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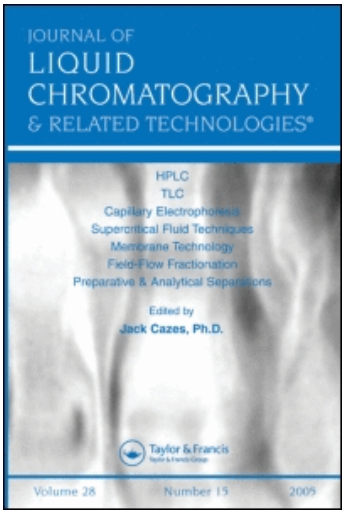

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TLC Study of the Binding of Nonionic Surfactants to the Corn Protein Zein

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ABSTRACT

The binding of 18 nonionic surfactants with various lengths of apolar ethylene oxide chains to the corn protein zein was studied by thin-layer chromatography (TLC) carried out on alumina layers covered with zein, and the effect of methanol, monovalent cations, and pH on the strength and selectivity of interaction was elucidated by using the spectral mapping technique and stepwise regression analysis. The binding of surfactants to zein has been demonstrated. It has been established that the number of hydrophilic ethylene oxide units in the surfactant molecule exerts the highest influence on the strength of interaction, and that the role of methanol and salt concentration is of secondary importance.

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The character of the cation and pH influenced both the strength and selectivity of the surfactant–zein interaction.

Key Words: Nonionic surfactant; Zein; Thin layer chromatography.

INTRODUCTION

Besides other physico-chemical methods such as equilibrium dialysis,[1] fluorimetry,[2] spectrophotometry,[3] thermogravimetry,[4] etc., various chromatographic techniques have also been extensively employed for the determination of molecular interactions.[5] The advantages of the chromatographic methods are the rapidity and low requirement on the purity of the solute because the impurities are separated during the chromatographic process.[6] The use of thin-layer chromatography (TLC) for the study of such interactions has marked advantages: the method is generally rapid, it is easy to carry out, it makes possible the simultaneous determination of numerous interactions on one plate, and the amount of the more hydrophobic interactive compounds is extremely low.[7]

Because of their advantageous physico-chemical characteristics, nonionic surfactants are frequently used in a wide variety of industrial processes, such as in agrochemical plant protection to increase the efficacy of the active ingredient,[8] in waste-water treatment to promote biodegradation of pollutants,[9] in biotechnology to accelerate fermentation processes,[10] in pharmaceutical formulation to enhance beneficial effects,[11] etc. However, nonionic surfactants show marked adversary effects too. Thus, they can promote the autolysis of *Listeria monocytogenes*,[12] induced hyperlipidemia,[13] promote percutaneous absorption of toxic chemicals,[14] and show cytotoxic and irritant effects.[15] The molecular base of the toxic effects is not entirely understood. It has been established that nonionic surfactants disorganize membrane structure[16] and modify enzyme activities.[17]

The spectral mapping technique (SPM), a multivariate mathematical-statistical method, can be employed for the evaluation of chromatographic retention data when separation of the strength and selectivity of the effect is required.[18] The method divides the information into two matrices using the logarithm of the original data. The first one is a vector containing the potency values related to the overall effect. The second matrix (selectivity map) contains the information concerning the spectra of activity (the qualitative characteristics of the effect).[19] Spectral mapping technique firstly calculates the logarithm of the members of the original data matrix, facilitating the evaluation of the final plots in terms of log ratios. Consecutively, SPM subtracts the corresponding column, mean and row-mean, from each

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logarithmic element of the matrix and calculates potency values. The source of variation remaining in the centered data set can be evaluated graphically (selectivity map). Spectral mapping technique has been previously employed for the elucidation of the relationship between the chemical structure and fungicidal activity of nonionic surfactants,[20] for the study of the inhibitory effect of surfactants on sunflower downy mildew,[21] and for the characterization of stationary phases in column liquid chromatography (HPLC).[22] As the selectivity matrices of SPM are generally multidimensional, they cannot be evaluated by visual methods. The nonlinear mapping technique (NLMAP) was developed for the reduction of the dimensionality of such matrices.[23] The technique projects the points scattered in multidimensional space on a plane in such a manner that the distances between the points on the plane approximate, maximally, their distances in the multidimensional space.

The objectives of this study were the determination of the binding of some nonionic surfactants to zein by TLC and to assess the correlation between the strength of binding and the structural characteristics of solutes and elucidation of the influence of monovalent cations and pH on the strength of surfactant–zein interaction using various multivariate mathematical-statistical methods. The investigation of the binding of nonionic surfactants to zein was motivated by the fact that zein is an important source of protein in many countries, and surfactants are pollutants in possible contact with zein. The elucidation of the mode of binding may facilitate not only a better understanding of the interactive forces between proteins and organic pollutants but also may promote the development of efficient environmental control procedures.

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EXPERIMENTAL

The chemical structures of nonionic surfactants are compiled in Table 1. They were purchased from Hoechst AG (Frankfurt, Germany) and used as received. Aluminium oxide 60 G neutral (type E) for TLC and methanol of HPLC quality were obtained from Merck KGaA (Darmstadt, Germany). Acetic acid, ammonium acetate, and inorganic salts of analytical purity were purchased from Sigma-Aldrich Kft (Budapest, Hungary). Zein-coated stationary phases were prepared by dissolving 0.5 g of zein in a mixture of 160 mL of *n*-propanol and 40 mL of water at 70°C under continuous gentle stirring. After the dissolution of the protein, 20 g of aluminium oxide was added and the mixture was stirred for 2 hr at the same temperature. Solvents were removed at 70°C in vacuum. Suspensions of 5 g of stationary phase and 14 mL of distilled water were prepared and plates (20 × 20 cm) were coated with a CAMAG TLC Plate Coater (Muttenz, Switzerland), the layer thickness being 250 μm. Surfactants were dissolved in methanol at a concentration of 20 mg/mL, and 4 μL of

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Table 1. Chemical structures of nonionic surfactants.

	Q-O(C_2H_4O)$_{ne}$—H (General structure)		
No.	Common name	Q (Hydrophobic moiety)	ne (average)
1	Arkopal N40	Nonylphenol	4
2	Arkopal N50	Nonylphenol	5
3	Arkopal N60	Nonylphenol	6
4	Arkopal N80	Nonylphenol	8
5	Arkopal N90	Nonylphenol	9
6	Arkopal N100	Nonylphenol	10
7	Arkopal N130	Nonylphenol	13
8	Arkopal N150	Nonylphenol	15
9	Arkopal N230	Nonylphenol	23
10	Arkopal N300	Nonylphenol	30
11	Sapogenate T60	Tributylphenol	6
12	Sapogenate T100	Tributylphenol	10
13	Sapogenate T110	Tributylphenol	11
14	Sapogenate T130	Tributylphenol	13
15	Sapogenate T138	Tributylphenol	13.8
16	Sapogenate T180	Tributylphenol	18
17	Sapogenate T300	Tributylphenol	30
18	Sapogenate T500	Tributylphenol	50

Note: ne, number of ethylene oxide groups per molecule.

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solutions were spotted on the plates. The mobile phases were bidistilled water and water–methanol mixtures containing 5, 10, and 15 vol.% of methanol. In order to assess the effect of salt concentration on the binding of surfactants to zein, developments were also carried out in aqueous solutions of 0.5, 1.0, 2.0, 3.5, 4.0, 4.5, and 5.0 M LiCl. The effect of pH and the type of cation was determined by using aqueous solutions of 4 M NaCl, 4 M KCl, 4 M RbCl, 4 M NH_4Cl, saturated $MgCl_2$, 4 and 0.1 M acetic acid, and 4 and 0.1 M ammonium acetate as mobile phases. Plates were developed in sandwich chambers ($22 \times 22 \times 3$ cm) at ambient temperature; the development distance was approximately 16 cm. After development, the plates were dried at 105°C, and the surfactants were detected by iodine vapors. Each experiment was run in quadruplicate. The R_M values characterizing the retention of solutes in reversed-phase thin-layer chromatography (RP-TLC) have been calculated by

$$R_M = \log\left(\frac{1}{R_f - 1}\right) \quad (1)$$

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for each surfactant in each mobile phase. When the coefficient of variation of the parallel determinations was higher than 6%, the R_M value was omitted from the subsequent calculations. It was supposed that a higher R_M value indicates higher affinity of the surfactants to zein, therefore, it can be used as a quantitative indicator of the strength of solute–protein interaction.

In order to assess the influence of methanol, the concentration of LiCl, and the structural characteristics of surfactants on their binding to zein, stepwise regression analysis was employed.[24] The dependent variable was the R_M value, and the independent variables were the concentrations of methanol and LiCl, the chain length of the apolar alkyl substituents in the hydrophobic moiety of surfactants, the average number of ethylene oxide groups per molecule (ne), and its logarithm. The inclusion of the last independent variable in the calculation was motivated by the assumption that the longer polar ethylene oxide chains can be in a folded state and, therefore, their length does not depend linearly on the number of ethylene oxide groups. As the linear character of the probable effect of the LiCl concentration on the surfactant–zein interaction has never been proven, the square of the LiCl concentration has also been included. In order to establish the influence of the length of ethylene oxide chain on the effect of LiCl, the combined independent variable LiCl × ne was calculated and included in the original data matrix. The number of accepted variables was not limited, and the acceptance level was set to the 95% significance level. In 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 automatically eliminates from the selected equation the dependent variables having no significant impact on the dependent variable, increasing in this manner the reliability of calculation.

Spectral mapping technique was performed on the data matrix consisting of the R_M values of surfactants measured in aqueous 4 M NaCl, 4 M KCl, 4 M RbCl, 4 M NH_4Cl, 4 and 0.1 M acetic acid, and 4 and 0.1 M ammonium acetate as mobile phases. Mobile phases were the variables, and the surfactants were the observations. As saturated $MgCl_2$ solution did not move on the zein layer, these results were omitted from the calculation. The dimensionality of the spectral map was reduced to two by NLMAP. Iterations for NLMAP have been carried out to the point where the difference between the last two iterations was lower than 10^{-8}.

Software for stepwise regression analysis was purchased from Compudrug Ltd. (Budapest, Hungary), and softwares for SPM and NLMAP were prepared by Dr. Barna Bordás (Plant Protection Institute of Hungarian Academy of Sciences, Budapest, Hungary).

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RESULTS AND DISCUSSION

Surfactants with short polar ethylene oxide chains (compounds 1, 2, 3, and 11 in Table 1) remained at the origin in each mobile phase system. This result suggests that they bind strongly to zein and the hydrophobic moiety accounts for the binding, while the highly water-soluble ethylene oxide chain increases the affinity of the solutes for the aqueous phase. The dependence of the R_M values of some surfactants on the concentration of methanol in the mobile phase is demonstrated in Fig. 1. Surfactants show regular retention behavior; the R_M value decreases linearly with increasing concentration of methanol in the mobile phase, indicating that the binding of solutes to the protein is less strong in the presence of methanol.

The parameters of the equation selected by the stepwise regression analysis are compiled in Table 2. The independent variables influence sigificantly the dependent variable, the significance level being over 99.9% (compare F_{calc} values with the tabulated one). The square of LiCl concentration, the combined variable LiCl × ne, and the length of the alkyl chain in the hydrophobic moiety of surfactant exerted no significant influence on the binding. This finding indicates that the effect of LiCl on the strength of surfactant–zein interaction is linear and does not depend on the length of the hydrophilic ethylene oxide chain. The inefficacy of alkyl chains to influence the binding can be tentatively explained by the supposition that the ring structure of surfactants interacts with the corresponding substructures in the zein molecule (stacking interactions), and the contribution of hydrophobic interactive forces to the binding is negligible. The normalized slope values (b_i % values) indicate that the impact of both methanol and LiCl concentrations on the strength of interaction is relatively low compared with the effect of the length of ethylene oxide chain, which effect is markedly nonlinear. This nonlinearity may be caused by the folding of the longer ethylene oxide chains, even in contact with the surface of the protein. The ratio of variance explained by the independent variables is relatively low (56.91%), indicating that other parameters not included in the calculation may exert a considerable effect on the strength of surfactant–zein interaction.

The potency values calculated by SPM are compiled in Table 3. They are related to the R_M values, and, therefore, a higher potency value indicates stronger surfactant–zein interaction. The data in Table 3 clearly show that both pH and the character of cation influence considerably the binding of surfactants to the protein. The impact of cations with higher ion radii is generally higher. This result suggests that the cations can probably bind to the surface of zein and inhibit competitively the adsorption of surfactants. Because of steric effects, cations with larger ion radii decrease the availability at a higher extent. Furthermore, the data demonstrate that acidic and alkaline mobile phase

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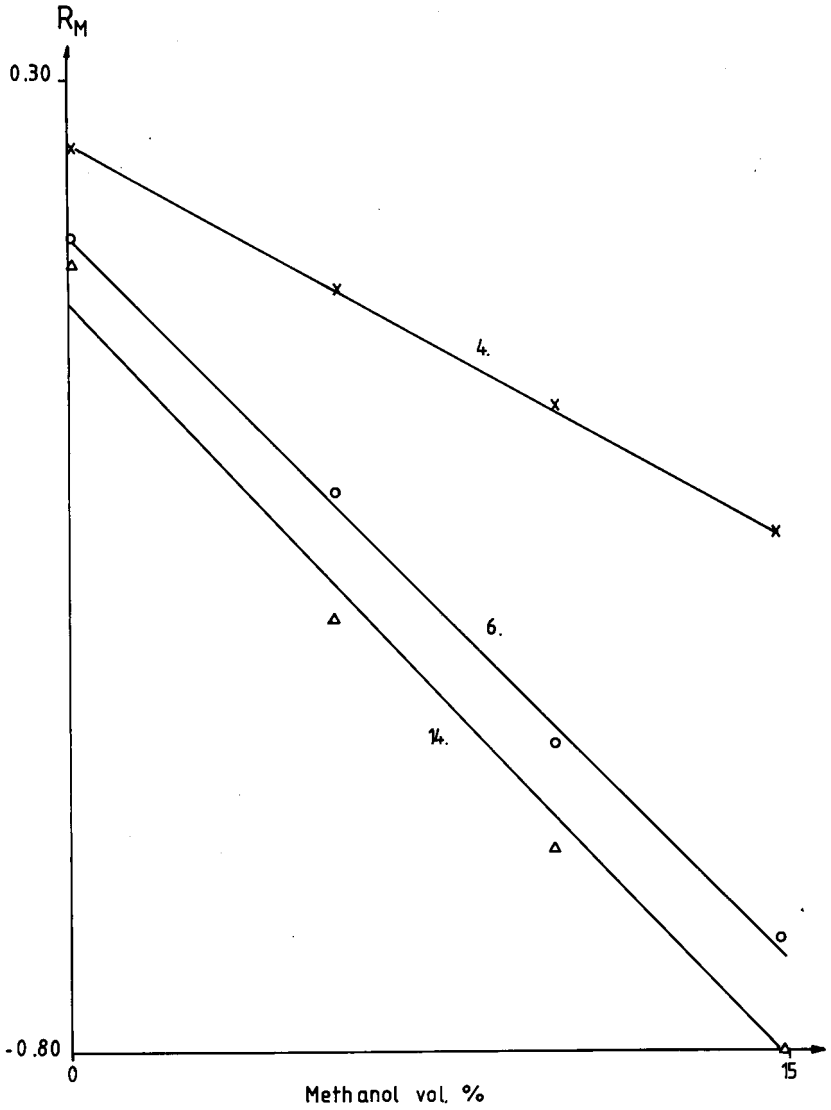

Figure 1. Dependence of the R_M values of some surfactants on the concentration of methanol in the mobile phase. Numbers refer to surfactants in Table 1.

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Table 2. Parameters of the relationship between the strength of surfactant–zein interaction (R_M), the concentration of methanol (vol.%), and LiCl (M) and the structural characteristics of surfactants.

	Methanol	LiCl	ne	log ne
b_i	−2.39	−3.28	5.12	−367
s_{b_i}	0.64	1.34	0.88	42
b_i (%)	7.50	4.86	34.91	52.73

$n = 139 \quad a = 312 \quad r^2(\%) = 56.91 \quad F_{calc} = 44.58 \quad F_{99.9\%} = 4.95$

$R_M = a + b_1 \cdot \text{methanol} + b_2 \cdot \text{LiCl} + b_3 \cdot \text{ne} + b_4 \cdot \log \text{ne}$

Note: Results of stepwise regression analysis.

additives exert, also, a marked influence on the strength of surfactant–zein interaction. Because the surfactants do not contain any polar substructures, the degree of dissociation of which may depend on the pH of the environment; this finding is somewhat surprising. It can be assumed that the acidic and alkaline mobile phase additives are in dissociated form and bind to the surface of the protein similarly to the dissociated ions of salts.

The effect of salts and pH on the selectivity of the surfactant–zein interaction is shown on the two-dimensional nonlinear selectivity map (Fig. 2). The scattering of data points entirely supports our previous qualitative conclusions. Neither salts nor acidic and alkaline components form a clear cut-cluster, indicating that each cation and anion exert a different influence on the selectivity of interaction.

Table 3. Effect of pH and the type of cation on the strength of surfactant–zein interaction.

Mobile phase additive	Potency
4 M LiCl	−85.15
4 M NaCl	79.60
4 M KCl	28.01
4 M RbCl	119.82
4 M NH_4Cl	19.14
0.1 M Acetic acid	−122.03
4 M Acetic acid	−78.21
0.1 M Ammonium acetate	−145.89
4 M Ammonium acetate	5.82

Note: Potency values calculated by SPM (arbitrary units).

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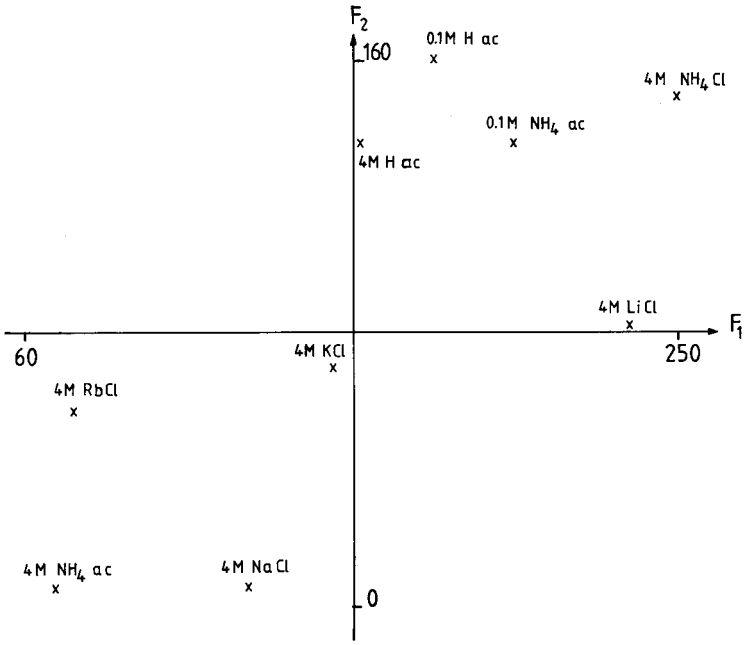

Figure 2. Selectivity of the effect of pH and cations on the strength of surfactant–zein interaction. Two dimensional nonlinear selectivity map. Number of iterations: 151; maximal error: 1.93×10^{-2}.

It can be concluded from the data, that the binding of nonionic surfactants to the corn protein zein can be successfully studied by RP-TLC carried out on zein-coated plates. The strength of interaction depends mainly on the length of the hydrophilic ethylene oxide chain, while the impact of organic modifier and salt concentration is relatively low. Concentration and character of salts and pH considerably influence the selectivity of such interactions.

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