

Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis

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Abstract

The objective of this work was to find a rapid, high-yield process to obtain an aqueous stable colloid suspension of cellulose nanocrystals/whiskers. Large quantities are required since these whiskers are designed to be extruded into polymers in the production of nano-biocomposites. Microcrystalline cellulose (MCC), derived from Norway spruce (*Picea abies*), was used as the starting material. The processing parameters have been optimized by using response surface methodology. The factors that varied during the process were the concentration of MCC and sulfuric acid, the hydrolysis time and temperature, and the ultrasonic treatment time. Responses measured were the median size of the cellulose particles/whiskers and yield. The surface charge as calculated from conductometric titration, microscopic examinations (optical and transmission electron microscopy), and observation of birefringence were also investigated in order to determine the outcome (efficiency) of the process. With a sulfuric acid concentration of 63.5% (w/w), it was possible to obtain cellulose nanocrystals/whiskers with a length between 200 and 400 nm and a width less than 10 nm in approximately 2 h with a yield of 30% (of initial weight).

Introduction

In recent years there has been an enormous increase in interest in producing composite materials with nanosized reinforcement, i.e. nanocomposites. This enthusiasm is due to the extraordinary properties that these kinds of materials exhibit as a result of the nanometric size effect from the reinforcement. However, especially for continuous and large-scale production, incorporating the reinforcement so that it is well-dispersed and without agglomerates in a continuous matrix has proved quite problematic. Most efforts have focused on the production of nanocomposites with inorganic reinforcements, but organic material has also been

used. At CERMAV-CNRS in France, cellulose whiskers, a name that refers to the needle-like structure of the crystallite, were separated from various sources such as wheat straw and tunicin, and were used as reinforcements in polymer matrices (Favier et al. 1995; Helbert et al. 1996; Dufresne and Cavaillé 1999; Anglès and Dufresne 2000). Incorporation of these nanosized elements into a polymeric matrix usually resulted in outstanding properties, at least with respect to their conventional microcomposite counterparts. Using cellulose crystallites as a reinforcement in nanocomposites has numerous advantages. Cellulose is the most abundant renewable polymer in the world; it is found in plant cell walls, but it can also

be synthesized by some bacteria (Ranby 1952a) and animals (Herzog and Gonell 1924; Ranby 1952b). Its reinforcing property is also remarkable. Calculations of Young's modulus along the chain axis for native cellulose gives a value of 167.5 GPa (Tashiro and Kobayashi 1991) and experimental analysis of elastic modulus for tunicin whiskers gives a value of 143 GPa (Sturcova et al. 2005). However, the drawbacks of using cellulose are that the crystallites have to be isolated, and incorporation of the crystallites in a matrix usually involves problems in controlling the dispersion level. Acid hydrolysis of cellulose is a well-known process used to remove amorphous regions. Several studies have been reported where cellulose crystallites/whiskers were identified and separated from various sources. Nickerson and Habrle (1947) talked about the use of hydrochloric and sulfuric acid hydrolysis to produce cellulose crystallites from cellulose materials, and in 1952c, Ranby reported the preparation of cellulose whiskers from microfibrils using acid hydrolysis. Marchessault and co-workers studied the hydrolysis of chitin and native, mercerized, and bacterial cellulose using acid hydrolysis, and reported birefringence (Marchessault et al. 1959, 1961). Proper treatment of microcrystalline cellulose (MCC) with sulfuric acid will not only give isolated cellulose whiskers but also a negatively charged surface resulting from the esterification of hydroxyl groups by sulfate ions, so that the cellulose suspension forms a stable colloid system (Marchessault et al. 1961; Favier et al. 1995).

The main goal of this study has been to optimize process conditions using the sulfuric acid hydrolysis method to separate individual crystallites/whiskers and prepare a stable colloidal water suspension of cellulose whiskers with a negative surface charge. As described earlier, acid hydrolysis of cellulose is a well-known process for the production of cellulose whiskers, but the production is often limited to amounts only sufficient for lab scale usage. However, the use of these whiskers as a nanoreinforcement in biopolymers requires a compounding process that consumes larger quantities of cellulose whiskers. This necessitates the optimization of production capacity in terms of both time and yield. Beck-Candanedo et al. (2005) have reported the effect of reaction time and acid-to-pulp ratio on nanocrystals and suspension properties for hydrolyzed black spruce acid sulfite pulp. In this study,

five factors were selected to investigate hydrolysis of the MCC; the initial concentration of the MCC, the sulfuric acid concentration, the duration and temperature of hydrolysis, and the duration of sonication. The responses measured were median size and yield of the hydrolyzed cellulose. Response surface methodology (MODDE 7, Umetrics) was used to investigate the effect of the selected factors. This study marks the first time response surface methodology has been used to investigate the effect of reaction conditions on the acid hydrolysis processing of cellulose. Response surface methodology is a collection of statistical and mathematical tools used in the empirical study of relationships between one or more responses, or product characteristics, with a group of input variables. It covers various issues in data exploration, experiment design, model building, and process optimization. Analyses that were not included in the response surface methodology model were the results from conductometric titrations, microscopic investigations (OM and TEM), and observations from birefringence. Conductometric titration was performed in order to quantify the amount of sulfate groups on the cellulose, which results from sulfuric acid hydrolysis. It is known that the insufficient hydrolysis of cellulose may result in larger particles (and therefore less surface area) with a lower surface charge, favoring particle–particle interaction (Dong et al. 1998). An optical light microscope (OM) was used to detect bigger particles and to get an overview at the microscopic level. A transmission electron microscope (TEM) was used to view and classify particles in the nanometer range. The observation of flow birefringence between two crossed polarizing films in a cellulose suspension is an indication of a nematic liquid crystalline alignment and reveals the existence of whiskers (Orts et al. 1998).

Experimental section

Sample preparations

The first step was to mix deionized water and MCC, derived from Norway spruce (*Picea abies*) (Borregaard ChemCell, Sarpsborg, Norway), with a particle size between 10 and 15 μm (Figure 1). The water/MCC-suspension was then put in an ice bath and stirred while concentrated sulfuric acid (Merck) was added by drop until the desired acid

concentration was reached. The suspension was heated while stirred. It took approximately 5 min for the suspension to reach the desired temperature for all temperatures. The suspensions were then washed with deionized water using repeated centrifuge cycles of (10 min at 12,000 rpm, Sorvall RC-5B), i.e. the supernatant was removed from the sediment and replaced by new deionized water and mixed. The centrifuge step was stopped after at least five washings, or until the supernatant became turbid. The last wash was conducted using dialysis with deionized water until the wash water maintained at constant pH. The samples were then sonicated (J.P. Selecta, Ultrasons) for the specified sonication time in an ice bath to avoid overheating, which can cause desulfation of the sulfate groups on the cellulose (Dong et al. 1998).

Characterization methods

Conductometric titration was used to measure the surface charge on the cellulose particles following the method described by Katz et al. (1984). A known amount of freeze-dried cellulose (approximately 0.5 g) was mixed with 45 ml of deionized water and 5 ml of 0.01 M NaCl before measurement. For the samples with poor or no sulfation, 3 ml of water was replaced by 0.01 M HCl in order to obtain a correct value for the amount of sulfate groups. A short, decreasing portion of the titration curve is characteristic for weak acid groups, and could be confused with a low concentration of strong acidic sulfate groups. NaOH, 0.1 M, was

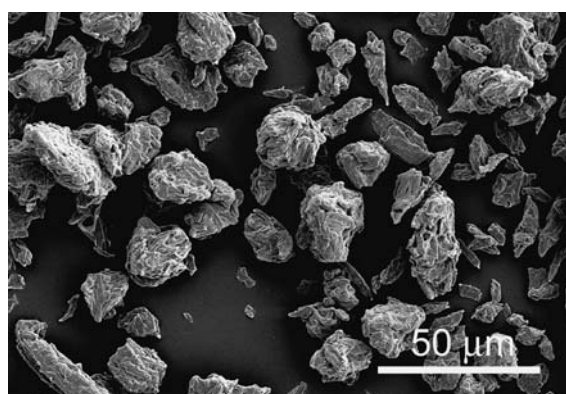


Figure 1. Scanning electron micrograph at 5.0 kV accelerating voltage showing MCC particles before acid hydrolysis (Hitachi S-4300).

added to the suspension periodically every 5 min while stirring continuously. The change in conductivity was recorded with a conductometer (Scientific Instruments, Consort K511).

Optical light microscope (OM) observations were performed using a Leica DMLB with magnifications of 100×, 200×, 500× and 1000× to detect bigger particles and to get an overview of the microscopic level. All samples were diluted to a concentration of 0.1 g/100 ml before examination.

Flow birefringence in the samples was investigated by using two crossed polarization filters. All samples were diluted to a concentration of 0.1 g/100 ml before examination.

Transmission electron microscope (TEM) observations were performed using a Philips CM30 operated at 100 kV. A droplet of suspension was placed on a copper grid covered by a thin carbon film and allowed to dry at 80 °C overnight.

Wide angle X-ray diffraction (WAXD) patterns of MCC and cellulose whiskers were obtained using a Siemens Diffractometer D5005. Cellulose whiskers samples were prepared by crushing the freeze-dried aqueous whisker suspension with a mortar. Both the MCC powder and the cellulose whisker samples were pressed into a cylindrical sample holder that was 25 mm in diameter and 2 mm high. The samples were exposed for a period of 11 s for each angle of incidence (θ) using a $\text{CuK}\alpha_{1,2}$ X-ray source with a wavelength (λ) of 1.541 Å. The angle of incidence was varied from $\sim 5^\circ$ to $\sim 30^\circ$ by steps of 0.06° . The periodical distances (d) of the main peaks were calculated according to Bragg's equation ($\lambda = 2d \sin \theta$). To eliminate preferential orientation, the specimens were rotated within the X-ray diffractometer as suggested by Eichhorn and Young (2001).

Yield was calculated as % (of initial weight) of MCC after hydrolysis. One-quarter of the total volume, typically 60 ml out of a total volume of 240 ml, was freeze-dried and compared to the initial weight of MCC.

Laser granulometry measurements were performed using a Coulter® LS-230 to get an approximate measure of the sizes of the cellulose particles/whiskers. This kind of analysis has been developed for measuring the dimensions of spherical particles, so that the measurement of materials that are shaped like whiskers will not result in a correct value for whiskers. The size registered for the whiskers will depend on their orientation in the

fluid. The main principle for size determination with this method is shading a laser beam that is being sent out in a suspension of particles. The shaded area, which originates from the particles in the suspension, is identified by detectors. This shaded area will be much less if the analyzed whisker is aligned parallel to the laser beam rather than perpendicular. Moreover, some suspensions will most probably contain both whiskers and spherical aggregates of cellulose whiskers. Nevertheless, in the absence of a good bulk analysis method for determine the sizes of whiskers, the values given from laser granulometry are still useful for internal comparisons. The median particle size, which divides the population into two equal halves, was used to characterize the suspensions.

Design of experiments

Response surface methodology (MODDE 7, Umetrics) was used to investigate the effect of different factors regarding the isolation process of the whiskers. Five different factors and two responses were used. The factors investigated were: initial concentration of MCC, sulfuric acid concentration, duration and temperature of hydrolysis, and duration of sonication. The factor intervals together with the corresponding orthogonally scaled factors have been provided in Table 1. These intervals and factors were selected according to earlier exploratory work and reports in the literature (Mukherjee and Woods 1953; Revol et al. 1992; Dong et al. 1998). The low ($u_{i,low}$), midrange ($u_{i,mid}$), and high ($u_{i,high}$) values for the parameters correspond to -1 , 0 , and 1 , respectively, for orthogonal variables, x_i , and are defined as:

$$x_i = \frac{2(u_i - u_{i,mid})}{u_{i,high} - u_{i,low}}.$$

The variation in the range for these factors was set fairly high in order to minimize the risk of

excluding any optimal conditions. At the same time, it is important to bear in mind that if the range is too large, the predictive power of the model runs the risk of being weak. The responses studied were median size (y_{size}) and yield (y_{yield}) of the cellulose whiskers/particles after the preparation step. A screening experiment was done using interaction models in order to identify important factors. A fractional factorial resolution V design was used to keep the amount of sample preparations down. This design is a half-fraction of a 25 factorial at two levels (low level and high level) and resulted in a total of 19 samples to investigate (16 runs in design and three replications at center points). The run order of the trials was randomized in order to prevent systematic errors.

Results and discussion

Models and model validations

The responses from the different sample runs, both the ones included in model and not included in model, are given in Table 2. It can be seen that the upper limit was reached using the factor values in sample runs 8 and 16. In these, the median size and yield were zero, which is a consequence of the reaction going too far and the cellulose being totally degraded. In regression analysis, it is advantageous if the data of a response variable are normally distributed, or nearly so, in order to improve the model (Montgomery 2001). This was achieved for our data with a logarithmic transformation ($\log [y + 1]$) for median size and a negative logarithmic transformation ($-\log [100 - y]$) for yield. The model was fitted to the data by using multiple linear regression (MLR) with orthogonally scaled factors. After elimination of negligible and insignificant factor effects, four stand out as being important and have been retained in the model: H_2SO_4 concentration (x_2),

Table 1. Parameter levels.

Parameter	Unit	Low level $x_i = -1$	Midrange level $x_i = 0$	High level $x_i = 1$
x_1 , MCC conc.	g/100 ml	5	10	15
x_2 , H_2SO_4 conc.	% (w/w)	44.1	55.1	64.8
x_3 , Hydrolysis time	min	10	65	120
x_4 , Temperature	°C	40	60	80
x_5 , Sonication time	min	10	20	30

Table 2. Experimental setup together with analytical results.

Sample no.	Orthogonal scaled factors					Factor responses		Factor responses not included in model		
	x_1	x_2	x_3	x_4	x_5	y_{size} (μm)	y_{yield} (%)	Sulfate groups (mmol/kg)	Agglomerates in light microscope	Flow birefringence
1	-1	-1	-1	-1	1	19.34	93	0	Numerous	Partial
2	-1	-1	-1	1	-1	9.12	88	12	Numerous	Partial
3	-1	1	-1	-1	-1	38.68	91	128	Numerous	No
4	-1	1	-1	1	1	10.82	47	297	Few	Partial
5	-1	-1	1	-1	-1	14.30	94	5	Numerous	Partial
6	-1	-1	1	1	1	7.01	92	85	Numerous	Partial
7	-1	1	1	-1	1	0.11	34	354	None	Yes
8	-1	1	1	1	-1	0	0	—	—	—
9	1	-1	-1	-1	-1	22.36	95	0	Numerous	Partial
10	1	-1	-1	1	1	8.53	90	11	Numerous	Partial
11	1	1	-1	-1	1	14.91	73	226	Numerous	Partial
12	1	1	-1	1	-1	12.69	23	393	Numerous	Partial
13	1	-1	1	-1	1	13.80	95	6	Numerous	Partial
14	1	-1	1	1	-1	6.88	86	83	Numerous	Partial
15	1	1	1	-1	-1	0.18	18	330	None	Yes
16	1	1	1	1	1	0	0	—	—	—
17	0	0	0	0	0	7.96	82	188	Numerous	Partial
18	0	0	0	0	0	8.15	78	157	Numerous	Partial
19	0	0	0	0	0	7.84	78	151	Numerous	Partial

hydrolysis time (x_3), temperature of hydrolysis (x_4), and interaction of H_2SO_4 concentration and hydrolysis time (x_2x_3). A normal probability plot of studentized residuals is a good tool to find experiments that deviate significantly from the norm, otherwise known as outliers. The warning limit is ± 3 standard deviations and any observation with a residual outside of this interval is potentially unusual with respect to its observed response (Montgomery 2001). Sample 3, with +3 standard deviations for median size and +5 standard deviations for yield, was assumed to be wrong and removed in order to improve the model. An analysis of variance (ANOVA) was used in order to identify the possible lack of fit of the proposed model, with the F -test used to compare the model and replicate errors with each other. No lack of fit was seen for yield ($p=0.257$) but, a lack of fit could be assumed for median size ($p=0.007$). This value is, however, just an artificial lack of fit, an assumption that was confirmed by the small scattering of the replicate response values. The model's statistics (coefficient of determination, R^2 , adjusted coefficient of determination, R^2_{adj} , and coefficient of prediction, Q^2) for median size ($\hat{y}_{\text{size}}$) and yield ($\hat{y}_{\text{yield}}$) are given in Table 3. The separation of R^2 and Q^2 is much less than 0.2

and R^2 , R^2_{adj} , and Q^2 are all greater than 0.9, which indicate excellent models for median size and yield.

The estimated coefficient effect of the parameters and their interactions on the responses are shown as coefficient plots in Figure 2. The plots interpret the coefficients for the models with a 95% confidence interval, and the data is centered and scaled to make coefficients comparable. The sizes of the coefficients represent the change in the response when a factor varies from a medium to a high level, while the other factors are kept at their averages. Coefficients of H_2SO_4 concentration (β_2), hydrolysis time (β_3), temperature of hydrolysis (β_4), and interaction of H_2SO_4 concentration and hydrolysis time ($\beta_2\beta_3$), all had p -values bellow 0.05 for both models, confirming significance. As expected, enhancement of H_2SO_4 concentration (x_2), hydrolysis time (x_3), and

Table 3. Model statistics for median size ($\hat{y}_{\text{size}}$) and yield ($\hat{y}_{\text{yield}}$).

Statistics	$\hat{y}_{\text{size}}$	$\hat{y}_{\text{yield}}$
R^2	0.977	0.974
R^2_{adj}	0.970	0.966
Q^2	0.955	0.944

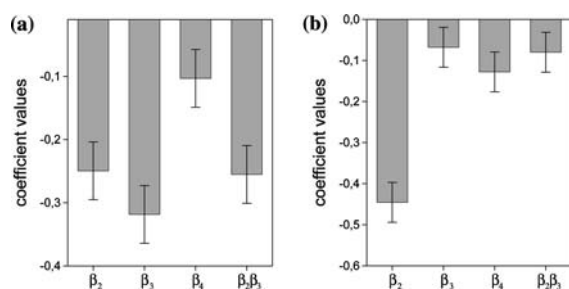


Figure 2. Main effects seen in a coefficient plot for (a) the median particle size and (b) the yield with a confidence interval of 0.95. Coefficients are H_2SO_4 concentration (β_2), hydrolysis time (β_3), temperature of hydrolysis (β_4), and interaction of H_2SO_4 concentration and hydrolysis time ($\beta_2\beta_3$).

temperature of hydrolysis (x_4) seemed to have a positive effect in decreasing the MCC size but at the expense of yield. More surprising is that sonication time (x_5) as single factor was not important. The importance of sonication for the separation of the cellulose is unclear from this investigation. Most probably the sonication equipment used in this study was not efficient enough to separate the cellulose whiskers. Marchessault et al. (1961) saw that ultrasonic vibration merely effected the deaggregation of side-by-side hydrogen bonded cellulose particles and used a sonication time of 30 min. Dong et al. (1998) obtained results that showed that a brief ultrasonic treatment (5 min) was adequate to disperse cellulose whiskers generated by sulfuric acid hydrolysis, and that further sonication was counterproductive. Start concentration of MCC did not seem to have an effect on either median size or yield, which proves that acid is largely in excess in the chosen ranges. Looking at the interaction of factors it is seen that H_2SO_4 concentration and hydrolysis time ($x_2 x_3$) have a noticeable influence on median size and yield. An enhanced sulfuric acid concentration is not enough for isolating the whiskers, an increase in hydrolysis time is also required.

Results not included in model

As a reference for the hydrolyzed MCC, the particle size for unhydrolyzed MCC was also measured by laser granulometry and had a median particle size of 37.24 μm . Comparing this result with the median particle size of sample 3

(38.68 μm), it is clear that sample 3 was correctly identified as an outlier.

Conductometric titration results (Table 2) showed that the esterification of hydroxyl groups by sulfate ions is very dependent on the treatment conditions. For example, using the lower limits of H_2SO_4 concentration, time and temperature of hydrolysis, no detection of strong acid groups was made. As the H_2SO_4 concentration, time and temperature of hydrolysis were raised, a gradual increase of amount of sulfate groups was seen. Using the high limit for H_2SO_4 concentration and time ($x_2=1$ and $x_3=1$), but the low limit for temperature ($x_4=-1$), a value of 354 and 330 mmol sulfate groups/kg cellulose was obtained for samples 7 and 15, respectively. This is notably higher than what has been reported by Araki et al. (1998, 2000), 84 and 140 mmol/kg.

Optical light microscope investigations give a clear indication of how well the MCC aggregates have been reduced in size with different treatment conditions. Analysis methods, such as TEM, show the structure down to nanolevel, but in these magnifications information about the suspension in general might be lost. Nanosized cellulose whiskers might very well be present in a suspension with lots of MCC agglomerates. In Table 2 the results from the optical light microscope investigation are given and categorized as numerous, few, and none particles seen in the samples. Samples treated with the lowest H_2SO_4 concentration ($x_2=-1$) at the lowest temperature ($x_4=-1$) showed a wide dispersion of particle sizes, including spherical aggregates similar to those seen in untreated MCC. As expected, intensifying the treatment conditions (i.e. increasing sulfuric acid concentration, temperature and time of hydrolysis) gave suspensions with decreasing particle sizes. From investigations of samples 7 and 15 (the samples with the best characteristics in terms of particle size measurements), no particles were distinguishable, even at the lowest magnification. This is evidence that the cellulose material is close to nanometer size, because the resolution in the light microscope is too poor.

A number of samples showed signs of flow birefringence, but just two samples, 7 and 15, clearly showed flow birefringence (Table 2). It is these same samples, according to median size analysis, that have the smallest particle size (whiskers).

Optimization

The models allow for the prediction of optimal treatment conditions regarding the isolation process for whiskers. The goal is an optimized isolation/separation of the cellulose whiskers with a maximum yield in a minimum hydrolysis time, sonication time, and H_2SO_4 concentration. The allowed interval for the factors and the desired responses are given in Tables 4 and 5, respectively. The upper factor interval limit for H_2SO_4 concentration and hydrolysis time has been extended by 25% from the experimental design in order to get the global optimal point. Table 5 shows the desired interval for the responses. The most important criterion is the production of cellulose nanocrystals/whisker, which is why the interval provided for the median size, based on measurements from laser granulometry and verified with TEM, is so narrow. A maximized yield is also desired, but our experience with the experimental runs shows that a value higher than 45% (w/w) is not reasonable. Anything less than 25% (w/w) is not accepted. The optimizer in MODDE 7 uses a Nelder Mead simplex method with fitted response functions to optimize an overall desirability function combining the individual desirability, d_i , and weight factor, w_i , of each response:

$$\log(D) = \frac{\sum w_i \log(d_i)}{\sum w_i}$$

Several sets of optimal treatment conditions were proposed by the MODDE 7 software; the one

Table 4. Allowed factor interval for optimized treatment conditions.

	Low limit	High limit
x_1 , MCC conc.	5	15
x_2 , H_2SO_4 conc.	44.1	81.0
x_3 , Hydrolysis time	10	150
x_4 , Temperature	40	80
x_5 , Sonication time	10	30

Table 5. Response criteria for optimization.

	Criteria	Min	Target	Max
Yield	Maximize	25	45	
Median size	Target	0.05	0.15	0.5

that gave the best results from analysis (as discussed in the next section) is shown in Table 6 and is the global optimal point. The value of the logarithm of desirability coefficient, $\log(D)$, equals zero when all responses are between the target and the limits. When the values of all responses approach the target, $\log(D)$ becomes negative, which is the case for our chosen optimal treatment conditions. The predicted values for the responses along with 95% confidence intervals are given in Table 7.

Analysis of optimized sample

Diffraction patterns of untreated MCC and a sample prepared from the optimized treatment conditions are shown in Figure 3. In order to determine the crystallinity of the samples, the ratio of the crystalline area to the total area can be calculated. However, to determine the crystalline area, the amorphous part must be known. This can be obtained by X-ray diffraction of a completely amorphous cellulose sample. This was not done in this study, and therefore no value for crystallinity is provided. However, by comparing the intensities of the diffraction peaks of both samples, it is possible to determine a change in crystallinity. The intensity of the peaks in the diffraction pattern of the optimized sample was significantly higher compared to the peak intensities in the diffraction pattern of MCC. Thus, the crystallinity had increased as a result of the optimized acid hydrolysis treatment. This indicated that the microcrystalline cellulose contained both crystallites and also amorphous regions, which were removed by the acid hydrolysis. Both diffraction patterns, believed to represent typical cellulose I diffraction patterns, showed a peak at $2\theta = 22.6^\circ$ and a shoulder in the region $2\theta = 14^\circ$ to 17° with two not well-defined peaks at $\sim 15.5^\circ$ and $\sim 16.4^\circ$.

The median particle size from laser granulometry of the sample prepared from the optimized treatment conditions was $0.2 \mu\text{m}$, well within the 95% confidence interval.

The yield of the sample prepared from the optimized treatment conditions was 30% (of initial weight). This value differs a bit from the predicted value and is close to the lower limit, but is still inside the 95% confidence interval. One reason why such a low yield was obtained could be that

Table 6. Optimized treatment conditions proposed by MODDE 7 software.

Temperature (°C)	H ₂ SO ₄ conc. % (w/w)	Time (min)	MCC conc. (g/100 ml)	Sonication time (min)	log (<i>D</i>)
44.0	63.5	130.3	10.2	29.6	-1.021

Table 7. Predicted values for optimized treatment conditions with a 95% confidence interval.

	Predicted	Lower limit	Higher limit
Median size (μm)	0.262	0.015	0.569
Yield (%)	40.35	24.85	52.65

there was still a significant amount of amorphous regions left in the starting material, which disintegrated during the hydrolysis. This is seen from WAXD analysis. Also, the MCC used in this study has in effect been subjected to two hydrolysis steps, with an intermediate drying step between, which might affect the accessibility of the crystalline cellulose. Edgar and Gray (2002) prepared cellulose whiskers from cotton pulp and used approximately the same sulfuric acid concentration and temperature (64 wt% and 45 °C, respectively) as for the optimized treatment conditions in this study. However, in their study a treatment time of 45 min was sufficient. Although the MCC most probably contains more crystalline cellulose than pulp the hydrolysis time is longer. As a consequence of the decreased accessibility, it is possible that some degradation of crystalline cellulose has occurred during the hydrolysis in order to obtain completely isolated whiskers.

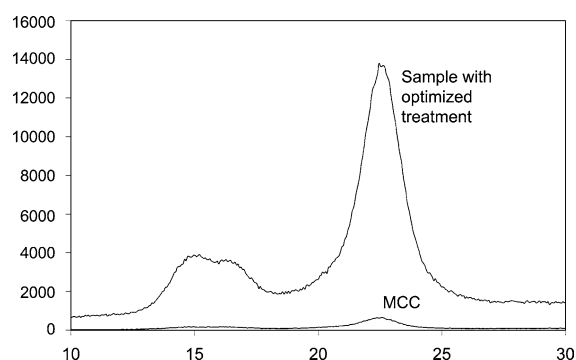


Figure 3. X-ray diffraction patterns of MCC and a sample produced with optimized treatment conditions.

Micrographs from optical light microscope at a magnification of 100× of the sample produced with the optimized treatment conditions and the untreated MCC are shown in Figure 4. No particles could be seen for the sample prepared with the optimized treatment conditions and the resolution in the light microscope is too poor to detect the cellulose particles. This indicates that there might be just nanosized cellulose whiskers in the suspension produced with the optimized treatment conditions.

Figure 5 shows a TEM image of the sample prepared with the optimized treatment conditions. Measurements from TEM images gave whiskers with a length between 200 and 400 nm and a width

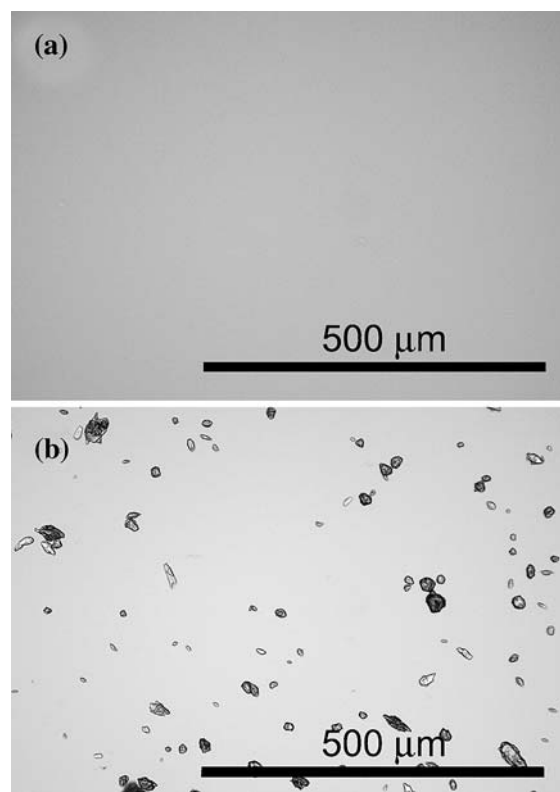


Figure 4. (a) Sample produced with optimized treatment conditions and (b) untreated sample seen at 100× in light microscope. Agglomerates of cellulose are seen for untreated MCC but are abundant in the optimized sample.

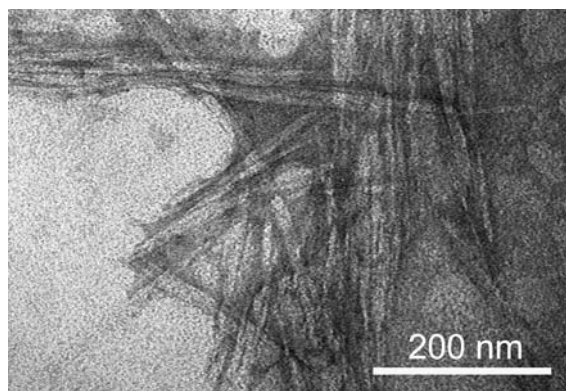


Figure 5. TEM image of the sample prepared with the optimized treatment conditions.

less than 10 nm. In Figure 5, the whiskers appear to be agglomerated, which most probably occurred during sample preparation that involved evaporation of water.

Figure 6 shows clear flow birefringence for the sample prepared from the optimized treatment

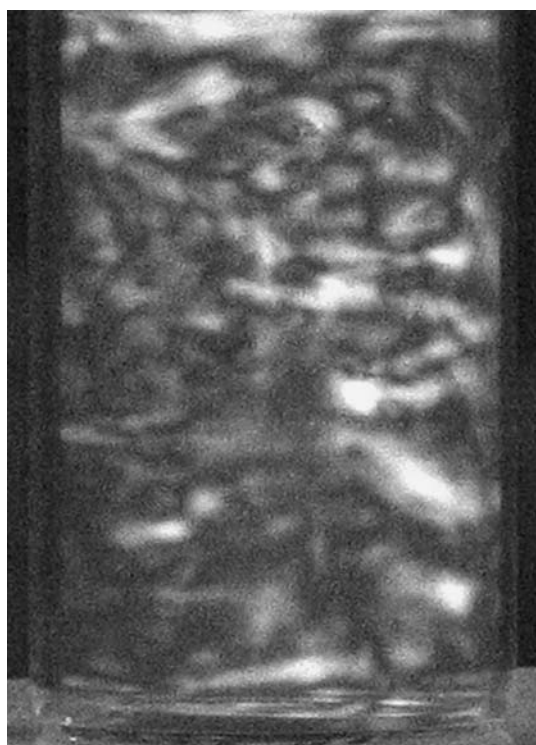


Figure 6. Flow birefringence for optimized sample seen between two crossed polarizing films. (Container is 24 mm in diameter).

conditions with a concentration of 0.1 g cellulose/100 ml in cross polarized light. This indicates a nematic liquid crystalline alignment and reveals the existence of whiskers (Orts et al. 1998).

Conclusions

The use of a response surface methodology in order to establish predictive models for median size and yield of sulfuric acid hydrolyzed MCC from Norway spruce (*Picea abies*) was very valuable. The time and temperature of hydrolysis together with sulfuric acid concentration are very important single factors in the process in order to obtain a suspension of negatively charged isolated cellulose whiskers in water. There is also an apparent interaction between hydrolysis time and H_2SO_4 concentration to consider. Both an enhanced sulfuric acid concentration and an increased hydrolysis time is required for the whisker isolation process. With a sulfuric acid concentration of 63.5% (w/w), it was possible to produce cellulose whiskers with a length between 200 and 400 nm and a width less than 10 nm in approximately 2 h and with a yield of 30% (of initial weight). Disintegration of amorphous regions and degradation of crystalline parts during hydrolysis is probably the explanation for the low yield. The whiskers produced under these treatment conditions would well be suited for incorporation into a polymer using an extrusion process where nanobiocomposites are produced. Optimized process parameters would most probably have been different if cellulose from other sources or without an intermediate drying step of MCC had been used.

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