Micellar Electrokinetic Chromatography: A Powerful Analytical Tool To Study Copolymerization Reactions Involving Ionic Species

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ABSTRACT: The radical copolymerization reactions of (2-hydroxyethyl methacrylate)−(2-acrylamido-2-methylpropanesulfonic acid), HEMA−AMPS, and (*N*,*N*-dimethylacrylamide)−(2-acrylamido-2-methylpropanesulfonic acid), DMAA−AMPS, are exhaustively studied by micellar electrokinetic chromatography (MEKC). Two new MEKC procedures are developed that allow us to follow the monomer consumption together with the copolymer synthesis for the two systems, i.e., HEMA−AMPS and DMAA−AMPS. The effects of the conversion and composition on the chemical composition distribution as well as on the molecular weight are analyzed by using this analytical technique. The large possibilities of MEKC for obtaining interesting information about synthesis progress, nature, and composition of the formed ionic copolymers are demonstrated. Moreover, it is shown that capillary electrophoresis instrumentation can be used to monitor the electrical conductivity of the reaction product obtained at different copolymerization stages of the HEMA−AMPS system in order to clarify the polymerization mechanism.

Introduction

The copolymerization reaction at high conversion of two or more monomers of different chemical structure lead, in general, to heterogeneous copolymers instead of homogeneous chains, considering not only the molecular weight and the molecular weight distribution but also the chemical or structural composition and the distribution of comonomer units along the macromolecular chains. For those systems that do not follow strictly the random copolymerization behavior where $r_1 = r_2 = 1$, or the azeotropic composition, as conversion advances, copolymer chemical composition enriches in the more reactive monomer, and the chemical feed composition overcomes a depletion of the monomer that is preferably incorporated. This gives rise to chemically heterogeneous copolymer macromolecules, and this phenomenon is particularly relevant when one of the comonomers contains ionic functional groups. According to the classical terminal kinetic model of copolymerization process, the relative reactivity of radicals and monomers are described by the reactivity ratios of the system. The chemical composition distribution of binary random copolymers gives much information about the copolymer chains: sequence distribution heterogeneity, instantaneous heterogeneity, and conversion heterogeneity. Although the analysis of such distributions along the copolymer (including the determination of charge heterogeneity in polyelectrolyte systems) can be theoretically predicted using copolymerization models, it frequently requires techniques that involve separation. For instance, the use of high-performance liquid chromatography (HPLC) has been described in the literature for this purpose.[1]

Different capillary electrophoresis (CE) procedures have been used for the elucidation of chemical composition distribution, despite the small number of articles registered in the bibliography about this topic. Thus, frontal analysis continuous capillary electrophoresis (FACCE),[2] capillary gel electrophoresis (CGE),[3] and isotachophoresis (ITP)[4] have been described to analyze different types of macromolecules, usually bearing electrical charge. We have recently shown that MEKC can be a very useful tool to separate copolymers having a variable number of hydrophobic groups.[5] By using this procedure, the chemical composition of the poly(*N*-vinylpyrrolidone-*co*-2-hydroxethyl methacrylate) was determined. The degree of interaction with sodium dodecyl sulfate, SDS, and the resulting electrophoretic mobility of the polymers were demonstrated to depend on the content of the more hydrophobic HEMA monomer.

Originally, MEKC was developed for the separation of nonionic compounds,[6] but it can also be used to analyze ionic molecules, combining in this way hydrophobic and ionic interactions between solutes and micelles. By this strategy the selectivity of the separation is considerably improved. MEKC can provide high efficiencies (10^5-10^6 theoretical plates per meter of column) in short times of analysis (less than 30 min). Moreover, it requires small volumes of sample (a few microliters). As such, it has considerable potential for characterizing ionic/nonionic copolymers.

In this work, we show the potential of MEKC for the analysis of copolymerization reactions of ionic with nonionic comonomers, to our knowledge, for the first time. The copolymerization mechanism, monomer consumption, and chemical composition distribution is exhaustively studied for the (2-hydroxyethyl methacrylate)−(2-acrylamido-2-methylpropanesulfonic acid) (HEMA−AMPS) and (*N*,*N*-dimethylacrylamide)−(2-acrylamido-2-methylpropanesulfonic acid) (DMAA−AMPS) systems by MEKC.

Materials and Methods

Reagents. 2-Acrylamido-2-methylpropanesulfonic acid, AMPS (99%), was supplied by Avocado and used as received. *N*,*N*-Dimethylacrylamide, DMAA (Aldrich), was carefully distilled under reduced pressure (>99%).

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Table 1. Molar Fractions of the Copolymers HEMA−AMPS (HA) and DMAA−AMPS (DA)

	PHEMA	HA90	HA70	HA50	HA30	HA10	PAMPS
$f_{\text{HEMA feed}}$	1	0.90	0.70	0.50	0.30	0.10	0
$F_{\text{HEMA copolymer}}$	1	0.89	0.67	0.47	0.27	0.10	0
$F_{\text{HEMA cop\_dial}}$[a]	1	0.92	0.80	0.67	0.51	0.23	0
	PDMAA	DA90	DA70	DA50	DA30	DA10	PAMPS
$f_{\text{DMAA feed}}$	1	0.90	0.70	0.50	0.30	0.10	0
$F_{\text{DMAA copolymer}}$	1	0.89	0.73	0.52	0.31	0.09	0
$F_{\text{DMAA cop\_dial}}$[a]	1	0.89	0.75	0.57	0.39	0.16	0

[a] Copolymer composition alter dialysis (cutoff 3500 Da).

2-Hydroxyethyl methacrylate, HEMA (Fluka), was purified according to the literature.[7] Briefly, the monomer was dissolved in H_2O (3:4), and $NaHCO_3$ (3% w/v in water) was added to eliminate the traces of methacrylic acid. The solution was stirred for 24 h. Hexane was added, and the aqueous phase was isolated and saturated with NaCl to remove the residual dimethacrylates. The monomer was extracted with diethyl ether, and the organic phase was collected and dried using anhydrous $MgSO_4$. The solvent was removed and the monomer carefully distilled at reduced pressure. The purity of the monomer after distillation was >99%.

Other reagents were extra pure grade and used as purchased.

Polymers. All the homopolymers and copolymers were obtained by free radical polymerization using water: 2-propanol (50:50) as solvent. As an example of the synthetic procedure, we describe the detailed preparation of one of the HEMA−AMPS copolymers: HA70 was obtained by free radical polymerization using 100 mL of water:2-propanol (1:1) as solvent. Monomers were mixed (2.733 g of HEMA and 1.865 g of AMPS, giving a HEMA feed molar fraction of 0.70), the initial monomers concentration being 0.3 M. After degassing by bubbling with pure N_2 for 30 min, the reaction was induced thermally at 50 °C by using azobis(isobutyronitrile) (0.246 g, 1.5×10^{-2} M) as free-radical initiator. After 24 h, the samples were lyophilized, and the isolated solid was dialyzed (using a Slide-A-Lyzer 3.5K Dialysis Cassette, 3500 molecular weight cutoff, PIERCE) against deionized water for 48 h to minimize the presence of low molecular weight molecules and residual unreacted monomer. Conversion before and after dialysis was 99.5% and 92%, respectively.

The synthesized products were characterized by ^{1}H NMR, and the molar fractions of the isolated samples are detailed in Table 1. The acronyms DA and HA followed by the initial feed molar percentage as it is written in the table were used to identify the different reactions for the DMAA−AMPS and HEMA−AMPS systems, respectively. Figures 1 and 2 show two ^{1}H NMR chosen as examples (DA70 and HA70 copolymers).

Kinetic Analysis. Copolymerization kinetics of different reactions were followed by capillary electrophoresis. Samples were directly collected (0.5 mL) from the reaction mixture using a syringe to avoid the oxygenation of the medium at different times. The reaction was stopped by adding a hydroquinone solution (20 μL, 0.375 M). All of the samples were stored at −20 °C until analysis. Samples were diluted 1:10 prior to injecting in the capillary column.

Polymer Characterization. ^{1}H NMR characterization was carried out using a Varian-XL 300 spectrometer working at 300 MHz. The spectra were recorded using 5% (w/v) deuterated water or dimethyl-d_6 sulfoxide solution, depending on solubility.

MEKC Analysis. MEKC measurements were carried out using a Beckman 5500 (P/ACE) electrophoresis apparatus controlled by a Pentium 100 MHz personal computer. A fused silica capillary (Composite Metal Services) with 50 μm i.d., 37 cm total length and 30 cm effective length (from injection point to the detector) was employed. The temperature of the capillary was maintained at 25 °C. The injection was carried out in the anode using N_2 pressure (0.5 psi) for 5 s. The separation voltage was 10 kV. All of the data were collected and analyzed using the System Gold software from Beckman. The detection took place at 200 nm. The composition was measured using a Beckman 5000 (P/ACE) instrument with a diode-array UV detector and a 200/220 nm ratio for quantifying the copolymer composition. Between injections, the capillary was washed with water for 1 min and buffer for 1 min.

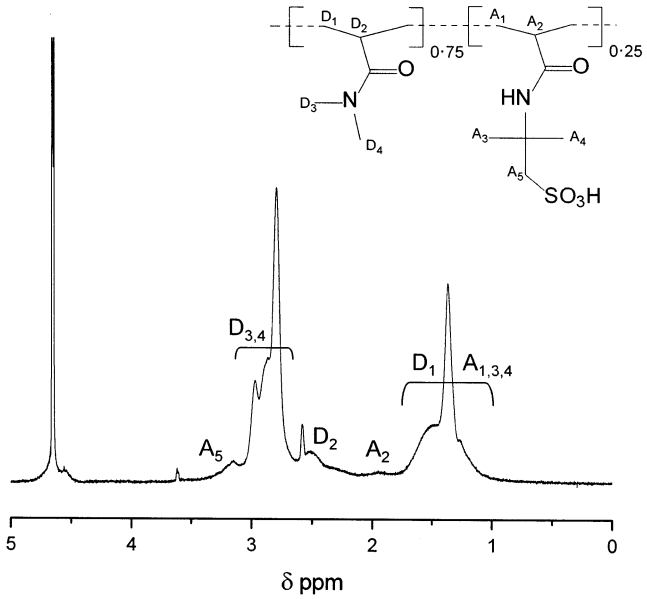

Figure 1. Chemical structure and ^{1}H NMR of a copolymer DMAA−AMPS (DA70).

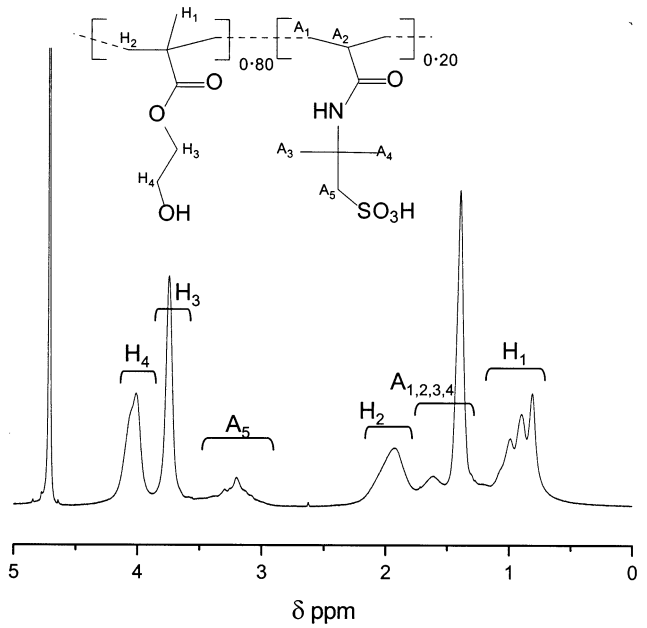

Figure 2. Chemical structure and ^{1}H NMR of a copolymer HEMA−AMPS (HA70).

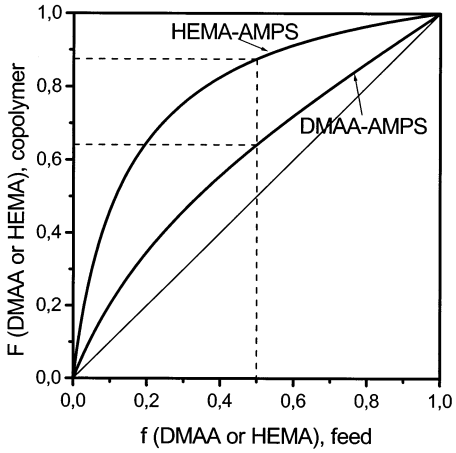

Figure 3. Composition diagrams (according to the terminal model) for both copolymerizations.

Buffers. Sodium tetraborate, boric acid, sodium dodecyl sulfate (SDS), and acetonitrile, all from Sigma, were used in the different MEKC buffers. All the reagents were of analytical purity (p.a.). Buffer solutions and all dilutions were prepared with water purified by a Milli-Q-System (Millipore). Poly(HEMA-*co*-AMPS) samples were analyzed using a 100 mM tetraborate, 80 mM SDS buffer at pH 9. The poly(DMAA-*co*-AMPS) system was analyzed using a 100 mM tetraborate, 100 mM SDS buffer at pH 9 with 5% v/v acetonitrile as organic modifier.

Microcones. A HA50 dialyzed water solution was centrifuged using a 10 000 molecular weight cutoff regenerated cellulose microcone (Microcone-YM10, Millipore Corp.). Filtrate and retentate were collected and processed in the same conditions mentioned above.

Conductivity Analysis. The electric current of the samples collected during the copolymerization of HEMA−AMPS (50:50) was measured under a voltage of 10 kV using the PACE 5500 instrument. Prior to any measurement, the capillary was rinsed with water for 15 min to ensure that all of the copolymer was removed. The capillary was filled with the sample and the voltage was applied, and after 3 min the electric current was registered.

Results and Discussion

Two copolymeric systems have been analyzed by MEKC in this work, namely, the HEMA−AMPS system and the DMAA−AMPS system. Their chemical structures are drawn in Figures 1 and 2. The three components have different hydrophilicities: DMAA is more polar than HEMA. (Although both monomers are hydrosoluble, poly-DMAA is soluble in water while poly-HEMA is not.) On the other hand, AMPS is a strong acid ionized over a wide pH range and is a well-known component of polyelectrolytes. In addition to these dissimilarities in polarity, both systems behave quite differently during copolymerization. The reactivity ratios, which govern the incorporation of the monomers in the growing macromolecular chains in binary copolymerizations (terminal model), are $r_{HEMA} = 6.81$ and $r_{AMPS} = 0.116$ for the HEMA−AMPS system and $r_{DMAA} = 1.50$ and $r_{AMPS} = 0.40$ for DMAA−AMPS, according to a previous publication.[8] The instantaneous copolymer molar fraction for a given feed molar fraction of both copolymerization reactions is shown in Figure 3, based on the reactivity ratios. For instance, the polymerization of an equimolar mixture of monomers (F-feed = 0.5) at low conversion leads to the formation of macromolecular chains with an AMPS molar content of 0.12 and 0.36 for the HEMA−AMPS and DMAA−AMPS, respectively.

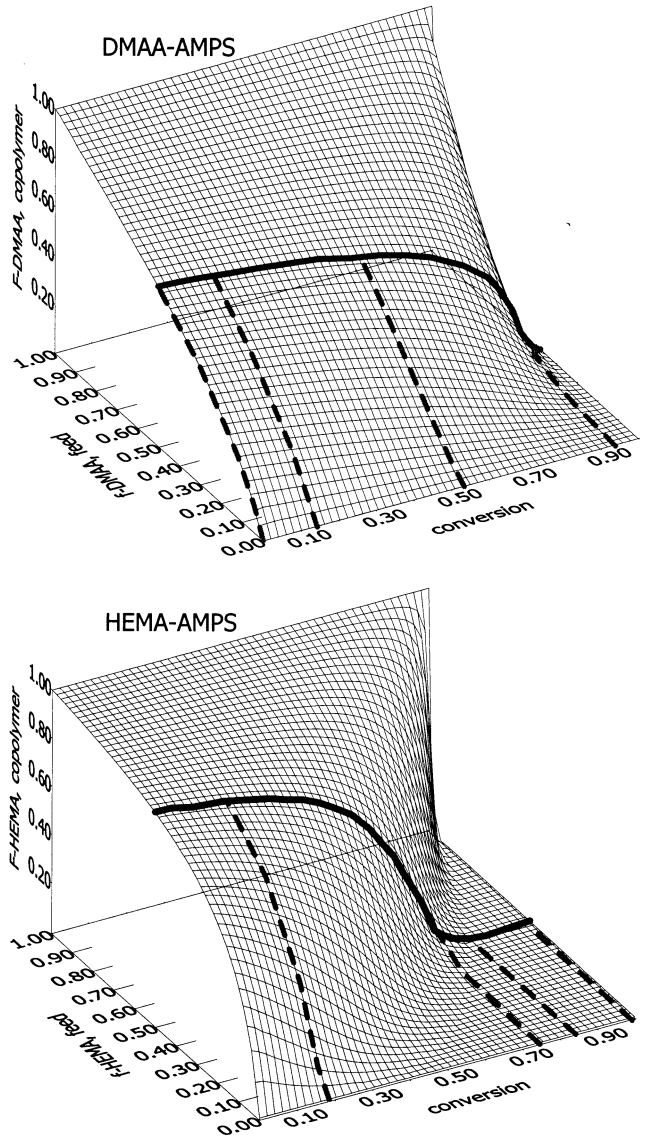

Figure 4. Three-dimensional diagrams for the relative variation of the instantaneous DMAA or HEMA molar fraction in the copolymer as a function of the initial feed molar fraction and the conversion degree. Dotted lines correspond to the electropherograms drawn in Figures 4 and 5 (0 conversion, 0 min; 0.10 conversion, 30 min; 0.50 conversion, 50 min; 0.94 conversion, 240 min for the DMAA−AMPS system; 0.18 conversion, 170 min; 0.74 conversion, 10 h; 0.84 conversion, 13 h; 0.98 conversion, 24 h for the HEMA−AMPS system).

There is a continuous drift on the monomer composition (toward higher AMPS feed molar fraction) because of the lower AMPS reactivity. In this sense, Figure 4 shows three-dimensional pictures of the variation of the instantaneous HEMA or DMAA molar fractions in the copolymer chains as a function of conversion and feed molar fraction.

Although AMPS is the less reactive compound in both systems (see Figures 3 and 4), the monomer incorporation is actually quite different. While HEMA is added with high preference in the HEMA−AMPS system (for an equimolar reaction), DMAA is incorporated just slightly faster for the DMAA−AMPS system. The relevance of this difference can be deduced from the theoretical prediction of Figure 4. Because the differential reactivity between the monomer pair is much higher in the HEMA−AMPS, the HEMA is initially quickly consumed, and as a consequence, two clear

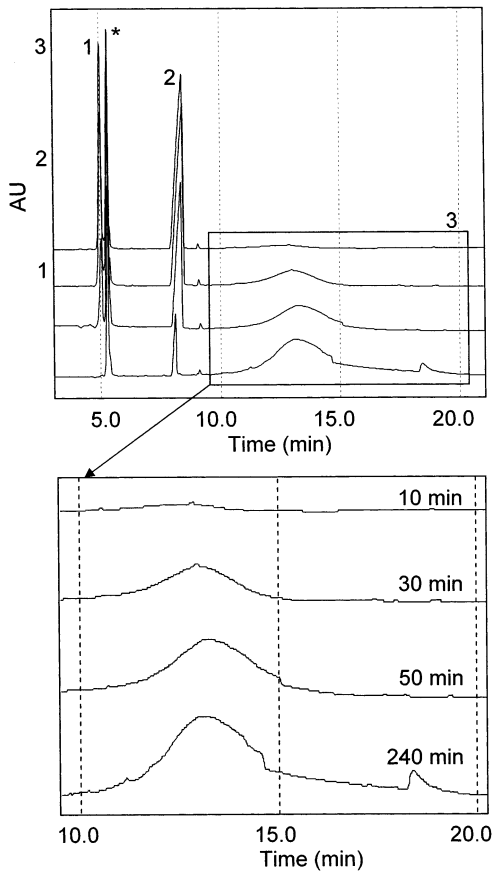

Figure 5. MEKC electropherograms of DA50 copolymers at the different reaction times indicated. Peaks: (1) DMAA monomer, (2) AMPS monomer, (*) hydroquinone, and (3) copolymer region.

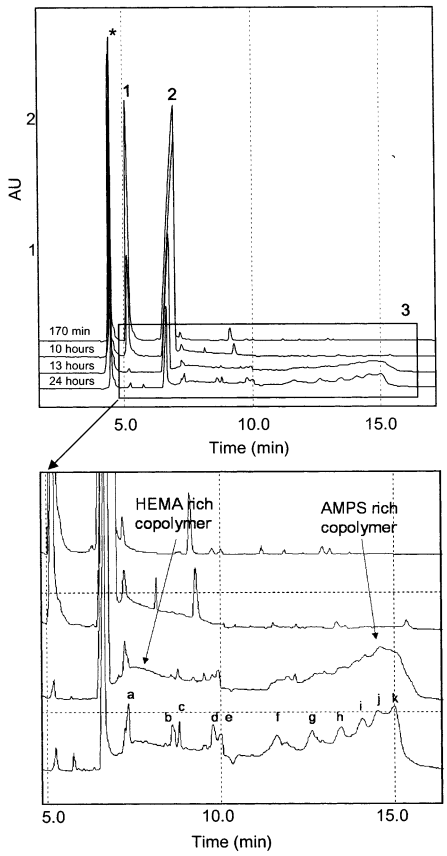

Figure 6. MEKC electropherograms of HA50 copolymers at the different reaction times indicated. Peaks: (1) HEMA monomer, (2) AMPS monomer, (*) hydroquinone, and (3) copolymer region.

regions are defined with a rather sharp transition between them. One corresponds to a region of macromolecules rich in HEMA units, at low conversions, and the second one corresponds to a region of macromolecules rich in AMPS units, at high conversions, with the generation of long chains or blocks of AMPS monomer. The course of the reaction forms few chains with intermediate composition, although when HEMA has been mostly consumed, the residual AMPS monomer begins to form copolymer chains with long AMPS sequences and eventually in the last stages homopolymerizes to a true poly-AMPS, as seen by the lower plateau drawn in Figure 4. However, the DMAA−AMPS system copolymerizes in a more homogeneous way until very high conversions at which there is a sharp variation toward copolymeric chains rich in AMPS (right-hand side of the DMAA−AMPS graphic in Figure 4).

MEKC Analysis of the Copolymerization Reactions. Several experiments have been performed monitoring the evolution of two copolymeric reactions used as reference and with an initial feed molar fraction of 0.5. At appropriate times, aliquots were taken out and analyzed by MEKC. The two MEKC methods used in this work have been developed in our lab by optimizing the running buffers and separation voltages. The electropherograms allow the determination of monomer consumption (and the conversion) by analysis of peaks 1 (monomer DMAA in Figure 5 and monomer HEMA in Figure 6) and 2 (corresponding to AMPS monomer in Figures 5 and 6). The peak with an asterisk in Figures 5 and 6 corresponds to hydroquinone which was

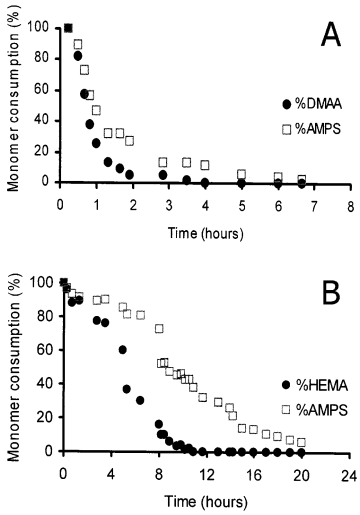

Figure 7. Monomers consumption as a function of the reaction time for (A) DMAA−AMPS system and (B) HEMA−AMPS system.

used simultaneously as a radical quencher and an internal standard. The variation of the monomer peak areas vs time is summarized in Figure 7. Some kinetic estimation can be taken out from these data: the DMAA−AMPS copolymerization proceeds to high conversion in about 6 h (see Figure 7), while the HEMA−AMPS takes approximately 24 h. Although a thorough kinetic study would provide valuable information on the reaction mechanism and/or monomer-radical interactions, is out of the scope of this work.

Next, we investigated the correlation between the formation of electrophoretically different species throughout the course of the reaction and the theoretical prediction of Figure 4. The surfaces in Figure 4 predict a relatively homogeneous copolymerization of DMAA−AMPS and the formation of two species in the case of HEMA−AMPS (HEMA- and AMPS-rich species). The electropherograms of the DMAA−AMPS reaction (see Figure 5) exhibit a single and broad peak during most of the reaction, in good agreement with the prediction of Figure 4. At long reaction time (i.e., 240 min), a small peak seems to appear at high retention times (18 min), which could be adscribed to a higher negative electrical charge that would correspond to the formation of AMPS-rich copolymer in the very last steps of the reaction, also in good agreement with the monomer consumption data of Figure 7.

In the case of the HEMA−AMPS copolymerization (see Figure 6), the macromolecular chains formed in the first steps of the reaction are mainly formed by HEMA, showing a low analysis time (i.e., minor anionic charge), and correspond to the upper plateau (HEMA-rich copolymer) in Figure 4. The peaks formed after the consumption of most of the HEMA monomer are mainly constituted of AMPS units (lower plateau of Figure 4) and have the higher analysis time (i.e., higher anionic charge). This also very well agrees with the monomer consumption data of Figure 7.

Thus, in a simplified way, copolymerizations of DMAA−AMPS (Figure 5) and HEMA−AMPS (Figure 6) at the beginning of the reaction produce copolymers rich in the neutral, most reactive monomer. For instance, the electropherogram of Figure 5 obtained at 30 min shows a broad peak that migrates closer to the poly-DMAA homopolymer the higher the DMAA feed ratio (see below). At longer reaction times (e.g., 240 min) a second peak appears that migrates close to the poly-AMPS homopolymer. The multiple peaks obtained in Figure 6 (peaks a−k) at longer reaction times (24 h) correspond to copolymers rich in AMPS or even poly-AMPS.

In a previous work,[9] we demonstrated the usefulness of CE instrumentation to measure critical micelle concentrations of ionic surfactants via the determination of conductivity variation with the concentration of detergent, using a minimum sample consumption in a fast and clean way. Following this idea, the determination of the conductivity of the reaction medium for the more heterogeneous system, HEMA−AMPS, should provide useful information on the polymerization mechanism of this ionic copolymer. Results from this experiment are shown in Figure 8. The initial decrease of the current intensity is related to the formation of HEMA-rich copolymers that incorporates AMPS molecules (phase I). This phase is related to a change in the electric conductivity σ due to the fact that AMPS has a very different value of σ in the monomeric form compared to the AMPS linked to a HEMA-rich copolymer (i.e., "parachute effect"[9]). In phase II, the current intensity is almost constant because of the formation of AMPS-rich copolymers. The inflection point correspond to the "cliff" of Figure 4 and to the consumption of the major part of HEMA in Figure 7. Therefore, this experiment supports the heterogeneous polymerization mechanism predicted in Figure 4.

Composition and Molecular Weight. The molecular weight also affects the separation of ionic species

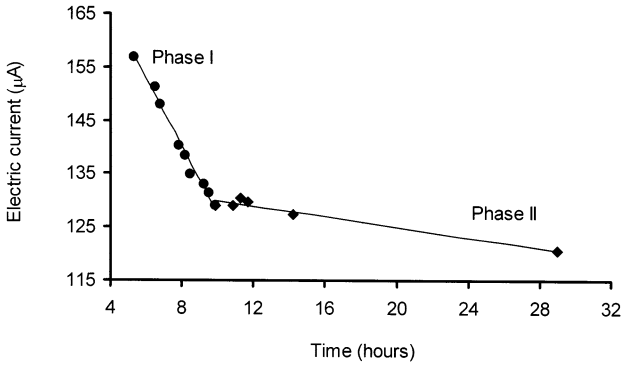

Figure 8. Electrical current as a function of the reaction time during the copolymerization of HEMA−AMPS (50:50). Voltage applied: 10 kV.

by MEKC, since ionic compounds are separated on the basis of their different charge/molecular weight ratio and hydrophobicity. For highly and uniformly charged polymers, it is well-known that low molecular weight chains can be electrophoretically separated using free solution capillary electrophoresis, until a chain length is reached (a few thousand daltons),[10,11] above which the charge/molecular weight ratio becomes constant. In the case of nonionic species and MEKC, the chain length can influence the separation because the hydrophobicity (and as a consequence, the electrophoretic mobility of analytes) changes with the molecular weight.[5]

The molecular weight range of these compounds was estimated by dialysis using a membrane with a nominal cutoff of 3500 Da. ^{1}H NMR analysis (before and after dialysis) demonstrated the presence of low molecular weight fractions in both cases. Table 1 shows the enrichment of the copolymers in HEMA or DMAA after the dialysis, which is consistent with a loss of AMPS-rich chains. This behavior is much more relevant for the HEMA−AMPS (see Table 1), which loses AMPS in most of the composition range while the DMAA−AMPS system has a significant variation at high AMPS initial content.

To understand the formation of low molecular weight species, a high conversion HA50 sample was dialyzed (using a membrane with a cutoff of 3500 Da) and further centrifuged through a 10 000 molecular weight cutoff microcone. Figure 9 shows the electropherograms of the untreated HA50 sample and the three fractions obtained from the previous treatment (<3500, > 3500 < 10 000, and >10 000). Dialysis of the original sample HA50 (Figure 9A) using a 3500 molecular weight cutoff membrane removes the peaks related to the monomers (peaks at migration times lower than 8 min, see Figure 9B). This dialyzed sample was centrifuged through a 10 000 molecular weight cutoff microcone. The filtrate with 10 000 > molecular weight > 3500 was analyzed by MECK, giving rise to the electropherogram shown in Figure 9C. This fraction is rich in AMPS as can be deduced from the longer migration time of the detected peaks. The electropherogram of the retentate with molecular weight >10 000 is shown in Figure 9D, with a broad peak (corresponding to copolymers rich in HEMA). Figure 9 shows that the dialysis (3500 molecular weight cutoff) does not change the electropherograms significantly; however, the centrifugation through the microcone (10 000 molecular weight cutoff) separates two very different fractions. Moreover, higher AMPS contents seem to be related to lower molecular

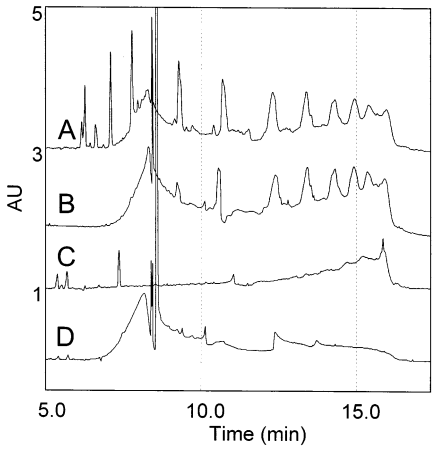

Figure 9. MEKC electropherograms of copolymer samples of the reaction HA50 (A) untreated, (B) dialyzed using 3500 Da cutoff membranes (>3500), (C) the filtrate (> 3500 < 10 000), and (D) retentate (>10 000) obtained after centrifuging with microcones.

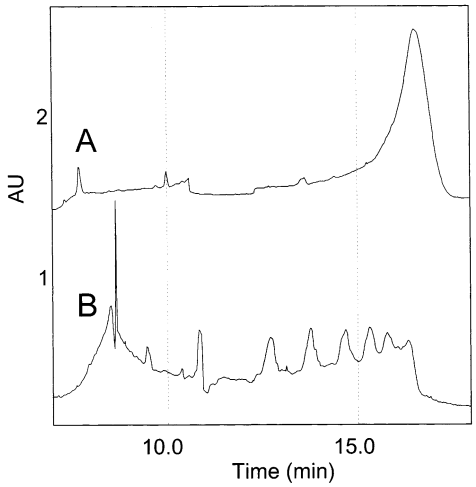

Figure 10. MEKC electropherograms of copolymer samples of the reaction HA50 obtained in (A) methanol/water (1:1) and (B) 2-propanol/water (1:1).

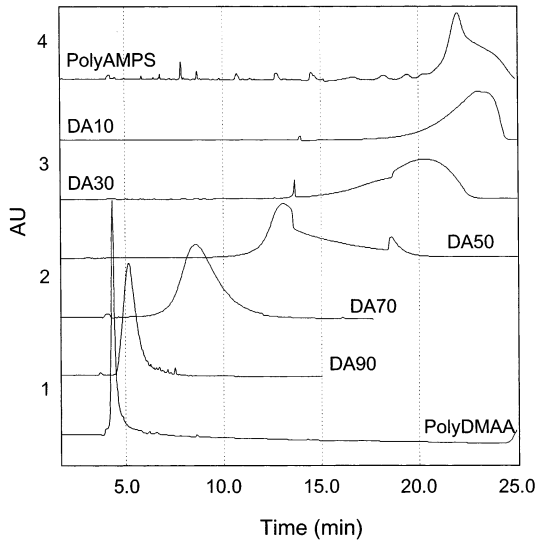

Figure 11. MEKC electropherograms of high conversion copolymer samples at various initial DMAA feed molar percentages (0, 10, 30, 50, 70, 90, and 100) for the DMAA−AMPS system or DA.

weights, whereas higher HEMA contents correspond to copolymers of larger molecular weight.

The formation of short macromolecular chains at high AMPS content can be related to the use of 2-propanol as solvent and to the low reactivity of AMPS in radical polymerization. 2-Propanol was chosen as the cosolvent because of the biomedical interest of the copolymers, which may be clearable by the kidney. To achieve a clearable polymer, the molecular weight must be less than the glomerular limit, which is around 30 000−40 000 Da.[12] This can be achieved using a solvent like 2-propanol with a high chain transfer constant (also has low toxicity). However, the reactivity of the AMPS monomer in radical copolymerization is low. In addition to its acrylamide nature, it has a bulky and ionized side group that polymerizes poorly in polar media because of electrostatic repulsions. The chain transfer to 2-propanol should increase with increasing amount of AMPS-ended radicals and with conversion because of the higher diffusion restriction of such a charged species in polar media. This increasing in the chain transfer due to a higher amount of AMPS-ended radicals occurs in the last stages of the reaction on the HEMA−AMPS (the bottom part of the "cliff" of Figure 4) or when the initial AMPS molar fraction is high in the DMAA−AMPS system.

To confirm the influence of the medium, an additional copolymerization of HEMA and AMPS was performed, under the same conditions as described except methanol was used instead of 2-propanol. (Methanol has a chain transfer constant that is about 2 orders of magnitude lower than that of 2-propanol.) The higher chain transfer in 2-propanol compared to that in methanol is well-known in polymer chemistry, and it is related to the presence of a tertiary hydrogen in the 2-propanol molecule. In this sense, we report, as comparative data taken from the *Polymer Handbook*,[13] transfer constant values for the free radical polymerization of structurally related monomers as methyl methacrylate (a methacrylic ester like HEMA) or acrylamide (AMPS is an acrylamide derivative), the transfer constant (C_s) values being $C_s \times 10^4$ for the polymerization of methyl methacrylate in 2-propanol, 1.91 (80 °C), and $C_s \times 10^4$ in methanol, 0.33 (80 °C). The reference $C_s \times 10^4$ values for the polymerization of acrylamide are 0.13 in methanol (30 °C) and 19 in 2-propanol (50 °C, we did not found data at the same temperature). Figure 10 shows the electropherograms of both copolymers. The electropherogram of the reaction carried out in methanol does not exhibit a splitting in several peaks of the AMPS-rich fraction, and the analysis time of this fraction is shifted to the highest times. Both facts agree well with a higher molecular weight and, therefore, a constant mobility value as described above.[10,11] Dialysis using membranes with cutoff of 3500 or 10 000 did not extract any low molecular weight material. This experiment corroborates that the splitting in several peaks of the AMPS-rich fraction is related to the formation of low molecular weight species.

The effects of composition and high conversion were also studied. Copolymerizations over the entire range of feed compositions were allowed to react until high conversion and then dialyzed (using a 3500 cutoff membrane) to remove the unreacted monomer and oligomers. Figures 11 and 12 show the electropherograms for DMAA−AMPS and HEMA−AMPS systems, respectively.

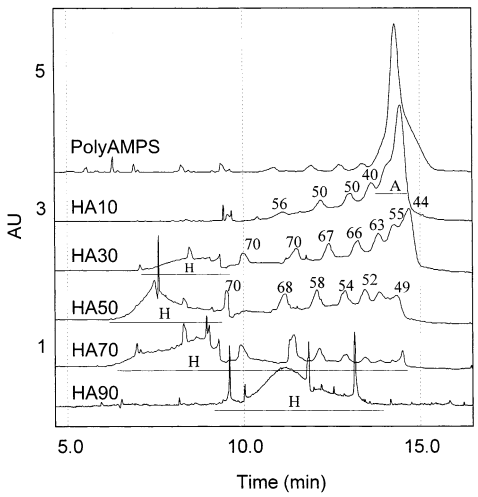

Figure 12. MEKC electropherograms of high conversion copolymer samples at various initial HEMA feed molar percentages (0, 10, 30, 50, 70, and 90) for the HEMA−AMPS system or HA. Peaks: (H) HEMA-rich copolymer; (A) AMPS-rich copolymer; the numbers assigned to some peaks are estimations of the composition obtained as described in the text.

DMAA−AMPS copolymerization (Figure 11) produces a homogeneous and random sequence distribution and a single broad peak whose analysis time shifts depending on the average composition. (Homopolymers poly-AMPS and poly-DMAA were used as reference.) The higher the AMPS content, the higher the average negative charge of the macromolecular species and the higher the analysis time (of the broad peak detected as a single peak, except the aforementioned small peak of DA50. This figure shows the usefulness of MEKC to distinguish between relatively homogeneous copolymers that are compositionally different.

However, the influence of the composition (that is, the increasing in analysis time with the ionic comonomer molar fraction) is limited by the "Manning theory" or counterion condensation,[14,15] which predicts a limiting charge density above which any increase in the number of charged species does not affect the mobility of the macromolecules (i.e., all have a constant mobility value). Figure 11 shows this behavior for the DMAA−AMPS system, where the more ionic copolymers (DA30 and DA10) migrate at times similar to the homopolymer poly-AMPS.

The HEMA−AMPS reaction, however, gives electropherograms with more complex patterns (see Figure 12) that also change with composition. In this case, the concentration of certain species increases whereas others decrease (and not just a shift of a single peak as in the DMAA−AMPS); that is, it seems that there is a change in the balance of concentrations of the different species. The higher the AMPS content, the higher the weight of the rich AMPS peaks in the electropherograms (those migrating at high analysis time). Poly-AMPS homopolymer was used as a reference (poly-HEMA is not hydrosoluble). In this case, there are several electrophoretically different species. MEKC can separate the various heterogeneous species formed during the reaction and monitor their evolution as a function of the initial feed composition.

Because of the different UV extinction coefficient (ϵ) of the components (ϵ of AMPS at $\lambda = 200$ nm is approximately twice higher that of DMAA and HEMA), the peak area of the AMPS-based fractions is higher

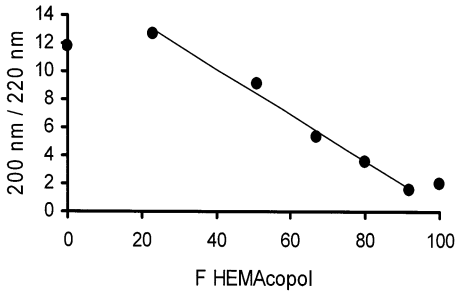

Figure 13. UV calibration curve using the 200 nm/220 nm ratio together with different HEMA−AMPS copolymers of known composition.

than the others. Moreover, the different UV spectra of the homopolymers poly-HEMA and poly-AMPS could be used to estimate the composition of each peak in Figure 12. To do this, the absorbance measures at 200 and 220 nm were employed for building the calibration plot of Figure 12, using real copolymers and homopolymers. From this, the chemical compositions of the different peaks detected in Figure 12 were estimated. This calculation is an approximate, because the calibration plot was obtained using "real" samples. As can be seen in Figure 13, the compositions close to the homopolymers do not show a linear behavior. Therefore, the calibration plot was used only for the intermediate compositions. Thus, in Figure 12 the calculated HEMA molar percentage of the copolymers is indicated for the different peaks analyzed, changing from 70% to 40%. Peaks indicated as H and A correspond to HEMA- and AMPS-rich copolymers whose compositions were not estimated due to the lack of linearity mentioned above. The molar composition (in AMPS) beyond which a constant electrophoretic mobility is observed is 50−60. This agrees well with the Manning theory.[12] Moreover, similar values (i.e., 50−60) were experimentally obtained by Whitlock and Wheller[4] for the HEMA−AMPS system that they studied using isotachophoresis.

From these approximate compositional data and as a general rule for the studied systems, the higher the AMPS content, the higher the analysis time. The peaks corresponding to the AMPS-rich fraction are mainly low molecular weight chains as shown in Figure 10.

The HEMA-rich fraction (H in Figure 12) does not have a trivial dependence on composition. Despite its slightly lower AMPS content, the H fraction in HA90 migrates at higher analysis time than HA70 and HA50. The ability of MEKC to isolate polymer chains as a function of their hydrophobicity (described previously for the characterization of nonionic copolymers[5]) must be taken into account to explain this behavior. These H fractions become quite hydrophobic (poly-HEMA is not hydrosoluble), and for such hydrophobic copolymers, the interaction with the surfactant becomes important. In this case, the higher the copolymer hydrophobicity, the higher the interaction with SDS, the higher the electrophoretic mobility, and therefore the higher the migration time.

Conclusions

This work demonstrates the large possibilities of MEKC to characterize copolymerizations involving ionic species. MEKC can be used to obtain information about the chemical composition of a copolymer.

References and Notes

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