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Nanotechnologies — Characterization of cellulose nanocrystals

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National foreword

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Nanotechnologies — Caractérisation des nanocristaux de cellulose

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Foreword

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The committee responsible for this document is ISO/TC 229, *Nanotechnologies*.

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Introduction

Cellulose nanomaterials, including cellulose nanocrystals (CNCs) and cellulose nanofibrils, are anticipated to have significant commercial impact. Cellulose nanocrystals are extracted from naturally occurring cellulose, primarily from wood and annual plants, by acid hydrolysis, or chemical or enzymatic oxidation.[1][2][3] Their production from cellulose sources, such as wood pulps makes them a candidate for use as a potentially non-toxic, biodegradable and sustainable nanomaterial. Furthermore, the recent demonstration of the feasibility of CNC production on a large scale and the availability of infrastructure for harvesting raw materials will facilitate their commercial development. CNCs and cellulose nanofibrils are produced in a number of countries on pilot, pre-commercial or commercial scales. Estimates of the market potential for cellulosic nanomaterials are as high as 35 million metric tons annually, depending on the predicted applications and the estimated market penetration.[4][5] Standards for characterization of CNCs are required for material certification to allow sustained commercial development and applications.

Cellulose nanocrystals are nanorods that have high aspect ratio, surface area and mechanical strength and assemble to give a chiral nematic phase with unique optical properties. They are smaller than cellulose nanofibrils and have a higher crystalline content. These properties, plus the ability to control CNC surface charge and chemistry for dispersion in a variety of matrices, lead to potential applications in many areas including nanocomposite materials, paints and adhesives, optical films and devices, rheology modifiers, catalysts and biomedical products. There are currently no International Standards for this emerging commercial nanomaterial, although an ISO/TC 229 project on terminology is in progress, a Canadian National Standard (CSA Z5100) was published in 2014 and two CNC reference materials were released in 2013. This Technical Report reviews information on sample preparation, data collection and data analysis/interpretation for the measurands that are predicted to be important for the development of commercial products containing CNCs. Information for the following CNC properties is included: composition (crystallinity, surface functional groups, degree of polymerization and contaminants), morphology as assessed by microscopy and light scattering methods, surface charge and specific surface area, viscosity and thermal stability. The Technical Report reviews various approaches that have been used for specific properties, but does not recommend standard methods or provide detailed information on the techniques. The coverage is restricted to CNCs as produced and does not extend to post-production modified CNCs or CNC-enhanced materials or products.

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Nanotechnologies — Characterization of cellulose nanocrystals

1 Scope

This Technical Report reviews commonly used methods for the characterization of cellulose nanocrystals (CNCs), including sample preparation, measurement methods and data analysis. Selected measurands for characterization of CNCs for commercial production and applications are covered. These include CNC composition, morphology and surface characteristics.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
agglomerate
collection of weakly or medium strongly bound particles where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example, van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.3]

2.2
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent bonds, or those resulting from sintering or complex physical entanglement, or otherwise combined former primary particles.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.4]

2.3
nanocrystal
nano-object with a crystalline structure

[SOURCE: ISO/TS 80004-2:2015, 4.15]

2.4
nanofibre
nano-object with two external dimensions in the nanoscale and the third dimension significantly larger

Note 1 to entry: The largest external dimension is not necessarily in the nanoscale.

Note 2 to entry: The terms nanofibril and nanofilament can also be used.

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2.5
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-2:2015, 2.2]

2.6
nanorod
solid nanofibre

[SOURCE: ISO/TS 80004-2:2015, 4.7]

2.7
nanoscale
size range from approximately 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

Note 2 to entry: The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

[SOURCE: ISO/TS 80004-2:2015, 2.1]

3 Symbols and abbreviated terms

For the purposes of this document, the following symbols and abbreviated terms apply.

AEC	anion-exchange chromatography
AFM	atomic force microscopy
BET	Brunauer-Emmett-Teller (method for determination of specific surface area)
CrI	crystallinity index (also CI)
CNC(s)	cellulose nanocrystal(s)
CP-MAS	cross polarization magic angle spinning
d_h	hydrodynamic diameter
DP	degree of polymerization
D_t	translational diffusion coefficient
DSC	differential scanning calorimetry
DLS	dynamic light scattering
ε	dielectric constant
EM	electron microscopy
FE-SEM	field emission-scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy

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GLC	gas-liquid chromatography
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	inductively coupled plasma-optical emission spectroscopy
ID	isotope dilution
IR	infrared
k	Boltzmann constant
PI	polydispersity
ssNMR	solid state nuclear magnetic resonance
SEC	size exclusion chromatography
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy free radical
TGA	thermogravimetric analysis
U_E	electrophoretic mobility
η	viscosity
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

4 Production of cellulose nanocrystals (CNCs)

Cellulose is a linear polysaccharide composed of anhydroglucose units linked by an oxygen atom between the C1 and C4 carbons of adjacent glucose rings. In cellulose biosynthesis individual, polysaccharide chains are assembled by an enzyme complex into an elementary fibril with stacked chains held together by hydrogen bonding. The number and organization of polymer chains is specific to the organism. These elementary fibrils are further assembled to give larger structures that contain ordered (crystalline), as well as disordered cellulose and other components that depend on the organism.

Cellulose nanocrystals are formed from one or more elementary fibrils and contain primarily crystalline and paracrystalline regions. CNCs have length and cross-sectional dimensions that depend on the cellulose source with typical aspect ratios between 5 and 50 and do not exhibit branching or network-like structures. The term nanocrystalline cellulose is synonomous with CNCs and the term nanowhiskers has also been used frequently in the literature. Cellulose nanofibrils are typically larger than CNCs and are branched, entangled and agglomerated structures. The nanofibrils have crystalline, paracrystalline and amorphous regions and can contain non-cellulosic components. They have cross-sections between 5 nm and 50 nm and aspect ratios that are greater than 50. An ISO/TC 229 project[6] aimed at standardizing the terminology for cellulose nanomaterials has recently been initiated.

Cellulose nanocrystals are produced from a variety of cellulose sources, primarily wood and other plants, but also algae, bacteria and tunicates.[2][3][7][8][9][10][11][12][13] Their extraction from cellulose-containing biomass begins with mechanical and/or chemical pre-treatment to remove non-cellulose components, reduce the particle size and increase the exposed surface area. This is followed by a hydrolysis or oxidation step that digests the more reactive amorphous cellulose and liberates CNCs from the larger cellulose fibrils (Figure 1). Acid hydrolysis with sulfuric acid is the most widely used method for CNC production in both research laboratories and pilot scale commercial facilities, although other

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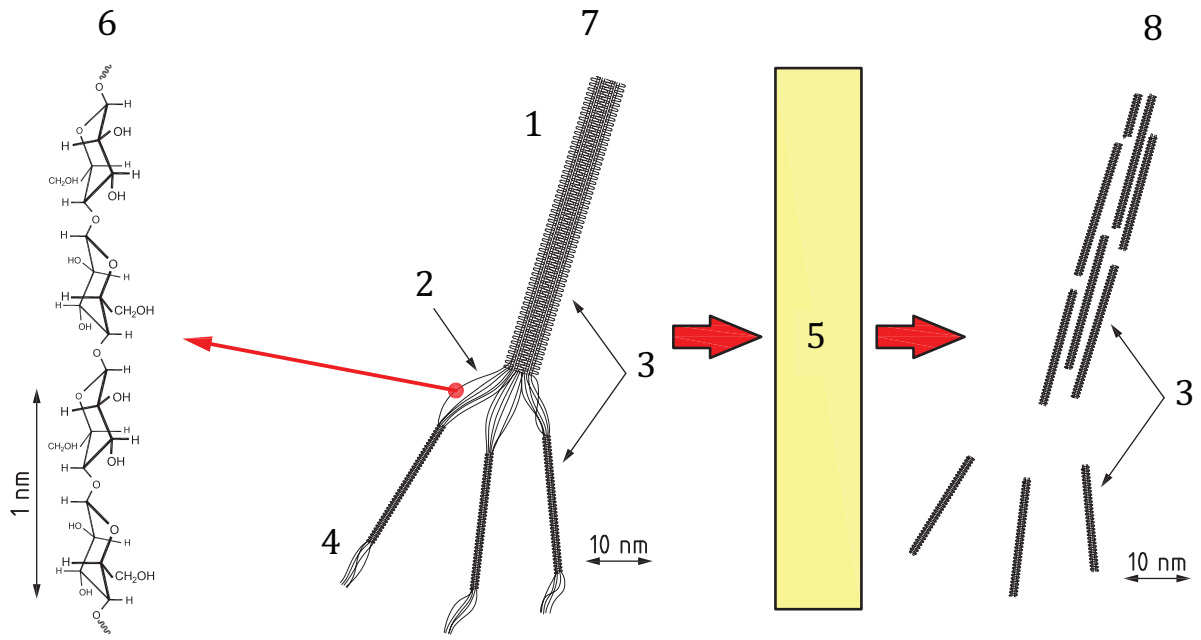
acids (e.g. hydrochloric, phosphoric, phosphotungstic) have also been employed.[2][3][7][8][9][14][15] In attempts to minimize the use of strong acids, a variety of other processes have also been examined including ultrasonication-assisted hydrolysis (with or without an iron chloride catalyst), enzymatic oxidation and ammonium persulfate oxidation.[16][17][18] After the acid hydrolysis or oxidation step, CNCs are purified by a combination of centrifugation or filtration and washing steps, followed by dialysis to remove residual salt and/or acids. A typical sequence for CNC production by acid hydrolysis is illustrated in Figure 2.



Key

1 micro-fibril
2 disordered
3 crystalline
4 elementary fibrils
5 hydrolysis or oxidation
6 cellulose
7 cellulose fibril
8 cellulose nanocrystals

Figure 1 — Cartoon description of the formation of CNCs from larger cellulose fibrils

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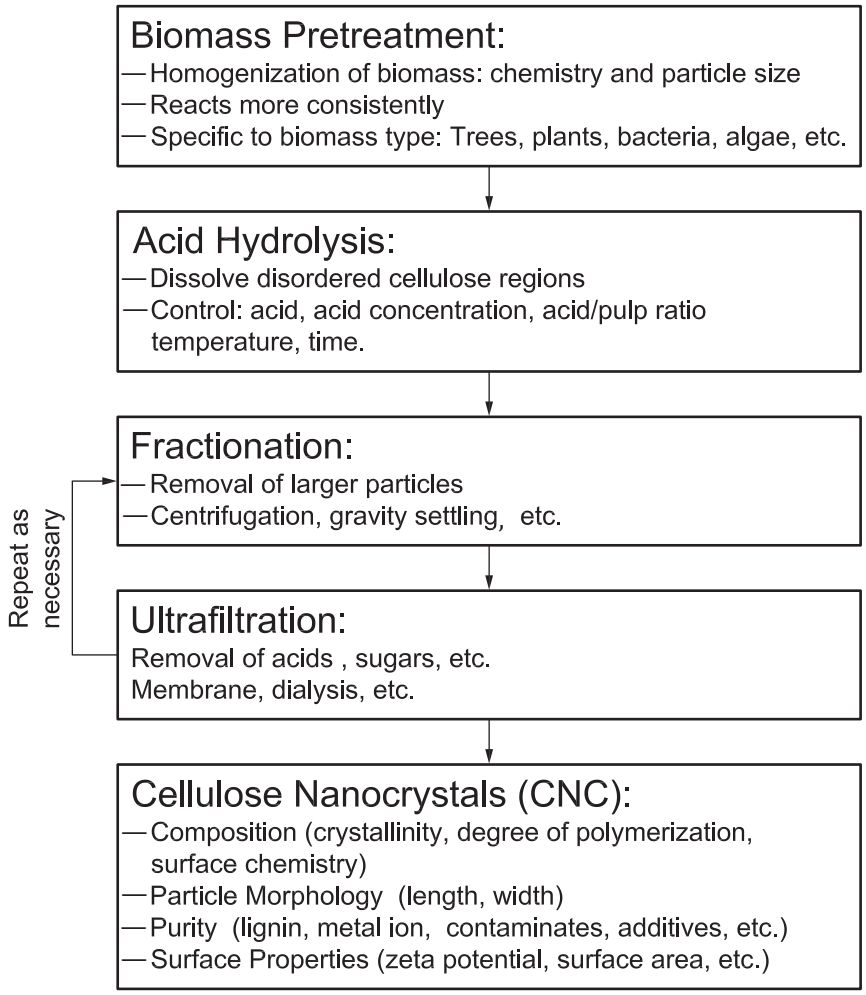

Figure 2 — Overview of a typical process for production of CNCs by acid hydrolysis

CNCs produced by sulfuric acid hydrolysis have negatively-charged sulfate half-esters on their surface which result in stable aqueous colloidal suspensions. Negatively charged CNCs are also formed by phosphoric acid hydrolysis, whereas hydrochloric acid gives uncharged CNCs with only surface hydroxyl groups. Oxidation catalysed by TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) can be used to convert surface hydroxyls to carboxylic acids for CNCs generated using either sulfuric or hydrochloric acid.[19][20] Oxidation with ammonium persulfate also generates carboxylated CNCs.[16] The CNC dimensions vary with the source of the cellulose; CNCs derived from wood pulps typically have average lengths of 100 nm to 200 nm and cross-sections of 4 nm to 9 nm, whereas those from bacterial and tunicate sources can be considerably larger, with lengths of 1 µm to 2 µm and cross-sections up to 50 nm (as reviewed in Reference [2]). The preparation method, acid or oxidant concentration, reaction time and temperature, and sonication steps during purification also affect the CNC dimensions and the overall yield and kinetics.[21][22][23][24][25][26][27]

The acidic CNC suspensions produced by acid hydrolysis can be used in never-dried form. However, in most cases the proton can be replaced by other cations by neutralizing the CNC suspension with aqueous bases, such as hydroxides (XOH) or carbonates (X_2CO_3), to give a salt form of the CNCs (X-CNC, where X is the *counterion* associated with the anionic group). The pH-neutral sodium form, Na-CNC, is most typically produced commercially and at large scale by in-line neutralization of H-CNCs with sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3). Advantages, such as the water-dispersability of the dried product,[28] allowing spray-dried or freeze-dried CNCs to be stored and shipped in the dry form at significantly lower cost and then re-suspended at the point of use, account for this preference. Proton counterions are most often exchanged for others by neutralization of the acidic groups with

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aqueous hydroxide bases,[29] but this can also be accomplished by treatment with the appropriate ion-exchange resin.[30]

Dry CNC samples are prepared from the initial aqueous suspensions by evaporation, oven-drying, freeze-drying (lyophilization), or spray-drying. Some characterization methods require dry samples, whereas others employ a dilute suspension of CNCs. If the CNCs are already available as an aqueous suspension, the sample can be diluted to the required concentration using deionized water or dilute buffer or salt (NaCl) solution. Dry samples can be redispersed in pure water; general guidelines for dispersion of powders in liquids can be found in ISO 14887[31]. Although an ultrasonic treatment step is typically used to break up aggregates and agglomerates, a lack of reproducibility might contribute to variability of results, as summarized in a recent study aimed at standardizing procedures for ultrasonic dispersion of nanoparticles.[32] It is not trivial to obtain redispersed samples of CNCs that have size distributions and levels of aggregates or agglomerates that are similar to those of a purified, but never-dried, sample. An early study showed that films of CNCs with fully protonated sulfate half-esters could not be redispersed after drying, whereas CNCs with monovalent cations, such as sodium were redispersed with mild ultrasonic treatment to give stable colloidal suspensions that were similar to those prior to drying.[29] Detailed procedures for the redispersion of the neutral sodium-form of CNCs prepared by evaporation, lyophilization or spray-drying have been reported.[28] The counterion and moisture content of the dry CNCs and the sonication conditions (energy, CNC concentration) were all shown to affect the CNC (re) dispersibility. While the sodium-form CNCs were fully dispersible when completely dried, the protonated CNCs were only fully dispersible above a threshold water content of 4 wt %.

In this Technical Report, emphasis is placed on CNCs manufactured using sulfuric acid, with sulfate half-ester groups on the cellulose surface (cellulose sulfate); unless otherwise noted, all examples are for this form of CNCs. This reflects the emphasis on this material, in both commercial and research laboratories. Most of the characterization methods are also applicable, in some cases with appropriate adjustments, to other chemical forms of CNCs or cellulose nanofibres. For example, the detection and quantification of surface functional groups is specific to the specific CNC production method. The nature of the CNC counterion is important for some measurands, notably determination of the surface charge due to sulfate half-ester or carboxylate groups by conductometric titration (see 5.2.1 and 5.2.2) and zeta potential (see 7.2). Unless otherwise mentioned, the particular counterion in the CNC sample does not affect the characterization methods discussed in this Technical Report.

Cellulose nanocrystals have specific physico-chemical properties associated with both the underlying cellulose particle and the surface chemistry imposed by its manufacturing process. At the point of commercialization, it is necessary to clarify the several descriptive systems that have been used in this field: the geometric forms in nanotechnology, the industrial production method, and the chemical form used in national regulations. All three are found in the recent approval under Canada's New Substances Notification Regulations[33] as it provides the following:

a) chemical description (cellulose, hydrogen sulfate, sodium salt with a total sulfur content greater than or equal to 0,5 % and less than or equal to 1,0 % by weight);

b) production method description (obtained from sulfuric acid hydrolysis of bleached pulp);

c) geometric description of length (nominal length of 100 nm ± 50 nm) and cross-section (cross-sectional dimensions of less than or equal to 10 nm). As suggested in ISO 12805, composition, length, diameter and surface area are the critical parameters to be considered first in setting specifications.[34]

5 Composition

5.1 Chemical composition

The chemical identity of CNCs as cellulose can be assessed by a qualitative identification test employed for microcrystalline cellulose; dispersion of dry CNCs in iodinated zinc chloride will result in a violet-blue colour.[35] Their composition can also be verified by elemental analysis, based on the formula $[(C_6H_{10}O_5)_n]$ and taking into account surface functional groups if their degree of substitution is known.

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Although elemental analysis provides some information on surface functionality (e.g. % S for sulfate half-esters), more detailed tests are typically used to quantify surface functional groups (see 5.2). The identity of inorganic metal counterions for CNCs with anionic surface groups can be determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) using the procedure outlined in 5.4.1 for sulfur. The density of CNCs has usually been assumed to be the same as other types of cellulose,[2] as confirmed by a recent determination of 1,56 g/cm^3 and 1,63 g/cm^3 for the densities of sulfated and unsulfated CNCs.[36]

5.2 Surface functional groups

5.2.1 Determination of sulfate half-esters

CNCs extracted by sulfuric acid hydrolysis have sulfate half-ester groups on their surface. The concentration of these negatively charged groups determines the CNC surface charge density and controls the colloidal stability of CNCs in aqueous suspension, along with the self-assembly behaviour and rheological properties. Two approaches have been used to determine the sulfate half-ester content. The first relies on measurement of total sulfur content by elemental analysis.[26][27] In cases where the sample has been purified to ensure removal of all residual unbound sulfate ions, the total sulfur content can be converted directly to the CNC sulfate half-ester content.[37] The second approach uses conductometric titration of the acidic sulfate half-ester groups on the CNC surface using an aqueous base. Both methods are described in this Clause, followed by a comparison of results for various CNCs.

Measurement of the total sulfur content can be accomplished by elemental analysis or by ICP-OES using a spectrometer equipped with a concentric nebulizer, a cyclonic spray chamber and a quartz torch with a quartz injector tube, optimized according to the manufacturer's specifications. The sample is completely solubilized by microwave assisted sample digestion using high purity nitric and hydrochloric acids in high pressure closed vessels or, by wet ashing with strong acids, such as nitric and perchloric.[38] A block digestion system can be used to evaporate excess acids after the initial digestion. Analyses are conducted by ICP-OES using the sulfur emission lines at 180,669 nm and 181,972 nm. Calibration is accomplished by the method of additions (to compensate for any residual matrix interferences) in which incremental spikes of sulfur as sulfate are prepared from a standard sulfur solution (e.g. a primary standard, such as NIST SRM 3154). Samples can be diluted to ensure linearity of response. Samples are gravimetrically spiked with at least two incremental levels of appropriate amounts of known calibration standard. The levels should be chosen such that the spike results in a one to twofold increase in the total sulfur concentration in the sample with each spike and the analytical response is linear. The calculation of the concentration of sulfur requires a three point (minimum) standard additions calibration. Since the slope of the standard additions calibration function for the sample and blank are likely not equivalent, separate calibrations for C_s and C_{blk} should be performed. Note that although total sulfur content can also be obtained by inductively coupled plasma mass spectrometry (ICP-MS, see 5.6.3) ICP-OES is the more reliable and straightforward method unless the sulfur concentration is very low.

The sulfate half-ester content can be determined by conductometric titration of the acidic sulfate half-ester groups on the CNC surface using an aqueous base, such as sodium hydroxide.[30][39] This is the most commonly used method for this purpose.[40] The sample should first be purified by extensive dialysis to remove any residual ions and then treated with H-form strong acid cation-exchange resin to ensure fully protonated sulfate half-esters. Commonly, CNC samples are diluted or re-dispersed with deionized water (typically to ≤1,5 wt %) and dialyzed against pure water until the pH and conductivity of the water surrounding the membranes no longer changes and approaches that of pure water. Hollow-fibre membrane dialysis systems can also be used, they reduce dialysis time by drastically increasing the exchange surface area and maintaining a large concentration gradient via counter-current sample and dialysate flow. Prior to CNC protonation, the H-form strong acid cation-exchange resin should be rinsed with a large excess of pure water until the filtrate is colourless and identical in pH and conductivity to the wash water. A large excess of resin should be added to dialyzed CNC suspension at a sufficiently low CNC concentration to ensure no coagulation, and the sample shaken to ensure uniform mixing. The resin is then removed by filtration. Multiple successive treatments with fresh resin might be required to achieve full protonation, particularly if the CNCs are in neutral salt form. Alternatively, passing the diluted suspension through a column filled with such resin results in faster treatment.[37] Such treatment ensures complete protonation of a pure CNC suspension containing

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no residual dissolved ions, yielding a 1:1 ratio of sulfate half-esters to protons and ensuring accurate titration results. The concentration of the final protonated CNC sample is determined gravimetrically, a sample of known volume is weighed and placed in a beaker with a dilute salt (NaCl) solution and titrated against dilute sodium hydroxide using titration conditions optimized from the literature.[30] [41] The sample conductivity is measured once the sample has equilibrated after the addition of each aliquot of NaOH. Equivalence points are determined from the intersection of the regression lines fit to the data points in the distinct regions of the titration curve (Figure 3).

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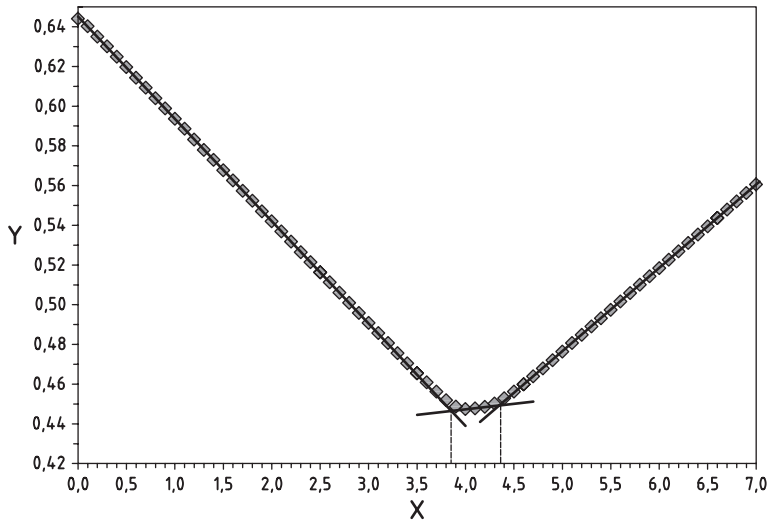
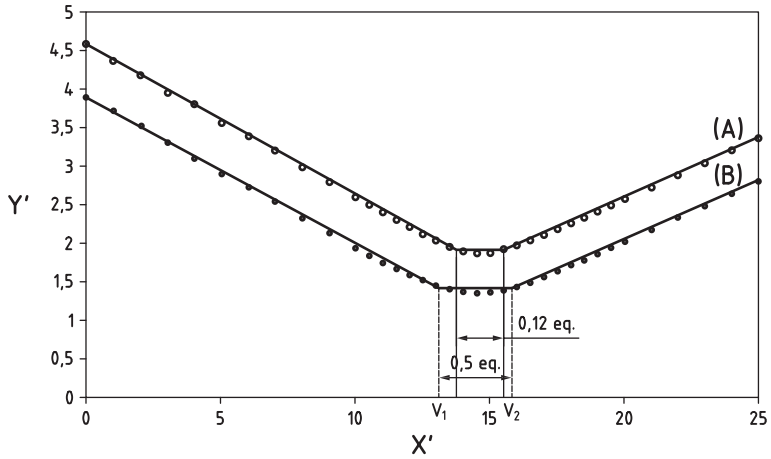


a) H-CNCs containing strong acid sulfate half-ester groups and a small quantity of weak acid carboxylic acid groups



b) TEMPO-oxidized CNCs generated with two different concentrations of oxidant

Key

X volume NaOH added (ml)

Y conductivity (µS/cm)

X′ volume NaOH (ml)

Y′ conductivity (mS/cm)

SOURCE Beck et al. 2014 and Habibi et al. 2006.[37][42]

Figure 3 — Schematic illustration of conductometric titration curves

When interpreting conductometric titration data, it is important not to confuse the protons that are actually detected (by neutralization with sodium hydroxide) with the sulfate half-ester content that is calculated from the titration results. The calculations are based on the assumption that the protons in the sample are in a 1:1 ratio with the sulfate half-ester groups. Despite this caveat, the titration method does not require specialized and expensive equipment, and can be very useful for quality control during CNC production.

Typical sulfate half-ester and sulfur content values for wood-based and other CNCs measured by titration and elemental analysis are shown in Table 1. The differences between values obtained by these two approaches have been discussed in the literature for CNCs of varying degrees of purification.

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[30][37][39][43][44] Titration values are often lower than those found by elemental analysis;[40] this is primarily due to insufficient sample preparation of the CNCs analysed by titration, notably failure to ensure that the CNCs are fully protonated following purification by dialysis. Sodium-form CNCs are an extreme example for which the sulfate half-ester content would not be measurable by conductometric titration but would be by elemental analysis, such as ICP-OES. Treatment with mixed bed ion-exchange resin (which contains hydroxide form anion exchange resin) has also been found to remove elemental sulfur from CNC samples, and as such it is recommended to completely avoid the use of mixed bed ion-exchange in CNC suspension purification; only dialysis should be used.[37] Alternatively, the presence of contamination in the form of sulfur-containing species, such as sulfate ions will yield erroneously high elemental analysis (and conductometric titration, if they are protonated) results. This illustrates the importance of dialysis for CNC suspension purification.

Differences between sulfate half-ester/sulfur contents measured by titration and elemental analysis methods, respectively, might also be caused by the presence of sulfate half-ester groups that are inaccessible to titrant[39] or other forms of sulfur, introduced during biosynthesis of the source cellulose.[45] Owing to these discrepancies, if elemental analysis is used to estimate CNC surface charge from sulfate half-esters, it is recommended to perform concurrent elemental analysis of the cellulose source of the CNCs being studied to obtain an estimate of sulfur. It is important to understand that total sulfur (measured by elemental analysis methods, such as ICP), titratable sulfur (protonated sulfate half-ester groups that are accessible to titrant), sulfate half-ester content contributing to surface charge (all surface sulfate half-ester groups) and total sulfate half-ester content (sulfate half-ester groups that are titrant-accessible and -inaccessible, if any) are not necessarily equivalent values. However, a recent study has shown that total sulfur and titratable sulfur are equivalent for softwood kraft pulp-derived CNCs, indicating that all sulfate half-esters are at the surface.[37] In general, elemental analysis of the source cellulose and CNCs combined with conductometric titration of protonated CNCs will give the most complete picture. Elemental analysis of CNCs extracted from the same source by HCl hydrolysis might also be helpful in determining the "base sulfur content" of the CNCs. The above recommendations are particularly important if knowledge of the precise quantitative surface charge or sulfur content is required. Sulfur contents determined by the different methods typically vary by no more than around 0,1 wt %, provided the CNCs contain no sulfur-containing impurities and are fully protonated if required.[37]

An additional complication in assessing sulfate half-ester content arises for samples that have been extracted by sulfuric acid hydrolysis and then subjected to TEMPO-catalysed oxidation to generate surface carboxylic acids. It is difficult to measure sulfate half-ester (strong acid) content by conductometric titration if significant levels of weakly acidic carboxylic acids are present, but sulfate half-esters can be determined in the presence of low quantities of carboxylic acid groups as shown in Figure 3 a) for a non-oxidized CNC sample with a small number of weak carboxylic acid groups.[39][43]

Finally, the surface sulfur content has also been measured by X-ray photoelectron spectroscopy (XPS).[20][40][46][47][48][49] Values typically range between 0,3 to 0,6 atomic % *S*, although in a few cases the sulfur content was reported to be too low to be detected. In several examples, the surface sulfur content has been compared to conductometric titration results for CNCs with sulfur content similar to the data in Table 1.[20][40][48] In one study, both methods showed lower sulfur content for desulfated CNCs than for the initial CNCs obtained by sulfuric acid hydrolysis.[40] However, quantitative agreement was poor, with the sulfur content showing a sixfold change by titration but only a twofold change by XPS. Although the film thickness was not reported, one should in principle obtain the same sulfur content for the two methods when films of a single monolayer of CNCs are measured by XPS, since the depth penetration of ~10 nm is greater than the particle cross-section (see 5.6.4 for more details on XPS measurements of CNCs).

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