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Pulp — Determination of cellulose nanocrystal sulfur and sulfate half-ester content

Pâte — Détermination de la teneur en soufre et en demi-ester de sulfate des nanocristaux de cellulose

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document, which establishes testing methodologies for measuring the total elemental sulfur and sulfate half-ester group contents of cellulose nanocrystals (CNCs), was developed in response to a need for a simple and rapid method for indirect quantification of CNC surface charge.

The main purpose of the two methods covered (inductively coupled plasma-optical emission spectroscopy (ICP-OES) and conductometric titration) in this document is to measure the surface charge of sulfated CNCs. Sulfate half-ester groups ($R–OSO_3H$) covalently bound at the nanocrystal surface are introduced during concentrated sulfuric acid hydrolysis by partial esterification of the cellulose hydroxy groups[1]. The anionic sulfate half-ester groups are strong acids, such that at neutral and basic pH values, the protons dissociate and the CNC surface is negatively charged ($R–OSO_3^-$). The pKa of the sulfate half-ester groups on CNCs is approximately 2,5 (as determined by potentiometric titration), implying that at very low pH the surface groups are protonated and CNCs have a net neutral charge[2]. This surface charge controls many important properties of CNC suspensions, including the colloidal stability, self-assembly and rheological behaviour, both in the pure state and in the presence of salts and other additives. As such, the CNC surface charge is a very important factor in the processing and development of commercial products containing CNCs. The sulfate half-ester (sulfur) content will also be a key entry on material specifications sheets which will accompany the commercial product, enabling different product grades to be distinguished from each other and from other companies' products.

ICP-OES and conductometric titration are both included in this document as they provide different but complementary ways of measuring the surface charge. ICP-OES measures elemental sulfur which is present in a 1:1 ratio with the charged sulfate half-ester groups, and does not depend on the nature of the counterion. Conductometric titration, on the other hand, measures only protons associated with the anionic $R–OSO_3^-$, but is much less complicated to carry out. The two analysis methods should yield equivalent results (see 5.1 and 6.1), or within 5 % to 10 % owing to sources of uncertainty/error such as transfer losses and slight differences in the purification and protonation steps. CNCs derived from different cellulose sources have shown different levels of agreement between the results from the two methods[3]. The objective of this document is to use this information in quantifying the CNC surface charge arising from the easily ionized sulfate half-ester moieties introduced during hydrolysis or post-sulfation.

The tests contained herein are based on literature methods and were developed over several years by a group of industry experts, and were identified as being those which can yield reproducible and accurate results. The tests are anticipated to be performed in a laboratory setting.

As with any laboratory procedure requiring the use of potentially hazardous chemicals, the user is expected to have received proper knowledge and training in the use and disposal of these chemicals.

This document contains footnotes giving examples of apparatus, reagents and sometimes the supplier(s) of those materials that are available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named. Equivalent products may be used if they can be shown to lead to the same results.

Annex A provides an alternative method of sample digestion for ICP-OES by wet ashing. Annex B provides an alternative method of sample protonation for conductometric titration by treatment with batches of ion exchange resin.

Pulp — Determination of cellulose nanocrystal sulfur and sulfate half-ester content

1 Scope

This document specifies procedures for the laboratory determination of the total elemental sulfur and the sulfate half-ester content of cellulose nanocrystals (CNCs) by inductively coupled plasma-optical emission spectroscopy and conductometric titration, respectively, including sample preparation, measurement methods and data analysis.

This document is applicable to the characterization of CNCs:

a) with all monovalent counterions (particularly hydronium and sodium cations);

b) which are either in the never-dried state in aqueous suspension, or have been redispersed from a dried form; and

c) which have been extracted from any naturally occurring cellulose source using a range of sulfuric acid hydrolysis conditions, or have been sulfated post-hydrolysis using sulfuric acid.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 14644-1, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1, ISO/TS 80004-2, ISO/TS 80004-6 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-1:2015, 2.1]

3.2
nano-object
discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.1)

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

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.3
nanocrystal
nano-object (3.2) with a crystalline structure

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

3.4
elementary fibril
structure, originating from a single terminal enzyme complex, having a configuration of cellulose chains specific to each cellulose-producing plant, animal, algal and bacterial species

[SOURCE: ISO/TS 20477:2017, 3.2.5]

3.5
cellulose nanocrystal
CNC
nanocrystal (3.3) predominantly composed of cellulose with at least one *elementary fibril* (3.4), containing predominantly crystalline and paracrystalline regions, with aspect ratio of usually less than 50 but usually greater than 5, not exhibiting longitudinal splits, inter-particle entanglement, or network-like structures

Note 1 to entry: The dimensions are typically 3 nm to 50 nm in cross-section and 100 nm to several µm in length, depending on the source of the cellulose nanocrystal.

Note 2 to entry: The aspect ratio refers to the ratio of the longest to the shortest dimension.

Note 3 to entry: Historically, cellulose nanocrystals have been called nanocrystalline cellulose (NCC), cellulose whiskers or cellulose nanowhiskers (CNW), and cellulose microfibrils; they have also been called spheres, needles or nanowires based on their shape, dimensions and morphology. Other names have included cellulose micelles, cellulose crystallites and cellulose microcrystals.

[SOURCE: ISO/TS 20477:2017, 3.3.5, modified — Note 3 to entry has been revised.]

3.6
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, 3.4]

3.7
analyte
element to be determined

3.8
calibration blank solution
solution prepared in the same way as the *calibration solution* (3.9) but leaving out the *analyte* (3.7)

3.9
calibration solution
solution used to calibrate the instrument, prepared from a *stock solution* (3.11) or a certified standard by adding acids, buffer, reference element and salts as needed

3.10
matrix blank solution
solution prepared in the same way as the *test sample solution* (3.14) but omitting the *test sample* (3.13)

3.11
stock solution
solution with accurately known *analyte* (3.7) concentration(s), prepared from pure chemicals such as a primary standard

3.12
quality control sample solution
solution of known composition within the range of the *calibration solutions* (3.8), but prepared independently

3.13
test sample
portion taken from the laboratory sample after, for example, homogenizing or dividing

3.14
test sample solution
solution prepared after extraction, dispersion, purification or other preparation of the *test sample* (3.13), such that it can be used for the envisaged measurement

4 Symbols and abbreviated terms

a	slope of the standard addition plot, in mg/kg
b	intercept of the standard addition plot, in mg/kg
c	concentration (titre) of sodium hydroxide, in mol/l
CAS	Chemical Abstracts Service, a division of American Chemical Society
CNC	cellulose nanocrystal
cps	counts per second
ICP-OES	inductively coupled plasma-optical emission spectroscopy
κ	conductivity corrected for dilution, in S/cm
m_{d}	mass of dry sample, expressed in g
$m_{\mathrm{int},\,i}$	mass of the internal standard added to sample aliquot number i, in g
m_{KHP}	mass of potassium hydrogen phthalate standard, in g
m_{o}	mass of original sample, in g
$m_{\mathrm{S},\,i}$	mass of the CNC sample present in sample aliquot number i, in g
$m_{\mathrm{std},\,i}$	mass of the sulfur standard added to sample aliquot number i, in g
m_{t}	oven-dry mass of resin-treated CNCs in the suspension being titrated, in kg

meq	milliequivalent
MWCO	molecular weight cut-off
NIST	National Institute of Standards and Technology
o.d.	oven-dry
ppm	parts per million
R_i	ratio of ICP-OES signals corresponding to the analyte and the internal standard
σ	CNC surface charge content, in meq/kg
S_{blk}	concentration of sulfur in the undiluted matrix blank solution, in mg/kg or mg/l
S_{std}	mass fraction of analyte (sulfur) in the sulfur standard added to each sample aliquot, in mg/kg
SAC	strong acid cation
v	added volume of titrant, in l
V_e	volume of sodium hydroxide solution required to reach the equivalence point, in l
V_t	initial volume of test sample solution being titrated, in l
w	mass fraction solids content, in percent
$[R–OSO_3H]$	quantity of protonated sulfate half-ester groups $[R–OSO_3H]$ present on the CNC surface, in moles per kg of dry CNCs
$[S]$	blank corrected concentration of sulfur in the CNC test sample, in mg/kg

5 Total elemental sulfur content — ICP-OES method

5.1 Principle

5.1.1 This method covers the determination of total elemental sulfur (S) content of cellulose nanocrystals (CNCs). Inductively coupled plasma-optical emission spectroscopy (ICP-OES) is used for analysis, following sample purification by dialysis to remove any sulfur-containing contaminants found in the water matrix of the aqueous CNC suspension, and sample digestion to ensure that most (all) of the S in the CNC sample is dissolved in the aqueous medium used for analysis.

5.1.2 Dialysis is typically used to purify CNC suspensions by removing dissolved ions, including residual sulfur-containing contaminants such as sulfuric acid or sodium sulfate, from the aqueous phase[4]. The final dialysed samples are freeze-dried prior to analysis by ICP-OES. Samples can also be digested and analysed beginning directly from aqueous CNC suspensions, but this method is less precise owing to the variations in solids content.

5.1.3 The aqueous sample containing the dissolved sulfur-containing ions is delivered by a peristaltic pump into an analytical nebulizer where it is atomized and introduced into a plasma flame. The sample is broken down into ions, which break up into their respective atoms, which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved. During analysis, light of wavelength around 180 nm to 182 nm (atom and ion lines) is emitted from the sulfur and onto a detector that measures the amount of light emitted. The emission intensity is a measure of the concentration of sulfur in the sample. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by a detector. The signals from the detector(s) are then

processed and controlled by a computer system. A suitable background correction technique is used to compensate for variable background contributions.

5.1.4 ICP-OES is used to measure the total elemental sulfur content in a CNC sample. Sulfur is not typically present in cellulose derived from native sources; the sulfur in the CNCs can therefore be assumed to originate only from the surface sulfate half-ester groups imparted during CNC production by sulfuric acid hydrolysis. However, if sulfur is known to be present in the original cellulose sample[5] [6], comparing the total S content of the CNCs with that of the original material and with CNCs produced from the same source by HCl hydrolysis (which do not contain sulfate half-ester groups) should give the concentration of sulfur derived from sulfate half-ester groups.

5.2 Reagents and apparatus

5.2.1 Water, ultrapure (deionized or distilled), conforming to Grade 2 of ISO 3696 or better[1)].

5.2.2 Nitric acid (HNO_3) solution, concentrated, trace metal grade (60 % – 70 % assay) (CAS number 7697-37-2).

5.2.3 Hydrochloric acid (HCl) solution, concentrated, reagent grade (36 %) (CAS number 7647-01-0).

5.2.4 Calibration blank, solution of acid used to digest samples (see 5.5.3).

5.2.5 Standard sulfur reference material, for quality control sample solution[2)].

5.2.6 Primary sulfur standard stock calibration solution, containing 1 000 ppm to 10 000 ppm S, and other elements[3)].

5.2.7 Solution of yttrium (Y) for internal standard, or other appropriate internal standard such as europium (Eu) which will not interfere with measurement of the sulfur wavelengths[4)].

5.2.8 Probe-type sonicator, with variable power output control, fitted with a probe of appropriate processing capability for the volume of sample to be treated[5)].

5.2.9 Plastic centrifuge tubes, 50 ml capacity.

1) Millipore Milli-Q® water purification systems are examples of suitable systems available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

2) CNCD-1 certified CNC reference material (National Research Council Canada) containing 8 720 mg/kg ± 140 mg/kg S in a matrix similar to the samples intended for analysis, and NIST bovine liver standard reference material 1577c containing 7 490 ± 340 mg S/kg are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Pre-mixed calibration stock solution composed of 27 elements including 1 000 ppm S (Delta Scientific) or NIST stock sulfur solution (10 300 mg/kg ± 30 mg/kg) are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

4) A 10 000 ppm Y solution from SCP Science is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5) A Sonics vibra-cell™ 130 watt ultrasonic processor with a 6 mm diameter probe is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.2.10 Dialysis membrane tubes, with molecular weight cut-off (MWCO) small enough to prevent CNCs escaping but large enough to allow rapid dialysis[6].

5.2.11 Dialysis clips.

5.2.12 Dialysis column or equivalent.

5.2.13 Freezer.

5.2.14 Freeze-dryer and freeze-dryer flasks.

5.2.15 Desiccator.

5.2.16 Oven, capable of maintaining a temperature of 105 °C ± 3 °C.

5.2.17 Balance, accuracy ± 0,000 1 g.

5.2.18 Aluminium weighing dishes.

5.2.19 Fume hood.

5.2.20 Volumetric flasks, 50 ml, 100 ml and 1 000 ml capacity.

5.2.21 Microwave digestion vessels, quartz or polytetrafluoroethylene (PTFE), with PTFE caps.

5.2.22 Stir bars, egg-shaped, sized to fit in microwave digestion vessels with enough clearance to move easily. Stir bars are not required with every microwave digestion system.

5.2.23 Microwave digestion system. Any pressurized closed vessel microwave digestion system equipped with temperature monitoring capabilities is suitable[7]. Alternatively, a conventional hotplate may be used (see Annex A).

5.2.24 ICP-OES system. Any inductively coupled plasma-optical emission spectroscopy system which can detect concentrations of sulfur ≥ 1 ppm is suitable[8].

5.2.25 Argon gas, high purity (CAS number 7440-37-1), for ICP-OES system.

5.3 Sample purification by dialysis

5.3.1 Carry out the entire procedure in duplicate on separate test specimens.

5.3.2 Around 0,25 g (o.d.) of CNCs are typically required to run the ICP-OES analysis, in order to obtain a sulfur content in the sample solutions that lies in the mid-range of the standard curve; this mass may

6) Spectra/Por® 4 Regenerated Cellulose membranes with MWCO of 12 kDa–14 kDa are an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

7) The CEM Discover SP-D is an example of a suitable instrument available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

8) The Thermo Scientific iCAP 6000 series is an example of a suitable instrument available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

be adjusted as required. The mass of CNC suspension required can be calculated by dividing the desired mass of CNCs for analysis by the mass fraction solids content of the CNC suspension.

5.3.3 Dilute never-dried CNC suspension containing at least 0,5 g (o.d.) of CNCs to around 0,55 % (mass fraction) with water (5.2.1).

It is not recommended that concentrated CNC suspensions [>1 % (mass fraction)] be dialysed, as diffusion rates will decrease due to the increased suspension viscosity, significantly reducing the dialysis efficiency.

5.3.4 Alternatively, redisperse at least 0,5 g (o.d.) spray-dried or freeze-dried CNCs to around 0,55 % (mass fraction) in water (5.2.1) by gradually adding to water in a container while stirring vigorously (e.g. with a magnetic stir bar). Cover and stir until all visible particles have disappeared. Continue stirring for 1 h.

5.3.5 Sonicate the redispersed dried CNC suspension to ensure full dispersion. A suitable procedure is described in Reference [3].

EXAMPLE A 30 g aliquot of 0,55 % (mass fraction) CNC suspension in a 50 ml plastic centrifuge tube is sonicated using a 6 mm diameter probe on a 130 W sonicator set at 60 % amplitude (7 W – 8 W power output), to a total energy input of 1 650 J (= 10 kJ per g CNCs in the sample). This operation is performed a total of three times to obtain a sonicated sample containing 0,5 g of CNC.

Ultrasonic baths are not powerful enough to achieve full dispersion of CNC agglomerates; sonicator probes directly immersed in the suspension shall be used.

Sonication may be omitted if unsonicated and sonicated samples show no difference in final results (e.g. for never-dried CNC suspensions). Each different type of sample should be tested before omitting sonication.

Sonicated suspensions may be filtered using GF/F glass microfibre filter paper to remove metal particles (normally titanium, aluminium or aluminium-containing alloys) released from the probe.

5.3.6 Soak dialysis membrane tubes in water (5.2.1) for 30 min, then rinse thoroughly inside and out with fresh water (5.2.1).

5.3.7 Place suspension in dialysis membrane tubes after clipping one end, ensuring the tubes are no more than two-thirds full, then clip the other end. Dialyse the samples against running deionized or better-quality water for at least 3 days. If using dialysis columns (tanks containing static water stirred with magnetic stir bar or equivalent), change the water (5.2.1) at least three or four times per day for at least 3 days, until constant pH and conductivity of the column water are reached for two consecutive water changes. Both pH and conductivity should be within ± 0,2 units and ± 5 µS/cm of the values for the water used for dialysis.

5.3.8 Freeze and lyophilize the dialysed CNC suspensions and store the freeze-dried CNC samples in a desiccator.

Dried cellulose nanocrystals are hygroscopic and adsorb atmospheric moisture rapidly; they should always be stored in a desiccator when not in use.

5.3.9 Accurately weigh (to ± 0,000 1 g) a minimum of approximately 0,20 g of the freeze-dried CNCs obtained in 5.3.8. Dry to constant mass at a temperature of 105 °C ± 3 °C. Cool the sample in a desiccator and weigh. Calculate w, the mass fraction solids content, expressed as a percentage, using Formula (1):

$$w = 100\left(1 - \frac{m_o - m_d}{m_o}\right) = 100\frac{m_d}{m_o} \quad (1)$$

where

w is the mass fraction solids content, expressed as a percentage, of the dialysed CNC suspension;

m_o is the mass, in grams, of the original sample;

m_d is the mass, in grams, of the dry sample.

The CNC sample may also be dried to constant mass by storing the sample in a desiccator over magnesium perchlorate for 8 days.

5.4 Microwave-assisted sample digestion and sample preparation

5.4.1 The sample is completely solubilized by microwave-assisted digestion using high purity nitric acid in high pressure closed vessels. Wet ashing digestion using a hotplate can provide similar results (see Annex A). The open glassware used in conventional wet ashing, however, poses a risk of cross-contamination if several samples are being heated at one time and "bumping" occurs in one or more samples. Additionally, losses due to volatilization are minimized in the closed vessels used for microwave-assisted digestion.

5.4.2 Pre-clean the microwave vessels by washing with soap, followed by 50:50 vol:vol hydrochloric acid/water solution, then rinse with ultrapure water (5.2.1). Dry in the oven at 105 °C ± 3 °C for ≥ 4 h. If necessary, add 5 ml of concentrated nitric acid and run the same microwave program as for the samples (5.4.10), then rinse with ultrapure water (5.2.1) and dry.

Alternatively, if the sample is digested for ICP-OES by wet ashing, the method given in Annex A shall be followed.

5.4.3 Measure the moisture content of each freeze-dried CNC sample immediately prior to analysis by weighing a portion before and after drying to constant mass as described in 5.3.9.

5.4.4 Accurately weigh (to ± 0,000 1 g) about 0,25 g (o.d.) of the freeze-dried CNCs obtained in 5.3.8 into a pre-cleaned microwave vessel, minimizing the amount of CNCs on the inner walls of the vessel.

5.4.5 Insert clean stir bar into the vessel if needed.

5.4.6 Slowly add 5 ml of concentrated nitric acid to the vessel, ensuring that the CNCs are rinsed down the inner walls. The amount of acid may be adjusted provided that the matrix composition of the test sample solutions and the calibration solutions is the same (see 5.4.12).

5.4.7 Set the sample aside for about 15 min to pre-digest in the acid. Do not stir the sample.

NOTE Freeze-dried CNC flakes are very light and tend to hold static charge. Therefore, they might fly onto the inner walls of the vessel during vigorous mixing and adhere there such that they are not exposed to the liquid acid during the subsequent steps, which could prevent them being fully digested.

5.4.8 Add 2 ml water (5.2.1) down the inner walls of the vessel to further rinse the CNCs into the acid.

5.4.9 Place the PTFE cap on the vessel and load the sample into the microwave system.

5.4.10 Digest the sample in the microwave according to the manufacturer's instructions or established protocol (see example).

EXAMPLE CNC microwave digestion protocol (operating parameters) for a CEM Discover SP-D.

Step	Temperature °C	Ramp time min	Hold time min	Pressure MPa	Power W	Stirring speed
1	100	4,0	1,0	2,76	300	Medium
2	90	2,0	1,0	2,76	300	Medium
3	180	6,0	3,0	2,76	300	Medium

5.4.11 After digestion, if there are undigested CNCs on the inner walls of the vessel or as visible solid particles in a transparent solution, repeat the digestion steps using the same method.

The acid used might need to be adapted to obtain complete dissolution (a clear solution with no solid residue), depending on the microwave system. A 3:1 vol:vol nitric acid/hydrochloric acid mixture has also been used to digest CNCs.

The microwave digestion program might need to be adapted depending on the microwave system. If the instrument manufacturer provides a suggested program for cellulose matrices, it can be used as a starting point if the above method does not work.

5.4.12 Cool the digested sample and transfer into a 100 ml volumetric flask by rinsing the vessel, including the cap, with ultrapure water (5.2.1). Reconstitute the sample with water (5.2.1) to give a test sample solution with a final volume of 100 ml (a 1:19 vol:vol ratio). The exact nitric acid concentration of the matrix (~5 %) will vary depending on the exact concentration (assay) of the concentrated nitric acid (5.2.2). As long as the same ratio of the volume of concentrated acid to the final volume of matrix is used for all test sample solutions, calibration solutions, etc., the matrix composition will be identical. The amount of acid used to digest the sample in 5.4.6, as well as the final volume, may be adjusted provided that the matrix compositions of the test sample solutions and those of the calibration solutions are the same.

The following optional drying and reconstitution steps may be taken:

— after cooling, transfer the contents of the vessels to a pre-cleaned polypropylene or PTFE vial;

— place on a hotplate in the fume hood and evaporate to near dryness to remove the acid;

— dissolve the residues in concentrated nitric acid;

— cool and dilute each sample with water (5.2.1) such that the matrix composition of the test sample solutions and the calibration solutions is the same.

5.4.13 Prepare a matrix blank solution by performing 5.4.2 to 5.4.12 without sample (same final volume).

5.4.14 Prepare quality control sample solution by performing 5.4.2 to 5.4.12 with standard sulfur reference material (5.2.5).

5.4.15 To evaluate the performance of the analytical procedure and compensate for any residual matrix interferences, samples should be spiked with at least three incremental levels of appropriate amounts of known primary sulfur standard stock calibration solution (5.2.6), which contains between 1 000 ppm and 10 000 ppm S.

5.4.15.1 Prepare a spiked sample solution series by dividing the (digested and diluted) test sample solution into four equal portions and placing them in four vials (A, B, C, D). Spike vial B with *x* g or ml of the primary sulfur standard stock calibration solution (5.2.6), vial C with 2*x* g or ml of the primary sulfur standard stock calibration solution (5.2.6) and vial D with 3*x* g or ml of the primary sulfur standard stock calibration solution (5.2.6) as shown in Table 1. To ensure each sample has the same volume, make up the balance by adding 5 % nitric acid. The internal standard (5.2.7) may be added to the unknown test sample solution prior to dividing it into four portions, or to each of the four portions separately.

NOTE It is assumed that each flask or vial contains more than enough sample volume for analysis.

Table 1 — Samples for standard addition analysis and plot

Vial	Sample, i	$m_{S,\,i}$	$m_{std,\,i}$	$m_{int,\,i}$
A	1	1	0	1
B	2	1	1	1
C	3	1	2	1
D	4	1	3	1

Symbols:

S = CNC sample

std = sulfur standard (5.2.6)

int = internal standard (5.2.7)

$m_{S,\,i}$ = mass, in grams, of the CNC sample present in each sample aliquot

$m_{std,\,i}$ = mass, in grams, of the sulfur standard added to each sample aliquot

$m_{int,\,i}$ = mass, in grams, of the internal standard added to each sample aliquot

5.4.15.2 Prepare a spiked sample series for both the undiluted matrix blank and the quality control samples.

The concentration of analyte in the spiking solution (primary sulfur standard stock calibration solution, 5.2.6) should be 50 to 100 times higher than the concentration of the analyte in the unspiked sample (sulfur from CNCs). This allows the minimum volume of standard to be added such that the sample is not diluted significantly (by more than ~5 %) if the balance is not made up by adding 5 % nitric acid.

The spiked concentration should be of the same magnitude as that of the sample itself. Spiking levels should be chosen such that the spike results in a onefold to twofold increase in the total sulfur concentration in the sample and the analytical response is linear. For example, a sample containing 10 ppm sulfur may be spiked with 10 ppm and 20 ppm of a sulfur standard, but not 0,001 ppm or 100 ppm. However, if the sample sulfur concentration is very low, it should be spiked at a level that is in the middle of the calibration range.

The concentration of the spiked sample should be within the calibration range. If necessary, dilute the spiked sample (after spiking).

5.5 Preparation of calibration solutions and blanks

5.5.1 Ensure that the matrix of all the calibration solutions and blanks matches that of the samples and contains the same final concentration of appropriate acid used to digest the samples.

5.5.2 Prepare sulfur calibration solutions of incremental concentration (e.g. 1 ppm, 10 ppm, 20 ppm, 50 ppm and 100 ppm or mg/l) from 1 000 ppm primary sulfur standard stock calibration solution by transferring aliquots (e.g. 0,1 ml, 1 ml, 2 ml, 5 ml and 10 ml) to 100 ml volumetric flasks. Fill about halfway with ultrapure water (5.2.1), add 5 ml of concentrated nitric acid (5.2.2) and dilute with ultrapure water (5.2.1) to 100 ml. Store in plastic bottles previously washed with a 50:50 hydrochloric acid/water solution.

Primary sulfur standard stock calibration solutions and test sample solutions may be diluted either gravimetrically or volumetrically; care shall be taken to ensure that the dilution method is applied consistently. Calibration solutions shall not be prepared by serial dilution, but rather from an appropriate aliquot of the primary standard stock solution.

5.5.3 Prepare calibration blank solution (acid blank) by diluting the acid used to digest the samples to the same concentration as the matrix of the samples to be analysed.

5.5.4 Prepare internal standard by diluting stock solution (5.2.7) to an appropriate concentration, e.g. 10 mg/l yttrium, following the procedure in 5.5.2.

5.6 Analysis of standards and samples by ICP-OES

5.6.1 Switch on the inductively coupled plasma-optical emission spectrometer and configure it for detection of sulfur according to the manufacturer's instructions.

The wavelength(s) used for sulfur detection (around 180 nm to 182 nm) may be varied slightly depending on the ICP-OES manufacturer and interference from sample matrix components.

5.6.2 Reference the analyte lines to the internal standard line (e.g. yttrium, wavelength 224,306 nm).

5.6.3 Rinse the instrument with calibration blank solution.

Ideally, the acid concentration of the rinse solution should match that of the samples, but a small variation is acceptable.

5.6.4 Run at least five incremental sulfur standard solutions and a calibration blank solution, rinsing the instrument with calibration blank solution between each sample. Plot the signal intensity against concentration and verify that the instrument response is linear in the range of sample measurements.

To determine if there is any memory effect, compare the signal with the first measurement of calibration blank (rinse) solution. If the signal is higher, keep running rinse solution until the signal is at the same level as the first measurement.

A calibration range from 1 ppm to 100 ppm or mg/l sulfur will help to ensure the samples are in the linear range of the calibration curve.

Always make sure that the sample signal intensity lies within the calibration range. If not, samples should be diluted or an additional standard with higher concentration prepared (making sure it is still in the linear range).

A minimum linear regression coefficient of 0,99 has to be achieved to ensure that a linear calibration function has been obtained.

5.6.5 Run a calibration blank or rinse solution to check for carryover. If there is carryover, increase rinse time until signal is at the same level as the first measurement of calibration blank or rinse solution.

5.6.6 Run sulfur standards as unknowns to check the calibration, each followed by a calibration blank or rinse solution. The calibration check standard concentrations shall cover both the low- and mid-range of the calibration curve, e.g. 1 ppm and 50 ppm or mg/l. The calibration check standards shall be purchased or prepared from a different primary stock solution, preferably from a different source than the primary sulfur stock solution (5.2.6).

5.6.7 Run matrix blank solution followed by a calibration blank or rinse solution.

5.6.8 Run spiked sample series for each unknown sample, the blank and the quality control samples, each series followed by a calibration blank or rinse solution. Check the calibration for drift when needed. If necessary to ensure that the signal strength is not out of range, dilute the samples while ensuring that the final acid concentration is the same.

5.6.9 After all samples have been analysed, run calibration blank solution or rinse solution to rinse the instrument.

5.7 Calculation of dry CNC total elemental sulfur content and CNC surface charge

Construct a standard addition plot for each CNC sample and for the quality control sample, using the following approach.

Since the slope of the standard addition calibration function for the sample and blank might not be equivalent, separate plots for the samples and the blanks should be constructed.

Prepare a standard addition plot for each series of spiked CNC (or quality control) samples:

$$y = b + ax$$

where

b is the intercept, in mg/kg, of the standard addition plot for the samples;

a is the slope, in mg/kg, of the standard addition plot for the samples.

The values for the y and x axes are given by:

$$y = S_{std}(m_{std,\,i}/m_{S,\,i}) \text{ for } i = 1, 2, 3, 4, \text{ mg/kg}$$

$$x = R_i(m_{int,\,i}/m_{S,\,i}) \text{ for } i = 1, 2, 3, 4$$

where

$m_{S,\,i}$ is the mass, in grams, of the sample present in each sample aliquot;

$m_{std,\,i}$ is the mass, in grams, of the sulfur standard added to each sample aliquot;

$m_{int,\,i}$ is the mass, in grams, of the internal standard added to each sample aliquot;

S_{std} is the mass fraction, in mg/kg, of analyte (sulfur) in the sulfur standard added to each sample aliquot;

R_i is the ratio of signals corresponding to the analyte and the internal standard.

The total elemental sulfur content in each sample, [S], expressed in mmol/kg of dry sample is obtained from Formula (2):

$$[S] = \frac{1}{32{,}06}\left(\frac{-100b}{w} - S_{blk}\right) \quad (2)$$

where

[S] is the total content, in mg/kg, of sulfur in the sample;

32,06 is the molar mass of sulfur, in mg/mmol;

w is the mass fraction solids content, expressed as a percentage, of the CNC or quality control sample taken for digestion, calculated using Formula (1) in 5.3.9;

S_{blk} is the concentration of sulfur, in mg/kg, in the undiluted matrix blank solution obtained from the average of the standard addition plots for the blank samples.

If volumetric dilution is used, replace all masses with volumes expressed in l, and replace all concentrations expressed in mg/kg with concentrations expressed in mg/l.

NOTE Assuming that all residual sulfate ions or other sulfur-containing contaminants were removed by dialysis, the total content of sulfate half-ester groups is equivalent to the total elemental sulfur content when expressed in mol per kg of dry CNCs.

The CNC surface charge content, σ, expressed in meq per kg of dry CNCs, is equivalent to the total elemental sulfur content [S] expressed in mmol/kg of dry sample.

Repeatability and reproducibility data for this method are given in Annex C.

5.8 Test report

The test report shall contain at least the following information:

a) a reference to this document, i.e. ISO 21400;

b) a reference to the method used;

c) complete identification of the sample including source, date of receipt, form of sample;

d) the results of the duplicate determinations;

e) the sulfur line and internal standard line used;

f) any details not specified in this document or which are optional;

g) any unusual features observed during the determination;

h) any deviations from this method and details of all circumstances which could have affected the results.

6 Sulfate half-ester content — Conductometric titration method

6.1 Principle

6.1.1 This method covers the determination of sulfate half-ester content of cellulose nanocrystals (CNCs). Conductometric titration is used for analysis, following sample purification by dialysis to remove any sulfur-containing and other ionic contaminants found in the water matrix of the aqueous CNC suspension, and sample protonation to ensure that most (all) of the sulfate half-ester groups in the CNC sample are in the acidic form and thus detectable by titration with sodium hydroxide during analysis.

6.1.2 Conductometric titration measures the protons associated with the anionic sulfate half-ester groups on the CNCs. If excess acid of any kind is present in solution, an erroneously high titration reading will be obtained. On the other hand, if some of the sulfate half-ester groups are not protonated (i.e. if sulfate half-ester groups have counterions other than protons, such as Na^+), a low reading will be obtained. A protonation step following purification is therefore vital to obtaining reliable and accurate conductometric titration results[3][7]. Protonation is performed by treating the CNC suspension with hydrogen form strong acid cation (SAC) exchange resins.

6.1.3 Conductometric titration is the most commonly used analytical technique for the quantification of sulfated cellulose nanocrystal surface charge[8], offering clearer end point determination than pH titration, and being more accessible than ICP-OES by virtue of the simpler equipment and chemicals it requires. Although conductometric titration measures the surface charge directly, it can provide the equivalent information to ICP-OES (total elemental sulfur content), provided the CNCs are pure and fully protonated as described above[3].

6.2 Reagents and apparatus

6.2.1 Water, ultrapure (deionized or distilled), conforming to Grade 2 of ISO 3696 or better[9].

6.2.2 Potassium hydrogen phthalate (KHP), primary standard (CAS number 877-24-7).

6.2.3 Sodium hydroxide (NaOH) solution, 0,010 M (CAS number 1310-73-2), standard grade.

6.2.4 Sodium chloride (NaCl) solution, 0,10 M (CAS number 7647-14-5), reagent grade.

6.2.5 pH standard solutions, pH 4, 7 and 10 or similar.

6.2.6 Desiccator.

6.2.7 Oven, capable of maintaining a temperature of 105 °C ± 3 °C.

6.2.8 Balance, accuracy ± 0,000 1 g.

6.2.9 Dialysis membrane tubes, with molecular weight cut-off (MWCO) small enough to prevent CNCs escaping but large enough to allow rapid dialysis[10].

6.2.10 Dialysis clips.

6.2.11 Dialysis column or equivalent.

6.2.12 Probe-type sonicator, with variable power output control, fitted with a probe of appropriate processing capability for the volume of sample to be treated[11].

6.2.13 Plastic centrifuge tubes, 50 ml capacity.

6.2.14 SAC exchange resin, hydrogen form, with an exchange capacity of around 1,8 meq per ml of resin in the hydrogen form[12].

6.2.15 Glass column with glass frit and tap, inner diameter 1,9 cm or similar.

6.2.16 Filter funnel.

6.2.17 Ashless filter paper or **GF/F glass microfibre filter**.

9) Millipore Milli-Q® water purification systems are examples of suitable systems available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

10) Spectra/Por® 4 regenerated cellulose membranes with MWCO of 12 kDa–14 kDa are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

11) A Sonics vibra-cell™ 130 watt ultrasonic processor with a 6 mm diameter probe is an example of a suitable instrument available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

12) Dowex™ Marathon C hydrogen form SAC exchange resin is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6.2.18 Buret, 50 ml graduated or automated titration system[13].

6.2.19 Conductivity probe and meter. A conductivity probe with a cell constant of up to 1 cm^{-1} is appropriate for the conductivity ranges typically measured during the conductometric titration.

6.2.20 pH probe and meter.

6.2.21 Stirrer (propeller mixer or **stir plate and magnetic stir bar)**.

6.2.22 Beakers.

6.2.23 Glass graduated cylinder, 100 ml to 250 ml capacity, tolerance ± 1 ml.

6.2.24 Nitrogen gas, pre-purified (CAS number 7727-37-9), for bubbling through samples during titration.

6.3 Sample purification by dialysis

6.3.1 Carry out the entire procedure in duplicate on separate test specimens.

6.3.2 Around 0,15 g (o.d.) CNCs are required for one conductometric titration. The mass of CNC suspension required for the desired number of replicates can be obtained by dividing the desired mass of CNCs for analysis by the mass fraction solids content of the CNC suspension. Purifying and treating excess suspension is desirable to compensate for transfer losses which may occur.

6.3.3 Dilute never-dried CNC suspension to around 0,55 % (mass fraction) with water (6.2.1).

It is not recommended that concentrated CNC suspensions [>1 % (mass fraction)] be dialysed, as diffusion rates will decrease due to the increased suspension viscosity, significantly reducing the dialysis efficiency.

Dialysis results in slight dilution of the CNC suspension; it is not necessary to determine an extremely accurate value of the mass fraction solids content before dialysis.

6.3.4 Alternatively, redisperse spray-dried or freeze-dried CNCs to around 0,55 % (mass fraction) by gradually adding to water (6.2.1) in a beaker while stirring vigorously with a magnetic stir bar. Cover and stir until all visible particles have disappeared. Continue stirring for 1 h.

6.3.5 Sonicate the redispersed dried CNC suspension to ensure full dispersion. A suitable procedure is described in Reference [3].

EXAMPLE A 30 g aliquot of 0,55 % (mass fraction) CNC suspension in a 50 ml plastic centrifuge tube is sonicated using a 6 mm diameter probe on a 130 W sonicator set at 60 % amplitude (7 W – 8 W power output), to a total energy input of 1 650 J (= 10 kJ per g CNCs in the sample).

Ultrasonic baths are not powerful enough to achieve full dispersion of CNC agglomerates; sonicator probes directly immersed in the suspension shall be used.

Sonication may be omitted if unsonicated and sonicated samples show no difference in final results (e.g. for never-dried CNC suspensions). Each different type of sample should be tested before omitting sonication.

13) A Metrohm Titrando titrator system is an example of a suitable instrument available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Sonicated suspensions may be filtered using GF/F glass microfibre filter paper to remove metal particles (normally titanium, aluminium or aluminium-containing alloys) released from the probe.

6.3.6 Soak dialysis membrane tubes in a container of several litres of water (6.2.1) for 30 min, then rinse thoroughly inside and out with fresh water (6.2.1).

6.3.7 Place suspension in dialysis membrane tubes after clipping one end, ensuring the tubes are no more than two-thirds full. Dialyse the samples against running deionized or better-quality water for at least 3 days. If using dialysis columns (static water stirred with magnetic stir bar or equivalent), change the water (6.2.1) at least three or four times per day for at least 3 days, until constant pH and conductivity of the column water are reached for two consecutive water changes. Both pH and conductivity should be within ± 0,2 units and ± 5 µS/cm of the values for the water used for dialysis.

6.3.8 Accurately weigh (to ± 0,000 1 g) approximately 10 ml of the dialysed CNC suspension obtained in 6.3.7. Dry to constant mass at a temperature of 105 °C ± 3 °C. Cool the sample in a desiccator and weigh. Calculate *w*, the mass fraction solids content expressed as a percentage, using Formula (3):

$$w = 100\left(1 - \frac{m_o - m_d}{m_o}\right) = 100\frac{m_d}{m_o} \quad (3)$$

where

w is the mass fraction solids content, expressed as a percentage, of the dialysed CNC suspension;

m_o is the mass, in grams, of the original sample;

m_d is the mass, in grams, of the dry sample.

This step may be omitted, provided that it is known approximately how much the dialysis step dilutes the original sample of known mass fraction solids content. An estimate is sufficient at this point.

6.4 Sample protonation by ion exchange

6.4.1 Ion exchange resin treatment may be performed by adding resin directly into the sample followed by mixing and removal of the resin by filtration (the batch method, described in Annex B), or by flowing the CNC suspension through a column containing a bed of the resin and collecting the treated eluate (the column method). Column treatment is analogous to an infinite series of batch steps; continuous contact between fresh resin and the sample to be treated favourably drives the exchange equilibrium. Column treatment is therefore significantly faster and more efficient than batch treatment[9].

6.4.2 Rinse hydrogen form SAC exchange resin with a large excess of ultrapure water (6.2.1) (generally 3 l to 5 l, depending on the quantity of resin to be used) until the filtrate is colourless and similar in conductivity to the original wash water (<5 µS/cm).

Hydrogen form SAC exchange resins can undergo decrosslinking of the polystyrene matrix during storage, which causes them to release small quantities of protonated sulfonated polystyrene oligomers into the CNC suspension, which would cause overestimation of the sulfate half-ester content by titration[10]. These resins shall therefore be thoroughly rinsed with large volumes of ultrapure water (6.2.1) immediately before use.

Mixed bed (H and OH form) ion exchange resin has been used in several publications with the purpose of both purifying and protonating the CNCs. Further study has shown that this practice reduces the total elemental sulfur content of the CNCs. For this reason, the use of mixed bed ion exchange resin is strongly discouraged[3].

6.4.3 Remove water by filtration with a filter paper or GF/F glass microfibre filter (plastic mesh filters may not remove fine resin fragments) until the resin is just slightly damp (similar to the resin in the container).

NOTE Around 21 g of damp resin per g (o.d.) of CNCs to be treated is sufficient for an SAC exchange resin with an exchange capacity of 1,8 meq/ml and a volume of 0,81 ml/g.

Varying water contents of the damp resin will affect the protonation capacity. If this appears to affect the results, use more damp resin.

6.4.4 Form a slurry of freshly rinsed resin in ultrapure water (6.2.1) and pour into a column with a fritted glass disk (or similar) at the bottom, ensuring no air bubbles remain in the resin.

The resin bed should preferably have an aspect ratio of at least 10:1 to ensure efficient ion exchange[11].

EXAMPLE For the preparation of 2,5 g (o.d.) of CNCs, equivalent to 500 ml of 0,5 % (mass fraction) CNC suspension, a column is prepared with 52,5 g of damp SAC resin with an exchange capacity of 1,8 meq/ml and a volume of 0,81 ml/g. The total column exchange capacity is therefore 52,5 g × 1,8 meq/ml × 0,81 ml/g = 76,5 meq. Assuming the CNCs contain 240 mmol/kg of sulfate half-ester groups, each associated with an Na^+ counterion, the sample will consume 0,002 5 kg × 240 meq/kg = 0,6 meq, or less than 1 % of the column's capacity. This will help ensure efficient ion exchange.

6.4.5 Rinse the resin quickly with at least 10 bed volumes of ultrapure water (6.2.1) and then with 10 bed volumes of ultrapure water (6.2.1) at a flow rate of around 1,3 ml/min/cm^2, or until the eluate conductivity is stable and close to that of the water used for washing (<5 µS/cm). Do not allow the water level to fall below the top of the resin bed.

The flow rate shall be slow enough to keep the exchange reaction in equilibrium.

NOTE A flow rate of 1,3 ml/min/cm^2 is equivalent to 3,69 ml/min for a column with an inner diameter of 1,9 cm.

6.4.6 Pass the dialysed CNC suspension through the resin column at a flow rate of around 1,3 ml/min/cm^2 and collect the treated suspension.

Small quantities (<100 ml) of CNC suspension may be treated using a batch method instead of the column method (see Annex B).

6.4.7 If unsure as to whether the sample is fully protonated, pass the treated suspension through the column again; the conductivity should be identical to the conductivity of the sample after the first pass.

NOTE The conductivity of the CNC suspension will increase during resin treatment as other cations are replaced by protons, which have a higher mobility.

6.4.8 Accurately weigh (to ± 0,000 1 g) 10 ml or more of the final resin-treated CNC suspension. Dry to constant mass at a temperature of 105 °C ± 3 °C. Cool the sample in a desiccator and weigh. Calculate *w*, the mass fraction solids content, expressed as a percentage, using Formula (3).

6.4.9 If the batch method of ion exchange resin treatment is used, the method described in Annex B shall be followed.

6.5 Sample analysis by conductometric titration

6.5.1 Calibrate the pH meter with fresh pH standards according to the manufacturer's instructions.

6.5.2 Standardize the sodium hydroxide solution against dry primary standard potassium hydrogen phthalate.

6.5.2.1 Dry potassium hydrogen phthalate for at least 4 h in an oven at 105 °C ± 3 °C.

6.5.2.2 Cool to room temperature in a desiccator.

6.5.2.3 Accurately weigh (to ± 0,000 1 g) a small amount of potassium hydrogen phthalate, such that the volume of sodium hydroxide solution required to neutralize can be accurately measured into a 250 ml beaker and add 200 ml of ultrapure water (6.2.1). Stir until dissolved.

6.5.2.4 Titrate potassium hydrogen phthalate solution against the fresh 0,010 M sodium hydroxide solution in triplicate and determine the precise concentration (titre) of sodium hydroxide from the volume of sodium hydroxide solution required to reach the equivalence point.

6.5.2.5 Calculate c, the concentration (titre) of sodium hydroxide expressed in mol/l using Formula (4):

$$c = \frac{m_{\mathrm{KHP}}}{204{,}22 V_{\mathrm{e}}} \tag{4}$$

where

c is the concentration, in mol/l, of sodium hydroxide;

m_{KHP} is the mass, in grams, of potassium hydrogen phthalate standard;

204,22 is the molar mass, in g/mol, of potassium hydrogen phthalate;

V_{e} is the volume, in l, of sodium hydroxide solution required to reach the equivalence point.

6.5.3 Prepare and calibrate the conductivity probe according to the manufacturer's instructions.

6.5.4 Accurately weigh (to ± 0,000 1 g) a known volume of SAC exchange resin-treated CNC suspension containing approximately 0,15 g (o.d.) of CNCs into a 250 ml beaker.

6.5.5 Add sufficient ultrapure (not DI) water (6.2.1) to give a total sample volume of 198 ml.

6.5.6 Add 2 ml of 0,1 M sodium chloride solution to the sample with mixing to give a test sample solution with a final sodium chloride concentration of 1 mM. Stir, ensuring that air bubbles are not created, for several minutes until the conductivity reading is stable. It is recommended that nitrogen gas be rapidly bubbled through the suspension to prevent carbon dioxide from being incorporated into the solution.

When stirring, ensure that air bubbles are not created, to avoid the incorporation of carbon dioxide into the sample, which would create carbonic acid and consume sodium hydroxide, thereby interfering with end point detection and giving an erroneously high equivalence point.

NOTE The sodium chloride is added to increase the sample conductivity and ensure that it lies in an acceptable range for accurate detection of conductivity differences by the conductivity meter.

6.5.7 Under constant stirring, perform conductometric titration of CNC samples, adding standardized 0,010 M sodium hydroxide in 0,05 ml to 0,10 ml increments, allowing the conductivity to stabilize between readings (a 30 s to 60 s equilibration time, which should be consistent throughout, is recommended if performing the titration manually).

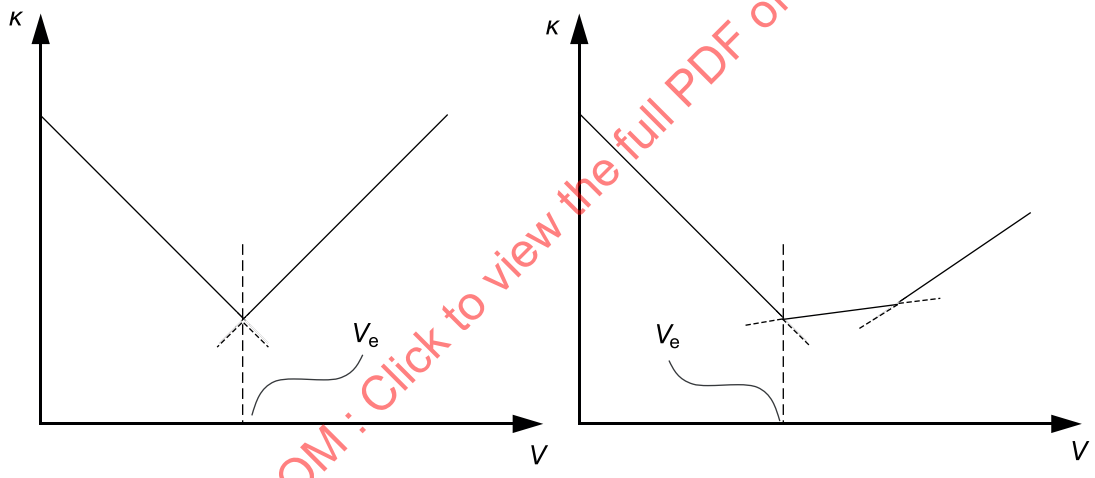
NOTE If the titration is not automated, larger aliquots of NaOH solution (e.g. 0,2 ml to 0,5 ml) can be used far from the equivalence point, and smaller aliquots (0,05 ml to 0,10 ml) can be used near the equivalence point to ensure sufficient resolution is achieved.

6.5.8 Once the equivalence region has been passed, continue adding aliquots of sodium hydroxide titrant until the conductivity value approaches the original value if possible, such that there are sufficient data points at a distance from the equivalence point(s) to allow accurate linear fits and extrapolation (see Figure 1).

6.6 Calculation of dry CNC sulfate half-ester content and CNC surface charge

6.6.1 Correct for dilution due to addition of the sodium hydroxide titrant, by multiplying the conductivity readings by the factor $(V_t + v)/V_t$, where V_t is the initial test sample solution volume expressed in l, and v is the added volume of titrant expressed in l.

6.6.2 Plot the corrected conductivities κ expressed in mS/cm versus the added volume of sodium hydroxide titrant v expressed in l to give a plot similar to one of the schematic conductometric titration curves in Figure 1.



a) For strong acids **b) For mixtures of strong and weak acids**

Key

V volume

κ corrected conductivity

V_e volume at equivalence point

Figure 1 — Schematic conductometric titration curves

6.6.3 If only strong acids (i.e. protonated sulfate half-ester groups) are present and a v-shaped curve is seen, extrapolate linear fits of the data close to zero added titrant volume and close to the final added titrant volume. If small quantities (<100 mmol COOH/kg CNC) of weak acids are also present, perform the second extrapolation from a linear fit of the data points in the more horizontal intermediate weak acid (i.e. carboxylic acid groups) region. The intersection of the extrapolated curves gives the volume of sodium hydroxide added at the equivalence point for the titration of the strong acid sulfate half-ester groups.

NOTE If small quantities (<100 mmol COOH/kg CNC) of weak acid groups are present, the graphical determination of equivalence points 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. Typical variation introduced into calculated sulfate half-ester content values is around 1 % to 2 %.

Small quantities (<100 mmol COOH/kg CNC) of weak acid groups such as carboxylic acids present on cellulose nanocrystals may be determined simultaneously with strong acid groups such as protonated sulfate half-esters by measuring both end points as described above. It is recommended to verify the total sulfur content of CNCs containing weak acid groups by ICP-OES analysis. Larger quantities of weak acid groups interfere with the detection of the strong acid end point. Conductometric titration is not recommended for the determination of sulfate half-ester groups on CNCs which also contain more than 100 mmol COOH/kg CNC.

6.6.4 Calculate the quantity of protonated sulfate half-ester groups [R–OSO_3H] present on the CNC surface, expressed in moles per kg of dry CNCs, using Formula (5):

$$\left[\mathrm{R-OSO_3H}\right] = \frac{V_e c}{m_t} \quad (5)$$

where

[R–OSO_3H] is the quantity of protonated sulfate half-ester groups present on the CNC surface, expressed in mol/kg;

V_e is the volume, in l, of added sodium hydroxide solution at the equivalence point;

c is the concentration (titre), in mol/l, of the NaOH solution;

m_t is the oven-dry mass, in kg, of resin-treated CNCs in the suspension being titrated.

NOTE Assuming that all residual sulfate ions were removed by dialysis, that all the sulfate half-ester groups were protonated by SAC exchange resin treatment, and that the carboxylic acid groups are fully deprotonated (neutralized) during titration, the quantity of sulfate half-ester groups, [R–OSO_3H], expressed in moles per kg of dry CNCs, is equivalent to the total elemental sulfur content measured by ICP-OES, expressed in moles per kg of dry CNCs. The quantity of sulfur, [S], associated with protonated sulfate half-ester groups, expressed in mg per kg of dry CNCs, is obtained by multiplying [R–OSO_3H] by 32,060 (the molar mass of sulfur expressed in mg/mol).

6.6.5 Calculate the CNC surface charge content, σ, expressed in meq/kg of dry CNCs by dividing [R–OSO_3H], the quantity of protonated sulfate half-ester groups expressed in moles per kg of dry CNCs, by 0,001 mol/meq.

NOTE Each sulfate half-ester group bears one negative charge.

6.6.6 Repeatability and reproducibility data for this method are given in Annex C.

6.7 Test report

The test report shall contain at least the following information:

a) a reference to this document, i.e. ISO 21400;

b) a reference to the method used;

c) complete identification of the sample including source, date of receipt, form of sample;

d) the results of the duplicate determinations;

e) any details not specified in this document or which are optional;

f) any unusual features observed during the determination;

g) any deviations from this method and details of all circumstances which could have affected the results.

Annex A
(normative)

Sample digestion by wet ashing

A.1 Reagents and apparatus

A.1.1 **Water**, ultrapure (deionized or distilled), conforming to Grade 2 of ISO 3696 or better.

A.1.2 **Perchloric acid ($HClO_4$) solution,** 65 % – 71 %, trace metal grade (CAS number 7601-90-3).

A.1.3 **Nitric acid (HNO_3) solution**, concentrated, trace metal grade (60 % – 70 % assay) (CAS number 7697-37-2).

A.1.4 **Hydrochloric acid (HCl) solution**, concentrated, trace metal grade (36 %) (CAS number 7647-01-0).

A.1.5 **Hydrochloric acid solution**, 6 M. Add 500 ml of concentrated hydrochloric acid to 1 l of water (A.1.1) to obtain a 6 M HCl solution.

A.1.6 **Conical oxidation flasks**, 300 ml capacity.

A.1.7 **Fume hood**, perchloric acid type, conforming to ISO 14644-1, class 10.

A.1.8 **Hotplate**.

A.1.9 **Volumetric flasks**, 100 ml and 1 000 ml capacity.

A.2 Digestion procedure

A.2.1 Carry out the entire procedure in duplicate on separate test specimens.

A.2.2 Rinse all glassware with 6 M hydrochloric acid (A.1.5) and water (A.1.1).

A.2.3 Immediately prior to analysis, measure and calculate the mass fraction solids content *w* of each dialysed then freeze-dried (5.3.8) CNC sample using Formula (1) as in 5.3.9.

A.2.4 Accurately weigh (to ± 0,000 1 g) about 0,25 g (o.d.) of the freeze-dried CNCs into a conical oxidation flask.

A.2.5 Add 50 ml concentrated nitric acid (A.1.3) to the sample, followed by 2 ml perchloric acid (A.1.2).

A.2.6 Place the sample on a hotplate in a perchloric acid type fume hood at low heat until the brown fumes subside. Increase the heat until the acid boils.

A.2.7 Continue heating until all the nitric acid is boiled off. When only perchloric acid remains and is giving off dense white fumes, heat for an additional 5 min.