Note

Preparative fractionation of fluorescent-dye-labeled poly(ethylene oxide) by aqueous size-exclusion chromatography

S. J. MUMBY*, P. M. COTTS and T. P. RUSSELL

IBM Research Laboratory, 5600 Cottle Road, San Jose, CA 95193 (U.S.A.)

(Received October 25th, 1984)

Recently a considerable amount of interest has been shown in the use of aqueous size-exclusion chromatography (SEC) for the characterization of water-soluble polymers. Aqueous SEC may be applied to a variety of synthetic and natural polymers as evidenced by recent reviews[1-5] and texts[6,7] that have been published in this area.

Much of the current impetus in the field of aqueous SEC has been created by the introduction of several high-performance SEC columns. The columns used in this investigation were of the commercially available TSK-PW type (Toya Soda, Tokyo, Japan). The exact structure of the gel contained in these columns has not been disclosed; however, it is known[8] to contain $-CH_2CHOHCH_2O-$ groups. Many different types of polymers such as proteins[9], poly(vinyl pyrrolidones)[8] and poly(acrylic acid)[10] have been characterized using these columns, which may be used with either tetrahydrofuran[8] or methanol[11] replacing water as the mobile phase. It has also been demonstrated[10] that polyethylene glycols can be successfully characterized using these columns. This is particularly important since it is known that the ether linkages in polyethylene glycols or poly(ethylene oxides) bind strongly to the more established solid SEC packing materials.

We report here the results of the preparative fractionation and characterization of a fluorescent-dye-labeled poly(ethylene oxide) using TSK-PW aqueous SEC columns. Fractions of the labeled poly(ethylene oxide) are to be used in an investigation of polymer diffusion by means of the fluorescence redistribution after pattern photobleaching technique[12]. Since the polymer samples have been synthesized to contain one fluorescent dye group in the center of each molecule it is not anticipated that they will behave perceptibly different to unlabeled poly(ethylene oxide) during the fractionation procedure.

EXPERIMENTAL

Preparation

The poly(ethylene oxide) specimens used in this study were kindly supplied by Professor E. T. Samulski at the University of Connecticut. Polymerization of the ethylene oxide was accomplished in a manner identical to that discussed previously for propylene oxide[13]. The reaction was initiated using 4-diethanolamino-9-nitro-

benzofurazan with zinc hexacyanocobaltate as a catalyst. The reaction produces a linear polymer with one dye molecule centrally located on each chain.

Apparatus

Four TSK-PW columns were used in series. Two of these columns contained 5000 PW grade Spherogel with the others packed with the 4000 PW and 3000 PW type material. All four columns were housed on top of a Waters Model GPC I liquid/gel permeation chromatograph (Waters Assoc., Milford, MA, U.S.A.) and were operated under ambient conditions. The mobile phase was high purity water (Burdick and Jackson, Muskegon, MI, U.S.A.) prefiltered through a 5-μm membrane filter and delivered at a rate of 1 ml/min by a Waters 6000 pump. Injections were made into the system using a Waters WISP Model 710B. Injection volumes were 500 μl of 2% (w/v) polymer solution for fractionation and 50 μl of 1% (w/v) polymer solution for calibration or characterization. Total elution time for the unfractionated polymer sample under these conditions was approximately 50 min. Elution of polymer from the columns was monitored by a dual detection system consisting of a Waters differential refractometer and a variable-wavelength detector with monochromator (Models SF 770 and GM 770, respectively, Kratos, NJ, U.S.A.) set to 498 nm. Fractions were collected by a programmable fraction collector (Model 201, Gilson, Villiers Le Bel, France). Progress of fractionation was continuously monitored using a Waters data module. The outputs of both the differential refractometer and variable-wavelength detector were also connected to a personal computer (Hewlett-Packard 87, Palo Alto, CA, U.S.A.) which was used in conjunction with Nelson Analytical chromatography software to determine the number- (M_n) and weight-average (M_w) molecular weights. By definition,

$$M_n = \frac{\sum_t n_t M_t}{\sum_t n_t} = \frac{\sum_t w_t}{\sum_t w_t/M_t}$$

$$M_w = \frac{\sum_t w_t M_t}{\sum_t w_t} = \frac{\sum_t n_t M_t^2}{\sum_t n_t M_t}$$

where n_t and w_t are the number and weight fractions, respectively, of species eluting at time t with molecular weight M_t. The columns were calibrated (see Fig. 1) using a series of narrow molecular weight distribution poly(ethylene oxide) standards.

RESULTS AND DISCUSSION

Use of both differential refractive index (RI) and visible-absorption detectors is essential during the fractionation of these labeled polymers. The RI detector responds to the presence of any polymeric material, whereas the visible-absorption detector responds only to the fluorescent chromophore. The RI detector measures
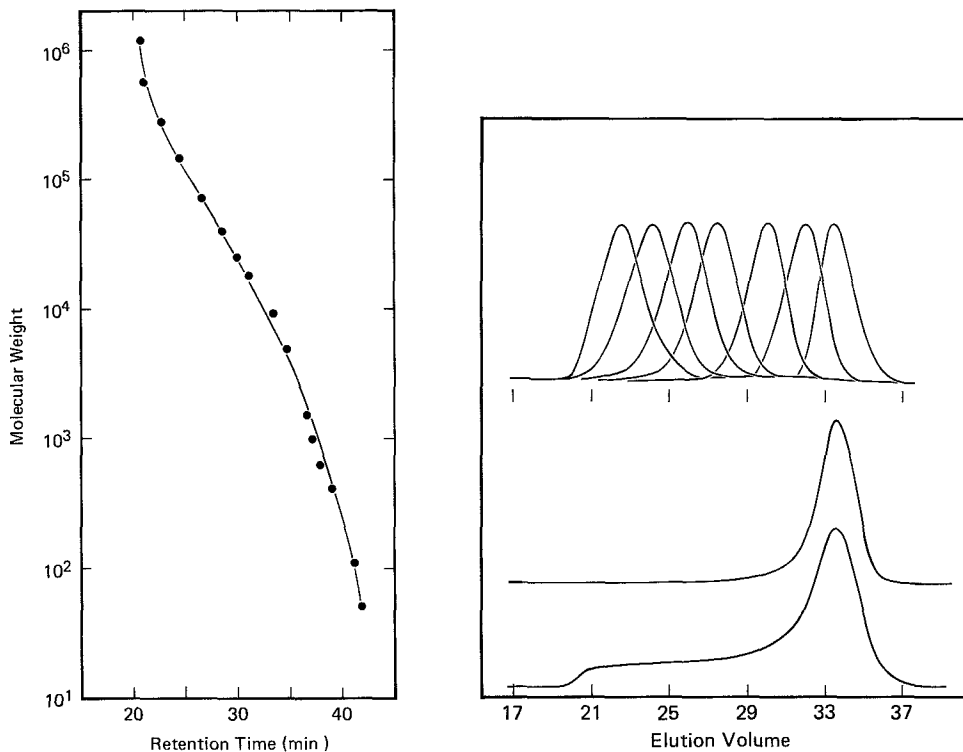
Fig. 1. Calibration curve of the SEC columns for poly(ethylene oxide) in high-purity water.

Fig. 2. Chromatograms of the initial poly(ethylene oxide) specimen as measured by the refractive index detector (lower) and visible-absorption detector (middle). The upper series of chromatograms are those of several fractions. (see text). Elution volume in ml.

the weight fraction w_t of polymer eluting at time t, while the visible absorption detector measures the number fraction n_t eluting at time t. Since

$$w_t = n_t M_t$$

where M_t may be determined from the calibration curve, the same distribution should be obtained from either chromatogram. While this was observed for the narrow fractions collected, the molecular weight averages obtained for the whole polymer were very different, as shown in Table I. While this could be due to unlabeled polymer in the high-molecular-weight tail, we do not believe this was the case since fractions collected at very high molecular weights did show absorption at 498 nm. The visible-absorption detector is very insensitive to the small weight fractions of high-molecular-weight species and can yield erroneously low and narrow distributions. Fig. 2 illustrates the outputs of the two detectors where the response is shown for the unfractionated polymer. The molecular weight distribution of the labeled polymer appears quite narrow from the chromatogram produced by the visible-absorption detector. However, from the response of the differential RI detector it is clear that

TABLE I

NUMBER-AVERAGE MOLECULAR WEIGHT, M_n, WEIGHT-AVERAGE MOLECULAR WEIGHT, M_w, AND HETEROGENEITY INDEX, $D = M_w/M_n$, OF THE FRACTIONS OF POLY-(ETHYLENE OXIDE) PREPARED AND THE STARTING SAMPLE (I)

All results shown are those obtained with the refractive index detector with the exception of that marked UV which was obtained with the UV/visible detector.

Fraction	M_n	M_w	D
I(UV)	6800	16,500	2.43
I	11,000	116,000	11.0
3	1,090,000	1,189,000	1.08
4	858,000	981,000	1.14
5	606,000	748,000	1.24
6	460,000	598,000	1.30
7	329,000	431,000	1.31
8	246,000	322,000	1.31
9	173,000	217,000	1.25
10	124,000	157,000	1.27
11	89,100	103,000	1.16
12	68,100	77,300	1.13
13	50,500	57,100	1.13
14	36,100	40,400	1.12
15	27,200	30,100	1.11
16	21,900	24,300	1.11
17	16,200	18,000	1.11
18	11,900	13,200	1.11
19	8350	9240	1.11
20	7590	8190	1.08
21	5760	6470	1.12
22	4920	5700	1.16
23	3620	4190	1.16
24	2550	2990	1.17

the heterogeneity index is substantially increased by the presence of a high-molecular tail.The response of the differential RI detector for several fractions (fraction numbers 7, 9, 11, 13, 16, 18 and 21 in Table I) are normalized and superimposed in Fig. 2 for illustrative purposes. It is clear from Fig. 2, and the data in Table I, that this aqueous SEC system described can be successfully used to fractionate an initial poly-(ethylene oxide) sample with a broad distribution of molecular weight into several fractions having substantially lower molecular weight dispersities and a wide range of average molecular weights. Furthermore, after somewhat in excess of 2 g of polymer had been passed through the columns no decrease in efficiency was detected and the measured plate count (9000 plates/m) was approximately equal to the initial value (9100 plates/m).

From the results shown it is evident that preparative SEC can easily be accomplished with these newly developed columns without affecting their overall efficiency. More importantly, for the polymer fractionated in this work, the necessity of utilizing both RI and visible-absorption detectors in series has been shown. Use of only the visible-absorption detector would have led to the erroneous conclusion that there was no polymer of molecular weight higher than *ca.* 100,000 to be fractionated, and that the polydispersity of the starting material was low.

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