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Organically Modified Silica Sol-Mediated Capillary Electrophoresis

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We describe in this paper the use of ormosil (organically modified silica) sols as additives to the run buffer for selectivity manipulations between solutes in capillary electrophoresis. CE systems that contain sol additives in the run buffer can be thought of as pseudocapillary electrochromatography. Three sols based on different types of silanes were studied. Methyltrimethoxysilane (MTMOS)-based sol was found to improve selectivities between various aromatic acids. Aminopropyltrimethoxysilane (APS) sol interacts differently with structural isomers of aromatic acids than does MTMOS. At low pH with APS sol in the run buffer, neutral solutes can be separated, as well. The separation of the neutral solutes seems to be facilitated by the formation of hydrogen bonds between the solutes and the APS sol. APS and *N*-[3-(trimethoxysilyl)propyl]-ethylenediamine (EDAS) affect the separation of the same compounds differently, thus indicating that even small changes of the functional groups of the sol have pronounced effect on the interactions between the sols and the solutes.

Capillary electrochromatography (CEC) is a recently developed separation technique and is a hybrid of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) that combines some of the best features of both techniques.[1−3] CEC has several advantages over either of its parent techniques: It is applicable to the separation of uncharged species, like HPLC. Charged solutes are affected by electric field and by interactions with stationary or pseudostationary phases. The combination of separation mechanisms provides new separation behavior of charged solutes as well as the separation of neutral compounds. In addition, it provides highly efficient separations on microvolumes of sample solution without the need for a high-pressure pumping system, like electrophoresis. Because the run buffer is transported through the capillary by electroosmosis and not by mechanical pumping, we get a flat flow profile that leads to significant reduction in plate height and greater separation efficiencies.

CEC can be classified into two variants. In one variant, the stationary phase is indeed stationary, either in packed capillaries (PCEC)[4,5] or in wall-coated open tubular capillaries (OTCEC).[6−11] The second variant of CEC has no true stationary phase. Rather, the run buffer contains an additional component, which is well-defined and is free to migrate through the capillary. The solutes can interact with this additive by partitioning, adsorption, electrostatic attraction, or by any other type of interactions. Different solutes interact to different extents with the additive, resulting in different effective partition coefficients. Therefore, the run buffer additive behaves as a pseudostationary phase. There are many examples to pseudostationary phase electrokinetic chromatography (PSEC) (see, e.g., refs 12, 13). Micellar electrokinetic capillary chromatography (MEKC)[14,15] is the most known member of the PSEC. In both variants of CEC, the purpose of the additive, either truly stationary or pseudostationary, is to provide additional interaction sites for the solutes. The additional interaction site is not an electrophoretic one.

In this paper, we describe the use of several novel organically modified silicates, prepared by the sol−gel process from different silane precursors, as pseudostationary phases for PSEC. The alkoxysilane precursors that we have used are methyltrimethoxysilane (MTMOS), aminopropyltrimethoxysilane (APS), and *N*-[3-(trimethoxysilyl)propyl]-ethylendiamine (EDAS). All of these alkoxysilanes have three hydrolyzable methoxy groups and an additional functional group that is covalently attached to the cross-linked siloxane backbone after sol formation. The synthesis was carried out in such a way that colloidal suspension (silica sol) was the final product of the reaction. The sol solution, dispersed in the run buffer, serves as a pseudostationary phase in capillary

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electrophoresis. Capillary electrophoresis enhanced by organically modified silicates can be thought of as another form of capillary electrochromatography.

The use of silica sol as run buffer additives is similar to the use of a sol−gel chromatographic phase in PCEC[6−9,16−19] and use of micelles in MEKC.[14,15] Sol−gel composites can be cast onto the inner walls of fused-silica capillaries and used as the stationary phase for open tubular capillary electrochromatography.[6−9] Another approach deals with monolithic silica columns for CEC.[18−20] In all of these cases, the purpose of the additive is to provide additional interaction sites for the solute. The approach to use silica sols as a pseudostationary phase resembles the use of nanoparticles in CE. Neiman et al.[21] recently reported on the use of colloidal gold nanoparticles dispersed in the run buffer for capillary electrophoresis separations. Pumera et al.[22] have extended the use of gold nanoparticles to chip-based capillary electrophoresis. Huber and co-workers[10,11] used polymer-based nanoparticles to coat fused-silica capillaries for use in CE. Fujimoto and Muranaka[23] used commercially available silica gel nanoparticles as run buffer additives. Gottlicher and Bachmann reported on the use of silica-based and polymer particles as pseudostationary phases in electrokinetic chromatography.[24]

Capillary electrophoresis enhanced by organically modified silicates offers some definite advantages over PCEC. In contrast to PCEC, the presence of silica sols in the buffer media obviates the need to pack the capillary with a stationary phase. Since there is no stationary phase, the need for frits and other retaining techniques is eliminated. The pseudostationary phase continuously refreshes itself, thus avoiding aging. Additionally, the moving-bed nature of the system can improve the separation capability (see, e.g., refs 25, 26).

There are also some important differences between MEKC and capillary electrophoresis enhanced by organically modified silicates: (a) Working with conventional micelles requires a minimum surfactant concentration that exceeds critical micelles concentration (CMC). This concentration limitation does not exist in the case of capillary electrophoresis enhanced by organically modified silicates. We should point out that in addition to the ormosils (organically modified silica) described here, there are polymeric micelles that do not require a minimum concentration.[27] (b) Because of the CMC requirement, the micelles in the solution are in a state of equilibrium with free surfactant molecules. As a result, the stability of the micellar phase is limited. The sol, on the other hand, is stable over a period of months. (c) Capillary electrophoresis enhanced by organically modified silicates extends the operable voltage range to much higher fields. The electric field strength in CE is limited, to a large extent, by heat-generation thermal effects induced by the applied voltage and the generated current. In general, the electric current in the silica sol systems is much less than with the micellar systems. (d) Finally, we can easily vary the chemical nature of functional groups attached to the silica-base sol, because there are a very large number of commercially available or readily synthesized ormosil precursors.

There are several reports by Palmer and co-workers[28−31] of using polymeric surfactants based on linear chain silicone (poly(dimethylsiloxane)) backbones as pseudostationary phases for electrokinetic chromatography. The poly(dimethylsiloxane) polymers, either with alkyl sulfate modifiers[29−31] or without[28] were used successfully to separate a large number of polynuclear aromatic hydrocarbons. These silicones differ from the siloxanes used by us by two main characteristics: (1) we use cross-linked sol−gel-derived siloxanes, rather than the linear-chain siloxanes used by Palmer et al.,[28−31] and (2) our sols are either neutral or positively charged, whereas their polymers are negatively charged.

The aim of the present work is to demonstrate that ormosil sols have selectivities that are different from CZE. Indeed, our results clearly indicate that ormosil sols-based pseudostationary phases lead to different selectivities and better resolutions, as compared to similar CZE systems without the sols. More importantly, we show that different sols provide for different selectivities. No attempts were made to optimize the efficiency of the separations or the peak shape of the solutes in any one of the CE modes studied here.

MATERIALS AND METHODS

Capillary Electrophoresis. Analyses were performed on a Bio-Rad BioFocus 2000 capillary electrophoresis system using a UV detector operated at 220 nm. Polyimide-coated fused-silica capillaries, 50-μm i.d., 375-μm o.d. (Polymicro), were employed in the study. The capillary total length was 26.8 cm; the effective length (from injection point to detector) was 22 cm. Samples were injected hydrodynamically by applying 5.0 psi head pressure for 0.4 s. Electrophoretic separations were performed at applied voltages of 10 kV. Between the runs, the capillary was washed sequentially with sodium hydroxide, water, and run buffer, each for a 1-min period. Once the capillary was treated with silica sol, it was washed between the runs only with the run buffer for 1 min. Data were recorded using a PC.

pK_b Measurements. Titration of APS and EDAS sols was performed using a pH meter (Mettler−Toledo AG, Switzerland) equipped with a Mettler−Toledo InLab413 pH electrode.

Electrospray Mass Spectrometry. A Finnigan (San Jose, CA) LCQ quadrupole ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface was used for data acquisition. The ESI was operated in positive ion mode. The spray voltage was set at 3.5 kV, and capillary voltage was 40 V. The source

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temperature was set to 100 °C after a detailed study that will be described elsewhere. Mass spectra were acquired by scanning the mass analyzer from m/z 50 to 2000 with 5 total microscans and a maximum injection time of 50 ms. The MS flow rate was 10 μL/min.

Small-Angle X-ray Scattering (SAXS). Measurements were obtained using Ni-filtered CuK radiation (SEIFERT ID 3000 generator), shone through an evacuated compact Kratky camera (Anton PAAR). A linear position-sensitive detector (MBRAUN) was used to record the scattering patterns.

Transmission Electron Microscopy (TEM). TEM was carried out using a Tecnai 211 (Philips, The Netherlands) microscope operated at 120 kV and equipped with a MegaView CCD camera (Soft Image System, Germany). The TEM samples were prepared by dip-coating a 300 mesh cupper grid, covered with Formvar and carbon, with the sol solution.

ζ-Potential Determination. Measurements were carried out on Zetasizer S model ZEM5 (Malvern Instruments Ltd., U.K.).

Chemicals. Nitrobenzene, benzenediols, *o*-chlorophenol, *o*-, *m*-, and *p*-aminophenols, toluic acids, and hydroxybenzoic acids were purchased from Aldrich (Israel). *m*- and *p*-Chlorophenols and *o*- and *m*-dinitrobenzenes were purchased from Fluka (Switzerland). Phenol was purchased from Baker (NJ). *o*-Nitrophenol was obtained from Merck (Germany). *m*- and *p*-Nitrophenols were obtained from Eastman (NY). MTMOS, APS, and EDAS were obtained from Aldrich (Israel).

The run buffer was prepared by adjusting the pH of a 0.02 or 0.04 M sodium phosphate monobasic monohydrate solution (Aldrich, Israel) to 3.5 with concentrated (15 M) phosphoric acid (Baker, Holland). The run buffer contained 15% methanol (Baker, Holland). For preparing buffer at pH 11, 0.04 M sodium phosphate dibasic heptahydrate solution (Aldrich, Israel) was titrated with 1 M sodium hydroxide solution (Frutarom, Israel). This buffer also contained 15% methanol.

The preparation of the buffers and samples used TDW quality water. The solutes were dissolved in the run buffer at concentration of 10^{-3} M. Buffers and sample solutions were filtered through 0.45-μm syringe filters. Sodium hydroxide for capillary washing was obtained from Frutarom (Israel). Hydrochloric acid for pK_b determination was purchased from Baker (NJ).

In most of the studies, mesityl oxide (Aldrich, Israel) was used as the neutral electroosmotic flow marker.

Preparation of Ormosil Sols. Silica sols were prepared by mixing different concentrations of the silane precursors with phosphate buffer with an initial pH of 3.5. The concentration of phosphate salt was 0.02 M, except in the experiment using APS sol at pH 3.5, in which the concentration of phosphate salt was 0.04 M. Methanol in buffer (15% v/v) served as a homogenization agent. The solutions were sonicated for ~5 min and refrigerated for 1 week, during which time the polymerization process was carried out. At the end of this period, the solutions were ready for use as run buffers. The concentrations of silane precursors were relatively low (up to ~2 M) so that the polymerization process ends at the stage of the sol formation. After mixing with the silane precursors, the pH of APS and EDAS solutions increased to 11 as a result of the protonation of the amine functional groups. The pH of MTMOS solution did not change and remained around 3.5. To examine electrophoretic separations at acidic pH, the solution of APS was titrated to pH 3.5 with 15 M phosphoric acid before being used as the run buffer. It should be mentioned that the percentage of methanol in the run buffer containing the sols exceeds 15 vol %, since methanol is generated during sol formation.

Intermediate pHs were not examined because in the pH range of ~9−5, the sol solutions are opaque as a result of the beginning of gel formation. These opaque solutions caused serious baseline fluctuations in the electrophoretic detection system and will have to be taken into account in the actual applications.

The different sol solutions used in the studies were obtained by the appropriate dilution of the sol reaction solutions. Because of the polydispersity of the sols, their concentrations in solution could not be determined accurately. Thus, the concentration values used throughout this study are based on the original *monomeric* concentrations. It should be noted here that the concentration of the sol monomer in the sol reaction is substantially higher than that of typical MEKC reagent; for example, 2 M, as compared to 50 mM in the case of sodium dodecylsulfate.

Buffers used for sample preparation did not contain the sols. However, the pH of the sample buffers was identical to that of the run buffer.

Initial Capillary Treatment. A 50-μm i.d. fused-silica capillary of the desired length was mounted into the CE system and washed sequentially with 1 M sodium hydroxide for silanol activation (10 min), water (10 min), and buffer (10 min). After the washing stage, the run buffer solution was pumped through the capillary and the high voltage (10 kV) was turned on for 1 h. At this stage, the capillary was ready for solute injections.

RESULTS AND DISCUSSION

Silica Sols Characterization. To gain a better understanding about the sols used in this study, we have examined the three ormosil networks by SAXS, TEM, electrospray ionization mass spectrometry (ESMS), and by ζ-potential measurements.

SAXS analysis of 1.7 M APS at pH 11, of 1.3 M EDAS at pH 11, and of 2.1 M MTMOS at pH 3.5 revealed logarithmic (fractal-like) intensity vs size dependence over the range 0.3−3 nm, with very small fractal dimensions (0.25−0.35), indicating that the ormosil particles are loosely packed and that all three ormosils exhibit wide distributions of particle sizes. The logarithmic intensity versus the size plots is available as Supporting Information.

A typical ESMS spectrum of 0.017 M APS-derived sol is presented in Figure 1. The size of the different oligomers is indicated in this spectrum. Fuller assignment is presented as Supporting Information, along with the corresponding spectra of the other sols used in this study.

The ESMS data show that the aminosilane sols attain steady-state distributions within <1 h. Subsequent ESMS studies revealed the same spectra after additional time intervals, which support and explain our observation on the stability of the sols over prolonged periods. The spectra also reveal wide distributions of low-molecular-weight oligomers that exist even after prolonged aging of the sol.

TEM studies provided further support for our conclusion regarding the noncompact, loose character of the sols. TEM studies of a thin sol−gel film deposited by dip-coating on copper

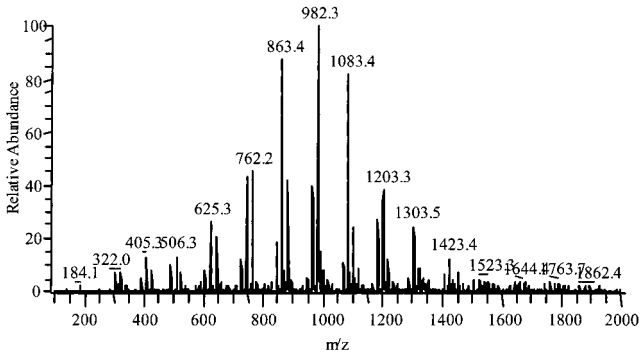

Figure 1. ESMS spectrum of 0.017 M APS-derived sol. Peak assignment is provided in the Supporting Information.

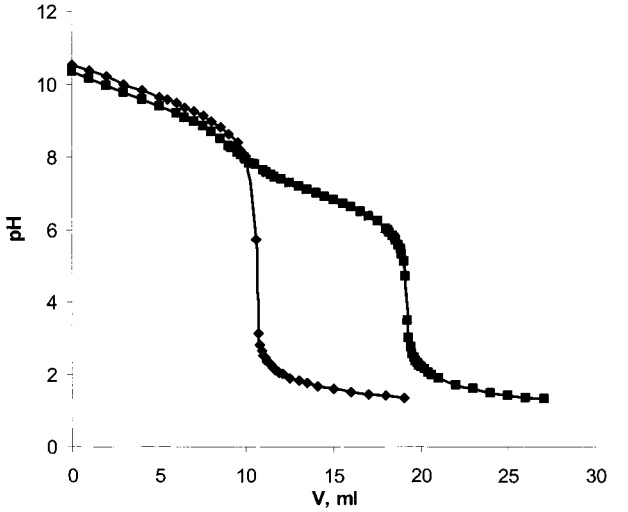

Figure 2. Titration curves of APS and EDAS. The concentration of APS was 0.28 M and of EDAS was 0.22 M.

grids did not reveal any distinct particle features that are usually associated with the formation of compact aggregates.

We believe that the open, not fully cross-linked, loose character of these sols is beneficial for capillary electrophoresis studies. Such a structure provides good accessibility of the solutes to the (amine) functional groups on the silicate backbone and also leaves a large number of silanols that may interact with the solutes and alter the electrophoretic mobility of the sols.

pK_b Determination. Of the sols used in this study, MTMOS has a hydrophobic methyl group and APS and EDAS have amine groups that can be ionized. The selectivities obtained with the various sols are a function of the sols' charges. To the best of our knowledge, there has been no prior reported effort to estimate the basic dissociation constants of the APS and EDAS derived sol. pK_b determination for these alkoxysilanes was carried out by the titration of the appropriate aqueous silane solutions with hydrochloric acid. The titration curves are shown in Figure 2. From the data, we calculate the pK_b of APS to be ~3.3. This pK_b value of APS is in close agreement with the pK_b value of *n*-propylamine.[32] In the case of EDAS, pK_{b1} is ~4.3, and pK_{b2} is ~6.7. These pK_b values are in good agreement with the values for some similar compounds, such as ethylenediamine.[33] The shape of the titration curves in Figure 2 indicates that the chemical environment of the sol molecules is not well-defined, and as a result, there is a relatively wide range of pK_b values for each amine group.

Electrophoretic Separations. Before discussing the effect of the silica sols on the behavior of the solutes, we need to define some mobility and selectivity terms. The electrophoretic mobility, μ_{ef}, is the mobility of the solute in the neat run buffer under the influence of an electric field. With sols in the run buffers, the migration of the solute can be modified by interactions with the additive in the buffer. The mobility in this case will be termed apparent mobility, μ_{ap}. The mobility of neutral compounds is the result of their interactions with the sols, and it will be termed acquired mobility, μ_{acq}. The observed mobility, μ_{obs}, signifies the mobility calculated from the experimental migration time, and it takes into account the electroosmotic flow as well; that is, $\mu_{obs} = \mu_{ap} + \mu_{eof}$ or $\mu_{obs} = \mu_{acq} + \mu_{eof}$. Mobilities with a positive sign indicate migration toward the cathode while a minus sign signifies an anodic migration.

The electrophoretic selectivity, α, is defined as the ratio of the electrophoretic mobilities of two neighboring solutes in the electropherogram. Similarly, the apparent selectivity, α_{ap}, is the ratio of two neighboring apparent mobilities; the acquired selectivity, α_{acq}, is the ratio of two neighboring acquired mobilities; and the observed selectivity, α_{obs}, is the ratio of two neighboring observed mobilities. In the presence of electroosmotic flow, the observed selectivity can be substantially different from the electrophoretic or apparent selectivity. The EOF vector can either enhance or diminish the electrophoretic or apparent selectivity.

Without the sols in the run buffer, the solutes migrate in the CE system as expected. The positively charged solutes migrate toward the cathode and reach the detector before the EOF marker [mesityl oxide, MO]. All of the negatively charged solutes studied except *o*-hydroxybenzoic acid, since their $|\mu_{ef}|$ is less than their μ_{eof}, also migrate toward the cathode, but reach the detector after MO. For *o*-hydroxybenzoic acid, $|\mu_{ef}| > \mu_{eof}$, and as a result, this solute migrates toward the anode. To observe *o*-hydroxybenzoic acid, the polarity of the electrodes needs to be reversed.

Methyltrimethoxysilane (MTMOS)-Based Sols. The studies with MTMOS were performed at pH 3.5. MTMOS is a neutral silane, and our expectation was that MTMOS sol would present a hydrophobic medium to the solutes. Moreover, we assumed that the electroosmotic flow might change in magnitude but not in direction. However, once MTMOS-based sol was introduced to the capillary in the run buffer, it was found that all neutral solutes, including MO, migrated together toward the anode. There are two possible explanations for the observed anodic mobility of the neutral solutes: (a) Some of the silanols in the MTMOS sol are ionized, thus rendering the sol negative. As such, the sol would migrate in the anodic direction, and if the neutral solutes partition between the sol and the run buffer liquid, they will be carried away with the sol toward the anode. (b) The MTMOS coats the inner wall of the capillary, and small amounts of cationic impurities in the sol make the adsorbed layer positively charged. As a result, the EOF will be reversed toward the anode. Evidence supporting the first hypothesis is the slightly negative charge of the MTMOS sol as observed with the Zetasizer; a 0.4 M solution of MTMOS sol at pH 3.5 had a ζ potential of −7.4 mV. However, the fact that

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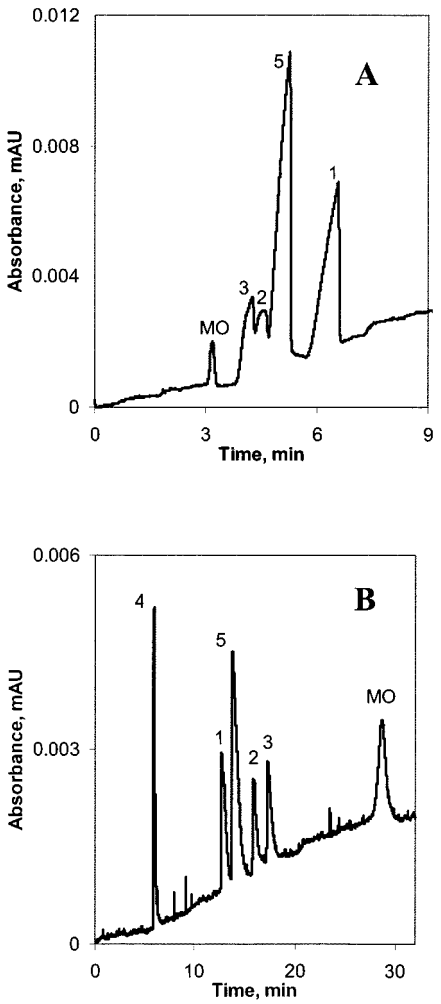

Figure 3. Electropherograms of toluic and hydroxybenzoic acids in (A) fused silica without an additive and (B) using a run buffer containing 1.69 M MTMOS-based sol. The concentration of phosphate salt in the run buffer was 0.02 M. The detector was on the cathodic side of the capillary in (A) and on the anodic side of the capillary in (B). Peak identification: (1) *o*-hydroxybenzoic acid, (2) *m*-hydroxybenzoic acid, (3) *o*-toluic acid, (4) *m*-toluic acid, (5) *p*-toluic acid. MO stands for the EOF marker mesityl oxide.

all the neutral solutes migrate at the same velocity, which means identical partition coefficients for all the solutes, tends to favor the explanation of an adsorbed MTMOS layer. In addition, the observation that the neutral solutes still migrate toward the anode when the MTMOS containing run buffer is replaced with a neat run buffer supports the adsorbed layer assumption. In fact, the migration behavior of all of the solutes tends to support the MTMOS adsorption mechanism. All of the positively charged solutes still migrate toward the cathode but at a lower observed mobility, and all of the negatively charged solutes migrate toward the anode at observed mobilities that are higher than those of the neutral solutes.

The negatively charged solutes were most affected by the presence of the MTMOS sol. Figure 3 shows the separation of the isomers of toluic and of hydroxybenzoic acids without MTMOS (Figure 3A) and with 1.69 M MTMOS sol in the run buffer (Figure 3B). At the pH of the study (3.5), the extents of ionization of these acids are 12, 15, and 28% for *p*-, *m*- and *o*-toluic acid, respectively, and 21 and 77% for *m*- and *o*-hydroxybenzoic acid. In the untreated capillary, Figure 3A, the electroosmotic mobility was greater (in absolute terms) than electrophoretic mobilities of the acids studied here, with the exception of *o*-hydroxybenzoic acid. As a result, the acids migrate in the cathodic direction, lagging behind the EOF marker, MO. The *o*-hydroxybenzoic acid does not appear in the electropherogram, since its electrophoretic mobility is greater than the electroosmotic mobility of the buffer, and thus, it migrates in the opposite direction to the anode.

We see from Figure 3A that without the silica sol in the buffer, the acids are only partially separated. The addition of MTMOS to the buffer caused a reversal of the electroosmotic flow, and as a result, the voltage polarity was switched to the $- \rightarrow +$ direction. The effect of the MTMOS sol on the observed behavior of the acids is evident from Figure 3B; the migration times are longer but the observed selectivities are much greater. In addition to the better resolutions, better peak symmetries were observed, as well. Note that the migration order is consistent with the degree of ionization of the acids. With the MTMOS in the run buffer, all the acids studied appear in the electropherogram, and all elute before MO. The lower signal observed in Figure 3B is most likely due to the presence of the sol in the run buffer.

We should stress that no attempt was made to optimize the separation performance of the CE system either without or with the sol in the run buffer. Our aim is to demonstrate the effect of the presence of the sol in the run buffer on the behavior of the solutes.

The presence of MTMOS in the run buffer has a significant effect on the apparent and the observed selectivities. For example, part A of Figure 4shows the apparent selectivity between some acidic solutes, whereas part B gives the observed selectivities between the same solutes. In part A, the apparent selectivities at zero MTMOS concentration (no sol in the run buffer) are actually the electrophoretic selectivities. We see from the figure that the apparent selectivities are substantially different from the electrophoretic selectivities. This last observation means that the presence of the sol in the run buffer influences significantly the mobilities of the solutes as a result of sol−solute interactions.

In Figure 4B, the observed selectivities at zero MTMOS concentration (no sol in the run buffer) are due to the interplay between the electrophoretic and electroosmotic mobilities. All other values in the figure are due to the interplay between the apparent and the electroosmotic mobilities. Since the electroosmotic flow changes direction in the presence of MTMOS, there is a reversal in the migration orders. Thus, those α_{obs} values that are >1 without the MTMOS in the run buffer become <1 in the presence of the sol, and conversely, α_{obs} values that are <1 become >1. We see from Figure 4B that the presence of the sol in the run buffer does change the observed selectivities between the solutes.

Aminopropyltrimethoxysilane (APS) and *N*-[3-(Trimethoxysilyl)propyl]-ethylenediamine (EDAS)-Based Sols at pH 11. Both APS and EDAS possess a primary amine functional group. In addition, EDAS also has a secondary amine group. Consequently, the pH of the sol solutions at the preparation stage is ~11, and this was the pH of the studies comparing the two sols. The APS sol system was investigated at pH 3.5, as well. The EDAS sol system was not investigated at pH 3.5 because of the

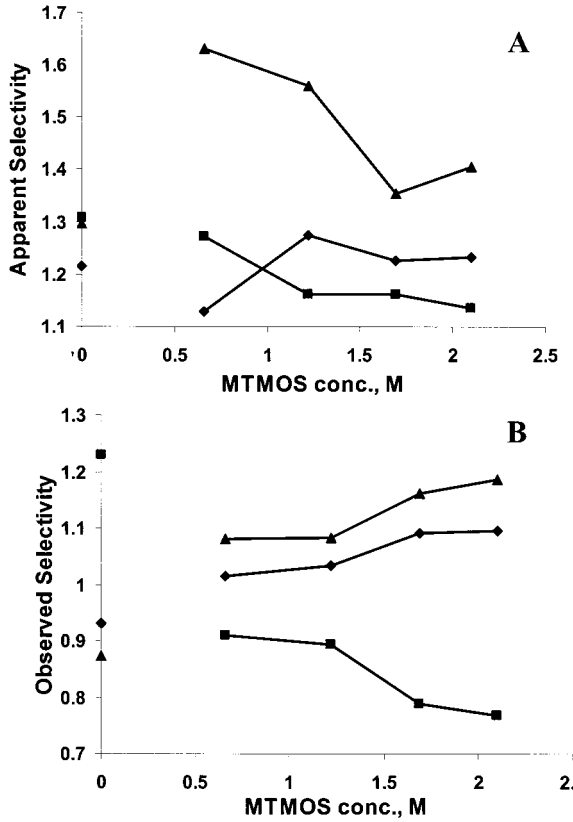

Figure 4. Apparent (A) and observed (B) selectivities of some acidic solute acids versus MTMOS sol concentration. The solute acid pairs are ◆, *m*-toluic/*p*-toluic; ■, *o*-toluic/*m*-hydroxybenzoic; and ▲, *m*-hydroxybenzoic/*m*-toluic acids.

large amount of acid needed to lower the solution's pH, which would have changed the ionic strength significantly. Intermediate pHs were not examined, because in the pH range of ~9−5, the sol solutions are opaque, most likely as a result of the beginning of gel formation. These opaque solutions caused serious baseline fluctuations in the electrophoretic detection system. With APS, since at each of the pHs studied here the nature of the sols is different, no attempt was made to compare directly the performances of the sol at the two different pHs studied.

At pH 11, APS is ~33% ionized, but EDAS is only ~5% ionized. The silanol groups on the capillary wall are completely ionized at pH 11. Therefore, the sols, once entered into the capillary, interact with the negatively charged silanols to produce a hydrophobic surface that is essentially uncharged. As a result, the EOF is quenched, and neutral solutes, including the EOF marker MO, do not reach the detector within reasonable time. Similarly, since the basic solutes that we have studied here are not ionized at pH 11, they do not migrate under the conditions of this experiment and could not be detected.

At pH 11, the APS sol can be used for the separation of acidic compounds. It is of interest to compare the separation obtained with the sol system and with conventional CE at this pH. Figure 5 shows several electropherograms of three isomers of toluic acid and of two isomers of hydroxybenzoic acid. The bottom electropherogram in Figure 5 was obtained using an untreated capillary. Without the sol in the run buffer, there is electroosmotic flow, which is greater in magnitude than the anodic electrophoretic mobility of all the solutes except *o*-hydroxybenzoic acid. Therefore,

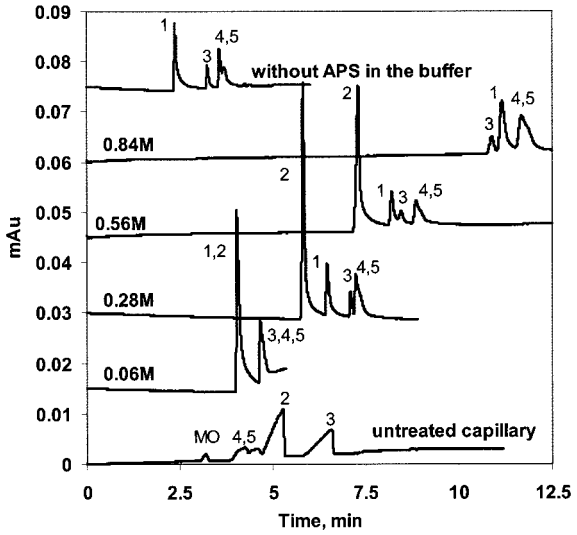

Figure 5. Electropherograms of toluic and hydroxybenzoic acids using run buffer containing different concentrations of APS sol. The concentration of phosphate salt in the run buffer was 0.02 M. The detector was on the cathodic side of the capillary in the electropherogram without the additive in the buffer and on the anodic side of the capillary in all APS-related electropherograms. Peak identification: (1) *o*-hydroxybenzoic acid, (2) *m*-hydroxybenzoic acid, (3) *o*-toluic acid, (4) *m*-toluic acid, and (5) *p*-toluic acid. MO stands for the electroosmotic flow marker mesityl oxide. Note that solute 2 was missing from the sample mixture in the case of the top two electropherograms.

without APS, the solutes migrate toward the cathode, except *o*-hydroxybenzoic acid, which migrates toward the anode and does not appear in the electropherogram. The shape of the peaks and the lack of resolution should be noted.

When the buffer containing APS is introduced to the capillary, the EOF stops, and all of the acidic solutes, being negatively charged, reverse their migration and move toward the anode. In Figure 5, all of the APS sol-related electropherograms were obtained by reversing the polarity of the electrodes. From Figure 5, we see that as we increase the concentration of the APS sol in the run buffer, the migration times increase proportionately. At the same time, there is a noticeable change in the migration pattern and in the selectivities between the solutes. As an example, we will follow the behavior of *o*-toluic acid. At an APS concentration of 0.06 M, *o*-toluic acid elutes together with the other two isomers of the acid. At 0.28 M APS, there is a partial separation between *o*-toluic acid and the other two isomers. At 0.56 M APS, *o*-toluic acid is completely resolved from the other two isomers. Increasing the concentration of APS to 0.84 M further improves the resolution between *o*-toluic acid and the other two isomers of the acid. Examination of Figure 5 reveals that although the migration times of all of the toluic acid isomers increase with increasing APS concentration, the rate of increase is the smallest for the ortho isomer. It should be noted that at an APS concentration of 0.84 M, *m*-hydroxybenzoic acid (peak 2) was not included in the mixture injected.

The rate of change in the migration time in the case of *o*-hydroxybenzoic acid is quite high. In fact, at a low concentration of APS in the run buffer, the apparent mobility of *m*-hydroxybenzoic acid is faster than that of *o*-toluic acid. However, at a high APS concentration, the apparent mobility of the *o*-toluic acid is faster than that of *o*-hydroxybenzoic acid, and the elution order

reverses. Note also that as we increase the APS concentration, we increase the resolution between *o*- and *m*-hydroxybenzoic acid, as well.

As mentioned above, with APS in the run buffer, at ph 11, there is no appreciable EOF. Therefore, the changes in the migration times of the solutes are due to the presence of APS in the run buffer and the interactions between the solutes and the sol. The sol is positively charged, but the solutes are negatively charged; therefore, the interactions between the sol and the solutes can be relatively strong. These interactions modify the mobilities of the solutes to give the apparent mobilities as discussed in the Electrophoretic Separations section above.

The top electropherogram in Figure 5 shows a separation of the acids in the same capillary as in the other electropherograms, with run buffer not containing APS sol. As in the case with 0.84 M APS, *m*-hydroxybenzoic acid was not included in the mixture of analytes. The EOF was still depressed, and the solutes kept migrating in the anodic direction, all of which indicates that APS sol is still adsorbed on the capillary wall. Even at this condition, we observe a separation between *o*-toluic acid and the other two isomers of that acid, but the *o*-hydroxybenzoic acid now migrates faster than the toluic acids. In general, all of the migration times decrease substantially. This last electropherogram demonstrates the most pronounced effect that the presence of APS sol in the run buffer has on the migration of the solutes. The extent of the separation is easily controlled by tuning the amount of APS in the run buffer.

As indicated above, APS sol contains a primary amine functional group. It is, therefore, useful to compare APS to another sol that also possesses amine groups to establish whether it is the amine group or the backbone that is responsible for the selectivities obtained. We decided to examine EDAS, which contains a primary and a secondary amine group. As solutes, we used phenol derivatives that at pH 11 are ionized. Part a of Figure 6 shows the separation of structural isomers of nitrophenols, chlorophenols, and benzenediols in APS, whereas part b shows the same using EDAS sol. The negatively charged phenol derivatives migrate toward the anode. From Figure 6, we see that the migration pattern in each sol system is different. For example, with APS, *p*-nitrophenol migrates faster than *o*-nitrophenol, whereas the opposite is the case with the EDAS sol. In addition, *p*-nitrophenol, *m*-nitrophenol, and *o*-chlorophenol are well-separated with the APS sol but not with the EDAS sol. In general, the peak shapes are better in the APS system. From the differences in the selectivities and in the migration patterns we conclude that the interactions between the solutes examined here and the two sols are substantially different. The migration behavior is dictated not only by electrostatic interactions but also by the interactions of the solutes with the polymeric network of the sol. Peterson and Palmer[34] and Wang et al.[9] among others also observed differences in the interactions between the same solutes and different polymeric media.

APS Sol at pH 3.5. We have examined the effect on the separation of using the APS sol at pH 3.5. At this pH, the amine groups of the sol are fully ionized. After the introduction of the run buffer containing the APS sol, the electroosmotic flow reverses its direction from the cathode to the anode. This reversal of the EOF is indicative of adsorption of the APS sol on the capillary wall. Unlike at pH 11, the adsorbed APS at pH 3.5 has residual positive charges, since the sol is completely ionized.

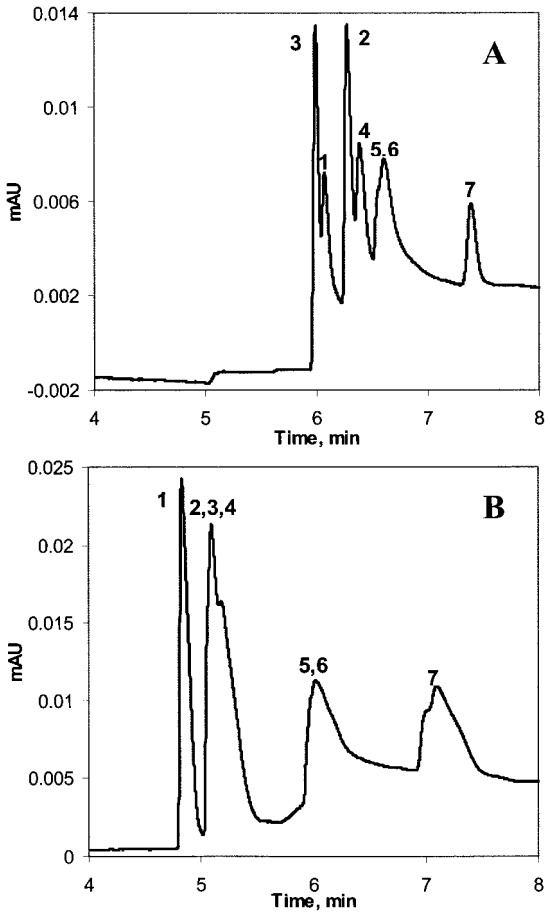

Figure 6. Electropherograms of some substituted phenols in (A) 0.28 M APS in the run buffer and (B) 0.22 M EDAS in the run buffer. In both cases the pH of the run buffer is 11. The concentration of phosphate salt was 0.02 M. The detector was on the anodic side of the capillary. Peak identification: (1) *o*-nitrophenol, (2) *m*-nitrophenol, (3) *p*-nitrophenol, (4) *o*-chlorophenol, (5) *m*-chlorophenol, (6) *o*-benzenediol, (7) *m*-benzenediol.

The presence of APS affects the apparent mobilities of ionized solutes. More importantly, we find that APS interacts with neutral solutes, causing them to migrate and to separate. The interaction between APS sol and neutral solutes presents difficulties in the determination of μ_{eof}, since MO is affected by the sol. As a result, we determined the EOF mobility using the methanol disturbance peak as the EOF marker. The methanol disturbance peak migrates faster than all other neutral solutes and the positively charged solutes. Figure 7 shows the separation of some nitro-substituted benzenes, MO, and the methanol disturbance peak. We should point out that although the observed mobilities of the neutral solutes are negative (indicating a migration toward the anode), the acquired mobilities of these solutes are positive (migration toward the cathode), as is the mobility of the APS sol.

In Table 1, the first column of values shows the electrophoretic mobilities of some of the solutes investigated. The second column of values gives, for the same solutes, the apparent (the charged solutes) and the acquired (the neutral solutes) mobilities when the sol is in the run buffer. The magnitude of the acquired mobility is related to the capacity factor, k', of the solutes between the sol

(34) Peterson, D. S.; Palmer, C. P. *J. Chromatogr. A* **2001**, *924*, 103−110.

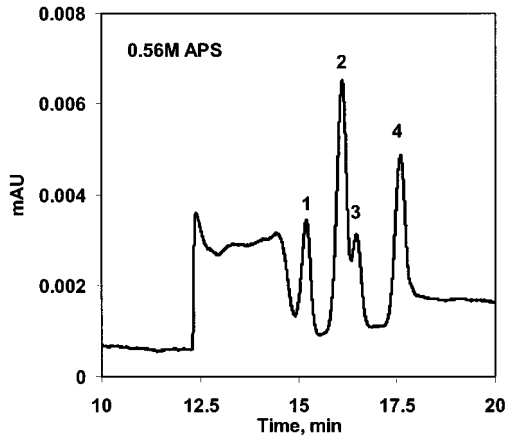

Figure 7. Electropherogram of neutral solutes using 0.56 M APS in the run buffer. Buffer pH is 3.5. The concentration of phosphate salt was 0.04 M. The detector was on the anodic side of the capillary. Peak identification: (1) MO, (2) *m*-dinitrobenzene, (3) nitrobenzene, and (4) *p*-dinitrobenzene. The first wide peak on the electropherogram corresponds to methanol.

Table 1. Electrophoretic, Apparent, and Acquired Mobilities[a] of Some Solutes Obtained without and with APS in the Run Buffer[b]

	run buffer without APS	APS in the run buffer, 0.28 M
electroosmotic mobility	3.4×10^{-4}	-9.1×10^{-5}
o-toluic acid	-1.5×10^{-4}	-7.0×10^{-5}
m-toluic acid	-1.1×10^{-4}	-5.4×10^{-5}
p-toluic acid	-1.0×10^{-4}	-4.5×10^{-5}
o-hydroxybenzoic acid		-1.2×10^{-5}
m-hydroxybenzoic acid	-1.3×10^{-4}	-5.4×10^{-5}
o-nitrophenol	0	8.9×10^{-6}
p- and *m*-nitrophenol	0	6.6×10^{-6}
o-benzenediol	0	5.0×10^{-6}
p- and *m*-benzenediol	0	2.9×10^{-6}
p-, *m*-, and *o*-chlorophenol	0	3.4×10^{-6}
o-dinitrobenzene	0	7.5×10^{-6}
m-dinitrobenzene	0	5.3×10^{-6}

[a] In $cm^2\ sec^{-1}\ V^{-1}$. [b] The pH of the phosphate (0.04 M) run buffer is 3.5. The electroosmotic mobility with the neat run buffer is 3.4×10^{-4} $cm^2\ sec^{-1}\ V^{-1}$ and with the APS containing run buffer, -9.1×10^{-5} $cm^2\ sec^{-1}\ V^{-1}$. The methanol disturbance peak is used as the EOF marker.

and the liquid run buffer and to the electrophoretic mobility of the sol, $\mu_{ef(sol)}$.

$$\mu_{acq} = \left(\frac{k'}{1+k'}\right)\mu_{ef(sol)} \quad (1)$$

It is of interest to note that although the various chemical groups are separated from each other (e.g. nitrophenols from chlorophenols), within a group, not all the isomers separate. For example, in the case of the nitrophenols, the ortho isomer is separated from the meta and para isomers, which comigrate. On the other hand, with the chlorophenols, all ofthe isomers comigrate. Furthermore, from the table, we see that in cases in which the structural isomers do separate, the ortho isomer has the largest acquired mobility, which indicates a larger partition coefficient and more time spent on the cathodically migrating APS sol than in the run buffer. Our results indicate that aromatic solutes with two functional groups in the ortho position, which are capable of forming hydrogen bonds with the sol, interact more strongly with the sol and are retained longer in the capillary than the meta or para isomers. Wang and co-workers,[9] in their study on polyamine coated capillaries, also have separated nitrophenols and benzenediols. At pH 8, they show a complete separation of these phenolic compounds. Depending on the nature of the coated polyamine, the ortho isomers migrated the slowest, as compared to the other structural isomers. Wang et al.[9] offer hydrogen bonds as a partial explanation of the observed migration pattern.

CONCLUSIONS

We have prepared organically modified silica sols (ormosils) by the sol−gel process. These sols are added to the run buffer as pseudostationary phases in PSEC. These sols, when in the run buffer, coat the walls of the capillary, as well. The presence of any of the ormosil sols changes the apparent mobilities of the charged solutes that were studied here. The change in the observed mobilities is due to changes in the electroosmotic mobility, resulting from the adsorption of the sols onto the capillary wall, and is also due to the interactions between the sols and the solutes. The changes in the apparent mobility of the solutes lead to improved selectivities, to better resolutions, and to migration order reversals. Often, the peak shape is also improved in the presence of the sol in the run buffer. Most importantly, it is shown that the migration patterns in various sol systems are different at the same buffer pH. The difference in the migration behavior between the various sols is due to specific interactions between the solutes and these sols. Neutral solutes can be separated with APS sol at pH 3.5. Aromatic solutes with two functional groups in the ortho position that can form effective hydrogen bonds with the APS sol elute after the other isomers of the compounds.

During the last two decades, there has been enormous progress in the synthesis of organic−inorganic hybrids by sol−gel technologies and their use in different fields of analytical chemistry. This research sheds light on yet another way by which separation and analytical sciences can benefit from recent advances in sol−gel science. Our results seem to indicate that different sols interact differently with similar solutes. Thus, future research should examine the possibility of tailor-designing sols for specific separations.

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SUPPORTING INFORMATION AVAILABLE

(1) List of oligomers of APS identifies by ESMS, (2) ESMS spectra of MTMOS and EDAS, and (3) SAXS spectra of MTMOS, APS and EDAS. This material is available free of charge via the Internet at http://pubs.acs.org.

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