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Wet-spun alginate/chitosan whiskers nanocomposite fibers: Preparation, characterization and release characteristic of the whiskers

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ABSTRACT

Incorporation of chitosan whiskers in alginate fibers was achieved by mixing homogenized chitosan whisker colloidal suspension with 6% w/v sodium alginate aqueous solution, followed by extrusion into fiber form by wet spinning. The chitosan whiskers were prepared by deacetylation of chitin whiskers which had been obtained by acid hydrolysis of chitin flakes from shrimp shells. The average length and width of the whiskers were 309 and 64 nm, respectively, with the average aspect ratio being ~4.8. Study on the mechanical properties of the neat alginate and the alginate/chitosan whiskers nanocomposite yarns (30 individual fibers) revealed that, at 1.0% w/w, noticeable improvement in the tensile strength of the nanocomposite yarns was observed, at the expense of the elongation at break. The release of the whiskers from the nanocomposite yarns was dose-dependent and it occurred based on the surface erosion phenomenon. The release half-lives of the nanocomposite yarns containing 0.2% and 0.6% w/w of the whiskers were about 6 h and less than 1 h, respectively. Incorporation of the chitosan whiskers within the alginate yarns imparted the antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* to the resulting alginate/chitosan whiskers nanocomposite yarns, which rendered them as effectual wound dressing materials.

1. Introduction

Chitosan is a natural biopolymer derived from deacetylation of chitin, a major component of shells of crustaceans such as crab, crawfish and shrimp. It has been widely used in biomedical, food and chemical industries (Knorr, 1984; Muzzarelli, 1997). The interests of chitosan and its oligomers result from their unique biological activities that are antimicrobial (Kendra & Hadwiger, 1984; Sekiguchi et al., 1994; Sudarshan, Hoover, & Knorr, 1992), antitumor (Suzuki et al., 1986; Tokoro et al., 1988) and hypocholesterolemic functions (Sugano, Yoshida, Hashimoto, Enomoto, & Hirano, 1992). The inherent antimicrobial and/or antifungal characteristics of chitosan and its derivatives (Chung, Wang, Chen, & Li, 2003; El-Ghaouth, Arul, Asselin, & Benhamou, 1992; Kim, Kim, & Choi, 1997; Papineau, Hoover, Knorr, & Farkas, 1991; Sudarshan et al., 1992) have resulted in their proposed uses as disinfectants. Comparing with other disinfectants, chitosan has several advantages: some of which are relatively high antibacterial activity, broad spectrum of activity and, in particular, low toxicity towards mammalian cells (Liu, Guan, Yang, Li, & Yao, 2001). Although chitosan can be fabricated into various forms, e.g., powder, films, beads, fibers and fabrics (Qin & Agboh, 1998; Qin, Agboh, Wang, & Gilding, 1997), production of pure chitosan fibers in a commercial capacity is still hard to realize. This is because of the relatively high processing costs and limited availability of the raw material in purified form. Poor solubility of chitosan in common solvents poses as another drawback that limits the use of this polymer. Chemical modification is a means that can be used to improve water solubility and, hence, its bioactivities (Kurita, Shimada, Nishiyama, Shimojoh, & Nishimura, 1998; Nishimura et al., 1998). Notwithstanding, various modification schemes are complicate and isolation of the products is both time- and resource-consuming. As a consequence, modification of chitosan with another polymer (i.e., blending) is more economical and the use of chitosan in the form of a colloidal suspension is an attractive choice, since chitosan still retains its solid nanocrystalline state so as to avoid the use of an expensive solvent.

Whiskers or crystalline nanofibrils are substances that can be made from self-assembling of basic building blocks or breaking-down of crystalline materials into nanocrystalline entities with specific shapes. Owing to the advent of nanotechnology, these materials have received tremendous interests in the research community, as the incorporation of whiskers within a polymer matrix results in a new class of materials, i.e., nanocomposites. Whiskers

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from natural resources that have already been investigated are those derived from cellulose (Favier, Chanzy, & Cavaille, 1995; Ljungberg, Cavaille, & Heux, 2006), starch (Dufresne, Cavaille, & Helbert, 1996) and chitin (Morin & Dufresne, 2002; Paillet & Dufresne, 2001). These whiskers are obtained from living organisms that are capable of synthesizing extracellular high-performance skeleton biocomposites, consisting of a matrix reinforced by fibrous biopolymers (Atkins & Keller, 1975; Neville, 1993; Preston, 1967). The use of whiskers as reinforcing nanofillers in a polymer matrix is interesting, because of their high aspect ratios and highly crystalline nature. Chitin is known to form microfibrillar arrangements in living organisms. Chitin whiskers can be prepared by the breaking-down of chitin superstructure by acid hydrolysis (Paillet & Dufresne, 2001). These whiskers have been used as reinforcing nanofillers in polymeric matrices of both synthetic (Morin & Dufresne, 2002; Paillet & Dufresne, 2001) and natural (Lu, Weng, & Zhang, 2004; Sriupayo, Supaphol, & Rujiravanit, 2005) origins. Between chitin and chitosan, chitosan exhibits greater antimicrobial activity than chitin. This is due to the greater number of the free amino groups, which, upon protonation, are responsible for the antimicrobial activity (El-Ghaouth et al., 1992; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). As a result, chitosan whiskers are of great interest in this study.

Alginate is a biopolymer derived from cell walls of certain brown algae (Clare, 1993). It is a linear block copolymer of $1 \rightarrow 4$-linked β-D-mannuronic and α-L-guluronic acid residues. Available in various fibrous and hydrogel products, alginate-based materials are extensively used in wound-care applications, because they offer many advantages, e.g., biocompatibility, haemostatic capability and gel-formability upon subjected to an aqueous environment (Jarvis, Galvin, Blair, & McCollum, 1987). Such gels prevent the wound bed from dehydration, as a moist wound environment has been known to promote healing, leading to a better cosmetic repair of the wounds (Winter, 1962). Alginate is water-soluble and, in the presence of divalent cations, e.g., Ca^{2+}, Mg^{2+}, etc., alginate gels can be formed as the cations act as ionic bridges between L-guluronic acid residues on adjacent chain segments (McDowell, 1974). Because of this reversible solubility, alginate can be fabricated in various forms (Agren, 1996; Dong, Wang, & Du, 2006; Li, Ramay, Hauch, Xiao, & Zhang, 2005), which translate into the availability of alginate-based dressings in various forms as well, such as gels (Paul & Sharma, 2004) and fibrous membranes (Agren, 1996). Despite their unique properties, alginate in its native form does not possess an antimicrobial property and wounds often provide favorable environments for colonization of microorganisms, which may lead to infection and delayed healing. Consequently, an alginate dressing with incorporated antimicrobial activity is desirable, provided that the antimicrobial agent of choice does not compromise the healing process (Guggenbichler, Boswald, Lugauer, & Krall, 1999).

In the present contribution, wound dressing materials in the form of fibers that combine the desirable properties of both alginate and chitosan were developed by wet spinning. Chitosan in the form of whiskers were prepared by deacetylation of chitin whiskers which had been obtained by acid hydrolysis of chitin from shrimp shells. The incorporation of chitosan whiskers within the alginate fibers was to achieve two main objectives: (1) to improve the mechanical properties of and (2) to impart antibacterial property to the alginate/chitosan whiskers nanocomposite fibers. Problems associated with the precipitation of chitosan (because of its high molecular weights) (Tamura, Tsuruta, & Tokura, 2002) can also be overcome by using chitosan in the form of whiskers. Here, the effect of chitosan whiskers on mechanical integrity and antibacterial efficacy of the nanocomposite fibers was investigated. In addition, the release characteristic of the incorporated whiskers was also studied.

2. Experimental details

2.1. 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/v 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). Calcium chloride dihydrate ($CaCl_2{\cdot}2H_20$; edible grade) was supplied from Asia Drug & Chemical Co., Ltd. (Thailand). Amido Black 10B was purchased from Wako Pure Chemical Industries, Co., Ltd. (Japan). Cibacron brilliant red 3B-A (also known as Reactive Red 4, C.I. 18105) was purchased from Sigma (Italy). Methanol (MeOH), ethanol and acetone (commercial grade) were purchased from Labscan (Asia) (Thailand). All other chemicals were used as received.

2.2. Preparation of chitin, chitin whiskers and chitosan whiskers

Decalcification and deproteinization of shrimp shells to obtain chitin flakes were done 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). Briefly, chitin whisker suspension was obtained by hydrolyzing the obtained chitin flakes with 3 N HCl at 104 °C for 6 h under vigorous stirring. The ratio of HCl aqueous solution (3 N) to chitin flakes was 30 $cm^3\ g^{-1}$. After the acid hydrolysis, the suspension was immediately diluted with distilled water, followed by centrifugation (10,000 rpm for 10 min) to separate the obtained chitin solid fraction from the aqueous medium. This process was repeated three times. To remove residual HCl in the suspension, the suspension was dialyzed in distilled water at room temperature for 3 days until pH = 6. Chitosan whisker suspension was prepared from the obtained chitin whiskers by deacetylation in 50% w/v NaOH aqueous solution containing 0.5% w/w sodium borohydride ($NaBH_4$), used as a reducing agent to prevent extensive depolymerization of chitosan. The ratio of chitin whiskers to NaOH aqueous solution was 1 g/10 mL. The deacetylation was performed at 121 °C for 20 min. The obtained product 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 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 refrigerated prior to further use.

2.3. Wet spinning of neat alginate and alginate/chitin whiskers nanocomposite fibers

An aqueous solution of sodium alginate at a fixed concentration of 6% w/v was first prepared as the base alginate spinning dope. The alginate/chitosan whiskers spinning dope suspensions were prepared by mixing the base alginate spinning dope with the as-prepared chitosan whisker suspension. The volumetric ratio of the chitosan whisker suspension to the base alginate spinning dope was varied to obtain the alginate/chitosan whiskers spinning dope suspensions with the weight ratios of chitosan whiskers to alginate ranging from 0.2% to 1.0% w/w. The alginate/chitosan whiskers spinning dope suspensions were left standing in a container at room temperature for degassing and later extruded through a spinneret (30 holes, the diameter for each 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 is a non-solvent for alginate, therefore it helps stabilize the fibers. 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.

2.4. Characterization of 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 diluted chitosan whisker suspension on formvar grids and the deposited drops were left to dry on the grids prior to TEM observations. The dimensions of the whiskers were determined from selected TEM images, from which at least 60 individual whiskers were measured for their lengths and widths using Sem-Afore 4.0 image-analytical software.

Chemical structure of the as-prepared chitosan whiskers was confirmed by a Thermo Nicolet NEXUS 670 Fourier-transformed infrared spectroscope (FTIR). The chitosan whiskers from the as-prepared chitosan whisker suspension were dried, mixed with KBr powder and pressed into a pellet. The scanning range was 4000–400 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 based on the method of Sannan, Kurita, Ogura, and Iwagura (1978).

Weight-average, number-average and zero-average molecular weights (i.e., M_w, M_n and M_z, respectively) and corresponding molecular weight distributions (i.e., M_w/M_n and M_z/M_w) of the as-prepared chitosan whiskers were determined by a Hitachi gel permeation chromatograph (GPC). The GPC equipment consisted of a Shodex OH Pak SB-804HQ column and a reflective index (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. The 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^{-1}. 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.

2.5. Characterization of alginate and alginate/chitin whiskers nanocomposite fibers

Chemical functionalities of the neat alginate and the alginate/chitosan whiskers nanocomposite fibers were investigated by FTIR with 64 scans at a resolution of 4 cm^{-1}. A horizontal attenuated total reflectance (H-ATR) accessory was used to obtain the spectra of all the fiber samples, which were placed on ZnSe crystals. For all sample types, the scanning range was 4000–650 cm^{-1}.

Mechanical integrity in terms of the tenacity and elongation at break of the neat alginate and the nanocomposite yarns of the 30 individual fibers was measured according to the ISO 2062:1993(E) standard test method using a Lloyd LR 100 K universal testing machine. The load cell, the gauge length and the displacement rate were 100 N, 50 mm and 50 mm min^{-1}, respectively. The yarns with initial lengths 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 recorded (i.e., 25 ± 2 °C and 55 ± 2%, respectively). The force and the extension at the breaking point were recorded: these values were used to calculate the tenacity and elongation at break of the yarns (n = 20).

Surface morphology of the neat alginate and the nanocomposite fibers was examined by a JEOL JSM-5200 scanning electron microscope (SEM), operating at an accelerating voltage of 10 kV capable of obtain SEM images at the magnification of 1500×. To assess the dispersion and distribution of chitosan whiskers within the nanocomposite fibers, dried yarns were immersed in 0.01% w/v Amido Black 10B (an amino acid staining diazo dye) aqueous solution for 12 h in order to stain the whiskers. The yarns were then washed with distilled water to remove excess dye and later observed for the dispersion and distribution of the chitosan whiskers by an Olympus BX50 optical microscope.

2.6. Release of chitosan whiskers from alginate/chitosan whiskers nanocomposite yarns

Quantification for the total amounts of chitosan loaded within the alginate/chitosan whiskers nanocomposite yarns was carried out based on the colorimetric quantification assay of Muzzarelli (1998). The essence of the method is based on the ability of chitosan in absorbing an anionic dye by its protonated amino groups. Based on this method, Cibacron Billiant Red 3B-A, a monochlorotriazine dye, was used as the reactive dye. A solution of the dye was prepared by dissolving 150 mg of the dye powder in 100 mL of deionized water. Aliquots of the dye solution (5 mL) were filled with 0.1 M glycine hydrochloride buffer to fill the final volume of 100 mL. The final concentration of the dye solution was 0.075 g L^{-1} (pH = 3.2). To obtain a calibration curve for the relationship between the amounts of chitosan and the dye, the following procedure was adopted. Varying amounts of the as-prepared chitosan whisker suspension were introduced into test tubes, followed by the addition of the buffer to fill the final volume of 0.3 mL. The exact solid contents of the chitosan whiskers within these solutions were 3.839, 7.677, 11.515, 15.354, 38.384, 46.061, and 172.727 μg mL^{-1}. Aliquots of the dye solution (3 mL) were then added into the tubes. The absorbance values were finally measured at 575 nm with a Shimadzu UV-2550 UV-VIS spectrophotometer. The buffer (0.3 mL) and the dye (3 mL) solutions were used to obtain the reference absorbance signals.

The release characteristic of chitosan from the nanocomposite yarns was investigated by total immersion release assay. Each specimen (about 0.1 g) was immersed in 20 mL of Tris–HCl buffer solution 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, 1 mL of the medium was withdrawn (hereafter, sample solution) and an equal amount of the fresh medium was refilled. The amount of chitosan whiskers within the sample solution was determined by first drying the 1 mL-sample solution, followed by the addition of 0.3 mL of 0.1 M glycine hydrochloride buffer and 3 mL of the dye solution, respectively. The absorbance values were measured spectrophotometrically at 575 nm. The obtained data were back calculated against the predetermined calibration curve to finally obtain the cumulative amounts of chitosan whiskers released from the yarns. The experiments were carried out in triplicate and the results were reported as average values.

2.7. Antibacterial evaluation

Antibacterial property of the neat and the alginate/chitosan whiskers nanocomposite yarns was evaluated based on the colony count method. The test was conducted against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Briefly, inoculum was prepared by transferring one colony of each microorganism into 20 mL of a broth solution. The mixture was cultured at 37 °C in a shaking incubator for 24 h. About 0.5 mL of the cell suspension of each microorganism was added into several vials of 4.5 mL of 0.85% sterile NaCl aqueous solution. Standard serial dilution method was used, i.e., 10^{-5} for *S. aureus* and 10^{-6} for *E. coli*. About 0.2 g of each of the yarn specimens was added into the mixture. The suspensions were shaken at 150 rpm. After the

contact time period of 3 h, 100 μL of these suspensions was dipped and spread on sterilized agar in Petri dishes. Bacterial growth was visualized after an overnight incubation at 37 °C in a shaking incubator for 24 h. The bacterial reduction rate (BRR) for each type of specimens against a microbe was calculated based on the difference in the number of colonies of the blank microbe suspension (N_1) and that of each of the sample suspensions (N_2) (i.e., $(N_1 - N_2)/N_1 \times 100$). The blank microbe suspensions were those prepared in the presence of the neat alginate yarns and the experiments were carried out in triplicate.

3. Results and discussion

3.1. Morphology and size of chitosan whiskers

The chitosan whiskers that had been prepared from deacetylation of the chitin whiskers displayed colloidal behavior, owing to the presence of positive charges induced on their surfaces by the protonation of amino groups ($-NH_3^+$) (Marchessault, Morehed, & Walter, 1959). The degree of deacetylation (%DD) of these chitosan whiskers was 50.5% and the solid fraction of these colloidal suspensions was 1.13% w/v. The greater %DD could not be achieved because further increase in the deacetylation time would compromise the integrity of the rod-like shape of the chitosan whiskers. This is in agreement with the work of Phongying, Aiba, and Chirachanchai (2007), who reported that prolonged and intense deacetylation of chitin whiskers (i.e., 7 h) led to the total disintegration of the whiskers and could self-assemble into a cotton-like fibrous nano-structure. A selected TEM image illustrating the physical appearance of the as-prepared chitosan whiskers is shown in Fig. 1.

Two populations of the whiskers are evident, i.e., individual and aggregated entities. The size of the individual whiskers distributed rather broadly. Specifically, the widths (d) of these whiskers ranged from 29 to 105 nm (with the average value being about 64 nm), while the lengths (L) ranged from 165 to 560 nm (with the average value being about 309 nm). These resulted in the average aspect ratio (L/d) of about 4.8. Certain amounts of the whiskers are present as aggregated entities, as shown in the inset to Fig. 1. The formation of the aggregates could be a result of the interactions between chitosan and the alkaline base, i.e., NaOH, utilized during the deacetylation procedure. This was earlier reported by Okano, Minagawa, Yang, Shimojoh, and Kurita (2009) in that, at a high pH, precipitation or gelation of chitosan tends to occur. The widths and the lengths of the obtained chitosan whiskers are different from those earlier reported by us for the chitin whiskers (i.e., d = 46 nm, L = 343 nm and L/d = 7.5 on average) (Watthanaphanit, Supaphol, Tamura, Tokura, & Rujiravanit, 2008). The likely reason for the shorter lengths of the chitosan whiskers should be due to the prolonged reaction with the rather concentrated NaOH aqueous solution, that may facilitate the cooperative chain scission, hence the shortening of the whiskers. On the contrary, the increase in the widths of the whiskers could be due to the increase in the number of free amino groups, which, upon protonation, would allow for greater accessibility of water molecules to penetrate into the amorphous parts of the whiskers, resulting in the swelling of the whiskers.

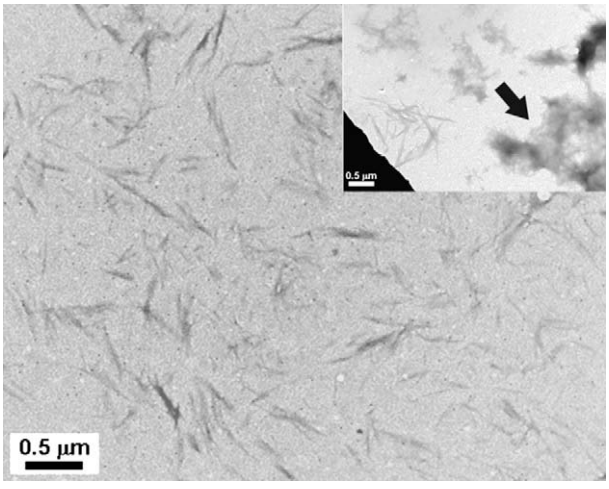

Fig. 1. Selected TEM image (magnification = 20,000×) of chitosan whiskers from the as-prepared colloidal suspension, illustrating their rod-like nature. These whiskers are present both as individual and aggregated entities (inset).

3.2. Molecular weight characteristics of chitosan whiskers

The obtained chitosan whiskers were characterized for their molecular weight characteristics. Their weight-average, number-average and zero-average molecular weights (i.e., M_w, M_n and M_z, respectively) were determined to be 59, 53 and 64 kDa, respectively. The corresponding molecular weight distributions were 1.11 (for M_w/M_n) and 1.09 (for M_z/M_w). Compared with chitosan that would normally be obtained from the same shrimp shells used to prepare the chitosan whiskers (i.e., M_w = 422 kDa), the molecular weights of chitosan in the whisker form were much lower and the molecular weight distributions were remarkably narrower. This should be a result of the rather homogeneous degradation of chitin and chitosan superstructures during the acid hydrolysis of chitin flakes to obtain the chitin whiskers and the subsequent deacetylation of the chitin whiskers to obtain the chitosan whiskers, as the degradation is likely to be taken place in the amorphous regions.

3.3. Preparation of neat alginate and alginate/chitosan whiskers nanocomposite fibers

One of the factors enabling both alginate and chitosan to be developed into biomaterials with tailored properties has been their potential to form a polyelectrolyte complex via ionic interactions between the carboxylate moieties of alginate and the protonated amino counterparts of chitosan (Watthanaphanit et al., 2009). Because of these ionic interactions, a direct blending of alginate and chitosan solutions would, however, coagulate or form gels, thus limiting the processability of the blend solutions into alginate/chitosan hybridized fibers. Chitosan in the form of an emulsion has recently been proposed by us and another colleague (Watthanaphanit et al., 2009) as a means to incorporate a high content of chitosan solution into an alginate solution so as to obtain the blend fibers without the problems associated with the formation of gels due to ionic complexation between the two polymers.

Here, chitosan in the form of whiskers is used as the chitosan source for the fabrication of alginate/chitosan hydridized fibers. The content of the chitosan whiskers within the obtained nanocomposite yarns ranged between 0.2% and 1.0% w/w (based on the dry weight of alginate). To confirm successful incorporation of the whiskers within the yarns, FTIR was utilized. The spectra of the neat alginate and the alginate/chitosan whiskers nanocomposite fibers along with that of the as-prepared chitosan whiskers are shown in Fig. 2. Tables 1 and 2 summarize the characteristic peaks of the as-prepared chitosan whiskers and the neat alginate fibers. According to Fig. 2, the FTIR spectra of all of the obtained fibers exhibited the characteristic peaks of alginate. The peaks associated with the chitosan whiskers were not clearly visible in the spectra of the fibers, likely a result of the extremely small amounts

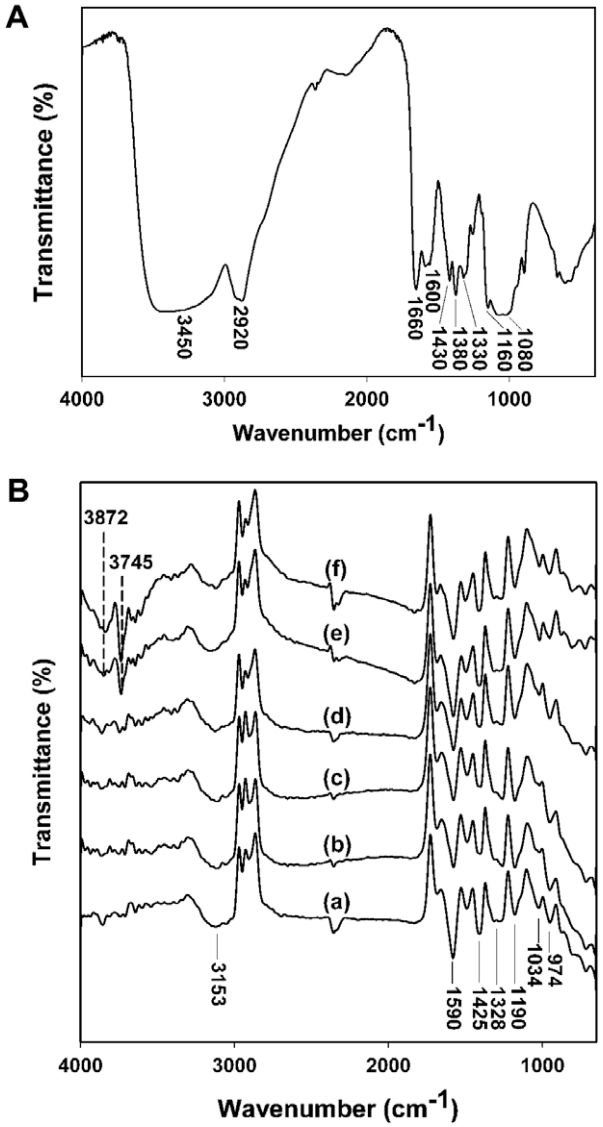

Fig. 2. (A) FTIR spectrum of chitosan whiskers and (B) ATR-FTIR spectra of (a) neat alginate fibers and alginate nanocomposite fibers containing (b) 0.2%, (c) 0.4%, (d) 0.6%, (e) 0.8% and (f) 1.0% w/w of chitosan whiskers (based on dry weight of alginate).

Table 1
Assignments of FTIR characteristic peaks 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

of them within the fibers. Notwithstanding, at 0.8% and 1.0% w/w of the chitosan whiskers, additional peaks at 3745 and 3872 cm^{-1} were clearly observed. While the presence of these peaks suggested the existence of certain kinds of interactions between the incorporated chitosan whiskers and the alginate matrix, their actual assignments are not certain at the present time.

Table 2
Assignments of FTIR characteristic peaks for neat alginate fibers.

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

3.4. Mechanical integrity of neat alginate and alginate/chitosan whiskers nanocomposite yarns

The tenacity and elongation at break of the neat alginate and the alginate/chitosan whiskers nanocomposite yarns are shown in Fig. 3. The change in the tenacity values of the nanocomposite yarns from those of the neat alginate yarns was not significant, despite the minima in the property values were observed at the whisker content of 0.6% w/w, until the amount of the whiskers reached 1.0% w/w, where the property values reached the maxima. Similarly, the change in the values of the elongation at break of the yarns from those of the neat yarns was not significant when the whisker contents were lower than or equal to 0.6% w/w, beyond which the property values decreased significantly to reach the minima at the whisker content of 1.0% w/w. The improvement in the stiffness of a composite is controlled not only by the size, the dispersion, the distribution and the alignment of the reinforcing

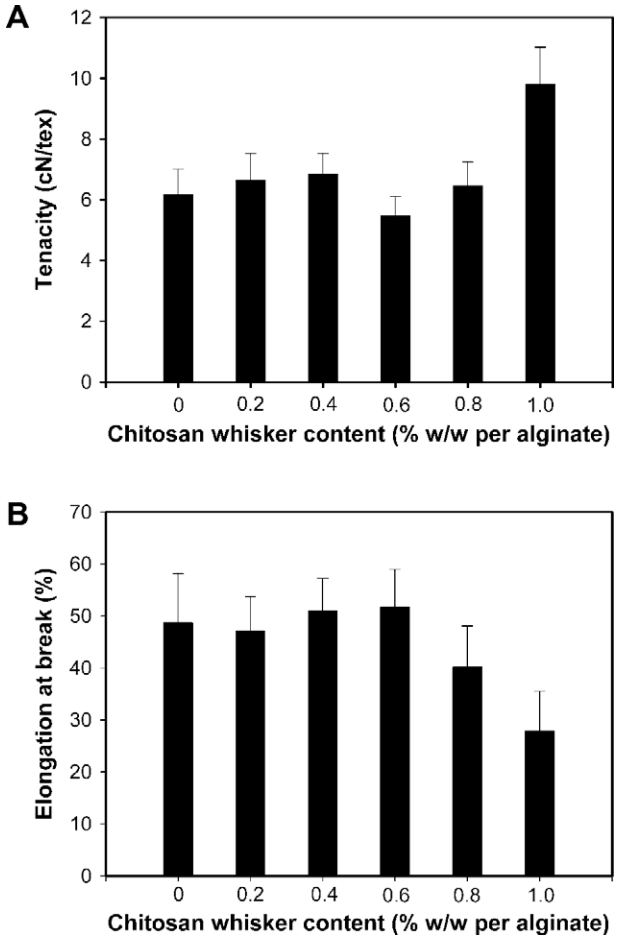

Fig. 3. (A) Tenacity and (B) elongation at break of neat alginate yarns and alginate nanocomposite yarns containing 0.2–1.0% w/w of chitosan whiskers (based on dry weight of alginate).

fillers within the matrix, but also by the ability of the load to be effectively transferred from the matrix to the fillers (Sretenovic, Muller, & Gindl, 2006). While the dispersion, the distribution and the alignment of the chitosan whiskers within the alginate/chitosan whiskers nanocomposite fibers were relatively difficult to quantify, possible interactions between alginate and the whiskers, as observed in the FTIR spectra of the fibers containing 0.8% and 1.0% w/w of the whiskers, may be indicative of the effectiveness in the ability of the whiskers to bear a portion of the load, hence the observed improvement in the stiffness of the nanocomposite yarns at 1.0% w/w of the whiskers. The improvement in the stiffness of the yarns could also be attributed to the decreased mobility of the alginate molecules as a result of the presence of the whiskers, hence the observed reduction in the elongation at break at 0.8% and 1.0% w/w of the whiskers (Premalal, Ismail, & Baharin, 2002).

3.5. Surface morphology of neat alginate and alginate/chitosan whiskers nanocomposite fibers

SEM images of the neat alginate fibers and the nanocomposite fibers containing 0.6% and 1.0% w/w of chitosan whiskers are shown in Fig. 4. Evidently, these fibers were straight, with streak patterns being observed on their surfaces. Both the roughness along the inner perimeters of the spinneret holes and the shrinkage upon drying of the fibers were postulated as the main reasons for the formation of these streaks (Watthanaphanit et al., 2008). Careful consideration of the streak patterns on the fiber surfaces revealed that the streaks that existed on the surfaces of the nanocomposite fibers were more obvious than those on the surfaces of the neat alginate fibers and the streaks became more noticeable with an increase in the whisker content. The incorporation of chitosan whiskers within the alginate matrix could change the way in which the fibers coagulated. Prior to the coagulation by Ca^{2+} in the coagulation bath, interactions between the negative charges of alginate molecules and the positive charges of the chitosan whiskers could facilitate the coagulation of the extrudates during their passage through the coagulation bath. Apart from these streaks, no evidence of aggregates of the whiskers was observed on the surfaces of the nanocomposite fibers, a result that suggested the total incorporation of the whiskers within the fibers.

3.6. Staining of neat alginate and alginate/chitosan whiskers nanocomposite yarns

The inclusion, the dispersion and/or the distribution of chitosan whiskers within the individual nanocomposite fibers could be assessed visually with an aid of an anionic dye, Amido Black 10B. The dye molecules would selectively adsorb onto the whisker surfaces, owing to the positively-charged nature of the whiskers. Fig. 5 illustrates photographic and the corresponding optical microscopic (OM) images of the neat alginate yarns and the nanocomposite yarns containing 0.6% w/w of the whiskers, after they had been immersed in the aqueous solution of the dye for 12 h. Results were compared with the neat chitosan yarns that had been prepared by wet spinning from the same apparatus utilized to fabricate the neat alginate and the nanocomposite yarns. It is evident that no specific interaction of the neat alginate yarns with the dye was observed, as the neat alginate yarns were not positively stained with the dye. On the contrary, the chitosan yarns, after staining, appeared in the characteristic dark blue color of the dye throughout the mass of the yarns. This confirmed the specific interactions between the negatively-charged moieties of chitosan and the dye molecules. The nanocomposite yarns, after being stained with the dye, appeared in light blue color however. Closer examination of the OM image of the individual nanocomposite fibers revealed that not all of the parts of the fibers were stained positive for the dye. Even though the stained areas distributed rather homogenously throughout the mass of the fibers, their dispersion within the fibers was not good, as very small and very large aggregates of the stained areas were observed throughout the mass of the fibers. The result indicated that parts of the incorporated chitosan whiskers existed as aggregated entities.

3.7. Release of chitosan whiskers from alginate/chitosan whiskers nanocomposite yarns

Iannuccelli et al. (1996) studied *in vivo* degradation of calcium alginate spheres and concluded that the degradation process of the spheres underwent three stages. The first stage involved hydration of the spheres, coupled with cleavage of some cross-links. The second stage involved partial dissolution of the soluble fragments of the spheres as originated by the ionic exchange between Ca^{2+} from the inside of the spheres and Na^{+} from the exterior of the spheres. The third stage involved physical disintegration of the spheres into small fragments. The degradation process of the calcium alginate spheres corresponded to the surface erosion phenomenon. Such a phenomenon could also occur to the alginate/chitosan whiskers nanocomposite yarns, as obtained by this work. It is hypothesized that, as the erosion occurs, chitosan whiskers could be let out from the confinement of the yarns.

Fig. 6 shows the release characteristic of the as-loaded chitosan whiskers from the nanocomposite yarns. The theoretical contents of the whiskers within the tested yarns were 0.2% and 0.6% w/w. The nanocomposite yarns having two different whisker contents were specifically chosen to represent the effect of the whisker content on the release characteristic of the whiskers from the yarns. Prior to investigating the release characteristic of the as-loaded whiskers, the actual amounts of the whiskers within the tested yarns needed to be determined and the results are shown in Table 3. The results showed excellent agreement with the theoretical values. These values were used as the basis for the calculation of the cumulative release profiles reported in Fig. 6.

According to the results shown in Fig. 6, the cumulative amounts of the whiskers released from the nanocomposite yarns containing 0.6% w/w of the whiskers, at most time points, were

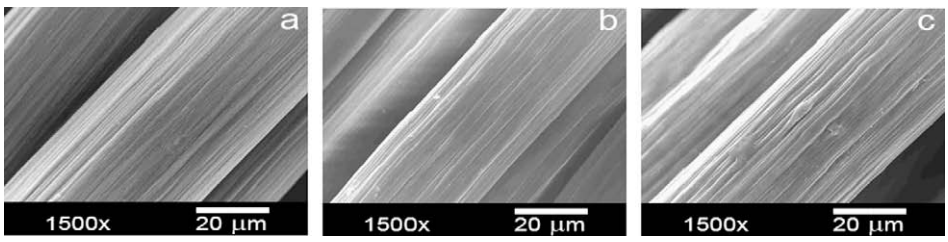

Fig. 4. Representative SEM images illustrating surfaces of (a) neat alginate fibers and alginate nanocomposite fibers containing (b) 0.6% and (c) 1.0% w/w of chitosan whiskers (based on dry weight of alginate).

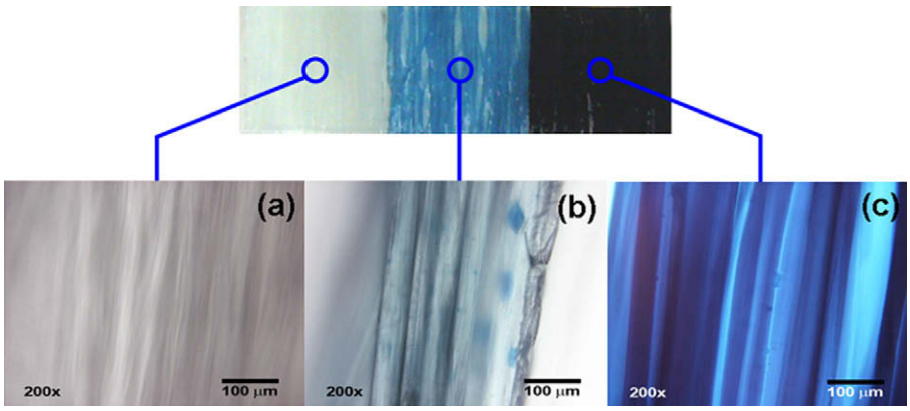

Fig. 5. Representative photographic (top panel) and corresponding optical microscopic (bottom panel; magnification 200×) images of (a) neat alginate yarns, (b) alginate nanocomposite yarns containing 0.6% w/w of chitosan whiskers and (c) neat chitosan yarns, after they had been stained with 0.01% w/v Amido Black 10B aqueous solution for 12 h.

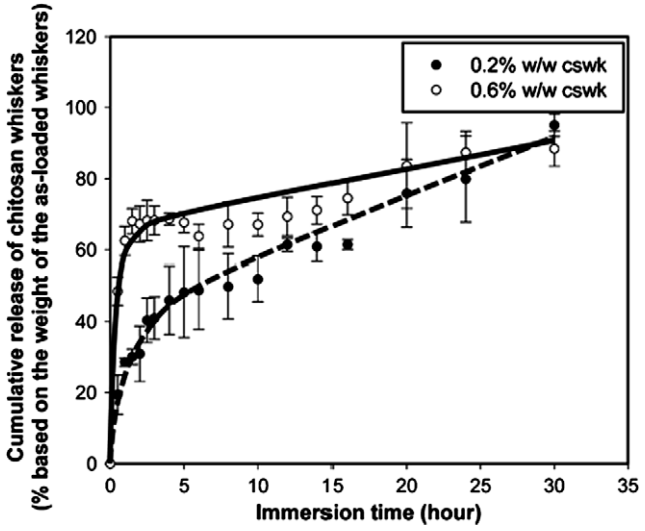

Fig. 6. Cumulative amounts of the as-loaded chitosan whiskers released from 0.2% and 0.6% w/w chitosan whiskers-filled alginate yarns as determined by total immersion method in Tris–HCl buffer solution (pH = 7.4) at the physiological temperature of 37 °C (n = 3). The lines drawn were to aid the visualization of each data set.

greater than those of the ones with 0.2% w/w. For the nanocomposite yarns containing 0.2% w/w of the whiskers, the cumulative amounts of the as-loaded whiskers released into the medium increased very rapidly during the first 6 h of immersion, as about half of the whiskers was released into the medium within this time period. After 6 h of immersion, such amounts increased further at a slower rate to finally reach the final value of about 95% at 30 h of immersion. For the nanocomposite yarns containing 0.6% w/w of the whiskers, the release of the as-loaded whiskers occurred in two steps. In the first step, the cumulative amounts of the whiskers released into the medium occurred very rapidly within the first 2 h (accounting to about 69% of the as-loaded whiskers). In the next step, such amounts increased very slowly to reach the maximum value of about 88% at 30 h of immersion. The greater cumulative amounts of the whiskers released from the nanocomposite yarns containing 0.6% w/w than those released from the ones containing 0.2% w/w is obviously due to the greater amounts of the as-loaded whiskers within the yarns (see Table 3).

As it was postulated that surface erosion is the main mechanism responsible for the release of the as-loaded chitosan whiskers from

Table 3
Actual amounts of chitosan whiskers in alginate nanocomposite yarns containing 0.2% and 0.6% w/w of chitosan whiskers, as determined based on the colorimetric quantification assay of Muzzarelli (1998) (n = 3).

Initial amount of chitosan whiskers in spinning dope (% w/w)	Weight of specimens (mg)	Actual amount of chitosan whiskers based on actual weight of specimens (mg)
0.2	102.2 ± 1.2	0.205 ± 0.002
0.6	104.3 ± 3.4	0.626 ± 0.021

the confinement of the alginate/chitosan whiskers nanocomposite yarns, it is logical to verify whether such the phenomenon could actually occur during the immersion of the yarns in the medium. In doing so, the nanocomposite yarns containing 0.6% w/w of the whiskers were immersed in 0.01% w/v Amido Black 10B aqueous solution for 3 d. Representative OM images of the immersed yarns were taken at two different magnifications (i.e., 40× and 200×) and the results are shown in Fig. 7. Clearly, a large number of aggregates of chitosan whiskers (appearing as light blue regions in the images) were observed on the surfaces of the fibers after they had been immersed in the dye solution. Since it was indicated by SEM results that chitosan whiskers were well encapsulated within the mass of the fibers, the observation of the whisker aggregates on the surfaces of the fibers after immersion in the dye solution suggested that surface erosion indeed occurred. Nevertheless, the amounts of the whiskers, despite after the fibers having been immersed in the dye solution for 3 days, remained high. By referring to the results shown in Fig. 7, almost all of the as-loaded whiskers (i.e., 88% w/w) have been found to release from the fibers after they had been immersed in Tris–HCl buffer solution for only 30 h. The likely explanation for such discrepancy could be due to the difference in the medium used in this experiment (i.e., the dye solution) from that used in the release assay (i.e., Tris–HCl buffer solution).

3.8. Antibacterial activity of alginate/chitosan whiskers nanocomposite yarns

Development of a wound dressing with embedded antibacterial activity is important because wounds often provide favorable environments for colonization of microorganisms which may lead to infection and delayed healing. Since chitosan is known for its antimicrobial property (Kendra & Hadwiger, 1984; Sekiguchi et al.,

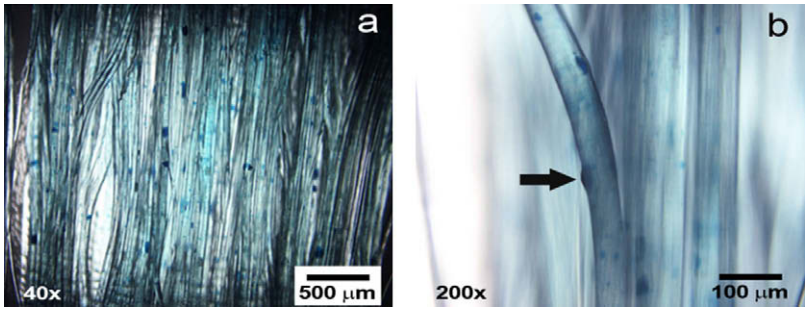

Fig. 7. Representative optical microscopic images at the magnifications of (a) 40× and (b) 200× of alginate nanocomposite yarns containing 0.6% w/w of chitosan whiskers after having been immersed in 0.01 w/v Amido Black 10B aqueous solution for 3 days.

1994; Sudarshan et al., 1992), it is hypothesized that the incorporation of chitosan whiskers within the alginate fibers would readily introduce such property to the obtained fibers. To prove the hypothesis, the antibacterial activity of the alginate nanocomposite yarns containing either 0.6% or 1.0% w/w of chitosan whiskers was tested against two commonly-studied microbes, i.e., Gram-positive *S. aureus* and Gram-negative *E. coli*, based on the colony count method. The neat alginate yarns were used as the negative control. The result of the analysis is shown in Fig. 8.

According to Fig. 8, after the neat alginate yarns had been in contact with the bacterial mixtures for 3 h, the numbers of the *S. aureus* and *E. coli* colonies were about 40 and 130, respectively. Such numbers for the nanocomposite yarns decreased significantly from those of the neat alginate counterparts. Such values for the two types of the investigated nanocomposite yarns against *E. coli* were significantly different, while those against *S. aureus* were not. Evidently, the bacterial reduction rate (BRR) values of the nanocomposite yarns containing 0.6% and 1.0% w/w of chitosan whiskers against *S. aureus* were about 40% and 43%, respectively, while those against *E. coli* were greater at about 66% and 84%, respectively. The result confirmed that chitosan whiskers incorporated within the yarns were responsible for the antibacterial activity of the resulting nanocomposite yarns and the activity is strong even if the concentration of the whiskers was low (i.e., $\leqslant$1.0% w/w). The strong antibacterial activity of the embedded whiskers is likely resulting from their small sizes that allow them to interact well with the microbes. As clearly seen in Fig. 8, the antibacterial efficacy of the nanocomposite yarns against *S. aureus* was lower than that against *E. coli*. This is in agreement with the work of Zheng and Zhu (2003) who reported that the antimicrobial activity of chitosan of varying molecular weights (i.e. <5 up to 305 kDa) against *S. aureas* increased with increased molecular weight of chitosan, while that against *E. coli* decreased. Additionally, they reported that the BRR values of 48.5 and 72.4 kDa chitosan samples (at the solid content of 0.25%) against *E. coli* were 30% and 5%, respectively, which were obviously greater than those against *S. aureus* that equaled 0% (Zheng and Zhu, 2003). Based on this, the greater antibacterial activity of the incorporated chitosan whiskers against *E. coli* than *S. aureus*, observed in the present contribution, is logical, as the M_w of the whiskers was 59 kDa.

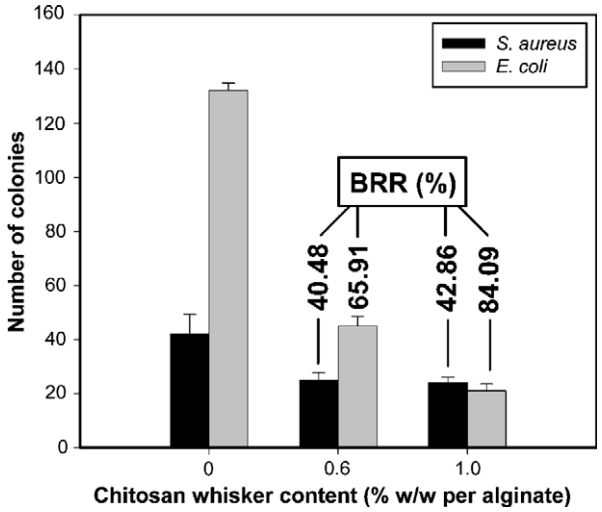

Fig. 8. Number of colonies of neat alginate yarns and alginate nanocomposite yarns containing 0.6% and 1.0% w/w of chitosan whiskers and the corresponding bacterial reduction rate (BRR) values against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*.

4. Conclusion

Preparation of chitosan whiskers was achieved by deacetylation of chitin whiskers *a priori* prepared from acid-hydrolyzed chitin flakes. The chitosan whiskers existed as both individual slender rods and aggregated entities. The average lengths and widths of the individual whiskers were 309 and 64 nm, respectively, with the average aspect ratio being about 4.8. Incorporation of the whiskers in the alginate yarns at 1.0% w/w improved the tensile strength of the nanocomposite yarns significantly, at the expense of the elongation at break. The improvement in the stiffness of the nanocomposite yarns (at 1.0% w/w of the whiskers) was postulated to be the results of the small sizes of the whiskers, the formation of specific interactions between the whiskers and the alginate matrix and the decreased mobility of the alginate molecules in the presence of the whiskers. SEM results indicated that the whiskers were encapsulated well within the individual fibers. The release of the whiskers from the nanocomposite yarns, as investigated by total immersion method in Tris–HCl buffer solution, was dose-dependent, with about half of the whiskers released from the yarns containing 0.2% w/w of the whiskers occurring within about 6 h as opposed to less than 1 h for the ones containing 0.6% w/w of the whiskers. The release of the whiskers from the nanocomposite yarns was based on the surface erosion phenomenon. Lastly, it is postulated that the obtained alginate/chitosan whiskers nanocomposite yarns could be used as effectual wound dressings, as the incorporated chitosan whiskers imparted the antibacterial activity against two commonly-studied microbial pathogens, i.e., Gram-positive *S. aureus* and Gram-negative *E. coli*.

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