Journal of Chromatography, 623 (1992) 315–322
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 433

Chromatographic investigations of macromolecules in the "critical range" of liquid chromatography

II☆. Two-dimensional separations of poly(ethylene oxide–block–propylene oxide)

H. Pasch and C. Brinkmann

Deutsches Kunststoff-Institut, Schlossgartenstrasse 6, W-6100 Darmstadt (Germany)

H. Much

Centre of Macromolecular Chemistry, Rudower Chaussee 5, O-1199, Berlin (Germany)

U. Just

Federal Institute of Materials Research and Testing, Department 3, Unter den Eichen 87, W-1000 Berlin 45 (Germany)

(First received March 26th, 1992; revised manuscript received June 12th, 1992)

ABSTRACT

A polyethylene oxide–polypropylene oxide block polymer was characterized with respect to molar mass distribution and block length of the individual blocks using two-dimensional chromatographic techniques. In the first dimension the block polymer was separated according to the length of the polypropylene oxide block by liquid chromatography at the critical point of adsorption. The resulting polypropylene oxide uniform fractions were subjected to supercritical fluid chromatography or size-exclusion chromatography and the average length and the molar mass of the polyethylene oxide blocks were determined for every fraction.

INTRODUCTION

The molecular heterogeneity of a polymer is characterized by three distribution functions: the molar mass distribution, the distribution in chemical composition and the functionality type distribution. The molar mass distribution may be determined by size-exclusion chromatography (SEC) and the determination of the chemical heterogeneity is possible using liquid adsorption or precipitation chromatography [1,2]. Until recently, the functionality type distribution could be determined only via preparative chromatographic separation into functionality fractions and spectroscopic determination of the functional groups.

The development of liquid chromatography at the critical point of adsorption by Entelis and coworkers [3–5] made it possible to determine the fuctionality type distribution of telechelic oligomers and polymers. Operating in the region between exclusion and adsorption modes of liquid chromatography, retention becomes independent of the

Correspondence to: Dr. H. Pasch, Deutsches Kunststoff-Institut, Schlossgartenstrasse 6, W-6100 Darmstadt, Germany.

☆ For Part I, see ref. 9.

0021-9673/92/$05.00

length of the polymer chain and separation is accomplished exclusively by the number and type of functional groups [6–8]. Accordingly, only the functional groups and not the polymer chain contribute to the retention time, *i.e.*, the polymer chain behaves like an "invisible" part of the molecule.

In Part I [9] it was shown that the same approach can be applied to the characterization of block polymers. For example, taking a block polymer A_nB_m the block A_n can be regarded as a functional group. Therefore, using the critical conditions of B_m for the chromatographic process the chemical structure of A_n can be characterized and *vice versa*.

This paper is aimed at using two-dimensional chromatographic techniques to characterize a block polymer of ethylene oxide and propylene oxide. The first (polypropylene oxide) block will be analysed by liquid chromatography at the critical point of adsorption whereas the second (polyethylene oxide) block is to be analysed by supercritical fluid chromatography (SFC) and SEC.

EXPERIMENTAL

The SFC experiments were conducted on a Dionex SFC 600D instrument using a 10 m × 50 μm I.D. SB Biphenyl-30 capillary column (Lee Scientific). The mobile phase was carbon dioxide (Scott). Flame ionization detection (FID) at 380°C was used, the initial oven temperature being 130°C. Timed split injection was carried out using a Valco injection valve. All samples were injected as 30% (w/w) solutions in methylene chloride.

High-performance liquid chromatographic (HPLC) separations were carried out on a system consisting of a Waters Model 501 HPLC pump, a manually operated six-port injection valve (Rheodyne) and an R-401 differential refractometer (Waters). The column was Nucleosil $5C_{18}$ (250 × 4 mm I.D.) (Macherey–Nagel) with a particle diameter of 5 μm. The mobile phase was acetonitrile–water (43:57, v/v) at a flow-rate of 0.5 ml/min. A 20-μl volume of a 10% polymer solution was injected for each separation.

The SEC investigations were performed on five 300 × 8 mm I.D. columns of Ultrastyragel, 1000 Å, 2 × 500 Å and 2 × 100 Å (Waters), using tetrahydrofuran (THF) as the mobile phase at a flow-rate of 1 ml/min. A Model R-410 refractive index detector (Waters) and a Model 501 pump (Waters) were used; the molar mass calculations were based on polyethylene oxide calibration standards. Volumes of 200 μl of 0.1% (w/w) polymer solutions were injected via a Rheodyne six-port injection valve.

The block polymer was prepared at the Central Institute of Organic Chemistry, Berlin, by anionic polymerization at 110°C using potassium glycolate as initiator.

RESULTS AND DISCUSSION

Polyethylene oxide (PEO) and polypropylene oxide (PPO) and their block polymers are important precursors of polyurethanes. Their detailed chemical structure, *i.e.*, the chemical composition, block length and molar mass of the individual blocks, may be decisive for the properties of the final product.

It was shown previously [9,10] that in block

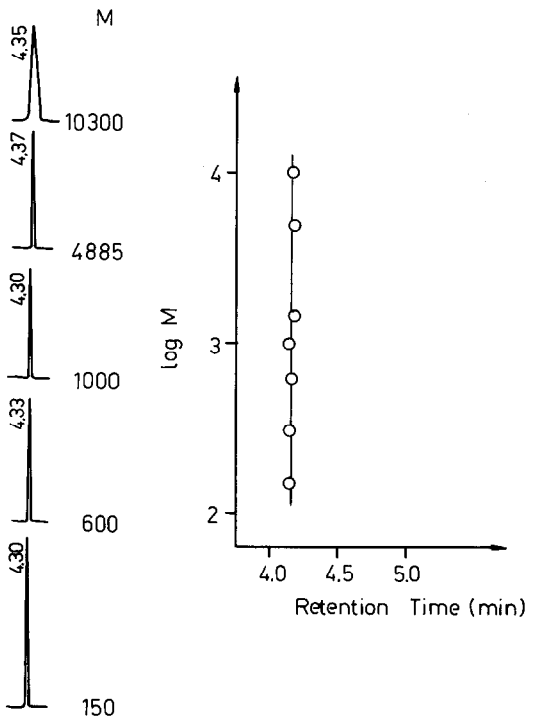


Fig. 1. HPLC of polyethylene oxide calibration standards at the critical point of adsorption and the corresponding critical diagram. Column, Nucleosil $5C_{18}$; mobile phase, acetonitrile–water (43:57, v/v); flow-rate, 0.5 ml/min; refractive index detection.

polymers the individual blocks may be characterized independently using liquid chromatography at the critical point of adsorption. Operating at the critical point of one block, the other block may be analysed and structural parameters may be calculated.

The mobile phase composition corresponding to the critical conditions for PEO was found to be acetonitrile–water (42:58, v/v) on a Chrompack RP-18 stationary phase [9]. As the present investigations were carried out on a Macherey–Nagel $5C_{18}$ column, the composition of the mobile phase had to be adjusted accordingly. Polyethylene oxide calibration standards of different molar masses were separated using acetonitrile–water of varying composition. The disappearance of the molar mass dependence of the retention time, corresponding to the critical conditions, was achieved at a composition of the mobile phase of acetonitrile–water (43:57, v/v); see Fig. 1.

Under these conditions a triblock polymer of ethylene oxide and propylene oxide, $HO(EO)_{n_1}(PO)_m(EO)_{n_2}OH$, was separated into fractions of different degrees of polymerization with respect to propylene oxide, regadless of the length of the ethylene oxide blocks (see Fig. 2). The assignment of the peaks was based on comparison with the chromatogram of a polypropylene oxide. The elution order and the retention time behaviour of the fractions, which are in agreement with the theoretically estimated distribution coefficient $K_d^{(m)} = [K_d^{(1)}]^m$ (see Part I [9]), where m is the degree of polymerization with respect to propylene oxide, suggests that the assignment given in Fig. 2 is correct.

The first peak corresponds to $m = 1$–3, the shoulder to $m = 4$, the peak at 2.5 ml to $m = 5$ and so on. Accordingly, every peak is uniform with respect to m but has a distribution in block length with respect to the polyethylene oxide blocks (n). Assuming that the refractive index responses of polyethylene oxide and polypropylene oxid oligomer series are similar, the amounts of fractions 1–8 may be determined (see Table I).

A complete picture of the microstructure of the block polymer can be provided by separating the fractions preparatively and subjecting them to a second chromatographic method. This method must separate the propylene oxide uniform fractions according to the oligomer distribution of the ethylene oxide blocks, thus providing the molar mass or

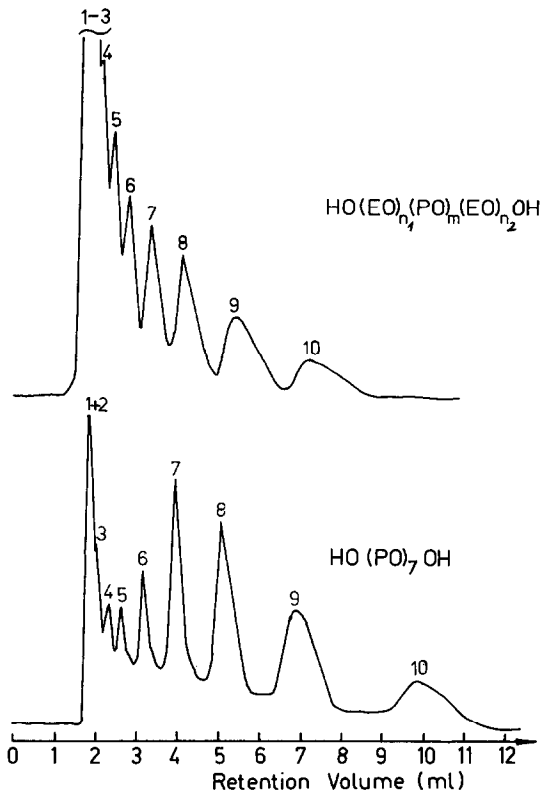


Fig. 2. HPLC of the block polymer. Chromatographic conditions as in Fig. 1.

TABLE I

RELATIVE AMOUNTS OF FRACTIONS 1–8 AFTER SEPARATION AT THE CRITICAL POINT OF ADSORPTION OF PEO

Fraction	m	Composition	Relative amount (%, v/v)
1	1	$HO(EO)_{n_1}(PO)(EO)_{n_2}OH$	41.9
	2	$HO(EO)_{n_1}(PO)_2(EO)_{n_2}OH$	
	3	$HO(EO)_{n_1}(PO)_3(EO)_{n_2}OH$	
2	4	$HO(EO)_{n_1}(PO)_4(EO)_{n_2}OH$	2.5
3	5	$HO(EO)_{n_1}(PO)_5(EO)_{n_2}OH$	8.5
4	6	$HO(EO)_{n_1}(PO)_6(EO)_{n_2}OH$	10.1
5	7	$HO(EO)_{n_1}(PO)_7(EO)_{n_2}OH$	12.1
6	8	$HO(EO)_{n_1}(PO)_8(EO)_{n_2}OH$	13.9
7	9	$HO(EO)_{n_1}(PO)_9(EO)_{n_2}OH$	9.0
8	10	$HO(EO)_{n_1}(PO)_{10}(EO)_{n_2}OH$	2.0

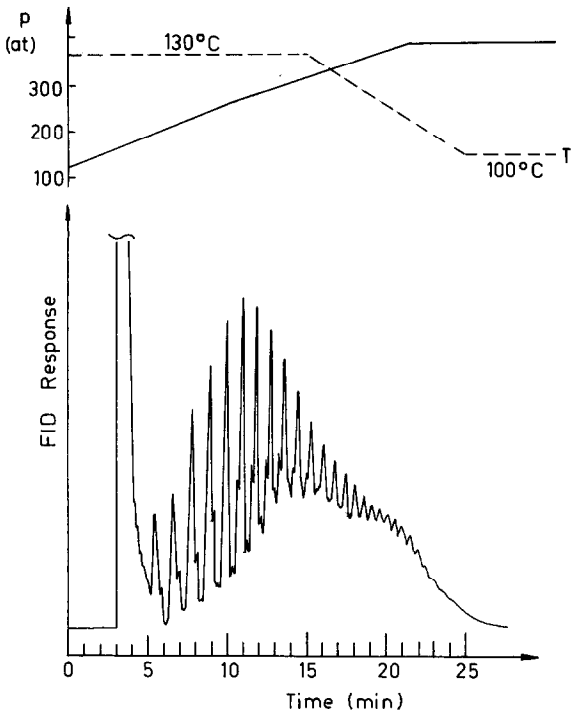


Fig. 3. SFC of fraction 1. Column, SB Biphenyl-30; mobile phase, carbon dioxide; FID.

block length distribution of the ethylene oxide blocks.

In previous investigations on the two-dimensional separation of telechelic oligomers, it was demonstrated that SFC is a very useful technique for separating polyethers according to their oligomer distribution [11,12]. Using highly efficient and selective capillary columns, oligomers may even be separated simultaneously according to the degree of polymerization and functionality.

Fig. 3 shows the SFC of fraction 1 using a pressure and temperature gradient. The oligomers are well separated and it can be seen that, in addition to the main oligomer series, peaks of minor intensity are also obtained. This is in agreement with our assumption that fraction 1 is a superposition of the oligomer series with $m = 1, 2, 3$. Based on the reaction mechanism and the retention behaviour of the fraction, it was concluded that the major peaks belong to the oligomer series with $m = 3$.

Using the same chromatographic conditions, fractions 3–8 were separated into their oligomers (see

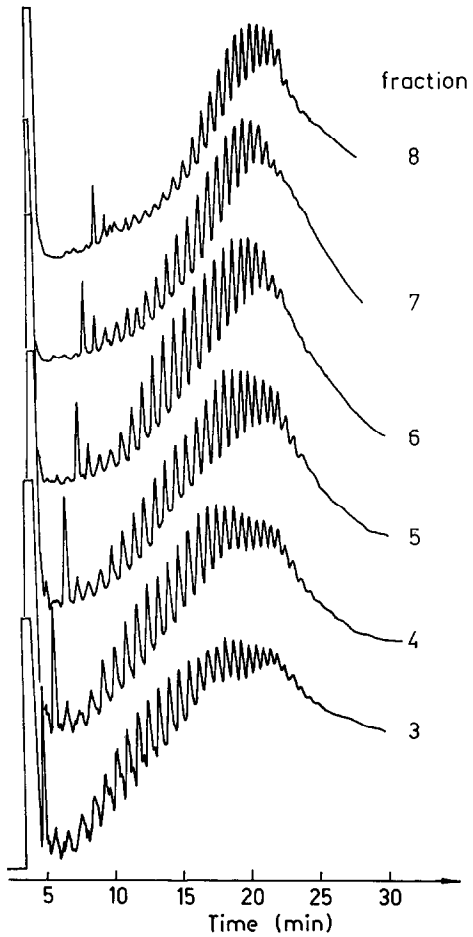


Fig. 4. SFC of fractions 3–8. Chromatographic conditions as in Fig. 3.

TABLE II

COMPARISON OF SFC RETENTION TIMES OF THE FIRST PEAKS IN FRACTIONS 3–8 WITH A COMMERCIAL PPO

n	Retention time (min)	
	$HO(PO)_nOH$	First peak
5	4.57	4.77 (fraction 3)
6	5.18	5.40 (fraction 4)
7	6.00	6.15 (fraction 5)
8	6.85	6.93 (fraction 6)
9	7.62	7.67 (fraction 7)
10	8.30	8.32 (fraction 8)

TABLE III

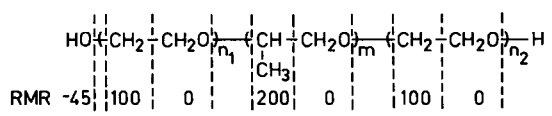
PEAK ASSIGNMENT IN SFC OF FRACTIONS 1–8 OF THE BLOCK POLYMER $HO(EO)_{n_1}(PO)_m(EO)_{n_2}OH$

$n_1 + n_2$	Retention time (min)						
	1 ($m = 3$)	3 ($m = 5$)	4 ($m = 6$)	5 ($m = 7$)	6 ($m = 8$)	7 ($m = 9$)	8 ($m = 10$)
0	[a]	4.77	5.40	6.15	6.93	7.67	8.32
1	4.48	5.52	6.32	7.10	7.83	8.50	9.12
2	5.32	6.33	7.28	7.88	8.67	9.30	9.85
3	6.52	7.40	8.10	8.77	9.47	10.10	10.62
4	7.75	8.33	8.95	9.58	10.17	10.90	11.30
5	8.90	9.22	9.82	10.38	10.95	11.55	12.00
6	9.95	10.03	10.62	11.17	11.73	12.25	12.70
7	10.88	10.83	11.42	11.93	12.50	13.00	13.40
8	11.80	11.63	12.20	12.70	13.28	13.75	14.08
9	12.67	12.43	12.97	13.47	14.07	14.52	14.80
10	13.53	13.20	13.73	14.24	14.83	15.27	15.53
11	14.37	13.97	14.48	14.97	15.58	16.00	16.20
12	15.18	14.72	15.25	15.72	16.32	16.72	16.85
13	15.97	15.48	15.95	16.42	17.00	17.38	17.48
14	16.70	16.20	16.63	17.10	17.67	18.02	18.07
15	17.38	16.88	17.27	17.73	18.30	18.63	18.65
16	18.02	17.52	17.88	18.33	18.90	19.23	19.20
17	18.60	18.15	18.48	18.92	19.47	19.80	19.73
18	19.05	18.73	19.08	19.48	20.03	20.37	20.25
19	19.52	19.28	19.65	20.02	20.58	20.95	20.75
20	20.02	19.83	20.18	20.55	21.13	21.45	21.28
21	20.97	20.38	20.68	21.05	21.62		21.78

[a] Eluted with solvent peak.

Fig. 4). These fractions consisted nearly exclusively of one oligomer series each, indicating that the preparative separation was of good quality. Because of its low concentration and the poor separation quality, fraction 2 could not be characterized by SFC. The assignment of the peaks was based on comparison with chromatograms of PPO and the retention behaviour of PEO. Thus, the first intense peak after the solvent peak in each chromatogram was identified as the propylene oxide oligomer without ethylene oxide units. The comparison of the retention times of the first peaks with a commercial PPO in Table II shows good consistency. The next peaks in the chromatograms may then be assigned to the oligomers containing one, two, three, and so on, ethylene oxide units. Because of the high chemical similarity, SFC did not resolve oligomers of the same gross composition but different ethylene oxide distribution, *e.g.*, $HO(EO)_4(PO)_m(EO)_2OH$ and $HO(EO)_3(PO)_m(EO)_3OH$. The complete assignment of the peaks is summarized in Table III.

One of the major problems in SFC is quantification. Normally, using FID the signal depends not only on the amount of the eluted component but also on its composition and chemical structure. However, in a number of instances, namely for homologuous series, the FID response may be calculated using increment methods [13]. As was shown in a previous investigation [12], relative mass response (*RMR*) numbers of structural units may be combined to give the relative FID response of a certain molecule:



$$RMR = 200\ m - 45 + 100(n_1 + n_2)$$

Accordingly, the *RMR* numbers for every oligomer may be calculated by the following formulae:

Fraction	m	*RMR*
1	3	$555 + 100(n_1 + n_2)$
2	4	$755 + 100(n_1 + n_2)$
3	5	$955 + 100(n_1 + n_2)$
4	6	$1155 + 100(n_1 + n_2)$
5	7	$1355 + 100(n_1 + n_2)$
6	8	$1555 + 100(n_1 + n_2)$
7	9	$1755 + 100(n_1 + n_2)$
8	10	$1955 + 100(n_1 + n_2)$

From the relative response of each component and the peak area in the SFC trace, the number of molecules n_i and the molar mass averages (M_n = number-average molar mass; M_w = weight-average molar mass) may be calculated:

$$n_i = A_i/RMR_i$$
$$M_n = \Sigma\, n_i M_i / \Sigma\, n_i$$
$$M_w = \Sigma\, n_i M_i^2 / \Sigma\, n_i M_i$$

As can be seen from the chromatograms, the lower oligomers of each homologuous series are separated very well by SFC. Therefore, the area of every peak may be determined quantitatively. As for the higher oligomers, where complete separation could not be achieved, quantification was carried out in the SEC mode. For each fraction a separate calibration graph of log M *vs.* retention time was used, based on the retention times of the lower oligomers, and extrapolated towards higher molar masses (see Fig. 5).

Using this approach, the molar mass averages of the fractions were calculated and are summarized in Table IV. In addition, from the concentration of the individual oligomers the average block length $n_1 + n_2$ with respect to ethylene oxide was determined.

In order to verify the accuracy of these calculations, an M_n value for the total sample was calculated and compared with M_n values obtained independently by SEC and NMR:

critical chromatography/SFC	$M_n = 870$
SEC (calibration with PEO)	$M_n = 940$
^{13}C NMR	$M_n = 1100$

Taking into account, that the quantification in SFC was based on increment schemes and extrapolation was necessary for the quantification of the higher-molar-mass oligomers, the results of two-dimensional separation critical chromatography *vs.* SFC are consistent with the SEC and NMR data.

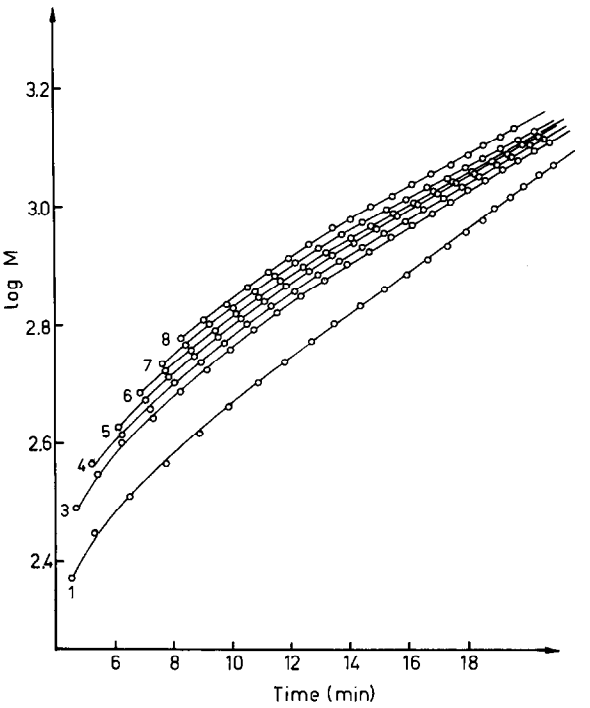


Fig. 5. SFC calibration graphs of log M *vs.* retention time for fractions 1–8.

For additional support of the results, another two-dimensional separation, but in this instance

TABLE IV

STRUCTURAL PARAMETERS OF THE BLOCK POLYMER FRACTIONS CALCULATED FROM SFC DATA, TWO-DIMENSIONAL SEPARATION CRITICAL CHROMATOGRAPHY *VS.* SFC

Fraction	$HO(EO)_{n_1}(PO)_m(EO)_{n_2}OH$		Composition	
	M_n	M_w	m	$n_1 + n_2$
1	640	770	3	10.3
3	940	1100	5	14.4
4	990	1110	6	14.1
5	1070	1170	7	14.7
6	1140	1220	8	15.0
7	1220	1290	9	15.4
8	1270	1320	10	15.2

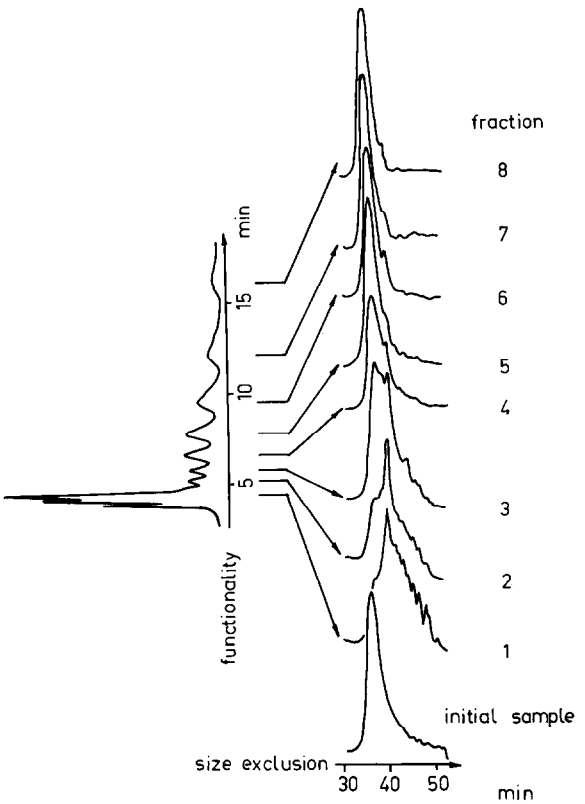


Fig. 6. Two-dimensional separation: critical chromatography *vs.* SEC of the block polymer.

critical chromatography *vs.* SEC, was performed. Again fractions were collected that were uniform with respect to the polypropylene oxide block (see Fig. 6). The fractions were subjected to SEC and the molar mass averages were calculated using a PEO calibration graph. Comparing these results with the values from the SFC experiments (see Tables IV and V), it was found that the M_w values of fractions 6–9 are significantly lower for the SFC experiments. This again might be due to poor resolution of the higher molar mass oligomers in SFC. However, comparing the calculated molar mass averages for the total sample, good agreement was obtained:

	M_n	M_w
critical chromatography *vs.* SFC	870	950
critical chromatography *vs.* SEC	830	1170

TABLE V

MOLAR MASS AVERAGES OF THE BLOCK POLYMER FRACTIONS CALCULATED FROM SEC DATA, TWO-DIMENSIONAL SEPARATION CRITICAL CHROMATOGRAPHY *VS.* SEC

Fraction	m	M_n	M_w
1	3	550	780
2	4	660	940
3	5	879	1050
4	6	900	1230
5	7	990	1500
6	8	1120	1680
7	9	1320	1780
8	10	1350	1820

CONCLUSIONS

Liquid chromatography at the critical point of adsorption has been shown to be a unique method for the separation of $A_nB_mA_n$ block polymers. Operating at the critical conditions of the first block the second block may be characterized according to the molar mass and block length. Using a second chromatographic method, preparatively separated fractions may be analysed with respect to the first block. Hence quantitative data on the molar mass distribution and the block length of the individual blocks may be obtained. Further investigations will focus on improving the accuracy and reproducibility of the chromatographic techniques. It would then be possible to determine block length distributions of the individual blocks.

REFERENCES

1 G. Glöckner, *Trends Anal. Chem.*, 7 (1988) 169.
2 S. Mori, Y. Uno and M. Suzuki, *Anal. Chem.*, 58 (1986) 303.
3 S. G. Entelis, V. V. Evreinov and A. V. Gorshkov, *Adv. Polym. Sci.*, 76 (1986) 129.
4 S. G. Entelis, V. V. Evrienov and A. I. Kuzaev, *Reactive Oligmers*, Khimiya, Moscow, 1988.
5 A. V. Gorshkov, V. V. Evreinov and S. G. Entelis, *Zh. Fiz. Khim.*, 59 (1985) 958.
6 A. V. Gorshkov, V. V. Verenikh and V. V. Evreinov, *Chromatographia*, 26 (1988) 338.

7 G. Schulz, H. Much, H. Krüger and C. Wehrstedt, *J. Liq. Chromatogr.*, 13 (1990) 1745.
8 A. V. Gorshkov, T. N. Prudskova, V. V. Guryakova and V. V. Evreinov, *Polym. Bull.*, 15 (1986) 465.
9 A. V. Gorshkov, H. Much, H. Becker, H. Pasch, V. V. Evreinov and S. G. Entelis, *J. Chromatogr.*, 523 (1990) 91.
10 H. Pasch, H. Much, G. Schulz and A. V. Gorshkov, *LC · GC Int.*, 5 (1992) 38.
11 H. Pasch, H. Krüger, H. Much and U. Just, *J. Chromatogr.*, 589 (1992) 295.
12 H. Pasch, H. Krüger, H. Much and U. Just, *Polymer*, (1992) in press.
13 R. G. Ackman, *J. Gas Chromatogr.*, 2 (1964) 173.