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COPOLYMER FRACTIONATION BY GRADIENT HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

GOTTFRIED GLÖCKNER*
Dresden University of Technology, Department of Chemistry, Dresden (G.D.R.)
and
JO H. M. VAN DEN BERG*
DSM Research and Patents, FPO, Geleen (The Netherlands)

SUMMARY

The size distribution and composition of statistical copolymers has been investigated by size-exclusion chromatography (SEC) and off-line gradient high-performance liquid chromatography of the SEC fractions. The copolymers were poly(styrene–acrylonitrile) (SAN) and poly(styrene–methyl methacrylate) (SMMA) model mixtures. SAN copolymers were separated with a suitable gradient of increasing dissolution power on normal-phase columns as well as on reversed-phase columns. In contrast, SMMA copolymers were eluted from a reversed-phase column by a solubility gradient as broad bands, whereas the same gradient gave excellent resolution on a normal-phase column. The elution characteristics of SMMA samples roughly parallelled their solubility characteristics in the system investigated but there was an extra contribution to retention on active columns.

INTRODUCTION

Cross fractionation (CF) of copolymers is a combination of their separation according to molecular size with the analysis of the chemical composition of each fraction[1], *i.e.*, the two-dimensional analysis of the molar mass distribution (MMD) and chemical composition. With classical fractionating techniques, the investigation of a copolymer requires 10–20 g of sample material and 8–16 weeks of experimental work. There have been few reports on classical CF, which does not reflect the importance of this technique in fundamental polymer science and copolymer technology.

Chromatographic investigation of copolymers usually involves analysis by size-exclusion chromatography (SEC) with dual detection.

This paper deals with CF of synthetic copolymers by the combination of SEC and high-performance liquid chromatography (HPLC). The method is similar to

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* Present address: Duphar B.V., Research Laboratories, Weesp, The Netherlands.

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"coupled-column chromatography" for the investigation of complex mixtures[2]. Copolymers have already been investigated by combinations of dry-column adsorption chromatography and SEC[3,4] or of SEC and thin-layer chromatography (TLC)[5,6]. SEC and turbidimetric titration of the fractions have also been employed[7–9]. Optimum results have been obtained by the combination of SEC and HPLC of the fractions[10,11].

EXPERIMENTAL

HPLC apparatus

System 1 comprised a liquid chromatograph 1090 A (Hewlett-Packard, Waldbronn, F.R.G.) with a ternary solvent-delivery system, Model DR5, a temperature-controlled column compartment, an autosampler, an autoinjector, a diode-array detector, Model HP 1040 A, an integrator, Model 3392 A, and a system controller Model HP 85 with double disc drive, HP 9121 D, and a plotter, HP 7470. Additionally, an analog recording device was connected to the analog port of the HP 1090 A.

System 2 comprised a high-pressure pump Model 5200 with an UV detector Model 8100 (Knauer, Bad Homburg, F.R.G.) and a laboratory-built gradient device using a metering pump MMC (Mikrotechna, Prague, Czechoslovakia) with pump head 2c for the controlled addition of solvent B to the mixture at the eluent inlet of the high-pressure pump[12].

System 3 comprised a liquid chromatograph Model 5020 (Varian, Palo Alto, CA, U.S.A.), equipped with a sampling valve Model 7105 (Rheodyne, Berkeley, CA, U.S.A.) and a variable-wavelength flow-through UV photometer Model SF 770 (Schoeffel, Kratos, Westwood, NJ, U.S.A.).

Columns: 1, 150 mm × 4.6 mm, Nucleosil CN, d_p = 5 μm, spherical; 2, 150 mm × 4.6 mm, Polygosil 60-5, d_p = 5 μm; 3, 150 mm × 4.6 mm, Polygosil 60-5 C_{18}, d_p = 5 μm; 4, 300 mm × 4.0 mm, μBondagel, E-1000, d_p = 10 μm; 5, 150 mm × 4.6 mm, LiChrospher Si 1000 C_8, d_p = 10 μm; 6, 60 mm × 4.0 mm (Cartridge), Nucleosil C_{18}, d_p = 5 μm; 7, 60 mm × 4.0 mm (Cartridge), Nucleosil 50, d_p = 5 μm; 8, 150 mm × 4.6 mm, LiChrosorb Si 4000 C_{18}, d_p = 10 μm.

Samples

The copolymers investigated had been prepared by radical polymerization under conditions ensuring a narrow range of chemical compositions. The specimens had been reprecipitated twice. For HPLC, they were dissolved in tetrahydrofuran (THF) stabilized with butylated hydroxytoluene. Some properties of the samples are listed in Table I.

Solvents

THF was distilled under nitrogen to remove the stabilizer and was kept under nitrogen to prevent peroxide formation. *n*-Hexane (pa quality) was obtained from VEB Berlinchemie (Berlin, G.D.R.) and isooctane (2,2,4-trimethylpentane, purum quality) from Fluka (Buchs, Switzerland). The solvents were filtered through a 0.45-μm filter and purged with nitrogen. Methanol was obtained from Fisons (Loughborough, U.K.) as HPLC quality. The gradients used are listed in Table II.

TABLE I

THE COPOLYMERS INVESTIGATED

	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
(1) Poly(styrene–acrylonitrile) (SAN) samples							
Acrylonitrile (mol. %)	27.4	36.9	44.6	52.9	59.4		
(wt. %)	16.1	23.0	29.2	36.4	42.5		
Molar mass (kg/mol)							
by osmosis	325	480	510	380	340		
by light scattering		825					
Content in mixture A (in μg per 30 μl)	7.2	14.1	11.1	16.35	13.95		
(2) Poly(styrene–methyl methacrylate) (SMMA) samples							
Methyl methacrylate (mol. %)	88.9	76.9	64.9	50.5	37.9	24.5	11.8
Molar mass (kg/mol)							
by light scattering	220	220	235	185	150	250	160
Content in mixture B (wt. %)	18.5	17.2	12.7	12.7	12.1	14.0	12.7
Content in mixture C (in μg per 9 μl)	–	5.8	–	3.3	–	2.0	–

HPLC conditions

The flow-rate was 1 ml/min, unless stated otherwise. Column temperature: 50°C. Detection, UV at 259 nm.

RESULTS AND DISCUSSION

Chromatographic cross-fractionation (CCF) of SAN (model mixture A and commercial samples) had been performed by SEC fractionation with THF as an eluent. Without any additional preparation the fractions were subsequently analyzed by HPLC on column 1 using gradient program 37b/84 (see Table II)[11].

Fig. 1 shows a similar separation performed on a model mixture of SMMA specimens. Here, the mixture of seven copolymers with a methyl methacrylate (MMA) content ranging from 12 to 89 mol. % was investigated by SEC using THF as an eluent, and by HPLC on a silica column. The gradient elution was performed with isooctane and THF, the concentration of the latter increasing at 5%/min.

In both CCF experiments, individual peaks for the specimens in the model mixtures were obtained. The difference in the patterns of the different SEC fractions is mainly due to the amount of sample contained. A given sample volume injected into the gradient HPLC system will yield a higher signal when taken from an SEC fraction near to the maximum of the SEC curve than from a fraction at the edges. Although the influence of molar mass is only small, in both examples the HPLC peaks appear earlier for SEC fractions of a higher fraction number, *i.e.*, for lower solute molar masses.

The flattening of the peaks with increasing retention is more pronounced with

TABLE II

GRADIENT PROGRAMS

n-hex = *n*-hexane; *i*-oct = isooctane; THF = tetrahydrofuran.

1/83	t/min	0	3.5	5.5	8	9	18.5	
	n-hex (%)	90	90	70	70	65	20	
	THF* (%)	10	10	30	30	35	80	
	(smoothly shaped gradient, HPLC No. 2)							
2/83	t/min	0	8	9.9	10	11		
	i-oct (%)	90	50		20	0		
	THF* (%)	10	50		80	100		
	Flow-rate (ml/min)	1		1	0.3	0.3		
28/84 30/84	t/min	0	1	1.2	1.8	2	22	23
	i-oct (%)	90	90	85	55	50	0	0
	THF* (%)	10	10	15	45	50	100	100
37b/84	t/min	0	10	15	17			
	i-oct (%)	50	0	0	0			
	THF (%)	50	100	50	50			
	Methanol (%)	0	0	50	50			
04/85	t/min	0	9.5	11.5	13			
	i-oct (%)	95	0	0	0			
	THF (%)	5	100	100	60			
	Methanol (%)	0	0	0	40			
58/85	t/min	0	14	16	17			
	i-oct (%)	90	20	0	0			
	THF (%)	10	80	100	0			
	Methanol (%)	0	0	0	100			

* With 10% (v/v) methanol added.

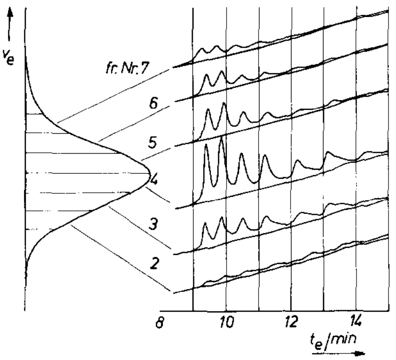
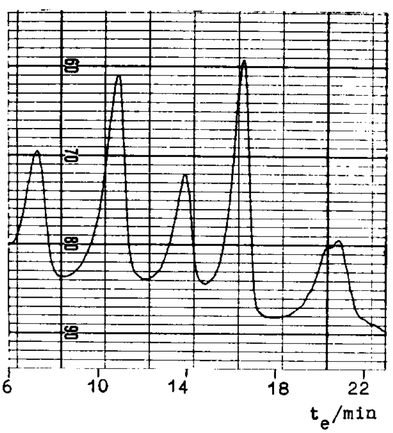


Fig. 1. Size-exclusion chromatography of 1.0 mg of SMMA mixture B (see Table I) and HPLC tracings of the SEC fractions on silica (column 7). Gradient No. 58/85 (see Table II).



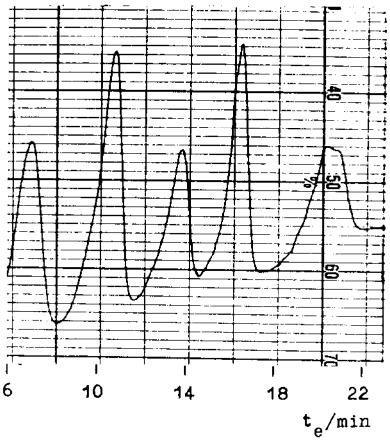


Fig. 2. Gradient chromatogram of SAN mixture A (see Table I) on silica (column 2): V_0 = 30 μl; m_0 = 62.7 μg; gradient No. 30/84. First peak is SAN I.

Fig. 3. Gradient chromatogram of SAN mixture A on reversed-phase C_{18} (column 3): V_0 = 30 μl; m_0 = 62.7 μg; gradient No. 30/84. First peak is SAN I.

the SMMA than with the SAN mixture. This might be simply due to the wider range of composition of mixture B in comparison with A (12–89 *vs.* 27–59%). On the other hand, there is a striking difference in the behaviour of SAN and SMMA in gradient HPLC, which also might be the reason for the flattening effect.

Fig. 2 shows the separation by composition of mixture A containing five SAN specimens on silica column 2 and Fig. 3 the corresponding results obtained on the reversed-phase C_{18} column 3. Fig. 4a and b show HPLC patterns obtained on repeated injections of the same mixture into the wide-pore reversed-phase C_{18} column 8.

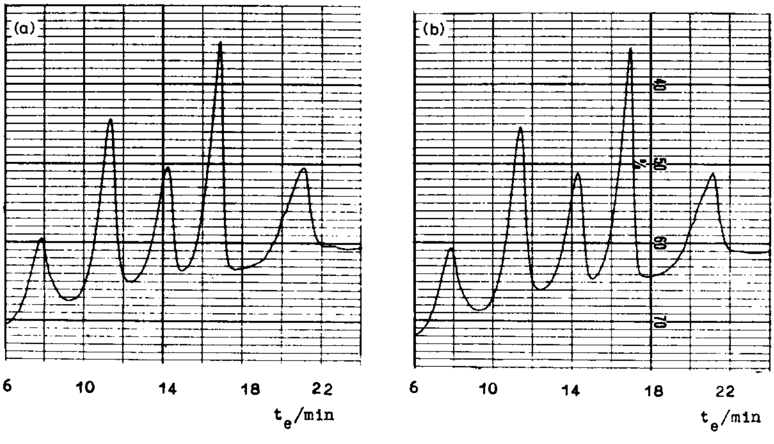
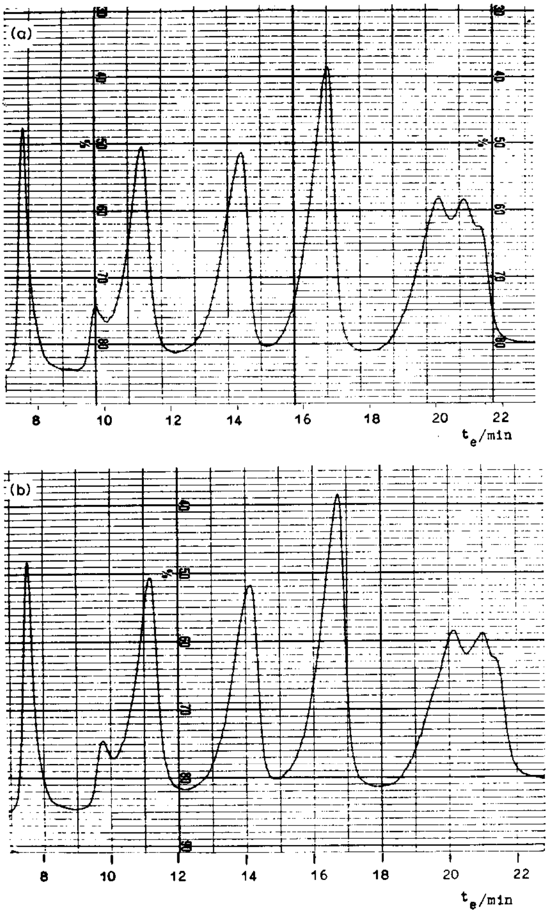


Fig. 4. Gradient chromatograms of repeated injections of SAN mixture A on reversed-phase C_{18} wide-pore material (column 8): V_0 = 30 μl; m_0 = 62.7 μg; gradient No. 30/84.



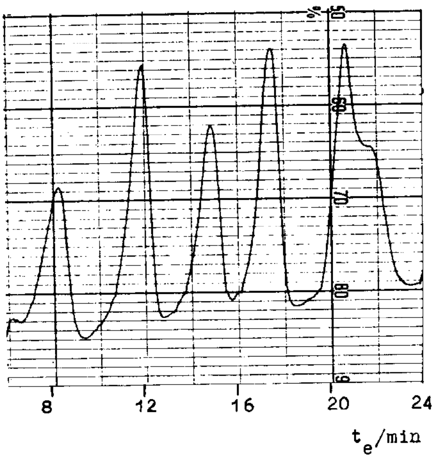


Fig. 5. Gradient chromatograms of repeated injections of SAN mixture A on CN bonded phase (column 1): V_0 = 30 μl; m_0 = 62.7 μg; gradient No. 28/84. First peak is SAN I. Paper feed rate: 10 mm/min.

Fig. 6. Gradient chromatogram of SAN mixture A on μBondagel (column 4): V_0 = 30 μl; m_0 = 62.7 μg; gradient No. 28/84. First peak is SAN I.

Fig. 5a and b present the chromatogram obtained by repeated injection of the test mixture A into the CN column 1, which had been also used for the cross fractionation of the SAN samples[11]. The separation of this mixture is also shown in Fig. 6 where the Bondagel column 4 was used.

The elution curves are reproduced without redrawing from the recordings of the analogue recorder in order to enable the patterns to be compared in detail.

In Table III the height/area ratios read from the chromatograms are compiled. The data for the peaks were taken from valley to valley.

Fig. 7 shows the elution behaviour of SMMA copolymers on the reversed-phase C_8 column 5. The tracings were obtained after separate injections of each copolymer. The gradient shape was determined experimentally with the help of an UV stained solvent B, which was tetrahydrofuran–methanol (9:1) in this case. The eluent composition was calculated from the first statistical moment of the peak position. The data are represented by triangles in Fig. 8.

TABLE III

HEIGHT/AREA RATIOS OF THE FIVE SAN PEAKS OBTAINED ON DIFFERENT COLUMNS

Peaks measured from valley to valley. Gradient program and all other conditions were identical.

	Peak number (= no. of SAN sample)				
	I	*II*	*III*	*IV*	*V*
Silica column (Fig. 2)	8.9	7.2	8.5	8.9	3.9
Reversed-phase C_{18} (Fig. 3)	6.7	6.5	6.8	8.6	3.9
RP C_{18} wide-pore column (Fig. 4a)	6.4	7.9	7.9	9.7	5.4
(Fig. 4b)	6.9	8.0	8.4	9.5	4.8
CN bonded phase, column 1 (Fig. 5a)	9.9	8.1	8.8	9.0	3.7
μBondagel column (Fig. 6)	7.4	7.4	7.6	7.9	4.8

Although no effort had been spared to prepare the copolymer samples as homogeneous as possible as regards both composition and molar mass, specimens of that kind are never chemical individuals in the sense of low-molecular-weight substances. Thus, a peak may be broader due either to the higher selectivity of a chromatographic system for certain sample properties or to a lower plate number. The former explanation is more likely if the peak exhibits additional features (see the shoulder on the last peak in Fig. 6 or on the second peak in Fig. 5a and b).

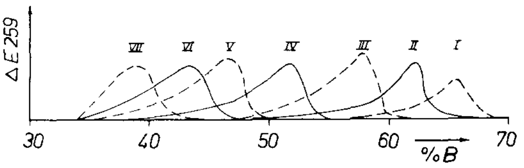


Fig. 7. Elution patterns of SMMA copolymers on reversed-phase C_8 (column 5). Separate injection of each copolymer; V_0 = 100 μl; m_0 = 20 μg; gradient No. 1/83^{20}. First peak is SMMA VII.

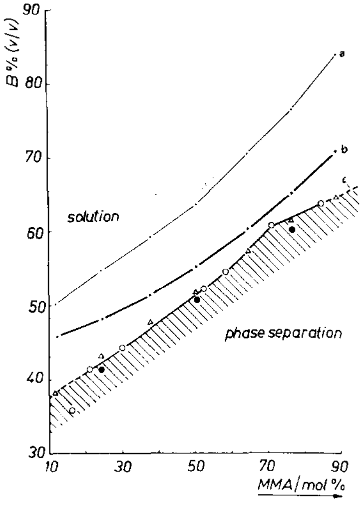


Fig. 8. Elution characteristics and solubility boundary *vs.* composition of SMMA copolymers in alkane–THF systems. Curves: a, peak position (first moment) from an experiment 38/83 (ref. 17) with the mixture of samples I–VII on silica (column 2), gradient No. 2/83 (solvent A = isooctane, B = THF + 10% methanol); b, first moment of the peak position from Fig. 1 for the central SEC fractions on silica column 7 (solvent A = isooctane, B = THF); c, solubility boundary according to turbidimetric titration with *n*-hexane of copolymer solutions in THF^{21} (○, experimental results). ●, First moment of the broad elution patterns of individually injected SMMA samples II, IV and VI; △, first moment of the elution patterns shown in Fig. 7 (solvent A = *n*-hexane, B = THF + 10% methanol).

A prerequisite for peak comparison in gradient HPLC is the equivalence of the gradients used. All gradients employed in the experiments shown in Figs. 2–6 were based on a linear increase in the THF content at 2.5%/min. The chromatograms showing the separation of the five copolymers in mixture A differ only slightly. The solvent composition at the SAN peak positions was almost independent of the chemical nature of the packings[13].

The most surprising result in the separation of SAN copolymers according to their acrylonitrile content was the equivalence of silica and reversed-phase columns (see Figs. 2 and 3, and also the results presented in refs. 14 and 15).

The gradients used in these experiments changed from a low to an high content of THF in alkanes (*n*-hexane or isooctane). In terms of polarity, they are normal-phase gradients. Applied to a normal-phase column, they should result in separations according to the polarity of the sample, as shown in Fig. 2 for the silica column. What is strange is the similarity of the results obtained when the same normal-phase gradient is used with a reversed-phase column (Fig. 3).

The alkanes *n*-hexane or isooctane are non-solvents for the SAN copolymers investigated. Since the elution of the specimens always occurred in mixtures containing the same amount of THF as that required for the dissolution of the samples, we have discussed these separations using the term high-performance precipitation LC (HPPLC)[14–16]. The column packing material had only a minor influence in these separations but a pronounced effect on the chromatography of SMMA copolymers.

Alkanes are also non-solvents for SMMA specimens, while THF is a solvent. The solubility of the copolymers decreases with increasing content of MMA units and can be measured by titrating dilute solutions in THF with a hydrocarbon non-solvent. The results of turbidimetric titrations of this kind are shown by open circles in Fig. 8. It determines the solubility boundary which separates the area of homogeneous solutions from the phase-separation area, where the copolymers coagulate. The gel phase formed contains solvent that is richer in THF than the surrounding liquid.

The triangles in Fig. 8 indicate the eluent composition that eluted the SMMA samples from the C_8 column 5, *cf.*, Fig. 7. Here and in the turbidimetric titrations, *n*-hexane was used as a hydrocarbon non-solvent. The triangles are close to curve c, thus indicating that the elution is due to precipitation and redissolution.

From the overlap of the curves in Fig. 7 it is concluded that the separation of the admixed specimens is not possible. On the other hand, rather good chromatograms of SMMA mixtures have been obtained using normal gradients of isooctane and THF (+ 10% methanol) on a silica column[17]. The elution characteristics of one of these separations (Fig. 2 in ref. 17) is represented by curve a in Fig. 8. It is obvious that the elution occurred at a THF concentration distinctly above the solubility boundary. The separation took place within the solution area, *i.e.*, under the influence of retention by adsorption. What is remarkable is the parallelism between the retention characteristic a and the solubility characteristic c.

The curve b shows the eluent composition at the position of the peaks from SEC fraction 4, *cf.*, Fig. 1. This gradient elution was performed on a silica column having an obviously lower activity than that of column 2. Nevertheless, the elution of the copolymer components required a higher concentration of THF than needed in the experiments with the reversed-phase columns. Other experiments with silica

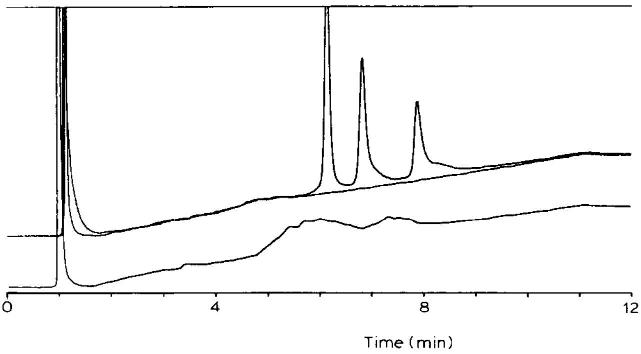


Fig. 9. Upper curve, gradient elution of SMMA mixture C (2.0 μg VI + 3.3 μg IV + 5.8 μg in V_0 = 9 μl) on silica (column 7). First peak is SMMA VI. The lower curve was obtained on C_{18} column 6. Gradient No. 04/85 was used in each case.

columns yielded elution characteristics in between the limits given by the curves a and c in Fig. 8, see also ref. 17.

The drawback of the SMMA investigations reported so far was that they had been performed at different places with different equipment and different brands of eluents. In order to come to a definite conclusion, we eluted the three SMMA samples II, IV and VI and their mixture C from the reversed-phase C_{18} column 6, employing the normal-phase gradient 04/85, comprising isooctane and THF. There was retention, but no resolution.

Immediately after that experiment, the mixture C was investigated under exactly the same conditions but on the silica column 7. Fig. 9 shows the rather good separation obtained. It also shows the elution curve of the mixture C on the C_{18} column. Obviously, the retention is longer on the silica than on the C_{18} column.

The THF concentration at the position of the first statistical moment of the broad elution curves obtained after separate investigation of the SMMA II, IV and VI on the C_{18} column is indicated in Fig. 8 by full circles. They are slightly below the solubility borderline, which was measured by turbidimetric titration with *n*-hexane. The difference can be explained by the higher temperature of the elution experiment (50°C instead of 20°C in the turbidimetric titration) and might be influenced also by the use of isooctane in the HPLC experiments.

The influence of the column packing material on separation in the elution of SMMA copolymers was observed independently also in gradient systems of cyclohexane and THF[18]. Silica columns have also been employed by Danielewicz and Kubin[19].

CONCLUSIONS

The gradient elution of SAN copolymers in general and the elution of SMMA copolymers on a reversed-phase column are determined by solubility phenomena. With the SMMA specimens, polar column packings cause additional retention which, although small, is essential for the separation by composition of these copolymers.

From a chromatographic point of view, a normal-phase gradient requires a normal-phase column for efficient separation. Thus, the behaviour of the SMMA copolymers is straightforward.

Taking into account the narrow solubility window of polymers on the other hand, the strong influence of the thermodynamic quality of the eluent is quite obvious. Further investigations with additional copolymer systems will reveal whether the behaviour observed with the SANs represents the rule or belongs to the exceptions.

At any rate, it is worthwhile to note that chromatographic cross-fractionation is possible with SMMA copolymers as well as with SANs —independent of the rather remarkable differences in retention mechanisms. This gives hope that coupled-column chromatography can generally be used for very much needed analyses of synthetic copolymers.

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