Extended thermodynamic approach to ion interaction chromatography: a thorough comparison with the electrostatic approach, and further quantitative validation

T. Cecchi*

Università degli Studi di Camerino, Dipartimento di Scienze Chimiche, 62032 Camerino, MC, Italy

Abstract

The most reliable literature experimental results, concerning retention behavior of charged molecules, in the presence of an ion-interaction reagent (IIR), were used to obtain a further quantitative validation of a new theory. The present work emphasizes the fact that the extent to which electrostatic interactions, ion pair formation in the adsorbed and the mobile phases, and adsorption competitions are one more important than the other depends on experimental conditions. Further insight into the meaning of the linearity of the log k vs. log [IIR] plot, which is common to many theoretical models, is given. The experimental conditions under which the linearity of this plot can be expected not only practically, but also theoretically, are elucidated. The dependence of the ratio of retention factors with and without IIR in the eluent on the analyte nature, which cannot be predicted by the electrostatic approach, was explained and tracked. The difference between the actual surface potential and that predicted by the electrostatic approach is also rationalized. The model is also theoretically shown to be able to elucidate the enantioselective retention mechanism, in the presence of chiral counter ions. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

The influence of ion-interaction reagents (IIRs) on retention of oppositely and similarly charged analytes has been well studied [1–15]. The distinguishing features that set superior models apart are their ability to explain observed behavior other models cannot explain and to reduce to previous models, under particular experimental conditions. We have

recently put forth an exhaustive thermodynamic retention model for ion-interaction chromatography (IIC) that is able to quantitatively predict retention of charged [2,3], neutral [4,5] and zwitterionic [6] analytes as a function of the IIR concentration both in the mobile and in the stationary phases. The importance of chemical equilibria was obtained from stoichiometric models, while thermodynamic, and not stoichiometric, equilibrium constants were used to take into account the chemical and physical modification of the interface.

From the epistemological point of view, this extended thermodynamic retention model is interest-

*Present address: ITIS Montani, Via Montani 7, I-62032 Fermo (AP), Italy.

E-mail address: teresacecchi@tiscalinet.it (T. Cecchi).

ing because it reduces to stoichiometric or electrostatic retention models, respectively, if the surface potential or pairing equilibria are neglected.

From a practical point of view, new retention equations are very important. They are quantitatively able to predict experimental evidence that cannot be rationalized even by the electrostatic approach, that is one of the most reliable thermodynamic retention models in IIC [8,9]. We have shown [2] that they can, for the first time, explain: (i) different theoretical curves when analyte retention is plotted against the surface concentration of the IIR, for different IIRs; (ii) the dependence of the retention factors ratios, for two different analytes, on the IIR concentration; (iii) better agreement between electrostatic approach expectations and experimental results, for analytes possessing the same charge as the IIR, compared to those oppositely charged to the IIR.

All these points strongly support the claims for a superior theory.

According to the present extended thermodynamic model, retention of an analyte does not depend exclusively on electrostatic interactions. The extent to which electrostatic and chemical interactions in both the mobile and stationary phases are one more important than the other is predicted to depend on experimental conditions. When chiral counter ions are used in the eluent (chiral IIC) it would be highly desirable [16] to know whether enantioselectivity is due to a chiral pseudostationary phase or to different adsorption of diastereomeric ion-pairs. Since the present model allows one to distinguish when retention is mainly caused by electrostatic attraction, ion-pair formation, or adsorption competitions, it merits practical consideration. Actually, the numerical and graphical estimates of the relative contributions to retention, arising from each of the mentioned phenomena, could shed light on the predominant enantioselective retention mechanism and allow the chromatographer to ascertain whether one interaction is more important than the other.

It is the aim of this work to demonstrate how the retention mechanism depends on experimental design. I also want to make a theoretical thorough comparison with the electrostatic approach. It is intended to emphasise the practical importance of the new model, which is able to predict further experimental evidence that cannot be rationalised even by the electrostatic approach.

2. Results and discussion

We have demonstrated [2] that the course of the analyte retention, upon IIR concentration in the mobile and in stationary phase, can be described, respectively, by the following two expressions:

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

$$k = \frac{d_1\left\{[LH]f + \left[([LH]f)^2 + 1\right]^{1/2}\right\}^{\pm 2|z_E|} + d_2[LH]_s^{1/b}}{(1 + d_3[LH]^{1/b})}$$
$$\times (d_4 - [LH]) \tag{2}$$

where a, b, and f are constants which depend on experimental conditions (a and b are related to the Freundlich isotherm), z_E, z_H are, respectively, the charges of the analyte E and of the IIR H, [H] and [LH] are, respectively, the mobile phase and stationary phase concentration of the IIR; c_1–c_4 or d_1–d_4 are the fitting parameters with clear physical meaning [2]: c_1 and d_1 are related to the capacity factor without IIR in the eluent, and hence to the pure electrostatic interaction with the charged surface (K_{EL}); c_2 and d_2 are related to the thermodynamic equilibrium constant for ion-pair formation in the stationary phase (K_{EHL}); c_3 and d_3 are related to the thermodynamic equilibrium constant for ion-pair formation in the eluent (K_{EH}); c_4 estimates the thermodynamic equilibrium constant for adsorption of the IIR onto the stationary phase (K_{HL}), and d_4 estimates the total ligand surface concentration.

If the charge status of the analyte and IIR is the same, ion pair equilibria do not apply in neither the stationary nor mobile phases, hence the c_2 and c_3 terms in Eq. (1), and d_2 and d_3 terms Eq. (2), are missing [2]. Analyte retention is expected to decrease with increasing IIR concentration. If the analyte is neutral, Eqs. (1) and (2) reduce to the previously developed retention equations for uncharged analytes (see Eqs. (28) and (36) of Ref. [4]).

The proposed new expressions will be further tested to show the influence of experimental conditions on the retention mechanism. The data set [13] that will be used is generally considered one of the most reliable in the field because the column was thermostatted, IIR concentration was below the

Table 1
Summary of parameter estimates, standard deviations (σ), correlation coefficients, standard errors, and number of data points

Analyte	c_2 (mM^{-1})	σc_2	$c_2/(a^{1/b})$	c_3 (mM^{-1})	σc_3	$c_3/(a^{1/b})$	c_4 (mM^{-1})	σc_4	r	SSE	No. of points
Morphine	$6.42 \cdot 10^{-1}$	$1.23 \cdot 10^{-1}$	$3.59 \cdot 10^{-2}$	$9.70 \cdot 10^{-3}$	$1.96 \cdot 10^{-3}$	$5.43 \cdot 10^{-4}$	–		0.9959	20.8719	9
Tyrosine amide	$3.74 \cdot 10^{-1}$	$6.97 \cdot 10^{-2}$	$2.09 \cdot 10^{-2}$	$1.35 \cdot 10^{-2}$	$2.95 \cdot 10^{-3}$	$7.55 \cdot 10^{-4}$	–		0.9936	5.5716	9
Adrenaline	$6.42 \cdot 10^{-2}$	$9.47 \cdot 10^{-3}$	$3.60 \cdot 10^{-3}$	$1.05 \cdot 10^{-2}$	$1.91 \cdot 10^{-3}$	$5.88 \cdot 10^{-4}$	–		0.9964	0.1236	9
p-Toluensulfonic acid	–			–			$2.46 \cdot 10^{-2}$	$5.75 \cdot 10^{-3}$	0.9777	3.1265	9
Benzensulfonic acid	–			–			$2.46 \cdot 10^{-2}$	$5.72 \cdot 10^{-3}$	0.9779	0.2500	9

Best fit of retention data digitized from Ref. [13] with permission from Elsevier Science, by Eq. (1).

critical micelle concentration [14] and counter ions were not adsorbophilic. The constant ionic strength ruled out salting-out effects. The surface potential increase was not influenced by ionic strength, and activity coefficient ratios were almost constant. From the eluent composition f was estimated to be $1.39 \cdot 10^6$ m^2 mol^{-1} (or $8.03 \cdot 10^3$ g mol^{-1}); a and b constants and their standard deviations were found to be 5.064 ± 0.423 μmol g^{-1} mM^{-b} and 0.563 ± 0.018, respectively, with a correlation coefficient $r = 0.99850$.

The fitting parameters, which are needed to contemporaneously obtain a good fit of retention data plotted against both the mobile and surface concentration of the IIR, are reported in Tables 1 and 2, respectively. If a parameter is missing, this means that it was unreasonable to include it (e.g., a negative estimate, correlation coefficient not increased by its inclusion); that is to say, in the chromatographic system, its influence was negligible. The number of adjustable parameters was two at maximum and their numerical estimate was very reasonable in all cases. In the fitting process, c_1 or d_1 were not considered adjustable parameters, since they were readily obtained from the capacity factor without IIR in the eluent (k_0, obtained from triplicate measurements with $\pm 1.5\%$ relative standard deviations [13]). How-

ever, if they were left as fitting parameters, the errors for the predicted k would be 3.20, 0.16, 0.23, 4.79, 3.48% for morphine (mor), tyrosine amide (tyr), adrenaline (adr), p-toluensulfonic acid (ptsa), and benzensulfonic acid (bsa), respectively. The predicted values are in complete agreement with the experimental ones, if an experimental error of about 5% is considered.

The parameters c_4 and d_4 are related to adsorption competitions [2]. As regards their absence in Tables 1 and 2, for analytes oppositely charged to the IIR, it can be remarked that, for the data set used [13], the highest IIR surface concentration is 86.42 μmol/g. If the surface area of the stationary phase is taken into account (173 m^2/g) we have ca. 0.50 μmol/m^2 as the maximum IIR surface concentration. This represents ca. 10% of the ODS alkyl silyl groups bonded to the silica surface (5 μmol/m^2 [17]). It follows that, even at the maximum IIR concentration, we are far from saturation, and adsorption competitions are negligible. This means that in Eq. (1) the second term in the right-hand factor of the denominator can be neglected, and in Eq. (2) [LH] can be neglected with respect to [L]$_T$; under this hypothesis $d_1 = k_0$ [2]. It has to be underlined that while K_{HL} (c_4) is related to the total free energy change for adsorption of the lipophilic charged IIR [2], the Freundlich a

Table 2
Summary of parameter estimates, standard deviations (σ), correlation coefficients, standard errors, and number of data points

Analyte	d_2 (g μmol^{-1})$^{(1/b)}$	σd_2	d_3 (g μmol^{-1})$^{(1/b)}$	σd_3	d_4 (μmol g^{-1})	σd_4	r	SSE	No. of points
Morphine	$2.95 \cdot 10^{-2}$	$3.58 \cdot 10^{-3}$	$4.73 \cdot 10^{-4}$	$5.71 \cdot 10^{-5}$	–		0.9988	5.8886	9
Tyrosine amide	$1.80 \cdot 10^{-2}$	$9.97 \cdot 10^{-4}$	$6.72 \cdot 10^{-4}$	$4.28 \cdot 10^{-5}$	–		0.9996	0.3766	9
Adrenaline	$3.02 \cdot 10^{-3}$	$2.43 \cdot 10^{-4}$	$5.08 \cdot 10^{-4}$	$4.89 \cdot 10^{-5}$	–		0.9992	0.0327	9
p-Toluensulfonic acid	–		–		$1.42 \cdot 10^2$	$1.68 \cdot 10^1$	0.9889	1.4860	9
Benzensulfonic acid	–		–		$1.51 \cdot 10^2$	$1.46 \cdot 10^1$	0.9937	0.0716	9

Best fit of retention data digitized from Ref. [13] with permission from Elsevier Science, by Eq. (2).

constant is related only to the lipophilic chain adsorption free energy [18]. Since the electrostatic part of the free energy runs counter further IIR adsorption because the surface potential and the IIR are of like signs, a small K_{HL} does not imply a negligible a constant, hence our findings are consistent with the existence of the IIR adsorption isotherm.

We have already shown [2] that, for the data set of the work of Knox and Hartwick [7], ion pair formation in the stationary phase and adsorption competitions were negligible. On the contrary, electrostatic interactions with the charged stationary phase and ion pair formation in the mobile phase were completely able to model the retention behavior. We aim to use data taken from Ref. [13] to show how experimental conditions make one kind of interaction more important than the other. For analytes oppositely charged to the IIR electrostatic interactions, ion pair formation in the mobile and stationary phases are all important in determining the shape of the k vs. [H] plot. If only electrostatic interactions and adsorption competitions are considered, as in the electrostatic approach [8,9], a poor fit and a negative estimate of K_{HL} are obtained. The importance of ion-pair formation in the stationary phase can be probably explained by the absence of methanol in the eluent. On the contrary, ion-pair formation in the stationary phase was negligible for the already discussed [2] data set in the work of Knox and Hartwick [7]: in the latter case, K_{EHL} is obviously lowered by the higher amounts of organic modifier (20%) in the eluent. A lower surface potential (compared to that relative to the already

discussed [2] data set taken from Ref. [7], see Table 3) can also explain the higher relative importance of ion-pair formation in the stationary phase, with respect to the electrostatic interactions of the analyte with the charged stationary phase, according to equilibria 5 and 1 of Ref. [2].

The estimates of K_{EHL} and K_{EH} from the fitting of retention data, plotted as a function of the eluent or surface concentration of the IIR, are in close agreement. The parameters d_2 and d_3 compare well with the $c_2/(a^{1/b})$ and $c_3/(a^{1/b})$ ratios, as expected if adsorption competitions are negligible [2].

The graphical estimates of the relative contributions to retention arising from electrostatic attraction, ion-pairing in the eluent, and ion-pairing in the stationary phase, obtained from the fitting of retention data, allow the chromatographer to ascertain the extent to which one interaction is more important than another. This would be particularly important in chiral IIC to elucidate the enantioselective retention mechanism. Figs. 1–3 outline the plot of k vs. [LH] for morphine, tyrosine amide, and adrenaline. They detail the retention model fitted to experimental data (curve A) and individual terms (curves B, C, D) as they contribute to the retention of the sample ion. The wrong concavity of the line is evident, if only electrostatic interactions are considered (curve B). However, it is clear that the electrostatic term is the most important contribution to k. The effect of the electrostatic attraction and ion-pairing at the stationary phase is to increase k, while ion-pairing in the eluent decreases retention, as demonstrated [19]. Even if ion-pairing in the eluent occurs to only a slight extent, is important for modeling experimental

Table 3
Best fit of retention data by the log k vs. log [H] relationship

Ref.	IIR	Analyte	Slope	Intercept	σ_{slope}	$\sigma_{intercept}$	r	SSE	No. of points	Ψ^0 (mV) from Eq. (4a) of Ref. [8]	Ψ^0 (mV) from Eq. (8)	k/k_0 experimental	k/k_0 from Eq. (1)
[7], Fig. 3	Octylsulfate	Tyrosine amide	$4.74 \cdot 10^{-1}$	$5.67 \cdot 10^{-1}$	$2.60 \cdot 10^{-2}$	$1.98 \cdot 10^{-2}$	0.9911	0.009	8				
[13], Fig. 8	Octylsulfate	Normetadrenaline	$4.77 \cdot 10^{-1}$	$4.15 \cdot 10^{-1}$	$1.67 \cdot 10^{-2}$	$1.28 \cdot 10^{-2}$	0.9963	0.004	9	-89	-115	32.1	31.2
[13], Fig. 2	Butylsulfonate	Morphine	$2.84 \cdot 10^{-1}$	1.23	$5.05 \cdot 10^{-3}$	$7.24 \cdot 10^{-3}$	0.9989	6×10^{-4}	9	-30	-34	3.3	3.3
[13], Fig. 3	Butylsulfonate	Tyrosine amide	$3.29 \cdot 10^{-1}$	$7.24 \cdot 10^{-1}$	$1.00 \cdot 10^{-2}$	$1.43 \cdot 10^{-2}$	0.9968	0.002	9	-35	-34	3.9	3.9
[13], Fig. 4	Butylsulfonate	Adrenaline	$3.46 \cdot 10^{-1}$	-8.72×10^{-5}	$1.04 \cdot 10^{-2}$	$1.56 \cdot 10^{-2}$	0.9973	0.002	9	-36	-34	4.1	4.1
[13], Fig. 5	Butylsulfonate	*p*-Toluensulfonic acid	-4.29×10^{-1}	1.18	$1.70 \cdot 10^{-2}$	$2.44 \cdot 10^{-2}$	0.9945	0.007	9	-46	-34	0.2	0.3
[13], Fig. 6	Butylsulfonate	Benzensulfonic acid	-4.31×10^{-1}	$6.32 \cdot 10^{-1}$	$1.83 \cdot 10^{-2}$	$2.63 \cdot 10^{-2}$	0.9937	0.008	9	-46	-34	0.2	0.3

Summary of data sources, parameter estimates, standard deviations (σ), correlation coefficients, standard errors, number of data points, maximum surface potentials, experimental, and calculated k/k_0 ratios. Surface potentials and k are those for the highest concentration of IIR. Data were digitized from Refs. [7,13] with permission from Elsevier Science.
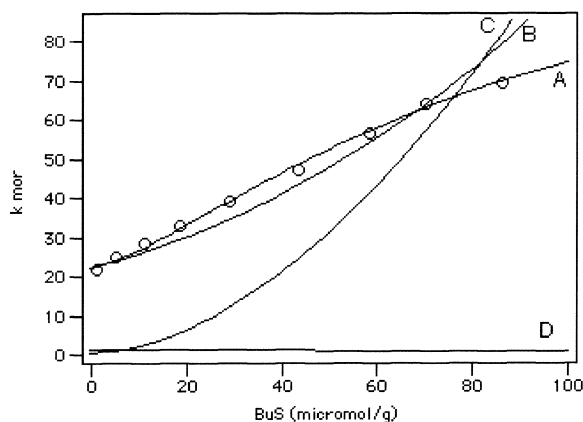
Fig. 1. Capacity factor of morphine vs. surface concentration of butylsulfate. Eq. (2) fitted to retention data (A). Contribution of electrostatic attraction (B), ion-pairing at the stationary phase (C), ion-pairing in the eluent (D) to retention. Data were digitized from Ref. [13] with permission from Elsevier Science.

Fig. 3. Capacity factor of adrenaline vs. surface concentration of butylsulfate. Eq. (2) fitted to retention data (A). Contribution of electrostatic attraction (B), ion-pairing at the stationary phase (C), ion-pairing in the eluent (D) to retention. Data were digitized from Ref. [13] with permission from Elsevier Science.

results. We have already demonstrated, for the first time, that this phenomenon is quantitatively able to explain the presence of different curves, for different IIRs, when analyte retention is plotted against the surface concentration of the IIR [2]. Instead, the electrostatic approach does predict only one curve [8]. To lend quantitative support to the present model, it should be emphasized that ion-pair formation constants in the eluent, estimated by c_3, are not
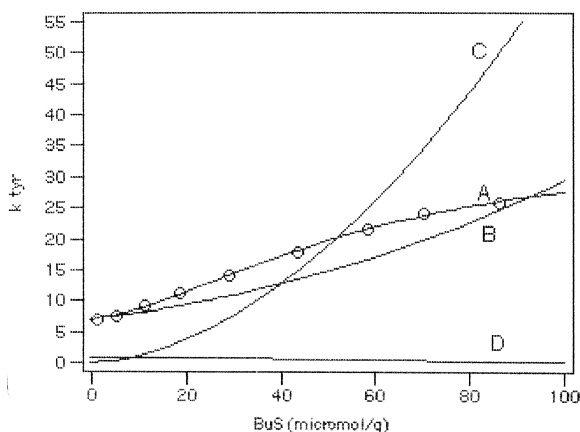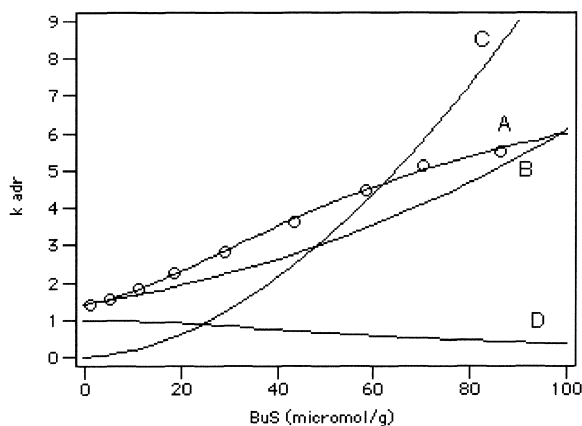

Fig. 2. Capacity factor of tyrosine amide vs. surface concentration of butylsulfate. Eq. (2) fitted to retention data (A). Contribution of electrostatic attraction (B), ion-pairing at the stationary phase (C), ion-pairing in the eluent (D) to retention. Data were digitized from Ref. [13] with permission from Elsevier Science.

very high stability constants but their values are in full agreement with those obtained by techniques other than chromatographic ones and reported in the literature for similar systems (terabutylammonium and alkylsulfonates) [20].

For analytes similarly charged to the IIR, adsorption competition phenomena must be taken into account to obtain a good fit for the experimental results. The lower potential and lower electrostatic repulsion, allow them to interact with the adsorbed IIR. The estimate of K_{HL} corresponds to $\Delta G^0 = -7.9$ KJ/mol, which is a very reasonable value for the standard free energy of adsorption of the IIR [18,21]. Moreover, the present estimate of K_{HL} for butylsulfonate, compares well with the estimate of K_{HL} for hexylsulfonate obtainable from the adsorption isotherm, under the same experimental conditions [15]. From the intercept of Fig. 7 of Ref. [15], we have: K_{HL} (hexylsulfonate) $= 0.096$ (mM)$^{-1}$, that corresponds to $\Delta G^0 = -11.3$ KJ/mol, with an increment of 1.7 KJ/mol per –CH2– with respect to butylsulfonate ΔG^0. It is in itself a very reasonable value (an increment of 1.9 KJ/mol per –CH2– is predicted from Ref. [18]), and is much more reasonable if one takes into account that a different probe is being used to obtain the estimates. The greater influence of adsorption competition on retention of similarly charged analytes, compared to oppositely

charged ones, is easily predicted. If the charge status of the IIR and the analyte is opposite, the attraction runs counter to competition phenomena. It can be confirmed [2] that adsorption competitions are obviously dependent on the analyte nature. This point begs the question of the intrinsic possibility of obtaining adsorption competition information [15] from the monolayer capacity estimated via the adsorption isotherm of the IIR. This is probably a valid procedure only if the charge status of the IIR and the analyte is the same.

It is now intended to shed light on the linear log k vs. log [H] relationship that can be derived from both stoichiometric [22,23] and thermodynamic [8,9,24,25] models suggested for IIC.

Retention models disagree on the slope of the log–log plot. The electrostatic approach that uses the linearized solution of the Poisson Boltzmann [8,9,24] does theoretically predict a slope of ± 0.5, if adsorption competitions are missing.

When analytes and IIR are oppositely charged, the log k vs. log [H] relationship (see Table 3) is almost linear for some analytes of Figs. 3 and 8 of Ref. [7] and for data taken from Ref. [13]. The slope, not surprisingly, is different than the theoretical one [8], because: (i) the potential that develops at the surface is always above 25 mV, and hence the linearized potential approximation, that is the presupposition [8] to obtain a linear log–log relationship, should not have been used [9]; (ii) ion pair equilibria should not have been neglected. Actually, Bartha and Stahalberg already remarked on the limitation of their approach as regards the use of the linearized potential approximation [24], but it is interesting that these deviations were found for low IIR concentrations. Hence, it can be inferred that they were probably related not only to item (i) but also to item (ii). Nevertheless, the practicality of their expressions is beyond dispute.

If only electrostatic interactions are taken into account, Eq. (24) of Ref. [2], from which Eq. (1) derives, reduces to the following expression, that can also be obtained from the electrostatic approach (see Eq. (4a) of Ref. [8]):

$$k = \phi[L]_T K_{LE} \frac{y_L y_E}{y_{LE}} \exp(-z_E F \Psi^0 / RT)$$

$$= c_1 \exp(-z_E F \Psi^0 / RT) \tag{4}$$

As already underlined for both the present data set and for the data set [2] taken from Ref. [7], the pure electrostatic approach, despite the use of the rigorous surface potential in Eq. (4), is not able to model experimental evidence. It follows that the linearity of the plot log k vs. log [H] does not imply that pure electrostatic interactions are operating,

The electrostatic theory predicts retention maxima and hence non-linear log–log relationship if adsorption competitions are operating [8]. At variance with this prediction, we have already demonstrated [2] that retention maxima can also be obtained because ion pair formation in the mobile phase withdraws the analyte from the stationary phase and reduces retention. Hence the rationalization offered by the electrostatic theory of the non-linearity of the plot does not always reflect the thermodynamics that solutes have undergone during separation.

For analytes of the same charge status as the IIR, the slope is similar to the theoretical one. It can be confirmed [2] from a different viewpoint, that the pure electrostatic theory works better if the analyte and the IIR are similarly charged, since in this case pairing equilibria are obviously missing. Unfortunately, most IIR applications describe how to separate analytes oppositely charged to the IIR.

According to the present extended thermodynamic retention model, the linearity of the log–log relationship is a limiting case that can be expected only:

(i) If ion pair equilibria in both the stationary and mobile phases and adsorption competitions are negligible and retention can be modeled according to Eq. (4). This is sometimes possible for analytes possessing the same charge as the IIR.

(ii) Experimental conditions must be such that the potential is quite high [3] and it can be approximated by the following expression, which can be derived from Eqs. (4)–(35) of Ref. [18] and that was already used by Deelder and Van Den Berg [26]:

$$\psi_0 = \alpha + \beta \ln [LH] \tag{5}$$

it is easily demonstrated [3,18] that:

$$\beta = -\frac{RT}{z_H F} \cdot \left(1 - \frac{1}{b}\right) \tag{6}$$

From Eqs. (4)–(6) one would obtain:

$$\log k = \log c_1 - \frac{z_E F \alpha}{RT} \log e + \frac{z_E}{z_H}(1 - 1/b) \log a$$

$$- \frac{z_E}{z_H}(1 - b) \log [H] \qquad (7)$$

This equation agrees with the general experimental evidence that the slope is usually less than unity [8,9,23,27,28], since b is usually less than unity [18]. The slope is sensitive to the charge status and charge values of both the analyte and IIR, as expected.

We decided to test Eq. (7) predictions for a data set that fulfils conditions (i) and (ii), that is the retention data of naphthalene-2-sulfonate, shown in Fig. 9 of Ref. [7], as a function of octylsulfate concentration in the eluent. From the fitting of the isotherm data for octylsulfate, a and b constants and their standard deviations were found to be 0.553 ± 0.016 μmol m^{-2} mM^{-b} and 0.435 ± 0.012, respectively, with a correlation coefficient of $r = 0.99873$. Data from Figs. 1 and 9 of Ref. [7] were used with permission from Elsevier Science. The slope resulting from the best fit of the log k vs. log [H] relationship (six points, $r = 0.9891$) is -0.579 ± 0.043. The predicted slope, according to Eq. (7) would be -0.565 ± 0.012. The agreement is very good. Again, it is noteworthy that the present model predictions reduce to predictions of other models (linearity of the log k vs. log [H] plot) under particular experimental conditions, with better agreement between expectations and experimental results. Moreover it is able to explain the great variability among experimental results.

Now let us consider yet another prediction of the electrostatic theory: the k/k_0 ratio should be independent of analyte nature if the IIR concentration and the experimental conditions are the same [8]. It is interesting to note in Table 3 that this ratio, as already observed for a different data set [29], is not constant, and that Eq. (1) is perfectly able to track variability of the k/k_0 ratio. The present theory is completely able to explain the dependence of this ratio on analyte nature. K_{EH}, K_{EHL}, are obviously dependent on the sample ion. However, K_{HL} depends on the nature of the analyte as well. The higher the effectiveness of the displacement equilibrium of H by E, the lower is the value of K_{LH} is [2]. This means that even if the analyte and the IIR are

similarly charged and ion-pairing is not operating, the k/k_0 ratio is still dependent on the nature of the analyte. The multi-site occupancy model of the electrostatic theory of ion-pair chromatography [29] implements the pure electrostatic retention model of IIC, by taking into account analyte surface requirements. It predicts that, when the surface area occupied by two analytes is the same, the ratio k/k_0 will be independent of the analyte nature, if the IIR concentration is the same. We decided to test this prediction that was not experimentally confirmed in the original paper [29]. It can be observed that for isomers of the same molecular area [23,30] experimental evidence is at variance with this theoretical prediction, hence the electrostatic theory needs an extension different from that offered by the multi-site occupancy model.

Two other points of interest are present in Table 3. First, when the surface potentials are empirically obtained, according to the electrostatic approach, from Eq. (4a) of Ref. [8], that is from the k/k_0 ratio, they are obviously very variable. Second, surface potentials empirically obtained by the electrostatic approach [8], are quite different from those calculated via the rigorous equation [2]:

$$\Psi^0 = \frac{2RT}{F} \ln \left\{ \frac{[LH]|z_H|F}{\left(8\varepsilon_0 \varepsilon_r RT \sum_i c_{0i} \right)^{1/2}} \right.$$

$$\left. + \left[\frac{([LH]z_H F)^2}{8\varepsilon_0 \varepsilon_r RT \sum_i c_{0i}} + 1 \right]^{1/2} \right\} \qquad (8)$$

that was used to obtain Eqs. (1) and (2).

Let us comment the electrostatic approach predictions. Firstly, we will focus on analytes possessing the same charge as the IIR, since pairing equilibria are obviously negligible and their thermodynamics are easier. We have found that adsorption competitions are important, for the present data set, to explain their retention behaviour. If one uses Eq. (4a) of Ref. [8] to obtain the surface potential, this means that adsorption competitions are not taken into account, hence the role they played in reducing analyte retention must be attributed to a higher electrostatic repulsion. It follows that a higher apparent surface potential is empirically estimated. For

analytes taken from the data set of Ref. [7] we demonstrated [2] that ion-pairing in the eluent decreases the effect of the electrostatic attraction, hence if ion-pairing is not taken into account, a lower apparent potential is obtained by the electrostatic approach. For analytes taken from the data set of Ref. [13], we have shown that both ion-pairing at the stationary phase and in the eluent concur to influence the effect of the electrostatic attraction. Since the former increases retention while the latter decreases it, the apparent potential is not very different from the actual one. Nevertheless ion-pairing interactions are fundamental to quantitatively explain analyte retention. We may generalize this principle by saying that, if interactions other than electrostatic ones are neglected, a surface potential that is different from the unique and real one, must be considered to explain analyte retention.

We may conclude by affirming that the electrostatic approach, which proved to be the most useful approach in the modeling of retention in IIC, is not faultless. Firstly, the electrostatic approach is not able to rationalize experimental evidence that underscores and involves complex formation. Secondly, the linearity of the log–log plot does not imply that the electrostatic approach assumptions are correct as underlined by the discrepancies between the theoretical and experimental slopes. In most cases, the linear log–log relationship of the electrostatic approach must be considered an empirical relationship without any general physical meaning but whose practicality is nevertheless very important. Thirdly, the use of a surface potential obtained from retention data, to obtain a retention equation for practical test [8], cannot compare with an ab initio, rigorous calculation of the surface potential.

The present extended thermodynamic retention model, which is consistent with fundamental physics, is able to track the versatility of IIC and to afford deep insight into the thermodynamics of IIC retention mechanism. This can be very useful to champion this separation strategy in chiral IIC.

Acknowledgements

References

[1] M.C. Gennaro, Adv. Chromatogr. 35 (1995) 343.
[2] T. Cecchi, F. Pucciarelli, P. Passamonti, Anal. Chem. 73 (11) (2001) 2632.
[3] T. Cecchi, F. Pucciarelli, P. Passamonti, Chromatographia 54 (2001) 589.
[4] T. Cecchi, F. Pucciarelli, P. Passamonti, Chromatographia 53 (2001) 27.
[5] T. Cecchi, F. Pucciarelli, P. Passamonti, J. Liq. Chromatogr. Rel. Technol. 24 (2001) 291.
[6] T. Cecchi, F. Pucciarelli, P. Passamonti, P. Cecchi, Chromatographia 54 (2001) 38.
[7] J.H. Knox, R.A. Hartwick, Mechanism of ion-pair liquid chromatography of amines, neutrals, zwitterions and acids using anionic hetaerons, J. Chromatogr. 204 (1981) 3.
[8] A. Bartha, J. Stahlberg, J. Chromatogr. A 668 (1994) 255.
[9] J. Stahlberg, J. Chromatogr. A 855 (1999) 3.
[10] F.F. Cantwell, J. Pharm. Biomed. Anal. 2 (1984) 153.
[11] J.-G. Chen, S. Weber, L.L. Glavina, F.F. Cantwell, J. Chromatogr. A 656 (1993) 549.
[12] J.J. Strahanan, S.N. Deming, Anal. Chem. 54 (1982) 2251.
[13] A. Bartha, H.A.H. Billiet, L. De Galan, Gy. Vigh, Studies in reversed-phase ion-pair chromatography III. The effect of counter ion concentration, J. Chromatogr. 291 (1984) 91.
[14] A. Bartha, Gy. Vigh, Z. Varga-Puchony, J. Chromatogr. 499 (1990) 423.
[15] J. Stahlberg, A. Bartha, J. Chromatogr. 456 (1988) 253.
[16] A. Karlsson, O. Karlsson, Chirality 9 (1997) 650.
[17] K.K. Unger, Porous Silica, Elsevier, Amsterdam, Oxford, New York, 1979.
[18] J.T. Davies, E.K. Rideal, in: Interfacial Phenomena, Academic Press, New York, London, 1961, Chapter 4.
[19] C.P. Terweij-Groen, S. Heemstra, J.C. Kraak, J. Chromatogr. 185 (1979) 225.
[20] Y. Yoshida, M. Matsui, O. Shirai, K. Maeda, S. Kihara, Anal. Chim. Acta 373 (1998) 213.
[21] M.J. Rosen, Surfactant and Interfacial Phenomena, Wiley, New York, 1978.
[22] P.J. Schoenmakerers, in: Optimization of Chromatographic Selectivity—A Guide To Method Development, Journal of Chromatography Library, Vol. 35, Elsevier, Amsterdam, 1986, p. 93.
[23] J.L.M. Van de Venne, J.L.H.M. Hendrikx, R.S. Deelder, J. Chromatogr. 167 (1978) 1.
[24] A. Bartha, J. Stahlberg, J. Chromatogr. 535 (1990) 181.
[25] F.F. Cantwell, S. Puon, Anal. Chem. 51 (1979) 623.
[26] R.S. Deelder, J.H.M. Van Den Berg, J. Chromatogr. 218 (1981) 327.
[27] H.A.H. Billiet, A.C.J.H. Drouen, L. de Galan, J. Chromatogr. 316 (1984) 231.
[28] A. Zein, M. Baerns, J. Chromatogr. Sci. 27 (1989) 249.
[29] J. Narkiewicz-Michalek, Chromatographia 35 (1993) 527.
[30] A. Bartha, J. Stahlberg, F. Szokoli, J. Chromatogr. 552 (1991) 13.