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)

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Published in Switzerland

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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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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.