

PREPARATION, MODIFICATION, AND APPLICATIONS OF CHITIN NANOWHISKERS: A REVIEW

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Abstract. This paper provides an overview of the most up-to-date information available relating to chitin nanowhiskers. This paper presents aspects about chitin nanowhiskers, including methods of extraction and preparation, chemical modification and applications. Chitin nanowhiskers can be obtained by hydrochloric acid hydrolysis, TEMPO-mediated oxidation, partial deacetylation with NaOH by fibril surface cationization, ultrasonication, electrospinning, aqueous counter collision treatment, a simple grinding treatment and gelation with 1-allyl-3-methylimidazolium bromide. An introduction into the methods used to prepare chitin nanowhiskers is given. The chitin nanowhiskers applications are used mainly as reinforcing polymer nanocomposites, but also to prepare scaffolds, hydrogels and wound dressings, as adsorbents in industry, water purification, for protein immobilization, transformation of bacteria by exogenous genes, stabilization of oil-in-water emulsion and nematic gels, formation of CaCO₃/chitin-whisker hybrids and as carbon precursors.

1. INTRODUCTION

Chitin is a natural, renewable and biodegradable polymer, the second most abundant natural polymer after cellulose [1]. Large amounts of this structural material can be found in animals, in exoskeleton shells of arthropods (crabs, shrimps and beetles), internal flexible backbone of cephalopods, worms, webs of spiders, cell walls of fungi and yeasts [2-4]. Despite its easy accessibility, chitin is still an underutilized resource because of its bulk structure and insolubility in water and common organic solvents [5].

Chitin is considered to be the main biomass resource [6], with more than 10¹¹ tones per year production in nature [5]. The shellfish processing industry (shrimp or crab shells) generates great amounts of waste from shells, which contain about 30% in chitin [4].

Chitin is non-toxic, odorless, biocompatible with living tissues, biodegradable [2], presenting antibacterial, moisture retaining and healing characteristics [7]. Chitin and chitosan (partially deacetylated chitin) can be utilized in water purification [8], additives in cosmetics [9,10], antibacterial agents [11,12], pharmaceutical adjuvants [11,13], paper production, textile finishes, photographic products, cements, heavy metal chelating agents, membranes, hollow fibers, and waste removal [14-16] and biomedical applications such as tissue engineering scaffolds, wound dressings, separation membranes, antibacterial coatings, stent coatings, and sensors [13,15- 18], since they are harmless for the human body [19].

Chitin is a high molecular weight linear polysaccharide consisting of copolymer repeated units of β -(1 \rightarrow 4)-2-acetamido-2-deoxy- β -D-glucose and β -(1 \rightarrow 4)-2- amino-2-deoxy- β -D-glucose [20], being

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highly basic. The β -(1 \rightarrow 4)-N-acetyl glycosaminoglycan structure with two hydroxyl groups and an acetamide group makes chitin very crystalline with strong hydrogen bonding [21].

Chitin is a semicrystalline biopolymer which forms microfibrillar arrangements in living organisms. Native chitin fibers are made up of thin filaments, crystalline fibrils called microfibrils, tightly bonded to each other through a large number of hydrogen bonds. These fibrils are typically embedded in a protein matrix and their diameters range from 2.5 to 2.8 nm, depending on their biological origins [14]. Chitin microfibrils are constituted of alternating crystalline and amorphous domains. Submitting the chitin to a strong acid hydrolysis treatment, causes the longitudinal cutting of these microfibrils, allowing dissolution of amorphous domains [22]. The microfibrils consist of nanofibers with 2–5 nm diameters [23,24]. Each chitin crystallite (otherwise called whisker i.e. highly crystalline chitin nanofibril) is composed of about 20 linear chains of N-acetylglucosamine, based upon the rod diameter and crystalline network dimensions [25].

Chitin is predominantly present as a fibrillar crystalline material. Based on infrared spectroscopy and X-ray diffraction data, native chitin can occur in one of the three crystalline forms [26]: α -chitin, β -chitin, and γ -chitin, respectively, depending on its origin. The molecules in α -chitin are arranged in an anti-parallel fashion, with strong intermolecular hydrogen bonding. α -chitin is the most abundant form existing in crabs, lobsters, krill and shrimps shells, insect cuticle, and fungal and yeast cells walls [3], having a crystallinity higher than 80% [27]. In β -chitin, present in squid pens and tube worms [28,29], the chains are arranged in a parallel fashion, while γ -chitin is the form in which the molecules are arranged in both parallel and anti-parallel manner. Due to the molecular packing, intermolecular interactions in β -chitin are weaker than those in α -chitin, making β -chitin more susceptible to dissolution in some solvents, and more reactive. α -Chitin is more abundantly present in nature compared with squid pen β -chitin and γ -chitin, purified α -chitins being commercially more readily available [30].

This paper details preparation methods and chemical modification, as well as applications of chitin nanowhiskers.

2. PREPARATION OF CHITIN NANOWISKERS

According with Watthanaphanit *et al.*, whiskers or crystalline nanofibrils are substances that can be made from the breaking down of crystalline materi-

als into nanocrystalline entities with specific shapes or self-assembling of basic building blocks [31].

Chitin nanowhiskers occur in biological tissues, according to structural hierarchies, jointly with proteins and inorganic compounds [32]. The purification step of chitin has to be optimized in order to remove remaining proteins and minerals that are present in the animal raw material and to take the best possible advantage of interwhiskers interaction, by favoring the development of a rigid chitin nanowhiskers network [28]. Chitin can be extracted from the biological tissues and dispersed in aqueous media to form colloidal suspensions [25].

Various methods have been employed for the preparation of chitin nanowhiskers (nanocrystals) or nanofibers including acid hydrolysis [7,14,22,28,29,31,33-42], TEMPO-mediated oxidation [27,43,44], ultrasonication [45], electrospinning [46], mechanical treatment [21,47,49], and gelation [6]. Acid hydrolysis was used to dissolve regions of low lateral order so that the water-insoluble, highly crystalline residue may be converted into a stable colloidal suspension by subsequent strong mechanical shearing action [40]. Chain cleavage occurring at random locations along the microfibrils form these partially acetylated whiskers that are rod-like or spindle-like particles that tend to align cooperatively and to develop rigid structures. Protonation of amino groups on the chitin nanowhiskers provide positive charges at their surface and stabilize the dilute colloidal dispersions of chitin nanowhiskers due to repulsive forces between crystallites [50]. It has also been reported that when such colloidal dispersions of acid-degraded chitin are dewatered to a critical increased concentration they can suffer an isotropic-anisotropic nematic transition [14,39,42].

Chitin nanowhiskers have been prepared from crab shells [21,22,35,36,40,42,47,51,52], squid pens [28], prawn shells [48,53], tubes of *Riftia pachyptila* worms [29] and shrimp shells [38,54]. The procedure used for the purification of chitin and the preparation of the suspensions of chitin nanowhiskers is quite similar in published procedures [14,28,29,34,50] with little modification due to the source of chitin.

The method for the preparation of chitin nanowhisiker reported by Morin [29] was as follows: (i) biological tissues (fragments of *Riftia pachyptila* tube worms) were suspended in a 5 wt.% aqueous KOH solution and boiled for 6 h under stirring in order to remove contaminating proteins. Then, the dispersion was rinsed with distilled water and filtered, the resulting paste being kept at room temperature

Table 1. Extraction methods and chitin sources on chitin nanowhisker size.

Nr.	Chitin source	Extraction method	Structural parameters of nanowhisker			Ref.
			Length (nm)	Width (nm)	Aspect ratio	
1	Crab shells	Hydrochloric acid hydrolysis	50-300(A=150)	6-8(A=10)	15	14
2	Crab shells	Hydrochloric acid hydrolysis	100-600(A=240)	4-40(A=15)	16	40
3	Crab shells	Hydrochloric acid hydrolysis	100-650(A=500±50)	10-80(A=50±10)	10±5	22
4	Crab shells	Hydrochloric acid hydrolysis	200-500	5-20	15-20	36
5	Chitin powder from crab shells	Hydrochloric acid hydrolysis	A=255±56	A=31±6	~8	56
6	Crab shells	TEMPO-mediated oxidation and subsequent ultrasonic treatment	340	8	-	27
7	Chitin powder from crab shell	Partial deacetylation with NaOH by fibril surface cationization and subsequent disintegration	A=250±140	A=6.2±1.1	-	44
8	Chitin powder from crab shells	Gelation with 1-allyl-3-methylimidazolium bromide, followed by regeneration with methanol	several hundred	20-60	-	6
9	Shrimp shells	Hydrochloric acid hydrolysis	150-800	5-70	-	
10	Shrimp shells	Hydrochloric acid hydrolysis	231-969(A=549)	12-65(A=31)	18	41
11	Shrimp shells	Hydrochloric acid hydrolysis	180-820(A=427)	8-74(A=43)	10	54
12	Shrimp shells	Hydrochloric acid hydrolysis	110-975 (A=343)	8-73(A=46)	~7.5	31
13	Squid pen	Hydrochloric acid hydrolysis	50-300 (A=150)	10	15	28
14	Squid pen	TEMPO-mediated oxidation	few microns	3-4	-	43
15	<i>Riffia</i> tubes	Hydrochloric acid hydrolysis	500-10000(A=2200)	~18	120	29

overnight under agitation. Subsequently, the boiling step was performed again, repeating also the washing and filtering steps. (ii) the chitin samples obtained after the above procedure, were bleached with a NaOCl_2 solution (17 g of NaOCl_2 in 1 L of 0.3 M sodium acetate buffer, pH 4.0), heated at 80 °C for 2 h under stirring. This procedure was repeated three times with rinsing. The resulting dispersion was centrifuged for 15 min. (iii) chitin was hydrolyzed with HCl boiling for 90 min under stirring and the product washed thoroughly with deionized water, followed by centrifugation (for 15 min) and decanting the supernatant. This process was repeated three times with the residue. The nanowhiskers were obtained by eliminating the amorphous parts of the microfibrils. Afterward, the suspensions of chitin nanowhiskers were transferred to a dialysis bag and dialyzed for 2 h in running water and, then, kept overnight in distilled water. (iv) the product was further subjected to a supplementary dialysis for 12 h, changing the distilled water every 2 h; the dialysis was performed until a pH=6 was reached. (v) the dispersion of nanowhiskers (0.15 wt.%) was accomplished further by three successive 2 min ultrasonic treatments. The dispersions were, subsequently, filtered to remove residual chitin nanocrystal aggregates. Next, appropriate volumes of HCl solution were added until a pH of about 2.5 was reached. The suspension, constituted from crystalline fragments of chitin, displayed a colloidal behavior, stabilized by positive charge (NH_3^+) at the surface of the whiskers resulting from the protonation of amino groups [42]. The particles were concentrated by dialysis against poly-(ethylene glycol). The solid fraction (0.22%) of this aqueous suspension was determined. It was kept at 6 °C in a refrigerator until used after adding chloroform to avoid microorganisms development [29].

The origin of chitin, namely the type of crystallinity, determines the structure and morphology of the chitin nanowhiskers [20]. The nanoparticles occur as rod-like or spindle-like nanowhiskers [27,43] with properties comparable to perfect crystals [4]. The structural characteristic of the chitin nanowhiskey extracted by different treatments from various chitin sources are summarized in Table 1. Chitin nanowhiskers extracted from various sources by a multi-stage chemical/mechanical separation are generally 4–80 nm in width and 50-10.000 in length. The dimensions for chitin whiskers obtained from crab shells are much shorter in length when compared with the chitin whiskers from *Riftia* tubes. The aspect

ratio is an important parameter, especially when chitin nanowhiskers are used to reinforce polymers; a higher aspect ratio usually results in greater reinforcement [55].

Chitin nanowhiskers dispersed in water were prepared by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) mediated oxidation of α -chitin in water at pH 10 with ultrasonic treatment. NaClO was added as co-oxidant in the reaction. Chitin was transformed into water-soluble polyuronic acid and water-insoluble chitin nanowhiskers. The TEMPO-oxidized chitin had crystallinity as high as that of the original α -chitin. A significant factor that affects the transparency of the dispersions, the resultant weight ratio of water-insoluble fractions, shape, length, and width of the chitin nanowhiskers obtained is the carboxylate content in the TEMPO oxidized chitins or the amount of NaClO added in the oxidation of α -chitin. The addition of 5.0 mmol of NaClO per gram of chitin seems to be optimal for preparation of mostly individualized nanowhiskers with high aspect ratios. The TEMPO-oxidized chitin nanowhiskers, prepared with 5.0 mmol of NaClO, had fiber widths smaller than 15 nm, and the average widths 8 nm. The anionic C6 carboxylate groups formed by TEMPO-mediated oxidation present only on the chitin whiskers surfaces may increase the individualization of the whiskers by simple mechanical treatment, such as sonication. Interwhisker linkages could be formed to some extent by electrical repulsion interactions between anionic C6 carboxylate groups and cationic C2 ammonium groups on the surfaces of chitin whiskers and/or through the osmotic effect [27].

Fan *et al.* [43] reported preparation of chitin nanowhiskers from squid pen β -chitin by TEMPO-mediated oxidation of native chitins, and subsequent mild mechanical agitation in water, at pH 3-4.8. Mostly individual and highly crystalline chitin nanowhiskers, 3–4 nm in width and few microns in length were successfully prepared. Cationization of the C2 amino groups in the β -chitin at pH 3-4 is keeping the stable dispersion state by interfibrillar electrostatic repulsion in water. This simple disintegration method for preparation of nanowhiskers is valuable in terms of safety issues, because the protocol involves no chemical modification. Nanowhiskers obtained by this method can be used in functional foods and cosmetics fields. The relatively low crystallinity of the nanowhiskers from squid pen is an important drawback. In addition, the biomass quantity of the pen is considerably lower than those of crab and shrimp shells.

Comparing with hydrochloric acid hydrolysis, TEMPO-mediated oxidation method is more controllable by the amount of NaClO and the yield of chitin nanowhiskers can reach 90%. *N*-deacetylation does not occur on the TEMPO-oxidized chitins [27].

Individual chitin nanowhiskers with average width and length 6.2 ± 1.1 and 250 ± 140 nm, respectively, have been obtained from partially deacetylated α -chitin by fibril surface cationization. Chitin nanowhiskers with *N*-acetylation values 0.74–0.70 were mechanically disintegrated in water at pH 3 to 4. In this method chitin nanowhiskers surfaces are positively charged, leading to electrical repulsion and nanowhiskers individualization [44].

Zhao *et al.* [45] developed a simple, versatile and environmentally friendly approach for extracting bionanowhiskers from various natural materials based on an ultrasonication technique. Bionanowhiskers, among which chitin nanowhiskers have been fabricated from various materials (chitin fibers, spider and silkworm silks, collagen, cotton, bamboo, and ramee and hemp fibers), with uniform diameters in the range of 25 to 120 nm, a useful size range for many tissue engineering and filtration applications. The ultrasonication causes the natural fibers to disassemble into nanowhiskers in water. The micron sized natural fibers are gradually disintegrated into nanowhiskers by the ultrasonic shock waves that cause erosion of the surface of the fibers which split along the axial direction. The disassembly rapidity depends on the intensity and frequency of the ultrasonic wave.

Min *et al.* [46] used an electrospinning method to fabricate chitin nanowhiskers. Electrospinning process is a technique that can produce polymer nanowhiskers with diameter in the range from several micrometers down to tens of nanometers, depending on the polymer and processing conditions. To improve chitin solubility, previous to electrospinning the chitin powder (100–500 nm) was depolymerized being packed in polyethylene bags and Co^{60} gamma irradiated, for 3 days. Chitin solutions with concentrations in the range from 3–6% were obtained. The electrospinning of chitin was performed with 1,1,1,3,3,3-hexafluoro-2-propanol as a spinning solvent. The electrospun chitin nanowhiskers were collected on a target drum which was placed at a distance of ~7 cm from the syringe tip and on which a voltage of 15 kV was applied to the collecting target by a high voltage power supply. The chitin nanowhiskers had a broad fiber diameter distribution, which ranged from 40 to 640 nm, the average diameter was 110 nm.

Kose and Kondo [49] prepared chitin nanowhiskers in an aqueous dispersion state from crab α -chitin powder, using an aqueous counter collision treatment (ACC). The ACC system is ejecting a liquid suspension of the sample from a pair of nozzles under a high pressure of 200 MPa, forming a pair of jets. The chitin fibers are not soluble in water, but chitin nanowhiskers can be dispersed in water by ACC method, that is able to cleave the facial interaction only by water jets without chemical modification of the molecules. The number of ejecting steps and ejecting pressure is adjusted to subject the sample to an appropriate degree of pulverization. Before the ACC treatment, the aqueous dispersion containing α -chitin powder was phase-separated. After the ACC treatment at 0, 1, 5, 10, 30, 60, and 120 ejecting steps, respectively, the chitin samples became turbid. Changing the number of ejecting steps and the desired pressure, the sample is supposed to be more downsized. Chitin particles having micro-size diameter were observed in polarizing light microscopy in the range from 0 to 10 ejecting steps. Chitin nanowhiskers having nanoscale diameter were observed in the range from 1 to 120 ejecting steps, their amount increasing with growing the ejecting steps number from 1 to 30. Also, the chitin nanowhiskers were homogeneously dispersed in water with increasing the ejecting steps number. The ACC treatment provided homogeneous aqueous dispersion of chitin nanowhiskers with 10–20 nm in width. The width of 10–20 nm was not significantly changed; by varying the treatment condition further pulverization did not take place any more. After chitin samples obtained by ACC treatment with 60 ejecting steps were kept for 3 months, the chitin nanowhiskers exhibited a favorable aggregation as a three-dimensional network formation.

Ifuku *et al.* [21,47,48] used mechanical disassembly of chitin to obtain highly uniform chitin nanowhiskers. Chitin nanowhiskers with 10–20 nm in width were prepared from wet and dried chitin obtained from crab shells [21,47] and prawn shells [48] by a simple grinding treatment after the removal of proteins and minerals. Because the exoskeleton of prawn has a finer structure, the nano-fibrillation of prawn shells is easier than that of crab shells, allowing the preparation of chitin nanowhiskers from prawn shells under neutral pH conditions, while the nanowhiskers from crab shells are obtained under acidic conditions. The cationization of small amounts of C2 amino groups (NH_3^+) on the chitin nanowhiskers surface, by the addition of an acid, promote the braking of the strong hydrogen bonds between the nanowhiskers, by electrostatic repul-

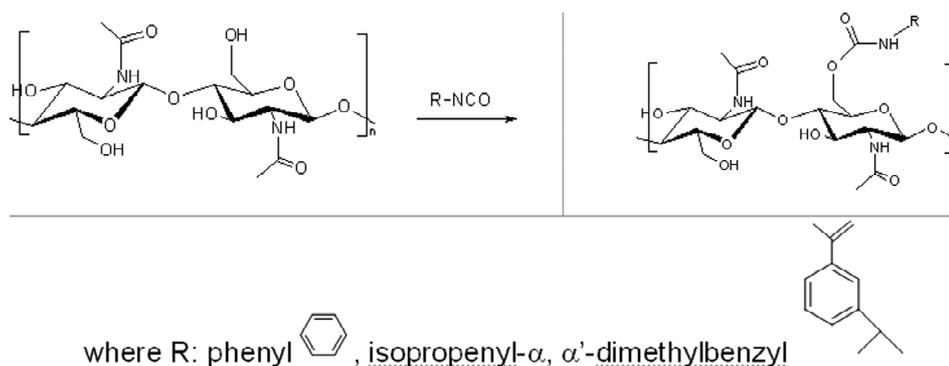


Fig. 1. Chemical modification of chitin nanowhiskers with isocyanate groups.

sions. The degree of substitution of the amino groups of only 3.9% was enough to break strong hydrogen bonds that protect the rigid chitin structure [47].

The suspensions of crude α -chitin were treated with a domestic blender. Then the slurry of 1% purified chitin was passed through a grinder. The mechanical treatment was used to isolate natural α -chitin nanowhiskers prepared in a never-dried state. These chitin molecules are aligned in an antiparallel manner that gives rise to α -chitin crystals in the form of bundles of highly uniform crystalline chitin nanowhiskers having a width of 2-5 nm. Furthermore, these nanowhiskers are wrapped in protein layers resulting chitin-protein fibers of around 100 nm diameter. Because strong hydrogen bonding between the bundles of dried chitin nanowhiskers, leads to difficulty in achieving thin and uniform nanowhiskers, the material was kept wet after the removal of the matrix [21].

Chitin nanowhiskers with a uniform structure and a long length were obtained by Ifuku *et al.* [48] from the cell walls of five different types of mushrooms, commonly used for human consumption *Pleurotus eryngii*, *Agaricus bisporus*, *Lentinula edodes*, *Grifola frondosa*, and *Hypsizygus marmoreus*. The chitin nanowhiskers were isolated by removing glucans, minerals, and proteins, and subsequent simple grinding treatment under acidic conditions. Depending on the species of mushroom, the width of chitin nanowhiskers was in the range 20–28 nm. It was noticed that the α -chitin structure was maintained and that on the chitin nanowhiskers surface a complex with glucans was formed.

Beside the excessive costs, the previously presented preparation methods based on chemical treatment of the raw materials have several other drawbacks, among which the low yield of the processes, dangerous handling of boiling HCl, disposal of the colored HCl solution, recovery of enormous quantities of slightly acidic water, difficult

adjustment of the pH value because of the strength of HCl, and so on [57].

Chitin nanowhiskers were easily produced by the gelation of a commercial chitin powder with 1-allyl-3-methylimidazolium bromide (AMIMBr), by soaking it in the ionic liquid at room temperature, followed by heating at 100 °C. Soaking the resulting gel in methanol and subsequent sonication gave chitin dispersion in which chitin nanowhiskers formations were regenerated [6]. This processing technique for the preparation of the chitin nanowhiskers is considered to have great advantages compared to the methods presented above because special equipments and chemical modifications are not necessary.

3. CHEMICAL MODIFICATION OF CHITIN NANOWHISKERS

Chitin nanowhiskers possess a reactive surface covered with hydroxyl groups, which provides the possibility of modification through chemical reaction. The purpose of chemical modification is to contribute to specific functions and to expand the applications of chitin nanowhiskers. A method for the processing of chitin nanowhiskers-based nanocomposites is their transformation through long chain surface chemical modification. The nanoparticles are modified based on the use of grafting agents bearing a reactive end group and a long compatibilizing tail [58].

Surface chemical modification of chitin nanowhiskers is a method to decrease their surface energy and disperse them in organic liquids of low polarity. Nair and Dufrense [52] investigated the surface chemical modification of chitin nanowhiskers with different reagents. The surface of chitin nanowhiskers - prepared by acid hydrolysis of chitin from crab shells - was chemically modified using a small molecule chemical reaction between

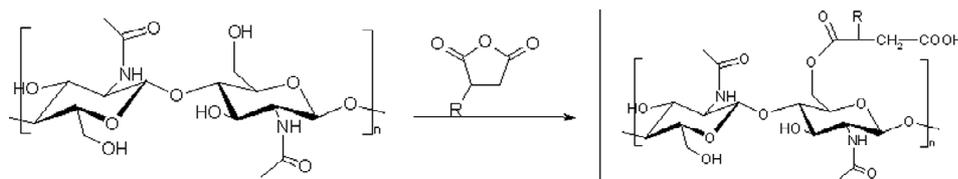


Fig. 2. Chemical modification of chitin nanowhiskers with alkenyl succinic anhydride.

hydroxyl groups ($-\text{OH}$) and isocyanate groups ($-\text{NCO}$) from phenyl isocyanate (PI) and isopropenyl- α , α' -dimethylbenzyl isocyanate (TMI) (see Fig. 1).

Alkenyl succinic anhydride (ASA) consisting of a mixture of oligomers of different sizes centered around C18 ($M_n = 300$) was used for the modification of chitin nanowhiskers [52] (see Fig. 2). The acylated whiskers can be dispersed in medium and low-polarity solvents. Nanowhiskers with different dispersibility could be obtained by controlling the time of the heating step [58]. FTIR spectroscopy, TEM, and contact angle measurements were performed to prove the occurrence of the surface modification without any major morphological changes [52].

The C6 primary hydroxyl groups on the chitin nanowhiskers surfaces are selectively oxidized to carboxylate groups *via* the aldehyde structure by TEMPO-mediated oxidation (see Fig. 3). The total contents of carboxylate and aldehyde groups at 5.0 and 10 mmol of NaClO per gram of chitin were 12% and 24% of the C6 primary hydroxyl groups of the original chitin to be oxidized to either carboxylate or aldehyde groups.

New β -(1 \rightarrow 4)-linked polyuronic acids (e.g. chitouronic acid) consisting of repeating units of the sodium salt of *N*-acetylglucosaminuronic acid, are obtained quantitatively by TEMPO oxidation. In order to oxidize only the C6 primary hydroxyl groups present on the chitin nanowhiskers surfaces the amount of NaClO added should be controlled. When a sufficient amount of NaClO is added to the chitin/water slurries in the oxidation, chitin can be converted to the corresponding water-soluble polyuronic acid with partial depolymerization at pH 10–11 [59,60].

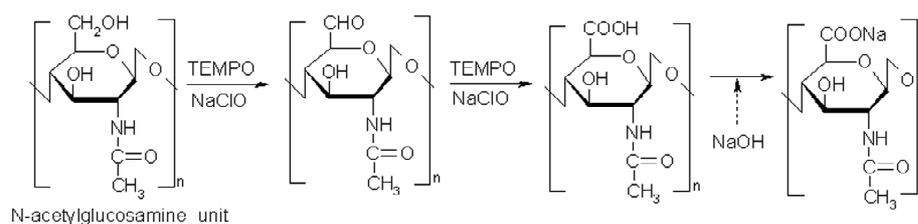


Fig. 3. TEMPO oxidation of chitin nanowhiskers.

4. APPLICATION OF CHITIN NANOWHISKERS

Since large quantities of crab and shrimp shells are produced annually as food waste, further utilization of chitins as functionalized materials is desired [27]. The chitin nanowhiskers are currently obtained as aqueous suspensions which are being studied and used as reinforcing additives for high performance environment-friendly biodegradable nanocomposite materials, as biomedical composites for drug/gene delivery, nanoscaffolds in tissue engineering and cosmetic orthodontics [6,17,27,29,40,41,51,52,61,62]. The reinforcing effect of chitin nanowhiskers results from the formation of a percolating network based on hydrogen bonding forces [58]. The chitin nanowhiskers with fungal origin can be used in antitumor application and immuno-modulating activity [48]. Current research on chitin nanostructures is important for preparing materials for medical and veterinary applications, as well [57].

4.1. Nanocomposites

Most of chitin nanowhiskers investigations have focused on hydrosoluble or latex-form polymers. Using surfactants or chemical grafting it is possible to disperse these nanowhiskers in non-aqueous media.

The addition of fillers to process a composite material is a standard method to improve the mechanical behavior of a material [29]. In the nanocomposite industry, a reinforcing particle is defined as having at least one of its linear dimensions smaller than 100 nm. Because of the recent rising of nanotechnology, chitin nanowhiskers have got significant attention, being promising reinforc-

ing materials for nanocomposites, due to their high stiffness and strength [31,63]. Nanocomposites are a class of materials that has aroused much interest in the last years since they exhibit ultrafine phase dimensions, they are renewable and ecologically friendly materials [27]. A large amount of the current nanofillers used to prepare nanocomposites with synthetic polymeric materials are inorganic [64]. The use of nanowhiskers from renewable resources as natural fillers, instead of traditional inorganic reinforcement materials (e.g. glass fibers, carbon, and talc) would be a much better choice providing numerous advantages including easy availability, nontoxicity, renewability, low density, low cost, good specific mechanical properties, biodegradability, good biocompatibility, reproducibility, and easy chemical and mechanical modification [65,66].

Chitin whiskers have been used as an innovative type of nanofillers as a reinforcing material in both natural [22,31,33,38,40,51,54] and synthetic polymeric matrices [6,21,28,29,41,47,48,67-72]. For obtaining of a good level of distribution of the fillers within the polymer matrix the use of either an aqueous suspension or an aqueous solution of the polymer is required. The reinforcing effect depends on the aspect ratio of the chitin whiskers [29].

Polymer/chitin nanowhiskers nanocomposite processing techniques that are usually employed are (i) *casting and evaporating technique* [22,28,29,31,38,40,50-52,54,56,72-74] in which polymer aqueous solution or dispersion is mixed with chitin nanowhiskers aqueous suspension resulting a homogenous dispersion. The dispersion is cast into a container and, by evaporation of water, a nanocomposite with chitin nanowhiskers incorporated is obtained, (ii) *freeze-drying and hot-pressing technique* [29,40,51] in which well-dispersed aqueous mixtures, of thermoplastic polymer and chitin nanowhiskers are freeze-dried to give nanocomposite powders, which are, subsequently, processed into specimen by hot-pressing, (iii) *polymer grafting* [69], a solvent free technique that use "graft from" strategy by which long chain surface chemical modification of polysaccharide nanoparticles consisting in grafting agents bearing a reactive end group and a long "compatibilizing" tail are taking place [58], and (iv) *nonaqueous solvent dispersion technique* [52] in which the hydroxyl groups from the chitin nanowhisiker's surface are modified by chemical reactions improving their hydrophobicity. Hydrophobic chitin nanowhiskers can form a good dispersion in nonaqueous solvents, such as toluene and form stable suspensions.

Nanocomposite films are obtained by removal of toluene.

4.1.1. Nanocomposites with Natural Polymers as Matrix

A particular group of biocomposites is green composites, in which polymers from renewable resources (bio-based polymers), representing the matrix of the composite, are reinforced by natural fibers. The study of "green composites" is an emerging area in polymer science. "Green composites" [75,76] is a term that indicates that both matrix and reinforcement material of the composite originate from renewable resources [77], their use being advantageous not only from an economical viewpoint, but also environmentally friendly.

Nair and Dufresne [40,51] investigated the effects of processing methods on the mechanical properties of natural rubbers (NR) reinforced with chitin nanowhiskers from crab shells nanocomposites. Chitin nanowhiskers reinforced NR nanocomposites were obtained from a colloidal suspension of chitin whiskers as the reinforcing phase and latex of both unvulcanized and prevulcanized natural rubber as the host matrix. The aqueous suspensions of chitin whiskers and rubber were mixed and stirred, solid composite films were obtained either by freeze-drying and hot-pressing method or by casting and water evaporation method. The samples prepared by casting and evaporation method showed higher reinforcing efficiency. Using this method the reinforcing effect of chitin nanowhiskers strongly depended on their ability to form a rigid three-dimensional network in the NR matrix, resulting from strong interactions such as hydrogen bonds between the whiskers during the evaporation method. The disruption of the network resulted in lowering or loss of this ability. Among the evidences for the existence of a three-dimensional rigid chitin network is better resistance of evaporated samples, than the hot-pressed ones, against swelling in an organic solvent medium, the values of diffusion coefficient, bound rubber content, and relative weight loss [40]. Latex from rubber trees (*Hevea brasiliensis*) is the source of all commercial natural rubber (cis-1,4- polyisoprene), one of the most important elastomers, an important and irreplaceable material in applications such as automotive (tires and engine mounts) and constructions [77,78], in industrial and technological areas [51].

Nair and Dufrense [52] investigated the incorporation of surface chemical modified chitin

nanowhiskers into natural rubber to obtain composite materials with enhanced mechanical properties and to enlarge the number of potential polymer matrixes. The surface of chitin whiskers from crab shells, was chemically modified with PI, ASA, and TMI. PI and ASA were used to improve adhesion between the NR used as a matrix and the chitin whiskers. The TMI was used to copolymerize with the unsaturations present in NR matrix. Stable suspensions of these chemically tailored chitin whiskers were obtained in toluene as a replacement for using aqueous suspensions. Nanocomposites were prepared using a toluene natural rubber solution in which the chitin nanowhiskers were dispersed. The various chemical treatments improved the adhesion between the filler and the matrix, but mechanical performances of the composites decreased after the chemical modification. The loss of mechanical performance, more obvious for the isocyanate treatments, could be owing to the partial or total destruction of the three-dimensional network of chitin whiskers after the surface modification which reduces the hydrogen bonding.

For unvulcanized NR latex based nanocomposites two processing methods were used, casting and water evaporation or freeze-drying and hot-pressing, whereas for vulcanized materials only the former method was used, in order to investigate the effect of the processing method on the properties of the material. The most important aspect that governs the mechanical behavior of the chitin whisker reinforced NR nanocomposites is the processing technique. Dynamic mechanical analysis showed the existence of a small percentage of crystallinity in unvulcanized NR prepared by the evaporation method, whereas no such evidence of crystallinity was detected either in unvulcanized NR prepared by hot pressing or in vulcanized rubber prepared by evaporation methods. The evaporation method is a slow step procedure, in which the chitin nanowhiskers get enough time and mobility to establish a rigid filler-filler network within the host matrix [51].

Jacobs *et al.* [33] have studied the introduction of chitin nanowhiskers as functional components into chitosan for nanocomposites preparation by electrospinning. Mostly individualized chitin nanowhiskers with spindle-like morphology and diameter ranges of 10-20 nm were developed as nanocomposite materials for the reinforcement of chitosan. The chitin nanowhiskers content in the resultant composites ranged from 1.25% to 5%. The average fiber diameter of neat chitosan nanofibres was 213 nm, and the chitin nanowhiskers loading

increased for 1.25 to 5% inducing a significant reduction in fibre diameter, down to a range of 143 – 171 nm. The results indicated a good interaction between the matrix and the chitin nanowhiskers that led to enhanced structural morphology, fibre diameter and thermo-mechanical properties of electrospun nanofibres.

The successful preparation and characterization of chitosan/ α -chitin whiskers with or without heat treatment has been reported. Films were cast from chitosan solutions containing dispersed α -chitin nanowhiskers in the range between 0 and 2.96%. Thermal stability and the apparent degree of crystallinity of the chitosan matrix were not much affected by the addition of α -chitin nanowhiskers. The tensile strength of α -chitin nanowhiskey-reinforced chitosan films was greater from that of the pure chitosan film, achieving a maximum at the nanowhiskey content of 2.96%. Water resistance of the nanocomposite films - marked by decreased weight loss and swelling in an aqueous medium – was improved by the addition of α -chitin whiskers and heat treatment [38].

Chitosan has been used in a film form for multiple applications, such as tissue engineering, wound dressing, controlled release, and food packaging [79-82]. The real utilization of chitosan films is limited due to its solubility in water and other aqueous solutions. The presence of α -chitin whiskers and heat treatment decreased the affinity to water of the chitosan/ α -chitin whiskers nanocomposite films, being more stable when used in an aqueous environment [38].

Glycerol plasticization was used to incorporate chitin nanowhiskers from crab shells as a filler to reinforce soy protein isolate (SPI) matrix for producing a class of environmentally friendly nanocomposites. SPI and different content of chitin were mixed and stirred to obtain a homogeneous dispersion. The dispersion was freeze-dried and 30% glycerol was added, then the resulting mixture was hot-pressed and, then, gradually cooled to room temperature. The SPI/chitin nanowhiskey composites, with thickness of about 0.4 mm, were thus obtained. Nanocomposites with lower whisker content displayed a relatively homogeneous dispersion in the SPI matrix than those with increased chitin whisker content. The composites show greater water-resistance as the chitin whiskers increase in the SPI matrix. Strong intermolecular hydrogen bonding interactions among different chitin whiskers and between filler and SPI matrix play a significant role in the improvement of mechanical properties which restrict the motion of the matrix and decrease

water sensitivity of the SPI-based nanocomposites without interfering with their biodegradability [22].

Biodegradable plastics, such as plastics from SPI can have important applications in rubbish and compost bags, mulch films, and disposable diapers [83,84]. Plastics from SPI have very high strength and good biodegradability. Their applications are limited because they are brittle and water sensitive [85]. By chitin nanowhiskers incorporation into SPI, the thermo-mechanical properties have been improved and the composites have shown greater water-resistance.

Glycerol plasticized-potato starch was mixed with chitin nanowhiskers to obtain fully natural nanocomposites by casting and evaporation. The results showed improvements in tensile strength, storage modulus, glass transition temperature, and water vapor barrier properties of the composite. However, at >5% loading, aggregation of the chitin nanowhiskers took place with negative effects [73].

4.1.2. Nanocomposites with Synthetic Polymers as Matrix

The possible applications of the chitin nanowhiskers with synthetic polymers, such as poly(vinyl alcohol) (PVA) and polycaprolactone (PCL) to give corresponding composite materials depend on the procedures that assure the compatibility between chitin and the polymeric matrix. Synthetic polymers are flexible materials with many industrial applications because of their excellent physical properties and chemical resistance. Some drawbacks of synthetic polymers are high cost, non-biocompatibility, poor mechanical or thermal performance of some polymers [86], and limited processability, biocompatibility, and biodegradability [41].

In some studies the preparation of composites or blends composed of chitin nanowhiskers and poly(vinyl alcohol) (PVA) have been accomplished [30,38,87-90]. The chitin nanowhiskers prepared by acid hydrolysis were incorporated in the composites with PVA [30]. Sriupayo *et al.* [38] prepared and characterized α -chitin whisker-reinforced chitosan nanocomposite films with or without heat treatment. The nanocomposite formed with PVA reinforced with α -chitin whisker presented enhanced thermal stability, tensile strength and water resistance. The contents of the nanowhiskers were less than 30%. Junkasem *et al.* [41] also obtained chitin whisker-PVA reinforced nanocomposite. The nanocomposites were fabricated by electrospinning a mixture between an aqueous solution of PVA and a suspension of α -

chitin whiskers prepared from chitin flakes from shells of *Penaeus merguensis* shrimps. Kadokawa *et al.* [6] described a method completely different from the previous techniques. Composite materials of chitin nanowhiskers with PVA were easily prepared by the gelation of a commercially available chitin powder with AMIMBr, followed by the regeneration with methanol. The SEM images of the composite (weight ratio of chitin to PVA = 1:0.3) illustrated that the nanowhisiker-like morphology was preserved. A relative immiscibility of chitin and PVA in the composite was specified, PVA components probably filled in spaces between the whiskers. Chitin nanowhisiker and PVA might be partially miscible at the interfacial area between the two polymers in the composites due to formation of hydrogen bonding between them or by the presence of a little amount of AMIMBr.

Ifuku *et al.* [47] examined the fibrillation properties of dry chitin and nanowhisiker homogeneity by preparation of optically transparent nanocomposites using chitin nanowhiskers and acrylic resin. Chitin nanowhiskers with a uniform width and a high aspect ratio were prepared from dried chitin, extracted from crab shell. The suspension, that contained 0.1% fibrillated chitin nanowhiskers dispersed in water, was vacuum filtered obtaining chitin nanowhisiker sheets, which were cut into 2 - 3 cm fragments and were impregnated with neat acrylic resin with 2-hydroxy-2-methylpropiophenone photo-initiator. The resin-impregnated sheets were UV treated resulting chitin nanowhisiker composite sheets. The optical losses of the transparent composites after the drying process were smaller than 2%, showing that dried chitin was fully fibrillated.

Nge *et al.* [67,68] studied the incorporation of chitin nanowhiskers in poly(acrylic acid) for preparation of composites, with unique optical properties. The chitin crystallites presented uniplanar orientation; the X-ray diffraction data revealed that molecular long axes were perpendicular to the direction of the magnetic field.

Using free-radical photopolymerization of acrylic acid in an unidirectional shearing aligned mesophase, a liquid chitin nanowhiskers/poly(acrylic acid) composite was fabricated, with unique optical properties. The composite, coated with a calcium fluoride substrate, was transparent. Its alignment was depending on the mesophase composition of the ternary dispersion composed of chitin microfibrils, water and acrylic acid. The mesophase behavior strongly influenced the degree of orientation and the molecular interactions [68].

One important application of PCL, a semicrystalline thermoplastic polymer, is to make suture thread. In order to keep the material's biodegradability, chitin whiskers are used as reinforcing phase for PCL. Morin and Dufrense [29] prepared nanocomposite materials from an aqueous suspension of high aspect ratio β -chitin whiskers, as the reinforcing phase, and a latex of PCL as the matrix. The colloidal microcrystalline dispersion was mixed with the suspension of latex in different amounts in order to obtain composite films with a weight fraction of chitin ranging between 0 and 10%. After mixing and stirring the two aqueous suspensions, solid films were attained by either freeze-drying and hot-pressing or casting and evaporating the preparations. The results showed that at high temperature and a percentage of above 5% nanowhiskers in the mixture, these nanowhiskers formed a rigid network assumed to be governed by a percolation mechanism, which stabilized the mechanical properties of the composite.

Squid pen chitin nanowhiskers were introduced as filler into an acrylic polymer matrix (average diameter of the particles around 150 nm), a copolymer of styrene and butyl acrylate, poly(styrene-co-butyl acrylate) and poly(S-co-BuA). The aqueous suspension of chitin microcrystals was mixed with an aqueous suspension of polymer (latex) containing spherical particles of poly(S-co-BuA) in different amounts in order to obtain nanocomposite films with a good level of dispersion and with a weight fraction of chitin ranging from 0 to 20 wt.%. Using vacuum, the air from the suspension was removed and the sample was casted in a Teflon mold, obtaining homogeneous, 1 mm thick films after water evaporation. These whiskers bring a reinforcing effect and improve the thermal stability of the composite. Dynamic mechanical analysis showed that the mechanical properties of these composites were substantially improved by increasing the amount of filler. For low whiskers content the filler-matrix interaction is the main phenomenon involved in the reinforcing effect of chitin whiskers. When the chitin whiskers were smaller than 10 wt.%, the films did not show consistent improved mechanical properties over the whole temperature range (between 200 and 425K) [28]. Chitin nanowhiskers can be used as environmentally friendly particulate fillers for thermoplastic nanocomposites films, which can be used for the processing of stiff small-size products.

Chitin whisker-graft-polycaprolactone (CHW-g-PCL) was prepared by ring-opening polymerization of the caprolactone monomer onto the chitin nanowhiskers surface under microwave radiation. It

involved surface chemical modification of the chitin nanowhiskers, based on the use of grafting agents with a reactive end group and a long compatibilizing tail, as the key of thermoforming. In order to preserve the mechanical properties instead of small molecules polycaprolactone long chains are grafted. This nanocomposite prepared using the "grafting from" strategy were thermoformed to fabricate CHW-g-PCL molded sheets with good mechanical properties. The increase of the PCL content in CHW-g-PCL, conducted to elevated strength and elongation, as well as high hydrophobicity of the nanocomposites [69].

Huang *et al.* [70] obtained waterborne polyurethane-based nanocomposites by casting and evaporating a mixture with waterborne polyurethane as matrix and small quantities of chitin nanowhiskers as nanofillers. The strength and Young's modulus of the nanocomposites were simultaneously improved and maintained ca. 500% elongation. The maximum tensile strength (28.8 MPa) and enhanced Young's modulus (6.5 MPa) that were ca. 1.8- and 2.2-fold over those of neat polyurethane were attained at chitin nanowhiskers loading of 3%. The active surface and stiffness of chitin nanowhiskers facilitated the interface for stress transferring formation and gave endurance to stress.

Environmentally-friendly, organic, solvent-free polyurethane (waterborne polyurethane) with low volatile organic compound levels and non-toxicity can be applied to leather and textile finishing, floor coverings, adhesives and pressure sensitive adhesives [91-95]. Chitin derivative [96] has been incorporated into waterborne polyurethane for reducing costs, better biodegradability, and increased mechanical performance.

Zeng *et al.* [74] prepared two series of nanocomposite films from waterborne poly(ester-urethane) and chitin nanowhiskers with and without ultrasound treatment. The effects of ultrasonication treatment and chitin nanowhiskey content on the chemical composition, crystallization behavior and miscibility were studied. When chitin nanowhiskey content was > 30% both nanocomposite films exhibited a certain degree of miscibility, resulting in higher thermal stability and tensile strength than in the case of the pure waterborne poly(ester-urethane) film. The nanocomposite films subjected to ultrasound treatment possessed better miscibility and mechanical properties (storage modulus, thermal stability and tensile strength) than those without ultrasound treatment over the entire composition range studied. The difference can be attributed to the relatively higher dispersion level of

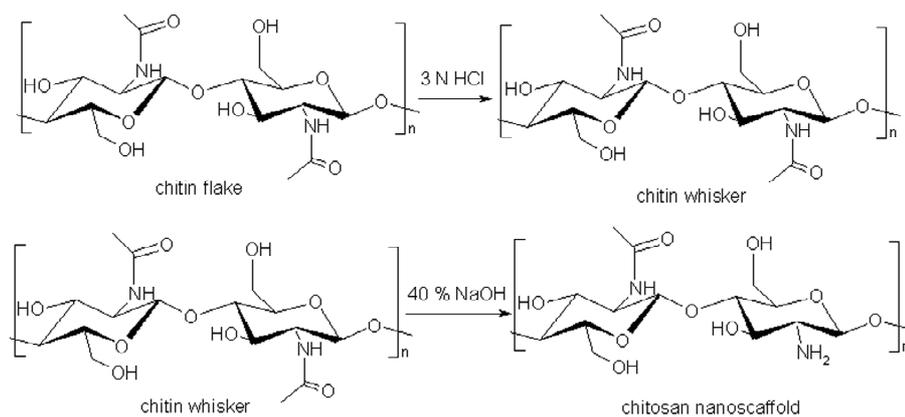


Fig. 4. Preparation of chitosan nanoscaffold from chitin flake.

nanowhiskers within poly(ester-urethane) matrix resulting in stronger interaction between both components. The structure, miscibility and mechanical properties of the nanocomposite films depended significantly on the preparation method.

Rizvi *et al.* [71], blended chitin nanowhiskey with polylactide to form melt-blended polylactide-chitin composites and Li *et al.* [72] prepared chitosan/chitin whisker/rectorite ternary films, both organic and inorganic composites being suitable for food-packaging applications.

4.2. Biomedical materials

Chitin nanowhiskers were incorporated in some potential biomedical materials, such as scaffolds, hydrogels and wound dressing in which the materials have to be cytocompatible. Hydrogels are physically or chemically cross-linked polymer networks capable to absorb large amounts of water. Hydrogels based on natural polymers present various applications in the field of tissue engineering [97].

Wongpanit *et al.* [54] studied the influence of chitin whiskers incorporation as nanofiller to silk fibroin sponge in order to improve its dimensional stability and to increase its compression strength. Silk fibers from the silkworm *Bombyx mori* have been used commercially as biomedical sutures for decades. Regenerated silk fibroin is biodegradable [98], and cells like L929 cells [99], endothelial cells [100], keratinocytes, osteoblasts fibroblasts [101], bone marrow stromal cells [102], and bone marrow-derived mesenchymal stem cells [103] grow well on its surface. The highly cytocompatible regenerated silk fibroin is an attractive scaffolding biomaterial applicable for a wide range of target tissues. Nanocomposite sponges filled with chitin whiskers were prepared by using a freeze-drying technique. All samples possessed an intercon-

nected pore network with an average pore size of 150 nm. Mouse fibroblast L929 cells were seeded onto the nanocomposites surfaces. Results indicated that silk fibroin sponges, both with and without chitin whiskers, were cytocompatible, making them possible materials for tissue engineering applications. The incorporation of chitin whiskers into the silk fibroin matrix was found to improve the dimensional stability and to promote cell spreading on the nanocomposite materials [54].

The scaffolds are used to obtain products with nano/micro pore structure appropriate for advanced applications, such as artificial extracellular matrices [104,105], micro-particles for drug delivery, and medical implants [106,107]. Functional scaffolding materials are used as templates for the attachment of cells for consequent tissue development in the process of tissue regeneration. Materials used for fabrication into scaffolds are synthetic polymers, such as polylactide, polyglycolide, and their respective copolymers, and natural polymers, such as collagen, gelatin, and alginate [108,109], as well. Because scaffolds made from natural polymers are mechanically weak blending, crosslinking, and compositing means are used to arrive at functional scaffolds with improved physical, mechanical, and biological integrities.

Phongying *et al.* [61] report for the first time that chitosan nanoscaffold can be directly prepared from the chitin nanowhiskey (see Fig. 4), by deacetylation in a highly concentrated alkaline solution for 21 h, yielding high amount of chitosan nanoscaffolds, because no organic solvents or chemicals were involved. The colloidal solution of nano-sized chitosan, with a degree of deacetylation of 95%, presented a fibrous network with a nanoporous structure and the pore diameter of ~ 200 nm. Although chitin whiskers showed a molecular weight

of 62,838 Da, the molecular weight of a chitosan nanoscaffold increased to 137,262 Da, probably due to the scaffold structure. This biocompatible material could potentially be used in scaffolding of a tissue-engineered vessel.

Efficient deacetylation of chitin nanowhiskers to a chitosan nanoscaffold in the form of a colloidal solution was obtained with a 60% alkaline solution, using a microwave technique, under a N₂ atmosphere, for only 3 h, seven times shorter than the treatment time of the conventional method. The degree of deacetylation of chitosan nanoscaffold was above 90%. The amorphous chitosan was obtained from the highly crystalline chitin whiskers. The aggregation of branched chitin whiskers initiates the formation of nanoscale “scaffold” morphology [20].

α -chitin-whisker-reinforced hyaluronan–gelatin nanocomposite scaffolds with enhanced physical, mechanical and biological performances were prepared with a 50 : 50 w/w blend of hyaluronan and gelatin, using α -chitin whisker as the reinforcing filler. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide was used as crosslinker. The CW-reinforced HA/Gel scaffolds were fabricated by a freeze-drying technique. The weight ratios of the chitin nanowhiskers to the blend were 0–30%, but the scaffolds with 10% chitin nanowhiskers showed the greatest cell viability, being the finest for supporting the proliferation of cultured human osteosarcoma cells, even higher than the native scaffolds. The inclusion of 2% chitin nanowhiskers in the scaffolds doubled their tensile strength. The average pore size of the scaffolds varied between 139 and 166 μ m, regardless of the chitin nanowhiskers content. Most of the incorporated chitin nanowhiskers improved the thermal stability and the resistance to biodegradation. A low proportion of the chitin nanowhiskers increased the tensile strength and enhanced the biocompatibility, conducting to attachment and proliferation of the cultured human osteosarcoma cells of the resulting scaffolds. Although the scaffolds that contained 10% chitin nanowhiskers showed great promise as substrates for bone cell culture, their actual utilization could be limited to a low-stress bearing area, such as the socket of a dental root [56].

Alginate-based materials (hydrogel and fibrous products) are widely used in wound-care applications. Some of their advantages are biocompatibility, haemostatic capability and gel-formability upon subjected to an aqueous environment [110]. Watthanaphanit *et al.* [31] prepared calcium alginate nanocomposite yarn (30 fibers) containing 0.05–2% chitin whiskers obtained from shells of *Penaeus merguensis* shrimp, by wet spinning

process. Alginate is a biopolymer resulting from cell walls of some brown algae [111]. Between the alginate molecules and the homogeneously dispersed chitin whiskers specific interactions are created, such as hydrogen bonding and electrostatic interactions. Apart from the nanocomposite fibers containing 2% whiskers that presented whisker aggregates on the fiber surface, in most of the nanocomposite fibers the chitin whiskers were embedded inside the fibers. The incorporation of a low amount (between 0.5 and 2%) of the whiskers in the nanocomposite fibers considerably improved the mechanical and thermal properties of the fibers and accelerated the biodegradation process of the fibers in the presence of lysozyme. The presence of Ca²⁺ ions in the Tris-HCl buffer solution improved the tenacity of the nanocomposite fibers.

Zhang *et al.* [112] introduced chitin nanowhiskers into supramolecular hydrogels based on cyclodextrin/polymer by inclusion, in order to improve mechanical strength and regulate drug release behavior. The *in vitro* cell viability of the extracted leached media from the nanocomposite and native hydrogels was assessed by the MTT method using the L929 cell line. The elastic modulus of the nanocomposite hydrogels raised due to the reinforcing function of the polysaccharide nanowhiskers. The presence of polysaccharide nanocrystals increased the stability of the hydrogel framework and inhibited the diffusion of bovine serum albumin (BSA). BSA served as a model protein drug in the nanocomposite hydrogels and showed important sustained release profiles. The results showed that incorporation of chitin nanowhiskers did not increase the cytotoxicity in comparison with the native hydrogel. The nanocomposite hydrogels could be used as injectable biomaterials due to their inherited shear-thinning property.

Muzzarelli *et al.* [17] integrated highly crystalline chitin nanowhiskers into wound dressings made of chitosan glycolate and dibutyl chitin. Non-woven dibutyl chitin was used as a biocompatible support. The obtained products were tested in murine wound models being applied in various traumatic wounds resulting in very good final healing.

4.3. Adsorbents in industry and water purification

Ma *et al.* [113] employed ultrafine chitin nanowhiskers with 5–10 nm diameters as barrier layers in a new class of thin-film nanocomposite membranes for water purification. The nanocomposite membranes presented high virus

adsorption capacity as demonstrated by MS2 bacteriophage testing, due to the very high surface-to-volume ratio. The low cost of raw chitin, the environmentally friendly fabrication process, and the impressive high flux indicate that such ultrafine nanofibril-based membranes can surpass conventional-membranes in drinking water applications.

The study of Dolphen and Thiravetyan [114] showed that chitin nanowhiskers prepared from shrimp shell waste is very promising for adsorption of melanoidins and other pigments from sugar syrup, being appropriate for application in sugar industry. The maximum adsorption capacities of melanoidins by chitin nanowhiskers at 20, 40, and 60 °C were 131, 331, and 353 mg/g, respectively. Chitin nanowhiskers presented an elevated affinity for melanoidins when compared to other chitin-derived adsorbents. The interaction between melanoidins and chitin nanowhiskers involved both electrostatic and chemical adsorption.

4.4. Protein immobilization

Na Nakorn [115] carried out protein immobilization with chitin nanowhisiker (the average diameter 420 nm with Nanosizer) and chitosan nanoparticles (the average diameter 215 nm with Nanosizer) in order to find suitable materials to fabricate an efficient biosensor, using for the optimization BSA as model protein. Before immobilization, chitin whiskers and chitosan nanoparticles were resuspended in deionized water and sonicated for 10 s. The immobilization time was 1 min and 15 min for BSA and chitin whiskers and chitosan nanoparticles incubation, followed by centrifugation. The concentration of remaining BSA was determined by UV absorption at 280 nm. BSA immobilization with chitosan nanoparticles for 15 min, pH 6 (acetate buffer) and 20–25 °C were the optimal conditions, because they provided the least remaining BSA. In this study, chitosan nanoparticles showed better BSA immobilization characteristics than chitin nanowhiskers, subsequently being used for the development of a glucose oxidase – chitosan electrode for the detection of glucose for a biosensor application.

4.5. Bioengineering

Mera *et al.* [7] investigated the frequency of plasmid uptake in penetration-intermediate *E. coli* cells through Yoshida effect [116] produced by chitin nanowhiskers. The optimum conditions required to create penetration-intermediates capable of

acquiring plasmid DNA for creating a genetic transformation method in *E. coli* dependent on chitin nanowhiskers and the Yoshida effect were determined. The chitin nanowhiskers surfaces possess amino groups due to acid hydrolysis-induced deacetylation. The protonation of these amino groups imparts a positive surface charge that interacts with plasmid DNA due to the negative charge of the nucleic acids to form ionic complexes. Chitin nanowhiskers were mixed with DNA oligonucleotides tagged at the 5' end. Under fluorescence microscope important amounts of chitin nanowhiskers aggregated and acicular crystalline materials were observed, concluding that the nucleic acid was adsorbed onto the chitin nanowhiskers surface. Similarly mixing pUC18 plasmid DNA with chitin nanowhiskers resulted in adsorption of pUC18 onto the surface of the nanowhisiker. The colloidal solution consisting of nano-sized acicular crystalline chitin containing pUC18 plasmid DNA and cells of *Escherichia coli* was placed on an agar hydrogel and stimulated by sliding friction at the interface between the agar hydrogel and polystyrene stir stick, facilitated the transformation of the *E. coli* cells to antibiotic resistant. The genetic transformation represents the effect of both chitin nanowhiskers and sliding friction. The formation of *E. coli* cells penetration-intermediates was a result of Yoshida effect induced by the chitin nanowhiskers. Chitin nanowhiskers form a complex that penetrated bacterial cells due to the driving force resulting from the sliding friction. Plasmid pUC18DNA was adsorbed onto the chitin nanowhiskers and then introduced into *E. coli* cells through the perforations. Intracellular uptake of exogenous pUC18 led to resistance of *E. coli* towards ampicillin. The transformation efficiency varied with both the number of recipient cells and amount of chitin nanowhiskers. Induction of the Yoshida effect with chitin nanowhiskers represents a simpler and efficiently comparable alternative to conventional chemical methods for introducing genes into bacteria.

4.6. Stabilization of oil-in-water emulsion and nematic gels

Chitin nanowhisiker aqueous dispersions shift towards a nematic gel-like behavior with increasing the solid particles concentration. Chitin nanowhiskers being charged rod-like colloids, at pH 3, form parallel alignments of anisotropic particles on entropic terms, as predicted by Onsager [117]. Between these nanowhiskers associative interactions occur, mostly of van der Waals type, which

could be responsible for sol-gel transition. Stronger gels are formed when these associative interactions are enhanced with increasing ionic strength, pH, temperature and time [34], or by adding whey proteins [35].

Tzoumaki *et al.* [118] studied the stabilizing properties of chitin nanowhiskers, colloidal rod-like particles obtained from crab shells, in oil-in-water (o/w) emulsions under varying conditions. Emulsions present broad occurrence in food, cosmetics and pharmaceutical industries [119]. An oil-in-water emulsion was prepared by homogenizing chitin nanowhiskers stock dispersion with corn oil and an aqueous solution, using an ultra-sonic homogenizer. The chitin nanowhiskers were effective in stabilizing o/w emulsions against coalescence, for one month period, even when the droplets were of relatively large size, due to the adsorption of the nanowhis-ker at the oil-water interface. The increase in chitin nanowhiskers concentration led to network formation in the emulsions, increased stability to creaming, and a gel-like behavior. Pronounced emulsion elastic responses and creaming stability was obtained by raising the temperature, NaCl concentration or pH (from 3.0 to 6.7). The chitin nanowhiskers adsorption at the o/w interfaces, resulted in the formation of inter-droplet network and a chitin nanowhiskers network in the continuous phase, representing a potential mechanism responsible for the o/w emulsion stabilization.

4.7. Formation of CaCO₃/chitin-whisker hybrids

Yamamoto *et al.* [36] prepared CaCO₃/chitin-whisker hybrids with hierarchical structures using the liquid-crystalline suspension of the chitin whiskers. Suspensions of chitin nanowhiskers, obtained by acid hydrolysis of chitin powder displayed lyotropic liquid-crystalline behavior. The casted suspension was immediately transformed into a gel when exposed to ammonium carbonate vapor. CaCO₃ crystals were created for 30 days in chitin gels, the crystals being deposited in the gels matrices to produce hybrids with three dimensional structures. This approach inspired by biomineralization is useful for the design of mechanically stable inorganic/organic hybrid materials.

4.8. Chitin nanowhis-ker aerogels, used as carbon precursors

Nogi *et al.* [53] prepared nanowhis-ker aerogels from nanowhiskers water suspensions by subjecting the

suspensions to solvent exchange and freeze-drying. Chitin nanowhiskers with reduced diameter did not aggregate with each other in carbon precursor aerogels and presented high thermal stability. These nanowhis-ker aerogels with fine and individual nanowhis-ker network were used as carbon precursors. After the carbonization of prawn chitin nanowhis-ker, the original fine nanowhis-ker network was preserved in the chitin carbon. Nanofibrillar chitin carbon is activated by physical or chemical treatment (H₂O or NaOH) attaining chitin carbon with a large surface area, with potential applications in filter media, electric double-layer capacitors and highly efficient catalysts.

5. CONCLUSIONS AND OUTLOOK

Chitin can be extracted from biological tissues and dispersed in aqueous media to form colloidal suspensions of chitin nanowhiskers. The utilization of chitin nanowhiskers contributes to a healthy ecosystem, chitin being a renewable resource. Chitin nanowhiskers have drawn attention in various applications due to their properties like nanosized dimensions, high surface area, high absorptivity, biodegradability, nontoxicity, renewability, low density and easy modification. The intrinsic rigidity of chitin nanowhiskers, special rod-like and spindle-like morphology, strong interfacial interactions, and the percolation network organized by nanowhiskers contribute to optimized mechanical performance, thermal properties, solvent absorption, and barrier properties. One key advantage is their high surface area that enables chitin nanowhiskers to interact effectively with cells, factors, proteins and other compounds. Chitin nanowhiskers possess a reactive surface covered with hydroxyl groups, giving the opportunity of chemical modification. Realization of functional modifications of chitin nanowhiskers under mild reactive conditions would greatly improve their practical utility for the future.

Chitin nanowhiskers can be used as environmentally friendly particulate biofillers being compounded with many different kinds of polymer matrices. The reinforcing effect strongly depends on the aspect ratio of the chitin whisker, and therefore on its origin, as well as on the processing technique of the composite. Chitin nanowhiskers can form viable materials for biomedical areas, such as scaffolding and immobilizing biomolecules in the process of producing novel biosensors by using waste and cheap materials obtained from other industries. They also have applications in the cosmetics industry,

mainly for the ordered regeneration of wound tissues and as dermal fillers.

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