CHAPTER III
FABRICATION, STRUCTURE, AND PROPERTIES OF CHITIN WHISKERS-REINFORCED ALGINATE NANOCOMPOSITE FIBERS

3.1 Abstract

Calcium alginate yarn (30 fibers) and calcium alginate nanocomposite yarn (30 fibers) containing 0.05-2.00% w/w chitin whiskers were both prepared by wet spinning process. The whiskers were prepared by acid hydrolysis of chitin from shrimp shells. The average length and width of the whiskers were 343 and 46 nm, with the aspect ratio being ~7.5. Incorporation of a low amount of the whiskers in the nanocomposite fibers improved both the mechanical and the thermal properties of the fibers significantly, possibly a result of the specific interactions, i.e., hydrogen bonding and electrostatic interactions, between the alginate molecules and the homogeneously dispersed chitin whiskers. Biodegradation of the calcium alginate fibers and the nanocomposite fibers was tested in Tris-HCl buffer solution and the same buffer solution that contained lysozyme. The addition of the chitin whiskers in the nanocomposites fibers accelerated the biodegradation process of the fibers in the presence of lysozyme, whereas the presence of Ca^{2+} ions in the Tris-HCl buffer solution helped to improve the tenacity of both the alginate and the nanocomposite fibers.

(**Key-words**: alginate; chitin whiskers; nanocomposite fiber; mechanical properties; biodegradability)

3.2 Introduction

Whiskers or crystalline microfibrils have got a great deal of interest in the research community, which is largely because of the recent uprising of nanotechnology. The interest in the use of whiskers as reinforcing nanofillers has been due to their high aspect ratio and highly crystalline nature. Incorporation of whiskers as reinforcing nanofillers in a polymer matrix results in a new class of

materials, i.e., nanocomposites. Whiskers that have already been investigated are those derived from cellulose (Favier *et al.*, 1995; Ljungberg *et al.*, 2006; Dufresne *et al.*, 1996) and chitin (Paillet and Dufresne, 2001; Morin and Dufresne, 2002). These whiskers are obtained from animals or plants that synthesize extracellular high performance skeletal biocomposites consisting of a matrix reinforced by fibrous biopolymers (Neville, 1993; Preston, 1967; Atkins, 1975).

Chitin, a structural polymer commonly found in exoskeletons of crustaceans, cuticles of insects, and cell walls of fungi, is, next to cellulose, the most abundant biopolymer (Singh and Ray, 2000). Its chemical structure is similar to that of cellulose but the 2-hydroxy group has been replaced by an acetamide one, resulting in mainly β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose structural units (Dumitriu, 1988). Chitin is well-recognized as a biocompatible material because of its low antigenicity, low toxicity, and biodegradability (Krajewska, 1991; Muzzarelli, 1980). In addition, it is a substrate for lysozyme digestion and, therefore, several research studies have reported on its use in medicine (Pulapura and Kohn, 1992), e.g., absorbable sutures (Nakajima *et al.*, 1986), drug carriers (Watanabe *et al.*, 1990), and veterinary concerns (Okamoto *et al.*, 1993). Specifically, the use of chitin in medicine is augmented by its wound healing ability (Prudden *et al.*, 1970; Watkins and Knorr, 1983).

Chitin is known to form microfibrillar arrangements in living organisms. These fibrils are usually embedded in a protein matrix and their diameters usually range from 2.5 to 2.8 nm, with chitin microfibrils from cuticles of crustaceans being found to be as large as 25 nm (Revol and Marchessault, 1993). Chitin whiskers can be prepared by acid hydrolysis of chitin (Paillet and Dufresne, 2001). These whiskers were used as reinforcing fillers in both synthetic (Paillet and Dufresne, 2001; Morin and Dufresne, 2002) and natural (Lu *et al.*, 2004; Sriupayo *et al.*, 2005) polymeric matrices. Lu *et al.* (2004) indicated that strong interactions among different chitin whiskers and between chitin whiskers and soy protein isolate (SPI) thermoplastic matrix play an important role in the improvement of mechanical properties and water resistance of the SPI-based nanocomposites without interfering with their biodegradability. Sriupayo *et al.* (2005) reported that the tensile strength of α-chitin

whisker-reinforced chitosan films increased from that of the pure chitosan film and both the addition of α-chitin whiskers and heat treatment improved water resistance (i.e., decreased weight loss and swelling in an aqueous medium) of the nanocomposite films.

Another polysaccharide heavily explored for its potential use as wound-care material is alginate, which is a biopolymer obtained from cell walls of brown algae, such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp. (Clare, 1993). Chemically, it is a linear block copolymer containing uronic acid residues, namely, β-D-mannuronic and α-L-guluronic acid, linked by (1→4)-linkages. An alginate-based product offers many advantages, e.g., biocompatibility and hemostatic capability. Upon exposure to wound exudates, it readily forms gel (Jarvis *et al.*, 1987). Such a gel prevents the wound bed from drying out as a moist wound environment has been known to promote healing, leading to a better cosmetic repair of the wound (Winter, 1962). Despite its solubility in water, alginate can readily form gel when being exposed to divalent cations, e.g., Ca^{2+} (McDowell, 1974). Because of this reversible solubility, alginate can be fabricated into various forms (Agren, 1996; Li *et al.*, 2005; Dong *et al.*, 2006) and, because of the aforementioned unique properties of alginate, alginate-based dressings are available as gel mats (e.g., AlgiSite®, Kaltostat®, and Tegagel®)(Paul and Sharma, 2004) and fiber mats (e.g., Algosteril®, Kaltostat®, and Sorbsan®)(Agren, 1996). Figure 3.1 shows chemical structures of sodium alginate/alginic acid and chitin.

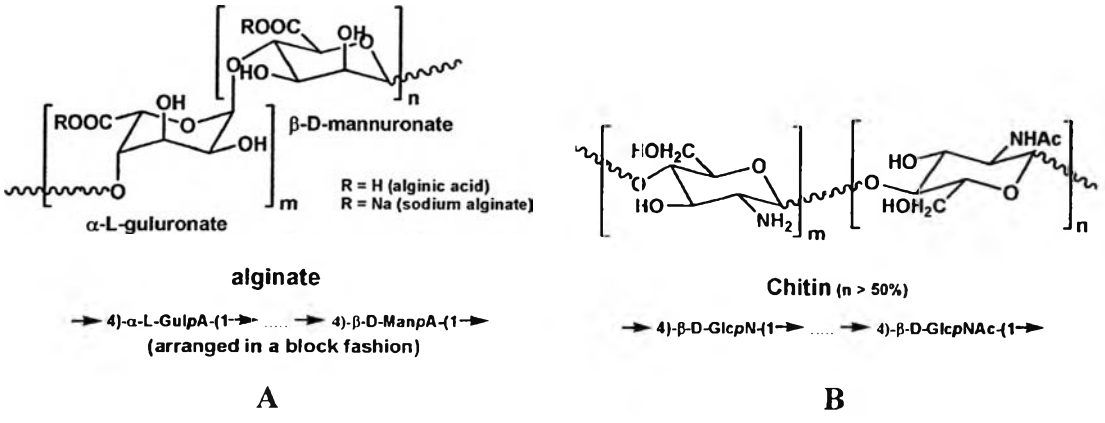


Figure 3.1 Chemical structures of (A) sodium alginate/alginic acid and (B) chitin.

In this contribution, possible wound-dressing materials in the form of fibers that combine the desirable properties of both alginate and chitin whiskers were developed. Chitin whiskers were used as nanoscopic reinforcing fillers to achieve two main objectives: (1) to improve the mechanical properties of the fibers and (2) to accelerate the healing of wounds as a result of the presence of chitin oligomers that were released from the fibers due to enzymatic hydrolysis of the whiskers by lysozyme (Chung *et al.*, 1994). The chitin whisker-reinforced alginate nanocomposite fibers were prepared by wet spinning from mixtures of an alginate solution and chitin whisker suspensions. The effect of chitin whiskers on thermal stability, mechanical behavior, and biodegradability of the chitin whisker-reinforced alginate nanocomposite fibers was investigated.

3.3 Experimental

3.3.1 Materials

Shells of *Penaeus merguiensis* shrimp were provided by Surapon Food Public (Samut Prakarn, Thailand). Sodium alginate (white powder) was purchased from Carlo Erba (Milano, Italy). Tris-HCl (molecular biology grade) was purchased from Scharlau Chemie (Barcelona, Spain). Chicken egg white lysozyme was purchased from Sigma-Aldrich (Deisenhofen, Germany). Sodium hydroxide (50% w/w aqueous solution) was supplied by KTP Cooperation (Nakhon Pathom, Thailand). Nitric acid (65% w/w, analytical reagent grade) and hydrochloric acid (37% w/w, analytical reagent grade) were purchased from Carlo Erba (Milano, Italy). Dehydrated calcium chloride (edible grade) was supplied from Asia Drug and Chemical (Bangkok, Thailand). Methanol, ethanol, and acetone (commercial grade) were purchased from Labscan (Bangkok, Thailand). All other chemicals were used as received.

3.3.2 Preparation of Chitin and Chitin Whiskers

Decalcification and deproteinization of shrimp shells to obtain chitin were carried out according to the procedure described by Shimahara and Takigushi (1988). Chitin whiskers were prepared from the as-prepared chitin based on the

method described by Paillet and Dufresne (2001). The whisker suspension was obtained by hydrolyzing chitin sample with 3N HCl at 104°C for 6 h under vigorous stirring. The ratio of the 3N HCl solution to chitin was 30 cm^3 g^{-1}. After acid hydrolysis, the suspension was immediately diluted with distilled water, followed by centrifugation to separate the chitin whisker solid residues from the aqueous medium and, because of the nanocrystalline nature of the as-prepared whiskers, the centrifugation could be achieved at 10,000 rpm for 10 min. This process was repeated three times. To remove HCl that could be remained in the suspension, the suspension was further dialyzed in distilled water at room temperature for 3 days until pH = 6. Homogeneity of the suspension was further achieved by sonication for 5 min and the suspension was subsequently filtered to remove residual aggregates and was kept in a refrigerator prior to further use.

3.3.3 Preparation of Alginate and Chitin Whisker-Reinforced Alginate Fibers

The dope solution for preparing the neat alginate fibers was 6% w/v sodium alginate aqueous solution, whereas the spinning dope suspensions for preparing of the alginate/chitin whisker nanocomposite fibers were prepared by mixing homogenized chitin whisker suspensions (diluted from the as-prepared chitin whisker suspension by distilled water, followed by sonication for 15 min) with the as-prepared alginate solution. The volumetric ratio of the chitin whisker suspensions to the sodium alginate solution was varied to obtain the spinning dope suspensions with the weight ratio of the chitin whiskers to alginate in the range of 0.05-2.00%. Both the neat alginate solution and the alginate/chitin whisker suspensions were left at room temperature for degassing before being extruded through a spinneret (30 holes, diameter of which was 0.02 mm) into the first coagulation bath containing 5% w/v $CaCl_2$ in 50% v/v MeOH aqueous solution and the second coagulation bath containing MeOH. MeOH was chosen because it is a nonsolvent for alginate, thus allowing beneficial stabilization to the obtained fibers. The obtained yarns of 30 individual fibers were drawn at a draw ratio of ~1.2 between two sets of rollers. Finally, they were collected on bobbins, dried, and extensively washed with MeOH.

3.3.4 Characterization

Morphology of the as-prepared chitin whiskers was observed by a JEOL JEM-200CX transmission electron microscope (TEM). Samples for TEM observations were prepared by depositing minute drops of a dilute chitin whisker suspension on formvar grids, which were left to dry on the grids before TEM observations. The average dimensions of the whiskers were determined from the selected TEM images, from which at least 60 whiskers were measured for their length and width using SemAfore 4.0 image-analytical software. Surface morphology of both the neat and the chitin whisker-reinforced alginate nanocomposite fibers was examined by a JEOL JSM-5200 scanning electron microscope (SEM), operating at an accelerating voltage of 10 kV to obtain a magnification of 1500x.

Chemical integrity of the chitin whiskers, the alginate fibers, and the chitin whisker-reinforced alginate nanocomposite fibers was investigated by a Thermo Nicolet NEXUS 670 Fourier-transformed infrared spectroscope (FTIR) with 64 scans at a resolution of 4 cm^{-1}. The chitin whiskers from the asprepared chitin whisker suspension were dried, mixed with KBr powder, and pressed into a pellet. On the other hand, a horizontal attenuated total reflectance (H-ATR) accessory was used for the measurement of all the fiber samples, which were placed on a ZnSe crystal. For all sample types, the scanning range was 4000 to 650 cm^{-1}.

Tenacity and elongation at break of both the neat and the chitin whisker-reinforced alginate nanocomposite yarns were measured according to the ISO 2062:1993(E) standard test method using a Lloyd LR 100K universal testing machine. The load cell, the gauge length, and the displacement rate used were 100 N, 50 mm, and 50 mm min^{-1}, respectively. The yarn samples with initial length of 25 cm were first dried in an oven at 40°C for 2 h. During the measurements, both the ambient temperature and the relative humidity were 25°C ± 2°C and 55% ± 2%, respectively. The force and the extension at the breaking point were recorded, and these values were used to calculate both the tenacity and the percentage of elongation at break of the yarns (n = 20).

Lastly, thermal behavior of the chitin whiskers, the alginate fibers, and the chitin whisker-reinforced alginate nanocomposite fibers was examined by a

Perkin–Elmer DSC-7 differential scanning calorimeter (DSC). Each sample weighed about 10 mg and each thermogram was recorded over a temperature range of 30-380°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere.

3.3.5 Biodegradation Studies

Biodegradability of the neat alginate and the nanocomposite yarns containing 0.10 and 2.00% chitin whiskers was evaluated using a Tris-HCl buffer solution (pH = 7.4) and the buffer solution containing 0.4 mg mL^{-1} of lysozyme (pH = 7.4). The use of lysozyme/Tris-HCl solution was to investigate the susceptibility of the chitin whiskers to depolymerization by lysozyme (Chung *et al.*, 1994). Specifically, the fiber samples were placed in 20 mL of the neat and the lysozyme containing buffer solutions in a shaking incubator at 37°C. At various time points, the fiber samples were removed and dried in an oven at 40°C for 2 h. SEM images of the fiber samples were taken to observe any change in the surface morphology. In addition, to simulate the effect of Ca^{2+} ions, which are cations commonly present in biological environment, during in vivo degradation of the neat alginate and the nanocomposite fibers containing 0.1 and 2.0% chitin whiskers, the buffer solution containing 5% $CaCl_2$ (pH = 7.4) was used. The mechanical integrity of the yarns after biodegradation tests was also observed. The reported value for each fiber sample was averaged from at least 10 specimens.

3.4 Results and Discussion

3.4.1 Morphology and Sizes of Chitin Whiskers

The as-prepared chitin whisker suspensions from hydrolyzed shrimp shells displayed colloidal behavior because of the presence of the positive charges induced on the surface of the crystallites by the protonation of the amino groups ($-NH_3^+$) (Marchessault *et al.*, 1959). The degree of deacetylation of these chitin whiskers was 20.4% and the solid fraction of these colloidal suspensions was 2.07% w/v. A selected TEM image illustrating the as-prepared chitin whiskers from a colloidal suspension is shown in Figure 3.2.

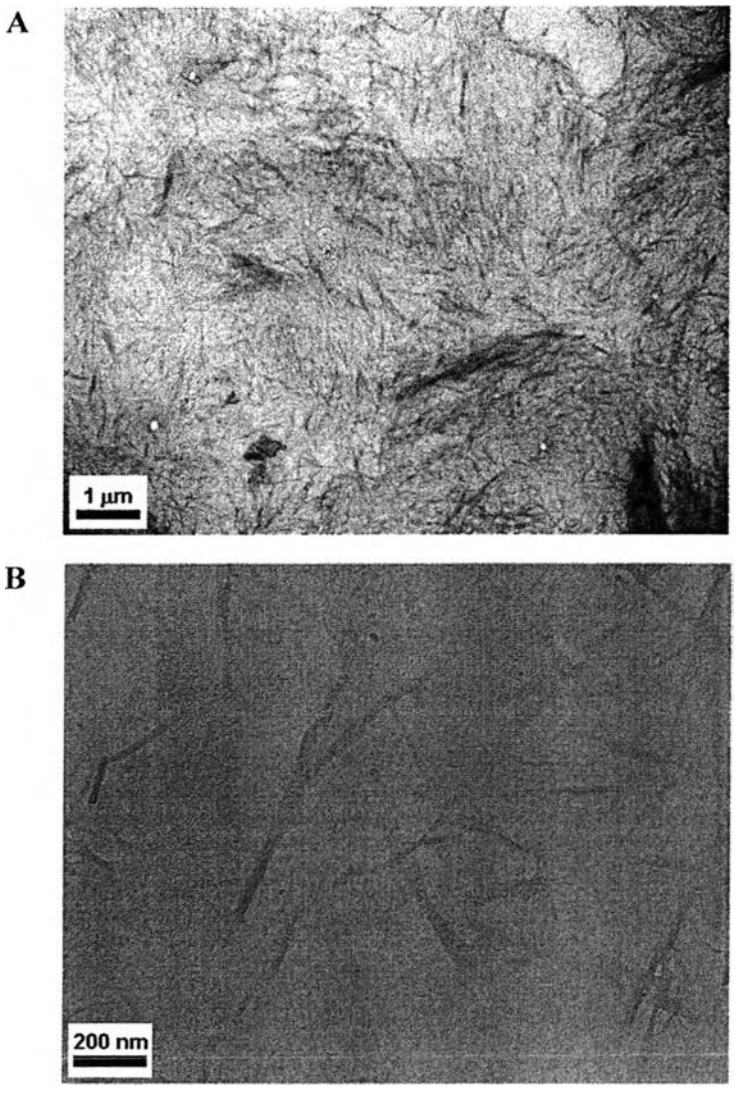


Figure 3.2 Selected TEM images of chitin whiskers from as-prepared colloidal suspensions at two different magnifications: (A) 20,000x and (B) 25,000x.

The suspension consisted of slender rods of crystalline fragments of chitin, which are present both in their individual and aggregated forms. These individual crystalline fragments of chitin exhibited a broad distribution in both of their width (d) and length (L). Specifically, the width of these chitin whiskers ranged from 8 to 73 nm (with the average value being ~46 nm), whereas the length ranged from 110 to 975 nm (with the average value being ~343 nm). The aspect ratio (L/d) of these whiskers was ~7.5. These dimensions are different from what was reported for chitin whiskers obtained from crab shells (Revol and Marchessault, 1993) and

squid pens (Paillet and Dufresne, 2001) in which the average width, the average length, and the aspect ratio were 10 nm, 150 nm, and 15, respectively.

3.4.2 Morphology of Neat Alginate and Nanocomposite Fibers

Selected SEM images of the neat calcium alginate fibers and the nanocomposite fibers that contained 2.00% w/w chitin whiskers are shown in Figure 3.3; those of the other nanocomposite fibers were not shown as they appeared to be similar to that of the alginate fibers. Evidently, these fibers were straight, with streak patterns being observed on their surface. Both the roughness along the inner perimeter of the spinneret holes and the shrinkage upon drying of the fibers were postulated as the main reasons for the formation of these streaks. Apart from these streaks, the surface of the neat alginate fibers and most of the nanocomposite fibers, except for the ones that contained 2.00% w/w of chitin whiskers, was smooth, suggesting the complete incorporation of the whiskers within the fibers. At 2.00% w/w chitin whiskers, the surface of the nanocomposite fibers was rough, with an evidence of the aggregates of the whiskers appearing on their surface.

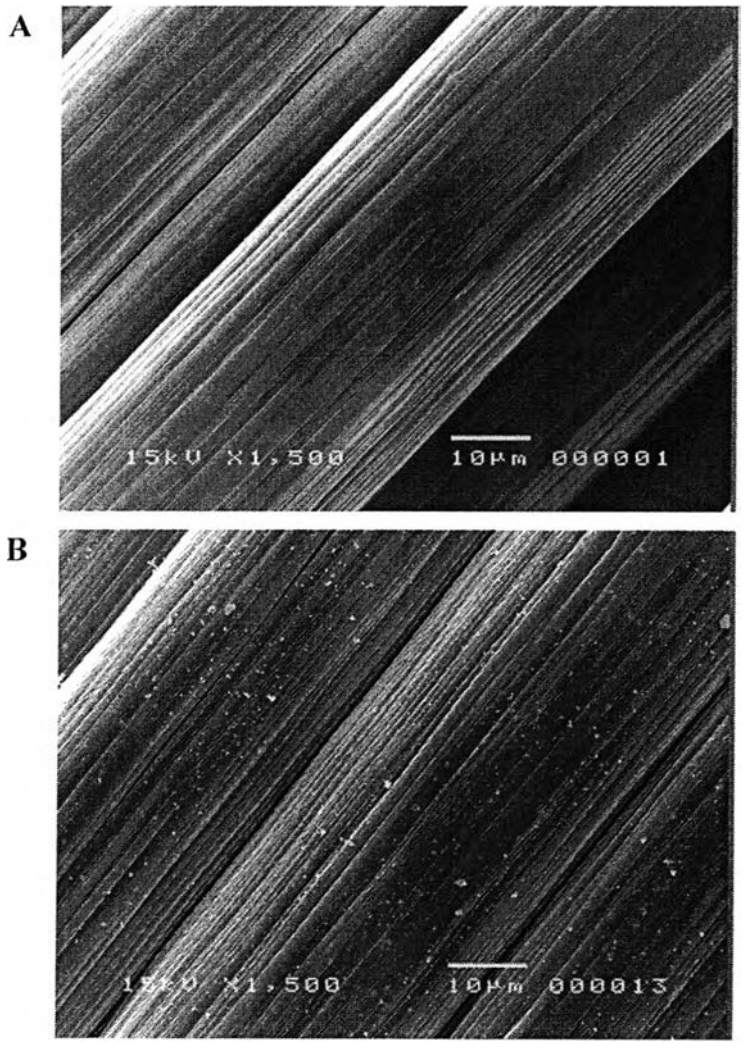
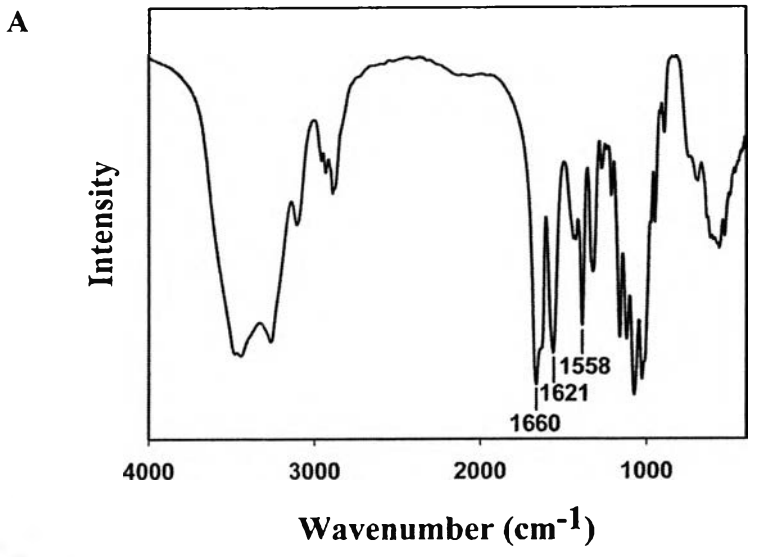
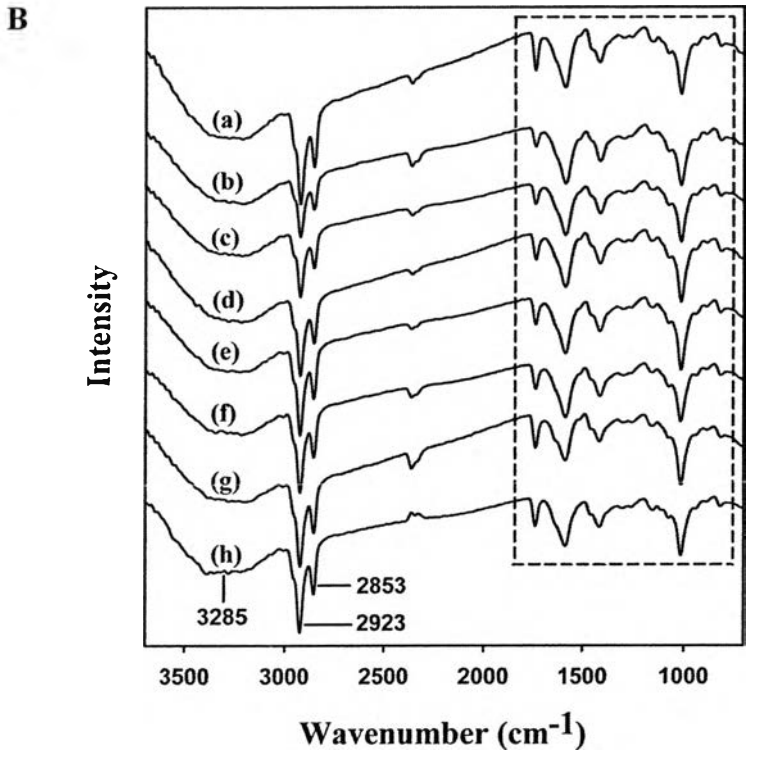


Figure 3.3 Selected SEM images of (A) calcium alginate fibers and (B) calcium alginate fibers containing 2.00% w/w of chitin whiskers (based on the weight of alginate).

3.4.3 Structural Characterization

FT-IR spectra of the chitin whiskers, the neat alginate fibers, and the nanocomposite fibers that contained chitin whiskers in various amounts (i.e., 0.05-2.00% w/w) are shown in Figure 3.4.


A
Intensity
1660
1621
1558
4000
3000
2000
1000
Wavenumber (cm-1)


B
Intensity
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
3285
2853
2923
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

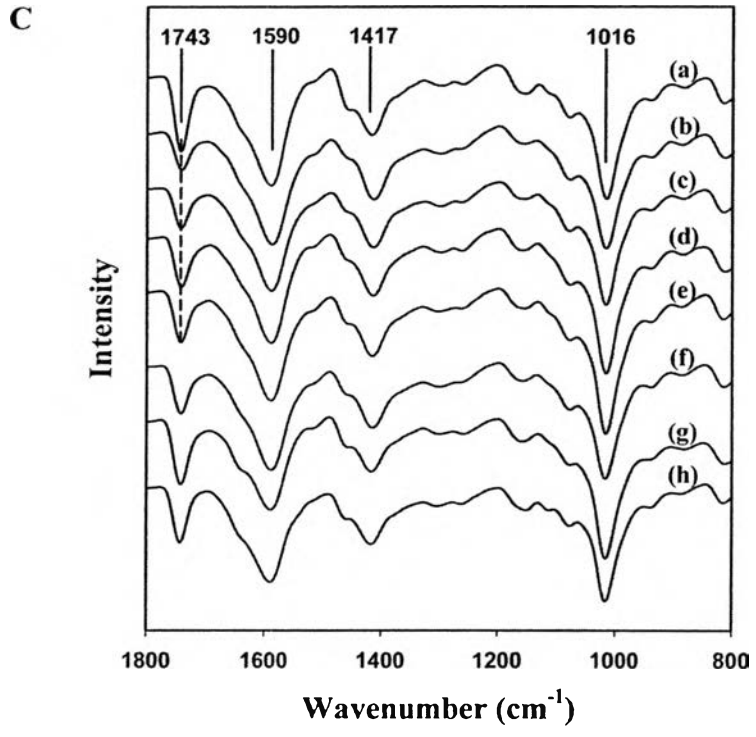


Figure 3.4 (A) FTIR spectrum of chitin whiskers and (B) ATR-FTIR spectra of (a) calcium alginate fibers and calcium alginate fibers containing (b) 0.05, (c) 0.10, (d) 0.15, (e) 0.20, (f) 0.40, (g) 1.00, and (h) 2.00% w/w chitin whiskers (based on the weight of alginate). The spectra in (B) are in the wavenumber range of 4000 to 650 cm^{-1}, while those in (C) highlighted from those in (B) in the wavenumber range of 1800 to 800 cm^{-1}.

The characteristic absorption bands of the chitin whiskers (see Figure 3.4A) were observed at 1660 and 1621 cm^{-1} (amide I bands; singly H-bonded and doubly H-bonded, respectively) and 1558 cm^{-1} (amide II bands) (Brugnerotto *et al.*, 2001). FT-IR spectrum of the neat alginate fibers (see Figure 3.4B,C) exhibited characteristic absorption bands at 1590 and 1417 cm^{-1} (asymmetric and symmetric stretching of COO^- groups of alginate, respectively) and 1743 cm^{-1} (C=O of ester groups) (Kadokawa *et al.*, 2005). The spectra of the nanocomposite fibers are characterized by the presence of the absorption bands typical of the pure components. All characteristic peaks of the alginate fibers were observed in all the spectra of the nanocomposite fibers. Interestingly, the intensity of the absorption

peak at 1743 cm^{-1} for the nanocomposite fibers at low whisker contents (i.e., 0.05-0.20% w/w) was lower than that of the neat ones [see Figure 3.4B,C (b-e)], possibly a result of the formation of intermolecular hydrogen bonds between alginate and the chitin whiskers. Moreover, due to the presence of COO^- on alginate molecules and NH_3^+ on chitin whiskers, electrostatic interaction between the chitin whiskers and the alginate matrix molecules might also occur (Fan *et al.*, 2005). With further increase in the whisker content between 0.20 and 2.00% w/w, the change in the intensity of the absorption peak at 1743 cm^{-1} was not significant, implying that the interactions between alginate and the chitin whiskers were not enhanced. Conclusively, both the intermolecular hydrogen bonding and the electrostatic interactions contributed to the strong interactions between alginate and the chitin whiskers, especially at low whisker contents.

3.4.4 Mechanical Properties

Since the amount of retained moisture in a hygroscopic material affects significantly its mechanical properties (Fan *et al.*, 2006), it is the reason why the amount of retained moisture in the yarn samples was controlled by simply drying at 40°C for 2 h and both the temperature and the relative humidity during testing were closely monitored. Figure 3.5 shows tenacity and elongation at break of the neat alginate fibers and the nanocomposite fibers that contained chitin whiskers in various amounts (i.e., 0.05-2.00% w/w).

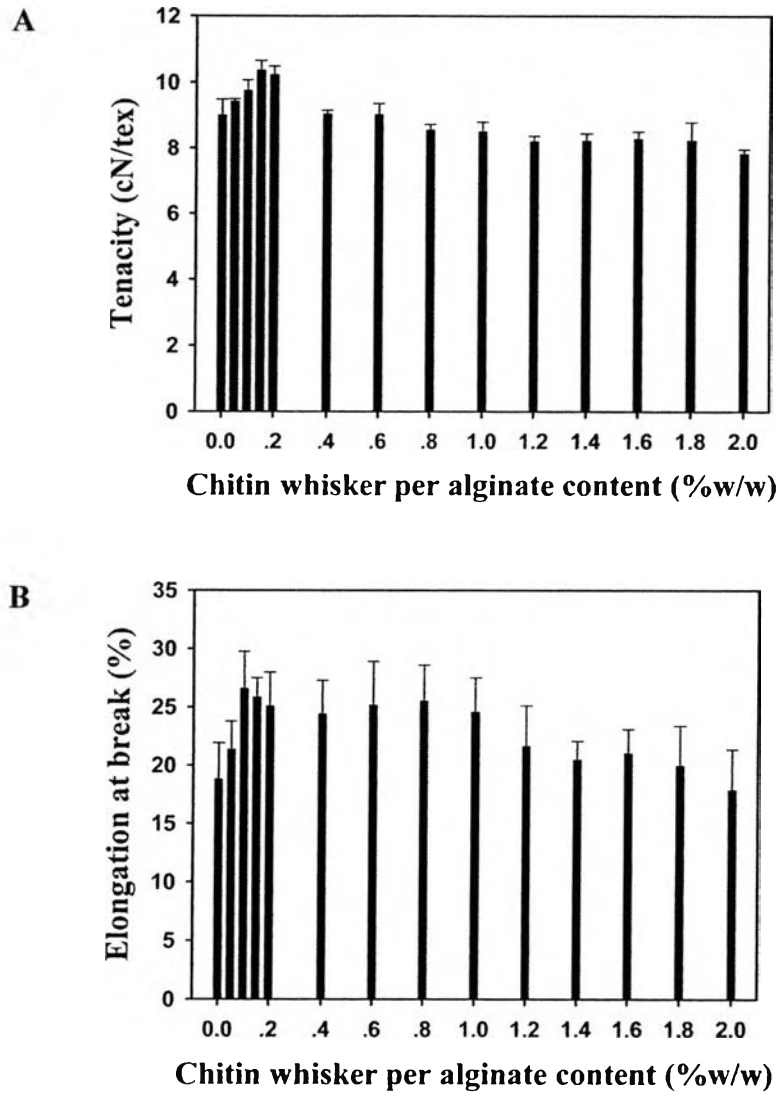


Figure 3.5 (A) Tenacity and (B) elongation at break of calcium alginate fibers and calcium alginate fibers containing 0.05-2.00% w/w chitin whiskers (based on the weight of alginate).

The tenacity of the nanocomposite fibers increased from that of the alginate fibers with initial increase in the whisker content to reach a maximum value at a whisker content of ~0.15% w/w and decreased with further increase in the whisker content. A similar trend was observed on the elongation at break of these fibers, with the maximum value being observed at a slightly different whisker content from that of the tenacity (i.e., at ~0.10% w/w). Mechanical properties of nanocomposites do not depend only on the amount, the size, the shape, and the

alignment of the fillers, but also on the ability of the matrix to transfer the stress to the fillers (Sretenovic *et al.*, 2006). The latter depends solely on the affinity between the matrix and the fillers. The initial increase in both the tenacity and the elongation of the nanocomposite fibers should be due to both the intermolecular hydrogen bonding and the electrostatic interactions between alginate molecules and the chitin whiskers at low whisker contents, while the decrease in the tenacity and the elongation of the nanocomposite fibers at the whisker contents greater than ~0.10-0.15% w/w could be due to the aggregation of the whiskers.

3.4.5 Thermal Behavior

Thermal behavior of the sodium alginate powder, the as-prepared chitin whiskers, the neat alginate fibers, and the nanocomposite fibers that contained chitin whiskers in various amounts (i.e., 0.05-2.00% w/w) was investigated by DSC and the results are shown in Figure 3.6.

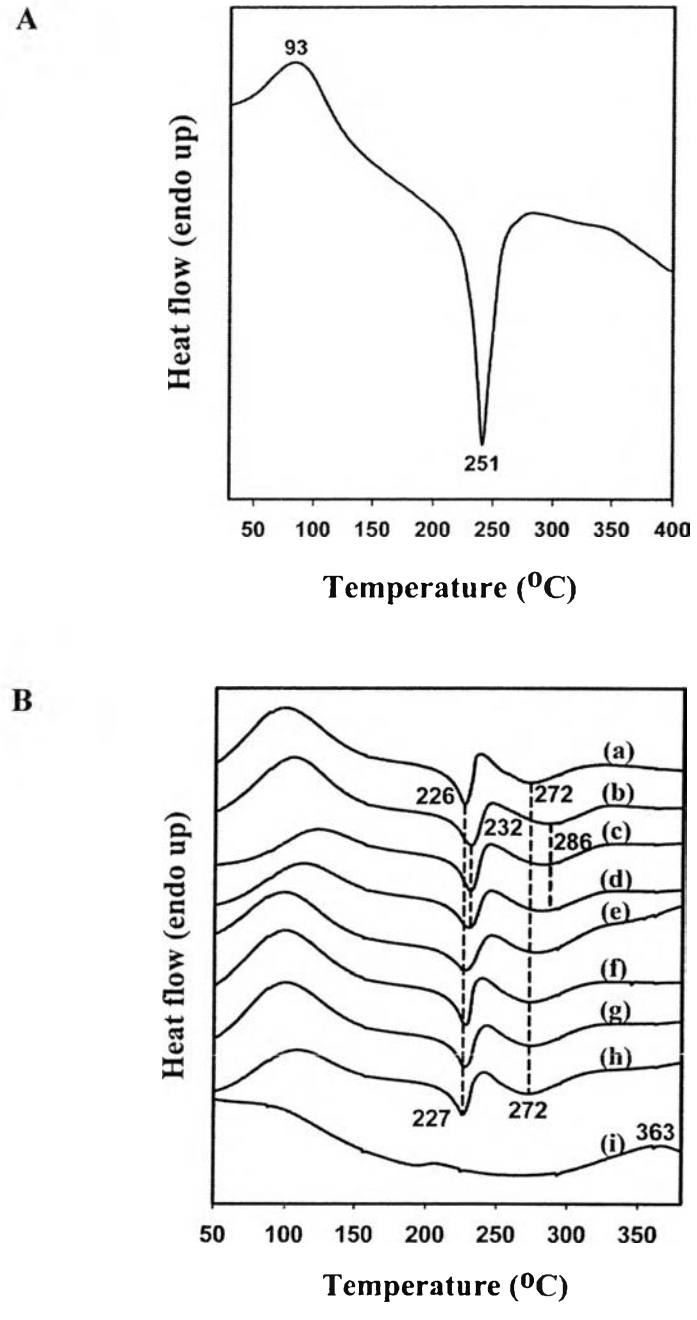


Figure 3.6 (A) DSC thermogram of native sodium alginate powder and (B) those of (a) calcium alginate fibers and calcium alginate fibers containing (b) 0.05, (c) 0.10, (d) 0.15, (e) 0.20, (f) 0.40, (g) 1.00, and (h) 2.00% w/w chitin whiskers (based on the weight of alginate) and (i) chitin whiskers.

The native sodium alginate powder exhibited an endotherm and an exotherm at ~93 and ~251°C, respectively (see Figure 3.6A). These compared well

with those reported by others (Miura *et al.*, 1999; Soares *et al.*, 2004; Smitha *et al.*, 2005). Soares *et al.* (2004) indicated that the endotherm at 93°C corresponded to dehydration while the exotherm at ~251°C corresponded to thermal decomposition of the material. The neat alginate fibers exhibited an endotherm over the temperature range of 63 to 134°C (with the peak temperature being observed at ~99°C) and two exotherms at ~226 and ~272°C (see Figure 3.6B). While the presence of the endotherm should be associated with the loss of moisture that was retained in the alginate fibers (due to its hygroscopic nature) (Zohuriaan and Shokrolahi, 2004), the two exotherms should correspond to the thermal decomposition of the material, despite the absence of the exotherms at ~251°C of the native sodium alginate. The shift in the thermal decomposition temperature of the neat alginate fibers from that of the sodium alginate is likely a result of the complexation of the alginate molecules with Ca^{2+} ions, while is in general accord with the observation by Ribeiro *et al.* (2005).

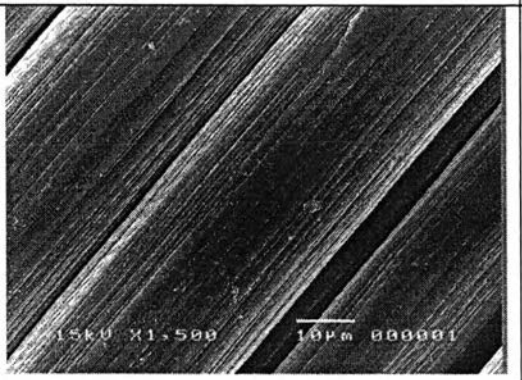
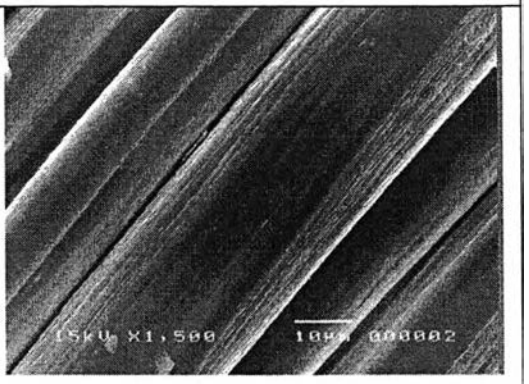
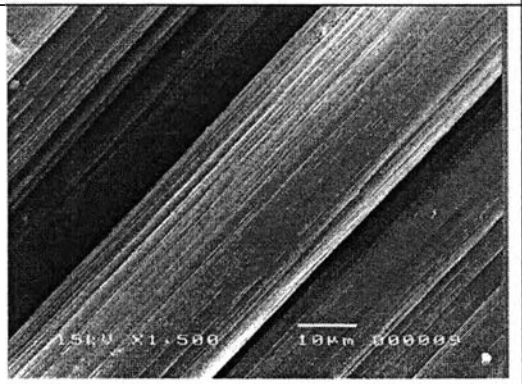
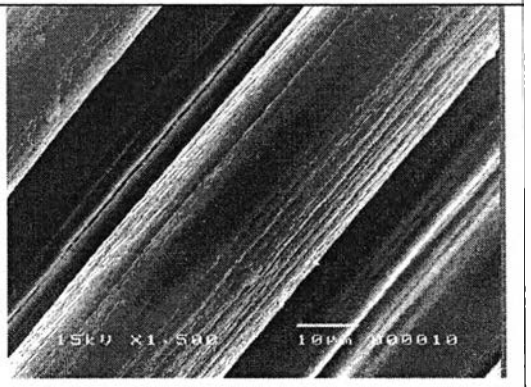
In case of the chitin whiskers, an endotherm at ~363°C should be attributed to the decomposition of the materials, while the DSC thermograms of all the nanocomposite fibers exhibited transition temperatures similar to those of the neat alginate fibers. The presence of the endotherms, with the peak temperatures locating at ~98 to ~130°C, indicated that incorporation of the chitin whiskers within the nanocomposite fibers did not affect the moisture-retention ability of the alginate matrix. On the other hand, incorporation of a small amount of the chitin whiskers (i.e., 0.05-0.15% w/w) caused the peak positions of the endotherm at ~99°C and the exotherms at ~226 and ~272°C of the native alginate to shift to higher values. Nakamura *et al.* (1995) reported that the crossing point of the baseline and the transition line of the endotherm of an alginate film containing either di- or trivalent cations at ~100 to ~130°C could also be associated with the glass transition temperature, which might be affected by the restricted motion of the main chains by such cross-linking. The fact that the increase in the peak positions of the native alginate of the nanocomposite fibers was greater at low whisker contents (i.e., 0.05-0.15% w/w) could be a result of the restriction of the alginate molecules due to the presence of the chitin whiskers (Nakamura *et al.*, 1995). At greater whisker contents

however, aggregation of the whiskers led to the increase in the ionic radius between the alginate molecules and the whisker aggregates, thus the restriction in the motion of the alginate molecules was reduced. Since it was previously shown that specific interactions between alginate molecules and the chitin whiskers were observed at low whisker contents, it is postulated that the observed improvement in the thermal stability of these nanocomposite fibers was also a result of such specific interactions.

3.4.6 Biodegradation Studies

For possible use in wound dressing application, the alginate fibers and the nanocomposite fibers containing chitin whiskers in various amounts (i.e., 0.05-2.00% w/w) were evaluated for their degradability in two types of media, i.e., Tris-HCl buffer solution and the buffer solution containing lysozyme. Table 3.1 shows selected SEM images of the neat alginate fibers and the nanocomposite fibers that contained 2.00% w/w chitin whiskers after submersion in Tris-HCl buffer solution or the buffer solution containing lysozyme for 5 days. Compared with that of the original fibers (see Figure 3.3), the surface of the alginate fibers and the nanocomposite fibers after degradation in both types of media was smoother, with the surface of the fibers after submersion in the buffer solution containing lysozyme being smoother than that of the fibers in the Tris-HCl buffer solution alone. The smoothening of the fiber surface should be a result of the erosion of the surface due to partial dissolution or degradation in the media.

Table 3.1 Selected SEM images of calcium alginate fibers and calcium alginate fibers containing 2.00% w/w of chitin whiskers after submersion in Tris-HCl buffer solution or the buffer solution containing lysozyme for 5 days

Type of fibers	Type of medium	
	Tris-HCl buffer solution	Tris-HCl buffer solution containing lysozyme
Alginate fibers		
Alginate fibers containing 2.00% w/w of chitin whiskers		

The partial dissolution or degradation of the alginate fibers and the nanocomposite fibers containing 0.10 and 2.00% w/w chitin whiskers after submersion in either Tris-HCl buffer solution or the buffer solution containing lysozyme for various time intervals is shown in Figure 3.7.

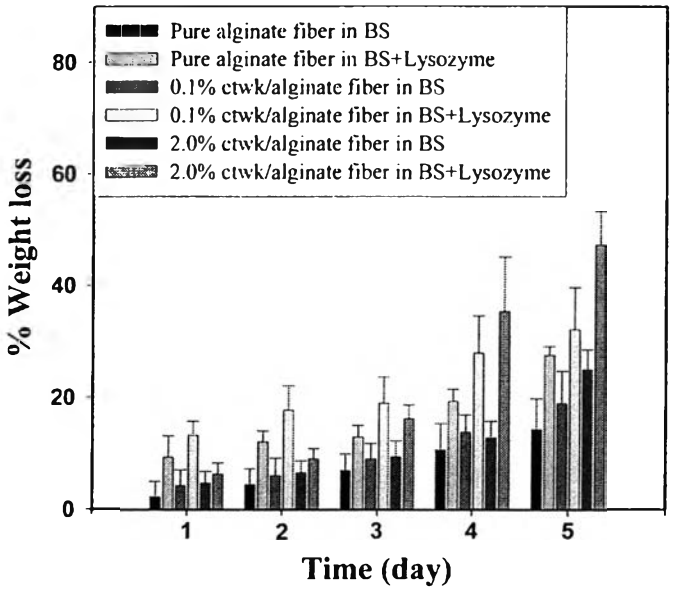


Figure 3.7 The loss in the weight of calcium alginate fibers and calcium alginate fibers containing 0.10 and 2.00% w/w chitin whiskers (based on the weight of alginate) after submersion in either Tris-HCl buffer solution (BS) or the buffer solution containing lysozyme (BS + Lysozyme).

At a given submersion time point, the loss in the weight of the fibers after submersion in Tris-HCl buffer solution generally increased with the addition and increasing amount of the chitin whiskers. A similar trend was also observed for the fibers in the buffer solution containing lysozyme on days 4 and 5, but the loss in the weight of the fibers containing 2.00% w/w chitin whiskers on days 1 and 2 was lower than that of the alginate fibers and the nanocomposite fibers containing 0.10% w/w of the whiskers, with the loss in the weight of the fibers containing 2.00% w/w of chitin whiskers on day 3 being greater than that of the alginate fibers, but lower than that of the nanocomposite fibers containing 0.10% w/w of the whiskers. Between the two types of media, the presence of lysozyme resulted in a significant

reduction in the weight of the fibers, which agreed well with the smoothening of the fiber surface shown in Table 3.1. Clearly, the presence of the chitin whiskers accelerated the biodegradation of the nanocomposite fibers in the buffer solution containing lysozyme.

The mechanical integrity in term of the tenacity of the neat alginate fibers and the nanocomposite fibers containing 0.10 and 2.00% w/w chitin whiskers after submersion in either Tris-HCl buffer solution or the buffer solution containing lysozyme for various time intervals is shown in Figure 3.8. The tenacity of the fibers prior to submersion in the media is also included for comparison. Surprisingly, the tenacity of all the fiber samples after submersion in both types of media was much greater than that of the original fiber samples, most likely a result of the multiple drying of the fiber samples. Generally, increasing the submersion time caused the tenacity of the fibers to decrease slightly, with an exception to the nanocomposite fibers containing 2.00% w/w chitin whiskers that showed a slight increase in the property value. In addition, the presence of lysozyme in the buffer solution caused tenacity of the fibers to decrease. Along with the greater loss in the weight of the fibers that had been submerged in the buffer solution containing lysozyme, the observed decrease in the tenacity of the fibers after submersion in this medium should be due to partial degradation of the whiskers (Chung *et al.*, 1994).

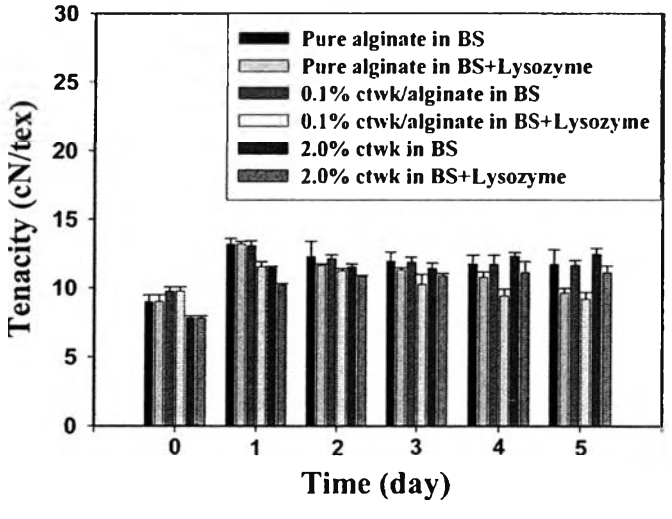


Figure 3.8 Tenacity of calcium alginate fibers and calcium alginate fibers containing 0.10 and 2.00% w/w chitin whiskers (based on the weight of alginate) after submersion in either Tris-HCl buffer solution (BS) or the buffer solution containing lysozyme (BS + Lysozyme).

The results obtained from the biodegradation studies are in agreement with the *in vivo* degradation study of calcium alginate spheres as reported by Iannuccelli *et al.* (1996). They concluded that the biodegradation process of the calcium alginate spheres underwent three stages. The first stage was hydration coupled with cleavage of some cross-links that could be attributed to both the surface erosion process and the drug diffusion. The second stage was likely due to the dissolution of the soluble polymer fragments originated by the ion exchange (the displacement of Ca^{2+} inside with Na^{+} outside). The third stage of the degradation involved physical disintegration of the spheres into small fragments. These reactions could be enhanced in the presence of lysozyme. Capon (1963) indicated that, in the presence of lysozyme, the intramolecular carboxyl groups accelerates the hydrolysis of 2-carboxy phenyl-β-D-glucopyranoside (the structure nearly the same as alginate). In a similar finding, Berger and Weiser (1957) demonstrated that lysozyme can hydrolyze the β(1→4) linkage of poly(*N*-acetylglucosamine) or chitin. These findings agreed with our postulation in that the observed decrease in the tenacity of

the fibers after submersion in the buffer solution containing lysozyme was a result of the partial degradation of the whiskers.

Lastly, the effect of the presence of Ca^{2+} ions in the Tris-HCl buffer solution on mechanical integrity of the neat alginate fibers and the nanocomposite fibers containing 0.10 and 2.00% w/w chitin whiskers after submersion in the buffer solution containing 5% $CaCl_2$ was investigated and the results are shown in Figure 3.9. This condition was to simulate the effect of Ca^{2+} ions which are commonly present in biological environment, e.g., in the plasma when these fibrous materials are to be used as wound dressings.

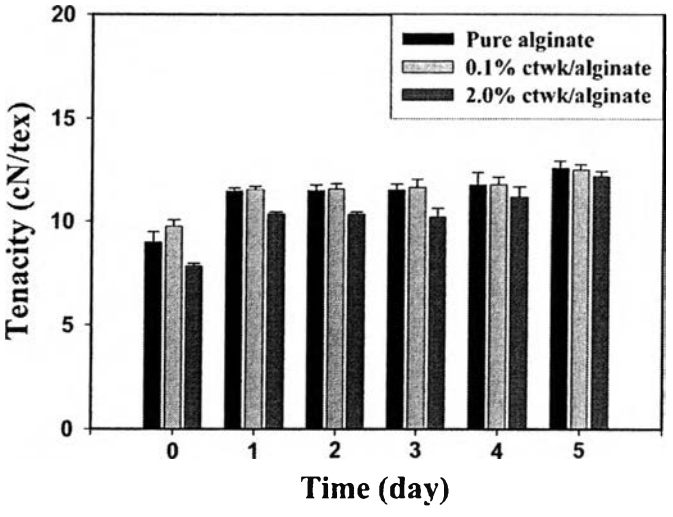


Figure 3.9 Tenacity of calcium alginate fibers and calcium alginate fibers containing 0.10 and 2.00% w/w chitin whiskers (based on the weight of alginate) after submersion in either Tris-HCl buffer solution (BS) containing 5% $CaCl_2$.

Clearly, all of the fibers that were submerged in the medium exhibited much greater tenacity than that of the original materials, likely a result of the multiple drying already mentioned and/or the enhancement in the degree of cross-linking of the fibers by Ca^{2+} ions upon the diffusion of the cations into the fibers. The latter was also responsible for the slight increase in the property values of all the fiber samples.

3.5 Conclusions

Wet spinning process was used to fabricate calcium alginate yarn (30 fibers) and nanocomposite yarn (30 fibers) based on chitin whisker-reinforced calcium alginate system. The chitin whiskers, consisting of slender rods with a broad distribution in both of their length and width (with the average length and width being 343 and 46 nm, resulting the aspect ratio of ~7.5), were prepared by acid hydrolysis from decalcified and deproteinized shrimp shells and the amount of the whiskers in the nanocomposite fibers ranged between 0.05 and 2.00% w/w. Incorporation of the chitin whiskers within the nanocomposite fibers was verified by Fourier-transformed infrared spectroscopy (FT-IR). Based on observation by scanning electron microscopy (SEM), the chitin whiskers in most of the nanocomposite fibers were embedded well within the fibers, except for the nanocomposite fibers containing 2.00% w/w of the whiskers that showed an evidence of whisker aggregates on the fiber surface. Improvement in both the mechanical and thermal properties was observed when the amount of the whiskers in the nanocomposite fibers was low. The significant increase in these properties was postulated to be a result of specific interactions, i.e., hydrogen bonding and electrostatic interactions, between the alginate molecules and the homogeneously-dispersed chitin whiskers. Biodegradation of the calcium alginate fibers and the nanocomposite fibers was tested in two types of media, i.e., Tris-HCl buffer solution and the buffer solution containing lysozyme. The addition of the chitin whiskers in the nanocomposites fibers accelerated the biodegradation process of the fibers in the presence of lysozyme, while the presence of Ca^{2+} ions in the Tris-HCl buffer solution helped improve the tenacity of both the alginate and the nanocomposite fibers.

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