High Osmotic Pressure Chromatography for Large-Scale Fractionation of Polymers

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ABSTRACT: We propose high osmotic pressure chromatography (HOPC) to separate a large amount of polydisperse polymeric materials into fractions with different molecular weights. A concentrated polymer solution is introduced into a chromatographic column packed with porous materials that provide the polymer with sufficient geometrical confinement. The injection is continued until the polymer is detected at the column outlet. Then, the injection is switched to the pure solvent, and the eluent is collected into different test tubes. The separation principle is based on the segregation that takes place between pore channels and the surrounding solution, as a high osmotic pressure of the concentrated polymer solution drives low molecular weight components preferentially into the pore channels. As the polymer solution is transferred along the column, enrichment of the highest molecular weight components in the mobile phase is repeated at the solution front until it reaches the outlet. It is followed by the next highest molecular weight components. The molecular weight of fractions collected at the column outlet monotonically decreases as the number of fractions increases, but it is accompanied by a broadening of the distribution. The molecular weight distribution is narrow, especially for initial fractions. We demonstrate HOPC using silica gels as the porous materials for broad-distribution polystyrene, polystyrene standards, and other amorphous polymers.

Introduction

In polymer science, the need for polymer fractions with a narrow molecular weight distribution has been frequently encountered. Anionic polymerization may yield a relatively narrow molecular weight distribution,[1] but it is unavailable for many polymers. The absence of calibration standards often forces the results of gel permeation chromatography (GPC) to be stated in terms of the polystyrene-equivalent molecular weight.[2]

Three methods have been often used to physically separate a broad-distribution polymer. The first method is fractional precipitation.[3] A nonsolvent is added to a dilute solution of the polymer. The highest molecular weight components precipitate first and are separated from the solution. The molecular weight of the precipitate decreases as the nonsolvent is added further. The second method is field-flow fractionation.[4−6] An external field is applied in a direction perpendicular to a thin channel in which the solvent flows. A dilute solution of polydisperse polymer is injected into the channel in a narrow band. The field, by distributing the polymer components into stream lines with different velocities, induces a differential elution. Low molecular weight components that tend to stay closer to the channel center elute earlier. The third method is preparative GPC,[7,8] a scaleup version of analytical GPC.[9] In conventional GPC, a small amount of dilute polymer solution is injected into a column packed with solvent-filled porous materials, and the column is subsequently flushed with pure solvent to elute the polymer sample. Partitioning of polymer chains between the pore channels and the surrounding solution causes an inherent band broadening, even for a perfectly monodisperse polymer. Higher concentrations induce additional interactions and result in a more serious overlapping of bands. To minimize the loss of resolution, the injected volume has to be small and the concentration has to be low (<0.1 wt %, depending on the molecular weight). In preparative GPC, a longer column with a larger diameter is used to allow a larger injection volume. The flow rate is increased proportionally.[7]

All of the existing physical separation techniques have only a limited processing capacity. To separate a given amount of polydisperse polymer, they consume a large volume of solvent. Furthermore, recovery of the solutes from dilute eluents is not easy. To overcome the low processing capacity, enhanced partitioning fractionation (EPF) was recently proposed.[10,11] It is based on the size exclusion principle and forced migration of the polymer into pore channels by the high osmotic pressure[12−14] of a semidilute polymer solution in which the concentration is higher than the overlap concentration, c^*. The overlap concentration c^* is defined by $c^*(2^{1/2}R_g)^3 = M/N_A$,[15] where R_g is the radius of gyration of the polymer chain, M is the molecular weight, and N_A is Avogadro's number. In EPF, a concentrated solution of polymer is poured into solvent-filled porous materials. The volume of the solution is comparable to the total pore volume. Net repulsions between polymer chains produce a high osmotic pressure, and it drives the polymer molecules, preferentially low molecular weight components, into the pore channels where the osmotic pressure is lower. After equilibrium is reached between the pore channels and the solution exterior to the pore, the external solution is separated to recover a fraction enriched with high molecular weight components. A pure solvent is subsequently added to the porous materials to drive out low molecular weight components. The external solution enriched with low molecular weight components is separated from the porous materials. The EPF was demonstrated to have a large separation capacity.[11] The processing time was, however, long, especially at high concentrations for large molecular weight samples. The resolution with respect to molecular weight was lower than that predicted by theory.[10] In addition, one EPF process separates only two fractions.

While we were trying to improve the resolution of EPF, we came across a novel method of separation that realizes EPF at every plate in the column as the mobile phase is transferred. We call the method high osmotic

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pressure chromatography (HOPC). HOPC combines the processing capacity of EPF and the resolution of column chromatography. This contribution is the first report on HOPC. We will first explain the separation mechanisms in the HOPC process. We then will show examples of the results for the separation of a variety of amorphous polymers. We will also compare the performance conducted under various conditions.

High Osmotic Pressure Chromatography

The HOPC system uses a column packed with solvent-imbibed porous materials. A concentrated solution of polymer is prepared. The concentration needs to be sufficiently high (at least several times as high as c^*). The solution is injected slowly into the column by a high-pressure pump until the polymer is detected at the column outlet. The detection can be done by using a concentration detector, such as a UV−visible detector, a refractive index detector, or a light scattering detector, or by observing drops of the eluent in a collecting bottle (Schlieren texture). As soon as the polymer is detected, the injection is switched from the polymer solution to the pure solvent. The eluent is collected into different fractions until it becomes pure solvent. The HOPC system then is ready for the next batch of processing.

In HOPC, enhanced partitioning between the mobile phase (exterior to the porous materials) and the stationary phase (interior of the porous materials) is repeated in each plate, as the mobile phase is transferred to the next plate. The front end of the transported solution is equilibrated each time with a pure solvent in the stationary phase, thereby eliminating lower molecular weight components. When the front end reaches the column outlet, it is enriched with the highest molecular weight components present in the injected solution. The next portion of the mobile phase that follows the front end exchanges the polymer chains between the mobile phase and the stationary phase by enhanced partitioning. Since low molecular weight components are already present in the stationary phase, removal of these components from the mobile phase is not as easy as for the front end. Thus, the peak molecular weight decreases and the distribution broadens as the number of fractions collected increases. When the injection is switched to the solvent later in a HOPC process, the mobile phase is equilibrated with a high concentration of the polymer in the stationary phase. High molecular weight components are preferentially released into the mobile phase. At the next plate, the mobile phase is equilibrated with a stationary phase that contains components higher in molecular weight than those in the mobile phase. The stationary phase will release the high molecular weight components, and in exchange the mobile phase will squeeze low molecular weight components into the stationary phase. EPF is repeated in this way until the mobile phase reaches the outlet, thereby sweeping the highest molecular weight components remaining in the column. Note that the concentration in the column does not decrease immediately because of the large volume injection of the concentrated solution. Therefore, the partitioning follows EPF for nearly the entire process of HOPC.

We also point out that possibility of nonequilibrium partitioning. When the flow rate is high or the porous materials have a large bead size, the concentration equilibrium may not be reached before the mobile phase is transferred to the next plate. This nonequilibrium may benefit the separation because the low molecular weight components have a larger diffusivity and therefore will be driven into the stationary phase more rapidly than the other components. We cannot rule out, however, the possibility of all different components being driven into the pore channels equally by a large difference in the osmotic pressure between the two phases.

Table 1. Characteristics of Silica Gels

sample	supplier	pore diameter (nm)	bead size (μm)	pore volume (mL/g)	surface area (m^2/g)
grade 644	Davison	15	100	1.15	300
grade 643	Davison	15	52	1.15	300
grade 642	Davison	15	37	1.15	300
grade 641	Davison	15	18	1.15	300
grade 632	Davison	6	37	0.75	480
Fluka 100	Fluka	9	35	0.90	400

HOPC may appear similar to GPC. In GPC, the concentration equilibrium partitions the majority of the polymer in the mobile phase, even for the lowest molecular weight components. The tendency is more pronounced as the molecular weight increases. In contrast, in HOPC, the high osmotic pressure drives the majority of low molecular weight components into the pore channels.

The advantages of HOPC over preparative GPC include a high sample loading capacity. To separate a given amount of polymer, HOPC consumes ca. one-thousandth of the solvent, which is often hazardous, and about one-hundredth of the porous materials required in preparative GPC. Thus, HOPC is environmentally friendly and cost-effective. Another advantage may be the resolution with respect to molecular weight. The inherent band broadening limits the resolution of GPC. The separation of HOPC is based on EPF, which was predicted to have a high resolution[10] sufficient to separate anionically prepared polymer samples. Since HOPC repeats EPF, its resolution is expected to be even better. Compared with EPF, which uses equilibration and produces two fractions in each process, HOPC rapidly produces many fractions in a single process. The fractionation procedure is easy. The performance of the separation can be optimized by changing experimental conditions such as pore size, column length, and concentration of the injected solution. The most important feature of HOPC lies in its universality. It can be applied to a whole spectrum of polymers, including water-soluble polymers such as proteins. The only requirement is that the polymer dissolves in a solvent at high concentrations.

Experimental Section

Materials. The porous materials used are silica gels manufactured by Davison of W. R. Grace and Fluka Chemical Corp. The characteristics of the silica gels (data supplied by the manufacturers) are shown in Table 1. The pore volume and surface area data are the targets in manufacturing. The pores in the silica gels have a highly interconnected network structure. The pore diameter was calculated for a porous medium with uniform cylindrical pores of the same pore volume and surface area. The bead size is the median of the upper and lower mesh sizes.

Chlorotrimethylsilane [$(CH_3)_3ClSi$], cyclohexanone [$C_6H_{10}(=O)$], tetrahydrofuran (THF, HPLC grade), and toluene (HPLC grade) were obtained from Aldrich and used as received. Methanol, from EM Science, was filtered before use.

A broad molecular weight distribution polystyrene and poly(methyl methacrylate) (PMMA) were purchased from Aldrich, polystyrene standards (PS2M and PS900K) were from Pres-

Table 2. Columns and Packing Materials

column	column length (mm)	column diameter (mm)	gel sample	bead size (μm)	pore diameter (nm)	volume ratio V_I/V_E
G1	500	3.0	644	100	15	1.44
G2	500	10.0	644	100	15	1.96
G3	250	6.6	643	52	15	0.91
G4	500	6.6	643	52	15	0.96
S1	300	3.9	642	37	15	1.44
S1′	300	3.9	642	37	15	0.97
S2	300	3.9	632	37	6	1.58
S2′	300	3.9	632	37	6	1.35
S3	300	3.9	F100	35	9	3.24
S3′	300	3.9	F100	35	9	2.82
S4	200	3.9	642	37	15	1.92
S4′	200	3.9	642	37	15	1.42
S5	500	3.9	642	37	15	2.15
S5′	500	3.9	642	37	15	1.76
S6	300	3.9	644	100	15	1.85
S6′	300	3.9	644	100	15	1.81
S7	300	3.9	643	52	15	1.55
S7′	300	3.9	643	52	15	1.52
S8	300	3.9	641	18	15	0.65
S8′	300	3.9	641	18	15	0.64

sure Chemical, and poly(hexyl isocyanate) (PHIC) was from Polysciences. Polycarbonate (PC105) was supplied by GE.

Empty stainless steel and glass columns were purchased from Phenomenex and Omnifit, respectively.

Silanation. To minimize the adsorption of polymer molecules onto the pore surface, the silica gels used in HOPC were all treated. When untreated silica gels were immersed in a polymer solution, the polymer concentration decreased in the supernatant solution above a packed bed of the silica gels. By displacing silanol groups with the trimethylsilanyl group, the silica gels no longer adsorbed the polymer. The pore channels of silica gels, therefore, provide only geometrical confinement.

The surface treatment of the silica gels was conducted in several steps.[16] Silica gel beads were soaked in concentrated nitric acid at 50−60 °C overnight to remove organic impurities and rinsed thoroughly with deionized water until neutral. Then the beads were soaked overnight in concentrated hydrochloric acid at room temperature to remove metal ions and to activate the silica surface for silanation. The beads were again rinsed with deionized water until neutral. The cleaned silica beads were dried in a convection oven at 50 °C for 6 h and at 90 °C for 24 h and then allowed to cool to ambient temperature. The silica beads were transferred into a three-neck flask with a thermometer, nitrogen inlet, and ventilation stopcock. A solution of chlorotrimethylsilane in toluene (2 M) was added to the flask with nitrogen sparge. The flask was heated to 50−60 °C. When the temperature had stabilized, the reaction flask was closed under nitrogen flow. The flask was kept at the temperature for 72 h. The minimum requirement of the silanation agent was calculated by assuming the surface density of silanol to be 1 functionality per 0.1 nm^2. The actual amount added was 15 times the minimum requirement. The silanation reaction was quenched by adding filtered methanol. The treated silica beads were washed with methanol until the filtrate was neutral. The beads were then dried in the convection oven at 50 °C overnight and at 210 °C in a vacuum oven for 1 h.

Column Packing. A one-end-capped, glass or stainless steel (SS) column was fixed to a vertical support. Surface-treated, freshly dried silica gels were added through the top, open end of the column until the column was full. The column was connected to a high-pressure liquid pump (Scientific Systems Inc., AcuFlow Series II) through Teflon or SS tubing. A polar solvent, such as THF and cyclohexanone, was circulated through the column at a flow rate of 9.9 mL/min. When a void space was created above the packed bed of silica gels in the column, the top fitting was removed and dried silica gels were added. Then the fitting was reconnected, and the solvent was circulated again. This procedure was repeated until no void space was left in the column.

The sizes of the columns used are listed in Table 2. The column diameter is the interior diameter, i.d. The silica gels in the columns are also listed with the bead diameter and the pore diameter. The last column is the ratio of the total pore volume V_I to the interstitial volume V_E. V_I was calculated by using $V_I = m_{gel}v_{pore}$, where m_{gel} is the mass of the gels and v_{pore} is the pore volume per unit mass. The interstitial volume V_E was computed from $V_E = V_{column} - V_{gel} - V_I$, where V_{column} is the column volume and V_{gel} is the total volume of the solid phase of the silica gels, calculated from $V_{gel} = m_{gel}/\rho_{silica}$ with the density of silica ρ_{silica}. A column name followed by a prime denotes a column that was packed with silica gels to yield the volume ratio given in the table, but developed a void space after highly concentrated solutions were injected and hence a larger pressure was applied. The column was subsequently packed, resulting in an increase in V_I/V_E. The void space did not develop afterward. We use a column name without a prime for this nearly perfectly packed column.

Instrumentation. Our high osmotic pressure chromatography system consists of a high-pressure liquid pump, one of the packed columns, a UV−visible detector (Alltech Model 450 UV), a fraction collector (Eldex Model 1243), and auxiliary tubing (stainless steel tubing of $^1/_{16}$ in. o.d. and 0.020 in. i.d. between the pump and the column, and Teflon tubing of 1.6 mm o.d. and 0.5 mm i.d. for other places, where o.d. denotes the outer diameter). Prior to injection of the polymer solution in each batch of HOPC, the column was washed with the same solvent that was used to dissolve the polymer. The solution was injected from a vial into the column through the high-pressure pump. The injection was continued at a constant rate. As soon as the polymer was detected at the outlet, the injection was switched to the pure solvent and the eluent was collected into different test tubes, unless otherwise specified. The solvent injection was continued at a constant rate until no more polymer was eluted. The eluent collected was concentrated, except for later fractions. We collected a small volume of the eluent (ca. 30 drops) in each of the initial fractions. The intermediate fractions collected ca. 50 drops each and the later fractions ca. 150 drops. All of the HOPC processing was performed at room temperature. The addition of excess methanol caused precipitation of the polymer in each test tube. The tube was left in the refrigerator for 2 h and centrifuged. The supernatant solvent was discarded. The precipitate was then dried in a vacuum.

The molecular weight distribution for all of the fractions of polymer, including those in the injection solutions, were analyzed by using a Waters analytical GPC system with a Model 510 HPLC pump and a Model 410 differential refractometer. Waters Styragel columns (HR 4E, HR 5E, and HMW 6E) were used for all of the analyses, except for the polystyrene standards and their fractionated products. For the latter, columns packed with Phenogel 5 μm of pore sizes 10^3, 10^4, and 10^5 Å (Phenomenex) were used. The columns were housed in a column heater thermostated at 35 °C. The mobile phase was THF, and the flow rate was 1.0 mL/min. The concentration of the polymer solution was low to avoid the molecular weight dependence of the chromatogram, typically between 0.10 and 0.02 wt %, depending on the molecular weight of the analyte. Each set of columns was calibrated with polystyrene standards of molecular weights from 1.36×10^4 to 2.16×10^6 purchased from Pressure Chemical.

Results and Discussion

At the early stage of our study of HOPC, we used glass columns that withstand a pressure of 900 psi (6.2×10^6 N/m^2). By using a solvent isorefractive with silica, such as cyclohexanone and an equal weight mixture of toluene and THF, we could observe how the air was removed in the imbibition. Another advantage is that the displacement of the mobile phase could be followed visually in the solution and solvent injections, especially for a polymer that has a refractive index greatly different from that of the solvent. In the solution injection, the front end moved uniformly toward the column outlet, although the boundary became more

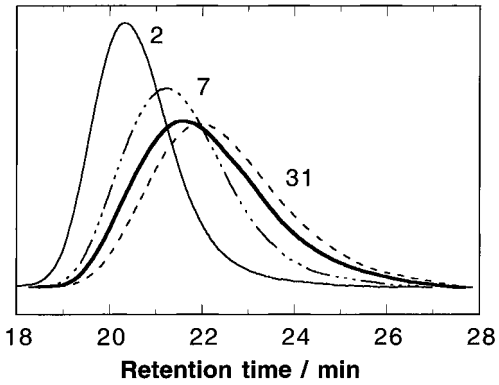

Figure 1. Examples of separation by HOPC. GPC chromatograms for fractions 2, 7, and 31 out of a total of 32 fractions obtained when 2.89 g of a 30.0 wt % solution of a broad-distribution polystyrene in THF was injected. The elution curve of the injected sample is also shown (thick solid line).

diffuse as it approached the outlet. When the polymer reached the outlet, the column was almost uniformly translucent except for the portion near the outlet. In the solvent injection, in contrast, the displacement was not uniform, and a fluid path more transparent than the rest of the column extended from the inlet. Eventually, the whole column regained transparency as the eluent dropped its concentration to zero.

Despite the preceding advantages, we could not use glass columns in most of the following experiments. A high back pressure due to the transport of a highly viscous fluid through the packed bed of small particles caused leakage from the Teflon fittings at the column inlet. We used mostly SS columns because they were free from leakage. The separation performance was similar between a Teflon column and a SS column compared at the same conditions.

Typical Separation Performance. We show a typical example of HOPC processing. In this example, column S5′ was used. The broad-distribution polystyrene was dissolved in THF at 30.0 wt %. The polystyrene had a weight-average molecular weight, M_w, of 2.5×10^5 and a number-average molecular weight, M_n, of 9.6×10^4 (polydispersity index, $M_w/M_n = 2.6$). The solution and solvent injection rates were 0.10 mL/min. The amount of the solution injected was 2.89 g. A total of 32 fractions was collected. The volume of the polymer solution injected is almost equal to the sum of V_I, V_E, and the dead volume in the pump head and tubing. The GPC chromatograms for fractions 2, 7, and 31 are shown in Figure 1 in comparison with a chromatogram for the injected sample. The curves are normalized so that the area above the baseline level is the same for all curves drawn. The earlier fractions have shorter elution times, indicating enrichment in the high molecular weight components. As the number of the fraction increases, the peak shifts to a lower molecular weight and broadens. The elution curves for most fractions collected are narrower than the curve for the injected sample. The purification effect is evident.

We characterized each chromatogram by the peak retention time, t_p, and the width, t_w, at half of the peak height. The limit of t_w available in our GPC system with Waters columns was 0.76−0.82 min, as identified in chromatograms for the polystyrene standards with $M_w \leq 6.7 \times 10^5$. Figure 2 shows t_p and t_w for fractions thus analyzed as a function of the cumulative mass of the fractions collected. In this example, 1.77 g of 20.7 wt % of the broad-distribution polystyrene solution in THF

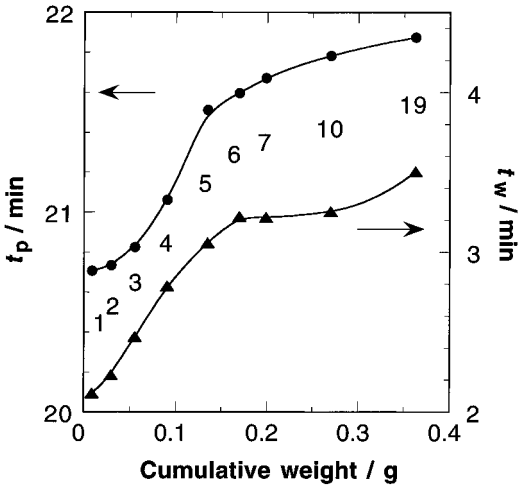

Figure 2. Peak retention time t_p (●) and half-width t_w (▲) of GPC chromatograms for fractions collected in HOPC, plotted as a function of the cumulative mass of the fractions. The arrows indicate t_p and t_w of the injected sample. The numbers of the fractions are given adjacent to the symbols.

was injected onto column S1. The solution was injected at 0.10 mL/min for 28 min and the solvent at 0.10 mL/min for 211 min to complete the elution of the polymer. The solvent was delivered at the rate set on the pump, but the solution injection was slower than the set value because of the difficulty in the delivery of the viscous liquid. A total of 22 fractions was collected. All of the polymer injected was recovered. The numbers in the figure denote the fraction numbers. A sigmoidal pattern is evident in the two curves, with t_p and t_w increasing monotonically. We did not analyze other fractions, but we expect that t_p and t_w for these fractions are located close to the curves drawn. The arrows shown in Figure 2 indicate t_p and t_w of the injected sample. As t_p of a collected fraction exceeds that of the injected sample, t_w becomes larger than that of the injected sample.

The tendency of t_p and t_w is reproducible for different batches of HOPC performed under the same conditions. In the following section we compare the performance of HOPC under different conditions by using the plot of t_w as a function of t_p. In particular, we pay attention to the span of t_p and the location of the t_w-t_p curve. A fractionation with its t_w-t_p curve located lower and with t_p values smaller for the initial fractions implies a better separation. Note that how the eluent is cut into different fractions affects t_p for the early and late fractions and t_w for all fractions. Therefore, small fluctuations in the span of t_p and in the location of the t_w-t_p curve from batch to batch cannot be avoided.

Repeated Application of HOPC. The molecular weight distribution can be narrowed further by repeatedly applying HOPC to the products obtained in the preceding batches. To demonstrate this narrowing, we first performed a couple of batches of fractionation on the original broad-distribution polystyrene in cyclohexanone by using column G1. We then made a 23.7 wt % solution of polystyrene in cyclohexanone by dissolving a few fractions with a similar molecular weight distribution obtained in the preceding batches. We injected 2.29 g of the solution into column G2 for the second processing. The solution and solvent injection rates were 0.01 and 0.06 mL/min, respectively. A total of 12 fractions was collected. Table 3 shows M_w, M_n, and their ratio for the original sample, the sample injected in the second HOPC, and fractions 2, 4, and 12. The second application of HOPC narrowed the molecular

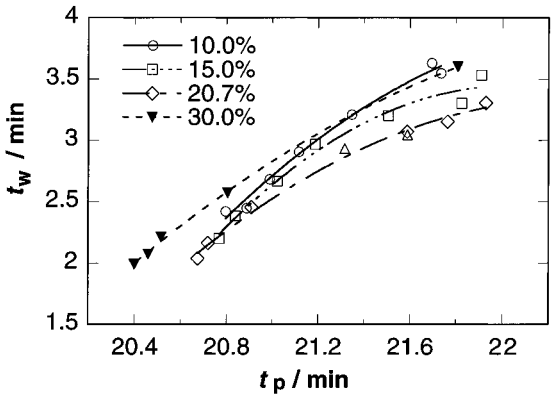

Figure 3. Concentration dependence of the t_w-t_p curve. Results for 10.0, 15.0, 20.7, and 30.0 wt % polystyrene in the injected solutions are shown. The lines are eye guides.

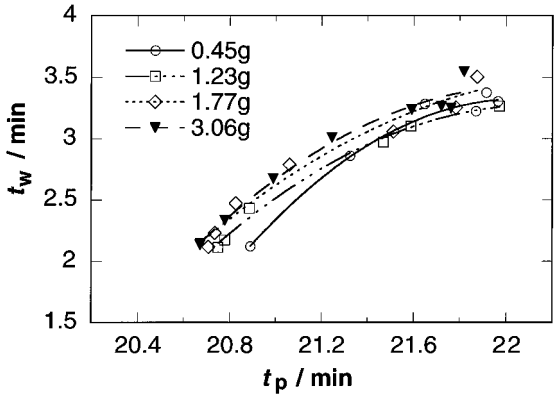

Figure 4. Injection volume dependence of the t_w-t_p curve. Results for injections of 0.45, 1.23, 1.77, and 3.06 g of a 20.7 wt % polystyrene solution are shown.

Table 3. Average Molecular Weights of the Fractions Obtained in the Second Application of HOPC

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
original	30.0	10.3	2.90
injected	44.4	28.5	1.55
2	67.1	49.2	1.36
4	59.1	42.0	1.41
12	36.5	24.1	1.52

weight distribution further, especially for the initial fractions.

Dependence on the Operational Conditions. The performance of HOPC as represented by the resolution and throughput can be affected by many parameters. The performance is expected to depend on the operational conditions, such as the polymer concentration in the injected solution, the injection volume, and the solution and solvent injection rates. The performance will also be a function of column conditions, such as the column length, pore size, particle size, pore volume, and pore size distribution. The column conditions will be examined later. In the following section, we first compare the HOPC results obtained under various operational conditions.

1. Concentration Dependence. We prepared solutions of the broad-distribution polystyrene in THF with concentrations of 10.0, 15.0, 20.7, 25.0, and 30.0 wt %. Column S1 was used for the five batches of HOPC. The solution and solvent injection rates were 0.10 mL/min. To avoid congestion, Figure 3 shows the t_w-t_p plot for only four concentrations. The curve for the 25 wt % injection (not shown) has a span of t_p and location similar to those for the curve for the 30.0 wt % injection. Later fractions have a slightly narrower t_w. In the figure, except for the 30.0 wt % injection, a decrease in concentration resulted in a narrower span of t_p and an increase in t_w, especially for later fractions, indicating a loss of resolution. As the concentration increases, the segregation boundary between high and low molecular weights shifts to a higher molecular weight. The increase in the concentration thus results in a shorter t_p in the initial fractions. The 30.0 wt % injection exhibited the shortest t_p in the initial fractions, but t_w was broader than that of the 20.7 wt % injection compared at the same t_p. When the concentration is too high, the stationary phase also accepts high molecular weight components, thus lowering the separation resolution.

The concentration of the mobile phase varies widely at different plates in the column and at different stages of HOPC processing. The resolution is optimized when the concentration is held to be several times as large as the overlap concentration for as many plates as possible in the column throughout the process.[10] Therefore, if the injected polymer solution is too concentrated, on the one hand, all of the fractions will suffer from a decrease in the resolution, except for initial fractions that take advantage of a diluted (but still above c^*) solution front in the mobile phase. On the other hand, if the polymer solution is too dilute, separation will deteriorate for all of the fractions. In particular, the initial fractions will lose resolution, as the front end of the mobile phase injected is too dilute for EPF to function properly. Then, the situation will be similar to that of GPC in which most of the analytes are partitioned in the mobile phase. Therefore, an optimal concentration exists for the injected solution. In addition, practical factors such as the solution viscosity might limit the maximum concentration because the highly viscous solution is more difficult to inject by a reciprocating piston pump and produces a higher back pressure. However, the dependence of the t_w-t_p curve on concentration is weak. HOPC accepts a rather broad range of concentrations of the injected solution.

2. Injection Volume Dependence. A 20.7 wt % solution of the broad-distribution polystyrene in THF was used. Except for different volumes of injection, none of the conditions changed from those used in the HOPC batches for Figure 3. The injection volumes (measured by mass) were 0.45, 1.23, 1.77 (injection was switched to THF when the polymer was detected at the column outlet), 2.50, and 3.06 g. Figure 4 shows their t_w-t_p curves. The 2.50 g injection performed similarly to the 3.06 g injection and is not shown. As the injection volume decreased, t_p in the initial fractions increased, and the span of t_p decreased. For the smallest injection volume, the second fraction's t_p was already as large as 21.3 min. The amount of polymer in the first fraction with $t_p \cong 20.8$ min was much smaller than that in the initial fractions of larger injection volume. It is apparent that the low volume injection causes the performance to deteriorate. If the transport of a less concentrated solution in the mobile phase is uniform and does not involve channel creation in the column when the solvent is being injected, then t_p and t_w of the initial fractions should not depend on the injection volume. The deterioration observed for the injection of the smallest volume indicates nonuniform transport. We consider that the solvent channel penetrated the solution band in the column before the front end of the polymer solution reached the outlet. Therefore, the concentration in the column was not sufficient to cause EPF at

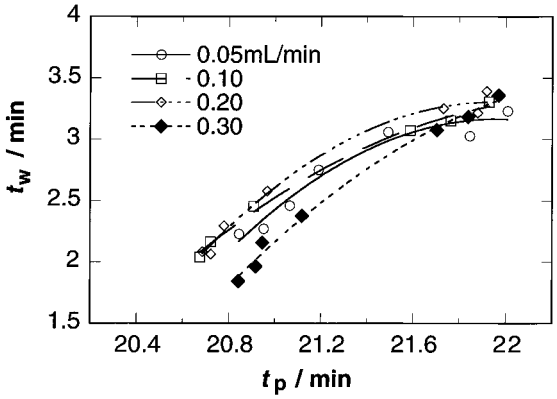

Figure 5. Dependence of the t_w-t_p curve on the solution injection rate. Results for 0.05, 0.10, 0.20, and 0.30 mL/min injection of a 20.7 wt % polystyrene solution are shown.

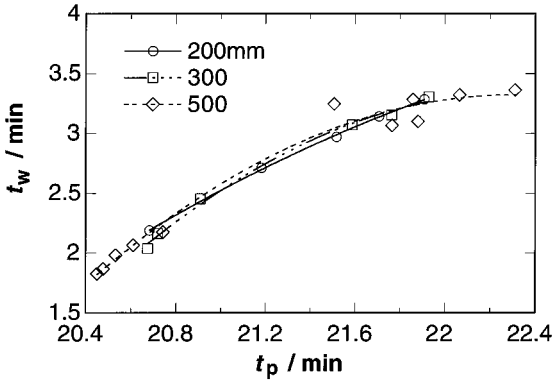

Figure 6. Column length dependence of the t_w-t_p curve. Results for 200, 300, and 500 mm columns are shown.

every plate throughout the HOPC process. In contrast, the initial fractions obtained in the overloading (3.06 g injection) are similar to those for the injections of a smaller volume as expected, although the overload tends to increase t_w for later fractions. HOPC allows overloading, thus increasing the processing capacity without sacrificing the resolution.

Figures 3 and 4 indicate that decreasing the concentration of the injected solution or decreasing the volume of injection, thus approaching the operational conditions of conventional GPC, causes deterioration in the resolution of HOPC. The separation principle is different between HOPC and GPC, although they may appear similar.

3. Dependence on the Solution Injection Rate. Column S1 was used to study the dependence of HOPC on the solution injection rate. A solution of the broad-distribution polystyrene in THF (20.7 wt %) was injected at 0.05, 0.10, 0.20, and 0.30 mL/min. The solvent injection rate was held constant at 0.10 mL/min. Figure 5 shows the t_w-t_p curves. The curves for 0.10 and 0.20 mL/min solution injection rates are similar. The curve for the 0.05 mL/min injection rate is located lower, but t_p begins at a larger value. The t_w-t_p curve for the 0.30 mL/min injection rate is the lowest, and the t_p of its first fraction is larger than that for the 0.10 and 0.20 mL/min curves. It is to be noted that, although t_p is large, early fractions have a small t_w. The amount of polymer recovered in early fractions was larger than that obtained with slower solution injection rates (0.10 and 0.20 mL/min), indicating that the exclusion of higher molecular weight components was more strict in the 0.30 mL/min injection. The observed dependence is ascribed to the nonequilibrium effect. The relatively longer equilibrium time available with the 0.05 mL/min injection rate, on the one hand, allowed the high osmotic pressure of the mobile phase to also push larger molecules into the pore channels, resulting in an increased t_p in the initial fractions. On the other hand, a higher flow rate transferred the mobile phase to the next plate before it reached equilibrium with the stationary phase. Since the low molecular weight components diffuse faster into the stationary phase than do the high molecular weight components, the quick transfer is considered to have resulted in a more stringent segregation. The threshold molecular weight in this nonequilibrium separation is lower than it is with the lower injection rates. Therefore, the initial fractions resulted a larger t_p, but their distributions were narrower. The higher injection rate also results in a shorter processing time. Thus, the higher injection rate is preferable as long as the system can tolerate the increased back pressure.

We also studied the dependence on the solvent injection rate. A 20.7 wt % solution of the broad-distribution polystyrene was injected at a common rate of 0.10 mL/min onto column S1. The solvent was injected at rates of 0.05, 0.10, 0.20, and 0.30 mL/min. Compared with the solution injection rate, the rate of solvent injection exhibited a less marked effect on the t_w-t_p curve over the whole range of t_p values. The 0.05 and 0.10 mL/min solvent injection rates had better results. A further increase in the solvent injection rate lifted the t_w-t_p curve slightly and decreased the t_p span.

Dependence on Columns. In the HOPC system, the column itself is a dominant factor in the resolution. Among the parameters that specify the column, the pore size and particle size of the packing materials can affect the performance. The column length will also be an important factor. In the following section, we will examine the performance of HOPC for various column conditions.

1. Column Length Dependence. Columns S4, S1, and S5 were used. The three columns have the same i.d. of 3.9 mm. The lengths are 200, 300, and 500 mm, respectively. A 20.7 wt % solution of the broad-distribution polystyrene dissolved in THF was injected onto the column at 0.10 mL/min. The solvent was injected at 0.10 mL/min. Figure 6 shows t_w-t_p plots for the three columns. The performance of the 500 mm column is the best. It shows the largest span of t_p values and the shortest t_p for initial fractions. The shortest column showed the worst performance. The amount of polymer in the first fraction was only one-fifth of that in the first fraction for the 300 mm column. The t_p value for the second fraction had already reached 21.2 min.

The dependence on the column length can be explained in terms of the number of plates in the column. The greater the number of plates, the better the resolution. The long column may, however, cause the concentration of the front end of the transported solution to decrease to a level much lower than c^* by the time it reaches the outlet. Then, plates close to the outlet may not practice EPF for the front end of the solution. Instead, a simple separation based on the conventional GPC principle may be carried out, with most of the polymers being excluded by the pore. Fortunately, this partitioning does not broaden the molecular weight distribution for the initial fractions that have dropped low molecular weight components in the early plates. The majority of the solution will, however, benefit from the increased plates that practice EPF.

We can avoid idling in these plates by injecting a more concentrated solution. Therefore, a longer column can possess a larger processing capacity than a shorter column, although it pays the penalty of increased back pressure. It is thus recommended that one use a column as long as possible and inject a solution sufficiently concentrated, if the HOPC system tolerates the increased pressure.

2. Pore Size Dependence. Columns S1, S2, and S3 were used. They were packed with silica gels of average pore diameters of 15, 6, and 9 nm, respectively. A solution of 20.7 wt % of the broad-distribution polystyrene in THF was injected at 0.10 mL/min, and the solvent was injected at 0.10 mL/min. In the t_w-t_p plot (not shown), the span in t_p was almost the same for the three pore sizes, but the range of t_p values shifted to a longer time as the pore size decreased. The smaller pore size excelled in the separation of low molecular weight components, while the larger pore did well in the separation of the high molecular weight components. It was expected that 6 nm pore size gels would work better for polymers with a small molecular weight that have comparable chain lengths. We ascribe the weak dependence to the broad pore size distribution in the silica gels. Use of better defined porous materials, such as nanochannel array glasses,[17] will exhibit more marked pore size dependence and improve HOPC resolution.

We also studied the particle size dependence. The diffusion of molecules in a medium determines the rate for the equilibration of polymer chains between the stationary and mobile phases. A larger particle size of the porous material will require a longer time. We expect that the result will be similar to that obtained with a faster injection rate. A 20.7 wt % solution of the broad-distribution polystyrene in THF was injected at 0.10 mL/min onto columns S8, S1, S7, and S6. They are packed with silica gels of average particle sizes of 18, 37, 52, and 100 μm, respectively. The pore diameter was fixed at 15 nm. The t_w-t_p plots (not shown) show a weak dependence of HOPC performance on particle size. The spans in t_p and the locations of the t_w-t_p curve are almost the same for the particle sizes tested. We do not know why the performance was similar.

3. Packing Density Dependence. The column packing density has two competing effects on the resolution. An increase in the packing density increases the number of plates, thereby improving the resolution. It accompanies, however, an increase in the volume ratio V_I/V_E. A large volume in the stationary phase compared with the volume in the mobile phase will result in a less efficient EPF, because there is not much space available for the high molecular weight components. The stationary phase therefore will be forced to accommodate more high molecular weight components than with a smaller V_I/V_E.[10] This second effect will lower the resolution of HOPC.

Columns S1′ and S1 (300 mm length), with V_I/V_E of 0.97 and 1.44, respectively, and S5′ and S5 (500 mm length), with $V_I/V_E = 1.76$ and 2.15, respectively, were used. These columns have the same packing material, but different packing densities. The 500 mm length columns have high ratios because of the high back pressure. A solution of 20.7 wt % of the broad-distribution polystyrene in THF was injected at 0.10 mL/min, and the solvent was injected at 0.10 mL/min. The t_w-t_p curves (not shown) show a similar tendency for the two different column lengths. The column with the lower V_I/V_E (or closer to unity) performed better than that with the higher V_I/V_E. The second effect is considered to be dominant in these long columns that already have a sufficient number of plates. We also investigated the packing density dependence for the 200 mm length column. The tendency was the opposite. We consider that the first effect was dominant in this short column.

Table 4. Average Molecular Weights of the Fractions Obtained from PS2M

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
injected	174	135	1.29
1	203	180	1.13
5	186	156	1.19
24	157	104	1.51

Table 5. Average Molecular Weights of the Fractions Obtained from PS900K

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
injected	92.6	85.0	1.09
2	95.9	89.1	1.08
18	91.7	81.9	1.12
20	74.7	63.8	1.17

Table 6. Average Molecular Weights of the Fractions Obtained from PMMA

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
injected	50.6	14.8	3.43
1	76.0	41.3	1.48
5	61.1	23.5	2.60
16	43.8	14.8	2.97

Polystyrene Standards. We applied HOPC to polystyrene standards that already have a narrow distribution of molecular weight. In the first example, 2.94 g of a 8.0 wt % solution of PS2M ($M_w = 1.74 \times 10^6$, $M_n = 1.35 \times 10^6$) in THF was injected at 0.10 mL/min onto column S5′, and the solvent was injected at 0.10 mL/min. The average molecular weights are shown in Table 4 for fractions 1, 5, and 24 out of a total of 24 fractions obtained. Compared with the separation for the broad-distribution polymer, the separation for PS2M appears worse. The molecular weight distribution for the first fraction, however, narrowed by a factor better than the square root. Because of the inherent band broadening, GPC analysis does not have sufficient resolution to quantify how much the distribution narrowed with $M_w/M_n \leq 1.1$. Nevertheless, the table shows that the fractions obtained have a retention time shorter or longer than that of the injected sample. Moreover, narrowing of the distribution was evident, and the low molecular weight tail was successfully curtailed for the initial fractions. The later fractions contained more of the low molecular weight tail.

We also applied HOPC to another polystyrene standard sample. A 1.66 g, 8.0 wt % solution to PS900K ($M_w = 9.26 \times 10^5$, $M_n = 8.50 \times 10^5$) in THF was injected onto column S1′ at 0.10 mL/min. The solvent was injected at 0.10 mL/min. The M_w, M_n, and their ratio for fractions 2, 18, and 20 out of a total of 20 fractions are listed along with those for the injected sample in Table 5. The amount of polymer in fraction 20 was small.

Other Amorphous Polymers. We applied HOPC to PMMA, PHIC, and PC. Table 6 shows the molecular weight data with reference to polystyrene for fractions 1, 5, and 16 out of a total of 16 fractions when HOPC was applied to 2.05 g of a 9.9 wt % solution of PMMA in THF. The solution and solvent were injected at 0.10 mL/min. Column S1′ was used. The molecular weight

Table 7. Average Molecular Weights of the Fractions Obtained from PHIC

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
injected	32.8	66.8	4.92
1	48.7	22.9	2.13
2	40.1	15.2	2.63
3	37.4	11.3	3.31
8	32.2	4.96	6.49

Table 8. Average Molecular Weights of the Fractions Obtained from PC105

sample	$10^{-4}M_w$	$10^{-4}M_n$	M_w/M_n
injected	4.73	2.47	1.92
1	8.34	5.86	1.42
3	6.16	3.37	1.83
13	4.16	1.44	2.89

distribution for the initial fraction narrowed by a factor better than the square root.

We also tried to separate a semirigid polymer, poly(hexyl isocyanate) (PHIC), by HOPC. The confinement effect on the partitioning and on chain diffusion is different from that for a flexible chain.[18] Column S1′ was used for this separation. A total of 1.62 g of a 7.86 wt % solution of PHIC in THF was introduced into the column at 0.05 mL/min, and the solvent was injected at 0.10 mL/min. The average molecular weights are listed in Table 7 for fractions 1, 2, 3, and 8 out of a total of 31 fractions. This result indicates that semirigid polymers can be fractionated by HOPC into a narrow molecular weight distribution. It is noted that isotropic solutions of a rodlike polymer usually have a higher viscosity than the solution of a flexible chain polymer, compared for the same molecular weight at the same concentration.[19] We could not increase the concentration of the injected solution because the resultant high viscosity prevented the solution from being delivered successfully by the reciprocating pump equipped with inlet and outlet check valves.

We applied HOPC to PC105 ($M_w = 4.73 \times 10^4$, $M_n = 2.47 \times 10^4$). Column S7′ was used. A total of 2.20 g of a 10.0 wt % solution of PC105 in cyclohexanone was introduced into the column at 0.10 mL/min, and the solvent was injected at 0.10 mL/min. The molecular weight data are shown in Table 8 for fractions 1, 3, and 13 (total 18 fractions). This result indicates that HOPC works with polycarbonate as well. The initial fraction picked up the high molecular weight components present in the injected sample.

Concluding Remarks

We have shown that HOPC can separate polymers with respect to molecular weight, as expected. This technology offers the advantages of high-capacity fractionation and high resolution compared with conventional methods.

We sometimes encountered a problem in the injection of a concentrated polymer solution. The problem was serious for high molecular weight polystyrene and PHIC. We could not increase the concentrations further, along an increased concentration may have produced better resolution. The outlet check valve of the reciprocating pump prevents counterflow by the ruby ball's returning back on its seat by gravity. If the liquid is too viscous, the check valve will be left open. As a result, solution injection was quite slow. We are now improving the injection method.

In a preliminary theoretical estimation of HOPC performance, we find that a single process of HOPC can bring the molecular weight distribution of a broad-distribution sample down to that of commercial polystyrene standards, if the porous material consists of straight cylindrical pores of uniform radius. The results we obtained here with silica gels are much worse than the estimate. It was also a surprise that the silica gels with the same pore size fractionated different polymer samples over a broad range of molecular weights equally well (from PC105 with $M_w = 4.73 \times 10^4$ to PMMA with $M_w = 5.06 \times 10^5$). The size-sensitive segregation principle of HOPC is expected to exhibit strong dependence on the solute dimension, if the pore size distribution is small. We are using another type of porous materials that have a narrower pore size distribution. The improved results will be reported in the near future.

Since the concentration of the injected solution for HOPC is high, the eluent still has a high concentration, except for the early and late fractions. Therefore, most of the eluent can be fed directly to the next HOPC column. By cascading, we will be able to further narrow the molecular weight distribution.

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