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TWO-DIMENSIONAL ANALYSIS OF COPOLYMERS BY SIZE-EXCLUSION CHROMATOGRAPHY AND GRADIENT-ELUTION REVERSED-PHASE PRECIPITATION CHROMATOGRAPHY

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SUMMARY

Poly(styrene–co-acrylonitrile) samples were fractionated by size-exclusion chromatography and subsequent high-performance precipitation liquid chromatography (HPPLC) of effluent fractions. HPPLC was performed on a reversed phase column by using a mobile-phase composition gradient with eluting power increasing from 2,2,4-trimethylpentane as a non-solvent to tetrahydrofuran as a solvent. A mixture of two model copolymers of 23 and 30 wt. % acrylonitrile, having molar masses of 825 and 190 kg/mol, respectively, and a commercial SAN copolymer were studied. The correction of retention volumes for the influence of molar mass was determined, and applied in the evaluation of the data for the commercial sample.

INTRODUCTION

Copolymers usually have a molar mass distribution (MMD), with a distribution function H(M), and a chemical composition distribution (CCD), with a distribution function H(x). Information on the complex distribution function H(M,x) can be obtained by fractionating the copolymer by molar mass and analyzing the distribution within each fraction by composition. The latter analysis should be performed in a direction perpendicular to that of the first separation. This is the principle of cross-fractionation, a powerful but time-consuming method in polymer analysis.

This paper deals with cross-fractionation by means of chromatographic methods. The evaluation of MMD and CCD by chromatographic techniques requires the combination of two methods, one which separates mainly by molar mass and the other mainly by composition. Inagaki and Tanaka[1] used a combination of column adsorption chromatography and size-exclusion chromatography (SEC), and Taga and Inagaki[2] used thin-layer chromatography (TLC) and SEC. Belenkii and Gankina[3] mentioned the combination of SEC (first fractionation) and TLC (second frac-

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tionation), which was also employed by Teramachi *et al.*[4]. Balke and Patel[5,6] performed two SEC analyses in which different mobile phases were used. Nakano and Goto[7] used a cross-fractionation based on crystallization on columns and SEC.

We used SEC for the first fractionation. With this technique about 1 mg of the copolymer could be fractionated. SEC fractions contain polymer molecules of about the same hydrodynamic volume, V_h. This quantity is related to molar mass, M, by

$$V_h = [\eta]M = KM^{1+a} \tag{1}$$

where $[\eta]$ is the intrinsic viscosity, which is related to M by

$$[\eta] = KM^a \tag{2}$$

If the Mark–Houwink coefficients K and a are not greatly influenced by composition, SEC can be considered as a fractionation by molar mass. Hence, the subsequent analysis of each fraction should reveal its chemical distribution. Solubility-based high-performance precipitation liquid chromatography (HPPLC) has been successfully employed for the investigation of SEC fractions of poly(styrene–co-acrylonitrile) samples (SAN copolymers) and poly(α-methylstyrene–co-acrylonitrile) specimens. HPPLC has the important advantage that SEC fractions (so-called slices) can be analysed without any additional pretreatment. Its disadvantage is that the solubility of copolymers is generally influenced by composition and by molar mass. In an earlier report[8] we stated that (under well chosen conditions) the molar mass dependence of HPPLC retention is smaller than the dependence on chemical composition. This paper presents new experimental data relating to this topic. It also describes the chromatographic cross-fractionation of a commercial SAN copolymer and reports on a first attempt to estimate the acrylonitrile (AN) content of its SEC fractions from their HPPLC patterns.

EXPERIMENTAL

The equipment and technique closely resembled those already described[8].

SEC

A Waters ALC/GPC Model 244 instrument (Waters Associates, Milford, MA, U.S.A.) was used together with a high-pressure pump (Model M-6000A) and an injection device (U6K) with a loop volume of 0.5 ml. The column effluent was monitored by means of a differential refractometer (Model R 401) and a fixed-wavelength (254 nm) UV detector (Model 440). A bank of five μStyragel (Waters) columns was used. The length of each column, $L = 0.30$ m; inner diameter, $d_C = 7.8$ mm. The nominal pore sizes of the packings were 5×10^2, 10^3, 10^4, 10^5 and 10^6 Å; the mean particle size was 10 μm.

Tetrahydrofuran (THF, pa grade from Baker, Deventer, The Netherlands) containing 0.025% butylhydroxytoluene as a stabilizer was employed as an eluent and as a solvent for sample injection. A flow-rate of 1 ml/min was used. The effluent fractions were collected at the indicated intervals (see Figures) without interrupting

the solvent flow. The concentration of the sample solutions was 0.2%, *i.e.*, about 1 mg of polymer was used as the starting material for a chromatographic cross-fractionation.

HPPLC

A liquid chromatograph (Type 5020; Varian, Palo Alto, CA, U.S.A.) suitable for gradient elution was used. The instrument was modified by inserting two serial mixing chambers (10 and 5 cm in length, 4.6 mm I.D.) between the pump and injection system. The first chamber, the larger, was filled with glass spheres (about 3 mm in diameter). The apparatus was equipped with an injection valve (Type 7105; Rheodyne, Berkeley, CA, U.S.A.) which enabled the injection of various volumes. The loop volume was 175 μl. Smaller injections could be accomplished by partly filling the loop. The detector was a variable flow-through UV photometer (Type SF 770 Schoeffel; Kratos, Westwood, NJ, U.S.A.), set at the wavelength of 259 nm. The reference cell was filled with 2,2,4-trimethylpentane (isooctane, purum grade; Fluka, Buchs, F.R.G.). The column used L = 0.15 m and d_C = 4.6 mm. It was slurry packed with LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.), mean particle size 10 μm.

The analyses were performed with the column kept at 50°C. 2,2,4-Trimethylpentane was used with the addition of a suitable trace amount of toluene (Merck) (solvent A). THF (Baker pa grade) was distilled under nitrogen in order to remove the stabilizer, and kept under nitrogen to prevent peroxide formation. In order to lessen the difference between the refractive index of isooctane (n_D = 1.392) and that of THF (n_D = 1.405), the latter solvent was mixed with 10% (v/v) methanol (n_D = 1.329, HPLC grade; Fisons, Loughborough, U.K.) (solvent B). The elution program is listed in Table I. Fig. 1 shows a baseline due to a gradient cycle together with a representation of the gradient elution program. The breakthrough time of the gradient was 5.6 min. The dead time for a non-retained sample was about 2 min, *i.e.*, it took 3.6 min for the eluent to reach the injection port. The detector deflection pattern shown in Fig. 1 is mainly caused by changes in refractive index. The deflection varies rather widely at the steep edges of the solvent program, but the baseline is almost flat without drift or long term noise in the part of the gradient that is relevant to the measurement. The injections were performed 2 min after the start of the program. In general, 100 μl of the respective SEC fractions were injected without any additional treatment of the eluate. For the more diluted fractions at the edges, portions of about 175 μl were injected. The solvent of the injected samples caused distinct pseudo-peaks. The stabilizer also appeared before the gradient.

Samples

The samples and the mixture investigated are listed in Table II. Sample I was

TABLE I

FLOW-RATE AND COMPOSITION GRADIENT OF THE MOBILE PHASE

Solvents: A, isooctane with a trace amount of toluene; B, THF with 10% methanol.

Time (min)	0	1	2	3	8	11	13	14	15	17	18	21.9	22	25	25.1	30
Solvent B (%)	10	18	46	60	65	70	75	80	90	90	10	———	———	———	———	10
Flow-rate (ml/min)	1	———	———	———	———	———	———	———	———	———	———	1	2	2	1	———

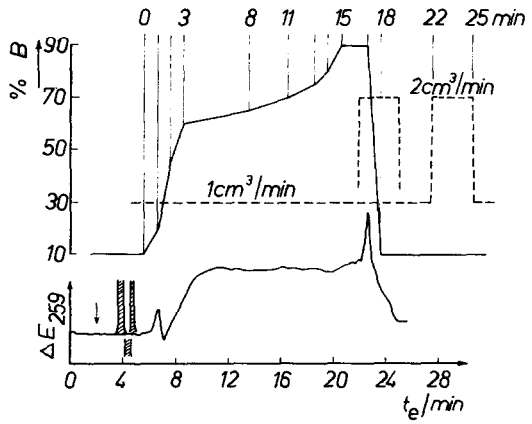

Fig. 1. Elution profile (%B *vs.* t, upper diagram) and baseline (ΔE_{259} *vs.* t_e, lower diagram) of a gradient cycle. The time-scale of the elution profile is shifted by the breakthrough time of the gradient (5.6 min). The dashed line shows the flow-rate. As this quantity will, of course, influence the detector signal without the delay, it is also indicated at the interval 22–25 min referring to the record of the baseline. The arrow indicates the moment of sample injection which would cause the deflections indicated at about 4 min elution time.

prepared by bulk polymerization and sample II by suspension polymerization, each to a low degree of conversion. Sample III was a commercial SAN.

RESULTS AND DISCUSSION

Fig. 2 shows the SEC elution curve of the mixture of two SAN samples and the HPPLC patterns of alternate slices. The difference in AN content between the constituents of the mixture investigated was 7% and the molar mass ratio was 1:4. This mixture was chosen to investigate the effect of composition as well as the adverse effect of molar mass on the solubility. The HPPLC patterns on the right-hand side of Fig. 2 show that the composition difference of 7% suffices for a separation according to composition which is by no means overridden by molar mass effects. It follows that separation according to composition is possible if a SEC fractionation precedes as an efficient pretreatment. In the HPPLC trace both copolymers are eluted

TABLE II

SAMPLE AND MIXTURE INVESTIGATED

The mixture investigated (Figs. 2 and 3) contained 21.5 mg of sample I and 25.3 mg of sample II dissolve in THF. Concentration: 0.2% (w/v).

Sample	*I*	*II*	*III*
AN (wt.%)	23	30	24
M_n (kg/mol)	480*	86**	110**
M_w (kg/mol)	825***	190**	230**

* By osmometry.
** By SEC with polystyrene calibration.
*** By light scattering.

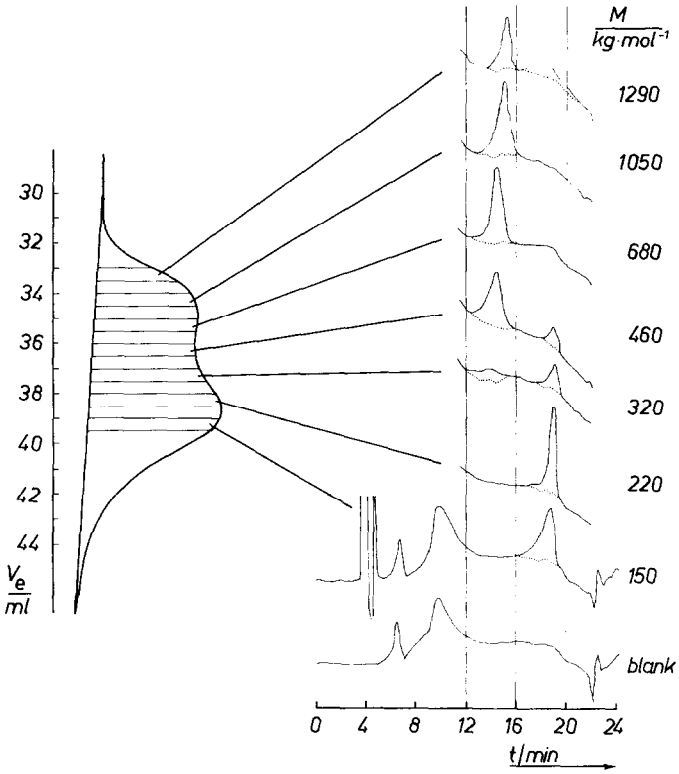

Fig. 2. SEC elution curve (RI detection) of the mixture specified in Table II, and the HPPLC patterns of the fractions indicated. The baseline of these chromatograms is redrawn at the bottom of the right-hand side. The broad deflection in the interval 9–12 min appears in each chromatogram. For the sake of clarity, it is only repeated in the trace of the fraction with 150 kg/mol and is otherwise omitted. The dotted line under the real peaks indicates the baseline in this region.

at well spaced intervals. The copolymer with 23% AN is eluted in 12–16 min and the 30% AN copolymer between 16 and 20 min. The influence of molar mass is of minor importance. For both copolymers, we calculated the average retention time, t_{av}, of the peaks

$$t_{av} = \frac{\sum_i h_i \cdot t_{e,i}}{\sum_i h_i} \tag{3}$$

where h_i are the differences in UV signal of the sample and the baseline at equally spaced elution times, $t_{e,i}$. From the gradient profile (Fig. 1) the corresponding eluent composition, φ_e, is determined.

In Fig. 3 these data are plotted *vs.* $M^{-0.5}$. The straight lines correspond to the equation:

$$\varphi_e = \alpha + \beta M^{-0.5} \tag{4}$$

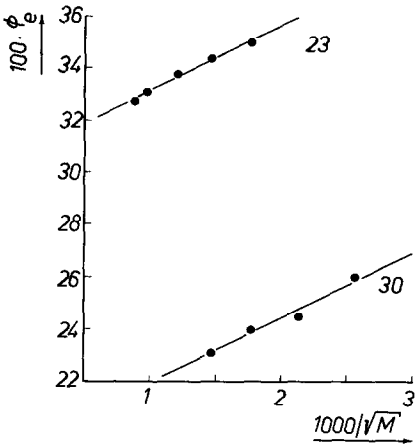

Fig. 3. Molar mass dependence of the volume fraction, φ_e, of non-solvent in the eluent at the point of elution of SAN samples containing 23 or 30% AN. The volume fractions were derived from the peak elution times shown in Fig. 2 by using eqn. 3 and the gradient profile listed in Table I.

Such a linear relationship was also empirically found by turbidimetric titration of fractions[9]. From Fig. 3 we derive $\beta = 25.1$. This value is larger than the corresponding one for SAN in THF–*n*-hexane[10]. In line with those previous results, Fig. 3 shows no distinct influence of the AN content on the slope factor, β. Of course, this statement is only justified in the limited composition range investigated and must be checked before application outside of this range. For our calculation we shall, for lack of further information, assume the same β value of AN contents over the whole range of our investigation (20–30 wt.% AN).

From the evaluation above one can infer the α and β values of eqn. 4 ($\beta = 25.1$) for copolymers of 23 and 30% AN, and from these the φ_e values for $M = 100$ kg/mol, *i.e.*, the volume fractions of eluent component A at which copolymers of 23 and 30% AN and reference molar mass 100 kg/mol are eluted. These volume fractions are: $\varphi_{e\ (30\%AN,\ M\ =\ 100\ kg/mol)} = 0.273$ and $\varphi_{e\ (23\%AN,\ M\ =\ 100\ kg/mol)} = 0.386$. Analysis of previous results on a SAN copolymer containing 19% AN leads to $\varphi_{e\ (19\%AN,\ M\ =\ 100\ kg/mol)} = 0.423$. Starting from these values, the following calibration relationship between % AN and φ_e can be calculated

$$\% \text{ AN} = 30 - \Delta\varphi\,[50 + 1{,}6 \exp\,[18\,\Delta\varphi)] \tag{5}$$

with $\Delta\varphi = \varphi_e - 0.273$.

Fig. 4 shows the SEC elution curve of a commercial SAN copolymer (sample III) and the HPPLC patterns of its fractions. These experiments were performed within 1 day and confirm the expectation that combination of SEC and HPPLC can reveal the MMD and CCD in genuine copolymers. Although it must be admitted that additional experiments are needed for a perfect calibration, we calculated the AN distribution within each slice by means of eqn. 5. For this purpose we evaluated the eluent compositions at HPPLC elution times at 0.2-min intervals and converted them into composition data valid for $M = 100$ kg/mol through eqn. 4 with $\beta =$

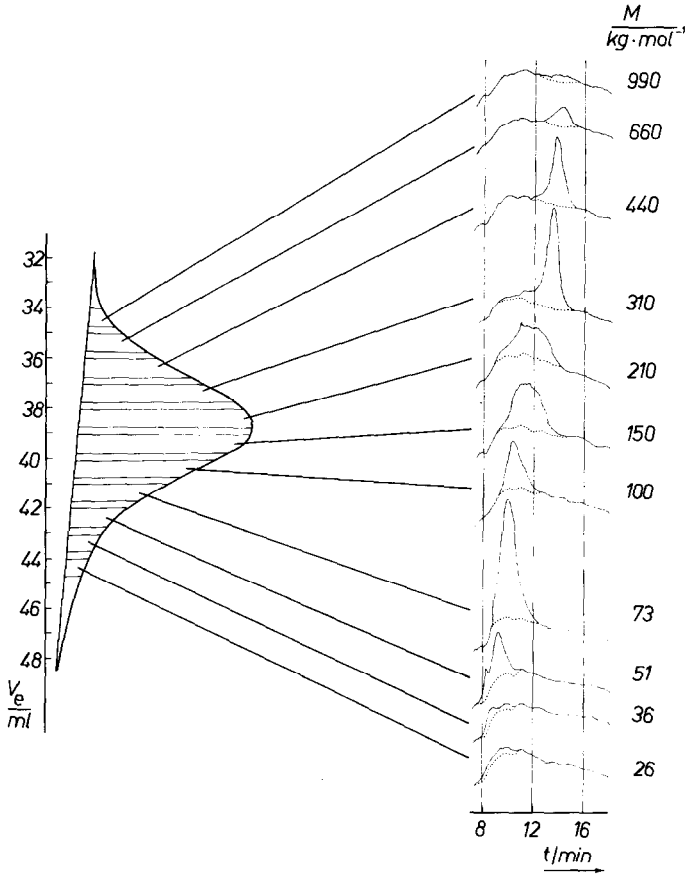

Fig. 4. SEC elution curve (RI detection) of a commercial SAN copolymer (sample III) and the HPPLC traces of the fractions indicated. This cross-fractionation was performed with a total sample mass of 1.095 mg.

25.1. The results are plotted in Fig. 5. From the combined molar mass and chemical composition distribution, as shown in this Figure, it is seen that the fractions with low molar mass have a rather sharp CCD and a relatively high AN content. The high molar mass fractions also have a sharp CCD but show a lower AN content. In the medium molar mass region (fractions with M = 150 and 210 kg/mol) there is a transition from higher to lower AN content, which results in a broader or even a slightly bimodal CCD. The change of average chemical composition with molar mass, *i.e.*, decreasing AN content with increasing molar mass, is in agreement with the results[11] we obtained by SEC with dual detection (RI and UV) on the same copolymer sample.

Teramachi and Esaki[12] investigated a poly(styrene–co-acrylonitrile) sample of 22.7% AN and $[\eta]$ = 1.382 by means of column adsorption chromatography and observed the same tendency with the average data for its fractions. Mori[13] performed pyrolysis gas chromatography of SEC fractions from a commercial SAN and also

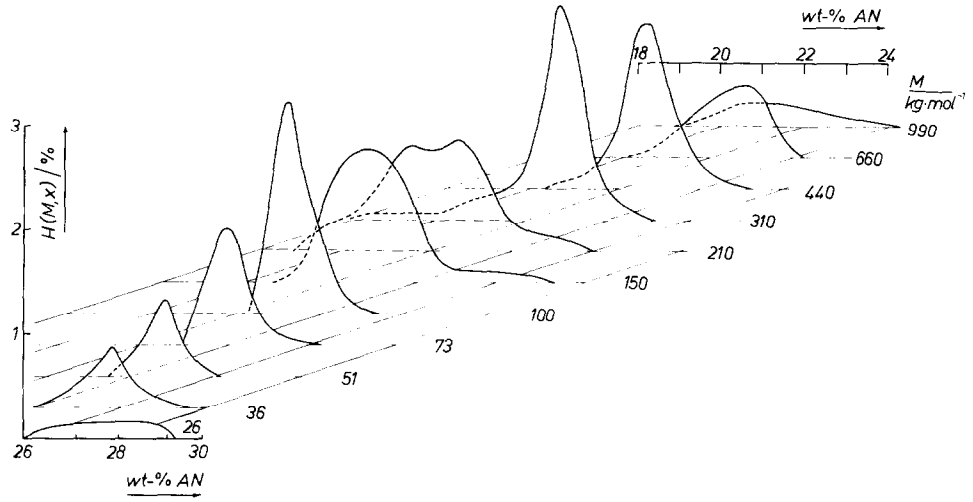

Fig. 5. Chemical composition distribution of the SEC fractions of a commercial SAN copolymer as estimated from the HPPLC traces shown in Fig. 4 and the areas of the corresponding slices of the SEC curve by using eqns. 4 and 5.

found an average AN content decreasing with increasing molar mass. The same conclusion was drawn by comparison of signals from UV and refractive index detectors. Garcia-Rubio *et al.*[14,15] also investigated SAN by means of SEC with dual detection. They found found an increase in AN content with increasing molar mass. This was also found by Wälchli *et al.*[16]. All these analyses were performed with so called "azeotropic" copolymers, which were believed to be homogeneous in composition. Our results confirm that such copolymers may consist of macromolecules differing in average AN content and, for the first time, give insight into the shape and broadness of CCD at different locations of MMD.

The evaluation of the commercial SAN sample clearly demonstrates the importance of the molar mass correction. The HPPLC peaks in Fig. 4 show a strong shift to higher elution volumes with higher molar masses. Without correction this would indicate an increasing AN content with increasing molar mass in this sample, but the correct evaluation leads to just the opposite result. The injection of SEC eluate into the HPPLC apparatus without adjustment of the solute concentration makes the investigations rapid and convenient. Another consequence is the fact that the amount of polymer fraction injected will differ in the series of HPPLC measurements. At the edges of an SEC elution curve the solute concentration is smaller than in the central part. To compensate partly for the dilution, we injected 175 instead of 100 μl of the middle fractions. In addition, some evaporation of solvent occurred during collecting and handling of the samples. Hence, the area under the HPPLC curves presented here is not proportional to the portion of polymer contained in the SEC fraction under investigation. Therefore we adjusted these areas to the size of the slices under the SEC curve, assuming a constant sensitivity of the RI detector, independent of molar mass and composition.

In general, application of large and different injection volumes might be expected to cause trouble. For this reason, we started our work by pre-concentrating

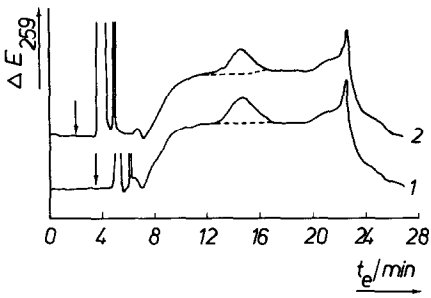

Fig. 6. Demonstration of the reproducibility of HPPLC. The repeated injection of 175 μl of a certain SEC fraction yielded curves which are almost identical in spite of a delayed injection in Experiment 1.

the SEC eluate and injecting small and constant volumes, but when examining the influence of injection volume, we observed no effect between 20 and 175 μl. This is plausible, because the first step in HPPLC is the precipitation of the sample at the top of the column. Although a larger injection volume will cause a broader precipitation zone, the difference in comparison with a smaller one will soon diminish, because the mobile phase gradient has a compressing effect. To a certain degree, it can even compensate for missing the correct moment of injection. The latter (unintentionally) happened with injection 1 in Fig. 6, which was performed 3.5 min (instead of 2 min) after the start of the gradient program. Immediately afterwards, the analysis was repeated with an injection at the right moment. The corresponding chromatogram is numbered 2 in Fig. 6. The appearance of the sample peak at 12.6 min was by no means disturbed by the delayed injection. The result support the hypothesis that the HPPLC of an excluded solute on a porous packing is based upon the different velocities of solute and solvent. On the basis of experience with differing injection volumes, the method of direct injection of SEC eluate was developed.

Fig. 6 also gives a nice example of reproducibility. We always obtained reproducible HPPLC chromatograms from samples of narrow distribution, especially from SEC fractions. (Of course, patterns like that in Fig. 6 can be expected only, if the baseline remains unchagend.) With commercial SAN copolymers that are not prefractionated we repeatedly observed changes in the patterns of consecutive analyses, *cf.*, Fig. 7. Until now we have not found the cause of this erratic behaviour, which never occurred with the prefractionated samples.

Even with the most careful calibration, relating retention volume, acrylonitrile

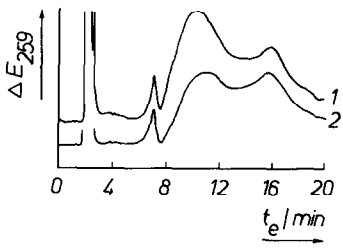

Fig. 7. Repeated analyses of a SAN copolymer that had not been prefractionated. The column was flushed with the starting eluent (A and 10% B) for 32 min before the first injection was made. The second injection was performed 30 min after the first one.

content and molar mass of standard samples, HPPLC basically is a separation by solubility differences. Normally, the data obtained can be transformed into the composition distribution of the sample under investigation, but one should be aware of the fact that in the case of additional change in polymer structure the solubility of a copolymer need not reflect only MMD and CCD. Fundamentally, the situation is equivalent to that of the evaluation of molar mass distributions from SEC curves. This method and its evaluation are valid as long as the separation is solely determined by size exclusion and any change in hydrodynamic volume is caused by a change in molar mass.

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