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Conversion of crystal structure of the chitin to facilitate preparation of a 6-carboxychitin with moisture absorption–retention abilities

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Abstract

Chitin has been subjected to regiospecific oxidation at C-6 with NaOCl in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaBr at room temperature in aqueous solution to yield fully soluble 6-carboxychitin. Several physical and chemical pretreatments of the original chitin changed its crystal structure from α to β. After this pretreatment of the chitin the oxidation was easier to effect and the yield was greatly increased from 36% to 97% and the molecular weight was about 4×10^4 which was ca. 8 times that from the unpretreated chitin. Infrared spectroscopy (IR), X-ray diffraction, ^{13}C NMR and solid-state NMR measurements, and thermal analysis techniques were used to characterize their molecular structures. The moisture absorption and retention abilities of these types of compounds were compared with those of sodium hyaluronan and carboxymethyl chitosan (CMCS) and were found to be superior. They therefore have the potential to substitute for hyaluronan for use in cosmetics and clinical medicine fields.

Keywords: Chitin; Crystal structure; 6-Carboxychitin; Moisture absorption; Moisture retention

1. Introduction

Chitin, commonly found in the exoskeleton or cuticles of many invertebrates and in the cell walls of most fungi and source algae, is one of the most abundant, easily obtained and renewable natural polymers, second only to cellulose (Li, Zhuang, Liu, Guan, & Yao, 2002). As an environmentally friendly material, it has attracted more and more researches (Bhunia, Jana, Basak, Lenka, & Nando, 1998; El-tahlawy, El-bendary, Elhendawy, & Hudson, 2005; Hirano, Noishiki, & Kinugawa, 1985; Yoon et al., 2000). Despite much recent research into utilization of chitin, its poor solubility in neutral water and in common organic solvents has so far prevented it from enjoying widespread utilization (Morita, Sugahara, Zbonai, & Takahashi, 1999).

Chemical modifications of chitin in order to obtain water-soluble products have therefore received much attention (Morimoto, Saimoto, & Shigemasa, 2002). The conversion of chitin into a water-soluble form can be achieved through oxidation at C-3, C-6 of chitin by introducing –COOH groups onto –OH along the chitin molecular chain. Since the 1950s, various works (Becher, Schlaak, & Strasdeit, 2000; Chen, Vassallo, & Chatterjee, 1985; Li et al., 2002; Tokura, Nishimura, & Nishi, 1983) have devoted their particular interest to O-carboxymethylation of chitin. In our previous work, 3,6-*O*-carboxymethyl chitin was successfully made by preparing an alkaline chitin solution before carboxymethylation (Chen, Du, Wu, & Xiao, 2002). The oxidation of chitin proposed by Horton and Just (1973) including the protection of the amino group as perchlorate salt, precipitation with excess perchlorate, suspension in acetic acid, and

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oxidation at C-6 with chromic anhydride has been applied occasionally. Oxidation of the chitin with NaOCl in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaBr at room temperature under mildest experimental regiospecific conditions has yielded novel biopolymers soluble over significant pH ranges (Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999). This reaction is of great importance as the fundamental chemical structure of the oxidation product is thereby brought much closer to that of hyaluronan than carboxymethyl chitin as shown by the molecular structures in Fig. 1. According to earlier work (Muzzarelli et al., 1999) the oxidation yield is only 36% and the molecular weight of the product is decreased to as low as 5000. In this study, pretreatment of the chitin before oxidation can improve the oxidation yield to 97% and the molecular weight can be better preserved at 4×10^4 which is eight times larger.

Hyaluronan (HA), which belongs to glycosaminoglycan type carbohydrate polymers, is unique for its moisture-retention ability and plays a key role in cosmetics and clinical medicine (Kennedy, Phillips, Williams, & Hascall, 2002a, 2002b). Although HA is ubiquitous in plants and animals, the total amount currently available is limited, and the price is very high (Bakos, Soldan, & Hernandez, 1999). Chitin derivatives appear to be more suitable than others for preparing HA-like substances (Matsumura, Cheng, Minami, Yoshikawa, & Kariyone, 1989). Our previous work has systematically studied the relationship between the molecular structures and the functions of carboxymethyl chitin (Chen et al., 2002; Chen, Du, & Zhen, 2003). In this work, oxidation was easy to effect and the yield was greatly increased due to the change of the crystal structure of chitin from alpha to beta, and the product exhibited interesting moisture-absorption and moisture-retention properties.

2. Experimental

2.1. Materials

Chitin was supplied by Yuhuan Aoxing Biochemistry Co. Ltd., Zhejiang, China. 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO) was obtained from Sigma–Aldrich China (Shanghai, China). Carboxymethyl chitosan (CMCS) was prepared in the lab, degree of deacetylation 0.8, degree of substitution 0.6, and molecular weight of which calculated from the GPC method was about 2.4×10^5. Sodium hyaluronate (cosmetic grade) was purchased from Shangdong Freda Biochem Co. Ltd., Ji'nan, China. All other chemicals were of reagent grade and used as received.

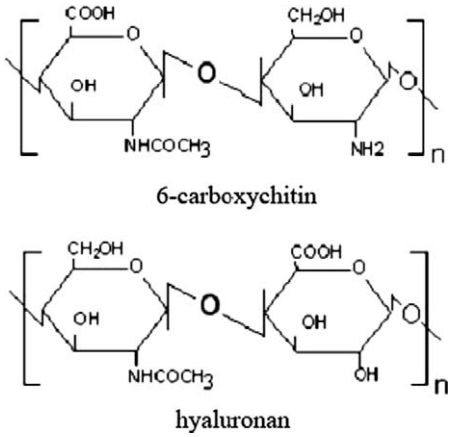

Fig. 1. The structure of 6-carboxychitin and hyaluronan.

2.2. Preparation of 6-carboxychitin

2.2.1. Conformational conversion of chitin

A series of physical and chemical methods were used to change the crystal structure of chitin: sample C_0 was chitin without any treatment, C_1 was chitin powder soaked in warm water at 50 °C for 4 h then dried at about 60 °C, C_2 was the acetylated product of chitosan, and C_3 was the chitin reprecipitated with water after having been dissolved according to the following procedure. A suspension of pure chitin (20 g) in distilled water (500 ml) was progressively mixed with cold concentrated H_2SO_4 (640 ml), the temperature being kept below 10 °C; the resultant viscous acid solution (100 ml) was filtered through glass wool into distilled water (900 ml) with continuous stirring.

2.2.2. Preparation of 6-carboxychitin (Muzzarelli et al., 1999)

The sample C_0 (1 g) was added to distilled water (50 ml) to form an aqueous suspension, then TEMPO (12 mg) and NaBr (0.4 g) were added, followed by 4% w/v aqueous NaOCl (24 ml). Immediately after the introduction of the latter, the pH was adjusted to 10.8 with 0.1 M NaOH and kept at this value for 30 min by the appropriate addition of drops of 4 M HCl. The solution was subjected to dialysis for at least four changes of demineralised water over 4 days. It was finally freeze-dried to yield a white powder signed with P_0. The same procedures were made to C_1, C_2, and C_3 separately. The products were marked as P_1, P_2, and P_3.

2.3. Moisture absorption and retention test (Matsumura et al., 1989)

Prior to the moisture-absorption testing, the samples were dried over P_2O_5 in vacuo for 24 h. The water-absorption ability was evaluated by the percentage of weight increase of dry sample (R_a):

$$R_a(\%) = 100 \times (W_n - W_0)/W_0,$$

where W_0 and W_n are the weights of sample before and after putting it into a saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and in a saturated K_2CO_3 desiccator (43% relative humidity) at 20 °C for 96 h.

In the moisture-retention test, wet samples were prepared by adding water to 10% to each sample. The moisture-retention ability was evaluated by the percentage of residual water of wet sample (R_h):

$$R_h(\%) = 100 \times (H_n/H_0),$$

where H_n and H_0 are the weights of water in the sample before and after putting in a desiccator with silica gel at 20 °C for 70 h.

2.4. Characterizations

FT-IR spectra were recorded as KBr pellets on a Nicolet FT-IR 360 spectrophotometer (Madison, Wisconsin, USA). Sixteen scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

X-ray diffraction patterns of the degraded chitosan fractions were measured by a Shimadzu LabX XRD-6000 (Japan) diffractometer and used a CuKα target at 40 kV and 50 mA at 20 °C. The relative intensity was recorded in the scattering range (2θ) of 8–40°.

Thermogravimetry (TG) and differential scanning calorimetry (DSC) were performed using a Setsys 16 TG/DTA/DSC (Setaram, Caluire, France), under a nitrogen atmosphere of 0.15 MPa, from 20 to 800 °C, at a heating rate of 10 °C/min.

^{13}C NMR spectra were recorded on a Varian mercury VX-300 (Palo Alto, CA, USA) spectrometer and chemical shifts were given by taking methanol as reference for ^{13}C NMR in D_2O at 323 K.

The solid-state NMR experiments were carried out at 9.4 T on a Varian (Palo Alto, CA, USA) Infinityplus-400 spectrometer at room temperature.

3. Results and discussion

3.1. Conversion of crystal structure from α-chitin to β-chitin

In these experiments, three methods including physical or chemical were used to pretreat the original material chitin C_0. The pretreatment made to C_1 and C_2 changed the higher degree of structure order of the chitin, and have greatly changed the crystal structure of chitin while that of C_3 has changed the crystal structure from α-chitin to β-chitin. These conclusions were made based on the characterization and analysis of the infrared spectroscopy (IR), X-ray diffraction, ^{13}C NMR and solid-state NMR measurements, and thermal analysis of them which were detailed in Sections 3.1.1, 3.1.2, 3.1.3, 3.1.4 and the summary of the conversion is also listed in Table 1.

Chitin is a (1,4)-linked linear polymer of *N*-acetyl-β-D-glucosamine and has at least three crystalline allomorphs, namely α-, β-, and γ-chitin. α-Chitin, the most abundant one, is also the most stable thermodynamically. Structurally, α-chitin is stabilized by two intramolecular hydrogen bonds, C(3)–OH···O–C(5) and C(6)–OH···O=C, and two intermolecular hydrogen bonds, NH···O=C and C(6)–OH···OH–C(6) (Zhang, Haga, Sekiguchi, & Hirano, 2000). β-Chitin consists of an array of poly-*N*-acetyl-D-glucosamine chains all having the same sense, which are linked together in sheets by N–H···O=C hydrogen bonding of the amide groups. In addition to the O(3)···HO(5) intramolecular hydrogen bond, analogous to that in cellulose, the CH_2OH side chain forms an intrasheet hydrogen bond to the carbonyl oxygen on the next chain (Gardner & Blackwell, 1975). Compared with α-chitin, β-chitin has much higher moisture-absorption and moisture-retention abilities (Kurita, Tomita, & Ishll, 1993a; Kurita, Tomita, & Tada, 1993b) and it is easier to be modified and to react. Table 1 lists out the different character between α-chitin and β-chitin in IR, XRD, and TG analyses.

3.1.1. FT-IR spectra

The FT-IR spectra of samples C_0, C_1, C_2, and C_3 are shown in Fig. 2. The most intense and useful absorption bands are the amide I vibration bends, which appear in the 1660 cm^{-1} region in sample C_0, and the band did not shift in sample C_1, C_2, and C_3, but in C_0 curve there is an additional absorption band near 1660 cm^{-1}, while the absorption intensity decreased and even disappeared in curves of C_1, C_2, and C_3. This concurred with an earlier

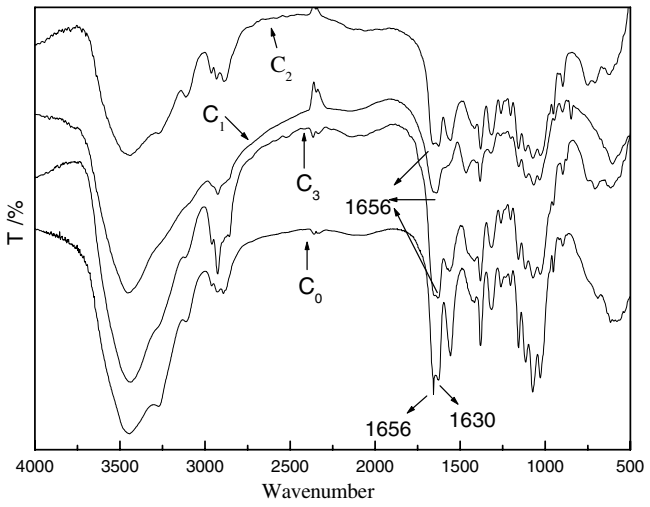

Fig. 2. FT-IR spectra of original chitin (C_0), chitin soaked before being dried (C_1), acetylated chitosan (C_2), and chitin reprecipitated after being dissolved (C_3).

Table 1
Different characteristics of α-chitin and β-chitin in IR, XRD and TG analysis

Spectrum	α-Chitin	β-Chitin	Ref.
IR	Amide I at 1660 cm^{-1}, additional absorption band near 1660 cm^{-1}	Amide I at 1660 cm^{-1}, No additional absorption band near 1660 cm^{-1}	Kurita et al. (1993a, 1993b)
XRD	High crystalline	More amorphism	Focher et al. (1992)
TGA	Water lost at 77 °C Degradation at 600 °C	Water lost at 95 °C Degradation at 720 °C	Jiang (2001)

study (Kurita et al., 1993a, 1993b). It seems to be that the crystal structure might change from α-chitin to β-chitin compare the curve C_3 with C_0.

3.1.2. X-ray analysis

In the X-ray diffractograms of C_0, C_1, C_2, and C_3 shown in Fig. 3, the diffraction spectra of C_0 exhibit three major crystalline peaks at 2θ 9.3, 12.4, and 19.1 (Focher, Naggi, Torri, Cosani, & Terbojevich, 1992), while after pretreatment the peaks presented at 9.3, 12.4 2θ values were significantly weaker and even disappeared in the spectrum for sample C_3. The peak at 2θ 19.1 was also decreased. The results indicate that the pretreatments have greatly changed the crystal structure of chitin.

3.1.3. Thermal analysis

The thermostability of chitin is related to its crystalline structure. The TG and DSC curves of sample C_3 were very different from those of sample C_0 in the three stages which are shown in Fig. 4. The first water-loss stage (C_3) reached a maximum at 60 °C with a weight loss of 10.02%. T_{max} (the temperature when weight loss reaches a maximum) of the first stage was higher than that of sample C_0. The T_{max} in the second stage of sample C_3 was similar to that of sample C_0. The third stage was the decomposition stage of chitin reaching maxima at 720 °C with a weight loss of 89.5% in sample C_3. T_{max} (646 °C) of sample C_0 was therefore lower than that of C_3 (Jiang, 2001). Thus, both the temperature when water was lost and the final decomposition temperature of α-chitin are lower than those of β-chitin which was quite coincident with the Jiang's reports. These results indicated that pretreatment most likely changed the higher degree of structure order of the chitin.

3.1.4. Solid-state NMR spectroscopy

Solid-state NMR spectroscopy is an effective tool to analyse the structure of chitin. The solid-state NMR spectrum of chitin samples C_0 and C_3 measured at 25 °C is shown in Figs. 5 and 6. The chemical shifts and the peaks ascription of the samples are marked in the figures. Comparison of the spectra of C_0 with the C_3 spectra shows that the C-3 and C-5 carbon atoms give two partially resolved peak for the chitin sample C_0 but only a single broad and asymmetric peak for the chitin sample C_3. This is the evident difference to identify chitin polymorphs by use of the C-3 and C-5 chemical shifts (Kameda, Miyazawa, Hiroshi, & Mitsuru, 2005; Rajamohanan, Ganapathy, Vyas, Ravikumar, & Deshpande, 1996; Steven, Henri, Marc, Jean, & Francoise, 1990).

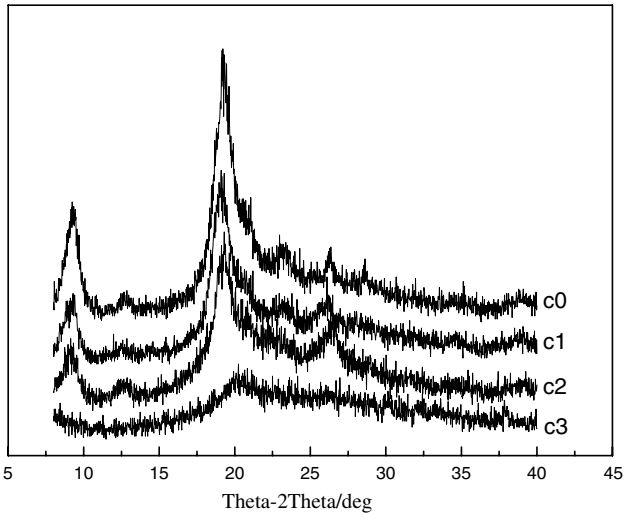

Fig. 3. X-ray diffraction patterns of original chitin (C_0), chitin soaked before being dried (C_1), acetylated chitosan (C_2), chitin reprecipitated after being dissolved (C_3).

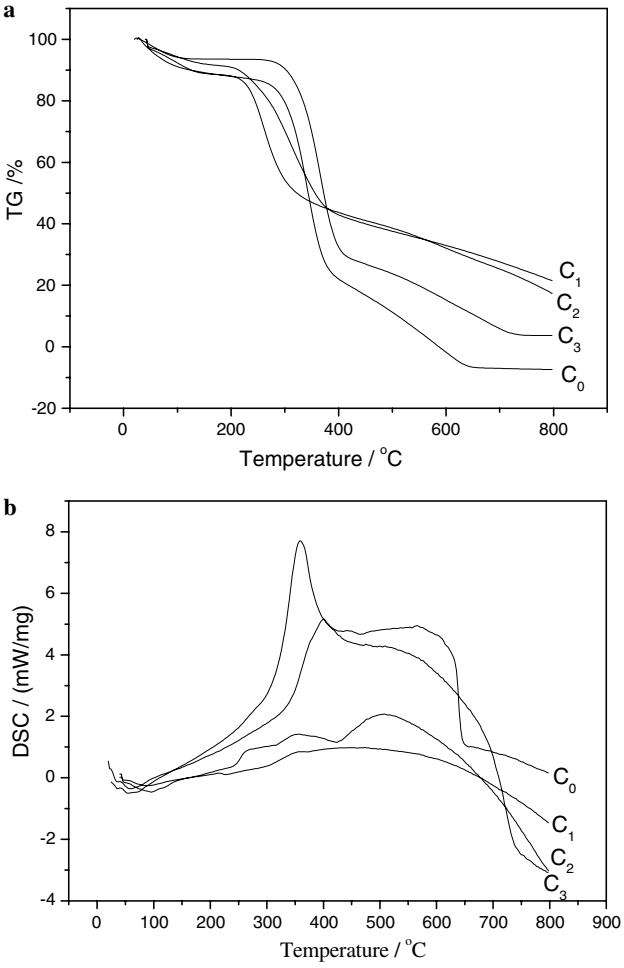

Fig. 4. Thermogravimetry (a) and Differential Scanning Calorimetry (b) curves of original chitin (C_0), chitin soaked before being dried (C_1), acetylated chitosan (C_2), and chitin reprecipitated after being dissolved (C_3).

3.2. Effect of pretreatment of chitin to the yield of oxidation

In this paper, we focused the effect of the pretreatment of the chitin on the oxidation, and the result is that the chitin which has been treated was easily oxidised and the yield of oxidation was greatly increased. Parameters such as DD and M_W of chitin may also affect the oxidation, although this was not taken into account in the original experiment design.

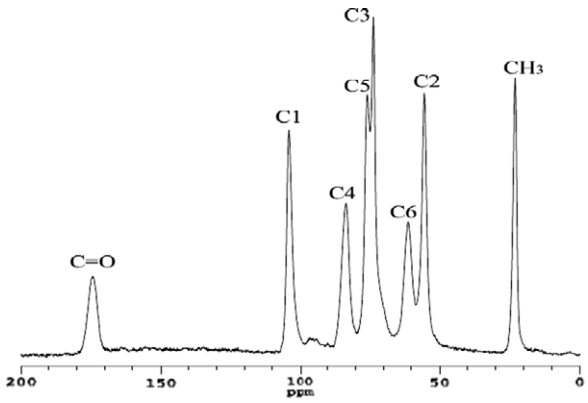

Fig. 5. Solid-state ^{13}C NMR spectrum of sample original chitin (C_0).

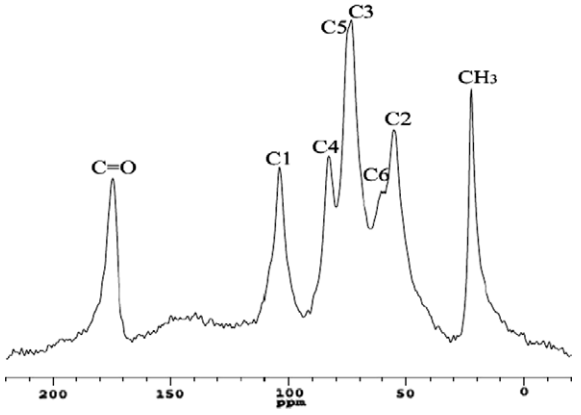

Fig. 6. Solid-state ^{13}C NMR spectrum of sample chitin reprecipitated after being dissolved (C_3).

The preparation of 6-carboxychitin has been detailed by Muzzarelli et al. (1999), but the yield was quite low due to the close arrangement of chitin molecules, regularity of molecular chain, the high rigidity, and the strong interaction existing in the intermolecular or intramolecular hydrogen bonds. In this study, pretreatment with different physical or chemical methods changed the turn of arrangement of the chitin molecular, disrupting the tightness of molecular chain and loosen the molecular of chitin, therefore it is easy to react. The yield of the oxidation is shown in Table 2. From this table combined with Fig. 3, it can be presumed that the reaction first took place preferentially in the amorphous region and with further oxidation, the crystalline structure was destroyed and the crystallinity disappeared.

α-Chitin is the dominating form of chitin, in which the unit cell is thought to be characterized by a $P2_12_12_1$ space group and to contain two antiparallel chains, whereas crystalline β-chitin has a monoclinic unit cell with $P2_1$ symmetry and a single chain located on the 2_1 axis, in which the chitin chains are therefore packed in parallel. Blackwell's studies (Blackwell, 1969) have proposed that β-chitin adopts sheet-like structures that are formed by hydrogen bonds linking the $–CH_2OH$ with the carbonyl groups on neighboring chains. When β-chitin swells in water, these sheets remain intact but move apart to include the water molecules, while the strong interaction of the molecular chains in α-chitin makes it difficult to include the water molecules, limiting purification, structure determination and chemical modification. On the other hand, β-chitin has a higher reactive ability than α-chitin due to the loose arrangement of molecule and the weak molecular interaction. Conversion of the conformation of chitin from α to β is therefore effective to make chemical modification easier.

Table 2
The yield of oxidation and the molecular weight of the products

Sample	C_0	C_1	C_2	C_3
Yield (%)	36	58	92	97
Product	P_0	P_1	P_2	P_3
M_W ($\times 10^4$)	0.5	1.8	3.5	3.9

3.3. Characterization of 6-carboxychitin

In the IR spectra of chitin and 6-carboxychitin sample P_3 (Fig. 7), the strong absorptions at 1661, 1571, and 1318 cm^{-1} in the spectrum chitin are assigned to the amide I, II, and III vibration bends. Comparing with the C–O stretching band at 1030 cm^{-1} corresponding to the primary hydroxyl group, it was decreased in the 6-carboxychitin curve. It indicated that the –COOH group was mainly substituted at the OH-6 position. The infrared spectrum showed intense bands at 1616 and 1454 cm^{-1}, assigned to asymmetric, symmetric stretching vibration, partially overlapping the 1661 and 1571 cm^{-1} bends typical for chitin (Bakos et al., 1999). All this confirmed a successful oxidation of chitin.

In the X-ray diffractograms of chitin and 6-carboxychitin (Fig. 8), it can be seen that major crystalline peaks at

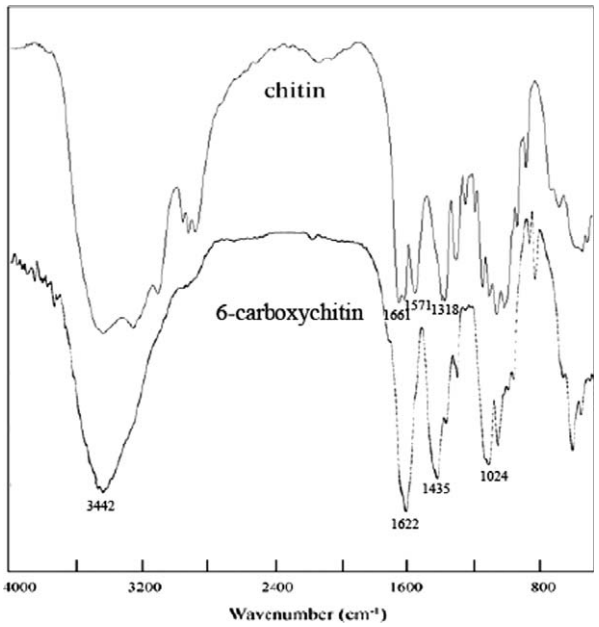

Fig. 7. FT-IR spectra of chitin and 6-carboxychitin (P_3).

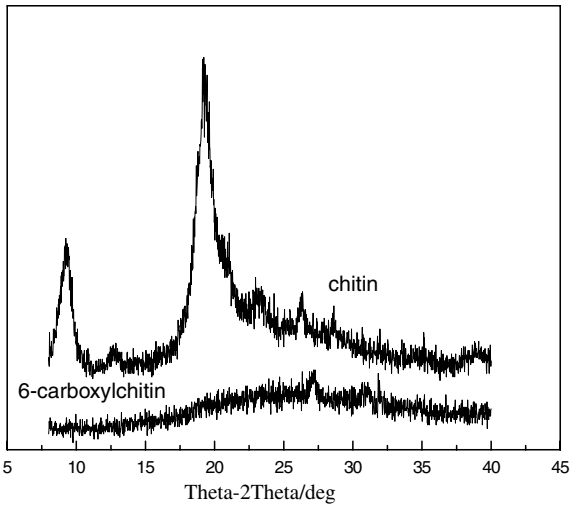

Fig. 8. X-ray diffraction patterns of chitin and 6-carboxychitin (P_3).

2θ 9.3, 12.4 (chitin) disappeared in the curve of 6-carboxychitin and at 2θ 19.1 the peak weakens and broadens compared with that of chitin graph. It indicated that the crystalline structure was destroyed and the crystallinity disappeared with oxidation and the product was amorphous.

Further evidence supporting a successful oxidation at the OH-6 position is provided by the ^{13}C NMR spectrum of 6-carboxychitin in Fig. 9. The signals for –COOH (oxidised C-6) are obvious at 178 ppm. The signals of the carbonyl groups attached to the N atoms were detected at 174 ppm. The three chemical shifts at 79, 74, and 72 ppm were attributed to C-4, C-5, and C-3, respectively. The signals at 101, 60, and 22 ppm are assigned to C-1, C-2, and CH_3. The C-6 signal was no longer detected at 62 ppm and a novel peak appeared at 178 ppm. The results were in agreement with the reported spectra (Muzzarelli et al., 1999).

3.4. Moisture-absorption and -retention properties

The moisture-absorption and -retention properties of dry and wet 6-carboxychitin samples were examined and compared with those of HA. Figs. 10 and 11 demonstrate that the moisture-absorption properties of 6-carboxychitin are quite similar to those of HA. The weight of moisture absorbed increase rapidly in the first stage, slowed down in the latter stage, and then become constant. The moisture-absorption ability is in the following sequence: $P_3 > P_1 > P_0 > CMCS > P_2 > HA$ (RH 81%), $P_3 > P_0 > P_1 > CMCS > P_2 > HA$ (RH 43%). All samples tested have higher moisture-absorption abilities than HA.

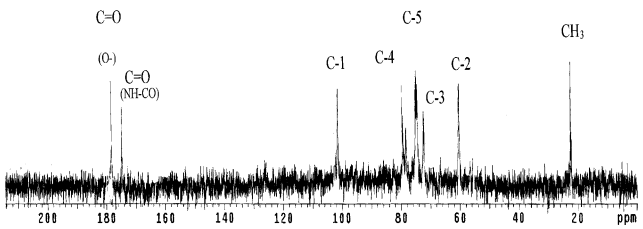

Fig. 9. ^{13}C NMR spectrum of 6-carboxychitin (P_3).

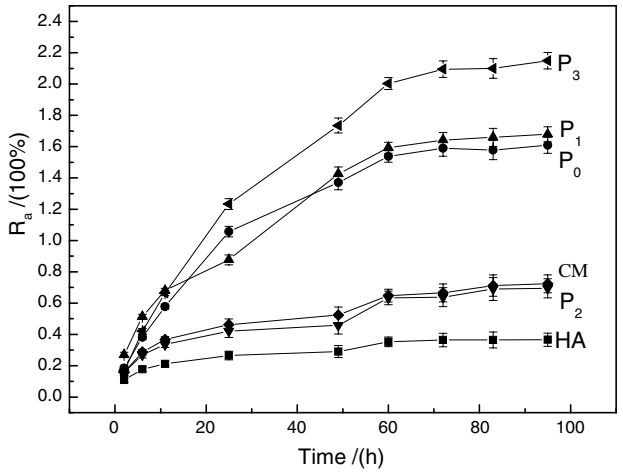

Fig. 10. Moisture-absorption ability of dry samples of 6-carboxychitins (P_0, P_1, P_2, and P_3 being oxidised products of C_0, C_1, C_2, and C_3), HA, and CM at 81% relative humidity at 20 °C.

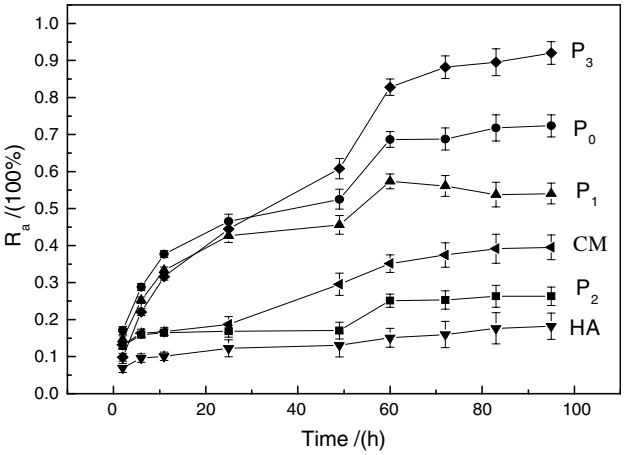

Fig. 11. Moisture-absorption ability of dry samples of 6-carboxychitins (P_0, P_1, P_2, and P_3 being oxidised products of C_0, C_1, C_2, and C_3), HA, and CM at 43% relative humidity at 20 °C.

In the results of the moisture-retention test in the saturated K_2CO_3 (RH43%) desiccator (Fig. 12), the weight of residual moisture in the wet samples increased with time and become constant 12 h later when the relative humidity was 43%. That means that all the samples tested have good moisture-retention ability. In the silica-gel RH 10% desiccator which can be seen in Fig. 13, all samples released water slowly, as well as HA. The moisture-retention ability follows the sequences of: $P_0 > CMCS > P_2 > P_1 > P_3 > HA$ (RH 43%); $P_2 > P_3 > CMCS > P_0 > P_1 > HA$ (silica-gel). All samples have higher moisture-retention ability than HA.

4. Conclusion

It is considered that physical or chemical pretreatment could change the crystal structure of chitin from α-chitin

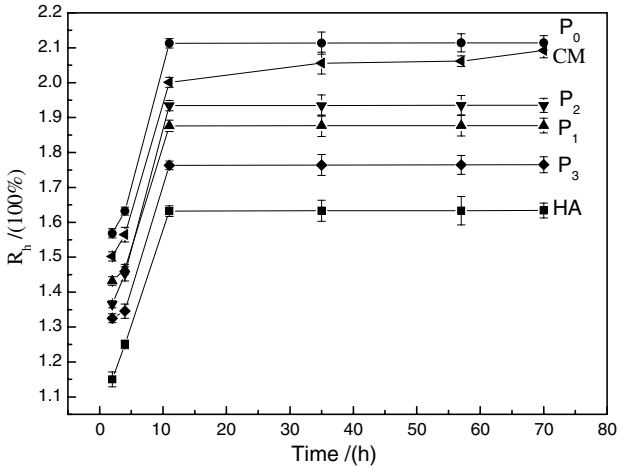

Fig. 12. Moisture-retention ability of wet samples of 6-carboxychitins (P_0, P_1, P_2, and P_3 being oxidised products of C_0, C_1, C_2, and C_3), HA, and CM at 43% relative humidity at 20 °C.

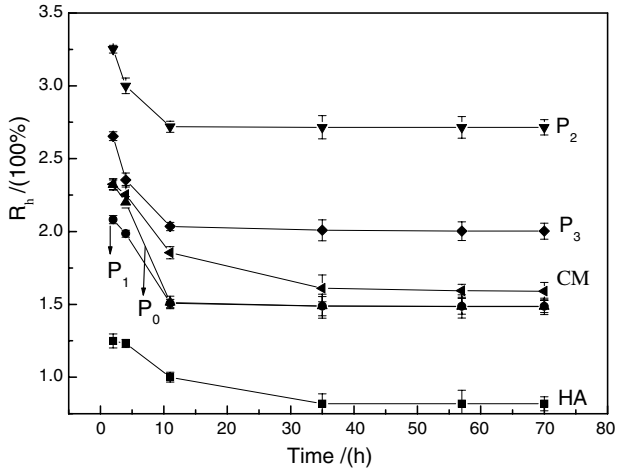

Fig. 13. Moisture-retention ability of wet samples of 6-carboxychitins (P_0, P_1, P_2, and P_3 being oxidised products of C_0, C_1, C_2, and C_3), HA, and CM over silica-gel at 20 °C.

to β-chitin, which results in the improvement of oxidation yield. It is also an effective method of introducing –COOH to the molecular chain of chitin to improve the moisture-absorption and -retention ability because of better structural similarity to HA. Therefore, the 6-carboxyl group in the molecular structure of chitin, i.e., the substitution at C-6 by carboxyl group, greatly enhanced the moisture-absorption and retention ability, so it is an active site for obtaining good moisture-absorption and -retention abilities. 6-Carboxychitin which has good moisture-absorption and -retention ability has the potential to substitute for HA for use in cosmetics and in clinical medicine.

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