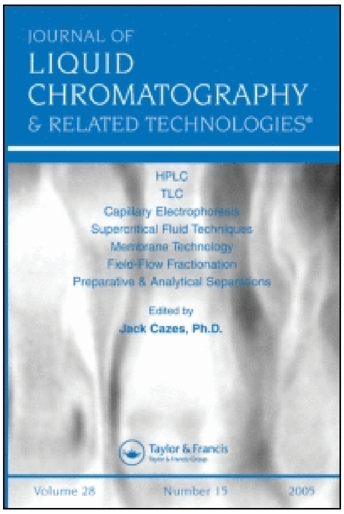

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ION INTERACTION CHROMATOGRAPHY OF NEUTRAL MOLECULES. USE OF A POTENTIAL APPROXIMATION TO OBTAIN A SIMPLIFIED RETENTION EQUATION

T. Cecchi[a]; F. Pucciarelli[a]; P. Passamonti[a]
[a] Dipartimento di Scienze Chimiche, Universitá degli Studi di Camerino, Camerino, Italy

To cite this Article Cecchi, T. , Pucciarelli, F. and Passamonti, P.(2001) 'ION INTERACTION CHROMATOGRAPHY OF NEUTRAL MOLECULES. USE OF A POTENTIAL APPROXIMATION TO OBTAIN A SIMPLIFIED RETENTION EQUATION', Journal of Liquid Chromatography & Related Technologies, 24: 3, 291 — 302

To link to this Article: DOI: 10.1081/JLC-100001334

URL: http://dx.doi.org/10.1081/JLC-100001334

J. LIQ. CHROM. & REL. TECHNOL., 24(3), 291–302 (2001)

ION INTERACTION CHROMATOGRAPHY OF NEUTRAL MOLECULES. USE OF A POTENTIAL APPROXIMATION TO OBTAIN A SIMPLIFIED RETENTION EQUATION

T. Cecchi,* F. Pucciarelli, and P. Passamonti

Università degli Studi di Camerino, Dipartimento di
Scienze Chimiche, Via S. Agostino, 1
62032 Camerino, Italy

ABSTRACT

The chromatographic behavior of neutral molecules in Ion Interaction Chromatography (IIC) was theoretically investigated. The use of a potential approximation to obtain a simplified retention equation was successfully tested. The most reliable experimental literature results, concerning the retention behavior of neutral molecules in IIC, were successfully fitted by the retention equation.

The wide variability among them was elucidated on the basis of the developed retention equation. A new separation strategy, to improve the selectivity of the chromatographic method, for difficult to separate mixtures of neutral eluates was proposed.

INTRODUCTION

While the effect of ion interaction reagents (IIRs) on the retention factor values of oppositely and similarly charged analytes has been well-studied (1–6), the influence of IIRs on the k values of neutral species has not been widely investigated.

For uncharged analytes no retention dependence on IIR concentration in the eluent was postulated (3-4) and experimentally confirmed (3,7–9). Even if this is generally accepted (2), some examples that point to the contrary are present in the literature (10–16).

In the last years, many examples of applications of IIC have been reported in the environmental, pharmaceutical, food, and clinical field (2). Unlike conventional ion exchange, IIC has been used to separate non-ionic and ionic or ionizable compounds in the same sample. We believe that the understanding of the retention behavior of uncharged analytes in IIC is a prerequisite for taylor-made separations.

Recently (17–18), we have developed an exhaustive, rigorous, thermodynamic retention model for IIC, which is able to predict experimental evidence that is not rationalized by the pure electrostatic theories (19–21) of IIC. To obtain the final retention equation, the solution of the Poisson-Boltzmann equation was used. Its simplified, linearized form can very seldom be used, since the calculated potential, which develops at the stationary phase surface, is usually above 25 mV (19), hence, the rigorous retention equation is obtained at the expense of a complex mathematical expression.

It is the aim of the present work to present and assess a simplified retention equation which can be used to predict the experimental behaviour of neutral analytes in IIC, even if the surface concentration of the IIR is not experimentally measured, for high, but chromatographically meaningful, surface potential.

THEORY

The adsorbed IIR ions are responsible for the electrical potential difference, Ψ_0, between the surface and the bulk solution. In the absence of any other charged species, the potential would decay linearly with distance into the solution from Ψ_0, at the charged surface, to zero in the bulk solution. However, counter ions of opposite sign are electrostatically attracted toward the charged surface, and similarly charged co-ions are repelled from the surface.

Two regions are distinguishable in the double layer: the compact part, in which the potential decays linearly, and the diffuse layer in which the potential decays almost exponentially because of screening effects. The higher is the ionic strength, the faster the potential decays.

In IIC systems, for a neutral eluate E, the main equilibria are the following:

(i) adsorption of the eluate E onto the stationary phase site L (hydrocarbonaceous ligand)

$$E + L \overset{K_{EL}}{\rightleftharpoons} EL \tag{1}$$

(ii) adsorption of the IIR H onto L

$$\mathrm{H + L \overset{K_{HL}}{\rightleftharpoons} HL} \tag{2}$$

(iii) displacement of H by E

$$\mathrm{E + HL \overset{K_{EL}/K_{HL}}{\rightleftharpoons} EL + H} \tag{3}$$

It is clear that stoichiometric equilibrium constants can not be considered constant after the addition of the IIR in the mobile phase, since it alters both the interfacial tension and the surface potential. On the contrary, thermodynamic equilibrium constants K_{EL}, K_{HL}, K_{EL}/K_{HL} are able (17) to take into account the modification of the surface properties.

Since equilibria (1), (2), and (3) are all dependent on each other, we are forced to conclude that the IIR adsorption equilibrium constant depends, as it was demonstrated (17), on the ability of the eluate to displace it. This point is at variance with predictions made by pure electrostatic theories (19–21), that postulate only one experimental curve of analyte retention vs surface concentration of the IIR, for different IIRs, since the displacement ability of the eluate is neglected.

The binding of the eluate and the IIR with the stationary phase is obviously supposed to take place independently since no electrostatic interactions can occur between them in the mobile phase.

The capacity factor of the neutral eluate, k, is defined in the usual way as:

$$\mathrm{k} = \phi \frac{[\mathrm{EL}]_s}{[\mathrm{E}]_m} \tag{4}$$

where ϕ is the phase ratio of the column $[EL]_s$ is the surface concentration of the eluate and $[E]_m$ is its mobile phase concentration.

It was demonstrated[17] that it holds:

$$\mathrm{k} = \phi[\mathrm{L}]_T \frac{K_{EL}\frac{\gamma_E\gamma_L}{\gamma_{EL}}\exp[(\sigma - \sigma_\circ)S_E/RT]}{\{1 + K_{HL}\frac{\gamma_L\gamma_H}{\gamma_{HL}}\exp(-z_H F\Psi_\circ/RT)\exp[(\sigma - \sigma_\circ)S_H/RT]a_H\}} \tag{5}$$

In the above expression F is the Faraday constant, σ and σ^0 are the surface tension with and without the IIR in the mobile phase, S_E and S_H represent the area on the surface occupied by 1 mol of the eluate and 1 mol of the IIR, respectively, and γ represents the activity coefficient of each species. a_H is the activity of the IIR in the mobile phase and it will be approximated by its concentration in the eluent.

Increasing amounts of adsorbed IIR cause a change in the chemical character of the stationary phase surface and, consequently, a change in γ_{EL} and γ_{HL}. In principle, the presence of IIR on the stationary phase could either increase or decrease the sorption of a neutral species. For example, residual silanols act as a very strong adsorbing site for alkylammonium ions: the first addition of these kinds of IIRs may result in a "dynamic end capping" (23). While the evaluation of activity coefficients in the mobile phase is straightforward, the activity coefficients for the eluate and the IIR in the adsorbed phase are not easily estimated (24). As a first approximation, we may assume that the activity coefficients ratios are almost constant.

Taking this for granted, the numerator describes the modification of the adsorption of the neutral eluate onto a stationary phase in presence of the IIR. The interfacial tension decreases with increasing IIR concentration and this results in a lower driving force for adsorption and lower retention, as also predicted by the solvophobic theory (22).

The denominator accounts for surface exclusion phenomena between the analyte and the IIR. If the IIR concentration in the eluent increases, k is predicted to decrease; however the influence of increasing IIR concentration is modulated by the surface potential, which develops since the adsorbed IIR is a charged lipophilic ion. Since z_H and Ψ_0 are of like sign, the tangent of the plot k vs. [H] is theoretically predicted to be steeper for lower IIR concentrations, since the surface potential, which runs counter further adsorption of IIR, is still low and IIR adsorption is more effective. This prediction is experimentally confirmed (see below).

Equation (5) underlines the characteristics of the IIR, which is both a surfactant which lowers the interfacial tension and a charged, lipophilic ion, which alters the surface potential.

It is evident that the course of neutral molecule retention upon IIR concentration in the eluent can be very complex and variable (an increase or a decrease of retention is possible) since it depends on a number of factors. The balance of them may also result in an apparent lack of retention dependence on IIR concentration in the eluent.

Let us try to obtain an equation for a practical test.

In a number of cases, if the IIR concentration in the mobile phase is not too high (25) the following empirical equation holds:

$$\Psi^0 = \alpha + \beta \ln[\mathrm{H}]_s \tag{6}$$

where α and β are constants and [H]s is the amount of IIR adsorbed on unit surface area. Under the same conditions, the potential modified Langmuir adsorption isotherm (25) reduces to a Freundlich isotherm

$$[\mathrm{H}]_s = a[\mathrm{H}]^b \tag{7}$$

where a and b are constants. The Freundlich isotherm was considered good enough for all isotherm data taken from selected references ($R > 0.998$).

The interfacial tension is well described by the Szyszkowski equation (26):

$$\sigma = \sigma^0 - RT\tau \ln(1 + [\mathrm{H}]/e) \tag{8}$$

where τ is the maximum surface excess concentration of the IIR, e is a constant. We can reasonably expect that interfacial tension effects can be negligible in presence of strong electrostatic interactions, as in the case of the IIR, while they can be more important for the neutral eluate.

By taking this into account, and by substitution of equations (6)–(8) into equation (5) we obtain:

$$\mathrm{k} = \frac{\mathrm{c}_1}{(1 = \mathrm{c}_2[\mathrm{H}])^{\mathrm{c}_3}(1 + \mathrm{c}_4[\mathrm{H}]^{\mathrm{c}_5})} \tag{9}$$

where:

$$\mathrm{c}_1 = \Phi[\mathrm{L}]_{\mathrm{T}}\mathrm{K}_{\mathrm{EL}}\frac{\gamma_{\mathrm{E}}\gamma_{\mathrm{L}}}{\gamma_{\mathrm{EL}}} \tag{10}$$

Hence, c_1 is equal to the retention factor when no IIR is present in the eluent (k_o) and it is often not an adjustable parameter.

$$\mathrm{c}_2 = 1/\mathrm{e} \tag{11}$$

$$\mathrm{c}_3 = \tau \mathrm{S}_{\mathrm{E}} \tag{12}$$

$$\mathrm{c}_4 = \mathrm{K}_{\mathrm{HL}}\frac{\gamma_{\mathrm{H}}\gamma_{\mathrm{L}}}{\gamma_{\mathrm{HL}}}\frac{\exp\left(-\frac{\mathrm{z_H F_\alpha}}{\mathrm{RT}}\right)}{\alpha^{\left(\frac{\mathrm{z_H F_\beta}}{\mathrm{RT}}\right)}} \tag{13}$$

$$\mathrm{c}_5 = \left(1 - \mathrm{b}\frac{\mathrm{z_H F}\beta}{\mathrm{RT}}\right) \tag{14}$$

since (25):

$$\beta = -\frac{\mathrm{RT}}{\mathrm{z_H F}}\left(1 - \frac{1}{\mathrm{b}}\right) \tag{15}$$

by substitution of equation (15) into equation (14) it is easily obtained that c_5 (b) is equal to the Freundlich constant b.

Equation (9) is a five-parameter equation, but both c_1 and c_5 can be experimentally obtained so it may become a three-parameter relationship.

It may be argued, on the basis of the experimental evidence (see below), that surface exclusion phenomena is the outstanding cause of the observed dependence of neutral molecule retention upon IIR concentration. So, equation (9) could be further simplified to obtain:

$$\mathrm{k} = \frac{\mathrm{c}_1}{1 + \mathrm{c}_4[\mathrm{H}]^{\mathrm{c}_5}} \tag{16}$$

If c_1 (k_o) and c_5 (b) are known, there is only one adjustable parameter (c_4) in this equation. It is noteworthy that, according to equation (16), if [H] is equal to zero, that no IIR is present in the eluent, k is equal to k_o.

DISCUSSION

A number of papers contain experimental results, which can be used to test a new theory on IIC.

In the present work, we have considered experimental results obtained:

(i) for IIR concentration below the critical micelle concentration (CMC) (27);

(ii) at constant ionic strength and IIR counter-ion concentration; in this way salting-out effects were ruled out, and when IIR concentration was changed only the relative concentrations of IIR and its counter ion were altered and the surface potential increase was not influenced by ionic strength effects. If the ionic strength was not constant, it was high enough to minimize the influence of the change of the IIR concentration;

(iii) at constant organic modifier concentration in the eluent;

The influence of ionic strength and organic modifier in the eluent will be accounted for in a subsequent paper.

Figure 1–6 show the graph of equation (16) fitted to representative experimental data for the capacity factor of neutral analytes as a function of the concentration of IIR in the eluent when interfacial tension effects can be neglected. As it is clear, there is a wide variability of the course of the capacity factor. Equation (16)

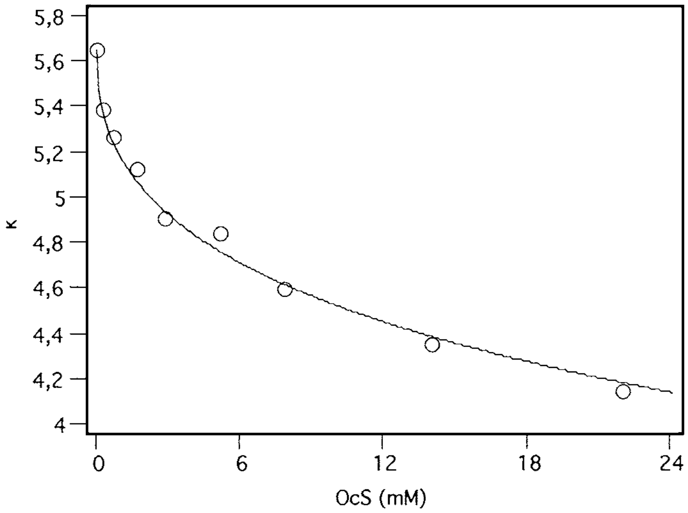

Figure 1. Capacity factor of benzyl alcohol vs. eluent concentration of Octyl sulphate. Eq. (16) fitted to data from Figure 9 of ref. (11) with permission from Elsevier Science.

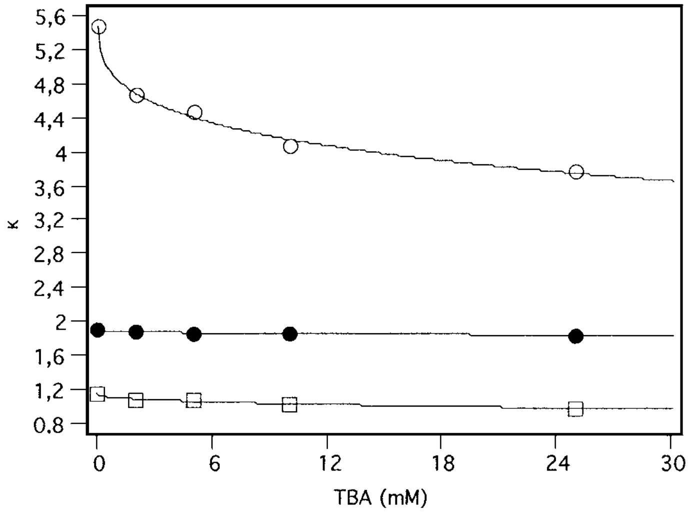

Figure 2. Capacity factor of Tetramethylurea (TMU open circles), Dimethylformamide (DMF, squares), Dimethylchetone (DMC, filled simbols) vs. eluent concentration of Tetrabuthylammoniumphosphate (TBA). Eq. (16) fitted to data reprinted from Table 3 and 4 of ref. (16) p. 977 by courtesy of Marcel Dekker, Inc. and unpublished results obtained with a 81.6 mM phosphate buffer pH 7.2-Methanol, 85:15.

is very versatile in the fitting of experimental data. It is interesting to observe that the theoretical prediction of a steeper tangent for lower IIR concentration is experimentally confirmed.

As regards Figure 1, 4, 5, 6, the mobile phase concentrations of the IIR which are reported in the x axis, were obtained from the surface concentrations via the adsorption isotherm.

Figure 2 underlines that retention decrease with increasing IIR concentration, for some analytes, can be so small that an apparent lack of retention dependence on IIR concentration can be claimed. This can explain the strong variability among experimental results.

One point of interest in Figure 3 is that IIC can be used to improve the resolution of uncharged analytes and to obtain a higher selectivity of the chromatographic method. To our knowledge, this separation strategy has not been deliberately exploited, but we believe that it can be helpful for difficult to separate mixtures.

It is interesting to compare retention data for the same analyte (phenol) obtained with butyl-, hexyl-, and octylsulfonate, in Figure 4–6. It is clear that the more lipophilic the IIR is, the more it is effective in reducing analyte retention because of surface exclusion phenomena. This is also quantitatively confirmed by the estimate of the K_{HL}, which is outlined in Table 1 (see below).

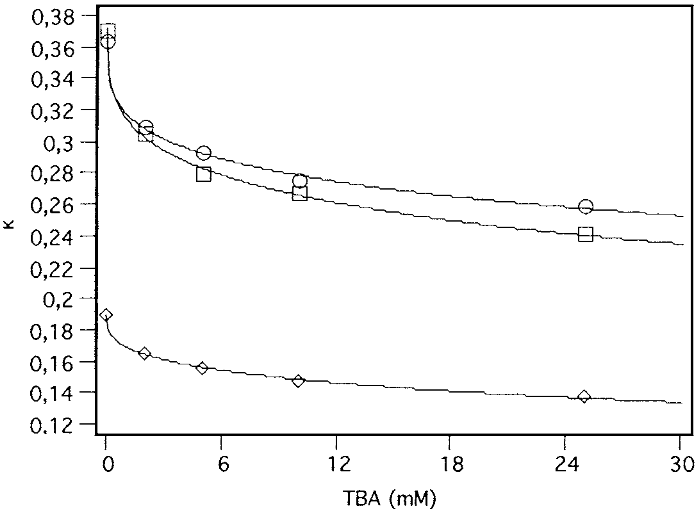

Figure 3. Capacity factor of Dimethylformamide (squares), Dimethylchetone (circles), Dimethylsolfoxide (rhombs) vs. eluent concentration of Tetrabuthylammoniumbromidee (TBA). Eq. (16) fitted to data reprinted from Figure 1 of ref. (15) p. 2428 by courtesy of Marcel Dekker, Inc.

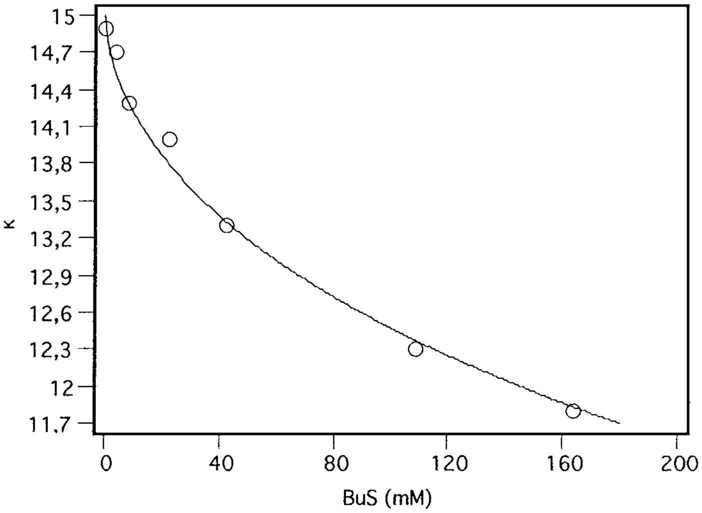

Figure 4. Capacity factor of Phenol vs. eluent concentration of Butylsulfonate. Eq. (16) fitted to data reprinted from Figure 8 of ref. (14) with permission from Elsevier Science.

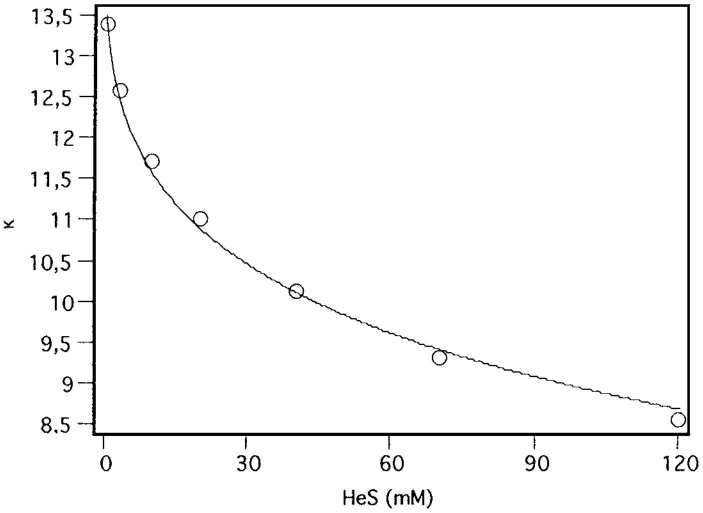

Figure 5. Capacity factor of Phenol vs. eluent concentration of Hexylsulfonate. Eq. (16) fitted to data reprinted from Figure 8 of ref. (14) with permission from Elsevier Science.

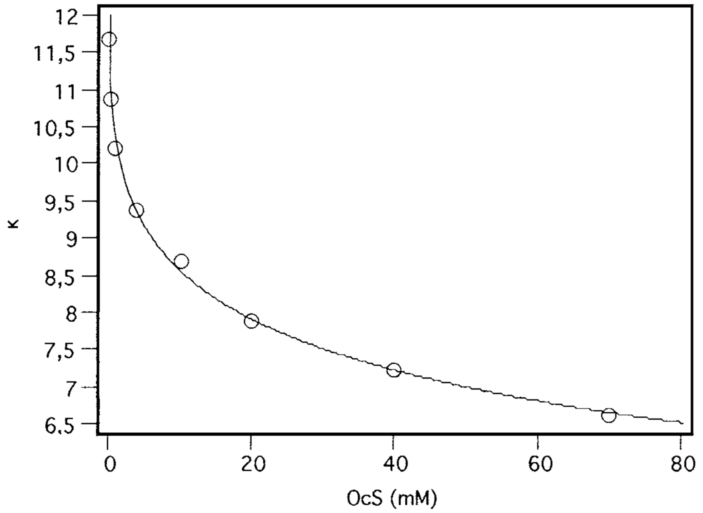

Figure 6. Capacity factor of Phenol vs. eluent concentration of Octylsulfonate. Eq. (16) fitted to data reprinted from Figure 8 of ref. (14) with permission from Elsevier Science.

Table 1. Summary of data sources, parameter estimates, correlation coefficients, standard errors, and number of data points

Reference	IIR	Eluite	pH	Methanol %	Fitted Eq.	*c1*	*c4* (1/mM)^b	*c5*	r	SSE	No. of Points
11, Fig. 9	octylsulphate	benzyl alcohol	6.0	20	16	–	0.0917	–	0.99625	0.0147	9
11, Fig. 4 left	decylsulphate	benzyl alcohol	6.0	20	16	–	0.3566	–	0.97211	0.2998	9
14, Fig. 8	butylsulfonate	phenol	2.1	0	16	–	0.0152	–	0.99591	0.0699	7
14, Fig. 8	hexylsulfonate	phenol	2.1	0	16	14.4	0.0953	–	0.99733	0.0977	7
14, Fig. 8	octylsulfonate	phenol	2.1	0	16	13	0.2512	–	0.99867	0.0597	8
16*	tetrabutylammonium	tetramethylurea	7.2	15	16	–	0.1274	0.4005	0.99672	0.0111	5
16*	tetrabutylammonium	dimethylformamide	7.2	15	16	–	0.0375	0.4707	0.99129	0.0003	5
16*	tetrabutylammonium	dimethylchetone	7.2	15	16	–	0.0098	0.3937	0.99359	3E-05	5
15, Fig. 1	tetrabutylammonium	dimethylformamide	7.2	15	16	–	0.1788	0.347	0.99882	2E-05	5
15, Fig. 1	tetrabutylammonium	dimethylchetone	7.2	15	16	–	0.1425	0.3302	0.99909	1E-05	5
15, Fig. 1	tetrabutylammonium	dimethylsulfoxide	7.2	15	16	–	0.117	0.3703	0.99897	3E-06	5
12, Fig. 5	DCSA	2-butanone	2.1	0	16	–	5.7999	–	0.97873	0.3847	7

Best fit of experimental data, from ref. [11], [14] with permission from Elsevier Science, and from ref. [12] with permission from Vieweg Publishing and from the authors, and from ref. [15] p. 2428 and [16] p. 977 by courtesy of Marcel Dekker, Inc., by equation (16).

*Data from Table 3 and 4 and unpublished results all obtained with Phosphate buffer 81.6 mM.

In the fitting of literature data by equation (16) the fitting parameters, which showed to be important to obtain a good fit of retention data are reported in Table 1. If a parameter is missing, this means that it was obtained by the experimental conditions. The number of adjustable parameters was two at maximum and their numerical estimate is very reasonable in all cases.

It can be observed that the estimates of K_{HL} (c_4) parallel the hydrophobicity of the IIR and increase with increasing chain length, as evident for data taken from ref. (11) and ref. (14). If the probe (analyte) by which K_{HL} is estimated is different, according to the displacement equilibrium (3), a different value of the c_4 parameter is obtained. The estimates of the c_5 parameter are also very reasonable (25). The high values of the correlation coefficients lend support to the present approach to obtain a simplified retention equation. A very good agreement between the experimental data and the predicted effect of the surface exclusion phenomena is obtained.

Finally, if an increase of the capacity factor of a neutral analyte is experimentally obtained, we have to conclude that the modification of the activity coefficient of the eluate in the adsorbed phase can not be neglected and the "chemical modification" of the stationary phase, due to the dynamic adsorption of the IIR, favors the partition of the analyte onto it. Taking into account the role played by the modification of the surface activity coefficient is a future challenge for model-makers.

We may conclude, that the fundaments of the IIC for neutral molecules are clearly far more complex than has been presumed. The present simplified retention model for neutral compounds in IIC considers the IIR both a surfactant and a lipophilic charged ions. The very simple retention equation that is obtained can be used even if the adsorption isotherm is unknown, or if the surface potential is not rigorously calculated.

ACKNOWLEDGMENTS

We thank MURST and CNR for financial support.

REFERENCES

1. Cecchi, T. In *Dekker Encyclopedia of Chromatography*, Marcel Dekker Inc.: New York, in press.
2. Gennaro, M.C. Adv. Chromatogr. **1995**, *35*, 343–381.
3. Bidlingmeyer, B.A.; Deming, S.N.; Price, W.P., Jr.; Sachok, B.; Petrusek, M. J. Chromatogr. **1979**, *186*, 419–434.
4. Bidlingmeyer, B.A. J. Chromatogr. Sci. **1980**, *18*, 525–539.

5. Thomlinson, E.; Jefferies, T.M.; Riley, C.M. J. Chromatogr. **1978**, *159*, 315–358.
6. Hung, C.T.; Taylor, R.B. J. Chromatogr. **1980**, *202*, 333–345.
7. Gelijkens, C.F.; De Leenheer, A.P. J. Chromatogr. **1980**, *194*, 305–314.
8. Sarzanini, C.; Bruzzoniti, M.C.; Sacchero, G.; Mentasti, E. J. Chromatogr. A **1996**, *739*, 63–70.
9. Kong, R.C.; Sachok, B.; Deming, S.N. J. Chromatogr. **1980**, *199*, 307–316.
10. Melin, A.T.; Ljungcrantz, R.A.; Schill, G.; J. Chromatogr. **1979**, *185*, 225–239.
11. Knox, J.H.; Hartwick, R.A. Mechanism of Ion-Pair Liquid Chromatography of Amines, Neutrals, Zwitterions and Acids Using Anionic Hetaerons. J. Chromatogr. **1981**, *204*, 3–21.
12. Bartha, A.; Vigh, Gy.; Billiet, H.; de Galan, L. Effect of the Type of Ion-Pairing Reagent in Reversed-Phase Ion-Pair Chromatography. Chromatographia **1985**, *20*, 587–590.
13. Bartha, A.; Vigh, Gy. J. Chromatogr. **1983**, *265*, 171–182.
14. Bartha, A.; Vigh, Gy. J. Chromatogr. Studies in Reversed-Phase Ion-Pair Chromatography. IV. The Role of Chain Length of the Pairing Ion. **1984**, *303*, 29–38.
15. Cecchi, T.; Pucciarelli, F.; Passamonti, P.; J. Liq. Chromatogr. & Rel. Technol. **1998**, *21* (16), 2423–2433.
16. Cecchi, T.; Pucciarelli, F.; Passamonti, P. J. Liq. Chromatogr. & Rel. Technol. **1999**, *22* (7), 969–979.
17. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Chromatographia. **2001**, *53*, 27–34.
18. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Anal. Chem. Submitted for publication.
19. Stahlberg, J. J. Chromatogr. A **1999**, *855*, 3–55.
20. Bartha, A.; Stahlberg, J. J. Chromatogr. A **1994**, *668*, 255–284.
21. Narkiewicz-Michalek, J. Chromatographia **1993**, *35*, 527–538.
22. Horvath, Cs.; Melander, W.; Molnar, I. J. Chromatogr. **1976**, *125*, 129–156.
23. Daignault, L.G.; Rillema, D.P. J. Chromatogr. **1992**, *602*, 3–8.
24. Lucassen-Reynders, E.H. In *Progress in Surface and Membrane Science*; Cadenhead, D.A.; Danielli, J.F., Eds.; Academic Press: New York, 1976; Vol. 10, 253.
25. Davies, J.T.; Rideal, E.K. *Interfacial Phenomena*; Academic Press: New York and London, 1961, Ch. 4.
26. Rosen, M.J.; *Surfactant and Interfacial Phenomena*; Wiley: New York, 1978.
27. Bartha, A.; Vigh, Gy.; Varga-Puchony, Z. J. Chromatogr. **1990**, *499*, 423–434.

Received July 21, 2000 Manuscript 5360
Accepted August 20, 2000

Downloaded At: 09:35 24 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JLC100001334