Separation of *stat*-Copoly(styrene / 2-Methoxyethyl Methacrylate) Samples According to Composition by Gradient High-Performance Liquid Chromatography

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Synopsis

Statistical copolymers of styrene (S) and 2-methoxyethyl methacrylate (MEMA) (13–87 mass % of the latter monomer) were investigated by gradient HPLC on columns with either silica or CN-bonded phase packings. The samples were injected in THF solution. The starting eluent was in each case a nonsolvent (isooctane or mixtures of isooctane with THF and methanol). Separation was achieved by increasing the concentration of THF or methanol in the eluent. A calibration mixture of four copolymers (26, 49, 62, and 87% MEMA) was investigated according to the principles of chromatographic cross-fractionation (CCF) by size exclusion chromatography and subsequent gradient HPLC. The influence of molar mass on HPLC retention was small and, for the samples investigated by CCF, independent of copolymer composition. The composition effect on detector signal was also studied. At 230 nm wavelength and with MEMA content in the range between 20 and 40%, the effect was small and caused the average composition calculated from CCF results to deviate from the directly measured value by not more than 0.2 or 0.8% for the two copolymers investigated.

INTRODUCTION

Gradient high-performance liquid chromatography (HPLC) is mostly performed as gradient elution, i.e., by using mixed eluents whose composition changes during the analysis. Effective elution gradients are characterized by increasing elution power. Usually, chromatographic strength is determined by polarity. In the so-called normal-phase gradient HPLC, the components of the sample are retained on a polar column and eluted by a gradient that starts with a mixture of low polarity and becomes richer and richer in the polar solvent. In addition to this recognized character, solubility effects are operative in gradient HPLC of polymers.[1-3]

Tetrahydrofuran (THF) is a powerful solvent for many copolymers whereas alkane hydrocarbons are usually nonsolvents. An elution gradient starting with, e.g., isooctane and going to a mixture with a high content in THF is a

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normal-phase gradient in terms of polarity and at the same time a gradient of increasing dissolution power. Such gradients have proved successful in separating by composition statistical copolymers containing units of different polarity, e.g., styrene and acrylonitrile (S–AN),[1-4] styrene and methyl methacrylate,[5] or styrene and ethyl methacrylate (S–EMA).[6]

The main objective of this paper is the evaluation of suitable conditions for chromatographic separation by composition of statistical copolymers prepared from styrene (S) and 2-methoxyethyl methacrylate (MEMA). These copolymers have already been subject to manyfold studies. The instantaneous heterogeneity of an azeotropic S–MEMA copolymer has been investigated by classical cross-fractionation.[7] The conversion effect on chemical composition distribution (CCD) has been studied by following the average copolymer composition and the composition of the residual monomer mixture as a function of conversion during the copolymerization reaction.[8] Furthermore, S–MEMA copolymers have been used successfully for acquiring fundamental knowledge on copolymer properties.[9,10]

Another objective of this study is the investigation of chromatographic cross-fractionation of S–MEMA specimens. Copolymers generally consist of macromolecules differing in molecular weight *and* chemical composition. The evaluation of both distributions can be performed by cross-fractionation which requires separation and analysis in two different directions. Classical cross-fractionation is performed by using two solvent/nonsolvent systems, one of them separating by molar mass and increasing content in one of the monomeric units, the other one by molar mass and decreasing content in this unit. Among the drawbacks of the classical procedure is the time required for the analyses (about 3 months labor per sample). Chromatographic cross-fractionation (CCF) implies the application of chromatographic techniques to copolymer analysis. Among the possible combinations of methods there is prefractionation by size exclusion chromatography (SEC) and subsequent analysis of the SEC fractions by means of gradient HPLC. This first separation should yield fractions graded in molar mass but, strictly spoken, SEC separates by hydrodynamic volume

$$V_h = [\eta] \cdot M = K \cdot M^{1+a} \tag{1}$$

where M = molar mass and $[\eta]$ = intrinsic viscosity. Varying sample composition usually modifies the parameters K and a of the Kuhn–Mark–Houwink (KMH) equation. For many polymers and THF as a solvent, the KMH constants are compiled in ASTM D-3593-80.[11] The variety of data for polystyrene (PS) in THF at 25°C is surprisingly broad. The graphical representation of $\log[\eta]$ vs. $\log M$ shows that the parameters $K = 12.5 \times 10^{-3}$ mL/g and $a = 0.713$ [12] determine a characteristic line near to the center of this sample of points.

These data have been used in a recent discussion.[6] In the present work we shall also use these parameters for PS. The KMH parameters for poly(2-methoxyethyl methacrylate) (PMEMA) in THF at 25°C are $K = 7.5_7 \times 10^{-3}$ mL/g and $a = 0.71$.[10] From these data it can be concluded that in the molar mass range of interest (i.e., around 40,000 g/mol) a PMEMA sample would have about 60% of the intrinsic viscosity of a corresponding PS sample.

The next question is concerned with the change in intrinsic viscosity $[\eta]$ or KMH parameters on transition from one of the parent homopolymers to the other. Data are available for, e.g., *stat*-copoly(S/methyl acrylate),[13] *stat*-copoly(S/*n*-octyl methacrylate),[14] and *stat*-copoly(S/butyl methacrylate)[14] and the respective homopolymers, but unfortunately not for the system under investigation. The quoted data show $[\eta]$ of statistical copolymers lying in between the values of the parent homopolymers of the same molecular mass. In the S/methyl acrylate system the real, or true viscosity of a 50 : 50 copolymer is higher than the linear interpolation, in S/octyl methacrylate somewhat lower, and in S/butyl methacrylate $[\eta]$ is almost equal to the interpolated value. Thus, in a first approximation it may be assumed that there is no extreme value of $[\eta]$ in the transition from PS to PMEMA.

Mixtures of PS and PMEMA represent the ultimate heterogeneity in the system under investigation. The copolymers J and K (cf. Table I) show limited chemical heterogeneity. Thus, the composition effect in SEC separation is estimated to cause no more than 10% uncertainty in molar mass.

What remains is the fact that copolymers of a given molar mass will show the higher $[\eta]$ values the richer in styrene the samples are. This connection will facilitate the SEC separation of species where both styrene content and molar mass vary in the same way but impair the separation of samples where high content in MEMA is linked to high molar mass. The latter holds true for the calibration samples A–I (cf. Table I). If the separation of a mixture of these samples can be achieved, analyses will be possible without difficulties.

The ultimate goal of the present paper is the evaluation and testing of the methodology for an investigation of two copolymers which were prepared via a certain monomer batch polymerization up to different degrees of conversion (samples J and K). This methodology must be reliable enough to allow mutual comparison of the specimens and comparison of the experimental results with theoretical expectations. This will be the subject of another paper.[15]

EXPERIMENTAL

Chemicals

THF without stabilizer (BASF, Ludwigshafen, F.R.G.) was distilled over potassium in a 2-m silver-coated column. The middle fraction was subsequently refluxed over potassium in a closed-circle apparatus, from which it was taken immediately to the HPLC system. Sample solutions for direct injection into the HPLC apparatus were prepared using analytical-reagent grade THF stabilized with 0.025% butylated hydroxy toluene (E. Merck, Darmstadt, F.R.G.). Isooctane and methanol were LiChrosolv grade (E. Merck). The sample code and their characteristics are given in Table I.

Size Exclusion Chromatography

SEC Apparatus. An HPLC pump from Biotronik (Maintal, F.R.G.), Model BT 3020, was connected to a Rheodyne injection valve, Model 7010 (Latek, Heidelberg, F.R.G.). The elution curves were monitored by a refractive index detector, Model 5178, from Knauer (Bad Homburg, F.R.G.), and a variable

TABLE I
Specification of *stat*-Copoly(styrene/Methoxyethyl Methacrylate) Specimens

Sample code	A	B	C	D	E	F	G	H	I	J	K
MEMA (mass %)	13.4	25.9	38.0	49.0	53.2	62.4	71.2	79.7	87.4	32.3	26.0
S (mol %)	90.0	79.8	69.3	59.0	54.9	45.5	35.9	26.0	16.6	74.4	79.8
$\overline{M}_n$ (kg mol^{-1})									185[a]	25.8[b]	37.8[b]
$\overline{M}_w$ (kg mol^{-1})	88[c]	96[c]	180[c]	137[c]	197[c]	173[c]	163[c]	164[c]	306[c]	37.2[b]	57.2[b]
Conversion (mass %)	3	20	3	29	20	24	26	29	6	10.1	86.4
Presence in mixture M1 (mass %)	24.4	—	26.8	—	—	26.8	—	—	22.0	—	—
Presence in mixture M2 (mass %)	—	32.9	—	26.7	—	23.0	—	—	17.4	—	—

[a] By osmometry.
[b] By size-exclusion chromatography with RI detection and calibration by polystyrene standards.
[c] By light scattering.

TABLE II
Survey of Gradient Programs in HPLC Investigation of S/MEMA Copolymers

Gradient no. 1

Time (min)	0	10	12	14	16
iso-Octane (%)	100	0	0	0	100
THF (%)	0	100	100	80	0
Methanol (%)	0	0	0	20	0
Flow (cm^3 min^{-1})	0.5	----------	0.5	2	0.5

Gradient no. 2

Time (min)	0	12	13	14	16.8	17.3	17.8	18
iso-Octane (%)	63	3		0	0	98	----------	
THF (%)	35	35		70	70	0	----------	
Methanol (%)	2	62		30	30	2	----------	
Flow (cm^3 min^{-1})	0.5	----------	0.5	2	----------		2	0.5

Gradient no. 3

Time (min)	0	10	12	12.5	13	14	15	16	16.5	17
iso-Octane (%)	68	18	0	0	0	0	0	98	----------	
THF (%)	30	30	30		100	0	100	0	----------	
Methanol (%)	2	52	70		0	100	0	2	----------	
Flow (cm^3 min^{-1})	0.5	----------	0.5	2	----------				2	0.5

wavelength UV detector, Model 3030 (Biotronik), operated at 254 nm wavelength.

SEC Columns. A set of two mixed gel columns GMH6 from Toyo Soda (Japan), each 600 × 7.8 mm was used; the particle size of the packings was d_P = 8–10 μm; the total interstitial volume was about 40 mL.

SEC Conditions. Flow rate 1 mL/min, volume injected 200 μL, sample concentration 0.5% in the preparative fractionations. Eluate fractions of 1 mL were collected under a cover of helium in 2.5 mL vials suited for the HPLC autosampler used in subsequent HPLC investigations. The vials were closed immediately after filling. Table III compiles some characteristics of the fractions.

Gradient High-Performance Liquid Chromatography

HPLC Apparatus. Liquid chromatograph Model HP 1090 Series A from Hewlett Packard (Waldbronn, F.R.G.) with auto-injector with 250 μL syringe and autosampler, with Ternary DR 5 pump, built-in diode array UV detector, and data processing unit, connected to a Thinkjet Printer HP 2225 A, a flexible disk drive HP 9121 D, and a graphic plotter HP 7470 A. A personal computer HP-85 was used as a system controller.

HPLC Columns. Cartridge columns 60 × 4 mm (Knauer), packed with silica Nucleosil 50, d_P = 5 μm, d_0 = 5 nm ("silica column") or Nucleosil CN, d_P = 5 μm ("CN column"). These columns were available from a broader investigation of stationary phase effects in the HPLC of copolymers. They were chosen due to the higher separating power of a 55 mm silica column in comparison with that of an otherwise equivalent column 150 mm in length.

HPLC Conditions. The flow rate was 0.5 mL/min, the injection volume in the range of 10–100 μL. The gradient lag time was 2.3 min both for the silica

TABLE III
SEC Fractionation of Mixture M2 and Copolymers J and K

Fraction no.	1	2	3	4	5	6	7	8	9
Mixture M2: amount injected 1.118 mg									
Volume (cm^3)	4.3	1	1	1	1	1	1	1	4.1
Relative area (%)	7.4	5.9	8.7	15.0	21.3	18.2	12.8	6.3	4.4
Molar mass (kg mol^{-1})	408	217	144	93.8	60.3	38.4	24.5	15.5	7.8
HPLC: amount in 100 μL^a (μg)	1.9	6.6	9.7	—	—	—	14.3	17.3	1.2
in 50 μL^a (μg)	—	—	—	8.4	11.9	10.2	—	—	—
Copoly(styrene/methoxyethyl methacrylate) sample J: amount injected 1.151 mg									
Volume (cm^3)	3.0	1	1	1	1	1	1	5	
Relative area (%)	2.9	17.0	20.2	24.5	19.3	11.3	3.9	1.0	
Molar mass (kg mol^{-1})	80.1	50.1	31.8	20.2	12.8	7.8	4.4	2.0	
HPLC: amount in 100 μL^a (μg)	1.1	19.6	—	—	—	4.4	4.4	0.24	
in 50 μL^a (μg)	—	—	11.6	14.1	11.1	6.5	—	—	
Copoly(styrene/methoxyethyl methacrylate) sample K: amount injected 1.151 mg									
Volume (cm^3)	2.0	1	1	1	1	1	1	4.1	
Relative area (%)	2.7	8.2	19.0	23.7	21.9	12.9	7.5	4.2	
Molar mass (kg mol^{-1})	130	88.2	56.6	36.1	23.0	14.5	9.0	4.4	
HPLC: amount in 100 μL^a (μg)	1.5	9.5	—	—	—	—	8.6	1.2	
in 50 μL^a (μg)	—	—	10.9	13.6	12.6	7.4	—	—	

[a] Figures given according to the injection volume used in gradient HPLC (either 50 or 100 μL).

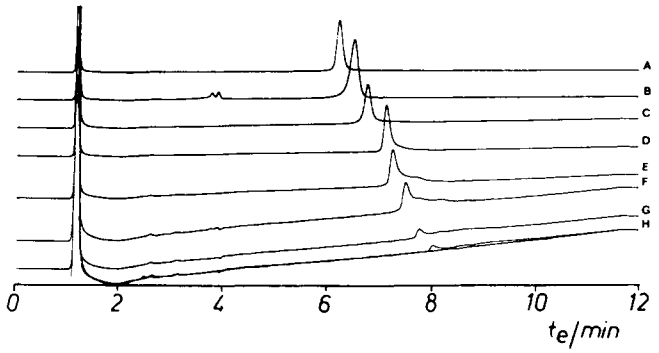

Fig. 1. Gradient elution of *stat*-copoly(styrene/2-methoxyethyl methacrylate) after separate injection of 10 μL each containing 10 μg copolymer (low-conversion samples A—H, see Table I). CN column, gradient no. 1, UV signal at 259 nm, attenuation graded according to signal size: (A) 600; (B) 500; (C, D) 300; (E) 150; (F, G, H) 75.

and the CN column. The gradient programs used are listed in Table II. The column temperature was 50°C.

RESULTS

Figure 1 shows the HPLC chromatograms of the samples A–H obtained after separate injections of 10 μg each. The traces were plotted with identical time scale (0–12 min) but individual values of attenuation. The inflection at about 1.2 min is caused by the THF used as a solvent for the sample solutions. The copolymers elute in the interval between 6 and 9 min. Figure 2 shows that the THF concentration in the eluent at peak position is related to the composition of the copolymers in a logical order. The dependence includes polystyrene homopolymer that gives the point on the *y*-axis.

The drawback of this approach is the small signal size that makes monitoring difficult, especially with samples rich in MEMA. Larger and possibly better balanced signals should be expected at shorter wavelengths. However, a

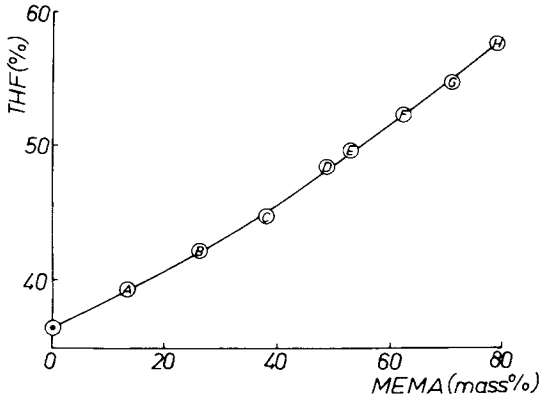

Fig. 2. Eluent composition at peak position in Figure 1 plotted vs. copolymer composition. The letters refer to the sample code, Table I. The point on the ordinate was measured with polystyrene.

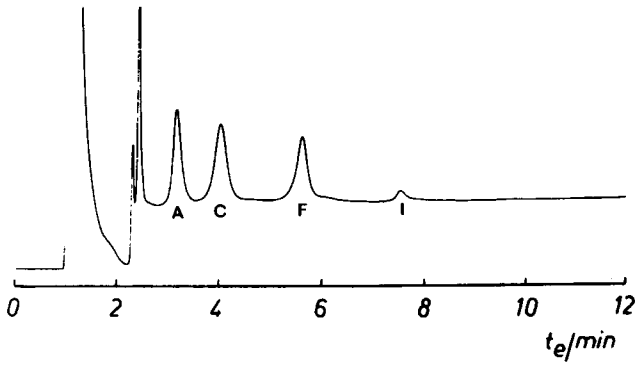

Fig. 3. Chromatogram of the mixture M1 of the copolymers A, C, F, and I (see Table I) obtained after injection of 20 μL containing 8.2 μg in total. Silica column, gradient no. 2, UV signal at 230 nm, 200 mAU full scale.

gradient formed by increasing concentration of THF would render this attempts difficult because this solvent is less transparent than isooctane. This shows up even at 259 nm (compare the rising baseline of the signals plotted at low attenuation, Fig. 1) and will be more pronounced at shorter wavelengths. Thus, we increased the polarity of the eluent by increasing the concentration of methanol (see Table II).

Figure 3 shows the chromatogram of mixture M1 developed through gradient no. 2 and monitored at 230 nm. Although the amount injected was only about 2 μg per copolymer, all four specimens can be seen. They elute in the

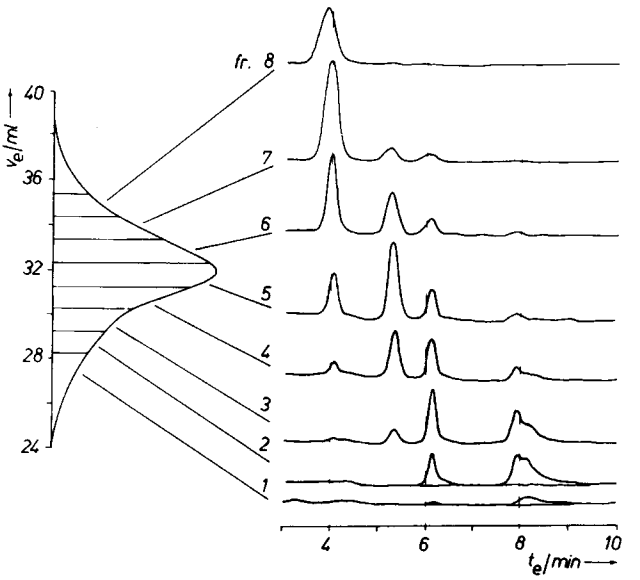

Fig. 4. Chromatograms of the SEC fractions from the calibration mixture M2 (see Tables I and III). CN column, gradient no. 3, UV signal at 230 nm, attenuation 100.

interval between 3 and 8 min. The leading three peaks are due to the samples A, C, and F containing 13.4, 38.0, and 62.4% MEMA, respectively.

The final goal of this investigation was an attempt to gain experimental evidence of the conversion effect on the CCD of copolymers formed under nonazeotropic conditions. Objects were the samples J and K whose average composition, 32.3 or 26.0% MEMA, respectively, lay in between that of the first and the second peak. These peaks are baseline-separated in the chromatogram shown in Figure 3. Thus, it appeared worthwhile to analyze in a corresponding manner a calibration mixture (M2) according to the principles of chromatographic cross-fractionation.

Figure 4 shows the chromatograms obtained from the SEC fractions of the mixture M2. The copolymers used differ rather widely in molar mass (see Table I). From Figure 4 it can be concluded that the SEC prefractionation was effective, although MEMA content and molar mass of the samples increased simultaneously. The SEC fraction no. 8 contained only the low-molar-mass species B, then the specimen D came, and in the high-molar-mass fractions no. 3, 2, and 1 the samples F and I eluted. The clear fractionation by molar mass in SEC confirms the expectations formulated in the concluding paragraphs of the Introduction.

Quantitative Aspects

Among the requirements for chromatographic cross-fractionation are quantitative retention of the injected polymer and its proper elution in the course of the gradient. With S–AN copolymers the required retention was obtained when at the moment of injection the mobile phase was a nonpolar nonsolvent, e.g., hexane or isooctane.

Recently a report was given that under certain conditions S–EMA copolymers eluted partly either (i) in the interstitial volume, (ii) in a peak immediately following the solvent peak, or (iii) together with the sample solvent.[16] This occurred when 100 μL of SEC fractions were injected on 60 × 4 mm columns packed with porous silica, CN bonded phase, or C18 bonded phase materials. The reason for this defect was obviously the low polarity of the S–EMA copolymers in combination with too high a ratio of sample volume to the pore volume of the column.

In the present CCF investigations we used columns of the same geometry (60 × 4 mm) and sample volumes of either 50 or 100 μL (depending on the polymer concentration in the respective SEC fraction). With polar columns (silica or CN) and injection into 98–100% isooctane no sign of incorrect elution was observed.

Signal Size vs. Sample Concentration

Signal size was evaluated by measuring the height of the elution curve over the experimental baseline at equidistant values of the elution time (about 20–60 data per peak). The total of this height data of a given peak was used as a measure of its area.

When the experimental baseline was straight and the peaks were baseline-separated, electronic peak integration was equivalent to this point-by-point evaluation. In more complex cases the time-consuming manual procedure

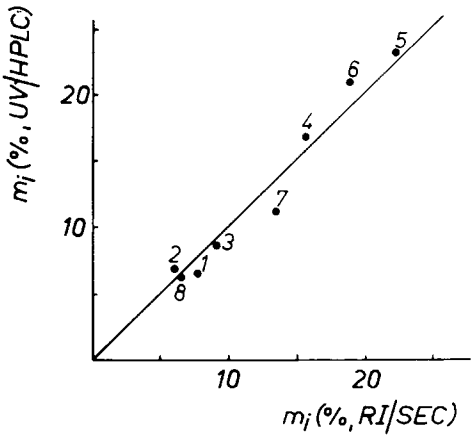

Fig. 5. Comparison of sample size and copolymer amount found in gradient HPLC: plot of relative signal area in HPLC of the SEC fractions 1–8 (UV signal) vs. relative area of the respective slice under the SEC curve (RI signal); fraction number indicated, data from Figure 4.

proved more reliable than automatic integration. With the gradient used in the experiments shown in Figure 4 the integrator readings correspond to the manual results.

Quantitative retention and elution implies equivalence between sample size and signal size. Provided that for the sample under investigation the composition effect on refractive index is negligible, the total signal area of a given SEC fraction must be in accordance with the amount of sample in this fraction. This amount was evaluated from the area between the SEC curve, the SEC baseline, and the straight boundary lines of the fractions which indicate how the eluate stream was cut (cf. the SEC scheme in Fig. 4). If solvent evaporation occurred during sample handling or if differing volumes were injected into the HPLC apparatus, the area of HPLC peaks would have to be corrected according to the real polymer content of the fraction under investigation.

Figure 5 shows that empirical correction was not necessary in the present study. The relative size of HPLC signals corresponds to the relative amount of polymer in each SEC fraction. (With 50 μL injections the HPLC signal found was multiplied by a factor 2, of course.)

Signal Size vs. Sample Composition

A UV detector measures only substances that absorb at the selected wavelength. At 259 nm the absorptivity of styrene/methacrylate copolymers is caused by the styrene units. At 230 nm the elution of poly(*t*-butyl methacrylate) homopolymer could be monitored by UV detection.[17]

In order to estimate the effect of sample composition on detector signal under the conditions of CCF, we separately calculated the total of the peak areas for sample B in the SEC fractions 1–8 and also those for samples D, F, and I. (In these evaluations again a factor 2 was applied to the signals obtained on 50 μL injections.) The totals were subdivided by the total amount of each sample in all SEC fractions which can be derived from the SEC injection (1.118 mg) and the known composition of the calibration mixture.

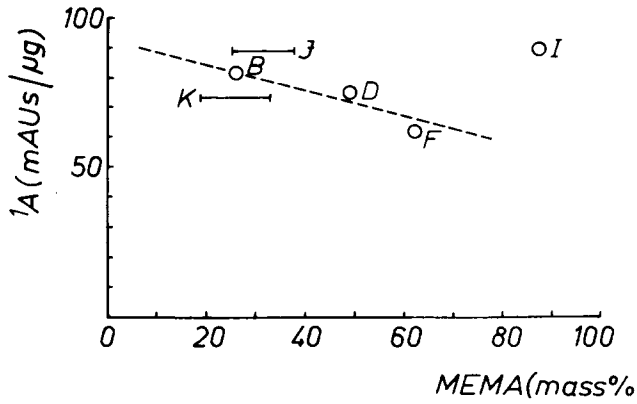

Fig. 6. Peak area per μg measured at 230 nm of the respective calibration sample (data from Fig. 4) vs. composition. Letters refer to the sample code in Table I.

Figure 6 shows the specific absorptivity (mAU/μg) plotted vs. MEMA content of the samples. The high data of sample I is unexpected. Fortunately, the deviation of this point will not impair the investigation of the samples J and K, whose composition distribution does not extend to the 62.4% MEMA content of sample I.

The limits of the experimentally determined CCD in sample J and K are indicated in Figure 6. For these samples the variation of specific absorptivity with copolymer composition is given by the slope of the dashed line in Figure 6. It amounts to about 4% decrease in detector signal by 10% increase in MEMA content. A correction of that size will be necessary in repeated investigations of a certain sample. Here we neglected the small concentration effect in a first approximation.

Molar Mass Effect on Separation by Composition

The molar mass effect on the gradient HPLC of S–MEMA copolymers was evaluated from the peak positions of calibration specimens and the characteristics of the respective SEC fractions. For this purpose the volume fraction of the nonsolvent isooctane, v_{NS}, was estimated from the peak retention time and the gradient program. This data was plotted vs. the square root of reciprocal molar mass according to the relation

$$v_{NS} = P + Q \cdot M^{-0.5} \tag{2}$$

which had been found empirically by turbidimetric titrations[18] and proved valid in HPLC behavior of several copolymer systems.[2,6]

Figure 7 shows the respective plot. The molar mass effect is extremely small, and in the range investigated, independent of the composition of the S–MEMA copolymers. The slope factor $Q = 1.754$ holds for all four specimens whereas the ordinate P has the values 0.584, 0.522, 0.483, or 0.394 for the calibration samples B, D, F, or I, respectively. In terms of elution time the corresponding data for samples of infinite molar mass reads (in the same sequence) $t = 4.22$, 5.46, 6.25, or 8.02 min. Figure 8 shows the calibration curve

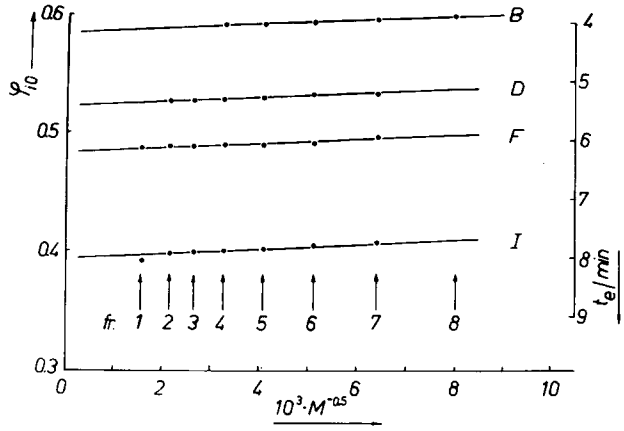

Fig. 7. Molar mass effect in gradient elution of S–MEMA copolymers by gradient no. 3. Data from Figure 4 plotted as volume fraction of the nonsolvent isooctane vs. reciprocal square-root of molar mass. Elution time scale at the right-hand side.

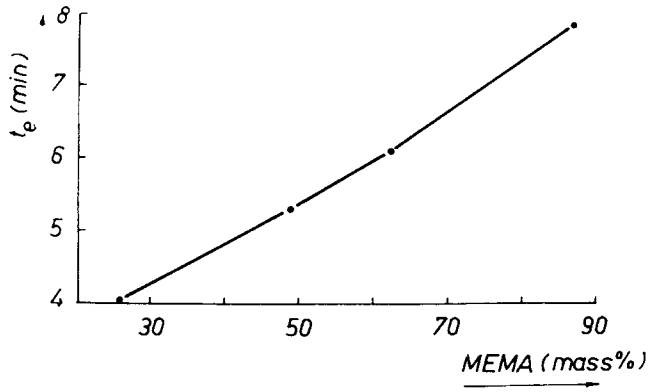

Fig. 8. Calibration curve for the gradient HPLC of copoly(S/MEMA) samples through gradient no. 3 read from Figure 7 for a molar mass of 40,000 g/mol.

for M = 40,000 g/mol. It is based on the data compiled in Figure 7 and enables the conversion of elution time into MEMA content to be performed. The knowledge of the molar-mass effect is indispensable when the calibration samples have molar-mass values which do not cover the range of values of the specimens to be analyzed. This situation must be considered in the experiments with the S–MEMA copolymers listed in Table I.

The information connecting MEMA content with elution time and sample amount with signal height can be used for the evaluation of HPLC curves of SEC fractions. Prerequisites are identical eluent quality and regular performance of gradient and column. Figure 9 shows the HPLC pattern of SEC fraction no. 2 of sample J and Figure 10 the corresponding one of the low-molar-mass fraction no. 7 of the same sample. The latter trace is significantly broader than the former. This is in accordance with theory which, at a low degree of conversion, predicts broader chemical distribution at lower molecular weight values than at higher ones.

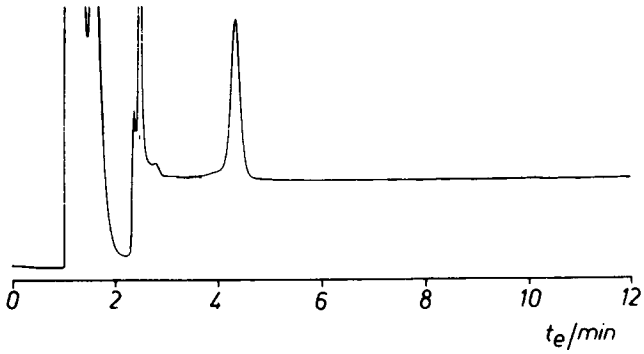

Fig. 9. Gradient chromatogram of SEC fraction no. 2 (M = 50,100) from sample J. CN column, gradient no. 3, injection volume 100 μL, UV signal at 230 nm, 100 mAU full scale.

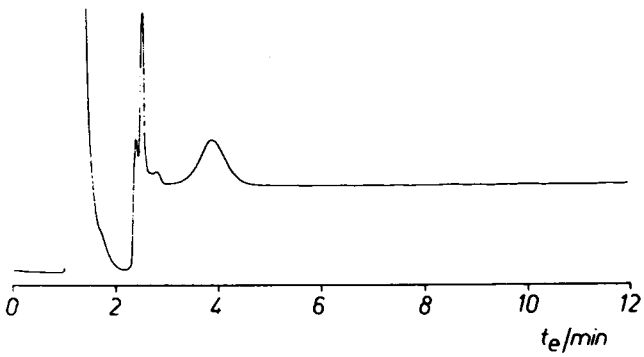

Fig. 10. Gradient chromatogram of SEC fraction no. 7 (M = 4,400) from sample J. Conditions as with Figure 9.

Furthermore, the peak in Figure 10 occurs at a shorter elution time than in Figure 9. This is only partly due to the low molar mass value of fraction J-7 and signifies indeed a lower MEMA content than fraction J-2 has.

The information drawn from all SEC fractions of a sample can be used for the construction of the contour-line maps of the 2-dimensional distribution in molar mass and chemical composition of the copolymer under investigation.

DISCUSSION

Precise determination of the complex distribution should better be performed only after repeated performance of CCF procedures—at best under modified conditions. This well-established rule which is commonly applied to much simpler analytical work is, of course, even more valid in such delicate evaluations as required here.

Nevertheless, we dare say that contour-line maps of different samples which are based upon HPLC investigations performed under strictly identical conditions in a mixed sequence of injections will show similarity to the real

distributions and, thus, eventually reveal significant difference between the samples.

We investigated the SEC fractions of the samples J and K, and the calibration mixture M2 in following sequence: blank/M2-4/M2-5/M2-6/J-3/J-4/J-5/J-6/K-3/K-4/K-5/K-6/blank/M2-1/M2-2/M2-3/M2-7/M2-8/M2-9/J-1/J-2/J-7/J-8/K-1/K-2/K-7/K-8/blank. This series of injections was performed by an autoinjector without any break under constant elution conditions within 8 h.

The calculation of the average MEMA content in the SEC fractions from their HPLC curves is more straightforward but nevertheless also dependent on proper measurements and reliable calibration. The average composition of the whole sample can be easily obtained from the mean composition of each fraction and its portion in the starting sample.

As already mentioned, we neglected the composition effect on detector sensitivity in a first approximation. A behavior as indicated by the dashed line in Figure 6 means that our approach underestimated portions rich in MEMA and overestimated portions rich in styrene. This deformation of the CCD curves will, in total, yield too low a value of MEMA content for the whole sample.

By evaluation of our CCF data we obtained an average MEMA content of 31.5 mass % in copolymer J and 25.8% in copolymer K. The corresponding results from refractometric analysis of the crude samples were 32.3 and 26.0%, respectively. Indeed, our CCF evaluation yielded too low values, but the differences are almost within the limits of experimental error. Hence, in the present investigation the neglection of the composition effect on detector sensitivity is not severely falsifying the CCF evaluation.

The weakest point in our investigation was the unfavorable ratio of injection volume to column pore volume. But, as we could not find any indication of insufficient retention, we believe that this preliminary results can be used for comparison of samples polymerized to different degrees of conversion under otherwise identical conditions. This will be the objective of another paper on S–MEMA copolymers.[15]

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