Journal of Chromatography, 368 (1986) 125–134
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 894

ISOTACHOPHORESIS OF SYNTHETIC ION-CONTAINING POLYMERS

SEPARATION OF COPOLYMER MIXTURES OF POLY(2-HYDROXYETHYLMETHACRYLATE-CO-2-ACRYLAMIDO-2-METHYLPROPANESULFONATE)

L. RONALD WHITLOCK* and LOUISE M. WHEELER*
Research Laboratories, Commercial and Information Systems Group, Eastman Kodak Company, Rochester, NY 14650 (U.S.A.)

(Received June 11th, 1986)

SUMMARY

Chemically heterogeneous copolymers prepared from 2-hydroxyethylmethacrylate and 2-acrylamido-2-methylpropanesulfonate are characterized by separation of the copolymer mixture into discrete zones by isotachophoresis. The separations are based on differences in electrophoretic mobilities of individual polymer chains. Polymer mobility is shown to be independent of molecular weight. It is governed primarily by the ratio of ionic to non-ionic repeat units in the chain for mole fractions of 0–0.6 ionic repeat units and by the extent of binding of counterions to the ionic groups for mole fractions of 0.6–1.0 ionic groups. Detection is achieved by sensing the change in potential between migrating zones. Compositions of the polymeric fractions in several copolymer samples are calculated from isotachophoretic zone dimensions and chemical analysis data.

INTRODUCTION

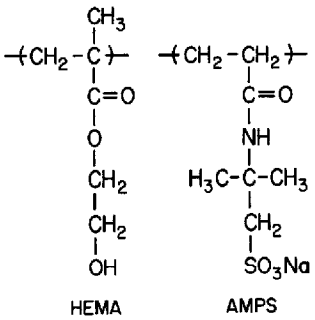
Isotachophoresis (ITP) offers an alternative to chromatographic methods for the separation and chemical characterization of high-molecular-weight synthetic polymers containing an ionizable functional group. Size-exclusion chromatography and, to a lesser extent, reversed-phase and adsorption high-performance liquid chromatography are recognized separation methods for synthetic polymers. Their application to ion-containing, water-soluble polymers can be troublesome because of unpredictable influences of the charge-bearing groups on the separation process. ITP is now a fairly advanced micro-separation method with applications predominately for small, ionic molecules. To a lesser extent ITP has contributed to biological macromolecular separations, including the profiling of protein mixtures[1–3] and the analy-

* Present address: Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, U.S.A.

0021-9673/86/$03.50

sis of enzymes[4,5]. These applications were recently reviewed by Boček[6] and Hjalmarsson and Baldesten[7].

Despite several potential advantages over chromatographic techniques, capillary ITP has not been generally applied to the problem of characterization of ion-containing synthetic polymers. In our previous paper of this series[8], ITP was used to characterize the amount and distribution of carboxymethyl substitution in carboxymethylcellulose. In this report, capillary ITP is applied to the quantitative characterization of chemically heterogeneous copolymers of 2-hydroxyethylmethacrylate (HEMA) and 2-acrylamido-2-methylpropanesulfonate, sodium salt (AMPS).



Chemical heterogeneity can be an important factor in controlling physical properties of copolymers when individual chains differ significantly in their relative concentrations of repeat monomer units. Measurements of chemical heterogeneity are frequently accomplished with a chromatographic separation or solvent fractionation, both of which are sensitive to molecular weight heterogeneity. Separations of copolymers by ITP lack this molecular weight dependence[8], which considerably simplifies their interpretation.

EXPERIMENTAL

Copolymer synthesis

Five copolymers were prepared with HEMA (Kodak Laboratory Chemicals, Rochester, NY, U.S.A.) and AMPS (Lubrizol Chemical Corp., Wickliffe, OH, U.S.A.) at weight ratios of 78:22, 40:40, 40:60, and 20:80 by free radical polymerization in water–ethanol (80:20) under nitrogen at 60°C. The reaction time was 20 h. A solution of the monomers was added to the polymerization vessel containing ammonium persulfate initiator (0.13%) for a period of 1 h. The resulting polymer solution was purified by diafiltration using a 2000 molecular weight-cut-off cellulose membrane to remove ethanol and residual monomers. AMPS homopolymer (designated AMPS-100) was prepared in a similar manner. Two of the five polymer solutions showed phase separation at polymer concentrations > 10% in water. For the phase separated samples, ITP analyses were performed on the individual phases.

The total AMPS content in each solution was determined by potentiometric titration of the sulfonate group with sodium hydroxide after ion exchange of the copolymers using Amberlite IR-10 ion-exchange resin.

Instrumentation

The separations were performed on a Shimadzu IP-2A isotachophoretic analyzer (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) equipped with a 60 mm × 1.0 mm I.D. PTFE first-stage capillary tube and a 100 mm × 0.5 mm I.D. fluorinated poly(ethylenepropylene) second-stage capillary tube. The compartment holding the capillaries and the potential-gradient detector cell was kept at 25°C by Peltier elements using a fluorinated-hydrocarbon cooling fluid. Zones were detected by sensing the potential gradient developed at the interface between migrating zones. The current-controlling anode and cathode were operated at potentials of 2000–10000 V and at currents of 125 or 150 μA. Operating parameters are noted in the figures with each isotachopherogram.

Electrolytes

The leading electrolyte was 0.01 *M* chloride buffered to pH 3.8 with β-alanine (Kodak Laboratory Chemicals) which was purified by recrystallization three times from ethanol. The electrolyte was prepared by diluting 100 ml of 0.1 *M* hydrochloric acid and 5 g of Triton X-100 surfactant (Rohm and Haas, Philadelphia, PA, U.S.A.) with 900 ml distilled water, and then approximately 0.95 g β-alanine was added to buffer the solution to pH 3.8. The surfactant minimized electroosmotic flow. The terminal electrolyte was 0.01 *M* *n*-hexanoic acid (Kodak Laboratory Chemicals). The *n*-hexanoic acid was purified by fractional vacuum distillation. The electrolyte was prepared by dissolving 1.16 g *n*-hexanoic acid and 5 g of Triton X-100 in distilled water and diluting to 1 l without pH adjustment.

Sample preparation

Copolymer samples were prepared for injection at 2000 ppm by dissolving 20 mg of the freeze-dried polymer in 10 ml of the leading electrolyte, or by diluting the original aqueous solution to 2000 ppm with the leading electrolyte.

RESULTS AND DISCUSSION

Molecular weight influence on the isotachrophoretic separation of polymers

The molecular weight dependence on the ITP separation of polymers was examined using six narrow-molecular-weight distribution sulfonated polystyrenes (Pressure Chemical Co., Pittsburgh, PA, U.S.A.) with weight-average molecular weights of 16000 to 780000 daltons. These polymers are prepared commercially by sulfonation of anionically polymerized styrene. The degree of sulfonation was determined by sulfur analysis on dialyzed samples, and by potentiometric titration of the sulfonate group after ion exchange to the sulfonic acid. Descriptive data for these polymers are summarized in Table I. The isotachopherograms in Fig. 1 show no significant variation in the observed effective electrophoretic mobilities. This lack of molecular weight influence for polyelectrolyte mobility is consistent with the work of Nagasawa *et al.*[9,10] and Meullenet *et al.*[11] for high-molecular-weight polymers using other electrophoretic techniques, and with our studies of carboxymethylcellulose[8].

TABLE I

MOLECULAR WEIGHT, COMPOSITION AND MOBILITY DATA FOR POLY(STYRENESULFONATE) SODIUM SALTS

*Lot No.**	$\bar{M}_w$** ($\times 10^{-3}$)	$\bar{M}_w/\bar{M}_n$***	*Sulfonation (%)*		*Effective mobility* (10^5 $cm^2/V\,s$)
			Sulfur analysis	*Titrimetric analysis*	
20	16	1.06	93.6	94.4	52.6
11	31	1.06	94.2	92.6	51.5
25	88	1.05	97.4	99.9	53.0
26	177	1.05	100.0	98.5	51.1
12	354	1.05	96.8	94.9	52.0
16	690	1.05	96.2	95.5	52.3

* Pressure Chemical Co., Pittsburgh, PA, U.S.A.

** Reported $\bar{M}_w$ from membrane osmometry, Pressure Chemical Co.

*** Reported values from size-exclusion chromatography, Pressure Chemical Co.

Quantitation of isotachopherograms

Quantitative evaluation of the isotachopherograms was performed by measuring zone lengths as the distance in millimeters between peak maxima of the differential potential-gradient detector signal. The relationship between the amount of AMPS homopolymer in the copolymer samples and the zone length was established from a calibration curve constructed from injections of 10–60 μg of the polymer dissolved in the leading electrolyte. The calibration isotachopherograms are shown in Fig. 2. A linear relationship was obtained.

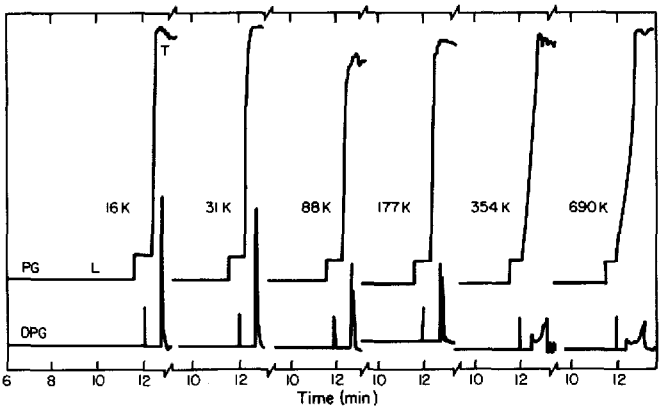


Fig. 1. Isotachopherograms of poly(styrenesulfonate) sodium salt of different molecular weights. Polymer descriptions in Table I. Leading electrolyte (L): 10 m*M* Cl^-, pH 3.8, β-alanine buffer, 0.5% Triton X-100 surfactant; terminating electrolyte (T): 10 m*M* hexanoic acid, 0.5% Triton X-100 surfactant; PG = potential gradient; DPG = differential potential gradient.

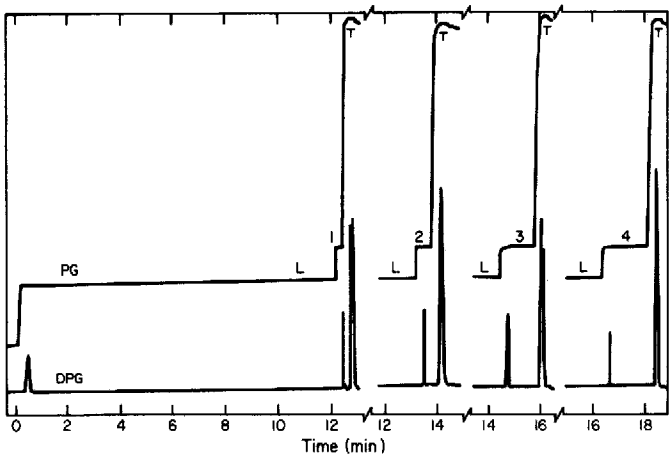


Fig. 2. Isotachopherograms of AMPS homopolymer at several concentrations for zone length calibration. Electrolytes, see Fig. 1. 1 = 9.5 μg AMPS (100); 2 = 19 μg; 3 = 38 μg; 4 = 58 μg.

Separations of HEMA–AMPS copolymers

Isotachopherograms were obtained on copolymers synthesized with HEMA-AMPS monomer feed ratios (wt.) of 78:22, 60:40, 40:60, and 80:20. Copolymer samples prepared at 60:40 and 40:60 monomer ratios were phase separated and are referred to as upper and lower phases. The phases are treated separately for quantitative analysis, but were recombined in dilute solution for the demonstration of mixture analyses by ITP that is described later.

Isotachopherograms of the seven polymer solutions are shown in Figs. 3–5. Copolymer 78:22 and both the upper and lower phases of copolymers 60:40 and 40:60 contain two distinct zones. One component in each copolymer had an effective mobility nearly identical to AMPS homopolymer and its zone length increased proportionately when authentic AMPS-100 was added. AMPS monomer has a lower effective mobility than the homopolymer and was easily distinguished from other components. None was detected in these dialyzed copolymers.

The concentration of AMPS-100 in each of the five copolymer samples was calculated using its zone length and the AMPS-100 calibration curve. The copolymer component in each sample appears to be of rather narrow compositional distribution, giving a zone that could not be further resolved into additional components using longer capillaries, other electrolyte systems, or injection of larger amounts of the copolymer. The compositions of each copolymer zone were calculated by subtracting the concentration of AMPS-100, detemined by ITP, from the total AMPS content, determined by potentiometric titration. The HEMA content was calculated by the difference,

$$\varphi_{\text{AMPS copolymer}} = \text{AMPS}_{\text{total}} - \text{AMPS}_{\text{homopolymer}}$$
$$\varphi_{\text{HEMA copolymer}} = 1 - \varphi_{\text{AMPS copolymer}}$$

where φ is the weight fraction of the copolymeric component.

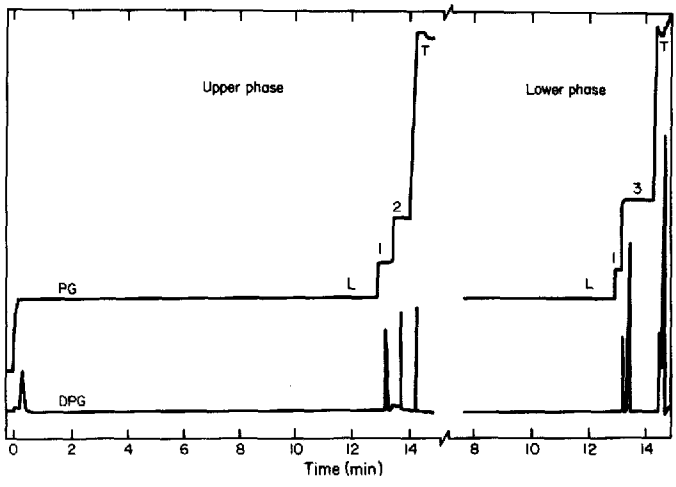


Fig. 3. Isotachopherograms of HEMA–AMPS copolymer 60:40, upper phase and lower phase. Electrolytes, see Fig. 1. 1 = AMPS (100); 2 = HEMA–AMPS (65:35); 3 = HEMA–AMPS (80:20).

The copolymer compositions prepared at each feed ratio are summarized in Table II. The phase-separated preparations consist of three distinct components. The lower phases were homopolymer and copolymer with an AMPS composition less than the monomer feed ratio. The upper phases were homopolymer and copolymer with AMPS composition greater than the monomer feed ratio. The two copolymers formed in each preparation were of such widely differing composition that they are incompatible in concentrated aqueous solution. This results in the formation of two liquid phases with unequal polymer concentration and composition.

This phase separation behavior for copolymers of like repeat unit composition, but different ratios of repeat units, has been observed for other ion-containing co-

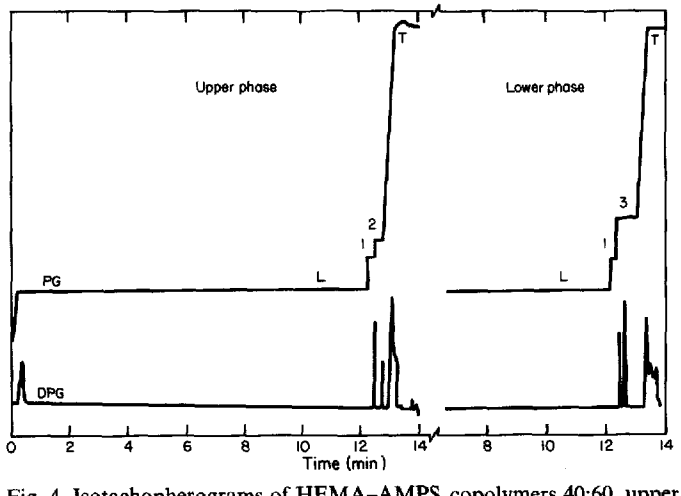


Fig. 4. Isotachopherograms of HEMA–AMPS copolymers 40:60, upper phase and lower phase. Electro lytes, see Fig. 1. 1 = AMPS (100); 2 = HEMA–AMPS (46:54); 3 = HEMA–AMPS (66:34).

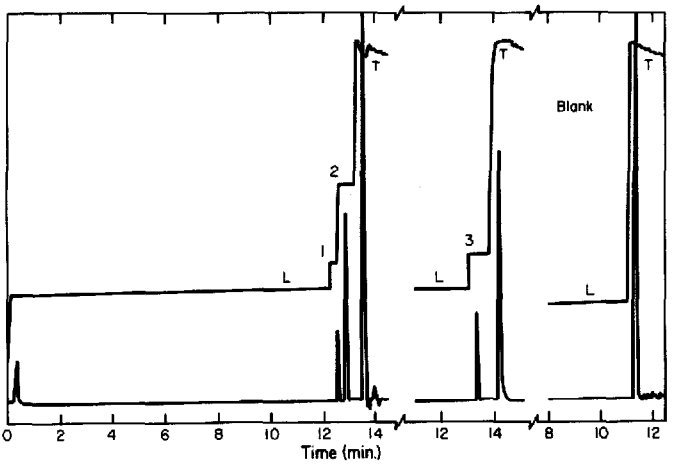


Fig. 5. Isotachopherograms of HEMA–AMPS copolymers, 78:22, 20:80, and electrolyte blank. Electrolytes, see Fig. 1. 1 = AMPS (100); 2 = HEMA–AMPS (84:16); 3 = HEMA–AMPS (25:75).

polymers including poly(ethylacrylate–co-methacrylic acid)[12], poly(methylmethacrylate–co-methacrylic acid)[13], and poly(styrene–co-maleic acid)[14].

The unusual copolymerization behavior of the HEMA–AMPS monomers to produce more than one distinct polymer composition during a single reaction is not readily explained by conventional solution chain growth processes. Instead, during the synthesis it is thought that high-molecular-weight, HEMA-rich copolymer forms early in the polymerization as a result of its greater monomer reactivity. This copolymer has low water solubility and may form a second microphase with unequal distribution of the AMPS monomer between these phases. As the HEMA monomer concentration becomes depleted, AMPS continues to polymerize to form AMPS homopolymer which can partition unequally in each of the phases because of limited compatibility with the copolymers.

TABLE II

COMPOSITION AND MOBILITY OF HEMA–AMPS COPOLYMERS

Polymer designation (wt. ratio)	*Total wt.% AMPS (titration)*	*% (rel.) AMPS as homopolymer (ITP)*	*Wt. ratio HEMA–AMPS*	*Apparent mobility (10^5 cm²/Vs)*	
				Zone A	*Zone B*
AMPS-100	100	100	0:100	53.1	*
HEMA–AMPS					
20:80	75.4	*	25:75	52.5	*
40:60 upper	60.4	24.5	46:54	53.8	46.9
40:60 lower	36.4	9.6	66:34	53.6	38.3
60:40 upper	42.0	27.1	65:35	53.8	39.1
60:40 lower	22.4	14.3	80:20	55.4	32.5
78:22	20.8	27.9	84;16	54.0	30.5

* Shows single zone in isotachopherogram.

Separation of copolymer mixtures

The effective mobilities of the copolymers may be calculated from zone dimensions using the equation[15],

$$\mu_i = \mu_L(E_L/E_i) = \mu_L(h_L/h_i),$$

where μ_L is the mobility of the leading ion (Cl, $79 \cdot 10^{-5}$ cm^2/Vs), E_L and E_i are the potential gradients, and h_L and h_i are the step heights in millimeters for the leading and sample zones, respectively. These values are shown plotted vs mole fraction AMPS in Fig. 6. Mobility is seen to change nearly linearly with AMPS content up to a mole fraction of 0.5–0.6 AMPS, beyond which little change occurs. This lack of change in mobility is principally in response to binding of counterions to the otherwise fully ionized polymeric sulfonate groups and it occurs only at compositions of copolymer with closely spaced ionic groups along the chain. This close charge-group spacing results in a very high local electrostatic charge density. Because of ion binding, the effective charge on the polyion is less than the number of analytically determined ionic groups present even though there is a fairly rapid exchange rate between free and bound counterions, as demonstrated by Gottlieb[16]. These coulombically attracted counterions move with the polyion in an electrophoretic experiment and, consequently, polymer mobility becomes less dependent on composition beyond 0.5–0.6 mole fraction of charge-bearing repeat units.

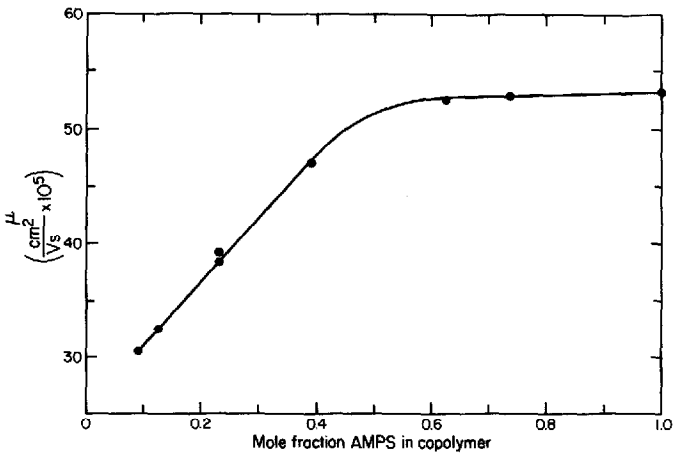


Fig. 6. Dependence of effective electrophoretic mobility of HEMA–AMPS copolymers on composition.

This behavior is consistent with the counterion-binding theory described by Manning[17–19] and confirmed experimentally for several types of polyelectrolytes by Manning[19] and Obubo and Ise[20], and for biomacromolecules by Record and coworkers[21,22]. Manning's theory suggests that the extent of counterion binding is dependent on several parameters, the most important ones being the average distance between charged groups along the polymer chain and the solvent dielectric constant. Counterion binding has considerable influence on the isotachophoretic separation of

charge-bearing macromolecules and a further evaluation of the influence of ion-binding on separations of certain carboxylic and sulfonic acid-containing copolymers will be presented in another paper.

The capability of ITP to separate complex mixtures of high-molecular-weight copolymers was evaluated by blending six copolymers and AMPS-100 into a single sample. The isotachophoretic separation is illustrated in Fig. 7. Five of the seven components are separated. Two copolymers, 60:40 upper phase and 40:60 lower phase were not resolved because their actual compositions and effective mobilities are nearly identical. AMPS-100 and copolymer 20:80 have mobilities that are nearly identical as a result of counterion binding and cannot be separated.

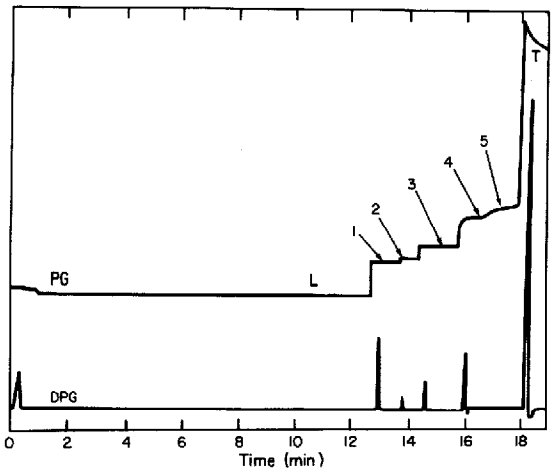


Fig. 7. Isotachopherograms of a mixture of HEMA–AMPS copolymers. 1 = AMPS (100) and 20:80; 2 = 40:60 upper phase; 3 = 60:40 upper and 40:60 lower phases; 4 = 60:40 lower phase: 5 = 78:22.

CONCLUSIONS

ITP provides a rapid and sensitive analytical separation of synthetic, charge-bearing polymers that is useful for the characterization of compositional mixtures. The independence of molecular weight on polymer chain mobility simplifies considerably the interpretation of an isotachophoretic separation when compared to polymer separations achieved by chromatographic processes. The separation is obtained without the aid of capillary packing materials or stabilizing media which eliminates the source of polymer–substrate interactions that can complicate the interpretation of polymer separations obtained from packed columns. Zone detection of the charge-bearing polymeric components is achieved regardless of composition using the potential gradient detection method. Zone lengths are shown to be proportional to the amount of polymer introduced into the capillary and are used in the calculation of copolymer compositions. The capability of ITP to resolve copolymer mixtures is strongly influenced by the relative difference in concentration of ionic repeat unit in the chains. The highest resolution is achieved for copolymers with the low average ionic group content and resolution is not possible for copolymers containing >0.6 mole fraction of a charge-bearing repeat unit.

ACKNOWLEDGEMENTS

We thank Mr. W. Bowman and Mr. R. Sutton who synthesized the copolymers and Ms. V. Childs who performed the chemical analyses and assisted in the isotachophoresis experiments.

REFERENCES

1 P. Delmotte, *Sep. Purif. Methods*, 10 (1981) 29.
2 R. Einarsson and R. Karlsson, *Anal. Lett.*, 18 (1985) 333.
3 P. Lange, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, 1980, p. 187.
4 H. Mikasa, K. Sasaki, J. Arata, Y. Yamamoto, T. Ohno and H. Kodama, *J. Chromatogr.*, 343 (1985) 179.
5 F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 91 (1974) 837.
6 P. Boček, P. Gebauer, V. Dolník, and F. Foret, *J. Chromatogr.*, 334 (1985) 157.
7 S. Hjalmarsson and A. Baldesten, *Crit. Rev. Anal. Chem.*, 11 (4) (1981) 261.
8 L. R. Whitlock, *J. Chromatogr.*, 363 (1986) 267.
9 M. Nagasawa, A. Soda and I. Kagawa, *J. Polym. Sci.*, 31 (1958) 439.
10 M. Nagasawa, I. Noda and M. Ota, *J. Amer. Chem. Soc.*, 86 (1964) 5075.
11 J. P. Meullenet, A. Schmitt and M. Driffort, *J. Phys. Chem.*, 83 (1979) 1924.
12 L. J. Hughes and G. E. Britt, *J. Appl. Polym. Sci.*, 5 (1961) 337.
13 J. V. Koleske and R. D. Lundberg, *J. Polym. Sci., Part A-2*, 7 (1969) 795.
14 K. Kosai and T. Higashino, *Nippon Setchaku Kyokai Shi.*, 11 (1975) 2.
15 T. Hirakawa and Y. Kiso, *J. Chromatogr.*, 252 (1982) 33.
16 M. H. Gottlieb, *J. Phys. Chem.*, 75 (1971) 1981.
17 G. S. Manning, *J. Chem. Phys.*, 51 (1969) 934.
18 G. S. Manning, *Acc. Chem. Res.*, 12 (1979) 443.
19 G. S. Manning, *J. Phys. Chem.*, 85 (1981) 1506.
20 T. Okubo and N. Ise, *Macromolecules*, 11 (1978) 439.
21 M. T. Record, C. F. Anderson and T. M. Lohman, *Q. Rev. Biophys.*, 11 (1978) 103.
22 C. F. Anderson and M. T. Record, *Ann. Rev. Phys. Chem.*, 33 (1982) 191.