

MEMBRANE FABRICATION VIA SHEAR INDUCED DEPOSITION AND
CHEMICAL STABILIZATION OF CELLULOSE NANOCRYSTALS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY



BY
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMICAL ENGINEERING

AUGUST 2023

Approval of the thesis:

**MEMBRANE FABRICATION VIA SHEAR INDUCED DEPOSITION AND
CHEMICAL STABILIZATION OF CELLULOSE NANOCRYSTALS**

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ABSTRACT

MEMBRANE FABRICATION VIA SHEAR INDUCED DEPOSITION AND CHEMICAL STABILIZATION OF CELLULOSE NANOCRYSTALS

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August 2023, 120 pages

This study shows membrane preparation using cellulose nanocrystals and their chemical crosslinking. Membrane preparation with colloids like cellulose nanocrystals is expected to lead to tuneable membrane structures which would have narrow pore size distribution.

AgNO₃ and KPS (potassium persulfate) were used for crosslinking for TEMPO (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) oxidation CNCs (cellulose nanocrystals). For 2 hours reaction, particle size was found to be stabilized with stabilization of turbidity. Additionally, 0.08 % KPS and 0.36 % AgNO₃ were found to be optimal crosslinker concentrations for the membrane application.

Membrane preparations were done with crossflow deposition of TEMPO CNC suspensions on a porous support with or without addition of NaCl in suspension during deposition. These layers were stabilized with AgNO₃ and KPS crosslinking. Membranes prepared with suspensions not containing any salt were stable for up to 1 week in ethanol solution and 2 days of continuous filtration experiments. It was also observed that the membrane rejection is partly caused by Donnan exclusion. To reduce internal stress in the membrane and prevent cracking, 100 mM NaCl was

added to the TEMPO CNC suspension, which increased the stability of the layer where 5 days of continuous filtration did not change the membrane performance, and higher than 95% blue dextran (5 kDa) rejection was observed for the membrane. Additionally, salt addition led to the formation of cholesteric self assembly on the TEMPO CNC layer. This layer showed visible wavelength Bragg's reflection in wet state where the pitch length decreased to ultraviolet range which could only be observed with scanning electron microscopy.

Keywords: TEMPO Cellulose Nanocrystal, Cholesteric Liquid Crystal, Ultrafiltration, Crossflow, Crosslinking

ÖZ

TEMPO OKSİDASYONLU SELÜLOZ NANOKRİSTALLERİN YÜZEYDE KESME KUVVETİ İLE BİRİKTİRİLMESİ İLE MEMBRAN ÜRETİMİ VE KİMYASAL STABİLİZASYONU

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Ağustos 2023, 120 sayfa

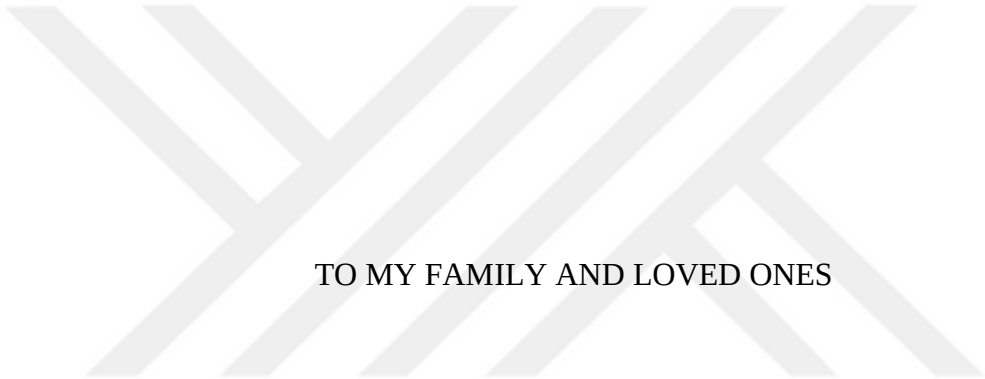
Bu çalışmada, selüloz nanokristalleri (SNK) ile bir membran oluşturma yöntemi ve kimyasal çapraz bağlanması gösterilmiştir. SNK gibi koloidler ile membran üretimiyle gözenek boyutlarının ayarlanabilmesi ve gözenek yapısı kristaller arası boşluklar olacağından dar bir gözenek boyu dağılımı elde edilmesi amaçlanmıştır.

TEMPO ((2,2,6,6-Tetramethylpiperidin-1-yl)oxyl) selüloz nanokristal süspansiyonları AgNO_3 ve potasyum persülfatla (KPS) çapraz bağlanmıştır. Çapraz bağlama sırasında parçacık boyutunun 2 saatin ardından stabilize olduğu bulanıklık ölçümü ile gözlenmiştir. Buna ek olarak, membran üretimi için optimal çapraz bağlama karışımının %0,08 KPS ve %0,36 AgNO_3 olduğu sonucuna varılmıştır.

Elde edilen TEMPO SNK süspansiyonları içine NaCl eklenerek veya eklenmeyerek gözenekli bir destek yüzeyine çapraz akı altında biriktirilmiş ve oluşan katman gümüş nitrat ve potasyum persülfatla çapraz bağlanarak stabilize edilmiştir. TEMPO SNK'ların yerleştirme sırasında tuz eklenmediği ve ardından çapraz bağlandığı durumda etanol solüsyonunda 1 haftaya kadar, devamlı filtrasyonda ise 2 gün stabil kaldığı görülmüştür. Desülfasyon ardından TEMPO

oksidasyona uğramış SNKlarla üretilen membranların mavi dekstran (5 kDa) performansının azaldığı gözlenmiştir. Bu azalma büyük ihtimalle desülfasyon sebebiyle yüzey yükünün azalması sonucu Donnan dışlaması etkisinin azalması kaynaklıdır. Membranlarda iç stresin azaltılması ve oluşan çatlakların azaltılabilmesi için süspansiyonlara depozisyon sırasında 100 mM NaCl eklenmiştir. Depozisyon ardından membranlar çapraz bağlanmış ve elde edilen membranların stabilitesinin 5 gün devamlı saf su filtrasyonunda ve günlük mavi dekstran (5 kDa) reddi testinde %95 red oranında sabit kalabildiği görülmüştür. Ayrıca, elde edilen membranların tuz eklenmesinin ardından kolesterik simetri davranışı gösterdiği görülmüştür. Elde edilen Kolesterik yapı ıslakken görünür renkte Bragg yansıması gösterirken, kurutulduğunda ise perde aralığının ultraviyole bölgeye geçtiği görülmüştür.

Anahtar Kelimeler: TEMPO Selüloz Nanokristal, Ultrafiltrasyon, Çapraz Bağlama, Çapraz Akış, Kolesterik Sıvı Kristal



TO MY FAMILY AND LOVED ONES

ACKNOWLEDGMENTS

I would like to thank my supervisor Prof. Dr. Pınar Zeynep Çulfaz Emecen and Assoc. Prof. Dr. Emre Büküşoğlu for guidance, support, and advice. I am grateful for the opportunity of working in the Membrane for Energy and Water Laboratory and Soft and Functional Materials Laboratory. It was an extraordinary experience for me, even if some of the study was during a chaotic COVID-19 pandemic.

I want to thank Membrane Energy and Water Laboratory members that I worked with; Bengisu Barış, Ecem Deveci, Onur Kaan Aydın, and İlker Kaygusuz for their help, support, opinions, and friendship. I would like to thank project partner Ceren Kocaman and all the Soft and Functional Materials Laboratory members for their help and opinion when I needed it.

I want to thank my friends İbrahim Burak Şen, Göktuğ Kahraman, Safiye Kahraman, Yağız Tezal, and Merve Tezal for being there when I need support and break.

I would also like to thank my parents, Yeşim İçten and Necati Tahir İçten, for their unconditional love, understanding, and support in every aspect of my life.

Last but not least, I would like to thank my love Toprak Çağlar for being there whenever I needed it from the first day of my study and giving me a reason to smile every day.

This study was funded by TUBITAK project no: 218M509 and METU BAP project no: GAP-304-2022-10827.

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LIST OF ABBREVIATIONS

ABBREVIATIONS

AgNO ₃	Silver Nitrate
APTES	(3-Aminopropyl) triethoxysilane
BTCA	1,2,3,4-Butanetetracarboxylic Acid
CNC	Cellulose Nanocrystal
CNF	Cellulose Nanofiber
DLS	Dynamic Light Scattering
DMSO	Dimethyl Sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDX	Energy Dispersive X-ray Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
GA	Glutaraldehyde
kDa	Kilodalton
KPS	Potassium Persulfate
MADLS	Multiple Angle Dynamic Light Scattering
MCC	Microcrystalline Cellulose
MWCO	Molecular Weight Cut-Off
Na ₂ S ₂ O ₃	Sodium Thiosulfate
NaBr	Sodium Bromide
NaCl	Sodium Chloride

NaClO	Sodium Hypochlorite
NMR	Nuclear Magnetic Resonance
PEG	Polyethylene Glycol
PES	Polyethersulfone
SAXS	Small Angle Light Scattering
SBKP	Soft Bleached Kraft Pulp
STAC	Stearyltrimethylammonium Chloride
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
THF	Tetrahydrofuran
TOPA	(2,4,5-trihydroxyphenylalanine)
XPS	X-ray Photoelectron Spectroscopy

LIST OF SYMBOLS

SYMBOLS

$\langle v \rangle$	Average velocity
A	Area
C_{bulk}	Bulk Concentration
C_{gel}	Gel Concentration
c_p	Permeate Concentration
c_r	Retentate Concentration
D_h	Hydraulic Diameter
D_{ij}	Diffusion Coefficient
g	Gravitational Acceleration
G'	Storage Modulus
G''	Loss Modulus
H	Half height
J	Membrane Flux
k_c	Mass Transfer Coefficient
L	Length
M_A	Mass of Substance A
P	Pressure
$\bar{P}$	Modified Pressure
Q	Volumetric Flow Rate

R	Rejection
Re	Reynolds Number
Sc	Schmidt Number
Sh	Sherwood Number
t	time
v	velocity
V_A	Volume of Substance A
v_{max}	maximum velocity
W	Width
μ	Viscosity
$\dot{\gamma}$	Shear Rate
δ	Boundary Layer Thickness
ρ	Density
τ	Shear Force

CHAPTER 1

INTRODUCTION

Separation processes such as distillation and extraction are among the most employed techniques in the chemical industry. These processes are energy intensive. As an alternative, membrane processes gained popularity as less energy intensive, low footprint, modular, and scalable separation processes (Baker, 2012).

Membranes are semi permeable barriers that distinguish between chemical species using driving forces such as pressure and concentration gradient by using species' properties, such as size, diffusion rate, solubility in membrane material, charge, etc. Membranes can be characterized by the size of separated species (Baker, 2012). Figure 1 shows membrane types and sizes of species that can be removed with those membranes.

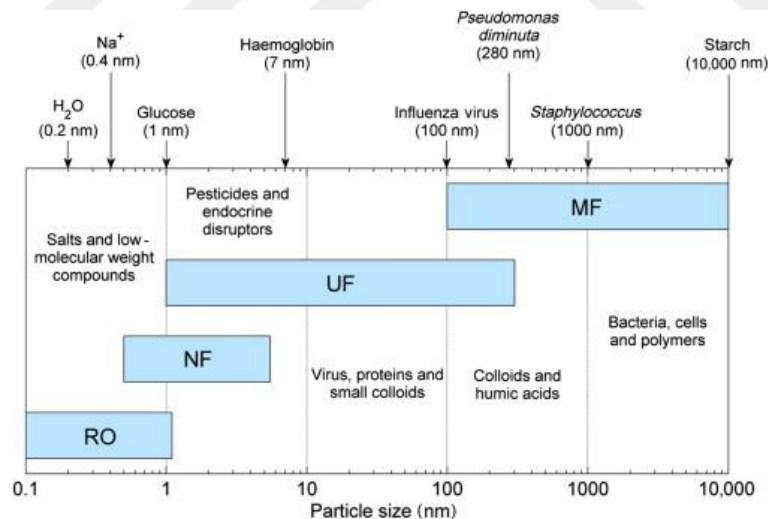


Figure 1. Characterization of membranes with respect to separated species (Madsen, 2014).

During membrane filtration, the concentration of the rejected species increases on the surface with a pressure effect while there is back diffusion caused by the

concentration difference between the membrane surface and bulk. This phenomenon is called concentration polarization. If the rejection for the rejected species is 100%, concentration on the surface of the membrane can be estimated with Equation 1. In the equation, k_c is the mass transfer coefficient, J is membrane flux, δ is boundary layer thickness, D_{ij} is the diffusivity of solute in solvent, and C is the concentration of the species either on the surface or in the bulk flow. If the concentration of the species reaches the solubility limit in the solvent, gel formation can be observed.

$$\frac{C_{gel}}{C_{bulk}} = \exp\left(\frac{J\delta}{D_{ij}}\right) = \exp\left(\frac{J}{k_c}\right) \quad \text{Equation 1}$$

Membrane filtration can be done with dead end filtration and crossflow filtration, as shown in Figure 2. In dead end filtration, the filtered mixture is pushed through a porous structure (membrane) without tangential flow. Concentration polarization and cake formation can be observed in this type of filtration. In crossflow filtration, filtered species flow through and tangentially to the membrane. Therefore, continuous sweep and increased mass transfer from the membrane surface can be obtained, which decreases the extent of concentration polarization and prevents cake formation.

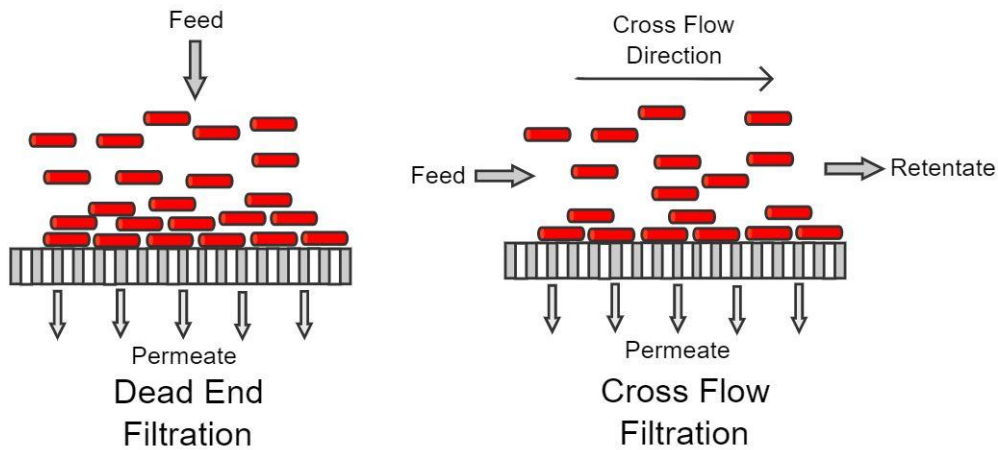


Figure 2. Membrane filtration modes.

In crossflow membrane filtration, there is a convectonal mass transfer caused by pressure driven flow and backflow caused by diffusive forces. Balance of those

mass transfer effects may increase concentration on the membrane surface. Increase in surface concentration comes with a decrease in membrane flux with respect to pure solvent flux. Flux can be increased with increased filtration pressure, where a deviation from pure solvent flux can be observed. Gel formation can be observed for increased surface concentration if the surface concentration reaches the solubility limit. In the case of gel formation, membrane flux reaches a constant value called “limiting flux.” An increase in membrane flux cannot be observed further for the limiting flux case, even if pressure increases. Instead, thickness of the gel increases. Figure 3 shows a representative sketch of flux behaviour during membrane filtration.

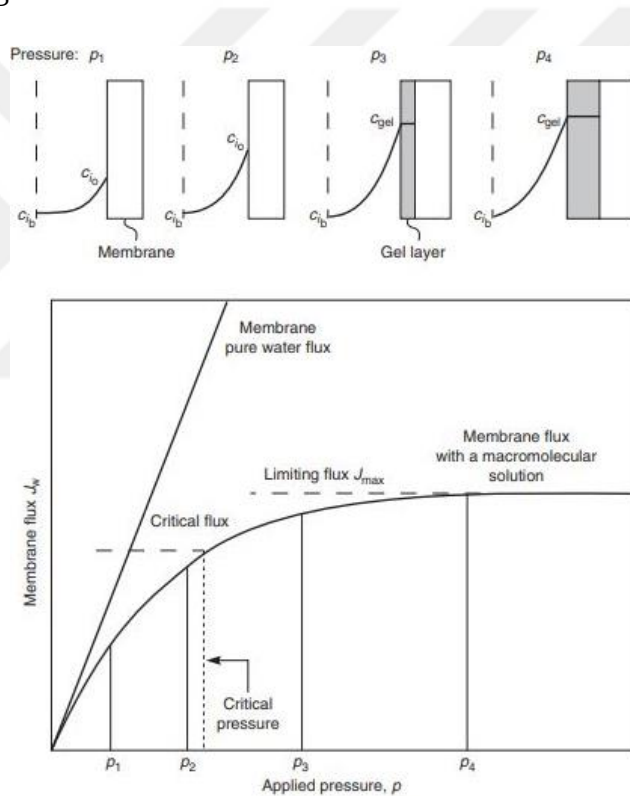


Figure 3. Fluxes during crossflow membrane filtration (Baker, 2012).

Ultrafiltration membranes are one of the most used membranes in filtration. These membranes are generally produced with coagulation of membrane solution in a nonsolvent bath which is called nonsolvent induced phase separation. The prepared membrane typically has a thin active layer which is the primary layer for

separation, and a support layer which is needed to support the active layer mechanically. The separation performance of membranes produced with nonsolvent induced phase separation depends on the preparation conditions, such as nonsolvent type, solvent type, polymer solution composition, etc. (Baker, 2012)

Even though membranes are considered greener processes caused by decreased energy needs for separation, harmful or toxic solvents are typically used during preparation. The need for using less harmful chemicals and recyclable or biodegradable polymers are essential goals for membrane industries. To reach those goals, incorporating solvents like dimethyl sulfoxide (DMSO) or water (Baig et al., 2020); or polymers like cellulose (Durmaz & Zeynep Çulfaz-Emecen, 2018; Sukma & Çulfaz-Emecen, 2018; J. Zhou et al., 2016), and their derivatives (Kocaman et al., 2021), can be done.

Cellulose is an important biopolymer that is abundant in nature. It can be obtained from plants, marine animals, algae, bacteria, invertebrates, amoebas, etc. Cellulose has low solubility in many conventional solvents (Bangar et al., 2022; Habibi et al., 2010; Trache et al., 2020). Low solubility in organic solvents makes cellulose a promising material for separations in these solvents, such as organic solvent nanofiltration or ultrafiltration.

Amorphous and crystal regions are present in the fibrillated nature of cellulose. Cellulose fibrils can be delaminated via physical methods to obtain a material called cellulose nanofiber (CNF), but most of the amorphous parts remain on the fibrils. To obtain fully crystalline cellulose, chemical hydrolysis methods can be employed. The most used method for chemical hydrolysis is sulfuric acid hydrolysis. Final crystalline structures are called cellulose nanocrystals (CNC). These crystal regions can withstand extreme pH and temperatures. These regions are also biodegradable (Ulaganathan et al., 2022), have high tensile strength, elastic modulus (J. Zhang et al., 2016), and unique optical properties (Wei et al., 2021). The mentioned properties make cellulose an interesting polymer for membrane (Kocaman et al., 2021) and sensor applications (Hu et al., 2022). Change in

appearance can be observed from SEM micrographs of native cellulose in Figure 4 and TEM micrographs of CNFs in Figure 5b, and CNCs in Figure 5a.

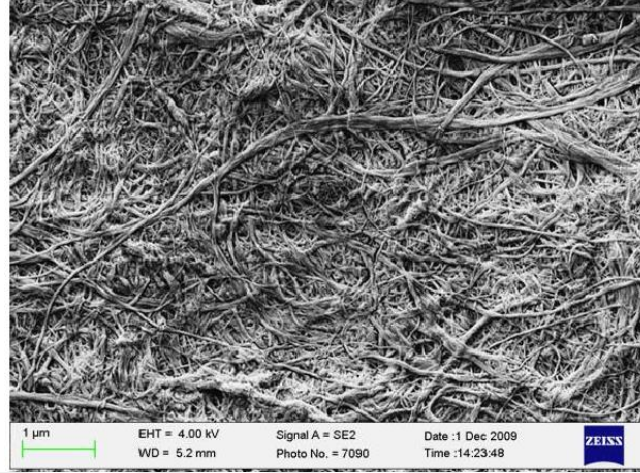


Figure 4. SEM micrograph of native cellulose (L. Zhang et al., 2010).

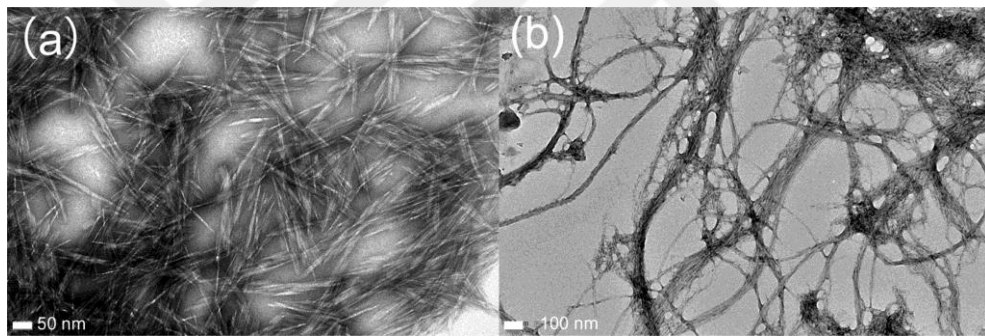


Figure 5. TEM micrographs of CNCs (a) and CNFs (b) (Xu et al., 2013).

Native cellulose contains only hydroxyl groups on its surface. Chemical hydrolysis of the cellulose leads to the grafting of new groups on surface while cleaving amorphous parts, such as sulfate half esters with sulfuric acid hydrolysis (Araki et al., 2000), carboxyl groups for nitric acid hydrolysis (Cheng et al., 2020), and phosphate groups for phosphoric acid hydrolysis (Amin et al., 2022), etc. These grafted groups increase the stability of the CNCs in suspension form, being able to suspend in medium, due to increased repulsive electrostatic interactions, which is needed for an application that uses CNC suspension as starting point (Habibi et al., 2010). Surface hydroxyl and grafted other groups also allow easy surface

modification according to needs. A useful modification method for native cellulose, CNF, and CNCs was previously mentioned as (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) oxidation which changes primary hydroxy groups into carboxyl groups on the cellulose surface. TEMPO oxidation uses TEMPO/NaBr/NaClO for the modification. The optimum reaction pH was reported as 10-10.5 (Fraschini et al., 2017). This modification was previously used for stabilization (Y. Zhou et al., 2018) and an intermediate step for further modification (Le Gars et al., 2020).

Liquid crystals are phase of a material where the particles show both liquid like properties such as fluidity and deformation with shear, and crystal like properties such as anisotropic electrical magnetic and optical properties (Andrienko, 2018). Rodlike particle dispersions may show a concentration of lyotropic liquid crystal, a sub definition for liquid crystals where liquid crystal properties are observed after reaching a critical concentration caused by the equilibrium between rotational and translational entropy (Parker et al., 2018). This behavior was demonstrated for CNCs in different studies (Habibi et al., 2010; Lagerwall et al., 2014). Therefore, lyotropic liquid crystals, such as CNCs, show isotropic behavior under critical concentration, dependent on surface charge, aspect ratio, particle size, etc. For increased concentrations, these CNC crystals start to show biphasic properties and can begin to show completely anisotropic properties with left handed cholesteric liquid crystal properties for increased concentrations (Mark Parker et al., 2017; Parton et al., 2022) (Figure 6). Cholesteric alignment can be characterized by a helical twist orthogonal to the individual alignment of CNCs. Formation of the helicoidal symmetry results in interesting optical properties, such as Bragg's reflection (Binet et al., 2000). Bragg's reflection of the layer can be observed with polarized optical microscopy as a colored appearance for left handed polarized light to characterize the pitch length, the distance between crystals with the same orientation with respect to the director (Figure 6d), and Bouligand arches, the characteristic look of the cholesteric self assembly (Majoinen et al., 2012), in scanning electron microscopy (SEM) micrographs (Figure 6e) for dried cholesteric

films. Recent studies showed that this left handed cholesteric liquid crystal behaviour could be modified via shear (Kocaman et al., 2021), magnetic field (Ganguly et al., 2022), electric field (Habibi et al., 2008), etc., to obtain nematic alignment, alignment of liquid crystals in the direction of the director (shear, electric field, etc.). For nematic aligned suspensions and films, polarized optical microscopy characterization may also be done for these films and suspensions. During characterization, a bright appearance may be observed caused by the formation of birefringence with nematic alignment with polarized optical microscopy. Optical birefringence can also be seen from the scattering of light more in one direction than another Figure 7. Another characterization may be obtained with rheological behaviour. CNC suspension shows three regions of different rheological behaviour under shear: Initial shear thinning behaviour, shear independent behaviour caused by the alignment of crystals nematically, and shear thinning behaviour after fully nematic alignment (Pignon et al., 2021).

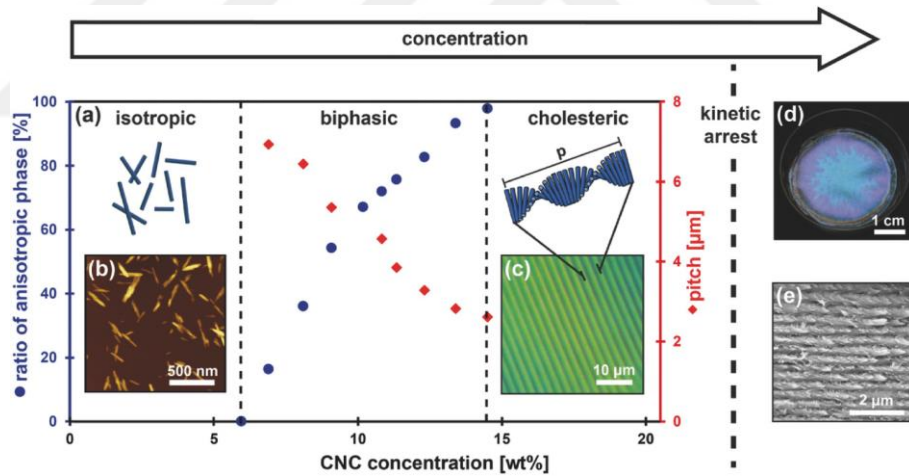


Figure 6. Concentration dependent lyotropic behavior of CNCs (a), AFM micrographs of CNCs, polarized optical microscopy images of fingerprint pattern, dried polarized optical microscopy images of the membranes (d), SEM images of the CNC film cross-section. (Mark Parker et al., 2017).

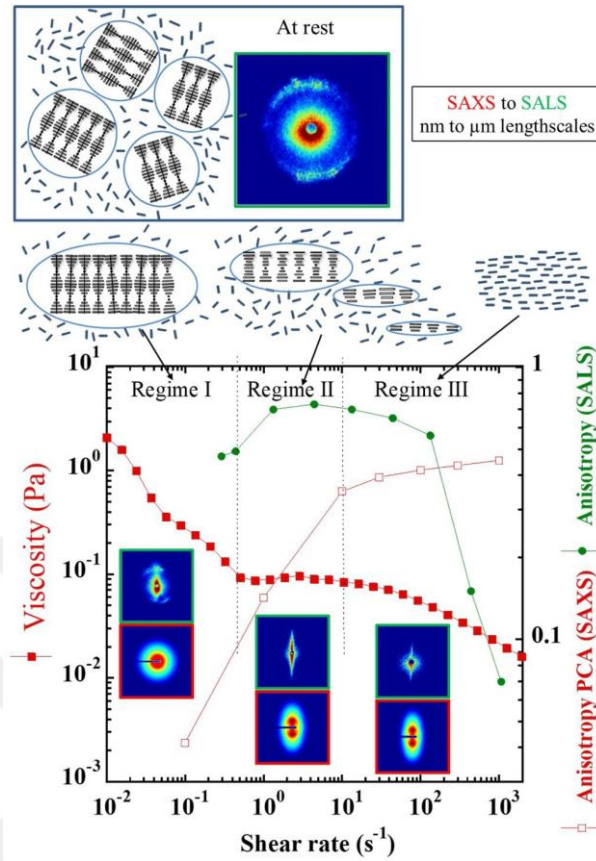


Figure 7. Shear dependent light scattering behavior of CNC suspensions. (Pignon et al., 2021)

CNC films were previously prepared by drying the CNC suspension on a surface, such as a petri dish or a glass substrate (Diaz et al., 2013), and it was previously shown that dried suspensions show initial liquid crystal properties for casted suspension. Figure 8 shows previously done membrane preparation methods with CNCs. Drying conditions during film preparation, surface conditions, temperature, and humidity of the environment, change final properties of the film. Therefore, homogeneity, film thickness, etc., are more challenging to control. Additionally, the initial suspension concentration should not be too high to eliminate the possibility of forming a kinetically arrested glassy state, which prevents self assembly and alignment. In the literature, filtration methods were also reported for the preparation of CNC films (Kocaman et al., 2021; Volkan, 2020).

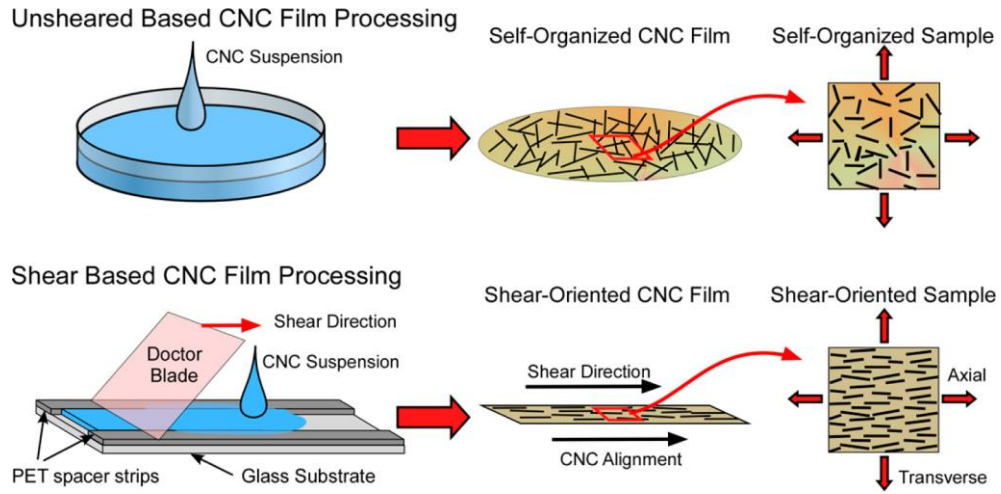


Figure 8. Film preparation methods for CNC suspensions. (Diaz et al., 2013)

The stability and liquid crystal properties of CNCs can be modified with addition of polymers and electrolytes. It was previously known that the electrical double layer can be suppressed by adding salts, which results in closer packing of CNCs with reduced repulsive forces (Lin et al., 2022). This destabilization effect for suspension and stabilization of the layer as a film instead was previously used to stabilize membranes prepared via deposition of CNCs (Kocaman et al., 2021; Volkan, 2020). In addition to the colloidal destabilization effect of salts, final membrane compactness was shown to be increased by the addition of low valence salts such as NaCl by suppressing the electrostatic repulsion between CNCs (Kocaman et al., 2022). Polyethylene glycol (PEG) polymers were also used for modification of cholesteric behaviours of CNC by making CNCs closer to each other and changing Bragg's reflection to red shift (Lin et al., 2022).

Membrane preparation methods with deposition of the membrane with dead and deposition (Volkan, 2020) and crossflow nematically aligned deposition (Kocaman et al., 2022) were previously mentioned in the literature. Still, both articles stabilize CNCs only physically with AlCl_3 permeation. Additionally, the long term stability of the prepared membranes was unknown. Therefore, the long term stability of the layer and the possibility of chemically linking CNCs to increase its stability was not previously studied. Also, the preparation of cholesteric layers with this method

may increase the predictability of the final layer, which was previously done in evaporation induced self assembly with need for reasonable environmental control.

In this study, TEMPO CNC membrane preparation methods with crossflow deposition and its crosslinking were studied. Crosslinking of TEMPO CNCs method were done with decarboxylation reaction with silver nitrate and potassium persulfate which were never tried for CNC suspensions. This crosslinking method were studied with TEMPO CNC suspensions and membrane layers. Membrane preparation using TEMPO CNC suspension during preparation (a cholesteric liquid crystal) would have better tunability for pore size, and final membranes have narrower pore size distribution since pores are formed by the gap between deposited aligned liquid crystal. Membrane performance was compared with physical stabilization at high ionic strength and chemical crosslinking for comparison with continuous filtration experiments. The cholesteric liquid crystal properties of the CNC layer with the addition of NaCl were also investigated.

CHAPTER 2

LITERATURE REVIEW

2.1 Preparation Methods of Cellulose Nanocrystals

Cellulose is the most abundant natural biopolymer. It is synthesized by plants, marine animals, fungi, and bacteria. During synthesis, amorphous and crystalline parts are formed, and these parts can be modified for different purposes (Habibi et al., 2010).

CNCs are conventionally prepared with acid hydrolysis of cellulose sources such as cotton (Park et al., 2019), kraft pulp (H. Wang et al., 2020), etc., and previously mechanically modified microcrystalline cellulose (Di Giorgio et al., 2020). During acid hydrolysis, cellulose source is added into concentrated hydrochloric acid (Araki et al., 2000), sulfuric acid (Elazzouzi-Hafraoui et al., 2008), phosphoric acid (Ding et al., 2017), etc. at high temperatures. Harsh conditions caused by hot acids lead to the removal of amorphous regions on the cellulose source and yield cellulose nanocrystals, which are nanorods of 180 ± 11 nm length and 8 ± 3 nm diameter for cotton linter (Kocaman, 2022) and showing liquid crystal properties at high concentrations (Parker et al., 2018). Each mentioned synthesis continues with quenching and purification steps such as centrifugation and dialysis to remove remaining acids and formed salts. In addition to removing amorphous regions, hydrolysis also leads to grafting new surface groups, such as sulfate half esters for sulfuric acid and phosphate groups with phosphoric acid. At the same time, there is no surface modification with hydrochloric acid hydrolysis. Cellulose is a hydrophilic material due to the presence of three free hydroxyl groups per anhydro glucose unit, which also leads to permanent agglomeration during drying (Bangar et al., 2022). Grafting of charged groups increases the colloidal stability of cellulose nanocrystal suspension due to electrostatic interactions.

Trends in environmental sustainability ask for less harsh chemicals and treatments in production, leading to experimentations on different methods to produce cellulose nanocrystals. A mix of strong and weak acids was used to reduce the harshness of strong acids. Wang et al. (2017) tried to reduce the amount of sulfuric acid in a hydrolysis medium by replacing it with a weak acid, acetic acid. By using acetic acid, the amount of strong acid is significantly reduced. In addition to that, the low boiling point of acetic acid lets one recover used acid with simple distillation. The addition of acetic acid to the reaction medium leads to grafting of acetoxyl groups. Even if replacing part of the strong acid with a weaker one was an environmentally conscious production method, reducing or eliminating chemical usage would be an ideal solution. Novo et al. (2016) offered a subcritical water hydrolysis method to produce CNC. In the method, high pressures and temperatures were utilized to cleave amorphous parts of cellulose. The yield of resultant crystals was found to be low with respect to acid hydrolyzed CNC. The thermal behavior of produced crystals was found to be close to pure cellulose, which is better than sulfuric acid hydrolyzed one due to elimination of desulfation of surface sulfate half ester groups in sulfuric acid hydrolyzed one. The crystallinity index of CNCs was also found to be close to pure cellulose. They also mention that the colloidal stability of obtained CNCs was like hydrochloric acid produced one since no surface group is grafted on the surface. Another method was offered as high pressure homogenization by Park et al. (2019). In their method, crystallinity was found to be increasing with increased temperatures and the number of passes. This method also does not graft any surface group on cellulose nanocrystals; colloidal stability should be low. Interestingly, they reported that obtained suspensions were colloidally stable, and no aggregate was observed for more than 1 year, but they did not mention any reason that might lead to stability.

2.2 Grafting New Surface Groups on CNCs

Most cellulose nanocrystal applications need stability in the organic or aqueous medium by being able to remain in the medium without sedimentation or agglomeration. In the mentioned studies, such as hydrochloric acid hydrolysis, it was seen that if there is no surface group grafted on the surface, colloidal stabilities of the suspensions are low (Araki et al., 2000). In such cases, additional modifications are needed, such as TEMPO oxidation. TEMPO oxidation of surface hydroxyl group to carboxyl groups was utilized for such cases. TEMPO oxidation utilizes the TEMPO/NaBr/NaClO system. The most accepted reaction mechanism is that the hypobromite ion forms first from the hypochlorite ion, which continues with the formation of nitrosonium salt from the TEMPO radical. After the formation of nitrosonium salt, primary alcohol on the cellulose surface oxidates into sodium carboxylate and releases hydroxylamine in the medium. The formed hydroxylamine reacts with nitrosonium and regenerates TEMPO radical. The General mechanism can be seen in Figure 9. In the reaction, two moles of NaClO are required per mole of primary alcohol. It was found that the optimum pH for the reaction is around ten. Therefore, it was recommended that pH should be in the range of 10-10.5 during the reaction (Fraschini et al., 2017). At the end of the synthesis, the reaction can be quenched by the addition of methanol or ethanol to eliminate remaining NaClO. Formed CNC suspension would be in deprotonated form due to the reaction pH, which can be turned into protonated form for needed applications by reducing pH to 1.5-2 before dialysis. Other than surface modification, TEMPO oxidation was also used before CNC preparation. Y. Zhou et al. prepared TEMPO oxidized CNC suspensions with TEMPO oxidized microcrystalline cellulose (MCC) and soft bleached kraft pulps (SBKP). To do that, SBKP and MCC were first modified with TEMPO oxidation, then sonicated with an ultrasonication system for 10 to 120 min. The study showed that the degree of polymerization of cellulose, hence length, decreases with TEMPO oxidation for SBKP while it only decreases marginally for MCC. After ultrasonication, the

degree of polymerization is also seen to be lowered. The ultrasonication study showed that the sonication length was too low and showed cellulose microfibrils, while 30 minutes and longer sonication led to CNC formation. It was also mentioned that the size of the crystals for MCC and SBKP reached the same value. TEMPO oxidation allows the further modification of the surface for other surface groups. Le Gars et al. (2020) modified carboxyl groups of TEMPO oxidized sulfuric acid hydrolysis CNC with 1-methyl-3-phenylpropylamine. They used that modification to improve the sorption of aromatic ring containing molecules. Obtained crystals showed better colloidal stability in acetone and dichloromethane, but sedimentation of crystals in these solvents was still observed.

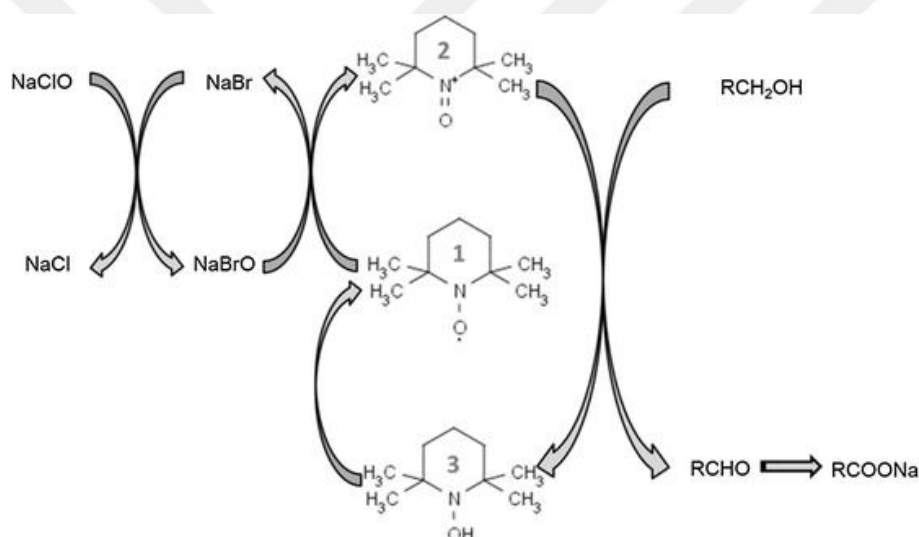


Figure 9. Accepted TEMPO oxidation mechanism (Fraschini et al., 2017).

As alternative, Fenton reaction is another process to add carboxyl groups onto surface. Fenton oxidation generally done with hydrogen peroxide and iron (II) which oxidates the organic compounds to obtain carboxyl and aldehyde groups (Cossu et al., 2018). Even if it is used for oxidation, this oxidation is a harsh process that can be even used for degradation of perfluorinated compounds (Tang et al., 2012). There are two main drawbacks of the process, which are wastage of oxidants due to radical scavenging and decomposition and continuous loss of iron ions (Babuponnusami & Muthukumar, 2014). Additionally, chemical consumption

of the conventional Fenton processes was reported as high due to that only small amount of the oxidant is available for the cellulose oxidation (Li et al., 2019).

Hydrophobization is another method to modify the CNC surface. It is generally done with the modification of surface groups with silanes. Khanjanzadeh et al. used (3-Aminopropyl) triethoxysilane (APTES) to modify the CNC surface. It was proposed that APTES modified CNCs may be used for nano absorbers to its amino groups.

Positively charged CNCs are another type of CNC that can be used in layer by layer deposition applications or increasing the affinity of CNCs with negatively charged polymers or surfaces. In literature, positively charged CNCs were generally prepared with quaternary ammonium salt modifications. Salajková et al. tried to modify TEMPO oxidized HCl oxidized CNCs with four different quaternary ammonium salt, which are stearyl trimethylammonium chloride (STAC), phenyl trimethylammonium chloride, glycidyl trimethylammonium chloride, and diallyl dimethylammonium chloride and final CNC dispersed in organic solvent. It was found out with STAC that the molar ratio of 1:2 carboxyl groups to ammonium salts was found as optimal in between 1:1, 1:2, and 1:4 ratios. Experiments with STAC modified CNCs showed that stable suspension with acetone and tetrahydrofuran could not be obtained while it was stable in toluene and chloroform. Increase in water contact angle was observed when CNCs were deposited on the Si surface, which shows an increase in the hydrophobicity of final crystals with respect to TEMPO oxidized ones.

2.3 Removal of Sulfate Half Ester Groups from CNCs

Removal of added groups was also tried on CNCs. Beck & Bouchard's (2014a) study on desulfation of sulfuric acid hydrolysis CNC suspensions showed that desulfation occurs at every temperature, but the rate of desulfation depends on the CNC form, either protonated or deprotonated and storage temperature. In the study,

they mention that desulfation is slow at 4 °C with respect to room temperature, and desulfation of deprotonated form CNC is almost nonexistent with respect to protonated form CNCs. When they tried at higher temperatures, it was seen that 66% of sulfate half esters were removed from the CNC surface in the protonated form, while only 4.1% of the sulfate half esters could be removed in deprotonated form with reaction at 70°C and 5 days. They proposed that the formation of sulfuric acid catalyses desulfation until the threshold CNC sulfur content is reached. Four different desulfation experiments were proposed to investigate this phenomenon: thermal desulfation, acid catalysed desulfation, alkaline desulfation, and solvolytic desulfation. (Lewis et al., 2016) worked on the thermal desulfation of CNC suspensions. In their experimental method, they heated 4% CNC suspension both in protonated and deprotonated form and heated between 60 to 120 °C up to 72 hours. For protonated form suspensions, gel formation was observed at 80°C for 20 h reaction time, but it was also observed that CNC suspensions might be burned at elevated temperatures like 150 °C. Elemental analysis of samples with different desulfation temperatures showed a plateau between 60 to 100 °C for 20 h reaction. For 120 °C and 20h reactions, it was not possible to get a reading for sulfate half ester content with the equipment they used, which might mean sulfate content was lower than the rejection limit of the equipment. Therefore, they said that increased desulfation was possible for increased temperatures. For deprotonated form suspensions, the same behaviour was observed with an elevated temperature of 110 °C at 20 h of desulfation. Gel formation might be caused by decrease in sulfate content or increase in ionic strength caused by increase in sulfuric acid in suspension. Jiang et al. (2010) used two different reaction paths to see the desulfation of CNCs. In the first path, desulfation was tried on with acid catalysis. In the experiment, 12.5 ml 1 N HCl was added into 500 ml 1% suspension, and the reaction was conducted for 225 min at 80 °C multiple times. At the end of each reaction, the suspension was dialyzed against pure water. After dialysis, the sulfate half ester content was estimated with conductometric titration. It was seen that sulfate half ester content density decreased with each step, but

complete desulfation could not be obtained with this procedure. Size of the particles in suspension was also measured with DLS, which shows particle size stays the same even with 7 times of desulfation step, which removed 81.2% of sulfate half esters. For the second path, solvolytic desulfation was tried. This reaction removes sulfate half esters by converting them to pyridinium salts. After this reaction, 98.3% of the sulfate half ester content could be removed, and the size of particles was also increased from 83 ± 1 to 329 ± 35 nm. For alkaline desulfation, Jordan et al. (2019) tried to model the final sulfate content of CNCs after alkaline desulfation with different CNC suspensions, different NaOH concentrations, time, and temperatures which were used for modelling of alkaline desulfation with those parameters. During each step, the charge of the CNCs was measured with conductometric titration. Conductometric titration is used when there is no indicator to see the end point of titration (Abitbol et al., 2013). Modelling of the alkaline system showed that alkaline desulfation could be done in broad conditions. Still, only minor differences above 0.1-0.2 M NaOH were observed for the efficacy of sulfate half ester removal, where reaction temperature and time are significant for desulfation.

2.4 Crosslinking of Cellulose Nanocrystals

This part of the literature review summarizes previously reported crosslinking strategies on cellulose, CNCs, or crosslinking studies that may work on modified CNCs.

Crosslinking can be done on materials physically and chemically. For physical crosslinking, weak physical interactions form a bond through polymer chains. These interactions might be van der Waals forces, coordinate bonding (Li et al., 2020), ionic or hydrogen bonding (Kim et al., 2021). These bonds are weaker with respect to chemical crosslinking, while these bonds are recoverable and self healing upon large deformation (W. Wang et al., 2017). Kim et al. (2021) used a hydrogen bonding promoter (2,4,5-trihydroxyphenylalanine) (TOPA) to arrest crack

propagation and poly ethylene glycol (PEG) as a plasticizer to mediate internal stresses to eliminate crack formation during the preparation of CNC films. In the end, crack free monolithic cholesteric films were obtained. Additionally, obtained films had improved ultimate tensile strength and toughness.

Both cellulose and CNCs have surface primary and secondary hydroxyl groups that may be crosslinked. Konca (2018) used both glutaraldehyde (GA) and 1,2,3,4-butanetetracarboxylic acid (BTCA). For crosslinking with GA, cellulose membranes were immersed in 0.5% GA 1% sulfuric acid solution at 40°C for 3 hours. Finally, membranes were washed with pure water. After modification, an increase in the number of C-H bonds was observed with estimation of areas under respective bond peaks on XPS spectra, which indicates the addition of GA to the surface, which means that crosslinking occurred. After crosslinking, there was almost no change in elastic modulus tensile strength or elongation at break, and the membrane's affinity toward solutes was similar. For BTCA crosslinking, membranes were first immersed in 2% BTCA and 1% monosodium phosphate for 15 minutes; then, it was cured in an 80 °C oven for an hour. Finally, membranes were washed with pure water. With FTIR analysis, the formation of a new peak around 1500-1600 cm^{-1} , which indicates a C=O bond, was observed. The addition of new peak indicates addition of BTCA to surface. Crosslinked membranes show higher elastic modulus, tensile strength, and elongation at break which were showed with universal testing machine tests. There was no significant change in ethanol permeance, Bromothymol Blue. At the same time, rejection of Crystal Violet was found to be decreased appreciably (88% to 75%), and Rose Bengal rejection was found to be increased (14% to 20%) caused by the formation of free carboxyl groups after the crosslinking reaction.

Vismara et al. (2019) crosslinked bacterial CNCs modified with glycidyl methacrylate and ethylene glycol dimethacrylate. To do that, 4.4% bacterial CNC solution was heated at 80 °C for 30 min. Then, hydrogen peroxide and Fenton reagent were added to the suspension and waited 1 h to activate cellulose. Finally, glycidyl methacrylate and ethylene glycol dimethacrylate were added

simultaneously to react for 30 min. As the reaction mechanism, they proposed that the Fenton reagent formed cellulose carbon centred radicals first scavenged by glycidyl methacrylate for surface modifications. Then ethylene glycol dimethacrylate formed crosslinks (Figure 10), purified with pure water by exhaustive washing to remove unreacted compounds and residual Fenton reagent. Crosslinking enhanced the mechanical resistance of dried bacterial cellulose nanocrystals with respect to non crosslinked ones.

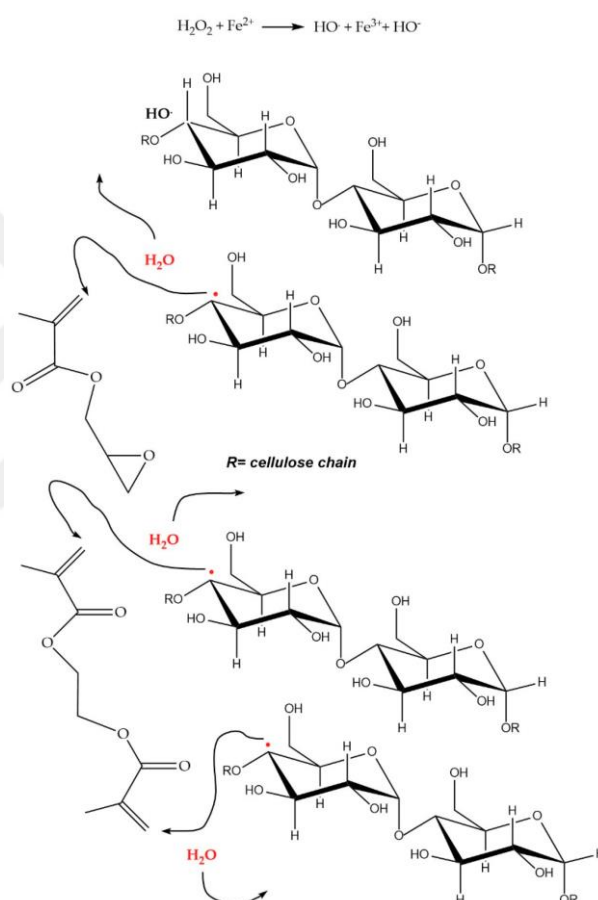


Figure 10. Reaction mechanism for glycidyl methacrylate and ethylene glycol dimethacrylate crosslinking reaction (Vismara et al., 2019).

Yang & Cranston showed an aerogel preparation method by crosslinking aldehyde modified CNC and hydrazide modified CNCs. After preparing both types, CNCs were mixed, which allowed CNCs with the two functional groups to crosslink and gelate. After hydrogel formation, aerogel continued with freezing, solvent

exchange, and critical point drying. Obtained aerogels are strong and remain intact in air and liquids.

Wang et al. (2022) used citric acid to crosslink CNFs and CNF/ ϵ -lysine each other. In their study, they observed that CNF/ ϵ -lysine aerogel which was not crosslinked, had lower tensile strength but higher elongation at break with respect to crosslinked one.

In our study, we used a method reported for crosslinking carboxyl containing polymers to crosslink TEMPO oxidized CNCs (Liu et al., 2023; Mendoza et al., 2023; Weng et al., 2017). Weng et al. (2017) showed a method for crosslinking of carboxyl contained polymers. They started with a known decarboxylation reaction with silver nitrate (AgNO_3) and persulfate and proposed that it can crosslink poly acrylic acid. After crosslinking, gel formation was observed. To show that behaviour, frequency sweep tests were done, and an increase in loss and storage modulus was observed. Still, the increase in storage modulus was higher, meaning increased elastic behaviour of the polymer due to crosslinking. This method can be used for crosslinking of TEMPO oxidated CNCs. 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) is another chemical that can crosslink amine groups and carboxyl groups of proteins. This chemical can also crosslink TEMPO oxidized and amine modified CNCs (Thermo Scientific, 2012).

2.5 Characterization of CNCs

CNCs are rodlike materials that can spontaneously self assemble into a cholesteric liquid crystal state in suspension when higher than its critical concentration. The ability of self assembly and overall stability of suspensions depends on a balance between attractive van der Waals forces and repulsive electrostatic interactions. The ionic strength of the medium can also change self assembly behaviour, hence pitch size. The emergence of spontaneous self assembly arises from a trade off between translational and rotational entropy (Lagerwall et al., 2014). Cholesteric

liquid properties of the CNC can be characterized by pitch, defined as the length of one complete turn (Figure 11a). Cholesteric order is characterized by the orientation of CNCs on a director axis which rotates around an axis perpendicular to the director axis, and characterization can be done with pitch length and distance along the axis after one complete rotation. This behaviour continues even if all the water evaporates (Lagerwall et al., 2014). Cholesteric order results in an interesting optical behaviour that can be observed with polarized optical microscopy in suspension form and SEM for dried films. In polarized optical microscopy, cholesteric symmetry can be characterized by fingerprint patterns or colours caused by Bragg's reflection (Figure 11b) (Binet et al., 2000). Another observation can be done with Bouligand structures to characterize the pitch via SEM for dried films (Majoinen et al., 2012).

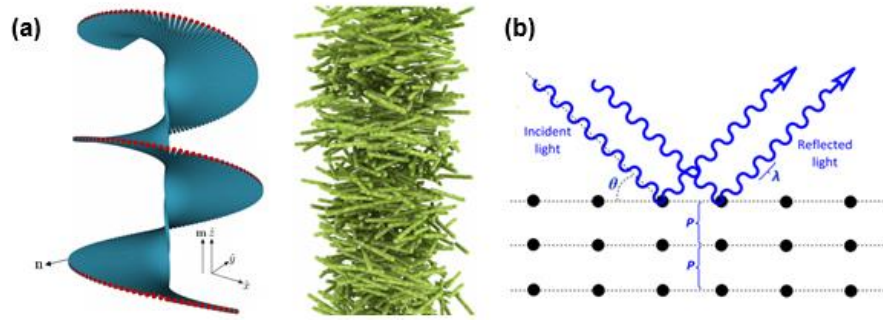


Figure 11. Sketch of left handed cholesteric self assembly (a), and Bragg's reflection with incident angle (θ) and reflected light wavelength (λ) with pitch (P) (Wei et al., 2021).

In addition to cholesteric alignment, nematic aligned CNCs can be obtained after critical concentrations with shear (Babaei-Ghazvini & Acharya, 2022; Diaz et al., 2013; Kocaman et al., 2021), electric field (Habibi et al., 2008), magnetic field (Cranston & Gray, 2006; Ganguly et al., 2022; Sugiyamaj et al., 1992). In nematic alignment, CNCs align in the direction of the force which is applied by the director. Nematic aligned CNC layers show birefringence caused by optical anisotropy in the longitudinal and axial axis of the crystal. Also, nematic aligned films show direction dependent properties such as mechanical strength, swelling (X. Zhang et al., 2022) etc. Characterization of the nematic alignment can be done with

polarized optical microscopy. For an isotropic material in a polarized optical microscope, no light should pass through if the analyser and polarizer are in crossed directions. When the birefringent material is inserted in between the analyser and polarizer at angles other than 0° , 90° , 180° , and 360° , light can pass through, and a bright appearance can be observed. For the CNC case, birefringence starts to occur after nematic alignment. Additionally, atomic force microscopy (AFM) was previously used for the characterization of nematic alignment for dried films (Kocaman et al., 2022).

For initial characterization, surface charge, crystallinity, and size (and aspect ratio) of the particles were generally specified with preparation methodology for the samples.

Size characterization was done with small angle X-ray spectroscopy (SAXS), transmission electron microscopy (TEM), and AFM analysis (Elazzouzi-Hafraoui et al., 2008). For an approximation, dynamic light scattering (DLS) and rheological analysis were also used. Diaz et al. described a method to estimate the aspect ratio of CNCs with an Ubblohde viscometer. In the method, electro viscous effects were eliminated via addition of NaCl, and Fedors plots were obtained to calculate the intrinsic viscosity of the samples, which were further used for the calculation of the aspect ratio of CNCs with Batchelor plot. They reported that this aspect ratio was a good estimation of the number averaged aspect ratio of the suspension. Boluk & Danumah (2014) used DLS for the measurement of diffusion coefficients. In their article, they also mention that more accurate and convenient results can be obtained via depolarized dynamic light scattering. They proposed that normal DLS can measure the translational diffusion coefficient, which can be used for the measurement of CNC length with additional measurement of particle width via AFM, TEM, etc.

Surface charges are characterized by zeta potential analysis and conductometric titration (Beck et al., 2015). Zeta potential is the electrical potential of the slipping plane for particles. This characterizes the surface charge of the particles and

suspension stability. For stability purposes, studied suspensions were characterized as unstable if the measured potential is between -30 to 30 mV; otherwise, it is assumed to be stable. This measurement directly depends on ionic strength, pH, and temperature; hence those should be described for the measurements. The isoelectric point is another parameter that might be used for the characterization of pH where the particle carries no net charge. It is defined as pH where zeta potential reaches 0. Conductometric titration is another method for the calculation of the direct surface charge of CNCs. This method is needed for surface charge characterization since surface charges were too low to change pH to result in color change by adding one or more indicators to see the end points of the titration. In the method, CNCs are added into a dilute NaCl solution, used as a baseline, and titrated with a dilute NaOH solution. At every addition, conductivity is recorded (and corrected for dilution caused by NaOH addition) during titration. The endpoint can be observed when linear trends of decreasing and increasing trends intersect for the titration (Beck et al., 2015).

Characterization of CNCs can be extended for needs and application. Rheologic analyses are one of the most used methods. For rheologic analyses, shear dependent viscosity measurements with given concentration is the most employed method for CNC characterization, while storage modulus and loss modulus were used in crosslinking and gel studies. CNCs show 3 different behaviors to shear when it is concentrated higher than its critical concentration. At low shear, CNC suspensions show shear thinning behavior. When shear increased, cholesteric alignments converted into nematic alignment, and shear independent behavior is observed during this alignment. When shear is increased further, all crystals align in the same direction, and shear thinning is observed again (Kim et al., 2022; Shafiei-Sabet et al., 2012). Three region behavior are shown in Figure 12 clearly. Adding salt suppressed some of the surface charges which result in more CNCs packed closer, leading to a clearer observation of these three regions. Another rheological characterization that was previously used was the characterization of gel formation with loss and storage modulus. The “gel” is defined as a nonfluid

colloidal or polymer network expanded throughout the whole volume by a fluid (Jones et al., 2009). Characterization of mentioned “gel” structure can be characterized by its storage modulus (G'), ability to store energy elastically, and its relationship with respect to loss modulus (G''), energy dissipated caused as heat. Those two moduli show the elastic (G') and viscous (G'') nature of a material, respectively (Ramli et al., 2022). For a material to be considered as gel, its storage modulus should be one order of magnitude higher than the loss modulus. If the storage modulus is higher but comparable in magnitude material is called as a “weak gel”. This approach was used for hydrogel studies to characterize the final structure behaviour (Lewis et al., 2016; Weng et al., 2017). Lewis et al., as mentioned in the modification of cellulose nanocrystals section, studied the hydrothermal desulfation methods for CNC suspensions to obtain CNC hydrogels. In the study, the main reason for the formation of hydrogel is decrease in surface charge, which makes CNCs closer to each other by decreasing electrostatic repulsion. After desulfation, frequency sweep was used for the characterization of gelation, and it was shown that suspensions treated with 80 °C and higher temperatures showed gel like characteristics which were decided by a steady storage/loss modulus ratio higher than 10. Weng et al. (2017), as mentioned in crosslinking of cellulose nanocrystals section, studied crosslinking carboxyl contained polymers and used time dependent dynamic rheology studies to observe gelation kinetics during crosslinking reaction. In their study, they showed that gelation time changes with a change in AgNO_3 concentration during crosslinking reaction from 48 min to 33 min with an increase from 0.03 wt.% to 0.12% with constant 0.3 wt.% ammonium persulfate for crosslinking of 20 wt.% polyacrylic acid solution.

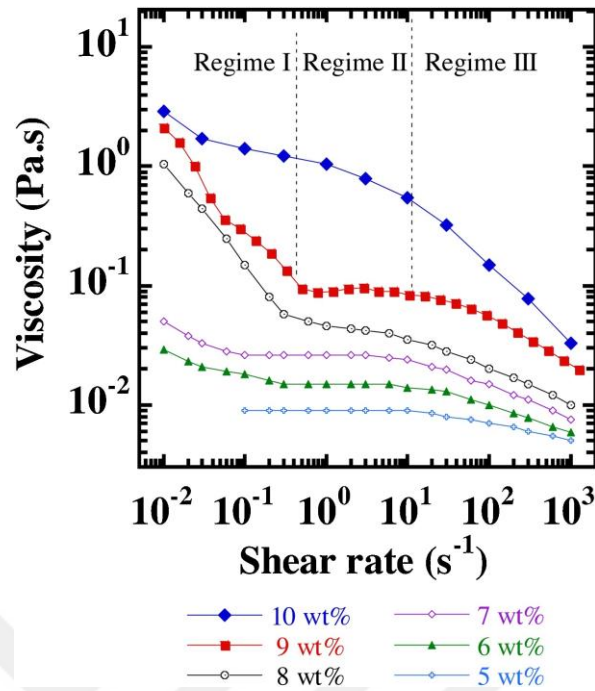


Figure 12. Shear dependent viscosity profile for 10 mM NaCl added CNC suspension (Pignon et al., 2021).

Characterization of bonds and surface groups can also be done with X-ray photoelectron spectroscopy (XPS), Fourier transforms infrared spectroscopy (FTIR), Raman spectroscopy, and nuclear magnetic resonance (NMR), where FTIR is the most used one. Even if those are qualitative characterization methods, there are still approximative methods for quantitative characterization. As an example, George & Montemagno (2017) used FTIR to analyse the surface sulfur content of CNCs by using FTIR. In their methods, the ratio of C-O-S peak to total C-H signal peak was used for the characterization of sulfur content for CNCs which were found to be close with respect to conductometric titration. Swasy et al. (2020) prepared amine functionalized CNC suspensions for the degradation of pesticides. In their study, they modified CNCs with polyethylene imine, carboxyl, ethylene diamine, and tris(2-aminoethyl) amine and showed that the addition of amine containing polymers increases N-H bond peaks while the addition of carboxyl leads to formation of C=O peaks instead of N-H with FTIR analysis.

2.6 Membrane Application of CNCs

CNCs were used for membrane preparation as filler or active layer previously. J. Zhou et al. (2016) used CNC crystals as fillers in a cellulose acetate membrane by firstly freeze drying CNCs and adding them into the dope solution. They showed that CNCs were incorporated into the membrane with the formation of an alcohol peak on FTIR analysis. After the addition of CNCs in the matrix, water contact angles of resultant membranes were found to be reduced for increased CNC concentration in membrane solution, but it was reported that high CNC concentration leads to decreased pure water flux after 0.5%. Bai et al., 2020 showed similar results with CNC added PES membranes. In the study, freeze dried CNCs were added to PES solution and dispersed before membrane fabrication. They reported that increasing the CNC amount led to thinner skin layer formation, which might be caused by increased hydrophilicity of the resultant membrane solution. Like a previously mentioned study, an increase in contact angle was observed due to the addition of hydrophilic particles to the surface. In addition to that, an increase in pure water fluxes up to a certain CNC concentration was observed, which is similar to CNC added cellulose acetate (CA) membrane. Finally, an increase in CNC concentration was found to increase humic acid and bovine serum albumin rejection while it stayed constant for sodium alginate.

Different methods can be employed to prepare CNC as a membrane active layer. The most basic method is the filtration of CNCs on a support surface and stabilizing the resultant CNC gel. In this method, 2 different methods were previously used, which were dead end deposition and crossflow deposition. Volkan (2020) used the dead end deposition method to prepare CNC membranes. CNC suspensions were filtered through cellulose acetate and PES supports, and deposited crystals were stabilized with AlCl_3 permeation. Instead of dead end filtration, Kocaman et al. (2021) employed crossflow membrane filtration as a deposition methodology to obtain a CNC gel by taking advantage of concentration polarization phenomena. In the study, it was shown that CNCs can be nematicall

aligned with crossflow deposition, and the performance of the membrane can be tuned by changing crossflow velocity, hence the degree of alignment, and it was observed that the membrane rejection performance was increasing as the degree of alignment increasing from around 80% to 92.7% for blue dextran (5 kDa). For the study, the CNC layer was also stabilized with 0.2 M AlCl_3 filtration, which makes the membrane more compact and increases the membrane resistance. In further study, NaCl was added to the CNC suspension during deposition, which led to the formation of a more tightly packed CNC layer (Kocaman et al., 2022). For the new membrane, pure water flux decreased and showed better rejection performance, increase from 92.7% to 98.5% for blue dextran (5 kDa).

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Materials

DMSO (99.9% analysis grade), ethanol (99%) from Isolab; Sulfuric Acid (95-98%); Cellulose (fibers, medium), Cellulose acetate (average molecular weight 50,000), Sodium Hydroxide (98-100.5%), Sodium Bromide (99.99% trace metal basis), potassium persulfate (99% ACS grade), TEMPO (99%), (3-bromopropyl) trimethyl-ammonium bromide (97%), PEG 400, Blue Dextran (average molecular weight 5 kDa) from Sigma-Aldrich, sodium chloride (99%), hydrochloric acid (fuming 37%) from Merck; aluminum chloride hexahydrate (99%) from Tekkim; Sodium hypochlorite from Aromel, PES from BASF, NanoVan™ TEM Stain from Nanoprobes.com.

Reverse osmosis water was used as nonsolvent in nonsolvent induced phase separation medium and washing, and pure water (conductivity of 18.3 MΩ.cm) was used in crosslinking solutions and dialysis.

3.2 Support Membrane Preparation

3.2.1 Polymer Solution Preparation

CA was dried for two days in vacuum oven before preparation of the casting solution, while PES were dried at 80 °C overnight. Dope solution for CA10 (10 wt. % CA, 90 wt. % DMSO) and support 2 (10 wt. % PES, 30 wt. % PEG 400 and 60 wt. % DMSO) prepared with given recipes. Then, solutions were mixed with magnetic stirrer and roller mixer until there was no visible phase difference. After

mixing, prepared solutions were also left still for 2 days to remove any bubbles that might be formed during mixing.

3.2.2 Membrane Fabrication

Flat sheet membranes were prepared as support structures for further modifications with a 250 μm casting bar at room temperature on a glass plate. Cast polymer films were immersed in a water bath directly to induce phase separation. After phase separation, support membranes were immersed in reverse osmosis water to wash the remaining solvent multiple times to reach complete coagulation. Membranes were stored in 20% ethanol to prevent bio formations (Figure 13).

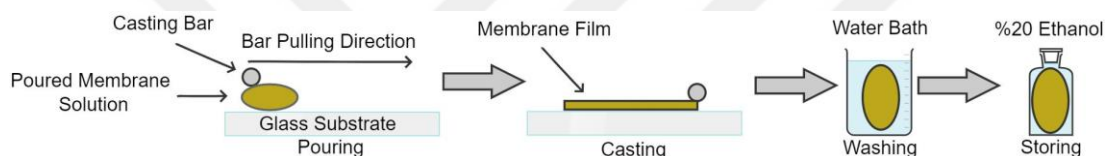


Figure 13. Support membrane preparation process.

3.3 Cellulose Nanocrystal Preparation and Modification

3.3.1 Preparation of CNC Suspensions

Cellulose was dried at 80 °C for 2 days and stored in a vacuum oven before CNC preparation. Initially, 80 ml pure water was mixed with 150 ml 96% sulfuric acid and heated until it reached 45 °C in a water bath. Dried 28.8g cellulose was hydrated with 58 ml pure water in another beaker, making the final acid concentration 50% with the addition of hydrated cellulose. Then, heated sulfuric acid was mixed with hydrated cellulose with rigorous mixing. The final solution was left reacting in a water bath for 30 minutes. Finally, the reaction was quenched with cold pure water (conductivity of 18.3 M Ω .cm) by diluting the mixture to 1 L.

CNC suspension was purified from the remainder of sulfuric acid and formed salts by first decanting and discarding the clear part until no phase separation occurred, then dialyzed against pure water (conductivity of 18.3 MΩ.cm) until dialysis water conductivity reached pure water conductivity.

3.3.2 Positively Charged Cellulose Nanocrystal Suspension Preparation

CNC suspension was mixed with (3-bromopropyl) trimethyl-ammonium bromide and 0.1 M NaOH while stirring at room temperature for 24 hours. After the reaction, no quenching step was implemented, and the suspension was dialyzed directly against pure water until dialysis water conductivity reached pure water conductivity (conductivity of 18.3 MΩ.cm).

3.3.3 TEMPO Oxidized Cellulose Nanocrystal Suspension Preparation

Previously prepared CNC solutions were further modified with TEMPO oxidation. 0.0295 g TEMPO, 0.324 g NaBr, and 6g of 12% NaClO equivalent were added into 1 g oven dried CNC basis suspension. The suspension was left to react for 3 hours, and the pH of the solution was adjusted into the 10-10.5 range with NaOH solution continuously. To quench the reaction, 3.7 ml ethanol was added to remove unreacted NaClO to form chloroform. Then, the suspension pH was adjusted to 1.5 to convert deprotonated form CNCs to protonated form for both carboxyl and sulfate half ester groups. Finally, the formed suspension is dialyzed against pure water until dialysis water conductivity reaches pure water conductivity.

3.3.4 Desulfation of Cellulose Nanocrystals

CNC suspensions were desulfated via thermal desulfation, acid catalyzed desulfation, and alkaline desulfation methods. The condenser apparatus was connected to the reaction vessel used to eliminate evaporation during desulfation.

In the thermal desulfation method, CNC suspensions were heated up to 60 °C and kept there for 20 hours. The suspension was cooled with a cold water bath. Then, the final suspensions were dialyzed against pure water until dialysis water conductivity reached pure water conductivity.

Acid catalysed desulfation was done by the addition of 0.05 M hydrochloric acid into suspension at 60 °C for 24 hours. The suspension was cooled with cold water bath, then the final suspensions were dialyzed against pure water until dialysis water conductivity reached pure water conductivity.

Alkaline desulfation was done by adding concentrated NaOH into suspension by making the final NaOH concentration 0.25, 0.5, or 1M and leaving the reaction for 30, 60, 90, or 180 minutes at 65°C. After desulfation, added NaOH was neutralized with fuming HCl, and the pH of the suspension was reduced in the region of 1.5-2 to transform deprotonated form CNC to protonated forms for further analysis. Then, suspensions were cooled and dialyzed against pure water until dialysis water conductivity reached pure water conductivity.

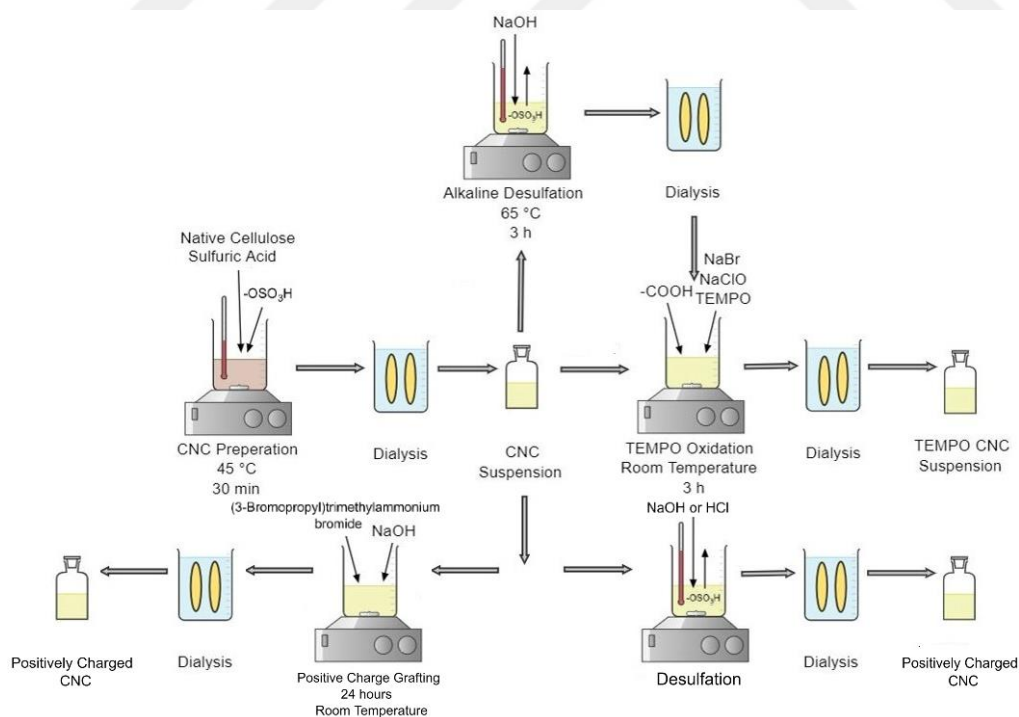


Figure 14. Schematic of employed CNC preparation methods.

3.4 Crosslinking of Cellulose Nanocrystals

3.4.1 Silver Nitrate Crosslinking

AgNO₃ crosslinking was done on both suspensions and TEMPO CNC membranes. For suspensions, 0.12 wt.%, 0.24 wt. %, and 0.36 wt. % AgNO₃ and 0.08 wt. %, 0.15 wt. %, 0.3 wt. %, 0.45 wt. %, and 0.60 wt. % KPS were added into TEMPO CNC suspensions and left reacting for 2 hours. For membrane crosslinking of the membrane in a crossflow cell, without discharging the membrane cell and tubing, 0.36 wt. % AgNO₃ and 0.08wt. % KPS crosslinking solution was permeated through the membrane for 2 hours. After 2 hours, the membrane cell and tubing were first emptied, and the final membrane was washed with pure water overnight.

3.5 Cellulose Nanocrystal Membrane Preparation

3.5.1 Crossflow Membrane Preparation

TEMPO CNC membranes were prepared by crossflow filtration of TEMPO CNC crystals on the support surface. As an exemplary procedure, 0.15 wt. % TEMPO CNC was prepared and sonicated with Bandelin Sonopuls mini (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. Then, the suspension was filtered through a support membrane by fixing the crossflow flow rate to 64 ml/min by measuring the retentate flow rate. Filtration continued until the limiting flux reached the constant pressure of 1 bar, where the filtration flux was known to be limiting flux. Finally, deposited TEMPO CNC particles were stabilized with 2 hour crosslinking with 0.36 wt. % AgNO₃ and 0.08 wt. % KPS solution. Tubing and membrane cells were not emptied before the addition of crosslinking solution to prevent delamination of deposited crystals. After crosslinking, the remaining solution was emptied, and the membrane was washed with pure water (Figure 15).

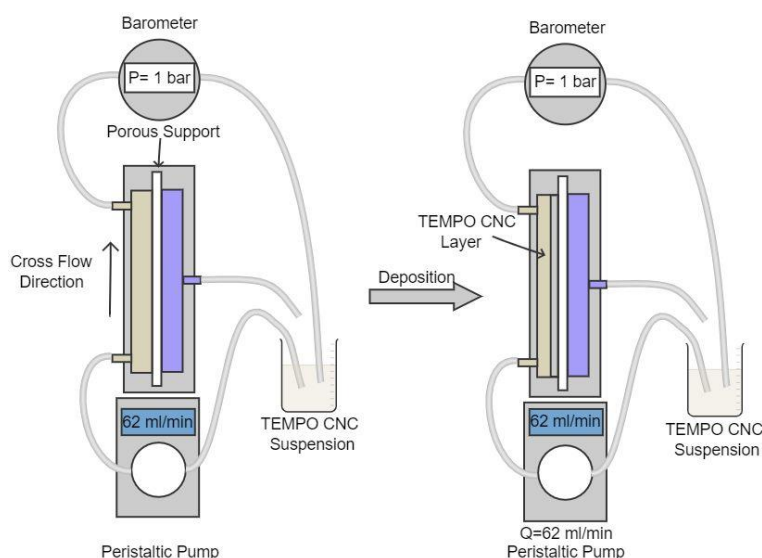


Figure 15. Crossflow membrane preparation methods

3.6 Characterization Methods

3.6.1 Analysis of Aluminium Chloride Stability of Suspensions

AlCl_3 Stability of 0.3 wt. % TEMPO CNC suspensions were analysed using Hanna Instrument HI88703 turbidimeter (Italy) with two methods. Before turbidity analysis, suspensions were sonicated Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. In the first method, salt was directly added to each sonicated suspension sample, and the turbidity of the suspension was recorded. In the second method, 0.4 M AlCl_3 was added to the suspension, and the turbidity of samples was collected by diluting that suspension with fresh TEMPO CNC suspension. Analysis was done to see aggregation and settling behaviour of the suspensions with addition of salt.

3.6.2 Analysis of Alignment of Suspensions and Films

Nematic alignment of CNC and TEMPO CNC suspensions was analysed with Zeiss Scope.A1 (Germany) optical microscope with the polarizer. Initially, CNC

and TEMPO CNC suspensions were concentrated to 2.5 wt. % by concentrating as a synthesized suspension with CA10 membrane filtration, then sonicated with Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. Analysis of nematic shear alignment was done in a 250 μm diameter capillary with a syringe pump with 400 s^{-1} and 800 s^{-1} shear rates on capillary walls. For NaCl added suspensions, 1 wt. % to 4 wt. % TEMPO CNC was again prepared by concentrating as a synthesized suspension with CA10 membrane filtration. Then, 100 mM NaCl is added to the prepared suspension. Analysis was done with 1.1 mm capillary with a syringe pump while wall shear rates were in the 0 s^{-1} to 400 s^{-1} . For 0 s^{-1} measurement, the capillary was first filled with flow rate where the wall shear rate was 10 s^{-1} , then the pump was stopped. After taking pictures of 0 s^{-1} , the pump was started again, and steady state images of 10, 20, 33, 50, 100, 200, and 400 s^{-1} shear rate flows were obtained by increasing the flow rate. Polarized optical microscopy images were taken at steady state flow and analysed with ImageJ.

3.6.3 Surface Charge Analysis with Conductometric Titration

Surface charge characterization of suspensions was done with conductometric titration. During titration, suspensions were added into 120 ml 1 mM NaCl solution. NaOH with known concentration was added with 1 ml of aliquots, and the conductivity of the sample was measured with each addition. After adding enough NaOH, recorded conductivity was corrected with dilution and plotted as NaOH consumption versus corrected conductivity. Endpoints were found by linearly extrapolating obtained two curves and finding intersection points of lines. For CNC suspension, only one endpoint was expected (Figure 16A). On the other hand, two endpoints were expected for TEMPO CNC suspensions (Figure 16B), and NaOH consumption caused by carboxyl groups can be found from the difference between the two endpoints. Surface charges can be calculated as follows:

$$SO_3H = \frac{m_{CNC}}{V_{1,NaOH}c_{NaOH}} \quad \text{Equation 2}$$

$$COOH = \frac{m_{CNC}}{V_{2,NaOH}c_{NaOH}} - \frac{m_{CNC}}{V_{1,NaOH}c_{NaOH}} \quad \text{Equation 3}$$

In the equation, m_{CNC} , $V_{x,NaOH}$, and c_{NaOH} were defined as the mass of dried basis CNC or TEMPO CNC, and NaOH volume was used to see the endpoints and concentration of NaOH solution respectively.

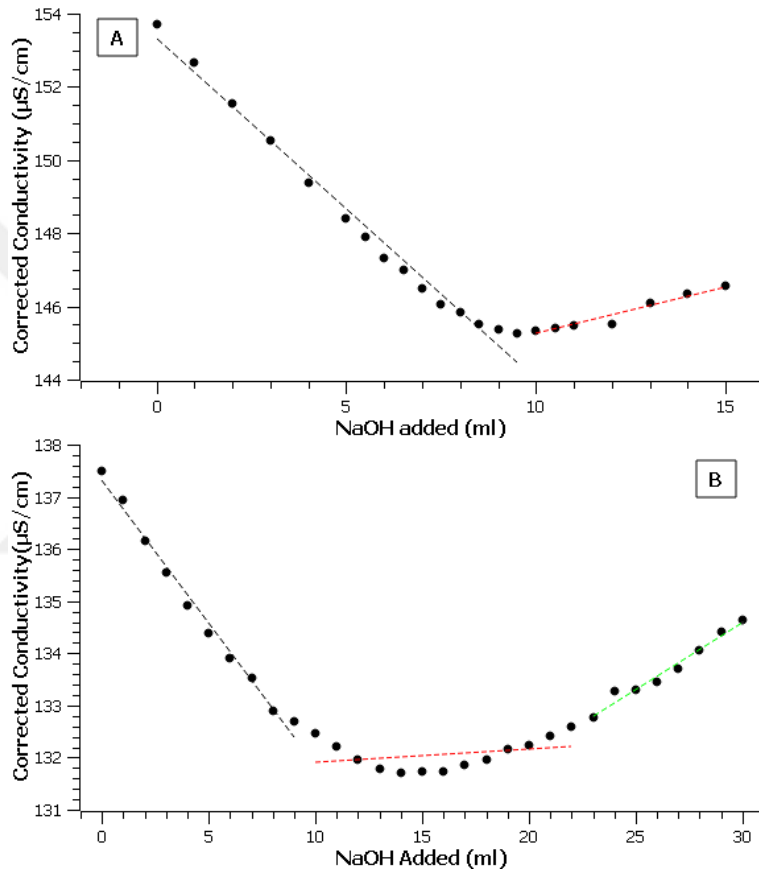


Figure 16. Conductometric titration example for CNC (A) and TEMPO CNC (B)

3.6.4 Zeta Potential and Isoelectric Point Analysis

Zeta potential analysis was done with Malvern Zetasizer Ultra for suspensions with DTS 10800 cuvette. CNC and TEMPO CNC samples were diluted to 0.10 wt. % for the analysis and sonicated with Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. Analyses were done in triplicate

without modifying the suspension's pH with 120 seconds of equilibration time at 25°C.

Isoelectric point analysis was done on suspensions. To find the isoelectric point, the pH of the suspension was adjusted, and the zeta potential was measured. Measurements were made with pH ranges between 2-10.

3.6.5 DLS Analysis

DLS analysis was used for the characterization of the size of particles with Malvern Zetasizer Ultra with Sarstedt 67.754 polystyrene cuvette. CNC and TEMPO CNC samples were diluted to 0.10 wt. % for the analysis and sonicated with Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. Analysis was done with 120 seconds of equilibration time in multiple angle dynamic light scattering (MADLS) mode without suppressing the electrical double layer and modifying pH with 3 repetitions at 25 °C.

3.6.6 Rheological Analysis

Rheological analyses were done with Anton Paar MCR 320 rheometer with Anton Paar CP50-2 cone and plate attachments with 2.008° cone angle and 0.21 mm spacing between cone and plate at minimum at 25 °C.

To obtain flow curves 2.5 wt. % CNC and TEMPO CNC samples were prepared with membrane filtration using CA10 support membrane. Concentrated samples were sonicated with Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes, and then flow curves were obtained between shear rates of 0.1 to 100 s⁻¹.

To obtain the loss and storage modulus of the crosslinked and non crosslinked samples, target concentrations of CNC and TEMPO CNC samples were prepared with membrane filtration using CA10 support membrane. After achieving target

concentrations, samples were sonicated with Bandelin Sonopuls mini20 (Germany) with MS 2.5 probe with 100 % amplitude for 20 minutes. After sonication, crosslinking experiments were conducted on the samples if needed. Finally, frequency sweep tests were done on final suspensions as the reaction time was achieved without purifying the sample. Frequency sweep tests were done for 0.63 to 100 rad/s angular frequencies and 5 % constant strain.

3.6.7 Thermogravimetric Analysis

Thermogravimetric analyses were done on dried TEMPO CNC and crosslinked TEMPO CNC films. Analysed films were first prepared with the previously mentioned membrane preparation method. Then films were removed from the surface, dried at room temperature, and opened to the atmosphere. Analyses were done between 25 to 900 °C in an N₂ environment with a 10 °C/min heating rate.

3.6.8 Glutaraldehyde Crosslinking

GA crosslinking was done with 0.25%, 0.5%, 1%, 2% glutaraldehyde, and 1% sulfuric acid addition into CNC suspension and left to react at 40 °C for 3 hours and 4 days. Final suspensions were used for size and gelation analysis.

3.6.9 Membrane Performance Tests

Membrane tests were done with Sterlitech CF042 crossflow filtration cell at 1 bar, where the effective membrane area was reported as 42 cm². Tests continued up until seeing a steady membrane flux. Pure water permeance was calculated with the following equation:

$$\frac{J}{\Delta P} = \frac{Q}{A \cdot t \cdot \Delta P} \quad \text{Equation 4}$$

In the equation, ΔP , Q , A , t , and J were defined as transmembrane pressure, permeate flow rate, membrane area, time, and flux, respectively, while the ratio of J and P were defined as permeance.

Rejections were determined using Blue Dextran (5 kDa) as solute with a concentration of 0.4 g/L with the same crossflow filtration cell at 0.4 bar to with the same crossflow rate deposition. For used flow rates, membrane pure water flux was found to be same with solute filtration fluxes, which means no concentration polarization. Rejection of the solute was measured by using Shimadzu UV-1601 spectrophotometer at 620 nm. Rejections were calculated with the following equation:

$$R = \left(1 - \frac{c_p}{c_r}\right) \cdot 100\% \quad \text{Equation 5}$$

In the equation, R , c_p , and c_r were defined as rejection, concentration of permeate, and retentate, respectively.

3.6.10 Polarized Optical Microscopy

Polarized optical microscopy images were obtained with Olympus BX53M equipped with an analyser and polarizer. Samples were prepared with the exact procedure mentioned in the membrane preparation steps. Then, the prepared membranes were put under the microscope and analysed while wet. Analysis was done for transmittance and reflectance modes.

3.6.11 Scanning Electron Microscopy

Scanning electron microscopy (SEM) micrographs of prepared membranes were obtained with TESCAN VEGA 3 electron microscope with secondary electron mode. Membrane samples were frozen and fractured with liquid nitrogen and sputter coated with Au/Pd coating of 40 nm with Leica EM ACE200 before obtaining micrographs. Surface micrographs were obtained with a working

distance of 6 mm, 10 kV high voltage, and beam intensity 8. Cross section micrographs were obtained with a working distance of 12 mm and beam intensity of 8 and 10 kV high voltage. Energy dispersive X-ray spectroscopy was done with TESCAN VEGA 3 in backscattered electron mode with a working distance of 6mm, beam intensity of 15 and 10 kV high voltage. Micrographs were analysed with ImageJ.

3.6.12 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) micrographs were obtained with FEI Tecnai G² Spirit BioTwin CTEM at METU Central lab at 120 kV. Suspensions with 0.02 g/L dropped on carbon coated TEM grids and left equilibrating for 2 minutes and wicked with filter paper. After that, NanoVanTM negative stain were dropped on a grid, left dyeing for 30 seconds, and washed with pure water. Micrographs were analysed with ImageJ.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of Cellulose Nanocrystals in Suspension

4.1.1 Initial Characterization of Cellulose Nanocrystal Suspension, TEMPO CNC Suspensions, and Desulfated TEMPO CNC Suspensions

CNC suspensions were prepared by sulfuric acid hydrolysis of cotton linter with 30 min of reaction time at 45 °C. During the reaction, sulfuric acid concentration in the liquid phase was 50% sulfuric acid. After reaching the allocated reaction time, the reaction was quenched with pure water while the final diluted suspension volume was 1 L. Diluted suspensions were found unstable caused of suppression of the electric double layer of CNCs caused by the low pH of the final suspension. In the case of increased ionic strength and low pH phase separation were observed for CNC suspensions. Therefore, the purification of CNCs can be started by decanting the upper layer, which is purely sulfuric acid, and adding pure water. After 3 days of decantation, the sedimentation rate was slower. The volumes of the suspensions were reduced as much as possible during final decantation to increase suspension concentration. Therefore, final purification was done with dialysis against pure water up until the conductivity of outer reached pure water conductivity (5 mS/cm). After purification, the pH of the final suspension was found to be in the region of 3-3.5, and the suspension concentration was found to be between 0.3 wt. % to 1.5 wt. % with respect to final suspension volume. The sulfate half ester content of the CNC suspensions was 393.9 ± 34.4 mmol/kg with conductometric titration (Figure 17A).

This CNC preparation method was previously used in our study group and detailed characterization were done (Volkan, 2020). XRD analysis were done according to peak height method to compare the height of amorphous peak of 18° and (002) plane which is around 21.5° and crystallinity index was found as 81%.

TEMPO oxidation was done on CNC suspension to modify primary alcohol groups into carboxyl groups. The modification was done with NaClO/NaBr/TEMPO reaction system for 3 h at room temperature while the pH of the system was adjusted to 10-10.5 region if it reduced below 10. After purification, the final pH of the suspensions was found to be in the region of 4-4.5, and the concentration of suspensions was found between 0.3 wt. % to 1.5 wt. % while it was lower than the initial CNC suspension concentration. The sulfate half ester contents of the CNC suspensions were found to be 388.8 ± 8.0 mmol/kg with conductometric titration. Carboxyl contents were found to be 500 ± 40 mmol/kg with 2 distinct end points, as it was expected from TEMPO CNC suspension modified from sulfuric acid hydrolysed CNCs (Figure 17B) (Fraschini et al., 2017). From conductometric titration, sulfate half ester content was found to be constant during TEMPO oxidation, which is expected, since there is no modification on sulfate half esters, while the total surface charge concentration was found to be increased caused by the addition of carboxyl groups.

As a third group, TEMPO oxidation was done on desulfated CNCs to modify primary hydroxyl groups of CNCs into carboxyl. CNC suspensions were desulfated with the alkaline desulfation method with 1M NaOH at 65°C 3h. After alkaline desulfation, the pH values of the suspensions were reduced to 1.5 with HCl, and dialysis against pure water was done as previously mentioned. After desulfation, sulfate half ester contents of suspensions were found as 28.7 ± 8 mmol/kg (Figure 17C). Then, the TEMPO oxidation procedure was done on desulfated suspension, and the dialysis procedure was performed again. Final suspensions carboxyl contents were found to be 338 ± 45 mmol/kg, assuming the sulfate half ester content remained constant (Figure 17D). Additionally, zeta potential for the desulfated TEMPO CNC suspension was found as 47.4 ± 0.4 mV, which was higher than

TEMPO CNC suspension (-59.5 ± 1.69 mV), which also means that the total surface charge of the TEMPO CNC was decreased.

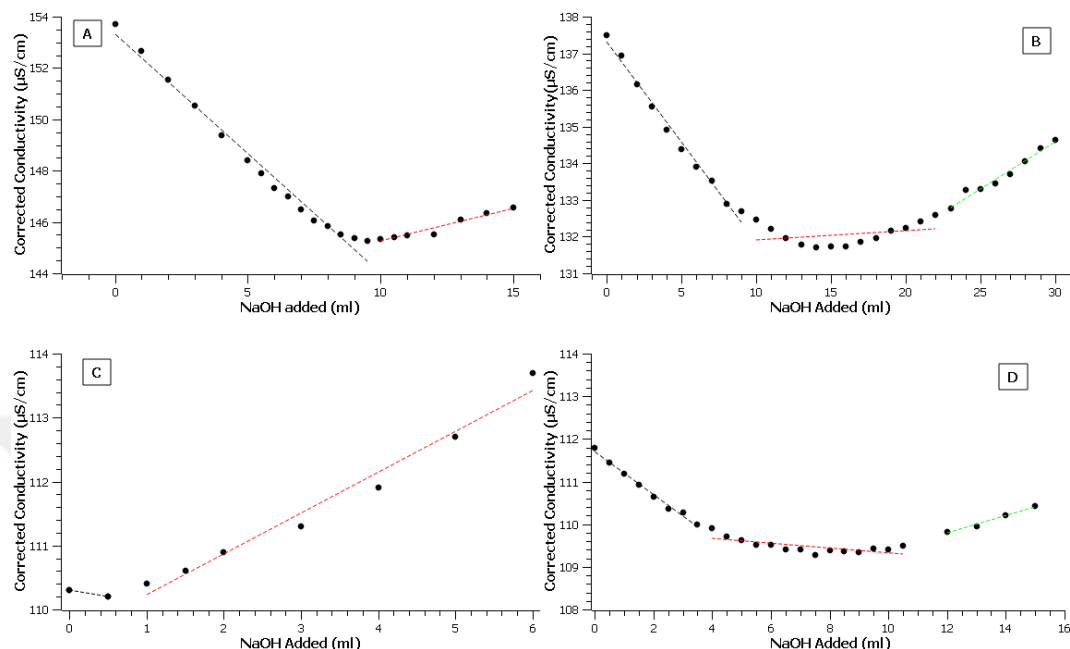


Figure 17. Exemplary conductometry results of CNC (A), TEMPO CNC (B), desulfated CNC (C) desulfated TEMPO CNC (D).

4.1.2 Size Analysis of CNC and Modified CNC Samples

Size analysis of the unmodified CNC, TEMPO CNC, and desulfated TEMPO CNC was done with TEM with and without NanoVanTM stain to see if it is possible to obtain micrographs without staining. After sample preparation, TEM micrographs were obtained with FEI Tecnai G² Spirit BioTwin CTEM with 120 kV voltage. TEM images without stain (Figure 18A) micrographs showed low contrast, making particles ambiguous and measurements hard due to low conductivity CNCs where stained samples show good contrast for the size measurements (Figure 18B). Therefore, NanoVanTM TEM stain was used for the TEM imaging for size analysis.

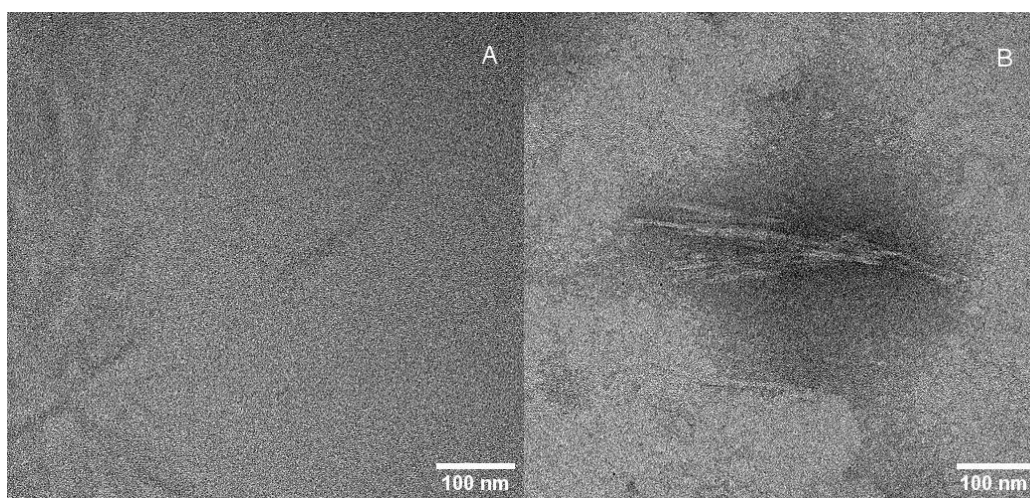


Figure 18. TEM micrographs of unstained (A) and stained (B) desulfated TEMPO CNCs.

TEM micrographs for CNCs prepared by sulfuric acid hydrolysis can be seen in Figure 19. The rodlike appearance of singular crystal can be observed from the figure, while some of the rods overlapped. When the sizes of the particles were analysed with ImageJ software, the length of CNCs was found as 122 ± 38 nm while the width was found as 10 ± 2 nm (Figure 19). This is expected for cotton cellulosic sources which was expected in the range of 100-150 nm in length and 5-10 nm in width (George & Sabapathi, 2015).

TEM micrographs for TEMPO CNCs can be seen in Figure 20. Again, the rodlike appearance of TEMPO CNCs can be observed with a dark background with overlapping. There are 2 different possibilities for the dark background. The first one is electron blockage caused by aggregated and highly charged TEMPO CNCs and stains, while the second one is impurities on suspension. Measurement of individual particles showed a length of 113 ± 17 nm and width of 8 ± 2 nm. The size of the TEMPO CNCs can be considered constant during modification. In the literature, TEMPO oxidation was mentioned as a mild reaction, and no size changes were observed for TEMPO oxidized CNCs suspensions (Fraschini et al., 2017).

TEM micrographs of desulfated TEMPO CNCs can be seen in Figure 21. Desulfated TEMPO CNCs also show rodlike behaviour with some overlapping with a less dark background with respect to TEMPO CNCs (Figure 20). Size change was not expected for desulfation and TEMPO oxidation, and measurement for the particles showed 148 ± 45 nm in length and 10 ± 2 nm in width, which is still in the expected range for cotton.

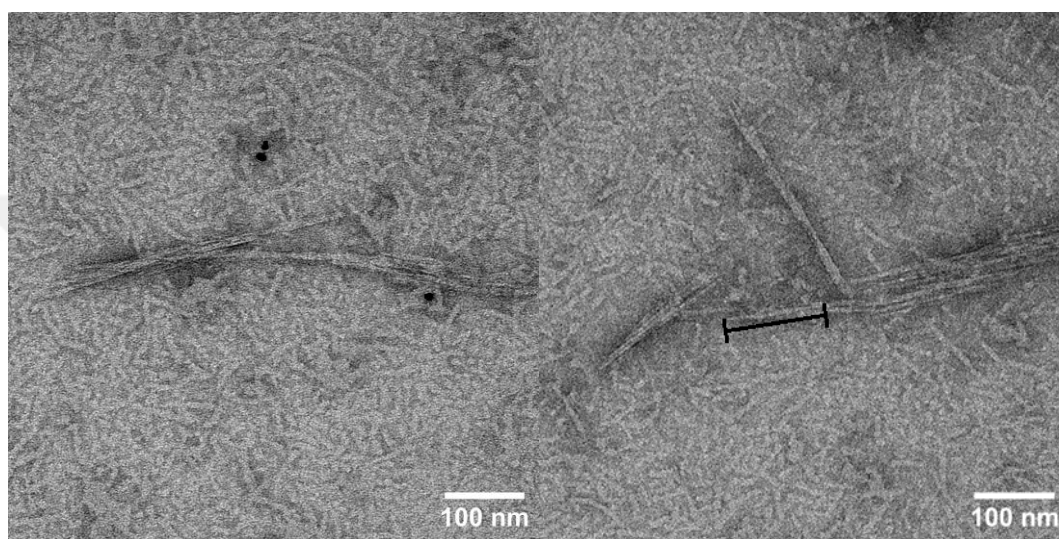


Figure 19. TEM micrographs of stained CNCs at 120 kV. A representative crystal was shown in the figure.

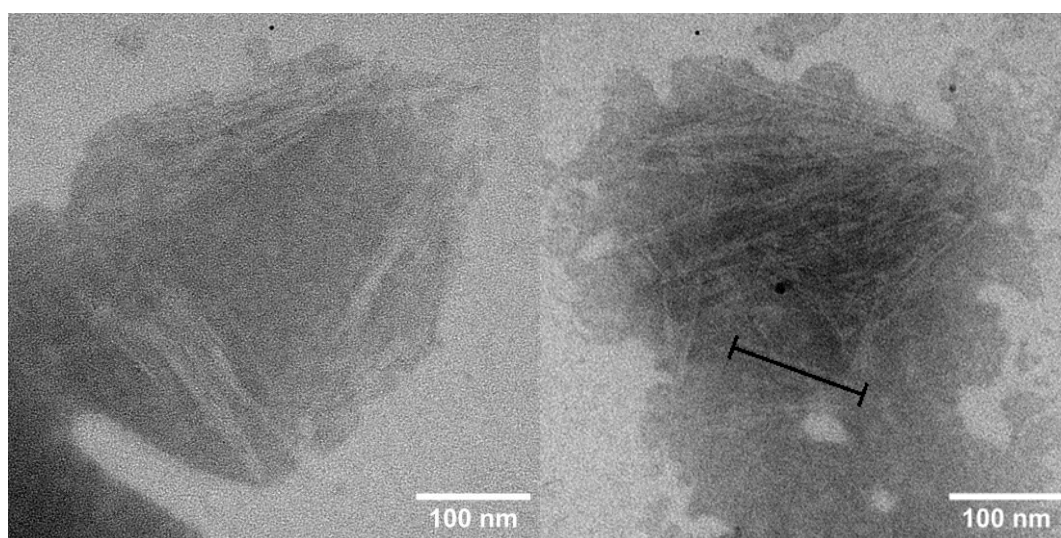


Figure 20. TEM micrographs of stained TEMPO CNCs at 120kV. A representative crystal was shown in the figure.

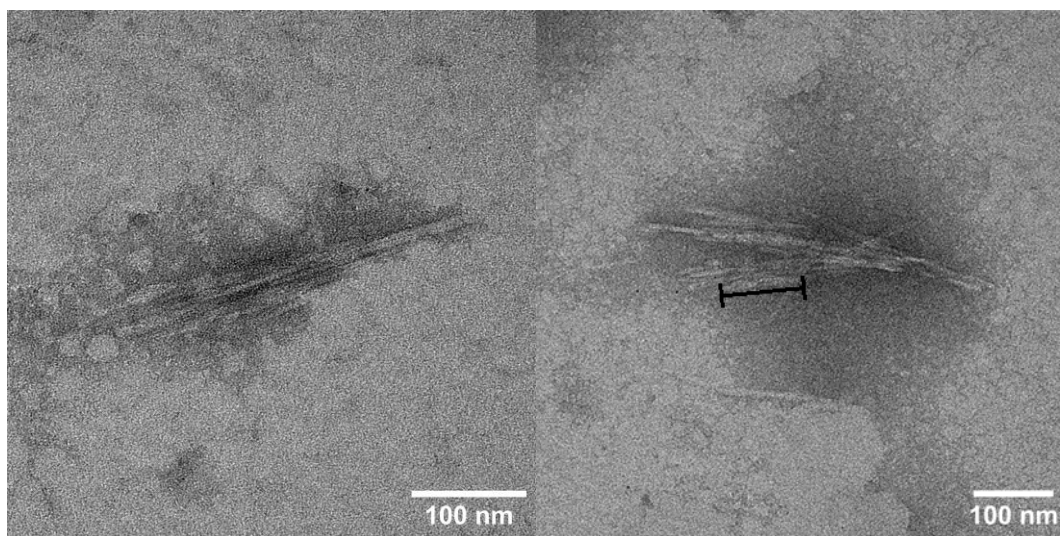


Figure 21. TEM micrographs stained desulfated TEMPO CNCs at 120 kV. A representative crystal was shown in the figure.

4.1.3 Isoelectric Points of CNCs and modified CNCs

The isoelectric point is defined as the pH where zeta potential reaches 0. To obtain the isoelectric point, zeta potential of suspensions was measured with Malvern Zetasizer Ultra, while the pH values of the suspensions were changed between measurements. Measurements were done in the range of 3-10 by changing pH with either HCl or NaOH, the limit of used cuvettes.

For measured TEMPO CNC and unmodified CNC samples, zeta potential with respect to pH graphs can be seen in Figure 22. For CNCs, pH after dialysis was measured as 3.35 for the sample with a zeta potential of -47.7 ± 1.2 mV, indicating a net negative charge for the CNCs as expected. For the sample, zeta potential stayed constant as the pH increased, while the decrease in zeta potential could be observed as the pH was decreased to 3 (red line). These results were in line with a previous literature finding (Eyley et al., 2015). The only difference with the literature finding is that zeta potential for the study is in an unstable region while our sample is in a stable region, indicating a higher surface charge. For the sample, no

isoelectric point was observed. The highly acidic nature of sulfate half ester grafted on cellulose is likely the reason.

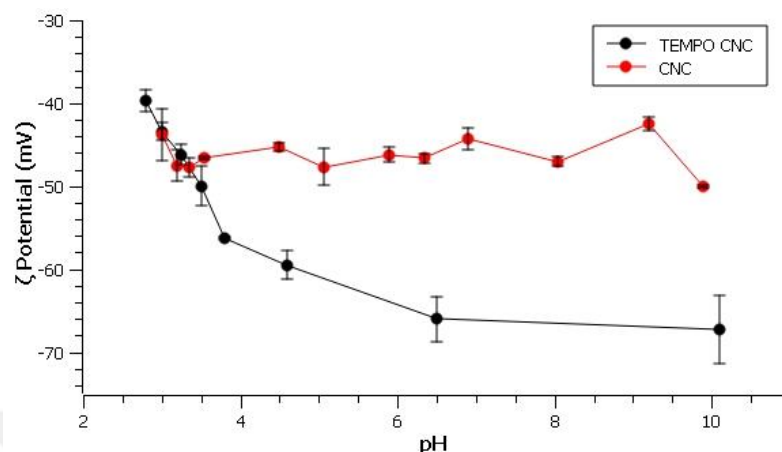


Figure 22. Isoelectric points scan results for CNC and TEMPO CNC suspensions. Error bars show three replicate zeta potential measurements of one sample.

For the TEMPO CNC sample, natural pH was measured as 4.6, and zeta potential is -59.5 ± 1.7 mV. Decreased zeta potential (or increased absolute value of zeta potential) with respect to increased pH was observed as opposed to the constant zeta potential of unmodified CNCs. At pH 4, zeta potential start to decrease faster with decreased pH, which might mean suppression of carboxyl groups which indicates pKa around 4 for the carboxyl. This observation was consistent with the pKa value of other carboxylic acids (Haslak et al., 2021). On the other hand, a decrease in pH led to a decrease in zeta potential at every pH, but no isoelectric point was observed for TEMPO CNCs. At pH values, no surface charge was suppressed, and zeta potential of TEMPO CNCs was consistently lower than normal CNCs, which means increased net negative charge for samples after TEMPO oxidation. Addition of carboxyl groups were expected to increase suspension stability, which was unwanted for membrane preparation, but it should be grafted for the planned chemical crosslinking method for membrane preparation.

4.1.4 Positively Charged CNC Preparation

Positive charging of cellulose nanocrystals was tried on previously prepared and dried hydrochloric acid hydrolysis in our study group (Volkan, 2020) and sulfuric acid hydrolysis CNCs suspensions. Positively charging of CNC crystals was done according to previously mentioned in the literature, as is with (3-bromopropyl) trimethyl-ammonium bromide and NaOH (Akcan, 2017). Before charging, hydrochloric acid hydrolyzed CNCs were in powder form, and no net charge was expected (Araki et al., 2000b), while sulfuric acid hydrolyzed CNCs were in suspension form with an initial zeta potential of -57.4 ± 1.3 mV. Zeta potentials of prepared samples were found as 10.4 ± 1.0 and -42.5 ± 1.7 mV for HCl and H₂SO₄ hydrolyzed CNCs respectively. Increase in measured zeta potentials indicates the formation of positively charged groups on CNCs but not sufficient to quantify.

4.1.5 Desulfation of Cellulose Nanocrystal Suspensions

Sulfuric acid hydrolysed cellulose nanocrystals are negatively charged caused by grafted sulfate half ester groups. These sulfate half ester groups were chemically bonded to the surface, but desulfation was previously observed at every temperature (Beck & Bouchard, 2014a). If needed, sulfate half esters can be removed with other methods. Desulfation can be done with 4 different methods mentioned before thermal desulfation (Lewis et al., 2016), alkaline desulfation (Jordan et al., 2019), acid catalysed desulfation (Beck & Bouchard, 2014a), and solvolytic desulfation (F. Jiang et al., 2010). In this study, we tried desulfation of CNCs on storage conditions, thermal desulfation, alkaline desulfation, and acid catalysed desulfation. Used CNCs were in the protonated form, which was known to desulfate easier with respect to the deprotonated form (Vanderfleet et al., 2019), and CNCs were dialyzed against pure water before conductometric titration to remove resulting sulfate ions in solute.

Conductometric titration results for desulfation at storing conditions (4°C in a refrigerator) can be seen in Figure 23. For the used batch, the initial sulfate half ester concentration of CNCs was measured as 350 mmol/kg and the concentration of the suspension was 1.53 wt. %. For storing conditions, sulfate half ester concentration for CNC suspension was found to be decreasing with time. In the second week, a sharp decrease in sulfate half ester concentration was observed and found as 208 ± 2 mmol/kg. For the 4th week, the charge concentration was found to be 220 ± 2 mmol/kg. After the 4th week, sulfate half ester content started to decrease more slowly. For the following measurements, concentrations were found as 204 ± 7 and 170 ± 16 mmol/kg for weeks 6 and 8, respectively, as expected. For those observations, it can be said that the sulfate half ester concentration of CNCs decreased with time, even if it was stored in low temperatures. These observations were in line with the literature (Beck & Bouchard, 2014a). In the literature, decreases in sulfate half ester concentration were minimized by converting protonated forms of CNCs into deprotonated form (Beck & Bouchard, 2014b) and also freeze dried (Bai et al., 2018), but CNCs should be converted into protonated form before each use in our study for this method, which increases the lead time with the addition of dialysis step to each experiment.

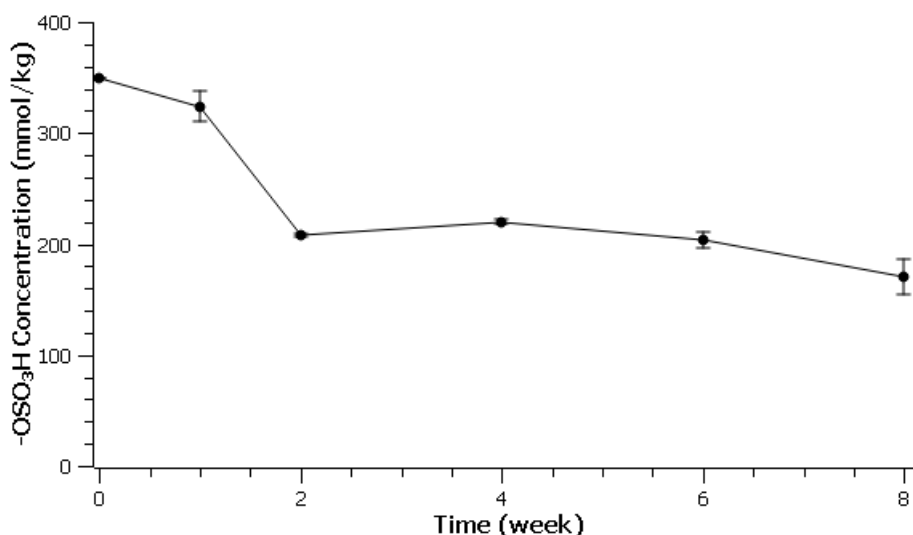


Figure 23. Time dependent sulfate half ester concentration for CNCs at storing conditions (refrigeration at 4 °C).

For the alkaline desulfation, thermal desulfation, and acid catalyzed desulfation of CNCs, a single batch CNC was synthesized with 2 L volume to make all the starting conditions the same for the following measurements but yield and resultant final concentration were found to be low for that batch, which made the starting concentration 0.18 wt. % and yield 6.3%. The sulfate half ester content of the batch was found as 393.9 ± 34.2 mmol/kg. Thermal desulfation was done at 65 °C for 3 and 20 hours. After dialysis of the desulfated samples, sulfate half ester content was found as 133.3 ± 2.6 mmol/kg and 111.5 ± 3.9 mmol/kg for 3 and 20 hours, respectively (Figure 24). As can be seen from these results, thermal desulfation is an effective desulfation method where 66% of the charges were removed in an initial 3 hours. If the forward reaction were defined as a desulfation reaction and the back reaction as a sulfation reaction, the reason for this decrease in reaction could be an increase in the back reaction rate and a decrease in the forward reaction rate caused by the formation of sulfuric acid from removed sulfate half ester groups. This means that desulfation might lead to the formation of sulfuric acid and act as an initial hydrolysis reaction to graft sulfate half esters back on the surface. Acid catalyzed desulfation is a one step further method for thermal desulfation, where it was done with almost the same procedure with the addition of HCl. Acid catalyzed desulfation was done at 65 °C for 20 hours, showing 103.8 ± 3.9 mmol/kg sulfate half ester content (red point in Figure 24). Therefore, it can be said that only a marginal difference was observed with the addition of HCl to CNC with respect to the thermal desulfated one. In literature, acid catalysis was intensively studied, and effectiveness was showed but, there was no study for comparison between thermal desulfation and acid catalysis (Beck & Bouchard, 2014a; F. Jiang et al., 2010), but thermal desulfation and acid catalysis were close to each other according to our study. It might be said that acid catalysis increases the desulfation performance of deprotonated form CNCs since the addition of acids converts deprotonated form CNCs into protonated forms which are more prone to desulfate. In this study, desulfation methods were not tried on concentrated samples. This marginal difference may be more meaningful for concentrated samples for acid

catalyzed desulfation. By employing thermal desulfation, sulfate half ester concentration could be decreased without need of waiting times of weeks. Therefore, longer desulfation times were not studied.

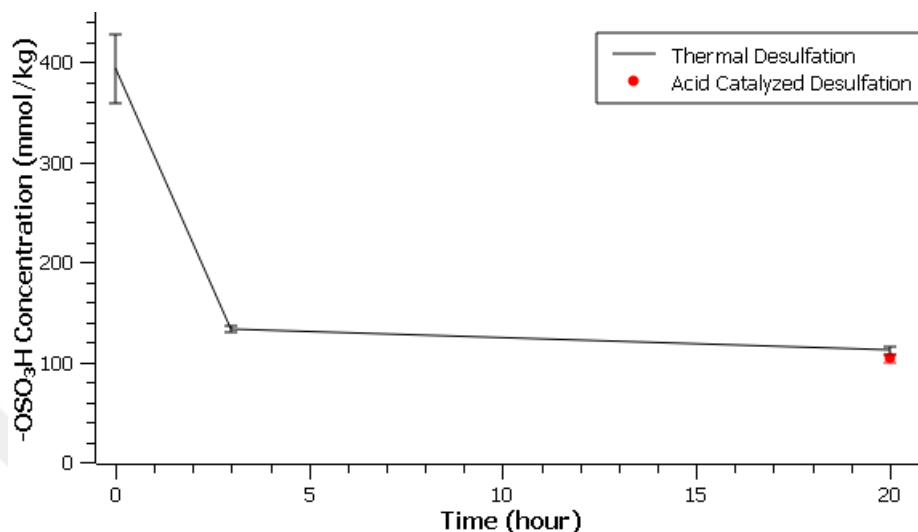


Figure 24. Time dependent sulfate half ester concentration for thermal desulfation and acid catalysed desulfation at 65 °C.

Alkaline desulfation was done at 65°C with 0.25 M, 0.5M, and 1M NaOH concentrations. Figure 25 shows the conductometric titration results for 0.25 M, 0.50 M, and 1M NaOH added alkaline desulfation results at 65 °C. As can be seen from the figure, desulfation occurred for the given concentration. At a 3 hour reaction time, the sulfate half ester content for the suspension was found to be 150±10.3 mmol/kg, while it was reduced to 135±10.4 mmol/kg after 3 hours for the 0.25 M NaOH concentration. For the 0.5 M NaOH added alkaline desulfation conductometric titration results, the figure shows that desulfation reaches a plateau at 30 min reaction where it reaches 69.4±27, 64.7±5.2 and 71.6±30 mmol/kg, respectively, for 30 min, 3 h, and 6 hours of reaction at 65 °C. For the 1 M NaOH added alkaline desulfation conductometric titration results, the same plateau can be observed on a lower sulfate half ester content for 1 M alkaline desulfation where surface concentration was found as 36.4±36.4 and 69.7±4.6 mmol/kg with 3 and 6 hours, respectively.

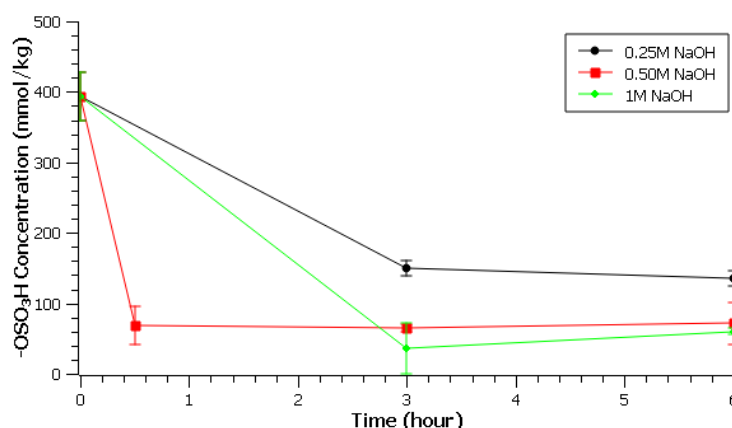


Figure 25. Time dependent sulfate half ester concentration of alkaline desulfation for 0.25 M NaOH, 0.50 M and 1 M at 65 °C.

Alkaline desulfation can be interpreted with replotting results with respect to the same time and different concentrations. Figure 26 shows alkaline desulfation results for 3 and 6 hours with different NaOH concentrations. To show comparison between thermal and alkaline desulfation, thermal desulfation result at the same temperature were included on the figure as 0 M NaOH concentration. For 3 hours of reaction time, the sulfate half ester content was found as 150 ± 10.3 , 64.7 ± 5.2 , and 59.7 ± 4.6 mmol/kg for 0.25, 0.50, and 1.00 M respectively, while thermal desulfation led to final surface charge of 133.3 ± 2.6 mmol/kg. Thermal desulfation was observed to be better than 0.25 M alkaline desulfation for the experimental setup, which may be caused by the formation of deprotonated form CNCs by adding NaOH. Still, as the concentration of the NaOH increased, alkaline desulfation appeared to be more effective than thermal one, even if the thermal desulfation reaction time was 20 hours. For 6 hours of reaction time, the sulfate half ester content was found as 135 ± 10.4 , 71.6 ± 30 , and 59.7 ± 4.6 mmol/kg for 0.25, 0.50, and 1.00 M, respectively. Therefore, it could be said that 3 h of reaction is enough for the reaction time for low concentration suspensions.

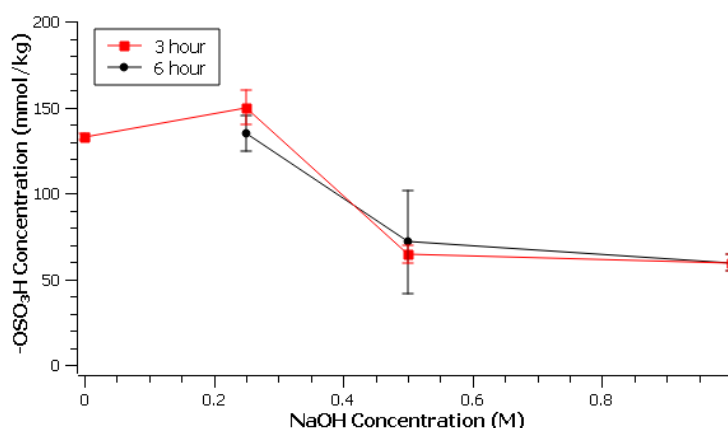


Figure 26. Concentration of alkaline dependent sulfate half ester concentration of CNCs for 3 h and 6 h reaction at 65 °C. For easier comparison, results for thermal desulfation at the same temperature were added as 0 M NaOH concentration.

Considering all the desulfation experiments, alkaline desulfation is the most effective method. This method allows the one to decrease sulfate half ester content of the CNCs to lower values than other methods for shorter reaction times for increased NaOH concentrations. In the study, alkali concentration dependent plateau regions were observed for the suspensions. These plateaus were also mentioned in the literature that the desulfation is much slower in low surface concentrations (Beck & Bouchard, 2014a). The concentration of the initial suspension was also found to be influential on desulfation performance. The desulfation performance of the system was modelled for time, suspension concentration, and alkaline concentration by (Jordan et al., 2019). No complete desulfation was observed for all mentioned methods in the study, which was also the case for the previous studies. In the literature, no study showed complete desulfation for alkaline, thermal, and acid catalysed desulfation; only solvolytic desulfation was used for complete desulfation (F. Jiang et al., 2010). Solvolytic desulfation is a method which is used for suspension forms. Therefore, it is not possible to employ this method after deposition. Additionally, suspensions are not stable since the crystals do not contain any surface charges after solvolytic desulfation. In this state, deposition would be problematic. Desulfation were used for membrane preparation also which can be seen in Appendix B.

4.1.6 Shear Alignment of Cellulose Nanocrystal and TEMPO Cellulose Nanocrystal Suspension.

Cellulose nanocrystal suspensions are lyotropic liquid crystals that show cholesteric symmetry after their critical concentration, which can be estimated with Onsager's model. In a previous study, the critical concentration for our CNC source was estimated as 2.0 wt. % while rheological characterization showed lyotropic nematic behaviour at 2.1 wt. % (Kocaman, 2022), where at this concentration biphasic region for cholesteric symmetry was observed. Based on Onsager's model, critical concentration is inversely proportional to length to diameter ratio. CNC and TEMPO CNC crystals prepared in our study have almost the same length to diameter ratio (Onsager, 1949). Additionally, the CNC source and method are identical to Kocaman's (2022) study. Therefore, it is reasonable to assume that the critical concentration of the CNC and TEMPO CNC suspensions are similar to the literature value.

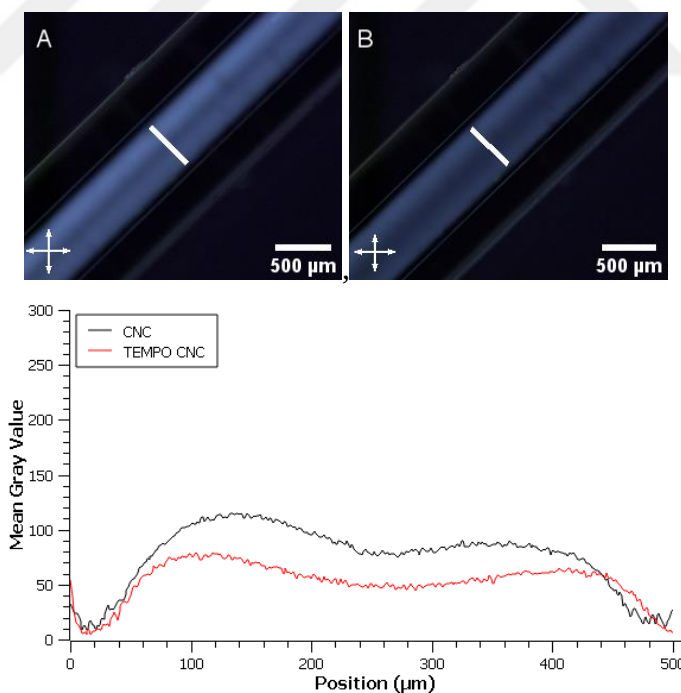


Figure 27. Polarized optical microscopy images and mean grey values for 2.5 wt. % CNC (A) and TEMPO CNC (B) suspensions under capillary flow with respective wall shear rate at 400 s^{-1} .

In our study, the nematic alignment of CNC and TEMPO CNC suspensions was done for 2.5 wt. % suspensions in a capillary with respective shear rates of 400 s^{-1} and 800 s^{-1} . In Figure 27 and Figure 28, polarized optical images of the capillaries under cross polarizers and mean grey values of images can be seen. As seen from the figures, CNC suspension has a brighter appearance with respect to TEMPO CNC suspensions for both 400 s^{-1} and 800 s^{-1} , which can be seen from the full mean grey value plot. These observations show that TEMPO CNC suspensions show a lower degree of nematic alignment with respect to unmodified CNC suspensions under the same shear rate conditions. The reason for the decreased alignment might be caused by increased surface charge, which also increases electrostatic repulsion between cellulose nanocrystals.

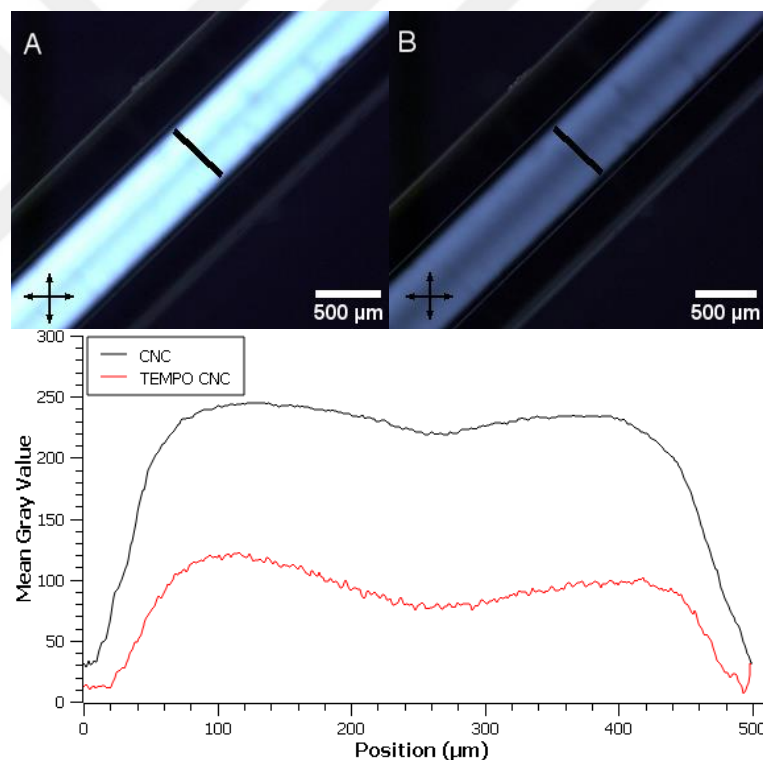
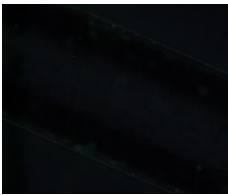
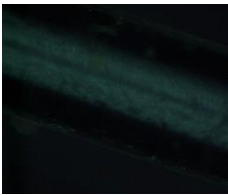
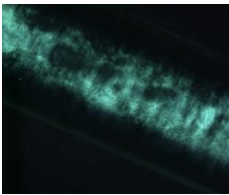
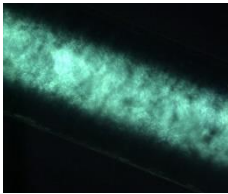
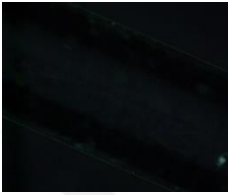
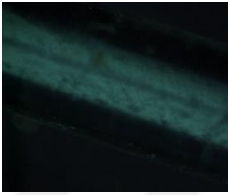
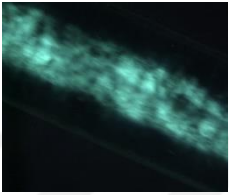
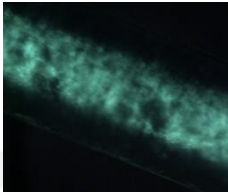
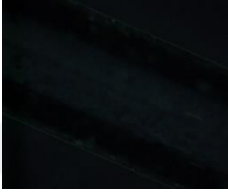
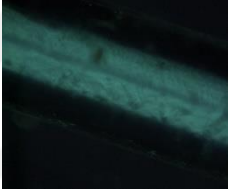
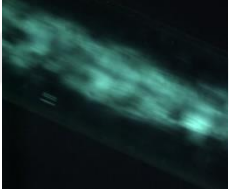
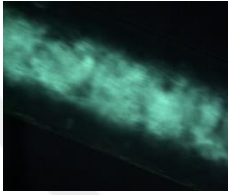
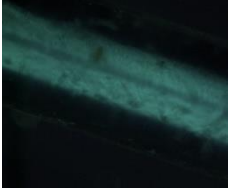
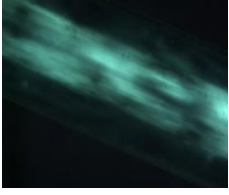
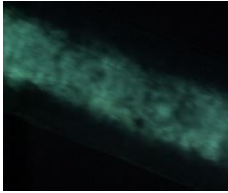
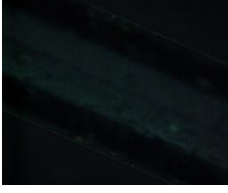
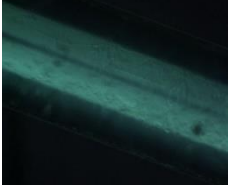
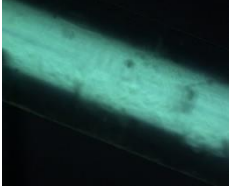
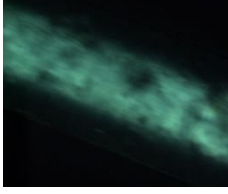


Figure 28. Polarized optical microscopy images and mean grey values for 2.5 wt. % CNC (A) and TEMPO CNC (B) suspensions under capillary flow with respective wall shear rate at 800 s^{-1} .

Another experiment was conducted for nematic alignment of 100 mM NaCl added TEMPO CNC suspensions which are later used in membrane fabrication. Figure 29

shows polarized optical microscopy images for the different TEMPO CNC concentrations with respect to shear rates. For the images, it was not possible to obtain images under the same exposure without making more birefringent images overexposed. This was needed to be able to make comparisons between different concentrations and shear rates. Therefore, exposures were halved and quartered for some images, shown in Figure 29. For the figure, all concentrations used in experiments showed birefringence caused by nematic alignment. Therefore, it can be said that the addition of salt, and increased ionic strength, lead to decrease electrostatic repulsion of TEMPO CNC particles which results in lowered critical concentration for liquid crystal behaviour. Polarized optical microscopy images for increased concentration of TEMPO CNC lead to the formation of aggregates which can be observed in lower shear rates. Formed aggregates were found to be broken when the shear rate increased. For broken aggregates, highly birefringent and clear suspensions were observed. Maximum mean grey values for images are shown in Table 1. Obtained mean grey values showed that birefringence was observed for every concentration. At 0 s^{-1} , no birefringence was expected for any of the suspensions, but it can be seen from both table and figures that there was some alignment at 2 wt. % and more concentrated solutions. There might be two reasons for that observation. Firstly, suspensions were pumped with shear rates at around 10 s^{-1} to fill the capillaries and stopped. This might be why the nematic alignment and relaxation may not be complete. Secondly, aggregates may be formed by suppressed electrostatic double layer with increased ionic strength. When the shear was applied to suspensions, increased brightness and birefringence were observed for both increased shear and suspension concentration, as expected.

	1%	2%	3%	4%
0 s^{-1}				 Half Exposure
10 s^{-1}				 Half Exposure
20 s^{-1}				 Half Exposure
33 s^{-1}				 Quarter Exposure
50 s^{-1}			 Half Exposure	 Quarter Exposure

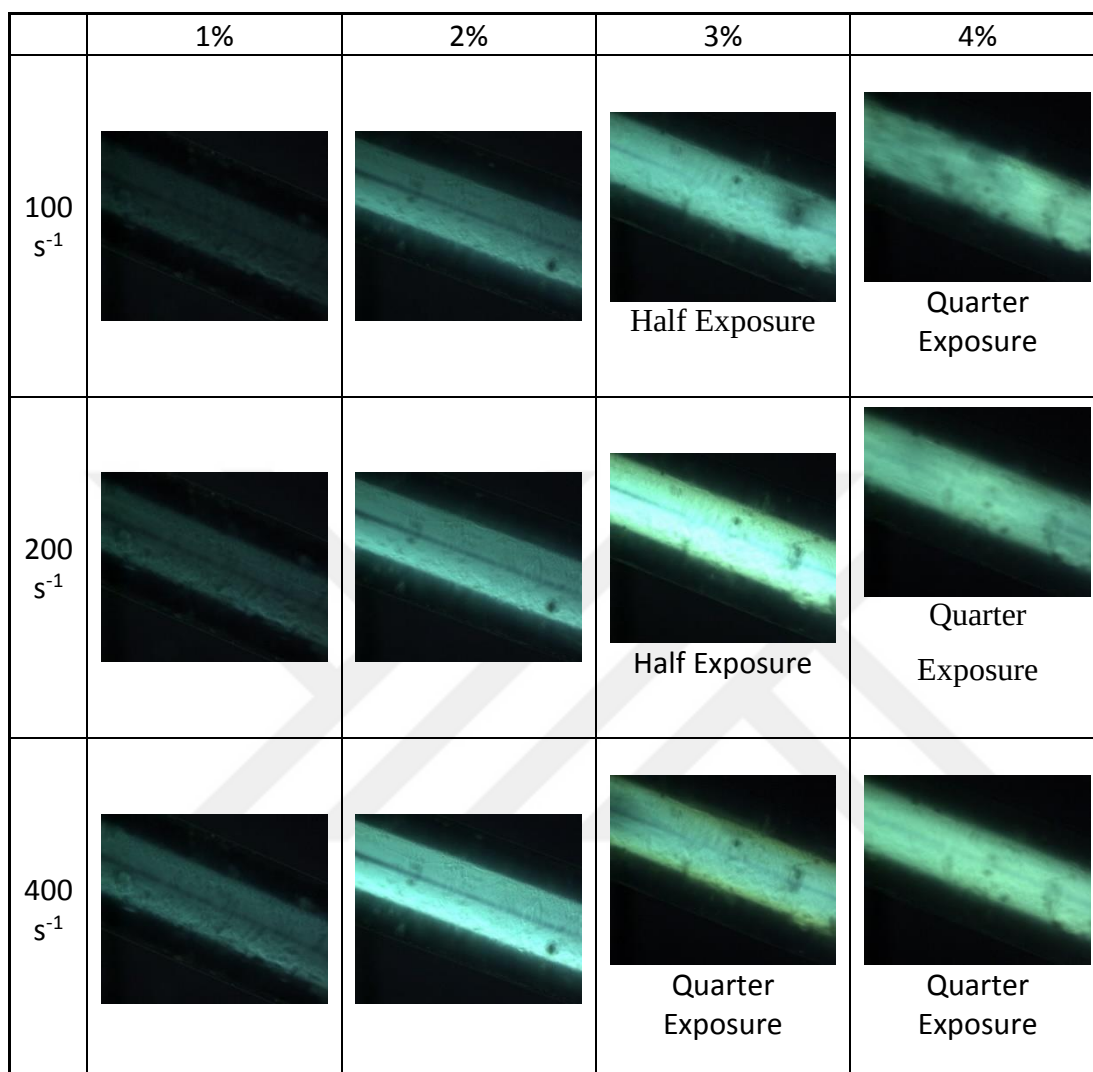
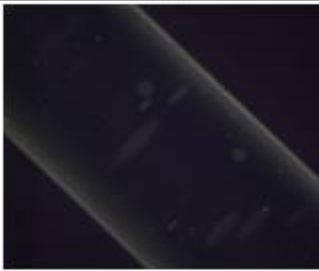
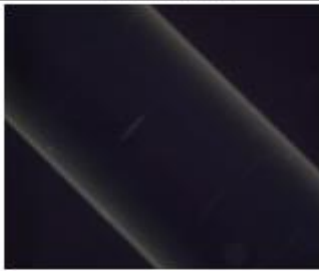
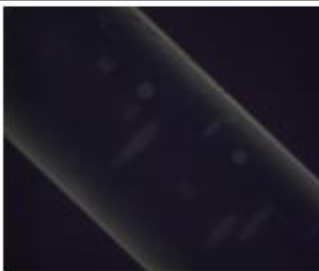
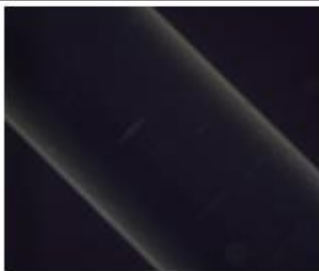
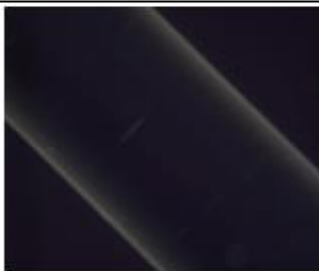


Figure 29. Polarized optical microscopy images for 100 mM NaCl added TEMPO CNC suspensions with given shear rate and concentrations.

Table 1. Maximum mean grey value for the respective polarized optical microscopy images (corrected for same exposure).

	0 s^{-1}	10 s^{-1}	20 s^{-1}	33 s^{-1}	50 s^{-1}	100 s^{-1}	200 s^{-1}	400 s^{-1}
1%	10	12	14	17	25	36	57	76
2%	48	69	80	90	105	125	155	215
3%	90	135	145	165	347	367	477	638
4%	177	197	317	388	498	578	698	758

	CNC	TEMPO CNC
0 s^{-1}		
10 s^{-1}		
20 s^{-1}		
33 s^{-1}		
50 s^{-1}		

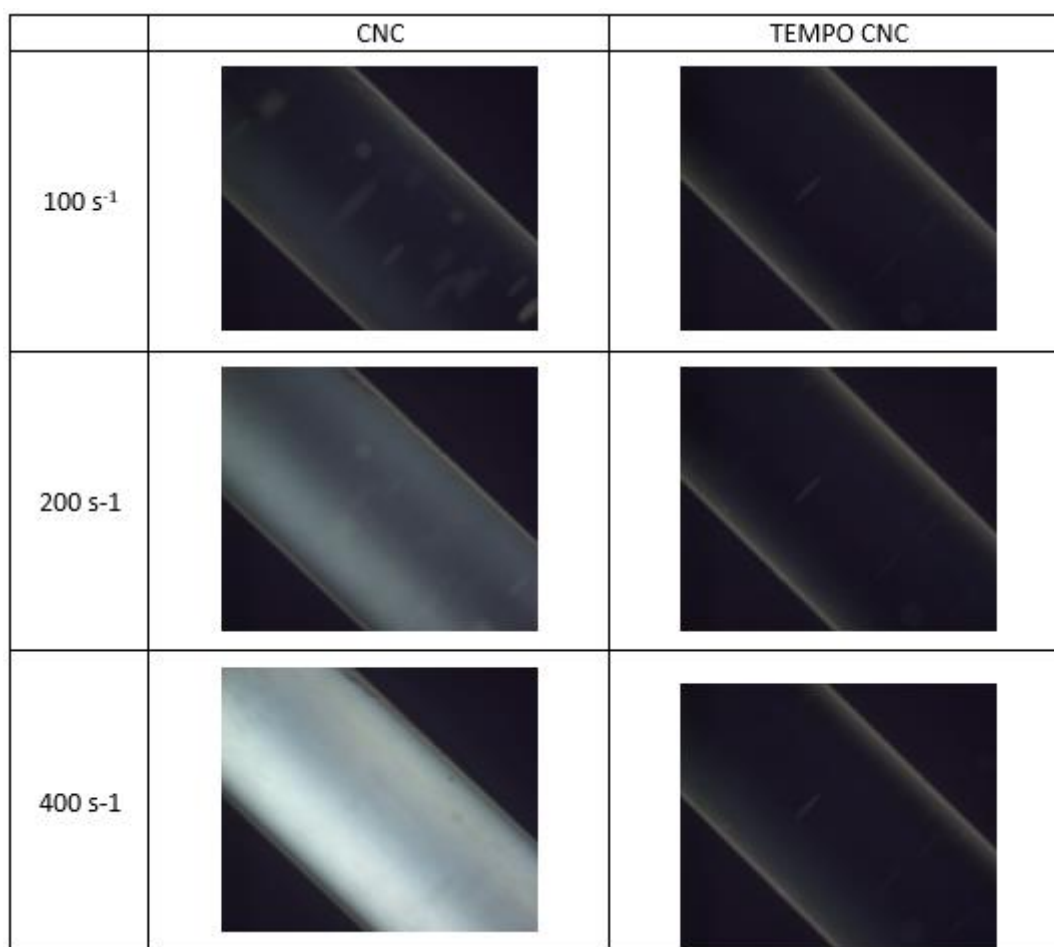


Figure 30. Polarized optical microscopy images for 2 wt. % CNC and TEMPO CNC suspensions with given shear rate.

Table 2. Maximum mean grey value for the respective polarized optical microscopy images (corrected with empty capillary).

	0 s^{-1}	10 s^{-1}	20 s^{-1}	33 s^{-1}	50 s^{-1}	100 s^{-1}	200 s^{-1}	400 s^{-1}
CNC	3	2	2	3	4	20	87	194
TEMPO CNC	3	1	3	3	4	4	6	26

Capillary studies were conducted under the same conditions to compare nematic alignments of CNC, TEMPO oxidized CNCs (Figure 30), and 100 mM NaCl added TEMPO oxidized CNCs (Figure 29). At 2 % concentration, CNC and TEMPO CNC showed birefringence as predicted, but TEMPO CNC showed low

birefringence that was only observed at the highest shear rate used in the study as 400 s^{-1} , which was too low. This observation was similar to previous observations for CNC and TEMPO CNCs, where CNCs show better nematic alignment with the same shear rates. Additionally, the addition of NaCl also increased the degree of alignment of TEMPO CNCs, since increasing the ionic strength of the medium would lead to suppression of electrostatic repulsion caused by surface charges.

4.1.7 Suspension Stability TEMPO CNCs with Addition of Salts

Salt stability of TEMPO CNC suspensions was studied for stabilization TEMPO CNC layer after deposition as a membrane layer. Turbidity and zeta potential analysis were used to see stability with the addition of salts. Turbidity of suspension is caused by solids suspended on a liquid, TEMPO CNC particles in our case. It was hypothesized that adding salt leads to agglomeration and increased turbidity. Additionally, the stability of a suspension can be measured by its zeta potential. Figure 31 shows the turbidity of TEMPO CNC suspensions concerning AlCl_3 concentration. Data of the figure were obtained by measuring the turbidity of TEMPO CNC suspension where 0.4 M AlCl_3 was added initially to the suspension, and consecutive measurements were done with the addition of pure TEMPO CNC to that suspension to decrease salt content for dilution case. On the other hand, addition case data were obtained by adding salt with respective concentrations to each suspension separately to measure turbidity. By looking at both figures, adding 0.001 M AlCl_3 led to an increase in turbidity which was reduced for increased concentration. The suppression of electrostatic repulsion and agglomeration caused the increase in turbidity, due to higher possibility of scattering light with formed CNC clusters. The only important difference between these measurement techniques is the region 5 mM and 50 mM, where the other data are consistent. All salt added suspensions were found to be settled after a while.

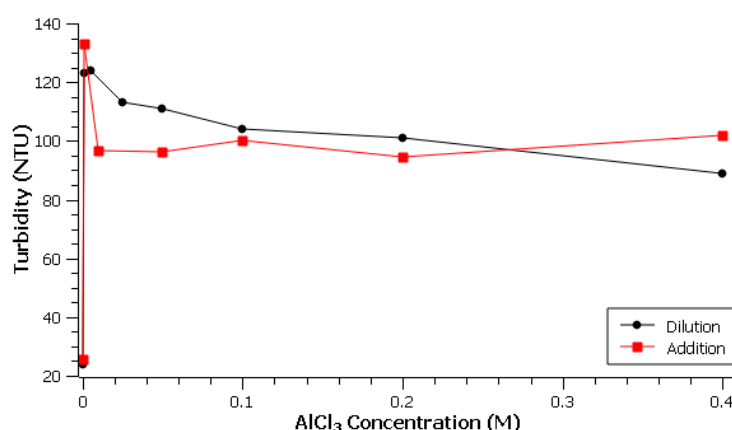


Figure 31. Turbidity of TEMPO CNC suspension with respect to AlCl_3 concentration measured with dilution of 0.4 M AlCl_3 added suspension, and addition of required AlCl_3 each suspension separately.

The stability of the suspension was measured for AlCl_3 and NaCl concentrations with zeta potential measurements. Table 3 shows zeta potential measurement results for salt added TEMPO CNC suspension. zeta potential for the pure TEMPO CNC suspensions was measured as -42.6 ± 2.6 mV for these measurements. For AlCl_3 addition, zeta potential decreased to unstable values after 1mM concentration, consistent with settling observed for salt added suspensions. For NaCl added suspensions, zeta potential was found to be decreasing for increased NaCl concentration. For 5, 50, and 100 mM NaCl concentration, zeta potentials were measured as -29.8 ± 2.8 , -23.3 ± 2.0 , and -18.8 ± 0.3 mV respectively.

Table 3. Zeta Potential of TEMPO CNC suspension with addition of NaCl and AlCl_3 .

	NaCl Added Zeta Potential (mV)	AlCl_3 Added Zeta Potential (mV)
0 M	-42.6 ± 2.6	-42.6 ± 2.6
1 mM	-34.7 ± 2.9	-3.4 ± 0.6
5 mM	-29.8 ± 2.8	15.2 ± 1.7
50 mM	-23.3 ± 2.0	No Result
100 mM	-18.8 ± 0.3	No Result

4.1.8 Rheological Analysis of CNC and TEMPO CNC Suspensions

Rheological analysis of TEMPO CNC and CNC suspensions was done for 2.5 wt. % concentration. For CNC suspensions, shear thinning regions were expected for low shear rates where it reaches the shear independent region at the middle region and shear thinning behaviour again for increased shear rates (Mark Parker et al., 2017). Only the first shear thinning and shear independent regions were observed in our study. At the shear rate of 0.1 s^{-1} , the viscosity of the suspensions was found as 1217 mPa.s and 814 mPa.s for CNC and TEMPO CNC, respectively, where shear thinning behaviour was found in between the shear rates of 0.1 and 17.8 s^{-1} . After 17.8 s^{-1} shear rates, shear dependent viscosity reached a plateau at 3.5 mPa.s and 2.2 mPa.s for TEMPO CNC and CNC, respectively, where alignment starts for respective suspension. A difference was observed for the CNC and TEMPO CNC suspensions which were found to be error margins for the measurements (Figure 32).

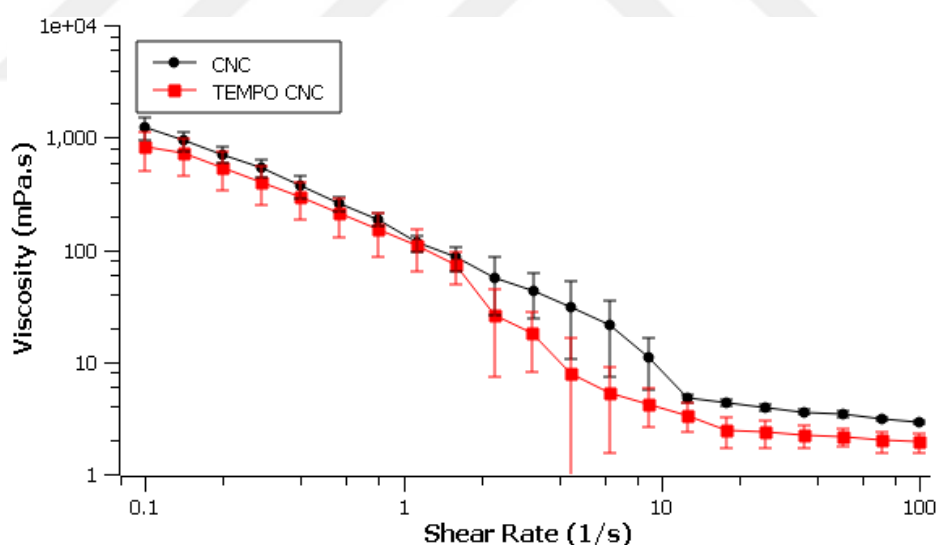


Figure 32. Shear dependent viscosity measurement results for 2.5 wt. % CNC and TEMPO CNC suspensions at 25 °C.

4.2 Characterization of Crosslinking of TEMPO CNC and CNC

4.2.1 Characterization of Crosslinking for TEMPO CNC Suspensions

TEMPO CNCs was crosslinked with silver catalysed decarboxylation reaction. In the reaction, Ag(I) and persulfate ions remove grafted carboxyl ions and radicals formed with decarboxylation links polymers (Weng et al., 2017). It was expected that the crosslinking would lead to an increase in the size of the suspension. The change in particle size in suspension caused by crosslinking was first characterized by turbidity since the increase in size leads to more light scattering. Figure 33 shows turbidity analysis during crosslinking for TEMPO CNC suspensions. As shown in the figure, the turbidity of the suspension increases as the KPS and AgNO₃ are added together. After initiation, turbidity of the suspensions was found to be increasing with time and KPS concentrations, which indicates crosslinking. Another observation is that turbidity of the suspensions reaches a plateau after 2 hours. Therefore, 2 hours of reaction time were selected for further studies.

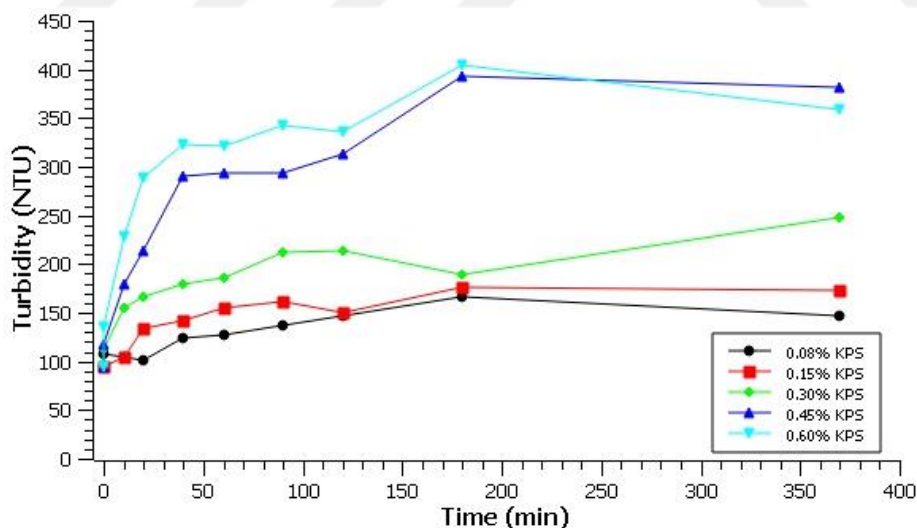


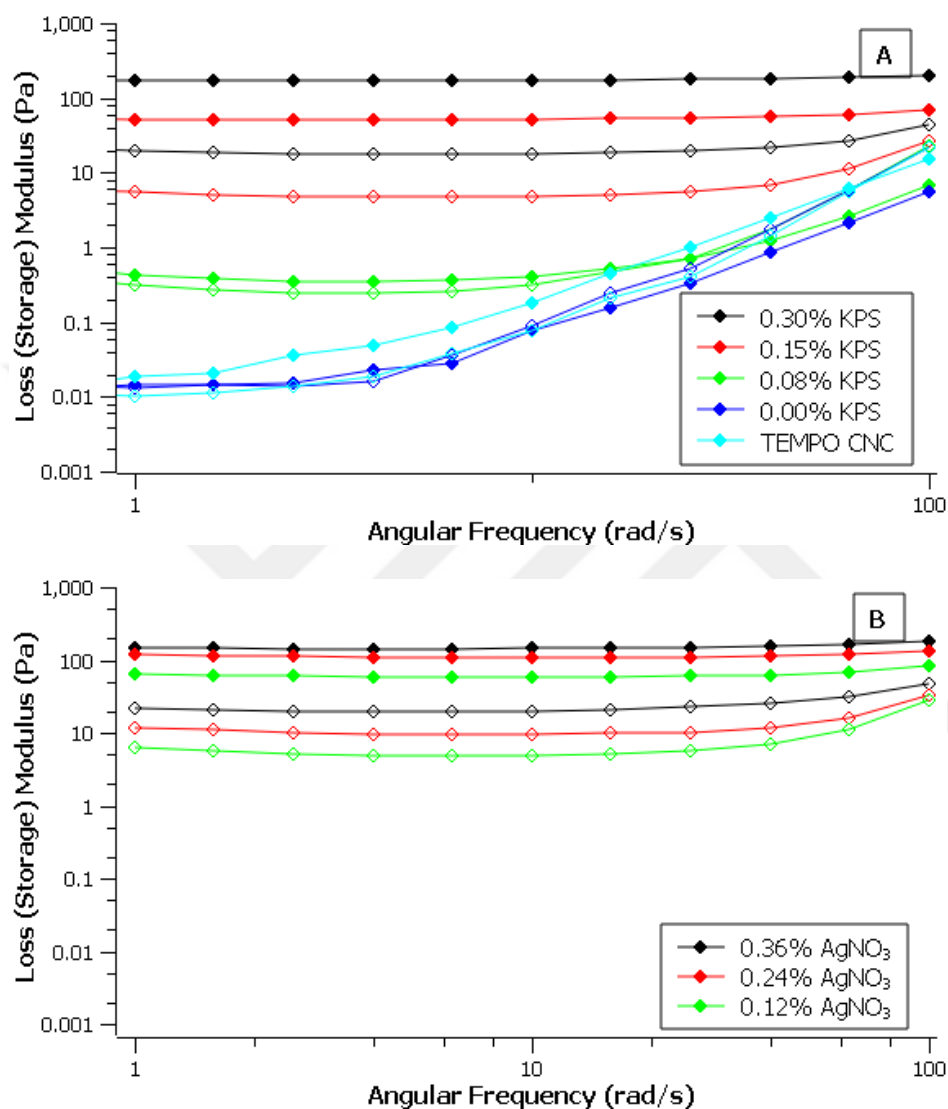
Figure 33. Turbidity of 0.3 wt. % TEMPO CNC suspensions during crosslinking for 0.12 wt. % AgNO₃ and variable KPS concentration at room temperature.

DLS analysis was conducted for the samples with 0.355 wt. % KPS and 0.12 wt. % AgNO₃, since those concentrations are used in literature as their highest

crosslinked samples (Weng et al., 2017). DLS analysis lets us observe if there is a change in size, although the hydrodynamic radius measured does not represent actual dimensions. The particle size was found to be increasing for given crosslinker concentrations from 191 ± 80 to 2050 ± 1330 nm when the reaction reached completion, 4 days of reaction time.

Frequency sweep tests were done for 2.1% TEMPO CNC for a constant 5% strain, crosslinked for 2 hours at room temperature. Frequency sweep on TEMPO CNC suspension showed that the storage modulus was higher than the loss modulus, which means TEMPO CNC is in weak gel form initially. Figure 34 shows all the results obtained for frequency sweep experiments for crosslinked TEMPO CNC suspensions. For 0.12 wt. % AgNO_3 concentration, crosslinking experiments were done with no KPS, 0.08 wt. % 0.15wt. % and 0.30 wt. % KPS which showed increased storage and loss moduli as the concentration of KPS increased. Even if both moduli increased, the storage modulus to loss modulus ratio was found to be increased other than 0.15 wt. % KPS case, where an increase in ratio means an increase in elastic behaviour, hence gelation, as the KPS concentration increased. Therefore, increasing the KPS concentration leads to increased crosslinking with constant reaction time. For the application in mind, increasing KPS concentration was not reasonable since it is a strong oxidant, and it was observed that KPS damages the cellulose acetate support membrane used in this study. Instead, it was decided to keep the KPS concentration constant and increase the AgNO_3 content. The literature mentioned that the increasing AgNO_3 content was also found to increase crosslinking density for constant reaction time. (Weng et al., 2017). Therefore, further tests were done for constant KPS concentrations of 0.15 wt. % and 0.08 wt. % with 0.12 wt. %, 0.24 wt. % and 0.36 wt. % AgNO_3 . For 0.15 wt. % KPS concentration, gel formation was observed for all AgNO_3 concentrations. On the other hand, 0.12 wt. % and 0.24 wt. % AgNO_3 concentration would not lead to “gel” formation for 0.08 wt. % KPS case, only 0.36 wt. % KPS lead to the formation of a gel. Therefore, it can be concluded that an increase in both KPS and AgNO_3 concentration resulted in better crosslinking for the studied range. For all

the measurements, the highest storage and loss moduli were found as 148.2 Pa and 21.4 Pa, respectively, at low angular frequencies (0.15 wt. % KPS and 0.36 wt. % AgNO_3) (Figure 34B).



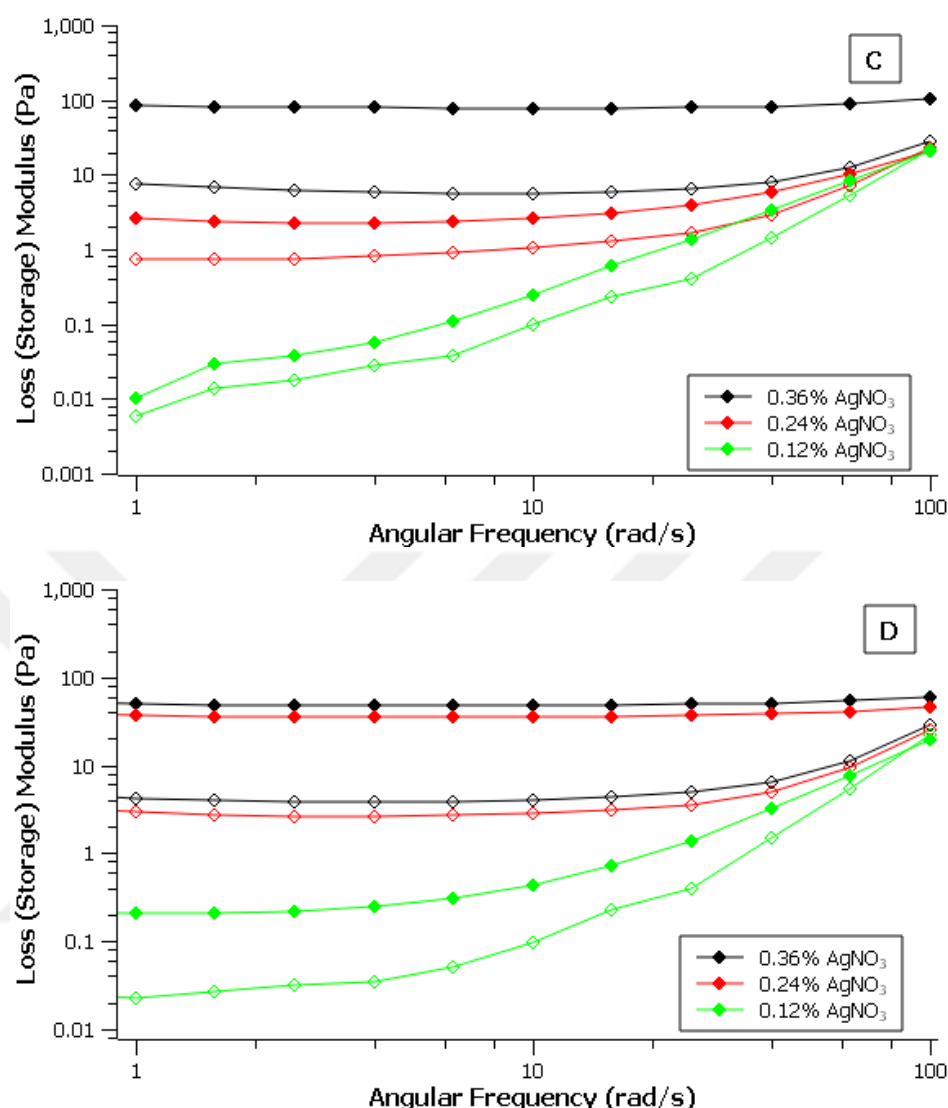


Figure 34. Frequency sweep results with 5 % constant strain for TEMPO CNC and 0.12 wt. % AgNO₃ crosslinking after 2 hours. TEMPO CNC shows TEMPO CNC without AgNO₃ or KPS addition (A). Frequency sweep results for TEMPO CNC and 0.15 wt. % KPS crosslinking with different AgNO₃ concentrations (B). Frequency sweep results for TEMPO CNC and 0.08 wt. % KPS crosslinking with different AgNO₃ concentrations (C). Frequency sweep results for desulfated TEMPO CNC and 0.08 wt. % KPS crosslinking with different AgNO₃ concentrations (D) at 5% constant strain. For the figures, filled diamonds show storage modulus while empty diamonds show loss modulus.

It was hypothesized that gelation would be possible for lower crosslinker concentrations if particles could approach closer to each other. Therefore, desulfated TEMPO CNCs were prepared as mentioned before, 3 h reaction time

with 1M NaOH at 65 °C. Desulfated TEMPO CNC suspension was used for crosslinking with 0.08 wt. % crosslinking with different KPS concentrations. It was observed that storage modulus to loss modulus ratios were found as 9.1, 14.3, and 12.5 (all can be considered as strong gel) for 0.12 wt. %, 0.24 wt. %, and 0.36 wt. % while the ratios were 1.7, 3.5, and 11.1 for the non desulfated counterparts (Figure 34C and Figure 34D). Therefore, it can be said that desulfation leads to better gel formation for our crosslinking case. It should be noted that TEMPO oxidation was done after desulfation; therefore, carboxyl content was not removed during the addition of alkaline and heating. It was previously reported as CNCs become closer to each other as the surface charge decrease (Kádár et al., 2021), and gel concentration would also increase with increased surface charge (Moud et al., 2022), which increases the possibility of crosslinking as the decarboxylation reaction occurs with KPS and AgNO₃.

All the results are summarized in Figure 35 and Figure 36 for the region where shear was low to deform three dimensional network so that characterization of gelation can be done. For 12 wt. % AgNO₃, 1 rad/s angular frequency results showed that 2.1% TEMPO suspensions were “weak gel” like when crosslinking was done with no KPS and 0.08 wt. % KPS where the ratio of storage modulus to loss modulus was found as 1.14 and 1.37, respectively. After 0.15 % KPS, the final suspension acts like “gel” as the ratio gets closer to 10, 9.5 for 0.15 wt. % and 8.6 for 0.3 wt. %. For “gel” like suspensions, storage moduli were measured as 51.6 and 169.6 Pa, while loss moduli were measured as 5.4 and 19.7 Pa, respectively, for 0.15 wt. % and 0.3 wt. % respectively. For 0.12 wt. % AgNO₃, therefore, it can be said that 0.15 wt. % and higher AgNO₃ concentration can be used for the most effective crosslinking. As mentioned before, increasing KPS content would damage the support membrane for the planned+ preparation method. Therefore, increasing AgNO₃ content would be more meaningful for increased AgNO₃ study and 0.15 wt. % KPS concentration, crosslinking always resulted in gelation where the storage to loss modulus ratio was found as 10.3, 9.9, and 6.9 for 0.12, 0.24, and 0.36 wt. % AgNO₃ concentrations. This ratio was reduced to 1.7, 3.4, and 11.4

when KPS concentration was reduced to 0.08%, but still 0.08 wt. % KPS and 0.36 wt. % AgNO_3 resulted in gelation. For the desulfated TEMPO CNCs, 0.08% KPS concentration worked better where the storage to loss modulus ratio becomes 7.9, 12.0, and 11.0 for 0.12, 0.24, and 0.36 wt.%, respectively.

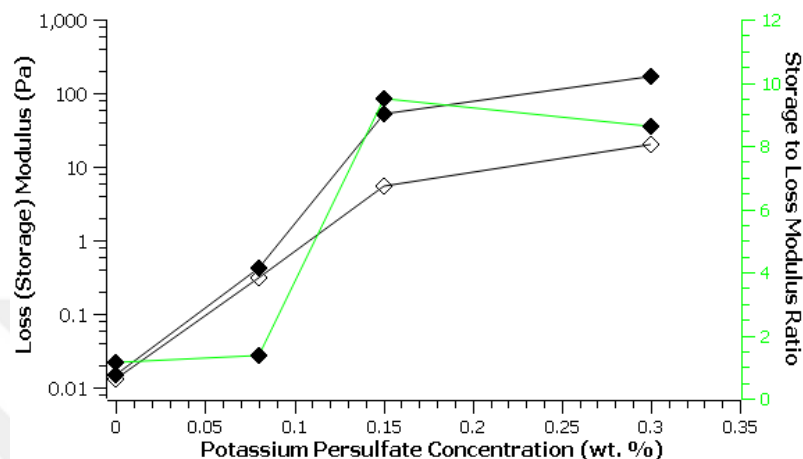


Figure 35. Storage modulus, loss modulus and storage to loss moduli ratio for 1 rad/s frequency and 5% strain for 2 hours of crosslinking for 0.12 wt % AgNO_3 concentration. For the figures, filled diamonds show storage modulus while empty diamonds show loss modulus. Green line shows the ratio of storage modulus to loss modulus.

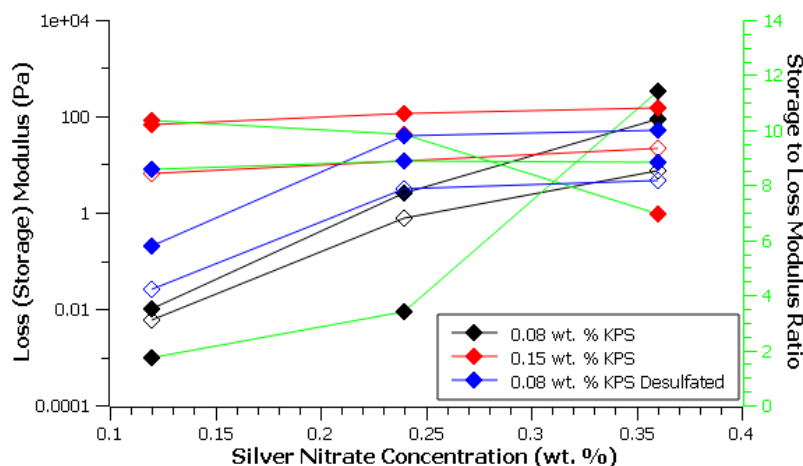


Figure 36. Storage modulus, loss modulus and storage to loss moduli ratio for 1 rad/s frequency and 5% strain for 2 hours of crosslinking. For the figure, filled diamonds show storage modulus while empty diamonds show loss modulus. Green lines show the ratio of storage modulus to loss modulus.

When all the results were combined, increasing both AgNO_3 and KPS resulted in increased storage and loss modulus, while the ratio of storage and loss moduli were decreased for the increased highest AgNO_3 and KPS case. Among the measured range, 0.08 wt. % KPS and 0.36 % AgNO_3 were found to be best for the application planned due to the strong oxidative properties of KPS. Desulfation was shown to increase the effectiveness of crosslinking by increasing the possibility of crosslinking. For further application in the study, it was decided that 0.36 wt. % and 0.08 wt. % KPS would be best with 2 hours of reaction time.

4.2.2 Characterization of Crosslinking for CNC Suspension with Glutaraldehyde

Glutaraldehyde was previously used to crosslink hydroxyl containing polymers (Gadhav et al., 2019). In this study, CNC suspensions were tried to be crosslinked. Reaction conditions were initially set for 1% sulfuric acid and 0.5 wt. % glutaraldehyde (GA) for 3h reaction while the reaction temperature was set to 40 °C. DLS analysis was done for the final suspension for these reaction conditions, where particle size was increased from 79.2 ± 1.9 nm to 415 ± 4.7 nm. Then the same reaction conditions were used for 1.6 wt. % CNC with 0.125 wt. %, 0.25 wt. %, 0.5 wt. %, 1 wt. % and 2 wt. % GA concentrations on frequency sweep analysis. The gelation of each suspension was observed as sulfuric acid was added to the suspension. Figure 37 shows frequency sweep results for GA crosslinking after a 3 h reaction. The figure can be interpreted as that addition of a crosslinker resulted in decreased loss and storage modulus while all the suspensions showed gel behavior. A decrease in loss and storage modulus by the addition of a potential crosslinker may be caused by the dilution of the suspension caused by the addition. It can also be said that no evidence was found for crosslinking for 1 % sulfuric acid added suspensions. Obtained gels may be caused by the addition of acid instead of crosslinking. Decreased sulfuric acid concentration (0.5 %) was used for the same reaction conditions with 3 h and 4 days of reaction times. Figure 38 shows the

results for 3 h of reaction for 0.5 % sulfuric acid with variable GA concentrations. For 0.5 wt. % sulfuric acid concentration, the difference between the GA concentration could be observed better. From the figure, it can be said that weak gel formation was observed for all the concentrations, while 0.25 wt. % GA concentration showed the highest storage modulus for these conditions as 1.65 Pa at 1 rad/s angular frequency. Adding extra glutaraldehyde only results in decreased storage and loss modulus, which can be attributed to dilution. Additionally, no “gel” formation was observed since the storage modulus to loss modulus was not higher than 10 in any case. Therefore, it can be said that some changes occurred during reaction time, but changes were minor; even dilutions caused by the addition of extra GA led to the elimination of the positive effects of the change.

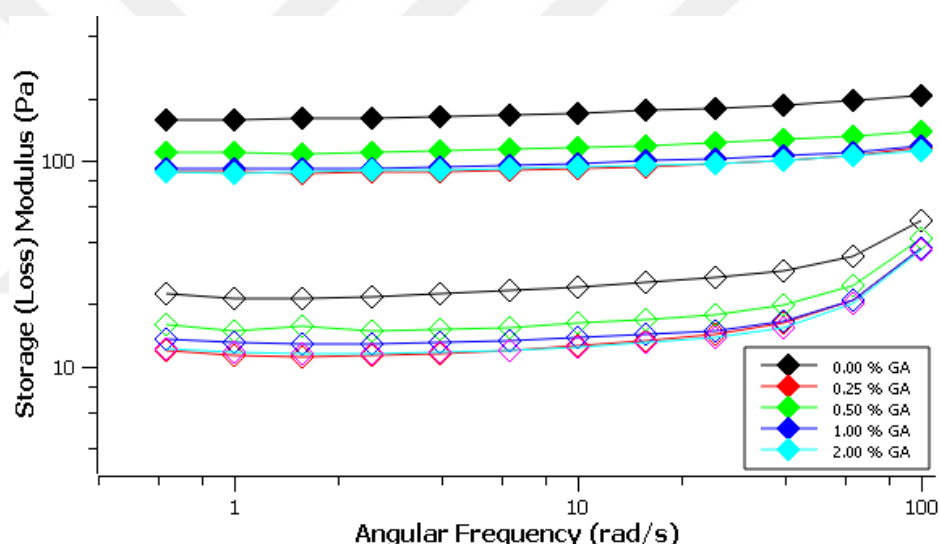


Figure 37. Frequency sweep analysis at 5% constant strain results for 1% sulfuric acid and variable glutaraldehyde concentration crosslinking results for 3 h reaction at 40°C. Filled diamonds show storage modulus while empty diamonds show loss modulus.

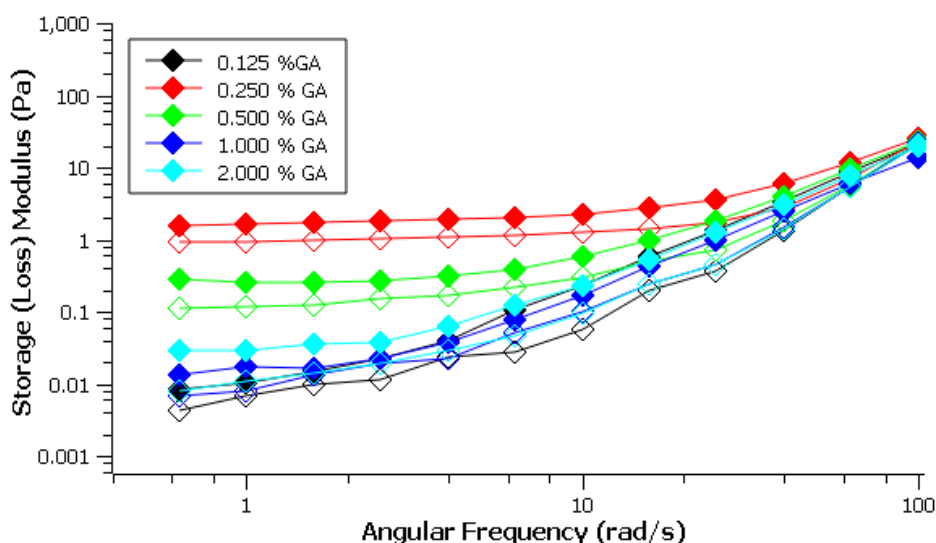


Figure 38. Frequency sweep result at 5% constant strain for 0.5 % sulfuric acid added glutaraldehyde crosslinking for 3 h reaction at 40 °C. Filled diamonds show storage modulus while empty diamonds show loss modulus.

Sulfuric acid can lead to the gelation of CNC suspension by completely suppressing the electrical double layer. To check this, different concentrations of sulfuric acids were added to 1 wt. % and 4 wt. % CNC suspensions and frequency sweep tests were done. Figure 39 and Figure 40 show frequency sweep results for 1 wt. % and 4 wt. % CNC concentrations. For both cases, the addition of sulfuric acid leads to a change in the behaviour of CNCs by increasing the storage and loss modulus of the CNC, where an increase in storage modulus always exceeds the loss modulus in each case, hence increased storage modulus to loss modulus ratio. Therefore, the addition of sulfuric acid resulted in increased gelation for each case. Another interesting observation of the study was that a sharp increase in loss and storage modulus was observed for both cases after adding 0.5 % sulfuric acid. Therefore, complete suppression of the electrostatic double layer was reached at 0.5 % sulfuric acid, which may mean reaching the isoelectric point of the suspension between 0.25% and 0.5% sulfuric acid concentration. These 0.25 % and 0.5 % sulfuric acid concentrations correspond to pH 1.329 and 1.027, which is lower than the Zetasizer cuvette recommended range.

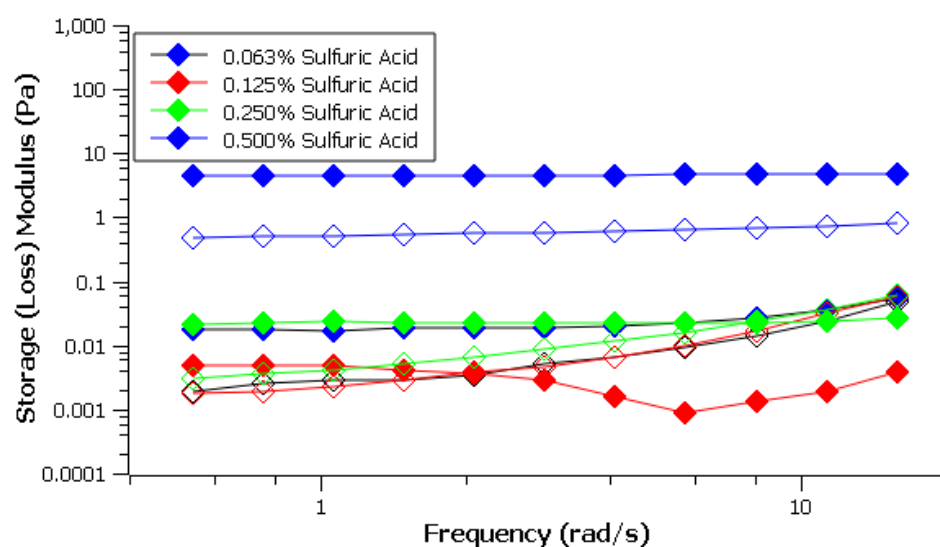


Figure 39. Frequency sweep results at 5% constant strain for only sulfuric acid added 1 wt. % CNC suspension. Filled diamonds show storage modulus while empty diamonds show loss modulus.

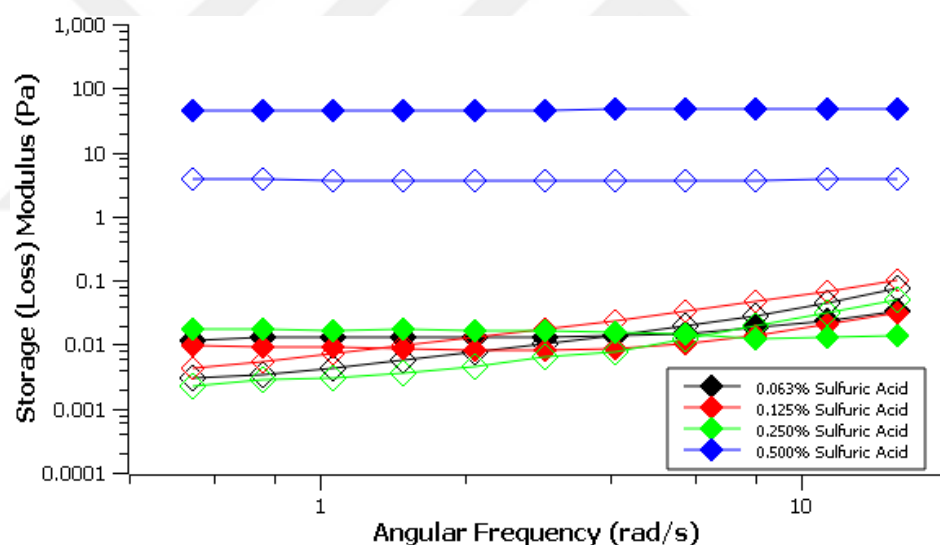


Figure 40. Frequency sweep results at 5% constant strain for only sulfuric acid added 4 wt. % CNC suspension. Filled diamonds show storage modulus while empty diamonds show loss modulus.

As a result, there needs to be more evidence that shows GA crosslinking was successful for this study, even if higher storage and loss moduli were observed for 0.5 % sulfuric acid and 0.25 % GA concentrations for 1.6 wt. % CNC for 3 h reaction at 40 °C. Adding 1 % sulfuric acid instead of crosslinking yielded stronger gels with respect to crosslinked ones. This study also showed that an acidic

environment can be utilized to stabilize a CNC layer for further membrane studies instead of crosslinking CNCs with glutaraldehyde. Also, it can also be said that the isoelectric point of CNC, and TEMPO CNC possibly, was in the region of pH 1.027 and 1.329.

4.3 Membrane Application of CNC and TEMPO CNC Suspensions

CNC and TEMPO CNCs suspensions were used for the preparation of membranes. CNCs and TEMPO CNCs were deposited on a support structure via crossflow deposition for membrane preparation. During membrane preparation, gel formation with concentration polarization phenomena was utilized in a crossflow membrane cell. During crossflow deposition, limiting flux was reached before progressing any further. In this section, employed membrane preparation methods and characterization will be mentioned. Exemplary filtration fluxes can be seen in Figure 41. After 1 bar, a constant limiting flux was reached, and prepared membranes were stabilized with the following methods in the following sections. Additionally, stabilization steps and the blue dextran rejection test can be seen in the following sections. Stabilization steps are essential for membrane preparation for the CNC and TEMPO CNC membranes since the formed gel layer is liquid like before the stabilization step and disperses once the pressure is released, which was the case for CNC and TEMPO CNC suspensions.

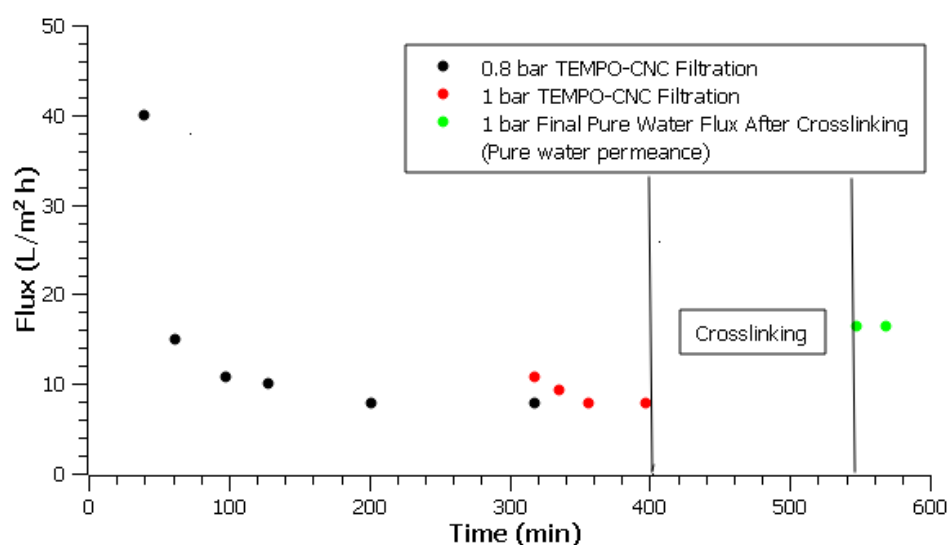


Figure 41. Exemplary 100 mM NaCl added 0.15 wt. % TEMPO CNC membrane preparation procedure at 62 ml/min crossflow rate. After 1 bar TEMPO CNC filtration, 2 hours of crosslinking reaction were done.

4.3.1 Characterization of CA10 Porous Support

CA 10 (10 wt. % cellulose acetate, 90 wt. % DMSO) support was selected for its optical transparency, high flux, and low molecular weight cut off (MWCO). Figure 42 shows a blue dextran rejection test for CA 10 support. Support showed 46 % blue dextran (5 kDa) rejection, and membrane permeance was 836 L/m²h per bar for pure water. During membrane preparation, CNCs and TEMPO CNCs were deposited on these supports. Therefore, increased blue dextran rejection and decreased membrane flux can be attributed to the prepared membrane. This membrane can be considered as ultrafiltration membrane on the tight side, but increasing pore size might lead to optically opaque membranes. Therefore, this membrane allows us both see changes in membrane performance and optically characterize to potential new layer.

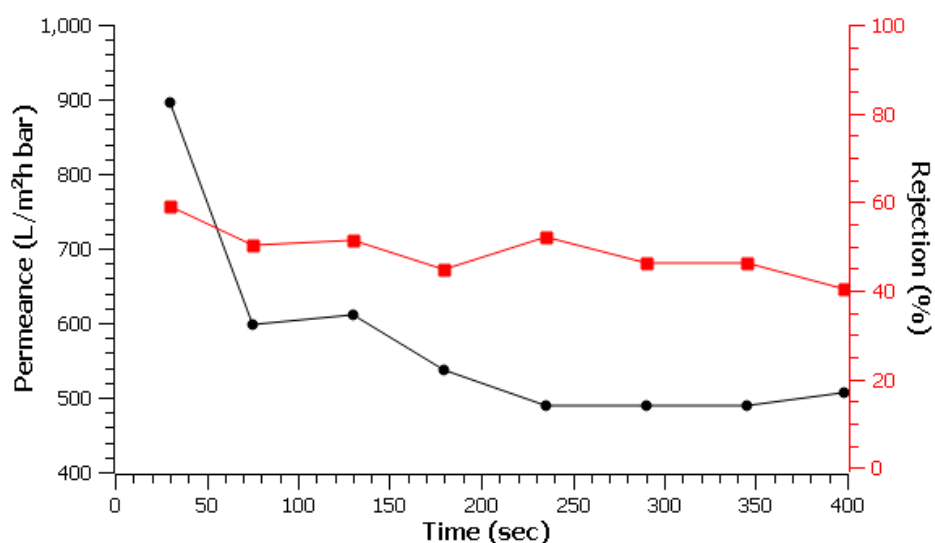


Figure 42. Blue dextran (5 kDa) test result for CA10. Blue dextran (5 kDa) rejection tests were done at dead end mode with mixing with 0.4 bar transmembrane pressure.

4.3.2 Stabilization of TEMPO CNCs with Silver Nitrate and Potassium Persulfate Crosslinking

Stabilization of TEMPO CNC layer were done with AgNO_3 and KPS crosslinking, which was employed right after the limiting flux was reached. After the crosslinking step, membranes were cleaned with pure water filtration. Then, pure water permeance and blue dextran rejection tests were done to characterize the membrane performance. For the crosslinked TEMPO CNC membrane studies, the crossflow rate was 62 ml/min, which means that the shear rate on the membrane surface was 29 s^{-1} (Appendix D), where nematic alignment was expected. For this flow rate no concentration polarization was observed for blue dextran (5 kDa) tests, which could be seen from same pure water permeance and solute filtration fluxes during the operation.

In this section, stability of the membrane layer was defined as ability of staying intact during filtration, hence ability to not delaminating and suspend in medium. For the colloidal application, it is opposite for colloidal stability where it means that the being able to suspend in the liquid medium and not forming aggregates.

Initially, membranes were prepared using TEMPO CNC suspensions of 0.3 wt. % concentration. In the tests, the limiting flux for the membrane was found to be 12.9 L/m²h at 1 bar, and the final membrane permeance was 11.4 L/m²h per bar. For this test, blue dextran rejection was found to be 85%. The membrane layer was also unstable when delaminated after being stored for 1 week in a 20% ethanol solution. The reason for delamination was hypothesized as internal stresses caused by the thickness of the layer. TGA were used for crosslinking of the delaminated film to characterize crosslinking (Appendix F). Therefore, the thickness of the layer was planned to be reduced by decreasing the deposition suspension concentration; for further studies, the suspension concentration was set to 0.15 wt. %. For 0.15 wt. % suspension deposition, a new limiting flux was found as 15.7 L/m²h at 1 bar, higher than 0.3 wt. % suspension as expected. The final membrane pure water permeance was 12.7±1.8 L/m²h per bar, and respective blue dextran (5 kDa) rejections were 95.3±1.9%. Both results showed that lowered concentration increased membrane performance both for blue dextran rejection and membrane flux. This membrane also delaminated in 1 week in ethanol solution.

Crosslinked membranes were prepared with 3h 1M NaOH desulfated TEMPO CNCs at 65 °C reaction temperature and were used for the exact membrane preparation method. For these membranes, the limiting flux was found as 13.6 L/m²h at 1 bar. Decreased limiting flux was expected since suspension stability should be decreased as the surface charge, hence electrostatic repulsion decrease, which was also expected to increase the thickness of the layer. This membrane's membrane permeance was 11.3±1.2 L/m²h per bar, and blue dextran (5 kDa) rejection was 90.7±3.8 %. By comparing TEMPO CNC and desulfated TEMPO CNC membranes, electrostatic interaction with negatively charged TEMPO CNC and negatively charged blue dextran (5 kDa) is an important parameter for this separation. Delamination was still observed with the desulfated TEMPO CNC membrane after 1 week of storage in 20% ethanol solution.

It was thought that salt addition would decrease the internal stress in the film and prevent delamination. Therefore, 100 mM NaCl was added to the suspension

during the deposition step. After each deposition step, a washing step should be employed to eliminate the formation of insoluble silver chlorides. This would decrease the crosslinking solution performance since silver ion concentrations would decrease with silver chloride formation. For the membrane limiting flux was found to be 7.6 ± 1.9 L/m²h. For the NaCl added depositions, membrane permeance was 11 ± 4 L/m²h per bar. The addition of NaCl increased the stability of the final film, which will be mentioned in this section. The three mentioned methods can be compared with Darcy's law to calculate resistance. For three mentioned crosslinked membranes, membrane resistances were shown in Table 4. As can be seen from the figure, the lowest membrane resistance was found for the TEMPO CNC membrane, while desulfated TEMPO CNC showed 10 % higher resistance. Even if the resistance of the desulfated TEMPO CNC layer were higher than the TEMPO CNC layer, separation performance was found to be a layer for blue dextran (5 kDa). The decreased separation performance can be explained by decreased surface charge of the suspension, which leads to decreased separation via electrostatic interaction (Donnan exclusion) between the layer and blue dextran (5 kDa). For 100 mM NaCl added membrane, resistance was 20 % higher than TEMPO CNC membrane, which also increased the separation performance of the layer for blue dextran (5 kDa), which might be expected.

Table 4. Membrane resistances for crosslinked TEMPO CNC, desulfated TEMPO CNC, and NaCl added TEMPO CNC membranes.

	Membrane Resistance (10^{13} m^{-1})
TEMPO CNC Membrane	2.9 ± 0.4
3h 1M 65 °C Desulfated TEMPO CNC Membrane	3.2 ± 0.4
100 mM NaCl added to Desulfated Membrane	3.5 ± 1.0

Interestingly, membranes prepared with the addition of NaCl showed structural colouring caused by cholesteric self assembly. Figure 43 shows the polarized

optical microscope images of wet membranes and dried SEM micrographs of the prepared membranes. Surface SEM micrographs of the membrane can be seen from Appendix C Polarized optical microscopy photographs of the wet membrane on the figure (insets) show the structural colouring of the layer caused by Bragg's reflection of the half pitch of cholesteric symmetry. As can be seen from the insets, prepared membranes showed distinct colours in the visible light range, red to blue, with visible tactoid formations and different coloured line like and dot like regions, while SEM micrographs showed Bouligand arches (Majoinen et al., 2012). This structural colour or swelling behaviour of the gel can be used for sensor applications (Appendix G). For dried membranes, no Bragg's reflection was observed. Table 5 shows the dominant colours on the wet membrane surface and measured pitch values from the SEM micrographs. Bragg's reflection was not observed for dried membranes, consistent with pitch values measured with SEM micrographs. Pitch measurement on SEM micrographs showed that all the half pitch values were lower than the visible wavelength range, in addition to the clear Bouligand arch structure. Therefore, Bragg's reflection could not be observed in the visible wavelength. Pitch measurements showed that all the membranes showed pitch close to each other, with a one standard deviation range; hence the method is reproducible where further optimization might be needed to decrease the standard deviation for the pitch values. Additionally, half pitch values can be correlated to structural colour that should appear with Bragg's law by assuming the average refractive index of the TEMPO CNC as 1.45 and using wet colour wavelength and resultant half pitch value ratio to correct dried membrane pitch to wet membrane. When wet colour wavelength to dried pitch value was taken as 3.07 for the Figure 43 membrane 1, 450 nm for blue, and 146.5 nm for measured average half pitch, all the other membranes can be characterized. Then, wet half pitch values can be found as 430 nm (blue), 344 nm (ultraviolet), and 395 nm (violet) for membranes 2, 3, and 4. Calculations showed that SEM micrographs and polarized optical microscopy were inconsistent with each other, even with correction. This might be

explained by the change in pitch length during drying, as seen in Figure 43, which shows the change in colour during membrane drying.

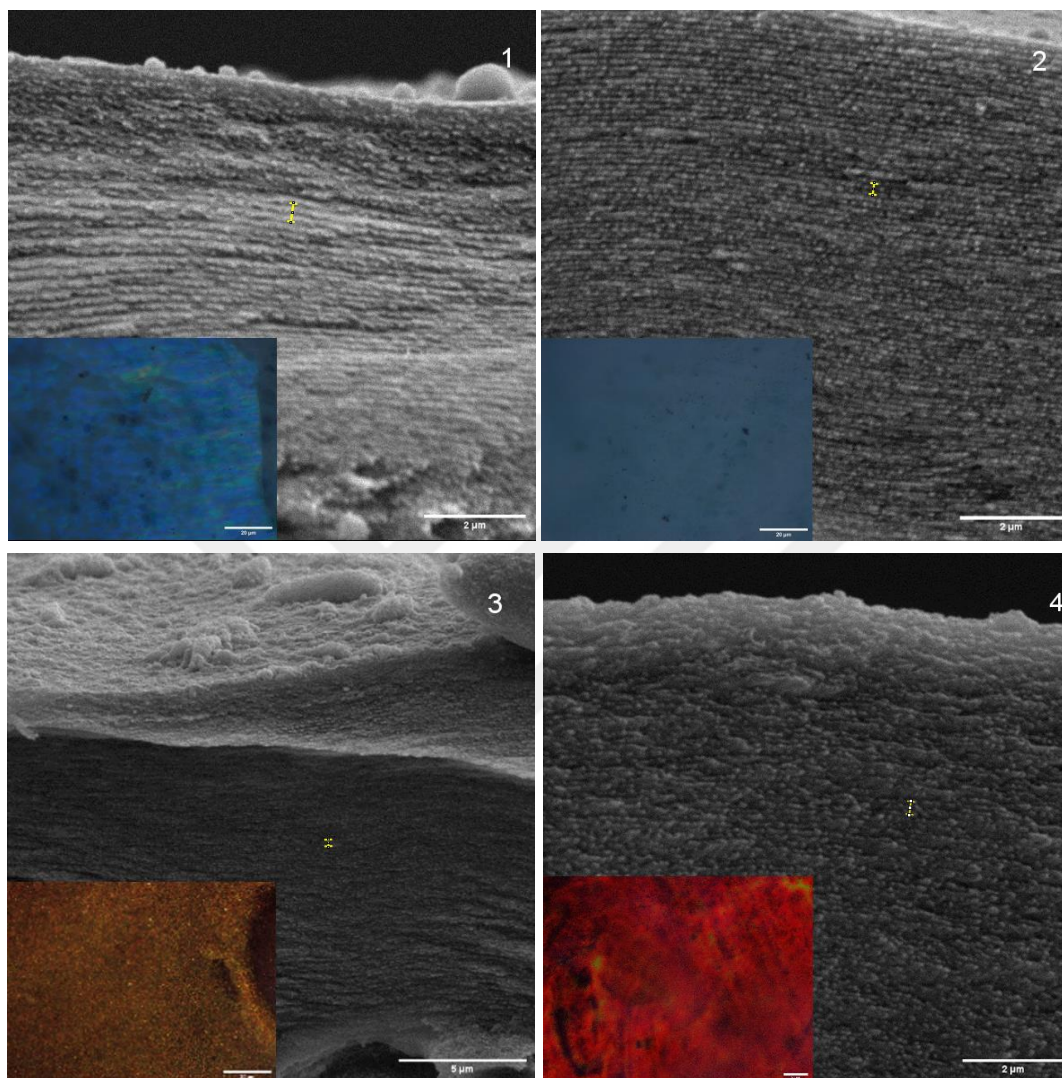
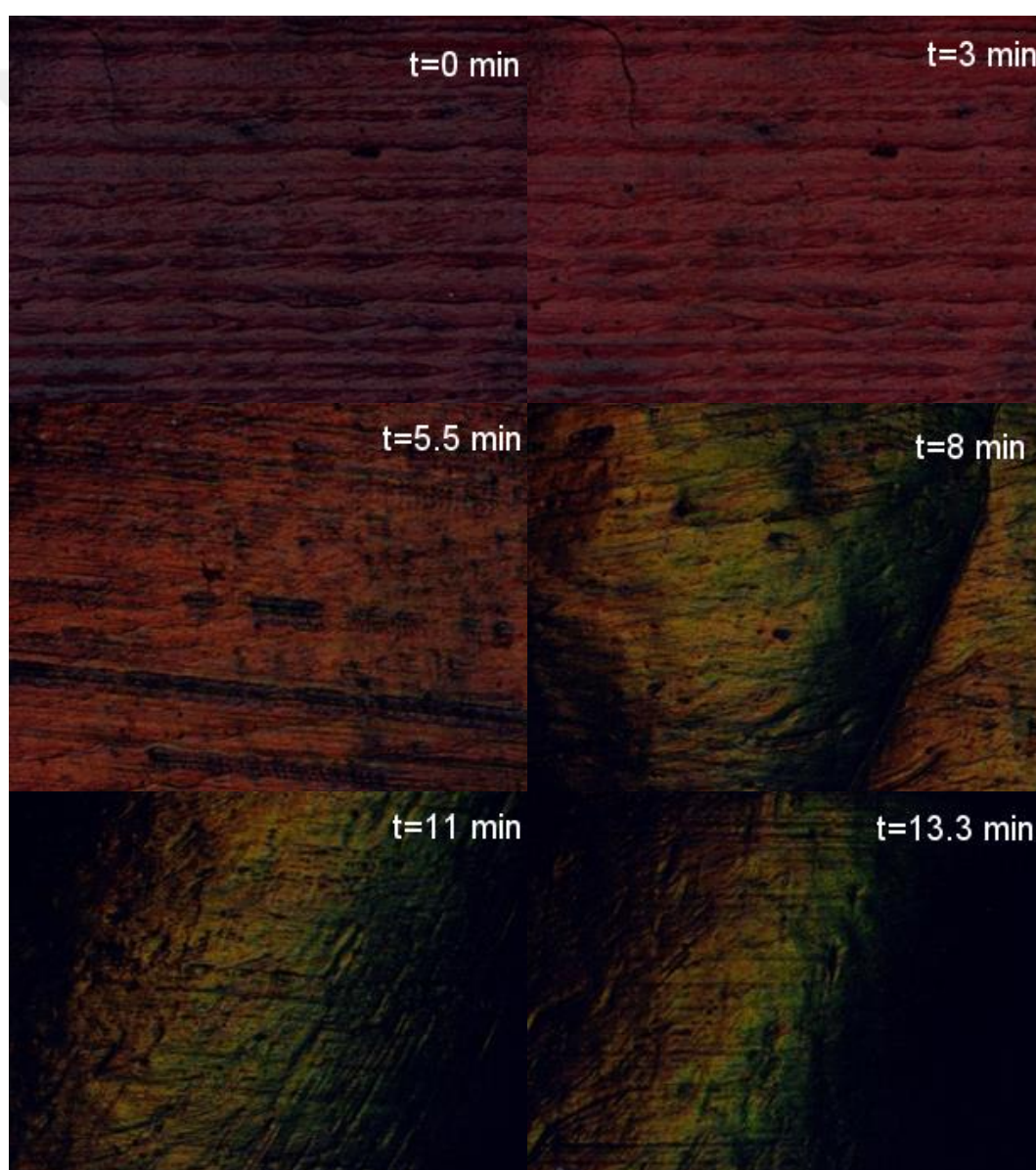


Figure 43. Wet polarized optical images (inset) and dried SEM micrographs of the 100 mM NaCl added 0.15 wt. % TEMPO CNC membranes.

Table 5. Colour of the membranes during wet and respective pitch length was analysed with SEM for the 100 mM NaCl added 0.15 wt. % TEMPO CNC membranes.

Membrane	Observed Colour	Layer Thickness (μm)	Pitch Length (nm)
1	Blue Green	9.1 ± 0.3	293 ± 65
2	Blue	9.9 ± 0.1	280 ± 65
3	Yellow	11.7 ± 1.1	224 ± 25
4	Red	13.8 ± 0.4	257 ± 60



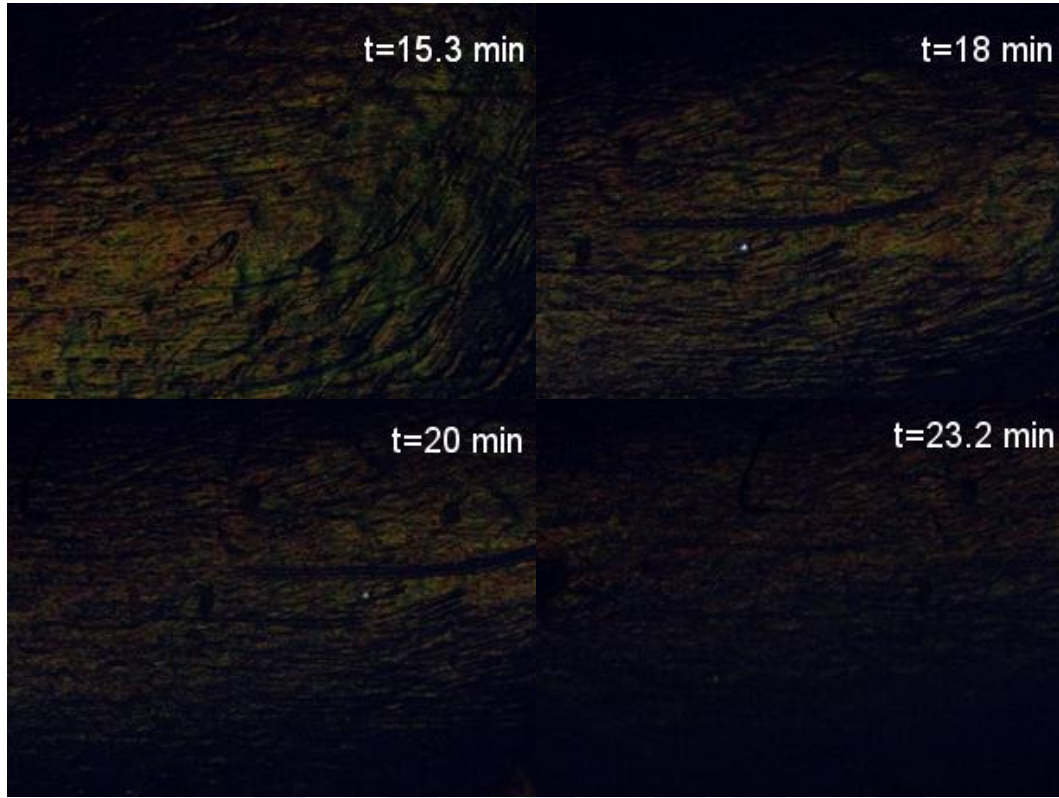


Figure 44. 4x reflection polarized mode optical microscope image of wet 100 mM NaCl added 0.15 wt. % TEMPO CNC membranes at during drying.

The thickness of the mass transfer boundary layer was estimated using the film theory and the definition of the boundary layer ($\delta = \frac{D_{AB}}{k_c}$) as 29 μm (Appendix E). This boundary layer describes where the concentration of the suspension starts to increase. After this thickness, the concentration should be at bulk concentration, which was lower than our case's critical concentration of TEMPO CNC. Therefore, no liquid crystal property should be expected after the boundary layer. In the mass transfer boundary, concentration should be between surface concentration and bulk concentration; because of that, critical concentration should be exceeded at some point in the mass transfer boundary, which leads to the formation of cholesteric symmetry or nematic alignment, if the director is stronger enough. Previous characterization, such as rheometry and capillary alignment tests, showed that the membrane should show nematic alignment in the mentioned shear rate (29 s^{-1} at the wall and 28 s^{-1} at the boundary layer) (Appendix D), but optical characterization

showed cholesteric behaviour. The reason for this might be the formation of self assembly in tactoids which could not be broken during shearing and deposition of self assembled tactoids on the surface.

Cholesteric TEMPO CNC film was also prepared by adding 5 mM NaCl to 5% TEMPO CNC suspension to see if tactoid could be observed better when equilibrated. Initially, 5 mM NaCl added TEMPO CNC suspension was equilibrated for 1 week before the filtration. After the equilibration step, the suspension was filtered at 0.2 bar on CA 10 in 10 ml dead end cell and dried. Figure 45 shows reflection mode polarized optical microscopy images of the film, showing reflected lights on the film surface. Therefore, these images only characterize a thin upper part of the film. On the films, different coloured sections were observed in the film which is characteristic of tactoid formation on a cholesteric structure (P. X. Wang et al., 2016). In different coloured sections, all visible wavelengths can be observed, meaning uneven pitch length for the TEMPO CNC films since colours directly measure pitch length with Bragg's law. Support under TEMPO CNC film was dissolved in acetone to obtain clearer images, and transmittance images were obtained for the film again. Figure 46 shows transmittance images of the structure, where transmittance images show lights passed through the film. Therefore, transmittance images characterize the film as a whole. Transmittance images show tactoids more clearly.

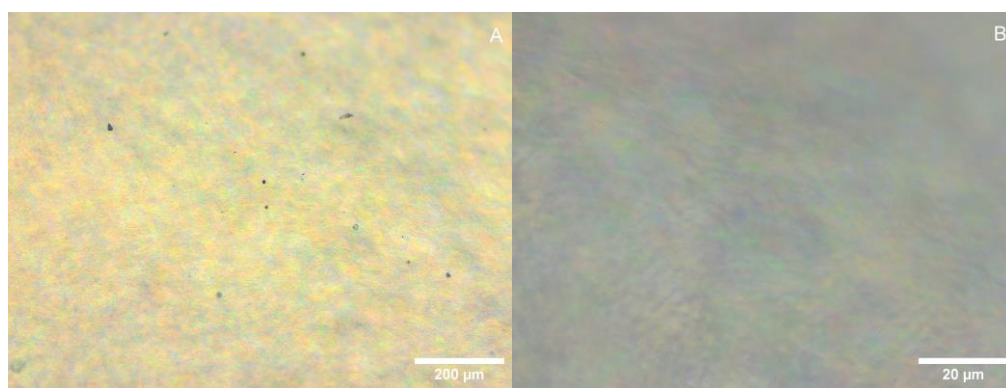


Figure 45. Polarized optical microscopy reflection mode images of dried TEMPO CNC film 10x (A) and 100x (B).

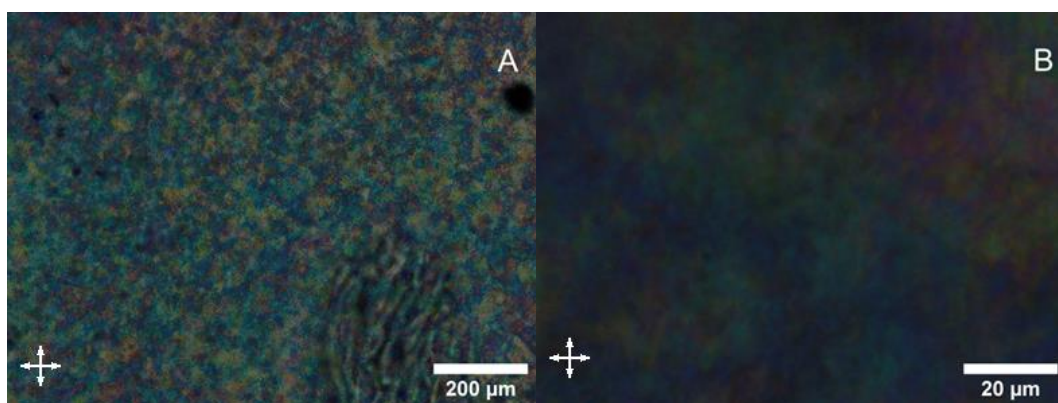


Figure 46. Polarized optical microscopy transmittance mode images of the dried TEMPO CNC film 10x (A) and 100x (B)

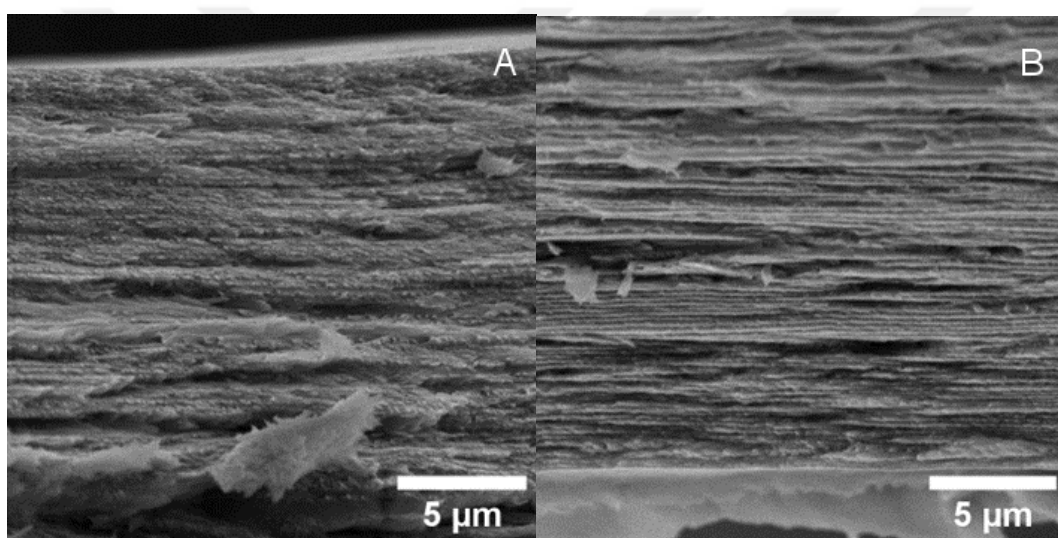


Figure 47. SEM micrographs of 5 mM NaCl added equilibrated 5 wt. % dried TEMPO CNC film.

Figure 47 shows SEM micrographs of the film. Pitches were measured, which shows the distance between the first and third lines between three consecutive lines. In the figure, pitch lengths were found as $0.74 \pm 0.11 \mu\text{m}$ (A) and $0.47 \pm 0.12 \mu\text{m}$ (B). These pitch values also showed nonuniform pitch along the film, parallel to the deposition surface. Observed SEM micrographs also indicate tactoid formation, consistent with polarized optical microscopy images. The average refractive index of CNCs was reported at 1.45 previously for cellulose I (Guo et al., 2021). For this value, Bragg's law (Nguyen et al., 2018) can be used to correlate

obtained pitch values from SEM to polarized optical microscopy images as 682 nm to 1090 nm. This pitch value describes colors for 391 nm to 545 nm wavelengths (blue to orange) that should be observed from polarized optical microscopy images. Therefore, it can be said that dried films Bragg's reflection correlated better with SEM measurements when the films were dried.

Long term stability of the membranes was studied on crossflow filtration. In this long term stability test, prepared membranes were used for continuous pure water filtration while the blue dextran (5 kDa) rejections were measured daily. To be able to compare, salt stabilized membranes were prepared for the same procedures other than stabilization steps, using AlCl_3 permeation for stabilization instead of crosslinker filtration. For tests, a crossflow rate of 62 ml/min was used. There was no change in pure water permeance and blue dextran filtration flux for any crossflow tests. Therefore, concentration polarization was minimized for the tests. Figure 48 shows long term stability results for the 0.15 wt. % TEMPO CNC deposition. Salt stabilized membrane showed 5 days of stable membrane flux and blue dextran (5 kDa) rejection performance. Oddly, membrane rejection was found to be lowered on the 2nd day but lowered performance did not persist, which was recovered for the day 1 rejection. On the other hand, crosslinked membrane only showed 2 days of stable membrane flux where visible delamination occurred between the 2nd and 3rd day. There was no way to see if there is a crack on the membrane in between those days, since the membrane cell is semi transparent on the more opaque side, but even if a crack was formed it was repaired during continuous filtration process. Both membranes showed higher than 90% blue dextran (5 kDa) rejection for those tests when they were stable. Figure 49 shows long term stability test results for 100 mM NaCl added crosslinked 0.15 wt. % TEMPO CNC deposited membranes. NaCl added during deposition and AlCl_3 stabilized membrane showed similar performance to AlCl_3 stabilized membranes without NaCl addition, with similar temporary performance drop as well. On the other hand, NaCl added crosslinked membrane showed better performance with respect to the crosslinked membrane without salt addition, and AlCl_3 stabilized one

for both flux and blue dextran (5 kDa) rejection. Additionally, the membrane did not show any performance drop during the experiment, and the stability of the membrane was like AlCl_3 stabilized membrane. Therefore, 100 mM NaCl added crosslinked membrane showed the best performance among all the prepared membranes for both flux and rejection while having similar stability to AlCl_3 stabilized membranes. Figure 50 shows photographs of the 100 mM NaCl added dried membranes. AlCl_3 stabilized membranes were delaminated and cracked, as seen from the inset (Figure 50A), while crosslinked membrane stayed intact during the drying step, but a black layer on the membrane was formed (Figure 50B). This black layer was interpreted as silver oxides that were sorbed and oxidized on the surface. To clean the black formation, sodium thiosulfate filtered through the membrane while it was wet, and it was seen that some of the black formations were cleanable with sodium thiosulfate due to the reaction of sodium thiosulfate with silver oxides to make non soluble silver compound soluble (Figure 50C).

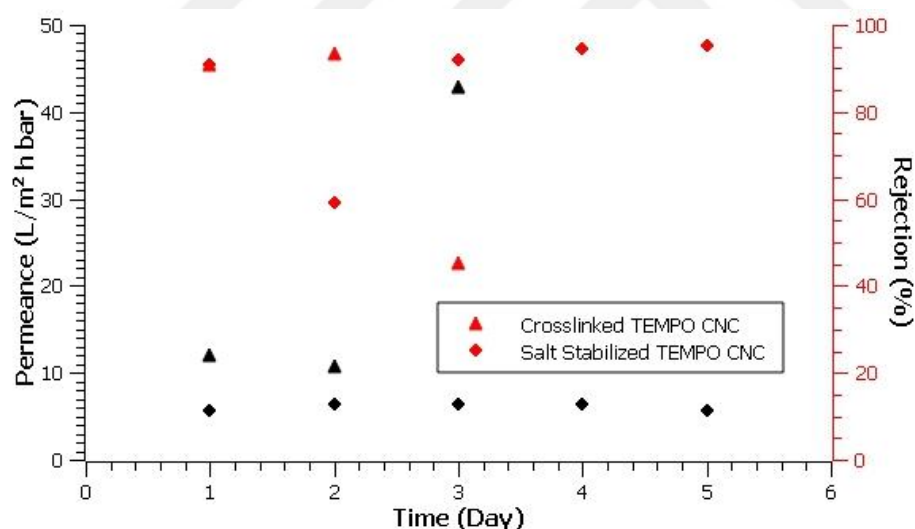


Figure 48. Long term stability results for crosslinked 0.15 wt. % TEMPO CNC deposition.

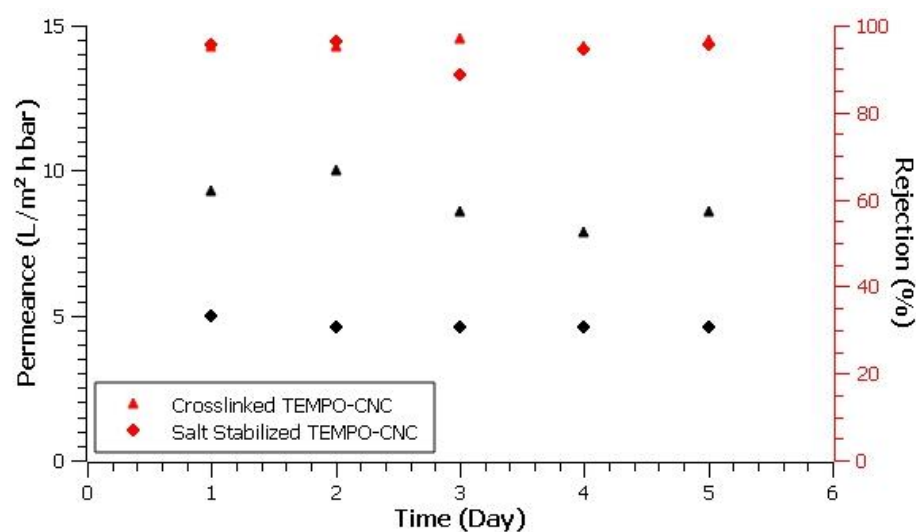


Figure 49. Long term stability result for 100 mM NaCl added crosslinked 0.15 wt. % TEMPO CNC results.

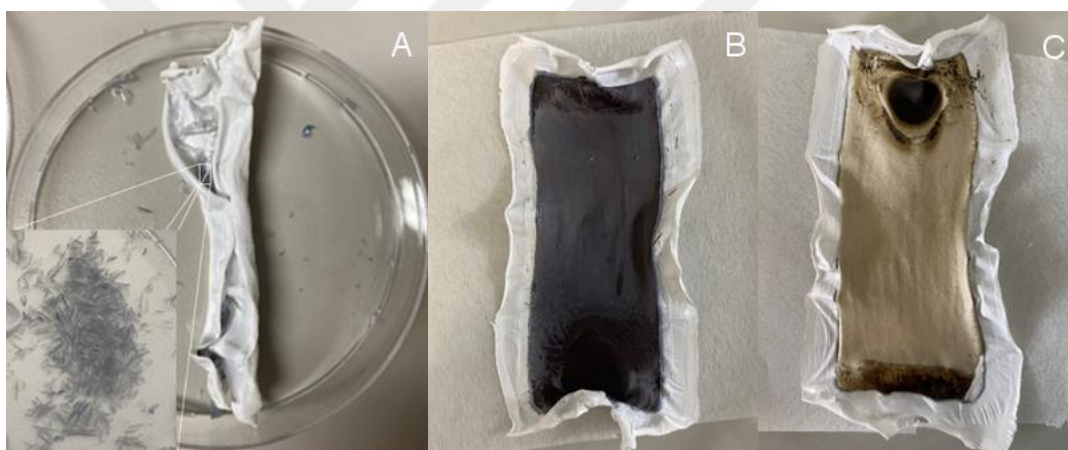


Figure 50. Photographs of dried membranes of 100 mM NaCl added 0.15 wt. % TEMPO CNC membrane for AlCl_3 stabilized and delaminated layer (inset) (A), crosslinked membrane (B), crosslinked and $\text{Na}_2\text{S}_2\text{O}_3$ treated membrane (C).

The dark appearance on the TEMPO CNC membrane surface was analysed with EDX analysis to see if there was silver on the surface which was deposited on or absorbed during the crosslinking step. Figure 51 shows the backscatter membrane surface micrograph used for the EDX analysis. Analysis results can be seen in Table 6. From the table, sodium, and silver atoms were available on the surface, sorbed on the membrane during the TEMPO CNC deposition and crosslinking

steps. Therefore, it can be said that the dark appearance of the dried membrane was caused by silver oxides adsorbed on the membrane during the crosslinking.

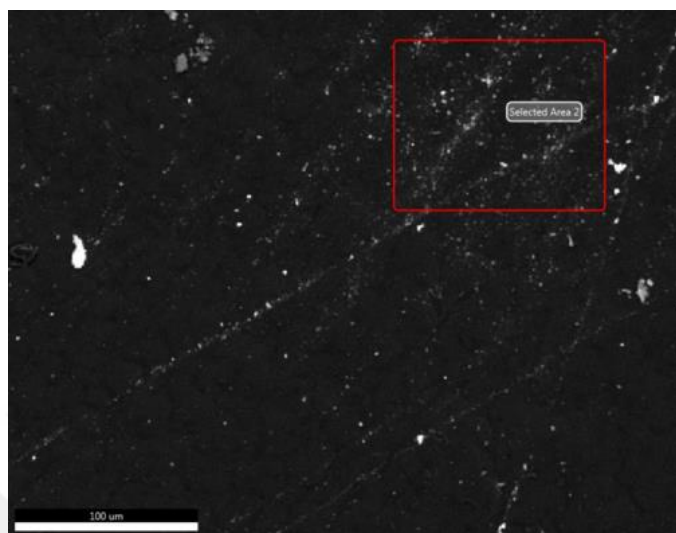


Figure 51. Backscatter SEM micrographs for EDX analysis.

Table 6. EDX analysis results of surface for crosslinked TEMPO CNC membrane.

Element	Concentration (wt. %)	Concentration (Atomic %)	Error (%)
C K	33	40.69	6.65
O K	60.49	56	8.05
Na K	4.76	3.07	9.97
Ag L	1.75	0.24	27.13

CHAPTER 5

CONCLUSION

In this study, the fabrication of a CNC and TEMPO CNC membrane and stabilization methods are shown. Using liquid crystalline colloids as membrane layers would make it possible to obtain tuneable membranes. In addition to that, pores for colloidal membranes formed between crystals, which leads to formation of narrower pore size distributions. For this study, CNC suspensions are prepared with sulfuric acid hydrolysis for 45 °C at 30 min with 50 % sulfuric acid solution. For TEMPO CNC membranes, obtained CNC suspensions are modified with TEMPO oxidation for 3 hours. For the suspensions, the length of CNCs is found to be 122 ± 38 nm while the width was measured as 10 ± 2 nm, and the length and width of TEMPO CNCs are measured at 113 ± 17 nm and 8 ± 2 nm, respectively. Therefore, it can be said that modification did not change the size of the particles, while the addition of carboxyl groups is observed with conductometric titration.

Stabilization tests planned for the membrane layer were tried on CNC and TEMPO CNC suspensions. For the desulfation tests, alkaline desulfation, acid catalysed desulfation, and thermal desulfation were tried. All the desulfation tests were shown to decrease surface sulfate half ester content. Thermal desulfation tests were done at 65 °C, which showed the removal of 66% of sulfate half ester in 3 hours, which was only improved to 72% with 20 hours of reaction. Acid catalysed desulfation for the same temperature did not result in almost any improvement over thermal desulfation. Alkaline desulfation tests showed that the increasing NaOH concentration led to increased removal of the sulfate half esters while a reaction time of 3 hours is enough to reach the plateau. Among all the desulfation tests, the best condition for desulfation was alkaline desulfation with 1M NaOH concentration at 65 °C for 3 hours reaction time where the removal of 83.6% of the surface sulfate half ester could be done. Chemical crosslinking with glutaraldehyde

and sulfuric acid was also tried on CNC suspension to crosslink hydroxyl groups. During the experiment, the formation of weak gel was observed. Further studies showed that the formation of the weak gel was mainly caused by the addition of sulfuric acid and suppression of electrostatic repulsion, where no direct evidence was found for crosslinking.

For TEMPO CNC suspensions, crosslinking with decarboxylation by the addition of AgNO_3 and KPSs was done. Particle size was increased for all the applied crosslinker concentrations, measured with turbidity change during the reaction. Changes in turbidity reached a plateau at 2 hours of reaction time, which were selected for the reaction length for the membrane study. The increase in the size of the particles was shown with DLS. Like glutaraldehyde crosslinking, gelation was also studied for suspensions. Gelation studies were done on 2.5 wt.% TEMPO CNC suspension. Gelation was first characterized with crosslinker concentrations of 0.12 wt.% AgNO_3 and variable KPS. Gel formation could not be observed for only KPS added samples which indicates both AgNO_3 and KPS are needed and gelation was caused by crosslinking. For variable KPS tests, 0.15 wt.% and 0.3 wt.% KPS concentration led to strong gel formation, but high KPS concentrations were found to be damaging the porous support. Instead, silver nitrate concentration were tried to be increased for low KPS concentrations. For 0.15 wt.% KPS, all tried crosslinked concentrations resulted in gelation while only 0.36 wt. % silver nitrate showed “strong” gel formation for the 0.08 wt.% KPS. In the experiments, increasing both AgNO_3 and KPS concentrations would result in gelation and increased storage modulus of the resultant suspension. For all used concentrations, 0.36 wt. % AgNO_3 and 0.3 wt. % KPS showed the highest storage modulus of 169.6 Pa. Even if increasing AgNO_3 and KPS concentrations increased storage modulus. Hence, reaction conditions were selected as 0.08 wt. % KPS 0.36 wt. % AgNO_3 for 2 hours for the membrane studies by considering stability of the porous support.

Membrane fabrication was done under shear in a crossflow membrane cell to deposit CNC or TEMPO CNC layer onto a porous support by taking advantage of

concentration polarization phenomena to make CNC and TEMPO CNC reach their gel concentration at the support wall. During membrane preparation, CNC layers were tried to be stabilized with desulfation, which was unsuccessful, whereas TEMPO CNC layers were stabilized with crosslinking with AgNO_3 and KPS. Prepared membranes were found to be stable in ethanol solution for up to 1 week, while continuous filtration resulted in 2 days of stable membrane performance. These membranes showed pure water flux of $12.7 \pm 1.8 \text{ L/m}^2\text{h}$, and blue dextran (5 kDa) rejection was found to be $95.3 \pm 1.9\%$. For comparison, desulfated TEMPO CNC was used for the exact membrane preparing procedure which showed $11.3 \pm 1.2 \text{ L/m}^2\text{h}$ and $90.7 \pm 3.8\%$ blue dextran (5 kDa) rejection. By comparing desulfated TEMPO CNC and TEMPO CNC membranes, it can be said that reduced surface charge is the reason for this rejection decrease, considering blue dextran (5 kDa) is also negatively charged. To further increase stability, 100 mM NaCl was added to the suspensions during deposition to decrease the possible internal stress formed during deposition. The addition of NaCl resulted in comparable results with AlCl_3 stabilized ones by remaining stable for 5 days of continuous filtration. For the 100 mM NaCl added membrane TEMPO CNC membrane $11.0 \pm 4.0 \text{ L/m}^2\text{h}$ with blue dextran (5 kDa) rejection higher than 95% for 5 days of consecutive blue dextran rejection tests. In addition to increased stability, membranes were dried without cracking and delaminating when crosslinked, whereas AlCl_3 stabilized membranes were cracked during drying. Additionally, NaCl addition led to the formation of cholesteric self assembly, which was observed with both Bouligand arches on SEM micrographs and Bragg's reflection on polarized optical microscopy images.



RECOMMENDATIONS

In this study, TEMPO CNC membranes were prepared with crossflow depositions of TEMPO CNC suspensions. Following recommendations can be done to better understanding and improve this method:

- Citric acid crosslinking can be tried on CNC membranes as a stabilization method to see if it is possible to obtain crosslinked CNC membranes. Being able to crosslink CNCs, crosslinked nematic membranes can also be obtained in addition to cholesteric membranes.
- TEMPO CNCs with different surface concentrations can be prepared by changing sulfate half esters to better understanding if its possible to obtain coloured dry films in this way. Additionally, this study may also explain if the different colours observed for the wet membrane are caused by change in surface charge during storage or not.



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APPENDICES

A. Characterization of Support 2 Porous Support

Support 2 (10 wt. % PES, 30 wt. % PEG 400, 60 wt. % DMSO) was used for the chemical stability of PES. Figure 52 shows dead end BD (5 kDa) test results for support 2, where blue dextran rejection was 97.8 % while membrane flux during the test was 62.8 L/m²h at 0.4 bar, making permeance 157 L/m²h per bar. Even if membrane permeance was found as 157 L/m²h per bar, this permeance was influenced by the concentration polarization effect since permeances were found for different support 2 membranes in the 200 to 2000 L/m²h per bar interval.

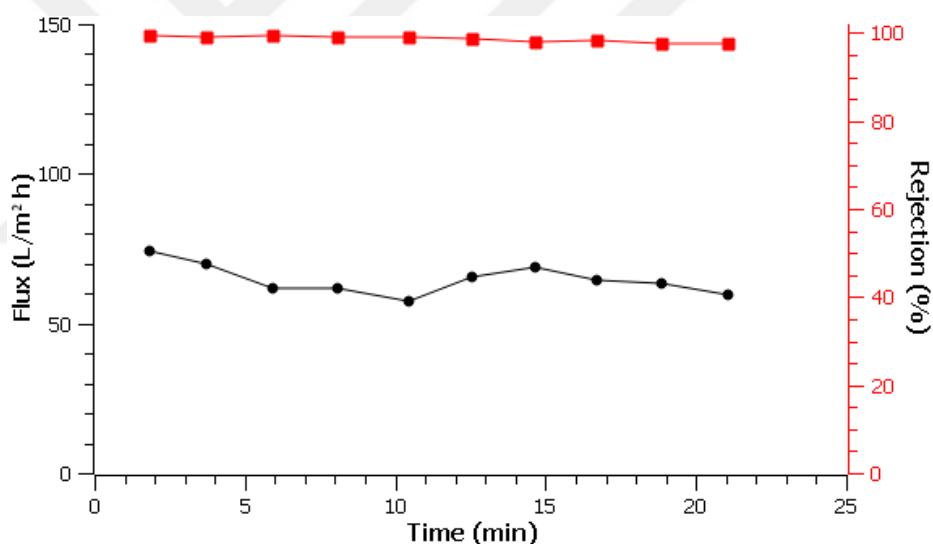


Figure 52. Blue dextran 5 (kDa) test results for Support 2. Blue dextran (5 kDa) rejection tests were done at dead end mode with mixing crossflow rate with 0.4 bar transmembrane pressure.

B. Stabilization of Membrane Layer on Support with Desulfation of CNCs

CNC suspensions were stabilized on Support 2 (Appendix A) to see if the obtained membranes were stable. Previously, stable membranes were reported for the CNC at 80 ml/min crossflow rate stabilized with AlCl_3 filtration (Kocaman et al., 2021). Membranes with the exact procedures were prepared, and only the stabilization methods were changed. For these membranes, alkaline desulfation and thermal desulfation were tried via filtration of either alkaline or pure water at 65 °C. Only the stability and pure water performance of the layer could be studied for the prepared membranes. Firstly, alkaline desulfation was tried on the membranes with filtration of 1M NaOH at 65 °C. Reaction time was selected to be short to eliminate damage to the support since membrane performance would be lowered if the support gets damaged. For the membrane, pure water permeance was found as 13.6 $\text{L/m}^2\text{h}$ per bar. The membrane was delaminated as it was put into an ethanol solution. Filtered heated alkaline was found to be problematic for the application; instead, thermal desulfation was tried on the membrane. Membrane flux was found to be 17.5 $\text{L/m}^2\text{h}$ per bar. Similar to alkaline desulfation, this membrane was also found to be unstable and delaminated as it was put into a 20% ethanol solution. Therefore, no stable membrane was found for the desulfation stability studies. Instead, chemical stabilization was tried to be employed.

C. Surface Analysis of NaCl added TEMPO CNC Membrane

Surface image of the 100 mM NaCl added 0.15 wt. % TEMPO CNC deposited and crosslinked membrane can be seen in Figure 53. Roughness on the membrane surface can be clearly seen from the micrographs, which were most probably caused by deposited TEMPO CNCs. Additionally, spherical impurities can be seen on top of the layer where these spheres might be silver oxide particles deposited on the membrane surface during crosslinking steps.

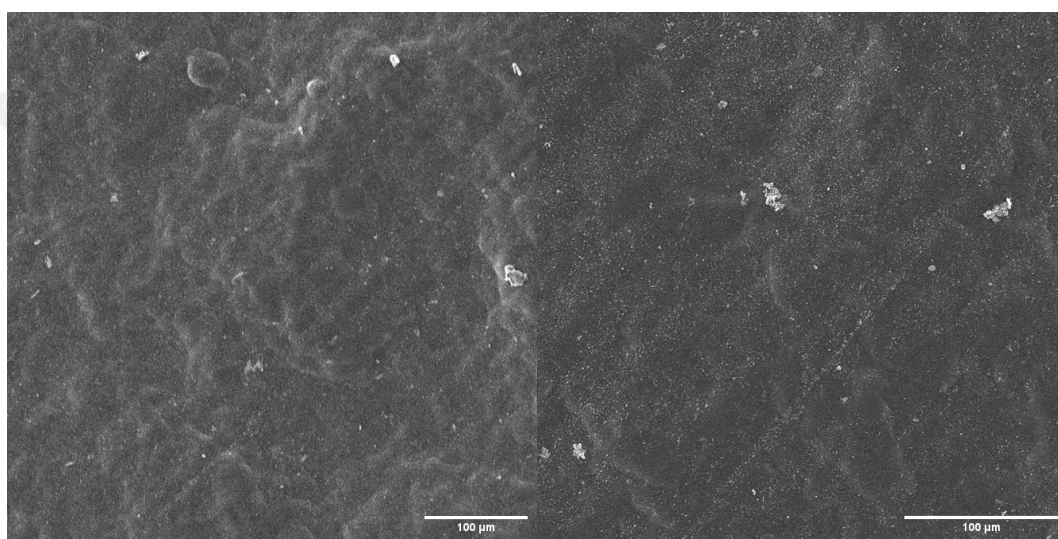


Figure 53. Surface SEM micrographs of crosslinked 100 mM NaCl added 0.15 wt. % TEMPO CNC deposited membrane.

D. Calculation of Shear Rate for the Module.



Figure 54. Representation of the system where center of the system was chosen as symmetry center.

Assumptions:

1. Steady state
2. Constant physical properties
3. Isothermal
4. Negligible end effects
5. Negligible permeates flux with respect to cross flow rate.
6. Wall effects are negligible for x direction. (Open channel from both sides)
7. There is no flow in the y and x directions.
8. Incompressible flow

CNC suspensions are non Newtonian; therefore, analysis should be done for non Newtonian systems.

Equation of Continuity:

$$\frac{\delta \rho}{\delta t} + \frac{\partial(\rho v_x)}{\partial x} + \frac{\partial(\rho v_y)}{\partial y} + \frac{\partial(\rho v_z)}{\partial z} = 0$$

For steady state and $v_x=v_y=0$, it becomes:

$$\frac{\partial(\rho v_z)}{\partial z} = 0$$

V_z only depends on the y.

Equation of Motion:

$$\tau_{zy} = \tau_{yz} = -\mu \frac{\partial v_y}{\partial z}$$

Where the other terms of shear force tensor are 0. Definition of modified pressure can be done as:

$$\frac{\partial \mathcal{P}}{\partial a} = \frac{\partial P}{\partial a} - \rho g_a$$

x-direction:

$$\begin{aligned} \rho \left(\frac{\delta v_x}{\delta t} + v_x \frac{\partial(v_x)}{\partial x} + v_y \frac{\partial(v_x)}{\partial y} + v_z \frac{\partial(v_x)}{\partial z} \right) \\ = -\frac{\partial \mathcal{P}}{\partial x} - \left(\frac{\partial(\tau_{xx})}{\partial x} + \frac{\partial(\tau_{xy})}{\partial y} + \frac{\partial(\tau_{xz})}{\partial z} \right) \end{aligned}$$

For steady state, $v_x=0$, and shear force terms=0:

$$\frac{\partial \mathcal{P}}{\partial x} = 0$$

y-direction:

$$\begin{aligned} \rho \left(\frac{\delta v_y}{\delta t} + v_x \frac{\partial(v_y)}{\partial x} + v_y \frac{\partial(v_y)}{\partial y} + v_z \frac{\partial(v_y)}{\partial z} \right) \\ = -\frac{\partial \mathcal{P}}{\partial y} - \left(\frac{\partial(\tau_{yx})}{\partial x} + \frac{\partial(\tau_{yy})}{\partial y} + \frac{\partial(\tau_{yz})}{\partial z} \right) \end{aligned}$$

For steady state, $v_y=0$, and shear force terms=0:

$$\frac{\partial \mathcal{P}}{\partial y} = 0$$

z-direction:

$$\begin{aligned} \rho \left(\frac{\delta v_z}{\delta t} + v_x \frac{\partial(v_z)}{\partial x} + v_y \frac{\partial(v_z)}{\partial y} + v_z \frac{\partial(v_z)}{\partial z} \right) \\ = -\frac{\partial \mathcal{P}}{\partial z} - \left(\frac{\partial(\tau_{zx})}{\partial x} + \frac{\partial(\tau_{zy})}{\partial y} + \frac{\partial(\tau_{zz})}{\partial z} \right) \end{aligned}$$

For steady state, $v_z = v_z(y)$, $\tau_{zy} = \tau_{yz} = -\mu \frac{\partial v_z}{\partial y}$ and other shear force terms =0:

$$\frac{\partial P}{\partial z} = -\frac{\partial(\tau_{zy})}{\partial y}$$

Unless the equation is exact no solution exists. Then:

$$\frac{\partial P}{\partial z} = -\frac{\partial(\tau_{zy})}{\partial y} = A$$

For modified pressure part integration gives:

$$P_z = Az + P_0$$

$$\frac{P_L - P_0}{L} = A$$

Which is also equal to shear force term:

$$\frac{\partial(\tau_{zy})}{\partial y} = -\left(\frac{P_L - P_0}{L}\right)$$

$$\tau_{zy} = -\left(\frac{P_L - P_0}{L}\right)y + C$$

Boundary Condition

At $y=0$ $\tau_{zy} = 0$

Which makes $C=0$

$$\tau_{zy} = -\left(\frac{P_L - P_0}{L}\right)y$$

For a power law shear thinning fluid

$$\tau_{zy} = -\eta \frac{\partial(v_z)}{\partial y}$$

$$\eta = m|D|^{n-1}$$

Where n is in between 0 and 1.

$$|D| = \sqrt{\frac{1}{2}(D:D)} = \sqrt{\Phi_v} = \left| \frac{\partial(v_z)}{\partial y} \right|$$

For $y > 0$

$$\left| \frac{\partial(v_z)}{\partial y} \right| = -\frac{\partial(v_z)}{\partial y}$$

For $y < 0$

$$\left| \frac{\partial(v_z)}{\partial y} \right| = \frac{\partial(v_z)}{\partial y}$$

For the center to the upper wall:

$$\tau_{zy} = -m \frac{\partial(v_z)}{\partial y} \cdot \left(\frac{-\partial(v_z)}{\partial y} \right)^{n-1} = m \left(\frac{-\partial(v_z)}{\partial y} \right)^n$$

$$m \left(\frac{-\partial(v_z)}{\partial y} \right)^n = -\left(\frac{P_L - P_0}{L} \right) y$$

$$v_z = -\left(\frac{A}{m} \right)^{\frac{1}{n}} \cdot y^{\frac{n+1}{n}} \cdot \frac{n}{n+1} + C$$

Boundary Condition

For $y=B$ $v_z=0$

$$v_z = \left(\frac{A}{m} \right)^{\frac{1}{n}} \cdot \frac{n}{n+1} \cdot B^{\frac{n+1}{n}} \cdot \left(1 - \left(\frac{y}{B} \right)^{\frac{n+1}{n}} \right)$$

$$v_{max} = \left(\frac{A}{m} \right)^{\frac{1}{n}} \cdot \frac{n}{n+1} \cdot B^{\frac{n+1}{n}}$$

Which is symmetric.

$$\dot{\gamma} = v_{max} \cdot \frac{n+1}{n} \cdot \frac{y^{1/n}}{B^{n+1/n}}$$

$$Q = 2 \cdot W \cdot B \cdot v_{max} \cdot \frac{n}{2n + 1}$$

$$v_{avg} = v_{max} \cdot \frac{n}{2n + 1} = \frac{Q}{A}$$

For the system

Q= 62 ml/min

B=0.00115 m

w=0.04572 m

n=0.73

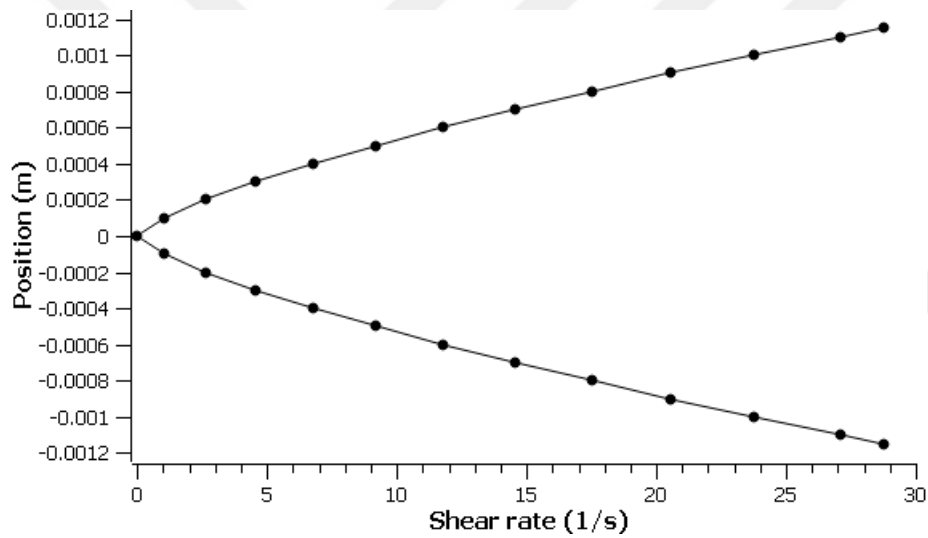


Figure 55. Shear rate profile for modelled system.

E. Calculation of Boundary Layer Thickness

For the CF042 cross flow module:

$$W=0.04572 \text{ m}$$

$$2H=0.23E-2 \text{ m}$$

$$Q=62 \text{ ml/min}=1.03E-6 \text{ m}^3/\text{s}$$

$$\mu=1E-3 \text{ Pa.s}$$

$$P=1010 \text{ kg/m}^3$$

$$4E-12 < D_{CNC} < 8E-12 \text{ (Shariatnia et al., 2022)}$$

$$L=0.092 \text{ cm}$$

For the system:

$$D_h = \frac{4A}{P} = \frac{1.052E-4}{9.604E-2} = 0.00438 \text{ m}$$

$$\langle v \rangle = \frac{Q}{A} = \frac{1.03E-6}{1.05E-4} = 9.8E-3 \text{ m/s}$$

$$Re = \frac{\langle v \rangle \rho D_h}{\mu} = \frac{9.8E-3 * 1010 * 4.38E-3}{1E-3} = 43.4$$

$$Sc = \frac{\mu}{\rho D_{CNC}} = \frac{1E-3}{1010 * 4 * E-12} = 247.5E3$$

$$\begin{aligned} Sh = \frac{k_c D_h}{D_{CNC}} &= 1.86 * \left[Re * Sc * \frac{D_h}{L} \right]^{\frac{1}{3}} = 1.86 * \left[43.4 * 247.5E3 * \frac{0.00438}{0.092} \right]^{\frac{1}{3}} \\ &= 148.7 \text{ (Tosun, 2007)} \end{aligned}$$

$$k_c = 1.36E-7$$

$$\delta = \frac{D_{CNC}}{k_c} = \frac{4E-12}{4E-7} = 2.94E-5 = 29.4$$

F. Thermogravimetric Analysis of Crosslinked and non-Crosslinked Films

Thermogravimetric analysis for crosslinked and non crosslinked TEMPO CNC films was done in a nitrogen atmosphere from room temperature to 900 °C with a 10 °C/min ramp. Dried films show an initial decrease in the initial moisture content of the film, whereas a sharp decrease followed it. The onset temperature for the decomposition decreased to 90 wt. % points at 215.9 °C for TEMPO CNC film and 199.3 °C for crosslinked TEMPO CNC film (Figure 56). First, Sharp decreases are caused by desulfation and decarboxylation, previously mentioned in the literature (Vanderfleet et al., 2019). Decreased offset temperatures were previously interpreted for crosslinked polymers for forming more volatile materials with crosslinking (D. D. Jiang et al., 1999), which is applicable to our case since carboxylic acid should be formed when decarboxylation occurs. Additionally, non volatile compounds at 900 °C were found to be increased for crosslinked TEMPO CNC films, but silver oxides were previously mentioned to be absorbed into final films, which might also increase the non volatile compound percentage (Weng et al., 2017).

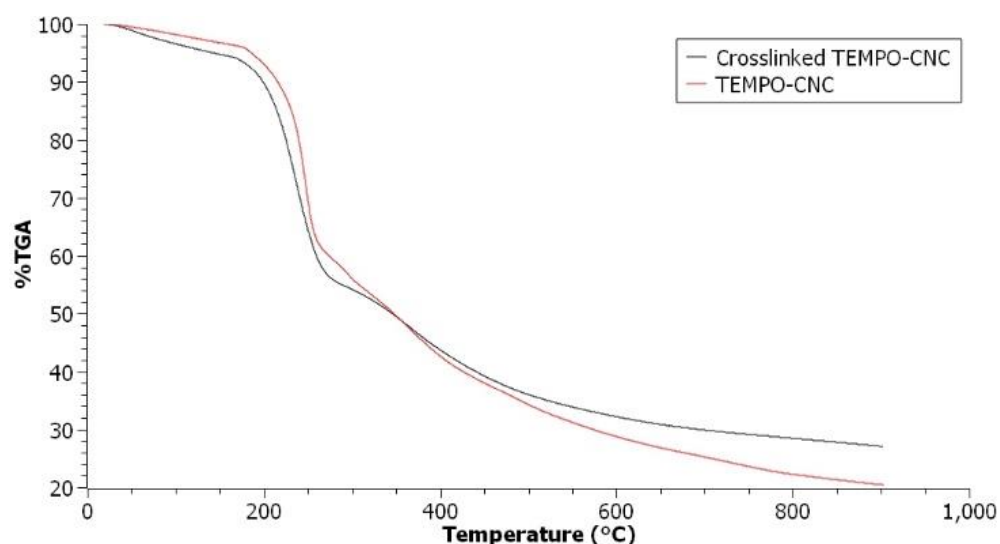


Figure 56. Thermogravimetric analysis for crosslinked dried TEMPO CNC film and non crosslinked dried TEMPO CNC film.

G. Characterization of Ethanol Solution with 100 mM NaCl Added Crosslinked TEMPO CNC Membranes

Literature previously showed cellulose nanocrystal films were used to characterize alcohols by change of colour on film, even in the dried state (Hu et al., 2022). From this observation, it was hypothesized that membrane performance might change performance for concentration change for different alcohols, where a change in alcohol concentration would change the pitch of the TEMPO CNC layer by differently swelling the film and modifying physical interactions. If the filtration performance changes measurably and consistently, the characterization of the filtered solution could be done. To test this hypothesis, ethanol solutions with different concentrations were filtered through the membrane, and steady state membrane fluxes were recorded. The viscosity of the ethanol solution changes drastically with concentration (Figure 57); therefore, characterizations were done for membrane resistance, which is a membrane specific property calculated using Darcy's law. Figure 58 shows the membrane resistance with respect to the filtered ethanol volume fraction. The figure shows a decreasing trend initially up to 75% ethanol, where increasing trends start. A sudden change from decreasing trend to an increasing trend may show limits of this membrane for this membrane, but recovery of the performance could be obtained when pure water filtration was done for 2nd time. In comparison, inconsistent resistance increases were observed after 75%, where the standard deviation of found resistance values was too high to be considered as a characterization method, and the slope of resistance was too low with respect to the standard deviation between studies. Therefore, the change in performance was observed with respect to ethanol concentration, but this method is not applicable to the characterization of solution concentration with this membrane.

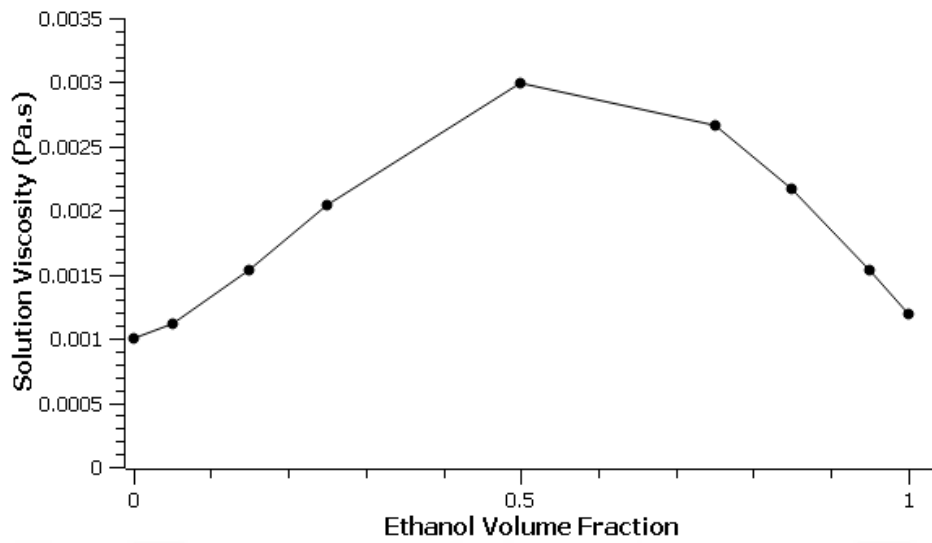


Figure 57. Literature reported viscosities for ethanol water mixture with respect to volume fraction (B. Zhang et al., 2019).

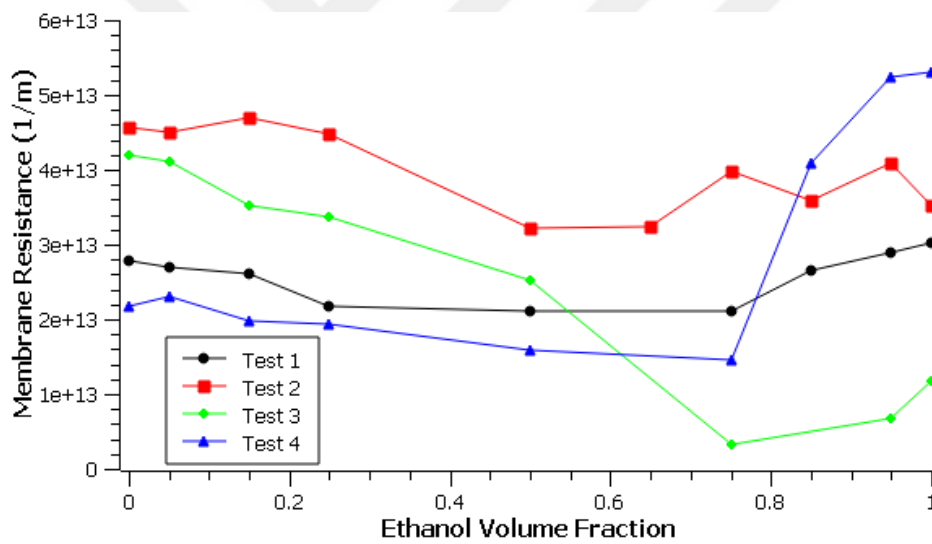


Figure 58. Calculated membrane resistance with respect to filtered ethanol volume fraction.