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Pseudo-Stationary Separation Materials for Highly Parallel Separations

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Pseudo-Stationary Separation Materials for Highly Parallel Separations

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Abstract

Goal of this study was to develop and characterize novel polymeric materials as pseudostationary phases in electrokinetic chromatography. Fundamental studies have characterized the chromatographic selectivity of the materials as a function of chemical structure and molecular conformation. The selectivities of the polymers has been studied extensively, resulting in a large body of fundamental knowledge regarding the performance and selectivity of polymeric pseudostationary phases. Two polymers have also been used for amino acid and peptide separations, and with laser induced fluorescence detection. The polymers performed well for the separation of derivatized amino acids, and provided some significant differences in selectivity relative to a commonly used micellar pseudostationary phase. The polymers did not perform well for peptide separations. The polymers were compatible with laser induced fluorescence detection, indicating that they should also be compatible with chip-based separations.

Contents

Nomenclature

EKC: Electro Kinetic Chromatoghraphy
PSP: Pseudo Stationary Phase
LIF: Laser Induced Fluorescence
AGENT: Allyl Glycidyl Ether N-methyl Taurine
SDS: Sodium Dodecyl Sulfate

1. Introduction

Miniaturized devices for the detection and identification of chemicals or biologicals in the environment are important for many applications of interest to national security and the DOE. This has been the motivation for the development of lab-on-a-chip devices at Sandia and other DOE National Laboratories. Typically, separations conducted in micro-channels on the chip-based device are essential to the utility of these devices. Thus, chemical technology that enables selective and efficient separations in capillaries or micro-channels is critical for the successful development and implementation of these devices.

Electrokinetic chromatography (EKC) is one of only a few high performance separation techniques that are compatible with the chip-based format. EKC is among the simplest techniques to adapt and implement in micro-channels, since it does not require a fixed matrix or stationary phase. Separations are achieved using an ionic pseudo-stationary phase (PSP) that migrates electrophoretically in an electric field applied along the length of the separation channel. Analytes are separated based on their relative affinity for the PSP. Since the PSP is dissolved in low viscosity solution, it can be introduced into and removed from separation channels by applying a low pressure.

EKC is not without limitations, however. The technique has a limited time window, the migration range, in which all analytes must be separated. To overcome this limitation, it is necessary to optimize the separations more completely. Thus it is critical that PSPs be available with varied selectivity and have high stability with respect to

analytical conditions. The commonly used micellar PSPs do not meet these requirements, and further restrict the use of EKC with mass spectrometric detection.

Polymeric PSPs are an alternative to micellar PSPs that address many of the limitations of EKC. These PSPs are stable under a variety of conditions, making it possible to optimize separations for a greater variety of analytes. Further, the polymers can provide unique separation selectivity not achievable with micellar media. Finally, the polymeric PSPs are compatible with mass spectrometric detection.

The current project has continued the development of novel polymeric PSPs, characterized the performance and selectivity of the PSPs as a function of structure, and evaluated the new PSPs for application to problems of interest at Sandia National Laboratory. The polymers have been evaluated for the separation of derivatized amino acids and peptides, and have been used in combination with laser induced fluorescence (LIF) detection. The results have shown that PSPs with varied selectivity can be synthesized on various polymer backbones, and that the materials should be compatible with micro-channel based separations with LIF detection. Highly efficient separations of derivatized amino acids were achieved with two polymer chemistries. However, high performance separations of peptides could not be achieved.

2. Siloxane Polymers

Siloxane polymers of the basic structure shown in Figure 1a and 1b have been studied extensively as PSPs for electrokinetic chromatography.[1-3] The phases are synthesized from hydrosiloxane polymers of known nominal molecular weight, and thus have the advantage that the final molecular weight is known. This approach also has the

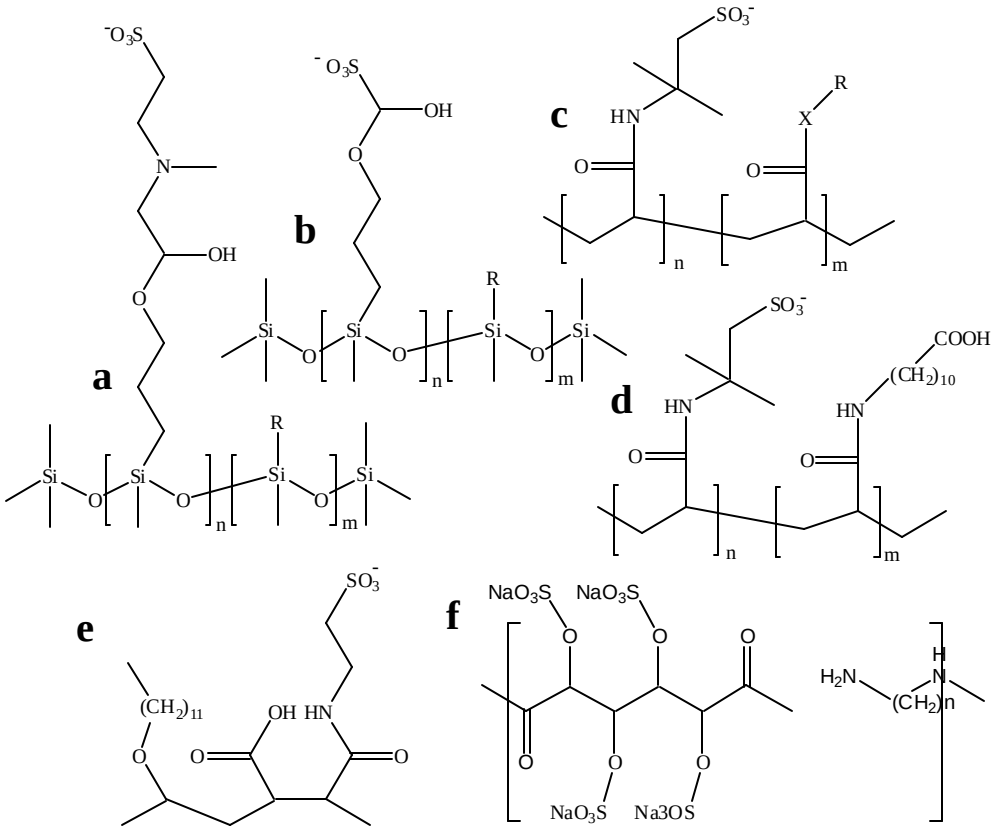

Figure 1: *Structures of the polymeric PSPs studied during the project. a. AGENT, b. AGESS, c. AMPS copolymers (X=O or NH), d. poly(AMPS/11-acrylamidoundecanoic acid) (poly(AMPS/AmU)), e. pSMADVE, f.*

advantage that polymers with a wide variety of ionic head group and pendant group chemistries can be easily synthesized with the same backbone chemistry. Siloxane polymers of this type may provide a vehicle for application of the wide variety of

silicone-based chemistries developed for gas and liquid chromatography over the past several decades.

The siloxane chemistry most studied over the course of the project was that shown in Figure 1a, with octyl-, dodecyl- or octadecyl- pendant groups at varying degrees of substitution. These polymers are synthesized by modification of the hydrosiloxane polymer with allyl glycidyl ether and the selected alkene. The epoxy ring of the glycidyl group was then opened by reaction with N-methyl taurine to introduce the sulfonic acid head group. The polymers were given the acronym AGENT for allyl glycidyl ether N-methyl taurine. This configuration provides polymers of sufficient aqueous solubility when the fraction of silicon centers modified with the ionic group exceeds 70%.[1]

By varying the density and the length of the alkyl chain, the hydrophobicity of these polymers can be varied from being less than SDS micelles to greater than SDS micelles.[1] The electrophoretic mobility of the polymers varies in a complex manner as the density and length of the alkyl chains added to the backbone is varied, possibly due to changes in ionization and/or conformation of the polymers as a function of the hydrophobic/lipophilic balance.[1] Both electrophoretic mobility and separation efficiency pass through a maximum at 10-20% substitution with the alkyl chains.[1] The polymers provide very different selectivity from SDS micelles, but selectivity does not vary greatly between polymers with different alkyl chain length or extent of substitution.[1] The dodecyl modified polymer with 15-20% substitution provided the best overall performance in terms of electrophoretic mobility, solubility, and efficiency.

Linear solvation energy relationship (LSER) studies were used to characterize the selectivity of these polymers in greater detail.[3] Selected results are presented in Table 1 and are labeled (C_nAGENT-#) where n refers to the alkyl chain length and # refers to the percentage substitution with the alkyl chain. A striking feature of the alkyl-modified siloxanes relative to most other PSPs is their very low propensity for interaction with polar or polarizable compounds (s-term). This does not seem surprising given the non-polar nature of the siloxane backbone. Another interesting feature is the ability of the polymers to interact strongly with hydrogen bond donors (a-term). This is most likely due to the presence of the tertiary amine in the AGENT linker arm. Finally, the siloxanes are more cohesive (m-term) than might have been expected given the relatively flexible siloxane backbone.

The siloxane polymer shown in Figure 1b (AGESS) was synthesized and its performance and selectivity compared to that of AGENT siloxanes of the same structure.[2-4] The AGESS polymers were found to provide significantly different chemical selectivity from AGENT polymers.[4] Selected results of LSER studies with AGESS polymers are presented in Table 1 above and are labeled C_nAGESS-#. While the

Table 1: LSER results for selected polymeric PSPs. n is the number of solutes used.

PSP	m	r	S	a	b	c	n
SDS	2.74 (0.11)	0.27 (0.08)	-0.37 (0.07)	-0.23* (0.13)	-1.82 (0.16)	-1.65 (0.11)	18
AGENT	2.1 (0.2)	0.76 (0.1)	-0.07* (0.1)	0.45 (0.09)	-1.9 (0.2)	-2.8 (0.2)	40
C_8AGENT-20	2.2 (0.3)	0.49 (0.2)	-0.90 (0.2)	0.11* (0.1)	-2.5 (0.2)	-1.5 (0.2)	40
C_{12}AGENT-10	1.3 (0.3)	0.58 (0.2)	-1.0 (0.2)	0.51 (0.1)	-2.0 (0.2)	-1.3 (0.3)	40
C_{12}AGENT-15	2.5 (0.3)	0.32 (0.2)	-0.86 (0.2)	0.21 (0.1)	-2.4 (0.3)	-1.8 (0.3)	40
C_{12}AGENT-20	2.4 (0.2)	0.59 (0.1)	-0.78 (0.1)	0.23 (0.07)	-2.4 (0.2)	-2.0 (0.2)	40
C_{18}AGENT-20	2.5 (0.3)	0.32 (0.2)	-1.1 (0.2)	0.33 (0.1)	-2.6 (0.3)	-1.8 (0.3)	40
C_{12}AGESS-8	2.0 (0.2)	0.40 (0.2)	-0.17 (0.1)	0.24 (0.08)	-2.1 (0.2)	-2.50 (0.2)	38
C_{12}AGESS-13	2.7 (0.1)	0.46 (0.06)	-0.43 (0.08)	0.27 (0.04)	-2.46 (0.09)	-2.40 (0.1)	38
pLMAt-15	3.65 (0.18)	0.43 (0.11)	-0.67 (0.16)	-0.274 (0.083)	-3.70 (0.22)	-2.84 (0.16)	20
pSMAt-16	3.78 (0.21)	0.65 (0.12)	-0.85 (0.18)	-0.495 (0.098)	-3.83 (0.26)	-2.73 (0.19)	20
pLAt-13	3.58 (0.26)	0.39 (0.15)	-0.40 (0.22)	-0.02* (0.12)	-3.52 (0.32)	-2.96 (0.23)	20
pLMAm-19	2.88 (0.13)	0.374 (0.075)	-0.32 (0.11)	0.254 (0.059)	-2.45 (0.16)	-2.69 (0.11)	20
PDHCHAt-33	3.40 (0.20)	0.65 (0.12)	-0.46 (0.17)	0.241 (0.093)	-3.20 (0.25)	-2.68 (0.18)	20
ptOAm-49	3.36 (0.16)	0.333 (0.096	-0.44 (0.14)	0.434 (0.075)	-3.22 (0.20)	-2.86 (0.14)	20
Poly(AMPS/AmU) pH 5.0	2.44 (0.26)	-0.12 (0.21)	0.20 (0.22)	0.24 (0.10)	-2.82 (0.21)	-2.26 (0.31)	27
Poly(AMPS/AmU) pH 8.0	1.51 (0.17)	0.46 (0.13)	-0.07 (0.14)	0.29 (0.07)	-1.30 (0.14)	-2.54 (0.20)	27

AGENT materials exhibit very low propensity for interaction with polar or polarizable compounds (s-term) this was not the case for the AGESS materials. Thus, the low polarity of AGENT materials is not entirely due to the non-polar nature of the siloxane backbone. It is possible that the shorter linker arm between the backbone of the siloxane and the ionic head group in the case of AGESS is the cause of its greater polarity relative to AGENT. Both of the siloxane polymers were observed to interact strongly with hydrogen bond donors (a-term). This can not be explained by the presence of the tertiary amine on AGENT, as it is absent in AGESS polymers. This behavior may thus be attributed to the backbone chemistry. Finally, all of the siloxanes are more cohesive (m-term) than might have been expected given the relatively flexible siloxane backbone.

The solvent characteristics of AGENT copolymers were also studied using fluorescence spectroscopy.[5] The AGENT polymer without pendant alkyl chains was found to solubilize pyrene in an environment with polarity similar to that of water. However, alkyl-modified AGENT copolymers provide a solvation environment much more nonpolar than SDS micelles, similar to *n*-butyl ether or 1-octanol. Consistent with the chromatographic studies,[3] the polymer modified with octadecyl chains was found to be more polar than that modified with dodecyl chains. This is counterintuitive, but may be a result of the more cohesive octadecyl polymer excluding the relatively large pyrene from the most hydrophobic solvation regions. The solvation pocket size and geometry with the AGENT copolymers was also found to be different from SDS micelles in that pyrene molecules are solvated so close to each other that they interact in the ground electronic state.

Some of the siloxane polymers were applied to the separation of hydrophobic compounds in buffers modified with organic solvents.[2] C_8AGENT-20, C_{12}AGENT-15 and C_{12}AGENT-25 were used in buffers containing up to 50% acetonitrile or 60% methanol for the separation of alkyl phenyl ketones and polynuclear aromatic hydrocarbons. The results were promising in that the polymers maintain large migration windows and high methylene selectivities in the organic-modified buffers. The addition of organic solvents also improved the separation efficiency for all but the most hydrophobic compounds. The siloxanes were used to separate 12 of 14 PAHs in acetonitrile-modified buffers, but separation of the PAHs could not be achieved in methanol-modified buffers. In general, the performance of these polymers in organic modified buffers was not as good as that of other polymeric PSPs studied to date.

3. Acrylamido Polymers

Copolymers of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and were developed and characterized as PSPs (Figure 1c).[6-10] These polymers were synthesized by free radical copolymerization of AMPS with a variety of (meth)acrylate and (meth)acrylamide comonomers. The effect of the comonomer chemistry and mole fraction on the performance and selectivity of the polymers was studied and reported. The performance of the polymers in organic modified buffers was studied, and the polymers were shown to be useful for high-speed separations and on-line preconcentration by sweeping.

The effect of the mole fraction of comonomer was studied in detail using copolymers of AMPS and lauryl methacrylamide (LMAm) made up of from 0.6 to 1 mole fraction AMPS.[8] As might be expected, the electrophoretic mobility of the polymers increased and the hydrophobicity decreased as the mole fraction of AMPS increased. Peak symmetries for more hydrophobic solutes decreased as the AMPS mole fraction increased. Considering the selectivity, separation efficiency and electrophoretic mobility, an AMPS mole fraction of 0.80 was considered optimum.

In efforts to study the effects of the pendant alkyl chain length and the backbone chemistry of the comonomer on the performance and selectivity of these phases, AMPS was copolymerized with octyl methacrylate (OMAt), lauryl methacrylate (LMAt), stearyl methacrylate (SMAt), lauryl acrylate (LAt), LMAm, stearyl amide, dihydrocholesteryl acrylate (DHCHAt) and t-octyl amide (tOAm).[6,10] LSER studies were used to characterize the differences in selectivity observed between these phases. Selected LSER

results are presented in Table 1 under the acronyms above followed by the mole percentage of the nonionic comonomer.

Significant differences in the selectivity of the AMPS copolymers and SDS micelles were observed. With few exceptions, the AMPS copolymers are the least cohesive of the polymeric phases studied to date, with most being less cohesive than SDS micelles. This is unusual, as the covalent stabilization of polymeric PSPs has generally been observed to result in more cohesive phases.

Significant differences in selectivity were observed between (meth)acrylamide and (meth)acrylate phases. Acrylamide copolymers are better able to donate and accept hydrogen bonds, and are more polar than their acrylate counterparts.[6] This is very much apparent for the hydrogen bond accepting ability (a-term), in which methacrylates are weaker bases than water, while acrylamides are stronger bases than water. Increases in the fraction of amide monomer also appear to increase the cohesiveness of the phases, possibly due to hydrogen bonding along the backbone of the polymers.[6] Increases in comonomer fraction and pendant chain length decrease the hydrogen bond accepting (a-term) and donating (b-term) ability of the polymers, and reduce the cohesiveness (m-term) of the polymers.[6] However, pendant alkyl chain length alone did not have a significant effect on the overall selectivity of the polymers.

Greater differences in the structure of the pendant group were studied with DHCHAt and tOAm comonomers.[10] These comonomers have semiplanar and tertiary pendant chemistry, respectively. No dramatic difference in the LSER parameters was realized with these polymers, although DHCHAt was the only acrylate AMPS copolymer with better hydrogen bond accepting strength than water, and tOAm was the only AMPS

copolymer more cohesive than SDS micelles. DHCHAt did appear to provide unique selectivity in the separation of planar PAHs from non-planar alkyl phenyl ketones. Although the selectivity differences were not dramatic, the performance of the DHCHAt/AMPS copolymer was very impressive, with separation efficiencies in excess of 190000 plates in 10 minutes or less. Representative separations utilizing the DHCHAt copolymer in acetonitrile-modified buffers are presented in Figure 2 below.

The strongly acidic sulfonate functionality, relatively high electrophoretic mobility, low cohesiveness and low polarity of the AMPS copolymers makes them ideal candidates as agents to effect online preconcentration of solutes by sweeping.

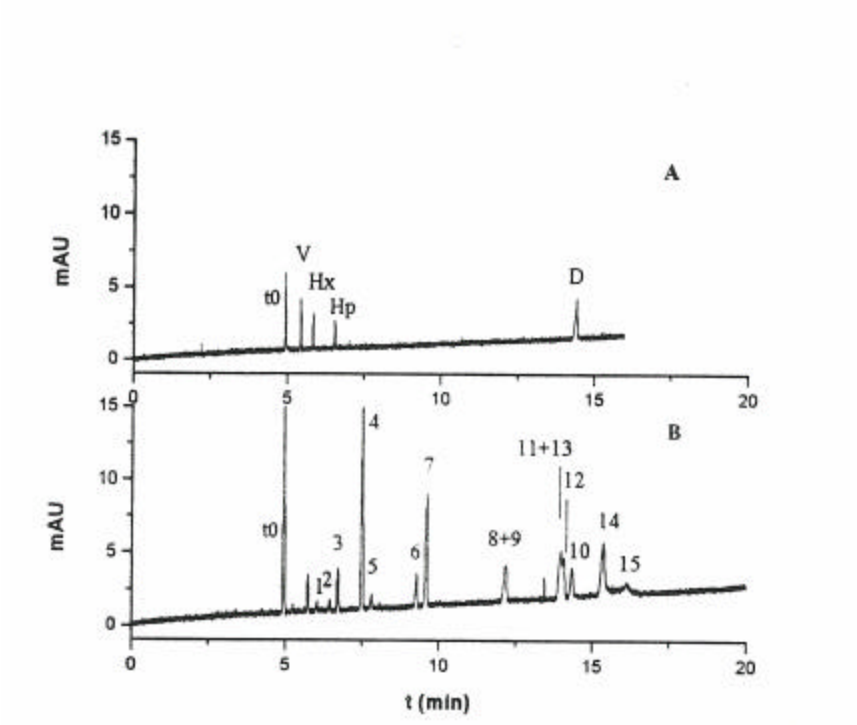

Figure 2: Separations by pDHCHAt-58. Polymer concentration: 0.72%, ACN%= 30%v/v, borate buffer: 35mM, (pH=9.2 before adding ACN). Column effective/total length: 45.5/53.9 cm, Voltage: 20 kV, Current: 11 µA. Column temperature: 25.0°C. UV: 254 nm. A. Alkyl-phenyl ketones, V. valerophenone, HX. hexanophenone, HP. heptanophenone, D. n-dodecanophenone. Injection: 1s at 1500Pa, B. PAHs, 1. acenaphthylene, 2. acenaphthene, 3. fluorene, 4 phenanthrene, 5 anthracene, 6 fluoranthene, 7 pyrene, 8 chrysene, 9 benz[a]anthracene, 10 benzo[a]pyrene, 11 benzo[e]pyrene, 12 benzo[k]fluoranthene, 13 benz[e]acephenanthrylene, 14 benzo[g,h,i]perylene, 15 dibenz[a,h]anthracene, injection: 3s at 5000Pa.

In the sweeping technique, analytes to be preconcentrated and separated are injected as a large plug in buffer media not containing the PSP.[11,12] The pH is adjusted to a low value, such that

electroosmotic flow is suppressed. Analytes are "swept" into a relatively narrow zone of high concentration as the PSP migrates through the sample zone. Using a combination of sweeping from a sample solvent of low organic modifier content and separation in a zone of high organic modifier content, the separation and detection of quinine and progesterone was achieved at concentrations as low as 12.5 ppb.[7]

The relatively low conductivity and high separation efficiency of the DHCHAt copolymer make it an ideal candidate as PSP for high-speed separations by EKC. As shown in Figure 3, the separation of 12 of 15 PAHs was achieved in less than 2.5 minutes using DHCHAt/AMPS copolymer in an acetonitrile-modified buffer using a 23 cm capillary and an applied voltage of 30 kV.[9] Plate numbers ranged from 20000 for the most hydrophobic solutes to 113000 for the less hydrophobic solutes.

The effect of polymer conformation on separation selectivity and performance was investigated in a systematic manner using the pH-responsive polymer poly(sodium 2-(acrylamido)-

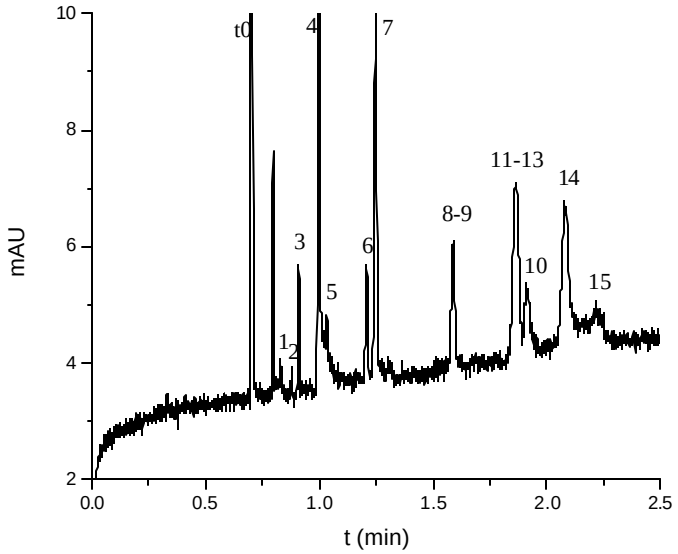

Figure 3: High-speed separation of 15 PAHs. 1. acenaphthylene, 2. acenaphthene, 3. fluorene, 4. phenanthrene, 5. anthracene, 6. fluoranthene, 7. pyrene, 8. chrysene, 9. benz[a]anthracene, 10. benzo[a]pyrene, 11. benzo[e]pyrene, 12. benzo[k]fluoranthene, 13. benz[e]acephenanthrylene, 14. benzo[g,h,i]perylene, 15. dibenz[a,h]anthracene. The peak immediately after t0 is an impurity. Separation conditions: pDHCHAt-58 (0.72 % w/v), sodium borate, 35 mM, ACN, 29.6% v/v, pH of the buffer before adding ACN was 9.2, column effective/total length, 23.0/31.2 cm, voltage, 30 kV, current, 38 μA, column temperature, 35.0°C, UV, 254 nm, injection, 2 s at 2500 Pa.

2-methylpropanesulfonate/11-(acrylamido)-undecanoic acid) (poly(NaAMPS/AmU)), shown in Figure 1d.[13,14] This polymer is similar to the AMPS copolymers described above, except for the presence of the carboxylate groups at the end of the pendant alkyl chains. The sulfonic acid groups remain ionic when the pH>2, while the carboxylate groups are not ionized at pH<5, and become ionized as the pH is increased from 5 to 8. Yusa *et al.* used static light scattering (SLS), quasi-elastic light scattering, viscometry, ^{1}H NMR spin-spin relaxation measurements and fluorescence probe studies to show that the ionization of the carboxylates alters the balance between ionic repulsion and hydrophobic association such that poly(NaAMPS/AmU) forms a compact intramolecular aggregate (unimer micelle) at pH≤5 and an open chain configuration at pH≥8.[13] The quantitative results of their studies are summarized in Table 2.

Table 2: Structure and properties of poly(NaAMPS/AmU) at pH 5 and pH 8.[20]

	pH 5	pH 8
M_w	$1x10^5$ g/mol	$1.4x10^5$ g/mol
R_g	7 nm	20 nm
R_h	7 nm	12 nm
NMR T_2	25 ms	75 ms
Pyrene I_3/I_1	0.82	0.65

The molecular weight, M_w, of poly(NaAMPS/AmU) as measured by GPC and light scattering is approximately 100 kDa, and the polydispersity (M_w/M_n) is 2.26. The measured molecular weight of the polymer does not change with pH, indicating that there are no changes in intermolecular aggregation. However, the radius of gyration (R_g, the root mean square displacement of mass from the center of gravity) and hydrodynamic radius (R_h, the Stokes radius, defined as the radius of a sphere that experiences the same viscous drag in solution as the

polymer) both increase dramatically at pH 8 relative to pH 5, indicating a significant change in conformation of poly(NaAMPS/AmU). Pyrene was found to be solvated in a much more non-polar environment at pH ≤ 5 relative to pH ≥ 8, and proton NMR spin-spin relaxation times (T_2) indicate that the pendant chains have greater motional freedom in the high pH open-chain conformation.

The chromatographic results obtained in this project indicate that conformation and ionization have a significant impact on the performance and selectivity of a PSP.[14] The compact low pH conformation had higher electrophoretic mobility and higher affinity for most of the solutes studied. This, in general, led to better resolution when the low pH conformer was employed. Linear solvation energy relationship results for the polymer at low and high pH are presented in Table 1. The solvation environment provided by the low pH conformation is more like that of micelles or other amphiphilic polymers than that provided by the high pH open conformation. Both conformations are able to interact with polar compounds, implying separate interaction sites for polar and hydrophobic compounds. The separation efficiency is often higher using the high pH conformation, but it was not possible to determine whether this was due to the change in polymer conformation. The results imply that amphiphilic self-associative polymers with a carefully selected balance between ionic and hydrophobic interactions are more likely to provide high resolution separations than more hydrophilic non-associative polyelectrolytes. The one exception to this rule may be the separation efficiency, which in this case was higher for the more highly ionized open conformation. The results also indicate that EKC can be used to characterize the changes in solvation environment provided by stimuli-responsive polymers as a function of conditions. This can be used to

predict the utility of such polymers as solvating agents in general or for specific compounds.

4. Other Polymers

The solvation environment provided by poly(sulfonyl maleic anhydride-*co*-dodecyl vinyl ether) (pSMADVE, Figure 1e) was characterized using electrokinetic chromatography and fluorescence spectroscopy.[15] This polymer is ionized to a different extent over the pH range of 4 to 10, with the sulfonic acid groups remaining anionic and the carboxylate groups being ionized at high pH. This leads to changes in the polymer conformation and solvent characteristics as a function of pH. Fluorescence measurements using pyrene and 1.3-bis(1-pyrenyl)propane were more sensitive to the changes in the solvation microenvironment with pH than were chromatographic measurements, and the results of the two approaches did not always agree. The polymer provides separations of substituted benzene and naphthalene compounds with good peak symmetry and efficiency, but only minor changes in separation selectivity as a function of pH. The chromatographic measurements also indicate that the polymers become more polar as the pH is increased. However, fluorescence measurements indicated that the polymers are less polar at low pH and high pH than they are at intermediate pH, and that the solvation pocket becomes smaller, less polar, more viscous and less heterogeneous at high pH. The results also show that PSMADVE polymers solubilize pyrene molecules in such a way that they are more likely to be closely associated with one another than they are in SDS micelles.

Polymers based on a carbohydrate backbone structure have also been synthesized and their utility as PSPs investigated. These polymers provide the advantages that the starting materials are inexpensive and readily available, the structure of the polymer is strictly controlled and chemically stable, and the backbone structure has the potential to provide

unique selectivity (including chiral selectivity). Additionally, the polymers can be designed and synthesized with a variety of carefully controlled backbone variations or pendant groups, making it possible to systematically vary the chemical selectivity and electrophoretic and chromatographic properties.

Figure 1f shows the general structure of the carbohydrate-based polymeric materials investigated. The polymers are polyamide condensation products derived from activated carbohydrate diacids (aldaric acids) and alkyl diamines. The resulting polymers are then derivatized, at the carbohydrate hydroxyl groups, by reaction with triethylamine sufur trioxide complex to introduce the ionic sulfate functionality. Depending on the carbohydrate used, the stereochemistry and structure of the polymer are different. Six such polymers derived from poly(dodecamethylenegalactaramide), poly(hexamethylene galactaramide), poly(dimethylene galactaramide), poly(dodecamethyleneglucaramide), poly(hexamethylene glucaramide) and poly(dimethylene glucaramide) were prepared and studied. These polymers were chosen to provide systematic variation in the polymer structure and hydrophobic character. The galactaryl unit has an extended, rigid, rod-like structure that tends to form crystalline molecules of low water solubility. The glucaryl unit, on the other hand, is bent and tends to form more amorphous structures of greater water solubility. Variation of the length of the alkyl chain linking the carbohydrate units also affects the water solubility, hydrophobicity and flexibility of the polymers.

None of the six polymers provided sufficiently strong interactions with substituted aromatic compounds in aqueous buffers to be suitable as PSPs. The dimethylene and hexamethylene materials provided little or no separation of substituted benzene or naphthalene compounds. Some separation was achieved between benzene and

naphthalene derivatives using the dodecamethylene materials. The dodecamethylene polymers did provide some separation based on the hydrophobicity of the solutes, but their general utility remained poor. In order for these polymers to be suitable PSPs, they will need to be synthesized with linkers having greater hydrophobicity.

5. Amino Acid and Peptide Separations

Two polymers, dodecy/allylglycidyl ether-sulfonate-siloxane (DAGESS) with 85% of the silicon centers modified with allylglycidyl ether-sulfonate and the remainder modified with dodecene[4], and a copolymer of AMPS and lauryl methacrylamide with 73 mole percent AMPS and 27 mole percent lauryl methacrylamide (LMAm-27)[8,15], were investigated as PSPs for the separation of derivatized amino acids and peptides.[16] Separations of fourteen NDA-derivatized amino acids using the two polymers are presented in Figure 4. Excellent efficiency, peak shape, and selectivity are observed with both systems, particularly when LIF detection is used. Not all of the amino acids are resolved on either system. Using AGESS with UV detection, the average plate number is 47,000±18000, while with LIF detection the average plate numbers are 129,000±44,000 and 49,000±17,000 for AGESS and LMAm, respectively. The lower efficiency with UV

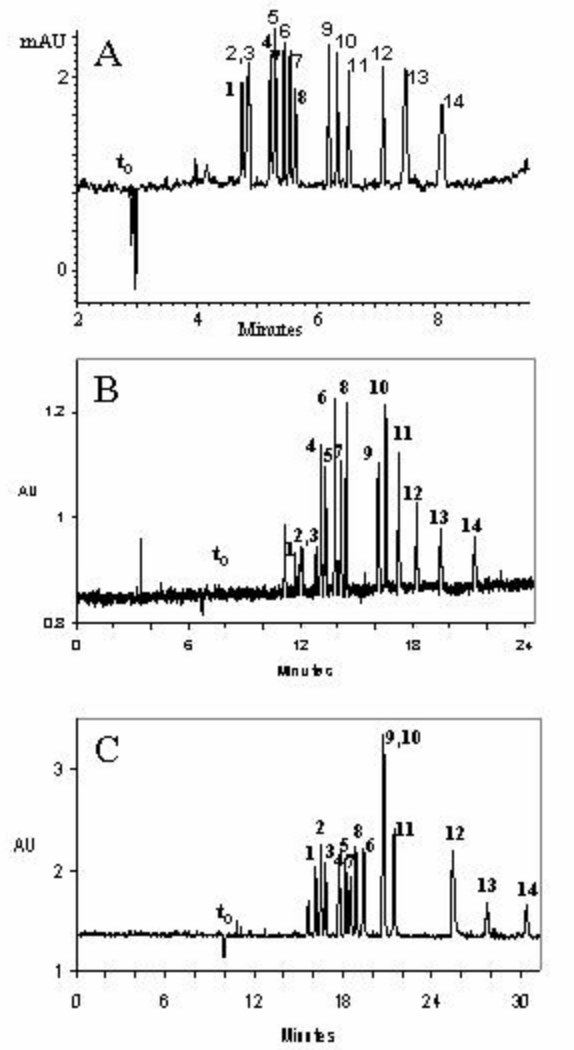

Figure 4: Separations of 14 NDA-amino acids. A. 0.02 mM of each amino acid, 1% AGESS polymer, UV detection at 200 nm. B. 4.3×10^{-5} mM of each amino acid, 1% AGESS polymer, LIF detection. C. 4.3×10^{-5} mM of each amino acid, 1% LMAm polymer, LIF detection. 1. histidine, 2. threonine, 3. serine, 4. alanine, 5. glycine, 6.tyrosine, 7. methionine, 8. valine, 9. isoleucine, 10. leucine, 11. phenylalanine, 12. arginine, 13. glutamic acid, 14. aspartic acid.

detection is likely due to electrophoretic mismatch and sample overloading at the higher derivatized amino acid concentrations. The general selectivity is as expected. The hydrophilic amino acids migrate first and more hydrophobic amino acids (Leu, Ile, Phe) migrate later. The doubly negatively charged glutamic acid and aspartic acid come last as electrophoresis dominates over partioning. Arginine migrates late in the separation, presumably due to strong electrostatic interaction with negatively-charged polymers. Both polymers are compatible with LIF detection, although there remains significant background. This background was greatly reduced when the polymers were washed with ether, implying that low levels of fluorescent impurities were responsible for at least part of the signal. It is also possible that some of the background is the result of scattering from polymer aggregates, and cannot be eliminated. Even with the background and associated noise, the averaged estimated detection limit ($S/N_{rms}=3$) for the amino acids on either system is $1x10^{-8}$M. Separations performed at lower concentration on the LIF system invariably displayed better efficiency and peak shape.

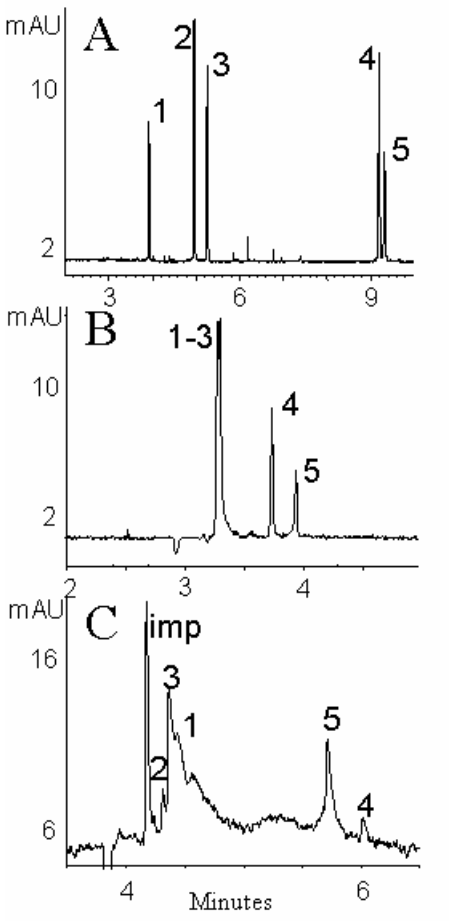

Figure 5: Peptide separations using A. 35 mM SDS, B. 1% AGESS and C. 1% LMAm in 50 mM borate buffer at pH 9.2. 1. ß-lipotropin (fragment 39-45), 2. thymopentin, 3. Arg-Lys-Glu-Val-Tyr, 4. a-Casein (fragment 90-95); 5. Methionine Enkaphalin-Arg-Phe.

The polymers were also investigated and compared to SDS for the separation of five peptides. These relatively small peptides range in

molecular weight from 698 to 877 g/mol. They do not have significant electrophoretic mobility and are not separated in the buffer system without added PSP (results not shown). The results of the separations with SDS, AGESS and LMAm are presented in Figure 5. SDS clearly outperforms the polymeric phases for this separation with better efficiency, selectivity, and resolution. Average plate numbers were 96,000±54,000, 78,000±1,000 and 47,000±10,000 using the SDS, AGESS, and LMAm, respectively. Higher concentrations of the AGESS polymer did not significantly improve the separation. The selectivity of the LMAm polymer is significantly different, but the efficiency and resolution is so poor that the utility of the polymer for this separation is severely limited. It is likely that SDS molecules form an ionic aggregate with the relatively large peptides, rather than the peptides interacting with preformed SDS micelles. The polymers, which have a fixed structure, are sterically unable to accommodate or interact strongly with the peptides in this fashion.

6. Conclusions

The results of these studies show conclusively that polymeric PSPs with a wide variety of chemical structures can be used for high-performance separations of small molecules by EKC. The selectivity and the performance of the polymers depends on the chemical structure and conformation in solution. The studies have determined the optimum structure for polymers based on acrylamide and siloxane backbones, and have provided significant fundamental characterization of the performance and separation selectivity afforded by these polymers. Fundamental studies conducted with pH responsive polymers have also shown that selectivity and performance is a function of polymer conformation, and that a lipophilic/hydrophilic balance that creates a collapsed "unimer micelle" conformation generally provides better performance. The polymers have been demonstrated to be useful for the separation of derivatized amino acids and to be compatible with sensitive LIF detection. Unfortunately, the studies also indicated that the polymers may not be suitable for the separation of larger analytes such as peptides.

The project has resulted in the publication of thirteen manuscripts in the peer reviewed literature. Citations 1-10 and 14-16 were all completed and published with support from this project. A further four review articles and one book chapter were published with partial support from the project. Four PhD students and one MS student were supported by the project for all or part of their studies. An additional seven undergraduate chemistry majors were supported by the project. Five undergraduates were included as coauthors on peer-reviewed publications.

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