CHROMSYMP. 2897

Retention behaviour of tributylphenol ethylene oxide oligomers on an alumina high-performance liquid chromatographic column

Esther Forgács and Tibor Cserháti*

Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest (Hungary)

ABSTRACT

The retention behaviour of tributylphenol ethylene oxide oligomer surfactants was studied on an alumina HPLC column using ethyl acetate–*n*-hexane mixtures as eluents. The surfactants contained various numbers of ethylene oxide groups per molecule and tributylphenol isomers as hydrophobic moieties. The column separated the surfactants according to the length of the ethylene oxide chain and the position of butyl substituents in one run, demonstrating the good separation power of alumina. A significant linear correlation was found between the logarithm of the capacity factor of each surfactant and the concentration of ethyl acetate in the eluent, but the dependence of the retention on the ethyl acetate concentration was low. Stepwise regression analysis indicated that the number of ethylene oxide groups per molecule has a greater effect than the position of the butyl substituents in the tributylphenol moiety on the retention.

INTRODUCTION

The application of silica or silica-based supports in HPLC is limited by the low stability of silica at alkaline pH [1] and by the undesirable electrostatic interactions between the polar substructures of the solutes and the free silanol groups not covered by the hydrophobic ligand [2,3]. To decrease or eliminate the effect of residual acidic silanol groups, the eluent has to be buffered or various additives have to be added to the eluent to mask the effect of silanol groups [4]. The drawbacks mentioned above necessitated a search for supports other than silica, such as alumina [5], octadecyl-coated alumina [6], zirconia [7,8] and various polymer-based supports [9]. Owing to its higher isoelectric point and higher stability in the alkaline pH range, alumina partially or totally overcomes the difficulties arising from the low stability of silica

[10], and therefore its application as a stationary phase for adsorption or after modification [11] for reversed-phase HPLC offers considerable advantages. The physico-chemical characteristics of alumina have recently been reviewed [12]. Good separations of various aromatic compounds [13,14], heroin derivatives, proteins [15] and drugs [16] have been achieved on alumina columns.

Many HPLC methods have been developed for the separation and determination of non-ionic surfactants [17,18]. As the separation power of ethylene oxide oligomer surfactants on common reversed-phase HPLC columns is relatively low, diol columns have also been used for the separation [19]. Non-ionic surfactants are generally separated on one HPLC column either according to the length of the ethylene oxide chain or according to the type of hydrophobic moiety. To carry out the separations in both senses, two different HPLC columns are needed [20]. Non-ionic surfactants have been separated according to the length of the ethylene oxide

---

* Corresponding author.

chain on a C_{18} HPLC column using typical reversed-phase eluents [21].

The objectives of this work were to study the retention behaviour of tributylphenol ethylene oxide oligomer surfactants on an alumina column, to elucidate the effects of various molecular substructures on the retention and to find the relationship between the retention parameters and solute characteristics.

EXPERIMENTAL

Alumina support material of 5-μm particle size was obtained from the Research Institute of the Hungarian Alumina Trust (Budapest, Hungary). A 25 cm × 4 mm I.D. column was filled in our laboratory using a Shandon (Pittsburg, PA, USA) analytical HPLC packing pump. The HPLC equipment consisted of a Liquopump Type 312 pump (LaborMIM, Budapest, Hungary), a CE-212 variable-wavelength UV detector (Cecil Instruments, Cambridge, UK), a Valco (Houston, TX, USA) injector with a 20-μl sample loop and a Model 740 integrator (Waters–Millipore, Milford, MA, USA). The flow-rate was 1 ml/min and the detection wavelength was set at 275 nm. Measurements were carried out at room temperature (22 ± 2°C). A sample of commercial tributylphenol ethylene oxide oligomer surfactants (Hoechst, Frankfurt, Germany) was dissolved in the eluent at a concentration of 0.5 mg/ml. The surfactant was a mixture of ethylene oxide oligomers (average number of ethylene oxide groups per molecule = 4) with the hydrophobic moiety containing a variety of tributylphenol isomers (probably 2,4,6-, 2,4,5- and 2,3,5-tributylphenol). This means that the commercial product contained a wide variety of solutes with similar chemical structures. The eluents were ethyl acetate–n-hexane mixtures, the ethyl acetate concentration being varied from 100 to 50 vol.% in steps of 10 vol.%. Each determination was run in quadruplicate.

Linear correlations between the logarithm of the capacity factors and the concentration of ethyl acetate were calculation:

$$\log k' = \log k'_0 + bC \qquad (1)$$

where k' is the actual capacity factor of tributylphenol ethylene oxide oligomers at 0 % (v/v) ethyl acetate, k'_0 is the theoretical capacity factor of a tributylphenol ethylene oxide oligomer at 0% (v/v) ethyl acetate (pure n-hexane), b is the change in the logarithm of capacity factor caused by a 1% (v/v) change in ethyl acetate concentration in the eluent (related to the hydrophilic surface area of the solutes [3]) and C is the concentration (vol.%) of ethyl acetate in the eluent.

To elucidate the effects of various molecular substructures on the retention parameters (slope and intercept values of eqn. 1), the relationship between the retention parameters and the number of ethylene oxide groups per molecule (n) and the position (PI) of butyl substituents was determined. Calculations were carried out by stepwise regression analysis [22]. In the common multivariate regression analysis, the presence of independent variables that exert no significant influence on the dependent variable lessens the significance level of those independent variables which do significantly influence the dependent variable. To overcome this difficulty, the stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables. The number of accepted variables was not limited and their acceptance limit was set to the 95% significance level.

The linear correlation between the slope and intercept values of eqn. 1 was calculated to investigate that from a chromatographic point of view the tributylphenol ethylene oxide oligomers represent a homologous series of solutes on an alumina column [23].

RESULTS AND DISCUSSION

A tributylphenol ethylene oxide sample was separated into fifteen fractions on the alumina column (Figs. 1 and 2); the relative standard derivation was lower than 1.5% in each instance. The peaks were symmetrical and they were clustered in groups of three peaks. We assume that each "triad" represents tributylphenol derivatives with an identical ethylene oxide number, and the three members of the triad
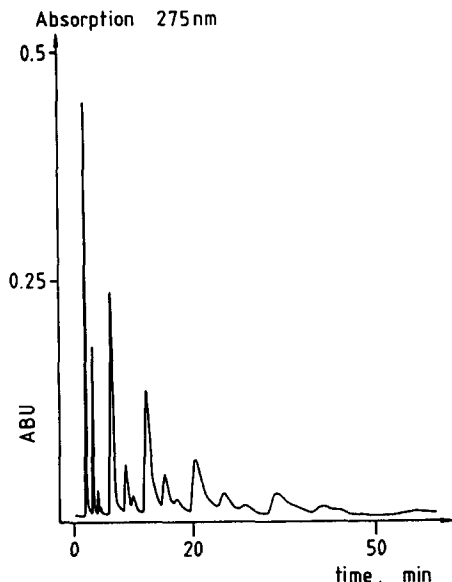
Fig. 1. Separation of tributylphenol ethylene oxide oligomer surfactants on an alumina column. Eluent, ethyl acetate–*n*-hexane (1:1, v/v); flow-rate, 1 ml/min; room temperature (22 ± 2°C); detection wavelength, 275 nm. ABU = Absorbance units.

represent the possible structural isomers of the tributylphenol moiety. These results indicate that the number of ethylene oxide groups and the
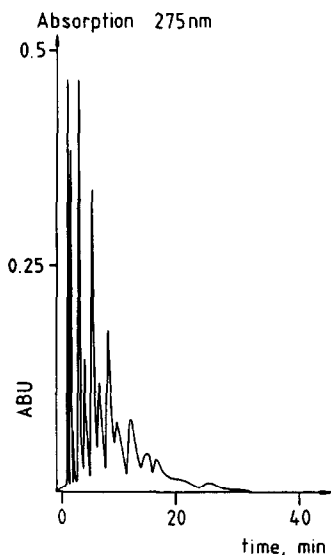

Fig. 2. Separation of tributylphenol ethylene oxide oligomer surfactants on an alumina column. Eluent, ethyl acetate; flow-rate, 1 ml/min; room temperature (22 ± 2°C); detection wavelength, 275 nm.

isomeric form of the hydrophobic moiety govern the retention of the surfactants on alumina. Steric considerations make it probable that the sterically less hindered 2,4,6-tributylphenol isomers are present in the greatest amount in the sample. Owing to the electron-withdrawing character of the substituents [24], we postulated the existence of 2,4,5- and 2,3,5-tributylphenol isomers. We must stress again that the assigment of positions to the various fractions is only hypothetical.

Although the strengths of the eluents are considerably different (ethyl acetate = 0.580 and ethyl acetate–*n*-hexane (1:1) = 0.295 (in arbitrary units) [25]), the differences in the retention times of the surfactants are still relatively low. Under these conditions changes in solvent strength do not have a considerable influence on the retention times of surfactants.

The linear correlations between the logarithm of the capacity factor and the ethyl acetate concentration in the eluent were significant for each fraction (Table I). However, as was qualitatively established from Figs. 1 and 2, the dependence of log k' on ethyl acetate concentration was considerably lower than with other HPLC systems [26].

The retention of surfactants increased with increasing length of the hydrophilic ethylene oxide chain. This indicates that the surfactants turn towards the stationary phase with their ethylene oxide chain, the hydrophobic moiety pointing away from the polar alumina surface. The specific hydrophilic surface area of surfactants containing the 2,4,6-tributylphenol hydrophobic moiety is smaller than that of surfactants containing the sterically more restricted 2,3,6-tributylphenol moiety. This finding can be hypothetically explained by the supposition that the symmetrical isomer is better solvated in the relatively hydrophobic mobile phase, drawing away the surfactant from the hydrophilic alumina surface.

The results of stepwise regression analysis are given in Table II. Both retention parameters of the tributylphenol ethylene oxide oligomer surfactants depended on the number of ethylene oxide groups per molecule and the position of the butyl substituents. The equations fit the

TABLE I

PARAMETERS OF LINEAR CORRELATIONS BE-TWEEN THE LOGARITHM OF CAPACITY FACTOR AND THE CONCENTRATION ON ETHYL ACETATE $[C, \% \ (v/v)]$ IN THE ELUENT: $\log k' = \log k_0' + bC$

k' = Capacity factor of surfactants at C vol.% ethyl acetate concentration; k_0' = theoretical capacity factor of a tri-butylphenol ethylene oxide oligomer at 0 vol.% ethyl acetate concentration, $C = \%$ (v/v) of ethyl acetate in the eluent and S_b = standard deviation of slope value.

Sample No.	Log k_0'	$-b \cdot 10^{-3}$	$S_b \cdot 10^{-3}$	r
1	−0.013	5.14	1.24	0.9899
2	0.189	5.01	1.39	0.9712
3	0.318	6.17	1.69	0.9805
4	0.526	5.52	1.48	0.9926
5	0.725	5.73	1.62	0.9845
6	0.862	6.85	1.66	0.9993
7	0.946	6.43	1.51	0.9752
8	1.061	6.43	1.52	0.9838
9	1.200	7.83	1.32	0.9974
10	1.293	8.03	1.65	0.9956
11	1.347	7.36	1.61	0.9726
12	1.458	8.49	1.45	0.9978
13	1.517	8.02	1.67	0.9802
14	1.615	8.11	1.61	0.9927
15	1.713	9.00	1.21	0.9832

experimental data well, the significance level being over 99.9% (see F values). The number of ethylene oxide groups per molecule (n) and the position of butyl substituents (PI) of solutes account for the 91.50% and 97.31% of the change in dependent variable (see r^2 values). The path coefficients indicate that the number of ethylene oxide groups per molecule has a greater effect on the retention than the position of the butyl substituents of the tributylphenol moiety (see $b_{1\%}$ and $b_{2\%}$ values). These results support our previous conclusions that the hydrophilic ethylene oxide chains of surfactants are in direct (probably electrostatic) interaction with the polar alumina surface, and the solvation state of the hydrophobic tributylphenol moiety only modifies the strength of the interaction.

A significant linear correlation was found between the slope and intercept value of eqn. 1:

$$b = 4.77 + 2.21 \cdot \log k_0' \qquad (2)$$

number of samples = 15; $r = 0.9336$; $S_b = 2.3 \cdot$

TABLE II

RELATIONSHIP BETWEEN THE RETENTION PARAMETERS OF TRIBUTYLPHENOL ETHYLENE OXIDE OLIGOMERS AND NUMBER OF ETHYLENE OXIDE GROUPS PER MOLECULE (n) AND POSITION OF THE BUTYL SUBSTITUENTS (PI)

Results of stepwise regression analysis. Number of samples = 15.

Parameter	Equation[a]	
	A	B
a	3.56	$-3.41 \cdot 10^{-1}$
b_1	$7.80 \cdot 10^{-1}$	$3.56 \cdot 10^{-1}$
S_{b1}	$7.30 \cdot 10^{-1}$	$1.17 \cdot 10^{-2}$
b_2	$5.21 \cdot 10^{-1}$	$1.28 \cdot 10^{-1}$
S_{b2}	$1.27 \cdot 10^{-1}$	$3.02 \cdot 10^{-2}$
r^2	0.9150	0.9731
$b_{1\%}$	78.17	82.79
$b_{2\%}$	27.83	17.21
F	64.65	217.65

[a] (A) $b = a + b_1 n + b_2 PI$
(B) $\log k_0' = a + b_1 n + b_2 PI$.

10^{-1}; $r_{99.9\%} = 0.7603$. The strong correlation indicates that from the chromatographic point of view the tributylphenol ethylene oxide oligomer surfactants can be considered as a homologous series of compounds. This result lends support to our previous conclusion that the retention of surfactants is mainly governed by the length of the ethylene oxide chains (identical in character in each oligomer) and that the isomeric form of the hydrophobic tributylphenol moiety has a secondary influence on retention.

We conclude that the alumina support is especially suitable for the separation of tri-butylphenol ethylene oxide oligomer surfactants, separating the solutes both according to the length of the ethylene oxide chain and the position of the butyl substituents in one run.

tal pollution by attachment and immobilization of bacteria at surfaces".

REFERENCES

1 J.P.B. Brunell, *Pure Appl. Chem.*, 50 (1978) 1211.
2 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
3 K.E. Bij, Cs. Horváth, W.R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
4 N.E. Tayar, H. Waterbeend and B. Testa, *J. Chromatogr.*, 320 (1985) 305.
5 C.J. Laurent, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 285 (1984) 161.
6 J.J. Sun and J.S. Fritz, *J. Chromatogr.*, 522 (1990) 95.
7 J.A. Blackwell and P.W. Carr, *J. Chromatogr.*, 549 (1991) 43.
8 J.A. Blackwell and P.W. Carr, *J. Chromatogr.*, 549 (1991) 59.
9 T. Takeuchi, W. Hu and H. Haraguchi, *J. Chromatogr.*, 517 (1990) 257.
10 R. Kaliszan, J. Petrusewitz, R.W. Blain and R.A. Hartwick, *J. Chromatogr.*, 458 (1988) 395.
11 J.J. Pesek, *Chromatographia*, 28 (1989) 565.
12 R. Poisson, J.P. Brunelle and P. Nortier, in A.B. Stiles (Editor), *Catalytic Supports, Supported Catalysis*, Butterworth, Boston, 1987, p. 11.
13 K.K. Unger, W. Messer and K.F. Krebs, *J. Chromatogr.*, 149 (1978) 1.
14 T. Cserháti, *Chromatographia*, 29 (1990) 593.
15 C.J.C.M. Laurent, H.A.H. Billiet, L. de Galan, F.A. Buytenhuys and F.P.B. van de Maeden, *J. Chromatogr.*, 287 (1984) 45.
16 H. Lingeman, H.A. van Muster, J.H. Beyben, W.J.M. Underber and A. Hulshoff, *J. Chromatogr.*, 352 (1986) 61.
17 T. Bán, E. Papp and J. Inczédy, *J. Chromatogr.*, 593 (1992) 227.
18 I. Zeman, *J. Chromatogr.*, 509 (1990) 201.
19 I. Zeman, *J. Chromatogr.*, 363 (1986) 223.
20 T. Okada, *J. Chromatogr.*, 609 (1992) 213.
21 Z.H. Wang and M. Fingas, *J. Chromatogr.*, 631 (1993) 251.
22 H. Hager, *Modern Regressionanalyse*, Salle Saulander, Frankfurt am Main, 1982, p. 135.
23 K. Valkó, *J. Liq. Chromatogr.*, 7 (1984) 1405.
24 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979, p. 1.
25 L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974, p. 286.
26 E. Forgács, K. Valkó and T. Cserháti, *J. Chromatogr.*, 631 (1993) 207.