Preparation of Water-Soluble Chitosan

Shaojie Lu,[1] Xuefeng Song,[2] Deyong Cao,[2] Yiping Chen,[2] Kangde Yao[2]

[1]*School of Science, Tianjin University, Tianjin 300072, People's Republic of China*
[2]*School of Materials Science and Engineering, Tianjin University, Tianjin 300072, People's Republic of China*

ABSTRACT: An improved method of preparing water soluble chitosan was studied by *N*-acetylation with acetic anhydride. Its merits were a simple processing technique, very short reaction time, little agent, high molecular weight of the product, and good water solubility. This article not only discusses the effect of several factors, such as the amount of reactants, concentration of hydrogen ions in the solution, and solvent system, on *N*-acetylation but also some influential factors on the water solubility of *N*-acetylated chitosan. Experiments showed that the amount of acetic anhydride was the most important factor affecting the *N*-acetylation degree of the chitosan. The effect of the means of adding materials and the amount of solvent on the reaction could not be ignored. The solubility of half *N*-acetylated chitosan was not changed with an increase in the molecular weight in water, and the water solubility decreased with increasing molecular weight in the alkaline region. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 91: 3497–3503, 2004

Key words: biopolymers; polysaccharides; compatibilization

INTRODUCTION

Chitosan, poly-β-(1→4)-D-glucosamine, is a deaceylated product of chitin. It has been used for various functional materials, including biomaterials.[1–3] However, when it is used in biological fields, its applications are restricted because it is insoluble in water and can only be dissolved in acid. To enhance the solubility of chitosan, many specialists and scholars have studied its preparation method and solubilization mechanism.[4–8] However, these methods have some defects, including tedious procedures long reaction times, the requirement of a large amount of solvent or reagent, and the low molecular weight of the final product. In this article, a modified method for the preparation of water-soluble half *N*-acetylated chitosan is described.

EXPERIMENTAL

Materials

Chitosan was purchased from Qingdao Haihui Industry, Ltd. (China). The deacetylation degree (DD) was 83 and 90%, respectively, and the viscous-average molecular weight was 1.5×10^5 to 6.0×10^5. Other chemicals were reagent-grade products of Tianjin Plant of Agents (China).

Correspondence to: S. Lu (jiesl@public.tpt.tj.cn).

Preparation of water-soluble chitosan by *N*-acetylation

The reaction of chitosan was carried out in a flask with a plug. Dried sample (Ig) was dissolved in 25 mL of 2.8% acetic acid; then, 25 mL of ethanol was added, and a little pyridine was dropped into the mixing solution until the solution became clear. Acetic anhydride was charged. After it was stirred at ambient temperature for a predetermined time, the reaction mixture was precipitated with ethanol, and the precipitate was washed with acetone to remove excess reactant. The *N*-acetylated chitosan was dried at 50°C.

Measurement of DD

The acid–base titration method was used to determine the DD from the amino group content in chitosan.[9] Dry chitosan (0.3 g) was dissolved in 30 mL of HCL standard solution (0.1 mol/L). Methyl orange and aniline blue mixing indicators were added. A standard solution of NaOH was used for titration until the solution became blue green. The following formulas were used to calculate the DD of the product:[10]

$$(-NH_2)\% = \frac{0.016(C_1 V_1 - C_2 V_2)}{W} \times 100$$

$$DD\% = \frac{203(-NH_2\%)}{16 + 42(-NH_2\%)} \times 100$$

where C_1, V_1, C_2, and V_2 are the concentrations and volumes for the HCL standard solution and NaOH

TABLE I
Effect of Different Concentrations of Chitosan–Acetic Acid Solution on Viscosity (η)

Sample	Concentration of acetic acid (mol/L)	$\eta \times 10^{-3}$ (Pa $\cdot$ S)
1	0.05	78
2	0.10	139
3	0.20	191
4	0.25	207
5	0.30	185
6	0.40	168
7	0.50	140

The concentration of the chitosan solution was 2×10^{-2}/kg/L.

standard solution, respectively, and *W* is the weight of the sample.

Measurement of molecular weight and apparent viscosity

The weight-average molecular weights of the chitosan samples were determined with a Photo-Gonio-Diffusometer CH_3COONa (0.1 mol. L^{-1}) and CH_3COOH (0.2 mol. L^{-1}) acted as solvent. Each sample was dissolved in the solvent and filtered through a G_5 glass funnel. The weight-average molecular weight was calculated by Zimm's drawing.[11] The apparent viscosity of the chitosan solution was measured by an NXS-111 rotational viscometer.
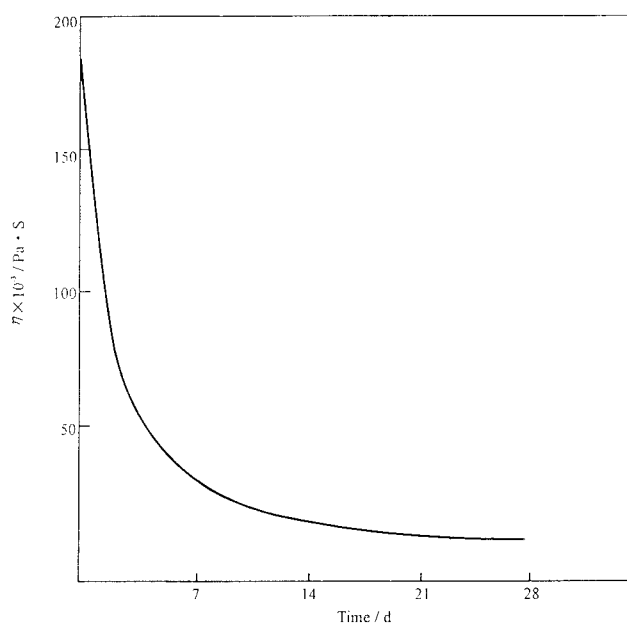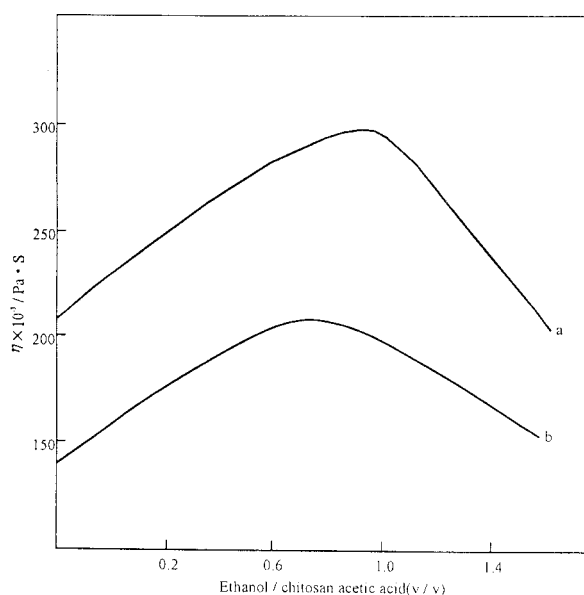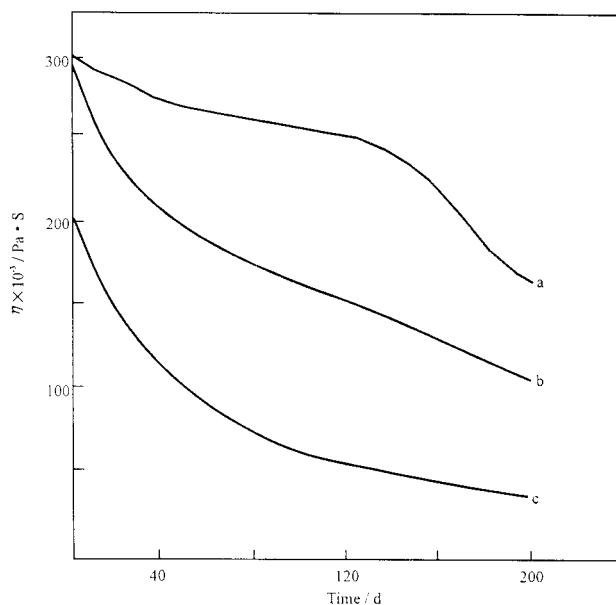

Figure 1 Stability of chitosan–acetic acid solution. Acetic acid concentration = 0.23 mol/L; chitosan–acetic acid solution concentration = 2%; and temperature = 50°C.

(A)

(B)

Figure 2 Effect of ethanol on the stability of chitosan-acetic acid solution: (A) Acetic acid concentration = 0.23 mol/L and chitosan–acetic acid solution concentration = 2%; viscograms of chitosan–acetic acid at (a) room temperature and (b) 50°C. (B) Acetic acid concentration = 0.23 mol/L; chitosan–acetic acid solution concentration = 2%; and temperature = 20°C; (a) added into ethanol (volume ratio of ethanol to 2% chitosan–acetic acid solution = 1.0), (b) added into methanol (volume ratio of methanol to 2% chitosan–acetic acid solution = 1.0), and (c) added into acetone (volume ratio of acetone to 2% chitosan–acetic acid solution = 1.0).

TABLE II
Effect of the Amount of Ac$_2$O on the *N*-Acetylation of Chitosan

Chitosan/Ac$_2$O (mol)	DD (%) at a reaction time (h) of			
	0.5	1	4	24
1:0.5	76.3	72.4	73.1	71.2
1:1	64.4	62.5	58.9	61.7
1:2	63.2	50.2	48.9	49.4
1:2.5	62.7	38.4	36.9	37.1

Chitosan (DD = 83%), = 1 g; 2.8% AcOH = 25 mL; ethanol = 25 mL; pyridine = 8 mL; reaction temperature = 25°C.

RESULTS AND DISCUSSION

Influential factors on the *N*-acetylated reaction of chitosan

Effect of the concentration of hydrogen ions in the solution

The concentration of hydrogen ions can effect the form of chitosan in the solution, and therefore, the process of the *N*-acetylated reaction is affected. The concentration of hydrogen ions in the solution primarily was determined by acetic acid. In a thin acid solution, the resolving process of chitosan started the continuous binding of hydrogen ions with free amine groups in the molecular chains of chitosan to form a cation ($-NH_3^+$). When almost all of the free amine groups in the solution became $-NH_3^+$ and there were few remaining hydrogen, the mutual exclusion of the electricity of the cation in the molecular chain caused the molecular chain of chitosan to stretch out and form a linear molecule, and the solution reached a high viscosity.[12] These conditions were beneficial to the *N*-acetylated reaction.

On the contrary, if the hydrogen ion concentration in the solution was too high, the number of negative ions increased. The negative ions gathered around $-NH_3^+$. This process reduced the repellency among positive ions and the molecular chains fold and, therefore, decreased the viscosity.

To sum up, the form of chitosan in the solution was recognized by its viscose change. About 2% of chitosan resolved in acetic acid-solution, which had a differential concentration, and a viscose change was observed, as shown in Table I. The viscosity increased with increasing acid concentration when the concentration of acetic acid was lower. Then, the viscosity of the chitosan solution reached a maximum value between 0.2 and 0.3 mol/L acetic acid. After that, the viscosity of the chitosan solution decreased with further increasing acid concentration. The experiment showed that an appropriate ratio of chitosan to acetic acid in the chitosan–acetic acid solution was necessary.

Effect of the solvent system

The *N*-acetylated reaction of chitosan was carried out in homogenous conditions and therefore, solvent was

TABLE III
Effect of the Reaction Temperature on the *N*-Acetylation of Chitosan

Temperature (°C)	DD (%) at a reaction time (h) of						
	0.5	1	2	4	8	12	24
25	63.2	50.2	46.7	48.9	48.2	47.4	49.4
30	64.7	49.2	48.7	50.1	50.9	49.8	47.2
35	62.7	47.9	50.1	47.2	46.9	49.4	50.3
45	63.4	51.1	48.4	48.2	47.9	46.1	49.6

Chitosan (DD = 83%) = 1 g; 2.8% AcOH = 25 ml; ethanol = 25 mL; pyridine = 8 mL; chitosan/Ac$_2$O (mol) = 1:2.

TABLE IV
Effect of Highly Deacetylated Chitosan on the *N*-Acetylated Reaction

Chitosan/Ac$_2$O (mol)	DD (%) at a reaction time (h) of			
	0.5	1	4	24
1:0.5	76.8	73.1	71.9	72.4
1:1	65.2	63.3	60.8	59.5
1:2	63.7	51.0	49.2	50.3
1:2.5	62.9	39.2	40.0	38.6

Chitosan (DD = 90%) = 1 g; 2.8% AcOH = 25 mL; ethanol = 25 mL; pyridine = 8 mL; reaction temperature = 25°C.

TABLE V
Effect of Different DD Values on the Water Solubility of
***N*-Acetylated Chitosan**

DD (%)	Water solubility	Solvable time at 25°C (h)
72.1	Insoluble in water	
64.4	Dissolved in water	≥10
62.5	Dissolved in water	≥10
58.7	Dissolved in water	>4
52.1	Dissolved in water	≤4
50.2	Dissolved in water	≤4
46.7	Dissolved in water	≥10
43.0	Insoluble in water	
38.4	Insoluble in water	

Molecular weight of samples = 0.28 million.

TABLE VI
Effect of Different Molecular Weights on the Water
Solubility of *N*-Acetylated Chitosan

Molecular weight of the sample		
Before the reaction	Molecular weight drop after the reaction (%)	Water solubility
1.8×10^5	6.67	Dissolved within 3.0 h
3.2×10^5	6.50	Dissolved within 3.5 h
6.3×10^5	5.0	Dissolved within 4.5 h

DD of samples = 50–52%; reaction time = 1 h.

very important for the reaction. This system had a complex solvent system: acetic acid–water–ethanol and a little pyridine. Acetic acid is a weak acid and is a very common solvent for chitosan. However, chitosan is not stable in acetic acid, and hydrolysis occurs: so, the molecular chains of chitosan decompose, and the viscosity decreases speedily (see Fig. 1). Therefore, it is necessary to keep the chitosan solution stable. Experiments found that the viscosity of the solution increased after ethanol was added into the chitosan–acetic acid solution, and the viscosity increased with increasing amounts of ethanol. However, the viscosity reached a maximum value when the volume ratio of ethanol to 2% chitosan–acetic acid solution was 1.0 [see Fig. 2(a)]. We performed other tests, too.

The results show that the stability of the chitosan–acetic acid solution was efficiently improved by the addition of ethanol. [see Fig. 2(b)]. However the mixture tended to form a gel during the course of *N*-acetylation in the solution, which was unfavorable for the reaction. Gelation was avoided when a little pyridine was added into the system. Pyridine is a good solvent, and it impelled acetylation to occur easily.[13]

Effect of other factors

The *N*-acetylated reaction of chitosan was carried out with acetic anhydride as an acetylated agent. Therefore, the amount of acetic anhydride was the most important factor affecting the *N*-acetylation degree of the chitosan. The effect is shown in Table II. The data indicate that DD decreased along with increasing amounts of acetic anhydride. The reaction rate of *N*-acetylation was fast. It took 1 h for a balance to be reached, and the *N*-acetylation degree did not change with time after an hour. Its mechanism remains to be studied carefully. When the molar ratio of chitosan to Ac_2O was 1/2, half *N*-acetylated chitosan was obtained.

The impact of the reaction temperature on the *N*-acetylation of chitosan was not obvious. Acetylation could be carried out at room temperature (see Table III).

The effects of the means of adding materials and the amount of pyridine on the reaction could not be ignored. We found that when acetic anhydride was added to the system first and pyridine was added afterward or both were added at the same time that gelation could occur. However, the reaction went off well in the reverse order. In addition, too much pyridine led gelation, too.

The results of the preparation of half *N*-acetylated chitosan with highly deacetylated chitosan (90%) under the same conditions are shown in Table IV. The effect of the DD on the *N*-acetylated reaction was not obvious; so, chitosan with different DDs could be used under the same reaction conditions for the preparation of water-soluble chitosan.

TABLE VII
Relationship Between the Water Solubility and the pH Value of *N*-Acetylated Chitosan

No.	Molecular weight	Water solubility (1 %)				
		pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
1[a]	4.6×10^3	100	100	100	100	100
2[b]	1.8×10^5	100	100	100	84	20
3[b]	3.2×10^5	100	100	100	60	12
4[b]	6.3×10^5	100	100	100	25	5

T% = transmittance. Water solubility of the *N*-acetylated chitosan was evaluated from the change in turbidity.
[a] DD = 85%.
[b] DD = 50–52%.

TABLE VIII
Effect of Different Preparation Routes on the Water Solubility of *N*-Acetylated Chitosan

DD (%)	Water solubility of chitosan	
	Regenerated from solution with alkali	Regenerated from solution with ethanol
62.5	Insoluble in water	Dissolved in water
52.1	Insoluble in water	Dissolved in water
50.2	Insoluble in water	Dissolved in water
46.7	Insoluble in water	Dissolved in water

Molecular weight of samples = 0.28 million.

Influential factors on the water solubility of chitosan

The *N*-acetylation degree of the chitosan determined its solubility in water. This is solid. For example, Table V shows the relationship between the water solubility of the samples and their DD values. The data revealed that the samples were soluble when the DD of chitosan was 46.7–64.4%. However, dissolution of half *N*-acetylated chitosan was easier than that of the other samples. Moreover, the water solubility was not changed with increasing molecular weight (e.g., Table VI). However, the water solubility decreased with increasing molecular weight in the alkaline region (see Table VII).

Whether DD was only influential factor for water solubility of chitosan or not we found that there were two routes for regenerating chitosan from solution. One was by coagulation on immersion in ethanol, and the other was precipitation with alkali. Chitosan regenerated from first method was soluble, and it was insoluble with alkali (see Table VIII). X-ray diffraction (XRD) pattern of samples implied that the insolubility of the sample may have stemmed from an increase in crystallinity caused by coagulation on immersion in alkali (see Fig. 3). In the pattern, Fig. 3(a) is the XRD pattern of half *N*-acetylated chitosan that was regenerated from solution by precipitation with ethanol. A broad peak of 2θ around 20° and another peak around 12° were ascribed to the diffraction of the plane of the crystal region in the chitosan structure. Diffraction intensity (I_{cps}) values were 100 and 80, respectively. When the route of the regeneration of chitosan was altered, a distinct spectrum was obtained for sample of precipitation with alkali [see Fig. 3(b)] In the pattern, three sharp peaks around 11–21° were observed. I_{cps} values were 132, 202, and 210, respectively. This showed that the crystallization of the sample increased. Crystallization caused by regeneration with alkali should have prevented water from entering the grown crystalline portion,[14] so the water-solubility of the sample decreased.

As mentioned previously, the chitosan sample precipitated from solution by coagulation on immersion in alkali had a high degree of crystallinity, and therefore, this led to water insolublity of chitosan. We surmised
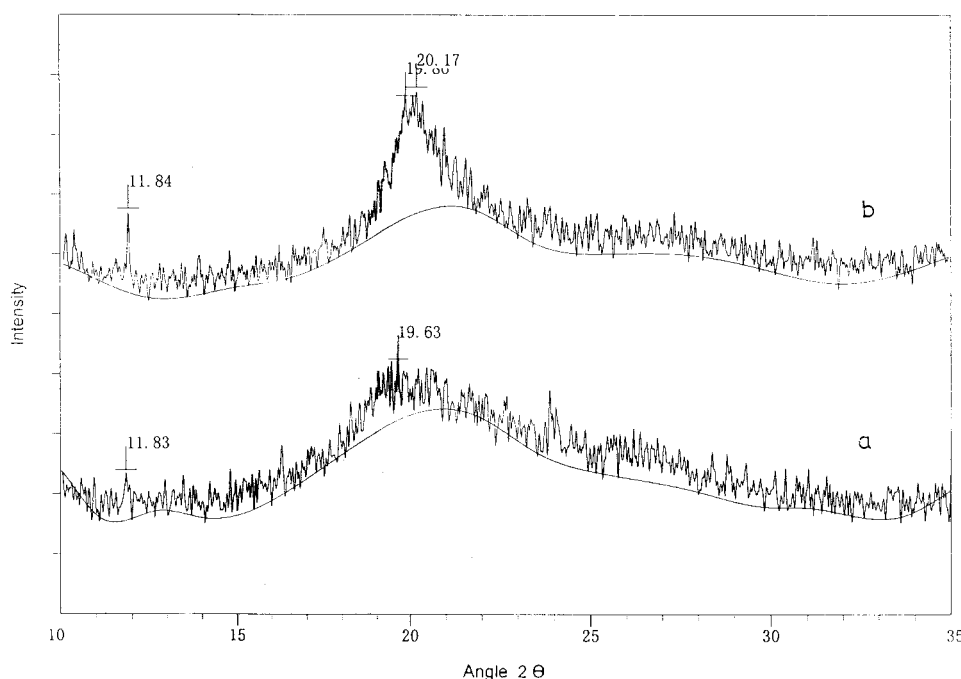

Figure 3 XRD patterns of half deacetylacted chitosan (2θ). Regeneration of chitosan from solution was by (a) coagulation on immersion in ethanol (2θ = 11.83°; I_{cps} = 80; 2θ = 19.63°; I_{cps} = 100) and (b) precipitation with alkali (2θ = 11.84°; I_{cps} = 132; 2θ = 19.68°; I_{cps} = 210; 2θ = 20.17, I_{cps} = 202).
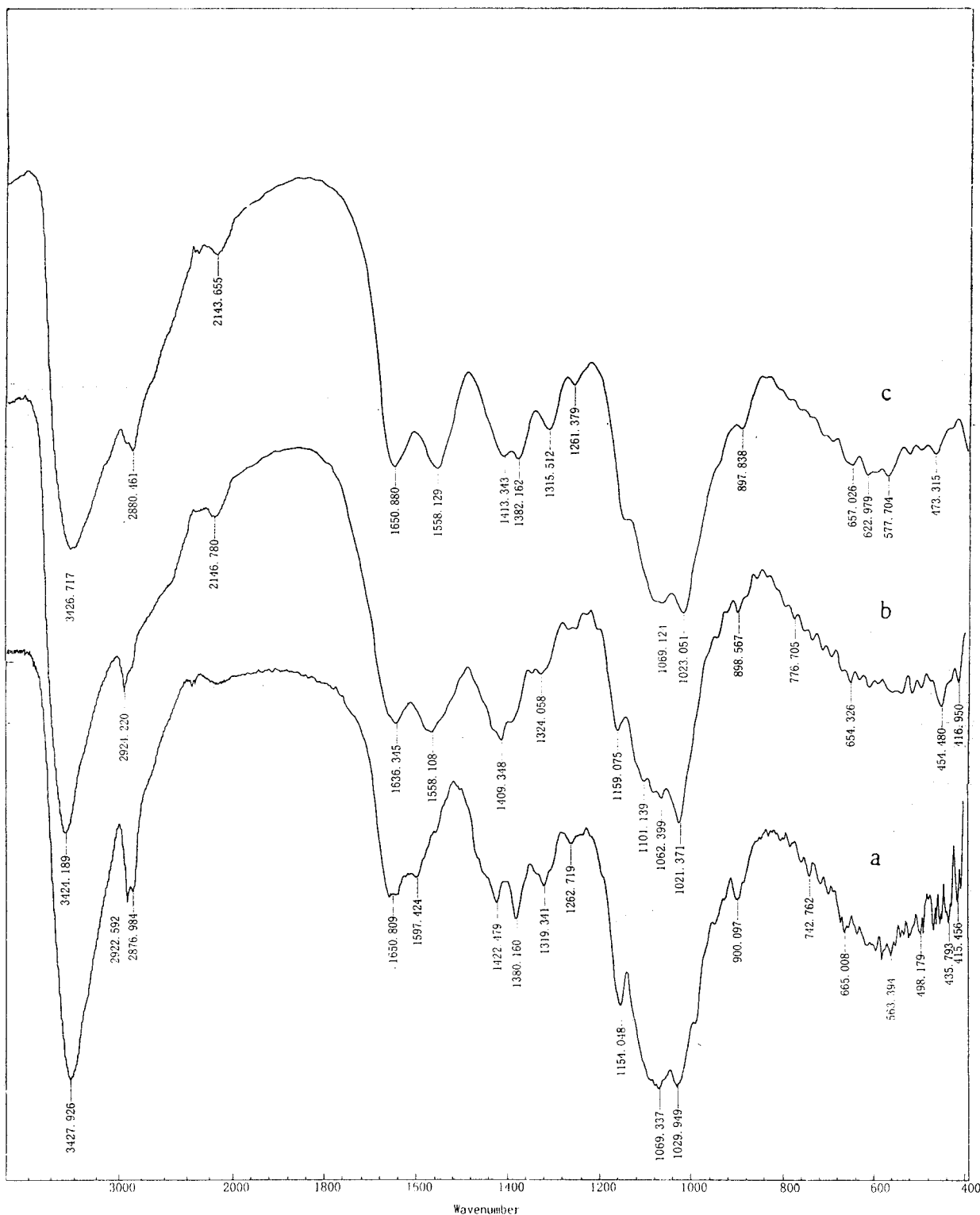
Figure 4   IR spectra of *N*-acetylated chitosan: DD = (a) 83%, (b) 52.7%, and (c) 46.7%.

that the chitosan sample with the amorphous form and loosely aggregated state was soluble.

IR analysis of soluble chitosan samples

Figure 4 shows the IR spectra of chitosan samples from different acetylation degrees. It shows the char-

acteristic absorption bands of chitosan at 1650 and 1597 cm^{-1} due to –CONH$_2$ stretching vibrations. The two peaks became more enhanced when chitosan was acetylated, especially concerning band II of the amide group observed. This showed that the acetylated reaction mainly occurred at the amino group of chi-

tosan.[15] In addition, the absorption peaks of C_6–OH and C_3–OH were weakened to some extent, and they disappeared at 1200–1030 cm^{-1}.

The N-acetylation of chitosan may have led to several effects on the amount and distribution of amino groups and N-acetyl groups in the molecular chains of chitosan. Destruction of the secondary structure of chitosan was the highest when chitosan formed half N-acetylated chitosan. As shown in Figure 4, the absorption peaks due to C–H stretching of methyl and methene were weakened at 2922 and 2876 cm^{-1} when half N-acetylated chitosan was prepared by N-acetylation with acetic anhydride. The result was that the crystallinity of chitosan was reduced.[16]

CONCLUSIONS

N-acetylated chitosan with whole water solubility was prepared via a simple processing techniques. When chitosan of a concentrated aquatic solution was used, the rate of N-acetylation greatly increased and made the process easy to complete in a very short time. The amount of acetic anhydride greatly decreased in such an aqueous system.

The essence of the dissolution of chitosan was the destruction of intermacromolecular hydrogen bonds and interchain hydrogen bonds, which altered the secondary structure of chitosan, decreasing its crystallinity and unfolding its molecular chains. When it formed a polyelectrolyte that dissolved in water, the chitosan became soluble in water. Water-soluble chitosan was regenerated from the solution by coagulation on immersion in ethanol. When alkali was added to the system, the chains of chitosan were deprotonated, and they packed together to aggregate with each other, and the chitosan became insoluble. This regeneration method should not be used.

References

1. Pariser, E. R.; Lombardi, D. P. Chitin Sonrcebook; Wiley: Chichester, England, 1989.
2. Dumitriu, S. Polysaccharides; Marcel Dekker: New York, 1998.
3. Suh, J. K. F.; Matthew, H. W. T. Biomaterials 2000, 21, 2589.
4. Sannan, T.; Kurita, K.; Iwakura, Y. Makromol Chem 1976, 177, 3589.
5. Kurita, K.; Koyoama, Y.; Nishimura, S.; Kamiya, M. Chem Lett 1989, 1597.
6. Kurita, K.; Sannan, T.; Iwakura, Y. Makromol Chem 1997, 178, 3197.
7. Kubota, N.; Tatsumoto, N.; Sano, T.; Toya, K. Carbohydrate Research, 2000, 324, 268.
8. Wang, X.; Ma, J.; Wang, Y.; He, B. Biomaterials 2001, 22, 2247.
9. Domszy, J. G.; Roberts, G. A. F. Makromol Chem 1985, 186, 1671.
10. Luo, P.; He, B. B.; Lin, X. J. Chem Res App 2000, 12, 677.
11. Zhewen, H. Texbook of Polymer Science; Huadong University of Science and Technology Press: Shanghai, 2001.
12. Jiang, T. D. Chitosan; Chemical Industry Press: Beijing, China, 2001.
13. Neckers, D. C.; Doyle, M. P. Organic Chemistry; Wiley: New York, 1977.
14. Iwamoto, R.; Miya M.; Mima, S. J Polym Sci Polym Phys Ed 1979, 17, 1507.
15. Wang, A. Q.; Yu, X. D. J Funct Polym (China) 1998, 11, 83.
16. Hughes, J. Biotechnol Tech 1990, 4, 55.