

SIZE EXCLUSION CHROMATOGRAPHIC COLUMN PACKED WITH REGENERATED CELLULOSE GELS*

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Abstract Microporous regenerated cellulose gel particles were prepared by mixing cellulose cuoxam with silk fibroin as pore former, and the mean pore size and pore volume of the particles were 525 nm and 7.27 mL g⁻¹, respectively. A preparative size-exclusion chromatography (SEC) column (550 mm × 20 mm) packed with the cellulose gel particles was used for the fractionation of two polysaccharides Dextran 07 ($M_w = 7.14 \times 10^4$, $d = 1.7$) and Dextran 50 ($M_w = 50.5 \times 10^4$, $d = 3.8$) in water phase. The fractionation range of the stationary phase covered M_w from 3×10^3 to 1.1×10^6 . The daily throughput was 2.9 g for Dextran 07 (D07) and 4.3 g for Dextran 50 (D50) with a flow-rate of 1.5 mL min⁻¹. The fractions obtained by using the SEC were analyzed by an analytical SEC combined with laser light scattering (LLS), and the polydispersity indices of fractions for Dextran 07 and Dextran 50 were determined to be 1.34–1.57 and 1.53–3.36, respectively. The preparative SEC is a simple, rapid, and suitable means not only for the fractionation of polysaccharides in water but also for other polymers in organic solvents.

Keywords Cellulose packing, Silk fibroin, Preparative chromatography, Polysaccharides

INTRODUCTION

Size-exclusion chromatography (SEC) is a rapid method for separation and characterization of the molecular weight distribution of polymers in industrial and laboratory procedures^[1]. It is worth noting that the column packing materials made from cross-linked dextran, agarose and polyacrylamide are too expensive to be used in industrial applications^[2]. The utilization of low-cost cellulose, as a chromatographic material, can not only protect the environment from pollution, but also conserve petroleum resources^[3]. Various cellulose chromatographic packings have been prepared from cellulose/17.5 wt% NaOH aqueous solution^[4] and cellulose derivatives^[5, 6]. In our previous work^[7, 8], a preparative SEC column packed with regenerated cellulose gel particles has been used for the fractionation of a dextran in water and *Poria cocos* in dimethyl sulfoxide. Good performance in fractionation was achieved, but the exclusion limit of molecular weight was only 7×10^5 .

Recently, we have prepared a microporous cellulose membrane by coagulating a mixture of cellulose cuoxam/silk fibroin with 10 wt% aq. NaOH solution^[9]. By adjusting the composition, molecular mass and content of the polymers or coagulation conditions, the pore size of microporous cellulose membranes and gel particles can be easily controlled. In this work, regenerated cellulose gel particles with larger pore size obtained by using silk fibroin as the pore former were studied. The pore size of the packing and the physical properties of the SEC column were measured. The fractions obtained from two kinds of polysaccharides were characterized by analytical SEC combined with laser light scattering (LLS).

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EXPERIMENTAL

Preparation of Regenerated Cellulose Gel Particles

Linters with a viscosity-average molecular weight (M_η) of 1.96×10^5 were supplied by Hubei Chemical Fiber Factory. Polyethylene glycol (PEG2000) with molecular weight 2000 was imported from Japan. Raw silk was degummed twice with 0.5 wt% NaHCO_3 solution at 100°C for 30 min and then washed with water, finally dried under vacuum at 40°C for 1 day and white silk fibroin was thus obtained. An 8 wt% cellulose cuoxam solution (I), 8 wt% silk fibroin cuoxam (II), and 20 wt% PEG2000 aq. solution (III) were prepared according to the previous method^[9, 10]. The mixture of I and II (1:1 *V/V*) was spun into 10 wt% aq. NaOH solution, and then regenerated in 5 wt% H_2SO_4 aq. solution to obtain clear cellulose fibers. The fibers were cut into small particles with a length of 0.5–1.5 mm, then washed with water. The cellulose gel particles prepared from solutions I, the mixture of I and III, and the mixture of I and II were coded as RCG-1, RCG-2^[7] and RCF-1, respectively.

The volume (V) of the wet particles was measured by a glass dilatometer, and the backbone density (ρ_g , namely the density of regenerated cellulose), mean pore volume (V_p) and porosity (P_r) were calculated as follows:

$$\rho_g = \frac{W_d}{V - (W_w - W_d) / \rho_{\text{H}_2\text{O}}} \quad (1)$$

$$V_p = \frac{V - W_d / \rho_g}{W_d} \quad (2)$$

$$P_r = \frac{V_p}{V_p + 1 / \rho_g} \quad (3)$$

where W_d and W_w are the weights of dry and wet particles, respectively. $\rho_{\text{H}_2\text{O}}$ is the density of water.

Scanning electron micrographs (SEM) were taken on a Hitachi S-570 microscope. The wet fibers were frozen in liquid nitrogen and snapped immediately, and then dried in vacuum. The surface and cross-section of the RCF-1 particles were coated with carbon and gold, and then observed and photographed. Picking out 20 gel particles in random, the particle size in the wet state was measured by a microscope, and estimated according to statistical method.

Characterization for Preparative SEC Column

The mixtures of RCG-1, RCG-2 and RCF-1 (2:2:1, *V/V*) were packed in a glass column (500 mm $\times$ 10 mm) to form a 450 mm long gel bed. 1 mL approximately 2×10^{-2} g mL^{-1} aqueous solution of the glucan standards (their weight average molecular M_w are 122×10^4 , 92.5×10^4 , 78.8×10^4 , 40.4×10^4 , 21.2×10^4 , 4.7×10^4 , 1.0×10^4 and 0.27×10^4 , respectively), which were gifts from the National Research Center in Beijing and purchased from Wako Co., except for the standard of 122×10^4 which was prepared in our laboratory, were separately injected into the column. The flow rate was maintained at 1.5 mL min^{-1} . The effluent was monitored at 200 nm by using an UV detector (UV-160, Shimadzu, Japan).

The preparative SEC column was equipped with an automatic fraction collector and a peristaltic pump. Dextran 07 (D07, $M_w = 7.14 \times 10^4$, Sigma, Norway) and Dextran 50 (D50, $M_w = 50.5 \times 10^4$, Sigma, Norway) were dissolved respectively in distilled water to prepare 6.0×10^{-2} g mL^{-1} solutions. Half the amount of the solution (2.5 mL) was injected into the column, and distilled water was used as the eluent at 25°C . The flow rate was adjusted to 1.5 mL min^{-1} during the runs. The column effluent and fractions were monitored by a UV detector at 200 nm.

Analysis of Fractions

SEC combined with LLS is used for the determination of the true molecular weight and its distribution of the fractions and the unfractionated D07 and D50. The SEC-LLS measurements were performed on the DAWN DSP multi-angle laser photometer combined with pump p100 equipped with a TSK-GEL G4000 PWXL column

(7.88 mm × 300 mm), a TSK-GEL G6000 PWXL column (7.88 mm × 300 mm) and a differential refractive index detector (RI-150) at 25 °C . The eluent was 0.2 mol/L NaCl aqueous solution with a flow rate of 1.0 mL min⁻¹. An Astra software was utilized for data acquisition and analysis.

RESULTS AND DISCUSSION

Figure 1 shows the SEM micrographs of the surface and cross-section of the cellulose gel particles for RCF-1. The physical properties of the gel particles are summarized in Table 1. The mean pore sizes of the gel particles are 135 nm for RCG-1, and 370 nm for RCG-2, and 525 nm for RCF-1. The surface and cross-section for RCF-1 showed obviously homogeneous pore structure and the pore size of RCF-1 is larger than that of RCG-2 due to the higher molecular weight and solubility of silk fibroin (SF) in alkali solution. The mean pore diameter based on the flow rate method is 58 nm for the membrane of SF as pore former^[9] and 41 nm for that of PEG2000 as pore former^[10], respectively. In addition, the regenerated cellulose membrane from cuoxam obtained by alkaline coagulation was used for hemodialysis, and it has dense morphology in the cross-sectional direction. In this case, a network structure of the cellulose molecules formed through the ligand exchange of the cupric ion from ammonia molecules into hydroxyl groups and poly-nuclear cupric chelate, resulting in larger pore size and smaller pore volume.

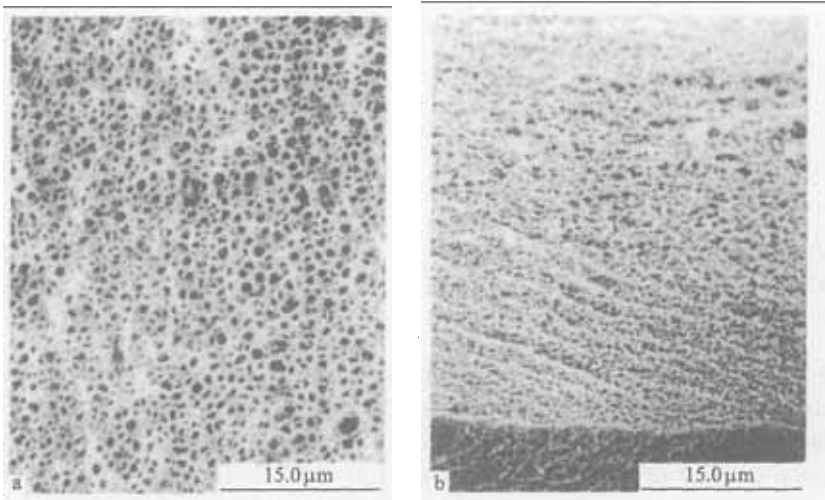


Fig. 1 SEM micrographs of the surface and the cross-section of the cellulose gel particles for RCF-1
a) Surface; b) Cross-section

Table 1. Physical properties of the gel particles

Gel	Mean particle size* (μm)	Mean pore size (nm)	Backbone density (g/mL)	Pore volume (mL/g)	Porosity (%)	References
RCG-1	320 × 1390	135	1.48	6.17	90.1	[7]
RCG-2	330 × 1180	370	1.54	8.12	92.6	[7]
RCF-1	400 × 1170	525	1.48	7.27	92.1	This work

* Diameter × length of the wet particle

The size-exclusion curve for the preparative SEC (500 mm × 10 mm) is illustrated in Fig. 2, and can be expressed as follows:

$$\lg M = 6.287 - 0.0492V_e$$

(4)

It indicates that the exclusion limit and fractionation range of the stationary phase of the preparative SEC

cover molecular weights of 1.1×10^6 and 3×10^3 – 1.1×10^6 , respectively. The distribution coefficient K_{SEC} , which determines the percentage of gel-phase available for each fraction independent of system geometry, was calculated by^[11]:

$$K_{SEC} = \frac{V_e - V_0}{V_i - V_0} \tag{5}$$

where V_e is the elution volume; V_0 , the void volume is 3.31 mL and V_i , the total pore volume is 53.25 mL. The molecular weight dependence of K_{SEC} is shown in Fig. 3. The pore size determines the range of molecular weight separation, and changes K_{SEC} strongly, therefore it influences the percentage of gel phase available to a given substance. In view of the fact mentioned above, the gel particles RCG-1, RCG-2 and RCF-1 with different pore sizes were successfully used as a stationary phase for the preparative SEC.

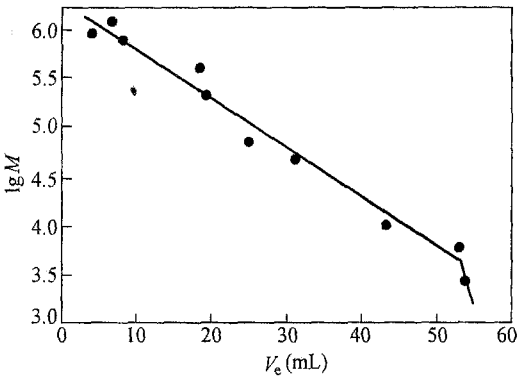


Fig. 2 Size exclusion curve of the preparative SEC for the glucan standards in water at 25°C

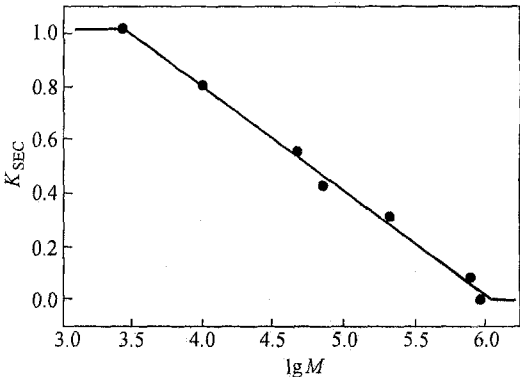


Fig. 3 Molecular weight (M_w) dependence of K_{SEC} of the preparative SEC for the glucan standards in water at 25°C

Figure 4 and 5 show the elution pattern of the preparative SEC (550 mm $\times$ 20 mm) for dextran D07 and D50 by using an UV detector. The values of M_w , M_n and polydispersity index $d(M_w/M_n)$ of each fraction obtained by SEC-LLS analysis are summarized in Table 2. The M_w values of the fractions decrease with the progress of fractionation, and the d values of the fractions are about 1.34–1.57 for D07 and 1.53–3.36 for D50, which are lower than that of the unfractionated dextran. The results indicate that preparative SEC has good fractionating efficiency and larger throughput with a daily throughput of 2.9 g for D07 and 4.3 g for D50 at a flow-rate of 1.5 mL min⁻¹ aqueous solution. It is well known that it is very difficult to obtain polysaccharide fractions with narrow distribution from a sample with $d < 2$ by the nonsolvent addition method.

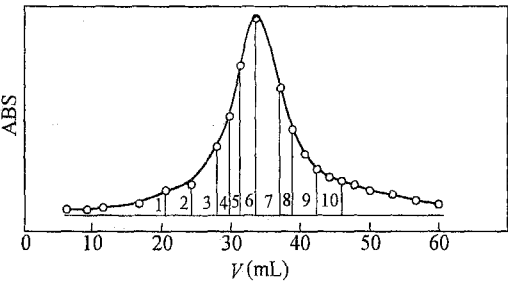


Fig. 4 Elution pattern and fractionation of dextran ($M_w = 7 \times 10^4$) in water by using the preparative SEC column

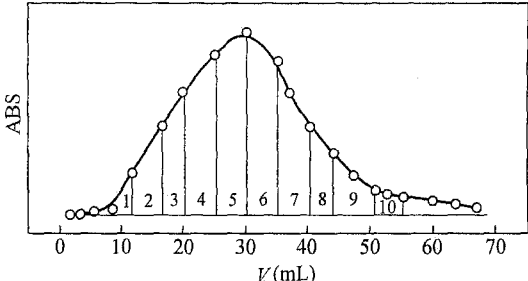


Fig. 5 Elution pattern and fractionation of dextran ($M_w = 50.5 \times 10^4$) in water by using the preparative SEC column

Table 2. Experimental results of M_w , M_n and d for dextran and its fractions by SEC analysis

Sample	$M_w (\times 10^{-4})$	$M_n (\times 10^{-4})$	d
D07	7.08	4.29	1.7
D07-1	8.98	6.70	1.34
D07-2	8.04	5.58	1.44
D07-3	7.63	5.30	1.44
D07-4	7.42	5.26	1.41
D07-5	7.32	5.01	1.46
D07-6	6.78	4.55	1.49
D07-7	6.42	4.17	1.54
D07-8	5.83	3.79	1.54
D07-9	5.07	3.23	1.57
D07-10	4.22	2.74	1.54
D50	50.2	13.2	3.8
D50-1	102.2	66.7	1.53
D50-2	94.9	38.7	2.45
D50-3	87.7	37.0	2.37
D50-4	76.6	28.4	2.70
D50-5	65.5	23.1	2.84
D50-6	56.5	18.8	3.01
D50-7	51.9	17.1	3.03
D50-8	46.4	14.3	3.25
D50-9	43.2	12.9	3.36
D50-10	30.8	9.8	3.13

CONCLUSION

A preparative SEC column packed with a mixture of microporous regenerated cellulose gel particles RCG-1, RCG-2 and RCF-1 ($V/V = 2:2:1$) was satisfactorily used to fractionate two polysaccharides, D07 ($M_w = 7.14 \times 10^4$, $d = 1.65$) and D50 ($M_w = 50.5 \times 10^4$, $d = 3.79$) in aqueous phase. RCF-1 was prepared by using silk fibroin as pore former, resulting in an improvement of the exclusion limit. The fractionating range of M_w of stationary phase is 3×10^3 to 1.1×10^6 . At a flow-rate of 1.5 mL min^{-1} , the daily throughput is 2.9 g for D07 and 4.3 g for D50, respectively. The fractions were analyzed by SEC-LLS, and the polydispersity indices of the fractions obtained are 1.34–1.57 for D07 and 1.53–3.36 for D50.

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