



## **Cellulose Light Interactions**

Relationship between Refractive Index and Surface Chemistry of Cellulose Nanocrystals

Master's thesis in MPISC

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CHALMERS UNIVERSITY OF TECHNOLOGY Gothenburg, Sweden 2021 www.chalmers.se

MASTER'S THESIS 2021

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Cover: Chemical structure of cellulose.

Typeset in LATEX Gothenburg, Sweden 2021 Cellulose Light Interactions Relationship between Refractive Index and Surface Chemistry of Cellulose Nanocrystals LEI WANG Department of Chemistry and Chemical Engineering Chalmers University of Technology

#### Abstract

Cellulose nanocrystals (CNC, CNCs) are a new type of biomass based nanomaterials derived from cellulose which is the most abundant biopolymer on Earth. Therefore, there is a need to have a defined and suitable battery of analytical methods to characterize their properties. Refractive index is an important parameter to describe CNC properties for analytical efforts and applications as well. However, refractive index of CNC is not conclusively reported. Therefore, this thesis is devoted in elucidating the refractive index on CNCs in suspension.

In order to demonstrate relationship of CNCs suspension concentration and refractive index, refractive index of diluted CNC suspensions under different concentrations was quantified. Meanwhile, on the purpose of studying the relationship between CNCs and surface properties, periodate oxidation and desulfation were adopted to alter surface physico-chemical properties of CNC suspensions, and their refractive index and refractive index increment evolution was monitored. Based on the results of experiments, potential applications are discussed.

Keywords: Cellulose nanocrystals, Refractive index, Periodate oxidation, Optical applications of CNCs.

#### Acknowledgements

At the moment of the completion of the work, I would like to thank Tiina Nypelö and Saül Llàcer Navarro for their help and support during my thesis work. During this period of time, Tiina gave me careful guidance in scientific research, constantly inspired me to strive forward and do my best in all work. Whenever I encounter scientific research questions, Saül always patiently answers my doubts for me, and lets me continue my work with confidence. I am very grateful to them for constantly guiding me and helping me in time so that I can finish the thesis work smoothly.

Secondly, I would like to thank everyone of the research group for their help in experiments and creating a great environment to work in, along with times during chatting and fika.

At the same time, I also want to thank my classmates in MPISC and friends for their great help in my life. Thanks to my parents for more than 24 years of nurturing, it was their dedication and support that made me finish my studies at ease.

Lei Wang, Gothenburg, June 2021

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# 1 Introduction

#### 1.1 Material: Cellulose Nanocrystals

#### 1.1.1 Properties of cellulose nanocrystals

Cellulose nanocrystals (CNC, CNCs) are a new type of biomass based nanomaterials derived from cellulose which is the most abundant biopolymer on Earth. Due to high crystallinity and high mechanical strength, CNCs are most widely suggested in polymer composites as nanofiller.[1][2] In addition, CNCs have properties typical of biomaterials such as low density, biodegradability, biocompatibility and renewability. Therefore, CNCs are a potential contributor for a more sustainable and environmentally friendly material future compared with non-biobased materials.[3]

The general size of CNC is  $50\sim2000$  nm in length and  $3\sim50$  nm in width. The length of CNC from wood based raw material is  $100\sim300$  nm and the width is  $3\sim5$  nm.[2][4]

High crystallinity is one of the properties that is seen beneficial for the future products from CNCs. Crystallinity indicates the proportion of crystalline volume and order in CNC. The general crystallinity of CNC is  $60\% \sim 85\%$ .[5] However, CNCs from various sources and preparation methods differ from each other. [6] For example, Herrera et al.[7] employed X-ray diffraction (XRD) to characterize two kinds of CNCs prepared by bleaching and homogenization methods. Crystallinities for cellulose nanowhiskers from microcrystalline cellulose (CNW-MCC) and cellulose nanowhiskers extracted from industrial bio-residue (CNW-BR) are 85% and 77% separately. Yu et al.[8] prepared CNC with alkaline pretreatment and TEMPO oxidation from jute. Characterized by wide-angle X-Ray scattering(WAXS), crystallinity of jute CNC is reported to be 71%.

#### 1.1.2 Preparation of cellulose nanocrystals

CNCs are typically obtained by acid hydrolysis from a cellulose source such as wood. In the process, non-cellulose components such as hemicellulose and lignin are first removed by chemical pretreatments. After pretreatment, the glycosodic bond of the pretreated cellulose fiber is subjected to acid hydrolysis process, the amorphous region or subcrystalline region of cellulose is hydrolyzed first, while the crystalline region remains. Therefore, after cellulose is hydrolyzed in the process of acid hydrolysis, rod-shaped cellulose crystals are produced. [9][5]

At present, there are many methods to prepare CNCs. Among them, sulfuric acid hydrolysis is the most common preparation method. CNCs can also be prepared with other acids, such as hydrochloric acid, phosphoric acid, hydrobromic acid, organic acid (formic acid), etc. it can also be prepared by mixing inorganic acid and organic acid in a certain proportion. [10] As early as 1947, Nickerson et al. [11] first used hydrochloric acid and sulfuric acid to hydrolyze wood fiber to prepare nanocellulose colloidal suspension. In 1995, Marchessault et al. [12] hydrolyzed MCC with sulfuric acid, not only successfully separated cellulose nanowhiskers, but also found that the surface of CNC was negatively charged, and the stable cellulose suspension system was formed due to electrostatic repulsion force. Filson et al. [13] used microcrystalline cellulose as raw material, innovatively used ultrasonic chemical assisted hydrolysis system of maleic acid, and reacted at 15 °C and 90% power for 9 minutes to prepare cylindrical CNC. Sadeghifar et al. [14] prepared CNC with diameter of 7-8 nm, length of 100-200 nm and high transverse crystallinity using HBr hydrolyzed cotton fiber. Liu et al. [15] successfully prepared CNC with phosphotungstic acid, which had better thermal stability than the same kind of CNC prepared with sulfuric acid.

Oxalic acid is the simplest kind of organic acid. In recent years, it has been used to replace inorganic acid to prepare CNC. Compared with other methods, oxalic acid is relatively weak in acidity, so it will not corrode the equipment, and is easy to recycle and reuse. Therefore, oxalic acid is suggested to replace strong acid hydrolysis as a way of large-scale preparation of CNC.[16] Solid acid hydrolysis method, such as phosphotungstic acid, can not only be recycled, simple operation and high efficiency, but also can realize the green and efficient preparation of nanocellulose. Moreover, there is no waste liquid in the preparation process, which has little corrosion to the equipment, so it may become a way of large-scale production of CNC in the future.[17]

In addition to treatment with mineral acids, CNCs can as well be prepared by other processes such as enzymatic hydrolysis, TEMPO oxidation, etc.[18] Due to the different pretreatments and different hydrolysis factors such as type of acids, reaction time and temperature, CNCs from different sources have different morphology, crystallinity and crystal structure.[19][6]

#### 1.1.3 Surface functional groups of cellulose nanocrystals

The main groups on the surface of CNCs are hydroxyl groups and sulfate groups. Celluloses are linear polymers composed of D-glucopyranosyl units linked by  $\beta$ -1,4-glycosidic bonds, with three free hydroxyl groups on the ring, as shown in Figure 1.1.

The three hydroxyl groups on the cellulose ring have different reaction abilities due to different stereochemical positions. C2 is the most vulnerable to attack and C3 is the least vulnerable to attack. In general, the reaction ability of primary hydroxyl group is higher than that of secondary hydroxyl group, especially when it reacts with larger groups. Due to the small steric hindrance, the reaction ability of primary hydroxyl group is higher. For example, the esterification reaction with chlorotoluene mainly occurs in primary hydroxyl group. However, the direct determination of substituents shows that the reactivity of each hydroxyl group of cellulose is different for different types of reactions. The reversible reaction mainly occurs in C6-OH, while the irreversible reaction mainly occurs in C2-OH. Therefore, for the esterification of cellulose, C6-OH has the highest reaction ability. The reaction ability of C2-OH is the highest when the fiber is etherified.[20]



Figure 1.1: Structure formula of cellulose

In recent years, there have been many different chemical modifications based on the abundant hydroxyl groups on the surface of CNCs, such as esterification,[21] etherification,[20] sulfonation,[22] oxidation[23] and so on. All the chemical modification has two main purposes: one is to introduce enough positive and negative charges to make CNC have good dispersitivity and stability, the other is to coordinate the surface potential energy of CNC to improve the compatibility with other substances, such as non-polar solvents, hydrophobic matrix and so on.[20]

Sulfate groups are another important groups on the surface of CNCs. The surfaces of CNCs prepared by sulfuric acid hydrolysis will produce sulfate groups.[24] These groups are obtained by partial esterification of hydroxyl groups on the surface of cellulose during hydrolysis.[25] The number of sulfate groups is low and has thermal instability. The existence of sulfate group is helpful to the dispersion of CNCs in water,[26] but the introduction of sulfate group will reduce the thermal stability of nanocrystals.[27] According to the research, the sulfation of the extract prepared by sulfuric acid hydrolysis, water-insoluble fiber material components are indirectly evaluated by the increase of sulfur content determined by element analysis method, or determined by the increase of strong acid group determined by conductometric titration method.[27]

#### 1.2 Light cellulose interaction

#### 1.2.1 Refractive index

Refractive index is an important parameter that is used for tuning its material optical properties but also in analytical purposes. In many industrial processes refractive index is used to define material fractions in dispersions or mixtures that can indicate purity and material concentration. At present, there are many methods to investigate refractive index of CNC, for instance, ellipsometry, reflectometry, nanoparticle tracking analysis (NTA), surface plasmon resonance (SPR) and immersion matching method. Different methods are suitable for different states of CNCs.[28][29] For suspensions of CNC, Niskanen et al.[28] prepared CNC suspension by mixing CNC powder with acetone and immersion oil. A method based on Beer-Lambert and immersion matching method was employed to measure refractive index of prepared CNC and the result is  $1.508\pm0.005$  at 400 nm and 1.489 at 700 nm.

For films of CNC, Reid et al.[30] measured refractive index of prepared CNC film by surface plasmon resonance which resulted in 1.55. Cranston et al.[31] used ellipsometry and angle-dependent optical reflectometry separately to determine refractive index of CNC film, resulting in  $1.51\pm0.01$  and  $1.516\pm0.001$  respective at 633 nm.

#### 1.2.2 Polarization

When a dielectric material is placed in the electromagnetic radiation field in the visible light range, the radiation polarization electric field causes the periodic displacement of the charged structural unit, and the radiation leads to the macro polarization of the material. Because the propagation of light is related to the polarization of medium, the response of medium to light wave field can be described by macroscopic physical quantity and polarizability.

According to Maxwell's electromagnetic wave theory, the propagation velocity of light in medium is shown in equation (1.1).

$$v = \frac{c}{\sqrt{\mu \cdot \epsilon}} \tag{1.1}$$

where c is the speed of light in vacuum,  $\epsilon$  is the polarizability of the medium,  $\mu$  is the permeability of the medium.

Therefore, for nonmagnetic materials whose permeability is defined as 1, relationship between refractive index and polarizability can be seen in equation (1.2).

$$n = \frac{c}{v} = \sqrt{\mu \cdot \epsilon} = \sqrt{\epsilon} \tag{1.2}$$

where n is refractive index, c is the speed of light in vacuum,  $\epsilon$  is the polarizability of the medium,  $\mu$  is the permeability of the medium, which is 1 for nonmagnetic materials.

Polarizability is a measure of the properties of electron clouds of molecules. The higher the polarizability, the easier the electron cloud is distorted in electric field or in light. When the radius of the ion increases, the polarizability increases, so the refractive index also increases. By changing the polarizability, the refractive index and hence the optical properties of the material can be changed.[32]

#### 1.2.3 Birefringence

Birefringence is defined as the difference between the refractive index of light polarized parallel to the optical axis and polarizations perpendicular to the optical axis. Birefringences are different depending on sources. For example, the birefringence of cellulose from cotton has been reported to vary from 0.045 to 0.062.[33] The values of birefringence can be different by changing measurement location. Cranston et al.[31] reported that for the same CNC film, center birefringence and edge birefringence are 0.013 and 0.009 respectively.

#### 1.3 Potential applications relying on information on light cellulose interaction

Since CNCs have properties typical of biomaterials such as low density, biodegradability, biocompatibility and renewability, in the past decade, with the further development of the research on CNCs, its application has been extended to biomedicine[34][35], wastewater treatment[36][37], electronics[38] and food industry.[39]

However, at present, compared to the applications in other fields, the applications of CNCs in optical field are still scarce. Among them, the vast majority of the optical applications are using the property that self-assembly of CNC dispersion occurs spontaneously, and a chiral nematic (cholesteric) liquid crystal structure is formed during the drying process. [26] The nanostructure of CNC chiral nematic liquid crystal can selectively diffract some wavelengths of light and make it present a certain structural color [40], so it is used in the fields of anti-counterfeiting [41] and decoration [42].

According to Bragg's Law, equation (1.3) can be derived. As can be seen in equation (1.3), the reflection wavelength depends on pitch, refractive index and incident light angle, which affect the color of the dried CNC. film.[43]

$$\lambda_{max} = n_{avg} \cdot P \cdot \sin\theta \tag{1.3}$$

where  $\lambda$  is the peak value of the wavelength center of the reflected light,  $n_{avg}$  is the average refractive index of the sample, P is the pitch,  $\theta$  is the the angle between the incident light and the plane perpendicular to the optical axis.

Based on different requirements of anti-counterfeiting, CNC film needs to change color under different sensing conditions such as temperature and humidity. For example, Gies et al.[44] doped thermotropic liquid crystals into CNC chiral nematic mesoporous materials. When temperature changes, the arrangement of liquid crystal in the mesoporous film changes, leading to the change of refractive index, and then the color change of CNC film. Youssef et al.[4] self assembled polyethylene glycol and CNC suspension to form a composite film with uniform structure color. Under different humidity conditions, the run up of polyethylene glycol leads to the change of pitch and color of the composite film.

#### 1.4 Objectives and hypothesis

While the refractive index is a parameter that is vital for many analytical efforts as well as application, it is not conclusively reported for CNCs. Therefore, this thesis is devoted in elucidating the refractive index on CNCs in suspensions.

There are three main objectives of this thesis. The first one is to demonstrate and explain alteration of the CNC suspension refractive index by modifying surface chemistry of CNC. The second one is to demonstrate linear relationship of CNC suspension concentration and refractive index and the effect of the surface chemistry on refractive index increment (dn/dc). The third one is to suggest potential applications relying on information obtained from experiments and data.

The hypothesis is that there is a relationship between CNC refractive index and its increment, and its surface physico-chemical properties.

## 2

### Theory

#### 2.1 Periodate oxidation

In order to modify the surface chemistry of CNCs, samples were required to be oxidized. According to the report[45], periodate oxidation is a highly specific oxidation reaction. Therefore, periodate oxidation was selected to be the method to oxidize CNC and desulfurized CNC samples to degree of oxidation (DO) 10 and 50 separately in this work. Periodate can oxidize hydroxyl groups to aldehydes by attacking and destroying the C2-C3 bond of glucose unit and selectively oxidizing the secondary hydroxyl group on the adjacent position of C2-C3 to aldehyde group. The reaction is demonstrated in Figure 2.1.



Figure 2.1: Oxidation of cellulose with periodate

#### 2.2 UV-vis

UV-vis, ultraviolet visible spectrophotometry, is a method to determine the absorbance of substances in the wavelength range of 190-800 nm, which is used for identification, impurity inspection and quantitative determination.[46]

In order to know when to stop the reaction of periodate oxidation to reach required degree of oxidation, UV-vis was used to measure the absorbance of the reaction solution after the reaction lasted for a specific time for DO 10 and DO 50 respectively. With the curve of absorbance to the concentration of NaIO<sub>4</sub> obtained from previous work which is demostrated in Appendix 1, aldehyde contents and degree of oxidation can be estimated.

#### 2.3 Hydroxylamine hydrochloride titration

UV-vis can only estimate the degree of oxidation, therefore, on the purpose of measuring DO of oxidized samples accurately, hydroxylamine hydrochloride titration was adopted as the method. According to the report[47], hydroxylamine hydrochloride can react with aldehyde groups to form oximes. The reaction is shown in equation (2.1). As can be seen in the reaction, when hydroxylamine hydrochloride is reacting, an HCl equivalent for each aldehyde residue will be released. The quantity of HCl produced in the reaction can be figured out by titration.

$$-(CHO)_n + H_2NOH \cdot HCl \longrightarrow -(CH = NOH)_n + H_2O + HCl$$
(2.1)

With the volume and molar concentration of consumed NaOH solution during titration, degree of oxidation of samples can be calculated from equation (2.2). Degree of oxidation shows the ratio of dialdehyde glucose units to total glucose units. Since a dialdehyde glucose unit contains 2 aldehyde groups, there is a coefficient 2 in denominator.

$$DO = \frac{V_{NaOH} \cdot N_{NaOH} \cdot M_{CNC}}{2 \times m_{CNC}} \times 100\%$$
(2.2)

where  $V_{NaOH}$  is the volume of consumed NaOH solution during titration in ml,  $N_{NaOH}$  is the molar concentration of NaOH solution, which is 0.01 mol/L,  $M_{CNC}$  is the molecular weight of CNC which is 162.16 g/mol,  $m_{CNC}$  is the weight of CNC in each sample which is 0.03 g.

#### 2.4 Dynamic light scattering

Dynamic light scattering (DLS) is a physical characterization method for measuring particle size distribution in solution or suspension. By scattering light from small particles, their geometrical structure and their state of motion can be measured.[48]

#### 2.5 Zeta potential

Zeta potential refers to the potential of shear plane, also known as electromotive potential or electromotive potential, is an important index to characterize the stability of colloidal dispersion system. The relationship between the stability of colloidal dispersion system and zeta potential is shown in table 2.1.[49] Measurement of zeta potential in this work was done by electrophoretic light scattering (ELS).

Zeta potential/mV	Stability
$0 \pm 5$	Rapid condensation
$\pm 5 \pm 30$	Unstable
$\pm 30 \pm 40$	Average stability
$\pm 40 \pm 60$	Good stability
more than $\pm 60$	Excellent stability

 Table 2.1: Relationship between stability of system and zeta potential

#### 2.6 X-ray photoelectron spectroscopy

For the purpose of quantitative analysis of elemental composition and carbon linkages, and simultaneously characterizing chemical modification of each sample, Xray photoelectron spectroscopy (XPS) of each sample were adopted separately. The principle of X-ray photoelectron spectroscopy (XPS) analysis is using X-rays to excite the inner electrons of atoms or molecules to become photoelectrons. Taking the kinetic energy or binding energy of the photoelectron as the abscissa and the relative intensity as the ordinate, the photoelectron spectrum is drawn. The samples can be analyzed by photoelectron spectrum.[50]

According to the theoretical instructions of XPS, carbon elements can be divided into four forms of combination, namely, single bond formed by carbon atom and carbon atom (C-C) whose chemical shift is 285.0 eV, single bond formed by carbon atom and oxygen atom (C-O) whose chemical shift is 286.5 eV, double bond formed by carbon atom and oxygen atom, including carbon atom and a carbonyl oxygen atom (C=O), and carbon atom connecting with 2 non carbonyl oxygen atoms (O-C-O) whose chemical shift is 288.0 eV, carbon atom connected with a carbonyl oxygen atom and a non carbon oxygen atom (O=C-O).

### Methods

#### 3.1 Materials

Cellulose nanocrystal powder was provided by CelluForce Canada.  $NaIO_4$  was purchased from Merck and used as received. Hydrochloric acid (37%), was purchased from Sigma-Aldrich and used as received. NaOH (99%) was obtained from VWR chemicals (Radnor, USA) and used as received. All other chemicals were of analytical or reagent grade.

#### 3.2 Preparation of samples

CNCs were prepared by oxidation and desulfation. Samples required in the experiments are shown briefly in table 3.1.

Nr	Chemicals	Degree of oxidation/%	Mass weight/g
1	$\operatorname{CNC}$	0	100
2	CNC	10	100
3	CNC	50	100
4	desulfated CNC (DSCNC)	0	100
5	desulfated CNC (DSCNC)	10	100
6	desulfated CNC (DSCNC)	50	100

 Table 3.1: Samples in the experiments

#### 3.2.1 Preparation of CNC samples

CNC (17.5g) was suspended in Milli-Q<sup>®</sup> water (482.5g) to make CNC suspension (500g) whose mass fraction was 3.5%. The suspension was stirred at room temperature for 24 hours.

#### 3.2.2 Preparation of desulfated CNC samples

CNC (25g) was suspended in Milli-Q<sup>®</sup> water (475 g) to make CNC suspension whose mass fraction was 5%. The suspension was stirred at room temperature for 24 hours. Then, a 12 mol/L hydrochloric acid (82.5 ml) was added to the CNC suspension, and the suspension was stirred at 90 °C for 2 hours. In order to remove chloride

ions from hydrochloric acid and sulfate ions from CNC molecules, the suspension was dialyzed by 12-14 kDA membranes until dialysate shares the same pH with the Milli-Q<sup>®</sup> water used to suspend CNC.

#### 3.2.3 Preparation of oxidized CNC samples

NaIO<sub>4</sub> (13 g) was suspended in Milli-Q<sup>®</sup> water (100 ml) to make NaIO<sub>4</sub> solution whose concentration was 0.13 g/ml. The solution was contained in a glass vial wrapped in silver paper in order to avoid the decomposition of the periodate in light. Then, the solution was stirred at room temperature for 30 minutes. NaIO<sub>4</sub> solution (17.5 ml) was added to two CNC suspension samples (100 g each) and two desulfated CNC suspension samples (100 g each) which were all contained in 250 ml Schott bottles wrapped in silver paper and stirred at room temperature.

According to the previous work of our group, both CNC and desulfated CNC could reach DO 10 and 50 after reacting 3.5 hours and about 40 hours respectively. In order to know more precisely when to stop the reaction, UV-vis was used to measure the absorbance of the reaction solution after the reaction lasted for 2.5 hours and about 39 hours for DO 10 and DO 50 respectively. 1 ml reaction solution was filtered by 0.45 micrometers PFTE filters to remove CNC and keep periodate in solution. 0.05ml filtrate was diluted by 2 ml Milli-Q<sup>®</sup> water and the absorbance of the diluted solution was measured. With the curve of absorbance to the concentration of periodate obtained from previous work which is shown in Appendix 1, aldehyde contents and degree of oxidation can be estimated. Each measurement interval is 30 minutes and there were two sets of repeats for each measurement.

Glycerol was added to stop the reaction as soon as the DO reached approximately the required value. In order to remove remaining  $NaIO_4$  and glycerol added, the suspension was dialyzed by 12-14 kDA membranes until dialysate shares the same pH with the Milli-Q<sup>®</sup> water used to suspend CNC.

#### **3.3** Gravimetric determination of concentration

After measuring the mass of the aluminum plate, 0.5 ml of the sample was dropped onto the plate and weighed again. The aluminum plate and sample were heated in oven at 100°C for 1 hour. Then, the aluminum plate and dried sample were weighed. Concentration of the measured sample can be calculated from equation (3.1). There were three sets of repeats for each measurement.

$$c = \frac{m_{p+d} - m_l}{m_{p+l} - m_l} \times 100\%$$
(3.1)

where c is the concentration of CNC in suspension sample,  $m_l$  is the weight of aluminum plate in g,  $m_{p+l}$  is the total weight of aluminum plate and suspension sample (0.5 ml) in g,  $m_{p+d}$  is the total weight of aluminum plate and dried sample in g.

#### 3.4 Hydroxylamine hydrochloride titration

Hydroxylamine hydrochloride (4.34 g) was suspended in Milli-Q<sup>®</sup> water (250 ml) to make hydroxylamine hydrochloride solution whose concentration was 0.25 mol/L. The pH of the solution was adjusted to 4. Then, each sample containing 0.03g dry mass of oxidized CNC or oxidized desulfated CNC was diluted by Milli-Q<sup>®</sup> water (10ml). The pH of the diluted sample was adjusted to 4 as well. After adding hydroxylamine hydrochloride solution (7.5 ml) to the diluted sample, each mixture was stirred at room temperature for 2 hours and titrated with sodium hydroxide solution (0.01 mol/L) until the pH of the mixture turned back to 4. The titration was done by Titroline@7000 SI Analytics (Schott, Germany) at room temperature.

#### 3.5 Dynamic light scattering

According to the report [50], each sample containing 0.05 g CNC was diluted with Milli-Q<sup>®</sup> water until the weight of mixture was 20 g. Each solution was sonicated for 1 minute and then adjusted pH to 4. Sonication was done by Chemical Instruments AB (Sollentuna, Sweden) with amplitude 20 % and pulse 15 seconds every minute. Dynamic light scattering was done by Zetasizer nano (Malvern, UK) at 25°C and at a scattering angle of 173°. There were three sets of repeats for each measurement.

#### 3.6 Zeta potential

According to the report[50], each sample containing 0.05 g CNC was diluted with Milli-Q<sup>®</sup> water until the weight of mixture was 20 g. Sodium chloride granules (11.7 mg) was added to each solution. Each mixture solution was sonicated for 1 minute and then adjusted pH to 4. Sonication was done by Chemical Instruments AB (Sollentuna, Swenden) with amplitude 20 % and pulse 15 seconds every minute. Zeta potential was done by Zetasizer nano (Malvern, UK) at 25 °C. There were six sets of repeats for each measurement.

#### 3.7 X-ray photoelectron spectroscopy

According to the report[50], freeze-drying was selected to be the method to turn samples of suspension into solid. After freezing in freezer for 48 hours, each sample (5 ml) were freeze-dried for 72 hours in freeze-drier, and then the samples became powder. The X-ray photoelectron spectroscopy (XPS) analysis was performed using a PHI 5000 VersaProbe III Scanning XPS Microprobe at an angle of 45°.

#### 3.8 Measurement of refractive index

Suspension of each sample was diluted to lower concentration and centrifugated to higher concentration to obtain samples of different concentrations expected. For

those whose concentrations were below 1 %, the samples were sonicated for 1 minute. For those whose concentrations were between 1 % and 3 %, the samples were sonicated for 3 minutes in ice. For those whose concentrations were beyond 3%, the samples were sonicated for 10 minutes in ice. Centrifugation was done by Hettick Zentrifugen Universal 32 (Schott, Germany) with 8000 RPM for 90 minutes for each sample. Sonication was done by Chemical Instruments AB (Sollentuna, Swenden) at room temperature with AMPL 20 % and pulse 15 seconds every minute. After sonication, refractive index of each sample of each concentration was measured by refractometer Abbemat 350/550 (AntonPaar, Austria) at 20 °C and at wavelength of 589 nm. There were three sets of repeats for each measurement.

## 4

### Results

#### 4.1 CNC suspension properties

Centrifugation was adopted to increase the concentrations (Appendix 2 Table B.1). The concentrated stock concentrations were then used to prepare dilutions for the refractive index analyses (Table 4.1).

Samples	Diluted concentrations/%
CNC	0.5, 1.0, 1.5, 2.0, 3.0, 3.5, 4.0, 4.5  and  5.0
CNC DO10	0.5, 1.0, 1.5, 2.0, 2.5  and  3.0
CNC DO50	0.5, 1.0, 1.5, 2.0, 2.5, 3.0  and  3.5
DSCNC	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5  and  5.0
DSCNC DO10	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5  and  4.0
DSCNC DO50	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0  and  4.5

Table 4.1: Dilutions of the CNC suspensions

The degree of oxidation was analyzed by hydroxylamine hydrochloride titration of oxidized CNC and oxidized desulfated CNC and is contrasted to the DO that was expected based on the consumption of periodate during the oxidation (Table 4.2).

**Table 4.2:** Degree of oxidation determined by UV absorbance (DO expected) and<br/>by titration (DO calculated)

Samples	DO expected/%	NaOH consumed/ml	DO calculated/%
CNC	10	2.2	8.5
CNC	50	13.7	54.4
DSCNC	10	3.0	8.7
DSCNC	50	15.9	46.5

As can be seen in Table 4.2, the degree of oxidation calculated from the results of hydroxylamine hydrochloride titration can be considered reasonable compared to the expected degree of oxidation. Therefore, it can be concluded that periodate oxidation reached the expectation and requirement.

The average values of hydrodynamic radius, r, by intensity and number, and average polymer dispersity index of each CNC suspension are shown in Table 4.3 (see also Appendix 3).

Samples	r by intensity/nm	r by number/nm	PDI
CNC	55	12	0.452
CNC DO10	94	16	0.486
CNC DO50	87	26	0.514
DSCNC	119	20	0.433
DSCNC DO10	89	20	0.245
DSCNC DO50	108	24	0.281

 Table 4.3: Hydrodynamic radius and polydispersity index (PDI) of CNC suspensions

As can be seen in Table 4.3, for hydrodynamic radius by intensity, the hydrodynamic radius of CNC was 55 nm, hydrodynamic diameter is 110 nm, which is similar to values reported in literature[4]. However, there is no relationship between hydrodynamic radius and degree of oxidation or desulfation development if only considering intensity distribution. However, when considering hydrodynamic radius by number distribution, it increases with increase of degree of oxidation for both CNCs and DSCNCs.

The larger PDI value is, the larger the range of particle size is; On the contrary, if PDI value is small, the particle size is concentrated. As can be seen in Table 4.3, PDI of all CNC suspensions are all below 0.8, which means that the CNCs are likely to be dispersed in the solvent. However, in general the changes between the CNC grade hydrodynamic radius are modest indicating that the physical properties of the CNCs are only minutely altered by the desulfation and oxidation.

Figure 4.1 displays the average values of zeta potential of each CNC suspension sample with standard deviation, where lines are guiding lines to show the tendency.



Figure 4.1: Average values of zeta potential of CNC suspensions with respect to degree of oxidation

As can be seen in Figure 4.1, the values of zeta potential of all CNC grades are negative, this is because negative charged sulfate groups in CNCs result in electrostatic repulsion between CNCs particles. Generally, if the zeta potential is greater than 30 mV, the system is considered stable. Therefore, CNC and DSCNC suspension are stable while suspensions of CNC DO50 and DSCNC DO50 are unstable.

The zeta potential decreases with increase of degree of oxidation. The main reason for this may be modulation of the complex electrical double layer consisting of the sulfate, hydroxyl and introduced aldehyde groups. As mentioned in section 2.5, the higher the zeta potential is, the more stable the system is. Therefore, the stability of the CNC suspensions decrease with increase of degree of oxidation. In section 4.3, hydrodynamic radius by number increases with increase of degree of oxidation for both CNCs and DSCNCs, which indicates that the stability of the CNC suspensions decrease with increase of degree of oxidation and further confirms the trend of zeta potential.

The value of zeta potential desulfated CNCs is lower than CNC under DO 10 and DO 50. The main reason is that negative charged sulfate groups were removed, thus absolute value of zeta potential decreases. However, DSCNC has a higher absolute value of zeta potential than CNC, which is in conflict with the previous results of hydrodynamic radius in section 4.3. This finding requires future work to be clarified.

The elemental composition of the CNC grades is shown in Table 4.4. The ratio of carbon content to oxygen content and the ratio of carbon content to sulfur con-

Samples	C/%	<b>O</b> /%	Na/%	$\mathrm{S}/\%$	C/O	C/S
CNC	61.18	38.15	0.30	0.37	1.60	165.35
CNC DO10	59.41	39.99	0.31	0.28	1.49	212.19
CNC DO50	59.40	40.30	-	0.30	1.47	198.00
DSCNC	61.45	38.37	-	0.18	1.60	341.39
DSCNC DO10	60.77	39.09	-	0.14	1.56	434.07
DSCNC DO50	61.48	38.44	-	0.08	1.59	768.50

tent are calculated and used for evaluating the DO and sulfation.

Table 4.4: Atomic percentage of elements in CNC suspensions

Compared to CNCs, C/S ratio of DSCNCs are higher (Table 4.4). This is because the sulfur contents of all DSCNC suspensions are lower compared to CNC suspensions, which indicates that the sulfate groups on the surface of CNCs are indeed removed by desulfation. Considering C/O ratio, with same degree of oxidation, DSCNC suspensions are higher compared to CNC suspensions. The reason is that sulfate groups containing oxygen of DSCNC suspensions were removed, leading to the decrease of oxygen content in DSCNC suspensions, and further leading to increase of the ratio.

For both CNCs and DSCNCs, C/O ratio should decrease with the increase of degree of oxidation because content of oxygen increases with increase of degree of oxidation. However, C/O ratio of DSCNC DO10 is lower than DSCNC DO50, which indicates that DSCNC DO50 contains less oxygen than DSCNC DO10. The reason could be that sulfates groups containing oxygen of DSCNC DO50 cleaved off during the long time oxidation, which leads to the reduction of oxygen content of DSCNC DO50. Highest C/S ratio and lowest sulfur content of DSCNC DO50 support this observation.

The XPS spectra of different CNC suspensions are presented in Appendix 4. The deconvolution of the carbon peak reveal four contributions that are shown in Table 4.5.

Samples	C-C/%	C-O/%	0-C-0, C=0/%	0=C-O/%
CNC	22.21	58.19	16.74	2.86
CNC DO10	16.87	61.32	17.72	4.09
CNC DO50	17.26	52.13	23.93	6.68
DSCNC	21.50	59.57	16.28	2.64
DSCNC DO10	18.08	59.41	19.16	3.36
DSCNC DO50	17.28	51.12	23.89	7.72

Table 4.5:	Deconvolution	$of \ high$	resolution	carbon	peak	intensity	of	CNC	XPS
			spectra						

Periodate can oxidize hydroxyl groups to aldehydes, thus the content of C=O increases while C-O decreases with the increase of degree of oxidation. Therefore, the ratio of content of C-O to the one of O-C-O and C=O can well reflect degree of oxidation. This ratio is visualized in Figure 4.2, where lines are guiding lines to show the tendency.



Figure 4.2: Ratio of content of C-O to C=O

Figure 4.2 shows that the ratio of content of C-O to C=O decreases with degree of oxidation for both CNC and DSCNC, which indicates that sodium periodate oxidizes some hydroxyl groups on the molecular chain of nanocellulose to aldehyde groups.

#### 4.2 Linear dependency of concentration and refractive index

Dependence of concentration and refractive index of CNC is shown in Table 4.6 as an example, and the other grades are presented in Appendix 5.

Conc diluted/%	Conc calculated/%	Refractive index/nD
0.5	0.4102	1.333674
0.5	0.4620	1.333676
0.5	0.5078	1.333678
1.0	0.9980	1.334445
1.0	0.9990	1.334447
1.0	1.0921	1.334448
1.5	1.5667	1.335173

 Table 4.6:
 Concentration and refractive index of CNC

1.5	1.5738	1.335174
1.5	1.5851	1.335179
2.0	2.0653	1.335918
2.0	2.0998	1.335919
2.0	2.1334	1.335921
3.0	3.0854	1.337359
3.0	3.1026	1.337380
3.0	3.1138	1.337374
3.5	3.3905	1.338016
3.5	3.3983	1.338024
3.5	3.4522	1.338043
4.0	3.8866	1.338547
4.0	3.9353	1.338568
4.0	4.0016	1.338657
5.0	4.9937	1.340164
5.0	5.0651	1.340167
5.0	5.0968	1.340177

The concentration versus refractive index dependence was exposed to the linear fitting. The linear fitting adopted was regular linear fitting, where intercepts of all the curves are 1.333, which is refractive index of water at 25 °C, and slopes of all curves are refractive index increments in ml/g. The refractive index increment of the non-oxidized, DO10 and DO50 are presented in Figure 4.3, 4.4 and 4.5.



Figure 4.3: Refractive index increment of non-oxidized CNC suspension samples



Figure 4.4: Refractive index increment of DO 10 CNC suspension samples



Figure 4.5: Refractive index increment of DO 50 CNC suspension samples

The refractive index increment of the CNC and DSCNC are presented in Figure 4.6 and 4.7.



Figure 4.6: Refractive index increment of CNC suspension samples



Figure 4.7: Refractive index increment of DSCNC suspension samples

Considering all the figures above, the dependence of concentration and refractive index of all CNC suspensions are almost linear, which indicates that CNCs are isotropic in CNC suspensions. However, the  $R^2$  values of curves of DSCNC and DSCNC DO10 are 0.979 and 0.981 respectively, which are lower than other curves. This indicates that curves of DSCNC and DSCNC DO10 do not fit well to the linear fitting. The main reason is that CNCs are not isotropic in DSCNC suspensions in high concentration. In order to show the linearity of DSCNC and DSCNC DO10 better, Figure 4.8 containing only refractive index of concentration below 2 %.



Figure 4.8: Refractive index increment and concentration of DSCNC suspension samples

Comparing two concentration ranges, if only consider concentrations below 2%, the refractive index increment of DSCNC and DSCNC DO10 are 0.00177 ml/g and 0.00123 ml/g respectively. However, if consider all range of concentrations, the refractive index increment of DSCNC and DSCNC DO10 are 0.00155 ml/g and 0.00142 ml/g respectively. The changes indicate that for DSCNC samples in DO0 and DO10, relationship between refractive index and concentration is linear when the concentration is low, however, particles begin to aggregate when the concentration is high, leading to the unlinear relationship in high concentration. For DSCNC DO50, refractive index increment for concentrations below 2 wt% and for concentrations of all range are all 0.00144 ml/g, which indicates that relationship between refractive index and concentration is almost linear.

The refractive index increment is contrasted to degree of oxidation and shown in Figure 4.9, where lines are guiding lines to show the tendency. As can be seen in the figure, if consider concentrations of all range, refractive index increments of CNC do not change significantly. For DSCNCs, refractive index increments decrease firstly and then increase with degree of oxidation, and vary between 0.00142 ml/g and 0.00155 ml/g. If only consider concentrations below 2%, the refractive index increments of DSCNCs firstly decrease significantly and then increase with degree of oxidation, and vary between 0.00123 ml/g and 0.00177 ml/g.



Figure 4.9: Refractive index increments of linear fitting curves

If assuming that the linear fitting curves apply to all concentrations of each CNC suspension sample, refractive index with 100 % concentration, which is the refractive index of each pure CNC suspension sample can be calculated. The refractive index increments of DSCNC samples are selected for both all range of concentrations. The results are shown in Table 4.7.

Samples	Refractive index/nD
CNC	1.476
CNC DO10	1.473
CNC DO50	1.481
DSCNC	1.488
DSCNC DO10	1.475
DSCNC DO50	1.477

Table 4.7: Refractive index when dn/dc is extrapolated to 100% concentration

As can be seen in figure 4.9 and Table 4.7, higher refractive index increments lead to higher values of refractive index with 100% concentration. Refractive index varies within 1.473 to 1.488 and refractive index increment between 0.0014 ml/g and 0.00177 ml/g considering concentrations below 2% and between 0.0014 ml/g and 0.00155 ml/g considering concentrations of all range.

Focusing on Table 4.7, among all CNC suspensions, the refractive index of DSCNC is highest, which is 1.488, the one of CNC DO10 is lowest, which is 1.473. The refractive index of CNC is 1.476, lower than the reported value at the same mea-

sured wavelength, which is 1.494 measured at wavelength of 589 nm.[28] This is mainly because that the linear fitting curves may not apply to all concentrations, which may lead to the difference. There is difference between each CNC suspension sample, which means that surface modification can lead to the alteration of refractive index of CNCs, and the potential applications proposed in section 1.3 is feasible.

Considering the relationship between refractive index and desulfation, it can be observed that refractive index increments and refractive index with 100% concentration of CNCs are lower than DSCNCs in both DO0 and DO10. This fact is contrary to theory. It is considered that there are sodium ions in CNC suspensions because the sulfate groups might contain sodium ions during sulfuric acid hydrolysis. However, there is no sodium ion in DSCNC suspensions because sulfate groups were removed. The difference of Na content can also be seen in elemental composition in section 4.5. The radius of sodium ion is larger than hydrogen ion. The polarizability increases when the radius of the ion increases, further leading to the increase of refractive index. Therefore, refractive index increments and refractive index with 100% concentration of CNCs should be higher than DSCNCs. However, only the fact of samples in DO50 is consistent with the theory.

Considering the relationship between refractive index and degree of oxidation, it can be seen that for both CNC and DSCNC suspensions, refractive index increments and refractive index with 100% concentration decrease firstly and then increase with the increase of degree of oxidation. The only difference between CNC and DSCNC is that for CNC, refractive index increment non-oxidized sample is highest while for DSCNC, the one of DO50 is highest.

Unusual performances that DSCNCs have higher refractive index has been observed but not been reasonably explained in this paper. Reasonable explanation may be proposed with further work. In addition, the tendency of refractive index and degree of oxidation has been observed but not been reasonably explained. In order to explain or improve results, more degrees of oxidation should be included to verify the trend.

# 4.3 Relationship between surface chemistry and refractive index increment

In order to further elucidate on the effect of the surface chemistry on refractive index increment, results of sulfur contents in section 4.5, ratio of content of C-O to the one of O-C-O and C=O in section 4.6 and hydrodynamic radius in section 4.3 are connected to refractive index increment and shown in Figure 4.10, 4.11 and 4.12 respectively.



Figure 4.10: Refractive index increment and sulfur contents



Figure 4.11: Refractive index increment and ratio of content of C-O to the one of O-C-O and C=O



Figure 4.12: Refractive index increment and hydrodynamic radius

As can be seen in the figures above, there is no obvious relationship between refractive index increment and parameters of surface chemistry of CNCs. Therefore, it can be concluded that refractive index increments of the suspensions are sensitive to the chemical treatment of CNCs. However, it is unclear still how to control it intentionally. In the further work, other modifications that would change the polarizability could be conducted to clarify this.

### Conclusion

In this thesis, periodate oxidation of CNCs was shown an effective method to oxidize CNC by attacking and destroying the C2-C3 bond of glucose unit. By controlling the time of reaction, samples can reach the degree of oxidation of 10 and 50 as expected respectively. In addition, adding hydrochloric acid of 12 mol/L into CNC and heating for two hours, the sulfate groups in CNC were effectively removed.

According to the characterization results, the hydrodynamic radius of the sample and the stability of the suspension are related to the surface properties of CNCs. Hydrodynamic radius increases with increase of degree of oxidation for both CNCs and DSCNCs, but there is no obvious regularity between hydrodynamic radius and desulfation of samples. When considering stability of the suspension, the stability of the samples decrease with increase of degree of oxidation, and it is likely to be the reason for increase in hydrodynamic radius as well.

For all the samples involved in our work, refractive index varies within 1.473 to 1.488 and refractive index increment between 0.0014 ml/g and 0.00177 ml/g considering the slope of fitting suspension concentrations below 2% and between 0.0014 ml/g and 0.00155 ml/g considering concentrations of all range. There is linear relationship between refractive index and concentration in CNC particle suspension concentrations where the isotropic suspensions transfer to liquid crystalline phases, which are 2-3% for CNCs.

Refractive index increment changes with the alteration of surface chemistry of CNCs, however, obvious relationship between refractive index increment and parameters of surface chemistry of CNCs cannot be expounded. Therefore, it can be concluded that refractive index increments of the suspensions are sensitive to the chemical treatment of CNCs. However, it is unclear still how to control it intentionally.

Based on the information from this thesis, surface modification can be used to change the refractive index of CNC. Therefore, oxidized CNC and desulfated CNC can be used as raw material instead of original CNC to produce films. In this way, materials from theses CNCs may entail the opportunity to modulate optical properties and allow use in, for example, anti-counterfeiting.

For further work, samples with more degree of oxidations need to be included in order to verify the trend, such as samples of DO 30. More characterization methods can be adopted, such as wide angle X-ray scattering (WAXS) to see crystallinity

and AFM to see CNC dimensions. Additionally, since this work is only devoted in CNC suspension, working on CNC films simultaneously may lead to a conclusive value for CNC refractive index.

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# A Appendix 1

The fitting curve of absorbance to the concentration of  $NaIO_4$  obtained from previous work is demonstrated figure A.1. As can be seen in figure A.1, the slope of the curve is 4.481.



Figure A.1: Curve of absorbance to the concentration of NaIO<sub>4</sub>

# В

## Appendix 2

Concentrations of CNC suspensions before dilution and after centrifugation are shown in Table B.1.

 
 Table B.1: CNC suspension concentrations before dilution and after centrifugation

Samples	Conc. before dilution/ $\%$	Conc. after centrifugation $/\%$
CNC	3.414	5.051
CNC DO10	2.385	3.238
CNC DO50	1.781	3.681
DSCNC	3.642	5.350
DSCNC DO10	2.529	4.217
DSCNC DO50	1.943	4.681

# C Appendix 3

Table C.1 displays the detailed data of 3 rounds' measurement of hydrodynamic size by intensity and polymer dispersity index (PDI) of different samples.

Samples	Size/nm	PDI	Pk 1/nm	Pk 2 / nm	Pk 3/nm
CNC 1	109,9	$0,\!452$	195,1	$36,\!51$	4832
CNC 2	109,8	$0,\!456$	175,2	4903	0,000
CNC 3	109,9	$0,\!447$	183,9	4487	$10,\!56$
CNCDO10 1	187,6	$0,\!485$	304,0	48,10	4633
CNCDO10 2	189,8	0,502	$376,\!9$	81,46	$20,\!25$
CNCDO10 3	188,4	$0,\!472$	287,0	$38,\!25$	4030
CNCDO50 1	170,2	0,560	218,7	4318	0,000
CNCDO50 2	171,4	$0,\!570$	212,0	4238	0,000
CNCDO50 3	182,8	0,411	175,2	2834	0,000
DSCNC 1	239,5	0,421	385,4	$53,\!86$	0,000
DSCNC 2	238,1	0,441	384,1	38,42	3984
DSCNC 3	238,6	0,436	388,0	$52,\!35$	4816
DSCNCDO10 1	176,2	0,244	242,6	30,63	0,000
DSCNCDO10 2	179,8	0,248	221,2	0,000	0,000
DSCNCDO10 3	176,3	0,244	244,3	0,000	0,000
DSCNCDO50 1	217,8	0,255	281,2	54,57	0,000
DSCNCDO50 2	216,1	0,275	261,9	4526	0,000
DSCNCDO50 3	214,5	0,313	264,4	5006	0,000

Table C.1: detailed data of hydrodynamic size and PDI

Figure C.1 displays size distribution by intensity of 3 rounds' measurement of CNC sample.



Figure C.1: Size distribution by intensity of CNC

Figure C.2 displays size distribution by intensity of 3 rounds' measurement of CNC DO10 sample.



Figure C.2: Size distribution by intensity of CNC DO10

Figure C.3 displays size distribution by intensity of 3 rounds' measurement of CNC DO50 sample.



Figure C.3: Size distribution by intensity of CNC DO50

Figure C.4 displays size distribution by intensity of 3 rounds' measurement of DSCNC sample.



Figure C.4: Size distribution by intensity of DSCNC

Figure C.5 displays size distribution by intensity of 3 rounds' measurement of DSCNC DO10 sample.



Figure C.5: Size distribution by intensity of DSCNC DO10

Figure C.6 displays size distribution by intensity of 3 rounds' measurement of DSCNC DO50 sample.



Figure C.6: Size distribution by intensity of DSCNC DO50

Figure C.7 displays size distribution by number of 3 rounds' measurement of CNC sample.



Figure C.7: Size distribution by number of CNC

Figure C.8 displays size distribution by number of 3 rounds' measurement of CNC DO10 sample.



Figure C.8: Size distribution by number of CNC DO10

Figure C.9 displays size distribution by number of 3 rounds' measurement of CNC DO50 sample.



Figure C.9: Size distribution by number of CNC DO50

Figure C.10 displays size distribution by number of 3 rounds' measurement of DSCNC sample.



Figure C.10: Size distribution by number of DSCNC

Figure C.11 displays size distribution by number of 3 rounds' measurement of DSCNC DO10 sample.



Figure C.11: Size distribution by number of DSCNC DO10

Figure C.12 displays size distribution by number of 3 rounds' measurement of DSCNC DO50 sample.



Figure C.12: Size distribution by number of DSCNC DO50

# D Appendix 4

Figures of photoelectron spectrum of CNC are demonstrated in figure D.1.



Figure D.1: Photoelectron spectrum of CNC  $\,$ 



Figures of photoelectron spectrum of CNC DO10 are demonstrated in figure D.2.

Figure D.2: Photoelectron spectrum of CNC DO10



Figures of photoelectron spectrum of CNC DO50 are demonstrated in figure D.3.

Figure D.3: Photoelectron spectrum of CNC DO50



Figures of photoelectron spectrum of DSCNC are demonstrated in figure D.4.

Figure D.4: Photoelectron spectrum of DSCNC



Figures of photoelectron spectrum of DSCNC DO10 are demonstrated in figure D.5.

Figure D.5: Photoelectron spectrum of DSCNC DO10



Figures of photoelectron spectrum of DSCNC DO50 are demonstrated in figure D.6.

Figure D.6: Photoelectron spectrum of DSCNC DO50

# E Appendix 5

Results of concentration and refractive index of CNC DO10 are shown in table E.1.

Conc diluted/%	Conc calculated/ $\%$	Refractive $index/nD$
0.5	0.4888	1.333753
0.5	0.5053	1.333762
0.5	0.5128	1.333766
1.0	1.0659	1.334421
1.0	1.1009	1.334422
1.0	1.1062	1.334424
1.5	1.4155	1.335024
1.5	1.4271	1.335030
1.5	1.5319	1.335044
2.0	1.8726	1.335382
2.0	1.9399	1.335412
2.0	1.9573	1.335421
2.5	2.3418	1.336390
2.5	2.4123	1.336392
2.5	2.4319	1.336419
3.0	2.7690	1.337143
3.0	2.7783	1.337145
3.0	2.8594	1.337152

 Table E.1: Concentration and refractive index of CNC DO10

Results of concentration and refractive index of CNC DO50 are shown in table E.2. **Table E.2:** Concentration and refractive index of CNC DO50

Conc diluted/%	Conc calculated/%	Refractive index/nD
0.5	0.5553	1.333779
0.5	0.5105	1.333779
0.5	0.5025	1.333778
1.0	0.9859	1.334499
1.0	0.9923	1.334501
1.0	1.0220	1.334506

1.5	1.4816	1.335184
1.5	1.4821	1.335186
1.5	1.5248	1.335188
2.0	1.9744	1.335912
2.0	1.9846	1.335920
2.0	1.9955	1.335934
2.5	2.5167	1.336687
2.5	2.5233	1.336688
2.5	2.5384	1.336694
3.0	2.9883	1.337452
3.0	3.0046	1.337487
3.0	3.0215	1.337512
3.5	3.5020	1.338201
3.5	3.5076	1.338213
3.5	3.5237	1.338223

Results of concentration and refractive index of DSCNC are shown in table E.3.

Conc diluted/%	Conc calculated/ $\%$	Refractive $index/nD$
0.5	0.3671	1.333757
0.5	0.3852	1.333767
0.5	0.3953	1.333768
1.0	0.8361	1.334454
1.0	0.8437	1.334458
1.0	0.9551	1.334516
1.5	1.4838	1.335603
1.5	1.4899	1.335654
1.5	1.5231	1.335758
2.0	1.9123	1.336496
2.0	1.9076	1.336488
2.0	1.8905	1.336462
2.5	2.5330	1.337336
2.5	2.5383	1.337345
2.5	2.5459	1.337402
3.0	3.1149	1.337902
3.0	3.1688	1.338090
3.0	3.2251	1.338238
3.5	3.4398	1.338346
3.5	3.4405	1.338366
3.5	3.4712	1.338393
4.0	3.7889	1.338885

4.0	3.9827	1.338916
4.0	4.0175	1.338958
4.5	4.5287	1.339653
4.5	4.5003	1.339635
4.5	4.4821	1.339604
5.0	4.9577	1.340325
5.0	4.9669	1.340476
5.0	4.9803	1.340650

Results of concentration and refractive index of DSCNC DO10 are shown in table E.4.

Conc diluted/%	Conc calculated/%	Refractive index/nD
0.5	0.5617	1.333752
0.5	0.6180	1.333756
0.5	0.6401	1.333757
1.0	1.1683	1.334457
1.0	1.2007	1.334480
1.0	1.2556	1.334488
1.5	1.7434	1.335133
1.5	1.7291	1.335118
1.5	1.6805	1.335115
2.0	1.8826	1.335925
2.0	1.9478	1.335930
2.0	2.1110	1.335948
2.5	2.5197	1.336546
2.5	2.5264	1.336590
2.5	2.5949	1.336647
3.0	3.0236	1.337332
3.0	3.0404	1.337356
3.0	3.0595	1.337379
3.5	3.4913	1.338027
3.5	3.5333	1.338078
3.5	3.5751	1.338135
4.0	4.0100	1.338841
4.0	4.0196	1.338874
4.0	4.0549	1.338876

 Table E.4: Concentration and refractive index of DSCNC DO10

Results of concentration and refractive index of DSCNC DO50 are shown in table

#### E.5.

Conc diluted/%	Conc calculated/%	Refractive index/nD
0.5	0.4400	1.333737
0.5	0.4865	1.333740
0.5	0.5133	1.333741
1.0	0.9645	1.334445
1.0	1.0457	1.334453
1.0	1.0867	1.334460
1.5	1.5442	1.335184
1.5	1.5173	1.335174
1.5	1.4736	1.335171
2.0	1.9928	1.335882
2.0	1.9992	1.335886
2.0	2.0081	1.335891
2.5	2.6174	1.336728
2.5	2.6179	1.336742
2.5	2.6279	1.336743
3.0	3.1037	1.337462
3.0	3.1134	1.337468
3.0	3.1148	1.337479
3.5	3.5681	1.338194
3.5	3.6588	1.338199
3.5	3.6957	1.338244
4.0	3.9864	1.338709
4.0	3.9820	1.338694
4.0	3.9602	1.339468
4.5	4.4783	1.339544
4.5	4.4989	1.339567

 Table E.5: Concentration and refractive index of DSCNC DO50

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