

General procedure for determining cellulose nanocrystal sulfate half-ester content by conductometric titration

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Abstract Charged groups on the surface of cellulose nanocrystals (CNCs) control the colloidal stability and electrostatic and rheological properties of aqueous CNC suspensions, as well as their ability to self-assemble into liquid crystalline structures with unique optical properties. CNCs extracted from wood pulp by sulfuric acid hydrolysis typically contain 200–300 mmol/kg of anionic sulfate half-esters introduced at some of the hydroxyl sites. Two analysis methods to determine CNC surface charge are presented in the published literature: total sulfur content determination by elemental analysis and protonated sulfate half-ester group determination by conductometric titration with sodium hydroxide. The main drawbacks to elemental analysis are the expensive and complicated instrumentation and sample preparation procedures it requires. Conductometric titration is a much simpler method, but requires complete protonation of the CNC samples in order to obtain an accurate value. Unfortunately, significant discrepancies between the sulfate half-ester contents measured with the two techniques are often observed in the literature, particularly with neutralized Na-CNCs. There are specific assumptions and pitfalls inherent to both analysis methods which must be taken into account and reconciled if comparable results are to be achieved by titration and elemental

analysis. In particular, sample preparation is crucial to obtaining accurate determinations of sulfate half-ester content by conductometric titration; however, methods differ widely among laboratories and are often not specified in detail, rendering published results meaningless. We have developed a rapid sample preparation protocol which allows quantitative and accurate determination of the sulfate half-ester content of CNCs from various cellulosic feedstocks (for H- and Na-CNCs, in both never-dried and redispersed dried forms) by conductometric titration, yielding results in good agreement with the total sulfur content determined by elemental analysis.

Keywords Cellulose nanocrystals · Sulfate half-esters · Conductometric titration · Ion exchange · Dialysis · Elemental analysis

Introduction

Cellulose nanocrystals (CNCs) are extracted from naturally occurring cellulose, primarily from wood and annual plants by acid hydrolysis. As a non-toxic, biodegradable and sustainable nanomaterial, they have evolved from a laboratory curiosity to an industrial material which has attracted considerable attention. CNCs are produced in a number of countries at pilot or pre-commercial scale and recently at commercial scale by CelluForce (Windsor, Québec).

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At the pilot and commercial scales, CNCs are typically extracted from bleached chemical wood pulps by sulfuric acid hydrolysis (Lin et al. 2012; Brinchi et al. 2013; Lagerwall et al. 2014). During the hydrolysis reaction, anionic sulfate half-ester groups are grafted at the sites of a fraction of the hydroxyl groups on the CNC surface, imparting a negative surface charge to the nanoparticles (Marchessault et al. 1961a, b).

The colloidal stability (and dispersibility) of CNCs in water is strongly dependent on charge-derived electrostatic repulsion between the nanocrystals (Beck et al. 2012; Zhong et al. 2012; Araki 2013). In addition, some of the unique exploitable properties of CNCs, such as their chiral nematic liquid crystal formation and self-assembly properties (Dong et al. 1996; Beck-Candanedo et al. 2005; Beck et al. 2011), rheological behaviour (Araki et al. 1999; Hasani et al. 2008; Shafiei-Sabet et al. 2012, 2013; Wu et al. 2014), and some surface modification properties (Salajková et al. 2012) are controlled by charged surface groups. The thermal stability of CNCs (Roman and Winter 2004) is also determined by the nature of the charged groups and their counterions.

Anionic surface groups on CNCs are most often either sulfate half-ester or carboxylate moieties. Sulfate half-ester groups are strong acids, and are always negatively charged in aqueous suspension. CNCs produced by sulfuric acid hydrolysis of wood pulp usually contain between 240 and 330 mmol sulfate half-esters per kg (Dong et al. 1996; Araki 2013). Carboxylate groups are weak acids, such that the surface charge of carboxylated CNCs is very pH dependent. CNCs extracted by HCl or sulfuric acid hydrolysis contain small amounts (ca. 20–30 mmol/kg CNC) of carboxylate groups (Araki et al. 1998, 1999), which can be greatly increased ($\leq 1,200$ mmol/kg CNC) by subsequent TEMPO-catalyzed oxidation of the primary hydroxyl groups (Araki et al. 2001). In addition, an oxidative process using ammonium persulfate produces highly carboxylated CNCs (Leung et al. 2011, 2012), and the hydrolysis of cotton with phosphoric acid yields slightly phosphorylated CNCs (Camarero Espinosa et al. 2013), both of which, while having a negative surface charge, do not contain sulfate half-ester groups.

Charged (ionic) sulfate half-ester groups at the CNC surface can be measured either indirectly as total elemental S content or directly as R-OSO₃⁻ content

(Marchessault et al. 1961b; Revol et al. 1994; Araki et al. 1998, 1999). There are inherent assumptions for both methods which must be taken into account.

Indirect CNC surface charge measurement employs elemental analysis to determine the total sulfur content of the CNCs after complete acid digestion. No specific sample pre-treatment is required other than purification (e.g. by dialysis) to remove free residual sulfate salts or acids. Using this method, all of the sulfur atoms in the CNC sample are measured; however, equating this quantity with the surface charge assumes that every sulfur atom is in the form of an anionic sulfate half-ester on the surface of the nanocrystal, which has not been previously unequivocally demonstrated. However, the major drawbacks to this method precluding its widespread use are the time-consuming, complicated, and expensive sample preparation and analysis procedures which require specialized chemicals and instrumentation which are not as widely available as the simpler materials needed for conductometric titration, particularly in industrial production facilities.

To date, CNC surface charge is most commonly directly quantified by conductometric titration of the acidic sulfate half-ester moieties against sodium hydroxide (Jiang et al. 2010). This method has several advantages, including faster sample preparation and analysis, simple and inexpensive instrumentation, and the ability to distinguish between strong and weak acid groups. The nature of the method gives rise to an inherent limitation: because it is the protons associated with the sulfate half-esters that are measured, and not the charged groups themselves (assuming one H⁺ per R-OSO₃⁻), other species such as OH⁻ may interfere with their detection. If not all the sulfate half-ester groups are protonated, a low reading will be obtained; if excess acid of any kind is present, a high reading is obtained. Sample preparation is therefore vital to obtaining reliable and accurate conductometric titration results (Abitbol et al. 2013).

Work published in the scientific literature usually employs one of the two analysis methods above (Marchessault et al. 1961b; Araki and Kuga 2001; Wang and Roman 2011) and makes the corresponding assumptions; where both methods are used together, discrepancies are observed between results obtained with the two methods above (Araki et al. 1998, 1999; Dong et al. 1998; Abitbol et al. 2013), making it a challenge to compare results among laboratories.

Because surface charge is a fundamental CNC property, there is a strong need to reconcile the two methods.

Some authors have suggested that sulfated CNCs contain sulfate half-ester groups that are not accessible to titrant (Araki et al. 1999) or that other non-acidic forms of sulfur are present in the native cellulose source, introduced during biosynthesis (Araki et al. 1999; Gu et al. 2013). In practice, many of the discrepancies between elemental analysis and titration results observed for CNCs appear mainly to be caused by a lack of full protonation of the samples prior to titration (Araki et al. 1998, 1999; Dong et al. 1998; Wang et al. 2007; Abitbol et al. 2013); elemental analysis typically yields slightly higher sulfur contents than conductometric titration. In many publications no protonation step is carried out following dialysis; despite this, “sulfur content” values based on titration are often reported (Araki et al. 1998, 1999; Wang et al. 2007). This is particularly important for CNC suspensions in the sodium form (Na-CNCs), which retain some sodium counterions after dialysis (Revol et al. 1998).

In the present paper, we re-evaluate and optimize CNC pre-treatment steps to ensure reproducible conductometric titration results which can be reliably related to total sulfur contents obtained by elemental analysis methods, particularly for sodium form Na-CNCs. The two main sample preparation steps are (1) purification, to remove free acid and other residual ionic contaminants so the only cations present are counterions associated with the sulfate half-ester groups; and (2) protonation, to allow detection of all the acidic sulfate half-ester groups during neutralization with NaOH for accurate results.

Cellulose nanocrystal suspension purification prior to titration has been performed by dialysis, mixed bed ion-exchange resin treatment, or a combination thereof (Dong et al. 1996, 1998; Araki et al. 1998, 1999; Wang and Roman 2011; Abitbol et al. 2013). Ensuring full protonation is more problematic, as one cannot simply add an excess of strong acid such as HCl as in the case of weak carboxylic acid group determination (Katz et al. 1984) because sulfate half-esters are strong acids and cannot be distinguished from the HCl by titration. It has been pointed out that inconsistent ion-exchange conditions used in the literature lead to inaccurate results, particularly for titration (Abitbol et al. 2013). Analysis by titration of Na-CNCs is

particularly challenging; however, this obstacle must be overcome given that spray-dried sodium form CNCs are produced at the commercial scale. Recently, solutions of phosphorylated cellulose were treated with a strong acid cation-exchange resin to convert the anionic groups to their protonated form prior to potentiometric and conductometric titration analysis (Sufflet et al. 2006). Strong acid cation-exchange resins have been used to convert Na-CNCs to protonated H-CNCs for conductometric titration, although the treatment was not optimized (Kloser and Gray 2010).

Data from conductometric titration (which measures titratable $S = R-OSO_3H$) in conjunction with data from elemental analysis by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (which measures total S) may also shed light on the physical distribution and nature of the sulfur-containing moieties in CNCs. Solving this matter may lead to a better understanding of CNC properties and surface chemistry which would be useful in developing new applications.

Experimental methods

Cellulose nanocrystal extraction

Northern bleached softwood kraft (NBSK) CNC suspensions were prepared in the FPIInnovations cellulose nanocrystal pilot plant using a commercial bleached softwood kraft pulp according to a sulfuric acid hydrolysis reaction procedure modified from the literature (e.g., Dong et al. 1998). Milled dried pulp (5 kg o.d.) was added to preheated ~ 64 wt% sulfuric acid (~ 6 mL acid/g pulp) at 45 °C, and mixed for 25 min. The reaction was quenched with reverse osmosis deionized (DI) water ($10\times$ acid volume), centrifuged and dialyzed to remove residual acid, and then homogenized to ensure good dispersion. The resulting suspension was filtered, neutralized with sodium hydroxide if desired, and concentrated to around 5 wt% CNCs (determined gravimetrically).

Northern bleached softwood kraft CNC suspensions were produced at 45, 50 and 65 °C. The sulfuric acid concentration was decreased by a few percent from 64 wt% H_2SO_4 used at 45 °C (Revol et al. 1998) to maximize the yield for the hydrolyses performed at higher temperatures (Hamad and Hu 2010). Increasing the hydrolysis temperature also increases the sulfate

half-ester content of the resulting NBSK CNCs: typical values are 210–220, 230–240, and 250–270 mmol/kg CNC at hydrolysis temperatures of 45, 50 and 65 °C, respectively.

A northern bleached softwood sulfite pulp (NBSS) CNC suspension was prepared in the FPIInnovations pilot plant using a commercial softwood dissolving pulp (Temalfa). Milled dried pulp (5 kg o.d.) was added to 63 wt% sulfuric acid (9 mL acid/g pulp) heated to 48 °C, and mixed for 25 min. The reaction was quenched with DI water (10× acid volume), and purified and homogenized as described above. The resulting suspension was filtered and concentrated to around 5 wt% CNCs.

A lab-scale batch of sisal CNCs was also prepared at FPIInnovations from milled dried bleached sisal fibre (40 g o.d.), which was added to 63 wt% sulfuric acid (8.75 mL acid/g fibre) at 45 °C and mixed for 35 min. The reaction was quenched with DI water, decanted, then centrifuged and dialyzed against DI water in a regenerated cellulose membrane (Spectra/Por 4) with a MWCO of 12–14 kDa. After homogenization, the suspension was filtered with a GF/F glass filter.

Cellulose nanocrystals produced at other facilities were generously provided as follows: Northern bleached softwood kraft (NBSK_{CF}) CNCs from René Goguen of CelluForce; northern bleached softwood kraft (NBSK_{AITF}) CNCs from Christophe Danumah of Alberta Innovates Technology Futures (AITF); southern softwood sulfite pulp (SBSS) CNCs from Alan Rudie of the United States Forest Products Laboratory (USFPL); and cotton (Buckeye) CNCs from Bruno Jean of the Centre de Recherches sur les Macromolécules Végétales (Cermav).

Cellulose nanocrystal neutralization and drying

Some NBSK CNCs were neutralized prior to drying. In these cases, aqueous NaOH (Sigma-Aldrich) was added to fully protonated acidic CNC suspensions until a stable pH of 7 was reached. Neutral Na-CNC suspensions (~5 wt%) were frozen at –65 °C and then lyophilized (VirTis Freezemobile 12SL) under vacuum (<50 mTorr) at room temperature to give white flaky solid freeze-dried (FD) CNCs. Alternatively, Na-CNC suspensions were spray-dried (atomizing inlet temperature 175 °C; outlet temperature 75 °C) to give white spray-dried (SD) CNC powder

similar to the dried CNCs produced at the commercial scale.

Cellulose nanocrystal pre-treatment conditions

The influence of sample pre-treatment conditions on the CNC sulfate half-ester content obtained by conductometric titration was investigated. Various combinations of dialysis, mixed bed (MB) ion-exchange resin (1:1 H⁺:OH⁻ forms), and strong acid cation (SAC) exchange resin (H⁺ form) treatments were applied to the CNC samples listed in Table 1.

Dialysis

Dialysis methods are typically used to purify CNC suspensions by removing dissolved ions, including residual sulfuric acid from hydrolysis, from the aqueous phase (Brinchi et al. 2013). Unless otherwise specified, CNC samples were diluted or re-dispersed with DI water to ≤1.5 wt% and extensively dialyzed (Spectra/Por 4 membrane, 12–14 kDa MWCO) against running DI water for at least 3 days to eliminate any free residual sulfate ions. Use of hollow-fibre membrane dialysis systems can greatly reduce dialysis time by drastically increasing the exchange surface area and maintaining a large concentration gradient via counter-current sample and dialysate flow. Suspensions of redispersed dried CNCs were sonicated to an energy input of 10 kJ/g CNC prior to further treatment.

Ion-exchange resin treatment of CNCs

Dowex Marathon C hydrogen form strong acid cation (SAC) exchange resin and Amberlite MB 6113 hydrogen and hydroxide form mixed bed (MB) ion-exchange resin (Sigma-Aldrich and Fluka Analytical, respectively) were used after thorough rinsing with ultrapure (milliQ) water.

In addition to dialysis, MB resins have also been used to further deionize CNC suspensions after dialysis by replacing all residual cations and anions with protons and hydroxide ions, respectively (Dong et al. 1998). Removal of any ions not associated with the sulfate half-ester groups at the CNC surfaces ensures that they are not exchanged for H⁺ during the protonation step, which would result in overestimation of the surface charge.

Table 1 CNC samples analyzed in this work

Sample ^a	Facility	Counterion	Drying method ^b
NBSK-A ₅₀ ^c	FPIInnovations	H ⁺	ND
NBSK-B ₅₀	FPIInnovations	Na ⁺	FD
NBSK-C ₄₅	FPIInnovations	Na ⁺	SD
NBSK-D ₅₀	FPIInnovations	Na ⁺	SD
NBSK-E ₆₅	FPIInnovations	Na ⁺ ^d	ND
NBSK _{CF}	CelluForce	Na ⁺	SD
NBSK _{AITF}	AITF	Na ⁺	ND
NBSS	FPIInnovations	H ⁺	ND
SBSS	USFPL	Na ⁺	ND
Sisal	FPIInnovations	H ⁺	ND
Cotton	Cermav	H ⁺	ND

^a Hydrolysis temperature of the NBSK CNC batch

^b ND never-dried, FD freeze-dried, SD spray-dried

^c Batch resin treatment; all others column treatment (see text)

^d Original never-dried H-CNC suspension sample neutralized to pH 7 with NaOH prior to dialysis/resin treatment

Hydrogen form SAC resin was used to remove sodium counterions and other cations and to fully protonate the CNC sulfate half-ester groups. Protonation was performed after mixed bed resin treatment as well as after dialysis. Sodium form CNC suspensions partially retain the Na⁺ counterions after dialysis, having a pH of around 4 after dialysis (Revol et al. 1998). H-CNC suspensions were also found to lose protons to a certain extent during dialysis. In dialyzed samples which have not been protonated post-dialysis, sulfur contents calculated from conductometric titration are significantly lower compared to those obtained by elemental analysis (Dong et al. 1998; Araki et al. 1999; Abitbol et al. 2013). Complete protonation of a pure CNC suspension containing no residual dissolved ions results in a 1:1 ratio of sulfate half-esters to protons, ensuring accurate titration results.

Batch and column methods were used to treat CNC suspensions with ion-exchange resin. The batch method is the addition of resin directly into the sample followed by mixing and removal of the resin by filtration; the column method is the flowing of the CNC suspension through a column containing a bed of the resin and collecting the treated eluate. Column treatment is significantly faster and more efficient than batch treatment (Bio-Rad Laboratories 2013) and is widely used in industrial applications such as water purification.

Batch method

The H⁺ form SAC exchange resin was rinsed with a large excess of ultrapure (milliQ) water until the filtrate was colourless and identical in pH to the wash water. Resin (1–18 g/g CNC, after rinsing and filtering) was added to dialyzed 1.0–1.5 wt% never-dried H-CNC suspension (sample NBSK-A₅₀) in sealed glass jars which were gently shaken to ensure uniform mixing. The resin was removed by filtration with GF/F glass microfiber filters (pore size 0.7 μm). Single treatments of 24 h duration were employed (Dong et al. 1998; Abitbol et al. 2013), as well as multiple successive treatments (2–5 × 2 and 6 × 0.5 h).

Column method

Strong acid cation and MB exchange resins were slurried in milliQ water and poured into glass columns (inner diameter 1.9 cm) with fritted glass disks at the bottom. The column was tapped to remove air bubbles and settle the resin. Excess water was drained slowly to the top of the resin column. Resin bed heights of about 28 cm were obtained with 52 g SAC resin and 34 g MB resin, respectively.

Prior to treatment, the columns of ion-exchange resin were rinsed with 1,000 mL (>20 bed volumes) of deionized (milliQ) water until the eluate was colourless and identical in pH and conductivity to the wash water. In particular, H⁺ form SAC exchange resins must be thoroughly rinsed with large volumes of DI water immediately before use, as they can undergo de-crosslinking of the polystyrene matrix during storage and thus release low levels of protonated oligomers of sulfonated polystyrene into the CNC suspension, which would cause overestimation of the sulfate half-ester content by titration (DOWEX 2013).

The usable CNC concentration was restricted by the increasing viscosity of the suspension due to the high effective ionic strength of the solution between the resin beads. A concentration of 0.5 wt% CNC gave reasonable flow rates with no CNC loss.

Cellulose nanocrystal suspensions (500 mL, 0.5 wt%) were fed from the top through a given column at a flow rate of 1.3–1.8 mL/min/cm² (3–4 mL/min), discarding the initial 1–2 bed volumes (1 BV = 30–40 mL in this case) of eluate to avoid excessive sample dilution. The resin-to-CNC ratios (g/g) were approximately 20.8 for SAC and 13.6 for

MB ion-exchange resin. It was confirmed experimentally that one pass through the resin column was sufficient to achieve constant conductivity of the treated sample. No loss of CNCs was observed after column treatment as determined by gravimetric measurement of CNC mass fractions.

Ion-exchange resins

Dowex Marathon C (SAC) resin in the hydrogen form has a volume of around 0.81 mL/g and an exchange capacity of 1.8 meq per mL of resin, giving a total H⁺ exchange capacity of around 76 meq for the column. Amberlite MB 6113 mixed bed resin has a volume of around 0.89 mL/g resin and a total H⁺ exchange capacity of around 21.5 meq.

Resin capacities used in these experiments are greatly in excess of what is required for full protonation of the samples. For a CNC sample containing 240 mmol –OSO₃[–] per kg CNC based on (ICP-AES) analysis, 2.5 g of Na-CNCs contain a maximum of 0.6 meq of Na⁺ ions to be exchanged for H⁺ ions, corresponding to up to 0.8–2.8 % of the columns' ion-exchange capacities. Similarly, the same amount of Na-CNCs consume up to 1.4 % of the resin's exchange capacity for a batch treatment using 12 g of SAC resin per g CNCs.

In this work, all ion exchange was carried out at room temperature to minimize the possibility of acid-catalyzed desulfation of the CNCs in the highly protic environment of the cation exchange resin beads (Jiang et al. 2010; Beck and Bouchard 2014).

Batch versus column resin treatment

Ion exchange is an equilibrium process, different resin forms having different affinities for certain ions (Winter 1956; DOWEX 2014). During batch treatment, the use of the resin's exchange capacity will be limited unless its selectivity for the ion in solution is far greater than for the exchangeable ion attached to the resin, which is why batch treatments generally must be repeated to approach complete ion exchange. For example, the selectivity ratio for Na⁺ over H⁺ ions in solution is ≤ 1.62 for Dowex Marathon C hydrogen form cation exchange resin, while the selectivity ratio for HSO₄[–] over OH[–] is around 85 (and is even higher for SO₄^{2–}) for Amberlite MB 6113 hydroxyl form anion exchange resin (DOWEX 2014). The (single)

batch resin treatments described in the literature therefore most likely do not result in complete demineralization and/or protonation of the CNC suspensions, which may partially explain the discrepancies observed between sulfur contents derived from titration and from elemental analysis (Araki et al. 1998, 1999; Dong et al. 1998; Abitbol et al. 2013). In contrast, passing a CNC suspension through a column containing a bed of ion-exchange resin is analogous to treating it in an infinite series of batch steps, as continuous contact between fresh resin and the sample to be treated favourably drives the exchange equilibrium. Column treatment is therefore much more efficient than batch treatment.

Resin bead porosity

Ion-exchange resins are porous 3D cross-linked networks of high molecular weight polymer chains such as sulfonated polystyrene (cationic) or functionalized styrene–divinylbenzene (anionic). Macroporous resins have pores 20–130 nm in diameter (Kaur 2010; de Dardel 2013), making them unsuitable for use with CNCs. The resins used in this work are microporous, or gel resins, with a pore size of 1–2 nm (de Dardel 2013), preventing loss of material by penetration of CNCs into the pores of the cross-linked resin. However, ion diffusion into and through the resin particle to interact with the exchange sites is slowed by the smaller pore size. If the suspension flows through the resin column too quickly, the counterions associated with the CNCs may not be able to diffuse into the resin beads and may only contact the outside surface of the resin beads, reducing the exchange efficiency. Slower flow rates during column treatment generally yield better exchange (Samuelson 1953; Winter 1956).

Column aspect ratio

The height-to-diameter ratio of the column can also affect the ion exchange efficiency; a 10:1 or 20:1 ratio is typically used (Kaur 2010). Larger ratios provide more theoretical plates per gram of resin in the column, allowing the sample to be treated to contact (and establish equilibrium with) more areas of fresh resin before exiting the column. The columns used in this work had a height-to-diameter ratio of around 13:6.

Total sulfur content by elemental analysis (ICP-AES)

Treated CNC suspensions were lyophilized and digested by wet ashing or microwave digestion, and the total sulfur content was measured by elemental analysis with ICP-AES. Analyses were performed in triplicate for each sample; the method standard deviation is $\pm 5\%$.

Acidic sulfate half-ester content by conductometric titration

Titration conditions were optimized from the literature (Katz et al. 1984; Abitbol et al. 2013) for CNC analysis. Ultrapure (milliQ) water was added to aliquots of resin-treated CNC suspension containing 150 mg of CNCs to give a total volume of 200 mL. An aliquot (2 mL) of NaCl solution (0.1 M, Sigma-Aldrich) was added with mixing to give a final NaCl concentration of 1 mM. Samples were titrated with 10 mM NaOH (Sigma-Aldrich) (~ 10 mL total volume, added in 0.1 mL increments over a period of 20–30 min), which was standardized against the primary standard potassium hydrogen phthalate, KHP. The pH meter must be calibrated with fresh pH standards immediately before standardizing the NaOH. It is essential that a fresh NaOH solution be used and that it be standardized immediately before titrating the sample. Titration conductivity values were corrected for dilution effects and plotted against added volume of sodium hydroxide. Analyses were performed in at least triplicate. Standard deviations were generally 1 % of the mean or lower, demonstrating the better reproducibility of this method compared to ICP-AES.

Equivalence points are determined graphically from the intersection of the least squares regression lines fit to the data points in the distinct regions of the conductometric titration curve. This naturally results in some operator bias in the selection of data points to constitute the small central weak acid (R-COOH) region, leading to possible differences in the slope of its trendline for different operators. However, it was found that even for a weak acid region only 0.4–0.5 mL (or 4–points) wide, variation in the selection of one or two points resulted in $<1\%$ variation in the value of the R-OSO₃H content given by the equivalence point. If slope variations are a

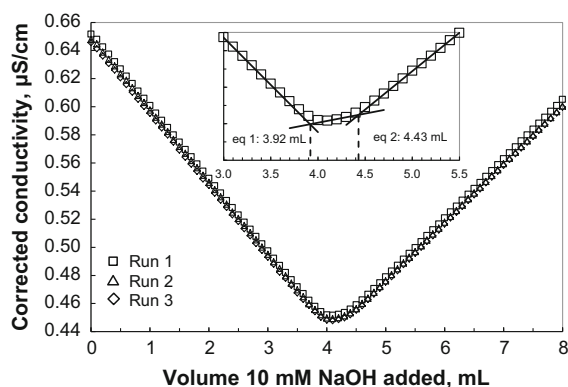


Fig. 1 Conductometric titration curves for dialyzed and protonated CNCs (sample NBSK-D₅₀). *Inset* equivalence point determination; the first equivalence point gives the strong acid (R-OSO₃H) content

problem, particularly in CNCs with a low degree of sulfation (DS), increasing the CNC sample size and/or decreasing the added aliquot size in the equivalence point region will generate more data points from which to graphically determine the equivalence points.

Figure 1 shows an example set of titration curves obtained for sample NBSK-D₅₀ (redispersed spray-dried Na-CNCs which were dialyzed then protonated with SAC resin). The mean sulfate half-ester content determined from the first equivalence point was 240 ± 1 mmol R-OSO₃H/kg CNC for this sample.

Results and discussion

Purification by dialysis: the need for (re-)protonation

Dialyzing Na-CNC suspensions only partially removes the sodium counterions (Revol et al. 1998), which results in very large discrepancies between sulfur contents determined by conductometric titration and elemental analysis, as shown in Table 2. Elemental analysis of Na-CNC samples after dialysis confirms the presence of significant quantities of sodium (data not shown). However, dialyzing H-CNC samples can also result in significantly lower apparent sulfur contents by conductometric titration compared to elemental analysis, particularly when running DI water is used, perhaps due to trace ions such as Ca²⁺ exchanging with the H⁺ counterions and accumulating in the CNC sample, as found by elemental analysis

Table 2 Effect of purification by dialysis alone on CNC sulfur contents measured by titration and ICP-AES

Initial CNC counterion	H ⁺		Na ⁺	
Dialysis water used	DI	milliQ ^a	DI	milliQ
Sample	NBSK-A ₅₀	NBSK-E ₆₅	NBSK-B ₅₀	NBSK-B ₅₀
<i>Conductometric titration</i>				
R-OSO ₃ H, (mmol/kg) CNC	140 ± 2	206 ± 2	50 ± 1	64 ± 1
<i>Elemental analysis (ICP-AES)</i>				
Total S, (mmol/kg) CNC	229 ± 11	267 ± 13	240 ± 12	240 ± 12

^a Dialyzed against 5 l of stirred ultrapure (milliQ) water; water was changed 5 times per day for 3 days

(5–10 mmol Ca/kg CNC). Even dialyzing H-CNCs against ultrapure (milliQ) water can result in an R-OSO₃H value by titration that is 10–25 % lower than the total elemental S content of the same sample (see Table 2 and sample S2 in Abitbol et al. 2013). Abitbol et al. (2013) suggest that SAC resin treatment of H-CNC samples may be “largely unnecessary” following dialysis (against ultrapure water); our results and others in the published literature (e.g. Araki et al. 1999) tend to contradict this suggestion. A protonation step is needed and highly recommended to ensure full protonation of the CNCs prior to conductometric titration.

Batch SAC resin treatment

A never-dried acidic H-CNC suspension (sample NBSK-A₅₀) having a total sulfur content of 229 ± 11 mmol S/kg CNC determined by elemental analysis (ICP-AES) was protonated with SAC exchange resin by the batch treatment method. A dialysis time of 72 h was found to be effective in removing excess sulfate and other ions remaining in suspension after hydrolysis. Initial batch resin treatment was performed on the dialyzed sample using the procedure described in the “Experimental methods”.

An initial 24-h treatment time was chosen as representing typical literature conditions (Dong et al. 1998), for which the quantity of resin was not given. Resin-to-CNC ratios between 4 and 13 g resin per g CNC were found to yield identical titration results for this treatment time and the titration conditions described in the “Experimental methods” (Fig. 2). However, the sulfate half-ester value obtained by titration (195 ± 2 mmol R-OSO₃H/kg CNC) greatly underestimates the total S content determined by ICP-AES (229 ± 11 mmol S/kg CNC), indicating that the

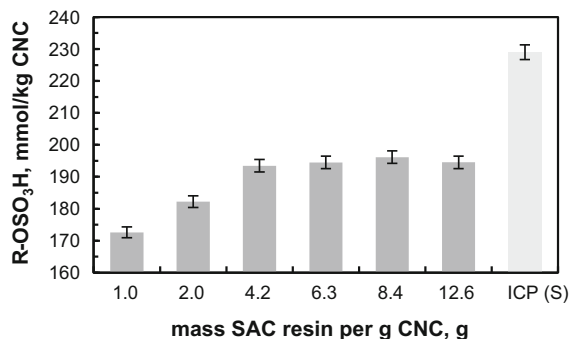


Fig. 2 Effect of resin-to-CNC ratio on sulfate half-ester content detected by conductometric titration compared to ICP-AES, for 24-h batch SAC resin treatment of sample NBSK-A₅₀

sample was not fully protonated at equilibrium. The low selectivity ratio of the SAC resin for exchanging H⁺ with common contaminant ions such as Na⁺ most likely prevents their full replacement, even when the total capacity of the resin far exceeds the requirements of the CNC sample. For all subsequent batch treatments, 12 g resin per g CNC was used to ensure an excess of resin capacity, similarly to Abitbol et al. (2013).

Based on the results in Fig. 2, a single batch resin treatment does not appear to be sufficient to achieve full protonation of the CNCs, even with a large excess of resin (e.g., Abitbol et al. 2013), assuming all sulfate half-ester groups to be accessible to titrant. Shorter repeated treatments of sample NBSK-A₅₀ with fresh SAC resin increased the sulfate half-ester contents measured by titration as shown in Fig. 3. Increasing the number of successive 2-h treatments from 2 to 5 resulted in a measured sulfate half-ester increase from 200 to 221 ± 2 mmol R-OSO₃H/kg CNC, whereas 6 treatments of 30 min each yielded an equivalence point of 227 ± 2 mmol R-OSO₃H/kg CNC, identical

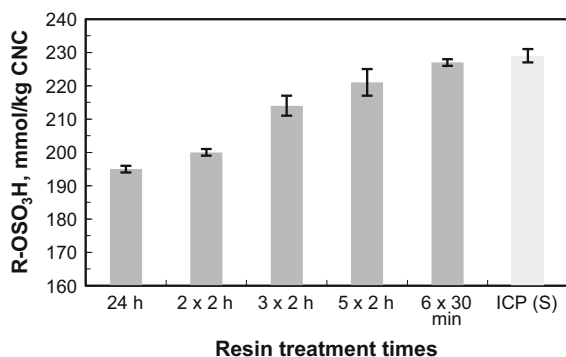


Fig. 3 Effect of multiple successive batch SAC resin (12 g/g CNC) treatments on sulfate half-ester content of sample NBSK-A₅₀ determined by conductometric titration compared to ICP-AES

to the value obtained by ICP-AES (Table 2). It is therefore possible, by increasing the number of batch SAC resin treatments of a CNC sample, for the sulfate half-ester content detected by conductometric titration to equal the total S content obtained by elemental analysis (ICP-AES).

As expected, the batch method requires repeated treatments with fresh SAC resin to reach maximum protonation. This method is very time-consuming and inefficient, as each treatment takes a minimum of 30 min plus filtration time, and the resin must be thoroughly washed and regenerated, either between treatments or at the end of the overall treatment, if resin quantity is to be minimized.

Column resin treatment

Until now, never-dried acid form H-CNCs have been available from laboratories or small pilot plants. Full re-protonation of H-CNCs following dialysis is relatively simple. However, industrial-scale production will most likely almost exclusively consist of sodium form CNCs (Na-CNCs) because it is the form that can

be dried and then redispersed in water (Dong and Gray 1997; Beck et al. 2012). These Na-CNCs require dialysis to remove the residual sodium sulfate produced during the neutralization step. Dialyzed Na-CNCs can be expected to be more difficult than H-CNCs to re-protonate, as the titration results in Table 2 suggest. Despite the use of SAC exchange resins to protonate sodium form CNCs for titration (Kloser and Gray 2010), there is no evidence from systematic study in the literature regarding the effectiveness of batch resin treatment on Na-CNCs compared to H-CNCs (Abitbol et al. 2013). Ion-exchange resin columns provide faster and more efficient treatment than the batch method. For example, using the column treatment conditions described in the “Experimental methods”, 2.5 g of dialyzed Na-CNCs (containing 50 mmol H⁺/kg CNC) can be fully protonated in 60 min using 52 g of SAC resin. In contrast, with the batch method, it takes at least 180 min to fully protonate 2.5 g of dialyzed H-CNCs (containing 140–200 mmol H⁺/kg CNC) using a total of 176 g of fresh resin.

Mixed bed ion-exchange resin: simultaneous purification and protonation?

Mixed bed ion-exchange resins in the H⁺/OH⁻ form are used to remove free cations and anions in solution and replace them with pure water (H⁺ + OH⁻ → H₂O). It is therefore plausible to envisage that in the case of CNCs, MB resins will demineralize the water in which the CNCs are suspended and in addition exchange all CNC counter-cations for protons, providing in one step a pure H-CNC suspension (Dong et al. 1996). Indeed, this appears to be the assumption many researchers do make, as MB resins are often used as the last purification step before titration with NaOH to determine the CNC surface charge (e.g., Dong et al. 1996). This would be particularly useful and efficient

Table 3 Effect of Na-CNC pre-treatment protocols on sulfur contents measured by conductometric titration and ICP-AES

Pre-treatment	Dialysis	MB resin	MB resin + SAC resin	Dialysis + MB resin	Dialysis + SAC resin
Sample NBSK-B ₅₀	1	2	3	4	5
<i>Conductometric titration</i>					
R-OSO ₃ H, (mmol/kg) CNC	50 ± 1	189 ± 2	210 ± 2	152 ± 2	242 ± 4
<i>Elemental analysis (ICP-AES)</i>					
Total S, (mmol/kg) CNC	240 ± 12	222 ± 11	222 ± 11	224 ± 11	241 ± 12

in the case of Na-CNC. The question is, therefore, can mixed bed ion-exchange resin column treatment be used as (a) a faster substitute for dialysis to remove residual electrolytes in solution, and (b) a means of simultaneously protonating CNCs in preparation for analysis by conductometric titration? Previous work suggests that it cannot (Abitbol et al. 2013) and our results confirm this.

Effect of MB resin on apparent sulfur content by conductometric titration

Table 3 shows the sulfate half-ester contents (conductometric titration) and total elemental sulfur contents (ICP-AES) determined for sample NBSK-B₅₀ (freeze-dried Na-CNCs re-dispersed in DI water) following various pre-treatments. It is clear that the sulfate half-ester (apparent sulfur) contents measured by titration for sample NBSK-B₅₀ after MB resin treatment are significantly lower than the total sulfur content measured by ICP-AES after the same MB resin treatment. This is consistent with the suggestion of Abitbol et al. (2013) that the drop in apparent sulfur content measured by titration after MB resin treatment is due to “consumption of protons [associated with sulfate half-esters] by quaternary ammonium beads” in the resin (in this case, Dowex MR-3 mixed bed resin).

The results for Na-CNCs in Table 3 strengthen previous observations (Abitbol et al. 2013) regarding the introduction of errors in subsequent conductometric titrations by deionization with mixed bed ion-exchange resin as follows:

- (1) Mixed bed resin treatment alone cannot be used as a substitute for both dialysis and protonation together prior to conductometric titration—MB resin treatment (2) gives a much lower sulfate half-ester content than dialysis followed by SAC resin (5);
- (2) Mixed bed resin treatment cannot be used as a substitute for dialysis when followed by protonation with SAC resin prior to titration—MB resin followed by SAC resin treatments (3) give a lower sulfate half-ester content than dialysis followed by SAC resin (5); and
- (3) MB resin treatment cannot be used to protonate CNCs after dialysis prior to titration—dialysis followed by MB resin treatment (4) gives a

much lower sulfate half-ester content than dialysis followed by SAC resin treatment (5).

We therefore recommend that CNCs always be purified by dialysis prior to analysis by conductometric titration.

Effect of MB resin on total elemental sulfur content

Interestingly, we have found that the total elemental S contents of the MB resin-treated samples [NBSK-B₅₀ (2)–(4)] are all approximately 17 ± 1 mmol S/kg CNC lower than the S content of the untreated sample NBSK-B₅₀(1). Conductometric titration of sample NBSK-B₅₀(1) also gives a much lower apparent sulfur content of around 50 mmol/kg CNC due to incomplete protonation of the Na-CNCs following dialysis. The only sample for which the elemental analysis and titration values coincide with each other and with the ICP-AES results for the untreated CNCs is sample NBSK-B₅₀(5), which was protonated with SAC resin following dialysis.

In addition to not imparting full protonation, mixed bed ion-exchange resin appears to remove a portion of the sulfur content from purified CNC suspensions. However, the apparent and real S loss values may not be identical. That is, mixed bed resin removes more protons than sulfur atoms, resulting in differences between titration and ICP-AES results for samples with and without MB resin treatment (see Table 3, NBSK-B₅₀(1), (2) and (3)). Elemental analysis of samples NBSK-B₅₀, -C₄₅, -D₅₀ and -E₆₅ reveals only a 15 ± 2 mmol/kg CNC decrease in elemental *sulfur* content after MB resin treatment across all the samples, as summarized in Table 4. The titration results are more variable and show a decrease in protonated *sulfate half-ester* content of around 50–90 mmol/kg CNC. Thus, in addition to the removal of sulfate half-ester groups, an additional 30–70 mmol/kg CNC of sulfate half-ester groups remain *unprotonated* following MB resin treatment.

Treating any CNC suspension with mixed bed ion-exchange resin must be avoided as it results in lower apparent sulfur contents by conductometric titration as compared to elemental analysis, both results being lower than the true value obtained by ICP-AES for CNCs not exposed to MB resin.

The above results support the conclusion that the purification step should be done by dialysis instead of

Table 4 Differences in measured CNC sulfate half-ester and elemental sulfur contents caused by MB resin treatment

Sample	SAC-MB ^a	NBSK-B ₅₀	NBSK-C ₄₅	NBSK-D ₅₀	NBSK-E ₆₅
<i>Conductometric titration</i>					
R-OSO ₃ H, (mmol/kg) CNC	$\Delta(\text{H}^+)_{\text{total}}$	88	54	47	60
<i>Elemental analysis (ICP-AES)</i>					
Total S, (mmol/kg) CNC	$\Delta(\text{R-OSO}_3\text{H})_{\text{total}}$	17	14	14	13
<i>Titration—ICP-AES</i>					
H ⁺ , (mmol/kg) CNC	$\Delta(\text{H}^+)_{\text{free}}$	71	39	33	47

^a Differences between dialyzed samples treated with SAC resin alone, and with MB resin followed by SAC resin

Table 5 Comparison of sulfate half-ester/sulfur determinations by conductometric titration and ICP-AES for NBSK CNC samples having different initial counterions and dried states

Sample	NBSK-C ₄₅	NBSK-A ₅₀	NBSK-D ₅₀	NBSK-B ₅₀
Initial counterion	Na ⁺	H ⁺	Na ⁺	Na ⁺
Drying method	SD	ND	SD	FD
<i>Conductometric titration</i>				
R-OSO ₃ H, (mmol/kg) CNC	216 ± 2	227 ± 2	240 ± 2	242 ± 4
<i>Elemental analysis (ICP-AES)</i>				
Total S, (mmol/kg) CNC ^a	207 ± 10	229 ± 11	230 ± 12	241 ± 12

^a The sulfate half-ester (total sulfur) content of CNCs increases with hydrolysis temperature (Hamad and Hu 2010)

SD spray-dried, ND never-dried, FD freeze-dried

MB resin treatment to avoid underestimating the CNC sulfate half-ester content, whether it is to be measured by titration or elemental analysis.

How does MB resin reduce the CNC sulfate half-ester content?

It is clear that mixed bed resin treatment is removing some sulfated species from the CNC suspensions, species which are not removed by prolonged dialysis. The fact that SAC resin column treatment does not result in the same loss of sulfur content suggests that the sulfur-containing species being removed are anionic. Both the mixed bed ion-exchange resin used in these experiments, Amberlite MB 6113, and the MB resin used by Abitbol et al. (2013), Dowex MR-3, have type I anion exchange resin beads containing quaternary ammonium groups with three methyl groups (Rohm and Haas Company 2008). Their affinity for HSO₄⁻ and SO₄²⁻ ions in solution is ≥85 times higher than for OH⁻ ions (DOWEX 2014). Small, negatively charged sulfated species could therefore exchange

efficiently with this resin. As discussed above, mixed bed resin removes approximately the same amount of elemental S (15–20 mmol S/kg CNC) from CNCs regardless of their hydrolysis temperature/grade, representing a 5–7 % loss of total sulfur.

Possible hypotheses for the removal of sulfated species by mixed bed resin include:

1. Exchange of OH⁻ for small sulfated oligosaccharides present in the CNC suspensions before dialysis (Beck et al. 2011), which are also known to non-specifically bind to Type I anion exchange resins (Bio-Rad Laboratories 2013; Bio-Rad Technical Support FAQ 2013); or
2. Solid-state desulfation of the CNCs driven by the high affinity of the Type I resin for HSO₄⁻ and SO₄²⁻ ions, and catalyzed by the high local concentrations of hydroxyl ions at the resin bead surfaces (Kloser and Gray 2010).

The CNC suspension concentration does not change noticeably upon contact with MB resin, and no differences are detected between the oligosaccharide

Table 6 Comparison of sulfate half-ester/sulfur determinations by conductometric titration and ICP-AES for CNC samples produced at different facilities from various native cellulose feedstocks

Sample	NBSK-A ₅₀	NBSK _{AITF}	NBSK _{CF}	NBSS	SBSS	Sisal	Cotton
Initial counterion	H ⁺	Na ⁺	Na ⁺	Na ⁺	H ⁺	H ⁺	H ⁺
Drying method	ND	ND	SD	ND	ND	ND	ND
Furnish sulfur content, (mmol/kg)	1.2 ± 0.1	–	–	1.0 ± 0.1	–	–	b.d.l.
<i>Conductometric titration</i>							
R-OSO ₃ H, (mmol/kg) CNC	227 ± 2	350 ± 1	234 ± 1	166 ± 4	278 ± 1	150 ± 2	173 ± 1
<i>Elemental analysis (ICP-AES)</i>							
Total S, (mmol/kg) CNC	229 ± 11	373 ± 19	234 ± 12	200 ± 10	312 ± 15	157 ± 8	194 ± 9

SD spray-dried, ND never-dried, b.d.l. below detection limit

(DP = 7–14) fraction of the DP profiles of the untreated and MB-resin-treated CNCs. Desulfation by the anion exchange resin beads therefore appears to be the most likely possibility. It is also consistent with the absence of decrease in mass and oligosaccharide content. We cannot, however, completely discard the first two hypotheses, as the small quantities of material involved may make it difficult to distinguish differences caused by the MB resin treatment.

Dialysis followed by strong acid cation exchange resin treatment

For accurate sulfate half-ester determination by conductometric titration, the CNC sample must be protonated (with H⁺ form strong acid cation exchange resin) after purification by dialysis, regardless of the initial counterion. To assess the reliability of the optimized sample pre-treatment conditions developed in this report, we treated and analyzed four NBSK CNC samples having different initial counterions and dried states. Table 5 lists the sulfate half-ester and sulfur contents obtained by titration and ICP-AES for these samples.

As shown in Table 5, the titration and ICP-AES results agree to within 5 % or better for the northern bleached softwood kraft pulp-derived CNC samples analyzed in this work. Never-dried, freeze-dried and spray-dried NBSK CNCs produced in our pilot plant all give similar results by both methods, demonstrating that drying does not interfere with the preparation and measurement protocol when the sample is fully redispersed. The initial counterion of the CNC samples does not affect the results obtained with the

protocol; both H-CNCs and Na-CNCs show nearly identical sulfur contents determined by conductometric titration and ICP-AES.

The data we have obtained from conductometric titration in conjunction with elemental analysis by ICP-AES indicate that all of the sulfur-containing groups in the well-dispersed NBSK CNCs produced in FPInnovations' pilot plant are accessible to titrant solutions, are probably located at the CNC surface, and are in the form of sulfate half-esters. To our knowledge, this is the first time evidence for this equivalence has been provided in the literature for a CNC sample.

CNCs extracted from other cellulosic feedstocks

As indicated in Table 6, we have found that despite the full protonation achieved using this protocol, CNCs prepared from a range of cellulose sources did show some discrepancies between elemental analysis and titration results. The variability in the DS across the CNCs arises from the different process conditions used at the different facilities, such as the ratio of acid to pulp, acid concentration and reaction temperature (Hamad and Hu 2010), as well as the different pulp furnishes and biomass used.

CelluForce CNCs are produced from northern bleached softwood kraft pulp at commercial scale using similar conditions to those used in FPInnovations' pilot plant. No discrepancies were found between the sulfur contents detected by conductometric titration and ICP-AES, which were comparable in value to the sulfur contents of the CNCs produced at FPInnovations.

Cellulose nanocrystals extracted at AITF from northern bleached softwood kraft pulp gave a total sulfur content of 373 mmol/kg CNC. The sulfate half-ester content of 350 mmol/kg CNC measured by titration was only 6 % lower (nearly identical, within experimental error), similarly to the results obtained for northern bleached softwood kraft pulp CNCs produced at FPIInnovations.

Cellulose nanocrystals derived from northern and southern softwood sulfite pulps gave CNCs with quite different degrees of sulfation. Northern softwood sulfite CNCs produced at FPIInnovations contained around 200 mmol S/kg CNC, whereas the southern softwood CNCs from the USFPL contained approximately 300 mmol S/kg CNC measured by ICP-AES. Titration of both sulfite pulp CNC samples detected ~ 34 mmol S/kg CNC (11–17 %) less than elemental analysis.

Within experimental error, identical sulfur contents of around 150 mmol S/kg CNC were obtained by titration and ICP-AES for CNCs extracted from sisal fibres.

The apparent sulfur content found by conductometric titration of the cotton CNCs is 21 mmol S/kg CNC (11 %) lower than the sulfur content found by elemental analysis.

An important distinction must be made between the elemental sulfur atoms detected by ICP-AES and the (acidic) sulfate half-ester groups detected by titration. It has been proposed that discrepancies between elemental analysis and titration arise from non-titratable sulfur-containing groups which fall under the classification of:

Non-acidic/non-ionic sulfur-containing groups

Other sulfur-containing moieties introduced by biosynthesis (Gu et al. 2013)

Sulfate half-ester groups that are not protonated, e.g. sodium salt form (Abitbol et al. 2013)

Sulfate half-ester groups that are protonated but are not accessible to titrant (Araki et al. 1999)

The results in Table 6 suggest that the sulfur present in the raw materials originating from biosynthesis would contribute negligibly to the CNC sulfur content, even if all of it remains in the CNCs after hydrolysis and purification. Certain results in the literature support this conclusion: Araki et al. (1999) observed negligible elemental S content (2 mmol/kg) in bleached kraft pulp CNCs isolated by hydrolysis

with HCl; CNCs extracted from commercial cotton fibres with HCl also did not contain S detectable by elemental analysis (Teixeira et al. 2010).

Non-protonated sulfate half-esters on the exposed CNC surfaces are likely minimal/negligible, given that we obtained identical ICP-AES and titration values for some CNC samples e.g. NBSK and sisal CNCs. The NBSK and NBSK_{CF} CNCs have a DS of around 230 mmol S/kg CNC, which is higher than the DS of some CNCs which do show a discrepancy in the ICP-AES and titration values, such as those derived from cotton. It is reasonable to assume that the CNCs with lower DS should be fully protonated by the same SAC resin treatment, and therefore that this is not the cause of the discrepancy.

Additionally, analysis of many separately prepared batches of NBSK CNCs (FPIInnovations) gave nearly identical S contents (within ~ 5 %) by ICP-AES and conductometric titration. Elemental analysis of the purified sodium or calcium forms of these CNC samples gave 1:1 ratios of S–Na⁺ or 1:2 ratios of S to Ca²⁺ counterions (data not shown). These results support the inference that all of the elemental S is present in the form of sulfate half-esters (titratable S) in the NBSK CNCs.

In some cases, a portion of the CNC sulfate half-esters may be unprotonated because of being inaccessible to the SAC exchange resin bead surfaces. Divalent cations such as calcium and magnesium are often present in the process water used in large-scale CNC extraction. These divalent cations can replace the protons and form ionic bridges between sulfate half-esters on adjacent CNCs. The cations bridging the sulfate half-esters in the interior surfaces of these aggregates or agglomerates may not be exchangeable for the protons in the SAC resin, leading to lower apparent sulfur content values by titration. We examined the calcium and magnesium contents of the CNC samples, but did not find a correlation with the differences in elemental sulfur and sulfate half-ester contents measured by the two methods.

It appears that the native cellulose source is important not only in determining the other characteristics of the nanocrystals extracted from it (size, morphology), but may also at least partially determine their sulfur content: In contrast to the results discussed above, Gu et al. (2013) detected ~ 41 mmol S/kg in CNCs extracted from Whatman CF11 cotton by HCl hydrolysis, which was essentially identical to the

sulfur content they found in the unprocessed starting material. This S content is comparable in order of magnitude to the ICP-AES/titration discrepancies we observed in the cotton and southern bleached softwood sulfite pulp CNC samples.

The general procedure to quantify CNC sulfate half-ester content described herein works very well with CNCs extracted from NBSK pulp by different producers, as well as from sisal. However, for an unknown reason, a discrepancy (never larger than 17 %) was found in CNCs extracted from bleached sulfite pulps and from cotton. The most significant finding of this work is that the purification and protonation protocol developed herein provides excellent correlation between elemental analysis and conductometric titration for both acidic and sodium form CNC suspensions. Thus, the sample preparation and analysis protocol developed in this report is generally applicable to CNCs extracted by sulfuric acid hydrolysis regardless of counterion, dried state, extraction temperature (DS) and cellulose feedstock or production facility.

Conclusions

We have developed and validated a rapid and general two-step sample preparation protocol for use prior to conductometric titration for the determination of the sulfate half-ester content of CNCs extracted by sulfuric acid hydrolysis.

The initial sample purification is best performed by dialyzing the CNC suspension; mixed bed ion-exchange resin containing Type I anion exchange resin cannot be used, because it gives uneven protonation and removes sulfur-containing material from CNC samples, causing erroneous and variable results.

Dialysis alone is insufficient to obtain accurate results, particularly for Na-CNCs but also for H-CNCs. Following dialysis, CNC samples are treated by passing them through a column of strong acid cation-exchange resin to ensure full protonation, and hence detection, of all the acidic R–OSO₃H groups. Although several successive batch treatments with SAC resin can give accurate results, it is more appropriate to use an ion-exchange resin column as a faster and more efficient method for producing H-CNC suspensions for analysis by conductometric titration.

With this protocol, accurate results are obtained for spray-dried or freeze-dried Na-CNCs (produced at the industrial scale) as well as the more fully protonated never-dried H-CNCs (generally produced at the laboratory scale). Thus, any form of well-dispersed CNCs may be fully protonated prior to analysis.

Our results show that care must be taken with analysis of sulfated CNCs—the total elemental sulfur content cannot automatically be equated with surface charge. Overall, the sample preparation and conductometric titration protocol developed in this paper yields reproducible CNC sulfate half-ester content values which are identical to the CNC elemental sulfur contents for well-dispersed CNC samples produced from northern bleached softwood kraft pulp, thus providing reliable and meaningful CNC sulfur content and surface charge data for commercial production of CNCs, with greater simplicity than ICP-AES.

The method we have developed may also be appropriate for the analysis of CNCs containing other strong acid groups such as phosphorylated CNCs (Sufflet et al. 2006; Camarero Espinosa et al. 2013).

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