

Homogeneous Acetylation of Chitosan in Ionic Liquids

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ABSTRACT: Homogeneous acetylation of chitosan was carried out with acetyl chloride in 1-allyl-3-methylimidazolium chloride (AmimCl) ionic liquid. The dissolution process of chitosan in AmimCl was observed by polarized optical microscopy. The optimal reaction conditions were achieved by screening the effect of temperature, reaction time, and acylating reagents on recovery yield of acetylated chitosan and degree of substitution, which was determined by infrared (IR) spectra analysis using baseline method. The acetylated chitosan could be separated easily by water precipitation followed by filtration. The acetylation process was further confirmed by IR spectra and scanning electron microscopy, and the crystal structure was changed after the acetylation detected by X-ray diffraction spectroscopy. Compared with traditional acetylation of chitosan, the homogeneous system, mild conditions, and simply workup make this methodology more accessible to the demand of sustainable green biomass chemistry. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 129: 28–35, 2013

KEYWORDS: catalysts; cellulose and other wood products; functionalization of polymers; ionic liquids

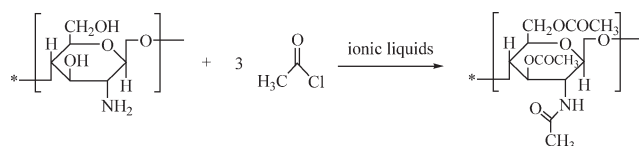
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INTRODUCTION

Nowadays, depleting nonrenewable resources have rendered a great crisis to our environment and human development. Therefore, people are increasingly making great efforts to exploit biomass such as natural polymer as the substitution of fossil resource.¹ For instance, polysaccharides widely existed in natural environment have been regarded as one of the most important renewable resources with the great potential as suppliers of energy.² Besides cellulose, chitin, the second most important natural polymer on earth, consisting of 2-acetamido-2-deoxy- β -D-glucose through a β (1 \rightarrow 4) linkage, is well known as biorenewable, biodegradable, and biofunctional natural mucopolysaccharide.³ Despite its huge annual production and easy accessibility, chitin is still an unutilized biomass resource and has not yet been developed on an industrial scale, primarily because of its intractable bulk structure, involved in the stiff molecules and close chain packing via numerous intermolecular and intramolecular hydrogen bonds,⁴ and this extremely debased its potential worth in practical applications.⁵ With regard to chitosan, the most important derivative of chitin, it is the *N*-deacetylated product of chitin although this *N*-deacetylation is almost never completed.⁶ Recently, chitosan has attracted much attention owing to its many important applications

(food, cosmetics, biomedical, and pharmaceutical applications) through a variety of physical and chemical processing and transformation.⁷ Derivation of chitosan perhaps is one of the most common and viable ways to improve its potential value owing to its multiple reactive sites, which can form up to hundreds of derivatives. For example, PEGylated chitosan derivatives are able to add new physicochemical properties to the cationic polysaccharide polymers, thereby overcoming some limitations, especially regarding their solubility and their use in drug and gene delivery (DNA and siRNA).⁸ Especially, acylated chitosan is one of the most common derivatives, and they are still growing species in the biological, medical, and other research and application fields.⁹ Up to now, product and property designed by chemical modification is limited on special hard conditions^{10–12} or heterogeneous conditions¹³ at least at the beginning of the conversion because of its rigid structure.¹ Although chitosan is soluble in dilute aqueous solutions of organic and mineral acids, an alkaline solution treatment process is necessary to remove the acid after dissolution.¹⁴ Anyway, the shortcomings of these processes including long reaction time, gelatin, complicated separation procedures, and the disadvantages of conventional solvents such as high volatility, severely contaminated, strongly causticity and the degeneration of chitosan resulted in



Scheme 1. Acetylation of chitosan with acetyl chloride in AmimCl IL.

environment pollution and energy consumption during these homogeneous functionalization of chitosan. Correspondingly, it violates inescapably the purpose of energy saving and restrains their wide applications and large-scale production. Therefore, the study to explore a new and direct green solvent for improving the acetylation of chitosan is of great significance.

Recently, ionic liquids (ILs) are in the focus of interests in various fields of research and development.¹⁵ Task-specific and biodegradable ILs have an attractive future in green chemistry because their utilization will greatly reduce the risk of environment. Furthermore, ILs, which are considered as desirable green solvents for great width of liquid range, excellent dissolution ability, low vapor pressure, and easiness to recycling, have been used to replace the organic solvents in many fields,¹⁶ such as dissolution of cellulose,¹⁷ hemicelluloses,¹⁸ wood powder,¹⁹ and all kinds of carbohydrates.²⁰ In view of the performance of good solubility and catalytic activity of ILs in biomass conversion, chitin/chitosan has also been introduced to intersection with ILs,²¹ including chitin/chitosan composite materials for gels and films,^{22–24} electrode,^{25,26} reversible sorbents of CO₂,²⁷ biosorbents for heavy metal ions,²⁸ and so on. However, very limited literatures about homogeneous chemical modification of chitin/chitosan have been reported by using ILs as solvents. For instance, Kadokawa and coworkers²⁹ published the acetylation

of α -chitin in 1-allyl-3-methylimidazolium bromide IL, and 1.86 of degree of substitution (DS) was achieved, but the detailed dissolution procedure was not yet reported. In addition, study on the homogeneous *N*-acylation of chitosan in aqueous ILs derived from natural amino acids has been reported by Ji.³⁰ However, the tedious workup treatments restricted the wide application of this method from the viewpoint of the energy efficiency.

During the course of preparation of high-value materials from biomass, we also found that imidazolium-based ILs have proved to be the excellent catalytic media and applied to many kinds of organic reactions owing to their inherent task-specific properties.³¹ This prompted us to investigate the homogeneous acetylation of chitosan in ILs in further research. We screened the acetylation of chitosan with acetyl chloride (Scheme 1) under different conditions in 1-allyl-3-methylimidazolium chloride (AmimCl) IL. Moreover, the structures of modified chitosan were checked by IR, scanning electron microscopy (SEM), and X-ray diffraction (XRD) spectroscopy. Unlike the previous studies on acetylation of chitosan,³² the reaction proceeded in homogeneous phase and in the absence of strong acids.

EXPERIMENTAL

Materials

All chemicals were analytical grade reagents and used as received without further purification. Chitosan powder (degree of deacetylation: 80.0–95.0%; Mw: 2.1×10^5 Da), allyl chloride, acetyl chloride, maleic anhydride, succinic anhydride, caprylyl chloride, and chloroacetyl chloride were purchased from Sino-pharm Chemical Reagent Beijing, Beijing. *N*-methylimidazole was supplied by J&K Chemical (in Beijing, China) and used as received.

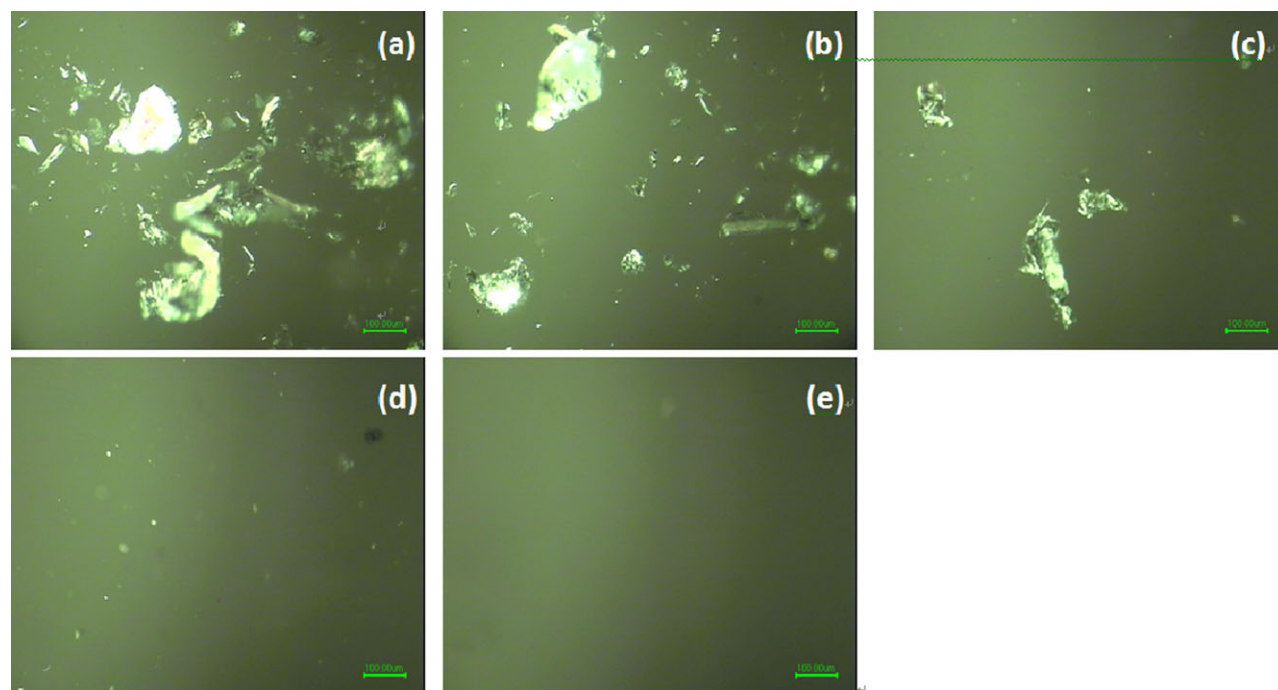


Figure 1. Polarized optical microscope images for dissolution experiment of 5 wt % chitosan with AmimCl at 85°C, (a) 0 h, (b) 1 h, (c) 4 h, (d) 8 h, and (e) 12 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

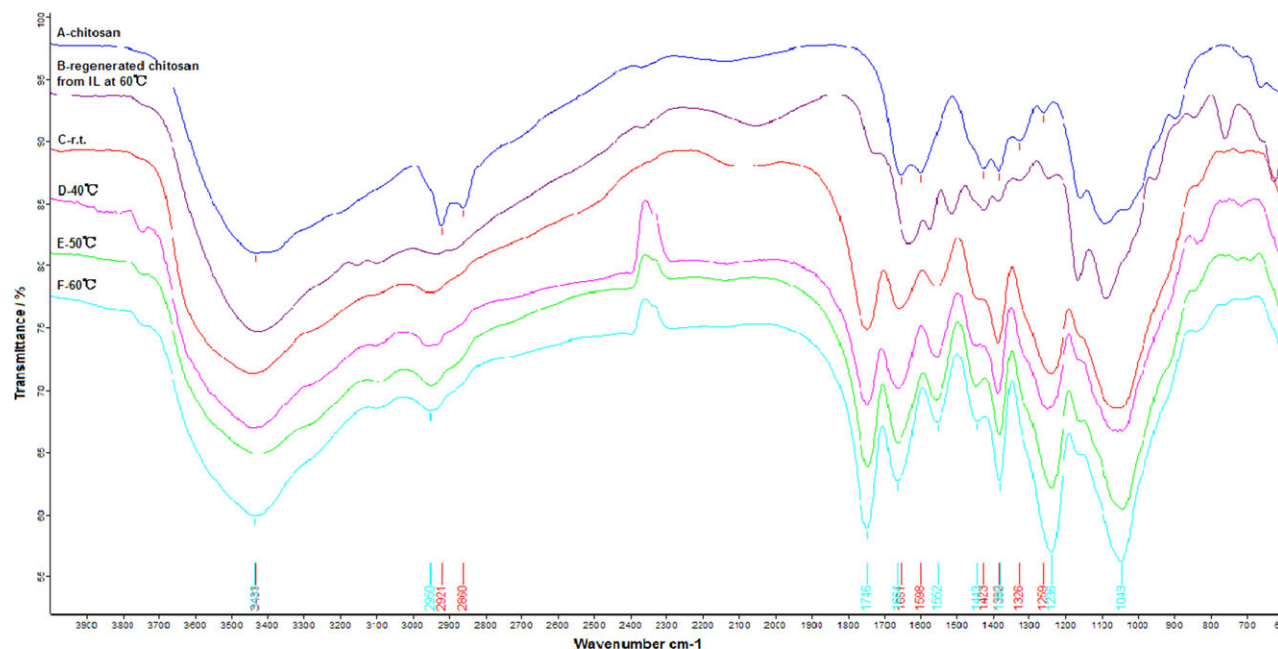


Figure 2. IR spectra of acetylated chitosan at different reaction temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Synthesis of AmimCl

AmimCl was prepared by reaction of *N*-methylimidazole with allyl chloride, according to the methods published in the previous literatures.³³ *N*-methylimidazole (100 mL) and allyl chloride (107 mL) at a molar ratio of 1 : 1.05 were added into a round-bottomed flask (500 mL), fitted with a reflux condenser for 8 h at 55°C with stirring to get amber viscous liquid. Ether (60 mL, three times) was added to wash out the unreacted chemicals and other impurities, and then the crude IL dissolved into CH₂Cl₂ was dried by anhydrous MgSO₄ for 2 h followed by the desiccant filtration and CH₂Cl₂ evaporation. Then, the anhydrous pure IL AmimCl was obtained after the treatment at 80°C under vacuum condition, which is slightly amber. The refractive index of AmimCl measured with an Abbe's refractometer is 1.5470.

Measurements

The structure of chitosan before and after reaction was identified with samples in KBr using Fourier transform infrared spectrometer (FTIR, Tensor 27) at room temperature by a pellet formed from 2 mg acetylated product and 100 mg of KBr. An Olympus-polarized optical microscopy was used to confirm the dissolution process of the polysaccharides in AmimCl. XRD measurements were conducted using a SHIMADZU XRD-6000 diffractometer in a $\theta/2\theta$ scan regime, with Ni-filtered Cu $K_{\alpha 1,2}$ radiation, step 0.04°, time per step 1 s and 2θ range 4–40°. SEM images were recorded on a Hitachi S-4100 electron microscope of the platinum-coated samples applying 5 kV acceleration voltage. DS determination was achieved by the quantitative determination of peak strength on IR spectra according to the baseline method.³⁴

Acetylation of Chitosan in AmimCl

A typical procedure for acetylation of chitosan was as follows: a mixture of chitosan (0.2 g, 1.2 mmol) in AmimCl (4.0 g, 25.2

mmol) was heated at 85°C for 12 h with stirring under nitrogen to dissolve chitosan. After the solution was cooled to room temperature, acetyl chloride (0.6 g, 7.5 mmol) was added and the mixture was heated at 60°C for 12 h. Then, three times of volume water was poured into the mixture to precipitate the acetylated chitosan, which was isolated by filtration, washed with ethanol, and dried under reduced pressure to give the product in 85.3% recovery yield.

Recycling and Reuse of AmimCl IL

After the acetylation, three times of volume water was poured into the reaction mixture to precipitate out the acetylated chitosan. After filtration, the filtrate containing [Amim]Cl was

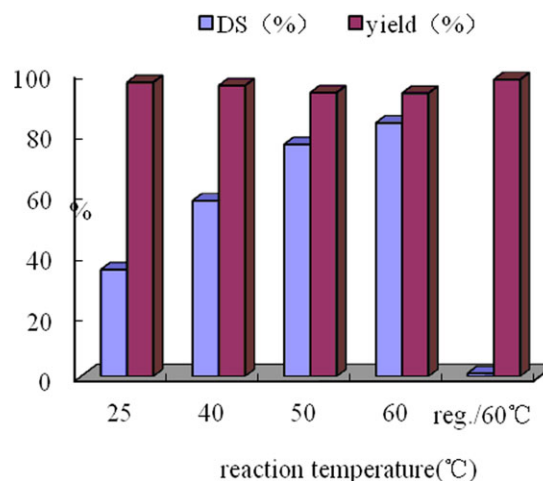


Figure 3. Effect of temperature on the acetylation of chitosan in AmimCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

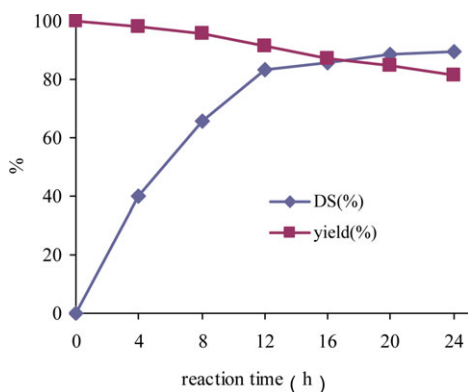


Figure 4. Effect of reaction time on the acetylation of chitosan in AmimCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

evaporated under reduced pressure at 80°C to get viscous liquid, which was then washed with diethyl ether and finally dried at 80°C under reduced pressure. After these treatments, the recovery IL was directly used for the next acetylation of chitosan.

RESULTS AND DISCUSSION

Dissolution of Chitosan in AmimCl IL

As for the dissolution of chitosan in IL, it is well known that the imidazolium-type ILs paired with a chloride counter anion such as 1-butyl-3-methylimidazolium chloride (BmimCl) were good solvents for carbohydrates, cellulose, wood, and so on, owing to the destroyed hydrogen bond network by Cl⁻.³⁵ This information has inspired us to survey the dissolution process of chitosan in AmimCl, which has the potential ability to interrupt the intramolecular and intermolecular hydrogen bonds of chito-

san, by reason of the lots of —NH₂ and —OH groups in the molecules.³⁶

As a consequence, the experiment was performed by addition of desired amounts of chitosan in IL at room temperature and heating the mixture at 85°C for desired time with stirring under an atmosphere of nitrogen. In view of the high degree of crystallinity, the dissolution process of chitosan in AmimCl can be simply observed by polarized optical microscopy. A drop of AmimCl contained with chitosan was sandwiched between two pieces of cover glasses and was viewed after certain time, and the monitored process of dissolution is shown in Figure 1.

As shown in Figure 1, it is clear that the solid crystal of chitosan still existed in IL after 1-, 4-, and 8-h dissolution at 85°C, but gradually diminished as time going on, and the clear liquid was formed till the dissolution process was carried out for 12 h. The results of the above observation suggested that 5 wt % chitosan solvated with AmimCl occurred at least after 12 h owing to its tight crystal structure.

Reaction Condition Optimization

Effect of Reaction Temperature on the Acetylation of Chitosan in AmimCl.

Initially, investigations have been conducted on the effect of reaction parameters, such as reaction time, temperature, and the molar ratio of acetyl chloride to glucosamine unit in AmimCl. The compared IR spectra of the native chitosan and acetylated chitosans at different reaction temperatures are shown in Figure 2. The curve A lay out the basic characteristics of native chitosan at: 3433 cm⁻¹ (O—H, N—H stretch), 2921 cm⁻¹, 2860 cm⁻¹ (C—H stretch), 1598 cm⁻¹ (N—H band), 1261 cm⁻¹ (O—H band), 1157 cm⁻¹ (bridge—O stretch), 1086 cm⁻¹ (C—O stretch). In all, 1651cm⁻¹ (acetamide I) is generally assigned to the stretching of C=O of acetamide group derived from the uncompleted deacetylation of chitin.³⁷

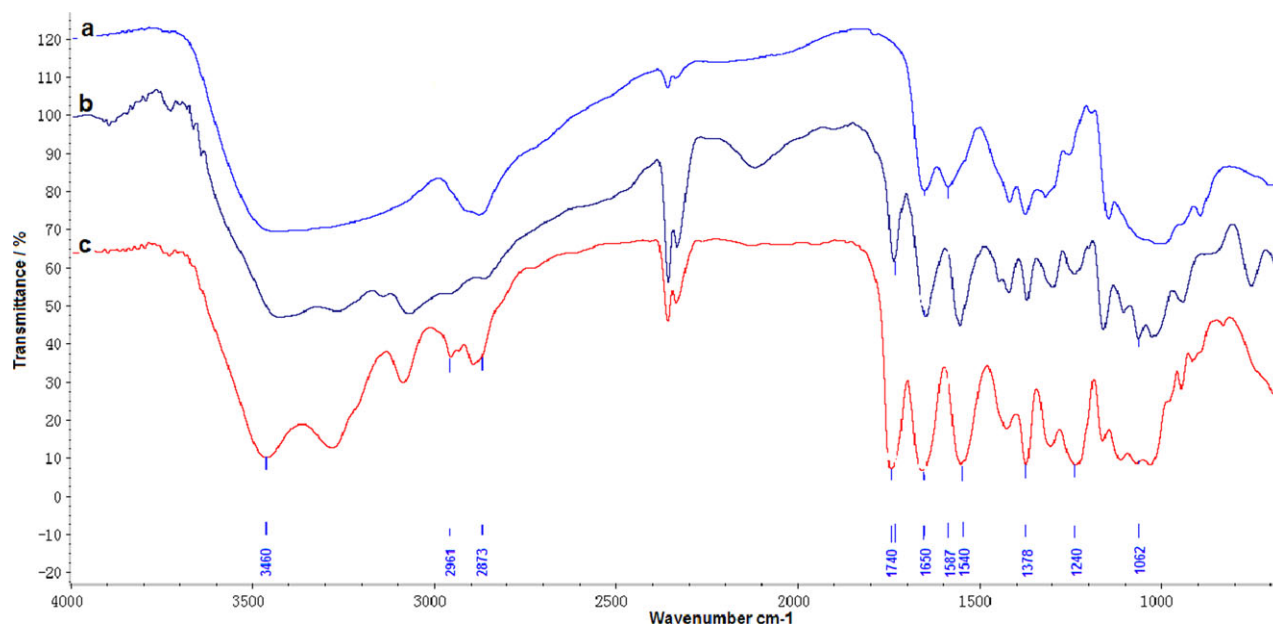


Figure 5. IR spectra of chitosan (a) original, (b) acetylated with three equivalents of CH₃COCl, (c) acetylated with five equivalents of CH₃COCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

As shown in Figure 2, the evident differences can be observed comparing the IR spectra of the acetylated chitosan at different temperatures in AmimCl. As along with the reaction temperature increasing from room temperature to 60°C, the reaction accelerated correspondingly. The peak at 1598 cm^{-1} (N—H band) was disappeared, and the absorption intensity of 1651 cm^{-1} (acetamide I), 1552 cm^{-1} (acetamide II), and 1382 cm^{-1} (C—CH₃ vibration) becomes stronger, which demonstrated that the *N*-acetylation occurred faster and faster. At the same time, the single bands at 1748, 1236, and 1043 cm^{-1} are emerged and the strength becomes stronger as the reaction temperature increased, which are belong to the stretching of C=O and C—O of ester group, respectively, resulted from the acetylation of —OH in the glucosamine unit of chitosan.

As for the DS of acetylation, the quantitative analysis was achieved by the peak strength analysis on IR spectra according to the base line method³⁴ and the results are shown in Figure 3. It can be seen that 35.2% of DS was achieved at 25°C, whereas 83.5% of DS was reached at 60°C under the condition of 12 h and six equivalents of acetyl chloride. For comparison, the regenerated chitosan from IL at 60°C was also investigated, and the barely detectable DS and negligible recovery yield demonstrated the minor influence of IL on the chitosan, perhaps derived from the free Cl⁻ in AmimCl IL. Thus, the optimal reaction temperature was selected at 60°C although 88.4% DS could be obtained at 80°C, on account of the recovery yield of acetylated chitosan decreased to 88.4% (data not shown), which indicates high temperature may lead to some decomposition of chitosan by HCl released from the acetylation owing to the reason that no neutralization reagent was added.

Effect of Reaction Time on the Acetylation of Chitosan in AmimCl. With these data in hand, the effect of reaction time on acetylation and DS was investigated in AmimCl IL using six equivalents of acetyl chloride at 60°C.

As shown in Figure 4, the DS of the acetylated product reached 39.9% within 4 h, 65.8% within 8 h, and 83.5% within 12 h. When reaction time was extended to 24 h, 89.7% of DS can be achieved. For further 12-h prolongation, only 6% of DS was added. It seemed that much longer reaction time had no effect on practically improving DS, but had an undesirable effect on

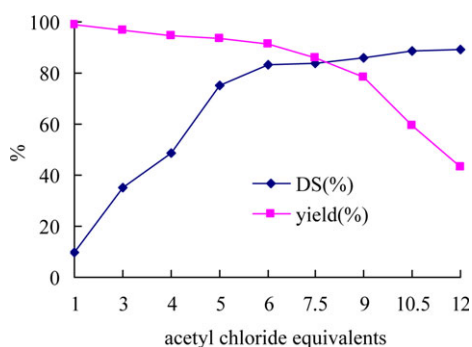


Figure 6. Effect of acetyl chloride amount on the acetylation of chitosan in AmimCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

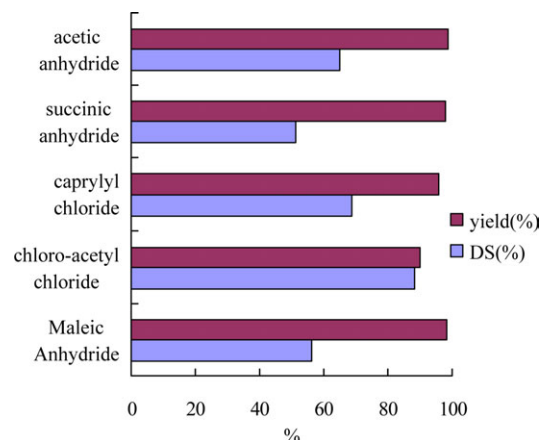


Figure 7. Acetylation comparison of chitosan with different acetylated reagents in AmimCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the recovery yield of derived product. As shown in Figure 4, the longer reaction time and slight lower recovery yields in the acetylation illustrated the decomposition of abovementioned chitosan, to some extent. Giving consideration to DS, 12 h was reasonable for optimal reaction time.

Effect of Acetyl Chloride Amount on the Acetylation of Chitosan in AmimCl. Undoubtedly, an excess of acetylated reagent is useful for the derivation of chitosan.²⁹ However, too high activity of acetyl chloride makes the cost-efficient ratio of acetyl chloride to glucosamine unit favorable for the practical application.

Figure 5 shows the IR spectra of acetylated chitosan with different amounts of acetylated reagent amount. Besides, the spectra

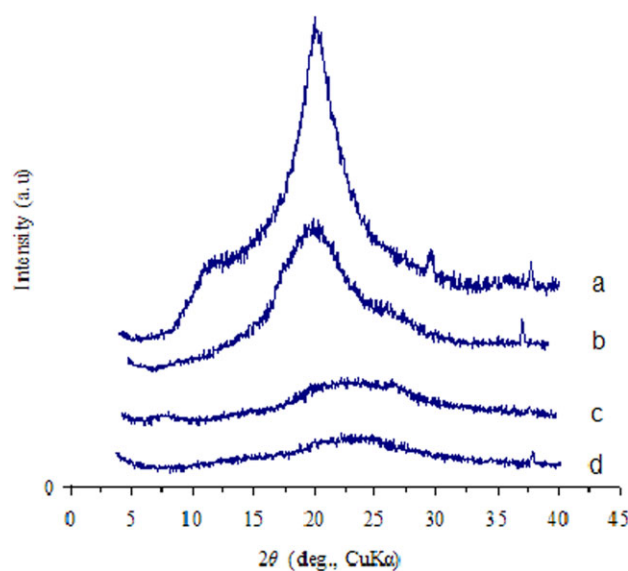


Figure 8. XRD profiles of chitosan powder (a), regenerated chitosan from 5 wt % chitosan/IL solution (b), acetylated chitosan with three equivalents of CH₃COCl (c), and acetylated chitosan with five equivalents of CH₃COCl (d). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

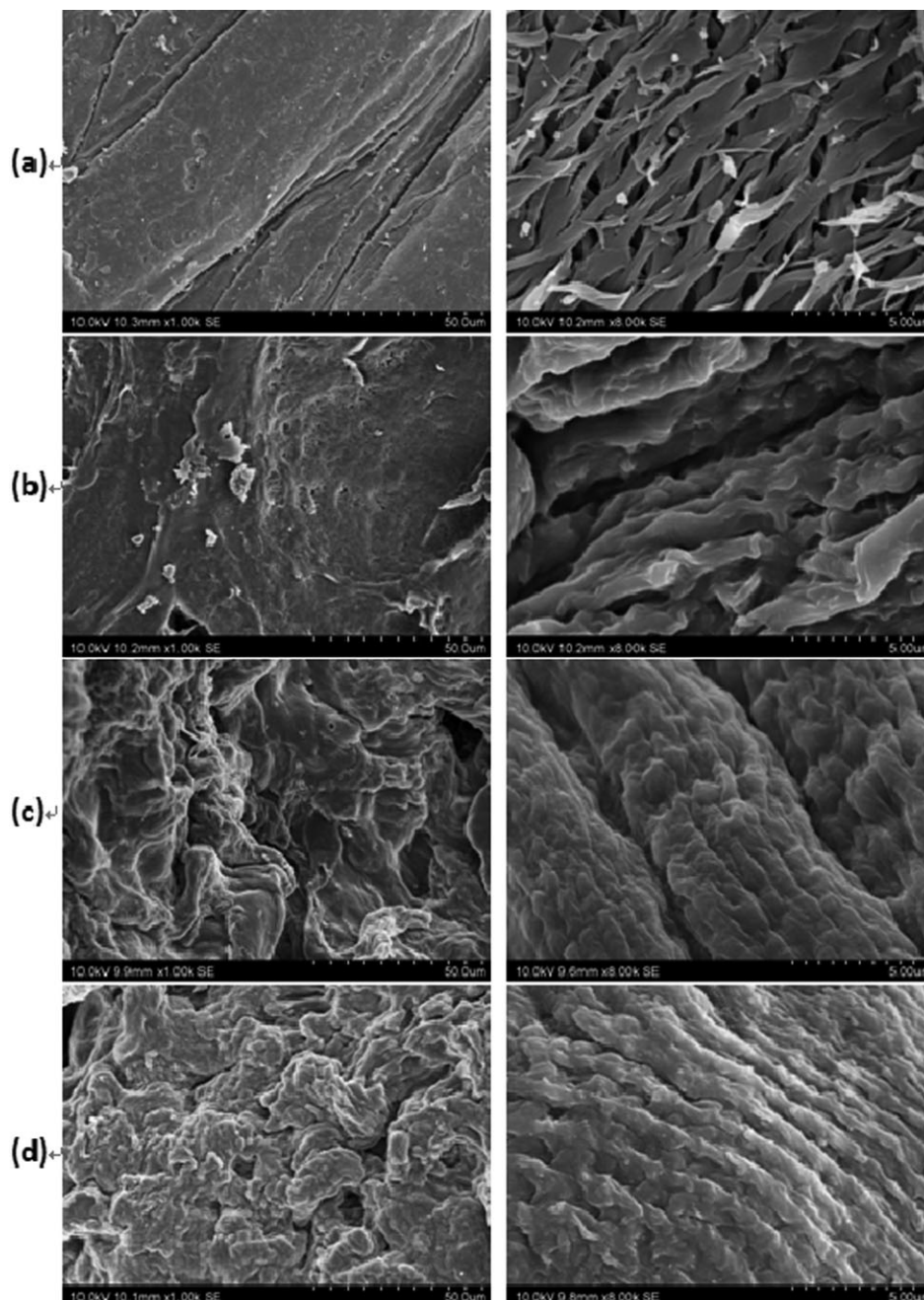


Figure 9. SEM images of chitosan (a), regenerated chitosan from AmimCl (b), acetylated chitosan using three equivalents of acetyl chloride (c), acetylated chitosan using five equivalents of acetyl chloride (d).

show the absorption strength at 1740 cm^{-1} (*O*-acetylated) increasing, and the peaks were gradually sharpened at $2920\text{--}2850\text{ cm}^{-1}$ (acyl chain),³⁷ which is attributed to the substitution degree increasing along with the increase in acetyl chloride amount.

As shown in Figure 6, it is clear that the DS of acetylated chitosan increased from 9.5 to 89.2% with increasing equivalent of acetyl chloride from 1 to 12. However, it looks like that too much excess of acetyl chloride was not good for the recovery yield, probably owing to the decomposition of chitosan by HCl

derived from the acetylation. In pace with the increase in amount of CH_3COCl , the recovery yield of acetylated product decreased gradually. When 12 equivalents of CH_3COCl were used, only 43.3% of recovery yield was obtained. Therefore, six equivalents of CH_3COCl were an appropriate compromise from which 83.5% of DS was achieved.

On the basis of the above viewpoints, attempts to perform the acylation of chitosan with relative mild reagents such as acetic anhydride, succinic anhydride, maleic anhydride, in IL have been subjected to the above-mentioned optimal conditions and

the results are shown in Figure 7. Obviously, caprylyl chloride and chloroacetyl chloride gave relative better DS. Although acetic anhydride, succinic anhydride, and maleic anhydride provided relative milder DS, acetic anhydride may be suitable for the acetylation of chitosan taking into the environmental protection account.

X-ray Spectra Analysis

To compare the chitosan before and after the derivation in IL, the crystal structure of original chitosan powder (a), regenerated chitosan from 5 wt % chitosan/IL solution (b), acetylated chitosan with three equivalents of CH_3COCl (c) and five equivalents of CH_3COCl (d) were investigated by XRD as shown in Figure 8. As shown in the curves of Figure 8, it is clear that original chitosan gives two kinds of diffraction patterns,³⁸ form I ($2\theta = 10^\circ$) shows a slight diffraction peak and form II provides a strong, sharp diffraction peak centered near $2\theta = 20^\circ$ (curve a). The regenerated chitosan from the 5 wt % chitosan/IL solution providing the relative weak diffraction peak in $2\theta = 20^\circ$ indicates that the crystal structure of chitosan has been affected by the dissolution process in AmimCl IL. However, the acetylated chitosan shows slight, broad diffraction pattern, and the strongest part has a tiny shift, which demonstrates that the crystal domains of chitosan have been destroyed by IL during the dissolving process regardless of how many equivalents CH_3COCl were added (c, d).

SEM Micrograph Analysis

In addition, SEM was used to image the microscopic surface structure of acetylated chitosan, and the comparative microphotographs are shown in Figure 9. Compared with the top row of images presenting the original chitosan magnified 1000 and 8000 times, respectively (a), the uncompact and friable structures can be observed from the SEM results (b, 1000 \times) of the regenerated chitosan, and the rigid crystal structure has decreased to some extent (b, 8000 \times). It was worth commenting that the regenerated chitosan is characterized by a relative rough surface with bulges and a porous interior structure. As for the acetylated chitosan (c, d), however, they have scaly pattern on the surface with millions of gullies, which contributed to the low crystallinity of acetylated chitosan.³⁹ Moreover, the surface structure in the acetylated chitosan with five equivalents (d) was obviously more disorder and lipophilic than those acetylated with three equivalents of acetyl chloride, and the difference could be attributed to the different DS of chitosan. The results of the above observation suggested that the surface structure of chitosan was changed during the acetylation, unlike that observed in the original and regenerated chitosan (a, b).

Recycling and Reuse of AmimCl IL

For the development of studies on chitosan with ILs, therefore, the recycle process was investigated starting with chitosan/IL solution after reaction. After the recycling treatments, the recovery IL was directly used in the next run. As summarized in Table I, to our delight, the IL could be reused at least for three times under the abovementioned optimum conditions just with slight activity loss (68.1% of DS, 85.0% of yield). However, the color of IL become gradually deeper and deeper as run times going

Table I. Reuse of [Amim]Cl Ionic Liquid in Acetylation of Chitosan^a

Run times	1	2	3	4
DS (%)	83.5	78.3	75.6	68.1
Yield (%)	91.3	89.7	87.2	85.0

^aConditions: 5 wt % chitosan in AmimCl/recycled AmimCl, six equivalents of acetyl chloride, 60°C, 12 h.

on, which may be owing to the more and more oxidized impurity.

CONCLUSIONS

In summary, room temperature IL AmimCl was found to be effective and the direct solvent for the homogeneous acetylation of chitosan. The formed transparent and viscous solution, on account of AmimCl, could interrupt the intramolecular and intermolecular hydrogen bond to accelerate the dissolution of chitosan, which was confirmed by polarized optical microscopy. The optimal degree of acetylated of 83.5% determined by the IR spectra analysis was obtained using six equivalents of acetyl chloride at 60°C for 12 h. Acetylated chitosan could be separated easily by water precipitation followed by filtration. Moreover, AmimCl could be reused several times just for the need of simple vacuum treatment. XRD analysis demonstrated that the crystal structure of chitosan has been influenced by the dissolution process in IL, and the acetylated chitosan shows slight, broad diffraction pattern, and the strongest part has a tiny shift, which demonstrates that the crystal domains of chitosan have been destroyed by the IL, which are in line with the SEM images. This method is far superior to others performed using acidic solution such as acetic acid, and the homogeneous system, mild condition, and simply workup make this methodology more accessible to the cost efficiency and green aspect, which are in line with the demand of sustainable chemistry.

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REFERENCES

- Pillai, C. K. S.; Paul, W.; Sharma, C. P. *Prog. Polym. Sci.* **2009**, *34*, 641.
- Lingle, S. E. In *Sustainability of the Sugar and Sugar–Ethanol Industries*, 2nd ed.; Eggleston G., Eds.; American Chemical Society: Washington, DC, **2010**; Vol. 1058, p 177.
- Wang, A. Q. *Chitin Chemistry*, 1st ed.; Science Press: Beijing, China, **2004**; p 12.
- Rinaudo, M. *Prog. Polym. Sci.* **2006**, *31*, 603.

5. Eugene, K.; Lee, Y. L. *Biomaterials* **2003**, *24*, 2339.
6. Ravi Kumar, M. N. V. *React. Funct. Polym.* **2000**, *46*, 1.
7. Ravi Kumar, M. N. V.; Muzzarelli, R. A. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. *J. Chem. Rev.* **2004**, *104*, 6017.
8. Casettaria, L.; Vllasaliu, D.; Castagnino, E.; Stolnik, S.; Howdle, S.; Illum, L. *Prog. Polym. Sci.* **2012**, *37*, 659.
9. Jayakumar, R.; Prabakaran, M.; Nair, S. V.; Tokura, S.; Tamura, H.; Selvamurugan, N. *Prog. Mater. Sci.* **2010**, *55*, 675.
10. Kafu, K.; Nishi, N.; Komai, T. *J. Polym. Sci. Polym. Chem. Ed.* **1981**, *19*, 2361.
11. Cai, J.; Zhang, L. *Macromol. Biosci.* **2005**, *5*, 539.
12. Min, B.; Lee, S.; Lim, J.; You, Y.; Lee, T.; Kang, P. *Polymer* **2004**, *45*, 7137.
13. Sashiwa, H.; Aiba, Sei-ichi. *Prog. Polym. Sci.* **2004**, *29*, 887.
14. Chen, Y. L. Preparation and characterization of water-soluble chitosan gel for skin hydration. Thesis of Degree of Master of Science, Universiti Sains Malaysia, Pulau Pinang, **2008**.
15. Holbrey, J. D.; Seddon, K. R. *Clean Prod. Proc.* **1999**, *1*, 223.
16. Welton, T. *Chem. Rev.* **1999**, *999*, 2071.
17. Fukaya, Y.; Hayashi, K.; Wada, M. *Green Chem.* **2008**, *10*, 44.
18. Peng, X. W.; Ren, J. L.; Zhong, L. X.; Sun, R. C. *Carbohydr. Polym.* **2011**, *86*, 1768.
19. Yuan, T. Q.; Sun, S. N.; Xu, F.; Sun, R. C. *J. Agric. Food Chem.* **2010**, *58*, 11302.
20. Zakrzewska, M. E.; Bogel-yukasik, E.; Bogel-yukasik, R. *Energy Fuels* **2010**, *24*, 737.
21. Xiao, W.; Chen, Q.; Wu, Y.; Wu, T.; Dai, L. *Carbohydr. Polym.* **2011**, *83*, 233.
22. Prasada, K.; Murakami, M.; Kaneko, Y.; Takadac, A.; Nakamura, Y.; Kadokawa, J. *Int. J. Biol. Macromol.* **2009**, *45*, 221.
23. Takegawa, A.; Murakami, M.; Kaneko, Y.; Kadokawa, J. *Carbohydr. Polym.* **2010**, *79*, 85.
24. Kadokawa, J.; Takegawa, A.; Mine, S.; Prasad, K. *Carbohydr. Polym.* **2011**, *84*, 1408.
25. Zeng, X. D.; Li, X. F.; Xing, L.; Liu, X. Y.; Luo, S. L.; Wei, W. Z.; Kong, B.; Li, Y. H. *Biosens Bioelectron* **2009**, *24*, 2898.
26. Zhu, Z. H.; Qu, L. N.; Li, X.; Zeng, Y.; Sun, W.; Huang, X. T. *Electrochim Acta* **2010**, *55*, 5959.
27. Xie, H. B.; Zhang, S. B.; Li, S. H. *Green Chem.* **2006**, *8*, 630.
28. Sun, X. Q.; Peng, B.; Ji, Y.; Chen, J.; Li, D. Q. *AIChE J.* **2009**, *55*, 2062.
29. Mine, S.; Izawa, H.; Kaneko, Y.; Kadokawa, J. *Carbohydr. Res.* **2009**, *344*, 2263.
30. Ji, H. H. Study on the new homogeneous synthesis method of N-acyl chitosan in ionic liquids. Thesis of Master degree, Qingdao University of Science & Technology, Qingdao, **2009**.
31. Kim, B.; Jeong, J.; Lee, D.; Kim, S.; Yoon, H.; Lee, Y. S.; Cho, J. K. *Green Chem.* **2011**, *13*, 1503.
32. Choi, C. Y.; Kim, S. B.; Ki Pak, P.; Yoo, D. I.; Chung, Y. S. *Carbohydr. Polym.* **2007**, *68*, 122.
33. Swatoski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. *J. Am. Chem. Soc.* **2002**, *124*, 4974.
34. Zhang, H.; Wu, J.; Zhang, J.; He, J. S. *Macromolecules* **2005**, *38*, 8272.
35. Wu, Y. S.; Sasaki, T.; Irie, S.; Sakurai, K. *Polymer* **2008**, *49*, 2321.
36. Shigemasa, Y.; Matsuura, H.; Sashiwa, H. *Int. J. Biol. Macromol.* **1996**, *18*, 237.
37. Samuels, R. J. *J. Polym. Sci. Polym. Phys.* **1981**, *19*, 1081.
38. Hirano, S.; Nagamura, K.; Zhang, M.; Kim, S. K.; Chung, B. G. *Carbohydr. Polym.* **1999**, *38*, 293.
39. Zhao, D.; Fei, Z.; Geldbach, T. J.; Scopelliti, R.; Laurenczy, G.; Dyson, P. J. *Helv. Chim. Acta* **2005**, *88*, 665.