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Comparison of the acidity of residual silanol groups in several liquid chromatography columns

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Abstract

The silanol acidity of Waters Resolve C_{18}, Waters Resolve silica, Waters Symmetry C_{18}, Waters Symmetry silica, Waters XTerra MS C_{18} and underivatized XTerra columns has been measured from the retention of $LiNO_3$ with a methanol/water (60:40) mobile phase buffered to different pH values. The Li^+ cation is retained by cationic exchange with the background cation of the mobile phase (Na^+) through the ionized silanols. The number of active silanols increases in the order: XTerra MS $C_{18} \ll$ Symmetry $C_{18} <$ underivatized XTerra $\ll$ Resolve $C_{18} <$ Resolve silica $\approx$ Symmetry silica. XTerra MS C_{18} does not present any residual silanol acidity up to ${}^{s}_{s}$pH 10.0 (pH in 60% methanol) as measured by $LiNO_3$. The underivatized XTerra packing and Symmetry C_{18} present active silanols only at ${}^{s}_{s}$pH values higher than 7.0. For the other three columns, two different types of silanols with different acidity (${}^{s}_{s}pK_a$ values about 3.5–4.6 and 6.2–6.8, respectively) have been observed. Symmetry C_{18} shows evidence of the presence of active basic sites that retain NO_3^- by anionic exchange.

Keywords: Silanol activity; Stationary phases, LC; Charge exclusion; Ion exchange; Acidity; Dissociation constants; Silica columns; Silica, *n*-octadecyl bonded

1. Introduction

Reversed-phase liquid chromatography (RPLC) is an analytical technique widely used for biomedical, pharmaceutical and environmental separations. The main separation mechanism is partition of the analyte between the mobile and stationary phases, which mostly depends on the properties of the analyte, pH and composition of the mobile phase and type of stationary phase. However, the packing of this stationary phase and the support employed play an important separation role too. In addition to partition, hydrophobic interactions, steric interactions, ion exchange, charge exclusion and hydrogen bonding are the most common types of interactions with the support that may significantly contribute to retention and separation.

Silica is the most used support for RPLC stationary phases because of its versatility [1–3]. Organic phases can be easily bonded to silica microparticles [4], which have good mechanical stability, allow an easy control of its properties and thus give high

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column efficiencies. However, silica supports have some disadvantages, specially in the analysis of basic compounds because of the strong interaction between base and support.

In the chromatographic elution of bases, asymmetric peaks, low column efficiency, poor reproducibility and retentions larger than expected are often observed. These effects are attributed to interactions with the silica support, rather than with the bonded phase [5–7]. The basic analyte may interact strongly with the acidic residual silanols of the support. Metal impurities also increase the acidity of the residual silanols and thus enhance the silanol interactions with basic analytes [8–10]. There are different types of silanol groups (vicinal, geminal or isolated) in the silica surface [4] that may have different acidity and may interact to a different degree with basic analytes.

Many studies have been carried out to block, remove or decrease the number of residual silanols in the silica surface [2,4,11,12]. The most common process is column endcapping. Endcapping produces an important decrease of the number of surface silanols, although it does not completely remove them [1,2,4,12].

Recently, a new generation of less acidic hybrid silica supports has been developed. These packings have silica–methylsilane hybrid surfaces with a lower number of free silanols. Also, basic mobile phases hydrolyse traditional silica surfaces and release a high number of silanols, whereas in these hybrid silicas, the released silanol groups are sterically and hydrophobically hindered by the methyl groups which prevents further attack of the silica surface by the basic mobile phase.

The study of the solute–silica interactions has led to the development of different characterization tests. Many tests are based on the retention of amines, which usually have an aromatic part that allows UV detection [3]. However, these compounds interact not only with the residual silanols by ionic exchange, but also with the bonded phase by hydrophobic interaction.

The change of the retention of lithium ions with the pH of the mobile phase that contains a constant concentration of sodium ions is a good method to determine the activity and acidity of the residual silanols [13]. Lithium is not retained by the organic bonded phase because of its small size and its charge.

In this paper, we compare the results obtained with this test for three Waters C_{18} columns with different silica supports and for the three corresponding unbonded packings. The main characteristics of the columns studied are given in Table 1. Resolve C_{18} is a non-endcapped column based on an older, less pure silica packing. Symmetry C_{18} is an endcapped C_{18} stationary phase bonded to a modern high-purity silica [2,4]. XTerra MS C_{18} is also an endcapped column, but the C_{18} stationary phase is bonded to a new generation packing based on a silicon inorganic–organic hybrid, which is expected to have a much lower residual silanol activity and can be used over an extended pH range [10].

Table 1
Physical characteristics of the waters columns studied

	Resolve C_{18}	Resolve silica	Symmetry C_{18}	Symmetry silica	XTerra MS C_{18}	Underivatized XTerra
Particle shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
Particle size (μm)	5	5	5	5	5	5
Pore size (ϕ) (Å)	90[a]	90	86[a]	90	125[a]	123
Surface area (m^2/g)	200[a]	200	346[a]	341	175[a]	169
Pore volume (ml/g)	0.50[a]	0.50	0.90[a]	0.90	0.70[a]	0.65
% Carbon load	10.2	0	19.6	0	15.5	6.8
Endcapped	No	No	Yes	No	Yes	No
Column length (mm)	150	150	150	150	150	150
Column diameter(mm)	3.9	3.9	4.6	4.6	4.6	4.6
pH range	2–8	2–8	2–8	2–8	1–12	1–12

[a] Data for the packings before derivatization.

2. Theory

2.1. Cation exchange

A silica surface, which has silanol groups partially or totally ionized, behaves as an ion exchanger. If the mobile phase contains A^+ as the unique cation of the background electrolyte, A^+ ions are retained by the ionized silanols (R_3SiO^-) and when a cationic analyte B^+ is introduced into the column, the following ion-exchange equilibrium should be observed:

$$R_3SiO^- A^+ + B^+ \Leftrightarrow R_3SiO^- B^+ + A^+$$

This equilibrium is ruled by the selectivity coefficient of B^+ in reference to A^+ ($K_{A^+}^{B^+}$), which is defined by Eq. (1):

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

where M and S subscripts refer to mobile and stationary phase, respectively.

The retention factor of B^+ ions can be related to the selectivity coefficient through the following equation where V_M and V_S are the volumes of mobile and stationary phase respectively:

$$k = \frac{[B^+]_S V_S}{[B^+]_M V_M} \quad (2)$$

Replacement of Eq. (1) into (2) gives

$$k = K_{A^+}^{B^+} \frac{[A^+]_S V_S}{[A^+]_M V_M} = K_{A^+}^{B^+} \frac{n_{A(S)}}{n_{A(M)}} \quad (3)$$

$n_{A(S)}$ and $n_{A(M)}$ are the number of A^+ ions in the stationary and mobile phase, respectively. Since $[A^+]_M \gg [B^+]_M$, $n_{A(S)}$ is equivalent to the number of silanol groups ionized and this depends on the overall number of silanols and on the degree of ionization of these silanols. In the previous work [13], it was first assumed that only one type of silanols was present in the silica surface. It was later demonstrated that at least two types of silanols with different acidities were present and the model was modified to account for these two types of silanols. Since there may be more than two types of silanols present, we shall consider here a general model for m different types of silanols. In this instance

$$n_{A(S)} = \sum_{i=1}^{m} n_i \alpha_i \quad (4)$$

where n_i is the overall number of silanols of type i and α_i is the degree of ionization of these silanols, which is related to the acidity of the particular type of silanols (pK_{ai}) and to the pH of the mobile phase through:

$$\alpha_i = \frac{K_{ai}}{K_{ai} + [H^+]} = \frac{1}{1 + 10^{pK_{ai} - pH}} \quad (5)$$

Eq. (3) can be then written as

$$k = \frac{K_{A^+}^{B^+}}{n_{A(M)}} \sum_{i=1}^{m} n_i \alpha_i \quad (6)$$

After replacement of Eq. (5) in (6) and rearrangement of terms, Eq. (7) that relates the retention of B^+ ion to the pH of the mobile phase is obtained.

$$k = \frac{K_{A^+}^{B^+} n_1}{n_{A(M)}} \sum_{i=1}^{m} \left(\frac{n_i / n_1}{1 + 10^{pK_{ai} - pH}} \right) \quad (7)$$

The retention factor of B^+ is calculated by the well-known relationship

$$k = \frac{t_R - t_M}{t_M} \quad (8)$$

where t_R is the retention time of B^+ and t_M the elution time of an appropriate hold-up time marker. However, due to the possible ion exclusion of markers of the same charge as the surface and to the retention of markers of the opposite charge, we use the retention time instead of the retention factor in the current study. Then, t_M can be a parameter measured in appropriate conditions (mobile phase pH) or fitted in the final equation. Both approaches have been used in this work.

Since $n_{A(M)}$ can be also related to t_M through the mobile phase flow-rate in the column (F_C) and the concentration of A^+ ion in the mobile phase

$$n_{A(M)} = [A^+]_M V_M = [A^+]_M t_M F_C \quad (9)$$

the following Eq. (10) that directly relates the retention time of the B^+ ion to the pH of the mobile phase is obtained:

$$t_R = t_M + \frac{K_{A^+}^{B^+} n_1}{[A^+]_M F_C} \sum_{i=1}^{m} \left(\frac{n_i/n_1}{1 + 10^{pK_{ai} - pH}} \right) \quad (10)$$

It must be noted that the degree of ionization of the silanols depends on the pK_a values of the different silanols in the particular mobile phase used and on the pH of this mobile phase. pK_a and pH values are solvent dependent parameters, and they change with the composition of the mobile phase. Some of us have widely discussed pH measurement in RPLC mobile phases [14–16] and recommend the use of the ^s_wpH and ^s_spH scales. The ^s_wpH value can be easily measured in the particular mobile phase after mixing aqueous buffer and organic modifier with an electrode system calibrated with the usual aqueous buffers. The ^s_spH value can then be obtained from the ^s_wpH by means of Eq. (11), where $\delta = 0.17$ for 60% methanol [14,16].

$$^s_s pH = {}^s_w pH - \delta \quad (11)$$

The acidity (pK_{ai}) values and the ratio of the different types of silanols (n_i/n_1), as well as the $K_{A^+}^{B^+} n_1/[A^+]_M F_C$ term, of Eq. (10) are obtained by non-linear regression from the retention times of B^+ and the measured pH values of the mobile phase. The pK_{ai} values obtained would be $^s_w pK_{ai}$ or $^s_s pK_{ai}$ depending on the pH values used (^s_wpH or ^s_spH, respectively).

2.2. Anion exchange

While ionization of acidic silanols leads to ion-exchange effects with sample cations, sample anions may also undergo retention, if protonated sites exist on the column surface. These protonated sites (RH^+) may act as anion exchangers between the anion of the background electrolyte (A^-) and the anions of the sample (B^-)

$$RH^+ A^- + B^- \Leftrightarrow RH^+ B^- + A^-$$

This equilibrium is governed by a selectivity coefficient $K_{A^-}^{B^-}$, equivalent to that of Eq. (1) for cationic exchange,

$$K_{A^-}^{B^-} = \frac{[B^-]_S [A^-]_M}{[A^-]_S [B^-]_M} \quad (12)$$

As in Eq. (3), the retention factor of B^- can be given as

$$k = K_{A^-}^{B^-} \frac{n_{A(S)}}{n_{A(M)}} \quad (13)$$

Since there may be more than one type of protonated sites in the stationary phase, Eq. (4) can be applied, but taking into account that

$$\alpha_i = \frac{[H^+]}{K_{ai} + [H^+]} = \frac{1}{1 + 10^{pH - pK_{ai}}} \quad (14)$$

Following the same steps described for cation exchange (Section 2.1), Eq. (15) is obtained.

$$t_R = t_M + \frac{K_{A^-}^{B^-} n_1}{[A^-]_M F_C} \sum_{i=1}^{m} \left(\frac{n_i/n_1}{1 + 10^{pH - pK_{ai}}} \right) \quad (15)$$

Notice that this equation is identical to Eq. (10) except for the $10^{pH - pK_{ai}}$ term, which has the opposite sign in the exponent.

3. Experimental

3.1. Apparatus

Two different equipment assemblies were used. One was a Waters 2690 Alliance chromatograph with a Micromass Platform II atmospheric pressure single quadrupole mass spectrometer controlled with Masslynx software. The mass spectrometer conditions were: source temperature 80 °C, probe voltage 4.5 kV for positive electrospray (ES+) and 3.9 kV for negative electrospray (ES−), counter electrode voltage 0.5 kV ES+ and 0.3 kV ES−, sample cone voltage 35 V ES+ and 50 V ES−, and skimmer lens offset 5 V. The masses were scanned from m/z 4 to 500 with a scantime of 1 s and an interscan time of 0.1 s. The second assembly was an ISCO Model 2350 dual-pump system with a 10 μl loop valve with an Ω Metrohm 690 ion conductivity detector. In this system, data were acquired with the ISCO Chem-Research data management program. The columns used are described in Table 1. C_{18} columns were used with precolumns of the same type (3.9×20 mm), but underivatized columns were used without precolumn.

Extracolumn times were measured with the same

assembly by replacing column and precolumn by a zero-volume connection.

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

3.2. Chemicals

The solvents used were methanol (Merck, for HPLC 99.9%) and water (Milli-Q plus from Millipore, with a conductivity lower than 0.05 μS cm^{-1}. Buffers were prepared from hydrochloric acid (Merck, for analysis 25%), sodium acetate (Carlo Erba, pure), sodium tetraborate decahydrate (Aldrich, A.C.S. Reagent) and sodium carbonate anhydrous (Merck, for analysis). Lithium nitrate was from Prolabo, 99% purified.

3.3. Procedure

The mobile phase used was methanol/water at a ratio of 60/40 buffered to different pH values. Acetate, borate and carbonate buffers were used. The sodium concentration was kept constant to 1 m*M* in the aqueous portion of the mobile phase. The mobile phase was filtered through a 0.45 μm Nylon (MSI) membrane and degassed for 15 min by a helium stream. The 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 dm^{-3} $LiNO_3$ in methanol/water (60:40) were prepared and filtered through 0.45 μm Nylon (MSI) syringe filters, and 5 μl of the $LiNO_3$ solution were injected into the HPLC systems.

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

4. Results and discussion

4.1. Measurement of the ion-exchange properties of bonded phases

Fig. 1 presents the mass spectrometry chromatograms obtained for the injection of $LiNO_3$ using the Resolve C_{18} column with a methanol/water (60:40) mobile phase buffered at different pH values with a

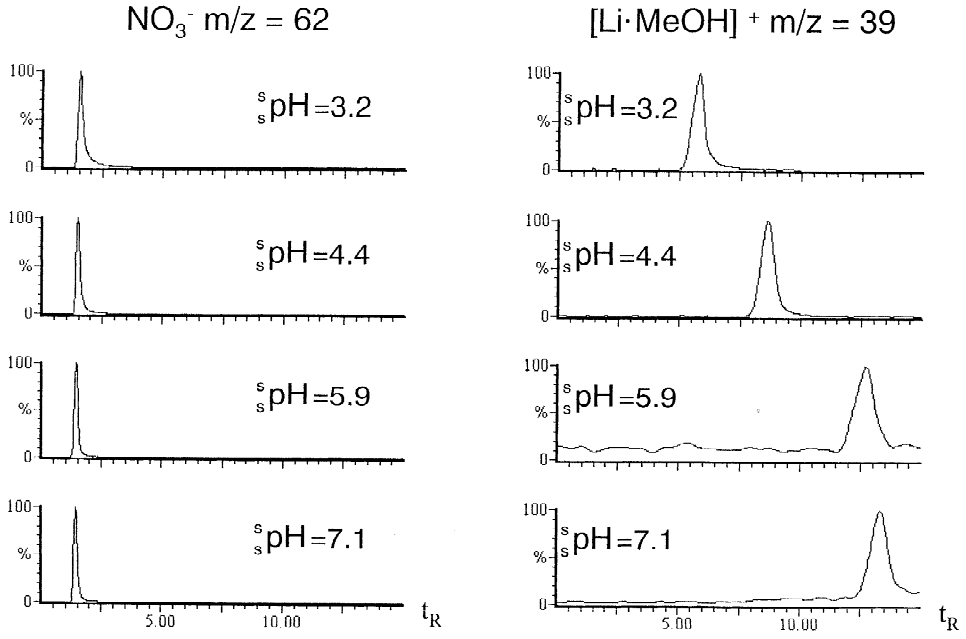

Fig. 1. Chromatograms for the elution of the $LiNO_3$ ion from the Resolve C_{18} column at several mobile phase pH values detected by mass spectrometry as NO_3^- (*m*/*z* 62) and $[Li{\cdot}CH_3OH]^+$ (*m*/*z* 39).

1 m*M* NaAc+HCl solution. Fig. 1 shows that the elution of NO_3^- changes little with the pH variation of the mobile phase. There is only a slight decrease of the elution time (including the extra-column volume) from 1.55 min at ^s_spH 3.2 to 1.42 min at ^s_spH 7.1 caused by a slight increase of the exclusion effect when the negative charge of the silica support increases with the pH of the mobile phase. However, the Li^+ ion (detected as its adduct with methanol, which is the predominant species under the gas phase conditions of the experiment) is notably retained by the ionized silanols of the stationary phase. When the degree of ionization of the silanols increases with the pH of the mobile phase, so does the retention of Li^+.

Mass spectrometry is a powerful detection technique that allows identification of the different peaks of the chromatogram. However, it is an expensive and time consuming technique and we have explored conductometric detection as an alternative technique. Fig. 2 presents the results obtained for the same experiments with conductometric instead of mass spectrometry detection. Elution of the two species can be determined with a single chromatogram for each experiment. The results obtained with mass spectrometry detection allow assigning the positive peak to the NO_3^- ion and the negative peak obtained at higher retention times to the Li^+ ion. A change of the sensitivity of the detector has been done just after the elution of NO_3^- because the signal of Li^+ is much lower than that of NO_3^-. The type of conductivity peaks obtained indicates that the conductivity of NO_3^- ion must be larger than that of the background Ac^- anion (positive peak), but the conductivity of Li^+ must be lower than that of the background Na^+ ion (negative peak). Although we do not know exactly the conductivities of these ions in 60% methanol, they are likely in the same order as in water, which are: NO_3^- 71.4×10^{-4} m^2 S mol^{-1}, Li^+ 38.7×10^{-4} m^2 S mol^{-1}, Ac^- 40.9×10^{-4} m^2 S mol^{-1} and Na^+ 50.1×10^{-4} m^2 S mol^{-1} [17]. Elution of NO_3^- as $NaNO_3$ increases the conductivity above that of NaAc, whereas the elution of Li^+ as LiAc decreases the conductivity below that of NaAc. These data support an ion-exchange process too.

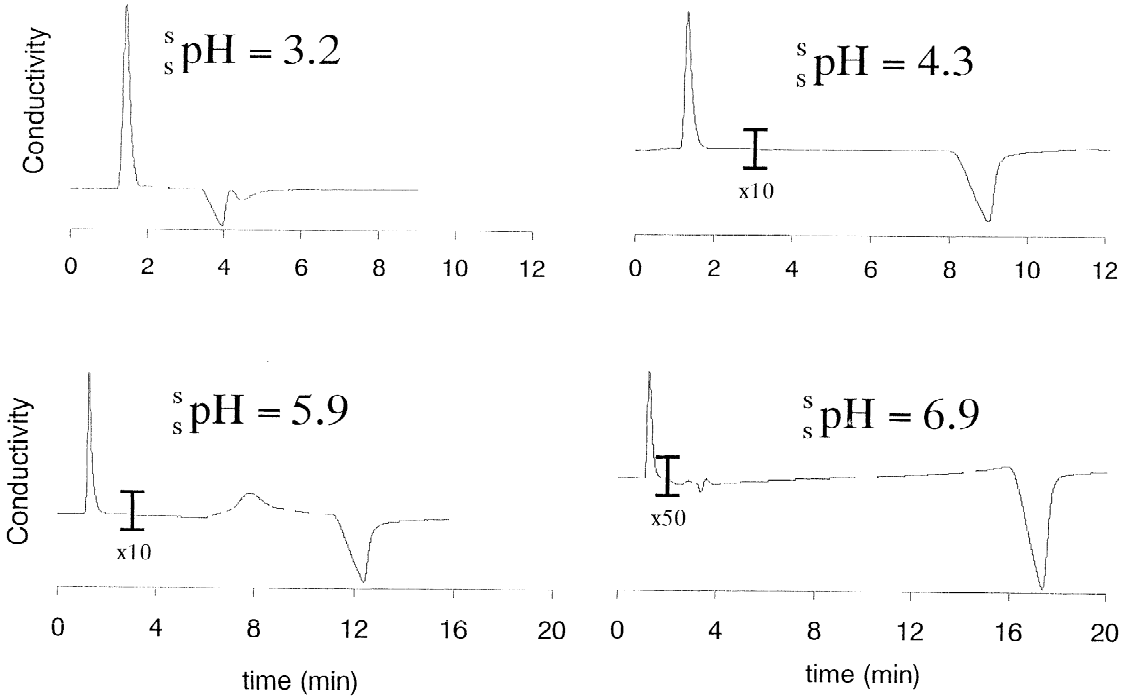

Fig. 2. Chromatograms for the elution of $LiNO_3$ from the Resolve C_{18} column at several mobile phase pH values detected by conductometry. The sensitivity of the detector has been increased just after the elution of the first peak (NO_3^-), except for ^s_spH 3.2.

In the experiments with the other columns, mass spectrometry detection was only used for peak identification at a few different mobile phase pH values. The column parameters of Eq. (10) were calculated by non-linear fitting to the retention times measured by conductometric detection in a variety of mobile phase pH values.

The mass spectrometry and conductometric results obtained confirm that there is an ion exchange between the Li^+ ion and the Na^+ ions of the background electrolyte retained in the column by the ionized silanols. Chromatograms similar to those of Figs. 1 and 2 have also been obtained for the other columns studied.

4.2. Acidity of underivatized silica and hybrid columns

Prior to the study of the residual silanol acidity of C_{18} columns, we have studied the acidity of the different silica supports used for preparation of the C_{18} bonded phases. The retention time of the Li^+ ion in the Resolve silica, Symmetry silica and underivatized XTerra columns for different mobile phase pH values was measured and fitted to the mobile phase ${}^{s}_{s}$pH values through Eq. (10). The pH range studied for each column and the fitting parameters and statistics obtained are given in Table 2. The retention plots are depicted in Fig. 3.

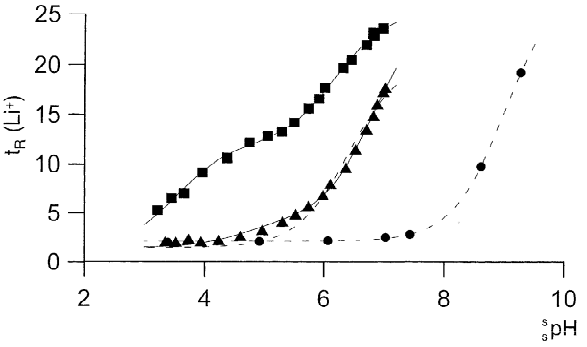

Fig. 3. Dependence of the retention of Li^+ in silica columns, (■) Resolve silica, (▲) Symmetry silica, (●) underivatized XTerra, with the pH of the methanol–(0.001 *M* NaAc+HCl) (60:40) mobile phase detected by conductometry. Fitting lines have been calculated according to the model of Eq. (10) for ion exchange assuming: (- - -) $m = 1$;(——); $m = 2$.

The shape of the t_R vs. ${}^{s}_{s}$pH plot for the Resolve silica column indicates that at least two different types of silanols are needed to explain the variation of the retention of Li^+ in the studied pH range from 3 to 7. The presence of different types of silanols (single, geminal, vicinal) with different acidities has been already discussed in detail by Nawrocki [7]. The concentration and acidity of the different types

Table 2
Parameters of Eq. (10) for the columns studied

	Resolve		Symmetry	
	Silica	C_{18}	Silica	C_{18}
${}^{s}_{s}$pH range	3.2–7.0	3.2–7.0	3.2–7.0	3.7–9.8
n_2/n_1	1.23±0.04	1.02±0.08	7.97±0.90	1.21±0.26
${}^{s}_{s}pK_1$	3.51±0.03	3.66±0.06	4.65±0.17	7.87±0.12
${}^{s}_{s}pK_2$	6.17±0.04	6.45±0.12	6.84±0.07	9.39±0.28
$\frac{K^{Li^+}_{Na^+} n_1}{[Na^+]_M F_c}$	10.76	9.27	2.78	2.76
$\frac{K^{Li^+}_{Na^+} n_2}{[Na^+]_M F_c}$	13.23	9.46	22.16	3.34
$\frac{K^{Li^+}_{Na^+}(n_1+n_2)}{[Na^+]_M F_c}$	23.99	18.73	24.94	6.10
r^2	0.998	0.992	0.998	0.990
SD	0.28	0.42	0.30	0.18
F	2612	623	2181	578

of silanol are dependent on the origin and thermal treatment of the silica [2,4,7–9]. The presence of two types of silanols with different acidity was also observed in previous work using the Merck LiChrospher 100 RP-18 column [13]. Application of Eq. (10) to Waters Resolve silica column gives ${}^{s}_{s}pK_a$ values of 3.5 and 6.2 in 60% methanol for these two types of silanols. The n_2/n_1 ratio shows a slightly lower proportion of the most acidic silanols (45%) in reference to the less acidic silanols (55%).

The shape of the t_R vs. ${}^{s}_{s}pH$ experimental plot for the Symmetry silica column seems to indicate only one unique type of silanol. However, when the retention data were fitted to the data through Eq. (10) considering two different types of silanols ($m = 2$), the fits obtained (presented in Table 2) were much better than those obtained for one unique type of silanols ($m = 1$, $r^2 = 0.979$, SD = 0.85, $F = 792$). Both fits are presented in Fig. 3. For $m = 2$, the ${}^{s}_{s}pK_a$ values obtained are 4.65 and 6.84, i.e. the two types of silanols of Symmetry silica are slightly less acidic than those of Resolve silica (Table 2). The second pK_a value is also in rough agreement with the data obtained by one of us [10], using a somewhat higher ionic concentration than the current study. The wider spreading of the data points in the previous study did not permit to see the second pK_a value.

It is known that metal impurities increase the acidity of the residual silanols [4,8,9]. Since Symmetry silica is much purer than Resolve silica, a lower presence of metal impurities is expected in Symmetry silica, which agrees with a lower acidity. This argument is also supported by the n_2/n_1 ratio, which shows that only an 11% of the silanols in Symmetry silica correspond to the more acidic type, in comparison with the 45% of Resolve silica. However, the overall number of silanols, as measured by the $K^{Li^+}_{Na^+}(n_1 + n_2)/[Na^+]_M F_C$ parameter given in Table 2, is similar in both columns. This is in agreement with expectations.

Silanols on the surface of the unbonded XTerra packing are much less acidic than those on the silica columns. This new generation of silica–methylsilane hybrid support does not show evidence of silanols in the ${}^{s}_{s}pH$ range studied for the other silicas. Since the unmodified XTerra packing is stable at pH values much larger than 7, we investigated the presence of more basic silanols. The data plotted in Fig. 3 show that the silanols present on the surface of the unbonded XTerra packing have ${}^{s}_{s}pK_a$ values about 9 or larger. This is also in agreement with the previous investigations [10].

4.3. Residual silanol acidity in C_{18}-bonded columns

The bonding of the C_{18} stationary phase to the different silica supports is expected to decrease the number of surface silanols and, based on the results above, may even alter their acidity. This has been investigated by application of the same method used for the silica columns to the corresponding C_{18}-bonded columns. The results obtained are presented in Table 2 and Fig. 4.

Bonding of C_{18} to Resolve Silica practically does not alter the acidity of the surface silanols. Two different types of silanols are obtained for Resolve C_{18} column, with pK_a values which do not differ much from the pK_a values obtained for Resolve Silica. The proportion of the two types of silanols is very close to 50%. As expected, bonding of C_{18} decreases the overall number of silanols, measured by the $K^{Li^+}_{Na^+}(n_1 + n_2)/[Na^+]_M F_C$ parameter, from 23.99 in Resolve Silica column to 18.73 in Resolve C_{18} column. Since Resolve C_{18} was measured with a precolumn and Resolve silica without precolumn, this means that Resolve C_{18} contains only about 70% of the silanols of Resolve silica. Comparison of the

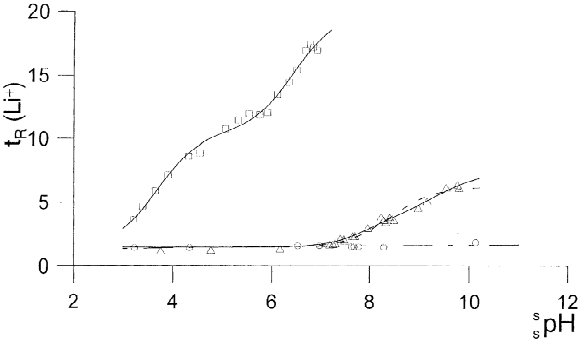

Fig. 4. Dependence of the retention of Li^+ in C_{18} columns: (□) Resolve C_{18}, (△) Symmetry C_{18}, (○) XTerra MS C_{18}, with the pH of the methanol–(0.001 *M* NaAc + HCl) (60:40) mobile phase detected by conductometry. Fitting lines have been calculated according to the model of Eq. (10) for ion exchange assuming: (- –– -) $m = 0$; (- - - - -) $m = 1$;(——) $m = 2$.

two n_2/n_1 ratios indicates that the bonding is slightly more effective for the second type of silanols (the less acidic), i.e. $K^{Li^+}_{Na^+}n_2/[Na^+]_M F_C$ decreases more than $K^{Li^+}_{Na^+}n_1/[Na^+]_M F_C$ in going from Resolve silica to Resolve C_{18}.

In contrast with Resolve columns, the bonding of the C_{18} silane to the Symmetry silica followed by end-capping reduces the number of surface silanols practically to zero in the pH range studied, i.e. up to 7.0 (see Fig. 4). We have explored the existence of more basic residual silanols in Symmetry C_{18} column up to ${}^s_s$pH 9.8 (Fig. 4 and Table 2). The results obtained indicate the presence of two small groups of silanols with ${}^s_s pK_a$ values of 7.87 and 9.39 in 60% methanol. A more likely alternative interpretation of the apparent population of silanols above pH 9 is the slow dissolution of the silica during the test. Due to this effect, the manufacturer recommends to use this phase only at pH values less than 8.

No evidence of residual silanols at ${}^s_s$pH values lower than 10 has been observed for XTerra MS C_{18} confirming the higher quality of the bonding to the hybrid packing. The mass spectrometry and conductivity chromatograms for $LiNO_3$ in this column are presented in Figs. 5 and 6, respectively. Mass chromatograms (Fig. 5) show that Li^+ is eluted at the same time as NO_3^-, giving a single peak in the conductivity chromatograms (Fig. 6). In fact, the acidity of XTerra MS C_{18} is even lower than the acidity of non-silica based polymeric columns, since cation-exchange sites have been reported for some polymeric columns at pH 7 [18]. The acidic sites of the polymeric columns are attributed to the introduction of charged species, such as carboxylate groups, into the polymer during the production process.

4.4. Basicity of C_{18}-bonded columns

Evidence of anion exchange due to basic sites has been observed only for the Symmetry C_{18} column. Fig. 7 depicts the MS/ES-detection chromatograms observed for $LiNO_3$ in this column at two different ${}^s_s$pH values. The retention time of Li^+ almost does not change with the pH because there is no cation exchange for Li^+ in the pH range 3–6 in this column. However, at ${}^s_s$pH 6.0, NO_3^- elutes just after Li^+, but at acidic pH values (${}^s_s$pH 3.4), the elution of NO_3^- (detected as $H(NO_3)_2^-$, the predominant species at acidic pH in the gas phase) occurs several minutes after that of Li^+. This indicates that NO_3^- is retained in the column by anion exchange. The anion exchange is confirmed by the MS chromatograms of the buffer anions. When NO_3^- is eluted, there is a decrease in the eluent concentration of Ac^- and Cl^- (measured in the ES− as sodium adducts).

The change of the retention time of the NO_3^- ion in the different columns is presented in Fig. 8. For Resolve silica, Symmetry silica, underivatized XTerra, Resolve C_{18} and XTerra MS C_{18}, the retention

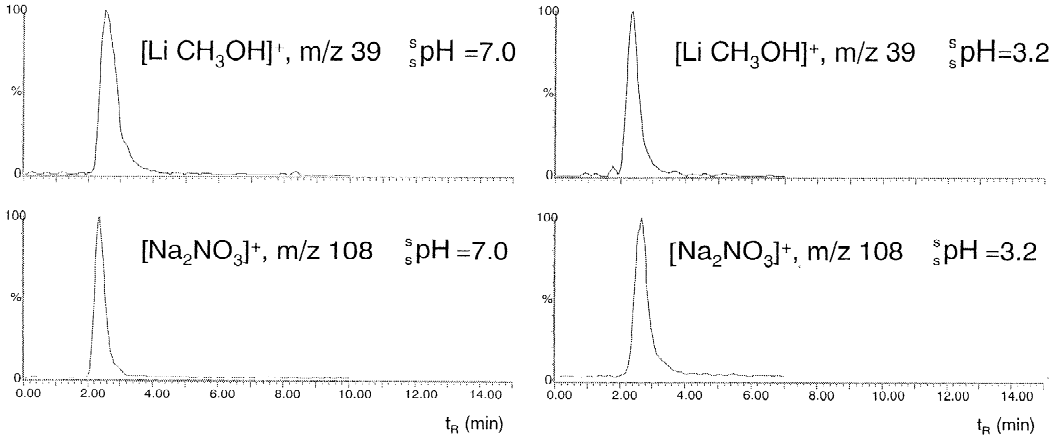

Fig. 5. Chromatograms for the elution of $LiNO_3$ from the XTerra MS C_{18} column at two mobile phase pH values detected by mass spectrometry. Li^+ has been detected as $[Li{\cdot}CH_3OH]^+$ (m/z 39) and NO_3^- as $[Na_2NO_3]^+$ (m/z 108).

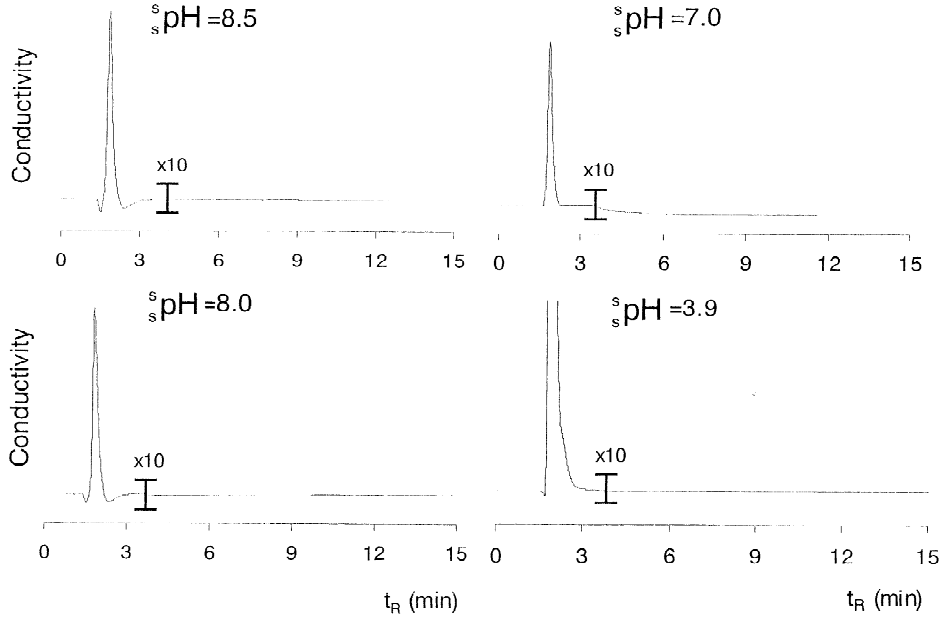

Fig. 6. Chromatograms for the elution of $LiNO_3$ from the XTerra MS C_{18} column at several mobile phase pH values detected by conductimetry. Sensitivity of the detector has been increased just after the elution of the first peak ($NO_3^- + Li^+$).

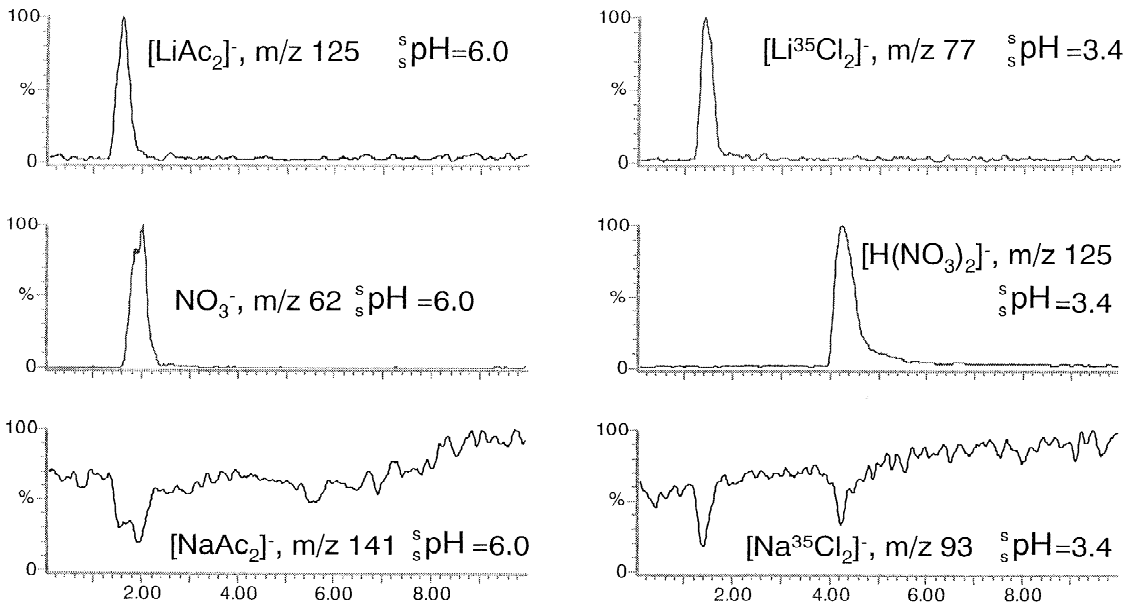

Fig. 7. Chromatograms for the elution of $LiNO_3$ (dissolved in the mobile phase) from the Symmetry C_{18} column at two mobile phase pH values detected by mass spectrometry.

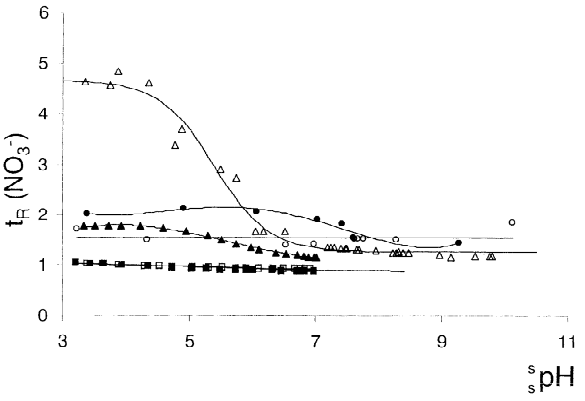

Fig. 8. Dependence of the retention of NO_3^- in silica and C_{18} columns with the pH of the methanol–(0.001 *M* NaAc+HCl) (60:40) mobile phase detected by conductometry. Symbols as in Figs. 3 and 4. Fitting line for Symmetry C_{18} is for $m=1$.

time of NO_3^- is about 2 min or less and it practically does not change with pH. However, the trend of the retention on Symmetry C_{18} is typical of anion exchange. Application of Eq. (15) for $m=1$ gives a $^s_s pK_a$ value of 5.40±0.07 with a $K^{B^-}_{A^-} n_1/[A^-]_M F_C$ term equal to 3.40±0.10. The parameter t_M has also been calculated from the retention and pH data, and a value of 1.26±0.04 has been obtained, which agrees with the retention times of Li^+ at acidic pH values. We suspect that these anion-exchange properties are due to residues of the base used in the bonding process.

5. Conclusions

The use of $LiNO_3$ seems to be a good method to characterize the acidity of silanols in C_{18}-bonded and underivatized silica columns. In most columns, the NO_3^- ion is excluded from the negatively charged silica support, and it elutes at a short retention time, whereas Li^+ is exchanged with the background cation retained by the ionized silanols (Na^+ in this study), and it elutes at larger retention times. The elution time of Li^+ increases with the pH of the mobile phase because ionization of the silanols increases too.

The analysis of the retention time of Li^+ demonstrates that in Resolve silica, Resolve C_{18} and Symmetry silica there are two different types of silanols. One type is very acidic and it has a $^s_s pK_a$ value between 3.5 and 4.6 and the other type is less acidic with a $^s_s pK_a$ between 6.2 and 6.8. The proportion of the two types of silanols is not very different in the Resolve columns, but the Symmetry column has only a very small proportion of the more acidic silanols.

Residual silanols have not been detected for the Symmetry C_{18}, the underivatized XTerra packing and XTerra MS C_{18} columns in the ^s_spH range 3–7. The Symmetry C_{18} column shows a low level of silanols in the pH range between 7 to 8, the latter value being the stability limit of the packing. The underivatized XTerra packing exhibits some silanol activity in the pH range 7–9. However, the bonded phase XTerra MS C_{18} does not present any evidence of residual silanols at all.

No evidence of anion exchange has been observed for all columns, except Symmetry C_{18}, which exhibits a measurable anion exchange at acidic pH values.

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