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Effect of reaction temperature and reaction time on the preparation of low-molecular-weight chitosan using phosphoric acid

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Abstract

Low-molecular-weight chitosan were prepared using 85% phosphoric acid at different reaction temperatures and reaction time. At room temperature, the viscosity average-molecular weights (M_v) of chitosan decreased to 7.1×10^4 from 21.4×10^4 after 35 days treatment. The degradation rate decreased with increasing hydrolysis time. The yields of chitosan also continuously decreased from 68.4 to 40.2% after 35 days. At 40, 60 and 80 °C, the molecular weight decreased to 3.70×10^4, 3.50×10^4 and 2.00×10^4 on 8 h hydrolysis, respectively. The yields of chitosan remain at a high level compared with that at room temperature and were 86.5, 71.4 and 61.3% at 40, 60 and 80 °C treatment, respectively. The different reaction time gave chitosan with different molecular weights. At 60 °C, the molecular weight of products decreased to 7.40×10^4 from 21.4×10^4 within 4 h, then decreased slowly to 1.90×10^4 in 15 h. It was also found that the water-solubility of chitosan increased as the molecular weight decreased. Results show the changes in yields and molecular weight of chitooligomers were strongly dependent on the reaction temperature and reaction time.

Keywords: Chitosan; Water-solubility; Molecular weight; Phosphoric acid; Degregation

1. Introduction

Chitin, chitosan and their derivatives have found a wide variety of application in the biomedical, pharmacological, agricultural and biotechnological industries (Brine, Sandford, & Zikakis, 1992). They were found to have biological medicinal and anti-fungal activities. The effect of molecular weight on some anti-bacterial and anti-fungal activities has been explored (Simojon & Fukushima, 1996; Tokura, Miuray, Johmen, Nishi, & Nishimura, 1994). Both chitin and chitosan are polymers with high molecular weights. It is of interest to degrade chitosan into different molecular weight under appropriate conditions and then compare the relationship between biological activity and molecular weight.

The methods for preparing low-molecular-weight chitosan are either enzymatic or chemical. More than 30 types of enzymes can be used for the degregation of chitosan, but there are still some difficulties for large-scale industrial processes (Chai, Zhang, & Jing, 1999; Hutadilok et al., 1995; Muzzarelli, Xia, Tomasetti, & Ilari, 1995). Horowitz and Roseman (1957) used concentrated hydrochloric acid to hydrolyze chitosan and following attempts include the use of nitrous acid (Tomaya, Yoshichi, & Akira, 1987), acetic acid (Todashi, 1990), concentrated sulfuric acid (Maksinov, Denisov, & Makarov, 1990) and hydrofluoric acid (Jacques, Andree, & Christion, 1990). However, the reaction conditions were so strong that it seemed difficult to obtain chitosan with molecular weights of 10 000–100 000 in high yields without large amounts of by-products. Two types of low degree of polymerization (DP) chitosan were prepared by homogeneous hydrolysis of chitosan in 85% phosphoric acid at room temperature for 1–6 weeks (Hasegawa, Isogai, & Onabe, 1993). The kinetics of the depolymerization of chitosan in dilute HCl solutions by nitrous acid have also been studied (Allan & Peyron, 1995). In the present paper, we have hydrolyzed chitosan with 85% phosphoric acid with different reaction time and temperature in anticipation of obtaining different low-molecular-weight chitosans.

2. Experimental

2.1. Chitosan samples

Chitosan derived from shrimp (Zhoushan Xinxing Medical Chemistry Factory, China) was used without

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Table 1
Effect of reaction time on properties of chitosan at ambient temperature (85% phosphoric acid; reaction temperature: room temperature)

Reaction time (d)	$[\eta]$ (ml/g)	M_v ($\times 10^{-4}$)	Yields (%)	Water-solubility (%)
0	323	21.4	–	1.04
4	231	14.5	68.4	1.22
8	184	11.3	64.5	1.99
14	166	10.0	58.0	2.16
21	159	9.6	49.2	2.38
28	140	8.3	47.7	2.89
35	123	7.1	40.2	2.99

further purification. The degree of deacetylation was 96% by potentiometry and the viscosity average-molecular weight (M_v) was 2.14×10^5 (methods described later).

2.2. Hydrolysis with phosphoric acid

Chitosan was hydrolyzed by a method based on Hasegawa et al. (1993). A chitosan (5 g) was placed in a 300 ml Erlenmeyer flask with a stopper and 85% phosphoric acid (100 g) was added. After stirring for a given time and temperature, chitosan was precipitated in excess ethanol. After stirring the mixture for 1 day, free phosphoric acid was removed with ethanol by decantation, and phosphoric acid forming salt at amine groups of hydrolyzed chitosan was removed by repeated treatments with 1% triethylamine in ethanol. The precipitate was thoroughly washed with ethanol and then was treated by adding 1000 ml of water at room temperature for 2 days. The precipitate was added by 1% aqueous sodium hydroxide to pH 10–11 for the complete removal of phosphate ions, and then was washed thoroughly with water to neutral. The low-molecular-weight chitosan was collected by filtration followed by drying in vacuum.

2.3. Degree of deacetylation determination

The potentiometry method reported by Lin, Jiang, and Zhang (1992) was followed. Dried chitosan of 0.5 g were accurately weighed and dissolved in $0.1\ \mathrm{mol\,l^{-1}}$ HCl. The solution was titrated with $0.1\ \mathrm{mol\,l^{-1}}$ NaOH. The degree of deacetylation was calculated as follows:

$$\mathrm{NH_2}\% = \frac{(C_1V_1 - C_2V_2)0.016}{G(100 - W)} \times 100$$

where C_1 is the concentration of HCl ($\mathrm{mol\,l^{-1}}$); C_2, the concentration of NaOH ($\mathrm{mol\,l^{-1}}$); V_1, the volume of HCl (ml); V_2, the volume of NaOH (ml); G, the sample weight (g); W, the water percentage of sample (%); and 0.16 is the weight of $\mathrm{NH_2}$ equal to 1 ml $0.1\ \mathrm{mol\,l^{-1}}$ HCl (g).

$$\text{Degree of deacetylation (\%)} = \mathrm{NH_2}\%/9.94\% \times 100\%$$

where 9.94% is the theoretical $\mathrm{NH_2}$ percentage.

2.4. Molecular weight determination

Dried chitosan of 0.5 g were accurately weighed and dissolved in $0.1\ \mathrm{mol\,l^{-1}}$ $\mathrm{CH_3COONa}$–$0.2\ \mathrm{mol\,l^{-1}}$ $\mathrm{CH_3COOH}$ solution. Five concentrations of chitosan solution were prepared. Relative viscosity was measured at 30 ± 0.5 °C in a constant temperature bath (Jianshu Changzhou Science Instrument Factory). Intrinsic viscosity is defined as $[\eta] = C(\eta_{\mathrm{red}}) \to 0$. It was obtained by extrapolating the reduced viscosity vs concentration data to zero concentration. The intercept on the ordinate is the intrinsic viscosity. The viscosity average-molecular weight was calculated based on the Mark Houwink equation as follows (Wang, Bo, Li, & Qin, 1991):

$$[\eta] = KM^a$$

where $K = 1.64 \times 10^{-30} \times \mathrm{DD}^{14}$, $a = -1.02 \times 10^{-2} \times \mathrm{DD} + 1.82$, and DD is the degree of deacetylation of chitosan.

2.5. Estimation of water-solubility

Dried chitosan of 4 g were accurately weighed and dissolved in 100 ml distilled water. The solution was allowed to stand for 15 h with intermittent stirring at room temperature. The solution was filtered with a sintered glass. The filter was dried to constant weight in an oven at 100–105 °C for 3 h. The water-solubility of chitosan was calculated.

2.6. Infrared spectra

Fourier transform infrared spectra were obtained with a SHIMADZU 470 Infrared spectrometer, using the KBr method.

3. Results and discussion

3.1. Hydrolysis with 85% phosphoric acid at room temperature

It usually took 1–2 days with intermittent stirring at room temperature for complete dissolution of chitosan, resulting in a brown viscous liquid and the subsequent hydrolysis seemed to proceed under homogenous conditions. This

Table 2
Effect of reaction temperature on properties of chitosan (reaction time, 8 h; 85% phosphoric acid)

Reaction temperature (°C)	[η] (ml/g)	M_v ($\times 10^{-4}$)	Yields (%)	Water-solubility (%)
40	69.0	3.70	86.5	2.96
60	66.4	3.50	71.4	3.08
80	40.9	2.00	61.3	3.18

solution was allowed to stand at room temperature for 1–6 weeks and at appropriate times, samples were removed for analysis. IR spectra of chitosan samples before and after degradation showed no significant differences. The degree of deacetylation both before and after degradation was 96%.

Table 1 shows the viscosity average-molecular weight (M_v) decreased with increasing hydrolysis time. However, the degradation rate also decreased with increasing hydrolysis time. After 4 days hydrolysis, the M_v decreased to 14.5×10^4 from 21.4×10^4, where after the next 4 days treatment, the M_v decrease to 11.3×10^4 from 14.5×10^4. The decrease in rate was 1.73×10^4 to 0.8×10^4 Da/d for 4, and 8 days treatment, respectively. After 35 days treatment, M_v decreased to 7.1×10^4. This process was accompanied by a decrease in viscosity of the solution from 323 to 122 ml/g. The yields of low-molecular-weight chitosan continuously decreased from 68.4 to 40.2% with increasing hydrolysis time in 85% phosphoric acid. This showed that more chitosan degraded to a water-soluble low-molecular-weight material, probably even as far as the monomer.

3.2. Effect of reaction temperature on preparation of low-molecular-weight chitosan

The results of degradation of the chitosan with 85% phosphoric acid in 8 h at 40, 60 and 80 °C, respectively, are listed in Table 2.

Both the viscosity and molecular weight of the chitosan decreased rapidly when heated. The table indicates that the viscosity of the chitosan decreased from 323 to 69.0 ml/g after only 8 h hydrolysis at 40 °C, while at ambient temperature viscosity remains 123 ml/g after 35 days hydrolysis. The molecular weight decreased from 21.4×10^4 to 3.70×10^4 after 8 h hydrolysis at 40 °C, while at ambient temperature the molecular weight only decreased to 7.1×10^4 after 35 days. Therefore, temperature plays indeed an important role in the degradation of the chitosan. Moreover, the viscosity and molecular weight of chitosan both decreased when the temperature gradually rises. For instance, the viscosity and molecular weight of the product were reduced to 40.9 ml/g and 2.00×10^4, respectively, at 80 °C. However, the yields of chitosan gradually decreased when the temperature rises, which indicates that the more chitosan is degraded into soluble chitosan or monomer at higher temperatures. The yields still remain at a high level compared with that at normal temperatures. The yield is only 40.2% when the molecular weight of chitosan is degraded to 7×10^4 at ambient temperature, while the yields could be as high as 77.2% when the molecular weight reaches 3.5×10^4 after degrading at 60 °C. Therefore, appropriate heating will help accelerate the hydrolysis reaction and raise the yields of low-molecular-weight chitosans.

Table 3
Effect of reaction time on properties of chitosan at 60 °C (reaction temperature, 60 °C; 85% phosphoric acid)

Reaction time (h)	[η] (ml/g)	M_v ($\times 10^{-4}$)	Yields (%)
1	145	16.4	89.3
2	137	15.5	87.9
3	132	15.0	83.6
4	127	7.40	79.2
5	120	6.90	77.2
6	109	5.10	68.4
7	107	5.00	71.2
8	66.4	3.50	71.4
9	59.6	2.40	66.7
12	52.6	2.10	58.2
15	49.3	1.90	49.2

3.3. Effect of reaction time on preparation of low-molecular-weight chitosan at 60 °C

The viscosity and molecular weight of chitosan recorded at different degradation reaction time with 85% phosphoric acid at 60 °C are listed in Table 3.

The result indicates that different reaction times will lead to chitosans with different molecular weights when degrading with 85% phosphoric acid at 60 °C. The degradation rate is a maximum in the first 4 h. The molecular weight decreases from 21.4×10^4 to 7.4×10^4 and then decrease slowly, finally reaching 1.9×10^4 after 15 h. The yields also decrease when the reaction time is lengthened, indicating that more chitosan is degraded to soluble chitosan or monomer. However, the yields of chitosan with a molecular weight of 3.50×10^4 after being degraded for 8 h still remains at a level as high as 71.4%. To sum up, increasing reaction temperature will speed up the reaction rate and different reaction times will result in chitosan with different molecular weights.

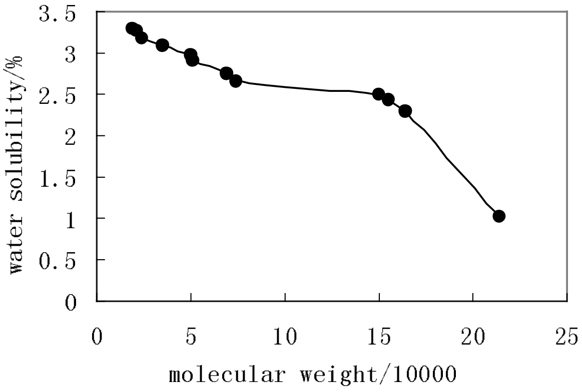

Fig. 1. Relationship between water-solubility and molecular weight of chitosan.

3.4. Relationship between molecular and water-solubility

The water-solubility of chitosan is usually evaluated in terms of turbidity. In this paper, water-solubility is measured directly. The water-solubility of 12 different chitosan with a 96% degree of deacetylation has been determined. Fig. 1 shows the relationship between water-solubility and molecular weight. The water-solubility increased with decreasing molecular-weight of chitosan. When the M_v decreased to 1.90×10^4 from 21.4×10^4, water-solubility increased to 3.20 from 1.04%. It is apparent that water-solubility is influenced by the molecular weight, lowering the molecular weight helps to improve solubility.

In conclusion, homogeneous hydrolysis of chitosan in 85% phosphoric acid was determined as a function of hydrolysis temperature and reaction time. Molecular weight between 20.0×10^4 and 1.90×10^4 of chitosan can be obtained by using 85% phosphoric acid at 60 °C in 1–15 h. The water-solubility increased with decreasing molecular weight of chitosan. These low-molecular-weight chitosan may be useful as a bio-active compounds.

References

Allan, G. G., & Peyron, M. (1995). Molecular weight manipulation of chitosan. 1. Kinetics of depolymerization by nitrous acid. *Carbohydrate Research*, *277* (2), 257–272.

Brine, G. J., Sandford, P. A., & Zikakis, J. P. (1992). *Advances in chitin and chitosan*, London: Elsevier Applied Science.

Chai, P., Zhang, W., & Jing, X. (1999). The new exploiting and researching trends of chitin and chitosan. *Huaixue Tongbao*, *7*, 8–11.

Hasegawa, M., Isogai, A., & Onabe, F. (1993). Preparation of low-molecular-weight chitosan using phosphoric acid. *Carbohydrate polymer*, *20*, 279–283.

Horowitz, S. T., & Roseman, S. (1957). The preparation of gluco-samine oligosaccharides. 1. Separation. *Journal of American Chemistry Society*, *79*, 5046–5049.

Hutadilok, N., Mochimasu, T., Hisamori, H., Hayashi, K., Tachibana, H., Ishil, T., & Hirano, S. (1995). The effect of *N*-substitution on the hydrolysis of chitosan by an endo-chitosanase. *Carbohydrate Research*, *268*, 143–149.

Jacques, D., Andree, G., & Christion, P. (1990). *Preparation of β-(1–6) oligomers of 2-acetamido-2-deoxyglacoses and galactoses as drugs*. FR, 2640628.

Lin, R., Jiang, S., & Zhang, M. (1992). The determination of degree of deacetylation. *Chemistry Bulletin*, *3*, 39–42.

Maksinov, V. I., Denisov, V. M., & Makarov, N. V. (1990). *Preparation of water-soluble oligosaccharides*. SU, 1571047.

Muzzarelli, A. A., Xia, W., Tomasetti, M., & Ilari, P. (1995). Depolymerization of chitosan and substituted chitosans with the aid of a wheat germ lipase preparation. *Enzyme and Microbial Technology*, *17*, 541–545.

Simojon, M., & Fukushima, K. (1996). Bactericidal activity of chitosan from seqid pen. *Kicbin, Kitosan, Kenku*, *2* (2), 90–91.

Todashi, I. (1990). *Preparation of water-soluble chitosan*. JP, 02 041301.

Tokura, S., Miuray, T., Johmen, M., Nishi, N., & Nishimura, S. I. (1994). Induction of drug specific antibody and the controlled release of drug by 6-*o*-carboxymethyl-chitin. *Journal of Controlled Release*, *28*, 235–241.

Tomaya, T., Yoshichi, A., & Akira, A. (1987). *Manufacture of water-soluble low-molecular-weight chitosan with narrow molecular weight distribution*. JP, 62 184002.

Wang, W., Bo, S., Li, S., & Qin, W. (1991). Determination of the Mark-Houwink equation for chitosan with different degree of deacetylation. *International Journal of Biological Macromolecules*, *13*, 281–285.