# Nonaqueous Capillary Electrochromatographic Separation of Synthetic Neutral Polymers by Size Exclusion Chromatography Using Polymeric Stationary Phases

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**In this paper, we report the separations of large, neutral, synthetic polymers using primarily a nonaqueous mobile phase without the use of a supporting electrolyte. The size- exclusion-based mechanism for separation was achieved on sulfonated polystyrene/divinylbenzene stationary phases. The effect of water, voltage, stationary phase exchange capacity, and pore size were investigated. The stationary phase and solvent interactions were studied by attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) and a possible mechanism for the generation of EOF in the THF/water system is provided. Linear calibration curves were obtained for polystyrenes ranging in MW from 5K to 2M, for columns made using a combination of high capacity ion exchanger and a neutral polystyrene/divinylbenzene material of varied pore sizes. Analysis of polyurethane, polystyrene, and other polymer samples using CEC correlated well with results obtained by conventional HPLC. The size exclusion CEC separations provide an alternative mode for determining the relative molecular weights of polymers, with reduced solvent consumption.**

Capillary electrochromatography (CEC) has gained much attention in recent years because of its capabilities for improved separation efficiencies, reduced analysis time, and its use as an alternative mode of separation, combining aspects of both HPLC and CE. The separation of both charged and neutral analytes is achieved in CEC by using mechanisms similar to HPLC; however, the driving force in CEC is electroosmotic flow (EOF), not hydrodynamic flow. The flat, plug-like flow profile present with EOF allows for lower reduced plate heights and, consequently, higher efficiency as compared to HPLC.

A survey of the current literature reveals applications of CEC for the separation of a wide range of compounds, including polyaromatic hydrocarbons,[1–3] proteins,[4–6] peptides,[7–10] inorganic anion,[11] and pharmaceuticals,[12–15] to name a few. The CEC separations reported to date utilize predominantly reversed-phase and ion-exchange modes, performed almost exclusively in buffered aqueous or an aqueous/organic mixed electrolyte systems. The columns are either packed with silica-based stationary phases or prepared using polymeric monoliths. A variety of acrylamide-, methacrylate-, and polystyrene/divinylbenzene-based monoliths have been used.[16–23]

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Separation of neutral, synthetic polymers such as polystyrenes, polycarbonates, and polyurethanes in the CEC mode poses a challenge in that these polymers are nonpolar and at higher molecular weights (MW) are insoluble in most organic, aqueous, and mixtures of organic and aqueous solvents. Typical HPLC separations of these polymers involve the use of size exclusion chromatography (SEC), in which molecules are separated according to their effective sizes (hydrodynamic volume) in solution. This is accomplished via the use of columns packed with pores of defined size; separation is achieved by the different degrees to which analytes of different sizes permeate the pores. Several columns of different, single pore sizes, connected in series, are often used to achieve the separation of a broad range of molecular weights.[24] Nonaqueous conditions are required because of the nonpolar nature of these samples. The mobile phases consist of solvents, such as *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), chloroform, dichloroethane, and methyl ethyl ketone, often associated with high-temperature chromatographic conditions.[25] Hence, a separation method that would allow for the reduction of organic solvent consumption while providing comparable or improved separation is highly attractive from environmental as well as economic points of view.

In recent years, there has been an increased interest in the use of nonaqueous conditions for separations by capillary electrophoresis (CE) because of the different selectivity obtained. Nonaqueous electrophoresis has been performed in the CZE mode using solvents such as methanol, acetonitrile, formamide, and DMF with various electrolytes.[26–28] The use of THF as the separation electrolyte/mobile phase beyond that as a modifier in a mixed aqueous–organic system has not been thoroughly explored. Its use in nonaqueous CEC can be beneficial because of its low viscosity and its UV transparency, thus allowing for lower detection limits. Despite the advantages nonaqueous conditions bring to certain separations, there are very few reports on its use for CEC, including its use for separation of enantiomers by normal-phase chromatography.[2,29–31] Similarly, size-based separations have not been fully exploited in CEC, excluding capillary gel electrophoresis,[32,33] which relies on sieving rather than a size exclusion mechanism. To our knowledge, the only true size-exclusion-based separations in a pure CEC mode for large-MW neutral polymers was reported by Venema et al. and Stol et al. for polystyrenes using bare silica columns.[31,34,35] Several different pore size stationary phases were examined, and the addition of sodium dodecyl sulfate and an electrolyte to the DMF mobile phase was required in order to stabilize the EOF. The increase in ionic strength resulting from the addition of the salt, however, impacted the pore and interstitial flows, affecting the resolution as well as the overall retention window. Therefore, for practical separations, the ionic strength needs to be carefully controlled and its effects, balanced. Similarly, Peters et al. demonstrated a separation of a mixture of low-polydispersity polystyrene standards on a methacrylate-based monolithic column.[36]

Packed polymeric stationary phases offer an attractive alternative to the limitations of the fixed-pore silica-based columns and monolithic columns. The polymerization chemistry can be readily manipulated to control the pore size and shape, allowing for the synthesis of materials with pore sizes on the order of several angstroms to hundreds of nanometers. The resulting materials have well-defined pore sizes and narrow pore distributions.[37,38] This offers the possibility of mixing several different pore sizes to produce individual columns with extended separation range. Since there is no pressure involved in generating flow in CEC, high flow rates can be used by manipulating the EOF without the fear of crushing the packed bed, which is a major disadvantage to the use of polymeric phases in HPLC. Another benefit of the packed columns over monoliths is that a great number of columns can be packed from a single batch of mixed polymer. The polymeric stationary phases offer an attractive alternative to silica-based packings.

In this paper, we report the separations of large, neutral, synthetic polymers using primarily a nonaqueous mobile phase without the use of a supporting electrolyte. The size-exclusion-based mechanism for separation was achieved on a sulfonated polystyrene/divinylbenzene stationary phase, and the effect of various parameters on the separations was investigated. The solvent interactions were studied by attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) and a possible mechanism for the generation of EOF in the THF/water system is provided. The size-exclusion-CEC-based separations provide an alternative mode for determining the relative molecular weights of polymers, with reduced solvent consumption. The use of a relatively volatile solvent, such as THF, and no salts, combined with the inherently low flow rates of CEC, provides an opportunity for coupling the technique with mass spectrometry for absolute molecular weight determinations.

## EXPERIMENTAL SECTION

**Chemicals and Materials.** THF, toluene, DMF, lithium bromide, and 2,2,4-trimethylpentane (isooctane) were purchased from Sigma-Aldrich (Milwaukee, WI). The methanol was from Fisher Scientific (Pittsburgh, PA). The monomers 2,3-epoxypropyl methacrylate (glycidyl methacrylate) and trimethylolpropane trimethacrylate, as well as the initiator α-methoxy-α-phenylacetophenone (benzoin methyl ether), were obtained from Aldrich. The monomers were purified on an ion-exchange column (Aldrich P/N 30,631-2) in order to remove polymerization inhibitors prior to

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**Table 1. Properties of the Polymeric Stationary Phases**

| | polymer[a] | particle size[a] ($\mu$M) | pore size[a] (Å) | capacity[a] (meq/g) | toluene[b] mobility ($\mu_{eo} \times 10^4$ cm$^2$/V sec) |
|---|---|---|---|---|---|
| PRP ×300 | sulfonated PSDVB | 3 | 100 | 0.20 | $0.142 \pm 0.003^c$ |
| | | | | | $0.149 \pm 0.002^c$ |
| PRP ×300 | sulfonated PSDVB | 3 | 300 | 0.13 | $0.224 \pm 0.002$ |
| PRP ×400 | sulfonated PSDVB | 7 | 300 | 2.5 | $0.452 \pm 0.006$ |
| PLGel C | PSDVB | 5 | 50−10$^4$ mixed | | |

[a] Data from Hamilton Company and Polymer Laboratories. [b] Column, 25 (33) cm × 75 $\mu$m; 25 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water, 25 °C. [c] Results from experiments performed on separate days.

use. All reagents were of the highest purity available. The water was purified using a Hydro Ultrapure filtration system (Picotech, Garfield, NJ).

The low polydispersity, less than 1.05, polystyrene (PS) standards of MW 3 900 000, 3 065 000, 2 320 000, 1 013 000, 488 400, 96 000, 50 400, 21 000, 13 100, 5000, 2960, and 1260 were obtained from Polymer Laboratories (Amherst, MA). A mixture of polystyrene standards, consisting of MWs 488K, 96K, 21K, and 5K each at a concentration of 2 mg/mL in THF was used for the column studies. Toluene, 0.2% (v/v), was added to the mixture as a marker, since it is of low MW and would, therefore, completely permeate the pores and be retained. It was used to calculate the normalized retention times of the polymers. The polystyrene, polycarbonate, and other polymer samples were provided by Hernan Cortes of the Dow Chemical Company (Midland, MI). The samples were prepared at 0.5 mg/mL, and 0.1% (v/v) toluene was added to each preparation. The mobile phase/run electrolyte was THF/water 98/2 by volume, or otherwise as indicated. The THF used throughout the studies was uninhibited, and hence, UV detection at low wavelengths, 214 nm, could be used to improve the sensitivity.

The porous ion-exchange stationary phases, sulfonated polystyrene/divinylbenzene copolymers PRP ×300 and ×400, were provided by Hamilton Company (Reno, NV). The resins are marketed for use in ion-exclusion chromatography by HPLC; thus, they are well-characterized and are readily available in bulk. The PLGel Mixed C phase was obtained from Polymer Laboratories (Amherst, MA) and consisted of polystyrene/divinylbenzene (PSDVB) beads of mixed pore sizes to provide linear calibration over a wide molecular weight range for SEC. The properties of these phases are provided in Table 1. The polyimide-coated, bare fused-silica capillaries were of 75 $\mu$m i.d. and 365 $\mu$m o.d. and were purchased form Polymicro Technologies (Phoenix, AZ).

**Instrumentation.** The CEC experiments were performed on a Hewlett-Packard (Palo Alto, CA) 3D capillary electrophoresis instrument equipped with a diode array detector. Data were collected and processed using the Hewlett-Packard 3D-CE Chemstation software. All separations were performed in the positive polarity mode, with a constant applied voltage of 25 kV or as indicated. A pressure of 10 bar was applied to both ends of the capillary using nitrogen gas for all experiments. Samples were introduced electrokinetically using a 25 kV/10 s injection or as indicated. The temperature of the capillary cassette was maintained constant at 25 °C or as indicated otherwise. Data were collected at 214 nm, and all results presented are an average of triplicate measurements. The resolution and number of theoretical

plates were calculated according to the half-width method. The number-average and the weight-average molecular weights, $M_n$ and $M_w$, respectively, were calculated according to the procedure outlined in ref 39.

The HPLC analysis of the wide-distribution polymer samples was performed using a Waters (Milford, MA) Alliance 2690 pump and an autosampler equipped with a Waters 2410 differential refractive index detector. The data acquisition and reduction was performed using Polymer Laboratories Caliber GPC/SEC software. A set of two PLGel Mixed C columns, 30 cm × 7.5 mm, coupled in a series with a guard column, were used. The flow rate was 1 mL/min, and the injection volume was 50 $\mu$L.

The infrared spectra were obtained using a Nicolet Nexus 670 FT-IR (Madison, WI) equipped with a zinc-selenide crystal horizontal attenuated total reflectance cell (Pike Technologies, Madison, WI) and OMNIC E.S.P. 5.1 software. The data acquisition was performed at a resolution of 1 cm$^{-1}$, and 64 scans/spectrum were collected.

**Column Fabrication.** For the fabrication of the columns, porous polymeric frits were made from poly(glycidyl methacrylate-co-trimethylolpropane trimethacrylate) polymer, GMA-co-TRIM, using an in situ photoinitiated polymerization reaction as described by Svec et al. with some modifications to the procedure.[37,40] A black-ray longwave UV lamp, model B100-A from UVP (Upland, CA), with an intensity of 8400 $\mu$W/cm$^2$ at 18 in. for 365 nm was used for the polymerization. The porogen mixture ratio was 70/30 isooctane/toluene, and the monomer mixture was 30/70, TRIM/GMA by weight. The porogen-to-monomers ratio was 50/50, and 2% benzoin methyl ether was used as the initiator.

The preparation of polymeric columns was as follows: An outlet frit was made by polymerization of glycidyl methacrylate and trimethylolpropane trimethacrylate monomers as described above. After the polymerization, excess porogen and reaction mixture were washed free, and the frits were tested for stability by flushing the capillary on an HPLC pump using methanol. The detection window was immediately after the outlet frit and was made by heating with a thermal wire stripper (Teledyne Kinetics, Los Angeles, CA). The packing solvent was THF, and a 50 mg/mL slurry of the stationary phase in THF was introduced into an empty glass-lined stainless steel tube (0.5 mm i.d., 1/8 in. o.d., 15 cm length, SGE, Austin,TX), fitted with HPLC column end fittings, which served as the slurry reservoir. The empty capillary

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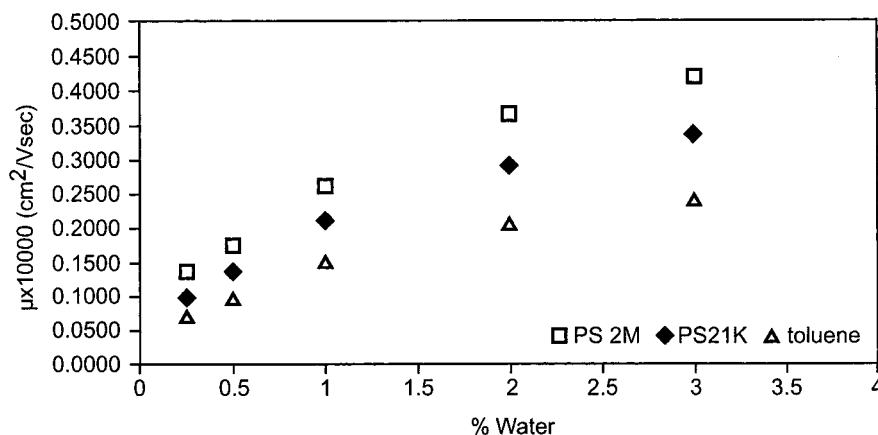
**Figure 1.** Effect of water on electroosmotic mobility of polystyrenes and toluene. Column: ×300, 300 Å, 25 (33) cm × 75 μm; 25 kV run voltage; 25 kV/10 s injection; mobile phase, THF with water; 25 °C.

was connected to the slurry reservoir using a PEEK tubing sleeve and a standard HPLC fitting. The columns were packed at 2000 psi using a Spectrasystem P4000 pump from ThermoSeparations (San Jose, CA). After the desired length of packed bed had formed (30−50 cm), the pump was turned off and the pressure allowed to return to zero. The column was then removed from the packing apparatus and cut to give 25 cm of packed bed (effective length) and 33 cm of total length. The inlet frit was formed using the same polymerization procedure described above. To prevent the column from drying out, the outlet end of the capillary was immersed in a vial of THF during the inlet frit formation. The outlet end of the column was then connected to an HPLC pump using a T-splitter with a restriction capillary at the other end of the tee. The column was flushed with the CEC mobile phase before installation into the CE instrument. All of the columns used were 75 μm i.d. × 365 μm o.d. with an effective length of either 25 cm (33 cm total length) or 45 cm (53 cm total length).

## RESULTS AND DISCUSSION

To achieve separations of polystyrene standards via size exclusion CEC (SE-CEC), several avenues were studied. Thus, in the initial studies, sulfonated polystyrene/divinylbenzene resins with different exchange capacities and pore sizes were examined to see if significant EOF could be generated using THF and the polymeric stationary phases.

**PLGel Columns.** The PLGel stationary phases are used for the HPLC separation of polymers under nonaqueous conditions. The neutral polystyrene/divinylbenzene phases consist of mixtures of different pore-size materials precisely blended to expand the mass calibration range to accommodate broad molecular weight distributions of the polymers. The PLGel Mixed C phase consists of pore sizes from 50 to 10⁴ Å and is designed for linear calibration of log MW vs elution volume up to 2 000 000 MW polystyrene.

To probe this stationary phase, a mixture of four polystyrene standards, ranging in MW from 5 to 488K, was used. Separation of the PS standards using THF as the mobile phase resulted in no peaks being detected. This may have been due to low EOF, since the PLGel phase is neutral, and hence, the only source for generation of the EOF was the surface silanols on the capillary column wall. The addition of 2% water to the THF, in a manner analogous to conditions used by Peters et al., allowed the PS

standards to elute, although at the expense of very long analysis times. The EOF was not constant from injection to injection, with retention times ranging from 60 to 120 min for consecutive injections of the same polymeric analytes.

To overcome the low electroosmotic flow, several options were explored. The flow in CE is influenced by the dielectric constant and the viscosity of the solvent. DMF has a higher dielectric constant than THF and, therefore, can generate higher EOF. Use of DMF, however, with the PLGel C columns resulted in no peaks being detected in 2 h. This may have been due to the higher viscosity of DMF (0.8c P for DMF vs 0.46 for THF).[41] It was concluded that the silanols on the walls did not provide sufficient EOF for CEC, and therefore, alternative methods for enhancing the EOF, affecting the ζ potential via the surface charge, were examined using THF as the mobile phase.

**Low-Capacity ×300 Columns.** Sulfonated polystyrene/divinylbenzene resins, ×300 series, from Hamilton were studied to determine if they could generate stable, reproducible EOF. The idea was to use the sulfonyl groups on the PSDVB phase to generate sufficient EOF to make SE-CEC practical. The resins are highly cross-linked, and therefore, the particles could be packed into capillary columns at high pressures without crushing.

Because the magnitude of the EOF is dependent on the surface charge, and the separation in SEC is based on pore size, resins with two different exchange capacities and pore sizes were studied. The first stationary phase had an exchange capacity of 0.13 meq/g and a pore size of 300 Å. The second resin was 100 Å with 0.20 meq/g exchange capacity. These resins were compatible with the nonaqueous solvent systems required for the polymer separations and were readily available in bulk.

*Influence of Water.* Initial experiments with 100% THF as the mobile phase on the 300 Å ×300 columns resulted in very low EOF. Addition of LiBr as an electrolyte to the THF did not improve the EOF. Addition of a small amount of water to the THF resulted in higher, stable, and reproducible EOF. Figure 1 demonstrates the effect of adding water to the THF on the polystyrene separations. As the amount of water increased from 0 to 3%, there was a significant increase in the electroosmotic mobility, and a corresponding decrease in the migration times. To determine the

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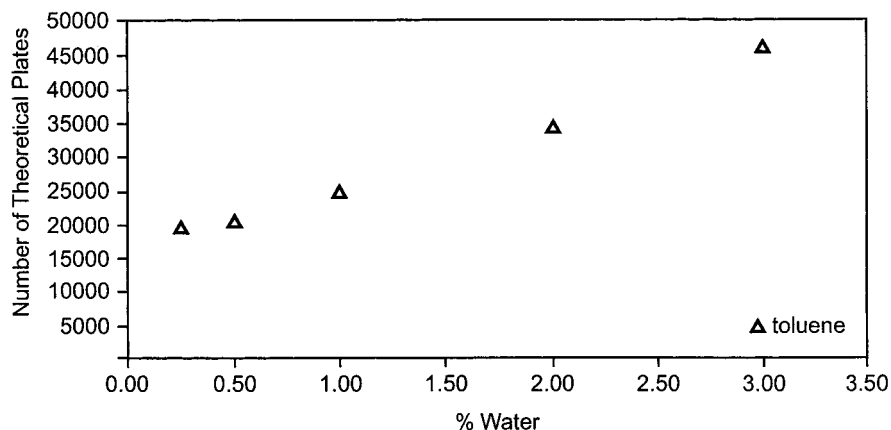
**Figure 2.** Effect of water on efficiency of toluene. Column: ×300, 300 Å, 25 (33) cm × 75 $\mu$m; 25 kV run voltage; 25 kV/10 s injection; mobile phase, THF with water; 25 °C.

EOF, the mobility of a totally excluded polymer, PS 2M, was used. Higher water concentrations could not be investigated because of the precipitation of the high molecular weight polystyrenes in the presence of water. The peak efficiencies for toluene also improved with the increase in water, as illustrated in Figure 2.

Polystyrenes of MW 5-, 21-, and 96K were resolved from each other, using the 300 Å ×300 column, whereas PS 96- and 488K were only partially resolved at all ratios of THF−water investigated. The resolution of the lower MW PSs improved slightly by increasing the concentration of water from 0.5% ($R_{96,21K}$ = 1.36, $R_{21,5K}$ = 1.56) to 3% ($R_{96,21K}$ = 1.61, $R_{21,5K}$ = 1.83). However, the resolution for the 96- and 488K was not affected. It appeared that the 488K PS was at the exclusion limit for this column. Although the resolution was also higher with 0.25% water in the mobile phase ($R_{96,21K}$ = 1.38, $R_{21,5K}$ = 1.69), the run times (80 min) were too long to be practical.

Generation of EOF in nonaqueous media without the use of added ionic species has been studied by several groups for CZE. Valko et al. reported that the ability of the solvent to accept protons played a crucial role in the development of EOF in studies with fused silica capillaries.[42] In addition, ionic impurities present in the solvent may also contribute to the generation of EOF.[27] This may account for the low EOF observed with the 100% THF as the mobile phase. Tjornelund and Hansen investigated the effect of small changes in water concentration on nonaqueous CE. They concluded that water added in concentrations up to 0.5% had only a minor effect on the EOF.[43] However, the solvents studied were dimethyl sulfoxide, *N*-methylformamide, acetonitrile, and methanol, which have been known to generate significantly higher EOF than THF. Thus, the relative changes in EOF with the addition of water in these solvents may not be appreciable.

To investigate further the enhancement of EOF with the addition of water, attenuated total reflectance FT-IR spectra of THF−water, stationary phase (×300) in THF, and stationary phase in THF−water systems were collected. In addition to the expected bands due to water, the spectra for the mixture of THF−water indicated a shift in the maximums for the asymmetric and symmetric C−O−C stretches (figure not shown). The band at
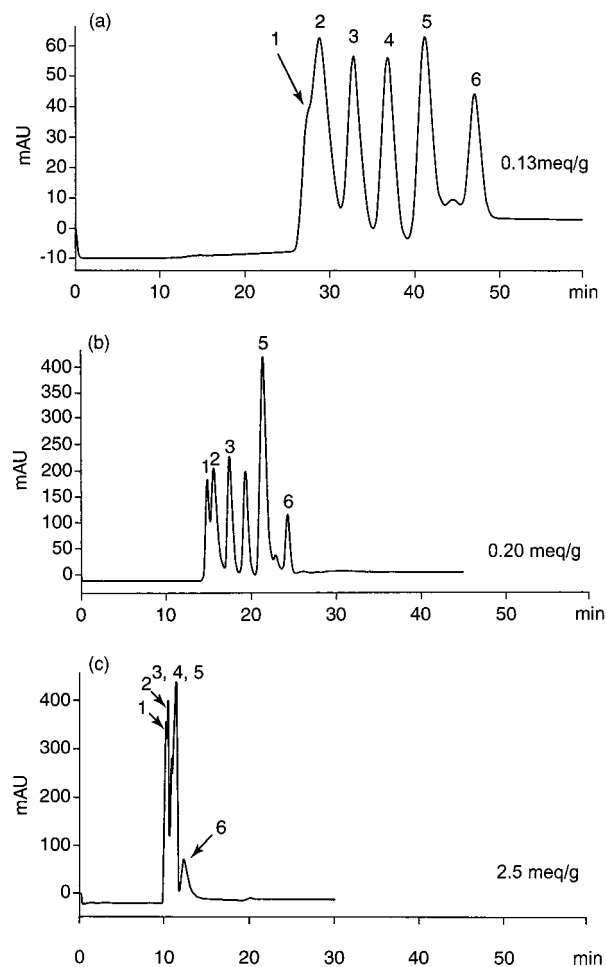
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**Figure 3.** Effect of varying the stationary phase capacity. Column: 25 (33) cm × 75 $\mu$m; 25 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water; 25 °C. Polystyrene standards: 1, 488K; 2, 96K; 3, 21K; 4, 5K; 5, blank; 6, toluene. a: ×300, 0.13 meq/g. b: ×300, 0.20 meq/g. c: ×400, 2.5 meq/g.

1066 cm$^{-1}$ in THF for the asymmetric C−O−C stretching was gradually shifted to 1059 cm$^{-1}$ and became broader as the concentration of water was increased from 0 to 10%. Similarly, the maximum for the symmetric C−O−C band at 908 cm$^{-1}$ was shifted to 904 cm$^{-1}$. This was perhaps due to the association of the THF with water. Hydrogen bonding results in a shift to a lower

wavenumber for the bands due to stretching.[44] Further evidence was provided by small shifts in the $CH_2$ deformation bands at 1459, 1365, and 1289 cm$^{-1}$ to increased wavenumbers. Katz et al. has suggested that mixtures of THF−water can be regarded as a ternary solvent system, with the three components being the free THF, free water, and THF−water complex.[45] Recently, Scott reported that in a THF−water system, as the volume fraction of water is decreased, the mixture changes from ternary in nature to largely binary, with mostly THF and THF−water complex; only a small amount of the water is present unassociated with THF.[46] This is in agreement with our IR data suggesting that the THF is associated with water.

Significant changes were also observed in the IR spectra of the stationary phase in the presence of THF water mixtures (figure not shown). The appearance of a pronounced peak at 1030 cm$^{-1}$, which was not observed in the THF−water or stationary phase− THF systems, could be due to the interactions occurring between the associated THF−water and the sulfonated PSDVB stationary phase. Hence, a reasonable explanation for the generation of EOF may be that it is due to the increased dielectric constant of the mobile phase and ionization of the sulfonic acid moieties of the polystyrene/divinylbenzene phase with the addition of water.

*Effect of Flow Rate.* To study the effect of flow rate changes, the voltages applied for the separations were varied. The Ohm's law plot of current versus voltage was linear ($r^2 = 0.990$). The resulting currents were very low, less than 1 $\mu$A. Thus, Joule heating with higher voltages did not appear to be an issue when using the THF−water system. The linear velocity, calculated using the retention time of PS 2M as the void volume, increased linearly ($r^2 = 0.999$). The run time of the analysis was reduced from 65 to 35 min, although at the expense of resolution with the increased flow (data not shown).

*Effect of Temperature.* The effect of temperature on the separation of the PS standards was also investigated. The electroosmotic flow increased slightly with increases in temperature from 10 to 40 °C. This may have been due to the decreased viscosity with higher temperatures. The resolution for the low-MW polymers decreased slightly with an increase in temperature (from 1.37 to 1.25 for $R_{21,5K}$). Such behavior could be attributed to changes in viscosity, which leads to changes in the mass transfer rate of the polymer molecules in and out of the pores and, hence, affects the separation.[39] The impact of temperature on higher-MW polymers was not as discernible (from 1.07 to 1.02 for $R_{96,21K}$). The larger polymers did not permeate the pores to the same extent as the smaller analytes and, thus, were not as affected. The stationary phase appeared to be stable in the temperature range studied.

*Influence of Pore Size.* The effect of pore size was studied by using columns made of 100 Å ×300 material. It should be noted that the capacity of this column was slightly higher, 0.2 meq/g; this would allow for higher EOFs. In comparing the 100-and 300-Å columns, there were only minor differences in selectivity and resolution. The primary difference appeared to be in the linear calibration range as indicated. The overall analysis time did

decrease, from 40 to 30 min, in going from the 300-to the 100-Å column. This was due to the higher capacity leading to higher flow.

*Calibration Curves.* The linear calibration range of the ×300 columns was determined by injection of polystyrene standards ranging in MW from 5- to 488K. On the basis of the curve (figure not shown), the 488K PS standard appeared to be at the exclusion limit of the 300-Å column, but the 100-Å material had an exclusion limit of 96K. The correlation coefficients for the linear regression fit for log MW versus the normalized migration times were determined to be higher than 0.99 for both resins. The normalized retention time, $\tau$, was calculated as the retention time of polystyrene divided by the retention time of toluene.[31] Since the calculations for MW determination in SEC are based on retention times, small changes in retention times resulting from changes in flow could lead to significant errors. Thus, by normalizing the retention times versus toluene, the errors due to variations in EOF were minimized. The retention times and the elution order of the peaks were confirmed by injection of individual MW PS standards.

**High-Capacity ×400 Resin.** To improve the separation, a higher-capacity resin, PRP ×400, was also examined. The hope was that the higher capacity would provide increased EOF and improved efficiency, as well as reduced analysis times. The apparent mobilities obtained using the different capacity sulfonated PSDVB resins are provided in Table 1 and serve to indicate the magnitude of the EOF. As expected, the general trend was an increase in flow with increased charge on the resin. The flow rate could be further manipulated by adjusting the voltage. Figure 3a−c shows the separation of PS on the three different exchange capacity resins. The very large peak (5) appearing after the PS peaks and before the toluene peak was a system peak due to the injection. It was present in all of the samples and blanks, appearing as either positive or negative peaks of varying intensity. The separation and resolution was poor for the high-capacity ×400 material, even though the pore size was the same, 300 Å, used with the lower capacity resin. The loss of selectivity may have been due to the increased flow rate. In size exclusion CEC, the separation is governed by the interparticle and intraparticle flow velocities. Venema and co-workers have reported that for electrically driven SEC separations, changes in the ratio of pore flow to interstitial flow can significantly impact the resolution.[35] In their studies, the ratio was affected by changes in the ionic strength of the mobile phase. The increased pore flow resulted in faster mass transfer and, hence, increased efficiency. On the other hand, the mass selectivity was negatively impacted because of the decreased retention window.[34,35] In our case, with the ×400 resin, the increase in flow led to the higher observed efficiency. At the same time, the decrease in the retention window ($\tau = 0.6-1.0$ for ×300 vs 0.8−1.0 for ×400) resulted in a loss of resolution. In addition, the higher particle size for the ×400 material may account for some loss of efficiency and, consequently, the resolution.

**Mixed ×400−PLGel Columns.** The results presented above clearly established the limitations of using resins with fixed pore size. The selectivity could be improved and the linear calibration range extended if a stationary phase containing a wider distribution of pore sizes was used. However, the distribution needs to be carefully controlled to obtain column-to-column reproducibility.

(44) Colthup, N. B.; Daly, L. H. *Introduction to Infrared and Raman Spectroscopy*, 3rd ed.; Academic Press: San Diago, 1990.
(45) Katz, E. D.; Ogan, K.; Scott, R. P. W. *J. Chromatogr.* **1986**, *352*, 67−90.
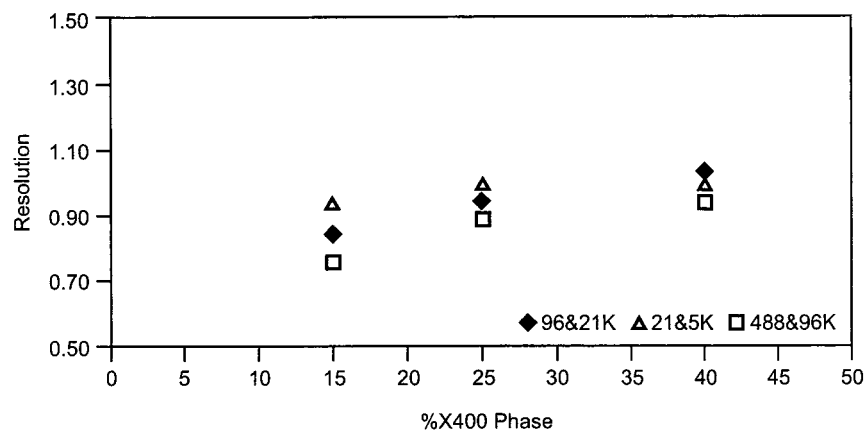(46) Scott, R. P. W. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 3083−3093.

**Figure 4.** Resolution of polystyrene standards versus column composition. Column: ×400−PLGel C, 25 (33) cm × 75 μm; 25 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water; 25 °C.
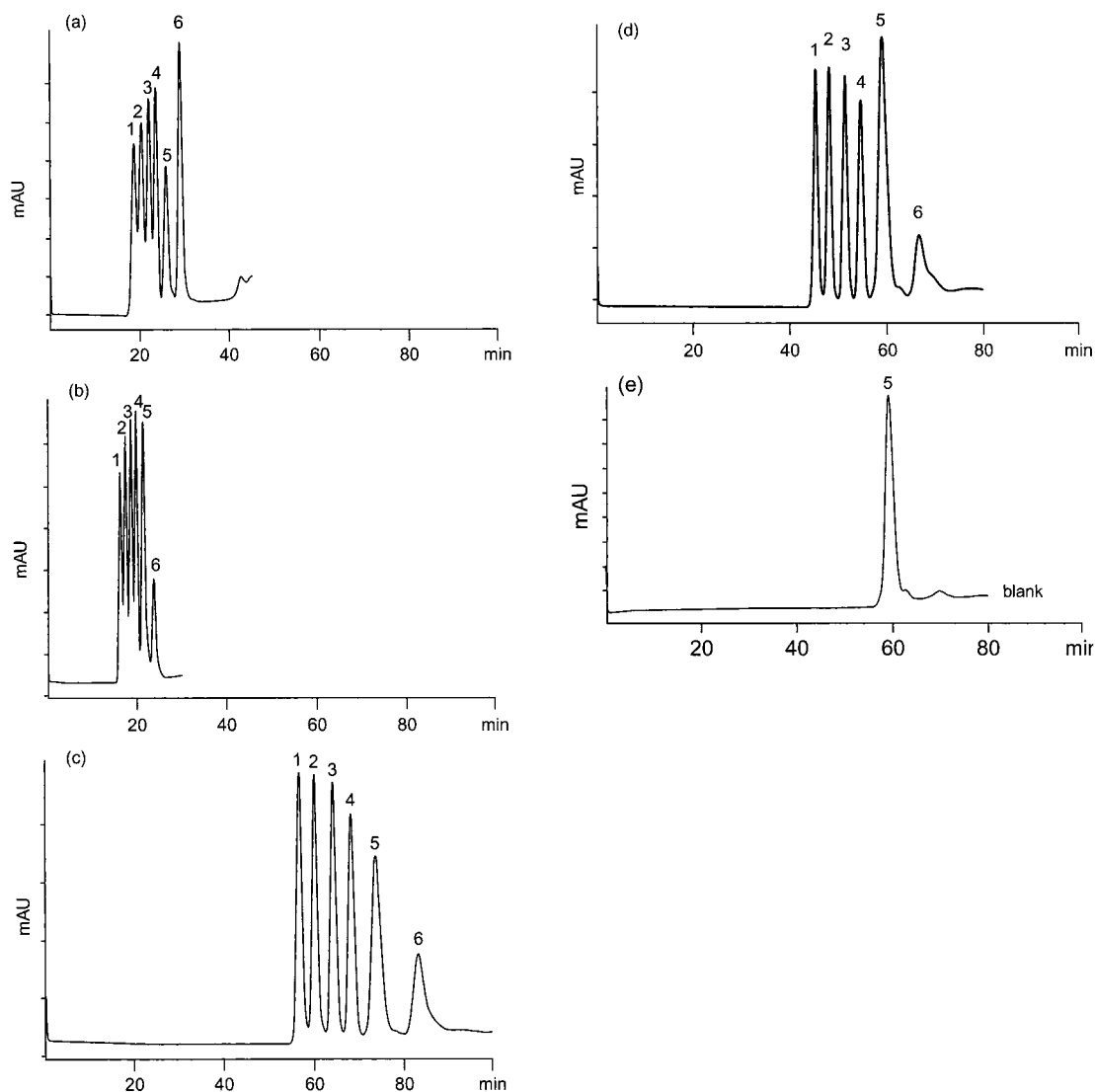


**Figure 5.** Influence of column composition, column length, and voltage on polystyrene separations. 25 kV/10 s injection; mobile phase, THF with 2% water; 25 °C. Polystyrene standards: 1, 488K; 2, 96K; 3, 21K; 4, 5K; 5, blank; 6, toluene. a: ×400−PLGel C (15:85), 25 (33) cm × 75 μm; 25 kV run voltage. b: ×400−PLGel C (40:60), 25 (33) cm × 75 μm; 25 kV run voltage. c: ×400−PLGel C (40:60), 45 (53) cm × 75 μm; 25 kV run voltage. d and e: ×400−PLGel C (40:60), 45 (53) cm × 75 μm; 30 kV run voltage.

The PLGel mixed stationary phases were ideal for this, because they are available in various pore sizes already optimized for polymer separations by HPLC. Since there was not sufficient EOF with the PLGel phase alone, columns packed with a mixture of PLGel C and the high capacity ×400 material at various ratios were studied next.

**Table 2. Analysis of Polymer Samples by HPLC and CEC[a]**

| | HPLC | | | CEC | | |
|---|---|---|---|---|---|---|
| sample | $M_n$ (SD) | $M_w$ (SD) | $M_w/M_n$ | $M_n$ (SD) | $M_w$ (SD) | $M_w/M_n$ |
| polycarbonate 1 | 13 133 (172) | 29 438 (319) | 2.24 | 16 308 (1081) | 32 081 (670) | 1.97 |
| polycarbonate 2 | 14 674 (210) | 32 777 (232) | 2.23 | 18 294 (680) | 35 884 (538) | 1.96 |
| styrene/acrylonitrile copolymer | 41 217 (312) | 84 814 (359) | 2.06 | 38 577 (2537) | 87 494 (4568) | 2.28 |
| polystyrene 1683 | 99 858 (374) | 260 499 (897) | 2.61 | 94 311 (2099) | 276 395 (10866) | 2.93 |
| thermoplastic polyurethane | 44 691 (504) | 83 739 (693) | 1.97 | 43 165 (2570) | 93 722 (3433) | 2.17 |

[a] SD = standard deviations. CEC column: ×400−PLGel C (40:60), 25 (33) cm × 75 $\mu$m; 20 kV run voltage; 20 kV/8 s injection; mobile phase, THF with 2% water; detection, UV 214 nm; 25 °C; samples, 0.4 mg/mL in THF. Results presented are average of triplicate injections. HPLC column: 2× PLGel C and guard, 30 cm × 7.5 mm; flow rate, 1 mL/min; 50 $\mu$L injection; mobile phase, THF; DRI detection; samples, 2 mg/mL in THF.
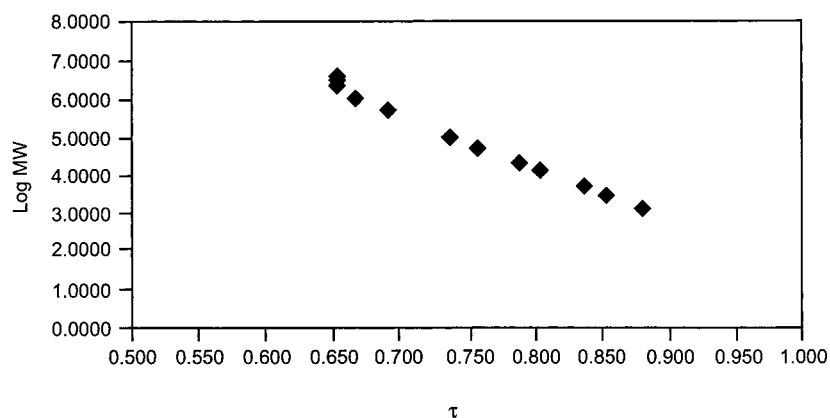


**Figure 6.** Mass calibration for mixed-bed column. Column: ×400−PLGel C (40:60), 25 (33) cm × 75 $\mu$m; 20 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water; 25 °C; samples, polystyrenes with MW 1K−4M.
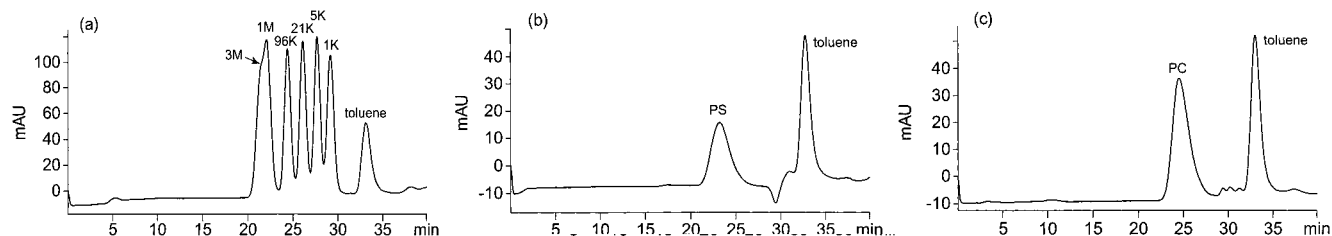


**Figure 7.** Typical electrochromatograms. Column: ×400−PLGel C (40:60), 25 (33) cm × 75 $\mu$m; 20 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water; 25 °C. a: polystyrene standards MW 3M, 1M, 96K, 21K, 5K, and 1K. b: polystyrene 1683. c: polycarbonate.

Figures 4 and 5a,b illustrate the effect of varying the ratio of ×400 phase on the resolution of PS using columns with an effective length of 25 cm. The resolution improved with the increase in the ×400 concentration as a result of sharper peaks. All of the four PS were separated, including the PS 2M (data not shown). The resolution was dramatically improved by increasing the column length from 25 to 45 cm, as demonstrated in Figure 5b,c. Since the flow is generated electroosmotically in CEC, long columns can be used without generating high back pressures, as in HPLC. The longer columns lead to increased analysis times, which could be improved by using higher voltages, as illustrated in Figure 5d. The presence of a peak in the injection of the blank in Figure 5e was discussed earlier.

On the basis of the results of the above experiments, a calibration curve was generated using optimized conditions on the mixed bed columns (Figure 6). The linear calibration range for the polystyrene was determined to be between 5000 and 2 000 000 molecular weight, with a correlation coefficient of >0.99. Samples of polystyrenes, polycarbonates, and other polymers were analyzed using the CEC method. The results correlated well with those obtained by conventional HPLC as presented in Table 2.[47] All of the samples were analyzed versus the polystyrene standards, and representative electrochromatograms are presented in Figure 7. It should be noted that some differences in the results may be attributed to sample nonhomogeniety.

The HPLC experiments were performed using a set of two 30-cm columns, whereas the CEC column had an effective length of 25 cm. Although the analysis times for the two methods were comparable, the reduction in solvent consumption was quite significant. The complete CEC analysis was performed using a total of 2 mL of solvent in the electrolyte reservoirs, whereas the HPLC analysis used over 800 mL.

*Reproducibility.* All of the data presented in this report was obtained by making triplicate injections. Although the retention/ migration times of the peaks varied somewhat from run to run, with relative standard deviations (RSD) of <2%, the relative

(47) Meunier, D.; Cortes, H. personal communication, 2001.

**Table 3. Retention Time Reproducibility**[a]

| polystyrene (MW) | retention time | | rel retention time | |
|---|---|---|---|---|
| | (min) | %RSD | ($\tau$) | %RSD |
| 3 065 000 | 21.64 | 0.43 | 0.65 | 0.32 |
| 1 013 000 | 22.06 | 0.41 | 0.67 | 0.32 |
| 96 000 | 24.41 | 0.32 | 0.74 | 0.19 |
| 21 000 | 26.12 | 0.29 | 0.79 | 0.15 |
| 5000 | 27.70 | 0.26 | 0.84 | 0.11 |
| 1260 | 29.18 | 0.27 | 0.88 | 0.11 |
| toluene | 33.15 | 0.20 | 1.00 | |

[a] Column: ×400−PLGel C, 25 (33) cm × 75 $\mu$m; 20 kV run voltage; 25 kV/10 s injection; mobile phase, THF with 2% water, 25 °C. Results are average of six injections performed over the course of a day.

retention times determined by normalizing against the retention time of toluene marker ($\tau$) consistently had RSDs <0.5% RSD. Table 3 represents data for a mixture of PS standards injected over the course of a day.

## CONCLUSIONS

In this report, we have demonstrated the potential of nonaqueous media, without the use of electrolytes, for the separation of large synthetic neutral polymers on polymeric packed columns by CEC. It was found that small amounts of water were required to produce sufficient EOF using THF. A mobile phase consisting of THF with 2% water produced stable and reproducible EOF and, hence, reproducible results when used with the sulfonated polystyrene/divinylbenzene stationary phases. Although the sulfonated resins (PRP X-series) are used primarily under aqueous conditions in HPLC, solvent compatibility under primarily nonaqueous conditions did not appear to be an issue. In addition, the salt-free mobile phases are of advantage when used with mass spectrometric detection. Thus, coupling of size exclusion CEC with MS may provide an absolute method of molecular weight characterization that overcomes some of the sample bias problems associated with direct MALDI-MS of polydisperse polymeric samples.[48]

(48) Wu, K. J.; Odom, R. W. *Anal. Chem.* **1998**, *70*, 456A−461A.

By varying the magnitude of the charge of the stationary phase via the exchange capacity, the flow rates for the CEC separations can be controlled. The increased flow generated by the higher capacity resin resulted in improved peak efficiencies, but the PLGel mixed pore size phase provided better resolution and extended the mass calibration range. Further modifications to the flow rate were made by changing the run voltage. Because of the low currents encountered with the nonaqueous system, Joule heating was not a concern at higher voltages.

The calibration curves for the molecular weight versus retention times were linear from 5K up to 2M for polystyrenes. The methods described in this paper provide an alternative to HPLC-based separations of neutral synthetic polymers.

## SUPPORTING INFORMATION AVAILABLE

Three graphs showing attenuated total reflectance FT-IR spectra of THF−water and stationary phase. This information is available free of charge via the Internet at http://pubs.acs.org.

## NOTE ADDED AFTER ASAP POSTING

This article was inadvertently posted ASAP without the Supporting Information paragraph. The corrected version was posted with the issue.