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Interaction of surfactants with homologous series of peptides studied by reversed-phase thin-layer chromatography[☆]

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Abstract

The relative strength of interaction between anionic (SDS) and nonionic surfactant (octaethoxylated oleyl alcohol, GEN) and homologous series of peptides was determined by reversed-phase thin-layer chromatography (RP-TLC) carried out on alumina layers impregnated with paraffin oil. The relative strength of interaction was calculated and was correlated with the physicochemical parameters of peptides. It was established that each peptide interacted with both surfactants and with their mixture (1:1, m/m). 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. The impact of individual parameters highly depended on the character of surfactant. The data prove that the retention order of peptides can be modified by adding different surfactants and surfactant mixtures to the mobile phase resulting in improved separation. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Peptides; Sodium dodecylsulfate; Octaethoxy oleyl alcohol; Surfactants

1. Introduction

Because of their advantageous application parameters surfactants are extensively employed in pharmaceutical [1,2] and agrochemical formulations [3,4] to enhance the efficacy of the active ingredient. Furthermore, surfactants have been successfully used in various biotechnological processes [5,6]. It has been established that surfactants increase the decomposi-

tion rate of polychlorinated biphenyls [7], polycyclic aromatic hydrocarbons [8,9], etc. Besides the beneficial effects surfactants also show marked toxicity [10,11]. Thus, they cause ocular [12,13] and skin irritancy [14,15].

The biological activity of surfactants has been partially explained by their binding to proteins [16,17] and enzymes [18–20]. Surfactants modify protein structure resulting in enhanced or deteriorated function depending on the character of protein–surfactant interaction.

The binding of surfactants to proteins and the consequent modification of the solubility and adsorption characteristics of proteins [21], peptides [22] and amino acids [23] has also been exploited in their chromatographic analysis. A considerable num-

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ber of chromatographic methods has been developed for the study of the interaction between various organic and inorganic compounds [24]. Chromatographic methods used for the study of molecular interactions are relatively rapid, one of the interacting components may be available in a very low amount and it does not need to be pure because the impurities are separated during the chromatographic process. Therefore, thin-layer chromatography (TLC) as a rapid, simple and versatile technique has been employed for the study of such interactions [25,26]. The advantages of TLC determination of interactions are the possibility of simultaneous measurement of a considerable number of interactions and the simplicity of the experimental design. The disadvantages of the method are that the stoichiometry of the complexation cannot be established and only the relative strength of interaction can be calculated from the retention data.

The objectives of the work were the measurement of the interaction of the anionic surfactant sodium dodecyl sulfate (SDS) and the nonionic surfactant Genapol O 80 (GEN) with homologous series of peptides, and the elucidation of the relationship between the relative strength of interaction and the structural and physicochemical parameters of the interactive compounds using stepwise regression analysis. The results can be used for the assessment of the theoretical background of the peptide–surfactant binding and can be successfully used for the improvement of the separation of homologous series of peptides by chromatographic methods and, perhaps by micellar electrokinetic chromatography.

2. Experimental

DC-Aluminiumoxide F₂₅₄ plates (Merck, Darmstadt, Germany) were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v) as previously described [27]. SDS, amino acids (Ala, Gly, Lys, Phe) and peptides (Ala₂, Ala₃, Ala₄, Ala₅, Gly₂, Gly₃, Gly₄, Gly₅, Lys₂, Lys₃, Lys₄, Lys₅, Phe₂, Phe₃, Phe₄, Phe₅) were purchased from Sigma (St. Louis, MO, USA) and used as received. Nonionic surfactant Genapol O 80 (ethoxylated oleyl alcohol containing eight ethylene oxide groups per molecule on average) was purchased from Hoechst

Aktiengesellschaft (Frankfurt am Main, Germany). Solutes were dissolved in the mobile phases at a concentration of 1 mg/ml and 5 µl of solutions was spotted onto the plates. As the objective of the experiments was the determination of the relative strength of interaction between peptides and surfactants and not the elucidation of the influence of surfactants on the separation of peptides, solutes were separately spotted on the plates. 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 motivated by the highly different retention of peptides on impregnated alumina. SDS, GEN and their mixture in a 1:1 molecular ratio (SDS+GEN) were separately added to the mobile phases in the concentration range of 0–100 mM. Developments were carried out in sandwich chambers (22×22×3 cm) at room temperature (20±1°C), with the distance of development at about 16 cm. After development the plates were dried at 105°C and the spots of solutes were revealed by ninhydrin reagent. In order to increase the sensitivity of detection the plates were sprayed with 2 M aqueous acetic acid prior to ninhydrin reaction. Each experiment was run in quadruplicate. Some plates were evaluated by a densitometer CD-60 (Desaga, Heidelberg, Germany) using reflectance mode and a 470-nm detection wavelength. The R_M value characterising the molecular hydrophobicity in reversed-phase thin-layer chromatography (RP-TLC) was calculated for each solute in each eluent:

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

When the relative standard deviation (RSD) of the parallel determinations was higher than 5% the R_M value was omitted from the following calculations. To separate the effects of methanol and surfactants on the lipophilicity of the solutes the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1 C_1 + b_2 C_2 \quad (2)$$

where $R_M = R_M$ value for a solute determined at given methanol and surfactant concentrations; $R_{M0} = R_M$ value extrapolated to zero methanol and surfactant concentrations; b_1 = decrease in the R_M value caused by a 1% increase in the methanol concen-

tration in the eluent (related to the specific hydrophobic surface area of the solutes) [28]; b_2 = decrease in the R_M value caused by a 1 mM concentration change of surfactants in the eluent (related to the relative strength of interaction); C_1 and C_2 = concentrations of methanol and surfactants, respectively. Eq. (2) was applied separately for mobile phase systems containing SDS, GEN and SDS + GEN.

The similarities or dissimilarities between the interactive capacity of SDS, GEN and SDS+GEN were assessed 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 surfactants was elucidated by stepwise regression analysis (SRA) [29]. 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 three times the dependent variables being the relative strength of peptide–surfactant interaction determined in the presence of SDS, GEN and SDS+GEN. 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. [30]. The combined variables (z values multiplied by the number of amino acid units, z_i No.) were also included in the calculation. The number of accepted independent variables was not limited, the acceptance level was set to 95% and 99.9% significance.

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

3. Results and discussion

Solutes Ala₂, Ala₃, Ala₄ and Ala₅ were very near to the front in the majority of mobile phases,

therefore, their interaction with surfactants cannot be assessed. Oppositely, Phe₅ showed very low mobility under the experimental conditions making impossible the determination of the relative strength of interaction. The effect of SDS and GEN on the reversed-phase mobility of Gly₃ is shown in Fig. 1. The densitograms indicate that both SDS and GEN decrease the retention of Gly₃ indicating interaction

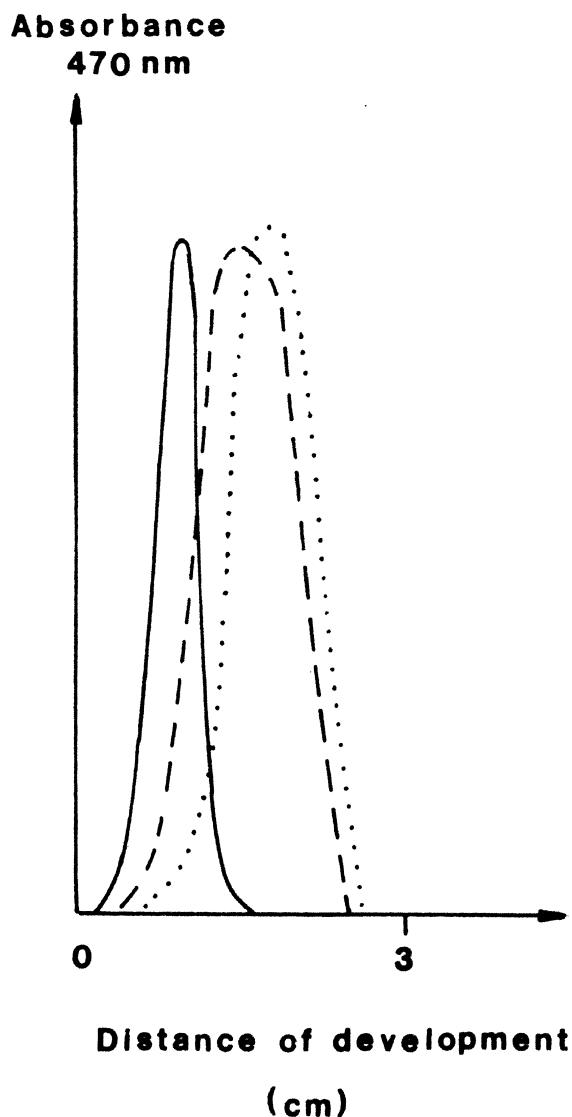


Fig. 1. Densitograms of Gly₃. Mobile phase: water–methanol (45:55, v/v). ---=Control; ---=100 mM SDS in the mobile phase; ···=100 mM GEN in the mobile phase.

between the two molecular species. The addition of surfactants do not change spot shape and spot symmetry that is surfactants do not deteriorate the reliability of the determination of R_F values used for the calculation of the relative strength of interaction. The influence of the composition of the mobile phase on the R_M value of Lys₃ is shown in Fig. 2. An increase in the concentration of surfactants caused a decrease in R_M values indicating that solutes become more hydrophilic in the presence of surfactants.

The effect depends on the type of surfactant too. This finding indicates that the retention of amino acids and peptides can be modified by the addition of surfactants to the mobile phase. Surprisingly, the concentration of methanol in the mobile phase exerts an anomalous effect on the retention of Ala, Gly and Lys peptides. Oppositely to the expected decrease of retention, the R_M value of these peptides increased with increasing concentration of methanol. This anomalous behaviour can be tentatively explained by the assumption that the highly polar dissociable substructures of solutes account for the effect. As the dissociation of the polar substituents is suppressed in the presence of methanol (lower dielectric constant), the apparent lipophilicity of solutes increases, resulting in higher retention. It can be assumed that the regular retention behaviour of Phe peptides may be due to the fact that the effect of the strongly hydrophobic side chain overshadows the effect of the dissociable polar substructures.

The relative strengths of surfactant–solute interactions and some statistical parameters are compiled in Tables 1–3. Significant correlation was observed between the retention of solutes and the concentration of surfactants in the mobile phase at a significance level of 95% in each instance (compare $F_{\text{calc.}}$ values with tabulated $F_{95\%}$ ones). The retention (apparent hydrophobicity) of each solute decreases with increasing concentration of surfactants (see negative b_2 values) indicating surfactant–peptide interaction. It can be further established that the relative strength of interaction shows high variation both among the surfactants and peptides. This result suggests that various surfactants and surfactant mixtures can influence differently the mobility of peptides in chromatographic systems, that means that the addition of surfactants and surfactant mixtures at various concentrations to the mobile phase may

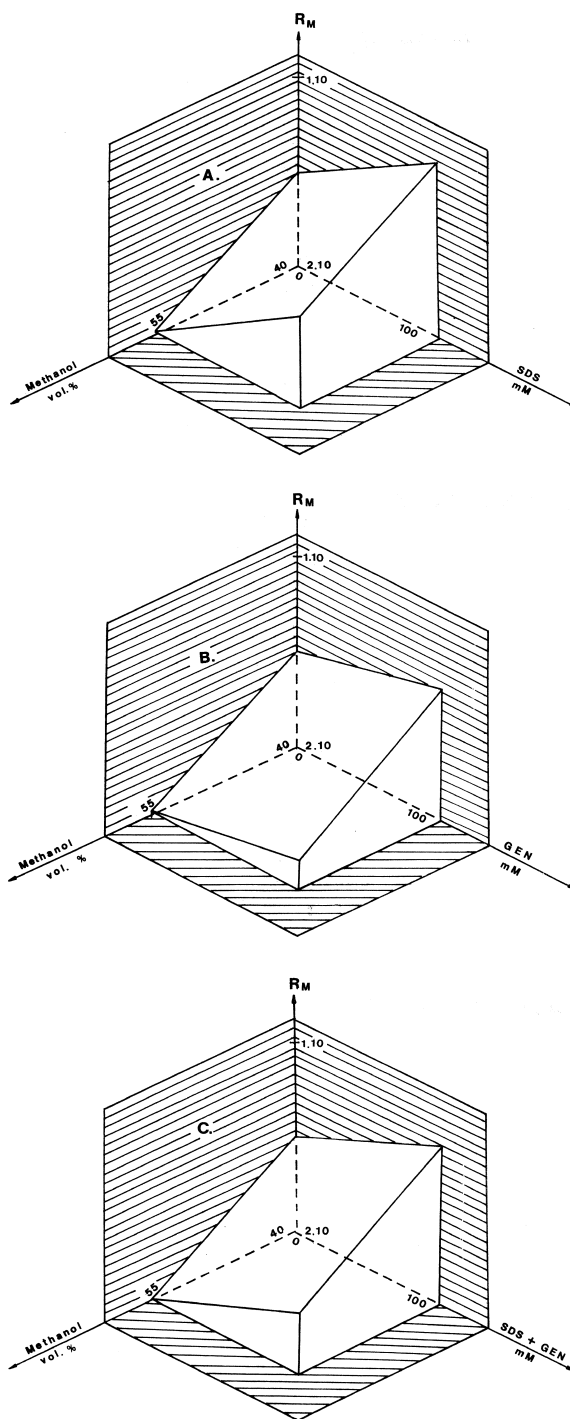


Fig. 2. Effect of methanol and surfactant concentrations on the R_M value of Lys₃ (A=SDS, B=GEN and C=SDS+GEN).

Table 1
Relative strength of interaction of amino acids and peptides with sodium dodecyl sulfate (b_2 values of Eq. (2))^a

Solute	Nos.	Relative strength of interaction		$F_{\text{calc.}}$	$F_{95\%}$
		Average ($-b_2 \cdot 10^3$)	Standard deviation ($\cdot 10^4$)		
Ala	20	1.26	2.06	37.16	4.38
Gly	20	1.53	2.18	49.14	4.38
Gly ₂	20	1.41	2.40	34.47	4.38
Gly ₃	20	1.48	3.08	23.06	4.38
Gly ₄	19	2.27	3.77	36.16	4.41
Gly ₅	19	3.87	5.17	56.06	4.41
Lys	20	1.87	2.86	42.52	4.38
Lys ₂	20	2.53	4.18	36.56	4.38
Lys ₃	15	3.95	6.22	40.41	4.67
Lys ₄	10	8.52	18.41	21.40	5.12
Lys ₅	17	6.55	15.89	16.99	4.54
Phe	14	0.66	2.25	8.56	4.75
Phe ₂	20	2.43	7.12	11.63	4.38
Phe ₃	26	2.40	9.42	6.47	4.26
Phe ₄	19	2.71	12.70	4.56	4.45

^a Nos., Number of measurements; $F_{\text{calc.}}$, calculated F value indicator of the deviation of the b_2 value from zero; $F_{95\%}$, tabulated F value determining the significance deviation of b_2 values from zero at the level of 95%.

improve the separation of peptides and any other solute molecule interacting with surfactants.

No significant linear relationship was found between the b_2 values determined in the presence of SDS and SDS+GEN ($r_{\text{calc.}}=0.1212$) and of SDS and GEN ($r_{\text{calc.}}=0.1537$). This finding supports our

previous qualitative conclusion that the character of surfactant exerts a considerable influence on the retention of peptides. Surprisingly, a highly significant correlation (significance level over 99.9%) was found between the b_2 values determined in the presence of SDS+GEN and GEN (Fig. 3). This

Table 2
Relative strength of interaction of amino acids and peptides with oleyl alcohol octaethoxylate (Genapol O 80) (b_2 values of Eq. (2))^a

Solute	Nos.	Relative strength of interaction		$F_{\text{calc.}}$	$F_{95\%}$
		Average ($-b_2 \cdot 10^3$)	Standard deviation ($\cdot 10^4$)		
Ala	16	2.37	5.35	19.64	4.54
Gly	16	1.68	6.52	6.62	4.54
Gly ₂	16	2.60	6.09	18.25	4.54
Gly ₃	16	2.91	7.25	16.10	4.54
Gly ₄	15	2.56	1.16	4.89	4.60
Gly ₅	17	0.50	0.23	4.60	4.54
Lys	20	0.84	3.21	6.90	4.38
Lys ₂	16	3.31	12.24	7.31	4.54
Lys ₃	17	1.68	7.42	5.15	4.49
Lys ₄	22	1.57	4.56	11.79	4.35
Lys ₅	11	2.42	9.34	6.73	4.96
Phe	16	0.66	6.71	95.45	4.54
Phe ₂	16	24.13	3.37	51.28	4.54
Phe ₃	13	24.11	4.63	27.11	4.75
Phe ₄	11	16.25	7.02	5.35	4.96

^a Nos., Number of measurements; $F_{\text{calc.}}$, calculated F value indicator of the deviation of the b_2 value from zero; $F_{95\%}$, tabulated F value determining the significance deviation of b_2 values from zero at the level of 95%.

Table 3

Relative strength of interaction of amino acids and peptides with the mixture of sodium dodecyl sulfate and oleyl alcohol octaethoxylate (1:1 molar ratio) (b_2 values of Eq. (2))^a

Solute	Nos.	Relative strength of interaction		$F_{\text{calc.}}$	$F_{95\%}$
		Average ($-b_2 \cdot 10^3$)	Standard deviation ($\cdot 10^4$)		
Ala	16	1.13	1.31	73.81	4.54
Gly	16	0.84	1.21	48.98	4.54
Gly ₂	14	0.62	2.80	4.93	4.67
Gly ₃	16	0.95	3.74	6.48	4.54
Gly ₄	15	1.19	3.67	10.42	4.67
Gly ₅	14	1.46	6.07	5.78	4.67
Lys	16	1.93	3.42	31.74	4.54
Lys ₂	16	2.50	3.52	50.52	4.54
Lys ₃	13	3.19	7.89	16.35	4.75
Lys ₄	23	2.21	6.65	11.06	4.30
Lys ₅	15	3.36	15.59	4.65	4.60
Phe	16	2.17	2.03	115.17	4.54
Phe ₂	16	7.03	8.30	71.69	4.54
Phe ₃	13	7.90	11.45	47.64	4.75
Phe ₄	16	5.16	16.30	10.02	4.54

^a Nos., Number of measurements; $F_{\text{calc.}}$, calculated F value indicator of the deviation of the b_2 value from zero; $F_{95\%}$, tabulated F value determining the significance deviation of b_2 values from zero at the level of 95%.

relationship indicates that the nonionic surfactant play a dominant role in its mixture with SDS and the effect of SDS on the interaction is of secondary importance.

The parameters of significant correlations between the physicochemical parameters of solutes and their capacity to interact with surfactants (results of step-wise regression, significance level 95%) are com-

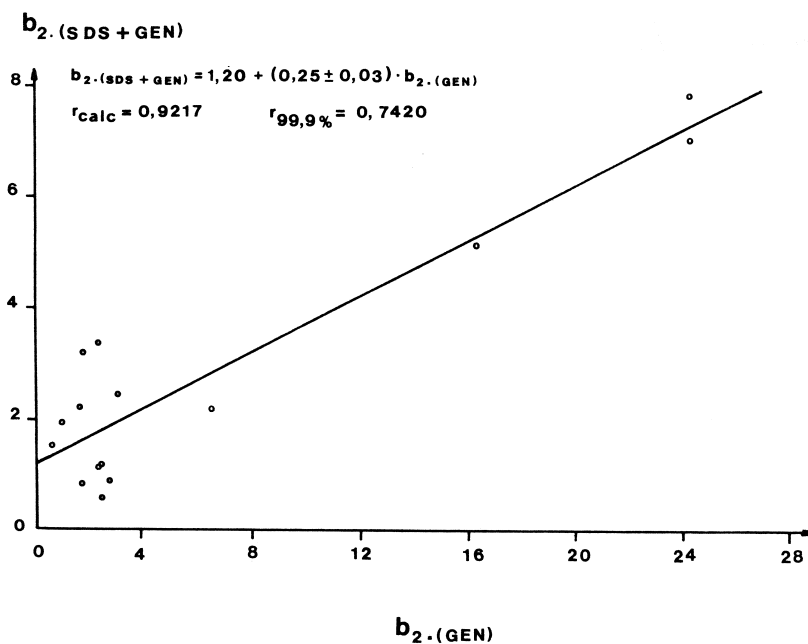


Fig. 3. Linear relationship between the relative strength of SDS+GEN-peptide and GEN-peptide interactions.

piled in Table 4 and one significant relationship is shown in Fig. 4. Each set of b_2 values depended significantly on the parameters included in the calculation (see F_{calc} values). The variance explained was fairly high, between 69 and 86% (see r^2 values), indicating that these parameters account for a considerable part of the relative strength of interaction. The selected dependent variables were different for each surfactant suggesting that different molecular forces are involved in the peptide–surfactant interactions. The number of amino acid units, the side chain bulk and the electronic properties equally influenced the binding of peptide to SDS. This result can be explained by the supposition that SDS preferably

binds to the bulky amino acid side chain (positive b_2 value) and electrostatic repulsive forces act between the dissociable head group of SDS and the hydrophilic substructures of peptides (negative b_3 values). Lipophilicity does not influence the interaction proving indirectly its polar character. Oppositely to SDS, only lipophilicity exerts a significant effect on the interaction of peptides with GEN. It can be assumed that the highly hydrophobic alkyl chain of oleyl alcohol interacts with the apolar parts of peptides and the ethoxy chain does not influence markedly the strength of interaction. As expected, both sterical and hydrophobic parameters influence the effect of surfactant mixture on the mobility of peptides indicating the possibility of ternary complex formation.

Table 4

Parameters of linear correlations between the physicochemical parameters of peptides and their capacity to interact with SDS, SDS + GEN and GEN ($b_{2,\text{SDS}}$, $b_{2,\text{SDS}+\text{GEN}}$, $b_{2,\text{GEN}}$)^a

Parameter	Equation		
	Eq. (3) (I)	Eq. (4) (II)	Eq. (5) (III)
A^b	0.49	3.47	8.87
b_3^c	0.74	−0.35	−2.27
$s_{b_3}^d$	0.19	0.13	0.39
b_4^c	0.07	0.42	—
$s_{b_4}^d$	0.03	0.14	—
b_5^c	−0.24	—	—
$s_{b_5}^d$	0.06	—	—
$b_3^r (\%)^e$	38.55	48.80	—
$b_4^r (\%)^e$	22.38	51.20	—
$b_5^r (\%)^e$	39.07	—	—
r^{2f}	0.8559	0.6856	0.7277
$F_{\text{calc.}}^g$	21.79	13.09	34.74

^a Results of stepwise regression analysis. Significant level 95%; Number of observations = 15.

$$b_{2,\text{SDS}} = A + b_3\text{No.} + b_4(z_2\text{No.}) + b_5(z_3\text{No.}) \quad (3)$$

$$b_{2,\text{SDS}+\text{GEN}} = A + b_3z_1 + b_4z_2 \quad (4)$$

$$b_{2,\text{GEN}} = A + b_3z_1 \quad (5)$$

Mobile phase additives: (I) sodium dodecyl sulfate; (II) sodium dodecyl sulfate–oleyl alcohol octaethoxylate (1:1, m/m); (III) oleyl alcohol octaethoxylate.

^b Intercept value of Eqs. (3–5).

^c Coefficients of regression.

^d Standard deviations of coefficients of regression.

^e Standard partial regression coefficients normalised to unity.

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

^g Calculated F value indicating the fitness of Eqs. (3–5) to the experimental data. For other symbols see Experimental.

4. Conclusions

It can be concluded from the data that both SDS and GEN bind to peptides modifying their mobility. Sterical, electrostatic and hydrophobic forces are equally included in the interaction, their relative importance depends on the character of surfactant added to the mobile phase.

5. Nomenclature

SDS	Sodium dodecyl sulfate
GEN	Oleyl alcohol octaethoxylate
SRA	Stepwise regression analysis
R_M	Characterises the molecular lipophilicity in reversed-phase thin layer chromatography at a given concentration of organic modifier in the mobile phase
R_{M0}	R_M value extrapolated to zero concentration of organic modifier in the mobile phase
b_i	Regression coefficients describing the effect of the individual independent variable on the dependent variable
C_1 and C_2	Concentrations of methanol (% , v/v) and surfactants (mM) in the mobile phase
No.	Number of amino acid units in the peptide molecule
Nos.	Number of measurements

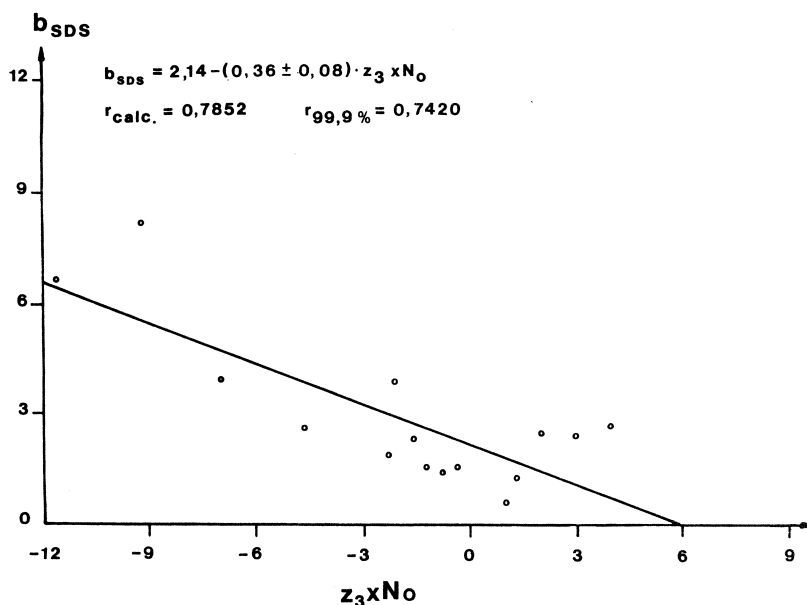


Fig. 4. Linear relationship between the z_3No value of peptides and their capacity to interact with SDS. Result of stepwise regression analysis. Significance level 99.9%; $n = 15$.

z_1	Hydrophobicity of parent amino acid
z_2	Side chain bulk of parent amino acid
z_3	Electronic properties of parent amino acid
A	Intercept value of Eqs. (3–5)
s_{b_i}	Standard deviations of regression coefficients b_i
b'_i (%)	Standard partial regression coefficients normalised to unity
r^2	Coefficient of determination indicating the ratio of variance explained by the independent variables
$F_{calc.}$	Calculated F value indicator of the deviation from zero
$F_{95\%}$	Tabulated F value determining the significance deviation from zero at the level of 95%.

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References

- [1] E. Toutou, F. Levi-Schaffer, N. Dayan, F. Alhaiquem, F. Riccieri, *Int. J. Pharm.* 103 (1994) 131.
- [2] J. Jansen, C. Treiner, C. Vaution, F. Puisieux, *Int. J. Pharm.* 103 (1994) 19.
- [3] J.D. Nalewaja, Z. Woznica, F.A. Manthey, *Weed Technol.* 5 (1991) 92.
- [4] T. Watanabe, I. Yamaguchi, *J. Pestic. Sci.* 18 (1993) 99.
- [5] J. Chen, S. Shimura, K. Kirimura, S. Usami, *Biosci. Biotechnol. Biochem.* 58 (1994) 773.
- [6] C.E. Forney, C.E. Glatz, *Biotechnol. Prog.* 11 (1995) 260.
- [7] B.N. Aronstein, J.R. Paterek, *Environ. Toxicol. Chem.* 14 (1995) 749.
- [8] F. Volkering, A.M. Breuere, J.G. vanAndel, W.H. Rulkens, *Appl. Environ. Microbiol.* 61 (1995) 1699.
- [9] H.J. Tsomides, J.B. Hughes, J.M. Thomas, C.H. Ward, *Environ. Toxicol. Chem.* 14 (1995) 953.
- [10] A.W. Hubbard, L.J. Moore, R.H. Clothier, H. Sulley, K.A. Rollin, *Toxicol. Vitro* 8 (1994) 689.
- [11] P.L. Casterton, L.F. Potts, B.D. Klein, *Toxicol. Vitro* 8 (1994) 835.
- [12] R.W. Lewis, J.C. McCall, P.A. Botham, R. Trebilcock, *Toxicol. Vitro* 8 (1994) 867.
- [13] W. Yang, D. Acosta, *Toxicol. Lett.* 70 (1994) 309.
- [14] K.P. Wilhelm, G. Freitag, H.H. Wolff, *J. Am. Acad. Dermatol.* 31 (1994) 981.
- [15] G.M. Shivji, L. Segal, R.C. McKenzie, D.N. Sauder, *Toxicol. Methods* 4 (1994) 193.

- [16] S. Levin, G. Tawil, J. Pharm. Biomed. Anal. 12 (1994) 499.
- [17] G. Prieto, J.M. del Rio, M.I. Paz Andrade, F. Sarmiento, M.N. Jones, Int. J. Biol. Macromol. 1 (1993) 343.
- [18] N. Kamiya, M. Goto, F. Nakashio, Biotechnol. Prog. 11 (1995) 270.
- [19] Q.L. Chang, H.Z. Liu, J.Y. Chen, Enzyme Microb. Technol. 16 (1994) 970.
- [20] P. Saudan, S.M. Zakeerrudin, M.-A. Malavallon, M. Gratzel, D.M. Fraser, Biotechnol. Bioeng. 44 (1994) 407.
- [21] G.M. Janini, H.J. Issaq, G.M. Muschik, J. Chromatogr. A 792 (1997) 125.
- [22] Z. Deyl, I. Miksik, J. Chromatogr. B 739 (2000) 3.
- [23] X. Páez, P. Prada, L. Hernández, J. Chromatogr. B 739 (2000) 247.
- [24] T. Cserhádi, K. Valkó, Chromatographic Determination of Molecular Interactions, CRC Press, Boca Raton, FL, 1994.
- [25] V. Lambroussi, S. Piperaki, A. Tsantili-Kakoulidou, J. Planar Chromatogr.-Mod. TLC 12 (1999) 124.
- [26] P.K. Zarzycki, M. Wierzbowska, J. Nowakowska, A. Chmielewska, H. Lamparczyk, J. Chromatogr. A 839 (1999) 149.
- [27] T. Cserhádi, B. Bordás, E. Fenyvesi, J. Szejtli, J. Chromatogr. 259 (1983) 107.
- [28] C. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.
- [29] H. Mager, in: Moderne Regressionsanalyse, Salle, Sauerländer, Frankfurt am Main, 1982, p. 135.
- [30] J. Jonsson, L. Eriksson, S. Hellberg, M. Sjöström, S. Wold, Quant. Struct.-Act. Relatsh. 8 (1989) 204.