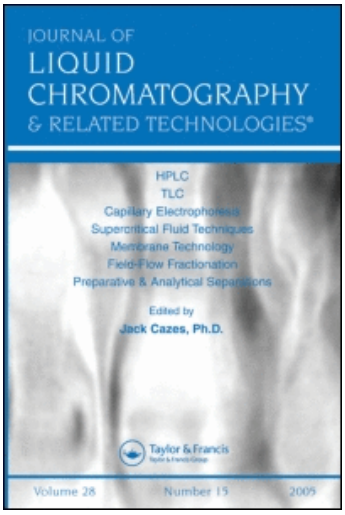

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SEPARATION OF STYRENE-ACRYLONITRILE COPOLYMERS BY STEPWISE GRADIENT ELUTION – HIGH-PERFORMANCE PRECIPITATION LIQUID CHROMATOGRAPHY

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ABSTRACT

With stepwise gradient elution of styrene-acrylonitrile copolymers from an ODS-silica column using chloroform - n-hexane mixtures as mobile phases, each copolymer was separated into six to eight peaks. The initial mobile phase was n-hexane and the content of chloroform in the mobile phase was increased 2% every five or ten minutes. Separated peaks were fractionated and the acrylonitrile (AN) content and molecular weight averages were measured. The copolymers were separated in order of increasing the AN content and molecular weight with a few exceptions. This explains that the fractions having lower molecular weight have lower AN content.

INTRODUCTION

Several attempts for the determination of chemical heterogeneity and chemical composition distribution for styrene-acrylonitrile copolymers P(S-AN) by high-performance liquid chromatography

(HPLC) have been reported. High-performance precipitation liquid chromatography (HPPLC), developed by Glöckner was applied to the separation of P(S-AN) according to composition (1,2). HPPLC uses a mixture of a good solvent and a non-solvent for the copolymer concerned as the mobile phase: tetrahydrofuran (THF) - n-hexane or THF - iso-octane in his work. The initial mobile phase consists of a non-solvent and the copolymer which is dissolved in a good solvent is injected into a column and is precipitated on top of the column. By applying a gradient elution as to increase the content of a good solvent in the mobile phase, the copolymer is redissolved and is eluted according to its solubility into the mobile phase.

The separation of P(S-AN) copolymers by HPPLC was found to be dependent on the combination of the stationary phase (RP-C18 or silica gel) and the mobile phase (a mixture of n-heptane and dichloromethane) (3). A system of silica gel with chemically bonded cyanoethyl groups / dichloroethane - heptane - acetonitrile has also been applied to the separation of P(S-AN) copolymers (4).

In our previous paper for the separation of P(S-AN) copolymers, the elution behaviour of P(S-AN) copolymers in HPPLC with mixtures of chloroform and n-hexane as mobile phases was evaluated (5). By applying isocratic elution with chloroform - n-hexane, next phenomena have been observed. The copolymers were eluted at retention volume corresponding to the interstitial volume (the volume of the mobile phase outside the gels in the column = the exclusion limit in size exclusion chromatography(SEC)) or retained in the column, depending on the compositions of the copolymers and of the mobile phases. The copolymers having much styrene could elute at the interstitial volume with the mobile phase of less chloroform. With increasing the content of n-hexane in the mobile phase, the copolymers were retained (precipitated) in the column. Besides the elution of the copolymers at the interstitial volume or precipitation of the copolymers in the column, the copolymers were eluted at the retention volume corresponding to the volume of the mobile phase in the column (the sum of the

interstitial volume and the pore volume of the packing materials in the column) with the mobile phase of the intermediate composition and this phenomenon was defined as the pre-precipitation state (a transition period).

In order to separate the copolymers according to composition, in other word, to observe different retention volumes among the copolymers having different composition, the linear gradient elution from 100% n-hexane to 100% chloroform has been performed and the elution was in order of increasing the acrylonitrile content in the copolymers. Although a linear relationship between retention volume and acrylonitrile content has been observed, the resolution between two copolymers was worse and the composition difference between the two copolymers should be more than 10% in the acrylonitrile content in order to get the complete resolution between two copolymers.

In the present report, stepwise gradient elution was performed for the purpose of increasing the resolution between two copolymers of which compositions were close each other. By this elution method, each copolymers could be separated into several peaks.

EXPERIMENTAL

HPPLC was performed with a high performance liquid chromatograph Model TRIROTAR-VI (Jasco Corp., Tokyo, Japan) equiped with an ultraviolet (UV) absorption detector Model UVIDEC-VI operated at 270 nm. The packing material was silica-ODS (Develosil, Nomura Chemical Co., Aichi, Japan) packed in a column of 250 mm x 4.6 mm I.D. The column was thermostated at 25 ^{o}C in a Model AO-30C column oven (Showa Denko Co., Tokyo, Japan).

Size exclusion chromatography (SEC) for the measurement of molecular weight averages of the fractions of P(S-AN) copolymers was performed with the same HPLC apparatus. A column for SEC was a Gelpak GL-W550 column (300 mm x 10.7 mm I.D.)(Hitachi Chemical

Co., Tokyo, Japan) packed with hydroxylated polymethacrylate gels. A UV detector was operated at 260 nm.

Samples used in this experiment were P(S-AN) copolymers prepared by suspension polymerization and supplied by Mitsubishi Monsant Co. (Yokkaichi, Japan). The acrylonitrile (AN) contents of these copolymers were measured by nitrogen analysis and were as follows: P(S-AN)-15, 14.8 wt%; P(S-AN)-20, 19.6 wt%; P(S-AN)-27, 26.2 wt%; and P(S-AN)-33, 32.3 wt%. These copolymers were dissolved in chloroform at a concentration of 0.1% for analytical separation and at a concentration of 0.5% for preparative separation. The injection volume for the sample solutions into the column was 0.1 mL.

The mobile phases for HPPLC were chloroform containing 1% ethanol as a stabilizer and n-hexan and their mixtures. Elution was performed by stepwise gradient. The initial mobile phase was n-hexane and ten or twelve minutes after the sample injection, the content of chloroform in the mobile phase was increased linearly in ten to seventeen minutes to specified concentration. Then the content of chloroform was increased 2% every five or ten minutes. The detailed programs are listed in Tables 1 (for analytical separation) and 2 (for preparative separation). The flow rate of the mobile phase was 0.5 mL/min.

The mobile phase for SEC was THF. Solvent in the fractions obtained by preparative separation was removed under reduced pressure and the residue was redissolved in THF.

RESULTS and DISCUSSION

In the previous report(5), adjacent peaks of the copolymers separated by linear gradient elution overlapped somewhat and in order to get complete resolution, the composition difference between the two copolymers should be more than 10 wt% in AN. A calibration curve of the peak retention volume vs. the composition of the copolymers can be constructed from Figure 6 in the previous

TABLE 1

Stepwise Gradient Program for Analytical Separation

Time, min	0	10*	20*	25	30	35	40	45	50	55
$CHCl_3$, %	0	0	20	22	24	26	28	30	32	34
Time, min	60	65	70	75	80	85	90	95	100	
$CHCl_3$, %	36	38	40	42	44	46	48	50	52	
Time, min	105	110	115	120	125	130	135	140		
$CHCl_3$, %	54	56	58	60	62	64	66	68		
Time, min	145	150	155*	175*						
$CHCl_3$, %	70	72	72	0						

* Linear gradient elution from 0% $CHCl_3$ to 20% $CHCl_3$ between 10 and 20 min and from 72% $CHCl_3$ to 0% $CHCl_3$ between 155 and 175 min.

report(5) and by using this calibration curve, the composition range of each copolymers can be calculated as follows: P(S-AN)-20, 16.5 - (34); P(S-AN)-27, 22.6 - (38)(AN wt%). The composition range calculated seems to be too broad and therefore, these peak widths must be affected not only by a composition distribution but also by a peak broadening effect during the elution in the column.

One of the possibilities to improve the peak resolution is to increase the programming time in the linear gradient elution. The difference in retention volume increased with increasing the programming time, but peak widths also increased and the improvement in the peak resolution was not much observed.

The second possibility to improve the peak resolution is to perform stepwise gradient elution. A stepwise gradient program operated in this work is listed in Table 1 for analytical separa-

TABLE 2

Stepwise Gradient Program for Preparative Separation

P(S-AN)-20 copolymer										
Time, min	0	12*	26*	36	46	56	66	76	86	96
$CHCl_3$, %	0	0	34	36	38	40	42	44	46	48
Time, min	106	116*	136*							
$CHCl_3$, %	50	50	0							
P(S-AN)-27 copolymer										
Time, min	0	12*	24*	34	44	54	64	74	84	94
$CHCl_3$, %	0	0	40	42	44	46	48	50	52	54
Time, min	104	114	124	134*	154*					
$CHCl_3$, %	56	58	60	60	0					
P(S-AN)-33 copolymer										
Time, min	0	12*	29*	39	49	59	69	79	89	99
$CHCl_3$, %	0	0	48	50	52	54	56	58	60	62
Time, min	109	119	129	139	149*	169*				
$CHCl_3$, %	64	66	68	70	70	0				

*Linear gradient elution between 12 and 26 min and between 116 and 136 min for P(S-AN)-20, between 12 and 24 min and between 134 and 154 min for P(S-AN)-27, and between 12 and 29 min and between 149 and 169 min for P(S-AN)-33.

tion and chromatograms obtained by the stepwise gradient elution are shown in Figures 1 and 2. Figure 1 is those for P(S-AN)-15 and P(S-AN)-20 and Figure 2 is those for P(S-AN)-27 and P(S-AN)-33. The programming time of stepwise gradient elution and the compositions of the mobile phase at the inlet of pump are also shown schematically. The compositions of the mobile phase listed in Table 1 and shown in Figures 1 and 2 are those at the system controller and the real compositions of the mobile phase in the column at the specified time are somewhat lower in the chloroform contents than those shown there. The monitoring at wavelength 260 nm caused the baseline drift and therefore, wavelength 270 nm was employed in this work.

P(S-AN)-20 copolymer was separated into 6 (+2) peaks and P(S-AN)-27 copolymer into 5 (+3) peaks by the stepwise gradient elution. Although some peaks of P(S-AN)-20 copolymer overlapped with those of P(S-AN)-15 copolymer, but all peaks of P(S-AN)-20 copolymer were not superimposed on those of P(S-AN)-27 copolymer. These results express that the range of composition for P(S-AN)-20 copolymer (and P(S-AN)-27 copolymer) is not wider than that calculated with a calibration curve mentioned above and peak broadening effect should be considered for the reason of broad peaks.

Stepwise gradient elution for analytical separation started at 20 min after the injection of the sample solution. This starting time affected retention volumes of separated peaks. In order to get good reproducibility for the elution positions of the resolved peaks, the starting composition of the mobile phase at a stepwise gradient elution should be lower in the chloroform content than the composition at which the copolymer started to elute.

Chloroform content in the mobile phase at which the copolymers started to elute from the column by linear gradient elution was different from that by stepwise gradient elution. P(S-AN)-15 copolymer started to elute at the composition of 75% chloroform in the mobile phase by linear gradient elution (5), while it started to elute at the composition of 34% chloroform in the mobile phase by stepwise gradient elution. Similarly, P(S-AN)-20

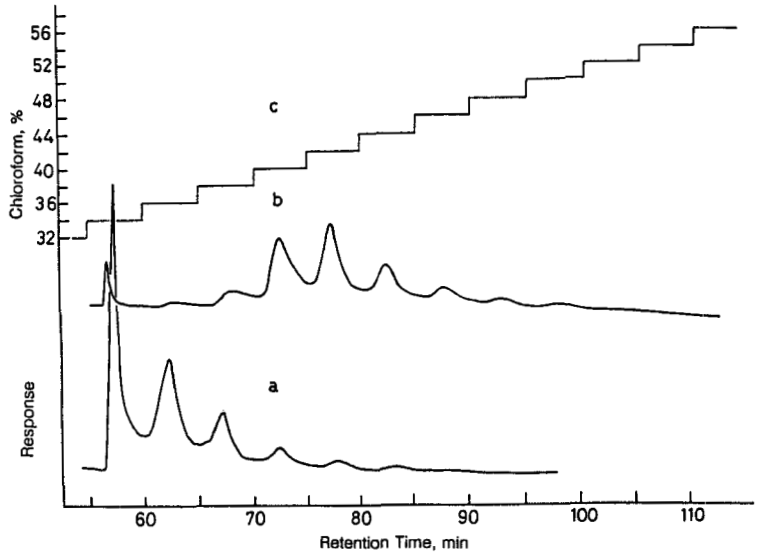

FIGURE 1. Chromatograms of P(S-AN) copolymers obtained by stepwise gradient elution (1). (a) P(S-AN)-15, (b) P(S-AN)-20, (c) composition profiles for the mobile phase; UV:270 nm, x0.16; sample concentration: 0.1%; injection volumne: 0.1 mL.

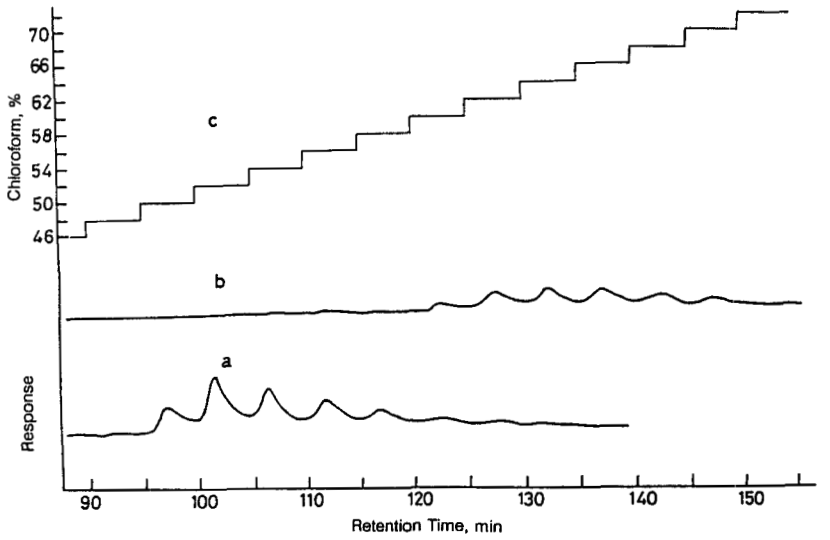

FIGURE 2. Chromatograms of P(S-AN) copolymers obtained by stepwise gradient elution (2). (a) P(S-AN)-27, (b) P(S-AN)-33, (c) composition profiles for the mobile phase; UV:270 nm, x0.16; sample concentration: 0.1%; injectiion volume: 0.1 mL.

copolymer started to elute at 82% chloroform in the mobile phase by linear gradient elution and 38% chloroform by stepwise gradient elution. Stepwise gradient elution required less good solvent than linear gradient elution. The composition in the mobile phase at which the copolymers started to elute by stepwise gradient elution (e.g., P(S-AN)-20, 38% chloroform, P(S-AN)-27, 50% chloroform) were nearly equal to those of mixtures of chloroform and n-hexane corresponded to cloud points (5) (e.g., P(S-AN)-20, 36% chloroform, P(S-AN)-27, 52.5% chloroform).

In order to measure the composition of each peak separated by the stepwise gradient elution, the peaks were fractionated by preparative separation. Gradient program for preparative separation is somewhat different from that for analytical separation and was indicated in Table 2. Chromatograms for P(S-AN)-20, P(S-AN)-27 and P(S-AN)-33 copolymers are shown in Figures 3, 4, and 5, respectively. Sample concentration was 0.5% and injection volume was 0.1 mL. No difference in peak retention volume was found in both sample concentrations, 0.1% and 0.5%. Chromatograms obtained with the 0.1% sample concentration are shown in Figures 3 and 4 for comparison purpose. Shaded parts in peaks indicate fractionated parts.

Four peaks of each samples were fractionated, the fractions were cast on a KBr disk, and then the infrared spectra of the fractions were measured by FTIR. A calibration curve was constructed by using the unfractionated P(S-AN) copolymers and the absorbance ratio of 2260 cm^{-1} and 1602 cm^{-1} was plotted against AN content. Composition of the fractions was determined by using this calibration curve. The results are shown in Table 3. From Table 3, it was found that P(S-AN)-33 copolymer was separated according to its composition and each peaks have about one percent difference in composition. Although fractions No. 1, 2, and 3 of P(S-AN)-20 copolymer were in order of increasing the AN content in the copolymer, fractions 3 and 4 were in reverse order. Fractions No. 1 and 2 of P(S-AN)-27 copolymer had the similar composition as well as those No. 3 and 4.

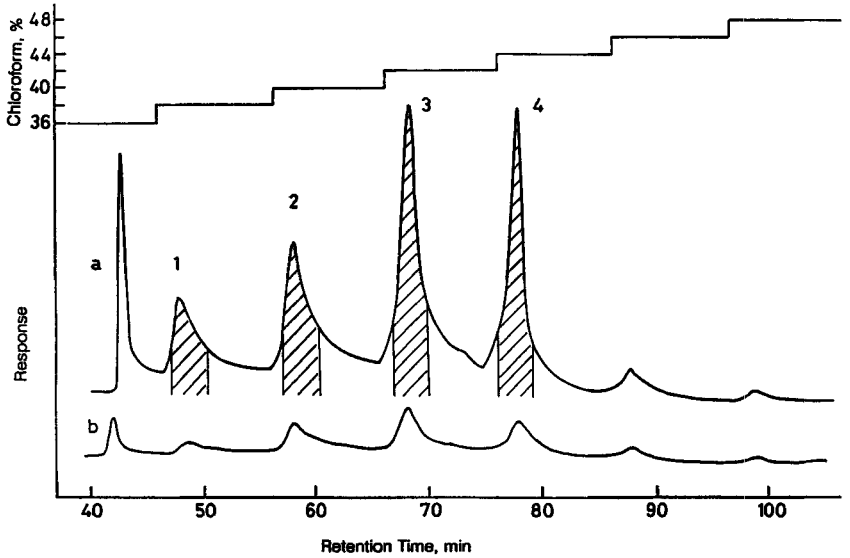

FIGURE 3. Chromatograms of P(S-AN)-20 copolymer obtained by stepwise gradient elution . Sample concentration: (a) 0.5%, (b) 0.1%; shaded parts in peaks indicate fractionation range and figures above the peaks represent the fraction numbers.

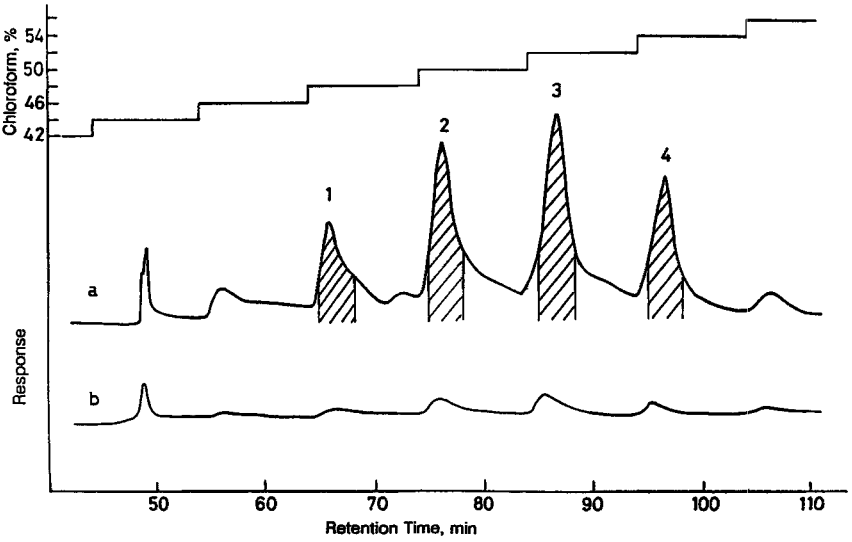

FIGURE 4. Chromatograms of P(S-AN)-27 copolymer obtained by stepwise gradient elution. Sample concentration: (a) 0.5%, (b) 0.1%; shaded parts and figures above the peaks were the same as in FIGURE 3.

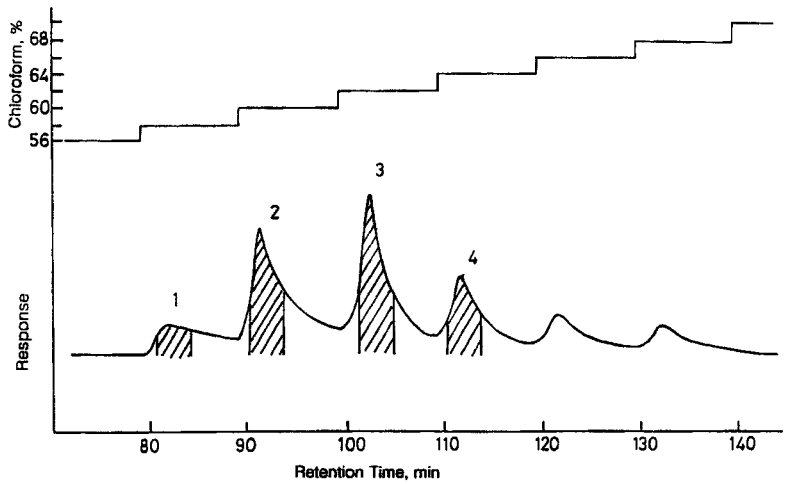

FIGURE 5. Chromatograms of P(S-AN)-33 copolymer obtained by stepwise gradient elution. Sample concentration: 0.5%; shaded parts and figures above the peaks were the same as in FIGURE 3.

TABLE 3

Composition of Fractions (AN, wt%)

Fraction number	P(S-AN)-20	P(S-AN)-27	P(S-AN)-33
1	18.0	25.4	30.5
2	19.2	25.8	31.8
3	21.8	27.3	32.8
4	20.5	27.3	34.0
Unfractionated	19.6	26.2	32.3

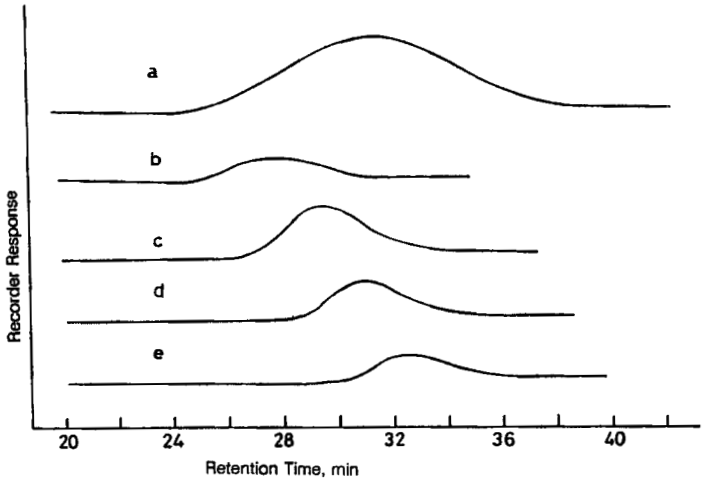

FIGURE 6. SEC chromatograms of P(S-AN)-20 copolymer and its fractions. (a):Unfractionated, (b): fraction number 4, (c):fraction number 3, (d):fraction number 2, (e):fraction number 1.

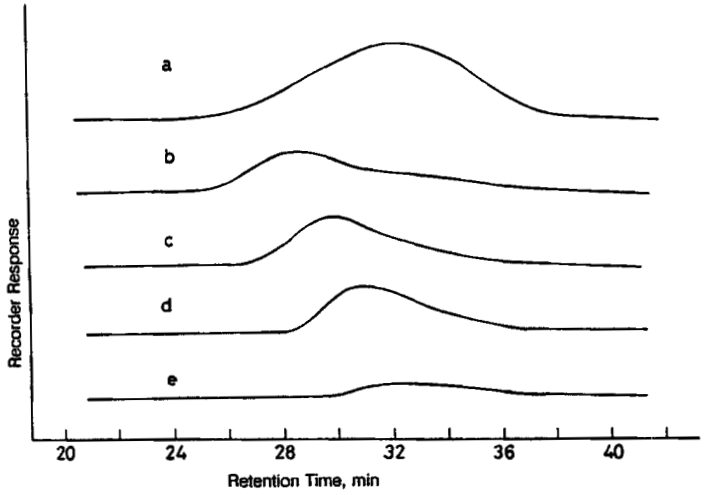

FIGURE 7. SEC chromatograms of P(S-AN)-27 copolymer and its fractions. a, b, c, d, and e are the same as in FIGURE 6.

TABLE 4

Molecular Weight Averages of Fractions as Polystyrene Equivalent

	Number average	Weight average
P(S-AN)-20	2.4×10^4	11.2×10^4
Fraction No. 1	2.8	4.7
Fraction No. 2	5.4	8.7
Fraction No. 3	11.7	15.5
Fraction No. 4	21.3	25.8
P(S-AN)-27	1.83	9.1
Fraction No. 1	2.6	5.2
Fraction No. 2	4.7	8.6
Fraction No. 3	6.6	12.9
Fraction No. 4	7.1	17.5

In order to consider this reverse order elution in composition, SEC of these fractions was performed and chromatograms are shown in Figures 6 and 7 and the molecular weight averages calculated are listed in Table 4 as polystyrene equivalent molecular weight. Elution of these fractions were in order of increasing molecular weight. It can be said that this elution method is affected by both the composition and the molecular weight of the copolymers. It is also possible to say roughly that the fractions having lower molecular weights have lower AN contents.

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