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Journal of Chromatography A, 959 (2002) 255–261

JOURNAL OF
CHROMATOGRAPHY A

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Novel alkyl-modified anionic siloxanes as pseudostationary phases for electrokinetic chromatography

III. Performance in organic-modified buffers

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Received 18 April 2001; received in revised form 11 April 2002; accepted 12 April 2002

Abstract

Anionic water-soluble siloxanes modified with different amounts of alkyl chains have been used as pseudostationary phases in electrokinetic chromatography. Ionic siloxane polymers with attached alkyl chains of C_8 and C_{12} and having different alkyl chain densities have been employed previously to achieve selective and efficient separations with a range of electrophoretic mobilities and methylene selectivities. In this study, the performance of three alkyl-modified siloxanes is examined in different organic-modified buffers and at differing amounts of organic modifier. The organic modifiers used are acetonitrile and methanol. The siloxanes are stable in these organic solvents and show good mobility and good methylene selectivities even at high concentration of organic solvent. Siloxanes have also been used to separate a mixture of 14 polynuclear aromatic hydrocarbons in an acetonitrile-modified buffer. © 2002 Published by Elsevier Science B.V.

Keywords: Pseudostationary phases; Electrokinetic chromatography; Buffer composition; Siloxanes; Polynuclear aromatic hydrocarbons; Alkyl phenyl ketones

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a type of capillary electrophoresis that is able to separate neutral analytes based on their partitioning between the aqueous mobile phase and a pseudostationary phase composed of micelles [1]. MEKC has been widely used and has been applied to a large number of separation problems [2–6]. Polymeric

surfactants have also been applied as pseudostationary phases in electrokinetic chromatography [7–11]. Polymers have many useful properties compared to micelles, including unique selectivity, ability to act without complications of co-micellization [12,13], and application with mass spectrometric detectors [14–16]. One particularly important property of polymeric surfactants is their stability in high concentrations of organic solvent [7–9,17,18]. This stability allows high concentrations of organic solvent to be added to the run buffer, which allows the separation of very hydrophobic analytes, such as polynuclear aromatic hydrocarbons (PAHs) [17–19]. It has been demonstrated that polymeric surfactants give better separations compared to other pseudo-

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stationary phases in organic-modified buffers [17,18].

Recently, we have demonstrated the applicability of water-soluble siloxanes as pseudostationary phases in electrokinetic chromatography [20–24]. Although siloxanes are generally considered as being hydrophobic much work has been done to make siloxanes water soluble [25,26]. Generally, water-soluble siloxanes can be prepared by attaching sufficient amounts of hydrophilic groups to the siloxane backbone to achieve water solubility [27–29]. We have produced a water-soluble siloxane that is capable of providing selective and efficient separations and is stable in the presence of large amounts of organic solvents [22]. We have also produced siloxanes with different anionic functionalities, which produce different electrophoretic and chromatographic properties [23]. In addition, alkyl chains can be added to the siloxane backbone to generate improved electrophoretic and chromatographic properties [23,24].

In previous studies, we have produced amino functional sulfonate siloxanes and have attached various alkyl chains to achieve different selectivities [20,24]. The alkyl chains that have been attached to the backbone include 1-octene (C_8), 1-dodecene (C_{12}), and 1-octadecene (C_{18}). These alkyl chains have been added to the siloxane backbones in concentrations ranging between 10 and 25%. The alkyl-modified siloxanes are soluble in water and have been demonstrated to achieve selective separations in electrokinetic chromatography in pH 9.2 and pH 7.0 buffers [20,24]. The electrophoretic mobility and the methylene selectivity of these siloxanes vary over a wide range. In addition, the selectivities of the siloxanes has been demonstrated to be very different from that of sodium dodecylsulfate (SDS), and the selectivity has been studied by linear solvation energy relationships (LSERs) [20]. It has been demonstrated that a family of polymers can be produced based on a single siloxane backbone [20,23,24].

In this study, we examine the performance of three alkyl-modified siloxanes in the presence of organic-modified buffers. We examine the performance in acetonitrile and methanol. In addition, we demonstrate the utility of the siloxanes to separate hydrophobic analytes such as PAHs.

2. Experimental

2.1. Synthesis and characterization

The siloxanes used in this study have sulfonate ionic groups and have alkyl chains attached to the siloxane backbone at varied densities. The alkyl groups used are octyl (C_8 , named OAGENT) with 20% substitution and dodecyl (C_{12} , named DAG-ENT) with 15 and 25% substitution. The structures of the siloxanes used are given in Fig. 1. The synthesis and characterization of these siloxanes have been described elsewhere [24]. Briefly, an epoxy functionality and the alkyl chain were attached simultaneously to a methylhydrosiloxane by a hydrosilylation reaction. The epoxy group was then reacted with *N*-methyl taurine to produce the ionic water soluble siloxane. The siloxanes were characterized by 1H NMR and FT-IR to confirm the structure and density of alkyl chains attached to the siloxane backbone.

2.1.1. Chromatography

Electrokinetic chromatography experiments were conducted on a Hewlett-Packard (Palo Alto, CA, USA) 3D CE capillary electrophoresis instrument. Fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA) with an inside diameter of 50 μm were used for all studies. The length was 50 cm and the effective length was 42 cm. The UV detector was operated at 244 nm for alkyl phenyl ketones and 215 nm for PAHs. Potentials of between 20 and 30 kV were applied for all separations. The temperature was maintained at 25 °C. The siloxanes were dissolved in the run buffers at a concentration of 1% (w/v) concentration, unless otherwise reported. The buffers were prepared by pipetting 2 ml of 125 mM sodium borate (Sigma, St Louis, MO, USA) buffer into a 5-ml volumetric flask. HPLC grade organic solvent [acetonitrile (Aldrich, Milwaukee, WI, USA) or methanol (Acros, Pittsburgh, PA, USA)] was pipetted in the desired amount and the buffer was diluted to the mark with distilled deionized water to produce a 50 mM borate buffer in all cases. All buffers were filtered through 0.45- μm filters (Whatman, Clifton, NJ, USA) prior to use. All solutes were obtained from Aldrich. Analytes were injected by applying a pressure of 30 mbar for 2 s, unless

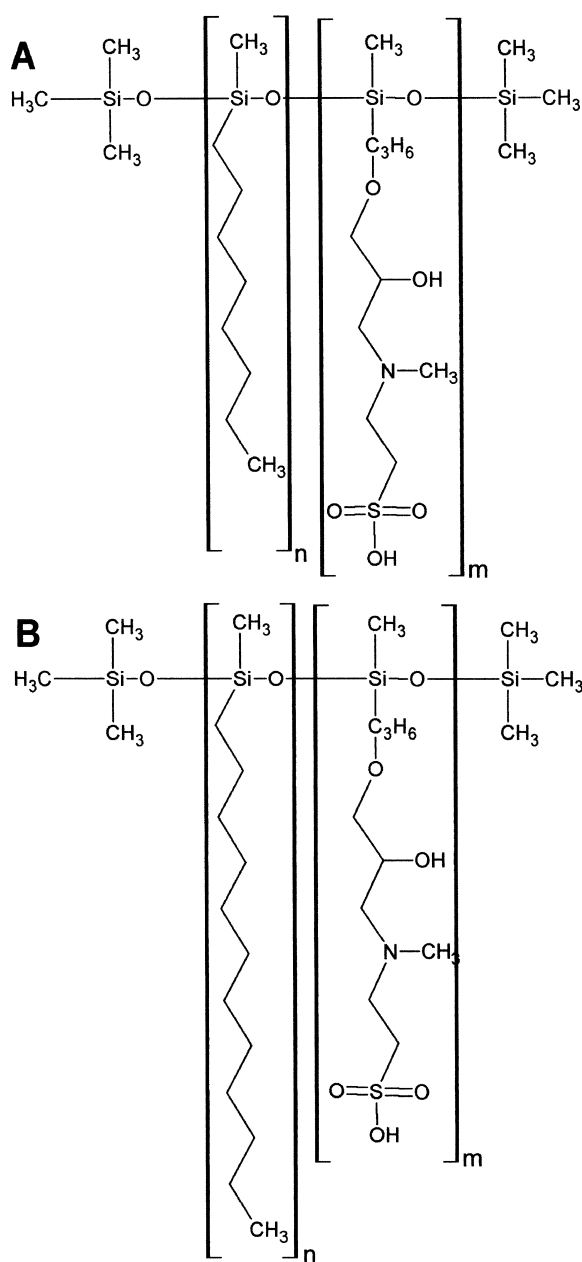


Fig. 1. Structure of anionic siloxane surfactants used in this study. (A) OAGENT (C₈), (B) DAGENT (C₁₂). OAGENT has a density of alkyl chain of 20%, while DAGENT has densities of 15 and 25%.

otherwise noted. Solutes for the PAH separations were identified using a spectral library. The reiterative calculation of Bushey and Jorgenson [30] was

used to determine the migration time of the polymers (t_{mc}), using nine alkyl phenyl ketones from butyrophenone to dodecanophenone. In some cases, especially at high organic modifier concentrations and with the octyl-modified siloxane, where the homologous series did not sample a sufficient fraction of the migration range, the reiterative calculation did not converge. In these cases, methylene selectivity and t_{mc} are not reported. The methylene selectivities were calculated from the slope (m) of the plot of the logarithm of the capacity factor versus carbon number ($\alpha_{CH_2} = 10^m$) and thus represent an average for the entire homologous series.

3. Results

3.1. Performance in organic solvents

The mobility of the three siloxanes in acetonitrile-modified buffers, as determined by reiterative calculation, are presented in Fig. 2A. It can be seen that the mobilities of all three siloxanes are very similar, especially at low concentrations of acetonitrile, and that the mobility does not vary substantially with increases in acetonitrile concentration. The value for t_R/t_0 was plotted versus percentage of acetonitrile (Fig. 2B), where t_R is the elution time of the last migrating homologous series analyte (dodecanophenone) and t_0 is the retention time of an unretained species (acetone). This parameter, the retention window, is a combined measure of the size of the migration window and the effect of organic modifier concentration on the partition coefficient for dodecanophenone. The value for t_R/t_0 gradually decreases as the amount of acetonitrile in the run buffer is increased. This is what would normally be expected and is what has been observed previously [22]. The value for t_R/t_0 for the three phases converges at high acetonitrile content. The methylene selectivity was calculated for the three pseudo-stationary phases studied and plotted versus percentage acetonitrile in the run buffer (Fig. 2C). The methylene selectivity for these siloxanes gradually falls off as more acetonitrile is added, which is what would be expected. It should be noted that the methylene selectivity is nearly identical for the three phases. The log values of the retention factors for

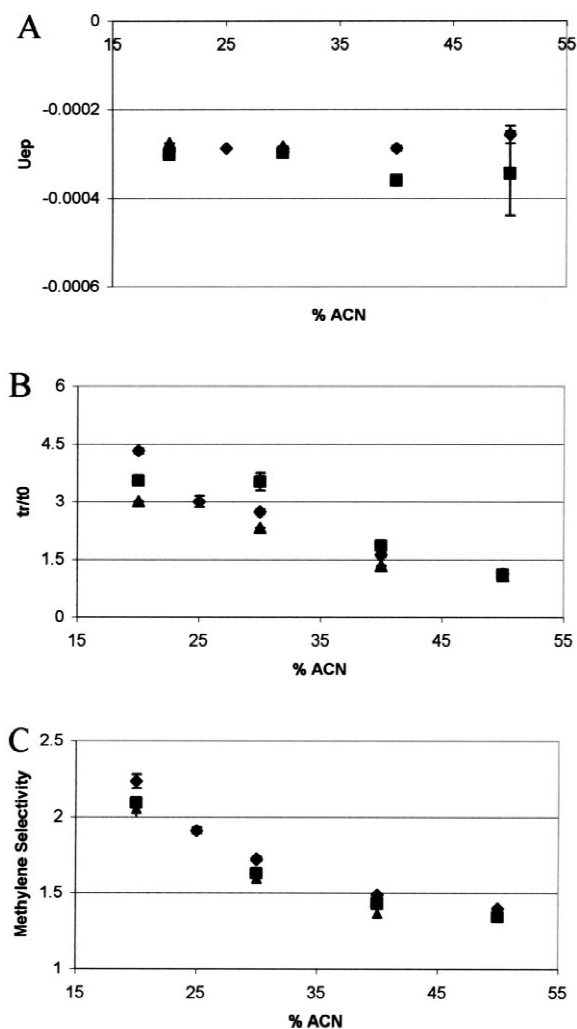


Fig. 2. Performance of siloxanes in acetonitrile (ACN). (▲) OAGENT, (◆) DAGENT-15, (■) DAGENT-25. (A) Electrophoretic mobility versus % ACN added; (B) t_R/t_0 versus % ACN added; (C) methylene selectivity versus % ACN added.

heptanophenone and dodecanophenone on 15% modified DAGENT are plotted versus the amount of acetonitrile in Fig. 3. A nearly identical plot is obtained for 25% modified DAGENT. This plot is as expected, with the log of the retention factor decreasing in a nearly linear fashion as organic modifier is added.

The performance of the pseudostationary phases was also examined in methanol at concentrations between 20 and 60%. The separation of alkyl phenyl

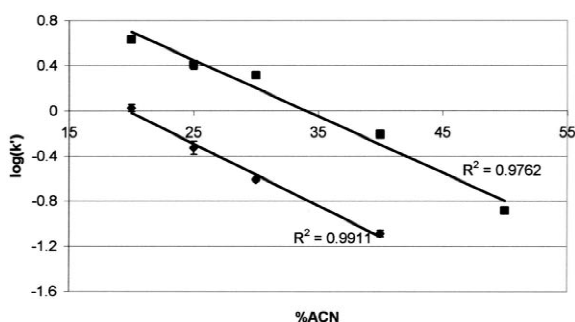


Fig. 3. Log k' on DAGENT-15 versus % ACN in run buffer. (◆) Heptanophenone and (■) dodecanophenone.

ketones using 15% modified DAGENT at various methanol concentrations is shown in Fig. 4. These separations show that the polymer remains retentive and provides a wide retention window up to 50% methanol, but that separation performance is diminished dramatically at 60% methanol. The average efficiency for the separations at 40 and 50% methanol is 105 000 plates. However, as has been reported previously for these polymers, efficiency suffers for later eluting compounds in the homologous series. The efficiency for hydrophobic compounds is im-

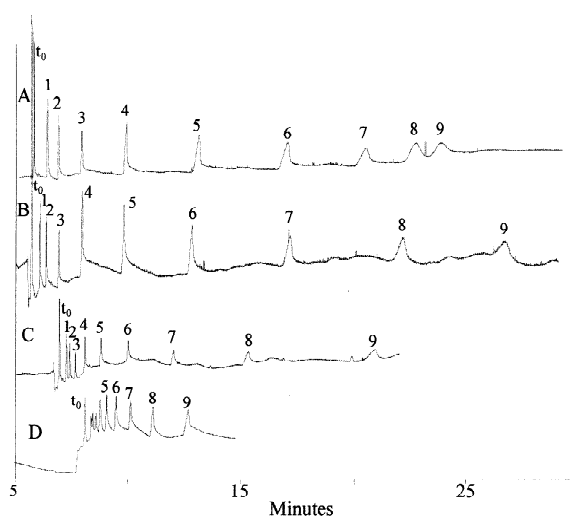


Fig. 4. Separation of alkyl phenyl ketone homologous series using DAGENT-15 in methanol-modified buffers. (A) 30% Methanol, 25 kV; (B) 40% methanol, 30 kV; (C) 50% methanol, 30 kV; (D) 60% methanol, 30 kV. 1, Butyrophenone; 2, valerophenone; 3, hexanophenone; 4, heptanophenone; 5, octanophenone; 6, nonanophenone; 7, decanophenone; 8, undecanophenone; 9, dodecanophenone.

proved with the addition of organic modifiers (much improved efficiency is observed for valerophenone, hexanophenone and heptanophenone relative to aqueous buffers), but remains low for the most hydrophobic solutes even at 50% methanol ($N=42\,000$ and $21\,000$ plates for undecanophenone and dodecanophenone, respectively). The electrophoretic mobility (Fig. 5A) for the DAGENT siloxanes goes through a minimum (absolute value) at approximately 40% methanol. The limited data for OAGENT

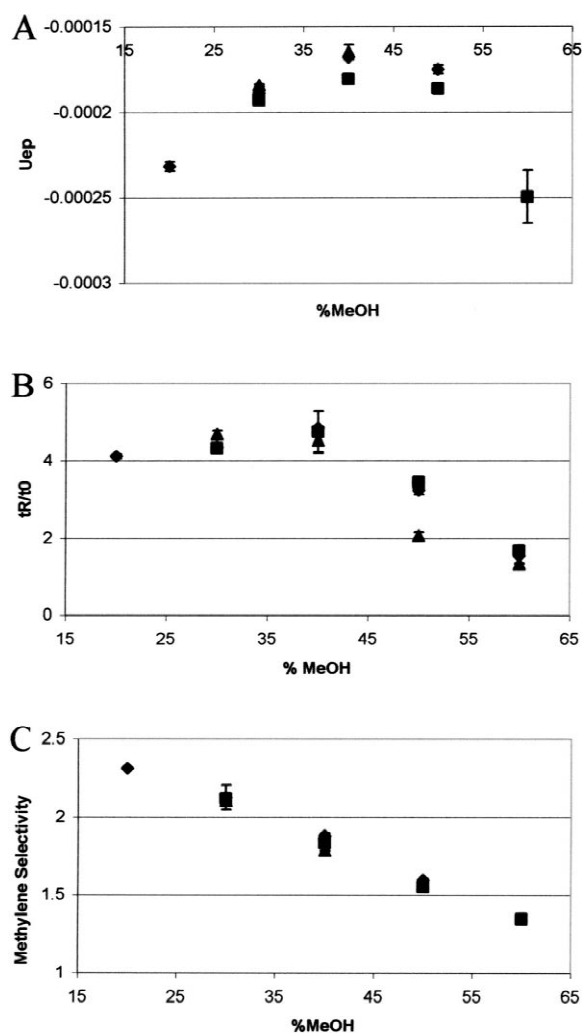


Fig. 5. Performance of siloxanes in methanol. (\blacktriangle) OAGENT, (\blacklozenge) DAGENT-15, and (\blacksquare) DAGENT-25. (A) Electrophoretic mobility versus % MeOH added; (B) t_R/t_0 versus % MeOH added; (C) methylene selectivity versus % MeOH added.

indicates that its mobility mirrors that of the DAGENT phases. The mobility increases substantially for 25% modified DAGENT between 50 and 60% methanol. This is not thought to be a viscosity effect because the electroosmotic flow decreases steadily as more methanol is added to the run buffer. The retention window for methanol (Fig. 5B) remains large until the methanol concentration is above 40%, after which the retention window decreases dramatically. The retention window at 40% methanol exceeds that observed at 40% acetonitrile by a factor of nearly three. The retention window remains sufficient to achieve separations of hydrophobic species throughout the methanol concentration range studied. It is surprising that the retention window increases between 20 and 40% methanol, the same range in which the mobility decreases. The methylene selectivity (Fig. 5C) remains good but gradually decreases as methanol is added to the run buffer. The methylene selectivity remains generally 15–25% higher in methanol-modified buffers than in acetonitrile-modified buffers. The effect of methanol on the logarithm of the retention factor of heptanophenone and dodecanophenone is presented in Fig. 6. As expected, a more or less linear decrease in $\log k'$ is observed with increase in methanol concentration. However, the result for dodecanophenone is very unusual. $\log k'$ remains constant or increases slightly from 20 to 40% methanol, and then decreases from 40 to 60% methanol. The siloxane polymer actually remains equally or more retentive of the most hydrophobic solutes as the methanol concentration increases from 20 to 40%, explaining the

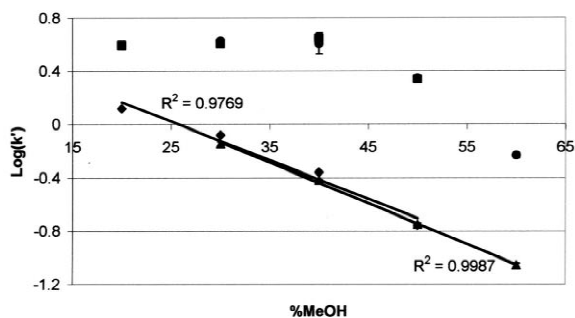


Fig. 6. $\log k'$ on DAGENT polymers versus % MeOH in run buffer. Heptanophenone with (\blacklozenge) DAGENT-15 and (\blacktriangle) DAGENT-25 and dodecanophenone with (\blacksquare) DAGENT-15 and (\bullet) DAGENT-25.

increased retention window in Figs. 4B and 5B. The cause of this behavior is not understood, but explanations consistent with increased retention factor and diminished mobility are that the polymer takes on a more open conformation with greater accessibility for hydrophobic solutes, or that the polymer forms an extended solvation sphere of high methanol content that gives it a larger effective volume.

These data demonstrate that the siloxanes are stable and useful in high concentrations of methanol, but may give the best performance at methanol concentrations below 60% where mobility, migration range, and methylene selectivity remain high. Separation efficiency for hydrophobic solutes is improved with the addition of methanol, but remains less than expected for EKC separations. The data also suggest that optimization of separations with methanol-modified buffers may be complicated at some methanol concentration ranges by the fact that retention factors remain constant or increase as methanol concentration increases. It may be possible to take advantage of this effect to improve resolution of hydrophobic compounds while at the same time improving their solubility in the buffer medium.

3.2. Separation of PAHs

Siloxanes were also studied to separate a mixture of hydrophobic analytes. The hydrophobic solutes used were 14 priority polycyclic aromatic hydrocarbon pollutants. Sample separations of these analytes are given in Fig. 7 for the 20% modified OAGENT and 15% modified DAGENT at acetonitrile concentrations of 40%. OAGENT (Fig. 7A) is able to resolve 11 of 14 components, with partial resolution of a 12th component. DAGENT (Fig. 7B) is able to resolve or partially resolve 12 of 14 analytes in less than 7 min. Very subtle differences in selectivity are observed between the two polymers. DAGENT is more effective at separating benzo[*e*]acephenanthrylene and benzo[*e*]pyrene. The separation efficiency is very good in both cases, particularly for the less hydrophobic compounds. The average efficiency for compounds acenaphthylene, acenaphthene, fluoranthene and pyrene is over 124 000 plates. Later migrating compounds, particularly dibenz[*a,h*]anthracene and

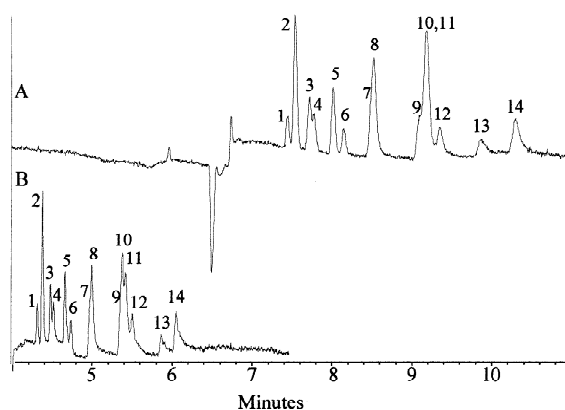


Fig. 7. Separation of PAHs using (A) OAGENT and (B) DAGENT-15. Conditions: 20 kV applied voltage, 40% acetonitrile in 50 mM borate buffer. Concentration of analytes 25–50 ppm. Peak t_0 , acetone; 1, acenaphthylene; 2, acenaphthene; 3, phenanthrene; 4, anthracene; 5, fluoranthene; 6, pyrene; 7, chrysene; 8, benz[*a*]anthracene; 9, benz[*e*]acephenanthrylene; 10, benzo[*e*]pyrene; 11, benzo[*k*]fluoranthene; 12, benzo[*a*]pyrene; 13, dibenz[*a,h*]anthracene; 14, benzo[*g,h,i*]perylene. Detection wavelength was 215 nm.

benzo[*g,h,i*]perylene, have tailing peaks and lower plate numbers (averaging 42 000 plates). This loss of efficiency and tailing may be caused by an insufficient solvation cavity for these large compounds in the polymeric phases. Attempts to separate these compounds in methanol-modified buffers were unsuccessful due to very long migration times, low efficiency, and peak asymmetry.

4. Conclusions

The performance of alkyl-modified water-soluble anionic siloxanes has been examined in organic-modified buffers. The buffers were modified with varying amounts of acetonitrile and methanol. The results are promising in that the siloxanes maintain high electrophoretic mobilities, large retention windows, and high methylene selectivities in organic-modified buffers. The efficiency for hydrophobic compounds is also improved with addition of organic solvents, but remains low for the most hydrophobic compounds. The retention factors of long chain alkyl phenyl ketones remain constant or increase slightly between 20 and 40% methanol, implying a change in solvation or conformation of the polymer in this

range. The siloxanes were also used to separate a mixture of 14 PAHs in acetonitrile-modified buffers. Not all of the PAHs were resolved, but the separations do demonstrate the utility and promise of phases of this type for the separation of similar hydrophobic compounds.

Acknowledgements

This work was funded by a Department of Energy (Sandia National Laboratories) Presidential Early Career Award for Scientists and Engineers.

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