Chromatographic Separation of Azeotropic and Low-Conversion Poly(styrene-*stat*-2-methoxyethyl methacrylate) According to Chemical Composition

M. KONÁŠ* and M. KUBÍN

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia

SYNOPSIS

Distribution of the chemical composition of statistical copolymers was studied by HPLC using azeotropic and low-conversion copolymers of styrene and 2-methoxyethyl methacrylate that are expected to exhibit only statistical chemical heterogeneity. Adsorption behavior of the copolymers was studied dynamically with a specially designed apparatus, using flow through a column packed with a macroporous silica and a mixed mobile phase 1,2-dichloroethane (DCE)–heptane (50 : 50 v/v) and tetrahydrofuran (THF). The shape of displacement isotherms indicates the existence of a region lying near the displacement critical composition, where the total amount of adsorbed copolymer is independent of the mobile-phase composition. Fractionation was achieved by step-by-step desorption of a copolymer sample previously adsorbed on silica packed into a chromatographic column, by means of a stepwise gradient of mobile-phase composition. The styrene content of isolated fractions decreased with increasing content of THF in the mobile phase. The experimentally determined integral weight distribution of chemical composition shows good agreement with theoretical curves. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Copolymers are, in general, polydisperse both with regard to molecular weight and chemical composition. Two types of chemical heterogeneity are known—statistical[1] and conversion[2] heterogeneity. In statistical copolymers—with the exception of azeotropic and low-conversion samples—the contribution of conversion heterogeneity is usually considered to prevail.[3] The importance of statistical heterogeneity decreases with increasing degree of polymerization and its quantitative characterization for samples with degree of polymerization $\cong 10^3$ represents a serious experimental challenge.[4,5] On the other hand, it is not always advisable to neglect statistical heterogeneity: Only copolymers with infinite degrees of polymerization are devoid of statistical chemical heterogeneity;[6] in low-conversion copolymers with lower molar mass, the individual

chains can differ[3] in their composition by as much as 20 wt %.

Examples of experimental determination of chemical composition distribution (CCD) of copolymers that exhibit exclusively statistical chemical heterogeneity do not abound in the literature. The resolving power of classical cross-fractionation of azeotropic copolymers styrene/methyl methacrylate[4] or styrene/2-methoxyethyl methacrylate[5] proved to be insufficient to separate such narrow-distribution samples according to chemical composition. Thin-layer chromatography was used in several attempts.[7] HPLC, a method of choice for studying the complex structure of copolymers, has not been used for the determination of statistical heterogeneity, with some notable exceptions.[8,9]

Copolymers of styrene with various acrylic or methacrylic esters are often used in studies aimed at assessing the potential of HPLC as a method for CCD characterization, mostly because the styrene building block absorbs in the UV region and, on the other hand, the (meth)acrylic units are capable of entering into specific interactions with a polar sor-

---

bent via hydrogen bonds. In our case, we used samples of statistical copolymers styrene/2-methoxyethyl methacrylate (S/MEMA). The chemical heterogeneity of this copolymer has already been studied by classical[5] and chromatographical cross-fractionation,[10,11] where in the second instance, CCD was determined for samples of relatively high chemical heterogeneity. It is the aim of the present paper to characterize by chromatographic methods samples with narrow CCD due exclusively to statistical chemical heterogeneity, by separating them first into fractions of appreciable mass and then determining their composition by an independent analytical method.

Chromatographic separation of copolymers differing sufficiently in their chemical composition is mostly performed by means of gradient elution with a continuous change of the mobile phase composition. This type of gradient, when applied to the analysis of a single polydisperse sample, may bring considerable difficulties, which are higher the lower the chemical heterogeneity of the studied sample. One method to avoid problems with reproducibility and sensitivity is to use a discontinuous, stepwise gradient consisting of segments with constant composition of the eluent, combined with a method of sample introduction into the column such that the entire surface of the sorbent is covered by the copolymer; in this manner, the amount of separated copolymer can be substantially enlarged.

EXPERIMENTAL

The investigated samples of poly(styrene-*stat*-2-methoxyethyl methacrylate) of different mean composition were prepared by radical copolymerization.[12,13] Characteristics of individual studied samples are listed in Table I.

Nonmodified (bare) macroporous silica with particle diameter $d_p = 5$ μm and specific surface area of 61 m^2 g^{-1} was used; the silica was prepared by means of hydrothermal treatment[14] from a commercial silica Separon SIX (Laboratory Instruments Works, Prague). Although it is sometimes assumed that, owing to the strong interactions of the polymer segment/surface, the copolymer chains can enter pores that would be otherwise inaccessible,[15] the mean pore size of the employed silica was selected to be so high as to enable free access of all copolymer coils present in the analyzed samples. Owing to this selection—and also to the use of very short columns—the effect of steric exclusion of polymer coils differing in their hydrodynamic diameter[16] was minimized.

The dimensions of the stainless-steel columns were 40 × 4 mm i.d. (columns employed for measuring the adsorption characteristics), 100 × 4 mm i.d. (adsorption liquid chromatography with a linear gradient of mobile-phase composition), and, finally, 100 × 6 mm i.d. (desorption fractionation). The columns were packed by means of a slurry technique

Table I Characteristics of Poly(styrene-*stat*-2-methoxyethyl methacrylate) Copolymers

Sample	c_s (wt %)	x_s	Ψ (wt %)	$M_n \times 10^{-5}$ [a]	$M_w \times 10^{-5}$ [b]	M_w/M_n
Azeotropic copolymer						
AZ38	37.6	0.455	[c,d]	[d]	1.73	[d]
Low-conversion copolymers						
NK13	12.6	0.166	5.70	1.85	3.06	1.65
NK23	22.6	0.288	5.61	1.38	2.14	1.55
NK29	29.3	0.364	4.25	[d]	1.63	[d]
NK46	46.1	0.542	3.06	0.71	1.08	1.52
NK62	62.0	0.693	2.54	0.58	0.96	1.66
NK76	76.1	0.815	2.48	0.63	0.89	1.41

c_s is the content of styrene in the sample in wt %; x_s, the mole fraction of styrene units; Ψ, the (weight) conversion in polymerization; and M_n and M_w, the number- and weight-average molecular weights, respectively.
[a] Osmometry.
[b] Light scattering.
[c] Higher conversion.
[d] Not measured.

at 20 MPa in the mixed solvent methanol–dioxan (50 : 50 by volume). The column efficiency measured with nitrobenzene in heptane with 0.1 vol % of 2-propanol varied from 28,000 to 35,000 theoretical plates per meter of column length.

Mixed mobile phases normally used for separation of copolymers consist mostly of a thermodynamically good and a thermodynamically poor solvent for the given sample. We considered as decisive for the mobile-phase selection, in addition to questions of solubility, the adsorption affinity to the silica surface. A mixture 1,2-dichloroethane (DCE)/heptane, 50 : 50 v/v—referred to as component A (a mixture of a good and poor solvent for S/MEMA, which is itself a good solvent), was used as a mobile phase of low eluting power; tetrahydrofuran (THF, component B) was used as a component of high eluting power (displacer).

Heptane (p.a. grade, 95%, Loba Chemie, Austria)

was freed of UV-absorbing impurities by repeated azeotropic distillation with methanol. Peroxides were removed from THF (pure grade, VEB Apolda, Germany) using CuCl and the solvent was then dried by KOH and distilled. DCE (puriss. p.a. Fluka Chemie, Switzerland) was distilled before use.

The instrument shown schematically in Figure 1 was used in dynamical studies of copolymer adsorption on silica packed into chromatographic columns. The multiple-port valves enabled one to lead one of three different mobile phases to the inlet of the chromatographic column C. Initially, the column was connected—via valve V2—to pump P3, which delivered the displacer, THF, to wash away any adsorbed impurity and/or any adsorbed copolymer from previous experiments. Only when the column was thoroughly washed with the displacer (after the detector base-line was stabilized) was pump P3 disconnected by means of valve V2 and a solvent mix-
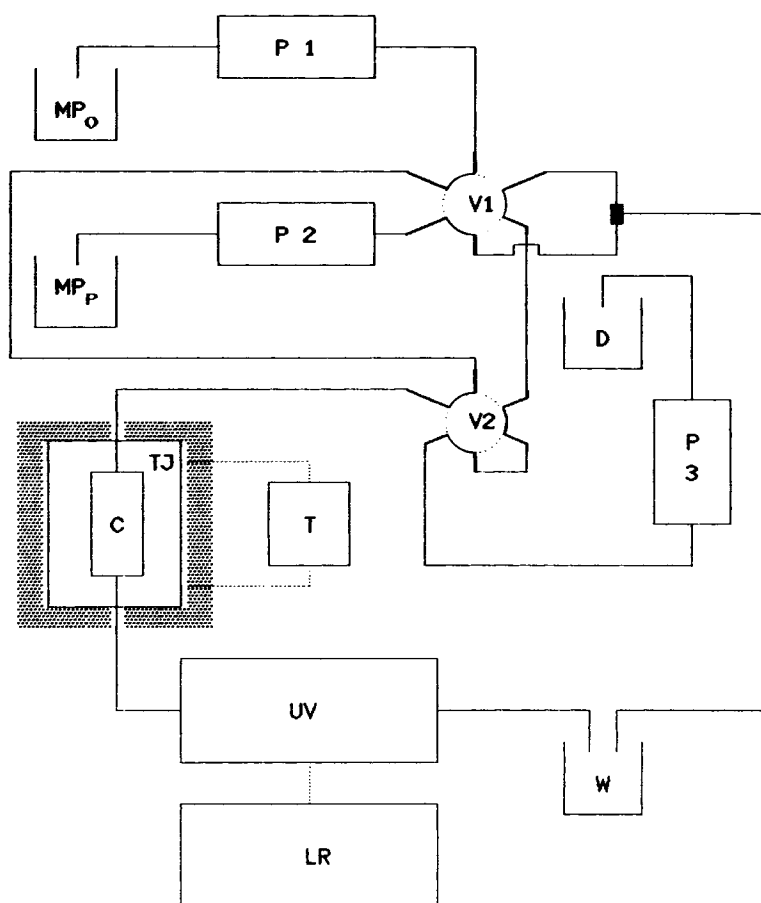

Figure 1 Apparatus used for studying copolymer adsorption. P1, P2, and P3 are pumps; V1 and V2, six-port valves; MP_o and MP_p, reservoirs of mobile phase and of mobile phase with dissolved copolymer; D, reservoir of displacer; C, column; TJ, thermostatting jacket; T, circulation thermostat; UV, UV detector; LR, line recorder.

ture delivered by either pump P1 or P2 allowed to enter the column. These two pumps delivered a mobile phase with the composition selected for the adsorption experiment; in addition, the mobile phase pumped by P2 contained the copolymer sample. One of the two pumps was connected to the column by means of the six-port valve V1: The column was first washed by the mobile phase not containing the copolymer (pump P1); 25 mL were considered to suffice for equilibration of columns 40 mm long. By rotating valve V1, the mobile phase with the dissolved copolymer sample was then pumped through the column by means of pump P2. The total amount of adsorbed copolymer was evaluated from the volume of the mobile phase that had passed through the column from the start to the first rise of detector signal; the volume was corrected by subtracting the dead volume of the column, determined by means of toluene.

The following pumps served as sources of pressurized mobile phases: P1, reciprocating piston micropump Waters Associates, Framingham, MA; P2, membrane micropump VCM 300 (Development Workshop, Czechoslovak Academy of Sciences, Prague); and P3, linear displacement pump VLD 30 purchased from the same manufacturer. Adsorption was followed by a variable-wavelength UV detector CE 2112 (Cecil Instruments, Cambridge, U.K.), connected to a two-channel recorder Servogor 220 (Goerz Electro, Wien, Austria). Columns were situated in a thermostatting jacket of our own design, connected to a throughflow water thermostat U1 (VEB Prüfgeräte Werk, Medingen, Germany). In adsorption experiments, the columns were kept at $15 \mp 0.2°C$. Saturation of the sorbent in columns 100×6 mm i.d. by the copolymer was performed at ambient. The flow rate was 0.8 mL min^{-1}.

A liquid chromatograph SP 8100 (Spectra Physics, San Jose, CA) with a UV detector SP 8400 and integrator SP 4200 was used in the chromatographic experiments. The shape of the stepwise gradient consisting of segments with constant mobile-phase composition is described below. The mobile phase consisted of component A (DCE + heptane, 50 : 50 v/v) and component B (THF). The difference in composition between the individual steps was varied from 1.5 to 2.5% B at a flow rate of 2 mL min^{-1}. HPLC experiments with a linear gradient of mobile-phase composition were performed with the same solvent system (A + B), using the following gradient: 0 min: 0% B; 15 min: 100% B; 25 min: 100% B; 27 min: 0% B; and 35 min: 0% B at a flow rate of 0.75 mL/min. In all cases, the detector was set to $\lambda = 260$ nm.

The composition of isolated fractions was also determined by spectrophotometry at 260 nm. The relevant calibration line was obtained by averaging results of several measurements of absorbancy of polystyrene standards differing in their molecular weight M. No dependence of absorbancy on M was detected between $M = 10^4$ and $M = 10^6$.

RESULTS AND DISCUSSION

Copolymer Adsorption

Prior to the fractionation proper of narrow-dispersion copolymers by means of controlled desorption from the silica surface, it was necessary to find optimum conditions of saturation by the copolymer of the sorbent packed in a chromatographic column. It has been ascertained by experiments performed with the described instrument that the total amount of adsorbed copolymer is independent of its concentration in the saturating solution. The flow rate may slightly influence the adsorbed amount, especially when a thermodynamically good mobile phase is used. Accordingly, all adsorption measurements were carried out at an identical flow rate of 0.8 mL min^{-1}. For designing an appropriate course of the stepwise gradient for desorption fractionation, it is necessary to establish the dependence of the adsorbed amount on the mobile-phase composition. The obtained curves are shown in Figure 2 for the azeotropic copolymer, and in Figures 3(a)–(d), for the low-conversion samples; in these experiments, component A (DCE : heptane 50 : 50 v/v), the basic mobile phase, contained variable amounts of com-
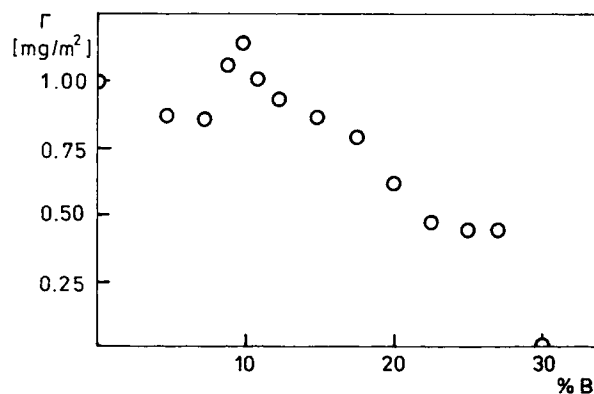
Figure 2 Displacement isotherm of the azeotropic copolymer S/MEMA. Mobile phase: DCE + heptane (50 : 50 v/v); B designates vol % of THF, and Γ (mg m^{-2}), adsorbed amount of copolymer AZ38.
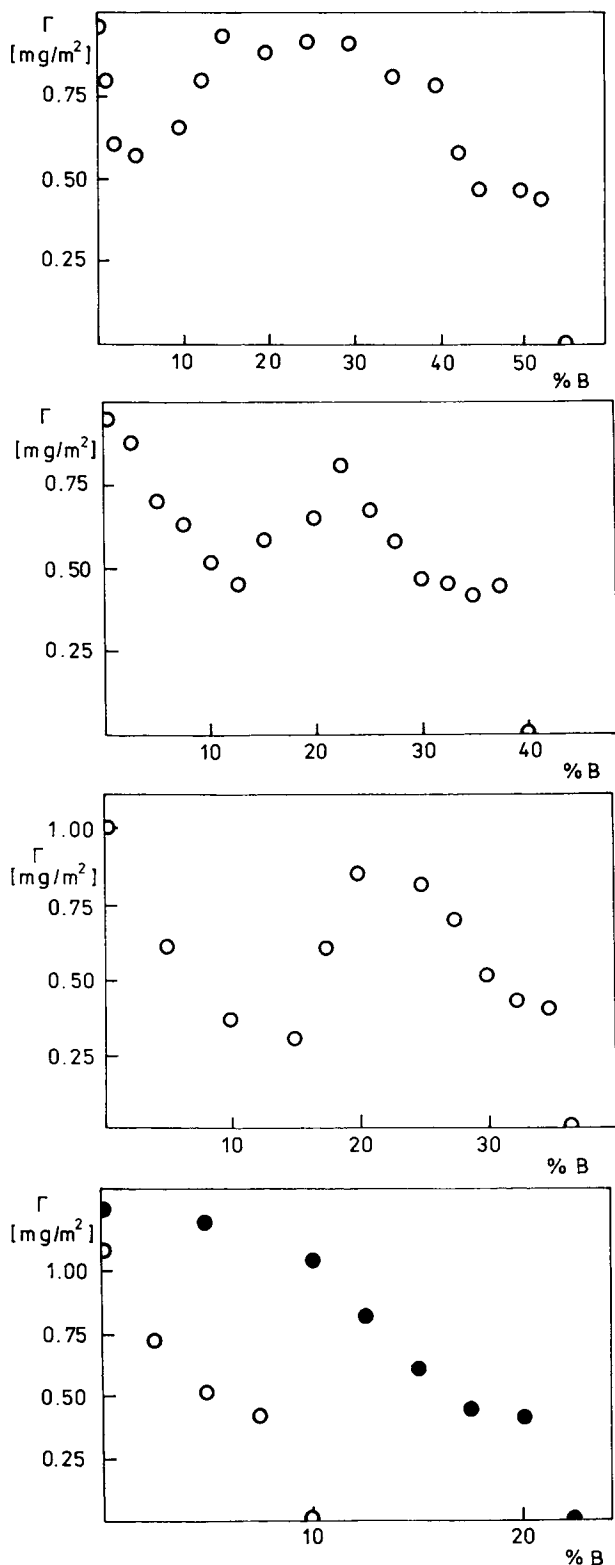
Figure 3 Displacement isotherms of low conversion copolymers. Mobile phase: DCE + heptane (50 : 50 v/v) with B designating vol % of THF; Γ (mg m^{-2}), adsorbed amount. (a) NK13; (b) NK23; (c) NK29; (d) (●) NK46 and (○) NK62.

ponent B (THF). The adsorbed amount was measured repeatedly for each mobile phase composition; the deviations did not exceed ∓3%.

The curves in Figures 2 and 3 represent displacement isotherms *sui generis*, but were measured not by the standard static (equilibrium) method, but dynamically, under chromatographic conditions. Accordingly, some specific features are to be expected. In case of classical displacement isotherms, the so-called critical solvent composition is taken as that composition where the total amount of adsorbed copolymer drops to zero; it depends on the interactions between individual components present in the system and varies between polymer chains differing in both length and composition.[17,18] The value obtained for a polydisperse sample will coincide with that measured for those chains that require for their desorption the highest concentration of the displacer. If the composition of the mobile phase corresponding to essentially zero adsorption of the sample is determined by the dynamic method, we can infer that the least retained chains of the copolymer in question just cease to be adsorbed in the mobile phase of that composition—very valuable information from the point of view of chromatography. This composition, which we propose to call the first critical composition, is experimentally inaccessible by the static method.

The available literature data for copolymers S/MEMA do not allow us to compare the values of the adsorbed amount reported here with data obtained by static experiments. However, a rough comparison can be made with the behavior of a similar copolymer, viz. styrene/methyl methacrylate. Kawaguchi et al.[19,20] measured the equilibrium amounts of that copolymer adsorbed from trichloroethylene and found that the values ranged from 0.9 to 1.3 mg m^{-2}, depending on the content of styrene units, in full agreement with our data. It seems that in the case of strongly interacting copolymer segments, represented by either methyl methacrylate or 2-methoxyethyl methacrylate units, the adsorption does not depend appreciably on the time of contact of the macromolecule with the surface. In assessing the magnitude of the adsorbed amount, one must take into account the incomplete saturation of the surface in dynamic measurements, due to the necessity to finish the saturation at the moment of first appearance of the copolymer at the column outlet, because only in this manner is it possible to prevent possible competition between adsorption of chains differing in length and composition, necessarily present in the polydisperse sample.

The displacement isotherms shown in Figures 2

and 3 are quite complex. From the practical point of view, it is important, however, that close to the first critical composition they exhibit a clearly apparent region where the adsorbed amount is independent of the mobile-phase composition (this region is only suggested for samples with the highest content of styrene building blocks). To explain the existence of such a region, one may identify it—following the Cohen-Stuart theory[17,18]—with that composition where the lattice model predicts the polymer concentration at the surface to be so low that the segment density in all layers of the lattice (with the exception of the surface layer) is the same as that in bulk solution. Accordingly, in this compositional region, only the first layer adjacent to the surface contributes to the free energy of adsorption. The amount of adsorbed polymer is then depressed in comparison with the case when other lattice layers also contribute to the overall adsorption owing to the prevalence of interactions between the segment and the adsorption site.

In the vicinity of the first critical composition, where the adsorbed amount is independent of the solvent composition, there is a very low probability that segments strongly interacting with the surface will be adsorbed in the order adsorption site—segment—chain segment without direct contact with the surface; on the other hand, this possibility cannot be excluded under conditions where interactions between the solvent molecules and the polymer segments are much weaker than are the interactions between the polymer segments and the adsorption sites on the surface. Although a possible multilayer adsorption would raise the total amount of adsorbed polymer, it could adversely affect the desorption fractionation. Saturation of the surface preceding the desorption fractionation must therefore be carried out from solutions having a composition close to the first critical composition.

The displacement isotherms of copolymer samples with styrene content not exceeding about 50% exhibit local extremes. Also, here, the Cohen-Stuart theory[17,18] offers a plausible explanation, as it predicts, for the case of nonzero interactions in a binary system solvent–displacer, the existence of two different critical compositions and a nonmonotonous course of the displacement isotherm; however, one must bear in mind that in our case we deal with a more complex system containing three solvents.

Figure 4 shows how the adsorbed amount depends on the copolymer composition. It is apparent from Figure 4(a) and (b) that the adsorbed amount is independent of the composition of the S/MEMA copolymer when the measurements are carried out

near the first critical solvent composition or in a pure component A—only the sample containing 62% styrene units exhibits a slight maximum exceeding the experimental error.

These results can be compared with the data obtained by the static method only for the analogous copolymer styrene/methyl methacrylate. Herd et al.[21] observed that the amount adsorbed from trichloroethylene remains the same over a broad range of solvent composition. On the other hand, Kawaguchi and co-workers,[20] who studied the same copolymer in the same solvent, found a maximum adsorbed amount at around 75% of styrene units in the copolymer, while in the region below 50% styrene, the adsorbed amount was approximately constant. The latter authors explained the dependence of the adsorbed amount on copolymer composition by assuming that above about 25% styrene not only the methacrylate but also the styrene units begin to take part in adsorption. Herd et al. refuted this possibility on the basis of previous experiments done by Thies[22] where adsorbed polystyrene was completely displaced by methyl methacrylate.

Our results seem to agree with the earlier results reported by Herd et al.,[21] but the reason may lie in the adopted dynamic method of measurement. Static experiments[20,21,23,24] have shown that not only methyl methacrylate but also styrene is adsorbed from both trichloroethylene and DCE (and the more so from the mixture DCE–heptane). Participation of styrene units in the adsorption of copolymer chains on silica cannot, therefore, be excluded a priori. However, we were able to demonstrate by separate experiments that measurable amounts of polystyrenes of widely different molecular weight were not adsorbed under the dynamic adsorption conditions employed, not even from the thermodynamically poorest solvent, i.e., component A. We may therefore reasonably assume that only the 2-methoxethyl methacrylate units are responsible for adsorption of the copolymer chains on silica under the dynamic experimental conditions employed here.

In assessing the curves shown in Figure 4, it must be taken into account that our experiments were carried out with a relatively complex mixture of solvents, where mutual interactions between the individual components will govern the overall adsorbed amount of the copolymer. This is confirmed by the data presented in Figure 4(c), where the dependencies of the adsorbed amount on the copolymer composition are plotted for different contents of THF in the mobile phase: The resulting curves differ considerably from the almost constant dependencies shown in Figure 4(a) and (b).
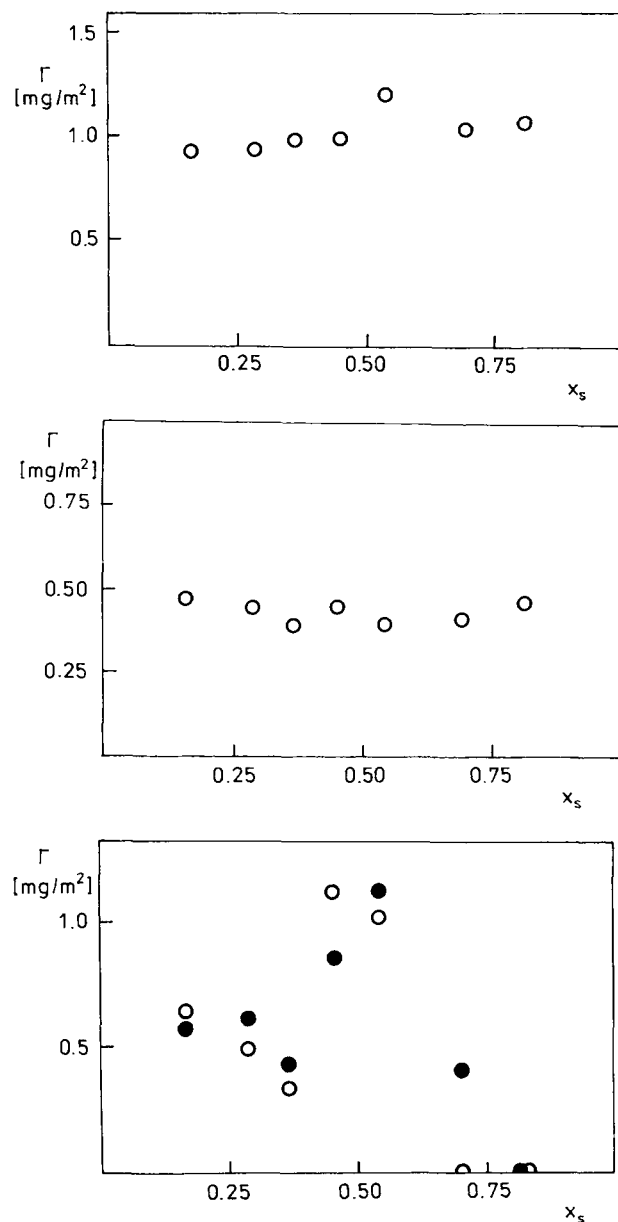
Figure 4 Adsorbed amount Γ (mg m^{-2}) of S/MEMA samples as a function of mole fraction x_s of styrene units in the copolymer: (a) mobile-phase DCE + heptane (50 : 50 v/v); (b) mobile-phase composition equal to the first critical composition of the given sample; (c) mobile-phase (●) 7.5 vol % THF and (○) 10.0 vol % THF in DCE + heptane (50 : 50, v/v).

Chromatographic Fractionation

Chromatographic fractionation of the copolymers was carried out with the already mentioned stepwise gradient of mobile-phase composition, using columns of dimensions 6 × 100 mm. The samples were adsorbed and fractionated at ambient. The column

was saturated by the copolymer dissolved in a mobile phase having a composition close to the first critical composition determined for the copolymer in question; the same composition was also used for the first step of the stepwise gradient.

An example of a preliminary fractionation of the azeotropic copolymer AZ38 is shown in Figure 5. It is apparent that the individual fractions indeed elute at an unequivocal mobile-phase composition: When the composition of the mobile phase was returned to a level already employed, the chromatogram shows no additional response. Figure 5 also demonstrates that the magnitude and number of obtained fractions can be easily controlled by changing the differences between the concentrations of individual steps.

Under the employed conditions, the overall adsorbed amount on the column was about 40 mg; the stepwise gradient was selected so as to separate this amount of copolymer into 7-8 fractions. Examples of chromatographic separations of samples AZ38, NK13, and NK62 are in Figures 6-8, where the course of the stepwise gradient employed is always shown directly on the chromatogram, along with the sensitivity of the record relative to the sensitivity of the chromatogram of sample NK13 (the number represents the detector sensitivity used for the copolymer in question, divided by the sensitivity used
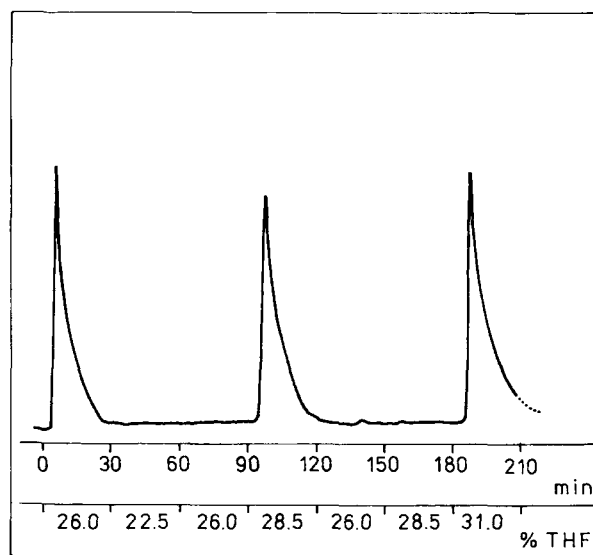

Figure 5 Results of fractionation of sample AZ38 by controlled desorption in a stepwise gradient with repeated steps: column 40 × 4 mm, UV detection at 260 nm, flow rate 1 mL/min, mobile-phase DCE + heptane (50 : 50 v/v, component A), THF (component B), initial composition A : B = 77.5 : 22.5 v/v.

for sample NK13). Analogous chromatograms were obtained by fractionation of the remaining samples by the desorption method.

Individual fractions were collected, dried, and characterized with regard to their mean composition. Each sample was fractionated three times under identical conditions; the results are listed in Table II. Spectrophotometric determination of composition of fractions met with some difficulties, however, due to the fact that the mass of isolated fractions was relatively small and impurities absorbing at the same wavelength as the styrene units of the copolymer were present (they were concentrated in the fraction because of the necessity to evaporate considerable amounts of the mobile phase). The contribution of impurities to the absorbancy was corrected for on the basis of a chromatographic analysis of individual isolated fractions (more about that below). Under the simplifying assumption that the molar absorptivities of styrene units and of the impurities are similar, and using the known weight of the dry residue, it was possible to evaluate and subtract the amount of impurities. However, this obvious drawback of spectrophotometry led us to an attempt to determine the mean composition of fractions by another, independent method.

Glöckner et al.[11] described a linear gradient of mobile-phase composition that they found suitable for separating, with a reasonable efficiency, a mix-

Figure 7 Fractionation of sample NK13 by controlled desorption: column 100 × 6 mm, mobile-phase DCE + heptane (50 : 50 v/v, component A), THF (component B), initial composition A : B = 50 : 50 v/v), flow rate 2 mL/min, UV detection at 260 nm, relative sensitivity 1.

ture of copolymers S/MEMA with mean composition similar to that of samples used in this study. We obtained analogous results with a gradient based on the above components A and B. The results are shown in Figure 9, where the retention times of
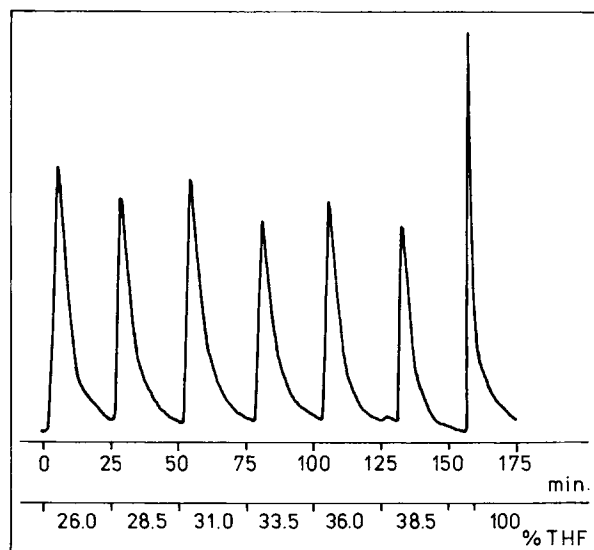

Figure 6 Fractionation of azeotropic sample AZ38 by controlled desorption: column 100 × 6 mm, mobile-phase DCE + heptane (50 : 50 v/v, component A), THF (component B), initial composition A : B = 77.5 : 22.5 v/v), flow rate 2 mL/min, UV detection at 260 nm, relative sensitivity 1.3.
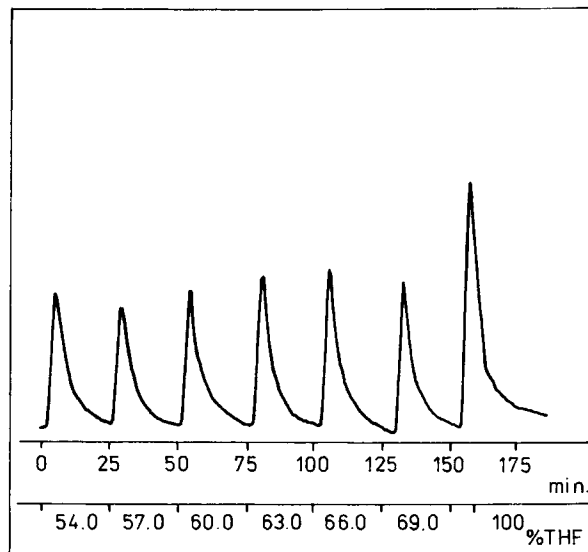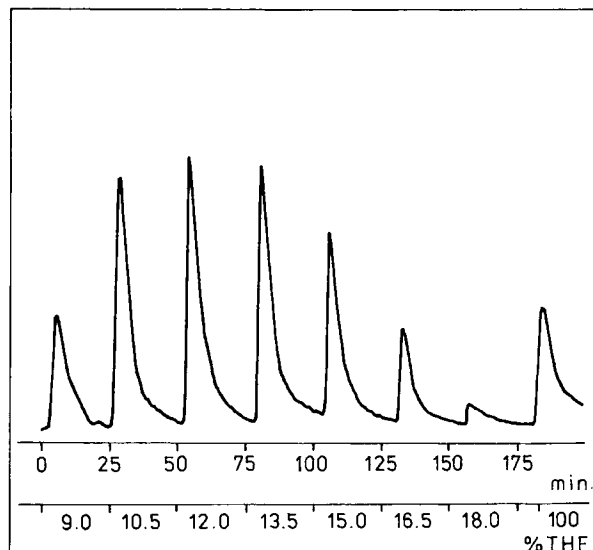

Figure 8 Fractionation of sample NK62 by controlled desorption: column 100 × 6 mm, mobile-phase DCE + heptane (50 : 50 v/v, component A), THF (component B), initial composition A : B = 92.5 : 7.5 v/v), flow rate 2 mL/min, UV detection at 260 nm, relative sensitivity 2.6.

Table II   Results of Chromatographic Desorption Fractionation

Sample		Fraction No.							
		1	2	3	4	5	6	7	8
AZ38	% B	26.0	28.5	31.0	33.5	36.0	38.5	100.0	
	c_s (UV)	40.6	38.6	36.8	36.8	35.5	34.1	32.1	
	c_s (LC)	42.3	40.3	39.8	39.2	38.3	36.9	34.3	
	v	0.150	0.156	0.162	0.151	0.143	0.137	0.101	
NK13	% B	54.0	57.0	60.0	63.0	66.0	69.0	100.0	
	c_s (UV)	14.4	13.4	12.7	11.5	10.6	9.7	—	
	c_s (LC)	15.0	13.4	11.5	11.1	10.8	10.4	9.4	
	v	0.110	0.077	0.166	0.151	0.144	0.152	0.199	
NK23	% B	46.0	48.5	51.0	53.5	56.0	58.5	62.0	100.0
	c_s (UV)	26.8	26.0	25.3	24.5	23.2	22.7	22.0	21.0
	c_s (LC)	23.8	20.8	20.8	19.9	19.1	18.7	17.8	18.2
	v	0.090	0.118	0.134	0.128	0.131	0.131	0.144	0.125
NK29	% B	35.5	38.0	40.5	43.0	45.5	48.0	50.5	100.0
	c_s (UV)	33.8	32.2	31.1	30.6	30.0	28.5	26.9	28.1
	c_s (LC)	33.2	32.1	31.7	30.7	30.0	29.7	28.8	28.2
	v	0.115	0.126	0.131	0.136	0.128	0.116	0.111	0.137
NK46	% B	20.0	22.5	25.0	27.5	30.0	32.5	100.0	
	c_s (UV)	47.7	45.0	44.3	42.5	42.9	41.5	41.2	
	c_s (LC)	47.9	45.0	44.1	43.6	42.9	42.2	41.4	
	v	0.148	0.200	0.217	0.188	0.133	0.063	0.051	
NK62	% B	9.0	10.5	12.0	13.5	15.0	16.5	18.0	100.0
	c_s (UV)	(73.2)	67.0	65.8	64.8	63.5	62.1	59.4	58.1
	c_s (LC)	65.6	64.3	63.5	62.8	61.8	60.8	—	—
	v	0.111	0.191	0.176	0.192	0.131	0.073	0.029	0.097
NK76	% B	4.0	5.5	7.0	8.5	10.0	11.5	100.0	
	c_s (UV)	(89.9)	80.9	78.7	(79.4)	75.6	69.8	68.6	
	c_s (LC)	79.3	76.8	74.5	73.0	71.1	70.0	68.8	
	v	0.121	0.288	0.269	0.172	0.082	0.030	0.037	

% B is the mobile phase composition necessary for eluting a given fraction (in vol % THF in component A); c_s is the chemical composition of the fraction in wt % of styrene units, measured by UV spectrophotometry (UV) or by adsorption liquid chromatography with linear gradient of mobile phase composition (LC); v is the weight fraction.

nonfractionated S/MEMA copolymers are plotted against their mean composition. The resulting linear calibration dependence was used for determining the composition of fractions isolated by means of the desorption method from individual polymer samples. It follows from Table II [where the composition of fractions determined in this manner is designated as c_s(LC)] that in most instances both methods give similar results, in spite of the discussed deficiencies of spectrophotometry.

Distribution of Chemical Composition

On the basis of the Mayo–Lewis copolymerization model,[21] Stockmayer[1] derived a bivariate mass fre-

quency function of a copolymer, where the variates are the degree of polymerization and the deviation of the composition of individual chains from the mean sample composition. For the case of unequal molecular weights of the two comonomers, the Stockmayer result was further generalized by Stejskal and Kratochvíl,[6] who, moreover, replaced the most probable distribution used by Stockmayer by a more general Schulz–Zimm two-parameter frequency function. If the chemical composition is expressed by means of weight fractions, the statistical chemical heterogeneity is described by the following marginal mass frequency function of chemical composition (a function that respects only chemical
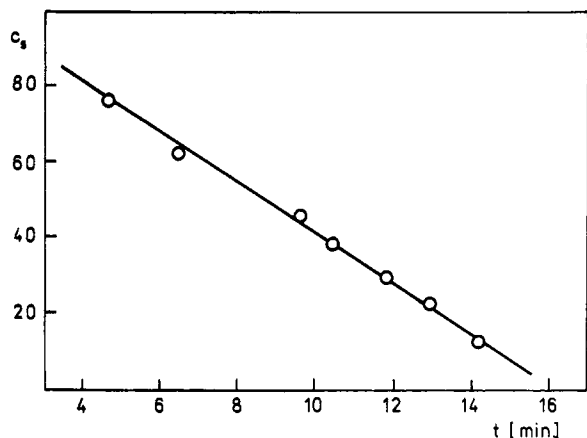
Figure 9 Retention times of nonfractionated S/MEMA copolymers in adsorption chromatography with linear gradient of mobile-phase composition, plotted as a function of copolymer composition, expressed as weight fraction of styrene units c_s.

heterogeneity and disregards differences in chain length)[6]:

$$W_s^+(w)$$

$$= \frac{\Gamma(a + \frac{3}{2})}{\Gamma(a + 1)\Gamma(\frac{1}{2})} \frac{D(w)}{\{1 + [z(w)]^2\}^{(a+3/2)}} \quad (1)$$

where

$$z(w) = \left\{\frac{P_n t}{2ak\bar{w}(1 - \bar{w})} \frac{(w - \bar{w})^2}{[(1 - t)\bar{w} + t]^2}\right\}^{1/2}$$

$$D(w) = \left[\frac{P_n t}{2ak\bar{w}(1 - \bar{w})}\right]^{1/2} \frac{(1 - t)\bar{w} + t}{[(1 - t)w + t]^2}$$

The parameters are defined as follows:

$$k = \left[1 + \frac{4t\bar{w}(1 - \bar{w})}{[(1 - t)\bar{w} + t]^2}(r_A r_B - 1)\right]^{1/2}$$

$$a = \left(\frac{P_w}{P_n} - 1\right)^{-1}$$

$$b = \frac{a}{P_n} = \frac{a + 1}{P} = (P_w - P_n)^{-1}$$

where r_A and r_B are the monomer reactivity ratios in the kinetic Mayo–Lewis model.[25] For the number- and weight-average degree of polymerization, P_n and P_w, we then have

$$P_n = \frac{M_n}{M_{ox}} = \frac{M_n}{M_{oA}\bar{x} + M_{oB}(1 - \bar{x})}$$

$$P_w = \frac{M_w}{M_{ow}} = \frac{M_w}{M_{oA}\bar{w} + M_{oB}(1 - \bar{w})}$$

where M_n and M_w are the number- and weight-average molecular weights, respectively. The mole (x) and the weight (w) fractions are related through the formula $x = w/[(1 - t)w + t]$, where $t = M_{oA}/M_{oB}$ is the ratio of molecular weights of the comonomers A and B. The barred quantities designate mean values. The marginal distribution function that describes only the chemical heterogeneity can be derived by integration of (1) between the limits 0 and w.

By the above formulae, one can express the distribution of chemical composition caused exclusively by statistical chemical heterogeneity on the basis of experimentally accessible values, assuming that the copolymerization obeys the Mayo–Lewis kinetics.

Figure 10 shows marginal distributions of chemical composition for the analyzed S/MEMA copolymers, derived from the mass of isolated fractions and their chemical composition as determined by the gradient adsorption chromatography. The dotted
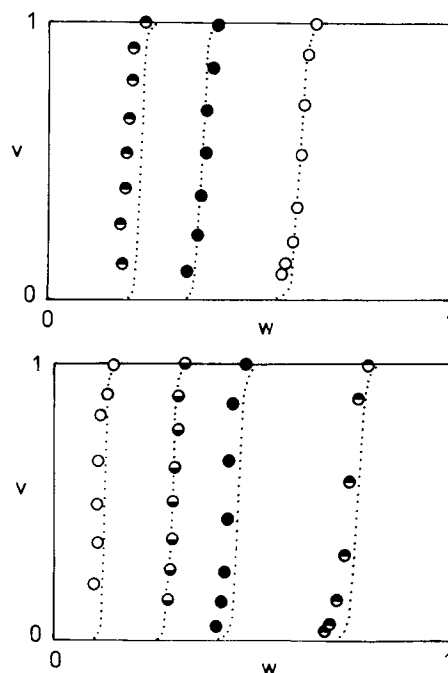

Figure 10 Marginal distributions of chemical composition of S/MEMA copolymers; w is the weight fraction of styrene units in the copolymer and v is the weight fraction of macromolecules. The dotted curves represent the theoretical distribution functions—see text. (a) Samples: (●) AZ38; (◒) NK23; (○) NK62.
(b) Samples: (○) NK13; (◒) NK29; (●) NK47; (◓) NK76.

curves represent the theoretical distributions calculated by integrating eq. (1) for r_A = 0.41 (styrene) and r_B = 0.48 (2-methoxyethyl methacrylate).[12]

It is apparent from the figures that for copolymers AZ39, NK13, NK29, and NK62 the experimental points coincide with the theoretical dependencies. For the remaining copolymers NK23, NK46, and NK76, the experimental data, although following the general trend of the calculated curves, are somewhat shifted toward a lower styrene content. This slight discrepancy may be caused either by deviations from linearity of the calibration dependence (Fig. 9), which could cause inaccurate determination of the mean composition of fractions, or by bias in the determination of the mean composition of nonfractionated copolymers. Nevertheless, the agreement between experiment and theory is seen to be good.

It is thus apparent that the proposed method of fractionation by controlled desorption enables one to separate copolymers with low chemical heterogeneity into fractions differing in their chemical composition. The total time required for one separation, including the sorbent saturation, is about 4 h. This time is relatively long from the point of view of modern HPLC, but is offset by the fact that the weight of sample that can be separated is relatively large—even with very short columns larger by a factor of about 100 more than the amount injected into a standard HPLC column—so that the separation is semipreparative.

The applicability of the proposed method to copolymers with broad distribution of chemical composition, graft, or block copolymers is studied here, along with the possible effect of molar mass distribution on the separation.

REFERENCES

1. W. H. Stockmayer, *J. Chem. Phys.*, **13**, 199 (1945).
2. V. E. Meyer and G. G. Lowry, *J. Polym. Sci. A*, **3**, 2843 (1965).
3. J. Stejskal and P. Kratochvíl, *J. Appl. Polym. Sci.*, **25**, 407 (1980).
4. S. Teramachi and Y. Kato, *Macromolecules*, **4**, 54 (1971).
5. J. Stejskal, P. Kratochvíl, and D. Straková, *Macromolecules*, **14**, 150 (1981).
6. J. Stejskal and P. Kratochvíl, *Macromolecules*, **20**, 2624 (1987).
7. H. Inagaki, H. Matsuda, and F. Kamiyama, *Macromolecules*, **1**, 520 (1968).
8. S. Teramachi, A. Hasegawa, Y. Simha, M. Akatsuka, and M. Nakajima, *Macromolecules*, **12**, 992 (1979).
9. H. Sato, H. Takeuchi, and Y. Tanaka, *Macromolecules*, **19**, 2613 (1986).
10. G. Glöckner, J. Stejskal, and P. Kratochvíl, *Makromol. Chem.*, **190**, 427 (1989).
11. G. Glöckner, M. Stickler, and W. Wunderlich, *J. Appl. Polym. Sci.*, **37**, 3147 (1989).
12. J. Stejskal, D. Straková, O. Procházka, and P. Kratochvíl, *Collect. Czech. Chem. Commun.*, **48**, 2656 (1983).
13. J. Stejskal and P. Kratochvíl, *Macromolecules*, **11**, 1097 (1978).
14. M. Kubín, B. Porsch, P. Špaček, S. Voska, and P. Holler, *Chem. Prum.*, **7**, 364 (1979).
15. M. Danielewicz and M. Kubín, *J. Appl. Polym. Sci.*, **26**, 951 (1981).
16. G. Glöckner, *Adv. Polym. Sci.*, **79**, 159 (1986).
17. M. A. Cohen-Stuart, G. J. Fleer, and J. M. H. M. Scheutjens, *J. Colloid Interface Sci.*, **97**, 515 (1984).
18. M. A. Cohen-Stuart, G. J. Fleer, and J. M. H. M. Scheutjens, *J. Colloid Interface Sci.*, **97**, 526 (1984).
19. M. Kawaguchi, I. Katsunori, S. Yamagiwa, and A. Takahashi, *Macromolecules*, **22**, 2204 (1989).
20. S. Yamagiwa, M. Kawaguchi, T. Kato, and A. Takahashi, *Macromolecules*, **22**, 2199 (1989).
21. J. M. Herd, A. J. Hopkins, and G. J. Howard, *J. Polym. Sci. C*, **34**, 211 (1971).
22. C. Thies, *J. Phys. Chem.*, **70**, 3784 (1966).
23. A. Takahashi and M. Kawaguchi, *Adv. Polym. Sci.*, **46**, 1 (1982).
24. G. J. Howard, *Surfactant Sci. Ser.*, **21**, 281 (1987).
25. F. R. Mayo and F. M. Lewis, *J. Am. Chem. Soc.*, **66**, 1594 (1944).