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FORMULATION DEVELOPMENT OF FIBRILLATED CELLULOSE BASED PASTES FOR ADDITIVE MANUFACTURING

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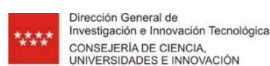
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Abstract

Nowadays, tons of clothes are wasted due to the fast fashion industry, which leads to environmental issues since the clothes are dumped in landfills or incinerated. However, clothes are composed of cellulose, a natural biopolymer that raises interest due to its variety of applications. This work proposes the development of a paste material based on fibrillated cellulose extracted from different sources of cotton for additive manufacturing. This solution contributes to sustainable fashion and circularity in the textiles value chain.

This dissertation aimed to formulate a paste that incorporates fibrillated cellulose, a natural hydrogel (sodium alginate) and water, that fulfills the requirements of a filament for additive manufacturing. Regarding the source of cellulose, it was analyzed two different types of cotton: (i) Fresh Cotton Linters (FCL) provided by Sigma-Aldrich, in which a pre-treatment of the sample was not required, since the diameter of the fibers was very small and (ii) Cotton Fibers (CF), cotton kindly provided by Polopiqué, a Portuguese textile company, where a combination of pre-treatments (cutting manually, milling and the use of NaOH to promote the disintegration of the sample) was essential to reduce the fibers' diameter. After that, fibrillation was carried out by chemical and mechanical treatments at different conditions of TEMPO catalyst, NaBr and NaClO, number of passes and pressures in the High Pressure Homogenizer. It was produced three different fibrillated cellulose suspensions: two fibrillated cellulose suspensions from FCL and one fibrillated cellulose suspension from FC.

Different formulations using water, fibrillated cellulose and sodium alginate were studied. Then, rheological measurements were conducted to study the printability of the pastes. It was essential to ensure that the paste had a shear thinning behavior, a yield point, a flow point and a high percentage of elastic recovery. The formulation that revealed the most promising results of printability has 2.3:5:92.7 wt.% of fibrillated cellulose, sodium alginate and water respectively. The same formulation was replicated with all the fibrillated cellulose, i.e. FCL and CF samples. The results of this proof of concept, producing a structure with at least two layers, revealed that the printed pastes maintained their shape fidelity, using a syringe of 0.684 and 2 mm. The last task of this work was the recycling test: a solution of sodium citrate tribasic dihydrate and water were added to the printed material, showing that the paste takes the rheological properties to be reprinted.

This work demonstrates the development of an innovative paste, since it was used from recycled materials from the textile industry, and it can be recycled giving life to a new paste for additive manufacturing.

Keywords: 3D printing; additive manufacturing; fibrillated cellulose; fast fashion clothes; nanocellulose.

Resumo

Atualmente, são desperdiçadas toneladas de vestuário oriundos da indústria de moda rápida, o que contribui para o agravamento dos problemas ambientais, uma vez que as roupas são depositadas em aterros ou acabam por ser incineradas. No entanto, as roupas são compostas por celulose, um biopolímero natural que devido à sua versatilidade de aplicações desperta muito interesse. Este trabalho foca-se no desenvolvimento de uma pasta, para impressão 3D, à base de celulose fibrilada extraída de diferentes fontes de algodão. Esta solução contribui para a existência de uma moda sustentável e para a circularidade na cadeia de valor do setor têxtil.

Esta dissertação teve como objetivo a formulação de uma pasta que incorporasse celulose fibrilada, alginato de sódio (um hidrogel natural) e água de forma a cumprir com os requisitos para poder ser utilizada como filamento em impressão 3D. Em relação à origem da celulose, esta foi proveniente de: (i) Sigma-Aldrich, designado de *Fresh Cotton Linters* (FCL) e (ii) *Cotton Fibers* (CF), fornecido pela Polopiqué, uma empresa têxtil Portuguesa, sendo que para se diminuir o diâmetro das fibras de algodão aplicaram-se alguns pré-tratamentos (corte manual, moagem e adição de NaOH para promover a sua desintegração). Posteriormente, procedeu-se à fibrilação através de tratamentos químicos e mecânicos, utilizando-se diferentes condições do catalisador TEMPO, NaBr e NaClO, número de passagens e pressões no Homogeneizador de Alta Pressão. Foram produzidas três suspensões de celulose fibrilada diferentes: duas suspensões a partir de FCL e uma a partir de CF.

Avaliaram-se diferentes formulações utilizando-se celulose fibrilada, água e alginato de sódio. Testes reológicos foram efetuadas para averiguar a capacidade de impressão das pastas. Era essencial assegurar que possuíam tinham um comportamento pseudoplástico, um ponto de cedência e de escoamento, e recuperação elástica considerável. A formulação que revelou os resultados mais promissores possuem uma composição de 2,3:5:92,7 wt.% de celulose fibrilada, alginato de sódio e água, respetivamente. A mesma formulação foi replicada a todas as amostras de celulose fibrilada, ou seja, FCL e CF. Os resultados desta prova de conceito, revelaram que podem ser impressos objetos até duas camadas, mantendo a sua forma, utilizando-se uma seringa de 0,864 e 2 mm. Por fim, uma solução de citrato de sódio tribásico di-hidratado e água foram adicionados ao material impresso, revelando que a pasta reúne as propriedades reológicas para ser reimpressa.

Este trabalho revela o desenvolvimento de uma pasta inovadora, visto que foi desenvolvida a partir de materiais reciclados da indústria têxtil. Além disso, pode ser reciclada dando vida a uma nova pasta para fabricação aditiva.

Palavras-Chaves: impressão 3D; fabricação aditiva; celulose fibrilada; roupas de moda rápida; nanocelulose.

Declaration

I hereby declare, under word of honor, that this work is original and that all non-original contributions are indicated, and due reference is given to the author and source.



(Filipa Daniela Ribeiro de Carvalho)

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Notation and Glossary

AG	aldehyde groups	mmol·g ⁻¹
AR	aspect ratio	
CD	cationic demand	meq·g ⁻¹
CG	carboxylic groups	mmol·g ⁻¹
G'	storage modulus	Pa
G''	loss modulus	Pa
GP	gel point	kg·m ⁻³
H	height	cm
PD	polymerization degree	monomers units
V	volume	cm ³

Greek Letters

τ	shear stress	Pa
η	viscosity	Pa·s ⁻¹
$\dot{\gamma}$	shear rate	s ⁻¹
ω	frequency	Hz

Listo of Acronyms

ABS	Acrylonitrile Butadiene Styrene
AM	Additive Manufacturing
BC	Bacterial Cellulose
CMF	Microfibrillated cellulose
CNC	Cellulose Nanocrystals
CNF	Cellulose Nanofibers
DIW	Direct Ink Writing
FDM	Fused Deposition Modeling Process
HPH	High Pressure Homogenizer
LSRE-LCM	Laboratory of Separation and Reaction Engineering-laboratory of Catalysis and Materials
LVE	Linear Viscoelastic Region
NC	Nanocellulose
OM	Optical Microscopy
OP	Oxidized pulp
PLA	Polylactic Acid
TEM	Transmission Electron Microscopy
TEMPO	2,2,6,6-Tetramethylpiperidin-1-oxyl
TMO	TEMPO-Mediated Oxidation
UCM	University Complutense of Madrid

1 Introduction

1.1 Framing and presentation of the work

Due to population growth, fashion consumption and standards are rising (Islam & Bhat, 2019). It is estimated that the global fashion market will expand from 1.5 trillion dollars in 2020 to 2.25 trillion dollars in 2025 (Centobelli et al., 2022; Islam & Bhat, 2019). Each year, million tons of clothing and textile are dumped in landfills. These clothes and textile residues have a significant environmental impact, as they contaminate groundwater and contribute to the formation of greenhouse gases during their decomposition. At the same time, it is estimated that people are buying 60% and wearing them for half as long (Islam & Bhat, 2019).

Despite the efforts to avoid wasting clothes through practices like second-hand sales, ultimately, these clothes still find their way to landfills. Since fast fashion is big business and the environmental costs are rising, a circular economy for textiles is another way. Although textile industries' efforts to incorporate recycled waste materials and contribute to a circular economy, this process is challenging, particularly for cotton, since its quality was not maintained after multiple cycles of use. Therefore, there is a social and environmental need to develop new recycling approaches for cotton wastes.

Due to the previously detailed reasons, this dissertation primarily aims to provide a second life to the wasted cotton derived from the fast fashion industry as a source of cellulose for the production of fibrillated cellulose. Fibrillated cellulose has unique properties and can be used in a widely range of applications, being a promising alternative to the filaments currently used in additive manufacturing (Thibaut et al., 2019). Therefore, this work aims the development of a paste that incorporates fibrillated cellulose, sodium alginate and water, with adequate rheological properties to be used in additive manufacturing. Sodium alginate is a natural hydrogel that presents the capacity of being reversible cross-linking via ion exchange. This property enables the production of a reprintable bio-based material for additive manufacturing (Sauerwein et al., 2020).

However, during the production of fibrillated cellulose, it was required to perform a chemical reaction pre-treatment prior to the mechanical defibrillation stage. Consequently, the resulting paste may not be suitable for use in certain areas, such as medicine and biomedicine devices, food industry and pharmaceutical industries.

1.2 Contribution of the author to the work

This dissertation was developed at Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM) at Faculty of Engineering University of Porto (FEUP) in collaboration with the Research Group of Cellulose, Paper and Advanced Water Treatments, from the Faculty of Chemistry of the Complutense University of Madrid (UCM). The development of this work required a secondment of two months at UCM.

At UCM, fibrillated cellulose was produced from two different sources: cotton fibers (CF), kindly provided by Polopiqué, a textile company in Portugal, and fresh cotton linters (FCL) purchased from Sigma-Aldrich, used as a reference. The cellulose pre-treatments and methodologies to produce fibrillated cellulose were proposed by Professors Angeles Blanco and Ana Balea. The production of fibrillated cellulose was performed with the help of Professor Ana Balea, PhD student Hongyu Xu and post-doctoral researcher Jose Luiz Sanchez-Salvador. Each fibrillated cellulose was fully characterized, to verify if it fulfills the requirements to be incorporated in the paste. Regarding the characterization, it was conducted by the author under the supervision and in collaboration of the UCM research group.

At FEUP, the formulations containing fibrillated cellulose, sodium alginate and water as well as the respective printability of the paste were studied by the author. Rheological measurements of the formulations were developed with the help of Doctor Margarida Brito and Doctor Yaidelin Manrique.

1.3 Organization of the dissertation

The present dissertation is structured in six chapters.

Chapter 1 includes the framing and the presentation of the objectives and the work that is detailed in subchapter 1.1 and the contribution of the author to the work in subchapter 1.2.

Chapter 2 provides the theoretical fundamentals essential for an overview of the explored research topic and for understanding the remaining chapters detailed in this dissertation. It is explored the cellulose topic in subchapter 2.1, nanocellulose in subchapter 2.2 and additive manufacturing and fibrillated cellulose in subchapter 2.3.

Chapter 3 contemplates the section of materials and methods to produce fibrillated cellulose (subchapter 3.1) and the formulation of pastes for additive manufacturing (subchapter 3.2).

Chapter 4 presents the results and their discussion concerning the characterization of oxidized pulps and fibrillated cellulose (subchapter 4.1), the evaluation of the production of fibrillated cellulose pastes for additive manufacturing (subchapter 4.2), the proof of concept for the

development of the fibrillated cellulose pastes and the potential of reprint ability of the fibrillated cellulose pastes (subchapter 4.4).

Chapter 5 assembles the main conclusions of the work accomplished.

Chapter 6 summarizes the objectives achieved (subchapter 6.1), other works carried out (subchapter 6.2), and the current limitations and future works (subchapter 6.3).

2 Context and State of the Art

2.1 Cellulose

Cellulose is the most widely biopolymer available in nature that possesses a density around $1.14\text{--}1.45\text{ g}\cdot\text{cm}^{-3}$ and a white appearance (Blanco et al., 2018; Gauss et al., 2021; Hashaikeh et al., 2015; Lavoine et al., 2012). It can be found in trees, plants, fungi, bacteria, and tunicates (Blanco et al., 2018). Particularly in plant sources, cellulose is in leaves (e.g. sisal), fruit (e.g. cotton) and stalk or rigid structure of the plants (e.g. wood) (Lavoine et al., 2012).

The cellulose chemical formula, shown in Figure 2.1, can be described as $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ where n is the degree of the polymerization associated with the number of glucose units. The degree of polymerization can vary from 600 for algae to 14 000 for cotton (Karpe, 2015).

Cellulose is a linear polymer, β -D-glucopyranose linked through β -1-4-glycosidic bonds, whereby cellobiose, a disaccharide of two glucose units joined by β -1,4 bonds, represents the monomer unit (Blanco et al., 2018; Karpe, 2015; Lavoine et al., 2012; Negro et al., 2020). Due to the β -1-4-glycosidic bonds established, cellulose is a combination of amorphous and crystalline regions. Each monomer incorporates three hydroxyl groups that can form intramolecular hydrogen bonds, that confer the crystallinity region and the physical properties (hydrophilicity, chirality, biodegradability) of the cellulose (Blanco et al., 2018; Gauss et al., 2021; Malladi et al., 2018). On the other hand, the amorphous regions are a consequence of the breakage and disorder of the hydrogen bonds, and every fibril contains crystals and amorphous in the chain combines to form each fiber of cellulose (Blanco et al., 2018; Malladi et al., 2018).

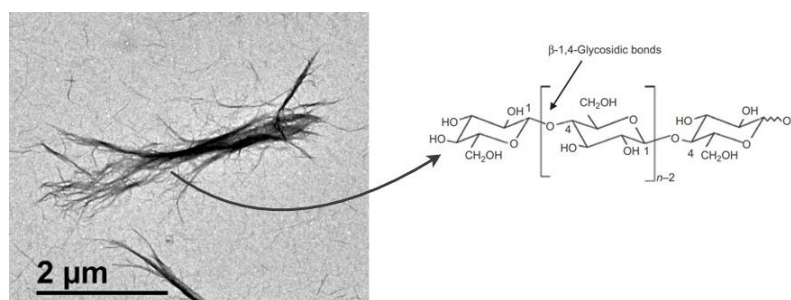


Figure 2.1. Chemical structure of cellulose and the respective microscope image. Adapted from (Blanco et al., 2018).

2.2 Nanocellulose

Nanocellulose (NC) can be described as cellulose in the form of nanostructures containing at least one dimension within the range of 1 to 100 nm (Negro et al., 2020).

In the last decades, the application of cellulose nanofibers (CNF) has been growing considerably due to their outstanding properties that result from the combination of cellulose and nanoscale

dimensions, including mechanical strength, high surface area, high aspect ratio, barrier properties, low thermal expansion coefficient, lightweight, biodegradability, renewability and sustainability (Balea et al., 2017; Negro et al., 2020; Sanchez-Salvador, 2021). These properties enable the use of NC in a wide range of applications, including papermaking, composites, packaging, films, electronic devices, filtering materials, rheological modifiers, paints and coatings, biomedicine and automotive (Balea et al., 2017; Xu et al., 2022). Despite their widespread use, it is estimated that only a fraction of the worldwide production, approximately 6×10^9 tons per year (in a total of 10^{10} to 10^{11} tons) is currently used in applications as paper, textile, nanocomposites, and chemical industries (Lavoine et al., 2012).

The terminology microfibrillated cellulose, microfibers and microfibrils (CMF) are commonly used as a generic definition of a cellulose structure with a 2-10 nm thickness and many microns in length, which includes microfibers. Nevertheless, the terms nanofibrillated cellulose, nanofibrillar cellulose, nanofibers, nanofibrils, cellulose nanofibrils and cellulose nanofibers (CNF) are widely utilized to specifically denote the presence of nanofibers within the structure (Blanco et al., 2018). In addition, the characteristics of CNF are influenced by various factors, including the properties of the cellulose source (chemical composition, morphology, swelling ability and crystallinity), type and intensity of the pre-treatment and the type and severity of the mechanical defibrillation process. The relationship between these variables is complex, but crucial for the final properties and fit-for-use applications (Balea et al., 2021). NC can be classified into three main categories: CNF, cellulose nanocrystals (CNC) and bacterial cellulose (BC) (Blanco et al., 2018).

2.2.1 Cellulose source

Fibrillated cellulose is formed by a mixture of CMF and CNF in suspension. It can be originated from a diversity of sources, including plants, namely seed fibers, such as cotton and coir and bast fibers of flax, hemp, jute, kenaf, and ramie, grasses, marine animals (tunicate), algae, fungi, invertebrates, and bacteria (Blanco et al., 2018; Lavoine et al., 2012; Negro et al., 2020; Sanchez-Salvador et al., 2021). However, although hardwood and virgin pulps are the most typical sources for the production of fibrillated cellulose at large-scale, alternative sources from agricultural wastes and non-wood plants are currently being used to contribute to circular economy and align with the availability of local market factors (Balea et al., 2017; Sanchez-Salvador et al., 2021).

The production of fibrillated cellulose from agricultural wastes can occur from several sources, including fruit peel and vegetables, such as: banana (Tibolla et al., 2018), cassava (Widiarto et al., 2019), potato (Liu et al., 2023), orange (Espinosa et al., 2022) and tomato (Ghadiri Alamdari et al., 2021), and from plants, including: corncob (Wang et al., 2019), sugarcane bagasse (Asem et al., 2023), wheat straw (Bian et al., 2022), bamboo (Wang et al., 2020) and hemp (Mhlongo et

al., 2022). Moreover, the production of fibrillated cellulose through coffee waste (Kanai et al., 2020), green tea waste (Dutta & Karak, 2019) and white and natural colored cotton fibers (Teixeira et al., 2010) is also reported. As previously detailed, currently, the most common source of NC production is wood, which consists of a combination of a mixture of cellulose, hemicellulose, lignin, inorganic salts, and extractives in a small amount. Consequently, it is necessary to apply various chemical and/or mechanical treatments before the production of fibrillated cellulose (Blanco et al., 2018). Therefore, it is only sustainable if they are produced in pulp plants.

2.2.2 Fibrillated cellulose production

As previously mentioned, the type of fibrillated cellulose production compromises the final application (Balea et al., 2021). For the production of fibrillated cellulose, mechanical treatments that involve processes of intensive disintegration are required, with the aim to promote the defibrillation of the CNF (Sanchez-Salvador et al., 2021). Therefore, the energy consumption associated to the cellulose structure breaking until a micro or nanoscale is very high and is usually associated with restricted nanofibrillation efficiency (Balea et al., 2017; Sanchez-Salvador et al., 2021; Xu et al., 2022). To facilitate the defibrillation process, reduce the energy costs and increase the NC yield, chemical, mechanical, enzymatic, or combined pre-treatments are applied (Balea et al., 2017; Negro et al., 2020).

2.2.2.1 Chemical pre-treatment

TEMPO-mediated oxidation (TMO) is a chemical pre-treatment developed by (Saito & Isogai, 2006) and co-workers, commonly used, that ensures the highest fibrillation degree and homogeneity of CNF production. The process involves the use of 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO) as catalyst, sodium bromide (NaBr) as co-catalyst and sodium hypochlorite (NaClO) as oxidant (Sanchez-Salvador et al., 2021; Xu et al., 2022). TMO ensures the modification of the cellulose structure by selectively oxidizing the C6 hydroxyl groups into aldehyde and subsequently to carboxyl groups. This process produces an electrostatic repulsion between the carboxylic groups which facilitates the mechanical defibrillation process. During the initial stage of the reaction, the NaClO is rapidly consumed within a few minutes, due to the high reaction rate. However, the reaction rate decreases after the pulp achieves a certain oxidation degree (Sanchez-Salvador et al., 2021).

During the TMO reaction, the pH was maintained between 10.5-11 to ensure that NaClO is used in the oxidation of the C6 primary hydroxyl groups to carboxyl groups, promoting the electrostatic repulsion between fibers and thus, the mechanical process to produce micro and nanofibers (Sanchez-Salvador et al., 2021; Xu et al., 2022). Besides the increase of the electrostatic repulsion between the fibers, carboxyl groups facilitate the formation of stable suspensions of NC in water (Xu et al., 2022). The scheme of the TMO reaction is shown in Figure 2.2.

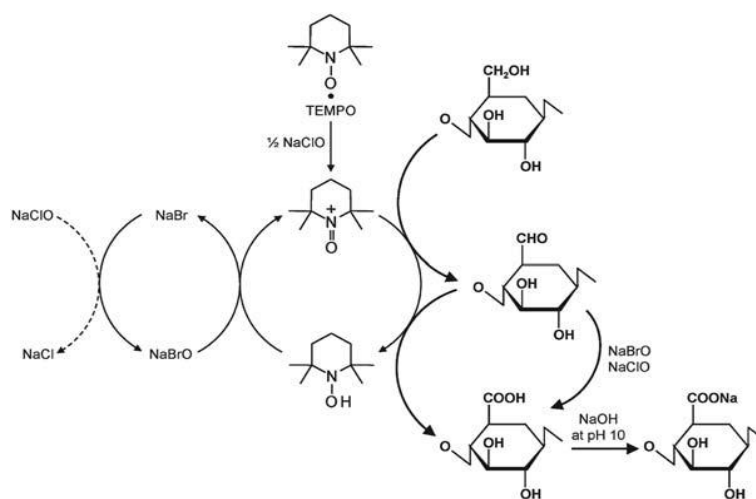


Figure 2.2. Scheme of TEMPO-mediated oxidation (Isogai et al., 2011).

2.2.2.2 Mechanical pre-treatment

Regarding the most efficient mechanical techniques for fibrillated cellulose production, the most relevant ones are High Pressure Homogenizer (HPH), microfluidization, refining and grinding. Other mechanical treatments to produce fibrillated cellulose are electrospinning, ultrasonication, cryocrushing, and steam explosion (Blanco et al., 2018). Regarding to HPH process, the cellulose suspension is forced to pass through a narrow space between the homogenizing valve and impact ring which leads to a decrease in pressure, but an increase of turbulence and temperature. As a consequence, the cellulose fibrillation process is ensured by the shear and impact forces (Blanco et al., 2018).

The influence of the size, aspect ratio and morphology of the fibrillated cellulose is defined by the homogenization conditions (particles collision, magnitude of the pressure drops, turbulent flow and high shear forces) while the degree of fibrillation is due to the pressure and the number of passes used in the HPH. Moreover, from HPH, there is an increase of the surface area and a decrease of the amorphous region of the fibers (Blanco et al., 2018).

2.2.3 Fibrillated cellulose application

Fibrillated cellulose finds application across various industrial sectors, including aerospace materials, textiles, plastics, environmental treatments, medical implants, tissue engineering, drug carries, wound dressings, cosmetics, hygiene products, pharmaceuticals, and food industry. It serves as a strength agent, stabilizer or construction-based materials and can be considered as a potential candidate for additive manufacturing (AM), since it offers lightweight, robust and recyclable structures for 3D printing, which can be applied as an additive for mechanical reinforcement and as a rheological modifier (Negro et al., 2020; Thibaut et al., 2019).

2.3 Additive manufacturing and fibrillated cellulose

2.3.1 Additive manufacturing techniques

Recently, AM, also commonly known by 3D printing, has gained significant attention from industry and academia, since it is associated with the fast production of a diverse range of customized objects with a defined geometry, through a layer-by-layer technique (Lamnini et al., 2022). This technique enables the creation of objects with complex shapes; however, it presents challenges that need to be addressed (Banasadi et al., 2021).

The success of the extrusion of a filament should integrate the following aspects: a constant flow to avoid nozzle clogging and filament break at a constant pressure; a homogeneous filament, and pressure according to the specifications required for the equipment. During the material printing, it is essential to maintain it in the vertical position, so it is crucial that its viscosity is high enough (Thibaut et al., 2019).

The AM printing system has a crucial function in the entire printing process that connects the digital AM models, the AM printing materials, and its final application. The AM printing used for cellulose materials is designated as extrusion-based AM, which presents an easy utilization. In extrusion-based AM procedure, the extruded material is released from a nozzle and deposited in a layer-by-layer technique, in which the second layer of the printed material can only be deposited after a structure fidelity of the first layer is ensured (Schwab et al., 2020; Wang et al., 2018). Regarding extrusion-based AM, its printing system can be piston-, pneumatic- and screw-driven (Schwab et al., 2020). Although for fluids with a viscosity up to 10^4 Pa·s is recommended the screw-driven system, for viscosities below 10^7 mPa·s, a piston- or pneumatic-based extrusion is indicated. Despite piston-based extrusion versatility, its deposition control, especially with heterogeneous inks is lower (Schwab et al., 2020). In Figure 2.3, is represented a scheme of the three types of extrusion mentioned before.

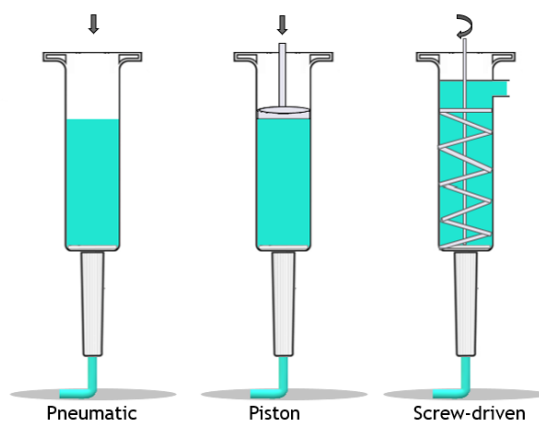


Figure 2.3 Illustration of the pneumatic, piston and screw-driven system for the extrusion printing. Adapted from (Schwab et al., 2020)

2.3.2 Materials printed from additive manufacturing

Nowadays, the most widely materials used in AM are polymers, composites, metals, ceramic, sand and wax. However, it is reported that some polymers such polylactic acid (PLA), acrylonitrile butadiene styrene (ABS) and nylon can lead to potentially hazardous situations. During the AM process, volatile compounds and ultrafine aerosol are emitted, which have been associated with potential harm to human health (Wang et al., 2018).

Natural biopolymer hydrogels, such collagen, sodium alginate ($\text{NaC}_6\text{H}_7\text{O}_6$), chitosan and hyaluronic acid are widely used in AM and revealed to be promising in tissue engineering and regenerative medicine applications, since they are renewable, and biodegradable (Wang et al., 2018). $\text{NaC}_6\text{H}_7\text{O}_6$ mixtures are widely used because of their ionic crosslinking ability and mechanical stability to mold the shape of the hydrogels. For this application, the rheological properties are highly important, and it is further explored in the section Materials and Methods.

Fibrillated cellulose has been incorporated into hydrogels for AM (Wang et al., 2018; Espinossa et al. 2019). Even at low concentration, the addition of fibrillated cellulose to hydrogels results in the substantial modification of the physical and chemical parameters, reflecting the increase of stiffness and shear-thinning behavior, giving better characteristics for printability and stability (Vel et al., 2022). Due to the fibrillated cellulose hydrogels shear-thinning and high zero shear viscosity properties, they can be considered as a potential candidate for extrusion-based AM. The development of cellulose-based for AM could serve as an eco-friendly alternative to the fossil-based plastics materials (Vel et al., 2022; Wang et al., 2018).

2.3.3 Application of fibrillated cellulose in additive manufacturing

Fibrillated cellulose has found wide applications in AM, particularly in the biomedical field for tissue engineering, implants, drug delivery systems, and cardiovascular devices. Researchers have developed bioink formulations with fibrillated cellulose, $\text{NaC}_6\text{H}_7\text{O}_6$ and poly(2-ethyl-2-oxazoline) (Trachsel et al., 2019) or with fibrillated cellulose, $\text{NaC}_6\text{H}_7\text{O}_6$ and polydopamine particles (Im et al., 2022) for extrusion-based AM. Another bioink formulation incorporating fibrillated cellulose, $\text{NaC}_6\text{H}_7\text{O}_6$, water and curcumin for drug delivery system was evaluated, with CNF concentration ranging from 1 wt.% to 5 wt.%, to identify a bioink paste with optimal rheological properties to be used in AM (Olmos-Juste et al., 2021). (Zhou et al., 2022) introduced an ink composed of fibrillated cellulose suspension derived from oil palm for thermal insulation and energy storage purposes. In another study, the development of a bio-ink for AM incorporating fibrillated cellulose from quince seed was reported (Baniasadi, Polez, et al., 2021). (Espinossa et al., 2019) conducted a study of an ink formulation composed by $\text{NaC}_6\text{H}_7\text{O}_6$ and fibrillated cellulose derived from *Pinus radiata kraft*. This ink can be used for AM, enabling the printing of porous structures that can be used as wound dressing devices.

3 Materials and Methods

Materials

In this work, two sources of cellulose were used as raw materials to produce fibrillated cellulose: Cotton Fibers (CF) kindly provided by Polopiqué, a Portuguese textile company, and Fresh Cotton Linters (FCL) supplied from Sigma Aldrich (CAS: 9004-34-6). Table 3.1 shows the composition of the two sources. The method to determine the ash content in this table is introduced in section 3.1.2.

Table 3.1. Composition of the raw material.

Raw Material	Supplier	Composition	Moisture (wt.%)	Ash Content (wt.%)
CF	Polopiqué	Cotton	2.9±0.1	1.79±0.01
FCL	Sigma-Aldrich	Cellulose	4.0±0.5	0.07±0.02

For TMO pre-treatment, TEMPO reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas NaBr and 12% (w/v) NaClO solution were purchased from Panreac AppliChem (Barcelona, Spain). Other reagents used for monitoring the reaction and for the oxidized pulps (OPs) and fibrillated cellulose characterization were NaOH, HCl, Na₂S₂O₃, KI and bis(ethylenediamine)copper(II) hydroxide solution, also supplied by Sigma-Aldrich (St. Louis, MO, USA). Besides, 0.1% (w/w) poly-L-lysine solution was purchased from Electron Microscopy Sciences (Hatfield, PA, USA).

Methods

This work is divided in two parts: (i) production of fibrillated cellulose performed in collaboration with the research group of Cellulose, Paper and Advanced Water Treatments, from UCM and (ii) formulation of pastes containing fibrillated cellulose for AM, developed in the LSRE-LCM (FEUP).

3.1 Production of fibrillated cellulose

3.1.1 Description of the process

Figure 3.1 shows a detailed description of the fibrillated cellulose production. Depending on the raw material, a mechanical pre-treatment before chemical TMO reaction can be required. After TMO reaction step, all the OPs (FCL-5T-OP-1, FCL-5T-OP-2, CF-10T-OP-1) are characterized and then, another mechanical treatment in the HPH generates fibrillated cellulose. At the end of the process, three different types of fibrillated cellulose (FCL-5T-CNF-1, FCL-5T-CNF-2, CF-10T-CNF-1) were produced and characterized.



Figure 3.1. Overall of the fibrillated cellulose production process.

Table 3.2 provides a summary of the different techniques employed in the production of fibrillated cellulose suspensions for each raw material, which includes the type of pre-treatment needed for the raw material, the pulp disintegration conditions, the chemical reagents doses used in the TMO reaction, and the consistency, number of passes and pressure applied in HPH.

Table 3.2. Production methods to obtain fibrillated cellulose.

Raw Material	FCL		CF
Pre-treatment	Not required		Cutting manually the sample Milling
Disintegration			
Weight of moist sample in distilled water at 50 °C	20.8 g of FCL in 1000 mL of water		30 g of CF in 1000 mL of water
NaOH added	Not necessary to add		1%
Procedure	When each sample is immersed in water and in some cases with NaOH, it was cooled for 24 h, until it reaches room temperature. Every pulp is placed into Messmer disintegrator twice for two cycles of 10 min each.		
TEMPO/NaBr/NaClO (mmol·g ⁻¹)	0.1/1/5		0.1/1/10
OPs nomenclature ⁽¹⁾	FCL-5T-OP-1	FCL-5T-OP-2	CF-10T-OP-1
Before HPH	Centrifugation at 4000 rpm for 10 min		Filtration Ultra-Turrax at 10000 rpm for 15 min
HPH:			
Consistency in HPH	≈3	≈3	≈4
Number of passes and pressure	3x300 bar + 3x600 bar	3x300 bar	3x300 bar
CNFs Nomenclature ⁽²⁾	FCL-5T-CNF-1	FCL-5T-CNF-2	CF-10T-CNF-1

(1) OPs were identified according to the following nomenclature, “raw material name - TMO pre-treatment - oxidized pulp - numeration of each sample produced”. For example, FCL-5T-OP-1 is equivalent to: Fresh Cotton

- Linters (FCL) - concentration of NaClO used in the TMO oxidation reaction (5T)- Oxidized pulp (OP)- (1) numeration of every cellulose fibers produced.
- (2) The fibrillated fibers were identified according to the following nomenclature, “raw material name - TMO pre-treatment - cellulose nanofibers - numeration of each sample produced”. For example, FCL-5T-CNF-1 is equivalent to: Fresh Cotton Linters (FCL)- concentration of NaClO used in the TMO oxidation reaction (5T)- Cellulose Nanofibers (CNF) -(1) numeration of every cellulose nanofibers produced.

Pre-treatment of the raw material

According to the raw material, different pre-treatment approaches and disintegration conditions were explored to promote the separation of the cotton fibers. In the case of FCL, a pre-treatment was not required. However, for CF sample, the following steps were performed: manual cutting of the sample, milling and NaOH addition for the disintegration of the fibers.

TMO reaction

TMO reaction was conducted in a Biostat B plus reactor with an impeller agitation of 250-300 rpm and pH was kept around 10-10.5 during the reaction. For FCL, TEMPO/NaBr/NaClO dosages were 0.1/1/5 mmol·g⁻¹. However, for CF raw material, the NaClO dose has increased to double (10 mmol·g⁻¹ NaClO) to obtain higher fibrillated cellulose.

Before starting the TMO reaction, the disintegrated pulp was diluted at 1 wt.% of consistency and mixed with TEMPO and NaBr reagents. Then, NaClO was slowly added to the reactor to maintain the pH in 10-10.5. For the OPs FCL-5T-OP-1 and FCL-5T-OP-2, a concentration of 7.12% (w/v) NaClO was used and for CF-10T-OP-1, a concentration of 6.86% (w/v) NaClO was used during the TMO reaction. When all the NaClO was inside the reactor, NaOH of 2 mol·L⁻¹ was slowly introduced to remain pH almost constant around 10-10.5.

In order to ensure the end of the reaction, a redox titration of the reaction medium was conducted to ensure that all the NaClO was consumed. The method to determine the medium's NaClO content was a multi-step method (Lewin & Avrahami et al., 1955). 25 mL of sample from the reactor were added in an Erlenmeyer flask with 15 mL of distilled H₂O, 20 mL of 2 mol·L⁻¹ HCl and 10% (w/w) KI solution. The mixture was titrated with 0.13 mol·L⁻¹ Na₂S₂O₃, adding 2 mL of 0.2 mol·L⁻¹ starch solution as color indicator until the sample became transparent. The NaClO content is calculated as one-half of the titrated thiosulfate content (Xu et al., 2022).

Then, the OPs were filtrated and then washed through filtration steps using 3 L of distilled H₂O. Finally, the OPs were stored at 4 °C for characterization purposes and homogenized to produce the fibrillated cellulose.

High Pressure Homogenizer

Fibrillated cellulose was produced using a Panda PLUS homogenizer at lab scale, manufactured by GEA Niro Soavi. The process involved passing the OP suspension, previously diluted until a

consistency of 3% in the case of FCL and 4% for CF (Table 3.2.), through the homogenizer multiple times, subjecting the suspension to high pressure and shear forces that fibrillated the cellulose fibers into micro and nanofibers. The conditions applied in the homogenization step, such as consistency, number of passes and pressure applied, depend on the specific sample and are detailed in Table 3.2. A few drops of glutaraldehyde solution of biocide were added to all fibrillated cellulose samples to prevent the growth of microorganisms, and finally each sample was stored at 4 °C.

3.1.2 Oxidized pulps and fibrillated cellulose characterization

The following aspects were considered to characterize each OP: *consistency*, *ash content*, *carboxylic groups (CG)*, *aldehyde groups (AG)*, *aspect ratio (AR)*, *polymerization degree (PD)*, and *Optical Microscopy (OM)*.

On the other hand, fibrillated cellulose is characterized regarding *consistency*, *ash content*, *cationic demand (CD)* and *Transmission Electron Microscopy (TEM)*.

Consistency

The solid content in the OPs and in the fibrillated cellulose was oven-dried at 65 °C for approximately 24 h to guarantee a constant weight. The consistency reported in this work results of an average for three samples (Xu et al., 2022) and can be determined by Eq. (3.1):

$$\text{Consistency} = \frac{m_{\text{dry}}}{m_{\text{moist}}} \times 100 \quad (3.1)$$

Where *consistency* is wt.% and m_{dry} is the mass of sample after 24 h in the oven and m_{moist} refers to the initial mass.

Ash content

The content of inorganic salts resulting from the cellulose source and the TMO reaction was according to (Xu et al., 2022), conforms ISO 1762 standard, where the dry mass of each sample after oven-dried at 65 °C for 24 h was placed in a muffle furnace at 525 °C. The ash content was determined from the average of three samples and is obtained by Eq. (3.2):

$$\text{Ash content} = \frac{m_{\text{ash}}}{m_{\text{dry}}} \times 100 \quad (3.2)$$

where *ash content* is wt.% and m_{ash} corresponds to the mass obtained after the muffle furnace and m_{dry} is the mass of sample after 24 h in the oven.

Carboxylic Groups (CG)

The amount of CG was determined by a conductimetric titration, and based on this parameter, the degree of oxidation reached after the oxidation reaction was determined. So, 0.15 g of dry

pulp was added 5 mL of NaOH $0.01 \text{ mol} \cdot \text{L}^{-1}$ and distilled water until a weight of 55-60 g. To adjust the pH around 2.5-2.8, a few drops of HCl $0.5 \text{ mol} \cdot \text{L}^{-1}$ were added to protonate the CG (Sanchez-Salvador et al., 2019; Xu et al., 2022). The conductivity values were recorded for every $0.1 \text{ mol} \cdot \text{L}^{-1}$ of NaOH added to the sample under continuous stirring. The typical curve of conductivity ($\text{mS} \cdot \text{cm}^{-1}$) as a function of NaOH (mL) added is shown in Figure 3.2.

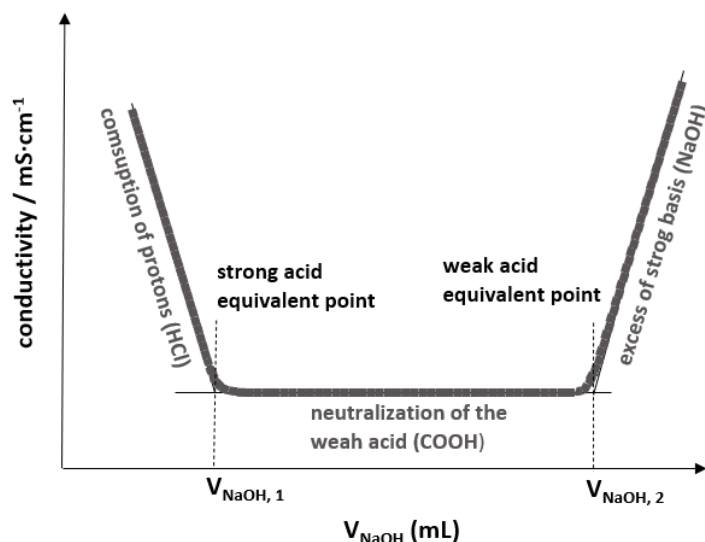


Figure 3.2. Typical conductivity curve of conductivity ($\text{mS} \cdot \text{cm}^{-1}$) vs NaOH (mL) of oxidized pulp. Adapted from (Foster et al., 2018).

The CG were determined according to Eq. (3.3):

$$CG = \frac{(V_{NaOH,2} - V_{NaOH,1}) \cdot NaOH}{\text{mass of dry pulp}} \quad (3.3)$$

where CG is $\text{mmol} \cdot \text{g}^{-1}$ of dry pulp, $V_{NaOH,1}$ and $V_{NaOH,2}$ corresponds to the volume, in mL, of NaOH in the initial and final values where the conductivity remains constant, respectively and NaOH is expressed in $\text{mol} \cdot \text{L}^{-1}$ (Xu et al., 2022). The CG was reported from triplicates.

Aldehyde Groups (AG)

The determination of AG was carried out with the reaction, where 0.05 g of dry oxidized cellulose fibers was diluted with distilled water until a weight of 19-20 g. In order to adjust the pH to 3.5, a solution of HCl $0.05 \text{ mol} \cdot \text{L}^{-1}$ was mixed and 0.5 mL of hydroxylamine hydrochloride (5 wt. %) was also added to the sample. To revert the pH to 3.5, NaOH of $0.0107 \text{ mol} \cdot \text{L}^{-1}$ was added until the pH solution did not change anymore (Xu et al., 2022).

The average determination of AG in mmol CHO per gram of dry oxidized cellulose fibers was determined by Eq. (3.4):

$$AG = \frac{V_{NaOH} \cdot NaOH}{m_{dry}} \times 10^3 \quad (3.4)$$

where AG is $\text{mmol}\cdot\text{g}^{-1}$ of dry pulp m_{dry} is determined from consistency of OP obtained from Eq. (3.1). NaOH is expressed in $\text{mol}\cdot\text{L}^{-1}$ and V_{NaOH} corresponds to the volume added, in mL.

Polymerization Degree (PD)

PD was performed in accordance with (Sanchez-Salvador et al., 2019; Xu et al., 2022), conforms ISO 5351 standard. For the determination of PD , 0.15 g of dry pulp was added to distilled water until an exact weight of 25 g was achieved. A volume of 25 mL of Bis(ethylenediamine)copper(II) hydroxide solution (CED) and 4 coins of 1 cent as source of copper was also mixed into the sample. The samples were stirred at a moderate agitation for 10-15 min to promote the homogenization. As a reference for time determination, a sample was also prepared and measured with only 25 g of distilled water and 25 mL of CED.

The PD determination is given by Eq. (3.5):

$$\eta_s = K \cdot PD^a \quad (3.5)$$

in which η_s corresponds to the intrinsic viscosity of the sample, in $\text{mg}\cdot\text{g}^{-1}$, and K and a are parameters that change according with PD value. In this particular case, $PD > 950$, $K = 2.28 \text{ mL}\cdot\text{g}^{-1}$ and $a = 0.76$. On the other hand, if $PD < 950$, $k = 0.42$ and $a = 1 \text{ mL}\cdot\text{g}^{-1}$. (Sanchez-Salvador et al., 2019; Xu et al., 2022).

Aspect ratio (AR)

The AR was determined through a simplification gel point (GP) measurement, in accordance with to the approach proposed by (Sanchez-Salvador et al., 2021).

To determine the GP , 0.2 wt.% of each OPs were mixed in 250 mL of distillate water and 100 μL of crystal violet 0.1 wt.% were also added to provide a better visualization of the suspension sedimentation. The samples were agitated for 15 s to promote the homogenization. During a few days, the sedimentation height (H_s), and the total height (H_0) were measured until a stable sedimentation height was reached. Figure 3.3 depicts the GP sediment height for different samples, during different days.

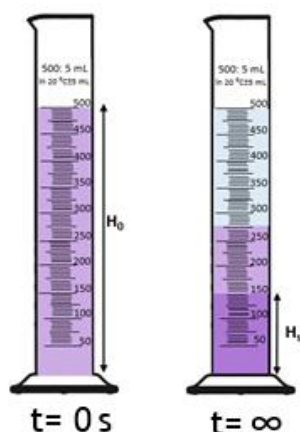


Figure 3.3. General overview of GP sediment height over the time. Adapted from (Sanchez-Salvador et al., 2022).

Once the constant sedimentation height is known, then the GP can be obtained from Eq. (3.6):

$$GP = \lim_{\frac{H_s}{H_0} \rightarrow 0} \left(\frac{dC_0}{d\left(\frac{H_s}{H_0}\right)} \right) = \frac{C_0(i)}{\left(\frac{H_s}{H_0}(i)\right)} \quad (3.6)$$

which GP is $\text{kg mmol} \cdot \text{m}^{-3}$, H_0 corresponds to the initial suspension height and H_s to the stable sediment height.

From the determination of GP , it becomes feasible to calculate AR , which can be obtained according to Eq. (3.7):

$$AR = 5.98 \sqrt{\frac{1000}{GP}} \quad (3.7)$$

AR was determined considering a Crowding number theory and a cellulose density of $1500 \text{ kg} \cdot \text{m}^{-3}$ (Sanchez-Salvador et al., 2020).

Microscopy analysis

For the *Optical Microscopy (OM)* analysis, a drop of every OP was placed onto a glass slide covered by a coverslip. The microscopy analyses were conducted in a Zeiss Axio Lab.A1 optical microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) at 5x and 10x magnification depend on the OP's fibers size (Xu et al., 2022).

Transmission Electron Microscopy (TEM) analysis was conducted in a JEM 1400 microscope (JEOL, Tokyo, Japan) from the National Centre of Electronic Microscopy at UCM (Madrid, Spain). Each fibrillated cellulose was diluted at 0.1 wt.% using distilled water (Xu et al., 2022).

Cationic Demand (CD)

CD is a parameter that enables the identification of the anionic character of the fibers and can be calculated from an inverse colloidal titration (Sanchez-Salvador et al., 2020; Xu et al., 2022). In general, Poly-DADMAC is the most used titrant, and the function of this cationic polymer is related with the neutralization of the particle suspension surface (Blanco et al., 2018).

For CD determination, 0.1 wt.% of fibrillated cellulose was mixed in 20 mL of distilled water and an excess volume (40-80 mL) of poly-DADMAC $0.001 \text{ mol} \cdot \text{L}^{-1}$ was added to the sample. Then, the sample was kept in agitation for 24 h. The remaining unreacted poly-DADMAC was titrated with PesNa $0.001 \text{ mol} \cdot \text{L}^{-1}$ using a Mütek PCD05 particle charge detector (BTG Instruments GmbH, Herrsching, Germany) (Serra-Parareda et al., 2021; Xu et al., 2022).

CD is determined from Eq. (3.8):

$$CD = (c_{poly-DADMAC} \cdot V_{poly-DADMAC}) - (c_{PesNa} \cdot V_{PesNa}) \quad (3.8)$$

where CD is $\mu\text{eq}\cdot\text{g}^{-1}$, $c_{\text{poly-DADMAC}}$ and c_{PesNa} are expressed in $\text{mol}\cdot\text{L}^{-1}$, $V_{\text{poly-DADMAC}}$ represents the excess of poly-DADMAC volume added to the sample and V_{PesNa} corresponds to the required titration volume. CD was reported from triplicates.

Transmittance

In order to conduct the *transmittance* analyses, each fibrillated cellulose was diluted to 0.1 wt.% with distilled water to a total mass of 10 g (Xu et al., 2022). The wavelength measurements were carried out at 400, 600 and 800 nm on a Cary 50 Conc UV-visible spectrophotometer (Varian Australia PTI LTD, Victoria, Australia) spectrophotometer. As a reference, a solution containing only distillate water was used.

3.2 Formulation of pastes for additive manufacturing

As already mentioned, this work aims to provide a solution for the tons of useless clothes derived from the fashion industry. Therefore, different sources of cotton were tested as proof of concept in the formulation of pastes for AM. As a reference, pastes with virgin cotton, that correspond to FCL were tested. The pastes formulation consisted of different weight compositions of fibrillated cellulose, $\text{NaC}_6\text{H}_7\text{O}_6$ (Sigma-Aldrich, CAS-No: 9005-38-3) and distillate water.

3.2.1 Description of the process of pastes

A detailed description of the pastes production process for AM is shown in Figure 3.4. Initially, different formulations of fibrillated cellulose, H_2O and $\text{NaC}_6\text{H}_7\text{O}_6$ were combined and its rheological properties (viscosity, yield stress, flow curve and elastic recovery) were evaluated, in order to determine which ones are able to AM printing.

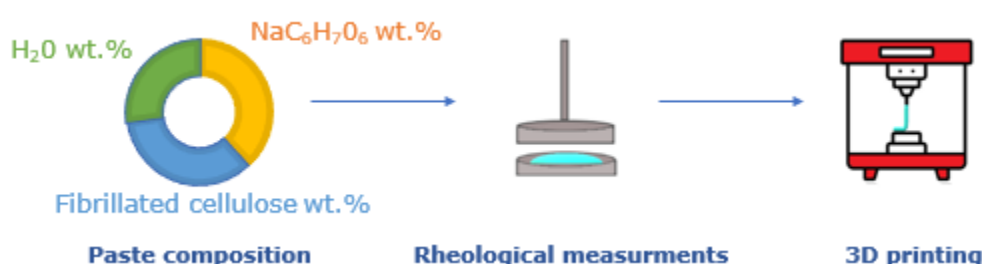


Figure 3.4. Overall of the production process production of the pastes. Adapted from (Nelson et al., 2021)

In the pastes' formulation, $\text{NaC}_6\text{H}_7\text{O}_6$ was used as a hydrogel for bioprinting, due to its ability to increase the water viscosity (Sauerwein et al., 2020). Moreover, since the $\text{NaC}_6\text{H}_7\text{O}_6$ provides the possibility of cross-linked via ion exchange, the reprint ability of the pastes is a feasible scenario, which is ensured by the presence of reversible ion cross-links in the sodium alginate. This is an excellent aspect of this work since it contributes to the recycling of pastes. For that, the printed material that initially possessed non-cross-linked ions is submerged in a solution of calcium chloride dehydrate 2 wt.% (VWR, CAS-No:10035-04-8) and occurred an ion exchange by the calcium

cation (Sauerwein et al., 2020). Studies reported that it was necessary to maintain the printed material submerged for 30 min (Sauerwein et al., 2020) and others for 24 h (Espinosa et al., 2019); however, it was noticed that the process of ion cross-linked was practically instantaneous and after 10 min the process was completed.

The first step was the study of five reference solutions of $\text{NaC}_6\text{H}_7\text{O}_6$ without cellulose fibers, and its compositions are summarized in Table 3.3. The mixing of $\text{NaC}_6\text{H}_7\text{O}_6$ and H_2O was promoted using a rotor-stator homogenizer (Micra GmbH D-9, Heitersheim, Germany) with the mixing time for 180 s for every sample.

Table 3.3. Composition of the reference solutions analyzed.

Sample	Mass of sodium alginate (g)	Mass of distilled water (g)
2.5 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$	0.6572	25.0036
5 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$	1.3167	25.0842
10 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$	2.6292	25.0888
15 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$	3.8540	25.0230
20 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$	5.2478	25.1570

Then, for the pastes' formulation, $\text{NaC}_6\text{H}_7\text{O}_6$ was added to the fibrillated cellulose, which consists of a solution of H_2O and cellulose fibers. Table 3.4 summarizes the pastes' formulation. Regarding its nomenclature, it was identified according to following structure: "name of the fibrillated cellulose, content of fibrillated cellulose: content of sodium alginate: content of water" present in the paste, in which the content for each component is in wt.%. For example, for FCL-5T-CNF-2, 2.3:5:92.7, means that the paste is obtained from FCL sample and is composed by 2.3 wt.% of fibrillated cellulose: 5 wt.% of sodium alginate and 92.7 wt.% of water. The nomenclature for the attribution FCL-5T-CNF-2 can be found in section 3.1.1.

The consistency of fibrillated cellulose used in the formulations, which can be found in section 4.1, is controlled by drying the sample in the oven at 65 °C or diluting the sample by adding water. Since for the fibrillated cellulose corresponding to FCL-5T-CNF-1 its consistency is 2.47 wt.%, for the development of the formulations FCL-5T-CNF-2, 5.2:2:89.8; FCL-5T-CNF-2, 5.9:2:92.1; FCL-5T-CNF-2, 5.9:10:84.1 and FCL-5T-CNF-2, 4.2:15:80.8 was necessary to concentrate the consistency, which was performed in an oven, at 65 °C.

In the case of CF-10T-CNF-1, 2.3:5:92.7 hydrogel formulation, since the original consistency is 3.29 wt.%, which can be found in Table 4.4, it was necessary to dilute the sample until reached a consistency around 2.47 wt.%.

Table 3.4. Fibrillated Cellulose, sodium alginate ($\text{NaC}_6\text{H}_7\text{O}_6$) and water compositions of every paste produced.

Sample	Consistency of the fibrillated cellulose (wt.%)	Fibrillated cellulose content (wt.%)	Sodium alginate (wt.%)	Water (wt.%)
FCL-5T-CNF-2, 2.3:5:92.7	2.47	2.3	5	92.7
FCL-5T-CNF-2, 2.4:2:95.6	2.47	2.4	2	95.6
FCL-5T-CNF-2, 5.2:2:89.8	5.48	5.2	5	89.8
FCL-5T-CNF-2, 5.9:2:92.1	6.07	5.9	2	92.1
FCL-5T-CNF-2, 5.9:10:84.1	6.56	5.9	10	84.1
FCL-5T-CNF-2, 4.2:15:80.8	4.95	4.2	15	80.8
FC-5T-CNF-1, 2.3:5:92.7	2.47	2.3	5	92.7
CF-10T-CNF-1, 2.3:5:92.7	2.47	2.3	5	92.7

Test for reprint ability of pastes for additive manufacturing

In order to evaluate the possibility of reprinting the paste, the printed material was immersed in a solution of 2 wt.% sodium citrate tribasic dihydrate ($\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$) (Sigma-Aldrich, CAS-No:6132-04-03) and H_2O . The printed material was grounded with a mortar and pestle to promote its milling before the immersion in a solution of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ immersed (Sauerwein et al., 2020). Table 3.5 shows the different proportions in wt%, of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and the printed material combined, with the aim to determine the composition of the components that ensure the reprint ability of the material. The rheological properties of the dissolved printed material were also analyzed, to check its reprint ability. This will further contribute to circular economy (Sauerwein et al., 2020).

In relation to FCL-5T-CNF-1, 2.3:5:92.7 and CF-10T-CNF-1, 2.3:5:92.7 two hypotheses have been tested: 1) remaining constant the content of sodium citrate tribasic dihydrate and 2) varying only the content of water added.

Table 3.5. Composition of the water (H₂O) added to each formulated paste, considering an addition of the addition of 50 wt.% of sodium citrate tribasic dihydrate (HOC(COONa)(CH₂COONa)₂·2H₂O).

Sample	Water (wt.%)
FCL-5T-CNF-2, 2.3:5:92.7	124
FCL-5T-CNF-1, 2.3:5:92.7	127
	249
CF-10T-CNF-1, 2.3:5:92.7	125
	249

3.2.2 Characterization of pastes - Rheological evaluation of the pastes

Quantitative rheological properties measurements were performed on the pastes formulations to characterize the printability, i.e. its extrudability and shape fidelity (Wang et al., 2018). For extrusion AM, the crucial parameters that determine a hydrogel paste as an acceptable candidate for AM are related with viscosity, shear stress, viscoelastic shear moduli and elastic recovery (Schwab et al., 2020). Figure 3.5 shows an extruder's sketch and identifies the role of each rheological measurement in the extruder process. At rest, the viscosity of the hydrogel formulation should be high since it is reported that an increase in the viscosity is related to a higher printing fidelity (Schwab et al., 2020). Moreover, it is crucial to develop a formulation that presents a shear-thinning behavior since it is associated to the easiness of extrusion and the initial preservation of the shape due to the decrease of viscosity as the result of the increase of the shear forces. Afterward the extrusion, the viscosity of the hydrogel formulation should increase due to the decrease of the shear rate, which enables to preserve the shape of the printed object.

The hydrogel formulation should also present viscoelastic properties since, during the extrusion, it is crucial that the paste flows through the nozzle with a low internal resistance. However, when the material is printed, the flow should immediately be stopped, and opposite internal forces to the deformation must prevail, contributing to the elastic shape retention. The viscoelastic properties are related to storage or elastic modulus (G') and loss or viscous modulus (G''). G' is associated with the amount of elastic energy stored during the deformation stage, that is related to the elastic shape retention. In contrast, G'' is associated with the amount of dissipated energy and, hence, is related to the viscous flow (Schwab et al., 2020).

Furthermore, the shape retention can be defined by the yield point, corresponding to the minimum stress applied for a deformation occur, in which G' and yield point are related to the amount of cross-linking or entanglements present in the paste developed (Murab et al., 2022; Schwab et al., 2020).

In the particular case of hydrogel pastes, the shape fidelity is determined by the flow behavior, but long-term shape stability is defined by the cross-linking established after the printed material is immersed in a calcium chloride solution.

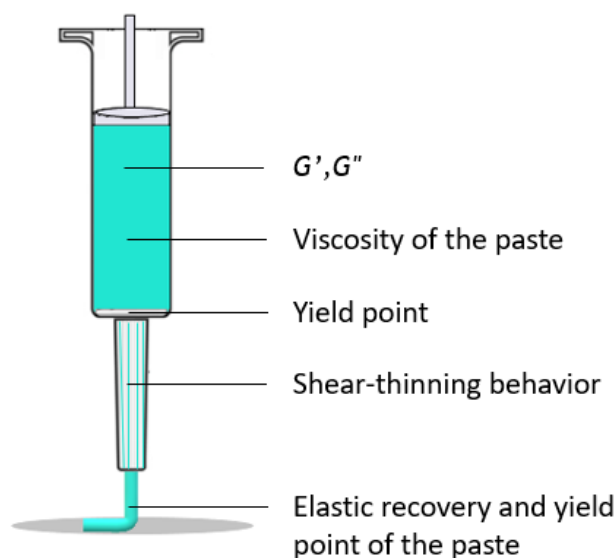


Figure 3.5. Rheological properties required for extrusion additive manufacturing to obtain a printable hydrogel ink and a shape fidelity of the printed paste. Adapted from (Schwab et al., 2020)

Table 3.6 summarizes the rheological properties measured, the role of each property for the extrudability and shape fidelity, and the rotational and oscillatory tests' conditions in this work.

A rheometer (Anton Paar, MCR 102e) using a cone plate system was used to study the rheological measurements with a diameter of 50 mm and an angle of 2°. The zero-gap established corresponds to 0.209 cm and a constant temperature of 20 °C was selected to perform all the measurements. The selected temperature for rheological measurements is the average temperature in the lab since the extrusion-based AM was the selected method.

Due to the limited quantity of fibrillated cellulose samples, rheological measurements were performed only once, and no triplicates were measured. However, the reliability of the results was assured by the recent equipment calibration for the supplier. Moreover, internal tests were conducted periodically to ensure the rheometer's calibration.

Rheological analyses of all the fibrillated cellulose produced in UCM (FCL-5T-CNF-1, FCL-5T-CNF-2, and CF-10T-CNF-1) and to the reference $\text{NaC}_6\text{H}_7\text{O}_6$ samples (2.5, 5, 10, 15 and 20 wt.%) were also conducted.

Table 3.6. Rheological properties required for extrusion printing.

Parameter	Purpose	Operating conditions	Graphical Representation
Flow behavior	For extrusion printing, a shear-thinning flow behavior, in where the viscosity decreases with the increase of shear rate ($\dot{\gamma}$) is indicated since it is related to extrusion easiness and initial shape fidelity (Schwab et al., 2020).	Rotational measurements with a shear rate between $0.01-1000\text{ s}^{-1}$	Viscosity (η), in $\text{Pa}\cdot\text{s}^{-1}$ as a function of the shear rate ($\dot{\gamma}$), in s^{-1} .
Yield stress	- Yield stress corresponds to the intersection of shear stress axis when a flow curve is performed, i.e., the shear stress condition for the flow; - Yield point corresponds to the limit of the linear viscosity range (LVE) in oscillatory tests; - In AM, yield stress can compensate the deformation induced by gravity or superficial tension; - It can provide information about an ink's ability to maintain its shape after extrusion (Schwab et al., 2020).	Flow Curve: Controlled of shear stress (τ) at $1 - 3000\text{ Pa}$ Viscoelastic Properties: Oscillatory measurements through a logarithmic ramp with a shear strain γ of $0.1\% - 100\%$ and a constant angular frequency (ω) of 1 Hz	Representation of the τ as a function of $\dot{\gamma}$. The amplitude sweep test is a plot of the storage modulus (G') and loss modulus (G''), in Pa, as a function of the shear stress (τ), in Pa.
Elastic Recovery	- Material's time response after submitted to shear deformation; - Provides recovery of the filament after extrusion outside the nozzle (Schwab et al., 2020).	Elasticity recovery test: strain deformation range at 0.5% , then at 200% and then 0.5% and a constant ω of 1 Hz	G' and G'' as a function of time, in s.

4 Results and Discussion

4.1 Characterization of oxidized pulps and fibrillated cellulose

The first part of this work studies the production of fibrillated cellulose from different sources of cotton: FCL and CF.

After the TMO reaction, each OP was characterized, and the results achieved for the various parameters are reported in Tables 4.1-4.3. Then, fibrillated cellulose was characterized, and the values obtained for each parameter are shown in Tables 4.4-4.5.

Table 4.1. Results of the characterization obtained for oxidized pulp.

Sample Name	Yield (%)	TMO time (h)	Consistency (wt.%)	Ash Content (wt.%)	CG (mmol·g ⁻¹)	AG (mmol·g ⁻¹)	PD Monomers units	AR (kg·m ⁻³)
FCL-5T-OP-1	73	1.5	2.97±0.01	5.5±0.7	1.18±0.22	0.27±0.03	168±5	22.0
FCL-5T-OP-2	77	1.1	4.32±0.07	11.3±0.9	1.21±0.41	0.29±0.01	124±4	20.4
CF-10T-OP-1	78	3	5.72±0.24	7.7±0.3	1.20±0.07	0.24±0.01	177±2	37.8

According to the yield results, Table 4.1, shows that all the pre-treatment steps to produce OPs possess a similar yield, which indicates a loss of cellulose fibers (around 25%) during the TMO reaction and after centrifugation, in the case of FCL, or during the TMO reaction and filtration, in the case of FCL sample. Depending on the raw material, the time required for TMO varies considerably. In the specific case of CF-10T-OP-1, the end of the reaction was assumed after 3 h, because when NaClO oxidant is used in a higher dose of 10 mmol·g⁻¹, after 5 h the reaction is still not completed. Moreover, for a reaction time too long, the pulp recovery decreases due to the dissolution of one part of the cellulose. In Appendix A, it is described the speed (rpm) used in the TMO for every sample, and the final volume necessary in the redox titration that was conducted to determine the end of the TMO.

In relation to the *consistency* reached OP, Table 4.1 reports the maximum values obtained after TMO.

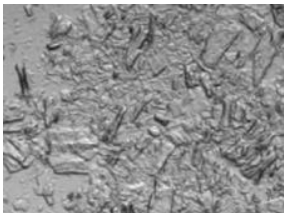
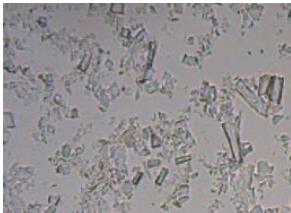
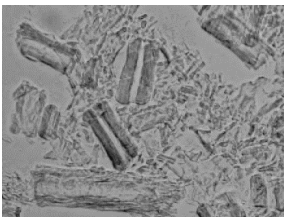
Regarding the *ash content*, this parameter could be considered negligible, if a reference percentage below 0.2% in relation to dry pulp is obtained. In the case of all OPs, since the *ash content* is above the reference percentage value, it cannot be considered insignificant and it means that NaCl, a by-product of TMO reaction, is formed and remained in the OPs, despite after the reaction all the OPs were washed with distilled H₂O.

(Xu et al., 2022) have reported that CG up to $1.1 \text{ mmol}\cdot\text{g}^{-1}$ in the OPs allow to produce highly fibrillated cellulose. In terms of CG amount, a high content of CG was achieved in the case of FCL-5T-OP-1. Consequently, when conducting a subsequent experiment using FCL raw material, the TMO conditions were kept and only the HPH conditions were modified. In relation to sample CF-10T-OP-1, TMO conditions used were ($0.1 \text{ mmol}\cdot\text{g}^{-1}$ TEMPO/ $1 \text{ mmol}\cdot\text{g}^{-1}$ NaBr/ $10 \text{ mmol}\cdot\text{g}^{-1}$ NaClO), because of previous experiments conducted, but there are not presented in this work, with a concentration of $5 \text{ mmol}\cdot\text{g}^{-1}$ of NaClO, the amount of CG obtained was very low and the value is $(0.78\pm0.22) \text{ mmol}\cdot\text{g}^{-1}$.

The amount of AG, which is produced during the intermediate reaction in the generation of CG, remains constant in the three cases, with values ranging between 0.24 and $0.29 \text{ mmol}\cdot\text{g}^{-1}$ of cellulose. This is because AG can form intra- and intermolecular hemiacetals with hydroxyl groups of cellulose. These hemiacetals exhibit resistance to further through TMO reaction due to steric hindrance, as explained by (Saito & Isogai, 2006).

During the OM analysis, different magnifications were used according to the raw material, as shown in Table 4.2. It was observed that the OPs from FCL were substantially smaller compared to CF samples, and due to this a magnification of 5x and 10x was used for FCL to obtain a better visualization of the cellulose fibers, while for CF only a magnification of 5x was required, since the cellulose fibers have larger dimensions. Additionally, since a large quantity of CG was present, indicating that an additional pre-treatment before HPH was not required to obtain fibrillated cellulose with a gel appearance. However, in the case of CF-10T-OP-1, the size of the fiber was relatively larger, and there was a concern regarding potential blockage during HPH. To mitigate this issue, an use Ultra-Turrax device was used to reduce the size of CF-10T-OP-1 fibers, ensuring proper homogenization.

Table 4.2. Optical Microscopy analyses results of the characterization of the oxidized pulp with 5x, for FCL-5T-OP-1, FCL-5T-OP-2 and CF-10T-OP-1 and 10x magnification for FCL FCL-5T-OP-1, FCL-5T-OP-2.

Sample	FCL-5T-OP-1	FCL-5T-OP-2	CF-10T-OP-1
OM 5x magnification			
OM 10x magnification			

Based on *AR* results, which is the ratio between the length and diameter of the fibers, it is possible to obtain the size of the fibers, using ImageJ software. Table 4.3 shows the length and diameter obtained for each OP. Based on the results, cellulose fibers from FCL-5T-OP-1 and FCL-5T-OP-2 contains similar length and diameter to each other, and its values are lower in comparison with CF-10T-OP-1.

Table 4.3. Length and diameter of each oxidized sample.

Sample identification	Average of length (μm)	Average of diameter (μm)
FCL-5T-OP-1	0.427	0.0194
FCL-5T-OP-2	0.428	0.0210
CF-10T-OP-1	1.917	0.0507

Table 4.4 summarizes *consistency*, *ash content*, *CD* and *transmittance* of the different fibrillated suspensions. *Ash content* of the fibrillated cellulose suspensions was ranging from 8.2 to 9.6 wt.% due to the presence of some by-products of the TMO reaction.

Table 4.4. Results of the characterization obtained for fibrillated cellulose.

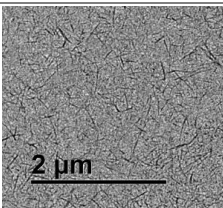
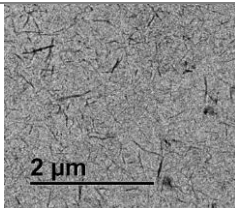
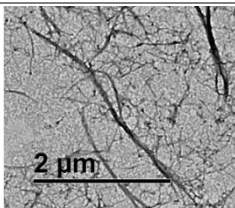
Sample Name	<i>Consistency</i> (wt.%)	<i>Ash Content</i> (wt.%)	<i>CD</i> ($\mu\text{eq}\cdot\text{g}^{-1}$)	<i>Transmittance</i> (%)		
				400 (nm)	600 (nm)	800 (nm)
FCL-5T-CNF-1	2.49 $\pm$ 0.01	8.6 $\pm$ 0.4	802 $\pm$ 1	93	90	80
FCL-5T-CNF-2	2.47 $\pm$ 0.05	9.6 $\pm$ 0.4	1.6 $\times$ 10 ³ $\pm$ 3	77	68	52
CF-10T-CNF-1	3.29 $\pm$ 0.04	8.2 $\pm$ 0.2	2.1 $\times$ 10 ³ $\pm$ 0.5	47	33	15

In terms of *CD*, it should be expected that samples of the same material with the same concentration of NaClO would exhibit similar *CD* values. Additionally, an increase in NaClO concentration from 5 to 10 mmol $\cdot$ g⁻¹, would like lead to a higher *CD* due to the increased amount of CG in the sample. These expectations align with the obtained results, where higher *CD* values were achieved at higher oxidation conditions. However, in FCL-5T-CNF-1 and FCL-5T-CNF-2, despite using the same raw material and oxidant concentration, *CD* values present some variations. On the other hand, CF-10T-CNF-1 achieved a higher *CD* value, attributed to the doubled concentration of oxidant used. Overall, although the expected trend of increasing *CD* with higher oxidant concentration holds true, some variations and dispersion in *CD* values were observed, particularly in the FCL-5T-CNF-1 and FCL-5T-CNF-2 samples.

In relation to the *transmittance*, FCL samples exhibit higher transmittance values compared to CF-10T-CNF-1. A possible reason that explains these differences may be related to the number of passes in the HPH that are detailed in Table 3.2. Specifically, FCL-5T-CNF-1, which undergoes a greater number of passes in HPH compared to FCL-5T-CNF-2, displays higher *transmittance* values,

revealing higher degree of homogeneity and dispersion of fibrillated fibers in the solution. On the other hand, for CF raw material, the *transmittance* values achieve at the same HPH conditions as FCL-5T-CNF-2 reveals lower values of *transmittance*, indicating that fibers are less fibrillated. Based on the results obtained, particularly for FCL-5T-CNF-1 and FCL-5T-CNF-2, the number of passes in HPH could influence the *transmittance*, which leads to a higher *transmittance* values, since both fibrillated cellulose were produced under the same cellulose source and TMO conditions, differing only each other with the HPH conditions (number of passes and pressure applied). *TEM* images confirm that the fibrillated cellulose obtained from FCL raw material possesses a more homogeneous and distributed fibrils, as evidence by the higher *transmittance* values (Table 4.4.). In contrast, *TEM* images of fibrillated cellulose from CF raw material reveal the presence of fibrils with varying sizes, indicating a heterogeneous size distribution of the fibers (Table 4.5). Therefore, the *TEM* images, coupled with the *transmittance* values, provide evidence of the distinct characteristics of fibrillated cellulose derived from FCL and CF raw materials.

Table 4.5. TEM analyses results of the characterization of fibrillated cellulose.

Sample	FCL-5T-CNF-1	FCL-5T-CNF-2	CF-10T-CNF-1
<i>TEM</i>			

From *TEM* analyses, it is verified that the samples provided by FCL raw material have a more homogeneous and distributed fibrillated cellulose, which differs from CF, in which fibrillated cellulose of different sizes are visible, evidencing the heterogeneity of size distribution of the fibers.

4.2 Evaluation of the production of fibrillated cellulose pastes for additive manufacturing

The formulation of cellulose-based paste and the rheological results are here described. In the next sections, the following parameters were studied: 1) the influence of $\text{NaC}_6\text{H}_7\text{O}_6$ in the rheological properties; 2) the influence of fibrillated cellulose; 3) the influence of the number of passes in the HPH; 4) the influence of cotton raw materials in the AM pastes. The first two studies were performed for FCL-5T-CNF-2.

Viscosity curves and amplitude sweep tests are reported for each formulation studied. For sake of summarizing, the flow curve is not represented here. However, the amplitude sweep tests report identical rheological properties as in flow curves. The elastic recovery was determined from

equation B.1 described in Appendix B using the results of the elastic recovery test. The elastic recovery of each sample is summarized for each paste formulation by the end of this section.

The measurement of rheological properties of $\text{NaC}_6\text{H}_7\text{O}_6$ solutions (reference case) and samples with fibrillated cellulose was the first task of this work. The rheological results of solutions of $\text{NaC}_6\text{H}_7\text{O}_6$ are described in Section 4.2.1. Appendix B shows the rheological measurements conducted to the samples of fibrillated cellulose obtained at UCM.

4.2.1 Influence of sodium alginate in additive manufacturing

The rheological behavior of solutions of $\text{NaC}_6\text{H}_7\text{O}_6$ is here studied. Figure 4.1 shows the viscosity curve, i.e. the dependence of the viscosity (η), in $\text{mPa}\cdot\text{s}$ with the shear rate ($\dot{\gamma}$), in s^{-1} . The viscosity curve shows that the initial viscosity, which corresponds to zero shear rate, increases with $\text{NaC}_6\text{H}_7\text{O}_6$ content. For concentrations equal to or below 5 wt.% of $\text{NaC}_6\text{H}_7\text{O}_6$, Figure 4.1 shows that the viscosity remains constant even with the increase of shear rate, which indicates a Newtonian fluid behavior. On the other hand, with the increase of $\text{NaC}_6\text{H}_7\text{O}_6$ content from 10 to 20 wt.%, the viscosity decreases substantially with the increase of the shear rate, showing the shear-thinning behavior of the formulations.

The LVE region, yield point and flow point were determined from the amplitude sweep test, as shown in Figure 4.2. Amplitude sweep tests were only carried out for formulations with a composition of $\text{NaC}_6\text{H}_7\text{O}_6$ between 10 to 20 wt.%, since these formulations correspond to non-Newtonian fluids. For a concentration between 10 and 15 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$, G'' is always greater than G' , i.e. samples present a liquid-like behavior. Furthermore, the inexistence of the intersection between G' and G'' shows that there is no flow point. On the other hand, 20 wt.% $\text{NaC}_6\text{H}_7\text{O}_6$ formulation exhibits a solid-like behavior, since G' is higher than G'' in the LVR, and there is the intersection of G' and G'' . Furthermore, the crossover of the G' and G'' indicates that the formulation has a flow point of approximately 1200 Pa, that corresponds to the threshold for the deformation permanently.

Considering the yield point, which coincides with the limit of LVE region and corresponds to the stress that needs to be exceeded for deformation to occur, the formulations with a concentration of 10, 15 and 20 wt.% of $\text{NaC}_6\text{H}_7\text{O}_6$ present a yield point near to 20, 100 and 220 Pa, respectively. In addition, an increase of $\text{NaC}_6\text{H}_7\text{O}_6$ is related to higher G' and G'' values, higher yield point, and a lower gap between G' and G'' .

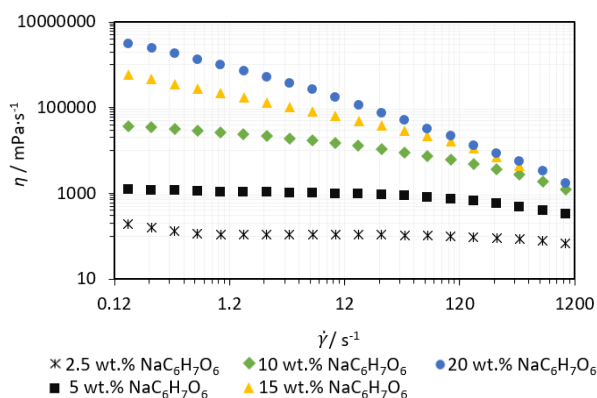


Figure 4.1. Evaluation of the rheological properties of the viscosity for different $\text{NaC}_6\text{H}_7\text{O}_6$ content in the hydrogel formulation.

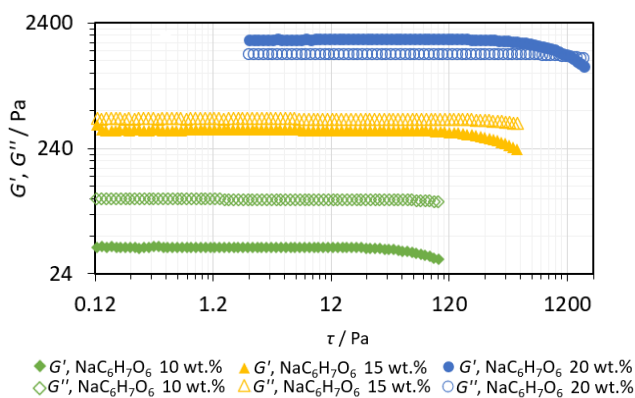


Figure 4.2. Evaluation of the rheological properties of the amplitude sweep test for $\text{NaC}_6\text{H}_7\text{O}_6$ hydrogel formulations between 10 and 20 wt.%.

4.2.2 Influence of sodium alginate in the hydrogel formulations

Formulation using fibrillated cellulose, H_2O and $\text{NaC}_6\text{H}_7\text{O}_6$ were tested, keeping the fibrillated cellulose and H_2O content constant and varying the amount of $\text{NaC}_6\text{H}_7\text{O}_6$. The influence of $\text{NaC}_6\text{H}_7\text{O}_6$ in the hydrogel formulations are 2.4:2:95.6 and 2.3:5:92.7; 5.2:2:92.1 and 5.9:10:84.1 (fibrillated cellulose: $\text{NaC}_6\text{H}_7\text{O}_6$: H_2O). The results in Figure 4.3 a) and Figure 4.4 a) show that the viscosity of the hydrogel formulations increases with the increase of $\text{NaC}_6\text{H}_7\text{O}_6$ content, which is related to a high print shape fidelity. Moreover, all the formulated hydrogel formulations show from the viscosity curve a shear-thinning behavior.

Figure 4.3 b) and Figure 4.4 b) show the amplitude sweep tests, where an increase of $\text{NaC}_6\text{H}_7\text{O}_6$ is related to a higher and closer value of G' and G'' . Moreover, an increase of $\text{NaC}_6\text{H}_7\text{O}_6$ is also associated to the increase of shear stress related to the LVE region, yield point and flow point. For 2.4:2:95.6, the limit of LVE or yield point is in the range between 4 and 8 Pa, and the flow point is around 40-50 Pa. For 2.3:5:92.7, the LVE region or yield point is around 10 Pa, and the flow point is in the range of 100-120 Pa, as shown in Figure 4.3 b). For 5.9:2:92.1, the LVE region and yield point are approximately 40-48 Pa, and a flow point is between 160 and 200 Pa. For 5.9:10:84.1, the LVE region or yield point is in the range of 48-64 Pa, and the flow point is approximately 560-800 Pa, as shown in Figure 4.4 b).

In all the formulations, except for 2.3:5:92.7 (Figure 4.3 b)), G'' exhibits a constant value in the LVE, followed by an increase until it reaches a maximum peak and then has a sharp decrease. This behavior is associated to the presence of micro-cracks, where these individual bonds break and separate from the network starting to flow. During the test, the micro-cracks grow, until a macro crack formation occurs, the viscous behavior prevails, and the sample starts to flow.

Results show that the increase of $\text{NaC}_6\text{H}_7\text{O}_6$ content increases the viscosity of the formulations, but also increases the LVE region, yield point and flow point.

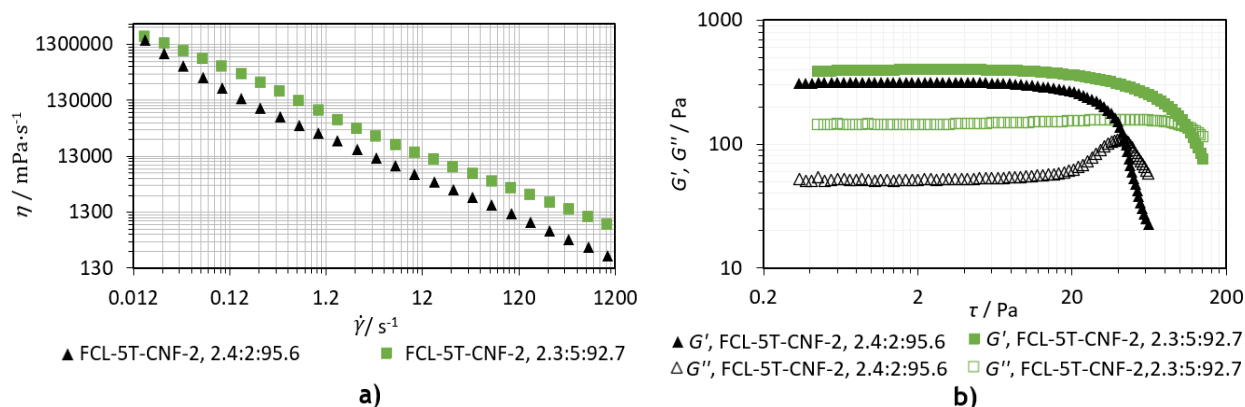


Figure 4.3. Evaluation of the rheological properties of varying the $\text{NaC}_6\text{H}_7\text{O}_6$ content (2% and 5 wt.%) in the hydrogel formulation for: a) viscosity curve and b) amplitude sweep test.

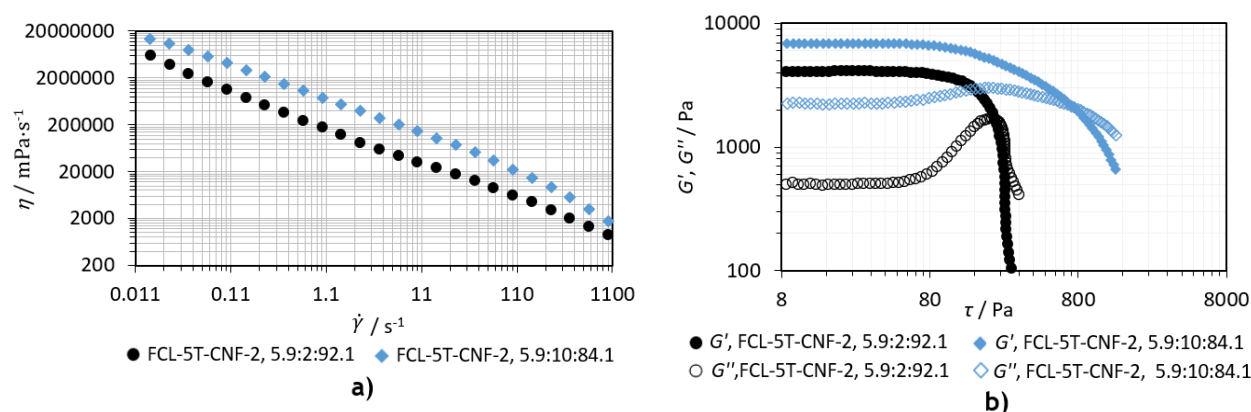


Figure 4.4. Evaluation of the rheological properties of varying the $\text{NaC}_6\text{H}_7\text{O}_6$ content (2% and 10 wt.%) in the hydrogel formulation for: a) viscosity curve and b) amplitude sweep test.

4.2.3 Influence of fibrillated cellulose in hydrogel formulation

Formulations with different contents of fibrillated cellulose, keeping constant the content of $\text{NaC}_6\text{H}_7\text{O}_6$ and H_2O , were tested to determine the effect of fibrillated cellulose. The formulations tested in this section are 2.3:5:92.7 and 5.2:5:89.9; 2.4:2:95.6 and 5.9:2:92.1, in which all the formulations were developed from FC-5T-CNF-2.

Figure 4.5 a) and Figure 4.6 a) show that increased fibrillated cellulose content in the hydrogel formulations is associated with higher viscosity. Therefore, higher content of fibrillated cellulose and $\text{NaC}_6\text{H}_7\text{O}_6$ contributes to a rise the zero viscosity, and so 5.2:5:89.8 and 2.3:5:92.7 formulations have a slight increase in viscosity in comparison to 5.9:2:92.1 and 2.4:2:95.6, respectively. For all formulations, the variation of fibrillated cellulose content does not change the fluid behavior, which is shear-thinning fluid. For 5.2:5:89.8, the LVE region or yield point is around 12.3-20.5 Pa, and the flow point is in the range of 287-410 Pa, as shown in Figure 4.5 b).

Figure 4.5 b) and Figure 4.6 b) show the amplitude sweep test. A high content of fibrillated cellulose is associated with a high value of G' and G'' .

From the experiments performed, a higher content of fibrillated cellulose in the hydrogel formulations increases the viscosity of the hydrogel, the shear stress of the LVE region limit or yield point, flow point and the values of G' and G'' .

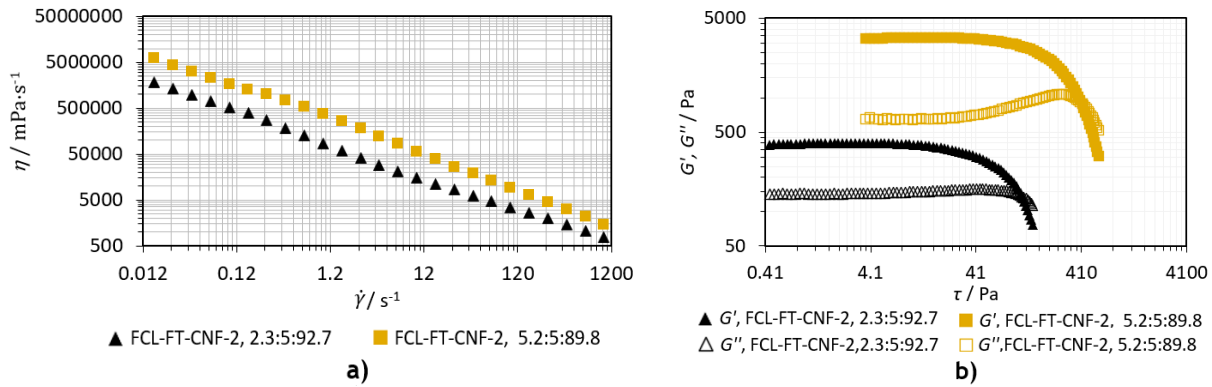


Figure 4.5. Evaluation of the rheological properties of varying the fibrillated cellulose content (2.3% and 5.2 wt.%) in the hydrogel formulation for: a) viscosity curve and b) amplitude sweep test.

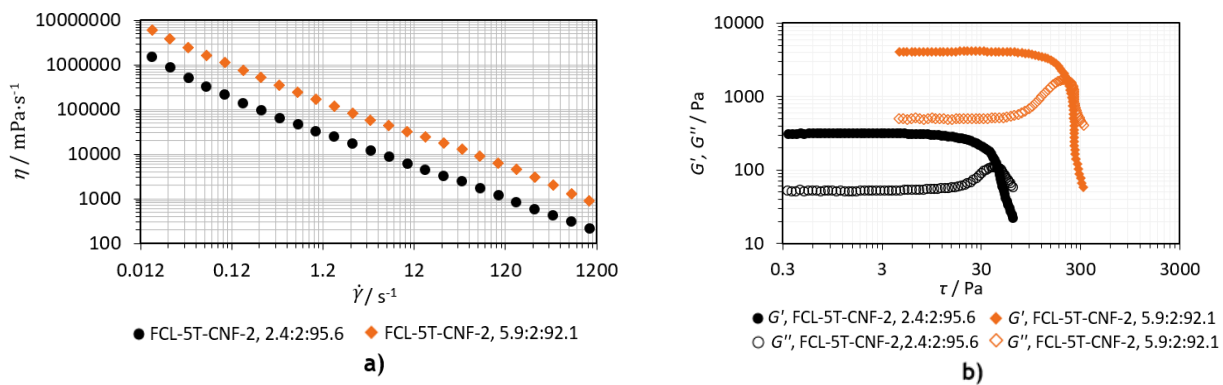


Figure 4.6. Evaluation of the rheological properties of varying the fibrillated cellulose content (2.4% and 5.9 wt.%) in the hydrogel formulation for: a) viscosity curve and b) amplitude sweep test.

4.2.4 Influence of High Pressure Homogenizer in the hydrogel formulations

The influence of working conditions in HPH on the rheological properties of pastes is studied here. Paste formulations with 2.3:5:92.7 were produced using fibrillated cellulose FCL-5T-CNF-1 and FCL-5T-CNF-2. The differences between FCL-5T-CNF-1 and FCL-5T-CNF-2 stem from the number of passes in HPH. FCL-5T-CNF-1 was produced for 3 passes at 300 bar followed by 3 passes at 600 bar. FCL-5T-CNF-2 was produced for 3 passes at 300 bar. This parameter is crucial since the energy boost associated with the HPH is too high.

Figure 4.7 a) shows, from the viscosity curve, that the number of passes in HPH does not change the fluid behavior. For both cases, the fluid has shear-thinning behavior.

Based on the amplitude sweep test in Figure 4.7 b), an increase in the number of passes in HPH is associated to a rise of G' , G'' , yield point and flow point, in which the flow point is approximately

130 Pa and, the yield point that coincides with LVE region limit is 12-15 Pa. On the other hand, the increase of number of passes at HPH, the existence of micro-cracks is observed.

Results show that the number of passes in HPH does not affect the rheological properties of the paste. It can be concluded that this factor does not affect and change the properties of hydrogels. Therefore, the conditions for 3 passes at HPH are enough to obtain rheological values similar to cellulose produced under 3 passes at 300 bar and 3 passes at 600 bar, enabling to decrease the energy consumption during the process.

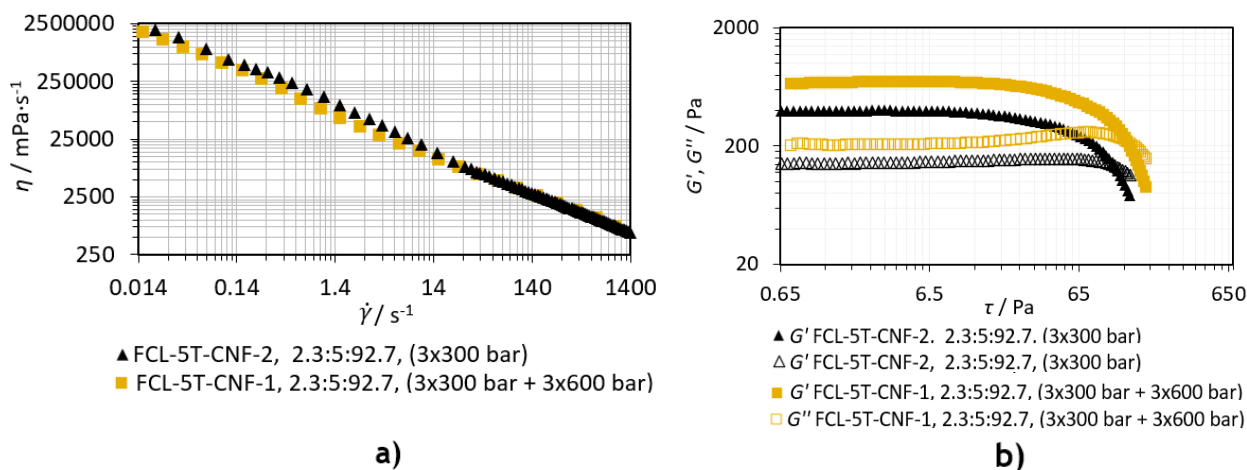


Figure 4.7. Evaluation of the rheological properties of varying the number of passes in High Pressure Homogenizer for FCL-5T-CNF-1 with 3 passes at 300 bar 3 passes at 600 bar and for FCL-5T-CNF-2 with only 3 passes at 300 bar, remaining the composition in the hydrogel formulation (2.3:5:92.7) for: a) viscosity and b) amplitude sweep test.

4.2.5 Influence of cotton raw materials in the hydrogel formulations

The effect of cotton raw material on the rheological measurements is evaluated in this section. CF-10T-CNF-1 fibrillated cellulose whose raw material was provided by a textile company was used as a filament for pastes and compared with the results for FCL-5T-CNF-2. The hydrogel formulation used in this work was 2.3:5:92.7. Both fibrillated cellulose samples were produced for 3 passes at 300 bar at HPH, but using different NaClO oxidant concentrations that were increased to the double. However, this aspect is only related to the amount of CG, and the use of $10 \text{ mmol}\cdot\text{g}^{-1}$ in CF-10T-CNF-1 enabled to obtain a similar quantity of CG as FCL-5T-CNF-2.

Figure 4.8 a) shows that both samples have identical viscosity values and a shear-thinning behavior. The amplitude sweep test in Figure 4.8 b) also shows that the rheological parameters do not considerably vary with the raw material source. In CF-10T-CNF-1, the yield point is 2.5-5 Pa, and the flow point is 50-100 Pa.

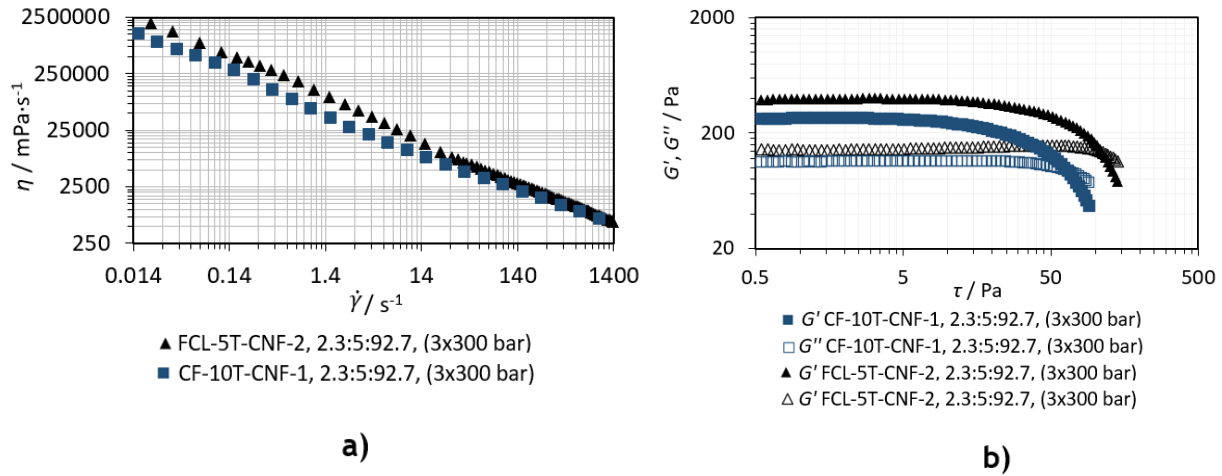


Figure 4.8. Evaluation of the rheological properties of varying the raw material (FCL and CF), remaining the composition in the hydrogel formulation (2.3:5:92.7) for: a) viscosity and b) amplitude sweep test.

Elastic recovery of the samples

The elastic recovery is really important for the AM pastes as previously mentioned. Table 4.5 summarizes the elastic recovery of the samples determined from equation B.1 demonstrating in Appendix B, using the parameters determined from the elastic recovery test.

Table 4.5. Percentages obtained for G' corresponding to elastic recovery test.

Hydrogel Formulation	G' (%)
FCL-5T-CNF-2, 2.3:5:92.7	61
FCL-5T-CNF-2, 2.4:2:95.6	56
FCL-5T-CNF-2, 5.2:5:89.8	65
FCL-5T-CNF-2, 5.9:2:92.1	58
FCL-5T-CNF-2, 5.9:10:84.1	58
FCL-5T-CNF-2, 4.2:15:80.8	59
FCL-5T-CNF-1, 2.3:5:92.7	63
CF-10T-CNF-1, 2.3:5:92.7	49

Based on the results in Table 4.5, the formulation of 2.3:5:92.7 for FCL-5T-CNF-1 has a high value of elastic recovery (63%). This sample was produced using the FCL sample for the following conditions at HPH: 3 passes at 300 bar and 3 passes at 600 bar.

Comparing the results for FCL-5T-CNF-2, all the hydrogel formulations present similar elastic recovery values, in a range between 56 and 65%.

However, it must be mentioned that the elastic recovery of 2.3:5:92.7 and 4.2:15:80.8 for FCL-5T-CNF-2 are similar to the results for FCL-5T-CNF-1 and so the first two formulations are the preference since they are associated to lower energy consumption. On the other hand, CF-10T-CNF-1 has the lowest values of elastic recovery.

4.3 Proof of concept for the development of fibrillated cellulose pastes

As a proof of concept, a hydrogel formulation with a composition of 2.3:5:92.7 using FCL-5T-CNF-2 was selected to verify its application as a fibrillate paste for AM. The rheological measurements performed for this formulation showed that this paste fills the requirements for printability: viscosity, G' , G'' , yield point, flow point, and elastic recovery considerably high. Moreover, this formulation has the following properties: i) the non-existence of micro-cracks in the amplitude sweep test; ii) the easy dissolution of $\text{NaC}_6\text{H}_7\text{O}_6$ in the hydrogel formulation; iii) the use of the initial consistency of CF-5T-CNF-2 sample, not requiring the adjust of consistency.

Then, the proof of the concept was extended to FCL-5T-CNF-1, 2.3:5:92.7 and CF-10T-CNF-1, 2.3:5:92.7 hydrogel formulations.

The printability of pastes was tested using two syringes with a diameter of 0.686 and 2 mm, considering that the diameter of the AM system is in between the value range of the syringes.

Figures 4.9-4.11 show the images of the pastes printed using the different syringes after approximately 10 min immersed in a solution of calcium chloride dehydrate 2 wt.%.

Results are a proof of concept that these pastes can be printed in an extruder-based AM system.

The proof of the concept reveals that the diameter of the syringe influences the thickness of the filament that is being dispensed to print the object, in which for a larger syringe (2 mm) the thickness is larger than for a syringe of 0.686 mm. Also, with a syringe of 2 mm, the filament was deposited at a constant flow, contrary to what happened to 0.686 mm syringe, in which a greater force had to be applied for the filament to flow.

Moreover, a proof of concept that consists in printing a four-layer structure was performed to FCL-5T-CNF-2; however, the structure collapsed.



Figure 4.9. Proof of the concept for 2.3:5:92.7 FCL-5T-CNF-2 sample: syringe diameter of 0.686 mm for 2-layer (left side) and syringe diameter of 2 mm for 2 and 3-layer structure (right side)

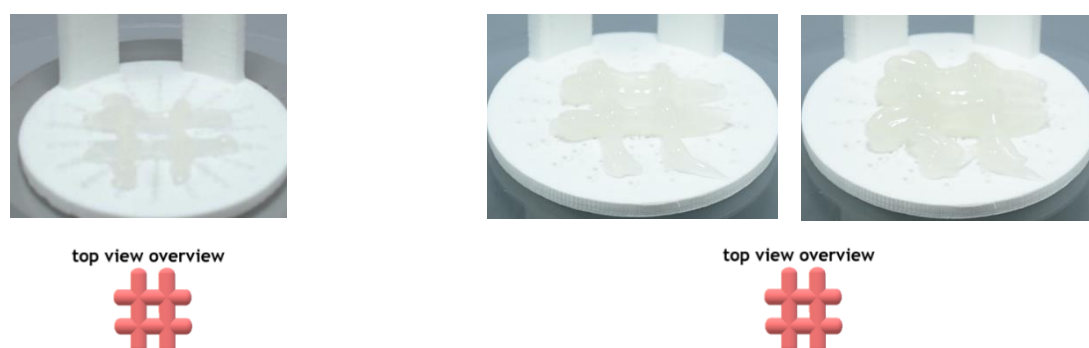


Figure 4.10. Proof of the concept for 2.3:5:92.7 FCL-5T-CNF-1 sample: syringe diameter of 0.686 mm for 2 layer structure (left side) and syringe diameter 2 mm for a 2-layer structure (right side).



Figure 4.11. Proof of the concept for 2.3:5:92.7 CF-10T-CNF-1 sample: syringe diameter of 0.686 mm for 2 layer structure (left side) and syringe diameter of 2 mm for a 2-layer structure (right side).

4.4 Regeneration of the fibrillated cellulose based pastes

The last task of this work consisted of the study of the potential of reprint ability of the extruded paste. Therefore, an aqueous solution of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ was prepared to add to the extruded material to regenerate the paste. With the finality to verify the reprint ability, rheological measurements were carried out for the hydrogel formulation of 2.3:5:92.7 for CF-10T-CNF-1, FCL-5T-CNF-1 and FCL-5T-CNF-2 fibrillated cellulose. The results were also compared to the corresponding hydrogel formulation previously reported.

Regarding the hydrogel formulation 2.3:5:92.7 using FCL-5T-CNF-2, the potential reprintable paste was only tested for 50 wt. % of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ in relation with the total mass of the printed object, and 124 wt.% of H_2O in relation to the total mass of the printed object.

Figure 4.12. shows that the regenerated paste has a yield point between 10 and 20 Pa and a flow point in a range of around 50 and 70 Pa. This result confirms the possibility of the reuse of pastes for extruder-based AM. Comparing these results with the initial paste after the extrusion in Figure 4.12., the regenerated paste has a lower flow point; however, the yield point increased slightly and the G' and G'' values increased around 100 times.

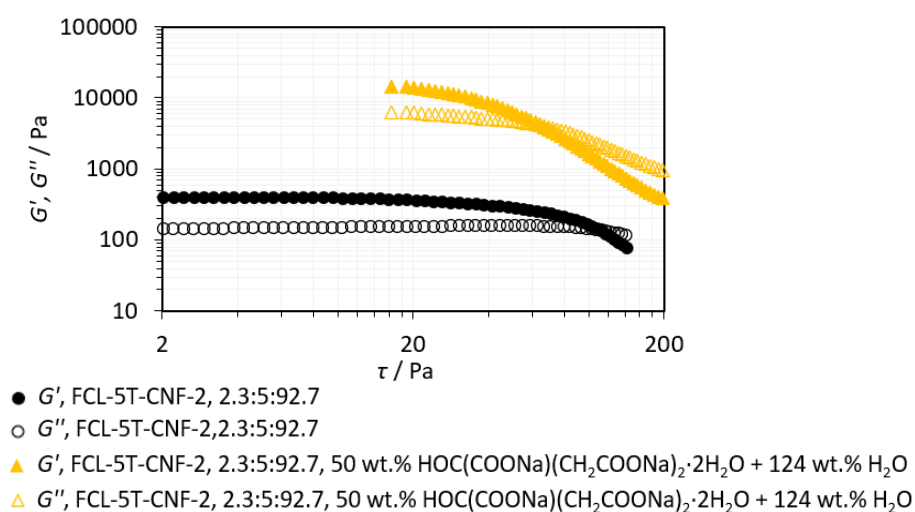


Figure 4.12. Evaluation of the amplitude sweep test for FCL-5T-CNF-2, with a composition of 2.3:5:92.7, considering the original printed paste and the regenerated paste with an addition of 50% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and 124% of H_2O in relation to the total mass of the printed object.

For the formulation of 2.3:5:92.7 using FCL-5T-CNF-1, the potential of reprint ability the paste was tested under two conditions: (1) addition of 50 wt.% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and 127 wt.% of H_2O and (2) addition of 50 wt.% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and 249 wt.% of H_2O in relation to the mass of the printed object. The aim of evaluating two pastes with different content of H_2O was an attempt to obtain the parameters of G' , yield point and flow point similar to the original paste, i.e. before the addition of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and H_2O . Figure 4.13 shows the representation of both regenerated pastes, as well as the original paste, in which reveals that the increase of H_2O content affects the rheological properties of the regenerated pastes, since it decreases G' , the yield point, and the flow point values.

Considering the condition of 50 wt.% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ and 127 wt.% of H_2O , the regenerated paste has a yield point at 4-5 Pa and a flow point between 35-45 Pa. In comparison to the original paste composed of 2.3:5:92.7, the regenerated paste has a lower yield point and flow point, the values of G' and are still above of the original paste formulation.

Regarding the regenerated paste with an addition of 249 wt.% of H₂O, the flow point is around 15-20 Pa. The inexistence of LVE region and thus the impossibility of identifying its limit and the yield point mean that it is not possible to conduct the test without compromising the structure of the formulation.

The elastic recoveries for the regenerated pastes are reported in Table 4.6. Based on the elastic recovery from the original paste, the regenerated paste of 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 127 wt.% of H₂O; 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 249 wt.% of H₂O presents an elastic recovery of G' higher.

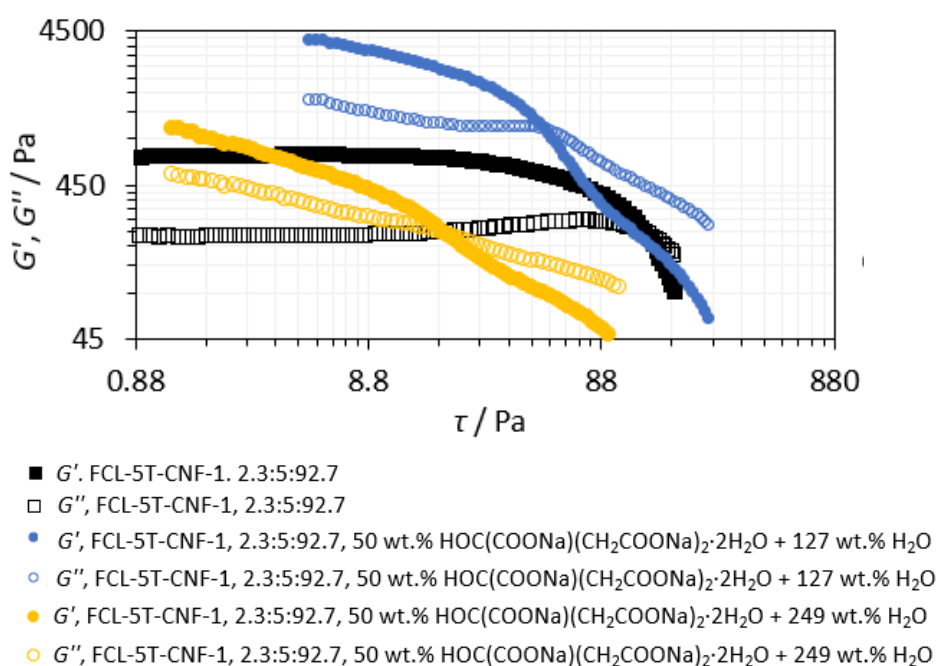


Figure 4.13. Evaluation of the amplitude sweep test for FCL-5T-CNF-1, with a composition of 2.3:5:92.7, considering the original printed paste, and regenerated paste with an addition of (1) 50% HOC(COONa)(CH₂COONa)₂·2H₂O and 124% of H₂O and (2) HOC(COONa)(CH₂COONa)₂·2H₂O and 249% of H₂O in relation to the total mass of the printed object.

Table 4.6. Percentages obtained for elastic recovery test corresponding for FCL-5T-CNF-1 fibrillated cellulose.

Paste regeneration	G' (%)
FCL-5T-CNF-1, 2.3:5:92.7	63
FCL-5T-CNF-1, 2.3:5:92.7, 50% of HOC(COONa)(CH ₂ COONa) ₂ ·2H ₂ O + 127% of H ₂ O	65
FCL-5T-CNF-1, 2.3:5:92.7, 50% of HOC(COONa)(CH ₂ COONa) ₂ ·2H ₂ O + 249% of H ₂ O	100

For the formulation of 2.3:5:92.7 using CF-10T-CNF-1, this sample was regenerated from two conditions: (1) addition of 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 125 wt.% of H₂O and (2) addition of 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 249 wt.% of H₂O in relation to the mass of the printed object, and as previous explained, the purpose of assessing two conditions varying

the content of H₂O was an attempt to obtain G' , yield point and flow point parameters similar to the original paste. Figure 4.14 shows the oscillatory sweep test conducted for both regenerated pastes, also containing the representation of the original paste without being added HOC(COONa)(CH₂COONa)₂·2H₂O and H₂O. The regenerated paste with a composition of 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 125 wt.% of H₂O has a yield point of 6-8 Pa and a flow point around 100 and 150 Pa. Based on the results, the regenerated paste has a yield point similar to the original paste, but its flow point increased. The G' and G'' values are still considerable higher than the original paste.

The results of the regenerated paste produced from 50 wt.% of HOC(COONa)(CH₂COONa)₂·2H₂O and 249 wt.% of H₂O showed the inexistence of yield point and the LVE limit. However, the flow point is around 50 and 60 Pa, which is similar to the original paste. Furthermore, G' and G'' values are still considerably higher than the original paste, and additional experiments could be performed to obtain other results more similar with the original paste. Besides that, as mentioned previously, an increase of H₂O decreases G' and G'' yield and flow points.

Table 4.7 provides the elastic recovery percentages for the regenerated and original pastes. The results show similar elastic recovery values for the regenerated pastes, in which the elastic recovery obtained for these pastes is higher than the original paste.

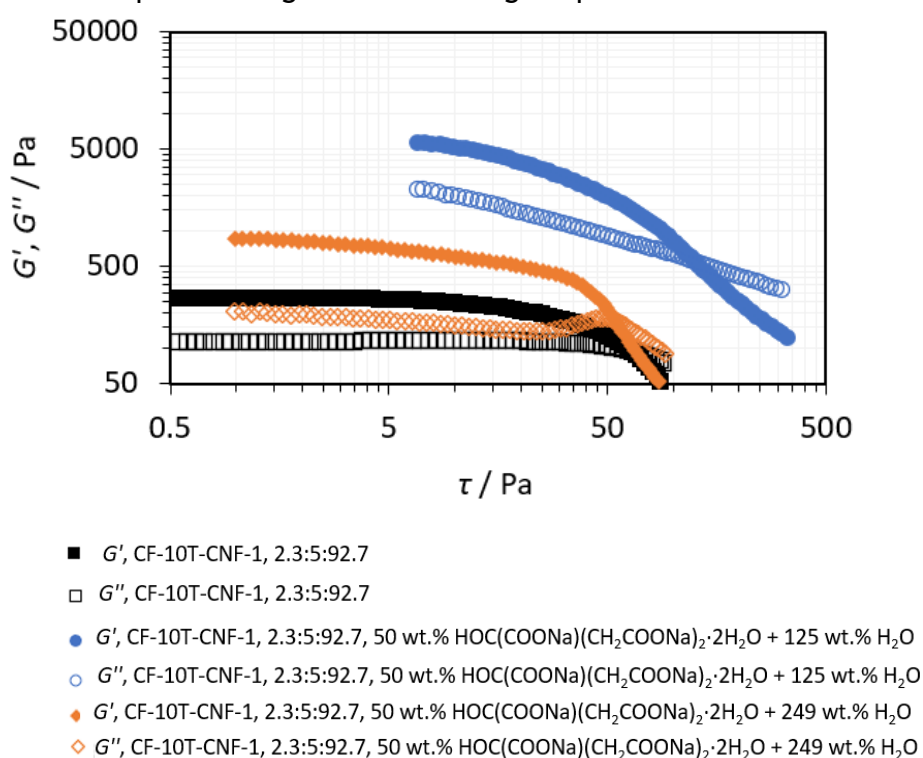


Figure 4.14. Evaluation of the amplitude sweep test for CF-10T-CNF-1, with a composition of 2.3:5:92.7, considering the original printed paste, and regenerated paste with an addition of (1) 50% HOC(COONa)(CH₂COONa)₂·2H₂O and 125% of H₂O and (2) HOC(COONa)(CH₂COONa)₂·2H₂O and 249% of H₂O in relation to the total mass of the printed object.

Table 4.7. Percentages obtained for G' and G'' corresponding to elastic recovery test for CFL-10T-CNF-1 fibrillated cellulose.

Paste regeneration	G' (%)
CF-10T-CNF-1, 2.3:5:92.7	49
CF-10T-CNF-1, 2.3:5:92.7, 50% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ + 125% of H_2O	55
CF-10T-CNF-1, 2.3:5:92.7, 50% of $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ + 429% of H_2O	53

5 Conclusion

In the present work, it was developed biobased and reprintable pastes for additive manufacturing from fibrillated cellulose, sodium alginate and water. The fibrillated cellulose was produced from two different sources of cotton: FCL, used as a benchmark, and CF provided by a Portuguese textile company.

The methodology to produce fibrillated cellulose from CF required, the implementation of pre-treatments (cutting manually and milling the sample) that revealed to be efficient in reducing the fibers' diameter. In contrast for FCL, no pre-treatment was necessary. Then, a chemical reaction, TMO, followed by High Pressure Homogenization allows the production of fibrillated cellulose, in which the concentration of the reagents used in chemical reaction and the pressure and number of passes applied in High Pressure Homogenization depends according to the source of cotton used. In this work three fibrillated celluloses suspensions were produced: two suspensions of fibrillated cellulose from FCL and one from CF.

The development of a paste with a composition of 2.3:5:92.7 wt.% (fibrillated cellulose: sodium alginate: water) using FCL-5T-CNF-1, FCL-5T-CNF-2 or CF-10T-CNF-1 showed to be the most promising formulations for extruder based additive manufacturing. The rheological measurements (viscosity curve, flow curve, amplitude sweep test and elastic recovery test) demonstrated that the formulated pastes fill the requirements for additive manufacturing.

The proof of concept consisted in printing an object with a diameter of two syringes of 0.686 and 2 mm. Results show that the hydrogels formulations can be used for additive manufacturing. Therefore, the developed paste emerges as an alternative to the current filaments used in additive manufacturing. Furthermore, it was explored the possibility of reuse of the produced pastes, adding a solution of sodium citrate tribasic dihydrate and water. The rheological parameters conducted revealed that the regenerated paste presents the rheological parameters required to be reused as a paste for additive manufacturing.

The development of this work revealed that the cotton derived from fast fashion industry is able to be used as a paste, in combination with sodium alginate and water, emerging as an alternative to the current filaments used in additive manufacturing. Moreover, it was proven the reprint ability of the paste when a solution of sodium citrate tribasic dihydrate and water are added to the paste. Therefore, with the work carried out, it is possible to contribute to a circular economy, since: it allows to give a second life to waste from the fast fashion industry, solving issues of environmental impact and, at the same time, the paste can be regenerated allowing its reuse.

6 Assessment of the work done

6.1 Objects achieved

This dissertation aimed to produce fibrillated cellulose from cotton to develop a formulation that can be used as a filament in additive manufacturing. In this work, it was used two sources of cotton: cotton from Sigma-Aldrich as benchmark and cotton provided by Polopiqué, a Portuguese textile company. The rheological tests of different hydrogel formulations using different sources of cotton for a composition of 2.5:5:92.7 (fibrillated cellulose: sodium alginate: water) showed to fulfill the requirements for a filament for additive manufacturing. The pastes were extruded using two syringes with different diameters, which is the proof of concept of this work. Finally, the regeneration of the pastes was tested using a solution of sodium citrate tribasic dihydrate of 2 wt.%. These results showed the possibility to reuse the hydrogel formulation as a filament. In sum, this dissertation enabled the development of a reprintable paste using cotton provided by a Portuguese textile company. The impact of this research stems from two aspects: 1) the capacity of recycling the produced pastes; ii) the contribution to the circular economy for textiles using the tons of cotton clothes that are not currently used as a consequence of the fast fashion industry.

6.2 Other Work Carried Out

In this work, other raw materials were tested. Sacia, a Portuguese textile company, supplied a recycled cotton sample. Fibrillated cellulose was for different number of passes at HPH, i.e. for 3 passes at 300 bar, 3 passes at 600 bar and 3 passes at 900 bar. However, this sample presented a challenge due to the presence of synthetic fibers, that clogging HPH equipment, which were extremely necessary to remove them to produce the pastes. So, a new approach, involving different pre-treatments, was required to produce fibrillated cellulose.

In addition to CF-10T-CNF-1, two another fibrillated cellulose samples were produced and characterized using the cotton provided by Polopiqué:

- CF-10T-CNF-2, using a similar methodology of CF-10T-CNF-1. However, the consistency before HPH was approximately 1 wt.%. In relation to the number of passes used, it was required 3 passes at 300 bar and 3 passes at 600 bar;
- CF-5T-CNF-3, using also a similar methodology as CF-10T-CNF-1 and CF-10T-CNF-2. The consistency of the OP before HPH was 1 wt.%. and number of passes in HPH required was 3 passes at 300 bar and 3 passes at 600 bar. Although, for this fibrillated cellulose, it was used a concentration of NaClO of 5 mmol·g⁻¹.

Finally, it was also produced fibrillated cellulose from a t-shirt made of 100% of cotton. The methodology to produce this fibrillated cellulose was similar to the production with FCL-10T-

CNF-2. The process also used a concentration of NaClO of 10 mmol·g⁻¹, a consistency before HPH was around 3 wt.% and number of passes at HPH required was 3 passes at 300 bar and 3 passes at 600 bar. However, due to time restriction, the characterization was not entirely completed for this fibrillated cellulose.

6.3 Limitations

Some limitations can be pointed out during this work. The pre-treatments of the raw materials are really time-consuming, especially the cotton provided from Polopiqué, which required the manual cutting of the cotton. In the future, a specific miller could reduce the size of the fibers, resulting in a reduction in the sample preparation time for the production of fibrillated cellulose.

Another limitation is related to the possibility of the growth of microorganisms in the fibrillated cellulose, which compromises this sample's utilization. The hypothesis of freezing the samples could be tested to avoid this problem.

Finally, and due to time restrictions, the extrusion of pastes was not tested in an AM system.

6.4 Future Work

As previously mentioned in this work, cellulose is a biopolymer that exists in nature in large quantities. Thus, in the future, the option of extending the formulations of pastes could be explored from other sources, especially from discarded sources.

In the future, the development of a paste and the evaluation of its potential as a filament for AM is a possibility could be evaluated. Due to time restrictions, and despite the production of fibrillated cellulose from recycled clothing and its characterization, it was not possible to evaluate and develop formulations with these nanofibers.

At last, in the future, pastes could be printed in an AM system, and different parameters can be evaluated and optimized: testing needles with different diameter, the distance between the needle and the surface and the velocity used to print the material.

7 Bibliographic References

- Asem, M., Noraini Jimat, D., Huda Syazwani Jafri, N., Mohd Fazli Wan Nawawi, W., Fadhilah Mohamed Azmin, N., & Firdaus Abd Wahab, M. (2023). Entangled cellulose nanofibers produced from sugarcane bagasse via alkaline treatment, mild acid hydrolysis assisted with ultrasonication. *Journal of King Saud University - Engineering Sciences*, 35(1), 24-31. <https://doi.org/10.1016/j.jksues.2021.03.003>
- Balea, A., Fuente, E., Tarrés, Q., Pèlach, M., Mutjé, P., Delgado-Aguilar, M., Blanco, A., & Negro, C. (2021). Influence of pretreatment and mechanical nanofibrillation energy on properties of nanofibers from Aspen cellulose. *Cellulose*, 28. <https://doi.org/10.1007/s10570-021-04109-w>
- Balea, A., Merayo, N., De La Fuente, E., Negro, C., & Blanco, Á. (2017). Assessing the influence of refining, bleaching and TEMPO-mediated oxidation on the production of more sustainable cellulose nanofibers and their application as paper additives. *Industrial Crops and Products*, 97, 374-387. <https://doi.org/10.1016/j.indcrop.2016.12.050>
- Baniasadi, H., Madani, Z., Ajdary, R., Rojas, O. J., & Seppälä, J. (2021). Ascorbic acid-loaded polyvinyl alcohol/cellulose nanofibril hydrogels as precursors for 3D printed materials. *Materials Science and Engineering: C*, 130, 112424. <https://doi.org/10.1016/j.msec.2021.112424>
- Baniasadi, H., Polez, R. T., Kimiaei, E., Madani, Z., Rojas, O. J., Österberg, M., & Seppälä, J. (2021). 3D printing and properties of cellulose nanofibrils-reinforced quince seed mucilage bio-inks. *International Journal of Biological Macromolecules*, 192, 1098-1107. <https://doi.org/10.1016/j.ijbiomac.2021.10.078>
- Bian, H., Yang, Y., Tu, P., & Chen, J. Y. (2022). Value-Added Utilization of Wheat Straw: From Cellulose and Cellulose Nanofiber to All-Cellulose Nanocomposite Film. *Membranes*, 12(5). <https://doi.org/10.3390/membranes12050475>
- Blanco, A., Monte, M. C., Campano, C., Balea, A., Merayo, N., & Negro, C. (2018). Chapter 5 - Nanocellulose for Industrial Use: Cellulose Nanofibers (CNF), Cellulose Nanocrystals (CNC), and Bacterial Cellulose (BC). In C. Mustansar Hussain (Ed.), *Handbook of Nanomaterials for Industrial Applications* (pp. 74-126). Elsevier. <https://doi.org/10.1016/B978-0-12-813351-4.00005-5>
- Centobelli, P., Abbate, S., Nadeem, S. P., & Garza-Reyes, J. A. (2022). Slowing the fast fashion industry: An all-round perspective. *Current Opinion in Green and Sustainable Chemistry*, 38, 100684. <https://doi.org/10.1016/j.cogsc.2022.100684>
- Dutta, G. K., & Karak, N. (2019). Waste brewed tea leaf derived cellulose nanofiber reinforced fully bio-based waterborne polyester nanocomposite as an environmentally benign material [10.1039/C9RA02973G]. *RSC Advances*, 9(36), 20829-20840. <https://doi.org/10.1039/C9RA02973G>
- Espinosa, E., Filgueira, D., Rodríguez, A., & Chinga-Carrasco, G. (2019). Nanocellulose-Based Inks- Effect of Alginate Content on the Water Absorption of 3D Printed Constructs. *Bioengineering (Basel)*, 6(3). <https://doi.org/10.3390/bioengineering6030065>
- Espinosa, E., Rincón, E., Morcillo-Martín, R., Rabasco-Vílchez, L., & Rodríguez, A. (2022). Orange peel waste biorefinery in multi-component cascade approach: Polyphenolic compounds and nanocellulose for food packaging. *Industrial Crops and Products*, 187, 115413. <https://doi.org/10.1016/j.indcrop.2022.115413>
- Foster, E. J., Moon, R., Agarwal, U., Bortner, M., Bras, J., Camarero Espinosa, S., Chan, K., Clift, M., Cranston, E., Eichhorn, S., Fox, D., Hamad, W., Heux, L., Jean, B., Korey, M., Nieh,

- W., Ong, K., Reid, M., Renneckar, S., & Youngblood, J. (2018). Current characterization methods for cellulose nanomaterials.
- Gauss, C., Pickering, K. L., & Muthe, L. P. (2021). The use of cellulose in bio-derived formulations for 3D/4D printing: A review. *Composites Part C: Open Access*, 4, 100113. <https://doi.org/10.1016/j.jcomc.2021.100113>
- Ghadiri Alamdari, N., Salmasi, S., & Almasi, H. (2021). Tomato Seed Mucilage as a New Source of Biodegradable Film-Forming Material: Effect of Glycerol and Cellulose Nanofibers on the Characteristics of Resultant Films. *Food and Bioprocess Technology*, 14(12), 2380-2400. <https://doi.org/10.1007/s11947-021-02734-8>
- Hashaiekh, R., Krishnamachari, P., & Abdul Samad, Y. (2015). Handbook of Polymer Nanocomposites. Processing, Performance and Application. In (pp. 229-248). https://doi.org/10.1007/978-3-642-45232-1_60
- Im, S., Choe, G., Seok, J. M., Yeo, S. J., Lee, J. H., Kim, W. D., Lee, J. Y., & Park, S. A. (2022). An osteogenic bioink composed of alginate, cellulose nanofibrils, and polydopamine nanoparticles for 3D bioprinting and bone tissue engineering. *International Journal of Biological Macromolecules*, 205, 520-529. <https://doi.org/10.1016/j.ijbiomac.2022.02.012>
- Islam, S., & Bhat, G. (2019). Environmentally-friendly thermal and acoustic insulation materials from recycled textiles. *Journal of Environmental Management*, 251, 109536. <https://doi.org/10.1016/j.jenvman.2019.109536>
- Isogai, A., Saito, T., & Fukuzumi, H. (2011). TEMPO-oxidized cellulose nanofibers [10.1039/CONR00583E]. *Nanoscale*, 3(1), 71-85. <https://doi.org/10.1039/CONR00583E>
- Kanai, N., Honda, T., Yoshihara, N., Oyama, T., Naito, A., Ueda, K., & Kawamura, I. (2020). Structural characterization of cellulose nanofibers isolated from spent coffee grounds and their composite films with poly(vinyl alcohol): a new non-wood source. *Cellulose*, 27. <https://doi.org/10.1007/s10570-020-03113-w>
- Karpe, A. (2015). *Biodegradation of winery biomass wastes by developing a symbiotic multi-fungal consortium*
- Lamnini, S., Elsayed, H., Lakhdar, Y., Baino, F., Smeacetto, F., & Bernardo, E. (2022). Robocasting of advanced ceramics: ink optimization and protocol to predict the printing parameters - A review. *Heliyon*, 8(9), e10651. <https://doi.org/10.1016/j.heliyon.2022.e10651>
- Lavoine, N., Desloges, I., Dufresne, A., & Bras, J. (2012). Microfibrillated cellulose - Its barrier properties and applications in cellulosic materials: A review. *Carbohydrate Polymers*, 90(2), 735-764. <https://doi.org/10.1016/j.carbpol.2012.05.026>
- Lewin, M., & Avrahami, M. (1955). The Decomposition of Hypochlorite-Hypobromite Mixtures in the pH Range 7-10. *Journal of the American Chemical Society*, 77(17), 4491-4498. <https://doi.org/10.1021/ja01622a014>
- Lin, L., Jiang, S., Yang, J., Qiu, J., Jiao, X., Yue, X., Ke, X., Yang, G., & Zhang, L. (2023). Application of 3D-bioprinted nanocellulose and cellulose derivative-based bio-inks in bone and cartilage tissue engineering. *Int J Bioprint*, 9(1), 637. <https://doi.org/10.18063/ijb.v9i1.637>
- Liu, X., Sun, H., Mu, T., Fauconnier, M. L., & Li, M. (2023). Preparation of cellulose nanofibers from potato residues by ultrasonication combined with high-pressure homogenization. *Food Chemistry*, 413, 135675. <https://doi.org/10.1016/j.foodchem.2023.135675>
- Malladi, R., Nagalakshmaiah, M., Robert, M., & Elkoun, S. (2018). Importance of Agricultural and Industrial Waste in the Field of Nanocellulose and Recent Industrial Developments of Wood Based Nanocellulose: A Review. *ACS Sustainable Chemistry & Engineering*, 6. <https://doi.org/10.1021/acssuschemeng.7b03437>

- Mhlongo, J., Nuapia, Y., Motsa, M., Mahlangu, O., & Etale, A. (2022). Green chemistry approaches for extraction of cellulose nanofibers (CNFs): A comparison of mineral and organic acids. *Materials Today: Proceedings*, 62. <https://doi.org/10.1016/j.matpr.2022.02.088>
- Murab, S., Gupta, A., Włodarczyk-Biegun, M. K., Kumar, A., van Rijn, P., Whitlock, P., Han, S. S., & Agrawal, G. (2022). Alginate based hydrogel inks for 3D bioprinting of engineered orthopedic tissues. *Carbohydrate Polymers*, 296, 119964. <https://doi.org/10.1016/j.carbpol.2022.119964>
- Negro, C., Balea Martin, A., Sanchez-Salvador, J., Campano, C., Fuente, E., Monte, M., & Blanco, A. (2020). NANOCELLULOSE AND ITS POTENTIAL USE FOR SUSTAINABLE INDUSTRIAL APPLICATIONS. *Latin American Applied Research - An international journal*, 50, 59-64. <https://doi.org/10.52292/j.laar.2020.471>
- Nelson, C., Tuladhar, S., Launen, L., & Habib, A. (2021). 3D Bio-Printability of Hybrid Pre-Crosslinked Hydrogels. *International Journal of Molecular Sciences*, 22, 13481. <https://doi.org/10.3390/ijms222413481>
- Olmos-Juste, R., Alonso-Lerma, B., Pérez-Jiménez, R., Gabilondo, N., & Eceiza, A. (2021). 3D printed alginate-cellulose nanofibers based patches for local curcumin administration. *Carbohydrate Polymers*, 264, 118026. <https://doi.org/https://doi.org/10.1016/j.carbpol.2021.118026>
- Saito, T., & Isogai, A. (2006). Introduction of aldehyde groups on surfaces of native cellulose fibers by TEMPO-mediated oxidation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 289(1), 219-225. <https://doi.org/10.1016/j.colsurfa.2006.04.038>
- Sanchez-Salvador. (2021). Increasing the Possibilities of TEMPO-Mediated Oxidation in the Production of Cellulose Nanofibers by Reducing the Reaction Time and Reusing the Reaction Medium. *Advanced Sustainable Systems*, 5. <https://doi.org/10.1002/adsu.202000277>
- Sanchez-Salvador, Balea Martin, A., Monte, M., Blanco, A., & Negro, C. (2019). Pickering Emulsions Containing Cellulose Microfibers Produced by Mechanical Treatments as Stabilizer in the Food Industry. *Applied Sciences*, 9, 359. <https://doi.org/10.3390/app9020359>
- Sanchez-Salvador, Lopez-Exposito, P., Tarrés, Q., Mutjé, P., Delgado-Aguilar, M., Monte, M. C., & Blanco, A. (2021). Enhanced Morphological Characterization of Cellulose Nano/Microfibers through Image Skeleton Analysis. *Nanomaterials (Basel)*, 11(8). <https://doi.org/10.3390/nano11082077>
- Sanchez-Salvador, J., Monte, M., Negro, C., Batchelor, W., Garnier, G., & Blanco, A. (2021). Simplification of gel point characterization of cellulose nano and microfiber suspensions. *Cellulose*, 28. <https://doi.org/10.1007/s10570-021-04003-5>
- Sanchez-Salvador, J. L., Balea, A., Monte, M. C., Negro, C., Miller, M., Olson, J., & Blanco, A. (2020). Comparison Of Mechanical And Chemical Nanocellulose As Additives To Reinforce Recycled Cardboard. *Scientific Reports*, 10(1), 3778. <https://doi.org/10.1038/s41598-020-60507-3>
- Sauerwein, M., Zlopasa, J., Doubrovski, Z., Bakker, C., & Balkenende, R. (2020). Reprintable Paste-Based Materials for Additive Manufacturing in a Circular Economy. *Sustainability*, 12(19). <https://doi.org/10.3390/su12198032>
- Schwab, A., Levato, R., D'Este, M., Piluso, S., Eglin, D., & Malda, J. (2020). Printability and Shape Fidelity of Bioinks in 3D Bioprinting. *Chem Rev*, 120(19), 11028-11055. <https://doi.org/10.1021/acs.chemrev.0c00084>
- Serra-Parareda, F., Aguado, R., Tarrés, Q., Mutjé, P., & Delgado-Aguilar, M. (2021). Potentiometric back titration as a robust and simple method for specific surface area

- estimation of lignocellulosic fibers. *Cellulose*, 28(17), 10815-10825. <https://doi.org/10.1007/s10570-021-04250-6>
- Teixeira, E., Corrêa, A., Manzoli, A., Leite, F., Ribeiro, C., & Mattoso, L. (2010). Cellulose nanofibers from white and naturally colored cotton fibers. *Cellulose*, 17, 595-606. <https://doi.org/10.1007/s10570-010-9403-0>
- Thibaut, C., Denneulin, A., Rolland du Roscoat, S., Beneventi, D., Orgéas, L., & Chaussy, D. (2019). A fibrous cellulose paste formulation to manufacture structural parts using 3D printing by extrusion. *Carbohydrate Polymers*, 212, 119-128. <https://doi.org/10.1016/j.carbpol.2019.01.076>
- Tibolla, H., Pelissari, F. M., Martins, J. T., Vicente, A. A., & Menegalli, F. C. (2018). Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: Characterization and cytotoxicity assessment. *Food Hydrocolloids*, 75, 192-201. <https://doi.org/10.1016/j.foodhyd.2017.08.027>
- Trachsel, L., Johnbosco, C., Lang, T., Benetti, E., & Zenobi, M. (2019). Double-Network Hydrogels Including Enzymatically Crosslinked Poly-(2-alkyl-2-oxazoline)s for 3D Bioprinting of Cartilage Engineering Constructs. *Biomacromolecules*, XXXX. <https://doi.org/10.1021/acs.biomac.9b01266>
- Vel, R., Bhatt, A., Priyanka, A., Gauthaman, A., Anilkumar, V., Safeena, A. S., & S, R. (2022). DEAE- Cellulose-based composite hydrogel for 3D printing application: Physicochemical, mechanical, and biological optimization. *Materials Today Communications*, 33, 104335. <https://doi.org/10.1016/j.mtcomm.2022.104335>
- Wang, J., Li, X., Song, J., Wu, K., Xue, Y., Wu, Y., & Wang, S. (2020). Direct Preparation of Cellulose Nanofibers from Bamboo by Nitric Acid and Hydrogen Peroxide Enables Fibrillation via a Cooperative Mechanism. *Nanomaterials*, 10(5).
- Wang, Q., Sun, J., Yao, Q., Ji, C., Liu, J., & Zhu, Q. (2018). 3D printing with cellulose materials. *Cellulose*, 25(8), 4275-4301. <https://doi.org/10.1007/s10570-018-1888-y>
- Wang, Y., Zhang, L., Liu, W., Cui, C., & Hou, Q. (2019). Fabrication of optically transparent and strong nanopaper from cellulose nanofibril based on corncob residues. *Carbohydrate Polymers*, 214, 159-166. <https://doi.org/10.1016/j.carbpol.2019.03.035>
- Widiarto, S., Pramono, E., Suharso, S., Rochliadi, A., & Arcana, I. M. (2019). Cellulose Nanofibers Preparation from Cassava Peels via Mechanical Disruption. 7, 44. <https://doi.org/10.3390/fib7050044>
- Xu, H., Sanchez-Salvador, J. L., Balea, A., Blanco, A., & Negro, C. (2022). Optimization of reagent consumption in TEMPO-mediated oxidation of Eucalyptus cellulose to obtain cellulose nanofibers. *Cellulose*, 29(12), 6611-6627. <https://doi.org/10.1007/s10570-022-04672-w>

Appendix A - Volume of titrant required in the titration reaction

To verify the TMO end, a redox titration was performed, using $\text{Na}_2\text{S}_2\text{O}_3$ as titrant and starch as solution indicator. Table A.1 shows the volume of titration, in mL, used for each OP, as well the speed, in rpm, used in TMO.

Table A.1. Volume of titrant used in the titration and speed used in TMO reaction.

Sample	$V_{\text{Na}_2\text{S}_2\text{O}_3}$ (mL)	Speed (rpm)
CF-5T-OP-1	0.25	250
CF-5T-OP-2	0.2	250
FCL-10T-OP-1	15.4	250

Appendix B - Rheological Properties of the fibrillated cellulose

In Figure B.1 and Figure B.2 are represented the rheological measurements (viscosity curve and amplitude sweep test) performed on isolated fibrillated cellulose for CF and FCL samples.

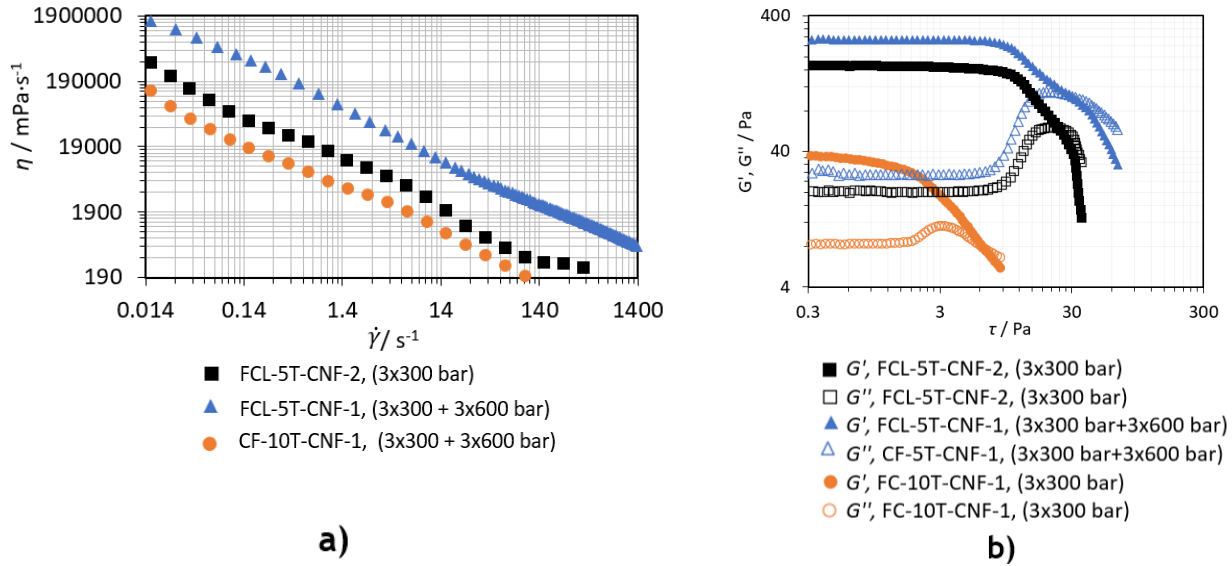


Figure B.1. Evaluation of the rheological properties for FCL-5T-CNF-1, FCL-5T-CNF-2 and CF-10T-CNF-1, according with the number of passes in High Pressure Homogenizer for: a) viscosity curve and b) amplitude sweep test.

Figure B.2 shows the elastic recovery test corresponding to FCL-5T-CNF-2,2.3:5:92.7. The determination of this test was performed according to

$$recovery (\%) = \frac{\text{average of final values of } G'}{\text{average of initial values of } G'} \times 100 \quad (B.1)$$

In which G' or G'' according with the storage modulus that allows the determination of recovery determination test.

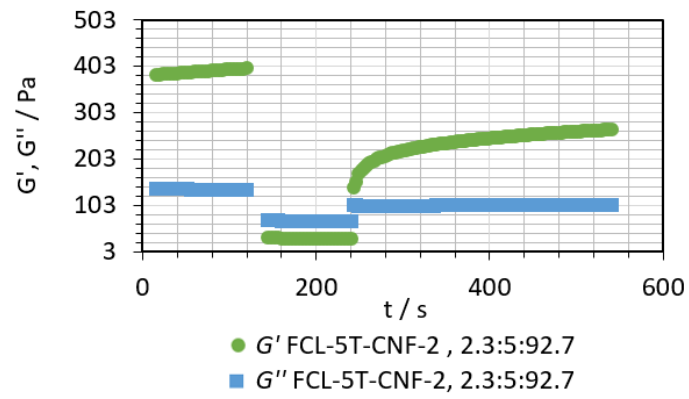


Figure B.2. Elastic recovery test corresponding to 2.3:5:92.7 hydrogel formulation.