Micellar Electrokinetic Chromatography Applied to Copolymer Systems with Heterogeneous Distribution

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ABSTRACT: The kinetic analysis and the characterization of copolymer systems at high conversion based on the free radical polymerization of *N*-vinylpyrrolidone, VP, and 2-hydroxyethyl methacrylate, HEMA, is analyzed on the basis of the reactivity ratios of these monomers as obtained by the Tidwell and Mortimer nonlinear method. Characterization of copolymers prepared at different conversion degrees was carried out by using micellar electrokinetic chromatography, MEKC, as well as by size exclusion chromatography, SEC. The experimental results obtained demonstrate a noticeable change of the composition of copolymer chains with the conversion degree and a good agreement between the approximation of the Skeist's kinetic treatment and the experimental data. Both MEKC and SEC provide valuable complementary information with respect to the average composition of copolymer chains and their macromolecular size and size distribution. To our knowledge this is the first time that MEKC has been applied to the characterization of high molecular weight copolymer systems.

Introduction

Copolymerization reactions provide an excellent way for the preparation of macromolecules with specific chemical structures and for the control of properties such as hydrophilic/hydrophobic balances, solubility, polarity, etc. These properties are regulated by the microstructural distribution of the sequences of monomeric units along the copolymer chains, which is controlled mainly by the average composition of the reaction medium and the relative reactivity of the corresponding monomers participating in the copolymerization reactions.[1−4] When monomers with similar reactivity are involved, the reaction medium remains relatively unchanged during all the reaction process, and from a microstructural point of view, homogeneous copolymers in a wide interval of conversions are obtained. However, this is not the case of monomeric systems with very different reactivity, which provides copolymers with average composition and microstructural distribution that could change with the conversion degree noticeably. In a limiting case, the formation of blocks of the monomer with lower reactivity could be expected.[5−7]

This characteristic could profit from the preparation of bioresorbable systems with a time-dependent resorption, controlled by the average distribution of copolymer chains prepared at high conversion from monomers with very different reactivity. This is the case for monomers such as vinylpyrrolidone, VP, and acrylic or methacrylic esters, which in addition present an interesting and demonstrated biocompatibility.[8−10]

Capillary electrophoresis (CE) has been broadly employed in the separation of biopolymers such as peptides, proteins, DNA fragments, and polysaccharides.[11−13] However, the application of this separation technique to the analysis of synthetic polymers has not received much attention. Thus, synthetic polymers such as for example poly(ethylene glycol)s,[14] poly(acrylic acid),[15] polystyrene,[16] polyaniline[17] and latex particles,[18] silica sols,[19] phenylacetylene dendrimers,[20] and poly(oxyalkylene)diamine polymers[21] have been separated by CE. These compounds have been mainly analyzed by using CE in the mode of free solution capillary electrophoresis (FSCE) or by capillary gel electrophoresis (CGE), which are based on their different charge/mass ratio or molecular size, respectively. However, in these two modes of CE, the polymer samples must bear electrical charge to accomplish their separation, what makes mandatory the preparation of derivatives of any neutral macromolecule prior to their analysis by FSCE or CGE in order to provide charge.[22,23] To solve this problem, the mode of CE denominated micellar electrokinetic chromatography (MEKC)[24] can be an adequate strategy because MEKC allows the separation of neutral compounds. In MEKC a surfactant is added to the separation buffer in concentrations usually higher than their critical micellar concentration (cmc), so that the separation of neutral compounds is based on their different partition coefficients between the micelle and the surrounding buffer. Thus, the surfactant works in MEKC as a pseudostationary phase. Despite these good capabilities, the use of MEKC has been scarcely applied to the separation of synthetic polymers.[25] One of the main features of this work is to demonstrate the possibilities of MEKC for the characterization of neutral copolymers of high molecular weight and different microstructural distribution.

Experimental Section

Chemicals. HEMA (purchased from Fluka) was purified according to the literature.[26] VP (supplied by Fluka) was distilled under reduced pressure. AIBN was purified by fractional crystallization from methanol (mp 104 °C).

Sodium dodecyl sulfate (SDS) and methanol were purchased from E. Merck. 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES) was purchased from Sigma Chemical Co. Boric acid was obtained from Aldrich. Other reagents were of extra pure grade and used as purchased.

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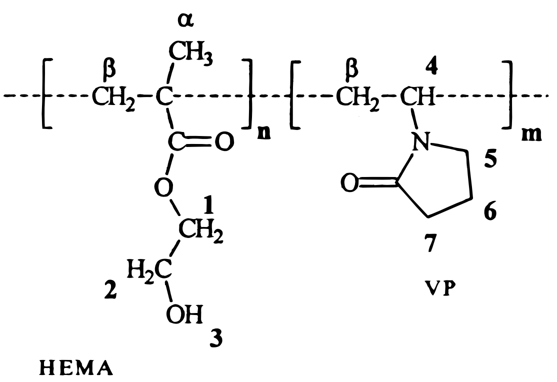

Figure 1. Chemical structure of hydroxyethyl methacrylate (HEMA) and vinylpyrrolidone (VP) units in copolymer chains.

Copolymerization. Copolymerization reactions were performed in water/ethanol solutions at 50 ± 0.1 °C in Pyrex glass ampules. Reactions were carried out in the absence of oxygen by bubbling twice nitrogen for 30 min before the sealing of the system. The monomer and initiator concentrations were 1.0 and 1.5×10^{-2} mol L^{-1}, respectively. The sealed ampules were immersed in a water bath maintained at the polymerization temperature. At appropriate reaction times, the ampules were removed from the bath, and the contents were immediately poured into a large excess of acetone for copolymers rich in VP or into acidic water for copolymers rich in HEMA. Copolymers of intermediate compositions were isolated by freeze-drying. The precipitated samples were washed with the precipitant mixture and dried under vacuum until constant weight was attained.

Polymer Characterization. The copolymers obtained from different mixtures of HEMA and VP were analyzed by ^{1}H NMR spectroscopy with a Varian XL-300 spectrometer working at 300 MHz. The spectra were recorded at 70 °C using 5% (w/v) deuterated dimethyl sulfoxide solutions.

Size Exclusion Chromatography. SEC measurements were performed by using DMF as eluent with a 0.1% (w/v) of LiBr, at 70 °C, and toluene as flow marker. The system consisted of a Perkin-Elmer Isocratic LC pump 250, working at a flow rate of 1 mL/mim. The system was thermostated at 50 °C by an oven (Perkin-Elmer model LC OVEN 101). The detector was a RI detector Waters 410 differential refractometer. Three polystyrene–divinylbencene pl-gel columns of nominal pore sizes 500, 10^4, and 10^5 Å (Polymer Laboratories) were used.

Capillary Electrophoresis. Separations were carried out using a P/ACE 5000 HPCE (Beckman Instruments Inc.) electrophoresis apparatus controlled by a PC computer. A fused silica capillary (Polymicro Technologies Inc.) with 75 μm i.d., 360 μm o.d., 47 cm total length, and 40 cm effective length (from the injection point to the detector) was used. The external temperature of the capillary was maintained at 20 °C. The injection was carried out in the anode using N_2 pressure (0.5 psi) for 1 s. The detection took place at 214 nm. Separation voltage was 12 kV. All the data were collected and analyzed using a System Gold software from Beckman running on a PC computer.

Results and Discussion

It is known that VP presents a relatively low reactivity with methacrylates in free radical polymerization.[27] For instance, the reactivity ratios for the copolymerization of methyl methacrylate (MMA) and VP are $r_{MMA} = 5$ and $r_{VP} = 0.02$.[28] However, the copolymer HEMA–VP is widely used as cross-linked hydrogel for biomedical applications,[29] mainly as a contact lens constitutive material.[30] In this way, there are many references on these cross-linked copolymeric systems, but not too much has been reported on the preparation and characterization of soluble, not cross-linked, material, which has a priori the advantages of their reabsorption in some biomedical applications, offering a wide range of possibilities for their use in the field of controlled release and drug targeting technologies. In this sense, the reactivity ratios of this comonomeric pair in free radical polymerization reactions was reported, to our knowledge, in 1985 by Reddy et al.[31] for the reaction in methanol, obtaining values of $r_{vp} = 0.06$ and $r_{HEMA} = 4.35$, and by Al-Issa et al.[32] for the reaction in ethanol, obtaining $r_{VP} = 0.05$ and $r_{HEMA} = 3.12$, which are in the range of other methacrylic–VP systems. Therefore, these results confirm the poor reactivity of VP in free radical copolymerization with HEMA. Figure 1 shows the schematic representation of HEMA–VP comonomeric units, with the identification of the carbon atoms used in the assignments of Figure 2.

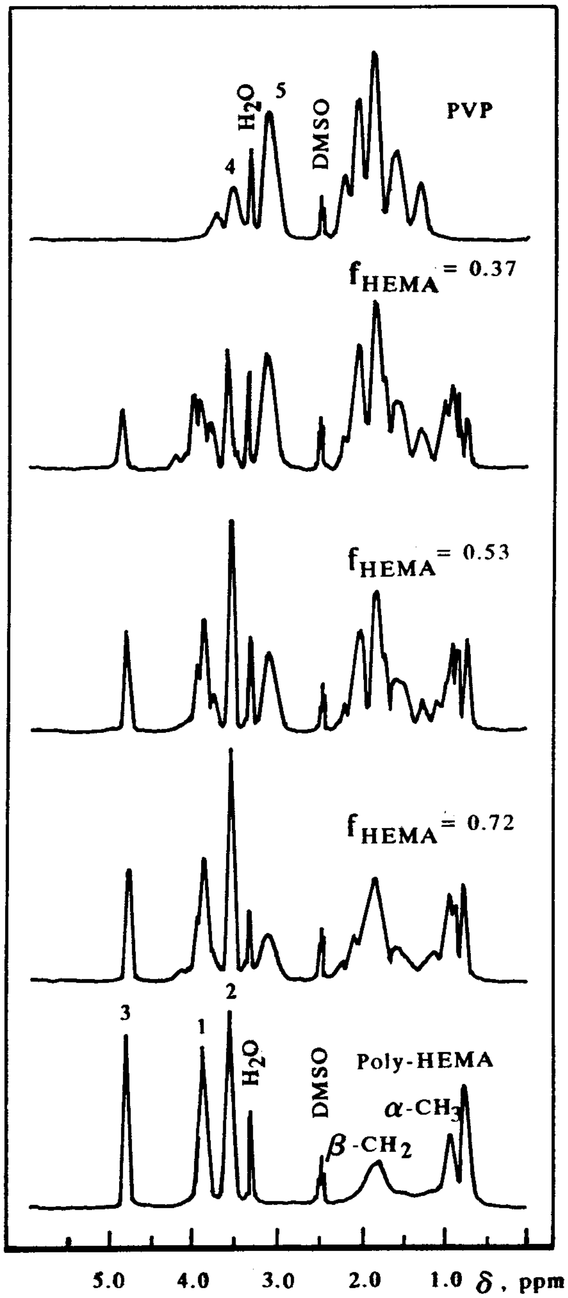

Figure 2. ^{1}H NMR (300 MHz) spectra of homopolymers PVP and poly-HEMA and random copolymers of VP–HEMA prepared at low conversion with different molar fraction of HEMA (f_{HEMA}) in the copolymer chains. Spectra recorded in DMSO at 70 °C.

Table 1. Composition and Conversion Data of the Free Radical Copolymerization of HEMA and VP in Water/Ethanol Solution at 50 °C[a]

reaction time (h)	hv13 ($F_{HEMA} = 0.13$)		hv27 ($F_{HEMA} = 0.27$)		hv46 ($F_{HEMA} = 0.46$)		hv56 ($F_{HEMA} = 0.56$)		hv77 ($F_{HEMA} = 0.77$)	
	f_{HEMA}	X (%)	f_{HEMA}	X (%)	f_{HEMA}	X (%)	f_{HEMA}	X (%)	f_{HEMA}	X (%)
0.5	0.59	2.9			0.87	3.9	0.92	4.1		
1	0.56	8.8	0.77	3.2	0.85[b]	13[b]	0.90	12	0.96	6.0
2	0.39	35	0.70	21			0.88	27	0.95	24
5	0.19	73	0.41	66	0.68	65	0.87	38	0.92	75
24	0.18	78	0.29	92	0.52	93	0.63[b]	88[b]	0.91	79

[a] X = molar conversion, f = copolymer molar composition, and F = feed molar composition. [b] Lyophilized samples.

Table 2. Copolymerization Parameters of the Free Radical Copolymerization of HEMA and VP

method	r_{HEMA}	r_{VP}	$r_{HEMA}r_{VP}$	$1/r_{HEMA}$	$1/r_{VP}$
Finemann−Ross	6.77 ± 0.3	−0.017 ± 0.07	−0.116	0.15	−58.8
Kelen−Tüdös	7.31 ± 1.4	0.046 ± 0.044	0.336	0.14	21.7
Tidwell−Mortimer	7.97	0.077	0.614	0.12	13.0

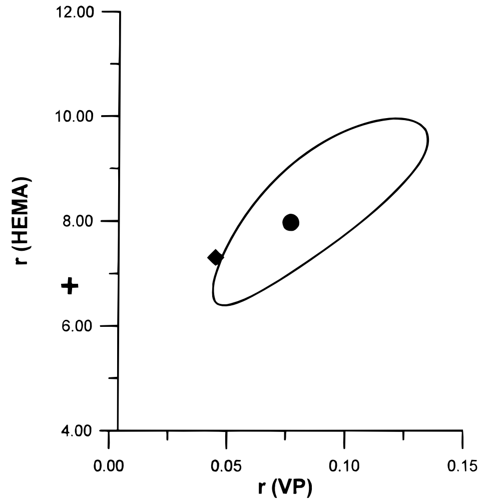

Figure 3. The 95% confidence diagrams for the reactivity ratios of VP and HEMA monomers, determined by the nonlinear least-squares method.[36] Experimental values of the reactivity ratios determined by the method of (+) Finemann−Ross, (◆) Kelen−Tüdos, and (●) Tidwell−Mortimer.

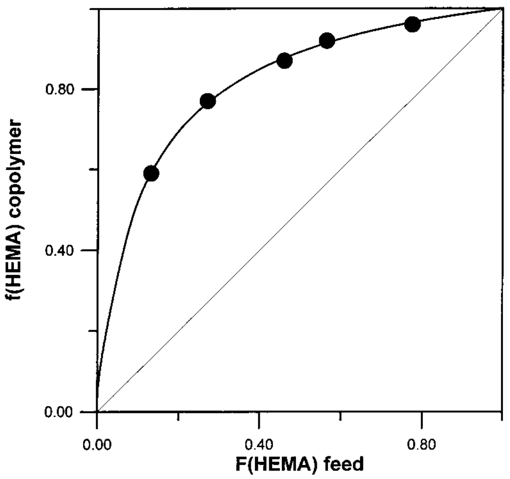

Figure 4. Average composition diagram for the free radical copolymerization of VP and HEMA at low conversion, in the experimental conditions described in the text.

In this work, copolymerizations of five mixtures of HEMA and VP (ranging from 13:87 to 77:23 mol % compositions) were carried out in the presence of AIBN as initiator, using a mixture of water/ethanol (1:1), at 50 °C and to different conversions, as indicated in Table 1. Water/ethanol was chosen as the medium, instead of other usual solvents in radical polymerization such as dioxane or DMF, because this medium is the most suitable for their biocompatibility properties. Homopolymers taken as reference were also synthesized at the same conditions. The resulting copolymer samples were characterized by ^{1}H NMR, SEC, and MEKC. Copolymers were isolated by precipitation in acetone or acidic water (depending of their composition) or by freeze-drying (those intermediate samples with precipitation problems).

Reactivity Ratios Determination. To calculate the reactivity ratios of this comonomeric pair in this reaction conditions, samples at conversions <5 wt % were taken to satisfy the differential copolymerization equation.[33] The molar fractions of monomer units incorporated in the copolymer were determined from the ^{1}H NMR spectra. Figure 2 shows the spectra of the homopolymers poly-HEMA and poly-VP together with the spectra of some copolymer samples. The analysis was performed by comparing the integrated intensities of the assigned resonance signals shown in Figure 2 for both comonomeric units. The obtained copolymer molar fractions are quoted in Table 1.

The reactivity ratios were determined according to the general copolymer composition equation by applying the Finemann−Ross[34] and Kelen−Tüdös[35] linearization methods and the Tidwell and Mortimer nonlinear least-squares analysis.[36] The results are shown in Table 2. We should stress here that the r values determined by the application of the analysis suggested by Tidwell and Mortimer are the most probable values for this system because of the well-known higher accuracy of this nonlinear method. Besides, the Finemann−Ross method gave a negative r_{VP} which has to be considered as an artifact unrelated to the underlying basic physical facts of the linear method.[27] In this sense, Figure 3 shows the 95% confidence limits of the Tidwell−Mortimer values, defined by the area of the elliptical diagram obtained by the treatment suggested by Bechnken[37] and Tidwell and Mortimer.[36] Dots of the reactivity ratios values obtained by the three methods are also drawn.

These values of reactivity ratios are close to those described by Reddy et al.,[31] and they are in the range of other copolymeric pairs methacrylates−VP (i.e., furfuryl methacrylate−VP,[38] $r_{FM} = 3.92$ and $r_{VP} = 0.004$; methyl methacrylate−VP,[28] $r_{MMA} = 5$ and $r_{VP} = 0.02$; glycidil methacrylate−VP,[39] $r_{GLI} = 3.8$ and $r_{VP} = 0.03$; phenyl methacrylate−VP,[40] $r_{PHM} = 4.49$ and $r_{VP} = 0.05$). For these systems an essential random copolymer rich in HEMA units can be expected at relatively low conversion, but at high conversion most of the residual

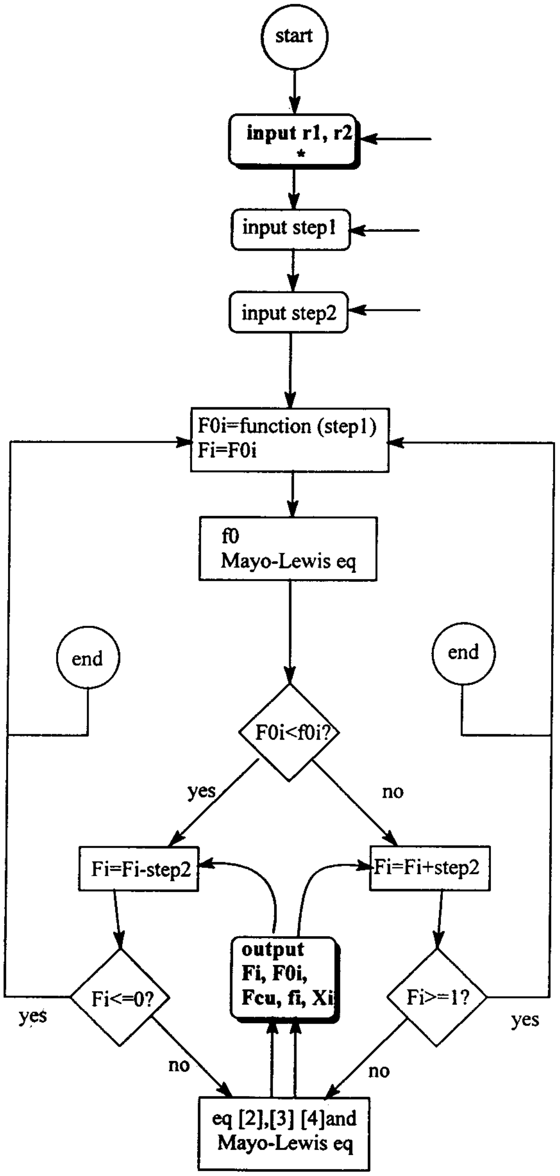

Figure 5. Schematic description of the algorithm applied for the integration of Skeist's equation and generation of the theoretical diagrams represented in Figures 6, 7, and 8, with *F* being the feed molar composition, *F*0 the initial feed molar composition, *f* the copolymer molar composition, *f*0 the initial copolymer molar composition, *X* the molar conversion, and *F*cu the cumulative molar composition in copolymer. (* nonvalid for azeotropic systems).

VP monomer molecules polymerize, mainly in the last steps of the reaction, giving rise to copolymer chains with relatively long blocks of VP units and a distribution of sequences that depends on the average composition of the reaction medium. The average composition diagram shown in Figure 4 has been drawn with the Tidwell–Mortimer r_{HEMA} and r_{VP} values using the Lewis–Mayo classical copolymerization equation. In addition, the experimental composition data obtained at low conversion (Table 1) which fit the theoretical diagram adequately are also depicted.

Kinetic Analysis. The kinetic analysis of the variation of the composition and microstructural distribution with conversion is quite interesting for this system because of the great differences in reactivity of the comonomeric pair VP and HEMA, which leads to high variations in the instantaneous feed and copolymer composition. A detailed kinetic analysis has to be carried out for explaining some characteristics and the experimental behavior of these systems. Moreover, from a practical point of view, copolymers have to be obtained at conversions as high as possible, and hence the kinetic study will also be very helpful.

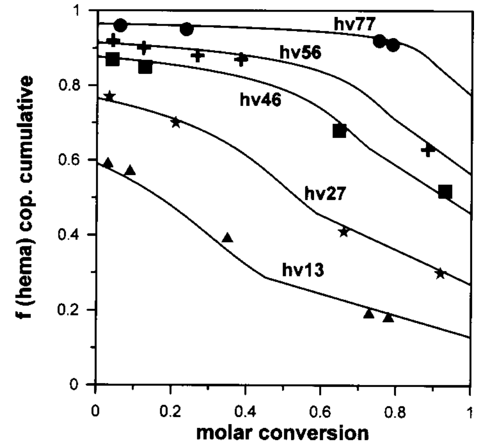

Figure 6. Variation of the cumulative composition of copolymer chains with the molar conversion degree. Lines correspond to the expected variation determined by means of the algorithm described in Figure 5. Points correspond to experimental data after the isolation and characterization of copolymer samples prepared at different conversion.

To compare the experimental data with the prediction obtained by the classical kinetic scheme of the copolymerization process, we have considered the treatment initially suggested by Skeist,[41] where the progress of the reaction is represented for the equation

$$\ln\left(\frac{M}{M_0}\right) = \int_{F_1^0}^{F_1} \frac{dF_1}{f_1 - F_1} \tag{1}$$

where M and M_0 represent the total numbers of moles present in the monomer feed at a given time t and at time 0, respectively. Meyer and Lowry[42] have shown that Skeist's equation can be readily integrated providing that f_1, the instantaneous copolymer composition, is expressed in terms of F_1, the corresponding instantaneous monomer composition, and the monomer reactivity ratios by means of the Mayo–Lewis[33] instantaneous copolymer composition equation. For $r_1 \neq 1$ and $r_2 \neq 1$ it is obtained

$$\frac{M}{M_0} = \left(\frac{F_1}{F_1^0}\right)^{\alpha}\left(\frac{F_2}{F_2^0}\right)^{\beta}\left(\frac{F_1^0 - \delta}{F_1 - \delta}\right)^{\gamma} \tag{2}$$

where

$$\alpha = \frac{r_2}{1 - r_2} \qquad \beta = \frac{r_1}{1 - r_1}$$

$$\gamma = \frac{1 - r_1 r_2}{(1 - r_1)(1 - r_2)} \qquad \delta = \frac{1 - r_2}{2 - r_1 - r_2}$$

From a simple mass balance consideration, it has been shown[43] that the molar fraction of monomer 1 in the feed at a given time of reaction, f_1, is relative to the cumulative average mole composition of the copolymer F_1 (cumulative) by means of the equation

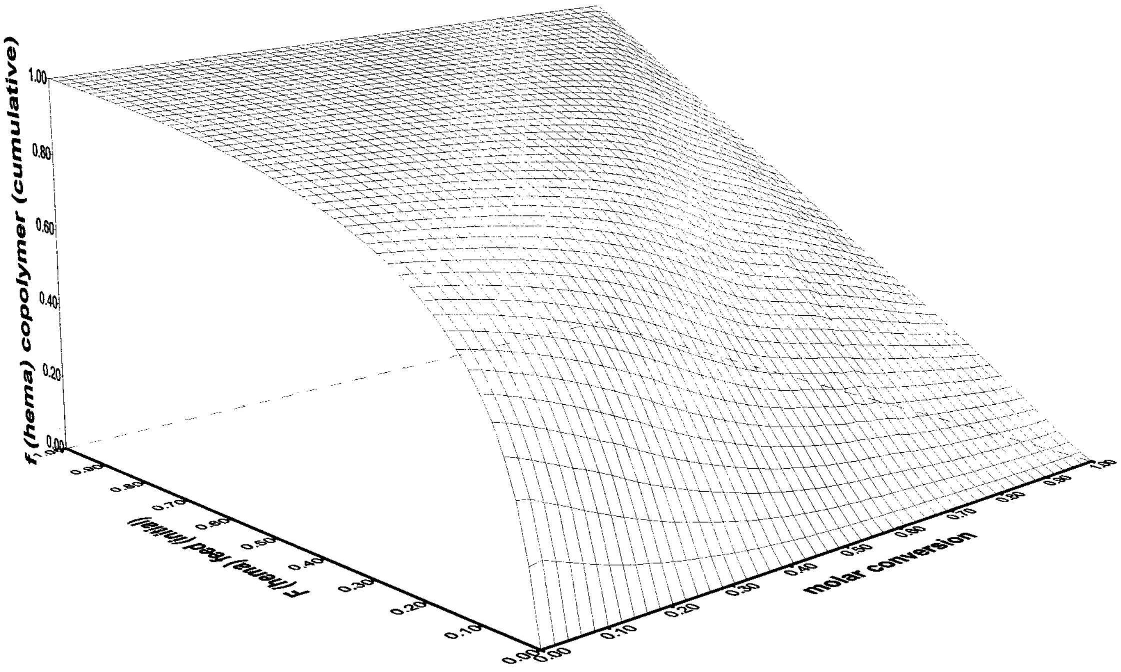

Figure 7. Tridimensional diagram for the relative variation of the HEMA molar fraction in the monomer feed *F*(HEMA) and the copolymer samples *f*(HEMA) with the conversion degree.

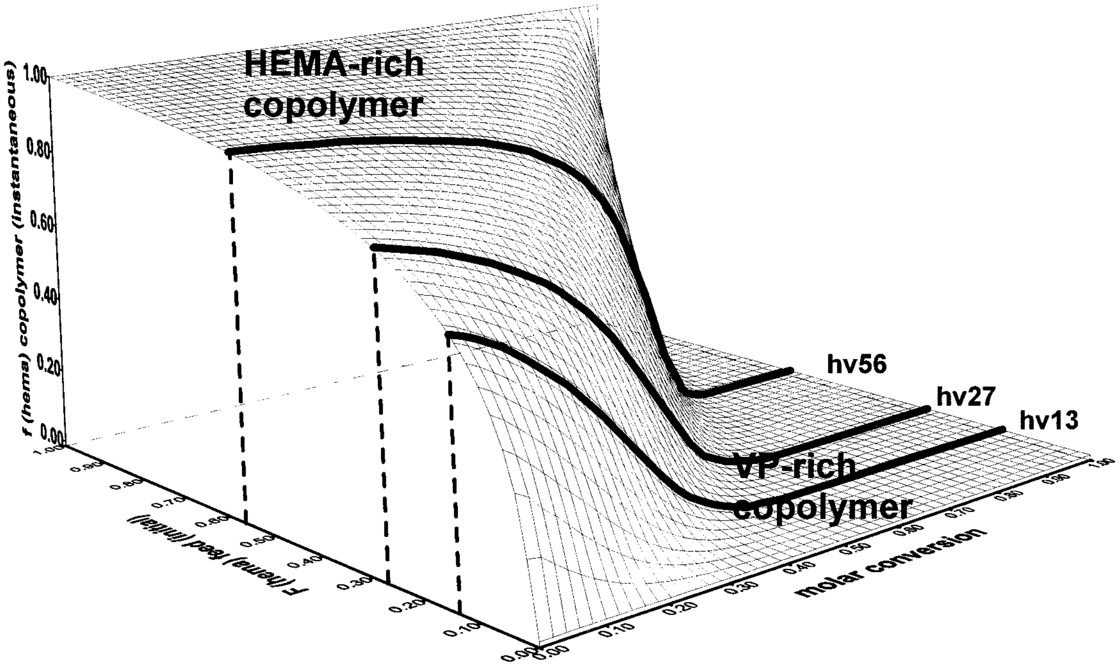

Figure 8. Tridimensional diagram showing the variation of the composition of particular copolymer formulations with the conversion degree.

$$f_1 = \frac{f_1^0 - XF_1(\text{cumulative})}{1 - X} \tag{3}$$

where X is the mole degree of conversion defined as

$$X = 1 - \frac{M}{M_0} \tag{4}$$

Equations 2−4 have been used to generate theoretical points of the different variables as a function of the conversion in order to predict the course of the reaction, by means of a computer program tailored by the algorithm shown in Figure 5. For such a copolymer system like HEMA−VP with the composition curve shown in Figure 4, the HEMA monomer, as it is more reactive, is consumed faster and, therefore, instantaneous F(HEMA) in the feed decreases with conversion until zero, while the f(HEMA) cumulative in copolymer decreases with conversion until a limit value for 100% conversion that corresponds to the initial feed value. The computer program, for different initial feed compositions (defined by step 1), takes successive instantaneous F values in a decreasing order until zero (with the desired decreasing factor, indicated as step 2), calculating for every input the conversion (X), the instantaneous molar fraction in copolymer, f_i, and the f(HEMA) cumulative in copolymer, as well as other variables if necessary.

Figure 6 shows the theoretical curve of predicted cumulative *f*(HEMA) as a function of the conversion for different experimental reactions, together with the experimental data (analyzed by means of ^{1}H NMR spectroscopy). The experimental data fit adequately the theoretical diagrams; this fact confirms the applied kinetic treatment.

Figures 7 and 8 show tridimensional pictures of the variation of the cumulative and instantaneous HEMA molar fractions in the copolymer chains as a function of conversion and feed molar fraction. These figures are quite illustrative and will help us to clarify the bimodal characteristics of this copolymer system observed by SEC and MEKC and their behavior in reabsorption in aqueous media. The *f* cumulative shows (Figure 7), as was described previously, a decrease in HEMA with conversion and a clear dependence with the feed ratio as was expected. However, this global analysis has to be considered as apparent because it does not show the true characteristics of the polymer which can be clearly seen in Figure 8 where the instantaneous copolymer composition is represented. From this figure we can consider that although a continuous gradient of the composition of copolymer system with the conversion degree could be expected, two clear regions are well defined with a more or less sharp transition between them. One corresponds to systems rich in HEMA units at low conversions and the second one to systems rich in VP units at high conversions with a clear generation of long chains or blocks of the VP monomer. The course of the reaction does not form practically chains with intermediate composition, but since HEMA is relatively consumed, the residual VP monomer begins to form copolymer chains with long VP sequences and eventually homopolymerizes giving a true PVP, mainly in the last steps of the reaction as can be seen in the lower plateau drawn in Figure 8.

SEC and MEKC Analysis. Separation of the neutral polymers by MEKC was optimized by modifying the buffer composition in terms of its nature, pH, and concentration of surfactant. Also, the separation voltage was optimized. The nature of the separation buffer had to be optimized because the solutes precipitated in aqueous buffers with or without high contents of surfactant. The results of this preliminary study demonstrated the necessity to employ a hydroorganic solution (i.e., 50% methanol–50% water) in order to dissolve the polymeric samples. It was also observed that by using a hydroorganic buffer without SDS no separation of the different polymers was accomplished, migrating the solutes together with the electroosmotic flow. However, when SDS at concentrations of 25 mM or higher were employed, the polymers could be separated. This result seems to support the hydrophobic mechanism behind the separation of this type of solute. The pH of the aqueous buffer was modified from 8 to 10.5, mixed with methanol (1:1), and after adding SDS the separation of the solutes was carried out at different running voltages, from 10 to 15 kV. The optimum conditions consisted of using a 1:1 methanol:50 mM boric acid/sodium tetraborate at pH 9.5 buffer plus 35 mM SDS at 12 kV. Under these conditions the repeatability of migration time and peak area was measured obtaining % RSD$_{n=5}$ values equal to 0.48% and 4.90%, respectively.

Copolymers were characterized by SEC and MEKC. Both chromatographic techniques are based on a different separation mechanism: while in SEC, a common technique for the characterization of macromolecules, different polymers chains are isolated as a function of their size (that is, for polymers structurally identical, the separation is a function of the molecular weight while the retention of chemically different polymers depends also of their coiling), in MEKC neutral macromolecules are separated as a function of their hydrophobicity. SEC and MEKC chromatograms of copolymer samples from the hv56, hv27, and hv13 reactions (see Table 1) are shown in Figures 9 and 10 and 11, respectively. The copolymer systems show a bimodal behavior in both SEC and MEKC chromatograms. However, MEKC resolves the copolymer in two well-separated peaks, which demonstrates the existence of, at least, two main species well differentiated in the copolymer.

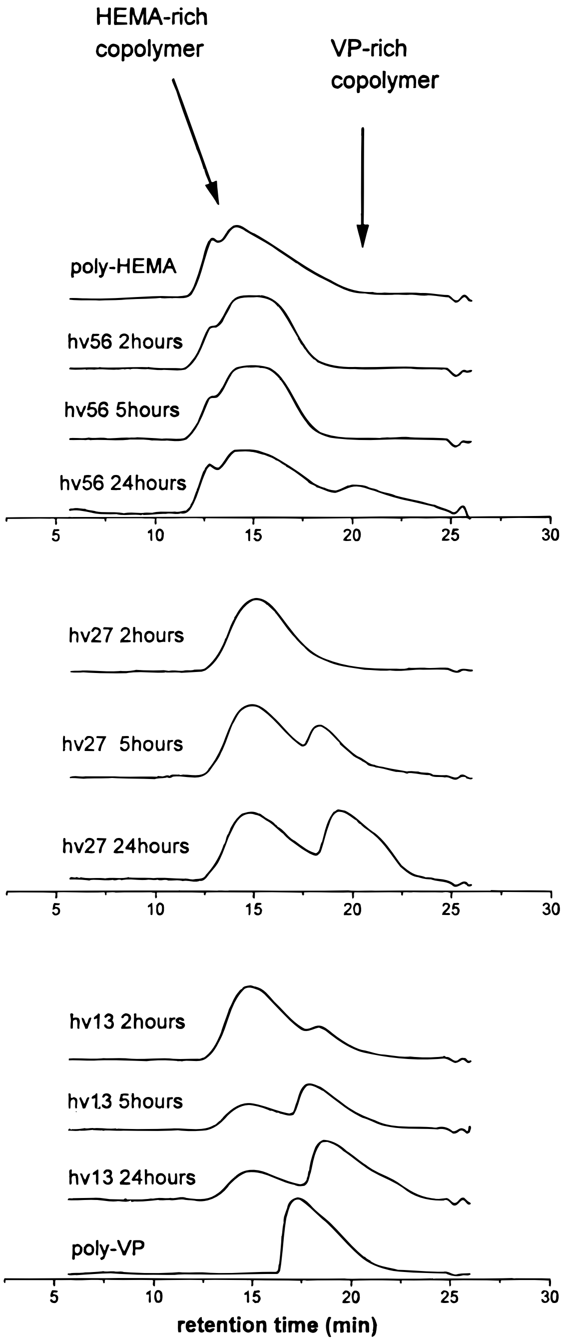

Figure 9. SEC chromatograms of copolymer samples prepared with various feed compositions at different reaction time.

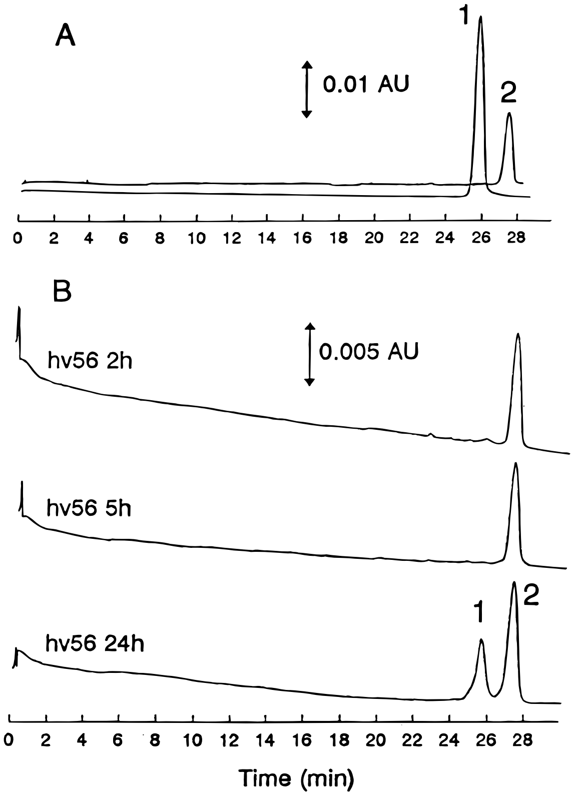

Figure 10. MEKC electropherograms of (A) homopolymer standards, PVP (1) and poly-HEMA (2), and (B) copolymer samples of the reaction hv56 at different reaction time.

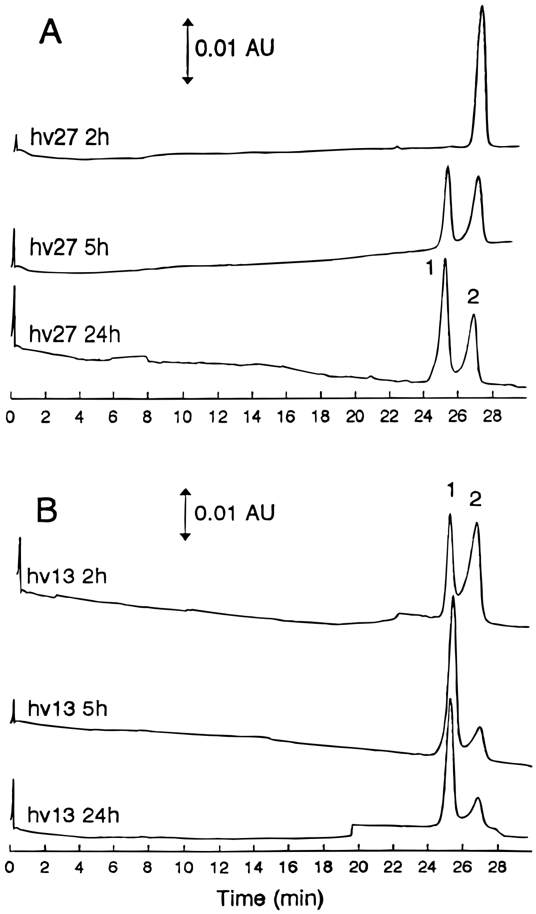

Figure 11. MEKC electropherograms of (A) copolymer samples of the formulation hv27 and (B) hv13 at different reaction time.

From the analysis of SEC chromatograms, no conclusion could be taken out rigorously, because of the multiparametric character of this technique: the variation in retention time depends not only on the molecular weight of the macromolecular chains but also on the chemical composition of the copolymers, the latter controlling in some extent the molecular weight, the coiling of the chains, and therefore the hydrodynamic volume of the macromolecular chains. Moreover, the high polydispersity brings about a large broadening of the peaks in SEC, making even more difficult the analysis as was reported by Reddy et al.[31] However, from the bimodal character of the curves and taking the chromatograms of the homopolymers as reference peaks (although the homopolymerization reaction could give polymers slightly different in macromolecular size), they could be tentatively assigned to copolymeric species rich in HEMA and VP as has been drawn in Figure 9. In general, all the samples analyzed present average molecular weight in the range $M_n = 50\,000$ to $M_n = 200\,000$ with polydispersities ranging from 2.5 to 4.5 depending on the composition of the feed and the conversion reached.

MEKC, however, gives an unambiguous analysis of the copolymeric reaction, because in this case the relative hydrophobic character of the species is the main factor implied in the retention mechanism. Moreover, it seems that polydispersity has a lower effect on peak broadening in MEKC than in SEC, which explains why MEKC gives better resolved diagrams compared to those obtained by SEC. From the MEKC analysis it is possible to conclude what could be guessed from the SEC analysis: the existence of two well-defined species in the copolymeric reaction which correspond to the two well-resolved peaks observed in MEKC. Moreover, if we compare these results with the electrophoregrams of the homopolymers (Figure 10A), it can be concluded that the two species are copolymers with a very high content in VP or rich HEMA species. These assumptions are confirmed by the kinetic analysis presented previously. It can be seen in Figure 10 that the hv56 reaction (copolymerization with $F(\text{HEMA}) = 0.56$ in feed, Figure 10B) showed after 2 and 5 h of reaction (which correspond to conversions of 27 and 38 mol %) only one peak which corresponds approximately to the elution time of the standard PHEMA (i.e., copolymer chains rich in HEMA units), while after 24 h (80 mol % of conversion), one peak appears at lower hydrophobicity which corresponds approximately to the PVP reference. This result is in agreement with the kinetic analysis shown in Figure 8 where the expected courses of these three reactions are drawn by thick lines. In this figure, the reaction hv56 goes from copolymer rich in HEMA (until approximately 60 mol % of conversion) to the formation of PVP or a copolymer very rich in VP at high conversion (>65 mol %), which corresponds to the points analyzed by MEKC. We have to point out the different response ratio of PVP and PHEMA in the UV detector in MEKC, because the ϵ_{PVP} (molar extinction coefficient) at 214 nm is 10 times higher than the ϵ_{PHEMA}. Samples from the hv27 copolymerization reaction (corresponding to a $F(\text{HEMA}) = 0.27$ in the feed) taken at 2, 5, and 24 h, which correspond to molar conversions of 21, 66, and 92 mol %, respectively, lead to the electropherograms shown in the Figure 11A. PVP or VP-rich copolymer appears in this case at lower conversions than in the case of the hv56 reaction. At high conversion, there is an increase in the relative amount of VP derivative, which is in good agreement with the results drawn in Figure 8. This tendency is raised in the case of the hv13 reaction (with a F(HEMA) = 0.13 in the feed) as expected from Figure 8. In this case VP rich polymeric derivatives appear at lower conversions (the chromatograms of Figure 11B correspond to samples of 35, 73, and 79 mol % conversion), and for similar conversions, the amounts of VP-rich copolymers are higher.

In conclusion, MEKC has shown to be very useful in the study of neutral copolymer systems, permitting the elucidation of the different species which correspond to different compositions of the copolymer chains, complementary to the SEC technique. Moreover, to our knowledge this is the first time that high molecular weight neutral copolymers have been separated based on their different hydrophobicity by using MEKC.

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