Optimisation of Nanocellulose and Xyloglucan Concentrations for Development and Testing of a Method Determining the Quality of Nanocellulose

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Abstract

In this study a method for measuring the specific surface area of nanocellulose, a type of nanocellulose, is developed. It employs xyloglucan adsorption to nanocellulose, spectrophotometry and the Beer-Lambert law. Optimal concentrations of the materials used are decided and the method is verified by being tested for several different qualities of nanocellulose. The conclusion is that the method works, although there is room for improvements of accuracy in order for the method and for nanocellulose to reach their full potentials.

Contents

1 Introduction

Research and development of new and superior materials is always necessary for technological advancement and the continued growth of society. Nanocelluloses and composites thereof are competitive new materials with qualities such as renewability and biodegradability, that can potentially replace unsustainable, oil based materials like plastic [16]. These nanofibres can create many different materials, e.g. aerogel, films with low oxygen permeability and very strong paper [11]. They can also be used in products such as sunscreen and food, as thickening agents amongst other things, due to their ability to adsorb vast amounts of water [12].

Nanocelluloses of varying qualities are unequally suitable for different purposes, due to different specific surface areas and thereby different affinities. A method measuring the specific surface area and determining the quality of the material is therefore useful when creating nanocomposites of the material. Such a method could also be used to price nanocellulose, reducing the risk of big companies exploiting the ignorance of their customers.

Normally, specific surface areas are measured using the Brunauer-Emmett-Teller (BET) theory, employing adsorption of gas present in abundance [1]. Through knowledge of the amount of gas adsorbed and the size of the gas molecules, the covered surface can be calculated [1]. The problem with this method is that the substance needs to be dry, and in the case of nanofibrillated cellulose approximately 98 wt% (i.e. weight percent) is water [12]. When drying a cellulose-based substance, it aggregates, and consequently the specific surface area decreases [17]. This makes the usual method very inaccurate or even unusable.

An alternative to drying the substance is to trade the solvent for one that can be sublimed against air, whilst the nanocellulose keeps its structure [18]. However, pores are created in this process and since the gas molecules that are to be adsorbed to the nanocellulose are so small, the gas molecules also cover the surface of the pores [13]. This

results in a false specific surface area, greater than the true [13].

Considering the aforementioned problems, a method enabling measurment of the specific surface area while the substance is still wet is preferable. The sodium salt Congo red has strong affinity to cellulose and adsorption of this could therefore be an option, but the ionic strength required causes aggregation of the nanocellulose [9, 4]. Consequently, the specific surface area decreases along with the measured value, giving a false impression of the specific surface area of the nanocellulose.

Due to the absence of an accurate method for measuring the specific surface area, the aim of the study was to introduce one. The idea was to adsorb xyloglucan to nanofibrillated cellulose (NFC) and through usage of spectrophotometry the surface area of the nanofibillated cellulose could be calculated. This study comprised optimisation of the concentration of nanocellulose and xyloglucan, respectively, to make differences in quality detectable. Subsequently, the method was verified by being tested on several different types and qualities of nanocellulose.

2 Background

2.1 Cellulose

Cellulose is most commonly found in the cell wall of plant cells. The compound is the main component in products such as tissues, parchment paper, cartons and wooden furniture. Composites of cellulose are difficult to make because the contact area between the different substances is small.[6]

Being a natural polysaccharide, cellulose consists of long chains of D-glucose bound together by $\beta(1,4)$-glycosidic bonds, see Figure 1, a structure which creates linear compounds that can be packed in parallel under the development of hydrophobic interaction and strong hydrogen bonds. This crystalline structure gives the organic compound great strength, although solely in one direction. Lest cellulose fibres become too rigid and brittle, regions of crystalline cellulose are interspersed with shorter regions of less crystalline

cellulose, where the cellulose chains are less densely packed and thus the compound is weaker and more flexible in these areas.[6]

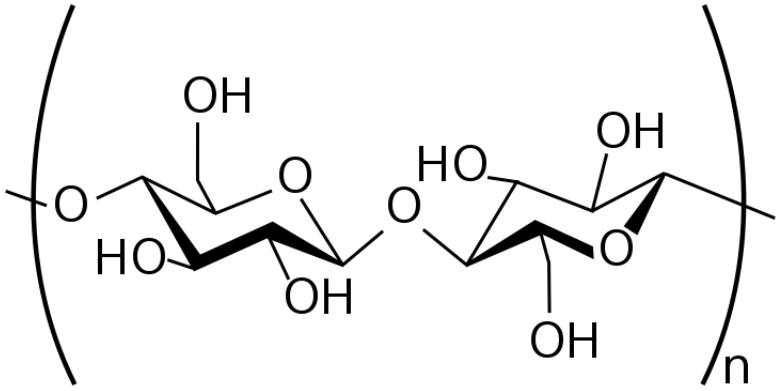


Figure 1: Basic structure of cellulose. A cellulose chain consists of many D-glucose residues where every second one is oppositely directed.[1]

2.2 Nanocellulose

Nanocellulose is a material consisting of cellulose fibrils and aggregates thereof with widths of $1 - 100$nm, combining the chemical properties of ordinary cellulose and nano-sized materials. These properties include crystalline structure, hydrophilicity and a large specific surface area compared to the volume, the latter increasing the adhesion and affinity in comparison with that of ordinary cellulose. Therefore, nanocellulose is more suitable for composites than ordinary cellulose, by binding to other substances and forming a joint material with enhanced features. [11]

[1]Wikimedia Commons. 2D skeletal diagram of cellulose with stereochemistry shown. 2007. 16.07.13. `https://commons.wikimedia.org/wiki/File:Cellulose-2D-skeletal.svg`

Nanocellulose is divided into three subcategories, based on the cellulose source and the production method. The subcategories are [11]:

1. Nanofibrillated cellulose (NFC), also known as microfibirillated cellulose (MFC), which comes from delamination (i.e. separation into layers) of e.g. wood, sugar beets or flax seeds.

2. Nanocrystalline cellulose (NCC), formed by acid hydrolysis of cellulose from various sources, such as wood, cotton and bacteria.

3. Bacterial nanocellulose (BNC), created by bacteria, from simple sugars and alcohols.

2.2.1 Production of Nanofibrillated Cellulose (NFC)

Prior to delamination, the cellulose fibres are treated either chemically or enzymatically [11]. The chemical pretreatment consists of the compounds being charged and thereby the electrostaic repulsion between the fibrils increasing [11]. The enzymatical pretreatment uses the enzyme monocomponent endoglucanase that splits the long cellulose chains, particularly in the less crystalling areas, into shorter ones [16, 5]. Both pretreatments serve to facilitate the disassembling of the fibres [11].

The delamination is achieved through homogenisation, a method where great pressure pushes aqueous cellulose fibres through the thin tunnels of a homogeniser, causing them to separate into nanofibres. The substance obtained in the process is thicker than the original substance due to the increased surface area which enables greater adsorbance of water and thereby creates a more gel like formula.[12]

2.2.2 Nanofibrillated Cellulose (NFC) qualities

Nanofibrillated cellulose of different types origin from different types of trees, softwood or hardwood trees, and have been processed in different ways, e.g. by being chlorite bleached and/or alkaline extracted [16]. The different qualities depend on the production of the material. For enzymatically treated NFC the amount of enzymes used can be varied and

for chemically modified NFC, e.g. through TEMPO oxidation, the charge density can vary. The more enzymes used or the greater the charge density, the easier the delamination. To which extent the material is delaminated also depends on the amount of energy used by the homogenisator, which is dependent on the number of passages through the tunnels and the pressure during the passage. For two NFCs of the same type, with the same sort and amount of pretreatment, the amount of energy used by the homogenisator is crucial for the difference in quality. The more energy used, the better the disassembling of the fibres and thereby the better the quality.

2.3 Xyloglucan

Like cellulose, the hemicellulose xyloglucan has a backbone of D-glucose residues bound together by $\beta(1,4)$-glycosidic bonds, but instead of having an unbranched chain, xyloglucan has plenty of side chains of monosaccharide residues. Found in abundance in the primary cell wall, xyloglucan has an α-helix structure and provides a link between cellulose fibrils. [3]

Because of the similarities between xyloglucan and cellulose, xyloglucan can dock to the cellulose by unfolding from the α-helix. Being a much larger compound than gases, xyloglucan does not enter potential pores of the nanocellulose and so it only covers the actual surface area.

2.4 Iodine Reagent

Together with starch, iodine can create coloured complexes and the iodine test is therefore commonly used to detect the presence of starch. Similar complexes can be created between xyloglucan and iodine, enabling detection of xyloglucan [2]. A high concentration of the complex darkens the colour, meaning the absorbance of light is greater. As described by the Beer-Lambert law,

$$A = \epsilon \cdot l \cdot c \tag{1}$$

the absorbance A, is proportional against the concentration c, regardless of the molar absorptivity (an intrinsic property) ϵ, and the distance the light passes through the solution l [14]. As a means of quantifying the xyloglucan concentration, equation (1) can be used in combination with spectrophotometry, a method for determining the absorption of light of a specific wavelength for a solution [15].

3 Method

The method was divided into two main parts. First the optimal concentration of a specific type and quality of nanocellulose and optimal concentrations of xyloglucan were decided as part of developing the method, see section 3.2. Subsequently the method was examined using several different nanocelluloses, see section 3.3.

3.1 Preparation of Stock Solutions

The dry content of three different xyloglucans, the difference being the molecular weight, see Table 1, was measured using an IR dryer (HB43-S, Mettler Toledo, SWE). Stock solutions with 1 wt% xyloglucan were created by dissolving xyloglucan in distilled water. The solutions were placed in water baths (approx. 60°C) on top of magnetic stirrers, to reduce the dissolution time. When dissolved, the solutions were refridgerated to minimise bacterial growth.

Solid potassium iodide and solid iodine were dissolved in distilled water, creating a solution with 1 wt% potassium iodide and 0.5 wt% iodine. To facilitate the dissolving of the iodine, the potassium iodide was dissolved first. The solution was placed on a magnetic stirrer (for 18 h). The iodine solution was mixed with aqueous sulfuric acid with a concentration of 20 wt% at a 1:5 ratio.

Aqueous nanocelluloses of different types and qualities, see Figure **??** and **??** and Table 2, were diluted to a concentration of 0.2 wt% nanocellulose. A dispersing instrument (Ultra Turrax ®T25 digital, IKA, GE; for 5 minutes, 12,200 rpm) was used to homogenise

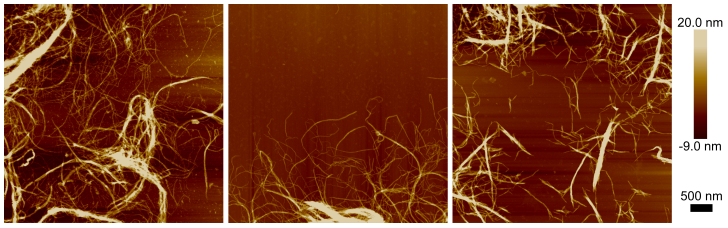
Table 1: Definitions of denotations given to the three different xyloglucans used. The denotations given refer to the molecular weight. The number average is the total weight of all polymers in the sample divided by the number of polymers in the sample. The weight average is the molecular weight of xyloglucan of a specific weight divided by the total molecular weight of the material, multiplied by the specific molecular weight being calculated on. Polydispersity describes the degree of heterogenity in a solution and it is calculated by division of the weight average with the number average. It shows the ratio between the two averages and is therefore unitless. The proportion states the ratio between different molecular weights in the material.

Xyloglucan denotation	**Number Average [u]**	**Weight Average [u]**	**Polydispersity**	**Proportion**
low	14,028	32,651	2.33	100.00 %
high	338,908	632,382	1.87	100.00 %
natural	418,826	779,351	1.86	88.50 %
	5,664	5,698	1.01	6.41 %
	2,093	2,119	1.01	5.09 %

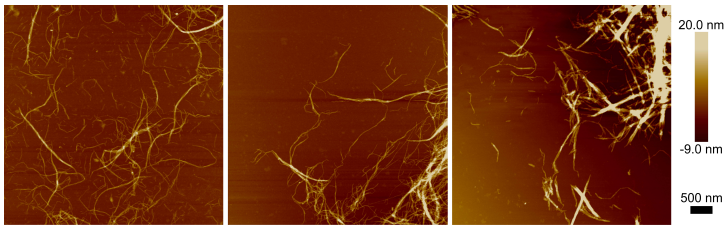
the solutions prior to placing them in the refrigerator, the latter to minimise the growth of bacteria.

Table 2: Definitions of denotations given to the different nanocellulose types and qualities tested. The different types of treatments were 0 = unbleached, 1 = chlorite bleached, 2 = chlorite bleached and alkaline extraction (wash with 10 wt% NaOH), 3 = TEMPO oxidated. "Number of passages" refers to the number of passages through the $200-100$nm tube of the homogeniser. All nanocelluloses passed through the $400-200$nm tube once.

Nanocellulose denotations	**Type of tree**	**Type of treatment**	**Number of passages**
SW_0 1p	softwood	0	1
SW_1 1p	softwood	1	1
SW_2 1p	softwood	2	1
HW_0 1p	hardwood	0	1
HW_1 1p	hardwood	1	1
HW_2 1p	hardwood	2	1
SW_0 4p	softwood	0	4
SW_1 4p	softwood	1	4
SW_2 4p	softwood	2	4
HW_0 4p	hardwood	0	4
HW_1 4p	hardwood	1	4
HW_2 4p	hardwood	2	4
T 1p	softwood	3	1
T 4p	softwood	3	4



(a) L-R: SW_0, SW_1, SW_2.



(b) L-R: HW_0, HW_1, HW_2.

Figure 2: Atomic-force microscopy (AFM) pictures of three of the nanocellulose types used.

3.2 Optimisation of Xyloglucan and Nanocellulose Concentrations

3.2.1 Finding Appropriate Xyloglucan Concentrations

The three stock solutions of xyloglucan were diluted to four different concentrations and 200µL of each was pipetted into 2mL Eppendorf tubes. To each sample 1mL of the iodine sulfuric acid solution was added, before being covered with aluminium foil for 30 minutes, to eliminate contact with light.

In order to find an appropriate concentration of xyloglucan, spectrophotometry was used. The spectrophotometer was calibrated against distilled water, for wavelengths ranging from 640nm to 680nm. The measurements were made at the wavelength 660nm and the absorbance was noted. The choice of wavelength was based on the spectrum for such solutions, showing 660nm in the middle of a plateau. Measurements of absorption at such a wavelength are less sensitive to slight shifts of wavelength, diminshing the impact of slight inaccuracies of the spectrophotometer.

Linear regressions were made, showing the relationship between the concentration of xyloglucan and the absorbance for the three xyloglucan types respectively. Through these

regressions, reasonable concentrations could be decided. A reasonable concentration is one where the absorption is around 1 because of the better accuracy of the spectrophotometer in that range. This is a consequence of the scale of absorption being logarithmic and an absorbance of 1 being defined as 1% of the light being absorbed.

3.2.2 Finding Optimal Concentrations of Xyloglucan and Nanocellulose

The three xyloglucan stock solutions were diluted, with both distilled water and 0.2 wt% aqueous nanocellulose (SW_1 4p in this case), to three different concentrations each. Also one of the stock solutions of aqueous nanocellulose (SW_1 4p in this case) was diluted, to four different concentrations. For every nanocellulose concentration, three different solutions were made, containing each of the three different types of xyloglucan. Refereneces were made for all concentrations of xyloglucan and nanocellulose, respectively. The Eppendorf tubes were placed in a thermal shaker (Multi-Therm, Benchmark, USA) (18 h, 24°C, 800 rpm).

Specimen were made in triplicates. Of each solution, 200µL was pipetted into Eppendorf tubes and subsequently 1mL of the iodine sulfuric acid solution was added. The samples were centrifuged (for 90 seconds, 14,500 rpm), wrapped in aluminium foil and left for 30 minutes.

The spectrophotometer (UV-2550 UV-vis spectorphotometer, Shimadzu, JP) was calibrated against distilled water, for wavelengths ranging from 400nm to 800nm and spectrum was measured for several different concentrations of xyloglucan, and for references of iodine sulfuric acid solution and xyloglucan solutions, respectively, diluted to concentrations matching those of the samples. The former was done to detect possible dispersion which could affect the result at a specific wavelength because of the different distribution across the spectrum. The latter two were measured to detect possible absorption by plain xyloglucan or xyloglucan adsorbed to nanocellulose which would reduce the actual absorption and give decepting values of the specific surface area unless discovered and accounted for. The remaining samples were only studied at wavelengths of 660nm and

the absorptions were noted.

A high absorbance means that there is a lot of iodine xyloglucan complex in the solution. This in turn means that only little xyloglucan has docked to the nanocellulose, indicating a small surface area.

Linear regressions showing the relationship between the absorption and the concentration of nanocellulose and xyloglucan, respectively, were made. Optimal concentrations of xyloglucan were chosen by studying the derivative of the formula. Concentrations considered optimal are ones where the derivative is high and constant, making differences in specific surface area and thereby differences in absorption easier to detect.

3.3 Tests on Different Nanocellulose Qualities

The nanocellulose stock solutions were pipetted into two Eppendorf tubes each, so that when diluted to 2mL the concentration was in accordance to the one determined during the optimisation. Xyloglucan was added in concentrations decided during the optimisation of concentration, and distilled water was also added, filling the Eppendorf tubes. Following trial and error, the tubes containing TEMPO oxidated nanocellulose only contained a third of the concentration of nanocellulose determined during the optimisation due to a significantly greater surface area. Also they were put in a vortex mixer due to being more difficultly dispersed. All samples were incubated (19 h, 24°C, 800 rpm).

Five specimen were created from each sample, containing 200µL of the sample and 1mL of the iodine sulfuric acid solution. The new samples were centrifuged (90 seconds, 14,500 rpm), wrapped in aluminium foil and left for 30 minutes.

The spectrophotometer was calibrated against distilled water and the tests were carried out at wavelengths of 660nm. The absorptions were noted and analysed. Calculations were performed, showing the average absorbance; the standard deviation; a 95 % confidence interval; the percentage xyloglucan adsorbed and the weight xyloglucan adsorbed. The dry content used for the caluculations was measured by placing some of the aqueous nanocellulose, two samples of each type and quality, in an oven and calculating the weight

percent left after it having dried.

4 Results

4.1 Optimisation of Xyloglucan and Nanocellulose Concentrations

For graphs showing how the absorbance of light depends on the concentration of xyloglucan and nanocellulose, see Figures 3 and 4. What this means in xyloglucan adsorption to the nanocellulose can be seen in Figures 8 and 9 in appendix A. For optimal concentrations of xyloglucan and nanocellulose, see Table 3. An optimal concentration for low molecular weight xyloglucan was not determined.

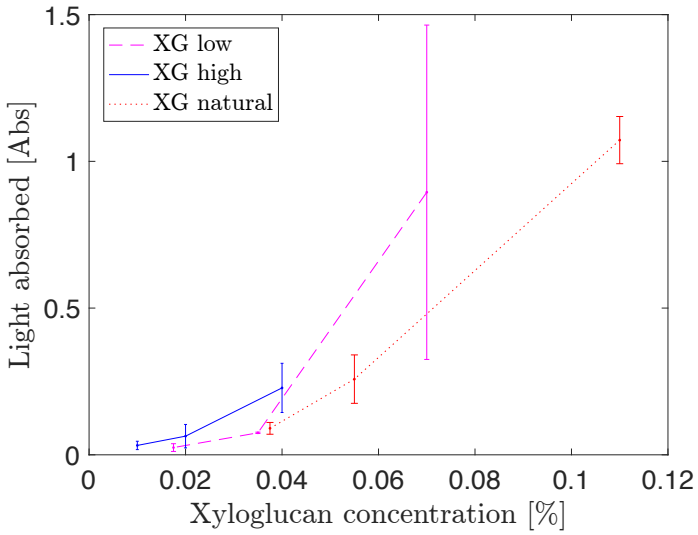


Figure 3: Average absorbance of light for different concentrations and different molecular weight xyloglucan, with the same concentration of nanocellulose and iodine sulfuric acid solution.

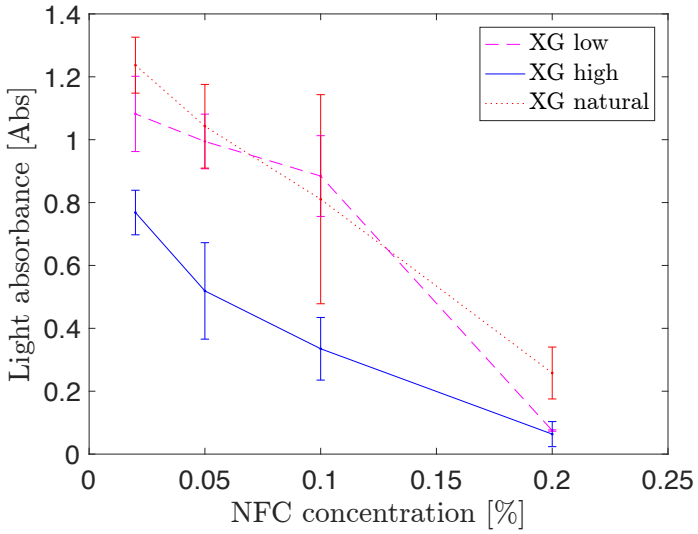


Figure 4: Average absorbance of light for different concentrations of nanocellulose (SW_1 4p) using the three different xyloglucans, with the same concentration of xyloglucan and iodine sulfuric acid solution.

Table 3: Optimised concentrations for distinguishing and determining different qualities of nanocellulose

Substance	**Concentration**
Nanocellulose SW_1 4p	0.05 %
XG low	-
XG high	0.04 %
XG natural	0.055 %

4.2 Tests on Different Nanocellulose Qualities

The amount of xyloglucan adsorbed by the nanocellulose varied depending on both the molecular weight of the xyloglucan and the type and quality of the nanocellulose, see Figures 5, 6 and 7.

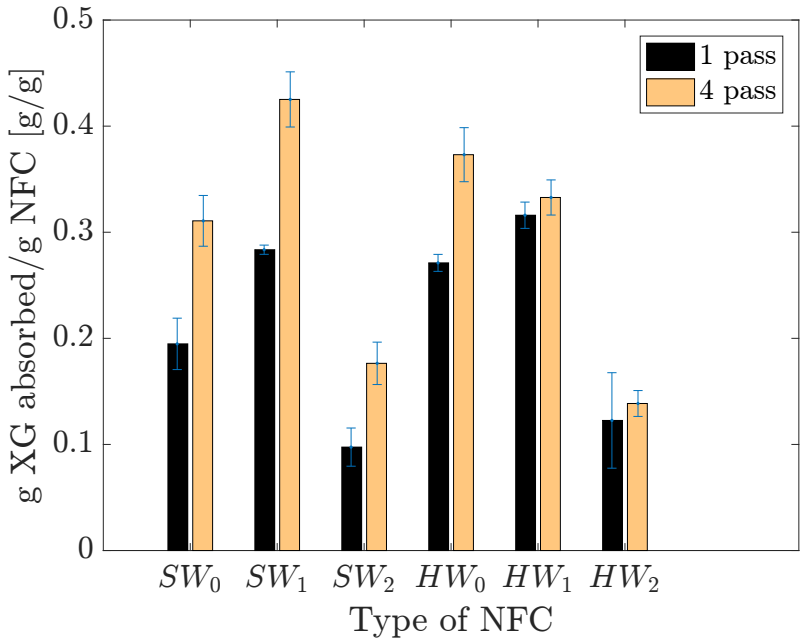


Figure 5: High molecular weight xyloglucan adsorbed by NFC in milligrams per milligram. The bars show the average amount adsorbed for different types and qualities of NFC and the error bars show the 95 % confidence interval.

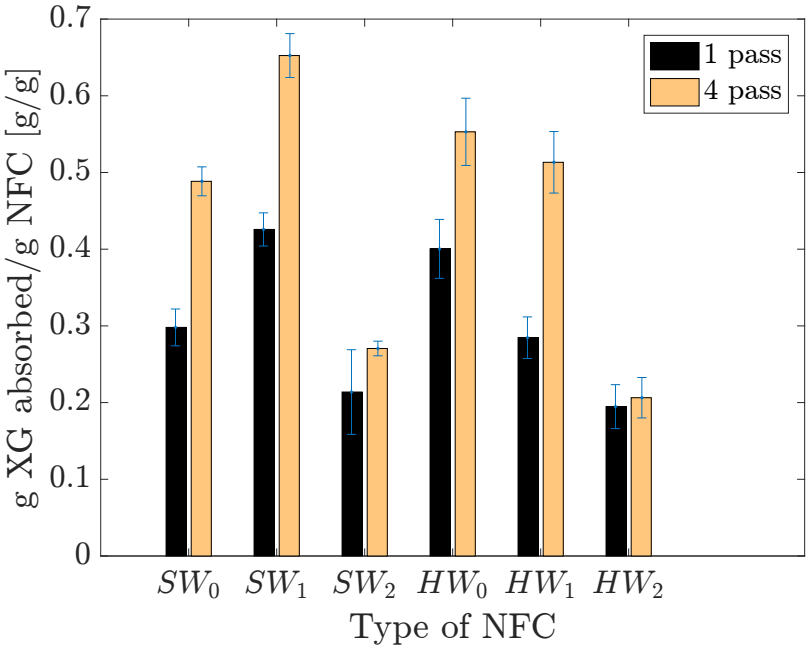


Figure 6: Natural distribution of molecular weight xyloglucan adsorbed by NFC in milligrams per milligram. The bars show the average amunt adsorbed for different types and qualities of NFC and the error bars show the 95 % confidence interval.

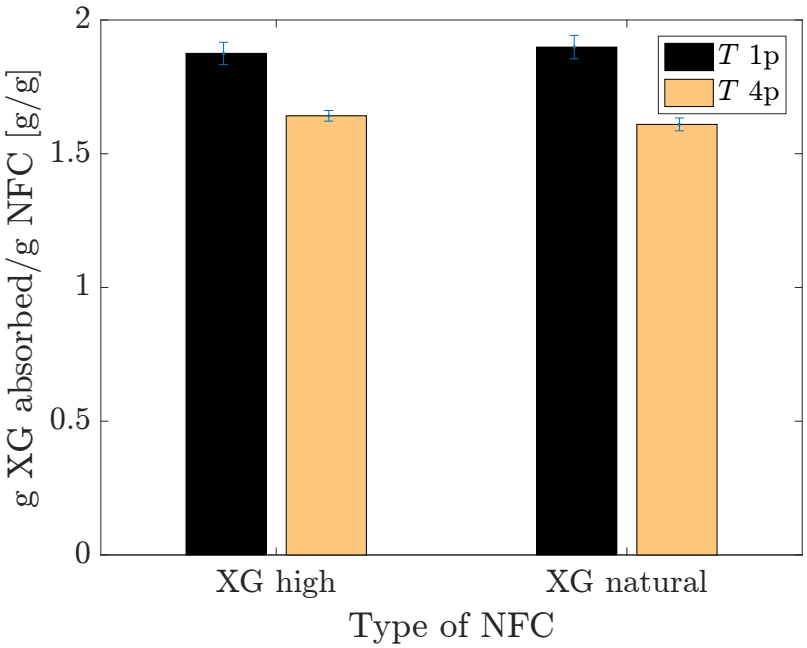


Figure 7: Xyloglucan adsorbed by TEMPO oxidated NFC in milligrams per milligram. The bars show the average amount adsorbed by the different qualities of NFC using different types of xyloglucan and the error bars show the 95 % confidence interval.

5 Discussion

Owing to the varying and inaccurate results given by the xyloglucan with low molecular weight when trying to optimise the concentration, it was excluded from further research and development of the method as it was not considered reliable. There needs to be a clear relationship between the concentration of the xyloglucan and iodine complex and the absorption of light, or else the concentration of the complex cannot be calculated with the help of spectrophotometry. If the concentration and therethrough the amount of the complex cannot be calculated, the amount of xyloglucan adsorbed to the nanocellulose cannot be calculated, making the method useless for deciding the specific surface area of nanocellulose.

Xyloglucan needs to be present in abundance for the method to work. If not, all xyloglucan will be adsorbed by the nanocellulose, making the difference in absorbance

between samples of the different types and qualities of nanocellulose indistinguishable. Although xyloglucan needs to be present in abundance, it cannot be too abundantly present. If it were to be, indifferent amounts would be adsorbed, again making the difference in absorbance of the xyloglucan and iodine complex indistinguishable between the different nanocelluloses. Also the absorbance would be much higher than 1, meaning the inaccuracy of the spectrophotometer as a source of error increases.

The concentration of xyloglucan that is considered optimal is one where the derivative of a formula describing the relationship between the concentration of the xyloglucan and iodine complex and the absorbance is high, making it possible to distinguish different types and qualities of nanocellulose. If more concentrations and more specimen of each concentration are tested, a more perfectly linear function can be adapted. As the derivative of a linear function is equal for all input values, any concentration following the relationship works. At the same time the absorbance should be around 1, because of the greater accuracy of the spectrophotometer in that range. In order to accomplish both, also the concentration of nanocellulose can be adjusted, which was done in this experiment. Optimal concentrations can vary between different nanocelluloses, which was seen when the method was tried on the TEMPO oxidated nanocelluloses. This is a consequence of a significantly differently surface area.

The relative calculated adsorbance of xyloglucan for the different nanocellulose types and qualities follows transmittance curves made for the same nanocelluloses. A higher adsorbance of xyloglucan means a greater surface area, and a higher transmittance indicates smaller compounds, making it easier for light to shine through the material. Thus, the transmittance values support the results of this study as well as the method itself.

The two TEMPO oxidated nanocelluloses were actual tests as they were produced by individuals outside of the original labteam. One would expect T 4p to have a greater surface area than T 1p, due to the greater amount of times passed through the $200-100$nm tunnel of the homogeniser. Despite this, the results show that, in this case, T 1p has a greater surface area, indicating either different amounts of pretreatment (it could indicate

different pretreatments altogether, but both are said to be TEMPO oxidated which is a chemical form of pretreatment) or one of the parties lying about their manufacturing process. Either way, it is for this exact reason that an accurate method for determining the specific surface area and the quality of nanocellulose must exist, or else consumers risk becoming thoroughly cheated.

5.1 Further Research

The method is not yet calibrated, and therefore it can only tell how the specific surface area of one nanocellulose is compared to another. The next step is to calibrate the method, making it possible to calculate the actual value. This can be done by measuring the specific surface area of a nanocellulose lacking pores, using the already calibrated method with adsorbation of gas. If there are few pores, the metod using adsorbation of gas molecules should be accurate, and the method using adsorbation of xyloglucan can therefore be calibrated against it.

An alternative way of calibration is to calculate the theoretical surface area of a nanocellulose quality consisting of only elementary fibrils of a known size. By comparing the theoretically calculated surface area with one achieved through this newly developed method, the latter can be calibrated.

Improving the accuracy of the method could be useful, so detection of differences in type and quality between similar nanocelluloses can more easily be detected. It seems that the difference in specific surface area between different types of nanocellulose is smaller when the quality is lower, making the method more accurate the higher the quality. If the accuracy of the method is improved it could potentially be used for evaluating the quality of ordinary, macroscopic cellulose and composites thereof as well.

In order to improve the accuracy, the sources of error need to be reduced in amount, size, or both. Major sources of error are the difficulties and inaccuracies of measuring very small amounts. The relative error easily becomes large when working with small amounts. Pipetting little quantities of viscous solutions is difficult and often too little of

the solution gets taken up by the pipette and too little gets pushed out of it. This leads to the practical concentration of some of the nanocelluloses being considerably smaller than others whilst the theoretical concentrations are the same, creating different conditions for different nanocelluloses. A more sophisticated way to make sure the concentrations are the same needs to be used. A possibility is making larger quantities of the solutions, to diminish the relative error. Another possibility is to calculate the density of the solution and therethrough measure the volume by using a scale with high accuracy.

Another major source of error is the varying amounts of iodine xyloglucan precipitation in the specimen. The samples were centrifuged to decrease the risk of pipetting the precipitation but it was clear it was not always enough. Since the percipitation is an accumulation of the complex, it absorbs very much light. Therefore the absorption between specimen is dependent on the amount of percipitation which it contains. This has contributed to large confidence intervals.

A problem that could occur for nanocelluloses of very high quality is the cellulose fibrils being smaller than the xyloglucan molecules. Measuring the size of a compound by adsorbing a larger one is not possible, because it will not be known how much of the compound that is actually adsorbed. This means that nanocelluloses of very fine quality will all adsorb the same amount of xyloglucan, making them indistinguishable using this method. In that case, a smaller compound needs to be adsorbed.

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A Percentage Xyloglucan Adsorbed

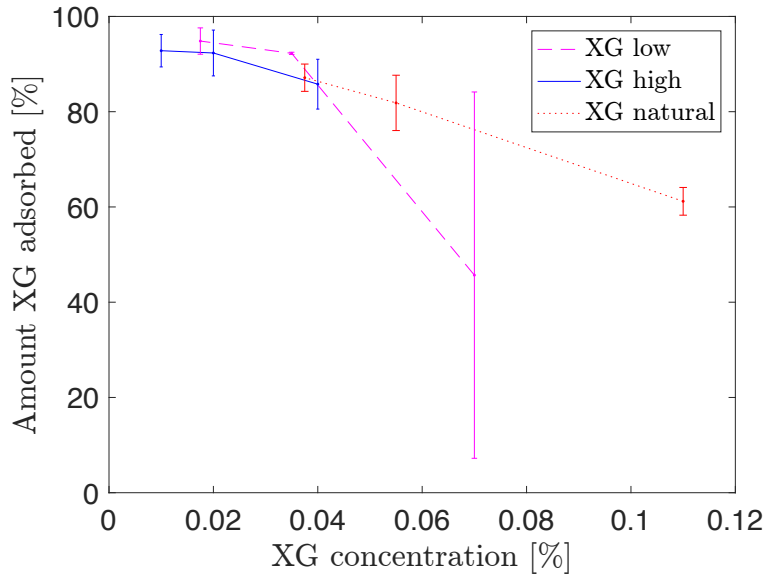

Figure 8: The percentage of xyloglucan adsorbed by nanocellulose (SW_1 4p) for varying concentrations and varying moelcular weight of xyloglucan.

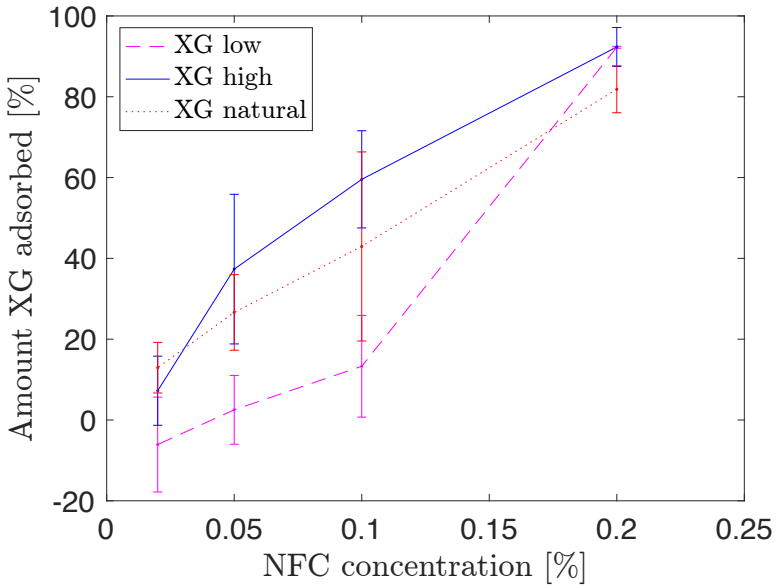

Figure 9: The percentage of xyloglucan, of the three different molecular weights, adsorbed by varying concentrations of the same nanocellulose (SW_1 4p).