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Polymer fractionation using chromatographic column packed with novel regenerated cellulose beads modified with silane

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Abstract

Novel microporous beads with the particle size of about 90 μm were prepared, for the first time, from cellulose and konjac glucomannan (RC/KGM3) in 1.5 M NaOH/0.65 M thiourea aqueous solution by emulsification method. The microporous beads were then modified with silane to avoid the adsorption of polymers containing hydroxyl groups, coded as RC/KGM3-Si. A preparative size-exclusion chromatographic (SEC) column (500 mm × 20 mm) was packed with RC/KGM3-Si, and its exclusion limit and fractionation range of the stationary phase were, respectively, weight-average molecular masses (M_w) of 4.8×10^5 g/mol and 5.3×10^3–4.8×10^5 g/mol for polystyrene in tetrahydrofuran. The preparative SEC column was used to fractionate poly(ε-caprolactone) (PCL, $M_w = 8.31 \times 10^4$ g/mol polydispersity index $d = 1.55$) in tetrahydrofuran and a polysaccharide PC3-2 ($M_w = 1.21 \times 10^5$ g/mol, $d = 1.70$) in 0.05 M NaOH aqueous solution, respectively. The M_w values of the fractions determined by analytical SEC combined with laser light scattering were from 1.2×10^4 to 1.84×10^5 for PCL and from 8.5×10^4 to 2.13×10^5 for PC3-2, as well as d from 1.2 to 1.5. The results indicated that the preparative SEC has good fractionation efficiency in both organic solvent and alkaline aqueous solution for the various polymers.

Keywords: Regenerated cellulose; Beads; Silane modification; Preparative SEC

1. Introduction

Size-exclusion chromatography (SEC) is an important technique in analysis and quality control of polymers [1,2]. The SEC column packed with polymeric packings such as methacrylate and vinyl types in their native forms are not suitable for analysis and separation of biopolymers because of their inappropriate surface properties [3]. Therefore, the chromatographic packing materials for biopolymer separation are mostly prepared from polysaccharides [4–6] or the inorganic packings coated with polysaccharides [7–10]. It has been reported that dextran gels were incorporated into macroporous solid particles [8], and cellulose derivatives were chemically bonded to silica-based gels [9] or coated on zirconia stationary phases [10] for biopolymer enantioseparations. Cellulose has great potential to be used as chromatographic packing because of the nature of good solvent-resistivity, biocompatibility, biodegradability and relatively low cost. Recently, fundamental research and industrial application drive more and more interests on the cellulose packings in the field of separation science and technology for biopolymers [11–13]. In our laboratory, preparative SEC columns packed with microporous regenerated cellulose gels have been used to fractionate biopolymers such as dextran [14], β-D-glucan from *Poria cocos* sclerotium [15] and enzyme [16]. However, during the above-mentioned fractionations the molecular mass of the first fraction is lower than that of the second fraction. This irregular elution pattern has indicated that some interaction exists between the stationary phases of the columns (cellulose gels) and the fractionated biopolymers [14]. Namely, the high molecular-mass parts of the polysaccharides were firstly adsorbed on the cellulose gel packings as a result of the hydroxyl groups, and relatively low

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molecular-mass parts were eluted as the first fraction, resulting in reduction of average molecular mass. Moreover, these cellulose gel packings were prepared by cutting regenerated cellulose fibers into small particles, and the technology is complex and time consuming [14–16]. Thus, developing of a simple and low-cost processing route to produce the cellulose packing is essential for industrial production.

Generally, the chromatographic column packed with sphere stationary has the highest fractionation efficiency. In our laboratory, a novel solvent of cellulose (NaOH/thiourea/water) has been developed [17], so we attempted to prepare new cellulose beads from this solvent. In this work, regenerated cellulose beads were prepared in NaOH/thiourea aqueous solution, for the first time, and then their surface were modified with trimethylchlorosilane to decrease the adsorption of polysaccharides. The structure and physical properties of the surface-modified beads were studied by Fourier transform infrared spectroscopy, scanning electron microscopy, test on solvent resistivity and adsorption measurement. A preparative SEC column packed with the cellulose beads was prepared, and the fractionation efficiency for synthetic polymer and polysaccharide in different solvents were evaluated.

2. Experimental

2.1. Preparation of cellulose beads

Cellulose (cotton linter pulp) was supplied by Hubei Chemical Fiber Co. Ltd. (Xiangfan, China), and the viscosity-average molecular mass (M_η) of this cellulose in cadoxen at 25 °C was determined to be $1.01 \times l0^5$ g/mol. Purified konjac glucomannan (KGM) was supplied by Zhuxi Konjac Institute of Hubei Province, China. Cellulose and KGM were dissolved, respectively, in 1.5 M NaOH/0.65 M thiourea aqueous solution by using a freezing-to-thawing process according to our patent [17]. The resulting cellulose solution was mixed with KGM solution (70:30, w/w) to produce mixture, which was stirred energetically at room temperature for 1 h and degassed at 5 °C. Forty milliliters of the mixture solution in a three-necked flask was stirred with a speed of 700 rpm at room temperature, and then 40 mL of 4% (v/v) span 80 liquid paraffin solution was added dropwise. The resulting slurry was stirred for 1 h before adding dropwise 60 mL 5 wt.% $CaCl_2$ aqueous solution to precoagulate. The pre-coagulation was performed for 1 h, and then the precipitate of the cellulose beads were regenerated with 2 wt.% HCl, washed in turn with ether and water, finally freeze-dried to obtain regenerated cellulose beads, coded as RC/KGM3.

Four grams of the regenerated cellulose RC/KGM3 beads suspended in 30 mL *N*,*N*-dimethylacetamide and 10 mL pyridine were added into a three-necked flask equipped with a condenser, and the mixture was stirred mechanically at 100 rpm for 1 h under a nitrogen atmosphere at 50 °C. Eight grams of trimethylchlorosilane was introduced dropwise into the resulting mixture by a syringe, and the system was kept at 50 °C for 3 h, to pour into methanol to stop the reaction. Finally the precipitate was collected by filtration and washed in turn with toluene, methanol and water three times. The obtained beads were coded as RC/KGM3-Si and stored in 20% isopropanol/2% formaldehyde aqueous solution before use.

2.2. Characterization of beads

FT-IR spectra were recorded with a spectrometer (1600, Perkin-Elmer Co., USA) using KBr-pellets at room temperature. To obtain Si content, RC/KGM3-Si was burned in a muffle oven at 800 °C for 6 h. The Si content was calculated as follows

$$\mathrm{Si\,(wt\%)} = \left(\frac{0.467 W_{\mathrm{SiO_2}}}{W_1}\right) \times 100\% \quad (1)$$

where the factor 0.467 is the mass fraction of silicon in silicon dioxide, W_1 the mass of the beads, and $W_{\mathrm{SiO_2}}$ is the SiO_2 mass of the inconsumable residue. Scanning electron microscopy (SEM) of the RC/KGM3 and RC/KGM3-Si beads was carried out on a Hitachi S-570 microscope (Hitachi, Japan). The beads were frozen in liquid nitrogen and vacuum-dried, then coated with gold, subsequently observed and photographed. Adsorption behaviors of the RC/KGM3 and RC/KGM3-Si beads toward polysaccharide were examined. One gram (W_2) of the dried beads and 20 mL (V) of the dextran ($M_w = 7.14 \times 10^4$ g/mol, $d = 1.94$, Sigma, Norway) aqueous solution with known concentration (C_0) were placed in sealed bottles, and the bottles were shaken at a constant speed (100 times/min) at 25 °C for 48 h. The concentration (C_i) of the dextran solution in the liquid phase after centrifugation was determined by ultraviolet–visible spectrometer (UV-160, Shimadzu, Japan). The adsorbability (q_e) was calculated as follows

$$q_e = \frac{(C_0 - C_i)V}{W_2}\,(\mathrm{mg/g}) \quad (2)$$

The dried RC/KGM3 and RC/KGM3-Si beads were immersed in 0.1 M NaOH aqueous solution and THF to determine their solvent resistivity (R_a), respectively. R_a of the beads was evaluated from the masses for the samples of before (W_d) and after treatment (W_T) by the following equation

$$R_a = \frac{W_T}{W_d} \times 100\% \quad (3)$$

2.3. Characterization of preparative SEC column

The RC/KGM3-Si beads were suspended in THF, and then packed in a glass column (600 mm × 20 mm) to form a 500 mm long bed. THF was the elution phase, and the flow rate was adjusted to 2 mL/min during the runs to stabilize the preparative SEC column for a week at room temperature. Acetone was used to determine the theoretical plate number (N) of the SEC column. The polystyrene (PS) standard samples (supplied by National Research Center of Standards,

Beijing, China) with weight-average molecular mass (M_w) of 1.92×10^6, 7.75×10^5, 3.90×10^5, 1.20×10^5, 3.00×10^4, 2.35×10^4, 1.10×10^4, 0.53×10^4 and 0.22×10^4 g/mol, respectively, were used to determine the exclusion limit and fractionation range of the stationary phase. A 0.5 mL solution of the standard samples in THF with a concentration of 2×10^{-3} g/mL was separately injected into the SEC column. THF with a flow rate of 1.2 mL/min was used as eluent in this case. The eluate for PS was monitored at 254 nm by using the UV detector.

2.4. Fractionation by preparative SEC column

Poly(ε-caprolactone) (PCL, $M_w = 8.31 \times 10^4$ g/mol, polydispersity index $d = 1.55$, and provided by Sigma, Norway) was dissolved in THF to prepare 0.1 g/mL concentration solution. Five milliliters of the solution was injected into the preparative SEC column at 25 °C. THF was the eluent and the flow rate was 1.2 mL/min. The eluate for PCL was monitored at 287 nm by the UV detector and separately collected. The collected solutions were precipitated with water, and then vacuum-dried to obtain six fractions.

A polysaccharide PC3-2 ($M_w = 12.1 \times 10^4$ g/mol and $d = 1.70$) [18], which was extracted from *Porica cocos* sclerotium, was dissolved in 0.05 M NaOH aqueous solution to prepare 0.02 g/mL concentration solution. Five milliliters of the solution was injected into the preparative SEC column at 25 °C. A 0.05 M NaOH aqueous solution was the eluent and the flow rate was 1.2 mL/min. The eluate for PC3-2 was monitored by the UV detector at 200 nm and separately collected, neutralized with 0.1 M HCl aqueous solution, and then dialyzed in distilled water, finally freeze-dried.

Analysis SEC combined with laser light scattering (SEC-LLS) measurement of the fractions and the unfractionated samples was carried out on a DAWN® DSP multi-angle laser photometer (λ = 633 nm; DAWN® DSP, Wyatt Technology Co., St. Babara, USA), combined with a P100 pump (Thermo Separation Products, San Jose, Japan), equipped with TSK-GEL G4000H6 (7.5 mm × 300 mm) for PCL and TSK-GEL G6000H6 (7.5 mm × 300 mm) for PC3-2 at 25 °C. A spectra system detector (RI-150, TSP, USA) was simultaneously connected. To determine the values of M_w and d, THF as eluent for PCL and dimethylsolfoxide (DMSO) as that for PC3-2 were used in the SEC-LLS with a flow rate of 1.0 mL/min. All the solutions with a polymer concentration of 1.0×10^{-3} to 2.0×10^{-3} g/mL were filtered first with a sand filter, and followed by a 0.45 μm filter (Whattman, England), then kept in sealed glass bottles before injected into the SEC column. The specific refractive index increments (d*n*/d*c*) of PCL in THF and PC3-2 in DMSO at 25 °C were measured on an optilab refractometer (DAWN®DSP, Wyatt Technology Co., USA) at 633 nm to be 0.079 and 0.058 mL/g, respectively. Astra software (Version 4.07.70) was utilized for the data acquisition and analysis.

3. Results and discussion

3.1. Physical properties of the beads

Fig. 1 shows the FT-IR spectra of the RC/KGM3 and RC/KGM3-Si beads. The broad band at around 953 cm^{-1} for RC/KGM3-Si is attributed to the stretching vibration of Si–O–C. The new absorption peak at 2907 cm^{-1} for RC/KGM3-Si belongs to methyl group of silane. In addition, the broad absorbency at 3000–3600 cm^{-1} for the hydroxyl group of RC/KGM3 beads weakens and shifts to a higher wavenumber after reacting with trimethylchlorosilane. The results from FT-IR indicate the reduction of hydroxyl number and inter-molecular hydrogen bonds. The Si content is 2.81%, lower than that of silylcellulose, which has been prepared from the reaction of cellulose and chloropropyltrichlorosilane in LiCl/*N*,*N*-dimethylacetamide [19]. So, it can be drawn that only the hydroxyl groups on the surface of the RC/KGM3 beads have been reacted with trimethylchlorosilane to form a silane layer on the bead surface. The reaction between RC/KGM3 and trimethylchlorosilane is schemed in Fig. 2.

Fig. 3 shows the SEM images of the RC/KGM3 and RC/KGM3-Si beads. The RC/KGM3 beads exhibit obvious microporous on the surface and large size with the diameter of about 90 μm. Our previous works [20,21] have proved that such kinds of materials possess homogeneous microporous structures, resulted from the aggregation and asymmetrical shrink of KGM during coagulation. Compared with the process of cutting regenerated cellulose fibers into gel particles [14,16], the preparation technology of the cellulose beads is simple, fast, cheap, and more suitable for industrial produc-

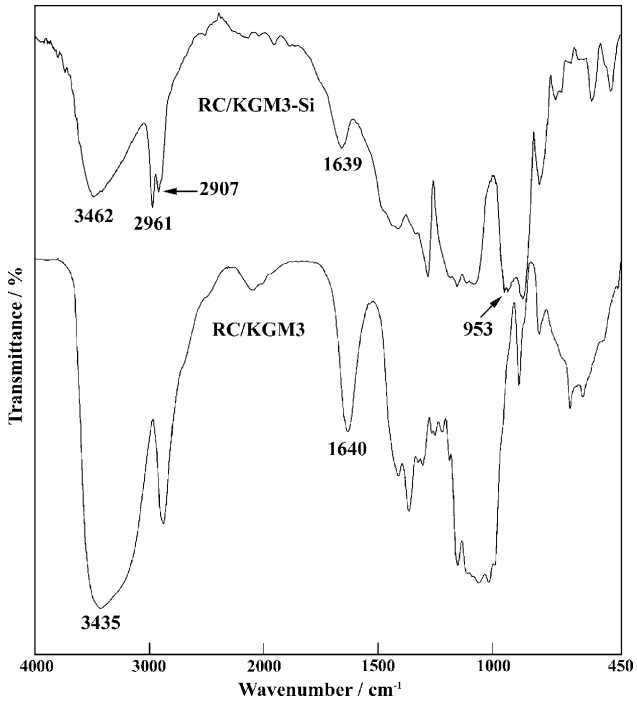

Fig. 1. The FT-IR spectra of RC/KGM3 and RC/KGM3-Si.

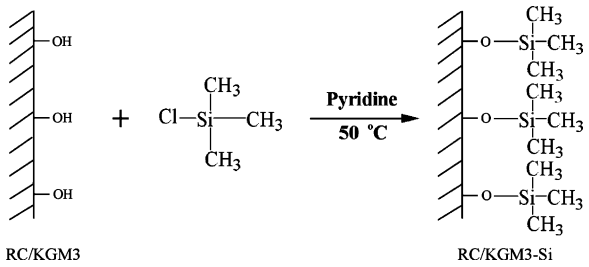

Fig. 2. The schema of the reaction between RC/KGM3 and trimethylchlorosilane on the surface of the bead.

tion. The SEM images of RC/KGM3-Si beads show that the microparticles are almost spheres with a lot of grooves, and the particle size is about 30–50 μm, which is much smaller than that of the RC/KGM3 beads. It can be explained that the surface of RC/KGM3 with a number of hydroxyl groups are swelled in water because of the good hydrophilicity. However, an increase of hydrophobicity for RC/KGM3-Si results in the shrink of particle size as well as the collapse of the bead surface, owing to the silane modification. This suggests that the hydrogen bonds on the surface of the RC/KGM3-Si beads have significantly weakened. Dextran was used as a model sample of polysaccharides to determine the adsorbability of the RC/KGM3 and RC/KGM3-Si beads. The q_e of RC/KGM3-Si is 2.1 mg/g, which is much lower than that of RC/KGM3 (q_e = 8.7 mg/g), indicating a significant decrease in the adsorption of polysaccharides on the beads surface modified with silane.

The solvent resistivity of RC/KGM3 and RC/KGM3-Si is illustrated in Fig. 4. The R_a values for RC/KGM3 in 0.1 M NaOH aqueous solution decrease rapidly from 100 to 89% within 96 h, because KGM and cellulose with lower molecular mass have been more or less hydrolyzed in the solvent [22,23]. However, R_a for RC/KGM3-Si in 0.1 M NaOH aqueous solution decreases slowly, and the leveling off of

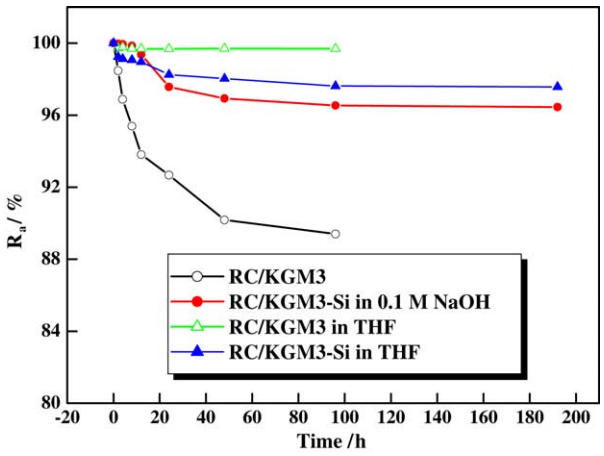

Fig. 4. The solvent resistant behavior of RC/KGM3 and RC/KGM3-Si in 0.1 M NaOH and THF at 25 °C.

the R_a values at time greater than 20 h is believed to indicate a stable state. This indicates that the alkali resistivity of the RC/KGM3-Si beads is improved by coating with silane. Moreover, all the R_a values for both RC/KGM3 and RC/KGM3-Si in THF keep a relatively high value of more than 98%, showing their good resistivity toward THF. Thus, RC/KGM3-Si can be used as a packing material not only in alkaline aqueous solution system but also in organic solvent system for the chromatographic applications.

3.2. Fractionation efficiency of the preparative SEC column

A general measurement of chromatographic efficiency is the theoretical plate number (N). The N value of the preparative SEC column (500 mm × 20 mm) packed with RC/KGM3-Si was measured to be 2730, indicating a good fractionation efficiency. The SEC curve of the column for the

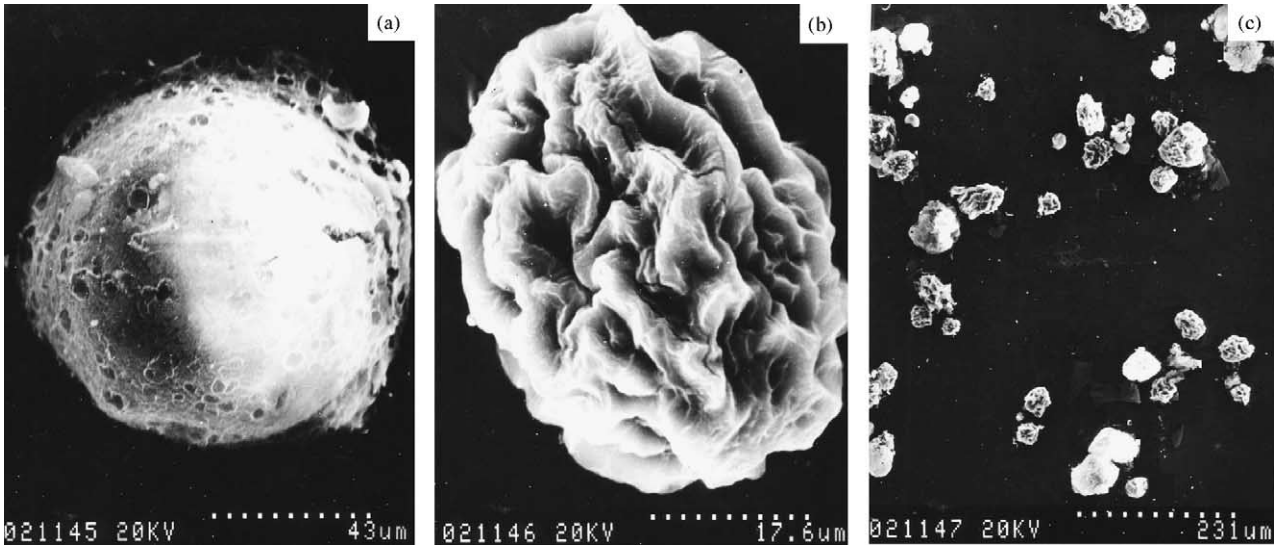

Fig. 3. SEM images of RC/KGM3 (a) and RC/KGM3-Si (b and c).

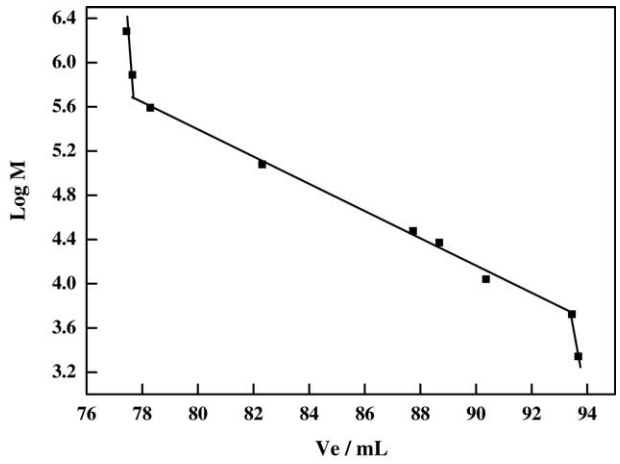

Fig. 5. SEC curve of the preparative SEC (500 mm × 20 mm) packed with RC/KGM3-Si for PS standard samples in THF at 25 °C, with a flow rate of 1.2 mL/min. Detector: UV at 254 nm.

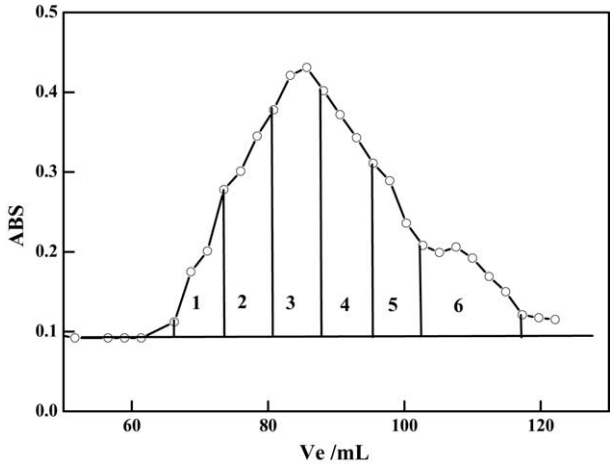

Fig. 6. Elution pattern of PCL measured on the preparative SEC column (500 mm × 20 mm) at 25 °C with THF as the eluent at a flow rate of 1.2 mL/min. Detector: UV at 287 nm.

PS standard samples in THF, with a flow rate of 1.2 mL/min by using UV detector at 254 nm, is illustrated in Fig. 5, and represented as follows

$$\log M = 15.27 - 0.123\, V_e \quad (4)$$

The exclusion limit and fractionation range of the stationary phase of the preparative SEC are determined to be molecular masses of 4.8×10^5 g/mol and 5.3×10^3–4.8×10^5 g/mol for polystyrene, respectively. In view of the results, the SEC column possesses a relatively high chromatographic efficiency and broad fractionation range.

Fig. 6 shows the elution pattern and fractionation of PCL in THF by using the preparative SEC at 25 °C. The elution patterns of the three injections are in the same shape on the whole. So the slicing indicates that the fractions collected from the whole injections were combined to get 0.1–0.3 g of the polymer (PCL-1, PCL-2, PCL-3, PCL-4, PCL-5 and PCL-6), and the total yield is 84.5%. The masses, yields, M_w and d values of the fractions are summarized in Table 1.

The M_w values of the fractions decrease from 18.4×10^4 for PCL-1 to 1.2×10^4 for PCL-6, and d values of the fractions are about 1.2 except PCL-6, which was collected from broad slicing. The M_{wcal} was calculated by

$$M_{w\,cal} = \sum_{i=1}^{6} w_i M_{wi} \quad (5)$$

in which w_i is the weight fraction of the fractions, and M_{wi} is the M_w of the fractions, The M_w values obtained is 9.40×10^4 g/mol. It is slightly higher than the experimental result of unfractionated PCL (8.31×10^4 g/mol). This can be explained that the low-molecular-mass part has been eluted out without collection, which also leads to 84.5% of total yield. The SEC chromatograms of PCL and its fractions measured by SEC-LLS are shown in Fig. 7. Each chromatogram of the samples contains one peak, and the peak positions of elution volume gradually shift to higher elution volume with the fractionation process, further indicating that the preparative SEC column possesses good fractionation efficiency.

Table 1
Mass, yields, M_w and d of the polymers and their fractions prepared by using the preparative SEC in different solvent at 25 °C

Sample	Solvent	Mass (g)	Yield (%)	$M_w \times 10^{-4}$	Polydispersity index (d)
PCL	THF	1.501	100	8.31	1.55
PCL-1		0.234	15.6	18.4	1.21
PCL-2		0.269	17.9	13.4	1.21
PCL-3		0.302	20.1	11.0	1.22
PCL-4		0.224	14.9	8.48	1.22
PCL-5		0.142	9.5	6.10	1.22
PCL-6		0.097	6.5	1.17	2.993
PC3-2	0.05 M NaOH aq. solution	0.401	100	12.1 [18]	1.70 [18]
PC3-2-1		0.049	12.2	21.3	1.44
PC3-2-2		0.062	15.5	14.2	1.38
PC3-2-3		0.057	14.1	11.6	1.21
PC3-2-4		0.050	12.5	10.7	1.28
PC3-2-5		0.050	12.5	9.2	1.21
PC3-2-6		0.032	7.9	8.5	1.51

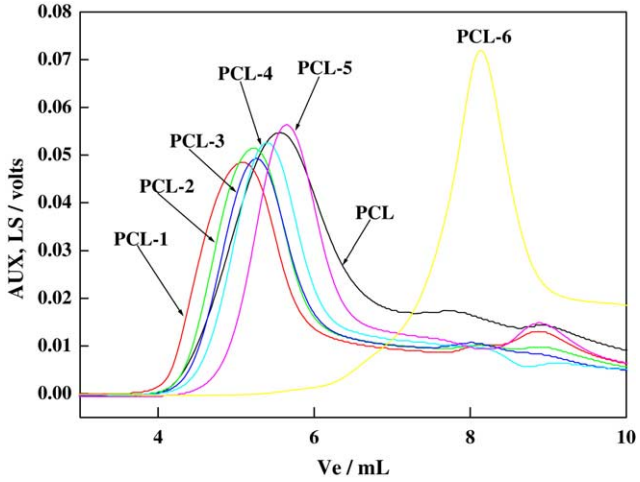

Fig. 7. SEC chromatograms of PCL and its fractions by using analytical SEC equipped with an TSK-GEL G4000H6 column and with laser light scattering. The eluent was THF with a flow rate of 1.0 mL/min at 25 °C.

Fig. 8 represents the elution pattern of PC3-2 by using the preparative SEC column in 0.05 M NaOH aqueous solution with a flow rate of 1.2 mL/min at 25 °C. Interestingly, there are double peaks in the SEC pattern, suggesting that PC3-2 contains fractions with different molecular masses because of some aggregates of the polysaccharide in water [15]. The elution patterns of the four injections are in the same shape on the whole. PC3-2 was fractionated into six fractions according to the slicing, and the fractions were respectively coded as PC3-2-1, PC3-2-2, PC3-2-3, PC3-2-4, PC3-2-5 and PC3-2-6. However, the M_w measurement of the fractions was carried out in DMSO, which can break the aggregates into single chains. The masses, yields, M_w and d values of the fractions are summarized in Table 1. Each fraction has been got to be 0.03–0.06 g. Similarly, the total yield is 75% because the low molecular mass part has been eluted out without collection. The M_w of the first fraction PC3-2-1 (2.13×10^5 g/mol) is

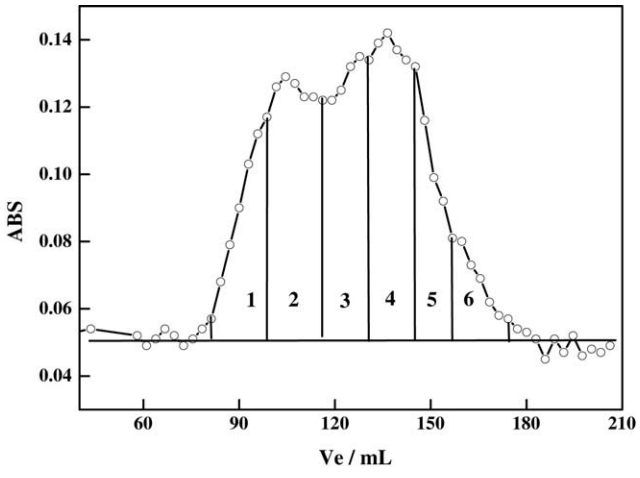

Fig. 8. Elution pattern of PC3-2 measured on the preparative SEC column (500 mm × 20 mm) at 25 °C with 0.05 M NaOH aqueous solution as the eluent, with a flow rate of 1.2 mL/min. Detector: UV at 200 nm.

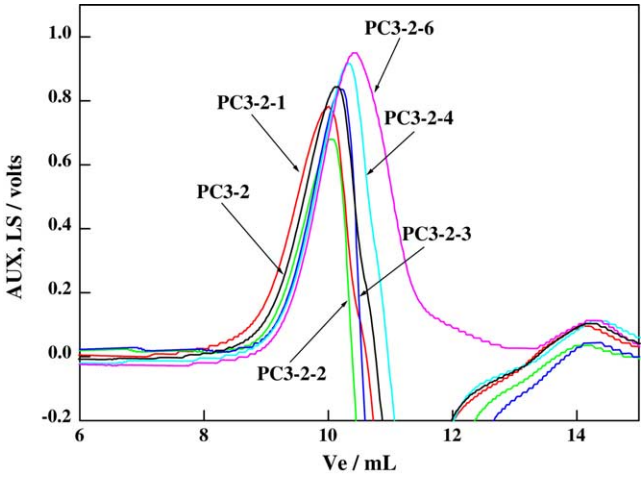

Fig. 9. SEC chromatograms of the PC3-2 fractions in DMSO by using analytical SEC equipped with an TSK-GEL G6000H6 column and with laser light scattering. The eluent was DMSO with a flow rate of 1.0 mL/min at 25 °C.

much higher than those of other fractions, further confirming that the fractions have been eluted out from high to low molecular mass to coincide with the size exclusion principle. It is worth mentioning that the molecular mass of the first fraction is not lower than that of the second because the coating silane layer prevents the adsorption of polysaccharides on the cellulose beads, different from the irregular fractionation behavior of the previous columns [14–16]. Meanwhile, the d values of the fractions are in the range from 1.2 to 1.5, which are lower than that of the unfractionated sample PC3-2. The SEC chromatograms of the PC3-2 fractions shown in Fig. 9 exhibit single peaks, and the peaks are obviously divided separately. It can be concluded that the adsorption of polysaccharide on the stationary phases of the preparative SEC column decreases, whereas the efficiency of the present column has been significantly improved. In view of the experimental results, the preparative SEC column is efficient for the fractionation of the polymers in both organic and alkali aqueous solvent systems. The fractions with narrow molecular mass distribution can be obtained with the preparative SEC column. A daily throughput of polymers is up to 6.0 g with a flow rate of 1.2 mL/min.

4. Conclusion

For the first time, the novel beads of RC/KGM3 were satisfactorily prepared from cellulose and KGM in 1.5 M NaOH/0.65 M thiourea aqueous solution by emulsification method. The regenerated cellulose beads were then modified on the surface by reacting with trimethylchlorosilane to form a silane layer to reduce the adsorbability of the RC/KGM3-Si beads toward polymers containing hydroxyl groups. The preparative SEC column packed with RC/KGM3-Si had a relatively high exclusion limit ($M_w = 4.8 \times 10^5$ g/mol) and a wide fractionation range ($M_w = 5.3 \times 10^3$–4.8×10^5 g/mol).

PCL in an organic solvent and polysaccharide PC3-2 in dilute alkali solution have been successfully fractionated into fractions with low polydispersity indices (d ranging from 1.2 to 1.5) by using the preparative SEC column, respectively. A daily throughput of polymers is up to 6.0 g with a flow rate of 1.2 mL/min. Therefore, the preparative SEC column has promising application in industrial process to separate, purify or fractionate biopolymers without adsorption.

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