CHROM. 25 505

Elution behaviour of styrene–acrylonitrile copolymers in high-performance liquid chromatography with mixtures of chloroform and *n*-hexane as mobile phases

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ABSTRACT

With isocratic elution of styrene–acrylonitrile copolymers from an ODS silica column using chloroform–*n*-hexane mixtures as mobile phases, the elution profiles of the copolymers with an acrylonitrile content from 15 to 33% showed four different patterns, depending on the compositions of the copolymers and the mobile phases: (i) elution with a retention volume corresponding to the interstitial volume of the column; (ii) elution with a retention volume corresponding to the sum of the interstitial volume and the pore volume of the packing materials in the column; (iii) elution with both retention volumes; and (iv) precipitation on the column. A transition period was found during the process in which the copolymer which dissolved in a good solvent precipitated when the latter became a poor solvent on adding a non-solvent, and this was defined as the pre-precipitation state. Each molecule was in a condensed form in this state. In order to separate the copolymers according to composition, gradient elution from 100% *n*-hexane to 100% chloroform, in 30 min was performed, and the copolymers were separated in order of increasing the acrylonitrile content. No molecular mass dependence of the separation was observed.

INTRODUCTION

Synthetic random copolymers, in general, show a molecular mass distribution and a chemical composition distribution, and the determination of both distributions is important for the characterization of the copolymers. Several attempts have been made to determine chemical composition distribution by high-performance liquid chromatography (HPLC). For example, poly(styrene–methyl acrylate) [1], poly(styrene–methyl methacrylate) [2–4], poly(styrene–acrylonitrile) [5] and poly(styrene–butadiene) [6] random copolymers have been separated according to their composition by HPLC.

Styrene–acrylonitrile random copolymers, P(S–AN), are of technical importance and the

determination of their chemical heterogeneity is of great interest. High-performance precipitation liquid chromatography (HPPLC), developed by Glöckner *et al.* [5], was first applied to the separation of P(S–AN) copolymers according to composition. HPPLC uses a mixture of a good solvent and a non-solvent for the copolymer concerned and the initial mobile phase consists of a non-solvent (isooctane in Glöckner *et al.*'s work) and, first, the copolymer which is dissolved in a good solvent and is injected into the chromatographic system is precipitated on top of the column. By applying gradient elution with a good solvent (tetrahydrofuran in their work), the copolymer is redissolved and is eluted according to its solubility (and composition).

The separation of P(S–AN) copolymers by HPPLC was found to be dependent on the stationary phase and the composition of the mobile phase [7]. By applying normal-phase

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gradient elution from n-heptane to dichloromethane with RP-C_{18} or polystyrene gel columns, the copolymers were separated in order of increasing acrylonitrile content, but the peaks were broad. Separation according to composition was also possible by normal-phase gradient elution from n-heptane–dichloromethane (20:80) to dichloromethane–2.75% methanol using 500-Å pore-size silica gel and the peaks were almost symmetrical.

In this work, the elution behaviour of P(S–AN) in HPPLC with mixtures of chloroform and n-hexane as mobile phases was evaluated. The copolymer was dissolved in chloroform in all instances. With isocratic elution with n-hexane–chloroform, the transition from elution from a column to precipitation on the column via the pre-precipitation state was clearly observed. Separation according to composition was possible by gradient elution from n-hexane to chloroform.

EXPERIMENTAL

The apparatus used was a Jasco (Tokyo, Japan) Trirotar-VI high-performance liquid chromatograph with a Uvidec-100 VI ultraviolet absorption detector operated at 260 or 270 nm. The packing material was ODS silica (Develosil) (Nomura Chemical, Aichi, Japan) packed in a column of 250 mm × 4.6 mm I.D. The number of theoretical plates was 2400, obtained by injecting 0.1 ml of a 0.1% solution of benzene in chloroform. The column was thermostated at 25°C in a Model AO-30C column oven (Showa Denko, Tokyo, Japan).

The samples used were styrene–acrylonitrile copolymers prepared by suspension polymerization and supplied by Mitsubishi Monsanto (Yokkaichi, Japan). The acrylonitrile contents of these copolymers were measured by nitrogen determination and were as follows: P(S–AN)-15, 14.8% (w/w); P(S–AN)-20, 19.6% (w/w); P(S–AN)-27, 26.2% (w/w); and P(S–AN)-33, 32.3% (w/w). These samples were dissolved in chloroform at a concentration of 0.05 or 0.1%. Chloroform is a good solvent for the copolymers and can dissolve them completely. The injection volume into the column was 0.1 ml, which was a

reasonable size in this experiment: no difference was observed in the elution behaviour between sample sizes of 0.1 and 0.01 ml, the peak being difficult to recognize in the latter instance. Polystyrenes (PS) having narrow molecular mass distributions (Pressure Chemical, Pittsburgh, PA, USA) were used for the measurement of the molecular mass dependence of the HPPLC separation.

The mobile phases were chloroform, n-hexane and their mixtures. Chloroform contained 1% of ethanol as a stabilizer and henceforth chloroform where mentioned contains 1% of ethanol unless specified otherwise. Elution was performed by isocratic or gradient elution. The flow-rate was 0.5 ml/min and the pressure drop of the column system was between 21 and 23 kg/cm^2 during elution.

RESULTS AND DISCUSSION

Isocratic elution

Samples were dissolved in chloroform, which was a good solvent for the copolymers. The sample solutions were introduced into the LC system where good or poor eluents for the sample copolymers were used as the mobile phases. When good eluents for the copolymers were used, they eluted at V_0 (= 2.0 ml) [the exclusion limit in size-exclusion chromatography (SEC)] and when poor eluents were used, they eluted at $V_0 + V_i$ (= 3.0 ml) (V_i = pore volume) or precipitated on the top of the column.

When chloroform was used as the mobile phase, all the copolymers eluted at a retention volume $V_R = 2.0$ ml. With up to 20% of n-hexane in chloroform, the copolymers eluted at $V_R = 2.0$ ml. The elution behaviour of the copolymers obtained with increasing n-hexane content in the mobile phase is discussed below. All the measurement were monitored by UV detection at 260 nm.

P(S–AN)-33. When the composition of the mobile phase was chloroform–n-hexane (70:30, v/v), the intensity of the peak at $V_R = 2.0$ ml decreased and a small peak was observed at $V_R = 3.0$ ml. At the composition chloroform–n-hexane (60:40, v/v), the peak at $V_R = 2.0$ ml disappeared and only the second peak at $V_R =$

S. Mori and M. Naito / J. Chromatogr. A 655 (1993) 185–190

187

3.0 ml was observed. On increasing the n-hexane content to 60% or 70%, the peak intensity at $V_R = 3.0$ ml became significantly larger. When n-hexane alone was used as the mobile phase, the second peak at $V_R = 3.0$ ml disappeared and only a small peak due to the solvent used for the sample solution was observed. All the copolymer precipitated on the column. The results are shown in Fig. 1. The small peak at $V_R = 3.0$ ml in Fig. 1a is not a sample peak but that due to the solvent used for sample solution.

P(S–AN)-27. With up to 30% of n-hexane in the mobile phase, all the copolymer eluted at $V_R = 2.0$ ml. With chloroform–n-hexane (60:40, v/v), the intensity of the peak decreased and a second peak appeared at $V_R = 3.0$ ml. At the composition chloroform–n-hexane (50:50, v/v), the first peak disappeared and only the second peak was observed. With chloroform–n-hexane (30:70, v/v), the intensity of the second peak increased significantly. When n-hexane alone was used as the mobile phase, the second peak decreased and most of the copolymer precipitated on the column. The results are shown in Fig. 2.

P(S–AN)-20. With up to 40% of n-hexane in the mobile phase, all the copolymer eluted at $V_R = 2.0$ ml. When the composition was chloroform–n-hexane (50:50, v/v), the intensity of the peak decreased and a second small peak appeared at $V_R = 3.0$ ml. At chloroform–n-hexane (40:60, v/v), only the second peak was observed. The intensity of the second peak increased
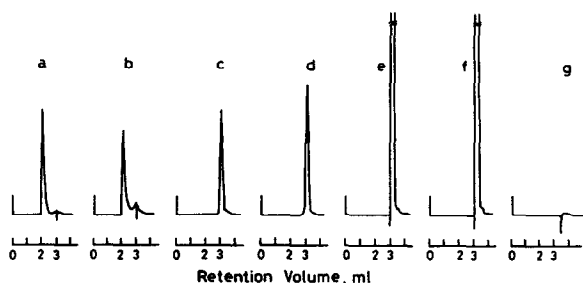

Fig. 2. Elution profiles of P(S–AN)-27 copolymer. Composition of chloroform–n-hexane mobile phase: (a) 70:30; (b) 60:40; (c) 50:50; (d) 40:60; (e) 30:70; (f) 0:100 (v/v). Other conditions as in Fig. 1.

significantly with n-hexane alone as the mobile phase. The results are shown in Fig. 3.

P(S–AN)-15. With up to 50% of n-hexane in the mobile phase, all the copolymer appeared at $V_R = 2.0$ ml. With chloroform–n-hexane (40:60, v/v), the peak became small and a second peak appeared at $V_R = 3.0$ ml. With chloroform–n-hexane (30:70, v/v), the first peak disappeared and only the second peak was observed. When n-hexane alone was used as the mobile phase, the intensity of the second peak increased significantly as with P(S–AN)-20. The results are shown in Fig. 4.

The retention volume of 2.0 ml in this system corresponds to the volume at the exclusion limit $(= V_0)$ in SEC and the solutes elute from the column through the interstices among the packing particles without entering the pores of the

Fig. 1. Elution profiles of P(S–AN)-33 copolymer. Sample concentration, 0.05%; UV detection at 260 nm, 0.64 AUFS. Composition of chloroform–n-hexane mobile phase: (a) 80:20; (b) 70:30; (c) 60:40; (d) 50:50; (e) 40:60; (f) 30:70; (g) 0:100 (v/v).
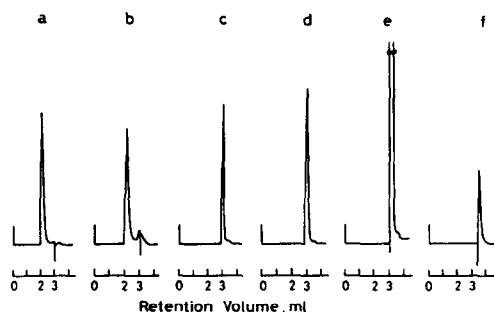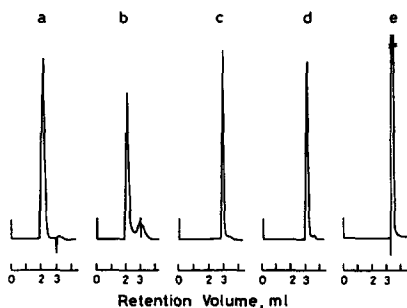

Fig. 3. Elution profiles of P(S–AN)-20 copolymer. Composition of chloroform–n-hexane mobile phase: (a) 60:40; (b) 50:50; (c) 40:60; (d) 30:70; (e) 0:100 (v/v). Other conditions as in Fig. 1.
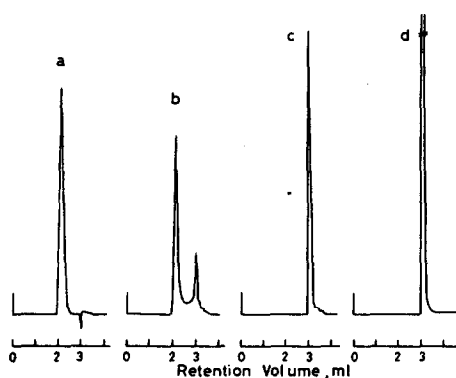
Fig. 4. Elution profiles of P(S–AN)-15 copolymer. Composition of chloroform–n-hexane mobile phase: (a) 50:50; (b) 40:60; (c) 30:70; (d) 0:100 (v/v).

particles. ODS silica packing materials prepared with silica gel having an average pore of 100 Å decrease the apparent average pore diameter to 30–40 Å and polymers having molecular masses over 1000 are totally excluded from the pores when they are eluted with a good solvent as the mobile phase [8]. The retention volume of 3.0 ml in this system corresponds to the sum of the interstitial volume, the pore volume of the packing materials in the column and the dead volume of tubing between the injection valve and the UV detector $(= V_0 + V_i)$. Small molecules are capable of entering deeply into the pores and elute at this retention volume.

Mobile phases having a low content of n-hexane in chloroform are still good solvents for P(S–AN) copolymers and the copolymers will elute at $V_R = 2.0$ ml with these mobile phases. Mobile phases having a high content of n-hexane are poor solvents for the copolymers and with increasing n-hexane content in the mobile phase the copolymers precipitate on the column, depending on their composition. A transition period (the pre-precipitation state) may exist during the process in which the copolymer that dissolved in a good solvent precipitated as the solvent became a poor solvent on adding a non-solvent to it. Each molecule was in a condensed form during this period. Therefore, the size of the copolymer became very small in this period and the copolymer eluted at $V_R = 3.0$ ml as in Fig. 1c and d, Fig. 2c and d, Fig. 3c and d and

Fig. 4c. These phenomena were different from those in HPPLC.

n-Hexane is a non-solvent for P(S–AN) copolymers and when it is used alone as the mobile phase, the copolymers should precipitate on the column according to the principle of HPPLC, as in Fig. 1g. On the other hand, a small peak at $V_R = 3.0$ ml is observed in Fig. 2f and the copolymers eluted from the column even with n-hexane as the mobile phase as in Figs. 3 and 4. The reason is considered to be as follows. As the copolymers were dissolved in chloroform as sample solutions, the copolymer molecules were surrounded tightly with chloroform molecules and the contact between n-hexane molecules and the copolymer molecules was interrupted for a while. Therefore, the copolymer eluted from the column without precipitating immediately, even though the mobile phase was n-hexane. This process depends, of course, on the composition of the copolymers and P(S–AN)-33 copolymer precipitated immediately in the n-hexane mobile phase.

A significant increase in the peak intensity with increasing n-hexane content in the mobile phase was observed, as in Fig. 1e and f, for example. The phenomena occurred just before the precipitation of the copolymers on the column. The reason is assumed to be that the transparency of the effluent containing the copolymer became poorer owing to the increase in turbidity, resulting in a decrease in the UV transmittance. At this moment some of the copolymer precipitated on the column (see Gradient elution, below). The peak areas at $V_R = 3.0$ ml were not constant, because the elution at this point was in a transition state and was influenced by the experimental conditions.

Turbidity measurement

The solubility of the copolymers decreases with increasing contents of acrylonitrile in the copolymers and of n-hexane in the mobile phase. The copolymers eluted at $V_R = 2.0$ ml at first, shifted to $V_R = 3.0$ ml with increasing n-hexane content in the mobile phase and were then retained, which is considered to be due to the transition of the state of the copolymers in the mobile phase. The copolymers are solvated with

a good solvent such as chloroform or chloroform with a small proportion of n-hexane, shrink in a poor solvent such as chloroform containing large amounts of n-hexane, and then are precipitated on the column in the non-solvent, n-hexane.

The solubility of the copolymers can be determined by turbidity measurement. A volume of 10 ml of a 0.1% copolymer solution in chloroform placed in a flask and was thermostated at 25°C in a water-bath and then visually titrated with n-hexane. For turbidity titration, the spectrometric method is preferable, but in order to understand the observations in this work, the visual technique may be sufficient. The volume of n-hexane when turbidity was first observed in the solution was taken as the cloud point. The volume of n-hexane required was as follows: P(S–AN)-15, 17.0 ml; P(S–AN)-20, 13.3 ml; P(S–AN)-27, 9.0 ml; and P(S–AN)-33, 6.0 ml. The n-hexane content in the solution required to precipitate the copolymers was plotted against the composition of the copolymers and is shown in Fig. 5 (closed circles).

The open circles in Fig. 5 indicate the n-
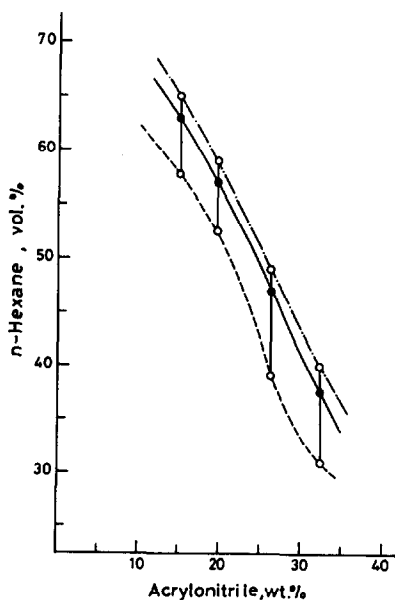

Fig. 5. Cloud point and elution characteristics plotted as n-hexane content in the mobile phase versus acrylonitrile content in the copolymer. ● = Cloud point; O–O = the range in which both peaks appeared at $V_R = 2.0$ ml and $V_R = 3.0$ ml, respectively.

hexane contents in the mobile phase at which the second peak at $V_R = 3.0$ ml appeared (bottom) and at which the first peak at $V_R = 2.0$ ml disappeared (top). Each cloud point is located between two open circles. The top open circles are close to the cloud points, indicating that the copolymers eluted from the column as if they were small molecules and they did not precipitate on the column at their cloud points. These results are different from those obtained by Glöckner and Van den Berg [9]. Mobile phases having compositions below the bottom open circles correspond to good solvents for the copolymer. The region between the bottom open circles and somewhat above the top open circles corresponds to the transition period (the pre-precipitation state) of the specified copolymer.

Gradient elution

The elution profiles of P(S–AN) copolymers obtained by isocratic elution were grouped into the following four patterns: elution at $V_R = 2.0$ ml, elution at $V_R = 2.0$ and 3.0 ml, elution at $V_R = 3.0$ and precipitation on the column. Elution between $V_R = 2.0$ and 3.0 ml or after $V_R = 3.0$ ml was not observed. Therefore, in order to separate the copolymers according to composition, gradient elution should be performed with n-hexane–chloroform, the concentration of the latter increasing linearly.

Linear gradient elution was performed with a gradient from 100% n-hexane to 100% chloroform in 30 min, and then the elution was continued with chloroform for a further 15 min. In order to minimize the baseline drift during the elution, the wavelength of the UV detector was changed to 270 nm. A sample volume of 0.1 ml was sufficient to detect peaks and no difference in the elution profiles was observed between sample volumes of 0.1 and 0.01 ml.

The results are shown in Fig. 6. Elution was in the order of increasing acrylonitrile content in the copolymers and there was a linear relationship between retention volume and acrylonitrile content. After the gradient elution, the mobile phase was changed to n-hexane, the flow of which into the column was continued for 70 min. By this treatment, it was possible to minimize the peak eluted at $V_R = 3.0$ ml except for P(S–
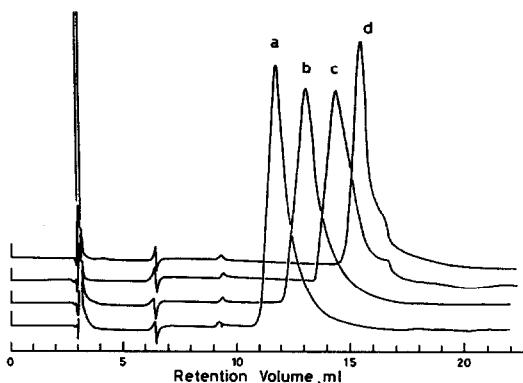
Fig. 6. Chromatograms of P(S–AN) copolymers obtained by gradient elution. Sample solution, 0.1%; volume injected, 0.1 ml; UV detection at 270 nm, 0.08 AUFS. Peaks: (a) P(S–AN)-15; (b) P(S–AN)-20; (c) P(S–AN)-27; (d) P(S–AN)-33.
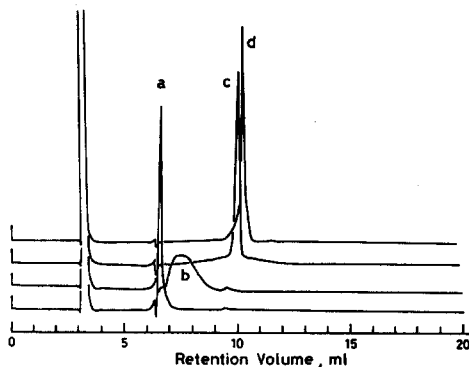

Fig. 7. Chromatograms of polystyrenes obtained by gradient elution. Sample solution, 0.05%; volume injected, 0.1 ml; UV detection at 270 nm, 0.16 AUFS. Peaks: PS of M_r (a) 2200, (b) 20 400, (c) 97 200 and (d) 411 000.

AN)-15, which still had a large peak there. Although P(S–AN)-15 copolymer had a large peak at $V_R = 3.0$ ml, part of the copolymer was also eluted at the appropriate retention volume by gradient elution, and thus the assumptions that the extremely large peak at $V_R = 3.0$ ml was due to the decrease in the transmittance of UV radiation caused by turbidity of the sample effluent and that part of the copolymer precipitated on the top of the column at this moment seem to be correct. The peak at $V_R = 3.0$ ml except for P(S–AN)-15 and that appeared at $V_R = 6.5$ ml are those due to the sample solvent.

In order to check the molecular mass dependence of the separation by gradient elution, polystyrene (PS) standards having a narrow molecular mass distribution were separated in the same manner as in Fig. 6 and the results are shown in Fig. 7. PS having $M_r > 10^5$ eluted at $V_R = 10.0$ ml, PS having $M_r = 2200$ eluted at $V_R = 6.5$ ml and PS having $M_r = 20\,400$ eluted after $V_R = 6.5$ ml as a broad peak. All the polymers had a large peak at $V_R = 3.0$ ml as with the P(S–AN) copolymers.

These results were obtained with PS samples and therefore similar experiments with P(S–AN) copolymer samples are required in order to estimate the molecular mass dependence. No data have appeared in the literature to indicate that the molecular mass dependence of elution

can or cannot be transferred from homopolymers to copolymers. If it could be, then it would be possible to state that no molecular mass dependence of the separation by gradient elution was observed for polymers and copolymers of high molecular mass, e.g., $>10^5$. P(S–AN) copolymers with a 4% acrylonitrile content eluted at 10.3 ml (data not shown). From these results and Fig. 6, it could also be possible to state that the P(S–AN) copolymers examined in this work eluted according to their composition and no molecular mass dependence was observed.

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