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Analysis of poly(styrene-co-methyl acrylate) and poly(styrene-co-butyl acrylate) by high-performance liquid chromatography

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SUMMARY

Poly(styrene-co-methyl acrylate) and poly(styrene-co-butyl acrylate) were separated according to their chemical composition by gradient elution. The chromatographic separation on silica was optimized for a gradient ranging from *n*-heptane as a non-solvent to dichloromethane containing a small amount of methanol as a strong solvent. The influence of different stationary phases, the chemical composition and molecular mass on the separation of the copolymers was investigated. From the results of different chromatographic and turbidimetric experiments it is concluded that the copolymer separation is controlled by both precipitation and adsorption mechanisms. The contribution of adsorption processes to the separation is only advantageous when normal-phase gradients are applied.

INTRODUCTION

In research on copolymers, liquid chromatographic cross-fractionation (CCF) is an important tool in the analysis of the molecular mass chemical composition distribution (MMCCD) of such components¹. The fractionation of copolymers according to molecular mass (MM) is usually performed by size-exclusion chromatography (SEC), followed by a normal- or reversed-phase gradient high-performance liquid chromatographic (HPLC) separation for the determination of the chemical composition distribution (CCD).

The gradient HPLC analysis of copolymers may be complicated by the starting and final eluents and the shape of the gradient, possible interferences from the sta-

tionary phases and the influence of the MM of the copolymers. In these analyses of copolymers, the gradient usually starts with a non-solvent, and subsequently the solvating power of the eluent is increased by the gradient.

For the separation of styrene-methyl acrylate (SMA) copolymers according to chemical composition, a gradient high-speed LC separation on silica as the stationary phase has been reported². No interference of the MM on the separation was observed. For the separation of poly(styrene-co-acrylonitrile)³⁻⁸ no influence of the applied stationary phases was reported, indicating that this separation is controlled by a precipitation mechanism rather than by an adsorption process. On the other hand, the separations of poly(styrene-co-methyl methacrylate (PSMMA))^{6,9-17} and poly(styrene-co-ethyl methacrylate)^{1,4,18} appeared to be influenced by the used normal-phase (NP) and reversed-phase (RP) stationary phases. Moreover, for NP packings Glöckner and Van den Berg^{6,9} showed that for the elution of a specific PSMMA sample with a typical gradient an increased percentage of the applied solvent, tetrahydrofuran (THF), was necessary in comparison with the results of turbidimetric titrations. This indicates that adsorption may also play a role in this separation process.

The detection of the copolymers was performed using UV absorption at 254 or 259 nm. At these wavelengths absorption is due to the aromatic styrene monomer parts of the polymers. This implies that the absorption coefficient increases with increase in the styrene content of a typical copolymer. On the other hand, the acrylonitrile or (meth)acrylate homopolymers are difficult to detect with this detection method.

In this study we developed and optimized an HPLC gradient system on silica as a stationary phase for PSMA, which also allowed the UV detection of the MA homopolymer. The investigated eluents were dichloromethane (DCM), dichloroethane (DCE) and distilled THF without stabilizer. As recommended by Snyder¹⁹, a few percent of methanol was added to DCM and DCE in order to increase their eluting strength to a value comparable to that of THF.

We also investigated the influence of a number of stationary phases, the chemical composition and the MM on the separation of the copolymers under study. Turbidimetric titrations were performed and compared with the chromatographic data to study the influence of the different stationary phases. Also, a similar separation system for poly(styrene-co-butyl acrylate) (PSBA), a copolymer in which the two monomeric units differ less in polarity compared with PSMA, was developed.

EXPERIMENTAL

Chemicals

The applied solvents *n*-heptane (extra pure), DCM (analytical-reagent or HPLC grade), methanol (HPLC grade) and THF with stabilizer (analytical-reagent grade) were obtained from Merck (Darmstadt, F.R.G.) and DCE (analytical-reagent grade) from Fluka (Buchs, Switzerland). Before use, the solvents were filtered through a 0.45- μ m HVLP membrane filter (Millipore, Bedford, MA, U.S.A.).

Samples

The polymer and copolymer samples with a narrow CCD were synthesized by

low-conversion solution polymerization in toluene at 62°C with 2,2'-azobis(isobutyronitrile) as an initiator under argon. The reaction mixture was poured into *n*-heptane after *ca.* 10% conversion. Subsequently the precipitated (co)polymer was dried under vacuum at 50°C. In Table I the (co)polymer samples are listed, including the number-average chemical composition and the weight-average molecular mass (M_w). The molecular masses of the samples were determined by SEC and the chemical composition by proton nuclear magnetic resonance spectroscopy.

Gradient HPLC

The eluent gradients were performed with a Model 720 system controller and two Model 510 HPLC pumps (Millipore-Waters, Milford, MA, U.S.A.). In all instances the linear gradients from time $t = 0$ to 18 min ranged from 10% to 100% of a typical solvent (a mixture of methanol with DCM or DCE) in the non-solvent (*n*-heptane). The eluent flow-rate was 0.4 ml/min. The samples were injected with a Waters Assoc. Model 710 Intelligent Sample Processor (WISP). UV detection was performed with a Waters Assoc. Model 490 multi-wavelength Detector at 235 and 260 nm. The chromatographic separations were carried out on a Chromsep 5- μ m

TABLE I
SYNTHESIZED LOW-CONVERSION SOLUTION (CO)POLYMER SAMPLES

Sample	Composition (% styrene)	M_w (kg/mol)
PS	100	43
PSMA 1	83	23
PSMA 2	83	22
PSMA 3	81	63
PSMA 4	77	20
PSMA 5	76	66
PSMA 6	67	72
PSMA 7	67	105
PSMA 8	64	20
PSMA 9	57	74
PSMA 10	49	32
PSMA 11	46	75
PSMA 12	46	23
PSMA 13	33	65
PSMA 14	31	113
PSMA 15	29	58
PSMA 16	27	30
PSMA 17	12	110
PMA	0	111
PBA	0	255
PSBA 1	12	177
PSBA 2	24	138
PSBA 3	35	103
PSBA 4	41	83
PSBA 5	44	99
PSBA 6	61	83
PSBA 7	69	—
PSBA 8	78	59

TABLE II
STATIONARY PHASES APPLIED IN THE GRADIENT HPLC OF SMA COPOLYMERS

<i>Stationary phase</i>	<i>Particle size (μm)</i>
Nucleosil Si-50	5
Nucleosil CN	5
Nucleosil NH ₂	5
LiChrosorb diol	5
LiChrosorb RP-18	5
LiChrosorb RP-8	5
Glass beads	0-20

silica cartridge column (10 cm \times 3.0 mm I.D.) (Chrompack, Middelburg, The Netherlands).

Laboratory-packed columns (10 cm \times 4.0 mm I.D.) (Knauer, Berlin, F.R.G.) were used for the investigation of the different stationary phases, listed in Table II. The eluent flow-rate was 0.8 ml/min. In these experiments methanol was also applied as a non-solvent. To study the influence of temperature on the separation of the copolymer samples, the column temperature was controlled with a water-jacket thermostatic bath at different temperatures ranging from 20 to 45°C.

Size-exclusion chromatography

The HPLC equipment used for the SEC analyses and fractionations of the (co)polymers consisted of a Waters Assoc. Model 510 HPLC pump, a WISP Model 712, a Model 440 absorbance detector operating at 254 nm, a Model 410 differential refractometer, a Waters Assoc. automated switching valve and a Cygnat fraction collector (Isco, Lincoln, NE, U.S.A.). The separations were performed on three Waters Assoc. μ Styragel columns (25 cm \times 7 mm I.D.; average particle size 10 μm ; nominal pore sizes of the packings 10², 10³ and 10⁴ nm). THF under helium was used as the eluent at a flow-rate of 0.6 ml/min. Analyses of the (co)polymers were performed with 20- μl injections of a solution of a typical polymer in THF (1 g/l) whereas for the fractionations 1000 μl of the same solutions were injected. The processing of the chromatographic data was performed on two SP 4100 computing integrators (Spectra-Physics, Santa Clara, CA, U.S.A.) equipped with a Kerr 4100 D minifile (Spectra-Physics).

Turbidimetric titrations

Turbidimetric titrations were performed by dissolving 25 mg of a typical polymer in 25 ml of solvent. Subsequently, this solution was titrated with small portions of a non-solvent (*n*-heptane or methanol) at ambient temperature with continuous stirring. Precipitation was monitored by measuring the absorption at 500 nm with an HP 8451 A diode-array spectrophotometer (Hewlett-Packard, San Diego, CA, U.S.A.). During the titration the solution was circulated through a bypass, connected to a 4 \times 1 \times 1 cm glass measuring cuvette in order to detect the absorption properties of the suspension. The precipitation points were calculated by the intersection of the tangent at the deflection point with the horizontal axis in a plot of the concentration-corrected absorption *versus* the percentage of non-solvent added.

RESULTS AND DISCUSSION

In order to allow the UV detection of PMA and PBA, solvents with a sufficiently low UV cut-off must be used. Of the three strong solvents investigated, DCM was the most suitable eluent with respect to a constant low-background UV absorption. The application of distilled THF was avoided as much as possible for safety and storage reasons. The lowest possible detection wavelength appeared to be 235 nm with a basic absorption of 0.3 absorbance.

For the optimum separation of PSMA on the Chromspher silica stationary phase, the influence of the following parameters was studied: eluent flow-rate, gradient speed, temperature (Fig. 1) and percentage of methanol in the strong solvents (Fig. 2). Without taking into account the width of the CCD of the copolymers, the separation efficiency was determined by calculating the average peak width at half-height for the separated copolymer standards expressed as a styrene percentage. The optimum eluent flow-rate and gradient shape were 0.4 ml/min and 5%/min, respectively. The data in Fig. 1 show the dependence of the retention times of the copolymers on temperature. From this it can be concluded that for accurate measurements the column temperature must be strictly controlled.

For an optimum separation efficiency the methanol fraction in the strong solvent should be as low as possible, but sufficient to elute the strongest adsorbing (co)polymer of the sample. It turned out that for PSMA and PSBA on the silica column the methanol concentration in DCM should be 4% and 1.2%, respectively.

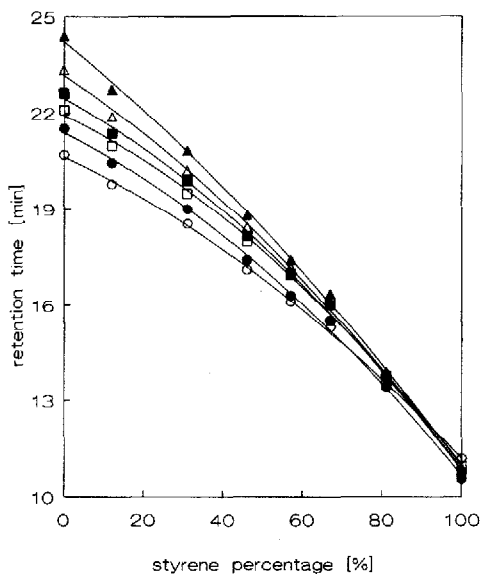


Fig. 1. Retention time as a function of the styrene percentage in PSMA at different temperatures, fitted with second-degree polynomials. Samples, PMA, PSMA 3, 7, 9, 11, 14 and 17, PS. Solvent, DCM + 4% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.4 ml/min; column, Chromspher silica, 100 × 3 mm I.D.; ○ = 20.2°C; ● = 25.1°C; □ = 30.2°C; ■ = 34.5°C; △ = 39.7°C; ▲ = 45.1°C.

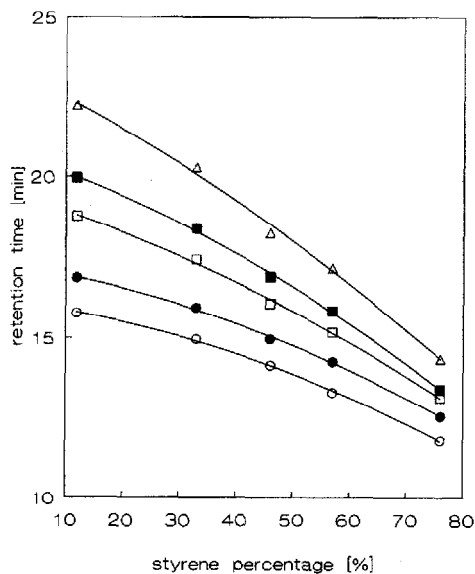


Fig. 2. Retention time as a function of the styrene percentage in PSMA at different compositions of the eluent, fitted with second-degree polynomials. Samples, PSMA 5, 9, 11, 13 and 17. Solvent, DCM + methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.4 ml/min; column, Chromspher silica, 100 × 3 mm I.D.; temperature, ambient. Methanol (%): ○ = 20; ● = 10; □ = 5; ■ = 4; △ = 3.

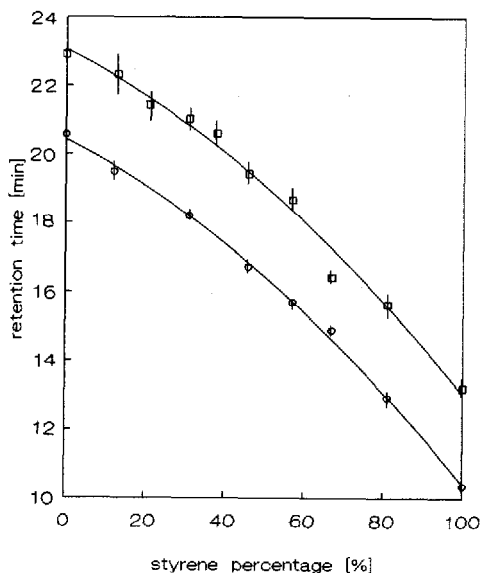


Fig. 3. Retention time as a function of the styrene percentage in PSMA and PSBA fitted with second-degree polynomials, with an indication of the peak width at half-height of the standards used. Samples, PSMA mixture, PBA, PSBA 1–8, PS. Solvents, DCM + 4% methanol for PSMA, DCM + 1.2% methanol for PSBA; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate 0.4 ml/min; column, Chromspher silica, 100 × 3 mm I.D.; temperature, ambient. ○ = PSMA; □ = PSBA.

For both PSMA and PSBA a similar relationship between the percentage of styrene in the copolymer and the retention time was observed (Fig. 3). Fig. 3 also indicates a decreased separation efficiency for PSBA, owing to a smaller difference in polarity between the two monomer components. An increase in the column temperature improved the separation efficiency of PSMA owing to an increased adsorption of PMA. On the other hand, the effect of increasing the column temperature may also be obtained, to a certain extent, by decreasing the methanol content of the eluent. The disadvantage of the latter possibility is the increased elution time of PS.

Several polymers (Table III) were fractionated according to molecular mass by SEC in order to investigate the influence of the molecular mass on gradient HPLC. The fractions were analysed with three different HPLC systems (Figs. 4-6). In these figures the influence of the molecular mass is shown in plots of the retention time *versus* $(MM)^{-1/2}$. According to Glöckner³ such a plot results in a straight line when the separation is dependent on the solubility. If the molecular mass influences the retention time it decreases linearly with $(MM)^{-1/2}$. However, under the optimum conditions for the separation of PSMA this influence is negligible, simplifying the interpretation of the data. The peak broadening of the copolymer standards at lower MM is mainly caused by the broader CCD at lower molecular masses, controlled by the copolymerization kinetics²⁰. The peak width increases with decreasing MM and reaches unacceptable values below an MM of 10 kg/mol for PSMA. At the same time the ratio of the UV absorption ratios (peak area or height) at 260 nm to 235 nm decreases strongly at the same low molecular masses. These two observations are shown in Fig. 7.

For accurate quantitative measurements, the relationship between the relative absorption coefficient and the composition of a specific copolymer must be known. To determine this relationship, a mixture of six SMA copolymers and both the homopolymers was prepared (PS, PSMA 3, 7, 9, 11, 14 and 17 and PMA) with exactly known relative amounts. This mixture was separated and the peak areas were measured. Fig. 8 shows the absorption coefficients of PSMA relative to polystyrene at 235 nm. The correlation coefficients of the two absorption curves are 0.995 at 260 nm and

TABLE III
MOLECULAR MASSES (kg/mol) OF SEC-FRACTIONS

$M_w \approx M_n$ (M_w = weight-average molecular weight; M_n = number-average molecular weight).

Sample	Fraction No.							
	1	2	3	4	5	6	7	8
PSMA 1	60	35	25	13	8.0	2.1		
PSMA 8	70	34	11	5.0	2.5	1.2		
PSMA 12	70	40	25	1.5	9.0	4.0		
PSMA 16	80	50	30	20	14	6.0		
PMA	300	140	70	42	24	16	11	2.1
Mixture (PS, PSMA 3,7, 9,11, 14 and 17, PMA)	220	120	82	59	40	25	13	
PSBA 2	330	210	150	110	82	53	23	
PSBA 6	200	130	92	68	48	31	16	

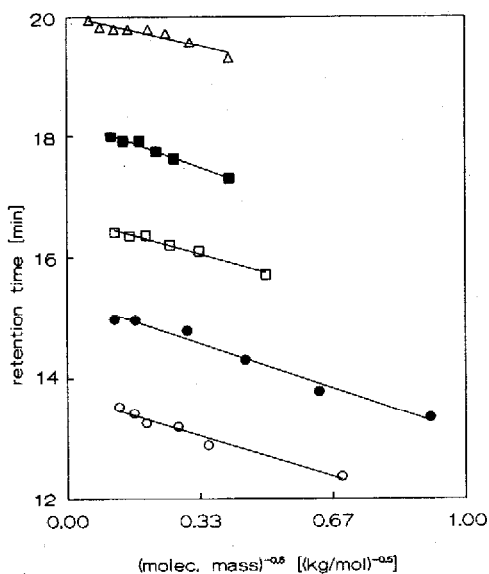


Fig. 4. Retention time as a function of $(MM)^{-1/2}$ of PSMA, fitted with first-degree polynomials. Solvent, DCE + 10% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.4 ml/min; column, Chromspher silica, 100 × 3 mm I.D.; temperature, ambient. ○ = PSMA 1; ● = PSMA 8; □ = PSMA 12; ■ = PSMA 16; △ = PMA.

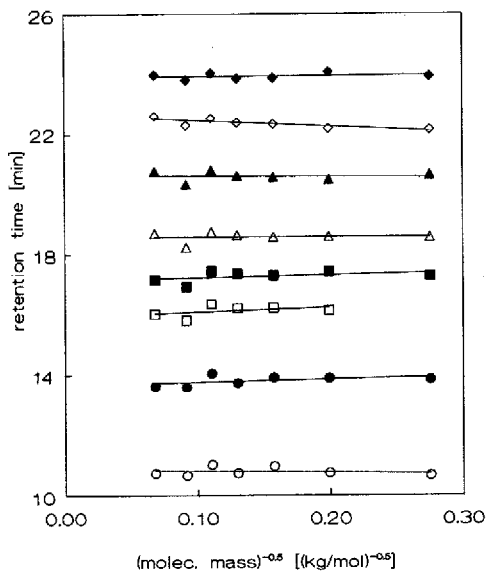


Fig. 5. Retention time as a function of $(MM)^{-1/2}$ of PSMA, fitted with first-degree polynomials. Solvents, DCM + 4% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.4 ml/min; column, Chromspher silica, 100 × 3 mm I.D.; temperature, 30°C. ○ = PS; ● = PSMA 3; □ = PSMA 7; ■ = PSMA 9; △ = PSMA 11; ▲ PSMA 14; ◇ = PSMA 17; ◆ = PMA.

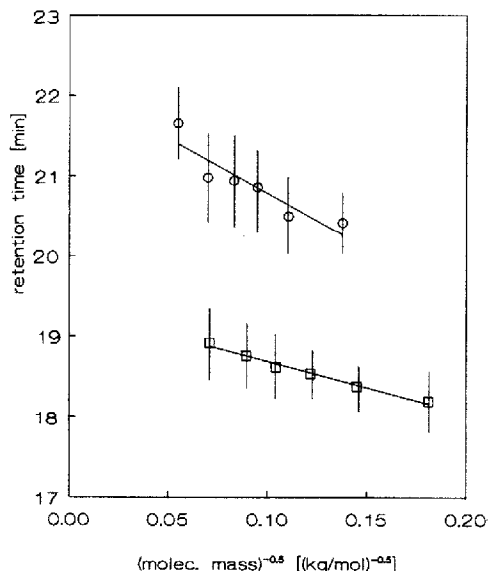


Fig. 6. Retention time as a function of $(MM)^{-1/2}$ of PSBA, fitted with first-degree polynomials, including an indication of the peak width at half-height. Solvent, DCM + 1.2% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.4 ml/min; column, Chromspher silica, 100×3 mm I.D.; temperature, ambient. \circ = PSBA 2; \square = PSBA 6.

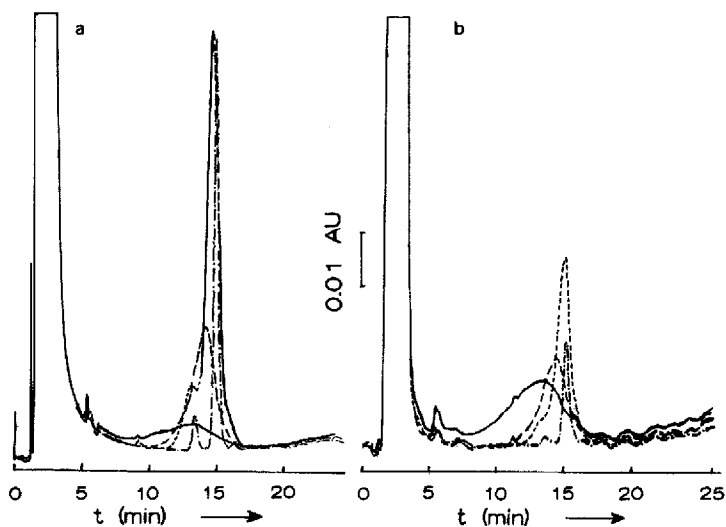


Fig. 7. Chromatograms of different SEC fractions of PSMA 8. Solvent, DCE + 10% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate 0.4 ml/min; column, Chromspher silica, 100×3 mm I.D.; temperature, ambient. (a) 260 nm; (b) 235 nm. Molecular mass: — = 1.2 kg/mol; --- = 5 kg/mol; = 11 kg/mol; - . - . - = 70 kg/mol.

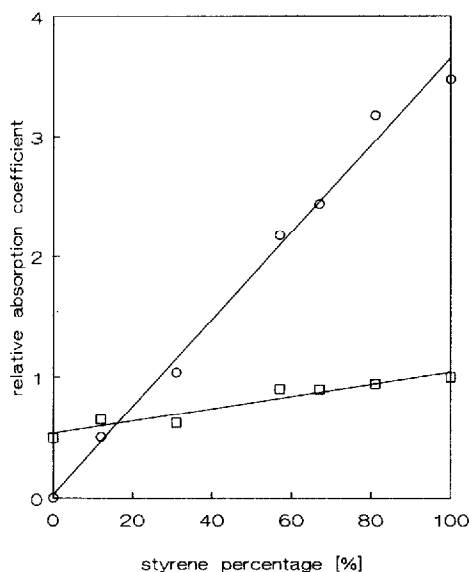


Fig. 8. Relative adsorption coefficient of PSMA as a function of the styrene percentage. Sample: mixture. ○ = 260 nm; □ = 235 nm.

0.96 at 235 nm. Application of the UV absorption at 235 nm proved to be impossible as a quantitative detection method because of two drawbacks: insufficient linearity of the UV absorption at 235 nm with respect to the chemical composition; and the decreasing ratio of the absorption ratio at 260 to 235 nm at decreasing molecular mass. These effects can be explained by the contribution of polymer end-groups being higher at a lower molecular mass. From this it can be concluded that copolymers with a low styrene fraction require another detection system in order to obtain accurate quantitative data.

For the study of the six different column packings in the NP mode, with a gradient from *n*-heptane to DCM with 5% methanol, the retention times of the sample peaks were expressed as the *n*-heptane content of the eluent leaving the column at the same time. It should be mentioned that the HPLC gradient was optimized for silica and was subsequently applied to the other stationary phases. Fig. 9 shows the curves (second degree polynomial fitting) of the percentage of *n*-heptane versus the percentage of styrene in PSMA, for the chromatographic and turbidimetric data. The same experiments were performed with three different stationary phases in the RP mode with a gradient from methanol to DCM (Fig. 10). The efficiency of the NP and RP separations is shown in Table IV, expressed as the average peak width at half-height for the PSMA 3, 5, 6, 9 and 11 samples.

For an NP gradient, applied with NP packings, it is shown that in addition to precipitation, the adsorption mechanism also plays a role in this particular chromatographic process. This can be observed by comparing the chromatographic and the corresponding precipitation data. The NP gradient analysis on C₁₈-modified silica and the titration experiments gave similar results. Also, an extremely low separation efficiency on this C₁₈-modified silica was observed. From this it can be concluded that

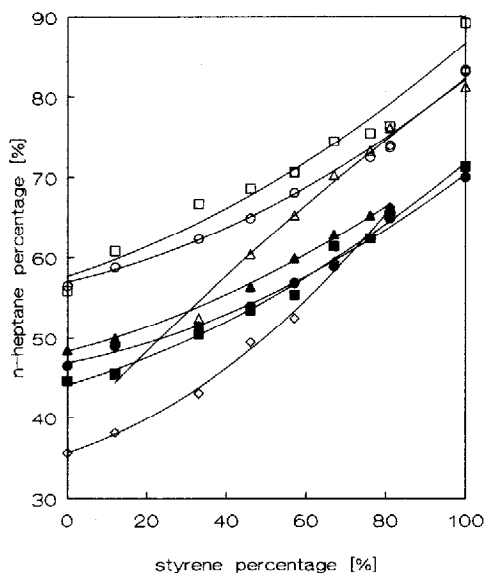


Fig. 9. Solvent composition at precipitation points and the eluent composition at elution times expressed in % *n*-heptane as a function of the styrene percentage of PSMA. Curves fitted with second-degree polynomials. Samples, PS, PSMA 3, 5, 6, 9, 11, 13 and 17, PMA. Solvent, DCM + 5% methanol; non-solvent, *n*-heptane; gradient, linear from 10% solvent in non-solvent to 100% solvent, 0–18 min; flow-rate, 0.8 ml/min; column, 100 × 4 mm I.D., stationary phases as indicated; temperature, ambient. ○ = Titration; ● = CN; □ = RP-18; ■ = diol; △ = glass; ▲ = NH₂; ◇ = Si.

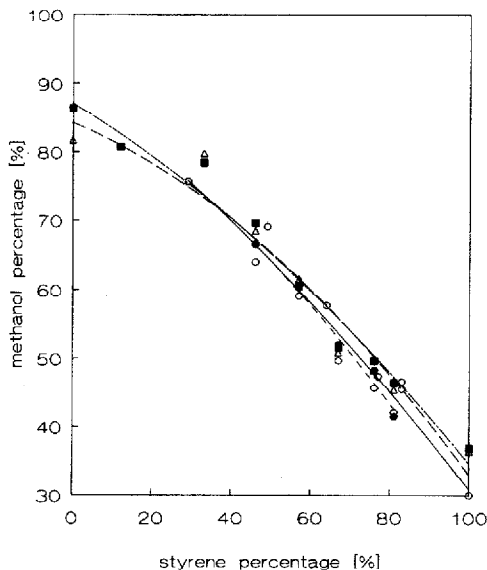


Fig. 10. Solvent composition at precipitation points and the eluent composition at elution times on different stationary phases expressed in % methanol as a function of the styrene percentage in PSMA. Curves are fitted with second-degree polynomials. HPLC samples, PS, PSMA 3, 5, 6, 9, 11, 13 and 27, PMA; titration samples, PS, PSMA 1–5, 7–11 and 15; non-solvent, methanol; solvent, DCM; gradient, linear from 100% non-solvent to 100% solvent, 0–18 min; flow-rate, 0.8 ml/min; column, 100 × 4 mm I.D., stationary phases as indicated; temperature, ambient. ○—○ = titration; ●—● = RP-8; △—△ = RP-18; ■—■ = CN.

TABLE IV

EFFICIENCY OF SEPARATION OF PSMA ON THE DIFFERENT STATIONARY PHASES IN THE NP OR RP MODE, EXPRESSED AS THE AVERAGE PEAK WIDTH AT HALF-HEIGHT, IN % STYRENE

<i>Stationary phase</i>	<i>Gradient</i>	<i>Separation efficiency</i>
Si	NP	4.5
CN	NP	4.5
NH ₂	NP	4.7
Diol	NP	6.3
Glass	NP	15
RP-18	NP	22
RP-18	RP	5.5
RP-8	RP	8.5
CN	RP	12

in this instance the separation is controlled by a precipitation mechanism and the contribution of adsorption is negligible. On the other hand, this implies that the adsorption process observed for NP packings contributes to acceptable separation efficiencies and higher retention times on the polar silicas.

For the RP gradient experiments the corresponding elution and precipitation data indicate that the separation is achieved by the precipitation mechanism. However, to obtain a satisfactory separation with an RP gradient it is necessary to apply RP packings, because on NP packings the separation is disturbed by adsorption processes.

CONCLUSIONS

The use of DCM with a suitable amount of methanol as a strong solvent mixture has proved to be successful in combination with *n*-heptane as a non-solvent for the separation by gradient HPLC of SMA and SBA copolymers according to their chemical composition. This eluent combination can be applied with both bare and polar modified silica. In addition to the separation of poly(styrene-acrylates), the proposed eluent system is also suitable for, *e.g.*, poly(styrene-methacrylates), PSAN and other copolymers with comparable solubility and adsorption properties. For each monomer combination the eluent system only has to be optimized according to the methanol content of the strong solvent.

Further studies will include the application of other detection systems to facilitate the quantitative analysis of copolymers with a low styrene content and the application of gradient HPLC to the characterization of emulsion copolymers and other copolymer systems.

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