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Connections between structure and performance of four cationic copolymers used as physically adsorbed coatings in capillary electrophoresis

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ABSTRACT

In this work, the properties of four cationic copolymers synthesized in our laboratory are studied as physically adsorbed coatings for capillary electrophoresis (CE). Namely, the four copolymers investigated were poly(*N*-ethyl morpholine methacrylamide-co-*N*,*N*-dimethylacrylamide), poly(*N*-ethyl pyrrolidine methacrylate-co-*N*,*N*-dimethylacrylamide), poly(*N*-ethyl morpholine methacrylate-co-*N*,*N*-dimethylacrylamide) and poly(*N*-ethyl pyrrolidine methacrylamide-co-*N*,*N*-dimethylacrylamide). Capillaries were easily coated using these four different macromolecules by simply flushing into the tubing an aqueous solution containing the copolymer. The stability and reproducibility of each coating were tested for the same day, different days and different capillaries. It is demonstrated that the use of these coatings in CE can drastically reduce the analysis time, improve the resolution of the separations or enhance the analysis repeatability at very acidic pH values compared to bare silica columns. As an example, the analysis of an organic acids test mixture revealed that the analysis time was reduced more than 6-times whereas the separation efficiency was significantly increased to nearly 10-times attaining values up to 595,000 plates/m using the coated capillaries. Moreover, it was shown that all the copolymers used as coatings for CE allowed the separation of basic proteins by reducing their adsorption onto the capillary wall. Links between their molecular structure, physicochemical properties and their performance as coatings in CE are discussed.

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1. Introduction

It is already well-known that capillary electrophoresis (CE) still shows some important drawbacks that need to be overcome before CE can reach the large expectations that this separation technique generated years ago. Thus, relatively low concentration sensitivity with mostly used UV-absorption detection, low quantitative repeatability, migration time drifts and the existence of analyte–capillary wall interactions are probably among the most important problems still to be solved in CE.

In CE, the electrical charge on the capillary wall controls the zeta potential and the electrostatic interactions, being responsible of electroosmotic flow (EOF) variations [1] and interactions between solutes and capillary wall that ruin the CE separation, mostly of compounds bearing a high positive charge [2–11].

Although many different approaches have been proposed to solve these problems [12–17], so far, the procedure most commonly used to improve repeatability between injections while reducing solute–capillary wall interactions is to coat the inner capillary wall. Since the first CE coatings reported in 1985 [18], many different approaches have been proposed [19] using covalently linked coatings [20–22] or physically adsorbed coatings (static or dynamic) [23–34]. The development of new coatings for CE still remains as an active area of research, as it is demonstrated through the publication of several papers in 2010 [35–39], including several reviews on coatings used for CE–MS [40] or for proteins separation [41].

Our research group has been developing for years new coatings for CE [2–5,9,11,21,42–44], demonstrating its suitability for the CE analysis of different analytes including peptides, proteins, DNAs, etc., or the compatibility of the coating with CE–MS [10]. As many other laboratories usually do, we have mainly followed a trial-error approach in order to obtain coatings with good CE capabilities. The novelty of the present work is not only based on the synthesis and testing of two new copolymers presented for the first time as CE coatings in this paper, but also on the comparative study among four slightly different copolymers trying to extract possible correlations between their physicochemical properties and their performance as CE coatings. Namely, in this work, four different cationic copolymers synthesized at our laboratory are comparatively investigated as physically adsorbed coatings in CE. The four copolymers (whose structures and physicochemical

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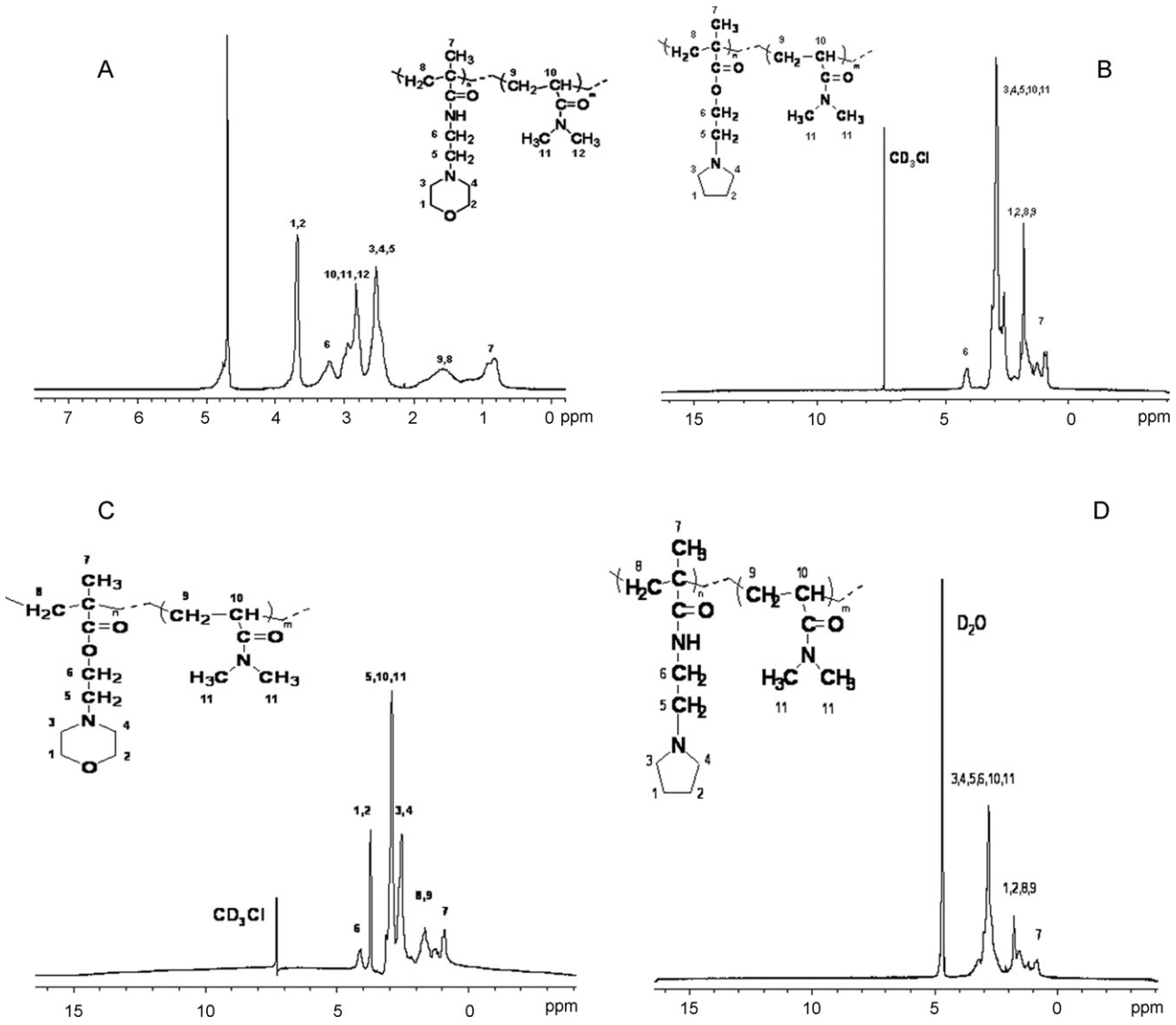

Fig. 1. ^{1}H NMR spectroscopy of the four copolymers used in this work, namely: (A) MAEM/DMA, poly(*N*-ethyl morpholine methacrylamide-co-*N*,*N*-dimethylacrylamide); (B) EPyM/DMA, poly(*N*-ethyl pyrrolidine methacrylate-co-*N*,*N*-dimethylacrylamide); (C) EMM/DMA, poly(*N*-ethyl morpholine methacrylate-co-*N*,*N*-dimethylacrylamide); (D) EPA/DMA, poly(*N*-ethyl pyrrolidine methacrylamide-co-*N*,*N*-dimethylacrylamide).

properties are given in Fig. 1 and Table 1) are the following ones: MAEM/DMA, poly(*N*-ethyl morpholine methacrylamide-co-*N*,*N*-dimethyl-acrylamide); EPyM/DMA, poly(*N*-ethyl pyrrolidine methacrylate-co-*N*,*N*-dimethyl-acrylamide); EMM/DMA, poly(*N*-ethyl morpholine methacrylate-co-*N*,*N*-dimethyl-acrylamide); EPA/DMA, poly(*N*-ethyl pyrrolidine methacrylamide-co-*N*,*N*-dimethyl-acrylamide). As mentioned, EMM/DMA and EPA/DMA copolymers are tested as CE coatings for the first time in this study. Advantages derived from the use of the four coated capillaries including faster separation speed, better resolution, improved repeatability and/or reduced analyte–capillary wall adsorption are discussed.

Table 1
Characterization of the copolymers used in this work: sample (indicating monomer mass percentage in the feed), *F* (molar fraction in the feed), *f* (molar fraction in the copolymer), η (yield of the copolymerization reaction), pK_a (acid–base dissociation constant), M_n (number average molecular weight determined) and polydispersity index determined by SEC.

Structure in Fig. 1	Sample	*F*	*f*	η (%)	pK_a	M_n	Polydispersity index
A	80 MAEM 20 DMA	0.67 0.33	0.69 0.31	84%	4.2	11,000	2.8
B	80 EPyM 20 DMA	0.68 0.32	0.68 0.32	82%	6.6	34,000	2.2
C	80 EMM 20 DMA	0.67 0.33	0.73 0.27	82%	4.0	16,000	3.7
D	80 EPA 20 DMA	0.68 0.32	0.70 0.30	83%	5.7	20,000	4.0

2. Materials and methods

2.1. Samples and reagents

Sodium hydroxide, acetone, formic acid and ammonium hydroxide were obtained from Merck (Darmstadt, Germany). 3-[Cyclohexylamino]-1-propanesulfonic acid (CAPS) and 2-[*N*-morpholino]ethanesulfonic acid (MES), lysozyme ($M_r \sim 14{,}600$; p*I* 10.9), bovine cytochrome C ($M_r \sim 12{,}300$; p*I* 10.5), horse cytochrome C ($M_r \sim 12{,}500$; p*I* 10.2), benzoic acid, L-ascorbic acid and sorbic acid, were supplied from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Boric acid was purchased from Riedel-de Haën (Hannover, Germany). Orthophosphoric acid was from Panreac (Barcelona, Spain). Water was deionized with a Milli-Q system from Millipore (Bedford, MA, USA).

The monomers MAEM, EMM, EPA and EPyM were synthesized as described elsewhere [45,46]. 2,2-Azobisisobutyronitrile (AIBN) from Fluka was purified by fractional crystallization from ethanol. The solvents used were tetrahydrofuran (THF) from Sharlau Chemie S.A. (Barcelona, Madrid) and ethanol from RP Normapur, Prolabo (Fontenay, France). *N*,*N*-dimethylacrylamide (DMA) from Aldrich, was vacuum distilled. Ammonium persulfate (Aldrich, Germany) was used as received. Isopropanol (Scharlau, Scharlab S.L., Spain, 99.5% purity) diethyl ether (SDS, France, 99.7% purity) hexane (mixture of hexane isomers, SDS, France) tetrahydrofuran (Scharlau, Spain, 99.8% purity) phosphorous pentoxide (Panreac, Spain, 98% purity), *N*,*N*′-dimethyl formamide (DMF) (Scharlau, Scharlab, Spain, 99% purity). NaCl (Panreac Química S.A., Spain, 99.5% purity) NaOH (Panreac Química S.A., Spain, 98%) and HCl (Analar Normapur, VWR International S.A.S., France, 37%) were used for polymers synthesis and characterization.

The monomers MAEM, EMM, EPA and DMA were copolymerized by free radical polymerization at 50 °C using ammonium persulfate ($[I] = 1.5 \times 10^{-2}$ mol l^{-1}) as radical initiator and distilled water for MAEM [44] and a mixture of distilled water/isopropanol (60/40) for EMM, EPA ($[M] = 1$ mol l^{-1}) as solvent. After 24 h reaction time copolymer samples were dialyzed against water in a Spectra/Por® (Spectrum Laboratories Inc., Houston, TX, USA) using membranes with a molecular weight cut-off 3500, and then lyophilized.

The monomers EPyM and DMA were copolymerized [9] by free radical polymerization at 50 °C using AIBN ($[I] = 1.5 \times 10^{-2}$ mol l^{-1}) as radical initiator and tetrahydrofuran (THF) ($[M] = 1$ mol l^{-1}) as solvent. The reaction mixture was precipitated in a large excess of a diethyl ether–hexane mixture, purified by re-precipitation, filtered off and vacuum-dried over phosphorous pentoxide.

2.2. Characterization of the copolymer systems

The four copolymers were analyzed by ^{1}H NMR spectroscopy using a Varian XLR-300 spectrometer operating at 300 MHz. All spectra were obtained at room temperature from 5% (w/v) $CDCl_3$ and D_2O solutions.

The pK_a of the copolymers were determined by acid–base titration of 100 mg of copolymer in 25 ml of a 0.1 M NaCl aqueous solution containing 2 ml of a 0.1 M HCl solution to ensure the ionization of the amine groups of the copolymers. Diluted aqueous solutions of NaOH were used to complete the titration using small volumes in order to avoid the modification of the ionic strength. The changes of pH were measured with a Schott GC841 pH meter (Schott, Germany). Determination of the corresponding compounds was carried out in triplicate. The standard deviation was in all the cases lower than 3%.

Average relative molecular mass and relative molecular mass distributions were determined by size exclusion chromatography (SEC) by using polymer solutions (5 mg/ml) in *N*,*N*′-dimethyl formamide (DMF). Measurements were carried out at 1 ml/min flow using Ultrastyragel columns of 500, 10^4 and 10^5 Å (Polymer Laboratories) at 70 °C and using a differential refractometer as detector. The calibration was performed with poly(styrene) (PS) standards in the range of $M_r \sim 2990$ and $M_r \sim 1{,}400{,}000$ and polydispersity values lower than 1.1.

2.3. Preparation of background electrolytes (BGEs) for CE experiments

To study the EOF mobility from the different coated capillaries at different pH, six different background electrolytes (BGEs) were employed using either normal or reversed polarity. These running buffer solutions were prepared by mixing adequate volumes of the following solutions: 1 M H_3PO_4, 1 M HCOOH, 0.5 M MES, 0.25 M H_3BO_3 and 0.25 M CAPS with 1 M NaOH. The final volume was adjusted to 50 ml, resulting the next six running BGEs at the indicated pH values: (i) 86 mM NaH_2PO_4/H_3PO_4 at pH 2.21; (ii) 75 mM NaHCOO/HCOOH at pH 3.60; (iii) 100 mM Na-MES/MES at pH 6.19; (iv) 22 mM NaH_2PO_4/Na_2HPO_4 at pH 7.18; (v) 100 mM NaB_4O_7/H_3BO_3 at pH 9.14; (vi) and 75 mM Na-CAPS/CAPS at pH 10.77.

2.4. Capillary coating procedure

Four cationic copolymers composed by 80/20 EPyM/DMA, 80/20 MAEM/DMA, 80/20 EMM/DMA and 80/20 EPA/DMA were tested as capillary coatings for CE. Firstly, 1 mg of each copolymer was accurately weighted and dissolved in 5 ml of a solution 100 mM formic acid in water. Once the solution was perfectly dissolved, an equivalent volume of 100 mM ammonium hydroxide was added in order to reach final copolymer concentrations of 0.1 mg/ml. To get reproducible capillary coatings, a procedure already developed in our group was applied [11]. Each new capillary was washed with 1 M NaOH for 30 min and then flushed with the final copolymer solution for 15 min and left to stand overnight at 4 °C. At the beginning of the day, the capillary was washed with copolymer solution for 15 min in order to adequately prepare the coating. Between runs, the capillary was washed using the next sequence: 3 min with copolymer solution, 2 min with water and finally 3 min using the selected running buffer.

2.5. CE separation conditions and samples

A P/ACE 2050 instrument with UV detection from Beckman Coulter Inc. (Fullerton, CA, USA) was used to perform the CE experiments. The capillaries used were made of fused silica with 50 μm i.d. and 360 μm o.d. (Composite Metal Services, Worcester, UK), with 370 mm of total length (300 mm detection length). For all the experiments the capillary was thermostated at 25 °C.

A solution of buffer/water/acetone (20/75/5) was employed as sample to measure the EOF mobility; it was injected for 3 s at 3450 Pa and detected at 254 nm. The voltage applied was 30 kV. Each run was performed by triplicate. For the study of the different coatings, the same sequence of BGEs was used (i.e., from the lowest to the highest pH) reproducing in that way any possible hysteresis effect.

Basic proteins sample consisted of lysozyme, bovine cytochrome C and horse cytochrome C all dissolved in buffer (75 mM NaHCOO/HCOOH at pH 3.6)/water (1/4, v:v) at 75 μg/ml. The proteins were analyzed at 30 kV (normal polarity) with the bare silica capillary and at −30 kV (reverse polarity) with the coated capillaries. The sample was injected for 5 s at 3450 Pa; the detection wavelength was set at 214 nm. The BGE employed was 75 mM NaHCOO/HCOOH at pH 3.6.

Table 2
RSD (%) values of the EOF mobility for bare silica and different coated capillaries at the different pH studied in this work. All copolymers used contained 20% DMA.

pH	RSD (%, $n = 5$) of EOF mobility values				
	Bare silica	MAEM/DMA	EPyM/DMA	EMM/DMA	EPA/DMA
2.21	17.2	0.8	0.9	1.4	1.0
3.60	2.9	1.9	1.4	2.0	1.1
6.19	3.4	7.6	9.0	3.8	5.0
7.18	2.7	4.5	6.0	3.5	4.5
9.14	0.7	0.8	0.7	0.7	1.7
10.77	0.4	0.9	0.8	1.1	1.5

Organic acids test mixture contained benzoic acid, L-ascorbic acid and sorbic acid all dissolved in buffer (75 mM NaHCOO/HCOOH at pH 3.6)/water (1/4, v:v) at 50 μg/ml. The analysis of the test mix was performed at 20 kV (normal polarity) with the bare silica capillary and at −20 kV (reverse polarity) with the coated capillaries. The detection wavelength used was 280 nm, and the mixture was injected for 3 s at 3450 Pa. The BGE used to analyze this test mix was 75 mM NaHCOO/HCOOH at pH 3.6.

3. Results and discussion

3.1. Characterization of the copolymers

The characterization of the four copolymers was performed by ^{1}H NMR spectroscopy by the signal assignments shown in Fig. 1.

3.1.1. Poly(MAEM-co-DMA)

The MAEM and DMA feed molar fractions were 0.67 and 0.33 (corresponding to MAEM and DMA mass percentage of 80% and 20% respectively). The signals used for the determination of composition of the copolymers are: 0.83 (α-CH_3, MAEM), 1.70 (–CH_2–, MAEM and DMA), 2.50 (3 × –CH_2–N–, MAEM cycle), 2.82 (2 × N–CH_3 and–CH– of DMA), 3.12 (–CH_2–NHCO– of MAEM) and 3.74 (–CH_2–O–CH_2– of MAEM cycle). The copolymers composition was determined by integration of the corresponding signals being the MAEM molar fraction 0.69 and 0.31 for DMA.

3.1.2. Poly(EPyM-co-DMA)

The EPyM and DMA feed molar fractions were 0.68 and 0.32 (corresponding to EPyM and DMA weight percentage of 80% and 20% respectively).

The signals used for the determination of composition of the copolymers are: 0.93 (α-CH_3, EPyM), 1.60 (–CH_2–, EPyM and DMA), 1.70 (–CH_2–CH_2–, EPyM cycle), 2.50 (3 × –CH_2–N–, EPyM cycle), 2.82 (2 × N–CH_3 and –CH– of DMA), 4.11 (–CH_2–O–CO– of EPyM). The copolymers composition was determined by integration of the corresponding signals being the EPyM molar fraction 0.68 and 0.32 for DMA.

3.1.3. Poly(EMM-co-DMA)

The EMM and DMA feed molar fractions were 0.67 and 0.33 (corresponding to EMM and DMA weight percentage of 80% and 20% respectively).

The signals used for the determination of composition of the copolymers are: 0.83 (α-CH_3, EMM), 1.50 (–CH_2–, EMM and DMA), 2.50 (3 × –CH_2–N–, EMM cycle), 2.82 (2 × N–CH_3 and –CH– of DMA), 3.74 (–CH_2–O–CH_2– of EMM cycle) and 4.11 (–CH_2–O–CO– of EMM). The copolymers composition was determined by integration of the corresponding signals being the EMM molar fraction 0.73 and 0.27 for DMA.

3.1.4. Poly(EPA-co-DMA)

The EPA and DMA feed molar fractions were 0.68 and 0.32 (corresponding to EPA and DMA weight percentage of 80% and 20% respectively).

The signals used for the determination of composition of the copolymers are: 0.93 (α-CH_3, EPA), 1.60 (–CH_2–, EPA and DMA), 1.70 (–CH_2–CH_2–, EPA cycle), 2.50 (3 × –CH_2–N–, EPA cycle), 2.82 (2 × N–CH_3 and –CH– of DMA), 3.12 (–CH_2–NHCO– of EPA). The copolymers composition was determined by integration of the corresponding signals being the EPA molar fraction 0.7 and 0.3 for DMA.

Table 1 shows some additional parameters of the synthesized copolymers whose structures can be seen in Fig. 1. Thus, as can be seen in Table 1, the reaction yields (η) were in all cases higher than 80%. Monomer molar fractions in the feed (F) and in the copolymer (f, determined by ^{1}NMR spectroscopy) were found to be similar at high conversions reactions. The ionisable character of the prepared polymers was studied by the determination of their acid–base dissociation constant, pK_a. The presence of tertiary amine groups in all the positive monomers EMM, MAEM, EPA and EPyM (see Fig. 1) confers the partial or total ionization of the polymers and with that their cationic character (*vide infra*). Number average molecular weights determined by SEC were found to be between 11,000 and 34,000 and polydispersity indexes determined by SEC between 2.2 and 4.0, typical of radical polymerization.

3.2. Coated versus bare fused silica capillaries. EOF and reproducibility studies

In Fig. 2, EOF is represented versus the pH of the background electrolyte (BGE) for the bare silica capillary and for the four capillaries coated using copolymers containing 20% of the neutral monomer DMA and 80% of the positive monomer (MAEM, EPyM, EMM and EPA).

According to the molecular structures of the four copolymers (see Fig. 1), the amine group in the monomers MAEM, EPyM, EMM and EPA is expected to provide some cationic character to these macromolecules what can be of interest from a CE point of view. Thus, as can be seen in Fig. 2, the EOF obtained for the bare fused-

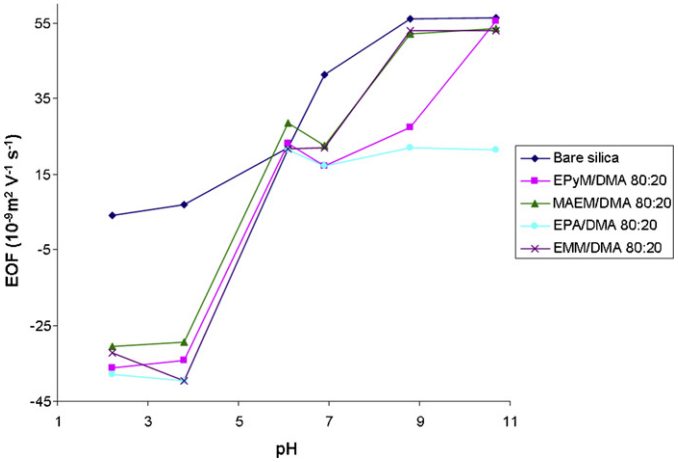

Fig. 2. Electroosmotic mobility as a function of pH and type of capillary.

Table 3
Reproducibility (RSD, %) of the EOF mobility for three different days and three different capillaries using bare silica columns and coated capillaries at the different pH studied in this work. All copolymers used contained 20% DMA.

pH	Bare silica		MAEM/DMA		EPyM/DMA		EPA/DMA		EMM/DMA	
	Three consecutive days	Three different capillaries	Three consecutive days	Three different capillaries	Three consecutive days	Three different capillaries	Three consecutive days	Three different capillaries	Three consecutive days	Three different capillaries
2.21	20.6	18.8	3.3	2.5	4.7	4.8	5.3	7.3	5.9	3.4
3.60	3.6	10.6	2.8	4.2	4.5	3.5	1.6	2.6	5.6	3.0
6.19	3.2	4.4	9.7	10.3	11.5	9.9	4.3	7.1	3.8	4.8
7.18	6.8	6.5	6.2	8.8	6.8	12.4	10.0	9.1	4.5	6.4
9.14	2.1	2.1	2.3	2.8	4.7	3.8	4.7	3.8	4.4	5.6
10.77	3.1	1.7	3.0	4.1	3.1	5.9	6.4	4.9	2.8	5.2

silica shows a typical dependence on the pH (i.e. EOF close to zero at very low pH, an increase of EOF at pH about 5 and nearly a constant EOF value at pH values higher than 8). As can be seen in Fig. 2, using the same running buffers with the coated capillaries the behaviour of the EOF versus pH is somewhat different. Thus, the coated capillaries show an anodal EOF at low pH values, a nearly zero EOF at running buffer at pH 5–6 and a low cathodal EOF at pHs higher than 8. This behaviour can be explained considering that the global electrical charge onto the capillary wall is due to both the silanol groups of the capillary (negatively charged) plus the amine groups of the polymer (bearing a positive electrical charge). Thus, under acidic pHs the amine groups are the predominant ionized groups bringing about a global positive charge and, as a consequence, an anodal EOF. Under very basic pHs the number of positive charges on the polymer decreases and the negative silanol groups become predominant, bringing about a cathodal EOF. It is noteworthy that this EOF is reduced using the coated capillaries what is a good indication of the shielding effect of the coating even at these basic pHs. As can be deduced from Fig. 2, the coating was successfully achieved in all the cases as can be deduced from the negative EOF obtained at pH values lower than 5 using the four copolymers.

In order to study the reproducibility that can be achieved with these coatings, RSDs (% RSD for $n = 5$ injections) were calculated for the analysis time of the EOF obtained at six different pHs from 2.21 to 10.77. Tables 2 and 3 show the RSD values obtained depending on the pH and type of capillary used (four coated capillaries plus a bare silica tube) for the same day, different days and different capillaries. Although the RSD values were better using the bare fused-silica capillary at pHs above 6.19, it can be observed that the use of coated capillaries clearly improved the RSD values at pH 2.21 and 3.60. This effect seems to be related to the magnitude of the EOF independently of its direction towards the anode or the cathode (i.e., the higher the EOF value, the better is the time reproducibility that can be achieved in CE).

3.3. Applications of coated capillaries

In order to demonstrate the possibilities of using cationic copolymers as coatings in CE, several applications were carried out. Thus, Fig. 3 shows a comparison of the CE separations of a group of organic acids achieved under identical conditions by using a bare fused-silica capillary (Fig. 3A) and four different coated capillaries (Fig. 3B–E) at pH 3.60. As it can be seen, the use of the coated capillaries offers an interesting advantage since they provide much faster separations at acidic pH values, making possible the baseline separation of the three organic acids in less than 3 min compared to the 18 min required by the bare silica tubing at the same pH. In this separation, due to the EOF generated into the coated capillaries at the selected pH, the polarity was reversed when using coated capillaries, what explains the different migration order obtained. Besides, the separation was not only improved in terms of speed but also in terms of separation efficiency probably due to the much lower molecular diffusion of the organic acids in a fast separation. In fact, using the coated capillaries, efficiencies up to 595,000 plates/m were obtained for L-ascorbic acid. These values significantly improved those obtained when using bare silica capillary that were below 64,000 plates/m. Among the different copolymers employed as coatings, no great differences were observed for this application. As it can be observed in Fig. 2, both the magnitude and the direction of the EOF were quite similar for all the coated capillaries at the working pH (pH 3.60).

An additional advantage of these coated capillaries is the possibility to use them to carry out separations of basic proteins in CE. One of the main problems during the separation of basic proteins by CE is the electrostatic interaction between the positively charged proteins and the negatively charged wall. Basic proteins can be sep-

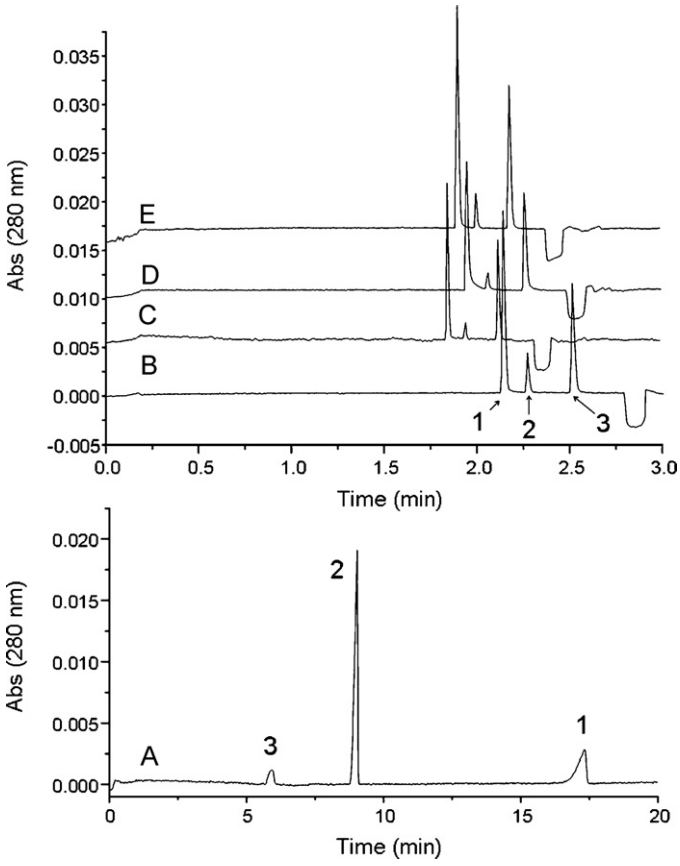

Fig. 3. Comparison of the separation of organic acids (1, benzoic acid; 2, L-ascorbic acid; 3, sorbic acid) at 50 μg/ml. (A) Bare silica capillary, (B) 80:20 MAEM/DMA coated capillary, (C) 80:20 EPyM/DMA coated capillary, (D) 80:20 EPA/DMA coated capillary, and (E) 80:20 EMM/DMA coated capillary. Separation conditions: capillary length, 37 cm; effective length, 30 cm; capillary i.d., 50 μm; background electrolyte, 75 mM NaHCOO/HCOOH at pH 3.60; voltage, −20 kV (except A, +20 kV).

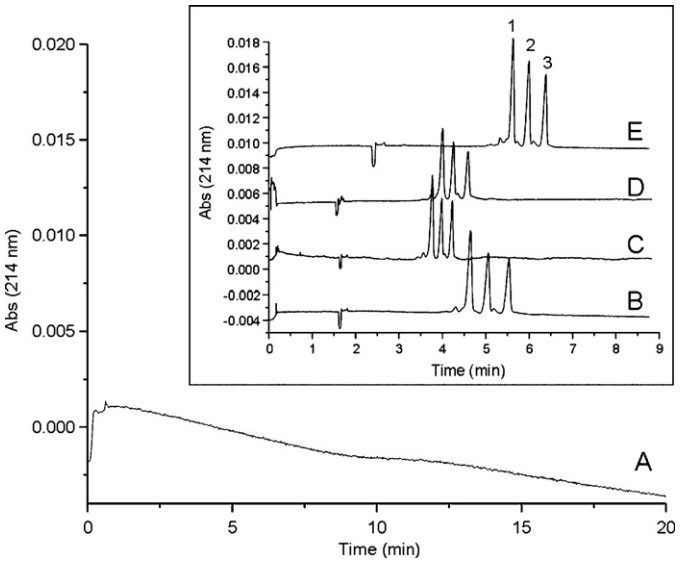

Fig. 4. Comparison of the separation of basic proteins (1, lysozyme; 2, bovine cytochrome C; 3, horse cytochrome C) at 75 μg/ml. (A) Bare silica capillary, (B) 80:20 MAEM/DMA coated capillary, (C) 80:20 EPyM/DMA coated capillary, (D) 80:20 EPA/DMA coated capillary, and (E) 80:20 EMM/DMA coated capillary. Separation conditions: capillary length, 37 cm; effective length, 30 cm; capillary i.d., 50 μm; background electrolyte, 75 mM NaHCOO/HCOOH at pH 3.60; voltage, −30 kV (except A, +30 kV).

arated at very low pH, decreasing the degree of ionization of the silica surface. Alternatively, the capillary can be coated to obtain a neutral or a positively charged surface allowing basic protein separation. The use of cationic coatings as the ones presented in this work provides a positively charged wall at pHs between 2 and 6 and whose EOF mobility can be tailored to optimize resolution and separation speed by choosing the adequate polymer composition and buffer pH. This is demonstrated in Fig. 4, where three basic proteins (lysozyme, horse cytochrome C, and bovine cytochrome C) are separated in a sodium formate buffer at pH 3.6, using both bare silica capillary (Fig. 4A) and the capillaries coated using the four copolymers abovementioned (Fig. 4B–E). As it can be observed in Fig. 4A, neither separation nor peaks were observed when using the bare silica capillary due to the irreversible adsorption of these proteins onto the capillary wall. At the same pH, the four coated capillaries provide high electroosmotic mobility (Fig. 2). Therefore, these capillaries should provide at that pH a fast separation, but also a highly positive charged surface, which should protect efficiently against proteins adsorption. This fact is demonstrated in Fig. 4, where the same three proteins at low concentration (75 μg/ml) could be well separated in less than 6 min showing a symmetrical peak shape in all the cases when coated capillaries were used. As it can be observed in Fig. 4, the migration behaviour was again similar using the four cationic copolymers, although better resolution among the proteins were obtained using the EMM/DMA coated capillary. Nevertheless, the fastest separation was obtained employing the EPyM/DMA coated capillary which also provided good resolution between the three proteins in less than 4.5 min. The efficiencies obtained for the four coatings were slightly higher than 100,000 plates/m.

3.4. Correlation between CE coating performance and copolymer structure

As it can be seen in Fig. 1, the four copolymers of this work have in common the use of the neutral monomer DMA varying the type of cationic monomer employed (MAEM, EPyM, EMM and EPA). The four copolymers seem to behave similarly since all of them are physically adsorbed onto the capillary wall and the coating is regenerated between injections just by flushing the capillary with a dilute solution containing the polymer. However, from the results given in Figs. 2–4, some interesting differences among their behavior can be observed depending on their structure. In general, the side chain group of the cationic monomer played an important role in the CE behaviour of the coating, because copolymers will be ionised (or not) at a given pH depending on the nature and pK_a value of the mentioned group. This dissociation constant value depends on the side chain group as can be deduced from Table 1 and Fig. 1 in which pyrrolidine (monomers EPA and EPyM) and morpholine (monomers MAEM and EMM) based copolymers show pK_a values close to 6 and 4, respectively. Moreover, within the same type of side chain group the amide (used in MAEM and EPA) or ester group (in EMM and EPyM) used to bind the side chain group to the main polymer chain also can modify, although more slightly, the pK_a value of these macromolecules. This effect seems to be more intense in the case of pyrrolidine based copolymers whose pK_a values go from 5.7 using the amide group to 6.6 using the ester group. As a result, pyrrolidine based copolymers show a stronger cationic character at acidic pH values (as can be deduced from the higher cathodal EOF values obtained at pH 2.21 as shown in Fig. 2) and corroborated by the fast separations obtained using EPA-DMA and EPyM-DMA copolymers (see Figs. 3 and 4). Furthermore, the higher pK_a values of the pyrrolidine monomers brings about an important shielding effect of the silanol groups at basic pHs, giving rise to lower EOF values as can be deduced from the results of Fig. 2.

4. Conclusions

In this work, the possibility of attaining reproducible cationic coatings physically adsorbed onto the capillary wall using MAEM/DMA, EPyM/DMA, EMM/DMA and EPA/DMA copolymers was demonstrated. The two latter copolymers have been tested for their use as coatings in CE for the first time in this study. The net charge of the coating depends on the copolymer nature, copolymer composition and working pH, determining the magnitude and direction of the EOF. Therefore, by modifying these parameters the EOF can be effectively tailored to solve a given application in CE introducing an additional and very useful parameter that can be optimized in CE. As it has been shown in this work, the adequate selection of these conditions can: (i) improve reproducibility; (ii) enhance both separation efficiency and resolution; (iii) increase analysis speed and; (iv) diminish the negative adsorption between solutes and capillary wall.

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