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# Preparation of Oligochitosan via *In Situ* Enzymatic Hydrolysis of Chitosan by Amylase in [Gly]$BF_4$ Ionic Liquid/Water Homogeneous System

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**ABSTRACT:** The preparation of oligochitosan with excellent performance via *in situ* enzymatic hydrolysis of chitosan by amylase in ionic liquid system is reported. It has been found that [Gly]$BF_4$ ionic liquid leads to the good solubility and assistant degradation for chitosan, as well as good biocompatibility for amylase. In the homogeneous system that contained 1.0 g chitosan (degree of deacetylation = 88.5%) and 99.0 g 2 wt % [Gly]$BF_4$ aqueous solution, oligochitosan with 2200 viscosity-average molecular weight has been obtained after 0.12 g amylase being used for 3 h at 50°C and pH 5.0. This result is superior to that conducted in acetic acid system. Moreover, [Gly]$BF_4$ can be easily separated from the product and reused with only slight performance loss (oligochitosan product with 2700 viscosity-average molecular weight has been obtained after [Gly]$BF_4$ being reused for five times). In addition, the mechanism for enzymatic hydrolysis of chitosan in [Gly]$BF_4$ ionic liquid has been described. The research on the moisture-absorption, -retention, and antibacterial activity of oligochitosan product shows that the smaller molecular weight would bring the better moisture-absorption and antibacterial properties. The oligochitosan product with 2200 viscosity-average molecular weight exhibits preferable antibacterial properties to *S. aureus* and *E. coli*. At the same time, the moisture-absorption and -retention capacity of the above product can reach 32% (relative humidity (RH) = 43%), 62% (RH = 81%), and 150% (RH = 43%), 35% (dry silica gel) respectively. The enzymatic preparation of oligochitosan through [Gly]$BF_4$ ionic liquid/water homogeneous system can be an efficient and environment-friendly method for academics and industry. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41152.

**KEYWORDS:** biodegradable; biopolymers & renewable polymers; degradation; ionic liquids

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## INTRODUCTION

Oligochitosan (chitooligosaccharide, chitosan oligomers), obtained from degradation of important biomass resource chitosan, is the water-soluble aminosugar derivative with 2–20 polymerization degree. During the process of degradation, high molecular chains of chitosan are truncated and a more out-of-order state of molecules is formed. As a result, water solubility, moisture-absorption and -retention performance of the biopolymer will be improved due to the increase of free —$NH_2$ and —OH.[1] Moreover, the degraded chitosan biopolymer can be digested and absorbed more easily by organism, and also have notable antibacterial properties.[2,3] At the same time, the accessibility of oligochitosan to the $O_2^-$ and OH free radical will promote the corresponding anti-oxidation activity.[4,5] In addition, oligochitosan still has the functions of activating the body immunity and increasing the body self-anti-cancer ability.[6] In short, as a sort of new functional oligosaccharide with specific immunity, oligochitosan is widely used in food,[7] pharmaceuticals,[8] health, and so on.

Physical degradation,[9,10] chemical degradation,[11] or biological degradation methods can be used in the preparation process of oligochitosan from chitosan. Among them, the biological degradation methods can overcome the disadvantages of physical methods such as uncontrollability, incomplete degradation, and of chemical methods such as poor selectivity, environment pollution.[12] So, it might be the most promisingly efficient, specific, and clean way to prepare oligochitosan with fine biocampatibility by cutting the $\beta$-1,4-glucosidic bond off selectively.[13]

Chitosanase, the specific degradation enzyme of chitosan, can contact interiorly with the $\beta$-1,4-glucosidic bonds with high activity to obtain oligochitosan specifically. However, the production cost of chitosanase is too high to apply in large-scale

industry owing to the lesser enzyme-producing ability and stability of zymogenic strain. And yet, amylase, cellulase, proteinase, lipase, and pentazyme all can catalyze non-specific enzymatic hydrolysis for chitosan. Among them, abundant and cheap amylase which has good affinity for chitosan can contact interiorly with the glucosidic bonds during the early reaction time to make chitosan degrade rapidly and mildly.[14] Accordingly, amylase is a promising biocatalyst for the degradation of chitosan.

In view of the poor dissolvability of crude stuff chitosan[15] and the environmental problems of usual small molecule acidic solvents, the key issue of preparing oligochitosan by enzymatic method is to seek for a solvent with not only proper dissolvability for chitosan but also fine biocampatibility for amylase in order to achieve the enzymatic hydrolysis of chitosan in the homogeneous system.

It has been found that ionic liquid (IL) had good dissolving capacity for natural high polymer.[16] Swatloski et al.[17] developed a novel way to dissolve cellulose by an IL at room temperature. Moulthrop et al.[18] demonstrated the disorder state of cellulose in [BMIM]Cl IL by high resolution $^{13}$C-NMR. Xie et al.[19] studied the dissolving capacity of chitin and chitosan in [BMIM]Cl IL, meantime, got homogeneous transparent solutions. Wu et al.[20] found that [BMIM]Ac could dissolve about 6 wt % chitosan at 110°C. Zhang et al.[21] realized acidic hydrolysis of chitosan in imidazolyl IL under mild conditions. As part of our systematic research on chitosan, we have paid more attention to the unique dissolving capacity of amino acid IL.[22] It has been found that the aqueous solution of [Gly]Cl IL can afford adequate dissolvability for chitosan, sequentially the film forming,[23] fiber forming,[24,25] and degradation[26,27] process on this platform can be conducted successfully.

IL with not only good dissolving capacity but also fine biocompatibility also have applied potential in biocatalysis aspect.[28,29] However, despite the good dissolving capacity for chitosan, $Cl^-$ with stronger electronegativity in [Gly]Cl molecule will interact with the interior group of enzyme and modify the structure of active sites during the enzymolysis reaction.[30,31] As a result, the activity of enzyme will be damaged quickly.[32] For these reasons, here we introduce a [Gly]$BF_4$ aqueous system with moderate viscosity to produce oligochitosan via amylase catalyzed chitosan hydrolysis. It has been found that [Gly]$BF_4$ possessed not only good dissolving capacity for chitosan but also fine biocompatibility to amylase. The homogeneous and controllable degradation of chitosan was actualized in the 2 wt % aqueous solution of [Gly]$BF_4$ which could be reused. Contrastively, the enzymolysis in the 2 wt % aqueous solution of [Gly]Cl under the same conditions showed poor activity due to the inactivation of enzyme caused by $Cl^-$. Therefore, oligochitosan product with eximious moisture-absorption and antibacterial properties can be prepared by an environment-friendly and efficient approach via [Gly]$BF_4$.

## EXPERIMENTAL

### General

Chitosan (molecular weight was about $3.9 \times 10^5$) was received from AK Biotech CO., Ltd. (Jinan, China) and directly used after being dried without further purification; amylase (BR) was obtained from Yuanye Biotech Co., Ltd. (Shanghai, China); all the other reagents were purchased from Aldrich.

### Preparation and Characterization of [Gly]$BF_4$ IL

A colorless transparent liquid of [Gly]$BF_4$ was prepared with 95% yield according to the report in the patent.[33] The structure of [Gly]$BF_4$ was verified by $^1$H-NMR, $^{13}$C-NMR, and FTIR spectroscopy.

$^1$H-NMR(500 MHz, $D_2O$, $\delta$): 3.778(s,2H), 4.746(s,1H). $^{13}$C-NMR(125MHz, $D_2O$, $\delta$): 40.069, 169.952.

IR (KBr): 3591($\upsilon$, —$NH_3^{*+}$—$CH_2$—COOH*), 3251($\upsilon_{as}$, —$CH_2$*—COOH), 1751($\upsilon_{as}$, —C=O*), 1613($\delta$, —$NH_3^{*+}$), 1508($\delta$, —$CH_2$*—CO—), 1435($\delta$, —COOH*), 1254($\upsilon$, —C—O*), 1117($\upsilon$, —N—C*), 903($\omega$, —O—H*), 861($\delta$, —N—H*), 638, 496.

### Dissolution and Reconstruction of Chitosan in [Gly]$BF_4$

About 0.1 g chitosan was gradually dissolved into 10.0 g [Gly]$BF_4$ under stirring to obtain a thick transparent solution. Then triple-mass absolute ethyl alcohol was added to make chitosan precipitate slowly. The precipitation was leached and dried. Another sample of chitosan was dissolved into HAc and reconstructed by the same way. The assistant degradation performances of both two solvents for chitosan were evaluated via the characterized results by fourier transform infrared spectroscopy (FTIS), scanning electron microscope (SEM), and X-ray diffraction (XRD).

### Biocompatibility Determination of [Gly]$BF_4$ to Amylase

[Gly]$BF_4$ aqueous solution with various concentrations (adjusting pH 5–6 by NaAc) was added into the flask with a reflux condenser. Then amylase was added into the system and preserved at 50°C. Timing sampling was conducted to determine the activity of amylase by DNS (3,5-dinitrosalicylic acid) method.[34]

### Enzymatic Hydrolysis of Chitosan and Recycling of Ionic Liquid

About 1.0 g chitosan and 99.0 g solvent (2 wt % [Gly]$BF_4$ aqueous solution, or 2 wt % HAc aqueous solution) were added in a round-bottom flask with reflux condenser to form a homogeneous colloid by agitation and heating slightly. After amylase being put into the system, the effects of pH, reaction time, temperature, dosage of enzyme, and deacetylation degree of chitosan were investigated to find optimum reaction conditions. The amylase was deactivated by placing the reaction system in boiled water bath for 5 min after reaction, then removed via filtering after cooling. Triple-mass absolute ethyl alcohol was put into the filtrate to make the product precipitate slowly. Afterwards, oligochitosan was obtained from the precipitation via filtering and drying.

The above residual filtrate was vacuum distilled until no steam generated any more. Then distilled water was refilled into the substrate to recover 99.0 g IL aqueous solution which would be reused in the enzymatic hydrolysis of chitosan by the same way as aforementioned. The molecular weights of products were measured by viscosity[35] to evaluate the reaction performance.

### Determination of Molecular Weight of Chitosan and its Degraded Production

The viscosity of sample was measured by Ubbleohde capillary viscometer (Ø = 0.5–0.6 mm) in an aqueous solution with 0.2 mol/L NaCl and 0.1 mol/L HAc at 25°C. Then the molecular weight was obtained using the classic Mark Houwink equation $[\eta] = 1.81\times10^{-3}M_v^{0.93}$.[33]

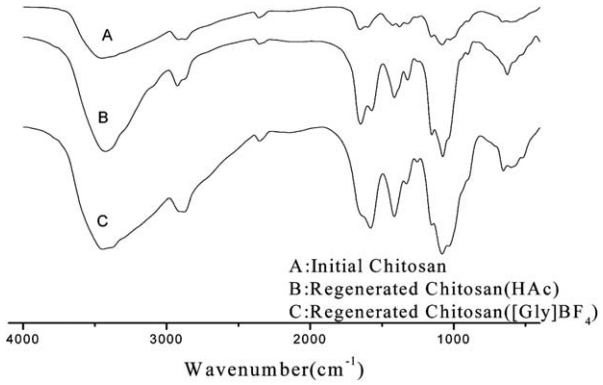


**Figure 1.** FTIR spectra of the initial and regenerated chitosan.

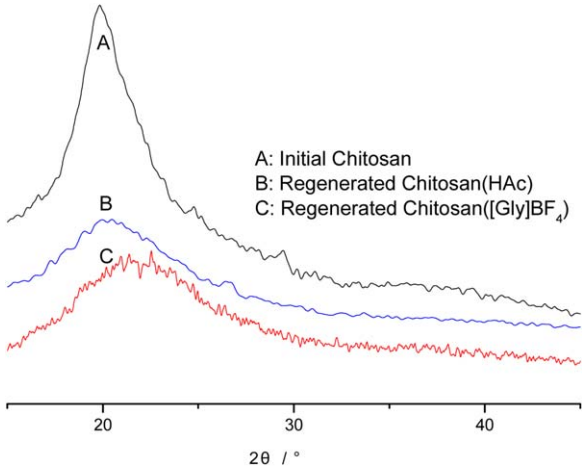


**Figure 2.** XRD spectra of the initial and regenerated chitosan. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

### Determination of Moisture-Absorption and -Retention Performance

All samples were disposed in dryer filled with phosphorus pentaoxide for 24 h.

**Determination of Moisture-Absorption Performance.** Two weighing bottles holding 0.5 g accurately weighed samples ($m_0$) were placed separately in one desiccator with saturated ammonium sulfate sodium(relative humidity = 81%), and the other one with saturated sodium carbonate (relative humidity = 43%). Experimental samples ($m_n$) after 12, 24, 36, 48, 60, 72, and 96 h were weighed to give moisture-absorption ratio (%): $100(m_n - m_0)/m_0$.

**Determination of Moisture-Retention Performance.** Two weighing bottles holding 0.5 g accurately weighed samples and 10 wt % water ($H_0$) were placed separately in one desiccator with saturated sodium carbonate (relative humidity = 43%), and the other one with dry silica gel. Experimental samples ($m_n$) after 12, 24, 36, 48, 60, 72, and 96 h were weighed up to give residual ratio of water (%): $100H_n/H_0$.

### Determination of Antibacterial Property

Antibacterial property was performed using representative *S. aureus* (Staphylococcus aureus, Gram-positive) and *E. coli* (Escherichia coli, Gram-negative) by disc agar diffusion (K-B method).

Bacterial suspension of *S. aureus* or *E. coli* diluted by physiological saline solution was well-proportionately coated onto the solid agar media in culture dish under amicrobic condition. Then 6 mm diameter round filter paper immersed with the HAc solution of oligochitosan sample was stuck onto the above culture dish. Antibacterial property of the oligochitosan sample was assessed via determining the diameter of inhibition zone after the sample being cultivated at 37°C in an incubator for 24 h.

## RESULTS AND DISCUSSION

### Assisted Degradation Performance of [Gly]$BF_4$ for Chitosan

Figure 1 shows the FTIR spectra of initial and regenerated chitosan. As can be seen from Figure 1, the main peaks in FTIR spectra of the regenerated chitosan from HAc [Figure 1(B)] and [Gly]$BF_4$ [Figure 1(C)] corresponds with that of initial chitosan [Figure 1(A)] on the whole, which confirms that no framework shift in chitosan molecule has taken place during dissolution. The intensifying of C—O stretching vibration around 1081 $cm^{-1}$ in Figure 1(B,C) (regenerated chitosan) is attributed to the break of glucosidic bond.[36] Furthermore, the intermolecular or intramolecular hydrogen bonds in chitosan are impaired after dissolution, leading to the reduction of molecular crystallinity and the increase of free amido. This is the reason why the N—H absorption peak around 1650 $cm^{-1}$ intensifies. Comparing Figure 1(B) with Figure 1(C), the greater peaks of

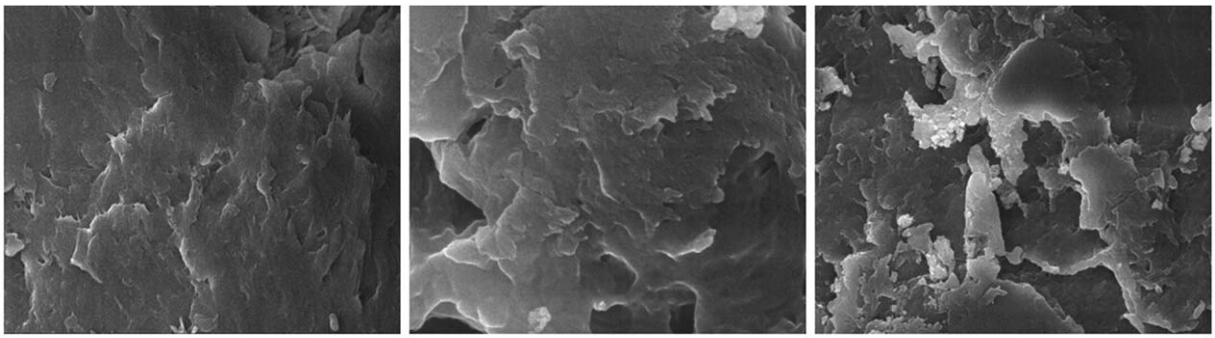


**Figure 3.** SEM of initial chitosan and regenerated chitosan.

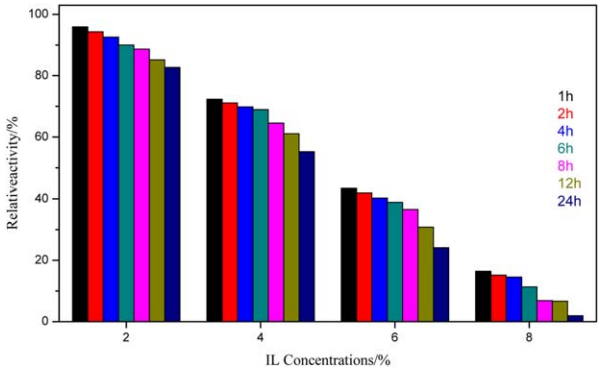


**Figure 4.** The influence of [Gly]$BF_4$ concentration on the enzymatic activity of amylase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

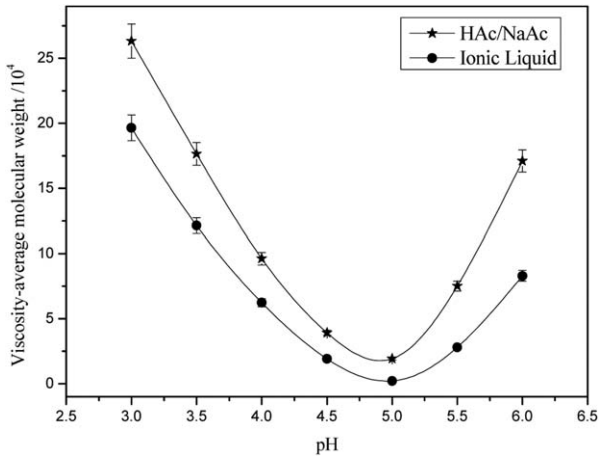


**Figure 5.** Effect of pH on enzymatic hydrolysis.

regenerated chitosan from IL than that of regenerated chitosan from HAc confirms that the assisted degradation performance of [Gly]$BF_4$ was superior to that of HAc via cracking hydrogen bonds more fiercely. Besides, the XRD results of initial and regenerated chitosan can be a further proof for the superior assisted degradation performance of [Gly]$BF_4$. In Figure 2, the

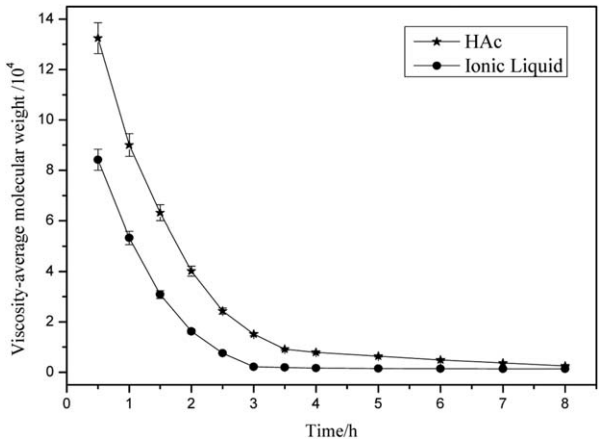


**Figure 6.** Effect of reaction time on enzymatic hydrolysis.

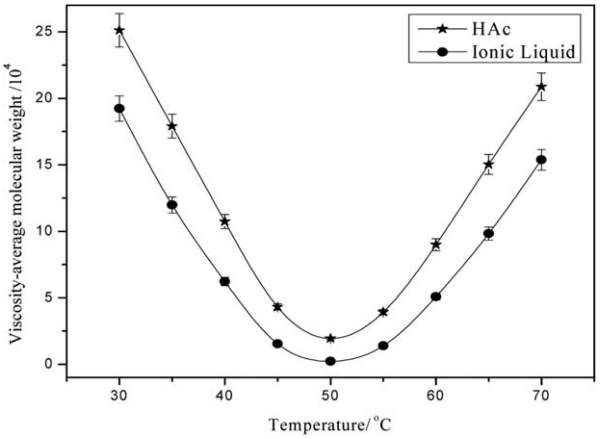


**Figure 7.** Effect of temperature on enzymatic hydrolysis.

broadened main peaks in XRD spectra of the regenerated chitosan from HAc [Figure 2(B)] and [Gly]$BF_4$ [Figure 2(C)] compared with that of initial chitosan [Figure 2(A)] indicates the decrease of crystallinity. Compared with that of Figure 2(B), the shift of main peak of Figure 2(C) to high-angle shows that regenerated chitosan with lower crystallinity can be obtained from [Gly]$BF_4$ than HAc.

SEM has been also used to observe the pattern of initial and regenerated chitosan (Figure 3). It can be seen that the pattern of initial chitosan is the perfect massive lamellar structure [Figure 3(a)]. Also lots of dislocations are observed on the pattern of regenerated chitosan [Figure 3(b,c)], especially the chitosan from [Gly]$BF_4$ in Figure 3(c). The ions of [Gly]$BF_4$ are supposed to enter the interior of chitosan molecules and break the intermolecular or intramolecular hydrogen bonds, leading to the more incompact structures. The SEM results, which correspond to those of FTIR and XRD spectra, show that [Gly]$BF_4$ is a good solvent for chitosan with favorable performance of dissolution and assistant degradation.

### Biocompatibility of [Gly]$BF_4$ with Amylase

The activities of amylases conserved in diverse concentration of [Gly]$BF_4$ aqueous solutions with time are exhibited in Figure 4. An 82.2% enzymatic activity can be reserved after amylase being in 2 wt % [Gly]$BF_4$ aqueous solution for 24 h. The effective

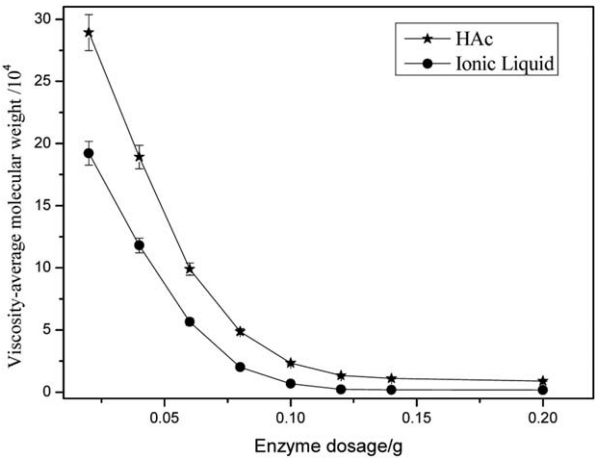


**Figure 8.** Effect of enzyme dosage on enzymatic hydrolysis.

**Table I.** Effect of Deacetylation Degree on Enzymatic Hydrolysis

| Deacetylation degree (%) | Viscosity-average molecular weight of oligochitosan ($10^4$) |
|---|---|
| 88.53 | 0.219 |
| 79.40 | 1.362 |
| 51.55 | 11.673 |

**Table II.** The Reusability of Ionic Liquid

| Repeated number | Viscosity-average molecular weight of oligochitosan |
|---|---|
| 1 | 2240 |
| 2 | 2310 |
| 3 | 2420 |
| 4 | 2570 |
| 5 | 2740 |

reservation of enzymatic activity is due to the deconcentration of $BF_4^-$ negative charge onto four fluorine atoms, which will reduce their hydrogen bonds interference with enzyme molecules.[37] However, stronger ionic strength in higher concentration [Gly]$BF_4$ aqueous solution will cause the descending of enzymatic activity through impacting the active sites of enzyme, even the denaturation of amylase.[38] Accordingly, a 2 wt % [Gly]$BF_4$ aqueous solution has been set in the following enzymatic hydrolysis reactions.

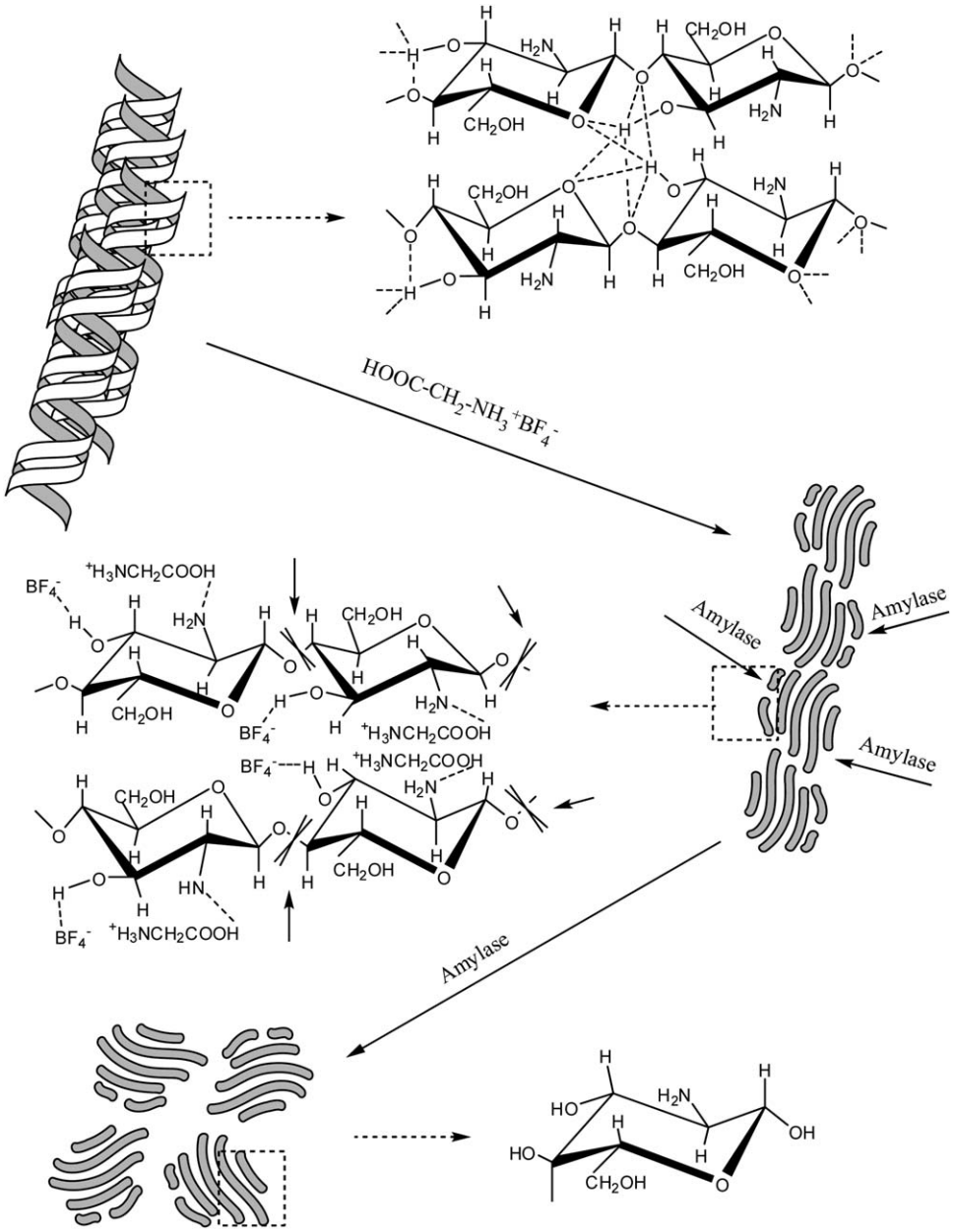


**Figure 9.** Reaction mechanism of enzymatic hydrolysis.

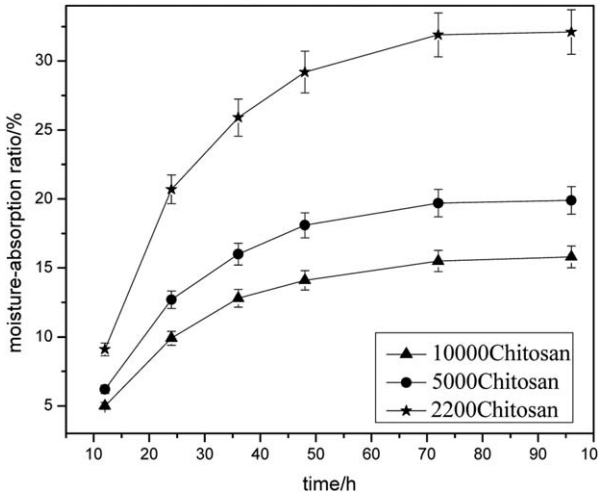


**Figure 10.** Moisture-absorption capacity of oligochitosan with different molecular weight (RH = 43%).

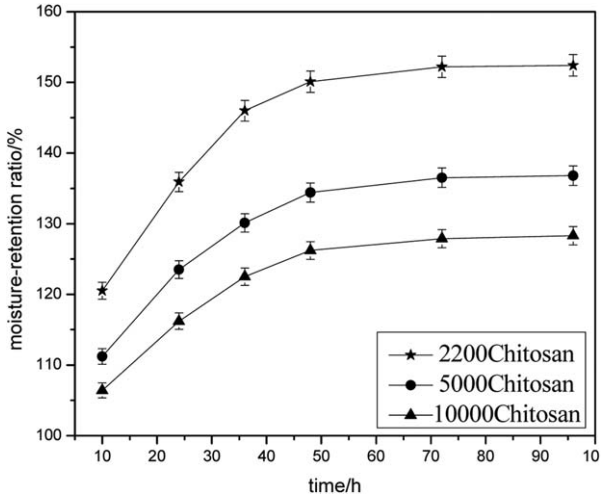


**Figure 12.** Moisture-retention capacity of oligochitosan with different molecular weight (RH = 43%).

### Degradation of Chitosan with Amylase Under Different Conditions

The pH of the reaction system will influence the form of active groups in amylase, at the same time determine the existential form of amino groups in chitosan molecules, which will account much for the dissolving state of chitosan and the accessibility of amylase to chitosan. Figure 5 shows that pH 5.0 is the optimal condition which can offer the product with minimum molecular weight in both ionic liquid and HAc systems. Moreover, the molecular weight of oligochitosan product obtained from ionic liquid system is lower than that obtained from HAc system, which can be attribute to the formation of new hydrogen bonds between —COOH, $BF_4^-$ in $[Gly]BF_4$ and —$NH_2$, —OH in chitosan. Furthermore, the fracture of chitosan self-hydrogen bonds promoted by IL will treat $\beta$-1,4-glucosidic bonds exposed, leading to active sites of enzymatic hydrolysis reaction increase.[39] The above result indicates that $[Gly]BF_4$ IL system is more propitious to enzymatic hydrolysis reaction than HAc system.

Effect of reaction time in Figure 6 exhibits that enzymatic hydrolysis has taken place mostly at the prophase of reaction with loss of molecular weight rapidly, which can be attributed to the interior contact degradation manner catalyzed by amylase.[14] Similarly with Figure 5, enzymatic hydrolysis reaction in $[Gly]BF_4$ system is faster than HAc system. Only after 3 h, the product with 2200 molecular weight can be obtained from the IL system.

It can be seen from Figure 7 that 50°C is the optimum enzymatic hydrolysis temperature in both $[Gly]BF_4$ and HAc system. Proper increasing of temperature can improve the enzyme activity and the collapsing force of solvents to the self-hydrogen bonds of chitosan. Nevertheless, the temperature over 50°C will make amylase denatured and deactive, accordingly the efficiency of enzymatic hydrolysis falls down.

From Figure 8, the increase of amylase dosage will accelerate enzymatic hydrolysis at the initial stage of reaction, as well decrease the oligochitosan molecular weight greatly. But on the premise of

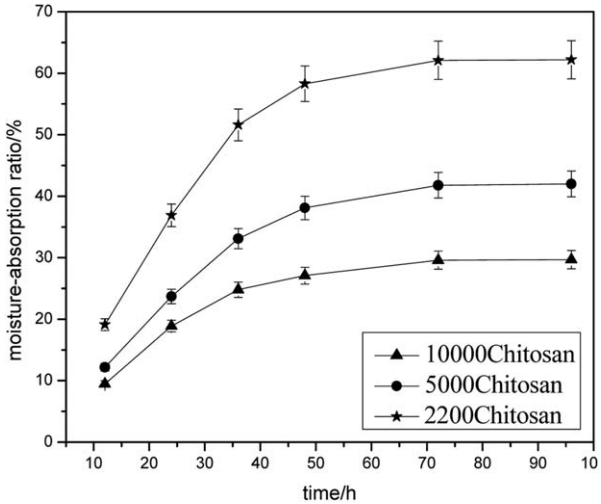


**Figure 11.** Moisture-absorption capacity of oligochitosan with different molecular weight (RH = 81%).

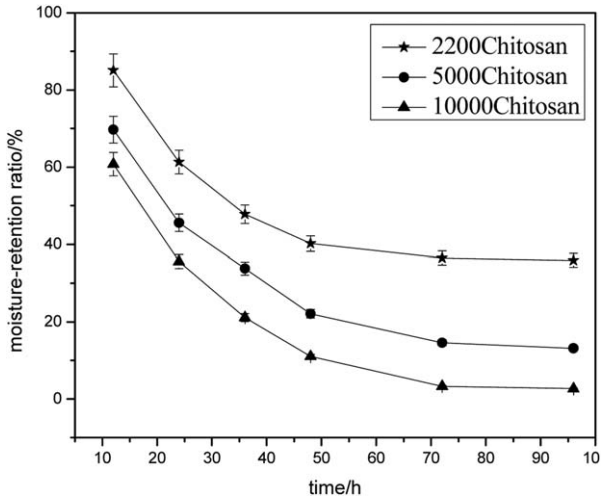


**Figure 13.** Moisture-retention capacity of oligochitosan with different molecular weight (silica gel).

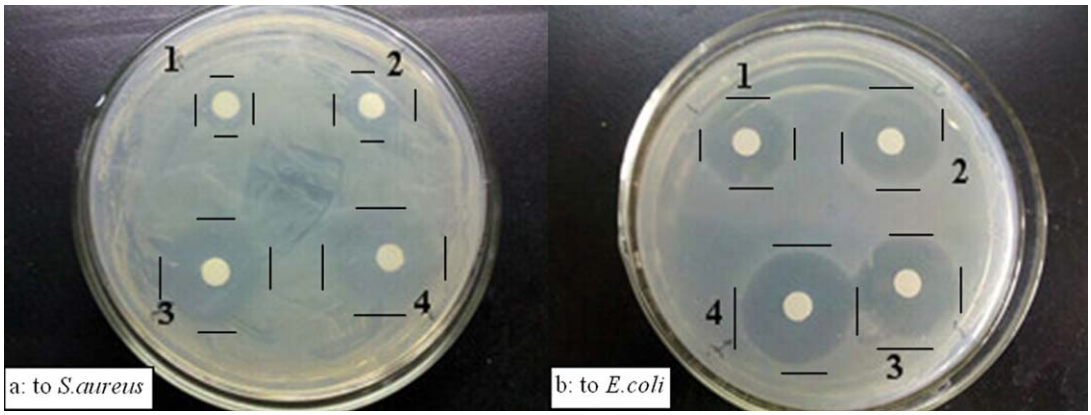


**Figure 14.** Diameter of inhibition zone of oligochitosan with different molecular weight (1 for blank; 2, 3, 4 for the samples with 10,000, 5000, 2000 molecular weight, respectively). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

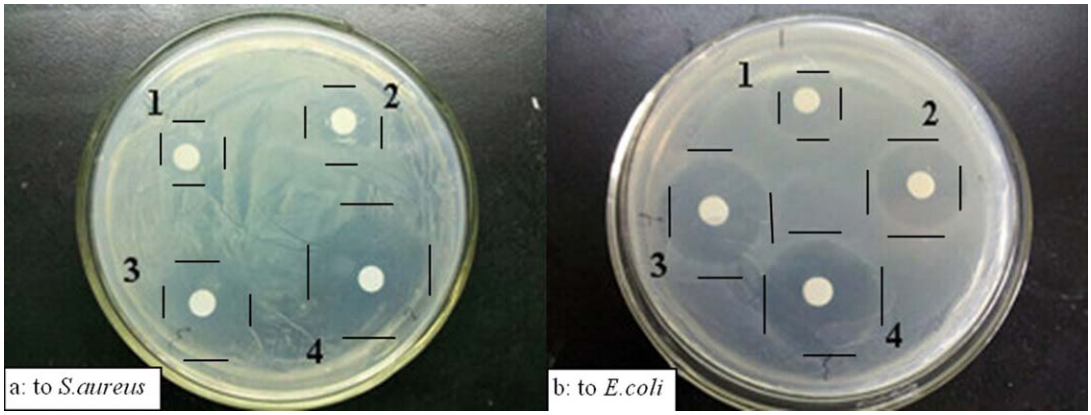


**Figure 15.** Diameter of inhibition zone of oligochitosan with different concentration (1 for blank; 2, 3, 4 for the samples with 0.5, 1.0, 1.5% concentration, respectively). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

invariable chitosan quantity, the accessibility of active sites to substrate will drive to saturation stage as the dosage of amylase exceeds over a given value. With a view to both reactive efficiency and economic factor, a 0.12 g dosage of amylase has been chosen.

Table I indicates that under the same conditions, the higher the deacetylation degree of chitosan substrate was, the smaller the product molecular weight was, i.e. the better enzymatic hydrolysis performance was. The better dissolution of chitosan mass with higher deacetylation degree would break self-hydrogen bonds more intensively, leading to $\beta$-1,4-glucosidic bonds exposed more adequately. As a result, the activity of enzymatic hydrolysis increases. Under the above optimum conditions, the reusability of $[Gly]BF_4$ IL in the enzymatic hydrolysis has been studied. According to Table II, only slight increase of product molecular weight is observed by reusing IL for five times. Hence, $[Gly]BF_4$ has a good reusable performance.

### The Mechanism of Enzymatic Hydrolysis of Chitosan by Amylase in $[Gly]BF_4$ Ionic Liquid

From all of the above results, the mechanism of enzymatic hydrolysis of chitosan by amylase in $[Gly]BF_4$ IL system is expressed in Figure 9. The first step is the dissolution process of chitosan in ionic IL. $H^+$ and $BF_4^-$ of IL can interact with —OH, $—NH_2$ of chitosan, respectively, to form new hydrogen bonds instead of self-hydrogen bonds of chitosan, which will lead to the exposure of $\beta$-(1,4)-glucosidic bonds. In the second step, amylase is mixed fully with the substrate, thus, the active groups of amylase can touch the $\beta$-(1,4)-glucosidic bonds of chitosan easily to promote the crack of glucosidic bonds. The $BF_4^-$ anions of IL will preserve the stability of amylase to a certain extent to insure the favoring degradation of chitosan.

### Moisture-Absorption and -Retention Performance of Oligochitosan

The moisture-absorption and -retention performance of oligochitosan with different molecular weight is exhibited in Figures

**Table III.** Diameter of Inhibition Zone of Oligochitosan to *S. aureus* (mm)

| Concentration of oligochitosan (%) | Molecular weight of oligochitosan | | |
|---|---|---|---|
| | 2000 | 5000 | 10,000 |
| 0.5 | 8.8 | 7.6 | 6.7 |
| 1.0 | 9.3 | 8.1 | 7.2 |
| 1.5 | 9.9 | 8.9 | 7.5 |

**Table IV.** Diameter of Inhibition Zone of Oligochitosan to *E. coli* (mm)

| Concentration of oligochitosan (%) | Molecular weight of oligochitosan | | |
|---|---|---|---|
| | 2000 | 5000 | 10,000 |
| 0.5 | 9.2 | 8.4 | 7.1 |
| 1.0 | 9.9 | 8.9 | 7.5 |
| 1.5 | 10.6 | 9.7 | 8.0 |

10–13. It has been shown that the lower the samples molecular weight are, the better the moisture-absorption and -retention performances are. Under both the conditions of RH = 43% and RH = 81% (Figures 10 and 11), oligochitosan with molecular weight below 2200 can absorb moisture rapidly until the moisture-absorption ratio reaches 32% and 62%, respectively. The results in Figure 12 show that not only no moisture is lost but also residual ratio of water increases under RH = 43%. Excellent residual ratio of water 150% and 35% are obtained in the samples with molecular weight below 2200 at RH = 43% and dry silica gel condition respectively (Figures 12 and 13).

During the degradation process, the self-hydrogen bonds of chitosan are weakened and the crystal structure is destroyed. As a result, the lower the product molecular weight is, the more —OH, —$NH_2$ polar hydrophilic groups, which tend to form new hydrogen bonds with $H_2O$, are exposed. This can explain the good moisture-absorption and -retention performance of oligochitosan with lower molecular weight.

**Antibacterial Properties of Oligochitosan**

Figures 14 and 15 exhibit illustratively the antibacterial performance of oligochitosan product to *S. aureus* and *E. coli*. The diameter of inhibition zone data of oligochitosan with various molecular weights and concentrations are listed in Tables III and Table IV. It can be seen that the samples with lower molecular weight as well as higher concentration have better antibacterial capability. In general, the negative polarity of compounds plays an important role in their antibacterial performance. The more exposed —OH, —$NH_2$ polar hydrophilic groups in the oligochitosan product with lower molecular weight may be also the main reason for their good antibacterial properties.

## CONCLUSIONS

[Gly]$BF_4$ IL has adequate dissolvability and assisted degradation capability for chitosan. A homogeneous system with appropriate viscosity and good biocompatibility can be provided by a 2 wt % [Gly]$BF_4$ aqueous solution, which can maintain 82.2% activity of amylase after 24 h. In the homogeneous system containing 1.0 g chitosan (deacetylation degree 88.53%) and 99.0 g 2 wt % [Gly]$BF_4$ aqueous solution, the oligochitosan product with about 2200 viscosity-average molecular weight can be obtained after 3 h under the condition of pH 5.0, 50°C, and 0.12 g amylase. A product with 2700 molecular weight still can be obtained from the IL system reused for five times.

The determination of antibacterial properties, moisture-absorption and -retention performance indicates that the molecular weight of oligochitosan product has a significant influence on the moisture-absorption and -retention capability, as well as antibacterial performance. The oligochitosan product with about 2200 molecular weight obtained from [Gly]$BF_4$ system can attain 32%, 62% moisture-absorption ratio under (RH) = 43%, 81%, as well, 150%, 35% residual ratio of water under (RH) = 43% and dry silica gel conditions. Besides, the inhibition zone diameter of 1.5% aqueous solution of oligochitosan sample with 2200 molecular weight to *S. aureus* and *E. coli* can reach 9.9 and 10.6 mm. Thus, amylase promoting hydrolysis of chitosan can be conducted in recyclable [Gly]$BF_4$ IL/water system to produce oligochitosan with superior performance.

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