TECHNICAL SPECIFICATION

ISO/TS 21346

First edition
2021-03

Nanotechnologies — Characterization of individualized cellulose nanofibril samples

Nanotechnologies — Caractérisation d'échantillons de nanofibrilles individualisées de cellulose

Reference number
ISO/TS 21346:2021(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 229, *Nanotechnologies*.

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

Cellulose nanomaterials derived from naturally occurring cellulosic fibres are renewable advanced materials with unprecedented properties. They are of wide variety in morphology, e.g. different shapes, branching and networking. Basic research related to cellulosic nanomaterials has been increasingly conducted worldwide. At the same time, manufacturing industries have already started to deliver cellulose nanomaterials to the market. Application industries are also becoming more and more interested in these new materials.

All native cellulosic fibres are composed of bundles in which the smallest fibril unit is an elementary fibril originating from a cellulose terminal enzyme complex. An elementary fibril is made of a certain number of cellulose molecules and contains crystalline regions predominantly. The size of an elementary fibril is specific to the native cellulose source. In wood pulp, the cross-sectional dimension of an elementary fibril is about 3 nm and its aspect ratio can reach more than 200. In native cellulose fibres, elementary fibrils do not exist as single fibrils but adhere to each other through hydrogen bonding and are densely packed to form a bundle of fibrils. Very recently, however, some novel methods to extract and separate these elementary fibrils, through chemical modification of the outer surface of the fibrils followed by mechanical treatment, were developed. The chemical modification methods include TEMPO-mediated oxidation and phosphorylation. Using the above treatments, each native elementary fibril can be converted to an individualized cellulose nanofibril (iCNF) with charges at its surface. An iCNF has the functional groups on the outer surface of the fibril, and iCNFs can be separated from each other, one by one, by the static repulsion due to the electrostatic charge of newly introduced functional groups. Refer to Annex B for more explanations on iCNFs.

Several manufacturing companies have already begun producing iCNFs. iCNFs are now delivered increasingly to the worldwide market for applications in the industrial fields of polymer composites, adhesives, additives, gels, etc. Some examples of iCNF-containing commercial products are diapers with deodorant performance and gel ink for ballpoint pens. In all applications, appropriate characterization of the iCNF samples is necessary so that desired products can be manufactured.

This document provides a sound basis for the commercialization as well as the research and development of iCNF materials.

Nanotechnologies — Characterization of individualized cellulose nanofibril samples

1 Scope

This document specifies characteristics to be measured of individualized cellulose nanofibril (iCNF) samples in suspension and powder forms and their measurement methods. In addition, it provides sample preparation, measurement and data analysis procedures.

This document does not apply to the characterization of iCNFs that have been modified after they are manufactured.

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-2 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
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 bacteria species

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

3.2
cellulose nanofibril
CNF
cellulose nanofibre composed of at least one *elementary fibril* (3.1), containing crystalline, paracrystalline and amorphous regions, with aspect ratio usually greater than 10, which may contain longitudinal splits, entanglement between particles, or network-like structures

[SOURCE: ISO/TS 20477:2017, 3.3.6, modified — The notes to entry have been deleted.]

3.3
individualized cellulose nanofibril
iCNF
discrete *cellulose nanofibril* (3.2) composed of one *elementary fibril* (3.1) with ionic functional groups on its surface

4 Abbreviated terms

AFM	atomic force microscopy
CNC	cellulose nanocrystal
FT-IR	Fourier transform infrared spectrometry
HPLC	high performance liquid chromatography
IC	ion chromatography
ICP-AES	inductively coupled plasma - atomic emission spectrometry
ICP-MS	inductively coupled plasma - mass spectrometry
ICP-OES	inductively coupled plasma - optical emission spectrometry
NMR	nuclear magnetic resonance
SEC-MALS	size-exclusion chromatography - multi-angle laser light scattering
TEM	transmission electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TGA	thermogravimetric analysis
UV-Vis	ultraviolet-visible
XRD	X-ray diffraction

5 Characteristics to be measured of iCNF samples and their measurement methods

5.1 General

The characteristics of iCNF samples listed in Table 1 are required to be measured or identified. The characteristics listed in Table 2 are recommended to be considered for measurement and identification based on the agreement between a buyer and a seller of an iCNF material in the market. The iCNF sample refers to a material taken from an iCNF product for characterization that contains iCNFs and other substances as impurities as well as solvent in the case of suspension.

The measurement methods listed in Tables 1 and 2 are required and recommended, respectively, to be adopted to determine the characteristics of an iCNF sample. Measurement protocols for characteristics in Tables 1 and 2 are separately provided in Annex A for the individual characteristics.

Test specimens for measurements shall and should be prepared from the sample as specified in each subclause in 5.2 and 5.3, respectively.

For each of the characteristics measured, the state of the test specimens, such as aqueous suspension and air-dried, freeze-dried or oven-dried powders, shall be reported in accordance with Clause 6. When the test specimen's dispersibility is considered an important factor for measurement, such as observation by electron microscopy, information about the dispersing methods used (e.g. sonication, homogenization, the use of surfactants) shall also be reported.

Table 1 — Characteristics of iCNF samples that are required to be measured or identified and their measurement methods

Characteristics	Measurement methods
Morphology and size	TEM or AFM
Total dry matter content	Oven drying and weighing
Crystal structure	X-ray diffractometry
Optical transmittance	UV-Vis spectrophotometry
Surface functional groups: Types	FT-IR
Surface functional groups: Content	Conductometric titration
Viscosity	Viscometry
NOTE The characteristics are arranged from general to specific for identification of iCNFs. The order indicates neither importance nor a flow of measurements.	

Table 2 — Characteristics of iCNF samples that are recommended to be measured or identified and their measurement methods

Characteristics	Measurement methods
Width and height	TEM or AFM
Length	TEM
Molecular weight distribution	SEC-MALS
Supernatant dry matter ratio	Centrifugation, oven drying and weighing
Crystallinity	Solid-state NMR
Thermal stability	TGA
Ash content	Combustion and weighing
Acid-soluble metal content	Incineration, wet chemical analysis and ICP-AES/OES or ICP-MS
Organic contaminant content	Solid-state NMR
Acetone-soluble matter content	Soxhlet extraction, oven drying and weighing
Constituent sugar content	HPLC or IC, and chemical analysis

5.2 Characteristics required to be measured or identified

5.2.1 Morphology and size

An iCNF is distinctive and unique in shape and size. The morphology of an iCNF sample refers to the shapes of iCNFs and other solid objects, such as bundle-formed cellulose nanomaterials that are not individualized, contained in the sample. The size refers to the width, height and length of iCNFs and other solid objects. The morphology and size are measured qualitatively to observe the presence of iCNFs and other solid objects contained in an iCNF sample.

Microscopic images of solid objects in an iCNF sample shall be obtained by TEM or AFM. When the sample is provided in powder form, a test specimen in aqueous suspension form is first prepared. The iCNF suspension is diluted by adding deionized water at an adequate concentration for the TEM and AFM measurements.

More than 10 images shall be provided at appropriate magnifications so that iCNFs can be clearly observed. Each image accurately represents the solid objects contained in an iCNF sample. The scale bar is shown on each image.

See examples of microscopic images of morphology and size in A.2.1.

Quantitative measurements of the size, width/height and length of iCNFs are separately described in 5.3.1 and 5.3.2, respectively.

5.2.2 Total dry matter content

An iCNF sample may contain solid components other than iCNFs as well as dissolved materials. The total dry matter content of an iCNF sample in suspension or powder form is the ratio of the mass of the iCNF sample after drying to that of the iCNF sample before drying.

The mass of total dry matter shall be measured by the oven drying method, which consists of drying the sample to constant mass at a temperature of 105 °C ± 2 °C and weighing.

The results of total dry matter content measurement shall be expressed in the unit of kg/kg.

See measurement protocols for the total dry matter content in A.2.2.

5.2.3 Crystal structure

The crystal structure is distinctive and unique for a crystalline solid and enables its identification. An iCNF derived from native cellulose has crystalline regions of cellulose I, which is a mixture of cellulose Iα and Iβ. It is also important to confirm if there are any solid objects having crystalline structures other than cellulose I in an iCNF sample.

The crystal structures shall be identified. Their amounts shall be qualitatively estimated by X-ray diffractometry for a dried test specimen. When the sample is provided in suspension form, a dried test specimen is prepared for the measurements.

The results shall be expressed as an X-ray spectral chart over the diffraction angles between 5° and 45° (as 2θ) to cover the peaks of cellulose I and other crystal structures included in an iCNF sample.

See an example of an iCNF X-ray spectral chart in A.2.3.

5.2.4 Optical transmittance

As a native cellulose material is physically reduced to individual iCNFs, the optical transmittance of the suspension increases due to a decrease of optical scattering by the cellulose fibrils. The optical transmittance in the UV-Vis range can be an indication of dispersibility of an iCNF sample. The optical transmittance is the ratio of the radiant flux of an optical beam that is transmitted through a test cell containing an iCNF suspension to that through a blank test cell with pure dispersant.

The optical transmittance shall be measured over the wavelength range from 200 nm to 750 nm by UV-Vis spectrophotometry. When the sample is provided in aqueous suspension form, a test specimen of a 1 % mass fraction total dry matter content is prepared by diluting or concentrating. When the sample is provided in powder form, a test specimen of aqueous suspension of a 1 % mass fraction total dry matter content is prepared. A cell with a 10 mm optical path length is used for the measurement.

The results of optical transmittance measurements shall be expressed as % or a dimensionless number and illustrated as a spectral chart of the optical transmittance versus wavelength over the specified wavelength range.

See examples of measurement results in A.2.4 and B.8.

5.2.5 Surface functional groups: Types

When iCNFs are manufactured from natural cellulose fibres, surfaces of the iCNF may be modified with functional groups. The type of functional groups depends on the manufacturing methods used. A test specimen in dried solid form shall be prepared and used for the measurement. The type of functional groups which are newly introduced during manufacturing iCNFs shall be identified by FT-IR. The spectral charts shall be shown in the range from 600 cm^{-1} to 3 800 cm^{-1}.

See examples of charts in A.2.5 and B.11.

5.2.6 Surface functional groups: Content

The surface functional group content of iCNF samples is the ratio of the amounts of the newly introduced and charged functional groups (such as carboxylic acids) to the mass of the total dry matter in a sample.

The amounts of the negatively charged functional groups in CNF samples, such as carboxylic acids and phosphoric acids, shall be measured by conductometric titration. When the sample is provided in suspension form, a test specimen in powder form is prepared from the suspension sample for the measurement.

The results of conductometric titration measurement for each negatively charged functional group shall be expressed in the unit of mmol/g.

See an example of measurement results for carboxylic acids in A.2.6.1.

NOTE When dissolved salts are contained in a suspension sample, they are also detected by the conductometric titration.

5.2.7 Viscosity

Viscosity is a rheological property of a fluid that expresses resistance to shearing flows. The viscosity of an iCNF suspension is a significant fundamental characteristic for liquid applications.

When an iCNF sample is provided in suspension form, a test specimen of 1 % mass fraction total dry matter content is prepared for the measurement by diluting or concentrating. When an iCNF sample is provided in powder form, an aqueous suspension test specimen of 1 % mass fraction total dry matter content is prepared for the measurement. The test specimen is stored sufficiently long prior to measurement in order to avoid the influence of the thixotropy of iCNF suspensions.

The viscosity shall be measured by the rotational viscometer at one shear rate in the range between 0,2 s^{-1} and 2 s^{-1} and can be optionally measured at other shear rates.

The viscosity results at 25 °C shall be expressed in the unit of Pa·s. The shear rates at which the viscosity measurements are taken, and the dispersion medium and viscometer type used (e.g. single cylinder, concentric cylinder, cone and plate or others) shall be reported with the viscosity results.

See an example of measurement results in A.2.7.

5.3 Characteristics recommended to be measured or identified

5.3.1 Width and height

Width and height measurements of fibrous objects contained in an iCNF sample can clearly distinguish iCNFs from other fibrous objects on a microscopic image. The cross-sectional dimensions of an iCNF are approximately 3 nm and are uniform along the fibre axis while those of other fibrous objects, e.g. CNF bundles, are much larger than those of iCNFs.

The width of a fibrous object is the distance on a two-dimensional image between the two edges on a cross-sectional line orthogonal to the longitudinal direction. The height of a fibrous object is the distance on a three-dimensional image between the top of the fibrous object and the substrate surface when the fibrous object is deposited laterally on the substrate. Since the cross-section of an iCNF or other fibrous object is not a perfect circle, the width and height measured on a microscopic image may vary depending on the viewing or probing angles of TEM or AFM to the fibres. The average of width and height can be obtained over randomly oriented viewing and probing angles.

One datapoint of width or height is obtained for each fibrous object. When the width or height varies along the fibre axis on an image, the largest width or height should be measured and recorded. The target fibrous objects to be measured should be representative of the fibrous solid objects contained in an iCNF sample, i.e. all types of fibrous objects on an image should be equally selected. The number of width or height data is more than 25.

Either the width or height may be measured for an iCNF sample. The width should be measured by TEM and the height by AFM. When the sample is provided in aqueous suspension form, the target concentration for a test specimen is obtained via dilution or concentration before testing, and then the test specimen is used for the measurement. When the sample provided is in powder form, an aqueous suspension test specimen is first prepared for the measurement.

The measurement results should be displayed as a histogram of the number of iCNFs and other fibrous objects versus width or height at the interval of 0,5 nm. Also, the average (median) of width or height data of iCNFs and other fibrous objects should be expressed in the unit of nm. It should be noted that the measurement results can be qualitative with increased uncertainty when the observed microscopic images are not representative of the sample.

See histogram examples in A.3.1.

5.3.2 Length

The length of a fibrous object not having branches is the longitudinal distance along the axis between its two ends on a two-dimensional image. When there are kinks along the fibre, the length of the fibrous object is the sum between adjacent kinks and a kink and an end. The target fibrous objects to be measured should be representative of the iCNF sample, i.e. all types of fibrous objects on an image should be equally selected. The number of length datapoints may be agreed between the buyer and seller of an iCNF material.

The length of fibrous objects not having a branch should be measured by TEM with the aid of image analysis techniques. When the sample is provided in powder form, a test specimen in aqueous suspension form is first prepared for the measurement.

The measurement results should be displayed as a histogram exhibiting length distribution of iCNFs and other fibrous objects. Also, the average (median) of fibrous object length data in an iCNF sample should be expressed in the unit of nm or µm.

See examples of the histogram in A.3.2.

5.3.3 Molecular weight distribution

An iCNF is a longitudinal sequence of cellulosic structures having the same cross-sectional dimensions. Although the length of an iCNF is not always equal to the lengths of the cellulose molecules composing the iCNF due to variation of enzymic reactions during biosynthesis of the elementary fibrils, longer fibrils can contain longer cellulose chains. Therefore, the molecular weight distribution of cellulose molecules in an iCNF sample could be strongly related to the length distribution of the iCNFs themselves.

The molecular weight distribution of an iCNF sample should be measured by SEC-MALS measurement after appropriate pre-treatment of the iCNF sample. A dried powder test specimen is prepared for the measurement.

The results should be expressed as M_w (weight-average molecular weight) as well as two-dimensional graphs obtained from SEC-MALS.

See an example of measurement results in A.3.3.

5.3.4 Supernatant dry matter ratio

Solid objects suspended in a fluid can be separated into lighter and heavier objects by centrifugation where the separation depends on the mass and density of individual solid objects. When an appropriate centrifugal separation is applied to an iCNF suspension sample, iCNFs and soluble matter remain in the supernatant while other heavier solid objects deposit as sediments.

The supernatant dry matter ratio is the ratio of the dry matter content of a supernatant of an iCNF test specimen after centrifugal separation to that of the iCNF test specimen before centrifugal separation.

When the sample is provided in suspension form, a test specimen prepared at a dry matter content of 0,1 % mass fraction should be used for centrifugal separation. When an iCNF sample is provided in powder form, a suspension test specimen is first prepared and then the same processes are followed as for the suspension samples.

The centrifugal separation is usually performed at more than 12 000 *g* for longer than 20 min. The mass of dry matter should be measured by the oven drying method as in 5.2.2.

The results of supernatant dry matter ratio should be expressed as % mass fraction or a dimensionless ratio.

See measurement protocols for the total dry matter content in A.3.4.

5.3.5 Crystallinity

An iCNF contains regions of highly ordered (crystalline) cellulose and regions of disordered (amorphous) cellulose. The fraction of crystalline cellulose depends on the cellulose source and the iCNF manufacturing processes. The crystallinity of an iCNF sample is the ratio of the mass of crystalline cellulose to that of the total (crystalline and amorphous) of cellulose.

The crystallinity of an iCNF sample should be measured by solid-state ^{13}C NMR. A test specimen for measurement is prepared in dried powder form from the powder or suspension form sample provided. The C4 peak of the iCNF in the cross polarization - magic angle spinning (CP-MAS) spectrum is separated from the C2, C3 and C5 peaks. The spectral charts should be shown in the range from 20 ppm[1)] to 200 ppm. The results of crystallinity measurement are calculated by the ratio of the area of the peak (87 ppm to 93 ppm region) assigned to C4 of crystalline cellulose to that of the peak (80 ppm to 93 ppm region) assigned to all C4 of cellulose.

See examples of solid-state NMR spectral charts obtained with cellulosic samples in A.3.5 and B.10.

5.3.6 Thermal stability

The thermal stability indicates the quality and the ability of the substances present in an iCNF sample to resist irreversible change in its chemical or physical structure by decomposition or depolymerization when subjected to high temperatures. The measurement of thermal stability of an iCNF test specimen refers to the mass loss of the specimen in dried powder form during heating to a sufficiently high temperature.

The thermal stability should be measured by TGA. When the sample is provided in suspension form, the dried test specimen is first prepared for the measurement. The mass loss of test specimen should be measured for both dynamic and isothermal conditions.

The result should be expressed as a thermogravimetric curve showing the mass as a function of temperature. Considering that the results depend on many experimental and instrumental variables, relevant measurement conditions are also reported including the atmosphere (e.g. air, N_2, O_2) and its flow rate, the method of sample drying (e.g. freeze-dried or air-dried), and the temperature programme used (e.g. heating ramp rate(s) and/or isothermal temperature(s)).

See examples of thermal stability measurement results in B.9.

5.3.7 Ash content

An iCNF sample is predominantly composed of cellulosic fibrils which can be eliminated by combustion. However, the sample may contain metals and inorganic constituents which are left as ash after combustion.

The ash content of an iCNF sample in suspension or powder form is the ratio of the mass of the residue after complete combustion of the sample to that of the total dry matter of the sample.

1) Chemical shift values are in parts per million (ppm) relative to tetramethylsilane.

The ash content should be measured by weighing the residue after combustion at 900 °C ± 25 °C using an electric furnace.

The results of ash content should be expressed as % mass fraction or in the unit of mg/kg.

See measurement protocols for the ash content in A.3.7.

5.3.8 Acid-soluble metal content

Metal constituents are likely introduced from the substrates used in manufacturing iCNFs.

The content of acid-soluble metals (magnesium, calcium, manganese, iron, copper, sodium and potassium) is the ratio of the mass of metallic element contained in the residue from incineration at 900 °C of a dried iCNF test specimen to that of the dried test specimen before incineration.

The acid-soluble metal content should be measured by using incineration followed by wet chemical digestion and ICP-AES/OES or ICP-MS. When the sample is provided in suspension form, a dried test specimen is prepared from the suspension for the measurements.

The measurement results should be expressed as % mass fraction or in the unit of mg/kg for each element present.

See measurement protocols for the acid-soluble metal content in A.3.8.

5.3.9 Organic contaminant content

An iCNF sample may contain organic compounds other than iCNFs, including lignin derived from wood pulp, as well as polymers and other components. A solid-state NMR spectrum can provide qualitative information about the presence of organic contaminants in an iCNF sample.

Organic contaminants should be identified by CP-MAS ^{13}C NMR. When the sample is provided in suspension form, a dried test specimen is first prepared for the measurement.

The measurement results should be expressed with a solid-state NMR spectral chart (0 ppm to 220 ppm) covering the peaks of iCNF and organic contaminants.

See measurement protocols for the organic contaminant content in A.3.9.

5.3.10 Acetone-soluble matter content

An iCNF sample may contain acetone-soluble matter including low molecular weight organic compounds which are intentionally added into the sample or which are introduced as contaminants during the manufacturing process. The acetone-soluble matter content is the ratio of the mass of low molecular weight organic compounds extracted from an iCNF sample by acetone to that of the total dry matter of the iCNF sample.

The mass of acetone-soluble matter in an air-dried iCNF powder sample should be measured by Soxhlet extraction with boiling acetone followed by weighing of the extraction residue. When the sample is provided in suspension form, a test specimen in air-dried powder form is first prepared and then the same processes are followed as for the powder samples.

The results of acetone-soluble matter content should be expressed as % mass fraction or in the unit of kg/kg.

See measurement protocols for the acetone-soluble matter content in A.3.10.

5.3.11 Constituent sugar content

When a wood pulp is the starting material for manufacturing iCNFs, an iCNF sample may include hemicelluloses depending on the purity of the pulp. The amount of hemicelluloses in an iCNF sample can

be estimated from the quantities of their constituent sugars. The constituent sugar content is the ratio of the mass of constituent sugar in an iCNF sample to that of the total dry matter of the iCNF sample.

The mass of constituent sugars in an iCNF air-dried powder sample should be measured by acid hydrolysis followed by sugar determination using a quantitative analysis for each monosaccharide. The separation and quantitative analysis of each sugar are conducted by using chromatography such as HPLC or IC, and the calibration curve method. When the sample is provided in suspension form, a test specimen in air-dried powder form is first prepared and then the same processes are followed as for the powder samples.

The measurement results of individual constituent sugars contents should be expressed as % mass fraction or in the unit of kg/kg.

See measurement protocols for the constituent sugar content in A.3.11.

6 Reporting

The characterization report shall include the following:

a) sample identification:

   1) sample name;

   2) manufacturer's name;

   3) lot number;

   4) sample source, e.g. type of plant, marine organism or bacteria;

   5) manufacturing method, e.g. TEMPO and others;

   6) sample form; suspension or powder;

   7) name of additives;

   8) storage conditions prior to testing;

b) name of characteristic measured or identified that is listed in Tables 1 or 2;

c) measurement method used for the individual characteristic;

d) test specimen prepared for measurement:

   1) suspension or powder;

   2) dry matter content of suspension;

   3) the solvent, such as deionized water, and dispersing method, if used, in the case that a suspension test specimen is prepared from a powder sample;

e) dates of measurement and name of organization that made the measurements for the individual characteristics;

f) quantitative and/or qualitative results of measurements with the sample state and dispersion method, if appropriate, for the reported characteristics;

g) information on the uncertainty of results;

h) additional information, if any, supporting the measurement results;

i) deviations: if there are any deviations from this document, the name of, and detailed information on, the measurement methods used and their justification.

Annex A
(informative)

Protocols for sample preparation, measurement and data analysis

A.1 General

The sample preparation and measurement procedures and data analysis methods that are generally used are provided in this annex for individual characteristics.

Sampling and sample homogeneity should be carefully considered in accordance with ISO 14488 to obtain accurate measurement results.

When the concentration of suspension is expressed as % mass fraction in this annex, it refers to the percentage of dry matter content.

A.2 Protocols for measuring required characteristics

A.2.1 Morphology and size

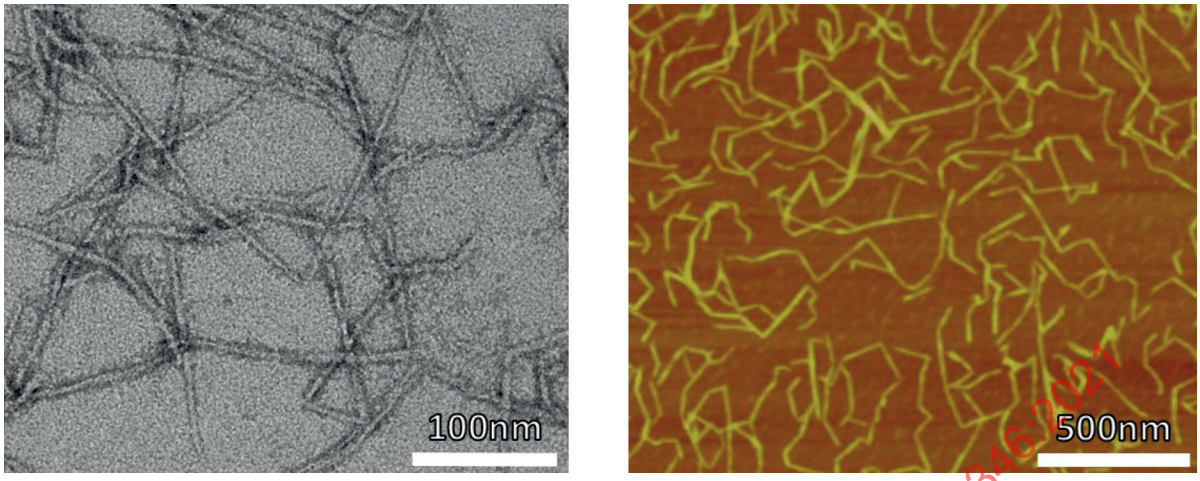
TEM, AFM and image processing using software are used for the visualization of morphology and size.

For morphology and size, either TEM or AFM can be used. TEM is recommended to determine the object width. AFM is used for measuring object height rather than width because of its tip broadening effect on the width measurements.

For TEM, an aqueous suspension (0,01 % mass fraction or less) of iCNFs is prepared. The suspension of an iCNF sample is dropped onto the surface of the elastic carbon film Cu microgrid pretreated by hydrophilic treatment. The wet iCNF on the grid is subjected to negative staining, and the sample is used for TEM observation after drying. The grid with dry iCNFs is set on the specimen mount holder of the TEM, and TEM observation is performed under the conditions of appropriate accelerating voltage.

For AFM, an aqueous suspension (0,01 % mass fraction or less) of an iCNF sample is dropped onto a freshly cleaved mica surface. The mica plate is used for AFM observation after drying. The grid with dry iCNFs is set on the specimen mount holder of the AFM, and AFM observation is performed using the tapping mode. Appropriate AFM images are acquired using magnification suitable for surveying the height and length of iCNFs and other fibrous objects.

Representative data for morphology and size are shown in Figure A.1. See also B.2 and B.3.



a) TEM b) AFM

Figure A.1 — Images of iCNFs

NOTE 1 ISO/TR 19716 provides information on CNC characterization and includes reference to diameter measurements by both TEM and AFM. ISO 13322-1 provides general guidance and a theoretical background of size measurement from images including TEM and AFM. ISO 14487:1997 provides information on standard water for physical testing (pulps).

NOTE 2 References [1] and [2] provide information on iCNF characterization and include reference to diameter measurements by AFM. Reference [3] provides information on iCNF diameter measurements by both TEM and AFM. Reference [4] provides information on the size evaluation of CNFs including iCNF by AFM.

NOTE 3 Reference [5] provides information on aspect ratios of iCNFs.

NOTE 4 Reference [6] provides information on sample preparation for measurement of CNC by AFM and TEM.

NOTE 5 References [7], [8] and [9] provide information on staining for TEM observation.

A.2.2 Total dry matter content

A glass container, oven, desiccator and precision balance are used for the measurement.

The measurement can be applied to an iCNF sample available in either aqueous suspension or powder form. For samples in aqueous suspension form, an adequate amount of test specimen is used to ensure that at a minimum 20 mg of solid material will remain after drying. For samples in powder form, a test specimen of 1 g to 10 g is normally used.

Before the test specimen is placed in the container, the empty container (body and lid) shall be previously heated to constant mass, cooled and weighed. The container with the test specimen is weighed. The lid is open, and the lid and container with the test specimen is placed in the oven. They are heated at 105 °C ± 2 °C for a sufficiently long period until constant mass is reached. After drying, the lid is put on the container. The container with test specimen is cooled in a desiccator to room temperature. The container is weighed after cooling to room temperature. Drying and weighing procedures are repeated until the difference between two consecutive weighing results is less than 1 % mass fraction of the test specimen mass after drying (i.e. constant mass is reached). At least two test specimens are used for determination of the total dry matter content.

The total dry matter content is calculated by using Formula (A.1):

$$C_D = \frac{(m_{CT\_after} - m_c)}{(m_{CT\_before} - m_c)} \times 100 \quad \text{(A.1)}$$

where

C_D is the total dry matter content (in % mass fraction);

m_C is the mass of the container (in g);

m_{CT_after} is the mass of the container with the test specimen after drying (in g);

m_{CT_before} is the mass of the container with the test specimen before drying (in g).

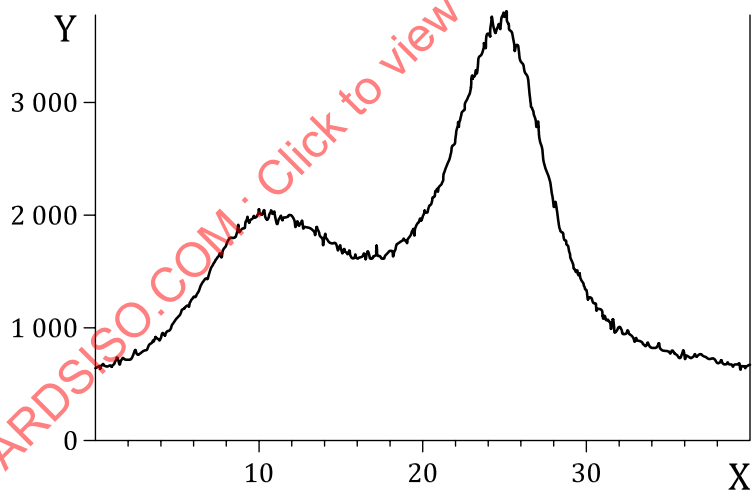
NOTE ISO 638-1 and ISO 638-2 specify protocols in more detail for the dry matter content measurement by the oven-drying method for powder and suspension samples, respectively.

A.2.3 Crystal structure

An X-ray diffractometer and Cu Kα (radiation source) are used for the measurement.

A freeze-dried iCNF sample of 0,1 g or more is pelletized by pressing for 1 min at a pressure of 750 MPa. A pellet sample having a thickness of about 1 mm is prepared. X-ray diffraction patterns of the pellet sample are obtained under the condition of measuring range 2θ from 5° to 45°, from which the crystal structures are identified.

Representative data for crystal structure are shown in Figure A.2.



Key

X diffraction angle, 2θ (°)

Y X-ray intensity (cps)

Figure A.2 — XRD pattern of an iCNF sample

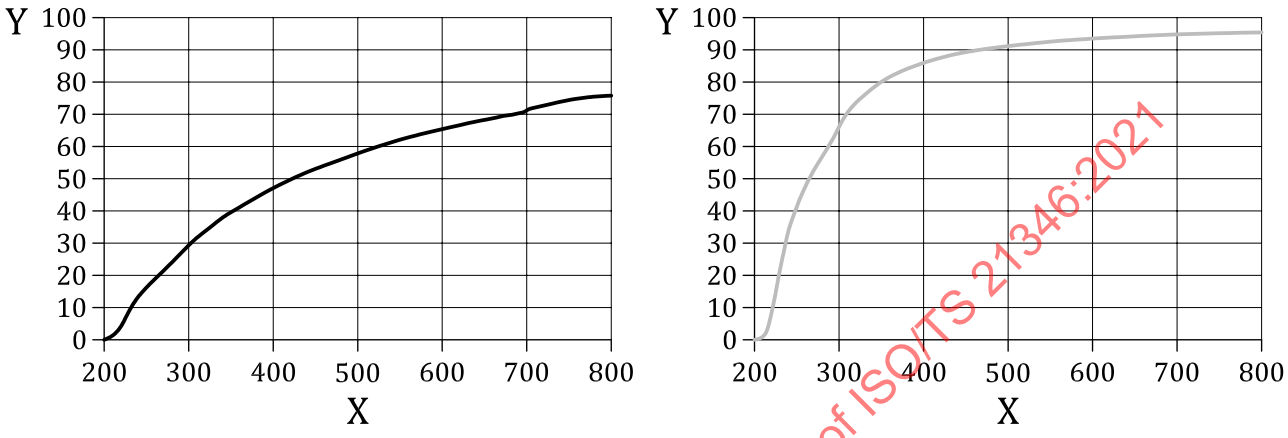
NOTE References [10] and [11] provide information on the use of XRD to confirm the crystal structure of iCNF samples.

A.2.4 Optical transmittance

A UV-Vis spectrophotometer, quartz cell with a 10 mm optical path length and precision balance are used for the measurement.

An aqueous iCNF sample suspension of 1 % mass fraction dry matter content is prepared by dilution or concentration. The suspension is poured into the quartz cell, and the cell is put into spectrophotometer sample holder. It should be confirmed that the suspension in the cell does not have any bubbles. The optical transmittance of the suspension is measured by using a light source of wavelength from 200 nm to 750 nm.

Representative data for optical transmittance are shown in Figure A.3. See also B.8.



Key

X wavelength (nm)

Y transmittance (%)

NOTE The iCNF sample represented by the plot on the right shows higher transmittance, and hence better dispersion.

Figure A.3 — Examples of plots of transmittance as a function of wavelength for differently prepared iCNF samples

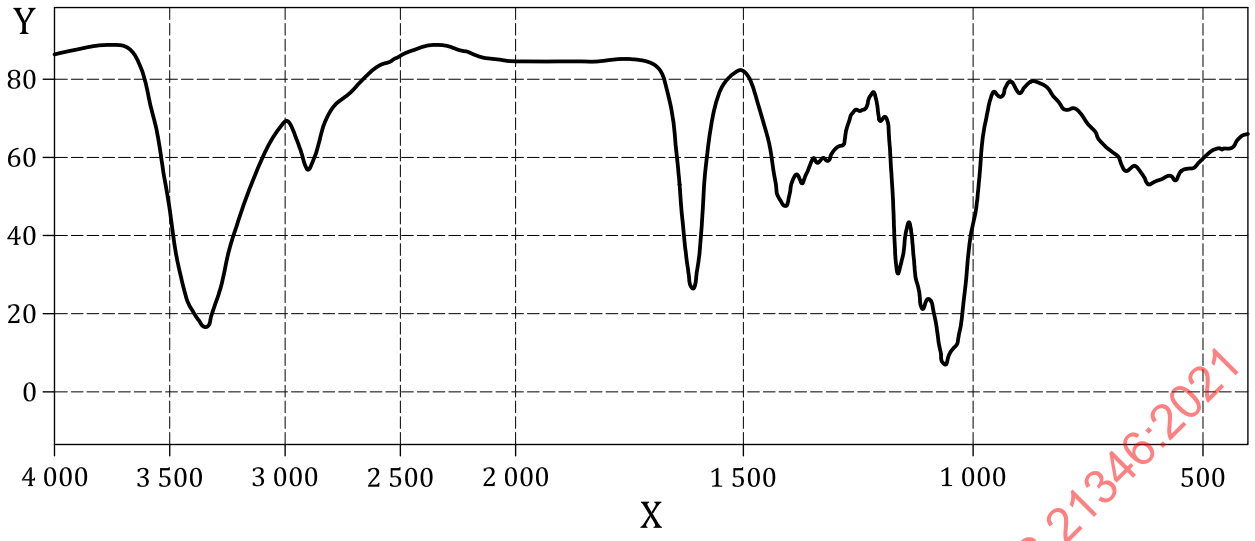
NOTE Reference [12] provides information on how to evaluate the dispersibility of iCNFs in iCNF samples.

A.2.5 Surface functional groups — Types

A Fourier transform infrared spectrometer is used for the measurement.

A dilute aqueous iCNF sample suspension (e.g. 0,2 % mass fraction) is prepared. This suspension is dried on a polytetrafluoroethylene (PTFE) Petri dish at room temperature to prepare a film of 5 µm to 30 µm in thickness. The film is equilibrated at a temperature of 23 °C and a relative humidity of 50 %, and is used for FT-IR measurement via the transmission method. It is recommended to use a resolution of 4 cm^{-1} and accumulate more than 256 scans. The spectral measurement will reveal the types of functional group that are present in the samples. Carboxylate (acid or salt form) or phosphate (acid or salt form) in CNF samples can be observed by FT-IR as newly introduced functional groups, which are not found in the original cellulosic materials.

Representative data showing the identification of functional groups by FT-IR are shown in Figure A.4. See also B.11.



Key

X wavenumbers (cm^{-1})

Y transmittance

Figure A.4 — FT-IR transmission spectrum of an iCNF sample between 500 cm^{-1} and 4 000 cm^{-1}

NOTE Reference [13] provides information on the detection and identification of functional groups on the surface of iCNFs in iCNF samples.

A.2.6 Surface functional groups — Contents

A.2.6.1 Quantitative analysis of carboxylic acids

An automatic titrator, automatic burette and conductivity meter are used for analysis.

A freeze-dried iCNF sample is prepared as a starting material for titration. 50 ml to 150 ml of deionized water is poured into the beaker to make an iCNF suspension of concentration between 0,2 % mass fraction and 0,4 % mass fraction. A 0,02 M solution of NaCl (5 ml) is added to the iCNF suspension and the suspension is mixed well. 0,1 M HCl (aq) is added to the iCNF suspension to adjust the pH value to 2,0 to 2,4. After mixing, 0,05 M NaOH (aq) is added dropwise to an iCNF suspension at a constant rate of 0,1 ml/min. The conductance and pH value of the iCNF sample suspension shall be continued to measure until the pH reaches around 11.

The conductometric titration consists of the following three stages:

— stage 1: neutralization of strong acid: HCl + NaOH;

— stage 2: neutralization of weak acid: cellulose-COOH + NaOH;

— stage 3: after the completion of neutralization.

Read the consumption of NaOH (0,05M NaOH aq) in stage 2 from the data of the pH curve and conductivity curve. The amount of carboxylic acids in the iCNF sample is calculated from the value by using Formula (A.2):

$$n_{CA} = 0{,}05 \times \left(\frac{V_{S2}}{m_D} \right) \quad \text{(A.2)}$$

where

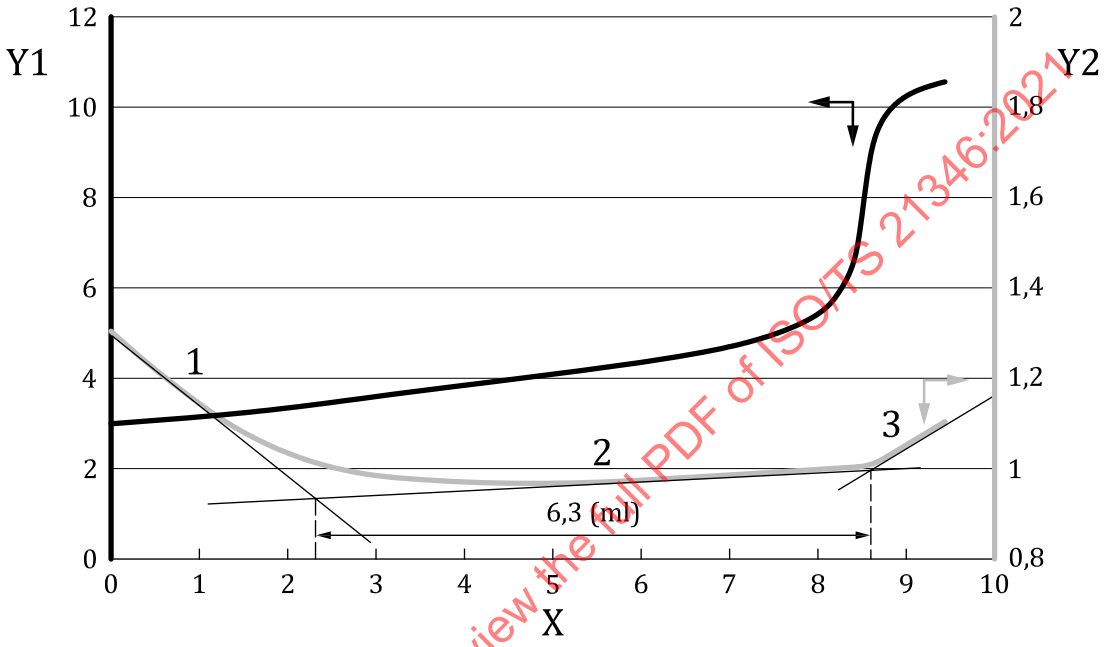
n_{CA} is the amount of carboxylic acids (in mol/g);

0,05 is in mol/l;

V_{S2} is the volume of NaOH aq in l) in stage 2;

m_D is the total dry matter mass of the iCNF sample (in g).

Representative data for carboxylic acid content determination are shown in Figure A.5.



Key

X 0,05 mol/l NaOH titre (ml)

Y1 pH

Y2 conductivity (mS/cm)

1 neutralization of strong acid: HCl + NaOH

2 neutralization of weak acid: cellulose-COOH + NaOH

3 after the completion of neutralization: NaOH

-COOH content = 0,05 [mmol/ml] × 6,3 [ml] / 0,239 1 [g, iCNF sample used] = 1,3 [mmol/g]

Figure A.5 — Plot of acid-base titration for carboxylic acids on the surface of an iCNF

NOTE Reference [14] provides information on the carboxylic acid content determination of TEMPO-oxidized iCNFs via titration.

A.2.6.2 Quantitative analysis of phosphoric acids

An automatic titrator, automatic burette and conductivity meter are used for the analysis.

An aqueous iCNF sample suspension (concentration of about 1 g/l) is prepared for the analysis. 10 vol% to 20 vol% of a strong-acid cation exchange resin is added into the iCNF sample suspension, and the mixture is stirred for 20 min to 30 min to allow ion exchange. After the treatment, the resin is removed from the iCNF sample suspension via filtration. About 200 g of the ion exchanged iCNF sample suspension is weighed, and 5 % mass fraction NaCl (aq) is added to the suspension in order to adjust the electrical conductivity of the suspension to 70 mS/m. The pH and electrical conductivity of the suspension are analysed while 0,1 M NaOH aq is added into the suspension at a constant injection rate of 0,6 ml/min. The analysis is continued until the pH of the suspension reaches 11.

There are three stages during the titration:

— the electrical conductivity decreases (stage 1);

— the conductivity increases slightly (stage 2):

— the conductivity increases sharply (stage 3).

From the data on the consumption of 0,1 M NaOH aq in stages 1 and 2, the amount of functional groups showing weak acidity and strong acidity is calculated by using Formulae (A.3) and (A.4), respectively:

$$n_{sa} = 0,1 \times \left(\frac{V_{S1}}{m_D} \right) \quad \text{(A.3)}$$

where

n_{sa} is the amount of functional groups with strong acidity (in mmol/g);

0,1 is in mmol/ml;

V_{S1} is the consumption volume of 0,1 M NaOH aq (in ml) in stage 1.

$$n_{wa} = 0,1 \times \left(\frac{V_{S2}}{m_D} \right) \quad \text{(A.4)}$$

where

n_{wa} is the amount of functional groups exhibiting weak acidity (in mmol/g);

0,1 is in mmol/ml;

V_{S2} is the consumption volume of 0,1 M NaOH aq (in ml) in stage 2.

NOTE 1 Reference [15] provides information on the quantitative analysis of phosphoric acids in iCNFs which are prepared through phosphorylation.

NOTE 2 Reference [16] provides information on the isolation of thermally stable CNCs prepared by phosphoric acid hydrolysis.

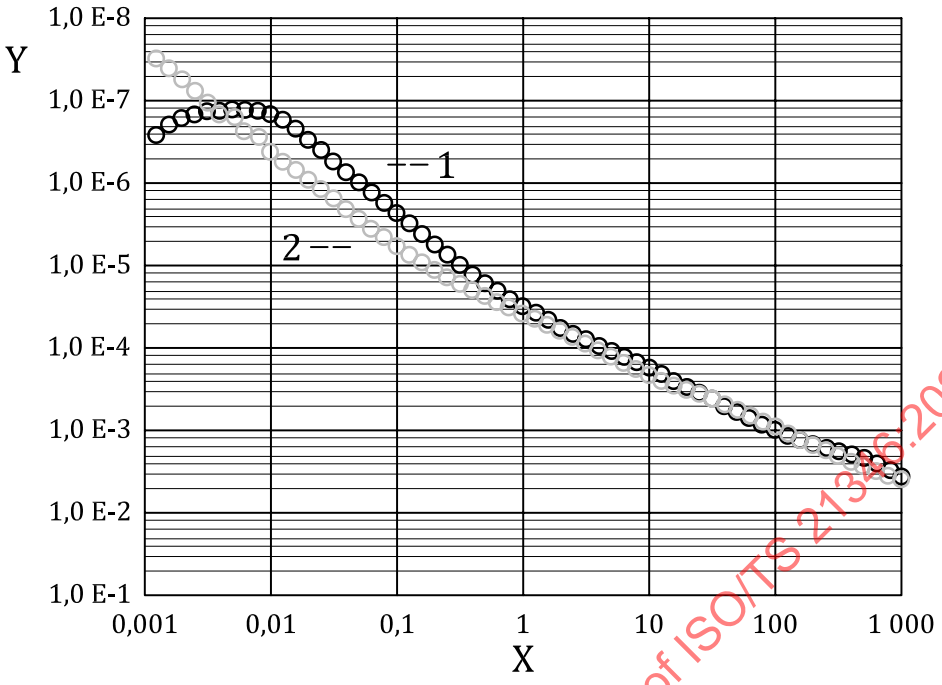
A.2.7 Viscosity

A rotational viscometer is used for the measurement.

The viscosity of an iCNF suspension is significant as a significant fundamental characteristic for liquid applications and the viscosity data taken at low shear rates are influenced by the length of iCNFs in the suspension.

A test specimen suspension of 1 % mass fraction total dry matter content is prepared by diluting or concentrating for the measurement. After confirming visually that the iCNFs are well dispersed, the iCNF sample suspension is stored at room temperature for more than 18 h and is used for the following measurement. The viscometer is used for measuring viscosity, and the data are collected at a temperature of 25 °C and a shear rate between 0,001/s to 1 000/s. By plotting a graph of viscosity (mPa.s) as a function of shear rate (s^{-1}), useful information about fluid properties may be obtained. The collected data should be described with experimental conditions used in detail. Such conditions include sample composition, sample concentration and measurement conditions.

Representative data for viscosity are shown in Figure A.6.



Key

X shear rate (s^{-1})
Y viscosity (mPa.s)
1 increasing
2 decreasing

Figure A.6 — 2D graph (viscosity versus shear rate) example of viscosity measurement for an iCNF sample

NOTE 1 Reference [17] provides information on how to measure the viscosity of iCNFs.

NOTE 2 References [18] and [19] provide information on how to measure the viscosity of polymers.

NOTE 3 References [1] and [20] provide information on the relationship between the length of iCNFs and viscosity of iCNF sample solution.

A.3 Protocols for measuring recommended characteristics

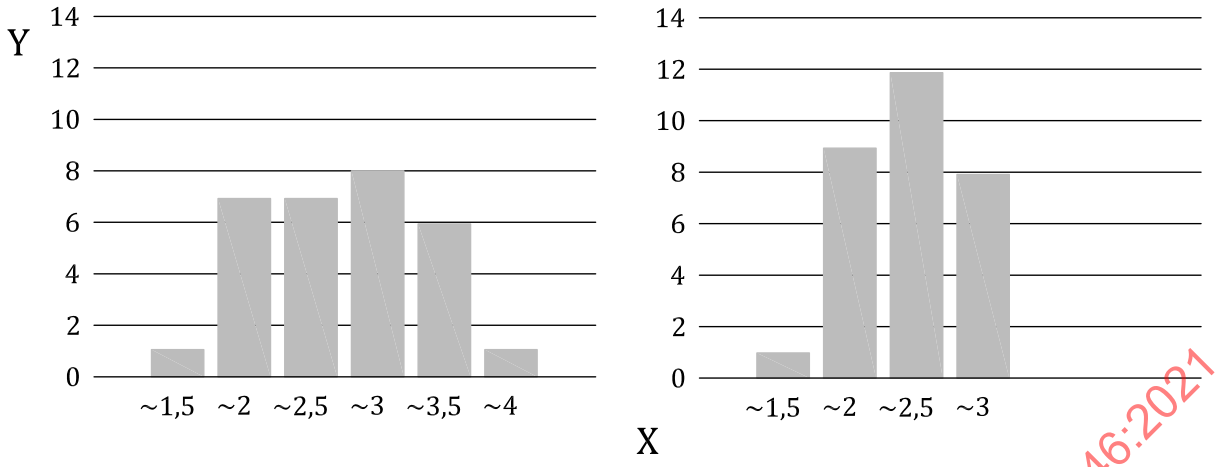
A.3.1 Width and height

When the width and height are quantitatively measured according to 5.3.1, an image processing software is used. In the measurement using software, the operator can choose one from two alternatives: the automatic measuring or the manual input processing. The width is measured once (the widest point) for each fibre. It is recommended that the total number of width determinations be at least 25.

See the description in A.2.1 for common measurement protocols. See also B.2 for typical TEM images.

Representative data for width are shown in Figure A.7.

The histograms in Figure A.7 show the width distributions of two differently prepared iCNF samples as measured by TEM. The x-axis shows the classes relevant to the width, while the y-axis shows the frequency of each class. In each sample, the widths of 30 iCNFs were measured. It can be observed that the iCNF width data are distributed around a value of 3 nm, which is the expected width of the native elementary fibril.



Key

X width of iCNFs (nm, measured by TEM)

Y frequency

Figure A.7 — Examples of histograms for iCNF width distributions

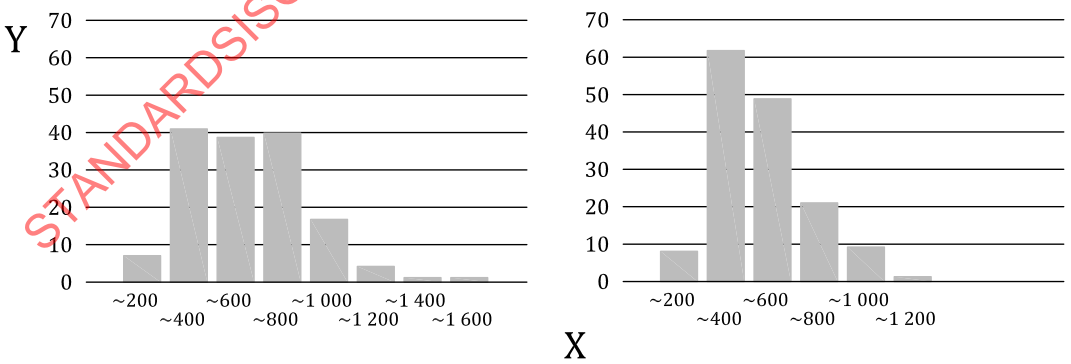
A.3.2 Length

When the length is quantitatively measured according to 5.3.2, an image processing software is used. In the measurement using software, the operator can choose one from two alternatives: the automatic measuring or the manual input processing. The number of length measurements as described in 5.2.1 should be decided via an agreement between the buyer and the seller.

See the description in A.2.1 for common measurement protocols.

Representative data for length are shown in Figure A.8.

The histograms in Figure A.8 show the length distributions of two differently prepared iCNF samples as measured by TEM. The x-axis shows the classes relevant to the length, while the y-axis shows the frequency of each class. In each sample, the lengths of 150 iCNFs were measured. It can be observed that the iCNF length data are distributed between values of 200 nm to 1 000 nm, and some of them reach over 1 µm.



Key

X length of iCNFs (nm, measured by TEM)

Y frequency

Figure A.8 — Examples of histograms for iCNF length distributions

The photos given in Figure A.9 are representative TEM images of iCNFs. When an iCNF is too long to be visualized within one image frame, its length can be measured by combining the several image frames in which that iCNF is observed. The concentration of the iCNF suspension and the sample deposition method are key factors to adjust in order to obtain images in which a single iCNF can be clearly distinguished for size analysis. A representative sample preparation method is provided in A.2.1.

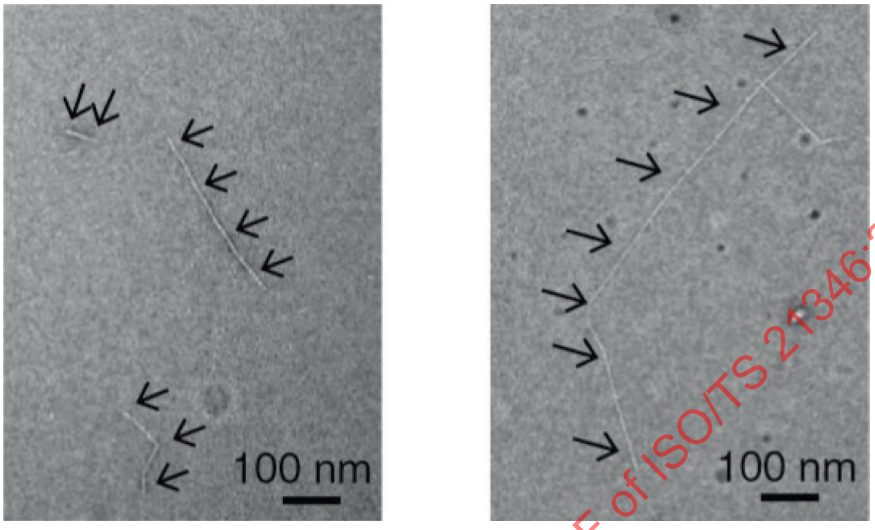


Figure A.9 — Examples of iCNF images for length measurement

NOTE Reference [21] provides information on TEM images of iCNFs.

A.3.3 Molecular weight distribution

Degassing apparatus, liquid supplying pump, auto-sampler, column for SEC, guard column, column oven, multi-angle light scattering and refractive index detector are used for the measurement.

Methylation of carboxyl groups at the C6 position of cellulose: To methylate the carboxyl groups for molecular weight measurement, 0,1 g (oven-dried mass) of an iCNF sample is weighed, and the sample is mixed with 50 ml of water until the sample is well dispersed. 1M HCl (aq) is added dropwise to the iCNF suspension, and the solution pH is adjusted between 1,5 and 2,0. The solution is stirred for 30 min at room temperature to allow the protonation of the C6 carboxyl groups. Protonated materials are washed with methanol several times using centrifugal separation and supernatant decantation. The supernatant is discarded and 30 ml of DMAc (N,N-dimethyl acetoamide) and 6 ml of methanol are added to the sample. Under a nitrogen gas stream, the suspension is mixed well. 0,5 ml of 2M TMSD (trimethylsilyldiazomethane) is added to the suspension, and the suspension is stirred for 1 h at room temperature under a nitrogen atmosphere. 1,5 ml of 5M acetic acid is added to the suspension to decompose excess TMSD, and to convert C6-COONa to C6-COOH. The suspension is washed by centrifugal washing using ethanol and t-butyl alcohol sequentially, and then dried by freeze-drying.

Measurement by SEC-MALS: 16 mg of the methylated sample is dissolved with 8 % LiCl/DMAc for 24 h. The solution concentration is adjusted to the intended concentration of 1 g/l against 1 % LiCl/DMAc, by dilution with DMAc. The sample solution and solvent are filtered through a 0,45 µm PTFE membrane. Basic conditions for SEC are as follows:

— sample concentration: 0,5 g/l to 1 g/l;

— solvent: 1 % LiCl/DMAc;

— injection volume: 100 µl;

— flow rate: 0,5 ml/min;

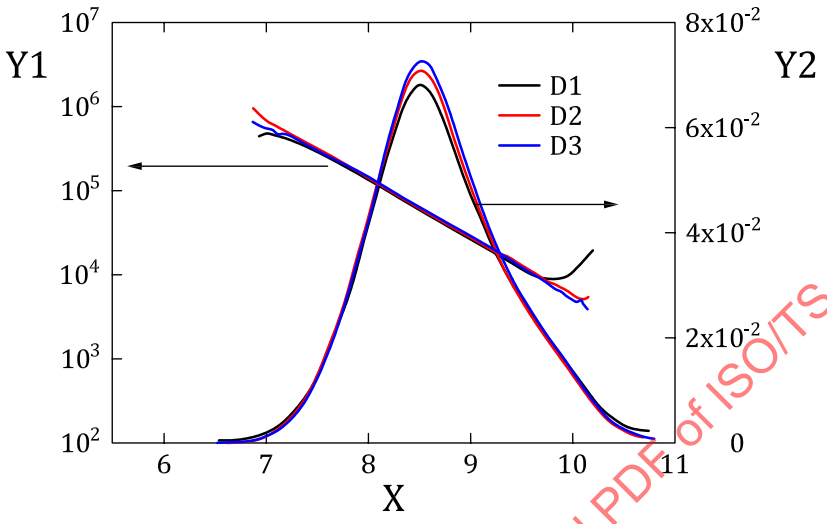
— column temperature: 40 °C;

— temperature of detector cell for MALS/RI: room temperature;

— column type: Shodex, KD-806M.

By using the SEC-MALS system, the weight average molecular weight (M_w) is measured (refractive index increment: dn/dc = 0,121).

Representative data for molecular weight distribution are shown in Figure A.10.



Key

X elution volume (ml)

Y1 molecular mass (g/mol)

Y2 concentration (mg/ml)

Figure A.10 — SEC elution patterns and molecular mass plots of carboxyl-methylated samples (D1, D2, D3) measured by SEC-MALS using 1 % LiCl/DMAc as an eluent

NOTE 1 Reference [22] provides information on the SEC-MALS measurement of TEMPO-oxidized celluloses via methylation of carboxyl groups.

NOTE 2 References [23] and [24] provide information on sample preparation for measurement of molecular weight distribution.

NOTE 3 References [25], [26] and [27] provide information on the definition and calculation procedure of M_w (average molecular weight).

A.3.4 Supernatant dry matter ratio

A centrifugal separator, centrifuge tube, UV-Vis spectrometer and quartz cell are used for the measurement.

An aqueous iCNF sample suspension of 1 % mass fraction is prepared in a well-dispersed state as a starting material. To 1 % mass fraction of the aqueous iCNF sample suspension, deionized water is slowly added under staring condition (e.g. 3 000 r/min) for 20 min, and then 0,1 % mass fraction of the aqueous iCNF sample suspension is prepared. Into the centrifuge tube (50 ml), 30 ml of the 0,1 % mass fraction of the iCNF sample suspension is transferred. This tube with the iCNF sample suspension (0,1 % mass fraction) is centrifuged under the condition of 12 000 *g* for 20 min, and 15 ml of supernatant is carefully extracted using pipette. The dry matter content of a supernatant is measured following the instructions described in A.2.2. The dry matter content of the suspension test specimen before centrifugation should be measured in the same way as in A.2.2.

The supernatant dry matter ratio is calculated as the ratio between the dry matter content of the supernatant and that of the suspension test specimen before centrifugation.

A.3.5 Crystallinity

An NMR spectrometer is used for the measurement of crystallinity.

Pretreatment: A freeze-dried sample of iCNF is prepared and conditioned at a temperature of 23 °C and a relative humidity of 50 %. After conditioning for more than 24 h, the sample is transferred into an NMR sample rotor.

^{13}C solid NMR measurement: A Larmor frequency of 50 MHz or more is used for ^{13}C nuclei. The acquisition is performed with the standard CP-MAS pulse sequence with a 2 ms contact time and 5 s delay time. The number of scans is 10 000 or more. The amount of sample is about 50 mg to 100 mg, but more sample can be used depending on the rotor size if required. The sample should be fully packed into the rotor (zirconium oxide MAS rotor fitting with a Kel-F or equivalent cap). Chemical shifts are referenced to hexamethylbenzene (17,4 ppm) or adamantane (29,5 ppm).

Determination of crystallinity: Customized peak resolution software using the pseudo-Voigt function is used for the measurement. The C4 peak of the iCNF in CP-MAS spectrum is separated from the C2, C3 and C5 peaks by pseudo-Voigt line shape deconvolution. The crystallinity of the iCNF is calculated by using Formula (A.5):

$$C = 100 \times \left(\frac{A}{B} \right) \tag{A.5}$$

where

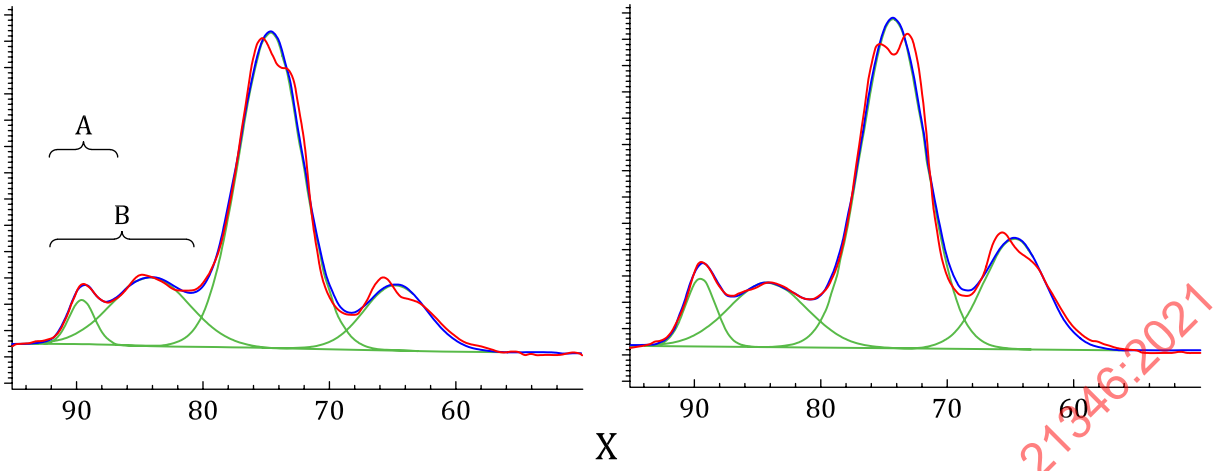
C is the crystallinity (in %);

A is the area of peak A (87 ppm to 93 ppm region assigned to C4 of crystalline cellulose);

B is the area of peak B (80 ppm to 93 ppm region assigned to all C4 of cellulose).

When comparing an iCNF with pulp (raw material of iCNF) in this measurement method, the area of peak A (87 ppm to 93 ppm region assigned to C4 of crystalline cellulose) of an iCNF is smaller than that of pulp. The reason is because iCNFs have larger surface area than pulp, and C4 on the surface of an iCNF as well as pulp is not contributing to crystallinity.

Representative data for crystallinity are shown in Figure A.11.



a) iCNF **b) Pulp**

Key

X chemical shift (ppm)

A peak A

B peak B

NOTE For the complete NMR spectrum, refer to Figure B.10.

Figure A.11 — C4 peaks between 82 ppm and 93 ppm observed by ^{13}C NMR

NOTE 1 Reference [28] provides information on how to determine the crystallinity of cellulose microfibrils by ^{13}C NMR.

NOTE 2 Reference [29] provides information on the iCNF crystallinity measured by NMR and XRD, respectively.

A.3.6 Thermal stability

A thermogravimeter is used for the measurement.

A freeze-dried iCNF test specimen is used for the measurement of thermal stability. Under a nitrogen atmosphere, the test specimen is heated from 50 °C to 600 °C at a rate of 10 °C/min. The relationship between temperature and sample mass is reported by a plot of relative mass (%, y-axis) as a function of temperature (°C, x-axis). The relative mass (%) is calculated relative to the initial mass (100 %).

Figure B.8 is an example of thermogravimetric curves of an iCNF.

NOTE Reference [30] provides an example illustrating the thermal stability of an iCNF.

A.3.7 Ash content

A crucible, muffle furnace, precision balance and desiccator are used for the measurement.

A dried iCNF test specimen is used for this measurement. It is recommended to use a dried test specimen of more than 1 g. A dried test specimen is added to the crucible at room temperature, and the mass of the crucible with the test specimen is measured. The crucible with the test specimen is put into the muffle furnace, and the temperature is slowly raised to 900 °C at a rate of 200 °C/h. The combustion temperature is kept for a predetermined number of hours at 900 °C. The incineration is continued until no black soot can be found in the crucible. The crucible is moved from the furnace to a desiccator and

cooled to room temperature. After cooling the crucible to room temperature, the crucible containing the combustion residue is weighed. The ash content is calculated by using Formula (A.6):

$$C_A = 100 \times \left(\frac{m_R}{m_S} \right) \quad \text{(A.6)}$$

where

C_A is the ash content in % mass fraction;

m_R is the (mass of crucible and combustion residue) – (tare mass of crucible);

m_S is the (mass of crucible and test specimen before combustion) – (tare mass of crucible).

NOTE ISO 2144 specifies measurement protocols for the ash content of cellulose nanomaterials at 900 °C.

A.3.8 Acid-soluble metal content

An inductively coupled plasma atomic emission spectrophotometer, precision balance and muffle furnace are used for the measurement.

Test specimen preparation: For the determination of major elements, including magnesium, calcium, sodium and potassium, a 1 g to 2 g test specimen (calculated as oven dry) is recommended. For minor elements, including manganese, iron and copper, test specimens of 5 g to 10 g are recommended. If trace levels of elements are needed, then it is recommended to use test specimen masses that are larger than 10 g.

Pretreatment of test specimens: Using the same method used for measuring ash content (see A.2.8), the organic matter in a freeze-dried test specimen is decomposed by incineration. After the incineration is complete, the dish is removed from the muffle furnace and is cooled. The dish is covered with a watch glass, and 40 ml to 50 ml of hydrochloric acid (1+1) is gently added to the dish. The watch glass is rinsed down to the dish with deionized water and is heated on a steam bath for 30 min. The cover is removed and rinsed. For another 30 min, heating is continued. Ten ml of hydrochloric acid (1+1) is added and deionized water is added to dissolve the salts. The solution is filtered into a 100 ml volumetric flask using filter paper. The residue is washed twice using dilute HCl. The total volume is adjusted to 100 ml with deionized water using a micro syringe. Blanks shall be prepared with the same quantities of reagents used in the test but omitting the test specimen.

Preparations of standard solution and test solution: The multi-element standard solution is prepared following each manufacturer's instruction. A calibration as to each metal will be performed to prepare the standard curve. A solution of 3 % to 5 % (volume fraction) nitric acid prepared from concentrated nitric acid shall be used as a calibration blank. Working standards, as required, are made by diluting 5 ml of multi-element primary standard to 500 ml with 3 % to 5 % nitric acid. Test solutions are prepared depending on the sample matrix or analyte concentrations.

Measurement: The instrument should be warmed up for at least 30 min before starting measurements. Correction of calibration curve is performed. Then, data collection of ICP-OES (ICP-AES) for the test specimens is implemented.

NOTE ISO 12830 specifies measurement protocols for acid-soluble metal content by ICP-OES.

A.3.9 Organic contaminant content

A solid-state NMR spectrometer and NMR probe are used for the measurement.

A freeze-dried iCNF test specimen is prepared for this measurement. The test specimen of 0,05 g to 0,07 g is compacted under 23 °C and a relative humidity of 50 % into an NMR sample rotor (e.g. 3 mm in diameter and 2 cm long made of zirconia), then CP-MAS ^{13}C NMR spectral data of the test specimen are acquired.

Representative CP-MAS ^{13}C NMR spectra of the original and TEMPO-oxidized celluloses are shown in Figure B.9. Contaminants such as lignin derived from plant materials can be qualitatively detected from the NMR spectral data.

A.3.10 Acetone-soluble matter content

A soxhlet extractor, heater, precision balance, weighing dish and solvent-proof oven are used for the measurement.

When the iCNF sample is provided as a suspension, a freeze-dried test specimen of 2 g to 10 g is prepared and used as the starting material. The freeze-dried test specimen is equilibrated at ambient conditions until the water content of the sample reaches that of the atmosphere. The uncertainty in the test specimen mass measurements should be less than 1 mg. The test specimen is added into a Soxhlet extractor and extracted with boiling acetone. The extraction procedure should be continued for at least 16 cycles of siphon extraction. It should be confirmed that any fibrous materials or other visible insoluble materials are absent from the acetone extraction liquid. Filtration is performed if necessary. The acetone extraction liquid is transferred to a weighing dish that has been previously weighed. The acetone extract in the weighing dish is air-dried in a draft chamber, and then dried again at 105 °C ± 2 °C in an oven for 2 h. After cooling the weighing dish to room temperature in a desiccator, the dry mass of acetone extract is weighed to within the uncertainty of 0,1 mg. The mass of acetone extract in the iCNF sample can be calculated by using Formula (A.7):

$$C_{AS} = \left(\frac{m_E}{m_{S\_before}} \right) \times 100 \tag{A.7}$$

where

C_{AS} is the acetone-soluble matter content (in % mass fraction);

m_E is the mass of dried acetone extracts (in g);

m_{S_before} is the mass of the dried test specimen before extraction (in g).

NOTE ISO 14453 specifies the determination of acetone-soluble matter for pulps.

A.3.11 Constituent sugar content

A high-performance liquid chromatograph or an ion chromatograph with detectors for detecting monosaccharides and autoclave is used for the measurement.

Preparation of acid hydrolysate: A freeze-dried iCNF sample of about 0,3 g is weighed and added into a 100 ml beaker. A solution of 72 % H_2SO_4 (precisely 3 ml) is added with a pipette to the iCNF sample. The beaker is immersed in a constant temperature water bath at 30 °C. The beaker contents are mixed with a glass rod intermittently as necessary. Water (84 ml) is slowly poured into the beaker and mixed well. The solution is transferred into a pressure resistant glass bottle. The bottle is capped tightly and placed in an autoclave for 1 h at 120 °C. After cooling the bottle to room temperature, the liquid is filtered through a glass filter which has been weighed in advance. Wash liquid is added to the filtrate to adjust the volume to 100 ml. This liquid is used for the measurement of sugars.

Sugar determination: A quantitative analysis of each monosaccharide is conducted through the separation by using either HPLC and IC and the calibration curve method.

Measurement of the residue ratio after pyrolysis: After filtration, the glass filter is dried at 105 °C for 2 h. The filter is transferred into a desiccator with silica gel and is cooled to room temperature. After

45 min, the mass of the filter is measured. The mass residue ratio after pyrolysis is calculated by using Formula (A.8):

$$C_S = \left(\frac{m_{F\_with} - m_{F\_without}}{m_{US}} \right) \times 100 \quad \text{(A.8)}$$

where

C_S is the constituent sugar content in % mass fraction;

m_{F_with} is the filter mass with samples;

$m_{F\_without}$ is the filter mass without samples;

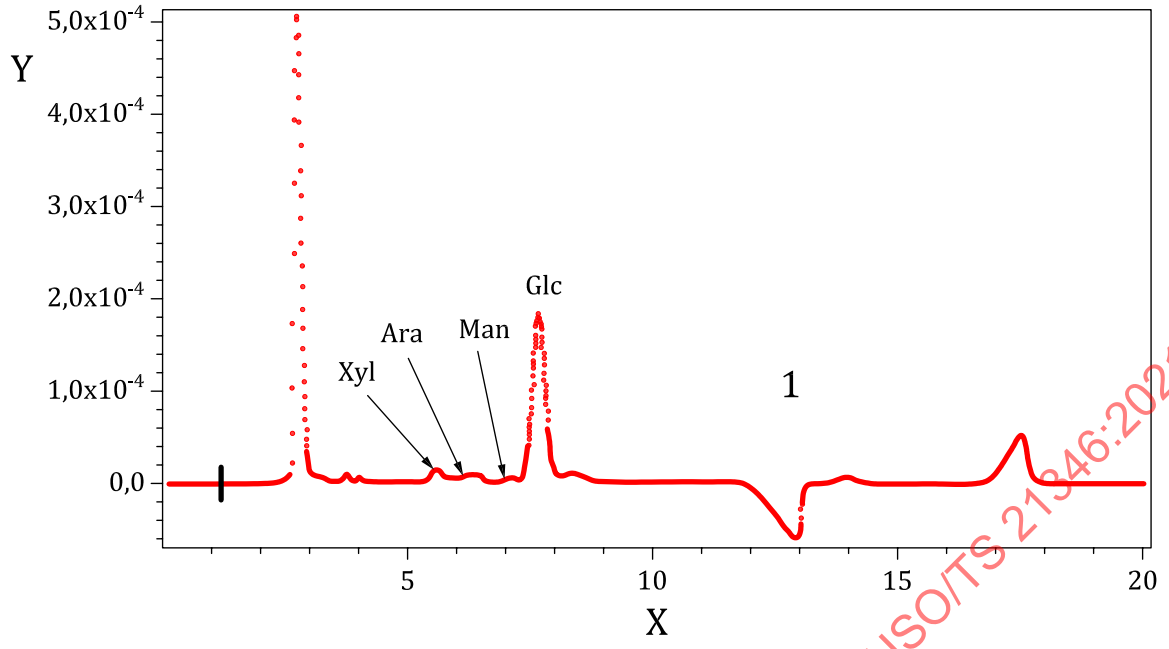
m_{US} is the mass of the used samples.

Method to determine the recovery ratio of each monosaccharide to correct for excessive decomposition: During a hydrolysis test, the sugars can decompose through an excessive decomposition reaction. The amount of each monosaccharide (component sugar) recovered is determined to correct for excessive decomposition of sugars. One of the duplicated solutions is used as the reference during the thermal decomposition experiment, i.e. it is subjected to the same test scheme as the method described in the above procedures including hydrolysis. After cooling, water is added to the autoclaved solution until the total volume reaches 100 ml (liquid a). Water is added to the other solution until the total volume reaches 100 ml (liquid b). According to the above procedure, the concentration of each monosaccharide in liquids a and b is analysed. After the hydrolysis, the recovery ratio is calculated for each monosaccharide. Correction for the excessive decomposition part of sugars is performed by multiplying the monosaccharide concentration before correction by the reciprocal of the recovery ratio. These data are referred to as the "corrected monosaccharide concentrations".

Scale factor: The scale factor from a monosaccharide to a polysaccharide is 0,9 for a hexose and 0,88 for a pentose.

Representative data for constituent sugars are shown in Figure A.12.

Representative constituent sugars of pulps are glucose(Glc), mannose(Man), xylose(Xyl) and arabinose(Ara).



Key

X time (min)

Y differential refractive index (RIU)

1 inositol (internal standard)

Figure A.12 — Example of constituent sugar analysis of iCNF samples by HPLC

NOTE Reference [31] provides information on the analytical procedure for determination of structural carbohydrates in biomass.

Annex B
(informative)

Description of individualized cellulose nanofibril (iCNF)

B.1 General

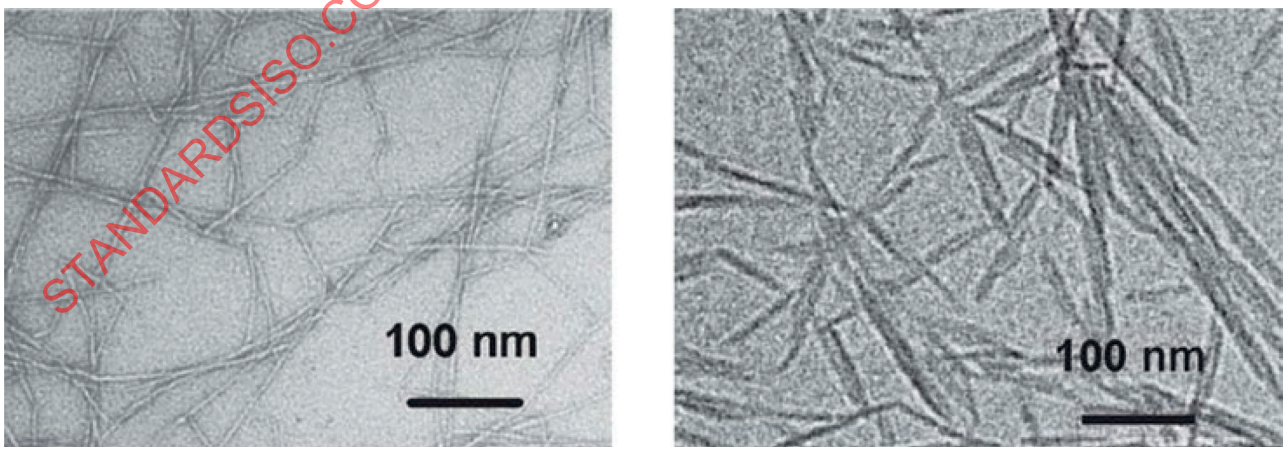
In the case of wood pulp, elementary fibrils with a cross-sectional dimension of about 3 nm can be observed, but they exist as a part of a cellulosic fibre in which the elementary fibrils adhere to each other by the intermolecular attraction called "hydrogen bonding".

iCNF refers to the artificial extraction of elemental fibrils from natural cellulose materials, such as pulp, consisting of a bundle of elemental fibrils. When an elementary fibril is extracted as an iCNF through TEMPO-mediated oxidation followed by mechanical treatment, the primary alcohols of the iCNF surface are converted to carboxylic acid groups. Due to the existence of carboxylic acids on the surface of iCNFs, iCNFs can be dispersed well in solvents such as water. There are other types of iCNFs, such as iCNFs having phosphate esters on the surface, which can also be dispersed well in water or other solvents.

Thus, when iCNFs are derived from a wood pulp, an aqueous suspension of iCNFs refers to the situation in which chemically modified cellulosic fibrils with about 3 nm diameters are well dispersed in water after a homogenization via a mechanical treatment. The width is the same as a natural elementary fibril. References [12], [14] and [32] provide several figures that explain the structure and properties of iCNFs.

B.2 Morphology and size of iCNF compared to CNC

When using wood pulps as starting materials, the width of iCNFs is about 3 nm, which is the same as the expected width of naturally occurring elementary fibrils. The width of iCNFs is smaller than that of CNCs, and the length of iCNFs is larger than that of CNCs. The breadth of the cross-section distribution is smaller for iCNFs than CNCs. iCNFs are individualized single fibrils with high dispersibility in water, while CNCs have a tendency to form aggregates. See Figure B.1.



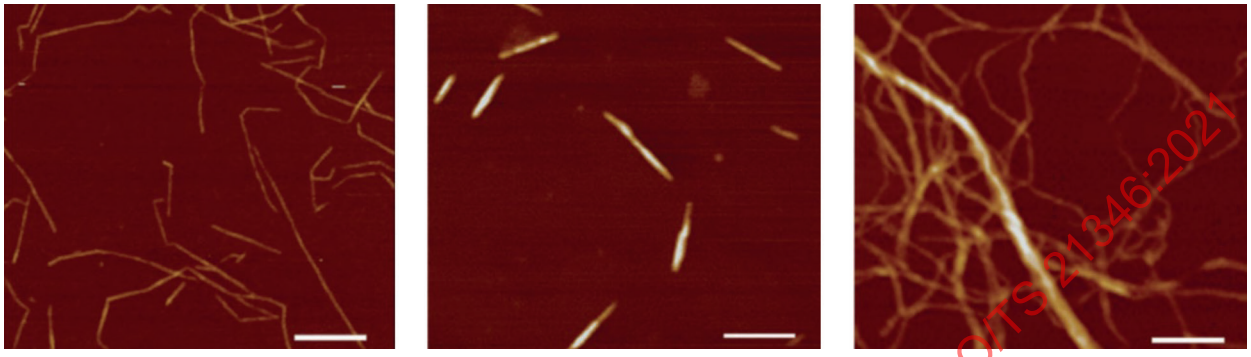
a) iCNFs **b) CNCs**

NOTE Reproduced from References [33], [34] and [35].

Figure B.1 — TEM images of wood

B.3 Differences between iCNFs, CNCs and CNFs

AFM images of iCNFs, CNCs and CNFs are shown in Figure B.2. When using wood pulps as a raw material, the widths of iCNFs, CNCs, and CNFs are about 3 nm, about 6 nm, and more than 10 nm, respectively. The cross-sectional size of iCNFs is the same as that of elementary fibrils of naturally occurring cellulose materials (about 3 nm, in case of wood). Although there are a variety of CNFs, a general type of CNFs is the mixture of fine and thick fibres. Their widths are larger than those of iCNFs.



a) iCNFs **b) CNCs** **c) CNFs**

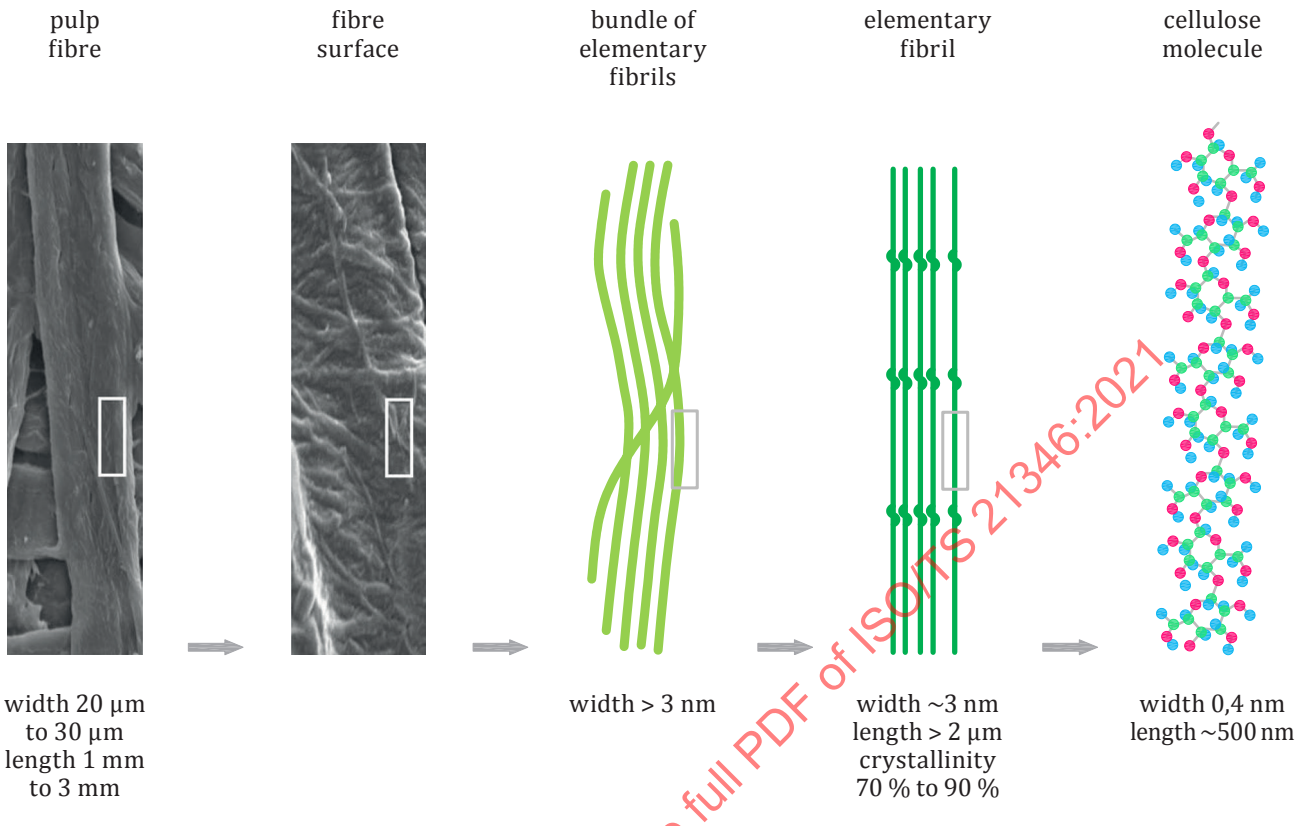
NOTE 1 The scale bar in each photo shows 200 nm.

NOTE 2 Reproduced from Reference [35].

Figure B.2 — AFM images

B.4 Native elementary fibrils

In the wood cellulose structural hierarchy, an elementary fibril is the structural unit which lies between bundles of elementary fibrils of width > 15 nm and cellulose molecules whose width is about 0,4 nm (see Figure B.3). The width of elementary fibrils derived from wood pulp is usually about 3 nm, and the length can reach more than 2 µm. Due to intermolecular attractive forces including hydrogen bonding, it is difficult for native elementary fibrils to be individualized. Reference [32] provides information about the hierarchical structure of wood cellulose.



NOTE Modified from a figure in Reference [32].

Figure B.3 — Hierarchical structure of wood cellulose

B.5 General procedure for preparing iCNFs

Individualized iCNFs can be made directly from natural cellulose materials including wood pulp, cotton and other crystalline cellulose, e.g. through TEMPO-mediated oxidation followed by a slight mechanical treatment. The cross-sectional sizes of iCNFs vary depending on the types of cellulose used as raw materials. When using wood pulps as a raw material, the width of the iCNF is about 3 nm. Other TEMPO derivatives can be also used as catalysts for iCNF preparation. In addition, it is reported that the iCNF can be prepared by using phosphoric acid. Other manufacturing methods could be developed in the future. During chemical treatment, the mixture of pulp (raw material) and reagents (oxidant and catalyst) can be converted to the mixture composed of iCNFs and spent/unspent chemicals. iCNFs and chemicals can be easily separated by simple treatments such as filtration and washing. Other special treatments such as fractionation and/or dialysis are not required to prepare iCNFs.

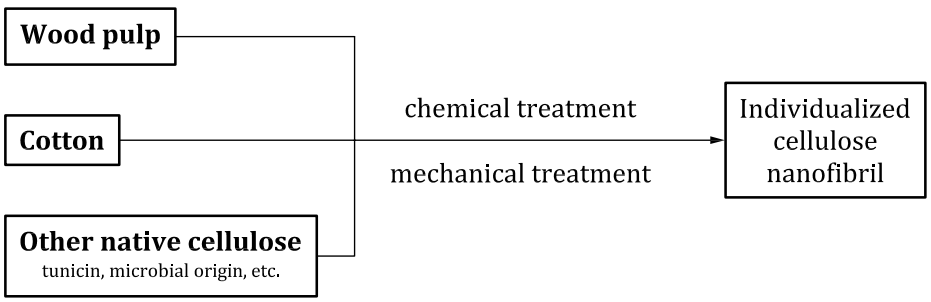
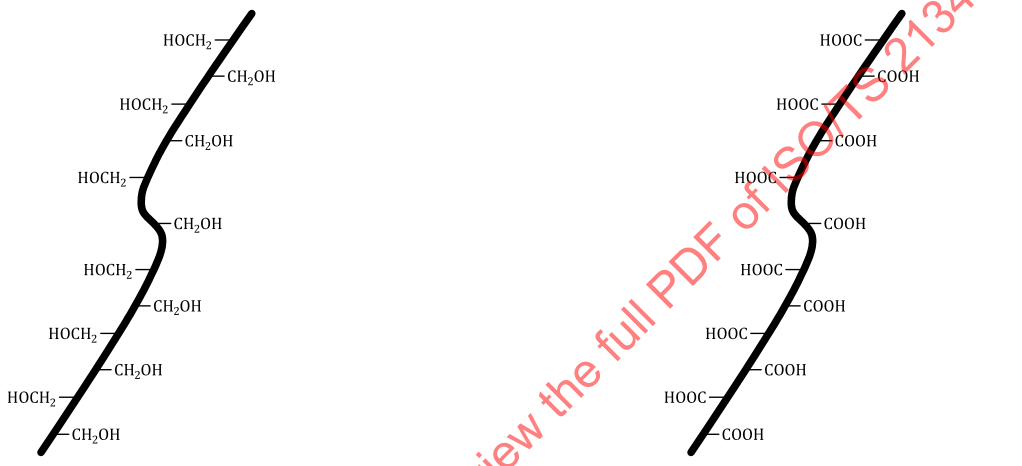


Figure B.4 — Example of the manufacturing processes of iCNFs

B.6 Differences in surface functional groups: elementary fibrils versus iCNFs

The difference between naturally occurring elementary fibrils and iCNFs is the presence of different functional groups on the fibrils' surface. The elementary fibril is made of only of cellulose molecules, so the functional groups on the surface of elementary fibrils are the primary alcohol and secondary alcohol groups of the glucose units composing the cellulose molecules. When iCNFs are artificially extracted from natural cellulosic materials including pulps, the surface of the iCNFs is partially or mostly modified by functional groups such as carboxylic acids or phosphate groups. For example, if TEMPO-mediated oxidation is used for the extraction of iCNFs, the primary alcohols of elementary fibrils are converted to carboxylic acids of iCNFs.

In Figure B.5, all of the primary alcohols of elementary fibrils are converted to carboxylic acids, but practically iCNFs could have small amount of the primary alcohols depending on the oxidizing conditions.



a) Native elementary fibril **b) iCNF made through oxidation**

Figure B.5 — Difference of functional groups on the fibril surface — Native elementary fibrils versus iCNFs

B.7 Dispersion force of iCNF

Naturally occurring elementary fibrils exist in bundle form within natural cellulosic fibres such as pulp fibres, and will never spontaneously separate, owing to hydrogen bonding interactions between them. When iCNFs are prepared from natural cellulosic fibres, they have newly introduced charged functional groups on the surface of the fibres. For example, when iCNF is prepared by TEMPO oxidation, primary hydroxyl groups on the iCNF surface are converted to carboxylic acid groups. Due to electrostatic repulsion between the charged functional groups, iCNFs can be dispersed to give stable suspensions in a medium such as water. The dispersion force of the iCNF depends on the amount of charged functional groups on its surface. Other functional groups on the surface of iCNFs which can be dispersed well in water include phosphate groups. See Figure B.6.

In the model illustration on the left of the figure, the gap between elemental fibrils is depicted larger than it really is. Reference [36] shows that natural elementary fibrils (microcrystalline structures) in cell walls and a gap between elementary fibrils can be clearly observed under the electron microscope by using appropriate staining.