Enhancement of the Wet Properties of Transparent Chitosan–Acetic-Acid–Salt Films Using Microfibrillated Cellulose

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This report presents a new route to enhance the wet properties of chitosan–acetic-acid–salt films using microfibrillated cellulose (MFC). The enhancement makes it easier to form chitosan–acetic-acid–salt films into various shapes at room temperature in the wet state. Chitosan with MFC was compared with the well-known buffer treatment. It was observed that films containing 5 wt % MFC were visually identical to the buffered/unbuffered films without MFC. Field-emission scanning electron microscopy indicated that MFC formed a network with uniformly distributed fibrils and fibril bundles in the chitosan matrix. The addition of MFC reduced the risk of creases and deformation in the wet state because of a greater wet stiffness. The wet films containing MFC were also extensible. Although the stiffness, strength and extensibility were highest for the buffered films, the wet strength of the MFC-containing unbuffered films was sufficient for wet forming operations. The effects of MFC on the mechanical properties of the dry chitosan films were small or absent. It was concluded that the addition of MFC is an acceptable alternative to buffering for shaping chitosan films/products in the wet state. The advantages are that the "extra" processing step associated with buffering is unnecessary and that the film matrix remains more water-soluble.

Introduction

Chitosan is a modification of the carbohydrate chitin, a component in the shells of crustaceans. One of the major reasons it is an interesting packaging material is that it has good gas barrier properties, which are in the same range as those of ethylene vinyl alcohol (EVOH) under dry conditions. Another typical feature is that films of acetic-acid-protonized chitosan (Chitosan A) become very flexible when exposed to water.[1] This opens up the possibility of forming a rigid chitosan film into various shapes in its wet state at room temperature. For this to work properly, two problems have to be solved: (1) when exposed to water, the film creases and adjacent surfaces adhere to each other, making it very difficult to retain a flat film and (2) the water-plasticized film has a very low strength, which prevents shaping involving large strains. A possible solution to these problems is the use of a fiber reinforcement (e.g., based on cellulose pulp) to enhance the wet stiffness and wet strength of chitosan.

The beneficial ionic interactions between chitosan, a cationic polyelectrolyte, and anionic cellulose are well-known.[2−8] Li et al.[9] found that chitosan was almost completely adsorbed onto the surface of cellulose fibers, and that this adsorption increased with an increasing degree of deacetylation. Mucha and Miskiewicz[10] found that chitosan, in blends with poly(vinyl alcohol) (PVAl) and gelatinized starch as fillers for papers, increased the strength by way of a strong ionic interaction. Chitosan has been considered a wet-end additive in paper-making systems for increasing the wet strength.[11] Chitosan additives have also been found to increase the strength of wet towels, grocery bags, and disposable diapers, and to increase the efficiency of the wood fiber beating process.[12] Thus the wet strength of cellulose pulp fibers has been increased by the addition of chitosan. The opposite would probably also work, that is, a small amount of pulp fibers might strengthen the chitosan matrix, but the addition of normal fibers will unfortunately reduce the transparency of the chitosan film.[13] This is a drawback in, for example, packaging applications where transparency is required. However, because of its small size, film transparency may be preserved if microfibrillated cellulose (MFC), rather than normal-sized fibers, is used. MFC has previously shown promising results as a reinforcing component in various composites.[14−19] In addition, Hosokawa et al.[20−23] have produced biodegradable MFC films with up to 50% chitosan including, in general, a plasticizer. The method of producing MFC was originally developed by Herrick et al.[24] and Turbak et al.[25] by subjecting cellulose pulps to high-pressure homogenization at an elevated temperature.

A common way to reduce the water sensitivity of the water-soluble chitosan–acetic-acid film is to treat it with an alkali buffer solution, followed by rinsing in water to remove the acetic acid residues.[26−28] This will, as in the case with MFC, increase the wet stiffness and strength and reduce the two problems mentioned above. One obvious drawback of using a buffer instead of MFC, if a high water solubility of the chitosan film is required, is that the film becomes less water-soluble. In addition, for commercial reasons, the buffer operation is undesirable since it is associated with an additional process step and the film has to be dried under a slight pressure in order to

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avoid distortion due to buffer-induced creasing.[13] Nevertheless, buffering provides improved gas and water vapor barrier properties, unaltered transparency, and a high fracture strain.[13,28]

The present investigation explores the possible improvement of the wet strength and stiffness of chitosan films by adding MFC and compares this with the alkaline buffer treatment. The properties of composites of chitosan and microcrystalline cellulose (MCC) have been investigated before, for example, to obtain coatings and gels for medical textiles and wastewater treatment.[29] However, this is, to our knowledge, the first study of the wet-strength and wet-stiffness enhancement of chitosan films using a small amount of MFC. In a parallel study, the mechanical properties of dry chitosan films containing 0−75 wt % MFC were investigated.[30] It was observed that the strength and stiffness did not change significantly with ≤10 wt % MFC. In order to have a suitable combination of optical clarity and strength/stiffness, it was decided to focus on a 5 wt % MFC composite in the present study.

Experimental

Materials. *Chitosan Films.* A 3 mL portion of acetic acid (glacial, p.a., 99.8%, Acros Organics, USA) was mixed, using a magnetic stirrer, with 250 mL distilled water in a glass beaker. Subsequently, 3 g of chitosan (2-amino-2-deoxy-(1-4)-β-D-glucopuranan; degree of deacetylation: 84.7%; viscosity: 200 mPa in 1% acetic acid; relative molar mass: $M_r \approx 400\,000$; received as platelets from Sigma-Aldrich Inc., Sweden) was added, and the solution was mixed with a magnetic stirrer for 4 h. The solution was then mixed in a Waring commercial blender 8011E (model 38BL41, Waring Products, USA) at the highest speed for 4 min. After this, the solution was subjected to magnetic stirring overnight. The next day, the solution was first filtered through a fine-meshed metal net (hole size: 500 μm × 500 μm) to remove the largest undissolved particles and then vacuum degassed for 30 min in order to remove entrapped air bubbles introduced during the mixing. Films were then prepared by pouring 35 g of solution into 14 cm diameter Petri dishes coated on the bottom with Teflon-covered aluminum Bytac foils.

The preparation of MFC, which was mixed with chitosan, is described in detail in the work of Pääkkö et al.[31] and is therefore only summarized here. Bleached sulfite softwood pulp (Domsjö ECO Bright; Domsjö Fabriker AB, Sweden) consisting of 40% pine (*Pinus sylvestris*) and 60% spruce (*Picea abies*) with a hemicellulose content of 13.8% (measured as solubility in 18% NaOH, R18) and a lignin content of 1% (estimated as 0.165 kappa number (SCAN C 1:00)) was used as a source for MFC. The pulp was used in its never-dried form. The cell-wall delamination was carried out by treating the pulp in four separate steps: refining using an Escher-Wyss refiner (Angle Refiner R1L, Escher-Wyss, Switzerland) in order to increase the accessibility of the cell wall to the subsequent enzyme treatment, an enzymatic treatment using monocomponent endoglucanase, a second refining stage, and finally allowing the pulp slurry to pass through a high-pressure fluidizer (Microfluidizer M-110EH, Microfluidics Corp., USA). A microbiocide, 5-chloro-2-ethyl-4-isothiazolin-3-one (Nalco AB, Sweden), was used in order to prevent microbial growth. The MFC suspension had a solids content of 2 wt %.

The MFC-containing films (hereafter designated Chitosan AMFC) were produced by first mixing the MFC with 30 mL of distilled water, which was taken from the 250 mL of distilled water intended for the dissolution of chitosan. The MFC was subsequently sonicated for 4 min using a Sonic's Vibra-Cell (Sonics & Materials, Inc., USA) and then poured into the filtered chitosan solution in an amount that gave 5 wt % dry MFC in the final films. The films without and with MFC were both flexible and easily handled.

As a comparison to the MFC composites, a chitosan film with 5 wt % "normal-sized" cellulose fibers (softwood kraft pulp) was also prepared: the chitosan solution was blended with the pulp fiber suspension, with a dry content of 35.4 g/dm^3, and subsequently poured into Bytac-coated petri dishes and dried at 23 °C and 50% relative humidity (RH).[13]

Buffer Treatment of Chitosan Films. Chitosan A and Chitosan AMFC films were treated in a Tris HCl buffer containing 24.22 g of tris(hydroxymethyl) aminomethane (purity 99.8%, Scharlau Chemie SA, Spain) dissolved in 100 mL of distilled water. When the dispersion was homogeneous, 12 mL of 0.1 M HCl (Acros Organics, USA) and additional distilled water were added to yield a 200 mL solution and a final pH of 7.6. The films were stored in Petri dishes filled with Tris HCl for 1 h before being carefully cleaned in distilled water for 5 min. The samples were subsequently stored under a slight pressure between paper towels (SAGA, C-fold Handtowel, MetsäTissue, Sweden) to dry into flat films. The buffered films, with and without MFC, are referred to as Chitosan BMFC and Chitosan B, respectively. The thickness of the resulting films was 20−50 μm as measured with, for example, a Mitutoyo 10C-1128 micrometer (Mitutoyo Scandinavia AB, Sweden).

Methods. *Tensile Testing.* The tensile testing was performed at 23 °C using a Zwick Z010 tensile tester (500 N load cell), controlled by testXpert 7.1 software, (Zwick GmbH & Co, Germany). The softest material (wet Chitosan A) and wet Chitosan AMFC were tensile tested using an Instron 5566 tensile tester (100 N load cell). Dumbbell-shaped specimens, with a narrow-section width and length of 4 and 16−20 mm, respectively, were used. The crosshead speed was 10 mm/min. Between 2 and 10 replicates were measured for each sample. The dry films were conditioned at room temperature in a silica-gel containing dessicator (ca. 15% RH) for at least 2 days, and the samples tested under wet conditions were subsequently immersed in water (23 °C) before being inserted into the clamps and tensile tested. Samples that were not subjected to the wet conditions are hereafter referred to as dry samples/films/materials. The buffered films were immersed for at least 20 min, and the unbuffered films were immersed for at least 10 s. The reason for this difference was the high water-sensitivity of the MFC-free unbuffered film. Because of the necessity to mount the specimen rapidly in the tensile tester and because of the weakness of the wet Chitosan A film, it was impossible to use extensometers, and the extension was therefore measured as the distance between the clamps. This may introduce some scatter in the calculation of the modulus and the elongation at break for the stiff dry films, but it does not affect the strength evaluations. Where needed, the sample data was compared pairwise with respect to the 95% confidence interval ($p = 0.05$), evaluated with the "Compare means, each pair Student's *t*" test using the JMP program (SAS Institute, Inc., USA). This method is referred to as Student's *t* below.

Field-Emission Scanning Electron Microscopy (FE-SEM). The fibril distribution was examined with a Hitachi S-4300 field-emission scanning electron microscope (Hitachi High Technologies America, Inc., USA). The samples were coated with gold using a high-resolution sputter coater (Agar, model 208RH, UK), for 60 s. Fiber/fibril lengths and thicknesses were measured on micrographs using the ClickGraph (Arizona Software, USA) or the Image J (National Institutes of Health, USA) software.

Optical Microscopy. Optical microscopy was performed using a Leitz Ortholux II POL-BK optical microscope, equipped with a Mettler hot stage FP 82HT and a camera, Leica DC300, controlled by a Leica IM50 v.1.20 computer program (Leica Microsystems AG, Germany). The fiber/fibril lengths and thicknesses were measured on micrographs with the same software used on the FE-SEM micrographs.

Ultraviolet−Visible (UV−Vis) Spectroscopy. UV−Vis spectra were obtained using a WPA lightwave UV/Vis diode array spectrophotometer (WPA, UK), which measures the UV−Vis absorption in the range from 200 to 825 nm.

Thermogravimetry (TGA). TGA was carried out using a Mettler-Toledo thermobalance (TGA/SDTA 851^e, Switzerland) at a 10 °C/min heating rate between 30 and 800 °C in N_2 on 3.15 ± 0.1 mg samples. The moisture content of dry samples was taken as the mass loss between 30 and 130 °C.

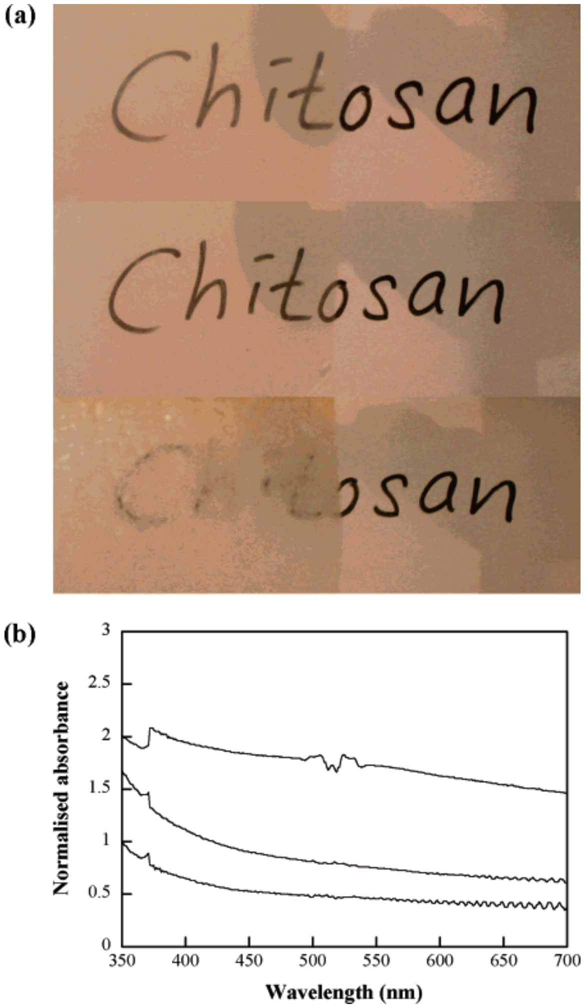

Figure 1. (a) Cast films of Chitosan A (top) and Chitosan AMFC (middle). As a comparison, a sample containing "normal-sized" cellulose fibers is shown (bottom). The films cover the left part of the text. Films were placed 17 cm above the text. The height of the "t" in "chitosan" is 2.5 cm. (b) UV−Vis spectra for the films shown in panel a: sample with normal sized fibers (top), Chitosan AMFC (middle), and Chitosan A (bottom). The spectra were normalized with respect to the film thickness and the absorbance of the Chitosan A film at 350 nm.

Results and Discussion

Figure 1a shows the visual similarities of chitosan films with and without MFC. The transparency was as good with MFC as without. In addition, the evenness in transparency over the film surface suggested that MFC, at least on a macroscopic level, was well dispersed. The buffered films (not shown) were visually the same as the unbuffered films. In fact, without labeling the films, it was impossible to distinguish between the buffered and unbuffered films, with or without MFC. UV−Vis spectroscopy indicated some differences among the samples: the sample with normal-sized cellulose fibers showed the highest absorption, and the pure Chitosan A film showed the lowest absorption (Figure 1b).

The length distribution of the MFC entities observed in the light transmission micrographs, mostly fibril bundles and fiber fragments, was broad; the average length was 35 (±29) μm, and the average thickness was 1 (±0.2) μm (Figure 2). Thus the aspect ratio of the optically observed entities was ~35. Entities with lengths up to 100−200 μm existed. The fibril/fiber entities were uniformly distributed, and they were apparently too small and too few to contribute to any significant reduction in transparency.

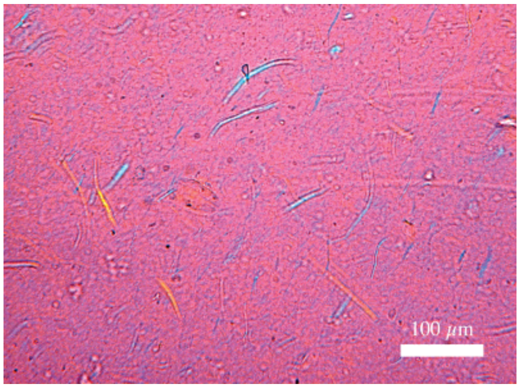

Figure 2. Optical micrograph of a chitosan film containing 5 wt % MFC.

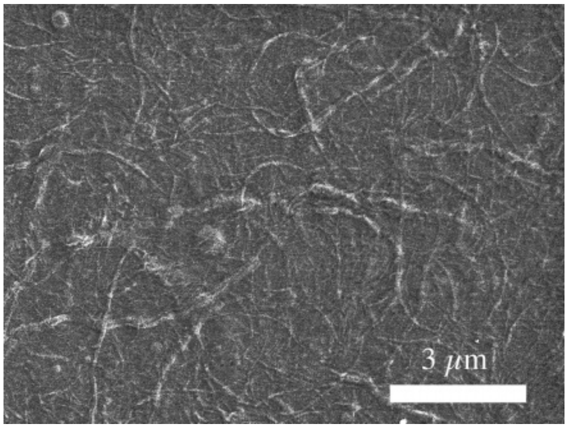

Figure 3. FE-SEM micrograph showing the Chitosan AMFC film surface.

FE-SEM revealed a finer structure (Figure 3): fibrils and fibril bundles (thicker entities) were evenly distributed and formed a percolated/interconnected network. It was assumed that this structure was representative also for the material below the surface. Percolation here refers to the idea that adjacent fibril/fibril bundles were in "contact" with each other at some point and that this led to a continuous network of fibril/fibril bundles in the matrix. Examination of the FE-SEM micrographs showed that the fibril/fibril-bundle length distribution was broad, with an average value of ~2600 (±1000) nm. The average thickness was ~56 (±20) nm, yielding an average aspect ratio of ~46. The calculated aspect ratio, as well as the average fibril length, was somewhat underestimated since the longest fibrils did not fit into a single micrograph. The thickness of the fibrils was similar to that measured on pure MFC.[31] To summarize, light transmission and FE-SEM microscopy revealed fibril/fibril-bundle and fiber fragment lengths that were on the order of 1−200 μm with aspect ratios, slightly underestimated, on the order of 35−45.

Figure 4 shows the effect of exposing a Chitosan AMFC film and a Chitosan A film to water. The Chitosan A film creased into a weak body (hydrogel) that fell apart if any attempt was made to flatten it out. The Chitosan AMFC film, on the other hand, creased significantly less, was stronger, and resisted extensive flexural deformation. Apparently, the fibrils restrict the deformation associated with exposure to water. MFC had the same beneficial effect as buffering. Although the main effect was probably the reinforcement with MFC, it cannot be ruled out that there could be some kind of chemical reaction between MFC, chitosan, and/or acetate groups, leading to, for example, some loss of acetic acid and consequently this beneficial effect.

Figure 4. Chitosan A (right) and Chitosan AMFC (left) films immersed in water for 10 s and left on a paper support.

Table 1. Young's Modulus, Stress at Break, and Strain at Break Together with the Standard Deviations for the dry and wet Chitosan Films

sample[a]	dry	wet	ratio[b]
		strength [MPa]	
Chitosan A	38 ± 5	5 ± 3	8
Chitosan AMFC	59 ± 9	14 ± 4	4
Chitosan B	88 ± 16	13 ± 6	7
Chitosan BMFC	89 ± 14	19 ± 8	5
		modulus [MPa]	
Chitosan A	3530 ± 500	13 ± 1	270
Chitosan AMFC	3790 ± 270	105 ± 14	36
Chitosan B	4790 ± 730	132 ± 64	36
Chitosan BMFC	4310 ± 610	153 ± 87	28
		strain at break [%]	
Chitosan A	4 ± 1	31 ± 10	8
Chitosan AMFC	4 ± 2	15 ± 5	4
Chitosan B	6 ± 2	234 ± 85	39
Chitosan BMFC	4 ± 1	146 ± 53	36

[a] A = Film produced from acetic-acid aqueous solution, B = buffered film, MFC = film with MFC. [b] Ratio between dry and wet films (strength and modulus) and wet and dry films (strain at break).

Table 1 presents the tensile data. When MFC was added to unbuffered chitosan, the dry strength increased by a factor of 1.5 (from 38 to 59 MPa), and the wet strength increased, although within the 95% confidence interval, by a factor of 2.8 (from 5 to 14). Buffering the film yielded a dry strength higher than and wet strength similar to that achieved by adding MFC (cf. Chitosan AMFC and Chitosan B). The combination of MFC and buffer treatment did not significantly enhance the dry strength or the wet strength beyond what was already achieved by using only the buffer.

Although the difference in stiffness between the dry Chitosan A and Chitosan AMFC films was insignificant, the stiffness in the wet state of the latter was approximately 8 times higher than that of the former! This difference was outside the 95% confidence interval. This explains the observed small creasing of the Chitosan AMFC film in water. The buffer treatment increased the stiffness of the dry film significantly (Student's *t*) (cf. Chitosan A and Chitosan B). The combination of both MFC and buffer treatment did not lead to any significant additional increase in stiffness in the dry state compared to what was achieved with the buffer alone.

The fracture strain was similar for all the dry films. Although it seemed as if the strain at break for the wet film decreased somewhat with the addition of MFC, the difference was within the 95% confidence interval (Student's *t*). However it was still significantly higher than that of the dry films (cf. Chitosan A and Chitosan AMFC). The fracture strain rose significantly, by almost an order of magnitude, when the films were buffered (Table 1).

It is noteworthy that the addition of MFC to the films did not increase systematically the standard deviation of the mechanical data, indicating that a homogeneous fibril distribution was achieved in the film. The generally small change in mechanical properties upon the addition of 5 wt % MFC to the dry films is not clear, but has been observed elsewhere.[30] It is also known that the improvement associated with using cellulose fibers in a glassy matrix is modest. In fact, the modulus of a PVAl film containing 5 wt % cellulose fibrils was not significantly different from that of a pure PVAl film.[32]

Among the most interesting effects, when 5 wt % MFC was added, were that (1) the wet strength increased from 12 to 25% of the dry strength (although within the 95% confidence interval) and (2) the wet modulus increased from 0.4 to 3% of the dry modulus. The wet strength in the presence of MFC was higher than 10 MPa, and the wet modulus was on the order of 100 MPa, values that were sufficient for forming chitosan products in the wet state. Overall, the role of MFC was more important in the presence of a "weak" wet chitosan matrix than in a strong dry matrix.

Figure 5a−c shows the fracture surfaces of tensile-tested dry samples with and without MFC. The presence of MFC yielded a fracture surface with a "layered structure" appearance, the layers being oriented along the film surface. This was probably because the fibrils were oriented in the plane of the film as a result of the casting process. A closer look at the fracture surface revealed a large amount of uniformly distributed short (<1 μm) fibril sections sticking out, yielding a warty/hairy appearance (Figure 5b). Only a few long (≈1−2 μm) fibril sections were sticking out (Figure 5a). The fracture surface of the chitosan sample without MFC (Figure 5c) was very grainy and apparently different from the MFC−chitosan fracture surface. Although somewhat smoother, the fracture surface of the wet MFC-containing sample was similar to that of the dry sample (cf. panels a and d of Figure 5). The fracture surface of the wet sample without MFC was smoother and more featureless than any of the other fracture surfaces (Figure 5e). It should be noted

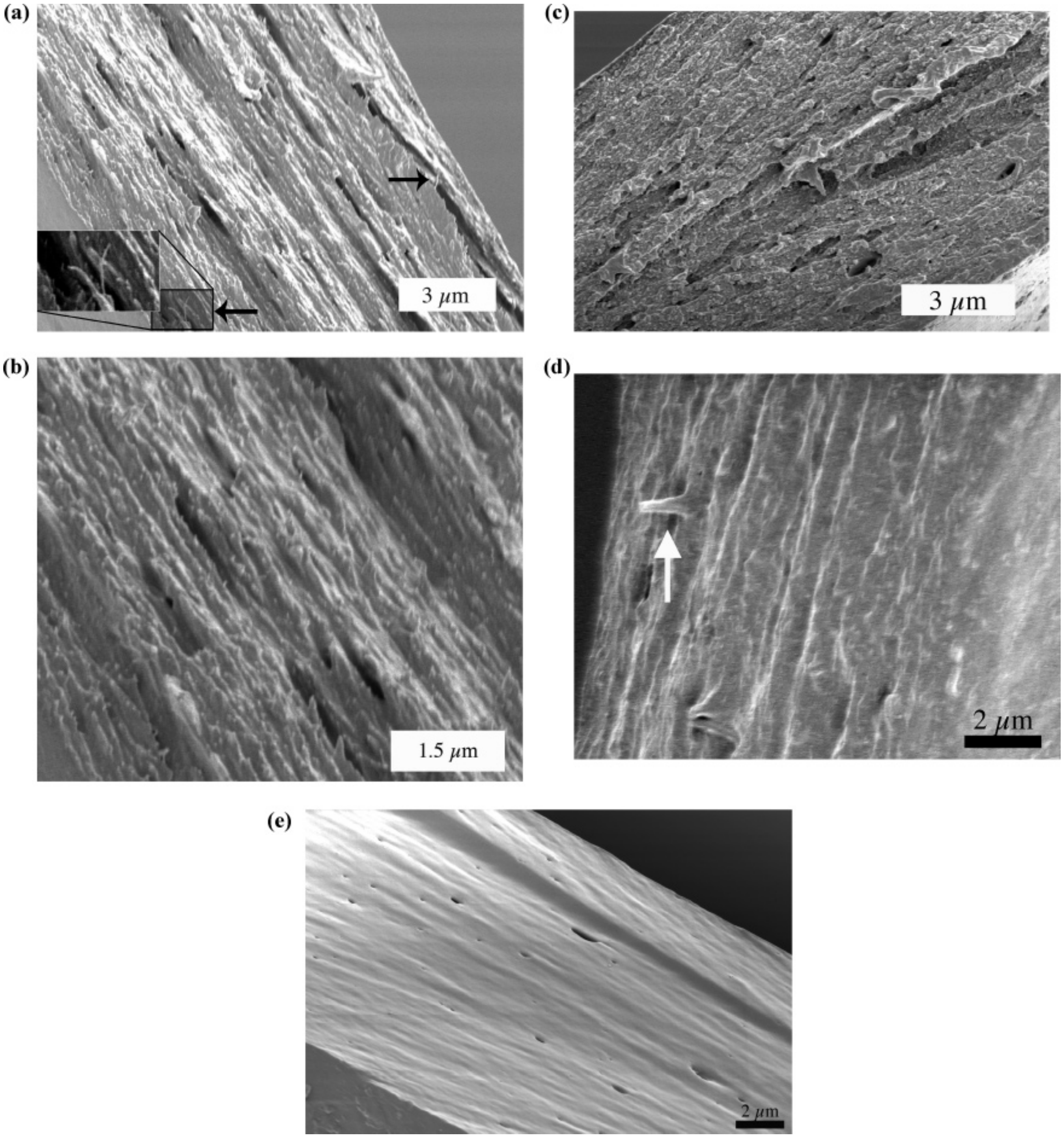

Figure 5. FE-SEM micrographs showing fracture surfaces of tensile tested dry films: (a) Chitosan AMFC, (b) magnified section of panel a, and (c) Chitosan A. Panels d and e correspond to the fracture surfaces of Chitosan AMFC and Chitosan A, respectively, drawn apart by hand in the wet state. The arrows point to fibrils/fibril-bundles sticking out.

that the holes observed here and in all fracture surfaces were probably a consequence of trapped air bubbles, pulled-out undissolved particles and/or moisture evaporation during the film drying and the sputtering operation. From Figure 5a,b,d it is concluded that, as the fracture propagated, most fibrils were broken along the fracture plane or slightly above, involving only a small degree of fibril/matrix delamination. This indicated strong bonding and good wettability/compatibility between the fibrils and the matrix. The smooth transition between the fibril and the matrix at the base of the fibril/fibril-bundle sticking out in Figure 5d (highlighted by the arrow) supports the idea of strong bonding and good wettability.

Consequently, the limited degree of fibril/matrix delamination could probably not be the sole reason for the small impact of the fibrils on the stiffness and strength of dry chitosan. The fibrils were mixed into chitosan in its never-dried state, and the composite films were never dried completely in, for example, a vacuum oven. Hence, remaining moisture in the fibrils of the dry material probably kept these in a plasticized state. It was observed in a previous study that MFC has the ability to retain "easily evaporating" moisture, that is, moisture leaving the sample below 130 °C as measured by TGA, in a MFC−amylopectin composite.[19] TGA on the dry chitosan films did not indicate any loss in moisture content when MFC was added. It seemed, therefore, that MFC contributed with enough moisture to keep the total moisture content constant in the composite. The average content of "easily evaporating" moisture with (Chitosan AMFC) and without MFC (Chitosan A) was 2.5-(±0.5)%, measured as the total mass loss below 130 °C. The moduli of cellulose crystals are estimated to be on the order of 130−140 GPa.[18] The MFC must have a significantly lower modulus since it is far from being a 100% crystalline material.[31] To conclude, there were several possible reasons for the small mechanical effects observed for the dry samples when MFC was added to the chitosan matrix: (1) fibrils were not purely crystalline, (2) fibrils were plasticized, (3) fibrils were randomly, rather than uniaxially, oriented in the film plane, and (4) fibrils delaminated to a small extent from the matrix during deformation. On the other hand, the observed reinforcing effect of MFC in the wet film must have been due to the fact that the moistureinduced plasticization of the chitosan matrix was significantly greater than that for the MFC component.[33] The modulus of the wet Chitosan A film was less than 1% of its dry value (Table 1) which, in the absence of wet MFC modulus data, can be compared with the modulus of wet isolated compression-wood spruce fibers, which was approximately one-third of the dry modulus.[34]

Conclusions

Transparent chitosan films that could be shaped in the wet state were obtained by the incorporation of MFC or by a buffer treatment. The uniform film transparency indicated that MFC did not scatter light to any significant degree because the thin fibril/fibril bundles were well dispersed. FE-SEM studies indicated that the fibril aggregates were evenly distributed on a finer scale. The combination of MFC and buffer treatment did not yield any significant increase in strength and stiffness compared to using only a buffer. The fracture strain in the dry state was independent of the addition of MFC or of a buffer treatment. Fractography indicated strong bonding and good wetting between the matrix and the fibril. In the wet state, the buffered films were significantly more extensible than the unbuffered films. The films with MFC remained extensible.

The mechanical data indicated that the MFC played a more important role in a “weak” wet chitosan matrix than in a strong dry matrix.

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