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Synthesis and evaluation of anionic polymer-coated capillaries with pH-independent electroosmotic flows for capillary electrophoresis

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Abstract

A capillary coating method was developed using a sodium-2-acrylamido-2-methylpropanesulfonate (NaAMPS) polymer. Capillaries coated with this anionic polymer exhibited pH-independent electroosmotic flows (EOFs). Capillaries with a particular EOF rate could be synthesized simply by changing the molar ratio of NaAMPS to neutral acrylamide in the polymer mixture. The pH-independent EOF of the coated capillary changed less than 1.5% over 20 days of routine, intermittent use. Capillary lifetime with a continuously applied electric field of 286 V cm^{-1} was about 60 h. Reproducibility of the coating procedure was 1.7% R.S.D. in observed EOF for independently synthesized capillaries. The pH-independent EOF of these capillaries as well as their selectable flow-rates (via synthesis) should prove useful in micellar electrokinetic capillary chromatography (MECC) at low pH values and for expansion of the separation window in MECC, as well as for more versatile separations of various species in the capillary zone electrophoresis mode.

1. Introduction

One of the most important features in capillary electrophoresis (CE) is the presence of electroosmotic flow (EOF), which because of its relatively flat flow profile [1–3], detracts little from the efficiency of a separation. A second feature of EOF is that it is often 5–10 times faster than the intrinsic electrophoretic mobilities of ions typically separated using capillary zone electrophoresis (CZE). This permits the migration of cations, anions and neutrals in one direction, allowing them to be detected at one end of the capillary within a reasonable time. Since the EOF affects the dwell time of a solute in the capillary, both the separation efficiency

and resolution are related to the magnitude of EOF [4]. Method development is complicated by the pH dependency of EOF, which when coupled with pK_a values of ionic moieties on the solute, can create complicated pH-resolution maps which exhibit critical behavior and can become extremely sensitive to localized electrolyte pH changes, contributing to poor overall precision. Since electroosmotic flow diminishes at pH values lower than 4 or 5, ion suppression of acidic molecules for separation by micellar electrokinetic capillary chromatography (MECC) is often not feasible. Further complications arise during electrokinetic injections, where the mass of solute injected is directly related to the EOF. Another problem with EOF is that, since concentration detectors are widely used in CE, reproducibility of quantitation using peak area is directly proportional to the reproducibil-

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ity of the EOF. While mathematical corrections can be applied on a post-run basis, in general, a constant EOF rate contributes greatly to the ruggedness and versatility of electrically based separations. This has made the control of EOF one of the fundamental problems in CE.

EOF is the bulk flow of solution due to the effect of the electric field on counterions in the diffuse layer adjacent to the shear layer residing next to the negatively charged capillary wall (assuming the use of fused silica at alkaline pH conditions). The potential at the shear layer is termed the ζ potential. When an electric field is applied, the cationic counterions migrate toward the cathode and drag solvent with them, resulting in a bulk flow of solution toward the cathode [5]. The velocity profile for EOF has been studied by numerous investigators [6,7]. The electroosmotic mobility can be expressed as [7]:

$$\mu_{\text{eo}} = \frac{-\epsilon\psi_0}{\eta} \quad (1)$$

where ϵ is the electrical permittivity of the solvent, ψ_0 is the electrical potential at the capillary–solution interface, and η is the viscosity at the wall. The surface charge density σ^* can be expressed as:

$$\sigma^* = \epsilon\kappa\psi_0 \quad (2)$$

where κ is equivalent to the inversion of double layer thickness (κ^{-1}) which is related to ϵ and ionic concentration (C). Thus Eq. 1 can be written in terms of the surface charge density:

$$\mu_{\text{eo}} = \frac{\sigma^*}{\kappa\eta} \quad (3)$$

or

$$\mu_{\text{eo}} = \frac{\sigma^*\kappa^{-1}}{\eta} \quad (4)$$

It can be seen that in order to control EOF, one can control either the charge density on the capillary wall (σ^*), the double layer thickness (κ^{-1}) or the viscosity of the solution adjacent to the capillary wall (η). Fujiwara and Honda [8,9] reported two methods to control EOF by reduc-

ing the double layer thickness: (1) increasing the electrolyte concentration in the run buffer, or (2) decreasing the permittivity of the run buffer by the addition of organic solvent. The limitations of these approaches are increased Joule heating within the capillary due to the increased ion concentration and altered electrolyte solubility due to the presence of organic solvents in the run buffer. EOF can also be altered by changing the buffer viscosity with addition of soluble polymers to the run buffer [10,11], such as methylcellulose.

Most research for the control of EOF has been focused on altering the surface charge density (σ^*) and viscosity (η) on the capillary wall. In situations where little or no EOF is desired, along with reduced solute adsorption, polymeric wall coatings such as methylcellulose [12], trimethylsilane [1,13], polyacrylamide [14], polyvinylpyrrolidone (PVP) [15], polyethylene glycol (PEG) [16], maltose [17], vinyl-bond polyacrylamide [18], polyethyleneimine (PEI) [19], polymethylglutamate [20], hydroxylated polyether [21], octyl, octadecyl [22] and epoxydiol [23] have been used. Another approach used to control EOF is that of applying a radial electric field across the capillary wall [24–26]. The applied radial voltage can be positive or negative, and hence the surface charge density may be enhanced or reduced. The EOF actually can be reversed by radial voltage or by using positively charged polymer coatings [19,27]. The dependence of EOF on buffer pH can also be reduced to some level by using non-ionic surfactant coatings [22,28].

For CE, an ideal surface modification would be one in which the surface charge density could be reproducibly synthesized to a desired level, have a charge density largely independent of pH, and be non-adsorptive to macromolecules. This would only be possible in capillaries with neutral charge or a constant charge at the surface [19]. Two approaches have been reported for the polymerization of 2-acryloylamido-2-methylpropanesulfonic acid monomer in capillaries at 120°C using azobisisobutyronitrile (AIBN) as a radical initiator [29,30]. In these two methods, capillaries were pretreated with trichloro-

vinylsilane [29] or 7-oct-1-enyltrimethoxysilane [30].

In this research a simple method is reported in which the sodium 2-acrylamido-2-methyl-1-propanesulfonate (NaAMPS) monomer has been polymerized or copolymerized with acrylamide at room temperature, thus producing a level of sulfonic acid groups bonded to the fused-silica wall surface. Capillaries coated by this anionic polymer have electroosmotic flows which can be varied from near zero (pure polyacrylamide) to a maximum value ca. 60% of the maximum flow of a bare silica capillary at alkaline pH. The EOF was found to be largely independent of pH, and highly reproducible. The stability, lifetime, coating and operating reproducibilities, as well as application potentials of these new capillaries are discussed.

2. Experimental

2.1 Chemicals

2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), β -cyclodextrin (β -CD), benzoic acid, 2-bromophenyl acetic acid, 4-bromophenyl acetic acid, 2-naphthalenesulfonic acid and methylphenol were obtained from Aldrich (Milwaukee, WI, USA). Acrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were purchased from Bio-Rad Labs. (Richmond, CA, USA). Dansyl-DL-amino acid samples and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, MO, USA). 3-Methacryloxypropyltrimethoxysilane was purchased from Huls America (Bristol, PA, USA) and benzenesulfonic acid sodium salt from Eastman (Rochester, NY, USA). All other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2 Apparatus

CE and MECC separations were performed on a laboratory-constructed instrument which included a Plexiglas box, a CZE 1000 PN 30 high-power supply (Spellman, Plainview, NY,

USA), and a fully digital controller for the CZE 1000 unit, designed by Chamonix (Johnson City, NY, USA). The controller is commercially available for a variety of high-voltage supplies. Detection was achieved via a Spectra 100 UV detector (Thermo Separation Products, Fremont, CA, formerly Spectra-Physics), using a commercial "flow cell" with a single ball lens, also purchased from Thermo Separation Products. The electropherograms were processed on a SP-4400 integrator (Thermo Separation Products). Detection wavelength was 210 nm.

2.3 Capillary coating

Approximately 0.8 g of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) was dissolved in 8 ml deionized water. A concentrated NaOH solution was added to neutralize this acidic solution to pH 7.0, and brought to a total volume of 10 ml with water. This 0.386 M NaAMPS solution (solution A) was degassed for 20 min using vacuum and sonication before polymerization. The 0.386 M acrylamide solution (solution B) was made by dissolving 0.275 g acrylamide in 10 ml deionized water. Pretreatment of the capillary using a bifunctional reagent was performed using the method developed by Hjertén [14]. Fused-silica capillaries (75 μ m I.D. \times 375 O.D., Polymicro Technology, Phoenix, AZ, USA) with detection windows were washed with 1 M NaOH solution for 30 min, then with deionized water for 15 min. Then these capillaries were filled with 1% (v/v) 3-methacryloxypropyltrimethoxysilane solution (pH has been adjusted to 3.5 by acetic acid). After 1 h, the silane solution was removed. These capillaries were ready to be coated after rinsing with water.

About 5 mg of ammonium persulfate and 5 μ l of TEMED were added to 5 ml of 0.386 M NaAMPS (solution A), then the mixed solution was pushed through the pretreated capillary and left in the capillary for 1 h. The capillary was rinsed with water and dried with air before use. Fig. 1 represents the scheme of this anionic polymer coating. To make capillaries with different EOF rates, solutions with different volume

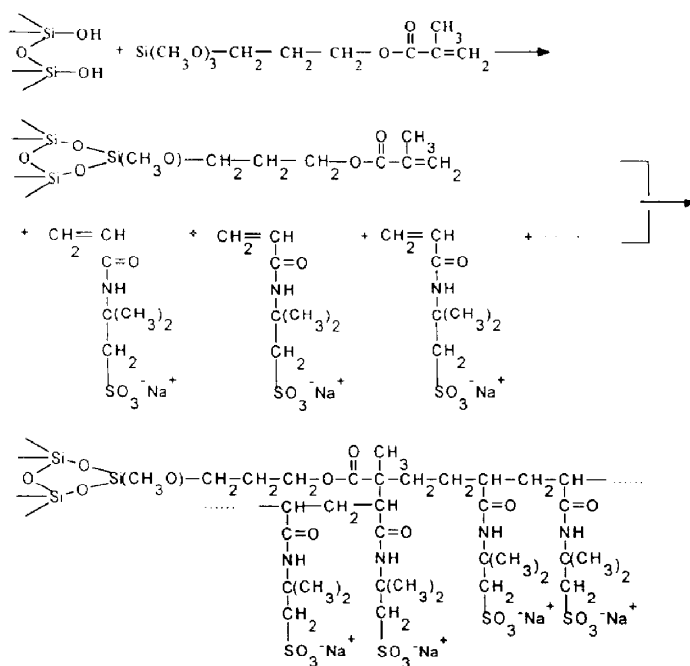


Fig. 1. NaAMPS polymer-coating procedure.

ratios of solution A to solution B were used to coat the capillaries. Methylphenol was used as a neutral marker to measure the EOF in this work.

2.4. Safety considerations

Caustic solutions are used in several steps of the synthesis. Suitable eye and skin protection should be employed for handling such solutions, realizing that splattering is possible as the solutions are forced through the capillaries under mild pressure.

3. Results and discussion

3.1. Effect of pH on EOF

Fig. 2 shows the effect of pH on EOFs for both coated and bare capillaries. NaAMPS polymer-coated capillary possesses a very stable flow over the pH range 2–9, while the bare capillary shows a strong dependence on pH. As described in Eq. 4, EOF is dependent on the surface

charge density (σ^*), the double layer thickness (κ^{-1}) and the solution viscosity (η). In this experimental case, the same buffer solution is used in testing the EOF for both bare and coated

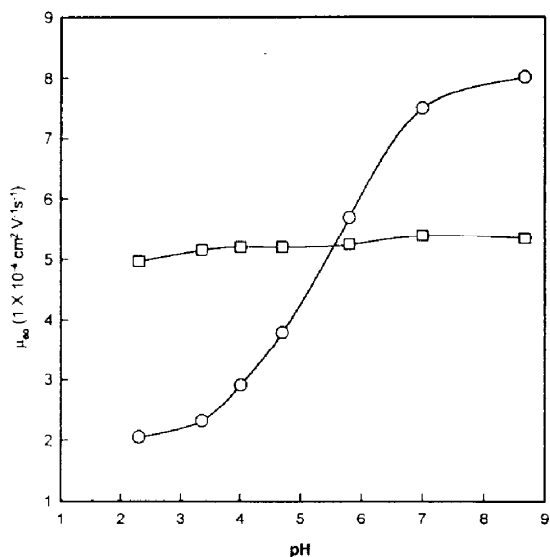
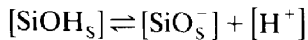


Fig. 2. pH effect on electroosmotic flow (μ_{eo}). \square = NaAMPS polymer-coated capillary; \circ = bare capillary.

capillaries. The major factor affecting the EOF is the surface charge density (σ^*). It results in dramatic difference in pH dependence between the two capillaries. For the bare capillary the surface charge density is controlled by the dissociation constant of the free hydroxyl groups on the silica surface. It can be calculated according to the following reaction [25]:



where the SiOH_2 and SiO_2^- are surface functional groups given as function groups per units area. The charge density can be expressed as [25]:

$$\sigma^* = \frac{\gamma}{1 + \frac{[\text{H}^+]}{K}} \quad (5)$$

where γ is the number density of surface SiOH group, K is the dissociation constant (ca. $4.2 \cdot 10^{-10}$) for surface hydroxyl groups on fused silica and $[\text{H}^+]$ is the bulk buffer hydrogen ion concentration. Since the $\text{p}K_a$ of the surface hydroxyl group is low, the ionization of this group will be vary at different pH. This results in the strong pH dependence of EOF.

For the NaAMPS polymer-coated capillary, the silica surface of the capillary is masked by a layer of NaAMPS polymer (Fig. 1). The sulfonic acid groups on the polymer are strongly acidic, and completely dissociate when pH is above 2. Consequently, the charge density on this polymer coating surface is very stable in pH range 2–9. This leads to a pH-independent EOF. Since the NaAMPS polymer coating has negatively charged sulfonate groups, this capillary exhibits a relatively fast EOF compared to the capillary coated with neutral polymers such as linear polyacrylamide [14]. In capillary modification using neutral or weakly charged polymer coatings, it is inevitable that an attempt to reduce sample-wall interaction will cause the loss of electroosmotic pumping. But EOF is needed in many practical cases to sweep the samples through the capillary to the detection end. The NaAMPS polymer coating is one method capable of solving this problem. The maximum EOF, $5.50 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, produced by this

new method is higher than that reported by Huang et al. [30], which is about $3.5 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. Another advantage of this new method is that it is simpler than other methods [29,30]. Instead of at high temperature (120°C), polymerization is accomplished at room temperature.

3.2. Stability and lifetime of the NaAMPS polymer-coated capillary

The stability of the NaAMPS polymer-coated capillary is tested by measuring the EOF periodically during the course of research work. Fig. 3 shows the EOFs measured in pH range 3–9 on different days. It can be seen that the pH-independent EOF remains stable after 18 days. The lifetime of the coating is measured by maintaining an applied electric field strength of 288 V/cm (see Fig. 4). EOF begins to deviate after 60 h. The authors strongly believe that with the improvement of this coating procedure the lifetime can be improved.

3.3. Desirable EOFs

The reduction of pH dependence of EOF has been reported by Dougherty et al. [22] and Towns and Regnier [28]. In their methods, the pH dependence of EOF has been reduced to

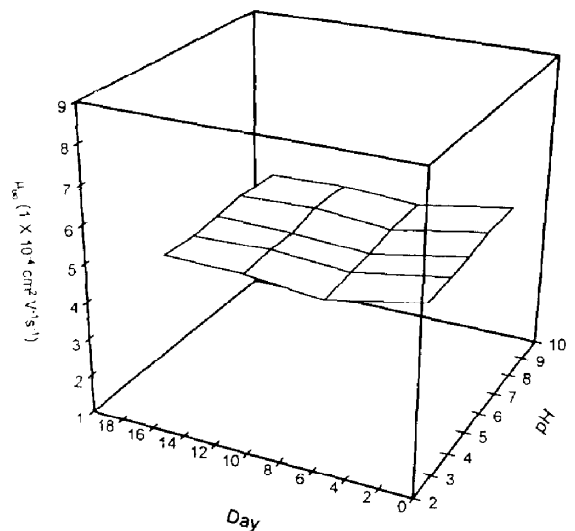


Fig. 3. Stability of NaAMPS polymer-coated capillary.

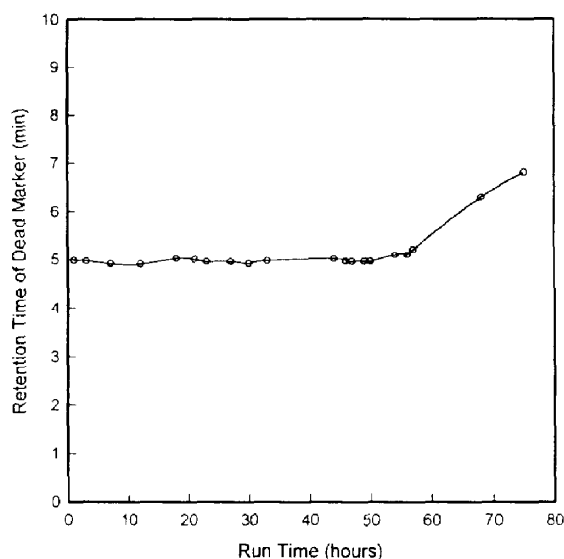


Fig. 4. Lifetime testing of NaAMPS polymer-coated capillary. Electric field: 18 kV; capillary: 63 cm total length and 42.2 cm effective length; buffer: 10 mM phosphate at pH 7.0; neutral marker sample: methylphenol in buffer.

some level by using different coatings. However, the EOF rates are still not very stable in the pH range of 3–10. Also, in both methods the modified capillaries have low EOFs. When negatively charged NaAMPS polymer is used to coat the capillary, a relatively high EOF can be obtained, and this flow is very stable in pH range of 2–9 (Figs. 2 and 3). Another advantage of this method is that capillaries with different EOFs can be made by simply adding acrylamide solution to the NaAMPS solution before polymerization. The surface charge density (σ^*) on the polymer coating can be adjusted by changing the molar ratio of negatively charged NaAMPS to neutral molecule acrylamide in the monomer solution. Fig. 5 shows the pH-independent EOFs obtained from coatings containing different NaAMPS molar fractions. pH-independent flow can be adjusted to a desired level by this method. The coating reproducibility of this method is illustrated in Table 1, which shows the results of capillaries made on three different days. It can be seen that the reproducibility of EOF is good. The relative standard deviation is 1.7%. Similar reproducibility is obtained for capillaries with

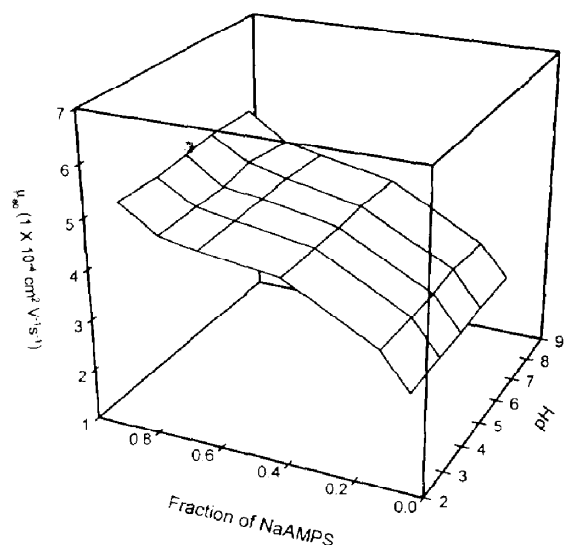


Fig. 5. Desirable electroosmotic flows of capillaries coated with polymers containing different NaAMPS molar fractions.

EOFs in the range reported in Fig. 5. This new method offers the possibility to make capillaries with desirable pH-independent EOFs. This allows one to vary pH to optimize selectivity without significantly affecting the EOF in CE separations.

3.4. Application potentials of NaAMPS polymer-coated capillary

In CE, acidic samples with low pK_a values are generally difficult to resolve at low-pH conditions on bare capillaries. Separations of 2-naphthalenesulfonic acid and benzenesulfonic acid on a bare capillary are represented in Fig. 6. At pH

Table 1
Coating reproducibility

No. of capillaries made at different days	μ_{co}^a ($1 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$)	R.S.D. (%)
1	5.50	1.7
2	5.34	
3	5.33	

^a All values of electroosmotic flows were the averages of three measurements.

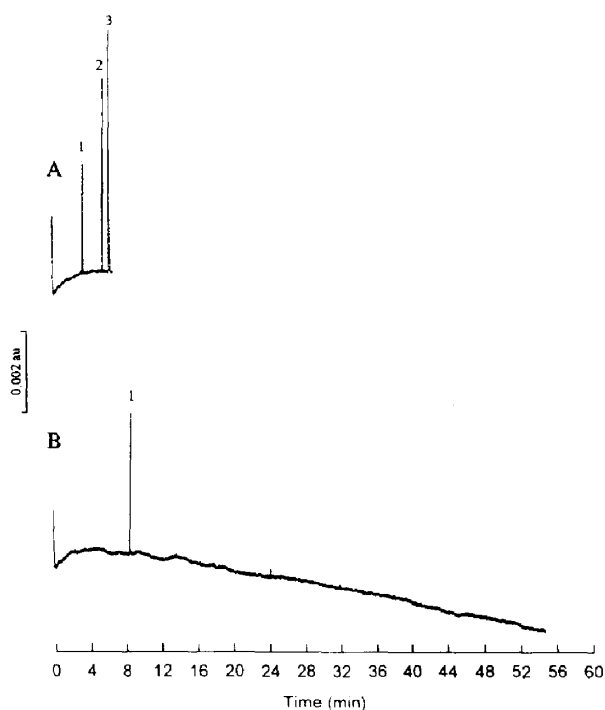


Fig. 6. Separations of acidic samples on bare capillary. Conditions: fused-silica capillary ($75 \mu\text{m}$ I.D. \times $375 \mu\text{m}$ O.D.) with 64.4 cm total length and 43.2 cm effective length; electric field, 288 V/cm. (A) pH 8.69 ; (B) pH 4.01 . Peaks: 1 = methylphenol; 2 = 2-naphthalenesulfonic acid; 3 = benzenesulfonic acid.

8.69 , the bare capillary generates a high EOF, and all sample peaks exhibit short retention times. At pH 4.01 the two acidic samples maintain strong negative charges while the EOF becomes very slow. Only the neutral marker peak is detected. The two acidic samples are not observed in 55 min (Fig. 6B). On the NaAMPS polymer-coated capillary, all the neutral marker and two acidic samples elute at pH 8.69 and 4.01 (see Fig. 7). Sample retention times are similar for the two different pH conditions due to the pH-independent flow of the coated capillary. This example illustrates the ability to optimize selectivity for the separation of samples with very different pK_a values by changing the pH without affecting EOF rate.

CE has proven to be a powerful new technique for enantiomer separations [2]. Currently, the most commonly used chiral selector is cyclodextrin (CD) [31]. Chiral molecules of many

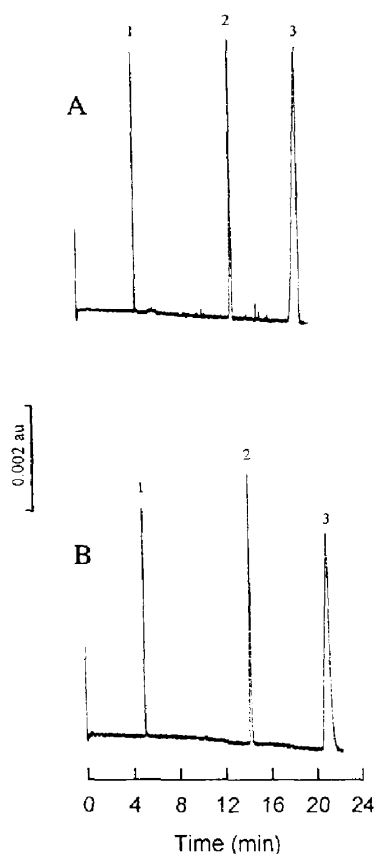


Fig. 7. Separations of acidic samples on NaAMPS polymer-coated capillary. Conditions as in Fig. 6, except that the capillary has a total length of 63 cm and effective length of 42.2 cm. (A) pH 8.69 ; (B) pH 4.01 . Peaks: 1 = methylphenol; 2 = 2-naphthalenesulfonic acid; 3 = benzenesulfonic acid.

pharmaceutical samples have been successfully separated using cyclodextrin as a run buffer additive. To obtain a good chiral separation, the mobility of the free sample species and the sample-CD complex must differ significantly from one another. Cross-linked polyacrylamide gel matrix [32] and dextran polymer network [33] have been used to achieve this requirement for the chiral separation of dansyl-DL-amino acids on coated capillaries. On a bare capillary, the EOF is relatively fast at pH 7 and the neutral β -CD molecules migrate with the EOF. The differences between the velocities of β -CD and the dansyl-DL-amino acid samples are too small

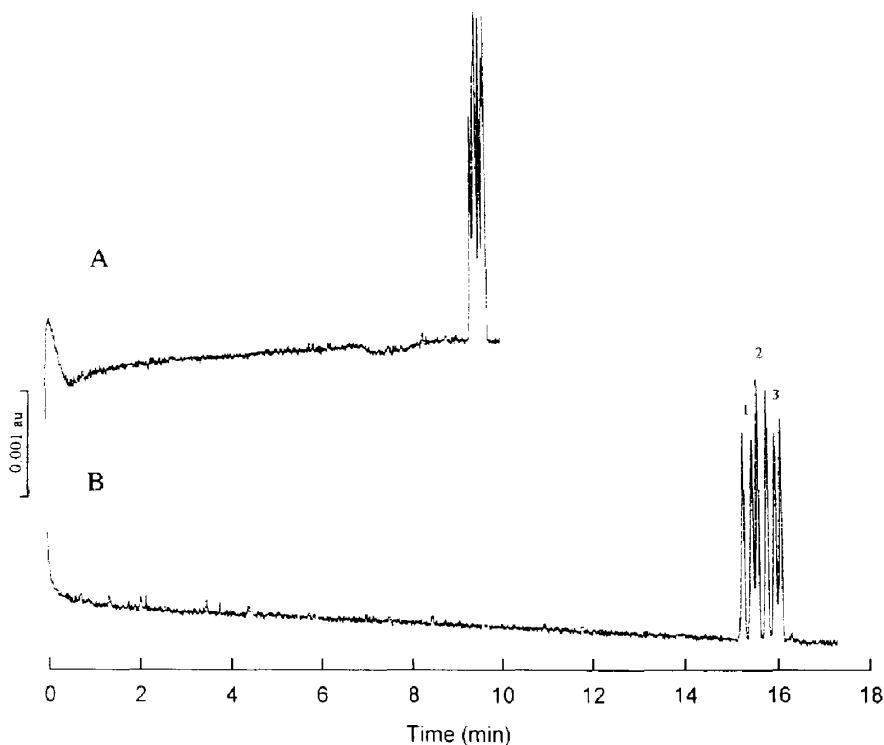


Fig. 8. Chiral separation of three dansyl-DL-amino acids. Conditions: electric field, 288 V/cm; buffer: 5 mM β -CD in 10 mM phosphate buffer at pH 7.0. (A) Bare capillary; (B) NaAMPS polymer-coated capillary. Peaks: 1 = dansyl-DL-norleucine; 2 = dansyl-DL-methionine; 3 = dansyl-DL-serine.

achieve chiral separation (Fig. 8A). A lower EOF is needed in this case to increase the difference between the free sample and CD-sample complex mobilities. This can be accomplished by using the NaAMPS polymer-coated capillary which possesses a lower EOF. Fig. 8B shows the chiral separation of three dansyl-CD-amino acids. All six enantiomers are resolved on this capillary at neutral pH.

Another application of the NaAMPS polymer-coated capillary with a pH-independent EOF is the use of MECC. MECC was first reported by Terabe et al. [34,35]. This method has provided an efficient means of separating a fairly wide variety of compounds of both biological and pharmaceutical significance [36]. One major limitation of MECC is the inability to perform separations at acidic pH ($< \text{pH } 5$) on a fused-silica capillary. In MECC, the net flow velocity

of the micelles is equal to the summation of their electrophoretic velocity and the EOF velocity. At a low pH, the EOF velocity is too low and the micelles migrate toward the anode. Separations of neutral samples cannot be achieved under these conditions. The new NaAMPS polymer-coated capillary has a pH-independent EOF and provides the ability to run MECC separations at very low pH. Fig. 9 shows the separations of three samples, benzoic acid ($\text{p}K_a = 4.20$), 2-bromophenylacetic acid ($\text{p}K_a = 4.05$) and 4-bromophenylacetic acid ($\text{p}K_a = 4.19$). At pH 3.15, these samples cannot be baseline resolved without the addition of SDS to the run buffer (Fig. 9A). The MECC separation of these samples is represented in Fig. 9B. Good separation is obtained by MECC under these conditions. However, sample peaks cannot be detected on the bare capillary under the same

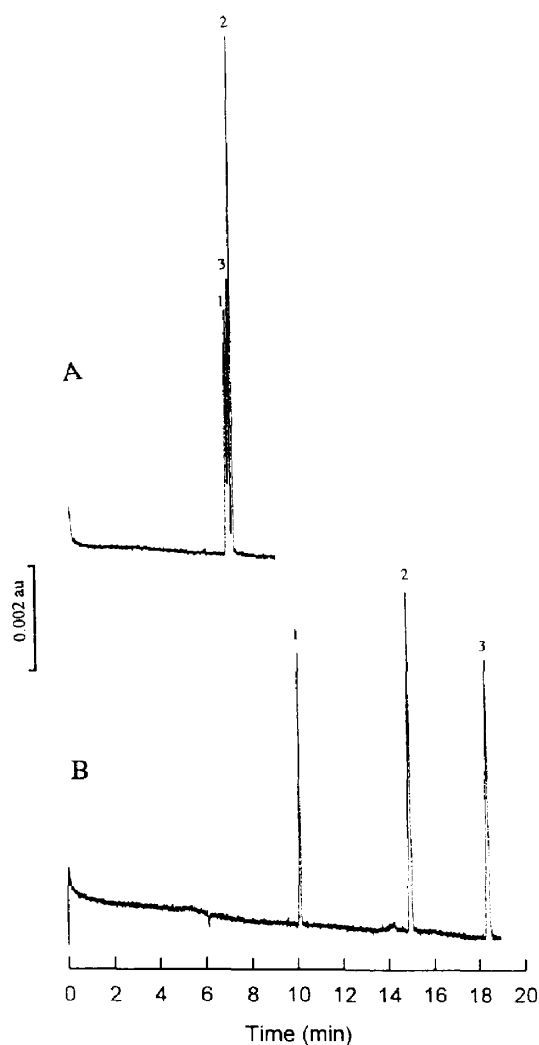


Fig. 9. Separations of three acidic samples on NaAMPS polymer-coated capillary. Conditions as in Fig. 7 except that run buffers are (A) 10 mM phosphate at pH 3.15 and (B) 50 mM SDS in 10 mM phosphate at pH 3.15. Peaks: 1 = benzoic acid; 2 = 2-bromophenylacetic acid; 3 = 4-bromophenylacetic acid.

conditions since the EOF is too low. Research is continuing in our laboratory to increase the applications of MECC using the new capillary coating. The preliminary results obtained so far [36] demonstrate that NaAMPS polymer-coated capillaries with desirable, pH-independent EOFs show very promising application potentials in MECC.

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