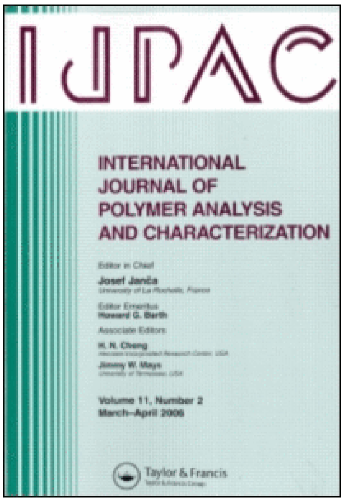

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Characterization of Styrene-Acrylonitrile Copolymers by Size Exclusion Chromatography/Stepwise Gradient Elution—Liquid Precipitation Chromatography

S. Mori[a]
[a] Department of Industrial Chemistry, Faculty of Engineering, Mie University, Tsu, Mie, Japan

To cite this Article Mori, S.(1996) 'Characterization of Styrene-Acrylonitrile Copolymers by Size Exclusion Chromatography/Stepwise Gradient Elution—Liquid Precipitation Chromatography', International Journal of Polymer Analysis and Characterization, 2: 2, 185 — 192

To link to this Article: DOI: 10.1080/10236669608233907

URL: http://dx.doi.org/10.1080/10236669608233907

Int. J. Polymer Analysis & Characterization
1996, Vol. 2, pp. 185–192
Reprints available directly from the publisher
Photocopying permitted by license only

Characterization of Styrene-Acrylonitrile Copolymers by Size Exclusion Chromatography / Stepwise Gradient Elution—Liquid Precipitation Chromatography*

S. MORI

Department of Industrial Chemistry, Faculty of Engineering Mie University, Tsu, Mie 514, Japan

(Received January 26, 1995; in final form March 10, 1995)

Styrene-acrylonitrile copolymer having acrylonitrile (AN) content of 19.4% was fractionated first by size exclusion chromatography (SEC) and SEC fractions were then separated by liquid precipitation chromatography (LPC), followed by SEC (SEC-LPC-SEC). LPC was performed by stepwise gradient elution with an octadecylsilane (ODS) silica column and mobile phase of mixtures of n-hexane and chloroform in which the chloroform content in the mobile phase was increased 2% every 5 min. The copolymer was separated by LPC according to both composition and molecular weight (MW); fractions having higher AN content had higher MW. The hyphenated technique of SEC-LPC-SEC gave detailed information in which the first SEC fractions having higher MW had lower AN content. In the same SEC fraction, the LPC fractions having higher MW had higher AN content.

KEY WORDS Styrene-acrylonitrile copolymer, size exclusion chromatography, stepwise gradient elution, liquid precipitation chromatography, chemical composition distribution, molecular weight distribution

INTRODUCTION

The determination of chemical heterogeneity and chemical composition distribution (CCD) for synthetic copolymers by high-performance liquid chromatography (HPLC) has been an active area of research in the last decade. Interactive liquid chromatography (ILC) in HPLC, such as adsorption chromatography and precipitation chromatography, has become as indispensable technique to characterize the copolymers. Hyphenated technique with size exclusion chromatography (SEC), such as ILC-SEC (or SEC-ILC), gives precise and detailed information on CCD of the copolymers in addition to a molecular weight distribution (MWD).

---

[*] Presented in part at the 7th International Symposium on Polymer Analysis and Characterization (ISPAC-7), Diablerets, Switzerland, May, 1994.

Separation in SEC is achieved according to the sizes of molecules in solution. Copolymers having the same molecular size but different molecular weights and composition appear at the same retention volume in SEC. Information on CCD and MWD should be obtained by separating the copolymer by composition independently of molecular weight and then determining MWD of each of fraction, or inversely, MWD is determined first, independently of composition and then CCD of the same molecular weight species is measured.

Several procedures of ILC for copolymers have been reported: liquid adsorption chromatography (LAC) [1], liquid precipitation chromatography (LPC) [2], normal-phase chromatography [3], reversed-phase chromatography [4], and LAC at critical condition [5].

In LPC, the initial mobile phase is a non-solvent for the copolymer concerned, so that the copolymer dissolved in a good solvent and injected into a column is precipitated at the inlet of the column. By using gradient elution, in which the content of a good solvent for the copolymer is increased, the copolymer that is precipitated first redissolves and elutes; thus the separation is based on copolymer solubility.

Poly(styrene-acrylonitrile) copolymers, P(S-AN), were separated on an octadecylsilane (ODS) silica column by gradient elution from 10% tetrahydrofuran (THF) in iso-octane to 90% THF—10% iso-octane [6]. The THF that was used contained 10% methanol. In addition, Schultz and Engelhardt [7] reported the separation of P(S-AN) with the solvent system of n-heptane-dichloromethane.

In our previous papers, we have reported the elution behavior of P(S-AN) by LPC [8,9]. With isocratic elution from an ODS-silica column using n-hexane—chloroform mixtures as mobile phases, five different elution profiles were observed and the transition from elution from a column to precipitation on the column via the pre-precipitation state was noted [8]. Separation according to the copolymer composition was possible by gradient elution of a mixture of n-hexane—chloroform from 100% n-hexane to 100% chloroform, though the resolution was not very satisfactory. In order to improve the resolution, stepwise gradient elution was performed [9] and each sample of P(S-AN) 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. The copolymers were separated in order of increasing the acrylonitrile content in the copolymer and also by molecular weight of the copolymer.

In this report, a hyphenated technique of SEC-LPC-SEC has been performed to characterize P(S-AN) copolymers precisely and in detail.

EXPERIMENTAL

Liquid Precipitation Chromatography

The packing material was ODS-silica (Develosil)(Nomura Chemical, Aichi, Japan) packed in a column of 250 mm × 4.6 mm i.d. which was thermostated at 25°C in a model AO-30C column oven (Showa Denko, Tokyo, Japan). LPC was performed with a model TRIROTAR-VI HPLC apparatus (Jasco, Tokyo, Japan) equipped with a model UVIDEC-100VI ultraviolet detector operated at 268 nm.

The sample used in this work was P(S-AN)-20 (Mitsubishi Monsant Co., Yokkaich, Japan) of which the acrylonitrile (AN) content was 19.6 (w/w)%. The bulk sample and

SEC fractions were dissolved in a mixture of n-hexane and chloroform (50:50) at a concentration of 0.2%. An injection volume of 0.1 mL was used. Polystyrene equivalent average molecular weights of the copolymer were: $M_W = 16.01 \times 10^4$ and $M_n = 5.41 \times 10^4$.

Elution was performed by isocratic or stepwise gradient elution. For stepwise gradient elution, the initial mobile phase was n-hexane alone. After 12 min from the sample injection, the content of chloroform in the mobile phase was increased to 30% in 15 min linearly and held for 5 min. After 32 min from the sample injection, the chloroform content was increased abruptly to 32%, and the elution was continued for another 5 min. The content of chloroform in the mobile phase was then increased 2% every 5 min. The flow rate was 0.5 mL/min. Chloroform used in this experiment contained 1% ethanol as a stabilizer.

Size Exclusion Chromatography

SEC was performed with the same HPLC apparatus. Shodex K-80M (300 mm × 8 mm i.d.) (Showa Denko) packed with polystyrene gel was used. Mobile phase was chloroform containing 1% ethanol as a stabilizer and the flow rate was 1.0 mL/min. Sample concentration was 0.3% for preparative fractionation and 0.1% for the determination of molecular weight averages. The sample was dissolved in chloroform and the injection volume was 0.1 mL. A calibration curve of the column system was constructed with polystyrene standards having a narrow MWD.

Fourier-Transform Infrared Spectroscopy

AN content of the fractions by SEC and LPC were measured by Fourier-transform infrared spectroscopy (FT-IR). Fractions were cast on a KBr plate and IR spectra were measured. Absorbance ratios of 2240 cm^{-1} and 1500 cm^{-1} were calculated and the AN content was determined using a calibration curve of the absorbance ratio vs. AN content obtained with P(S-AN) copolymers of known AN content. The copolymer standards for calibration were obtained from the same source of P(S-AN)-20 copolymer, and the AN content of the standards was measured by nitrogen determination.

RESULTS AND DISCUSSION

Selection of a Solvent for Sample Solutions

When the copolymers containing 15 to 33% AN were dissolved in chloroform, which is a good solvent, five different elution profiles were observed with isocratic elution using mixtures of n-hexane and chloroform as mobile phases, depending on the compositions of the copolymers and the mobile phase [8]: (I) elution with a retention volume corresponding to the interstitial volume of the column; (II) elution with a retention volume corresponding to the volume of the mobile phase in the column; (III) elution with both retention volumes; (IV) elution with the same retention volume as in (II) but with the significantly increased peak intensity; and (V) precipitation on the column.

In the case of P(S-AN)-20, however, profile V was not observed even with 100% n-hexane. The copolymer showed profile IV when it was eluted with n-hexane [8]. n-Hexane is a non-solvent for P(S-AN) copolymers and when it is used alone as the mobile phase, the copolymer should precipitate on the column. The reason of the elution of the copolymer from the column with n-hexane is considered to be as follows: the copolymer molecules are tightly solvated with chloroform molecules, allowing the elution of the copolymer from the column without precipitating immediately. This profile is defined as the pre-precipitation state.

When the copolymer P(S-AN)-20 was dissolved in a mixture of n-hexane—chloroform (50:50), however, profile IV was not observed and the copolymer precipitated on the column when n-hexane was used as the mobile phase. The results are shown in Figure 1. Since it is preferable to precipitate the sample copolymer on the column initially before eluting from the column by gradient elution for PLC, samples were dissolved in a mixture of n-hexane—chloroform (50:50) in this experiment.

Stepwise Gradient Elution

Chromatograms of the copolymer obtained by stepwise gradient elution are shown in Figure 2. The programing of the stepwise gradient elution and the composition of the mobile phase at the inlet of the pump are also shown schematically. (The composition of the mobile phase shown in Figure 2 were those at the system controller of the pump and the real composition of the mobile phase in the column at the specified time were somewhat lower in chloroform content than those shown here.) The copolymer was separated into six peaks. The peak at 15 mL was not a sample peak but impurities, or low-molecular weight materials in the copolymer, because the same peak appeared at the same position even when only the solvent (a mixture of n-hexane and chloroform) was injected.

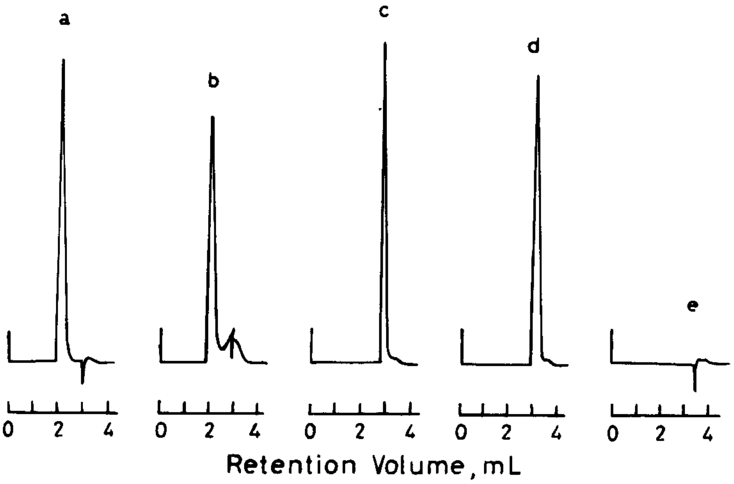

FIGURE 1 Elution profiles of P(S-AN)-20 copolymer. Composition of mobile phase, n-hexane—chloroform (a) 40:60; (b) 50:50; (c) 60:40; (d) 70:30; (e) 100:0. Sample was dissolved in a mixture of n-hexane—chloroform (50:50).

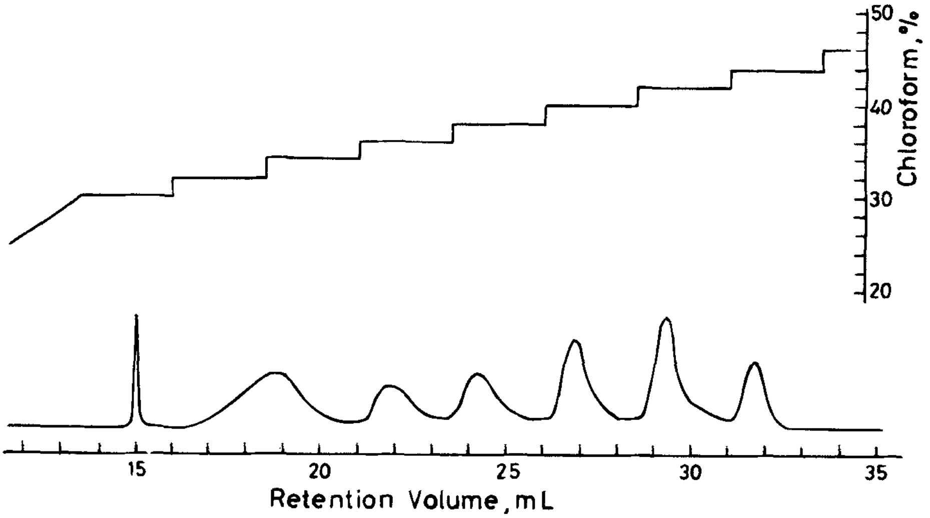

FIGURE 2 LPC chromatogram of P(S-AN)-20 copolymer obtained by stepwise gradient elution and composition profiles for the mobile phase. Attenuation of UV: ×0.08 AUFS.

Although the programing time in this experiment was different from the previous experiment [9], we can assume that the peaks appearing earlier have low AN content and low molecular weight as compared to later eluting peak. For detailed characterization of the copolymer, fractionation by SEC was done first.

Fractionation by Size Exclusion Chromatography

In order to characterize the copolymer in terms of composition and molecular weight precisely and in detail, the copolymer was fractionated first by SEC. An SEC chromatogram and a calibration curve constructed with narrow MWD polystyrenes are shown in Figure 3. The chromatogram was divided into three equal portions as shown in the chromatogram and three fractions were obtained. This fractionation was repeated about one-hundred times and about 10 mg of each fraction was obtained. Solvent in the fractions was removed under reduced pressure, the remaining polymer was redissolved in 10 mL of a mixture of n-hexane—chloroform (50:50) and 0.1% solutions were used for LPC.

Peak molecular weights (polystyrene equivalent) and compositions of these three fractions were determined by SEC and by FT-IR, respectively. The results are shown in Table I. Designations F1, F2 and F3 in Table I correspond to Figure 3. The amount in each fraction was calculated from peak areas of the LPC chromatograms, in which the detector response was corrected to take into account different copolymer compositions.

Liquid Precipitation Chromatography of the SEC Fractions

LPC chromatograms of three SEC fractions are shown in Figure 4. Each fraction was separated into four to five peaks and some of peaks from different fractions have the same retention volumes (e.g., F1-b, F2-d, and F3-e; F1-a, F2-c, and F3-d). Each peak in these

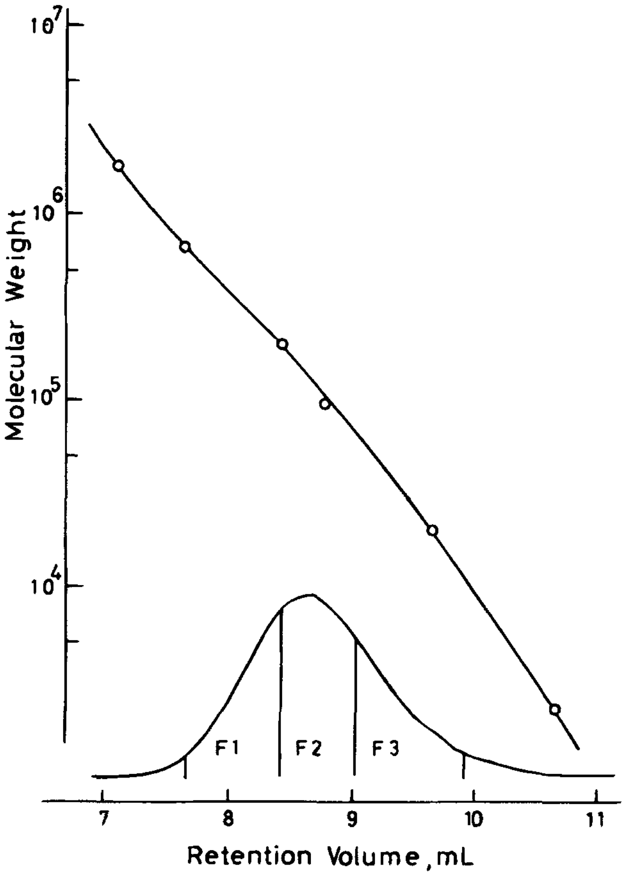

FIGURE 3 SEC chromatogram of P(S-AN)-20 copolymer and a polystyrene calibration curve of the SEC system.

three fractions were collected and the peak molecular weights and composition was determined by SEC and FT-IR, respectively. The results are shown in Table I. Examples of SEC chromatograms are shown in Figure 5.

Elution of SEC fractions by LPC was in the order of increasing AN content, except for F3-a, which had the smallest molecular weight, but had a higher AN content than F3-b and F3-c. The elution was also in the order of increasing molecular weight. Therefore, from these results, P(S-AN) copolymer was separated by LPC according to both composition and molecular weight and that fractions having higher AN content have higher molecular weight.

However, SEC fractions having higher molecular weight had lower AN content and in the same SEC fraction, the LPC fractions having higher molecular weight had higher AN content. Moreover, peaks obtained by LPC of these three SEC fractions eluted at the same retention volumes, but have different molecular weights and AN contents (see F1-a, F2-c, and F3-d; F1-b, F2-d, and F3-e). Fractions having higher molecular weight and lower AN content and those having lower molecular weight and higher AN content were eluted together.

TABLE I

Molecular weight and AN content of fractions by SEC and LPC

Fraction	Molecular weight	AN content, wt%	Amount, %
F1*	30.4×10^4	18.9	100
F1**-a	17.9	—	6.6
-b	24.8	18.7	44.4
-c	38.0	19.1	47.5
-d	—	—	1.5
F2*	15.3×10^4	19.4	100
F2**-a	—	—	2.0
-b	10.3	18.5	11.3
-c	11.8	19.4	28.2
-d	20.5	19.9	50.8
-e	28.9	—	7.7
F3*	6.25×10^4	20.5	100
F3**-a	3.29	20.0	35.4
-b	5.61	18.7	17.0
-c	9.41	19.0	21.4
-d	12.9	21.2	18.0
-e	—	—	8.2
P(S-AN)-20***	13.8×10^4	19.4	

*Fractions by SEC; **fractions by LPC; ***unfractionated sample.

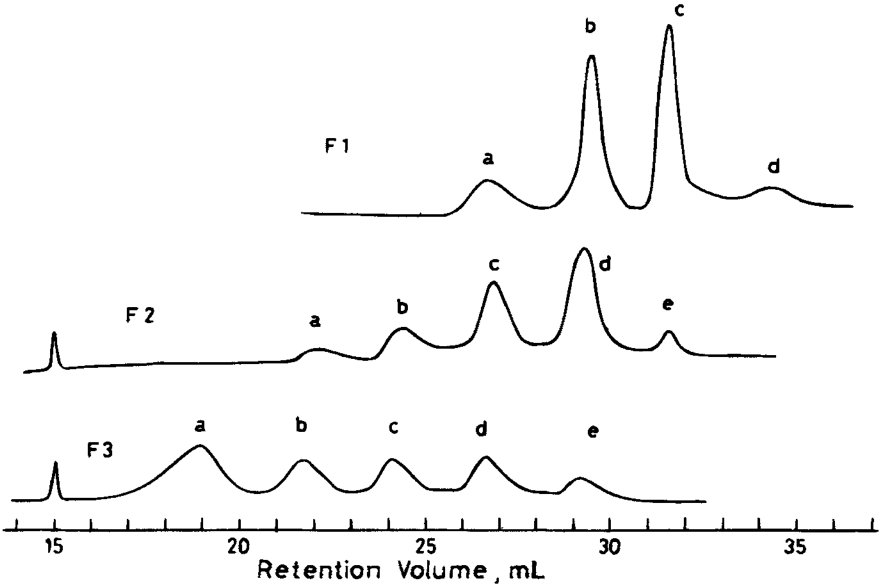

FIGURE 4 LPC chromatograms of SEC fractions of P(S-AN)-20 copolymer. Gradient conditions are the same as in Figure 2.

CONCLUSION

Elution by LPC in this system is influenced by both composition and molecular weight. However, it is still useful to characterize P(S-AN) copolymers by SEC and LPC. SEC-LPC-SEC gives precise and detailed information on the relationship of molecular weight

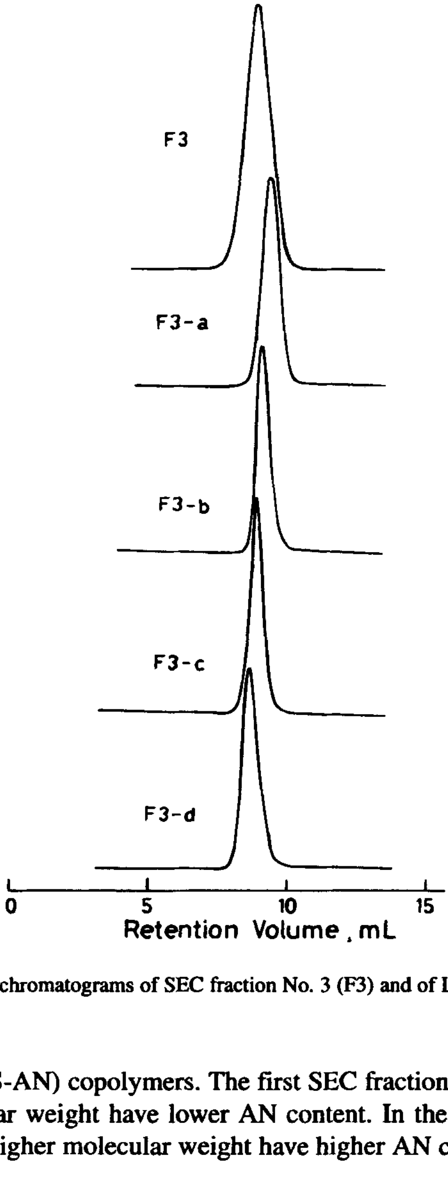

FIGURE 5 SEC chromatograms of SEC fraction No. 3 (F3) and of LPC fractions of F3.

and composition of P(S-AN) copolymers. The first SEC fractions show that the fractions having higher molecular weight have lower AN content. In the same SEC fraction, the LPC fractions having higher molecular weight have higher AN content.

References

1. S. Mori and Y. Uno, *Anal. Chem.*, **59**, 90 (1987).
2. G. Glöckner, H. Kroschwitz and Ch. Meissner, *Acta Polymerica*, **33**, 614 (1982).
3. S. Teramachi, A. Hasegawa, and K. Motoyama, *Polym. J.*, **22**, 489 (1990).
4. H. Sato, K. Mitsutani, I. Shimizu, and Y. Tanaka, *J. Chromatogr.*, **447**, 387 (1988).
5. H. Pasch, C. Brinkmann, H. Munk, and V. Just, J. *Chromatogr.*, **623**, 315 (1992).
6. G. Glöckner, J. H. M. van den Berg, N. L. J. Meijerink, T. G. Scholte, and R. Koningsveld, *Macromolecules*, **17**, 962 (1984).
7. R. Schultz and H. Engelhardt, *Chromatographia*, **29**, 325 (1990).
8. S. Mori and M. Naito, *J. Chromatogr.*, **A655**, 185 (1993).
9. S. Mori and H. Taziri, *J. Liq. Chromatogr.*, **17**, 3055 (1994).