CHAPTER IV
MICROWAVE TECHNIQUE FOR EFFICIENT DEACETYLATION OF CHITIN NANOWHISKER TO CHITOSAN NANOSCAFFOLD

4.1 Abstract

A chitosan nanoscaffold in the form of a colloidal solution was achieved from the deacetylation of chitin whisker in alkaline conditions by using the microwave technique, in only 1/7 of the treating time of the conventional method. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{1}H-NMR) techniques confirm the degree of deacetylation to be above 90% within 3 h. The wide angle X-ray diffraction (WAXD) pattern clearly shows that the highly crystalline chitin whisker is changed to amorphous chitosan. SEM micrographs show the aggregation of branched nanofibers, whereas the TEM micrographs reveal the scaffold morphology.

Keywords: Chitin-chitosan, Whisker, Microwave technique, Nanoscaffold

4.2 Introduction

Chitin is the second-most abundant natural polysaccharide, next to cellulose, and is obtained from the cell walls of fungi and yeast and from the shells of crustaceans such as crabs and shrimp.[1] In general, chitin consists of copolymer repeat units of β(1-4)-2-acetamido-2-deoxy-β-D-glucose and β(1-4)-2-amino-2-deoxy-β-D-glucose. As chitin dissolves only in specific solvents—such as *N,N*-dimethylacetamide (DMAC)-LiCl[2,3], hexafluoroacetone, and hexafluoro-2-propanol[4]—its practical uses are limited.[5] Deacetylating chitin to chitosan to increase the units of β(1-4)-2-amino-2-deoxy-β-D-glucose is one way to develop chitin-chitosan-based material because of the good solubility in carboxylic acids.[6] Up to now, most reports on chitosan have been about potential materials with biocompatibility, biodegradability, bioactivity, and non-toxicity.[7-10] Despite the many

unique properties for utilization, chitosan acid solutions are, sometimes, not ideal for the preparation of the practical materials. However, chitosan derivatives (especially water-soluble chitosans and organo-soluble chitosans) have been reported for novel applications.

Advances in characterization techniques have allowed us to observe materials at nano-scale and the development of material from the molecular level has become a challenging theme. Currently, the changing of chitin and chitosan from flakes or powder to the nano-scale, along with possible novel applications, is being variously reported. For example, Gopalan Nair and Dufresne reported chitin whisker by the acid hydrolysis of chitin from crab shell and its use as nanofiber reinforcing material for natural rubber.[11] For chitosan, chemical modification to obtain nanoparticles[12] and electrospinning to obtain nanofibers[13,14] are some reported cases.

For the past few years, our group has been focusing on various approaches to achieving chitosan nanomaterials, including studies on their unique properties and feasible applications. We succeeded in preparing chitosan nanospheres by conjugating hydrophilic and hydrophobic groups on chitosan.[10] We also found that, by extending the deacetylation after obtaining chitin whisker, we could obtain nano-sized chitosan.[15] At that time, we found that the uniqueness of deacetylating chitin whisker to chitosan is that the obtained chitosan had a fibrous network with nanoporous structure.

Although obtaining chitosan with nanopores via a simple treatment is attractive (where many advanced applications related to the high surface area and high absorptivity are the goal), we accept the fact that treatment in a concentrated alkaline solution at almost reflux temperature for more than 20 h is quite severe and may not be practical for large scale production. As the microwave technique is known to be an effective system for either synthesis[16-18] or depolymerization[19] due to its instantaneous heating and vibrational energy at the molecular level,[20,21] we herein consider this technique for the efficient deacetylation of chitin whisker.

4.3 Experimental

4.3.1 Materials

Chitin in flake form from shrimp shell was provided by Seafresh (Lab) Company Limited, Thailand. Sodium hydroxide (NaOH) was purchased from Fluka Chemicals, Switzerland, and hexafluoroisopropyl alcohol (HFIP) was obtained from Central Glass Co., Ltd., Japan. Hydrochloric acid (HCl) was purchased from Labscan, Ireland, and the deuterated acetic acid (CD_3COOD) and deuterium oxide (D_2O) were purchased from Sigma Aldrich, the Netherlands. All chemicals were AR grade and used without further purification.

4.3.2 Instruments and equipment

A Milestone Ethos microwave solvent extraction labstation, at a frequency of 2.45 GHz, was used to treat the chitin whisker in N_2 at 150 °C. Qualitative and quantitative Fourier transform infrared spectra were obtained with a Nicolet NEXUS 670 with 32 scans at a resolution of 2 cm^{-1} in a frequency range of 4000–400 cm^{-1}. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were collected from a Bruker Avance 400 spectrometer with 512 scans by using CD_3COOD/D_2O (2% v/v) as a solvent. Wide angle X-ray diffraction (WAXD) patterns were obtained over a 2 to 60° 2θ range by a RIGAKU RINT 2000, using Cu Kα as an X-ray source, equipped with a Ni filter and operating at 40 kV and 30 mA. A Perkin Elmer thermogravimetric-differential thermal analyzer was applied using a N_2 flow rate of 20 mL/min with a heating rate of 10 °C/min starting from 50 to 550 °C. The morphology was analyzed by a JEOL JSM-5410LV scanning electron microscope (SEM) at 15 kV and a H-7650 Hitachi transmission electron microscope (TEM) at 100 kV. Tohso HLC-8220 gel permeation chromatography (TSK gel Super HM-N and Super H-RC columns; Tohso Co., Japan) was used to evaluate the molecular weight of the chitosan. The conditions were an operating temperature of 40 °C, using hexafluoroisopropyl alcohol (HFIP) as an eluent, and an eluent flow 0.2 mL/min.

4.3.3 Preparation of chitin whisker

Chitin whisker was prepared by modifying the conditions based on the report from Gopalan Nair and Dufresne.[11] Chitin, **1** (3 g, 0.0148 mol) was hydrolyzed with 3N HCl (300 mL) at 105 °C for 3 h. After washing the product thoroughly by deionized water, the alkaline treatment was further treated in 3N HCl (300 mL) at 105 °C for 6 h. The product was dialyzed in distilled water until neutral (pH = 7) to obtain chitin whisker, **2**.

FTIR (KBr, cm^{-1}): 3000–2800 cm^{-1} (C-H stretching); 1661 and 1624 cm^{-1} (amide I), and 1557 cm^{-1} (amide II).

4.3.4 Preparation of chitosan nanoscaffold

Chitin whisker, **2**, in aqueous solution (solid content ~ 5.91%, 5 mL) was treated in aq. NaOH (40% w/v, 25 mL) at 150 °C in the microwave chamber under N_2 atmosphere. The sample was collected and dialyzed in distilled water until it was neutral (pH=7), after treating times of 1, 3, and 6 h, to obtain chitosan **3**. Similarly, treatment using aq. NaOH for 50% and 60% w/v was also carried out.

FTIR (KBr, cm^{-1}): 3500–3300 cm^{-1} (OH); 3000–2800 cm^{-1} (C-H stretching); 1661 cm^{-1} (amide I); and 1595 cm^{-1} ($-NH_2$).

^{1}H-NMR (δ, ppm): 1.9 ppm (NH-Ac); 3.15 ppm (H-2 of GLcN unit in chitosan), and 3.5–4.0 ppm (H-3 to H-6 of pyranose ring).

4.3.5 Evaluation of degree of deacetylation

The degree of deacetylation (%DD) was examined by using ^{1}H-NMR spectrum. The peak areas of the *N*-acetyl group at 1.9 ppm and the H-2 at 3.15 ppm were considered for substituting in the following equation to calculate %DD:

$$\%\,DD = \left\{1 - \left[\frac{(I_{CH_3}/3)}{(I_{H2})}\right]\right\} \times 100$$

The %DD was also quantitatively analyzed by FTIR using OPUS spectroscopic software, version 2.0. The peaks of interest were 1658 cm^{-1} (-CONH-) and 895 cm^{-1} (C-O-C).

4.4 Results and Discussion

4.4.1 Chemical structure analysis

Chitin flake, **1**, was treated in hydrochloric acid (3N) (as reported by Gopalan Nair and Dufresne[11]) before being further treated with NaOH. Compared to the FTIR spectrum of **1** (Figure 4.1a), that of **2** shows sharp characteristic peaks in all ranges, as seen from the peaks at 3500–3300 cm^{-1} (-OH); 2900–2800 cm^{-1} (C-H stretching); and 1661, 1624, and 1557 cm^{-1} (amides I and II) (Figure 4.1b). This implies a highly crystalline chitin whisker.

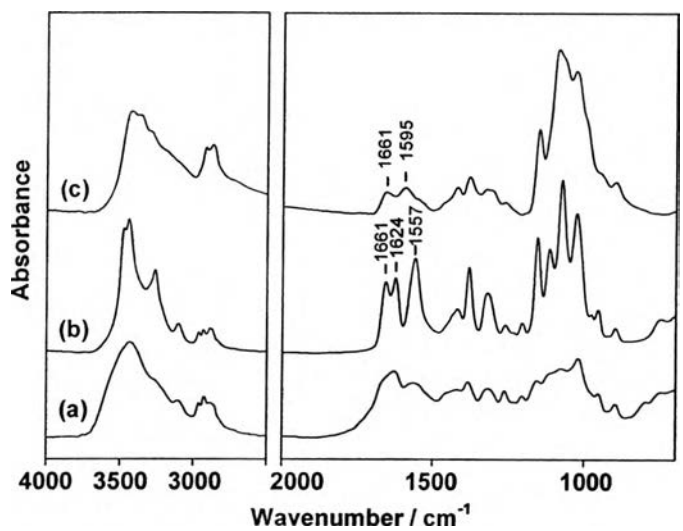

Figure 4.1 FTIR spectra of (a) **1**, (b) **2**, and (c) **3** under 60% alkaline solution for 6 h treating time.

Phongying *et al.*[15] demonstrated the preparation of chitosan by treating chitin whisker in NaOH 40% for 21 h to obtain a degree of deacetylation of 95%. In that condition, a colloidal solution was obtained. Here, **2** was deacetylated by using a 40–60% alkaline solution to obtain **3** in the form of a colloidal solution. For the 60%

alkaline treatment, the FTIR spectrum (Figure 4.1c) shows a significant decrease in the peak of 1661 cm^{-1} with a new peak at 1595 cm^{-1} ($-NH_2$), as compared to **2** (Figure 4.1b). This indicates that the acetyl group of chitin was significantly removed.

Figure 4.2 shows the chemical shift at 1.9 ppm ($-CH_3$ in chitin unit), 3.15 ppm (H-2 of pyranose ring), and 3.5–4.0 ppm (H-3 to H-6 of pyranose ring), which can be calculated for degree of deacetylation to be 96%.

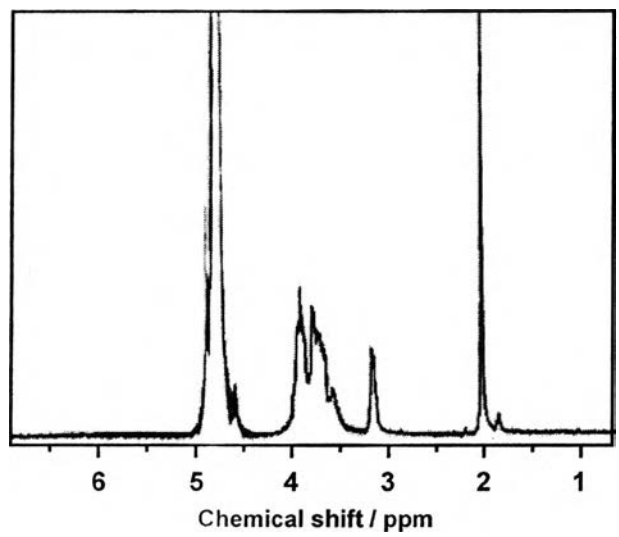

Figure 4.2. ^{1}H-NMR spectrum of **3** under 60% alkaline solution treating for 6 h.

4.4.2 Optimal condition of microwave technique for deacetylation

In order to find the optimal conditions for the deacetylation of chitin whisker, the alkaline concentration and the treating time were varied. The products obtained were evaluated by FTIR and ^{1}H-NMR.

An example of curve fitting at 1658 (amide I) and 895 (internal standard peak) cm^{-1} is shown in Figure 4.3A. When the treating time was varied from 1, 3, and 6 h at 60% alkaline solution (Figure 4.3A a–c), the gradually decreasing peak at 1658 cm^{-1} can be clearly identified. The plot of the peak ratio between 1658 and 895 cm^{-1} indicates how amide I is decreased as the deacetylation proceeds (Figure 4.3B). This peak ratio shows a rapid decrease during the first 3 h, and maintains a similar level after that until 6 h. This implies that the deacetylation occurred effectively in the microwave system.

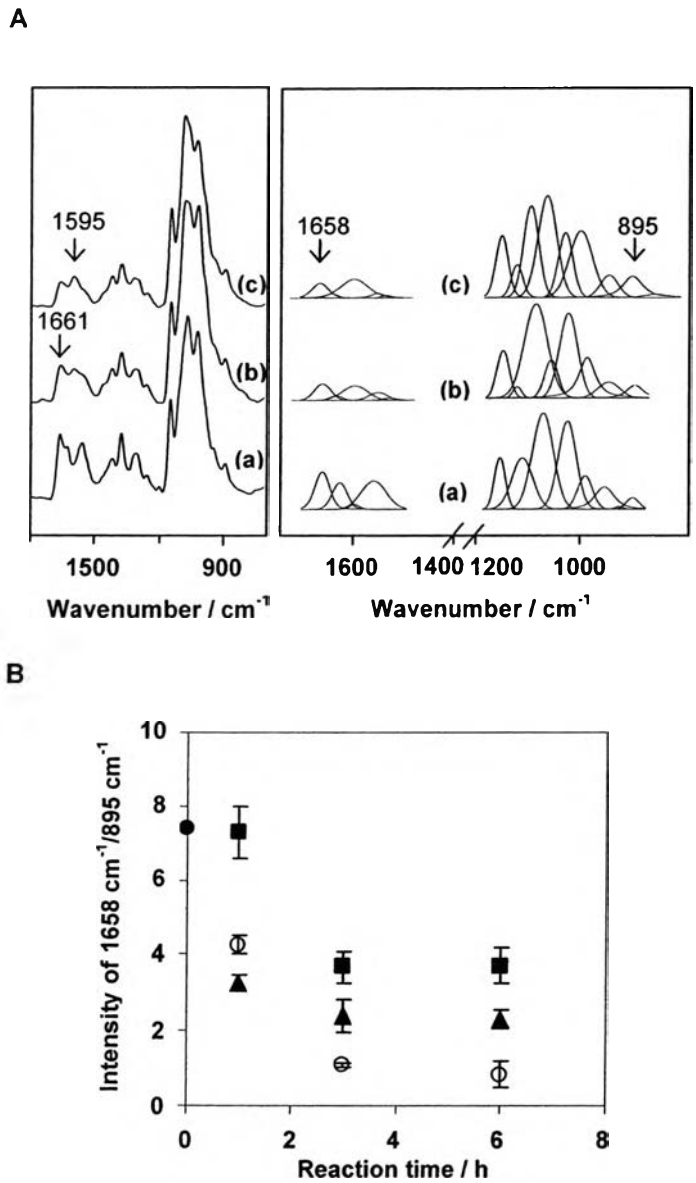

Figure 4.3. (A) FTIR spectra and curve fitting of **3** under 60% alkaline solution for (a) 1 h, (b) 3 h, and (c) 6 h; and (B) quantitative analysis based on (A) for (●) **2** and **3** under various alkaline concentrations in (■) 40%, (▲) 50%, and (○) 60% for 1, 3, and 6 h treating time.

^{1}H-NMR was also used to determine the degree of deacetylation (%DD). Figure 4.4 shows the relationship between %DD with reaction time at various alkaline concentrations compared to the result obtained from the conventional method. The plot confirms the increment of %DD with reaction time and the alkaline concentration, which is relevant to the result obtained from quantitative FTIR.

Previously, we found that we needed at least 40% alkaline solution treating at reflux for 15 h to obtain chitosan with a %DD above 90. Here, as shown in Figure 4.4, the %DD is clearly increased in less than 10 h for both the 40 and 50% alkaline concentration. Moreover, we found that the %DD could be significantly improved to 90–98% by increasing the alkaline concentration from 50 to 60% (w/v). It is important to note that the microwave technique allows us to enhance the treatment in a very high alkaline concentration (higher than 40% (w/v)), which is difficult to do in the conventional method using glassware. Here, we confirm efficient chitin whisker deacetylation via the microwave apparatus. The reaction time for achieving a %DD above 90 was decreased from 21 h to only 3 h using a 60% (w/v) alkaline concentration.

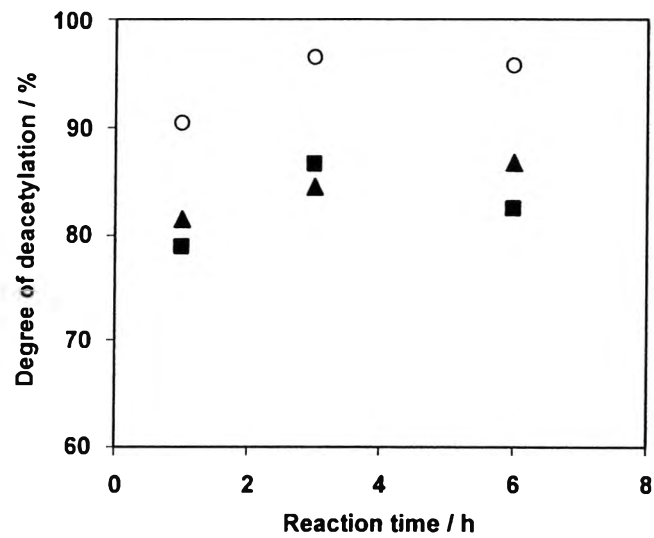

Figure 4.4. % DD of **3** under various alkaline concentrations in (■) 40%, (▲) 50%, and (○) 60% for 1, 3, and 6 h treating time.

4.4.3 Morphological studies

It is important to note that both chitin and chitosan have a strong inter- and intra-molecular hydrogen bond; the specific morphology in whisker form is, thus, an enhanced crystalline structure. In the past, we found that the alkaline treatment of chitin whisker not only induced a degree of deacetylation but also changed the

whisker morphology to a fibrous network.[15] Here, the morphological studies were carried out by WAXD, SEM, and TEM.

Figure 4.5b shows sharp major peaks at 9° and 19° 2θ of **2**, confirming the crystalline morphology as compared to the starting chitin (Figure 4.5a). After alkali treatment in the microwave to obtain **3**, the peaks shift to 11°, 20°, and 22° 2θ, and become broad. The result is relevant to our previous work, especially the result regarding the loss of the packing structure of the highly crystalline morphology of the whisker **2** after being treated with the alkaline solution.[15]

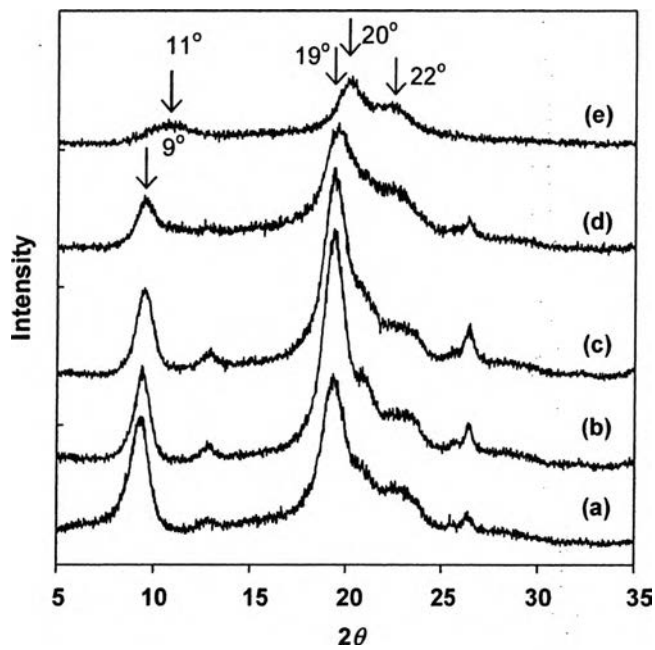

Figure 4.5. WAXD patterns of (a) **1**, (b) **2**, and **3** under various alkaline concentrations in (c) 40%, (d) 50%, and (e) 60% for 6 h treating time.

SEM micrographs show significant changes from the starting chitin flakes to **2** and **3**. Figure 4.6A shows the changes in **2** when it was gradually deacetylated in the microwave with 40%, 50%, and 60% alkaline solution. It is important to note that one may expect to see the nanofiber whisker maintained while being deacetylated to chitosan. Figure 4.6A (a) clearly shows the aggregation of nanofibers. After treating with 40% and 50% alkaline solutions, the morphology was changed to a nanofiber aggregation, implying that the packing structure was destroyed, as shown in Figure

4.6A (b) and (c). In the other words, the whisker was swollen and the fibrous networks were generated (Figure 4.6A d). This might be due to the differences in the hydrogen bond networks between chitin and chitosan.

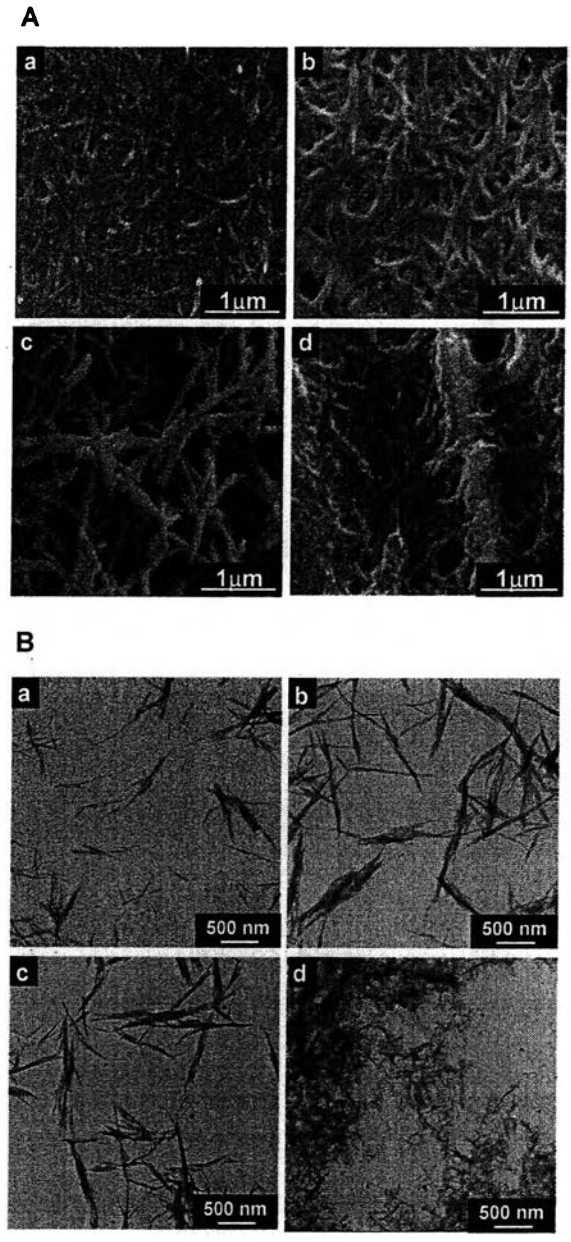

Figure 4.6. (A) SEM micrographs and (B) TEM micrographs of (a) **2** and **3** under various alkaline concentrations in (b) 40%, (c) 50%, and (d) 60% for 6 h treating time.

Gopalan Nair and Dufresne[11] reported the uniqueness of chitin whisker with an aspect ratio of 16. Figure 4.6B (a) confirms our chitin whiskers have an aspect ratio of 16, which corresponds to that of Gopalan Nair and Dufresne's report.

Figure 4.6B (b) and (c) show that chitosan, after being treated with 40% and 50% alkaline solution, is in whisker form. However, the morphology is totally changed to a scaffold when it is treated with a 60% alkaline solution for 6 h. As the morphology was finally changed to a fibrous network, the aspect ratio of chitosan cannot be identified. In the past, we reported a unique nanoscaffold chitosan, obtained from traditional alkaline treatment in glassware, of chitin whisker[15]; here, we also find a similar result, even though the alkaline treatment was carried out in a microwave reactor.

4.4.4 Thermal Stability

Thermal stability based on the degradation temperature (T_d) is important information for determining how the packing structure was changed, as compared to the starting structure. The suspension of **2** and **3** was lyophilized to obtain a fibrous material.

Table 4.1 Degradation temperature (T_d) of **2** and **3**

Compound	T_d (°C)
2	369
3 under 40% alkaline treatment for 6 h	359
3 under 50% alkaline treatment for 6 h	347
3 under 60% alkaline treatment for 6 h	293

Compound **2** shows a T_d as high as 369 °C, which reflects high crystallinity with strong inter- and intra-molecular hydrogen bond. Table 4.1 shows the decrease in T_d with an increase in alkaline concentration. For example, when the alkaline concentration was as high as 60%, the T_d was decreased from 369 °C to 293

°C. This also supports the morphological result regarding crystalline deformation to obtain **3**.

4.4.5 Molecular weight

It should be noted that the molecular weight of **3**, determined by GPC, shown in Figure 4.6B (d) was 147,740 Daltons, which is higher than those in Figure 4.6B (b) and (c), which were 110,433 and 85,670 Daltons. We suspect that as **3** (Figure 4.6B d) was in a networked scaffold form, the swelled polymer network might lead to an increase in molecular weight. This corresponds to our previous result[15] where we concluded the effect of the significant radius of gyration when the chitosan was in the scaffold structure.

4.5 Conclusions

Chitin whisker was successfully modified to chitosan with a %DD as high as 95 from one-pot deacetylation with a 60% alkaline solution by using the microwave technique under N_2 for only 3 h. The microwave technique is 7 times shorter than the conventional method. The nanoscaffold structure of chitosan showed a milky aqueous solution, as seen by the naked eye. WAXD and TEM techniques confirmed the change in morphology from crystalline to amorphous, or whisker to fibrous; thus we termed it a nano-scale "scaffold".

4.6 Acknowledgments

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4.7 References

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