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Determination of the retention behaviour of some non-ionic surfactants on reversed-phase high-performance liquid chromatography supports by spectral mapping in combination with cluster analysis or non-linear mapping

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Abstract

The retention of tributylphenol-ethylene oxide oligomer surfactants was determined in reversed-phase high-performance liquid chromatography using various supports (C_1, C_2, C_6, C_8, C_{18}, polyethylene-coated silica, polyethylene-coated alumina and alumina-based C_{18}). The retention data matrix was evaluated by spectral mapping followed by non-linear mapping and cluster analysis. The retention capacity of polyethylene-coated silica and alumina as well as C_1 column was similar. Surfactants formed distinct clusters on the two-dimensional non-linear map of the spectral map according to the length of the ethylene oxide chain. Significant linear correlation was found between the carbon loading and the retention capacity of columns, indicating the preponderant role of the quantity of hydrophobic alkyl chains in the determination of retention capacity.

1. Introduction

Most analysis with high-performance liquid chromatography (HPLC) is carried out in reversed-phase (RP) separation mode; therefore many reversed-phase chromatographic supports have been developed with various hydrophobic ligands covalently bonded to the surface of inorganic (silica or alumina) or organic supports [1,2]. The retention of solutes in RP-HPLC depends considerably on both the physicochemical parameters of the support and the solutes [3,4], solute hydrophobicity being the most relevant parameter. However, the acidic or basic groups of supports not covered by the hydrophobic ligand may influence the retention of polar solutes, resulting in a retention order different from that predicted according to the molecular lipophilicity [5,6]. The influence of free silanol groups on the retention was defined as the 'silanophil effect' [7]; it decreases with increasing density of covalently bonded alkyl chains [8,9], and it is generally low or absent on polymer (i.e. polystyrene)-coated silica [10]. Not only polystyrene but also other polymers such as octadecyl polyvinylalcohol co-polymers [11], polytrifluorostyrene [12] and polyethylene [13] have been tested as coating agents of silica for the preparation of reversed-phase supports. These polymer-coated supports show enhanced mechanical and pH stability.

The objectives of this study were to determine the retention behaviour of some non-ionic surfactants on various RP-HPLC supports and to

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find the similarities and dissimilarities between the retention characteristics of supports and solutes by using multivariate mathematical-statistical methods such as spectral mapping [14], cluster analysis [15] and non-linear mapping [16,17]. The simultaneous application of non-linear mapping and cluster analysis was motivated by the fact that these methods are theoretically similar: both calculate and visualize the relative distances between the members of a data matrix. According to our knowledge the comparison of their suitability for the evaluation of chromatographic retention data has never been carried out.

2. Experimental

The HPLC equipment consisted of a Gilson gradient analytical system (Gilson Medical Electronics, Villiers-le-Bel, France) with 2 piston pumps (Model 302), detector (Model 116), Rheodyne injector with 20-μl sample loop (Cotati, CA, USA), and a Waters 740 integrator (Milford, MA, USA). The flow-rate was 0.5 ml/min, and the detection wavelength was 235 nm, at which each surfactant shows adequate absorption. Eluent was methanol–water (80:20, v/v). Columns of 25 cm × 4 mm I.D. were used in each experiment. They were filled with silica-based C_1 (Column A, particle size 10 μm), C_2 (Column B, 10 μm), C_6 (Column C, 5 μm), C_8 (Column D, 10 μm), C_{18} (Column E, 5 μm) reversed-phase supports as well as with polyethylene-coated silica (Column F, 10 μm) and alumina (Column G, 10 μm) and alumina-based C_{18} (Column H, 10 μm). The polyethylene-coated supports were prepared in our laboratory, and their retention characteristics have been reported elsewhere [13]. The surfactants were ethoxylated tributylphenol derivatives containing on average 4 (further S4), 6 (S6), 8 (S8), 10 (S10), 11 (S11), 13 (S13), 18 (S18), 30 (S30) and 50 (S50) ethylene oxide groups per molecule (Hoechst, Frankfurt, Germany). They were dissolved in the eluent at a concentration of 0.05 mg ml^{-1}. The retention time of each compound was measured on three consecutive determinations. The capacity factor and the coefficient of variation of the capacity factor were calculated for each compound on each column.

To separate the retention strength and retention selectivity of both HPLC columns and solutes, spectral mapping followed by non-linear mapping and cluster analysis was applied. In order to calculate the retention capacity and selectivity of columns as well as the retention strength and selectivity of solutes, the spectral mapping technique was used twice:

I. The surfactants were the variables, and the capacity factors of the surfactants on the eight columns were the observations.

II. The capacity factors were the variables, and the surfactants the observations.

To find the relationship between the carbon load (X) and retention capacity (Y) of the various RP-HPLC columns, a linear correlation was calculated between these two variables:

$$Y = a + bX. \quad (1)$$

The carbon load of supports was determined by the traditional micro-analytical method [18].

3. Results and discussion

Each column was suitable for the separation of non-ionic surfactants according to the character of the hydrophobic moiety. The retention increased with increasing length of the bonded alkyl chain of the supports (Fig. 1). Surfactants contained different quantities of impurities (Fig. 2). This finding suggests that the quality and quantity of impurities may differ in the various products, variably influencing the surface activity and biological efficiency of the surfactant preparation. As the surfactants cannot be well separated in RP-HPLC according to the length of ethylene oxide chain, the fractions represent surfactants with a different hydrophobic moiety.

The $\log k'$ values of surfactants and the relative standard deviation (R.S.D.) of $\log k'$ on various RP-HPLC columns are compiled in Table 1. As the standard deviation of the RP-HPLC measurements was low, the majority of differences is significant, although only three

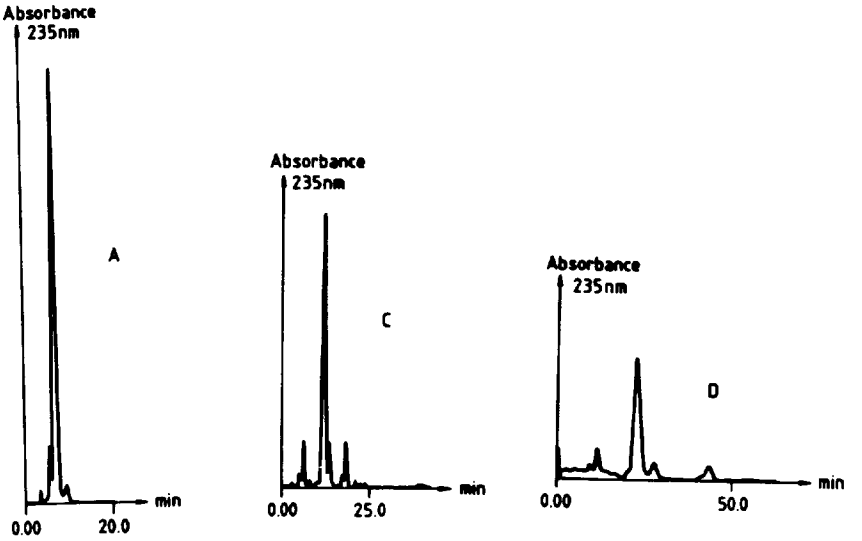

Fig. 1. Separation of tributylphenol-ethylene oxide oligomer surfactant (S4) on column A, column C and column D. Eluent: methanol–water (8:2, v/v), flow-rate: 0.5 ml/min, detection wavelength 235 nm.

parallel determinations were used for the determination of each $\log k'$ value. Considerable differences were observed between the retention capacities of columns, those of polyethylene-coated silica and alumina columns as well as the C_1 column being the lowest. The retention of surfactant increased with increasing length of the apolar hydrocarbon chain covalently bonded to the silica surface. However, some exceptions have been observed (S4, S30 and S50 on C_6 column). We do not have any valid hypothesis to explain these anomalies. We assume that a better understanding of the folded state of ethylene oxide chains in contact with the highly hydrophobic alkyl chains will make possible a more precise explanation of the retention behaviour observed. The similarity between the retention capacity of polymer-coated and C_1 columns can be tentatively explained by the supposition that the polyethylene coating probably lies parallel to the support surface, the end groups being in close contact with the adsorption centres of the support. Only the surface of the polyethylene coating pointing towards the eluent is available to the solutes. It is a hydrophobic layer similar to that of the C_1 coating in thickness but differs considerably from the 'brush-like' coatings where the alkyl chains more or less penetrate the mobile phase and more than one–CH_2–group can interact with the hydrophobic substructure of the solute.

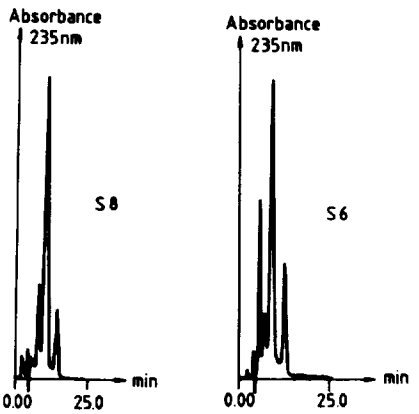

Fig. 2. Separation of tributylphenol-ethylene oxide oligomer surfactant (S6) and (S8) on column A. Eluent: methanol–water (8:2, v/v), flow-rate: 0.5 ml/min, detection wavelength 235 nm.

The results of spectral mapping entirely support our previous qualitative conclusions (Table 2). The differences between the retention capacities of hydrocarbon-bonded silicas are very large, indicating the marked impact of the alkyl

Table 1
Retention of tributylphenol-ethylene oxide oligomers on RP-HPLC columns

Surfactant	HPLC column							
	A		B		C		D	
	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)
S4	−7.40	0.12	50.03	0.23	45.81	0.32	65.04	0.18
S6	−7.01	0.27	40.94	0.11	45.31	0.56	66.98	0.17
S8	−6.71	0.27	40.64	0.32	44.68	0.22	66.92	0.12
S10	−5.82	0.16	40.88	0.40	44.80	0.28	65.27	0.75
S11	−5.11	0.19	40.68	0.67	43.65	0.37	66.50	0.18
S13	−5.05	0.49	40.13	0.32	41.58	0.49	41.58	0.56
S18	−5.11	0.24	39.91	0.39	40.36	0.31	63.83	0.50
S30	−3.25	0.12	37.77	0.28	29.44	0.41	61.67	0.22
S50	−0.82	0.45	32.77	0.21	29.44	0.47	58.35	0.63
	E		F		G		H	
	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)	$\log k'(\times 10^{-2})$	R.S.D.(%)
S4	100.16	0.19	−24.53	0.78	−23.79	0.45	67.04	0.87
S6	101.29	0.21	−33.42	0.47	−25.06	0.23	61.67	0.45
S8	102.61	0.40	−30.79	0.39	−24.85	0.16	59.42	0.31
S10	103.98	0.34	−20.50	0.54	−25.06	0.34	58.77	0.69
S11	104.24	0.55	−21.61	0.41	−25.06	0.21	57.38	0.10
S13	104.08	0.17	−39.97	0.23	−27.51	0.12	57.86	0.45
S18	104.18	0.22	−38.94	0.29	−25.06	0.31	60.52	0.23
S30	105.60	0.31	−38.66	0.24	−26.16	0.21	56.98	0.11
S50	108.56	0.23	−39.22	0.39	−25.28	0.37	49.95	0.55

For symbols see Experimental; $n = 3$, R.S.D. = relative standard deviation.

Table 2
Retention capacity of RP-HPLC columns and retention strength of non-ionic surfactants (arbitrary units). Results of spectral mapping technique

Column	Retention capacity	Surfactant	Retention strength
A	−0.14	S4	0.96
B	1.21	S6	0.89
C	1.24	S8	0.89
D	1.93	S10	0.93
E	3.12	S11	0.92
F	−0.96	S13	0.84
G	−0.76	S18	0.87
H	1.77	S30	0.82
		S50	0.76

For symbols see Experimental.

chain length on the retention of non-ionic surfactants. The retention strengths of surfactants also differ; however, they do not depend linearly on the number of ethylene oxide groups per molecule. It can be supposed from this result that the polar ethylene oxide chain of surfactants is more or less in a folded state. The folding of the ethylene oxide chain influences the solvation of the surfactants, that is, their affinity to the mobile phase, resulting in anomalous retention behaviour.

Surfactants formed two distinct clusters on the two-dimensional non-linear selectivity map according to the length of the ethylene oxide chain (Fig. 3). It is probable that the shorter ethylene oxide chains are not in folded state (cluster B), and the folding of the polar chain increases with increasing number of ethylene oxide groups, resulting in modified retention selectivity (cluster A). As the ethylene oxide chains change conformation at about a length of 9 in water, our data indicate that the conformation change in methanol–water (80:20, v/v) eluent moves towards longer ethylene oxide chains (ca. 13) per molecule. This phenomenon may be due to the lower dielectric constant of the methanol–water mixture.

The two-dimensional non-linear selectivity map of RP-HPLC columns entirely supports our previous conclusions. Not only the retention capacities but also the retention selectivities of

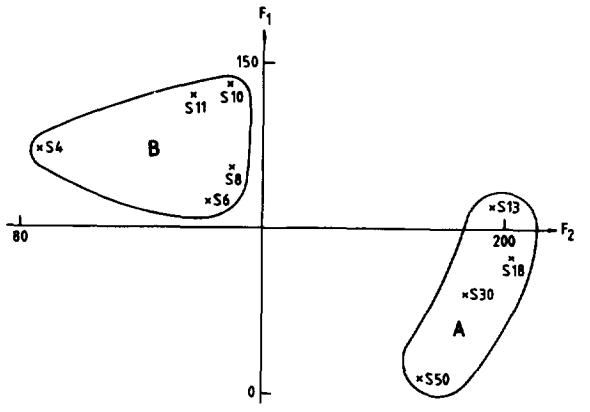

Fig. 3. Two-dimensional non-linear selectivity map of surfactants. Number of iterations: 460. Maximum error: 1.8×10^{-3}. For symbols see Experimental.

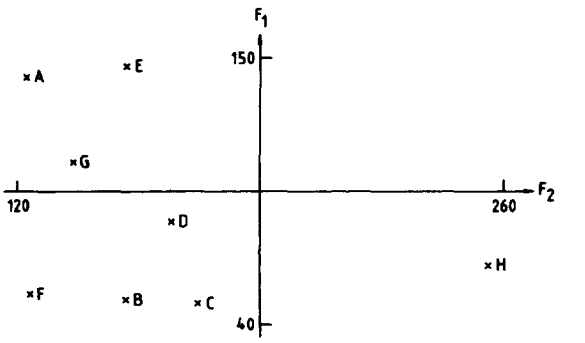

Fig. 4. Two-dimensional non-linear selectivity map of RP-HPLC columns. Number of iterations: 377. Maximum error: 2.1×10^{-3}. For symbols see Experimental.

RP-HPLC columns differ considerably (Fig. 4). The columns do not form distinct clusters, indicating that the length of the apolar alkyl chain, the polymer character of the hydrophobic coating and the original nature of the supports have a similar impact on the selectivity. The selectivity of the alumina-based C_{18} column (Column H) considerably differed from that of the other RP-HPLC columns. This can be tentatively explained by the supposition that the active centres of the original supports not covered by the hydrophobic ligands retain their original adsorptive capacity, influencing the retention of solutes. The cluster dendrogram of RP-HPLC columns calculated from the spectral map is shown in Fig. 5. The results of cluster analysis are in good agreement with those of the two-dimensional non-linear map, indicating that both techniques can be successfully used for the visualization of multi-dimensional data matrices.

Significant linear correlation was found between the carbon loading (X) and retention capacity (Y) of RP-HPLC columns:

$$Y = 6.59 - (0.95 \pm 0.02)X,$$

$$n = 8, \quad r = 0.9395, \quad r_{99.9\%} = 0.9249.$$

The equation fits well to the experimental data, the significance level being over 99.9%. Thus, the retention capacity of the modified supports significantly depends on the carbon content of the hydrophobic coating. This relationship suggests that each–CH_2–group contributes to the hydro-

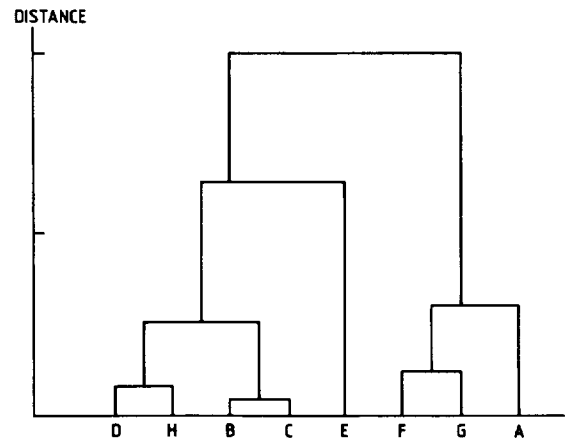

Fig. 5. Cluster dendrogram of RP-HPLC columns. For symbols see Experimental.

phobic binding of solutes to the surface of the RP-HPLC supports, which means that the availability of the different $-CH_2-$groups is similarly independent of the length of the alkyl chain.

4. Conclusion

It can be concluded from our data that spectral mapping followed by two-dimensional non-linear mapping or cluster analysis is suitable for the comparison of RP-HPLC columns and for the classification of solutes. It must be emphasized that the conclusions discussed above are based on calculations carried out on one special data matrix and are not the results of theoretical considerations. Therefore, they have to be applied to different data matrices with extreme caution.

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