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PRECIPITATION AND ADSORPTION PHENOMENA IN POLYMER CHROMATOGRAPHY

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SUMMARY

Poly(styrene-co-methylmethacrylate) samples have been separated according to their chemical composition on silica columns by gradient elution with 2,2,4-trimethylpentane, tetrahydrofuran and methanol as mobile phase components. The eluent composition at peak position for separation on silica and on reversed-phase packings is compared with the solubility measured by turbidimetric titration in a similar solvent-non-solvent system. From these results and also from high-performance liquid chromatographic experiments with varying initial conditions for the solvent gradient, it can be concluded that, in silica columns, solubility and adsorption phenomena govern the separation of styrene methylmethacrylate copolymers according to their composition.

INTRODUCTION

Polymer chromatography is often assumed to be solely based on size-exclusion chromatography (SEC). This technique is generally performed in good solvents well above the precipitation threshold and, ideally, SEC separation is due only to the size of the sample molecules. Energetic interactions, such as coulombic forces or adsorption, would badly disturb this separation. Basically, SEC is separation by hydrodynamic volume, $V_h = M[\eta]$. Here, M is the molar mass and $[\eta]$ the intrinsic viscosity of the polymer sample. For a given solvent and molar mass value, the intrinsic viscosity of different polymers or copolymers is influenced by their chemical composition. Thus, retention in SEC is not independent of polymer structure although better separation according to composition can be obtained by other chromatographic methods, e.g., by adsorption chromatography.

Separation of poly(styrene-co-acrylonitrile) samples (SAN) according to their composition has been performed with excellent resolution by gradient elution using a suitable solvent-non-solvent combination¹. The elution gradient employed was a gradient of increasing thermodynamic quality (= solvent power). With 2,2,4-tri-

methylpentane as a non-solvent and tetrahydrofuran (THF) as a solvent, the elution of SAN copolymers of given acrylonitrile content always occurred at about the same composition of eluent mixture, regardless of whether the column used was packed with a reversed-phase material, with a nitrile- or a diol-bonded phase, or even with bare silica². This surprising result showed that the separation was not due to adsorption or solvophobic interaction, because the mechanisms would yield different retention values due to the polarity of the packing material. A SAN copolymer of a given acrylonitrile content left the column in the eluent mixture in which it was on the verge of precipitating. This observation and the fact that retention was independent of column polarity led to the conclusion that the separation was obtained by means of high-performance precipitation liquid chromatography (HPPLC)².

Precipitation phenomena are certainly of more general importance in liquid-solid chromatography of polymers. The chromatographic processes require contacts between the solute and the surface of the packing material. In order to establish these contacts, the eluent must not be too good a solvent for the polymer. The measures taken to ensure solvophobic interaction between the polymer and the packing material may easily lead to an eluent quality that no longer meets the narrow solubility range of the polymer.

Even if precipitation phenomena are the main reasons for chromatographic separation (*e.g.*, as with SAN in THF-alkane hydrocarbon), additional effects may contribute to the separation. A number of topical papers report on multi-mode mechanisms, which are especially advantageous in the chromatographic separation of biological compounds^{3,4}.

This paper deals with the separation of random poly(styrene-co-methylmethacrylate) samples (SMMA). With these polymers, the problem of multi-mode retention in polymer HPLC arises (see Results and discussion).

SMMA copolymers have been separated according to composition by means of thin-layer chromatography (TLC). Belenkii and Gankina^{5,6} performed TLC of SMMA with binaries containing chloroform as a solvent and acetone, methyl ethyl ketone, or diethyl ether as a displacer. Inagaki⁷ obtained separation by TLC using chloroform-ethyl acetate mixtures. Teramachi *et al.*⁸ separated SMMA according to composition by means of gradient TLC. They used a polar liquid, a mixture of dichloromethane with 6% (v/v) 2-propanol in cyclohexane and linearly increased the content of the polar mixture from 25 to 44.7% (v/v) in the course of each development.

Column HPLC was applied to the separation of SMMA according to composition, by Danielewicz and Kubin⁹. They used silica columns and performed gradient elution with mixtures of 1,2-dichloroethane (DCE) and THF. With the help of a laboratory-built mixing device, the THF content was increased exponentially from 3 to 20% (v/v) in the course of a run. Reproducible retention characteristics were obtained when the activity of the silica packing before each run was controlled by flushing with pure THF (at least ten column volumes). According to Snyder's table of solvent properties¹⁰, DCE ($\epsilon^\circ = 0.49$ on Al_2O_3) should be stronger than THF ($\epsilon^\circ = 0.45$). In contrast to this, Danielewicz and Kubin⁹ observed that retention is irreversible for all SMMA copolymers in pure DCE and that the THF concentration necessary for the elution of a certain copolymer increases with the methylmethacrylate (MMA) content of that copolymer.

This surprising behaviour has also been observed by Mori¹¹, who studied the chromatographic elution of SMMA copolymers from silica by mixtures of DCE and chloroform ($\epsilon^\circ = 0.40$ on Al_2O_3). No elution at all was obtained in pure DCE. With increasing MMA content, the eluent had to contain more and more chloroform for elution. Samples with 51% (or more) MMA units were not eluted even by pure chloroform.

The aim of this work was separation of SMMA copolymers according to their composition by means of gradient HPLC in the system THF-alkane hydrocarbon that has been successfully applied to the separation of SAN copolymers.

EXPERIMENTAL

Samples

The poly(styrene-co-methylmethacrylate) samples investigated are listed in Table I. These samples had been prepared by radical polymerization under conditions ensuring a narrow chemical composition distribution. The samples were dissolved in stabilized THF.

Solvents

THF (analytical grade from Baker, Deventer, The Netherlands, and from VEB Laborchemie Apolda, G.D.R.) was distilled under nitrogen to remove the stabilizer (butylated hydroxytoluene) and was kept under nitrogen to prevent peroxide formation. *n*-Hexane (analytical quality) was obtained from VEB Berlinchemie (G.D.R.) and iso-octane (2,2,4-trimethylpentane, purum quality) was purchased from Fluka (Buchs, F.R.G.). Methanol was obtained from Fisons (Loughborough, U.K.) as HPLC quality and from VEB Laborchemie as analytical quality. The solvents were degassed by nitrogen purge and filtered through a 0.45- μm filter (Sartorius Type SM 11-106) immediately before use. The nitrogen was oxygen-free.

In the text below, iso-octane is referred to as solvent A and THF [sometimes with 10% (v/v) methanol] as solvent B.

Equipment

Several liquid chromatographs were used in the course of this study. They are designated here as HPLC I-IV.

HPLC I comprised a Varian liquid chromatograph (Type 5020, Varian, Palo Alto, CA, U.S.A.) equipped with a sampling valve (Type 7105, Rheodyne, Berkeley, CA, U.S.A.) and a variable flow-through UV photometer (Type SF 770, Schoeffel, Kratos, Westwood, NJ, U.S.A.)¹.

HPLC II was a Hewlett-Packard liquid chromatograph (Type 1090 A, Hewlett-Packard, Waldbron, F.R.G.) with a ternary solvent delivery system (Type DR5), a thermostated column compartment, an autosampler, an autoinjector, a diode-array detector (Type HP 1040 A), an integrator (Type HP 3392 A), a personal computer (Type HP 85 B) with double disk drive (HP 9121 D), and a plotter (HP 7470 A).

HPLC III consisted of two high-pressure pumps (Type 708.64), an HPLC programmer (Type 50 B 716.50), a sampling valve (Type 721.63) and a UV detector (Type 714.87), all from Knauer (Bad Homburg, F.R.G.).

HPLC IV was constructed from a high-pressure pump (Type 5200, Knauer)

TABLE I
 SURVEY OF SMMA SAMPLES INVESTIGATED
 Molar mass of samples I-VII obtained by lightscattering, and VIII-XIV by membrane osmometry.

No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
MMA (% m/m)	88.5	76.2	64.0	49.5	37.0	23.8	11.4	84.8	71.4	58.6	52.7	30.1	21.4	16.6
Conversion (%)								2.3	1.7	7.8	10.8	10.4	11.8	11.3
M (kg/mol)	220	220	235	185	150	250	160	86	56	160	160	133	133	120

with a UV detector (Type 8100) and a laboratory-built gradient device in the low-pressure part of the equipment.

In every case, the HPLC signal was monitored at 259 nm. The columns were kept at 50°C and the volumetric flow-rate was 1 cm³/min unless stated otherwise. The gradient programmes are given in the legends of the figures. The initial eluent was rich in alkane and the THF content was increased during the run. The samples, dissolved in stabilized THF, were injected by means of a sampling valve, the injection volume generally being 20 μl.

Three columns were used in this study: (a) 150 × 4.6 mm I.D., packed with Polygosil 60-5 silica (Macherey & Nagel, Düren, F.R.G.) with a mean particle size of 5 μm; (b) 150 × 4 mm I.D., packed with LiChrosorb Si 60 silica (Merck, Darmstadt, F.R.G.) with a mean particle size of 7 μm; (c) 150 × 4.6 mm I.D., packed with a reversed-phase material, LiChrosphere 1000 RP-8 (Merck), an experimental batch kindly provided by Dr. Krebs (Merck), with a mean particle size of 10 μm.

Turbidimetric titrations were performed with a Zeiss colorimeter Type Specol (VEB Carl Zeiss Jena, G.D.R.) with a modified turbidity measuring device TK at wavelength 500 nm and at a temperature of 20 ± 0.2°C. Each 10 cm³ of a sample solution in THF, containing 0.4 mg of SMMA copolymer, was introduced into the cuvette. The non-solvent *n*-hexane was added in steps of 0.2 cm³ at intervals of 2 min. The turbidity was measured 90 s after each addition of precipitant. Stirring was extended beyond the non-solvent addition for 60 s.

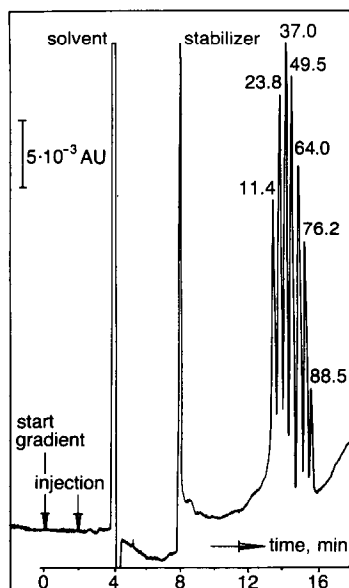


Fig. 1. HPLC of SMMA copolymers in an iso-octane-THF gradient. Sample: mixture of copolymers I-VII (see Table I) in THF (1.6 mg/cm³). Column: (a) (see Experimental) Polygosil 60-5. Gradient programme: $t = 0$ min, 10% (v/v) solvent B [THF + 10% (v/v) methanol]; $t = 8$ min, 50% (v/v) solvent B; $t = 10$ min, 80% (v/v) solvent B; $t = 11$ min, 100% (v/v) solvent B. Figures near peak top give the MMA content of the sample.

RESULTS

SMMA samples can be separated according to composition by using THF-alkane hydrocarbon mixtures and silica columns. A solvent gradient must be applied; its slope determines the widths of the peaks and, ultimately, the appearance of the chromatograms. The rather steep gradient used for the experiment shown in Fig. 1 produces a short chromatogram with narrow peaks of the seven SMMA copolymers in the sample. At first glance, this looks like an HPLC separation of a low-molecular-weight sample. The copolymer samples are eluted in order of increasing MMA content. In Fig. 2, the gradient programme is similar but at $t = 10$ min the flow-rate is reduced from $1 \text{ cm}^3/\text{min}$ to $0.3 \text{ cm}^3/\text{min}$. This yields well-shaped peaks of virtually baseline-separated copolymers. For all seven samples, the eluent composition at peak position, as derived from Fig. 1, differs only slightly from those derived from Fig. 2.

In several experiments (including those which produced Figs. 1 and 2), the polar eluent B was THF with an admixture of 10% (v/v) methanol. The latter has a low refractive index (1.3288) and was added in order to diminish the difference between the value of iso-octane (1.391) and the mobile phase component B. (The refractive index of pure THF is 1.4050.) A smaller difference in refractivity reduces the baseline deflections occurring with some UV detectors.

Full compensation would require 18.4% (v/v) methanol. The addition of 10%

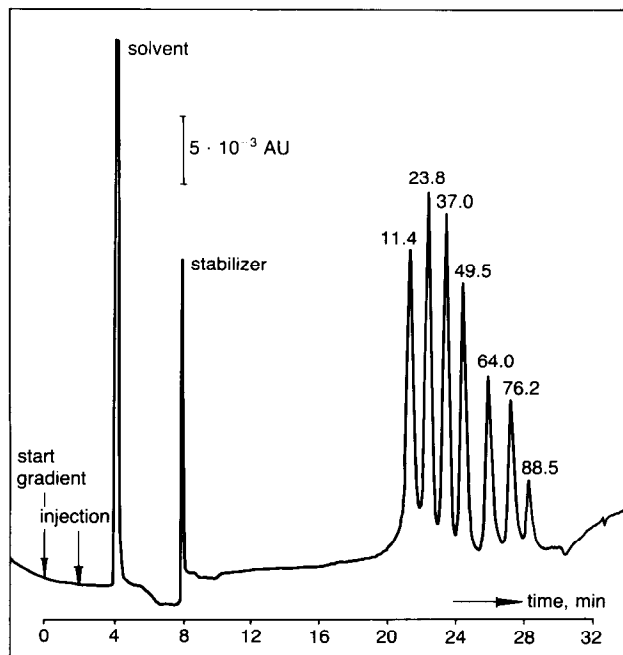


Fig. 2. HPLC of SMMA copolymers. Experimental conditions: see Fig. 1. Gradient and flow programme: $t = 0$ min, 10% (v/v) solvent B, flow-rate $1 \text{ cm}^3/\text{min}$; $t = 8$ min, 50% (v/v) solvent B; $t = 9.9$ min, flow-rate $1 \text{ cm}^3/\text{min}$; $t = 10$ min, 80% (v/v) solvent B, flow-rate $0.3 \text{ cm}^3/\text{min}$; $t = 11$ min, 100% (v/v) solvent B. Figures near peak top give the MMA content of the sample.

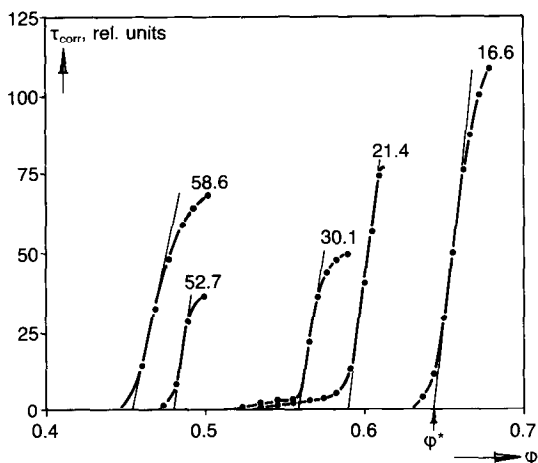


Fig. 3. Turbidimetric titration curves of SMMA copolymers in THF with *n*-hexane as a precipitant. Temperature, 20°C; ϕ = volume fraction of *n*-hexane; τ_{corr} = turbidity corrected for dilution, $\tau_{\text{corr}} = \tau/(1 - \phi)$. Samples: SMMA copolymers X–XIV; polymer concentration at the point of precipitation: 23, 23, 22, 22, 20 mg/dm³, respectively. Experimental work: E. Claus, 1983. Figures give the MMA content of the sample.

sufficed for practical purposes and disturbed separation neither here nor in the investigation of SAN copolymers. With the latter, we found that small amounts of methanol non-solvent surprisingly improved the solubility of SAN in THF–*n*-hexane mixtures. With SMMA samples, we did not succeed in performing turbidimetric titrations by adding methanol to solutions in THF; thus, it can be assumed that methanol does not disturb the solubility of SMMA in THF–hydrocarbon mixtures.

The five curves drawn in Fig. 3 have been obtained separately. They show the increased intensity of light scattered at a 90° angle, caused by increasing addition of *n*-hexane non-solvent to dilute solutions of each of the SMMA copolymers mentioned in the legend. The tangents at the inflection points of each curve yield intersections with the abscissa, ϕ^* , which are characteristic of the sample investigated. The higher the MMA content of a copolymer, the less alkane hydrocarbon is required for precipitation of the sample. In Fig. 4, the THF concentration calculated from the ϕ^* values is plotted vs. copolymer composition. Fig. 4 also contains the solvent composition at the chromatographic elution of SMMA copolymers, as derived from Figs. 1 and 2, and corresponding results from other chromatographic experiments. Among these was the investigation of SMMA copolymers on a reversed-phase RP-8 column. The points indicate the position of the centre of rather broad peaks and they were obtained by separate injection of each crude copolymer. The eluent system was *n*-hexane–THF with 10% (v/v) methanol. In contrast with HPPLC of SAN copolymers, where the column packing has only a minor influence on the chromatographic result, the separation of SMMA samples in this eluent system is rather poor on reversed-phase columns but excellent on silica packings. The same has been observed independently by Teramachi¹² when using cyclohexane–THF gradients.

Fig. 5 shows the chromatographic behaviour of three SMMA samples with 23.8, 49.5 or 76.2% (w/w) MMA on a silica column, as obtained by gradient elution with pure THF and iso-octane. When the injection had been performed into an eluent

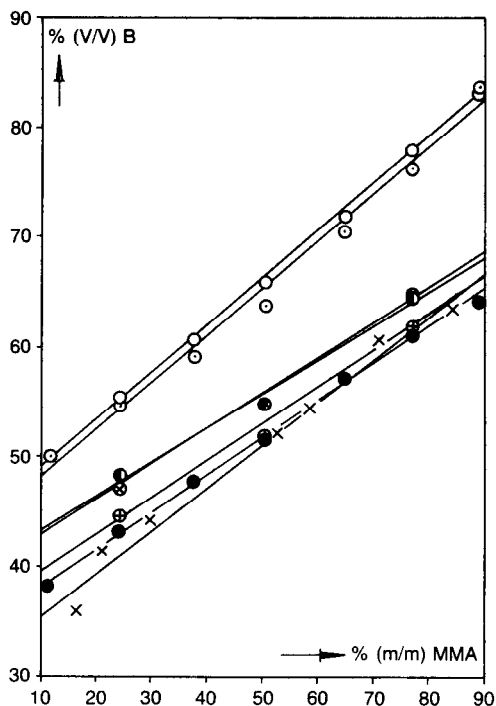


Fig. 4. Solvent composition at chromatographic peak elution (circles) or precipitation points (crosses) vs. MMA content of the copolymers investigated. (○) HPLC on silica column (a); date: run 73, September 30, 1983; equipment: HPLC I; conditions: see Fig. 1. (⊙) HPLC on silica column (a); date: run 38, October 3, 1983; equipment: HPLC I; conditions: see Fig. 2. (⊗) HPLC on silica column (a); date: run 54, September 6, 1984; equipment: HPLC II; samples: II, IV, VI; gradient: $t = 0$ min, 42% (v/v) solvent B (THF); $t = 10$ min, 44% (v/v) solvent B; $t = 16$ min, 100% (v/v) solvent B. (⊕) HPLC on silica column (b); date: run 42, September 5, 1984; equipment: HPLC II; samples: II, IV, VI; gradient: $t = 0$ min, 40% (v/v) solvent B [THF + 10% (v/v) methanol]; $t = 20$ min, 42% (v/v) solvent B; $t = 25$ min, 80% (v/v) solvent B. (●) HPLC on silica column (b); temperature: 22°C; date: run D2, March 20, 1985; equipment: HPLC III; samples: II, IV, VI; gradient: $t = 0$ min, 30% (v/v) solvent B; $t = 24$ min, 90% (v/v) solvent B; solvent A: *n*-hexane; solvent B: THF. (●) HPLC on reversed-phase packing column (c); equipment: HPLC IV; samples: I–VII; gradient: $t = 0$ min, 10% (v/v) solvent B; separating range 33–80% (v/v) solvent B within 10 min; solvent A: *n*-hexane; solvent B: THF + 10% (v/v) methanol; experimental work: D. Ilchmann, 1983. (x) Precipitation points from turbidimetric titration in THF–*n*-hexane at 20°C (see Fig. 3); samples: X–XIV; experimental work: E. Claus, 1983.

containing 44% (v/v) THF, perfect retention and separation of all three copolymers was observed. (As always, the sample was dissolved in THF, containing 0.025% butylated hydroxytoluene stabilizer.) The elution of the first peak occurred at retention time 13.2 min, just after the slope of the gradient had been increased from 0.2 to 9.0% (v/v) B/min. Fig. 5 also shows that the first peak [due to the copolymer containing 23.8% (m/m) MAA] diminished or even disappeared when the injection had been performed into an initial eluent containing 45 or 46% (v/v) THF. The missing portion of the sample did not appear in the chromatogram; it was either hidden in the solvent peak or remained on the column, modifying the activity of the silica. The injections had been made in an uninterrupted sequence (1st: 46%, 2nd:

45%, 3rd: 44%). They followed each other immediately and can therefore be compared directly.

Fig. 6a shows what will normally happen if a polymer is injected into an eluent that is too strong to cause retention: the polymer leaves the column in front of the eluent peak as it is excluded from the pores. We repeatedly observed this behaviour also in HPPLC of SAN when the initial eluent did not contain a sufficient amount of hydrocarbon non-solvent.

Fig. 6b shows the result of an experiment that is closely connected to the preceding run no. 54, the retention data of which are included in Fig. 4. From the gradient programme, the retention time of the three peaks in run 54, and $t_{\text{lag}} = 3.0$ min for the whole system (HPLC II, column 150×4.6 mm I.D., packed with Polygosil 60-5), the eluent composition at peak position can be calculated: 48.0, 54.7 or 64.7% (v/v) THF eluted the samples with 23.8, 49.5 or 76.2% (m/m) MMA, respectively. No methanol was added. Run no. 55 (Fig. 6b) was performed only 25 min later, with the objective of finding out what would happen if the same sample was injected into an eluent with an initial THF content of 48% (v/v). A very flat gradient of 0.2% (v/v) B/min should approximate isocratic elution very closely. The chromatogram in Fig. 6b shows that the copolymer in question indeed appeared where it was expected: immediately after the solvent peak. In spite of the extreme flatness of the gradient, this polymer peak is nearly as narrow as the peaks of the two subsequent copolymers of 49.5 and 76.2% (m/m) MMA, which are located in the steep

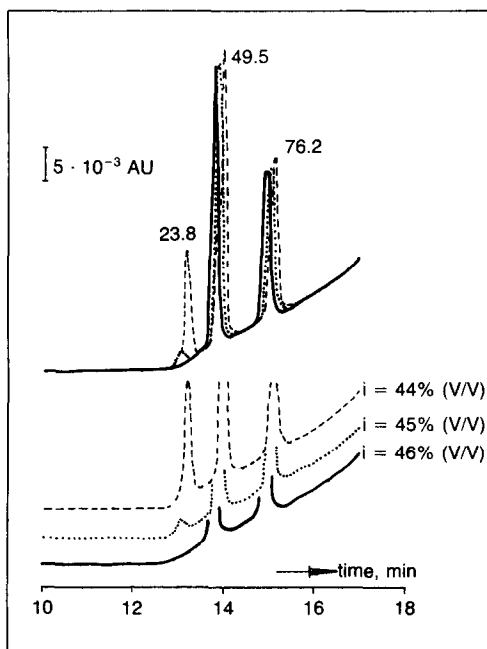


Fig. 5. HPLC of three SMMA samples (II, IV, VI) in iso-octane-THF gradient column (a). Initial content of THF: $i = 44, 45$ or 46% (v/v). Gradient: $t = 0$ min, $i\%$ (v/v) solvent B; $t = 10$ min, $i + 2\%$ (v/v) solvent B; $t = 16$ min, 100% (v/v) solvent B. Equipment: HPLC II. Figures near peak top give MMA content of the sample.

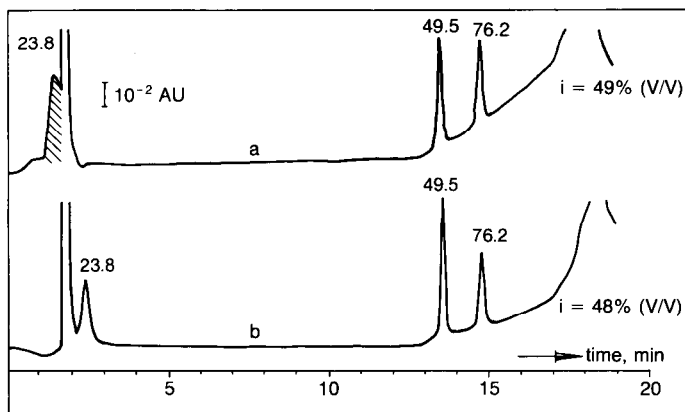


Fig. 6. HPLC of three SMMA samples. Conditions and samples as in Fig. 5, but initial content 48 or 49% (v/v) THF; partial exclusion with $i = 49$ (cross-hatched area). Figures near peak top give MMA content of the sample.

part of the gradient [8.33% (v/v) B/min]. If we bear in mind the strong influence of the gradient slope on peak width (*cf.* Figs. 1 and 2), the slenderness of this peak is remarkable. So far, this chromatogram is our only observation of the virtually isocratic elution of a polymeric sample. We believe migration of this kind to be possible only under very-well-balanced conditions.

DISCUSSION

Among the peculiarities of HPPLC of SAN, the most surprising one is the possibility to perform the separations on silica as well as on a reversed-phase column. Especially with the latter, it is essential that the initial eluent composition contains enough non-solvent in order to ensure precipitation and retention of the polymer. This is the reason why we usually started with 90% (v/v) iso-octane, in spite of the fact that the solubility thresholds of typical SAN copolymers are in the range of 20–50% (v/v) iso-octane only. The higher the initial content of non-solvent, the lower the risk that the sample will be rushed through the column and will be eluted earlier than the solvent (*cf.* Fig. 6a). Columns with packings that interact with the polymer are safer with respect to this failure.

In contrast with the behaviour of SAN in THF–hydrocarbon mobile phase systems, good separation of SMMA samples with increasing retention of MMA-rich specimens can be obtained only on polar columns. This points to separation by adsorption.

Solubility effects *per se* cannot yield effective chromatographic separation of SMMA copolymers according to composition, at least not in the systems investigated, *i.e.*, alkane (or cyclohexane¹²) hydrocarbons–THF (with or without addition of methanol). This must be concluded from the rather poor resolution on reversed-phase columns in comparison with the excellent chromatograms that could be obtained with the help of silica-packed columns. More precisely, these are results from copolymer samples that had not been prefractionated by SEC. With SEC fractions of SMMA samples, isolated peaks could be seen on a reversed-phase column.

Adsorption effects are obviously sufficient for separating SMMA copolymers according to composition. This is demonstrated by the work of Danielewicz and Kubin⁹. They used DCE and THF as eluent components, which are both thermodynamically good solvents for SMMA. Let us finally discuss the question of whether the excellent separation shown in Figs. 1 and 2 is mainly or even solely due to adsorption effects. In Fig. 4, the lines of the results from different experiments run roughly parallel. We calculated the slope factor for each set of points by means of linear regression and found values within the limits 0.305 and 0.428. The lowest value (0.305) was from the experiments with the LiChrosorb Si 60 column (b) and THF-*n*-hexane, and the highest one (0.428) from run no. 73 on the Polygosil 60-5 column (a) with iso-octane-THF + 10% (v/v) methanol. In run no. 42, we found a value of 0.331 on Polygosil 60-5 with iso-octane-THF + 10% (v/v) methanol and in run no. 54, under corresponding conditions but without methanol, we found a value of 0.319. The coefficient of determination, r^2 , was always better than 0.98, sometimes better than 0.99. The worst value was $r^2 = 0.984$ for the points from turbidimetric titration. This analysis confirms the graphic impression that there is a pronounced shift between several sets of data but no marked increase of the slope related to this shift.

The results from the turbidimetric titration indicate that the fact that more THF is required for a copolymer with higher MMA content is connected with solubility. The chromatographic runs on the RP-8 column yielded points that are close to the turbidimetric results. The parallelism between these and all other lines is surprising and suggests solubility influence in all experiments. Runs 38 and 73 on the silica column from 1983 required *ca.* 15% (v/v) more B than the elution from an RP-8 column. This gives evidence of retention from solution, caused by a highly active surface. After one year, the same column showed reduced adsorption activity. We can think of several reasons for this change, but have insufficient information to discuss this in detail. More important for the question raised is the fact that even with the reduced activity, the column produced slender and well-resolved peaks (Figs. 5 and 6 may serve as an illustration.) All respective points are in the field of stable solutions in Fig. 4. Provided that the boundary of this area is not influenced by the presence of an active surface, the retention would be due solely to adsorption. The roughly parallel lines in Fig. 4 suggest a balance between solvating and adsorbing forces, *i.e.*, mixed-mode retention by adsorption at the verge of precipitation.

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