



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA MECÂNICA

JÉSSICA HELINE LOPES DA FONSÊCA

**Rheological behavior of cellulose-based
nanocomposite hydrogels aiming extrusion-
based bioprinting**

**Comportamento reológico de hidrogéis
nanocompósitos baseados em celulose
visando a bioimpressão baseada em
extrusão**

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Dissertation presented to the School of Mechanical Engineering of the University of Campinas in fulfillment of the requirements for the degree of Master in Mechanical Engineering, in the area of Materials and Fabrication Processes.

Dissertação apresentada à Faculdade de Engenharia Mecânica da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestre em Engenharia Mecânica, na área de Materiais e Processos de Fabricação.

Orientador: Prof. Dr. Marcos Akira d'Ávila

Este exemplar corresponde à versão final da Dissertação defendida pela aluna Jéssica Heline Lopes da Fonsêca e orientada pelo Prof. Dr. Marcos Akira d'Ávila.

CAMPINAS

2019

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Área de Engenharia e Arquitetura
Luciana Pietrosanto Milla - CRB 8/8129

F733r Fonsêca, Jéssica Heline Lopes da, 1993-
Rheological behavior of cellulose-based nanocomposite hydrogels aiming extrusion-based bioprinting / Jéssica Heline Lopes da Fonsêca. – Campinas, SP : [s.n.], 2019.

Orientador: Marcos Akira d'Ávila.
Dissertação (mestrado) – Universidade Estadual de Campinas, Faculdade de Engenharia Mecânica.

1. Hidrogéis. 2. Reologia. 3. Impressão tridimensional. 4. Nanocristais de celulose. 5. Carboximetilcelulose. I. D'Ávila, Marcos Akira, 1972-. II. Universidade Estadual de Campinas. Faculdade de Engenharia Mecânica. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Comportamento reológico de hidrogéis nanocompósitos baseados em celulose visando a bioimpressão baseada em extrusão

Palavras-chave em inglês:

Hydrogels

Rheology

3D Printing

Cellulose-nanocrystal

Carboxymethylcellulose

Área de concentração: Materiais e Processos de Fabricação

Titulação: Mestra em Engenharia Mecânica

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Data de defesa: 26-07-2019

Programa de Pós-Graduação: Engenharia Mecânica

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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA MECÂNICA
COMISSÃO DE PÓS-GRADUAÇÃO EM ENGENHARIA MECÂNICA
DEPARTAMENTO DE ENGENHARIA DE MANUFATURA E
MATERIAIS

DISSERTAÇÃO DE MESTRADO ACADÊMICO

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A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica da aluna:

Campinas, 26 de julho de 2019.

Dedication

To my dear grandfather Archimimo Pereira (in memory), who taught me to be a better person, for the dedication, unconditional love and have been my greatest motivator.

To my mothers Maria Helena and Gertrudes Lopes, for their love, comprehension, and support at all my life.

To my nephews André and Laís, who are growing and will soon be able to see that every dream is possible. You can do more!

To Rêner for having accompanied me during this journey, and for making my dreams, yours.

With all my love,

Jéssica Heline

Acknowledgments

I want to express my sincere gratitude to all the people and institutions who have contributed to the completion of this dissertation:

To Prof. Dr. Marcos Akira d'Ávila for the guidance and full support provided to this research. I would also like to thank you for the opportunity, confidence, and by the motivate constant.

To José Luís Dávila for the support in the final moments of this research, for the patience and availability to help whenever I needed it.

To the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq-Brazil) for the master scholarship N° 131986/2018-0 and financial support from the projects N°308660/2015-3, N°421745/2016-9.

To the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP-Brazil) for the financial support from the project (2017/23776-5).

To Prof. Dr. Pedro Duarte Novaes and Flávia Sammartino Mariano, for allowing the polarized light microscopy analyzes to be performed in Microscopy and Imaging Center (CMI) of the Piracicaba Dental School – UNICAMP

To Prof. Dra. Maria Helena Andrade Santana and the Carla França, for making available the Zetasizer equipment at the *Laboratório de Desenvolvimento de Processos Biotecnológicos* da FEQ.

To my mothers, Gertrudes, and Maria Helena, and my siblings, César, Bruno, Cristiane, and Gabriel, for all support, affection, and motivation.

To Rêner Pontes, for patience, attention, for never letting me give up and by for believing that I can always do better.

To my older and dear friends Hedrey Narcos and Elkiane Frazão for affection, attention, and motivation at all times.

To my friends Marjorie, Martinha, Nathi, Juca, Gi, and Lucas. Thank you for having welcomed me and made me feel loved and protected in this city. You are God's gifts in my life.

To my friends and labmates João de Deus, Ana Flávia, Rosana, Eronildo, Rosemeire, Gabriel, Ingri and Andreliza for the discussions and their suggestions.

“Do everything in love”

1 Corinthians 16:14

Resumo

Hidrogéis são redes poliméricas reticuladas capazes de absorver e reter uma grande quantidade de água de maneira reversível devido à sua natureza hidrofílica. Hidrogéis à base de celulose têm recebido grande interesse nos últimos anos atribuído às propriedades atrativas da celulose como: grande disponibilidade na natureza e seu potencial de uso em diversas aplicações. Neste trabalho, hidrogéis nanocompósitos de carboximetilcelulose (CMC) e nanocristais celulose (CNC) foram obtidos, e seu comportamento reológico foi estudado visando aplicações em impressão tridimensional de *scaffolds* para aplicações na engenharia de tecidos. Inicialmente, o comportamento reológico de dispersões aquosas de CMC/CNC em baixas concentrações foi investigado. O mecanismo de interação entre as hastes de CNC e as cadeias aniônicas do CMC foi estudado por microscopia de luz polarizada (POM), e por medidas dinâmicas de espalhamento de luz (DLS) e potencial zeta (ZP). Imagens POM mostraram que a microestrutura CMC/CNC é composta por regiões isotrópicas e líquidas-cristalinas. Aglomerados líquido-cristalinos dispersos na fase isotrópica puderam ser observados desde as concentrações mais baixas e, em maiores concentrações observou-se que esses aglomerados líquido-cristalinos tinham formato de gotículas. Os valores de ZP indicam suspensões estáveis e fortes forças repulsivas. O diâmetro hidrodinâmico do CNC aumentou na presença de cadeias de CMC, como resultado da floculação por depleção. Floculação por depleção foi identificada como a principal interação entre as cadeias de CMC e as partículas do CNC, responsável pelas fortes mudanças nas propriedades reológicas do sistema CMC/CNC. Após compreender as interações polímero/partícula desde dispersões aquosas ao estado de gel, hidrogéis nanocompósitos de CMC/CNC foram preparados, caracterizados reologicamente e avaliado a capacidade de impressão desses géis em um segundo estudo de caso. Os géis foram obtidos a partir de diferentes formulações de CMC/CNC em água. As interações entre CMC e CNC promoveram fortes mudanças nas propriedades reológicas. Todas as amostras exibiram um forte comportamento de afinamento e um comportamento inicial de espessamento a baixas taxas de cisalhamento. Quanto às propriedades viscoelásticas, todas as amostras apresentaram comportamento elástico predominante ao viscoso em todas as faixas de frequências analisadas ($G' > G''$). Géis físicos macios foram obtidos. A capacidade de impressão foi avaliada por testes de impressão de filamentos usando microscopia óptica. Testes de formação de filamentos mostraram a formação gradual do filamento. Gotas foram obtidas em concentrações mais baixas de géis e em concentrações mais altas a qualidade do filamento melhorou. A composição de 1:10 wt.% CMC/CNC foi definida como concentração para os testes de impressão, devido suas propriedades reológicas aprimoradas e à qualidade do filamento obtido. A velocidade de extrusão de $45,3 \text{ mm s}^{-1}$ e a velocidade de impressão de 20 mm s^{-1} foram usadas para imprimir as estruturas. As estruturas impressas apresentaram boa resolução, mesmo com maior número de camadas, confirmando a capacidade de impressão do gel CMC/CNC.

Palavras chaves: hidrogéis, reologia, impressão tridimensional, nanocristais de celulose, carboximetilcelulose.

Abstract

Hydrogels are cross-linked polymeric networks able to absorb and retain a large amount of water in a reversible manner due to their hydrophilic nature. Cellulose-based hydrogels have received great interest in the last years because of attractive properties of cellulose, large availability in nature and potential use in several applications. In this research, nanocomposite hydrogels of carboxymethyl cellulose (CMC) and cellulose nanocrystals (CNC) were obtained, and their rheological behavior was studied aiming applications in scaffolds extrusion three-dimensional printing for tissue engineering applications. Initially, the rheological behavior of aqueous dispersions of the CMC/CNC at low concentrations was investigated. The mechanism of interaction between the CNC rod-like and the anionic CMC chains was studied using polarized optical microscopy (POM), dynamic light scattering (DLS) and zeta potential (ZP) measurements. POM images showed the CMC/CNC microstructure is composed of isotropic and liquid crystalline regions. Liquid-crystalline agglomerates dispersed in the isotropic phase can be observed from the lowest concentrations, and in higher concentrations, this liquid-crystalline agglomerates presented droplets format. Zeta potential values indicate stable dispersions and strong repulsive forces. The hydrodynamic diameter of CNC increased in the presence of CMC chains, as a result of depletion flocculation. Depletion flocculation was identified as the main interaction between CMC chains and CNC particles, responsible for the strong changes in rheological properties of CMC/CNC dispersions. After understanding the polymer/particle interactions from aqueous dispersions to gel state, CMC/CNC nanocomposites gels were prepared, studied rheologically, and was evaluated the printability such gels in a second case study. The gels were obtained from different formulations of CMC/CNC in water. The interactions between CMC and CNC promoted strong changes in rheological properties. All samples exhibited a strong shear-thinning behavior and an initial shear-thickening behavior at low shear rates. As for the viscoelastic properties, all samples presented elastic behavior predominant to the viscous in all frequency range analyzed ($G' > G''$). At higher concentrations, G' and G'' were almost frequency independent, which is typical of physical gels. Thus, soft physical gels were obtained. The printability was evaluated by filament printing tests using optical microscopy. Filament formation tests showed the gradual formation of the filament. Droplets were obtained in lower concentrations of gels and at higher concentration the quality of filament improved. The composition 1:10 wt.% CMC/CNC was defined as concentration to the printing tests due to its enhanced rheological properties and obtained filament quality. The extrusion speed of 45.3 mm s^{-1} and the print speed of 20 mm s^{-1} were used to printing structures. The printed structures presented a good resolution even with greater numbers of layers confirming the printability of nanocomposite hydrogel CMC/CNC.

Keywords: *hydrogels, rheology, 3D printing, cellulose-nanocrystal, carboxymethylcellulose*

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List of Symbols, Acronyms, and Abbreviations

Mathematical symbols, variables, and parameters

C_N Nanocrystal cellulose concentration

C_p Polymer concentration

wt. % Percentage by weight

η Viscosity

η_{sp} Specific viscosity

η_0 Zero-shear viscosity

η_∞ Infinite shear viscosity

γ Strain

$\dot{\gamma}$ Shear rate

$\dot{\gamma}_c$ Critical shear rate

τ Shear stress

ω Angular frequency

G' Storage modulus

G'' Loss modulus

$\tan(\delta)$ Damping factor

m Consistency index

n Power-law index

t Time

L Nozzle tip length

R Nozzle tip radius

v_e Extrusion speed

v_p Printing speed

v_{ps} Piston speed

Acronyms and Abbreviations

3ITT - Three-interval thixotropy tests

3D - Three-dimensional

CMC - Carboxymethylcellulose

CNC - Nanocrystal cellulose

GNF - Generalized Newtonian fluid

LVE Linear viscoelasticity range

pH - Potential of hydrogen

POM - Polarized optical microscopy

SAOS - Small amplitude oscillatory shear

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1 INTRODUCTION

In this chapter, a general overview of the subject of this work is presented; further, the objectives of this research are defined.

1.1 Overview

Hydrogels are highly hydrophilic natural or synthetic polymers, which are able to retain large amounts of water due to the three-dimensional chain networks formed by intermolecular crosslinks. In many cases, they are biocompatible with structural similarity to biological tissues and the extracellular matrix (ECM), which may mimic some of the physical and chemical properties of other physiological systems (PEPPAS; HOFFMAN, 2013). Due to these characteristics, the development of hydrogels has grown significantly in novel biomedical applications such as wound dressing (CAPANEMA *et al.*, 2018), drug delivery systems (PARSA; PAYDAYESH; DAVACHI, 2019) and the scaffolds for tissue engineering (FU *et al.*, 2019; VARAPRASAD *et al.*, 2017).

Cellulose-based hydrogels have received significant interest in the last years due to cellulose properties such as low-toxicity, biocompatibility, biodegradability and large availability in nature. Besides, the chemical modification of cellulose structure allows for obtaining numerous cellulose ether/esters and micro/nanosized particles with unique properties. Carboxymethyl cellulose (CMC) is an anionic water-soluble ether cellulose derivative. It is a biodegradable polysaccharide that has become widely used as superabsorbent hydrogels, with smart behavior in response to the physiological environment (SANNINO; DEMITRI; MADAGHIELE, 2009). Cellulose nanocrystals (CNC) is a rod-like nanoparticle with a width of 5-10 nm and length up to 300 nm. They are *produced* by isolation of crystalline cellulose chains through acid hydrolysis. CNCs attract attention since they are biocompatible and exhibit unique properties such as high aspect ratio, high crystallinity, and high mechanical properties, which are attractive for the mechanical strengthening of hydrogels and are recognized to have

potential use in several applications in the biomedical field. Moreover, with the advance of additive manufacturing technology, the development of cellulose-based hydrogels for 3D printing is one of the most recent and promising developments in this field (LIU *et al.*, 2019; SULTAN *et al.*, 2017).

In the last years, additive manufacturing process (AM) has been studied aiming novel applications in the biomedical field. The AM process, also known as 3D printing, is a technique to obtain three-dimensional structured objects through the controlled layer-by-layer deposition of different materials such as metals, ceramics, polymers, composites, gels and hydrogels (DAI *et al.*, 2019; GROLL *et al.*, 2016; LIU *et al.*, 2019). In the biomedical field, 3D printing technologies of biomaterial ink are known as bioprinting, where one of the main applications is the manufacturing of three-dimensional scaffolds for tissue engineering applications (HABIB; KHODA, 2018). Extrusion-based printing is an AM technology based on extrusion of a soft material by a pneumatic system, a piston or a screw. It offers advantages over other techniques since it is possible to obtain scaffolds of high biocompatibility with different geometries. Hydrogels scaffolds are of great interest due to their high hydrophilicity and biocompatibility, presenting rheological behavior that can be tuned for extrusion printing, such as shear-thinning, viscoelasticity and thixotropy. However, it is recognized that suitable rheological properties are crucial for a successful extrusion printing of hydrogel scaffolds (SULTAN *et al.*, 2017). Therefore, rheological characterization is an essential tool to study the structure-properties relationships of hydrogels and predict hydrogel printability during extrusion printing, which is fundamental to obtain an adequate resolution and printing fidelity.

Hydrogels with high zero shear viscosity and strong shear-thinning behavior are attractive, since they present low viscosity upon high shear stresses, enabling extrusion through the nozzle tip, while high zero shear viscosity allied to elasticity can maintain the shape fidelity after printing, preventing filament deformation when shear is removed (SULTAN *et al.*, 2017). In general, printable hydrogels should present suitable viscoelastic response forming stable networks that can resist compressive stresses from capillary forces and, consequently, maintaining their shape, avoiding structure deformation.

Usually, the rheological properties of conventional hydrogels are not suitable for 3D printing; thus, different hydrogel systems have been studied. Nanocomposite hydrogels arise as

promising candidates for extrusion printing since the combination of nanoparticles with the polymeric matrix can result in physiochemical and enhanced rheological properties, which enable their use for 3D printing of gels.

The incorporation of CNC rod-like particles in the CMC polymer matrix may result in nanocomposite gels with rheological properties that can be suitable for extrusion printing. Both cellulose derivatives can be used as rheological modifiers for 3D printing inks. The CMC is often used as viscosity modifier due to its viscosity thickening capability and thixotropy (HABIB; KHODA, 2018; MULAKKAL *et al.*, 2018) and CNC dispersions can be used to enhance shear-thinning and ensure viscoelastic response required for filament printing, offering the possibility to control pore structure and shape in scaffolds extrusion printing. Oguzlu, Danumah, and Boluk (2016) reported gels formation in CMC/CNC aqueous dispersions at low concentrations. As mentioned above, both materials present interesting characteristics for 3D printing, besides the low cost and easy handling. The combination of mechanical properties that makes CNC an excellent rheological modifier with the characteristics of CMC polymer and the ability to gel formation, indicates an attractive alternative for printable hydrogel-inks. However, until now, the combination of CMC/CNC has not been explored for 3D extrusion-based printing process.

1.2 Objectives

1.2.1 General objectives

The main objective of this work was to study the rheological behavior of system carboxymethylcellulose (CMC) and cellulose nanocrystals (CNC) aiming to obtain nanocomposite hydrogels with satisfactory characteristics for the 3D extrusion-based printing process.

1.2.2 Specific objectives

- Evaluate the rheological behavior of carboxymethyl cellulose solutions and cellulose nanocrystals dispersions.
- Study the rheological behavior and colloidal interactions of CMC/CNC gels in aqueous dispersions.
- Obtain nanocomposite hydrogels with rheological properties suitable for the 3D extrusion-based printing process and study the influence of rheological properties and processing condition on gel printability.

1.3 Outline of the dissertation

This dissertation is structured in five chapters. Initially, **Chapter 1** showed a general overview of the hydrogels and cellulose-based hydrogels, followed by objectives of this study. **Chapter 2** presents a literature review deepening the main fundamentals of hydrogels, nanocomposite hydrogels, and cellulose-based hydrogels. The characteristics and properties of the materials used throughout this work are also presented. Moreover, in **Chapter 2**, the rheological properties related to structural features of hydrogels are discussed. **Chapter 3** presents a rheological study of aqueous dispersions of the CMC/CNC system at low concentrations, in which the mechanism of interaction between the CNC rod-like and the anionic CMC chains, as well as the influence of CNC as a rheological modifier in CMC solutions, were investigated. This study was fundamental to understand the polymer/particle interactions since aqueous dispersions to the state of gel. In **Chapter 4**, the rheological behavior of CMC/CNC gels was studied aiming extrusion printing applications. Extrusion printing tests were performed, evaluating the characteristics of the filaments formed and printability such gels. Finally, **Chapter 5** presents the conclusions of this research.

2 LITERATURE REVIEW

In this chapter, the main characteristics, classification, and obtention of the hydrogel, nanocomposite hydrogels, and cellulose-based hydrogels, with a focus in the polymer and rheologic modifier used in the research are presented. Finally, the rheological properties are discussed.

2.1 Hydrogels

Hydrogels are three-dimensional polymeric structures with the ability to swell and retain a notable fraction of water in its structure. A hydrogel is a macromolecular polymer gel with a network of crosslinked chains. Water is present in the hydrogel up to 90%, and this high hydrophilicity is due to hydrophilic polymers crosslinked either by covalent bonds or held unitedly via physical intramolecular and intermolecular attractions (AHMED, 2015; EL-SHERBINY; YACOUN, 2013).

Hydrogels can be prepared from natural or synthetic polymers. Both polymers have significant advantages and disadvantages. Generally, hydrogels from natural polymers have enhanced biological properties such as biocompatibility, biodegradability while exhibiting low mechanical properties. On the other hand, synthetic-based hydrogels present lower biological properties but provide better tuning and control of mechanical and degradation properties (AHMED, 2015; PEPPAS; HOFFMAN, 2013).

Hydrogels are classified depending on their origin, preparation methods, physicochemical structural features and, crosslinking type, which can be seen in Figure 2.1.

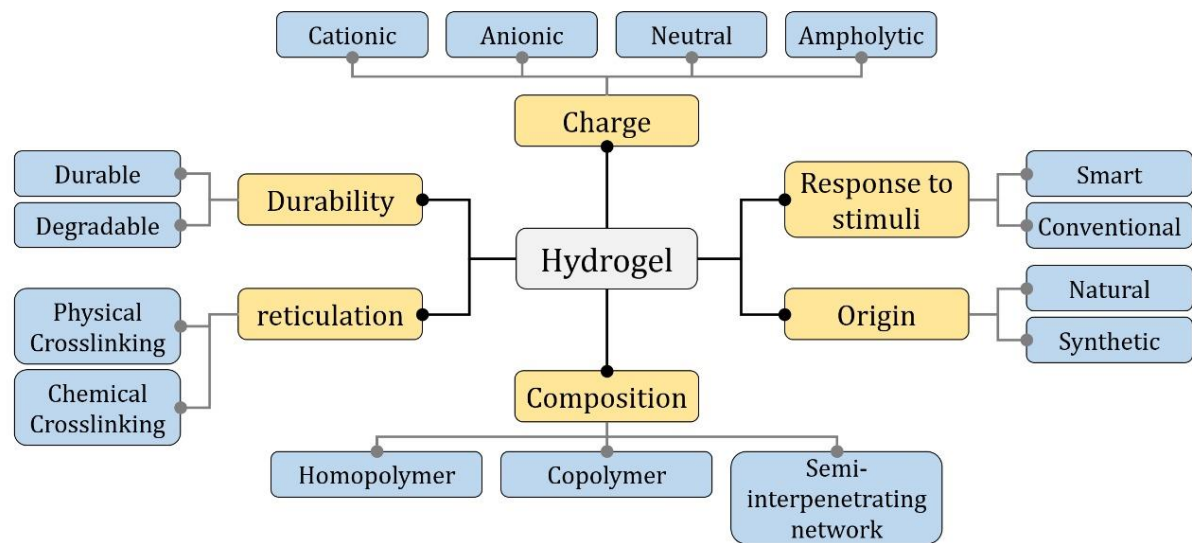


Figure 2.1: Schematic representation of the most common classes of hydrogels

Sources: (EL-SHERBINY; YACOUB, 2013).

As observed, according to the crosslinking, hydrogels are classified in chemical hydrogels and physical hydrogels. The type and degree of crosslinking influence many network properties, such as swelling, elasticity, and transport properties (HORKAY; DOUGLAS, 2018). It is shown in Figure 2.2 a schematic representation of chemical and physical hydrogels.

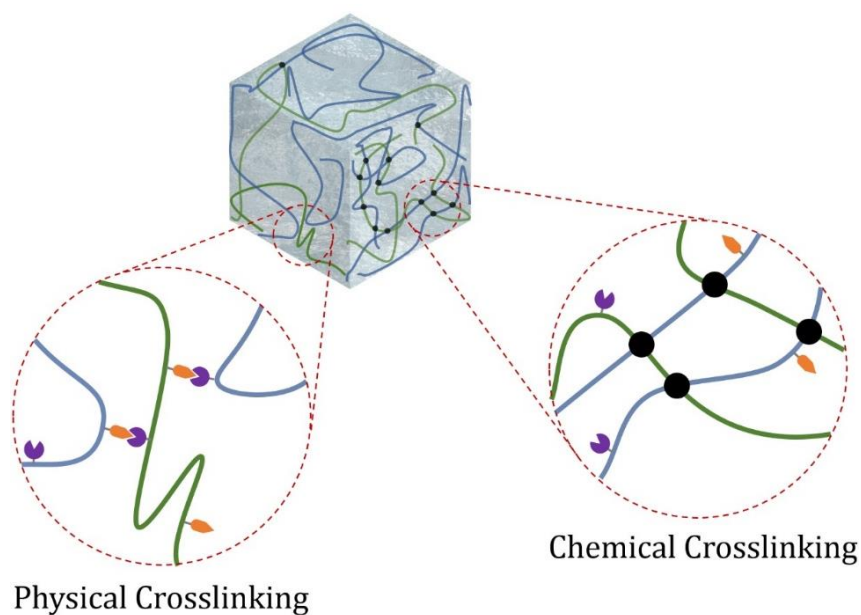


Figure 2.2: Schematic representation of chemical and physical hydrogels.

Sources: (PEPPAS; HOFFMAN, 2013)

Chemical hydrogels are formed by chemically crosslinked permanent junctions resulting from strong covalent bonds leading to stable gels. Chemical crosslinking results from the direct reaction of a linear or branched polymer with a crosslinking agent. These hydrogels attain an equilibrium swelling state which depends on the polymer-water interaction parameter and the crosslink density (AKHTAR; HANIF; RANJHA, 2016; MATANOVIĆ; KRISTL; GRABNAR, 2014).

On the other hand, physical hydrogels are formed by transient junctions or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions. All these interactions are reversible and can be disrupted by variations in physical conditions such as ionic strength, pH, temperature or application of mechanical stresses, so the gels resulting from these interactions are considered reversible and weak (EL-SHERBINY; YACOUB, 2013; HOFFMAN, 2012). Even so, for some applications, the use of physically crosslinked gels is preferred since crosslinking agents are not required for their preparation.

In this way, a hydrogel is a hydrophilic polymeric network crosslinked somehow to produce a soft elastic/viscoelastic structure, and different methods can be used to form a crosslinked structure. Therefore, the water holding capacity and permeability are important characteristics that make hydrogels attractive for different research areas, mainly in biomedical applications.

The first registry of applications of hydrogels in the medical area dates back to 1960 when Wichterle and Lim (1960) developed and investigated poly-2-hydroxyethyl methacrylate (PHEMA) hydrogels as a biocompatible material useful for contact lens applications. In the 1980s the investigations gained more prominence when Lim and Sun (1980) demonstrated the application of sodium alginate microcapsules in cell encapsulation and Yannas together with co-workers (1989) evidenced the possibility of incorporation of natural polymers into hydrogels for wound-healing. Since then, hydrogels have been used as biomaterials in various biomedical applications.

In the last years, hydrogels have become increasingly studied as matrices for tissue engineering due to their ability to retain a great amount of water, good biocompatibility, low interfacial tension, and minimal mechanical and frictional irritation, all appealing features from the perspective of mimicking a bio-environment (MOTALLAH; KEHR, 2017;

VARAPRASAD *et al.*, 2017; ZHANG *et al.*, 2017).

Despite the many advantages of using conventional crosslinked hydrogels, their application domain is restricted due to their low mechanical properties. To overcome this, recent trends indicate a significant and growing interest in producing nanocomposite hydrogels for different biomedical applications.

2.1.1 Nanocomposite Hydrogels

Recent progress in the synthesis and functionalization of nanoparticles have made possible a significant increase in biomedical applications, emphasizing the release and delivery of drugs (SUKTHAM *et al.*, 2018), biosensors for diagnosis and prevention of diseases (PIRICH *et al.*, 2017) and regenerative medicine (GOENKA; SANT; SANT, 2014).

The International Organization for Standardization defines nanoparticles (NPs) as a broad class of materials, including particulate substance ranging in size from 1 to 100 nm (ISO/TS 80004-2:2015). These nanoparticles can be classified into different nano-systems depending on their morphology, size, and chemical properties (KHAN; SAHED; KHAN, 2017; MCNAMARA; TOFAIL, 2017). Based on the physical and chemical characteristics, nanoparticles can be classified in metallic, carbon, polymer nanoparticles and inorganic nanoparticles. Nanoparticles have unique characteristics and excellent mechanical properties that can be controlled and modified through chemical and structural changes on their surface (AZIZI *et al.*, 2013; YOHAN; CHITHRANI, 2014).

Combining the advantageous characteristics of the nanoparticles with the hydrogels networks opens new possibilities of applications. Recent trends show the increase in scientific research to incorporate different particles into conventional hydrogel.

Nanocomposite hydrogels can be defined as polymeric networks, either physically or covalently crosslinked with each other or with nanoparticles or nanostructures (GAHARWAR; PEPPAS; KHADEMHOSEINI, 2014). Nanocomposite hydrogels can be engineered to present superior physical, chemical, electrical, and biological properties opening possibilities

in the development of advanced biomaterials for applications that would not be possible with conventional hydrogels.

As previously stated, a large range of nanoparticles can be used with the natural or synthetic polymers to take nanocomposite hydrogels. The interactions between nanoparticles with the polymeric chains result in novel properties to the nanocomposite system.

Studies have reported the improvement of properties when combining nanoparticles with natural polymers. Xavier *et al.* (2015) developed bioactive nanocomposite hydrogels of silicate nanoparticles within collagen-matrix for application in bone tissue and reported the enhanced several mechanical, physical, chemical, and biological properties. Similarly, T. Zhang, Zuo, Hu, & Chang (2017) created a dual physically cross-linked hydrogels based on poly (acrylic acid-co-acrylamide) and tunicate cellulose nanocrystals, and the results show the toughness of dual crosslinked hydrogel was higher than that of the mono-cross-linked hydrogel, which was higher than that of PAAAM hydrogel.

In recent studies, Khalilipour and Paydayesh (2019) developed nanocomposite hydrogels incorporating nano zinc oxide (ZnO) into polyvinyl alcohol (PVA) for wound dressings. The nanocomposite hydrogel obtained presented better mechanical, structural, physical, and antibacterial properties. Besides, the viability of the cells increased with the progressive increase of ZnO nanoparticle.

Parsa, Paydayesh and Davachi, (2019) synthesized nanocomposite hydrogels based on polyvinyl alcohol (PVA) and nanoparticles of chitosan (NC) capable of transporting drugs (tetracycline) and conduct controlled release in the physiological environment. The effect of NC add on the physicochemical and antimicrobial properties was analyzed. The authors reported: (i) positive interaction of the PVA/NC/tetracycline system, (ii) structural properties such as swelling rate and gel fraction were influenced by drug and (iii) cell and antibacterial viability approved. Therefore, the hydrogel has the essential characteristics for use as a dressing, as well as having potential in controlled drug delivery systems.

Other studies on nanocomposite hydrogels are summarized in review articles Sharma *et al.* (2018), Nascimento *et al.* (2018), Vashist *et al.* (2018), Karimi and Wan Daud (2017), Jeznach, Kołbuk and Sajkiewicz (2018) and Motealleh and Kehr (2017).

2.2 Cellulose-based hydrogels

Cellulose is the most abundant organic biopolymer found in nature, with a wide presence in plants, dry wood, animals, and microorganisms (KAMIDE; KAMIDE, 2005; LI *et al.*, 2015). Besides, it exhibits excellent mechanical properties, biodegradability, hydrophilicity, and biocompatibility, which are responsible for the wide use of cellulose in different applications such as agriculture, medical science, and other industrial applications.

Cellulose is a semi-crystalline polysaccharide macromolecule, composed of D-glucose units that are linked together by β -(1–4)- glycosidic bonds and forms crystalline microfibrils and fibers with strong intra and intermolecular hydrogen bonds (OGUZLU; DANUMAH; BOLUK, 2017; ZHANG *et al.*, 2014). The basic chemical structure of cellulose is shown in Figure 2.3. The cellulose chain forms a complex structure of layers that have different functions, physical-chemical properties, and is practically insoluble in water and most organic solvents. Moreover, cellulose consists of both crystalline and amorphous regions. Therefore, the properties are strongly dependent on the crystalline organization and polymorphism of cellulose, besides chemical and thermal stability of the material (H.P.S. *et al.*, 2017; MARIANO, 2016).

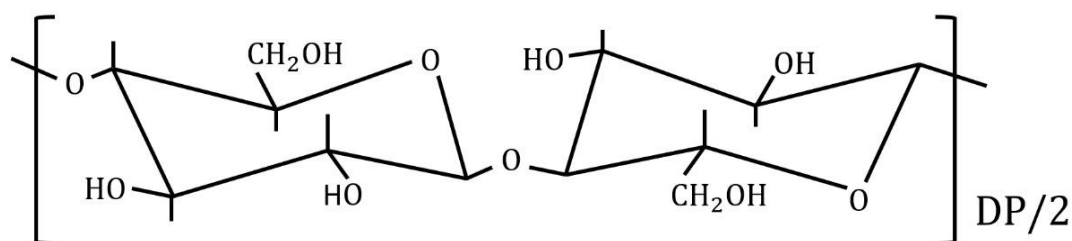


Figure 2.3: Basic chemical structure of cellulose.

Source: (BEMILLER, 2019)

Due to abundant hydroxyl groups, cellulose can be used to prepare hydrogels with fascinating structures and properties. These hydroxyl groups can form hydrogen bonding linked network easily in a cellulose solution, generating physically crosslinked cellulose hydrogels. However, the highly extended hydrogen bonded structure, which hinders the dissolution of the cellulose, limits the preparation of pure cellulose hydrogels (KABIR *et al.*, 2018).

On the other hand, the rigid structure of cellulose allows numerous chemical and physical reaction that converts the pure cellulose in cellulose ether-esters. Cellulose derivatives including methylcellulose (MC), hydroxyethyl cellulose (HEC), carboxymethyl cellulose (CMC), cellulose sulfate (CS), and cellulose phosphate (CP) have been used to obtain reversible or stable hydrogels (CHANG; ZHANG, 2011; HYNINEN *et al.*, 2018; KABIR *et al.*, 2018; RAUCCI *et al.*, 2015; SU *et al.*, 2010). Among cellulose ethers, CMC, CS, and CP are polyelectrolytes, which show sensitivity to pH and ionic strength.

Cellulose-based hydrogels are promising for applications in the biomedical field such as drug delivery and tissue engineering. Besides, cellulose can be converted to cellulose nanofibers (CNF) and cellulose nanocrystals (CNC) by top-down procedures (CLASEN; KULICKE, 2001; OGUZLU; DANUMAH; BOLUK, 2017). Cellulose nanoparticles are commonly used as reinforcing agents in nanocomposite hydrogels due to their unique properties such as high strength, low density, biodegradability, and biocompatibility.

2.2.1 Carboxymethylcellulose

Among the cellulose derivatives converted by etherification, carboxymethyl cellulose (CMC) is highlighted due to applications in food, cosmetics, pharmaceutical, and detergents industries. Carboxymethyl cellulose is an anionic polyelectrolyte, biodegradable and biocompatible with large water solubility. The water-solubility is obtained by introducing carboxymethyl groups along the cellulose chains, which makes hydration of the molecule possible.

CMC is produced by two main reactions the alkalization, followed carboxymethylation in the cellulose. The first is the impregnation of cellulose with aqueous sodium hydroxide solution. This reaction is of the acid/base type in which the hydroxyls of the cellulose react with a strong base occurring the formation of alkali-cellulose. The second step is the reaction between alkali-cellulose and monochloroacetic acid to form carboxymethyl cellulose ethers (ELLIOT; GANZ, 1974). In Figure 2.4, it is shown the process of obtaining carboxymethyl cellulose.

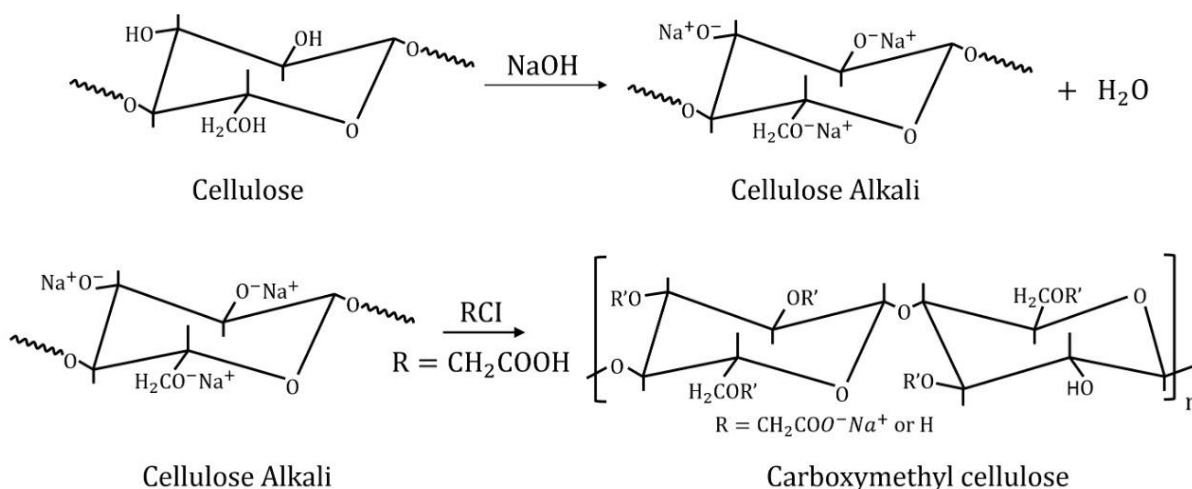


Figure 2.4: Process for obtaining carboxymethyl cellulose.

Source: (BEMILLER, 2019)

Therefore, the CMC molecular structure is based on β -(1–4)-D-glucopyranose polymer of cellulose. Different preparation methods may lead to different degrees of ionizable group substitution (DS) (HEINZE; KOSCHELLA, 2005). The DS value is defined as the number of moles of functionalization per mole of cellulose and reflects the proportion of hydroxyl groups which have been functionalized by a carboxymethyl group (KOMOROWSKA; RÓŻAŃSKA; RÓŻAŃSKI, 2017; VALAPPIL *et al.*, 2013). The water solubility, stability, clarity of the solutions are characteristics that are strongly dependent on DS. Also, the DS influences the rheological behavior of solutions, and these rheological properties can then serve as a guide for the selection of CMC type to achieve the desired characteristics in the final system.

Carboxymethylcellulose-based hydrogels are prepared from aqueous solutions with several crosslinking methods (ZHENG *et al.*, 2015). In the case of physical crosslinking, the network structure of the CMC hydrogel is constructed along with the formation of hydrogen bonds between the neighboring CMC polymer chains. In the chemical crosslinking, crosslinking agents such as polycarboxylic acids, epichlorohydrin, and N, N'-methylene-bisacrylamide (MBA) are commonly used (FEKETE *et al.*, 2017).

Carboxymethylcellulose is a negatively charged polyelectrolyte. Complex hydrogels can be created by adding other positively charged polyelectrolytes in the presence of CMC. In addition, the mixture of polyelectrolytes of opposite charges may gelate or precipitate depending on concentrations, ionic strength, and pH of the solution (CIOLACU; SUFLET,

2018; KABIR *et al.*, 2018). These hydrogels are especially used in oral pharmaceutical formulations as a disintegrant for capsules, tablets, and granules.

Carboxymethylcellulose-based hydrogels are suitable candidates as a stimuli-responsive biomaterial exhibiting pH and ionic strength-responsive property due to abundant acidic functional groups in hydrogel networks. Gao *et al.* (2014) synthesized and tested a series of biodegradable pH-sensitive hydrogels composed of acrylate-grafted carboxymethyl cellulose and poly(acrylic acid) for oral insulin delivery (GAO *et al.*, 2014). These hydrogels exhibited pH-responsive swelling behaviors, leading to pH-dependent enzymatic degradation properties and insulin release profiles. Because of their unique responsiveness to the gastrointestinal environment, these biodegradable and pH-sensitive hydrogels hold great promise as versatile vehicles for oral insulin delivery.

Summarizing, carboxymethyl cellulose is a water-soluble cellulose derivative. The water solubility, non-toxicity and low-cost make it is widely utilized as a natural ingredient of the hydrogels, and the properties of the CMC hydrogel can be affected by different parameters as molecular mass, solute concentration, pH, type of salt or ionic strength of the swelling solution.

Even with the abundant applications and advantages of CMC-based hydrogel, their use is limited in applications in which mechanical properties are undemanding such as drug delivery. However, to prepare hydrogels with improved mechanical performance and other attractive properties, cellulose nanocrystals can be used as fillers to reinforce polymeric hydrogels due to their favorable mechanical properties and intrinsic biocompatibility (FU *et al.*, 2019; NASCIMENTO *et al.*, 2018).

2.2.2 Cellulose nanocrystal

As mentioned in the previous sections, cellulose is a linear polymer comprised entirely of β -(1–4) linked anhydro-D-glucopyranose units. The chains of these biopolymers are self-assembled into microfibrils composed of amorphous and crystalline domains, which are known to alternate in the cellulose fiber structure.

The microfibril can be considered a string of cellulose crystals, linked along the chain axis by amorphous domains. These highly ordered crystalline domains can be isolated from microfibrils by means of chemically induced treatment, in the form of well-defined nanosized particles, denominated as cellulose nanocrystals (CNCs). This is represented in Figure 2.5. These cellulose nanocrystals exhibit mechanical properties that can vary based on their morphology, source, geometrical dimensions, crystal structure, the degree of crystallinity as well as the synthesis process.

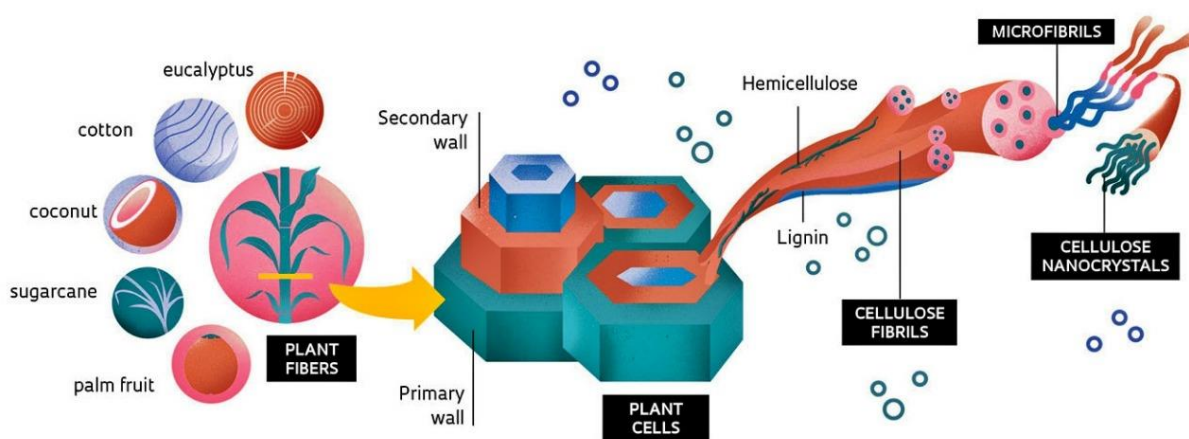


Figure 2.5: Hierarchical structure of cellulose extracted from plants.

Source: (CAMPOS; MALAGOLI, 2017)

As mentioned above, the isolation of CNC from cellulose substrate occurs using various types of acid hydrolysis, including hydrochloric, acetic, and sulfuric acid. During the acid hydrolysis process, the hydronium ions (H_3O^+) can penetrate the cellulose chains in the amorphous domains causing the hydrolytic cleavage of the glycosidic bonds and releasing individual crystallites (BRINCHI *et al.*, 2013; PIRES; NETO, 2017; TRACHE *et al.*, 2017).

The first works that reported the acid hydrolysis to obtain crystalline cellulose began in the late 1940s. Nickerson and Habrle (1947) noted that the sulfuric acid penetrated the amorphous regions of the fibers, resulting in smaller cellulose crystallites. The studies were confirmed by Rånby (1951) when he produced stable aqueous colloidal dispersions of cellulose crystals, isolated by sulfuric acid hydrolysis of cotton and wood pulp. Until the late 1990s, the term cellulose whiskers were used for cellulose nanocrystals, because of their needle-like morphology and high crystallinity.

The hydrolysis using Sulfuric acid is the most used process in CNC preparation. The choice of sulfuric acid is due to the substitution of hydroxyl groups (OH) of the cellulose by sulfate groups (OSO_3^-) during the reaction. Figure 2.6 shows a schematic of the extraction process of CNC from cellulose structure by sulfuric acid hydrolysis. These groups are negatively charged, leading to the formation of a negative electrostatic layer covering the nanocrystals, promotes their dispersion in water and allows obtaining stable colloidal dispersions, due to repulsive forces between individual nanoparticles (PIRES; NETO, 2017; QIAO *et al.*, 2016a).

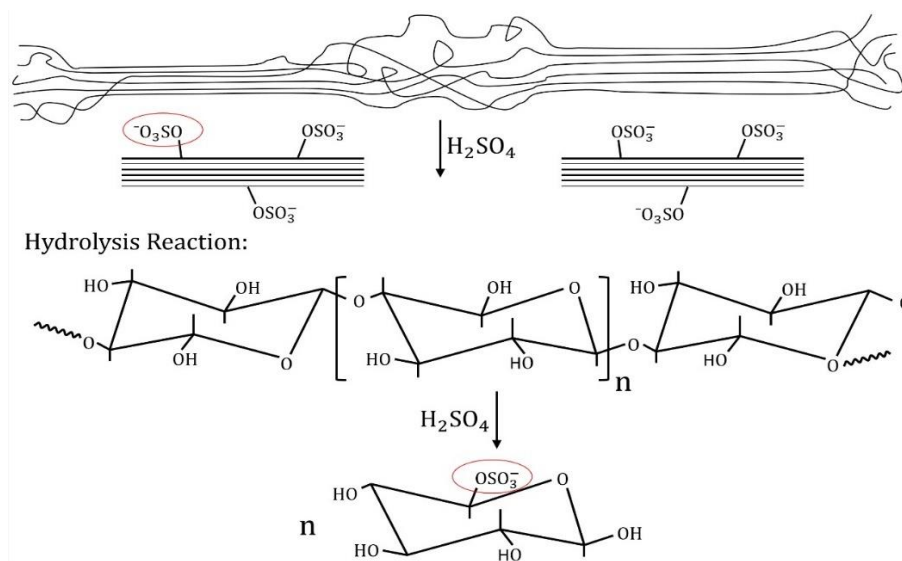


Figure 2.6: Sulfuric acid hydrolysis reaction producing cellulose nanocrystal with negatively charged sulfate on its surface.

The stability of the colloidal dispersions is determined by the DLVO theory (Derjaguin, Landau, Verwey, and Overbeek). The DLVO theory considers that the stability of a colloidal dispersion is determined by the balance of attractive van der Waals forces and the electrical double layer forces, which are repulsive (BHATTACHARJEE, 2016; LEONG; ONG, 2003; OGUZLU; DANUMAH; BOLUK, 2017).

Charged colloidal particles such as sulfated CNC are stabilized by the electrostatic repulsive force between the particles. When two particles become close, the electric double layer around the particles prevents their aggregation and keeps them apart.

The electrical potential on particle surface characterizes the electrostatic repulsive potential that counters the attractive potential and, indirectly, is a measure of the magnitude of

the van der Waals potential. This surface potential is not measured directly. However, the electric potential at the slipping plane near the particle surface can be measured experimentally and is therefore proportional to the surface potential (BHATTACHARJEE, 2016; LEONG; ONG, 2003; LI, 2004). Thus, the surface potential is an important parameter for the evaluation of colloidal dispersions stability. The surface of a charged particle is represented schematically Figure 2.7.

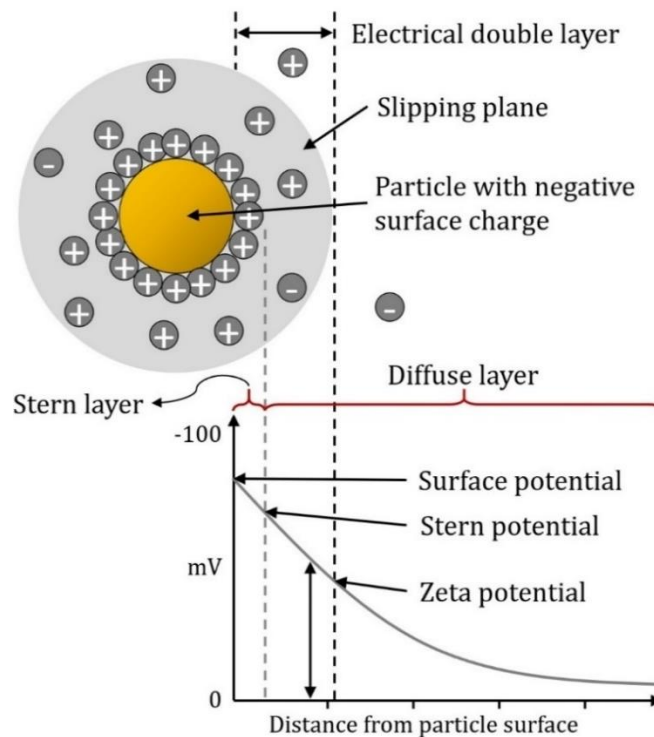


Figure 2.7: Schematic representation of the double electric layer and the zeta potential.

Source: (PAR; SEO, 2011)

The magnitude of the zeta potential indicates the stability of the colloidal system. In general, the colloidal dispersion is stable if it has a zeta potential greater than or equal to +30 mV or less than or equal to -30 mV (HEURTAULT *et al.*, 2003).

In addition, to the modifications that may occur during the isolation of the CNCs during the acid hydrolysis. The cellulose source and extraction conditions affect directly in the morphology, length (L), diameter (D), and crystallinity of produced CNC. In general, CNCs extracted from cotton, coconuts, sugarcane bagasse, etc., have a lower length than CNC isolated from tunicates. Table 2.1 shows the variation of crystallinity and dimensions of CNC provided from some sources.

Table 2.1: The crystallinity and dimensions of CNC provided from sources.

Source	Length (mm)	Diameter (mm)	Crystallinity (%)
Cotton	100-300	5-10	70-90
Alga	1000	20	-
Wood pulp	100-300	3-15	70-85
Hard wood	140-150	4-5	43-65
Bacterial	100-1000	10-50	72-74
Tunicate	100-4000	10-30	83-90

Source: (MOON *et al.*, 2011).

The degree of crystallinity of CNC is expected to be very high and may is considered a crystal no apparent defect. So, the values for this parameter can be range from 46 to 96%. Also, the degree of crystallinity is correlated with CNC mechanical properties.

CNC has a rod-like or needle-like morphology. The morphology and dimensions of CNC depend on its cellulosic source and can vary widely, with a diameter in the range of 5–50 nm and length in the range of 100–500 nm. CNC is often described in terms of its aspect ratio (L/D), which is defined as the ratio between the length and diameter of the rod-like particles. The aspect ratio is a parameter of great influence on the rheological behavior and the capacity of reinforcement of the nanocrystal.

Yang *et al.*, (2014) studied how the aspect ratio of CNCs affected mechanical reinforcement in nanocomposites of cellulose nanocrystals/poly(acrylamide) and reported the CNCs with a greater aspect ratio contributed to higher mechanical properties. Similarly, Wu *et al.*, (2014) studied the influence of CNCs aspect ratio on its dispersions steady and dynamic rheological behavior over a wide range of CNC concentrations. CNC dispersions with higher aspect ratio formed a hydrogel at lower concentrations than the CNC dispersions with a lower aspect ratio.

Compared to most of the current nanoparticles, CNCs have unique properties such as renewability, high aspect ratio, excellent mechanical properties, biodegradability, good

processability, high water holding capacity, low density, high crystallinity as well as lower cost versus common nanomaterials, leading to an increase in its applications. These properties added to their biocompatibility and low-toxicity are also attractive for applications as biomaterials.

An important application of CNCs is as a rheological modifier. In addition to the aspect ratio, the rheological properties of CNC can be affected by other parameters such as source, morphology, ionic strength, temperature, surface charge, solvent interaction, and others (CHING *et al.*, 2016; QIAO *et al.*, 2016a).

In general, CNC-hydrogel interactions may be physical, chemical, or a combination of both. CNC can be used as a major component or only be dispersed within the hydrogel structure. The synthesis of the hydrogel may involve just a step, as simultaneous polymerization and crosslinking, or several steps, as chemical modification of CNC, followed by crosslinking.

Hydrogels scaffolds are of great interest due to their high hydrophilicity and biocompatibility, presenting rheological behavior that can be tuned for extrusion printing, such as shear-thinning, viscoelasticity and thixotropy.

2.3 Bioprinting

According to the International Organization for Standardization, additive manufacturing (AM) is a process of joining materials to make objects from 3D model data, layer upon layer, (ISO/ASTM 52900, 2015). This process has grown rapidly, has been used in many industrial applications, and in recent years, different research groups have combined the additive manufacturing process with the biomedical field. Thus, emerging the Biofabrication term. Biofabrication was defined by Groll and coworkers (2016), as “the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through Bioprinting or Bioassembly and subsequent tissue maturation processes” (GROLL *et al.*, 2016). Therefore, Bioprinting is one of the two main strategies of Biofabrication and is utilized in order to produce bio-engineered structures with three-dimensional organization serving in regenerative medicine, pharmacokinetic and basic cell biology studies

(GROLL *et al.*, 2016; GUILLEMOT; MIRONOV; NAKAMURA, 2010).

In this way, different additive manufacture techniques such as laser-assisted bioprinting, stereolithography, inkjet-based bioprinting and extrusion-based bioprinting are utilized in the scaffold fabrication, cell behavior studies and tissue repair (LI *et al.*, 2016). Among these techniques, extrusion-based bioprinting has been used to obtain 3D scaffolds because allow the use of different bio-inks resulting in scaffolds with high biocompatibility, different geometries, and better structural resolution due to the continuous deposition of filaments (AHLFELD *et al.*, 2017; CHUNG *et al.*, 2013; MORONI *et al.*, 2018; SARKER *et al.*, 2019).

In this method, inks, usually a gel or hydrogel-based material, are dispensed from a deposition system, under the control of a computer, resulting in the controlled deposition of continuous filaments obtaining 3D custom-shaped structures. The fluid-dispensing system can be controlled by a pneumatic system, a piston or screw-driven as shown in Figure 2.8. Pneumatic system uses pressurized air and can occur delay in compressed gas volume. Piston-driven deposition provides more direct control over the material flow, and the screw-driven system is beneficial for dispensing materials with higher viscosities (OZBOLAT; HOSPODIUK, 2016).

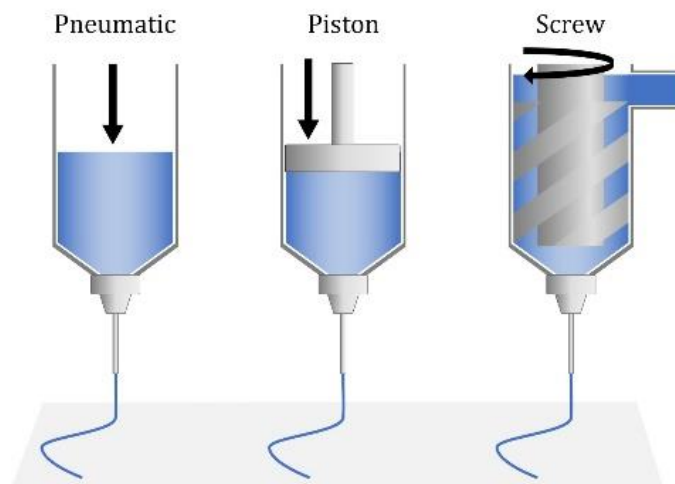


Figure 2.8: Schematic representation of the fluid-dispensing systems for 3D extrusion-based printing.

To secure printing of different geometries with high structural integrity it is necessary to evaluate the rheological properties because these are crucial in the 3D extrusion-based printing process. Some of the essential rheological properties such as viscosity, shear thinning and thixotropy are illustrated in Figure 2.9.

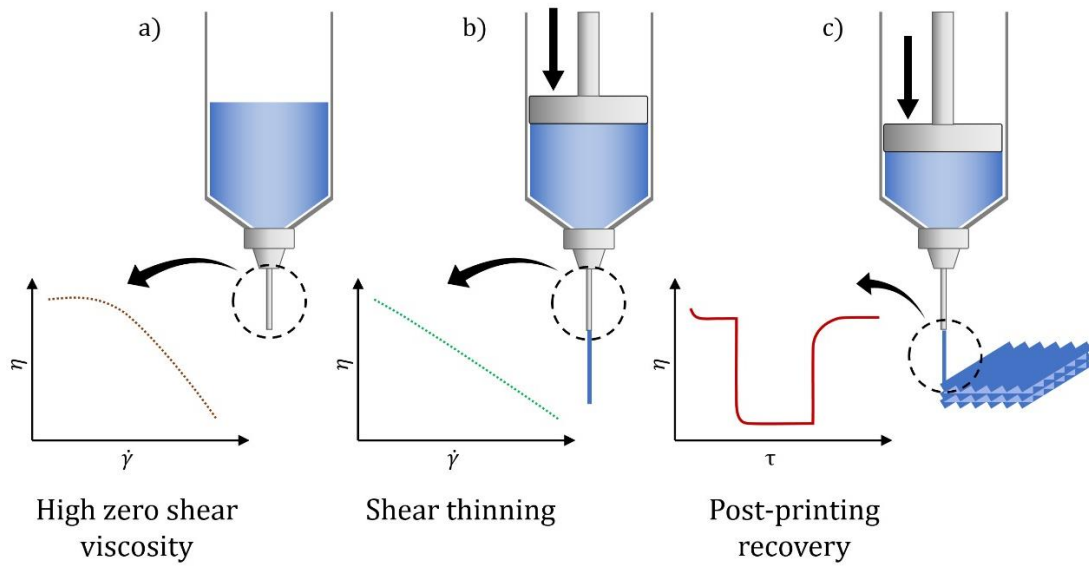


Figure 2.9: Rheological properties essential for 3D extrusion-based printing process: (a) high zero shear viscosity, (b) strong shear-thinning and (c) post-printing recovery.

Therefore, the rheological characterization provides information on the structural characteristics of hydrogels. In the next section, the fundamental details associated with rheological behavior are presented.

2.4 Rheological behavior

Rheology is the study of the flow properties and deformation behavior of matter. Rheological properties are the mechanical properties of materials that flow (liquids) and deform (solids) and are expressed in terms of the effects of stress, strain, and time.

Polymeric systems, foods, biological systems, dispersions, emulsions, and pastes are examples of complex systems which show mechanical responses intermediate between those of liquids and solids because they are composed of both viscous and elastic components. So, the relationships between stress and deformation for these materials differ from Newton's law of viscosity, which describes the shear behavior for normal liquids, and also do not follow Hooke's law of elasticity used for metals and other elastic materials (MORRISON, 2001).

Hydrogels, polymer solutions, and dispersions are complex materials, and their

rheological characterization represents an important tool to investigate their structural characteristics that are fundamental to evaluate their potential use and performance in various applications, mainly hydrogels with use potential in the extrusion 3D printing.

A common way of characterizing a liquid is by measurement of its viscosity. Viscosity is the resistance of a fluid to sliding motion. The non-constant value of $\eta(\dot{\gamma})$ at different shear rates are usually the first indication that the fluid is non-Newtonian. The viscosity is defined as

$$\eta(\dot{\gamma}) \equiv \frac{-\tau_{21}}{\dot{\gamma}_0} \quad 2.1$$

where $-\tau_{21}$ is a constant stress and $\dot{\gamma}_0$ is a constant shear rate.

Most polymeric solutions exhibit non-Newtonian behavior, where viscosity varies with the shear rate. The viscosity of a polymer solution is influenced by the size, flexibility, degree of hydration of polymer molecules, solution concentration, and the conformations they adopt in the solvent system and all of which influence of the viscosity versus shear-rate behavior. Figure 2.10 shows the schematic representation for three different viscosity behavior versus shear rate.

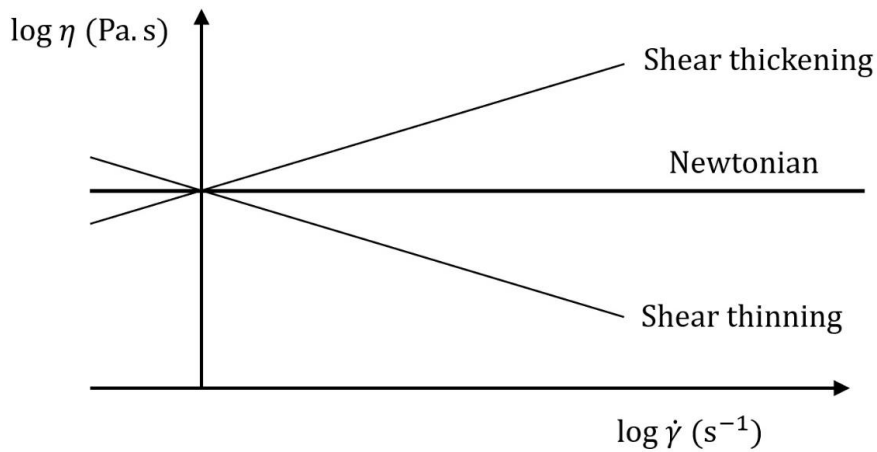


Figure 2.10: Schematic representation of viscosity behavior.

Source: based on (MORRISON, 2001)

The most common non-Newtonian effect in polymeric systems is shear-thinning. Shear-thinning is the tendency of some materials to present a viscosity decrease when they are driven to flow at high rates. Some materials show the opposite effect, that is, they exhibit higher viscosity with the shear rate increase; this is called shear-thickening.

2.4.1 Shear-thinning behavior

Shear-thinning is observed in concentrated polymer solutions. The schematic representation of the molecular structure during shear-thinning behavior is shown in Figure 2.11.b. At low rates of shear, the molecules are randomly entangled, so they have difficulty sliding past each other, resulting in high viscosity. This constant high viscosity is referred to as a Newtonian plateau. As the shear rate is increased, the polymer chains begin to disentangle and orient in along the flow. This phenomenon is called shear-thinning behavior, depicted in Figure 2.11.a. Finally, after oriented, the molecules can no longer stretch, resulting in a second Newtonian plateau.

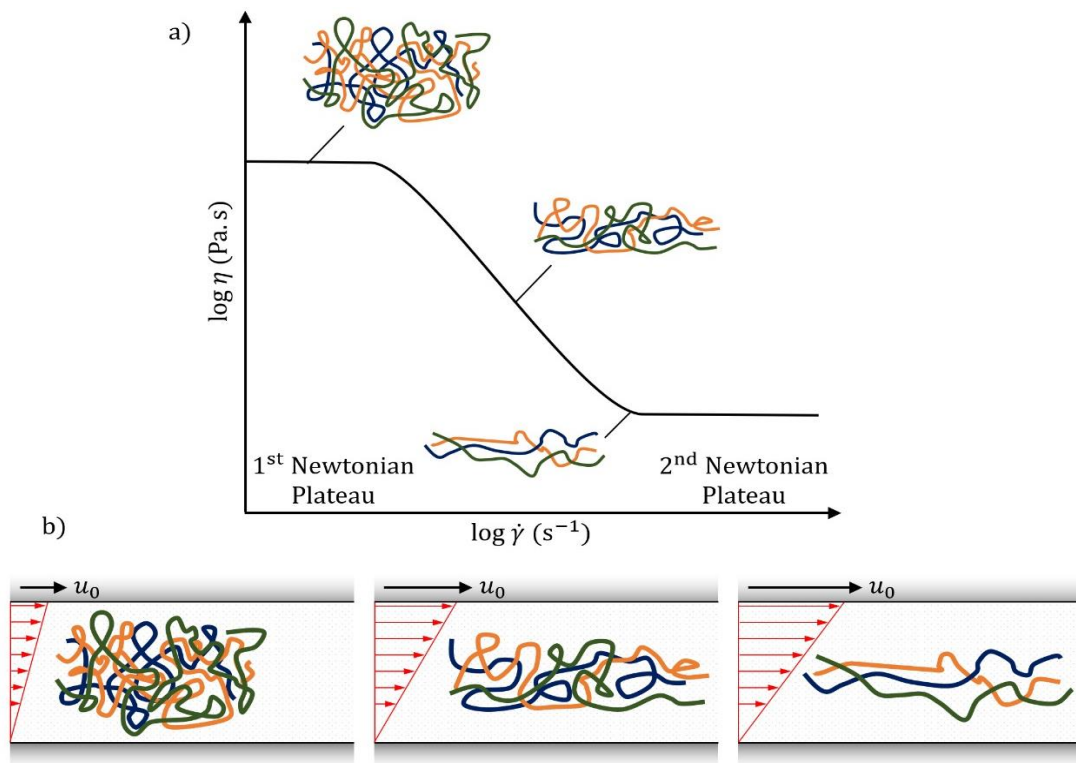


Figure 2.11: Schematic representation of shear-thinning behavior a) shear-thinning (b) molecular structure with increased shear rate. Source: based on (OSSWALD; RUDOLPH, 2015)

Rheology modifiers such as CNC can be added to polymer solution leading to shear-thinning behavior at low polymer concentrations. Hence, the viscosity profile can be adapted from moderated shear-thinning to strong shear-thinning depending on the concentration. This effect is shown in Figure 2.12.

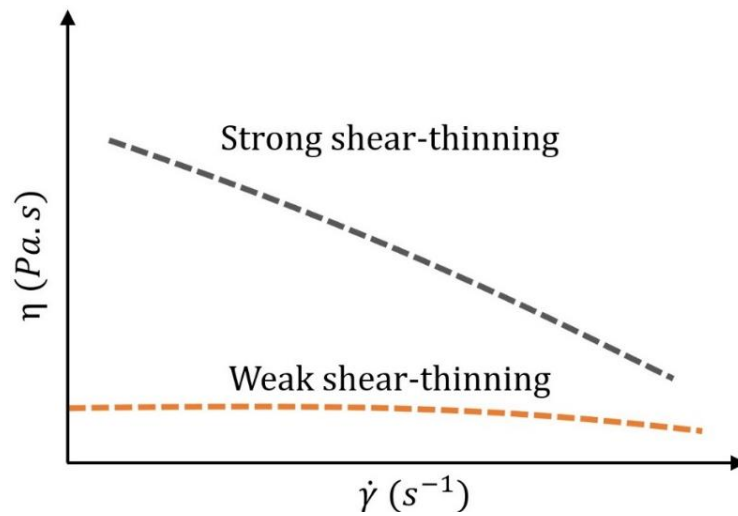


Figure 2.12: Comparison between shear-thinning behavior of polymer solution in dilute state and solution rheological modified.

Several polymeric biomaterials, such as hydrogels and polymer solutions, show marked shear-thinning. Shear-thinning can be linked to diverse structural processes, among which, the breakup of interchain connections for physical hydrogels represents the predominant mechanism induced by increasing stresses and strains within nonlinear conditions.

In Table 2.2, different mathematical models used to describe viscosity curves showing shear-thinning behavior are presented.

Table 2.2: Mathematical models for viscosity curves fitting

Model	Equation	Reference	Parameters
Ostwald-de Waele	$\eta(\dot{\gamma}) = m\dot{\gamma}^{n-1}$	(MORRISON, 2001)	m consistency index n power-law index
Carreau	$\frac{\eta(\dot{\gamma}) - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = [1 + (\lambda\dot{\gamma})^2]^{\frac{n-1}{2}}$	(CARREAU, 1972)	η_0 zero-shear viscosity η_{∞} infinite shear viscosity
Carreau-Yasuda	$\frac{\eta(\dot{\gamma}) - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = [1 + (\lambda\dot{\gamma})^a]^{\frac{n-1}{a}}$	(YASUDA; <i>et al.</i> , 1981)	$\eta_{\infty} = 0$, simplified model
Cross	$\frac{\eta(\dot{\gamma}) - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = 1 + (\lambda\dot{\gamma})^{-n}$	(CROSS, 1965)	λ time constant of the fluid a transition parameter

2.4.2 Viscoelastic properties

In an oscillatory rheological test, a sample is subjected to low-frequency stress or strain input, which is recorded along with the strain or stress response. In this test, it is possible to separate the elastic contribution of the viscous contribution on the total property of the polymer with respect to amplitude and frequency. The elastic behavior is associated with the storage modulus (G') and the viscous behavior with the loss modulus (G'').

These material functions can be analyzed through amplitude and frequency sweep tests. In the amplitude sweep test, illustrated in Figure 2.13, the angular frequency remains constant while the strain or stress amplitude increases as a function of the time. The strain and stress sweeps are described as follows:

$$\gamma(t) = \gamma_A \sin(\omega t) \quad (2.2)$$

$$\tau(t) = \tau_A \sin(\omega t) \quad (2.3)$$

where γ and τ are the strain and stress respectively, (A) is amplitude (t) is the time and (ω) is the angular frequency.

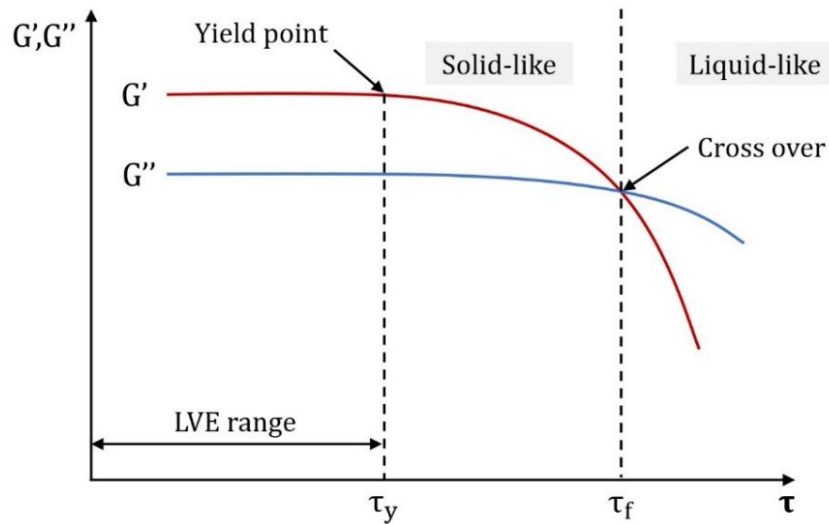


Figure 2.13: Amplitude sweep test illustrating the linear viscoelasticity range (LVE), yield stress (τ_y) and the transition from the solid-like to the liquid-like behavior, which takes crossover.

As observed, in the amplitude sweep test, the linear viscoelasticity range (LVE) is defined. The LVE is the region where the viscoelastic properties do not change with strain or

deformation. Yield stress is the limiting value of the LVE range, and Crossover defines the transition to liquid-like behavior ($G'' > G'$).

After knowing the LVE, frequency sweep tests are performed to determine the viscoelastic behavior of the material. As mentioned above, $G'(\omega)$ describes the storage modulus that gives information about the elasticity or the energy stored in the material during a shearing process, upon release this energy can be used completely for the recovery of the deformation, which makes it reversible. On the other hand, $G''(\omega)$ describes the viscous character or the energy dissipated of the material. Figure 2.14 shows examples of viscoelastic behavior of samples at different concentrations.

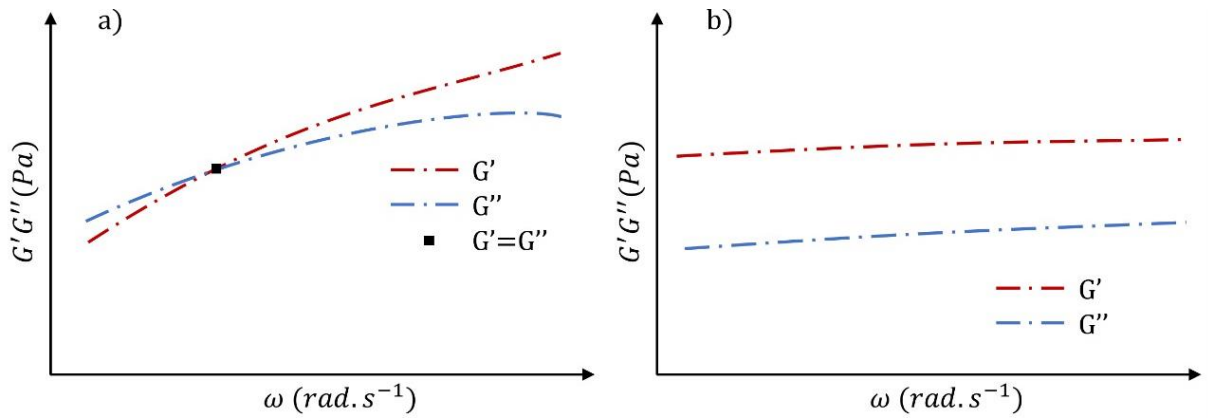


Figure 2.14: Frequency dependence of the elastic (storage) modulus G' and viscous (loss) modulus G''

Generally, concentrated solutions or entangled networks at low-frequencies present viscous behavior ($G'' > G'$), while at high frequencies there is the transition of viscous to elastic behavior ($G' > G''$), as observed in Figure 2.14.a. On the other hand, Figure 2.14.b illustrate the viscoelastic behavior different from gels. In this case, the modules are almost frequency independent. There is no transition between them and the elastic modulus is higher than that viscous.

Ideal viscous materials are changed by deformation, which makes the process irreversible. The ratio between the viscous modulus and the elastic modulus is denominated damping and it is described by:

$$\tan(\delta) = \frac{G''}{G'} \quad (2.3)$$

When $\tan(\delta) < 1$ the solid-like is predominant, G' dominates G'' . In a liquid-like, $\tan(\delta) > 1$, so, G'' dominates G' completely. However, when the viscous and the elastic behavior are equal to $\tan(\delta) = 1$, the material is making a transition from liquid to solid or vice versa.

Oscillatory tests are important to evaluate the viscoelastic properties and the dependence of viscoelastic moduli upon the frequency and so to discriminate between solution and gels.

2.4.3 Thixotropic behavior

Thixotropy is a phenomenon where the viscosity decreases as the rate of shear increases, but it changes occurred as a function of the time, rather than instantaneously. After cessation of shear, thixotropic fluids regain their original viscosity, but again only after a measurable time interval, which can range from fractions of a second to hours. Hence, thixotropy is a time-dependent phenomenon (BEMILLER, 2019; MEWIS; WAGNER, 2009; ŻOŁEK-TRYZNOWSKA, 2016). The most common test methods for evaluating the thixotropy are 3 intervals thixotropy test (3ITT) and hysteresis area. Both tests are illustrated in Figure 2.15.

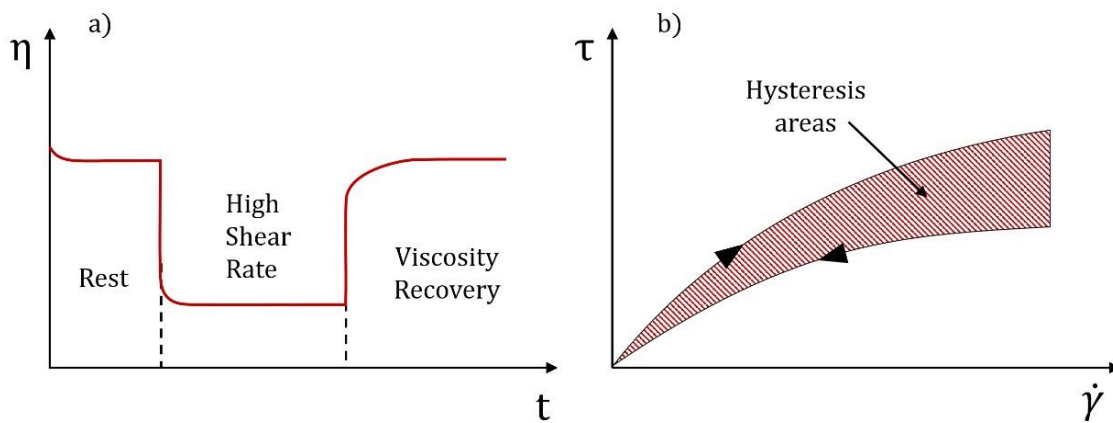


Figure 2.15: Test methods for thixotropy testing (a) time-dependent viscosity of a sample with thixotropic behavior (3ITT); (b) flow curve showing the hysteresis area.

Three intervals thixotropy test consists of 3 intervals of application of the shear rate, performed at two different shear rates. The first and last intervals are performed at a low shear

rate, and the second interval is performed at a high shear rate. Figure 2.15.a shows the schematic representation of this test. In the first interval, a very low shear rate is applied to simulate the behavior at rest. Then, in the second interval, the sample is strongly sheared simulating the structural breakdown of the sample. Next, in the third interval, the low shear rate is applied again by a higher period. In this latter, it is simulated the structural recovery at rest. This test type can be used very well in most cases to simulate the conditions of application processes. There are different methods for analyzing the structural regeneration after 3ITT; the most used is the recovery ratio after a given time, where it is defined times in which the viscosities of the structural recovery are compared to the viscosity of the rest interval.

$$\% Recovery = \frac{\eta_{sr}}{\eta_r} \times 100 \quad (2.4)$$

where η_{sr} is the viscosity of structural recovery and η_r is the viscosity in rest.

Hysteresis test consists of systematically increasing and decreasing the shear rate between zero and a maximum value, generating an upwards and other downwards ramp. So, the area between the ramps is measured. If the upwards and downwards ramp does not differ from each other, the behavior of the samples is time-independent when shearing. However, if the upwards ramp shows a higher shear stress reading than the downwards ramp, the behavior of the samples is time-dependent under shear load. The area within the hysteresis loop represents the energy consumed in structure breakdown. Figure 2.15.b shows the schematic representation of this test. Hysteresis area evaluates the amount of structural breakdown under high shear conditions but does not evaluate structural recovery under low shear conditions. Therefore, this method does not fully evaluate the thixotropic behavior.

Solutions of polysaccharides such as alginate and carboxymethyl cellulose (CMC) can exhibit thixotropic behavior. In the CMC, the degree of thixotropy is a function of the degree of substitution (DS), the uniformity of substitution, and the degree of polymerization (DS) of the polymer (BEMILLER, 2019; EDALI; ESMail; VATISTAS, 2001).

PENG *et al.*, (2018) studied the thickening properties and steady rheological behavior of CNC dispersions into different systems containing polymers of varying charge properties and molecular structures. The results showed that due to the higher aspect ratio, CNC rod-like

particles are more efficient in viscosity enhancement compared to classic spherical models, the addition of CNC resulted in improved rheological properties in all systems studied.

Oguzlu, Danumah, and Boluk (2016) investigated the rheological behavior of CMC/CNC dispersions. The addition of CNC particles (0.33 at 2.0 vol. %) significantly increased the viscosity of the polymer in dilute and semi-dilute concentration state (0.5 at 3 vol.%). The authors reported the increase in viscosity at low shear rates in the function of the increase in the concentration. This increase was associated with nematic flocculation of CNCs in all samples.

The incorporation of CNC into hydrogel formulations promotes improvements in properties and a significant increase in the range of applications. The mechanical properties are improved due to particle/particle and particle/polymer interactions and the high concentration of the particle may induce an increase in the viscosity of the system and reduction in the mobility of the polymer chains, favoring the formation of a non-homogeneous polymer network during crosslinking (NASCIMENTO *et al.*, 2018).

3 RHEOLOGICAL BEHAVIOR OF CMC/CNC DISPERSIONS

In this chapter, the rheological behavior of carboxymethyl cellulose (CMC) solutions and CMC/cellulose nanocrystal aqueous dispersions were studied. In CMC solutions, shear-thinning behavior was observed for all samples; solutions in the dilute state (0.5 and 1.0 wt. %) exhibit a near-Newtonian behavior and shear-thinning behavior progressively with concentration increase. Similar behavior was observed for CNC dispersions. The experimental data for CMC solutions were well represented by the Cross-viscosity model. Finally, the influence of the progressive addition of low CNC concentrations (0.5 to 2 wt. %) in the rheological behavior of CMC dilute solutions (0.5 and 1.0 wt. %) were analyzed. The addition of CNC increased drastically the viscosity, and the subtle shear-thinning of CMC solutions was altered to strong shear-thinning. Frequency sweep tests were conducted to determine the viscoelastic behavior of the material, only the lowest concentration showed predominant viscous behavior ($G'' > G'$), while the other concentrations showed a transition from viscous to elastic behavior ($G' > G''$). Thixotropy tests were performed, and CMC/CNC dispersions presented time-dependence. Particle stability was studied in terms of dynamic light scattering (DLS) and zeta potential measurements. The negative magnitude of zeta potential increased, which indicates stable dispersions and strong repulsive forces. Results from DLS revealed an increase in the hydrodynamic diameter of suspended CNC due to the presence of CMC chains. The phase transition from the isotropic phase to the anisotropic phase was determined using polarized optical microscopy (POM). Liquid crystalline droplets dispersed in the isotropic phase were observed in all concentrations studied. Depletion flocculation was identified as the main interaction between CMC chains and CNC particles, which is responsible for the strong changes in rheological properties of system CMC/CNC.

3.1 Experimental Design

3.1.1 Materials

Sodium carboxymethyl cellulose (CMC) with an average molar mass $M_w = 250.000 \text{ g.mol}^{-1}$ and degree of substitution $DS = 0.70$ was purchased from Sigma-Aldrich. Cellulose nanocrystals (CNC) aqueous dispersion (12 wt. % of CNC) were purchased from the US Department of Agriculture Forest Product Lab (Lot number 2016-FPL-CNC-098) and supplied by the University of Maine. The degree of sulfonation and mass concentration was 0.95 wt. % sulfur on dry CNC and 12.1 wt. % dry CNC, respectively.

3.1.2 Preparation of solutions and dispersions

CMC solutions with concentrations from 0.25 to 3.0 wt. % were prepared by adding CMC powder to deionized water with $pH = 7$ and magnetic stirring for 6 hours at room temperature until complete solubilization. CNC dispersions in the range of 0.5 – 2.0 wt. % were prepared by adding DI water in the original CNC dispersion under stirring. Next, CNC dispersions with concentrations of 0.5, 1.0, 1.5, and 2 wt. % were added at the CMC solutions of 0.5 and 1.0 wt. %. Each dispersion was stirred overnight. Finally, the samples were sonicated using a USC 700 ultrasonic bath for 10 minutes to ensure a homogeneous mixture. All samples were stored for 4h at room temperature 23 °C before the rheological tests. Figure 3.1 shows the process of preparation of CMC solution, CNC, and CNC/CMC dispersions.

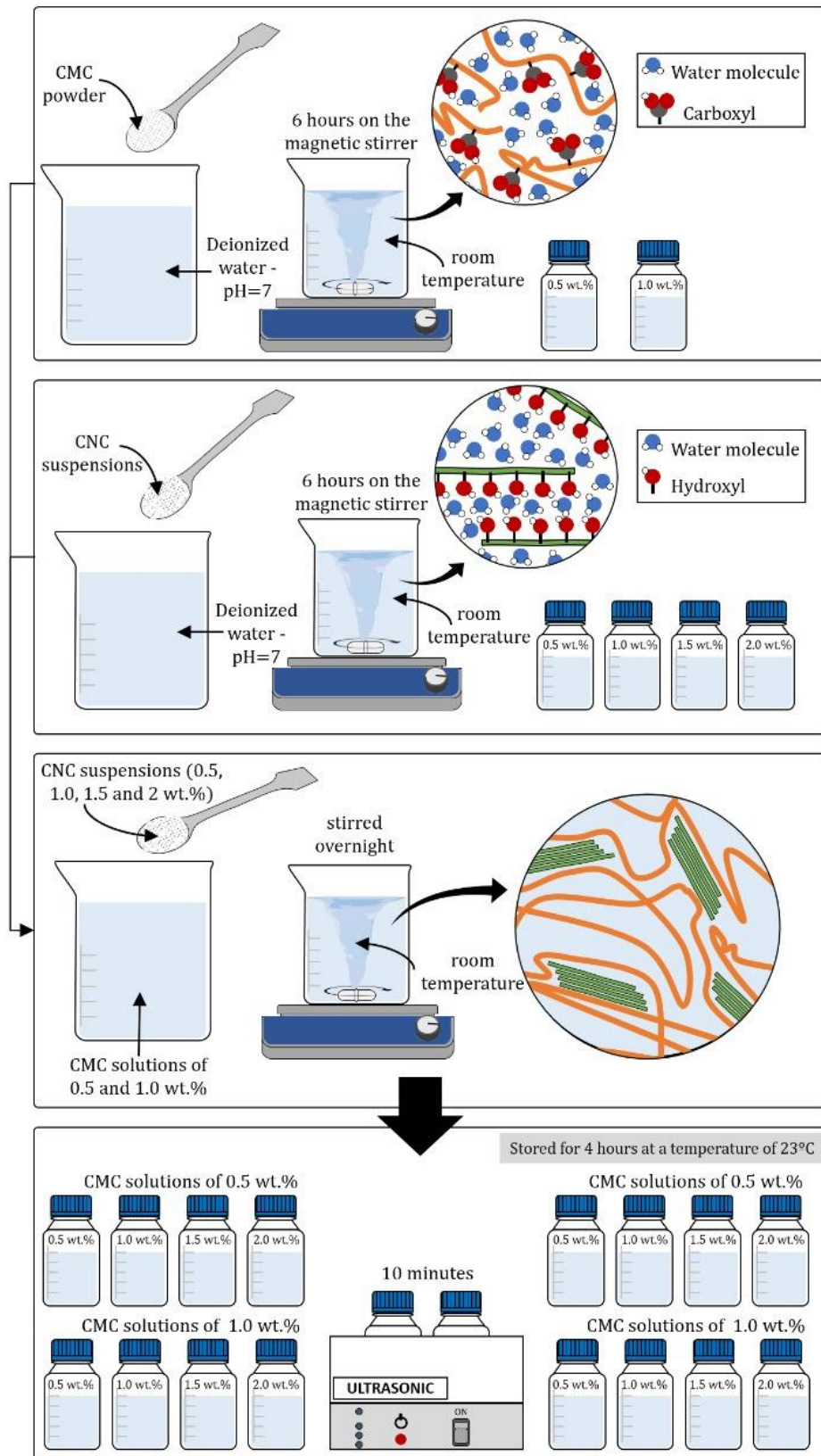


Figure 3.1: Preparation of CMC solution, CNC, and CNC/CMC dispersions.

3.1.3 Polarized optical microscopy (POM)

The microstructure of the CMC/CNC system was observed using a polarized light optical microscope. Polarized optical microscopy (POM) was performed using a Leica DMLP Polarizing Microscope equipped with 5 to 20x magnification lenses and an Optical View video camera (Mannheim, Germany).

3.1.4 Zeta Potential

Zeta potential measurements were carried out in dilute CMC, CNC and CMC/CNC dispersions. The samples with and without polymers were previously diluted (1:30) and sonicated for 10 *min*. The zeta potential was measured using a Malvern Zeta sizer Nano-S instrument with a detection angle of 173 °. All measurements were performed in triplicate and performed at a temperature of 25 °C.

3.1.5 Dynamic Light Scattering

Size distribution experiments in dilute CNC and CNC samples in the presence of CMC were performed in triplicate, and the hydrodynamic diameter was obtained in terms of Z-average (Equation 3.1) using a dynamic light scattering (DLS) evaluator Zeta sizer Nano-S, Malvern instrument at 25 °C.

$$Z - average = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (3.1)$$

where n is the number of particles with a given diameter, d.

3.1.6 Rheological characterization

Rheological measurements were performed at 25 °C using an Anton Paar MCR-102 Modular Compact Rheometer. A cone-plate geometry (CP50-1) with 50 mm in diameter, the cone angle of 0.9815 and truncation of 97 μm was used for all samples. Before each test, a pre-shear of 200 s^{-1} for 200 s followed by a rest period of 300 s was carried out in order to avoid any memory effect. Steady shear viscosity curves versus shear rate were obtained for each sample for shear rates in the range of 0.01 to 1000 s^{-1} . Amplitude sweep tests were conducted at an angular frequency of 10 rad.s^{-1} with a shear strain from 0.01 to 100% to obtain the linear viscoelasticity (LVE) range ($\gamma_0 = 1\%$ for all samples). Next, frequency sweep tests were performed in the range of 0.1 to 240 rad.s^{-1} . Hysteresis loop tests were conducted to analyze the energy consumption during steady shear. The shear rate was consecutively increased from 2 to 1000 s^{-1} during the first 165 s, was maintained at for 50 s, and was again decreased to 2 s^{-1} over 165 s. Further, three-interval thixotropy tests (3ITT) were carried out to characterize the viscosity recovery. A rest interval a low shear rate of 1 s^{-1} was applied for 25 s, followed by a sudden increase to 100 s^{-1} for 50 s, and back to 1 s^{-1} for 250 s. All rheological measurements were triplicated. Figure 3.2 shows a schematic representation of rheological characterization.

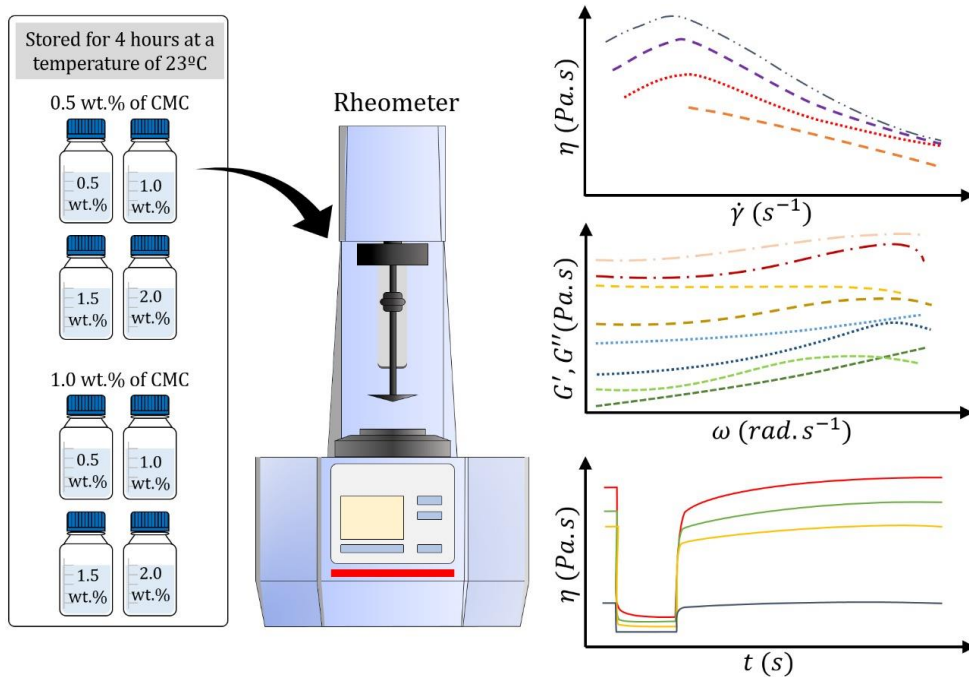


Figure 3.2: Rheological characterization of CMC solution, CNC, and CNC/CMC dispersions.

3.2 Results and discussion

3.2.1 Polarized Optical Microscopy

As previously mentioned, CNC suspensions can present different properties according to their crystalline structure organization. For example, at low concentrations, it can present liquid crystalline and isotropic domains, whereas gel-like states appear at high concentrations (UREÑA-BENAVIDES *et al.*, 2011). Formation of structures of CNC suspensions in CMC solutions was analyzed using polarized optical microscopy (POM). In Figure 3.3, it is shown POM images for solutions with 0.5 wt. % of CMC with CNC concentration from 0.5 to 2 wt. %.

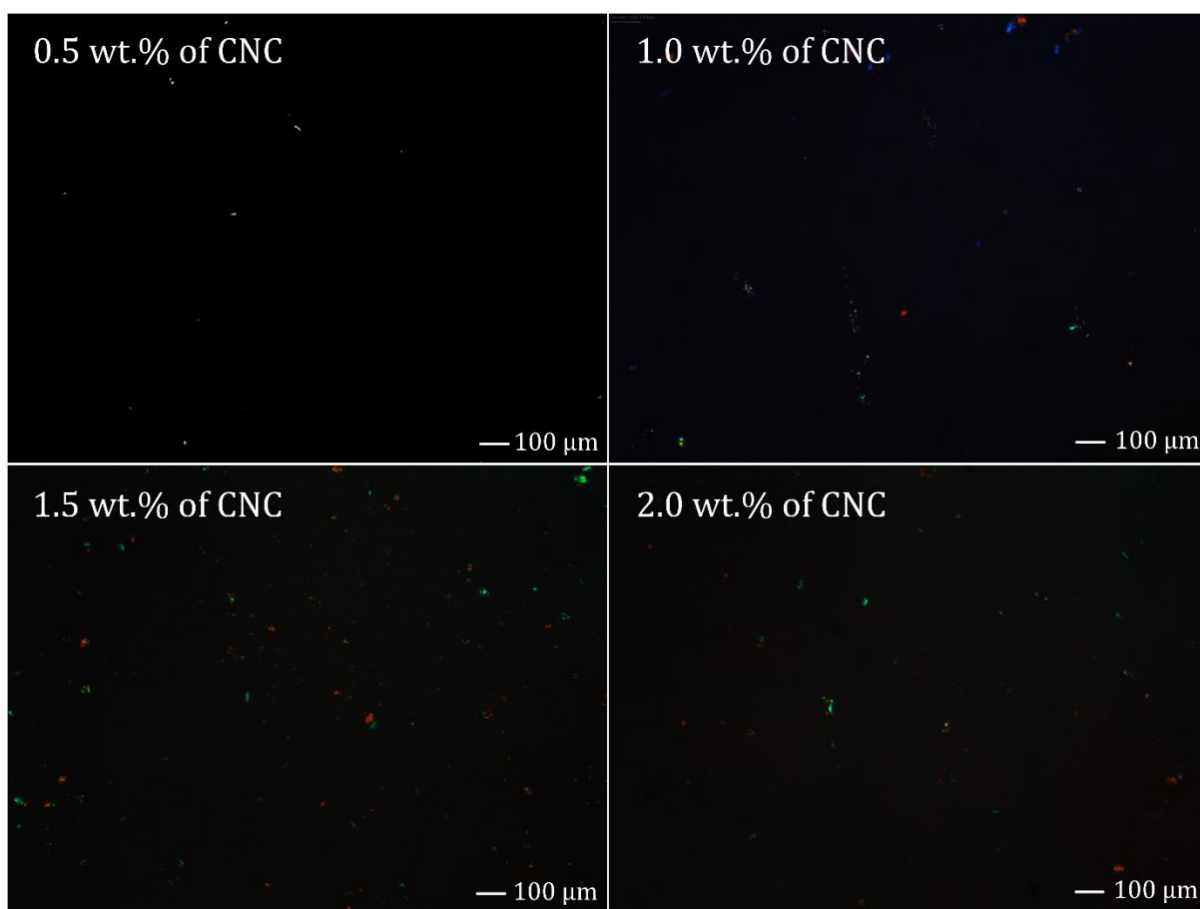


Figure 3.3: Polarized optical microscopy of 0.5 wt. % of CMC solutions with a gradual increase in CNC concentrations at 25 °C.

It is observed, anisotropic phases in all concentrations studied. Both CMC and CNC without the adding of the nanoparticle or polymer, respectively, at these concentrations, present isotropic phase (the POM analyses for CNC without CMC are provided in Appendix A). By adding only 0.5 wt. % of CMC, liquid crystalline agglomerates dispersed in the isotropic phase can already be observed.

As can be seen, in Figure 3.4, this microstructure composed of isotropic and liquid crystalline regions is best observed when the CMC concentration was increased to 1.0 wt. %, as the liquid crystalline agglomerates increase. At the higher CNC concentration, it is possible to observe that the crystalline liquid agglomerates presented droplets format with a mean diameter of $27.17 \pm 8.98 \mu\text{m}$. According to Oguzlu and Boluk (2017), the increase of the liquid crystalline domains is associated with the increased orientation of the CNC particles due to their flocculation. In this way, this flocculation is related to electrostatic interactions between CNC rods and CMC chains.

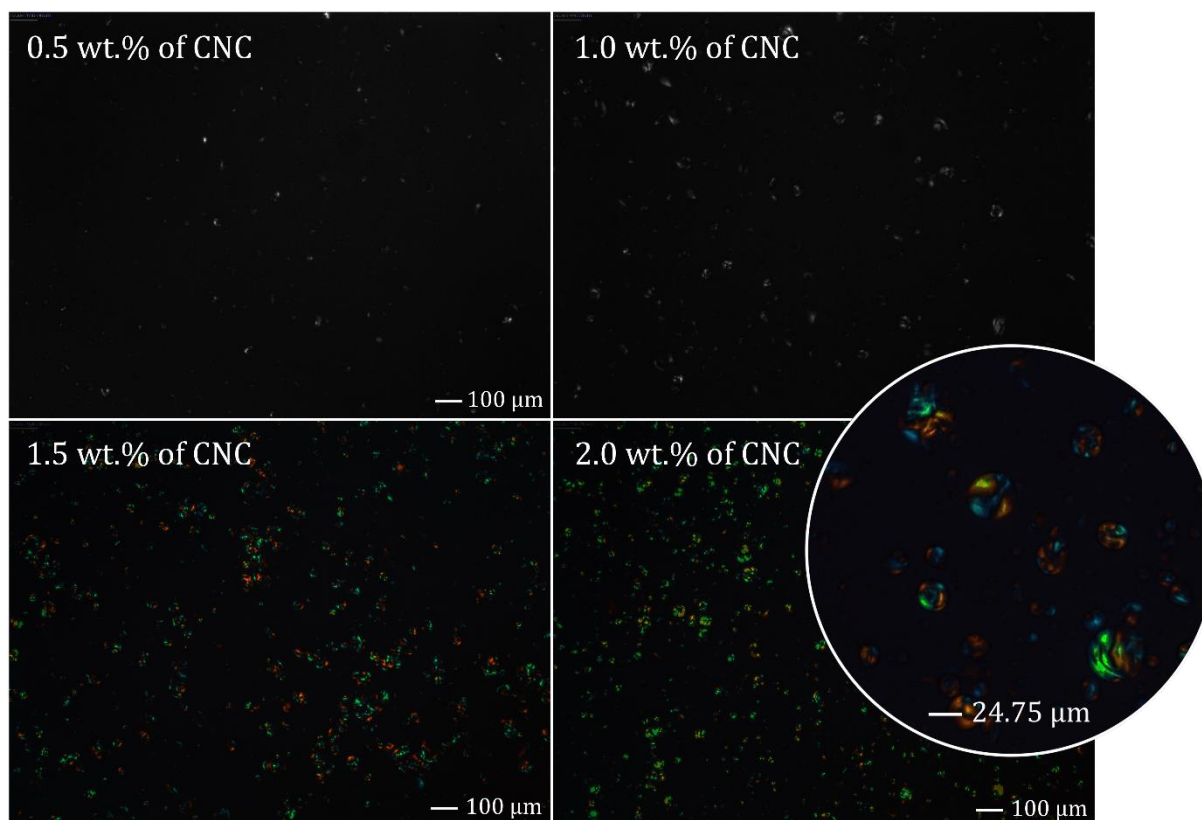


Figure 3.4: Polarized optical microscopy of 1.0 wt. % of CMC solutions with a gradual increase in CNC concentrations at 25 °C.

CNC surface is highly negatively charged due to sulfate half-ester groups introduced during hydrolysis. Charged surfaces could cause the adsorption of polyelectrolytes; however, CMC is a negatively charged polyelectrolyte, and a strong electrostatic repulsion between polymer chains and particles occurs. Due to electrostatic repulsion, anionic CMC chains do not adsorb on CNC particles. The presence of non-adsorbing polymers into the dispersion induces attractive forces between the particles, resulting in a solvent-rich region between the particle surfaces. The osmotic pressure in the fluid exceeds that attraction forces, leading the particles to be close to each other (SIMON BIGGS; DENNIS C. PRIEVE; DAGASTINE, 2005). This mechanism is known as depletion interaction. Therefore, the change in phase behavior observed for the aqueous dispersions of CMC/CNC is explained by flocculation of the particles caused by the depletion interactions. Recent studies reported similar behaviors for CMC polymer and the cellulose nanoparticles and nanofibers (OGUZLU; BOLUK, 2017; SOUZA *et al.*, 2019).

3.2.2 Zeta Potential and Dynamic Light Scattering analyzes

The stability of the CMC/CNC system was analyzed in terms of zeta potential. The zeta potential reflects the interaction energy between particles and relates to system stability in the medium it is dispersed in, based on DLVO theory (BHATTACHARJEE, 2016; LEONG; ONG, 2003; OGUZLU; DANUMAH; BOLUK, 2017).

Results of zeta potential for CMC/CNC mixtures is shown in Figure 3.5. For CNC dispersion, the average zeta potential obtained was $-42.6 \pm 1.74 \text{ mV}$ at neutral pH. This negative potential is relatively high and is related to the presence of the sulfate groups derived from the esterification of the hydroxyl groups present on the CNC surface. Similarly, Xu, Atrens, and Stokes, (2018) reported a zeta potential of $-45 \pm 0.8 \text{ mV}$ for aqueous suspension of CNC, obtained by hydrolysis of the wood pulp. Generally, systems with zeta potential values higher than $+ 30 \text{ mV}$ or less than $- 30 \text{ mV}$ are considered stable (HEURTAULT *et al.*, 2003).

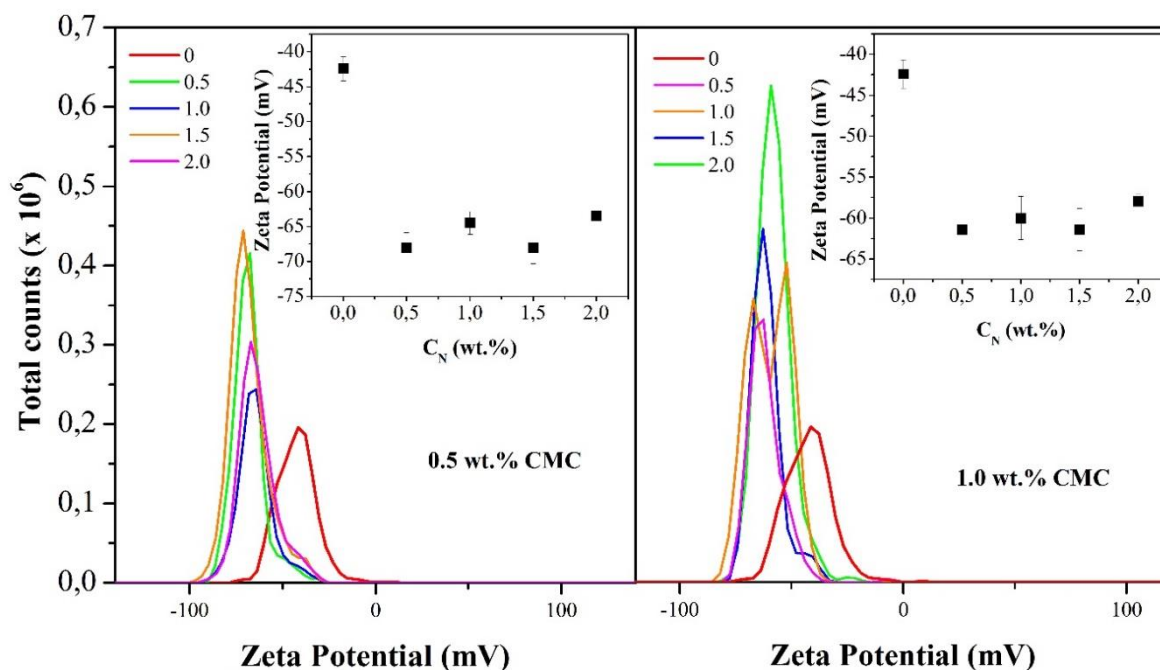


Figure 3.5: Zeta potential for systems CMC/CNC calculated from the electrophoretic mobility determined for Zeta sizer equipment

In all samples studied, the zeta potential was negative and, in the range that indicate stable dispersions. The adding of anionic carboxymethyl cellulose caused a significant increase in the negative magnitude of zeta potential. This increase in the negative magnitude of zeta potential indicates a stable dispersion and can be associated with the electrostatic repulsion between the negatively charged carboxyl groups in the CMC molecular chain interacting with the sulfate ester groups surface of CNC particles. The repulsive electrostatic force was observed at all samples studied. Zhong and coworkers (2012) studied the colloidal stability of negatively charged cellulose nanocrystals in aqueous systems and reported the same effect in the presence of CMC solutions.

The strong electrostatic repulsion can result in structural changes on the particle-polymer surface, and increased zeta potential in absolute value is observed. As previously seen, the depletion interactions indicate that the polymer chains cover up the nanoparticle agglomerates. So it is suggested when the CMC cover up the CNC, it may be that the carboxyl group ($-\text{COOH}$) which is negatively charged "turns" to the surface and the methyl group (CH_3) interact with the particles since it is more hydrophobic in relation of hydroxyl group (OH). A schematic representation of the structural changes in the surface of polymer-particle is shown in Figure 3.6.

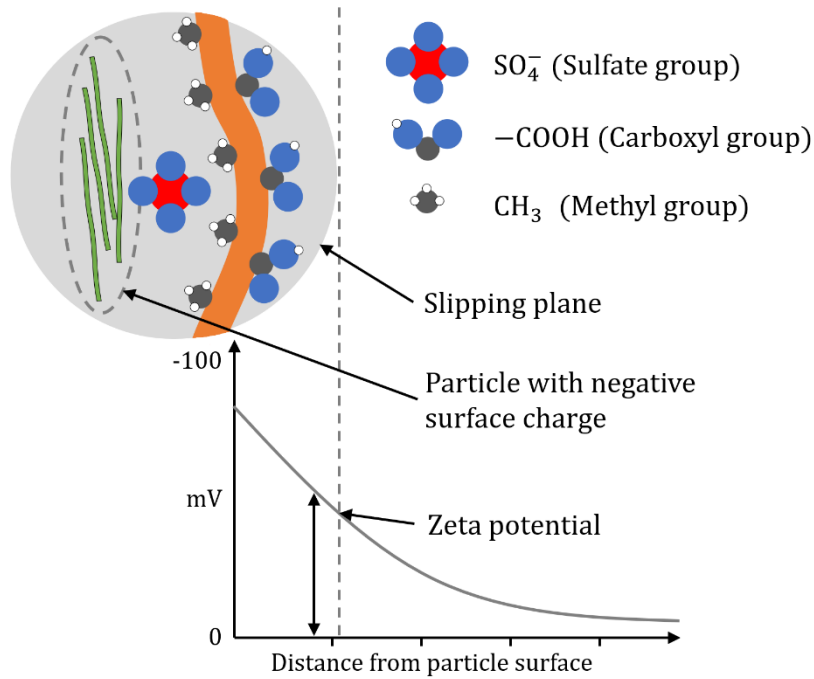


Figure 3.6: Schematic representation of the structural changes in the surface of polymer-particle.

Already the hydrodynamic diameter size and the polydispersity of the system were analyzed by dynamic light scattering (DLS). DLS measures the random motion of particles (Brownian) due to collisions with the surrounding liquid molecules and relates the motion with the size of the particles (BHATTACHARJEE, 2016). The velocity of the Brownian motion is defined by the translational diffusion coefficient and is related to the size of particles by the Stokes-Einstein Equation.

$$d(H) = \frac{kT}{3\pi\eta D} \quad (3.2)$$

where $d(H)$ is hydrodynamic diameter, k is Boltzmann constant, T is absolute temperature, η is viscosity and D is translational diffusion coefficient.

The mean hydrodynamic diameter size and polydispersity for the CNC dispersion and CMC/CNC system is shown in Table 3.1. The addition of CMC promoted an increase in the hydrodynamic diameter size. This increase can be the result of particles flocculation. The increase in the concentration of CNC and CMC did not show significant differences in the mean particle diameter. All CMC/CNC formulations showed the polydispersity index (PdI) near to

0.2, demonstrating that these dispersions were monodisperse in size. The polydispersity index (PdI) is a parameter used to define the size distribution of particles. Samples with PdI of > 0.7 indicate that the sample has a very broad particle size distribution in solution while dispersions with PdI values of 0.1 to 0.7 represents a nearly monodisperse sample. (DEHGHANKHOLD *et al.*, 2018; STETEFELD; MCKENNA; PATEL, 2016).

Table 3.1: Mean diameter and polydispersity of CNC/CMC dispersions containing 0.5 and 1.0 wt. % of CMC diluted (1:30)

CNC/CMC ratio	Mean Diameter (nm)	Polydispersity (PdI)
CNC	107.30 ± 1.084	0.186 ± 0.060
0.5 CMC		
1.0:1	218.9 ± 2.836	0.205 ± 0.013
2.0:1	226.8 ± 2.844	0.203 ± 0.006
3.0:1	218.1 ± 11.38	0.207 ± 0.04
4.0:1	230.3 ± 16.76	0.211 ± 0.06
1.0 CMC		
0.5:1	$220,50 \pm 21.12$	0.172 ± 0.163
1.0:1	211.30 ± 11.55	0.125 ± 0.081
1.5:1	227.65 ± 17.75	0.192 ± 0.032
2.0:1	279.10 ± 27.51	0.136 ± 0.057

3.2.3 Steady-state shear characterization

In Figure 3.7.a it is shown steady shear viscosity versus the shear rate of CMC solutions. The shear-thinning behavior can be observed for all CMC solutions, even at low concentrations. Solutions with concentrations up to 1.0 wt. % presented near-Newtonian behavior with small shear-thinning at higher shear rates. Above 1.0 wt. % up to 2.5 wt. % the solutions exhibit a shear-thinning behavior with a Newtonian plateau at low shear rates, while in 3 wt. % the shear-thinning behavior is predominant. Shear-thinning is caused by the disentanglement of the polymer coils in solution or the increased orientation of the polymer coils in the flow direction. This latter is a well-known behavior of polymers solutions, as previously reported in the literature (AL-SHAMMARI *et al.*, 2011; GÓMEZ-DÍAZ; NAVAZA, 2003; LOPEZ *et al.*, 2017).

Based on the steady-state shear viscosity measurements for CMC solutions, a master curve was constructed by shifting the data over the horizontal and vertical axes using the 2.5 wt.% CMC concentration as a reference for the calculation of the shift factors. It can be observed in Figure 3.7.**Erro! Fonte de referência não encontrada.**b the viscosity master curve, which exhibits that the samples have a common mechanism that governs their viscoelasticity. The experimental data were fitted and well represented by the Cross-viscosity model. This model allows effectively describing the viscosity behavior at different shear rates and is given by:

$$\eta(\dot{\gamma}) = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + \left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^n} \quad (3.3)$$

where η_{∞} is the infinite shear viscosity, η_0 the zero-shear viscosity, $\dot{\gamma}$ is the shear rate, $\dot{\gamma}_c$ is the critical shear rate and n is a dimensionless parameter indicating the degree of dependence of the viscosity on the shear rate in the transition region between the Newtonian plateau and power-law region.

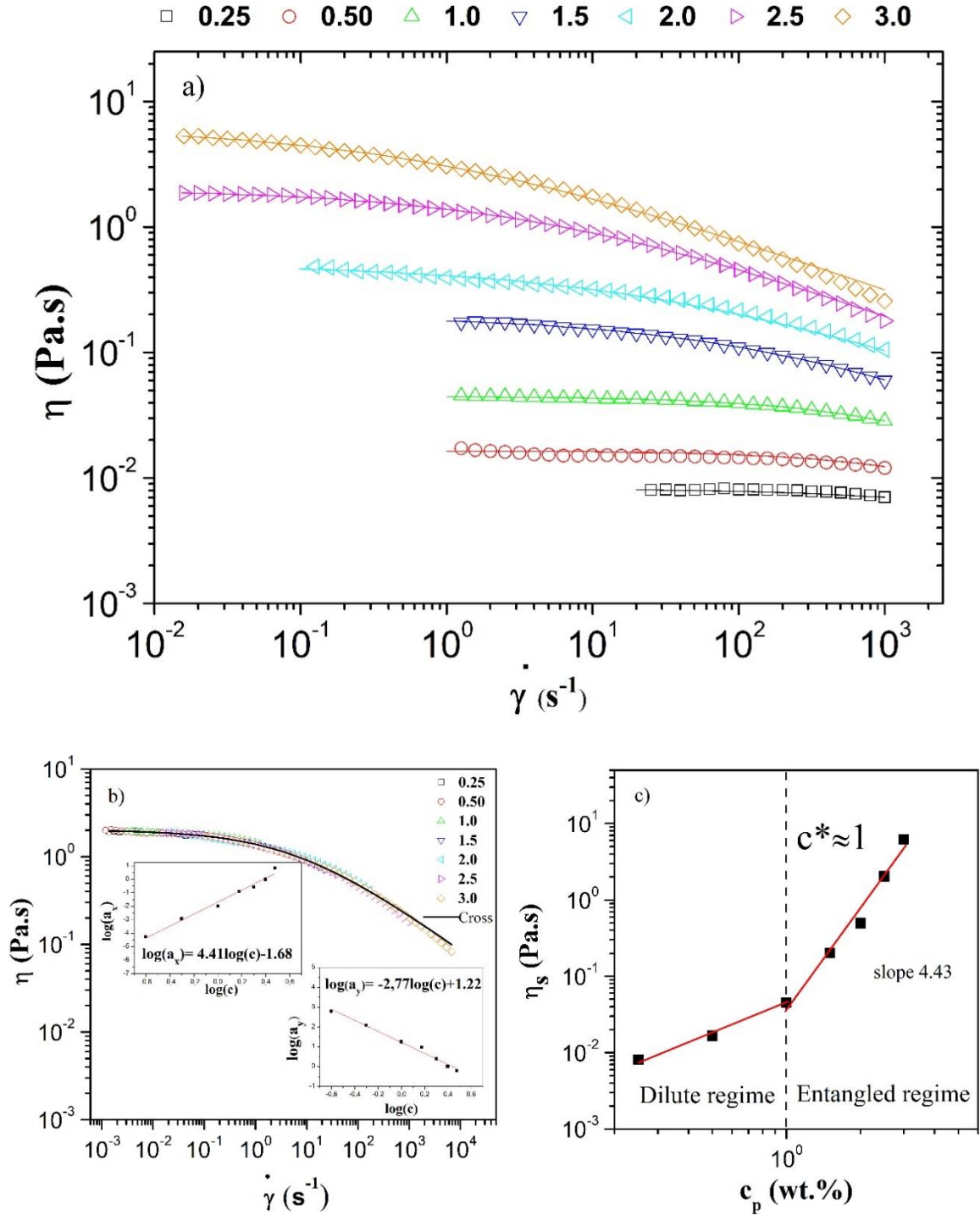


Figure 3.7: The steady-state viscosity curves of (a) CMC solutions, the solid line corresponds to the Cross-model fitting for CMC, and open symbols correspond to experimental data (b) dependence of specific viscosity for CMC solutions (c) dependence of specific viscosity on concentration for CMC in pure water at 25 °C.

The fits to the experimental data, using the Cross model, are exhibited as a solid line, and the shift factors used for the construction of the master curve are given in Table 3.2.

Table 3.2: Shift factors for experimental viscosity master curve and fitting parameters for the Cross model of CMC solutions at 25 °C

Experimental							
c_p (wt. %)	0.25	0.5	1.0	1.5	2.0	2.5	3.0
a_x	0.00055	0.00115	0.00234	0.03418	0.1688	1	6.76
a_y	238.94	123.184	43.093	10.332	3.897	1	0.323
Fit							
c_p (wt. %)	n	η_0 (Pa.s)	η_∞ (Pa.s)	$\dot{\gamma}_c$ (s ⁻¹)	R^2		
0.25	0.9558	0.008094	0.006326	641.8	0.9991		
0.50	0.6366	0.01652	0.0005907	5003	0.9989		
1.0	0.6122	0.04484	0	2443	0.9994		
1.5	0.4413	0.2007	0	154.3	0.9999		
2.0	0.3843	0.4956	0	45.83	0.9999		
2.5	0.4336	2.035	0	5.719	0.9994		
3.0	0.416	6.149	0	0.8805	0.9992		
Master curve	0.4246	2.022	0	6.304	0.9956		

It is observed in Figure 3.7.c that by increasing the concentration, a significant increase in zero shear viscosity occurred for CMC solutions. The viscosity dependence on polymer concentration is associated with the distinct states of the solution. The specific viscosity (η_{sp}) of CMC solutions was plotted against concentration and is shown Figure 3.7.c, in which η_{sp} describes the interactions of the polymer in solution at different concentrations. The specific viscosity is given by:

$$\eta_{sp} = \frac{\eta_0 - \eta_s}{\eta_s} \quad (3.4)$$

where η_0 is the zero-shear viscosity and η_s is the solvent viscosity.

Clearly, two distinct areas were observed in Figure 3.7.c, the first corresponds to the dilute regime in which charge repulsion dominates keeping the chains apart and random, so the flow behavior is mainly Newtonian. As the CMC concentration increases, the conformation of individual chains starts to overlap and entangle, indicating increasing of intermolecular associations, this second part is denominated semi-dilute entangled regime. The transition between the dilute solution and semidilute entanglement solution is well defined by a marked change in the concentration dependence of solution viscosity. The polymer concentration marking this transition is called the critical or coil overlap concentration (c^*). Therefore, CMC concentration of 1.0 wt. % characterized the entanglement concentration, which is in agreement with other data reported previously for CMC solutions (BENCHABANE; BEKKOUR, 2008; WU *et al.*, 2009).

Viscosity versus shear rate curves of CNC dispersed in deionized water in a concentration range from 0.5 to 2.0 wt. % is depicted in Figure 3.8.

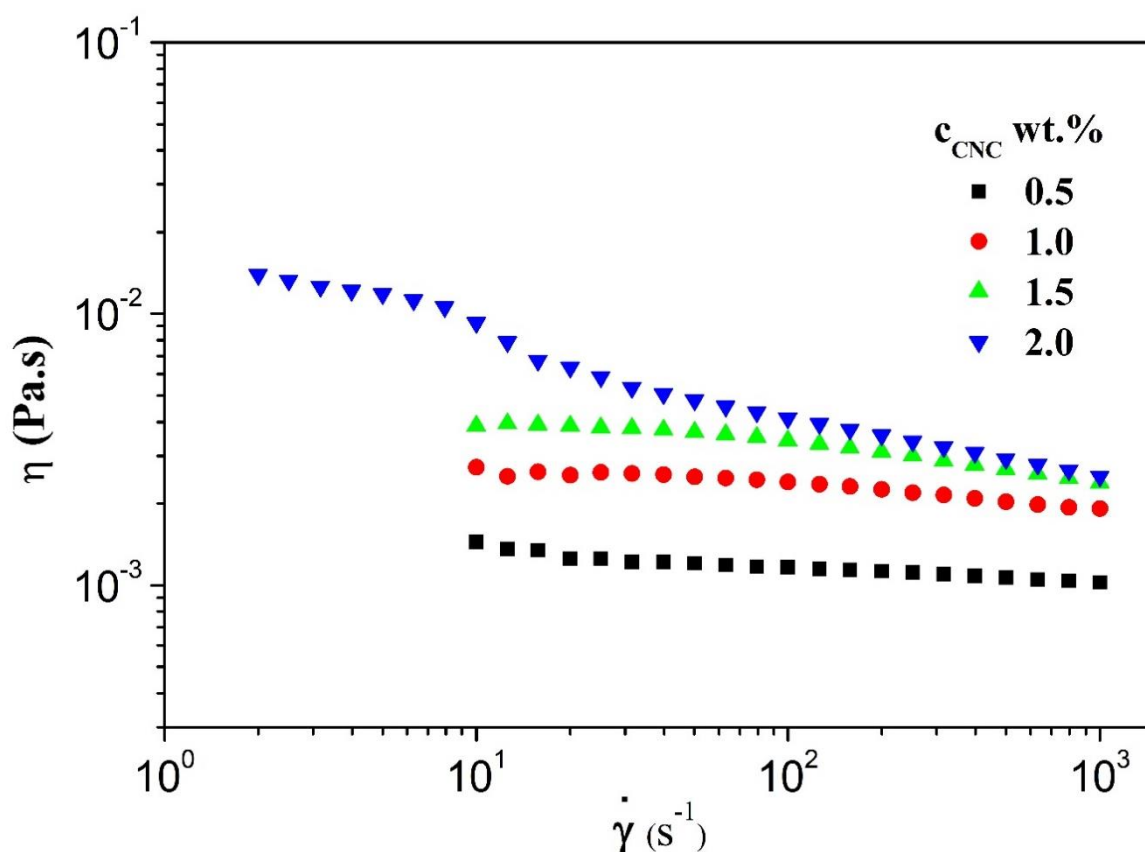


Figure 3.8: The steady-state viscosity curves of CNC solutions

The samples containing 0.5 wt. % and 1.0 wt. % of CNC exhibit a near-Newtonian behavior, the flow curves show minimal shear-thinning. A slight shear-thinning at high shear rates is observed for a concentration of 1.5 wt. %. Already, at 2.0 wt. %, two shear-thinning regions can be observed. Viscosity curves for the CNC dispersions presented an overall viscosity increase and a higher shear-thinning behavior with the increase of CNC concentration. That is, at lower shear rates, the nanocrystals are assumed to randomly organize while with increasing shear rate, the rods are progressively aligned in the flow direction. Similar rheological behavior has been reported in other CNC systems (LI *et al.*, 2015; QIAO *et al.*, 2016b; XU; ATRENS; STOKES, 2017). These different rheological behaviors of CNC dispersions are associated with aspect ratio and the ability to self-organize in the dispersion, thus, generating distinctive dispersion states and phases interactions by increasing the CNC concentration.

The influence of CNC dispersions in CMC solutions was studied. Viscosity curves show the result of the progressive addition of low CNC concentrations into 0.5 and 1.0 wt. % CMC solutions. The viscosity curves are shown in Figure 3.9.a.b.

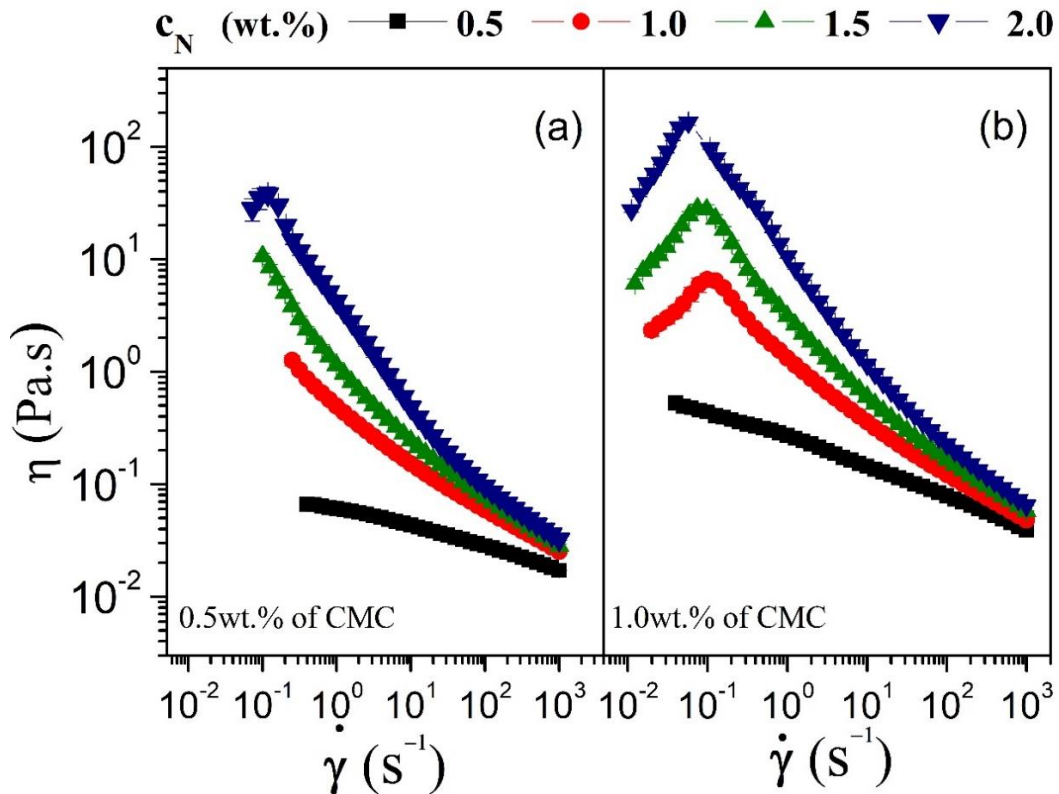


Figure 3.9: The steady-state viscosity curves of CMC/CNC mixtures (a) CMC/CNC dispersions containing 0.5 wt. % of CMC and (b)) CNC/CMC dispersions containing 1.0 wt. % of CMC

Compared with the polymer solutions, the addition of CNC has to lead to a significant viscosity increase at low shear rates. Also, it can be observed a strong shear-thinning behavior, which is attributed to the disentanglement of the polymer chains and align of the CNC rods along with the flow due to high shear rates (PENG *et al.*, 2018). Moreover, the high viscosity increase can be associated with the microstructure and electrostatic interactions between CNC particles and CMC chains. The CNC surface is highly negatively charged due to sulfate half-ester groups introduced during hydrolysis, so, when interacting with the carboxyl groups of CMC chains, strong electrostatic repulsion between them is induced. Increasing the CMC concentration from 0.5 to 1.0 wt. % resulted in a progressive increase in viscosity due to CMC chain entanglement and percolated blob structures of CNC. Thus, higher CMC concentrations caused stronger depletion interactions contributing to viscosity increase.

On the other hand, it is shown in Figure 3.9.b that the samples, except 1.0 and 0.5 wt. % CMC/CNC, exhibit shear-thickening at low shear rates. The same behavior is exhibited in the sample 0.5 – 2.0 wt. % CMC/CNC (Figure 3.9.a). When increasing the shear rate, the apparent viscosity increases up to a maximum value, and then the viscosity decreases again with shear rate exhibiting a shear-thinning behavior. This effect is related to the increase in the intermolecular interactions caused by concentration increase (QIAO *et al.*, 2016b). The most accepted interpretation for this phenomenon in the polymer solutions is the “flow-induced formation of macromolecular associations” (LIU; YU; LIN, 2007). According to Benchabane and Bekkour (2008), CMC solutions with the $M_w = 700.000 \text{ g.mol}^{-1}$ and critical concentration of 1% exhibit an initial shear-thickening behavior above the critical concentration, where shear-thickening for CMC solutions was observed only in high concentrations (above 5.0 wt. %). Therefore, depletion interactions due to the addition of CNC in dilute solutions of CMC might have induced shear-thickening.

3.2.4 Small amplitude oscillatory measurements

The storage modulus (G') and the loss modulus (G'') are associated with the linear viscoelastic behavior of materials. The damping factor parameter $\left(\tan(\delta) = \frac{G''}{G'}\right)$ can be used as an additional parameter to analyze the predominance of elastic or viscous behavior. When

$\tan(\delta) < 1$ the solid-like behavior is predominant, and when $\tan(\delta) > 1$ the liquid-like behavior predominates.

Figure 3.10.a displays the dependence of storage and loss modulus on the angular frequency (ω) for CMC aqueous solutions. For all samples, CMC solutions presented a liquid-like behavior ($G'' > G'$) for all frequency range. For high frequencies, the interval between storage and loss modulus decreases with CMC concentration. The rise in CMC concentration causes chain entanglements, restricting the motion of the polymer in the solution; thus, the viscous properties decrease in favor of the elastic properties. However, for the studied concentrations and frequency range, it was not observed the transition to solid-like behavior ($\tan(\delta) < 1$). The damping factor ($\tan(\delta) = \frac{G''}{G'}$) in the function of the angular frequency (ω) is shown in Figure 3.10.b.

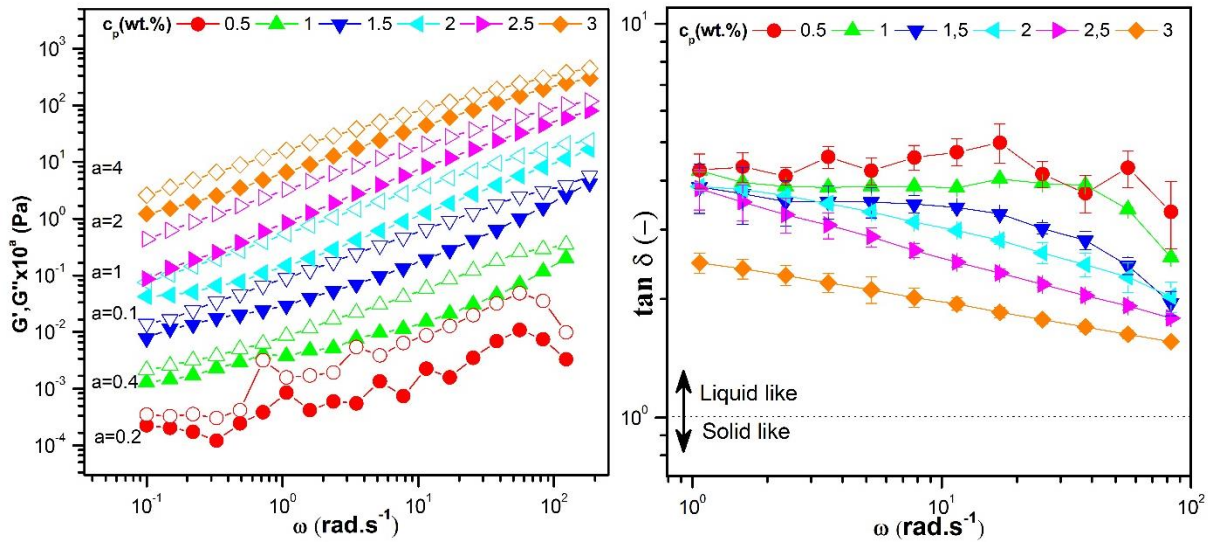


Figure 3.10: (a) Dependence of the storage modulus (G') (solid symbols) and the loss modulus (G'') (open symbols) on the angular frequency (ω) for carboxymethylcellulose solutions (data are vertically shifted to avoid overlapping); (b) damping factor ($\tan(\delta)$) as a function of the angular frequency (ω) for CMC solutions

On the other hand, Figure 3.11.a.b. show the storage and loss moduli as functions of the angular frequency when CNC dispersions were added in CMC solutions. Regardless of CMC concentration, at lower concentrations of the CMC/CNC dispersion, the loss modulus was higher than the storage modulus, indicating a typical behavior of an entangled solution liquid ($G'' > G'$). However, with the increase in CNC concentration, the magnitudes of both moduli

increased and the tendency to solid-like behavior can be observed. By contrast, at higher frequencies, the elastic moduli decrease, and crossover points ($G' = G''$) arise defining the transition from a gel-like to a liquid-like structure. As can be seen in Figure 3.11a.b. the crossover frequency shifts toward higher values in the dispersions containing higher CNC/CMC concentrations, because, the strong electrostatic repulsion between CNC particles and CMC chains the system become with more gel-like character. Consequently, the transition for liquid-like occurs only in higher frequencies.

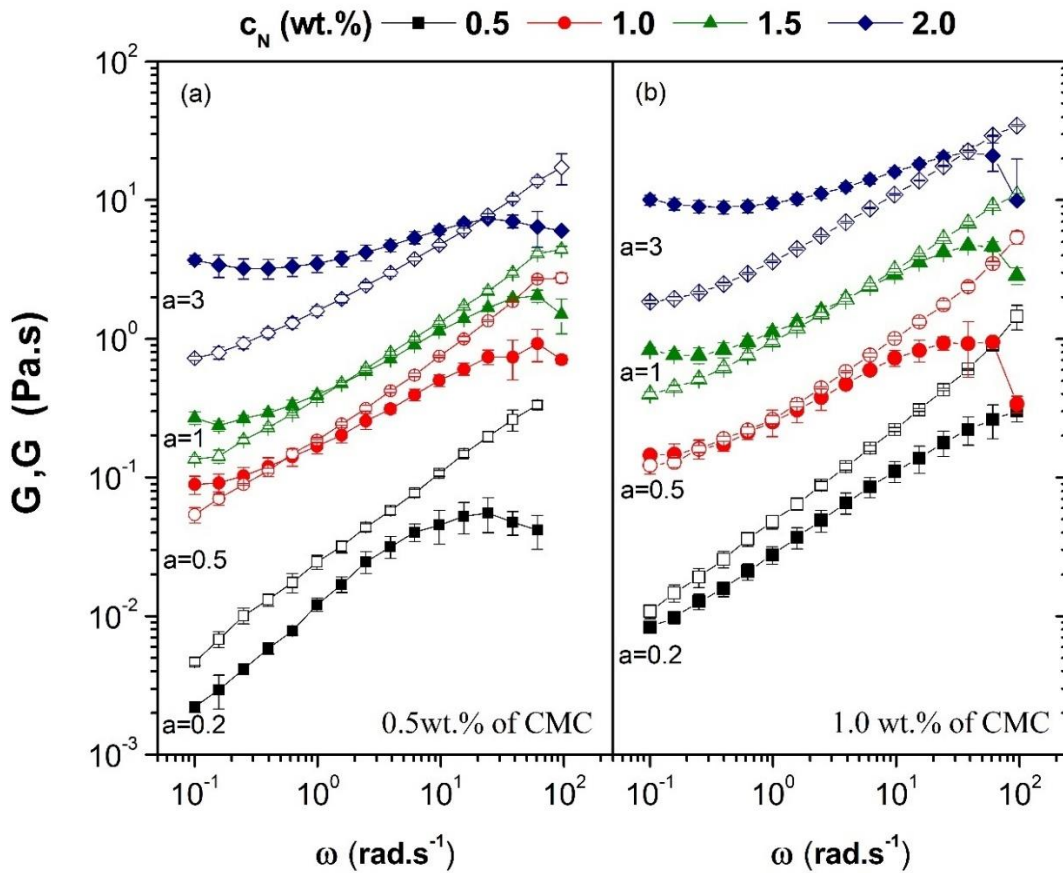


Figure 3.11: Dependence of G' (solid symbols) and G'' (open symbols) on the angular frequency for samples CMC/CNC (a) CNC/CMC dispersions containing 0.5 wt. % of CMC and (b) CNC/CMC dispersions containing 1.0 wt. % of CMC

The influence of CMC concentration on CNC dispersions properties is observed by diminution the liquid-like behavior with increasing CMC. Figure 3.12 shows the ratio of loss moduli to storage moduli plotted against the angular frequency. It is notable that the system solid-like character continuously increases with increasing CMC concentration.

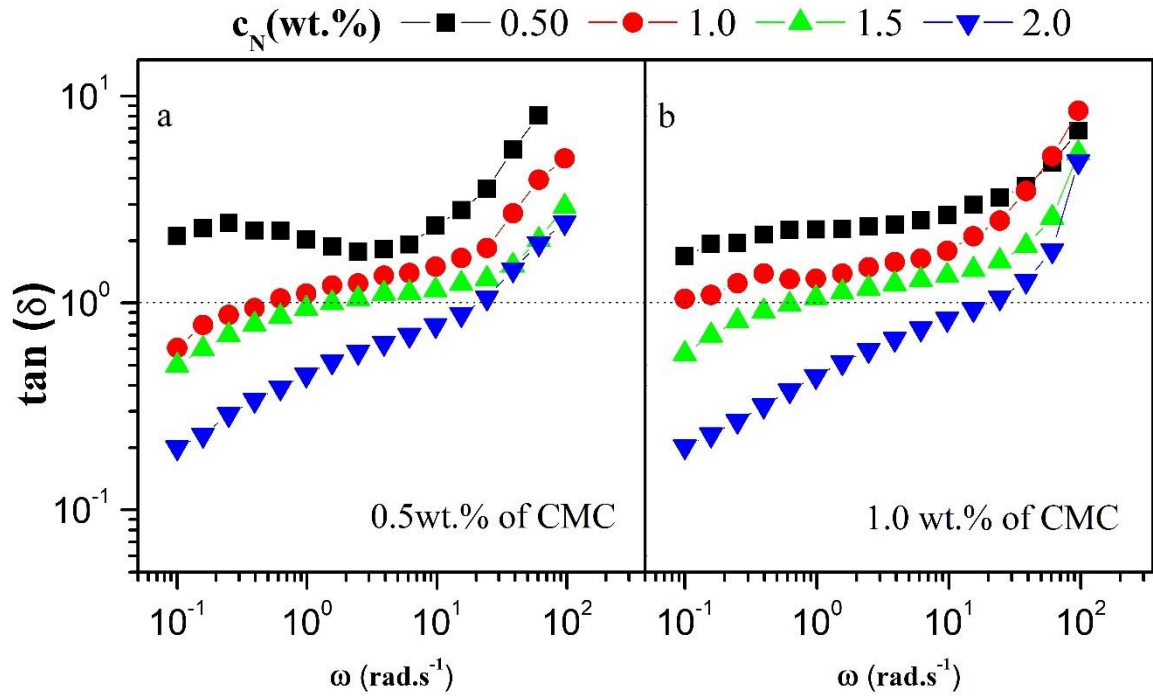


Figure 3.12: Damping factor ($\tan(\delta)$) as a function of the angular frequency (ω): (a) CNC/CMC dispersions containing 0.5 wt. % of CMC and (b) CNC/CMC dispersions containing 1.0 wt. % of CMC.

3.2.5 Thixotropy tests

To study the time-dependent rheological properties of CMC/CNC, the samples were analyzed through two measuring method, including hysteresis loop test and three intervals thixotropic test. Hysteresis loop tests were used to verify the viscosity behavior during a gradual increase of shear rate, followed by a gradual decrease. The hysteresis loop curves are shown in Figure 3.13.a.b. For all samples, it is observed that the upward curves showed higher stress than the downward flow curves, evidencing the shear-thinning behavior and the time-dependence when shearing.

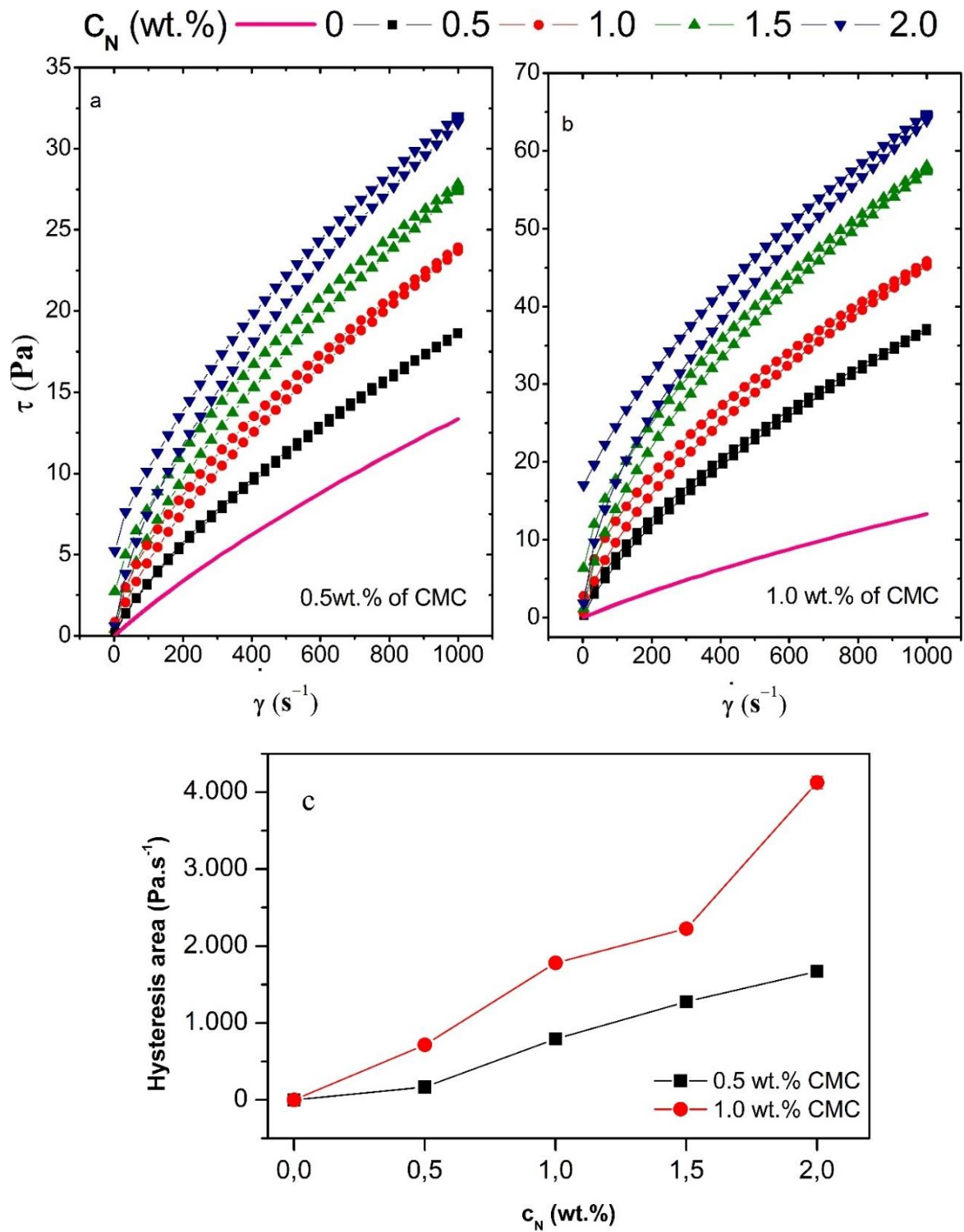


Figure 3.13: Shear stress as a function of shear rate for CNC/CMC dispersions containing (a) 0.5 wt. % of CMC (b) 1.0 wt. % of CMC (c) hysteresis loop area for structure recovery.

In Figure 3.13.c it is observed that the hysteresis loop area significantly increases when the concentrations of CNC and CMC are increased, due to increase in shear-thinning and of intermolecular interactions that also increased requiring more energy to deform the material, and the time of the test was not enough to reorganize the structure. The time-dependence in CMC solutions was reported in other studies (BENCHABANE; BEKKOUR, 2008; EDALI; ESMAIL; VATISTAS, 2001; GHANNAM; NABIL ESMAIL, 1997), nevertheless in higher concentrations. The pure solutions in the dilute state are time-independent; hence, when CNC is added, the time-dependence behavior is accelerated due to intermolecular interactions between CNC particles and CMC chains.

The 3ITT tests were used to determine the structural regeneration of the sample after a shear or how long time the recovery takes. This evaluation method can be used to determine the structural regeneration of the sample after a shear or the recovery times. Results for the viscosity recovery test are presented in Figure 3.14.

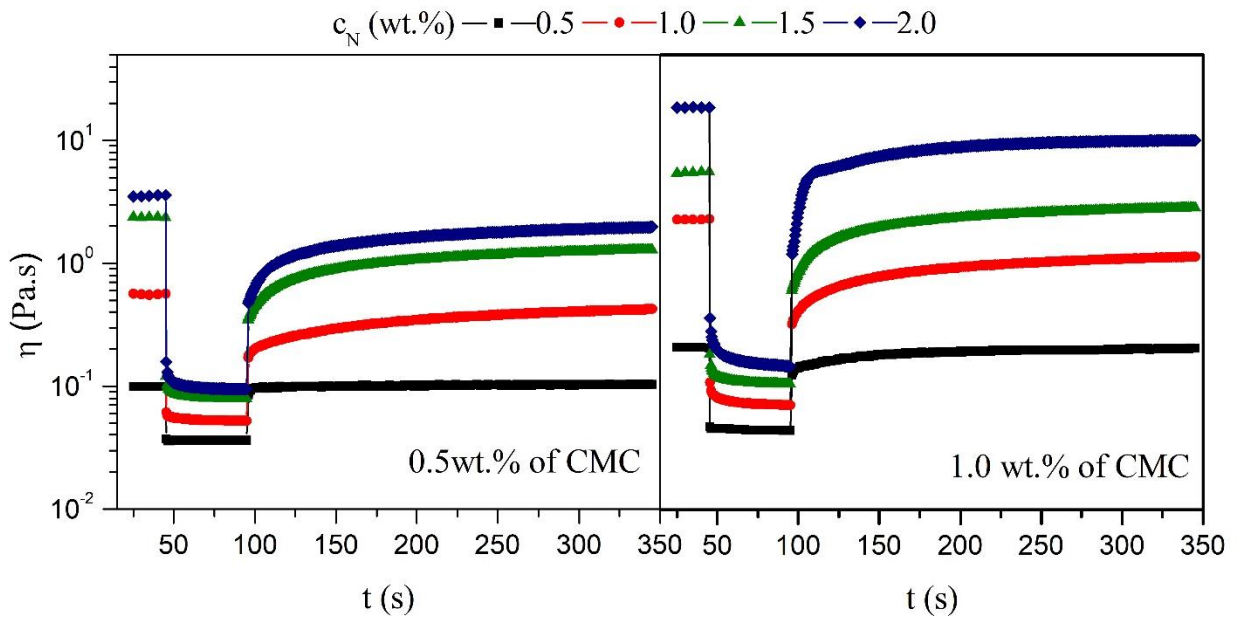


Figure 3.14: Viscosity curves as a function of the time for the recovery test (a) CNC/CMC dispersions containing 0.5 wt. % of CMC (b) CNC/CMC dispersions containing 1.0 wt. % of CMC.

As shown in Figure 3.14 the viscosity remained stable in the reference interval, with a shear rate of $\dot{\gamma} = 1 \text{ s}^{-1}$, then falls to a lower value during the high-shear interval, when the samples were submitted to a shear rate of $\dot{\gamma} = 100 \text{ s}^{-1}$ for 50 s, followed by an overshoot became stable in the regeneration interval, the same shear rate $\dot{\gamma} = 1 \text{ s}^{-1}$ was applied to

evaluate the regeneration of the structures.

Figure 3.15 shows the viscosity recovery percentage according to the concentration. The recovery viscosity was calculated using Equation 2.4. It can be seen that the viscosity at lower concentrations takes less time to stabilize in the recovery interval while in the higher concentrations, the time for recovery is larger. However, the initial structure did not recover completely, only a partial recovery was observed, except in the concentrations with 0.5 CNC. As observed in Figure 3.15.a.b that the samples with 0.5 wt.% CNC, the viscosity recovers almost instantaneously, presenting a time-independent viscosity behavior.

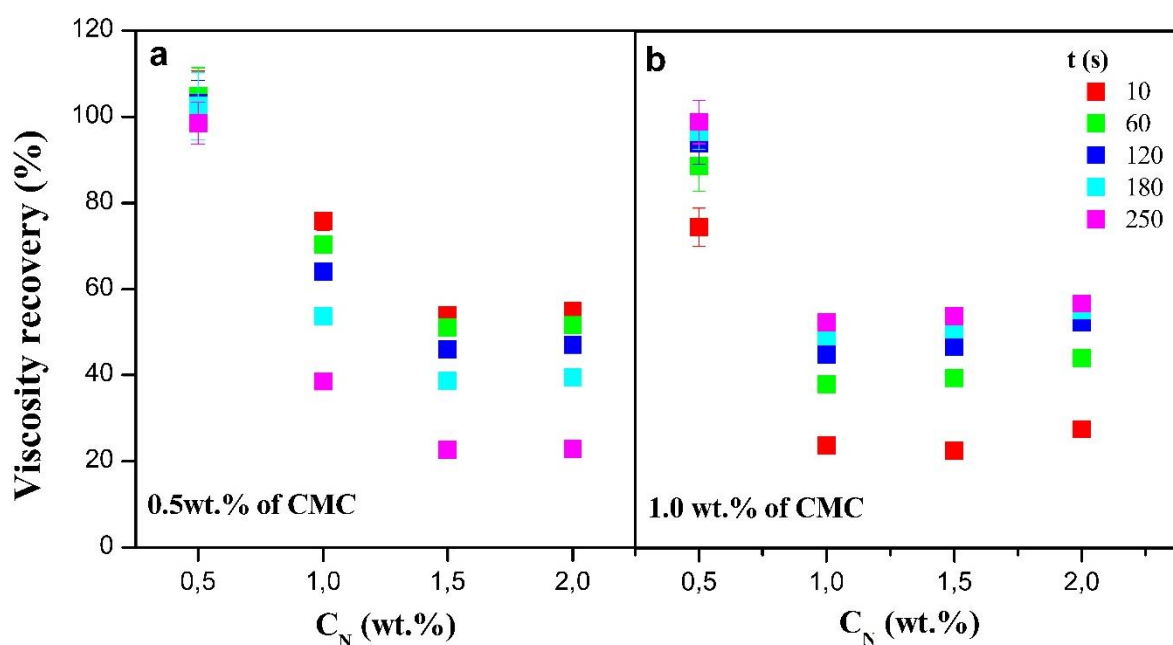


Figure 3.15: Viscosity recovery percentage as a function of CNC concentration (a) CNC/CMC dispersions containing 0.5 wt.% of CMC (b) CNC/CMC dispersions containing 1.0 wt.% of CMC.

4 RHEOLOGY AND 3D EXTRUSION-BASED PRINTING OF CELLULOSE-BASED NANOCOMPOSITE HYDROGELS

In this chapter, the rheological behavior of nanocomposite hydrogels of carboxymethyl cellulose (CMC) and cellulose nanocrystals (CNC) was studied. Different formulations of CMC/CNC hydrogels in water were prepared and steady-state viscosity and small amplitude oscillatory shear (SAOS) measurements were performed. All samples exhibited a pronounced shear-thinning behavior. Also, an initial shear thickening behavior at low shear rates was observed with increase in polymer concentration. Both behaviors are associated with the interaction between CNC particles in the presence of nonadsorbing CMC polymer chains. Frequency sweep tests were performed to analyze the viscoelastic properties of the mixtures. All samples presented elastic behavior predominant to the viscous in all frequency range analyzed ($G' > G''$). At higher concentrations, G' and G'' were almost frequency independent, which is typical of physical gels. Therefore, physical gels were obtained for all concentrations studied. The printability of these gels was evaluated. Gels with higher concentrations of CNC showed filament formation ability and the composition of 1: 10 wt. % CMC/CNC was defined as optimal hydrogel ink, due to their rheological behavior and the good filament formation. The best printing parameters were defined and scaffolds with different layers were printed using the optimal hydrogel ink.

4.1 Experimental Procedure

4.1.1 Material

Sodium carboxymethyl cellulose (CMC) with an average molar mass $M_w = 250.000 \text{ g} \cdot \text{mol}^{-1}$ and degree of substitution $DS = 0.70$ was purchased from Sigma-Aldrich. Cellulose nanocrystals (CNC) aqueous dispersion (12 wt. % of CNC) were purchased from the US Department of Agriculture Forest Product Lab (Lot number 2016-FPL-CNC-098) and supplied by the University of Maine. The degree of sulfonation and mass concentration was

0.95 wt. % sulfur on dry CNC and 12.1 wt. % dry CNC, respectively.

4.1.2 Preparation CMC/CNC gels

CMC/CNC nanocomposite hydrogels with different concentrations of dispersed CNC and a constant polymer concentration were prepared. Initially, CNC dispersions were obtained by adding deionized water with $pH = 7$ in the original CNC dispersion and kept under magnetic stirring for 4 h at room temperature. Next, CMC was dissolved in CNC dispersions. Each solution was stirred overnight. Subsequently, vortex stirring was carried out for 10 min in order to ensure a homogeneous mixture (Figure 4.1.a). The compositions of the nanocomposite hydrogels are listed in Figure 4.1. All samples were stored for 6 h at room temperature 23 °C before the rheological tests.

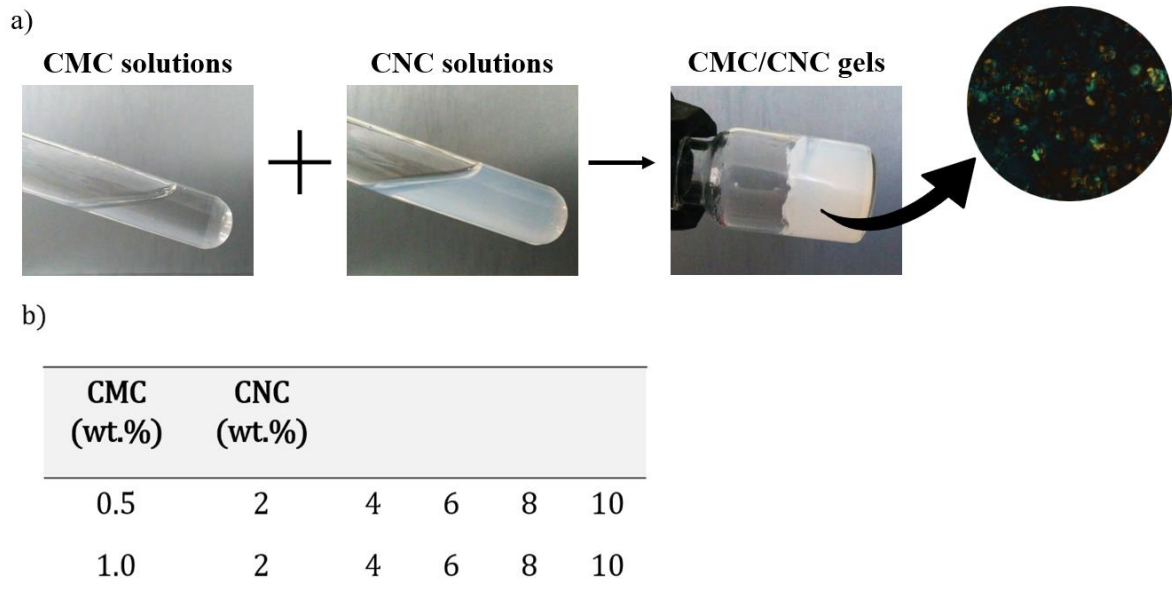


Figure 4.1: Schematic representation of nanocomposite gels preparation: (a) samples preparation (b) compositions the nanocomposite hydrogels studied.

4.1.3 Rheological characterizations

The rheological behavior of CMC/CNC gels was analyzed using an Anton Paar MCR-102 Modular Compact Rheometer at 25 °C. Plate-plate geometry (PP50-1) with 50 mm diameter and distance of the plates 1 mm was used for all measurements. Before each test, a pre-shear of 200 s^{-1} applied for 300 s followed by a rest period of 300 s was performed. Steady shear viscosity versus shear rate curves were obtained for each sample for shear rates in the range of 0.01 to 1000 s^{-1} . Amplitude sweep tests were carried out at a constant angular frequency of 10 rad. s^{-1} with a shear strain in the range from 0.01 to 100% to obtain the linear viscoelasticity (LVE) range ($\gamma_0 = 1\%$ for all samples). Finally, frequency sweep tests were conducted in the range of 0.1 at 240 rad. s^{-1} . All rheological measurements were triplicated.

Three-interval thixotropy tests (3ITT) were performed to evaluate the viscosity recovery after the shear application. For these tests, was used the hydrogel concentration that exhibits satisfactory characteristics for 3D printing. A low shear rate of 1 s^{-1} was applied for 25 s, followed by a constant shear rate ($100 - 700\text{ s}^{-1}$) applied for the 50 s and back to 1 s^{-1} for 250 s.

4.1.4 3D printing tests

The 3D printing tests were performed following the protocol proposed by Dávila and d'Ávila (2019), using a 3DCloner Lab printer with a home-made printing head for gel printing, which operates with the Marlin firmware and RAMPS1.4/Arduino electronics.

Firstly, the filament formation was analyzed using an optical microscope coupled to the 3D printing head. The software Repetier-Host V2.1.3 was used to generate a piston speed of 0.526 mm min^{-1} (flow rate of 0.2 ml min^{-1}). The filament formed at a small flow rate is associated with the material rheological properties. The extrusion was performed using a 22G nozzle tip of 25 mm in length and diameter of 0.70 mm purchased from Injex, Brazil.

After the filament formation tests, the printing parameters of CMC/CNC gels were analyzed. Piston speeds of 0.526, 1.052 and 1.578 $mm\ min^{-1}$ and printing speeds in the range of 10 to 30 $mm\ min^{-1}$ were tested using the software Repetier-Host V2.1.3. BioScaffoldsPG, software to generate 3-D continuous printing paths for the fabrication of tissue engineering scaffolds (DÁVILA *et al.*, 2015), was used to create the g-code files with the printing paths. Before the tests, the sample was centrifuged at 4000 *rpm* for 5 *min* in a Kasvi K14-4000 centrifuge to eliminate air bubbles. Then, one layer of material was deposited and subsequently observed in a microscope Leica DM-ILM. The width of the deposited material was measured using the software ImageJ.

4.2 Results and discussion

4.2.1 Steady-state shear characterization

In Figure 4.2 it is shown the steady shear viscosity as a function of the shear rate of CMC solutions, CNC dispersions, and CMC/CNC nanocomposite hydrogels. Shear-thinning is observed for all samples studied, which is attributed to the polymer chains disentanglement and CNC rods alignment due to high shear rates (PINTO; DÁVILA; D'ÁVILA, 2019). It can be observed that this behavior is influenced due to both CMC and CNC concentrations. At higher concentrations, shear-thinning is more pronounced, while at lower concentrations only a subtle shear-thinning is observed at higher shear rates. In CMC solutions, shear-thinning depends on concentration, which can be seen in Figure 4.2.a. The CMC solutions with 0.5 and 1.0 *wt. %* have near-Newtonian behavior since these solutions are in the dilute regime. On the other hand, above the critical overlap concentration, 1.0 *wt. %*, the solutions exhibit more pronounced shear-thinning as well as higher viscosity, due to the entanglement and overlap of the chains. This is the typical behavior of polymer solutions. It is observed in Figure 4.2.b the viscosity profile of the CNC aqueous dispersions. The CNC dispersions present viscosity dependence on the concentration and pronounced shear-thinning above 4 *wt. %*. In Figure 4.2.c.d, the viscosity curves of CNC/CMC solutions show the influence of cellulose nanocrystal on CMC rheological behavior at 0.5 and 1.0 *wt. %*.

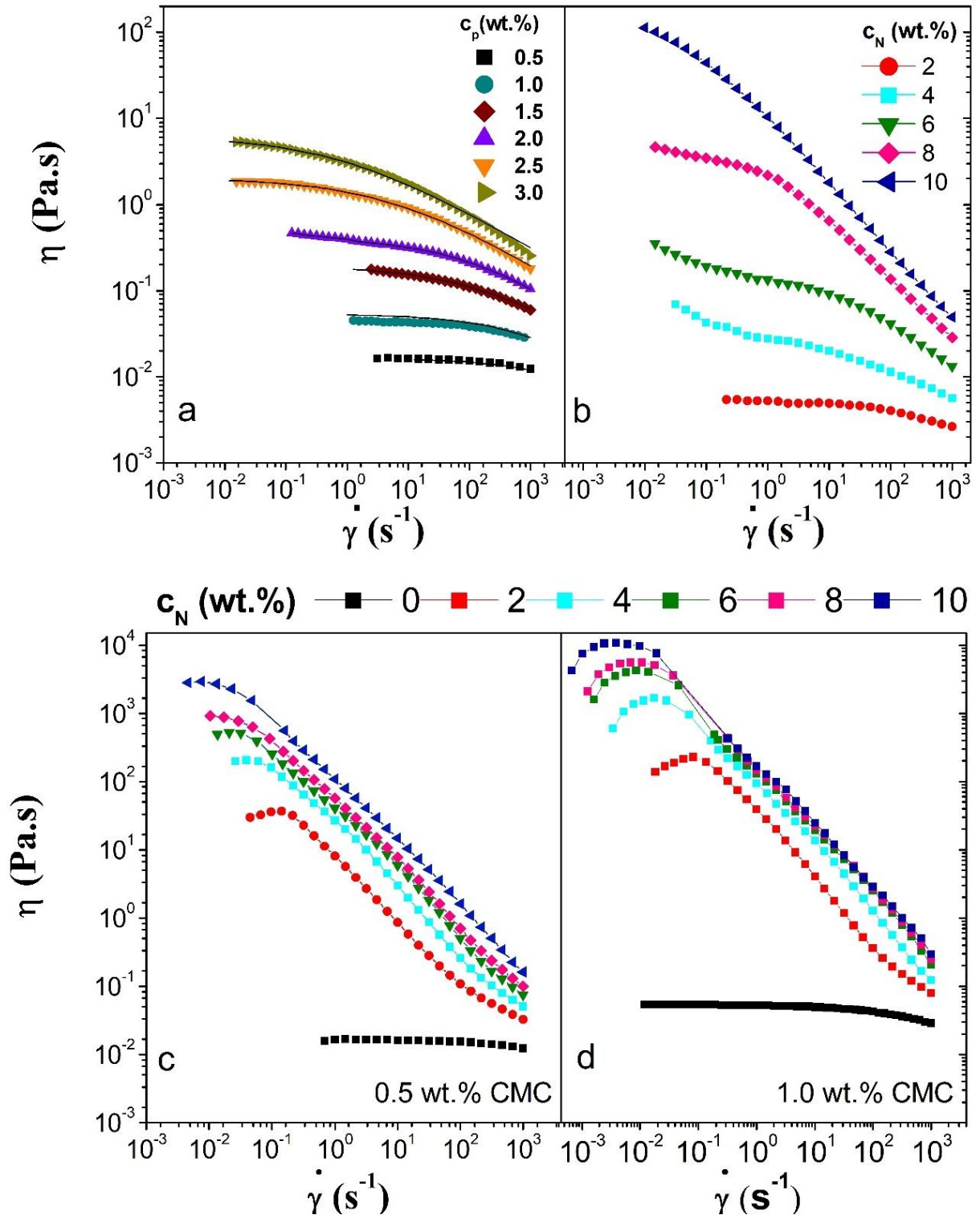


Figure 4.2: The steady-state viscosity curves of: (a) CMC solutions and (b) CNC dispersions (c) CMC/CNC gels containing 0.5 wt.% of CMC and (d) CMC/CNC gels containing 1.0 wt.% of CMC.

In the samples with 0.5 wt. % of CMC, a significant increase in viscosity at low shear rates followed by strong shear-thinning behavior were observed, when compared to CMC solution without CNC particles (Figure 4.2.c). Also, a slight shear-thickening behavior was observed at low shear rates. Shear-thickening behavior is commonly observed in CMC solutions above the critical concentration (1 wt. %) (BENCHABANE; BEKKOUR, 2008; EDALI; ESMAIL; VATISTAS, 2001). In this study, the shear-thickening for CMC solutions was observed only at high concentrations (above 5.0 wt. %). Therefore, this effect is attributed to structural changes caused by the addition of CNC in dilute solutions of CMC, which induced shear-thickening. On the other hand, in the samples with 1.0 wt. % of CMC and different CNC concentrations (Figure 4.2.d), shear-thickening is observed, where after reaching a maximum viscosity, it suddenly decreases with pronounced shear-thinning.

In Figure 4.2.c.d, it can be observed a high viscosity increase at low shear rates by adding only 2.0 wt. % of CNC particles. This is associated with electrostatic interactions between CNC rods and CMC chains. Due to the presence of negative charges in the CNC surface and the anionic CMC polymer, a strong electrostatic repulsion is generated, and the CMC chains do not adsorb in CNC particles. The presence of non-adsorbing polymers into the dispersion induces the flocculation of the particles. This mechanism is known as depletion interaction. Therefore, the rheological behaviors observed for CMC/CNC gels and the dramatic increase in viscosity are explained by depletion flocculation (OGUZLU; BOLUK, 2017; SOUZA *et al.*, 2019).

4.2.2 Small amplitude oscillatory measurements

CMC solutions at 0.5 and 1.0 wt. % showed a liquid-like ($G'' > G'$) behavior for all frequency range. In all CMC samples studied, it was not observed crossover point between the viscous to the elastic modulus. The addition of CNC promoted strong changes in viscoelastic properties. Figure 4.3.a.b shows the dependence of the viscoelastic moduli as a function of the angular frequency for CMC/CNC gels. In the samples, the storage modulus is higher than viscous ($G' > G''$) for all angular frequency range, indicating that all systems studied behave rheologically as gels. Except in the gels with 2 wt. % of CNC that a crossover point defines the transition from a solid-like domain ($G' > G''$) to a liquid-like domain ($G'' > G'$) in higher

angular frequency.

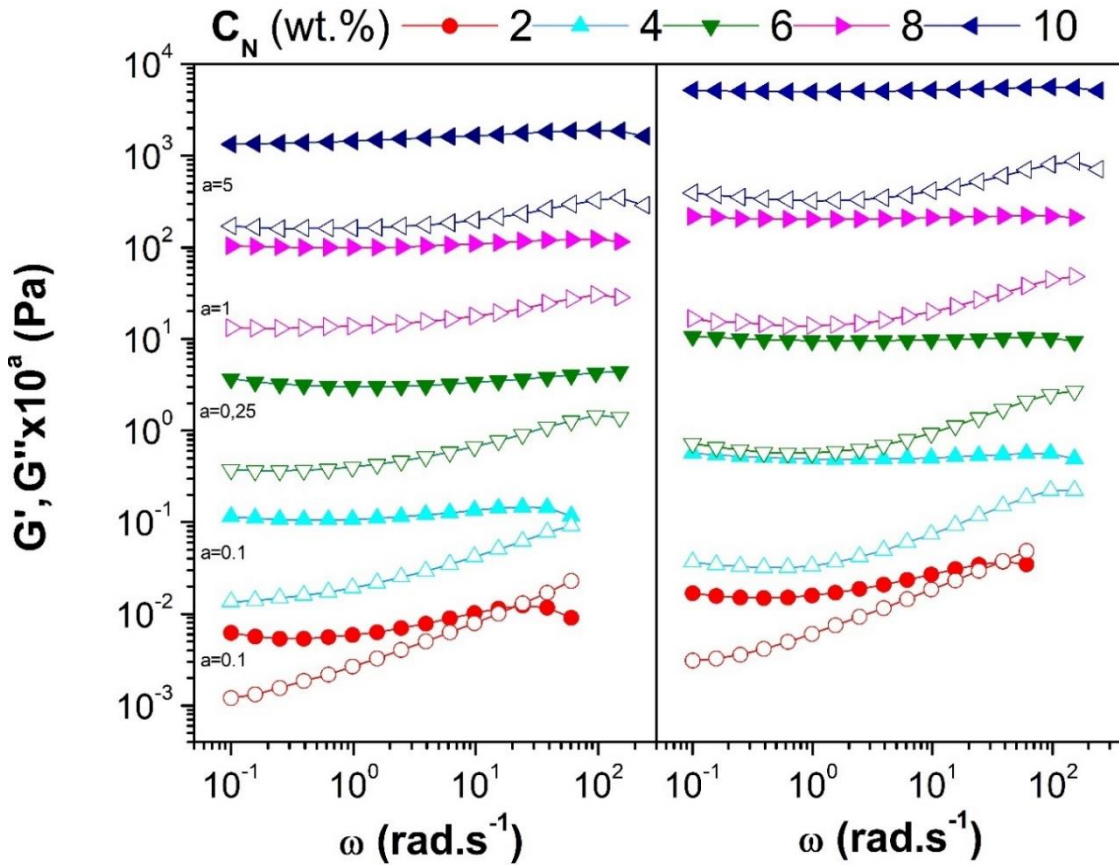


Figure 4.3: Dependence of the storage modulus (G') (solid symbols) and the loss modulus (G'') (open symbols) on the angular frequency (ω) for CMC/CNC gels (a) CMC/CNC gels containing 0.5 wt.% of CMC and (b) CMC/CNC gels containing 1.0 wt.% of CMC (data are vertically shifted to avoid overlapping).

Increasing the CNC concentration, the electrostatic repulsion between negatively charged CNC particles and anionic CMC chains become stronger, influencing the viscoelastic properties of the CMC/CNC gels. The storage and loss modulus become almost independent of the angular frequency, which is the characteristic of gel formation with physical bonds. Increasing the CMC concentration from 0.5 wt. % to 1.0 wt. %, the magnitudes of both moduli increase. Nonetheless, significant changes in viscoelastic behavior were not observed.

The damping factor for the CMC solutions is shown in Figure 4.4.a. It is observed that the liquid-like behavior is predominant, and the transition to the solid-like behavior did not occur for all concentrations. In Figure 4.4.b, the damping factor for the CNC samples is

presented. As observed, with 8 wt. % of CNC, the liquid-like behavior is predominant. When the concentration is increased to 10 wt. % , it can be observed a transition to the solid-like behavior at an angular frequency of 1.46 rad s^{-1} . For the concentration of 12 wt. %, the damping factor is completely in the solid-like region. On the other hand, as observed in Figure 4.4.c and 4.4.d, when CMC is added, the solid-like character is predominant for CNC concentrations above 4 wt. %. The solid-like behavior is promoted by the presence of CMC and when the CNC concentration increases. Thus, with CNC concentrations above 4 wt. %, the CNC produces physical gelation in CMC solutions. The CMC/CNC gels with 1 wt. % of CMC presented a stronger solid-like behavior compared with 0.5 wt. %. This fact is associated with electrostatic interactions with the increase in CNC concentration, where the repulsive forces between negatively charged CNC particles and anionic CMC chains become stronger to generate the elastic behavior and physical gelation in CMC/CNC system.

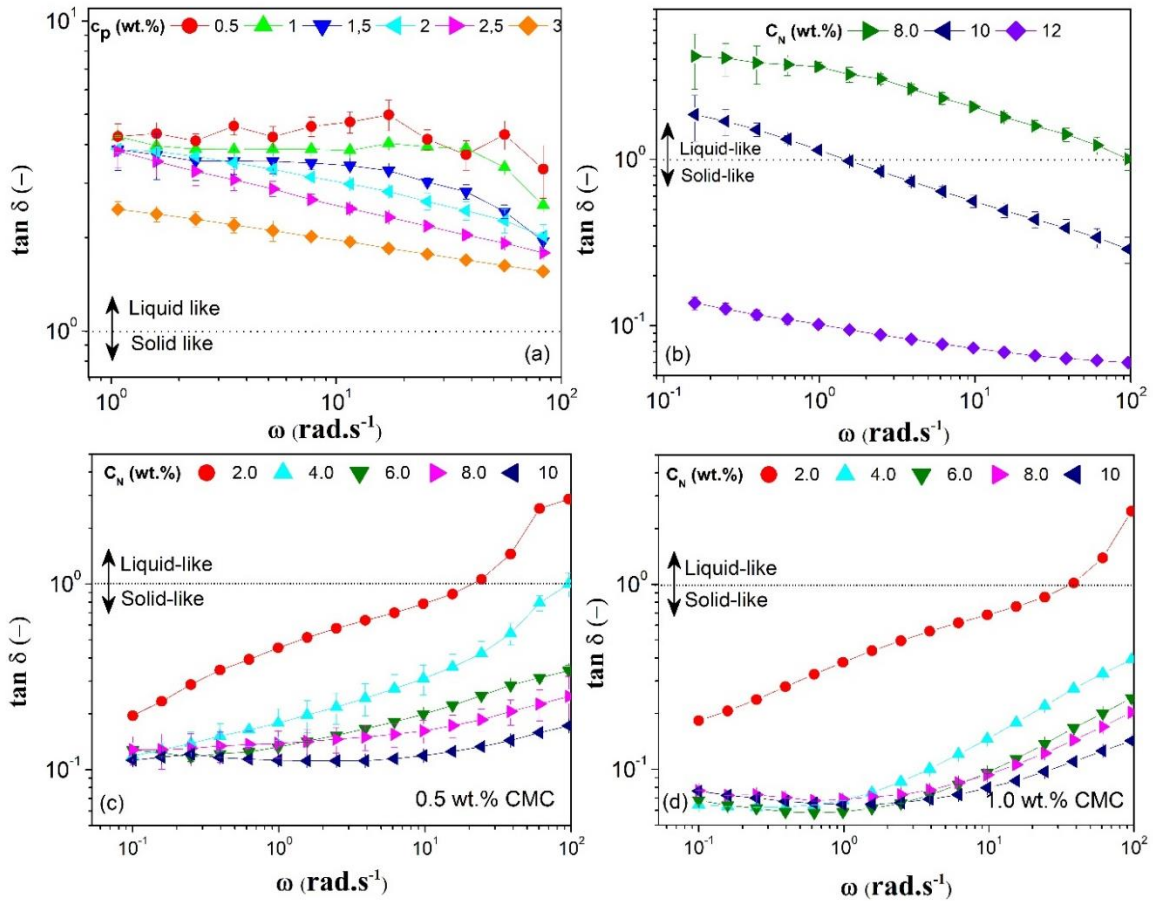


Figure 4.4: Damping factor ($\tan(\delta)$) as a function of the angular frequency (ω) for: (a) CMC solutions, (b) CNC dispersions, (c) CMC/CNC gels with 0.5 wt.% of CMC, and (d) CMC/CNC gels with 1.0 wt.% of CMC.

4.2.3 Evaluation of the gels for 3D printing tests

Filament formation tests were performed for all samples studied. In Figure 4.5a.b it is shown optical microscopy images for the 0.5 and 1.0 *wt. %* of CMC solutions, respectively, with a gradual increase in CNC concentration from 0 to 10 *wt. %*. The CMC solution without the addition of CNC showed the well-defined droplet shape at both concentrations. When CNC particles are added, the influence of the CNC on these solutions is observed. The droplets shapes are progressively modified as the concentration of CNC is increased, until filaments are obtained. The continuous filament is formed from the 8 *wt. %* of CNC concentration for 0.5 *wt. %* CMC solution and since 6 *wt. %* of CNC in 1.0 *wt. %* of CMC solution. Among the filaments formed, it is observed that the composition of 1.0 *wt. %* of CMC with 10 *wt. %* of CNC was that best maintained the fidelity of the filament.

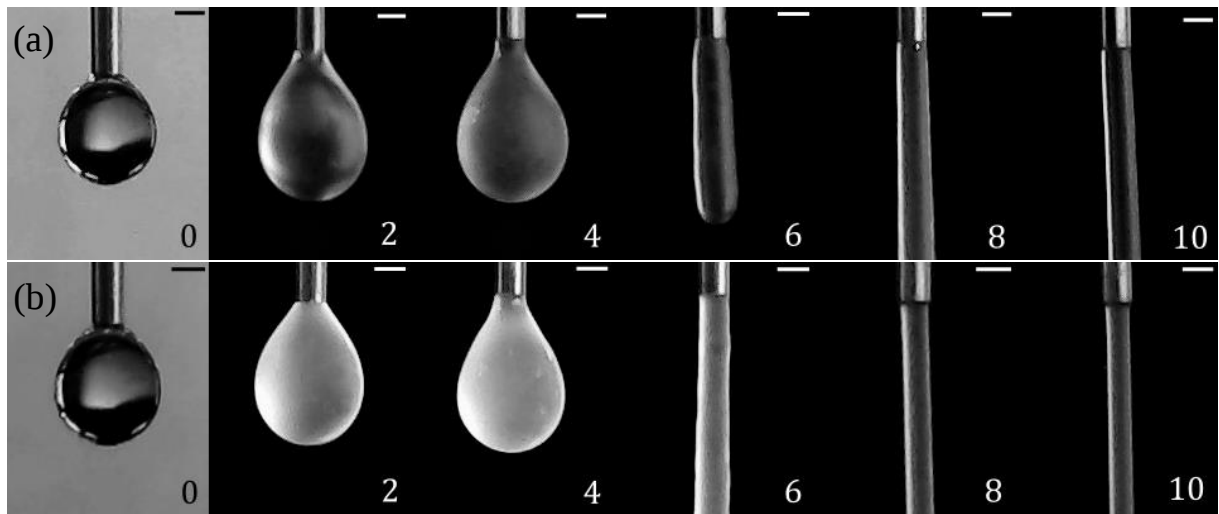


Figure 4.5: Optical microscopy images showing the filaments formation tests of CMC/CNC gels in the CMC solutions with a gradual increase in CNC (a) 0.5 *wt. %* of CMC and (b) 1.0 *wt. %* of CMC. The scale bar represents 1 mm.

Viscosity curves of the concentrations that formed continuous filaments were modeled using the Ostwald-de Waele viscosity model, which is given by:

$$\eta(\dot{\gamma}) = m\dot{\gamma}^{n-1} \quad (4.1)$$

where m is the consistency index and n the power-law index; m is associated with the magnitude of the viscosity and n defines the viscosity behavior: (i) Newtonian if $n = 1$, (ii)

shear-thinning if $n < 1$ or (iii) shear-thickening if $n > 1$ (MORRISON, 2001).

It is observed in Figure 4.6 the viscosity curves fitted and the parameters obtained with the Ostwald-de Waele model.

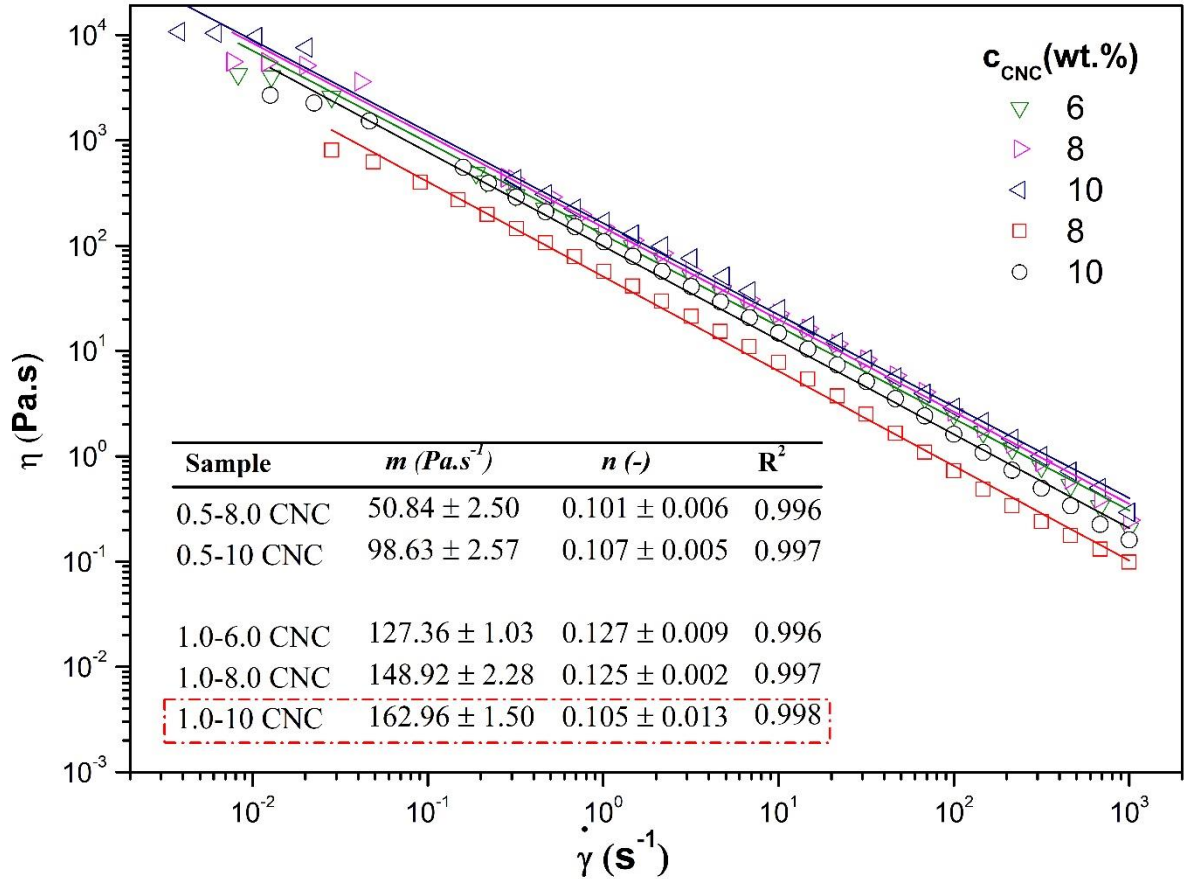


Figure 4.6: Ostwald-de Waele model fitting for CMC/CNC gels, and open symbols correspond to experimental data.

It can be observed that the consistency index m increases with the CNC and CMC concentrations, whereas n decreases with the increasing of CNC concentration, defining a strong shear-thinning behavior in all samples studied. This strong shear thinning is fundamental for extrusion printing, since with the increase of shear stresses the viscosity strongly decreases, allowing the gel extrusion through the needle. Moreover, suitable viscosity is essential due to its influence in resolution and printing fidelity. Gels with low viscosity are extruded easily, however, droplets or filament with low definition are obtained (Figure 4.5.a). As previously seen in Figure 4.5.b, gels obtained with higher concentrations of CMC and CNC (1:8 and 1:10 wt.%) presented better print resolution. The good resolution and shape fidelity after

printing are due to the high zero shear viscosity allied the viscoelastic properties these gels.

According to the data, the composition of 1 wt.% of CMC and 10 wt.% of CNC was defined as the optimal gel for 3D printing tests due to the strong shear-thinning, high viscosity, viscoelastic properties and the ability to maintain the shape.

The 3ITT tests were performed in the optimal gel composition to determine the structural regeneration of the sample after shear and the recovery time. The results are presented in Figure 4.7. It is observed that the sample needs a short time to recover the initial structure, in all shear rates analyzed. So, the viscosity recovery has no dependence on the shear rate applied during the process. The recovery viscosity was calculated using Equation 2.4. The viscosity recovery is around 70% after 10 s and after 180 s the viscosity recovery is in the range between 80 and 90%. Recent studies reported that an initial viscosity recovery of 80% is significant for 3D printing (PEAK *et al.*, 2018).

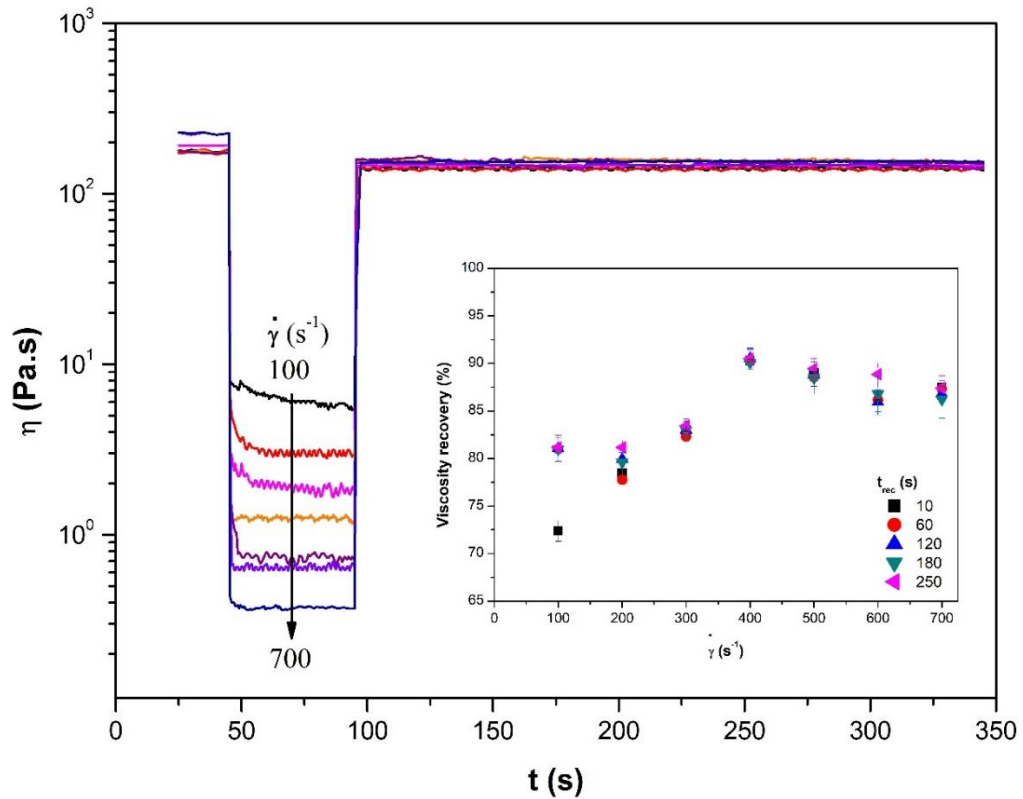


Figure 4.7: Viscosity curves (η) as a function of the time (t) for the recovery test for optimal gel ink. The shear rate ($\dot{\gamma}$) increases from 100 to 700 s $^{-1}$. The inset plot depicts the viscosity recovery percentage as a function of shear rate.

4.2.4 3D extrusion-based printing

In addition to rheological properties, the ideal 3D printing parameters for hydrogel inks must be evaluated, in each case, to ensure the printing resolution. So, the influence of print and extrusion speeds in shape fidelity of printed filament were analyzed. The extrusion speed was calculated based on the piston speed, needle radius, needle length, the power-law coefficients (n and m) and the equation for of velocity profile along a tube by power-law generalized Newtonian fluid (GNF) model (a full description of the calculation process is provided in APPENDIX B). The piston speeds used followed the protocol proposed by Dávila and d'Ávila (2019). Figure 4.8 summarizes the printing parameters evaluated.

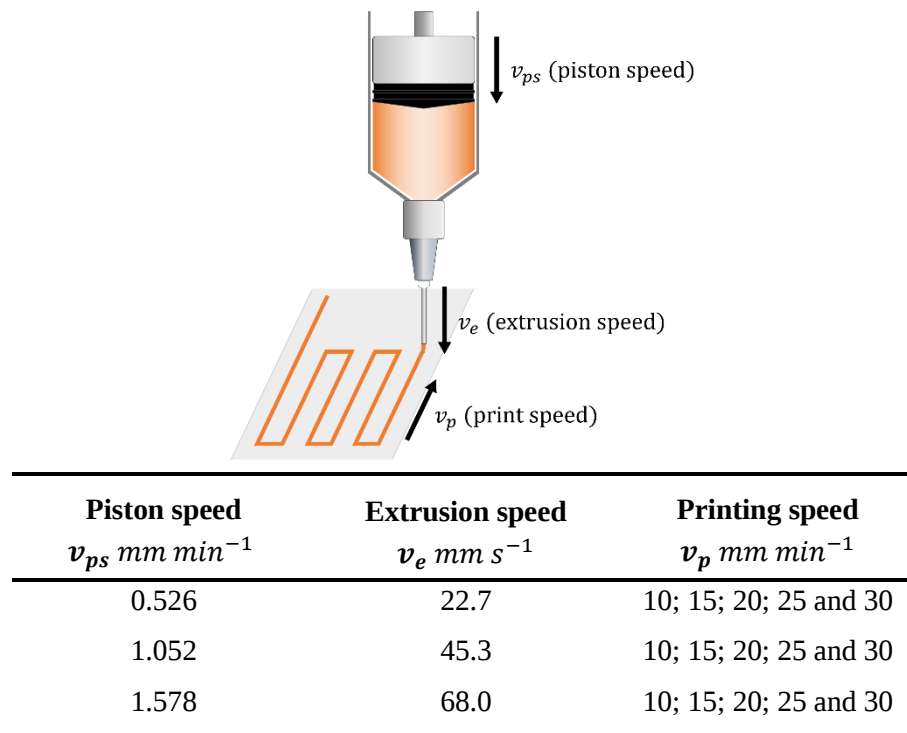


Figure 4.8: Printing parameters evaluated in 3D extrusion-based printing using the optimal hydrogel ink.

The print and extrusion speeds affect the printed filament diameter, directly. At high extrusion speeds and low print speed, the filament width is higher due to the high deposition of material. On the other hand, at high printing speeds with a low extrusion speed, the filaments are stretched, decreasing the diameter these filaments, and in some cases, the deposition of the material may be interrupted. Therefore, the extrusion and printing speeds must be correlated so

that the material is continuously deposited securing better print fidelity to the printed filament. In Figure 4.9 the filament width obtained after measurements of printed samples under different conditions is illustrated.

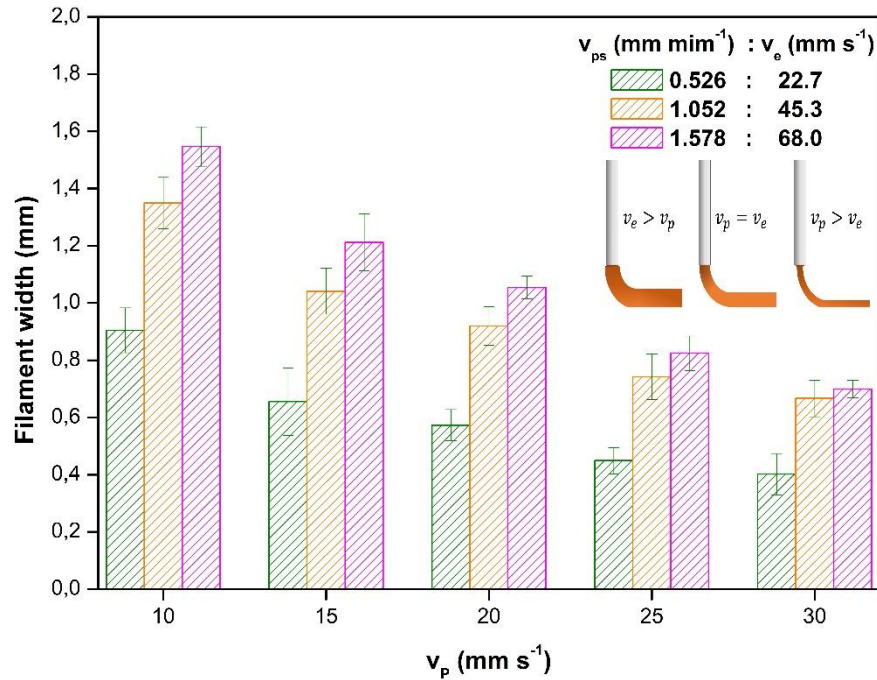


Figure 4.9: Width of filament as a function of the printing speed (v_p) for different extrusion speeds (v_e).

It is observed that as extrusion speed increases, the width of the filaments obtained are higher, which is due to the higher material content deposited per unit of time. Moreover, it is also possible to observe that the filament diameter gradually decreases with increasing print speed. This occurs because higher print speed promotes the formation of elongated filaments. Among the studied parameters, the extrusion speed of 45.3 mm s^{-1} and the print speed of 20 mm s^{-1} were chosen as the best printing conditions, since these conditions ensured better print fidelity. After the printing tests, the optimal hydrogel ink was printed with different numbers of layers. Figure 4.10 shows the printed structures.

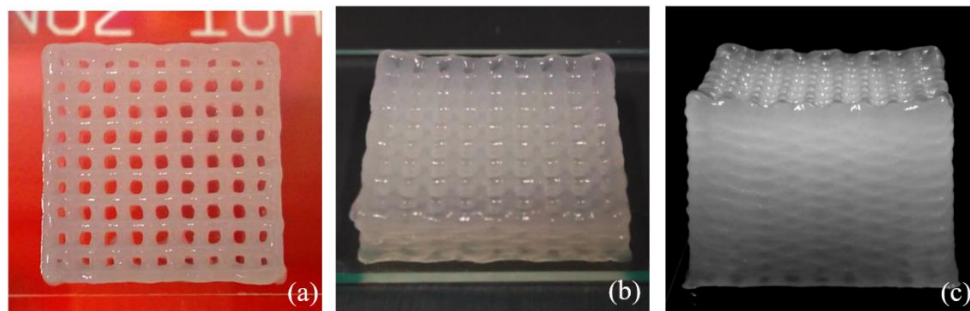


Figure 4.10: Samples printed on different layers: (a) 10 layers (b) 15 layers (c) 50 layers

As shown in Figure 4.10.a-c the hydrogel ink was continuously dispensed, the rheological properties of the gel ink allowed to obtain scaffolds with good shape fidelity that did not collapse even after 1 hour of the printing process. It is possible to observe that the filaments did not merge with each other (Figure 4.10.a) and that the increase of the number of layers did not affect the integrity of filaments (Figure 4.10.b). However, the number of filaments was a limiting parameter for printing process, because bending of the top layers were observed in printed scaffolds with 50 layers (Figure 4.10.c).

The ability to maintain the integrity of the structure is important for future steps using the gel, such as crosslinking and cell culture aiming tissue engineering applications with good printing fidelity.

5 CONCLUSIONS

Rheological studies performed in CMC, CNC, and CMC/CNC dispersions allowed analyzing as rheological properties of these materials were influenced by interactions between anionic CMC chains and CNC rod-like. In all compositions were observed the transition from a near-Newtonian behavior of the CMC solutions to a strong shear-thinning behavior, accompanied by a drastic viscosity increase, when CNC particles were added. Frequency sweep tests revealed that in low concentrations of CNC particles, the system CMC/CNC behaves as liquid-like ($G'' > G'$), and with increased concentration the mixtures behave solid-like at low frequency ($G' > G''$) while at high frequency a viscous behavior is observed ($G'' > G'$). Already with higher CNC concentrations (above 4.0 wt.%), the intermolecular interactions induce physical gelation of the system CMC/CNC. Thus, soft physical gels were obtained. The changes in viscosity profile, drastic increase in viscosity and the transition in viscoelastic properties are attributed the depletion interaction of CMC chains and CNC particles.

Evaluating printability of the gels, it was observed the gradual formation of the filament as the concentration was increased. Therefore, based on rheological properties such as high viscosity, strong shear-thinning, high storage modulus, almost instant structure recovery after applying shear, and filament formation ability the composition 1:10 wt.% CMC/CNC was defined as hydrogel ink for printing scaffolds. Testing the printing parameters showed the influence of printing and extrusion speeds on the printed object. The extrusion speed of 45.3 mm s^{-1} and the print speed of 20 mm s^{-1} were used in scaffolds printing. Scaffolds with different layers were printed using the hydrogel ink. The printed structures presented a good resolution even with greater numbers of layers. Hence, confirming the printability of nanocomposite hydrogel CMC/CNC.

Future works can be oriented to:

- Complementary studies on the mechanism of interaction between CNC particles and CMC polymer chains.
- Perform chemical crosslinking of the hydrogel after the printing process.
- Evaluate the printability of other compositions of CMC/CNC gels with different

geometries.

- Studies of different printing parameters associated with rheological behavior.
- Biological studies as: in vitro, cell viability, degradation, swelling.
- Evaluate the ability to use in tissue engineering applications.

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APPENDIX A –Polarized optical microscopy

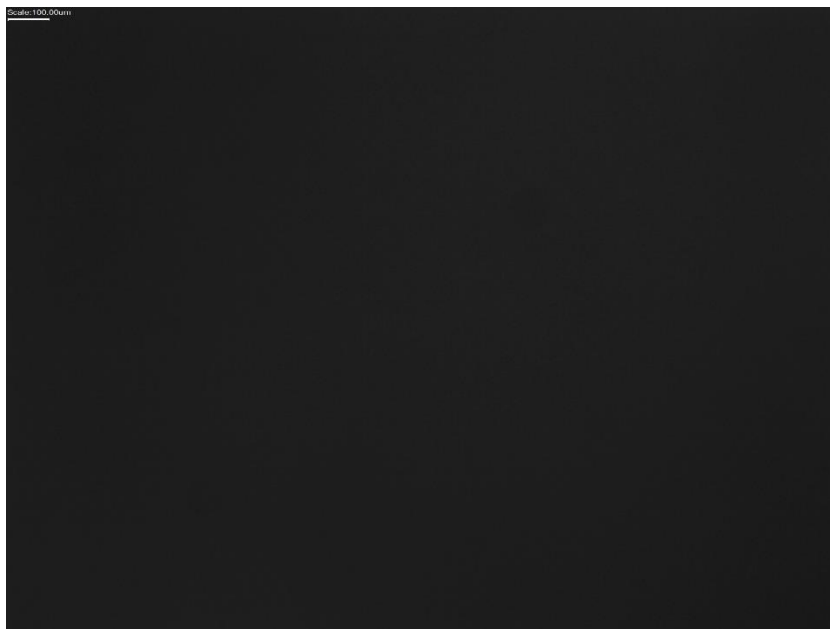


Figure A. 1: Polarized optical microscopy of 2.0 *wt. %* of CMC solutions.



Figure A. 2: Polarized optical microscopy of 2.0 *wt. %* of CNC dispersions.

APPENDIX B –Printing extrusion speed

According to Morrison (2001), the velocity profile along a tube of in power-law generalized Newtonian fluids can be calculated via the Equation:

$$v_z(r) = R^{\frac{1}{n}+1} \left(\frac{\Delta p}{2ml} \right)^{\frac{1}{n}} \left(\frac{n}{n+1} \right) \left[1 - \left(\frac{r}{R} \right)^{\frac{1}{n}+1} \right] \quad (\text{B. 1})$$

where η is the viscosity, Δp is the pressure difference between the inlet and outlet of the nozzle tip (extrusion pressure), r is a distance of velocity profile, m and n are derived from the power law, R is the radius of needle and L is the total length of the needle. And the flow rate is given by:

$$Q = \left(\frac{\Delta p R}{2ml} \right)^{\frac{1}{n}} \left(\frac{n\pi R^3}{1+3n} \right) \quad \text{B. 2}$$

Based on the flow rate and piston speed, the pressure difference between the inlet and outlet of the nozzle tip was calculated and finally, the extrusion velocity profile can be obtained. Figure B. 1 shows the extrusion velocity profile in the needle.

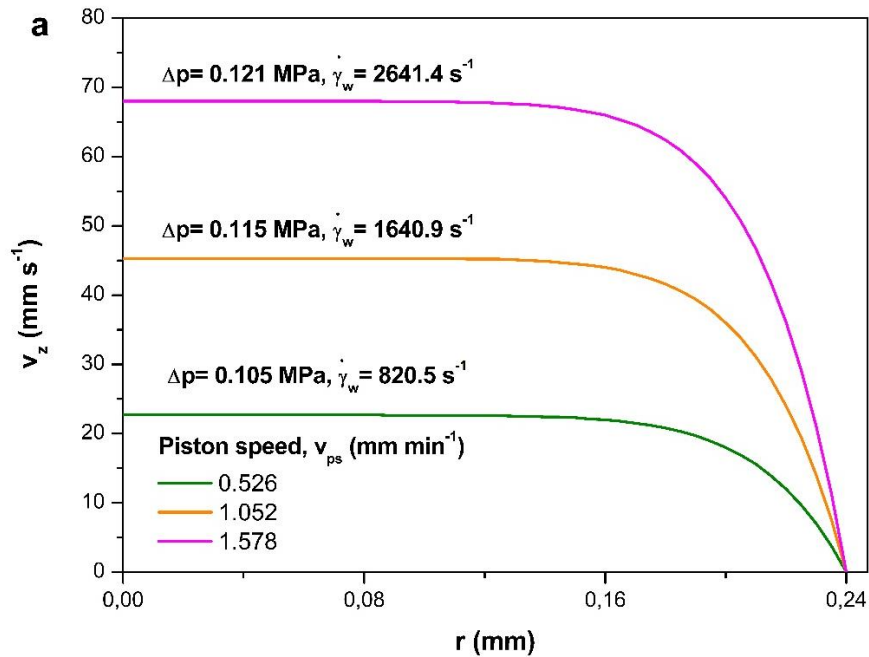


Figure B. 1: Extrusion velocity profile inside the needle.