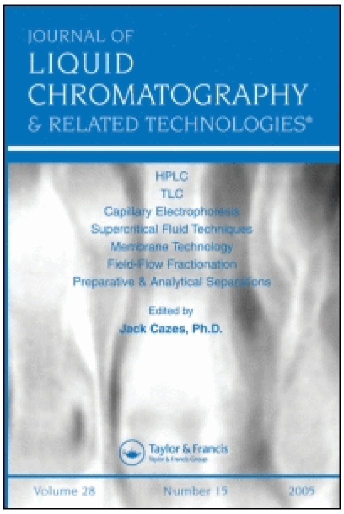

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Extended Thermodynamic Approach to Ion Interaction Chromatography: Effect of the Electrical Charge of the Solute Ion

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

To cite this Article Cecchi, T. , Pucciarelli, F. and Passamonti, P.(2004) 'Extended Thermodynamic Approach to Ion Interaction Chromatography: Effect of the Electrical Charge of the Solute Ion', Journal of Liquid Chromatography & Related Technologies, 27: 1, 1 — 15

To link to this Article: DOI: 10.1081/JLC-120027082

URL: http://dx.doi.org/10.1081/JLC-120027082

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 1, pp. 1–15, 2004

Extended Thermodynamic Approach to Ion Interaction Chromatography: Effect of the Electrical Charge of the Solute Ion

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

Università degli Studi di Camerino, Dipartimento di Scienze Chimiche,
Camerino, Italy

ABSTRACT

The chromatographic behavior of multiply charged analytes in ion interaction chromatography (IIC) was theoretically investigated. Practical equations that describe the relationship between the retention factor and the concentration of the ion interaction reagent (IIR) were developed. They can be used to model analyte retention as a function of both the mobile and stationary phase concentrations of the IIR. A comparison between the retention behaviour of singly and doubly charged analytes is given.

Key Words: Ion interaction chromatography; Ion interaction concentration; Electrical charge effect; Solute ion.

---

[*] Correspondence: T. Cecchi, Università degli Studi di Camerino, Dipartimento di Scienze Chimiche, Via S. Agostino 1, I-62032 Camerino, Italy; E-mail: teresacecchi@tiscalinet.it.

DOI: 10.1081/JLC-120027082
1082-6076 (Print); 1520-572X (Online)

www.dekker.com

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

INTRODUCTION

Reversed-phase ion interaction chromatography (IIC) is an intriguing separation mode of HPLC[1–11] because a number of variables, including ion interaction reagent (IIR) concentration and lipophilicity, organic modifier percentage, pH, and ionic strength, can be easily tuned to obtain tailor-made separations.

Retention models are often sought in the course of optimization procedures to help the chromatographer to perform an educated guess to prove its optimization criteria.

Retention models for IIC can be divided into stoichiometric[3,5] and thermodynamic mondels.[6–11] The former, pioneered this technique even if the retention mechanism was a matter of controversy, as witnessed by the different terminology, which is found in literature to denote this separation mode. In a corner stone paper Know and Hartwick demonstrated that both the two fundamental stoichiometric approaches (the so called dynamic ion-exchange and ion-pair chromatography) lead to identical retention equations.[5] Unfortunately, even if the practicality of these relationships promoted the use of IIC, they lacked a firm foundation in physical chemistry, because they were not able to account for the surface potential that develops at the stationary phase as a consequence of the adsorption of the lipophilic IIR ions. Thermodynamic approaches, on the other hand, are able to account for this modification of the stationary phase surface, but disregard all chemical equilibria in the chromatographic system. We have recently developed an extended thermodynamic retention model for IIC that is able to quantitatively predict retention of charged, neutral and zwitterionic analytes[12–20] as a function of the IIR concentration both in the mobile and in the stationary phases. It takes into account chemical equilibria between the solute ion and the IIR from a thermodynamic and not stoichiometric point of view, and it also considers the electrostatic interaction between the charged analyte and the charged stationary phase. New retention equations are quantitatively able to predict experimental evidence that cannot be rationalized by the most reliable thermodynamic retention models.[12,17,19] Moreover the present theory is able to predict when other models' prediction are at variance with experimental results.

It is the aim of the present work to develop and test retention equations for multiply charged analytes.

THEORY

We will use the Stern–Gouy–Chapman model of the electrical double layer[21] that develops at the chromatographically interesting interface between the stationary and the mobile phases. A large experimental evidence demonstrates that IIRs adsorb onto the stationary phase, and the adsorbed IIR ions are responsible for surface charge that give the electrical potential difference, Ψ_0, between the surface and the bulk solution. Its sign and magnitude depends on the charge status of the IIR, at a fixed IIR surface concentration, according to the following complete expression.[22]

$$\Psi_0 = \frac{2\mathrm{R}T}{z_\mathrm{H}\mathrm{F}} \ln\left\{ \frac{[\mathrm{LH}]|z_\mathrm{H}|F}{(8\varepsilon_0\varepsilon_r \mathrm{R}T\sum_i c_{0i})^{1/2}} + \left[\frac{([\mathrm{LH}]z_\mathrm{H}F)^2}{8\varepsilon_0\varepsilon_r \mathrm{R}T\sum_i c_{0i}} + 1\right]^{1/2} \right\} \tag{1}$$

In Eq. (1) ε is the dielectric constant (or relative permittivity) of the medium; ε_0 is the vacuum permittivity; F is the Faraday constant, R is the gas constant; T is the temperature, and Σc_{0i} is the mobile phase concentration of electrolyte ions; [LH] is the stationary phase concentration of the IIR, H.

Equation (1) parallels Eq. (25) of Ref.[12], in which the charge of H (z_H) was omitted because it was assumed to be singly charged.

If we indicate:

$$f = \frac{[\mathrm{LH}]|z_\mathrm{H}|F}{(8\varepsilon_0\varepsilon_r \mathrm{R}T\sum_i c_{0i})^{1/2}} \tag{2}$$

we easily have

$$\Psi_0 = \ln([\mathrm{LH}]\,f + (([\mathrm{LH}]\,f)^2 + 1)^{1/2})^{2\mathrm{R}T/z_\mathrm{H}\mathrm{F}} \tag{3}$$

Solute ions with opposite (similar) charge to the IIR are attracted (repelled) by the charged surface and their retention is predicted to increase (decrease) with increasing IIR surface concentration. The higher the charge on the analyte, the stronger the electrostatic interaction, hence, multiply charged analytes are easily predicted to show larger changes in retention than singly charged solute ions.

If the charge status of the analyte and IIR is the same, ion pair equilibria in both the stationary and mobile phases do not apply. If the analyte is oppositely charged to the IIR, but it is multiply charged, electrostatic interactions between the analyte and the charged stationary phase are predicted to predominate, while multi-body ionic associations are probably residual (see below Eq. (16) to take them into account).

At constant ionic strength and organic modifier concentration, by substitution of Eq. (1) into Eqs. (24) and (33) of Ref.[12] the course of the analyte retention, upon IIR concentration in the mobile ([H]) and in stationary phase ([LH]), can be described, respectively, by the following two expressions that parallel Eqs. (28) and (34) of Ref.[12], except c_2, d_2, and c_3, d_3 are missing since they are related to ion-association at the stationary phase and in the mobile phase, respectively.[12]

$$k = \frac{c_1(a[H]^b f + ((a[H]^b f)^2 + 1)^{1/2})^{(-2(z_E/z_H))}}{1 + c_4[H](a[H]^b f + ((a[H]^b f)^2 + 1)^{1/2})^{-2}} \tag{4}$$

$$k = d_1([\mathrm{LH}]\,f + (([\mathrm{LH}]\,f)^2 + 1)^{1/2})^{(-2z_E/z_H)}(d_4 - [\mathrm{LH}]) \tag{5}$$

where a and b are constants related to the Freundlich adsorption isotherm[23]

$$[\mathrm{LH}] = a[\mathrm{H}]^b \tag{6}$$

that gives [LH] as a function of [H] (its use is not empirical[19]); z_E is the charge of the analyte E, and

$$c_1 = \phi[L]_T K_{EL}\frac{\gamma_E\gamma_L}{\gamma_{EL}} \tag{7}$$

c_1 is the retention factor when the IIR is not present in the eluent (k_o), and it is related to K_{EL} that is the thermodynamic equilibrium constant for the adsorption of the solute ion onto the stationary phase. k_o can be obtained by experimental results, hence, it is not always a fitting parameter;

$$c_4 = K_{HL}\frac{\gamma_H\gamma_L}{\gamma_{HL}} \tag{8}$$

where K_{HL} is the thermodynamic equilibrium constant for adsorption of the IIR onto the stationary phase

$$d_1 = \phi K_{EL}\frac{\gamma_E\gamma_L}{\gamma_{EL}} \tag{9}$$

d_1 is equal to (k_o/d_4), hence, it is not an additional fitting parameter, if k_o is known; if [HL] is negligible with respect to $[\mathrm{L}]_\mathrm{T}$, this term is included in d_1, hence, $d_1 = c_1 = k_o$

$$d_4 = [\mathrm{L}]_\mathrm{T} \tag{10}$$

where $[\mathrm{L_T}]_\mathrm{T}$ estimates the total ligand surface concentration.

As may be observed from the second term of the denominator of Eq. (4), the potential that develops at the stationary phase always runs counter further adsorption of the IIR, since the surface potential is of the same sign as z_H. From the exponent of the numerator of both Eqs. (4) and (5) it is clear that, if the charge status of E and H is the same, k is expected to decrease with increasing IIR concentration, while the opposite is predicted for oppositely charged analyte and IIR. The magnitude of the electrostatic interaction is predicted to depend on the actual number of charges on E and H.

If adsorption competitions are residual, compared to the strong electrostatic interaction, Eq. (4) further reduces to

$$k = c_1(a[H]^b f + ((a[H]^b f)^2 + 1)^{1/2})^{(-2(z_E/z_H))} \tag{11}$$

Under the same assumptions Eq. (5) becomes

$$k = d_1([\mathrm{LH}]\, f + (([\mathrm{LH}]\, f)^2 + 1)^{1/2})^{(-2(z_E/z_H))} \tag{12}$$

It was demonstrated[17] that, if pairing equilibria and adsorption competitions can be neglected, as in the case of multiply charged analytes, the following approximate expression may describe the relationship between k and [H] for non-zero IIR concentration:

$$\begin{aligned}\log k = {} & \log c_1 - \frac{z_E \mathrm{F}\alpha}{\mathrm{R}T}\log e + \frac{z_E}{z_H}\left(1 - \frac{1}{b}\right)\log a \\ & - \frac{z_E}{z_H}(1 - b)\log [\mathrm{H}]\end{aligned}$$

It is clear that the slope of this linear $\log k - \log [\mathrm{H}]$ relationship is sensitive not only to the sign and number of charges of the analyte but also to the global charge status of the IIR, as expected.

If we cannot a priori rule out that multi-body ion-associations of the following kind:

$$\mathrm{E} + z_E\mathrm{H} + \mathrm{L} \overset{K_{\mathrm{EH}_{z_E}\mathrm{L}}}{\longleftrightarrow} \mathrm{EH}z_E\mathrm{L} \tag{14}$$

$$\mathrm{E} + z_E\mathrm{H} \overset{K_{\mathrm{EH}_{z_E}}}{\longleftrightarrow} \mathrm{EH}z_E \tag{15}$$

are residual for multiply charged (z_E) analytes (E), following a procedure similar to that one in Ref.[12], we obtain the general forms of Eq. (4):

$$k = \frac{c_1(a[\mathrm{H}]^b f + ((a[H]^b f)^2 + 1)^{1/2})^{(-2(z_E/z_H))} + c_2[H]^{z_E}}{(1 + c_3[H]^{z_E})(1 + c_4[H](a[H]^b f + ((a[H]^b f)^2 + 1)^{1/2})^{-2})} \tag{16}$$

where:

$$c_2 = \phi[\mathrm{L}]_\mathrm{T} K_{\mathrm{EH}z_E\mathrm{L}} \frac{\gamma_E \gamma_H{}^{z_E} \gamma_L}{\gamma_{\mathrm{EH}z_E\mathrm{L}}} \tag{17}$$

$K_{\mathrm{EH}_{z_E}\mathrm{L}}$ is the thermodynamic equilibrium constant for the multi-body ion-association in the stationary phase

$$c_3 = K_{\mathrm{EH}z_E} \frac{\gamma_E \gamma_H{}^{z_E}}{\gamma_{\mathrm{EH}z_E}} \tag{18}$$

where $K_{\mathrm{EH}_{z\mathrm{E}}}$ is the thermodynamic equilibrium constant for the multi-body ion-association in the eluent.

EXPERIMENTAL

Instrumentation

Chromatographic runs were performed with a Phenomenex Synergi Hydro-RP (150 mm × 4.6 mm I.D., 4 μm particle size, 80 Å pore size, bonded phase coverage 4.05 $\mu\mathrm{mol/m^2}$). The high performance liquid chromatograph consisted of a Series 200 LC pump (Perkin Elmer, San José, CA), a 785A programmable Absorbance Detector (Applied Biosystems), connected in series. The injector was a Rehodyne, Model 7125NS-005 (Cotati, CA), with a 20 μL external loop.

A water jacket (Alltech, Milan, Italy) and a Criotherm 190 thermostat (ISCO, Fizzonasco, Italy) were used to thermostat columns at 25.0 ± 0.1°C. A three-way ball valve Kel-F seat (Perker Hannifin plc, Devon, England) was used to switch the eluent flow from a backpressure regulator (Varian, Walnut Creek, CA) to the column.

The Turbochrom 4.0 software (Perkin Elmer, San José, CA) was used for data acquisition.

pH measurements were done by a Methrom 645 pH meter (Herisau, Switzerland).

Chemicals

All chemicals were obtained from Sigma-Aldrich (Milan, Italy).

The water was deionized and further purified on a Milli-Q 185 Plus water purification system from Millipore (Bedford, MA).

HPLC Analysis

The standard eluent was phosphate buffer 37.10 mM KH_2PO_4 and 4.29 mM Na_2HPO_4, calculated to provide a pH of 6.0. After addition of the desired amount of sodium 1-hexanesulfonate, NaCl was added so that the total sodium concentration was 50 mM and the ionic strength was constant.

The individual behavior of each analyte was investigated in isocratic mode to study the effect of increasing IIR concentration at constant ionic strength. The flow rate was 1.0 mL/min.

Each analyte was prepared in the eluent to be used in the chromatographic run (to avoid system peak). Each concentration was 500 ppm. All samples were filtered via 0.2 μm Nylon filters (Lida, WI). At least three measurements were averaged and used for the *k* calculations.

Mobile phases were degassed under a constant flow of helium (100 mL/min). All eluents were filtered via 0.2 μm Cellulose Acetate filters (Advantec MFS Inc, CA). The dielectric constant was considered that of water (78.49).

Adsorption Isotherms

The detailed procedure to obtain the adsorption isotherm of the 1-hexanesulfonate and to desorb the column is described elsewhere.[20]

Software

Analysis of raw data and fittings were performed using the software MacCurveFit 1.5.4, Copyright © 1991–2000, Kevin Raner Software.

RESULTS AND DISCUSSION

To obtain a clear test of the proposed new expressions, the experimental design provided for constant ionic strength conditions. In this way (i) salting effects could be ruled out; (ii) the activity coefficient ratios in Eqs. (7)–(9) were almost constant; and (iii) the surface potential increase was not influenced by ionic strength effects according to Eq. (2). Moreover, the IIR concentration was always below its critical micelle concentration and its counterion was not adsorbophilic, hence, it did not influence the surface potential.

The f constant in Eq. (2) was calculated to be 1.90E+06 $\mathrm{m}^2/\mathrm{mol}$ from experimental conditions. The fitting of experimental data according to the Freundlich adsorption isotherm gave the following parameter estimates $a = 1.90\mathrm{E}-01 \pm 3.90\mathrm{E}-03 \quad \mu\mathrm{mol}\cdot\mathrm{m}^{-2}\cdot\mathrm{mM}^{-b}$, $b = 5.10\mathrm{E}-01 \pm 6.60\mathrm{E}-03$ ($r = 0.9991$). It can be confirmed[12,17,19] that the Freundlich isotherm (Eq. (6)) properly describes the adsorption isotherm of IIRs.

Figures 1–4 show the fitting of experimental data according to Eqs. (11) and (12) as detailed in the captions, for doubly charged analytes. In Figs. 5–8 the retention behaviors of singly charged analytes are shown to draw a parallel. It is clear that the retention decrease with increasing IIR

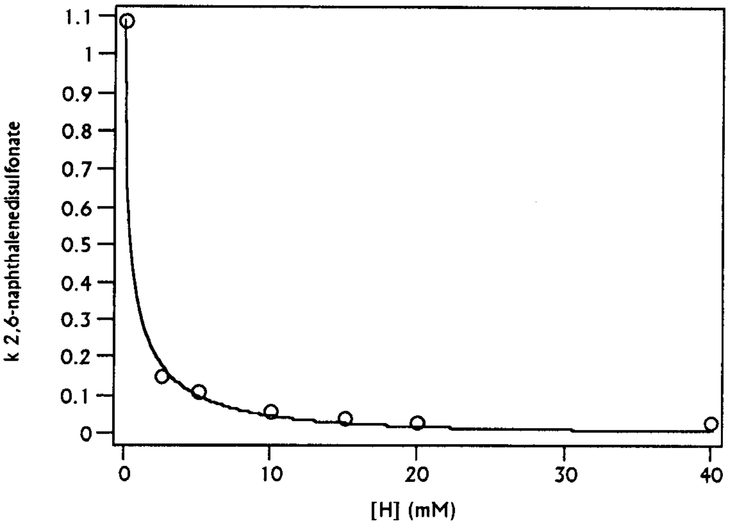

Figure 1. Dependence of k for 2,6-naphthalenedisulfonate upon mobile phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (11).

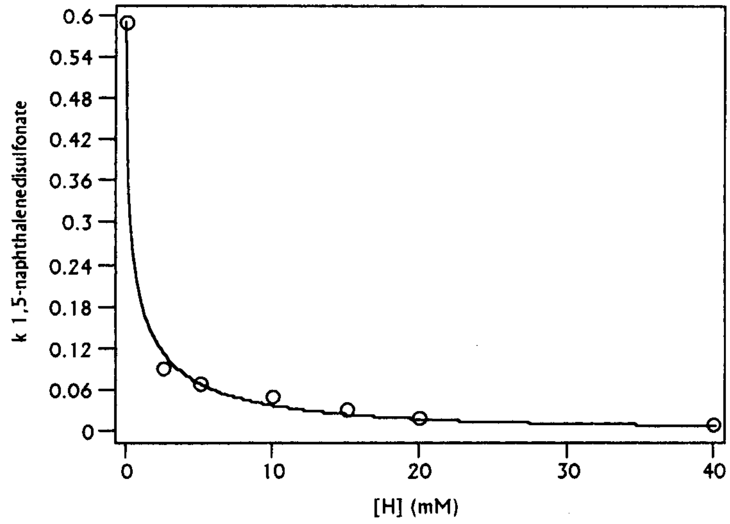

Figure 2. Dependence of k for 1,5-naphthalenedisulfonate upon mobile phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (11).

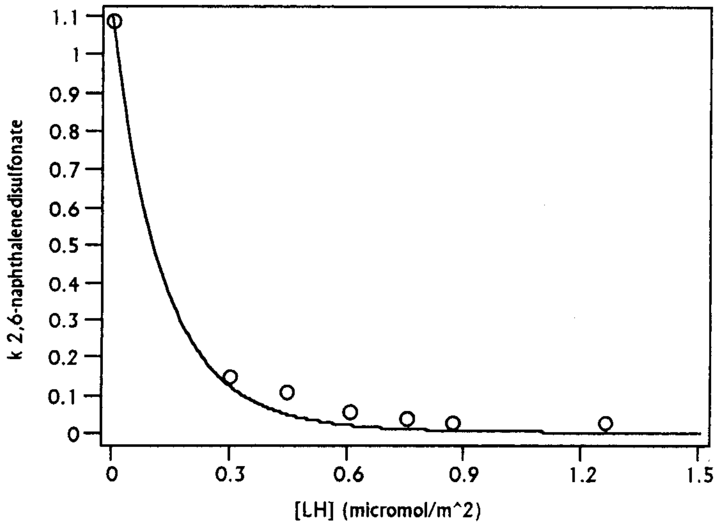

Figure 3. Dependence of k for 2,6-naphthalenedisulfonate upon stationary phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (12).

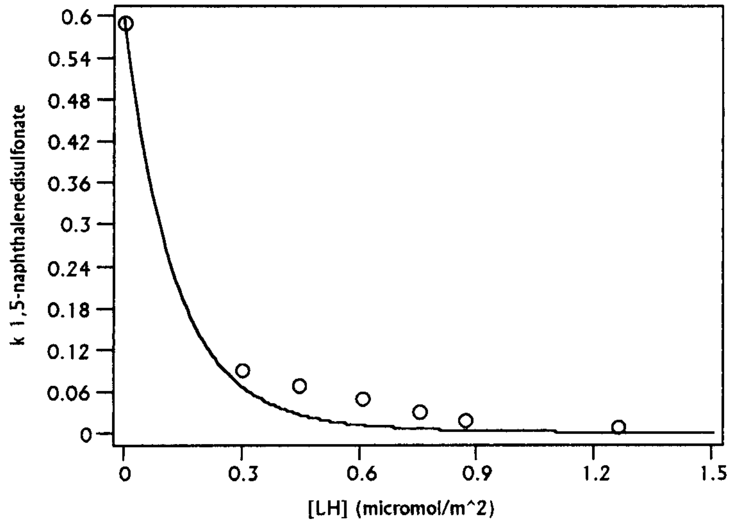

Figure 4. Dependence of k for 1,5-naphthalenedisulfonate upon stationary phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (12).

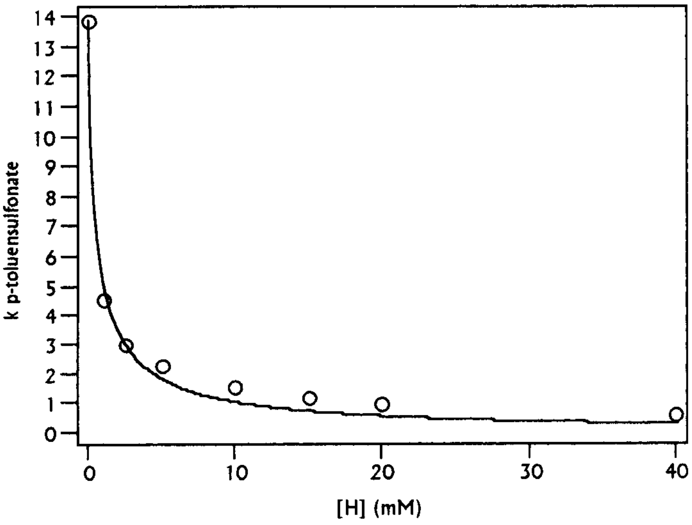

Figure 5. Dependence of k for p-toluenesulfonate upon mobile phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (4).

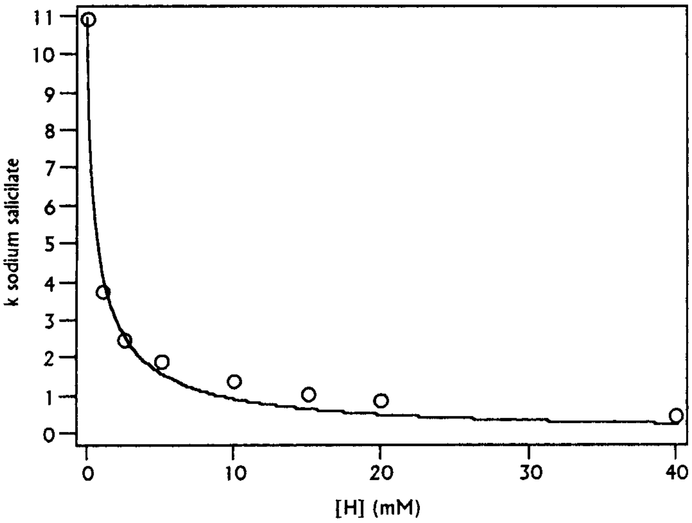

Figure 6. Dependence of k for sodium salicylate upon mobile phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (4).

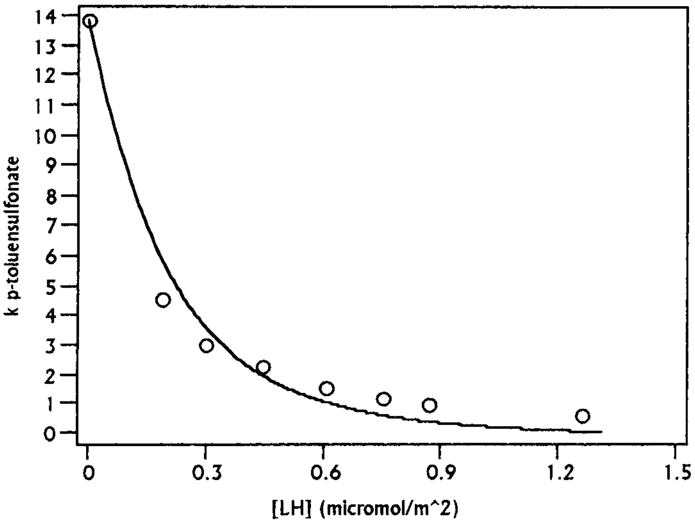

Figure 7. Dependence of k for *p*-toluenesulfonate upon stationary phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (5).

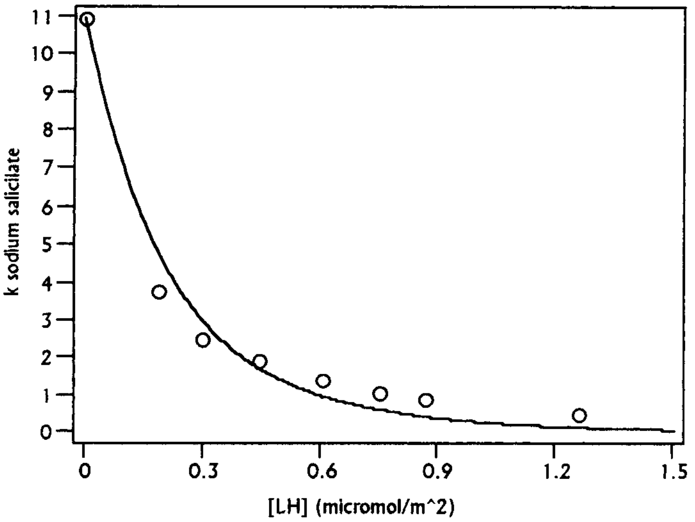

Figure 8. Dependence of k for sodium salicylate upon stationary phase concentration of 1-hexanesulfonate. Experimental data were fitted by Eq. (5).

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

concentration is steeper for doubly charged analytes (Figs. 1–4), compared to singly charged solute ions (Figs. 5–8), as expected. The C18 Synergi Hydro-RP phase was selected because it provides extreme retention of the analytes used in the present study, that would be otherwise too poorly retained.

The best fit of Eqs. (4) and (11), (5) and (12) to experimental data gave the parameter estimates, correlation coefficients, standard deviations, and sum of square errors detailed in Tables 1 and 2, respectively. For doubly charged analytes, c_4 and d_4 parameters are missing because the correlation coefficient was not increased by their inclusion at 95% confidence level, that is, in the chromatographic system, the physical phenomenon of adsorption competition was negligible compared to the strong electrostatic repulsion between this multiply charged analyte and the charged stationary phase. It follows that Eqs. (11) and (12) could be properly used. In Tables 1 and 2, the only fitting parameter for doubly charged analytes was c_1 that represents k_0. We wish to emphasize that the percent error between the experimental and calculated k_0 is very low, thereby confirming the assumption that adsorption competitions may be neglected compared to the strong electrostatic interaction. For singly charged analytes, experimental k_0 was used to keep the number of adjustable constants at the minimum, hence, c_1 and d_1 are missing since they were not considered fitting parameters.

Since the values of the constants obtained from the non-linear regression has an essential meaning to witness the adequacy of a retention model, it should be pointed out that, in Table 2, the estimates of the total ligand surface

Table 1. Summary of fitted equation, parameter estimates, standard deviations (σ), correlation coefficient, sum of square errors (SSE), and number of points, for the best fit of experimental retention data as a function of the mobile phase concentration of the IIR (Figs. 1, 2, 5, and 6).

	Analyte			
	2,6-Naphthalene disulfonate	1,5-Naphthalene disulfonate	*p*-Toluenesulphonate	Salicylate
Equation	11	11	4	4
c_1	1.10E + 00	5.96E − 01	—	—
σc_1	3.51E − 02	2.75E − 02	—	—
c_4 (mM^{-1})	—	—	6.29E − 01	4.88E − 01
σc_4	—	—	1.44E − 01	1.40E − 01
r	0.9958	0.9911	0.9950	0.9935
SSE	0.0075	0.0046	1.3369	1.0637
N	8	8	8	8

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 2. Summary of fitted equation, parameter estimates, standard deviations (σ), correlation coefficient, sum of square errors (SSE), and number of points, for the best fit of experimental retention data as a function of the mobile phase concentration of the IIR (Figs. 3, 4, 7, and 8).

	Analyte			
	2,6-Naphthalene disulfonate	1,5-Naphthalene disulfonate	*p*-Toluenesulphonate	Salicylate
Equation	12	12	5	5
d_1	1.10E + 00	5.96E − 01	—	—
σd_1	3.62E − 02	2.80E − 02	—	—
d_4 (μmol m^{-2})	—	—	1.31E + 00	1.62E + 00
σd_4	—	—	3.93E − 01	5.95E − 01
r	0.9956	0.9907	0.9874	0.9872
SSE	0.0080	0.0048	3.3803	2.0849
N	8	8	8	8
Error (%)	0.6	0.9	—	—

concentration (d_4) compares very well with the calculated bonded phase coverage of the Synergi column that is 4.05 μmol/m^2. The estimated $[L]_T$ is lower than the calculated value, probably because not all sites are accessible, or because of the model assumption,[12] nevertheless, the order of magnitude is correct and the estimates make sense physically. It is also rewarding to see that the standard free energy of adsorption that correspond to the mean estimate of K_{HL} (c_4 in Table 1) is $\Delta G^\circ = -15.6$ KJ/mol, which is a very reasonable value for the standard free energy of adsorption of the IIR.[24]

As regards analytes oppositely charged to the IIR, there is wide experimental evidence that confirms the linearity of the log k vs. log [H] plot (see Eq. (13)) for multiply charged analytes and/or multiply charged IIRs, as it may be observed in Fig. 6 of Ref.[6], in Figs. 4–5 of Ref.[7] and in Fig. 2 of Ref.[3]. It is interesting to observe in Fig. 6 of Ref.[6] that the slope of singly charged analytes is lower than that of doubly charged ones as expected on the basis of Eq. (13). However it should be borne in mind that the latter is an approximate expression of practical value.

Good agreement between the experimental data and predicted effects of the charge is generally obtained, as witnessed by good correlation coefficients, hence, the predictive capabilities of retention equations can be used to optimize the mobile phase composition during method development. The very low percent errors, and the physical reliability of the parameters estimates validate the present approach to take into account the charge status of the analyte and IIR in IIC.

ACKNOWLEDGMENTS

We thank MURST and CNR for their financial support.

REFERENCES

1. Cecchi, T. Ion interaction chromatography. In *Dekker Encyclopedia of Chromatography*; Cazes, J., Ed.; Marcel Dekker Inc.: New York, 2001; 457–460.
2. Gennaro, M.C. Reversed-phase-in-pair and ion-interaction chromatography. Adv. Chromatogr. **1995**, *35*, 343–381.
3. Terweij-Groen, C.P.; Heemstra, S.; Kraak, J.C. Distribution mechanism of ionizable substances in dynamic anion-exchange systems using cationic surfactants in high-performance liquid chromatography. J. Chromatogr. **1978**, *161*, 69–82.
4. Bidlingmeyer, B.A.; Deming, S.N.; Price, W.P.; Sachok, B., Jr.; Petrusek, M. Retention mechanism for reversed-phase ion-pair liquid chromatography. J. Chromatogr. **1979**, *186*, 419–434.
5. 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.
6. Bartha, A.; Stahlberg, J. Electrostatic retention model of reversed-phase ion-pair chromatography. J. Chromatogr. A **1994**, *668*, 255–284.
7. Bartha, A.; Stahlberg, J. Retention prediction based on the electrostatic model of reversed-phase ion-pair high-performance liquid chromatography: effect of pairing ion concentration. J. Chromatogr. **1990**, *535*, 181–187.
8. Stahlberg, J.; Furangen, A. An experimental verification of the electrostatic theory for ion pair chromatography. Chromatographia **1987**, *24*, 783–789.
9. Cantwell, F.F. Retention model for ion-pair chromatograph based on double-layer ionic adsorption and exchange. J. Pharmaceut. & Biomed. Anal. **1984**, *2*, 153–164.
10. Chen, J-G.; Weber, S.; Glavina, L.L.; Cantwell, F.F. Electrical double-layer models of ion-modified (ion-pair) reversed-phase liquid chromatography. J. Chromatogr. A **1993**, *656*, 549–576.
11. Strahanan, J.J.; Deming, S.N. Thermodynamic model for reversed-phase ion-pair liquid chromatography. Anal. Chem. **1982**, *54*, 2251–2256.
12. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Extended thermodynamic approach to ion interaction chromaotography. Anal. Chem. **2001**, *73*, 2632–2639.

13. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Ion interaction chromatography of neutral molecules. Chromatographia **2001**, *53*, 27–34.
14. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Ion interaction chromatography of neutral molecules: a potential approximation to obtain a simplified retention equation. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24* (3), 291–302.
15. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Extended thermodynamic approach to ion interaction chromatography for high surface potential: use of a potential approximation to obtain a simplified retention equation. Chromatographia **2001**, *54*, 589–593.
16. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Extended thermodynamic approach to ion interaction chromatography for low surface potential: use of the linearized potential expression. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24* (17), 2551–2557.
17. Cecchi, T. Extended thermodynamic approach to ion interaction chromatography: a thorough comparison with the electrostatic approach and further quantitative validation. J. Chromatogr. A **2002**, *958*, 51–58.
18. Cecchi, T.; Pucciarelli, F.; Passamonti, P.; Cecchi, P. The dipole approach to ion interaction chromatography of zwitterions. Chromatographia **2001**, *54*, 38–44.
19. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Extended thermodynamic approach to ion interaction chromatography. The influence of the organic modifier concentration. Chromatographia **2003**, *58* (7/8), 411–419.
20. Cecchi, T.; Pucciarelli, F.; Passamonti, P. The fractional charge approach to ion interaction chromatography of zwitterions. J. Chromatogr. A. Submitted for publication.
21. Grahame, D.C. The electrical double layer and the theory of electrocapillarity. Chem. Rev. **1947**, *41*, 441–501.
22. Stahlberg, J. The Gouy–Chapman theory in combination with a modified langmuir isotherm as a theoretical model for ion-pair chromatography. J. Chromatogr. **1986**, *356*, 231–245.
23. Davies, J.T.; Rideal, E.K. Adsorption at liquid interfaces. In *Interfacial Phenomena*; Academic Press: New York and London, 1961; Chap. 4.
24. Rosen, M.J. Adsorption of surface-active agents at interfaces: the electrical double layer. In *Surfactant and Interfacial Phenomena*; Wiley: New York, 1978; Chap. 2.

Received August 6, 2003
Accepted September 14, 2003
Manuscript 6197