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Preparative fractionation of polysaccharides by columns packed with regenerated cellulose gels

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Abstract

Two kinds of regenerated cellulose microporous gel particles with mean pore sizes of 135 nm and 370 nm were satisfactorily prepared by mixing cellulose cuoxam with polyethylene glycol (PEG) as pore former. A preparative size-exclusion chromatography (SEC) column (550 mm×20 mm) packed with the gel particles mentioned above was used for the fractionation of a dextran in water. The exclusion limit and fractionation range of the stationary phase were molecular masses $7\cdot10^5$ and $3\cdot10^3$~$7\cdot10^5$, respectively. The dextran (weight-average molecular mass=$7.14\cdot10^4$, polydispersity=2) was fractionated into ten fractions with molecular masses range from $4\cdot10^4$ to $1\cdot10^5$, which were analyzed by an aqueous phase analytical SEC. A daily throughput of 6.5 g of the dextran was given with a flow-rate of 2.88 ml min^{-1}. The preparative SEC is simple, fast and cheap, and suitable for large-scale fractionation of the polysaccharides and other biopolymers.

Keywords: Cellulose packings; Preparative chromatography; Polysaccharides

1. Introduction

The preparative fractionation of polysaccharides and other biopolymers by size-exclusion chromatography (SEC) is becoming more important in industrial processes and in laboratories [1–3]. The column packing materials have conventionally been made of cross-linked dextran, agarose and polyacrylamide, which are too expensive to be used in industrial applications [2]. The cellulose membranes have been found extensive commercial applications in membrane separation techniques such as dialysis, ultrafiltration and fractionation, because of their relatively low cost, good compatibility with biological compounds and their remarkable hydrophilic properties [4,5]. Therefore, cellulose as a chromatographic material has promising application. Various chromatographic packing prepared by cellulose aqueous calcium thiocyanate solution [2], viscose solution [6] and cellulose/17.5% NaOH aqueous solution [7] have been reported.

In order to investigate the solution properties and the correlation of molecular mass to bioactivities of the polysaccharides, the fractions with different molecular masses are needed. A nonsolvent addition method has been long used to fractionate the polymer in organic solvent. In our previous works [8,9], the glucans from Auricularia auricular-judae and Poria cocos sclerotium were fractionated, respectively, by nonsolvent addition method, however, no satisfactory fractions were obtained, because of gelatinization of the glucan aqueous solution in the

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precipitant process or aggregation in aqueous solution. Moreover, the polydispersity of the fractions for lacquer polysaccharide prepared by nonsolvent addition method was even larger than that of unfractionated sample [10]. The purpose of this study was to develop a simple and fast fractionation method of the polysaccharides to substitute for the low efficiency and time-consuming nonsolvent addition method. Recently, the regenerated cellulose microporous membranes were satisfactorily prepared by mixing cellulose cuoxam with polyethylene glycol (PEG) as pore former [11]. When 50% PEG2000 was added to the cellulose cuoxam, the value of mean pore diameter ($2r_f$) of the cellulose membrane is more than four times than that of non-mixing membrane. Based on the method, we prepared regenerated cellulose gel particles and developed a large-scale preparative chromatograph for the polysaccharides in aqueous solution.

2. Experimental

2.1. Preparation of regenerated cellulose gel particles

The linter used was supplied by Hubei Chemical Fiber Manufacture, and its viscosity-average molecular mass ($M\eta$) was determined to be $1.96\cdot10^5$. Polyethylene glycol (PEG2000) with molecular mass 2000 was imported from Japan. A 6% (w/w) cellulose cuoxam solution (I) was prepared according to previous method [12]. The 20% (w/w) PEG2000 was dissolved in water (II). The mixture of I and II (1:1, w/w) was spinned to a wire of a diameter of 0.3 mm, and coagulated in 10% NaOH aqueous solution, then regenerated in dilute aqueous H_2SO_4. The clear cellulose fibers were cut to small particles with length of 0.5–1.5 mm, then washed by water, finally stored in 20% isopropanol/2% formaldehyde aqueous solution. The cellulose gel particles prepared by I and the mixture of I and II were coded as RCG-1 and RCG-2, respectively, and their preparation process was same.

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

$$\rho_g = \frac{w_d}{V-(w_w-w_d)/\rho_{H_2O}} \quad (1)$$

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

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

where w_d and w_w are masses of dry and wet particles, respectively. The ρ_{H_2O} is density of water.

The scanning electron micrographs (SEM) of the RCG-1 and RCG-2 were made on a SEM ISI-SX-40. The surface and cross section of the particles were observed and photographed. The wet particle sizes (diameter and length) were measured by using a microscope, and estimated according to statistical method.

2.2. Preparative SEC

To determine the exclusion limit of the stationary phase, the cellulose gel particles of RCG-1 and RCG-2 (1:3, v/v) suspended in distilled water were packed in a glass column (500 mm×10 mm), in which the consumption of the polysaccharide standards could be reduced, to form a ca. 450 mm long gel bed. The 1 ml approximately $2\cdot10^{-2}$ g ml^{-1} aqueous solutions of the glucan standards (weight-average molecular masses, M_w, $70\cdot10^4$, $56.3\cdot10^4$, $38.5\cdot10^4$, $14.4\cdot10^4$, $6.87\cdot10^4$, $2.98\cdot10^4$, $1.51\cdot10^4$, $0.27\cdot10^4$), which were a gift of National Research Center for Certified Reference Materials in Beijing, and D-cellobiose (Wako Co.) were separately injected into the column, and the flow-rate was adjusted to 1.1 ml min^{-1} and kept constant. The effluent was monitored at 200 nm by using UV detector (UV-160, Shimadzu, Japan).

To prepare fraction, the cellulose gel particles of RCG-1 and RCG-2 (1:3, v/v) suspended in distilled water were packed in a glass column (550 mm×20 mm) to form a ca. 500 mm long gel bed. The preparative SEC column so obtained was equipped with automatic fraction collector. The dextran produced by strain No. B-512 ($M_w=7.14\cdot10^4$, Sigma, Norway) was dissolved in distilled water to prepare

0.057 g ml^{-1} concentration. The 3.5 ml dextran solutions were injected into the column, and distilled water was used as eluent at 25°C. The flow-rate was adjusted to 2.88 ml min^{-1} by using peristaltic pump during the run. The column effluent and fractions were monitored by UV detection at 200 nm.

2.3. Characterization of fractions

Viscosity of the fractions and unfractioned dextran were measured at 25±0.1°C by using a modified capillary viscometer supplied by the Institute of Industrial Science, Tokyo University. Huggins and Kraemer plots were used to estimate the intrinsic viscosity [η].

SEC analysis of the fractions and unfractionated dextran as well as pullulan standards (P-5, P-10, P-20, P-50, P-100, P-200, P-400, and P-800) were performed on a HPLC apparatus (LC-6A, Shimadzu) equipped with aqueous phase analytical SEC column packed with silca gel (Institute of Jilin Chemical Industrial Co., China) and with refractive index detector. The eluent was 0.2 *M* Na_2SO_4–0.01 *M* NaH_2PO_4–Na_2HPO_4 buffer (pH 7.03), and the flow-rate was 1.0 ml min^{-1} at total pressure of 40 kgf cm^{-2}. The calibration curve obtained from pullulan standards shows in Fig. 1, and was represented as following:

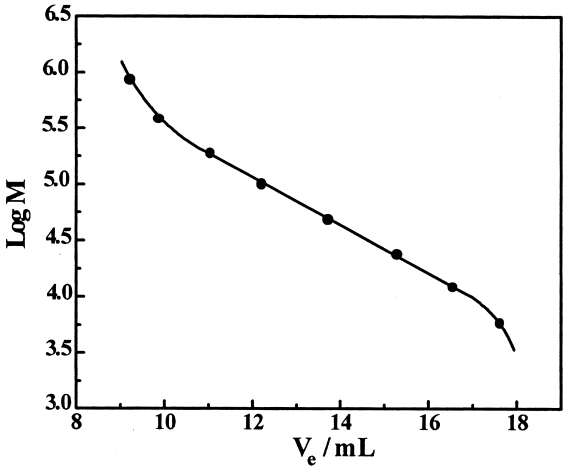

Fig. 1. Calibration curve for pullulan standards in 0.2 *M* Na_2SO_4–0.01 *M* NaH_2PO_4–Na_2HPO_4 buffer by using LC–6A HPLC equipped with silica gel column for SEC and with a refractive index detector at a flow-rate of 1.0 ml min^{-1}.

$$\text{Log } M = 7.616 - 0.214V_e \quad (4)$$

where V_e is elution volume. Using this calibration curve the weight average molecular mass M_w of the dextran was determined to be $7.08 \cdot 10^4$ (see Table 2) and similar to the given data ($7.14 \cdot 10^4$). Therefore, SEC universal calibration was not used in this work.

3. Results and discussion

Fig. 2 shows SEM of the surface and cross section of the cellulose gel particles of RCG-1 (A) and RCG-2 (B). The pore sizes were 90 nm (surface) and 180 nm (cross section) for RCG-1, and 140 nm (surface) and 600 nm (cross section) for RCG-2, respectively, namely mean pore size 2r is 135 nm for RCG-1 and 370 nm for RCG-2. The physical properties of the gel particles are summarized in Table 1. The size-exclusion curve for the preparative SEC (500 mm×10 mm) is illustrated in Fig. 3, and was represented as following:

$$\text{Log } M = 9.756 - 0.139V_e \quad (5)$$

It was determined that the exclusion limit and fractionation range of the stationary phase of the preparative SEC were molecular masses $7 \cdot 10^5$ and $3 \cdot 10^3 \sim 7 \cdot 10^5$, respectively. The distribution coefficient K_{SEC}, which determines the percentage of gel-phase available for each fraction independently on system geometry, was calculated by [13]

$$K_{SEC} = (V_e - V_0)/(V_i - V_0) \quad (6)$$

where V_e is the elution volume, V_0 is the void volume and V_i is the total pore volume. The molecular mass dependence of K_{SEC} is shown in Fig. 4. The pore size dictates the range of molecular mass separation changing K_{SEC} strongly, therefore influencing the percentage of gel phase available to a given substance. In view of mentioned above, the gel particles RCG-1 and RCG-2 with different pore sizes were successfully used as a stationary phase on the preparative SEC.

Fig. 5 shows the elution pattern of the preparative SEC (550 mm×20 mm) for the dextran monitored by UV absorbance. The slicing indicates that the fractions collected 12 injections were combined to

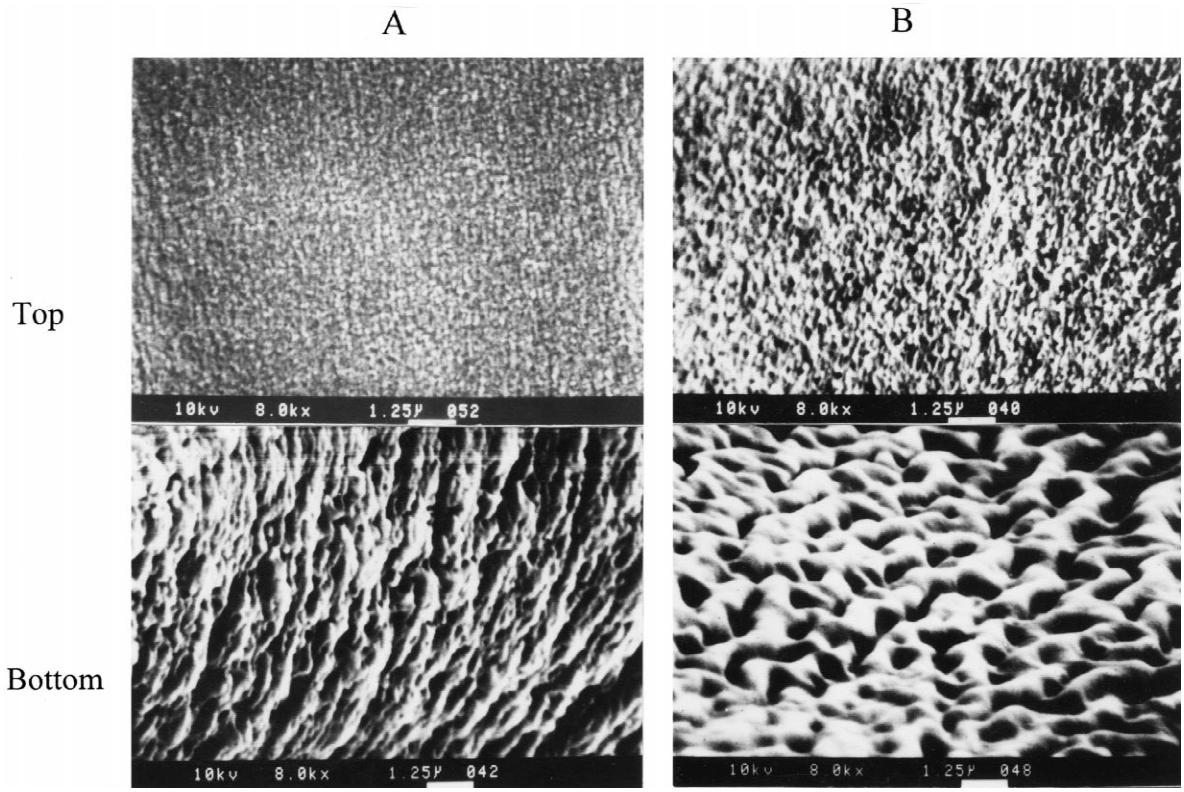

Fig. 2. SEM of the surfaces (top) and the cross sections (bottom) of the cellulose gel particles for RCG-1 (A) and RCG-2 (B).

get 0.15–0.25 g of each fractions (DF-1~DF-9, except DF-10), the total yield was 87.6%. The $[\eta]$ of the fractions are listed in Table 2. Except DF-1, the $[\eta]$ decreases with increasing elution volume, and the $[\eta]_{cal}$ calculated by $\Sigma w_i[\eta]_i$ from all fractions, where w is weight fraction of the fractions, was 24.6 $cm^3\ g^{-1}$ close to that measured from unfractionated dextran, suggesting a good fractionation effect. The values of M_w, number-average molecular mass (M_n) and polydispersity index d (M_w/M_n) of each fraction obtained by SEC analysis are also summarized in Table 2. The $M_{w\ cal}$ calculated by $\Sigma w_i M_i$ from all fractions was $7.05 \cdot 10^4$, which is in good agreement with the value measured by SEC from the unfractionated dextran. The d values of the fractions are about 1.4 except DF-8, DF-9 and DF-10, which were collected from broad slicing. It is worth noting that first fraction DF-1 was given anomalous data, which showed lower values of M_w, M_n and $[\eta]$ than those of DF-2, DF-3, DF-4. It can be explained that some interaction exists between the stationary phase and dextran caused by their hydroxyl groups, so that the

Table 1
Physical properties of the gel particles

Gel	Mean particle size[a] (μm)	Mean pore size (nm)	Backbone density ($g\ ml^{-1}$)	Pore volume ($ml\ g^{-1}$)	Porosity (%)
RCG-1	320×1390	135	1.48	6.17	90.1
RCG-2	330×1180	370	1.54	8.12	92.6

[a]diameter×length of the wet particle.

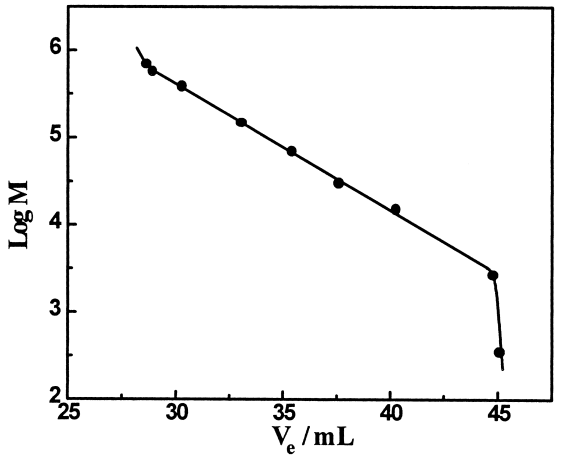

Fig. 3. Size-exclusion curve of the preparative SEC (500 mm×10 mm) packed with a mixture of RCG-2 and RCG-1 (3:1, v/v) for the glucan standards in water at 25°C with a flow-rate of 1.1 ml min^{-1} by using UV detector at 200 nm.

high-molecular-mass part of DF-1 was first adsorbed on the gel particles and retarded, resulting in reduction of its average molecular mass. The interpretation was supported by the d value (1.46) of DF-1, which should have the *d* value of 1.6 as same as DF-8, DF-9, and DF-10 due to collection from broad slicing, and the uncompleted recovery of total product after fractionation.

It is well known that size-exclusion HPLC with TSK G3000 SW and G4000 SW columns has been

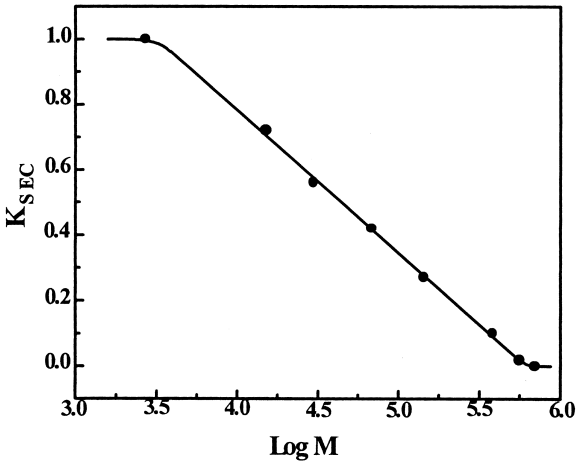

Fig. 4. Molecular mass (*M*) dependence of K_{SEC} of the preparative SEC. The conditions are same as Fig. 3.

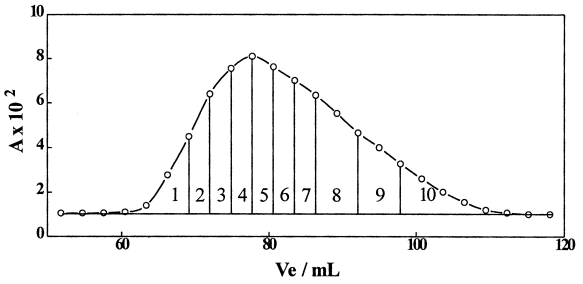

Fig. 5. Elution pattern and fractionation of dextran in water at 25°C by using the preparative SEC (550 mm×20 mm) packed with RCG-2 and RCG-1 (3:1, v/v) with a flow-rate of 2.88 ml min^{-1}. The absorbance (A) of the effluent was monitored at 200 nm by using a UV detector.

widely used in the fractionation of biopolymers [3,14,15]. In our previous study [16], the lacquer polysaccharides ($M_w = 10.9 \cdot 10^4$, $d = 1.96$) were isolated into 12 fractions by aqueous-phase preparative SEC (Toyo Soda). It was equipped with TSK G3000 SW and TSK G4000 SW column (600 mm×22 mm), in which 0.08 *M* KCl/0.01 *M* NaAc aqueous solution was used as eluent, and the flow-rate was 4 ml min^{-1}. These data of SEC and $[\eta]$ cited were listed in Table 3, where 11 fractions were analyzed by SEC and viscometry. These results indicate that the preparative SEC studied in this work has good fractionating efficiency and large throughput, in which a daily throughput of 6.5 g for dextran is reached with a flow-rate of 2.88 ml min^{-1} aqueous solution. It is worth noting that the cellulose gel particles are much cheaper than G3000 SW, G4000 SW, cross-linked dextran, agarose as well as polyacrylamide, since cellulose is the most abundant natural material [2]. Therefore, the preparative SEC packed with these cellulose gel particles have promising application in industrial process to produce fractions with different molecular masses or structure for polysaccharides and other biopolymers, because of its simple, fast and cheap feature. Furthermore, utilizing cellulose as material can not only reduce loss of limited petroleum resources, but also protect the environment.

4. Conclusion

Two kinds of cellulose gel particles RCG-1 and

Table 2
Experimental results of $[\eta]$, M_w, M_n and d for dextran and its fractions by viscosimetry and SEC analysis

Fraction	Mass (g)	$[\eta]$ ($cm^3\ g^{-1}$)	$M_w\times10^{-4}$	$M_n\times10^{-4}$	d
Dextran		23.85	7.08	3.65	1.94
DF-1	0.2392	26.46	8.19	5.61	1.46
DF-2	0.1530	29.22	9.93	6.94	1.43
DF-3	0.1528	27.12	9.33	6.61	1.41
DF-4	0.1824	26.76	9.18	6.51	1.41
DF-5	0.1750	26.22	8.17	5.83	1.40
DF-6	0.1618	25.75	7.71	5.43	1.42
DF-7	0.1590	24.10	7.45	5.25	1.42
DF-8	0.2478	23.78	6.67	4.17	1.60
DF-9	0.2096	22.85	4.99	3.10	1.61
DF-10	0.4294	19.14	4.06	2.51	1.62

RCG-2 with mean pore sizes of 135 nm and 370 nm were prepared by cellulose cuoxam and by mixing cellulose cuoxam with polyethylene glycol as pore former, respectively. The preparative SEC packed with the mixture of RCG-2 and RCG-1 (3:1, v/v) was satisfactorily used to fractionate dextran, which was divided into 10 fractions with the molecular masses ranging from $4\cdot10^4$ to $1\cdot10^5$ and polydispersity indexes of ca. 1.4 except 3 fractions. The fraction range of the stationary phase is $3\cdot10^3\sim7\cdot10^5$, and the daily throughput of the SEC column (550 mm×20 mm) with a flow-rate of 2.88 ml min^{-1} is 6.5 g for the dextran in aqueous solution at 25°C. Therefore, the cellulose gel particles should be suitable not only for fractionation of the polysaccharides, but also for other biopolymers in industrial process.

Table 3
Data of SEC and $[\eta]$ of lacquer polysaccharides fractionated by Toyo Soda SEC instrument equipped with TSK-G3000 SW and G4000 SW [16]

Sample	SEC			$[\eta]$ ($cm^3\ g^{-1}$)
	$M_w\times10^{-4}$	$M_n\times10^{-4}$	d	
FR1	14.8	8.42	1.76	11.5
FR2	13.7	7.35	1.86	10.7
FR3	12.3	6.72	1.83	9.52
FR4	11.6	7.19	1.61	9.68
FR5	10.1	6.32	1.60	9.50
FR6	8.16	5.36	1.52	8.03
FR7	6.28	4.03	1.56	7.45
FR8	5.50	3.93	1.40	6.92
FR9	5.09	3.43	1.48	6.18
FR10	4.95	3.36	1.47	5.76
FR11	4.57	2.72	1.68	—
Unfractionated polysaccharide	11.0	5.61	1.96	8.46

References

[1] D. Lecacheux, G. Brigand, Carbohydr. Polym. 8 (1988) 119–130.
[2] S. Kuga, J. Chromatogr. 195 (1980) 221–230.
[3] S.C. Churms, J. Chromatogr. A 720 (1996) 151–166.
[4] J.H. Hannemaji, T. Robberstsen, T.V. Boomgaard, C. Olieman, P. Both, D.G. Schmidt, Desalination 68 (1988) 93–108.
[5] F. Moror, M. Oliver, Sep. Sci. Technol. 24 (1989) 353–367.
[6] S. Okuma, K. Yamagichi, M. Hara et al., Jpn. Kokai Tokkyo Koho 63 92 603 (1988).
[7] H. Inagak, T. Miyamoto, S. Takenishi et al., Jpn. Pat., 2-235944 (1990).
[8] L. Zhang, L. Yang, Biopolymers 36 (1995) 695–700.
[9] L. Zhang, Q. Ding, P. Zhang, R. Zhu, Y. Zhou, Carbohydr. Res. 303 (1997) 193–197.
[10] L. Zhang, X. Qiu, J. Xiu, Y. Du, B. Qian, J.F. Kennedy, Carbohydr. Polym. 19 (1992) 161–162.
[11] G. Yang, L. Zhang, J. Membrane Sci. 114 (1996) 149–155.
[12] L. Zhang, G. Yang, S. Yan, H. Liu, Chinese Pat., CN 1091144A (1993).
[13] W.W. Yan, J.J. Kirkland, D.D. Bly, Modern Size Exclusion Liquid Chromatography, Wiley, New York, 1979.
[14] R. Oshima, J. Kumanotani, Carbohydr. Res. 127 (1984) 43–57.
[15] M. Calero, J. Escribano, F. Soriano, A. Grubb, K. Brew, E. Mendez, J. Chromatogr. A 719 (1996) 149–157.
[16] L. Zhang, Y. Du, J. Kumanotani, Chin. J. Polym. Sci. 7 (1989) 252–257.