

Preparation of Bionanomaterials and their Polymer Nanocomposites from Waste and Biomass

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Abstract Nature is gifted with several nanomaterials which could be easily prepared from animals and plants. Cellulose, chitin and starch are abundant, natural, renewable and biodegradable polymers. By intelligent processing techniques they could be used as classical nano reinforcing elements in polymers. They are often called whiskers. These whiskers are almost defect free and as a result, their properties are comparable to perfect crystals. In most cases, aqueous suspensions of these nano crystallites are prepared by acid hydrolysis process. The object of this treatment is to dissolve away regions of low lateral order so that the water-insoluble, highly crystalline residue may be converted into a stable suspension by subsequent vigorous mechanical shearing action. The reinforcing ability of these natural whiskers stem from their chemical nature and hierarchical structure. During the past decade, many studies have been devoted to mimic biocomposites by blending natural whiskers from waste and biomass sources with various polymer matrices. In this review article, the recent advances on the preparation and characterization of nanowhiskers from waste and biomass and their polymer nanocomposites have been reported. Finally the emerging applications are also discussed.

Keywords Cellulose nanowhiskers · Chitin nanowhiskers · Starch nanowhiskers · Bionanocomposites · Characterization

Introduction

Cellulose was discovered in 1838 by the French Chemist Anselme Payen, who isolated it from plant matter and determined its chemical formula [1, 2]. Cellulose was used to produce the first successful thermoplastic polymer, celluloid, by Hyatt Manufacturing Company in 1870. Hermann Staudinger determined the polymer structure of cellulose in 1920. The compound was first chemically synthesized (without the use of any biologically-derived enzymes) in 1992, by Kobayashi and Shoda [3]. Cellulose is the most abundant natural biopolymer and is readily available from renewable resources. It is an excellent fiber. Wood, cotton, and hemp rope are all made of fibrous cellulose. Cellulose is made of repeat units of the monomer glucose. This is the same glucose which in our body metabolizes in order to live, but we cannot digest it in the form of cellulose. Because cellulose is built out of a sugar monomer, it is called a polysaccharide. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand $\beta(1 \rightarrow 4)$ linked D-glucose units [1, 4]. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae. Some species of bacteria secrete it to form biofilms. About 33% of all plant matter is cellulose (the cellulose content of cotton is 90% and that of wood is 50%) [5]. In addition to cellulose, plant fibers contain different natural substances mainly hemicelluloses, lignin, pectins and waxes [6–8].

Cellulose derived from plant is unpurified cellulose associated with other kinds of natural fiber like lignin and hemicellulose while bacterial cellulose (BC) is nearly-purified cellulose. Cellulose is considered as a nearly inexhaustible raw material with fascinating structures and properties for the remarkable demand for environmentally

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friendly and biocompatible products. As is well known, cellulose is insoluble in many solvents, which leads to a limitation in its reactivity and processability for utilization. Preparation of microcrystalline cellulose from materials other than wood and cotton such as water hyacinth, coconut shells, sugar cane bagasse, ramie, wheat and rice straws, jute, flax fibers and flax straw and soybean husk is receiving a lot of interest. Natural cellulose fibers are gaining attention as a reinforcing phase in thermoplastic matrices [9–11]. Its low density, a highly reduced wear of the processing machinery, and a relatively reactive surface may be mentioned as attractive properties, together with their abundance and low price. Moreover, the recycling by combustion of cellulose-filled composites is easier in comparison with inorganic filler systems. Nevertheless, such fibers are used only to a limited extent in industrial practice, which may be explained by difficulties in achieving acceptable dispersion levels. The reinforcing ability of the cellulose whiskers lies in their high surface area and good mechanical properties [12, 13]. However, to obtain a significant increase in material properties the whiskers should be well separated and evenly distributed in the matrix material. Different processing methods aided with a variety of chemicals (compatibilizers, surfactants, etc.) have been explored to fulfill these requirements [14].

Chitin is a polymer that can be found in anything from the shells of beetles to webs of spiders. Chitin is the second most abundant natural polymer after cellulose [15]. It is present all around us, in plants and in animal creatures. Chitin and cellulose are molecular similar polysaccharide compounds, cellulose contains a hydroxyl group, and chitin contains acetamide group. Crabs, beetles, worms and mushrooms contain large amount of chitin. Chitin is a very stable material, and it helps to protect an insect against harm and pressure. Depending on its thickness, chitin can be rigid or yielding. Often, insect coats contain thick, stiff layers of chitin. It protects Crustaceans, Parasites, Fungi and other Pathogens from the adverse effects of their environments. Shellfish containing industry waste (Shrimp or Crab shells) in which chitin content range between 8% and 33% constitutes the main source of this polymer.

Chitin is a high molecular weight linear polysaccharide, specifically β -(1–4) (*N*-acetyl-D-glucosamine). Chitin is natural, non-toxic, non-allergenic, anti-microbial and biodegradable it is insoluble in water and resistant to acid, alkalis, and many organic solvents [16]. The shellfish processing industry generates great amounts of waste from shell, which represent about 30-wt% in chitin. World wide, about 105 metric tone of chitin coming from waste are available yearly for industrial uses, the main sources being shrimp and crab waste material. Chitin has found applications in many areas other than food such as in biosensors [17]. Main uses of chitin film and fiber are in medical and

pharmaceutical applications as wound-dressing material [18, 19] and controlled drug release [20, 21]. Chitin has versatile biological activity, excellent biocompatibility and complete biodegradability in combination with low toxicity. α -chitin is more abundant than β -chitin and χ -chitin [22]. Chitin has been known to form microfibrillar arrangements embedded in a protein matrix and these microfibrils have diameters ranging from 2.5 to 2.8 nm [23]. Crustacean cuticles possess chitin microfibrils with diameters as large as 25 nm [24, 25]. The chitin-protein fibers are arranged in horizontal planes forming a typical twisted plywood structure or Bouligand pattern. Chitin can easily be isolated from crab shell, where it is found to be highly thixotropic and liquid crystalline [26, 27] Dufresne and coworkers have successfully isolated the crystalline regions of chitin whiskers from the crab shells and squid pens by hydrochloric acid hydrolysis [28, 29]. It was reported that the reinforcing effect strongly depends on the aspect ratio of the chitin whiskers [30–32].

Starch is a well known polymer naturally produced by plants in the form of granules (mainly from potatoes, corn, and rice). Starch granules vary from plant to plant, but in general are composed of a linear polymer, amylose (in most cases about 20% of the granule), and a branched polymer amylopectin [27, 28, 33, 34]. Amylose is a semi-crystalline biopolymer and is soluble in hot water, while amylopectin is insoluble in hot water during its biodegradation. Starch undergoes enzyme-catalysed acetal hydrolysis, the α -1,4 link in amylopectin is attacked by glucosidases [33]. Starch or amyllum is a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. Starch is produced by all green plants as an energy store and is a major food source for humans. Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules, linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20–25% amylose and 75–80% amylopectin [35]. Glycogen, the glucose store of animals, is a more branched version of amylopectin. Starch can be used as a thickening, stiffening or gluing agent when dissolved in warm water, giving wheat paste.

Structure of Cellulose, Chitin and Starch

From the molecular structure of cellulose given in Fig. 1, it can be seen that cellulose is a homopolymer of D-anhydroglucopyranose monomeric units connected through β (1–4) glycosidic linkages. In general, cellulose can be seen as a long chain polymer with D-glucose, a sugar, as its repeating units. Since the glucose units are 6 member rings within cellulose chain, they are known as pyranoses. These

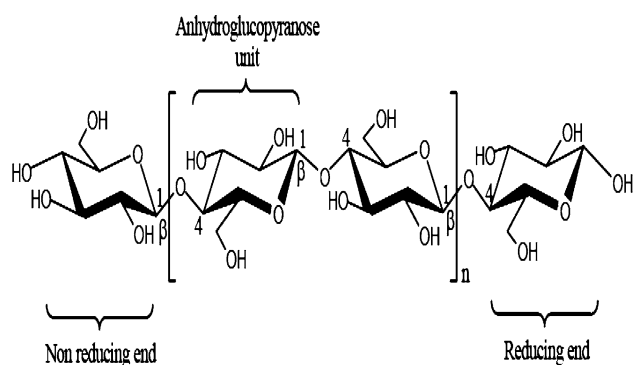


Fig. 1 Molecular structure of cellulose

pyranose rings are joined by single oxygen atoms, acetal linkages, between the C-1 of one pyranose ring and the C-4 of the next ring. The glucose units in cellulose polymer are referred to as anhydroglucose units. Often in nature, cellulose is associated and mixed with other substances such as lignin, pectins, hemicelluloses, fats, and proteins. Cellulose that is produced by plants is referred to as native cellulose, which is found in two crystalline forms, cellulose I and cellulose II [36]. Cellulose II, generally occurring in marine algae, is a crystalline form that is formed when cellulose I is treated with aqueous sodium hydroxide [37–39]. Among the four different crystalline polymorphs cellulose I, II, III, and IV, cellulose I is thermodynamically less stable while cellulose II is the most stable structure.

Liquid ammonia treatment of cellulose I and cellulose II gives crystalline cellulose III form [40, 42], and the heating of cellulose III generates cellulose IV crystalline form [42]. Recently a non crystalline form known as nematic ordered cellulose has been described [43]. From a solution in lithium dimethylacetamide highly ordered cellulose is produced which is crystalline. The $\beta(1-4)$ glycosidic linkage gives cellulose the linearity that results in a rigid rod-like molecule. The hydroxyl groups on the cellulose chain that are in the equatorial position protrude laterally along the extended molecule. This position makes them readily available for intramolecular and intra-strand hydrogen bonding (Fig. 2). The hydrogen bonds cause the chains to group together in highly ordered crystal-like structure and hold the network flat. Figure 3 shows a representation of cellulose chains that are usually longer than the ordered crystalline regions and are thought to pass through several different crystalline regions which also have areas of amorphous regions in between them. Within the crystalline regions, the extensive and strong inter-chain hydrogen bonds give the resultant fibers good strength and insolubility in most solvents. This also prevents cellulose from melting at elevated temperatures. In the less ordered regions, the chains are further apart and more available for hydrogen bonding to other molecules, such as water

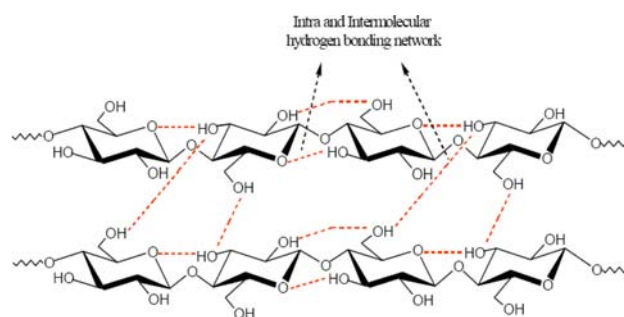


Fig. 2 Representation of the extensive intra- and intermolecular hydrogen bonding network occurring in cellulose

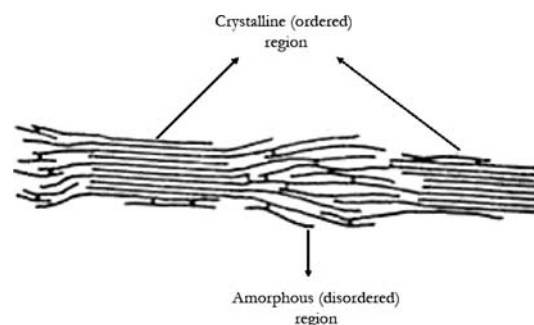


Fig. 3 Crystalline and amorphous regions in cellulose microfibril

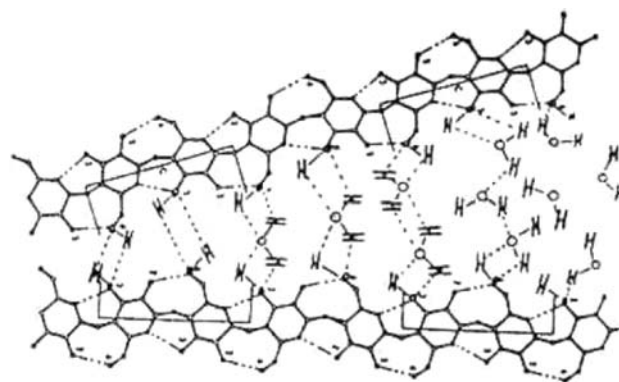
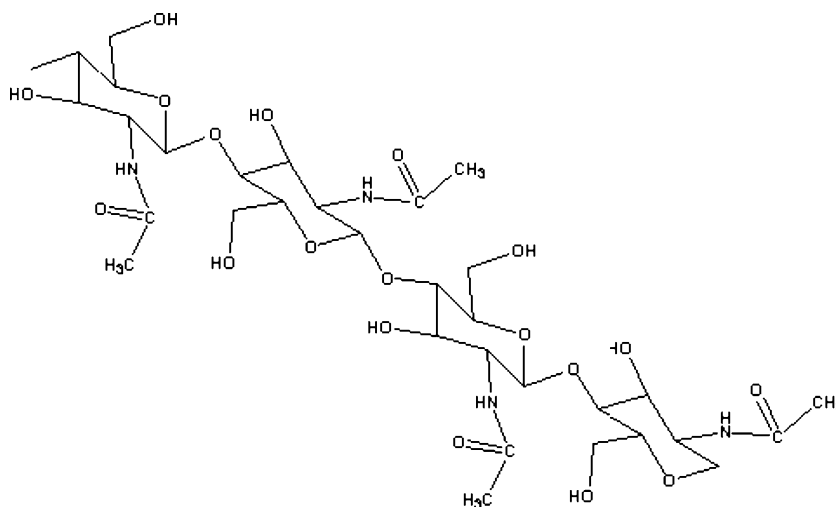


Fig. 4 Structure of cellulose microfibril, showing hydrogen bonding with water

(Fig. 4). Most cellulose structures can absorb large quantities of water and is thus very hygroscopic. As a result cellulose swells, but does not dissolve in water.

The molecular structure of chitin is shown in Fig. 5. The crystallography of chitin has been investigated for a long time [44–47]. Marguerite [22] has reported in his reviews on chitin and chitosan; at first glance the powder X-ray diagrams of chitins from shrimp shell (α -chitin) and anhydrous squid pen (β -chitin) appeared nearly the same. Further information on the crystalline structure of α - and β -chitin is obtained by analysis of electron diffraction

Fig. 5 Molecular structure of chitin



patterns of highly crystalline samples. The crystallographic parameters of α and β -chitin reveal that there are two antiparallel molecules per unit cell in α -chitin, whereas only one is present in β -chitin, which consists therefore of a parallel arrangement. Despite this difference, it appears that *N*-acetyl glycosyl moiety is the independent crystallographic unit in both allomorphs.

The observation of diffraction patterns of various α -chitin samples indicates some discrepancy in their diffraction patterns. In particular the X-ray pattern of lobster tendon chitin presents a marked 001 diffraction spot [47] which is absent in the more crystalline sagitta chitin [48–50]. Therefore, it appears that more work is required to resolve these ambiguities about the crystal structure of α -chitin. In contrast, the structure of anhydrous β -chitin appears to be well established. However, the crystal structure of the β -chitin hydrate remains to be refined, as some uncertainty exists, even as to its unit cell parameters [51, 52]

A number of studies have been reported in the literature about the infrared spectra of chitin [53–58] C=O stretching region of the amide moiety, between 1600 and 1500 cm^{-1} , the amide I band is split at 1656 and 1621 cm^{-1} , the amide II band is in at 1556 cm^{-1} . The band at 1656 cm^{-1} , which occurs at similar wavelengths in polyamides and proteins, is commonly assigned to stretching of the C=O group hydrogen bonded to N–H of the neighboring intra-sheet chain. The band at 1621 cm^{-1} , which is not present in polyamides and proteins, its occurrence may indicate a specific hydrogen bond of C=O with the hydroxymethyl group of the next chitin residue of the same chain [58, 59]. This is reinforced by the presence of only one band in this region for *N*-acetyl D-glucosamine [59]. For Chitin, the band at 1621 cm^{-1} is modified in deuterated water, whereas the band at 1656 cm^{-1} remains nearly unaffected [57]. Band at 1621 cm^{-1} could be either a combination

band or due to an enol form of the amide moiety [56]. Definition of the molecular structure of α -chitin and its inter-sheet hydrogen bonding does not allow us to give a definitive explanation for this band.

Starch molecules arrange themselves in plants in semi-crystalline granules. Each plant species has a unique starch granular size: rice starch is relatively small (about 2 μm) while potato starches have larger granules (up to 100 μm). Although in absolute mass only about one quarter of the starch granules in plants consist of amylose, there are about 150 times more amylose molecules than amylopectin molecules. Amylose is a much smaller molecule than amylopectin. A typical feature of starch is that it becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granules. This process is called starch gelatinization. During cooking, starch becomes a paste and gets its viscosity. During cooling or prolonged storage of the paste, the semi-crystalline structure partially recovers and starch paste thickens. This is mainly caused by the retrogradation of the amylose. This process is also responsible for staling, hardening of bread and water layer on top of a starch gel. Some cultivated plant varieties have pure amylopectin starch without amylose, known as waxy starch. Waxy starch have less retrogradation; the viscosity of the paste will be more stable. Also high amylose starch, amylo maize, is cultivated for the use of its gel strength. The structure of starch can be studied with several techniques (X-ray, AFM and TEM). The amorphous and partially crystalline rings start from the center, called hilum, and follow each other alternatively. The amorphous rings are amylose, while the partially crystalline ones are amylopectin. The macromolecules are oriented in the radial direction. The amorphous region of a partially crystalline rings are formed by those parts of the amylopectin macromolecule where the chain

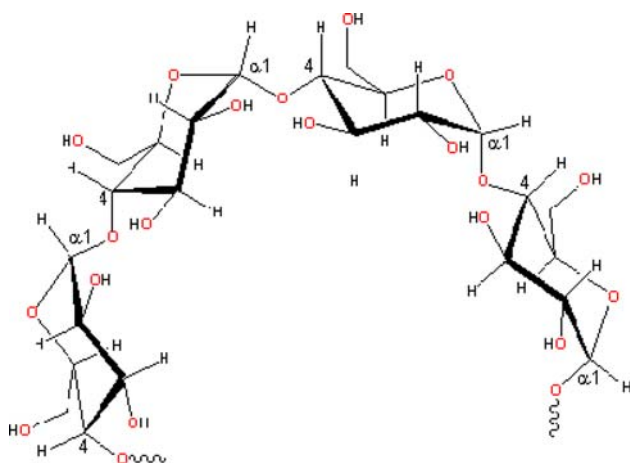


Fig. 6 Structure of amylose

branches. The crystalline part consists of the oriented, double helix molecular chain parts of amylopectin. The structure of amylose and amylopectin are given in the Figs. 6 and 7. Different starch plant species and its amylose, amylopectin and crystallinity content are given in Table 1.

Properties of Cellulose, Chitin and Starch

Cellulose has no taste, is odourless, hydrophilic, chiral and biodegradable. It is insoluble in water and in most organic solvents. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. The multiple hydroxyl groups on the glucose

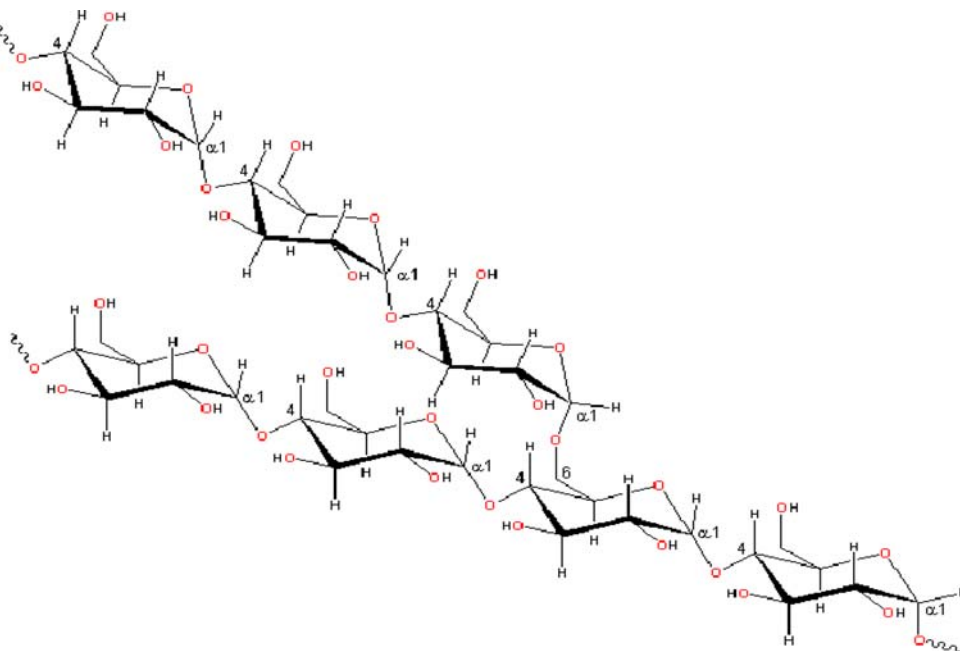
Table 1 Properties of starch for different biomass species

Starch	Amylose content (%)	Amylopectin content (%)	Crystallinity (%)
Wheat	26–27	72–73	36
Maize	26–28	71–73	39
Waxy maize starch	<1	99	39
Amylomaize	50–80	20–50	19
Potato	20–25	74–79	25

residues from one chain form hydrogen bonds with oxygen molecules on the same or on a neighbour chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This strength is important in cell walls, where the microfibrils are meshed into a carbohydrate matrix, conferring rigidity to plant cells. It has interesting sound insulation properties and low thermal conductivity

Most of the naturally occurring polysaccharides e.g. cellulose, dextrin, pectin, alginic acid, agar, agarose, carrageenans are neutral or acidic in nature, whereas, chitin is an example of highly basic polysaccharide. Their unique properties include polyoxysalt formation, optical structural characteristics, ability to form films, chelate metal ions and optical structural characteristics. Cellulose is a homopolymer, while chitin is a heteropolymer. Neither random nor block orientation is meant to be implied for chitin. Like cellulose, it naturally functions as a structural polysaccharide, but differs from cellulose in the properties. Chitin is highly hydrophobic and is insoluble in water and most organic solvents. It is soluble in hexafluoroisopropanol,

Fig. 7 Structure of amylopectin



hexafluoroacetone, and chloroalcohols in conjugation with aqueous solutions of mineral acids and dimethylacetamide containing 5% lithium chloride. Chitin is a highly crystalline, interactable material and only a limited number of solvents are known which are applicable as reaction solvents. Chitin degrades before melting, which is typical for polysaccharides with extensive hydrogen bonding. For each solvent system, polymer concentration, pH, counter ion concentration and temperature effects on the solution viscosity must be known. The comparative data from solvent to solvent are not available. As a general rule, researchers dissolve the maximum amount of polymer in a given solvent that still retained homogeneity and then regenerated in the required form. The nature of the coagulant is also highly dependent on the solvent and solution properties as well as the polymer used.

Starch has received considerable attention during the past two decades as a biodegradable thermoplastic polymer and as biodegradable particulate filler. Indeed, products from agricultural sources such as starch, offers an attractive and cheap alternative in developing degradable materials. Starch is not truly thermoplastic as most synthetic polymers. However it can be melted and made to flow at high temperatures under pressure. If the mechanical shears become too high, then starch will degrade to form products with low molecular weight. Addition of water or other plasticizers enables the starch to flow under milder conditions and reduces the degradation considerably. However, the thermo chemical stability is strongly due to the addition of plasticizers [60]. By itself, starch is a poor choice as a replacement for any plastic. It is mostly water insoluble, difficult to process, and brittle. In principle some of the properties of starch can be significantly improved by blending it with synthetic polymers. Physical incorporation of granular starch or starch derivatives as a functional additive and filler into synthetic polymers during processing has been largely used, since the first announcement of using starch in combination with synthetic polymer either as starch gel blends with ethylene acrylic acid copolymer by Westhoff et al. [61] or as a particulate starch dispersion in polyolefin by Griffin and Priority [62].

Preparation of Nanowhiskers

Many researchers extracted nanocellulose whiskers from various sources of micro cellulose fibers (derived from waste and biomass) using very simple process. From the literature, it can be seen that most of the scientists have used hydrolysis and dialysis methods for the preparation of nano cellulose whiskers. Favier et al. [63] extracted nanocellulose crystals from tunicate *Microcosmus fulcatus*. These sea animals are having diameter between 5 and

10 cm with a 1 cm thick cellulose tunic. After simple hydrolysis and dialysis process, they prepared nanocellulose whiskers parallelepiped rods with lengths ranging from 100 nm to several micrometers and widths on the order of 10–20 nm. Oksman and Bondeson [64] extracted nanocellulose whiskers from spruce (*Picea abies*) [65–67] microcrystalline cellulose. They also used the simple hydrolysis method and dialysis for the preparation of nanowhiskers. In another study from the group of Oksman et al. [68], nanocellulose from micro crystalline cellulose (MCC) having 10 nm width and length between 200 and 400 nm has been extracted. It was pure microcrystalline cellulose and the particle size was between 10 and 15 μm . Grunert and Winter [69] have prepared nanocrystals by acid hydrolysis of BC microfibrils. Nanocrystals with dimensions of 50 by 8 nm in width and thickness, respectively, and a few hundred to several thousand nanometers in length, have been reported by Tokoh and coworkers [70]. They obtained nano crystals by sulphuric acid hydrolysis with boiling 2.5 N sulphuric acid as suggested by Ranby et al. [71].

Chitin nano whisker extraction was also nearly the same method as of cellulose. Based on the procedure adopted by Michel and Dufresne [72] samples were first boiled and stirred in a 5% KOH solution for 6 h to remove most of the proteins. This suspension was subsequently kept at room temperature overnight under stirring, filtered, and washed several times with distilled water. Chitin samples were then bleached with 17 g of NaClO_2 in 1 L of water containing 0.3 M sodium acetate buffer for 6 h at 80°C. The bleaching solution was changed every 2 h followed by abundant rinsing the sample with distilled water. After bleaching, the suspension was kept in a 5% KOH solution for 72 h to remove residual protein. The resulting suspension was centrifuged at 3000 trs/min for 20 min. Chitin whisker suspensions were prepared by hydrolyzing the purified chitin sample with 3 N HCl at the boil for 1.5 h under stirring. The ratio of 3 N HCl to chitin was 30 mL/g. After acid hydrolysis, the suspensions were diluted with distilled water followed by centrifugation (10,000 trs/min for 5 min). This process was repeated three times. Next, the suspensions were transferred to a dialysis bag and dialyzed for 24 h against distilled water until a pH 6 was reached. The pH was subsequently adjusted to 3.5 by adding HCl. The dispersion of whiskers was completed by a further 2.5 min ultrasonic treatment (B12 Branson sonifier) for every 40 mL aliquot. Morin and Dufresne [29] also prepared the nano chitin whiskers from *Riftia*. The diameter was 18 nm and length around 120 nm. In another study Gopalan and Dufresne [73] extracted nanochitin whisker from crab shell. They successfully extracted 100–600 nm length and 4–40 nm width nanocrystal form 500–1000 μm chitin micro crystal. Rujiravanit and coworkers [74] have

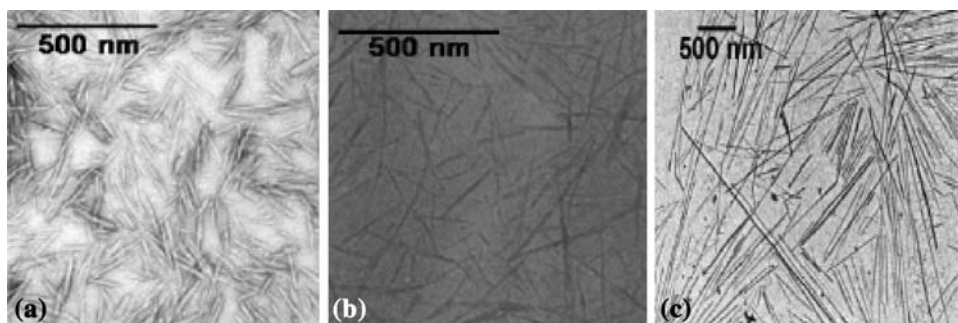
reported preparation of chitin whiskers by acid hydrolysis of shrimp shells. The nanochitin whiskers consisted of slender rods with sharp points that had broad distribution in size. The length of the chitin fragments ranged from 150 to 800 nm, the width ranged from 5 to 70 nm. More than 75% of the whiskers however had a length below 420 nm. From the group of Zhang and coworkers [75] nanochitin whiskers were prepared from crab shell. It was spindle shaped with broad distribution in length (L) ranging from 100 to 650 nm and diameter (D) ranging from 10 to 80 nm. The average of length and diameter were estimated to be 500 and 10 nm, respectively.

Angellier et al. [76] have extracted nano starch crystals from macro maize granules. According to the extraction procedure, 36.72 g of native waxy maize starch granules were mixed with 250 ml of 3.16 M H_2SO_4 for 5 days at 40°C, with a stirring speed of 100 rpm. The resulting suspension was washed by successive centrifugations with distilled water until neutrality. The aqueous suspensions obtained had a weight concentration of about 3.4 wt%. Waxy maize starch nanocrystals consist of platelet-like particles with a thickness of 6–8 nm, a length of 40–60 nm, a width of 15–30 nm, and a density, F_s , measured by theory of Brunauer, Emmett, and Teller (BET) of 1.55 g cm^{-3} . Such nanocrystals are generally observed in the form of aggregate.

Characterization of Nanowhiskers

Transmission Electron Microscopy (TEM) is very good tool for characterizing the structure of nanosized materials. Samir et al. [14] extracted nano whiskers from macro cellulose. Figure 8 shows transmission electron micrographs (TEM) obtained from dilute suspensions of cotton, sugar-beet pulp, and tunicin (the cellulose extracting from tunicate) whiskers. The length and lateral dimension are around 200 nm and 50 Å and 1 nm and 150 Å for cotton and tunicin whiskers, respectively. Grunert et al. [69] have reported on the size of the cellulose extracted from Bacterial using TEM images. Many scientists have [77, 78] used TEM for characterization of cellulose nano whiskers.

Fig. 8 Transmission electron micrographs (TEM) of dilute suspensions of cotton, sugar-beet pulp, and tunicin starch nanowhiskers



Atomic Force Microscopy (AFM) is also another good tool for detecting the size of the nano sized cellulose particles. Figure 9 shows the AFM image of cellulose whiskers after drying on a mica surface. Oksman and co-workers [78] observed that AFM analysis of the cellulose whiskers is a good alternative to electron microscopy, without any limitations regarding contrast and resolution. The shape of the whiskers appeared, however, different from that observed in TEM.

Scanning Electron Microscopy (SEM) is generally employed for the more extensive morphological inspection. It consists of the observation of fractured surface films at liquid nitrogen temperature. This technique allows for conclusions about the homogeneity of the composite, presence of voids, dispersion level of the whiskers within the continuous matrix, presence of aggregates, sedimentation, and possible orientation of whiskers. Their diameter determined by SEM is much higher than the whiskers diameter. This results from a charge concentration effect due to the emergence of cellulose whiskers from the observed surface. Figure 10 shows the SEM image of nanowhiskers from bamboo fiber

Gopalan et al. [79] have extracted nano chitin whiskers from crab shell. Figure 11 shows a transmission electron

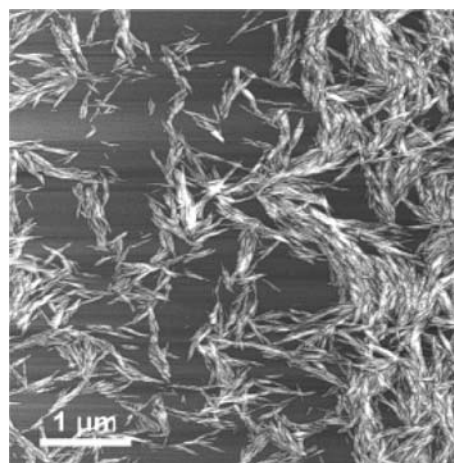


Fig. 9 AFM topography image of cellulose whiskers

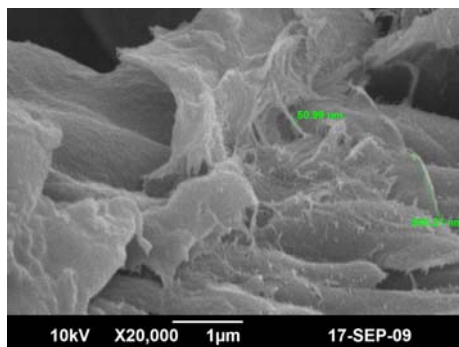


Fig. 10 SEM image of cellulose nanowhisker from bamboo fiber

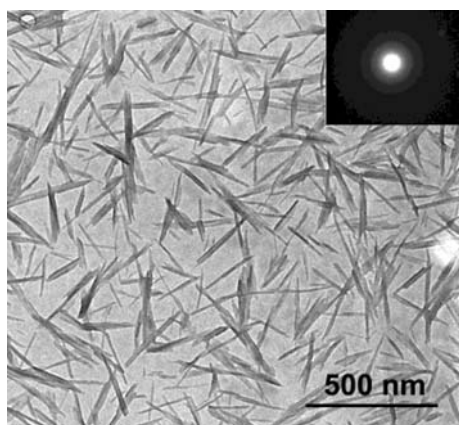


Fig. 11 TEM image of chitin whiskers from crab shell

micrograph of a dilute suspension of hydrolyzed crab shell chitin. The suspension contains chitin fragments consisting of both individual micro crystals and associated or collapsed micro crystals. These chitin fragments consist of slender rods with sharp points that have a broad distribution in size. Rujiravanit and coworkers [74] also showed the size of chitin whisker from shrimp shells by using TEM. Michel and Dufresne [72] also showed transmission electron micrographs of nano chitin whiskers from squid pen chitin. AFM analysis of the whiskers was found to be a good alternative to TEM without any limitations regarding contrast and resolution. The shape of the whiskers appeared, however, different than that observed in TEM and FESEM.

X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials. Very recently Mathew et al. [80] reported on the crystal studies of chitosan/chitin crystal nanocomposites. The chitosan (a) exhibits a highly amorphous nature with broad and ill-defined signals at $2\theta = 9\text{--}10^\circ$ and $18\text{--}20^\circ$ (Fig. 12). The chitin nanocrystals (b) show a strong peak at $2\theta = 8.8$ and 19° and shoulders at $2\theta = 20$ and 22° , confirming its crystalline structure as

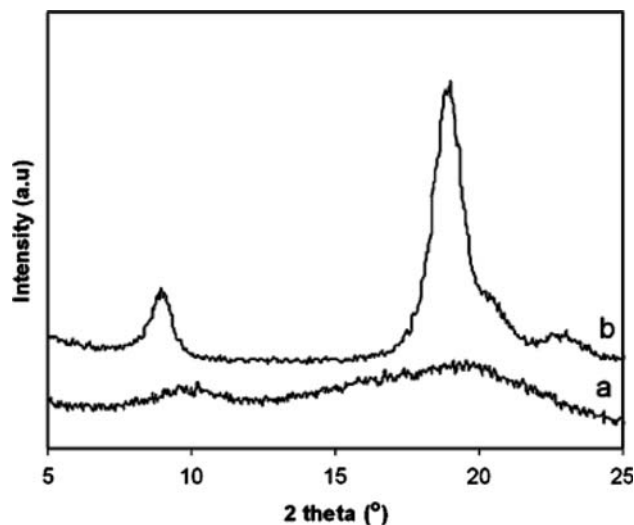


Fig. 12 X-ray analysis data of chitosan/chitin crystal nanocomposites

α -chitin. Oksman [68] performed the WAXD patterns of raw materials such as PLA, PLA_{DMAc}, microcrystalline cellulose and their nanocomposites. They mentioned that PLA exhibits a small peak at $2\theta = 16.4$ and is due to its semicrystalline nature. PLA_{DMAc} also shows a prominent peak at $2\theta = 16.4$ together with other less prominent peaks and exhibits more crystalline nature than pure PLA. Microcrystalline cellulose shows peaks at $2\theta = 15.4, 16.2$ and 22.5 . The peaks were more prominent and sharp for microcrystalline cellulose showing the crystalline nature of this reinforcement. The *d* values associated with $2\theta = 15.4, 16.2$ and 22.5 are 6.14, 5.46 and 3.95 Å which is corresponding to cellulose I polymorph structure. The WAXD pattern of the composites showed peaks at $2\theta = 15.2$ and 22.5 indicating cellulose I structure.

Figure 13 shows that the TEM image of the starch nanowhiskers [76]. Waxy maize starch nanocrystals consist of platelet-like particles with a thickness of 6–8 nm, a length of 40–60 nm, a width of 15–30 nm, and a density, F_s ,

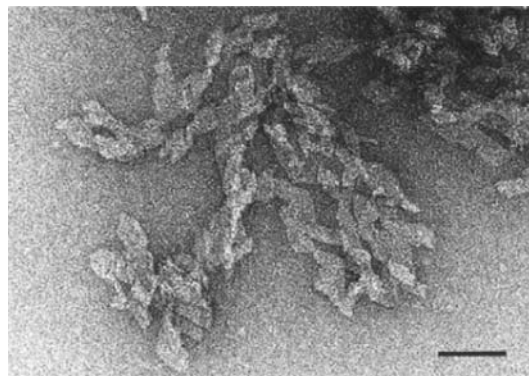


Fig. 13 TEM image of starch nanowhiskers waxy maize starch granules

measured by theory of BET of 1.55 g cm^{-3} , in agreement with the density reported in the literature for the crystalline part of starch. Starch nanocrystals are generally observed in the form of aggregates (Fig. 13) having an average size around $4.4 \mu\text{m}$, as measured by laser granulometry. The specific surface measured by BET was found to be equal to $3.23 \text{ m}^2 \text{ g}^{-1}$, proving that platelets are not individual but also not compact spherical aggregates with a diameter of $4.4 \mu\text{m}$. Angellier et al. [76] have chemically modified the starch nanocrystals. The two grafting agents used were commercial alkenyl succinic anhydride and phenyl isocyanate. The modified particles appeared as a powder after extraction.

Manufacture of Nanocomposites

Cellulose nanowhiskers have been used as reinforcement in many polymer matrixes, such as plastics and rubbers using different methods. Most of the work had been done to understand the influence of processing conditions and the effect of whisker content on the morphology and properties. Hajji et al. [81] prepared nanocomposites of cellulose whiskers using styrene copolymers latex. They used different weight fraction of cellulose ranging from 0 to 6 wt%. Composites were manufactured by using three different processing methods such as (i) casting by direct water evaporation, (ii) freeze drying and hot pressing and (iii) freeze drying, extruding (cylindrical extrudates were randomly dispersed in the mold) and then hot pressing. Favier et al. [63] prepared the nanocomposites of cellulose whiskers and suspensions from the copolymerization of styrene (35% w/w), butyl acrylate (65% w/w), and a small amount of acrylic acid. The suspensions were poured into poly (tetrafluoroethylene) molds and allowed to dry slowly for one month at room temperature. Cellulose based nanocomposites were successfully prepared Grunert and Winter [69] using cellulose acetate butyrate by solution casting method. Samples were prepared containing 0, 2.5, 5.0, 7.5, and 10.0 wt% cellulose crystals. The resulting films were transparent. Oksman and team [68] prepared nanocomposites based on polylactic acid (PLA) and three different types of cellulose reinforcements, microcrystalline cellulose (MCC), cellulose fibers (CFs), and wood flour (WF). They used a twin-screw extruder and injection-molding techniques. They in fact treated micro cellulose with *N,N*-dimethylacetamide (DMAc) containing lithium chloride (LiCl) in order to swell the micro cellulose and partly separate the cellulose whiskers. The suspension of whisker was pumped into the polymer melt during the extrusion process. Samir et al. [82] prepared nanocomposite materials from poly (Oxyethylene) (POE) as the matrix and a stable aqueous suspension of cellulose

nanocrystals extracted from tunicate as the reinforcing phase. Kulpinski [83] reported on the cellulose nanofibers obtained by the electrospinning process from spinning drops containing cellulose dissolved in an *N*-methylmorpholine-*N*-oxide/water system. Under different electrospinning process conditions, a nonwoven fiber network, and a cellulose membrane were obtained. Torres et al. [84] prepared a nanocomposite material formed by BC networks and calcium-deficient hydroxyapatite (HAP) powders.

Morin and Dufresne and prepared [31] nanocomposite materials from a colloidal suspension of high aspect ratio chitin whiskers as the reinforcing phase and poly (caprolactone) as the matrix. The chitin whiskers, prepared by acid hydrolysis of *Riftia* tubes, consisted of slender parallelepiped rods with an aspect ratio close to 120. Films were obtained by both freeze-drying and hot-pressing or casting and evaporating the preparations. Amorphous poly (styrene-*co*-butyl acrylate) latex was also used as a model matrix. In another work [79] nanocomposite materials were obtained from a colloidal suspension of chitin whiskers as the reinforcing phase and latex of both unvulcanized and prevulcanized natural rubber as the matrix. The solid composite films were obtained either by freeze-drying and hot-pressing or by casting and evaporating the preparations. Lu et al. [75] prepared environmentally friendly thermoplastic nanocomposites using a colloidal suspension of chitin whiskers as a filler to reinforce soy protein isolate (SPI) plastics. SPI of desired weight and various content of chitin were mixed and stirred to obtain a homogeneous dispersion. The dispersion was freeze-dried, and 30% glycerol was added. The resulting mixture was hot-pressed at 20 MPa for 10 min at 140°C and then slowly cooled to room temperature. Rujiravanit and coworkers [85] prepared α -Chitin whisker-reinforced poly (vinyl alcohol) (PVA) nanocomposite films by solution-casting technique.

Casting technique leading to the formation of films was used for the preparation of latex based starch nanocomposites [76]. According to the procedure, the aqueous suspension of starch nanocrystals and the NR latex were mixed in various proportions in order to obtain dry films between $200 \mu\text{m}$ and 1 mm thick depending on the test and with weight fractions of dry starch nanocrystals (*w*_s) within the NR matrix ranging from 0 to 50 wt%. After mixing, the mixtures were stored under vacuum and stirred on a rotavapor during about 10 min in order to degasse the mixture and thereby avoid the formation of irreversible bubbles during the water evaporation step. Then, the films were cast in Teflon molds and evaporated at 40°C in a ventilated oven for 6–8 h (depending on the water content) and then heated at 60°C under vacuum for 2 h. Resulting dry films were conditioned at room temperature in desiccators containing P_2O_5 salt until being tested.

Characterization of Nanocomposites

Hajji [81] et al. characterized cellulose whiskers filled poly (styrene-co-butyl acrylate) nanocomposites by DSC, DMA and UTM. The thermo mechanical properties of these nanocomposites have been investigated, and the influence of processing conditions and the effect of whisker content have been considered. The thermo mechanical behavior has been enhanced by increasing the filler content. Differential scanning calorimetry was used to determine T_g (Glass Transition temperature). The T_g of the composites systems had been found to be relatively insensitive to different cellulose content (average value of $T_g = -2^\circ\text{C}$). They have concluded that T_g is nearly independent of both filler content and processing conditions. The modulus increased with increasing cellulose whiskers. Oksman and Bondenson [64] characterized polylactic acid/PVOH cellulose whisker nanocomposites by using SEM, TEM and DMA. TEM analysis showed that the whiskers were better dispersed in the nanocomposite produced with liquid feeding; Analysis of microtomed and fractured samples in FE-SEM showed that PLA and PVOH formed two immiscible phases with a continuous PLA phase and a discontinuous PVOH phase. The thermal stability of the nanocomposites was not improved compared to its unreinforced counterpart, probably because the majority of the whiskers were located in the PVOH phase and only a negligible amount was located in the PLA phase. The small improvements for the nanocomposites in tensile modulus, tensile strength, and elongation to break were noted compared to its unreinforced counterpart. Samir et al. [82] used SEM, polarized optical micrographs for morphological studies of POE based nanocomposites and TGA, DMA, DSC for thermal, mechanical and crystallization behavior respectively. The glass–rubber transition temperature of POE was

not influenced by the cellulosic filler. The melting temperature and degree of crystallinity which were found to decrease for highly filled (10 wt% and above) materials. This restricted crystallinity was confirmed by dynamic cooling crystallization experiments and polarized optical microscopic observations. It was ascribed to both strong interactions between the POE chains and cellulosic surface and increased viscosity of the melt composite. Cellulose/POE interactions were quantified using heat flow microcalorimetry measurements. The mechanical behavior of tunicin whiskers/POE nanocomposites was evaluated in the linear range over a broad temperature range from dynamic mechanical analysis. The main effect of the filler was a thermal stabilization of the storage modulus for the composites above the melting temperature of the POE matrix.

In Fig. 14 Oksman and co-workers [64] presented the DMTA curves of the PLA–PVOHdry and PLA–PVOHwet) and the nanocomposites (PLA–PVOH/Cellulose 5% dry and PLA–PVOH/Cellulose 5% wet). In this figure polymer transitions in the PLA phase and the contribution from the PVOH phase, with or without whiskers, are too small to influence the storage modulus and $\tan\delta$ curve (Fig. 14a and b respectively). Cellulose whiskers are expected to cause a change of the $\tan\delta$ peak towards higher temperature due to restricted chain mobility of PLA at the PLA/Cellulose interphase. Figure shows that the PLA with wet fed additives show a slight lowering of $\tan\delta$ peak temperature. The reason for this behavior is difficult to explain, it can be due to residual water in the system which act as plasticizer or causes a degradation of PLA.

The properties of high performance chitin filled natural rubber nanocomposites were carefully analyzed by Gopalan et al. [28]. It was concluded that the whiskers form a rigid network in the NR matrix which is assumed to be governed by a percolation mechanism. A percolated filler–

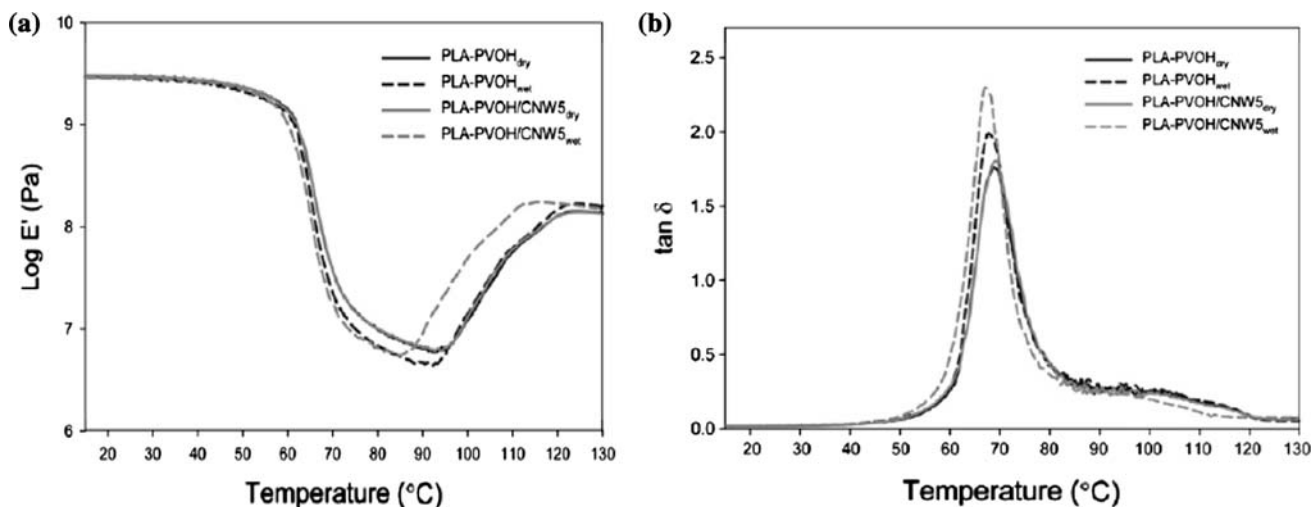


Fig. 14 DMTA (storage modulus and $\tan\delta$) curve cellulose based nanocomposites

filler net work is formed by hydrogen bonding interaction between chitin particles above the percolation threshold. The values of diffusion coefficient, bound rubber content, and relative weight loss also supported the presence of a three-dimensional chitin network within the NR matrix. The mechanical behavior of the composites gives additional insight and evidence for this fact. Rujiravanit and co-workers [85] studied thermal stability of the chitin nanocomposites by TGA. The presence of the whiskers did not affect much the thermal stability and the apparent degree of crystallinity of the chitosan matrix. The tensile strength of α -chitin whisker-reinforced chitosan films increased from that of the pure chitosan film with initial increase in the whisker content to reach a maximum at the whisker content of 2.96 wt% and decreased gradually with further increase in the whisker content, while the percentage of elongation at break decreased from that of the pure chitosan with initial increase in the whisker content and leveled off when the whisker content was greater than or equal to 2.96 wt%. They also studied [85] crystallinity of the PVA/chitin nanocomposites; the presence of the whiskers did not have any effect on the crystallinity of the PVA matrix. They suggested that the cast PVA film was essentially amorphous for the α -chitin whiskers, their WAXD pattern exhibits two major scattering peaks at 2θ angles of about 9 and 19°, respectively for the resulting α -chitin whisker-reinforced PVA films. The WAXD patterns were intermediate to those of the pure components with the strong scattering peaks of α -chitin whiskers (i.e. at about 9 and 19°) being more pronounced with increasing whisker content. To verify whether or not incorporation of α -chitin whiskers into PVA resulted in an increase in the crystallinity of the PVA matrix, FT-IR spectra, were considered. The peak at 1144 cm^{-1} (C–O of doubly H-bonded OH in crystalline regions) was useful for indication of the crystallinity of PVA. Apparently, the relative intensity of this peak was not found to increase with increasing whisker content, indicating that incorporation of α -chitin whiskers did not have an effect on the crystallinity of the PVA matrix. Lu et al. [75] showed that the chitin filled SPI composites showed an increase in Young's modulus and tensile strength from 26 to 158 MPa and 3.3 to 8.4 MPa with increasing chitin content from 0 to 20 wt%. As the chitin whiskers increase in the SPI matrix, the composites showed greater water-resistance. The improvement in all of the properties of these novel SPI/chitin whisker nanocomposites may be ascribed to three-dimensional networks of intermolecular hydrogen bonding interactions between filler and filler and between filler and SPI matrix.

Rujiravanit [85] and co-workers have determined the TGA thermograms of pure PVA, α -chitin whiskers, and α -chitin whisker-reinforced PVA nanocomposites films having whisker content of 14.8 and 29.6 wt%, respectively. All

of the samples investigated showed initial weight loss at about 60–80°C, due to the loss of moisture upon heating. Figure 15 shows that the moisture content in these samples was almost similar (i.e. about 8%). According to the derivative TGA curves, pure PVA film exhibited a major degradation peak at 274°C while as-prepared α -chitin whiskers showed a major degradation peak at 347°C curve (d). The major degradation peaks for PVA films reinforced with 14.8 and 29.6 wt% α -chitin whiskers were intermediate to those of the pure components, with the thermal stability of the nanocomposite films increased with increasing α -chitin whisker content.

Solid nanocomposite films made from NR latex and starch nanocrystals were characterized using scanning electron microscopy, water and toluene absorption experiments, differential scanning calorimetry, and wide-angle X-ray diffraction analysis by Angellier et al. [76]. In their work the nanocomposite films NR/starch nanocrystals were characterized by X-ray diffraction. The diffraction patterns recorded for a film of pure waxy maize starch nanocrystals obtained by pressing freeze-dried nanocrystals displayed typical peaks. By adding starch nanocrystals into NR, the peaks corresponding to amylose allomorph become stronger and stronger, as expected. This shows that an increase of the starch content results in an increase of the global crystallinity of the composite material. The diffraction patterns of the various NR/starch nanocrystals do not exactly correspond to a simple mixing rule of the diffractograms of the two pure parent components. The barrier properties of the nanocomposites to water vapor and oxygen were also investigated, and the effect of surface chemical modification of starch nanocrystals was studied. By introduction of starch nanocrystals in NR, the swelling by toluene decreased and the swelling by water increased. It was assumed that these phenomena were due to the formation of a starch nanocrystals network through hydrogen linkages between starch nanoparticles clusters

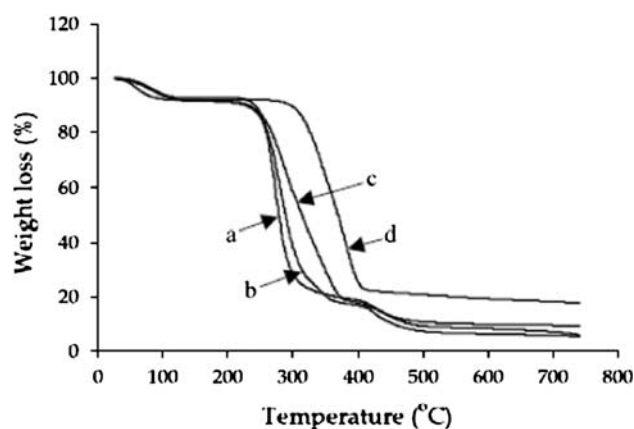


Fig. 15 Thermogravimetric analyses of chitin whisker, PVA and their nanocomposites

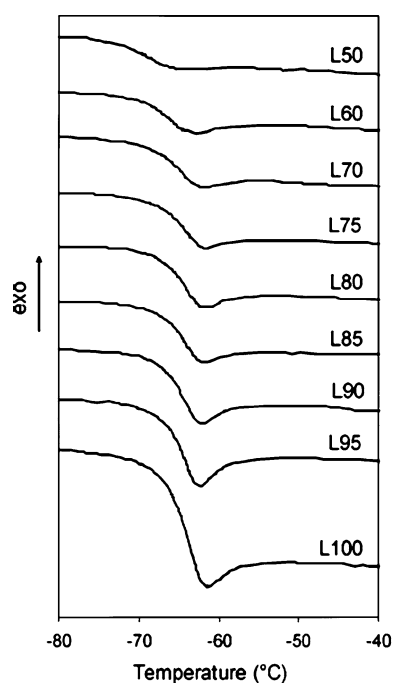


Fig. 16 DSC thermograms of starch nanocrystals/NRnanocomposite films

and also to favorable interactions between the matrix and the filler. As explained earlier, the formation of the network of starch nanocrystals is governed by a percolation mechanism. According to the authors, the critical volume fraction of starch nanocrystals at the percolation should be around 6.7 vol% (i.e., 10 wt%). The platelet-like morphology of starch nanocrystals seems to be responsible for the decrease of both the permeability to water vapor and oxygen of natural rubber filled films.

Angellier et al. [76] repeated on the thermal behaviour of NR/unmodified starch nanocrystals using Differential scanning calorimetry (DSC) measurements. The measurements were performed for all the NR/unmodified starch nanocrystals compositions. For all the samples, two successive temperature scans were recorded and they could be perfectly superimposed and reproducible. The DSC traces corresponding to the first temperature scan are shown in Fig. 16. The glass-rubber transition of unfilled NR occurred at an onset temperature T_{g1} of -66.6°C and was followed by an endothermic peak. By adding starch nanocrystals, the magnitude of the specific heat increment at T_g obviously decreased as well as the endothermic peak. It is simply ascribed to the decreasing amount of NR matrix.

Applications

Bionanocomposites combine plant and animal nanofibers (derived from waste and biomass) with resins and other

polymers like plastics and rubbers to create natural based composite materials. A variety of plant fibers with high tensile strength can be used including kenaf, industrial hemp, flax, jute, sisal, coir etc. Fibers can be combined with traditional resins or newer plant based resins. The result is a plant based alternative for many traditional steel and fiberglass applications. Advantages of bionanocomposites over traditional composites are reduced weight, increased flexibility, greater moldability, reduced cost, sound insulation and renewable nature. For environmental awareness and the international demand for green technology, nanobiocomposites have the potential to replace present petrochemical-based materials. They represent an important element of future waste disposal strategies. In true bionanocomposites, both the reinforcing material such as a natural fiber and the matrix are biodegradable. Cellulose, chitin and starch are the most abundant organic compounds in nature; they are also inexpensive, biodegradable, and renewable. They obviously receive a great attention for non-food applications. The use of natural fibers instead of traditional reinforcement materials, such as glass fibers, carbon, and talc, provides several advantages including low density, low cost, good specific mechanical properties, reduced tool wear, and biodegradability. The important applications include packaging, wide-ranging uses from environment-friendly biodegradable composites to biomedical composites for drug/gene delivery, tissue engineering applications and cosmetic orthodontics. They often mimic the structures of the living materials involved in the process in addition to the strengthening properties of the matrix that was used but still providing biocompatibility, e.g. in creating scaffolds in bone tissue engineering.

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