X-Ray Diffraction as a Measurement Tool for Biodegradability of Cellulose Nanocrystals

Abstract. *Parameters of X-ray diffraction (XRD) patterns of cellulose micro-crystals (CMC) and cellulose nanocrystals (CNC) exposed to artificial lung fluid for up to seven days are used to determine their biodegradability. It is observed that on exposure to lung fluid, both Segal crystallinity and crystallite size for CNC systematically decrease with increase in exposure time, whereas CMCs are largely unaffected. Other effects observed are decreases in the intensities of certain XRD spectra lines with exposure time for CNC. These observations establish XRD as a valuable tool for determining biodegradability of cellulose CNC.*

Keywords. Cellulose, nanocrystals, diffraction, crystallite, crystallinity, measurement.

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Introduction. Cellulose is a biopolymer made of β-1-4-linked D-glucose units with neighboring units cork-screwed 180° and inter-chain coupling provided through a hydrogen-bonded network [1,2]. Differences in the hydrogen-bonding network lead to two different unit cells: I_α cellulose fibrils from less mature sources (algae, bacteria) that crystallize in the triclinic unit cell, and the more stable I_β form, with a monoclinic unit cell, that exists in plant-based sources. Because of the crystalline nature of cellulose, X-ray diffraction (XRD) is one of the important techniques for characterizing cellulosic materials. Defects in the cellulose chains often result from distortion of chains in the microfibrils, which affects cellulose crystallinity. These distortions break crystalline symmetry and produce the amorphous component of cellulose. Depending on the procedures used for extracting cellulose from bio-sources, cellulose micro-crystals (CMC), cellulose nanocrystals (CNC), and cellulose nanofibers (CNF) are often distinguished in the literature [1,2]. The widths and lengths of commercial CMCs are about 10 by 50 μm,

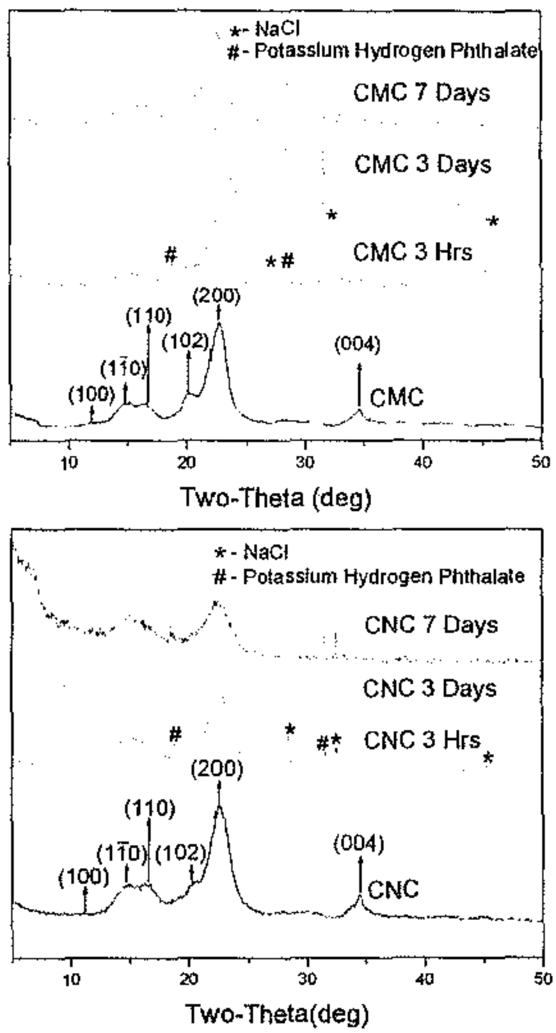

Figure 1. Comparison of XRD patterns of CMC (top) and CNC (bottom) samples exposed to artificial lung fluid for 3 hours, 3 days, and 7 days vis-à-vis the non-exposed samples. The (hkl) values are the calculated Miller indices for the monoclinic Iβ structure of cellulose.

whereas widths and lengths of CNCs and CNFs are about 5 by 100 nm. Commercial CMCs with their inert characteristics have found numerous uses in consumer products.

Because of their polymeric crystalline nature, cellulose nanomaterials possess high strength and directional rigidity, high surface area, high aspect ratio, low density, and good thermal stability. As such, they are increasingly receiving attention as a potential building block for developing non-petroleum based materials with low environmental impact. As with any new material, cellulose nanomaterials must be evaluated to ensure their safety for workers and the public. High-aspect-ratio nanoparticles such as CNC are of special interest because of previous experiences with lung injury from exposure to mineral fibers. One factor influencing fiber toxicity is persistence in the lung (biopersistence). To understand biopersistence, a sufficiently sensitive metric of material stability is required; however, for nanomaterials, including CNCs, the choice of an appropriate metric is not always clear. Crystallinity is considered an important property of nanomaterials [3]. Here, we present data to support the utility of crystallite size and the relative intensities of certain lines as well as crystallinity as sensitive metrics of CNC biopersistence.

Methodology. In this study, CNC materials were evaluated along with a CMC material (used as a benchmark). Each cellulose material was suspended (1.5 mg/mL) in artificial lung airway epithelial lining fluid (ALF) with pH 7.4. At three hours, three days, and seven days, the suspensions were centrifuged and the cellulose pellet-dried and mounted on a silicon plate. X-ray diffraction was used to determine material properties; analytical parameters were: CuKα source (λ=1.54185Å), 2Θ range 5° to 50° (where most of the strong lines from cellulose are expected), step size of 0.06°, and count time of 5 sec per step. To determine the crystallinity (XCR) of cellulose, the Segal method was used, which defines XCR in terms of the peak height I_{200} of the (200) Bragg line near 2Θ = 22.5° and amorphous component I_{am} near 2Θ = 18.5° (Fig. 1). The crystallite size D of cellulose was determined using the full-width at half-maximum B (in radians) of the strong (200) line and the Scherrer relation $D = 0.9\ \lambda / (B \cos\Theta)$. Line intensities of the (004) and (102) lines were also compared qualitatively. It is now known that magnitudes of X_{CR} determined by the Segal method are systematically overestimated by about 10% [4,5], although the simplicity of the method is very convenient and overall conclusions drawn from sample-to-sample comparison from such an analysis are not affected.

Results. The XRD patterns of the non-exposed CMC and CNC samples and those of the same samples exposed to ALF are shown in Fig. 1. For the non-exposed samples, the numbers (hkl) shown on the XRD patterns are the Miller indices of the lines based on the monoclinic structure of I_β cellulose. For the samples exposed to lung fluid, sharp lines marked with * and # are respectively due to NaCl and potassium hydrogen phthalate, which are components of the lung fluid. Despite washing, some of these salts remained in the samples. Nevertheless, these lines do not interfere with the lines due to cellulose, enabling definite conclusions to be drawn as discussed below.

For the CMC samples, all the original lines observed in the non-exposed sample were also observed in the three samples exposed to ALF. In particular, the weaker lines (102) and (004) can still be observed in the exposed samples. In contrast, for the CNC samples, the weaker (102) line, although present in the non-exposed sample, is absent in the samples exposed to ALF. Moreover, the intensity of the (004) line continues to decrease with increase in exposure time. Using the procedures outlined earlier for the calculations of X_{CR} and D, magnitudes determined from the strongest (200) line for the non-exposed samples and the samples exposed for 3 h, 3 days, and 7 days respectively are as follows: for CMC, X_{CR}(%) = 91, 94, 93, and 90; D (nm) = 4.7, 4.8, 4.8, and 4.7; and for CNC, X_{CR}(%) = 88, 90, 83, and 74 and D(nm) = 4.3, 3.9, 4.0, and 3.3. From the above changes in X_{CR} and D with exposure time to ALF, it is evident that CMC are largely unaffected by exposure to lung fluid for up to seven days. On the other hand, the CNC biodegrade because the magnitudes of both X_{CR} and D systematically decrease with increasing exposure time, implying a decrease in cellulose crystallinity.

Conclusions. Results presented here have shown that the values of X_{CR} and D calculated from the XRD patterns of cellulose show degradation of CNC in ALF, thus providing a useful measure of CNC biodegradability. The biodegradability of CNC may transfer into effective lung clearance and low health risk to humans. However, the larger micro-crystals of CMC are generally unaffected when exposed to ALF for up to seven days.

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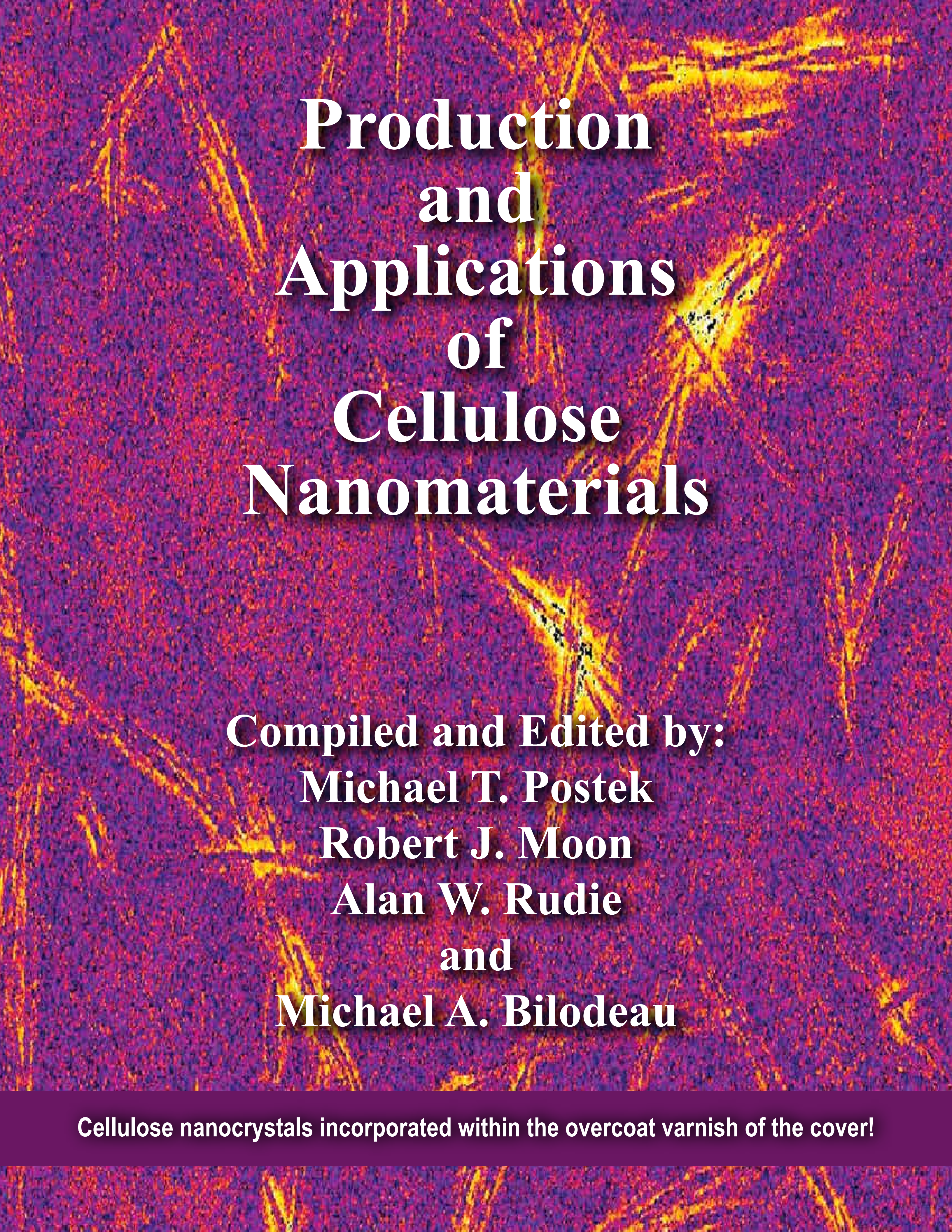

Production and Applications of Cellulose Nanomaterials

Compiled and Edited by:
Michael T. Postek
Robert J. Moon
Alan W. Rudie
and
Michael A. Bilodeau

Cellulose nanocrystals incorporated within the overcoat varnish of the cover!

Cover: Transmission electron microscope (TEM) image of CNCs extracted from microcrystalline cellulose; original micrograph from Moon *et al.*, “Cellulose Nanocrystals—a material with unique properties and many potential applications,” colorized by Michael Postek.

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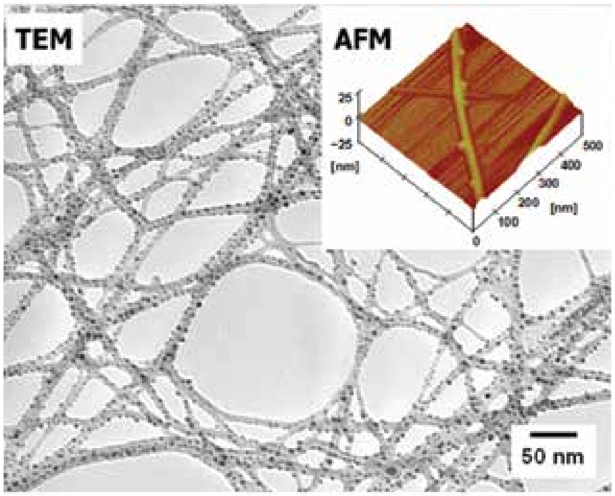

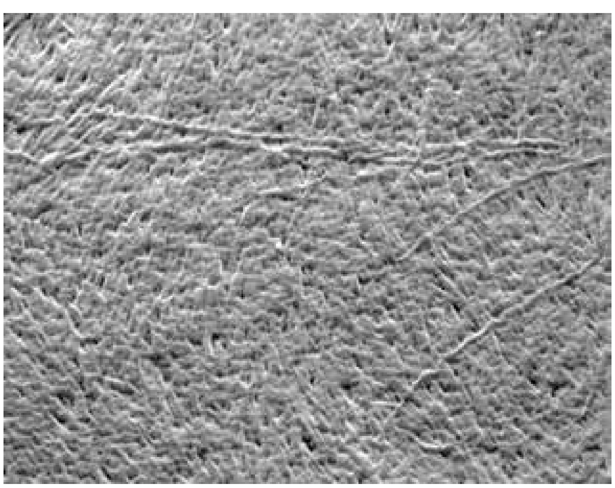

Production and Applications of Cellulose Nanomaterials

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June 2013

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Foreword

"Production and Applications of Cellulosic Nanomaterials" was intended to help organize and highlight the wide range of research being conducted worldwide on the science and technology of cellulose nanomaterials. The format of this book consists of short research summaries, targeted for a level where they can be understood by non-specialists in the research fields, and with a lot of figures and pictures to help convey the science. Although we have tried to be thorough and inclusive in searching out authors, the world is still a big place in the 21st century, and we can guarantee that we have missed a lot of good science. The book has 106 contributions from about 45 institutions and 10 countries. Science on cellulose nanomaterials that is not included in the book is simply the result of limited time and limited resources. We believe there is sufficient on-going science on cellulose nanomaterials to support two or three books of this nature, maybe more, and encourage others to take up that challenge.

The book is organized into two main chapters, based on the two general cellulose nanoparticle types used to date: cellulose nanocrystals (rod-like particle types) and cellulose nanofibrils (fibril-like particle types). Each chapter is itself divided into several main sections: Preparation and Characterization, Health and Safety, Coatings-Films-Optics, and Composites. The chapter on cellulose nanocrystals also contains a section on modeling. This deviates somewhat from the main chapters in that most of the summaries deal with molecular modeling of cellulose crystals, but several papers deal rather with models of composite products and the interface. This latter group tends to be agnostic on material form and in general works well within the CNC chapter, but anyone with interests in composite models should make sure to check the modeling section of the CNC chapter.

Coupled with the irrational exuberance of taking on a book, the editing job is at times exhilarating, at times exasperating, exhausting, and ultimately when complete, rewarding, not in a personal or financial sense but with a real sense of achievement and contribution. Those emotions were increased in all of us when we realized that the initial response to the request for summaries was a book of 300 to 400 pages, approximately twice the initial target. But that response is a testament to the level of interest within the scientific community, and that knowledge helped propel us through that list of E's (exuberance, emotion, exasperation and exhausting). We hope that we have served this community of scientists well. We thank them all for their contributions and for bearing with us as we tried to assemble the book. Now it is for you to decide whether the effort was worthwhile. We hope you enjoy and learn.

This project has been a joint cooperation between the USDA Forest Products Laboratory (FPL), DOC National Institute of Standards and Technology (NIST), the University of Maine, and the Technical Association of the Pulp and Paper Industry (TAPPI). Inspiration for this book came from Michael Postek of NIST, while connections within the community and expertise with cellulose nanomaterials came from Robert Moon and Alan Rudie of FPL and Mike Bilodeau of the University of Maine. Having embraced TAPPI as publisher, the editors also loosely based the original distribution list of the call for summaries on the contributors to the TAPPI International Conference on Nanotechnology for Renewable Materials. In addition, the TAPPI Nanotechnologies Division became the formal book sponsor, and we would like to thank the Chairman, Sean Ireland, and the Division Council for their enthusiastic support.

Cellulosic Nanomaterials: Sustainable Materials of Choice for the 21st Century

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"Production and Applications of Cellulosic Nanomaterials" is intended to bring together current leading-edge knowledge and information on cellulosic nanomaterials from worldwide expert sources. This has been a joint cooperation between the USDA Forest Products Laboratory (FPL), DOC National Institute of Standards and Technology (NIST), the University of Maine, and the Technical Association of the Pulp and Paper Industry (TAPPI). This book is needed because during the past several years, discovery of the properties and performance of these materials has accelerated. However, by comparison with some other nanomaterials, the level of funding and effort has been modest, largely because most scientists and government bodies are unaware of their existence. The scope and breadth of the knowledge and information outlined in this book span the range of cellulosic nanomaterials research, process and product development, and commercial exploitation and include standards development and environmental, health, and safety issues. It is hoped that this book will help spread the knowledge of cellulosic nanomaterials and lead to further efforts in the broader scientific communities.

It is difficult to imagine anything on our planet more ubiquitous and environmentally friendly than plants—they grow using sunlight, carbon dioxide, water, and soil nutrients. What many people may not know is that plants, from the smallest algae cell to the largest redwood tree, contain cellulose. Cellulose is the most abundant polymer on Earth, representing about 1.5 x 10^{12} tons of total annual biomass production [1]. It consists of glucose-glucose linkages arranged in linear chains where C-1 of every glucose unit is bonded to C-4 of the next glucose molecule as shown in Fig. 1 [2,3]. These chains aggregate along the chain direction with intermolecular hydrogen bonds and hydrophobic interactions. They form fibrous structures called nanofibrils 2 to 20 nm wide depending on biological species. These nanofibers make up the structure of all plants as well as some fungi, animals, and bacteria [4]. Because these cellulosic nanodimensional building blocks have crystalline regions, they have unique distinguishing properties. They have strength properties greater than Kevlar®, piezoelectric properties equivalent to quartz, can be manipulated to produce photonic structures, possess self-assembly properties, and are remarkably uniform in size and shape. In addition, because of their abundance, we can sustainably and renewably produce them in quantities of tens of millions of tons.

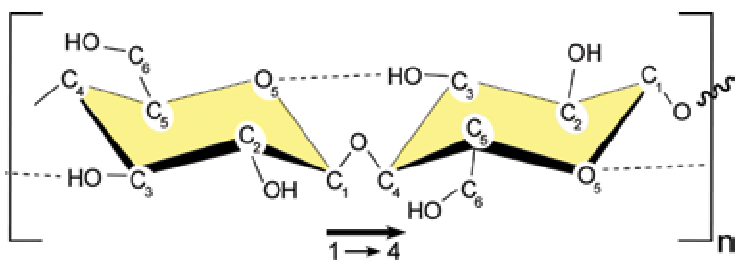

Figure 1. *Cellulose Schematic. Adapted from reference [3].*

Plants have been a major source of raw materials and products for humankind for millennia. For example, products derived from trees, such as wood and paper, have been with us so long and are used so widely in society that they are largely taken for granted as part of traditional industries with no new science to learn. However, the opposite is true. Because of the complex cascading hierarchical structure of wood (Fig. 2), many of the technologies used in the forest products industry were first developed through experience. The complexities of wood are just now yielding to newer and more robust qualitative and quantitative analytical tools. We are beginning to see and track

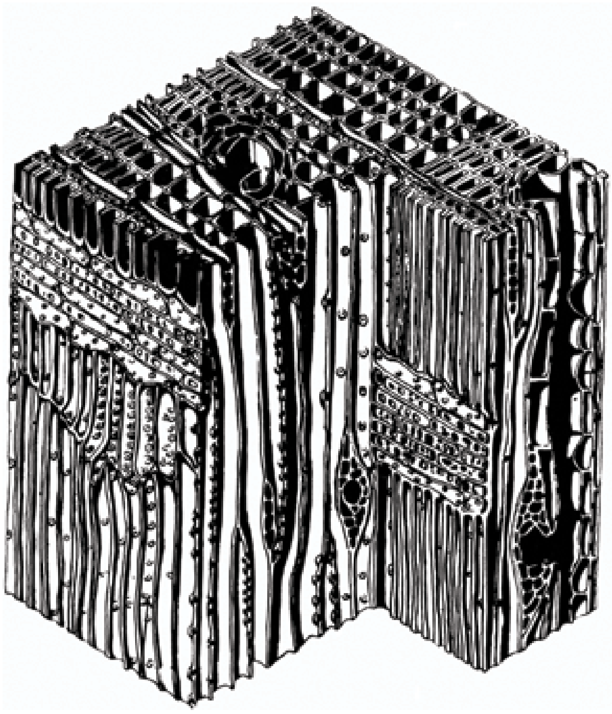

Figure 2. *Wood structure schematic*

how the mechanical, optical, and other physical properties of wood are related to its discrete hierarchical structures ranging from nanoscale to microscale to macroscale. As a result, we are now seeing growing but disjointed efforts worldwide to move research, development and deployment forward to commercialize cellulosic nanomaterials. Several small-scale pilot and pre-prototype facilities have been built to produce working quantities of cellulosic nanomaterials to support research and product applications development. Several different forms of cellulosic nanomaterials are being pursued. One form, cellulose nanocrystals, consists almost exclusively of nanodimensional cellulose crystals. Another form, cellulose nanofibrils, consists of regions of crystalline as well as amorphous cellulose. In addition, production methods that include acid hydrolysis, enzymatic treatments, chemical treatment, and mechanical treatment are being pursued. All this activity and more is leading to exciting but challenging times in the commercial development of cellulosic nanomaterials.

Materials of the 21st Century Revisited

As we move forward in the 21st Century, we are seeing an explosion in demand for materials, energy, food, and water driven by growing world population and the

Cellulose is the most abundant polymer on Earth, representing about 1.5 x 10^{12} tons of total annual biomass production

emergence of large numbers of middle-class consumers in emerging economies wishing to consume at Western-world levels. The supply of material building blocks as we know them today will not be sufficient, and we will have to revisit and look to materials from forest and agricultural-based resources as major sources of materials for products. In addition, for similar reasons, the rapidly increasing demand for higher-quality food types will require enhanced packaging performance to minimize loss of food in the supply chain. Sustainable, renewable cellulose-based nanomaterials have excellent oxygen barrier properties and can fill this need. Concerns about climate change are leading to a resurgence of interest in cellulose due to the increased focus on renewable materials that meet the material needs of society while at the same time sequestering carbon. The use of cellulose-based materials to produce products in a sustainable and ecologically preferable manner is furthered by the need to adhere to the principles of Green Chemistry and Green Engineering [5]. The forest products industry has substantial infrastructure already in place to harvest sustainably grown trees and transport them to centers for debarking, chipping, and pulping. Such a sustainable supply base will enable the rapid scaleup of nanocellulosic materials based on this existing platform.

Nanocellulose as a Green Material

Society requires scientists and manufacturers to focus research on sustainable materials and develop them so they are easy to manufacture, affordable to the consumer, and widely available. A term, “Green”, has been developed not just as a label, but as a new measure of materials, technologies, and products. “Green” generally refers to materials, technologies, and products that have less impact on the environment and/or are less detrimental to human health than traditional equivalents [6]. For example, green products might be produced from sustainable raw materials, be manufactured in a more energy-conservative or environmentally friendly way, pose

"Green" generally refers to materials, technologies, and products that have less impact on the environment and/or are less detrimental to human health than traditional equivalents

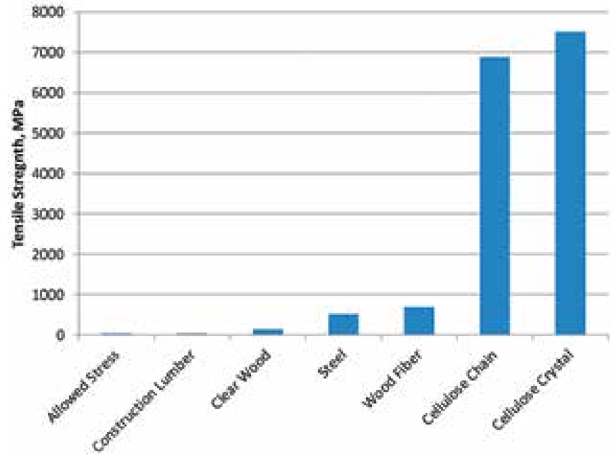

Figure 3. *Tensile strength properties of selected wood-based products and constitutive hierarchical structures versus steel. Note—When using a linear scale, the allowable stress for lumber and the tensile strengths of construction lumber, clear wood, and wood fiber are barely distinguishable on the Y-axis.*

few, if any, health and safety problems, sequester carbon, be recyclable, be compostable, be supplied to the market using less material, or all the preceding. Cellulosic nanomaterials have the capability to meet almost all the requirements for being "green" and with further responsible and thoughtful research, development, and deployment, have the opportunity to become sustainable materials of choice for the 21st Century.

Nanotechnology can also play an important role in the production of liquid biofuels from lignocellulosic biomass. For example, nanoscale cell-wall structures within trees could be manipulated so they are more easily disassembled into their constitutive materials through bio-conversion, thermo-conversion, or catalysis. Another approach would be to use nanocatalysis to break down recalcitrant cellulose. Recalcitrant cellulose is on the order of 15–25 percent of wood, and failure to convert this to sugars reduces bioconversion yields.

Nanocellulose Form and Function

The various forms of nanomaterials that can be produced from cellulose are often collectively referred to as cellulosic nanomaterials or nanocellulose. For example, the extraction of cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC) from plants, bacteria, and some animals (e.g., tunicates) is leading to a wide array of worldwide research to use these nanomaterials in product applications [3, 7–10]. Examples include using CNFs as reinforcing agents in composites due to their high strength properties, relative low cost, and availability, or CNCs due to their incredibly high strength (Fig. 3), renewability, lightweight, high surface area, and unique photonic characteristics.

As you will see when reading this book, research and development is currently taking place worldwide within academia, industry, and government agencies to study, characterize, and use these highly complex cellulosic nanomaterials. Nanocellulose in its various forms contains unique structures and self-assembly features that we can exploit to develop new nano-enabled green products. A specific example is use of cellulosic nanomaterials in lightweight, high-performance composites. Such nanocellulose-enabled composites could eventually replace carbon fiber mats and strands by weaving cellulose-derived nanomaterials and fiber into mats. This could lead to replacement of the nonrenewable and fossil-based materials currently used to make automotive parts such as dashboards, seats, floor mats, and even body panels or frames. The world may not be ready yet to step back into a wooden airplane, but the day will come when aircraft will have wings and fuselage components containing lightweight, high-performance nanocellulose-enabled composites. Fiberglass is a common composite with which most people have experience. It is used to manufacture diverse products including tool handles, sporting goods, bike frames, boats, and even the bodies of some sports cars. Fiberglass cannot be made transparent and is a heavy material for a composite. Replacing fiberglass mat with nanocellulose-containing mat could lead to new lighter-weight materials and the eventual replacement of nonrenewable products with sustainable and renewable cellulosic materials

Another valuable feature of cellulosic nanomaterials is their compatibility with human tissue, as evidenced by a number of research studies focused on their use as a tissue scaffold [10].

The area of nanomanufacturing science and technology has not received sufficient attention despite its being one of the most critical pathways to applying the benefits of nanotechnology. It is absolutely critical to build the nanomanufacturing science and technology base to the point where nanomaterials exhibiting unique nanoscale properties can routinely be placed into components or systems, retaining and combining their unique properties in a matrix of other materials and resulting in superior and controllable composite performance.

Partnering Nationally and Internationally

To scientists, everything meets at the atom. All of us, whether we are scientists, engineers, materials producers, industrial product producers, or consumers, have something to learn from new technological advancements in nanoscale and atomic-scale science. These new advancements cannot come to fruition without focused and responsibly targeted efforts in research, development, and deployment led by government and industry in conjunction with academia. This also requires increased international cooperation due to the worldwide importance of trade, the need to engage collectively the best minds to achieve rapid use of cellulosic nanomaterials for the benefit of humankind, the need to meet the needs of all people sustainably, and the shared responsibility we all have to live within the carrying capacity of our planet Earth.

Nanotechnology and the development of the science and technology for producing and using cellulosic nanomaterials, although promising, are still high-risk and expensive. Cooperation, pooling of resources, and openly sharing of pre-competitive information is critical to moving the science and technology for exploiting cellulose nanomaterials expeditiously forward. In North America, Europe, and Asia, governmental agency partnerships with industry and academia are becoming common. For example, in the United States (U.S.), Federal government emphasis on renewable materials has led to increasing emphasis on these specific materials. The U.S. National Nanotechnology Initiative (NNI), which brings together 25 federal agencies and departments, serves as a natural focal point for government and industry to work collaboratively [11]. Within NNI, the U.S. Department of Agriculture (USDA) Forest Service is the lead federal agency advocating for cellulosic nanomaterials from forest biomass. The forest products industry, through the Agenda 2020 Technology Alliance, has also formed a relationship with the NNI. In addition, the NNI has recently developed a sustainable manufacturing "signature" initiative which includes renewable and sustainable cellulosic nanomaterials [12]. These NNI signature initiatives are aimed at enhancing the commercialization of nanomaterials and nano-enabled products for the benefit of humankind.

....to create new business opportunities using renewable forest resources and the advances made in nanotechnology, especially cellulosic nanomaterials, to develop novel or superior products with enhanced performance attributes

In Canada, ArboraNano has served as the focal point for public-private partnerships [13]. ArboraNano is the Canadian Forest NanoProducts Network, made possible through the Government of Canada's Business-led Centres of Excellence program, FPInnovations, and NanoQuébec. Its mission is to create new business opportunities using renewable forest resources and the advances made in nanotechnology, especially cellulosic nanomaterials, to develop novel or superior products with enhanced performance attributes.

Similarly, in Finland, the Finnish Centre for Nanocellulose Technologies was established as a public-private partnership by the federal VTT Technical Research Center of Finland, Aalto University, and UPM (one of the world's leading forest products groups) [14]. The focus of the Center is to create new applications for nanocellulose as a raw material, substance, and end product.

To translate fundamental knowledge developed by investment in nanotechnology into manufacturing and create jobs, it will be necessary for industry to partner with national laboratories and academia both nationally and internationally. This is a critically important linkage. National laboratories and academia have the expensive infrastructure in place to conduct needed work on nanoparticles as well as the ability to carry out basic research. University faculty and students also bring enormous intellectual capacity to bear in providing innovative solutions and advancing the underlying science. To be effective, the work of academia and national laboratories must be focused and adequately funded. With respect to funding, government often supplies the bulk of the funding for the basic underlying science and technology. Industry often supplies leadership to focus government spending in a manner that leads through research to development and manufacturing for the consumer. Additionally, industry involvement ensures that material development will be in alignment with modern manufacturing processes and workplace and consumer product regulatory requirements. When technically and economically viable pathways to commercialization become clearer the result is that, industry funding increases exponentially and government funding decreases exponentially as the science moves into commercial development and deployment. Industry generally funds commercial deployment, which is very expensive, on the order of 12 to 20 times the cumulative research and development costs.

The Path Forward

In responsibly and efficiently moving cellulosic nanomaterials through research, development, and deployment in partnership, we need to concentrate our efforts in the following five general areas:

- ***Economically viable and environmentally preferable production of the various forms of cellulose nanomaterials***
- ***Characterizing cellulosic nanomaterial morphology and properties***
- ***Exploring new applications for using cellulosic nanomaterials and tailoring them to perform well in those applications***
- ***Elucidating and quantifying EHS (environment, health, and safety) and ELSI (ethical, legal, and social implications) information for responsible use, recycling, and disposal***
- ***Developing national and international codes and standards to support responsible use and trade***

There is ample opportunity for national and international cooperation, sharing resources and avoiding needless duplication of efforts to develop and commercialize uses of cellulose nanomaterials. TAPPI, through its annual International Conference on Sustainable Nanomaterials, has created a welcoming forum to share information and convene like-minded people seeking to advance research, development, and deployment of cellulosic nanomaterials to make them a material of choice for the 21st Century [15].

The uniqueness, abundance, and potential low cost of cellulosic nanomaterials from trees will serve many industrial materials needs. In our immediate future, we can envision automobiles and trucks made with cellulosic nanomaterials, wind turbines producing green power, ships crossing the oceans, and medicines and medical diagnostics. Electronic devices, including photovoltaics, electrical storage devices, and sensors, all will be made with cellulose nanomaterials produced from trees. We hope the vision that we and others share will open your mind to the potential opportunities presented by this new material. Hundreds of millions of dollars are being spent worldwide in a race to discover and patent the capabilities of cellulosic nanomaterials. Small-scale facilities to produce limited quantities of cellulosic nanomaterials have already been built in Asia, North America, and Europe. Governments cannot stand by and leave the rewards to international competitors; academia cannot do research without support; and industry has to step up and work cooperatively with others to exploit these materials. We need to reach out to others, work collaboratively, and share information. The potential benefits of using cellulosic nanomaterials are too great for us to fail to harness them for the benefit of humankind.

Everything meets at the atom—unless you are a physicist, in which case, it all comes down to quarks...

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Chapter 1

Cellulose Nanocrystals – A Material with Unique Properties and Many Potential Applications

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Introduction

Cellulose nanocrystals (CNCs) are cellulose-based nanoparticles that can be extracted by acid hydrolysis from a wide variety of natural source materials (e.g., trees, annual plants, tunicates, algae, bacteria) [1-7]. These rod-like or whisker-shaped particles (Fig. 1, 3–20 nm wide, 50–2000 nm long) have a unique combination of characteristics: high axial stiffness (~150 GPa), high tensile strength (estimated at 7.5 GPa), low coefficient of thermal expansion (~1 ppm/K), thermal stability up to ~300°C, high aspect ratio (10–100), low density (~1.6 g/cm^3), lyotropic liquid crystalline behavior, and shear-thinning rheology in CNC suspensions. The exposed –OH groups on CNC surfaces can be readily modified to achieve different surface properties and have been used to adjust CNC self-assembly and dispersion for a wide range of suspensions and matrix polymers and to control interfacial properties in composites (e.g., CNC-CNC and CNC-matrix). This unique set of characteristics results in new capabilities compared to more traditional cellulose-based particles (wood flakes, pulp fibers, etc.) and the development of new composites that can take advantage of CNCs' enhanced mechanical properties, low defects, high surface area to volume ratio, and engineered surface chemistries. CNCs have been successfully added to a wide variety of natural and synthetic polymers [2] and have been shown to modify composite properties (mechanical, optical, thermal, barrier). Additionally, CNCs are a particularly attractive nanoparticle because they have low environmental, health, and safety risks, are inherently renewable, sustainable, and carbon-neutral like the sources from which they are extracted, and have the potential to be processed in industrial-scale quantities at low costs.

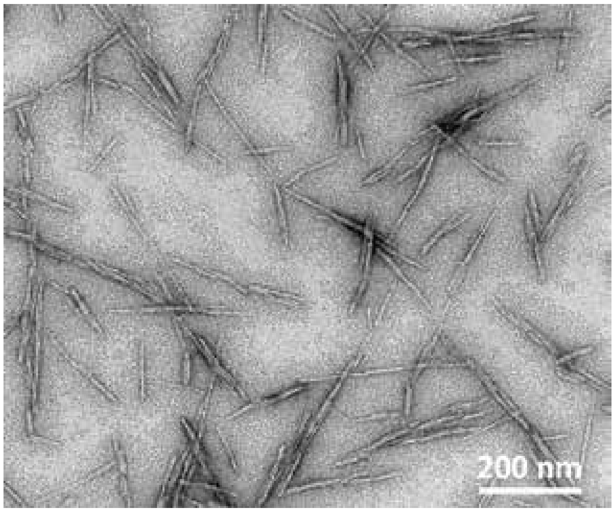

Figure 1. *Transmission electron microscopy (TEM) image of CNCs extracted from microcrystalline cellulose.*

Processing of Cellulose Nanocrystals

Although there are many variants of the process to isolate CNCs from a given cellulose source material, this process generally occurs in two primary stages. The first stage is a purification of the source material (plants, tunicates, algae, bacteria, etc.) to remove most of the non-cellulose components in the biomass. These include lignin, hemicellulose, fats and waxes, proteins, and inorganic contaminants. The second stage uses an acid hydrolysis process to deconstruct the "purified" cellulose material into its crystalline components. This is accomplished by preferentially removing the amorphous regions of the cellulose microfibrils [3,8]. The resulting whisker-like particles (3–20 nm wide, 50–2000 nm long) are ~100% cellulose, are highly crystalline (62%–90%, depending on cellulose source material and measurement method), and have been referred to in the literature as cellulose nanocrystals (CNCs), nanocrystalline cellulose (NCC), and cellulose nanowhiskers (CNW) to name a few. The variations in CNC characteristics (e.g.,

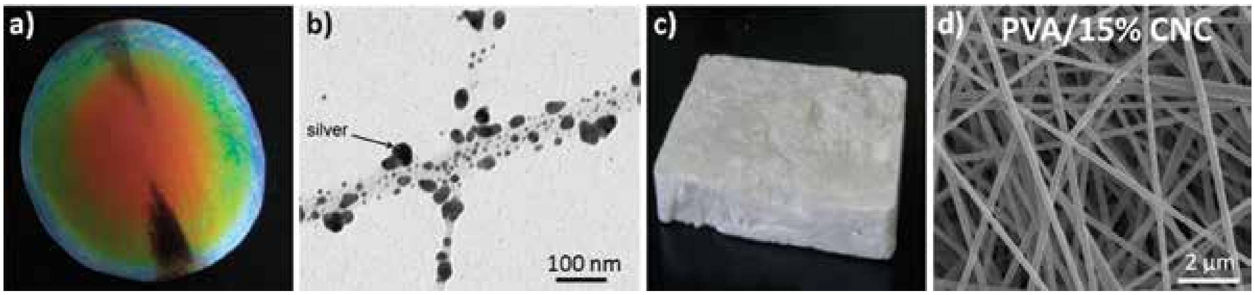

Figure 2. a) Photograph of CNC film showing iridescent/pearlescent optical behavior [courtesy of FPInnovations], b) TEM image of a tunicate CNC surface functionalized with silver nanoparticles[16], c) Photograph of CNC foam [courtesy of Shaul Lapidot, Hebrew University of Jerusalem, Israel], d) Scanning electron microscopy (SEM) image of 15wt% CNC-polyvinyl alcohol (PVA) electrospun continuous fibers [courtesy of Prof. Orlando Rojas [15]].

particle morphology, surface chemistry, percent crystallinity, etc.) are strongly linked to the cellulose source material and the acid hydrolysis processing conditions. Subsequent chemical treatments can be carried out to alter the CNC surface chemistry.

CNCs are a particularly attractive nanoparticle because they have low environmental, health, and safety risks, are inherently renewable, sustainable, and carbon-neutral like the sources from which they are extracted, and have the potential to be processed in industrial-scale quantities at low cost.

Research Areas in Cellulose Nanocrystals

Research on CNC materials covers a wide range of topics, including, but not limited to, CNC extraction processes, CNC suspension (dispersion, modification, liquid crystallinity, rheology, etc.), CNC surface functionalization (chemical, polymer grafting, nanoparticles, metal cations, DNA, etc.), CNC structural and property characterization, CNC composite processing (self-assembly, dispersion, network formation, interface engineering, films, continuous fibers, foams, etc.), CNC composite properties (mechanical, optical, thermal, barrier properties, etc.), predictive modeling (multi-length scale, structure, properties, etc.), life-cycle analysis, and environmental health and safety. Many of these topics will be covered in subsequent summaries within this section of the book.

Potential Applications

Potential applications of CNCs can be loosely grouped based on some unique combinations of CNC characteris-tics; several of these are listed below.

Rheology modifiers. Addition of CNCs can alter the rheology [9] of various media (liquids, polymer melts, particle mixtures) that are used in many industrial applications, such as paints, coatings, adhesives, lacquers, food, cosmetics, drugs, and cements.

Reinforcement for Polymer Materials. Addition of CNCs to various polymer matrix materials alters the mechanical properties of the resulting composites and can be used in the development of robust, flexible, durable, lightweight, transparent, and dimensionally stable films which may be used in packaging or structural composite applications.

Barrier Films. CNC-based composites incorporating tailored CNC surface chemistry and spacing between CNCs have attracted interest as barrier films with potential uses in selective filtration, batteries, and packaging applications [2-4,6].

Optical Films or Coatings. The liquid crystallinity of CNC suspensions, coupled with the birefringent nature of the particles, leads to interesting optical phenomena

> The exposed –OH groups on CNC surfaces can be readily modified to achieve different surface properties, and have been used to adjust CNC self-assembly and dispersion for a wide range of suspensions and matrix polymers and to control interfacial properties in composites

which can be exploited for the development of iridescent/pearlescent optical behavior for unique optical patterning of surfaces (Fig. 2a) [5,9,10].

CNC-Hybrid Composites. CNC composites that integrate inorganic nanoparticles (or chemical species) onto CNC surfaces (Fig. 2b) and/or into CNC networks have added chemical functionality which could be of use in biosensors, catalysis, photovoltaics, drug delivery, filters, and antimicrobial applications [4,6].

CNC Foams. CNC foams (e.g., aerogels) are highly porous materials (densities = 0.01–0.4 g/cm^3, surface area = 30–600 m^2/g) [11–13] and could be used in lightweight packaging, lightweight core-skin structures, and thermal or vibration insulation applications (Fig. 2c).

CNC Continuous Fibers. Continuous CNC-composite fibers (Fig. 2d) have been produced through typical fiber spinning techniques (e.g., electrospinning, dry and wet spinning) [14,15] and could be used in textile development and long and short fiber-reinforcement applications.

Summary

The unique set of characteristics of CNCs and CNC suspensions and the recent advances in CNC production capability have accelerated fundamental and applied research and development of CNC materials for a number of industrial applications. In the following pages, scientists working with CNCs will summarize aspects of their research on the properties and applications of this fascinating material.

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Cellulose Nanocrystals: Extraction from Bio-Residues

Abstract. *The aim of this study is to explore the use of industrial bio-residues as a source of raw material, for the industrial production of cellulose nanocrystals. For this purpose, cellulose nanocrystals have been isolated from bio-residues from ethanol and specialty cellulose production to analyze their properties.*

Keywords. Cellulose nanocrystals, bio-residues.

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Introduction. The continuously increasing amount of industrial bio-residues and the rising cost of their management are forcing us to make better use of the residues of the bio-based industries. The economics of forest-based industries like ethanol and specialty cellulose production can be improved with the co-production of chemicals and bio-based products from their residues. The most common co-products obtained from the ethanol industry are lignin, furfuryl, phenolic, epoxy, and isocyanate resins, as well as heat recovery for the main process [1]. However, another interesting co-product from these bio-based industries may be cellulose nanocrystals (CNC). CNC are the crystalline part of the cellulose, which is the structural component of the cell wall of green plants and some algae [2,3]. CNC are rod-shaped nano-sized crystals which can be separated by acid hydrolysis from cellulosic materials and residues from forest-based industries [4-6]. These crystals have attracted great interest as a novel nanostructured material during recent years and are expected to be used as reinforcement in polymers, in pharmaceutical products, and in barrier films [7,8].

Methodology. The methodology followed for the isolation of CNC from ethanol residues (CNCER) was previously reported by Oksman *et al*. in 2011 [4]. The first step is the purification of the bio-residue to remove the extractives. For this purpose, a Soxhlet apparatus is used, as described in TAPPI test method T204 [9]. The samples are extracted for 6 h at 150°C using a mixture of toluene and ethanol in the ratio 2:1. The process is stopped and allowed to cool after 6 h. The sample is then placed in a Buckner funnel and vacuum-dried at room temperature to remove excess solvents. The sample is then re-extracted under the same conditions after drying with ethanol to remove traces of toluene. Finally, the sample is vacuum-dried at room temperature for 24 h to remove traces of residual solvents. Then the sample is reweighed and placed in a conical flask to proceed with the bleaching step. A conical flask containing 700 ml deionized water is preheated to 70°C, after which 1.5 ml of acetic acid and 6.7 g of sodium chlorite are added, and the samples are kept at 70°C for 12 h. During the 12 h, four further additions of acetic acid and sodium chlorite are made at 2 h intervals, and after the fourth addition, the mixtures are kept at 70°C for 12 more hours. After 24 h, deionized water is added to the mixture, and centrifugation is then continued to remove any excess residual chemicals. The cellulose nanocrystals are isolated from the purified cellulose using high-pressure homogenization at a pressure of 500 bars.

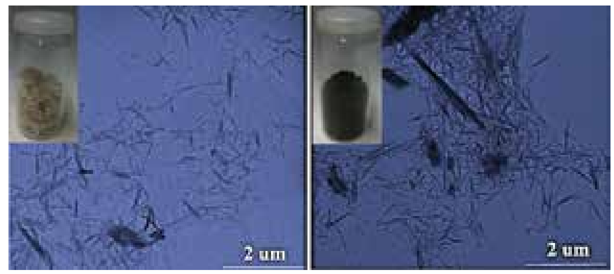

Figure 1. *Photo and TEM images of reject cellulose and CNCER (left), and ethanol residue and CNCER (right).*

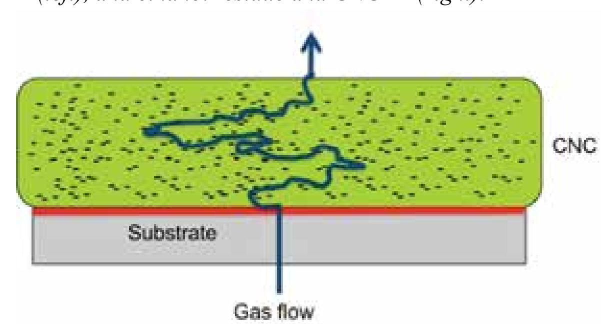

Figure 2. *Diagram showing the increase in tortuosity of films due to the addition of cellulose nanocrystals.*

To separate CNC from reject cellulose (CNCRC) from specialty cellulose production, the procedure reported by Bondeson *et al*. in 2006 [7], with minor differences,

Table 1 Summary of Characteristics.				
Source	Length (nm)	Crystallinity (%)	Degradation onset temperature (T_0) (˚C)	Peak degradation temperature (T_{max}) (˚C)
CNCRC	377 ± 132	85.8 ± 4.6	198.2 ± 20.7	287 ± 5
CNCER	301 ± 126	77.7 ± 7.0	218.5 ± 6.0	296 ± 21

was followed. The first step was to place the rejected cellulose in a solution of 65% sulfuric acid at 40°C under mechanical stirring for 30 min. The suspension was then diluted with deionized water and centrifuged several times in cycles of 5 min at 6,000 rpm. The supernatant was removed from the sediment and replaced by new deionized water and mixed. The centrifuge step was stopped after at least five washings or when the supernatant became turbid. This turbid supernatant was collected and dialyzed against deionized water until reaching a constant pH. The samples were then sonicated for 2 minutes in an ice bath to avoid overheating.

Flow birefringence was used to confirm the presence of isolated nanowhiskers in the suspension.

Results. The first result obtained was the observation of flow birefringence in both samples. With this test, it could be proved that cellulose nanocrystals were obtained from the isolation processes in both materials [6].

After a naked-eye inspection of the flow birefringence, the CNC were observed in a transmission electron microscope (TEM). With these images, a similar morphology could be observed in both samples. Both CNCER and CNCRC had a whisker appearance, as shown in Figure 1. The length of the crystals could also be determined from these images, yielding the data shown in Table 1. Both crystals had similar lengths, approximately 300 nm for CNCER and 377 nm for CNCRC. However, the length distribution of CNCRC was in the range of 375–449 nm and that of CNCER between 300 and 374 nm [6].

UV/Vis spectroscopy revealed that the films were not transparent in the UV and visual spectra and that CNCRC displayed more interference in all the ranges studied, confirming the presence of longer crystals as observed in the TEM study [6].

The results of X-ray diffraction analysis showed that both materials exhibited cellulose I structure. The crystallinity of the crystals extracted from reject cellulose, as shown in Table 1, was approximately 86%, and that of the CNC from ethanol residues was somewhat lower (78%) [6].

The thermo-gravimetrical analysis (TGA) data, shown in Table 1, indicate that the crystals extracted from ethanol residues were more thermally stable than those extracted from reject cellulose. The reason might be that several washing steps were used in the extraction of the CNCER [6].

Conclusions. The properties of cellulose nanowhiskers separated from two different industrial residues, sludge from cellulose production and lignin residues from wood bioethanol production, were studied. The nanocrystal isolation procedures used on these sources varied according to the specific needs of each bio-residue. Sulfuric-acid hydrolysis was used for the reject cellulose, and bleaching and high-pressure homogenization were used for the ethanol residues.

This work demonstrates that reject cellulose from specialty cellulose production and residual ethanol from wood bioethanol production can potentially be used as raw materials to produce value-added products from bio-residues, (i.e., cellulose nanocrystals) thereby increasing the value of forest resources. The results are relevant for bringing added value to the forest resource. Our aim is also to use these nanocrystals as gas barriers or gas separation membranes. The idea is to increase the tortuosity in the path of the gas through the film by increasing the CNC content, as illustrated in Figure 2. [11].

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