Journal of Chromatography, 499 (1990) 645–654
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1641

USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE CHARACTERIZATION OF SYNTHETIC COPOLYMERS

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SUMMARY

The evaluation of copolymer distribution as a function of molecular weight and chemical composition requires two separations, known as cross-fractionation. This procedure can be substantially improved by using high-performance liquid chromatographic (HPLC) techniques, including size-exclusion chromatography for the separation by molecular size. The separation by chemical composition can be achieved by gradient HPLC. Examples are given for copolymers of styrene and ethyl methacrylate (S–EMA). Gradient HPLC of these and similar copolymers can be performed in both normal-phase and reversed-phase modes with inversion of elution order. In isooctane–tetrahydrofuran (THF) mixtures on polar columns, the elution of S–EMA samples occurs at a higher THF concentration than required by solubility, *i.e.*, with a distinct contribution of adsorption to retention. In contrast, reversed-phase elution with methanol–THF mixtures takes place almost exactly at the solubility borderline of the system.

INTRODUCTION

Synthetic polymers are not the most attractive type of samples for analysis by high-performance liquid chromatography (HPLC). They are composed of such an enormous number of components that, at present, even under favorable conditions scarcely more than the *distribution* of the constituents can be evaluated experimentally. However, no matter how accurate and precise statistical average molecular weights are, polymers cannot be sufficiently characterized by average values alone, because any mean value can be connected to an unlimited number of different distributions. Samples that have identical average data may behave very differently in certain applications because of differences in the distribution of constituents. As a result, efficient separation methods for mixtures as complex as polymers are urgently required.

0021-9673/90/$03.50

The basic property of a polymer molecule is the chain length (or degree of polymerization (DP), *i.e.*, the number of repeat units per macromolecule). A typical polymer contains molecules with hundreds or thousands of different DP values. Only with oligomers is there a chance of separating individual species. One of the finest studies in this field is the separation of ethylene oxide oligomers published by Melander *et al.*[1] a decade ago. With oligomer samples of higher molecular weight (MW), baseline separations of individual homologues deteriorate at the high-MW end of the distribution.

The most versatile technique for evaluating the chain-length distribution is size-exclusion chromatography (SEC). Modern equipment consists of columns with inert packings of 3–5 μm particle diameter, pumps which deliver solvents at precise flow-rates and, more recently, molecular-weight-sensitive detectors, based on light scattering or viscosity. Although SEC can be applied satisfactorily to homopolymers, difficulties arise during the SEC analysis of copolymers because copolymer macromolecules are made of more than one kind of repeat unit. The chemically differing units can be composed of blocks or branches, they can alternate or be distributed statistically along the polymer chain. Because SEC separates on the basis of hydrodynamic volume rather than molecular weight, the contents of the detector cell at each elution volume increment consist of a mixture of structurally and chemically different components of the same hydrodynamic volume.

Statistical copolymers, produced by copolymerizing monomer mixtures, have a molecular weight distribution (MWD) similar to that of homopolymers and, in addition, a chemical composition distribution (CCD). Further, there is a sequence distribution which is a distribution along a polymer chain, related to the average composition of the respective chain by the rules of statistics.

Copolymers can be separated by chain length and by composition. Separation by average sequence length is, through the rules of statistical copolymerization, related to a separation by composition. However, any separation by individual sequence lengths, *e.g.*, diads, triads, tetrads, maybe possible only after destructive degradation of the polymer, as is done in pyrolysis–gas chromatography[2].

The properties of copolymers are determined by the complex distribution in chain length and composition. Chemical compositional heterogeneity can arise from fluctuations of batch composition in the vicinity of a growing chain end. This instantaneous heterogeneity will diminish with increasing MW because, during the sufficiently long lifetime of a growing chain, random fluctuations become more effective.

If, for kinetic reasons, the average composition of a copolymer differs from the batch composition, the latter is depleted in the preferentially consumed monomer. Hence, subsequent portions polymerize from a different batch composition. This gives rise to the chemical heterogeneity due to conversion, which increases with the difference in composition of the starting monomer mixture and of the copolymer formed at the very beginning of the reaction. Mixing problems and changes in reaction conditions by drifting temperature or decreasing initiator concentration may further affect the chemical composition distribution.

The complex MWD/CCD of a binary copolymer can be evaluated by cross-fractionation in bulk solution which requires at least two solvent–non-solvent combinations[3]. (It is not necessary that one combination fractionates, on addition of the

non-solvent, strictly according to MW and the other strictly according to composition.)

Other workers[4,5] have treated the method theoretically in terms of the Flory–Huggins theory of polymer solutions. Unfortunately, effective solvent–non-solvent combinations are not readily available, which may be one reason why only a small number of publications have reported experimental results of cross-fractionation. Another reason may be the time-consuming procedure of fractionation in one direction, subsequent separation of each fraction in another direction and, finally, the characterization of some dozens of subfractions with regard to amount of polymer, molecular weight and chemical composition. In this way, several months of labor-intensive studies are required for the evaluation of the MWD/CCD of a copolymer sample.

Chromatographic cross-fractionation employs the potential of HPLC for separating the copolymer in two different directions[6]. In this way, the limits set by solvent–non-solvent properties can be overcome through specific interactions of the individual solutes with the stationary phase. As an added attraction, the sample size is reduced from $\geqslant 10$ g to about 1 mg per copolymer and the time for characterization is decreased from about 10 weeks per sample to about four samples per day[7–9]. Among the possible combinations of methods for chromatographic cross-fractionation are those which employ SEC for separation by molecular size, followed by separation by composition by gradient HPLC[10].

An interesting version of chromatographic cross-fractionation, called orthogonal chromatography, was introduced by Balke and Patel[11,12]. In this technique, the sample is first fractionated by SEC. The fractionated components are then eluted through a second series of SEC columns, utilizing a different mobile phase that will either change the conformation of the polymer or encourage partitioning.

Investigators who have reported on the chemical composition distribution of styrene–acrylate copolymers by HPLC include Danielewicz and Kubin[13], Teramachi and co-workers[10,14], Sato and co-workers[15,16], Mourey[17] and Mori and co-workers[18–25].

The applicability of gradient elution HPLC to synthetic polymers is not as commonly used as expected. In view of this, the purpose of this paper is to report studies involving gradient elution HPLC of styrene–ethyl methacrylate (S–EMA) random copolymers with the hope that chromatographers can use this approach to characterize these and other types of copolymers.

EXPERIMENTAL

Samples

Statistical copolymers of styrene (S) and ethyl methacrylate (EMA) were prepared by radical copolymerization in bulk, as described elsewhere[26]. Sample (codes as used in Figs. 1 and 4) A: 4.7% (w/w) EMA (MW $51.6 \cdot 10^3$), C: 32.2% ($63.1 \cdot 10^3$), E: 54.6% ($65.2 \cdot 10^3$), G: 68.0% ($83.6 \cdot 10^3$), I: 92.5% ($61.6 \cdot 10^3$).

Solvents

Tetrahydrofuran (THF) without stabilizer (BASF, Ludwigshafen, F.R.G.) was distilled over potassium in a silver-coated column. The middle fraction was sub-

sequently refluxed over potassium continuously in a closed apparatus and used as needed in the HPLC and SEC systems. Sample solutions were prepared using analytical-reagent grade THF with 0.025% stabilizer (butylated hydroxytoluene; E. Merck, Darmstadt, F.R.G.). Isooctane (iOct) and methanol were of LiChrosolv grade (Merck). In the HPLC solvent reservoir, the eluents were continuously sparged with helium.

Gradient HPLC

The liquid chromatograph was a Model 1090 A (Hewlett-Packard, Waldbronn, F.R.G.) with a ternary solvent delivery system (Model DR5), equipped with an autosampler and autoinjector, diode-array detector and data-processing unit. The system was controlled by an HP85 personal computer.

The following columns were used: cartridge columns, 60 x 4 mm I.D. (Knauer, Bad Homburg, F.R.G.), packed with either Nucleosil CN (pore diameter, $d_0 \geqslant 5$ nm, particle diameter $d_p = 5$ μm, column 1), Nucleosil 50 ($d_0 = 5$ nm, $d_p = 5$ μm, column 2) or Nucleosil C_{18} ($d_0 \geqslant 5$ nm, $d_p = 5$ μm, column 3). Column 4 (150 x 4.6 mm I.D.) was packed with Polygosil 60-5 ($d_0 = 6$ nm, $d_p = 5$ μm).

The gradient conditions were as follows: gradient 1, iOct–THF with 1% methanol throughout, THF concentration = 0% at time zero, 30% (1 min), 70% (9 min), flow-rate 0.5 ml/min; gradient 2, iOct–(THF + 10% methanol), THF + 10% methanol concentration = 10% at time zero, 50% (8 min), 80% (10 min), 100% (11 min), flow-rate 1 ml/min, reduced to 0.3 ml/min between 9.9 and 10 min; gradient 3, iOct–methanol, injection of iOct–methanol (98:2) solution, followed by a sudden transition in THF content to the indicated level (see Fig. 4), after which a 5%/min linear increase in methanol concentration was used at a flow-rate of 0.5 ml/min.

Size-exclusion chromatography

A Model BT 3020 HPLC pump (Biotronik, Maintal, F.R.G.) was connected to a Model 7010 injection valve (Rheodyne, Latek, Heidelberg, F.R.G.), a bank of two GMH6 mixed-gel columns (Toyo Soda, Japan), each 600 × 7.8 mm I.D., $d_p = 8$–10 μm) and a Model 51.78 refractive index detector (Knauer). The following conditions were used: THF flow-rate, 1 ml/min; injection volume, 0.2 ml; and sample concentration, 0.5% for preparative fractionations.

RESULTS

Normal-phase gradient HPLC of statistical S–EMA copolymers [stat-copoly(S–EMA)]

A model mixture of five S–EMA samples was prefactionated by SEC and the fractions were analysed by gradient HPLC (see Fig. 1). The elution curve gives no indication of a mixed sample. This can be understood from the similarity of the MWs, which are within the limits 51 600 (sample A) and 83 600 (sample G). The presence of different copolymers is clearly visible in the HPLC traces. In the normal-phase (NP) system employed, retention increases with increasing EMA content. The comparatively high MW of sample G is reflected in the appearance of this component in the HPLC trace of SEC fraction 1, which contains the high-MW portions of the mixture and by its predominance in SEC fraction 2. The predominance of Sample A with MW

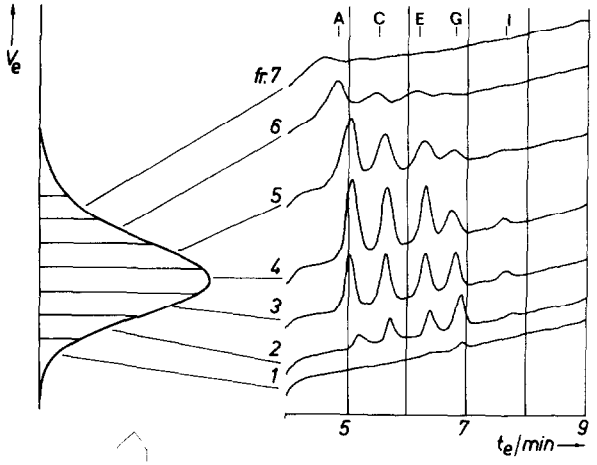

Fig. 1. Separation of a mixture of five *stat*-copoly(styrene–ethyl methacrylate) samples by SEC and normal-phase gradient HPLC on CH-bonded phase (column 1) by gradient elution with iOct–THF (No. 1). Temperature, 50°C; flow-rate, 0.5 ml/min; injection volume for gradient HPLC, 100 µl; detection at 259 nm. V_e = Elution volume; t_e = elution time; fr = fraction.

51 600 in the low-MW SEC fractions 5–7 is also straightforward. (In comparing the HPLC peaks in Fig. 1, it must be acknowledged that only styrene units are monitored at 259 nm. This accounts for, *e.g.*, the small peak area of sample I.)

Without SEC prefractionation, the copolymers were not baseline separated under the conditions used for Fig. 1. This can be understood as the superimposition of a molecular-weight effect. Separate injections of the individual copolymers yielded well shaped peaks. Their first moment was used for calculating the eluent composi-

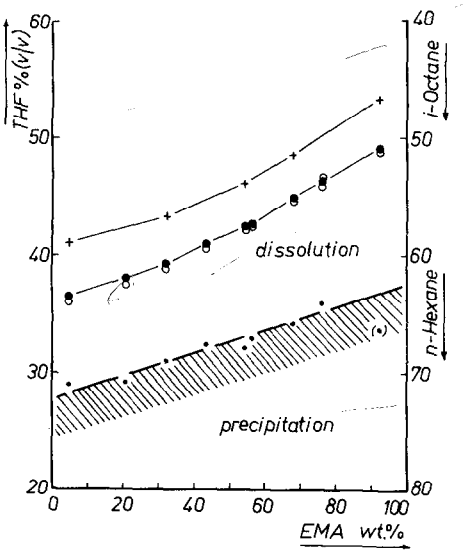

Fig. 2. Elution characteristics in iOct–THF gradients and solubility of *stat*-copoly (styrene–ethyl methacrylate) samples. + = Silica column 2, gradient 1; ○, ● = gradient elution from two CN-bonded phase columns of the same size (see column 1) with iOct–THF (0–100% in 10 min). Solubility borderline determined by turbidmetric titration (dots) with *n*-hexane at 20°C. (From ref. 27, with permission from Wiley).

tion at the elution of a given sample. These data (obtained in two laboratories) are shown as open and filled circles in Fig. 2. The curve marked by crosses is from a similar investigation but on a silica column. The higher activity of the latter accounts for the fact that *ca.* 5% more THF is needed for elution from silica than from a nitrile-bonded phase.

The elution characteristics of both columns are well above the solubility borderline. Although the latter had been determined with *n*-hexane non-solvent and at 20°C, both solvent systems are comparable because of the similar solubility parameters of *n*-hexane (14.9 $MPa^{0.5}$) and iOct (15.3 $MPa^{0.5}$)[27].

The separation of S–EMA copolymers according to composition is more difficult than the separation of *stat*-copoly(styrene–methyl methacrylate) (S–MMA) samples. This can be appreciated from a synoptic presentation of the elution characteristics (see Fig. 3) of S–MMA (curva a), S–EMA (curve b) and *stat*-copoly (styrene–*tert.*-butyl methacrylate) samples (S–TBMA, curve c). The shielding effect of the alkyl groups increases with the bulkiness of the latter and diminishes the adsorption interactions between the ester linkages and polar groups on the surface of the stationary phase.

Normal-phase HPLC with separate adjustment of solubility and polarity

The differences between elution characteristics and solubility line in Fig. 2 indicate that retention is due to adsorption. However, the parallelism between the two different types of curves suggests that solubility behavior may also play a role in

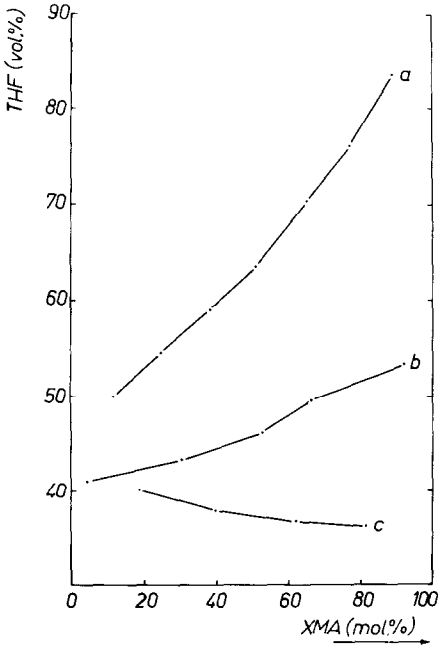

Fig. 3. Shielding effect of alkyl groups in poly(methacrylate esters) on the adsorption of styrene copolymers. Elution characteristics as in Fig. 2 on silica columns. (a) S–MMA, column 4, gradient 2; (b) S–EMA; (c) S–TBMA; (b) and (c) on column 2 with iOct–THF gradient (0–100% in 10 min).

retention. (From past studies[27], we believe that polymer HPLC is generally governed by solubility and adsorption effects. A pure adsorption mechanism or a pure precipitation–redissolution mechanism are limiting cases.)

Usually, we performed normal-phase gradient elution with THF as gradient eluent B. THF is more polar than iOct, which was used as eluent A, but THF is at the same time a better solvent for all of the copolymers investigated. Thus, in the usual gradient technique we increased simultaneously the dissolution power and elution strength of the eluent. The chromatograms shown in Fig. 4 were obtained under modified conditions, where solubility and displacement effects could be adjusted separately. A linear gradient of increasing methanol concentration was used after a sudden increase in THF content from zero to a selected level. The starting eluent was iOct, which contained 2% methanol in order to suppress adsorption of any impurities during the pre-run period.

The starting eluent contained no THF. As a strong precipitant and poor eluent, it should enable the injected polymer to be retained at the beginning of the column. Of course, the sudden transition in THF concentration caused disturbances of the baseline but, fortunately, the latter became stable again before the sample components were eluted (the trace at 20% THF concentration shows this by the flat section between 3 and 3.5 min). At 20% THF content, all five components were properly eluted when methanol was used, but the resolution was poor. Optimum resolution was found at 25–30% THF. At 35% THF content, the first component of the model mixture disappeared in the 'noise' which had been caused by the transition in THF concentration. The elution in iOct–methanol–THF (63:2:35) is in accordance with the elution characteristics of this copolymer sample in iOct–THF (see Fig. 2).

The independent adjustment of polarity at a selected level of solubility has several advantages in optimizing a separation. In the present instance, *i.e.*, with THF as the solvent and UV detection, elution at a constant THF level has the additional

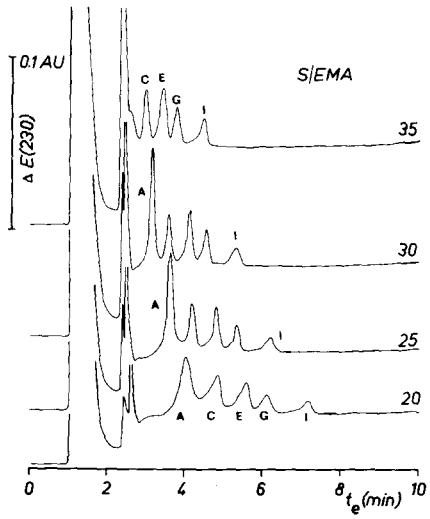

Fig. 4. Separation of a mixture of five *stat*-copoly (styrene–ethyl methacrylate) samples by linear gradients of iOct–methanol after a sudden increase in THF content from 0% to the indicated level (CN column 1, gradient 3). Detection at 230 nm; flow-rate 0.5 ml/min.

benefit that detection is possible at shorter wavelengths. The chromatograms in Fig. 4 were monitored at 230 nm, where THF usually shows adsorption. In the gradients used for Fig. 4, the THF absorption causes a difference in baseline level only before and after the addition of THF, but no baseline rise. The change in baseline can be suppressed within certain limits, whereas a rising baseline renders quantitative evaluation more difficult. Further, for the present system, the effect of copolymer composition on signal size is less severe at 230 than at 259 nm (compare the peaks of sample I in Figs. 4 and 1).

Reversed-phase chromatography with inversion of elution order

Low-MW and polymer HPLC may have the same physico-chemical basis[28,29]. Some peculiarities of the latter can be understood as a consequence of the narrow solubility window of polymers, the possibility of multiple attachment of flexible chains on a rigid surface and the fact that synthetic polymers are not chemically uniform substances but mixtures of a huge number of similar homologs. It should be noted, however, that Boehm and Martire[30] have recently developed a theory that suggests that homopolymer HPLC cannot be predicted from small-molecule results.

Gradient HPLC of low-MW substances can be performed in normal-phase (NP) or reversed-phase (RP) modes. Retention in NP chromatography increases with increasing polarity of the samples. The NP retention of S–EMA copolymers is in accordance with the general rule. In RP gradient HPLC, samples of equal molecular

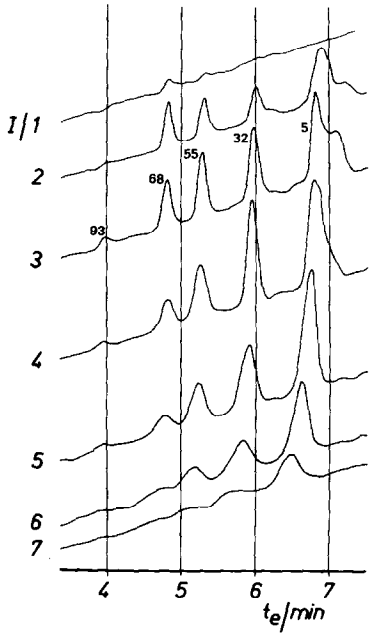

Fig. 5. Reversed-phase separation of the mixture of five *stat*-copoly(styrene–ethyl methacrylate) samples after prefractionation by SEC. Same sample as in Fig. 1; C_{18}-bonded phase (column 3), methanol–THF gradient (0–100% in 10 min); flow-rate 0.5 ml/min; temperature 50°C; detection at 259 nm. Numbers refer to the % (w/w) of ethyl methacrylate.

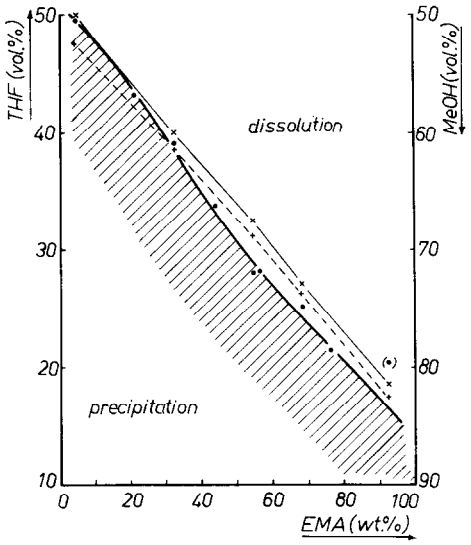

Fig. 6. Elution characteristics in methanol–THF gradients and solubility of *stat*-copoly(styrene–ethyl methacrylate) samples. × = elution characteristic of fraction I/2 in Fig. 5; + = same for fraction I/5. Solubility borderline determined by turbidimetric titration with methanol (MeOH) at 20°C (dots). (From ref. 27, with permission from Wiley).

size have greater retention the less polar they are, *i.e.*, with S–EMA copolymers, the less EMA units they contain.

S–EMA samples have been chromatographed on RP C_{18} columns with methanol–THF gradients[31,32]. Fig. 5 shows that retention indeed increased with decreasing EMA content. An analogous inversion of elution order in NP- from that in RP-HPLC has been independently observed with *stat*-copoly(S–MMA) samples[14,15].

Fig. 6 shows the elution characteristic of SEC fractions I/2 and I/5 from Fig. 5, together with the solubility line. In contrast with the behavior on polar columns, S–EMA copolymers are eluted almost precisely at the solubility borderline.

CONCLUSIONS

Interactive HPLC of SEC-fractionated samples is a useful approach for the characterization of copolymers. By analyzing components of similar hydrodynamic volumes, the uncertainty due to the superimposition of molecular weight and composition is removed. By a judicious choice of mobile phases and gradient conditions, as outlined in this paper, separations of copolymers can be effected. Also, the applicability of this approach for protein characterization has recently been reported[33]. In addition to NP and RP separations, it should also be possible to use ion-exchange chromatography, combined with aqueous SEC, to cross-fractionate polyelectrolytes.

ACKNOWLEDGEMENTS

Thanks are due to Drs. K. Albrecht, M. Stickler and W. Wunderlich, Darmstadt, for the preparation of S–EMA copolymers and their prefractionation by SEC,

to Dr. A. Müller, Mainz, for supplying THF of the required quality, and to Mrs. Ch. Meissner, Dresden, for light-scattering and turbidimetric measurements. Gottfried Glöckner is indebted to the Sonderforschungsbereich 41 der Johannes-Gutenberg-Universität Mainz for the opportunity to perform the HPLC investigations.

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