

PARTIAL CARBAMOYLATION OF CELLULOSE MICROSPHERES: A NEW METHOD TO PREPARE ADSORBENTS FOR LIQUID CHROMATOGRAPHY*

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Abstract Micron-sized cellulose microspheres were prepared through sol-gel method using NaOH/urea solution to dissolve cellulose, then cross-linked by 1,6-hexanylene diisocyanate (HDI), toluene 2,4-diisocyanate (TDI) and 1,4-phenylene diisocyanate (PDI), respectively. The reaction conditions for partial modification of the microspheres were studied. The degree of substitution (DS) in cellulose was controlled by adjusting the reaction conditions. HDI-crosslinked microspheres were partially modified with phenyl isocyanate to obtain chiral stationary phases (CSPs). The CSPs of a lower degree of crosslinking (DC) showed better chiral recognition ability than those of a higher DC. Meanwhile the CSPs prepared by pre-modification exhibited better chiral recognition ability than those prepared by pre-crosslinking.

Keywords: Cellulose; Microspheres; Functionalization; Chiral stationary phase; Chiral recognition.

INTRODUCTION

As a most abundant natural polysaccharide, cellulose has attracted more attentions due to its regeneration and unique structure^[1–5]. After solution processing, it was widely used as separation membranes, carriers for controlled release of drugs and magnetic microspheres for separating biomacromolecules^[6–8]. Besides, its derivatives have also been used as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) owing to their helical chains and the corresponding cavities^[9–10]. Since the existed strong intra- and intermolecular hydrogen bonds lead to the insolubility of cellulose in usual solvents, how to dissolve cellulose during its processing and modification is a major challenge. Recently, Zhang *et al.*^[11, 12] developed some new solvent systems to dissolve cellulose rapidly. These solvents are the solutions of urea or thiourea in NaOH or LiOH aqueous solutions. By employing a cellulose solution, they prepared cellulose microspheres as chromatographic packing material for liquid chromatography^[13]. In order to widen the applications in HPLC, cellulose microspheres need to be partially benzoylated and carbamoylated for their enantioselectively separating chiral compounds^[10]. In the present work, the new methods to crosslink and partially carbamoylate cellulose microspheres were developed. As shown in Fig. 1, cellulose microspheres (CMs) were prepared through sol-gel method using NaOH/urea solution to dissolve cellulose, then cross-linked by 1,6-hexanylene diisocyanate (HDI), toluene 2,4-diisocyanate (TDI) and 1,4-phenylene diisocyanate (PDI), respectively (designated as CCMs 1–4). The residual hydroxyls on HDI-crosslinked microspheres with different degrees of crosslinking (DC) were

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partially carbamoylated with phenyl isocyanate to obtain **CSPs 1–2**. **CSPs 3** was prepared by crosslinking the pre-modified cellulose microspheres using phenyl isocyanate as the modifier and HDI as the crosslinker. The enantioseparation capability of these **CSPs** was also preliminarily evaluated.

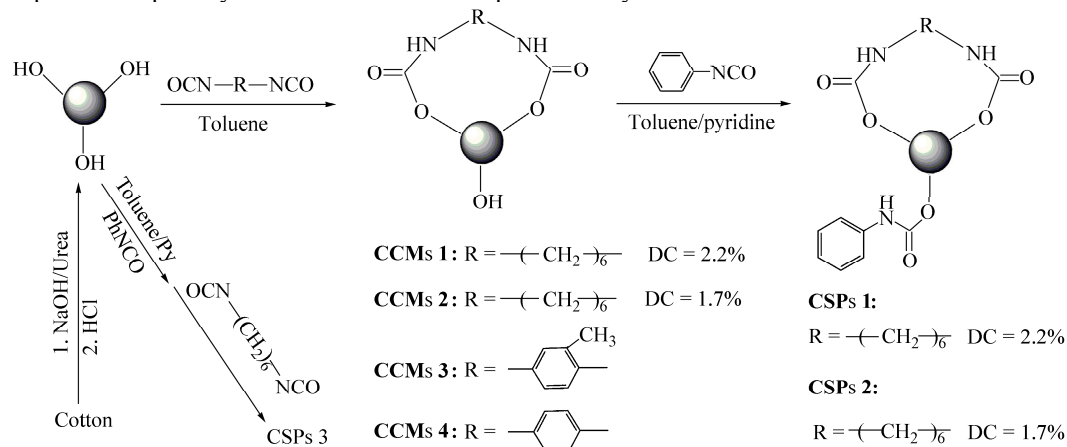


Fig. 1 Crosslinking of cellulose microspheres and the preparation of **CSPs 1–3**

EXPERIMENTAL

Materials

Cotton linter pulp (α -cellulose, > 95%) was available from Hubei Chemical Fiber Group Ltd. (Xiangyang, China), with a viscosity-average molecular weight M_v of 9.0×10^4 . Paraffin oil was purchased from Tianjin Bodi Chemical Co., Ltd (Tianjin, China). 1,4-Phenylene diisocyanate (PDI) was available from Jiangsu Xingyi Pesticide Company (China), and recrystallized from toluene before use. 1,6-Hexamethylene diisocyanate (HDI) and toluene 2,4-diisocyanate (TDI) were, respectively, purchased from BASF (Germany) and Bayer (Germany). Phenyl isocyanate was obtained from Sigma-Aldrich (USA). Pyridine was dried with NaOH and CaH_2 in sequence, and redistilled. Toluene was refluxed with sodium and re-distilled. All other chemicals were of analytical grade and used as received.

Instruments and Measurements

IR spectra were recorded on a Nicolet FTIR instrument (USA) with KBr pellets. ^1H -NMR measurements were performed on a Varian INOVA 500 spectrometer (USA) with deuterated trifluoroacetic acid (d -TFA) as the solvent. The volume-average diameter (D_{av}) of cellulose microspheres was measured on a Microtrac S3500 laser particle size analyzer (USA) using deionized water as the fluid. The cellulose microspheres were coated with gold and observed on a JSM-5510LV scanning electron microscope (SEM, Japan) at accelerating voltage of 30 kV. The thermogravimetric analysis (TGA) was conducted on a thermogravimetric analyzer (TGA Q50 V20.10 Build 36, USA). The microspheres (approximately 10 mg) were placed in a Pt pan, and then were heated from 25 to 800 °C at a rate of 10 K/min. The surface areas of cellulose microspheres were measured with a JW-BK specific surface analyser (USA) using nitrogen adsorption method. The as-prepared **CSPs** were packed into stainless steel columns (150 mm $\times$ 4.6 mm) with a model of 1666 Alltech slurry packer (USA). Enantioseparation was performed on an Agilent 1100 chromatograph (USA), consisting of Agilent G1365B DAD, Agilent G1311A Quat Pump, Agilent G1379A degasser and Agilent G1313A ALS autosampler.

Preparation of Cellulose Microspheres (CMs)

Cellulose solution (4.3 wt%) was prepared with NaOH/urea solution as a solvent^[12, 14]. Cellulose microspheres were prepared according to the reported method with some improvements^[15]. After drying in vacuum, the microspheres were dispersed in water by sonication, and washed with water and acetone and filtered. The microspheres were obtained as white powder with D_{av} of 8.3 μm . In case of the preparation of 30 μm microspheres, cyclohexane was used instead of paraffin oil.

Drying of Cellulose Microspheres

The prepared cellulose microspheres were mixed with benzene in a round bottom flask, which was equipped with a water separation apparatus. The resulting mixture was heated to reflux till the organic layer in the apparatus became clear. The microspheres were filtered and the removal of water was repeated again. The pre-treated microspheres were dried in a vacuum desiccator.

Crosslinking Cellulose Microspheres with Diisocyanate

Cellulose microspheres (3.00 g) and HDI (0.21 g) were added in toluene (20 mL). The suspension was heated to 85 °C and stirred for 18 h under nitrogen atmosphere. After reaction completion, the microspheres were washed with toluene and acetone by centrifuge. The crosslinked microspheres, **CCMs 1**, were obtained as white powder (3.04 g) after removing volatiles. EA (%): C 40.19, H 6.71, N 1.48.

Similar with **CCMs 1**, **CCMs 2** (3.04 g) were prepared with cellulose microspheres (3.00 g) and HDI (0.11 g). EA (%): C 41.73, H 6.83, N 1.06. **CCMs 3** (0.91 g) were prepared with cellulose microspheres (1.00 g) and TDI (0.14 g). EA (%): C 41.17, H 6.39, N 1.06. **CCMs 4** (1.98 g) were prepared with cellulose microspheres (2.00 g) and PDI (0.25 g). EA (%): C 41.73, H 6.34, N 1.10.

Phenylcarbamoylation of Cellulose Microspheres

CSPs 1 were prepared as following: **CCMs 1** (2.49 g) were mixed with pyridine (12 mL), toluene (4 mL) and phenyl isocyanate (7.5 mL). The resulting mixture was heated to 85 °C for 1 h, and then another portion of toluene (10 mL) was added in the mixture. The solid was collected by centrifugation and washed with acetone in a soxhlet for 12 h, 2.89 g of **CSPs 1** were obtained after removing the volatiles in vacuum. IR (KBr, cm^{-1}) ν : 1718 cm^{-1} ($-\text{C}=\text{O}$), 1598 cm^{-1} ($-\text{NHC}=\text{O}$), 1532 cm^{-1} ($-\text{NH}$); EA (%): C 47.39, H 6.33, N 1.65.

Similarly, **CSPs 2** (2.91 g) were prepared with **CCMs 2** (2.49 g) and phenyl isocyanate (7.5 mL). IR (KBr, cm^{-1}) ν : 1714 cm^{-1} ($-\text{C}=\text{O}$), 1598 cm^{-1} ($-\text{NHC}=\text{O}$), 1536 cm^{-1} ($-\text{NH}$); EA (%): C 44.48, H 6.20, N 1.12.

CSPs 3 were prepared by pre-modifying and crosslinking as the previous work^[16].

Enantioseparation of CSPs 1–3

The **CSPs** were mixed with the mixture of *n*-hexane/isopropanol (90/10, *V/V*) to form slurries, and then packed into three empty stainless steel columns with *n*-hexane as displacer solvent under a pressure of 28.1 MPa. The chiral sample solutions were prepared by dissolving the chiral analytes in ethanol. The sample solutions and all mobile phases were filtered through 0.25 μm membrane, and the column temperature was set at 25 °C.

RESULTS AND DISCUSSION

Pre-treatment of Cellulose Microspheres

Figure 2 shows the TGA curves of cellulose microspheres. Obviously, the water in **CMs** was 8 wt%. Since isocyanate is very sensitive to moisture, it is necessary to remove the water in **CMs** before modification and crosslinking. However, the water is difficultly removed thoroughly by traditional methods because the hydroxyls in the microspheres strongly interact with water. In this work, **CMs** were dried twice through azeotropic distillation with fresh benzene as the solvent. When the **CMs** were dried for the third time, the distillate was clear, revealing that there is no need to remove the water again.

Reaction Conditions for Carbamoylation of CMs

Typically, cellulose is arylcarbamoylated with aryl isocyanate in pyridine, causing an increment of molecular weight^[17, 18]. As the hydroxyls in cellulose are completely substituted, the carbamoylated cellulose is soluble in the solvent. Therefore, it is important to control the partial arylcarbamoylation of **CMs** in order to keep their morphological structure for their application as the adsorbents of HPLC. The effects of experiment conditions on phenylcarbamoylation of **CMs** are summarized in Table 1.

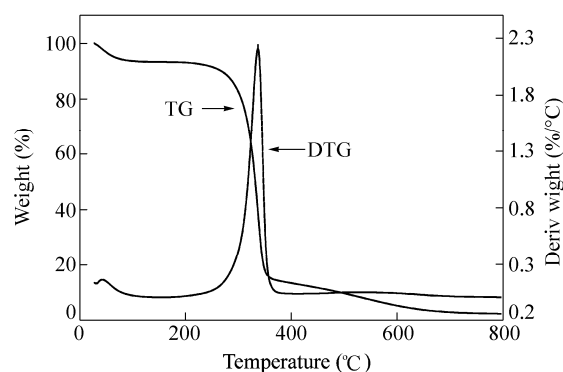


Fig. 2 TGA curves of CMs

Table 1. Phenylcarbamoylation of cellulose microspheres under different reaction conditions

Entry	Particle size (μm)	Microspheres weight (g)	Toluene/pyridine (mL/mL)	Reaction time	Phenyl isocyanate (mL)	Reaction temperature (°C)	Weight after reaction (g)
1	8.3	3.99	5/10	18 h	20	70	4.10
2	8.3	4.03	5/10	12 h	12	70	4.02
3	8.3	2.02	5/10	24 h	15	70	2.01
4	8.3	2.06	3/10	4 h	6	90	2.02
5	8.3	2.06	3/10	12 h	6	90	1.92
6	8.3	2.07	3/10	10 min	6	90	2.15
7	8.3	2.10	3/10	30 min	6	90	2.67
8	8.3	1.99	0/10	15 min	6	85	3.66
9	8.3	2.00	0/10	15 min	0.6	85	2.13
10	30.1	2.07	3/10	18 min	6	90	gelled
11	30.1	2.06	3/10	10 min	6	90	2.68
12	30.1	2.06	10/10	10 min	6	90	2.38

As shown in Table 1, there was almost no increment in weight after arylcarbamoylation of cellulose at 70 °C (see Entries 1–3), indicating no arylcarbamoylation took place at the temperature. As phenyl isocyanate was added in toluene and the resulting mixture was stirred overnight at room temperature, a white solid that was the dimmer of phenyl isocyanate formed. Furthermore, the white solid appeared fast in the presence of pyridine. When the mixture was heated above 80 °C, the solid disappeared. In order to avoid the formation of the dimmer of phenyl isocyanate, the carbamoylation of cellulose should be performed at above 80 °C.

Reaction time is also important for carbamoylation. For entries 4 and 5, the weight of the phenylcarbamoylated CMs decreased as the reaction temperature was 90 °C and reaction time was 4 h and 12 h, indicating there was the formation of soluble phenylcarbamoylated cellulose. On the contrary, as the reaction time was changed to 10 or 30 min, the weight of the modified CMs increased (Entries 6 and 7). Thus, the carbamoylation of CMs with an appropriate degree of substitution (DS) can be achieved by adjusting the reaction duration and reaction temperature.

During carbamoylation, pyridine not only acts as a swelling agent but also as a catalyst. After CMs were swelled, phenyl isocyanate molecules accessed into CMs, and reacted with hydroxyls on the surface of CMs. As pyridine was used only, CMs are quickly carbamoylated (entries 8 and 9). Based on the dilution effect, the mixed solvents, such as a mixture of toluene and pyridine could be used for partial carbamoylation of CMs in order to control the DS of CMs.

Additionally, the carbamoylation is related to the size of CMs. Comparing entry 6 with 11, the larger CMs were easily carbamoylated than the smaller ones due to the looser structures of the larger CMs.

Figure 3 is SEM images of **CMs**, **CCMs 2** and **CSPs 2**. Obviously, all of them were spherical even **CMs** were crosslinked with HDI and then were carbamoylated with phenyl isocyanate. Similarly, **CSPs 1** and **CSPs 3** also remained spherical in shape. Usually a regular shape is essential for materials to be used as adsorbents of HPLC.

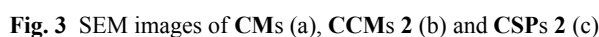


Fig. 4 FTIR spectra of CMs and CCMs 1–4

¹H-NMR spectra of **CCMs 3** and **CCMs 4** are shown in Fig. 5. When **CCMs 3** and **CCMs 4** were subjected to ¹H-NMR measurement, they were suspended in CF₃COOD because of their insolubility in common solvents. After ultrasonicated for 2 to 4 days time to time, **CCMs 3** and **CCMs 4** gradually swelled and dissolved. The chemical shifts corresponding to the hydrogen atoms in aromatic ring and ethylene unit of hexamethylene separate from those of cellulose skeleton. The DC was estimated by the ratio of integral areas of the H of aromatic H and the H of D-glucose units, and the DC values of **CCMs 1–4** are listed in Table 2. Obviously, HDI has higher reactivity than TDI and PDI due to its flexible and linear structure, and the DC of **CCMs** could be controlled by the fed amount of HDI.

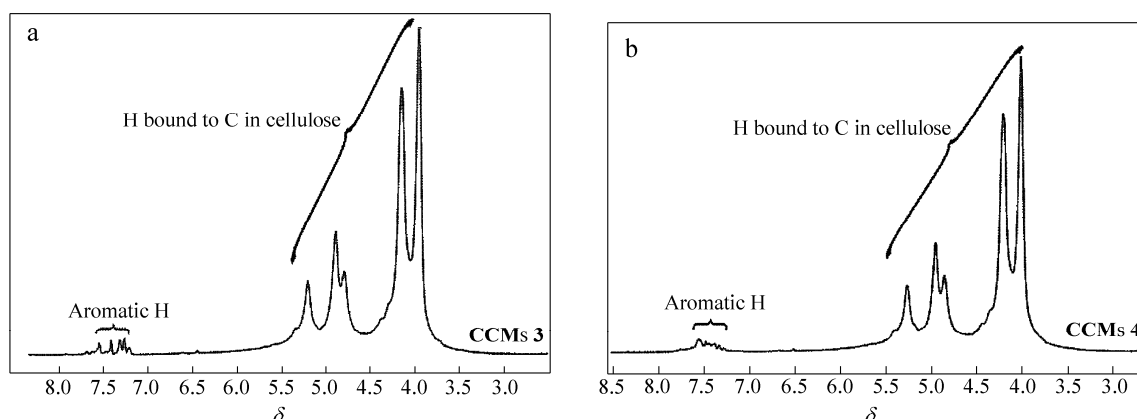


Fig. 5 ^1H -NMR spectra of CCMs 3 (a) and 4 (b)

Table 2. DC values of CCMs 1–4

Crosslinked microspheres	CCMs 1	CCMs 2	CCMs 3	CCMs 4
Crosslinking agent	HDI	HDI	TDI	PDI
Amount of crosslinker (mmol/g)	0.42	0.22	0.80	0.78
DC (%)	2.2	1.7	3.1	3.5

CSPs with DC less than 3% are favorable for enantioseparation^[21], therefore CCMs 1 and 2 were further carbamoylated with phenyl isocyanate to prepare CSPs. Based on their DC values, the average molecular weight (M_{unit}) of repeating units in CCMs 1 and 2 were calculated by the Eq. (1):

$$M_{\text{unit}} = 162 \times (1 - \frac{1}{3} \times \text{DC}) + \frac{1}{3} \text{DC} (162 + \frac{3}{2} \times 168) \quad (1)$$

where 162 is the molecular weight of the repeating unit ($\text{C}_6\text{H}_{10}\text{O}_5$) in cellulose, and 168 is the molecular weight of HDI. The M_{unit} of CCMs 1 and 2 were calculated as 163.8 and 163.4, respectively.

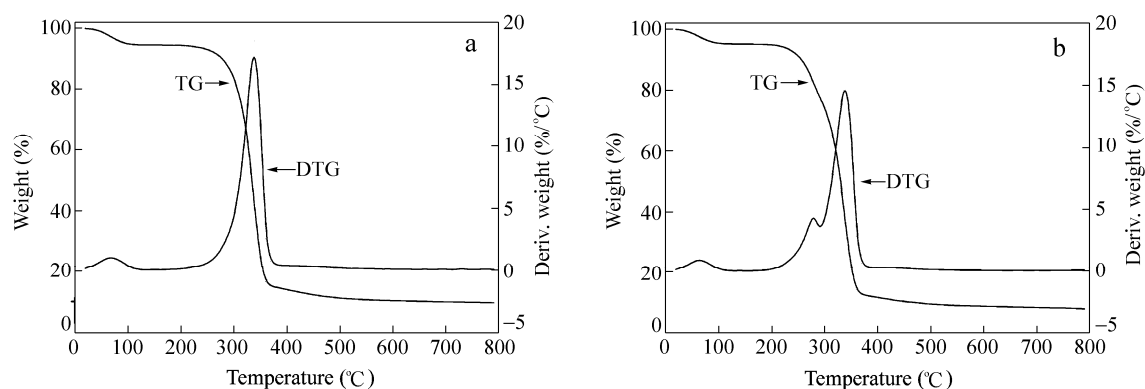


Fig. 6 TGA curves of CCMs 2 (a) and CSPs 2 (b)

Further, the actual contents of cellulose and the derivatized cellulose were available from TGA, which is representatively demonstrated by Fig. 6. The DS of the hydroxyls is defined as the molar percentage of the modified hydroxyls in CMs 1 and CMs 2.

The DS of the hydroxyls that are 8.4% and 8.3%, respectively, in CSPs 1 and 2, were calculated according to Eq. (2):

$$DS(\%) = \frac{\frac{m_2 \times C_2 \% - m_1 \times C_1 \%}{119}}{\frac{m_1 \times C_1 \%}{M_{\text{unit}}}} \times 3 \quad (2)$$

where m_1 is the mass of CCMs 1 or CCMs 2 fed for modification reaction; m_2 is the mass of the obtained CSPs 1, 2; 119 refers to the molar weight of phenyl isocyanate; C_1 refers to the actual content of CCMs 1 and CCMs 2 in the microspheres; C_2 presents the actual contents of derivatized cellulose in CSPs 1 and 2. The DS of CSPs 3 is 11.6%^[16].

Chromatographic Separation Evaluation of CSPs 1–3

The chiral recognition capability of CSPs 1–3 was evaluated by using structurally various chiral compounds (Fig. 7). Table 3 summarizes the enantioseparation results of chiral analytes resolved by CSPs 1–3 under the same mobile phase conditions. The chromatographic results exhibit that CSPs 1–3 separated increasing chiral analytes in turn. CSPs 1 has biggest DC, but has bad chiral recognition capability. As for polysaccharide type CSPs, if the derivatives of cellulose or amylose are covalently immobilized on silica gel, the recognition capability of the CSPs decreases^[19]. Crosslinking is similar to covalent immobilization that may change the supramolecular structures of these derivatives, resulting in the decrease of chiral recognition capability^[20].

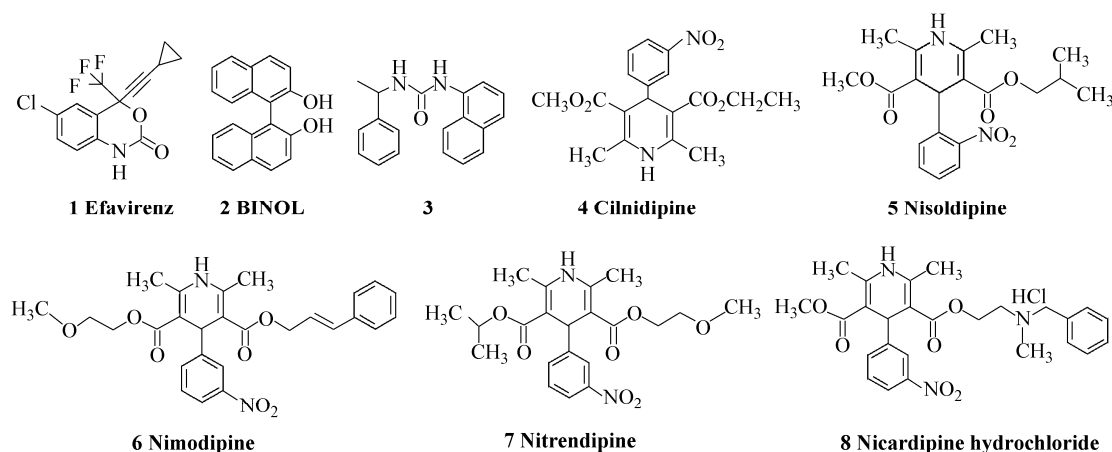


Fig. 7 Structures of the chiral analytes resolved by CSPs 1–3

Table 3. The evaluation in chiral recognition capability of CSPs 1–3

Chiral analytes	CSPs 1				CSPs 2				CSPs 3			
	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent
1	0.47	1.48	0.82	A (50/50) ^b	0.60	1.54	0.72	A (70/30) ^c	1.07	2.13	1.02	A (70/30) ^c
2	1.36	1.30	0.50	A (30/70) ^a	0.44	1.48	0.47	A (10/90) ^a	0.24	1.34	0.66	B (10/90) ^a
3	No Separation				No Separation				0.27	1.22	0.65	B (10/90) ^a
4	No Separation				0.25	1.32	0.55	A (30/70) ^a	0.49	1.33	0.65	A (10/90) ^a
5	No Separation				No Separation				1.06	1.27	0.67	B (30/70) ^a
6	No Separation				0.15	1.36	0.57	A (10/90) ^a	0.25	1.27	1.17	B (10/90) ^a
7	0.58	1.47	0.68	A (30/70) ^a	0.15	1.42	0.57	A (10/90) ^c	0.43	1.47	0.73	A (10/90) ^b
8	No Separation				No Separation				0.44	1.24	0.69	B (10/90) ^a

Eluent: A: *n*-hexane/isopropanol; B: *n*-hexane/ethanol

The wavelength of UV detection (nm): ^a235; ^b245; ^c254

Because the DC of **CSPs 1** is higher than that of **CSPs 2** and **3**, the supramolecular structure of the modified cellulose in **CSPs 1** might be changed more. Thus, the chiral recognition capability of **CSPs 1** is the lowest. **CSPs 2** was prepared by using a “pre-crosslinking-then-modifying” method, while a “pre-modifying-then-crosslinking” method was used for the preparation of **CSPs 3**. The supramolecular structure of the derivatized cellulose in **CSPs 3** was less changed as compared with **CSPs 2**. Therefore, **CSPs 3** showed better chiral recognition capability.

CONCLUSIONS

The reaction conditions to modify and crosslink cellulose microspheres with isocyanate were studied. By varying reaction duration, the fed amount of isocyanate or the fed ratio of toluene and pyridine that were used as the solvents for cellulose carbamoylation, the DS of the hydroxyls could be adjusted. By extension, functionalized cellulose microspheres can be applied as absorbents for liquid chromatography. Depending on applications, cellulose microspheres should be modified with appropriate substituents and crosslinked at an appropriate DC in order to enhance their strength. In the future work, the adsorbents of cellulose microspheres should be prepared using the “pre-modifying-then-crosslinking” method with a higher DC.

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