



Technical Specification

ISO/TS 23361

Nanotechnologies — Crystallinity of cellulose nanomaterials by powder X-ray diffraction (Rietveld analysis)

*Nanotechnologies — Cristallinité des nanomatériaux à base de
cellulose par diffraction aux rayons X sur poudre (analyse de
Ruland-Rietveld)*

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Foreword

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Introduction

Cellulose nanomaterials (CNM) are a family of emerging bioproducts with significant commercial impact. Their production from abundant cellulose sources such as wood pulps makes them a candidate for use as a potentially non-toxic, biodegradable and sustainable nanomaterial for a wide range of applications, including those that currently use petroleum-based components. Several types of CNM are currently produced in a number of countries on pilot, pre-commercial or commercial scales. Realizing the full potential of these materials requires standard methods for characterization of a range of material properties, including crystallinity. Crystallinity is an indication of material quality, success of processing, and degradation of the material during processing, which can affect performance for various applications, particularly for use in nanocomposites. Crystallinity is also important for distinguishing between CNC grades and products and ensuring batch control and repeatability, and can provide information on the cellulose source and production method.

Crystallinity of CNM is defined as the fraction of the material composed of crystallites. There are several approaches for measuring crystallinity of materials, including powder X-ray diffraction, differential scanning calorimetry and solid-state nuclear magnetic resonance. Each of these techniques obviously has its merits and limitations. Use of crystallinity for quality control purposes for CNM production and further processing requires a measurement method that can be quickly, routinely and reproducibly implemented and that is easily accessible and can be run in most laboratories with the same level of proficiency. Powder X-ray diffraction addresses these criteria. This technical specification describes the measurement of CNM crystallinity by powder X-ray diffraction using deconvolution of the diffraction pattern based on Rietveld analysis. The Rietveld method allows a diffraction profile to be modelled based on known diffraction peaks for a specific crystallographic structure by attempting to minimize the difference between the calculated and observed patterns by the least-squares method.

The Rietveld method requires that the powder diffraction pattern be representative of the sample, which requires careful sample preparation and assumes a randomly oriented sample. The instrument configuration and detector sensitivity are also important. This document provides guidance on both sample preparation and instrument configuration and operating parameters. The method is applicable to all types of CNM, including cellulose nanocrystals, cellulose nanofibrils and cellulose filaments.

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Nanotechnologies — Crystallinity of cellulose nanomaterials by powder X-ray diffraction (Rietveld analysis)

1 Scope

This document specifies the determination of the bulk crystallinity (crystalline contribution relative to the total crystalline and amorphous contributions in the material) of cellulose nanomaterials using powder X-ray diffraction followed by deconvolution of the diffraction patterns based on Rietveld analysis. It is applicable to all types of cellulose nanomaterials, assuming a representative sample.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 Core nanotechnology terms related to cellulose nanomaterials

3.1.1

nanoscale

length range approximately from 1 nm to 100 nm

[SOURCE: ISO 80004-1:2023, 3.1.1]

3.1.2

nanomaterial

material with any external dimension in the *nanoscale* (3.1.1) or having internal structure or surface structure in the nanoscale

Note 1 to entry: Certain types of nanomaterials include engineered, manufactured and incidental nanomaterials.

Note 2 to entry: The nanoform of a material is a nanomaterial.

[SOURCE: ISO 80004-1:2023, 3.14, modified — Note 1 to entry has been adapted to this document.]

3.1.3

nano-object

discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.1.1)

[SOURCE: ISO 80004-1:2023, 3.1.5]

3.1.4

nanofibre

nano-object (3.1.3) with two similar external dimensions in the *nanoscale* (3.1.1) and the third dimension significantly larger

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

[SOURCE: ISO 80004-1:2023, 3.3.5]

3.1.5

nanocrystal

nano-object (3.1.3) with a crystalline structure

[SOURCE: ISO 80004-1:2023, 3.1.15]

3.2 Non-nanotechnology terms related to cellulose nanomaterials

3.2.1

crystalline

having a solid structure with a three-dimensional arrangement of ions, molecules, or atoms with long range order

[SOURCE: ISO/TS 20477:2023, 3.2.1]

3.2.2

amorphous

regions within a polymeric material that, on the basis of X-ray diffraction or other suitable techniques, do not show any evidence of crystalline structure

[SOURCE: ISO 472:2013, 2.50]

3.2.3

paracrystalline

having short and medium range ordered lattice structure and lacking long range order in at least one direction; in the intermediate state between *crystalline* (3.2.1) and *amorphous* (3.2.2)

[SOURCE: ISO/TS 20477:2023, 3.2.2]

3.2.4

cellulose

linear polymeric chains of β (1 \rightarrow 4) linked D-glucopyranose units

[SOURCE: ISO/TS 20477:2023, 3.2.3]

3.2.5

elementary fibril

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

[SOURCE: ISO/TS 20477:2023, 3.2.4]

3.3 Terms specific to cellulose nanomaterials

3.3.1

cellulose nanomaterial

CNM

material composed predominantly of *cellulose* (3.2.4), with any external dimension in the *nanoscale* (3.1.1), or a material having internal structure or surface structure in the nanoscale, with the internal structure or surface structure composed predominantly of cellulose

Note 1 to entry: Some cellulose nanomaterials can be composed of chemically modified cellulose.

Note 2 to entry: This term is inclusive of cellulose nano-object and cellulose nanostructured material.

[SOURCE: ISO/TS 20477:2023, 3.3.1]

3.3.2

cellulose nanofibre

nanofibre (3.1.4) composed predominantly of *cellulose* (3.2.4)

Note 1 to entry: This definition is a description of the morphology and the size of an object. It should not be confused with wood fibres or wood pulp fibres which typically have diameters of tens of micrometres.

[SOURCE: ISO/TS 20477:2023, 3.3.4]

3.3.3

cellulose nanocrystal

CNC

nanocrystal (3.1.5) predominantly composed of *cellulose* (3.2.4) containing predominantly *crystalline* (3.2.1) and *paracrystalline* (3.2.3) regions, with at least one *elementary fibril* (3.2.5), not exhibiting longitudinal splits

Note 1 to entry: The aspect ratio of cellulose nanocrystals is usually smaller than 50 but usually greater than 5, where aspect ratio refers to the ratio of the longest to the shortest dimensions.

Note 2 to entry: Cellulose nanocrystals do not exhibit interparticle entanglement or network-like structures.

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

[SOURCE: ISO/TS 20477:2023, 3.3.5, modified — Note 4 to entry has been deleted.]

3.3.4

cellulose nanofibril

CNF

cellulose (3.2.4) *nanofibre* (3.1.4) composed of at least one *elementary fibril* (3.2.5) that can contain branches, a significant fraction of which are in the nanoscale

Note 1 to entry: The dimensions are typically 3 nm to 100 nm in cross-section and typically up to 100 µm in length.

Note 2 to entry: CNF can form entanglements between particles or network-like structure when the distance between CNF fibres is sufficiently close.

Note 3 to entry: Cellulose nanofibrils from plant sources, produced by mechanical processes, can be accompanied by hemicellulose, and in some cases lignin.

Note 4 to entry: Some cellulose nanofibrils can have functional groups on their surface as a result of the manufacturing process.

Note 5 to entry: The terms nanofibrillated cellulose (NFC), nanofibrillar cellulose (NFC), microfibrillated cellulose (MFC), microfibrillar cellulose (MFC), cellulose microfibril (CMF) and *cellulose nanofibre* (3.3.2) have been used interchangeably with cellulose nanofibril. The terms microfibrillated cellulose (MFC), microfibrillar cellulose (MFC), cellulose microfibril (CMF) have also been used incorrectly to describe cellulose nanofibrils.

Note 6 to entry: The term cellulose nanoribbon has been used to describe cellulose nanofibrils from bacterial sources.

[SOURCE: ISO/TS 20477:2023, 3.3.6, modified — Note 5 to entry has been adapted to this document and Note 7 to entry has been deleted.]

3.3.5

**individualized cellulose nanofibril
iCNF**

discrete *cellulose nanofibril* (3.3.4) composed of one *elementary fibril* (3.2.5) with ionic functional groups on its surface

[SOURCE: ISO/TS 20477:2023, 3.3.7]

4 Principle

Crystallinity of cellulose nanomaterials (CNM) is defined as the mass fraction of the material composed of crystallites. This document describes the measurement of CNM crystallinity by powder X-ray diffraction using deconvolution of the diffraction pattern based on Rietveld analysis.^{[1],[2]} The scattering due to the crystalline peaks is separated from background scattering, including any amorphous component, and the background is fit over the entire angular range.^[3] The Rietveld method fits either known crystallographic structures or peaks, or both, to collected powder diffraction data, attempting to minimize the difference between the calculated and observed patterns by the least-squares method. As such, the method adopts the original diffraction pattern in its entirety, unlike the widely used Segal method which uses the intensity of the (200) peak as an indicator of crystalline content and the minimum between the (100) and (200) peaks as the indicator of amorphous content.^[4] The minimum, which is assumed to represent the maximum of amorphous scattering, has been reported to depend on crystallite size for small crystals.^[5]

A commonly overlooked and poorly understood requirement is that the powder diffraction pattern must be representative of the sample if the Rietveld method is to be employed. This is not a trivial matter and requires stringent sample preparation.^[6] In general, the sample must be in powder form, with particles between 1 µm to 5 µm in size, packed in random orientations (theory requires infinite depth). Proper instrument alignment and sample placement are also important. Selecting instrument components with higher spatial resolution or better resolved options can also improve the data quality. For example, using a silicon strip position sensitive detector on the X-ray diffractometer rather than a sodium iodide (NaI) scintillation point detector results in vastly improved signal to noise ratios within a given time and, consequently, improved data and models.

Once the sample is properly prepared and a representative data set is obtained, a large number of corrections can be applied to obtain a better fit during Rietveld modelling. These corrections range from instrumental parameters to finer crystallographic details of each particular sample. Since it is difficult to completely remove preferred orientation for samples with high aspect ratio particles, it is important that the modelling procedure be able to correct for preferred orientation and crystallite size anisotropy. The modelling process is involved and requires crystallographic understanding and is thus impacted by the subjectivity of the analyst. A fitting description of the Rietveld modelling process is given in Reference [7].

The availability of crystallographic information files (CIF) for cellulose I α and I β and Cellulose II and III allows the crystal structure to be input directly into the Rietveld modelling program.^{[8] - [10]} The space group, unit cell dimensions and atomic coordinates of a material are used to construct the CIF and are routinely output from single crystal diffraction software. The use of a CIF eliminates the need for a peak fitting method which would require that individual peaks representing cellulose peak positions be input into the modelling program. That is not a simple procedure because cellulose samples result in broad and overlapped peaks which are difficult to model. The availability of CIF files significantly simplifies the modelling procedure and provides higher crystallographic accuracy.

The raw powder X-ray diffraction patterns are deconvoluted based on the Rietveld approach using known diffraction patterns for cellulose from the appropriate CIF. When modelling percent crystallinity using the degree of crystallinity method, the crystalline and amorphous areas are defined, and their total area is used to calculate the percentage of crystallinity. In general, the crystalline peaks are the well-defined peaks (although there can be considerable peak overlap due to broadened peaks). Amorphous phase contributions normally appear as very broad humps in the same region as background signals from other sources. A number of factors can contribute to the background signal. Minimizing or eliminating these contributions is critical to avoid a situation where secondary background-related factors significantly contribute to the amorphous content. The following factors can affect the background contribution:

- air scatter, particularly at lower angles;
- an uneven sample surface;
- fluorescence;
- contributions from the sample holder, mount or mounting medium.

Refer to Reference [11] for a summary and examples of the relative merits and limitations of the Rietveld modelling approach as compared to the Segal method and an amorphous subtraction method. Reference [11] discusses the various approaches that have been used to model the amorphous component, specifically the use of a small cellulose crystallite or a user-input function.

5 Sample preparation

5.1 General considerations

The method described in this document has been tested with different types of wood-derived-CNM, including cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and individualized cellulose nanofibrils (iCNF) in an interlaboratory comparison (see [Annex A](#)) and may also be applicable to CNM derived from other sources. The CNM samples used in this study were received as dry powders that had been dried by either spray drying (CNC) or freeze-drying (CNF, iCNF). The CNF and iCNF samples were milled using a laboratory Wiley Mill with a 0,5 mm sieve opening to ensure uniform particle size. Using randomly packed dry powdered samples with uniform particle sizes in the range of 1 μm to 5 μm will average out orientational effects in individual crystals. Independent of the CNM source or the method used to prepare samples, the sub-sample(s) used for XRD analysis must be representative of the bulk sample.

A range of sample preparation methods have been used in literature studies, including preparation of thin films or sheets and pellets. Different sample preparation methods can lead to issues with orientational effects that require larger corrections than those in the interlaboratory comparison (ILC) study (see [Annex A](#)). Similarly, CNM generated from other sources can lead to larger degrees of orientation, depending on the particle morphology. Although it is desirable to ensure that the sample has a uniform particle size, unnecessary or excessive grinding of the sample should be avoided in order to minimize possible damage to the crystalline structure.

As for any hygroscopic material, care should be employed to ensure that the effects of moisture content in the sample are taken into account either by recording the environmental conditions during sample preparation and XRD measurements or by using sample holders specifically designed for environmentally sensitive samples. While studies that focus on the moisture content effects on the XRD data from cellulosic materials are limited, it is known that the moisture content in various types of lignocellulosic samples (including wood, cotton and even CNC) will directly influence the diffraction peak position [12],[13] and, consequently, the apparent crystallinity can decrease if the water content is higher. [14],[15] Thus, it is important to prepare the samples to be compared in the same environmental conditions; this can be accomplished by equilibrating the sample at a specific relative humidity and temperature for 24 h. Likewise, the room conditions during the XRD measurements should be recorded, which is particularly important if the sample holders used allow for any moisture to enter the samples.

5.2 Preparation of samples from powders

Mix the sample to ensure homogeneity prior to removing a specimen of powder for powder X-ray diffraction measurement. Pack the powdered CNM into the sample holder. A zero-background cell is preferred to minimize background signals, but other typical sample cells (e.g. 25 mm plastic, Al sample holder) can also be used. Level the surface that will be exposed to the X-ray beam with a blade. This is the normal sample preparation method for data collection in the Bragg-Brentano configuration for Rietveld modelling.

NOTE This sample preparation method works well for fine powdered samples (e.g. spray-dried CNC). Samples that have interconnected fibrous structures (e.g. freeze-dried CNC, CNF or iCNF) do not pack as well, leading to a rough surface that can increase background signals and peak broadening. It is recommended to pre-treat such samples by milling with a laboratory mill with an appropriately sized sieve opening to ensure a uniform particle size.

6 Instrument and software requirements

6.1 Diffractometer

The protocol described here is appropriate for powder X-ray diffractometers capable of being used in the Bragg-Brentano configuration and equipped with a copper X-ray tube and a position sensitive detector. The instrument configuration should be capable of removing $\text{CuK}\beta$ radiation from the measurement, for example by using a Ni-filter. Other X-ray sources are acceptable provided that the resolution and angular range are similar. Benchtop XRD instruments have lower power and slightly reduced resolution. However, as they allow use of more of the X-ray source beam, giving higher scattering intensity, they can also be suitable.

A rotating specimen holder should be used for data collection, if available.

NOTE A silicon strip position sensitive detector provides better signal-to-noise ratios than point detectors (e.g., NaI scintillation or Xe proportional detectors) which is important for these measurements.

6.2 Software

Software that is capable of Rietveld analysis is provided with some instruments, including TOPAS from Bruker, HighScorePlus from Malvern and SmartLab Studio II from Rigaku. There are also free software packages such as MAUD, a Java based package for material diffraction, JCryst for visualization and calculations, GSASII for Rietveld refinement of crystal structure and MTex, a Matlab toolbox for quantitative texture analysis. This list is not meant to be inclusive of all software packages suitable for Rietveld analysis. Most commercial XRD analysis software with Rietveld capabilities is likely to contain the required analytical capabilities, although there can possibly be some differences in implementation. More than one routine or algorithm can be needed for free software.

An important software requirement is that the X-ray powder line profile fitting must take into account the contribution of the instrument configuration to the peak shape and width for the diffraction pattern. This can be accomplished using the fundamental parameters approach which is available in some software packages (e.g. TOPAS and SmartLab Studio II). The fundamental parameters approach uses physically based models to generate the line profile shapes.^{[16],[17]} Fits can be obtained over the whole 2θ range using the known properties of the diffractometer, such as the slit sizes and diffractometer radius, and the emission profile. Alternately, the user shall measure a standard crystalline sample with the same instrument geometry to be used for the cellulose samples and fit the diffraction pattern to the Cagliotti equation to obtain the instrument response (e.g. HighScorePlus).¹⁾

6.3 Crystallographic information files

Selection of the proper CIF file should be based on either the material source or processing, or both. Wood pulp derived CNM (i.e. the samples used in the ILC summarized in [Annex A](#)) and other plant-based CNM will generally exhibit a cellulose I β crystal structure, whereas CNM derived from algae and bacterial cellulose have a cellulose I α structure. Small crystallites of Cellulose II or IV have been suggested as appropriate models for the amorphous component.^[11]

NOTE Cellulose I α and I β CIF are available from the Crystallography Open Database (<http://www.crystallography.net>) and are based on neutron data for tunicate obtained by Nishiyama.^{[8],[9]} Cellulose II and III^[14] CIF are also available. An overview of powder diffraction patterns for various cellulose polymorphs based on the published atomic coordinates and unit cell dimensions contained in modified CIF has been provided in Reference [\[18\]](#).

7 Data collection

The data collection process should be as follows:

- a) Set up the instrument in accordance with the manufacturer's instructions.

1) TOPAS, HighScorePlus, SmartLab Studio II, MAUD, JCryst, GSASII and MTex are examples of suitable products available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

- b) Measure a standard (or a material with known diffraction peak positions within the accessible range of the instrument) to ensure that the instrument is properly calibrated. Alumina is a suitable sample.
- c) Configure the instrument slits as specified in the user manual. The instrument parameters shall be adjusted to have as much of the beam as possible incident on the sample, while avoiding the sample holder. The following examples of instrument configurations are provided for reference:
- Bruker D8-Advance powder diffractometer in Bragg-Brentano configuration, with a 1,0 mm divergent slit, 8,0 mm anti-scatter slit, 2,5° Soller slits and 3,7° window opening.
 - Malvern PANalytical X'Pert powder diffractometer in Bragg-Brentano configuration with 0,04 rad Soller slits, anti-scatter slits with a fixed 1° aperture, anti-diffusion slits with a 2° aperture, and 20 mm mask.
 - Rigaku SmartLab powder diffractometer in Bragg-Brentano configuration with 1/6° incident slit, 2,5° Soller slit, fully-opened receiving slit, anti-scatter slit.²⁾
- d) Collect scattering data for the CNM sample from an angular range of 5° to 90° in 2 θ , with a step size (sampling interval) of $\Delta 2\theta = 0,04^\circ$. The counting time per step should be selected so as to collect a minimum of 30 000 counts at the position of maximum intensity in the area corresponding to d-spacings of 6 Å to 3,5 Å (15° to 25° 2 θ with 1,5406 Å CuK α radiation). The number of counts required will depend on the noise level. A measurement of 10 000 counts is typically considered sufficient such that random noise will contribute $\leq 1\%$ to the signal; therefore a minimum of 30 000 counts is recommended to avoid noise issues.
- e) Collect an environmental background scan under the same conditions but with an empty sample holder to account and correct for contributions such as air scatter that can possibly contribute to the intensity and can be confused with amorphous signal. Note that a zero-background sample holder does not eliminate the environmental background. Additional background signals can be related to the use of a standard sample holder or the choice of slits.
- f) Repeat measurements in triplicate using three separate sub-samples of the material. Fewer replicates can be adequate for repeat measurements of similar samples if the repeatability has been previously demonstrated to be adequate for the stated purpose.
- g) Measure scattering from a standard crystalline sample (e.g. quartz) if required to determine the contribution of instrumental factors to line broadening (see 6.2).

NOTE 1 Both data collection and analysis software must be configured for fixed slits.

NOTE 2 An angular range of 5° to 60° 2 θ is adequate for cellulose samples and can be used to reduce data collection time.

NOTE 3 In some cases, it can be necessary to subtract a reduced fraction of the background signal to avoid systematically negative data after correction. If the instrument is stable, it can be possible to use a single background collection for each sample that was measured in the same sample holder.

NOTE 4 If for any reason it is not possible or practical to record a background scan, preparation of samples using the same method will help to ensure that the background signals are consistent and comparable across different samples, allowing for analysis of trends in the data.

8 Data processing and analysis

8.1 General considerations

The results obtained from the Rietveld method on properly prepared samples can be quite precise. Sample preparation is important as there is no point to attempt to model a scan that is not representative

2) Bruker D8-Advance powder diffractometer, Malvern PANalytical X'Pert powder diffractometer, and Rigaku SmartLab powder diffractometer are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

(the results would differ from different data collections on the same sample). However, this less exacting expectation allows or tolerates a pursuit of modelling on non-powdered cellulose-type samples to look for trends indicating that ideal Rietveld sample preparation cannot be achieved and that errors are possible.

When modelling percent crystallinity using the degree of crystallinity method, the crystalline and amorphous areas are defined, and their total area is used to calculate the percentage of crystallinity. In general, the crystalline peaks are the well-defined peaks, although there can be considerable peak overlap due to broadened peaks. Amorphous peaks normally appear as very broad humps that lie in the background of the scan.

8.2 Modelling procedure

The modelling procedure should be as follows:

- a) Read in the diffraction data file and CIF files(s).
- b) Adjust the scale factor and crystal size and peak width for the CIF, so that the sample diffraction pattern and CIF files can be compared.
- c) Apply corrections for sample displacement and absorption.
- d) Correct the raw data by subtracting the environmental background scan recorded in [Clause 7 e](#)) prior to data refinement. Subtraction of a blank run possibly will not eliminate all background signals (for example, that due to inelastic scattering), in which case a background component modelled as a second-order polynomial can be added during Rietveld modelling. In some cases, the blank background data can be input into the Rietveld modelling software.
- e) Select one of the following two options to model the amorphous component.
 - Option 1: Insert broad peaks at 17° to 21° and 8° to 10° 2θ to represent the amorphous component commonly designated in cellulose diffraction patterns. Model the amorphous component with a polynomial function. A Chebyshev polynomial with coefficients 0,1 (so this parameter does not vary significantly across the models) is frequently used.
 - Option 2: Use Cellulose II^[10] with a 12 Å crystallite size to model the amorphous component.

For either option 1 or 2, start the model at 10° 2θ , with a fitting range from 10° to 60° 2θ .

NOTE 1 Both the starting point and additional low angle amorphous peak will affect the percentage of crystallinity results.

NOTE 2 The broad peaks (option 1) are based on the fundamental parameters geometry with a maximum crystal size limit of 1,5 nm and 1,0 nm for the 8° and 17° peaks, respectively. An additional minimum crystal size of 0,3 nm is used for the 17° peak to ensure that this peak has an amorphous peak shape instead of a shape that looks like modelling background. In general, model-based Rietveld analysis is also used to model the amorphous profile using the 17° peak (e.g. SmartLab Studio II). Using other software (e.g. HighScorePlus), the broad peak is based on peak shape parameters determined by measuring a standard crystalline sample. The width should be constrained so that it does not become so broad that it starts to model background.

- f) Allow the unit cell of the cellulose structures to refine within reasonable parameters. Track the goodness of fit parameter to determine whether the model improves.

NOTE 3 Use of the absolute values of the residuals is not recommended as a method to assess whether the model is suitable or not.^[19] However, this approach can be used to monitor the course of the modelling process. The parameters available to monitor the goodness of fit vary with the software. The available parameters must be monitored at each step in the refinement and a record kept of the order in which the parameters are adjusted. This will help to avoid overfitting of the data as new variables are added. Suggested allowable variations for the various parameters are noted in [A.4](#).

- g) Refine the crystallite size and peak shape employing a double-Voigt approach, correcting for both Lorentzian and Gaussian contributions to crystallite size peak broadening. The proportion of Lorentzian and Gaussian contributions depends on the software. As a starting point, one can select 0,75 Lorentzian

and 0,25 Gaussian. If possible, keep this proportion parameter fixed during the refinement or allow it to vary with $\pm 0,01$ of the desired value.

NOTE 4 Other approximations for correction are possible as an alternative to the double-Voigt approach (e.g. pseudo-Voigt), and will work well for most cases.

h) Correct for orientation and anisotropic broadening.

A preferred orientation correction is required for samples that have some alignment of particles.^{[20],[21]} This can be accomplished with one of two options in most software packages. The March-Dollase correction (5 parameter) is simpler and is adequate in most cases; this approach is recommended for use with MAUD software. Alternately, the spherical harmonics correction with order of 6 can be used and is recommended with TOPAS. A refinement cycle using one of the two corrections should be used to correct for possible orientation effects.

Some software packages include an option to correct for anisotropic broadening,^{[5],[22]} which can be tested in a final refinement step. A model to correct for this effect is available.^[5]

NOTE 5 Some spray-dried, packed CNC samples show a sharp feature at $\sim 35^\circ 2\theta$. The (004) peak in this region overlaps with several other peaks and can appear as a sharp feature in samples that exhibit some level of preferred orientation. A similar feature has been observed previously in diffraction patterns of cotton fibres obtained using synchrotron radiation; the feature is assigned to the (004) meridional and its intensity varies with the degree of orientation.^[23]

i) Calculate the percentage of crystallinity and crystallite size.

Calculate the percent crystallinity of the sample by dividing the crystalline area (A_{cry}) over the sum of the crystalline and amorphous (A_{amor}) areas, determined by the deconvolution approach.

$$\% \text{ crystallinity} = (A_{\text{cry}} / (A_{\text{cry}} + A_{\text{amor}})) \times 100 \quad (1)$$

Plot raw data and the final results of Rietveld analysis (raw data, calculated, cellulose I β , background, amorphous and difference between raw data and calculated).

The reported crystallite size (the technically accurate term is “domain size”) is based on the integral breadth (IB) of the peak.

$$\beta_i = \frac{\lambda}{L_{\text{vol}} \cos \theta} \quad (2)$$

where

β_i is integral breadth as proposed by Stokes and Wilson,^[24] addressing domain-size broadening independent of crystallite shape;

L_{vol} is the volume-weighted mean column height;

λ is the radiation wavelength (1,542 Å);

θ is the Bragg diffraction angle, corresponding to the (200) plane in the present case.

Conceptually, [Equation 2](#) is identical to the Scherrer equation with the constant K set to 1:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (3)$$

where

D is the “apparent crystallite size”;

β is the full width of the diffraction peak measured at half maximum height (FWHM) of the instrument corrected line profile.

Calculate the crystallite size using [Equation 3](#).

NOTE 6 Some software packages (e.g. MAUD) give percentage of crystallinity values that are different from those calculated by taking the areas under the curves.

NOTE 7 Some software packages do not use the Scherrer equation, which can be considered a first approximation to crystallite size. More sophisticated routines are built into the software and it is not straightforward to obtain a Scherrer result without exporting the data and measuring peak widths.

9 Uncertainty

[Annex A](#) provides a summary of an ILC that recorded and analyzed powder X-ray diffraction data for three CNM. [Annex A](#) also provides an assessment of the reproducibility of results across laboratories for these samples. The raw data collected by ten laboratories was in agreement, except for some differences at low angles due to background-related issues which varied between laboratories and related to differences in signal-to-noise due to instrumental factors. The ILC labs used different diffractometers and detectors, so that the results were representative of most of the common instrumental setups for collection of powder X-ray diffraction data. The final results of Rietveld modelling were used to obtain ILC consensus values for both the percentage of crystallinity and crystallite size (see [Table A.1](#)) using the NIST consensus builder. [25] The expanded uncertainties for percentage of crystallinity were 8,5 %, 13,3 % and 8,6 % for CNC, CNF and i-CNF, respectively. These values are substantially larger than the standard deviations for individual samples in each laboratory (mean standard deviation for ten laboratories of 2,9 %, 3,3 % and 4,4 % for CNC, CNF and i-CNF, respectively), necessitating the inclusion of a dark uncertainty component to estimate the ILC consensus values. This result indicates that there are factors that are not well-controlled or accounted for in the protocol, leading to a relatively large variation between laboratories. Nevertheless, the better repeatability within a given laboratory indicates that the protocol can be useful for quality control purposes within a single laboratory. The ILC consensus values and uncertainties indicate that a larger difference between samples is required in order to conclude that two samples exhibit a different percentage of crystallinity.

Since the raw data agree reasonably well for the ten laboratories, it appears that most of the laboratory-to-laboratory variance is related to the modelling procedure. Issues with packing of samples and some inherent variability in the samples (both of which are more likely for CNF and iCNF) are also possible contributors. A number of sources of uncertainty can be identified in the Rietveld modelling procedure. The choice of amorphous component is an important factor and there was not a consensus as to whether the use of a user-input function on a small crystallite of, for example, Cellulose II was the better option. The various software packages differ somewhat in the available modelling options which can lead to variation in the modelling results. The background also varies from instrument to instrument and represents a larger source of uncertainty in some instrumental setups; this highlights the importance of careful adjustment of the instrument parameters to ensure a minimum contribution from background signal sources. Currently, the available CIF for the crystalline cellulose component is for a tunicate sample, rather than a wood-pulp derived sample as used in this ILC.

[A.4](#) also summarizes results obtained by re-analysis of one CNC data set for each laboratory by a single analyst using the same software and procedure to the extent possible. These results indicate that the variation between laboratories can be reduced using one software package and a similar set of parameters, consistent with the above conclusion that most of the issues with reproducibility between laboratories arise from differences in Rietveld modelling. Overall, this study indicated that the following factors require further standardization:

- amorphous component;
- correct fitting range;

- minimization of background contributions;
- the allowable variation of the lattice parameters for the crystalline component during modelling.

Although it is premature to claim that the parameters selected for the re-analysis are the optimal ones for general use, this test clearly indicates that it is possible to significantly reduce laboratory-to-laboratory variability by employing one software package and a standard modelling approach. Future studies should address the correct method of modelling the amorphous component, the preferred orientation correction, the correct model for the peak shapes, and appropriate background corrections.

10 Test report

The test report shall include details of the following information:

- a) Sample.
- b) Analyst.
- c) Date of data collection.
- d) Method of sample preparation.
- e) Diffractometer (manufacturer, model).
- f) Source and filter.
- g) Detector.
- h) Slits.
- i) Environmental conditions (temperature, humidity).
- j) Sample holder and rotation.
- k) Data collection range, step size, etc.
- l) Modelling software (including the version).
- m) Orientation correction.
- n) Other corrections.
- o) Provide plots of modelling results, including raw data, calculated data, background, crystalline cellulose, amorphous component, fitted data and difference between raw data and fitted.
- p) Crystallite size (include individual replicates, mean and standard deviation).
- q) Percentage of crystallinity (include individual replicates, mean and standard deviation).
- r) Reference to ISO 23361:2024.

Annex A (informative)

Results of interlaboratory comparison

A.1 Background and objectives

This interlaboratory comparison was conducted under VAMAS Technical Working Area 34 (nanoparticle populations) with the goal of testing a protocol for data acquisition and determination of percentage of crystallinity and crystallite size for CNM using powder X-ray diffraction and Rietveld analysis. The ILC was organized in three phases.

Phase 1 recruited participants and obtained their input on the initial protocol, which was based on experiments carried out at FPInnovations in Vancouver, BC, Canada.

Phase 2 tested the protocol for Rietveld analysis using a data set consisting of diffraction data for three samples:

— A CNC reference material released by the National Research Council Canada and produced by sulfuric acid hydrolysis of softwood pulp followed by neutralization with NaCl, generating sulfate half-ester groups on the surface. Detailed characterization of this material is summarized in References [26] and [27].

— A CNF sample purchased from University of Maine and obtained from wood pulp as a freeze-dried powder.

— An iCNF sample kindly provided by Nippon Japan and produced from wood pulp by TEMPO-oxidation and freeze-dried.

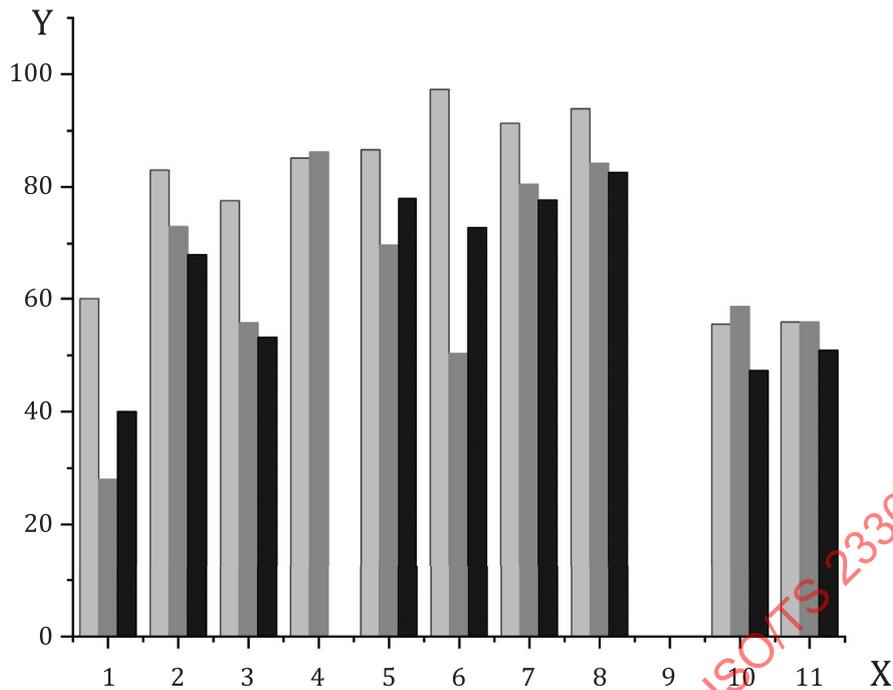
These three samples were sent to phase 3 participants for data acquisition and analysis, with a request to measure three replicates per sample.

Ten participating institutions from Asia, Europe and North and South America completed phase 3 of the ILC and several others participated in the initial stages of the ILC. The final ten laboratories for phase 3 included four universities, one industrial laboratory and five government laboratories. This annex summarizes the ILC results and full details will be published in Reference [28].

A.2 Phase 2 results

The Rietveld modelling procedure was tested by sending powder X-ray diffraction data recorded at FPInnovations for each of the three samples (described in A.1) to participants. The results of these tests are shown in Figure A.1. There was a relatively large number of sources of variability in the Rietveld modelling for this step, which included the CIF used, the approach for modelling the amorphous component, corrections implemented, and fitting range. At least in part, this leads to the laboratory-to-laboratory variability that is observed for the percentage of crystallinity shown in Figure A.1. As a result of this test of the Rietveld modelling procedure, several changes were implemented in the protocol, such as:

- more detail on the recommended software packages;
- specification of the CIF for cellulose I β to use for modelling (available from the Crystallography Open Database, and based on neutron data given in Reference [8]);
- two options for modelling the amorphous component;
- restriction of the fitting range;
- two possible methods to deal with background and orientation corrections.



Key

- X participant number
- Y percentage of crystallinity
- data for CNC
- data for CNF
- data for iCNF

NOTE: Participant 9 did not return results. The mean percentage of crystallinity and standard deviation for each sample are as follows: CNC (78 ± 15) %; CNF (62 ± 20) %; iCNF (61 ± 15) %.

SOURCE: Reference [28], reproduced with the permission of the authors.

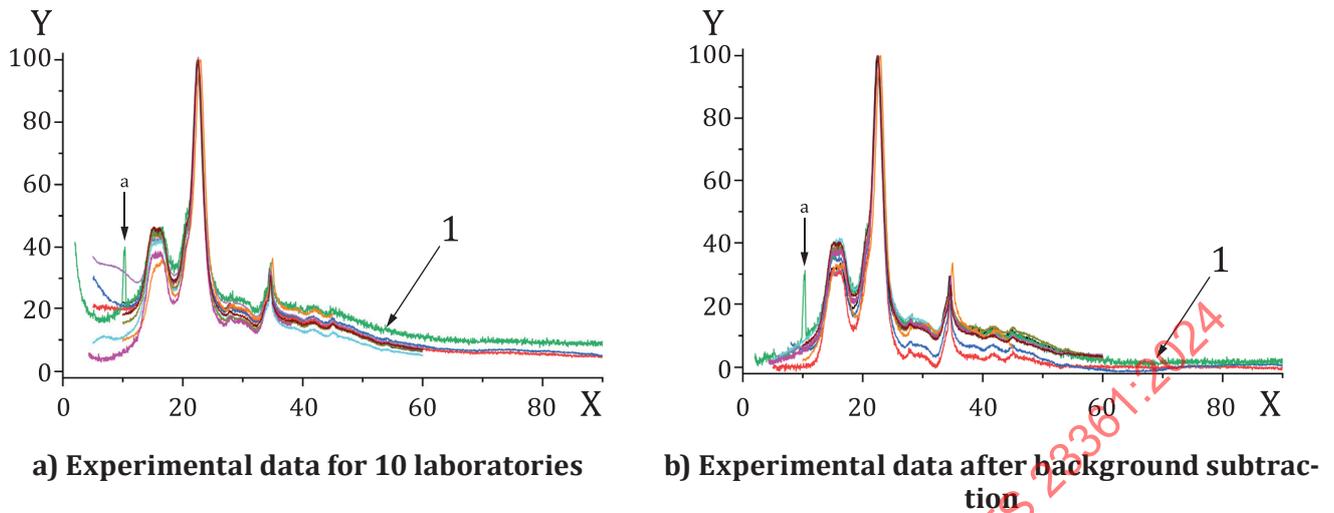
Figure A.1 — Percentage of crystallinity measured in Phase 2

A.3 Phase 3 results

The data collection for Phase 3 by participants employed diffractometers from three commercial suppliers; all but one laboratory used a Cu X-ray source with a Ni filter (laboratory 7 used a Co source) and all but one laboratory used a 1D detector (laboratory 8 used a NaI scintillation detector). Both zero-background cells and standard sample holders were used, most participants (6 of 10) rotated the sample and most recorded an instrument blank, although in some cases this was only used for optimization, rather than subtracted from the raw data. A variety of different options were used for the final instrument configuration (primarily, the slits), which reflected the different diffractometers used. Data was collected from 5° to 90° 2θ in most cases, although a smaller range was used for 3 laboratories and all laboratories used the recommended 0,04 (or smaller) step size. Overall, the experimental conditions used for data collection followed the protocol reasonably well and represented the most common instrument configurations used for powder X-ray diffraction measurements. Full details are available in Reference [28]. Two laboratories tested both dry powder and pellet samples, although only the powder samples are reported here for consistency across laboratories.

The diffraction patterns for the ten participating laboratories were very similar except for some variation at low angles due to background signals. This is illustrated in Figure A.2 for one replicate for each laboratory for CNC, with data before and after background correction shown. Each laboratory is assigned a code (L#);

note that the codes are different from those for Phase 2 tests. The results indicate good reproducibility of the powder X-ray diffraction data across laboratories.



Key

X scattering angle, 2θ

Y intensity

1 data for L8

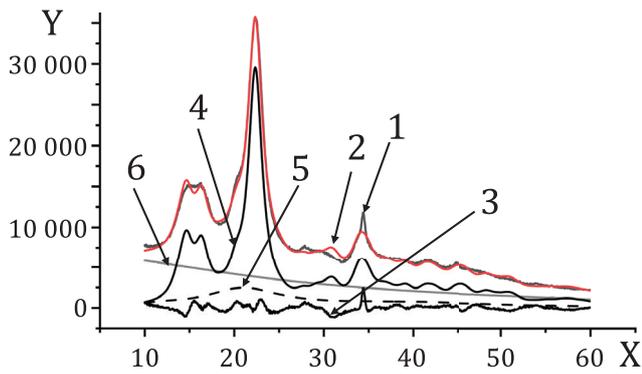
a Additional signal for L8 at 10° that is due to the sample holder used

NOTE The data for each laboratory has been normalized based on the main peak at $\sim 22^\circ 2\theta$. The data for L7 was collected using a Co X-ray source but converted to Cu for display purposes.

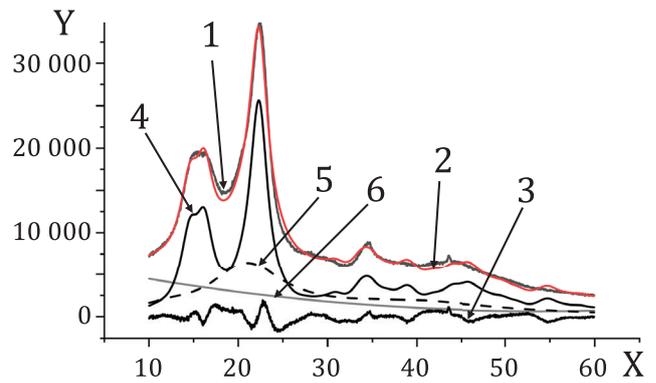
SOURCE Reference [28], reproduced with the permission of the authors.

Figure A.2 — Comparison of diffraction patterns for one CNC replicate

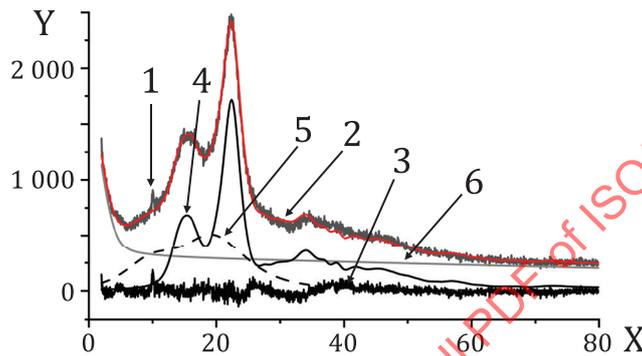
Rietveld modelling of the diffraction data was done using three commercial software packages (provided by the diffractometer manufacturers), MAUD, a freely available software package, and two in-house designed software packages. Examples of the final results for three labs for a single CNC replicate are shown in [Figure A.3](#). For consistency and ease of duplication, results obtained with the three commercial packages and MAUD were considered in the final ILC analysis where possible. One laboratory used in-house software and also compared results to a commercial software package; the data obtained with the commercial software package were used in the final ILC analysis. Only in-house software results were available for laboratory 7. In both cases the in-house software gave considerably lower percentage crystallinity values than were obtained using one of the other software packages.



a) Rietveld modelling results for CNC for L5



b) Rietveld modelling results for CNF for L4



c) Rietveld modelling results for iCNF for L8

Key

X scattering angle, 2θ

Y intensity

1 experimental data

2 final calculated modelling results

3 difference between experimental data and final calculated modelling results

4 crystalline cellulose

5 amorphous components

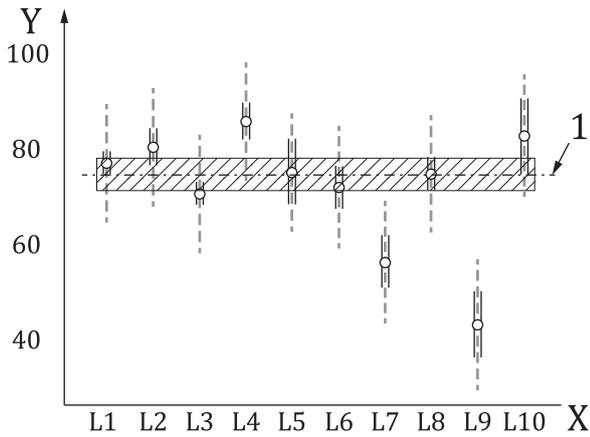
6 background components

SOURCE Reference [28], reproduced with the permission of the authors.

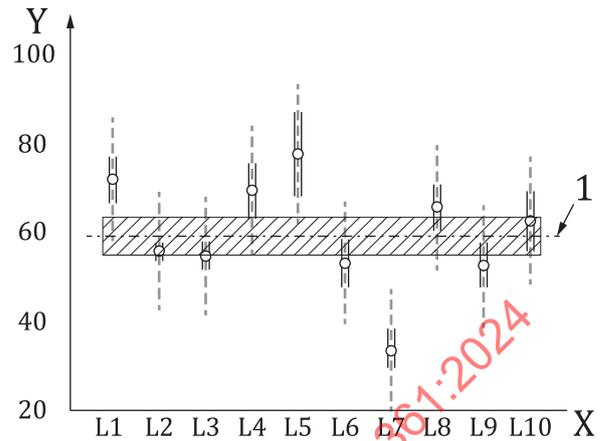
Figure A.3 — Representative Rietveld modelling for one sample replicate for CNC, CNF and iCNF

The approach to Rietveld modelling varied across laboratories, with differences in the manner in which the amorphous phase was modelled, the availability of a background correction, the type of orientation correction used and other factors, including whether or not an anisotropic correction was implemented. The final results for percentage of crystallinity and crystallite size for each of the three samples are shown in [Figures A.4](#) and [A.5](#) and were calculated using the NIST consensus builder with the Bayes-Laplace model, [25] which provides the uncertainty weighted median as a consensus value. This is an appropriate approach when potential outliers will skew the mean value. [Figures A.4](#) and [A.5](#) show the individual laboratory results as the mean plus standard deviation (circles and vertical lines) as well as the final ILC consensus values (1) and the standard uncertainty (hatched area). There is considerable variation between laboratories for each of the three samples as shown by the consensus values and expanded uncertainty for both measurements summarized in [Table A.1](#). However, the changes made as a result of the Phase 2 modelling tests have had some impact in reducing the laboratory-to-laboratory variability. In addition to the uncertainty listed in [Table A.1](#), there is a relatively large dark uncertainty component that is required for estimation of the final

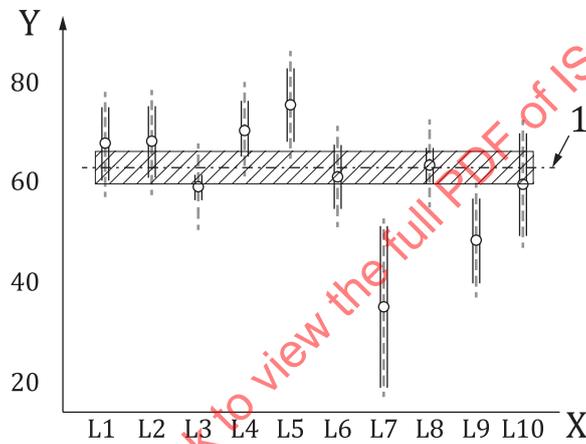
consensus value. This is shown in the plots (vertical dashed lines for each data point) and accounts for a number of unknown effects that contribute to the variation between laboratories.



a) CNC consensus plot



b) CNF consensus plot



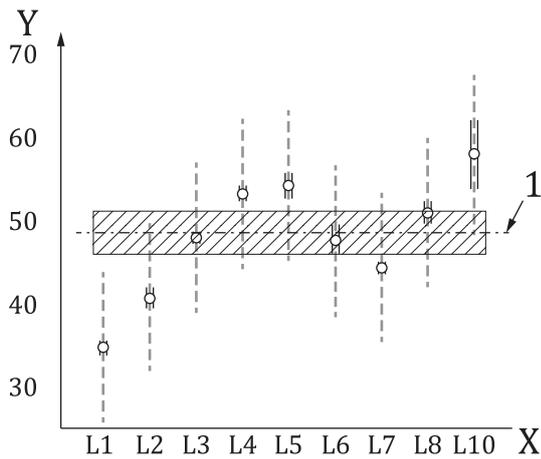
c) iCNF consensus plot

Key

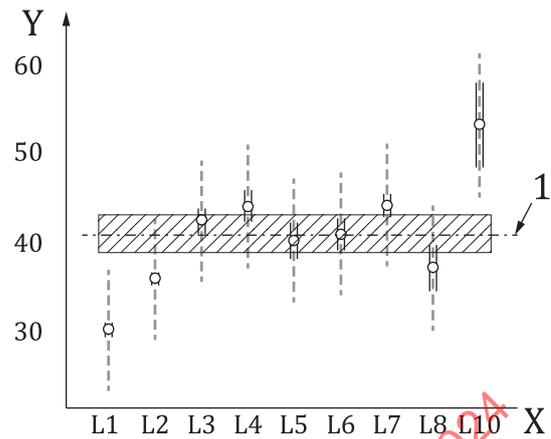
- X participating laboratory number
- Y measured percentage of crystallinity
- 1 ILC consensus value
- mean values for all replicates for each laboratory
- || laboratory standard deviation
- ▨ standard uncertainty
- - - dark uncertainty

SOURCE Reference [28], reproduced with the permission of the authors.

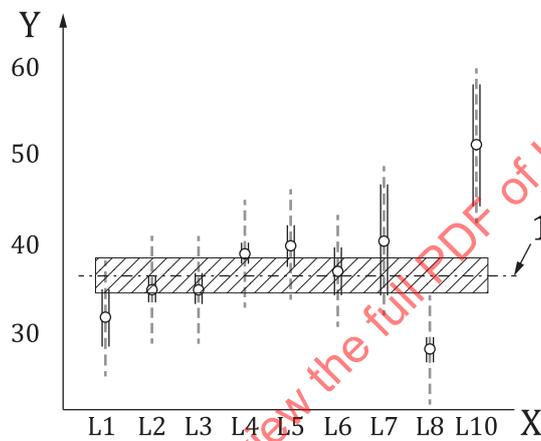
Figure A.4 — NIST Consensus Builder plots of percentage of crystallinity for CNC, CNF and iCNF



a) CNC consensus plot



b) CNF consensus plot



c) iCNF consensus plot

Key

- X participating laboratory number
- Y measured crystallite size (Å)
- 1 ILC consensus value
- mean values for all replicates for each laboratory
- || laboratory standard deviation
- ▨ standard uncertainty
- ⋯ dark uncertainty

SOURCE Reference [28], reproduced with the permission of the authors.

Figure A.5 — NIST Consensus Builder plots for crystallite size (Å) for CNC, CNF and iCNF

Table A.1 provides a summary of final consensus values and expanded uncertainty (based on the 95 % confidence interval) for the three CNM samples. Data were estimated with the NIST consensus builder using the Bayes-Laplace model.

Table A.1 — Summary of results

Sample	Percentage of crystallinity		Crystallite size, Å	
	Consensus value	Expanded uncertainty	Consensus value	Expanded uncertainty
CNC	75,1	6,4	48,6	5,1
CNF	59,6	7,9	40,8	3,9
iCNF	62,6	5,4	30,5	2,8

Despite the differences between laboratories, the individual standard deviations for each laboratory (see [Figure A.4](#) and [A.5](#)) are smaller than the expanded uncertainty for the overall ILC. This indicates that it is possible to achieve a lower standard deviation within a specific laboratory when the same modelling procedure is used, and to more readily make comparisons between samples prepared using different methods. Nevertheless, one of the most important findings of this study is a clear demonstration of the level of agreement and variability that can be expected between laboratories if different software and slightly different procedures are used for the Rietveld analysis. The observed variability between laboratories is particularly important given the relatively large number of literature reports that attempt to draw conclusions based on small differences in measured crystallinity. Despite the fact that Rietveld modelling can be expected to yield results with significant (e.g. 10 %) variation in reproducibility across laboratories, the method is based on consideration of the total signals associated with crystalline and amorphous components and is therefore an improvement over the Segal method which considers only two intensity measurements at specific 2θ values to determine crystallinity.^[4] The addition of a sample equilibration step at prescribed values for relative humidity, temperature and time can be useful to reduce variability between laboratories.

A.4 Re-analysis of CNC data by one analyst

In order to better understand the variability between laboratories, one CNC data set for each laboratory was re-analysed by a single analyst. The first CNC replicate was selected for each laboratory, independent of any assessment of the final fitted results. The data were used as received with subtraction of a blank scan (where available) before modelling. A constant fitting range (10° to 50°) was used except for cases where the raw data did not cover the entire range. The crystalline component was modelled using the Cellulose I β CIF provided with the ILC protocol^[8] and all lattice parameters were allowed to vary. The amorphous component was modelled using the Cellulose II CIF with fixed crystal size (12 Å) and crystal lattice parameters.^[10] All data sets were fitted with MAUD with a polynomial background (linear or quadratic). Initial tests compared results for isotropic and anisotropic crystal sizes and, since the differences were small, an isotropic crystal size was used for all data sets. Initial tests also determined that the fit did not improve beyond 10 or 11 free variables, so the number of variables was kept constant in subsequent fittings. Results for random and preferred orientations were compared using the March-Dollase 001 correction for the latter. This comparison was done for both linear and quadratic polynomial backgrounds. For each combination, the percentage of crystallinity was obtained both from the MAUD fits and by summing the areas under the curves for each component. The Rwp parameter was monitored as the number of free parameters varied to assess the fit.

The MAUD results for percentage of crystallinity are shown in [Table A.2](#). The variation in the number of parameters to fit the background suggests that at least a second degree polynomial is needed to correctly fit the data in most cases. This was more critical if the low-angle data included significant background contributions or if there was no data acquired below 10 degrees. Overall, the results indicate that correcting for preferred orientation did not significantly change the percentage of crystallinity for the CNC sample. Interestingly, the final values for the average percentage of crystallinity have a standard deviation that is more than a factor of two smaller than observed for the original data sets but similar to the expanded uncertainty determined from the NIST consensus builder method for estimating the ILC consensus value. The re-analysis of a single data set per laboratory is incompatible with the NIST consensus builder, but one can hypothesize that reanalysis of all data for a specific sample will lead to a reduced expanded uncertainty.