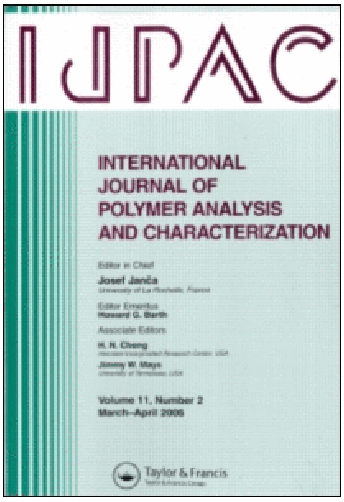

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Solubility and Chromatographic Separation of Styrene/Methacrylate Copolymers in Ternary Eluent Systems

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Chemically heterogeneous polymers can be separated according to composition by liquid chromatography. Usually, binary gradients are used where solvent strength and polarity are changed simultaneously. A new method employs three-component gradients: the starting eluent is a nonsolvent which ensures proper retention of the injected sample. A solvent of intermediate polarity is added next, the amount of which does not suffice for elution and is kept constant during the development of the chromatogram. The solvent is added in one step, polymer solubility is increased by a sudden transition to the desired solvent level. Finally, elution is triggered by the addition of a component which need not be a solvent but whose polarity fits the rules of liquid chromatography. Since there are many more nonsolvents than solvents for polymers, the new procedure offers a wider choice of suitable liquids. Further advantages are improved separation, independent control of solubility and adsorption, and suppression of unfavorable solvent effects. With copoly(styrene-*stat*-methyl methacrylate) separation according to composition was performed through acetonitrile, dichloromethane, and *n*-heptane in normal and reversed phase mode. The chromatographic results were compared with the solubility in the ternary system.

KEY WORDS High performance liquid chromatography, Gradient elution, poly(styrene-*co*-ethyl methacrylate), poly(styrene-*co*-methyl methacrylate), solubility in ternary liquid mixtures

INTRODUCTION

Solubility thermodynamics

The condition of mixing is the decrease in Gibbs free energy function, $\Delta G_{mix} < 0$. A negative change in ΔG_{mix} can, according to the Gibbs-Helmholtz equation

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} \tag{1}$$

be caused by a sufficient increase in the entropy of mixing, ΔS_{mix}, or by a large decrease in the enthalpy of mixing, ΔH_{mix}.

The dissolution of substances with low molecular weight (MW) is favored by a large increase in entropy. The basic constituents of a crystalline solid are regularly arranged. Dissolution yields a random distribution of the constituents. The decrease in order is accompanied by a substantial increase in entropy which, through multiplication with the absolute temperature T, is often large enough to allow dissolution even if the change in enthalpy is positive. There are many examples of endothermic mixtures.

With high-MW compounds, the conditions of mixing are also given by Equation (1) and the negative change in ΔG_{mix}, but the change in entropy is much smaller than in low-MW systems. Polymer chains maintain a one-dimensional order on dissolution; thus, the gain in entropy due to increasing degree of freedom of macromolecules is relatively small. Furthermore, increased entropy can be counteracted by solvation of solvent molecules along the polymer chains causing higher order and restricted mobility of solvent molecules as compared with the pure solvent. Thus, dissolution of polymers can be achieved only if the enthalpy of mixing is small.

Solubility Parameters

Low enthalpy of mixing can be expected when solute and solvent have (nearly) equal values of Hildebrand solubility parameter δ. The solubility parameters of most commercial polymers are about 16–20 $(\text{J/cm}^3)^{0.5}$. The solubility parameters of some popular solvents for polymers are: toluene 18.2, tetrahydrofuran (THF) 18.6, and dichloromethane (DCM) 19.8 $(\text{J/cm}^3)^{0.5}$. Although solubility parameters allow only a rough estimation, a compilation of data [1] indicates that the solubility parameters of solvents usually differ from the values of the respective polymers by less than 2 $(\text{J/cm}^3)^{0.5}$. Larger differences are characteristic of nonsolvents. It is also obvious that low-MW systems show miscibility at larger solubility parameter differences, for example, THF + *n*-hexane at a difference of $18.6 - 14.9 = 3.7$ or THF + methanol (MeOH) at $18.6 - 29.7 = -11.1$ $(\text{J/cm}^3)^{0.5}$. As a consequence, the list of solvents is much shorter for a polymer than for a low-MW solute of similar structure. The solubility window of polymers is narrower than that of low MW substances.

Fractionation

The solubility of a polymer depends upon its chemical structure and decreases with increasing MW. This dependence can be used for fractionation. Precipitation fractionation is mostly performed by controlled addition of a nonsolvent to a dilute solution of the polymer to be fractionated. For solutions having intermediate values of solubility parameter, e.g., around 18 $(\text{J/cm}^3)^{0.5}$, the nonsolvent can be chosen either from the lower or from the upper end of a list where the liquids are arranged according to their solubility parameter. At the lower end, nonpolar liquids are to be found, for example, *n*-heptane (Hp) with 15.1 $(\text{J/cm}^3)^{0.5}$, at the upper end polar ones, for example, acetonitrile (AcN) with 24.3 $(\text{J/cm}^3)^{0.5}$. Fractionation according to MW occurs in both systems in the same direction, that is, precipitation starts with the highest MW, but fractionation according to chemical composition is with a nonpolar precipitant different from fractionation with a polar precipitant. The latter starts with the precipitation of the least polar macromolecules whereas fractionation with a nonpolar nonsolvent starts with the precipitation of the most polar macromolecules. Usually, fractionation according to chemical composition is superimposed by a MW effect which is small in comparison with the composition effect.

Normal phase vs. reversed phase chromatography

Liquid chromatography was first performed using a polar stationary phase (e.g., silica) and a less polar mobile phase. The procedure was called "normal phase" (NP) chromatography since afterwards another mode of chromatography was developed, where a nonpolar stationary phase (e.g., C18 bonded phase) and a polar mobile phase were used. This mode was called "reversed phase" (RP) chromatography. In both modes, mixed eluents of two or more components are preferred because a pure liquid seldom has the proper eluting power.

Gradient Elution

NP chromatography (NPC), as well as RPC, can be performed isocratically with a mobile phase whose composition remains constant throughout the chromatographic run. The alternative is gradient elution where the composition is altered and thus, the eluting strength of the mobile phase increases in the course of the run. In NPC, gradient elution starts with an eluent of low polarity and proceeds with increasing polarity of the mobile phase, for example, with a Hp-DCM mixture whose DCM content is continuously raised. In RPC, gradients start with an eluent of high polarity and continue with decreasing polarity of the mobile phase. Gradient chromatography is capable of separating chemically heterogeneous polymers, for example, copolymers, blends, etc., according to composition [2–6].

With low-MW samples, gradient elution is dominated by polarity. With high-MW samples, the solubility restrictions often implement additional precipitation/redissolution effects. Generally, complete retention of the injected sample is prerequisite of quantitative chromatography. With polymers, this often requires a starting mobile phase whose elution strength is so low that the sample will be precipitated on injection. The same holds true with proteins of low solubility. Fortunately, the precipitation usually does not cause column blocking or related problems, provided that the sample mass is kept within reasonable limits, for example, 20–50 μg per injection on a column with an inside diameter of at least 4 mm.

In the case of precipitation liquid chromatography, both the polarity rules of NP and RP chromatography and the solubility rules of polymers in solvent/nonsolvent mixtures are effective. In most gradient experiments performed so far with polymers, both polarity and solvent strength of the mobile phase were altered simultaneously by the addition of a strong eluent.

Sudden-transition Gradients

In a new gradient regime [7,8] the starting eluent is poor and provides quantitative retention of the injected sample. After injection, the solvent power is increased by the sudden addition of a good solvent of intermediate polarity. The amount of solvent is adjusted to the condition that the sample still remains in the column. Elution occurs only when another component is added that gradually changes the polarity of the eluent in the direction required in NPC or RPC. Surprisingly, even nonsolvents can, in sudden-transition gradients, be capable of eluting and separating retained polymers according to the polarity rules of liquid chromatography. In NPC, the component having the lowest polarity is eluted first whereas in RPC the lowest polar solute elutes last.

Sudden-transition gradients offer several advantages as compared to binary gradients:

1. Improvement of separation and peak shape.
2. Adjustment of elution time and chromatogram length by separate control of solubility level and the rate of polarity gradient.
3. Possibility of monitoring the elution without disturbance by changing solvent concentration (e.g., employing UV detectors at short wavelengths where the solvent already absorbs).

Sudden-transition gradients have been employed so far for the separation according to composition of statistical copolymers from styrene (S) and methoxy ethyl methacrylate [7,9], styrene and ethyl methacrylate (EMA) [7,10,11], styrene and methyl methacrylate (MMA) [7,12], or styrene and acrylonitrile (AN) [13].

EXPERIMENT

Equipment

HP1090 liquid chromatograph (Hewlett Packard, Waldbronn, Germany) with ternary eluent system, autosampler, diode-array detector HP1040A, work station HP79999A, equipped with a Winchester hard-disc HP9133, a Think Jet Printer, and a plotter were used. An evaporative light scattering detector (ELSD) model 750/14 (Zinsser—Applied Chromatography Systems), was connected to the chromatographic system and operated at 14 psi air pressure and an evaporator temperature which was set by turning the switch labeled "evaporation set" (EVS) to "EVS 90" (see, e.g., Ref. [21]). The columns used in this study were: column 1: Cartridge column 60 × 4-mm I.D. (Knauer, Germany), packed with Nucleosil nitrile bonded phase (Macherey & Nagel, Germany), particle size 5 μm; column 2: Cartridge column 60 × 4-mm I.D. (Knauer, Germany), packed with silica, particle size 5 μm; column 3: 250 × 4.1-mm I.D., packed with silica-based cyanopropyl bonded phase, particle size 10 μm; column 4: 250 × 4.1-mm I.D., packed with silica-based RP C18 bonded phase, particle size 10 μm.

Samples

Styrene/ethyl methacrylate (S/EMA) and styrene/methyl methacrylate (S/MMA) copolymers were obtained by free-radical copolymerization to a low degree of conversion (about 5%). S/EMA copolymers were polymerized in bulk and precipitated twice from solution in THF by pouring into an excess of petroleum ether [15]. For sample data, see Table I.

Solvents

Acetonitrile (AcN) for HPLC, dichloromethane (DCM) pure, and *n*-heptane (Hp) pure were purchased from Riedel de Haen, Germany. Tetrahydrofuran (THF) without stabilizer (BASF, Germany) was refluxed over potassium in a silver-coated column for about 24 h. The pure solvent was subsequently kept boiling over potassium in a closed-loop apparatus from which it was taken immediately to the HPLC instrument. *Iso*-octane (iOct) and methanol (MeOH) were LiChrosolv grade (E.Merck, Germany).

TABLE I

Copolymer composition and characteristics

S/EMA copolymers					
Sample code	**A**	**C**	**E**	**G**	**I**
100 wt% EMA	4.7	32.2	54.6	68.0	92.5
$10^{-3}M_w$	51.6	63.1	65.2	83.6	61.6
mo(μg/10 μL mixt.)	8.6	8.9	7.8	10.0	10.5
S/MMA copolymers					
Sample code	**A**	**C**	**E**	**G**	**I**
100 wt% MMA	14.1	34.1	48.1	62.2	83.7
mo(μg/10 μL)	6.76	5.54	5.28	5.48	5.02
styrene content[a]	5.81	3.65	2.74	2.07	0.82
$10^{-3}M_w$ (SEC)	91.0	85.0	99.0	95.0	173.0
$10^{-3}M_w$ (light sctg)[b]	47	150	109	119	182
$10^{-3}M_w$ (SEC)[b]	55	88	94	112	144
M_w/M_n (SEC)[b]	1.58	1.66	1.54	1.65	1.69

a) Calculated from *mo* and wt% MMA
b) Provided by St.Pauly, TU Dresden

Chromatographic Conditions

I. Flow rate $F = 0.5$ mL/min; column temperature $T = 50$ °C; injection volume $V_0 = 10$ μL; sample solutions in analytical-reagent grade THF, stabilized with 0.025% butylated hydroxy toluene (E.Merck). II. $F = 1$ mL/min; $T = 35$ °C; $V_0 = 10$ μL; sample solvent DCM.

Turbidimetric Titration

For titration in the area of solvent/nonsolvent composition where RP elution was achieved, 2.5 mL of a stock solution containing 201.6 mg/L of S/MMA copolymer E in DCM was diluted by 2.5 mL AcN and 0.75 mL Hp. (Thus, the starting position S1 in Figure 10 represented a solution in the mixture of 43.5% DCM, 43.5% AcN, and 13% Hp.) AcN was added at a constant rate of about 3 mL/min and the apparent extinction measured with a colorimeter "Specol" (VEB Carl Zeiss Jena, Germany) modified by a home-made temperature-controlled cell of a 30-mL capacity. The intersection of the tangent at the turbidity curve with the abscissa (volume fraction of titrant) yielded the precipitation point. Since the addition of AcN leaves the ratio of DCM:Hp unchanged, the titration follows a straight line towards the AcN corner of the diagram (indicated in Figure 10). For titration in the area of NP elution, 5 mL of the same stock solution was diluted by 5 mL Hp and 2 mL AcN. The starting mixture S2 contained 41.7% DCM, 41.7% Hp, and 16.7% AcN. Hp was added at a constant rate of about 1.5 mL/min and the apparent extinction measured and evaluated as before.

RESULTS AND DISCUSSION

General Information

Figure 1 shows the merged plot of four chromatograms measured with the mixture of five S/EMA copolymers differing in composition by about 20% EMA each. Sample G differs from I by 24.5% but from E by only 13.4% EMA. This is reflected in the position of peak

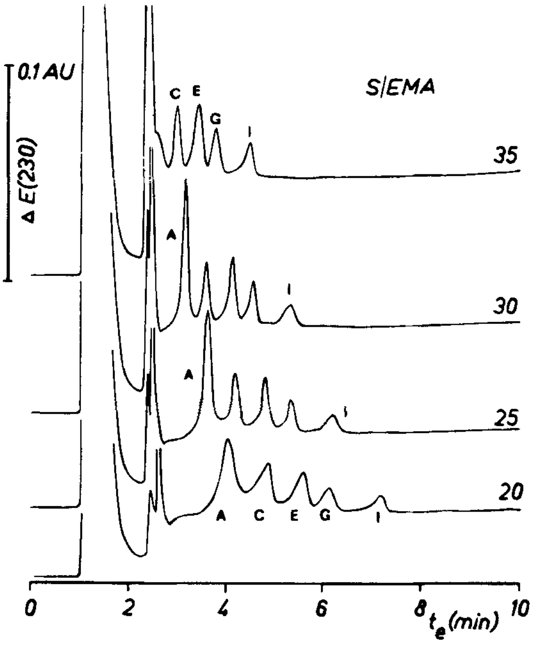

FIGURE 1 Separation of the mixture of S/EMA copolymers A, C, E, G, and I on CN column # 1 using sudden-transition gradients *i*-octane/methanol (5%/min) at constant THF concentration 20, 25, 30, or 35 vol%, UV 230 nm; chromatographic conditions # I [4].

By courtesy of Springer Verlag, Heidelberg, Germany.

G, which is closer to E than to I. The large inflection of the UV signal between 1.0 and 1.8 min is caused by the solvent (THF) and stabilizer (butylated hydroxy toluene) of the sample solution. It is not a polymer peak. The same holds true for the inflection between 2.2 and 2.8 min which is due to the sudden transition from 0 to 20, 25, 30, or 35% THF (indicated at the respective tracing). The transition is programmed at the start of the recording, but it lasts more than 2 min until the composition step reaches the detector [7,8]. If the elution is monitored by an ELS detector, both inflections are invisible, of course. However, UV tracings contain more information and indicate whether or not the solvent transition was finished before the polymer reached the detector cell.

The elution of the sample was in each case performed by the gradual substitution of methanol for the starting eluent (iOct+2% MeOH). The gradient (iOct+2% MeOH)/MeOH (5%/min) was the same in each chromatogram, but performed at different a level of THF concentration, as indicated. THF is a solvent for S/EMA copolymers, iOct and MeOH are nonsolvents.

Improvement of Separation

Whereas baseline separation could be achieved with an optimized sudden-transition gradient, a plain binary gradient iOct/THF [14] was not that effective in separating the mixture of S/EMA copolymers A–G, see Figure 2. The binary gradient could only partly separate copolymer A from C. The objection that the binary gradient might have been less

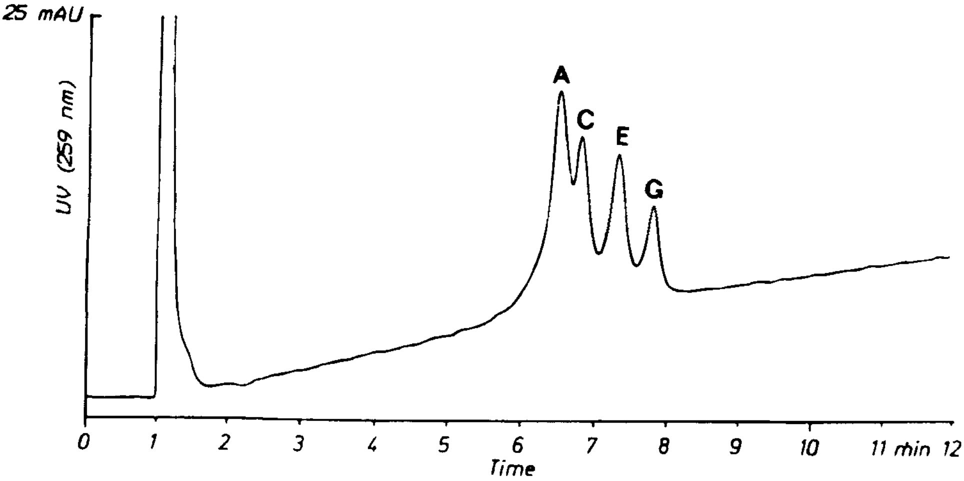

FIGURE 2 Separation of the mixture of S/EMA copolymers A, C, E, and G on silica column # 2. Sample of 10 μg eluted by a binary gradient *i*-octane/THF (20–80% in 12 min); chromatographic conditions # I [14].
By courtesy of Elsevier Scientific Publishers, Amsterdam, Netherlands.

effective because the column packing was silica can be answered by comparing Figures 6 and 7 of a previous paper [15]. Both figures show gradient chromatograms of fractions obtained by size exclusion chromatography (SEC) of the mixture of S/EMA copolymers A, C, E, G and I. The SEC fractions and the gradient iOct/THF (5%/min), with 1% MeOH constant throughout the elution of the polymers were the same in both cases, but the chromatograms were measured either on a silica column or on CN bonded phase.

The comparison shows that the main difference between silica and CN bonded phase is the longer retention on the former due to its higher activity. The graphical superimposition of the chromatograms of all SEC fractions would, for the whole sample, show a rather poor separation on the silica as well as on the CN bonded phase column. Since even with 1% MeOH in the starting eluent the separation did not reach the quality achieved with a sudden-transition gradient iOct/MeOH at 30% THF (see Figure 1), we can say that, in suitable cases, sudden-transition gradients indeed can improve separation and peak shape against the results of binary gradients. (The columns employed in the quoted work [15] were exactly the same as those used in Figures 1 and 2 of the present paper.)

Adjustment of Analysis Time

Figure 1 of the present paper also demonstrates the influence of solvent level on analysis time. The higher the solvent concentration, the earlier the elution starts and the shorter the chromatogram is. With 35% THF, the peak of component A disappeared in the disturbance during the solvent transition. The component was eluted by THF and not by the subsequent gradient iOct/MeOH. Thus, a THF concentration of 35% was too high for the sample mixture under investigation. However, at 20% THF concentration, the four leading peaks were not baseline separated. The best separation could be obtained with THF concentration around 30%.

Of course, solvent quality has also a substantial influence on analysis time. Figure 3 shows sudden-transition gradient separation of S/EMA copolymers on CN column # 3

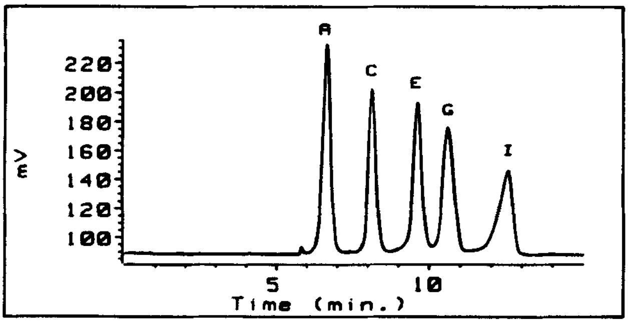

FIGURE 3 Separation of the mixture of five S/EMA copolymers on CN column # 3 using a gradient *n*-heptane/methanol (2.5%/min) after the addition of 20% dichloromethane; chromatographic conditions # II [10].
By courtesy of Vieweg Verlag, Wiesbaden, Germany.

through a Hp/MeOH gradient after adjustment of solubility by the addition of DCM. The nonsolvents iOct and Hp are chromatographically equivalent in NP chromatography of S/EMA copolymers. The amount of solvent required for an adequate solubility level is the same with iOct or with Hp as a starting eluent, but DCM is a more powerful solvent than THF. Only 20% DCM was necessary for optimum separation instead of 30% THF in Figure 1. With a gradient rate of 2.5% MeOH/min, one of best resolved chromatograms of the system could be measured [10].

Control of Adsorption and Solubility

The most important feature of sudden-transition gradients is the possibility of (almost) independent control of solubility and polarity. With an adequate solvent concentration, the elution of the sample can be triggered just by changing the polarity of the eluent. Since the number of nonsolvents for polymers is larger than the number of solvents, the variation of polarity can be performed by a nonsolvent which possibly favors separation by specific interactions between solute and stationary phase.

Figure 4 demonstrates that, at an adequate level of solvent concentration, the polarity and not the solvent power of subsequent eluent effects elution to a greater extent. The upper chromatogram shows the elution of five S/EMA copolymers through the strongly polar nonsolvent MeOH, the lower chromatogram is obtained using THF, which is a good solvent for the samples under investigation. The starting eluent (Hp), the amount of rapidly added solvent (DCM), and the gradient rate (5%/min) were the same in both cases. With the nonsolvent MeOH, elution started earlier (at 5.4 min) than with THF (6.9 min), the chromatogram was shorter (3.0 min instead of 3.8 with THF) and the peak height was about 50% larger than with THF [10]. The nonsolvent MeOH was a more powerful eluent than the solvent THF.

Elimination of Baseline Shift

Detection in gradient chromatography should monitor sample components without any disturbance by the change in eluent composition. UV detection is possible with absorbing solutes and UV-transparent liquids. There are many reports in the literature on the gradi-

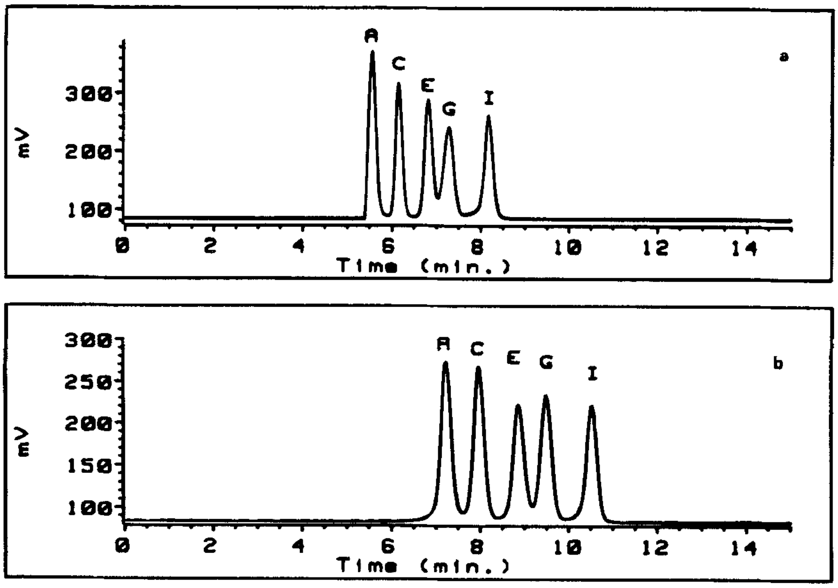

FIGURE 4A Same separation as in Figure 3 using a gradient *n*-heptane/methanol (5%/min) after the addition of 20% dichloromethane [10].

4B Same separation but with a gradient of *n*-heptane/tetrahydrofuran (5%/min) [10].

By courtesy of Vieweg Verlag, Wiesbaden, Germany.

ent elution of styrene copolymers monitored at a wavelength around 260 nm where styrene units have maximum absorption. In this region, THF may also have some absorption, thus, binary gradients with THF as eluting component may exhibit a rising baseline, see Figure 2. At a shorter wavelength, for example, at 230 nm, THF of the same quality would cause a baseline rise, which could lead to reduction of sensitivity. Sudden-transition gradients can eliminate this problem: THF solvent is added in a sudden step, its concentration kept constant during the subsequent polarity gradient. When the latter is performed by a liquid of the same UV transparency as the starting eluent, a horizontal baseline is achieved. Only the level of the baseline is shifted by the absorbing solvent. This influence can be seen in Figure 1: The difference between the initial signal in front of the solvent peak (0–1 min) and the trailing signal after the elution of all sample components increased with the concentration of THF in the eluent, but did not disturb the signal; the chromatograms could be monitored with a uniform detector sensitivity.

Sudden-transition Gradients in NP and RP Mode

In a previous paper [11], we reported the first example of separation through sudden-transition gradients in the RP mode. The copolymer system was S/EMA. A sample mixture was injected into AcN and, after addition of an adequate volume of DCM or THF for adjusting the solubility level, eluted by Hp. Although *n*-heptane is a powerful precipitant for S/EMA copolymers it was capable of separating the mixture. As expected, the sequence of peaks was inverted against the sequence in NP chromatography. The surprising efficiency of Hp in sudden-transition gradients once more stresses that, at an appropriate level of solubility, the polarity of the last component indeed determines its eluting power.

Recently, RP separation according to composition of S/MMA copolymers by sudden-transition gradients in NP and RP mode was published [12]. Figure 5 demonstrates the separations achieved in both modes of sudden-transition gradients.

Sudden-transition Gradients and Common Binary Gradients in RPC of S/MMA

Figure 6 permits the comparison of RP separation achieved by a binary gradient AcN/DCM and by a sudden-transition gradient AcN/Hp at constant DCM concentration. DCM is known to cause deviations of ELSD signals [16,17]. In Figure 6a, the concentration of DCM increased in the course of the run. A surprising increase in peak height and a baseline drop may be the consequences. In Figure 6b, 30% DCM was present throughout the separation. Thus, sudden-transition gradients can help to improve the chromatograms (*vide supra*) not only with optical detectors, but also with ELS detectors.

Elution Characteristics

A synoptic presentation of sudden-transition gradient results can be given by the eluent composition at each peak position. The lines connecting the points measured with a copolymer of given composition yield the elution characteristics of the sample. Usually, three-component mixtures are depicted in an equilateral triangle. Rectangular triangles with the solvent position at the upper corner and the polar and nonpolar nonsolvents at the edges of the base offer the advantage that systems with constant concentration of the nonsolvent, which is depicted on the abscissa of the diagram, yield *perpendicular* elution characteristics. Along perpendicular elution characteristics, the total of the solvent and the second nonsolvent (e.g., AcN) is constant as well, that is, a given volume of the second nonsolvent is equivalent in elution power to the same volume of solvent. Both liquids have equal eluotropic capacity in the system under investigation (for further details, see refs. [8] and [10]). Another advantage of this presentation is the possibility of adopting a different scale on the ordinate and abscissa without loosing the feature of a rectangular triangle.

Figure 7 shows the results measured with S/MMA copolymers by sudden-transition gradients in the NP mode. The elution characteristics of copolymers **I**, **G**, **E**, and **C** are similar. They exhibit a slightly negative slope which indicates that a given reduction in DCM content could not be totally compensated for by the addition of the same volume AcN. The elution characteristics of copolymer **A** and of polystyrene have, in their upper part, a positive slope. These samples were eluted in mixtures where the solvent strength was obviously so high that the polarity of AcN made this nonsolvent a stronger eluent than DCM. (This behavior is similar to that discussed in connection with Figure 4.)

Basically, the results of sudden-transition gradient elution in RP mode could be plotted in the same graph. For that reason the abscissa were to be extrapolated towards the left, but since in RP elution Hp was added (and not AcN as in NPC), the reference line for eluotropic equivalence would no longer run perpendicularly but parallel to the line indicated by "AcN = 0".

In order to maintain the advantage of perpendicular elution characteristics for liquids of equal eluotropic strength, in Figure 8 the AcN concentration was plotted on the abscissa and the line "Hp = 0" adopted as the right-hand borderline of the triangle. In Figure 8 all

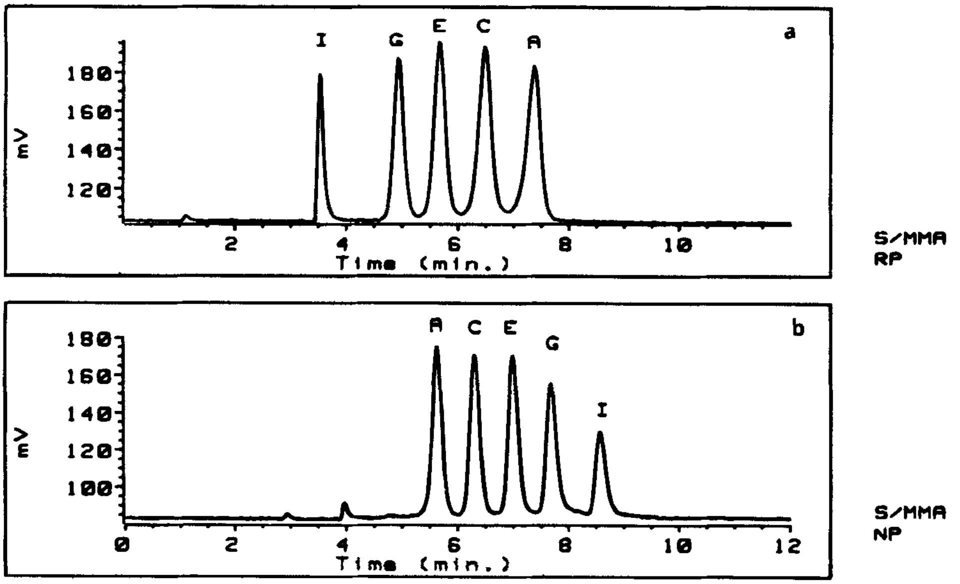

FIGURE 5 Separation of the mixture of five styrene/methyl methacrylate copolymers through a sudden-transition gradient in reversed-phase (5a, C18 column #4) or normal-phase modes (5b, CN column # 3). Both separations were achieved after adjustment of dichloromethane concentration to 30%; chromatographic conditions # II [12].

By courtesy of Vieweg Verlag, Wiesbaden, Germany.

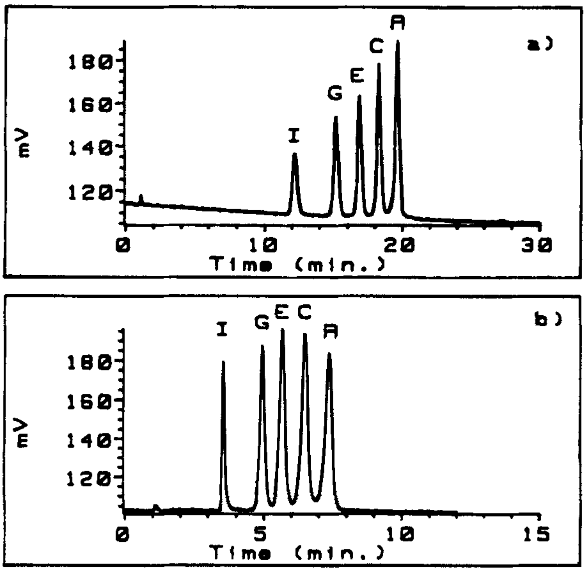

FIGURE 6A Reversed-phase separation of the mixture of five styrene/methyl methacrylate copolymers by a common binary gradient acetonitrile/dichloromethane (5%/min) on RP C18 column # 4, copolymer composition 14–84 wt% MMA, see Table Ib, chromatographic conditions #II, ELS detection.

6B Separation of the same mixture by a sudden-transition gradient acetonitrile/*n*-heptane (5%/min) on the same column after addition of 30 vol% dichloromethane, chromatographic conditions #II [12].

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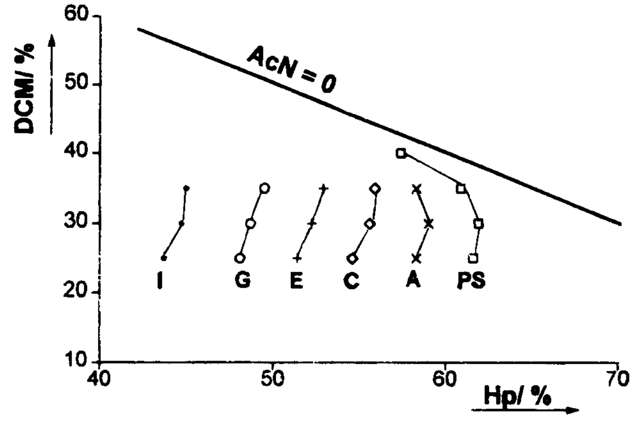

FIGURE 7 Elution characteristics of S/MMA copolymers in normal-phase sudden-transition gradients *n*-heptane/acetonitrile, solvent: dichloromethane [12].

By courtesy of Vieweg Verlag, Wiesbaden, Germany.

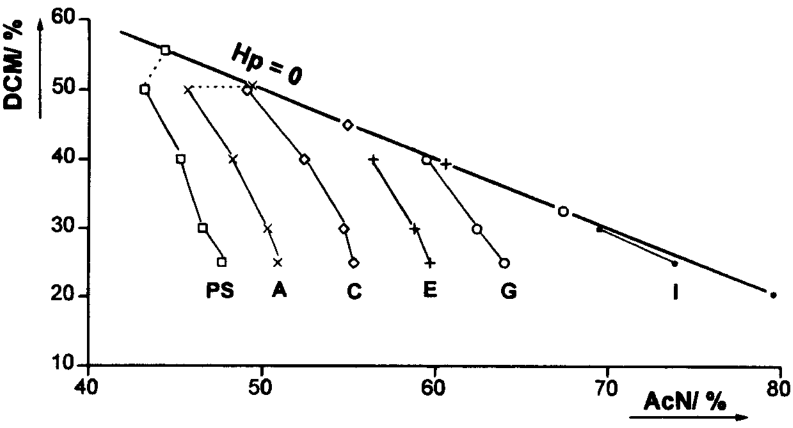

FIGURE 8 Elution characteristics of S/EMA copolymers in reversed-phase sudden-transition gradients acetonitrile/*n*-heptane, solvent: dichloromethane [12].

By courtesy of Vieweg Verlag, Wiesbaden, Germany.

but one copolymer yield lines with a positive slope indicating higher efficiency of Hp than DCM in RP systems, which is plausible considering the polarity of both eluents. The strange position of copolymer I suggests improper retention of this component. It was certainly swept through the column by DCM, similar to S/EMA copolymer A in Figure 1 at 35% THF concentration. (As already mentioned, an ELSD response cannot reveal the disturbance of eluent composition as would a UV detector.)

NP and RP Retention of S/MMA Copolymers vs. Solubility

Recently we published the first investigation [12] where both modes of sudden-transition gradient chromatography, NPC as well as RPC, were performed with the same set of eluents: DCM as a solvent of moderate polarity, Hp as a nonpolar nonsolvent and AcN as a polar nonsolvent. Thus, the experiments provide for the first time the chance of comparing sudden-transition elution characteristics for NPC and RPC in a joint graph.

Figure 9 shows the composition triangle for the ternary system DCM solvent and both nonsolvents Hp and AcN. The S/MMA copolymers A–I and polystyrene were eluted within two distinct areas, each covering not more than about one tenth of the overall composition range. In both areas, the graphical sequence of samples was the same, with copolymer I at the left side and polystyrene at the right-hand border of each group. This is the sequence of decreasing polarity of the polymers, corresponding in direction to the decrease in eluent polarity along the baseline of Figure 9.

The influence of stationary phase was certainly among the reasons why separation in the RP mode was achieved in an eluent composition area aside from that of NP elution. Elution characteristics of RP separations are gathered in the polar area of the eluent composition graph. This result suggests that RP retention on nonpolar phases requires a high-polar starting eluent. However, NP retention on polar columns is achieved only with weakly polar starting eluents. Thus, distinct areas for both modes of retention can be understood as the consequence of adsorption interactions.

With numerous synthetic copolymers, correspondence has been found between elution characteristics and solubility borderlines. With S/EMA copolymers in MeOH/THF [18], elution characteristics and solubility borderlines are almost identical curves in the same position. A behavior of that kind represents the highest degree of correspondence. In other systems, where elution characteristics and solubility borderlines run more or less parallel, the shift between both parallel lines reveals eventually the contribution of adsorption [19]. With S/AN copolymers, RP elution was possible only under conditions determined by solubility [20]. Thus, the question remains whether or not the elution characteristics in Figure 9 might indicate also solubility borderlines having similar position and slope.

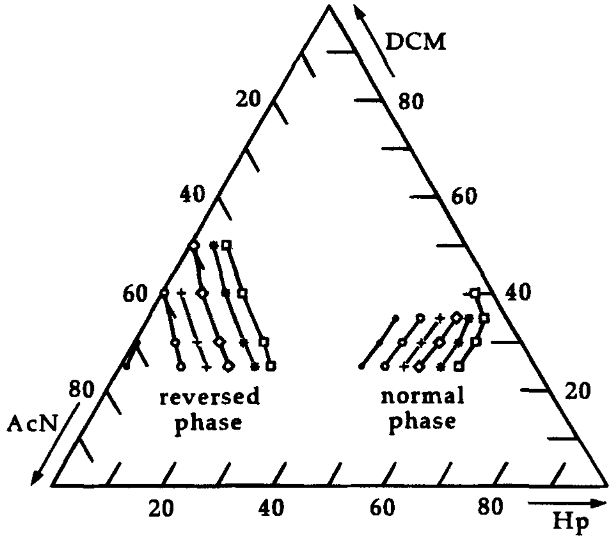

FIGURE 9 Composition triangle acetonitrile/*n*-heptane/dichloromethane with elution characteristics of S/MMA copolymers in normal-phase and reversed-phase sudden-transition gradients, •: copolymer I, o: G, +: E, ◇: C, *: A, □ polystyrene. (Data from Figures 7 and 8, respectively.)

In order to experimentally check this question, turbidimetric titrations were performed in the ternary system with copolymer **E** as a solute. The results are shown in Figure 10. In addition to the procedure described in the experimental section, attempts were made to measure precipitation points of S/MMA copolymer E at a given solvent concentration, for example, at 25 or 30% DCM. In spite of several difficulties, it was possible to measure a value in the region rich in Hp (included in Figure 10). Together with the precipitation points measured in the binary systems DCM/AcN and DCM/Hp, the results determine two sections of the solubility borderline.

AcN and Hp are only partly miscible. The gap becomes narrower on the addition of DCM. With 25% or more DCM, miscibility was complete at room temperature [11]. All polymers considered here are soluble in the upper region of Figure 10, that is, in the area immediately below the DCM solvent corner. The addition of small amounts of Hp or AcN will, of course, impair solubility but not yet cause precipitation. For instance, with about 40% Hp or 40% AcN in DCM, the polymers are still soluble. Thus, in the upper region, solubility extends to both the left and right sides of the eluent triangle. With decreasing concentration of DCM, the solvent power of binary mixtures of DCM and one of the non-solvents will reach a precipitation threshold. Thus, the area of homogeneous solutions will be reduced towards the center of the triangle.

The beginning of the borderline bending from both the left and right sides of the triangle towards the inner area can clearly be seen in Figure 10. Homogeneous solutions cannot exist in the region adjacent to the baseline where, even without polymer, the liquids do not yield homogeneous mixtures. Thus, the miscibility gap determines the lower limit of homogeneous solutions. This section of the solubility borderline will probably merge with the sections of the borderline at the left and right sides of the phase diagram. The total

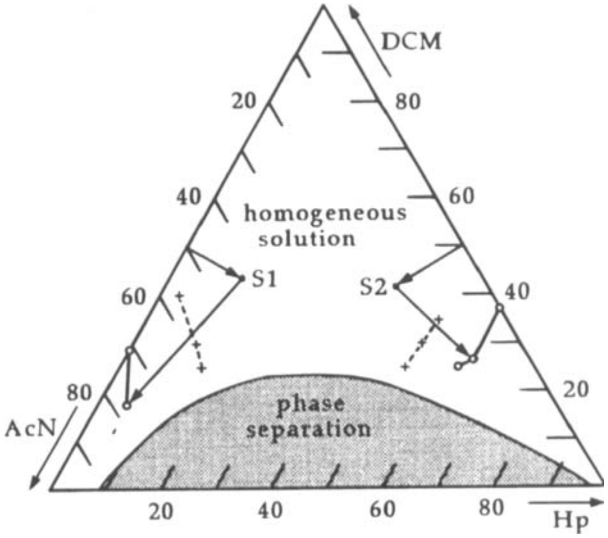

FIGURE 10 Phase diagram of acetonitrile/*n*-heptane/dichloromethane indicating the miscibility gap, o: precipitation points measured by turbidimetric titration of S/MMA copolymer E, +: elution characteristics of this copolymer, redrawn from Figure 9.

borderline determines a solubility window which obviously is similar to the elution characteristics of the copolymer. A closer look at Figure 10 reveals that at a given solvent level, say 25% DCM, the elution characteristics at its left branch indicates about 15% more Hp than the broken solubility borderline. This can be understood as the contribution of the column to RP retention on a nonpolar packing in a highly polar eluent system (containing about 60% AcN). At the right branch, about 10% more AcN is needed for elution than for solubility. This can, in a corresponding manner, be understood as the contribution of sample adsorption to NP retention on a polar column.

The similar shape of the elution windows of the samples investigated indicates that solubility was certainly involved in all experiments described here. Taking the measured line of copolymer E as a guide, one can determine corresponding elution windows for the other samples as well.

CONCLUSIONS

Many polymers are, in mixtures of a solvent with two nonsolvents differing in polarity, likely to exhibit solubility behavior analogous to that discussed in Figure 10. Hence, HPLC separation through sudden-transition gradients should, with a common ternary eluent system, generally be possible in the NP mode as well as in the RP mode. Both modes of separation should be achievable near the respective side of the solubility borderline. The location of the elution characteristics in a composition diagram similar to Figure 10 will certainly, in any case, show different areas for NP or RP mode of separation. Investigations of this kind will not only offer the chance of improving separations with only small additional effort but also contribute to a better understanding of the mechanisms of polymer chromatography.

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