

# Synthesis and Characterization of Amphoteric Xylan-type Hemicelluloses by Microwave Irradiation

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**S** Supporting Information

**ABSTRACT:** In this study, a novel amphoteric macromolecule was synthesized by sequential incorporation of carboxymethyl and quaternary ammonium groups into the backbone of xylan-type hemicelluloses under microwave irradiation. The reaction parameters such as the molar ratio of reagent (NaOH or 3-epoxypropyltrimethylammonium chloride)/anhydroxylose unit in hemicelluloses, the temperature, and the reaction time were investigated to optimize the reaction condition. The maximum degrees of substitution (DS) of carboxymethyl and quaternary ammonium groups under the optimum reaction condition were 0.90 and 0.52, respectively, exhibiting a higher efficiency as compared to the conventional heating method. Moreover, the thermal stability and weight-average molecular weight of amphoteric hemicellulosic derivatives decreased as compared to the native hemicelluloses. The viscosity, elastic modulus, and loss modulus of the amphoteric biomacromolecules increased with the increasing DS of quaternary ammonium groups in aqueous solution due to stronger electrostatic attraction. This study provides an efficient and rapid method to prepare amphoteric biomacromolecules.

**KEYWORDS:** *amphoteric macromolecule, xylan-type hemicelluloses, microwave irradiation, carboxymethyl group, quaternary ammonium group*

## INTRODUCTION

Hemicelluloses, which are branched polysaccharides formed by a number of different neutral sugar units such as xylose, arabinose, glucose, galactose, and mannose, to a small extent, and also D-glucuronic acid units and their 4-O-methyl derivatives, are considered to be the second most abundant macromolecules in the plant kingdom. Xylan-type hemicelluloses are the main hemicellulosic components of the cell walls of hardwoods and herbaceous plants (constituting about 20–35 wt % of the biomass) and thus are available in huge amounts as byproducts from forestry, agriculture, and pulp and paper industries. Recently, the applications of hemicelluloses in drug delivery, tissue engineering, and food packaging have spurred great interest because of their bioactivity, biocompatibility, renewability, and oxygen barrier.<sup>1</sup> Chemical modification is an important strategy to tailor the properties of hemicelluloses as desired macromolecules for their specific applications. In the past few years, a number of functional biomacromolecules and biomaterials have been successfully developed from hemicelluloses.<sup>1–4</sup>

Amphoteric macromolecules, which contain both anionic and cationic groups in the macromolecular backbone, possess polyampholyte characteristics and pH or salt-sensitivity, nonspecific adsorption resistance, and functionality from the pendant charged groups.<sup>5</sup> These macromolecules have received considerable attention in biomedical, cosmetic, pharmacological, agricultural, and biotechnological fields during the past decades.<sup>6</sup> Of particular recent interest is the development of new amphoteric biomaterials for the applications in medical devices, drug delivery system, and separation of ionic drugs and proteins.<sup>5,6</sup> Amphoteric macromolecules had been used to prepared charged membranes for protein separation and

absorption because of their strong antifouling and ion-exchange properties.<sup>7</sup> The presence of positive and negative charges in the amphoteric macromolecules allows biomaterials (e.g., hydrogels, capsules) to respond to changes in environmental pH and ionic strength in a highly reversible way, and thus affords multiple routes for encapsulation and the controlled release of proteins and drugs.<sup>8,9</sup> The amphoteric macromolecules and their biomaterials can interact electrostatically with the phosphate groups of deoxyribonucleic acid (DNA) and thus can be employed as gene carriers.<sup>10</sup> In addition, amphoteric macromolecules can be applied as a bioprobe for live cell imaging.<sup>11</sup>

Preparing adsorbents for removal of toxic metal ions and organic substances is another important application of amphoteric macromolecules. Adsorbents prepared from amphoteric macromolecules not only offer enough adsorption sites for metal ions, and acidic or basic dyes,<sup>12,13</sup> but also allow themselves to be used across a wide pH range due to their peculiar electrical character.<sup>14</sup> Amphoteric macromolecules can also find their attractive applications in the preparation of nanoparticles,<sup>15</sup> and inorganic–organic hybrid composites.<sup>16</sup> Therefore, amphoteric macromolecules, which have a wide application perspective, can serve as a versatile platform for functional polymers and materials.

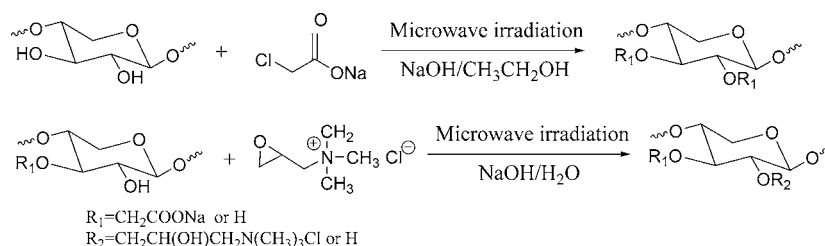
Microwave irradiation has spurred great interest in chemical reaction due to its rapid heating and energy homogeneous penetration manner as compared to conventional heating method.<sup>17</sup>

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**Figure 1.** Reaction scheme of the quaternized carboxymethyl xylan-type hemicelluloses.

Our previous work on microwave-induced synthesis of xylan-type hemicellulosic derivatives also presented encouraging results.<sup>4</sup> In the present study, our interest mainly focused on the microwave-induced synthesis of a novel amphoteric macromolecule with carboxymethyl and quaternary ammonium groups from bamboo xylan-type hemicelluloses. The structure and weight-average molecular weight of the amphoteric xylan-type hemicelluloses were characterized by Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (<sup>13</sup>C and DEPT-135 NMR), and gel permeation chromatography (GPC). The rheological characterization and thermal stability were also studied by dynamic and flow curve analysis and simultaneous thermal analyzer. This study provides an efficient way for the preparation of amphoteric biopolymers.

## MATERIALS AND METHODS

**Materials.** Xylan-type hemicelluloses were isolated from *Dendrocalamus membranaceus* Munro according to a previous procedure.<sup>4</sup> Briefly, xylan-type hemicelluloses were isolated using 10% KOH at 23 °C for 10 h with a solid to liquid ratio of 1:20 (g/mL) from holocellulose, which was obtained by delignification of the extractive-free *Dendrocalamus membranaceus* Munro (40–60 mesh) with sodium chlorite in acidic solution (pH 3.7–4.0, adjusted by 10% acetic acid) at 75 °C for 2 h. The sugar analysis showed the following sugar composition (relative weight percent): 89.4% xylose, 5.8% arabinose, 1.9% glucose, 0.7% galactose, 1.8% glucuronic acid, 0.6% galactose acid. Sodium monochloroacetate and cationic moiety 2,3-epoxypropyltrimethylammonium chloride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Sodium hydroxide and ethanol were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

### Preparation of Carboxymethyl Xylan-type Hemicelluloses.

The typical procedure of the preparation of carboxymethyl xylan-type hemicelluloses is as follows. 0.33 g of xylan-type hemicelluloses powder was dispersed in water (10 mL) and heated to 80 °C under stirring for 30 min before being cooled to room temperature. Subsequently, a known quantity of sodium hydroxide (accounting for 50% all amount sodium hydroxide) was added dropwise to the solution for 20 min at 30 °C, and then ethanol and water were added to keep the total volume up to 15 mL under the condition of the 4:1 volume ratio of ethanol to water. Followed by the addition of sodium monochloroacetate and the remaining sodium hydroxide, the microwave program (XH-100B, 200 W, Beijing XiangHu Sci.-Tech. Dept. Co. Ltd., Beijing, China) immediately was performed with simultaneous magnetic stirring at the required temperature. After the desired reaction time, the mixture was first cooled to room temperature, and then neutralized with diluted acetic acid, and the resulting mixture was filtered and washed with 50 mL of 70% ethanol aqueous (v/v) once and 50 mL of 95% ethanol four times. The products obtained were dissolved in distilled water and freeze-dried at –50 °C. The reaction scheme is shown in Figure 1.

**Preparation of Quaternized Carboxymethyl Xylan-type Hemicelluloses.** The quaternization of carboxymethyl xylan-type hemicelluloses was conducted using cationic moiety 2,3-epoxypropyltrimethylammonium chloride as the quaternizing agent. To a

carboxymethyl xylan-type hemicelluloses (0.33 g) aqueous solution (10 mL) was added the definite amount of sodium hydroxide, and then the solution was placed in the microwave oven for microwave irradiation (microwave power was 200 W). When the temperature was raised to the required value, cationic moiety 2,3-epoxypropyltrimethylammonium chloride was added dropwise under stirring (the amount of cationic moiety 2,3-epoxypropyltrimethylammonium chloride was calculated by different molar ratios of cationic moiety 2,3-epoxypropyltrimethylammonium chloride/carboxymethyl xylan-type hemicelluloses). Upon completion of the reaction, the product was precipitated by 95% ethanol and washed three times with 95% ethanol. The obtained quaternized carboxymethyl xylan-type hemicelluloses were purified by dialysis against distilled water and then freeze-dried at –50 °C. The reaction scheme is shown in Figure 1.

**Determination of Degree of Substitution (DS) of Carboxymethyl Groups and Quaternary Ammonium Groups.** The degree of substitution of carboxymethyl groups (DS<sub>C</sub>) in carboxymethyl xylan-type hemicelluloses was determined by the acidometric titration method, which has been applied in the determination of the DS of carboxymethyl starch and carboxymethyl hemicelluloses, and was previously described in detail.<sup>4,18</sup> All of the titrations were carried out in triplicate, and standard deviations were less than 4.0%.

The degree of substitution of quaternary ammonium groups (DS<sub>Q</sub>) in quaternized carboxymethyl xylan-type hemicelluloses was calculated by elemental analysis.<sup>19</sup> The nitrogen and carbon contents in the quaternized carboxymethyl xylan-type hemicelluloses were determined with an elemental analyzer (vario EL, Elementar Analysensysteme GmbH, Hannover, Germany).

All samples were dried at 60 °C for 24 h before measurement. To reduce errors, each experiment was performed in duplicate, and mean values were presented in all of the cases studied. The DS<sub>Q</sub> was calculated in the following equation:<sup>2</sup>

$$DS_Q = \frac{(60 + 24 \times DS_C) \times N\%}{14 \times C\% - 72 \times N\%}$$

where N% and C% are the nitrogen and carbon contents of the products determined by elemental analysis, and the terms of 14, 72, 60, and 24 are the total molecular weights (g/mol) of nitrogen, carbon content in the products, carbon element in xylose unit in hemicelluloses, and carbon content of carboxymethyl group, respectively.

**Characterizations of Quaternized Carboxymethyl Xylan-type Hemicelluloses.** FT-IR transmission spectra of xylan-type hemicelluloses and quaternized carboxymethyl xylan-type hemicelluloses were measured by using a Nicolet 750 spectrophotometer (Thermo Fisher Nicolet, FL) within the wavenumber range 400–4000 cm<sup>-1</sup>, and the 1% finely ground samples were mixed with KBr to press a plate for measurement.

The solution-state <sup>13</sup>C NMR and DEPT-135 NMR spectra were obtained on a Bruker AVIII 400 MHz spectrometer (Bruker Corp., Rheinstetten, Germany) operating in the FT mode at 100.6 MHz after 15 000 scans. The sample (80 mg) was dissolved in 1 mL of D<sub>2</sub>O. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.36 s acquisition time, and 2 s relaxation delay time were used.

The molecular weights of carboxymethyl xylan-type hemicelluloses and quaternized carboxymethyl xylan-type hemicelluloses were determined by GPC on a 300 mm × 7.7 mm PL (Polymer Laboratories Ltd., Shropshire, UK) and calibrated with PL pullulan

**Table 1. Effects of Reaction Condition on the DS<sub>C</sub> and M<sub>w</sub> of Carboxymethyl Xylan-type Hemicelluloses**

sample no.	time (min)	molar ratio <sup>a</sup>	molar ratio <sup>b</sup>	temp (°C)	DS <sub>C</sub>	M <sub>w</sub>	M <sub>w</sub> /M <sub>n</sub>
xylan-type hemicelluloses						47 200	4.66
1	10	5:5	2:1	60	0.49	34 600	4.12
2	10	5:5	3:1	60	0.57	33 200	3.95
3	10	7:5	2:1	60	0.65	33 500	3.98
4	20	7:5	2:1	60	0.84	30 300	3.82
5	20	5:5	3:1	60	0.87	30 000	3.72
6	20	7:5	3:1	60	0.90	29 500	3.70

<sup>a</sup>Represents the molar ratio of NaOH to anhydroxylose units in xylan-type hemicelluloses, xylose unit M<sub>w</sub> = 132. <sup>b</sup>Represents the molar ratio of sodium monochloroacetate to anhydroxylose units in xylan-type hemicelluloses, xylose unit M<sub>w</sub> = 132.

polysaccharide standard (average peak molecular weights of 783, 12 200, 100 000, 1 600 000). A flow rate of 0.5 mL/min was maintained. The eluent was 0.02 N NaCl in 0.005 M sodium phosphate buffer (pH 7.5). Samples were dissolved with 0.2 N NaCl in 0.005 M sodium phosphate buffer (pH 7.5), at a concentration of 0.1%.

Thermal analysis was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (TGA Q500, TA Instruments, New Castle, U.S.). The apparatus was continually flushed with nitrogen. The sample was weighed between 9 and 11 mg and was heated from room temperature to 600 °C at a heating rate of 10 °C per minute.

The dynamic rheological properties of xylan-type hemicelluloses and quaternized carboxymethyl xylan-type hemicelluloses were measured between a 40 mm diameter steel parallel plate and a Peltier plate in an AR 2000 rheometer (TA Instruments, New Castle, U.S.) at room temperature. All samples were dissolved in water with a magnetic stirrer for 30 min. A thin layer of paraffin oil was applied on top of the sample to avoid evaporation. The values of the strain amplitude were checked to ensure that all oscillatory shear experiments were performed within the linear viscoelastic regime, where the dynamic elastic modulus (*G'*) and viscous modulus (*G''*) are independent of the strain amplitude. All measurements were done twice. Flow data were collected over shear rates from 10<sup>-2</sup> to 10<sup>3</sup>/s, frequency from 10<sup>-1</sup> to 10<sup>2</sup> rad/s, respectively.

## RESULTS AND DISCUSSION

**Influence of Reaction Condition on the DS<sub>C</sub> of Carboxymethyl Xylan-type Hemicelluloses and the DS<sub>Q</sub> of Quaternized Carboxymethyl Xylan-type Hemicelluloses.** In the present work, the interest was focused on investigating the synthesis of quaternized carboxymethyl xylan-type hemicelluloses from carboxymethyl xylan-type hemicelluloses using microwave irradiation. Carboxymethyl xylan-type hemicelluloses with various DS<sub>C</sub>, which served as the precursors for preparation of quaternized carboxymethyl xylan-type hemicelluloses, were obtained by changing the reaction conditions according to our previous work.<sup>4</sup> Table 1 reveals that carboxymethyl xylan-type hemicelluloses with DS<sub>C</sub> of 0.49–0.90 could be obtained within 20 min using microwave irradiation, which is a much shorter time scale as compared to the conventional heating method (75 min).<sup>20</sup> In addition, a higher DS<sub>C</sub> (0.90) could be achieved by microwave-induced synthesis than conventional-heated synthesis.<sup>20</sup> These results suggest that microwave irradiation has the ability to remarkably enhance the carboxymethylation efficiency due to its homogeneous and rapid heating manner,<sup>17</sup> superheating effect, and agitation, which improves molecular transport.<sup>21</sup>

An increase in the molar ratio of sodium monochloroacetate/xylan-type hemicelluloses from 2:1 to 3:1 (sample 1 vs sample 2, or sample 4 vs sample 6) or the molar ratio of NaOH/xylan-type hemicelluloses from 5:5 to 7:5 (sample 1 vs sample 3, or sample 5 vs sample 6) resulted in an increment in DS<sub>C</sub>, indicating that a higher sodium monochloroacetate or NaOH/

xylan-type hemicelluloses ratio facilitates the chemical reaction. Prolonging the reaction time from 10 to 20 min (sample 2 vs sample 5, or sample 3 vs sample 4) also significantly increased the DS<sub>C</sub> of the carboxymethyl xylan-type hemicelluloses.

Conventionally, the homogeneous reaction of cationic moiety 2,3-epoxypropyltrimethylammonium chloride and hemicelluloses was carried out in dimethyl sulfoxide (DMSO) or alkaline solution using the conventional heating method. However, a higher DS could be obtained in alkaline solution.<sup>19,22</sup> Therefore, the reaction was carried out in alkaline solution in this study. Table 2 shows the influences of reaction conditions on the DS<sub>Q</sub> of quaternized carboxymethyl xylan-type hemicelluloses. The DS<sub>Q</sub> of the products increased from 0.20 to 0.29 when the reaction time was prolonged from 10 to 40 min; thereafter, DS slightly decreased. This observation indicates that a reaction time of 40 min was sufficient for the reaction under the microwave irradiation.

NaOH used as a nucleophilic catalyst in this study showed an influence on DS<sub>Q</sub>. Increasing the molar ratio of NaOH/carboxymethyl xylan-type hemicelluloses from 0.4:1 to 0.8:1 obviously promoted the reaction efficiency, as shown by the increase of DS<sub>Q</sub> from 0.22 to 0.30. A higher NaOH ratio can activate carboxymethyl xylan-type hemicelluloses better, and thus produces a high DS<sub>Q</sub>. The further increase of the molar ratio of NaOH/carboxymethyl xylan-type hemicelluloses (from 0.8:1 to 1.6:1) resulted in a slight decrease in DS<sub>Q</sub> (from 0.30 to 0.25). This can be attributed to the increase in the hydrolysis of cationic moiety 2,3-epoxypropyltrimethylammonium chloride to the corresponding diol at a higher NaOH/carboxymethyl xylan-type hemicelluloses ratio.<sup>19</sup> The DS<sub>Q</sub> increased from 0.23 to 0.54 as the cationic moiety 2,3-epoxypropyltrimethylammonium chloride/carboxymethyl xylan-type hemicelluloses molar ratio increased from 1:1 to 6:1.

An increase in the reaction temperature from 40 to 70 °C resulted in DS<sub>Q</sub> increasing from 0.37 to 0.62 due to diffusion and compatibility of the reaction ingredients and the mobility of the reactant molecules. Further rise of temperature to 80 °C led to a slight fall in DS<sub>Q</sub> from 0.62 to 0.57, which may be attributed to the degradation of carboxymethyl xylan-type hemicelluloses and more side reactions during the quaternization.<sup>23</sup>

The influence of DS<sub>C</sub> on the DS<sub>Q</sub> (samples 19–24) was also investigated. DS<sub>Q</sub> decreased from 0.62 to 0.45 as the DS<sub>C</sub> increased from 0.49 to 0.84, and thereafter it showed little difference as the DS<sub>C</sub> was higher than 0.84. In a conventional heating method, a maximum DS<sub>Q</sub> of quaternized hemicelluloses (hemicelluloses without carboxymethylation) of 0.54 could be obtained in 5 h.<sup>19,22</sup> A DS<sub>Q</sub> of 0.62 obtained in 40 min in this work exhibited a higher efficiency of microwave irradiation method in the synthesis of quaternized carboxymethyl

Table 2. Effects of Reaction Condition on the  $DS_C$  and  $M_w$  of Quaternized Carboxymethyl Xylan-type Hemicelluloses

sample	$DS_C$	molar ratio <sup>a</sup>	molar ratio <sup>b</sup>	$T$ (°C)	time (min)	$DS_C$	$M_w$	$M_w/M_n$
xylan-type hemicelluloses							47 200	4.59
carboxymethyl xylan-type hemicelluloses	0.49						34 600	4.12
1	0.49	1.2:1	2:1	60	10	0.20	30 000	2.98
2	0.49	1.2:1	2:1	60	20	0.25	28 600	2.83
3	0.49	1.2:1	2:1	60	30	0.26	25 700	2.79
4	0.49	1.2:1	2:1	60	40	0.29	24 000	2.64
5	0.49	1.2:1	2:1	60	50	0.28	23 600	2.68
6	0.49	0.4:1	2:1	60	40	0.22	23 900	2.72
7	0.49	0.8:1	2:1	60	40	0.30	23 900	2.67
8	0.49	1.2:1	2:1	60	40	0.29	24 000	2.64
9	0.49	1.6:1	2:1	60	40	0.25	22 900	2.69
10	0.49	0.8:1	1:1	60	40	0.23	24 500	2.79
11	0.49	0.8:1	2:1	60	40	0.30	23 900	2.67
12	0.49	0.8:1	4:1	60	40	0.42	23 200	2.60
13	0.49	0.8:1	6:1	60	40	0.54	23 900	2.34
14	0.49	0.8:1	6:1	40	40	0.37	26 800	2.75
15	0.49	0.8:1	6:1	50	40	0.39	25 300	2.79
16	0.49	0.8:1	6:1	60	40	0.54	23 900	2.34
17	0.49	0.8:1	6:1	70	40	0.62	21 300	1.81
18	0.49	0.8:1	6:1	80	40	0.57	19 200	1.73
19	0.49	0.8:1	6:1	70	40	0.62	21 300	1.81
20	0.57	0.8:1	6:1	70	40	0.56	21 000	1.84
21	0.65	0.8:1	6:1	70	40	0.53	20 200	1.96
22	0.84	0.8:1	6:1	70	40	0.45	19 500	1.75
23	0.87	0.8:1	6:1	70	40	0.44	19 200	1.86
24	0.90	0.8:1	6:1	70	40	0.44	18 500	1.82

<sup>a</sup>Represents the molar ratio of NaOH to anhydroxylose units in carboxymethyl xylan-type hemicelluloses, xylose unit,  $M_w = 132 + (M_{CH_2COONa} - 1) \times DS_C$ . <sup>b</sup>Represents the molar ratio of quaternized carboxymethyl xylan-type hemicelluloses to anhydroxylose units in carboxymethyl xylan-type hemicelluloses, xylose unit,  $M_w = 132 + (M_{CH_2COONa} - 1) \times DS_C$ .

xylan-type hemicelluloses, especially when less hydroxyl groups (only about 1.51 hydroxyl groups per xylose unit in carboxymethyl xylan-type hemicelluloses can be available to cationic moiety 2,3-epoxypropyltrimethylammonium chloride, instead of 2.0 hydroxyl groups per xylose unit in xylan-type hemicelluloses) were taken into account in the case of carboxymethyl xylan-type hemicelluloses. Therefore, quaternized carboxymethyl xylan-type hemicelluloses could be rapidly and efficiently synthesized by microwave irradiation.

**Average Molecular Weight.** To evaluate the influences of chemical reaction condition on the chemical structure of the products, the molecular weights ( $M_w$ ) were determined by GPC, and the results are present in Figure S1 (Supporting Information) and Tables 1 and 2. The molecular weights (29 500–34 600 g/mol) of the carboxymethyl xylan-type hemicelluloses (Table 1) became lower than that of xylan-type hemicelluloses (47 200 g/mol), which indicates the degradation of xylan-type hemicelluloses during chemical reaction under the alkaline conditions. All of the molecular weights of quaternized carboxymethyl xylan-type hemicelluloses listed in Table 2 were lower than that of xylan-type hemicelluloses (47 200 g/mol) and carboxymethyl xylan-type hemicelluloses (34 600 g/mol), as shown by the  $M_w$  ranging from 18 500 to 30 000 g/mol. As reaction time increased from 10 to 50 min,  $M_w$  decreased from 30 000 to 23 600 g/mol. A higher reaction temperature also resulted in a remarkable fall in  $M_w$ , as shown by the decreasing  $M_w$  from 26 800 to 19 200 g/mol. On the other hand, NaOH and cationic moiety 2,3-epoxypropyltrimethylammonium chloride showed less significant influence on  $M_w$ .

**FT-IR Spectra.** The chemical structures of xylan-type hemicelluloses, carboxymethyl xylan-type hemicelluloses, and quaternized carboxymethyl xylan-type hemicelluloses were characterized by FT-IR spectra, as shown in Figure 2. For

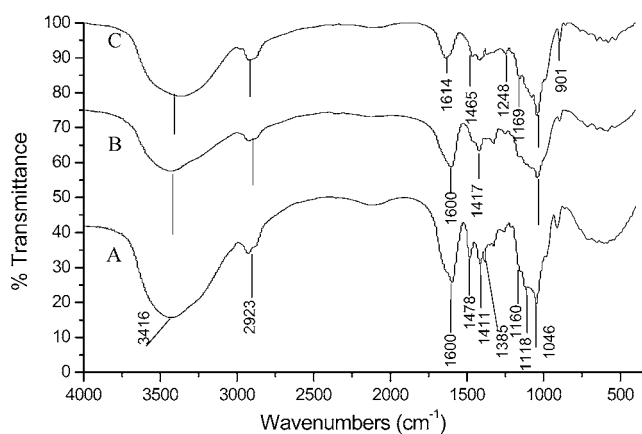
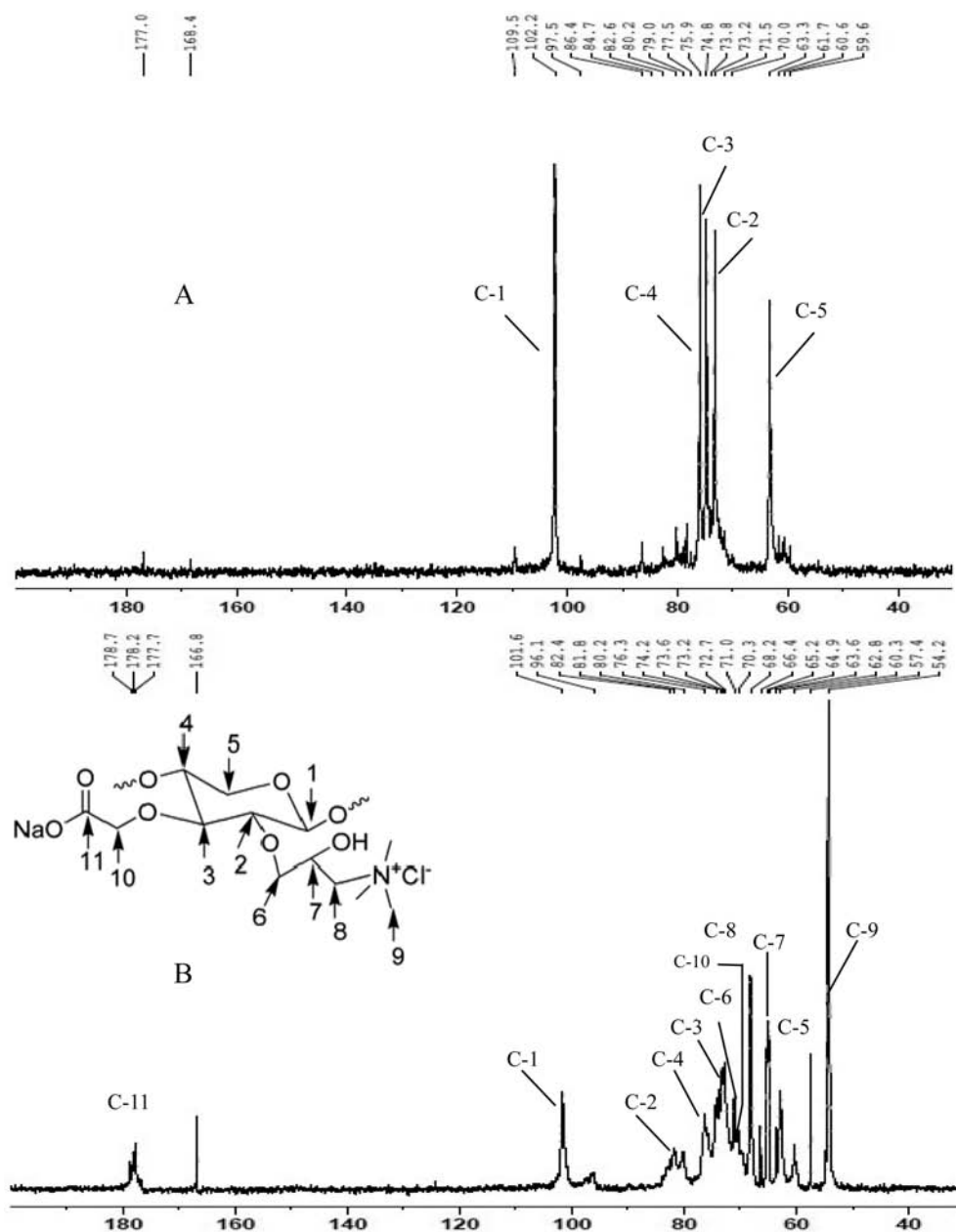


Figure 2. FT-IR spectra of xylan-type hemicelluloses (spectrum C), carboxymethyl xylan-type hemicelluloses (spectrum B, sample 1 in Table 1), and quaternized carboxymethyl xylan-type hemicelluloses (spectrum A, sample 13 in Table 2).

xylan-type hemicelluloses (Figure 2C), the absorptions at 3416, 2923, 1614, 1465, 1411, 1248, 1169, 1046, and 901  $cm^{-1}$  are assigned to xylan-type hemicelluloses. In spectrum B (carboxymethyl xylan-type hemicelluloses), two strong peaks at 1600 and 1417  $cm^{-1}$  are ascribed to the asymmetrical and



**Figure 3.**  $^{13}\text{C}$  spectra of xylan-type hemicelluloses (spectrum A) and quaternized carboxymethyl xylan-type hemicelluloses (spectrum B, sample 13 in Table 2).

symmetrical stretching of  $\text{COO}^-$  groups. These new bands in the spectra of carboxymethyl xylan-type hemicelluloses confirmed that carboxymethylation occurred to xylan-type hemicelluloses. The characteristic absorptions at  $1118$  and  $1046\text{ cm}^{-1}$  due to the  $\text{C}-\text{O}-\text{C}$  stretching of the ether groups were observed in spectrum A (quaternized carboxymethyl xylan-type hemicelluloses). The new absorption peak at  $1478\text{ cm}^{-1}$  corresponds to the  $\text{CH}_2$  bending mode and methyl groups in the long chain alkyl ammonium of quaternized carboxymethyl xylan-type hemicelluloses. The appearance of the band at  $1411\text{ cm}^{-1}$ , which is assigned to the  $\text{C}-\text{N}$  stretching vibration, is evidence of quaternary ammonium groups.<sup>19</sup>

**$^{13}\text{C}$  NMR and DEPT-135 NMR Spectra.** In the  $^{13}\text{C}$  NMR spectrum of xylan-type hemicelluloses (Figure 3A), the main 1,4-linked-D-xylopyranosyl units are characterized by five strong signals at  $\delta$  102.2, 75.9, 74.8, 73.2, and 63.3 ppm, which are

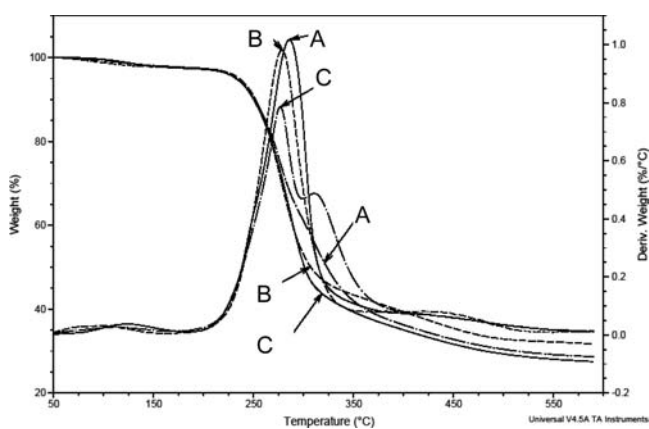
assigned to C-1, C-4, C-3, C-2, and C-5 positions of the  $\beta$ -D-xylp units, respectively. The signals at  $\delta$  109.5, 86.4, 80.2, 77.5, and 61.7 ppm correspond to C-1, C-4, C-2, C-3, and C-5 positions of the  $\alpha$ -L-arabinofuranosyl residues linked to  $\beta$ -D-xylans, respectively.<sup>24</sup> Two signals at  $\delta$  177.1 and 168.4 ppm are indicative of the carbonyl signal of the esterified ferulic or *p*-coumaric acids in the hemicelluloses, which show the esterified ferulic acids linked to lignin by ester bonds.<sup>25</sup> The C-1, C-4, and C-6 of glucose residue in the xylan backbone are identified by three signals at  $\delta$  97.5, 82.6, and 60.6 ppm, respectively. The signal at  $\delta$  59.6 ppm originates from 4-O-methyl-D-glucuronic acid residue in the xylan.

In Figure 3B (quaternized carboxymethyl xylan-type hemicelluloses), the signals of the carboxyl groups appear at  $\delta$  178.2 and 166.8 ppm (C-11). The signals at  $\delta$  68.6 and 71.0 ppm are assigned to the methylene carbon atoms of the carboxymethyl

groups (C-10). These observations proved the carboxymethylation of xylan-type hemicelluloses. The signals at  $\delta$  72.7, 64.9, 68.2, and 54.2 ppm are assigned to the carbon resonances of C-6, C-7, C-8, and C-9 moieties, respectively, which confirmed the occurrence of quaternization again by  $^{13}\text{C}$  NMR.

DEPT-135 NMR spectrum of quaternized carboxymethyl xylan-type hemicelluloses was used to further confirm the chemical structure of quaternized carboxymethyl xylan-type hemicelluloses because the carbon signals in  $^{13}\text{C}$  NMR spectra (especially some overlapping signals) can be further distinguished. In the DEPT-135 NMR spectrum, positive signals indicate  $-\text{CH}$  and  $-\text{CH}_3$  groups, negative signals indicate  $-\text{CH}_2$  groups, and no signals appear for quaternary carbons. The DEPT-135 spectrum of quaternized carboxymethyl xylan-type hemicelluloses is shown in Figure S2 (Supporting Information). Peaks of the  $-\text{CH}_2$  groups at positions 5 ( $\delta$  62.8 ppm), 6 ( $\delta$  74.2 ppm and  $\delta$  71.0 ppm), 8 ( $\delta$  68.1 ppm), and 10 ( $\delta$  69.7 ppm) are shown by negative intensities and can easily be distinguished from  $-\text{CH}$  and  $-\text{CH}_3$  moieties. The two signals from positions 6 observed at  $\delta$  74.2 and 71.0 ppm might result from the substitution of the hydroxyl group at positions 2 and 3 of the xylan residue.<sup>26</sup> The three methyl groups of the ammonium moieties (position 9) show an intensive signal at  $\delta$  55.0 ppm.

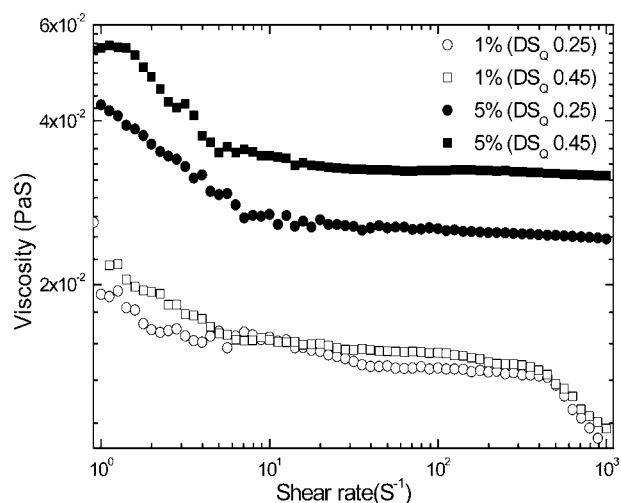
**Thermal Analysis.** Figure 4 shows the typical TGA/DTA curves of xylan-type hemicelluloses, carboxymethyl xylan-type



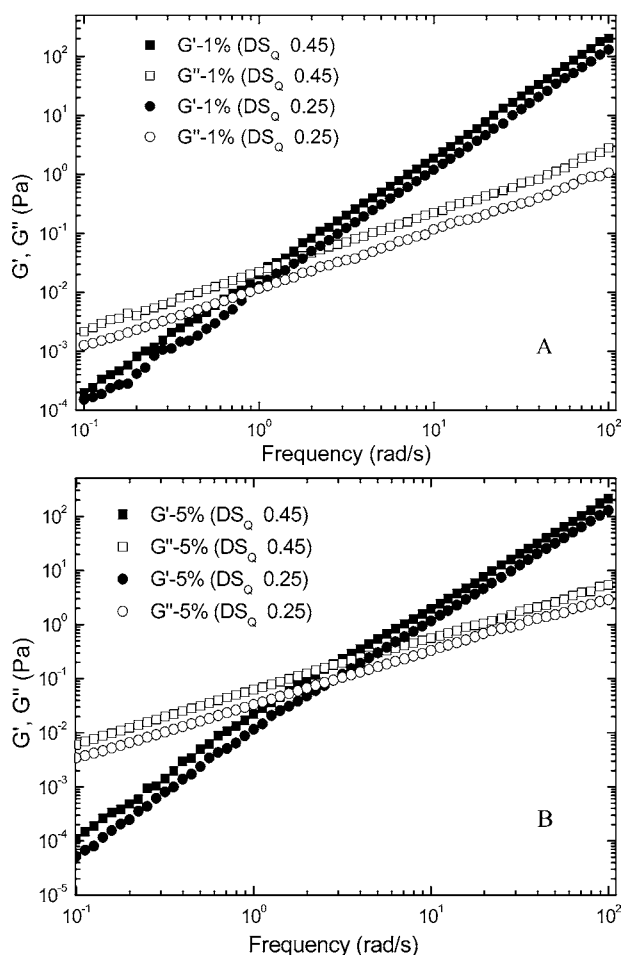
**Figure 4.** TG/DTA curves of xylan-type hemicelluloses (spectrum A), carboxymethyl xylan-type hemicelluloses (spectrum B,  $\text{DS}_\text{C}$  of 0.49 in Table 1), and quaternized carboxymethyl xylan-type hemicelluloses (spectrum C, sample 13 in Table 2).

hemicelluloses, and quaternized carboxymethyl xylan-type hemicelluloses. The main degradation temperature of the three samples ranged from 200 to 370 °C. The maximum decomposition rates differed, which is indicated by the  $T_{\text{max}}$  (the decomposition temperature corresponding to the maximum weight loss rate) in Figure 4. The xylan-type hemicelluloses, carboxymethyl xylan-type hemicelluloses, and quaternized carboxymethyl xylan-type hemicelluloses showed their  $T_{\text{max}}$  at 287, 278, and 277 °C, respectively, suggesting that chemical modification leads to lower thermal stability mainly due to more hydrogen bonds, and molecular structure was destroyed.

**Rheological Properties.** The rheological behaviors of quaternized carboxymethyl xylan-type hemicelluloses (samples 7 and 13 in Table 2) are shown in Figures 5 and 6. Because quaternized carboxymethyl xylan-type hemicelluloses can well



**Figure 5.** Shear rate-dependent viscosity of quaternized carboxymethyl xylan-type hemicelluloses (samples 7 and 13 in Table 2) solutions at concentrations of 1% and 5% (w/w).



**Figure 6.** Frequency-dependent elastic modulus ( $G'$ ) and loss modulus ( $G''$ ) of quaternized carboxymethyl xylan-type hemicellulosic (samples 7 and 13 in Table 2) solutions at 1% concentration (A). Frequency-dependent elastic modulus ( $G'$ ) and loss modulus ( $G''$ ) of quaternized carboxymethyl xylan-type hemicellulosic (samples 7 and 13 in Table 2) solutions at 5% concentration (B).

be dissolved in water and form a viscous solution, the rheological measurements were carried out in distilled water

at concentrations of 1% and 5% (w/w). The viscosity of the quaternized carboxymethyl xylan-type hemicelluloses decreased with the increase of shear rate (especially at the shear rate  $\leq 10$ /s), implying that quaternized carboxymethyl xylan-type hemicellulosic solutions exhibit pseudoplastic or shear-thinning behavior due to the destroying of the entangled macromolecules network at higher shear rate. The shear-thinning behaviors are commonly observed in other carbohydrate polymers.<sup>27,28</sup>

A higher concentration (5%) resulted in a higher viscosity, indicating more entanglements among macromolecules form at higher concentrations. The viscosity of quaternized carboxymethyl xylan-type hemicelluloses with  $DS_Q$  of 0.54 was generally higher than that of quaternized carboxymethyl xylan-type hemicelluloses with  $DS_Q$  of 0.30 at 1% concentration, which was more remarkable at 5% concentration. This suggests that quaternized carboxymethyl xylan-type hemicelluloses with more quaternary ammonium groups have stronger intermolecular interactions among their molecules due to the electrostatic attraction between carboxymethyl groups and quaternary ammonium groups in aqueous solution. It was found that strong electrostatic interaction between positively charged starch and negatively charged xanthan gum resulted in higher viscosity of starch.<sup>29</sup> In contrast, the viscosity of starch with negatively phosphate groups greatly decreased when negatively charged gums were added to the starch solution due to the electrostatic repulsion.<sup>30</sup> Carboxymethyl xylan-type hemicelluloses were found to show a lower viscosity than xylan-type hemicelluloses in alkaline solution due to the repelling forces between negatively charged carboxymethyl xylan-type hemicellulosic molecules.<sup>4</sup>

Viscoelastic properties can be used to characterize the three-dimensional network structure of macromolecules in solution. Figure 6 shows the frequency-dependent  $G'$  and  $G''$  of quaternized carboxymethyl xylan-type hemicelluloses solutions at concentrations of 1% and 5%. At 1% concentration,  $G'$  was higher than  $G''$  at low frequencies, but went above  $G''$  when the tested frequency was higher than 2 rad/s (Figure 6A). A similar trend was also observed at 5% concentration in Figure 6B. Therefore, quaternized carboxymethyl xylan-type hemicellulosic solution shows a viscous behavior at low frequencies and an elastic behavior at higher frequencies. Figure 6 also shows that both  $G'$  and  $G''$  of QCMH with  $DS_Q$  of 0.54 were higher than those of quaternized carboxymethyl xylan-type hemicelluloses with  $DS_Q$  of 0.30, indicating that quaternized carboxymethyl xylan-type hemicelluloses with more quaternary ammonium groups show stronger electrostatic attraction in aqueous solution. This observation agreed well with the results obtained from the viscosity in Figure 5. Therefore, the presence of quaternary ammonium groups in quaternized carboxymethyl xylan-type hemicelluloses molecular chain will cause electrostatic attraction between carboxymethyl groups and quaternary ammonium groups.

In summary, amphoteric hemicelluloses with carboxymethyl groups and quaternary ammonium groups were efficiently synthesized by sequent incorporation of carboxymethyl and quaternary ammonium groups into the xylan-type hemicelluloses backbone using microwave irradiation. Carboxymethyl xylan-type hemicelluloses with  $DS_C$  of 0.49–0.90 could be obtained within 20 min using microwave irradiation method, exhibiting a higher efficiency synthesis as compared to the conventional heating method. Reaction parameters such as the molar ratio of reagent (NaOH or cationic moiety

2,3-epoxypropyltrimethylammonium chloride)/anhydroxylose unit in hemicelluloses, the temperature, the reaction time, and the  $DS_C$  of carboxymethyl xylan-type hemicelluloses influenced the  $DS_Q$ . The maximum  $DS_Q$  under the optimum reaction condition was 0.52. Average molecular weight indicated that the prolonging time and elevated temperature as well as the action of microwave irradiation led to the degradation of hemicelluloses. Chemical modification resulted in lower thermal stability of quaternized carboxymethyl xylan-type hemicelluloses. The quaternized carboxymethyl xylan-type hemicelluloses showed shear-thinning performance and an elastic behavior at higher frequencies in aqueous solution. The viscosity, elastic modulus, and loss modulus of quaternized carboxymethyl xylan-type hemicelluloses increased with the increasing  $DS_Q$ . This study represented a first attempt to synthesize amphoteric hemicelluloses using microwave irradiation, and provided a very efficient way for the preparation of amphoteric biopolymers.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Molecular weight distributions of xylan-type hemicelluloses (Figure S1), and DEPT-135 NMR spectrum of quaternized carboxymethyl xylan-type hemicelluloses (Figure S2). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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