Carbohydrate Polymers 198 (2018) 270–280

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Characterization of pulp derived nanocellulose hydrogels using AVAP® technology

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ARTICLE INFO

Keywords:
Nanocellulose
Nanofibrils
Nanocrystals
Blends
Characterization

ABSTRACT

Bioinspiration from hierarchical structures found in natural environments has heralded a new age of advanced functional materials. Nanocellulose has received significant attention due to the demand for high-performance materials with tailored mechanical, physical and biological properties. In this study, nanocellulose fibrils, nanocrystals and a novel mixture of fibrils and nanocrystals (blend) were prepared from softwood biomass using the AVAP® biorefinery technology. These materials were characterized using transmission and scanning electron microscopy, and atomic force microscopy. This analysis revealed a nano- and microarchitecture with extensive porosity. Notable differences included the nanocrystals exhibiting a compact packing of nanorods with reduced porosity. The NC blend exhibited porous fibrillar networks with interconnecting compact nanorods. Fourier transform infrared spectroscopy and X-ray diffraction confirmed a pure cellulose I structure. Thermal studies highlighted the excellent stability of all three NC materials with the nanocrystals having the highest decomposition temperature. Surface charge analysis revealed stable colloid suspensions. Rheological studies highlighted a dominance of elasticity in all variants, with the NC blend being more rigid than the NC fibrils and nanocrystals, indicating a double network hydrogel structure. Given these properties, it is thought that these materials show great potential in (bio)nanomaterial applications where careful control of microarchitecture, surface topography and porosity are required.

1. Introduction

Bioinspiration from complex, hierarchical structures found in natural, biological molecules is a useful tool to aid scientists develop contemporary advanced functional materials for a plethora of applications. Of these, cellulose has received much attention due to increased demand for high-performance materials with tailored mechanical, physical and biological properties (Abitbol et al., 2016). Cellulose, a polysaccharide composed of D-glucopyranose linked by β−1,4 glycosidic bonds (Endes et al., 2016), is the most abundant, renewable and biodegradable polymer in the world, found in plant cell walls, algae, marine organisms and is produced by bacteria. By natural, photosynthetic processes, plants synthesise cellulose, accounting for approximately 40% of lignocellulosic biomass. Cellulose has three hydroxyl groups (−OH): a primary hydroxyl at the C-6 position, and two secondary hydroxyls at the C-2 and C-3 positions, which play an important role in the compactness of the crystalline structure and determine its physical properties. Extensive inter- and intramolecular

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https://doi.org/10.1016/j.carbpol.2018.06.091
Received 1 March 2018; Received in revised form 30 May 2018; Accepted 20 June 2018
Available online 22 June 2018

hydrogen bonding between −OH groups imparts structural integrity and mechanical strength to the material.

Nanocellulose (NC) is an emerging class of advanced nanomaterials possessing unique physical, structural, chemical and biological properties. NC can be divided into three types of materials: (i) biomass-based cellulose nanocrystals (CNCs) or nanowhiskers, (ii) biomass-based cellulose nanofibrils, and (iii) bacterial cellulose nanofibrils. Hybrid composite blends can also be created where CNCs and fibrils are mixed to enhance physicochemical and biological properties, making them more suited to certain applications. Unique properties of NC include extraordinarily high stiffness (100–200 GPa) and strength, low density, flexibility, reactive surfaces, functionalisability, high surface area and aspect ratio, excellent biocompatibility and non-toxicity. As a result, NC research in the last decade has grown exponentially.

Production of NC range from 'top-down' approaches involving chemical, mechanical and/or enzymatic (Yarbrough et al., 2017) methods which can isolate the materials from wood and agricultural residues, to 'bottom-up' methods using bacteria to produce cellulose from glucose. The resulting nanoparticles offer unique properties with varied surface chemistry, crystallinities and mechanical properties (Abitbol et al., 2016). Mechanical processing, typically through refining or homogenization, is relatively simple but has high energy consumption. Chemical pre-treatments, such as applying 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) functionality before mechanical treatment can minimise energy consumption. However, methods like TEMPO are associated with increased consumable costs. Bacterial cellulose has its uses (Markstedt et al., 2015) and can be produced with high purity, free from lignin and hemicelluloses. Bacterial cellulose has shown promise for tissue engineering applications due to its biocompatibility, nanostructure, water-holding capacity, high strength and morphological similarities to collagen, thereby providing good cell support (Ahrem et al., 2014; Dugan, Gough, & Eichhorn, 2013; Markstedt et al., 2015; Paakko et al., 2007). Several studies have also demonstrated that the rheological properties of bacterial cellulose make it a suitable bioink for 3D bioprinting. However, the major drawbacks of high substrate costs, low yield of NC products and concerns regarding residual bacterial toxins/epitopes makes this method less desirable and warrants investigation of alternative sources if NC is to become a commercially viable biomaterial (Paakko et al., 2007).

Acid hydrolysis is the most widespread chemical method to extract nanocellulose from lignocellulosic biomass (Lin, Bruzzese, & Dufresne, 2012), creating CNCs/nanowhiskers from cellulose fibers (hydrolysis of the less ordered domains of cellulose with persisting intact crystalline domains) (Abdul Khalil, Bhat, & Ireana Yusra, 2012). The hydrolysis kinetics between crystalline and amorphous regions of cellulose are different, leading to hydrolysis of the amorphous regions and production of CNCs (Habibi, Lucia, & Rojas, 2010).

NC produced using American Value Added Pulping (AVAP®) technology chemically pre-treats the wood-pulp derived biomass to remove hemicelluloses, lignin and the amorphous regions of cellulose (Nelson & Restsina, 2014). Lignin is separated from cellulose and hemicelluloses using the delignifying agent, sulfur dioxide. Mechanisms of this process have previously been reported (Iakovlev, You, van Heiningen, & Sixta, 2014). Resins and extractives are dissolved using ethanol. In addition, the degradation of crystalline cellulose is also reduced (Nelson & Restsina, 2014). During the delignification process, strong lignosulfonic acids help hydrolyse the amorphous regions of cellulose (Nelson & Retsina, 2014). Fractionation to NC fibrils dissolves approximately 90% of lignin and 90% hemicelluloses, whilst fractionation to CNCs additionally hydrolyses amorphous cellulose (approximately half of cellulose). However, a lignin-carbohydrate complex is hydrolysed in both cases and the fractionation liquor contains dissolved lignin, lignosulfonic acids and hemicellulose sugars. The resulting NC yield (with respect to softwood biomass, NC fibrils: 40–60%; CNCs: 20–30%; NC blend: intermediate yield values) and morphology depends on optimized experimental parameters such as time and temperature. The AVAP® process is based on the "tunability" of the pre-treatment step. For each feedstock, the pre-treatment conditions (time and temperature) are selected to give the desired levels of fibrillation and removal of amorphous cellulose, as indicated by the degree of polymerization (DP). American Process Inc. (API), GA, USA have shown, using eucalyptus, softwood and cane straw, that two unique DP targets exist for the production of NC fibrils and CNCs after mechanical treatment. Between the two targets, a blend of NC fibrils and CNCs is produced (Nelson & Retsina, 2014; Nelson et al., 2016). The morphological structure of NC is highly dependent on the efficient removal of non-cellulosic regions and hydrolysis of amorphous domains (Lu et al., 2013; Mondal, 2017). Hence AVAP technology is a sustainable platform that uses low cost raw biomass and pre-treatment chemicals, a small number of process steps, low operating and capital costs, low energy consumption and creates revenue from the ethanol co-product (Nelson & Restsina, 2014; Nelson et al., 2016).

Although the cellulose molecular backbone is common to all; surface morphology, size, chemical and physical properties can vary greatly depending upon the material source and extraction methods used (Mao et al., 2017). The objective of this study was to examine the physicochemical properties of NC fibrils, CNCs and a novel blend of these materials produced via the AVAP® technology. The product containing fibrils and CNCs is produced *in situ* during production and is not a *de facto* blending of separate fibrils and CNC products. However, it is herein referred to as a blend for simplicity. The physical properties of these materials were characterized in terms of surface morphology (TEM, AFM and cryoSEM), chemical functional groups (FTIR), crystalline structure (XRD), thermal stability (TGA) and rheology. Determining these properties is a key aspect for structure-function relationships which could widen NC applications. For example, assessing physicochemical properties enables better prediction of cellular interactions when considering the use of NC as a biomaterial for tissue engineering. It is also important when planning functionalization with biologically active groups to improve biocompatibility and tissue generation. Linking NC structure and surface chemistry with the rheological properties of the material may also allow fine tuning of the biomaterial for 3D bioprinting applications (Kyle, Jessop, Al-Sabah, & Whitaker, 2017).

2. Materials and methods

2.1. Production of NC fibrils, crystals and blend

NC fibrils and CNCs were produced from wood chips using the patented AVAP® technology which fractionates biomass into cellulose, hemicelluloses and lignin using ethanol and sulfur dioxide. NC products were processed by American Process Inc, Atlanta, GA (Nelson & Restsina, 2014; Nelson et al., 2016; Nelson, Retsina, Pylkkanen, & O'Connor, 2015). The final nanocellulose product morphology (NC fibrils (3 wt.% solids), CNCs (6 wt.% solids), or a novel blend of NC fibrils and CNCs (3 wt.% solids)) was controlled by the time and temperature (i.e. severity) of the pre-treatment step.

2.2. Transmission electron microscopy (TEM)

Each sample (2 mg) was dispersed in 5 mL of deionized water and sonicated for 30 min. After sonication, 50 µL of the sample was immediately taken and further dispersed in 1 mL of deionized water to prevent coalescence. This solution (10 µL) was added to 300 mesh copper grids coated with lacey carbon film. The grid was allowed to air dry prior to staining with a 1.5% uranyl acetate solution. For staining, a drop of the uranyl acetate solution was placed on a parafilm strip and the grid inverted onto the droplet for a few seconds. The samples were then allowed to air dry. Analysis was performed on a Jeol 2100 TEM operating at 200 kV.