

Separation of a diblock–triblock copolymer mixture by phase fluctuation chromatography

T. Fujiwara^a, Y. Kimura^a, I. Teraoka^{b,*}

^aDepartment of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

^bHerman F. Mark Polymer Research Institute, Polytechnic University, Six MetroTech Center, Brooklyn, NY 11201, USA

Received 10 March 2000; received in revised form 26 May 2000; accepted 12 June 2000

Abstract

A crude mixture of an A–B–A triblock copolymer, an A–B diblock copolymer, and B homopolymer was separated by phase fluctuation chromatography, where A is poly(L-lactic acid) and B is poly(ethylene glycol). The preparative separation method utilizes composition fluctuations occurring in a concentrated solution of the mixture. Injection of the viscous solution in a large volume into a column packed with a chemically modified stationary phase separated the mixture by the chemical composition and the number of blocks. The separation performance depended on the surface moieties, the solvent, the pore size, and the concentration. When the surface preferred B blocks and the solvent preferred A blocks, the early eluent was enriched with lactate and a triblock copolymer. The later eluent was low in the lactate content. We could not, however, find an appropriate combination of the surface moieties and the solvent that reverses the trend. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Triblock copolymer; Phase fluctuation chromatography; Surface moieties

1. Introduction

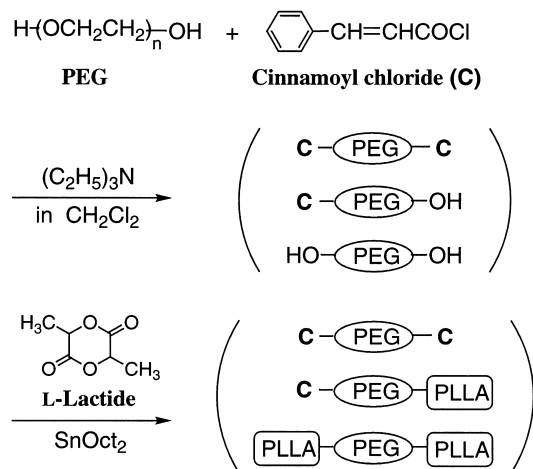
Phase fluctuation chromatography (PFC) was used in the past to separate on a preparative scale a random copolymer of styrene and acrylonitrile by the chemical composition [1]. PFC is a corollary to the high osmotic pressure chromatography developed to separate a large amount of polymer by the molecular weight [2–4]. The procedures of the two chromatographic methods are similar. In PFC, a concentrated solution of the copolymer is injected into a column packed with solid porous materials with specific surface modifications until the whole column is filled with the solution. Combination of the high concentration and large-volume injection renders a high processing capacity. It is considered that PFC utilizes large composition fluctuations naturally occurring in a concentrated solution of a copolymer [5–8]. When the solution is brought into contact with a pore surface that has different interactions with the two monomers that constitute the copolymer, a phase or a region of the solution enriched with surface-preferred components occupies the pore space, partially helped by the high osmotic pressure in the concentrated solution. The other phase will remain in the exterior solution. Thus, the inhomogeneous

solution is partitioned between the two spaces according to the chemical composition difference. In chromatographic separation, segregation by the chemical composition between the stationary phase and the mobile phase is repeated as the solution is transported along the column. The front end of the transported solution becomes increasingly enriched with the components more strongly repelled by the pore surface. The early eluent therefore has a high content of those components. In the separation of poly(styrene-*co*-acrylonitrile) [1], deterioration of the separation performance as the concentration was lowered demonstrated a need to have large composition fluctuations in the solution. The surface and solvent were also important to produce a desired separation. Diphenyl surface retained styrene-rich components, whereas cyanopropyl surface retained acrylonitrile-rich components. A selective solvent poorer to the retained components produced a better resolution.

Unlike HPLC widely used for analytical separations, PFC does not rely on the interaction of each analyte molecule with the surface moieties that may result in adsorption for some of the components. In PFC, regions enriched with one of the components interact with the surface to be partitioned between the stationary phase and the mobile phase. The separating medium, therefore, needs to have a pore size comparable to the typical dimensions of the composition

* Corresponding author. Tel.: +1-718-260-3466.

E-mail address: teraoka@poly.edu (I. Teraoka).



Scheme 1.

fluctuations in the solution injected, rather than to provide as large a surface area as possible.

The present work is the first to report separation of block copolymers by the chemical composition as well as by the number of blocks. It often occurs that, by synthetic methods, preparation of a pure diblock copolymer or a pure triblock copolymer is difficult. Precipitation of one of the components by solvent and non-solvent does not always separate the mixture efficiently. Here we want to show that PFC is a viable method to separate the block copolymer mixture into desired fractions.

The copolymer we worked on in the present study is a mixture of an A–B diblock copolymer and an A–B–A

triblock copolymer [9–13], where A = poly(L-lactic acid) (PLLA) and B = poly(ethylene glycol) (PEG). A small amount of PEG homopolymer is also present in the mixture. Unlike statistical copolymers, there is a three-way correlation between the overall composition, the length of the whole chain, and the total number of blocks on the chain. The relationships are, however, not straightforward, because of length distributions of the PLLA block and, to a lesser extent, of the PEG block.

Purification of the triblock copolymer will be facilitated if the size exclusion and the selective surface interaction cooperate. In other words, a pore with a small pore diameter and surface moieties that attract PEG blocks and repel PLLA blocks will produce early fractions enriched with the triblocks and/or components rich in L-lactate (LLA). As a consequence, late fractions will be enriched with diblocks and/or components rich in oxyethylene.

In contrast, reversing the trend to enrich diblocks in the early eluent would be difficult. For this purpose, longer chains have to be taken into the pore space. A porous material with a large pore diameter is necessary to avoid size exclusion effects, but it accompanies a smaller surface area to interact with the copolymer, resulting in a poorer resolution.

2. Experimental

2.1. Samples

Polyethylene glycol was purchased from Aldrich. Size exclusion chromatography (SEC) using pure water as

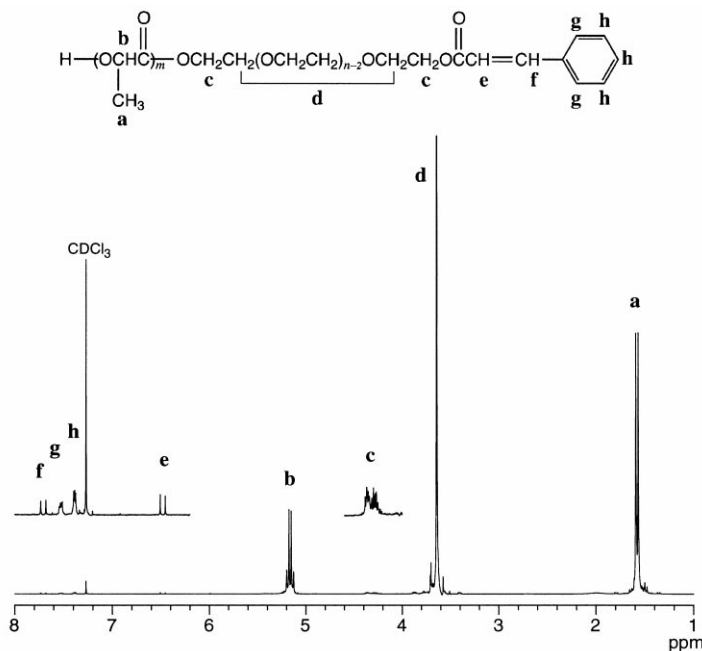


Fig. 1. ^1H NMR spectrum of the PLLA–PEG–C mixture. The symbols on the peaks are assigned to the protons shown in the figure.

Table 1
Surface modifications of controlled pore glasses

Chemical formula	Surface functionality	Abbreviation
HO–	Silanol	OH–CPG
(CH ₃) ₃ SiO–	Trimethylsiloxy	TM–CPG
CN(CH ₂) ₃ Si(CH ₃) ₂ O–	3-cyanopropyltrimethylsiloxy	CN–CPG
C ₈ H ₁₇ Si(CH ₃) ₂ O–	Octyldimethylsiloxy (octyl)	C8–CPG
(C ₆ H ₅) ₂ Si(CH ₃)O–	Diphenylmethylsiloxy (diphenyl)	DPM–CPG
HOOCCH ₂ OCH ₂ CONH(CH ₂) ₃ Si(O–) ₃	N-[(carboxymethyl)oxyacetyl]-3-aminopropylsilyltriethoxysilane (carboxyl)	CML–CPG
HOCH ₂ CH(OH)CH ₂ O(CH ₂) ₃ Si(O–) ₃	3-[(2,3-dihydroxypropyl)oxy] propylsilyltriethoxysilane (glyceryl)	GLY–CPG

mobile phase and Shodex columns (OHpakSB803, 804, 805) calibrated with PEG standards (Scientific Polymer Products) showed that the PEG has a number-average molecular weight $M_n = 4.53 \times 10^3$ and a polydispersity index $M_w/M_n = 1.07$, where M_w is a weight-average molecular weight. This M_n gives the number-average degree of polymerization $N_{\text{PEG}} = 103$, which is shared by the PEG blocks in the diblock and triblock copolymers. Solvents used for the separation were 1,4-dioxane (Acros), dimethyl formamide (DMF, Fisher), and 1,2-dichloroethane (DCE, Fisher).

2.2. Syntheses of block copolymers

As shown in Scheme 1 (synthesis of PLLA–PEG diblock copolymer and PLLA–PEG–PLLA triblock copolymer), terminal hydroxyl groups of PEG were acylated with cinnamoyl chloride. Into a solution of 50 g of PEG (10.9 mmol) and 1.324 g of triethylamine (13.1 mmol) in 100 ml of dichloromethane, 1.81 g of cinnamoyl chloride (10.9 mmol) dissolved in 50 ml of dichloromethane was added at 0°C, and the mixture was kept stirred at 30°C for 12 h. The product was then isolated by reprecipitation into diethyl ether. The precipitated product was then dissolved in 500 ml of benzene to remove the by-product, triethylamine hydrochloride by filtration. The benzene solution filtered was subsequently concentrated and lyophilized to obtain a solid product of cinnamoyl PEG; 300 MHz ¹H NMR (CDCl₃) δ 3.6–3.7 (m, CH₂CH₂O for PEG), 4.3–4.4 (m, COOCH₂ for the oxyethylene unit next to the cinnamoyl group), and 6.5 (d), 7.4 (m), 7.5 (m), and 7.7 (d) (for the cinnamoyl group). The product is a mixture of di- and monocinnamoyl PEG and unmodified PEG. The small differences in properties among the three components made it all but impossible to separate them.

A 45 g ([OH] = ca. 13.8 mmol) of the cinnamoyl PEG was reacted with 64.7 g (449 mmol) of L-lactide with Sn(Oct)₂ (10 mol% relative to [OH] involved in the cinnamoyl PEG) as a catalyst at 120°C for 7 h. The product was then subjected to reprecipitation with chloroform (solvent)/diethyl ether (precipitant) system. PLLA–PEG–C mixture; 300 MHz ¹H NMR (CDCl₃; see Fig. 1) δ 1.56–1.6 (d, CH₃ for PLLA), 3.6–3.7 (m, CH₂CH₂O for PEG), 4.3–4.4 (m, COOCH₂ for the oxymethylene next to the PLLA and the cinnamoyl units), 5.1–5.2 (q, CH for PLLA), and 6.5 (d),

7.4 (m), 7.5 (m) and 7.7 (d) (for the cinnamoyl group). Its SEC in chloroform mobile phase with Toso columns (TSK gel G4000H8 and G2500H8) and a refractive index detector revealed $M_n = 1.50 \times 10^4$ and $M_w/M_n = 1.39$ with reference to polystyrene standards. There is a slight difference in the differential refractive index between PEG and PLLA. Therefore, the molecular weight data and chromatograms presented in the present paper are primarily for reference purposes.

2.3. Solubility

Separation of the PLLA–PEG–C mixture by PFC requires that the injected solution be sufficiently concentrated. We therefore looked at the solubility in different solvents at high concentrations. The solubility was the best with chlorinated solvents such as chloroform, DCE, and 1,1,2,2-tetrachloroethane, producing a clear solution at room temperature. The solubility was also good with dioxane. The mixture dissolved in dimethylsulfoxide, dimethyl acetamide, and DMF at elevated temperatures, forming a clear solution. When left at room temperature, these solutions became cloudy and gel-like. We expect that these solutions have some heterogeneity even when they are clear. We do not know the dimension of the heterogeneity, however. Tetrahydrofuran and methylethylketone could not dissolve the polymers at high concentrations.

2.4. Separation method

Columns (3.9 mm interior diameter \times 300 mm length) were packed with controlled pore glasses (CPG; manufactured by CPG, Inc. or prepared by W. Haller at NIST) of various pore sizes. CPG used was either unmodified (surface was rich with silanol) or modified with functional groups covalently bonded to the silica surface. Table 1 lists the native CPG and the surface-modified CPG used in the present study. The first four modifications after the native silanol in the table were done in house. The procedure of the surface modification is described elsewhere [1–3]. For the last two, already modified CPG was obtained from CPG, Inc. We use the code OH–CPG_{xxx} for a column packed with silanol (native) CPG with a mean pore diameter $xxx \text{ \AA}$, for instance. The distribution of the pore diameter

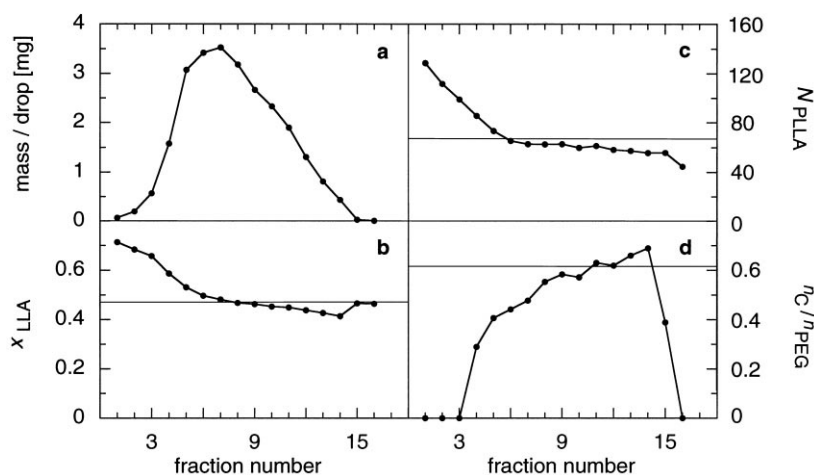


Fig. 2. A typical separation result: (a) Mass of solid polymer per drop of the eluent; (b) x_{LLA} ; (c) N_{PLLA} ; and (d) $n_{\text{C}}/n_{\text{PEG}}$ are plotted as a function of the fraction number. A 25.0 wt% solution of the PLLA–PEG–C mixture dissolved in dioxane was separated by a column filled with CML–CPG156. Horizontal lines represent values in the original mixture.

is narrow, typically within several percent of the mean value.

A uniform, concentrated solution (25 wt% unless otherwise specified) of the PLLA–PEG–C mixture was prepared on a hotplate. One of the packed columns was placed in a thermostatted column oven (Timberline, TL-430) and washed with the same solvent as the one used to dissolve the polymer. The column temperature was 80°C unless otherwise specified. The solution was injected directly through the pump head of a single-head HPLC pump (SSI, Acuflo II) into the column at a nominal flow rate of 0.20 ml/min. The injection continued until the first polymer was detected at the column outlet by pouring the eluent into diethyl ether. Then, the injection was switched to the pure solvent, and the eluent was collected by a fraction collector (Eldex) into different test tubes. The first 10 fractions collected 20 drops each, fractions 11 and 12 collected 40 drops each, fractions 13 and 14 collected 100 drops each, and fractions 15 and 16 collected 300 drops each. Collected fractions were dried by blowing hot nitrogen and then in a vacuum oven overnight. The amount of the solution injected was between 2.3 and 3.2 g, depending on the surface and solvent. After each batch of separation, the column was washed with dioxane at 80°C.

2.5. Composition analysis

The compositions of the original PLLA–PEG–C mixture and separated fractions were analyzed by using a ^1H NMR spectrometer. The spectra were analyzed with MacNuts on a Macintosh computer. Three integral peak heights were used. The integral $I_{3.7}$ at 3.65 ppm is due to methylene protons in PEG, the integral $I_{5.2}$ at 5.16 ppm is by methine protons in PLLA, and the combined integral I_7 for the four peaks at 6.48, 7.40, 7.53, and 7.70 ppm is by 7 protons on cinnamoyl groups (5 on phenylene and 2 on C adjacent to phenylene). Other peaks, although some were high, could not be used

because of overlapping with other peaks. The average mole fraction x_{LLA} of LLA in each fraction was calculated according to

$$x_{\text{LLA}} = \frac{I_{5.2}}{I_{5.2} + I_{3.7}/4} \quad (1)$$

Fractions of diblocks and triblocks were estimated as follows. Let ν_1 , ν_2 , and ν_3 be the number fractions of PEG, PEG–PLLA diblock copolymer, and PLLA–PEG–PLLA triblock copolymer, respectively ($\nu_1 + \nu_2 + \nu_3 = 1$). The ratio of $I_7/7$ to $I_{3.7}/(4N_{\text{PEG}})$ gives the ratio of cinnamoyl units, n_{C} , to the number of PEG blocks, n_{PEG} :

$$\frac{n_{\text{C}}}{n_{\text{PEG}}} = 2\nu_1 + \nu_2 = \frac{I_7/7}{I_{3.7}/(4N_{\text{PEG}})} = \frac{I_7}{I_{3.7}} \times 58.9 \quad (2)$$

Note that $n_{\text{C}}/n_{\text{PEG}}$ ranges between 0 for pure triblocks and 2 for pure PEG. Likewise, the ratio of $I_{5.2}/N_{\text{PLLA}}$ to $I_{3.7}/(4N_{\text{PEG}})$ gives the ratio of the number of PLLA blocks, n_{PLLA} , to n_{PEG} :

$$\frac{n_{\text{PLLA}}}{n_{\text{PEG}}} = \nu_2 + 2\nu_3 = \frac{I_{5.2}}{I_{3.7}} \times \frac{4N_{\text{PEG}}}{N_{\text{PLLA}}} \quad (3)$$

where N_{PLLA} is the number-average degree of polymerization of LLA in a PLLA block. Here it is assumed that the PLLA block length is common between the diblock and triblock copolymers.

First we apply this analysis method to the original PLLA–PEG–C mixture. Let α be the conversion yield for a terminal alcohol of PEG to cinnamoyl ester. Assuming an uncorrelated conversion of the two chain ends

$$\nu_1 = \alpha^2, \quad \nu_2 = 2\alpha(1 - \alpha), \quad \nu_3 = (1 - \alpha)^2 \quad (4)$$

With $I_7/I_{3.7} = 0.0105$ and Eq. (2), α is estimated as 30.9%. Thus we find the composition of the original PLLA–PEG–C mixture as: $\nu_1 = 9.6\%$; $\nu_2 = 42.7\%$; $\nu_3 = 47.7\%$. Then with Eq. (3) and $I_{5.2}/I_{3.7} = 0.224$, we find $N_{\text{PLLA}} = 66.7$. Other relevant quantities for the original mixture are $x_{\text{LLA}} = 0.472$ and $n_{\text{C}}/n_{\text{PEG}} = 0.619$.

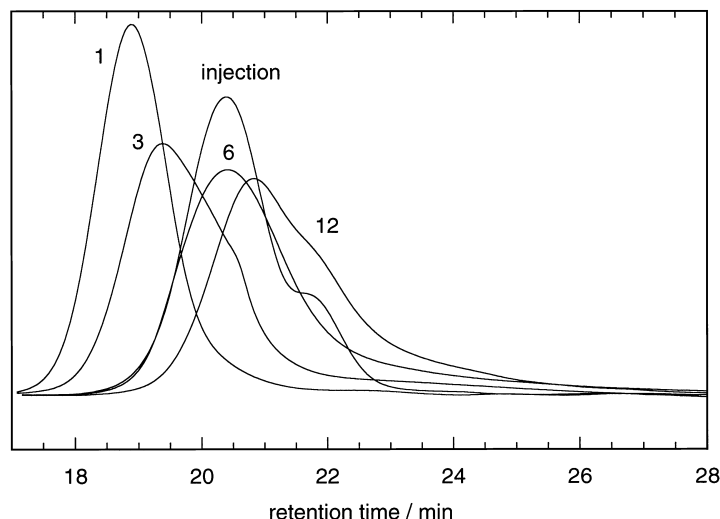


Fig. 3. Normalized SEC chromatogram for the PLLA–PEG–C mixture and some of the separated fractions. The number indicates the fraction number.

PLLA block has a substantial polydispersity, as seen in the increased polydispersity of pure triblocks compared with the parent PEG. Therefore, N_{PLLA} of a separated fraction may be different from the one evaluated for the original mixture. Fortunately, addition of Eqs. (2) and (3) gives an estimate of N_{PLLA} as

$$N_{\text{PLLA}} = 4N_{\text{PEG}} \times \frac{I_{5.2}/I_{3.7}}{2 - (I_7/I_{3.7}) \times 58.9} \quad (5)$$

3. Results and discussion

3.1. Typical separation performance

Fig. 2 shows a typical separation performance. (a) Mass of solid polymer per drop of the eluent; (b) x_{LLA} ; (c) N_{PLLA} ; and (d) $n_{\text{C}}/n_{\text{PEG}}$ are plotted as a function of the fraction number. For this batch of separation, 2.76 g of a 25.0 wt% solution of the PLLA–PEG–C mixture dissolved in dioxane was injected into a column filled with CML–CPG156. The injection required 16.4 min. The eluent concentration was the highest in the middle fractions, almost comparable to the concentration of the solution injected. The last two fractions are insignificant. A total 0.689 g of the polymer was recovered in 16 fractions. The mass amounts to 98% of the solid polymer injected, indicating a complete recovery within experimental errors. In part b of the figure, x_{LLA} decreased nearly monotonically with an increasing fraction number. The thin horizontal line represents x_{LLA} of the original mixture. The early fractions have a high content of lactate-rich components. Late fractions have x_{LLA} slightly smaller than that of the original. In part c, the length of the PLLA block shows a similar tendency. In part (d), $n_{\text{C}}/n_{\text{PEG}}$ was zero for the first three fractions, indicating a high

purity of triblocks. The ratio increased gradually and, in late fractions, exceeded the ratio for the original mixture. Thus, we find that late fractions are enriched with diblocks and/or PEG. In parts b and c of the figure, the averages of the relevant quantities for the fractions collected appear to be around the quantities of the original mixture, a reasonable result. In part d, however, the average for the fractions collected is substantially lower. We speculate that some of cinnamoyl esters underwent some changes, thereby resulting in an unusually low count of cinnamoyl units in the NMR spectra. Therefore, the estimates of N_{PLLA} and $n_{\text{C}}/n_{\text{PEG}}$ obtained with a help of the $I_7/I_{3.7}$ reading are not as reliable as the estimate of x_{LLA} .

We characterized the molecular weight distribution for the original PLLA–PEG–C mixture and the separated fractions by using SEC with chloroform mobile phase. Some of the results are shown in Fig. 3. The number adjacent to each curve indicates the fraction number. Each chromatogram is normalized by the area under the peak. Fraction 2 has a similar chromatogram (not shown) to that of fraction 1, but its peak is shifted by about 0.3 min toward the longer retention time. Later fractions including those shown in the figure gradually shifted in the same direction with increasing fraction number. Fractions 7–16 have essentially the same chromatogram. These results agree with the decrease in N_{PLLA} in Fig. 2(c). Apparently, fractions 1 and 2 collected the highest end of the molecular weight distribution of the original mixture. We expect that, in the early fractions (1–3), a copolymer consisting predominantly of triblocks was separated by the molecular weight. The overlapping chromatogram in late fractions suggests that there are collected components with a similar molecular weight. Together with the results shown in Fig. 2, we expect that they are enriched with diblock copolymers.

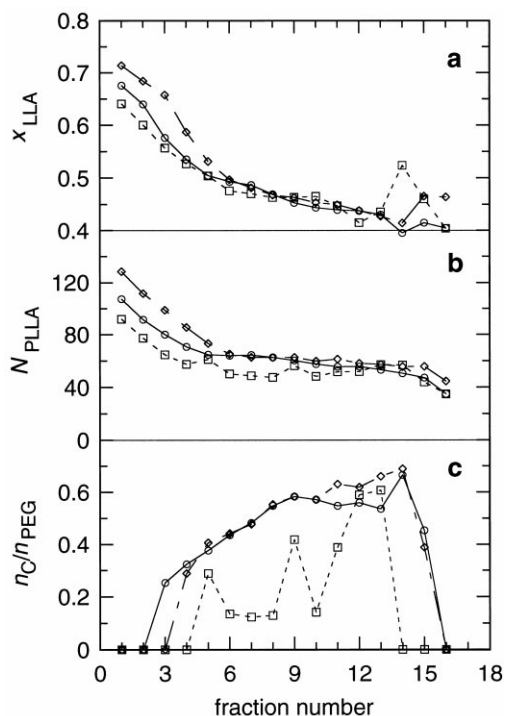


Fig. 4. Comparison of the separation results for different concentrations of the injected solution: (a) x_{LLA} ; (b) N_{PLLA} ; and (c) n_C/n_{PEG} are plotted as a function of the fraction number. Rhombuses, circles, and squares represent results for 25, 15, and 5.0 wt%. The column used was CML-CPG156, and the solvent was dioxane.

3.2. Concentration effect

Fig. 4 compares the results obtained for dioxane at concentrations of 5.0, 15, and 25 wt%. The last result is the same as the one shown in Fig. 2. The other conditions such as the column (CML-CPG156) were identical. The amounts of injection were 3.01 and 2.88 g for the 5.0 and 15 wt% solutions, respectively. The higher concentration produced early fractions with a higher x_{LLA} and a longer PLLA block. A good separation requires sufficiently high concentrations, in agreement with the prior results obtained for poly(styrene-*co*-acrylonitrile) [1]. The n_C/n_{PEG} remained low nearly for the entire fractions obtained from the 5.0 wt% solution. We do not know why. We also used 30 wt% solution, but the result was worse compared with the 25 wt% solution, and close to that of 15 wt%. The 30 wt% solution became cloudy when left at room temperature overnight, which may explain why this higher concentration resulted in a worse performance. The optimal concentration was thus found to be at around 25 wt% for dioxane. This concentration was used in the following study.

We note that the lowest of the three concentrations tested here is about two orders of magnitude as high as the concentration commonly practiced in HPLC for polymer composition analysis [14]. We did not attempt analytical separation of the mixture by injecting a dilute solution in a small volume.

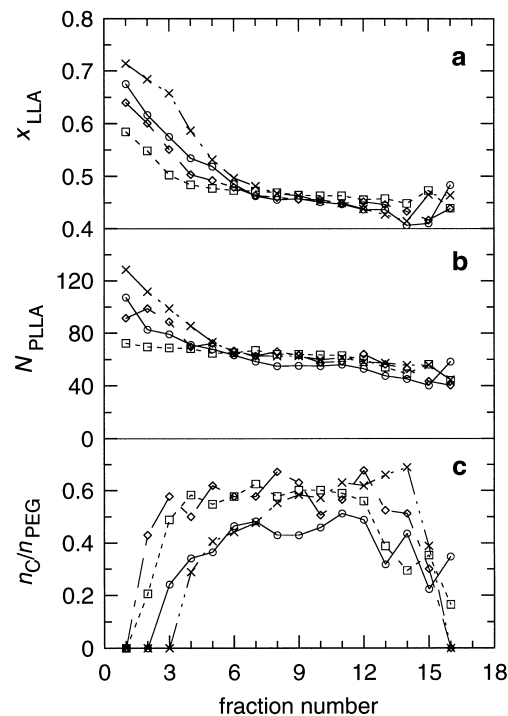


Fig. 5. Comparison of the separation results for different surface moieties of CPG: (a) x_{LLA} ; (b) N_{PLLA} ; and (c) n_C/n_{PEG} are plotted as a function of the fraction number. Crosses, circles, rhombuses, and squares represent results for CML-CPG156, OH-CPG156, TM-CPG156, and DPM-CPG156, respectively. A 25 wt% solution of the mixture in dioxane was injected.

3.3. Surface chemistry

Next we look at the effect of surface chemistry. All of the seven surface modifications listed in Table 1 were compared. The pore diameter was 156 Å except for CN-CPG177 and GLY-CPG115. A 25.0 wt% solution of the mixture in dioxane was used. Fig. 5 shows some of the results. The carboxyl (CML) surface produced early fractions with the highest x_{LLA} and the longest PLLA block. The results for glyceryl (GLY) and octyl (C8) surfaces were between those for trimethyl (TM) and diphenyl (DPM). The results for cyanopropyl (CN) were close to those for DPM.

With GLY-CPG115, n_C/n_{PEG} was small for the entire fractions, lower than that of OH-CPG156. We speculate that PEG-rich components were adsorbed onto the diols and failed to come out of the column or that the cinnamoyl group decomposed on the surface. We did not measure the mass of the polymer recovered. As described later, the smaller pore size of the GLY CPG might have weakened the enrichment effect in early fractions. We therefore exclude this surface from comparison.

The high purity of LLA in the early fractions with the carboxyl surface can be ascribed to specific interactions between PEG and the terminal of the surface moieties. PEG/poly(acrylic acid) and PEG/poly(methacrylic acid) are known to form a complex [15,16]. It is likely that

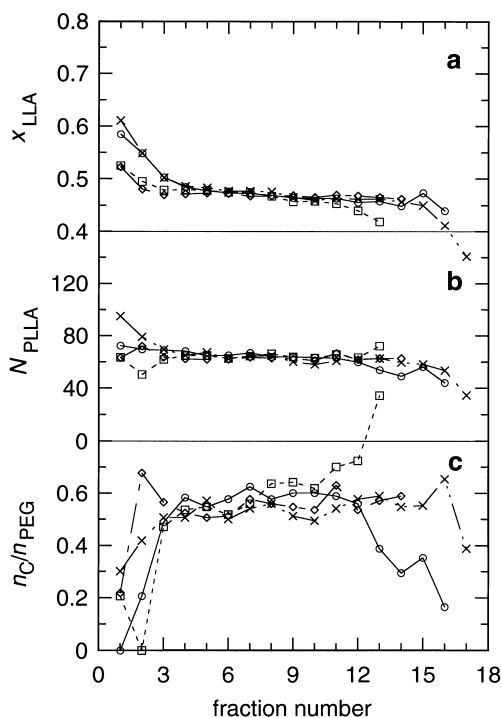


Fig. 6. Comparison of the separation results for different solvents: (a) x_{LLA} ; (b) N_{PLLA} ; and (c) n_C/n_{PEG} are plotted as a function of the fraction number. Circles and squares represent results for dioxane and DMF, respectively. The column used was DPM-CPG156. Also shown by crosses and rhombuses are the results obtained in DCE and DMF, respectively, with DPM-CPG497.

oxyethylene-rich components were attracted to carboxyl groups grafted at a high density onto the silica surface. Release of all the polymer injected indicates that the association between the two were not sufficiently strong to permanently retain oxyethylene-rich components. Use of dioxane that dissolves PLLA more easily than PEG should have contributed to retaining LLA-rich components in the mobile phase.

In contrast to CML, diphenyl and cyanopropyl surfaces appear to prefer lactate to oxyethylene, and, to a lesser extent, the octyl surface prefers lactate. It is interesting to see a near flat N_{PLLA} for all fractions when the surface was DPM or CN, although early fractions have a slightly higher x_{LLA} compared with the later ones. The cinnamoyl to PEG unit ratio was also held high except for the very early and very late fractions that are negligible in terms of mass.

The results obtained here allow us to arrange the strength of selective interaction that prefers oxyethylene to lactate: carboxyl > hydroxyl > trimethyl > octyl > cyanopropyl, diphenyl.

3.4. Solvent

The peculiar results obtained with the diphenyl surface and dioxane raised a hope that the trends of the higher x_{LLA} and N_{PLLA} in early fractions might be reversed by using a

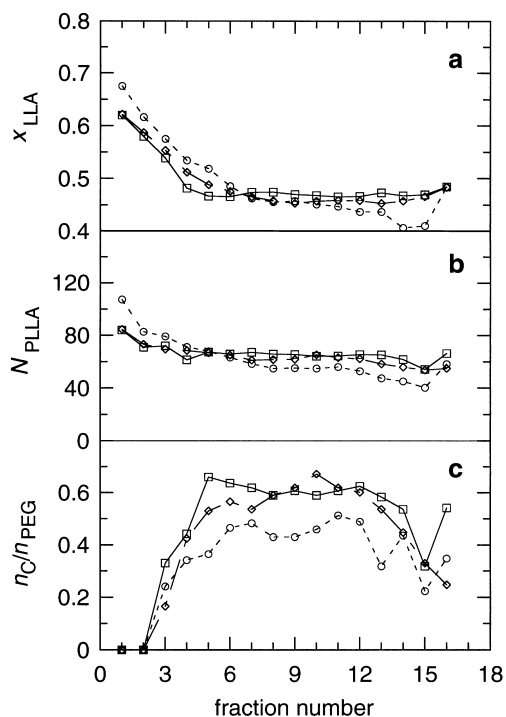


Fig. 7. Comparison of the separation results for different pore diameters (a) x_{LLA} ; (b) N_{PLLA} ; and (c) n_C/n_{PEG} are plotted as a function of the fraction number. Circles, rhombuses, and squares represent results for OH-CPG156, OH-CPG130, and OH-CPG85.

different solvent or CPG of a different pore diameter but the same surface. Fig. 6 compares the results obtained for dioxane and DMF. The same column, DPM-CPG156, was used. The concentration of the DMF solution was 18 wt%, because of a limited solubility. Apparently, DMF produced flatter x_{LLA} and N_{PLLA} . The high polarity of the solvent may have helped partition oxyethylene-rich components to the mobile phase. Yet, the preference of lactate-rich components by the surface and preference of oxyethylene-rich components by the solvent were not sufficiently strong to suppress the size exclusion effect.

Then, we used DMF and the same surface but with a larger pore diameter (497 Å) to decrease the effect of size exclusion. The result was not much different from the 156 Å pore, but a dip in the plot of x_{LLA} in early fractions gives a hint of a reversed trend. We used CPG with an even larger pore diameter (697 Å) and the same surface chemistry. The result was not much different. Here we find a trade-off between suppressing size exclusion and weakening surface interaction as the pore size increases. Separation with DPM-CPG497 and DCE at 25 wt% produced fractions similar to those obtained DPM-CPG156 and dioxane. A good solvent such as DCE does not promote partitioning of oxyethylene-rich components to the mobile phase.

3.5. Pore diameter

The effect of the pore diameter is compared in Fig. 7 for

three pore diameters (85, 130, 156 Å) with the silanol surface. A 25.0 wt% solution of the mixture in dioxane was used. There is apparently a size exclusion effect. The largest pore diameter did a better job in purifying high- x_{LLA} components in the early fractions that tend to have a higher molecular weight. Consequently, late fractions gained more in enriching oxyethylene-rich components when the pore diameter was greater. We therefore expect that, also in the separation with CML–CPG156, size exclusion effect helped produce high-lactate content early fractions and low-lactate content late fractions.

We also studied the effect of the column temperature for CML–CPG156 and dioxane. Comparison of separations at 25, 40, 60, and 80°C did not show a clear difference.

3.6. Separation mechanism

The results obtained in various separation conditions corroborate the PFC mechanism of separation for the copolymer mixture. If the separation had occurred according to the difference in the interaction of each polymer chain with the surface moieties, then use of the smaller pore diameter and injection of the less concentrated solution would have produced a better separation. PFC requires a concentrated solution that has sufficiently large composition fluctuations. To separate the heterogeneous solution, the pore diameter must be sufficiently large.

4. Concluding remarks

We demonstrated that PFC can separate a mixture of an A–B–A triblock copolymer, an A–B diblock copolymer, and a B homopolymer by the chemical composition and the number of blocks (A = PLLA, B = PEG). Use of surface groups that attract B blocks and a solvent that preferentially solvates A blocks produced early fractions rich in A and triblocks. Size exclusion effect also helped this trend. The best performance was obtained when a 25 wt% solution of the mixture in dioxane was injected into a column packed with carboxyl-modified CPG with a pore diameter of 156 Å. For a certain combination of the pore surface and the solvent, we could see a hint of the reversed trend, but it did not go far enough to enrich B

blocks in the early eluent. If the purpose of separation is to secure a large amount of diblocks, as it often is, then collection of late fractions after triblock-rich components were eluted in the early fractions may provide an efficient route. We are currently using this latter method to increase the purity of diblocks [17]. Preliminary results indicate that this route is more efficient than the solvent/non-solvent method is in purifying the diblocks.

Acknowledgements

T.F. and I.T. thank financial support from NSF-DMR9458055. Most of the experiments were conducted at Polytechnic University when T.F. stayed in New York in the summer of 1999. T.F. and Y.K. acknowledge the support of a Grant-in-aid for Scientific Research on Priority Area, “Sustainable Biodegradable Plastics”, No. 11217210 (1999) from the Ministry of Education, Science, Sports and Culture.

References

- [1] Xu Y, Teraoka I. *Macromolecules* 1998;31:4143.
- [2] Luo M, Teraoka I. *Macromolecules* 1996;29:4226.
- [3] Luo M, Teraoka I. *Polymer* 1998;39:891.
- [4] Xu Y, Teraoka I, Senak L, Wu CS. *Polymer* 1999;40:7359.
- [5] de Gennes PG. *Scaling concepts in polymer physics*. Ithaca, NY: Cornell University Press, 1979.
- [6] Swift BW, de la Cruz MO. *Europhys Lett* 1996;35:487.
- [7] Koňák Č, Fleischer G. *Macromolecules* 1997;30:1457.
- [8] Koňák Č, Helmstedt M, Bansil R. *Macromolecules* 1997;30:4342.
- [9] Kimura Y, Matsuzaki Y, Yamane H, Kitao T. *Polymer* 1989;30:1342.
- [10] Zhu KJ, Song B, Yang S. *J Polym Sci, Polym Chem Ed* 1989;27:2151.
- [11] Cohn D, Younes H. *J Biomed Mater Res* 1988;22:993.
- [12] Gref R, Mibamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. *Science* 1994;263:1600.
- [13] Scholz G, Iijima M, Nagasaki Y, Kataoka K. *Macromolecules* 1995;28:7295.
- [14] Berek D. *Macromolecules* 1999;32:3671.
- [15] Jeon SH, Ree T. *J Polym Sci, Polym Chem Ed* 1988;26:1419.
- [16] Maunu SL, Kinnunen J, Soljamo K, Sundholm F. *Polymer* 1993;34:1141.
- [17] Fujiwara T, Kimura Y, Teraoka I. *J Polym Sci, Polym Chem Ed* 2000;38:2405.