

**Liquid to Soft Solid Transition of Cellulose Nanocrystal Suspensions in
Presence of Micelle Forming Surfactants**

by

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Presence of Micelle Forming Surfactants**

Koç University

Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a master's thesis by

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and have found that it is complete and satisfactory in all respects,
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*This thesis is dedicated to my supportive parents,
Sibel and Kemal Kuşhan.*

ABSTRACT

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Cellulose nanocrystals (CNC) are versatile nanomaterials benefited widely for both industrial and scientific purposes. Their abundance, non-toxicity, biodegradability, extraordinary mechanical, optical, and thermal properties make them suitable for a wide range of applications. Particularly, high aspect ratio, hydrophilicity, easy modification of surface properties make CNC ideal rheology modifiers. In presence of salts, polymers and surfactants, thixotropic features of aqueous CNC suspension alter drastically, and liquid to soft solid phase transition is often observed. Even though rheological properties of neat aqueous CNC suspensions are widely studied and understood, there is an undiscovered area about gelation in presence of macromolecules, like polymers and surfactants. Understanding gelation mechanism is crucial to tailor mechanical properties of the complex CNC mixtures. Our study is focused on surfactant – CNC interactions especially when the surfactant concentration is higher than critical micelle concentration.

This thesis is the result of two different studies. In the first one, the bulk rheology (performed in both linear and transition from linear to non-linear viscoelastic region) of aqueous CNC suspensions in the presence of cationic surfactants of 1-decyl-3-methyl imidazolium chloride and 1-decyl-3-methyl imidazolium ferric tetrachloride was studied separately. The head and tail groups of these surfactants are identical, the only difference is the counterions, which led to formation of identical micelles of identical size and shape but with varying interparticle interactions. Micelles of $[C_{10}mim][Cl]$ are strongly positively charged and stabilized via long-range Coulomb repulsion whereas $[C_{10}mim][FeCl_4]$ surfactants form nearly neutral micelles interacting with short-range hard-sphere type interaction. We, therefore, evaluate directly, for the first time, the effect of micelle charge on the gelation of CNC suspensions using the same surfactant system. At intermediate surfactant concentrations (slightly above CMC), positively charged micelles electrostatically interact with negative CNC and adhere to their surface; therefore, micelles behave as crosslinking points and gel network becomes strong. However, at high surfactant concentrations well above critical micelle concentration, the charged micelles repel each other and result in much weaker gel networks. Such a drastic weakening effect was absent in presence of neutral micelles.

In the second study, thermo-responsive block copolymer with the molecular structure of $(PEO)_{100}-b-(PPO)_{65}-b-(PEO)_{100}$ (which is also a non-ionic surfactant commercially named Pluronic F127) is mixed with aqueous CNC suspensions and liquid to soft solid phase transition was observed as micellization takes place. The micelles are neutral due to nature of block copolymer. Pluronic F127's aqueous solutions are thermo-responsive; they undergo gelation due to Pluronic F127 micelle formation taking place at above

critical micelle temperature (CMT), near the room temperature. CMT and therefore gelation temperature go down as Pluronic F127 concentration increases. Since the critical temperature is lower than body temperature yet still high enough for processability at room temperature, Pluronic F27 is suitable for biomedical applications. Gel point of the complex is increased with increasing CNC concentration and thermal and mechanical reversibility of the complex is also preserved. The resultant Pluronic F127 / CNC solutions are promising materials for especially dual drug therapy in which both Pluronic F127 and CNC can be utilized as drug carriers.

Our studies demonstrate that addition of ionic and non-ionic surfactants and polymers to aqueous CNC suspensions can have a dramatic influence on the phase change due to network formation by electrostatic, hydrophilic, or hydrophobic interactions. Designed by manipulating these interactions, CNC-based hydrogels can be used in tissue engineering, drug and gene delivering systems in medicine as well as protective coatings in the industry.



ÖZETÇE

Selüloz Nanokristal Süspansiyonlarının Misel Oluşturan Sürfaktanlar Aracılığıyla Sıvıdan Yumuşak Katı Formuna Geçmesi

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Selüloz nanokristalleri endüstriyel ve bilimsel amaçlar için kullanılabilen çok yönlü nanomalzemelerdir. Zehirli olmayan, biyolojik bozunma gerçekleştirebilen bu yaygın nanomalzemeler sıra dışı mekanik, optik ve termal özellikleri sayesinde birçok uygulamada kullanılabilmektedir. Selüloz nanokristallerinin yüksek en boy oranı, hidrofilik kimyası ve modifiye edilebilen yüzeyi onları mükemmel reoloji ajanları haline getirir. Tuz, polimer ve sürfaktan varlığında selüloz nanokristallerinin sulu çözeltilerin tiksotropik özellikleri belirgin bir şekilde değişir ve hatta bu çözeltilerin bu kimyasallar aracılığıyla sıvıdan yumuşak katı formuna ani geçişleri yaygın bir şekilde gözlemlenir. Selüloz nanokristallerinin saf sulu çözeltileri üzerine çok fazla çalışma yapılmış ve davranışları anlaşılmış olmasına rağmen makro moleküllerin (sürfaktanların ve polimerlerin) bu çözeltilerle karıştırılması sonucu oluşan kompleksler (jeller) fazla çalışılmamıştır. Selüloz nanokristallerinin sulu çözeltilerinin jelleşme mekanizmalarını anlamak bu jellerin mekanik özelliklerini akort edebilmek adına önemlidir. Çalışmamız genel itibarıyla sürfaktanların özellikle konsantrasyonları kritik misel konsantrasyonu üzerindeyken selüloz nanokristalleriyle gerçekleşen etkileşimleri üzerinedir.

İki çalışmadan oluşan bu tezin ilk çalışmasında selüloz nanokristallerinin 1-desil-3-metil imidazolyum klorit, $[C_{10}mim][Cl]$, ve 1-desil-3-metil imidazolyum ferrik tetraklorit, $[C_{10}mim][FeCl_4]$, sürfaktanlarını içeren sulu süspansiyonlarının yığmsal reolojik analizi ayrı ayrı gerçekleştirilmiştir. Bu iki sürfaktanın baş ve kuyruk gruplarının aynı olmasına rağmen tersyükünleri farklıdır, bu da benzer boyuta ve şekle sahip olan fakat farklı partiküller arası etkileşim gösteren iki farklı misel oluşumuna yol açmıştır. $[C_{10}mim][Cl]$ sürfaktanının oluşturduğu miseller pozitif yüklü ve bu miseller arasındaki etkileşim uzun etki alanlı Coulomb etkileşimidir. Fakat $[C_{10}mim][FeCl_4]$ sürfaktanının oluşturduğu miseller neredeyse nötr ve kısa etki alanlı sert küre etkileşimi gerçekleştirmektedir. Çalışmamızda benzer kimyasal yapıdaki sürfaktanların oluşturduğu farklı yüzey zeta potansiyeline sahip miselleri inceleyerek ilk defa misel yükünün sulu selüloz nanokristallerinin süspansiyonlarının jelleşmesi üzerindeki etkisini değerlendirdik. Kritik misel konsantrasyonu civarında ve hafif üzerinde, pozitif miseller negatif selüloz nanokristalleriyle elektrostatik olarak etkileşime geçer ve negatif yüzeye yapışan pozitif miseller bir çapraz bağlanma noktası gibi davranırlar. Sonuç olarak negatif selüloz nanokristallerinin birbirlerine bağlanmasına sebep olup güçlü bir jel ağı yaratırlar. Daha yüksek konsantrasyonlarda oluşan serbest pozitif miseller çapraz bağlanma noktası olarak jel ağını güçlendiren diğer pozitif miselleri de iterek jel ağının sağlamlığının azalmasına sebep olur. Diğer tarafta ise nötr $[C_{10}mim][FeCl_4]$ miselleri jel ağının yapısı üzerinde pozitif misellerin göstermiş olduğu bu güçlü zayıflatıcı etkiyi göstermemiştir.

Tezde yer alan ikinci çalışmada ısıya duyarlı (PEO)₁₀₀-*b*-(PPO)₆₅-*b*-(PEO)₁₀₀ molekül yapısına sahip bir blok kopolimer ve aynı zamanda noniyonik bir sürfaktan olan ticari ismiyle Pluronic F-127'in sulu selüloz nanokristalleriyle olan karışımları incelenmiştir. Bu sürfaktanların da misel oluşturmasıyla sulu selüloz nanokristalleri süspansiyonlarının sıvıdan yumuşak katı formuna geçtiği gözlemlenmiştir. Blok kopolimerin yapısından dolayı oluşan miseller de nötraldir. Pluronic F127'nin sulu çözeltileri kendinden ısıya duyarlıdır. Sulu çözeltiler kritik misel sıcaklığından yüksek sıcaklıklarda (oda sıcaklığına yakın sıcaklıklar) Pluronic F127 moleküllerinin misel oluşturmaya başlamasıyla beraber jelleşirler. Blok kopolimer konsantrasyonun artması kritik miselleşme sıcaklığını ve aynı zamanda sulu çözeltilerin jelleşme sıcaklığını da düşürür. Sulu Pluronic F127 çözeltilerinin vücut sıcaklığından çok düşük olmayan sıcaklıklarda sıvı olması ve işlenebilmesinden dolayıısıyla biyomedikal uygulamalarda kullanılabilmeye uygundur. Selüloz nanokristalleri ile Pluronic F127'nin oluşturduğu kompleks de artan sıcaklıkla faz değişimi göstermektedir; fakat faz değişimi gösterdiği sıcaklık selüloz nanokristalleri konsantrasyonunun artmasıyla beraber de yükselmektedir. Selüloz nanokristallerinin faz değişim sıcaklığı üzerindeki bu etkisine karşın kompleksin termal ve mekanik tersine dönebilirliği korunmaktadır. Oluşan kompleks kendini oluşturan her iki elementin de ilaç taşıyabilme yeteneğine sahip olmasından dolayı özellikle çifte ilaç terapisi için gelecek vaat etmektedir.

Bu iki çalışmamız da misel oluşturan iyonik ve noniyonik sürfaktanların sulu selüloz nanokristalleri süspansiyonlarına katılmasının neticesi olarak karışımların sıvıdan yumuşak katı formuna geçişini göstermektedir. Bu geçiş selüloz nanokristallerinin ve sürfaktanların elektrostatik, hidrofilik ve hidrofobik etkileşimleri sonucunda oluşan jel ağıyla mümkündür. Etkileşim mekanizmalarını yöneterek tıp alanında kullanabilmek üzere selüloz nanokristalleri temelli biyolojik olarak uyumlu hücre dışı matrisler, ilaç ve gen transferi yapabilen sistemler ve endüstriyel alanlarda kullanabilmek üzere ise koruyucu kaplamalar yapabilmek mümkün olmaktadır.

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ABBREVIATIONS

CNC	Cellulose Nanocrystals
CMC	Critical Micelle Concentration
CMT	Critical Micelle Temperature
CNF	Cellulose Nanofibrils
CTAB	Centrimonium Bromide
DEC	Diethyl Carbonate
DLS	Dynamic Light Scattering
DLVO	Derjaguin, Landau, Verwey, Overbeek
DMAPS	3- (N, N Dimethylmyristylammonio) Propanesulfonate
DP	Degree of Polymerization
DTAB	Dodecyltrimethylammonium Bromide
EC	Ethylene Carbonate
EISA	Evaporation Induced Self-Assembly
FE-SEM	Field Emission Scanning Electron Microscope
ITC	Isothermal Titration Calorimetry
LAOS	Large Amplitude Oscillatory Shear
LCST	Lower Critical Solution Temperature
LVER	Linear Viscoelastic Region
MIM	Methyl Imidazolium
PEO	Polyethylene Oxide
PLA	Polylactic Acid
PNIPAM	Poly (N-isopropylacrylamide)
PPO	Polypropylene Oxide
PPZ	Poly (Organophosphazene)
PVA	Polyvinyl Alcohol
SANS	Small Angle Neutron Scattering
SAOS	Small Amplitude Oscillatory Shear
SDBS	Sodium Dodecylbenzene Sulfonate
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscope
SLAS	Sodium Lauroyl Sarcosinate
SLES	Sodium Laureth Sulfate

UCST	Upper Critical Solution Temperature
TDS	Total Dissolved Solids
TTAC	Tetradecyltrimethylammonium Chloride
XRD	X-ray Diffraction



Chapter 1: INTRODUCTION

1.1 *Motivation of the Thesis*

The science of soft matter deals with complex materials exhibiting distinctive characteristics in the spectrum of solid - liquid. The very first studies about soft matter started in the mid twentieth century; however, the great emphasis about the subject emerged in the last century and led to a Nobel prize in physics awarded to Pierre-Gilles de Gennes "*for discovering that methods developed for studying order phenomena in simple systems can be generalized to more complex forms of matter, in particular to liquid crystals and polymers*" in 1991. He described the soft materials as a class of materials displaying large response to small deformations and he underlined two major features of soft materials which are *complexity* and *flexibility* in his Nobel lecture. [1] Briefly and more straightforwardly, materials deforming under mild external stimuli such as mechanical, thermal, electrical, and magnetic are classified as soft matter. There are three common characteristics of soft matter: (i) the structural components at the mesoscopic scale determine the macroscopic behavior of these materials, (ii) the order between material's building blocks is short-ranged in comparison to the order of crystalline structures, and (iii) perhaps more importantly, these materials are more susceptible to temperature changes than hard materials are. [2] A wide variety of materials ranging from hard plastics, foams, adhesives, gels, polymeric solutions, to foods, and biological fluids and structures falls within this category. Environmentally sensitive nature of soft materials makes them useful for many applications where the material can behave "smartly". Therefore, they are often used in sensors, actuators, electro-mechanical devices, smart textile products, wearable electronics, smart medical instruments, drug carriers, hydrogels, biomimetic and bioinspired materials, and artificial tissues. [3]

New findings and advancements in soft matter allowed the development of stimuli-responsive materials such as stimuli-responsive hydrogels widely benefited both in industrial and medical applications. Hydrogels, formed by either covalent crosslinking or physical entanglements of hydrophilic polymers, have emerged as an important class of soft materials with numerous potential applications in industry and medicine. As they can

hold water up to 99 % by weight without sacrificing their structural integrity [4], they are excellent candidates for mimicking the structure and mechanical properties of the biological microenvironments in synthetic systems. [5] They can be physically, chemically, and biochemically responsive [6] depending on the type of the polymer forming the network, conjugated chemical moieties and even crosslinking agents. The broadly studied stimuli-responsive hydrogels [6] are pH-sensitive [7], temperature-sensitive [8], electro-sensitive [9], and light-responsive [10, 11]. These environmental effects basically change the short-ranged interaction between the components and causes huge difference in macroscopic properties (such as volume and stiffness). Since hydrogels are used for drug [12], cell [13], gene [14] delivery, dye and metal ion removal [15] and used as scaffolds in tissue engineering [16], their mechanical and rheological features are of great importance in their applicability. [6, 17, 18]

Hydrogels can be originated from both natural (proteins [19], polysaccharides of alginate [20], chitosan [21], dextran [22], and cellulose [23, 24]) and synthetic polymers. However, great biodegradability, biocompatibility, low cytotoxicity features of natural hydrogels and economic, and environmental concerns make them more alluring than synthetic ones. Although natural polymers have numerous advantages, it is also noteworthy that they have some limitations such that mechanical properties of natural hydrogels are slightly poor. [25] Hence, there is a need for utilization of natural polymers like cellulose possessing more rigid structures to keep the integrity. Cellulose is the most abundant natural polymer in the world synthesized and degraded about 10^{10} to 10^{11} tones, annually. [26] Its neat form has been used from the beginning of the mankind. It has an extraordinary toughness and stiffness due to the dense crystalline regions which are called as cellulose nanocrystals (CNC). They are excellent structural materials and can be used to form the hydrogel scaffold. [27] Besides the enhancement of mechanical properties, the large surface area of CNC allows conjugation of responsive chemical moieties, and sulfate ester, hydroxyl groups on the surface support physical crosslinking. All in all, CNC can implicitly grant smartness to the hydrogels.

With the intention of creating less environmentally risky, non-toxic, smart hydrogels, non-chemical methods are preferred. [28-30] Reversible physical bonds bring tailorability to the hydrogels and make them susceptible to environmental stimuli. Since surfactants are outstanding macromolecules displaying both hydrophilic and hydrophobic features and behaving differently at various ionic strengths, temperatures, and concentrations, they

are generally utilized as agents. For inducing gelation in CNC suspensions and donating responsiveness to the resultant hydrogels, the use of surfactant is quite appropriate. There are many recent studies about CNC – surfactant interactions and their composite hydrogels. [31] In the literature, gelation mechanism of CNC suspensions via addition of cationic [32], anionic [33], and non-ionic [34] surfactants were proposed to a certain extent; however, there are some undiscovered areas about gelation mechanism for the concentrations above critical micelle concentration (CMC). Even though some responsive behavior were achieved in CNC-based hydrogels, [30] further investigation about creating temperature, electrical or magnetic response is required.

Throughout my thesis term, I aspired to comprehend CNC – surfactant interactions for both below and especially above CMC and create smart CNC-based hydrogels by using the fundamental knowledge I obtained. My thesis combines two distinct projects serving for the same aim. In my first project, I analyzed the CNC-based hydrogels formed by addition of cationic surfactants of $[C_{10}mim][Cl]$ and $[C_{10}mim][FeCl_4]$ (which is a magneto-responsive surfactant [35]) to aqueous CNC suspensions. Hydrogels containing same surfactant group but different counterions ($[Cl]^-$, $[FeCl_4]^-$) displayed varying mechanical performances for both below and above CMC as proven by rheology experiments. Another important finding of this project is that magneto-responsive surfactant lost its ability when it is dispersed in water; therefore, magnetic susceptibility was not adapted to the CNC-based hydrogel. In my second project, I desired to develop thermo-responsive and injectable CNC-based hydrogels. The thermo-responsive block copolymer of Pluronic F127 was added to aqueous CNC suspensions. The thermal response (between 10 – 50 °C) of the F127 containing hydrogels and their mechanical properties were analyzed by performing steady and oscillatory shear rheology experiments. By means of completing these two projects, interactions between CNC and both cationic and non-ionic surfactants, especially when micellization occurs, were investigated and interpretations about gelation mechanism were made. As a result, my findings can answer some key fundamental questions about CNC – micelle, and micelle – micelle interactions and surfactants' role when they are used as agents in CNC-based hydrogels.

1.2 *Outline of The Thesis*

- Chapter 2 starts with the description of cellulose, cellulose nanocrystals (CNC) and continues with studies about aqueous CNC suspensions and CNC-based hydrogels. Works related to surfactants, which are used as gelation agents, and their CNC mixtures are provided also in this chapter.
- Chapter 3 contains materials and synthesis, experimental methods, results and discussion parts of the first project named as “Surfactant Driven Liquid to Soft Solid Transition of Cellulose Nanocrystal Suspensions”. Through light scattering, small angle neutron scattering (SANS), and oscillatory rheology analyses, characterizations of [C₁₀mim][Cl] – CNC and [C₁₀mim][FeCl₄] – CNC containing hydrogels were performed. The findings are presented in this chapter.
- Chapter 4 includes materials, experimental methods, results and discussion sections of the second project named as “Temperature Induced Gelation of Cellulose Nanocrystal Suspensions”. Via light scattering, steady shear with temperature ramp and oscillatory shear rheology experiments, F127 – CNC containing hydrogels’ gelation behavior was analyzed between 10 – 50 °C. The mechanical properties of these hydrogels were investigated using both large amplitude oscillatory shear (LAOS) and small amplitude oscillatory shear (SAOS) applications. The discoveries of these analyzes are provided in this chapter.
- Chapter 5 is the final chapter in which all conclusions drawn from both projects are given. Gelation behavior of aqueous CNC suspension were analyzed for the addition of both cationic and non-ionic surfactants. The analysis of resultant hydrogels’ mechanical features with comparison are also given in this part in a nutshell.

Chapter 2:

LITEARURE REVIEW

2.1 Cellulose

Cellulose is the most plentiful natural polymer in our planet. It is the main component of the cell wall of all plant cells, and besides that it is found in bacteria, tunicates, oomycetes and algae. [36, 37] Their role in human lives is traced back to the beginning of humankind and has been used as a necessary material for construction, cloth and paper making for centuries. The very first modern studies about cellulose and its derivatives began in the beginning of 19th century and therefore, their potential applications besides their classical utilizations were started to be discovered. [38] It is a linear homopolymer constructed by hundreds or thousands of D-glucose molecules connected to one another with β -1,4 glycosidic bond. [39] Each glucose residue being the building block of cellulose polymer is rotated 180° corresponding the neighbor glucose residue. This isotactic duo, which is called cellobiose, becomes the repeating unit of long helical cellulose molecules. The hydroxyl groups adjacent to 2nd, 3rd, and 6th C atoms in the ring plane leads the hydrogen bond formation. [36] Appearance of both intra and inter molecular hydrogen bonding produces an attraction between long spiral chains, which leads the formation of cellulose fibrils. Depending on the degree of hydrogen bonding and attraction between long cellulose molecules, crystalline, semi-crystalline and amorphous regions develop in bulk cellulose. [40, 41]

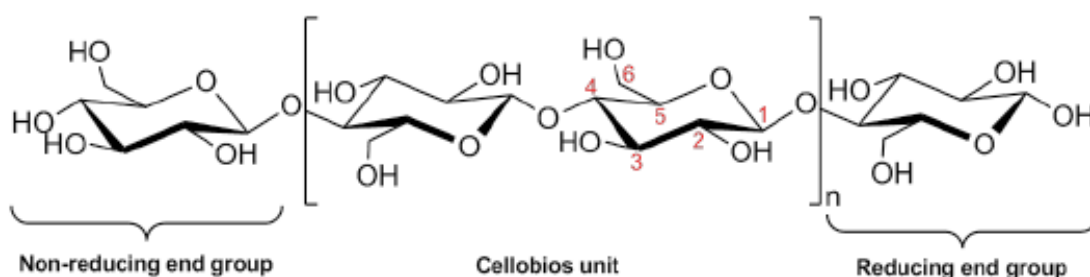


Figure 2.1: Molecular Structure of Cellulose [36].

Cellulose is a polydisperse natural polymer and its degree of polymerization differs among the source from where cellulose is isolated. For example, native cotton has long cellulose chains with degree of polymerization (DP) up to 15000 whereas cellulose isolated from wood pulp has a DP around 300 – 1300. [41-43] Inherently, the variance among the DP of cellulose chains cause the formation of different macro molecules and even crystalline order. The native cellulose polymorphs are named as cellulose I α and cellulose I β . They coexist in nature, yet their amounts differ specie to specie. Cellulose I α , which is predominantly seen in algae and bacteria, has a triclinic crystalline system [44] while cellulose I β found mainly in plants has a monoclinic crystalline system. [45] The alkali treatment or urea treatment made on native cellulose causes the formation of various cellulose polymorphs, which are known as cellulose II [46] and cellulose III [47], respectively. The main variation between cellulose I group and cellulose II or III stems from the hydrogen bonding occurring between different numbered carbon atoms on the ring.

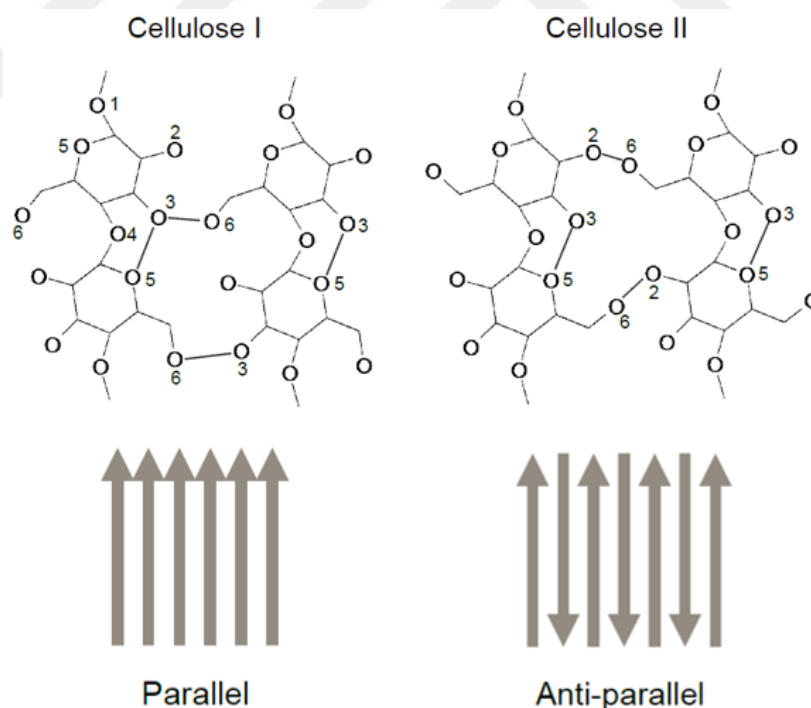


Figure 2.2: Cellulose I and Cellulose II Polymorphs [48].

Bundles of long spiral cellulose chains are named as cellulose microfibrils. These cylindrical structures are regarded as the smallest structural entity within the plant cell wall, and their sizes are around 10 – 35 nm. Microfibril packages build macrofibrils and

macrofibrils construct micron sized cellulose fibers. [43] All these structural units comprise both crystalline and amorphous regions and they are not the only component of rigid plant cell walls. Besides cellulose fibers, primary cell wall includes a heteropolymer of hemicellulose and pectin; and secondary cell wall structure possess both hemicellulose and lignin. [49]

Owing to the presence of other organic molecules within both plant cell and wood pulp cellulose isolation necessitates some extra chemical treatment. Dewaxing, alkali treatment and bleaching are fundamental steps made for cellulose isolation. Wax and extractives are removed in dewaxing step with the help of organic solvents such as acetone and ethanol. Secondly, alkali treatment at a high temperature (around 80°C) is performed by using bases such as sodium or potassium hydroxide. Hemicellulose and lignin are removed in this step. The bleaching step involves sodium chlorite treatment. The residual lignin is removed, and the resultant isolated cellulose fibers become white after completing this step. [50] Certainly, the amounts or the chemicals used through purification might be changed depending on the source of the cellulose. The obtained cellulose fibers are mechanically strong structures. Though, the chemical activity of these structures is somewhat limited. There is a need for additional chemical modifications on cellulose and creation of various cellulose derivatives to utilize them in semi-synthetic industrial purposes. Cellulose ethers, cellulose esters and cellulose nitrates are mainly utilized in non-conventional applications such as coating, textile, plastic film and packaging manufacture, pharmaceuticals, sensor, adhesives, and cosmetics. [51] Especially crystalline regions of cellulose have more superior characteristics than bulk cellulose itself does.

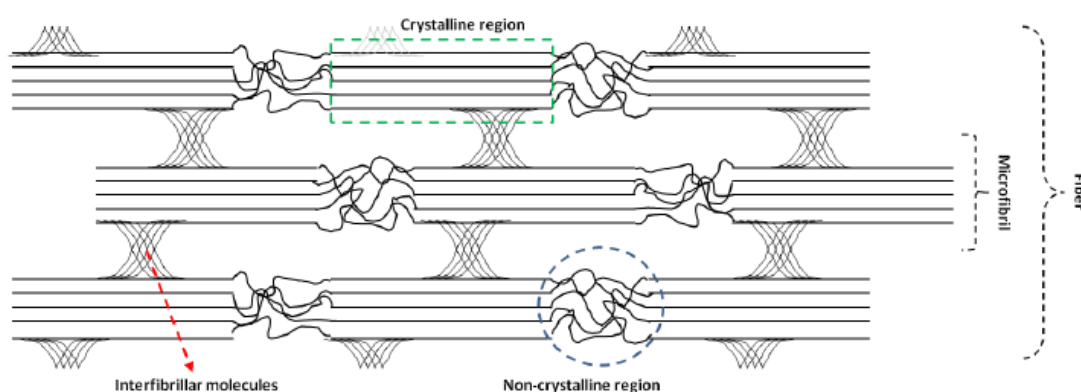


Figure 2.3: Cellulose Fiber with Crystalline and Non-Crystalline Regions [36].

High crystallization comes with high strength, high aspect ratio, large surface area, great electrical and thermal properties. Isolated crystalline regions of cellulose are cellulose nanocrystals (CNC), variously named as cell nano or micro whiskers, or cellulose nanorods, or nanofibrils (CNF). These names are generally used interchangeably but some research use different names due to the differences in size and source of these crystalline structures.

2.2 Cellulose Nanocrystals (CNC)

The necessity for renewable, non-toxic and non-petroleum-based materials makes scientists interested in cellulose. Studies about cellulose demonstrated that there is possibility to obtain more crystalline sub-structures from bulk cellulose. These nanostructured materials known as cellulose nanocrystals (CNC) have various new potential utilizations. Following the mechanical processes of high-pressure homogenization or widely used chemical treatment method of sulfuric acid treatment, undesired amorphous regions of cellulose are eliminated, and the resultant crystalline regions are acquired. These regions have a Young's modulus around 110 – 200 GPa [52], which is higher than the concrete's (30 GPa [53]); therefore, they are selected for development of novel composite materials. CNC have also fibrillar structures. The fibrillar length differs from 100 nm to several micrometers, the diameter of a CNC rod differs from 1 to 50 nm. The source of cellulose causes the formation of different sized CNC rods. Strong acidic treatment is generally implemented on cellulose as it is less energy consuming. 64 weight % sulfuric acid attacks the glycosidic bond, hydrolyses it. This reaction triggers a bond cleavage especially in less dense and less complex regions of bulk cellulose (amorphous regions). Then complex regions (crystalline regions) become free and sulfonated. Besides sulfuric acid treatment, methods such as enzymatic hydrolysis, TEMPO or periodate oxidation, liquefaction are applied. The advantages of CNC include larger surface area, high aspect ratio, advanced mechanical, thermal, electrical, barrier, and rheological features. The larger specific surface area is an important parameter in the creation of porous forms, CNC display surface area as large as 500 square meter per gram. The large surface area additionally allows extensive chemical modifiability. As CNC utilized as a scaffold material in hydrogels or aerogels,

the features of the final structure might be tuned by introducing different chemical moieties on this large surface. The hydroxyl groups on surface can undergo numerous chemical modifications of polymer grafting, silylation, cationization, esterification, and acetylation.

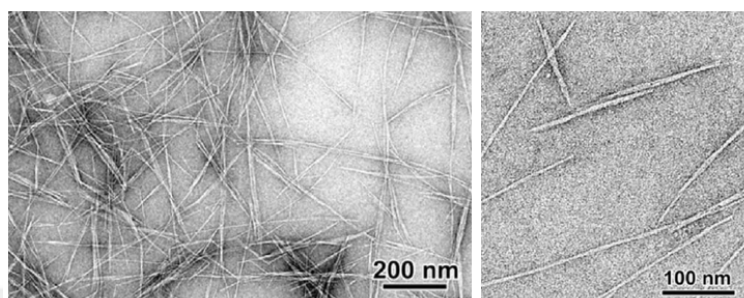


Figure 2.4: TEM Images of CNC Isolated From Soy Hulls [54].

The mechanical properties of cellulose-based micro or nanocomposite materials are reliant on mechanical percolation phenomenon. CNC with high aspect ratio (around 50) has low percolation network concentration. Therefore, at the same concentration, cellulose nano whiskers exhibit better mechanical performances in comparison to other reinforcing components. [55-57] The tensile modulus, which was reported between 56 – 220 GPa, is higher than steel's and similar to Kevlar's. The average value of tensile modulus is 130 GPa. Even though CNC has a low density (its density is around 1.5 – 1.6 g/cm³), its mechanical performance is compared with steel having density of 8.05 g/cm³.

The crystalline nature gives CNC a low thermal expansion coefficient. Thus, CNC has a role as a thermal expansion reducing agent in some nanocomposites up to its degradation temperature.

Interestingly, usage of CNC in optics is also expanding. The dilute suspensions of CNC are isotropic, the concentrated suspensions display cholesteric liquid-crystal behavior. A long-range order of spindle-shaped CNC is observed in semi-concentrated CNC suspensions (around 4 wt. %). Upon evaporation induced self-assembly (EISA) applications, nanocrystals aggregate to form ordered stacks and these stacks become the main component of the resultant iridescent films. [29] Depending on the rate of evaporation, chemical moieties conjugated on CNC surface or the medium CNC dissolved, various birefringence films can be obtained. [58] These films might also be utilized as sensors due to their stimuli-responsive properties. [59]

The isotropic dilute CNC mixtures are classified as shear thinning viscoelastic liquids, whereas complexes having CNC concentration higher than 5 wt. % exhibit gel behavior unless sonication is applied. [60] Low CNC concentrations (below 3 – 4 wt. %) are stable mixtures due to the sulfate ester groups on CNC surface. Owing to these groups, CNC acquires a negative zeta potential causing the formation of stable suspensions. This stability is too susceptible to environmental changes. Even addition of insignificant amounts of long chain molecules or of ionic components might efface this stability and lead the formation of gels irreversibly. The sudden sol-gel transition without any chemical bond formation is useful in numerous applications. Physical CNC-based hydrogels are the products of this alternation. In addition to CNC's fast gelation response, their usage as a rheology modifying agent in cosmetics and pharmaceuticals prove that CNC has exceptional rheological properties.

2.3 *CNC Composites, Mixtures and Their Applications*

Composite materials are produced by the combination of at least two different materials showing different physical and chemical characteristics. By means of this blending, alternative materials possessing major characteristics of all components are developed. Therefore, advances in composite and nanocomposite material technologies allow development of novel smart and active materials. A subgroup of composites of nanocomposites are complexes containing at least one nano sized particle, fiber or structural component in a matrix of metal, polymer or ceramic. The interaction between nano scale material and matrix permits formation of new properties. These properties rely on several diverse factors such as processing methods, size and shape of the nano scale material, quality of dispersion, the orientations of nano scale components, the strength of interaction between components. The abundant and non-toxic nanocellulose components are great candidates for development of innovative materials due to their inherent features. Upon addition of these nanofibers in both natural and synthetic polymer matrices, great advances were observed. Xu et. al. demonstrated that increased CNC concentration in waterborne epoxy resins induced an increase almost 2 times in Young's modulus. The elongation rate of the nanocomposites does also increase via addition of CNC. [61] Kupka et. al. proved that polyurethane – CNC and polyurethane – PEG grafted

CNC nanocomposites show enhanced modulus by 50 % and strength by 25 %. By means of grafting different chemical moieties on CNC surface, solvent free or organic solvent containing processing methods might also be performed as Kupka et. al. performed. The studies about nanocomposites of PVA/CNC, PLA/CNC, PEO/CNC, polyurethane/CNC basically validate that CNC has superior reinforcing filler features. [62] Owing to this fact, CNC is preferred in paper-manufacturing and packaging.

Nanocellulose itself does not reflect electrical properties yet their composites with conductive polymer, ionic liquids or graphene possess electroconductivity. These materials are processed with the chemical methods of doping, blending, or coating. CNC forms the matrix and allows the travel of ions with a controlled pace; therefore, electrical conductivity is achieved. [63] Depending on the chemical structure of CNC or processing methods, different mesh sized matrices are obtained. These alternations change the conductivity performance of the resultant material. Their applications comprise biosensors, energy storage devices, capacitors, electromechanical devices. Hansel et. al. validated that CNC can also be used as single ion conductor in Lithium ion batteries. In their work, evaporation induced self-assembled nanocellulose structures soaked in ethylene carbonate: diethyl carbonate (EC:DEC) did not let the accumulation of Li⁺ ions within the medium. They had broad electrochemical stability range. [64] This recent paper is a good illustration of CNC's prospective role in our lives.

Besides using CNC as a mechanical reinforcing agent in composites, their usage as a rheology modifier is common. CNC's impact on rheology was investigated for several systems. CNC possessing concentrations higher than 0.5 wt. % has a viscosity modifier effect in cement admixtures. [65] Additionally, CNC in cement paste works as a great agent for hydration and flexural strength. [66] Another industry in which rheology modifying is an important aspect is gas and well drilling. The fluids used for these purposes necessitate high shear thinning behavior to remove and suspend the cuttings of the well and seal the well wall with a good stability. Even though bentonite containing water-based muds are widely accepted in drilling processes, they can form thicker filter cakes and harm the well. Li et. al. suggested CNC as a rheology modifying agent for these applications and they showed that CNC containing water-based muds were better in filtration. [67] Non-toxicity of CNC enlarges the extent of CNC applications in cosmetics. They can be utilized as a both reinforcer and scaffold material. Even the bulk cellulose derivatives of hydroxyethyl cellulose and hydroxypropyl methyl cellulose are the main

ingredients of commercially available thixotropic agents, which are largely utilized in cosmetics, of Liporamnosan, Natrosol 250 MR and Methocell M312. [68]

There are numerous review papers about CNC utilization in biomedical applications. Especially combinations of CNC with biodegradable natural polymers of starch, alginate, chitosan permit development of biomedical devices specialized for drug delivery, wound dressing and tissue engineering. The invention of hydrogels, which is traced back to the 1960s, is a ground-breaking point in biomedical. Human body contains water up to 60 wt. %. Therefore, it is not abnormal to regard the human body as a giant, complex hydrogel. Any laboratory grown tissue material or tissue-resembling drug carrier should contain right amount of water to achieve material-body compatibility. The work of Mendes et. al. proves that platelet lysate hydrogels used in regenerative medicine can have improved porosity and significantly improved mechanical features (around 100 times increased Young's modulus) upon addition of 0.61 wt. % CNC without any increase in toxicity. [69, 70] Sampath et. al.'s findings about CNC's impact on semi-interpenetrating chitosan hydrogels is clear. The resultant hydrogels become more elastic and mimic human tissue better. [71] CNC/alginate [72, 73] and CNC/gelatin [74] hydrogels used as drug carriers exhibit better stability, pH insensitivity and controlled drug release, which is an aimed task in treatments. CNC-based or supported hydrogels have many examples. Depending on the CNC crosslinking methods, physical or chemical hydrogels useful for medical, household or industrial purposes can be obtained. Development of CNC-based hydrogels necessitate great understanding of the rheology of CNC water suspensions as CNC dispersion in water is the very first step in hydrogel fabrication.

2.3.1 Aqueous CNC Suspensions

CNC is isolated from bulk cellulose generally by sulfuric acid treatment taking place in aqueous medium. Following the treatment, nanocellulose crystals constitute stable aqueous CNC suspensions due to negative sulfate ester groups on the CNC surface. These suspensions are sometimes dried and CNC powders ready-for-dispersing are obtained. Depending on the dispersed concentration, CNC suspensions can display isotropic, chiral nematic liquid crystal and gel-like phases. To utilize CNC as a reinforcing or structural

material, it is necessary to begin the process by dispersing it in water. Therefore, rheology of aqueous CNC suspensions should be understood. Morphology of the crystals, groups on the surface, concentration and temperature are determining factors. However, it is eligible to express all CNC suspension as shear-thinning non-Newtonian fluids.

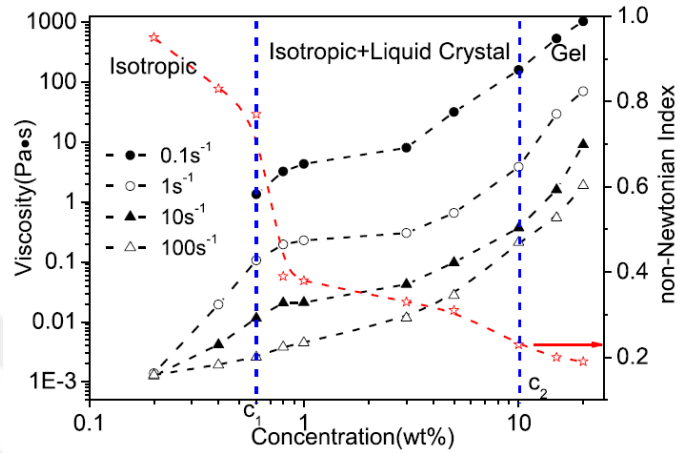


Figure 2.5: Apparent Viscosity and Non-Newtonian Index versus CNC (with Aspect Ratio of 13) Concentration [75].

According to Qiao's work [75], suspensions having low CNC concentrations tend to exhibit Newtonian behavior. The first critical CNC concentration shown as c_1 in Figure 2.5 is the overlapping concentration of CNC. This number is a function of aspect ratio of single crystals. The critical overlapping concentration is calculated by:

$$c^* = \frac{\rho \pi d^2}{4L^2} \quad (2.1)$$

The density is 1.52 g/cm^3 [76] and the aspect ratio is 13 [75]. Overlapping concentration becomes $7.06 \times 10^{-3} \text{ g/cm}^3$, which is roughly equal to 0.7 wt %. Alternatively, the rate of change of non-Newtonian index versus concentration can be used to find both the first and the second critical concentrations. It is possible to see isotropic, biphasic and gel phases occurring at distinct concentrations for different sourced CNC. Wu and coworkers [76] demonstrated that the biphasic range occurs between 0.4 wt. – 1.5 wt. % for switchgrass-based CNC and 1 wt. – 3 wt. % for cotton-based CNC.

The viscosity of CNC suspensions is determined by CNC concentration. At low shear rates, the effect of CNC is more substantial. As the shear rate increases, alignment of crystals eases the flow and causes a reduction in viscosity. Shafiei-Sabet et. al.'s work [60] expresses that the unsonicated suspensions having 5 wt. and 7 wt. % CNC have signs of gel formation. 2 – 4 wt. % CNC containing suspensions have viscosity profiles including three distinct regions resemble liquid crystal behavior. [77]

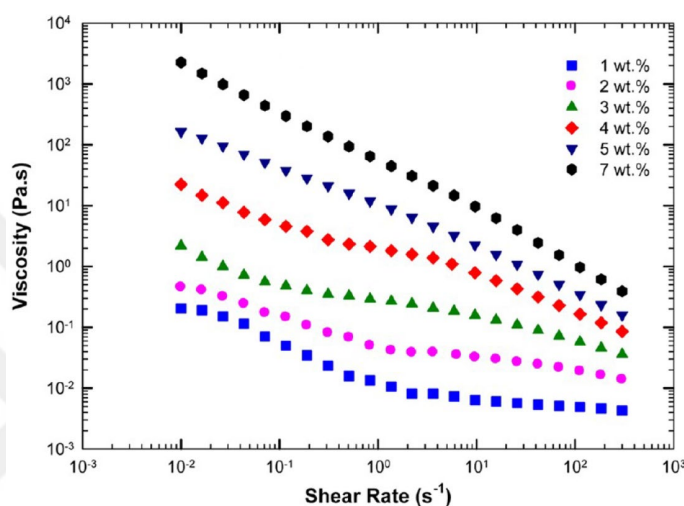


Figure 2.6: Viscosity Profile of CNC Suspensions (1, 2, 3, 4, 5, and 7 wt. %) [60].

2.3.2 CNC Hydrogels

Hydrogels are polymeric networks able to retain water up to 90 %. The water retention capacity stems from the hydrophilic nature of the constituents. The natural polymer of cellulose and more specifically its crystalline regions of CNC are excellent building blocks for hydrogels. The hydroxyl groups on cellulose increases the water affinity. The stiffness of crystalline regions allows the formation of 3-D network.

Physical CNC Hydrogels

Neat CNC suspensions displaying liquid-crystal behavior can undergo sol-gel transition easily by addition of small amounts of salts, surfactants, or polymers. Dilute cellulose nanocrystals suspensions are regarded as polymeric solutions. Electrostatic repulsion between single crystals controls the suspension stability. However, further addition of CNC reduces the electrostatic repulsion between the crystals. At this level, a

component acting as a crosslinking agent or eliminating the remaining repulsions provokes the gel formation. Gels formed because of these types of physical interactions and entanglements of polymers called as physical gels. Covalent bonding does not take place in physical gel's gelation.

Fundamentally, salts affect the electrostatic repulsion and induce phase transition. Chau et. al. [78] and coworkers investigated the gelation of aqueous CNC suspensions facilitated by ions. In their work, they showed that addition of cations reduced the electrostatic screening effect and shortened the Debye length of nanocrystals. Attractive forces of van der Waals and hydrogen bonding started to dominate owing to the diminished electrostatic repulsion between nanocrystals. Cation's charge number, solubility of the salt and size of the cation are directly effective in the resultant gel's stiffness and mesh size. These findings show agreement in DLVO theory. [79] Besides Chau et. al.'s study, the effect of cations in CNC gelation were analyzed by Xuan et. al. [80] and Lenfant et. al. [81]. An excellent instance of physical and stimuli responsive gelation was shown by Way et. al. [30] In their work, amine functionalized CNC can be dispersed at low pH whereas CNC aggregation or in other words gelation is observed at higher pH. The reverse was seen for carboxylic acid functionalized CNC.

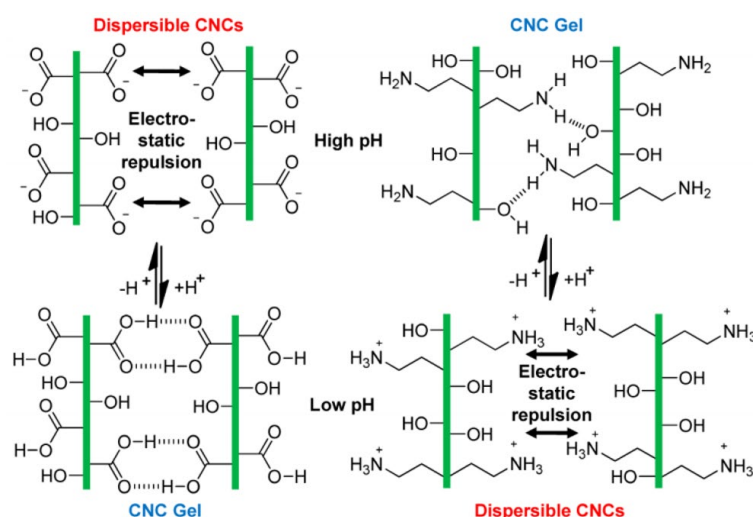


Figure 2.7: Mechanism of pH-responsive Gelation of Carboxylic Acid and Amine Functionalized CNC [30].

Surfactants, especially ionic ones, are also used for creating physical CNC hydrogels. The attraction between ionic groups of surfactants and nanocellulose surface lessens

electrostatic screening between single rods as salt molecules do. Tardy and coworkers' [31] review takes these interactions into consideration classifying the surfactants as cationic, anionic, and, non-ionic. The work summarizes CNC-surfactant chemistry as the summation of surfactant structure, electrostatics, hydrophilicity and concludes that CNC-surface complexes might have limitless possibilities. In addition to electrostatic effect of ionic groups, the steric repulsions between long hydrophobic surfactant chains take role in physical CNC hydrogel creation. Dhar et. al.'s work [82] investigated nanocellulose and tetradecyl trimethyl ammonium bromide (TTAC) affinity by interpreting isothermal calorimetric titration (ITC) experiments and they proposed a mechanism for CNC gelation upon addition of cationic TTAC surfactant. A similar gelation mechanism was also proposed by Hossain et. al. [32]

Processing method performed on aqueous CNC suspension might also be adequate to obtain physical CNC hydrogels. In the study of Lewis et. al. [28], freeze-thawing method was applied on 1 wt. %, 2 wt. %, 3 wt. %, and 4 wt. % aqueous CNC suspensions and hydrogels with differing complex modulus were acquired. Freezing induces aggregation of nanocellulose crystals due to formation of ice crystals. Each freeze-thawing cycle creates more rigid CNC aggregates. Consequently, more rigid crystal aggregates generate strict scaffolds and therefore hydrogel.

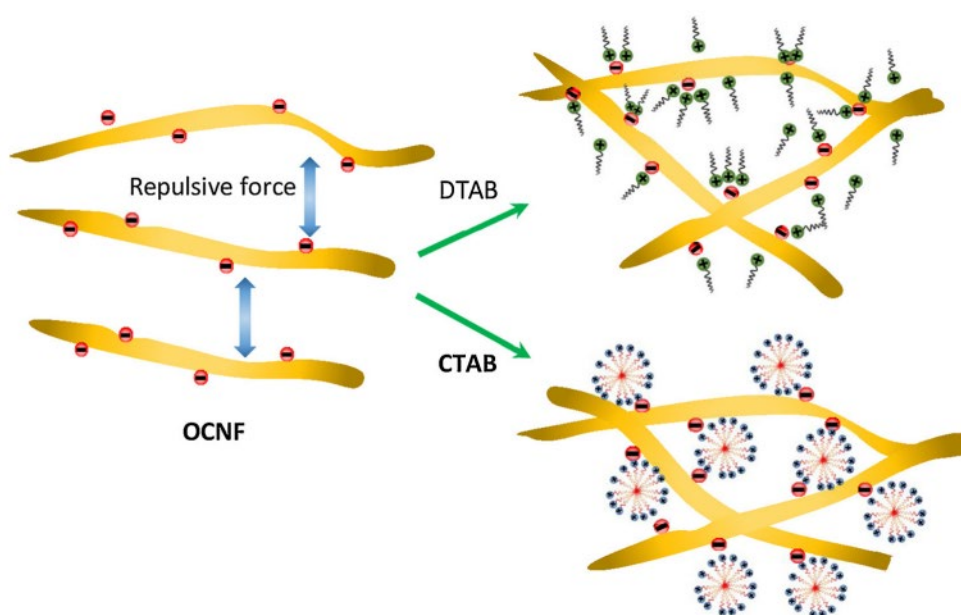


Figure 2.8: Cationic Surfactant Driven Gelation Mechanism (Interaction between Tempo-Oxidized CNC and Cationic Surfactants of CTAB and DTAB) [32].

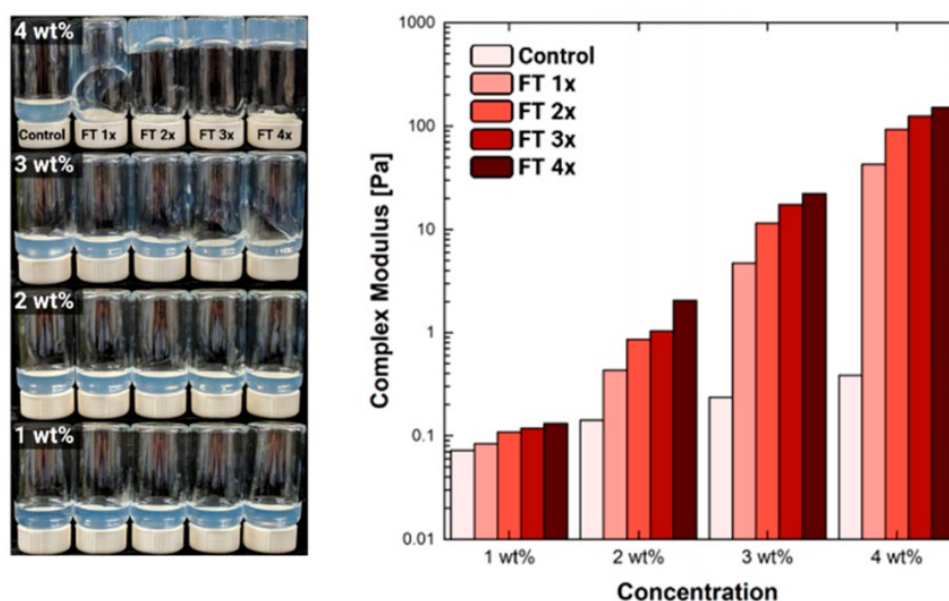


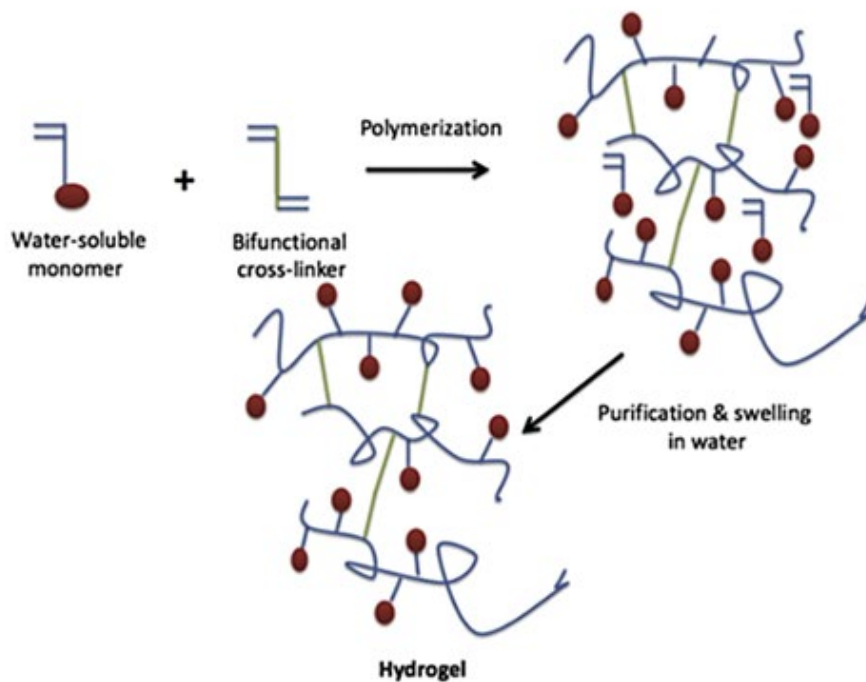
Figure 2.9: Photos of Freeze-Thawed Aqueous CNC Suspensions and Their Complex Modulus vs Concentration Graph (FT #x: Number of Freeze-Thaw Cycle) [28].

Chemical CNC Hydrogels

Hydrogels having scaffold elements connected one another with covalent bonding are called chemical or chemically crosslinked hydrogels. These hydrogels are usually stiffer than physical hydrogels; however, they are less responsive to environmental stimuli. To obtain chemical CNC hydrogels, it is necessary to chemically modify the isolated single crystals. The chemically modified crystals can be crosslinked by either polymerization of hydrophilic monomers on the surface or direct crosslinking of water-soluble polymer chain. These hydrogels are also called nanocomposites. [83] Depending on the crosslinking density, the stiffness and swelling ratio of these materials might be altered. Any chemical modification allowing the covalent bond formation between CNC surface and crosslinking monomer or polymer can be implemented.

In the study of Yang et al. [84], silane treatment was performed on CNC prior to free radical polymerization on the surface. After the treatment, polymerization of acrylamide started to come about with the addition of initiator. The resultant flexible and resilient CNC hydrogel had 20 – 30 % swelling ratio.

(a)



(b)

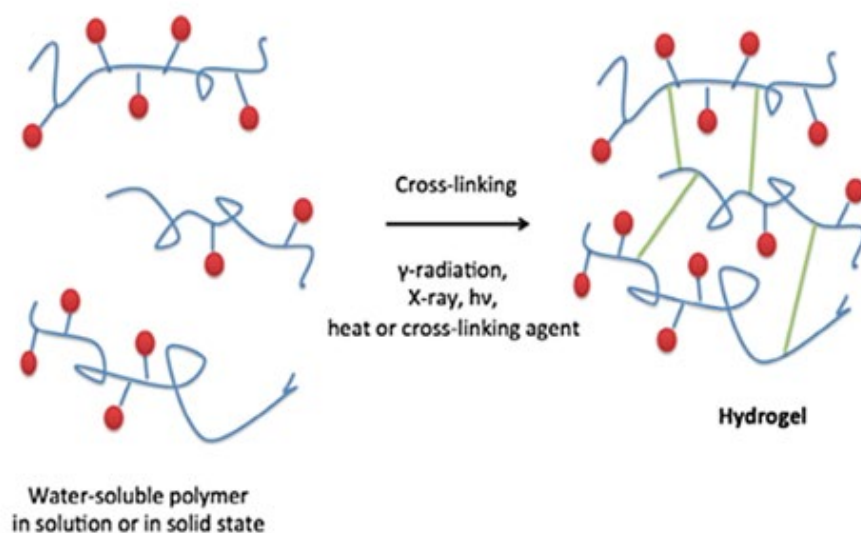


Figure 2.10: Chemically Crosslinked Hydrogel Synthesis. (a) Polymerization of Water-Soluble Monomer (b) Direct Polymerization of Water-Soluble Polymer [83, 85].

In Xu et. al.'s work [86], CNC was firstly functionalized with aldehyde groups. The natural polymer of chitosan was used as crosslinker, there was not a polymerization

reaction. The Schiff base reaction occurring between amine groups of chitosan and aldehyde groups of CNC allow the formation of bio-orthogonal drug delivering CNC/chitosan hydrogels.

Composites	CNC (%)	CS (%)	CNC:CS
CNC/CS1	51.0	49.0	1:1
CNC/CS2	33.0	67.0	1:2
CNC/CS3	20.2	79.8	1:4

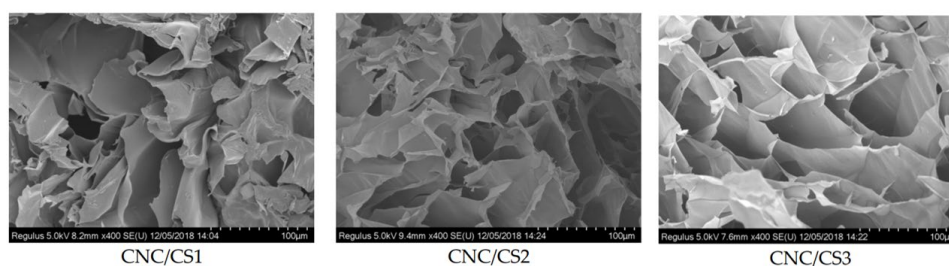


Figure 2.11: FE-SEM Images of CNC/Chitosan Hydrogels [86].

2.4 Surfactants

Surfactants are elements changing the free energy of surfaces and interfaces even used in low concentrations. These molecules are made of both hydrophilic and hydrophobic parts. Hydrophobic part is generally a long hydrocarbon, and rarely ring structure or siloxane chain. [87] Hydrophilic part is the ionic or highly polar side of the molecule. In aqueous solutions, surfactants alter the hydrogen bonding between the water molecules and are pushed to the phase boundaries. As the interaction between water molecules and hydrophobic part is not entropically favorable, the self-aggregation of surfactants take place. The most investigated form of these self-aggregates is micelles. They might be spherical, ellipsoid or cylindrical. Aside from micelles, monolayer and bilayer vesicles can be formed upon aggregation of surfactants. These structures start to form when the concentration of surfactant molecules exceeds a certain concentration called critical micelle concentration (CMC) at temperatures higher than Krafft temperature. Upon formation of micelles, the energy of system decreases due to the formation of these crystal-like forms. Micelle concentration and free surfactant

concentration are in equilibrium. The association features of surfactants differ depending kind of hydrophobic and hydrophilic parts of molecules, pH, ionic strength, and temperature of environment. The number of molecules forming micelles are usually in the range 50 – 100 molecules. [87]

The size and shape of self-aggregates are crudely determined by critical packing parameter, P_c . To calculate this parameter, volume of hydrophobic chain v , length of full extended hydrophobic tail l_c in a fluid environment, and minimum interfacial area of hydrophilic head a_0 must be known. [87]



Figure 2.12: Structure of A Surfactant Molecule with Parameters of v , l_c , a_0 [87].

$$P_c = \frac{v}{l_c a_0} \quad (2.2)$$

Critical packing parameter	Approximate molecular shape	Expected aggregate structure
<0.33		(Normal)
0.33 - 0.5		
0.5 - 1.0		
1.0		
>1.0		(Inverted)

Figure 2.13: Critical Packing Parameter and Expected Aggregate Structures [87].

Surfactants are typical used in aqueous systems and therefore, their classification is based on the type of hydrophilic group. The surfactants are classified as anionic, cationic, non-ionic and zwitterionic. Anionic and cationic names come from the charge of the surface-active section. Non-ionic surfactants do not have charge on surface-active

portion, yet these surfactants have highly polar heads. The polarity is generally given by poly(oxyethylene) or sugar groups. Zwitterionic ones possess both positive and negative charges on the head section.

Table 2.1: Surfactant Types and Examples.

Surf. Type	Molecular Structure	Examples
Anionic		Sodium Dodecyl Sulfate (SDS), Sodium Lauroyl Sarcosinate (SLAS)
Cationic		Dodecyl Trimethylammonium Bromide (DTAB), 1-Decyl-3-Methylimidazolium Chloride
Non-ionic		Pluronics, Polioksietilen Sorbitan Monooleat (Tween 80)
Zwitterionic		3- (N, N-Dimethylmyristylammonio) Propanesulfonate (DMAPS)

2.5 CNC – Surfactant Mixtures

CNC is a superior material as a structural component, there is a necessity to modify the CNC surface by chemical modifications for further applications. The environmental cost of chemical modifications might sometimes not be tolerable. Therefore, physical methods should be utilized. Surfactants are great candidates for physically modifying the CNC surface and recent studies about CNC – surfactant interactions prove that they possess tremendous potential in novel and smart material development. These interactions and applications are assessed taking the surfactant type as basis.

2.5.1 Anionic Surfactant – CNC Mixtures

Anionic surfactants basically have alkyl sulfate and carboxylate groups. Detergents and cosmetic products generally contain this kind of surfactants such as SDS, SLAS, SLES. Both CNC and anionic surfactants are negatively charged. Hence, the interaction between these two is electrostatically not favorable at all. There are two interaction mechanisms suggested for anionic surfactant adsorption on the surface. The first mechanism suggests that hydrophobic chain of the surfactant adsorbs on the hydrophobic parts of the CNC. The second mechanism asserts that the positively charged counterion or a cation belonging to the electrolyte behaves like a bridge between negative head group and negatively charged CNC surface. According to Ranjbar et. al. [33], suspension stability is achieved by the summation of these two mechanisms. As the adsorption of anionic surfactants is limited, more anionic surfactants is required for sol-gel transition in comparison to non-ionic and cationic ones. [34]

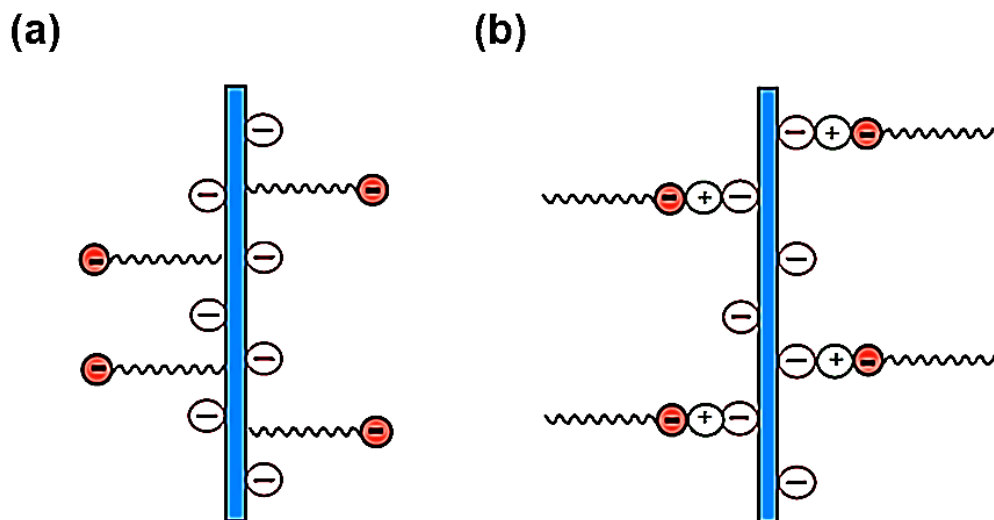


Figure 2.14: Schematic of Anionic Surfactant – CNC Interaction Mechanism. (a) Hydrophobic Interaction (b) Cation Bridging [33].

Another research comparing the adsorption of anionic sodium dodecylbenzene sulfonate (SDBS) and non-ionic Triton X-100 (TX-100) surfactants on secondary fiber structures proposes a similar mechanism for SDBS. [31] Additionally, micellization of both anionic and non-ionic surfactants does not favor adsorption. [88]

2.5.2 Cationic Surfactant – CNC Mixtures

There are negatively charged sulfate ester groups on CNC due to the prior sulfuric acid treatment. Therefore, it is expected to see more strong affinity between cationic surfactants and CNC. The most prevalent interaction in these mixtures is electrostatic interaction as shown by several papers. [31-33, 89] Owing to the predominant mechanism, concentration of salt, pH and counterion are also decisive in the resultant's rheological characteristic. According to the findings of Prathapan et. al. [89], firstly positively charged surfactants adsorb and hemimicelles form on the surface. Followingly, hydrophobic attractions take place and free surfactant monomers are attracted to surfactants extended from the surface. These aggregates on CNC are called admicelle. Their existence was understood through zeta potential measurements. [32, 89] Further addition of surfactants results in formation of free micelle. It is probable to observe sol-gel transition even with the addition of cationic surfactants in small amounts, however extra surfactant addition might disturb the adsorption of surfactants.

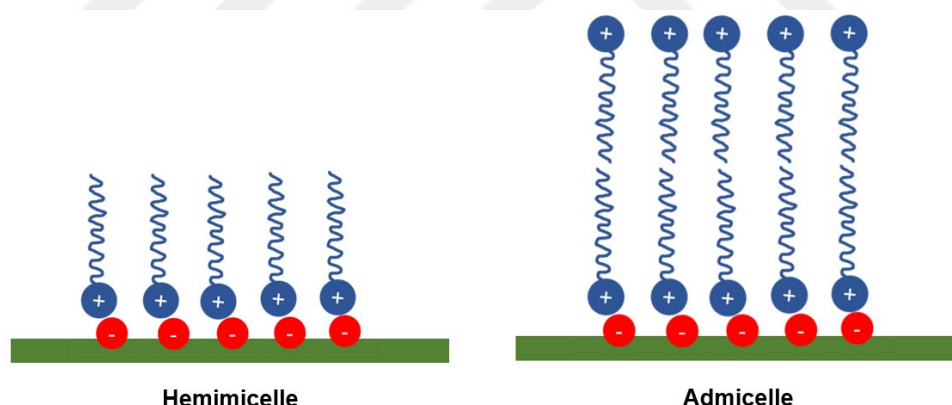


Figure 2.15: Hemimicelle and Admicelle Formation on CNC.

2.5.3 Non-ionic Surfactant – CNC Mixtures

Non-ionic surfactants possess hydrophilic ethoxy groups besides long hydrocarbon chains. Quennouz et. al.'s work [34] investigated the cellulose nanofibrils (CNF) – Pluronic F68 and TX-100 suspensions separately. The mixtures containing 0.6 wt. % CNF were analyzed for both non-ionic and anionic surfactants. Storage modulus of SLES containing ones were as much as 10 times higher than Pluronic F68 and TX-100

containing mixtures for the same surfactant amount. The attraction of non-ionic surfactants to CNC is gained through ethoxylated hydrophilic head. Even though there is a strong adsorption inclination at low surfactant concentrations, not further adsorption is monitored above CMC. [90]

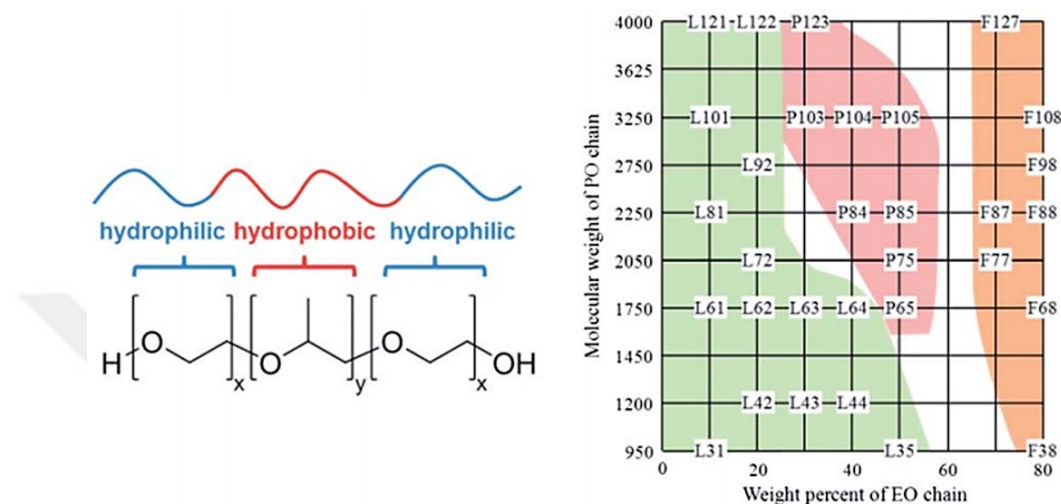


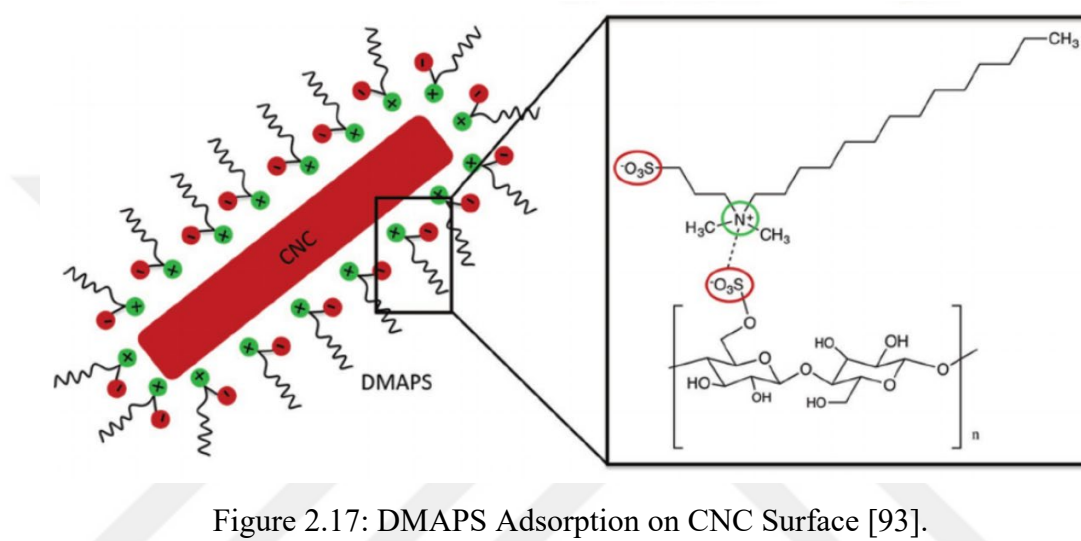
Figure 2.16: Pluronic Molecular Structure and Map of All Pluronics with Compositions (Colors symbolizes the phase: Green - Liquid, Red - Paste, Orange - Solid) [91].

Amphiphilic triblock copolymers – which are commercialized with Pluronics name – are also regarded non-ionic surfactants. Pluronics have the molecular structure as $[\text{PEO}]_x - [\text{PPO}]_y - [\text{PEO}]_y$. These synthetic polymers are capable of undergo temperature induced self-aggregation. Depending on the number of both hydrophilic monomer $[\text{PEO}]$ and hydrophobic monomer $[\text{PPO}]$, these long chains form self-aggregates shaped ranging from micelles to vesicles. Emami and coworkers [92] wanted to utilize Pluronic L61 and L121 as an agent allowing dispersion of CNC in epoxy matrix. Their work is also a good example for proving the affinity between ethoxylated groups and CNC.

2.5.4 Zwitterionic Surfactant – CNC Mixtures

Zwitterionic (amphoteric) surfactants have both anionic and cationic groups on the hydrophilic head. The cationic group is generally quaternary ammonium, and the anionic groups can be phosphate, sulfate, or carboxylate. In Guidetti et. al.'s work [93], adsorption of 3- (N, N-Dimethylmyristylammonio) Propanesulfonate (DMAPS) on CNC surface

was desired to produce flexible CNC films. Interaction mechanism between zwitterionic surfactant and CNC is the same of the one between cationic surfactant and CNC. The adsorption of DMAPS gave viscoelasticity to the CNC film due to long hydrocarbon chains and resumed the negative zeta potential of the suspension prior to evaporation. By changing quantity of surfactant or pH of the mixture, iridescent films with different flexibility were obtained following EISA.



Chapter 3:

SURFACTANT DRIVEN GELATION OF CELLULOSE NANOCRYSTAL SUSPENSIONS

3.1 *Introduction*

The ever-growing demand for renewable nanomaterials with tailorable mechanical characteristics has increased attraction towards cellulose nanocrystals (CNC). [45] The most abundant natural polymer of cellulose possesses both highly ordered (crystalline) and slightly disordered (amorphous) regions with differing ratios based on the source. Following the mechanical, chemical or enzymatic treatments, highly ordered crystalline regions, which constitute 25 - 75 % of the bulk cellulose, [61] called as CNC are isolated. These needle-like structures owe their structural integrity to strong intermolecular and intramolecular forces between fibrils, [94] their sizes differ 2 - 70 nm in width and 100 nm to several micrometers in length. [95] Owing to their crystallinity and surface chemistry, they possess superior mechanical features [96], exceptional optical characteristics [97], ultrafast ionic conduction [98] and unique thermal behavior. [99] Besides these intriguing features, they are biocompatible, environmentally friendly, lightweight and eligible for surface modifications. Therefore, they are excellent alternatives for both petroleum-based and ceramic-based nanomaterials.

CNC is a water-insoluble, hydrophilic nanomaterial whose aqueous suspensions are isotropic and stable up to concentrations around 5 % by weight. [60, 76] Due to significant number of hydroxyl groups, CNC disperse well in water and the stability is sustained by the negative surface ester groups on the surface. [100, 101] Additionally, larger surface area with chemical moieties enables further surface modifications such as esterification [102, 103], cationization [104], acetylation [105] and polymer grafting [106], which permits us to tune viscoelastic behavior and add stimuli-responsivity to the nanomaterial. Neat CNC suspensions' phase behavior, rheological properties and gelation are well studied and understood. [28, 107] However, aqueous CNC mixtures comprising competing interactions caused by the presence of salts, polymers, and surfactants are not fully reviewed. The assessment of complex CNC mixtures gains great importance as the

addition of chemicals might have strong influence on both phase behavior and rheological properties.

Several studies showed that addition of salt to neat CNC suspensions distorts the suspension's stability as positive ions of salt electrostatically interact with negative sulfate ester groups. [80, 81] Cation's size, ion charge, ion amount and even ion pair dissociation have impacts in sol-gel transition of CNC suspensions. Peddireddy and coworkers demonstrated that higher salt concentrations lead to shorter gel time. [108] Furthermore, addition of non-ionic polymers and surfactants might also trigger phase transition as they lead to bridging between CNC owing to hydrophilic and hydrophobic interactions. [109] Due to the reversible nature of physical interactions, numerous studies have concentrated on creating stimuli-responsive physical CNC-based hydrogels. Based on their findings, especially cationic surfactants are great macro molecules for inducing physical gelation as they can manipulate both electrostatic and hydrophobic interactions within the suspension. They are also responsive to external stimuli such as temperature and concentration. At low concentrations, cationic surfactants' positively charged head groups bound to negatively charged sulfate ester groups and hydrophobic tails stretching out of surface interact with adjacent surfactant groups adhered on CNC surface. If the number of surfactant-coated CNC is adequate to constitute a network, gelation is observed. Several studies done about dodecyl trimethyl ammonium bromide (DTAB) and cetyl trimethyl ammonium bromide (CTAB) and CNC interactions proved an electrostatically bound surfactant layer via observing a decrease in surface zeta potential's absolute value with the addition of surfactants. [93] At intermediate concentrations, at which surfactants starts to form self-assemblies called micelles, CNC network with various strengths form due to micelle bridging. The strength of the network is strongly related to charge, size and the number of micelles. At high concentration far from critical micelle concentrations (CMC), free micellization takes place in the complex CNC mixture, their formation does not impose a significant rheological effect on the mixture. Quennouz et. al. studied the interaction between cellulose nanofibrils (CNF) and non-ionic (Pluronic F68, TX100) and ionic (DTAB, SDS) surfactants. Dynamic light scattering experiments allowed them to observe the decay of autocorrelation function and variations in CNF sizes. [34] A micelle driven bridging mechanism was proposed by them suggesting that cationic micelles caused stronger networks. Ranjbar et. al. assessed the effect of anionic surfactant on CNC suspensions' phase transition and they found that the

responsible mechanism for gelation was both counterion bridging and hydrophobic interactions between CNC' hydrophobic sides and surfactant tails. Their findings are also great proofs that anionic surfactant CNC interactions are not as favorable as cationic surfactant CNC interactions to achieve phase transition. [33]

Although numerous studies about CNC surfactant interactions express the effect of surfactant charge on gelation, they missed to analyze the effect of micelle charge on sol-gel transition. The previous studies comprise effect of micelles in sol-gel transition, yet this effect has not been systematically analyzed and therefore, concluding micelles' effect based on the previous studies' proposals might not be correct. We used small angle neutron scattering (SANS), dynamic light scattering (DLS), and rheology to assess the effect of cationic 1-decyl trimethyl imidazolium chloride $[C_{10}mim][Cl]$ and 1-decyl trimethyl imidazolium ferric tetrachloride $[C_{10}mim][FeCl_4]$ on aqueous CNC suspension's rheological behavior and gelation. These surfactants have the same chemical structure but different counterions. Even though both CMCs and the shape of the micelles of these surfactants are identical, they exhibit distinct inter-micelle interactions due to presence of different counterions. This allows us to clarify the role of micelles charge in gel's structure and rheology.

3.2 *Materials and Synthesis*

Nanocrystalline cellulose in spray dried form was provided by Celluforce (Montreal, Canada). The chemical name of the product is expressed as cellulose hydrogen sulphate sodium salt in the product safety data sheet. As reported by the supplier, overall crystalline density is 0.88 (XRD) and hydrodynamic diameter is 70 nm (DLS). 1-decyl-3-methyl imidazolium chloride ($[C_{10}mim][Cl]$) with 96 % purity and iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Reagent grade) with 98 % purity were purchased from Sigma-Aldrich (St. Louis, MO).

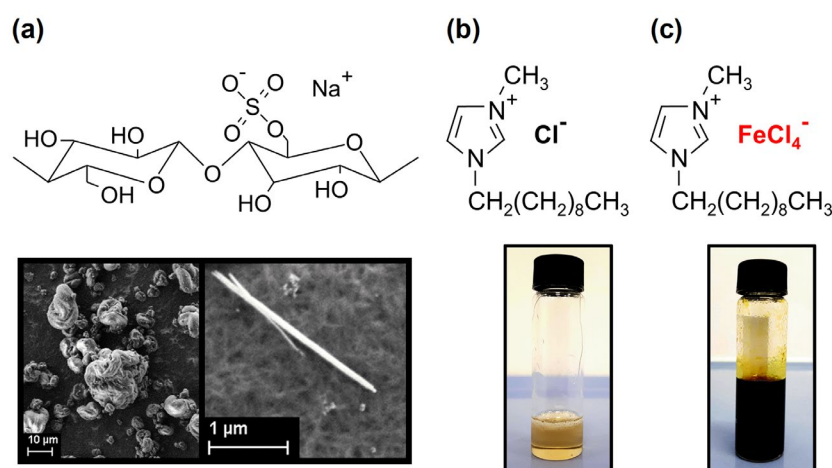


Figure 3.1: Materials (a) Molecular Structure of CNC and SEM Image of Spray Dried and Dispersed Forms (b) Molecular Structure of 1-Decyl-3-Methyl Imidazolium Chloride (c) Molecular Structure of 1-Decyl-3-Methyl Imidazolium Tetrachloroferrate.

Magnetic ionic liquid of 1-decyl-3-methyl imidazolium tetrachloroferrate ($[\text{C}_{10}\text{mim}][\text{FeCl}_4]$) was synthesized following the protocol of Brown et.al. [35]. Equimolar ionic liquid of 1-decyl-3-methyl imidazolium chloride and ferric chloride hexahydrate were mixed in ethanol at 25°C for 12 hours. After allowing the evaporation of ethanol in fume hood at room temperature, the mixture was placed in vacuum oven to remove all residual ethanol. Brown-colored 1-decyl-3-methyl imidazolium tetrachloroferrate was obtained after leaving the mixture in vacuum oven at 80°C , full vacuum for 12 hours.

3.3 Experimental Methods

3.3.1 Electrical Conductivity Measurements

Conductometry was used for finding critical micelle concentration (CMC) of both $[\text{C}_{10}\text{mim}][\text{Cl}]$ and $[\text{C}_{10}\text{mim}][\text{FeCl}_4]$ ionic liquids. The rate of change of electrical conductivity with respect to concentration was monitored by using ISOLAB pH, mV, conductivity, TDS meter. Before each measurement, the meter was calibrated with its calibration solution. Measurements were carried out at least three times at every single point ensuring that temperature is $24 \pm 1^\circ\text{C}$.

3.3.2 Hydrodynamic Size and Zeta Potential Measurements

Both hydrodynamic sizes and zeta potentials were measured by using Malvern ZS Zetasizer. All measurements were carried out at 25°C. The concentration of CNC suspension was 1 mg/ml. Surfactant concentrations were set to a value higher than their CMCs. The concentration of both [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] aqueous solutions were 100 mM. Measurements were performed 4 times for each sample.

3.3.3 Small Angle Neutron Scattering (SANS) Experiments

SANS experiments were completed on NGB 30 SANS beamline at the NIST Center for Neutron Research (NCNR). Surfactants of [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] were mixed with deuterium oxide (D₂O) with surfactant concentrations ranging from 5 to 194 Mm. Mixtures were rested overnight after loading them into 1 mm-thick titanium cells with quartz window. The analyses were completed at sample-to-detector difference of 1 – 4 m, covering the wavevector ($Q = 0.01$ to $0.5 \text{ \AA}^{-1}$) at 25 °C. 1-D isotropic absolute intensity profile was gained after correcting the scattering intensity for background, empty cell, and sample transmission. Igor Pro software was used for data analysis.

3.3.4 Sol-Gel Transition Experiments

To assess the sol-gel transition, widely recognized method of tube inversion was implemented. Before developing CNC – surfactant mixtures, aqueous stock solutions of CNC, [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] were separately prepared. 5 % w/v CNC solution was prepared by adding 5 gram of spray dried CNC powder on stirred 100 ml water slowly (the rate of addition is 0.2 gram/minute) obeying the manufacturer's protocol. The aqueous suspension was mixed overnight to obtain full homogeneity. 50 ml, 500 mM [C₁₀mim][Cl] and 50 ml, 500 mM [C₁₀mim][FeCl₄] stock solutions were prepared. These stock solutions were mixed with various ratios to obtain 1 ml of CNC – surfactant complexes. Finally, 12 CNC – [C₁₀mim][Cl] and 12 CNC – [C₁₀mim][FeCl₄] complexes (mixtures) were obtained. Each mixture was swirled for 20 seconds and inverted. If no flow is recorded, sample is recorded as gel; if flow is recorded, sample is recorded as solution.

3.3.5 *Rheology Experiments*

Oscillatory rheology experiments were performed on a Malvern Pro Kinexus rheometer. Frequency and amplitude sweep measurements were performed only on gel samples at 25 °C. These samples were placed between parallel plates. The diameter of the upper one was either 20 mm or 40 mm depending on the sample amount. Initially, amplitude sweep tests were performed at both 0.1 Hz and 100 Hz separately to find the upper bound (the highest strain value in the linear viscoelastic region) of the linear viscoelastic region (LVER). These experiments were stopped soon after the boundary of LVER had been discovered to eliminate further damage on gels. If the upper bound values were different among these frequency values. The smaller one was preferred for the frequency sweep experiments taking place between 0.1 Hz and 100 Hz. Frequency sweep experiments allowed us to confirm that investigated samples are gel. Followingly, amplitude sweep experiments were performed at 10 Hz between 0.01 and 100 % strain using the same equipment.

3.3.6 *Scanning Electron Microscopy (SEM) Imaging*

Prior to SEM imaging, solution samples were dried on a carbon grid at room temperature in full vacuum overnight. Gel samples were dried at room temperature in full vacuum for 15 minutes priori. Samples were not coated. Images were taken by using Zeiss Ultra Plus Field Emission SEM operated at 1 kV, 2 kV and 10 kV.

3.4 *Results and Discussion*

3.4.1 *Critical Micelle Concentration (CMC) Determination*

The increase in surfactant amount causes the formation of self-assembled surfactant aggregates in aqueous solutions. Due to formation of these macro-molecules, the physical features (turbidity, electrical conductivity, surface properties) of these solutions start to change. In our work, we observed the change of electrical conductivity with respect to surfactant concentration. Addition of surfactants leads to an increase in the electrical conductivity with a constant rate until CMC. After micellization starts to take place,

composition of solution and therefore electrical conductivity of solution change. [110] The point where conductivity starts to increase with a different rate is designated as CMC.

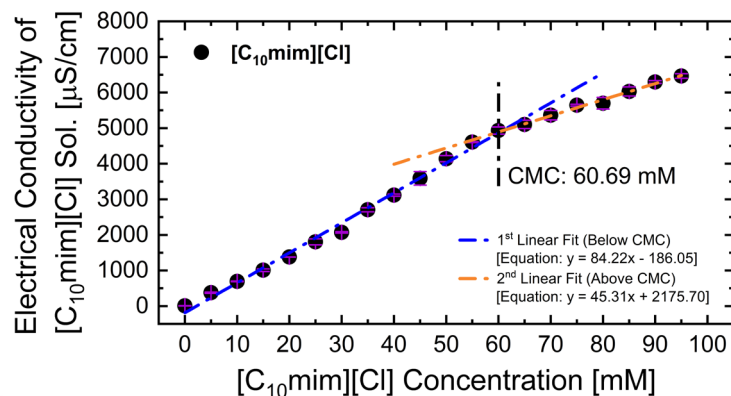


Figure 3.2: Finding CMC of $[C_{10}mim][Cl]$ - Electrical Conductivity versus Concentration.

As seen in Figure 3.2, two distinct lines were fitted for both concentrations below CMC and above CMC. The intersection of these two lines is the CMC of $[C_{10}mim][Cl]$, which is 60.90 ± 2.5 mM, which is in great agreement with literature. [111]

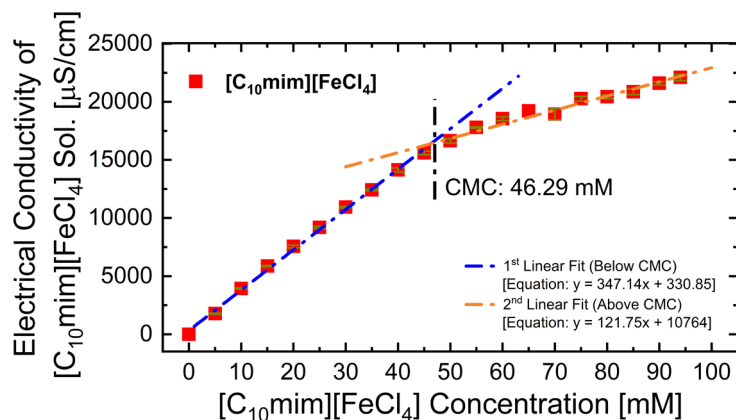


Figure 3.3: Finding CMC of $[C_{10}mim][FeCl_4]$ - Electrical Conductivity versus Concentration.

As seen in Figure 3.3, two distinct lines were fitted for both concentrations below CMC and above CMC. The intersection of these two lines is the CMC of

[C₁₀mim][FeCl₄], which is 46.29 ± 2.5 mM, which is found as 40.6 mM by Brown et. al. [112]

3.4.2 Dynamic Light Scattering (DLS) Analysis

Dynamic light scattering allows us to find the size distribution and surface zeta potential of small particles. The number weighted size distribution of single cellulose nanorods, and [C₁₀mim][Cl], [C₁₀mim][FeCl₄] micelles was demonstrated in Figure 3.4. Since CNC has a rod-like shape, its hydrodynamic size distribution is broader than spherical or ellipsoid micellar structures. Both ionic liquids (surfactants) have similar hydrodynamic size distributions. The slight shift observed for [C₁₀mim][FeCl₄] micelles in hydrodynamic size distribution graph might be related to the size of negative [FeCl₄] counterion. Its thermochemical radius is 0.317 ± 0.019 nm whereas the negative [Cl] counterion's is 0.168 ± 0.019 nm. [113] The number mean sizes of [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] micelles are 1.54 ± 0.04 nm and 1.89 ± 0.01 nm, respectively. CNC's number mean hydrodynamic size is measured as 35.12 ± 11.53 nm, which was reported as 44 – 108 nm in length, L , and 2.3 – 4.5 nm in diameter, d , (having an aspect ratio, L/d , around 20) in manufacturer's report using AFM. This discrepancy between the measurement and manufacturer's report is ascribed to non-spherical shape of CNC.

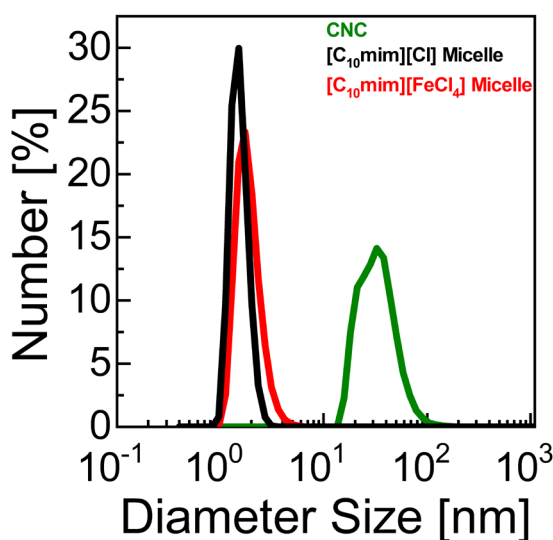


Figure 3.4: Number Weighted Distribution of Hydrodynamic Sizes of CNC, Surfactant Micelles.

Knowing surface zeta potentials of these small particles allows us to interpret the colloidal stability and electrostatic interactions within the mixture. These measurements are performed 5 times at 25 °C. The surface zeta potential of CNC is found -45.24 ± 0.86 mV, which is reported as -37 mV by the manufacturer. This zeta potential value was anticipated because of the negative sulfate ester groups on the surface. Interestingly, surface zeta potentials of [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] micelles are very different. The former's zeta potential is 24.92 ± 3.25 mV whereas the latter's is 1.65 ± 1.16 mV. This shows that counterion effect is drastic.

3.4.3 Small Angle Neutron Scattering (SANS) Analysis

Small-angle neutron scattering (SANS) is performed to analyze the size and shape of the [C₁₀mim][Cl] and [C₁₀mim][FeCl₄] micelles. Therefore, the analysis was performed for concentration below, above and highly above CMC. Owing to the significant difference in neutron scattering cross-sections of hydrogen and deuterium atoms, hydrogen containing micelles in deuterium dioxide become more visible to neutrons. In Figure 3.5, intensity profiles for both surfactants at related scattering wavevector are given. The angle θ is between the incident and scattered beam, and λ is the neutron wavelength. Structural features are not observed at lower surfactant concentrations, which is the indicator that surfactants do not form micelles until 45 – 50 mM concentrations. Following increasing the concentration up to 60 – 70 mM, well-defined scattering intensity profiles of micelles are acquired. According to SANS findings, CMC is around 50 – 70 mM for [C₁₀mim][Cl] and CMC is around 45 – 60 mM for [C₁₀mim][FeCl₄], which are in great agreement with the conductivity data. Marginally above CMC, the micelles are not interacting, therefore the structure factor contribution in the intensity is only 1. SANS intensity is the multiplication of both structure and form factor, $I(Q) = P(Q) * S(Q)$, therefore, intensity around these concentrations are the result of only form factor, $(P(Q))$.

As the surfactant concentration increases, number of micelles increases, and intensity profiles become different due to interactions between these micelles. [C₁₀mim][Cl] micelles exhibit a peak related to structure factor, which is the result of Coulombic repulsion between positively charged micelles. Further increase in surfactant

concentration creates narrower peaks in the profile. The peak shifts from $Q = 0.07 \text{ \AA}^{-1}$ to $Q = 0.1 \text{ \AA}^{-1}$, the equivalent Bragg distances, calculated with this formula $d = 2\pi/Q$, are 9 nm and 6.3 nm. On the other side, structure factor peak was not observed in $[\text{C}_{10}\text{mim}][\text{FeCl}_4]$ containing solutions. As the negative counterion, $[\text{FeCl}_4]^-$ stays on the micelle surface, a positive electrical double layer was not detected. $[\text{C}_{10}\text{mim}][\text{FeCl}_4]$ micelles display hard-sphere interaction at higher concentrations as suggested by Brown et. al. . Surface zeta potential results are also in agreement with this deduction.

Ellipsoid micelle form factors with Hayter-Penfold structure form factor for $[\text{C}_{10}\text{mim}][\text{Cl}]$ and with Percus-Yevik structure form factor for $[\text{C}_{10}\text{mim}][\text{FeCl}_4]$ are fitted to the scattering profiles. The fitted lines are shown as solid lines in Figure 3.5. Surfactants form prolate shaped micelles and the sizes differ from 1 nm to 1.4 nm in equatorial radius and 2.5 to 3.9 nm in polar radius. Hydrodynamic diameter found by DLS analysis is also in this size range. Even though $[\text{C}_{10}\text{mim}][\text{Cl}]$ micelles are slightly more elongated than $[\text{C}_{10}\text{mim}][\text{FeCl}_4]$ micelles, it is possible to express that size of the micelles is similar. SANS analysis implicitly expresses that micelles are identical in the sense of size, shape and CMC, yet the only difference is the surface zeta potential of the micelles.

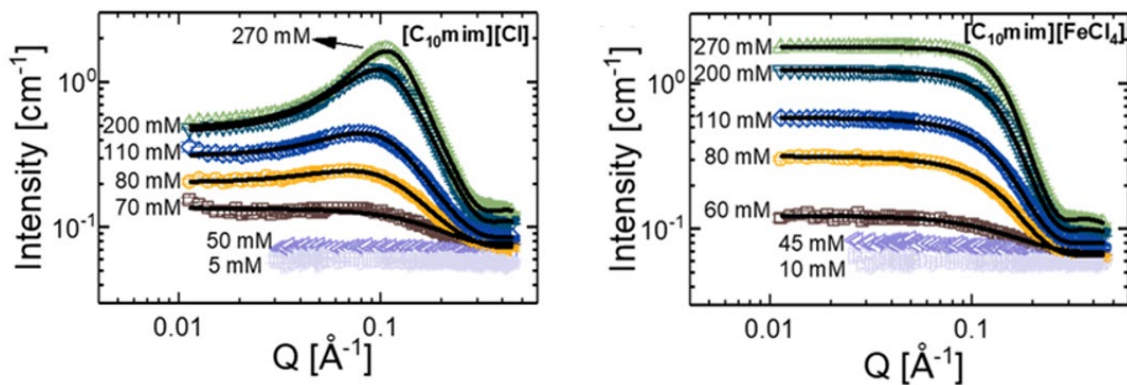


Figure 3.5: Intensity Profiles from SANS.

3.4.4 Sol – Gel Transition Analysis

As proven by numerous studies, CNC colloids undergo sol-gel phase transition upon addition of surfactants. These molecules alter the electrostatic, hydrophilic, and hydrophobic interactions between negatively charged cellulose nanorods and cause

formation of networks. To understand the boundaries of gelation and assess the effects of concentration and micellization of $[C_{10}mim][Cl]$ and $[C_{10}mim][FeCl_4]$ on CNC colloids, a widely acknowledged method of tube inversion was implemented. Initially surfactant stock solution and distilled water were mixed in a vial, and formation of micelles was aimed. Then, CNC stock solution was added on this mixture. Each vial was blended gently for at least 30 seconds. Finally, each tube was inverted. If the sample stuck at the top, the sample was recorded as gel, otherwise it was recorded as sol. The gel ones were also analyzed in oscillatory rheology experiments. The photos of samples were demonstrated in Figure 3.6.

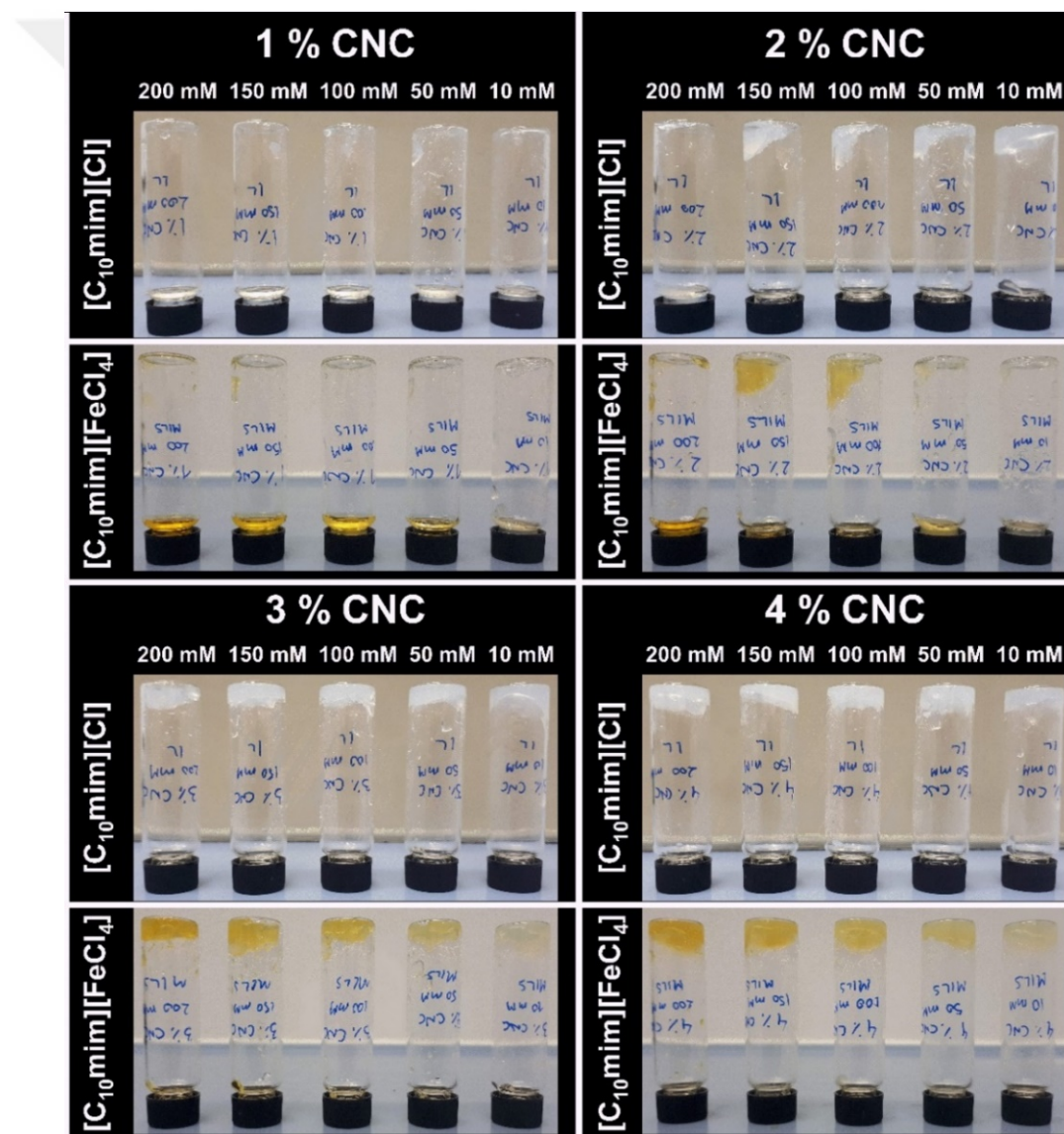


Figure 3.6: Photos of CNC - $[C_{10}mim][Cl]$ and CNC - $[C_{10}mim][FeCl_4]$ Complexes after Tube Inversion.

Alternatively, phase of these complexes was shown on Figure 3.7. 2 % was the lowest CNC concentration at which phase transition was observed. Neat 3 and 4 % CNC colloids were viscoelastic liquids, and small amounts of surfactants were enough to instigate gelation.

Interestingly, there is a discrepancy regarding gelation of $[C_{10}mim][Cl]$ and $[C_{10}mim][FeCl_4]$ at 2 % CNC concentration. Lower $[C_{10}mim][Cl]$ concentrations of 10 and 50 mM were adequate for creating physical hydrogels, whereas $[C_{10}mim][FeCl_4]$ was not successful about gelation at the same concentration. At lower concentrations, which are also below CMC, there is no micelle formation. Both surfactants' cationic head and anionic counterion dissociate in water and therefore positive head groups become ready to bind electrostatically on CNC surfaces. In these mixtures, hydrogen bonding occurs between different groups such as cation – water, anion – water and water – water. As demonstrated by Niazi et. al., size of counterion plays a role in hydrogen bond formation among anion – water. [114] The smaller anionic counterion of $[Cl]^-$ has a better accessibility to water molecules. Hence, ion pair dissociation of $[C_{10}mim][Cl]$ becomes more than $[C_{10}mim][FeCl_4]$ due to higher number of hydrogen bonding taking place between $[Cl]^-$ and water. This also means that higher number of $[FeCl_4]^+$ stays with the surfactant head and causes the reduction of number of electrostatically adsorbed $[C_{10}mim][FeCl_4]$ groups on negative CNC surface. As a result of this reduced number of surfactant adsorption, gelation was not observed at lower $[C_{10}mim][FeCl_4]$ concentrations.

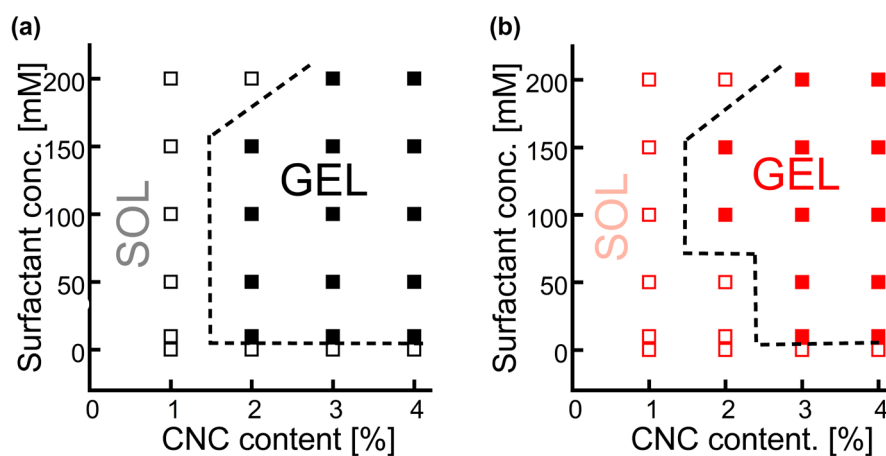


Figure 3.7: Phases of (a) CNC - $[C_{10}mim][Cl]$ and (b) CNC - $[C_{10}mim][FeCl_4]$ Complexes.

At higher surfactant concentrations of 100, 150 and 200 mM, micelles were already formed before CNC addition. Following the insertion of negatively charged nanorods, dynamic species of micelles were disrupted, and some surfactant groups adsorbed on the surface and some stayed within self-assembled aggregates. Both single surfactants adsorbed on CNC surface and micelles bridging CNC groups have impact in gelation. Above CMC, positive $[C_{10}mim][Cl]$ micelles electrostatically adsorb on CNC surface and behaves like a crosslinking point. On the other hand, neutral $[C_{10}mim][FeCl_4]$ micelles' attraction is hydrophilic, which is less effective than the electrostatic. Formation of excessive number of micelles are unfavorable in physical gel development since micelles jeopardizes constructive interactions between the nanorods. $[C_{10}mim][Cl]$ micelle has positive charge and $[C_{10}mim][FeCl_4]$ micelle has neutral charge. The former creates long range electrostatic repulsion, the latter has short range electrostatic repulsion. Both complexes were not able to undergo gelation at 200 mM surfactant and 2 % CNC owing to this negative influence of large number of micelles. This negative influence was not seen in 3 and 4 % CNC containing complexes as they do have less micelle formation due to abundance of CNC surfaces allowing adsorption of more surfactants. Further oscillatory rheology experiments were performed to understand the interactions and effect of micelles within the 3 and 4 % CNC containing gels.

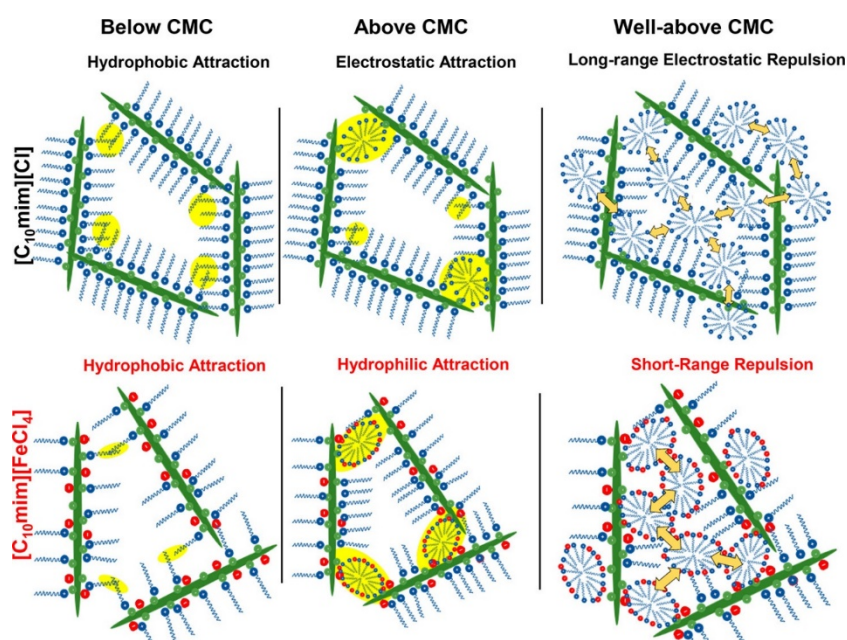


Figure 3.8: CNC - $[C_{10}mim][Cl]$ and CNC - $[C_{10}mim][FeCl_4]$ Interaction Mechanisms at Below CMC, Above and Well-Above CMC [115].

3.4.5 Oscillatory Rheology Analysis

Rheology, which is the branch of physics, is the science of flow and deformation. It is interested in mainly non-Newtonian fluids and solids which deform under applied stress and flow rather than deform elastically. From the molecular viewpoint, the materials being the subject of rheology are basically mixtures containing macromolecules. In rheology, there is not a definite distinction between solids and liquids, the behavior of materials is dependent on the timescale and applied stress. They can exhibit either solid-like or liquid-like property under certain conditions.

Hydrogels are the most common examples of soft matters. These materials exhibit dominant elasticity with less viscous dissipation. Therefore, assessing viscous and elastic features is ultimately important to understand hydrogel's mechanical capabilities. These properties can be analyzed by oscillatory rheology experiments in which the material is sandwiched between two parallel plates, one is moving, and the other is stationary, and the response of material to a sinusoidal shear deformation was analyzed. The stress resulted from the deformation has two components. One component is elastic in phase with the strain, the other component is viscous and out of the phase with the strain. The ratio between these two components allows us to interpret the solid-like and liquid-like features of the material. [116, 117] To understand the time-dependent behavior of the sample, frequency sweep experiments must be performed. In these experiments, frequency varies from high-to-low or low-to-high at a constant strain or stress without deforming the structure. Therefore, they should be completed within the linear viscoelastic region (LVER). To understand the microstructure of the material, non-linear viscoelastic region should also be discovered. When high shear deformation is applied on the material, network junctions start to deform and the rate of deformation or change of elastic and viscous modulus with respect to the increasing deformation give information about the structure.

Frequency Sweep Tests

It is necessary to find the boundaries of LVER to perform frequency sweep tests. Therefore, we firstly did amplitude sweep experiments at extreme frequencies of 0.1 and 100 Hz and boundaries of LVER (which are strain values differing from 0.1 to 1 %) was found. Frequency sweep tests were performed at the highest possible strain (LVER's

upper boundary) to eliminate noise. In frequency sweep tests, lower frequencies enable us to interpret samples' behavior in the longer timescales, higher frequencies enable us to interpret samples' behavior in the shorter timescales. Via performing these tests, the samples' phase at the rest can easily be found. Hydrogels exhibit frequency independence though solutions' modulus changes with differing frequency. As it can be seen in Figure 3.9 and Figure 3.10, 3 % and 4 % CNC containing complexes have frequency independence in LVER. Neat CNC solutions are viscoelastic liquids, they exhibit solid-like property at higher frequencies whereas liquid-like property is observed at lower frequencies. [118] Neat CNC solution's behavior is consistent with the findings of Shafiei-Sabet and coworkers. [60] The complexes containing 10 mM $[C_{10}mim][Cl]$ displayed the lowest storage and loss modulus. Besides this complex, modulus of the others is close to one another. Lower storage modulus means that degree of physical crosslinks within hydrogel is less. Low surfactant concentration leads to lower modulus; however, there is an exception regarding 10 mM $[C_{10}mim][FeCl_4]$ containing gel's modulus. At the same concentration, $[C_{10}mim][FeCl_4]$ containing gel's modulus is greater than the modulus of $[C_{10}mim][Cl]$ containing one. This can be explained by the fact that some portion of dissociated $[FeCl_4]^-$ ions adsorbs on CNC and lead a role as a bridge between nanocrystals. Besides this finding, frequency sweep tests performed at small amplitudes are not adequate to thoroughly understand the microstructure of the hydrogels. There is a need for large amplitude oscillatory shear (LAOS) experiments showing also non-linear viscoelastic regions.

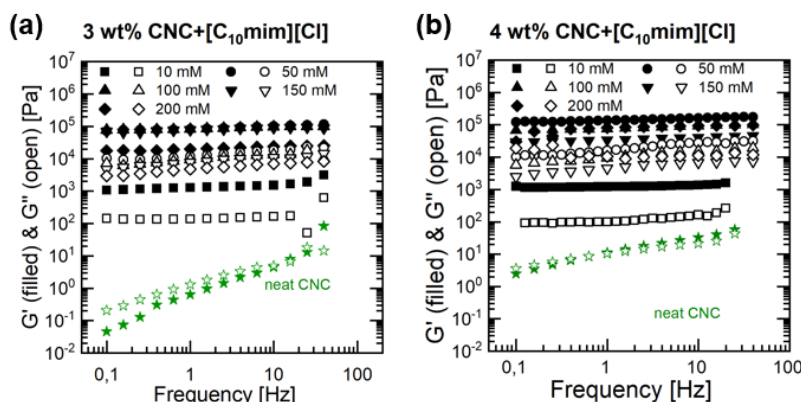


Figure 3.9: Frequency Sweep Tests of (a) 3 w/v % CNC - $[C_{10}mim][Cl]$ and (b) 4 w/v % CNC - $[C_{10}mim][Cl]$.

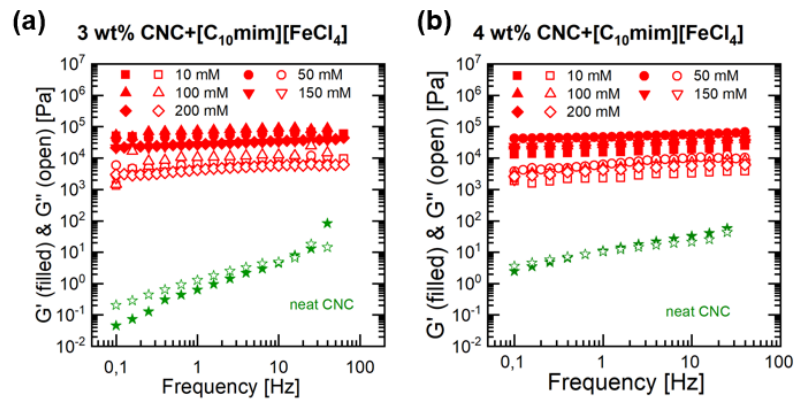


Figure 3.10: Frequency Sweep Tests of (a) 3 w/v % CNC - $[C_{10}mim][FeCl_4]$ and (b) 4 w/v % CNC - $[C_{10}mim][FeCl_4]$.

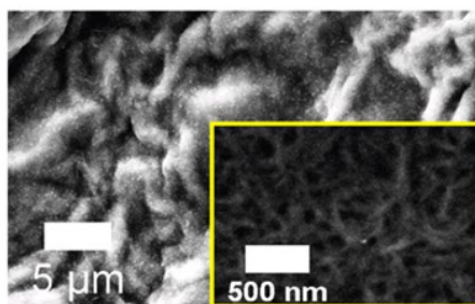
Amplitude Sweep Tests

Amplitude sweep tests were performed on 3 % and 4 % CNC containing gels. These tests are crucial for understanding rheological properties and therefore deducing structural features of the materials. Both linear and non-linear viscoelastic regions were analyzed at 10 Hz monitoring the gels response to varying strain from 0.01 to 100 %. For the full characterization, small amplitude oscillatory strain (SAOS) tests and large amplitude oscillatory strain (LAOS) tests were performed. Besides understanding gels behavior in linear viscoelastic region (LVER) by SAOS, non-harmonic behavior of gels in LAOS allowed us to interpret deformation within gels. [119]

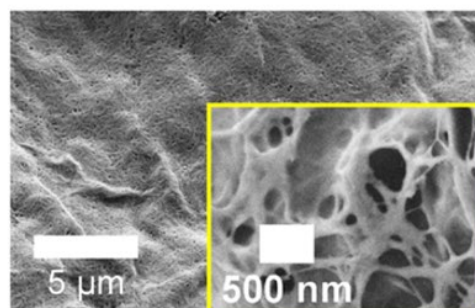
In LVER, neither storage (G') nor loss modulus (G'') does not change with increasing strain amplitude. G' is always higher than G'' within LVER, this shows that the material is a gel-like solid while it is rested. [118] Fundamentally, there are two critical strain values should be read from amplitude sweep tests:–yield point (strain) and flow point (strain). Yield point (γ_y) is the strain value where G' leaves the plateau and starts to decrease; it is generally calculated by certain software. It is also the strain corresponding to 90 or 95 % of the $G'_{plateau}$ value. Flow point (γ_f) is the strain where G' is equal to G'' , and it is generally easier to find this point. Noteworthy, yield point can be 5 or 10 % less than flow point. [118] In LVER, no change occurs within the gel even though some small strain is applied. Between the yield point and the flow point, microcracks form, and at strain values higher than flow point macrocracks form and gel structure is destroyed. Mezger expressed that the ratio between flow and yield point give us idea

about the brittleness of the material assuming that the closer the ratio to 1, the more probable that the structure is brittle. [118] Since these complexes have sophisticated intermolecular interactions, flow strain to yield strain ratio is useful for determining the predominant interaction type. Ratio closer to unity demonstrates that material is brittle and therefore predominant interaction is electrostatic. When the main attraction is electrostatic, applied strain causes the repulsion between the same charged ions and fast crack propagation. [120] This repulsion is an inhibiting factor for material to bear high strain amplitudes, and it therefore breaks at lower strains. Besides understanding the prevalent interaction type, it is also important to quantify number of these interaction. The higher the $G'_{plateau}$, the more probable that gel is more crosslinked.

The SEM images of the gels are given in Figure 3.11. These images are proofs that mesh sizes are different; thus, gel's degree of crosslinking and predominant interaction mechanisms within gels are also different. For better understanding, it is necessary to interpret the amplitude sweep tests (SAOS to LAOS transition).



CNC - [C₁₀mim][Cl]



CNC - [C₁₀mim][FeCl₄]

Figure 3.11: SEM Images of Uncoated and Dried Hydrogels with 4 % CNC and 200 mM Surfactant Concentration.

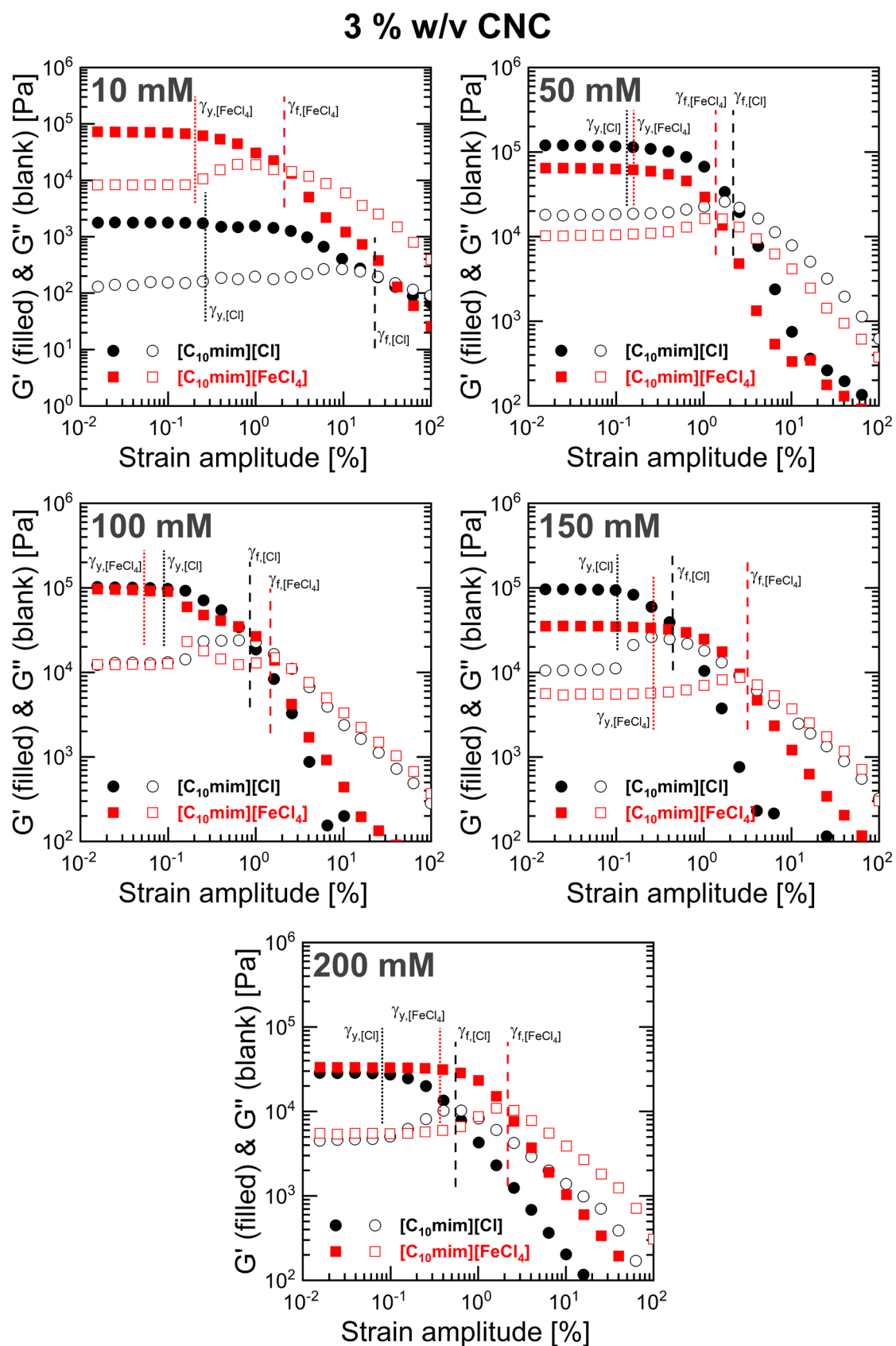


Figure 3.12: Modulus vs Strain Amplitude of 3 % w/v CNC Gels at 10 Hz.

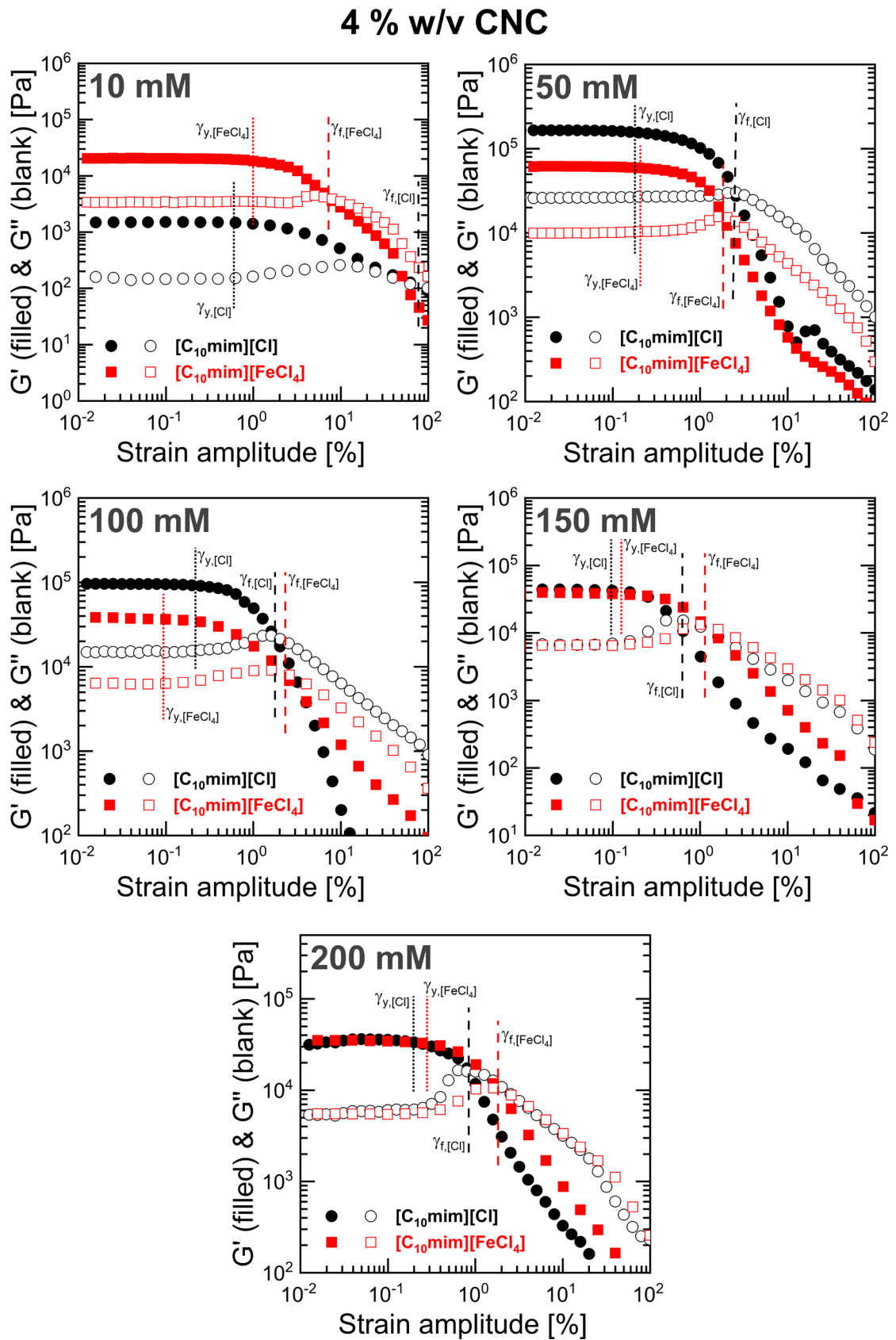


Figure 3.13: Modulus vs Strain Amplitude of 4 % w/v CNC Gels at 10 Hz.

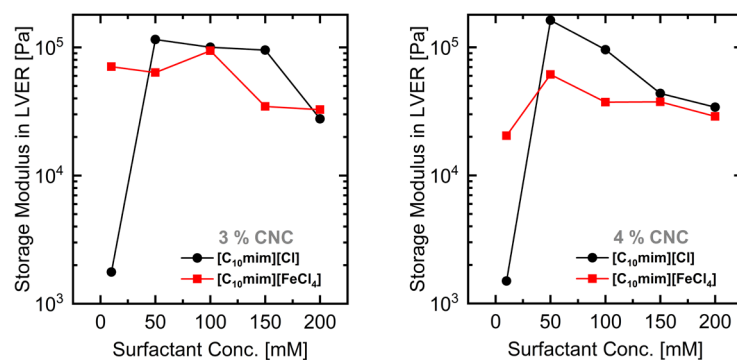
Figure 3.14: Storage Modulus in LVER (G'_{plateau}) of 3 %, 4 % CNC Containing Gels.

Table 3.1: Yield, Flow Point and Brittleness Index of 3 % CNC - Surfactant Gels.

[C ₁₀ mim][Cl] Concentration (mM)	Yield Point, γ_y (%)	Flow Point, γ_f (%)	Ratio, γ_f/γ_y	[C ₁₀ mim][FeCl ₄] Concentration (mM)	Yield Point, γ_y (%)	Flow Point, γ_f (%)	Ratio, γ_f/γ_y
10	0.25	23.41	93.64	10	0.10	2.45	24.50
50	0.15	2.37	15.80	50	0.17	1.55	9.12
100	0.10	0.91	9.10	100	0.06	1.59	26.50
150	0.11	0.41	3.73	150	0.25	2.98	11.92
200	0.10	0.54	5.40	200	0.33	2.19	6.64

Table 3.2: Yield, Flow Point and Brittleness Index of 4 % CNC - Surfactant Gels.

[C ₁₀ mim][Cl] Concentration (mM)	Yield Point, γ_y (%)	Flow Point, γ_f (%)	Ratio, γ_f/γ_y	[C ₁₀ mim][FeCl ₄] Concentration (mM)	Yield Point, γ_y (%)	Flow Point, γ_f (%)	Ratio, γ_f/γ_y
10	0.88	78.12	88.77	10	0.61	7.56	12.39
50	0.18	2.50	13.89	50	0.21	1.86	8.86
100	0.23	1.78	7.74	100	0.09	2.3	25.56
150	0.11	0.54	4.91	150	0.13	1.2	9.23
200	0.16	0.84	5.25	200	0.2	1.93	9.65

As viscoelastic behavior is generally restricted to small deformations, [121] further analysis of LAOS (outside linear viscoelastic region) might be required for a better analysis. According to Hyun and coworkers, complex fluids might be classified depending on their LAOS performance. The four kinds of complex fluids suggested by Hyun et. al. are Type I (strain thinning), Type II (strain hardening), Type III (weak strain overshoot), and Type IV (strong strain overshoot). [119, 122] As seen in both Figure 3.10 and Figure 3.11, storage modulus leaves the plateau and constantly decreases owing to loss of physical crosslinks. At high strain values, the gel is not elastic and intact anymore. The increase in loss modulus is due to the energy dissipated during physical crosslinks being pulled off. After reaching the peak value, at which the viscosity is the highest because of the formation of unstable CNC and surfactants flocs [123], loss modulus starts to decrease since these clusters cannot be further separated. [124] Type III behavior, which is known as exhibited by natural polymer suspensions such as Xanthan Gum, and starch solutions [120], is observed in both $[C_{10}mim][Cl]$ and $[C_{10}mim][FeCl_4]$ containing complexes.

At 10 mM surfactant concentration, $[C_{10}mim][Cl]$ containing gels have fewer physical crosslinks than $[C_{10}mim][FeCl_4]$ containing ones, this can be understood by comparing their $G'_{plateau}$ values. In addition, $[C_{10}mim][FeCl_4]$ containing ones are more brittle than $[C_{10}mim][Cl]$ containing ones according to the ratio. Besides hydrophobic attraction, electrostatic attraction is existent in $[C_{10}mim][FeCl_4]$ containing complexes at 10 mM surfactant concentration. The most flexible and the least brittle gels are the ones having the lowest surfactant amount.

At 50 mM surfactant concentration, surfactants cannot form free micelles and they adsorb on CNC surface. Thus, the surface contains the highest number of surfactants. As seen in Figure 3.14, the stiffest gels at the rest are formed when the concentration is 50 mM due to presence of large number of surfactants on the surface. Via hydrophobic attraction (tail-to-tail), the highest number of crosslinks is reached. $[C_{10}mim][Cl]$ containing gels are more flexible than $[C_{10}mim][FeCl_4]$ containing gels due to hydrophobic attraction between surfactant tails. $[C_{10}mim][FeCl_4]$ containing ones are more brittle as negative counterion settles on CNC surface. Increase in concentration increased also reduced the yield strain to flow strain ratio.

When the concentration reaches above CMC, the effect of micelle charge also starts to make a difference. At 100 mM surfactant concentration, the brittleness of

[C₁₀mim][FeCl₄] gels decrease drastically due to formation of neutral micelles. Predominant interaction type becomes hydrophilic interaction between CNC surface and neutral micelles. In contrast, at the same concentration, [C₁₀mim][Cl] containing gels become more brittle owing to the formation of positively charged micelles. Their stiffness also reduced, which is an indication that number of crosslinks reduced.

At concentrations, which are well above CMC, such as 150 and 200 mM, more micellization takes place. Additional micelle formation is not in favor of gel network enhancement. The adverse effect of positive micelle formation is more apparent according to Figure 3.14. As positively charged free micelles start to form, long-range of repulsion between positive micelles is observed. This repulsion inhibits the crosslinking and makes the gel more brittle. A severe drop in storage modulus was not observed in [C₁₀mim][FeCl₄] gels as formation of free neutral micelles does not have a dramatic influence on gel network.

The storage modulus values prove that micelle formation adversely affects the network formation and decreases the number of interactions. At above CMC, surfactants leave the surface and form their aggregates. To have a more elastic gel, it is better to eliminate micelle formation. It is also worth mentioning that elasticity and flexibility are two different concepts. Elasticity is related to the strength of the crosslinks; an elastic material is expected to save its original shape when the strain is moved. Even though flexible material might bear large strain values, it may not come back to its original shape. These results are pivotal in our understanding of the rheology and phase behavior of CNC–surfactant (micelle) mixtures and will support us to construct advanced functional cellulosic materials.

Chapter 4:

TEMPERATURE INDUCED GELATION OF CELLULOSE NANOCRYSTAL SUSPENSIONS

4.1 *Introduction*

The advancements in stimuli-responsive hydrogels are promising, especially in medicine. Hydrogels are the materials able to retain significant amount of water due to the hydrophilic structure formed by chemically or physically cross-linked hydrophilic polymers. Besides their water retaining ability, they can also be biodegradable and biocompatible and might adopt stimuli-responsiveness. These aspects make them ideal materials in tissue engineering, gene and drug delivery applications. [61, 125-127] Stimuli-responsiveness is a necessity for particularly drug, protein or gene delivery systems as only stimuli-responsive materials can achieve the gradual release of the desired substance in the targeted tissues. [128, 129] If stimuli-responsive moieties are conjugated to or stimuli-responsive macromolecules are embedded in the hydrogel network, hydrogels can become susceptible to external stimulus of pH [7], light [130], electric [131], magnetic field [132, 133], and temperature [134, 135]. Upon exposure to any external stimulus, chemical and physical interactions holding the structure intact might change and these leads to release of trapped active agents. Additionally, stimuli-responsiveness is required if injectability is desired in the material. [136] Injectable hydrogels are preferable to the pre-formed ones as injectable hydrogel can fill the asymmetrical places and does not necessitate surgical operations. [137] These hydrogels are fluid prior to injection and upon exposure to body temperature following the injection, they solidify and become gel like. Thermo-responsive chemical moieties or large molecules cause this sol-gel transition. PNIPAM [138-140], Pluronic F127 [141, 142] and poly(organophosphazene) (PPZ) [143, 144] are thermo-responsive polymers; there are numerous studies about aqueous solutions of these polymers. When these polymers are inserted in the hydrogel network as main or supplementary components, the complex exhibits liquid to soft solid phase transition as temperature exceeds a critical temperature called lower critical solution temperature (LCST).

Pluronic F127 is a non-toxic triblock copolymer with the structure of poly (ethylene oxide) – poly(propylene oxide) – poly(ethylene oxide) (PEO-PPO-PEO). Pluronic F127 molecules disperse well in water at lower temperatures. As temperature increases, poly propylene oxide groups (relatively hydrophobic groups in the chain) start to accumulate and constitute a dense poly propylene oxide core, whereas hydrophilic polyethylene oxide groups in the chain stretch out. The resultant shape is a spherical micelle with PPO core and PEO corona. [145] PEO groups in contact with water can touch with other PEO groups and the gel structure is formed via this affinity. At higher temperatures and concentrations, the crystalline order is constituted by Pluronic F127 micelles. Furthermore, the idea of using Pluronic molecules in drug delivery applications is firstly suggested by Dorn et. al. [146] They mixed hydrophobic drug and block copolymer at a lower temperature and they proved that hydrophobic drug started to accumulate in the hydrophobic Pluronic core upon heating the complex. Drug accumulation in the core is effective when the gradual release on the target is desired. [147, 148]

The natural polymer cellulose derivative of cellulose nanocrystals (CNC) is also utilized in drug delivery applications. CNC is a biocompatible nanomaterial that does not alert the human immune system and therefore is able to convey chemically conjugated or physically adsorbed drug molecule to the targeted tissue without a significant immune response. [149-151] They are thin rod-like large molecules with dimensions of 2 – 70 nm in width and 100 nm to several micrometers in length. [95, 152] Aqueous suspensions of neat CNC are not responsive to environmental stimuli, yet stimuli-responsiveness can be adopted by chemical or physical treatments. CNC is relatively small for the detection of mononuclear phagocyte system and at the same time large for removal from the blood circulation. Therefore, residence time of CNC in the circulation is long. Furthermore, the hydrophilic nature of CNC restrains the adsorption of opsonin proteins, which causes the delayed phagocytosis. [151] Another advantage of CNC in drug delivery systems is that hydroxyl groups on the surface are suitable for both physical adsorption and chemical conjugation of bioactive molecules.

Dual drug therapy is more effective in treatment of numerous diseases, particularly in treatment of cancer. Drug resistance is a potential obstacle when one type of drug is used in the treatment. Supply of a specific drug with large amounts can stimulate the gene mutation in tumor cells and threatens the elimination of cancer. [151, 153, 154] Therefore, dual or multiple drug release with independent releasing mechanisms is

regarded more successful. Moreover, some tissue engineering applications, in which more than one type of growth factor is necessitated [155] or angiogenesis is desired [156], require carriers able to entrap different bioactive agents or drugs in the meanwhile.

In the literature, there are several studies about Pluronic F127 utilized in drug delivery systems. Cicada and coworkers investigated the drug delivering performance of Pluronic F127 combined with alginate microparticles. [157] In the study of Yeh et. al., the cell viability in gelatin and Pluronic F127 containing complexes were analyzed. [142] Additionally, a dual drug delivery system with dual responsive Pluronic F127 and polysaccharide-based hydrogel developed by Chatterjee et. al. [134] is a great instance for responsive systems. In our study, we aspired to propose an injectable, thermo-responsive, and biocompatible hydrogel combining Pluronic F127 and CNC for the dual drug delivery. We analyzed the rheological response, shift in the gelation temperature, thermo-reversibility and mechanical characteristics of these composite gels using both block copolymer and CNC as a matrix of the composite. The interaction of Pluronic F127, which is a non-ionic surfactant, with CNC was comprehensively analyzed. Pluronic F127/CNC complex was proposed as an injectable thermo-responsive hydrogel possessing a high potential for biomedical applications.

4.2 Materials

Nanocrystalline cellulose in spray dried form was provided by Celluforce (Montreal, Canada). The chemical name of the product is cellulose hydrogen sulphate sodium salt in the product safety data sheet. As reported by the supplier, overall crystalline density is 0.88 (XRD) and hydrodynamic diameter is 70 nm (DLS). Pluronic F127 in powder form was purchased from Sigma-Aldrich (St. Louis, MO). Pluronic F127 is a block copolymer with the molecular structure of $(\text{PEO})_{100}\text{-}b\text{-(PPO)}_{65}\text{-}b\text{-(PEO)}_{100}$. Both materials were used without further purification.

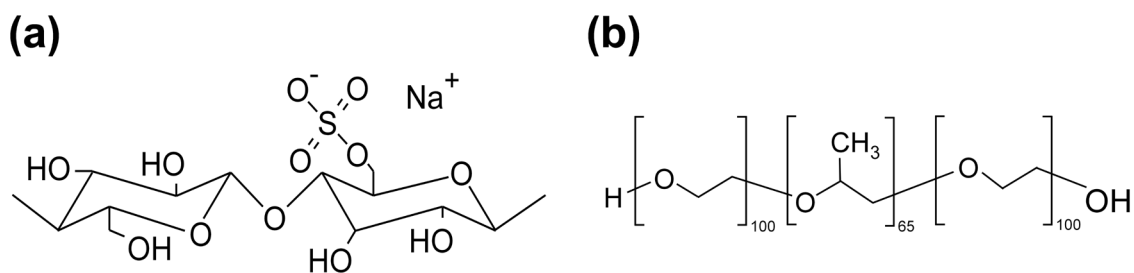


Figure 4.1: Molecular Structure of (a) CNC and (b) Pluronic F127.

4.3 Experimental Methods

4.3.1 Hydrodynamic Size and Zeta Potential Measurements

Hydrodynamic sizes and zeta potentials were measured by using Malvern ZS Zetasizer. All measurements were carried out at 10 °C, 15 °C, 37 °C, and 50 °C for each sample. The concentration of CNC suspension was 1 mg/ml. The concentration of aqueous Pluronic F127 solution was 10 mg/ml (1.01 wt. %). This concentration was chosen for Pluronic F127 as it is feasible to observe both unimer (at 10 °C, 15 °C) and micelle phases (at 37 °C, 50 °C).

4.3.2 Sample Preparation

Aqueous CNC suspension with 6 % w/w concentration was prepared following the manufacturer's protocol. To eliminate sudden gelation, CNC powder was added to distilled water with the rate of 0.2 gram/minute while the CNC/water mixture was being stirred. Firstly, required amount from stock CNC suspension was taken and it was diluted with distilled water. To reach higher Pluronic F127 concentrations, F127 stock solution was not prepared and powder F127 was directly added on diluted aqueous CNC suspension. As the block copolymer is thermo-responsive and dissolves better at lower temperatures, samples were blended on a tube rotator at 4 °C. The resultant complexes containing both CNC and Pluronic F127 collectively had CNC with 0, 1, 2, 3, 4, 5 % w/w and Pluronic F127 with 12.5, 15, 17.5, 20 % w/w concentrations. The concentration calculations were done using following formulas.

$$\% F127 (w/w) = \frac{Weight_{F127}}{Weight_{water} + Weight_{F127}} \times 100 \quad (4.1)$$

$$\% CNC (w/w) = \frac{Weight_{CNC}}{Weight_{water} + Weight_{CNC}} \times 100 \quad (4.2)$$

4.3.3 Steady Shear Rheology Experiments

Neat CNC, neat F127 and CNC – F127 complexes were analyzed using Malvern Kinexus Pro rheometer under steady shear. The apparent viscosities of all samples were analyzed within a temperature interval of 10 – 50 °C. Before starting the measurement, rheometer was cooled down to 10 °C. The sample was placed under the cone and plate geometry which has a diameter of 40 mm and the angle of 4°. Shear stress was set to 1 Pa and temperature ramp rate was set to 0.5, 1 and 5 °C/min for different measurements. Viscosity versus temperature and viscosity on temperature cycle results were obtained following steady shear rheology experiments.

4.3.4 Oscillatory Shear Rheology Experiments

Neat CNC, neat F127 and CNC – F127 complexes were analyzed using Malvern Kinexus Pro rheometer under oscillating shear. Viscoelastic properties of resultant gels such as storage and loss modulus, complex viscosity were analyzed. The sample was placed under the cone and plate geometry which has a diameter of 40 mm and the angle of 4°. Prior to performing the experiments, the temperature was set to 37 °C at which all samples were gel. Firstly, the boundaries of LVER were found by frequency sweep tests. Followingly, amplitude sweep tests were performed to fully characterize microstructures of gels at 10 Hz. The injectability feature of gel were also analyzed performing recovery tests in which elastic and viscous modulus of samples were measured shortly after a high strain application.

4.4 Results and Discussion

4.4.1 Dynamic Light Scattering Analysis: Hydrodynamic Size and Zeta Potential Determination

To observe formation of neutral Pluronic F127 micelles, dynamic light scattering analysis was performed. The critical micelle temperature (CMT) of dilute Pluronic F127 (0.725 % w/w) solutions is 25 °C [147] and further increase in the block copolymer concentration leads to decline in CMT. 10 mg/ml (1.01 wt. %) Pluronic F127 containing solution was prepared and the change in hydrodynamic size was observed. In Figure 4.2, the self-assembly of Pluronic F127 at high temperatures is seen. At 10 °C and 15 °C, hydrodynamic sizes are 4.45 ± 0.40 nm and 4.67 ± 0.25 nm, corresponding to the unimers. At 35 °C and 50 °C, hydrodynamic sizes are 15.30 ± 0.81 nm and 17.48 ± 0.30 nm, and reflect the sizes of the individual micelles. According to Atwood et. al., hydrodynamic size of the Pluronic F127 micelle is dependent on concentration and their finding is 10.2 ± 0.1 nm at 35 – 45 °C (using aqueous 4 mg/ml Pluronic F127 solution as a sample). They calculated this data from diffusion study (using a different method). [158] Zeta potentials were measured as -0.53 ± 0.28 mV, 0.06 ± 0.14 mV, -0.48 ± 0.03 mV, -0.50 ± 0.19 mV at 10 °C, 15 °C, 37 °C and 50 °C, respectively. This verifies that both unimers and micelles are neutral, as expected.

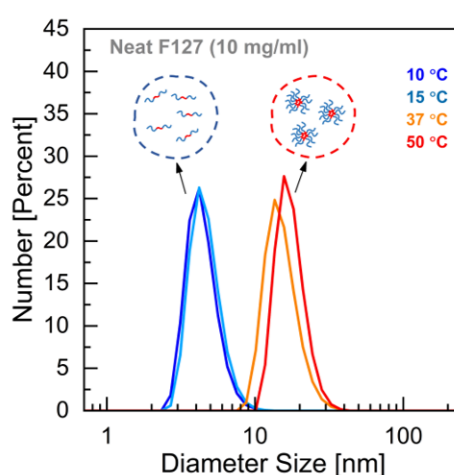


Figure 4.2: Number Weighted Distribution of Hydrodynamic Sizes of Pluronic F127 Micelles.

4.4.2 Steady Shear Rheology Analysis: Apparent Viscosity Determination with Temperature Ramp & Temperature Cycle

Apparent viscosity of all samples at a single imposed stress of 1 Pa were measured in the temperature range of 10 – 50 °C. To understand the injectability of these complexes, effect of CNC on injectability and on the gelation temperature, it is required to perform an apparent viscosity versus temperature analysis. As it is seen in Figure 4.3, viscosity of the neat aqueous CNC suspensions decreases monotonically with temperature and it is strongly dependent on CNC concentration. The 4 % and 5 % w/w CNC containing solutions are already in the mixed state of both isotropic and liquid crystal phases. Formation of liquid crystals causes great increase in viscosity. Dilute CNC solutions, on the other hand, are isotropic with low viscosity. [60]

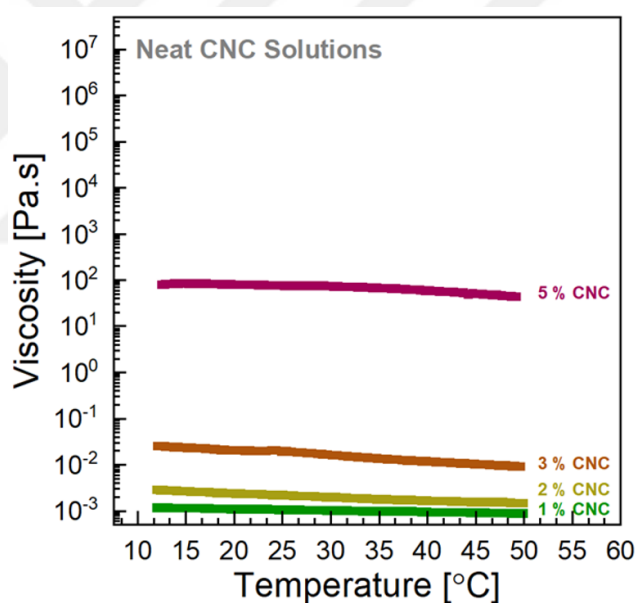


Figure 4.3: Apparent Viscosity vs Temperature of Neat CNC Suspensions.

Viscosities of the neat Pluronic F127 solutions with 12.5 %, 15 %, 17.5 % and 20 % w/w concentrations exhibit change with the increasing temperature. This is expected due to formation of close packing of Pluronic F127 micelles. Even though there is an increase in the viscosity of the 12.5 % w/w Pluronic F127 solution, number of micelles are not adequate to complete phase transition and constitute a full packed crystal structure. Phase transition occurs in the elevated concentrations.

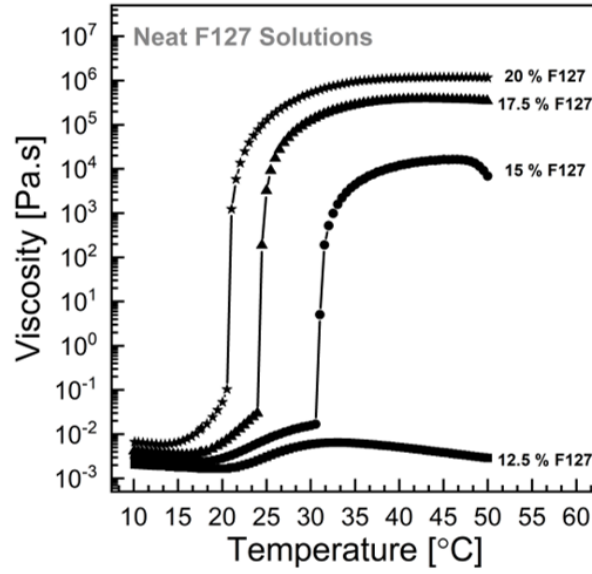


Figure 4.4: Apparent Viscosity vs Temperature of Neat Pluronic F127 Solutions.

The rapid increase in the viscosity is an indication that sol-gel transition takes place. The temperature at which there is a drastic viscosity rise is called as phase transition temperature, gelation temperature or gel point. In Figure 4.4, the gel point is clear since the increase is asymptotic. It is noteworthy that sometimes this rise might not be seen easily and not be asymptotic. Even though there are alternative ways to find the gel point, in this work, gel temperature was recorded as the temperature at which the highest viscosity jump occurs as suggested by Schoolaert et al. [159] The following numerical formulas were not given by them, yet to find the highest viscosity jump, it is necessary to calculate the second derivative of each point and compare the calculated values. The numerical formulas used for calculation of second derivative are as follows. The normalization is used to find the highest jump.

$$f''(T_0) = \frac{\mu_1 - 2\mu_0 - \mu_{-1}}{(T_0 - T_{-1})^2} \quad (4.3)$$

$$f''(T_1) = \frac{\mu_2 - 2\mu_1 - \mu_0}{(T_1 - T_0)^2} \quad (4.4)$$

$$\text{Normalized Value at } T_1 = \left| \frac{f''(T_1) - f''(T_0)}{f''(T_0)} \right| \quad (4.5)$$

If the highest normalized value is found at T_1 , then the phase transition temperature is acknowledged as T_1 . It is another crucial point that if all normalized values are in the same order of magnitude, then it is not possible to mention a single gel point. Phase transition temperatures of 15 %, 17.5 % and 20 % w/w Pluronic F127 containing solutions are 30.55 °C, 23.99 °C and 20.55 °C. The higher the block copolymer concentration, the easier it becomes for the complex to undergo liquid to soft solid transition. Besides the concentration, temperature ramp rate affects the gelation temperature [141], and the findings we report here are for the temperature ramp rate of 0.5 °C/min.

Change of viscosity upon heating was analyzed for all complexes and the graphs are presented in Figure 4.5. In Figure 4.5a, viscosity change of 12.5 % w/w Pluronic F127 containing samples with differing CNC concentration was plotted. Every sample displays a viscosity increase after 20 °C due to micellization. However, the effect of micellization is not enough to induce phase transition. The only sample which demonstrates soft solid characteristics is 5 % w/w CNC containing one as the neat sample is already viscous and the effect of Pluronic F127 micellization allows the completion of phase transition.

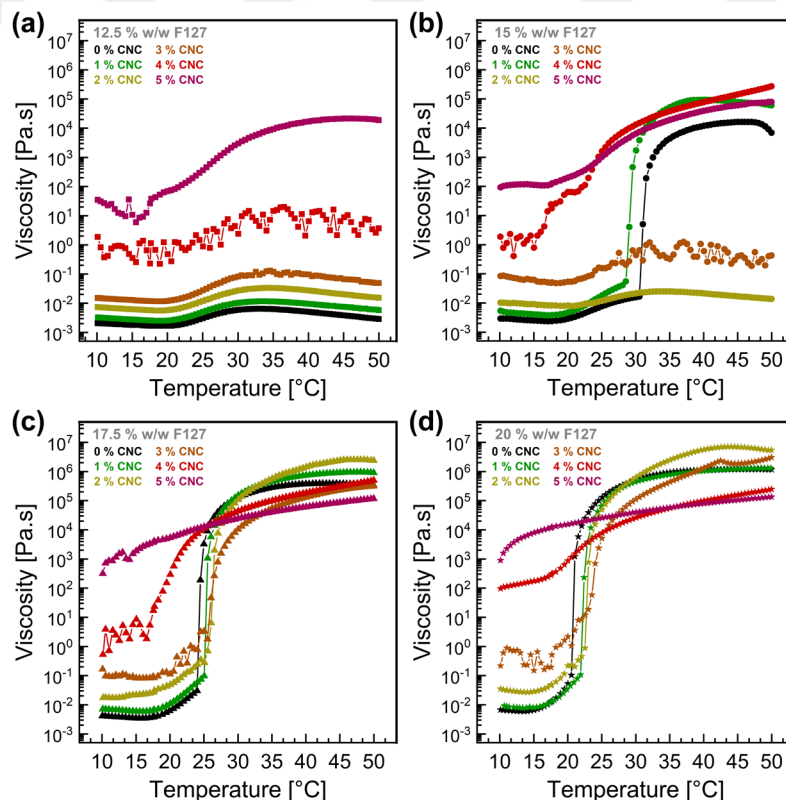


Figure 4.5: Apparent Viscosity vs Temperature for All Pluronic F127 and CNC Complexes.

In Figure 4.5b, 15 % w/w Pluronic F127 complexes are demonstrated. Besides neat 15 % w/w Pluronic F127 containing one, 1 % CNC, 15 % Pluronic F127 containing sample undergoes phase transition. Even though the final form of both 4 % and 5 % CNC holding samples are soft solid, there is not a single transition temperature. CNC blocks the connectivity of Pluronic F127 micelles and therefore generally leads to delay in gelation; however, the effect of 1 % CNC in 15 % Pluronic F127 complex is different than the expected. The gelation temperature of 1 % CNC holding one is 28.56 °C, which is less than 30.55 °C.

In Figure 4.5c and 4.5d, the inhibiting effect of CNC is apparent. Due to high amount of Pluronic F127, all samples undergo phase transition. Apart from 4 and 5 % CNC containing samples, abrupt viscosity rise is observed for all other samples and gel points are found. Pluronic F127 micelles' hydrophilic corona attracts one another and this results in packed crystal structure. Addition of CNC leads to adsorption of block copolymer's hydrophilic sides on the CNC; and as a result, number of micelles goes down and additionally, CNC blocks the connectivity between the micelles. The inhibiting influence of CNC caused the increase in phase transition temperature for 17.5 % and 20 % w/w Pluronic F127 samples. Tube-inversion tests also confirm our findings and show that 17.5 % Pluronic F127 samples are all soft solid at higher temperatures. The pictures of tube-inversion tests and the gel points of all complexes are given in the following figures.

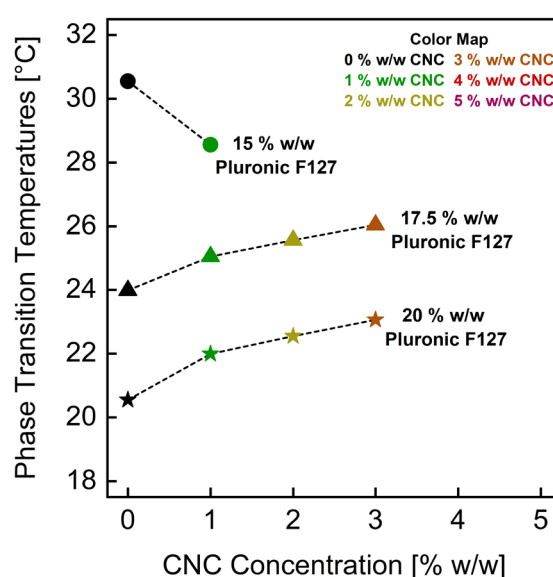


Figure 4.6: Phase Transition Temperatures of Pluronic F127 and CNC Complexes.

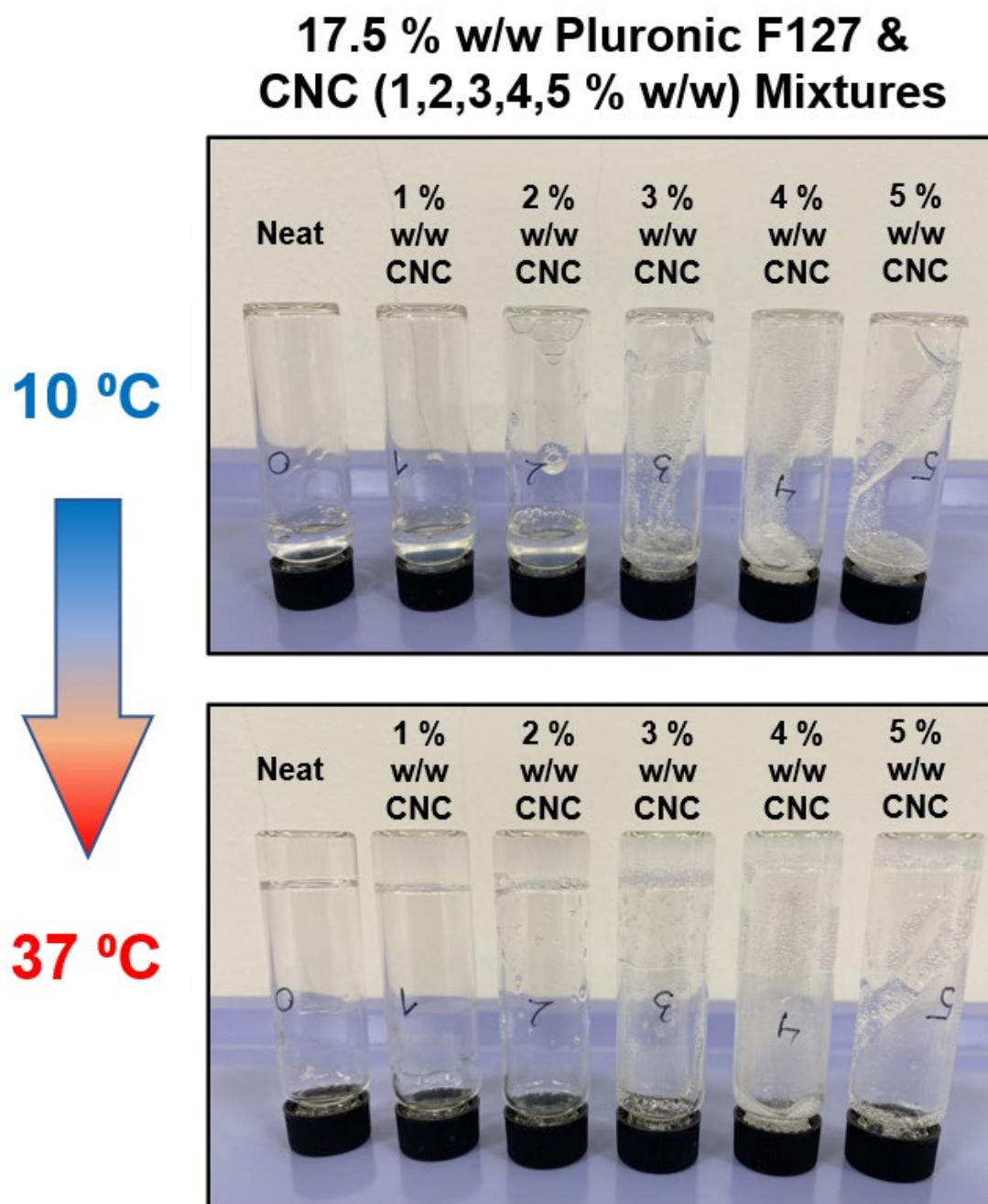


Figure 4.7: Tube Inversion Tests for 17.5 % w/w Pluronic F127 Containing CNC Mixtures.

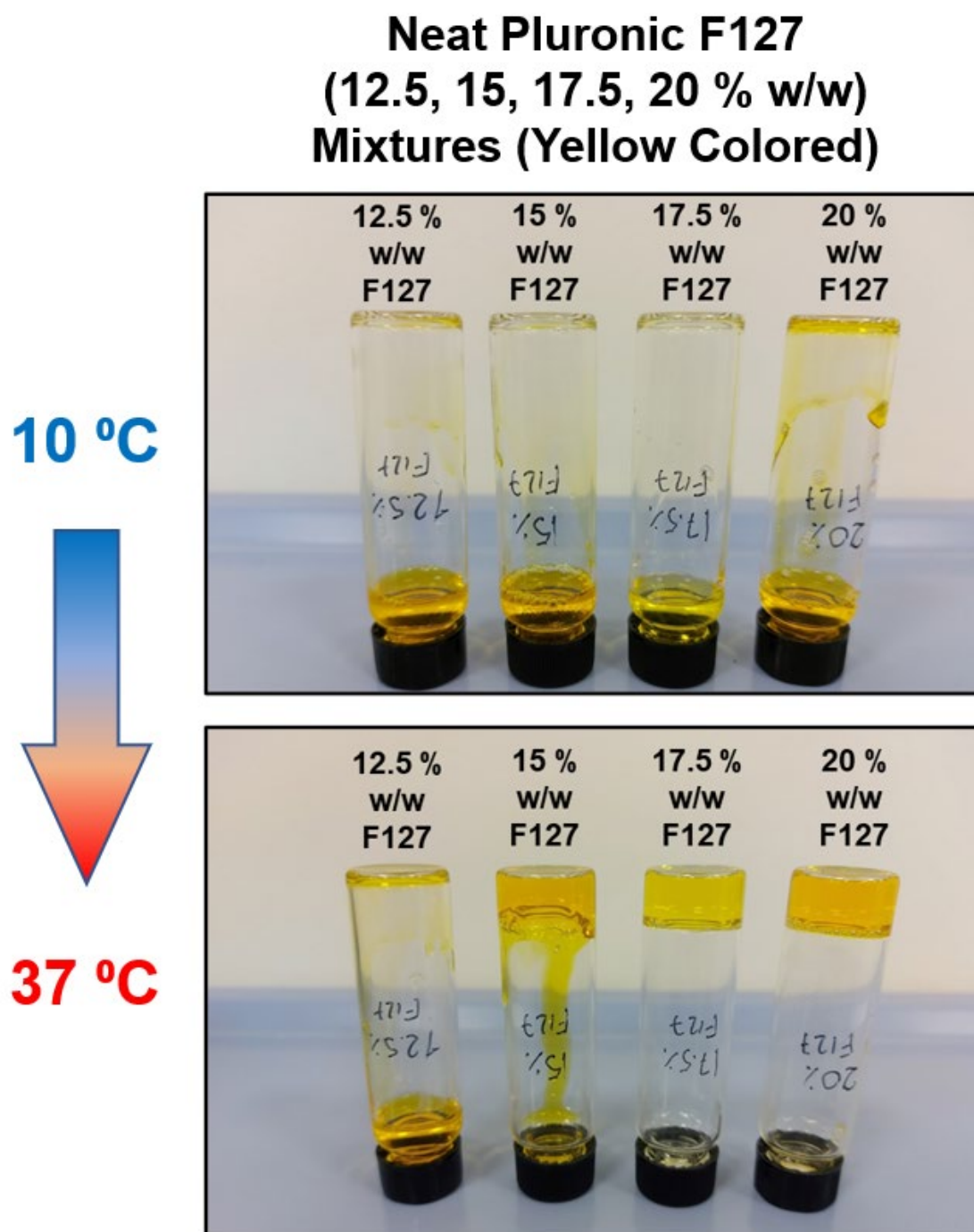


Figure 4.8: Tube Inversion Tests for 17.5 % w/w Pluronic F127 Containing CNC Mixtures.

In addition to finding the phase transition temperatures, steady shear rheology was used to find the thermo-reversibility of hydrogels. Pluronic F127 solutions are known as thermo-reversible, however, the influence of CNC on thermo-reversibility was not

known. As 17.5 % w/w Pluronic F127 having all complexes undergo phase transition, thermo-reversibility of these complexes was analyzed. The viscosity of samples was measured at a single imposed stress of 1 Pa over the same temperature range with the temperature ramp rate of 5 °C/min. Although there are some differences between the neat and CNC containing forms in the heating and cooling path. The final viscosity value of each sample is equal to its pre-experimental viscosity. This clearly shows that CNC addition does not alter thermo-reversibility but only changes the path of viscosity change as the dynamics within the complex is different than the dynamics in the neat form.

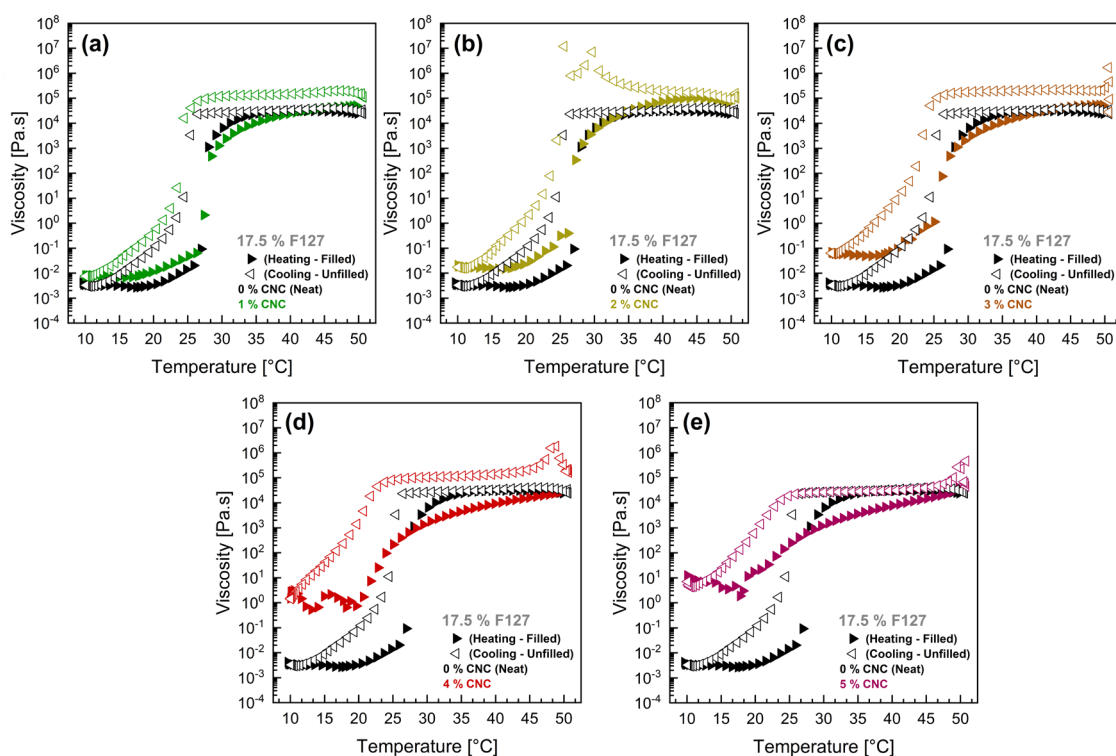


Figure 4.9: Reversibility of 17.5 % w/w Pluronic F127 Containing Complexes.

4.4.3 Oscillatory Shear Rheology Analysis: Frequency Sweep, Amplitude Sweep and Recovery Tests

Oscillatory shear rheometer applies a sinusoidal strain on a material which is sandwiched between two parallel plates. The time-dependent sinusoidal strain, $\gamma(t) = \gamma_0 * \sin(\omega t)$, applied on the material is dependent on the frequency, ω , of the oscillation. As a result of strain application, material gives a response of torque to the upper plate.

The quantified stress, $\sigma(t)$, measured from this torque value is analyzed and both elastic and viscous contributions are assessed by the rheometer. The stress component in phase with the applied strain is elastic component, the other component, which is out of phase with the strain, is viscous component. [116] Oscillatory shear rheology analysis allows evaluation of time-dependent response of material and presents better understanding about material's mechanical response and microstructure.

Firstly, frequency tests were performed to analyze time-dependent behavior of the complexes. These tests are completed at non-destructive LVER regime applying the constant strain rate with alternating frequency. High values probe short timescale behavior of the material, low values give ideas about long timescale behavior of the material. The aim is to find the mechanical characteristics of the resultant gels; therefore, these tests were performed at 37 °C, which is also the body temperature, with the 1 % strain amplitude for only 17.5 % w/w F127 containing complexes.

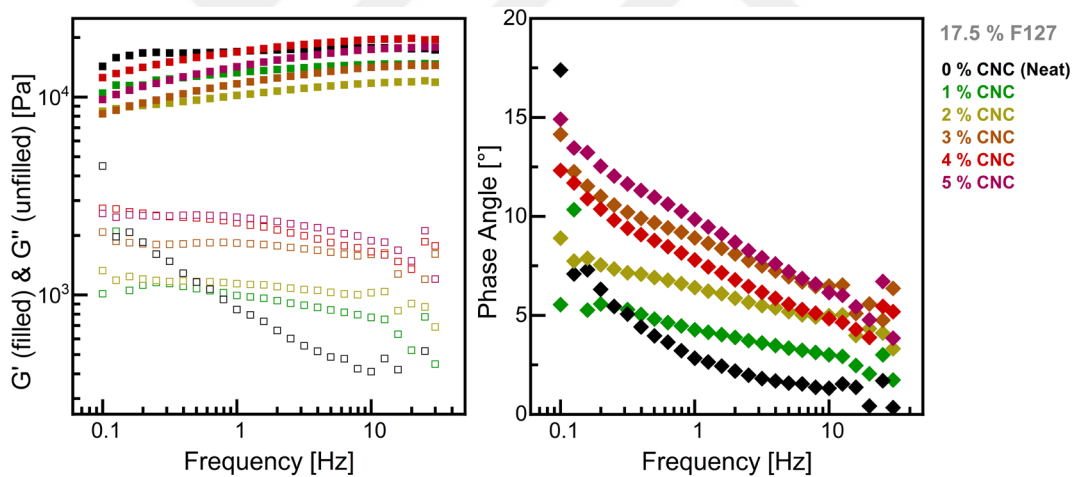


Figure 4.10: Frequency Sweep Tests and Phase Angle vs Frequency.

In Figure 4.10, storage, G' , loss modulus, G'' , and phase angle, δ , versus frequency are given. Phase angle is simply $\delta = \arctan(G''/G')$, if it is less than 45°, it means that storage modulus is bigger than loss modulus and in other words elastic response outweighs viscous response. Storage modulus of all samples is loosely dependent on the frequency, which is an indication that the final phase is soft solid.

Rheological information might be gathered from both small amplitude oscillatory shear (SAOS) deformation and the transition to the large amplitude oscillatory shear

(LAOS) deformation. Sometimes, information from SAOS (application of a non-destructive shear) – is not sufficient to understand the microstructure. Network is under equilibrium when SAOS is applied and modulus is the outcome of the number of crosslinks forming the scaffold. [160, 161] Besides knowing the number of crosslinks, it is also necessary to assess the strength of these crosslinks and therefore, the strength of the whole network. The reason of applying destructive strains and observing the complexes' behavior in SAOS to LAOS transition is to classify the gel's behavior. Hyun et. al. [52, 119, 122] categorized the complex fluids into four groups. Pluronic F127 and Pluronic F127 & CNC mixtures in soft solid phase exhibit type III behavior, weak strain overshoot. In this type of non-Newtonian fluids, storage modulus decreases, and loss modulus firstly increases and then decreases while strain rate grows. To mention type III behavior, a network must be present. At lower strains, non-destructive effect is seen. Both storage and loss modulus become stable. Followingly, some defects take place with the increasing strain in the microstructure and storage modulus slightly drops, but gel is still intact. At intermediate strains, gel starts to lose its integrity due to loss of crosslink points, the loss of crosslinking points means increase in loss modulus. At higher strains, gel integrity is totally lost, whole structure elements form clusters and these clusters flow with the shear. In Figure 4.11, amplitude sweep tests of 17.5 % w/w Pluronic F127 containing gels at 37 °C and at an intermediate frequency of 10 Hz are provided. Plateau elastic modulus, $G'_{plateau}$, of neat Pluronic F127 is higher than 1 %, 2 % and 3 % w/w CNC containing ones. This hard gel feature of the neat one is the result of cubic array order of micelles. [162] Number of crosslinks is higher in neat Pluronic F127 mixture. Addition of CNC resulted in adsorption of block copolymer on CNC [163] and therefore reduction in number of micelles. Plateau values and critical strain values are given in Figure 4.12. The critical strain is also the lowest when there is no CNC in the mixture. As the order of micelles is highest in the neat form, small strains may also disrupt the array and constitute new micelle layers. This behavior is called as slip-stick and studied by Hamley et. al. [164] Soft solid form of neat Pluronic F127 gel is more brittle. Since CNC added ones are less brittle, the highest non-destructive strain is inclined to increase due to less crosslink points and less orderly scaffold. However, this condition changes when the CNC concentration becomes higher than 3 %. At higher CNC concentrations, CNC plays the leading role in hydrogel formation and Pluronic F127 supports the gels' integrity. Critical strain seems to be slightly affected at higher CNC concentrations since the

network is not dependent on the array of Pluronic F127 micelles. Gelation mechanism is proposed as shown in Figure 4.13.

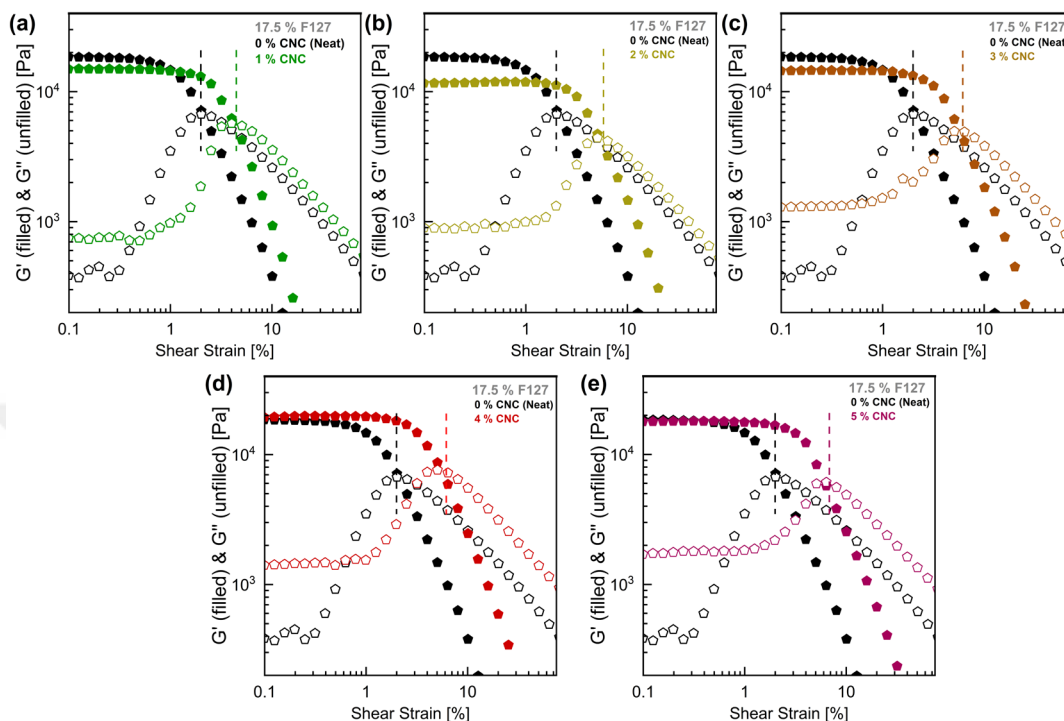


Figure 4.11: Amplitude Sweep Tests of 17.5 % w/w Pluronic F127 Containing Complexes.

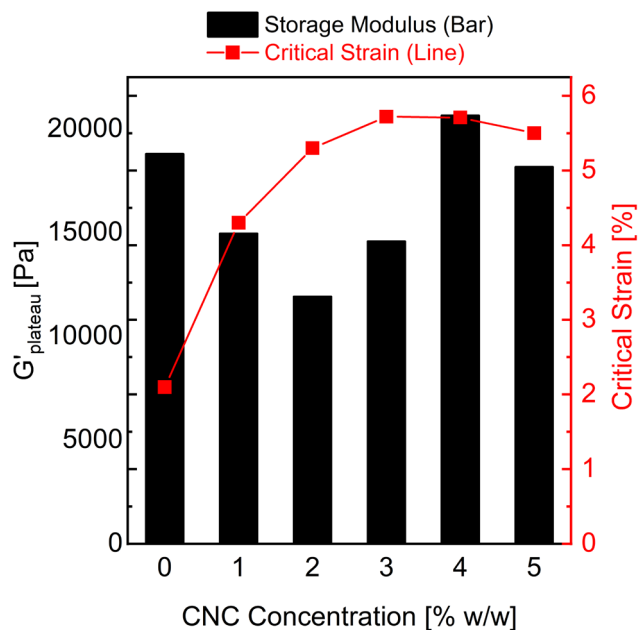


Figure 4.12: Plateau Storage Modulus and Critical Strain vs CNC Concentration.

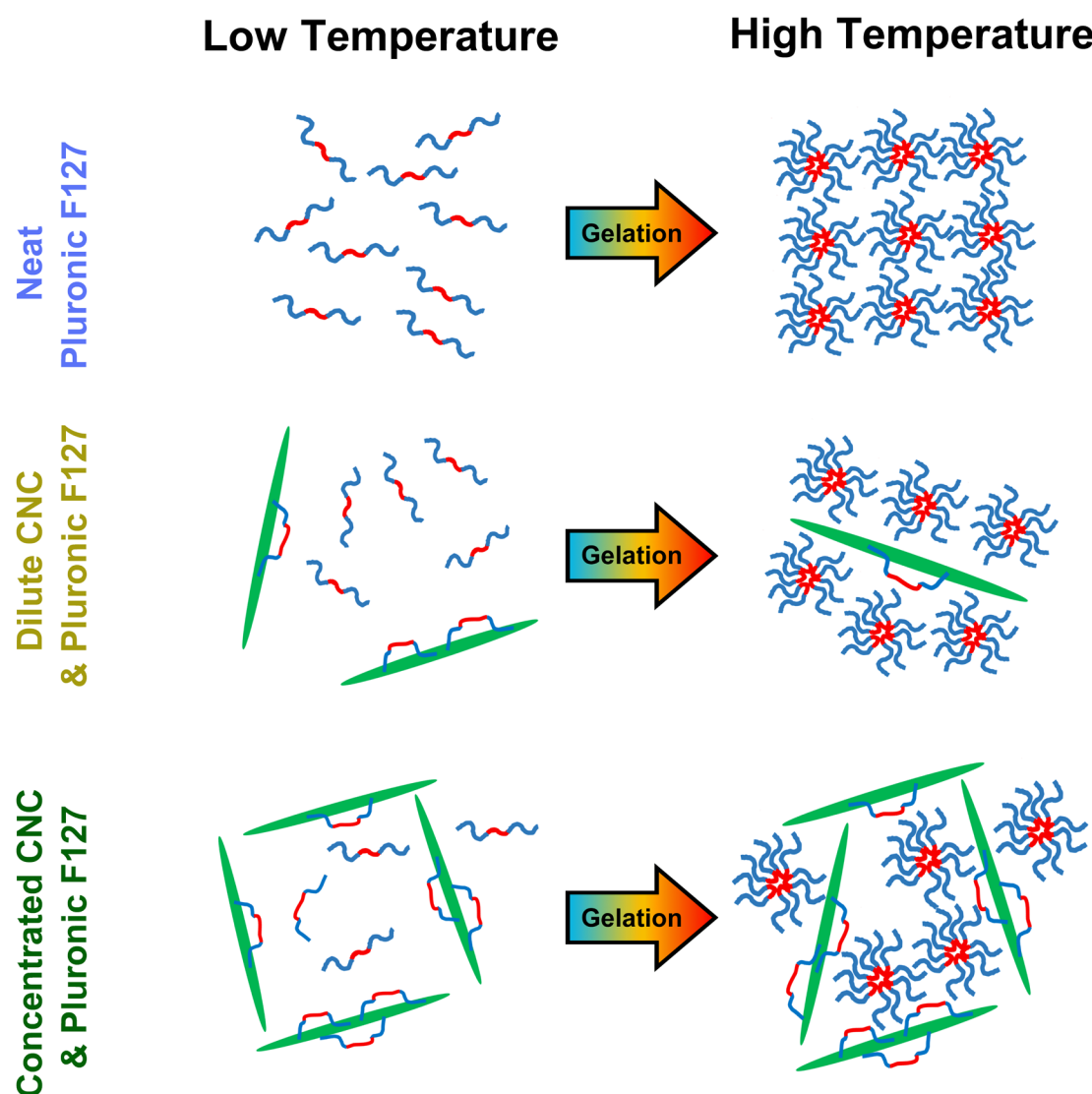


Figure 4.13: Gelation Mechanism of Pluronic F127 and CNC Based Complexes.

Since it is aimed to develop an injectable and thermo-responsive hydrogel, the recovery of hydrogel shortly after a deformation must also be analyzed. A deformation-recovery experiment was performed following a similar approach used by Pang and coworkers. [165] Before applying a higher strain of 20 %, the storage modulus of 1 % CNC and 17.5 % F127 containing hydrogel under a lower strain of 0.1 % was measured for 30 seconds. The storage modulus was found 16000 GPa. For 2 minutes, 20 % strain is applied on the gel, loss modulus surpassed the storage modulus during the application. This is the indication that gel became liquified. Following reducing the strain back to 0.1

%, hydrogel is mechanically recovered. This analysis demonstrates that hydrogel is injectable at the body temperature.

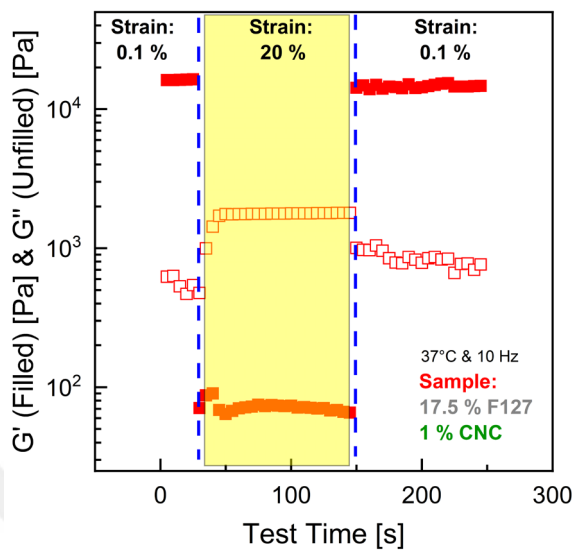


Figure 4.14: Recovery Test done for 1 % CNC and 17.5 % Pluronic F127 Containing Hydrogels.

Chapter 5:

CONCLUSIONS AND OUTLOOK

In this study, we aimed to understand gelation mechanism of aqueous CNC suspensions in presence of micelles formed by assembly of cationic and non-ionic surfactants. CNC is an ideal nanomaterial for the manufacture of biocompatible hydrogels specialized for medical and industrial applications. They are non-toxic, lightweight, hydrophilic. High surface area with sulfate ester groups and hydroxyl groups are promising for chemically conjugation and physical adsorption of bioactive molecules. Especially, CNC-based physically crosslinked hydrogels attracted great interest due to easy of manufacture and non-chemical modification demanding nature.

It is extremely important to tune the mechanical features of a hydrogel as these features possess great importance generally in tissue engineering applications. The stiffness of the hydrogel mimicking extracellular matrix can decide the differentiation direction of the stem cell or the rate of drug release. Our objective in the project named “Surfactant Driven Liquid to Soft Solid Transition of Cellulose Nanocrystal Suspensions” is to analyze the effect of charge of the micelle (positive vs neutral) in aqueous CNC suspensions’ gelation and in resultant gels’ stiffness. Even though both $[C_{10}mim][Cl]$ and $[C_{10}mim][FeCl_4]$ have the identical size, shape and CMC, the network formed by negative CNC and positive $[C_{10}mim][Cl]$ micelles were stronger than the network formed by negative CNC and neutral $[C_{10}mim][FeCl_4]$ micelles at intermediate concentrations. When excessive free micelles start to form in the complex (which means surfactant concentration goes high), network weakens due to repulsion between positive micelles. In contrast, increasing the number of $[C_{10}mim][FeCl_4]$ micelles lead to neither weaker nor stronger network formation. It is also beneficial to perform a further study to understand the effect of counterion valence in CNC gelation. By means of it, we might be able to confirm existing interactions between head groups, counterions, nanocrystals and medium and discover the unforeseen ones. As a summary, tuning only counterion of a surfactant may change the overall mechanical features of the hydrogel significantly.

In the second project named as “Temperature Induced Gelation of Cellulose Nanocrystal Suspensions”, thermo-responsive and injectable hydrogels are aimed to be developed combining thermo-responsive Pluronic F127 block copolymer and CNC.

Similarly, phase transition is achieved by micellization of the non-ionic surfactant and gelation mechanism is proposed comparing bulk rheology of Pluronic F127 and CNC containing complexes. The adsorption of hydrophilic sides of Pluronic F127 to CNC causes a delay in gelation (which means gelation occurs at a higher temperature than neat Pluronic F127 at the same concentration undergoes at). At lower CNC concentrations, the scaffold of hydrogel is mostly Pluronic F127. As CNC concentration rises, network becomes the product of connected CNC network. The resultant product is thermo-reversible, and it can be mechanically recovered after the application of a strain. This complex can be used as a dual drug delivery system as both Pluronic F127 and CNC are able to bring drugs to the applied tissue. Although the scope of the project is limited to investigation of bulk rheology of Pluronic F127 and CNC complexes, further experiments can be conducted to analyze the dual drug releasing performance of both components and discover the performance of different Pluronics with varying molecular compositions in similar applications.

In summary, the effect of addition of cationic and non-ionic surfactants (at both below and above CMC) to aqueous CNC suspensions' gelation and to complexes' final mechanical features were investigated. Our study proposes mechanisms for interaction between $[C_{10}mim][Cl]$ – CNC, $[C_{10}mim][FeCl_4]$ – CNC and $(PEO)_{100}-b-(PPO)_{65}-b-(PEO)_{100}$ – CNC and lends assistance to new studies about CNC-based hydrogels crosslinked by macromolecules of polymers and surfactants.

BIBLIOGRAPHY

- [1] P.-G. de Gennes, "Soft Matter (Nobel Lecture)," *Angewandte Chemie International Edition in English*, vol. 31, no. 7, pp. 842-845, 1992, doi: 10.1002/anie.199208421.
- [2] L. S. Hirst, *Fundamentals of soft matter science*, Second edition. ed. Boca Raton, FL: CRC Press, 2020.
- [3] J. S. Leng, p. r. p. f. t. t. I. S. o. P. Selected, and D. H. K. Fabrication of Advanced Materials, "Multi-Functional Soft Smart Materials and their Applications," *Advanced Materials Research*, vol. 410, p. 25, 2012.
- [4] H. J. van der Linden, S. Herber, W. Olthuis, and P. Bergveld, "Stimulus-sensitive hydrogels and their applications in chemical (micro)analysis," *The Analyst*, vol. 128, no. 4, pp. 325-331, 2003.
- [5] S. Sant, M. J. Hancock, J. P. Donnelly, D. Iyer, and A. Khademhosseini, "Biomimetic gradient hydrogels for tissue engineering," *The Canadian Journal of Chemical Engineering*, vol. 88, no. 6, pp. 899-911, 2010, doi: 10.1002/cjce.20411.
- [6] M. Bahram, *An Introduction to Hydrogels and Some Recent Applications*, [sine loco]: IntechOpen, 2016.
- [7] M. Rizwan *et al.*, "pH Sensitive Hydrogels in Drug Delivery: Brief History, Properties, Swelling, and Release Mechanism, Material Selection and Applications," *Polymers*, vol. 9, no. 4, 2017, doi: 10.3390/polym9040137.
- [8] G. Grassi *et al.*, "Temperature-sensitive hydrogels : Potential therapeutic applications," *American Journal of Drug Delivery*, vol. 3, no. 4, pp. 239-251, 2005, doi: 10.2165/00137696-200503040-00004.
- [9] Y. Qiu and K. Park, "Environment-sensitive hydrogels for drug delivery," *Advanced Drug Delivery Reviews*, vol. 64, no. Supplement, pp. 49-60, 2012, doi: 10.1016/j.addr.2012.09.024.
- [10] C. T. Huynh, M. K. Nguyen, G. Y. Tonga, L. Longé, V. M. Rotello, and E. Alsberg, "Photocleavable Hydrogels for Light-Triggered siRNA Release," *Advanced Healthcare Materials*, vol. 5, no. 3, pp. 305-310, 2016, doi: 10.1002/adhm.201500778.

- [11] C. Li, A. Iscen, L. C. Palmer, G. C. Schatz, and S. I. Stupp, "Light-Driven Expansion of Spiropyran Hydrogels," *Journal of the American Chemical Society*, vol. 142, no. 18, pp. 8447-8453, 2020, doi: 10.1021/jacs.0c02201.
- [12] T. R. Hoare and D. S. Kohane, "Hydrogels in drug delivery: Progress and challenges," *Polymer*, vol. 49, no. 8, pp. 1993-2007, 2008, doi: 10.1016/j.polymer.2008.01.027.
- [13] B. Oh, R. B. Melchert, and C. H. Lee, "Biomimicking Robust Hydrogel for the Mesenchymal Stem Cell Carrier," *Pharmaceutical research*, vol. 32, no. 10, pp. 3213-27, 2015, doi: 10.1007/s11095-015-1698-y.
- [14] W. Chen *et al.*, "Gene-Hydrogel Microenvironment Regulates Extracellular Matrix Metabolism Balance in Nucleus Pulposus," *Advanced Science*, vol. 7, no. 1, 2020, doi: 10.1002/advs.201902099.
- [15] Z. Sekhavat Pour and M. Ghaemy, "Removal of dyes and heavy metal ions from water by magnetic hydrogel beads based on poly(vinyl alcohol)/carboxymethyl starch-g-poly(vinyl imidazole)," *RSC Advances*, vol. 5, no. 79, pp. 64106-64118, 2015, doi: 10.1039/C5RA08025H.
- [16] B. Chan and K. Leong, "Scaffolding in tissue engineering: general approaches and tissue-specific considerations," *European Spine Journal*, vol. 17, no. Supplement 4, pp. 467-479, 2008.
- [17] P. Priyadarshani, Y. Li, S. Yang, and L. Yao, "Injectable hydrogel provides growth-permissive environment for human nucleus pulposus cells," *Journal of Biomedical Materials Research Part A*, vol. 104, no. 2, pp. 419-426, 2016, doi: 10.1002/jbm.a.35580.
- [18] S. Žigon-Branc *et al.*, "Impact of Hydrogel Stiffness on Differentiation of Human Adipose-Derived Stem Cell Microspheroids," *Tissue Engineering. Part A*, vol. 25, no. 19-20, pp. 1369-1380, 2019, doi: 10.1089/ten.tea.2018.0237.
- [19] W. Junhua *et al.*, "Rationally designed synthetic protein hydrogels with predictable mechanical properties," *Nature Communications*, doi: 10.1038/s41467-018-02917-6.
- [20] F. Flores-Céspedes, M. Villafranca-Sánchez, and M. Fernández-Pérez, "Alginate-based hydrogels modified with olive pomace and lignin to removal organic pollutants from aqueous solutions," *International journal of biological*

- macromolecules*, vol. 153, pp. 883-891, 2020, doi: 10.1016/j.ijbiomac.2020.03.081.
- [21] A. Borzacchiello *et al.*, "Chitosan-based hydrogels: Synthesis and characterization," *Journal of Materials Science: Materials in Medicine : Official Journal of the European Society for Biomaterials*, vol. 12, no. 10-12, pp. 861-864, 2001, doi: 10.1023/A:1012851402759.
- [22] W. E. Hennink, O. Franssen, W. N. E. van Dijk-Wolthuis, and H. Talsma, "Dextran hydrogels for the controlled release of proteins," *Journal of Controlled Release*, vol. 48, no. 2, pp. 107-114, 1997, doi: 10.1016/S0168-3659(97)00047-3.
- [23] C. Chang and L. Zhang, "Cellulose-based hydrogels: Present status and application prospects," *Carbohydrate Polymers*, vol. 84, no. 1, pp. 40-53, 2011, doi: 10.1016/j.carbpol.2010.12.023.
- [24] L. H. Fu, C. Qi, M. G. Ma, and P. Wan, "Multifunctional cellulose-based hydrogels for biomedical applications," *Journal of materials chemistry. B*, vol. 7, no. 10, pp. 1541-1562, 2019, doi: 10.1039/c8tb02331j.
- [25] M. C. Catoira, L. Fusaro, D. Di Francesco, M. Ramella, and F. Boccafroschi, "Overview of natural hydrogels for regenerative medicine applications," *Journal of Materials Science: Materials in Medicine : Official Journal of the European Society for Biomaterials*, vol. 30, no. 10, pp. 1-10, 2019, doi: 10.1007/s10856-019-6318-7.
- [26] H. Zhao, J. H. Kwak, Z. Conrad Zhang, H. M. Brown, B. W. Arey, and J. E. Holladay, "Studying cellulose fiber structure by SEM, XRD, NMR and acid hydrolysis," *Carbohydrate polymers.*, vol. 68, no. 2, pp. 235-241, 2007.
- [27] W. Y. Hamad, *Cellulose Nanocrystals : Properties, Production and Applications*, 2nd ed. ed. Somerset: John Wiley & Sons, Incorporated, 2017.
- [28] L. Lewis, S. G. Hatzikiriakos, W. Y. Hamad, and M. J. MacLachlan, "Freeze–Thaw Gelation of Cellulose Nanocrystals," *ACS Macro Letters*, pp. 486-491, 2019, doi: 10.1021/acsmacrolett.9b00140.
- [29] K. Osamu, T. Andy, L. Lev, Y. H. Wadood, and J. M. Mark, "Unwinding a spiral of cellulose nanocrystals for stimuli-responsive stretchable optics," *Nature Communications*, vol. 10, no. 1, pp. 1-7doi: 10.1038/s41467-019-08351-6.

- [30] A. E. Way, L. Hsu, K. Shanmuganathan, C. Weder, and S. J. Rowan, "pH-Responsive Cellulose Nanocrystal Gels and Nanocomposites," *ACS Macro Letters*, vol. 1, no. 8, pp. 1001-1006, 2012, doi: 10.1021/mz3003006.
- [31] B. L. Tardy *et al.*, "Nanocellulose-surfactant interactions," *Current Opinion in Colloid & Interface Science*, vol. 29, pp. 57-67, 2017, doi: 10.1016/j.cocis.2017.02.004.
- [32] K. M. Z. Hossain *et al.*, "Cationic surfactants as a non-covalent linker for oxidised cellulose nanofibrils and starch-based hydrogels," *Carbohydrate Polymers*, vol. 233, 2020, doi: 10.1016/j.carbpol.2019.115816.
- [33] D. Ranjbar and S. G. Hatzikiriakos, "Effect of Ionic Surfactants on the Viscoelastic Properties of Chiral Nematic Cellulose Nanocrystal Suspensions," *Langmuir : the ACS journal of surfaces and colloids*, vol. 36, no. 1, pp. 293-301, 2020, doi: 10.1021/acs.langmuir.9b03437.
- [34] N. Quennouze, S. M. Hashmi, H. S. Choi, J. W. Kim, and C. O. Osuji, "Rheology of cellulose nanofibrils in the presence of surfactants," *Soft matter*, vol. 12, no. 1, pp. 157-64, 2016, doi: 10.1039/c5sm01803j.
- [35] P. Brown *et al.*, "Properties of new magnetic surfactants," *Langmuir : the ACS journal of surfaces and colloids*, vol. 29, no. 10, pp. 3246-51, 2013, doi: 10.1021/la400113r.
- [36] M. Börjesson and G. Westman, "Crystalline Nanocellulose — Preparation, Modification, and Properties," in *Cellulose - Fundamental Aspects and Current Trends*: InTech, 2015.
- [37] M. Jawaid and F. Mohammad, *Nanocellulose and nanohydrogel matrices : biotechnological and biomedical applications*, Weinheim, Germany: Wiley-VCH, 2017.
- [38] D. Klemm, H.-P. Schmauder, T. Heinze, E. J. Vandamme, S. De Baets, and A. Steinbüchel, "Cellulose," 2005, doi: 10.1002/3527600035.bpol6010.
- [39] G. Johnsy and S. N. Sabapathi, "Cellulose nanocrystals: synthesis, functional properties, and applications," *Nanotechnology, Science and Applications*, pp. 45-54, doi: 10.2147/NSA.S64386.
- [40] Y. Pu, D. Zhang, P. M. Singh, and A. J. Ragauskas, "The new forestry biofuels sector," *Biofuels, Bioproducts and Biorefining*, vol. 2, no. 1, pp. 58-73, 2008, doi: 10.1002/bbb.48.

- [41] B. B. Hallac and A. J. Ragauskas, "Analyzing cellulose degree of polymerization and its relevancy to cellulosic ethanol," *Biofuels, Bioproducts and Biorefining*, vol. 5, no. 2, pp. 215-225, 2011, doi: 10.1002/bbb.269.
- [42] L. Berglund, "Cellulose-Based Nanocomposites," in *Natural Fibers, Biopolymers, and Biocomposites*: CRC Press, 2005.
- [43] T. Wüstenberg, *Cellulose and Cellulose Derivatives in the Food Industry : Fundamentals and Applications*, Baden-Württemberg, Germany: Wiley-VCH, 2015.
- [44] A. Mihranyan, "Cellulose from cladophorales green algae: From environmental problem to high-tech composite materials," *Journal of Applied Polymer Science*, vol. 119, no. 4, pp. 2449-2460, 2011, doi: 10.1002/app.32959.
- [45] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, "Cellulose nanomaterials review: structure, properties and nanocomposites," *Chem. Soc. Rev.*, vol. 40, no. 7, pp. 3941-94, 2011, doi: 10.1039/c0cs00108b.
- [46] P. K. Gupta, V. Uniyal, and S. Naithani, "Polymorphic transformation of cellulose I to cellulose II by alkali pretreatment and urea as an additive," *Carbohydrate Polymers*, vol. 94, no. 2, pp. 843-849, 2013, doi: 10.1016/j.carbpol.2013.02.012.
- [47] L. Y. Yatsu, T. A. Calamari, and R. R. Benerito, "Conversion of Cellulose I to Stable Cellulose III," *Textile Research Journal*, vol. 56, no. 7, pp. 419-424, 1986, doi: 10.1177/004051758605600704.
- [48] B. L. Pelegrini *et al.*, "Cellulose Nanocrystals as a Sustainable Raw Material: Cytotoxicity and Applications on Healthcare Technology," *Macromolecular Materials and Engineering*, 2019, doi: 10.1002/mame.201900092.
- [49] B. B. Buchanan, W. Gruissem, and R. L. Jones, *Biochemistry & molecular biology of plants*. Rockville, Md.: American Society of Plant Physiologists (in English), 2000.
- [50] W. Chen, H. Yu, Y. Liu, Y. Hai, M. Zhang, and P. Chen, "Isolation and characterization of cellulose nanofibers from four plant cellulose fibers using a chemical-ultrasonic process," *Cellulose*, vol. 18, no. 2, pp. 433-442, 2011.
- [51] T. Heinze, W. G. Glasser, P. American Chemical Society. Cellulose, and D. Textile, *Cellulose derivatives : modification, characterization, and nanostructures* (ACS symposium series, 0097-6156 ; 688). Washington, DC: American Chemical Society (in English), 1998.

- [52] G. R. Majid and G. Behnouch, "Mechanical Properties of Cellulose Nanocrystal (CNC) Bundles: Coarse-Grained Molecular Dynamic Simulation," *Journal of Composites Science*, vol. 3, no. 2, doi: 10.3390/jcs3020057.
- [53] T. Ayub, S. U. Khan, and F. A. Memon, "Mechanical characteristics of hardened concrete with different mineral admixtures: a review," *TheScientificWorldJournal*, vol. 2014, p. 875082, 2014, doi: 10.1155/2014/875082.
- [54] A. Dufresne, "Nanocellulose Processing Properties and Potential Applications," *Current Forestry Reports*, vol. 5, no. 2, pp. 76-89, 2019, doi: 10.1007/s40725-019-00088-1.
- [55] M. N. Belgacem and A. Gandini, *Monomers, polymers and composites from renewable resources*, 1st ed. ed. Amsterdam ;: Elsevier, 2008.
- [56] A. Dufresne, "Comparing the mechanical properties of high performances polymer nanocomposites from biological sources," *Journal of nanoscience and nanotechnology*, vol. 6, no. 2, pp. 322-30, 2006.
- [57] V. Favier, G. R. Canova, J. Y. Cavaillé, H. Chanzy, A. Dufresne, and C. Gauthier, "Nanocomposite materials from latex and cellulose whiskers," *Polymers for Advanced Technologies*, vol. 6, no. 5, pp. 351-355, 1995, doi: 10.1002/pat.1995.220060514.
- [58] D. G. Gray, "Order and gelation of cellulose nanocrystal suspensions: An overview of some issues," *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, vol. 376, no. 2112, 2018, doi: 10.1098/rsta.2017.0038.
- [59] T. Hiratani, W. Y. Hamad, and M. J. MacLachlan, "Transparent Depolarizing Organic and Inorganic Films for Optics and Sensors," *Advanced Materials*, vol. 29, no. 13, 2017, doi: 10.1002/adma.201606083.
- [60] S. Shafiei-Sabet, W. Y. Hamad, and S. G. Hatzikiriakos, "Rheology of nanocrystalline cellulose aqueous suspensions," *Langmuir : the ACS journal of surfaces and colloids*, vol. 28, no. 49, pp. 17124-33, 2012, doi: 10.1021/la303380v.
- [61] S. Xu, N. Girouard, J. C. Meredith, G. Schueneman, and M. L. Shofner, "Mechanical and thermal properties of waterborne epoxy composites containing

- cellulose nanocrystals," *Polymer (United Kingdom)*, vol. 54, no. 24, pp. 6589-6598, 2013, doi: 10.1016/j.polymer.2013.10.011.
- [62] V. Kupka *et al.*, "Well-dispersed polyurethane/cellulose nanocrystal nanocomposites synthesized by a solvent-free procedure in bulk," *Polymer Composites*, vol. 40, pp. E456-E465, 2019, doi: 10.1002/pc.24748.
- [63] Z. Shi, G. O. Phillips, and G. Yang, "Nanocellulose electroconductive composites," *Nanoscale*, vol. 5, no. 8, pp. 3194-201, 2013, doi: 10.1039/c3nr00408b.
- [64] C. Hänsel, E. Lizundia, and D. Kundu, "A Single Li-Ion Conductor Based on Cellulose," *ACS Applied Energy Materials*, vol. 2, no. 8, pp. 5686-5691, 2019, doi: 10.1021/acsaem.9b00821.
- [65] F. Montes, T. Fu, J. P. Youngblood, and J. Weiss, "Rheological impact of using cellulose nanocrystals (CNC) in cement pastes," *Construction and Building Materials*, vol. 235, 2020, doi: 10.1016/j.conbuildmat.2019.117497.
- [66] T. Fu, F. Montes, P. Suraneni, J. Youngblood, and J. Weiss, "The Influence of Cellulose Nanocrystals on the Hydration and Flexural Strength of Portland Cement Pastes," *Polymers*, vol. 9, no. 9, 2017, doi: 10.3390/polym9090424.
- [67] M. C. Li, Q. Wu, K. Song, Y. Qing, and Y. Wu, "Cellulose nanoparticles as modifiers for rheology and fluid loss in bentonite water-based fluids," *ACS Applied Materials and Interfaces*, vol. 7, no. 8, pp. 5009-5016, 2015, doi: 10.1021/acsami.5b00498.
- [68] V. Rossatto, T. Picatonotto, D. Vione, and M. Eugenia Carlotti*, "Behavior of Some Rheological Modifiers Used in Cosmetics Under Photocatalytic Conditions," *Journal of Dispersion Science and Technology*, vol. 24, no. 2, pp. 259-271, 2003, doi: 10.1081/DIS-120019977.
- [69] H. Du, W. Liu, M. Zhang, C. Si, X. Zhang, and B. Li, "Cellulose nanocrystals and cellulose nanofibrils based hydrogels for biomedical applications," *Carbohydrate Polymers*, vol. 209, pp. 130-144, 2019, doi: 10.1016/j.carbpol.2019.01.020.
- [70] B. B. Mendes, M. Gómez-Florit, R. A. Pires, R. M. A. Domingues, R. L. Reis, and M. E. Gomes, "Human-based fibrillar nanocomposite hydrogels as bioinstructive matrices to tune stem cell behavior," *Nanoscale*, vol. 10, no. 36, pp. 17388-17401, 2018, doi: 10.1039/c8nr04273j.

- [71] U. G. Sampath, Y. C. Ching, C. H. Chuah, R. Singh, and P. C. Lin, "Preparation and characterization of nanocellulose reinforced semi-interpenetrating polymer network of chitosan hydrogel," *CELLULOSE -ANDOVER-*, vol. 24, no. 5, pp. 2215-2228, 2017.
- [72] N. Lin, A. Gèze, D. Wouessidjewe, J. Huang, and A. Dufresne, "Biocompatible Double-Membrane Hydrogels from Cationic Cellulose Nanocrystals and Anionic Alginate as Complexing Drugs Codelivery," *ACS applied materials & interfaces*, vol. 8, no. 11, pp. 6880-9, 2016, doi: 10.1021/acsami.6b00555.
- [73] J. Supramaniam, R. Adnan, N. H. Mohd Kaus, and R. Bushra, "Magnetic nanocellulose alginate hydrogel beads as potential drug delivery system," *International Journal of Biological Macromolecules*, vol. 118, no. Part A, pp. 640-648, 2018, doi: 10.1016/j.ijbiomac.2018.06.043.
- [74] S. Y. Ooi, I. Ahmad, and M. C. I. M. Amin, "Cellulose nanocrystals extracted from rice husks as a reinforcing material in gelatin hydrogels for use in controlled drug delivery systems," *Industrial Crops & Products*, vol. 93, pp. 227-234, 2016, doi: 10.1016/j.indcrop.2015.11.082.
- [75] C. Qiao, G. Chen, J. Zhang, and J. Yao, "Structure and rheological properties of cellulose nanocrystals suspension," *Food Hydrocolloids*, vol. 55, pp. 19-25, 2016, doi: 10.1016/j.foodhyd.2015.11.005.
- [76] Q. Wu *et al.*, "Rheological behavior of cellulose nanocrystal suspension: Influence of concentration and aspect ratio," *Journal of Applied Polymer Science*, vol. 131, no. 15, 2014, doi: 10.1002/app.40525.
- [77] M. Srinivasarao, "RHEOLOGY AND RHEO-OPTICS OF POLYMER LIQUID CRYSTALS," *International journal of modern physics. B, Condensed matter physics, statistical physics, applied physics.*, vol. 9, no. 18-19, p. 2515, 1995.
- [78] M. Chau *et al.*, "Ion-Mediated Gelation of Aqueous Suspensions of Cellulose Nanocrystals," *Biomacromolecules*, vol. 16, no. 8, pp. 2455-62, 2015, doi: 10.1021/acs.biomac.5b00701.
- [79] C. N. Likos, "Effective interactions in soft condensed matter physics," *Physics Reports*, vol. 348, no. 4, pp. 267-439, 2001, doi: 10.1016/S0370-1573(00)00141-1.
- [80] T. Phan-Xuan, A. Thuresson, M. Skepö, A. Labrador, R. Bordes, and A. Matic, "Aggregation behavior of aqueous cellulose nanocrystals: the effect of inorganic

- salts," *Cellulose*, vol. 23, no. 6, pp. 3653-3663, 2016, doi: 10.1007/s10570-016-1080-1.
- [81] G. Lenfant, M.-C. Heuzey, T. G. M. van de Ven, and P. J. Carreau, "A comparative study of ECNC and CNC suspensions: effect of salt on rheological properties," *Rheologica Acta*, vol. 56, no. 1, pp. 51-62, 2017, doi: 10.1007/s00397-016-0979-7.
- [82] N. Dhar, D. Au, R. C. Berry, and K. C. Tam, "Interactions of nanocrystalline cellulose with an oppositely charged surfactant in aqueous medium," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 415, pp. 310-319, 2012, doi: 10.1016/j.colsurfa.2012.09.010.
- [83] E. Caló and V. V. Khutoryanskiy, "Biomedical applications of hydrogels: A review of patents and commercial products," *European polymer journal*.
- [84] J. Yang *et al.*, "Synthesis and characterization of mechanically flexible and tough cellulose nanocrystals-polyacrylamide nanocomposite hydrogels," *Cellulose*, vol. 20, no. 1, pp. 227-237, 2013, doi: 10.1007/s10570-012-9841-y.
- [85] N. Kayra and A. O. z. Aytekin, "Synthesis of Cellulose-Based Hydrogels: Preparation, Formation, Mixture, and Modification," in *Cellulose-Based Superabsorbent Hydrogels*, (Polymers and Polymeric Composites: A Reference Series 2510-3466: Cham : Springer International Publishing : Springer, 2019, pp. 407-434.
- [86] X. Qinghua, J. Yunzhong, S. Qiucun, F. Yingjuan, X. Yongjian, and J. Liqiang, "Fabrication of Cellulose Nanocrystal/Chitosan Hydrogel for Controlled Drug Release," *Nanomaterials*, vol. 9, no. 2, doi: 10.3390/nano9020253.
- [87] D. Myers, *Surfactant science and technology*, 2nd ed. ed. New York: VCH (in English), 1992.
- [88] Z. Tang *et al.*, "Adsorption characteristics of surfactants on secondary wood fiber surface," 2016.
- [89] R. Prathapan, R. Thapa, G. Garnier, and R. F. Tabor, "Modulating the zeta potential of cellulose nanocrystals using salts and surfactants," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 509, pp. 11-18, 2016, doi: 10.1016/j.colsurfa.2016.08.075.

- [90] L. H. Torn, L. K. Koopal, d. A. Keizer, and J. Lyklema, "Adsorption of nonionic surfactants on cellulose surfaces : adsorbed amounts and kinetics," *Langmuir*, vol. 21, no. 17, pp. 7768-7775, 2005.
- [91] A. Pitto-Barry and N. P. E. Barry, "Pluronic® block-copolymers in medicine: From chemical and biological versatility to rationalisation and clinical advances," *Polymer Chemistry*, vol. 5, no. 10, pp. 3291-3297, 2014, doi: 10.1039/c4py00039k.
- [92] Z. Emami, Q. Meng, G. Pircheraghi, and I. Manas-Zloczower, "Use of surfactants in cellulose nanowhisker/epoxy nanocomposites: effect on filler dispersion and system properties," *Cellulose*, vol. 22, no. 5, pp. 3161-3176, 2015, doi: 10.1007/s10570-015-0728-6.
- [93] G. Guidetti, S. Atifi, S. Vignolini, and W. Y. Hamad, "Flexible Photonic Cellulose Nanocrystal Films," *Advanced materials (Deerfield Beach, Fla.)*, vol. 28, no. 45, pp. 10042-10047, 2016, doi: 10.1002/adma.201603386.
- [94] S. Kalia, B. S. Kaith, and K. Inderjeet, *Cellulose fibers : bio- and nano-polymer composites ; green chemistry and technology*, Berlin ;: Springer, 2011.
- [95] A. María Graciela *et al.*, "Isolation and Characterization of Cellulose Nanocrystals from Rejected Fibers Originated in the Kraft Pulping Process," *Polymers*, vol. 10, no. 10, doi: 10.3390/polym10101145.
- [96] Y. Oishi, M. Nakaya, E. Matsui, and A. Hotta, "Structural and mechanical properties of cellulose composites made of isolated cellulose nanofibers and poly(vinyl alcohol)," *Composites: Part A, Applied Science & Manufacturing*, vol. 73, 2015.
- [97] H. Kargarzadeh, M. Ioelovich, I. Ahmad, S. Thomas, and A. Dufresne, "Methods for Extraction of Nanocellulose from Various Sources," in *Handbook of Nanocellulose and Cellulose Nanocomposites*: Wiley-VCH Verlag GmbH & Co. KGaA : Weinheim, Germany, 2017, pp. 1-49.
- [98] T. Li *et al.*, "Cellulose ionic conductors with high differential thermal voltage for low-grade heat harvesting," *Nature Materials*, vol. 18, no. 6, pp. 608-613, 2019, doi: 10.1038/s41563-019-0315-6.
- [99] A. Raisipour-Shirazi, Z. Ahmadi, and H. Garmabi, "Polylactic acid nanocomposites toughened with nanofibrillated cellulose: microstructure,

- thermal, and mechanical properties," *Iranian Polymer Journal*, vol. 27, no. 10, pp. 785-794, 2018, doi: 10.1007/s13726-018-0651-4.
- [100] M. A. Hubbe *et al.*, "Rheology of nanocellulose-rich aqueous suspensions: A review," *BioResources*, vol. 12, no. 4, pp. 9556-9661, 2017, doi: 10.15376/biores.12.4.Hubbe.
- [101] H. Oguzlu, C. Danumah, and Y. Boluk, "The role of dilute and semi-dilute cellulose nanocrystal (CNC) suspensions on the rheology of carboxymethyl cellulose (CMC) solutions," *The Canadian Journal of Chemical Engineering*, vol. 94, no. 10, pp. 1841-1847, 2016, doi: 10.1002/cjce.22597.
- [102] A. Leszczyńska, P. Radzik, E. Szefer, M. Mičušík, M. r. Omastová, and K. Pielichowski, "Surface Modification of Cellulose Nanocrystals with Succinic Anhydride," *Polymers (20734360)*, vol. 11, no. 5, 2019.
- [103] B. M. Trinh and T. Mekonnen, "Hydrophobic esterification of cellulose nanocrystals for epoxy reinforcement," *Polymer*, vol. 155, pp. 64-74, 2018, doi: 10.1016/j.polymer.2018.08.076.
- [104] S. Xiang *et al.*, "Cellulose Nanocrystal Surface Cationization: A New Fungicide with High Activity against *Phycomycetes capsici*," *Molecules (Basel, Switzerland)*, vol. 24, no. 13, 2019, doi: 10.3390/molecules24132467.
- [105] Y. Bespalova, D. Kwon, and N. Vasanthan, "Surface modification and antimicrobial properties of cellulose nanocrystals," *Journal of Applied Polymer Science*, vol. 134, no. 18, 2017, doi: 10.1002/app.44789.
- [106] S. Wohlhauser *et al.*, "Grafting Polymers from Cellulose Nanocrystals: Synthesis, Properties, and Applications," *Macromolecules*, vol. 51, no. 16, pp. 6157-6189, 2018, doi: 10.1021/acs.macromol.8b00733.
- [107] E. E. Ureña-Benavides, G. Ao, V. A. Davis, and C. L. Kitchens, "Rheology and phase behavior of lyotropic cellulose nanocrystal suspensions," *Macromolecules*, vol. 44, no. 22, pp. 8990-8998, 2011.
- [108] K. R. Peddireddy, I. Capron, T. Nicolai, and L. Benyahia, "Gelation Kinetics and Network Structure of Cellulose Nanocrystals in Aqueous Solution," *Biomacromolecules*, vol. 17, no. 10, pp. 3298-3304, 2016.
- [109] Z. Hu, E. D. Cranston, R. Ng, and R. Pelton, "Tuning cellulose nanocrystal gelation with polysaccharides and surfactants," *Langmuir : the ACS journal of surfaces and colloids*, vol. 30, no. 10, pp. 2684-92, 2014, doi: 10.1021/la404977t.

- [110] K. Nesměrák and I. Němcová, "Determination of Critical Micelle Concentration by Electrochemical Means," *Analytical Letters*, vol. 39, no. 6, 2006.
- [111] F. I. El-Dossoki, "Micellization Thermodynamics of Some Imidazolium Ionic Liquids in Aqueous Solutions—Conductometric Study," *Journal of Solution Chemistry*, vol. 42, no. 1, pp. 125-135, 2013, doi: 10.1007/s10953-012-9947-8.
- [112] P. Brown *et al.*, "Magnetic Control over Liquid Surface Properties with Responsive Surfactants," *ANGEWANDTE CHEMIE -INTERNATIONAL EDITION IN ENGLISH-*, vol. 51, no. 10, pp. 2414-2416, 2012.
- [113] H. K. Roobottom and H. D. B. Jenkins, "Thermochemical radii complex ions," *Journal of Chemical Education*, vol. 76, no. 11, 1999.
- [114] A. A. Niazi, B. D. Rabideau, and A. E. Ismail, "Effects of water concentration on the structural and diffusion properties of imidazolium-based ionic liquid-water mixtures," *The journal of physical chemistry. B*, vol. 117, no. 5, pp. 1378-88, 2013, doi: 10.1021/jp3080496.
- [115] E. Kushan, C. Demir, and E. Senses, "Surfactant Driven Liquid to Soft Solid Transition of Cellulose Nanocrystal Suspensions," *Langmuir*, vol. 36, no. 32, pp. 9551-9561, 2020, doi: 10.1021/acs.langmuir.0c01555.
- [116] C. W. Macosko, *Rheology : principles, measurements, and applications* (Advances in interfacial engineering series). New York: VCH (in English), 1994.
- [117] A. I. A. k. Malkin and A. I. Isayev, *Rheology : concepts, methods, and applications*, 3rd edition. ed. Toronto: ChemTec Publishing, 2017.
- [118] T. G. Mezger, *The Rheology Handbook : 4th Edition*, Hannover: Vincentz Network, 2012.
- [119] K. Hyun *et al.*, "A review of nonlinear oscillatory shear tests: Analysis and application of large amplitude oscillatory shear (LAOS)," *Progress in Polymer Science*, vol. 36, no. 12, pp. 1697-1753, 2011, doi: 10.1016/j.progpolymsci.2011.02.002.
- [120] T. Doi, E. Uhlmann, I. Marinescu, and a. O. R. M. C. Safari, *Handbook of Ceramics Grinding and Polishing, 2nd Edition*, 2nd edition. ed. [Erscheinungsort nicht ermittelbar]: William Andrew, 2015.
- [121] M. Barczewski, K. Lewandowski, M. Schmidt, and M. Szostak, "Melt fracture and rheology of linear low density polyethylene - calcium carbonate composites,"

- Polymer Engineering & Science*, vol. 57, no. 9, pp. 998-1004, 2017, doi: 10.1002/pen.24477.
- [122] K. Hyun, S. H. Kim, K. H. Ahn, and S. J. Lee, "Large amplitude oscillatory shear as a way to classify the complex fluids," *Journal of Non-Newtonian Fluid Mechanics*, vol. 107, no. 1, pp. 51-65, 2002, doi: 10.1016/S0377-0257(02)00141-6.
- [123] M. Parthasarathy and D. J. Klingenberg, "Large amplitude oscillatory shear of ER suspensions," *Journal of Non-Newtonian Fluid Mechanics*, vol. 81, no. 1, pp. 83-104, 1999, doi: 10.1016/S0377-0257(98)00096-2.
- [124] S. R. Raghavan and S. A. Khan, "Shear-induced microstructural changes in flocculated suspensions of fumed silica," *Journal of Rheology*, vol. 39, no. 6, pp. 1311-1325, 1995, doi: 10.1122/1.550638.
- [125] S. Chatterjee and P. Chi-leung Hui, "Stimuli-Responsive Hydrogels: An Interdisciplinary Overview," in *Hydrogels [Working Title]*: IntechOpen, 2018.
- [126] H. Dai *et al.*, "A Temperature-Responsive Copolymer Hydrogel in Controlled Drug Delivery," *Macromolecules*, vol. 39, no. 19, pp. 6584-6589, 2006, doi: 10.1021/ma060486p.
- [127] M. C. Koetting, J. T. Peters, S. D. Steichen, and N. A. Peppas, "Stimulus-responsive hydrogels: Theory, modern advances, and applications," *Materials Science & Engineering R*, vol. 93, pp. 1-49, 2015, doi: 10.1016/j.mser.2015.04.001.
- [128] H. Katono, A. Maruyama, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, "Thermo-responsive swelling and drug release switching of interpenetrating polymer networks composed of poly(acrylamide-co-butyl methacrylate) and poly (acrylic acid)," *Journal of Controlled Release*, vol. 16, no. 1, pp. 215-227, 1991, doi: 10.1016/0168-3659(91)90045-F.
- [129] S. Purushotham and R. V. Ramanujan, "Thermoresponsive magnetic composite nanomaterials for multimodal cancer therapy," *Acta Biomaterialia*, vol. 6, no. 2, pp. 502-510, 2010, doi: 10.1016/j.actbio.2009.07.004.
- [130] H. Haiqin, Q. Xiaole, C. Yanhua, and W. Zhenghong, "Thermo-sensitive hydrogels for delivering biotherapeutic molecules: A review," *Saudi Pharmaceutical Journal*, vol. 27, no. 7, pp. 990-999, doi: 10.1016/j.jsps.2019.08.001.

- [131] G. S. Longo, M. Olvera De La Cruz, and I. Szleifer, "Controlling swelling/deswelling of stimuli-responsive hydrogel nanofilms in electric fields," *Soft Matter*, vol. 12, no. 40, pp. 8359-8366, 2016, doi: 10.1039/c6sm01172a.
- [132] Y. Li *et al.*, "Magnetic Hydrogels and Their Potential Biomedical Applications," *Advanced Functional Materials*, vol. 23, no. 6, pp. 660-672, 2013, doi: 10.1002/adfm.201201708.
- [133] Z. Liu, J. Liu, X. Cui, X. Wang, L. Zhang, and P. Tang, "Recent Advances on Magnetic Sensitive Hydrogels in Tissue Engineering," *Frontiers in chemistry*, vol. 8, p. 124, 2020, doi: 10.3389/fchem.2020.00124.
- [134] S. Chatterjee, P. C.-I. Hui, C.-w. Kan, and W. Wang, "Dual-responsive (pH/temperature) Pluronic F-127 hydrogel drug delivery system for textile-based transdermal therapy," *Scientific Reports*, vol. 9, no. 1, pp. 1-13, 2019, doi: 10.1038/s41598-019-48254-6.
- [135] L. Klouda and A. G. Mikos, "Thermoresponsive hydrogels in biomedical applications - a review," *European journal of pharmaceuticals and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V.*, vol. 68, no. 1, pp. 34-45, 2008, doi: 10.1016/j.ejpb.2007.02.025.
- [136] S. Lü, B. Li, B. Ni, Z. Sun, M. Liu, and Q. Wang, "Thermoresponsive injectable hydrogel for three-dimensional cell culture: chondroitin sulfate bioconjugated with poly(N-isopropylacrylamide) synthesized by RAFT polymerization," *Soft Matter*, vol. 7, no. 22, p. 10763, 2011, doi: 10.1039/c1sm06053h.
- [137] J. H. Lee, "Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering," *Biomaterials Research*, vol. 22, no. 1, 2018, doi: 10.1186/s40824-018-0138-6.
- [138] M. Cao *et al.*, "Reversible Thermoresponsive Peptide-PNIPAM Hydrogels for Controlled Drug Delivery," *Biomacromolecules*, vol. 20, no. 9, pp. 3601-3610, 2019, doi: 10.1021/acs.biomac.9b01009.
- [139] Y. Chen *et al.*, "A thermo-/pH-responsive hydrogel (PNIPAM-PDMA-PAA) with diverse nanostructures and gel behaviors as a general drug carrier for drug release," *Polymer Chemistry*, vol. 9, no. 29, pp. 4063-4072, 2018, doi: 10.1039/c8py00838h.

- [140] J. Gan *et al.*, "Biodegradable, thermoresponsive PNIPAM-based hydrogel scaffolds for the sustained release of levofloxacin," *RSC Advances*, vol. 6, no. 39, pp. 32967-32978, 2016, doi: 10.1039/C6RA03045A.
- [141] E. Gioffredi *et al.*, "Pluronic F127 Hydrogel Characterization and Biofabrication in Cellularized Constructs for Tissue Engineering Applications," *Procedia CIRP*, vol. 49, pp. 125-132, 2016, doi: 10.1016/j.procir.2015.11.001.
- [142] M.-Y. Yeh *et al.*, "Reverse thermo-responsive hydrogels prepared from Pluronic F127 and gelatin composite materials," *RSC Advances*, vol. 7, no. 34, pp. 21252-21257, 2017, doi: 10.1039/C7RA01118K.
- [143] J.-K. Cho, H.-J. Kuh, and S.-C. Song, "Injectable poly(organophosphazene) hydrogel system for effective paclitaxel and doxorubicin combination therapy," *Journal of Drug Targeting*, vol. 22, no. 8, 2014.
- [144] K. H. Hong, Y. M. Kim, and S. C. Song, "Fine-Tunable and Injectable 3D Hydrogel for On-Demand Stem Cell Niche," *Advanced science (Weinheim, Baden-Wurttemberg, Germany)*, vol. 6, no. 17, p. 1900597, 2019, doi: 10.1002/advs.201900597.
- [145] B. Shriky *et al.*, "Pluronic F127 thermosensitive injectable smart hydrogels for controlled drug delivery system development," *Journal of Colloid And Interface Science*, vol. 565, pp. 119-130, 2020, doi: 10.1016/j.jcis.2019.12.096.
- [146] C. G. Gebelein, C. E. Carraher, and V. R. Foster, "Overview of Synthetic and Modified Natural Bioactive Polymers," in *Applied Bioactive Polymeric Materials*: Springer US : Boston, MA, 1988, pp. 1-15.
- [147] R. Basak and R. Bandyopadhyay, "Encapsulation of hydrophobic drugs in Pluronic F127 micelles: effects of drug hydrophobicity, solution temperature, and pH," *Langmuir : the ACS journal of surfaces and colloids*, vol. 29, no. 13, pp. 4350-6, 2013, doi: 10.1021/la304836e.
- [148] G. S. Kwon and K. Kataoka, "Block copolymer micelles as long-circulating drug vehicles," *Advanced drug delivery reviews*, vol. 64, pp. 237-245, 2012.
- [149] P. David, L. Kevin, J. John, and B. Helen, "A review of nanocellulose as a novel vehicle for drug delivery," *Nordic Pulp & Paper Research Journal*, vol. 29, no. 1, pp. 105-118, 2014, doi: 10.3183/npprj-2014-29-01-p105-118.
- [150] J. Huang, *Polysaccharide-based nanocrystals : chemistry and applications*, Weinheim, Germany: Wiley-VCH, 2015.

- [151] M. Roman, S. Dong, A. Hirani, and Y. W. Lee, "Cellulose Nanocrystals for Drug Delivery," in *Polysaccharide Materials: Performance by Design*: American Chemical Society : Washington DC, 2010, pp. 81-91.
- [152] R. Z. Khoo, W. S. Chow, and H. Ismail, "Sugarcane bagasse fiber and its cellulose nanocrystals for polymer reinforcement and heavy metal adsorbent: a review," *Cellulose*, vol. 25, no. 8, pp. 4303-4330, 2018, doi: 10.1007/s10570-018-1879-z.
- [153] L. Ma, M. Kohli, and A. Smith, "Nanoparticles for combination drug therapy," *ACS nano*, vol. 7, no. 11, pp. 9518-25, 2013, doi: 10.1021/nn405674m.
- [154] P. Nittayacharn and N. Nasongkla, "Development of self-forming doxorubicin-loaded polymeric depots as an injectable drug delivery system for liver cancer chemotherapy," *Journal of Materials Science: Materials in Medicine : Official Journal of the European Society for Biomaterials*, vol. 28, no. 7, pp. 1-13, 2017, doi: 10.1007/s10856-017-5905-8.
- [155] L. Cao *et al.*, "Synergistic effects of dual growth factor delivery from composite hydrogels incorporating 2-N,6-O-sulphated chitosan on bone regeneration," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 46, no. sup3, pp. S1-S17, 2018, doi: 10.1080/21691401.2018.1488721.
- [156] C. M. Riley, P. W. Fuegy, M. A. Firpo, X. Zheng Shu, G. D. Prestwich, and R. A. Peattie, "Stimulation of in vivo angiogenesis using dual growth factor-loaded crosslinked glycosaminoglycan hydrogels," *Biomaterials*, vol. 27, no. 35, pp. 5935-5943, 2006, doi: 10.1016/j.biomaterials.2006.08.029.
- [157] M. T. Cidade, D. J. Ramos, J. Santos, H. Carrelo, N. Calero, and J. P. Borges, "Injectable Hydrogels Based on Pluronic/Water Systems Filled with Alginate Microparticles for Biomedical Applications," *Materials (1996-1944)*, vol. 12, no. 7, 2019.
- [158] D. Attwood, J. H. Collett, and C. J. Tait, "The micellar properties of the poly(oxyethylene) - poly(oxypropylene) copolymer Pluronic F127 in water and electrolyte solution," *International Journal of Pharmaceutics*, vol. 26, no. 1, pp. 25-33, 1985, doi: 10.1016/0378-5173(85)90197-8.
- [159] E. Schoolaert *et al.*, "Waterborne Electrospinning of Poly(N - isopropylacrylamide) by Control of Environmental Parameters," *ACS Applied Materials & Interfaces*, vol. 9, no. 28, pp. 24100-24110, 2017, doi: 10.1021/acsami.7b05074.

- [160] V. D. Andrey and Y. C. Jan-Michael, "Universality in Nonlinear Elasticity of Biological and Polymeric Networks and Gels," *Macromolecules*, vol. 44, no. 1, 2011.
- [161] S. Vural, K. B. Dikovics, and D. M. Kalyon, "Cross-link density, viscoelasticity and swelling of hydrogels as affected by dispersion of multi-walled carbon nanotubes," *Soft Matter*, vol. 6, no. 16, pp. 3870-3875, 2010, doi: 10.1039/c0sm00099j.
- [162] K. Mortensen, "PEO-related block copolymer surfactants," *Colloids and surfaces. A, Physicochemical and engineering aspects.*, vol. 183, no. 1, p. 277, 2001.
- [163] Y. Li, H. Liu, J. Song, O. J. Rojas, and J. P. Hinestroza, "Adsorption and association of a symmetric PEO-PPO-PEO triblock copolymer on polypropylene, polyethylene, and cellulose surfaces," *ACS applied materials & interfaces*, vol. 3, no. 7, pp. 2349-57, 2011, doi: 10.1021/am200264r.
- [164] I. W. Hamley, J. A. Pople, C. Booth, L. Derici, M. Imp  rator-Clerc, and P. Davidson, "Shear-induced orientation of the body-centered-cubic phase in a diblock copolymer gel," *Physical Review E*, vol. 58, no. 6, pp. 7620-7628, 1998, doi: 10.1103/PhysRevE.58.7620.
- [165] Y. Pang *et al.*, "Endoscopically Injectable Shear-Thinning Hydrogels Facilitating Polyp Removal," *Advanced science (Weinheim, Baden-Wurttemberg, Germany)*, vol. 6, no. 19, p. 1901041, 2019, doi: 10.1002/advs.201901041.