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Salt-assisted acid hydrolysis of chitosan to oligomers under microwave irradiation

Ronge Xing,[a,b] Song Liu,[a,b] Huahua Yu,[a,b] Zhanyong Guo,[a,b] Pibo Wang,[a,b] Cuiping Li,[a,b] Zhien Li[a] and Pengcheng Li[a,*]

[a]*Institute of Oceanology, The Chinese Academy of Sciences, Qingdao 266071, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*

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Abstract—The effect of inorganic salts such as sodium chloride on the hydrolysis of chitosan in a microwave field was investigated. While it is known that microwave heating is a convenient way to obtain a wide range of products of different molecular weights only by changing the reaction time and/or the radiation power, the addition of some inorganic salts was shown to effectively accelerate the degradation of chitosan under microwave irradiation. The molecular weight of the degraded chitosan obtained by microwave irradiation was considerably lower than that obtained by traditional heating. Moreover, the molecular weight of degraded chitosan obtained by microwave irradiation assisted under the conditions of added salt was considerably lower than that obtained by microwave irradiation without added salt. Furthermore, the effect of ionic strength of the added salts was not linked with the change of molecular weight.

FTIR spectral analyses demonstrated that a significantly shorter time was required to obtain a satisfactory molecular weight by the microwave irradiation-assisted inorganic salt method than by microwave irradiation without inorganic salts and conventional technology.

Keywords: Salt-assisted; Acid hydrolysis of chitosan; Microwave irradiation; Chitosan oligosaccharides; Ionic strength

1. Introduction

Chitosan oligomers have distinct physiological activities, and they are widely used in medicines, in plant physiology, in foods and cosmetics, as well as in other applications.[1–3] Because of the excellent solubility of chitosan oligomers, their applications are numerous and varied in contrast to chitin and chitosan.

Several methods have been suggested for the preparation of chitosan oligomers, but most are limited to acidic hydrolysis and traditional heating methods. Most methods have been well established. In an earlier study, chitosan oligomers with a degree of polymerization (DP) 2–5 were prepared by partial hydrolysis of chitosan with concentrated hydrochloric acid.[4] However, the chitosan oligomers in the hydrolysate obtained by this procedure were accompanied by a large amount of D-glucosamine as the reaction time increased. Therefore, hydrolysis by concentrated hydrochloric acid has usually been modified by working with 35% HCl at 80 °C for a short time.[5–7] In the process, chitosan oligomers were mainly composed of DP 1–15 and DP 20–40 as determined by analysis on Bio-Gel P-30 chromatograms.[8] In addition, partial hydrolysis with 0.5 M and 1.3 M HCl at 100 °C produced chitosan oligomers with average M_ws of 6000 and 3000, respectively.[9] Oxidative degradation in concentrated nitrous acid[10–13] provided chitosan oligomers with a DP of 9–18, but it was difficult to produce oligomers with DP below 10, and the final products contained 2,5-anhydromannose residues by deamination with nitrous acid. A method for preparing chitosan oligomers with hot phosphoric acid has been reported recently.[14]

* Corresponding author. Tel.: +86 532 82898707; fax: +86 532 82968951; e-mail addresses: pcli@ms.qdio.ac.cn; xingronge@ms.qdio.ac.cn

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The yields were reported as 10–20% for products with DP 6–8, and neutralization and desalting steps were recommended. In addition, two types of chitosan oligomer with DP 7.3 and 16.8 were prepared also by homogeneous hydrolysis of chitosan in 85% phosphoric acid at room temperature, but long reaction times of more than four weeks were required.[15] Fluorohydrolysis of chitosan in anhydrous hydrogen fluoride seemed to afford a more convenient route than conventional chemical depolymerization and produced products of DP of 2–10 in good yield.[16,17] However, this process also had practical limitations because of the necessity to perform an additional step for defluorination. Because of the limitations of chemical hydrolysis methods, research into a new method that can overcome those limitations was necessary.

In recent years, the reaction speed and the selectivity of product formation have been demonstrated to be greatly improved when chemical reactions are performed under microwave irradiation, and as a result, microwave chemistry has received much attention. Researchers not only devote themselves to expanding its application in each reaction system, but also continually to explore the mechanism of the microwave action. Shao et al.[18] prepared medicinal oligoglucosamine by the oxidative degradation of chitosan with neutral hydrogen peroxide under microwave irradiation and obtained a water-soluble oligoglucosamine. However, the degradation reaction of chitosan was not sufficient by this heterogeneous reaction method, and the molecular weight of the products was not well-proportioned. This paper applies microwave technology assisted by the addition of salts under homogeneous reaction conditions to prepare chitosan oligomers and achieves better results than those heretofore reported.

2. Experimental

2.1. Materials and apparatus

Chitosan from shrimp shells (Qingdao Baicheng Biochem. Corp., China), which had a degree of deacetylation (DA) of 0.85 and average molecular weights (M_w) of 560 KD, was used. HCl and other reagents were of analytical reagent grade and were used without further purification. All solutions were prepared with distilled water. A domestic microwave oven of 2.45 MHz (Tianjin Yuejin Electric and Electrical Appliance Limited Liability Company, WD850CMG-5588SDTW) was used.

2.2. Analytical methods

Viscosity changes during the reaction were measured by a falling-ball viscometer. The average viscometric molecular weight of chitosan was estimated from the intrinsic viscosity determined in the solvent 0.1 M CH_3COOH–0.2 M NaCl using the Mark–Houwink parameters $a = 0.96$, $K_\eta = 1.424$ at 25 °C when the intrinsic viscosity was expressed in mL/g. FTIR spectra were measured by a Nicolet Magna–Avatar 360 apparatus with samples in KBr disks.

2.3. Degradation of chitosan under microwave radiation

Inorganic salts containing 0.15 mol/L [Cl^{-1}] were added to a 300-mL Erlenmeyer flask containing 100 mL of chitosan solution in HCl (2% w/v) or HAc (2% w/v) with swirling to get gelatinous chitosan. The Erlenmeyer flask containing the mixture of reactant was placed on the center of the turntable of the microwave oven. To control the reaction temperature to ~100 °C, another 50-mL Erlenmeyer flask containing a higher boiling solvent was also placed on the turntable in the microwave oven. Different irradiation times (0.5–25 min) and power (480–800 W) were set. After the irradiation ceased, the reaction mixture was immediately cooled to room temperature. The viscosity of the resultant mixture was successively measured by falling-ball viscometry at the same intervals. The reaction was not stopped until change of viscosity was negligible. Then, the viscometric average molecular weight of the mixture was determined with an Umstatter viscometer. The solution was adjusted to pH 7–8 by the addition of 2 N NaOH. The product was then precipitated by the addition of Et_2O, and a white precipitate was obtained, filtered off, washed with Et_2O (85%), and dried overnight in vacuum at 60 °C.

3. Results and discussion

3.1. The molecular weight is affected by microwave and conventional heating

Table 1 shows the results of degraded chitosan under both microwave and conventional heating and the influence of the added salts. Entry 1 shows that the molecular weight of the degraded chitosan is about 10×10^4 in 25 min under microwave irradiation. Entry 2 shows that the molecular weight of the degraded

Table 1. The molecular weight of resultant under microwave and conventional heating

Entry	Ionic strength (I)	Molecular weight ($\times 10^4$)		
		NaCl	KCl	$CaCl_2$
1	0	10.5		
2	0.01	3.52	2.79	3.09
3	0.01[a]	51.5	50.7	51.0

[a] With conventional heating to replace microwave irradiation to maintain the reaction volume for 2 h.

chitosan is about 3×10^4 in 25 min under microwave dielectric heating assisted by inorganic salts. Entry 3 shows that the molecular weight of the degraded chitosan is about 51×10^4 in 2 h by means of conventional heating. The above-mentioned results show that microwave radiation accelerated the degradation of chitosan, and the molecular weight of chitosan was considerably lower than 51×10^4. Moreover, as shown in Table 1 the molecular weight changed abruptly when an inorganic salt was added to the reaction; namely, the molecular weight with added salt decreased about 7×10^4 Da (i.e., from $\sim 10 \times 10^4$ to $\sim 3 \times 10^4$ Da) compared to that without salt. The results suggest that the metal halide's ability to promote the hydrolysis of chitosan is due to the salt's ability to cause superheating of the solution.[19] Microwave heat involves a direct interaction with certain classes of absorbing molecules. This direct absorption can lead to localized introduction of energy to a region from the remote microwave source and raises the solution temperature.[20] Moreover, the addition of salts to solvents can increase their conductivity and has a dramatic influence on their rate of heating.[21] Furthermore, the presence of salts in polar solvents frequently enhances dielectric loss effects and microwave coupling of the solvents. Consequently, superheating effects can be magnified by the addition of such ions as NaCl. It is also possible that localized superheating effects could lead to a small but significant increase in the reaction rate and the selectivity.[22] The hydrolysis of chitosan can thus be completed in a short time in the presence of metal halides under microwave radiation.

3.2. Viscometric variation of chitosan under the same electrolyte and different ionic strength

Figures 1–3 show that for the same electrolyte of different ionic strengths the trend in viscometric variation of chitosan is almost uniform with increasing reaction time. Moreover, as shown in Figures 1–3, the influence of the ionic strength was rather limited for the viscometric variation of chitosan, especially for NaCl. With increasing reaction time, the effects of the strong ionic strength of KCl and $CaCl_2$ both become less obvious, and the order of chitosan viscosity observed for KCl and $CaCl_2$ changed to the order of $0.03 > 0.06 > 0.01$ in ionic strength.

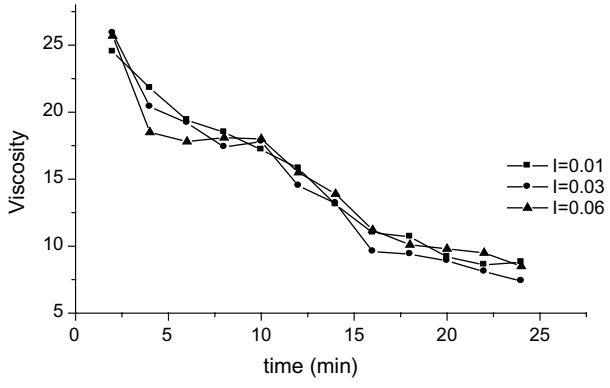

Figure 1. Viscosity changes of chitosan with addition of NaCl and different ionic strengths.

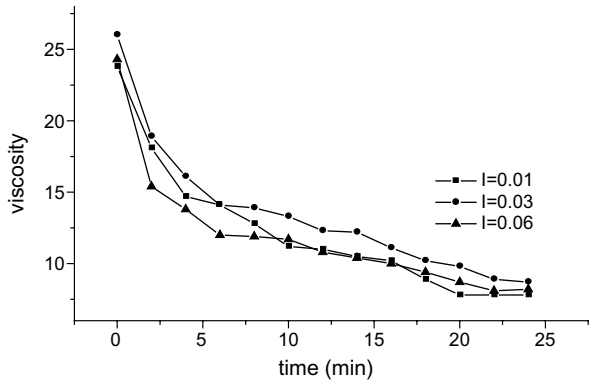

Figure 2. Viscosity changes in chitosan with addition of KCl and different ionic strengths.

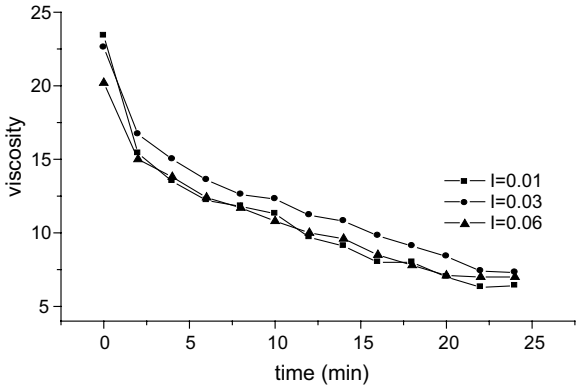

Figure 3. Viscosity changes of chitosan with addition of $CaCl_2$ and different ionic strengths.

3.3. Viscometric variation of chitosan under the equal ionic strength of different electrolytes

For chitosan solutions of equal ionic strength but of different electrolytes, the rate of degradation and hence the viscosity of the solutions is somewhat affected by different electrolytes. Figure 4 shows that the composition of the electrolyte influences the order of chitosan degradation as follows: $K^+ > Ca^{2+} > Na^+$. This result may relate to the ionic radius of the metals. Order of their ionic radius is $K^+ > Ca^{2+} > Na^+$. However, the mechanism by which the different electrolyte influences chitosan degradation needs to be further researched.

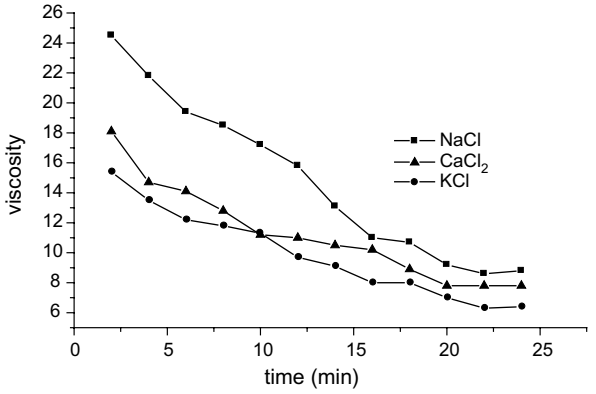

Figure 4. Different electrolytes with $I = 0.01$ influences chitosan viscosity.

3.4. FTIR analysis

The FTIR spectra determined for both chitosan and the degraded products were relatively unchanged except for the intensity of some peaks. Characteristic absorptions of chitosan and chitosan oligosaccharides at 3410 cm^{-1} (ν_{O-H} and ν_{N-H}), 2924 cm^{-1} (ν_{C-H}), 1623, 1513 cm^{-1} (δ_{N-H}), 1088 cm^{-1} (ν_{C-N}), 651 cm^{-1} (δ_{NH2}), 1153 cm^{-1} (δ_{C-O-C}) and 895 cm^{-1} (β-δ_{C-H}) are easily observed.[18] Moreover, the peak at 895 cm^{-1}, proved that the cyclic pyranosyl rings were not destroyed by microwave irradiation, nor were the amino groups at 651 cm^{-1}.

4. Conclusions

Investigations of the salt-assisted acid hydrolysis of chitosan have established that hydrolysis is accelerated significantly by the addition of inorganic salts in a microwave irradiation field. It was found that microwave heating-assisted inorganic salt is a convenient way to obtain a wide range of products of different molecular weight only by changing reaction time or/and radiation power. Moreover, microwave heating-assisted degradation in the presence of inorganic salts does not affect the pyranose rings of chitosan oligosaccharides as compared to products of traditional technology. Compared with conventional heating and microwave irradiation without inorganic salts, microwave dielectric heating assisted by inorganic salts is an efficient procedure that produces low-molecular-weight chitosan in a shorter time. Furthermore, the effect of ionic strength was not related to a change in molecular weight of the products.

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