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The Relationship between Crystallinity and Degree of Deacetylation of Chitin from Crab Shell

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Abstract

Crab shells from local culture farm were treated by acid and alkali for deminerlization and deproteination to obtain the sample of chitin. The deacetylation was performed under strong alkali condition at ambient temperature with different time of treatments and characterized by IR spectroscopy and HPLC method to determine degree of deacetylation. Different degree of deacetylation of chitin/chitosan samples were obtained for analysis and study of the crystallinity patterns. It was found that the depletion of crystallinity of chitin was appeared during the increasing of degree of deacetylation until reaching the transition forming of chitosan. Then, the patterns were gradually shifted, the distance between the two main peaks at $2\theta = 10^0$ and 20^0 were narrowed down, at the same time the angle of peaks at 20^0 (2θ) were moved in lower angle position. This implied some changes of lattice plane occurred during the formation. Meanwhile, the appearance of chitosan was also increasing.

Keywords : Chitin, Chitosan, Deacetylation, Crystallinity, Crab shell

Introduction

Chitin, a linear polysaccharide composed of β-(1-4)–linked–2-deoxy–acetamido-D-glucose unit (Glu-$NHCOCH_3$) is a microfibrillar materials with distinct crystal morphologies. Generally, chitin occur as a component of crustacean exoskeleton (shrimp, crab and cuttlefish), insect exoskeleton, fungel cell walls, and plankton (Urbanczyk, *et al.* 1994; and Kawada, *et al.* 1998). The different sources of raw materials contain different in both quality and quantity of chitin. Chitosan, a main derivative of chitin obtained by the process of alkali deacetylation. Different conditions of alkali

treatment yield different degree of deacetylation in the chitosan products (Kawada, *et al.* 1998; and Muthumar, 1984).

The terms chitin and chitosan do not refer to specific compounds but two ranges of copolymers, containing the two "monomer" residues, anhydro-N-acetyl-D-glucosamine and anhydro-D-glucosamine. The former is the predominat component in chitin and the latter is the predominant component in chitosan. The two ranges of copolymers form a continuum and classification of a sample which is normally done on basis of its solubility (chitosan) or insolubility (chitin) in dilute acid such as acetic acid, propionic acid, butyric acid etc. (Mathur, *et al.* 1990; Robert, 1994; and Hirano, 1996). Therefore, the study on degree of deacetylation will lead to chemical modification and application of chitin and chitosan.

The aim of this study is to find the relationship between the increasing of degree of deacetylation and the crystallinity patterns of chitin from crab shells during the deacetylation process. The crystallinity patterns were measured by X - Ray diffractometer while the degree of deacetylation was measured by IR–spectroscopy in comparison to the method of High Performance Liquid Chromatography (HPLC). (Baxter, *et al.* 1992; and How, 1996).

Materials and Methods

The crude chitin was obtained from crab shell of *Carcinus*. The crab shells were collected from the crab culture farm in Ranong province, southern part of Thailand. The deproteination of the shells was done in dilute alkali solution (5%NaOH) and followed by demineralization by dilute acid (5%HCl).

Deacetylation process of chitin was carried out in 50%(w/w) NaOH solution at ambient temperature. The different times of treatment were varied at 1, 2, 3, 4, 5, 6, and 7 days. The treated samples were then collected and throughly washed until reaching the neutral pH. After drying, the samples were ready for further analysis.

The measurement of degree of deacetylation was done by two different techniques namely IR-spectroscopy (Baxter, *et al.* 1992) and HPLC. (How, 1996) The X-Ray diffractograms of different alkali-treated chitin were recorded by using Philips PW3710 Diffractometer using CuK α radiation and generated at 40 kV and 30mA. With scanning speed at 0.02 $^{0}2\theta$/min.

Result and discussion

Physical and chemical characteristics of chitin and deacetylated samples obtained from various times of N-deacetylation is presented in

Table 1, a typical IR spectra and X-ray diffraction patterns are shown in Figure 1 and 2 , respectively.

Determination of Degree of Deacetylation

In Figure 1 shows the IR-spectra of chitin and deacetylated chitin samples, the splitting of $\nu_{C=O}$ band at 1661 and 1652 cm^{-1}, in α-chitin spectra which does not occur in β-chitin. This indicates the presence of different types of carbonyl groups. The observed band at $\nu_{C=O}$ band at 1652 cm^{-1} and δ_{N-H} band at 1559 cm^{-1} of starting chitin shows the most noticeable variation with the increase of %DD. A change in wavenumber and increased broadening occur and making it difficult to accurately determine the position of the band.

However, the ν_{C-H} band at 2891 cm^{-1} of starting chitin shows an increased broadening while the %DD is increase. The same is observed for the ν_{C-O} bands at 1157, 1030 and 1072 cm^{-1} in starting chitin, deacetylated samples with treatment times at 1 and 2 days and increased broadening occur when the increased of treatment times.

As expected for probe bands, the intensity of the $\nu_{C=O}$ band at 1661 cm^{-1} shows decrease with the increase of treatment times. This band practically disappears for the most deacetylated samples. Similarly, the δ_{N-H} band at 1559 cm^{-1} decreases with the increase of treatment times, becoming practically absent for the most deacetylated samples. It is important to note that this band disappears faster than the $\nu_{C=O}$ band.

The %DD was mearsured from IR spectra compared with HPLC method. In Table 1, shown DD value calculated by the method of Alasdiar Boaxter, *et al.* (1992) and the other was obtained by HPLC method (How, *et al.* 1996). The %DD from IR spectra measure in range of 16.5 to 72.3 while the %DD from HPLC method measure in range of 8.6 to 76.1. A good correlative of %DD is also found when IR data from time that 3 to 7 days are compare with HPLC data as shown in Figure 3.

The Observation of X-ray diffraction

In Figure 2 shows the diffractograms of starting chitin (1) consist of two major peaks at 9.46 and 19.18^0 2θ which have similar formation to the diffractograms of deacetylated samples with the treatment times of 1 and 2 hours. Similarly, the diffractograms of deacetylated chiin samples at treatment times of 3, 4, 5, 6, and 7 days show a similar formation and differ from starting chitin. And when the chitin sample was treatment with alkali condition, however, reduced crystallinity was observed.

The percent relative crystallinities of all samples was determined the ratio of crystalline fraction of deacetylated chitin to chitosan and calculated on the basis of the width at half-maximum intensity(WHMI), which they are

presented in Table 1. X-ray powder diffractograms of starting chitin and deacetylated samples are shown in Figure2. Comparison of the X-ray patterns in Figure2 found that line broadening and width at half-maximum intensity of the composite at 19.18^0 (2θ) increased with increase of the treatment times. From the Figure4 the crystallinity of the samples and showed that the alkali treatment reduced the crystallinity of the samples. The crystallinity change as a function of treatment times is due to deacetylation taken place in the swollen crystalline in the samples (Figure5). It is important to note that the percent relative crystallinity shows to increase when the treatment times is reach 7 days.

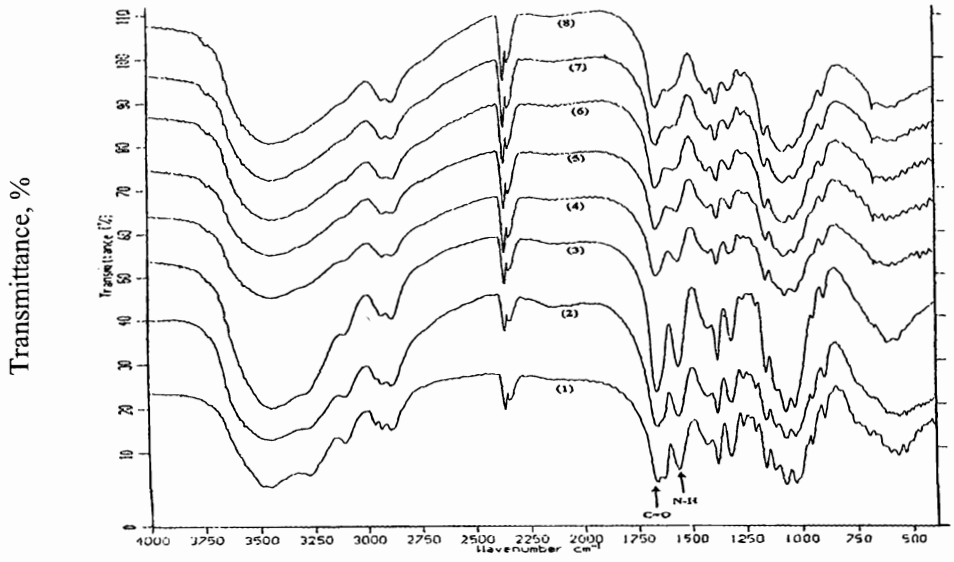

Figure 1 IR-spectra of starting chitin(1) and deacetylated samples in 50%NaOH at ambient temperature and various of treatment times(days) include 1(2), 2(3), 3(4), 4(5), 5(6), 6(7) and 7(8)

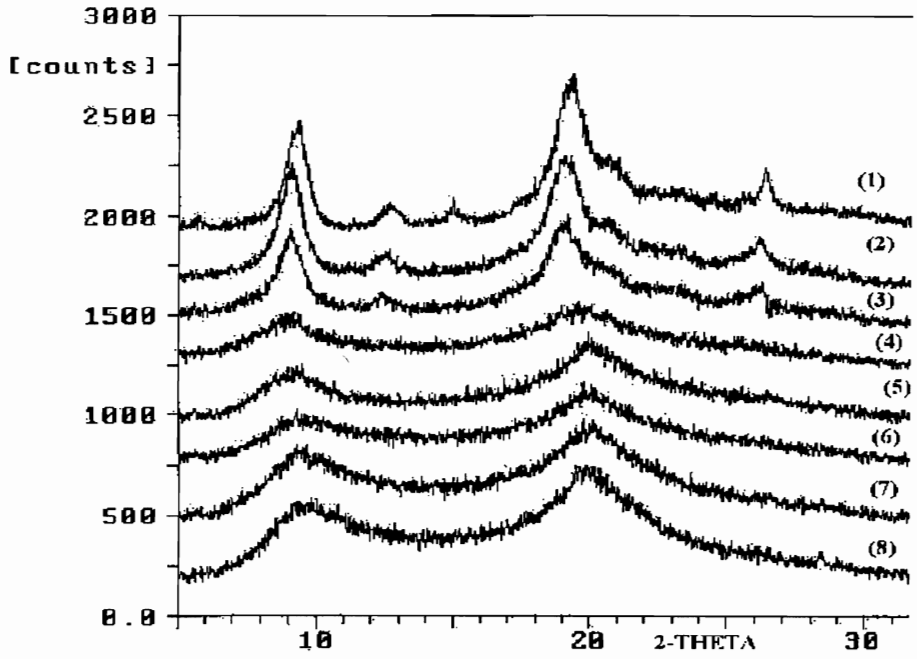

Figure 2 X-ray difractometer pattern of starting chitin(1) and deacetylated samples in 50%NaOH at ambient temperature and various of treatment times(days) include 1(2), 2(3), 3(4), 4(5), 5(6), 6(7) and 7(8)

Table 1 Chemical and Physical characteristics of chitin and deacetylated chitin

Deacetylation (days)	Degree of Deacetylation(%DD)		Ash content (%wt)	%Relative crystallinity
	IR spectra	HPLC method		
0	16.5	8.6	9.69	100
1	36.11	17.5	0.54	80
2	45.4	22.6	0.52	66.6
3	51.9	51.5	0.35	36
4	58.2	60.4	0.27	46.7
5	68.3	69.9	0.30	36
6	68.9	73.5	0.25	60
7	72.3	76.1	0.16	70.7

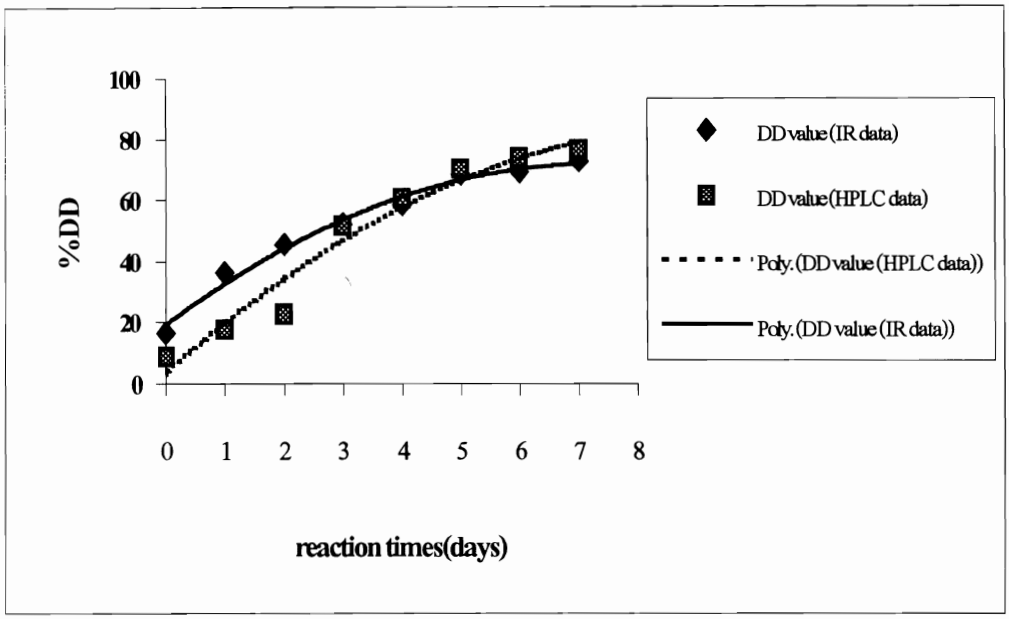

Figure 3 Plot of %DD from IR-spectroscopy vs. HPLC method

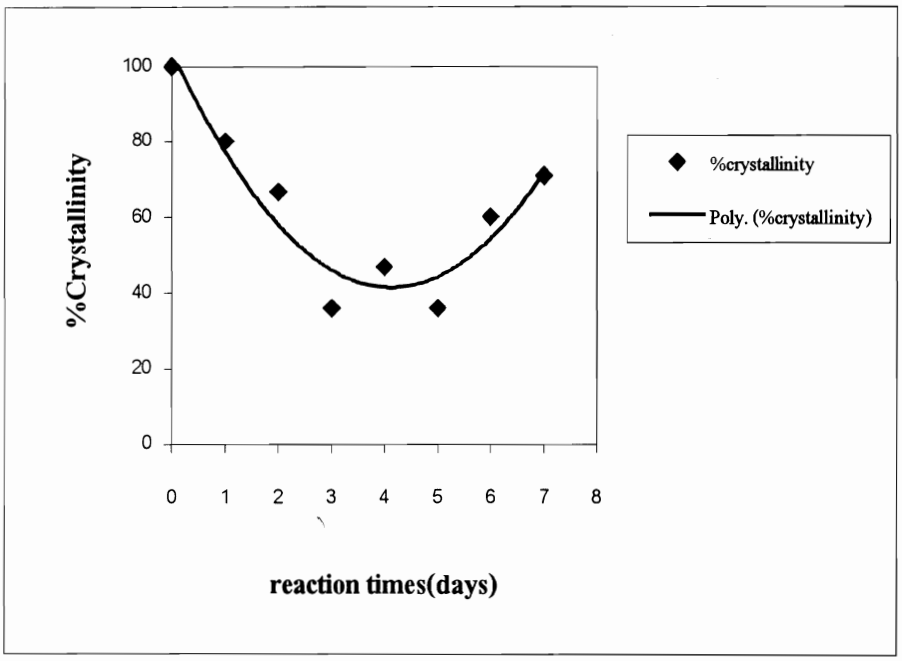

Figure 4 Percent relative crystallinity of alkali-treated chitin samples at various times

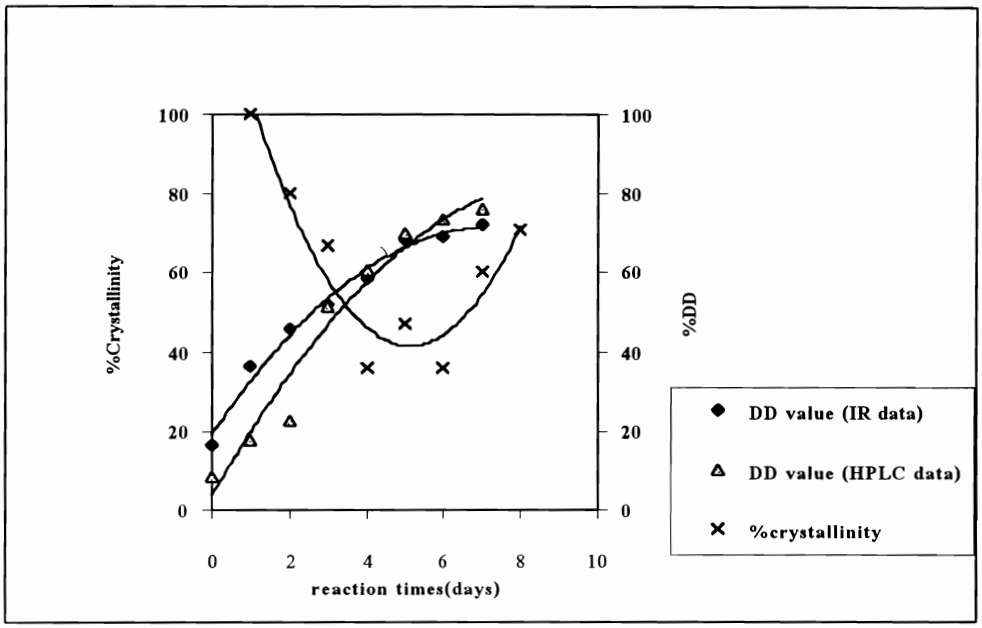

Figure 5 The relationship between crystallinity and degree of deacetylation of alkali-treated chitin samples at various times

Conclusion

The degree of deacetylation of chitin from crab shells was increased with increasing time of treatment. Meanwhile, the percentage of relative crystallinity of chitin was decreased down to the transition period of chitin to chitosan which is about 51% of degree of deacetylation. Consequently, the crystallinity value was changed in the direction of gradually increasing along with the degree of deacetylation of chitosan.

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