ELSEVIER

Journal of Chromatography A, 910 (2001) 187–194

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Retention of ionizable compounds on high-performance liquid chromatography
VII. Characterization of the retention of ionic solutes in a C_{18} column by mass spectrometry with electrospray ionization

Martí Rosés*, Fadoua Z. Oumada, Elisabeth Bosch

Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain

Received 17 July 2000; received in revised form 1 November 2000; accepted 24 November 2000

Abstract

The elution of ions from a C_{18} column with mobile phases containing methanol (60%, v/v) and aqueous buffers is studied by mass spectrometry. It is demonstrated that the anions are excluded from the stationary phase by the ionized silanols. However, the ionized silanols interact strongly with cations, which are retained in the column. These cations are later eluted from the column by ion exchange with the cations present in the pH buffered mobile phase. The size of the ions, the mobile phase cation concentration and the mobile phase pH are the main parameters that affect elution of the retained cations. It is also demonstrated that there are at least two different types of ionizable silanols, with different acidities, that contribute to the retention of cations. An estimate of the pK_a values of these two groups of silanols in 60% methanol is given.

Keywords: Charge exclusion; Mobile phase composition; Retention behavior; Inorganic cations; Tetrabutylammonium bromide

1. Introduction

Reversed-phase liquid chromatography is nowadays a commonplace laboratory tool widely used for the separation of many analytes. The main separation mechanism is partition of the neutral analyte between the mobile and stationary phases [1]. Stationary phases are chemically bonded reversed-phase packings, and among these the most used are long-chain alkyl compounds bonded to a silica support. Because of the residual silanol groups, these packings are in most instances charged and when ionic or ionized analytes and buffer components are present in the mobile phase, other mechanisms such as charge exclusion or ion exchange contribute significantly to retention and separation of analytes [2–8]. Nawrocki has published an excellent review about the properties of silanols and their role in liquid chromatography [8].

Ionic and ionized solutes are quite common in reversed-phase liquid chromatography separations. Mobile phase pH is a major factor for the separation of analytes with acid–base properties [1], and therefore these analytes are partially or even completely

*Corresponding author. Tel.: +34-93-4021-796; fax: +34-93-4021-233.

E-mail address: marti@apolo.qui.ub.es (M. Rosés).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)01192-4

ionized in some mobile phases. Ionic solutes are also effectively used as hold-up time markers [1,7,9–12]. In particular, in retention studies of ionizable analytes, the behavior of an ionic hold-up time marker matches that of the ionized form of the analyte much more closely than that of any other non-ionic hold-up time marker [12].

As a part of a series of papers devoted to the retention of ionizable compounds on reversed-phase liquid chromatography [12–17], we have investigated the chromatographic elution of some ionic compounds on a Merck LiChrosphere 100 RP-18 column by mass spectrometry with electrospray ionization. Mass spectrometry is a powerful detection technique that allows characterization of the elution of each individual ion present in the high-performance liquid chromatography (HPLC) mobile phase.

2. Experimental

2.1. Apparatus

The chromatograph was a Waters 2690, Alliance equipped with a 25 cm×4 mm I.D. Merck LiChrosphere 100 RP-18 (5 μm) column and a similar type 4 mm×4 mm I.D. pre-column.

The detector was a Micromass, Platform II, atmospheric pressure triple quadrupole mass spectrometer controlled with Masslynx software. The mass spectrometer conditions were: source temperature 80°C, probe voltage 4.47 kV for positive electrospray (ES+) and 3.88 kV for negative electrospray (ES−), counter electrode voltage 0.49 kV ES+ and 0.31 kV ES−, sample cone voltage 25 V ES+ and 49 V ES−, skimmer lens offset 5 V, and the mass spectrometer was scanned from m/z 0 to 500.

pH was measured with a Crison, MicropH 2002 potentiometer and an Orion 8102 Ross combined glass electrode.

2.2. Chemicals

The solvents used were methanol (Merck, for HPLC 99.9%) and water (Culligan, ultra Gs, with a resistivity of 18.3 MΩ cm). Buffers were prepared from hydrochloric acid (Merck, for analysis 25%) and sodium acetate (Merck, for analysis). The test solutes were: lithium nitrate (Prolabo, purified 99%), sodium nitrate (Probus, 99%), potassium bromide (Merck, for spectroscopy), and tetrabutylammonium bromide (TBABr) (Fluka, for analysis).

2.3. Procedure

The mobile phase used was methanol–water or aqueous buffer (60:40) filtered through a 0.22-μm nylon (MSI) membrane and degassed for 15 min by a helium stream. Mobile phase flow was 1 ml min^{-1} and a 1/20 flow splitter was used to introduce the mobile phase into the mass spectrometer.

Solutions of 0.01 mol l^{-1} of the test solutes in the same mobile phases used were prepared and filtered through 0.45-μm nylon (MSI) syringe filters. A 5-μl volume of the $LiNO_3$, $NaNO_3$ and KBr or 20 μl of the TBABr test solutions were injected into the HPLC system.

In all experiments, the column was first equilibrated with the mobile phase for at least 30 min.

3. Results and discussion

3.1. Elution of ionic solutes with ionic mobile phases

Table 1 presents the elution times obtained for KBr in methanol–water (60:40) mobile phases containing several concentrations of $NaNO_3$. When the mobile phase does not contain $NaNO_3$, the unique ions present are K^+ and Br^- from the sample. Since some silanols of the silica support of the stationary phase are ionized, Br^- is excluded from the stationary phase and it elutes at a short time. The K^+ ion is

Table 1
Elution times (min) of ions obtained from 0.01 *M* KBr with several ionic mobile phases

Mobile phase (60:40)	t_R (min)		
	K^+	Br^-	NO_3^- [a]
Methanol–water	1.42	1.39	–
Methanol–0.001 *M* $NaNO_3$	7.62	1.49	1.47
Methanol–0.002 *M* $NaNO_3$	4.74	1.50	1.50
Methanol–0.005 *M* $NaNO_3$	2.98	1.63	1.62

[a] Vacancy peaks.

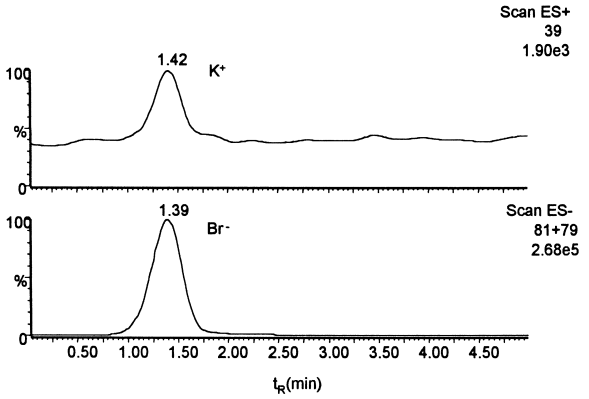

Fig. 1. Chromatograms for all ions eluted for 5 μl of 0.01 *M* KBr in a C_{18} column with a methanol–water (60:40) mobile phase.

eluted at the same time to keep the electroneutrality of the mobile phase (Fig. 1 and Table 1).

However, the elution of KBr with mobile phases containing $NaNO_3$ is more complex. The ionized silanols retain Na^+ as counterion, which can be exchanged by K^+ from the sample. In this instance, the elution of both ions of the test solute occur at different times (Fig. 2). The anion of the test solute (Br^-) is eluted at a short time because of the charge exclusion from the ionized silanol groups. At the same time a vacancy peak is observed for the NO_3^- ion of the mobile phase buffer, which contributes to maintain the electroneutrality of the mobile phase. The cation of the test solute (K^+) is retained by the negatively charged ionized silanol groups and it is eluted later than the anion. The retention of K^+ is likely accompanied by elution of Na^+ ions previously retained as counterions by the ionized silanols, and when K^+ is eluted there should be a decrease in the mobile phase Na^+ concentration. However, this is almost unnoticed in the chromatogram because of the large amount of Na^+ present in the mobile phase and the large retention of K^+ which widens the peaks.

The elution time of the Br^- ion slightly increases with the concentration of the buffer because the increase in the ionic strength of the mobile phase minimizes charge exclusion effects.

Retention of K^+ cations decreases more or less exponentially with the buffer concentration. This behavior can be explained by an ion exchange equilibria between the buffer cation (Na^+) retained

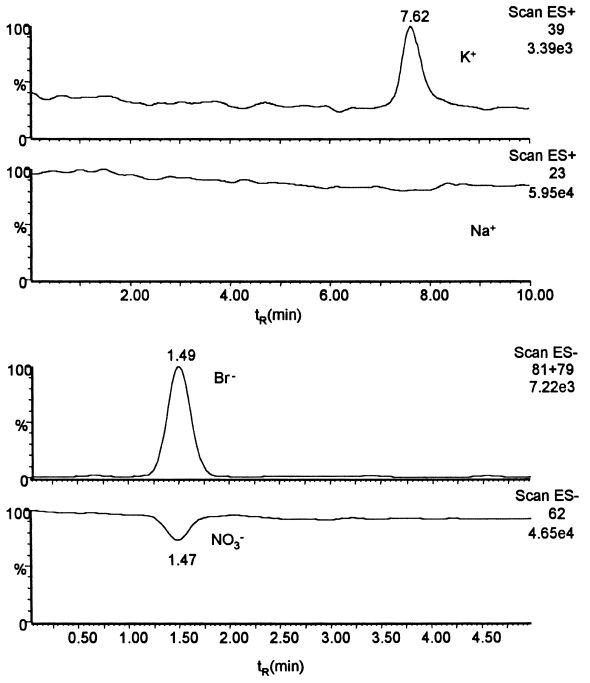

Fig. 2. Chromatograms for all ions eluted for 5 μl 0.01 *M* KBr in a C_{18} column with a methanol–0.001 *M* $NaNO_3$ (60:40) mobile phase.

by the silanol group and the solute cation (K^+) [2,8,18]:

$$R_3Si{-}O^-Na^+ + K^+ \Leftrightarrow R_3Si{-}O^-K^+ + Na^+$$

This equilibrium is ruled by the selectivity coefficient of K^+ in reference to Na^+ ($K_{Na^+}^{K^+}$) according to the following equation, where the subscripts S and M indicate concentrations in the stationary and mobile phases, respectively:

$$K_{Na^+}^{K^+} = \frac{[K^+]_S[Na^+]_M}{[Na^+]_S[K^+]_M} \quad (1)$$

The retention factor (k) of K^+ ion can be related with the selectivity coefficient through the following:

$$k = [K^+]_S V_S / [K^+]_M V_M$$
$$= K_{Na^+}^{K^+} V_S [Na^+]_S / V_M [Na^+]_M \quad (2)$$

where V_S and V_M are the volumes of mobile and stationary phase, respectively. Since the K^+ concentration is much lower than the Na^+ concentration,

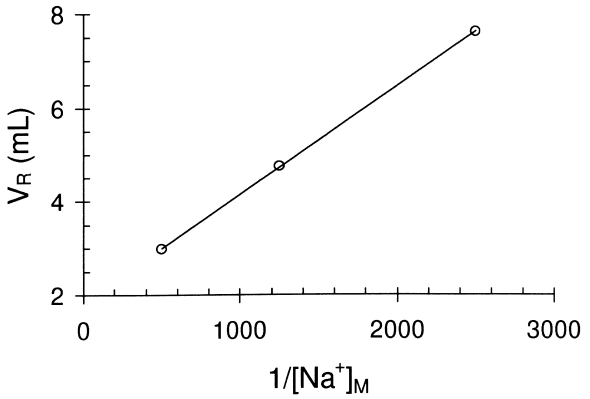

Fig. 3. Variation of the retention volume of K^+ in a C_{18} column with the concentration of Na^+ in the mobile phase methanol–$NaNO_3$ (60:40).

$[Na^+]_S$ is constant and Eq. (2) predicts a proportionality between the retention factor of K^+ and the reverse of the Na^+ concentration in the mobile phase.

Retention factor is related to the retention volume (V_R) through the mobile phase volume according to:

$$k = (V_R - V_M)/V_M \quad (3)$$

and the retention volume of KBr with mobile phases containing $NaNO_3$ should be linearly related to the reverse of the Na^+ concentration in the mobile phase (Fig. 3) [2,3,8]:

$$V_R = V_M + K^{K^+}_{Na^+} V_S [Na^+]_S [Na^+]_M{}^{-1} \quad (4)$$

3.2. Elution of ionic solutes with pH buffered mobile phases

The most common mobile phases in reversed-phase liquid chromatography contain aqueous pH buffers, that keep the mobile phase pH close to a certain value. These buffers are also ionic solutions that participate in charge exclusion and ion exchange processes with the stationary phase. We have studied the behavior of three test solutes ($LiNO_3$, KBr, and TBABr) with mobile phases buffered with equimolar mixtures of acetic acid (HAc) and sodium acetate (NaAc). Namely, the buffers prepared were $5\cdot10^{-4}$ *M* HAc + $5\cdot10^{-4}$ *M* NaAc, $1\cdot10^{-3}$ *M* HAc + $1\cdot10^{-3}$ *M* NaAc, $2\cdot10^{-3}$ *M* HAc + $2\cdot10^{-3}$ *M* NaAc, $5\cdot10^{-3}$ *M* HAc + $5\cdot10^{-3}$ *M* NaAc. These aqueous buffers were later mixed with methanol to obtain the 60:40 methanol–aqueous buffer mobile phase, and the test solutes were injected, after equilibration of the column with the mobile phase. The results obtained are presented in Table 2, including the elution times for the ions of the test solutes with unbuffered methanol–water (60:40), and they are similar to those obtained for KBr with $NaNO_3$ in the mobile phase.

For unbuffered mobile phases, all ions elute at the same time (1.40±0.02 min) because ion exchange effects are not an influence. Also the principle of electroneutrality requires the cation and the anion of the test solute to be eluted at the same time.

However, for pH buffered mobile phases the elution of both ions of the test solute is at different times (Fig. 4). The anion of the test solute (NO_3^- in

Table 2
Elution times (min) of ions with mobile phases prepared with methanol (60%) and equimolar mixtures of acetic acid and sodium acetate aqueous buffers (40%)

Buffer[a] (*M*)	Solute									
	$LiNO_3$				KBr			TBABr		
	Li^+	NO_3^-	Na^+[b]	Ac^-[b]	K^+	Br^-	Ac^-[b]	TBA^+	Br^-	Ac^-[b]
0.000	1.40	1.40	–	–	1.42	1.39	–	1.38	1.38	–
0.001	11.19	1.42	–	1.42	16.32	1.42	1.42	–	1.42	1.42
0.002	7.37	1.46	–	1.47	9.30	1.46	1.47	44.85	1.46	1.46
0.004	4.65	1.50	–	1.51	6.50	1.51	1.50	27.96	1.49	1.51
0.010	2.94	1.62	2.89	1.62	3.85	1.62	1.60	11.17	1.62	1.62

[a] Overall aqueous buffer concentration of sodium acetate + acetic acid.
[b] Vacancy peaks.

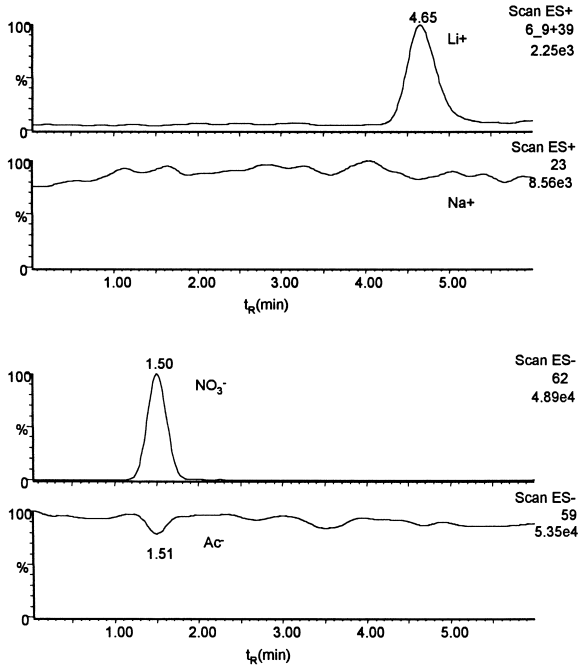

Fig. 4. Chromatograms for all ions eluted for 5 μl 0.01 *M* $LiNO_3$ in a C_{18} column with a methanol–(0.002 *M* HAc + 0.002 *M* NaAc) (60:40) mobile phase.

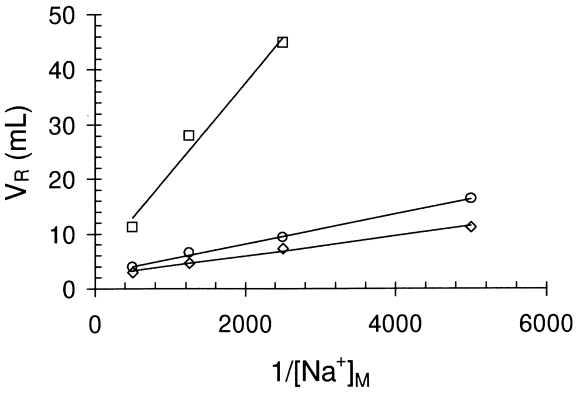

Fig. 5. Variation of the retention volume of several ions in a C_{18} column with the concentration of Na^+ in mobile phases prepared from methanol (60%, v/v) and aqueous equimolar mixtures of acetic acid and sodium acetate. Ions: (◊) Li^+, (○) K^+, (□) TBA^+.

Fig. 4) is eluted at a short time because of the charge exclusion from the ionized silanol groups, and a vacancy peak is observed for the Ac^- ion of the mobile phase buffer at the same elution time. The cation of the test solute (Li^+ in Fig. 4) is retained by the negatively charged ionized silanol groups and it is eluted later than the anion.

Fig. 5 presents the retention volumes of the cations of the test solutes against the reverse of the Na^+ concentration in the mobile phase. Straight lines are obtained for the three cations, Li^+, K^+, and TBA^+, which shows that the retention mechanism is by ion exchange, as in the case of K^+ with $NaNO_3$ buffers (Eq. (4)). The slopes of the lines increase in the order of the ion sizes: $Li^+ < K^+ < TBA^+$, which indicates that the selectivity coefficient also increases in this order.

3.3. Ionization of the silanol groups with the pH of the mobile phase

The charge exclusion and ionic exchange effects observed with mobile phase acetate buffers depend on two different characteristics of the mobile phase. On one hand, the type and concentration of the ions present in the mobile phase, which interact with the ionized silanol groups. On the other hand, the pH of the mobile phase which ionizes in a certain degree the silanol groups of the silica support [2,8]. The latter factor has been characterized by measuring the elution time of $LiNO_3$ in several buffers of different pH value, but constant concentration of Na^+ in the mobile phase.

The pH has been measured in three different scales, already discussed for methanol–water mobile phases [15]. The first scale is aqueous pH, measured in the aqueous buffer before mixing it with methanol ($^{w}_{w}$pH). The second scale is pH in methanol–water but referred to water as standard state for the ionic activity coefficients ($^{s}_{w}$pH). In practice, this scale agrees with the pH measured in the mobile phase with the pH electrode system calibrated with aqueous buffers [15]. The third pH scale is pH measured in methanol–water and referred to methanol–water as standard state ($^{s}_{s}$pH), which agrees with calibration of the pH electrode system with buffers prepared in methanol–water. The latter two scales are more rigorous than the $^{w}_{w}$pH scale and they can be easily related through the constant δ term, which for 60% of methanol in volume is equal to 0.17 [15,17]. In the calculations we have used the rigorous $^{s}_{s}$pH scale.

The buffers have been prepared from a solution of sodium acetate by addition of concentrated hydrochloric acid until the desired pH. This procedure keeps the Na^+ concentration of the mobile phase constant, and therefore the retention of Li^+ depends only on the ionization of the silanol groups. Typical chromatograms for all ions involved are presented in Fig. 6. NO_3^- is eluted at a short retention time because of charge exclusion and at the same time vacancy peaks are obtained for the Ac^- and Cl^- ions of the buffer. Li^+ is retained by the ionized silanol groups and it elutes at a larger time, and at the same time a vacancy peak is observed for the Na^+ ion of the buffer. The retention of Li^+ increases with the increase of the pH of the mobile phase because of the increase in ionization of the silanol groups. The results obtained are presented in Table 3.

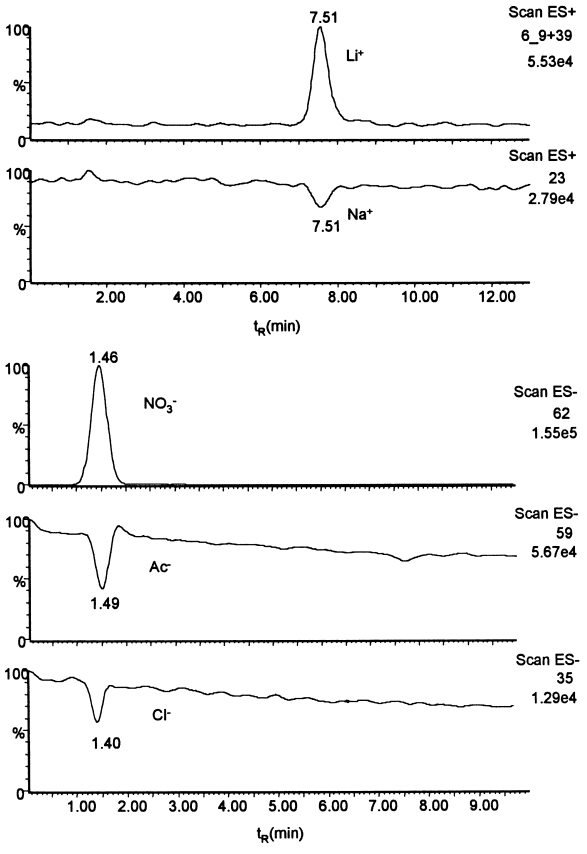

Fig. 6. Chromatograms for all ions eluted for 5 μl 0.01 *M* $LiNO_3$ in a C_{18} column with methanol–(0.001 *M* NaAc+HCl to ${}^{w}_{w}$pH 4.51) (60:40) mobile phase of ${}^{s}_{s}$pH 5.51.

Table 3
Elution times (min) of ions from $LiNO_3$ with mobile phases prepared with methanol (60%) and mixtures of sodium acetate and hydrochloric acid (40%) at several pH values

pH scale			t_R (min)				
${}^{w}_{w}$pH	${}^{s}_{w}$pH	${}^{s}_{s}$pH	Li^+	NO_3^-	Na^{+a}	Ac^{-a}	Cl^{-a}
2.94	3.40	3.23	4.32	1.53	4.28	–	1.51
3.41	4.02	3.85	5.83	1.47	5.79	–	1.51
4.03	5.08	4.91	6.87	1.47	6.85	1.56	1.56
4.51	5.68	5.51	7.51	1.46	7.51	1.49	1.40
5.02	6.15	5.98	7.62	1.46	7.64	1.45	1.42
5.45	6.38	6.21	8.03	1.46	7.97	1.46	–
6.02	6.88	6.71	8.06	1.46	8.06	1.47	–
6.71	7.07	6.90	8.16	1.46	8.17	1.46	–

[a] Vacancy peaks.

The retention of Li^+ is caused by ion exchange with Na^+ and therefore an equation similar to Eq. (2) should apply to it. This equation can be written as follows:

$$k = K_{Na^+}^{Li^+} n_{Na(S)} / n_{Na(M)} \quad (5)$$

where $n_{Na(S)} = V_S[Na^+]_S$, and $n_{Na(M)} = V_M[Na^+]_M$. They are the number of mols of sodium ion retained in the stationary phase and in the mobile phase, respectively. This number equals to the number of mols of ionized silanol groups, which depends on the overall number of silanol groups (n_{SiOH}) and on the degree of ionization of them (α):

$$k = K_{Na^+}^{Li^+} \alpha n_{SiOH} / n_{Na(M)} \quad (6)$$

The degree of ionization is related with the concentration of H^+ in the mobile phase and the ionization constant (K_a) of silanol through the following equation:

$$\alpha = \frac{K_a}{K_a + [H^+]} = \frac{1}{1 + 10^{pK_a - pH}} \quad (7)$$

From Eqs. (6) and (7), we obtain the equation:

$$k = \frac{K_{Na^+}^{Li^+} n_{SiOH}}{n_{Na(M)}} \cdot \left(\frac{K_a}{K_a + [H^+]} \right) \quad (8)$$

The equation can be linearized to the form:

$$k^{-1} = \frac{n_{\mathrm{Na(M)}}}{K_{\mathrm{Na^+}}^{\mathrm{Li^+}} n_{\mathrm{SiOH}}} + \frac{n_{\mathrm{Na(M)}}}{K_{\mathrm{Na^+}}^{\mathrm{Li^+}} K_a n_{\mathrm{SiOH}}} \cdot [\mathrm{H^+}] \quad (9)$$

We have checked this equation by calculation of the retention factors by Eq. (3) using a value of 1.47 min as estimation of the hold-up time, which is the elution time (Table 3) of the anion marker, NO_3^-, excluded from the stationary phase. Fig. 7 shows two straight lines, which suggest that at least two different ionization equilibria of silanol groups are needed to explain the retention of Li^+ ions. Therefore, the model has to be modified to include two different degrees of ionization (α_1 and α_2) that come from two different pK_a values (pK_{a1} and pK_{a2}) corresponding to two structurally different silanol groups. The overall number of mols of each silanol type will be indicated by n_1 and n_2, with $n_{\mathrm{SiOH}} = n_1 + n_2$:

$$k = \frac{K_{\mathrm{Na^+}}^{\mathrm{Li^+}}}{n_{\mathrm{Na(M)}}} \cdot (n_1\alpha_1 + n_2\alpha_2) \quad (10)$$

$$k = A \cdot \left(\frac{1}{1 + 10^{\mathrm{p}K_{a1} - \mathrm{pH}}} + \frac{n_2/n_1}{1 + 10^{\mathrm{p}K_{a2} - \mathrm{pH}}} \right) \quad (11)$$

with

$$A = \frac{K_{\mathrm{Na^+}}^{\mathrm{Li^+}} n_1}{n_{\mathrm{Na(M)}}} \quad (12)$$

The four parameters, A, pK_{a1}, pK_{a2}, and n_2/n_1 of Eq. (11) have been estimated by non-linear regression from the k and ${}^{\mathrm{s}}_{\mathrm{s}}$pH data and we have obtained

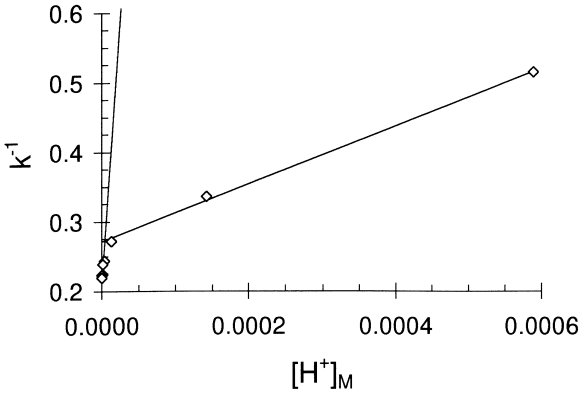

Fig. 7. Variation of the retention factor of Li^+ with the hydrogen ion concentration of the methanol–(0.001 *M* NaAc+HCl) (60:40) mobile phase.

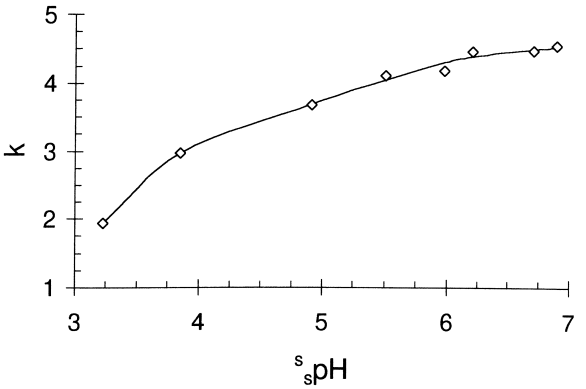

Fig. 8. Variation of the retention factor of Li^+ with the pH of the methanol–(0.001 *M* NaAc+HCl) (60:40) mobile phase. Line calculated according to the model with two ionization constants for the silanol groups (see text for details).

$A = 3.53$, p$K_{a1} = 3.15$, p$K_{a2} = 5.49$, and $n_2/n_1 = 0.29$. Fig. 8 shows the fit obtained with these parameters to the experimental data.

The presence of different types of silanols (single, geminal, vicinal) with different acidities has been discussed in detail by Nawrocki [8]. The concentration and acidity of the different types of silanol seems to be very dependent on the origin and thermal treatment of the silica base [3,8,19]. Our results indicate that about 80% of the residual silanols of LiChrosphere 100 RP-18 are very acidic, with a pK_a in 60% methanol of about 3.2, which is similar to the first pK_a of phosphoric acid, whereas that about a 20% of the silanols are less acidic, with a pK_a of about 5.5 in 60% methanol which is close to that of acetic or benzoic acids [12]. These results are somewhat surprising because the average pK_a of silanol groups is generally accepted to be around 7, and although it is also well accepted that some silanols can have a pK_a of less than 3 [8,20], most workers believe that these high-acidity silanols are only a small proportion of the total silanols. We do not have yet any explanation for the presence of this large proportion of high-acidity silanols in the LiChrosphere column, although they are likely isolated non-hydrogen-bonded silanols, which acidity may have been considerably enhanced by metal impurities [20,21]. In fact, the explanation of the retention of Li^+ as a function of pH in terms of only two pK_a values is only an approximation of reality. The

residual silanols on the silica support probably have a continuum of pK_a values in the pH range specified by the two pK_a values. Work is in progress in our lab to confirm the acidity of the silanols by other techniques and to extend the HPLC study to other columns and supports.

4. Conclusions

The elution of ions in reversed-phase liquid chromatography with C_{18} columns is mostly ruled by charge exclusion of the sample anions from the ionized silanol groups of the silica support and ionic exchange between the sample cations and the mobile phase buffer cations retained by the ionized silanol groups.

The peak elution of the sample cations is accompanied by vacancy peaks of the ions of the same type of charge from the mobile phase buffer. This mechanism keeps the electroneutrality of the mobile phase.

Sample cation size and mobile phase cation concentration and pH are relevant parameters that affect elution of sample cations. The larger the sample cation size, the larger its retention. An increase in the mobile phase cation concentration decreases retention of sample cation. There is a linear relationship, derived from ion exchange models, between the retention time of the sample cation and the reverse of the cation concentration in the mobile phase. An increase in the mobile phase pH increases retention of sample cations because it favors ionization of the acidic silanol groups of the column. However, at least two different types of silanol groups with different acidity are needed to explain the retention behavior of sample cations in C_{18} columns with methanol–water (60:40) mobile phase.

Mass spectrometry has been proved to be a very useful technique to characterize the interactions between the different ions and the column because it allows specific detection of the different ions involved.

Acknowledgements

The financial support from the DGICYT of the Spanish Government (projects PB97-0878 and PB98-1257) and from the Catalan Government (Grant 1999SGR00047) is gratefully acknowledged. We also thank the Scientific and Technical Service of the University of Barcelona for allowing us to carry out the experiments in their facilities, and in particular the helpful assistance from Dr. Isidre Casals. F.Z.O. work was supported by a grant from the Agencia Española de Cooperación Internacional.

References

[1] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, Amsterdam, 1991.
[2] R.W. Stout, G.B. Cox, T.J. Odiorne, Chromatographia 24 (1987) 602.
[3] G.B. Cox, R.W. Stout, J. Chromatogr. 384 (1987) 315.
[4] B.A. Bildingmeyer, J.K. Del Rios, J. Korpi, Anal. Chem. 54 (1982) 442.
[5] O.A.G.J. van der Houwen, R.H.A. Sorel, A. Hulshoff, J. Teeuwsen, A.W.M. Indemans, J. Chromatogr. 209 (1981) 393.
[6] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 183.
[7] O.A.G.J. van der Houwen, J.A.A. van der Linden, A.W.M. Indemans, J. Liq. Chromatogr. 5 (1982) 2321.
[8] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[9] G.E. Berendsen, P.J. Schoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony, J. Inczédy, J. Liq. Chromatogr. 3 (1980) 1669.
[10] K. Jinno, N. Ozaki, T. Sato, Chromatographia 17 (1983) 341.
[11] L.G. Daignault, D.C. Jackman, D.P. Rillema, Chromatographia 27 (1989) 156.
[12] M. Rosés, I. Canals, H. Allemann, K. Siigur, E. Bosch, Anal. Chem. 68 (1996) 4094.
[13] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[14] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.
[15] I. Canals, J.A. Portal, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 1802.
[16] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 5193.
[17] I. Canals, F.Z. Oumada, M. Rosés, E. Bosch, J. Chromatogr. A, submitted for publication.
[18] R.L. Smith, D.J. Pietrzyk, Anal. Chem. 56 (1984) 610.
[19] Y. Bereznitski, M. Jaroniec, J. Chromatogr. A 828 (1998) 51.
[20] D.V. McCalley, LC·GC Eur. 12 (1999) 638.
[21] G.B. Cox, J. Chromatogr. A 656 (1993) 353.