CHAPTER V
PREPARATION AND CHARACTERIZATIONS OF WET SPUN ALGINATE FIBER HOLDING CHITOSAN WHISKERS

5.1 Abstract

Holding of chitosan whiskers in alginate fibers was achieved by mixing the homogenized chitosan whisker colloidal suspension with 6% w/v sodium alginate aqueous solution. The wet spinning process is the economical method used for the preparation of the target fibers. The obtained chitosan whiskers were prepared by deacetylation of chitin whiskers which were obtained by hydrolyzing of chitin from shrimp shells. The average length and width of the chitosan whiskers were 309 and 64 nm, with the aspect ratio being ~4.8. Study on the mechanical properties of the neat and nanocomposite fibers revealed that, the embedded chitosan whiskers lead the nanocomposite fibers to increase in the tensile strength but lowering the elongation at break. Remarkable occurrence of the streak pattern on the nanocomposite fiber surface containing high whisker content postulated that pre-interactions between alginate aqueous solution and chitosan whiskers suspension occurred before coagulation with Ca^{2+}. Comparing the release profiles of the nanocomposite fibers, influence of chitosan whisker content on the rate and extent of the release of whiskers was evident. Release behavior of 0.2% chitosan whisker-reinforced alginate nanocomposite fiber was gradually increase by the time since the initial time of incubation until the 30 h period of incubation was reached with the content of chitosan whisker released ~95%. On the other hand, release behavior of 0.6% chitosan whisker-reinforced alginate nanocomposite fiber was rapid for the first hour of incubation and further released by increasing in a continuous way until ~84% of chitosan whiskers were released at 30 h of incubation. Release data also indicated the influence of erosion of polymer matrix on chitosan whiskers released from the nanocomposite fibers. Incorporation of antibacterial activities in alginate fiber was also successful by the addition of chitosan whiskers. The chitosan whiskers-reinforced alginate nanocomposite fibers show antibacterial efficacy against both gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*.

(**Key-words**: Alginate; Chitosan whisker; Mechanical property; Release behavior; Antibacterial activity)

5.2 Introduction

Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crawfish. It has received considerable attention for its commercial applications in biomedical, food, and chemical industries (Knorr, 1984; Muzzarelli, 1991). Chitosan and chitosan oligomers have attracted considerable interest due to their biological activities, that is, antimicrobial (Kendra and Hadwiger, 1984; Sekiguchi *et al.*, 1994; Sudarshan *et al.*, 1992), antitumor (Suzuki *et al.*, 1986; Tokoro *et al.*, 1988), and hypocholesterolemic functions (Sugano *et al.*, 1992). The natural antimicroial and/or antifungal characteristics of chitosan and its derivatives (Sudarshan *et al.*, 1992; Chung *et al.*, 2003; El-Ghaouth *et al.*, 1992a; Kim *et al.*, 1997; Papineau *et al.*, 1991) have resulted in their use in commercial disinfectants. Chitosan has several advantages over other types of disinfectants in that it possesses a higher antibacterial activity, a broader spectrum of activity and a lower toxicity for mammalian cells (Liu *et al.*, 2001).

Although chitosan can be produced in powder, film, bead, fiber and fabric forms (Qin and Agboh, 1998; Qin *et al.*, 1997), products made from pure chitosan fibers have not been commercially viable due to the high processing costs involved and the availability of such purified material is still insufficient for large industrial scale fiber production. Poor solubility also limited its applications as a polysaccharide drug. Chemical modification has been attempted to improve water solubility and bioactivities of chitosan (Kurita *et al.*, 1998; Nishimura *et al.*, 1998), however, the reaction course is complicated and the isolation of products is difficult. As a consequence, mixing of chitosan with the other polymeric substances is preferred and chitosan in the form of colloidal suspension is considered as fillers, to avoid the restriction of the solubility.

Whiskers or crystalline microfibrils are colloidal suspension substances that have caught a great deal of interest in the research community due to the recent

uprising of nanotechnology. Incorporation of whiskers as fillers in a polymer matrix results in a new class of materials, i.e., nanocomposites. Whiskers that have already been investigated are cellulose (Favier *et al.*, 1995; Ljungberg *et al.*, 2006), starch (Dufresne *et al.*, 1996), and chitin whiskers (Morin and Dufresne, 2002; Paillet and Dufresne, 2001). These whiskers are obtained from animals or plants that synthesize extracellular high-performance skeleton biocomposites consisting of a matrix reinforced by fibrous biopolymers (Atkins and Keller, 1975; Neville, 1993; Preston, 1967). The interest in the use of whiskers as reinforcing nanofillers has been due to their high aspect ratio and highly crystalline nature. Chitin is known to form microfibrillar arrangements in living organisms, thus, chitin whiskers can be prepared by acid hydrolysis of chitin (Paillet and Dufresne, 2001). These whiskers were used as reinforcing fillers in both synthetic polymeric matrices (Morin and Dufresne, 2002; Paillet and Dufresne, 2001) and natural ones (Lu *et al.*, 2004; Sriupayo *et al.*, 2005). However, chitosan is much more considerable in term of antimicrobial activities compare with chitin because of the higher amount of amine group, therefore, higher antibacterial activities. In the present contribution, chitosan whiskers were prepared by deacetylation of chitin whiskers.

Alginate on the other hand is a natural biopolymer obtained from cell walls of brown algae (Phaeophyta) such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp (Clare, 1993). It is a linear block copolymer consisting of uronic acid residues, namely β-D-mannuronic and α-L-guluronic acid, linked by (1→4)-linkages. Among the various fibrous and hydrogel products, alginate-based products are currently the most popular ones used in wound management, since they offer many advantages, e.g., biocompatibility, haemostatic capability, and readily form gels upon absorption of wound exudates (Jarvis *et al.*, 1987). Such gels prevent 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). Alginate is water-soluble and, in the presence of divalent cations, e.g., Ca^{2+}, alginate gels can be formed due to ionic cross-linking via calcium bridges between L-guluronic acid residues on adjacent chains (McDowell, 1974). Because of this reversible solubility, alginate can be fabricated in various forms (Agren, 1996; Dong *et al.*, 2006; Li *et al.*, 2005) and because of the above-mentioned 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). However, alginate itself does not possess an antimicrobial property and wounds often provide favorable environments for colonization of microorganisms, which may lead to infection and delayed healing. Therefore, in designing a material that promotes wound healing, the ability of the material in providing an adequate antimicrobial activity is desirable, provided that the antimicrobial agent present does not compromise its healing abilities (Guggenbichler *et al.*, 1999).

In this study, wound dressing materials in the form of fibers that combine the desirable properties of both alginate and chitosan whiskers were developed. Chitosan whiskers were used as nanoscopic reinforcing fillers and were oriented by holding with alginate fiber. The incorporation of chitosan whiskers was in order to achieve two main objectives: 1) to improve the mechanical properties and 2) to incorporate antibacterial ability in the alginate fibers. Problem of precipitation of chitosan (because of its high molecular weight) in the presence of calcium ions (Tamura *et al.*, 2002) can also be overcome by using chitosan in the form of whiskers, therefore, higher level of chitosan incorporated in alginate fiber was allowed. Moreover, antibacterial effect with initial use and the ability to sustain antibacterial components of the nanocomposite fibers were expected. The chitosan whisker-reinforced alginate nanocomposite fibers were prepared by the wet spinning from mixtures of an alginate solution and chitosan whisker suspensions. The effect of chitosan whiskers on mechanical behavior and antibacterial activity of the chitosan whisker-reinforced alginate nanocomposite fibers was investigated. In addition, release characteristic of the presence chitosan whiskers was observed.

5.3 Experimental

5.3.1 Materials

Shells of *Penaeus merguiensis* shrimp were provided by Surapon Food Public Co., Ltd. (Thailand). Sodium alginate (white powder) was purchased from Carlo Erba (Italy). Tris-HCl (molecular biology grade) was purchased from Scharlau Chemie (Spain). Sodium hydroxide (50% w/w aqueous solution) was supplied by KTP Cooperation Co., Ltd. (Thailand). Nitric acid (65% w/w, analytical reagent grade) and hydrochloric acid (37% w/w, analytical reagent grade) were purchased from Carlo Erba (Italy). Dehydrated calcium chloride (edible grade) was supplied from Asia Drug & Chemical Co., Ltd. (Thailand). Cibacron brilliant red 3B-A (also known as Reactive Red 4, C.I. 18105) was purchased from Sigma (Milano, Italy). Amido Black 10B was purchased from Wako Pure Chemical Industries, Ltd. Methanol, ethanol, and acetone (commercial grade) were purchased from Labscan (Asia, Thailand). All other chemicals were used as received.

5.3.2 Preparation of Chitin, Chitin Whiskers, and Chitosan 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 obtained chitin based on the method described by Paillet and Dufresne (2001). The whisker suspension was obtained by hydrolyzing chitin sample with 3 N HCl at 104°C for 6 h under vigorous stirring. The ratio of the HCl solution (3 N) to chitin was 30 cm^3g^{-1}. After the acid hydrolysis, the suspension was immediately diluted with distilled water, followed by centrifugation to separate the obtained chitin solid fraction from aqueous medium and because of the small size of the as-prepared chitin the rate of centrifugation used was at 10,000 rpm (for 10 min). This process was repeated three times. To remove the HCl remaining in the suspension, the suspension was then dialyzed in distilled water at room temperature for 3 d until pH=6. Chitosan emulsion was prepared from the obtained chitin whiskers by deacetylation of chitin whiskers in 50% w/v NaOH aqueous solution containing 0.5% w/w sodium borohydride ($NaBH_4$) acting as a reducing agent to prevent depolymerization of chitosan. The ratio of chitin whiskers

to NaOH aqueous solution was 1 g of chitin whiskers in 10 ml of NaOH solution. The deacetylation was performed at 121°C for 20 min. The product obtained was diluted with distilled water, followed by centrifugation at 10,000 rpm for 10 min. This process was repeated three times. The suspension was then dialyzed in distilled water for 3 d until pH=6. Homogeneity of the suspension was further achieved by a 5 min treatment in a sonication bath and the suspension was subsequently filtered to remove residual aggregates and was kept in a refrigerator prior to further use.

5.3.3 Neat Alginate Fiber and Chitosan Whisker-Reinforced Alginate Nanocomposite Fiber Preparation

6% w/v sodium alginate aqueous solution was prepared as the original doped solution for the preparation of neat alginate fiber. The spinning dope suspensions of chitosan whisker-reinforced alginate nanocomposite fibers were prepared by mixing homogenized chitosan emulsion (diluted from the as-prepared chitosan emulsion by distilled water, following by a 15 min treatment in a sonication bath) with 6% w/v sodium alginate aqueous solution. The volumetric ratio of the chitosan emulsion to the sodium alginate solution was varied to obtain the spinning dope suspensions with the weight ratio of the chitosan whiskers to alginate ranging from 0.2 to 1.0%. The spinning dope suspensions were left standing in a container at room temperature for degassing and later 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 used for coagulation because it is the non-solvent for alginate, therefore allowing stabilization of the fiber. The obtained yarns (consisting of 30 individual fibers) were drawn at a draw ratio of ~1.2 between two sets of rollers. Finally, the yarns were collected on bobbins, extensively washed with MeOH, and dried.

5.3.4 Characterizations of the As-Prepared Chitosan Whiskers

5.3.4.1 Morphology of the As-Prepared Chitosan Whiskers

Morphology of the as-prepared chitosan 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 chitosan emulsion on formvar grids and were left to dry on the grids prior to TEM observations. The average dimensions of the whiskers were determined from selected TEM images from which at least 60 rods were measured for their length and width using SemAfore 4.0 image-analytical software.

5.3.4.2 Chemical Structure and Degree of Deacetylation of the As-Prepared Chitosan Whiskers

The chemical structure of the chitosan whiskers was confirmed by a Thermo Nicolet NEXUS 670 Fourier-transformed infrared spectroscope (FT-IR). The chitosan whiskers from the as-prepared chitosan emulsion was dried, mixed with KBr powder, and pressed into a pellet. The scanning range was 4000 to 400 cm^{-1} with 64 scans at a resolution of 4 cm^{-1}. The degree of deacetylation of the as-prepared chitosan whiskers was determined using the obtained FTIR spectrum by the method of Sannan *et al.* (1978).

5.3.4.3 Average Molecular Weight (M_w, M_n, M_z) and Molecular Weight Distribution (M_w/M_n, M_z/M_w) of the As-Prepared Chitosan Whiskers

Weight-average molecular weight (M_w), number-average molecular weight (M_n), zero-average molecular weight (M_z) and molecular weight distribution (M_w/M_n and M_z/M_w) of sample were measured by GPC. The GPC equipment consisted of Shodex OH Pak SB-804HQ columns and RI detector. The eluent was 0.2 M CH_3COOH/0.1 M CH_3COONa (4:1). The sample concentration was 0.4% w/v. Eluent and chitosan sample solutions were filtered through 0.45 μm PTFE filters. The column temperature was 40 °C and the flow rate was maintained at 0.8 ml/min. The standard used to calibrate the column was Shodex pollulan P-82. All data provided by the GPC system were collected and analyzed using the Hitachi software package.

5.3.5 Characterizations of Neat Alginate Fiber and Chitosan Whisker-Reinforced Alginate Nanocomposite Fibers

The chemical structures of neat alginate fiber and chitosan whisker-reinforced alginate nanocomposite fibers were confirmed by a Thermo Nicolet

NEXUS 670 Fourier-transformed infrared spectroscope (FT-IR) with 64 scans at a resolution of 4 cm^{-1}. 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}.

The tenacity and the elongation at break of both the neat and the chitosan 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 $mm \cdot min^{-1}$, 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 ± 2°C and 55 ± 2%, respectively. The force and the extension at the breaking point were recorded: these values were used to calculate both the tenacity and the percentage of elongation at break of the yarns (n = 20).

Surface morphology of both the neat and the chitosan whisker-reinforced alginate nanocomposite fibers was examined by a JEOL JSM-5200 scanning electron microscope, operating at an accelerating voltage of 10 kV to obtain a magnification of 1500x. Lastly, staining of the yarns was achieved by immersing the dried yarns in 0.01% w/v Amido Black 10B aqueous solution for 12 h, washed with water to remove the excess dye on the fiber surface and Olympus BX50 light microscope was then used for microscopic observation on the appearance of chitosan whiskers in the nanocomposite fibers.

5.3.6 Release of Chitosan Whiskers from Chitosan Whisker-Reinforced Alginate Nanocomposite Yarns

5.3.6.1 Colorimetric Determination of Chitosan

Determination of the amount of chitosan was done by the method of Muzzarelli (1998)-Colorimetric determination. Briefly, Chitosan is an adsorbent of dyes because protonated amino groups of chitosan can act as cationic sites for anionic dyes. By this method Cibacron brilliant red 3B-A was used as a reactive dye. A solution of the dye was prepared by dissolving the dye powder (150

mg) in demineralized water (100 ml). Aliquots of the dye solution (5 ml) were made up to 100 ml with 0.1 M glycine hydrochloride buffer. The final concentration of the dye was 0.075 g/liter with the final pH of 3.2.

To obtained calibration curve the following procedure was adopted: chitosan emulsions were introduced into test tubes, followed by different volumes of buffer until reach 0.3 ml, to obtained the following concentration of chitosan: 3.839, 7.677, 11.515, 15.354, 38.384, 46.061, and 172.727 μg/ml. Then, aliquots of dye solution (3 ml) were added to each. The absorbance values were measured at 575 nm with a Shimadzu UV-2550 UV-VIS spectrophotometer. Buffer (0.3 ml) and dye (3 ml) solutions were used to prepare the reference solution.

5.3.6.2 Chitosan Whisker-Release Assay

The release characteristic of chitosan whiskers in the chitosan whisker-reinforced alginate nanocomposite yarns was investigated by total immersion release assay. Each specimen of the weight ~0.1 g was immersed in 20 ml of the medium at the physiological temperature of 37°C in a shaking incubator at a speed of 70 rpm. At a specified immersion period ranging between 0 and 30 h, either 1 ml of a sample solution was withdrawn and an equal amount of the fresh medium was refilled. The amount of chitosan whiskers in the chitosan whisker-reinforced alginate nanocomposite yarns was determined by drying 1 ml of the withdrawn sample solution, followed by adding 0.3 ml of 0.1 M glycine hydrochloride buffer and 3 ml of dye solution, respectively. The absorbance values were measured at 575 nm with a Shimadzu UV-2550 UV-vis spectrophotometer. The data were then back calculated from the obtained data against a predetermined calibration curve for chitosan whiskers to determine the cumulative amount of chitosan whiskers released from the specimens at each immersion time point. The experiments were carried out in triplicate and the results were reported as average values.

5.3.7 Antibacterial Activities

Antibacterial property of the neat alginate yarn and chitosan whisker-reinforced alginate nanocomposite yarns were evaluated based on the viable cell counting method. These were test against gram-positive *Staphylococcus aureus* (*S. aureus*) and gram-negative *Escherichia coli* (*E. coli*). Briefly, Inoculum was

prepared by transferring one colony of each microorganism into 20 ml of broth solution. The mixtures were cultured at 37°C in a shaking incubator for 24 h. 0.5 ml of original cell suspension of each microorganism was added into several 4.5 ml of 0.85% sterile NaCl aqueous solution. Diluted with standard serial dilution method was used; 10^5 for *Staphylococcus aureus* and 10^6 for *Escherichia coli*. ~0.2 g of the yarns was added into the mixture. These mixtures were shaken at 150 rpm, and after contacting for 3 h, 100 μL of these suspensions were dipped and spread in standard flat bottomed petri dish containing sterilized nutrient agar. Bacterial growth was visualized after overnight incubation at 37°C in a shaking incubator for 24 h. The plate counting was carried out and the concentrations of viable cell for different samples were determined as colony forming unit (CFU). The Bacterial Reduction Rate (BRR) was calculated according to the following equation.

$$\text{Bacterial Reduction Rate (BRR)} = \frac{N_1 - N_2}{N_1} \times 100$$

Where N_1 = Number of colonies of blank control

N_2 = Number of colonies of positive control

The neat alginate yarn was used as blank control. 0.6% and 1.0% chitosan whisker-reinforced alginate nanocomposite yarns were used as positive control. Each type of yarn was evaluated three times.

5.4 Results and Discussion

5.4.1 Morphology and Sizes of Chitosan Whiskers

The as-prepared chitosan whisker suspensions from deacetylation of chitin whiskers displayed colloidal behavior, due to 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 chitosan whiskers was 50.54% and the solid fraction of these colloidal suspensions was 1.13% w/v. The higher degree of deacetylation is not allowed because, by increasing the time of deacetylation, slender rod shape of chitosan whiskers was

disrupted. This result was in agreement with the work of Phongying *et al.* (2007) that using the longer time of deacetylation of chitin whiskers (i.e. 7 h) leading to the formation of direct chitosan nanoscaffold. A selected TEM image illustrating the as-prepared chitosan whiskers from a colloidal suspension is shown in Figure 5.1. The suspension consisted of 2 parts. Figure 5.1a shows slender rods of crystalline fragments of chitosan which are present their individual forms. These individual crystalline fragments of chitosan exhibited a broad distribution in both of their width (*d*) and length (*L*). Specifically, the width of these chitosan whiskers ranged from 29 to 105 nm (with the average value being ~64 nm), while the length ranged from 165 to 560 nm (with the average value being ~309 nm), resulting in the aspect ratio (*L*/*d*) of ~4.8. Another part of the as prepared chitosan whiskers (see Figure 5.1b) exhibited aggregated forms. The occurrence of the latter part is the result from the interactions between chitosan and alkaline base (i.e. NaOH) during the preparation which was reported by Kurita (1998) that at high pH, precipitation or gelation of chitosan tends to occur and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in the gel formation.

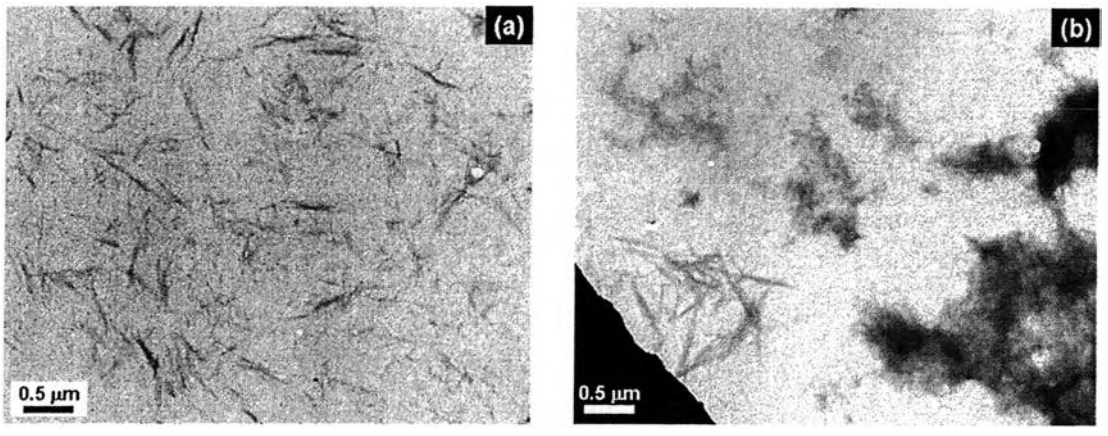


Figure 5.1 Selected TEM image (magnification = 20,000x) of chitosan whiskers from as-prepared colloidal suspensions.

5.4.2 Average Molecular Weight (M_w, M_n, M_z) and Molecular Weight Distribution (M_w/M_n, M_z/M_w) of the As-Prepared Chitosan Whiskers

An attempt to clarify the chitosan whiskers in terms of molecular weight was carried out to confirm the size and the molecular weight distribution of the obtained chitosan whiskers. The obtained chitosan whiskers have the weight-average molecular weight (M_w), number-average molecular weight (M_n), and zero-average molecular weight (M_z) equal to 53000, 59000, and 64000, respectively, while, molecular weight distribution M_w/M_n and M_z/M_w equal to 1.11 and 1.09, respectively. It is be clarified that the obtained chitosan whiskers have low molecular weight (compared with the original chitosan obtained from the same shrimp shells which the molecular weight is ~422,240 Da) and also show good molecular weight distribution. As a result, these obtained chitosan whiskers is beneficial for using as fillers for nanocomposite due to their small size and unique properties.

5.4.3 Chemical Integrity of the As-Prepared Chitosan Whiskers, Neat and Chitosan Whisker-Reinforced Alginate Nanocomposite Yarns

One of the factors that have enabled the development of tailored biomaterials using alginate and chitosan has been their potential to form a polyelectrolyte complex through ionic interaction. It is assumed that the carboxylate moieties on alginate will ionically interact with the protonated amines on chitosan to form a three dimensional matrix. In order to confirm alginate-chitosan whisker interactions, samples were analyzed by FTIR spectroscopy. Figure 5.2 shows spectra of (A) chitosan whiskers, and (B) neat alginate fiber and alginate fibers containing various contents of chitosan whiskers. Tables 5.1 and 5.2 give detailed absorption band assignments for chitosan whiskers and neat alginate fiber, respectively.

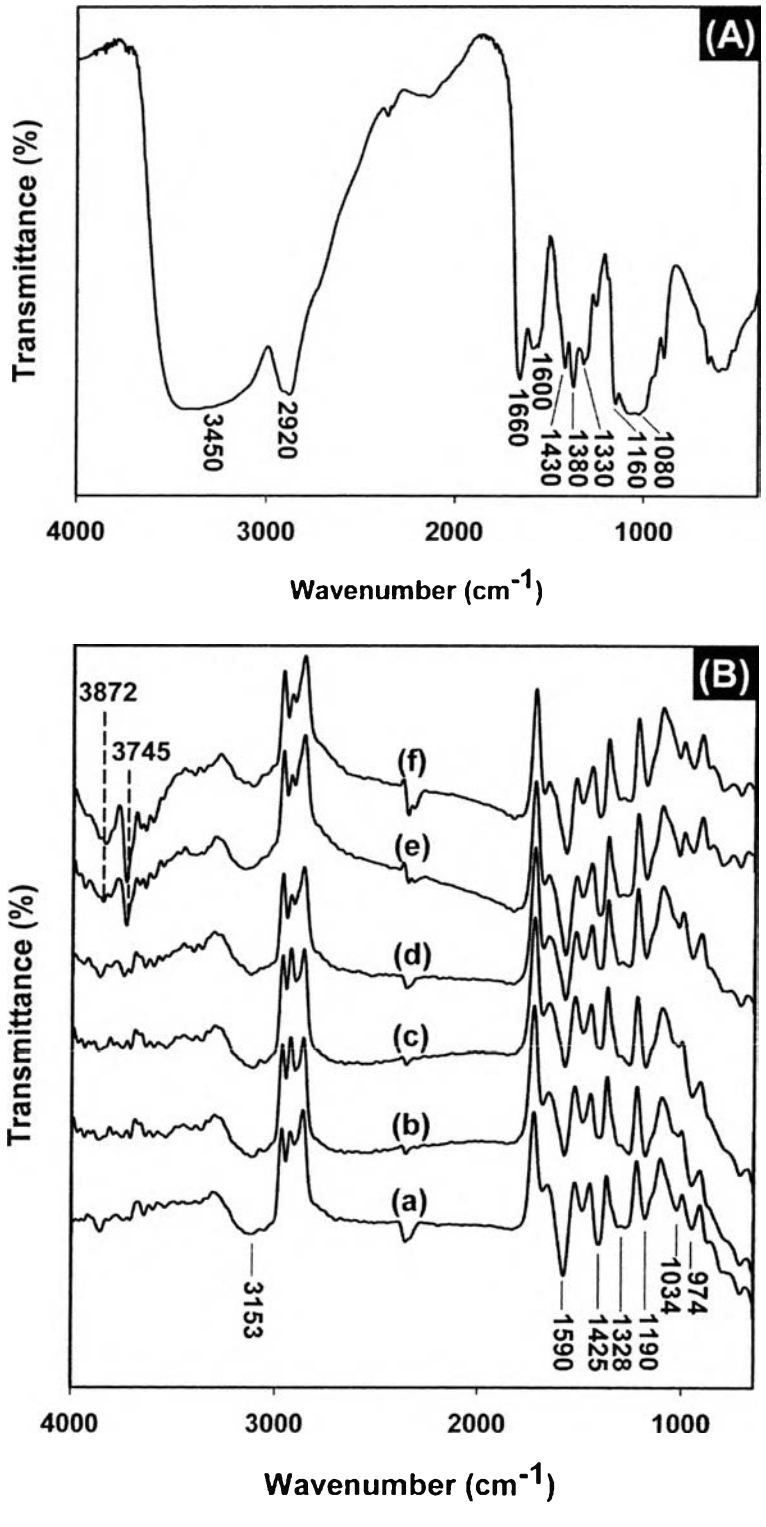


Figure 5.2 (A) FTIR spectrum of chitosan whiskers and (B) ATR-FTIR spectra of (a) neat alginate fiber and alginate fibers containing (b) 0.2%, (c) 0.4%, (d) 0.6%, (e) 0.8%, and (f) 1.0% w/w chitosan whiskers (based on the weight of alginate).

Table 5.1 Assignments of FTIR absorption bands for chitosan whiskers

Wavenumber (cm^{-1})	Assignment
3450	O-H and N-H stretching
2920	C-H stretching
1660	amide I
1600	N-H bending from amine and amide II
1430	CH_2 bending
1380	CH_3 symmetrical deformation
1160	antisymmetric stretch C-O-C and C-N stretching
1080	skeleton vibration of C-O stretching

Table 5.2 Assignments of FTIR absorption bands for neat alginate fiber

Wavenumber (cm^{-1})	Assignment
3153	O-H stretching
1590	COO^- stretching (asymmetric)
1425	COO^- stretching (symmetric)
1328, 1190	C-O stretching
974	C-O stretching, C-H stretching

The bands in the FTIR of all alginate nanocomposite fibers correspond to the species $-COO^-$ and of chitosan whiskers correspond to $-NH_3^+$ were observed, but their position is not different from each of the single components. However, the additional peaks at ~3872 and ~3745 cm^{-1} was observed in 0.8% and 1.0% nanocomposite fibers suggesting that some interactions between these two polymers occurred, nevertheless, a more precise definition of these peaks could not be determined.

5.4.4 Mechanical Integrity of Neat and Chitosan Whisker-Reinforced Alginate Nanocomposite Yarns

Figure 5.3 shows tenacity and elongation at break of the alginate yarns and the nanocomposite yarns that contained chitosan whiskers in various contents (i.e., 0.2-1.0% w/w). The tenacity of the nanocomposite yarns increased from that of the neat alginate yarn with initial increase in the whisker content until reach a maximum value at a whisker content of 1% w/w. Contrary to the tenacity, the percentage of elongation at break remained constant with initial increase in the whisker content until reach a whisker content of ~0.6% w/w and decreased with further increase in the whisker content. The control of the mechanical properties of these nanocomposites is not only associated with the alignment of the fillers, but also with optimizing the stress transfer from the matrix material to the fillers (Sretenovic *et al.*, 2006). The increase in the tenacity of the nanocomposite yarns is due to the small particle size and high surface area of the chitosan whiskers. This is the reason why the beneficial stress transfer from the matrix to the fillers is allowed. As the surface area is increased, the filler-matrix adhesion is improved, resulting in a decrease in the mobility of the macromolecules. Therefore, the percentage of elongation at break tends to reduce gradually. On the other hand, if the filler matrix adhesion is very strong, fillers restrict the mobility of the matrix molecules (Premalal *et al.*, 2002).

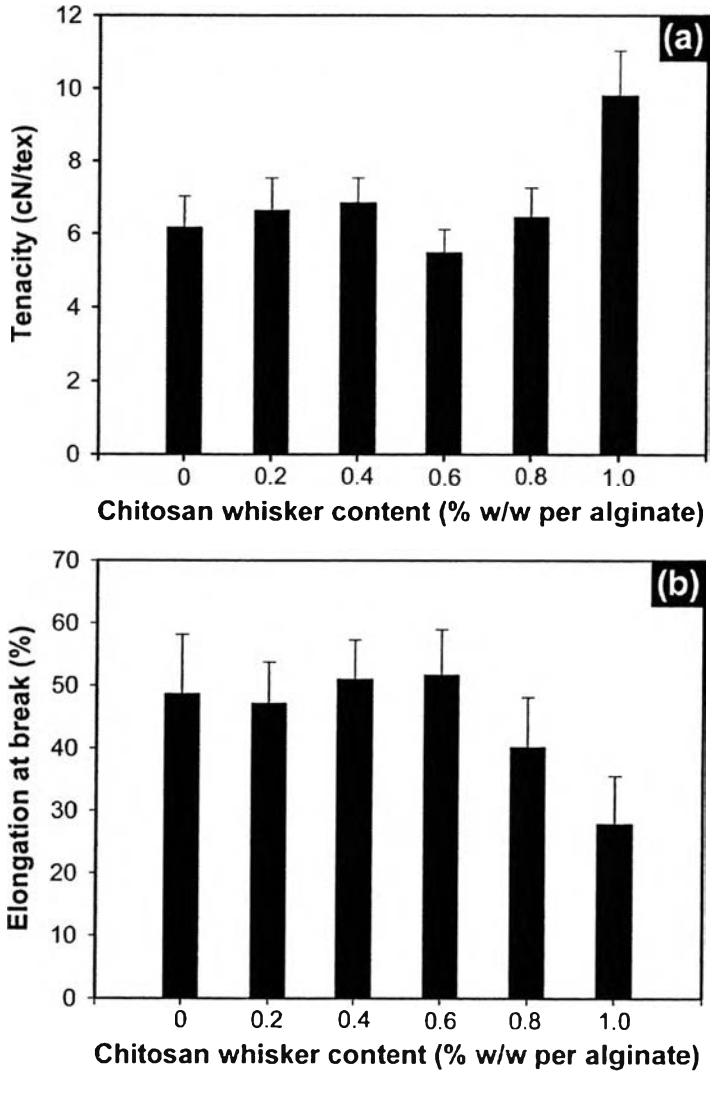
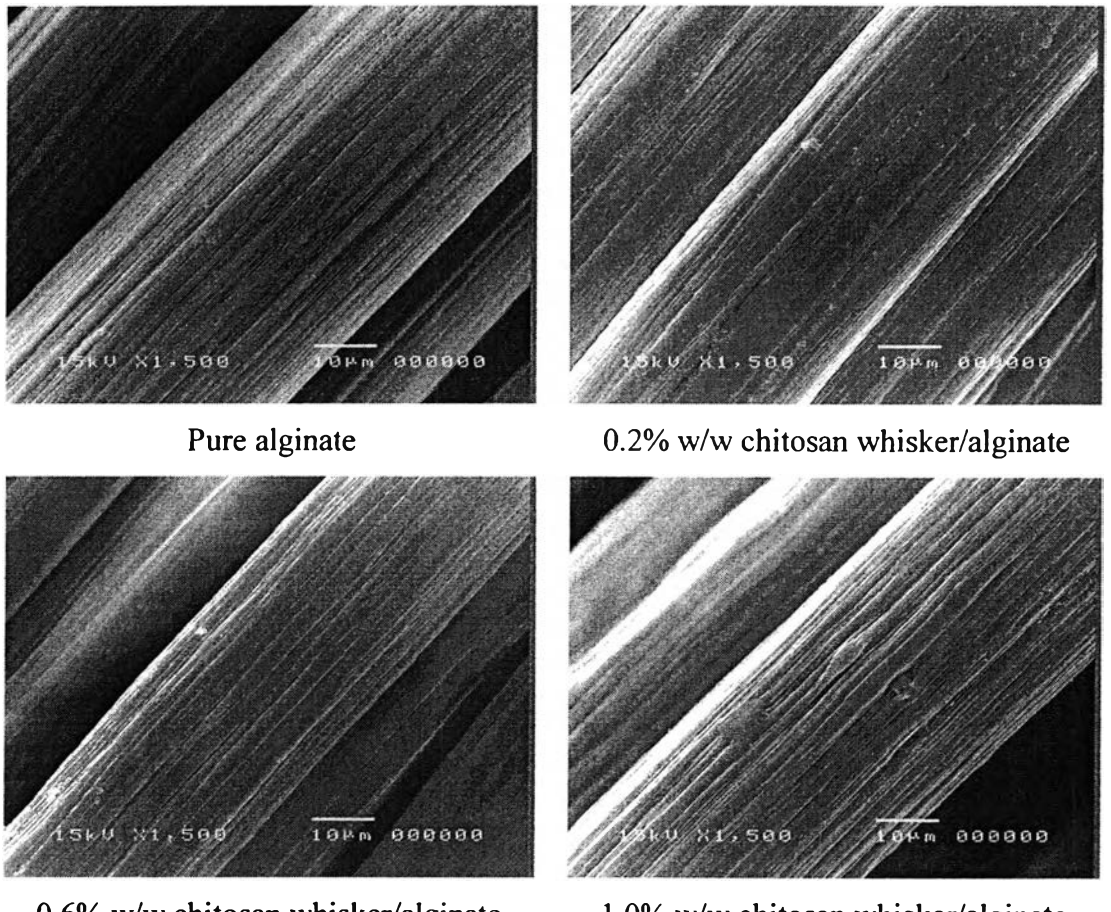


Figure 5.3 (a) Tenacity and (b) percentage of elongation at break of alginate yarns and alginate yarns containing 0.2-1.0% w/w chitosan whiskers (based on the weight of alginate).

5.4.5 Surface Morphology of Neat and Chitosan Whisker-Reinforced Alginate Nanocomposite Fibers

SEM images of the neat alginate fiber and the nanocomposite fibers that contained 0.2%, 0.6%, and 1.0% w/w chitosan whiskers are shown in Figure 5.4. 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. Comparison between the occurrence of the streaks of the nanocomposite fibers with different chitosan whisker contents shows that, streaks of the nanocomposite fibers are remarkable than that of the neat fiber and streaks of the nanocomposite fibers with higher chitosan whisker contents also exhibited more noticeable appearance than that of the one which chitosan whisker contents are low. This result suggests that, incorporation of chitosan whiskers in alginate fibers leading to the change of coagulation property of the fibers. In this vein, before coagulation, the interactions between negatively charges of alginate aqueous solution and positively charges of chitosan whisker suspension may be occurred. These pre-interactions allowed the nanocomposite fibers to coagulate more rapid during passed through the first coagulation bath. Apart from these streaks, the surface of the alginate fibers and most of the nanocomposite fibers was smooth, suggesting the complete incorporation of the whiskers within the fibers.



Pure alginate | 0.2% w/w chitosan whisker/alginate

0.6% w/w chitosan whisker/alginate | 1.0% w/w chitosan whisker/alginate

Figure 5.4 Selected SEM images of neat alginate fiber and alginate fibers containing 0.2%, 0.6%, and 1.0% w/w of chitosan whiskers (based on the weight of alginate).

5.4.6 Staining of the Yarns

Staining is the technique that can be used to better visualize the reactive components. Therefore, staining technique was used to clarify the appearance of the chitosan whiskers in the nanocomposite fibers. Briefly, because of the different between ionic property of alginate and chitosan (anionic and cationic properties, respectively), chitosan (but not alginate) can act as cationic sites for anionic dyes. By this vein, Amido Black 10B, an anionic dye which was reported to attach strongly to cationic groups in the fiber directly, was used as the reactive dye. Figure 5.5 illustrates photographs (top) and microscopic images (bottom) of (a) neat alginate yarns, (b) 0.6% w/w chitosan whisker-reinforced alginate nanocomposite

yarns, and (c) neat chitosan yarns after stained with Amido Black 10B aqueous solution for 12 h.

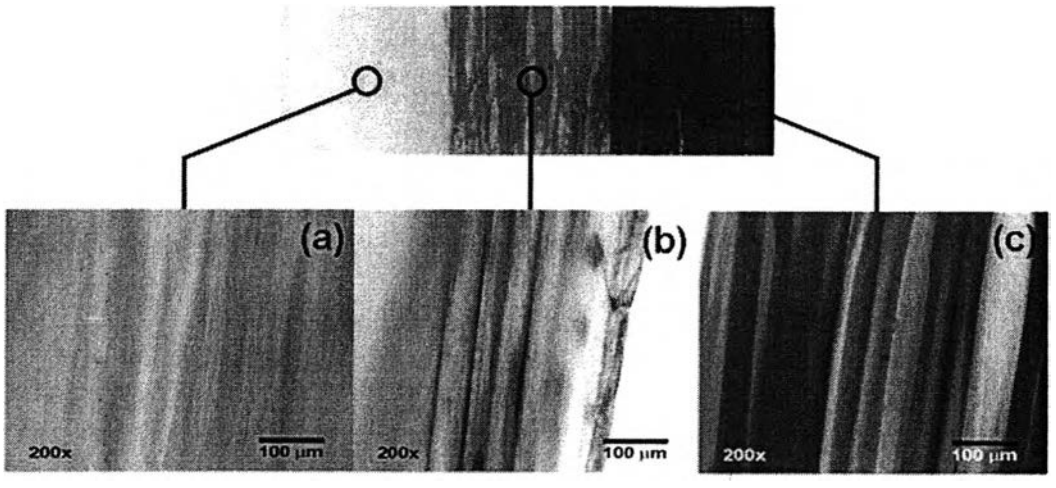


Figure 5.5 Photographs (top) and microscopic images (bottom) at a magnification of 200x of (a) neat alginate yarns, (b) 0.6% w/w chitosan whisker-reinforced alginate nanocomposite yarns, and (c) neat chitosan yarns after stained with Amido Black 10B aqueous solution for 12 h.

Visual analysis of these images shows that alginate yarns did not interact with the dye thus the color of the stained yarns did not change. In contrast, chitosan yarns strongly interacted with the dye therefore the yarns' color was changed to dark blue. In-between, the nanocomposite stained yarn exhibits light blue color while microscopic image shows that the occurrence of this color is in combination between the highly dispersed part and some aggregation part of chitosan whiskers in the fibers. It has been confirmed that, dispersing the chitosan whiskers inside the fiber was successful and chitosan whiskers also appeared at the surface of the fibers.

5.4.7 Release of Chitosan Whiskers from Chitosan Whisker-Reinforced Alginate Nanocomposite Yarns

In vitro release behavior of chitosan whiskers from chitosan whisker-reinforced alginate nanocomposite yarns is shown in Figure 5.6. 0.2% and 0.6%

chitosan whisker-reinforced alginate nanocomposite yarns were used as the representative in this test in order to compare the effect of chitosan whisker content (low and high contents, respectively) in the alginate fiber on the release behavior of the yarns. The release characteristic of chitosan whiskers from 0.2% chitosan whisker-reinforced alginate nanocomposite yarns was gradually increased during the 30 h incubation period with total amount of chitosan whiskers released equal to ~95%. Whereas, for 0.6% chitosan whisker-reinforced alginate nanocomposite yarns, chitosan whiskers appeared to be released in a biphasic way; an initial release was rapid for the first hour and then began to increase in a continuous way for up to 30 h, reaching percentage of cumulative release close to ~84%. Conclusively, the accumulative release amount of chitosan whiskers is dependent on the initial chitosan whisker content in the yarns; low chitosan whisker content exhibited gradually released by the time while high chitosan whisker content lead to faster release of the whiskers at the initial period but sustained the release for the further incubation time.

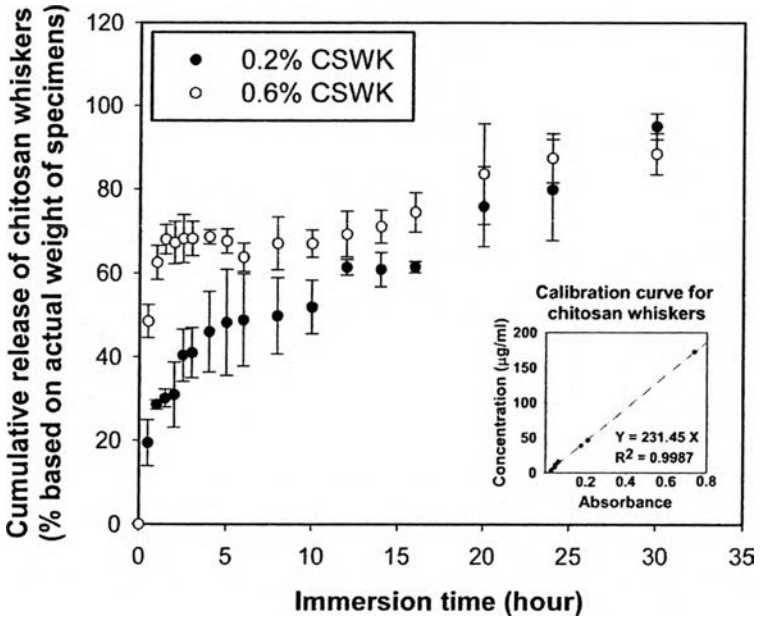


Figure 5.6 Chitosan whiskers release curves of 0.2% and 0.6% w/w chitosan whisker-reinforced alginate nanocomposite yarns in Tris-HCl buffer solution (pH = 7.4).

Iannuccelli *et al.* (1996) studied the *in vivo* degradation of calcium alginate spheres and 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 involved physical disintegration of the spheres into small fragments. Therefore, it is possible that chitosan whiskers can be released out from alginate fiber by this phenomenon. Figure 5.7 illustrates the imagination image of the oriented chitosan whiskers in 0.2% and 0.6% chitosan whisker-reinforced alginate nanocomposite fibers from the obtained release behavior and fiber staining results. As a consequence, release behavior of 0.2% chitosan whisker-reinforced alginate nanocomposite fibers was gradually increased by the time since the initial time of incubation because of the lower amount of the chitosan whiskers at the fiber surface and the larger thickness of the fiber's wall that allowing more time for erosion phenomena and ~95% of chitosan whiskers were released since the 30 h period because of the low total amount of chitosan whiskers. On the other hand, release behavior of 0.6% chitosan whisker-reinforced alginate nanocomposite fibers was rapid for the first hour because of the higher amount of the chitosan whiskers at the fiber surface and the thinner fiber's wall. After that, the whiskers were further released by increasing in a continuous way until 30 h of incubation and only ~84% of chitosan whiskers were released. The sustained release of 0.6% chitosan whisker-reinforced alginate nanocomposite fibers in the latter step was reached because, the increase of the chitosan whisker fraction in the fiber leading to the lower fraction of Ca^{2+} cross-linked in the alginate fibers thus, calcium displacement process was decreased. Tris-HCl buffer solution (pH 7.4) was used in this test because this buffer solution lack of Na^{+} therefore calcium displacement process occurred was due solely to the Na^{+} present in the environmental solution. As a result, the embedded chitosan whiskers in the alginate fibers had successfully been released continuously in Tris-HCl buffer solution of pH 7.4.

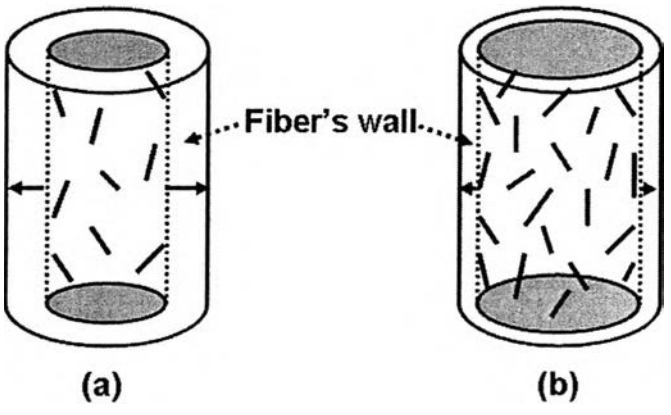


Figure 5.7 Imagination images of the oriented chitosan whiskers in (a) 0.2% and (b) 0.6% w/w chitosan whisker-reinforced alginate nanocomposite fibers from the obtained release behavior result.

Figure 5.8 shows microscopic images of 0.6% chitosan whisker-reinforced alginate nanocomposite yarns which were allowed to immerse in the Amodo Black 10B aqueous solution for 3 d. It has been confirmed that, incorporation of only a little amount of the chitosan whiskers in the nanocomposite fibers exhibited strong occurrence of the embedded chitosan whiskers (see Figure 5.8a). Erosion phenomena also been verified because of the noticeable chitosan whiskers at the fiber surface (see Figure 5.8b).

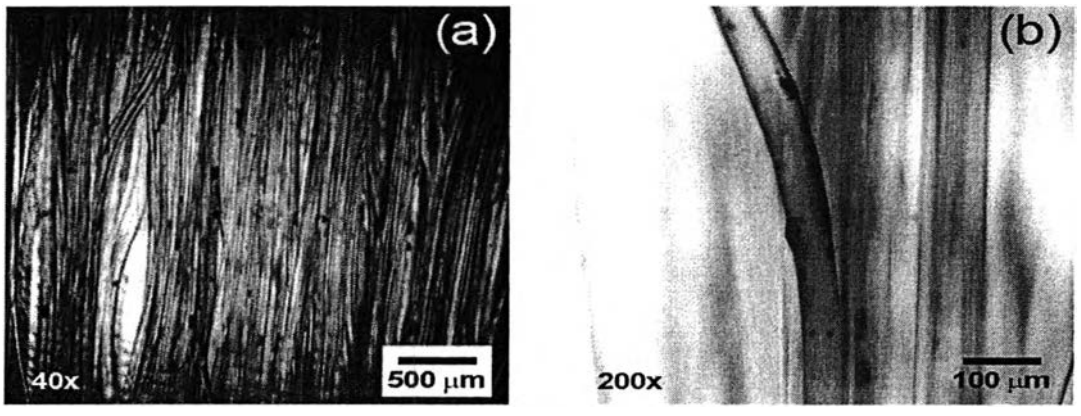


Figure 5.8 Microscopic images of 0.6% chitosan whisker-reinforced alginate nanocomposite yarns which were allowed to immerse in the Amodo Black 10B aqueous solution for 3 d.

5.4.8 Antibacterial Activities

Because wounds often provide favorable environments for colonization of microorganisms which may lead to delayed healing, therefore, antibacterial ability of the wound dressing is important. The antibacterial activities of the neat alginate yarn and chitosan whisker-reinforced alginate nanocomposite yarns against gram-positive *S. aureus* and gram-negative *E. coli* were quantitatively investigated by observing the colony forming unit (CFU) of the bacterial culture after contacting with the neat and the nanocomposite yarns. The results are shown in Figures 5.9 and 5.10.

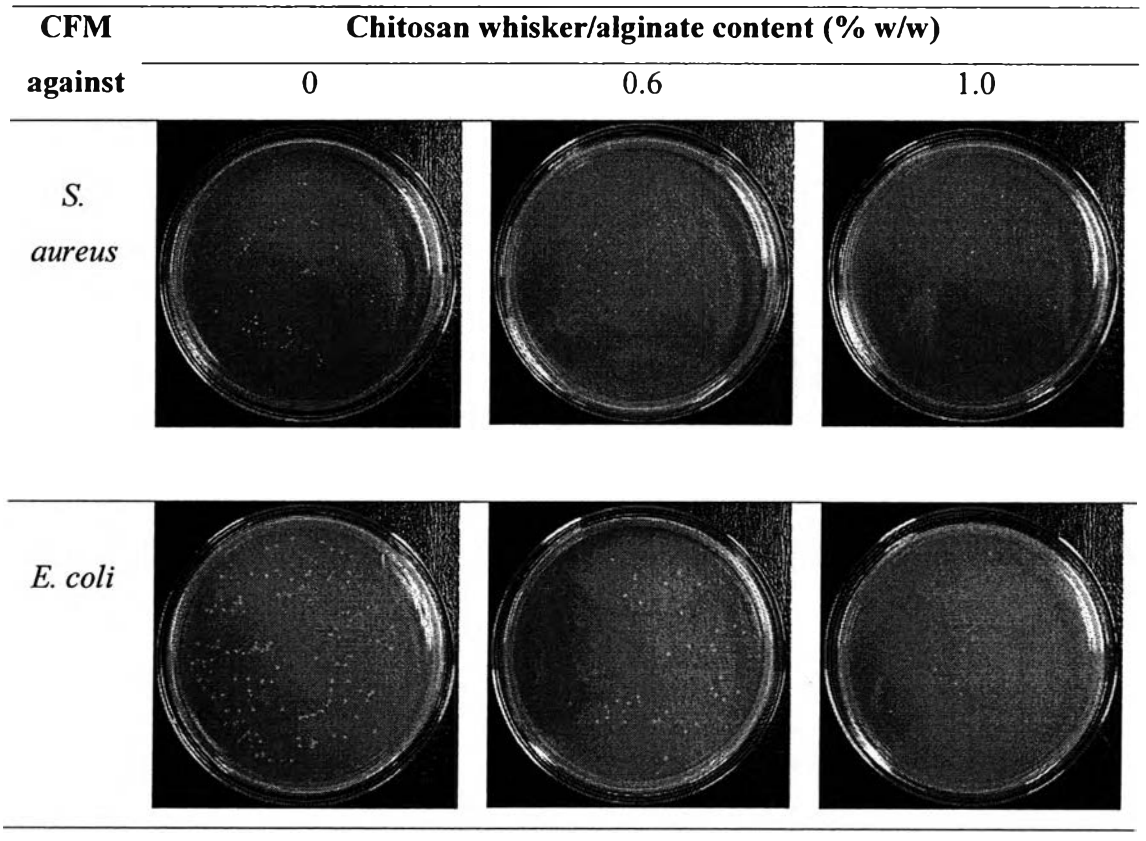


Figure 5.9 Test results of antibacterial activity of the neat alginate yarn and chitosan whiskers-reinforced alginate nanocomposite yarns against gram-positive *S. aureus* and gram-negative *E.coli*.

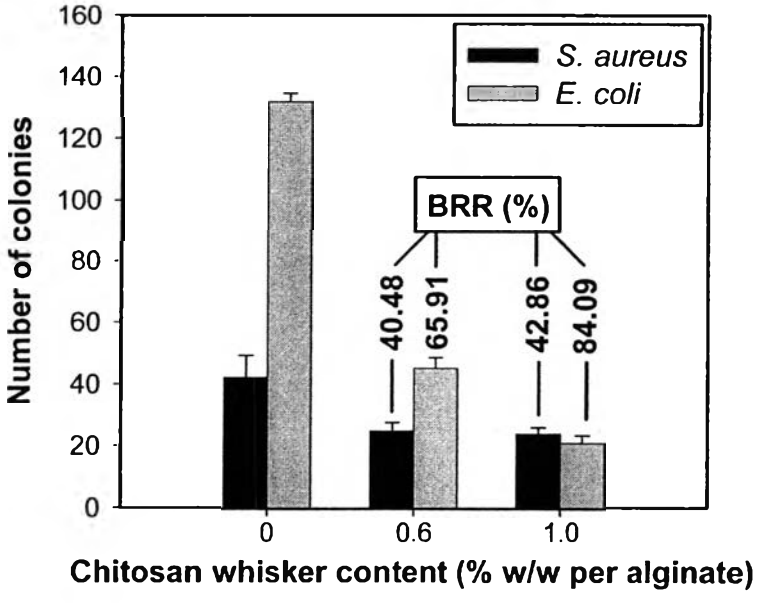


Figure 5.10 Number of colonies of the test samples and the corresponding Bacterial Reduction Rate (% BRR) of chitosan whiskers-reinforced alginate nanocomposite yarns against gram-positive *S. aureus* and gram-negative *E.coli*.

According to Figures 5.9 and 5.10, BRR of 0.6% and 1.0% chitosan whisker-reinforced alginate nanocomposite yarns against gram-positive *S. aureus* are 40.48% and 42.86%, respectively while the same property values against gram-negative *E. coli* are 65.91% and 84.09%, respectively, indicating that the embedded chitosan whiskers are responsible for the antibacterial activity of the nanocomposite yarns and the activity is quite strong with only a little amount of this component. A strong antibacterial activity of these whiskers is probably due to their small size that allowed them to penetrate out from the alginate based material and thus be able to interact with bacterial cells. Relatively, the antibacterial efficacy of the nanocomposite fibers against *S. aureus* is lower than that against *E. coli*. This contribution is in agreement with the work of Zheng and Zhu (2003) who studied on antimicrobial activity of chitosan with different molecular weights and proved that, as the molecular weight of chitosan increased the antimicrobial effect against *S. aureus* was enhanced. In contrast, the antimicrobial effect against *E. coli* was enhanced when the molecular weight of chitosan decreased. The present as-prepared chitosan whiskers have the average molecular weight equal to 53,000 which is in the

range of their study. In this range of molecular weight the antimicrobial effect against *E. coli* is higher than that of against *S. aureus*.

5.5 Conclusions

Preparation of chitosan whiskers was success. The key of this preparation was the control of the rod like shape of the whiskers. The obtained chitosan whiskers consisted of both slender rods and aggregated part. Slender rod chitosan whiskers exhibited broad distribution in both of their length and width (with the average length and width being 309 and 64 nm, resulting in the aspect ratio of ~4.8). Incorporation of the chitosan whiskers in the alginate fibers significantly improved the tensile strength of the nanocomposite fibers but lowering the elongation at break. The significant change in these properties was postulated to be a result of the small particle size and high surface area of the chitosan whiskers. Based on the observation by scanning electron microscope (SEM), the chitosan whiskers in all nanocomposite fibers were embedded well within the fiber. In addition, the remarkable occurrence of the streak pattern on the nanocomposite fiber surface containing high whisker content evidence that pre-interactions between alginate aqueous solution and chitosan whisker suspension occurred before coagulation with Ca^{2+}. The accumulative release amount of chitosan whiskers is dependent on the initial chitosan whisker contents in the fibers; low chitosan whisker content exhibited gradually released by the time while high chitosan whisker content showed release characteristic in a biphasic way, i.e., release of the whiskers was fast at the initial period but sustained for the further incubation time. Incorporation of antibacterial activities in alginate fiber was also successful, the chitosan whiskers-reinforced alginate nanocomposite fibers show antibacterial efficacy against both gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli.*

It should be postulated that, chitosan whisker-reinforced alginate nanocomposite fiber represents a type of effective wound dressings. It can be prepared without a complex process and the definition of success of wound dressings are achieved: moist wound environment, good mechanical properties, and antibacterial activities.

5.6 Acknowledgements

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