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Modification of chitosan with monomethyl fumaric acid in an ionic liquid solution

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ABSTRACT

Antibacterial and antioxidant monomethyl fumaric acid (MFA) was selected to modify chitosan, using aqueous solution of an ionic liquid as a homogeneous and green reaction media. The chemical structures of resulting polymers were systematically characterized by ^{1}H NMR, diffusion ordered spectroscopy, solid ^{13}C NMR and wide-angle X-ray diffraction. The results show that two kinds of MFA modified chitosan materials with totally different chemical structures have been synthesized. One product was a MF-chitosan salt composed of chitosan cation and MFA anion, which was obtained with the mediation of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide. The other one synthesized with the mediation of EDC was a MF-chitosan amide in which MFA and chitosan are covalently attached. Solubility of chitosan has been improved, and MF-chitosan salt can be readily dissolved in water. The antioxidant activity has been enhanced with the introduction of MFA, irrespective of the chemical structure.

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1. Introduction

Chitosan, the linear cationic (1-4)-2-amino-2-deoxy-β-D-glucan is industrially produced from marine chitin (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Both polysaccharides are attracting attention owing to their characteristic properties, among which are antibacterial activity, biocompatibility and absence of allergenicity (Busilacchi, Gigante, Mattioli-Belmonte, & Muzzarelli, 2013; Muzzarelli, 2009; Muzzarelli, 2010; Muzzarelli et al., 1990). Unfortunately, due to the strong intramolecular and intermolecular hydrogen bonds (Hirase, Higashiyama, Mori, Takahara, & Yamane, 2010; Martino, Pollet, & Avérous, 2011; Qian & Zhang, 2010; Wu et al., 2004), it is insoluble in common organic solvents except acidic solution, and the antibacterial activity of chitosan is limited to acidic condition.

To overcome these challenges, various chitosan derivatives, such as hydroxyethyl chitosan and carboxymethyl chitosan, have been prepared (Feng & Xia, 2011). Though the solubility of chitosan has been effectively improved, the antibacterial activity of chitosan has been reduced in different extents. Therefore, antibacterial functional groups, such as quaternary ammonium (Qin et al., 2004) and guanidyl (Cai, Sun, Yang, & Zhu, 2012), have been introduced into the molecular chains of chitosan to compensate the loss in antibacterial properties: though the antibacterial activity of chitosan has been effectively enhanced (Xu et al., 2011; Cai, Sun, Zhu, Zhao, & Yue, 2013), the substitution degree of the functional groups shouldn't be high because of their toxic nature. For example, it has been reported that the hydroxypropyltrimethyl ammonium chloride chitosan with a substitution degree of 44% has cytotoxicity and poor compatibility (Peng et al., 2010).

Monomethyl fumaric acid (MFA) is an antibacterial agent with wide antibacterial spectrum, powerful antioxidant activity and nontoxicity. In this work, MFA was chosen to improve the solubility and antioxidant activity of chitosan while retaining the antibacterial activity.

Feng and Xia (2011) employed antibacterial fumaric acid to modify chitosan. Both the solubility and antibacterial activity of chitosan have been improved by the introduction of fumaryl group. It is a pity that H_2SO_4 has been used as the reaction media and protective agent of amino groups, causing serious pollution.

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On the other hand, ionic liquids, as a new generation of green solvents, have attracted increasing attention recently owing to their low vapor pressure and tunable solubility by varying the structures of cations and anions (Klingshirn, Broker, Holbrey, Shaughnessy, & Rogers, 2002; Zhao, Jackson, Song, & Olubajo, 2006). Recently, it has been reported that some ionic liquids or their aqueous solutions can successfully dissolve chitosan and chitin (Barber, Griggs, Bonner, & Rogers, 2013; Li et al., 2012; Xiao, Chen, Wu, Wu, & Dai, 2011). Therefore, it provides a brand-new platform for the utilization of chitosan. Furthermore, the breaking tenacity of chitosan fiber spun from aqueous solution of an ionic liquid is reported to be four times higher than that from acetic acid system (Li et al., 2012). However, very limited information is available on the modification of chitosan or chitin in ionic liquids, except the acetylation of α-chitin (Mine, Izawa, Kaneko, & Kadokawa, 2009) and graft of chitosan with polycaprolactone (PCL) performed by our group (Wang et al., 2013).

In this work, chitosan was modified by MFA, which was mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) with or without *N*-hydroxysuccinimide (NHS), using aqueous solution of an ionic liquid as a homogeneous and green reaction media.

The chemical structures of the polymers were systematically characterized by nuclear magnetic resonance spectroscopy (NMR), diffusion ordered spectroscopy (DOSY), and wide-angle X-ray diffraction (WAXD). Solubility and antioxidant activity of MFA modified chitosan have been studied by solubility test and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and correlated with their structures.

2. Materials and methods

2.1. Materials

Chitosan (M_w = 52 kDa) with a degree of deacetylation of 85% was bought from Golden-shell Biochemical Co. Ltd. (Zhejiang, China) and dried in vacuum at 60 °C for 12 h before use. MFA and EDC were purchased from Adamas (Switzerland). NHS was bought from Alfa Aesar (USA). 1-sulfobutyl-3-methylimidazolium trifluoromethanesulfonate ([BSMIM]CF_3SO_3) was obtained from Chengjie Chemical Reagent Co. Ltd. (Shanghai, China). DPPH was supplied from Sigma-Aldrich (USA). All the other chemicals and solvents were analytical grade and used as received without further purification.

2.2. Synthesis of MF-chitosan salt and MF-chitosan amide

MF-chitosan salt, whose structure will be confirmed by ^{1}H NMR and DOSY later on, is the product from the reaction of MFA with chitosan in the presence of EDC and NHS, while MF-chitosan amide is the product with the mediation of EDC.

Typically, 0.2 g of chitosan was dissolved in 10 mL of [BSMIM]CF_3SO_3 aqueous solution (4 wt%) to obtain the chitosan solution. Predetermined amount of MFA was dissolved in 10 mL of distilled water. After complete dissolution of MFA, predetermined amount of EDC and NHS were added in sequence. Fifteen minutes later, this solution was dripped into the above chitosan solution. The reaction mixture was stirred for 24 h at 25 °C. The resulting polymer of MFA-chitosan salt was dialyzed using dialysis membrane (MWCO: 8–14 kDa) against distilled water for 72 h followed by freeze-drying to remove the EDC, NHS, MFA and side products.

For the synthesis of MF-chitosan amide, whose structure will be testified by ^{1}H NMR, DOSY and ^{13}C NMR later on, only EDC was added into the chitosan solution and pH of the reaction system was adjusted to 6.

2.3. Characterization

2.3.1. NMR

^{1}H NMR and solid ^{13}C NMR were performed on Bruker 400 MHz and 600 MHz instruments at ambient temperature, respectively. D_2O/CF_3COOD (95:5; v/v) was used as the solvent for chitosan and MF-chitosan amide, and D_2O was used as the solvent for MFA and MF-chitosan salt.

A Bruker AVANCE 600 NMR spectrometer with a TBO probe using stebpgp1s pulse sequence was used to conduct the DOSY experiments. The big delta time Δ was 0.15 s. The little delta δ was 9.2 ms. Diffusion coefficient (D) for each sample was obtained by fitting each curve using the equation below:

$$I = I_0 \exp(-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)) \quad (1)$$

where, I is signal intensity, I_0 is the reference intensity, D is diffusion coefficient, γ is the gyromagnetic ratio of the observed nucleus, g is the gradient strength, the little delta δ is the length of the gradient and the big delta Δ is the diffusion time.

2.3.2. Wide-angle X-ray diffraction (WAXD) analysis

WAXD was obtained on a Ragaku Model D/max-2B diffractometer using Cu-Kα radiation (40 kV, 200 mA) at room temperature. The scanning region was 3–60°; the scanning rate was 4° min^{-1}.

2.4. Solubility test

Five milligram of chitosan or derivative sample was dispersed in 5 mL of distilled water or organic solvents and stirred at room temperature for 24 h.

2.5. Antioxidant test

The radical scavenging activity was evaluated using DPPH according to the references (Chen et al., 2010; Tang et al., 2013). DPPH was dissolved in the mixed solvent of ethanol and distilled water (1:1; v/v) to obtain a concentration of 0.1 mmol/L. 1.0 mL of sample solution (0.2 mg/mL) was added into 2.0 mL of DPPH solution. The solution was left for 30 min at room temperature in the dark. Absorbance was measured with a UV-Vis spectrophotometer (Hitachi, Tokyo, Japan) at 517 nm. DPPH radical scavenging ratio was calculated as an inhibition percentage based on the following equation:

$$\text{Radical scavenging ratio } (\%) = (1 - A_{\text{sample}}/A_0)100 \quad (2)$$

where, A_{sample} is the absorbance of the unmodified or modified chitosan, A_0 is the absorbance of the solvent.

3. Results and discussion

3.1. Synthesis and NMR analysis

The ^{1}H NMR spectra and the corresponding assignments of chitosan, MF-chitosan salt, MF-chitosan amide and MFA are shown in Fig. 1. It can be found that the ^{1}H NMR spectra of MF-chitosan salt and MF-chitosan amide demonstrate all the characteristic peaks belonging to chitosan. Furthermore, the signal at 3.82 ppm for H^{γ} of MFA can be observed in Fig. 1b and c. It should be mentioned that the resonance signal occurring at 6.87 ppm, originating from protons on carbon-carbon double bonds of fumaryl group in Fig. 1d, splits into two groups of peaks around 6.36 ppm and 6.66 ppm in Fig. 1b and c. Just one signal for H^{α} and H^{β} in the reactant is attributed to their very similar chemical environments. After linking with chitosan, the difference in chemical environments of H^{α} and H^{β} has been expanded, and thus two groups of peaks are

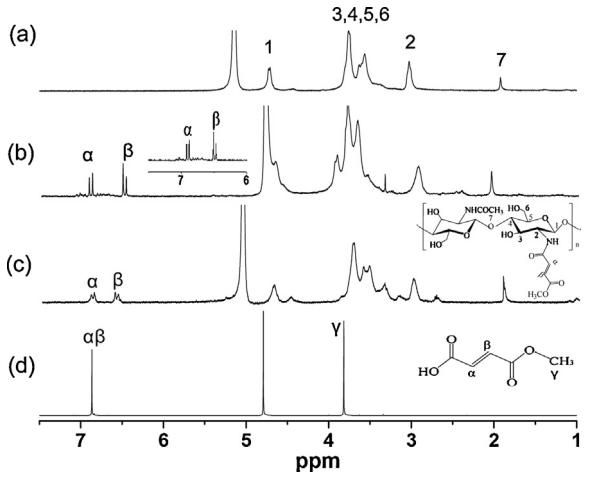

Fig. 1. ^{1}H NMR of chitosan (a), MF-chitosan salt at degree of complex of 0.17 (b), MF-chitosan amide at degree of substitution of 0.20 (c) and monomethyl fumaric acid (d).

resulted. Similar results have been observed by Feng and Xia (2011) for the *O*-fumaryl-chitosan.

Based on the above results, it can be safely concluded that ^{1}H NMR spectra of MF-chitosan salt and MF-chitosan amide show all the characteristic signals of chitosan and MFA residue, and MF-chitosan salt and MF-chitosan amide are not simple mixtures of chitosan and MFA. However, whether the structure is salt (also known as complex) or covalent bonded compound is still unknown.

Diffusion ordered spectroscopy (DOSY) is a pseudo two-dimensional spectrum that displays chemical shifts along the horizontal axis and diffusion coefficients (D) along the vertical dimension (Cohen, Avram, & Frish, 2005; Macchioni, Ciancaleoni, Zuccaccia, & Zuccaccia, 2008; Pregosin, Kumar, & Fernandez, 2005). Recently, DOSY has been proved to be a useful and handy tool for analysis for a variety of complex mixtures (Balayssac et al., 2009; Han et al., 2012; Primikyri et al., 2012;) and aggregates (Hamdoun et al., 2014; Kotzé, Gerber, Wu, & Koch, 2013; Li, Hopson, Li, Liu, & Williard, 2008; Li, Sun, et al., 2008; Li, Keresztes, Hopson, & Williard, 2009) as well as for the study of the hydrogen bonds between chitosan and poly(vinyl alcohol) (Lejardi et al., 2014). It can resolve diffusion data for each component and permits a fingerprint of complex mixture to be obtained quickly.

In order to characterize the structure more accurately, DOSY experiments were carried out on MFA, MF-chitosan salt and MF-chitosan amide. The purpose of this measurement was to determine the average value of D of every group. Since protons can be classified and assigned according to D, these results can be correlated with the components of these systems.

Fig. 2 shows DOSY spectra of MFA, MF-chitosan salt and MF-chitosan amide. And the values of D have been calculated and summarized in Table 1. It can be found that H^{α}, H^{β} and H^{γ} in MFA have the same value of D of $5.8e^{-10}$. In contrast, H^{α}, H^{β} and H^{γ} in MF-chitosan salt demonstrate evidently slower D around $3.2e^{-10}$. It suggests that there is some interaction between MFA and chitosan, which restricts the mobility of H^{α}, H^{β} and H^{γ}. However, there is evident difference between values of D for the protons of MFA and chitosan in MF-chitosan salt. The latter has evidently slower D ranging from $3.3e^{-11}$ to $3.7e^{-11}$. If the two components are linked by covalent bond, they will shift simultaneously in the solution and have the same value of D. Therefore, it can reach a conclusion that MF-chitosan salt is not a compound linked by amide bond, but a complex of chitosan and MFA, which dissociates into chitosan cation and MFA anion in solution, as displayed in Fig. 3.

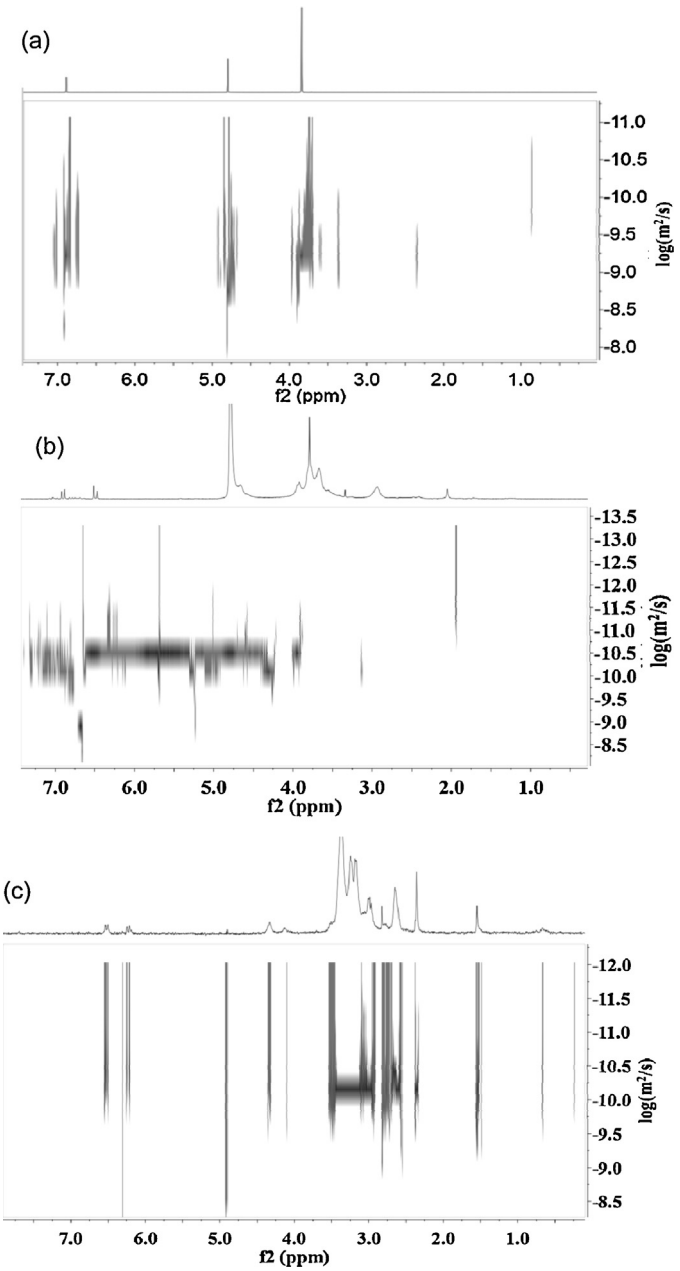

Fig. 2. DOSY spectra of monomethyl fumaric acid (a), MF-chitosan salt at degree of complex of 0.17 (b), and MF-chitosan amide at degree of substitution of 0.20 (c).

It is known that chitosan, with a repeating unit of glucosamine, which possesses unique polycationic nature in acidic condition, can form complex with anions. For example, Cai, Jiang, Chen, et al. (2009); Cai, Jiang, Tu, Wang, and Zhu (2009) prepared sodium dodecyl sulfate–chitosan complex (SCC), which substantially enhances the solubility of chitosan in organic solvents. And it

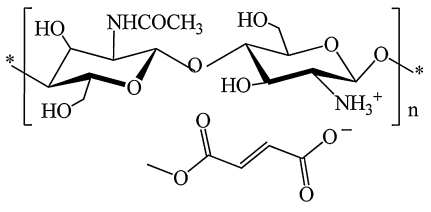

Fig. 3. Chemical structure of MF-chitosan salt.

Table 1
Diffusion coefficient (D) of the various groups in MFA, MF-chitosan salt and MF-chitosan amide.

D (m^2/s)	$H(^{\alpha}$	$H(^{\beta}$	H^1	H ($^{\gamma}$ or ($H^{\gamma}+H^{3-6}$)	H^2
Chemical shift (ppm)	7.1–6.8	6.7–6.5	4.7–4.4	4.2–3.1	3.1–2.8
D (MFA)	$5.8e^{-10}$	–	$5.8e^{-10}$	–	
D (MF-chitosan salt)	$3.3e^{-10}$	$3.2e^{-10}$	$3.3e^{-11}$	$3.7e^{-11}$	$3.7e^{-11}$
D (MF-chitosan amide)	$4.7e^{-11}$	$3.6e^{-11}$	–	$6.4e^{-11}$	$5.5e^{-11}$

MFA, monomethyl fumaric acid.

Table 2
Synthesis data and results of MF-chitosan salt under different conditions.

Sample	MFA/chitosan (mol/mol)	EDC/NHS/MFA (mol/mol/mol)	t (h)	T (°C)	DC
1	0.5	0/0/1	24	25	0
2	0.5	1.5/1.5/1	24	25	0.07
3	0.7	1.5/1.5/1	24	25	0.08
4	1.0	1.5/1.5/1	24	25	0.17

MFA, monomethyl fumaric acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NHS, *N*-hydroxysuccinimide; DC, Degree of complex: determined by ^{1}H NMR.

should be noted that SCC is a good intermediate for regioselectively grafting polymers onto chitosan through hydroxyl groups.

Generally, it tends to form amide bonds through the coupling reaction between amino groups on chitosan and carboxyl groups on MFA mediated by EDC and NHS as indicated in Fig. 4. But in the present work, it fails to obtain the covalent linked derivative. The reasons may be interpreted as follows.

It has been reported that NHS activated ester is unstable under basic condition (Nakajima & Ikada, 1995; Nojima, Iguchi, Suzuki, & Sato, 2009; Zhang & Li, 2014). The hydrolysis rate of poly(ethylene glycol)-NHS is relatively slow at near neutral condition (pH 7.4), but it increases 34.2 fold under alkaline conditions (pH 9.0) (Nojima et al., 2009). The optimal pH for amide formation mediated by EDC-NHS is 4–6 (Dhar, Akhlaghi, & Tam, 2012; Nakajima & Ikada, 1995). But the present reaction system is basic with pH higher than 8 due to the inherent basic nature of EDC. Therefore, the NHS activated MFA (compound 2 in Fig. 4) is very unstable under this reaction condition. It hydrolyzes quickly into NHS and MFA, and loses catalytic activity. Consequently, it fails to form amide linked derivative, but a complex, composed of chitosan cation and MFA anion.

Degree of complex (DC) of MFA on chitosan has been calculated according to the following equation:

$$\mathrm{DC} = \frac{A_{6.4-7.0}/2}{(A_{3.4-4.2} - 1.5A_{6.4-7.0})/5} \quad (3)$$

where, $A_{6.4-7.0}$ denotes the integral areas of H^{α} and H^{β} originating from MFA, $A_{3.4-4.2} - A_{6.4-7.0}$ represents the integral areas of H^{3-6} arising from chitosan.

Influence of reaction conditions on DC has been studied and the results are given in Table 2. It can be found that DC is zero and no complex is found without the mediation of EDC and NHS, implying that EDC and NHS have some catalytic activity toward the formation of salt based on chitosan and MFA. Furthermore, DC increases from 0.07 to 0.17 with increasing feed ratio of MFA to chitosan from 0.5 to 1.0.

Therefore, the second strategy was adopted to synthesize the amide bonded compound based on chitosan and MFA. Only EDC was used as the media, and pH of the reaction system was adjusted to 6 by the ionic liquid. The resulting compound was also characterized by DOSY, and the results are revealed in Fig. 2c and Table 1. It can be found that the values of D for the protons of chitosan and MFA in MF-chitosan amide have the same order of magnitude and no evident difference between the absolute values has been found. Thus, it can be safely concluded that MF-chitosan amide is a covalently bonded compound.

The chemical structure of MF-chitosan amide was further confirmed by ^{13}C NMR spectrum. Due to the limited solubility of MF-chitosan amide in CF_3COOD/D_2O, it is very hard to obtain high concentrated solution for the ^{13}C NMR measurement. As an alternative, solid ^{13}C NMR was employed to characterize the chemical structure of MF-chitosan amide. The small signals occurring at 134.7 ppm (δC^{10}) and 131.4 ppm (δC^{11}) (Fig. 5) belong to carbon atoms on carbon–carbon double bonds of fumaryl group. The signals locating at 169.2 ppm (δC^{9}) and 166.4 ppm (δC^{12}) originate from carbonyl carbon atoms of fumaryl group. Meanwhile, the very typical resonance signals around 97.2 ppm (δC^{1}) and 76.3 ppm (δC^{2-5}) are assigned to carbon atoms on glucosamine of chitosan. The characteristic signals at 22.1 ppm (δC^{8}) and 170.8 ppm (δC^{7}) evidence the residue of acetyl groups of chitosan in the derivative. Thus, the solid ^{13}C NMR spectrum of MF-chitosan amide reveals all the characteristics signals belonging to chitosan and MFA.

Table 3
Synthesis data and results of MF-chitosan amide under different conditions.

Sample	MFA/chitosan (mol/mol)	EDC/MFA (mol/mol)	DS
1	0.5	1.5	0.13
2	1	1.5	0.20
3	1.5	1.5	0.32
4	0.5	3	0.18
5	1	3	0.34
6	0.5	5	0.19

MFA, monomethyl fumaric acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; DS, degree of substitution: determined by ^{1}H NMR.

Therefore, it can be undoubtedly concluded that MFA has been successfully introduced to the molecule of chitosan and the two units are linked by amide bond in MF-chitosan amide.

The reaction mechanism is depicted in Fig. 6. First, MFA reacts with EDC and EDC activated MFA (MFA-EDC) is formed. Then MFA-EDC reacts with chitosan and amide bonded MF-chitosan amide is produced. Degree of substitution (DS) of MFA on chitosan has been calculated from ^{1}H NMR according to the following equation:

$$\mathrm{DS} = \frac{A_{6.4-7.0}/2}{(A_{3.4-4.2} - 1.5A_{6.4-7.0})/5} \quad (4)$$

where, $A_{6.4-7.0}$ denotes the integral areas of H^{α} and H^{β} originating from MFA, $A_{3.4-4.2} - A_{6.4-7.0}$ represents the integral areas of H^{3-6} arising from chitosan.

Influence of synthesis parameters on DS of MF-chitosan amide has been investigated, and the results are summarized in Table 3. It can be found that MF-chitosan amide with DS ranging from 0.13 to 0.34 has been synthesized. DS of MF-chitosan amide increases linearly with increasing the feed ratio of MFA to chitosan. This trend can be explained by the fact that the probability of

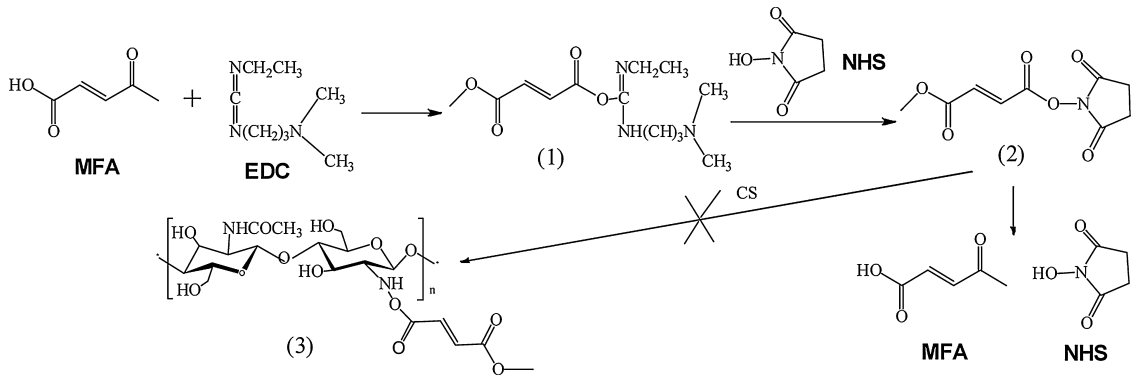

Fig. 4. Proposed reactions occurred during the synthesis of MF-chitosan salt.

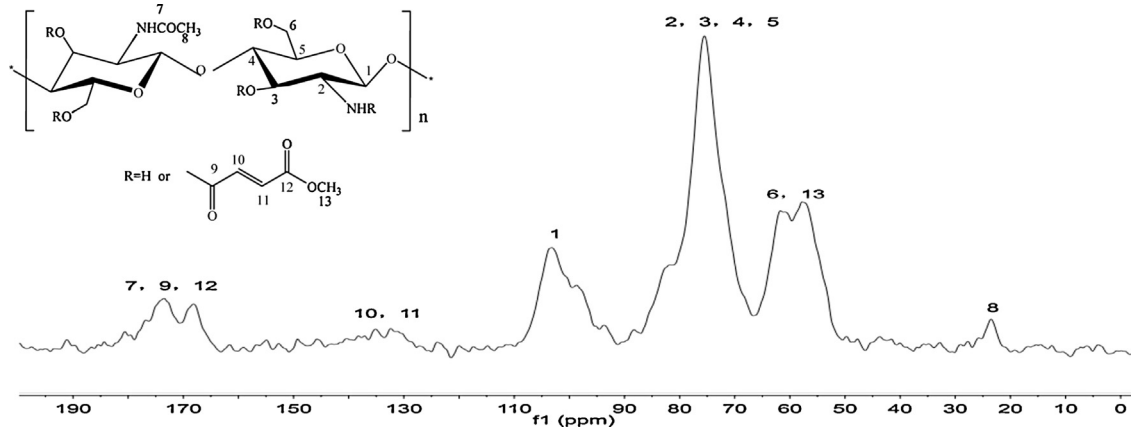

Fig. 5. Solid ^{13}C NMR of MF-chitosan amide at degree of substitution of 0.17.

collision and reaction between MFA molecules and D-glucosamine units increases when more MFA is fed. Similar results have been reported by Du, Lu, Zhou, Yuan, and Hu (2010) and Lin, Chen, Liu, Chen, and Chang (2011).

The EDC is a significant media for the coupling reaction between MFA and chitosan, and the influence of EDC on DS has also been studied. As indicated in Table 3, DS increases evidently with increasing molar ratio of EDC to MFA before it achieves a plateau level at a molar ratio of 3. It can be ascribed to the fact that with increasing EDC ratio, the reaction between MFA and EDC tends to be complete. The plateau level at higher EDC level is also related with the hydrophobic nature and low solubility of EDC in water.

As compared with the acetylation of α-chitin with acetic anhydride (Mine et al., 2009) and graft of chitosan with PCL (Wang et al., 2013), there are several advantages of the present work. First, pure ionic liquids are used as solvents in both cases and dissolution time reaches up to 24 h or 6 h. The employment of aqueous solution of the ionic liquid in the present work largely reduces the production cost and shortens dissolution time (dissolution time is 0.5 h). Meanwhile, large excess of acetic anhydride or ε-caprolactone (5–50 equiv. for repeating unit of chitin or chitosan) is needed for previous modification. But only 0.5–1 equiv. amount of MFA is used in the present work.

3.2. Wide-angle X-ray diffraction (WAXD)

WAXD experiments were further performed to study the effect of introduction of MFA on the crystallinity. The WAXD pattern of chitosan is also given for comparison. The corresponding results are summarized in Fig. 7. Evidently, chitosan shows two sharp diffraction peaks at *ca* 11.0° and 20°, attributing to crystal form I and crystal form II of the rhombic crystal of chitosan (Duan, Dong, Yuan, & Yao, 2004; Dung, Milas, Rinaudo, & Desbrieres, 1994; Zhang, Ping, Zhang, & Shen, 2003). In contrast, the intensities of the diffraction

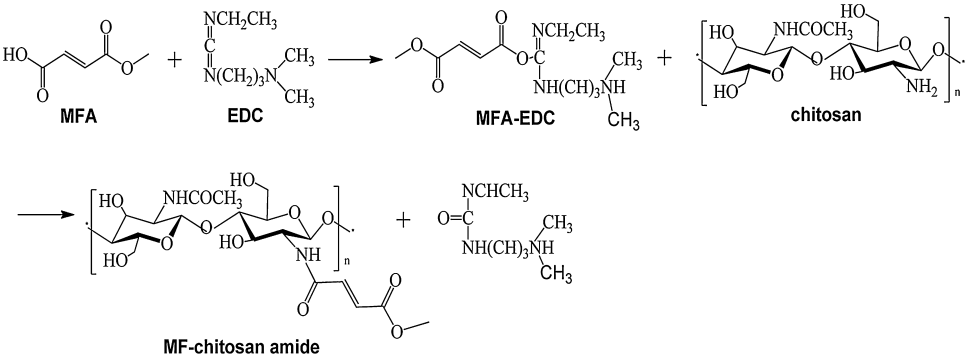

Fig. 6. Proposed reaction mechanism of MF-chitosan amide.

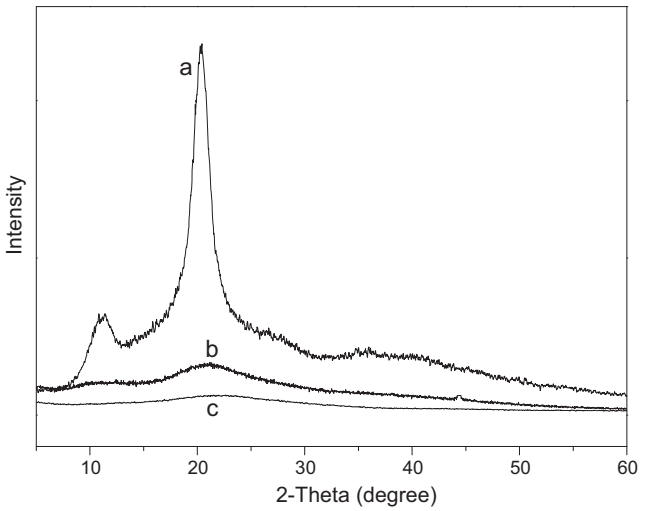

Fig. 7. WAXD patterns of chitosan (a), MF-chitosan salt at degree of complex of 0.17 (b) and MF-chitosan amide at degree of substitution of 0.20 (c).

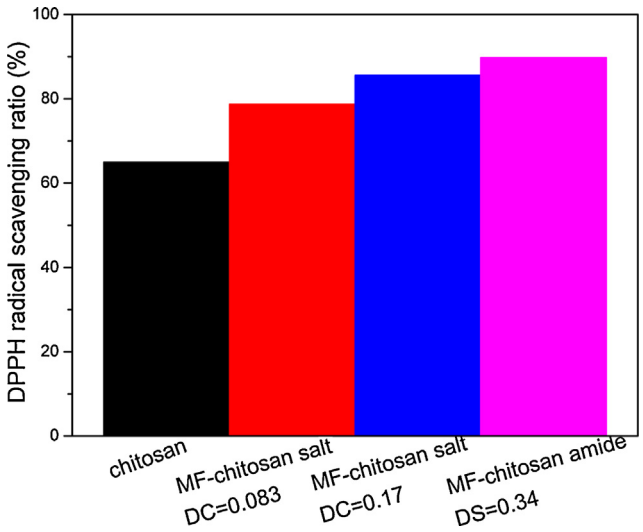

Fig. 8. DPPH scavenging activities of chitosan, MF-chitosan salt and MF-chitosan amide.

Table 4
Solubility of chitosan, MF-chitosan salt and MF-chitosan amide in different solvents.

Sample	H_2O	DMSO	DMF	$CHCl_3$	Toluene	Acetic ester
Chitosan	–	±	–	–	–	–
MF-chitosan salt DC = 0.17	+	±±	±±	±	±	±
MF-chitosan amideDS = 0.18	±±	±±	±±	±	±±	±

+, Soluble; ±±, partially soluble or highly swelled; ± swelled; – insoluble; DC, degree of complex; DS, degree of substitution.

peaks for MF-chitosan salt and MF-chitosan amide are much lower, implying significantly reduced crystallinity. These results can be ascribed to the facts that the introduction of MFA breaks the hydrogen bonds and destructs the ordered crystal structure of chitosan.

Generally, the formation of complex or covalent bond decreases the crystallinity of chitosan. Decreased crystallinity has also been observed for the acetylated chitosan (Liu, Zhou, Wang, Xu, & Sun, 2013), *N*-succinyl-chitosan (Zhu, Chen, Yuan, Wu, & Lu, 2006), and chitosan-metal complexes (Pornsunthorntawee, Katepetch, Vanichvattanadecha, Saito, & Rujiravanit, 2014).

3.3. Solubility

It is well-known that it is extremely difficult to dissolve chitosan in common solvents except acid solution due to the high density of hydrogen bonds. One of the purposes of this work is just to improve the solubility of chitosan. Therefore, the solubility of MF-chitosan salt and MF-chitosan amide has been investigated and the results have been presented in Table 4. Chitosan is insoluble in H_2O and most organic solvents. In contrast, MF-chitosan salt can be readily dissolved in distilled water and swelled by various organic solvents.

As far as MF-chitosan amide is regarded, though it cannot be dissolved completely in H_2O, it can be swelled by most organic solvents. These results suggest that solubility of chitosan has been effectively improved by the introduction of MFA and solubility increases in the order of MF-chitosan salt > MF-chitosan amide > chitosan.

As compared to acetylated chitin (Mine et al., 2009) or PCL grafted chitosan (Wang et al., 2013), MF-chitosan salt has an evident advantage of solubility, since it has been reported that only acetylated chitin with DS higher than 1.8 can be dissolved in DMSO, while all the PCL grafted chitosan is organic swellable.

The improved solubility is attributed to the fact that MFA breaks hydrogen bonds, lowers the density of hydrogen bonds and reduces the crystallinity of chitosan, as confirmed by WAXD. The difference between the solubility of MF-chitosan salt and MF-chitosan amide in water is originated from their different chemical structures. As discussed before, the former is a complex, which disassociates into cation of chitosan and anion of MFA, and dissolves in water. The latter is a MF-chitosan amide in which the two units are linked by amide bonds. Since MFA residue is organic soluble, MF-chitosan amide cannot be dissolved completely in water, but swellable in organic solvents. Conversely, the different solubility of MF-chitosan salt and MF-chitosan amide further testifies the different chemical structure of the two materials.

3.4. Antioxidant activity

The antioxidant activity was measured by DPPH assay and expressed as radical scavenging ratio (%) (RS). The results are given in Fig. 8. It can be found that RS of any MF-chitosan salt or MF-chitosan amide is higher than that of unmodified chitosan due to the incorporation of antioxidant MFA. And the antioxidant activity increases regularly with increasing DS, irrespective of chemical structure. Thus, it can reach a conclusion that MFA enhances the antioxidant activity. However, only a moderate RS is obtained even at the DS of 0.34.

4. Conclusions

Antibacterial and antioxidant MFA was selected to modify chitosan and two kinds of chitosan materials have been synthesized with the mediation of EDC with or without NHS in aqueous solution of an ionic liquid. Chemical structures of the two materials were systematically characterized by ^{1}H NMR, DOSY, solid ^{13}C NMR and WAXD. The results show that a MF-chitosan salt, composed of chitosan cation and MFA anion, was prepared when EDC and NHS were used as the media. While a MF-chitosan amide, in which MFA and chitosan are linked by covalent bonds was synthesized using EDC as the media. MF-chitosan salt with DC ranging from 0.07 to 0.17, and MF-chitosan amide with DS varying from 0.13 to 0.34, have been successfully synthesized. Solubility of MF-chitosan salt or MF-chitosan amide is better than that of the native chitosan. MF-chitosan salt can be readily dissolved in the H_2O, but MF-chitosan amide just swells in H_2O. Antioxidant activity has been improved after modification with MFA. Due to the good solubility in water and enhanced antioxidant performance, MF-chitosan salt has a great

potential to be used in the field of ordinary life such as ingredients for food preservation and cosmetic.

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