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Composition analysis of poly(ethylene glycol)–poly(L-lactide) diblock copolymer studied by two-dimensional column chromatography

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Abstract

We used two-dimensional column chromatography to analyze the composition of a sample of presumably a diblock copolymer of poly(ethylene glycol) (PEG) and poly(L-lactide) synthesized from monomethoxy-terminated PEG. The first dimension of the separation is phase fluctuation chromatography to prepare fractions that contain various components of the copolymer in different ratios. The second dimension is size-exclusion chromatography, NMR, and HPLC at the critical condition of PEG. The PEG initiator has small amounts of diol-terminated dimeric components. We found that the copolymer sample contains a triblock copolymer and low-molecular-mass components in addition to the main part of the diblock copolymer. The SEC chromatograms show that the main part consists of two components with distinct peak lengths for the PLLA block. The low-molecular-mass components have a broad distribution in chemical composition. Phase fluctuation chromatography enriched the triblock copolymer and the diblock copolymer with the longer PLLA block in early fractions when the column was packed with carboxymethyl-modified porous silica. When the porous medium was PLLA-grafted silica, size exclusion dominated, but the low-molecular-mass components were separated according to their chemical composition.

Keywords: Phase fluctuation chromatography; Poly(ethylene glycol); Poly(L-lactide); Diblock copolymer

1. Introduction

Block copolymers prepared from monofunctional poly(ethylene glycol) (PEG) are widely used in drug delivery systems [1,2] and for other applications [3]. It is difficult to prepare copolymer samples free from distributions in the number of blocks or the length of each block. Copolymer samples with as narrow distributions as possible are desired for the optimal performance of the delivery system [4]. Earlier, we used two-dimensional (2D) chromatography to analyze the molecular mass distribution and the terminal chemistry of presumably monomethoxy-terminated PEG (MePEG) [5], a typical monofunctional PEG often used to build a diblock copolymer. Using columns of a specific pore size in size-exclusion

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chromatography (SEC) revealed that commercial MePEG samples contain dimeric and other multimeric components. Their mass fraction was 8 to 20%, depending on the source. The presence of multimeric components leads to copolymers with a PEG block twice and three times as long as the main components. Furthermore, if the dimeric components have diol terminals, a triblock copolymer will be a part of the final copolymer. The first dimension of the 2D separation was high osmotic pressure chromatography [6–8] to prepare fractions that contain these components in different ratios. The second dimension was SEC and high-resolution NMR. Decomposition of the SEC chromatogram coupled with isolation of the peak ascribed to methoxy terminals in the NMR spectrum in each of the separated fractions led to the unique determination of the terminal chemistry of the components. We found that the main, monomeric component is monomethoxy-terminated and the dimeric and other multimeric components are nearly pure diols.

The present contribution builds upon our findings on the MePEG initiator. We applied 2D chromatography to a supposedly diblock copolymer of PEG–PLLA prepared from one of the samples of MePEG investigated earlier. The first dimension is phase fluctuation chromatography (PFC) [9–11] to prepare fractions that contain various components in different ratios. The preparative separation is based on segregation of a concentrated, heterogeneous solution of the copolymer with respect to the chemical composition between the stationary phase and the mobile phase. The stationary phase will be filled with domains that contain components preferred by the pore surface. Within the column, segregation of the solution is repeated at each theoretical plate, enriching the early eluent with components strongly rejected by the pore, i.e. by the surface interaction and/or size exclusion. The preparative separation produced a sufficient amount of polymer in each fraction for analysis by SEC and NMR in the second dimension. We also used HPLC [12–17] to investigate the length distribution of the PLLA block in each fraction. Regular two-dimensional column chromatography [18] does not allow analysis of each fraction by the three methods in the second dimension.

In our preceding separation study of PEG–PLLA copolymers [11], we used a carboxymethyl-modified stationary phase and a PLLA-grafted stationary phase, among others. The carboxymethyl surface retained PEG-rich components, resulting in early elution of lactate-rich components. In contrast, the PLLA surface retained lactate-rich components longer. Our focus was on the overall chemical composition of the separated fractions. We did not pay attention to possible complications that may arise from the impurities of the PEG initiator and other side reactions.

There was another attempt [15] to analyze the composition heterogeneity in a diblock copolymer. In the method, the diblock copolymer was separated first by HPLC, at the critical condition of one of the blocks, according to the length of the other block, followed by mass spectrometry of each fraction. This method allows one to find an exact correlation between the two block lengths, if the sample only contains a diblock copolymer. The latter may not be the case, as we demonstrate in the present article.

2. Experimental

2.1. Materials

Monomethoxy-terminated poly(ethylene glycol) (MePEG) was purchased from Aldrich (USA; lot No. 11229PG). L-Lactide (LLA) from Purac Biochem (Netherlands) and tin(II) 4-ethylhexanoate [4-$Sn(Oct)_2$] from Nacalai Tesque (Japan) were used to synthesize the diblock copolymer poly(ethylene glycol)–poly(L-lactide) (PEG–PLLA). 1,4-Dioxane and deuterated benzene were from Acros (USA). Tetrahydrofuran and acetonitrile were from Fisher Scientific (USA).

We used three columns (CML75B, PLLA170B, and PLLA350B) from those used in our preceding study [11]. The base packing materials were controlled pore glasses (CPG) obtained from CPG. The porosimetry results before chemical modification of the surface are listed in Table 1. The surface silanol of CML75B was modified with *N*-[(carboxymethyl)oxyacetyl]-3-aminopropylsilanetrioxy. Brushes of PLLA chains were grown on the surface silanol of porous silica to prepare PLLA170B and PLLA350B. Details are given in Ref. [11]. Briefly,

Table 1
Characteristics of controlled pore glasses

Code	Pore diameter (Å)	Particle size (mesh)	Surface area (m^2/g)	Pore volume (mL/g)	Loading (g/g CPG)	DP[a]
CML75B	82	120/200	224.5	0.37	–	–
PLLA170B	182	120/200	112.7	0.97	0.163	19
PLLA 350B	343	120/200	67.5	0.97	0.070	14

[a] Degree of polymerization of PLLA brushes.

the surface silanols of CPG initiated ring-opening polymerization of the cyclic dimer, L-lactide, in *p*-xylene with 2-$Sn(Oct)_2$ (Aldrich) as catalyst. Unreacted silanols were end-capped with trimethylmonomethoxysilane. The surface modification was characterized quantitatively by infrared absorption spectroscopy for a packed bed of PLLA-grafted CPG immersed in carbon tetrachloride. The latter solvent is free of hydrogen and nearly index-matched with silica. The PLLA loading and the degree of polymerization (DP) of the grafted PLLA thus estimated are listed in Table 1.

2.2. Preparation of PEG–PLLA

The synthesis of PEG–PLLA is described elsewhere [11]. In brief, the ring-opening polymerization of L-lactide with a hydroxy terminal of MePEG (91% monomethoxy-terminated) as a macroinitiator led to a presumably diblock copolymer. The copolymer was then dissolved in chloroform and precipitated in diethyl ether. This sample of PEG–PLLA was redissolved in chloroform and filtered through a paper filter (Whatman 1; USA). The precipitate formed by adding excess diethyl ether was recovered by filtration (Whatman 1). The average mole fraction of lactate decreased from 0.164 to 0.112 in the last step, as estimated by NMR analysis. The decrease occurred mostly in the PLLA homopolymer as evidenced by SEC.

2.3. Phase fluctuation chromatography

Phase fluctuation chromatography (PFC) was performed on PEG–PLLA in the same way as reported previously [11]. Briefly, a concentrated solution of PEG–PLLA in dioxane was injected at 0.2 mL/min onto a column (300×3.9 mm) packed with CML75B, PLLA170B, or PLLA350B until the whole column was filled with the solution. The concentration was 30 wt% for the CML75B and PLLA170B columns and 25 wt% for the PLLA350B column. High concentrations are required for optimal separation [10]. The columns filled with the polymer solution were eluted by the pure solvent, and fractions were collected by counting the drops of the eluent. In separations with CML75B and PLLA170B, 20 drops each were collected in fractions 1 to 10; 40 drops in fractions 11 and 12; 100 drops in fractions 13 and 14; and 300 drops in fractions 15 and 16. In the separation with PLLA350B, 20 drops were collected in fractions 1 and 2; 10 drops in fractions 3 to 17; 20 drops in fractions 18 to 22; 40 drops in fractions 23 to 25; and 520 drops in fraction 26.

2.4. Size exclusion chromatography (SEC)

The original PEG–PLLA and the fractions separated in PFC were analyzed by SEC. The chromatographic system contains a Waters 510 pump and a Waters 410 refractive index detector (35 °C). We primarily used a set of two columns with a fixed pore size (500 Å; 10×250 mm; Jordi Associates, Bellingham, MA, USA). An inline filter (0.2 μm; stainless steel) was placed before the columns. The column packing materials (diameter 5 μm) were not prepared by cross-linking polystyrene with divinylbenzene, but rather by cross-linking (mostly) divinylbenzene. For comparison purposes only, we also used another set of two mixed-bed columns of the same dimensions from the same manufacturer. The columns were thermostatted at 35 °C. The mobile phase was THF at 1 mL/min. The 500 Å pore columns were calibrated with PEG standards (Scientific Polymer Products; Ontario, NY, USA) of molecular mass from $1.43 \cdot 10^3$ to $4.10 \cdot 10^4$ g/mol. The mixed-bed columns were calibrated with PEG standards of

molecular mass from $1.43 \cdot 10^3$ to $2.50 \cdot 10^5$ g/mol. Each chromatogram was corrected for flow-rate fluctuations by the solvent peak at around 29.0 min.

2.5. NMR analysis

A Bruker DPX-300 spectrometer was used to obtain proton NMR spectra for the original PEG–PLLA and the separated fractions. The solvent was deuterated chloroform. The average mole fraction of lactate in each sample was calculated as $x_{\mathrm{LLA}} = I_{5.2}/(I_{5.2} + I_{3.6}/4)$, where $I_{5.2}$ is the integral of the methine protons in lactate at around 5.2 ppm, and $I_{3.6}$ is the integral of the methylene protons in oxyethylene at around 3.6 ppm.

2.6. HPLC at the critical condition of PEG

Some of the separated fractions were analyzed by HPLC at the critical condition of PEG [12–15]. We followed the method devised by Lee et al. [15]. At the critical condition for PEG, the size exclusion on the PEG block and the attractive interaction of the stationary phase with the block in a given mobile phase compensate each other, thereby masking the PEG block from recognition by the stationary phase. As a result, retention of the copolymer is determined solely by the PLLA block length [15–17]. A column packed with octadecyl-modified silica gel (Luna C_{18}, Phenomenex; 4.6×250 mm) and an isocratic mobile phase of acetonitrile–water (60:40, v/v) at 68 °C provided the PEG block with the necessary critical conditions. In this condition, the copolymer with a longer PLLA block elutes later because of favorable interactions of the block with the stationary phase. The flow-rate was 1.0 mL/min. A 20 μL solution with a concentration between 0.6 and 1.0 wt% was injected. The detector was a refractive index detector at 35 °C.

3. Results and discussion

3.1. Chemical composition of the block copolymer

The SEC chromatogram of the original PEG–PLLA obtained with the 500 Å pore columns (solid line in Fig. 1) consists of four components. Component 1 is eluted at around 14.5 min on the left shoulder of the main peak. The latter can be decomposed into components 2 and 3 eluting at around 15.2 and 15.8 min. Component 4 is the tail portion of the main peak.

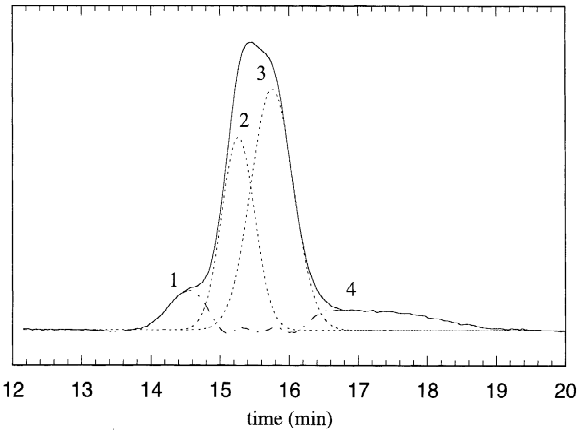

Fig. 1. The SEC chromatogram (———) of the original PEG–PLLA was decomposed into four components by curve fitting. The main peak was fitted with two Gaussians (- - -; 2 and 3). The residual (– · –) has higher- and lower-molecular-mass components (1 and 4).

We also analyzed the same sample with the mixed-bed columns as we did earlier for MePEG [5], but component 1 appeared only as a small shoulder on the leading edge of the main peak. Components 2 and 3 were not distinguishable. Component 4 was almost immersed in the baseline. The resolution of the mixed-bed columns is not sufficient to characterize the molecular mass distribution of PEG–PLLA.

We decomposed the normalized chromatogram $y(t)$ into two steps. In the first step, we fitted a good portion of the main peak by a sum of two Gaussians, $A_2 f(t;t_2,\sigma_2) + A_3 f(t;t_3,\sigma_3)$, with $t_2 < t_3$, where $f(t;t_i,\sigma_i)$ is a normalized Gaussian profile with a mean of t_i and a standard deviation of σ_i for component i:

$$f(t;t_i,\sigma_i) \equiv (2\pi\sigma_i^2)^{-1/2} \exp[-(t-t_i)^2/(2\sigma_i^2)] \qquad (1)$$

and A_i is the area of the component. The two Gaussian functions are shown as dashed lines in Fig. 1. The standard deviation is $\sigma_2 = 0.237$ min and $\sigma_3 = 0.305$ min. In the second step, we displayed the residual, $y(t) - [A_2 f(t;t_2,\sigma_2) + A_3 f(t;t_3,\sigma_3)]$, shown

Table 2
Components in PEG–PLLA

Component	Area	$M_p/10^2$ (g/mol)[a]	$M_{PLLA}/10^2$ (g/mol)[b]	DP_{PLLA}[c]	x_{LLA}[d]
1	0.070	138.9	18.3	25	0.085
2	0.316	86.4	21.4	30	0.185
3	0.507	63.6	4.4	6.4	0.044
4	0.107	–	–	–	0.253

[a] Peak molecular mass with reference to PEG.
[b] Molecular mass of PLLA blocks with reference to PLLA.
[c] Degree of polymerization of PLLA blocks.
[d] Mole fraction of lactate units.

as the dash-dotted line. The relative areas A_1 and A_4 for the first and fourth components were calculated as the areas under the peak to the left of the first zero point and under the peak to the right of the last zero point, respectively. The peak retention time of component 1 was obtained as the peak time in the residual plot. The residual curve for component 4 was too broad to evaluate its peak retention time reliably. Table 2 lists the area and the peak molecular mass (M_p) with respect to PEG standards for each component.

In our preceding study [5], we analyzed the chromatogram of MePEG (from the same bottle as that used for copolymer synthesis) by decomposing it into different components as described above. Our findings relevant to the present contribution are summarized in Table 3. The sample has a dimeric component, PEG_d, and low-molecular-mass components, in addition to the main component, PEG_m. The rest is trimeric and tetrameric components with M_p three and four times as high as that of PEG_m and diol-terminated. We can neglect it here because its mass fraction is as small as 0.008.

We compare the M_p of PEG_d and PEG_m with the M_p of components 1, 2, and 3 of PEG–PLLA. The difference ($2.35\cdot10^3$ g/mol) between PEG_d and component 1 is similar to the difference ($2.86\cdot10^3$ g/mol) between PEG_m and component 2. The M_p of component 3 is slightly greater than that of PEG_m. Furthermore, the ratio of the mass fraction of PEG_d to that of PEG_m is close to $A_1/(A_2+A_3)$. From these results, we conclude that component 1 was made from PEG_d, and components 2 and 3 were made from PEG_m with different lengths of PLLA chains attached. We consider that component 4 is a mixture of PEG homopolymer, PLLA homopolymer, and their block copolymer.

From the M_p of the PEG block and the PLLA block, the chemical composition, represented as the mole fraction of lactate, x_{LLA}, can be estimated for each component. We estimate the M_p of the PLLA block by subtracting the M_p of the PEG block from the M_p of the whole copolymer (the result is with reference to PEG standards) and then convert it into the true molecular mass.

To find the conversion formula, we obtained the PEG-equivalent molecular mass for three fractions of PLLA homopolymer with a known, true molecular mass (PolySciences). Their weight-average molecular masses (M_w) are $1.09\cdot10^5$, $5.70\cdot10^4$, and $2.05\cdot10^3$ g/mol. We analyzed them by using the two mixed-bed columns. The M_w of PLLA with refer-

Table 3
Major components in MePEG

Component	Mass fraction	M_p[a]$/10^2$ (g/mol)	Structure
Monomeric (PEG_m)	0.825	57.8	$CH_3–(OC_2H_4)_{131}–OH$
Dimeric (PEG_d)	0.079	115.4	$H–(OC_2H_4)_{262}–OH$
Low molecular mass	0.087	–	$CH_3–(OC_2H_4)_x–OH$

[a] Peak molecular mass.

ence to PEG standards thus estimated was plotted as a function of its true M_w (not shown). The curve fitting resulted in M_w (with respect to PEG) = M_w (true) × 1.33; $R^2 = 0.993$. The same relationship should hold for any measure of the molecular mass for PLLA. The true molecular mass of the PLLA block, M_{PLLA}, thus estimated is listed in Table 2 together with the degree of polymerization of the PLLA block, DP_{PLLA}.

Once the molecular mass of the PLLA blocks for components 1, 2, and 3 in PEG–PLLA have been estimated, x_{LLA} in each component is calculated as

$$x_{LLA,i} = \frac{M_{PLLA,i}/72}{M_{PEG,i}/44 + M_{PLLA,i}/72} \quad (i = 1,2,3) \qquad (2)$$

where PEG,1 = PEG_d, PEG,2 = PEG,3 = PEG_m, and $M_{PLLA,i}$ is the true molecular mass of the PLLA block in component *i*. The values of $x_{LLA,i}$ ($i = 1,2,3$) thus estimated are listed in Table 2.

Because of the diol termination of PEG_d, component 1 is a PLLA–PEG–PLLA triblock copolymer with an average block length (DP) of PLLA = 25/2 = 12.5. Components 2 and 3 are diblock copolymers. Their weighted average of the PLLA block length is 15.5. The nearly equal length of the PLLA block between the triblock copolymer and the diblock copolymer leads us to two conclusions. (1) The growth mechanism of the PLLA block is common to PEG_d and PEG_m. The OH terminals of PEG_d and PEG_m have the same reactivity. Lactide monomers attach to different components of PEG at the same rate. (2) Transesterification may have occurred during polymerization, especially at a late stage, resulting in components 2 and 3 for the diblock copolymer.

Eq. (2) does not apply to component 4. Its average lactate content, $x_{LLA,4}$, was estimated as follows. The peak area *A* of the SEC chromatogram of a given polymer above the baseline is proportional to the product of dn/dc, the specific refractive index (R.I.) increment of the polymer, and the mass of polymer injected. When the polymer sample consists of several components with different compositions, the mass of component *i* is given by the ratio of the area A_i of the component in the chromatogram to its dn/dc. Therefore, the following relationship holds:

$$\sum_{i=1}^{4} \frac{A_i}{(dn/dc)_i} = \frac{1}{(dn/dc)_0} \qquad (3)$$

where A_i is normalized to $A_1 + A_2 + A_3 + A_4 = 1$, and $(dn/dc)_i$ and $(dn/dc)_0$ are the specific R.I. increments of the *i*th component and the diblock copolymer sample injected into the SEC columns, respectively. We assume the following relationship for $(dn/dc)_i$:

$$(dn/dc)_i = w_{LLA,i}(dn/dc)_{PLLA} + (1 - w_{LLA,i})(dn/dc)_{PEG} \quad (i = 0,1,2,3,4) \qquad (4)$$

where $w_{LLA,i}$ is the mass fraction of lactate in component *i*, and $(dn/dc)_{PLLA}$ and $(dn/dc)_{PEG}$ are the specific R.I. increments of the PLLA homopolymer and the PEG homopolymer, respectively. This relationship is exact if the blocks are sufficiently long. We introduce $\nu \equiv (dn/dc)_{PEG}/(dn/dc)_{PLLA}$. Then, Eq. (3) is rewritten as

$$\sum_{i=1}^{4} \frac{A_i}{w_{LLA,i} + (1 - w_{LLA,i})\nu} = \frac{1}{w_{LLA,0} + (1 - w_{LLA,0})\nu} \qquad (5)$$

To estimate ν, we obtained SEC chromatograms for PEG homopolymer solutions and PLLA homopolymer solutions with known concentrations at a fixed injection volume. From the ratio of the peak areas and the concentrations, we obtained $\nu = 1.25$. This means that the R.I. of PLLA is between that of THF and that of PEG.

The following equation links the mass fraction of lactate to the mole fraction of lactate for each component as well as for the copolymer sample injected:

$$\frac{72x_{LLA,i}}{72x_{LLA,i} + 44(1 - x_{LLA,i})} = w_{LLA,i} \quad (i = 0,1,2,3,4) \qquad (6)$$

The $x_{LLA,0}$ of the copolymer sample was determined from its NMR spectra. Together with $x_{LLA,i}$ ($i = 1,2,3$) in Table 2, we have $w_{LLA,i}$ for $i = 0,1,2,3$. Then, we can solve Eq. (5) for one unknown, $w_{LLA,4}$, from which we obtained $x_{LLA,4}$ (Table 2). The high value of $x_{LLA,4}$ indicates the presence of a large amount of PLLA homopolymer, even after its removal by precipitation in ether.

3.2. Separation of PEG–PLLA by PFC

From what we have learned from the decomposition of the SEC chromatogram for the original PEG–PLLA, we can list what is expected in the PFC separation of the copolymer sample using surface-modified porous materials. (1) Component 1 and the main peak (components 2 and 3) have nearly the same average chemical composition. Separation of component 1 from the rest will be based on the total molecular mass of the polymer, not on the chemistry. (2) Components 2 and 3 have different chemical compositions. The higher-molecular-mass component has a greater lactate content. Separation of the two components will be based both on the total molecular mass and the chemical composition. (3) Separation of component 4 from the rest may be based on size exclusion, but the separation within component 4 will be based on the chemical composition. Its molecular mass will be too small to effectively distinguish different components by molecular mass itself. (4) Separation within each of components 1, 2, and 3 may occur. Because of the narrow distribution of the PEG block length, the separation will be primarily with respect to the PLLA block length. It will be based both on size exclusion and on surface interaction.

3.2.1. Overall composition

Each separated fraction was analyzed using NMR. Fig. 2 shows the values of x_{LLA} thus obtained, plotted as a function of the cumulative number of drops collected since the detection of the first polymer in the eluent. Each point corresponds to one of the fractions. The results are similar to those we reported earlier for a slightly different PEG–PLLA [11]. The overall trend of x_{LLA} was governed by the interaction with the surface. With the CML75B column, x_{LLA} decreased monotonically, except for the last two fractions. The carboxyl surface retained ethylene glycol-rich components longer. The opposite trend was observed in the separations with the PLLA surface. An exception was observed in the early fractions with a decreasing x_{LLA}. We consider that the size-exclusion effect dominated the surface effect among the polymer molecules in the early fractions [11]. Components with a longer PLLA block, although chemically preferred by the surface, were excluded by the pore.

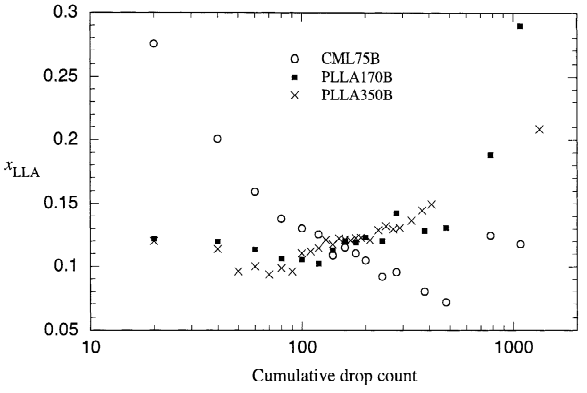

Fig. 2. Mole fraction of lactate, x_{LLA}, plotted as a function of the cumulative drop count for fractions obtained in the separations with CML75B (circles), PLLA170B (squares), and PLLA350B (crosses).

3.2.2. SEC analysis of separated fractions

Fig. 3a–c compare normalized SEC chromatograms for fractions obtained in separations with the CML75B, PLLA170B, and PLLA350B columns, respectively. These chromatograms clearly show that at least four components are present in the diblock copolymer. Their positions hardly move from fraction to fraction or from separation to separation. The relative abundance of each component is different from fraction to fraction. This result indicates that the two components of the main peak in the SEC chromatogram of the original PEG–PLLA are not artifacts of the curve fitting.

The chromatograms of fraction 1 have a relatively large area in the first two components in all of the separations. Each separation shows a different degree of removal of component 3 in fraction 1. CML75B is the best in that respect. With increasing fraction number, component 1 diminishes in height, and then the peak shifts from component 2 to component 3, common to all separations, indicating that the size-exclusion effect was dominant in the separation of component 1 from the rest and in the separation of components 2 and 3 within the main peak. Also, notice the difference in component 4. It was enriched in the early fractions with CML75B, whereas it was in the late fractions with PLLA170B and PLLA350B.

Component 4 has the lowest molecular mass and the highest lactate content (Table 2). Enrichment of

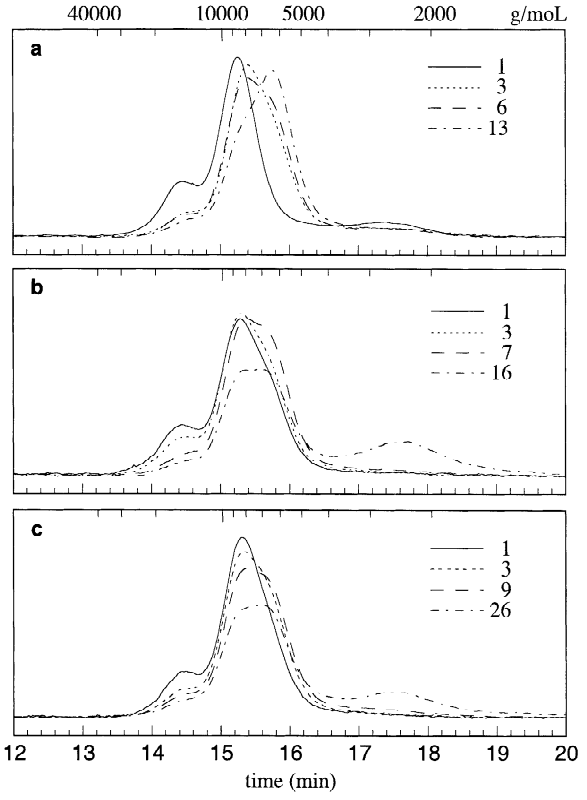

Fig. 3. SEC chromatograms for some of the fractions obtained in PFC separation with (a) CML75B, (b) PLLA170B, and (c) PLLA350B. The top axis indicates the molecular mass of the PEG standards. The fraction numbers are indicated.

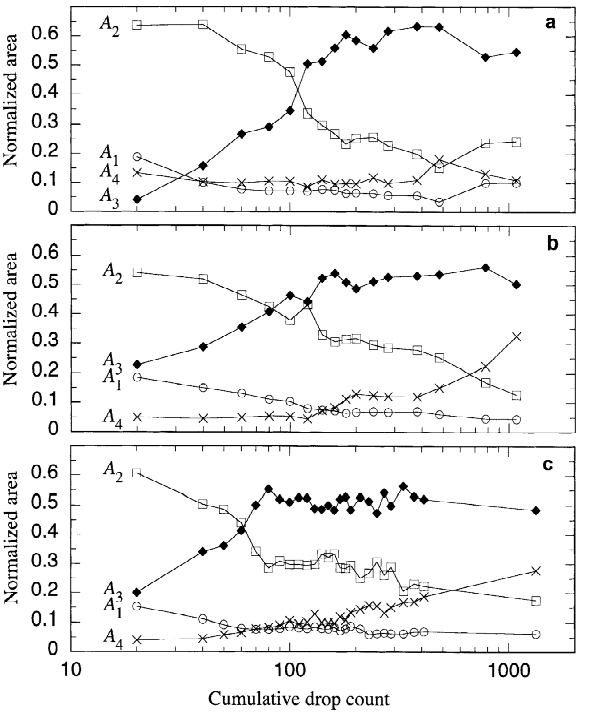

Fig. 4. Areas A_1 (circles), A_2 (squares), A_3 (rhombuses), and A_4 (crosses) of the four components in the SEC chromatogram for each of the fractions obtained in PFC separation plotted as a function of the cumulative drop count. (a) CML75B, (b) PLLA170B, and (c) PLLA350B. Lines are to guide the eye.

component 4 in fraction 1 of the CML75B separation indicates that the CML surface excludes lactate-rich, low-molecular-mass components against the size-exclusion effect. In contrast, PLLA columns efficiently enriched component 4 in late fractions due to the cooperation of the surface interaction and size exclusion.

3.2.3. Change of composition in separated fractions

The SEC chromatogram for each fraction was decomposed into four components as described earlier for the original PEG–PLLA. Fig. 4 shows the areas of individual components in all of the separated fractions as a function of the cumulative drop count. The trends of A_1, A_2, A_3, and A_4 are consistent with the changes in the chromatogram observed in Fig. 3.

In all separations, A_1 decreased nearly monotonically. In Fig. 4a, there is an increase in A_1 in the last two fractions. PLLA170B maintained a large A_1 in the early fractions, indicating the strongest size exclusion of component 1 by the pore. This is corroborated by the difference in the injection volumes in the three separations. The mass of solution injected was 2.67, 1.77, and 2.67 g in separations with CML75B, PLLA170B, and PLLA350B, respectively. The small injection volume indicates that the polymer in the front end of the transported solution stayed mostly in the mobile phase. The small pore size of PLLA170B after growing the polymer brush and the absence of attractive surface–polymer interactions such as those present in CML75B contributed to the exclusion of component 1 by the pores.

Separation between components 2 and 3 is best with CML75B. In terms of A_2, the other two columns do not have the counterparts of fractions 1

and 2 obtained with CML75B. The decrease in A_2 and the increase in A_3 are nearly persistent in all separations, except for some irregularities in late fractions. A cooperative effect of size exclusion and surface repulsion on component 2 in CML75B is evident. With the PLLA surface, especially with PLLA170B, size exclusion was cancelled, more or less, by the surface effect, resulting in a poorer enrichment of component 2 in the early fractions.

Partitioning of component 4 to the stationary phase was more effective with the PLLA surface, especially with the smaller pores, compared to CML75B. With PLLA170B, A_4 increased stepwise to reach a high value in the last fraction. With PLLA350B, the increase was gradual. With CML75B, A_4 was high in the first fraction and some late fractions, but not much different from that of the original copolymer in the other fractions.

In all of the separations, we observed a plateau region in the middle fractions for all the components. This was caused by overloading of the sample solution in PFC, which was needed for the uniform transport of a viscous solution through a packed bed of CPG. The plateau is most obvious in the separation with PLLA350B and has the same composition as that of the original copolymer (Table 2). Fractions 15 and 16 in the CML75B separation have almost the same composition as that of the original copolymer. We consider that a small amount of copolymer was adsorbed on the surface of the CML75B column and eluted at the end.

3.2.4. Peak retention times of components 1, 2, and 3

In Fig. 5, peak retention times t_1, t_2, and t_3 of the first three components are plotted as a function of the cumulative drop count for the three separations. This plot allows evaluation of the separation within each component.

All of the separations show a general trend of an increase in t_1, indicating that size exclusion dominated in the separation within component 1. This trend was most distinct in the separation with CML75B. Again, the cooperative effect is evident for partitioning of higher-molecular-mass components with a longer PLLA block to the mobile phase by both size exclusion and surface repulsion. The size-exclusion effect was less obvious for t_2 and t_3.

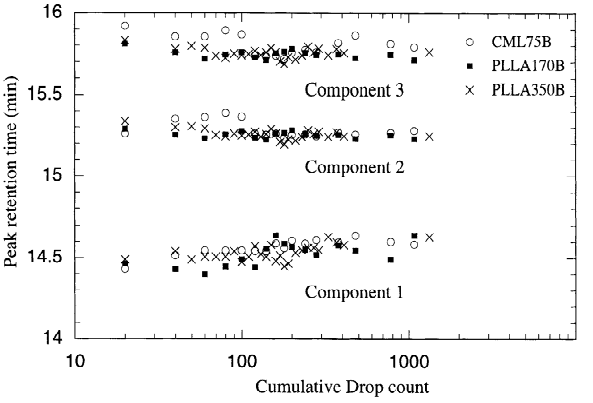

Fig. 5. Peak retention times t_1, t_2, and t_3 of the three components, plotted as a function of the cumulative drop count for separations with CML75B (circles), PLLA170B (squares), and PLLA350B (crosses).

With PLLA170B and PLLA350B, t_2 and t_3 were almost unchanged, except for a slight decrease in early fractions. In these fractions, ethylene glycol-rich components with a lower molecular mass eluted earlier, being repelled by the PLLA surface. With CML75B, t_2 and t_3 were longer in fractions 1 to 5 compared with the other fractions. This result can only be explained by the presence of a PEG–PLLA component with a short PEG block. This component will have a lower molecular mass and therefore a higher lactate content, which is selectively rejected by the CML surface to elute as early fractions.

3.2.5. x_{LLA} of the fourth component in the separated fractions

Assuming that the chemical compositions of the first three components are the same as those in the original copolymer, we calculated $x_{LLA,4}$ in each fraction in the same way as for the original copolymer. The results for the three separations are plotted as a function of the cumulative drop count in Fig. 6. The data are scattered and exceed the allowed range of $0 < x_{LLA,4} < 1$, probably because of the errors in the curve fitting and the assumptions made. CML75B separation shows a decreasing trend, whereas PLLA170B and PLLA350B separations show an increasing trend, parallel to x_{LLA} in Fig. 2. The range of variation is, however, greater in $x_{LLA,4}$. Component 4, which we consider to be a mixture of homopolymer PLLA, homopolymer PEG, and a

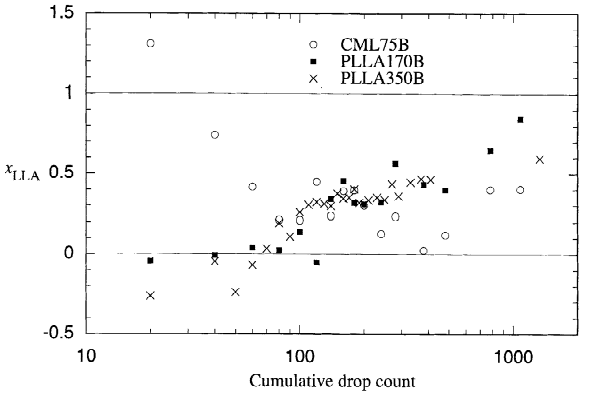

Fig. 6. Mole fraction of lactate in component 4, $x_{LLA,4}$, plotted as a function of the cumulative drop count for separations with CML75B (circles), PLLA170B (squares), and PLLA350B (crosses).

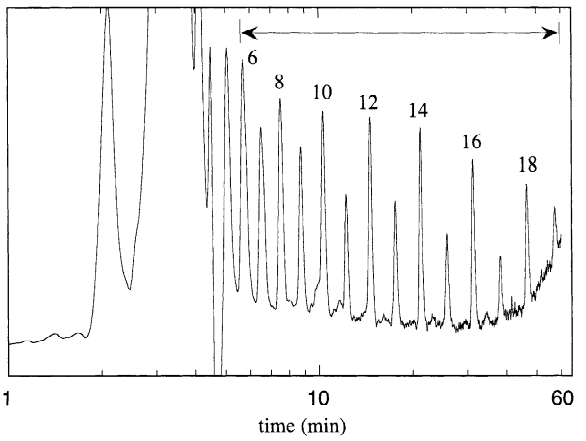

Fig. 7. HPLC chromatogram of PEG–PLLA (original) at the critical condition of PEG. The number adjacent to each peak represents DP_{PLLA}. The peaks in the range indicated by the arrow were used in the subsequent analysis. To show as many peaks as possible, the time is on the logarithmic scale. The solvent peak appears at around 3.1 min.

short diblock copolymer, was mostly separated by chemical composition, without being compromised by size exclusion. Therefore, a large contrast was observed between the two surfaces.

3.2.6. Length distribution of the PLLA block

The copolymer was separated according to the total DP of the PLLA blocks by using HPLC at the critical condition for the PEG block. For components 2 and 3, it is the block length, but for component 1, it is the sum of the two block lengths. Fig. 7, obtained for the original PEG–PLLA, shows an example of the HPLC chromatogram. The retention time is on a logarithmic scale to place the peaks nearly equidistant apart; each peak represents a specific number of lactate units in the polymer chain, as indicated in the figure [15]. The peak for PEG–LLA_{18} ($DP_{PLLA} = 18$) appears as narrow as the peak for PEG–LLA_{10}, but the actual peak on a linear scale is 3.4 times as broad. The large peak at around 3.1 min is due to the solvent; it was also observed for the PEG homopolymer. We identified the DP_{PLLA} of each peak from the proportionality (not shown) between DP_{PLLA} and $\ln(t_R - t_0)$, where t_R is the peak retention time for the component and $t_0 = 2.11$ min is the peak retention time for a component that always stays in the mobile phase [19]. Alternate even-numbered and odd-numbered peaks are evident in the chromatogram. Ideally, the PLLA block should have even-numbered peaks only, because cyclic dilactate molecules were added to the chain end one by one. The presence of odd-numbered peaks indicates transesterification during the reaction. The latter may explain the bimodal length distribution in the PLLA block.

To determine the change in the relative peak areas, we show in Fig. 8 a plot of the peak areas for even-numbered peaks only (six to 20 lactate units), normalized by the peak area for PEG–LLA_{12} as a

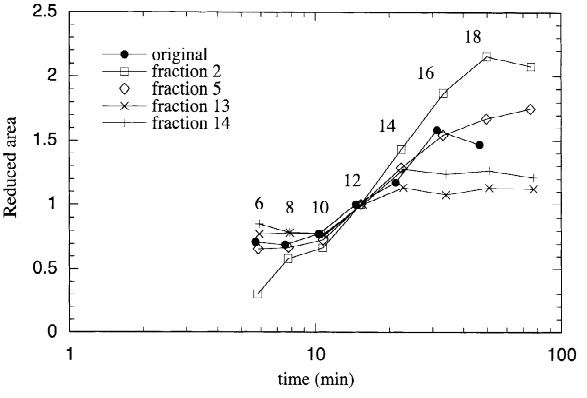

Fig. 8. Areas of the even-numbered oligomer peaks for some of the fractions obtained in the PFC separation with CML75B. The numbers adjacent to the symbols indicate DP_{PLLA}. In each fraction, the peak areas are normalized by the peak area of PEG–LLA_{12}. Lines are to guide the eye.

function of the retention time. The earlier peaks were not well resolved from each other, and the later peaks were too broad to reliably evaluate the peak area. The plot is for CML75B separation. Fractions 2, 5, 13, and 14 are compared with the original copolymer.

Early fractions, such as fraction 2, have more long PLLA components, caused mostly by enrichment of components 1 and 2. Fractions 13 and 14 have more short PLLA components compared with fractions 2 and 5, in agreement with the smaller A_1 and A_2 in Fig. 4a.

We also characterized the fractions obtained in separations with PLLA170B and PLLA350B. The plots of the normalized peak area for different fractions almost overlapped with the plot for the original copolymer (not shown). The absence of an enrichment effect is partly due to a poorer separation with the PLLA surface, but may also be due to the limited DP_{PLLA} range that we could analyze using our method.

4. Concluding remarks

We used 2D column chromatography to characterize different components of presumably a diblock copolymer of PEG–PLLA. Three components with a distinct distribution in each block length were found in addition to low-molecular-mass components that vary widely in chemical composition. Our characterization method can also be applied to other block copolymers. The first step is to characterize the macroinitiator, which is one of the blocks, and the second step is to characterize the whole block copolymer. In this way, we can obtain a comprehensive view of the block copolymer.

Using phase fluctuation chromatography to prepare a PEG–PLLA copolymer free of triblock components is not efficient. The smallest A_1 value was 0.036 in the separation with CML75B, but the mass of polymer in that fraction was only 5.3 mg, 0.66% of the mass of the polymer injected. Rather, eliminating the dihydroxy-terminated component in the macroinitiator MePEG would be more efficient. This study will be reported in a future publication [20].

Acknowledgements

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