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Synthesis and Antibacterial Activity of Alkylated Chitosan Under Basic Ionic Liquid Conditions

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ABSTRACT: A novel method to synthesize alkylated chitosan with [Bmim]OH basic ionic liquid as alkaline reagent is reported for the first time. Chitosan was alkalized by [Bmim]OH basic ionic liquid before it was reacted with various alkyl halides, and a series of alkylated chitosans with different carbon chain substituents were prepared. The structure of alkylated product was characterized by IR, ^{1}H-NMR, and XRD. The reusability of the ionic liquid was also investigated, and the experimental results indicated that there was no noticeable change for the degree of substitution (DS) of alkylated chitosan after the ionic liquid was reused for three times. The antibacterial activities of the target products were investigated according their minimum inhibitory concentration (MIC) against *K. pneumoniae, P. aeruginosa, E. aerogenes, E. coil, S. epidermidis,* and *S. aureus*, and the results indicated that E-CTS, B-CTS, and D-CTS could exhibit excellent antibacterial activities against *P. aeruginosa*, and their MIC were 128, 128, and 256 μg mL^{-1}, respectively. Meanwhile, the antibacterial activities of alkylted chitosans were decreased with the growth of the DS or the growth of the carbon chain. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40052.

KEYWORDS: ionic liquids; applications; recycling

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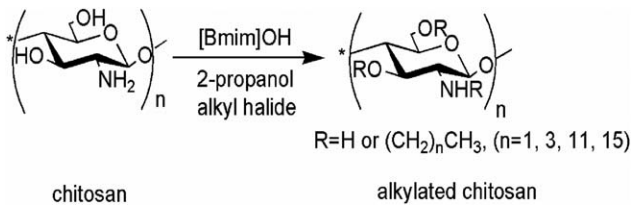
INTRODUCTION

Chitosan (CTS), with a repeating structure unit of β-(1, 4)-2-amino-2-deoxy-β-D-glucose, is a fully or partly deacetylated product of chitin, which is an abundant natural resource.[1] Because of its biodegradability, nontoxicity, and antimicrobial activity,[1–3] CTS has been widely used for biomedical applications, such as tissue engineering scaffolds, drug delivery, wound dressings, and antibacterial coatings.[1,4,5] However, the existence of strong intramolecular and intermolecular hydrogen bonds between hydroxyl groups and amino groups makes it insoluble in most solvents. To overcome this limitation, some researchers have set out to modify chitosan to gain derivatives with water solubility and higher activity.[6–10] Among various methods for the chemical modification of chitosan, the alkylation is one of very important ways and it could be used to prepare a variety of alkylated chitosan derivatives.[11,12]

Because of their good antimicrobial activity, adsorption capacity, antithrombogenicity, and biodegradability, alkylated chitosans are often used as antibacterial agent, pesticides, and extraction adsorption carrier.[4,5] The traditional method for the preparation of alkylated chitosan is carried out using sodium hydroxide to destroy the stereoregularity and molecular internal strong hydrogen bonds of chitosan, then the basified chitosan is reacted with alkylating agents.[13] This method not only consumes a lot of sodium hydroxide, but also has the disadvantages of equipment corrosion, environmental pollution, recovery difficulty, and other shortcomings. Therefore, it is necessary to prepare alkylated chitosan using green alkaline reagent.

Ionic liquids (ILs) are promising alternatives of "green solvents" for their properties such as negligible vapor pressure, broad liquid regions, high thermal stabilities, no burning or explosive point, and recyclability.[14] As one of novel green solvents and catalysts, ILs have been widely used in various process of organic synthesis.[15] In recent years, basic ionic liquid has aroused great concern. In some alkali-catalyzed organic reactions, the basic ionic liquid, which is utilized as both a solvent and a catalyst, has a higher catalytic activity than inorganic base.[16,17]

In this article, 1-Butyl-3-methylimidazolium hydroxide, as one of basic ionic liquid, was used to alkalize chitosan for preparing alkylated chitosan. Meanwhile, the effects of carbon numbers of alkyl substituted on chitosan and its DS on the antibacterial



Scheme 1. Synthesis of alkylated chitosan.

activities of alkylated chitosan were also investigated in this article. The preparation of alkylated chitosan is presented in Scheme 1.

EXPERIMENTAL

Materials and Instruments

Chitosan was supplied by Golden Shell Biochemistry (Zhejiang, P.R. China), which deacetylation degree was 90.2% and average viscometric molecular weight (Mv) was estimated to be 50,000 based on Mark-Houwink equation.[18] N-methylimidazole, bromoethane, 1-chlorobutane, bromododecane, and bromohexadecane were purchased from Sinopharm Chemical Reagent (Shanghai, P.R. China). Dialysis bag (Mw cut-off 500) was purchased from Biosharp (USA). *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia aerogenes* (*E. aerogenes*), *Escherichia coil* (*E. coil*), *Staphylococcus epidermidis* (*S. epidermidis*), and *Staphylococcus aureus* (*S. aureus*) were purchased from Thermo Fisher of American and were inoculated on nutrient broth medium before use. All other chemicals used were of regent grade.

The IR spectra were taken on a Nicolet IS10 FTIR spectrometer (Nicolet, Madison). The ^{1}H-NMR spectra were recorded on a Bruker AV-300 nuclear magnetic resonance spectrometer (Bruker, Karlsruhe, Germany) with D_2O or $CDCl_3$ as solvent and TMS as internal standard. The elemental analysis was performed using a Vario EL-III elemental analyzer (Elementar, Hanau, Germany). X-ray diffraction (XRD) was recorded on a philips X'pert Pro X-ray diffractometer (Philips Company, Holand) using a Cu Kα radiation ($\lambda = 0.154\,056$ nm).

Preparation of 1-Butyl-3-methylimidazolium Hydroxide ([BmIm]OH)

Synthesis of 1-Butyl-3-methylimidazolium Chloride. 1-Butyl-3-methylimidazolium Chloride ([Bmim]Cl) was prepared according to the method developed by Huddleston et al.[19] Equal molar 1-chlorobutane and 1-methylimidazole were added to a round-bottomed flask fitted with a reflux condenser and reacted at 100°C until two phases formed under stirring (the duration ca. 72 h). Then the upper phase containing unreacted starting material was decanted, and ethyl acetate (its volume was approximately equal to half of that of the bottom phase) was added into reactant with thorough mixing. The ethyl acetate was decanted before another fresh ethyl acetate was added, and this process was repeated twice to ensure that the unreacted materials were removed sufficiently from the bottom phase. The remained ethyl acetate was removed by distillation using rotary evaporator under vacuum at 70°C. The remainder was dried in vacuum at 70°C for 8 h and the [Bmim]Cl was obtained.

IR: $v = (cm^{-1})$: 3143.59, 2960.35, 2951.66, 2873.57 (C—H), 1572.78, 1465.71 (C=N), 1382.78 (—C—CH_3); 1168.73, 1109.24 (C—N); 865.92, 755.99 (C—H).

^{1}H-NMR (300 MHz, D_2O, δ): 8.67 (s, 1H, —NCHN—), 7.36 (d, 1H, CH_3NCHCHN-), 7.03 (d, 1H, CH_3NCHCHN-), 4.09 (t, 2H, -N$\underline{CH_2}CH_2CH_2CH_3$), 3.78 (s, 3H, -$NCH_3$), 1.74 (m, 2H, -$NCH_2\underline{CH_2}CH_2CH_3$), 1.23 (m, 2H, -$NCH_2CH_2\underline{CH_2}CH_3$), 0.82 (t, 3H, -$N(CH_2)_3CH_3$).

Anal. Calcd for $C_8H_{15}ClN_2$: C 55.01, H 8.66, N 16.04; found: C 54.64, H 8.98, N 15.35.

Synthesis of 1-Butyl-3-methylimidazolium Hydroxide. The synthesis of 1-Butyl-3-methylimidazolium Hydroxide ([Bmim]OH) was carried out according to literature.[20] Briefly, potassium hydroxide (2.3 g, ca. 41.1 mmol) was added into [Bmim]Cl (8.8 g, 40 mmol) dissolved in methylene chloride (20 mL), and the mixture was stirred vigorously at room temperature for 10 h. The precipitated KCl was filtered off, and the filtrate was evaporated. [Bmim]OH was obtained after the remainder was washed with ether for three times and dried at 90°C for 10 h.

IR: $v = (cm^{-1})$: 3416.09 (O—H); 3078.9, 2959.03, 2933.19, 2872.77 (C—H), 1572.91, 1462.84 (C=N); 1169.79, 1114.98 (C—N), 877.39, 755.29 (C—H).

^{1}H-NMR (300MHz, $CDCl_3$, δ): 0.83 (t, 3H, -$N(CH_2)_3CH_3$), 1.14~1.34 (m, 2H, -$N(CH_2)_2\underline{CH_2}CH_3$), 1.76~1.89 (m, 2H, -$NCH_2\underline{CH_2}CH_2CH_3$), 3.17~3.25 (s, 1H,-$CH_2OH$), 4.02 (s, 3H, -$NCH_3$), 4.26 (t, 2H, -N$\underline{CH_2}(CH_2)_2CH_3$), 7.46 (d, 1H, CH_3NCHCHN-), 7.59 (d, 1H, CH_3NCHCHN-), 10.18 (s, 1H, -NCHN-).

Anal. Calcd for $C_8H_{16}ON_2$: C 61.5, H 10.32, N 17.93; found: C 61.39, H 10.95, N 17.62.

The Preparation of Alkyl Chitosan by [Bmim]OH

Chitosan (0.02 mol) was added into flask and swelled in 2-propanol (50.0 mL) at room temperature for 1h under stirring. Then [Bmim]OH (0.02 mol) was added into the bottle. The mixture was heated to 40°C under stirring and alkalized for 1 h before alkyl halide (0.04 mol) was added. The mixture was heated to 80~100°C and allowed to react at this temperature for 8~10 h. After the reaction ended, the reactant was filtered and the filter residue was washed with ethanol and anhydrous methylene chloride in sequence. The alkylated chitosans (a-d) were obtained after the filter residue was dried at 50°C in vacuum till constant weight.

Ethyl Chitosan (a E-CTS). IR (KBr): $v = (cm^{-1})$: 2915 (2838 (-CH_3, -CH_2); 1623 (C=O); 1434 (C—H); 1327 (C—N); 3359, 1012 (-OH).

^{1}H-NMR (300MHz, D_2O, δ): 4.58 (d, 1H, -OC$\underline{H}$-), 3.52-3.80 (t, 2H, -C$\underline{H_2}$OH), 3.67 (m, 1H, CH_2OH-C$\underline{H}$-), 3.55 (m, 1H, -C$\underline{H}$OH), 2.80 (m, 1H, -C$\underline{H}$NH-), 2.59 (m, 2H, -NHC$\underline{H_2}$-), 2.15 (t, 1H, -CH_2O$\underline{H}$), 1.96 (d, 1H, -OH, 1.66 (m, 1H, -C$\underline{H}$-), 1.29 (m, 2H, -$CH_2\underline{CH_3}$), 1.00 (t, 3H, -CH_3).

Butyl Chitosan (b B-CTS). IR (KBr): $v = (cm^{-1})$: 2920, 2848 (-CH_3, -CH_2); 1645 (C=O); 1438 (C—H); 1332 (C—N); 3455, 1015 (-OH); 728 ((-CH_2-)$_3$).

^{1}H-NMR (300MHz, D_2O, δ): 4.64 (d, 1H, -OCH-), 3.53-3.78 (t, 2H, -CH_2OH), 3.65 (m, 1H, CH_2OH-CH-), 3.54 (m, 1H, -CHOH), 3.38-3.63 (d, 2H, -CHCH_2O-), 2.82 (m, 1H, -CHNH-), 2.55 (m, 2H, -NHCH_2-), 2.18 (t, 1H, -CH_2OH), 1.98 (d, 1H, -OH), 1.66 (m, 1H, -CH-), 1.41 (m, 2H,-NHCH_2CH_2-), 1.33 (m, 2H,-NHCH_2CH_2CH_2-), 0.96 (m, 3H, -CH_2CH_3).

Dodecyl Chitosan (c D-CTS). IR (KBr): $v = (cm^{-1})$: 2923, 2853 (-CH_3, -CH_2); 1640 (C═O); 1450 (C—H); 1340 (C—N); 3470, 1020 (-OH); 725 ((-CH_2-)$_{11}$).

^{1}H-NMR (300MHz, D_2O, δ): 4.56 (d, 1H, -OCH-), 3.54-3.79 (t, 2H, -CH_2OH), 3.68 (m, 1H, CH_2OH-CH-), 3.51 (m, 1H, -CHOH), 2.78 (m, 1H, -CHNH-), 2.54 (m, 2H, -NHCH_2-), 2.14 (t, 1H, -CH_2OH), 1.95 (d, 1H, -OH), 1.66 (m, 1H, -CH-), 1.40 (m, 2H,-NHCH_2CH_2-), 1.29 (m, 16H, -NHCH_2CH_2-(CH_2)$_8$-CH_2-), 1.33 (m, 2H, -CH_2CH_3), 0.98 (t, 3H, -CH_3).

Cetyl Chitosan (d C-CTS). IR (KBr): $v = (cm^{-1})$: 2933, 2857 (-CH_3, -CH_2); 1645 (C═O); 1458 (C—H); 1353 (C—N); 3420, 1015 (-OH); 722 ((-CH_2-)$_{15}$).

^{1}H-NMR (300MHz, D_2O, δ): 4.60 (d, 1H, -OCH-), 3.50-3.85 (t, 2H, -CH_2OH), 3.72 (m, 1H, CH_2OH-CH-), 3.58 (m, 1H, -CHOH), 3.42-3.58 (d, 2H, -CHCH_2O-), 2.85 (m, 1H, -CHNH-), 2.55 (m, 2H, -NHCH_2-), 2.17 (t, 1H, -CH_2OH), 1.93 (d, 1H, -OH), 1.66 (m, 1H, -CH-), 1.41 (m, 2H,-NHCH_2CH_2-), 1.33 (m, 2H, -CH_2CH_3), 1.1 (m, 24H, -NHCH_2CH_2-(CH_2)$_{12}$-CH_2-), 0.95 (t, 3H, -CH_3).

Regeneration and Reutilization of [Bmim]OH

After the alkylation reaction, the filter liquor and washing liquid were gathered into a flask, and then were distilled. During the distillation, the solution became thicker until no solvent was steamed out. At this time, the distillation was terminated and the flask was allowed to cool to room temperature.[21,22] The [Bmim]OH was regained in the residue, and here it was assumed that the [Bmim]OH was not lost during the process of recuperation. According to the method mentioned in the section 1.3, the alkylated chitosan was prepared again with the recycled [Bmim]OH as alkalization reagent and its DS was determined.

Evaluation of Antibacterial Activities

The agar plate method was used to determine the minimum inhibitory concentration (MIC) of alkylated chitosan. The samples were prepared at a concentration of 0.0512 g mL^{-1}, then they were autoclaved at 121°C for 30 min. Duplicate two-fold serial dilutions of each sample were added into nutrient broth (beef extract 3 g, peptone 10 g, NaCl 5 g and agar 15 g to 1000 mL deionized water, PH 7.0) for final concentration of 0.0256, 0.0128, 0.0064, 0.0032, 0.0016, 0.0008, 0.0004, and 0.0002 g mL^{-1}. Some samples were prepared by decuple diluting method. The cultured bacterium was diluted by deionized water to obtain bacterium suspension. Adjust the density of the organism suspension to 10^8 CFU·mL^{-1} by adding sterile deionized water. Each bacterium suspension was inoculated on nutrient medium with sample and control sample. After inoculation, the plates were incubated at 37°C for 24 h.[23,24] The colonies were counted and the MIC values were obtained.

Determination of Alkylated Chitosan DS

The determination of DS of alkylated chitosan was carried out according to literature.[25]

RESULTS AND DISCUSSIONS

The Structural Characterization of the Product

FTIR and ^{1}H-NMR. The FTIR spectra of CTS and alkylated chitosan were presented in Figure 1. The absorption bands at 1655, 1593, 1323, 1381 cm^{-1} in the spectrum of CTS were assigned to amides I, II, III, and –CH_3 vibration bends. Both characteristic peaks for CTS at 3359 and 1070 cm^{-1} could be attributed to the O—H and C—O vibration, respectively. In the spectrum of alkylated chitosan, the C—H stretching vibration absorption peak intensity at 2853 and 2923 cm^{-1} were obviously enhanced compared with chitosan material. Characteristic peaks of amine NH vibration deformation appeared at 1595 cm^{-1} for chitosan. Note that this peak was weakened in chitosan derivatives, caused by formation of alkyl group at C-2 in the chitosan and a new C—H distortion absorption peak appears at 1452 cm^{-1}. Characteristic peaks of alcohol and second alcohol between 1350 and 1030 cm^{-1} weakened, which confirmed the introduction of an alkyl group at C-3 and C-6 in the chitosan. But the amino is still the main substituted group because of the hydroxyl's low reaction degree.

The ^{1}H-NMR spectrum of CTS, E-CTS, B-CTS, D-CTS, and C-CTS were shown from Figure 2 to Figure 6. The signals at 4.56–4.64, 2.78–2.85, 3.50–3.58, 1.66, 3.65–3.72, and 3.50–3.85 ppm in the ^{1}H-NMR spectrum of alkylated chitosan were assigned to the hydrogen of C-1, C-2, C-3, C-4, C-5, and C-6, respectively. The signal at 2.15–2.18 ppm was attributed to the protons of C6-OH. The signal at 1.93–1.98 ppm was assigned to the proton of C3-OH. Compared with CTS, the signals at 0.95–1.0 ppm and 1.1–1.41 ppm in the ^{1}H-NMR spectrum of alkylated chitosan were correspondingly assigned to -CH_3 and -CH_2. Among them, the peak area of methylene group was significantly greater than that of the methyl group. These indicated the presence of long carbon chain. The result was consistent with the infrared spectroscopy.

X-ray Analysis. The X-ray diffraction of CTS and alkyl chitosans were shown in Figure 7. It could be seen that there were some differences of peak height, width, and position between them. CTS mainly consisted of two major peaks at 11°and 20°. Compared with CTS, the peak of alkylated chitosan at 11° did not appear and the peak at 20° became widened. But between 30° and 40° appeared a series of new diffraction peaks. As the alkylation reaction proceeded, the introduction of alkyl groups significantly weakened the intramolecular and intermolecular hydrogen bonds. The original ordered crystal structure of chitosan was destroyed and tended to be disordered. Thus the diffraction peaks changed.

In addition, the crystallinity of CTS, alkalized CTS, E-CTS, B-CTS, D-CTS, and C-CTS were 72.6, 27.9, 44.1, 49.5, 58.7, and 53.2% calculated by the "Jade" software. Compared with CTS, the crystallinity of alkalized CTS was decreased due to the destruction of chitosan stereoregularity. Besides, under the condition of the alkylated chitosan with the same DS, the

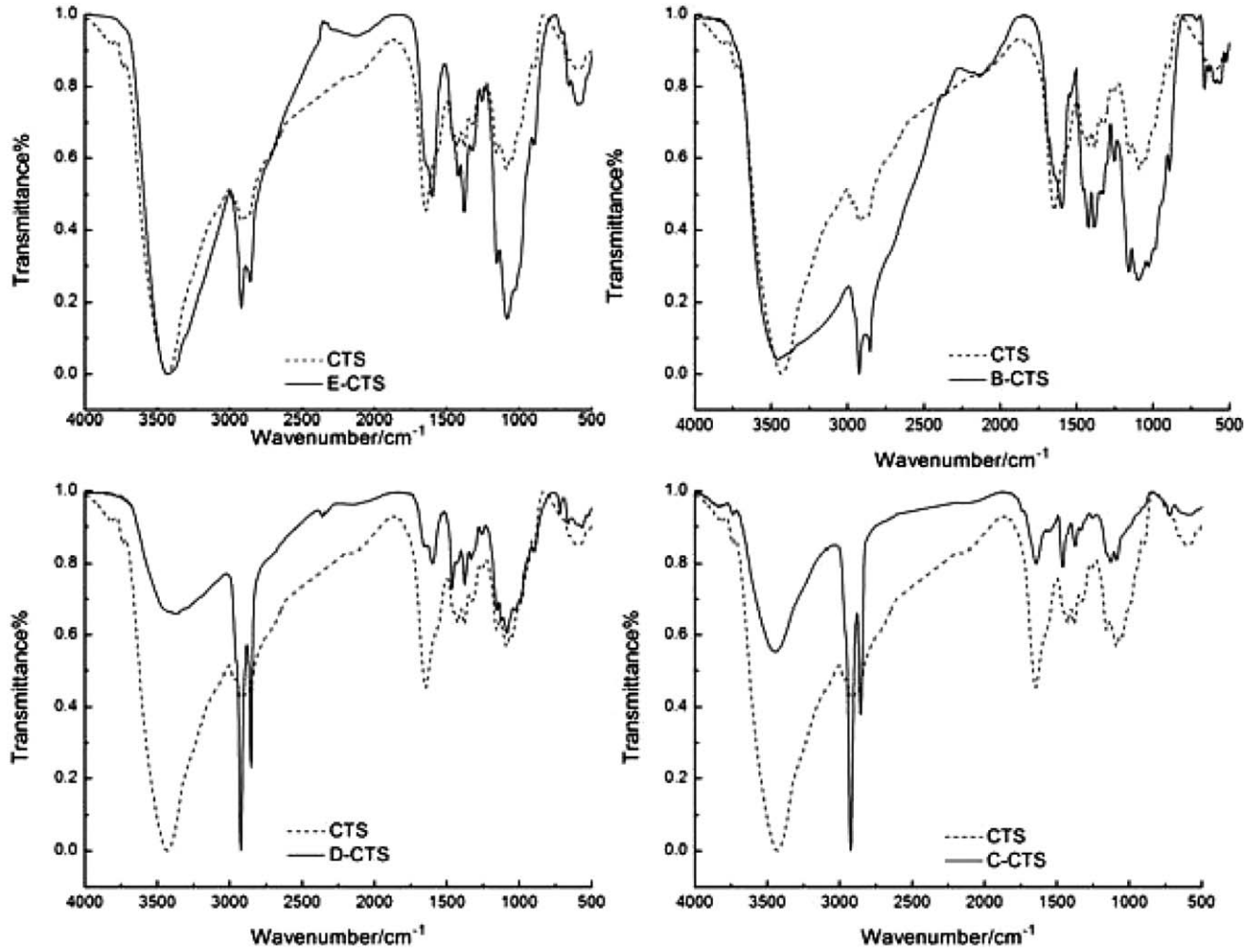


Figure 1. FTIR spectra of CTS, E-CTS, B-CTS, D-CTS, and C-CTS.

crystallinity of E-CTS, B-CTS, and D-CTS were increased with the growth of carbon chain. While the crystallinity of C-CTS was degraded slightly due to the destruction of the C-CTS stereoregularity, caused by the extension of reaction time and the rise of the reaction temperature to gain the C-CTS with the same DS.

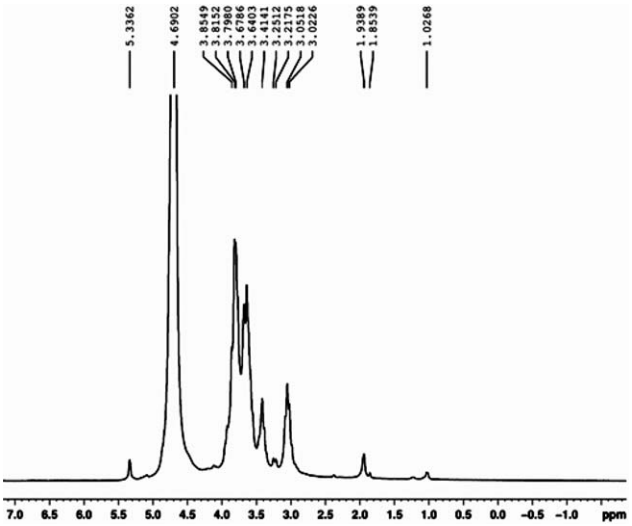


Figure 2. ^{1}H-NMR spectrum of CTS.

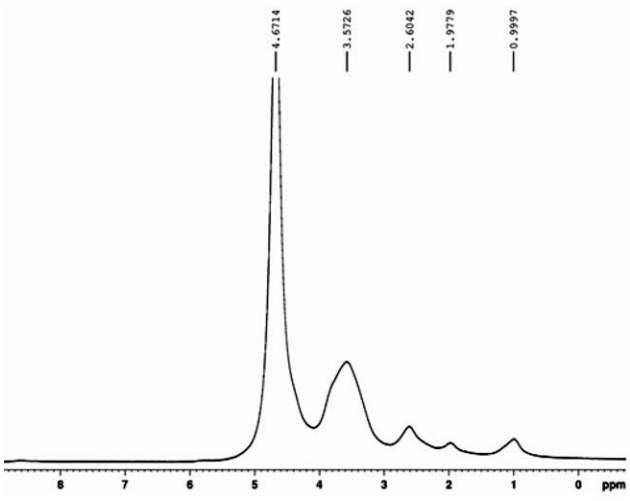


Figure 3. ^{1}H-NMR spectrum of E-CTS.

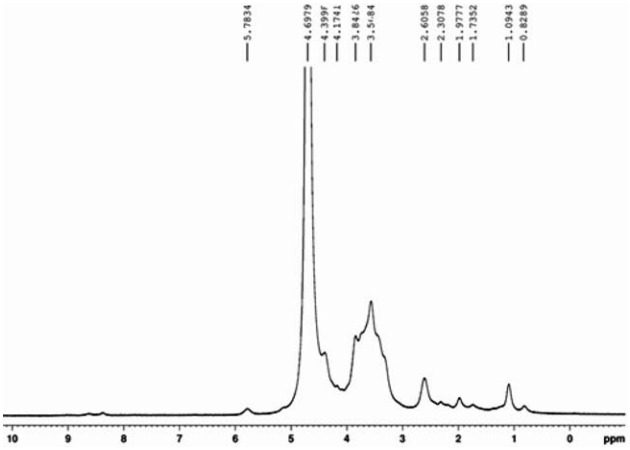


Figure 4. ^{1}H-NMR spectrum of B-CTS.

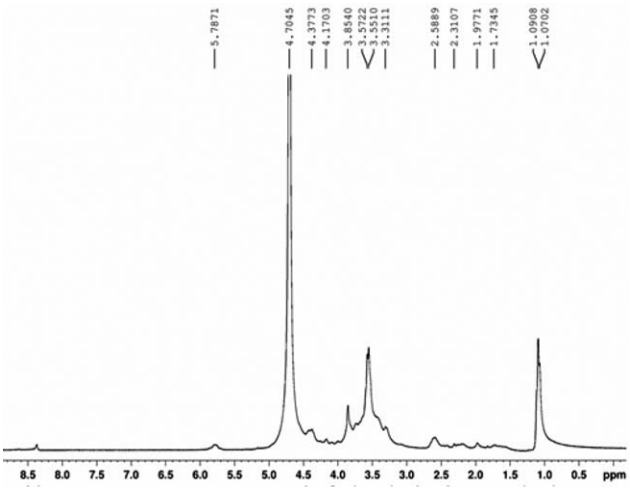


Figure 5. ^{1}H-NMR spectrum of D-CTS.

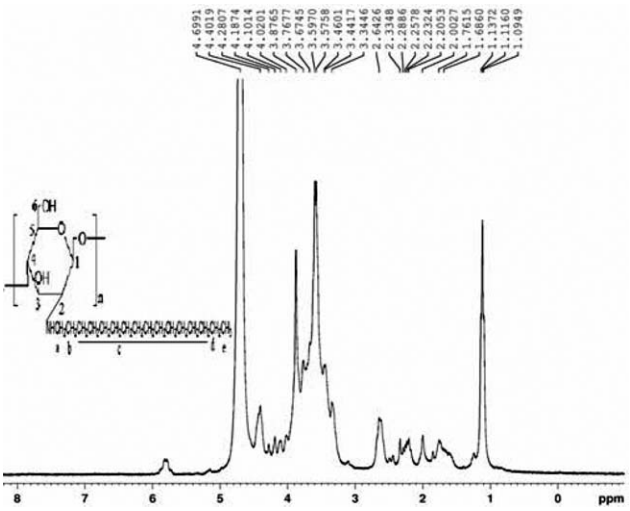


Figure 6. ^{1}H-NMR spectrum of C-CTS.

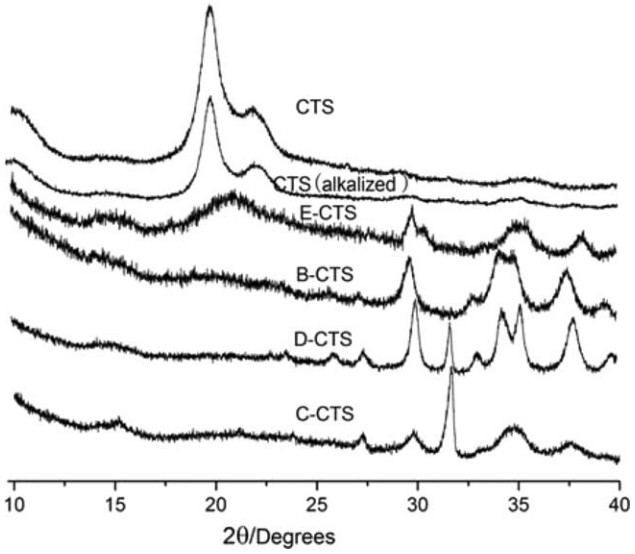


Figure 7. X-ray diffraction patterns of CTS, alkalized CTS, E-CTS, B-CTS, D-CTS, and C-CTS.

Effect of Alkalizing Agent on the DS of D-CTS

The traditional alkalization method for preparing the D-CTS is as follows: n (NaOH): n (chitosan)=1.0 : 1.0, at 40 °C alkalized for 1 h, n (bromodecane) : n (chitosan)=1.0 : 1.0, at 80 °C alkylated for 8 h. In what follows we investigated separately the effects of NaOH and [Bmim]OH on dodecyl chitosan DS. And the results could be found in Table I.

From the Table I we could know that the DS of dodecyl chitosan was the largest when it used the [Bmim]OH as alkalization reagent. The catalytic performance of [Bmim]OH as quaternary amine alkali was better than that of NaOH and it could be recycled. The reason might be ascribed to the fact that methylimidazolium type IL can partially dissolve chitosan and thus effectively destroy its intermolecular and intramolecular hydrogen bonding. As a result, the reaction accessibility of chitosan was greatly enhanced.[26] So [Bmim]OH was used as the alkalization reagent of chitosan.

Regeneration of Utilized [Bmim]OH

The regenerated [Bmim]OH was used as a alkalization reagent to alkalize chitosan to prepare alkyl chitosan derivatives. The DS of alkylated chitosan prepared by means of the recycled [Bmim]OH are shown in Table II. From Table II, we could know that there was no noticeable change for the DS of alkylated chitosan after the ionic liquid was reused for three times. It is indicated that [Bmim]OH had excellent stability and could be recycled. However, during the regeneration process, the loss of [Bmim]OH could not be avoided. So the effect of [Bmim]OH alkalizing chitosan was decreased. As a result, the DS of the alkylated chitosan was slightly decreased. In order to keep the alkalization effect of [Bmim]OH, the [Bmim]OH

Table I. Effect of Different Alkalization Reagents on DS

	[Bmim]Cl	NaOH	[Bmim]OH
DS%	15.9	48.5	58.4

Table II. The DS of Alkylated Chitosan from Regeneration of [Bmim]OH as the Alkalization Reagent (n ([Bmim]OH): n (chitosan) = 3.0 : 1.0, at 45°C alkalized for 1 h, n (bromodecane): n (chitosan) = 3.0 : 1.0, at 80°C alkylated for 8 h)

	DS of the alkylated chitosan (%)		
Alkalization reagent	1	2	3
[Bmim]OH	78.4	78.6	78.3
the regenerated [Bmim]OH	75.6	75.5	75.2

should be regenerated when the DS of the alkylated chitosan was decreased by 10%. Before the regeneration, the [Bmim]OH was reused seven times in the experiment.

The Antibacterial Activities of Derivatives

The viscosities and MIC values of CTS, E-CTS, B-CTS, D-CTS, and C-CTS were listed in Table III. The results showed that CTS had the lowest antimicrobial activity against the tested bacteria under the experimental condition primarily due to its poor solubility in the neutral condition. Compared with CTS, the viscosities of alkylated CTS with different DS and different carbon chain substituents were decreased. As a result, the antibacterial activities of alkylated CTS were greatly increased. From the Table III, we could know that the antibacterial activities of E-CTS, B-CTS, D-CTS, and C-CTS against *S. epidermidis* and *S. aureus* were much better than that against *K. pneumoniae, P. aeruginosa, E. aerogenes,* and *E. coil.* The fact may be attributed to their different cell walls. *S. epidermidis* and *S. aureus* are Gram positive bacteria, and their cell walls are fully composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to enter the cell without difficulty. But the *K. pneumoniae, P. aeruginosa, E. aerogenes,* and *E. coil* are Gram negative bacteria, the cell walls of which are made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein and phospholipids. As a result of the bilayer membrane structure, the outer membrane becomes a potential barrier against foreign molecules.

From Table III, we could know that the antimicrobial activity of compound against *E. coli* was superior to commercially available fungicides Bromogeramine, and its MIC was 64 μg mL^{-1}. The antimicrobial activities of compounds a, b, and c against *P. aeruginosa* were much better than that of ampicillin sodium against the bacteria, and their MIC were 128, 128, and 256 μg mL^{-1}, respectively. In Table III, the effect of the derivatives DS on antibacterial activity was preliminarily investigated with D-CTS as example. With the increasing of the derivatives DS, the replacement degree of the positively charged amino-group in the chitosan material was increased, and the electropositivity of the chitosan molecule was decreased, so the MIC of the compound against the bacteria was also increased, and the antibacterial activities of the derivatives against the bacteria was degraded. This is consistent with the reported literature that chitosan containing -NH_2 has antibacterial effect.[27]

From investigating the antibacterial activities of E-CTS, B-CTS, D-CTS, and C-CTS with the same DS, we could know that the MIC was decreased with the growing of the alkyl substituent carbon chain introduced into the chitosan molecules. It is because the dissolution performances of derivatives were decreased with the growth of the carbon chain, and the difficulty for the positively charged derivatives to enter the bacterial cell interior for affecting the negatively charged DNA increased. So the antibacterial activities were decreased.

CONCLUSIONS

The basic ionic liquid [Bmim]OH was used to replace the traditional alkalization reagent for preparing the alkylated chitosan. The reusability of the ionic liquid was also investigated, and the experimental results indicated that there was no noticeable change for the DS of alkylated chitosan after the ionic liquid was reused for three times. The antibacterial activities of alkylated chitosan against *S. epidermidis* and *S. aureus* were much better than that against *K. pneumoniae, P. aeruginosa, E. aerogenes,* and *E. coil.* The antibacterial activity of E-CTS against *E. coil* was much better than that of Bromogeramine against the same bacteria, and its MIC was 64 μg mL^{-1}. The antibacterial activities of the compounds E-CTS, B-CTS, and D-CTS against *P. aeruginosa* were superior to the commercially

Table III. Antibacterial Activities of Compounds a-d (μg·mL^{-1})

Compounds	DS%	Viscosity(mpa·s)	A	B	C	D	E	F
c D-CTS	35.3	19.1	128	32	32	256	256	>256
c D-CTS	51.5	20.7	128	64	64	256	>256	>256
c D-CTS	64.4	29.3	>256	128	128	256	>256	>256
c D-CTS	77.2	37.9	>256	>256	256	>256	>256	>256
CTS	—	40.3	>256	256	256	>256	256	>256
a E-CTS	51.8	17.4	64	32	16	128	256	256
b B-CTS	50.7	18.7	128	64	32	128	256	>256
d C-CTS	50.5	23.6	256	128	256	>256	>256	>256
bromogeramine	—	—	256	8	8	8	128	64
ampicillin sodium	—	—	64	4	8	>256	>256	64

A, *E. coil*, B, *S. aureus*, C, *S. epidermidis*, D, *P. aeruginosa*, E, *E. aerogenes*, F, K. pneumonia.

available ampicillin sodium, and their MICs were 128, 128, and 256 $\mu g\ mL^{-1}$, respectively. Meanwhile, the antibacterial activities of alkylated chitosans were decreased with the growth of the DS or the growth of the carbon chain.

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REFERENCES

1. Tang, H.; Zhang, P.; Kieft, T. L.; Ryan, S. J.; Baker, S. M. *Acta Biomater* **2010**, *6*, 2562.
2. Fu, R. H.; Liu, S. P.; Ou, C. W.; Huang, C. M.; Wang, Y. C. *Acta Biomater* **2010**, *6*, 3621.
3. Souza, R. D.; Zahedi, P.; Allen, C. J.; Micheline, P. M. *Biomaterials* **2009**, *30*, 3818.
4. Badawy, M. E. I.; Rahea, E. I.; Rogge, T. M.; Stevens, C. V.; Smagghe, G.; Steurbaut, W.; HÖfte, M. *Biomacromolecules* **2004**, *5*, 589.
5. Stenger, F. C.; Cechinel-Filho, V.; Meyre-Silva, C.; Bresolin, T. M. B.; Rodrigues, C. A.; *Chromatographia Supplement* **2009**, *69*, 183.
6. Chen, D. M.; Li, W.; Wu, Y. R.; Zhu, Q.; Lu, Z. J.; Du, G. X. *Chem. Eng. J.* **2013**, *221*, 8.
7. Wang, Z. D.; Zheng, L. C.; Li, C. C.; Zhang, D.; Xiao, Y. N.; Guan, G. H.; Zhu, W. X. *Carbohydr. Polym.* **2013**, *94*, 505.
8. Verheul, R. J.; Amidi, M.; Van der Wall, S.; Van Ribt, E.; Jiskoot, W.; Hennink, W. E. *Biomaterials* **2008**, *29*, 3642.
9. Diab, M. A.; El-Sonbati, A. Z.; Al-Halawany, M. M.; Bader, D. M. D. *Open J. Polym. Chem.* **2012**, *2*, 14.
10. Croisier, F.; Jérôme, C. *Eur. Polym. J.* **2013**, doi: 10.1016/j.eurpolymj.2012.12.009.
11. Robles, E.; Villar, E.; Alatorre-Meda, M.; Burboa, M. G.; Valdez, M. A.; Taboada, P.; Mosquera, V. *J. Appl. Polym. Sci.* **2013**, doi: 10.1002/app.38870.
12. Peng, Y. F.; Han, B. Q. *Carbohydr. Res.* **2005**, *340*, 1846.
13. Wang, A. Q.; Yu, X. D. *J. Funct. Polym.* **1998**, *11*, 83.
14. Yue, C. B.; Fang, D.; Liu, L.; Yi, T. F. *J. Mol. Liq.* **2011**, *163*, 99.
15. Wang, C.; Zhao, W. J.; Li, H. R.; Guo, L. P. *Green Chem.* **2009**, *11*, 843.
16. Xu, J. M.; Liu, B. K.; Wu, W. B.; Qian, C.; Wu, Q.; Lin, X. F. *J. Org. Chem.* **2006**, *71*, 3991.
17. Ranu, B. C.; Banerjee, S.; Jana, R. *Tetrahedron* **2007**, *63*, 776.
18. Gupta, K. C.; Ravi Kumar, M. N. V. *Biomaterials* **2000**, *21*, 1115.
19. Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Roqers, R. D. *Green Chem.* **2001**, *3*, 156.
20. Brindaban, C. R.; Subhash, B. *Organic Lett.* **2005**, *7*, 3049.
21. Forbes, D. C.; Weaver, K. J. *J. Mol. Catalysis A: Chem.* **2004**, *214*, 129.
22. Zhang, S. G.; Zhang, Q. L.; Zhang, Z. C. *Ind. Eng. Chem. Res.* **2004**, *43*, 614.
23. Tang, Y. L.; Shi, Y. H.; Zhao, W.; Hao, G.; Le, G. W. *Food Control.* **2009**, *20*, 149.
24. Andrews, J. M. *J. Antimicrobial Chemotherapy.* **2001**, *48*, 5.
25. Domard, A.; Rinaudo, M.; Tarassin, C. *Int. J. Biol. Macromol.* **1986**, *8*, 105.
26. Zhang, X. X.; Zhang, W.; Tian, D.; Zhou, Z. H.; Lu, G. H. *RSC Adv.* **2013**, *3*, 7722.
27. Li, X. F.; Feng, X. Q. Fu, G. Q.; Yang, S.; Su, Z. X. *Nat. Product Res. Dev.* **2010**, *22*, 373.