Journal of Chromatography, 547 (1991) 429–433
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 189

Short Communication

Sample remaining in an ODS column after compositional fractionation of copolymers by high-performance liquid chromatography

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(First received November 5th, 1990; revised manuscript received February 8th, 1991)

ABSTRACT

High-performance liquid chromatography is very effective for the compositional fractionation of copolymers. Octadecyl-modified silica gel (ODS) has been used as a packing material for fractionation in the reversed-phase (RP) adsorption mode. In the compositional fractionation of the samples of copoly-(methyl methacrylate–styrene) by RP gradient elution through ODS column, it was found that small peaks were observed with blank elution just after the sample measurement and a perfect baseline was recovered after several blank elutions.

INTRODUCTION

High-performance liquid chromatography (HPLC) has been widely used for the compositional fractionation of copolymers in both adsorption [1] and phase-separation (selective precipitation and dissolution) [2] modes. In the compositional fractionation of copolymers by the adsorption mechanism, both normal-phase (NP) and reversed-phase (RP) modes [3–6] have been used succesfully. Octadecyl-modified silica gel (ODS) has commonly been used as a packing material for the RP fractionation.

Although no mention of any sample remaining after elution was made in the papers cited, we observed small peaks with blank elution just after sample measurement in the compositional fractionation of the samples of statistical and graft copoly(methyl methacrylate–styrene) using ODS columns. As this is a serious problem for the accurate determination of the chemical composition distribution of copolymers using ODS columns, we present here a brief of the phenomenon.

EXPERIMENTAL

Statistical and graft copolymers of methyl methacrylate (MMA) and styrene (S) were used as samples. The former samples were prepared by radical copolymerization in bulk using benzoyl peroxide as the initiator. Styrene contents determined by elemental analysis and number-average molecular weights determined by osmometry are given in Table I. The conversions of the samples were low, so that the chemical composition distributions of the samples should be sharp. The graft copolymer samples, which were supplied by T. Tsukahara of Nagoya University, were prepared by radical copolymerization of ω-methacryloyl polystyrene macromonomer with MMA [7]. Styrene contents of the samples determined by ^{1}H NMR spectroscopy are shown in Table II.

TABLE I

SAMPLES OF STATISTICAL COPOLYMERS

Code	Styrene content (mol%)	$M_n \times 10^{-5}$
SMMA-81-25(2)	21.0	1.22
-87-30	29.0	2.01
-81-50(2)	48.7	1.27
-88-61	60.8	2.23
-88-73	72.5	2.41
-81-75(1)	84.9	1.40
PS (Tosoh)	100	1.86

Tetrahydrofuran (THF) and acetonitrile (ACN), used as eluents for HPLC, were of chromatographic grade from Wako (Tokyo, Japan) and Nacalai Tesque (Kyoto, Japan), respectively.

Two sets of HPLC instruments were used. One (A) was composed of two Model-510 pummps and a Model-680 controller (Waters Assoc., Milford, MA, USA), a CO-8000 column oven (Tosoh, Tokyo, Japan) and a Model ERC-7211 UV detector (Erma, Tokyo, Japan). The other (B) was composed of two LC-6A pumps, an SCL-6A controller, an SPD-6A UV detector (Shimadzu, Tokyo, Japan) and an SSC-3510 column oven (Senshu Scientific, Tokyo, Japan). In both sets, Rheodyne Model 7010 injectors were used.

TABLE II

SAMPLES OF GRAFT COPOLYMERS

Code	Styrene content (wt.%)
PMMA-g-PS-A	27.0
-B	46.3
-C	74.4

TABLE III

GRADIENT PROTOCOLS

Gradient No.	Time (min)	THF (vol.%)	ACN (vol.%)
1	0–15	10–60	90–40
	15–20	60	40
	20	60–100	40–0
	20–30	100	0
	30–45	100–10	0–90
2	0–15	15–60	85–40
	15–18	60	40
	18	60–95	40–5
	18–28	95	5
	28–43	95–15	5–85

Two ODS columns and also a phenyl-modified silica gel column (phenyl column) were used: ODS-1251-K (Senshu Scientific) (25 cm × 4.6 mm I.D., particle diameter $d = 5\ \mu m$, micropore size of starting silica gel, $d_p = 10$ nm); ODS-120T (Tosoh) (25 cm × 4.6 mm I.D., $d = 5\ \mu m$, $d_p = 12$ nm) and μBondasphere 5 μm Phenyl-100A (Waters Assoc.) (15 cm × 3.9 mm I.D., $d = 5\ \mu m$, $d_p = 10$ nm).

HPLC measurements were carried out on two samples. One was an equi-weight mixture of six samples of statistical copolymers and a polystyrene (Table I) dissolved in THF at a total concentration of 1.05 mg/cm^3 (STAT) and the other was an equi-weight mixture of three samples of the graft copolymers (Table II) dissolved in THF at a total concentration of 1.5 mg/cm^3 (GRAFT). In all measurements, the injection volume was 0.1 cm^3, the flow-rate was 1.0 cm^3/min, the column temperature was 30°C and the wavelength of the UV detectors was 254 nm. The gradient protocols of the eluents are given in Table III, in which the samples were separated by a linear gradient from 10 or 15 to 60 vol.% THF. The combinations of the HPLC instrument, the column, the gradient protocol and the sample are given in Table IV.

RESULTS AND DISCUSSION

The chromatograms of the STAT sample mixture obtained by a combination of HPLC set A, ODS-1251-K column and gradient protocol No. 1 are shown in Fig. 1. Chromatogram (a) obtained by the sample injection has seven clearly separated

TABLE IV

COMBINATION OF EXPERIMENTAL CONDITIONS

No.	HPLC set	Column	Sample	Gradient No.
1	A	ODS-1251-K	STAT	1
2	A	ODS-120T	STAT	1
3	A	Phenyl	STAT	1
4	B	ODS-1251-K	GRAFT	2

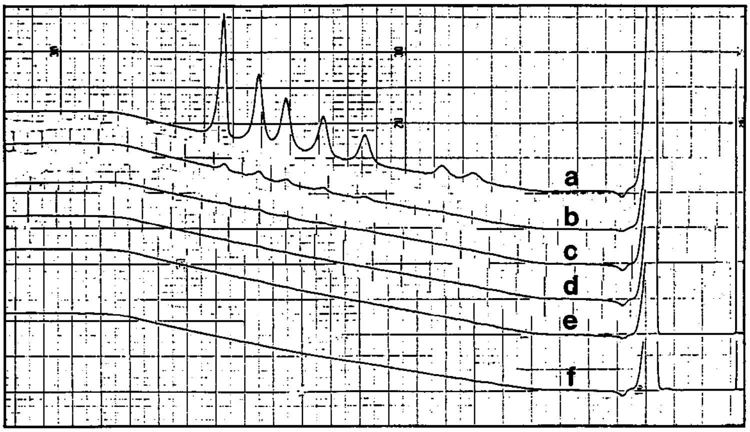

Fig. 1. Chromatograms of a mixture of statistical copolymers (Table I) and blank elutions obtained by combination No. 1. (Table IV). (a) Sample elution; (b)–(e) 1st–4th blank elutions after the sample elution; (f) baseline obtained by blank elution just before the sample elution. For further details, see text.

peaks, which correspond to the respective samples in order from low to high styrene content in accordance with the mechanism of reversed-phase adsorption. The peak assignment was made by single injections of the respective samples. Chromatograms (b)–(e) were obtained by consecutive blank elutions with the same gradient and pure solvent injection just after the sample elution. Curve (f) is the baseline obtained with the same gradient just before the sample measurement. In the first and second blank elutions, clear, small peaks for the respective components were observed at the same positions as in the sample measurement. Even in the third blank elution, the peaks were not completely eliminated, although the peak heights gradually decreases as the

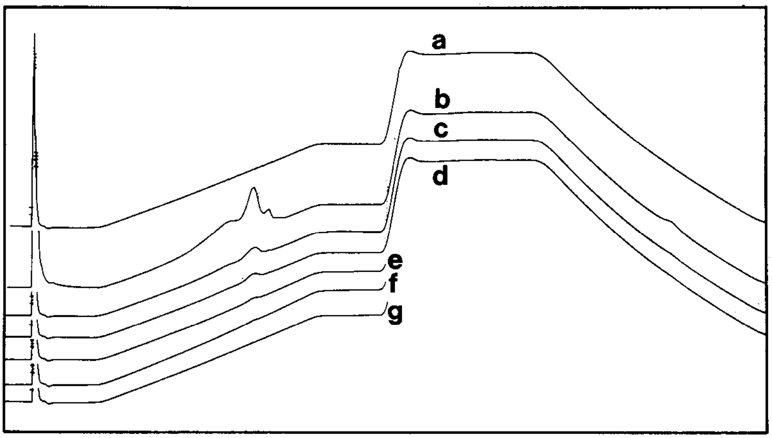

Fig. 2. Chromatograms of a mixture of graft copolymers (Table II) and blank elutions obtained by combination No. 4 (Table IV). (a) Baseline obtained by blank elution just before the sample elution; (b) sample elution; (c) 1st, (d) 2nd, (e) 4th, (f) 7th and (g) 10th blank elutions just after the sample elution. For further details, see text.

blank elution was repeated. The fourth blank elution (e) finally exhibited the complete baseline as in (f).

The same experiments were carried out changing the column from ODS-1251-K to ODS-120T and also to the phenyl column. The results with the ODS-120T column (not shown) were the same as those with the ODS-1251-K column, whereas no peak was observed with blank elution through the phenyl column.

The chromatograms shown in Fig. 2 are for the GRAFT sample, obtained by a combination of HPLC set B, ODS-1251-K column and gradient protocol No. 2. Chromatogram (a) is the baseline obtained just before the sample measurements. Chromatogram (b) obtained by sample injection appears to contain three peaks, but they do not correspond to the respective original samples. As shown elsewhere [7], the first peak corresponds to the mixture of samples A and B, the second is sample C and the last is homopolystyrene. Chromatograms (c)–(g) were obtained by blank elutions. For the graft copolymer, it was more difficult than for the statistical copolymer to remove the small peaks by repeated blank elutions. As shown in Fig. 2, the tenth blank elution finally exhibited the perfect baseline (g). Further, during the return to the starting composition, a peak was observed at an eluent composition near to that of the main peak not only with the sample elution but also with the first and the second blank elutions, although no peak on the returning gradient was found for the statistical copolymer.

In these experiments, the injectors of the HPLC instruments were carefully rinsed with pure THF before the blank elutions, and the injection syringe used for the pure solvent in blank elutions was carefully rinsed and was not also used for injection of sample solutions. Therefore, it is clear that the small peaks on the chromatograms obtained by blank elutions are not due to samples injected accidentally, but represent sample components remaining in the ODS columns. It is also obvious that the sample remaining is particular to an ODS column, as the phenomenon was not observed with the phenyl column. As shown in a previous paper [6], both adsorption and phase-separation mechanisms coexist in the compositional fractionation of the statistical copolymer by the present combination of column and eluent. However, it is difficult to explain the phenomena reported here by these mechanisms. Although partitioning is regarded as the main mechanism for the separation of low-molecular-weight compounds by ODS columns, it is not clear whether the mechanism can explain the present phenomena.

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