Evaluating the Surface Charge of C18 Stationary Phases

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Abstract

The surface charge of four C18 stationary phases was investigated by measuring the flow induced streaming potential, a well known electrokinetic property of charged surfaces. Three of the stationary phases (Symmetry, Gemini, and Xterra-MS) had significantly positive streaming potentials at both pH 3 and 4.5. The fourth (Zorbax-SB) appeared to be essentially neutral at pH 3 and became negative at pH 4.5. Apparent zeta potentials ranged from approximately +16 to –4 mV. The retention behavior was also investigated using chloride as model anion and glycinamide (in its protonated form) as model cation. When the retention factor (k) of glycinamide was subtracted from k of chloride anion, the resulting delta k values showed very similar trends as apparent zeta potential values, suggesting that the simple chromatographic method could be used to estimate zeta potential values, or that the zeta potential values could be useful for ranking columns according to ion exchange or exclusion behavior. The anion exchange capacity of the Symmetry and Gemini columns was also estimated, using a published chromatographic procedure, and the results suggest about 2 microEq. capacity per gram of packing.

Introduction

Recently, evidence for a positive charge on commercially available C18 stationary phases has been reported. In several cases, significant retention of inorganic anions and exclusion of various cationic analytes has been observed for several C18 columns (1–3). Because this evidence is based solely on chromatographic retention properties, it would be useful to have an alternative non-chromatographic method to examine the surface charge of these columns. Recently, it has been shown that the flow induced streaming potential, a well known electrokinetic property, is useful for characterizing the surface charge of open and packed capillaries used for capillary electrochromatography (4).

However, streaming potential or streaming current measurements have been rarely used in HPLC, despite the suitability of an HPLC system to perform such measurements. It has been shown that streaming current can be used as a detection method in HPLC (5), or that monitoring a streaming potential sensor is effective for evaluating flow irregularities produced by HPLC pumps (6). In another study, electrokinetic current measure-

ments of several derivatized silica materials were used to calculate ζ potentials. The materials included C18 derivatized silica, for which a strongly negative ζ potential value of –104 mV was reported. In this case, the investigators used a solvent system of 1:9 (v/v) acetone–heptane (7).

This report summarizes experiments in which the streaming potential method was used to study the surface charge of C18 stationary phases with typical reversed-phase liquid chromatography (RP-LC) mobile phases, containing a mixture of water and acetonitrile. Two pH values (3 and 4.5) were tested, using four commercially available C18 stationary phases (Symmetry, Gemini, Xterra-MS, and Zorbax-SB). The chromatographic behavior of the same C18 columns was also tested with the same mobile phases, using two ionic analytes of opposite charge, to see if there was a correlation between the electrokinetic properties and the chromatographic elution behavior of the charged probes. In addition, a published chromatographic procedure was used to estimate the ion exchange capacity for the Symmetry and Gemini columns.

Experimental

HPLC instrument

The chromatographic system consisted of an Alliance 2695 separations module and 996 photodiode array UV detector (Waters Corp., Milford, MA). Empower chromatography software (Waters) was used for instrument control, data acquisition, and data processing. The PDA spectrum was acquired in a wavelength range of 192 to 300 nm using a spectral bandwidth of 2.4 nm and a sampling rate of 5 Hz. Chromatograms were later extracted at suitable UV wavelengths from the electronically stored PDA data. Columns were maintained at 30°C unless otherwise stated.

Chromatographic experiments

For studying retention and exclusion behavior, one neutral compound (urea) and two charged compounds (chloride anion and glycinamide cation) were used as probes. In order to facilitate UV detection of the weakly absorbing probes, acetonitrile (MeCN) was used as organic solvent, and ammonium phosphate as buffer. Two different mobile phase pH values (pH 3 and pH 4.5) were studied. pH 3 was chosen as the low pH because it is the lowest possible pH where ionic strength (I) does not exceed

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1mM. The low I value was important in order to reduce electrostatic screening effects, thereby making ion exclusion phenomena easier to observe. pH 4.5 was selected as the high pH so that two simplifying assumptions could be applied. The first assumption was that the only significant anion present in mobile phase was dihydrogen phosphate. At pH values greater than 4.5, one must expect the amount of the doubly charged monohydrogen phosphate anion will become significant and add an additional variable to the system, as well as complicating the calculation of ionic strength values. The second simplifying assumption involved the glycinamide probe. It was desired that glycinamide remain fully protonated and quantitatively in cationic form. Although the pK_a of glycinamide cation in pure water is reported as 8.1 (8), it is known that amino-type compounds will undergo a shift towards lower pK_a when organic solvent is added to the medium, typically one pK_a unit as organic solvent content reaches 50% (9). It was therefore assumed that in 33% MeCN the pK_a of glycinamide is approximately 7.4, meaning at pH 4.5 the assumption of fully protonated glycinamide is valid (only about 0.1% of glycinamide in neutral form and the remainder in the desired cationic form).

Chemicals and mobile phase preparation

Chemicals were reagent grade or better. MeCN was HPLC grade and water was purified using a Purelab Ultra system (Elga Labwater, Lowell, MA). Mobile phases were prepared using calculated amounts of acid and salt to produce the target pH and ionic strength (I) in 33% MeCN, using pKa value of 2.90 for phosphoric acid in 33% MeCN based on interpolation of literature values (10). The pH 3 mobile phase was 1.8 mM H_3PO_4 in 33% MeCN (theoretical pH 3.00, I = 1.00). The pH 4.5 mobile phase was 1mM in $NH_4H_2PO_4$ and 0.05 mM in H_3PO_4, also in 33% MeCN (theoretical pH 4.52, I = 1.03 mM).

HPLC C18 stationary phases

Four different C18 HPLC stationary phases were used in this study. The stationary phases were Gemini (Phenomenex, Torrance, CA), Xterra-MS (Waters), Symmetry (Waters), and Zorbax-SB (Agilent Technologies, Palo Alto, CA). For the flow induced streaming potential measurements, the materials were 5-μm nominal particle diameter. HPLC experiments were also conducted, using columns containing both 3 μm and 5 μm nominal particle size for each type stationary phase.

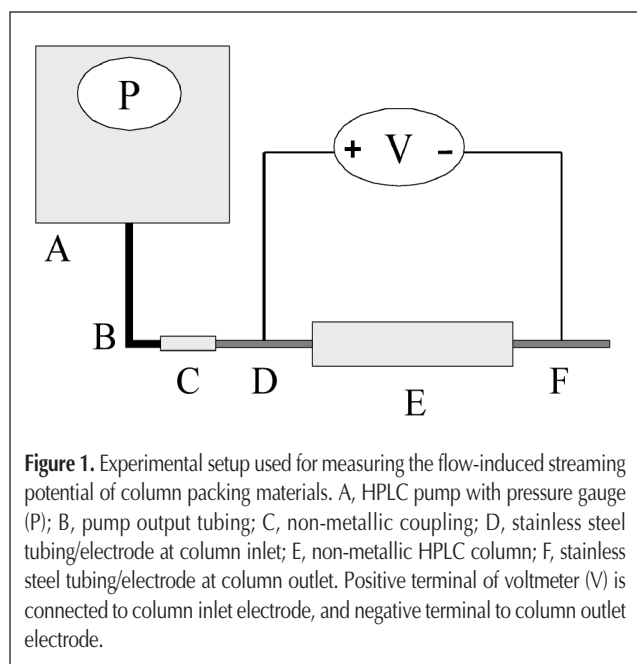### Flow induced streaming potential measurements

Streaming potential measurements were made as shown in Figure 1. A non-metallic HPLC column was packed with the C18 material under study. An HPLC pump (Alliance 2695, Waters) was used to control the flow of mobile phase and to measure the pressure applied to the column. The column was connected to 10-cm lengths of standard sized HPLC tubing (1/16 in o.d., 0.02 in i.d.) at each end, which also served as electrodes for measuring the voltage potential. The use of stainless steel material for electrodes has been shown by others to give satisfactory results for streaming potential studies (11). The voltage potential was measured with an Orion 3 Star pH meter (Thermo Electron Corp.), which was connected to the electrodes via a cable consisting of a BNC connector, two 75-cm lengths of insulated 18 gauge stranded wire, and two alligator clips. As is the common convention of streaming potential measurements, the positive (+) voltmeter terminal was connected to the electrode on the inlet (high pressure side) of the column, and the negative (–) voltmeter terminal was connected to the electrode on the outlet (low pressure side) of the column (11).

Conductivity measurements were made using a model WD-35607-10 conductivity meter (Oakton). The meter was calibrated with a standard of 10.0mM KCl, having a theoretical value of 1413 μS (12). A lower value standard was also checked (1.00mM KCl), and the meter reading was within 2% of the theoretical value (147 μS). Measurements were conducted at 25°C.
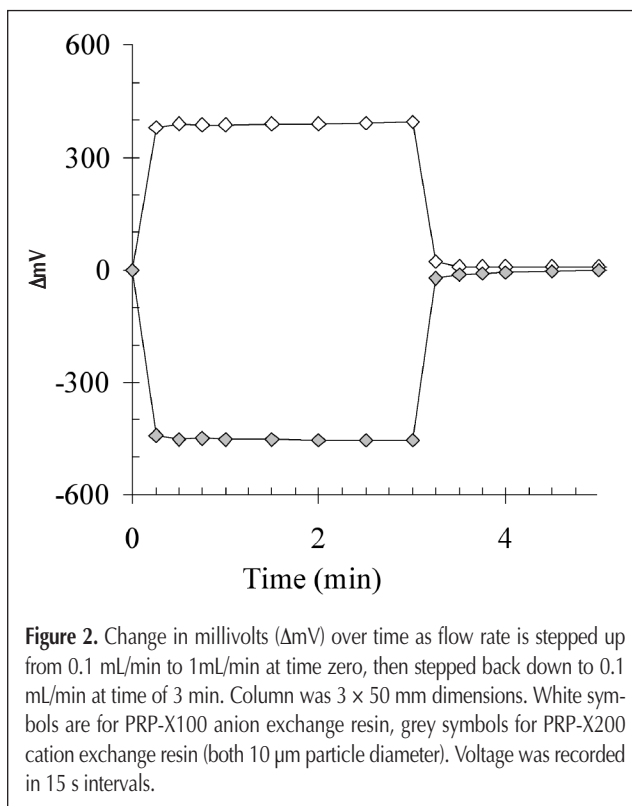
Results and Discussion

Flow induced streaming potential measurements of HPLC packing materials

Commercial instruments are available for measuring streaming potentials, but for this study HPLC, equipment was used to perform the measurements as shown in Figure 1. For preliminary experiments, the column was 3×50 mm, made of PEEK material, with end fittings containing integral frits (0.5 μm) also made from PEEK material (Omega modular column system, Upchurch Scientific, Oak Harbor, WA). The column was first packed with an anion exchange resin (PRP-X100, 10 μm, Hamilton Co., Reno, NV), and then a subsequent experiment was run using resin of the opposite charge (PRP-X200 cation exchange resin, 10 μm, Hamilton). Mobile phase of 33% MeCN (pH 3, I = 1mM) was used. The voltage potential was monitored as the flow rate was varied. The results are shown in Figure 2, where the change in voltage was recorded as the flow rate was stepped up from 0.1 to 1 mL/min, held for 3 min, and stepped back down to 0.1 mL/min again. Note that the response is very rapid and a plateau is reached in about 15 s or less. Also, the sign

Figure 1. Experimental setup used for measuring the flow-induced streaming potential of column packing materials. A, HPLC pump with pressure gauge (P); B, pump output tubing; C, non-metallic coupling; D, stainless steel tubing/electrode at column inlet; E, non-metallic HPLC column; F, stainless steel tubing/electrode at column outlet. Positive terminal of voltmeter (V) is connected to column inlet electrode, and negative terminal to column outlet electrode.

of the voltage potential was consistent with the expected surface charge (+ or –) of the resins, with the PRP-X100 material (containing cationic quaternary ammonium groups) showing a positive voltage potential and the PRP-X200 material (containing anionic sulfonate groups) showing a negative voltage potential. However, a significant problem became apparent when checking to see if the column itself caused any bias in the electrical potential. When experiments were run with the column only (without any packing) at pH 3 and pH 4.5 (I = 1mM), a significant bias was observed. For flow rate of 1 mL/min, the measured potential of the empty column was typically about +220 mV at pH 3, and about –80 mV at pH 4.5. It is possible that this background potential was due to the inlet and outlet frits of the column, which are made of polymeric material. Another column was,

therefore, used for subsequent experiments. The alternative column was a non-metallic column obtained by removing the existing packing from a previously used 4.6 × 100 mm monolithic column (Chromolith Performance RP-18e, Merck, Darmstadt, Germany). To reduce the space between the packing material and the electrodes, the end fittings of the column were modified by drilling a 1/16 in diameter through hole, and then substituted standard stainless steel HPLC column frits instead of the original non-metallic frits. This allowed the stainless steel tubing to be inserted so that essentially no space existed between the electrically conducting frit and tubing. When this column was tested without packing, no electrical bias was detectable at pH 3, and at pH 4.5 the bias was less than +30 mV at a flow rate of 1 mL/min. Based on the much lower background potential, the second column was subsequently used for testing the C18 packing materials.

C18 packing materials of 5 µm particle diameter were used for the streaming potential measurements. In each case, the potential was measured at several flow rates. This is common practice for streaming potential studies (11,13–15). This procedure minimizes errors from background potential which may be present. For each determination, the voltage was measured at flow rates of 0.1, 0.25, 0.5, and 1 mL/min, then measured again for the same flows in descending order. With one exception, the observed voltage changes were 400 mV or higher. For Symmetry at pH 4.5, the voltage at 1 mL/min exceeded the capacity of the pH meter (±2 volts) and a lower flow rate range (0.05 to 0.5 mL/min) was used. The data were evaluated by first calculating the slope of V/F (where V is voltage and F is flow rate in mL/min), which generally showed good linearity. Correlation coefficients (r^2) were 0.996 or better in the majority of cases. Examples are shown in Figure 3, which illustrates an experiment in which the potential was measured using the standard procedure, then the direction of column was reversed and the procedure was repeated. This is illustrated for Symmetry at pH 3 and for Zorbax-SB at pH 4.5. Note that when the column was reversed, the entire curve was shifted slightly up or down, which reflects the small background potential. However, the slopes obtained from regression analysis were very similar, differing by less than 5% for the Symmetry and less than 3% for the Zorbax-SB. This illustrates the effectiveness of using the slope obtained from several flow rates to cancel out background bias.

In streaming potential studies, measurements are typically expressed in the form of voltage potential as a function of pressure (11,13–15). Therefore, the slope values of V/F were converted to voltage as a function of pressure (V/P) according to:

$$(mV/F) \times (F/P) \qquad \text{Eq. 1}$$

where P was the pressure observed at the 1 mL/min flow rate during the measurement. Before the calculation, the pressure reading was first corrected for background pressure from the empty column and tubing alone. For the 4.6 × 100 mm column containing 5 µm

Figure 2. Change in millivolts (ΔmV) over time as flow rate is stepped up from 0.1 mL/min to 1mL/min at time zero, then stepped back down to 0.1 mL/min at time of 3 min. Column was 3 × 50 mm dimensions. White symbols are for PRP-X100 anion exchange resin, grey symbols for PRP-X200 cation exchange resin (both 10 µm particle diameter). Voltage was recorded in 15 s intervals.

Table I. Experimental and Literature Values Used for Calculating Apparent Zeta Potential (ζ_A) for C18 Type HPLC Packing Materials (Nominal 5 µm Particles)*

pH	Packing material	V/P (mV/Pa) × 10⁴	σ (µS/cm)	ε	η (Cp)
3	Symmetry	3.6	280	67	0.95
	Gemini	2.2			
	Xterra	0.59			
	Zorbax-SB	–0.03			
4.5	Symmetry	5.0	94	67	0.95
	Gemini	3.8			
	Xterra	0.71			
	Zorbax-SB	–2.6			

* Voltage/pressure relationship (V/P) and conductivity (σ) were experimentally measured. ε is based on interpolation of values from the literature (16), and η from the literature (17). The V/P values were measured at the temperature of the laboratory (23°C). Other values were measured or reported for a temperature of 25°C.

C18 materials, pressures were typically about 500 PSI (~3500 kP$_a$) at 1 mL/min.

The experimental values for V/P are shown in Table I, along with several properties of the solvent system being used for the measurements. The V/P values shown in Table I are the overall mean of several determinations. For the Symmetry material, testing was conducted on five different days. Each day of testing at least two replicates were performed and the mean value was calculated for each day. When the mean values obtained from the five different days were evaluated as a group, the relative standard deviation was 6% for both pH 3 and pH 4.5. Similarly, Gemini material was tested on four different days, and the relative standard deviations were 8% (pH 3) and 10% (pH 4.5). Xterra-MS material was tested on two different days. The relative difference between the two determinations was 5% (pH 3) and 3% (pH 4.5). Zorbax-SB material was also tested on two different days. For pH 3, the observed potential was slightly negative on one day and slightly positive on the other. The actual measured potentials were very low (only approximately –50 mV for one determination, and +25 mV for the other at 1 mL/min) suggesting that this material is essentially neutral at the pH 3 condition. At pH 4.5, the difference between the two determinations was 15%.

Estimating zeta potential values of packing materials

In principle, the V/P values can be used to calculate ζ potential values. To do this, it is also important to know the properties of the solvent. In particular, knowing the conductivity (σ) of the solution is required. As σ is increased, the observed streaming potential will be reduced. This is understandable considering the higher conductivity will essentially act as a short circuit and make it more difficult to build up a voltage potential across the column. This becomes an important issue when comparisons are being made between solutions having different composition. Even if the ionic strength of the solutions are the same, the conductivity may be different due to the differences in mobility of the ions involved, and may lead to erroneously high or low ζ potential values. In this case, the cation is 1mM hydronium ion at pH 3, whereas it is primarily ammonium ion in the pH 4.5 buffer. Conductivity measurements showed that the conductivity of the pH 3 solution was significantly higher than for pH 4.5 (by a factor of approximately 3, Table I), which is reasonable considering the expected higher mobility of hydronium ions. This explains why the V/P values observed at pH 4.5 were significantly higher than the ones at pH 3, even though surface charges normally become less positive and more negative as pH is increased. The remaining factors required to calculate ζ potentials are also shown in Table I, which were obtained from literature values (16,17).

An apparent ζ potential ($ζ_A$) can be calculated according to the equation:

$$ζ_A = (V/P) \times (η σ / ε ε_0) \qquad \text{Eq. 2}$$

where the term (V/P) is the experimentally measured voltage change as a function the applied pressure, and the other terms consist of viscocity (η), conductivity (σ), dielectric constant (ε), and permittivity constant ($ε_0$). The equation gives an "apparent" value because it neglects surface conductivity effects, which are known in some cases to have a significant impact (13). Nevertheless, it is interesting to make a side by side comparison
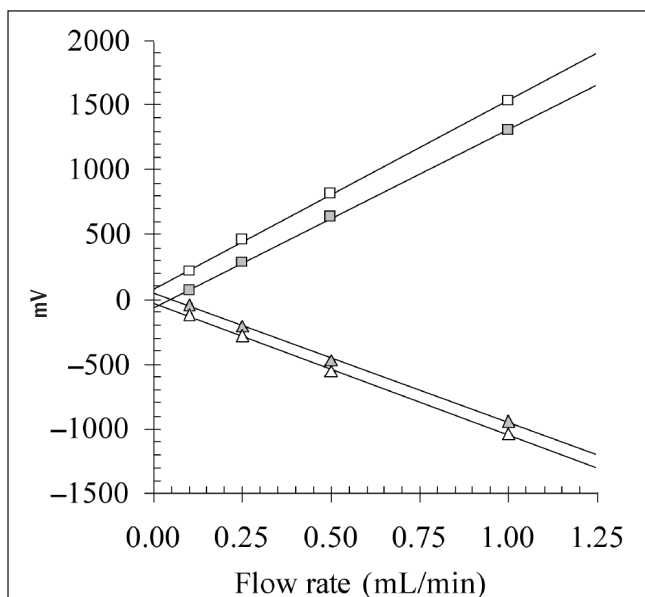
Figure 3. Effect of reversing column direction on the mV vs. flow rate for Symmetry C18 at pH 3 (squares) and Zorbax SB C18 at pH 4.5 (triangles). Lines show the linear regression obtained for each set of four mV readings at flow rates of 0.1, 0.25, 0.5, and 1 mL/min. Grey symbols show results of first determination, white symbols show results of second determination obtained after reversing direction of column.
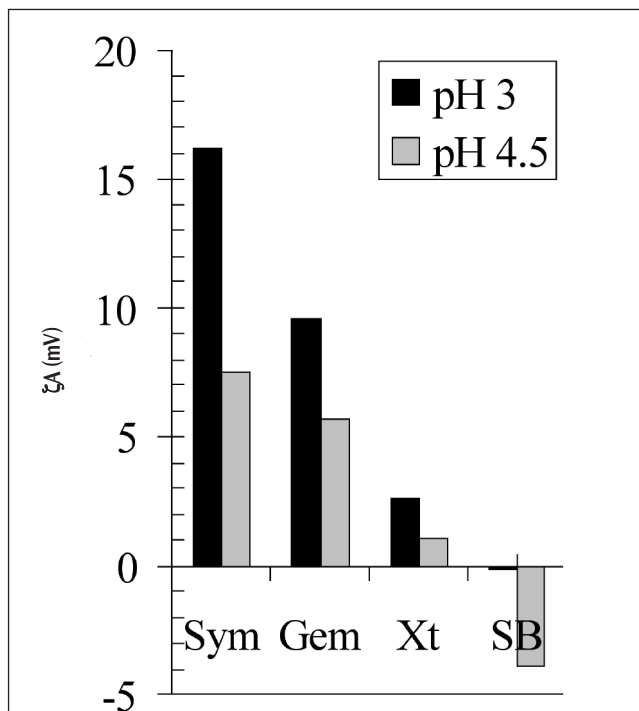
Figure 4. Apparent zeta potential values ($ζ_A$) obtained using streaming potential measurements. All four packing materials (Symmetry, Gemini, Xterra-MS, and Zorbax SB) are C18 type with 5 μm nominal particle diameter. Mobile phases were ionic strength of 1mM.

between the different C18 packing materials, even if only to get an approximation of the sign and magnitude of apparent charge. Figure 4 shows values calculated with equation 2, using the values in Table I. Note that, at pH 3, the Symmetry column shows a significantly positive charge. This is consistent with several chromatographic studies which suggest that this column has an appreciable positive character, based on the tendency to retain anions and exclude cations in mobile phases of low pH and low ionic strength (1–3). The Gemini and Xterra-MS columns also showed positive values at pH 3. The Zorbax-SB column, on the other hand, showed essentially no charge at pH 3. When switching to pH 4.5, all of the ζ_A values shifted towards a less positive (more negative) value. The values for Symmetry, Gemini, and Xterra-MS all remained positive in sign, but became smaller. For the Zorbax-SB, the potential became significantly negative.

Evaluating column charge by retention and exclusion of ionic analytes

The apparent charge of the stationary phases was explored further utilizing a simple chromatographic approach. The procedure involved injecting two charged probes, one positive and one negative, and observing any retention or exclusion which occurred under the weak ionic strength conditions. This is similar to an approach explored by other investigators, where inorganic ions were studied with MS and conductivity detection (1). In the current study, a UV detection mode was employed. Chloride was used as anionic probe, and glycinamide as cationic

probe. By employing UV transparent phosphate buffers, detection was satisfactory at the lowest detector wavelength of 193 nm. Because the glycinamide solutions were prepared from the hydrochloride salt, equimolar amounts of both chloride anion and glycinamide cation were injected simultaneously. For Gemini, Symmetry, and Xterra-MS, the two probe compounds were well separated and could be observed in the same chromatogram (examples shown in Figure 5). For these columns, the elution order was always glycinamide followed by chloride. Also, the positively charged probe eluted before a neutral void marker (urea), resulting in a negative k value. This suggests exclusion from some of the volume within the column, presumably due to a repulsive force from the positively charged stationary phase. On the other hand, the negatively charged probe eluted after the void marker, resulting in a positive k value and suggesting an attractive force between analyte and column. For the Zorbax-SB column, the charged probes both eluted very close to the neutral void marker and were only partly separated from each other. Therefore, separate injections of KCl were made to obtain the chloride peak retention, and the glycinamide peak was detected at a slightly higher wavelength (205 nm), thereby tuning out interference from chloride. For Zorbax-SB, the elution order of the two probes reversed when the pH was switched from 3 to 4.5. At the lower pH, the order was the same as with the other columns (glycinamide cation eluted first), but at pH 4.5 chloride eluted first. This was the only column which showed this behavior. To further evaluate the retention and exclusion data, the following formula was used

$$\Delta k = k_{Cl} - k_G \qquad\qquad Eq. 3$$

which is the difference in retention factor for the chloride anion (k_{Cl}) and glycinamide cation (k_G). The resulting Δk values for the four columns are shown in Figure 6. Columns of 3 μm and 5 μm particle size were tested. The sign and magnitude of the Δk values showed a very similar trend as the ζ_A values in Figure 4. However, the Zorbax-SB column showed a slightly negative Δk value at pH 3, in contrast to the ζ_A potential which was close to zero. If the surface of the column is truly neutral as indicated by the streaming potential, the chromatographic behavior suggests that the chloride ion is somewhat more retained than the glycinamide cation, even on a neutral surface, and this inherent retention difference causes the discrepancy between ζ_A potential and Δk for the Zorbax-SB at pH 3. At pH 4.5, the Δk value for Zorbax-SB became negative, in agreement with the sign of ζ_A potential. The similarity in trends between the ζ_A potential and Δk values suggests that either method could be useful for ranking the columns in terms of relative surface charge. A disadvantage to the streaming potential method is that for normal stainless steel columns, the packing material would have to be removed from the column, whereas for the chromatographic method the column could still be used after the test was conducted.
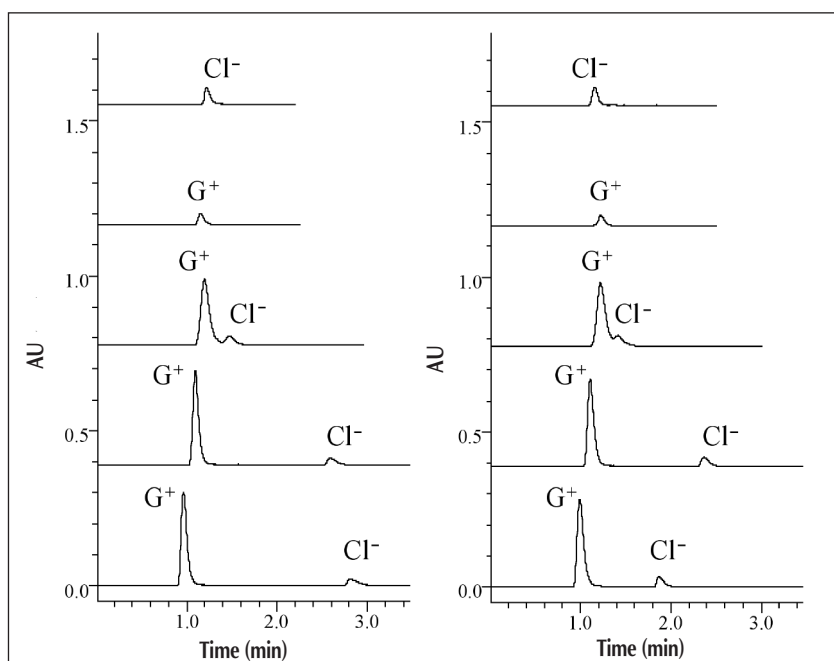
Figure 5. Example chromatograms showing glycinamide cation (G+) and chloride anion (Cl–) with four different C18 stationary phases. Mobile phases contain 33% MeCN with ionic strength of 1mM. Left panel is pH 3, right panel is pH 4.5. Flow rate 0.5 mL/min, all columns are C18 columns with dimensions 3.0 × 150 mm and nominal particle diameter of 3 μm. Chromatograms are (Bottom to top) Symmetry (injection of glycinamide HCl, 193 nm), Gemini (injection of glycinamide HCl, 193 nm), Xterra-MS (injection of glycinamide HCl, 193 nm), Zorbax-SB (injection of glycinamide hydrochloride, detection at 205 nm to eliminate Cl– interference), and Zorbax-SB (injection of KCl, 193 nm). Retention times for urea void marker (not shown) were 1.27 min (Symmetry), 1.42 min (Gemini), 1.37 min (Xterra-MS), and 1.24 min (Zorbax-SB).

Estimation of anion exchange capacity for Gemini and Symmetry at pH 3

A published experimental procedure was used to estimate the ion exchange capacity of the two columns (Symmetry and Gemini) which showed the most significant positive charge (18). The method involves HPLC experiments employing two different anions, using one as analyte and the other as competing ion in the mobile phase. After retention is determined under these conditions, experiments are conducted with the roles of the ions reversed. One can then obtain the ion exchange capacity of the column using the following equation:

$$\text{Column exchange capacity} = \{(k_A V_0 [B])(k_B V_0 [A])\}^{1/2} \qquad \text{Eq. 4}$$

where k_A is the retention of ion A obtained with concentration [B] in the mobile phase, and k_B and [A] are analogously defined. V_0 is the dead volume of the column. Assuming V_0 is the same in both cases, and recognizing that the quantities $k_A[B]$ and $k_B[A]$ are the slopes of k_A vs. $[B]^{-1}$ or k_B vs. $[A]^{-1}$ plots according to the derivation of equation 4 (18), one can derive the following alternate equation:

$$\text{Column exchange capacity} = V_0\,(m_A m_B)^{1/2} \qquad \text{Eq. 5}$$

where m_A is slope of k_A vs $[B]^{-1}$ obtained when injecting A into B mobile phase, and m_B is the slope obtained using reverse conditions.

For the ion exchange capacity determinations, the pH was controlled by using mobile phases containing constant 1mM concentration of strong acid (HCl or HNO_3). The corresponding KCl or KNO_3 salt was then added to further increase the ion concentration in the mobile phase, thereby assuring that the pH was kept constant at 3. For two of the columns (Symmetry and Gemini), experiments were run using 1, 2, and 3mM concentrations of competing ion in the mobile phase. Nitrate ion was easily detectable by UV when injected into mobile phases containing HCl–KCl. For injections of chloride into mobile phases containing HNO_3–KNO_3, the chloride ion was not detected directly, but the chloride peak (and potassium peak) could be detected by changes in the background UV absorbance from the nitrate in the mobile phase, which is a well established detection method for ion exchange LC applications (19). The potassium peak eluted prior to the void time of the column (consistent with an ion exclusion effect). The peak was positive for potassium, presumably due to the excess nitrate required to maintain charge balance. The chloride ion could be detected as a vacancy peak which occurred later in the chromatogram, having approximately the same peak area as the positive potassium peak. Examples are shown in Figure 7, which again shows the substantial amount of retention obtained for both of the inorganic anions.

The results are shown in Table II, where the slope values of k vs. reciprocal competing ion concentration are used to calculate ion exchange capacity of the column (in μEq) according to equation 5. The excellent linearity of the k vs. reciprocal competing ion concentration (Table II) again supports the view of an apparent ion exchange type of interaction between negatively charged analyte and positively charged stationary phase. Both columns showed a value of about 1 μEq. Considering that columns of this dimension will contain about 0.6 grams of packing (3), this suggests an ion exchange capacity of roughly 2 μEq. per gram of packing, which is about two to four orders of magnitude lower than typical porous ion exchange materials (20). This low value explains why the ion exchange effects are only observable at very low ionic strength mobile phases.
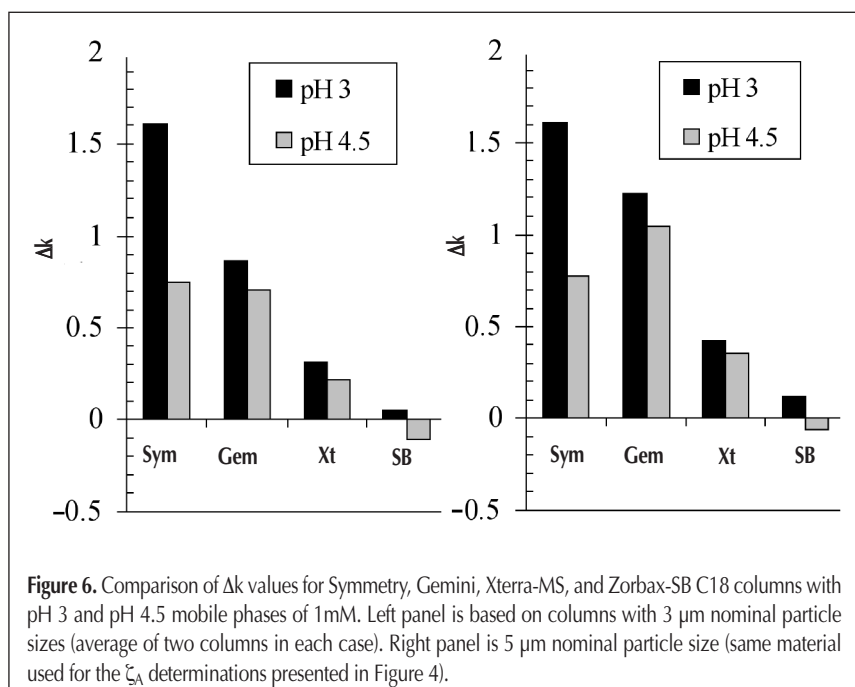
Table II. Linear Regression Analysis for k_A vs. 1/[B] and k_B vs. 1/[A] Experiments*

Column (V_0, mL)	k_A vs. 1/[B]			k_B vs. 1/[A]			
	Slope	Intercept	r^2	Slope	Intercept	r^2	Capacity μEq
Gemini (0.606)	1.46	–0.162	0.9998	2.03	–0.124	> 0.9999	1.04
Symmetry (0.530)	1.16	–0.110	0.9994	1.92	–0.078	> 0.9999	0.79

*A = chloride and B = nitrate. Mobile phase concentrations of 1, 2, and 3mM were used in each case. Values of V_0 are also shown.

Figure 6. Comparison of Δk values for Symmetry, Gemini, Xterra-MS, and Zorbax-SB C18 columns with pH 3 and pH 4.5 mobile phases of 1mM. Left panel is based on columns with 3 μm nominal particle sizes (average of two columns in each case). Right panel is 5 μm nominal particle size (same material used for the ζ_A determinations presented in Figure 4).

Cause of ion exchange behavior

One possibility for the apparent ion exchange behavior is the presence of a contaminant on the surface of the stationary phase. While metal contamination was common for earlier generation silica gel mate-

rials, modern silica gel materials (including Symmetry C18) have been shown to be essentially free of metal impurities, using both spectroscopic analysis for metal content, and chromatographic testing using a metal chelating analyte (21). An alternative explanation to trace impurities is that the apparent positive charge of the surface is due to the preferential adsorption of cations (from the mobile phase) relative to anions (22). It is known that adsorption of ions such as surfactants will significantly alter both the streaming potential of surfaces (13,23) and the chromatographic retention behavior of ionic analytes (24). Even the nature of organic solvents may influence the sign and magnitude of the observed streaming potential (14). Also, it has been pointed out that a general tendency exists for a variety of hydrophobic surfaces to exhibit similar isoelectric points, typically in the range of pH 3 to 4, despite the lack of any obvious ionogenic chemical entity on the surfaces (25). Thus, the apparent positive charge observed in this study may be a general phenomenon which occurs at the interface of hydrophobic C18 packing materials and acetonitrile–water mobile phases, when the pH and ionic strength are sufficiently low. A possible reason for the different columns showing different degrees of charge is variations in the degree of coverage of the underlying silica support. The three columns which showed the most positive character are all considered as endcapped columns (26,27), but Zorbax-SB is not considered as endcapped, but rather a sterically protected type stationary phase (28). This difference may be a contributing factor to the lack of positive character of Zorbax-SB. However, the number of columns used in this study is too small to establish any clear relationship between the nature of stationary phase and surface charge.

Conclusions

The flow-induced streaming potential of four different C18 stationary phases was measured using mobile phases of pH 3 and 4.5, with 1mM ionic strength. For Gemini, Symmetry, and Xterra-MS, the sign of the streaming potential was positive, affording ζ_A values ranging from about +3 mV (Xterra-MS) to +16 mV (Symmetry) at pH 3. At pH 4.5, values remained positive, but were reduced to about half the pH 3 values. A fourth stationary phase (Zorbax-SB) appeared to be essentially neutral at pH 3, and at pH 4.5 the sign of the streaming potential became negative, affording a ζ_A value of about –4 mV.

When injecting two relatively polar compounds of opposite charge (chloride anion and glycinamide cation) into the low ionic strength mobile phases, and obtaining the algebraic difference between the retention factors ($k_{Cl} - k_G$), the resulting $\varnothing k$ values showed very similar trends to the ζ_A values. This method could in principle be used to evaluate the charge of stationary phases, so that it would be possible to predict how the retention of charged analytes will be affected by increasing or decreasing ionic strength during method development, and how different columns will have different tendencies to retain anions or cations. A previously published chromatographic procedure was also used to estimate the ion exchange capacity of Gemini and Symmetry columns, and the result indicated a total capacity of about 1 µEq for columns of 3×150 mm dimensions, equivalent to about 2 µEq per gram of packing material.

In general, the trend in electrokinetic properties of the various columns appears to mirror the chromatographic retention behavior of charged analytes. An exact chemical cause for the positive character for three of the columns, and the lack of positive character for the fourth, remains unclear. However, despite the very low apparent surface charges (based on the ion exchange capacity estimates), the effect of the positive charge towards the retention of charged analytes appears to be quite significant when the ionic strength of the mobile phase is sufficiently low.

Acknowledgments

Figure 7. UV chromatograms used to determine ion exchange capacity for Gemini C18 column. Left side shows injections of potassium chloride into 33% MeCN mobile phases containing (top to bottom) 1, 2, and 3mM nitrate ion. Right side shows injections of potassium nitrate into 33% MeCN mobile phases containing 1, 2, and 3mM chloride ion. Mobile phases contained 33% MeCN and pH was kept constant at 3. Wavelengths were 230 nm (left) and 210 nm (right). Flow rate was 0.6 mL/min, column temperature was 35°C.

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