Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Research 340 (2005) 1351–1356

Carbohydrate
RESEARCH

# Preparation of carboxymethyl chitosan in aqueous solution under microwave irradiation

Hua-Cai Ge* and Deng-Ke Luo

*College of Chemistry, South China University of Technology, Guangzhou 510640, China*

Received 16 September 2004; received in revised form 8 February 2005; accepted 14 February 2005
Available online 23 March 2005

**Abstract**—Carboxymethyl chitosan was prepared by reacting chitosan with chloroacetic acid in water under microwave irradiation. The effect of the reaction conditions was investigated and optimal conditions were identified. The influence of mass ratio of chloroacetic acid to chitosan, microwave power and pH on the degree of substitution or intrinsic viscosity were further studied. The degree of substitution of the carboxymethyl chitosan synthesized exceeded 0.85.

*Keywords:* Chitosan; Carboxymethylation; Aqueous solution; Microwave radiation

## 1. Introduction

As one of the most abundant natural and biodegradable polymers, chitosan has been widely applied in many fields as a multi-functional material including biomedical materials.[1,2] However, its applications are restricted because it is essentially insoluble in water at neutral pH. To improve the solubility of chitosan, chemically modified chitosans have been prepared including carboxymethyl chitosan.[3] Carboxymethyl chitosan (CMC) has been prepared by reacting pre-alkalized chitosan in alcoholic solvents with chloroacetic acid for several hours.[4] The use of the alcohol solvents is undesirable given environmental pollution concerns; however, if the solvent changed to water, the yield of the product is very low under standard conditions that involve traditional heating.[4]

Microwave irradiation has been widely used in organic synthesis because it can speed up the reaction rate by orders of magnitude over conventional heating and can markedly reduce the amount of solvent needed and thus the amount of waste produced.[5–8] Recently, microwave irradiation has been extended for use in the chemical modification of polysaccharides such as starch and chitosan.[9] We have also carried out studies on chitosan and obtained some significant results.[10,11] In this paper, we report the carboxymethylation of chitosan in aqueous solution under microwave irradiation, which because no organic solvents are required, represents a more environmentally-friendly method for modification of chitosan.

## 2. Experimental

### 2.1. Materials and equipment

Biochemical reagent grade chitosan (deacetylation degree >90%, viscosity <100 cps) from Shanghai Yuanju Biology Technology Ltd, Shanghai, China, was used. All other chemicals were of reagent grade and were used without further purification. A Galanz microwave oven (WP700L17), produced by Shunde Galanz Electric Appliances Factory, Ltd, China, was employed in these studies after modification as outlined below. Measurements of solution pH were done on a pH meter (PHS-25) produced by Shanghai Weiye Instrument Plant, China. IR spectra were recorded on a Bruker FT-IR spectrometer (Vector 33).

---

* Corresponding author. Tel.: +86 2087 112419; fax: +86 2087 112906; e-mail: chhcge@scut.edu.cn

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.02.025

### 2.2. Modification of microwave oven

A diagram of the microwave oven used in these studies is shown in Figure 1. Three holes were drilled into the oven to provide the inlet and outlet for recycling thermostat water, and the inlet for nitrogen gas. The holes were jacketed with copper tubing to avoid microwave leakage. The recycling thermostat water was provided by a thermostated water bath. Using this apparatus, the highest reaction temperature was 100 °C and temperature variation could be controlled under low or middle microwave power.

### 2.3. Preparation of carboxymethyl chitosan

Chitosan (0.640 g) was added to 10 M aqueous NaOH (10 mL) with stirring. The mixture was kept for several hours at room temperature and was stirred every 30 min to avoid aggregation of chitosan. After treatment with base, the chitosan mixture was transferred into an Erlenmeyer flask and 7 M aqueous chloroacetic acid was added dropwise over 5 min. Then, while stirring 1 M aqueous acetic acid was added dropwise into the mixture until the solution reached the correct pH. The reaction mixture flask was then placed into the water bath in the microwave oven and the temperature was adjusted by the recycling thermostated bath water. The reaction mixture was heated for some minutes under microwave irradiation. After cooling and filtering, the solution was concentrated and adjusted to the neutral pH by adding chloroacetic acid. Acetone was added to the solution, which resulted in the formation of a white precipitate, which was filtered and washed with a 3:1 solution of MeOH/water. White CMC was obtained after drying.

For purposes of comparison with the microwave radiation method, the traditional heating method (microwave power of 0 W) was also done. All other steps were the same as mentioned above except that the reaction was carried out in a thermostated water bath.

### 2.4. Determination of degree of substitution

The degree of substitution of CMC was determined by using potentiometric titration.[12] CMC (0.2 g) was dissolved in distilled water (40 mL). The solution was adjusted to pH < 2 by adding hydrochloric acid. Then, the CMC solution was titrated with 0.1 M aqueous NaOH and the pH value of the solution was simultaneously recorded. The amount of aqueous NaOH was determined by the second order differential method. The degree of substitution (DS) can be calculated as follows:

$$\mathrm{DS} = \frac{161 \times A}{m_{\mathrm{CMC}} - 58 \times A}$$

$$A = V_{\mathrm{NaOH}} \times c_{\mathrm{NaOH}}$$

where $V_{\mathrm{NaOH}}$ and $c_{\mathrm{NaOH}}$ are the volume and molarity of aqueous NaOH, respectively, $m_{\mathrm{CMC}}$ is the mass of CMC (g), and 161 and 58 are the respective molecular weights of glucosamine (chitosan skeleton unit) and a carboxymethyl group.

### 2.5. Determination of viscosity

Carboxymethyl chitosan was dissolved in 0.1 M aqueous NaCl. The viscosity was determined at 30 °C. The

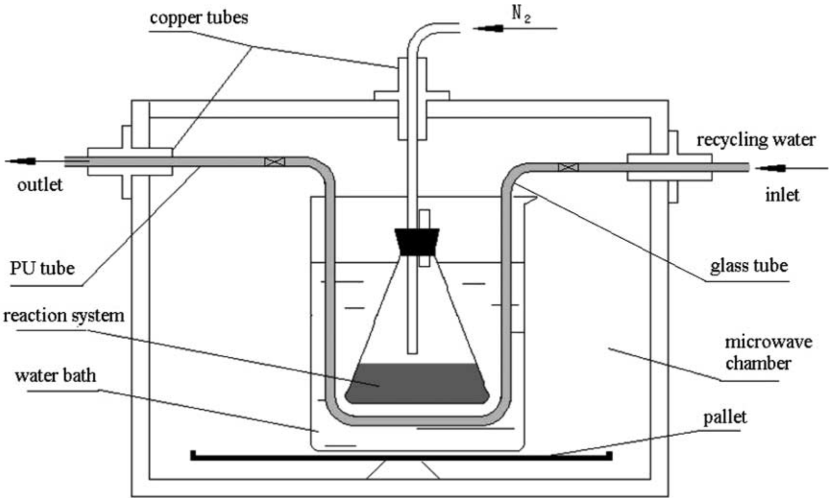

**Figure 1.** Schematic of modified microwave oven.

viscosity and molecular weight can be calculated as follows:[13–15]

$$\eta_r = t/t_0$$

$$\eta_{sp} = \eta_r - 1$$

$$[\eta] = \frac{4\eta_{sp}^{1.02} \times \ln \eta_r}{c^{1.01}(3\eta_{sp} + \ln \eta_r)}$$

$$[\eta] = 7.92 \times 10^{-5} M_r^{1.00}$$

where $t$ and $t_0$ are the delivery time of CMC solution and the solvent, $c$ is the concentration (g/mL), $\eta_r$ and $[\eta]$ are the relative viscosity and intrinsic viscosity, and $M_r$ is the viscosity average molecular weight of CMC.

## 3. Results and discussion

### 3.1. Optimization of reaction condition

The optimum conditions for the carboxymethylation reaction of chitosan were investigated by independent variation of five parameters: microwave power (*A*), reaction temperature (*B*), heating time while under microwave irradiation (*C*), alkalization time (*D*), and pH (*E*). For each parameter, the three values were investigated, as listed in Table 1. Reference to the experimental design theory, the orthogonal array $L_{27}$ ($3^{13}$) was selected to arrange the test program. The degree of substitution (DS) of CMC was the criterion of each synthesis. The test results are shown in Table 2.

The order of influence of each variable on DS appears to be $A \gg E > C \sim B > D$. Thus, the microwave power has the greatest influence. The optimum level of each variable is *A*-3, *B*-1, *C*-2, *D*-3, *E*-3. However, for alkalization time, there was no significant difference between DS for those samples treated for 2 h versus 3 h. Therefore, the optimum reaction conditions were as follows: microwave power, 260 W; reaction temperature, 100 °C; microwave heating time, 20 min; alkalization time, 2 h; pH, 13.5. Under these conditions, the degree of CMC substitution exceeded 0.85 and the product could be easily dissolved in water.

### 3.2. FT-IR analysis of carboxymethyl chitosan

The IR spectra of the raw chitosan and CMC prepared under the optimal conditions are shown in Figures 2 and 3. Figure 2 shows that the main characteristic peaks of chitosan are at 3455 $cm^{-1}$ (O–H stretch), 2879 $cm^{-1}$ (C–H stretch), 1600 $cm^{-1}$ (N–H bend), 1327 $cm^{-1}$ (C–N stretch), 1155 $cm^{-1}$ (bridge O stretch), and 1092 $cm^{-1}$ (C–O stretch). Figure 3 shows the main characteristic peaks of CMC and the intrinsic peaks of carboxy group (1737 $cm^{-1}$). The bands at 1599 $cm^{-1}$ and 1401 $cm^{-1}$ correspond to the carboxy group (which overlaps with N–H bend) and carboxymethyl group, respectively. Compared with the peaks of chitosan, the peaks of CMC at 1599 $cm^{-1}$ and 1324 $cm^{-1}$ increase, thus indicating carboxymethylation has occurred on both the amino and hydroxyl groups of chitosan.

### 3.3. Effect of the ratio of chloroacetic acid to chitosan

We examined the effect of the mass ratio of chloroacetic acid to chitosan on the reaction by looking at three different ratios of the reactants (chloroacetic acid:chitosan = 5:1, 8:1, and 10:1), while keeping the other reaction variables at the optimal levels (alkalization time, 2 h; pH, 13.5; the reaction temperature, 100 °C; microwave power, 260 W). For each experiment, the relationship of DS and intrinsic viscosity as a function of time was investigated and the results are shown in Figures 4 and 5.

Figure 4 shows that after 20 min the DS does not increase significantly regardless of the chloroacetic acid:chitosan ratio. However, the DS does increase as the chloroacetic acid:chitosan ratio is increased from 5:1 to 8:1, but that at the greater ratios (10:1) no further increase is observed. Figure 5 shows that the relationship between intrinsic viscosity and chloroacetic acid:chitosan is similar to the DS. However, the intrinsic viscosity decreases when the reaction time exceeds 20 min. This phenomenon might be attributed to greater degradation of the higher DS CMC. Therefore, the proper mass ratio of chloroacetic acid:chitosan should be 8:1 and the reaction time should be limited to about 20 min.

### 3.4. Effect of microwave radiation

To study the effect of microwave radiation on the reaction, the other reaction variables were adopted as the

**Table 1.** Investigated variables and their levels

| Levels of each variable | *A*: microwave power (W) | *B*: reaction temperature (°C) | *C*: heating time by microwave (min) | *D*: alkalization time (h) | *E*: pH |
|---|---|---|---|---|---|
| 1 | 0 | 100 | 15 | 1 | 11 |
| 2 | 120 | 80 | 20 | 2 | 7 |
| 3 | 260 | 60 | 10 | 3 | 13.5 |

**Table 2.** Orthogonal experimental arrangement and test results

| Experiment number | | *A*[a] | *B*[a] | *C*[a] | *D*[a] | *E*[a] | DS |
|---|---|---|---|---|---|---|---|
| 1 | | 1 | 1 | 1 | 1 | 1 | 0.258 |
| 2 | | 1 | 1 | 2 | 2 | 2 | 0.227 |
| 3 | | 1 | 1 | 3 | 3 | 3 | 0.320 |
| 4 | | 1 | 2 | 1 | 2 | 3 | 0.336 |
| 5 | | 1 | 2 | 2 | 3 | 1 | 0.319 |
| 6 | | 1 | 2 | 3 | 1 | 2 | 0.067 |
| 7 | | 1 | 3 | 1 | 3 | 2 | 0.117 |
| 8 | | 1 | 3 | 2 | 1 | 3 | 0.258 |
| 9 | | 1 | 3 | 3 | 2 | 1 | 0.172 |
| 10 | | 2 | 1 | 1 | 2 | 3 | 0.694 |
| 11 | | 2 | 1 | 2 | 3 | 1 | 0.675 |
| 12 | | 2 | 1 | 3 | 1 | 2 | 0.424 |
| 13 | | 2 | 2 | 1 | 3 | 2 | 0.511 |
| 14 | | 2 | 2 | 2 | 1 | 3 | 0.645 |
| 15 | | 2 | 2 | 3 | 2 | 1 | 0.555 |
| 16 | | 2 | 3 | 1 | 1 | 1 | 0.506 |
| 17 | | 2 | 3 | 2 | 2 | 2 | 0.474 |
| 18 | | 2 | 3 | 3 | 3 | 3 | 0.567 |
| 19 | | 3 | 1 | 1 | 3 | 2 | 0.749 |
| 20 | | 3 | 1 | 2 | 1 | 3 | 0.892 |
| 21 | | 3 | 1 | 3 | 2 | 1 | 0.805 |
| 22 | | 3 | 2 | 1 | 1 | 1 | 0.781 |
| 23 | | 3 | 2 | 2 | 2 | 2 | 0.748 |
| 24 | | 3 | 2 | 3 | 3 | 3 | 0.842 |
| 25 | | 3 | 3 | 1 | 2 | 3 | 0.880 |
| 26 | | 3 | 3 | 2 | 3 | 1 | 0.815 |
| 27 | | 3 | 3 | 3 | 1 | 2 | 0.562 |
| Average | Level 1 | 0.230 | 0.560 | 0.537 | 0.488 | 0.543 | Total average = 0.526 |
| | Level 2 | 0.561 | 0.534 | 0.561 | 0.543 | 0.431 | |
| | Level 3 | 0.786 | 0.483 | 0.479 | 0.546 | 0.604 | |
| Variance (average) | | 0.556 | 0.077 | 0.082 | 0.058 | 0.173 | |

[a] *A*: microwave power; *B*: reaction temperature; *C*: heating time by microwave; *D*: alkalization time; *E*: pH.

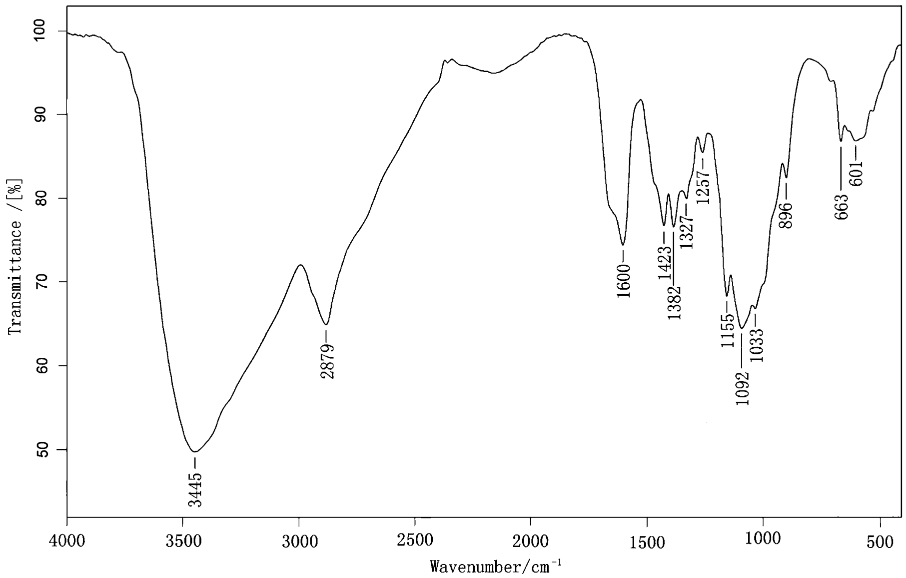

**Figure 2.** IR spectrum of chitosan.

above mentioned conditions: alkalization time, 2 h; mass ratio of chloroacetic acid to chitosan, 8:1; pH, 13.5; reaction temperature, 100 °C and the microwave power was adjusted. Because higher microwave power might cause the temperature of reaction system to be difficult to control, the highest power used was 260 W.

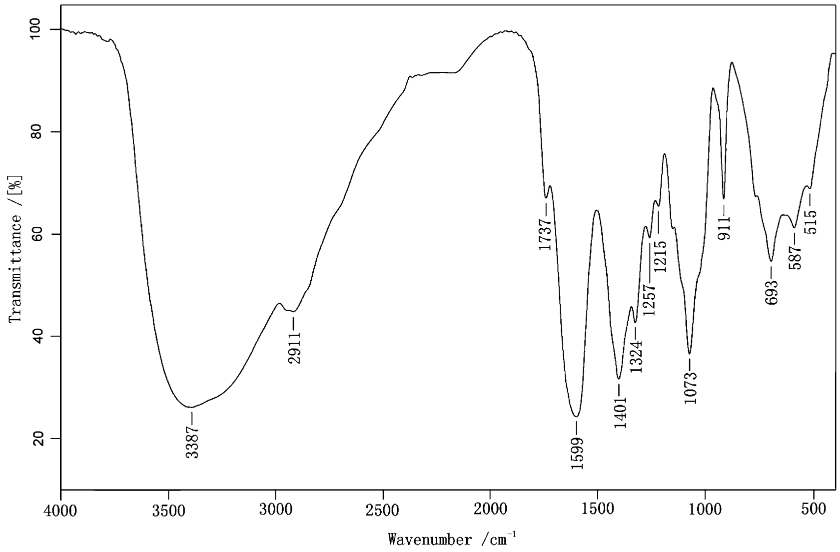

**Figure 3.** IR spectrum of carboxymethyl chitosan.

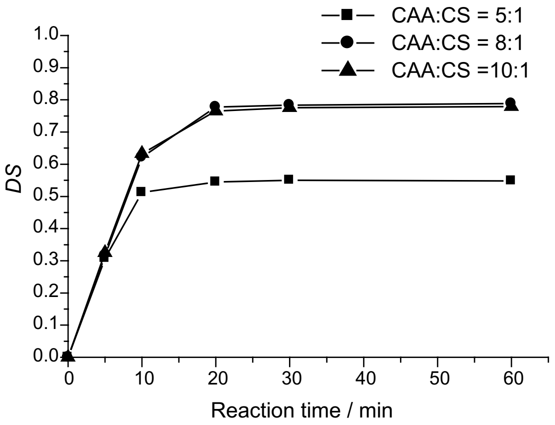

**Figure 4.** Effect of mass ratio of chitosan to chloroacetic acid on the degree of substitution.

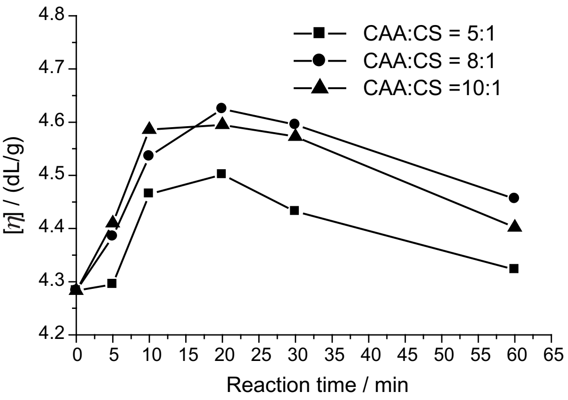

**Figure 5.** Effect of mass ratio of chitosan to chloroacetic acid on intrinsic viscosity.

The relationship between DS and intrinsic viscosity and microwave power are given in Figures 6 and 7.

Figure 6 shows a clear effect of microwave irradiation on the reaction. Under traditional heating conditions (Power 0 W), the reaction time exceeds 240 min and the DS is about 0.7. With microwave irradiation, however, the reaction time is only about 20 min and the DS exceeds 0.8. In addition, Figure 7 shows that the viscosity under microwave irradiation is larger than when conventional heating is used. These results mean that the DS increases under microwave radiation and that the microwave method in the water solvent can be compared to the traditional method in the alcohol solvent.[3,4] We propose that microwave irradiation increases the rate of mass transport in the reaction system, which in turn increases the contact between chloroacetic acid and the reacting functional groups on the chitosan, and the shorter reaction time can avoid the degradation of CMC. Based on the relationship between intrinsic

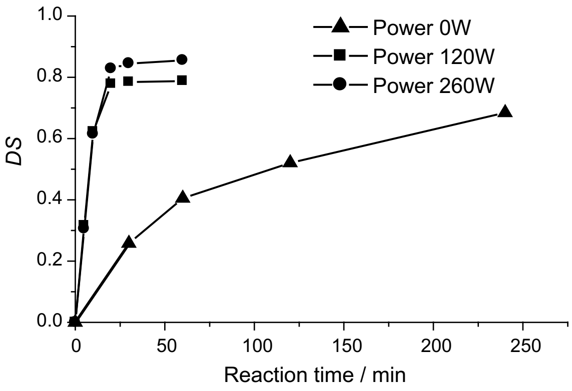

**Figure 6.** Effect of microwave power on the degree of substitution.

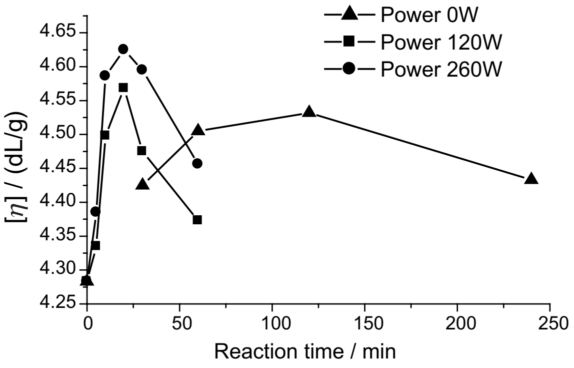

**Figure 7.** Effect of microwave power on viscosity.

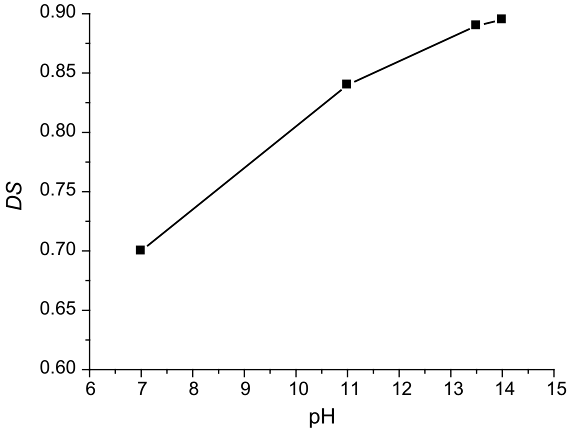

**Figure 8.** Relation of DS to pH.

viscosity and the molecular weight, we conclude that CMC with larger molecular weight and narrower molecular weight distribution can be prepared by controlling the reaction time.

### 3.5. Effect of pH

The effect of pH on the reaction was also investigated while the other variables were kept at the above mentioned conditions: alkalization time, 2 h; mass ratio of chloroacetic acid to chitosan, 8:1; reaction temperature, 100 °C; microwave power, 260 W; reaction time, 20 min. The relationship between DS and pH is given in Figure 8 and indicates that an increase of pH favors an increase in DS. However, the experiment shows that at high pH some degradation of CMC may occur and therefore the optimum value of pH should be about 13.5, which provides a DS of 0.89.

## 4. Conclusion

Carboxymethyl chitosan was prepared by reacting the pre-alkalized chitosan with chloroacetic acid in aqueous solution under microwave radiation. The optimum reaction conditions were as follows: alkalization time, 2 h; mass ratio of chloroacetic acid to chitosan, 8:1; pH, 13.5; reaction temperature, 100 °C; microwave power, 260 W; reaction time, 20 min. Using this microwave irradiation method, the reaction rate was increased by an order of magnitude over the conventional method, and the use of organic solvents could be reduced. The degree of substitution of CMC exceeded 0.85 and thus this is a valuable preparative method.

## References

1. Li, Q.; Dunn, E. T.; Grandmaison, E. W.; Goosen, M. F. Application and Properties of Chitosan. In *Applications of Chitin and Chitosan*; Goosen, M. F. A., Ed.; Technomic Publishing: Lancaster, PA, 1997; pp 3–29.
2. Kurita, K. *Prog. Polym. Sci.* **2001**, *26*, 1921–1971.
3. Krause, T. J.; Goldsmith, N. K.; Ebner, S.; Zazanis, G. A.; McKinnon, R. D. *J. Invest. Surg.* **1998**, *11*, 105–113.
4. Chen, X. G.; Park, H. J. *Carbohydr. Polym.* **2003**, *53*, 355–359.
5. Gedye, R.; Smith, F.; Westaway, K. *Tetrahedron Lett.* **1986**, *27*, 279–282.
6. Kappe, C. O. *Curr. Opin. Chem. Biol.* **2002**, *6*, 314–320.
7. André, L. *C.R. Chim.* **2004**, *7*, 103–112.
8. Wang, L. Y.; Zhang, X. G.; Shi, Y. P.; Zhang, Z. X. *Dyes Pigments* **2004**, *62*, 21–25.
9. Balint, K.; Stephen, P. M. *J. Polym. Environ.* **2002**, *10*, 93–104.
10. Cao, Z. Y.; Ge, H. C.; Lai, S. L. *Eur. Polym. J.* **2001**, *37*, 2141–2143.
11. Zheng, D. F.; Ge, H. C. *Huanan Ligong Daxue Xuebao/J. South China Univ. Technol. (Nat. Sci.)* **2003**, *12*, 51–54.
12. Han, X.; Tan, T. W. *Beijing Huagong Daxue Xuebao/J. Beijing Univ. Chem. Technol.* **2000**, *3*, 1–4.
13. Roberts, G. A. F. *Chitin Chemistry*; Macmillan: London, 1992; pp 173–186.
14. Nishimura, S. I.; Nishi, N.; Tokura, S.; Nishimura, K.; Azuma, I. *Carbohydr. Res.* **1986**, *146*, 251–258.
15. Zhao, Z. H.; Sun, H. Z.; Sun, M. K. *Haiyang Shuichan Yanjiu/Mar. Fisheries Res.* **2002**, *3*, 41–43.