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Creation of regenerated cellulose microspheres with diameter ranging from micron to millimeter for chromatography applications

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ABSTRACT

Regenerated cellulose microspheres (RCM) with different diameters were prepared from cellulose solution using 7 wt% NaOH/12 wt% urea aqueous solvent pre-cooled to −12 °C by the sol–gel transition method via a "green" process. By varying the hydrophile–lipophile balance, the amount of the surfactants, the proportion of the water to the oil phase and the stirring speed, the mean diameter of the cellulose microsphere with nano-scale pore size could be controlled easily from 5 μm to 1 mm. The structure and physicochemical properties of the microspheres were characterized by FT-IR spectroscopy, scanning electron microscopy, X-ray diffraction, mercury intrusion-porosimetry and particle size analyzer. The RCM microspheres exhibited spherical shape with the cellulose II structure. A preparative size-exclusion chromatography (SEC) column packed with the cellulose microspheres was used for the fractionation of a polyethylene oxide (PEO) in water, which indicated high efficiency for the fractionations and a large daily throughput of 4 g. Moreover, they had good adsorption capacity to dye particles through physical interaction. The cellulose microspheres would have potential applications in the fields of purification, separation and fractionation of polymers as chromatography packing and adsorbent both at laboratory and industrial scale.

1. Introduction

Polymer microspheres have wide applications in the fields of chromatography, separation technology, sorbent, biocatalyst immobilization and carriers. Moreover, the microspheres with carbonyl, hydroxyl, or amino groups can be used to immobilize antibodies and other proteins through covalently bonding [1,2]. However, numerous production and wide applications of synthesized polymer packing have led to environment problems due to the non-biodegradability of these materials. To minimize environmental pollution that has become an unquestionable threat for the plant and the quality of human life, novel environmentally friendly processes and materials prepared from renewable resources, especially biodegradable polymers are gaining increased interest in recent years [3]. Cellulose is the most abundant natural polymer with an annual production rate of 10^{11} to 10^{12} tons [4], and it has very attractive properties, such as biocompatibility, biodegradability, and thermal and chemical stability [5]. Regenerated cellulose microspheres possess large surface area, porosity, hydophilicity, and –OH groups, thus showing potential applications in the fields of chromatographic separation, purification and sorption. In the early 1950s, O'Neil first developed a method to manufacture cellulose beads based on the jet injection process of cellulose xanthate viscose [6]. Subsequently, many studies relating to the preparation and applications of spherical cellulose have been performed. However, the viscose route requires additional facilities to treat the hazardous by-products (CS_2) and aqueous waste emissions. In the past decade, new cellulose solvents for cellulose, such as ionic liquid [7], *N*-methylmorpholine-*N*-oxide (NMMO) [8], LiCl/dimethyl acetamide (DMAc) [9] have been developed, and the regenerated porous cellulose beads have been used as column packing materials for liquid chromatography, and as matrix for further derivatization for ion exchange or affinity chromatography [10]. Moreover, the cellulose exchangers or adsorbents, and magnetic beads [11,12] have been potentially employed for enzyme immobilization [13,14], base catalysis [15], protein separation and purification [16], adsorption of precious metal ions for recovery or as catalysts after reduction [17], waste water treatment to remove heavy metal ions [18] and acidic dyes [19].

It is noted that size-exclusion chromatography (SEC) is a rapid method for separation and fractionation of both synthetic polymers and biopolymers [20,21], and has become a conventional method for determination of molecular mass distribution (MMD) of numerous polymeric materials [22]. Thus, SEC has long been an important technique in the analysis and quality control of polymers [23,24]. In modern polymer science, a wide range of methodologies based on SEC have been elaborated for the analysis and characterization

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of complex polymers [22,25,26]. One of the required experimental efforts can be in part carried out in industry by SEC column manufacturers, namely providing better packing materials [27]. The chromatographic packing materials for biopolymer separation are mostly prepared from polysaccharides [23,28]. Preparative SEC columns packed with microporous regenerated cellulose gels have been used to fractionate biopolymers such as dextran [29], β-D-glucan from *Poria cocos* sclerotium [30] and enzyme [31].

In our laboratory, a new solvent for cellulose has been developed, showing that cellulose can be dissolved rapidly in the aqueous solution of NaOH/urea at low temperature to form a transparent solution [26]. Moreover, from the cellulose solution, cellulose microspheres have been prepared to create magnetic materials [32]. A worthwhile endeavor would be to create environmental friendly cellulose packing materials produced *via* a "green" process for the separation applications. Both fundamental research and industrial applications drive our interest to prepare the cellulose microspheres having different particle sizes. In the present work, various regenerated cellulose microspheres were prepared from cellulose solution in NaOH/urea aqueous system by using the sol–gel transition method (SGT) at room temperature. The structure and physicochemical properties of the cellulose microspheres were investigated to evaluate their separation efficiency and fractionation capacity on polymers. We provided here a new design and fabrication method of cellulose microspheres with various particle diameters for chromatography applications.

2. Experimental

2.1. Materials

Cotton linter pulp (α-cellulose, >95%) was provided by Hubei Chemical Fiber Group Ltd. (Xiangfan, China). According to the Mark–Howink equation $[\eta] = 3.72 \times 10^{-2}\ M_w^{0.77}$ [33], its viscosity-average molecular weight (M_η) was determined as 8.1×10^4 by using an Ubbelohde viscometer in LiOH/urea aqueous solution at 25 ± 0.05 °C. The cellulose sample was shredded and dried for 8 h in a vacuum oven at 65 °C, and then stored in a desiccator until used. Polyethylene oxide (PEO) (Alfa Aesar Co., $M_w = 1 \times 10^5$) was used here. All other chemicals, which were of analytical grade, were purchased domestically and used as received.

2.2. Preparation of RCM

The sol–gel process involves the transition of a system from a liquid ('sol') into a solid ('gel') phase. In a typical sol–gel process, the precursor is subjected to a series of hydrolysis and polymerization reactions to form a colloidal suspension, and then the microspheres aggregate in a new phase to transform into the gel [34]. The cellulose solution was prepared according to the previous method [35] A solution of NaOH/urea/H_2O (7/12/81, by weight) was pre-cooled to −12.3 °C, and then 8 g of cellulose was immediately dispersed into the solvent (200 mL) under vigorous stirring for 3 min at ambient temperature (below 20 °C) to obtain a transparent cellulose solution. The cellulose solution was degassed by centrifugation at 8000 rpm for 15 min at 5 °C [36]. This solution was dropped in the solution of Span-80 in paraffin oil within 1 h and stirred in a scheduled stirring speed. The suspension in the vessel was stirred at the same speed and temperature for an additional 3 h. The regenerated cellulose microspheres were formed when the pH value of the suspension was adjusted to 7.0 by the addition of dilute hydrochloric acid with stirring. The suspension was allowed to stand until it was separated into two layers. The upper layer organic phase was washed with water, and then recovered. The lower layer aqueous phase was rinsed with deionized water and washed with acetone three times to obtain regenerated cellulose microspheres, coded as RCM.

2.3. Staining of microspheres with dyes

To test dye-adsorption behavior of the microspheres, the RCM samples (0.01 g) were suspended in a 0.1% congo red, methyl orange or reactive red (20 mL). After shaking the mixture suspension at room temperature for 3 h, the stained RCMs were washed thoroughly with distilled water (300 mL) for the congo red-treated microspheres or ethanol (300 mL) and then water (50 mL) for the methyl orange or reactive red-treated microspheres by filtration [37]. Then the stained microspheres without drying were observed with an optical microscope (DP-20 and BX-50, Olympus, Japan).

2.4. Fractionation by preparative SEC

To evaluate the separation capacity and fractionation efficiency, the RCM microspheres suspended in distilled water were packed in a glass column (550 mm × 20 mm) to form a ca. 500 mm long gel bed. The resulting preparative SEC column was equipped with an automatic fraction collector. The PEO sample was dissolved in distilled water to prepare a solution of about 0.05 g mL^{-1} concentration. 5 mL of the PEO solution was injected onto the column to fractionate, and distilled water was used as eluent at 25 °C. The flow rate was adjusted to 3 mL min^{-1} by using a peristaltic pump during run. The column effluent and fractions were monitored by UV detection at 200 nm. To measure the molecular weight of the PEO fractions prepared via the preparative SEC, analytical SEC combined with laser light scattering (SEC-LLS) measurements of both the fractions and the unfractionated samples were carried out on a DAWN® DSP multi-angle laser photometer (λ = 633 nm; DAWN® DSP, Wyatt Technology Co., St. Santa Babara, USA), combined with a P100 pump (Thermo Separation Products, San Jose, Japan), equipped with TSK-GEL G4000H6 (7.5 mm × 300 mm) and TSK-GEL G600H6 (7.5 mm × 300 mm) at 25 °C. A detector (RI-150, TSP, USA) was simultaneously connected. The PEO solutions were filtered first with a sand filter, followed by a 0.45 μm filter (Whatman, England), then kept in sealed glass bottles before injecting into the SEC column. The Astra software (Version 4.07.70) was utilized for the data acquisition and analysis.

2.5. Characterization

The RCM in water was observed with a digital camera (Canon A630). The definite size distribution of the wet RCM microspheres was determined with a Malvern Mastersizer 2000 laser particle size analyzer (Malvern, UK). Scanning electron microscopy (SEM) was performed on a FESEM (SEM, SiRION TMP, FEI) by using an accelerating voltage of 20 kV. The RCM microspheres in the wet state were frozen in liquid nitrogen, and then freeze-dried by using Lyophilize (CHRIST Alpha 1-2, Germany). All the microspheres were coated with Pt for the SEM observation. The surface of the microspheres was observed and photographed. Fourier-transform infrared spectra (FT-IR) of the microsphere were recorded on a FT-IR spectrometer (model 1600, Perkin-Elmer Co.) with KBr pellets. Wide-angle X-ray diffractograms (XRD) were recorded employing an XRD diffractometer (D8-Advance, Bruker). The patterns with Cu Kα radiation (λ = 0.15406 nm) at 40 kV and 30 mA were recorded in the 2θ region of 10–70°. Samples were ground into powder and dried in a vacuum oven at 60 °C for 48 h. The crystallinity χ_c (%) of the native cellulose and RCM was estimated by Rabek's method [38], using the following relationship:

$$\chi_c = \frac{S_c}{S_c + S_a} \times 100 \quad (1)$$

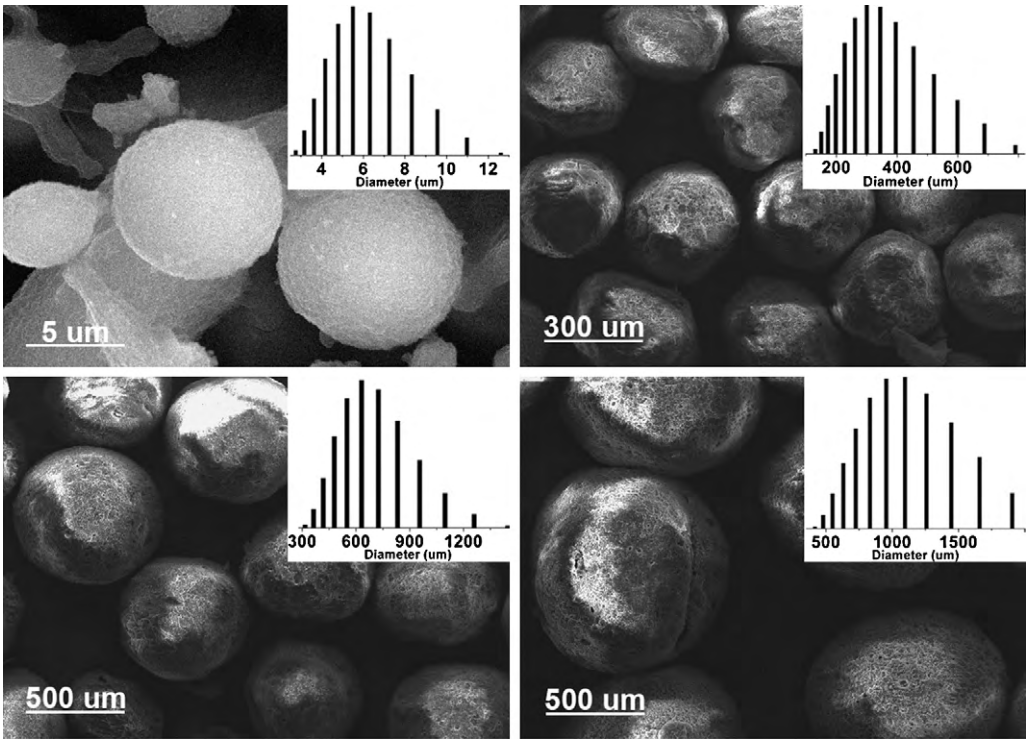

Fig. 1. SEM images of the RCM microspheres with different particle diameters and the size distributions as measured by Mastersizer 2000 (insets).

where S_c and S_a are the areas of crystalline and amorphous diffraction peaks of the samples, respectively. Thermo gravimetric analysis (TGA) was carried out on thermo gravimetric analyzer (Netzsch, German). The microsphere samples were ground into powder, and about 5 mg of the powder was placed in a platinum pan and heated from 20 to 600 °C at a rate of 10 k min^{-1} in ambient atmosphere.

Determination of the physical properties of RCM was performed according to our previously reported method [29]. The volume (V) of the wet microspheres was measured with drainage method by a dilatometer, which usually is used to measure the volume of polymer piece in the experimental method. The V value of the wet microspheres equals the total volume including the wet microspheres and original water minus that of original water in the dilatometer. The backbone density (ρ_g, namely density of the regenerated cellulose), mean pore volume (V_p) and porosity (P_r) were calculated using the relations:

$$\rho_g = \frac{w_d}{V - (w_w - w_d)/\rho_{H_2O}} \quad (2)$$

$$V_p = \frac{V - w_d/\rho_g}{w_d} \quad (3)$$

$$P_r = \frac{V_p}{V_p + 1/\rho_g} \quad (4)$$

where w_d and w_w are the mass of dry and wet microspheres, respectively. ρ_{H_2O} is the density of water. Pore size distribution was measured by AutoPore IV 9500 mercury intrusion-porosimetry (MIP$_y$, Micromeritics Instrument Corporation, GA, USA). The specific surface area (S) of the microspheres was determined by nitrogen adsorption using the BET method (Model ASAP 2400 Micromeritics Instruments, USA). The average pore radius (R) of the cellulose microspheres was calculated as:

$$R = \frac{2V_p}{S} \quad (5)$$

The flow properties of the microspheres in water were characterized in terms of the angle of repose [39]. For the determination of angle of repose (θ), the microspheres were poured through the walls of a funnel, which was fixed at a position so that its lower tip was at a height of exactly 2.0 cm above hard surface. The microspheres were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. The tan g^{-1} (θ) of the height of the pile/radius of its base gave the angle of repose.

3. Results and discussion

3.1. The effect of preparation conditions on particle size

Fig. 1 shows the SEM images of the RCM microspheres with different particle diameters and size distributions measured by Mastersizer 2000. The RCM microspheres exhibited spherical shape with mean diameter of 5.5, 315, 631 and 1096 μm, depending on the preparation conditions. The processing parameters and mean particle diameters of the RCM microspheres, coded as RCMA, RCMB, RCMC and RCMD, are summarized in Table 1. By changing various processing conditions such as dispersant dosage (1–10%), oil–water ratio (3:1–10:1) and stirring speed (200–1000 rmp), we prepared regenerated cellulose microspheres with particle diameter from 5 μm to 1 mm. With decreases of the dispersant dosage, oil–water ratio and stirring speed, the microsphere size increased rapidly. Therefore, we could regulate the cellulose bead size and its distribution by varying preparation conditions. It has been reported that

Table 1
The processing parameters and mean diameter of the RCM microspheres.

Sample	Dispersant dosage (%)	Oil–water ratio	Stirring speed (rmp)	Mean diameter (μm)
RCMA	10	10:1	1000	5.5
RCMB	6	6:1	800	315
RCMC	3	5:1	600	631
RCMD	1	3:1	200	1096

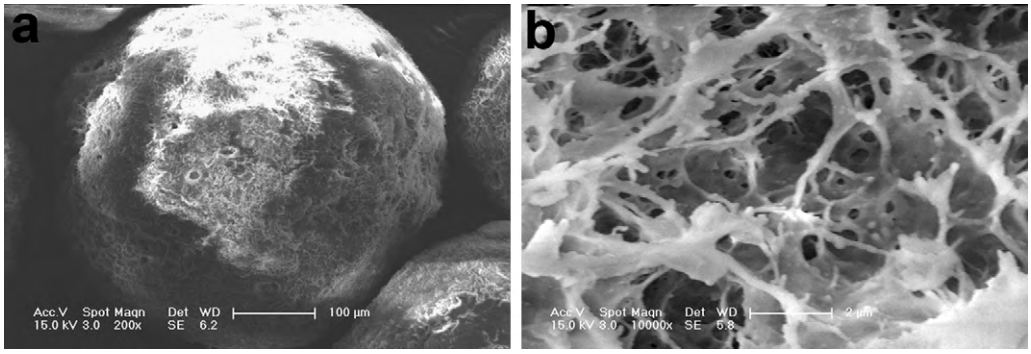

Fig. 2. SEM images of the morphology (a) and surface structure (b) of RCMB.

the microspheres shape, particle size and distribution of the microspheres are influenced by the optimum HLB value, the type and the amount of the surfactant, the proportion of the water phase and the oil phase, and stirring speed [40]. Moreover, in our findings, there was no evaporation of any chemical agents during dissolution of cellulose in NaOH/urea aqueous at low temperature, and the preparation method for RCM is simple and safe. Therefore, this is a "green" process, and the cellulose microspheres with various sizes will be of greater importance to both fundamental researchers in laboratory and industrial applications for various purposes.

3.2. Physical properties of the cellulose microspheres

Fig. 2 shows the SEM images of morphology and surface structure of RCMB (a) and the enlarged view (b). The microspheres exhibited good spherical shape and homogeneous surface with porous structure. Since the microspheres were regenerated directly from cellulose 'solution' in a quasi-gel state and formed mainly by physical cross-linking and hydrogen bonding interaction [33], the sol–gel transition led to the porous structure in the cellulose microspheres. Fig. 3 shows the digital photographs of the RCMB microspheres in water. The mean diameter of the wet RCMB microspheres was 315 μm (Table 1), and their size distribution curve fits the Gaussian one with relatively narrow range on the whole (Fig. 1). Generally, the chromatographic column packed with sphere stationary phase has the highest fractionation efficiency.

The physical properties of the RCMB are summarized in Table 2. Clearly, the RCMB microspheres displayed high water content (w), wet density (ρ_p), porosity (P_r), pore volume (V_p), the specific surface area (S). The mean pore diameter (D) of the microsphere was in nano-scale. The pore volume (4.16 mL/g) of RCMB obtained by MIP was smaller than that calculated by Eq. (3), due to the high pressure. The results indicated that the RCMB microspheres had good porous structure. Moreover, the RCMB samples had good settlement and flow properties. The flow properties of the microspheres can be judged from the angle of repose. The angle of repose < 30° indicated free flowing material and > 40° with poor flow properties [41]. In our findings, the value for the angle of repose of RCMB was found to be 24.2°, indicating that the RCMB microspheres possessed free flow properties.

As shown in Fig. 2(b), there were a large number of hollow patterns on the surface of the microspheres, as a result of the rough surface generated during the preparation process, and many nano-size pores in the microsphere. Therefore, the large pore resulted from the rough surface of the microspheres should be neglected when the pore size was calculated from the results of SEM and MIP. We have analyzed the nano-scale pores by using the SEM image processing without surface defects to get the apparent (D) value (about 280 nm). Fig. 4 shows the pore size distribution, $f(D)$, of the RCMB microspheres by mercury intrusion-porosimetry. The values of apparent mean pore diameter (D) obtained by MIP and SEM are listed in Table 2. The D values from both MIP and BET were similar, indicating a consistent result. The mean pore diameter of the microspheres ranged from 50 to 300 nm, which is suitable as the SEC chromatographic packing for the polymer fractionation. By virtue of the hydrophilic character of cellulose, the cellulose microspheres may be particularly suitable as water phase chromatographic column packing.

3.3. The structure and chemical properties of the cellulose microspheres

Fig. 5 shows the FT-IR spectra of cellulose (a), RCMA (b), RCMB(c), RCMC (d) and RCMD (e). Cellulose (a) exhibited characteristic adsorption of cellulose I [7], whereas the RCM regenerated

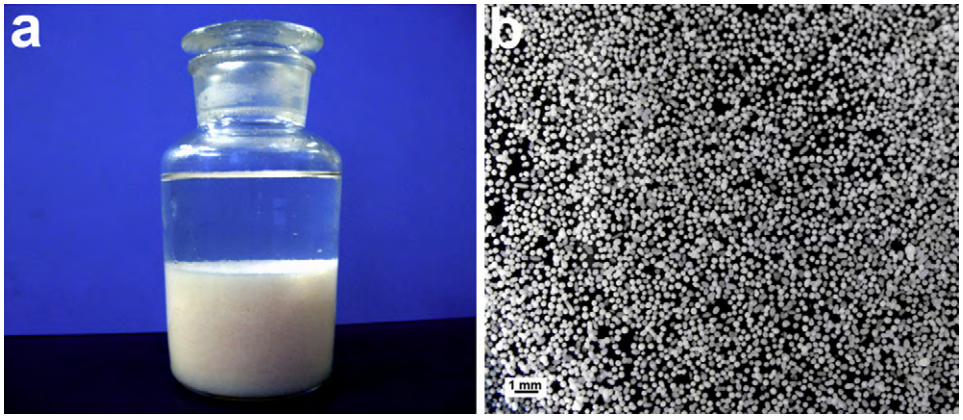

Fig. 3. Photographs of the RCMB microsphere in water.

Table 2
The physical properties of the RCMB microspheres.

Sample	ω (%)	ρ_g (mg/mL)	P_r (%)	V_p (mL/g)	S (m^2/g)	D (nm)		
						BET	MIP	SEM
RCMB	92.3	1.02	95.6	11.9	16.6	168	180	280

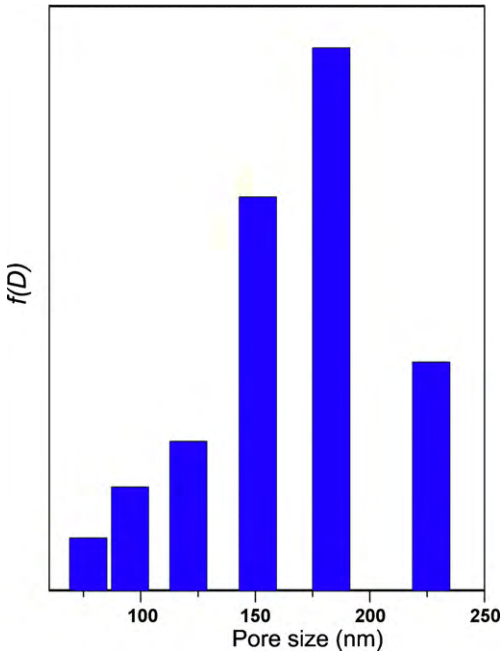

Fig. 4. Pore size distribution, $f(D)$, of the RCMB microspheres by mercury intrusion-porosimetry.

cellulose microspheres (b–e) showed characteristic peaks of cellulose II [42]. The band at 890 cm^{-1} is assigned to the β-linked glucose of cellulose. The band at 1421 cm^{-1} in RCMB is involved in the change of the conformation of CH_2OH at the C_6 position in cellulose from *trans-gauche* (*t-g*) to *gauche-trans* (*g-t*) [42–44]. The band at 3440 cm^{-1} in RCM was clearly broadened and shifted to a larger wavenumber compared with the native cellulose sample, suggesting the enhancement of the intermolecular hydrogen bonding in the cellulose microspheres [32]. During the dissolution process of cellulose, the intra- and intermolecular hydrogen bonds of cellulose were broken, and rearrangement of the hydrogen bonding of the regenerated cellulose occurred during the coagulation. The results of FT-IR spectra revealed that the structure of the cellulose II was formed in the RCM samples.

Fig. 6 shows the XRD diffraction patterns of cellulose (a), RCMA (b), RCMB (c), RCMC (d) and RCMD (e). The diffraction peaks at $2\theta = 14.8°$, 16.3°, and 22.6° for (1 $\bar{1}$ 0), (1 1 0) and (2 0 0) planes are characteristic for cellulose I crystal, and those at $2\theta = 12.1°$, 19.8°, and 22.6° for (1 $\bar{1}$ 0), (1 1 0) and (2 0 0) planes are characteristic for cellulose II crystal [45]. The cellulose raw material (Fig. 6(a)) had typical crystalline peaks of cellulose I, whereas the RCM (Fig. 6(b)–(e)) samples exhibited peaks of the cellulose II. Interestingly, the χ_c values of the RCM samples were much lower than those of the cellulose sample (a), and the values of the RCMA–RCMD (Fig. 6(b)–(e)) were similar (0.21–0.29). These results indicated that the water-swollen microspheres have more amorphous regions, as a result of the formation of porous structure. In view of the results from FT-IR and XRD, it is obvious that the crystal structure of cellulose changed from cellulose I to cellulose II in the process of dissolution and regeneration. Despite the small differences in the physical properties (particle size, pore size and wet density) of the cellulose microspheres from RCMA to RCMD, their chemical structure changed hardly.

Fig. 7 shows the thermogravimetric (TG) traces and differential thermogravimetry (DTG) of RCM. Since the data of all the microspheres (RCMA–RCMD) were almost identical, only one thermogravimetric trace was presented here. Compared with the cellulose raw material [46], an initial peak between 50 and 160 °C, corresponds to a mass loss of absorbed moisture [47] of approximately 8%. The major decomposition peak at about 350–360 °C could be attributed to decomposition and oxidative degradation of cellulose (mass loss 85%), and the small peak at about 540 °C (mass loss 30%) could be attributed to oxidation of the charred residue carbon. These results indicated a good thermal stability of the cellulose microspheres. The cellulose decomposition in air was complete and proceeded at a low temperature (below 550 °C) [48]. The thermal stability of the cellulose microspheres with the different particle sizes and pore sizes was almost similar. In addition, the mechanical intensities of the microspheres were better, and could

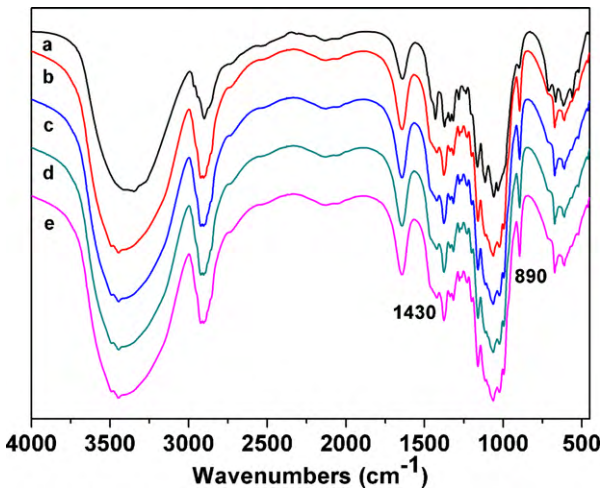

Fig. 5. FT-IR spectra of cellulose (a), RCMA (b), RCMB (c), RCMC (d) and RCMD (e).

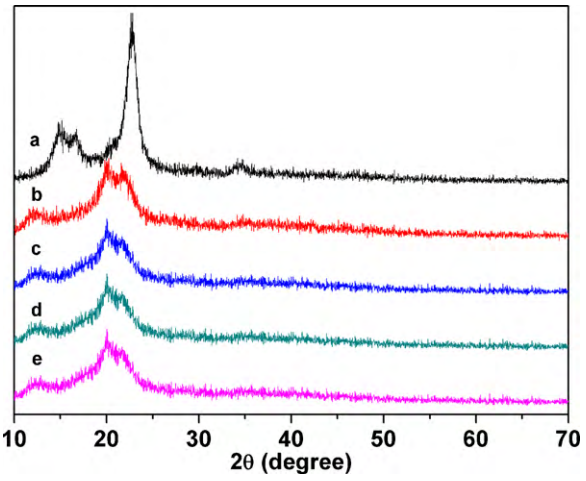

Fig. 6. The powder X-ray diffraction patterns of cellulose (a), RCMA (b), RCMB (c), RCMC (d) and RCMD (e).

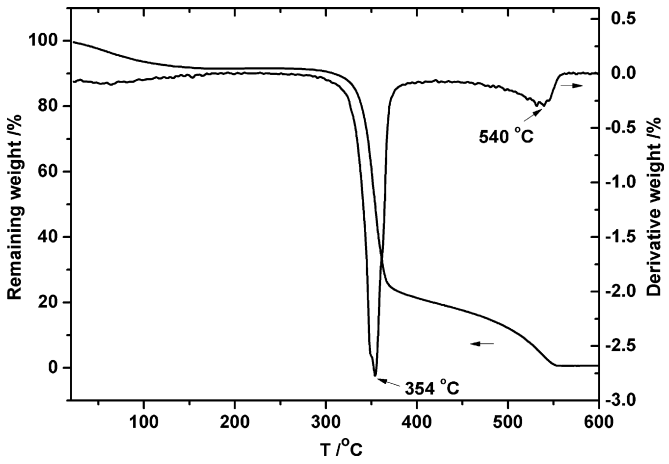

Fig. 7. TG traces and DTG curves of RCMB.

be enhanced by cross-linking with cross-linking agent (*e.g.* glutaraldehyde, epichlorohydrin, ethylene glycol glycidyl ether, *etc*) [49] to expand its applications at higher pressure.

3.4. Adsorption-separation behavior of the cellulose microspheres

To study on the adsorption behavior of the cellulose microspheres, dyes of congo red and methyl orange as well as reactive red were used here. Fig. 8 shows the optical micrographs of RCMB (a) and the RCMB samples treated with congo red (a), methyl orange (b) and reactive red (c). Usually, the congo red molecules preferentially adsorb on hydrophobic sites of cellulose [50], whereas those of methyl orange are fixed to anionic sites of cellulose by electrostatic interactions [51]. The RCMB sample treated with the congo red displayed red color via physical interaction, and it was well stained by methyl orange through van der Waals forces between cellulose and methyl orange molecules. However, the RCMB sample was almost not stained by reactive red, suggesting that it contained pure cellulose II and no other residue groups, impurity or catalysts, which may react with reactive red, existed in the RCMB microspheres. As verified by FT-IR and XRD spectra, there were only the hydroxyl groups in the cellulose microspheres. The SEM images of the surfaces of the RCMB microspheres treated with congo red (a), methyl orange (b) or reactive red (c) are shown in Fig. 9. The surface of RCMB stained with congo red and methyl orange exhibited the well-dispersed dyes pigments, suggesting that a strong interaction between dyes and cellulose existed in the microspheres. The results revealed that the microspheres have good adsorption capacity to the dye particles through physical interaction, rather than chemical reaction. The surface of the RCMB microspheres stained with reactive red (Fig. 9(c)) hardly changed, compared with the RCMB surface (Fig. 2(b)). Therefore, the RCM microspheres can be used to separate the organic substances through physical adsorption.

3.5. Fractionation efficiency of the chromatographic column

Fig. 10 shows the elution pattern on the preparative SEC (550 mm × 20 mm) packed with the RCMB microspheres for the PEO samples in water detected with an UV detector. The elution patterns of the three injections are in the same shape on the whole. From the preparative SEC, 6 fractions of PEO were obtained. The values of the weight-average molecular weight (M_w), number-average molecular weight (M_n) and the polydispersity index d (M_w/M_n) of each fraction determined by SEC-LLS analyses are summarized in Table 3. The M_w and M_n values of the fractions decreased with the progress of fractionation, and the d values of the fractions ranged from 1.16 to 1.49, which were much lower than that of the unfractionated PEO. It was demonstrated that the fractionation by the preparative SEC was successful. Moreover, the slicing indicated that the fractions collected 3 injections were combined to get 0.1–0.2 g of each fractions (PEO-1–PEO-6), the total yield was 86.6%. The M_w and M_n values of the PEO fractions are listed in Table 3. On the basis of the molecular weight definition, the M_w and M_n values of the PEO

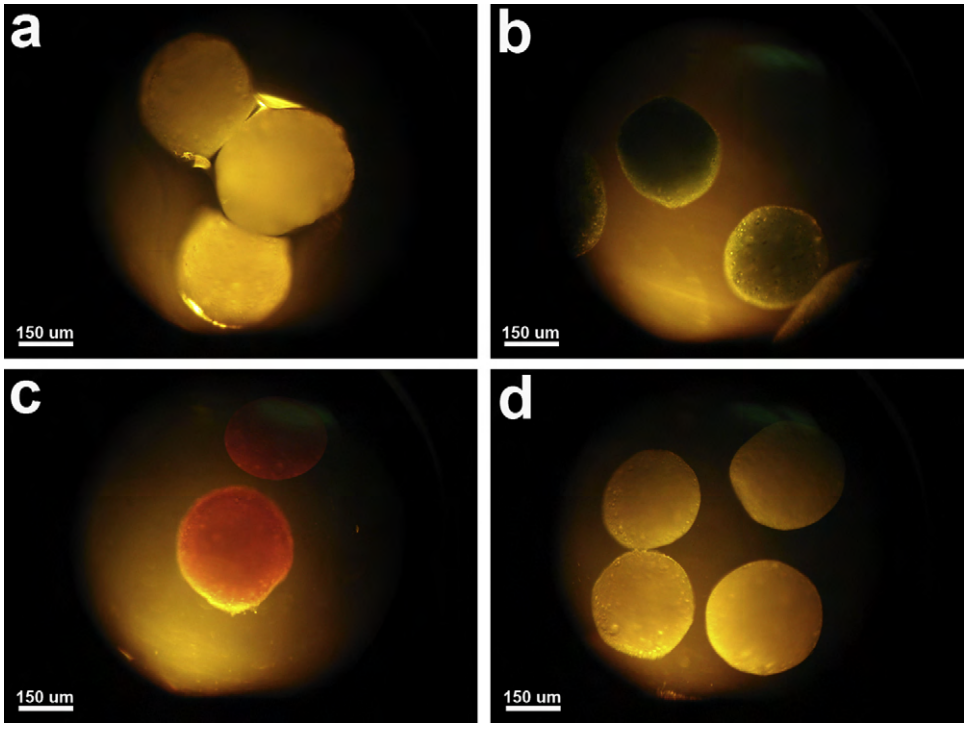

Fig. 8. Optical micrographs of RCMB (a) and the RCMB samples treated with congo red (b), methyl orange (c) and reactive red (d).

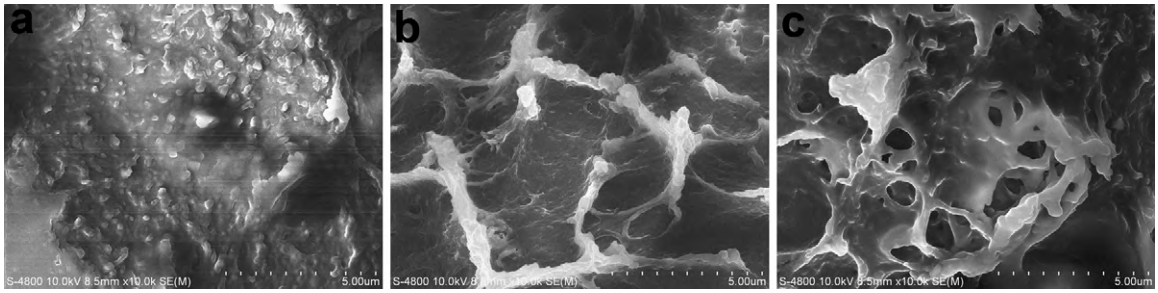

Fig. 9. SEM images of the RCMB samples treated with congo red (a), methyl orange (b) or reactive red (c).

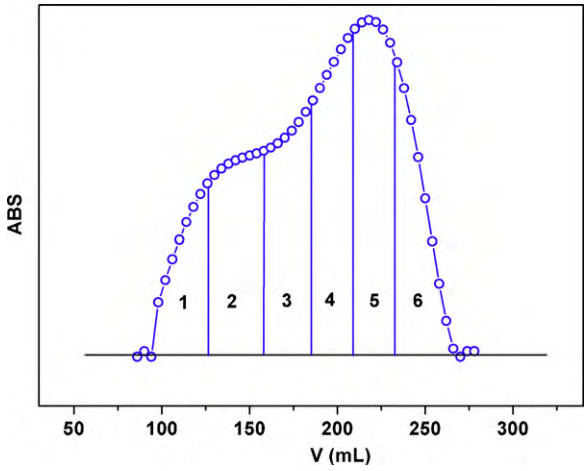

Fig. 10. Elution pattern on the preparative SEC (550 mm × 20 mm) packed with the RCMB microspheres for the PEO samples in water detected with an UV detector.

samples can be calculated from that of all fractions, as:

$$M_w = \sum_{i=1}^{6} W_i M_{w,i} \quad (6)$$

$$M_n = \frac{1}{\sum_{i=1}^{6} W_i / M_{n,i}} \quad (7)$$

where W_i is weight fraction of the i fraction, $M_{w,i}$ and $M_{n,i}$ are, respectively, weight-average molecular weight and number-average molecular weight of the i fraction. The mean value of M_w and M_n of the unfractionated PEO could be calculated by Eqs. (6) and (7) to be 10.1×10^4 g/mol and 6.37×10^4 g/mol. The calculated values of molecular weight were in agreement with that measured PEO by SEC-LLS. The results demonstrated that the preparative SEC packed with RCMB had good fractionation efficiency. Moreover, the preparative SEC displayed large throughput, in which a daily throughput of 4 g for PEO was reached with a flow rate of 3 mL/min aqueous solution. Usually, it is very difficult to obtain the polysaccharide fractions with narrow distribution from a sample with $d < 2$ by the non-solvent addition method [52]. Therefore, we have developed new chromatographic packing materials for SEC to obtain polymer fractions with narrow distribution and a high daily throughput.

Table 3
Experimental results of W_i, M_w, M_n and d for PEO and its fractions by SEC analysis.

Sample	W_i (%)	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	d
PEO-0		9.51	5.31	1.79
PEO-1	13.2	13.95	10.84	1.29
PEO-2	15.4	12.00	7.32	1.64
PEO-3	15.8	10.51	7.35	1.43
PEO-4	20.3	9.03	6.67	1.35
PEO-5	23.2	8.44	5.60	1.51
PEO-6	12.1	7.79	5.58	1.40

4. Conclusions

Regenerated cellulose microspheres (RCM) with diameters ranging from micro to millimeter were prepared successfully from the cellulose dope dissolved in NaOH/urea aqueous system at low temperature by sol–gel transition. It was a "green" process for the production of the regenerated cellulose microspheres from renewable raw materials, and the cellulose microspheres were safe and biodegradable. By changing process parameters, the spherical regenerated cellulose microspheres and beads, which possessed the cellulose II crystal structure and with diameters range from 5 μm to 1 mm, could be created, and they can find applications at in both laboratory and industrial scale. With a decrease in the dispersant dosage, oil–water ratio and stirring speed, the size of the microspheres increased rapidly. The RCM microspheres exhibited a good spherical shape, nano-scale pore, as well as better flow properties and adsorption capacity for the dyes. The preparative chromatographic column packed with these cellulose microspheres displayed good fractionation efficiency and large throughput. Therefore, the RCM microspheres can find applications as chromatographic packing materials, biocarrier and biosorbent.

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