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Preparation of narrow-distribution polyvinylpyrrolidone by multi-stage high osmotic pressure chromatography ☆

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Abstract

Separation of polyvinylpyrrolidone, a water-soluble polymer of neutral charge, by high osmotic pressure chromatography (HOPC) is presented. HOPC, suitable for preparative separation of polymer by molecular weight, has been applied to various organic-soluble polymers. We demonstrate here that HOPC can also separate water-soluble polymers and is capable of separating fractions of narrower polydispersity compared to preparative gel permeation chromatography (GPC). Typically fractions with a polydispersity index (PDI) of 1.4–2 were generated from broad-distribution polymers (PDI ~ 5) in significant quantities from a single separation. By multi-stage separation, standard-grade fractions were obtained. Single- and multi-pass fractions were characterized by both GPC with narrow-standard calibration and GPC in tandem with a multi-angle laser light scattering detector.

Keywords: Polyvinylpyrrolidone; High osmotic pressure chromatogaphy; Multi-angle light scattering detector

1. Introduction

High osmotic pressure chromatography (HOPC) was developed a few years ago as a technique for large-scale separation of polydisperse polymers by molecular weight (MW) [1–3]. A solution of polymer, at least several times as concentrated as the overlap concentration, is injected into a column packed with solid porous materials until the whole column is filled with the solution. Then, the injection is switched to the pure solvent, and the eluent is collected into different fractions. Injection of a large volume of a concentrated solution renders a high processing capacity and low solvent consumption.

It is believed that HOPC's separation is based on segregation of polymer chains between the pore spaces (stationary phase) and the surrounding solution (mobile phase) [1–3]. At low concentrations, each polymer chain is partitioned independently according to its dimension. The partition coefficient, defined as the ratio of the concentration in the pore to that in the surroundings, decreases as MW increases (size exclusion). At higher concentrations, interaction between chains changes the partitioning rule. The high osmotic pressure of the solution forces polymer chains, especially low-MW components, to enter the pore spaces with a proportion much higher than at low concentrations [4,5]. Low-MW components are enriched in the stationary phase and depleted in the mobile phase [6,7]. Note that, at low concentrations, the mobile phase never becomes deficient in any component. Segregation of polymer chains between the two phases is repeated as the concentrated solution is transported along the column, thereby enriching the front end with high-MW components [1]. Thus, the early eluent has a MW distribution centered on the highest end of the distribution of the original polymer. Typically the polydispersity index, defined as M_w/M_n, where M_w and M_n are the weight-average MW and the number-average MW, decreases in the early fractions to 1/3–1/5 power of the index of the original polymer, for instance, from 2.0 of the original to 1.20 [2]. It was also predicted in theory that HOPC's resolving power is higher than the one available in regular gel permeation chromatography (GPC) [3]. HOPC may therefore offer a versatile method to prepare standard-grade polymer fractions for GPC calibration and other purposes.

In the past, HOPC was employed exclusively to separate organic-soluble polymers such as polystyrene, poly(methyl methacrylate), and polycaprolactone in organic media using silanized porous silica [1–3]. In this article we show that HOPC can also separate water-soluble polymers in aqueous media. Water-soluble polymers are increasing their

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Table 1
Characteristics of PVP

Polymer	K-value	Molecular weight (g mol^{-1})	R_g (nm)
PVP K-15	13–19	12 000	5
PVP K-30	26–35	57 500	14.5
PVP K-60	50–62	400 000	29

importance and presence in many applications. A narrow-distribution polymer fraction is essential in capillary gel electrophoresis for high-resolution DNA sequencing [8], for instance. As a typical water-soluble neutral polymer, we chose polyvinylpyrrolidone (PVP), available in several viscosity grades. PVP is a linear flexible polymer [9] and is widely used in adhesives, personal care products, paper manufacturing, and in synthetic fibers. At atmospheric pressure, the PVP–water system remains in a clear solution up to 100% PVP from 0 to 100°C [10]. It was possible to prepare standard-grade fractions of PVP in a three-stage separation. Analysis of the fractions in GPC with an on-line multi-angle laser light scattering (MALLS) detector demonstrated that HOPC surpasses GPC in resolution.

2. Experimental

2.1. Materials

Three grades of PVP samples (K-15, K-30, and K-60) obtained from International Specialty Products were used as received. The characteristics of these PVP samples provided by the manufacturer are listed in Table 1. Another PVP K-30 sample was obtained from Fluka and was coded as PVP K-30F. The K-value assigned to each grade of PVP represents the dependence of the solution viscosity on the concentration. The larger the value, the viscosity is greater compared at the same concentration. The root-mean-square radius of gyration, R_g, was obtained in GPC-MALLS at the peak of the light scattering intensity. Water used as the mobile phase and the solvent in HOPC was obtained from a water purifier, Easypure UV (Barnstead).

Controlled pore glasses (CPG) [11] with different pore sizes were used as separation media. The pore surface was either acid-washed or treated with chlorotrimethylsilane. For both these cases, CPG was first immersed in nitric acid under stirring at about 60°C and kept overnight and then rinsed with deionized water until it was neutralized. It was then dried in the oven. The silanization procedure is described elsewhere [1]. The manufacturer-supplied characterization data for various grades of CPG used are listed in Table 2. In the present article, we code the acid-washed CPG as CPGxxxY-OH and trimethylsilanol-substituted CPG as CPGxxxY-TMS, where CPGxxxY denotes the sample name of the original CPG. Earlier, it was shown that the performance of HOPC does not depend appreciably on the particle size. We therefore used two sizes ($Y = B$ for 120/200 and C for 200/400 mesh sizes), depending on the availability.

2.2. HOPC system

The HOPC system is similar to the one used in the earlier study [1]. The system consists of a high pressure liquid pump (SSI, AcuFlow series II), a stainless steel column packed with one of the CPGs, a fraction collector (Eldex, model 1243) with a drop counter, and a differential refractometer (Waters, R401). Prior to injection of the polymer solution in each batch of HOPC, water was circulated through the column for at least 1 h. A concentrated solution of PVP was injected from a vial into the column through the pump at a constant injection rate. When the polymer was detected at the outlet, the injection was switched to pure water. The eluent of the column was collected as follows: when a column of 3.9 mm (interior diameter, ID) by 300 mm (length) was used, 15 drops were collected in each of the fractions 1–6, 30 drops in fractions 7–10, and 150 drops in fractions 11–14. When the column dimension was 7.8 mm (ID) by 300 mm (length), approximately four times as many drops were collected in each fraction. The amount of solution injected was about 1.8 and 6.0 g for the two column dimensions, respectively. The flow rate of 0.1 and 0.3 ml min^{-1} was used for the two column dimensions.

2.3. Gel permeation chromatography

To characterize the MW distribution for the original and fractionated samples, we used two different GPC systems. The first GPC system, used for routine characterization, consists of a Waters model 510 HPLC pump, a model 410 differential refractive index (DRI) detector, and three Shodex columns (OH-Pak SB803, SB804, and SB805). The columns were housed in a column heater thermostatted at 35°C. The mobile phase was 0.1 M KCl aqueous solution,

Table 2
Characteristics of CPG

Sample	Supplier	Mean pore diameter (Å)	Pore volume (ml g^{-1})	Particle size (mesh size)
CPG75B	Dr. Haller	85	0.59	100/200
CPG120C	CPG, Inc.	130	0.68	200/400
CPG170B	Dr. Haller	177	1.11	100/200
CPG240B	CPG, Inc.	242	0.89	120/200
CPG350C	CPG, Inc.	364	0.77	200/400
CPG500B	CPG, Inc.	500	0.97	120/200

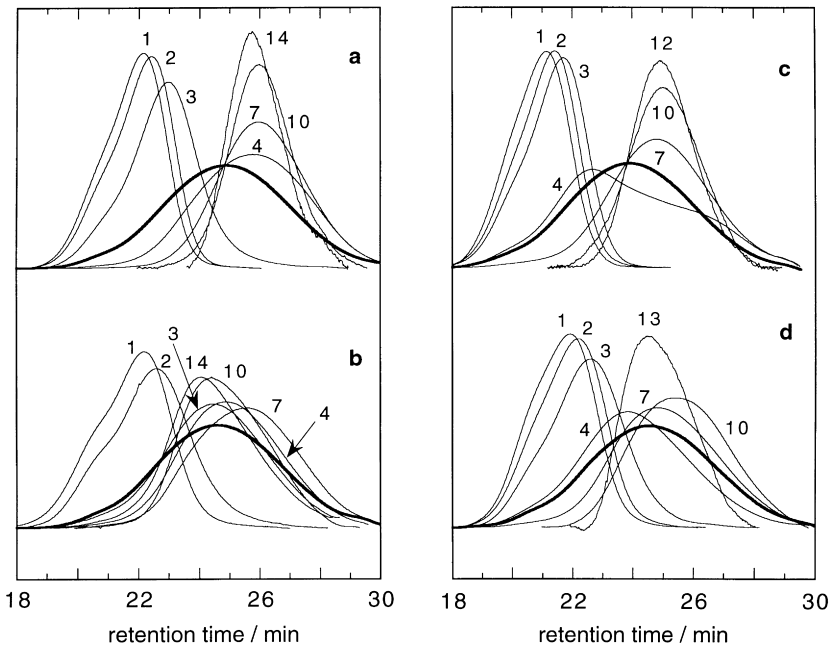

Fig. 1. GPC chromatograms for some of the fractions obtained in HOPC separation of PVP K-30F in a 30 wt.% aqueous solution: (a) the first batch and (b) the sixth batch with a column packed with CPG120C-TMS; (c) the first batch and (d) the fourth batch with a column packed with CPG120C-OH. The thick lines represent a chromatogram for the injected sample. The numbers adjacent to the lines indicate fraction numbers.

and the flow rate was 1.0 ml min^{-1}. The concentration of the polymer solution was low to avoid overloading, typically between 0.02 and 0.10 wt.%. The columns were calibrated with poly(ethylene glycol)/poly(ethylene oxide) standards of MW from 970 to 250 000 purchased from Scientific Polymer Products.

To prove the high resolution of HOPC, we also analyzed some of the fractions by using another GPC system with an on-line MALLS detector. The system comprises a Waters 590 HPLC pump, a Waters 410 DRI detector, a Wyatt Dawn MALLS detector, and two Shodex OH-Pak SB80-MHQ linear columns. The two detectors are connected in series. The mobile phase was 0.1 M $LiNO_3$ aqueous solution, and the flow rate was 0.5 ml min^{-1}. A Zimm plot constructed from the detector signals at 10 scattering angles between 51.5° and 152.6° was used to calculate M_w of the polymer in the eluent in a narrow slice of retention volume. If the MW distribution is sufficiently narrow, M_w equals the absolute MW, which is the advantage to combining GPC with light scattering detection.

3. Results and discussion

3.1. Effect of the pore surface

We compared the separation performance for a trimethylsilanol surface (CPG-TMS) and a silanol surface (CPG-OH). The former is slightly hydrophobic, and the latter is hydrophilic. Separation of organic-soluble polymers in HOPC has been done by using CPG-TMS of various pore diameters [1–3].

A 30 wt.% solution of K-30F in water was injected into each of a column packed with CPG120C-TMS and a column packed with CPG120C-OH. The column dimension was 300×3.9 mm^2 in both cases. The separation was repeated several times to examine the reproducibility. Fig. 1(a) shows the chromatograms for some of the fractions obtained in the first batch with the CPG120C-TMS column. The chromatograms were obtained using the three-column GPC system with a DRI detector. The chromatogram of the original K-30F injected is drawn in a thick line. The number adjacent to each curve indicates the fraction number. Each chromatogram is normalized by the area under the peak. Early fractions have a MW distribution centered at the high end of the distribution of the original sample. This feature is the same as the one observed in separation of many organic-soluble polymers in organic solvents [1–3]. The figure, however, shows equally good separation in late fractions centered at the low end of the original distribution. The latter was not observed in HOPC with organic solvents [1–3]. In the subsequent batches of separation with the same column, however, the performance was worse. Fig. 1(b) shows the result for the sixth batch. Early fractions have a low MW tail compared to those of the first batch. Later fractions, including fraction 3, are not much different from the original sample.

The situation was different for the separation with the CPG120C-OH column. Fig. 1(c) shows the chromatograms for some of the fractions obtained in the first batch. The peaks of the first three fractions are narrow and centered

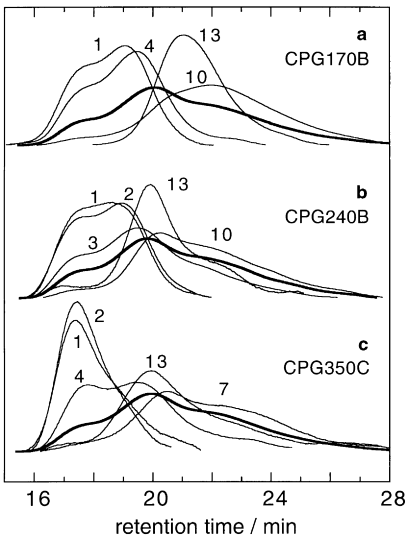

Fig. 2. GPC chromatograms for some of the fractions obtained in HOPC separation of PVP K-60 dissolved in water. Columns used were packed with: (a) CPG170B-OH; (b) CPG240B-OH; and (c) CPG350C-OH, respectively.

at the highest end of the original distribution. Their peak heights exceed those in Fig. 1(a). Late fractions have as narrow a MW distribution as that in the counterparts in Fig. 1(a). When the column was fresh, the overall performance between the two pore surfaces was similar. As with the CPG120C-TMS column, the performance was worse in the second batch, but the performance stabilized in the subsequent batches. Fig. 1(c) shows the result for the fourth batch. The deterioration in the performance from the first to the fourth batch is smaller compared with the one experienced using the other column. The early fractions (1 and 2) are still free of low-MW components. The last fraction also has a narrow MW distribution.

Reproducibility test was conducted using other columns also. For instance, a column packed with CPG170B-OH was used to separate a 20 wt.% solution of K-60 in water. Comparing the first and sixth batches, we find that there was virtually no difference, especially in the early fractions (1–3). We did not test the reproducibility with CPG170B-TMS. All of the following separations was done by using acid-washed CPG.

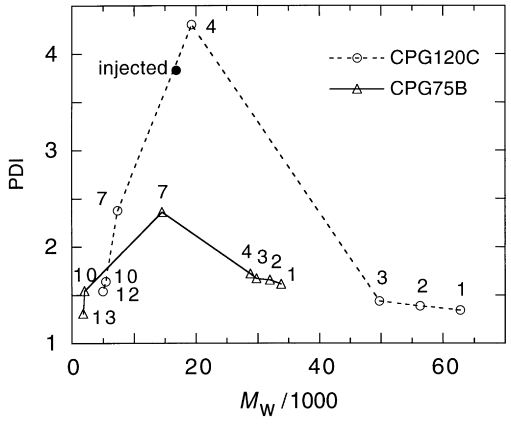

Fig. 3. M_w/M_n is plotted as a function of M_w for fractions obtained in HOPC separation of PVP K-30F at 30 wt.% in water by using columns packed with CPG120C-OH (open circles), and CPG75B-OH (open triangles). The closed circle indicates the original K-30F. The numbers adjacent to the symbols indicate the fraction numbers.

3.2. Effect of the pore size

In nonaqueous HOPC, it was shown that the separation performance for a given polydisperse polymer depends on the pore size. We used here three columns of a dimension of 3.9 × 300 mm^2, each packed with CPG170B-OH, CPG240B-OH, or CPG350C-OH (mean pore diameters are 177, 242, and 364 Å, respectively) to separate K-60 in a 25 wt.% aqueous solution. Fig. 2(a)–(c) compare the GPC chromatograms obtained for some of the fractions separated by the three columns. These columns had been used several times before. The original K-60 chromatogram, shown in a thick line, reveals a multi-modal MW distribution with three peaks around 17.5, 20, and 22 min, indicating that K-60 is a mixture of a few unimodally distributed fractions different in the peak MW. Fractions 1–4 obtained in the separation with CPG170B-OH exhibited two peaks. The lower-MW peak, which constitutes the majority component in these fractions, shifted to a longer retention time as the fraction number increased. The peak in fraction 1 at about 19 min is different from the second peak (~ 20 min) in the original K-60. It appears that separation by HOPC uncovered a component hidden in the broad distribution of the original polymer. The second peak of fraction 4 is considered to have resulted from a mixture of the newly revealed component and the component responsible for the peak at about 20 min. Fractions 7–10 mainly contained the low-MW component of the original K-60. When CPG240B-OH was used, fractions 1 and 2 consisted of the two components (17 and 19 min) present in fraction 1 in part (a) of the figure. The two peaks now have an almost equal height. The other fractions exhibited multi-modal peaks, not much different from those of original K-60. When CPG350C-OH was used, the first three fractions consisted nearly of the highest-MW component only (fraction 3 was similar to fraction 1, but the peak retention time was slightly longer), but the amounts of polymer recovered were less when compared with those obtained from the other columns. Fraction 4 contained the first two components of the original K-60, fraction 7 the last two components, and fraction 13 mainly the second component. Comparison of the three results shows that the MW distribution in the early fractions narrowed and the average MW increased with increasing pore size. In contrast, late fractions' purity was better with the smallest pore. These results are in agreement with those observed in nonaqueous HOPC [2,3]. The last fraction (fraction 13; virtually no polymer was detected in fraction 14) returned to the higher MW for all of the three columns. The recoiling phenomenon was also observed in the separation of poly(methyl

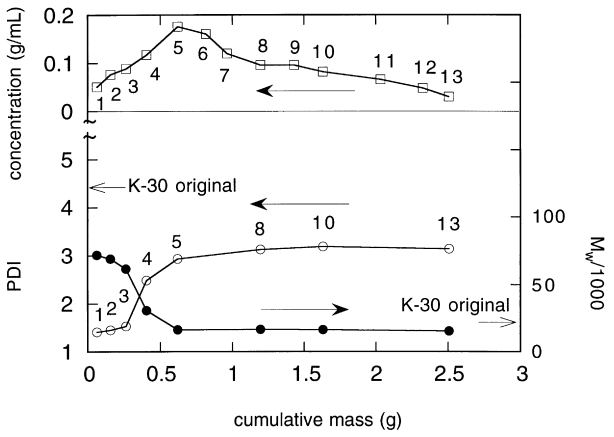

Fig. 4. Concentration (squares), PDI (open circles), and M_w (closed circles) of the fractions obtained in the separation of K-30 (40 wt.% in water), plotted as a function of the cumulative mass collected. The numbers adjacent to the symbols indicate the fraction number. The PDI and M_w of the original K-30 are indicated by arrows.

methacrylate) in organic solvents when the pore surface was not sufficiently repulsive [12]. We are not certain, at this moment, if the recoiling in PVP is caused by adsorption or a kinetic effect. Further study with different solvents and mobile phases is necessary.

We also tested the pore size dependence for the separation of K-30F in an aqueous solution at 30 wt.%. Two columns packed with CPG120C-OH and CPG75B-OH were used. The polydispersity index, PDI $= M_w/M_n$, is shown as a function of M_w in Fig. 3 for some of the fractions analyzed by GPC. The MW values are with reference to poly(ethylene oxide) standards. The numbers adjacent to the symbols represent fraction numbers. The original K-30

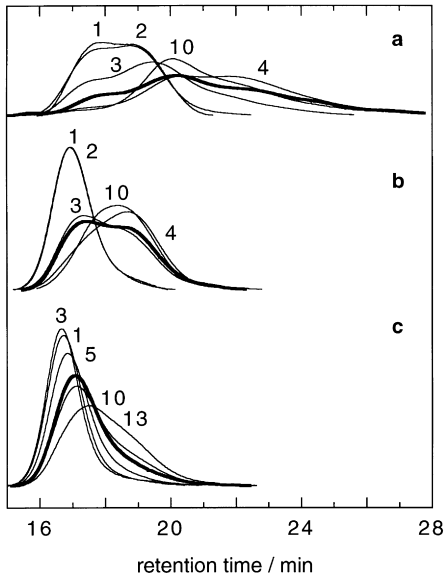

Fig. 5. GPC results of multi-stage separation of PVP K-60: (a) the first stage, (b) the second stage, and (c) the third stage. The numbers adjacent to the lines represent fraction numbers.

injected is indicated by a closed circle. Both results are obtained in the first batch with the column. The result for CPG120C-OH is taken from the chromatograms shown in Fig. 1(c). The larger pore (130 Å) produced initial fractions with a higher M_w and a smaller PDI, but the amounts recovered in these fractions were less compared with similar fractions obtained with the other column. In later fractions, the smaller pore outperformed the other, collecting fractions purer in low-MW components.

In HOPC, low-MW components are preferentially driven into pore channels by the high osmotic pressure of the concentrated mobile phase. When the pore size is too large, only the highest-MW components will be partitioned in the mobile phase. Therefore, peak retention time of the initial fractions, obtained with CPG350C for K-60 and CPG120C for K-30, was shorter, but the amount recovered was less compared with the smaller pores. In contrast, when the 177 Å pore size was used for the separation of K-60 and the 85 Å pore for K-30, a large portion of the polymer, including relatively low-MW components, was partitioned in the mobile phase. Therefore, the initial fractions had a broader distribution and a smaller M_w. To collect as large an amount as possible in early fractions at the expense of a broader MW distribution, a smaller pore size should be used. Otherwise, a larger pore size can do a better job in collecting the highest end in the MW distribution of the original polymer.

3.3. Quantity of each fraction

Feasibility of HOPC depends on the amount of polymer collected in the early fractions that have a sufficiently narrow MW distribution. The amount is critical in the multi-stage separation to prepare narrow-distribution fractions. We measured the mass of each fraction obtained in the HOPC separation of K-30. A 6.87 g of a 40 wt.% aqueous solution of K-30 was injected into a column of 7.8 × 300 mm^2 packed with CPG120C-OH that had been used 12 times before. The solution was injected at 0.3 ml min^{-1} for 38 min followed by solvent injection at the same rate to complete the elution of the polymer. A total of 13 fractions was collected. 15 drops were collected in each of the fractions 1–7, 100 drops each in fractions 8–10, and 250 drops each in fractions 11–13. The solution in each test tube was weighed. After drying, each test tube was again weighed. It was found that all of the polymer injected was collected. The polymer concentration in each fraction thus estimated is shown in the upper portion of Fig. 4. The number adjacent to each data point indicates the fraction number. The polymer concentration increased rapidly from 0.05 to 0.18 g ml^{-1} in the first five fractions, then decreased rapidly to 0.1 g ml^{-1} in fraction 8, followed by a gradual decrease to 0.03 g ml^{-1} in fraction 13. Some of the fractions were characterized in GPC. The lower portion of Fig. 4 shows M_w and PDI as a function of the cumulative mass of the dried polymer. The values for the original K-30 are indicated by arrows.

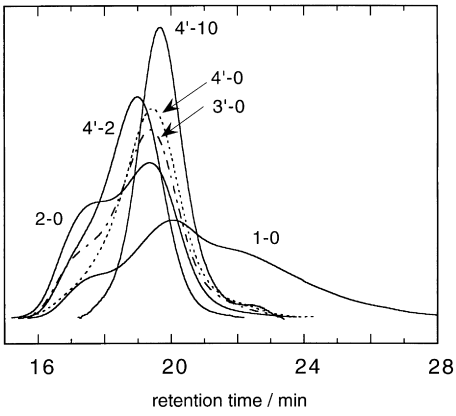

Fig. 6. GPC chromatograms for some of the fractions obtained in four-stage separation of PVP K-60. The number '*i-j*' adjacent to each line represents fraction *j* obtained in the *i*th-stage separation. $j = 0$ represents the sample injected in the relevant stage.

Fractions 1–3 have a PDI less than 1.5. Combined they claim about 10% of the total mass of the polymer injected which is 2.75 g. It indicates that HOPC is an efficient method to obtain a large amount of polymer with a narrow MW distribution at the high end of the original distribution.

3.4. Multi-stage separation

The MW distribution of the early fractions obtained can be further narrowed by repeatedly applying HOPC. The large amount of polymer recovered in these fractions makes the repeated application feasible. To demonstrate the narrowing, multi-stage fractionation was performed on K-60, K-30, and K-15.

In the first-stage separation of broad-distribution K-60, we used a column ($3.9 \times 300\ mm^2$) packed with CPG240B-OH. A solution of K-60 dissolved in water at 25 wt.% was injected. Fig. 5(a) shows a typical result. A chromatogram of the injected sample is shown in a thick line. Figs. 2(b) and 5(a) are from different batches but are close to each other, indicating reproducibility in successive batches under the similar conditions. The first two fractions collected were the two high-MW components of multimodal K-60, and were devoid of low-MW components that exhibited peaks at around 20 and 22 min in K-60's chromatogram. Early fractions that have a chromatogram similar to that of fraction 1 or 2 in Fig. 5(a), obtained in several batches, were combined to make a concentrated solution for injection in the second-stage separation. Attention was paid to make the solution as viscous as the 25 wt.% solution of K-60; the concentration, however, was not measured. As the average MW of the combined fractions was higher compared with that of K-60, another column of the same dimension packed with CPG350C-OH was used in the second stage. Fig. 5(b) shows a typical result. The injected sample, shown in a thick line, has a bimodal MW distribution. Fractions 1 and 2 (almost identical) consist mostly of the higher-MW component, whereas later fractions (4–10) consist mostly of the lower-MW component. Fractions that have a chromatogram similar to that of fraction 1 or 2 in Fig. 5(b), obtained in a few batches of the second-stage separation, were combined to make a concentrated solution for the third-stage separation. A third column of the same dimension packed with CPG500B-OH was used, and the result is shown in Fig. 5(c). The injected sample is unimodal with a low MW tail. Fractions 1–4 have a similar chromatogram with a high peak MW and a narrow distribution. With increasing fraction number, the peak MW decreases and the distribution broadens. The pattern is similar to the one observed for separation of polystyrene and other polymers in THF or toluene [1,2]. Apparently, the MW distribution of the sample injected in the third-stage separation was close to unimodal. We thus find that the first two stages were consumed to resolve multimodal peaks, whereas the third stage further narrowed the MW distribution of the high-MW component. It is not fair, however, to evaluate the separation performance of the third stage solely from the chromatograms in Fig. 4(c), because the chromatograms of the early fractions are already narrow and may be artificially broadened by the inherent band broadening of the GPC columns. We will address this problem later when we show the analysis results obtained in the other GPC system with a MALLS detector.

An attempt was also made to purify the second peak (at around 19 min) in the sample injected at the second stage. In Fig. 6, 1-0 is the chromatogram of K-60, and 2-0 indicates the chromatogram of the polymer injected in the second stage, slightly different from the counterpart in Fig. 5(b). Late fractions from two second-stage batches were combined to prepare a concentrated solution for injection in the third stage (3′-0). In the latter, CPG350C-OH was used. Late fractions were again combined to prepare a solution (not as concentrated as others) for injection in the fourth stage (4′-0). This time CPG240B-OH was used. Chromatograms for its second (4′-2) and tenth (4′-10) fractions are shown in the figure. The latter has a peak close to the one at about 20 min in the chromatogram of K-60 and is considered to be nearly pure in that component. The MW distribution narrowed as separation was repeated, but its efficiency was not as good as the separation shown in Fig. 5. Moreover, the amount recovered was not as large. This result supports the fact that HOPC's strength is in collecting a large amount of high-MW components in the early fractions.

Separation of K-30 was done in three stages. A 40 wt.% solution of PVP K-30 was injected into a column ($7.8 \times 300\ mm^2$) packed with CPG120C-OH in the first stage. The result was similar to the one shown in Fig. 1(d). Initial two fractions of several batches, having similar GPC chromatograms, were combined to make a solution injected for the second-stage separation. The same column as the one used in the first stage was used. The peak MW decreased and the distribution broadened as the fraction number increased, a pattern similar to Fig. 5(c). Then the first two

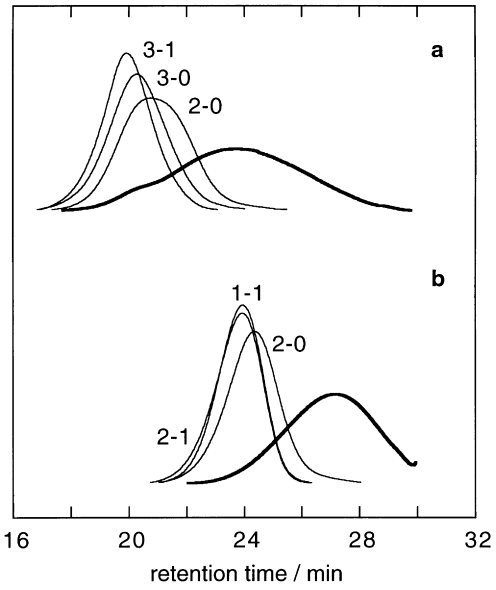

Fig. 7. GPC results of multi-stage separation of: (a) PVP K-30 and (b) PVP K-15. Thick lines represent the original K-30 and K-15. The number '*i*-*j*' adjacent to each line represents fraction *j* obtained in the *i*th-stage separation. $j = 0$ represents the sample injected in the relevant stage.

fractions obtained in the single batch were combined to make a solution for the third-stage separation which was conducted with another column (3.9 × 300 mm^2) packed with CPG240B-OH. The pattern in the chromatogram change was similar to that of the second stage. Chromatograms for the separated fractions showed a distinct pattern. The first three fractions were almost identical, with a narrower peak at a shorter retention time compared with the injected sample. The late fractions (8–10) were also narrower, but the peak MW was lower. The intermediate fractions were not much different from the injected sample. We believe that an already narrow MW distribution of the injected sample resulted in the pattern observed for the third stage.

Fig. 7(a) compares the chromatograms for the original K-30 (thick line), the combined fractions for injection in the second and third stages (2-0 and 3-0, respectively), and fraction 1 (3-1) obtained in the third stage. The MW distribution of the early fractions narrowed at each stage, but not as efficiently as we saw in the separation of K-60, especially in the second stage. The latter should have been done with CPG of a larger pore size in place of the same size as the one used in the first stage.

PVP K-15 was separated in two stages. A 50 wt.% aqueous solution of K-15 was injected into a column (7.8 × 300 mm^2) packed with CPG75B-OH. The pattern of separation was similar to the one seen in Fig. 1(b), but chromatograms of the early fractions were much narrower. From fraction 1–3, the peak retention time increased while the broadness changed little. Early fractions of two batches were combined to prepare a solution for injection in the second stage. A thinner column (3.9 × 300 mm^2) with the same packing material was used. Fig. 7(b) compares the chromatograms for the original K-15 (thick line), fraction 1 obtained in the first stage (1-1), the combined fractions for injection in the second stage (2-0), and fraction 1 obtained in the second stage (2-1). The low-MW end for original K-15 could not be resolved with the GPC system used. In the second-stage separation, the peak MW and the distribution of the early fractions were similar to those of the early fractions obtained in the first-stage separation, but the characteristics of the later fractions were different (not shown). The peak MW was smaller, yet the distribution was narrower compared with the sample injected (2-0), as in the third-stage separation of K-30. The narrowing in MW distribution for the early fractions in the single-stage separation of K-15 is comparable to those obtained in the three-stage separation of K-30 and K-60. Single-stage fractionation was perhaps sufficient for K-15 to prepare narrow-distribution fractions. Mixing the few early fractions undermined the fractionation effort, thereby forcing the second-stage separation to be just a remedial so long as the early fractions are concerned.

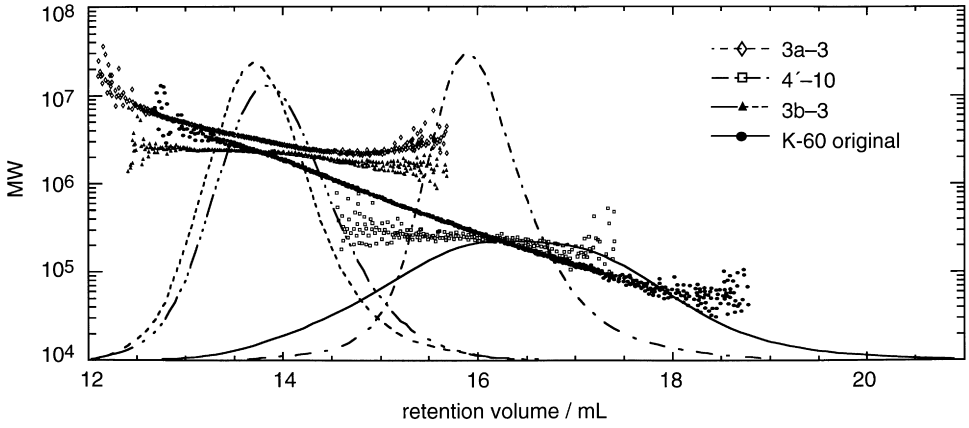

Fig. 8. Plot of MW as a function of retention volume for the original K-60 (closed circles), and three fractions obtained in the third- or fourth-stage HOPC, 3a-3 (rhombuses), 4′-10 (squares), and 3b-3 (closed triangles). The results were obtained in the GPC system with a MALLS and a DRI detectors. Lines indicate signals of the DRI detector.

The high efficiency in the multi-stage separation of K-60 can be ascribed to its multi-modality. When the polymer to separate is bimodal or, in general, multi-modal, it is possible to crop a few early fractions that have a similar MW distribution. Combining these fractions to prepare a concentrated solution for injection in the next stage does not broaden the MW distribution.

3.5. Analysis by MALLS detector

Some of the fractions obtained in the third-stage separation of K-60 were also characterized by using the GPC system with a MALLS detector. Symbols in Fig. 8 show a plot for MW of polymer in the eluent for K-60 original and three fractions (3a-3, fraction 3 in Fig. 5(c); 4′-10 in Fig. 6; 3b-3 obtained in another third-stage separation). Large errors at both ends of the MW data are due to weak scattering of light by the diluted eluent. Lines indicate a signal of the DRI detector and are shown for reference. Each curve is normalized by the area under the peak.

The MW distribution of the original K-60 ranges over two decades. Its log(MW) plot is sloped and almost a linear function of the elution volume, as expected for the linear columns. The MW plots for the separated fractions are, in contrast, nearly horizontal, similar to those observed for polystyrene standards in organic media [13]. In particular, the MW distribution ranges only over 0.2 decades for 4′-10 and 3b-3. Further, these plots are not a part of the plot for the K-60. Apparently, chromatograms for these fractions were artificially broadened by the inherent band broadening of the size exclusion columns when the fractions had a MW distribution narrower than the resolution of the columns.

Preparative GPC is often used to fractionate a polydisperse polymer. GPC produces fractions with a MW distribution that is essentially a product of the distribution function of the injected sample and the band broadening function. The latter can be evaluated by analyzing a fraction with a sufficiently narrow MW distribution in the same GPC system. The analytical GPC system is estimated to have a band broadening of about one decade, as seen from the comparison between the MW plot and the DRI chromatogram in Fig. 8. Multi-stage separation therefore does not help in GPC. If K-60 were separated in GPC, it would be difficult to obtain fractions with a MW distribution as narrow as in the fractions we obtained in multi-stage HOPC. This result attests the high resolution of HOPC.

4. Concluding remarks

We have demonstrated that HOPC can separate water-soluble PVP with an aqueous mobile phase, and its resolution exceeds that of GPC. Standard-grade fractions can be prepared by a couple of stages of separation. HOPC's large processing capacity, low solvent consumption, and high resolution make it a much better method than preparative GPC.

It is not clear, at this moment, why the resolution in the separation of PVP in water was better compared with the separation of organic-soluble polymers in organic solvents. We suspect that repulsions between the pore surface and the polymer are responsible. Further studies are needed, for instance, separation of PVP in other solvents such as methanol and separation with other surface modifications.

We are extending the scope of HOPC to other water-soluble polymers, such as poly(ethylene oxide), poly(vinyl alcohol), and proteins. The focus is how to overcome the problem that some water-soluble polymers tend to form complexes via strong hydrogen bonding or ion–ion interactions. Complex formation and aggregation are detrimental to HOPC that is based on the molecular size.

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References

[1] Luo M, Teraoka I. Macromolecules 1996;29:4226.
[2] Luo M, Teraoka I. Polymer 1998;39:891.
[3] Teraoka I, Luo M. Trends Polym Sci 1997;5:258.
[4] Daoud M, de Gennes PG. J Phys (Paris) 1977;38:85.
[5] de Gennes PG. Scaling concepts in polymer physics. Ithaca, NY: Cornell University Press, 1979.
[6] Teraoka I, Zhou Z, Langley KH, Karasz FE. Macromolecules 1993;26:6081.
[7] Teraoka I, Zhou Z, Langley KH, Karasz FE. Macromolecules 1993;26:3223.
[8] Gao Q, Yeung ES. Anal Chem 1998;70:1382.
[9] Senak L, Wu C-S, Malawar EG. J Liq Chromatogr 1987;10:1127.
[10] Molyneux P. Water-soluble synthetic polymers: properties and behavior, 1. Boca Raton, FL: CRC Press, 1984.
[11] Haller W. Nature 1965;206:693.
[12] Dube A, Teraoka I. Isolation and purification 1999;in press.
[13] Wyatt PJ, Villalpando DN, Alden P. J Liq Chromatogr 1997;20:2169.