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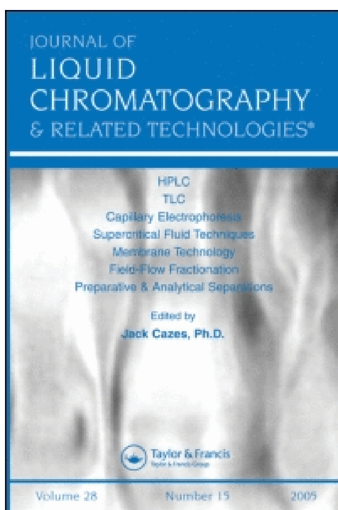

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Applications of Physically Adsorbed Polymer Coatings in Capillary Electrophoresis

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Applications of Physically Adsorbed Polymer Coatings in Capillary Electrophoresis

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Abstract: Physically adsorbed polymer coated capillaries are used to enhance separations in capillary electrophoresis (CE) by preventing adsorption of species to the fused silica surfaces and/or by modifying the electro-osmotic flow (EOF) in the capillaries. In this review, the application of the physically adsorbed coating in CZE, MEKC, NACE, CGE, chiral CE, and CIEF is explored. Emphasis is given to the more recent developments in the field.

Keywords: Capillary electrophoresis, Polymer coating, Physically adsorbed coating, Surface modification

INTRODUCTION

Physically adsorbed polymer coatings are used to enhance capillary electrophoresis (CE) separations by modifying the electro-osmotic flow (EOF) and minimizing solute adsorption in fused silica capillaries. These coatings are formed by simply introducing a polymer containing solution into the capillary where the polymer adsorbs to the silica surface. Adsorption occurs through the formation of physical forces with the silica surface, such as hydrogen bonds, electrostatic or hydrophobic interactions. In contrast to covalent polymer coatings, the generation and regeneration of a coating is a

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fast process as no time consuming synthetic procedures are involved. Static and dynamic physically adsorbed polymer coatings are used.

Static coatings are exposed to the capillary surface for a period of time during which adsorption occurs. The excess polymer is removed from the capillary and analysis is performed with the polymer immobilized on the capillary surface. Regeneration of the statically adsorbed coating occurs between runs or after a number of runs. Since no polymer is contained in the buffer, mass spectrometry (MS) is a feasible detection technique.

Dynamic coatings can be applied to the capillary surface but are also present in the buffer, while analysis proceeds. The presence of polymer in the buffer replenishes any polymer that desorbs from the capillary surface. Disadvantages of dynamic coatings are that solute-polymer and additive-polymer interactions can occur in the buffer, competition may arise between the solutes and the polymer for silanol sites, and coupling to MS is less straightforward.

The magnitude and direction of the EOF generated in fused silica capillaries are modified by the presence of an adsorbed polymer coating. Modifications of the cathodal EOF generated in fused silica capillaries by adsorbed polymer coatings include creating a stable EOF, a pH independent EOF, suppressing, increasing, or reversing the EOF (generation of anodal EOF).

The adsorbed polymer coatings reduce solute adsorption by covering surface silanol groups. In order to minimize the degree of solute interaction with the capillary wall, the adsorbed polymer coating must provide sufficient coverage of the surface silanols. The coating must also provide a surface that the solute does not adsorb to. Cationic polymers have been widely used for the analysis of basic proteins below the pI value where the cationic surface repels the cationic protein electro statically.

The subject of surface modification has been reviewed before.[1–4] In this paper, the applications of physically adsorbed coatings with respect to the major modes of CE are reviewed. Capillary zone electrophoresis (CZE),[5–63] micellar electrokinetic chromatography (MEKC),[64–69] non-aqueous capillary electrophoresis (NACE),[70–74] chiral CE,[75–86]capillary gel electrophoresis (CGE),[87–104] and capillary isoelectric focusing (CIEF).[105–108]

APPLICATIONS

Capillary Zone Electrophoresis (CZE)

Neutral Polymers

Polyvinylalcohol (PVA)

Static and dynamic PVA coatings have been used for protein analysis.[5–7] Dynamic coatings created by the addition of 0.05% (w/w) PVA to the buffer, created an EOF that was stable from pH 5 to 8 and suppressed compared to an uncoated capillary. The dynamic coating created efficient protein separations; however, the separation performance was noted to vary when

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fused silica capillaries from different manufacturers were used and optimal results were attained when the coating was regenerated after each run.[6] Static PVA coatings were enhanced by thermal immobilization; the PVA coated capillary was heated to 140°C under nitrogen flow for several hours. The PVA becomes water insoluble with thermal treatment and adsorbs strongly to the silica surface.[6,7] The static coating suppressed the EOF in the pH range 3 to 10 and no effect of capillary manufacturer was observed. The dynamic and static coating had similar effects at low pH, but the static coating could be used at higher pH values more effectively.[6]

Polymethylglutamate (PMG)

PMG coated capillaries, formed in situ, were investigated as capillary coatings.[8] The EOF was suppressed compared to an uncoated capillary and at pH 7.0 efficient separations of myoglobin, carboanhydrase B, and lactalbumin were attained. However, the same response in the peak efficiency of bovine serum albumin (BSA) was observed on coated and uncoated capillaries. BSA was the most acidic, hydrophobic, and largest protein analyzed, and this response was attributed to the hydrophobic character (methyl ester groups) in the coating.

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Polyvinylpyrrolidone (PVP)

PVP[9] was used as a static capillary coating for the analysis of inorganic anions. The PVP coating reduced the EOF about 10 times less than that of an uncoated capillary that was stable from pH 6 to 8. The EOF suppression was enhanced by the use of higher molecular weight (MW) PVP. An inorganic anion separation was performed and with the suppressed EOF, the analysis was performed in only a few minutes. The electrophoretic mobilities of the anions calculated from the data closely matched the absolute electrophoretic mobilities, which indicated the lack of interaction between the coating and the anions.

Polyethyleneoxide (PEO)

PEO has been investigated as both a dynamic[10,11] and static[10] adsorbed coating. Acid washing the fused silica capillary surface prior to the application of the PEO coating enhances the hydrogen bonding attachment of the PEO layer. In the initial study of PEO as a dynamic coating,[10] 0.2% PEO of relative molecular mass 8,000,000 was added to the buffer and poor peak shapes of basic proteins were attained. In a recent study,[11] when 1% PEO MW 600,000 was used as a dynamic coating, excellent separations of oligosaccharide isomers were attained. The static PEO coating gave good separations of basic proteins in the pH range 3 to 7 with migration time RSD values of 1.4% to 3.4% ($n = 5$). The coating and regeneration procedure for the static coating involved etching the capillary with 1M hydrochloric acid (HCl), rinsing with a 0.2% PEO solution that contained 0.1 M HCl, and washing the capillary with buffer.

EOTrol™ and UltraTrol™

EOTrol™ and UltraTrol™ are commercially available coating systems based on N-substituted acrylamide co-polymer solutions that are strongly attracted to silica through hydrophobic interactions. These coatings reduce the EOF dramatically and have been used for the protein isoform analysis.[12,13] The low cathodal EOF created by the coatings results in the protein spending a longer time in the capillary and resolution of the isoforms is attained. In the study by Chang et al.,[12] the performance of covalently coated PEO capillaries or uncoated fused silica capillaries was very poor compared to the EOTrol™ LN-coated capillary. In another study,[13] a static UltraTrol™ LN-coated capillary was used in conjunction with CZE-MS to determine the glycoform characterization of the protein erythropoietin.

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Epoxy Poly (AG-AA)

Epoxy poly (AG-AA) (polyacrylamide-*co*-allyl α-D-glucopyranoside-co-allylglycidyl ether) was designed as a capillary coating for use in protein analysis.[14] It was desired to create a polymer that has sufficient hydrophobicity to adsorb well to the surface, yet with enough hydrophilic character to function well as a coating. In addition to being an effective static coating that is simple to apply and regenerate, the coating is stable in high pH, temperature, and in the presence of urea. The coating was a more effective suppressor of EOF than methyl cellulose, poly (AG-AA), and polydimethylacetylamide (PDMA). In comparison to PDMA, faster and more efficient separations of acidic proteins were obtained.

Polydimethylacetylamide (PDMA) Derivatives

In a series of PDMA derivatives explored as capillary coatings, epoxy-poly (dimethylacetylamide) (EPDMA)[15,16] and 2% hydrolyzed epoxy-poly (dimethylacetylamide) (HPDMA)[16] provided stable static adsorbed coatings. EPDMA contains a PDMA backbone with oxirane groups attached. In HPDMA, the epoxy groups are hydrolyzed into diol groups, therefore, HPDMA is a more hydrophilic coating. In the analysis of the R-phycoerythrin protein (PHYCO), the EPDMA coating had a similar performance to polyacrylamide in terms of EOF suppression, peak width, and peak asymmetry. Issues arose for EPDMA coatings due to instability in low ionic strength buffers, the presence of SDS, and with the analysis of a hydrophobic analyte.[15] EPDMA, 20% EPDMA, and 2% HPDMA all reduce the EOF. The 20% EPDMA or 2% HPDMA coatings are less stable but have improved peak asymmetry compared to EPDMA.[16]

Poly-*N*-hydroxyethylacrylamide (PHEA)

Poly-*N*-hydroxyethylacrylamide (PHEA), also known as polyDuramide™,[17] was assessed for protein separations by CZE. The polymer coating suppressed the EOF compared to uncoated capillaries. The stability of the coating was very good with over 275 consecutive runs at pH 8.4 and more than 800 runs at pH 4.4. Enhanced stability at basic pH was obtained by adding 0.1% PHEA to the buffer. High efficiency values were attained for all the proteins analyzed. In comparing the performance of the PHEA coated capillary to a linear polyacrylamide (LPA) coated capillary and a PDMA coated capillary for protein separations, the PHEA capillary had a similar performance to LPA and improved performance to PDMA.

Cellulose Derivatives

Cellulose based coatings include methyl cellulose,[18] cellulose acetate,[19] hydroxycellulose,[20] and hydroxypropylcellulose.[21] The methyl cellulose (MW of about 200,000) was mixed with formic acid and formaldehyde and applied to the capillary surface. The capillary was then heated at 120°C in order to crosslink the methyl cellulose and render it less soluble to water. A cellulose acetate coating[19] provided efficient separations of basic proteins in the low pH range 3 to 4.5. The cellulose acetate coating was not stable above pH 7.5. Sanzgiri et al.[21] recently used a hydroxypropylcellulose adsorbed coating to resolve the charge heterogeneity of an intact monoclonal IgG antibody.

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Charged Anionic Polymers

Dextran Sulfate (DS), Polyvinylsulfonate (PVS)

The anionic polymers, dextran sulfate (DS), and polyvinylsulfonic acid (PVS) were used as dynamic coatings for food protein analysis.[22] Although the protein peaks were discernible using PVS as a dynamic coating, enhanced separation was clearly obtained with the DS coating. For the PVS coating, a broad electrophoretic profile was obtained for proteins in avian muscle tissue. The more hydrophilic DS coating had a shorter analysis time and efficient peak efficiencies of 1.2×10^5 to 1.8×10^5 theoretical plates.

Charged Cationic Polymers

Polydiallyldimethylammonium Chloride (PDADMAC)

Polydiallyldimethylammonium chloride (PDADMAC) coated capillaries have been used both as static and dynamic coatings.[22–25] Fritz et al.[23]

used the PDADMAC statically to provide very reproducible separations of anions. The EOF remained stable from pH 2.5 to 12.0. The authors evaluated three molecular mass ranges of PDADMAC (100,000–200,000, 200,000–350,000, 350,000–400,000) and determined all to be suitable as capillary coatings. The high molecular mass polymer performed better at pH 12.

The performance of polybrene was compared with PDADMAC for the separation of the polypeptide molecules insulin like growth factors (IGF) I and II by Roche et al.[24] For this separation, PDADMAC provided improved reproducibility, durability, and lower detection limit (by a factor of eight) compared to a polybrene coated capillary. Drawbacks of using PDADMAC as a dynamic coating were the noise level of the electropherogram, baseline fluctuations, and the inability to combine solid phase extraction (SPE). However, these issues were resolved when a static coating was used.

A PDADMAC coated capillary was also used for the analysis of proteins in food.[22] The coating was applied statically coated and also added to the run buffer. Addition of an ion pairing agent improved the resolution of the protein separation, but it is unclear whether ion-pairing to the PDADMAC occurred and affected the hydrophobicity of the capillary coating.

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Chitosan

Chitosan, ((1 $\rightarrow$ 4)-2-amino-2-deoxy-β-D-glucan)) is a hydrophilic polyelectrolyte that was used by Yao et al.[26] as both a dynamic and static capillary coating. The cationic polymer was used for protein separations and was used in the pH range 3.0 to 5.5, to reduce interaction of the proteins with the capillary wall. Huang et al.[27] created a chitosan based multiple polymer layer coating that had enhanced stability compared to the monolayer coating.

Polyethyleneimine (PEI)

Polyethyleneimine (PEI) has been investigated for use as a static[28,29] and dynamic capillary coating.[29–31] PEI was used as a dynamic coating for the analysis of peptides,[30] but better separation performance was obtained with the smaller buffer additives ethylenediamine and 1,7-diaminoheptane.[30] In the analysis of poly(amidoamine) (PAMAM) dendrimer generations, a dynamic PEI coating (0.05% (w/v)) effectively separated seven generations of the spherical polymers at neutral pH.[31]

Erim et al.[28] used static PEI coated capillaries for the analysis of peptides and proteins. PEI of high molecular mass (6×10^5 to 1×10^6) was used in varying percentages (0.1 to 10%). The same general EOF profile was attained from each one for the pH range 3 to 11; however, the 0.1% coating had a higher EOF. The coating produced efficient separations of basic proteins (3×10^5 to 5×10^5 plates per meter). The separation performance of acidic proteins was poor due to interaction with the coating.[28]

Polyarginine

A high molecular weight cationic peptide polyarginine was used as a capillary coating[32] and provided very high efficiency separations of basic proteins. It was investigated as both a static and dynamic coating with similar efficiency values being attained for both. A dilute solution of 0.005% (w/w) polyarginine was determined as optimal between providing high efficiency separations and acceptable absorbance values. Extremely high efficiency peaks (over 1 million plates per meter) were obtained for a basic protein separation at pH 5. The drawback to the use of the polyarginine capillary is the lack of stability below pH 4 and above pH 9.

Polybrene (Hexadimethrine Bromide (HDB))

Polybrene (hexadimethrine bromide (HDB)) is a cationic polymer coating[24,25,30,32–35] that effectively reverses the EOF and forms a very stable static coating. Wiktorowicz and Colburn[33] used a static polybrene coating for the analysis of the isoforms of the proteins lactate dehydrogenase (LDH), trypsinogen, and the multi-acetylated histone H4 at neutral pH. A static polybrene coated capillary was used in conjunction with a volatile buffer for the separation of hemoglobin variants by CE-MS in 10 to 15 minutes.[34] A dynamic HDB coated capillary was used for the first determination of plant hormones from bio fertilizer.[35]

In comparison to other coatings under the same conditions, polybrene has performed very well.[25,32] In the analysis of a lysozyme charge ladder, polybrene and PEI had a faster EOF, faster coating time, and shorter analysis time than poly (methoxyethoxyethyl)ethyleneimine and PDADMAC.[25] In comparison to a polyarginine coated capillary for the analysis of basic proteins at pH 5, polyarginine provided more efficient separations; however, the polybrene coating had better stability.[32]

eCAP Amine™

The commercially eCAP ™ amine coating was investigated by Assi et al.[36] for the separation of a pharmaceutical product and the related impurities. The eCAP amine™ coated capillary system creates a cross-linked polymer with quaternary ammonium groups. The developed method was validated and tested for stability. The coating was assessed for more than 600 sample injections in a 6 month period. The RSD values for migration time and peak area were 2.86 and 3.62%, respectively. The coating has a large pH range of stability (from pH 2 to 10) and was simple to regenerate between runs.

PolyE-323

Poly E-323[37] is a cationic polymer of 1,2-bis(3-aminopropylamino)-ethane and epichlorohydrin. Poly E-323 contains short aliphatic blocks of

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combined 2 and 3-carbon length and hydroxyl groups and has been used as a static coating. The EOF is reversed and stable from pH 4 to 8. The stability of capillaries prepared the same day is 2.0% (n = 5), and for those prepared on different days is 3.4% (n = 4). The EOF of the capillary was observed to be stable for 60 injections and thereafter started to decrease. Four basic proteins were analyzed at pH 7 and very sharp and efficient peaks were observed (efficiency values of 2.64×10^5 to 5.84×10^5).

Poly-LA 313

Poly LA 313[38] is a cationic polymer of 3,3′-diamino-*n*-methyldipropylamine and 1,4-butanediol diglycidyl ether. In comparison to Poly-323,[37] there is increased distance between the positive charges in the polymer, which was designed to improve interaction with the silica surface. The generation and regeneration of the capillary coating was a simple process, and straightforward, since the synthesized polymer was stable for over one year. The coated capillaries produced a stable, reversed EOF in the pH range 2 to 10, and were resistant to the presence of methanol and acetonitrile modifiers. Four basic proteins were analyzed at pH 5 and had peak efficiency values of 1.7×10^5 to 3.9×10^5 plates per meter. The coated capillary was used with an electrospray ionization mass spectrometer (ESI-MS) and excellent separations of peptides and proteins were obtained. The CE-ESI-MS indicated the stability of the coating as desorption of the polymer from the capillary surface was not observed.

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Fibrinogen

Fibrinogen was used as a capillary coating by Van Tassel et al.[39] A layer of fibrinogen was adsorbed onto the capillary surface as a static coating. The EOF was 30% reduced compared to an uncoated capillary. The fibrinogen was thermally treated and observed to remain stable on the capillary surface. It was shown that desorption of fibrinogen from the surface occurred within even 10 minutes if the thermal treatment was not used.

Cationic Starch Derivatives

Cationic starch derivatives were investigated as dynamic coatings.[40] The coating reversed the EOF with the 2-hydroxy-3-(trimethylammonio)propyl ether chloride, 2-hydroxypropyl ether derivative (2H) having the highest EOF and, therefore, being selected as most suitable for fast analysis. The usable pH range of the coating was from pH 3 to 9, where the EOF remained essentially stable. The separations of four basic proteins at pH 7.5 (0.1% (w/v) 2H) on the 2H coated capillary, had efficiency values of 9.8×10^4 to 2.76×10^5 plates per meter and a migration time RSD range of 0.61% to 1.67%.

Multilayer Anionic Polymer Coatings

CEofix^{TM}

CEofix^{TM} (also known as CElixir^{TM}) is a commercially available dynamic double-layer coatings system developed by Chevigne and Janssens[41] and manufactured by Analis. CEofix^{TM} has been used extensively for clinical[42–46] and drug[47–51] analysis. It is available in a variety of configurations depending on the pH of the required separations. It has found particular use for the analysis of basic pharmaceuticals that require low pH conditions. The low and irreproducible EOF created in fused silica capillaries lead to long run times and poor reproducibility. CEofix^{TM} uses a base wash to clean the fused silica surface, then the initiator (a polymeric cationic solution) is applied to the capillary surface. A polymeric anionic solution (the accelerator) is incorporated in the running buffer and coats the cationic capillary surface. By maintaining an anionic surface, the EOF reduces the migration time of positively charged analytes, and migration times are greatly reduced compared to fused silica.

The analysis of benzodiazepine pharmaceuticals was performed using the CEofix^{TM} system with MS detection by Vanhoenacker.[47] The limits of detection were 100 ppb and for the compound diazepam less than 50 ppb. MS detection requires the use of a volatile buffer and formic acid was used instead of the phosphate based buffer provided with CEofix^{TM}. The addition of TFA to the accelerator further enhanced the sensitivity. Altria[48] also used CEofix^{TM} for the analysis of basic pharmaceuticals with UV detection.

Forensic applications of CEofix^{TM} have been performed by Boone[49] and Lurie.[50,51] Boone used the CEofix^{TM} system to screen 73 basic pharmaceuticals for use with systematic toxicological analysis (STA). Lurie has used the CEofix^{TM} system for forensic drug screening, including opium alkaloids, lysergic acid (LSD),[50] and heroin.[51] Lurie added cyclodextrins effectively to the CEofix^{TM} system to attain selectivity of several of the alkaloids.[50]

Clinical applications of CEofix^{TM} have also been investigated.[42–46] Lanz et al. have performed a series of studies on carbohydrate-deficient transferring (CDT) in human serum with the CEofix^{TM} system.[43–45] The initial study on CEofix^{TM} was a comparison with other dynamic coating agents.[43] The suitability of CEofix^{TM} was confirmed. Using this system, the quantitative determination of CDT and additional information concerning the transferrin pattern in serum was attained. Further research and refinements to the original research was performed in 2003 using the then newly available CEofix^{TM} CDT-kit from Analis.[44,45] Another clinical study was performed by Sirén who monitored glycohemoglobin A_{1C} in the blood samples of diabetics.[42] The developed method produces a fingerprint profile of glycohemoglobins. The migration time of the HbA_{1C} was only 1.77 min; the run time with regeneration of the coating was only 3.70 minutes. The reproducibility of the migration time had an RSD of 0.56% for 40 runs of clinical samples.[42]

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Polybrene-Dextran Sulfate Coatings

Katayama et al.[52,53] developed the process of multiple layer coatings using polybrene and dextran sulfate (DS). The process is referred to as SMIL (successive multiple ionic polymer layers). The presence of sulfonic groups on the surface gave pH independent EOF in the pH range of 2 to 11. CZE separations at low pH were effectively performed and enabled pKa determinations to be performed, as the effective mobility at each pH could be determined. The SMIL coating lasts for over 100 runs and is easily regenerated.

Polybrene-Polyvinyl Sulfonate (PVS) Coatings

Double layer coatings using a polybrene cationic layer and a polyvinylsulfonate (PVS) layer have been developed.[54–56] Polybrene-PVS coatings have been used for the analysis of proteins at medium pH[54] and peptides at low pH.[55] At low pH, the migration time drift was reduced by the addition of 0.01% PVS to the run buffer for UV detection, and a static coating was used for CE-MS experiments successfully.[55]

A direct comparison for a set of model proteins insulin, α-lactalbumin, β-lactoglobulin, β-lactoglobulin A was performed for the polybrene-PVS coating, the CEofix™ coating system, and an uncoated capillary.[54] The coated capillary systems both provided fast reproducible methods. The polybrene-PVS coated capillary with optimal buffering conditions separated a protein mixture in 11 minutes with plate counts of 1.5×10^5–2.5×10^5. The peak areas (corrected for migration time) have an RSD less than 5% and the migration time RSD was lower than 0.8%. The polybrene-PVS coating had good long term stability (over a number of days) with RSD of migration time remaining within 1%. Under the same conditions, on the uncoated capillary the peak area RSD was more than 30%, migration time RSD values were often higher than 5%, and plate counts were lower. The CEofix™ coating separated the proteins in 10 minutes; the RSD of migration time was 0.4 to 0.6% and plate numbers of 2.5×10^5 to 3.1×10^5 were obtained. However, the accelerator solution in the CEofix™ coating was replaced with 300 mM Tris phosphate at pH 7.

PDADMAC-Single Walled Carbon Nanotubes (SWCNTs)

PDADMAC treated capillaries were used to adsorb acid treated single walled carbon nanotubes (SWCNTs).[57] This unique double layer coating reduced the EOF compared to an uncoated capillary. The presence of the EOF is attributed to the presence of –COOH groups on the carbon nanotubes that result from the process of etching in acid. In the SWCNT coated capillary, baseline resolution of a series of aniline derivatives was obtained. Hydrophobic interaction of the derivatives, as well as the pKa of the solutes, was postulated to contribute to the separation mechanism. The separation

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efficiency values ranged from 7,000 to 51,000 plates and the run-to run RSD of 0.45% for 12 runs. The durability of the coating was also high with over 70 hours of analysis being performed without deterioration of separation performance.

Multilayer Cationic Polymer Coatings

Polybrene

A polybrene coated capillary was made by creating a three layer coating of polybrene, dextran sulfate, and then another layer of polybrene.[58] This coating had much improved stability compared to a single layer coating of polybrene. The coating remained stable for 600 replicate analyses and remained stable against 1 M NaOH and 0.1 M HCl. The coated capillary was compared to polyethyleneglycol (PEG), PVA, and acrylamide coated capillaries for the analysis of rat plasma where it was advantageous since it could be flushed with NaOH, removing any extraneous adsorbed sample between runs. The coating was also used to separate 4 beta-blocker drugs by CE-MS.

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Polystyrene Nanoparticles

Novel coatings of polystyrene (PS) nanoparticles derivatized with α-ω diamines (ethylene diamine (EDA) and 1,10-diaminodecane (DAD)) were successfully used as capillary coatings for the analysis of peptides and proteins.[59] These coatings separated 9 proteins in pH 3.1 phosphate buffer in under 6 min. The peak efficiency values increased by a factor of 2.4 compared to uncoated capillaries. The EOF in these coated capillaries remain cathodic, therefore, the charge density of the positively charged particles was not high enough to counteract the charge from the surface silanols. The RSD values of migration time and peak efficiency were 2.92% and 1.9%, respectively, for ribonuclease A on 6 coated PS-EDA capillaries.

In a single capillary for cytochrome c, the RSD for migration time and peak areas were 0.43% and 2.1%, respectively, for 20 runs. The long term stability was also good, with protein and peptide separations being performed for more than 2 months without deterioration in efficiency being observed. Efficiency values of were on average increased by a factor of 9.4 when PS-EDA was converted to PS-EDA-diol, with four of the five proteins having efficiency values greater than 1 million plates.

PDADMAC-polystyrenesulfonate

A novel deposition process was used to create polyelectrolyte multilayers (PEMs) on the silica surface.[60] The process of depositing these thin films

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had excellent control of layer thickness. Successive layers of PDADMAC and polystyrenesulfonate (PSS) were combined to form the stable multilayer coating. The ability to choose to select an anionic or cationic coating makes this method of creating the coating very versatile. Efficient separations of basic proteins were attained.

Tunable Coatings

Poly(DMA-EpyM)

Polymer coatings consisting of different percentages of ethylpyrrolidine methacrylate (EpyM) and *N,N* dimethylacrylamide (DMA) monomers have been used as effective adsorbed capillary coatings.[61,62] The coatings have a pH tunable electrical charge. The EOF is anodal at low pH and cathodal at high pH. By selecting the relative amounts of the monomers, the degree of positive charge on the copolymer is varied and, therefore, so is the charge on the polymer coating. For polymer coatings with 20 to 40% EpyM, zero EOF is at pH 6, whereas for coatings containing 60, 80, and 100% EpyM, zero EOF is attained at about pH 7.5. In all cases, the EOF is reduced relative to uncoated capillaries. The coating has been used successfully for the analysis of acidic and basic proteins. Figure 1 shows the separation of 3 basic proteins using a 60% EpyM coating at pH 3.6 and the corresponding poor result on an uncoated capillary.

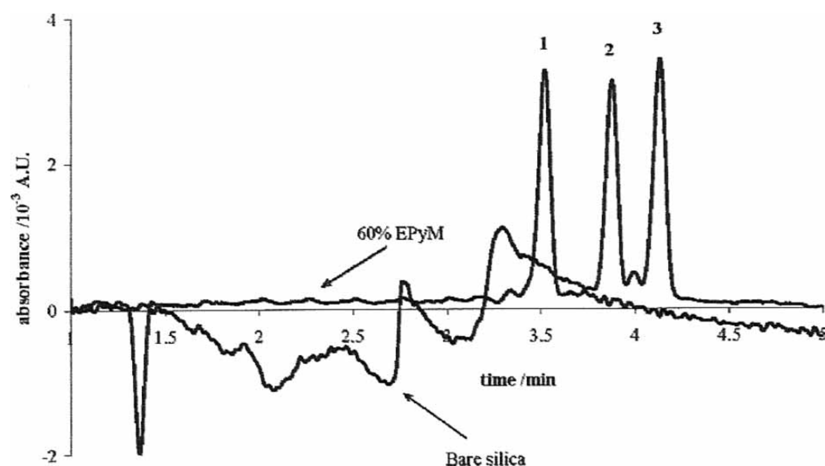

Figure 1. Separation of basic proteins using a bare silica capillary and a 60–40 EpyM-DMA coated capillary. Running buffer: 25 Mm NaOH, 50 Mm acid formic at pH 3.6. Running voltage 30 kV. Injection of (1) lysozyme, (2) rabbit cytochrome C, and (3) bovine cytochrome C for 10 s at 0.5 psi, all of them at 0.05 mg/mL. Reprinted with permission from reference 62.

Coupled Chitosan

A chitosan based multiple polymer layer coated capillary was created by Huang et al.[27] The capillary wall was coated with a layer of chitosan, which was then coupled to gluteraldehyde, another layer of chitosan was adsorbed on top. The coating is referred to as coupled chitosan (COCH). This coating is amphoteric, carrying a positive charge below pH 4.5 and negatively charged above pH 4.5. The EOF profile increases with increasing pH but is stable above pH 9. The coated capillary was chemically stable for at least 500 runs at pH 1.8 and 150 runs at pH 12, and tolerant to washing with organic solvents. In comparison to the chitosan coated capillary and an uncoated capillary, the COCH capillary provided superior separation performance for the analysis of a plant extract. The COCH coated capillary prevented solute adsorption and could withstand the necessary washing with base needed to create a reproducible separation.

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Polyaniline

A double-strand complex of polyaniline (PAN) and poly (methacrylate-co-acrylic acid) (P(MA-AA)) termed (PAN: P(MA-AA)[63] was used for the separation of advanced glycation end products (AGE) formed from the reaction of N-α-L-lysine with reducing sugars. The two layers of the novel polymer complex are oriented side-by-side; the layers are not covalently bonded. The PAN changes from being hydrophilic to hydrophobic between pH 6 and pH 7. At low pH, the complex is positively charged due to protonation of the amine groups, as the pH is increased the carboxylic acid groups become negatively charged while the PAN backbone becomes diffusely positively charge, at high pH the PAN backbone becomes neutral. A greater number of AGE products were observed using the coated capillary, the optimal efficiency was obtained at pH 6 and analysis time was reduced relative to an uncoated capillary.

MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC)

In recent years, physically adsorbed anionic coated capillaries have led to an increase in low pH MEKC separations.[51,56,64–68] MEKC separations require the presence of an EOF, as the micellar phase must be drawn to the detector. Fused silica capillaries have low EOF at low pH and, therefore, unfeasible for low pH MEKC. The anionic coatings are attached to the capillary surface through a cationic polymer layer. PDADMAC,[64–67] polybrene,[56] PEI,[68] and the cationic polymer from the CEofix™ system[51] have all been used as intermediate layers. The anionic layer is provided either as a polymer

layer such as PVS,[56,64,65] PSS,[66] or the surfactant sodiumdodecylsulfate (SDS).[51,66–68]

Hansen et al.[64,65] investigated PDADMAC-PVS coatings for the analysis of acyl gluconaride metabolites[65] and the impurity profile of the pharmaceutical compound bromazepam.[64] The coated capillary was used to separate the isomers and hydrolysis products of the acyl gluconarides using a phosphate buffer, which contained lithium dodecyl sulfate and the organic modifier 1-butanol. The MEKC separation of the bromazepam impurity standards was successful, however, when a sample at 3 mg/mL concentration was injected precipitation occurred, therefore, MEKC was not pursued for this particular application.[64]

The use of SDS as the anionic layer attached to a cationic polymer has become a popular approach.[51,66–68] This approach was first developed by Rodríguez-Delgado et al.[68] A PEI coated capillary used with a SDS containing buffer, produced a stable EOF higher than that generated in an uncoated fused silica capillary under the same conditions. The PEI coated capillary was used to optimize the MEKC separation of a series of polyphenolic compounds (at pH 1.5) in terms of both the SDS concentration and organic modifier content. The reproducibility of migration times, both run to run and day to day were good (less than 2% RSD for most solutes).[68]

The analysis of adulterants in heroin was performed using the cationic polymer from the CElixir™ system and was used in conjunction with a SDS layer to create a double layer coating for the screening of acidic, weakly basic, and neutral adulterants in heroin.[51] The optimal pH for the adulterant analysis was pH 6.5 and the 8 main adulterants were analyzed in under 8 min. The increased EOF reduces the analysis time dramatically; the migration time of phenobarbital was 33.3 minutes with an uncoated capillary and 6.8 min using a coated capillary, under the same conditions. A 2 min flush with run buffer between injections gave a migration time precision range of 0.1% to 0.5% RSD and a peak area precision of 0.6% to 1.2% RSD.

Pranaitytė et al.[67] studied SDS-induced EOF with PDADMAC coated capillaries. It was determined that a stable EOF is produced at or just below the critical micelle concentration (CMC) of the electrolytes that are used. A pH independent EOF was generated above the CMC value of the SDS and EOF suppression by the presence of organic modifiers and was greater for the coated capillary system compared to an uncoated capillary.

In order to be effective as an anionic coating in MEKC, the polymer must remain adsorbed. In the study by Bendahl et al.,[56] the observed migration time increase (4-6% over 54 runs) of the solutes was attributed to SDS molecules competing with the PVS polymer for sites on the polybrene cationic layer. In the study by Pranaitytė[66] where a PDADMAC-PSS coating system was used, the PSS layer was replaced by SDS during the analysis. Figure 2 shows the separation of 4 common photographic developing agents on the coated capillary system.[66]

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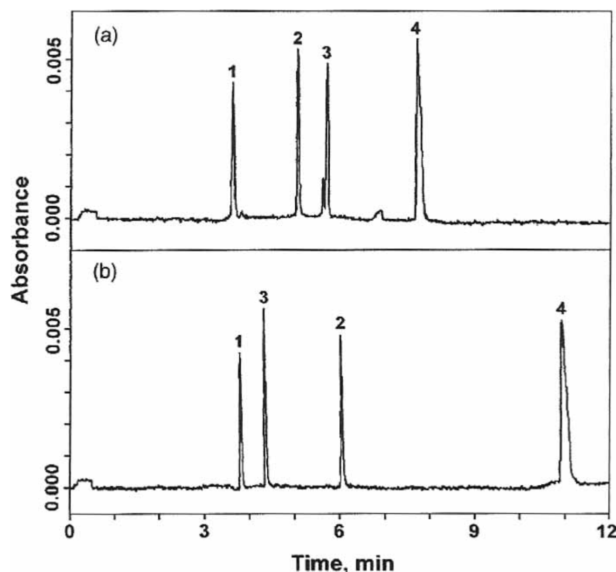

Figure 2. Separation of 4 developers in (a) the uncoated capillary and (b) the PDADMA coated capillary. Electrolytes: (a) 25 $mmol^{-1}$ $Na_2B_4O_7$, 50 $mmol^{-1}$ SDS, pH 9.0 and (b) 25 $mmol^{-1}$ H_3PO_4, 50 $mmol^{-1}$ SDS, pH 3.0. Conditions: voltage, 25 kV; UV detection at 214 nm. Peaks: 1 = hydroquinone, 2 = phenidone; 3 = pyrocatechol; 4 = pyrogallol. Reprinted from Reference 66, Copyright (2004), with permission from Elsevier.

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In a different type of application, the double-strand polyaniline complex of polyaniline with polyacrylic acid (PAA) and poly (methacrylate-co-acrylic acid) (PAN: P(AAMA))[69] was used to increase the resolution of a MEKC separation of nucleobases by reducing, but maintaining, the EOF. This increase in resolution is offset by the increase in analysis time.

NON-AQUEOUS CAPILLARY ELECTROPHORESIS (NACE)

In NACE,[70] the capillary is filled with an electrolyte containing organic solvent system. Organic solvents have very different chemical and physical characteristics from each other and aqueous solutions and large changes in electrophoretic mobilities can be achieved with the use of NACE. NACE is perfectly suited for combination with MS detection due to the absence of non-volatile buffers and additives. Polymer coated capillaries are used in NACE to prevent solute adsorption, but the primary use is to modify the EOF. Optimization of the separation performance involves selecting the appropriate combination of coating, organic solvent, and electrolyte.

PVA and PEG coated capillaries and uncoated capillaries were studied in methanolic electrolytes.[71] The EOF of the PVA, PEG, and uncoated capillary

were compared in 20 mM ammonium acetate in methanol. The EOF was completely suppressed in the PVA coated capillary, reversed in the PEG coated capillary, and a small amount of cathodic EOF existed in the uncoated capillary. After about 40 injections, the separation performance in the PEG coated capillary decreased in terms of changing migration times and reduced peak efficiency. PVA coated capillaries were stable for more than 100 injections under the same conditions.

The reversed EOF generated by PEG coated capillaries was further studied and determined to be caused by the complexation of the PEG coating with cations in the background electrolyte.[72] Reversed EOF was observed in methanol/water and acetonitrile/water solutions containing ammonium acetate (NH_4OAc) with high percentages of organic modifier present. The EOF was also observed to increase as the concentration of NH_4OAc increased.[72] PEG coated capillaries were also used for the analysis of enkephalin peptides with electrochemical detection.[73]

Polybrene (HDB), the cationic polymer is readily soluble in organic solvent and has also been used to create reversed EOF conditions. The separation and analysis of aspirin and three metabolites in urine and plasma was performed in less than 5 min by using a methanol, acetonitrile, sodium acetate, ammonium acetate electrolyte.[64] In another study,[74] HDB coated capillaries were used to separate the oligomers present in KM 20, a mixture of non-ionic surfactants. HDB coated capillaries were prepared both statically and dynamically, and both of these were compared to the commercially available eCAP™ amine coated capillary. The eCAP™ amine coated capillary gave lower EOF than the HDB coated capillaries, required regeneration between runs, and although good resolution was obtained, efficiency values were the lowest of all the three capillaries studied. In comparing the static and dynamically coated HDB capillaries, the static treatment had lower EOF than the dynamic coating but higher resolution and efficiency values, and therefore, was the coating of choice. The EOF influenced the oligomer separation strongly. The final optimized method corresponds well to results obtained by HPLC, the more typical method of analysis.[74]

CHIRAL CAPILLARY ELECTROPHORESIS

Chiral separations are an area in which CE has excelled. CE provides fast, highly efficient separations and chiral selectors can be easily incorporated into the buffer. Physically adsorbed polymer capillaries have been used to minimize the interactions between chiral solutes and the capillary wall, or chiral selectors and the capillary wall. The critical influence of EOF on the resolution of enantiomers and the role of the coated capillary has been examined by Cunãt-Walter et al.[75]

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Chiral Solutes

The commercial CEofix® solution kit/dynamic coating kit was used by Souverain et al.[76] for the enantiomeric separation of amphetamine and related compounds (10 solutes in total). A higher EOF was attained compared to fused silica, which created a significant reduction in migration (factor of 2.5) and run times (factor of 2.7), and increased plate counts. The larger EOF created the predicted reduction in resolution; however, the resolution values remain excellent for the 10 compounds. The use of the coating also provided exceptional migration time stability. The coefficient of variation for uncorrected migration times was 0.1% for all enantiomers except one, which was 0.2%.

Phinney et al.[77] improved the enantiomeric separations of eight commonly used basic pharmaceuticals using the CElixir system. The chiral selector in this case was Heptakis (2,6-di-*O*-methyl)-β-cyclodextrin. The EOF generated by the double layer system was higher than an uncoated capillary and a 30% to 50% reduction in migration times was observed. Peak shape was also improved on the coated capillary and peak tailing was reduced. The resolution of enantiomers was improved for all but one enantiomer. The basic compounds in this study are protonated at the low pH used, and although the coating is anionic, it appears to repel the solutes much more effectively than fused silica.

PVA was used as a dynamic coating in the separation of a selection of basic pharmaceutical compounds with sulfated-β-cyclodextrin compounds.[78] Optimal separation conditions were at high pH (11.6) where the majority of compounds were neutral or negatively charged and a concentration of 0.1% PVA was used. At low pH (2.5), good separations of selected solutes were achieved, however, strong electrodispersion forces for some solutes gave severe peak tailing that not even the addition of the PVA could eliminate. The PVA reduced the EOF and prevented solute adsorption to the capillary wall.[78]

In order to improve the peak shapes of enantiomeric separations of basic drugs, Du et al.[79,80] created dynamic double layer coatings that incorporated chiral selectors. The capillary was first coated with the cationic polymer polybrene (HDB) followed by the chiral selector used in each separation. Chondroitin sulfate[80] and colomic acid (Col A)[79] were used. In both cases, an EOF is generated as the capillary wall is cathodal. For the Col A coating, the EOF profile undergoes a smaller change with buffer pH and improved reproducibility of migration times compared to an uncoated capillary. The Col A coating is also extremely durable, with little fluctuation in migration times occurring even after washing the capillary with acid or base. The peak symmetry of the basic drugs was improved by a factor of about 10 using the Col A coated capillary.

Chiral Selectors

Polybrene (HDB) was used as a capillary coating by Kang et al.[81–83] with the chiral selector vancomycin. Vancomycin adsorbs strongly to fused silica

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capillaries, which reduces the peak efficiency of the chiral separations. In addition to coating the capillary with HDB before analysis, HDB was added to the buffer. The positively charged capillary surface created by the HDB reduces the adsorption of vancomycin, improves the peak efficiency, and provides fast analysis of anionic solutes. In the separation of 12 derivatized amino acids on the HDB coated capillary, excellent resolution of enantiomers was attained (1.4 to 6.7) with a run time of about 4.5 minutes.[81] The reproducibility of the efficiency was (RSD 0.32% to 0.62%) on a run-to-run basis and (RSD 3.2% to 6.5%) on a day-to-day basis.[81] The same approach was used to separate the carboxylic group containing analogues of the drug dimethyl diphenyl bicarboxylate (DDB).[82]

An extremely fast separation system combining the partial filling technique (PFT), a coating to obtain reversed EOF, and the short-end-injection technique was used to analyze dansylated amino acids.[83] Using a HDB coating to reverse the EOF and partially filling the capillary with vancomycin, separations were obtained in under 3.5 minutes. When the short-end-injection technique was used to introduce a 4 cm plug of vancomycin, the separation of dansyl-α-amino-n-butyric acid was attained in only 50 seconds (see Figure 3).[83]

In another study, a HDB coated capillary was used with the PFT to ascertain the chiral selectivity of the glycopeptides balhimycin and bromobalhimycin.[84] These selectors were evaluated individually and compared to vancomycin for the separation of 16 acidic racemic analytes. Bahlimycin and

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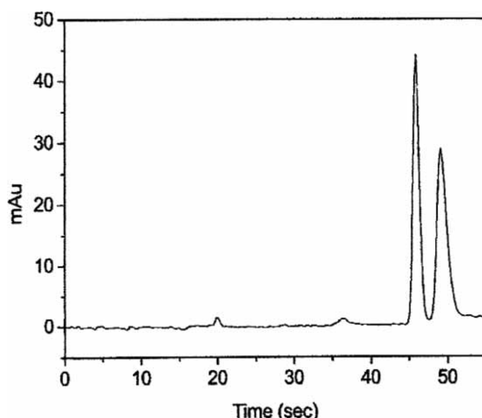

Figure 3. A typical electropherogram of a very fast enantiomeric separation by combination of co-electroosmotic flow electrophoresis, the partial filling technique, and the short-end injection technique. Conditions: running buffer 50 mM Tris-phosphate buffer (pH 6.2) containing 0.001% w/v HDB; 2 mM vancomycin solution was charged into the capillary from the outlet end of the capillary by a pressure of −40 mbar for 40 s; a voltage of 15 kV was applied; dansyl-ABA was injected by a pressure at −10 mbar for 4 s from the outlet end of the capillary. Reprinted with permission from reference 83.

bromobalhimycin were determined to both have high enantiorecognition properties. Overall bahlimycin had the strongest ability for enantioresolution; however, tiaprofenic acid was best resolved by vancomycin.[84]

An assessment of the polymers epoxy poly (AG-AA), epoxy poly (DMA), and poly (DMA) as adsorbed coatings for chiral separations using the PFT, showed the polymers performed well compared to covalently bonded coatings and uncoated capillaries.[85] The chiral selectors used in the investigation were vancomycin, human serum albumin (HSA), riboflavin binding protein, and a cationic cyclopeptide. For the vancomycin and cyclopeptide selectors, the epoxy poly (AG-AA) and poly (DMA) provided good migration time, reproducibility, and peak shape of derivitized amino acids. For the protein chiral selectors, poly (DMA) provided acceptable results for the riboflavin binding protein and excellent results for the HSA, using basic drugs as the test compounds.[85]

Poly (DMA-EpyM) polymers[62,86] were also used for the analysis of fluorescein isothiocyanate (FITC) derivitized amino acids in conjunction with the PFT. The study by Erny[62] used a vancomycin selector with a 20% EpyM coated capillary and separation of FITC derivitized proline and glutamic acid were attained. Simo et al.[86] performed CE-MS of 15 derivitized amino acids using a beta-cyclodextrin selector, for the application of screening adulterated juice.

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CAPILLARY GEL ELECTROPHORESIS (CGE)

CGE is used to separate molecules based on size and is widely used for the analysis of DNA fragments. CGE is performed with capillaries that contain a molecular sieving matrix, such as a gel or a dilute entangled polymer solution. Polymer coatings are used for the purpose of reducing the EOF and as self-coating sieving matrixes that function both as a sieving matrix and as a dynamic EOF modifying capillary coating.

Self-Coating Sieving Matrixes

PDMA

PDMA is an effective self-coating sieving matrix. In the study by Madabhushi,[87] a PDMA (98 kDa) matrix was used to separate about 600 bases in 2 hours. One hundred successive runs were performed by simply replacing the matrix after each run. The thermal stability of the matrix could be enhanced by increasing the molecular weight. Matrixes of 200 kDa were stable at 70°C, whereas, 98 kDa PDMA was stable at 50°C. The EOF suppression of PDMA was greater than PVP, PEG, polyacrylamide, and poly (N-isopropylacrylamide) PNIPAM.[87]

Short-chain PDMA was used for the separation of complex DNA samples.[88] In nondenaturing conditions at pH 7.8, over 800 injections were made before sample efficiency decreased. Using denaturing conditions, at pH 8.3, only 200 injections were made before this effect was observed.[88] High molecular weight PDMA (5.2×10^6 g/mol) had enhanced coating, stability, and comparable separation abilities to polyacrylamide of the same molecular weight and concentration.[89] Eight hundred bases and 1000 bases with resolution limits of 0.5 and 0.3, respectively, were separated in 96 minutes at room temperature.[89]

A mixture of PDMA with polymers[90,91] or additives is another approach that has been investigated for self-coating sieving matrixes. Highly efficient separations of PCR reaction products were attained using a mixture of 0.2% PDMA (molecular mass 8000) with 2.5% polyacrylamide (molecular mass 2.2×10^6).[90] A PDMA/hydroxypropylmethylcellulose (HPMC) matrix provided efficient separations of mutated double strand DNA fragments.[91] The capillary lifetime of the capillary coated with sieving media (PDMA/HPMC) compared to polyacrylamide was 6 to 7 times longer. The coating stability of the polymer mixture was 700 runs at pH 7, 600 runs at pH 8, and only 200 runs at pH 8.5.[91] The addition of montmorillonite clay to low molecular weight PDMA enhanced the sieving characteristics of the PDMA.[92] The addition of 5.0×10^{-5} g/mL clay into 5% (w/v) PDMA of molecular mass of 100 K increased the resolution and efficiency of base pairs of DNA fragments, while maintaining the same migration time. The presence of the clay is believed to effectively increase the molecular weight of the PDMA by working as a dynamic cross-linking plate.[92]

The synthesis of copolymers to modify PDMA has also been performed.[93–96] Song et al.[93] created polymers containing copolymers of acrylamide (AM) and (DMA), P (AM-*co*-DMA). Optimal conditions were determined as 2.5% (w/v) P(AM-*co*-DMA) 3:1, and separations of one base resolution of 0.55 up to 699 bases and 0.30 up to 963 bases were achieved in ambient conditions and in under 80 min.[93] Hydro-EPDMA[94] has the excellent properties of a self-coating sieving matrix, low viscosity, EOF suppressing ability, high sieving capacity (100 base pair resolution). Comb-like co-polymers developed by Barbier et al.,[95] which had a polyacrylamide backbone and PDMA sidechains were used to separate DNA in denaturing conditions. The co-polymers provided comparable performance to polyacrylamide as a sieving matrix and one of the co-polymers developed (P (AM-PDMA)-2) had improved performance to the polyacrylamide above 600 bases.[95]

A study of the influence of hydrophobicity of PDMA based self-coating sieving matrixes was performed by Albarghouthi et al.[96] Linear polyacrylamide, PDMA, and a series of polymers that contained polydiethylacrylamide (PDEA) and polydimethylacrylamide groups were compared in terms of sieving capability. The hydrophilic linear polyacrylamide had the best sieving capability, followed by the PDMA and the polymers containing PDEA.

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Poly(N-isopropylacrylamide) (PNIPAM)

The hydrophilicity of poly(N-isopropylacrylamide) (PNIPAM) is between that of acrylamide and DMA, therefore, it has good potential as a self coating sieving matrix.[97–99] In the study by Zhou,[97] PNIPAM was dissolved with TBE (Tris, boric acid, EDTA) buffer that contained mannitol. Figure 4 shows the separation with various amounts of polymer and mannitol. The sieving properties of the media were observed at 4% mannitol. The relation of mannitol on the separation is attributed to interaction with the boric acid, which lowered the pH of the solution to 6.3. Separations performed at pH 6.3 without mannitol present were also successful.[97] In a

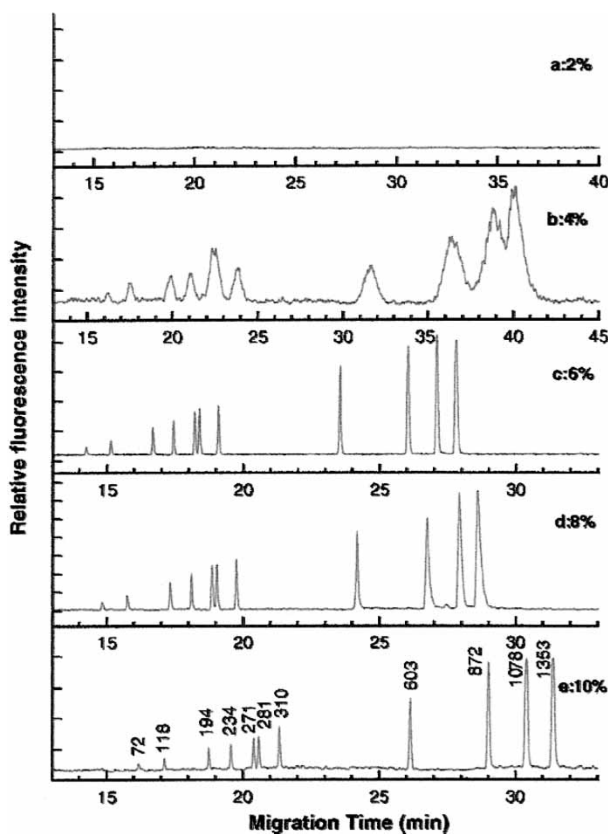

Figure 4. Separation of ΦX174/*Hae*III DNA by CE with (a) 1.5% PNIPAM + 2% mannitol; (b) 1.5% PNIPAM + 4% mannitol; (c) 1.5% PNIPAM + 6% mannitol; (d) 1.5% PNIPAM + 8% mannitol; (e) 1.5% PNIPAM + 10% mannitol. Capillary, 75/365 μm i.d/o.d., and 32/40 cm efficient/total length; inject, 5 s at −8 kV; separation electric field strength 220 V/cm. Reprinted from Reference 97, Copyright (2005), with permission from Elsevier.

recent study of the effect on pH on PNIPAM, it was determined that enhanced hydrogen bonding between silica and the polymer occurred at lower pH values.[98]

PNIPAM copolymers densely grafted with short PEO chains, (PNIPAM-*g*-PEO)[99] have also been studied as a self coating sieving matrix. The polymer had good coating characteristics, good resolution (one base pair), fast separation times, and was easy to inject into the capillary. The polymer was limited to low temperature separations since detrimental conformational changes in the polymer occurred at higher temperatures.[99]

Phea

PHEA (poly-N-hydroxyethylacrylamide) is a self-coating sieving matrix with excellent performance.[100] The polymer resolved 620 bases within 3 hours. The PHEA matrix suppressed the EOF for over 600 runs performed in conditions analogous to those of a DNA sequencer. In addition, the level of EOF suppression was greater than that of an uncoated capillary, a polyacrylamide coated capillary, and a PDMA coated capillary.[100]

PVP

PVP has also been used as a self-coating sieving matrix for DNA genotyping[101,102] and sequencing.[101,103] PVP is very water-soluble, has low viscosity, and effectively reduces the EOF. It is straightforward to prepare, apply, and easy to regenerate upon the capillary surface. In the study by Gao and Yeung,[101] regenerating the matrix between runs allowed the capillary to be effectively used for at least 30 injections. A high molecular weight fraction of the commercial PVP was extracted and explored as a self-coating sieving matrix. It was observed that the extracted PVP extended from 350 bases to 530 bases in the single-base separation.[101]

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Coatings

In recent years, the study of major importance in the field of wall coatings for CGE established the critical factors for high performance dynamic wall coatings, using a set of acrylamide based polymers and copolymers.[104] The polymers based on *N,N*-dimethylacrylamide (DMA) and *N,N*-diethylacrylamide (DEA) had a range of hydrophobicity. PDMA, PDEA, linear polyacrylamide (LPA), and copolymers containing different ratios of DMA to DEA were all studied. These polymer wall coatings were evaluated using linear polyacrylamide as a sieving matrix. In this study, the EOF suppressing effects were investigated as a function of hydrophobicity, electrokinetic layer thickness, polymer conformation, and polymer contour length. The less hydrophobic polymer PDMA and PDEA10 had the thick adsorbed layers and

suppressed the EOF the most. The more hydrophobic polymers had the thinnest layers, the largest surface excess concentration, and suppressed the EOF the least. These results were related to the conformation of the polymers in the adsorbed layers, with the more hydrophilic polymers creating a "loopy" conformation that suppresses the EOF more effectively. EOF suppression was significantly improved when the polymer (PDMA and PDEA30) contained more than 15,000 monomer units. For polymers with equivalent EOF suppressing ability, the more hydrophobic polymer had reduced separation efficiency for more hydrophobic (larger) DNA fragments in linear polyacrylamide.

CAPILLARY ISOELECTRIC FOCUSING (CIEF)

In CIEF, the role of the polymer coating is to reduce EOF, minimize solute adsorption, and provide a uniform capillary surface that does not change with pH. Hydrophilic adsorbed polymer coatings have been used including cellulose derivatives,[105] PVA,[105,106] PEG,[107] poly (methyl methacrylate).[108]

Shen and Smith[105] investigated hydroxyethyl cellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), and compared the performance to physically adsorbed PVA and linear polyacrylamide. Stable static hydroxyalkyl-substituted celluloses coated capillaries were created by thermal immobilizing the polymers on the capillary surface. The coated capillaries were stable for over 100 runs of analysis. In the analysis of hemoglobin variants using a carrier polyampholyte with a pH range of 3 to 10, HPC and HEC coated capillaries had a resolving power of 0.013 pI units, whereas PVA and linear polyacrylamide had a resolving power of 0.019 pI units.

CIEF analysis of microorganisms was performed using dynamic PEG coated capillaries.[107] The PEG 4000 was added to the catholyte, anolyte, and the sample pulse in a pH gradient, which was from pH 2 to 5. The PEG coated capillary reduced the EOF and the microorganism cultures were successfully separated in about 25 min.

Palm et al.[108] compared the performance of a covalently bonded PVP coating to that of a physically adsorbed poly (methyl methacrylate) (plexiglass) coating for the analysis of a set of protein standards. The plexiglass coating took only 40 min to prepare and had a capillary lifetime of about 30 runs. The PVP capillary took about 2 days to generate, was stable for over 100 runs, and had better reproducibility of migration time, peak height, and area compared to the plexiglass coating. Both coatings resolved the protein mixture well in a similar analysis time of about 25 min.

Statically coated polyvinyl alcohol (PVA) coated capillaries were used to enhance the CIEF analysis of proteins in whole human blood and cerebrospinal fluid.[106] The PVA capillaries were coated according to the method

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devised by Gilges et al.[6] In an innovative manner, the salt ions present in the samples were removed on-line before the focusing was started, by applying a slow voltage ramp. The coated capillaries suppressed the EOF, and migration time reproducibility of protein standards were less than 4% (n = 8). Migration times monitored over 21 consecutive injections, using another set of proteins standards, differed less than 1% indicating the stability of the coating. The on-line desalting and analysis of the physiological samples was successfully performed.

CONCLUSION

In all the major modes of CE, the use of physically adsorbed coatings has continued to grow. The modification of the silica surface can minimize solute-wall interactions, additive-wall interactions, as well as determining the direction, magnitude, or stability of the EOF. Although, not as durable as covalently modified coatings, the speed and simplicity of the coating process and the ability to easily regenerate the coatings provides and maintains the properties at the silica surface from run-to run. The commercial availability of coating solutions indicates the demand for enhanced capillary surface properties and the continued growth of the field. The major developments have been the design and synthesis of self-coating sieving matrixes for CGE, the growth of low pH MEKC by the use of anionic coatings, and the development of robust, commercially available coating systems.

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