation of the influence of the composition of surfactant mixtures on the relative strength of interaction, and the assessment of the relationship between the binding forces and the structural and physicochemical parameters of the interactive compounds using multivariate mathematical statistical methods.

2. Experimental

The experimental technique used followed that applied in our preceding paper [18]. Briefly, DC-Aluminiumoxide F₂₅₄ plates (Merck, Darmstadt, Germany) were impregnated by overnight pre-development in *n*-hexane–paraffin oil (95:5, v/v) as previously described [19]. Sodium dodecylsulfate (SDS), amino acids and their low molecular mass

homopeptides (Ala, Gly, Lys and Phe di- to tetra- or pentapeptides) were purchased from Sigma (St. Louis, MO, USA) and used as received. Commercial nonionic surfactant Genapol ×20 (tridecyl alcohol diglycolate, further GNX) was purchased from Hoechst (Frankfurt, Main, Germany). Solutes were dissolved in the mobile phases at a concentration of 1 mg ml⁻¹ and 5 µl of solutions were spotted into the plates. As the objective of the study was the measurement of the relative strength of interaction between peptides and surfactant mixtures and not the elucidation of the influence of surfactants on the separation of peptides, solutes were separately spotted on the plates (for the theoretical background see our preceding paper [18]). Methanol–water mixtures were used as mobile phases, the methanol concentration varying between 10 and 90% (v/v). The employment of this wide range of methanol concentration was necessitated by the highly different retention of peptides on the stationary phase. Mixtures of SDS and GNX at the molar ratios of 8:2, 6:4, 4:6 and 2:8 (further mixtures I, II, III and IV, respectively) were separately added to the mobile phases at the concentration range of 0–100 mM. Developments were carried out in sandwich chambers (22×22×3 cm) at ambient temperature the distance of development being about 16 cm. After development the plates were dried at 105°C and the spots of solutes were revealed by the ninhydrin reagent. In order to increase the sensitivity of detection the plates were sprayed with 2 M aqueous acetic acid prior ninhydrin reaction. Each experiment was run in quadruplicate. In order to elucidate the effect of surfactants on the peak shape and peak symmetry some spots were evaluated by Densitometer CD-60 (Desaga, Heidelberg, Germany) applying reflectance mode. Spots were detected at 470 nm.

The R_M value characterizing the molecular lipophilicity in reversed-phase TLC was calculated for each solute in each eluent and for each eluent additive:

$$R_M = \log(1/R_F - 1) \quad (1)$$

When the coefficient of variation of the parallel determinations was higher than 5% the R_M value was omitted from the subsequent calculations. To separate the effects of methanol and surfactant mixtures

on the lipophilicity of the solutes the following equation was fitted to the experimental data:

$$R_M = R_{M_0} + b_1 C_1 + b_2 C_2 \quad (2)$$

where R_M is the value for a solute determined at given methanol and surfactant mixture concentrations; R_{M_0} is the R_M value extrapolated to zero methanol and surfactant mixture concentrations; b_1 is the decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent (related to the specific hydrophobic surface area of the solutes) [20]; b_2 is the decrease in the R_M value caused by a 1 mM concentration change of surfactant mixtures in the eluent (related to the relative strength of interaction); C_1 and C_2 are the concentrations of methanol and surfactant mixtures, respectively. Eq. (2) was applied separately for mobile phase systems containing mixtures of SDS and GNX at the molar ratios of 8:2, 6:4, 4:6 and 2:8. The similarities or dissimilarities between the interactive capacity of various surfactant mixtures were elucidated by calculating linear relationships between the corresponding b_2 values of solutes.

The relationship between the physicochemical parameters of solutes and their capacity to interact with surfactant mixtures was elucidated by stepwise regression analysis (SRA) [21]. In the traditional multivariate regression analysis the presence of the independent variables that exert no significant influence on the dependent variable lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables increasing in this manner the information power of the calculation. SRA was carried out four times the dependent variables being the relative strength of peptide–surfactant interaction determined in the presence of surfactant mixtures I, II, III and IV. The independent variables were the number of amino acid units in the peptide molecule (No), the hydrophobicity (z_1), side chain bulk (z_2) and electronic properties of amino acids (z_3). The molecular parameters were taken from Ref. [22]. The combined variables (z values multiplied by the number of amino acid units, $z_i \cdot \text{No}$) were also included in the calculation. The number of

accepted independent variables was not limited; the acceptance level was set to 95% significance.

Software for stepwise regression analysis was purchased from CompuDrug (Budapest, Hungary).

3. Results and discussion

Similarly as in our previous investigation of peptides Ala₂, Ala₃, Ala₄ and Ala₅ were very near to the front in the majority of mobile phases, and, consequently, their interaction with surfactants was impossible to assess. Also similarly to the results of our previous investigations with SDS and octaethoxylated oleyl alcohol, GEN [18] Phe₅ showed very low mobility under the experimental conditions making impossible the determination of the relative strength of interaction. Solutions of GNX were not transparent at low methanol and high GNX concentrations indicating the inadequate dissolution of GNX. SDS added to the mobile phase of methanol–water (45:55, v/v) at 20 mM concentration resulted in a clear and transparent solution of 80 mM of GNX. This phenomena can be tentatively explained by the supposition that SDS promoted the solubility of GNX by forming complexes or mixed micelles with it. The influence of SDS and GNX mixtures on the spot shape of Ala and Lys₂ are demonstrated in Figs. 1 and 2, respectively. The densitograms show that both surfactant mixtures increase the chromatographic mobility of both Ala and Lys₂ suggesting the existence of interaction between solutes and mobile phase additives. The spot shape and spot symmetry did not change in the presence of surfactant mixtures suggesting that surfactant mixtures do not lessen the reliability of the determination of R_f values employed for the calculation of the relative strength of interaction.

The simultaneous effect of methanol and surfactant mixtures on the R_M value of Phe₂ and Gly₂ is shown in Figs. 3 and 4, respectively. In the case of Phe₂ the increase in the concentrations of both methanol and surfactant mixtures resulted in the decrease of apparent lipophilicity. The impact of surfactant mixture on the lipophilicity depended on its composition (compare Fig. 3a and 3b) indicating that various surfactant mixtures may influence the retention differently. Interestingly, higher methanol

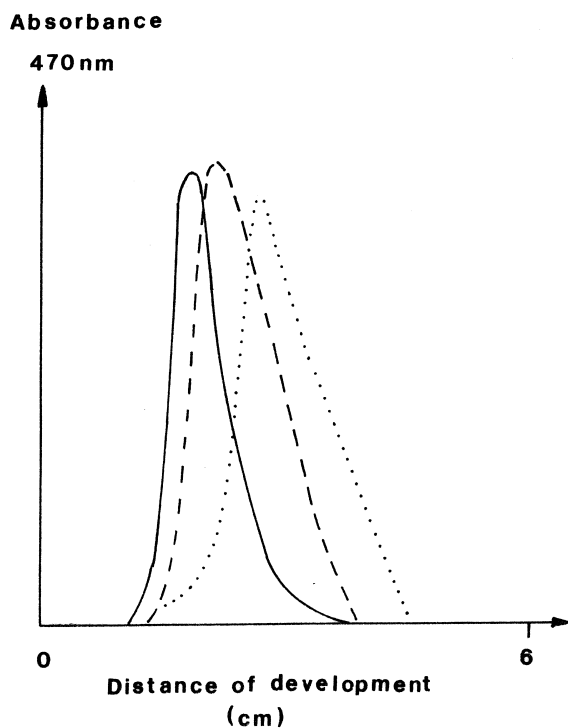


Fig. 1. Densitograms of Ala. Mobile phase: water–methanol (60:40, v/v). ----- = control; - - - = 20 mM SDS and 80 mM GNX in the mobile phase; = 80 mM SDS and 20 mM GNX in the mobile phase.

concentration increased the R_M value of Gly₂ proving that it exerts a different effect on the retention of Gly₂ than on that of Phe₂. Similar anomalous retention behaviour of peptides [23] and amino acids [24] has been previously observed and it was explained by the side effect of the adsorption centers on the surface of the support not covered by the hydrophobic ligand [25]. The influence of surfactant mixtures depended also in this case on the composition.

The relative strength of interactions (b_2) between solutes and surfactant mixtures and some validation parameters such as standard deviation of b_2 values and the calculated and tabulated F values are compiled in Tables 1–4. The b_2 values are also the regression coefficients of Eq. (2) indicating the magnitude of the decrease of lipophilicity caused by unit change of the concentration of surfactant mixtures in the mobile phase. It has to be emphasized that the b_2 values are not identical numerically with

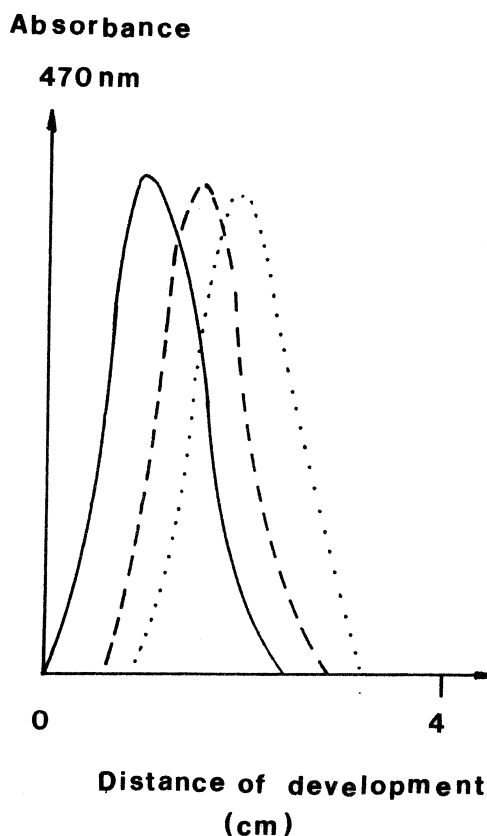


Fig. 2. Densitograms of Lys₂. Mobile phase: water–methanol (60:40, v/v). ----- = control; - - - = 20 mM SDS and 80 mM GNX in the mobile phase; = 80 mM SDS and 20 mM GNX in the mobile phase.

the traditional complex stability values but they are linearly related with them [26,27]. The comparison of the calculated F values with the tabulated ones indicating 95% significance level proves that each solutes significantly increased with each mixture of surfactant. The b_2 values either increased monotonously with the concentration of GNX in the surfactant mixtures indicating that neither synergistic nor antagonistic effects occur. The relative strengths of solutes composed of Ala, Gly and Lys units decreased at increasing ratio of SDS in the surfactant mixtures the effect being the highest with Lys peptides. Solute containing Phe showed opposite tendency. This finding may be due to the fact that the polar head group of SDS binds to the hydrophilic NH₂ substructures of solutes and the strength of this

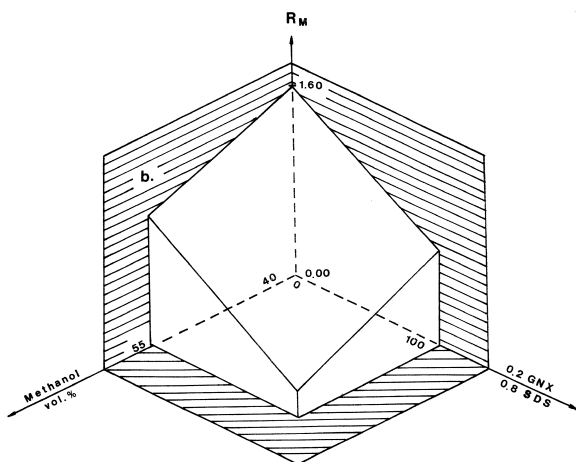
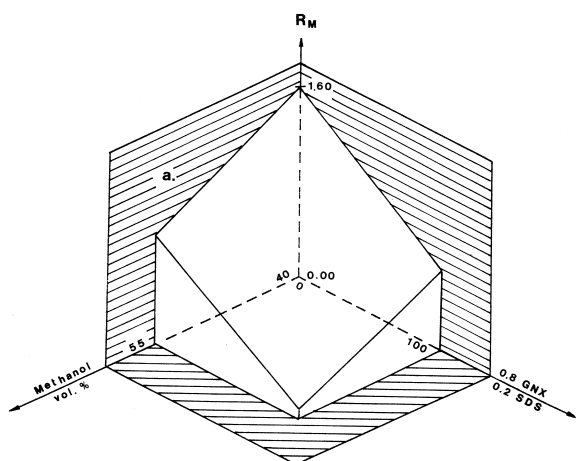


Fig. 3. Effect of methanol and the concentration of surfactant mixtures on the R_M value of Phe₂; (a): GNX–SDS (8:2, m/m), (b): GNX–SDS (2:8, m/m).

electronic interaction governs the solute–surfactant binding.

This assumption has been supported by the higher interactive strength of the Lys peptides. The more hydrophobic GNX probably binds to the apolar ring structure of Phe and the strength of this interaction overshadows the strength of electrostatic interactive forces between the polar ethylene oxide chain of GNX and the polar head group of Phe.

The parameters of significant relationships between the physicochemical characteristics of solutes and their capacity to interact with surfactant mixtures are compiled in Table 5. The linear correlation was significant for each surfactant mixture (see F_{calc} .

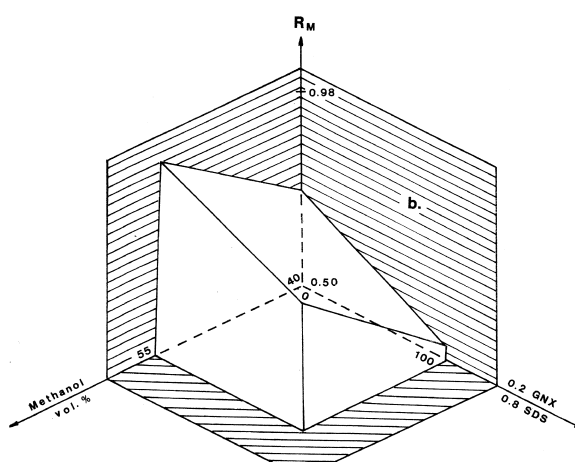
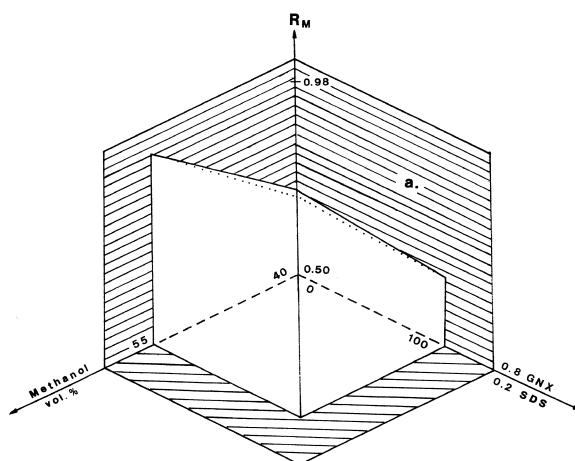


Fig. 4. Effect of methanol and the concentration of surfactant mixtures on the R_M value of Gly₂; (a): GNX–SDS (8:2, m/m), (b): GNX–SDS (2:8, m/m).

values), and the variance explained varied between 55 and 92% (see r^2 values). The good statistical parameters suggest that the dependent variables included in the calculation can be employed for the prediction of the interactive strength for other peptides not included in the experiments. As expected from the different composition of surfactant mixtures the selected dependent variables were different for each surfactant mixture indicating the involvement of different molecular forces in the interactions. The relative strength of interaction depended on the number of amino acid units and on their bulkiness at the lowest concentration of GNX in the surfactant

Table 1

Values of the b_2 parameter of Eq. (2) (relative strength of interaction of the analytes under investigation with surfactant mixture I (sodium dodecylsulfate–tridecylalcohol diglycolate, 8:2, m/m)

Solute	n^a	Relative strength of interaction $-b_2 \cdot 10^3$ standard deviation $\cdot 10^4$		$F_{\text{calc.}}$	$F_{95\%}$
Ala	12	1.65	1.53	116.88	4.96
Gly	12	1.75	0.76	532.28	4.96
Gly ₂	12	1.66	2.36	49.70	4.96
Gly ₃	12	1.87	2.53	55.06	4.96
Gly ₄	11	1.90	4.10	21.36	5.12
Gly ₅	11	3.07	3.92	61.19	5.12
Lys	12	2.56	4.19	37.43	4.96
Lys ₂	12	3.15	3.43	84.47	4.96
Lys ₃	13	4.41	6.48	46.36	4.84
Lys ₄	15	4.99	8.89	31.56	4.67
Lys ₅	13	5.56	16.71	11.06	4.84
Phe	12	2.14	3.53	36.49	4.96
Phe ₂	12	7.26	11.28	41.44	4.96
Phe ₃	18	7.22	24.16	8.91	4.49
Phe ₄	13	5.88	19.72	8.90	4.84

^a n = Number of measurements.

mixture. The significant effect of the number of amino acid units suggest that the long SDS molecule can bind to more than one amino acid forming more stable complexes and the binding is influenced by the sterical correspondence of the interactive molecular species. The lipophilicity and electronic properties of

amino acids influenced significantly the interaction at the highest concentration of GNX proving again the mixed character of the interactive forces.

The correlation coefficients of the linear relationships between the relative strength of solute–surfactant mixture interactions are compiled in Table 6.

Table 2

Relative strength of interaction (b_2 values of Eq. (2)) of amino acids and peptides with surfactant mixture I (sodium dodecylsulfate–tridecylalcohol diglycolate 6:4 m/m)

Solute	n^a	Relative strength of interaction $-b_2 \cdot 10^3$ standard deviation $\cdot 10^4$		$F_{\text{calc.}}$	$F_{95\%}$
Ala	12	1.30	1.18	121.62	4.96
Gly	12	1.17	1.09	117.01	4.96
Gly ₂	12	0.86	2.12	16.57	4.96
Gly ₃	12	1.03	2.09	24.00	4.96
Gly ₄	11	1.25	3.88	10.78	5.12
Gly ₅	11	2.40	4.45	29.02	5.12
Lys	12	2.01	0.44	20.84	4.96
Lys ₂	12	2.40	3.45	48.69	4.96
Lys ₃	9	3.95	8.80	20.13	5.59
Lys ₄	15	4.63	9.61	23.18	4.67
Lys ₅	13	5.37	17.71	9.20	4.84
Phe	12	3.04	3.38	80.97	4.96
Phe ₂	12	7.34	9.32	62.04	4.96
Phe ₃	18	8.00	22.69	12.42	4.49
Phe ₄	12	8.07	18.67	18.66	4.96

^a n = number of measurements.

Table 3

Analogous data as in Tables 1 and 2 for the sodium dodecylsulfate–tridecylalcohol diglycolate (4:6, m/m) mixture

Solute	n^a	Relative strength of interaction $-b_2 \cdot 10^3$ standard deviation $\cdot 10^4$		$F_{\text{calc.}}$	$F_{95\%}$
Ala	12	1.03	1.09	88.61	4.96
Gly	12	0.95	1.09	76.60	4.96
Gly ₂	12	0.44	1.93	5.16	4.96
Gly ₃	16	0.43	1.75	6.00	4.60
Gly ₄	16	0.54	2.37	5.29	4.60
Gly ₅	11	0.86	2.71	10.02	5.12
Lys	12	1.44	3.98	13.02	4.96
Lys ₂	12	1.93	3.22	35.74	4.96
Lys ₃	9	2.63	10.33	6.48	5.59
Lys ₄	13	4.12	8.86	21.61	4.84
Lys ₅	13	6.71	19.03	12.42	4.84
Phe	12	2.84	2.26	158.02	4.96
Phe ₂	12	8.16	8.75	86.99	4.96
Phe ₃	12	7.12	15.97	19.86	4.96
Phe ₄	16	7.47	21.19	12.41	4.60

^a n = number of measurements.

The results entirely support the previous conclusions that neither synergistic nor antagonistic effects can be observed. The data prove that the various sets of b_2 values are similar but not identical the differences being highest for the mixtures containing 80 and 20 mol% GNX.

4. Conclusions

It can be concluded from the results that amino acids and peptides interact with surfactant mixtures; the strength of interaction depends on both the composition of the surfactant mixture and the

Table 4

Analogous data as in Table 1 for the sodium dodecylsulfate–tridecylalcohol diglycolate (2:8, m/m) mixture^a

Solute	n	Relative strength of interaction $-b_2 \cdot 10^3$ standard deviation $\cdot 10^4$		$F_{\text{calc.}}$	$F_{95\%}$
Ala	12	0.75	1.04	51.52	4.96
Gly	12	0.37	0.53	50.04	4.96
Gly ₂	16	0.36	1.49	5.73	4.60
Gly ₃	16	0.43	1.88	5.31	4.60
Gly ₄	21	0.63	2.44	6.69	4.38
Gly ₅	11	0.63	2.28	7.72	5.12
Lys	12	0.96	3.44	7.84	4.96
Lys ₂	12	1.05	3.99	6.92	4.96
Lys ₃	13	1.11	4.40	6.38	4.84
Lys ₄	14	2.93	9.81	8.91	4.75
Lys ₅	11	4.32	17.92	5.80	5.12
Phe	12	2.69	2.68	100.91	4.96
Phe ₂	12	8.69	9.64	81.21	4.96
Phe ₃	9	11.33	14.83	58.37	5.59
Phe ₄	11	8.24	18.39	20.09	5.12

^a n = number of measurements. Relative strength of interaction (b_2 values of Eq. (2)) of amino acids and peptides with surfactant mixture I (sodium dodecylsulfate-tridecylalcohol diglycolate 2:8 m/m).

Table 5

Linear correlations between the physicochemical parameters of peptides and their capacity to interact with surfactant mixtures I, II, III and IV – results of stepwise regression analysis

Parameter	No. of equation			
	1	2	3	4
A^a	2.40	1.73	3.63	3.79
B_1^b	0.60	0.97	0.78	-1.55
s_{B1}^c	0.23	0.16	0.19	0.32
B_2^c	0.54	-0.44	-	-1.70
s_{B2}^c	0.12	0.08	-	0.70
B_3^b	-	0.18	-	-
s_{B3}^c	-	0.03	-	-
$B_1' \%^d$	36.92	32.27	-	66.32
$B_2' \%^d$	63.08	31.47	-	33.68
$B_3' \%^d$	-	36.26	-	-
r^{2e}	0.6732	0.9219	0.5543	0.7216
$F_{calc.}^f$	12.36	43.30	16.17	15.55

^a Significant level 95%; $n=15$

$$b_{2.I} = A + B_1 \cdot No + B_2 \cdot z_2 \quad (1)$$

$$b_{2.II} = A + B_1 \cdot No + B_2 \cdot z_1 + B_3 (z_2 \cdot No) \quad (2)$$

$$b_{2.III} = A + B_1 z_2 \quad (3)$$

$$b_{2.IV} = A + B_1 z_1 + B_2 z_3 \quad (4)$$

Intercept value of Eqs. (1)–(4).

^b Coefficients of regression.

^c Standard deviations of coefficients of regression.

^d Standard partial regression coefficients normalized to unity.

^e Coefficient of determination indicating the ratio of variance explained by the independent variables.

^f Calculated F value indicating the fitness of Eqs. (1)–(4) to the experimental data.

physicochemical characteristics of solutes. It can be assumed that the rational selection of surfactant mixtures may result in better pharmaceutical and agrochemical formulations and may promote the chromatographic separation of peptide mixtures [28].

Table 6

Similarities between relative strengths of peptide–surfactant mixture interactions^a

		No. of surfactant mixture		
		I	II	III
No. of surfactant mixture	II	0.9621		
	III	0.9433	0.9699	
	IV	0.8917	0.9428	0.9236

^a Correlation coefficients of the linear relationships between different sets of interactive values. Higher values indicate higher similarity. Roman numbers refer to surfactants mixtures in Experimental [I=SDS–GNX (8:2, m/m); II=SDS–GNX (6:4, m/m); III=SDS–GNX (4:6, m/m); IV=SDS–GNX (2:8, m/m)].

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