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Scale-up study of high osmotic pressure chromatography for separation of poly(ε-caprolactone)

Dean Lee, Iwao Teraoka*
Herman F. Mark Polymer Research Institute, Polytechnic University, Six MetroTech Center, Brooklyn, NY 11201, USA

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Abstract

Methods to prepare fractions of poly(ε-caprolactone) with a narrow molecular mass distribution in large quantities have been examined using high osmotic pressure chromatography under the theta condition. Effects of column dimension and coupling columns in series on the separation resolution were studied. We found that use of a thicker column can improve the resolution if adverse effects of viscous fingering are avoided. We also demonstrated that coupling the columns results in a better separation if the second column does not adsorb high-molecular-mass components purified in the first column.

Keywords: High osmotic pressure chromatography; Theta solvent; Preparative chromatography; Poly(ε-caprolactone)

1. Introduction

Poly(ε-caprolactone) (PCL) has been studied intensively in recent years as a biodegradable, semicrystalline polymer. Ring-opening ionic polymerization (ROP) by metal–organic complex catalysts and lipase have been used to prepare PCL [1–6]. One focus of the polymerization studies was to establish a protocol to synthesize PCL of as high a molecular mass (M_r) as possible and in as narrow a M_r distribution as possible. Typically, ROP yields PCL with $M_w < 10^5$ g/mol (polystyrene-equivalent) and M_w/M_n from 1.3 to 1.7, where M_w and M_n are the mass-average M_r and the number-average M_r, respectively. The low average M_r and the broad distribution are ascribed to transesterification. Lowering of M_w/M_n to below 1.1 was achieved by employing bulky catalysts that may prevent transesterification [2].

All of the catalyst-based synthesis methods have a problem of residual catalyst in the polymer. Furthermore, scale-up is not easy for ionic polymerization. Catalyst-free polymerization of PCL, followed by separation using liquid chromatography, may be an efficient alternative route to preparation of PCL with a high M_r and a narrow M_r distribution in large quantities. High osmotic pressure chromatography (HOPC), developed for preparative separation of polymer by M_r, may be useful for this purpose [7,8].

HOPC uses a column packed with rigid porous particles that have a pore size too small to accommodate polymer at low concentrations by a size-exclusion mechanism. At high concentrations, a high osmotic pressure of the solution forces polymer chains, especially shorter chains, into the pore to the extent to have the shorter chains at a higher con-

*Corresponding author. Tel.: +1-718-260-3466; fax: +1-718-260-3125.
E-mail address: teraoka@poly.edu (I. Teraoka).

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centration in the pore compared with the interstitial volume. In effect, polymers are segregated by M_r between the pore and the interstitial volume. When the concentrated solution is fully loaded into the column, the segregation is repeated, thereby narrowing the M_r distribution for the early fractions. As in displacement chromatography used for purification of low-M_r substances [9], a high purity is seen only in early fractions. Late fractions can be almost indistinguishable from the polymer injected. Injection of a large volume of a concentrated solution renders a high processing capacity for columns of analytical size.

HOPC has an edge over preparative-scale size-exclusion chromatography (SEC). In a recent study [10], we identified multi-modal peaks in the M_r distribution of a commercial monomethoxy-terminated polyethylene glycol. In addition to the main component with the nominal M_r, components with M_r twice, three times, and four times as high as that of main component were found. In contrast to separation by preparative SEC, the retention time of each component obtained by HOPC barely changed from fraction to fraction. Instead, the relative amount of each component changed. The early fractions were enriched with high-M_r components. Terminal-group analysis by nuclear magnetic resonance (NMR) allowed us to successfully determine the terminal group chemistry of each component.

In the past, HOPC was extensively practiced in a good solvent with repulsive surface. In our preceding article, we compared the performance of separation of PCL by HOPC in good solvent and in theta solvent [11]. Dioxane is a good solvent to PCL, toluene is a theta solvent with the upper critical solution temperature at around 15 °C. Separation under near the theta condition dramatically improved the efficiency of HOPC. In the study, a 0.23 g/ml solution in either solvent was injected into a column (300×3.9 mm) filled with porous packing materials at 30 °C. The injection was continued until the whole column was filled with the concentrated solution. The injection volume into the thin column was 1.9 to 3.6 ml, depending on the solvent and the separation medium. When the porous packing did not adsorb PCL as in the separation by octyldimethyl-modified controlled pore glass with pore diameter 130 Å, the injection volume was small. Fractions obtained in toluene had a narrower M_r distribution compared with those obtained in dioxane, in agreement with the results of partitioning studies in different solvent conditions by lattice Monte Carlo simulations [12]. The latter studies found that the missing second virial coefficient allows the solution in the pore to retain low-M_r components in a higher purity compared with the good solvent in a broad range of concentrations including the high concentration with a high osmotic pressure. The separation was only slightly worse at 80 °C; it is known that the solvent quality depends weakly on the temperature [13].

A slightly adsorptive surface, typically trimethyl-substituted surface, made the separation performance in toluene even better. In separation of a PCL sample with $M_w/M_n=1.66$ using a column packed with controlled pore glasses (octyldimethyl-modified, 81 Å pore diameter; this surface was slightly adsorptive because of a lower degree of substitution), for instance, M_w/M_n of the fractionated polymer started with 1.10 in fraction 1 and increased only to 1.20 when about 20% of the injected polymer eluted. The amount of PCL adsorbed and retained by the packing material in toluene elution was more than sufficient to coat the pore surface in thickness of a monolayer. It means that the pore diameter should have decreased during separation as more polymer molecules moved in to coat the surface. We considered that this mechanism of adjustable pore size contributed to holding down the M_r distribution even for middle fractions that should be indistinguishable from the original polymer were it not for the retention and the concomitant narrowing of the pores.

The study promised efficient separation of PCL in the theta condition with weakly adsorptive surface for large-scale separation, although only single, thin columns of dimension 300×3.9 mm were used in all batches of separation [11]. For HOPC to be used in production, it needs to be able to separate PCL with at least a comparable resolution in a scaled-up version. The present contribution investigates the effects of column dimensions and serial connection of columns on the separation performance. Possible complications include non-uniform transport of the concentrated polymer solution and the solvent in the packed bed that may be more evident in thicker columns. Longer columns may not directly lead to

improved separation, as the partitioning and adsorption in each plate depends on the concentration. In the past, thicker columns were used in HOPC to separate poly(vinyl pyrrolidone) in water [7] and poly(methyl methacrylate) in tetrahydrofuran (THF) [8], both in good solvent conditions, but the chromatography was not analyzed as thoroughly as was done here.

2. Experimental

2.1. Materials

The packing materials used for HOPC in the present study are various grades of controlled pore glasses (CPGs) from CPG (Lincoln, NJ, USA). Their characteristics, supplied by the manufacturer, are listed in Table 1. The letter in the code indicates the particle size. The surface modification of CPG was done in the same way as described in the literature [8,11]. Reaction with chlorotrimethyl silane (Acros, Geel, Belgium) changed surface silanols into trimethyl silanols; Reaction with *n*-octyl dimethylchlorosilane (Gelest, Tullytown, PA, USA) prepared octyl silanol-modified CPG. We denote the two surface-modified CPGs by CTMS and C8, respectively. Together with the nominal pore diameter and the particle size, CTMS-modified CPG is called CTMS120B here, for instance.

We used two commercial samples of PCL from Aldrich (St. Louis, MO, USA), the same samples as those used in our preceding study [11]. In the latter, we called them PCL22K and PCL72K, because we knew polystyrene-equivalent M_r only. In the present study, we could evaluate their true M_r, as described below. We call the two samples PCL10K and PCL33K, respectively. Their weight-average and number-average M_r, M_w and M_n, and the polydispersity M_w/M_n are listed in Table 2. Separation study was conducted for PCL10K only to minimize complications arising from kinetic effects in partitioning.

Table 1
Characteristics of controlled pore glasses

Code	Particle size (mesh)	Pore size (Å)	Pore volume (ml/g)	Surface area (m^2/g)
CPG75B	120/200	81	0.49	197
CPG75C	200/400	81	0.49	197
CPG120B	120/200	130	0.68	130

Table 2
Molecular masses of poly(ε-caprolactone) samples

Sample	$M_w/10^4$ (g/mol)	$M_n/10^4$ (g/mol)	M_w/M_n
PCL10K	1.02	0.61	1.66
PCL33K	3.34	1.95	1.71

2.2. Size-exclusion chromatography

The SEC system used for M_r analysis consists of a Waters 510 pump, a Waters 410 differential refractometer, and a bank of three columns (Phenogel, 10^3, 10^4, 10^5 Å) from Phenomenex (Torrance, CA, USA). The columns and the detector were thermostatted at 35 °C. The mobile phase was THF at 1.0 ml/min. The retention time in each SEC chromatogram was corrected by the solvent peak.

Because reliable PCL standards are not commercially available, we calibrated the columns with polystyrene (PS) standards of M_r from $1.05\cdot10^3$ to $9.49\cdot10^5$ g/mol from Pressure Chemical. For each chromatogram, we first evaluated PS-equivalent M_r and then used a conversion formula to change it to the true M_r.

To obtain the formula, we used another SEC system (SEC–MALLS) that consists of a Waters 510 pump, a Wyatt Dawn DSP multi-angle laser light scattering (MALLS) detector, a Wyatt Optilab DSP differential refractometer (632.8 nm), and the same Phenomenex columns. The columns and the detectors were set to 35 °C. The specific refractive index increment dn/dc of PCL in THF was evaluated as 0.073 ml/g by using one of the narrow-distribution PCL fractions separated by HOPC to ensure a well-defined baseline in the chromatogram of the refractive index. The light scattering data were collected every 1/100 of a minute. Fourteen angles from 31° to 147° were used to calculate M_w for each slice. The light scattering detector is not sensitive to low-M_r components. For PCL, the lower limit is about $1\cdot10^4$ g/mol in injection of 100 μl of 0.5 wt.% solution. Two plots of $\log_{10}[M_w/(g/mol)]$, one for a broad

distribution PS sample and the other for PCL33K, as a function of the retention time t_R obtained by SEC–MALLS are parallel to each other except for the end portions where the polymer concentration in the eluent is low. The two plots (not shown) share about 2 min of retention time in which the data of M_w are reliable. Curve fitting resulted in $\log[M_w/(g/mol)]=9.665-0.220t_R/min$ for PS and $\log[M_w/(g/mol)]=9.168-0.212t_R/min$ for PCL. Using the mean of the two slopes, we fitted the same data again with $\log[M_w/(g/mol)]=A-0.216t_R/min$ with A being an adjustable parameter to obtain $A=9.585$ for PS and 9.250 for PCL. Thus, we obtained (true PCL M_r)=(PS-equivalent M_r)×0.462. Although the coefficient may have only two significant figures, this conversion formula is enforced for the entire range of M_r throughout the present report. The small coefficient is reasonable, because PCL has a lower M_r per main-chain atom compared with PS.

2.3. High osmotic pressure chromatography

We used the same HOPC system as the one used earlier [11]. It consists of an HPLC pump (SSI, State College, PA, USA; 0.01 ml/min resolution), a fraction collector (Eldex, Napa, CA, USA), and one or two columns packed with the materials listed in Table 1. No sample loop was used. The columns were thermostatted in a column oven. A concentrated solution of PCL in toluene was injected at 0.20 ml/min, unless otherwise specified, through the pump head into the column at 30 °C. The eluent was dropped into ether to detect the first polymer. When precipitate was observed and thus we knew that the whole column was filled with the solution, the injection was switched to the pure solvent, and the eluent was diverted to the fraction collector. Table 3 lists the condition of each separation. The schedule of fraction collection by counting drops is also listed. In schedule A, for instance, 20 drops were collected in each of fractions 1 to 20, 40 drops each in fractions 11 and 12, and so forth. Recovery gives the percentage of polymer collected in the test tubes, relative to the polymer injected. The rows in italics indicate the separations reported in our preceding article [11] and referred to here for comparison. The column was washed in excess dioxane at 80 °C after each use to remove the adsorbed polymer. The numbers with ''b'' indicate the percentage collected with the additional washing in dioxane. Washing was continued without collection to ensure complete removal of PCL.

Typical concentration of the injected solution was 25 wt% or 0.228 g/ml, which is much higher than

Table 3
HOPC conditions

Column dimensions (mm×mm)	Packing material	Concentration		Flow-rate (ml/min)	Injection volume (ml)	Collection protocol	Recovery (%)
		Wt.%	g/ml				
300×3.9	*CTMS120B*	*25.0*	*0.228*	*0.2*	*3.45*	*A*	*84.5*[a]
300×3.9	*CTMS75B*	*24.9*	*0.227*	*0.2*	*3.41*	*A*	*75.9*[a]
300×3.9	*C8-75B*	*25.2*	*0.229*	*0.2*	*2.16*	*A*	*85.1*[a]
300×7.8	CTMS120B	25.0	0.228	0.2	12.0	B	83.2[a]
300×7.8	CTMS120B	25.0	0.228	0.4	12.1	B	83.4[a] 98.5[b]
300×7.8	CTMS120B	25.0	0.228	0.8	12.5	C	82.5[a] 98.4[b]
300×3.9	CTMS120B	25.0	0.228	0.05	3.46	A	86.9[a]
300×3.9	CTMS75C	25.0	0.228	0.2	3.52	A	81.9[a]
300×7.8	CTMS75C	25.0	0.228	0.2	12.6	B	–
600×3.9	CTMS120B	26.0	0.237	0.2	6.61	D	83.7[a] 97.4[b]
300×3.9×2	CTMS120B/C8-75B	25.0	0.228	0.2	4.80	D	82.8[a] 99.2[b]
300×3.9×2	CTMS75B/C8-75B	25.0	0.228	0.2	5.14	D	80.5[a] 99.3[b]

A: 1–10 (20 drops); 11–12 (40 drops); 13–14 (100 drops); 15–16 (300 drops) in toluene. B: 1–20 (40 drops); 21–24 (80 drops); 25–28 (200 drops); 29–34 (400 drops) in toluene. C: 1–20 (40 drops); 21–24 (80 drops); 25–28 (200 drops); 29–32 (400 drops) in toluene. D: 1–20 (20 drops); 21–24 (40 drops); 25–28 (100 drops); 29–32 (300 drops) in toluene. More fractions were collected in dioxane (300 drops each) when recovery with dioxane is shown.

[a] Recovery from elution by toluene at 30 °C.

[b] Recovery from elution by dioxane at 80 °C.

the overlap concentration c^*. We can estimate c^* as follows: the radius of gyration, R_g, of PCL10K is estimated, using its PS-equivalent $M_w = 2.2 \cdot 10^4$ g/mol, as 4.6 nm [14]. Then, $c^* = (2^{1/2} R_g)^{-3} (M/N_A) =$ 0.058 g/ml, where N_A is the Avogadro's number and M is the true M_r. This estimate gives a higher value compared with the reciprocal of the intrinsic viscosity, another measure of c^*. In fact, the injected solution was much more viscous than toluene was.

3. Results and discussion

3.1. Column thickness

The preceding study used exclusively 300×3.9 mm I.D. columns and a flow-rate of 0.2 ml/min [11]. Use of a thicker column increases processing capacity. Compared at the same column length, separation performance will be similar, if the transport of solution and solvent in the column is uniform across its cross section. Among various columns used earlier, a column packed with CTMS120B produced one of the best performances [11].

Here we used a 300×7.8 mm column of the same packing material. A 25 wt.% solution of PCL10K in toluene was injected into the column at a flow-rate of 0.8 ml/min that gives the same linear velocity as the 0.2 ml/min injection into the thinner column. The same solution was injected into the same column at 0.2 and 0.4 ml/min. The times needed for injection at nominal flow-rates of 0.8, 0.4, and 0.2 ml/min were 16, 29, and 56 min, respectively. As seen in Table 3, the injection volume (breakthrough volume in frontal analysis) does not depend on the flow-rate and is slightly less than four times as large as the one loaded into the thinner column. The result is reasonable, when we take into account the volumes in the pump head and the tubing. The three recovery percentages are almost identical.

We prepared a plot of PCL concentration in the eluent as a function of the cumulative elution volume since the injection started, which we call a HOPC retention curve. Fig. 1 compares the HOPC retention curves for 0.2 and 0.8 ml/min. The curve for 0.4 ml/min (not shown) is close to that of 0.2 ml/min. The curve for the thinner column and its replica, with the elution volume multiplied by 3.5 (the ratio of the injection volumes), are shown for reference. It is common to the three flow-rates that the con-

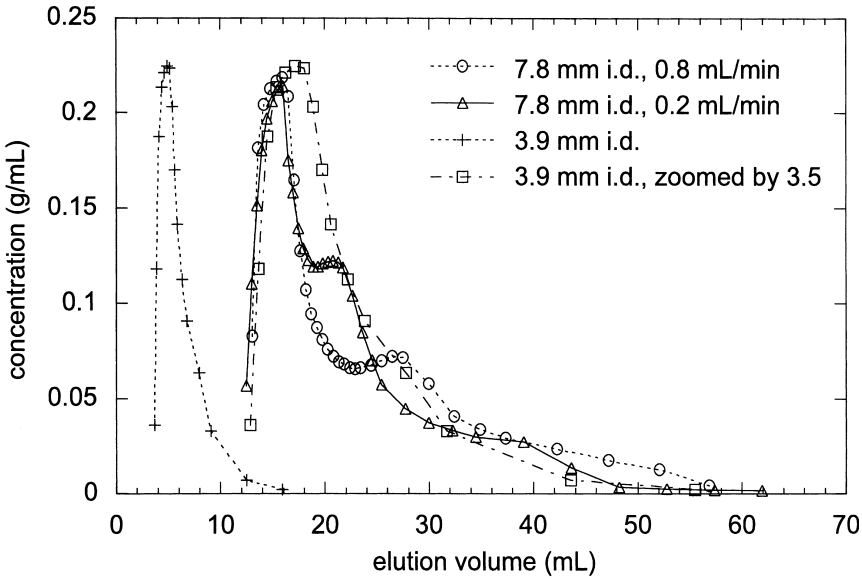

Fig. 1. HOPC retention curves in separation of PCL10K with a 300×7.8 mm column packed with CTMS120B. Flow-rates of 0.8 ml/min (circles) and 0.2 ml/min (triangles) are compared. The retention curve obtained in the separation of PCL10K with a 300×3.9 mm column packed with the same materials (crosses) and its replica with the elution volume multiplied by 3.5 (squares) are shown for reference.

centration increases in several fractions to a plateau level that is almost equal to the concentration of the injected solution (0.23 g/ml). The sharp rise in the concentration occurs at around the same elution volume for the three flow-rates. The width of the plateau is insensitive to the flow-rate and is about a half of the width observed in the thinner column (zoomed). Unlike the thinner column, the thicker column shows a hump on the trailing side of the peak at a different location, depending on the flow-rate.

The hump and the narrow plateau suggest non-uniform displacement of viscous polymer solution by non-viscous solvent when the column was washed with the solvent. Upon entering the solution-filled column, the solvent may create a channel of a lower concentration. The subsequent part of the solvent will find it easier to follow the same path, avoiding the packed bed imbibed with the viscous solution. Once the stable channel is formed, exchange of polymer molecules in the transported solution will not occur efficiently. This phenomenon is called ''viscous fingering'' and is widely observed at the interface between an immiscible pair of viscous and non-viscous liquids in a backed bed [15]. Viscous fingering will be more serious in a thicker column that can accommodate more solvent channels. Creation of solvent channels resulted in a premature drop in the eluent concentration at 16–17 ml of the elution volume. The drop was the greatest and the tailing of the retention curve was the longest at 0.8 ml/min. In contrast, advancement of the viscous solution replacing non-viscous solvent appears to have occurred uniformly and nearly in the same way in columns of the two diameters at different flow-rates, as indicated by the overlap of the retention curves on the rising side of the peaks.

SEC chromatograms (not shown) were obtained for 18 to 21 fractions in each separation. The pattern of transition in the SEC chromatogram from early to late fractions was similar among the three flow-rates and similar to the one observed in the thinner column (Fig. 3b in Ref. [11]) except that a multi-modal peak was not visible in late fractions at any of the three flow-rates in the thicker column. The latter result again suggests non-uniform transport for the late eluent and inefficient partitioning.

The difference of the transition pattern in the chromatograms can be better represented in the plots of M_w and M_w/M_n of the fractions as a function of the cumulative mass of the polymer collected (Fig. 2). The results are shown for 0.2 and 0.8 ml/min only to avoid congestion; those for 0.4 ml/min were mostly between the corresponding curves for the other two flow-rates. For reference, the results of the thinner column are also shown with the cumulative mass multiplied by 3.5. Note that not all the fractions were analyzed by SEC, although all of them were weighed. A better separation has a greater span in M_w and a lower-lying plot of M_w/M_n. It is apparent that the slower flow-rate separates the polymer in a narrower M_r distribution and with a slightly higher M_w in early fractions. In separation at 0.2 ml/min, about 0.35 g of the collected PCL claims M_w/M_n below 1.2, more than 3.5 times as large as the amount obtained by the thinner column. Note that the fractions with $M_w/M_n<1.2$ were obtained mostly before the eluent concentration started to fall in Fig. 1; These fractions were not affected by the viscous fingering. If the goal of separation is to collect as much polymer as possible with $M_w/M_n<1.2$, use of the 7.8 mm I.D. column and 0.2 ml/min gives the right separation condition. If the goal is to lower the polydispersity in early to late eluent, then the thicker column does not have an advantage.

The better separation in early fractions at a slower flow-rate can be ascribed to better exchange of solute molecules between the pore and the interstitial space. Hindrance to mass transfer is a major factor that contributes to an increase in the plate height at a faster flow-rate in liquid chromatography. The problem of mass transfer is more serious in equilibration of concentrated polymer solution, as the diffusion of polymer is slowed by entanglement. We did not try an even slower flow-rate; the time needed for injection would be excessively long.

There is a jump in the plot of M_w/M_n at around cumulative mass of 0.9 g for the two flow-rates in Fig. 2. The jump occurs when the eluent concentration falls below 0.17–0.15 g/ml after the peak. In contrast, the thinner column does not exhibit the jump. It appears that these jumps are related to the viscous fingering. Another jump at the cumulative mass of around 2.3 g is due to the adsorbed polymer released in washing by dioxane.

After these studies, we returned to the thinner

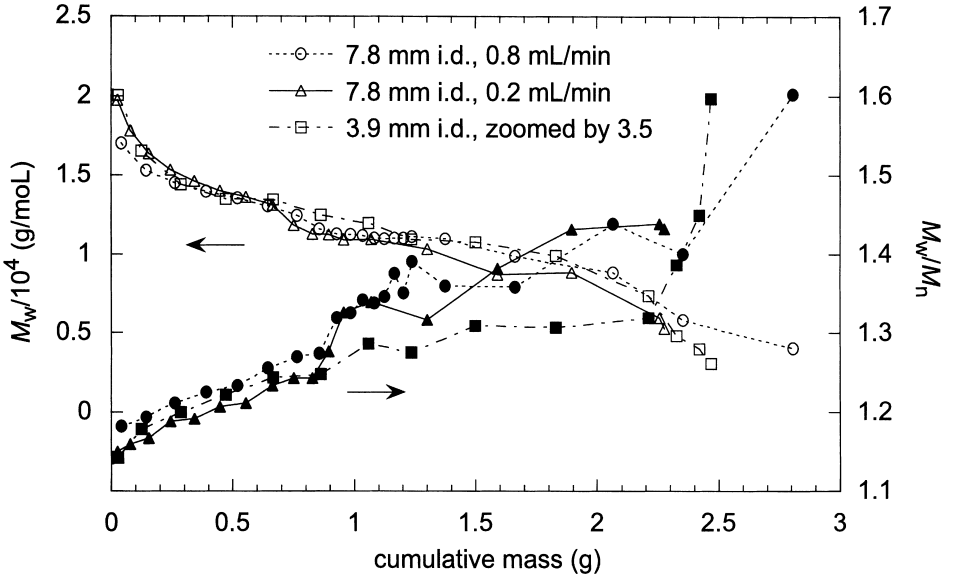

Fig. 2. M_w (open symbols) and M_w/M_n (closed symbols) for fractions obtained in HOPC separations of PCL10K with the CTMS120B column, plotted as a function of the cumulative mass of the polymer collected. Results are shown for separations by the 300×7.8 mm column at flow-rates of 0.8 ml/min (circles) and 0.2 ml/min (triangles). For reference, hypothetical results obtained with the 300×3.9 mm column at 0.2 ml/min, with the abscissa multiplied by 3.5 are also shown (squares).

column to examine the effect of the flow-rate. We injected the PCL solution of the same concentration into the 300×3.9 mm column at 0.05 ml/min that gives the same linear velocity as 0.2 ml/min in the thicker column. The results of the 0.05 ml/min (not shown) were nearly the same as those obtained at 0.2 ml/min, except that the HOPC retention curve rose more quickly and that M_w was higher in fractions 2–6.

We also compared separations by 300×3.9 mm and 300×7.8 mm columns packed with CTMS-75C, both at 0.2 ml/min. The plots of M_w and M_w/M_n of the thicker column (not shown) were nearly overlapped with those of the thinner column when the cumulative mass of the latter was multiplied by the ratio of the injection volumes. Apparently, the smaller particle size improved the mass transfer, resulting in no difference between the two separations.

3.2. Column length

Another way to increase the processing capacity is to use a longer column. The latter is always possible unless the back pressure is too high to maintain the flow. The longer column may provide a better resolution, since it will contain more theoretical plates. We compared the performance by the 300 and 600 mm columns (both have a 3.9 mm I.D.) packed with CTMS120B for separation of PCL10K in toluene. The injection volume of the 26 wt.% solution into the longer column was slightly less than twice as large as the one into the shorter column (Table 3). The injection time at 0.2 ml/min was 31 min, as opposed to 17 min in the 300 mm column, indicating no slowing down of linear velocity from the nominal flow-rate. The recovery percentages of the polymer in the two column lengths were similar.

The two HOPC retention curves are compared in Fig. 3. Also shown as a reference is a hypothetical retention curve obtained from the one for the shorter column by multiplying the elution volume by 1.92, the ratio of the two injection volumes. Compared with the hypothetical curve, the one for the longer column rises more sharply, has a broader plateau at the concentration of the injected solution, and falls more quickly. The rapid fall of the eluent concentration indicates efficient exchange of polymer

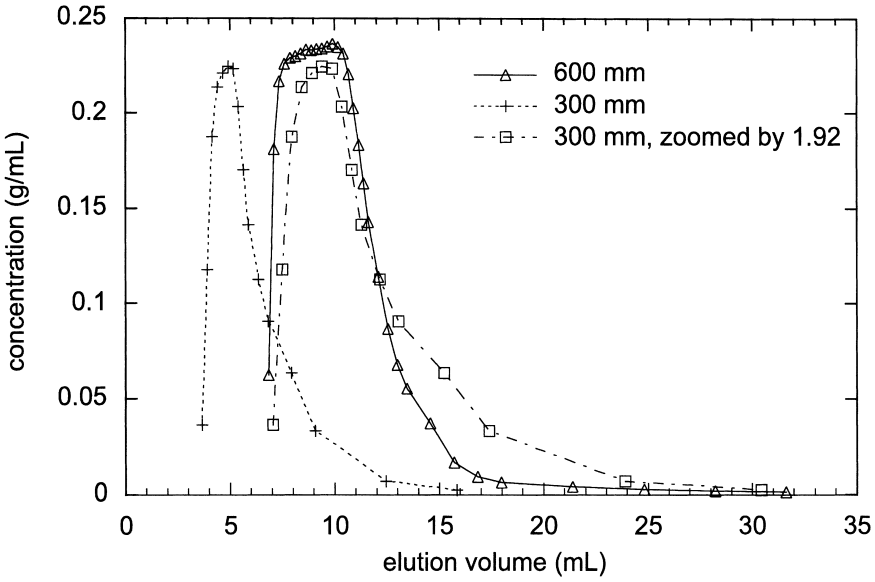

Fig. 3. HOPC retention curve for a 600×3.9 mm column packed with CTMS120B. For reference, a retention curve obtained in the separation with a 300×3.9 mm column (crosses) and its replicas with the elution volume multiplied by 1.92 (squares) are shown.

between the pore and the interstitial space when the solvent was forced into the column. It means that the column of 300×3.9 mm is also subject to solvent channels, although their problem is less serious compared with the 300×7.8 mm column.

Fig. 4 compares the plots of M_w and M_w/M_n for the two column lengths. Hypothetical plots, prepared from the plots of the shorter column by multiplying the cumulative mass by 1.92, are also shown. Except for fraction 1, the longer column outperforms the

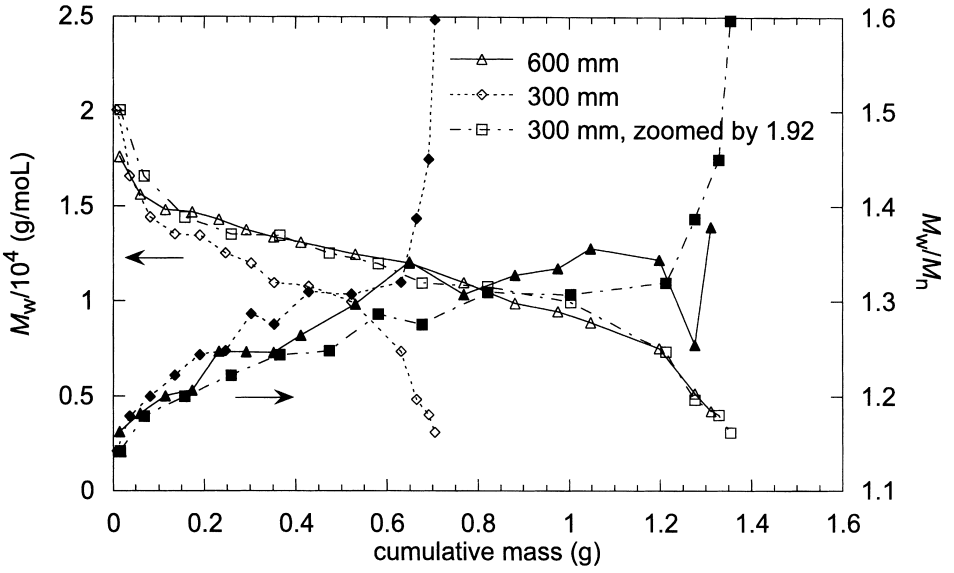

Fig. 4. M_w (open symbols) and M_w/M_n (closed symbols) for fractions obtained by the 3.9×600 mm column (triangles). For reference, results obtained with the 300×3.9 mm column (rhombuses) and their replicas with the abscissa multiplied by 1.92 (squares) are also shown.

shorter column, but the gain in the length does not translate into the gain in resolution.

The sharp increase in the eluent concentration and the broad plateau make one think that dilution did not occur for the front end of the transported polymer solution. What happened is the other way around. Recall that the injection volume into the CTMS120B column was larger compared with the one into the non-adsorbing C8-120B column because of the retention of PCL by the CTMS surface [11]. We divide the longer column into two sections. What passes the boundary between the two sections is essentially identical to what comes out of the shorter column. The front end of the solution that passes the boundary will contain high-M_r components in a high purity, as seen in the low M_w/M_n for fraction 1 obtained by the shorter column. The same applies to the portion that immediately follows the front. The large injection volume into the longer column, nearly twice as large as the one into the shorter column, indicates that adsorption occurred for these high-M_r components in the second half of the column, resulting in a lower M_w and a larger M_w/M_n in fraction 1 compared with the counterparts in the shorter column. If the high-M_r components were not retained in the second half, then the polymer would have come out of the column much earlier, and the early eluent would have been at least as pure in high-M_r components as the early fractions obtained by the shorter column. We consider that the retained polymer in the second half of the longer column is mostly high-M_r components, in contrast to mostly low-M_r components retained in the first half.

We expect that the same result would be obtained by coupling two 300 mm columns in series, both packed with CTMS120B. To avoid the damage by the second half of the longer column or the second 300 mm long column in the series, the latter must be packed with porous materials that do not retain the high-M_r components. Small pores such as CTMS75B and C8-75B and repulsive surfaces such as C8-120B will qualify. We will examine these serial connections below.

3.3. Serial connection of columns

In SEC, coupling columns in series is widely practiced to increase the number of theoretical plates or to extend the linear range of analysis. We tried separations by CTMS120B/C8-75B and CTMS75B/C8-75B with the first code denoting the first column, as listed in Table 3. We chose C8-75B for the second column, because it was the best-performing column when used alone [11], and it showed a weak adsorption, as seen in the small injection volume. The injection volume into the coupled columns was significantly less than the sum of the injection volumes for the individual columns: for CTMS120B/C8-75B, it was 4.80 ml as opposed to the sum 5.61 ml, and for CTMS75B/C8-75B, it was 5.14 ml as opposed to the sum 5.57 ml. The difference between the two injection volumes was greater than the one we observed in the preceding section. The injection volumes did not add up, because the front-running high-M_r components from the first column were partitioned mostly to the mobile phase in the second column. The second column retained more polymer when used alone to separate PCL10K. In fact, the difference of the injection volume into the coupled columns from the one in the first column alone is 1.35 ml and 1.73 ml, respectively, in the two separations. These volumes are smaller than the injection volume in the C8-75B column alone in a good solvent (dioxane) with no adsorption.

In both couplings, the actual flow-rate was less than 4% different from the nominal flow-rate of 0.2 ml/min. Thus, we find there was no problem of increased back pressure.

The HOPC retention curves of the two separations are shown in Fig. 5. Note a gradual increase in the concentration, indicating absence or weak retention of high-M_r components by the second column. The curve in each separation was between two hypothetical curves (not shown) obtained from the retention curves of individual columns in the series by multiplying the elution volume by the ratio of the injection volumes, except for the tail portion; the retention curve of the coupled columns fell more quickly.

The SEC chromatograms in Fig. 6 are for CTMS75B/C8-75B. Each chromatogram is normalized by the area under the peak. The chromatograms of early fractions are similar to those of C8-75B single column separation. Middle fractions removed low-M_r components more efficiently. Late fractions show multimodal peaks. Note a skewed distribution: the leading edge is more tailed than the trailing edge.

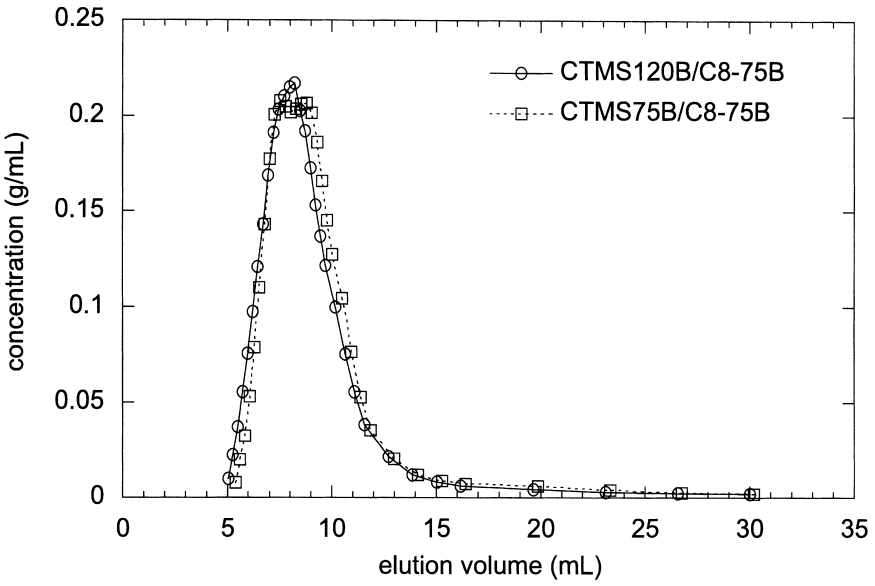

Fig. 5. HOPC retention curves for separations by CTMS120B/C8-75B (circles) and CTMS75B/C8-75B (squares) coupled columns.

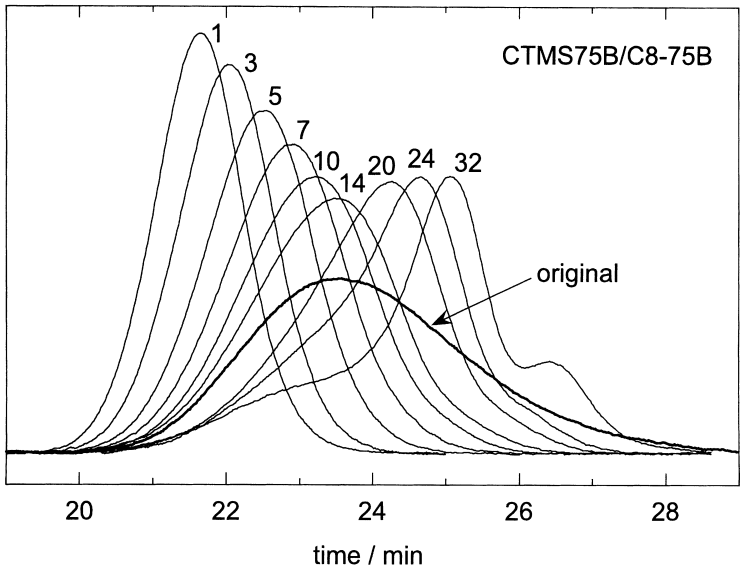

Fig. 6. SEC chromatograms for some of the fractions obtained in separation of PCL10K by CTMS75B/C8-75B columns. The number adjacent to each curve indicates the fraction number. The chromatogram of PCL10K is shown as a thick line.

This asymmetry is characteristic of HOPC by a weakly adsorbing medium. Cutting the trailing edge increased M_n, thus decreasing M_w/M_n.

Improvement in resolution by the serial coupling is evident in the plots of M_w and M_w/M_n as a function of the cumulative mass of polymer (Fig. 7). Results for the two separations as well as the result obtained by a single column of C8-75B are shown. The span of M_w is greater at both ends of M_w in the coupled columns. Fraction 1 in the separation by the coupled columns has a higher M_w than fraction 1 in separation by any of the single constituent columns does. The transported solution left behind low-M_r components further in the second column, enriching the early eluent further with high-M_r components. In either separation by the coupled columns, about 0.25 g of PCL has $M_w/M_n<1.2$ as opposed to a mere 0.16 g in the separation with the 600 mm column.

We compared the plots of M_w and M_w/M_n for the coupled columns with two hypothetical curves prepared from the plots obtained for each of the two columns by multiplying the cumulative mass by the injection volume ratio. In the comparison for CTMS75B/C8-75B, the plots for the coupled columns run between the corresponding hypothetical plots; the plot of M_w/M_n is closer to the better one (C8-75B). In CTMS120B/C8-75B, both M_w and M_w/M_n plots of the coupled columns nearly overlap with the better of the two hypothetical curves. It means that adding the second column did not degrade what was attained by the first column. Rather, the resolution was boosted by the increased column volume, in contrast to a meager increase by the longer column packed with CTMS120B. We consider that connecting a second column that does not retain the high-M_r components in the early eluent from the first column is responsible for the good separation.

Table 4 lists the amount of PCL in each of the fractions with $M_w/M_n<1.2$, obtained in separations by the coupled columns. Even with these narrow columns, we could obtain fractions in a narrow M_r distribution without any other peaks in the SEC chromatograms, each in 2 to 50 mg. Use of thicker columns, when operated at a slower flow-rate, will increase the amount in each fraction without degrading the resolution, as we demonstrated in the present work. For instance, two coupled columns of 300× 7.8 mm, packed with CTMS75B and C8-75B, will produce fractions with $M_w/M_n<1.2$, each in 8 to 200 mg, in one batch.

PCL with $M_w/M_n \cong 1.12$ was obtained only for the

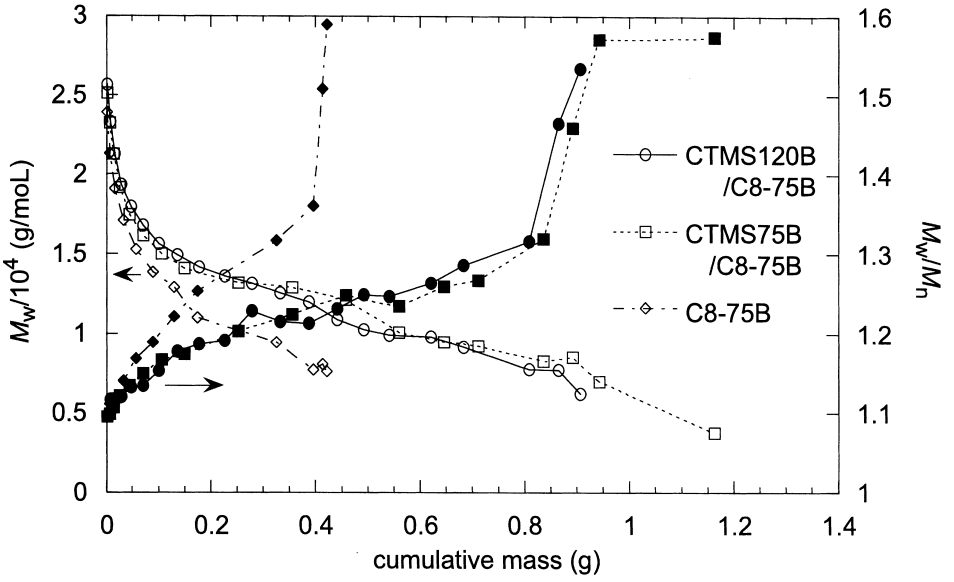

Fig. 7. M_w (open symbols) and M_w/M_n (closed symbols) for fractions obtained by the CTMS120B/C8-75B columns (circles) and CTMS75B/C8-75B columns (squares). For reference, results obtained by a single column of C8-75B (rhombuses) are also shown.

Table 4
Amount of PCL in each fraction

Column	Fraction	Amount (mg)	$M_w/10^4$ (g/mol)	M_w/M_n
CTMS120B/C8-75B	1	2.3	2.56	1.096
	2	5.2	2.33	1.117
	3	8.6	2.13	1.118
	4	13.1	1.93	1.120
	5	18.1	1.79	1.133
	6	23.5	1.68	1.135
	7	29.5	1.56	1.154
	8	35.3	1.49	1.179
	9	42.1	1.41	1.187
	10	48.3	1.36	1.191
CTMS75B/C8-75B	1	1.8	2.51	1.095
	2	4.6	2.32	1.099
	3	7.4	2.12	1.107
	4	12.3	1.92	1.122
	5	18.5	1.74	1.135
	6	26.1	1.61	1.150
	7	34.4	1.50	1.167
	8	43.7	1.41	1.174
	9	50.3	1.35	1.183

very early fractions. It is possible to collect late fractions to re-inject into the same column(s) and obtain fractions with M_w/M_n close to 1.10 and with a lower M_w than the one obtained in the first run. Similar fractions can be obtained in a single-step separation of a broad-distribution PCL sample with a lower M_w. The two methods need to be compared in cost effectiveness.

4. Conclusions

We demonstrated the capability of HOPC to scale up easily by using a thicker column and a serial connection of columns. Some precautions are needed. Use of a thicker column may improve the separation, but a slower linear velocity may be required to minimize non-uniformness in transport of the polymer solution and the solvent. Coupling columns in series improves the separation, if the second column does not retain high-molecular-mass components purified in the first column. Obviously, it is important to use a porous packing material that gives the optimal separation performance for a given polymer. We will address the issue in our forthcoming publication.

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