

# Preparation Methods for Nanocrystalline Cellulose

- Acid hydrolysis and various cellulose  
sources

Bachelor Science Thesis

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Preparation Methods for Nanocrystalline Cellulose  
- *Acid hydrolysis and various cellulose sources*

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Göteborg, Sweden 2015

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Cover:

AFM pictures of nanocrystalline cellulose prepared from sulphuric acid with two different starting materials, microcrystalline cellulose and cellulose extracted from barley husk.

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## **ABSTRACT**

The aim of this project has been to prepare nanocrystalline cellulose (CNC) from hydrochloric acid hydrolysis and optimise the process. CNC was also prepared from sulphuric acid hydrolysis to use as a reference. The starting material in both these processes was microcrystalline cellulose (MCC), therefore another cellulose source was used to try and prepare CNC from. The cellulose was extracted from barley husk in another project performed in the same division which is usually considered a waste product.

Four different procedures were performed using hydrochloric acid, where the first three procedures followed various studies in literature and the parameters of the fourth procedure was determined after evaluating the results from the first three. After analysing the samples with atomic force microscopy (AFM) it was found that no clear CNC had been obtained.

Preparing CNC from sulphuric acid hydrolysis was shown to be an optimised process with successful results. Characteristic rod-like crystals were obtained and could be seen after using AFM. Therefore this process was used in the attempt of preparing CNC from cellulose that was extracted from barley husk. Two different procedures were evaluated, one starting with wet cellulose and one with dry cellulose. It was found that preparing CNC from dry cellulose with sulphuric acid hydrolysis was possible. The AFM picture showed that some of the crystals seemed to be a bit too long to be nanosize which implies that the process needs to be evaluated and adjusted.

The conclusions of this project is that further studies must be performed on preparing CNC from hydrochloric acid hydrolysis to obtain an optimised process. It is possible to prepare crystals from cellulose extracted from barley husk although the process must be further evaluated to obtain parameters that accommodates the cellulose source. It has been shown that preparing CNC from sulphuric acid hydrolysis is an optimised and established process.

Keywords: Nanocrystalline cellulose, acid hydrolysis, barley husk, hydrochloric and sulphuric acid

# SAMMANFATTNING

Syftet med detta projekt har varit att framställa nanokristallin cellulosa (CNC) genom saltsyra hydrolys samt optimera denna process. CNC framställdes också genom svavelsyra hydrolys för att användas som referens. Startmaterialet i båda dessa processer var mikrokristallin cellulosa (MCC), därför undersöktes det om det gick att framställa CNC från ytterligare en cellulosa källa. Cellulosan extraherades från kornskal och anses vara en avfallsprodukt, den framställdes i ett annat projekt som utfördes på samma avdelning.

Fyra olika procedurer utfördes med saltsyra, där de första tre procedurerna följde olika studier i litteratur och parametrarna för den fjärde proceduren bestämdes utefter resultaten från de tre första. Efter analys med atomic force microscopy (AFM) konstaterades det att ingen tydlig CNC hade erhållits. Framställning av CNC genom svavelsyra hydrolys visade sig vara en optimerad process där man kan få framgångsrika resultat. Karaktäristiska stav-liknande kristaller erhöles och kunde observeras efter analys med AFM.

Därför användes även denna process i försöket att framställa CNC från en cellulosa som extraherats från kornskal. Två olika procedurer utvärderades där den ena startade med blöt cellulosa och den andra med torr cellulosa. Det konstaterades att det var möjligt att framställa CNC genom svavelsyra hydrolys med torr cellulosa som startmaterial. AFM bilderna visade att somliga av de erhållna kristallerna verkade vara lite för långa för att vara i nanostorlek vilket indikerade att processen måste studeras vidare och justeras.

Slutsatserna av detta projekt är att fortsatta studier måste utföras när det gäller att framställa CNC genom saltsyra hydrolys för att erhålla en optimerad process. Det är möjligt att framställa kristaller från cellulosa som extraherats från kornskal, dock måste processen studeras vidare för att få fram parametrar som passar cellulosa källan. Det har också visat sig att framställa CNC genom svavelsyra hydrolys är en optimerad och etablerad process.

## ABBREVIATIONS

CNC	Nanocrystalline cellulose
MCC	Microcrystalline cellulose
AFM	Atomic Force Microscopy
XRD	X-ray Diffraction
FT-IR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance

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# 1. INTRODUCTION

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## 1.1 BACKGROUND

Cellulose is an interesting and sought of material due to current demands for green chemistry, more environmentally friendly resources and renewable raw material. The use of cellulose is therefore highly attractive because of it being renewable, biodegradable and non-toxic [1]. In recent years, nanotechnology and especially nanocellulose has received a lot of attention and is one of the most interesting and promising areas for future development [2]. Nanocrystalline cellulose (CNC) has unique characteristics and can be used as a reinforcement in polymer nanocomposites to highly enhance mechanical properties [3].

Before being able to fully apply CNC, various preparation methods must be evaluated to be able to obtain a stable and usable material. Today's most common method for extracting CNC is through controlled acid hydrolysis where sulphuric acid is the most used because it creates a stable aqueous suspension of CNC. When using sulphuric acid, charged sulfate groups attach to the surface of the CNC after the hydrolysis which induces a significant decrease in thermal stability [3]. There is therefore an interest in using an acid that would not create charged CNC such as hydrochloric acid.

A less stable aqueous suspension will be obtained using hydrochloric acid but the thermal stability will increase. As a consequence, there is a delicate balance to find a thermal stable CNC that at the same time gives a stable suspension. It is therefore important to study various types of acids and preparation methods to gain more knowledge about the differences in properties of the CNC and to obtain the highest compatible CNC for use in polymer matrices. It is also important to compare and evaluate different starting material and various cellulose sources, to obtain material that can be used in sustainable industrial processes.

## **1.2 OBJECTIVES**

The aim of this project was to optimise the production of CNC extracted through acid hydrolysis using hydrochloric acid and create a reliable process for future use. Part of the project was to produce CNC using sulphuric acid to use as a reference.

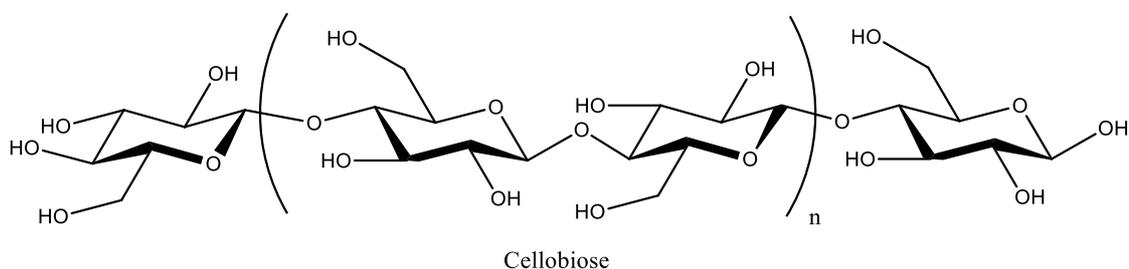
The aim was also to examine whether CNC could be obtained from an alternative cellulose source such as annual plants. In a parallel bachelor project at the department, extraction of hemicellulose from annual plants (barley husk), cellulose was obtained as a by-product. The cellulose extracted from barley husk was collected and analysed to find out if it was crystalline enough to be used for CNC preparation. Solid state NMR and XRD were used in order to characterise the cellulose and for the nanocrystals AFM, IR and XRD were used.

## 2 THEORY

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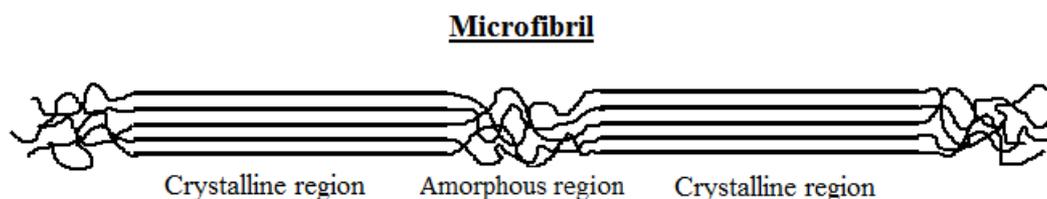
### 2.1 CELLULOSE

Cellulose is one of the most abundant natural biopolymer in the world. It can be found in a variety of sources such as trees, plants, animals and some bacteria [2], but the most important and common source is wood which contains up to 50% cellulose. Cellulose is a linear homopolysaccharide that consist of anhydroglucopyranose units linked together by  $\beta$ -1,4-glycosidic bonds where the repeating unit is two glucose monomers known as cellobiose, see figure 1 [4].



*Figure 1. Molecular structure of the repeating unit of cellulose.*

Every glucose monomer consists of three hydroxyl groups which gives the cellulose molecule high functionality and thus makes it an interesting and important material to study [1]. Due to the hydroxyl groups, cellulose molecule chains attach to each other through hydrogen bonding and form larger units called elementary fibrils. These pack into even larger units called microfibrils, which are composed into fibres and this whole contexture creates a morphological hierarchy formed by the hydrogen bond network. The microfibrils has a diameter of 5-50 nm and several micrometers in length and within these fibrils there are highly ordered crystalline regions and disordered amorphous regions as can be seen in figure 2 [4,5].



*Figure 2. Crystalline and amorphous regions within a microfibril.*

The crystalline regions contain complicated intra- and intermolecular interactions while the amorphous regions are regularly scattered. By isolating and extracting the crystalline regions, nanocrystalline cellulose can be obtained.

## **2.2 NANOCRYSTALLINE CELLULOSE**

When extracting CNC using controlled acid hydrolysis, the amorphous regions will initially decompose due to them being more accessible compared to the crystalline regions higher resistance. Thereafter rod-like cellulose crystals with typical diameter of 2-20 nm and length from 100 to 600 nm will be obtained [2]. During acid hydrolysis, parameters such as cellulose source, type of acid, reaction time and temperature are vital for the characterisation of the nanocrystals. These parameters will determine the properties of the nanocrystals, partially due to crystalline and amorphous regions that change significantly depending on the cellulose source [6].

As mentioned earlier, CNC is highly interesting as reinforcement in polymer nanocomposites. This is due to their low density, high aspect ratio, low toxicity, good mechanical properties and reactive hydroxyl groups attached to the surfaces [7]. Although CNC has a lot of unique characteristics there are some disadvantages such as difficulty in dispersing in organic solvents and tendency to aggregate during drying processes due to the hydroxyl groups. Therefore a lot of interest in surface modification of nanocrystals have attracted attention to improve the dispersibility and make the nanocrystals more compatible with different polymer matrices.

Addition of surfactants and modification by surface grafting are two common methods for this purpose [8]. If the shortcomings of CNC can be managed, there are a lot of applications to be considered such as in paper and packaging products, construction, furniture, electronics, pharmacy and so on [2]. A great benefit for these applications are that only a percentage of nanocrystals are required to reinforce the material when applied in composites.

## **2.3 CELLULOSE SOURCES**

As previously mentioned it is important to consider different cellulose sources when extracting CNC through acid hydrolysis. The various parameters of the hydrolysis must be adjusted to suit the cellulose starting material. The properties of the nanocrystals will also turn out differently depending on the source. Therefore several studies have been made with different cellulose sources such as bamboo [6], curaua fibers [9], sugarcane bagasse [7] and so on. Considering various cellulose sources is also vital to obtain sustainable and economically feasible industrial productions. This is one of the main problems that has to be evaluated to be able to implement CNC more today.

Therefore a different cellulose source was evaluated in this study instead of using industrially made microcrystalline cellulose (MCC) which is too expensive when producing CNC in larger scales.

The cellulose was produced as a by-product when extracting hemicellulose from barley husk in another bachelor project performed at the same department [10]. Barley husk is a material that is considered a waste product and is often discarded. Instead of throwing away this material, other applications are examined such as extracting hemicellulose for use in biocomposites. The cellulose that is extracted as a by-product is not further used and is usually discarded. By examining its crystallinity and the possibility of producing nanocrystals a more sustainable process is created when recycling within the department. The cellulose was prepared following a method studied by Höije et al. with some adjustments [11].

## **3 EXPERIMENTAL**

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### **3.1 MATERIALS**

#### **3.1.1 Chemicals**

Sulphuric acid from Sigma Aldrich

Hydrochloric acid from Honeywell

#### **3.1.2 Other material**

Microcrystalline cellulose, Avicel PH-101 from FMC Biopolymer

Dialysis membrane, Standard RC Tubing from Spectrum Labs (part no. 132684)

#### **3.1.3 Apparatus**

Centrifuge: Megafuge 40 centrifuge.

Sonicator: Sonics vibra cell from Chemical instruments AB.

Conductometric Analyser: EcoScan from Eutech instruments.

FT-IR: Spectrum One from PerkinElmer instruments.

XRD: Diffractometer D5000 from Siemens.

AFM: NanoScope III from Digital Instruments Santa Barbara, California.

### **3.2 METHODS**

#### **3.2.1 Dialysis**

Dialysis is used to wash away excess ions. It was performed using a dialysis membrane that was prepared by being put in deionised water for 15 minutes. Thereafter one end was tied together and the suspension was added to the membrane through a funnel and the other end was tied together. The membrane was put in a container with deionised water.

#### **3.2.2 Preparation of CNC through sulphuric acid hydrolysis**

The starting material MCC was hydrolysed with 64% w/w sulphuric acid. 40 g of MCC was added to 250 ml of sulphuric acid and the hydrolysis was carried out at 45°C for 2 hours under constant mechanical stirring. The suspension was then diluted into 7 litres of deionised water and centrifuged

at 4300 rpm for 15 minutes. The supernatant was removed and the solid phase was diluted with 2 litres deionised water and put on dialysis until the conductivity measured below 5  $\mu$ S. After cancelling the dialysis, the suspension was sonicated at an amplitude of 40% for 6 x 7 minutes.

### **3.2.3 Preparation of CNC through hydrochloric acid hydrolysis**

Preparation of CNC through hydrochloric acid hydrolysis was carried out in four different procedures following studies in literature with some adjustments. Procedure 1 was prepared by following a study on curaua fibers [9], procedure 2 followed a dissertation on nanocomposites based on nanocellulose whiskers [12] and procedure 3 followed a study on facile extraction of thermally stable CNC [13]. Procedure 4 was established and determined after analysing the results from procedure 1, 2 and 3. The starting material in all procedures was microcrystalline cellulose.

#### **Procedure 1**

MCC was hydrolysed with 4 M hydrochloric acid. 5 g of MCC was added to 100 ml hydrochloric acid and the hydrolysis was carried out at 45°C for 90 minutes under constant stirring. The suspension was then diluted into 500 ml deionised water and centrifuged at 4300 rpm for 15 minutes. The supernatant was removed and the solid phase was diluted into 600 ml deionised water and the suspension was vigorously stirred for 1 hour. Thereafter the suspension was put on dialysis until the conductivity measured below 5  $\mu$ S. After cancelling the dialysis, the suspension was centrifuged at 4300 rpm for 15 minutes and the solid phase was collected, filtered using Microfunnel Plus filter unit 0.45  $\mu$ m and dried.

#### **Procedure 2**

5 g of MCC was hydrolysed with 361 ml of 4 M hydrochloric acid in a water bath shaker at 80°C for 8 hours. The suspension was diluted into 1200 ml deionised water to terminate the reaction. The cellulose settled overnight and was then centrifuged until the pH of the supernatant was around 3. Thereafter the suspension was put on dialysis until the conductivity measured below 5  $\mu$ S. After the dialysis, the suspension was sonicated at an amplitude of 40% for 2 minutes and then centrifuged at 3000 rpm for 5 minutes. The turbid supernatant was decanted and the solid phase was diluted into 400 ml deionised water, sonicated and re-centrifuged. This procedure was supposed to be repeated until the supernatant became clear. After the procedure was repeated several times and no clear

supernatant could be observed, the solid phase was collected and the turbid supernatant was filtered. Both the solid phase and the filtered supernatant was dried.

### **Procedure 3**

MCC was hydrolysed with 6 M hydrochloric acid. 5 g of MCC was added to 200 ml of hydrochloric acid and sonicated at an amplitude of 40% for 3 x 2 minutes. The hydrolysis was carried out at 100°C for 2 hours under constant mechanical stirring. The suspension was diluted into 1000 ml deionised water and centrifuged at 4300 rpm for 15 minutes. The solid phase was diluted into 300 ml deionised water and put on dialysis until the conductivity measured below 5  $\mu$ S. After the dialysis, the suspension was centrifuged at 4300 rpm for 15 minutes, filtered and dried.

### **Procedure 4**

5 g of MCC was added to 200 ml of 6 M hydrochloric acid and hydrolysed in a water bath shaker at 80°C for 3 hours. The suspension was diluted into 2 litres deionised water and left overnight. The suspension was then centrifuged at 4300 rpm for 15 minutes where the solid phase was diluted into 100 ml deionised water and transferred to a dialysis membrane. When the conductivity measured below 5  $\mu$ S the dialysis was terminated and the suspension was sonicated at an amplitude of 40% for 2 x 2 minutes.

#### **3.2.4 Preparation of CNC using cellulose extracted from barley husk**

The nanocrystals from cellulose extracted from barley husk was prepared through sulphuric acid hydrolysis using 64% w/w sulphuric acid. 10 g of cellulose was added to 87,5 ml acid and hydrolysed at 45°C for 2 hours under constant mechanical stirring. The reaction was terminated by diluting the solution in 1750 ml deionised water and then centrifuged at 4300 rpm for 15 minutes. After decanting, the solid phase was diluted into 500 ml deionised water and transferred to a dialysis membrane. The dialysis ended when the conductivity measured below 5  $\mu$ S and then sonicated at an amplitude of 40% for 5 x 7 minutes.

## 4 ANALYSIS AND CHARACTERISATION

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### Fourier transform infrared spectroscopy (FT-IR)

FT-IR was performed to determine functional groups and examine different peaks that can indicate cellulose crystallinity. 2 mg of each sample was mixed with 300 mg of potassium bromide, finely ground and pressed under vacuum to form pellets. The FT-IR spectrum was then recorded in the range of 4000-400  $\text{cm}^{-1}$  and were set to 16 scans per sample. Crystallinity index was calculated by taking the ratio between band intensity at around 1425  $\text{cm}^{-1}$  and band intensity at around 900  $\text{cm}^{-1}$  [14]. This is due to cellulose being sensitive to crystal structure at absorbance values around the 850-1500  $\text{cm}^{-1}$  region. With higher crystallinity index values, the crystallinity and ordered structure in a sample increases [7, 15].

### Powder X-ray Diffraction

XRD were used to analyse crystallinity and calculate a crystallinity index. Each sample was grinded into a fine powder and placed on a sample holder to obtain total X-ray exposure. The X-ray diffractometer operated at 45 kV and 40 mA. The patterns were obtained over the angular range  $2\theta = 10-60^\circ$  and the crystallinity was calculated using the following equation, calculations can be found in appendix B, [14, 16]:

$$CrI(\%) = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100 \quad (1)$$

Where  $I_{002}$  is the maximum intensity of the crystalline peak at a diffraction angle around  $2\theta = 22^\circ$  and  $I_{amorphous}$  is the minimum intensity scattered by the amorphous part at a diffraction angle around  $2\theta = 18^\circ$ . The intensities can be seen highlighted in figure 3.

### Atomic Force Microscopy

AFM was performed to examine the dimensions of the nanocrystals by viewing them individually. Each sample was diluted to 0.005% with deionised water. Thereafter the samples were put in ultrasonification for 10 minutes. One drop from each sample was placed on a mica plate and dried for about 30 minutes. The AFM analysis was performed by Anders Mårtensson, Polymer Technology, Chalmers.

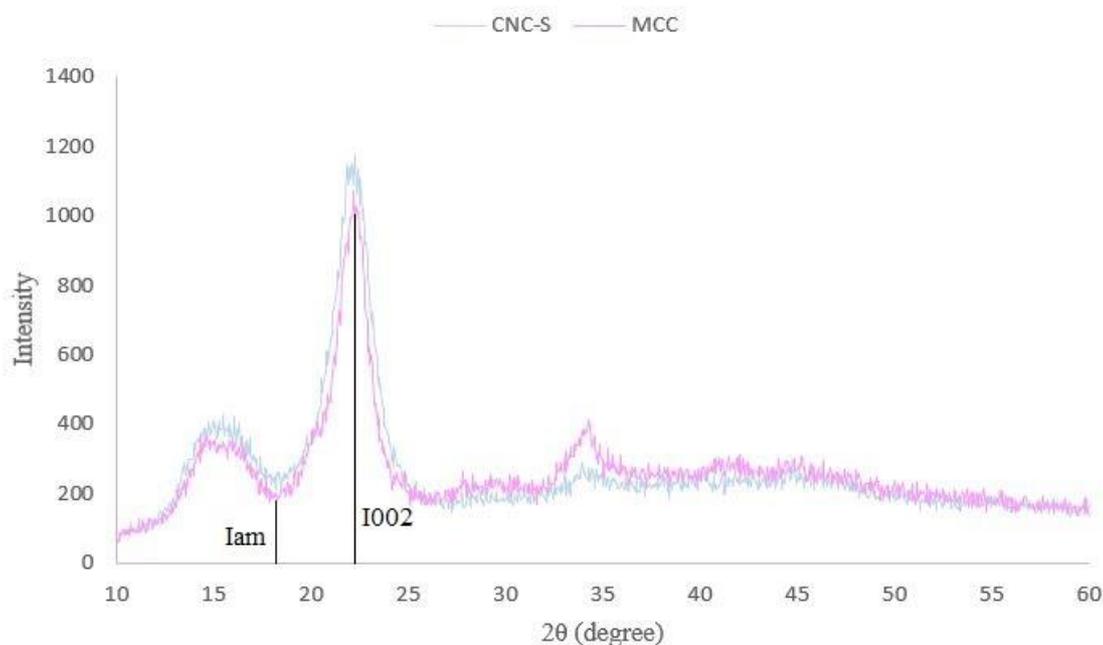
## 5 RESULTS AND DISCUSSION

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### 5.1 PREPARATION AND CHARACTERISATION OF CNC THROUGH SULPHURIC ACID HYDROLYSIS

The hydrolysis was carried out at 45°C for 2 hours and after the hydrolysis was completed, the solution had a discoloured appearance. After being put on dialysis the suspension was white and had cloudlike patterns that implied there was small particles in the suspension. These observations indicated that the hydrolysis had succeeded. After the suspension had been sonicated, 50 ml were freeze-dried.

A few milligrams of the freeze dried sample was analysed with XRD. The result can be seen in figure 3, where CNC-S stands for CNC from sulphuric acid hydrolysis, together with the XRD result of the starting material MCC.

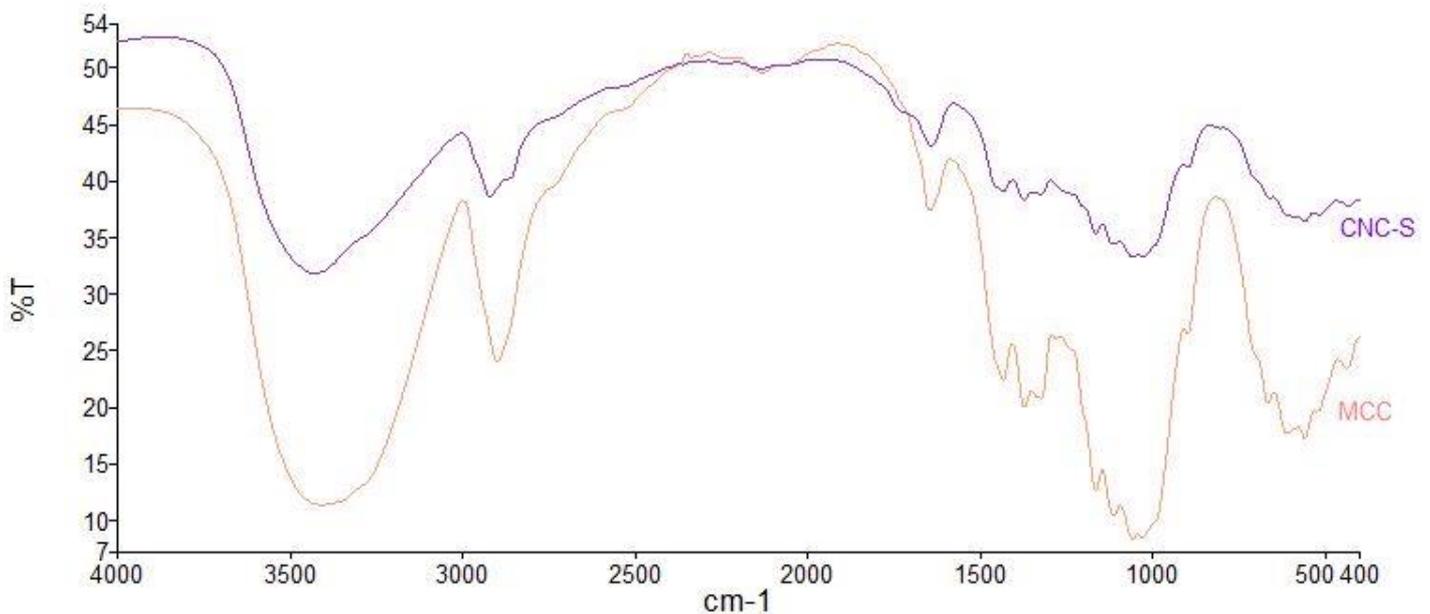


*Figure 3. XRD pattern of CNC from sulphuric acid hydrolysis (CNC-S) and starting material microcrystalline cellulose (MCC) with the peaks  $I_{002}$  and  $I_{amorphous}$  highlighted.*

The XRD pattern showed that the CNC had the typical pattern of crystalline cellulose with a high peak at around  $2\theta = 22^\circ$  [8, 14]. It can also be observed that the peaks of CNC is broader than the peaks of MCC, which implicates that the nanocrystals are more crystalline than the microcrystals.

When looking at the pattern for CNC there is a barely noticeable peak at around  $2\theta = 34^\circ$  compared to a clearly visible peak for MCC. This is due to sulphuric acid being a strong acid that can damage the crystalline domains during the acid hydrolysis [13]. The crystallinity was calculated using equation 1 that can be found in chapter 4. The crystallinity of CNC was calculated to be 82,2% while MCC was calculated to be 83,7%. This can be a result of difficulties in grinding the freeze-dried sample into a fine powder or that CNC loses some of its properties when fully dried.

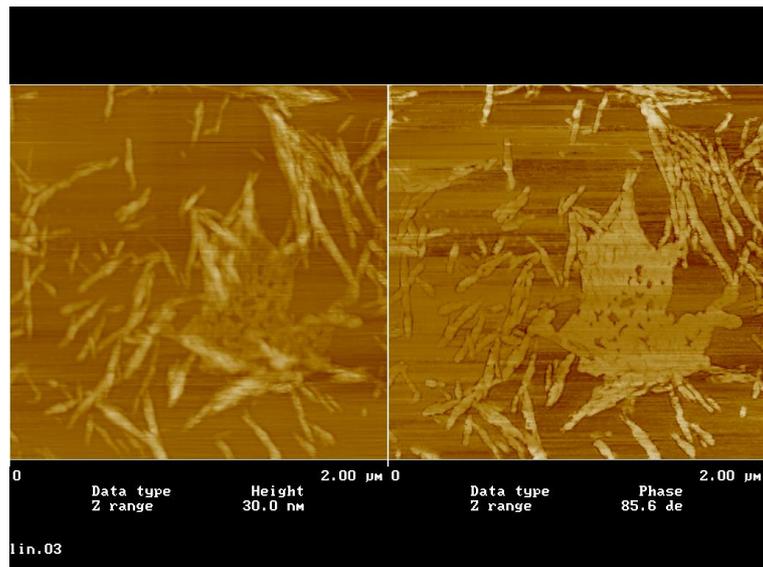
The freeze dried sample was also analysed with FT-IR which can be seen in figure 4. It can be observed that the peak at around  $3400\text{ cm}^{-1}$  is further down for MCC than for CNC, which indicated that there were less amorphous regions for CNC. This can be due to the hydrogen bonding being less flexible when the material is more crystalline [14].



**Figure 4.** FT-IR spectra of CNC from sulphuric acid hydrolysis (CNC-S) and starting material microcrystalline cellulose (MCC).

The peak on CNC at around  $3400\text{ cm}^{-1}$  is also slightly sharper than for MCC which is an additional indication of nanocrystals [17]. The crystallinity index for CNC was calculated to be 0,85 and the index for MCC was calculated to be 0,60 which also implied that the CNC was more crystalline. How this was calculated can be found in chapter 4.

The suspension was analysed with AFM which showed the dimensions of the nanocrystals. As can be seen in figure 5, the CNC had the characteristic rod-like shape and showed slight agglomeration which can depend on the suspension being stored for about a month. This showed that nanocrystalline cellulose in fact had been obtained.



*Figure 5. AFM picture of a CNC suspension from sulphuric acid hydrolysis.*

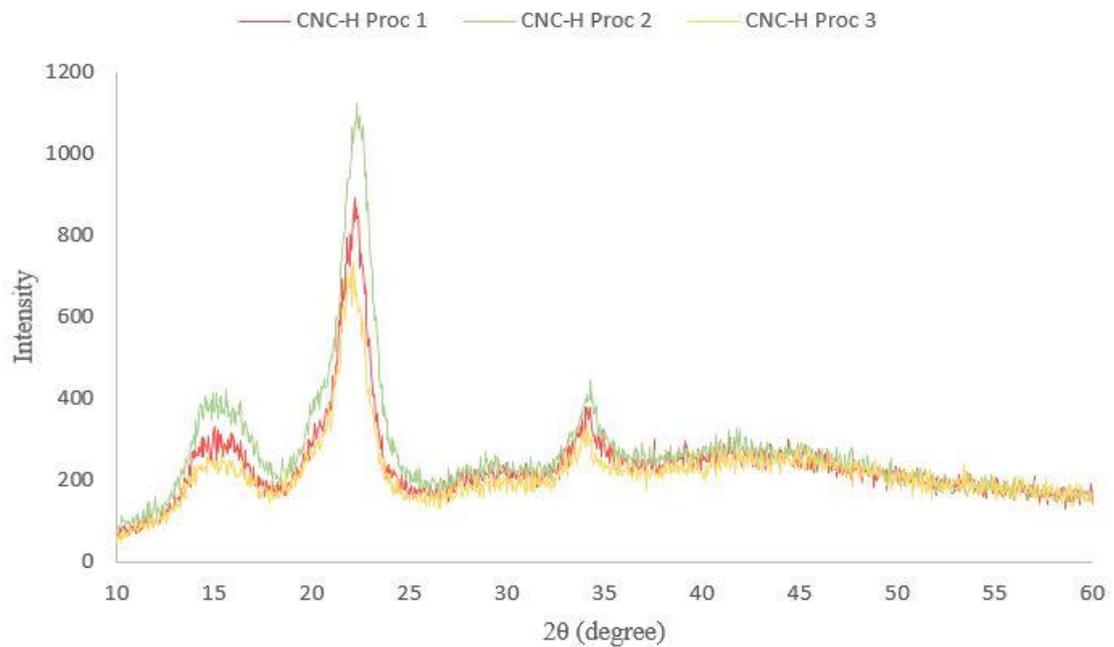
## 5.2 PREPARATION AND CHARACTERISATION OF CNC THROUGH HYDROCHLORIC ACID HYDROLYSIS

The preparation of CNC through hydrochloric acid hydrolysis was carried out in four different procedures with different parameters which can be seen in table 1. The first three procedures was following studies in literature while procedure 4 was performed after evaluating the results from the first three procedures. Procedure 2 had a dark brown colour after the hydrolysis which indicated that the reaction had gone too far and the acid probably attacked the nanocrystals.

*Table 1. Four different preparation methods and parameters of CNC through hydrochloric acid hydrolysis.*

Procedure	Temperature (°C)	Time of hydrolysis (min)	Concentration of acid (M)	Acid/pulp ratio (ml/g)	Appearance
1	45	90	4	20	White
2	80	480	4	72,2	Slightly yellow
3	100	120	6	40	Dark brown
4	80	180	6	40	Discoloured

The first three procedures were analysed with XRD, see figure 6 below, where CNC-H stands for CNC from hydrochloric acid. All three procedures followed the same pattern which match the typical cellulose crystallinity pattern. When evaluating the result it can be observed that the graph of procedure 2 had slightly broader peaks than the two other procedures. This implied that procedure 2 had more crystalline content and was the most likely to have succeeded.



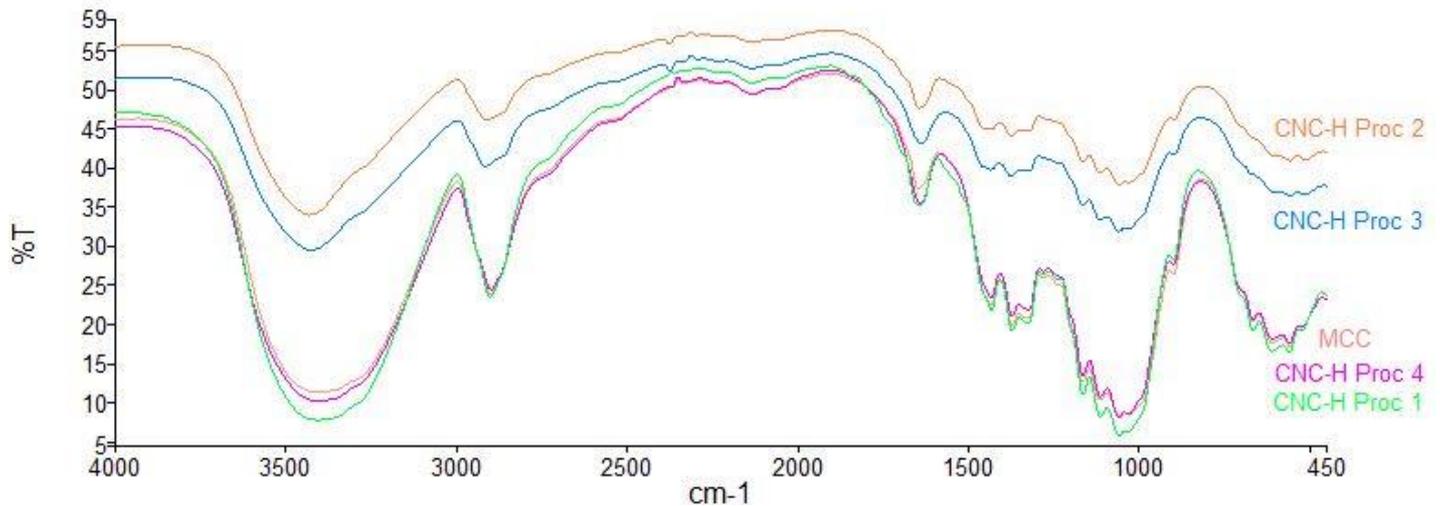
**Figure 6.** XRD pattern of CNC from hydrochloric acid hydrolysis in three different procedures.

The crystallinity index was calculated using equation 1 and the result can be seen in table 2 where it is compared to the crystallinity calculated for MCC. The calculation showed that neither of the procedures had succeeded to obtain CNC through hydrochloric acid and that MCC was more crystalline than any of them.

**Table 2.** Crystallinity index of MCC and four different procedures of CNC from hydrochloric acid hydrolysis.

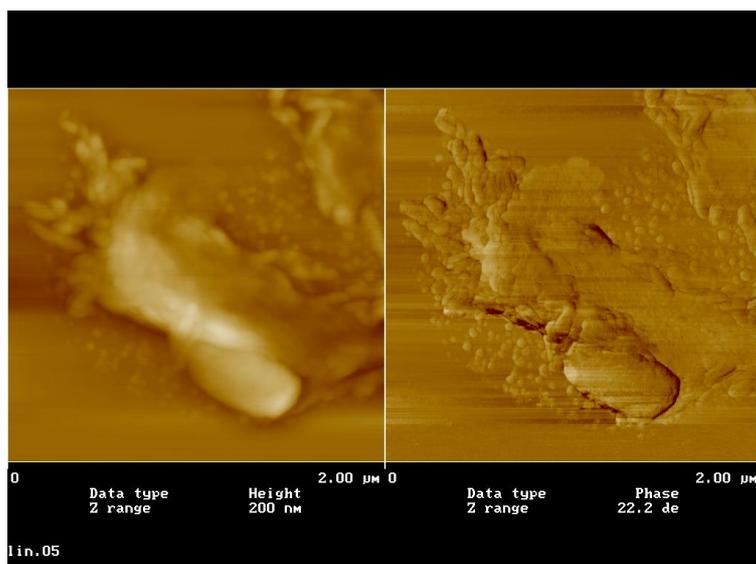
Sample	CrI(%) from XRD	Crystallinity index from FT-IR
MCC	83,7	0,60
Procedure 1	82,1	0,53
Procedure 2	83	0,85
Procedure 3	80,6	0,82
Procedure 4	85,7	0,80

The procedures were also analysed using FT-IR and the results can be seen in figure 7. The FT-IR analysis showed that procedure 2 and 3 indicated to be more crystalline than MCC by observing the shape of the graphs. This is due to, as previously mentioned, a sharper peak at around  $3400\text{ cm}^{-1}$  that is not as far down as for MCC. Procedure 1 followed almost the exact same pattern as MCC and it is therefore implied that this procedure had not been successful.

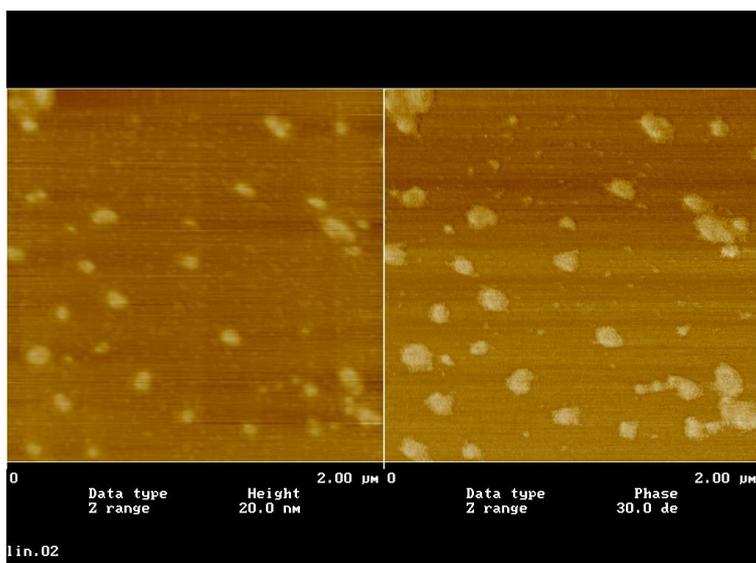


**Figure 7.** FT-IR spectra of MCC and CNC from hydrochloric acid hydrolysis in four different procedures.

The crystallinity index for the different procedures was calculated and is shown in table 2. By evaluating the various index results, procedure 2 indicated to have the most crystalline structure. After analysing with XRD and FT-IR it was determined that procedure 2 gave the best result and was slightly more crystalline than the other samples from procedure 1 and 3. Therefore a larger batch was produced following procedure 2 and was analysed with AFM together with samples from procedure 1 and 3. The AFM picture of procedure 1 showed barely any result at all and can be seen in appendix A. By observing the AFM pictures of procedure 2 and 3, as can be seen in figures 8 and 9, the following indications can be found: procedure 2 seemed to have agglomerated and not been hydrolysed sufficiently while procedure 3 showed small particles that implicated that the hydrolysis had gone too far.

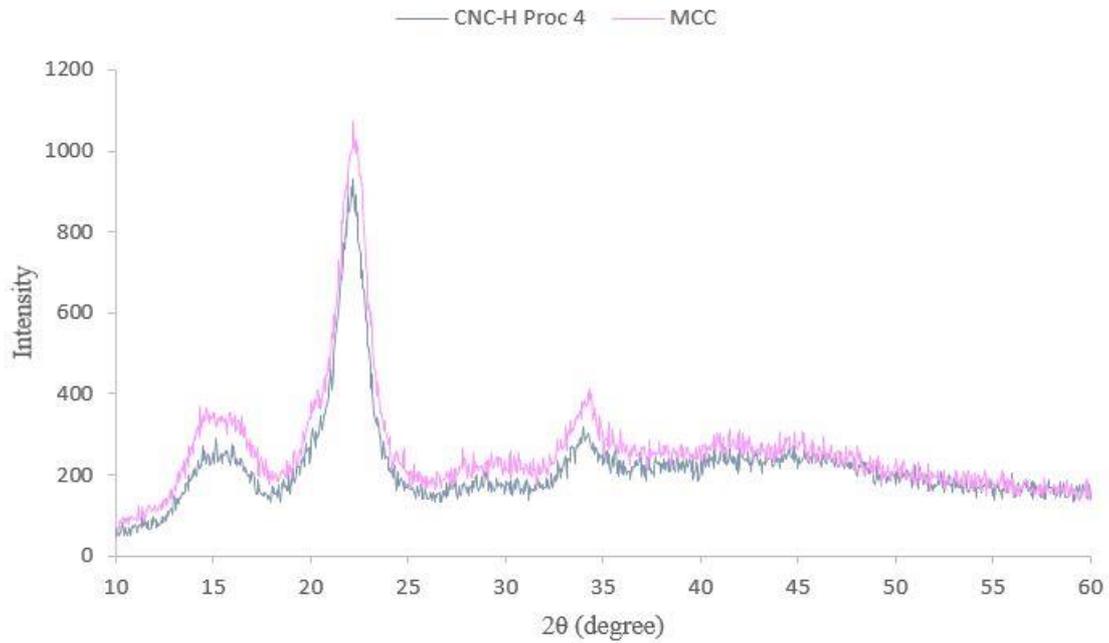


**Figure 8.** AFM picture of a CNC suspension from hydrochloric acid hydrolysed MCC following procedure 2.



**Figure 9.** AFM picture of a CNC suspension from hydrochloric acid hydrolysed MCC following procedure 3.

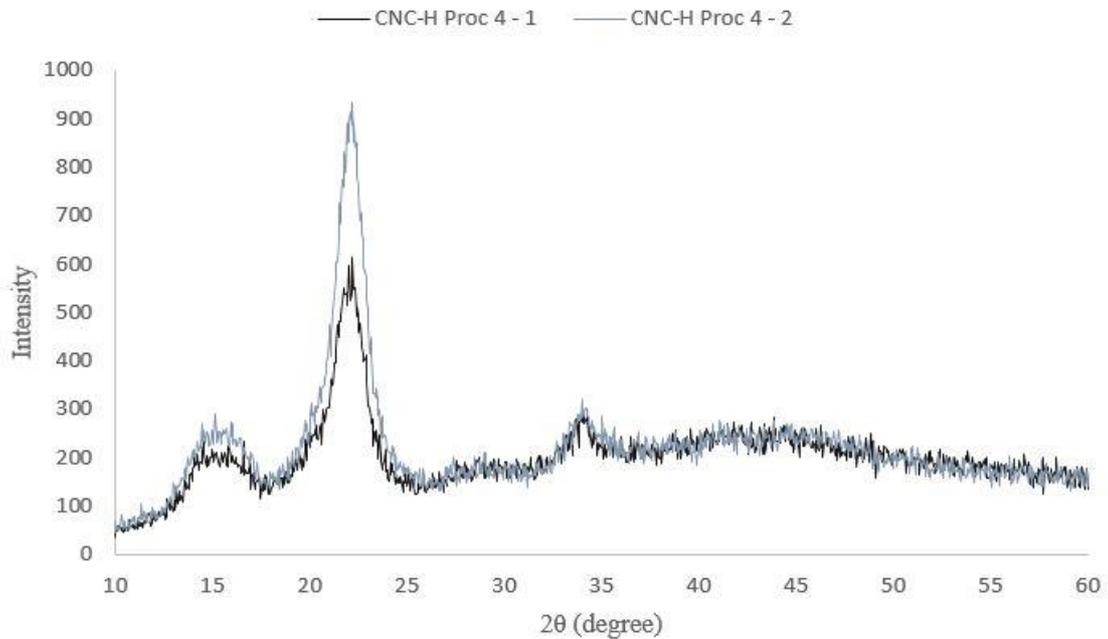
By evaluating the results of the first three procedures, a conclusion could be drawn that procedure 4 should have hydrolysis parameters in the area between procedure 2 and 3, see table 1. This is due to procedure 2 showing tendency to have not been fully hydrolysed and procedure 3 showing indications to have been hydrolysed too much. Procedure 4 was analysed with FT-IR, see in figure 7, which showed a pattern similar to MCC although the crystallinity index was calculated to be higher than for MCC. The procedure was also analysed with XRD and the result can be seen in figure 10.



**Figure 10.** XRD pattern of MCC and CNC from hydrochloric acid hydrolysis following procedure 4.

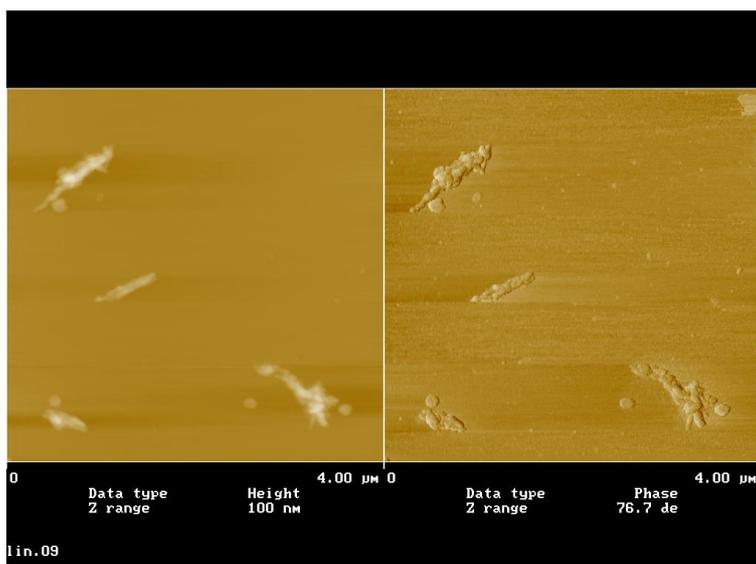
The XRD pattern for MCC was slightly broader than for procedure 4 which implies that MCC is more crystalline. Nevertheless, as for the FT-IR result, the calculated crystallinity was higher for procedure 4 compared to MCC, see table 2. An interesting observation was made when analysing the sample with XRD. It was found that sonicating had an impact on the XRD pattern of the sample. Two patterns can be seen in figure 11, CNC-H Proc 4 – 1 is before the suspension was sonicated and CNC-H Proc 4 – 2 is after the suspension was sonicated.

The peak of the sonicated suspension is not only clearly broader but also had the highest calculated crystallinity, 85,7% while it was calculated to be 81,1% before sonicating.

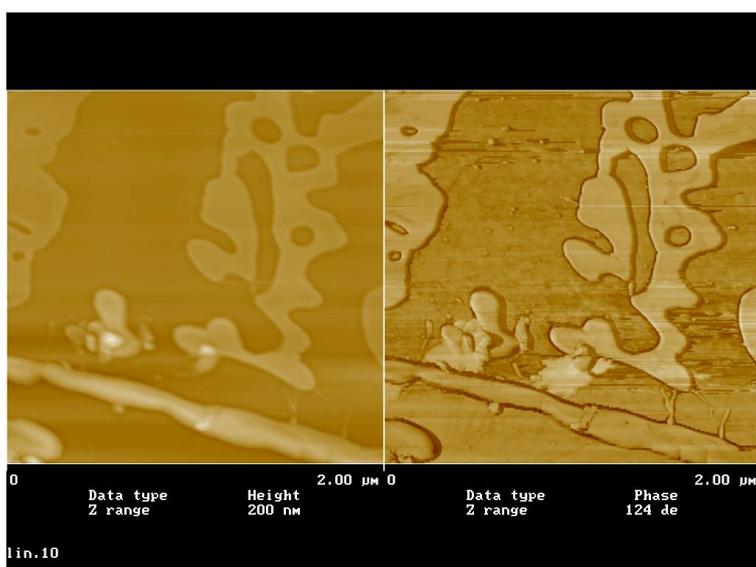


**Figure 11.** XRD pattern of CNC from hydrochloric acid hydrolysis following procedure 4, before and after sonication.

This can be assumed to depend on agglomeration that holds the crystals together and therefore they are not detected when analysing with XRD. Sonicating divides the agglomeration and makes the suspension more homogeneous and therefore it might be easier for the XRD to detect crystallinity. Finally, AFM analysis were performed to obtain a picture of the material. Two pictures were taken and they can be seen in figures 12 and 13.



**Figure 12.** AFM picture of a CNC suspension from hydrochloric acid hydrolysis following procedure 4.



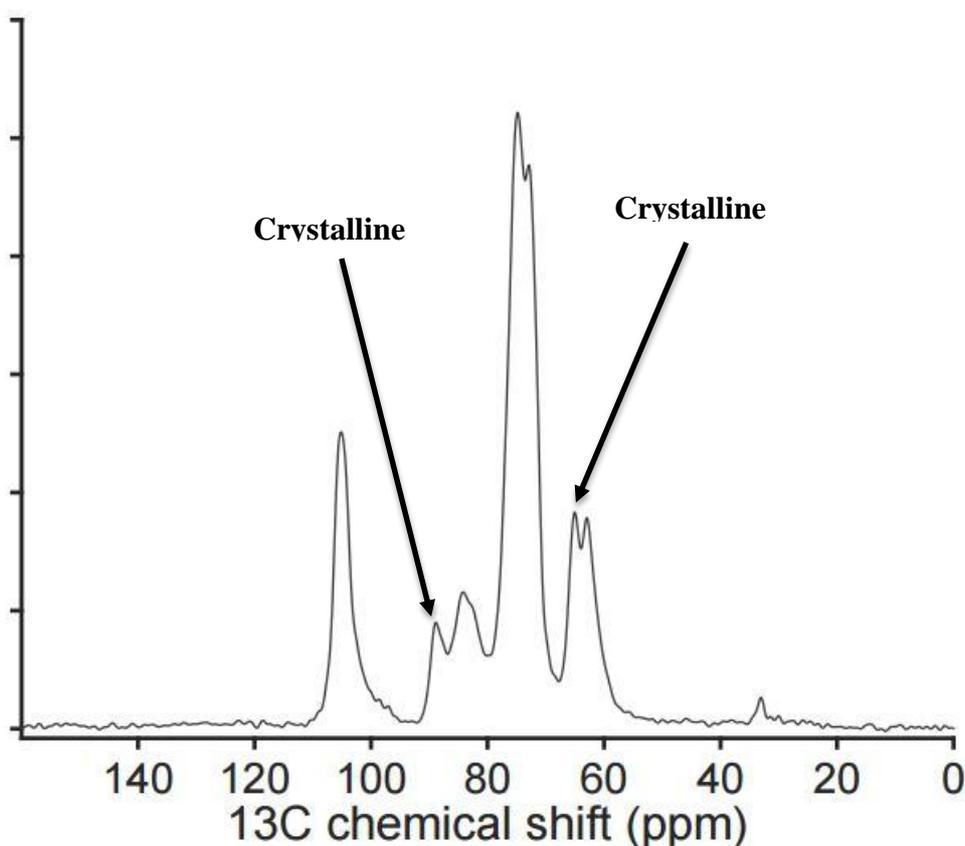
**Figure 13.** AFM picture of a CNC suspension from hydrochloric acid hydrolysis following procedure 4.

Both pictures showed a lot of agglomerated areas that did not have any specific crystals. This might have indicated that the suspension should have been sonicated longer to obtain a more homogeneous and dispersed solution. Figure 13 had some tendency of nanocrystals in the lower right corner which implied that there might have been some nanocrystalline cellulose that had agglomerated.

## 5.3 PREPARATION AND CHARACTERISATION OF CNC FROM BARLEY HUSK

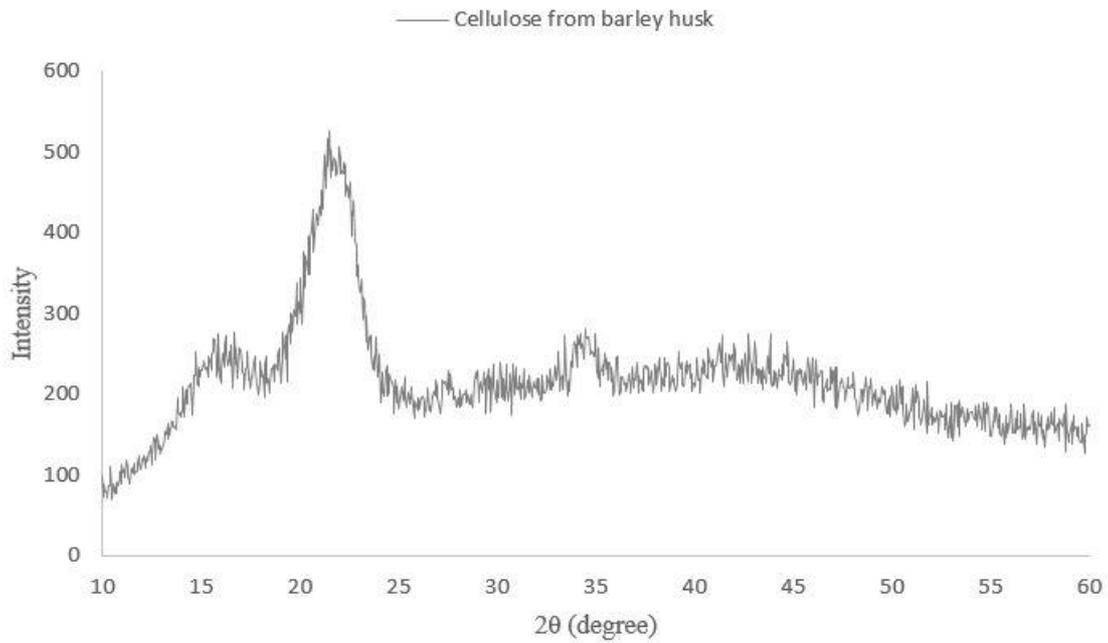
### 5.3.1 Cellulose crystallinity

Solid state NMR was performed on the cellulose extracted from barley husk and showed that the cellulose was crystalline as can be seen in figure 14. This is due to the typical crystalline cellulose peaks at around 65 ppm and 90 ppm that are not overlapping as they would be if the sample was amorphous [18]. The crystalline peaks are highlighted in figure 14 and if the cellulose would have been fully amorphous they would not have existed.



*Figure 14. Solid state NMR on cellulose extracted from barley husk with crystalline peaks highlighted.*

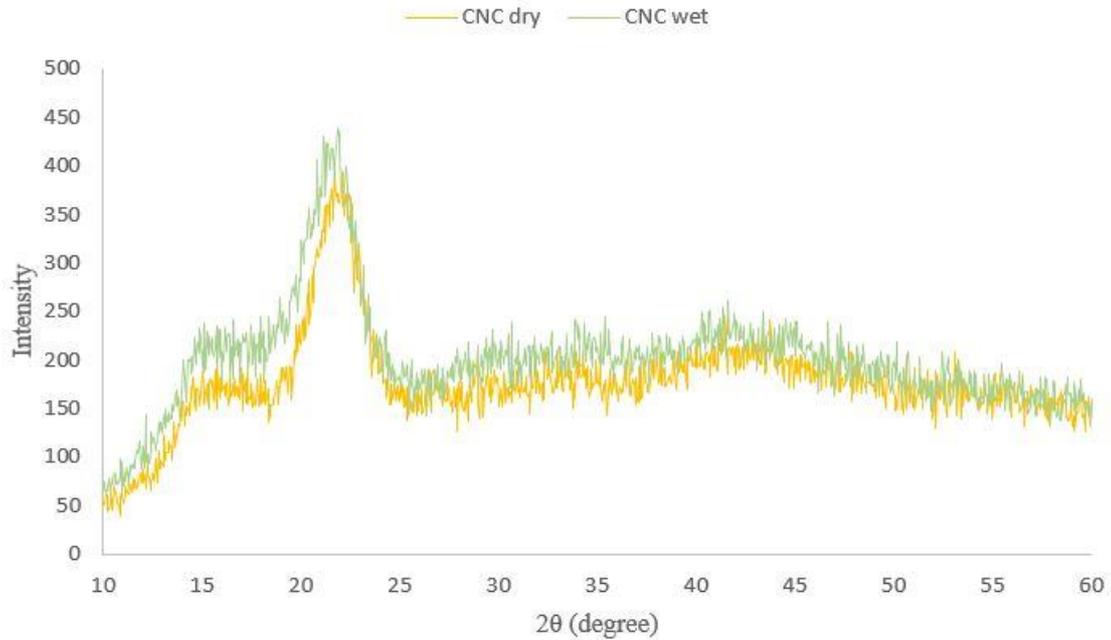
The crystallinity of the cellulose was also examined using XRD, see figure 15, and the result followed the typical pattern of crystalline cellulose with a high peak around  $2\theta = 22^\circ$ . The calculated crystallinity, using equation 1, was 62,9% and this was enough crystallinity to make an attempt to produce CNC.



*Figure 15. XRD pattern of cellulose extracted from barley husk.*

### **5.3.2 Characterisation of CNC**

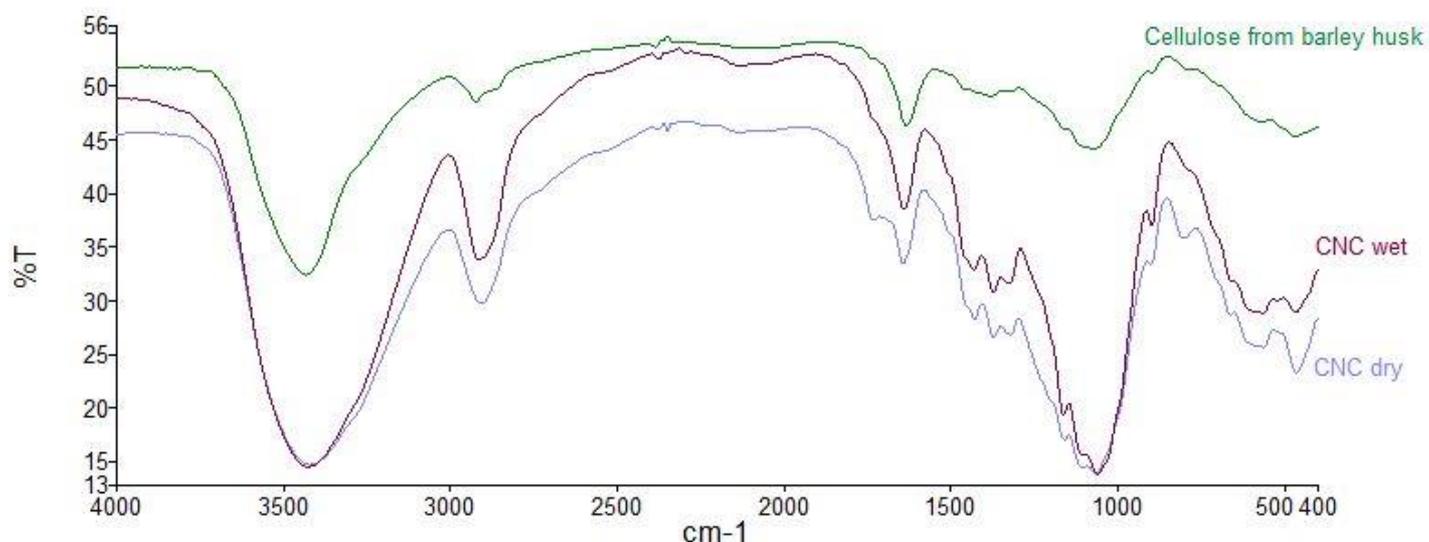
CNC from barley husk was prepared following the same procedure as nanocrystals prepared through the optimised sulphuric acid hydrolysis procedure. Two batches were made, one with small pieces of completely dried cellulose and one with wet cellulose that had been washed with ethanol before hydrolysis. Both batches were analysed with XRD and the result can be seen in figure 16.



**Figure 16.** XRD pattern of CNC from sulphuric acid hydrolysis prepared from dry and wet cellulose extracted from barley husk.

The XRD pattern for CNC from wet cellulose was slightly broader than CNC from dry cellulose which implied that CNC-wet was more crystalline. Although the calculated crystallinity can be seen in table 4 where CNC-dry was calculated to be the most crystalline and that indicated that this batch had succeeded in becoming more crystalline than the cellulose source. The XRD pattern can be affected by the samples not being a fine powder and that would explain why the pattern of CNC-wet is broader when it is not calculated to be more crystalline than CNC-dry.

The samples were also analysed with FT-IR, see figure 17. As can be seen both CNC samples followed the same pattern while the cellulose sample differed. The crystallinity index was calculated for all three samples and can be seen in table 4.



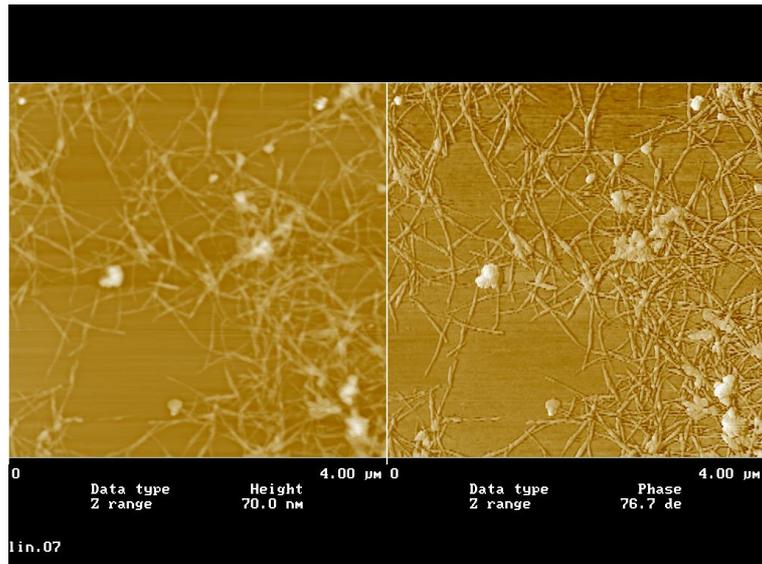
**Figure 17.** FT-IR spectra of cellulose extracted from barley husk and nanocrystalline cellulose from dry and wet cellulose.

The calculated index showed similar indications as the results obtained from XRD, which was that CNC prepared from dry cellulose had the most crystalline structure. When observing the different patterns in figure 17 it can be seen that the graph of CNC-dry does not seem to be more crystalline than the cellulose, although the calculated index indicates that it is. This can depend on difficulty when preparing the sample for FT-IR because the cellulose could not be grinded into a fine powder.

**Table 3.** Crystallinity index of cellulose extracted from barley husk and CNC prepared from wet and dry cellulose, calculated after analysis with XRD and FT-IR.

Sample	CrI(%) from XRD	Crystallinity index from FT-IR
Cellulose from barley husk	62,9	0,87
CNC from wet cellulose	59,4	1,12
CNC from dry cellulose	66,5	1,22

To determine the dimensions of the nanocrystals AFM analysis were executed. The AFM picture of CNC-wet showed only a layer of something unknown on the mica plate, it did not show any crystals or particles at all and the picture can be seen in appendix A. The AFM picture of CNC-dry, see figure 18, showed a lot of isolated crystals and some agglomeration.



*Figure 18. AFM picture of CNC prepared from dry cellulose extracted from barley husk.*

When observing the picture it can be found that some of the crystals are about 1  $\mu\text{m}$  long which is somewhat too long to be nanocrystals. This can depend on the cellulose not being hydrolysed enough so that there are still some amorphous regions left. Nevertheless, the AFM analysis showed a result which was more crystalline than the starting cellulose.

## 6 CONCLUSIONS

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Preparing CNC from sulphuric acid hydrolysis has been shown to be an optimised process where there is clear instructions available for obtaining a good result. The preparation of CNC went well and nanocrystals were obtained.

One objective of this study was to optimise the preparation method of CNC through hydrochloric acid hydrolysis. The results did not show that any CNC had been obtained through the different evaluated procedures, which can be seen in table 4. Nevertheless a platform has been created where the hydrolysis parameters have been encircled and further studies can carry on from this. The next step would be to continue evaluating and optimising procedure 4 due to its indication to be on the right course with some positive results. This study has shown that preparing CNC from hydrochloric acid hydrolysis is challenging and needs to be studied further in the future to obtain another optimised preparation method through acid hydrolysis.

*Table 4. Four different procedures hydrolysed with hydrochloric acid and the result of the hydrolysis.*

<b>Hydrochloric acid hydrolysis</b>	<b>Hydrolysed</b>
Procedure 1: 4 M, 45°C, 90 min	Not enough
Procedure 2: 4 M, 80°C, 480 min	Not enough
Procedure 3: 6 M, 100°C, 120 min	Too much
Procedure 4: 6 M, 80°C, 180 min	Not enough

This project showed that it is possible to obtain crystals, although longer than nanocrystals, from a cellulose source that otherwise would be disposed and it is therefore possible to obtain a more sustainable production of CNC. It is important to consider that the sulphuric acid hydrolysis process is efficient when using MCC as a starting material. Therefore further studies must be performed to obtain a certain process when using cellulose from barley husk. MCC is an industrially produced material which probably withstand more than cellulose from barley husk and it is therefore significant to evaluate different hydrolysis parameters so they accommodate the cellulose source. It can also be interesting to examine preparation of the barley husk cellulose to obtain a more purified cellulose. Even pre-treatment before hydrolysis can be studied to be able to obtain crystals in nanosize.

## 7 FUTURE PERSPECTIVES

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Containing nanocrystals through acid hydrolysis using sulphuric acid is a developed and efficient extraction method. This study has shown that extracting nanocrystals using hydrochloric acid is a method that needs to be developed and optimised further to be able to use it in a future perspective. After developing an optimised process the next step would be to start producing larger scales with various cellulose sources and still be able to obtain a good result.

Concerning the preparation methods it is important to further improve the process and make it more efficient. This needs to be accommodating to be able to lower the costs and enlarge the production. As of today it is highly expensive to produce nanocrystals and it is therefore difficult to establish the process. When using hydrolysis as a preparation method it is necessary to examine different acids, cellulose sources and hydrolysis parameters. This is to gain more knowledge about production outcome when it comes to uniform size, aspect ratio and surface chemistry. This will create a controlled preparation method for nanocrystalline cellulose and make the process standardised and by that more available and trustworthy [4].

One important aspect that has not been an objective in this project but could be an extension, is the drying of CNC. This is a challenge because CNC tend to agglomerate when dried due to hydrogen bonds that generates which can be irreversible. This can change size and characteristics of the CNC. It is essential to continue studies when it comes to drying process to be able to maintain the size of the CNC and reduce transportation cost that increase with the weight of the CNC [2, 4].

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At last I would like to thank my fellow thesis workers, Ali Amirjani, Lea Jerre, Robert Lindberg and Mona Svantesson for all the help and companionship, it has been a pleasure working with you.

## 9 REFERENCES

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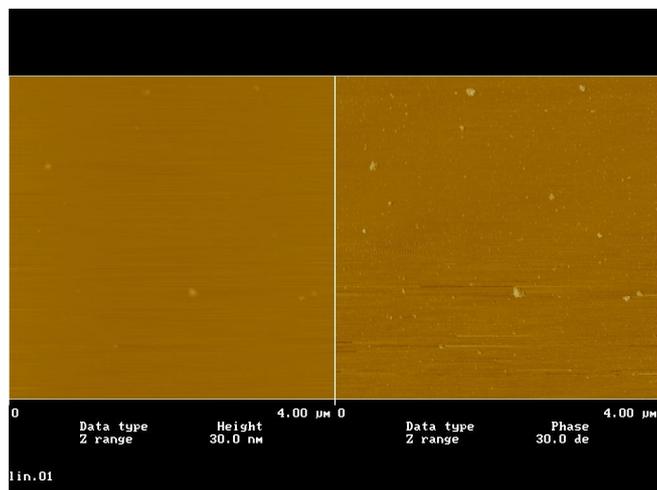
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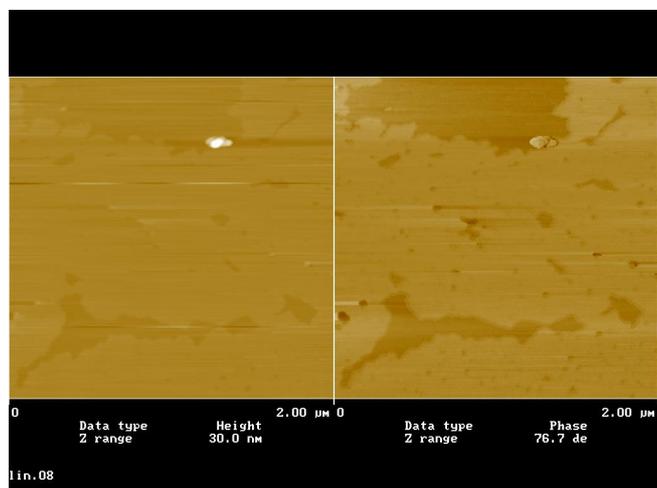
# APPENDIX A

## A. AFM PICTURES

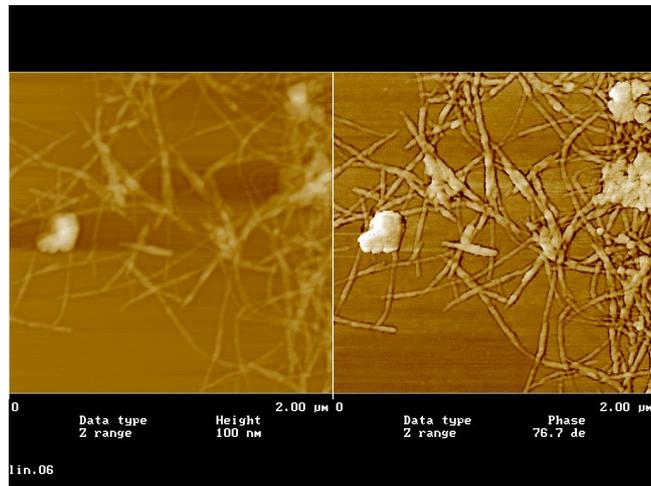
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*A 1. AFM picture of CNC prepared from hydrochloric acid hydrolysis following procedure 1.*



*A 2. AFM picture of CNC prepared from wet cellulose extracted from barley husk.*



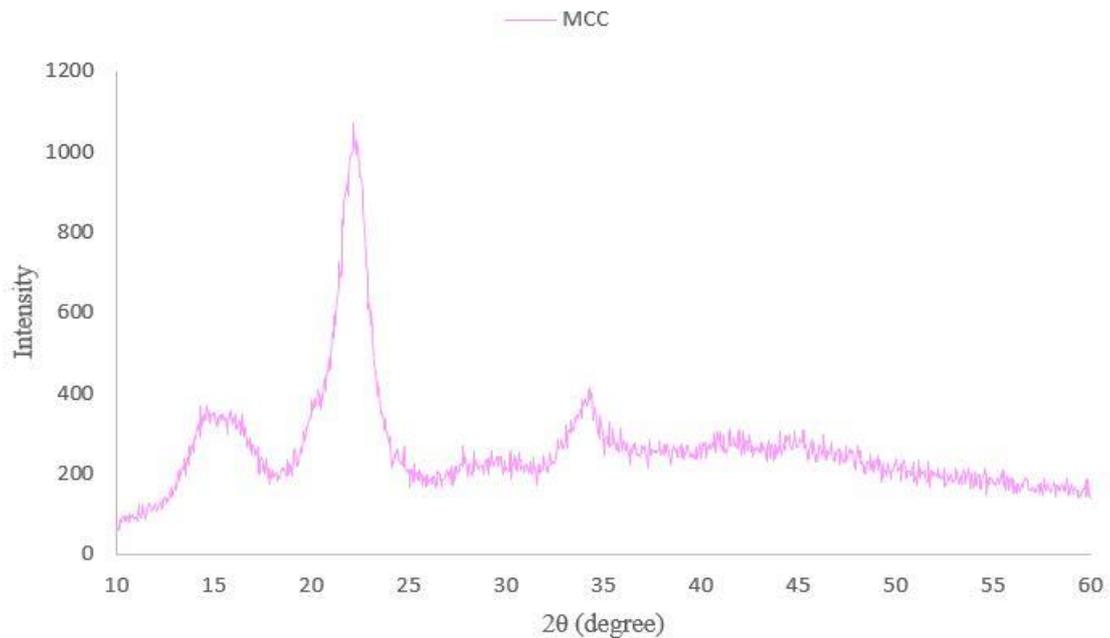
*A 3. AFM picture of CNC prepared from dry cellulose extracted from barley husk.*

# APPENDIX B

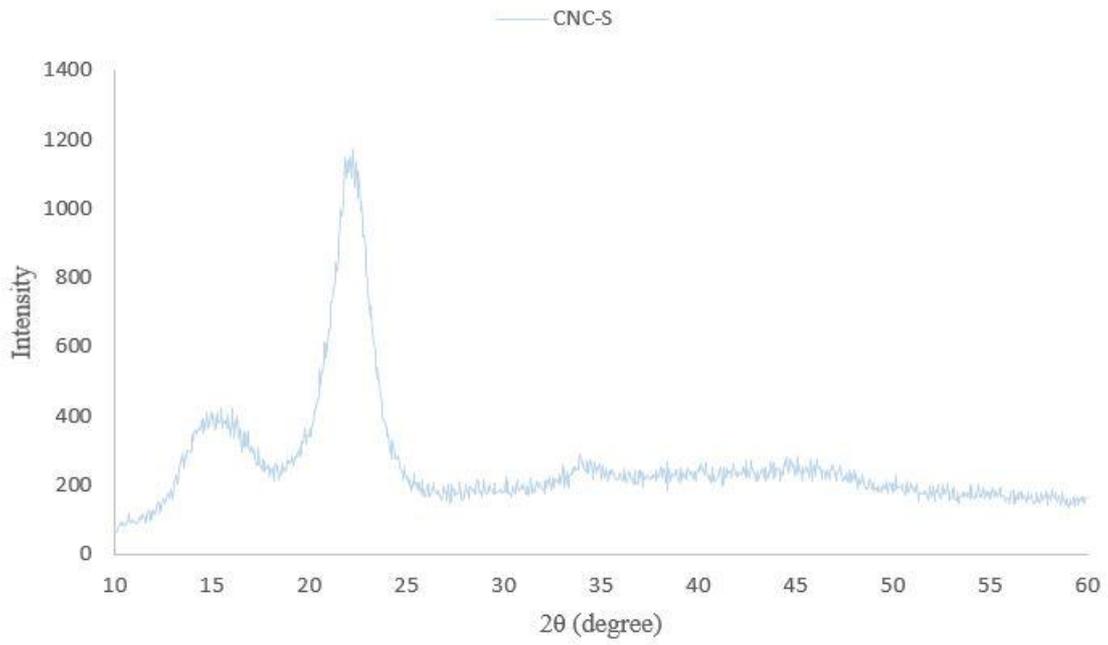
## B. X-RAY DIFFRACTION

*Table b 1. Calculated crystallinity of all performed procedures after XRD analysis.*

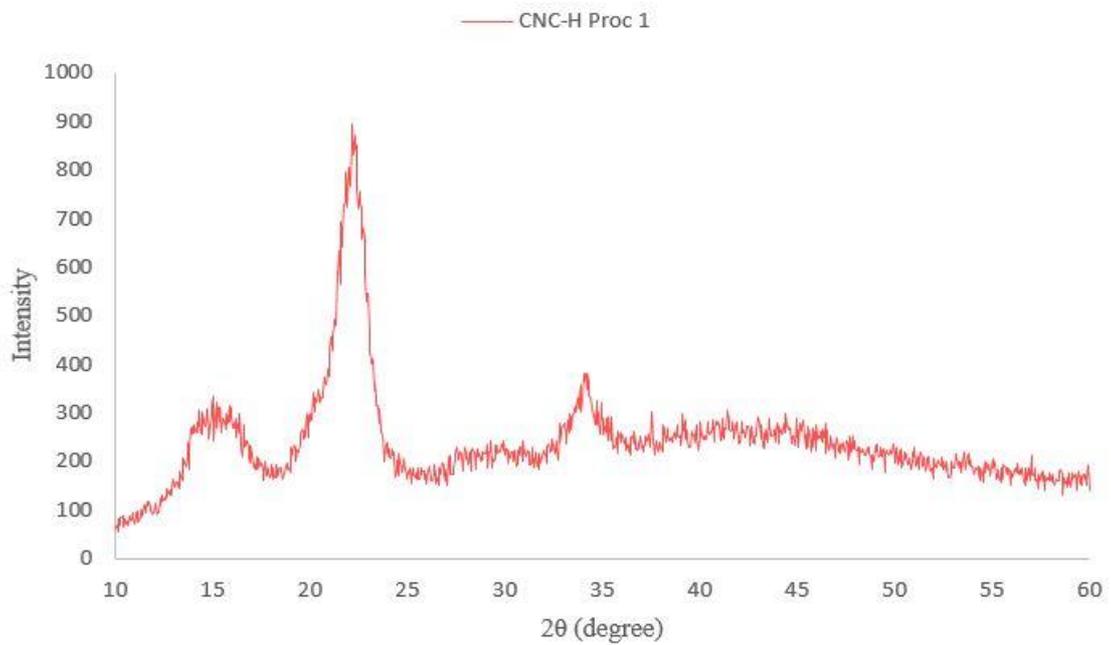
Sample	$(I_{002} - I_{amorphous})/I_{002}$	CrI(%)
MCC	$(1073 - 175)/1073$	83,7
CNC from H <sub>2</sub> SO <sub>4</sub>	$(1175 - 209)/1175$	82,2
CNC from HCl Procedure 1	$(893 - 160)/893$	82,1
CNC from HCl Procedure 2	$(1124 - 191)/1124$	83
CNC from HCl Procedure 3	$(738 - 143)/738$	80,6
CNC from HCl Procedure 4	$(933 - 133)/933$	85,7
Cellulose from barley husk	$(526 - 195)/526$	62,9
CNC from wet cellulose	$(439 - 178)/439$	59,4
CNC from dry cellulose	$(406 - 136)/406$	66,5



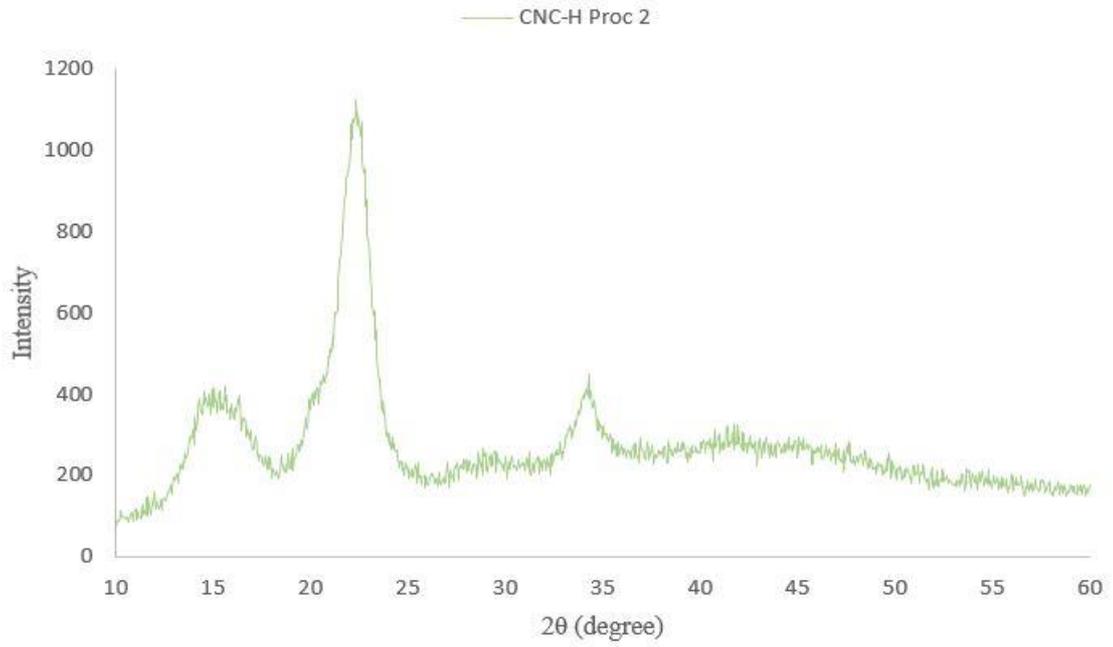
*B 1. XRD pattern of microcrystalline cellulose (MCC).*



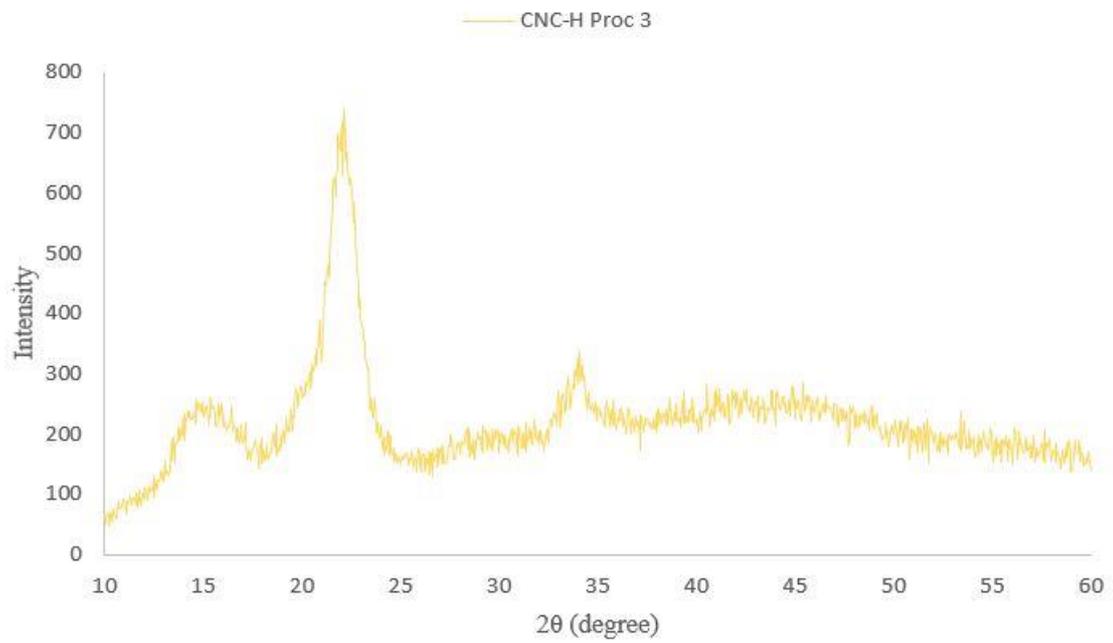
**B 2.** XRD pattern of CNC from sulphuric acid hydrolysis.



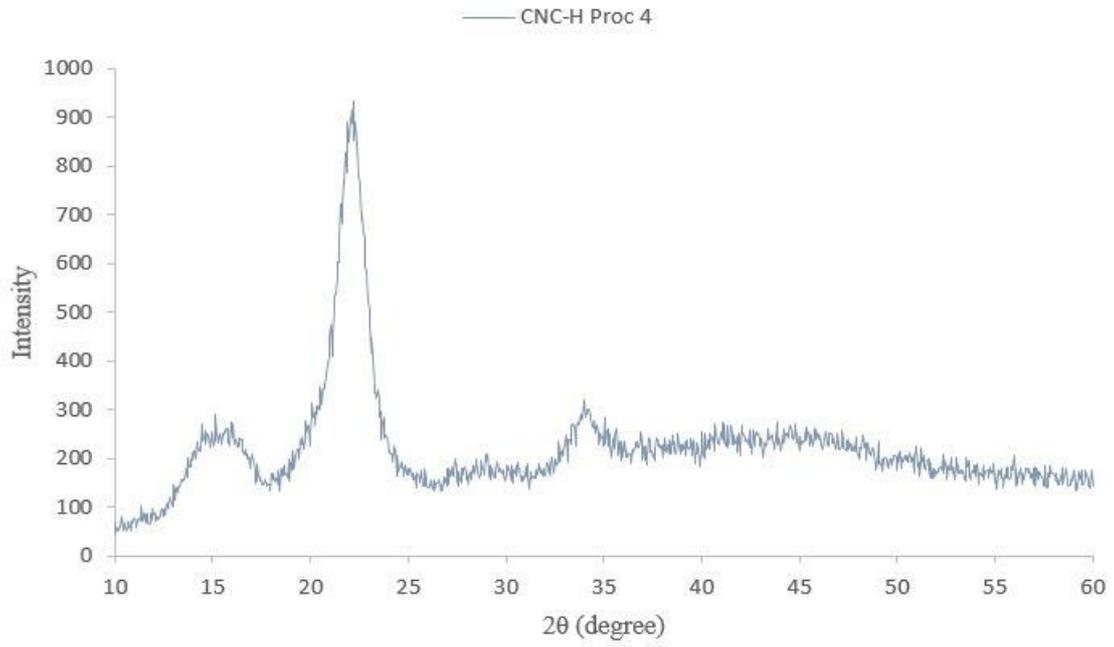
**B 3.** XRD pattern of CNC from hydrochloric acid following procedure 1.



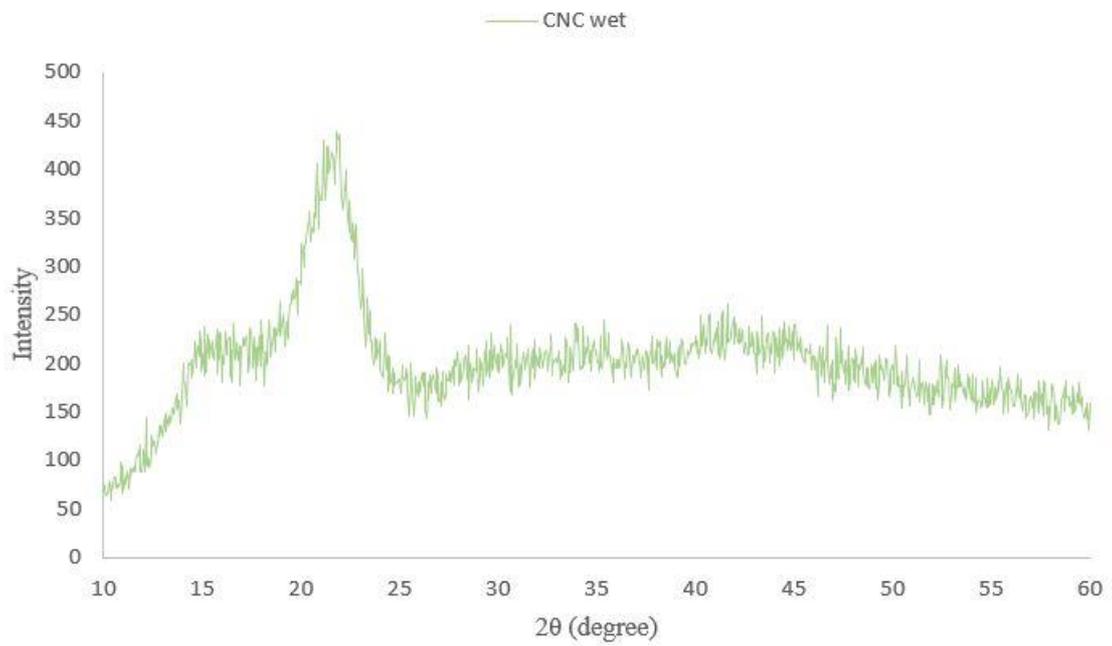
**B 4.** XRD pattern of CNC from hydrochloric acid following procedure 2.



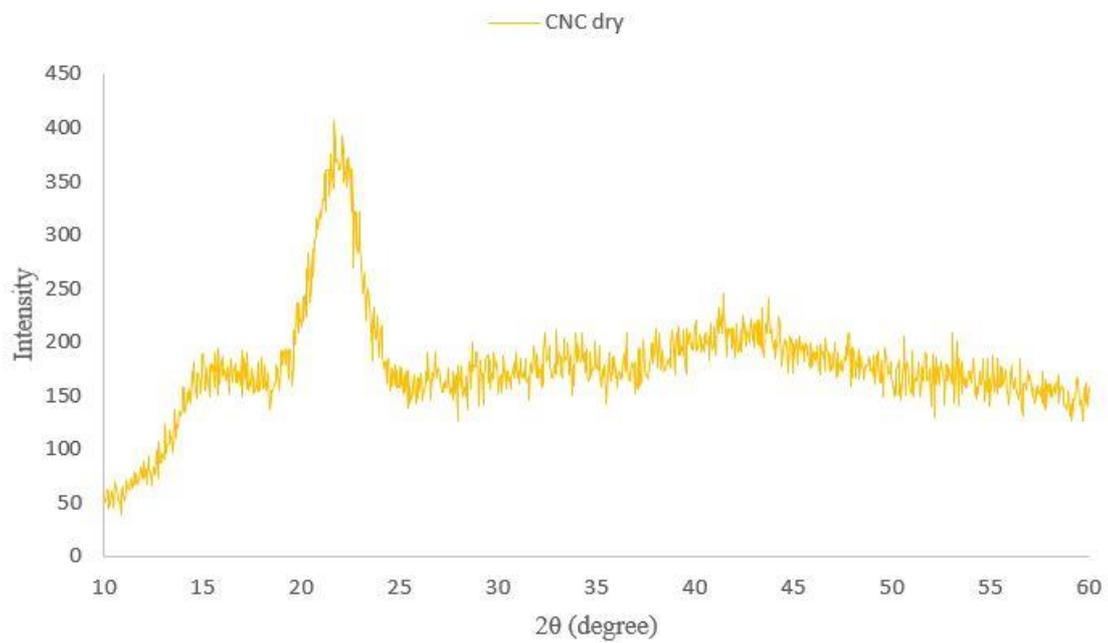
**B 5.** XRD pattern of CNC from hydrochloric acid following procedure 3.



**B 6.** XRD pattern of CNC from hydrochloric acid hydrolysis following procedure 4.



**B 7.** XRD pattern of CNC from sulphuric acid hydrolysis prepared from wet cellulose extracted from barley husk.

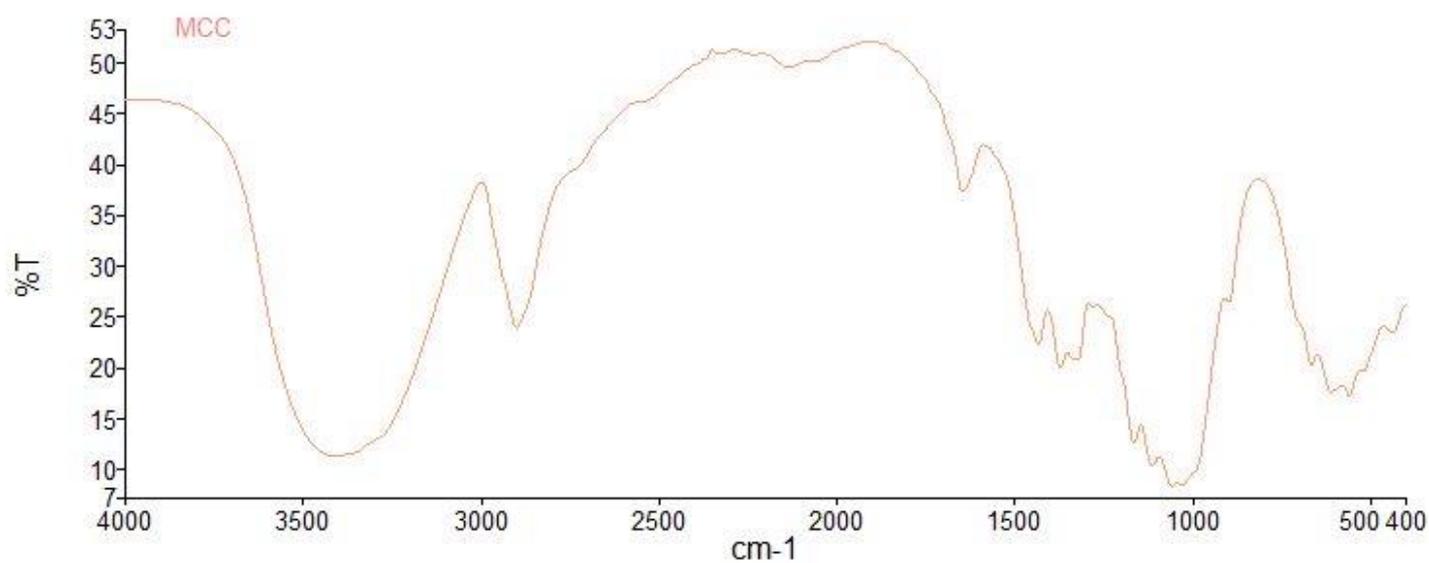


**B 8.** XRD pattern of CNC from sulphuric acid hydrolysis prepared from dry cellulose extracted from barley husk.

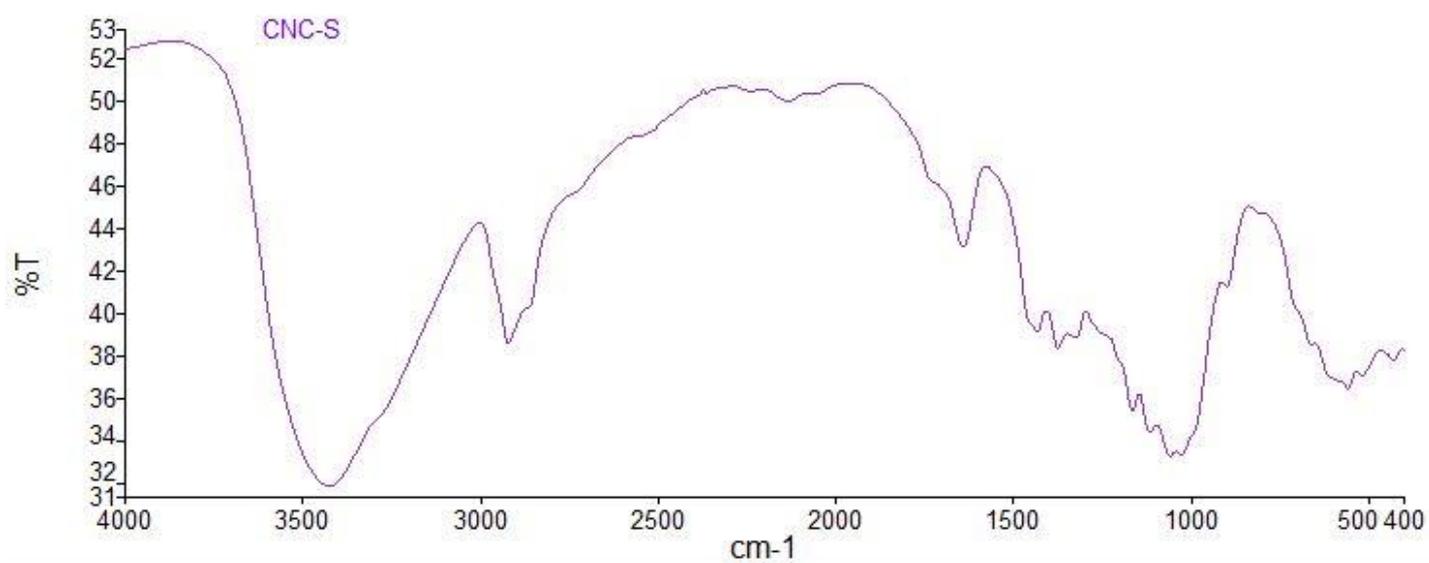
# APPENDIX C

## C. FT-IR SPECTRA

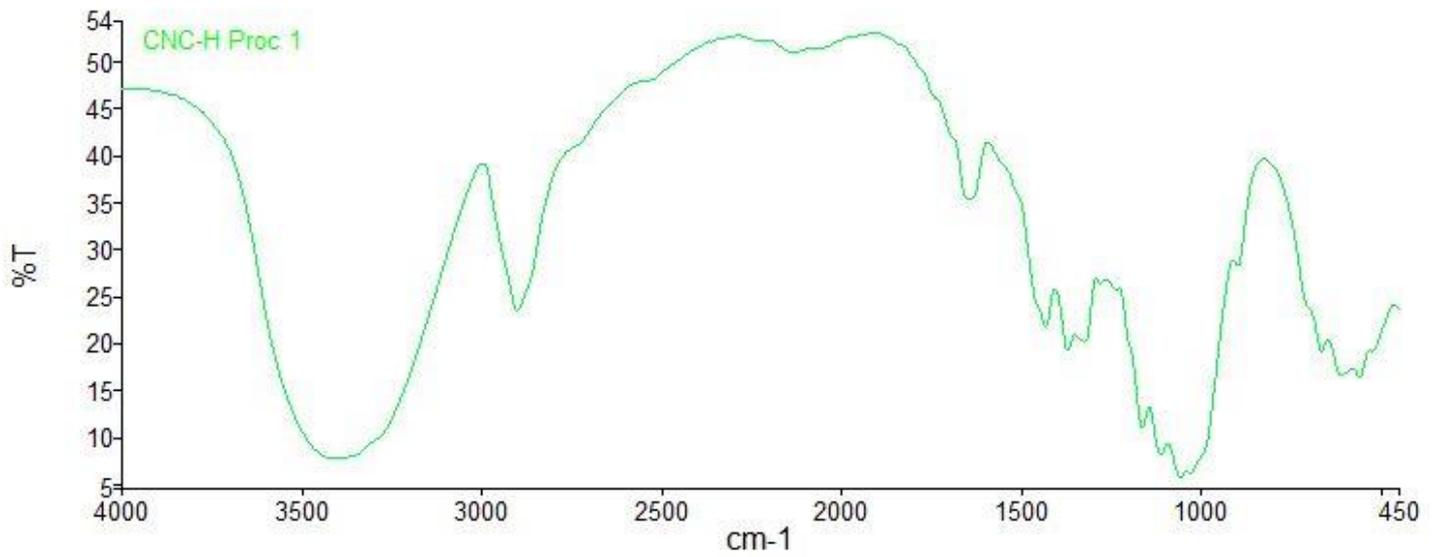
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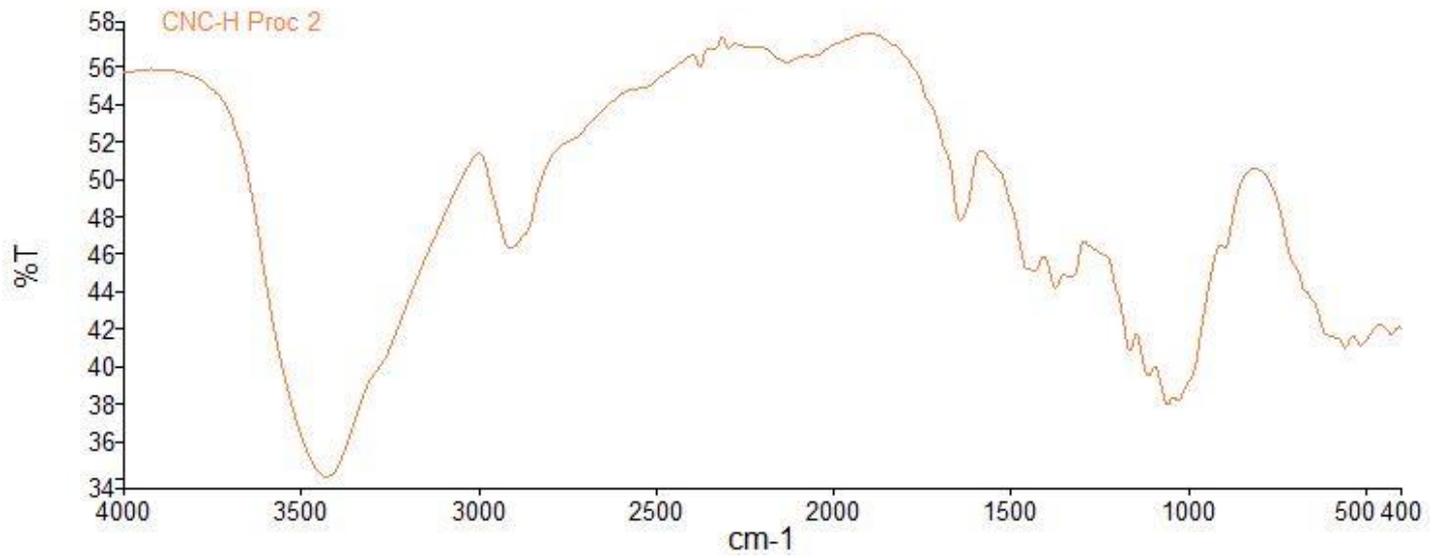
C 1. FT-IR spectra of microcrystalline cellulose (MCC).



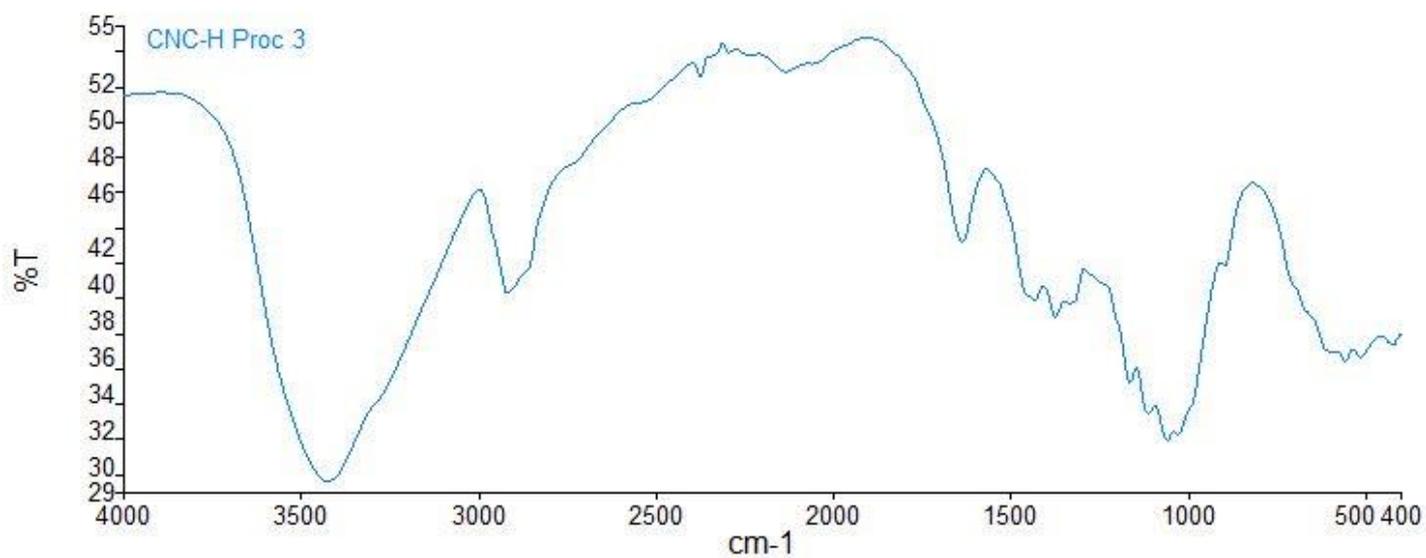
C 2. FT-IR spectra of CNC from sulphuric acid hydrolysis.



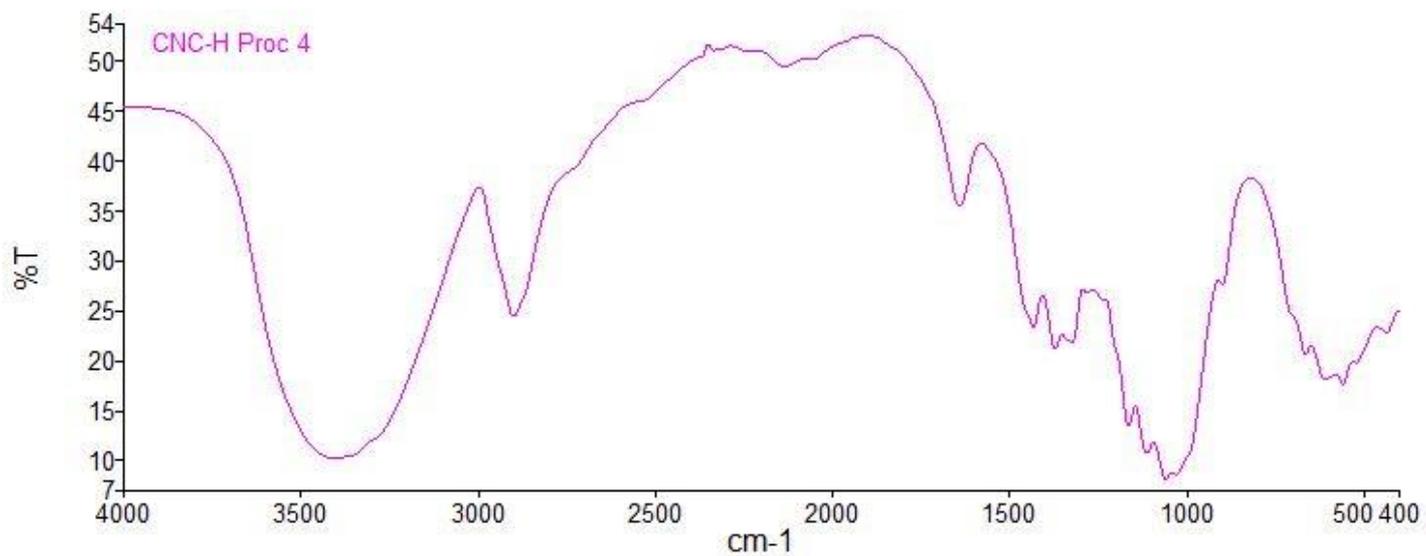
C 3. FT-IR spectra of CNC from hydrochloric acid hydrolysis following procedure 1.



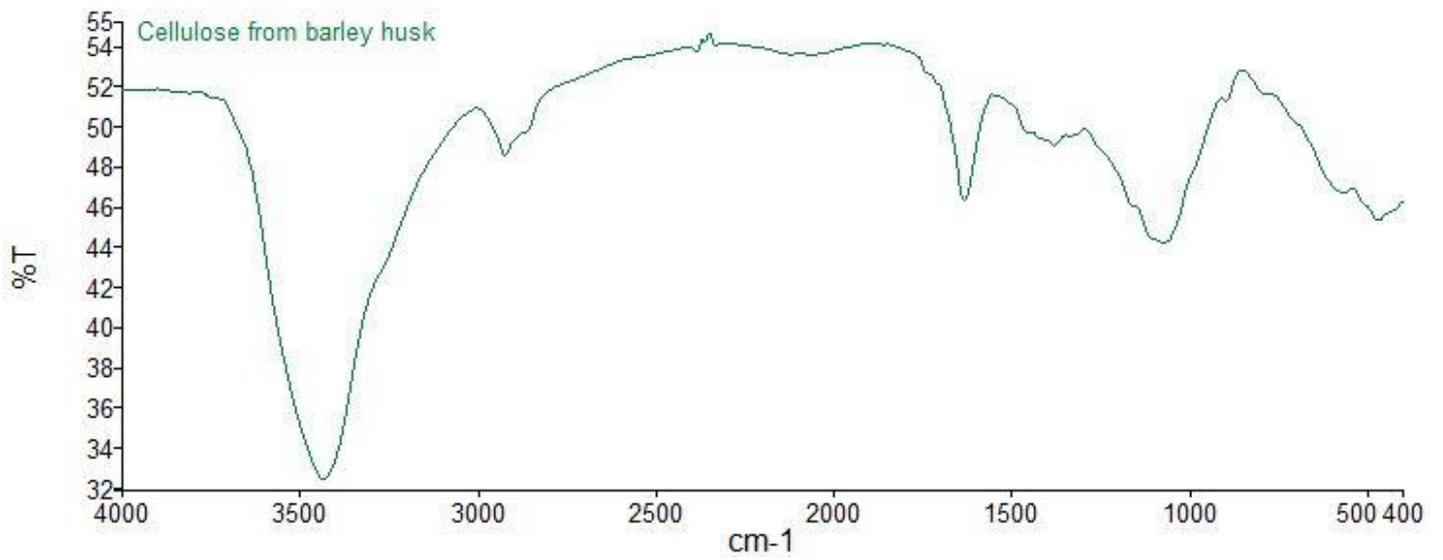
C 4. FT-IR spectra of CNC from hydrochloric acid hydrolysis following procedure 2.



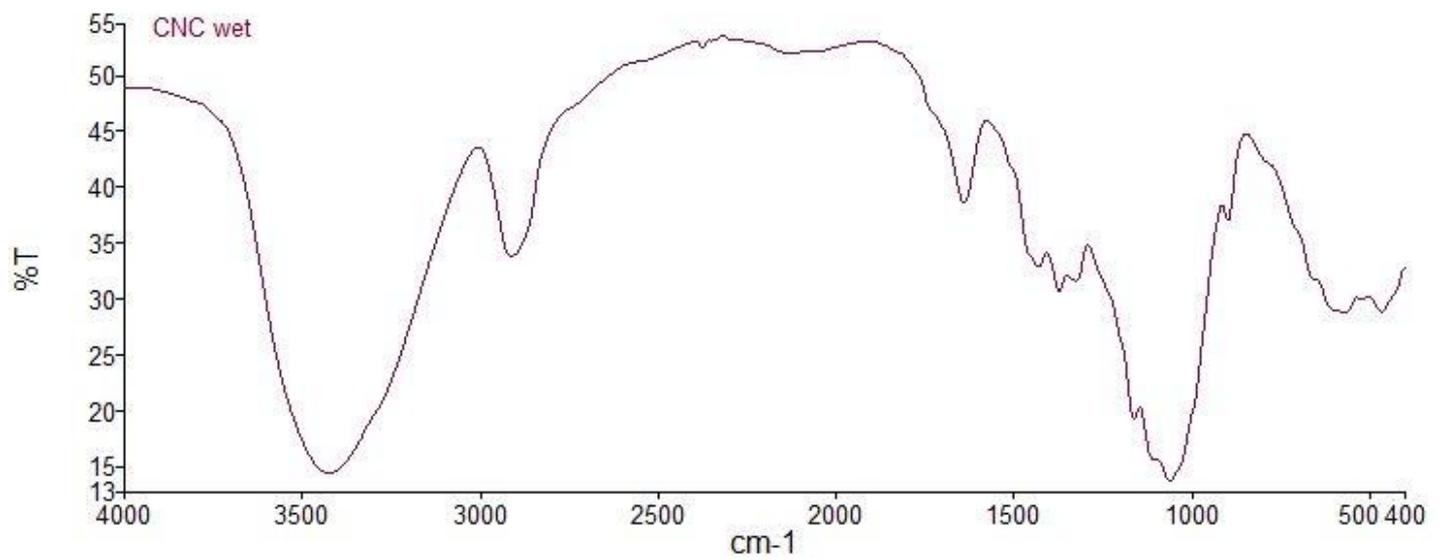
C 5. FT-IR spectra of CNC from hydrochloric acid following procedure 3.



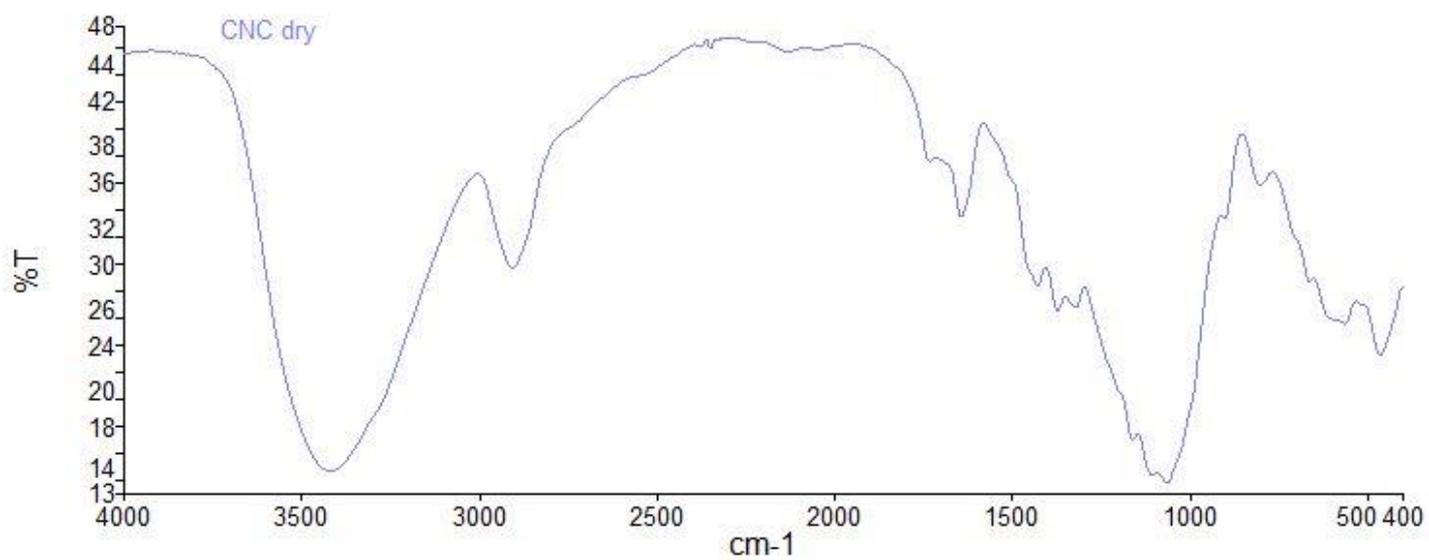
C 6. FT-IR spectra of hydrochloric acid hydrolysis following procedure 3.



C 7. FT-IR spectra of cellulose extracted from barley husk.



C 8. FT-IR spectra of CNC from sulphuric acid hydrolysis prepared from cellulose extracted from barley husk.



C 9. FT-IR spectra of CNC from sulphuric acid hydrolysis prepared from cellulose extracted from barley husk.