

Study of the elution mechanism of sodium aryl sulfonates on bare silica and a cyano bonded phase with methanol-modified carbon dioxide containing an ionic additive

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Abstract

The elution mechanism of sodium sulfonates on both Deltabond cyanopropyl and bare silica stationary phases with an isocratic mobile phase composed of methanol-modified CO₂ wherein an ammonium salt additive was dissolved in the methanol has been studied. The presence of the additive was crucial concerning elution of the sulfonate salts. Solid state ²⁹silicon nuclear magnetic resonance spectroscopy provided some insight concerning the interaction of the mobile phase additive with the silica-based stationary phase. Computational calculations concerning the charge distribution on various ammonium salts were performed in an effort to explain the elution behavior. Ammonium ions are believed to deactivate available silanol sites on both phases. In addition, ammonium ion is speculated to interact with the cyano groups on the bonded phase. For concentrations of additive greater than 2 mM, stationary phase coverage of ammonium ion is anticipated to exceed one monolayer for both bare and bonded silica. The acetate counter-ion is thought to facilitate elution of the anionic sulfonates from the positively charged stationary phase in a pseudo ion exchange mechanism.

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1. Introduction

Traditionally, the application of packed column supercritical fluid chromatography (SFC) was thought to be limited to non-polar and moderately polar analytes because of (a) the weak solvating power of the mobile phase, which typically was supercritical carbon dioxide, and (b) the strong irreversible interaction between analytes and residual active silanol groups associated with silica-based bonded stationary phases.

The active silanol sites on silica-based stationary phases can irreversibly adsorb highly polar solutes, especially when the mobile phase does not have sufficient solvating power. End-capping with trimethylchlorosilane has been used to

reduce the number of residual silanol sites. Due primarily to steric hindrance and a highly porous solid support, all silanol sites on the silica surface cannot be removed by this treatment. Polymer-coated, bonded stationary phases have been shown to provide a higher degree of deactivation. Berger et al. [1] and Ashraf-Khorassani et al. [2], for example, reported that a polymer-coated cyanopropyl (e.g. Deltabond) phase significantly improved chromatographic peak shapes of eluted phenols, anilines, and benzylamines compared to a non-deactivated cyanopropyl phase. This was thought to be due to the presence of fewer accessible active silanol sites on the polymer-coated phase.

An alternative strategy in this chromatographic area has been to increase the solvating power of supercritical CO₂ by the addition of small amounts of polar organic mobile phase modifiers, such as methanol, 1-propanol, and acetonitrile [3–7]. While these binary mobile phases significantly

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improved the elution of polar analytes in SFC, they are still not sufficiently solvating to elute highly polar or ionic compounds due to the fact that the organic solvents that are miscible with carbon dioxide are not sufficiently polar.

The addition of a small amount of a very polar component to the mobile phase modifier has been shown to further enhance chromatographic performance. More specifically, solvatochromic studies showed that, for example, a low concentration of trifluoroacetic acid (TFA) dramatically improved the solvating power of methanol-modified CO₂ [8]. Such very polar, low molecular weight compounds, called additives, improved chromatographic peak shape and allowed the elution of solutes that would otherwise be very strongly retained by silica-based packed column stationary phases. Both low molecular weight organic acids and bases have been utilized as mobile phase additives in SFC to facilitate elution of moderately acidic and basic analytes [8–16]. In addition to enhancing the solvating strength of the mobile phase, suppression of analyte ionization by the polar additive was believed to be a competing mechanism of action [1,11].

In addition to (1) enhancing the solvating power of the mobile phase and (2) deactivating the stationary phase, ion-pairing of the ionic analyte with the additive in the supercritical fluid mobile phase has been successfully demonstrated to a limited degree [17,18]. Successful elution of propanol hydrochloride from a cyano packed column with 25 mM sodium heptane sulfonate (HSNa) in methanol-modified CO₂ has been reported. Without HSNa in the mobile phase, the analyte failed to elute. Solubility of the ion-pairing reagent (e.g. additive) in the mobile phase was suggested to be a critical feature in the success of the ion-pair SFC elution mechanism.

In another study, ammonium acetate, ammonium formate, and ammonium carbonate were each recently reported to be effective SFC mobile phase additives for the successful elution of several organic ions from packed columns, which up to that point were either very strongly or irreversibly retained on the stationary phase [19]. The authors suggested two possible roles for the ammonium salts: (1) charge neutralization via ion-pairing formation between the solute and additive and (2) charge introduction to the stationary phase by exchange of silanol hydrogen for ammonium ion followed by anion exchange of the analyte.

In our previous research [20], we successfully eluted three sodium aryl sulfonates with various ammonium salts added to the primary modifier at a concentration of 2.5 mM from Deltabond cyanopropyl, conventional cyanopropyl, and bare silica phases. In some cases, we found that lithium acetate as an additive was even effective in promoting elution. We discovered that different stationary phases yielded different analyte retention properties when the same mobile phase additive was used. Moreover, with the same analyte and stationary phase, various ammonium salts gave rise to different analyte retention times. This study addresses our desire to understand more thoroughly the retention and

elution mechanisms of ionic sulfonates on both a deactivated cyano phase and a bare silica phase with ionic mobile phase additives.

Solid state nuclear magnetic resonance (NMR) spectroscopy, with the help of cross polarization and magic angle spinning (CP-MAS), has been widely used to provide valuable structural information concerning the surface of bare and bonded silica stationary phases [21–25]. Three types of silicon atoms can be identified on the bare silica surface by solid state ²⁹Si NMR (i.e. silicon bonded to two, one, or no hydroxyls). Thus, we decided to use this technique to investigate the interaction between ionic mobile phase additives and the silica stationary phase.

In this work, we have studied the elution mechanism of sodium sulfonates on both Deltabond cyanopropyl and bare silica phases with three ammonium salts as the mobile phase additive. Solid state ²⁹Si NMR spectroscopy provided some insight concerning the interaction of the mobile phase additive with the silica-based stationary phase. Computational calculations concerning the charge distribution on various ammonium salts were also performed in an effort to explain the elution behavior of ionic sulfonates observed with the various additives.

2. Experimental

2.1. Chemicals

Methanol was HPLC grade, (EMD, Durham, NC, USA). The carbon dioxide was SFE/SFC grade (Air Products and Chemicals, Inc., Allentown, PA, USA) with no helium head pressure. Ammonium acetate (AA) (99%, ACS grade), tetramethylammonium acetate (TMAA) (90%, tech. grade), and tetrabutylammonium acetate (TBAA) (97%) were obtained from Sigma Aldrich (Milwaukee, WI, USA). Silica packing material with a particle size of 5 μm was donated by Supelco (Bellefonte, PA, USA).

Sodium *para*-normal dodecylbenzene sulfonate (tech. grade), sodium 4-octylbenzene sulfonate (97%), and sodium *para*-toluene sulfonate were purchased from Sigma Aldrich. A solution of each sample was prepared in methanol at a concentration of approximately 0.5 mg/mL.

2.2. SFC-UV instrumentation

The SFC system was a Berger Instruments Analytical SFC Instrument (Newark, DE, USA) with a Hewlett Packard (Little Creek, DE, USA) Model 1050 Diode Array Detector, which employed a 13 μL high pressure flow cell (10 mm path length) and Berger Instruments 3D SFC ChemStation software, version 3.4.

The chromatographic columns were Deltabond Cyano (Thermo Hypersil-Keystone, Bellefonte, PA, USA), and Supelcosil LC-Si. The column dimensions were 25 cm in length and 4.6 mm I.D., with a particle size of 5 μm for each

stationary phase. Supelcosil LC-Si had a pore size of 120 Å, while Deltabond Cyano had a pore size of 200 Å.

Unless otherwise specified, chromatographic conditions were: injection volume 10 µL, mobile phase flow rate 2 mL/min (measured in the liquid state), column outlet pressure 120 bar, and column oven temperature 40 °C. The isocratic mobile phase composition was 15% modifier in CO₂, unless specified. The modifier consisted of either pure methanol or methanol with 2.5 mM ammonium salt. In order to establish stable chromatographic conditions, the isocratic mobile phase was allowed to flow through the packed column for 30 min prior to injection of the sample.

Between each change of mobile phase additive, the stationary phase was regenerated with 90/10 methanol/CO₂ for 15 min (about nine column volumes) in order to purge previous additive solution from the system, or to remove analyte that had interacted with the stationary phase. After this time period, the next additive solution was introduced to the SFC system, and the column was equilibrated again for 30 min prior to injection.

2.3. Solid state CP-MAS NMR spectroscopy

²⁹Si CP-MAS spectroscopy was performed on a Bruker MSL300 operating at 59.601 MHz. Magic Angle Spinning parameters were 45° pulse with proton decoupling and a recycle delay of 15 s. For CP-MAS, a recycle delay of 5 s and a contact time of 4 ms were used.

The weights of samples placed in the rotor were as follows: 143.1 mg for untreated silica gel; 169.3 mg of silica gel treated with MeOH/NH₄OAc; 205.8 mg of silica gel treated with MeOH/TMAA, and 227.5 mg of silica gel treated with MeOH/TB AA. All NMR spectra employed a fixed number of scans (27 000).

The supercritical fluid treated samples were prepared by packing a 2.5 mL extraction vessel about 40% full with silica packing material. The rest of the space was filled with a glass rod. The vessel was flushed for 1 h with supercritical CO₂ containing 15 or 20% modifier (e.g. pure methanol or methanol containing 2.5 mM of additive) with an outlet pressure of 120 bar and an oven temperature of 40 °C.

2.4. Computational chemistry

The structures of three ammonium additives, AA, TMAA, and TBAA, were sketched into the CAChe Worksystem Pro Version 6.1 (Fujitsu CAChe Group, Beaverton, OR, USA) on a Dell Dimension 2350 computer running Microsoft Windows 2000 operating system. The optimized geometry of the molecules was calculated based on the postulate that the molecules take the form with the lowest energy. The optimized geometry of individual atom was generated by three different methods: AM1 geometry; AM1 geometry including solvent (water) effect; and B88-LYP DFT geometry. The first two methods are semi-empirical methods and the third one is

a DGauss calculation that provides an approximate solution to the Schrödinger equation using only mathematical approximation [26].

3. Results and discussion

3.1. Additive concentration study

Due to the fact that ionic compounds have been shown not to elute in SFC even with methanol-modified CO₂ as the mobile phase [19], we wanted to determine the lowest concentration of ionic additive whereby the ionic sulfonates would elute. We chose sodium dodecylbenzene sulfonate as the probe analyte and ammonium acetate as the probe additive.

A systematic study concerning the elution of the probe analyte with various concentrations of ammonium acetate in methanol was performed on both Deltabond Cyano and bare silica columns. Sodium dodecylbenzene sulfonate did not elute in 25 min from either stationary phase with 15% modifier containing either 0.01 or 0.1 mM NH₄OAc. The analyte, however, eluted isocratically from the Deltabond Cyano column with 15% methanol modifier containing 0.25 mM NH₄OAc as a very broad peak at about 11 min, Fig. 1. With increased concentration of additive in 15% methanol, the analyte eluted even earlier from the bonded phase column and with a sharper peak shape. For example, at 2.5 mM additive the analyte eluted in about 3 min compared to 11 min with 0.25 mM additive. Interestingly, we found that the sulfonate analyte eluted much earlier with 0.25 mM additive on the silica column than on the bonded-phase column. In contrast on silica, retention actually increased to a point and levelled off when the concentration of NH₄OAc in methanol was increased. For example at 15% methanol containing 0.25 mM additive, elution from the bare silica column was slightly less than 4 min. While at 15% methanol containing 2.5 mM

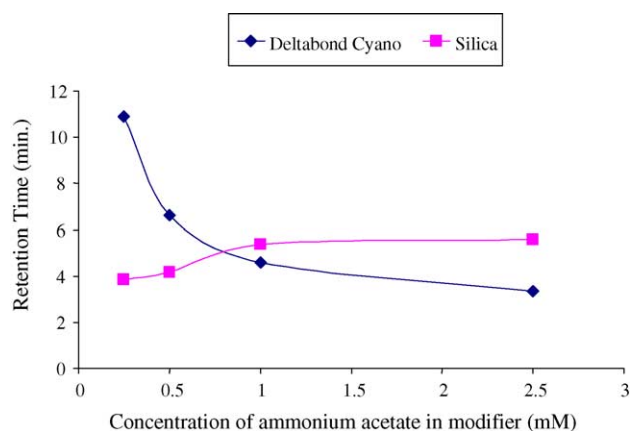


Fig. 1. Retention time of sodium dodecylbenzenesulfonate on bare silica and Deltabond Cyano columns with different concentrations of ammonium acetate as the mobile phase additive. Outlet pressure: 120 bar; oven temperature: 40 °C and flow rate: 2 mL/min.

additive, elution of the analyte increased to approximately 6 min.

In order to attempt an explanation of these findings a consideration of the factors that influence retention in the absence of ammonium acetate should be initially made. We envision at least two contributors to retention: (1) an irreversible interaction of the sulfonate analyte with the bare silica or the silica support in the Deltabond column case and (2) a dispersion (nonpolar–nonpolar/dipole-induced dipole) interaction of the alkyl chain and/or aryl ring of the analyte with the cyano groups and the siloxane polymer of the cyano-modified polysiloxane coating on the Deltabond phase.

On the Deltabond Cyano column after all the silanol protons that would cause irreversible retention are exchanged for ammonium salts, ion-pairing formation between the sulfonate and ammonium cation may be the dominant mechanism. In this case, higher additive concentrations may lead to formation of more ion-pairs and thus faster elution would be achieved. Alternatively, as the ammonium acetate concentration in the mobile phase rises, it increasingly interacts with the cyano-modified polysiloxane coating. Cyano groups (e.g. permanent dipoles) would interact with ammonium cations, which then would interact with acetate ions. From the analyte's perspective, the stationary phase would present fewer and fewer bare cyano groups and more and more cyano units that are associated with ammonium cations. The cyano groups would be less available to interact directly with the analyte and thus cause retention via the alkyl/aryl part of the sulfonate to decrease.

On the other hand, with a bare silica phase, modification of the stationary phase by the additive may be the dominant mechanism, Fig. 2. In other words, an ammonium cation might displace a proton and attach itself to the silanol oxygen thereby deactivating the silica stationary phase. We then speculate that sulfonate anion would then form weaker ion

pairs with the partially charged ammonium-modified stationary phase than with the fully protonated silanol site. As the ammonium acetate concentration increases in the mobile phase, one soon reaches the point where all the silanol protons that would cause irreversible retention are exchanged for ammonium salts. Irreversible retention no longer takes place and analytes elute with good peak shape. The ammonium ions bound to the silanols still retain a partial positive charge, and likely interact with acetate and/or sulfonate analyte ions in the mobile phase. The relatively large amount of acetate anion in the mobile phase is then hypothesized to replace any sulfonate from the ammonium-modified stationary phase resulting in elution of the sulfonate as shown again in Fig. 2. Unlike the irreversible interaction that probably exists between the sulfonate and the bare silica surface silanols, the interaction between NH_4^+ (or tetraalkylammonium ion) that is attached to the silica stationary phase and the sulfonate is more reversible.

In the silica study, the higher concentration of ammonium acetate in the mobile phase led to a longer retention time for the sulfonate in contrast to the similar study just discussed with the Deltabond Cyano phase. An explanation for this phenomenon is not straightforward. As the ammonium acetate concentration increases further, we likely form a pseudo-bonded phase (or layer) of acetates that interact with stationary phase “bound” ammonium ions. The nonpolar acetate tails would interact with the analytes by dispersion. This would cause retention to increase slightly until all the silica surface is covered by this pseudo-phase. An alternate hypothesis is that more than a monolayer of ammonium ion coverage on the silica is possible via interaction with the layer of acetates that cover the pseudo-stationary phase. Subsequent layers of ammonium ion would carry a greater positive charge than the cation immediately attached to the silanol oxygen with the consequence that sulfonate would be retained longer

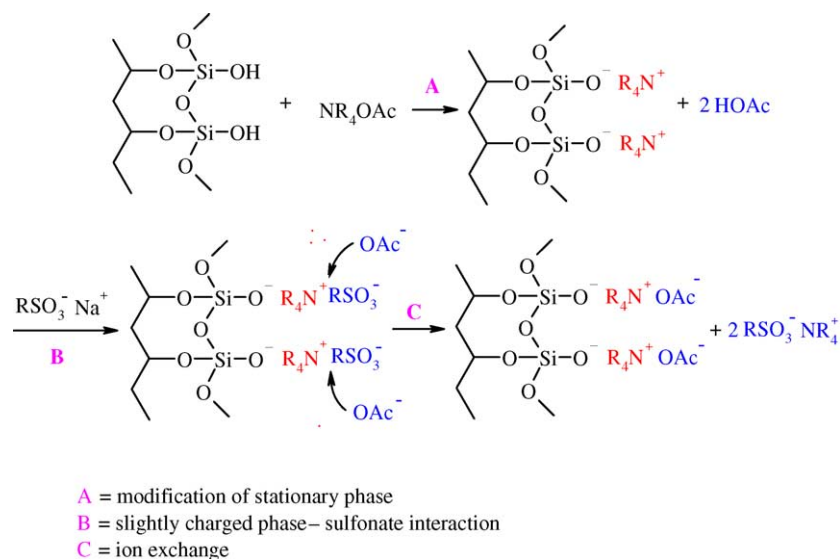


Fig. 2. Proposed mechanism for modification of bare silica phase by ionic additive.

with higher concentrations of ammonium in the mobile phase.

Uptake and adsorption of the mobile phase by the stationary phase in supercritical fluid chromatography has been known for some time. Strubinger and Parcher [27] investigated this phenomenon using mass spectrometric tracer pulse chromatography. Below the critical point, an increase in the adsorption of CO₂ is observed with increasing pressure. Above the critical point, the reverse is true. At supercritical pressures, the excess adsorbed CO₂ increases with temperature at constant pressure. This is the reverse of what is observed for subcritical pressures. Modifiers have been found [28] to have a more pronounced effect on the stationary phase than CO₂. Polar additives are expected to exert a strong influence as well.

3.2. Memory effect on silica phase

Modification of the bare silica phase by ammonium cations was proposed to be an important step in the overall elution process. In an effort to test this hypothesis, repeated injections of sodium *p*-toluene sulfonate on the silica column were performed. Prior to the first injection, pure methanol-modified carbon dioxide was allowed to flow through the SFC system and column. At the first of fourteen injections, the modifier pump was prompted to deliver 15% 2.5 mM (additive) modifier into the SFC system. Fig. 3 shows the retention time measured for each of the fourteen repeated injections with ammonium acetate as the probe additive.

Several chromatograms corresponding to selected injections are presented alongside the retention time versus injection plot. The retention time quickly decreased from 12.79 min for the first injection to 8.66 min for injections 2–5. This suggested that it took about 15 min (i.e. run time of one injection) for the column and mobile phase to reach equilibrium. At the beginning of the sixth injection, pure methanol modifier was re-introduced to the SFC system. When we changed the modifier, the tubing that connected modifier bottle and the system was not filled with new modifier. Thus, it took about 8 min for the new modifier (i.e. pure methanol) to get into the column under the experimental conditions. This means that when the 6th injection was made, the mobile phase in the column was still 15% 2.5 mM (additive) methanol, therefore the sulfonate still eluted around 8 min at the sixth injection. With subsequent injections, retention time increased to approximately 10 min and levelled off. Even though the retention time did not change appreciably after ammonium acetate was removed, the peak shapes were degraded and tailed badly without the additive in the mobile phase. Failure of the retention time to return to its original near 13 min suggested that NH₄OAc not only modified the silica surface, but was retained on it. If necessary the silica column could be regenerated with 90% methanol/10% carbon dioxide for 15 min. After this treatment, however, the sulfonate failed to elute with pure methanol as the modifier, which suggested that the additive eventually could be displaced. At lower concentrations of methanol-modifier, the additive probably can be removed, but more column volumes are needed.

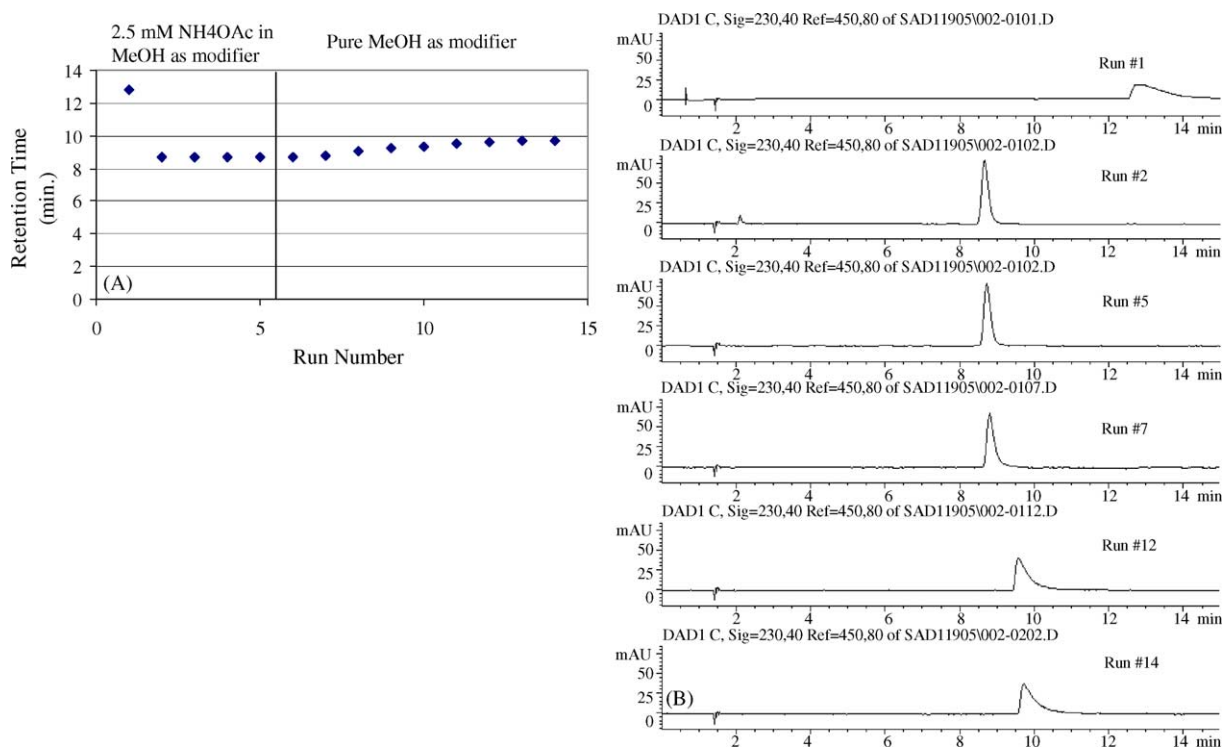


Fig. 3. (A) Retention times for 14 repeated injections of a sodium *p*-toluene sulfonate solution on a silica column. Injections 1–5: modifier contained 2.5 mM ammonium acetate; Injections 6–14: modifier was pure methanol. (B) Selected chromatograms from the fourteen separations.

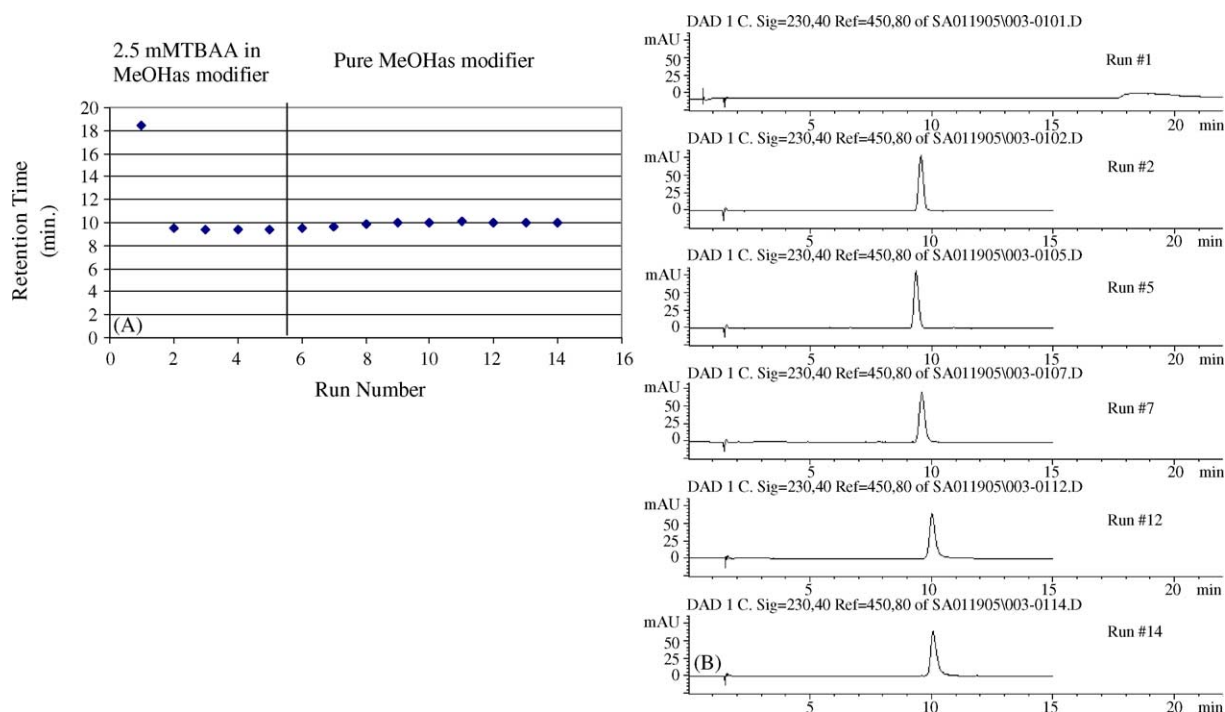


Fig. 4. (A) Retention times for 14 repeated injections of a sodium *p*-toluene sulfonate solution on a silica column. Injections 1–5: the modifier contained 2.5 mM TBAA; injections 6–14: modifier was pure methanol. (B) Selected chromatograms from the 14 injections.

Similar experiments were performed on the silica column with the tetraalkylammonium acetate salts as mobile phase additives, TBAA and TMAA. Fig. 4 shows the measured retention time for the 14 repeated injections with TBAA as probe additive. As in the previous case, TBAA was initially introduced to the modifier pump at the first injection and was removed at the sixth injection. The *p*-toluene sulfonate analyte isocratically eluted at about 18 min for the first injection. The retention time was shortened to about 9.5 min and fairly good reproducibility was achieved with the third, fourth, and fifth injections. This may suggest that it takes about 30 min for the column and the mobile phase to reach equilibrium. After TBAA was removed from the mobile phase, a strong memory effect similar to that found with ammonium acetate was observed. Retention time slightly increased from near 9.3 min to only 10 min for the subsequent nine injections. The greatest change in retention time after the removal of the TBAA additive was between injections 6 and 8 (e.g. 9.35–9.92 min).

Fig. 5 shows the memory effect observed with TMAA as mobile phase additive on the silica column. From our previous study [20], TMAA was observed to provide the longest retention time for selected sulfonates on the silica column. This could be due to the greater residual surface charge on TMAA as opposed to TBAA and the ammonium ion, *vide infra*. A greater surface charge on the stationary phase would result in a slightly longer sulfonate retention time. When equilibrium was reached in this case, retention time for *p*-toluene sulfonate was about 16.6 min. However, it took a much longer time for the column and mobile phase to reach equilibrium with TMAA as the additive than with ammonium acetate or

TBAA. When TMAA was removed from the modifier after the fifth injection, a memory effect was again observed as with the other two additives such that sulfonate analyte could be eluted with pure methanol as the modifier.

Similar experiments involving repeated injections were performed on the Deltabond Cyano column with ammonium acetate as the ionic additive (Fig. 6). After purging the modifier pump with methanol, 15% 2.5-mM NH_4OAc in methanol modifier was introduced as soon as the first injection was initiated. Once reproducible chromatograms were achieved, the mobile phase modifier was changed back to pure methanol (after the fifth run). Again, due to the modifier delay time, the mobile phase in the column contained NH_4OAc such that the sulfonate eluted in run 6 in similar fashion to the previous injection. By the seventh injection, however, the mobile phase on the column no longer contained NH_4OAc , and sulfonate no longer eluted. Thus, no memory effect was observed with the Deltabond Cyano phase, in striking contrast to the bare silica phase. In this case, ion-pairing formation between the analyte and NH_4OAc is a reasonable elution mechanism.

3.3. Solid state NMR study of silica phase

Solid state NMR of bare silica was studied to provide some insight concerning the additive-stationary phase interaction. By using magic angle spinning (MAS) with cross polarization, proton magnetization can be transferred to diluted hetero nuclei such as ^{29}Si . This allows various surface species on silica to be distinguished. Structural elements of bare silica consist of Q^2 , Q^3 , Q^4 species where the superscripts indicate

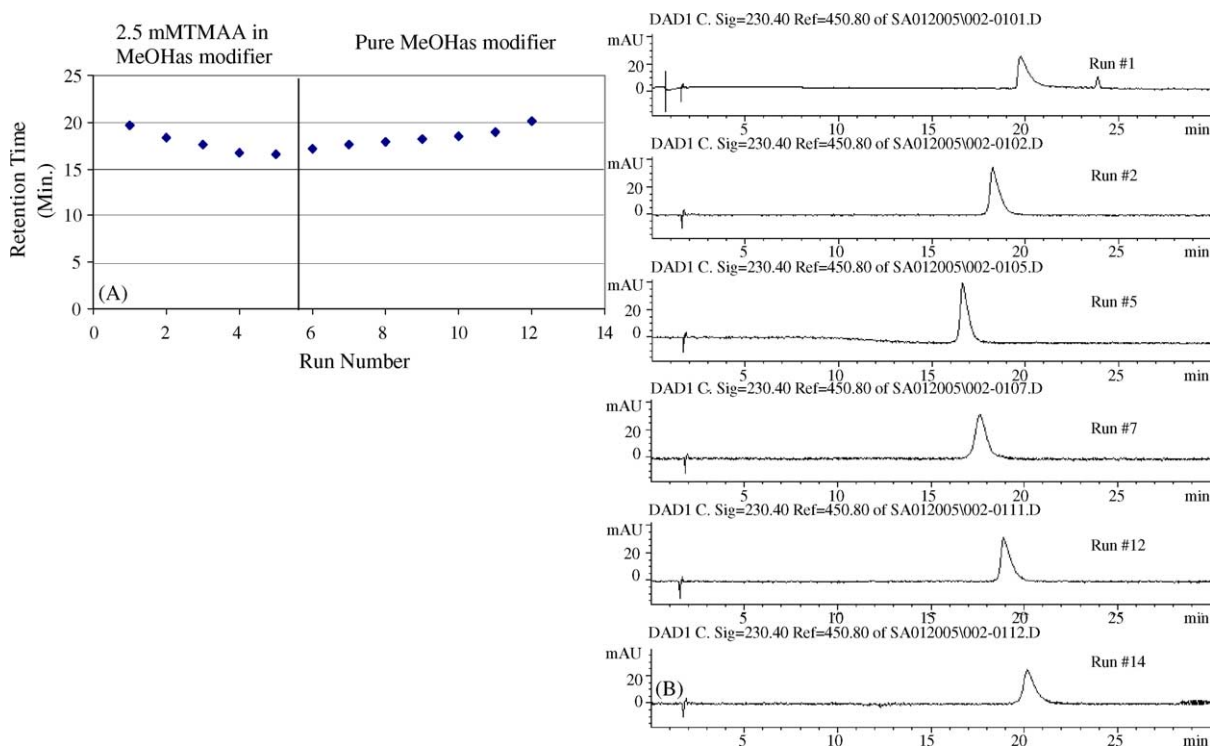


Fig. 5. (A) Retention times for 12 repeated injections of a sodium *p*-toluene sulfonate solution on a silica column. Injections 1–5: the modifier contained 2.5 mM TMAA; injections 6–12: modifier was pure methanol. (B) Selected chromatograms from the fourteen injections.

the number of Si–O–Si linkages (Fig. 7). These three species can be easily distinguished by their corresponding chemical shifts at -92 , -101 , and -110 ppm, respectively [23]. Since all of the spectra were obtained using a fixed number of scans, the peak intensity can be used to compare the abundance of each group. The S/N ratio of Q^3 ($-\text{Si}-\text{OH}$ groups) was measured and was normalized to the peak intensity per mg of each sample. The result is found in Table 1. After being flushed with supercritical CO_2 modified by 15% methanol containing 2.5 mM ammonium acetate for about 60 min, the protons on about 28% of total Si–OH groups were no longer detected. The corresponding values for tetramethylammonium acetate and tetrabutylammonium acetate were 25 and 22%, respectively. We presume that the protons were no longer detected because they were exchanged with ammonium ions. All other chromatographic conditions were the same as previously mentioned. Ammonium acetate modified the largest percentage of free silanol groups and TBAA modified the smallest percentage. This might be explained by steric effects since TBAA is the largest cation, and ammonium the smallest.

3.4. Computational calculation of charge distribution

From our previous research [20], the behaviour of TMAA was quite different from that of AA or TBAA. When negatively charged sulfonates were the probe analyte, they were retained much longer on the silica column with TMAA than with the other two ammonium salts. On the other hand, TMAA provided more rapid elution of the same sulfonates on the Deltabond Cyano column. We believe that modification of the stationary phase is the dominant elution mechanism on the silica column and ion-pair formation is the dominant elution mechanism on the deactivated Deltabond Cyano column. The observed elution behaviours with the various ammonium salts cannot be explained by lipophilic interaction between the analyte and additive-modified-stationary phase or between the ion-pairs and the cyanopropyl stationary phase. It might be explained by different charge distribution on the individual ammonium ion.

CaChe is a computer-aided chemistry modelling program for predicting molecular structure, properties, spectra,

Table 1
 Q^3 peak intensity per mg of silica and the percentage of free silanol groups covered by ammonium additive

	Q^3 peak intensity $I = S/N$	Q^3 peak intensity/mg of sample ($\times 10^{-2}$)	% Of modified Si–OH groups (%)
Untreated silica	31.1	21.7	–
Silica treated with AA	26.4	15.6	28
Silica treated with TMAA	33.3	16.2	25
Silica treated with TBAA	38.5	16.9	22

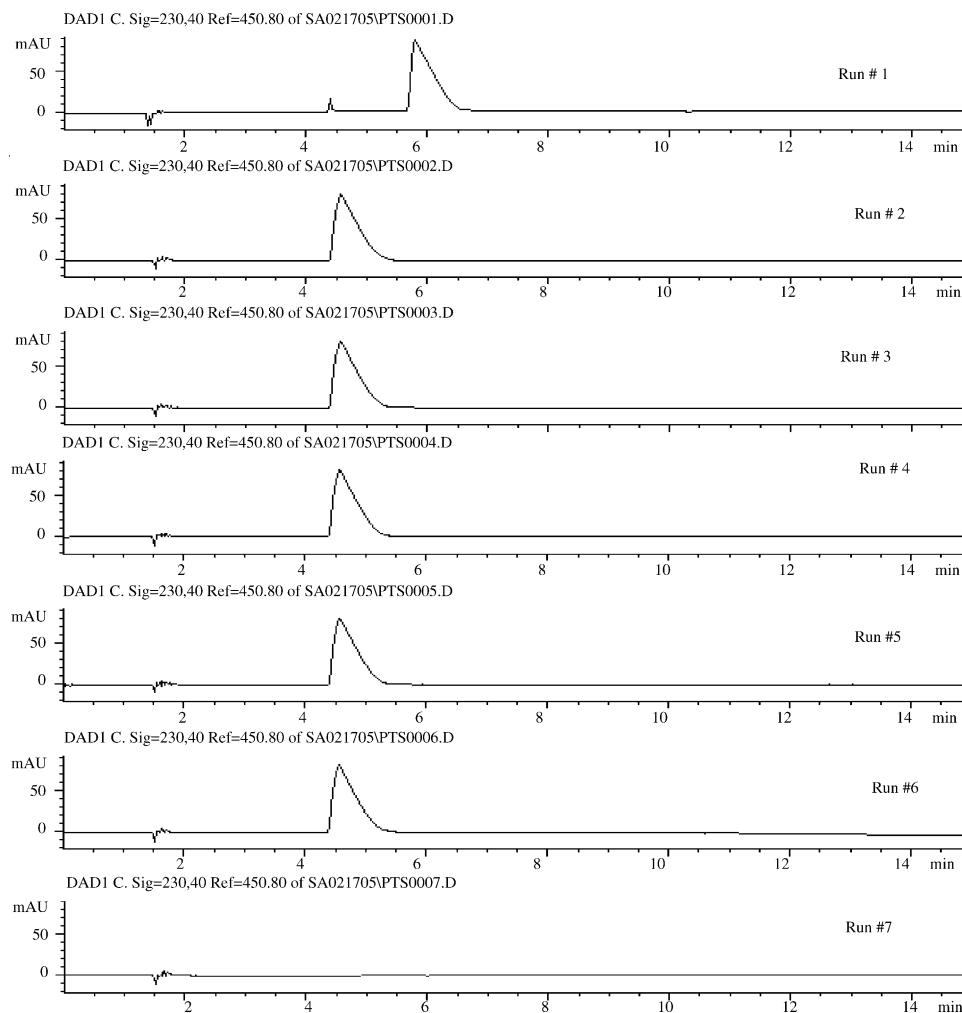


Fig. 6. Repeated injections of sodium *p*-toluene sulfonate on a Deltabond Cyano column. Injections 1–5: the modifier contained 2.5 mM ammonium acetate; injections 6–14: modifier was pure methanol.

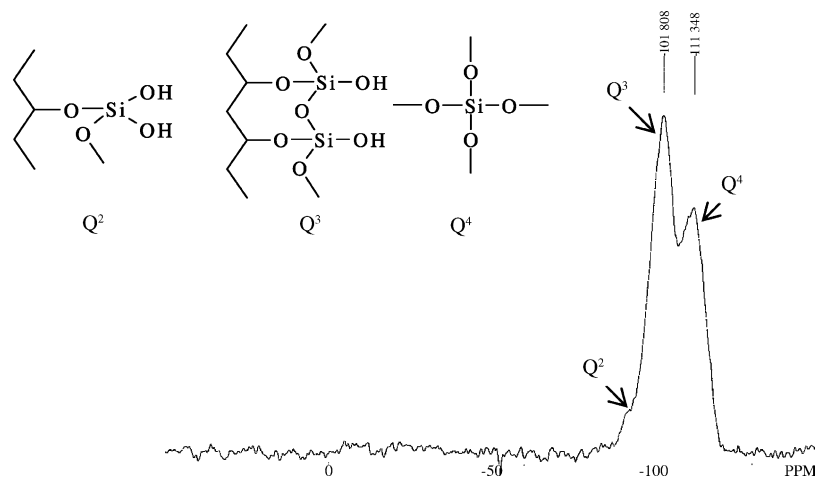


Fig. 7. ²⁹Si CP-MAS NMR spectrum of bare silica packing material.

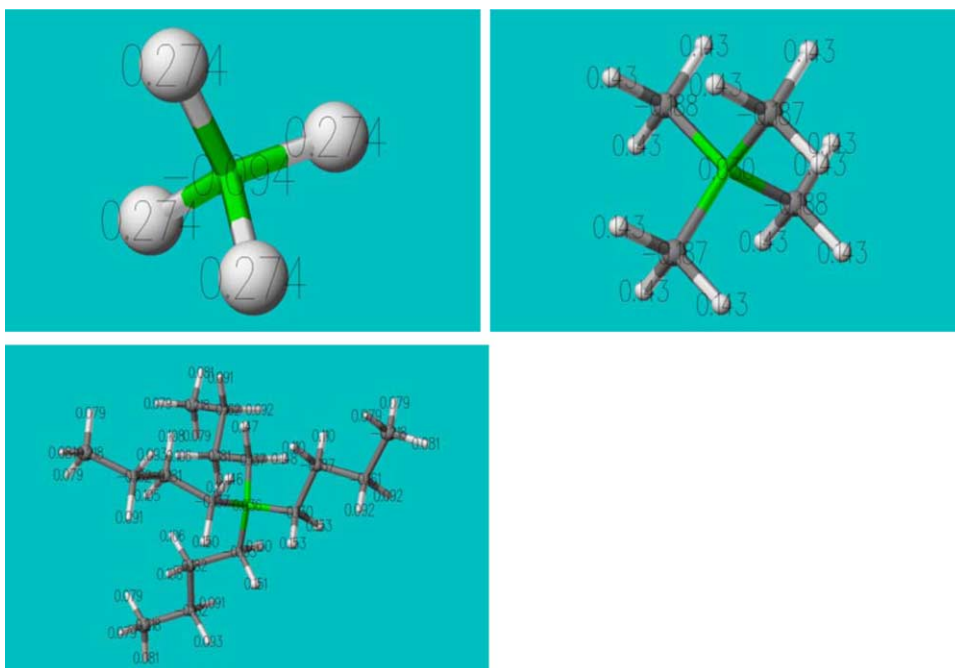


Fig. 8. Optimized geometry of AA, TMAA, and TBAA molecules and partial charges on each atom.

reactions, thermodynamics and kinetics [20]. CaChe allows calculations based on different theories and various methods. The CaChe MOPAC application is a semi-empirical, quantum mechanics package. It determines both an optimum geometry and the electronic properties of molecules by solving the Schrödinger equation using AM1 (Austin Model 1) parameters, MNDO, PM3 and PM5 parameters [26,29]. Molecular geometry and partial charge distribution on the probe ammonium salts were thus calculated using CaChe MOPAC with AM1 parameters, Fig. 8. This calculation is based on gas state parameters. Another method that also uses MOPAC with AM1 parameters but in aqueous solution was also used to calculate the charge distribution on AA, TMAA, and TBAA. This might be a better approximation than the gas-state-based calculations. The use of AM1 parameters neglects the diatomic differential overlap. To gain a more accurate calculation, a DGauss/B88-LYP calculation was performed. DGauss calculations use DFT (density functional theory) methods that provide an approximate solution to the Schrödinger equation using only mathematical approximations. This method is more computationally intensive than MOPAC. The results of the calculations of charge distribution on the probe ammonium cations are listed in Table 2.

All three methods indicated that the tetramethylammonium cation has the largest surface charge of the three ammonium salts. These results helped to explain the chromatographic behaviour. On the silica column, ammonium cations may cause the stationary phase to become positively charged. Since TMAA has the largest surface charge, TMAA-modified silica may retain negatively charged sulfonate analytes longer than silica modified with the other ammonium salts. On the deactivated Deltabond Cyano phase, TMAA may form stronger ion-pairs with sulfonate analytes and help to elute them more rapidly.

The above chromatographic experiments, ^{29}Si solid state NMR on the pressurized fluid treated stationary phase, and computational calculations on the three ammonium salts have shed light on the elution and retention mechanisms of specifically sodium *p*-toluene sulfonate with ammonium salts as CO_2 -based mobile phase additives. Modification of the stationary phase active silanol sites by ammonium ions has been demonstrated to be a major contributor to the overall mechanism. On bare silica, where abundant silanol sites are present, modification of the stationary phase may be the dominant effect, while ammonium ion-induced dipole interaction with the cyano function may be dominant with the more deactivated Deltabond Cyano phase.

Table 2
Partial charge distribution on the three ammonium salts calculated with different theories

Additive	MOPAC/AM1 gas phase	MOPAC/AM1 in water	DGAUSS/B88-LYP
Ammonium	1.096 ^a	1.076 ^a	1.792 ^a
Tetramethylammonium	1.716	1.732	3.144
Tetrabutylammonium	1.067	0.956	2.562

^a Sum of the charges on the terminal hydrogen atoms.

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