Certificate of Analysis

NRC·CNRC Certified Reference Material

CNCD-1

Cellulose Nanocrystal Powder Certified Reference Material

The following tables show those constituents for which certified, reference and information values have been established for this cellulose nanocrystal certified reference material (CRM).

The expanded uncertainty (U_{CRM}) in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM Guide [1] and k is the coverage factor. A coverage factor of two (2) was applied. It is intended that U_{CRM} accounts for every aspect that reasonably contributes to the uncertainty of the measurement.

Table 1: Certified value for mass fraction of elemental sulfur in CNCD-1

Mass fraction,[a,b] mg/kg
8720 ± 140[c]

[a] Measured by standard addition inductively-coupled plasma atomic emission spectroscopy (ICP-AES).
[b] Expressed on a dry mass basis.
[c] The expanded uncertainty of the weighted mean from 2 laboratory results was calculated by the BOB method [2].

Table 2: Reference value for massic amount of sulfate half ester in CNCD-1

Massic amount,[a,b] mmol/kg
253.6 ± 7.5[c,d]

[a] Measured by conductometric titration for CNCD-1 that has been dialysed and protonated with strong acid cation exchange resin.
[b] Expressed on a dry mass basis.
[c] The expanded uncertainty of equally weighted mean of 2 laboratory means calculated by BOB method [2].
[d] For comparison, the massic amount of sulfate half ester in CNCD-1 corresponds to an elemental sulfur mass fraction (as sulfate half ester) of 8132 ± 240 mg/kg.

Table 3: Reference values for CNCD-1 suspension from dynamic light scattering (DLS) measurements [a]

Quantity	Value
Z-average,[b] nm	70.0 ± 1.4[d]
polydispersity[c]	0.18

[a] A 0.5 mg/g suspension of CNCD-1 in 5 mM NaCl.
[b] Z-average is the intensity-weighted equivalent spherical hydrodynamic diameter obtained by cumulants analysis.
[c] Polydispersity is a dimensionless parameter determined by cumulants analysis of DLS measurements; information value.
[d] Expanded uncertainty of the mean calculated by unbalanced ANOVA.

Table 4: Reference values for CNCD-1 from atomic force microscopy measurements [a]

Quantity	Value, nm
Mean height	3.4 ± 0.8[b]
Width of height distribution	1.1 ± 0.4[b]

[a] Samples were prepared by spin coating a dilute CNC suspension on poly-lysine coated mica and were imaged dry; 5 independently prepared samples were imaged with analysis of approximately 300 individual CNCs/sample, excluding aggregates. The maximum particle height is reported.
[b] Expanded uncertainties of the equally weighted mean of 5 measurement means of height and width of height distribution were calculated with $k = 2.8$ and $k = 2.1$, respectively.

Table 5: Information values for CNCD-1 from microscopy measurements

Method	Quantity	Value, nm
AFM[a]	Mean length	76[b]
AFM	Width of length distribution	32[b]
TEM[c,d]	Mean length	87[e]
TEM	Width of length distribution	35[f]

[a] Samples were prepared by spin coating a dilute CNC suspension on poly-lysine coated mica and were imaged dry; 5 independently prepared samples were imaged with analysis of approximately 300 individual CNCs/sample, excluding aggregates. The maximum length along the long axis of the particle is reported.
[b] Standard deviation of sample means of 5 samples was 5 nm for both length and the width of the length distribution.
[c] Samples were prepared by depositing a dilute CNC suspension on a carbon-coated copper grid and staining with uranyl acetate. Data from two labs are reported (5 samples, 1909 particles; 3 samples, 600 particles). Only individual CNCs were analyzed.
[d] A mean width of 7.3 nm was obtained by TEM. The difference between the TEM width and AFM height may be due to lateral aggregation of particles.
[e] Mean of 2 laboratory means: 82 and 92 nm with standard deviations of 5 and 19 nm, respectively.
[f] Mean of 2 laboratory means: 36 and 33 nm with standard deviations of 5 and 6 nm, respectively.

Table 6: Information value for zeta potential for CNCD-1 suspension [a]

Method	Value, mV
Electrophoretic mobility	–37[b]

[a] A 0.5 mg/g suspension of CNCD-1 in 5 mM NaCl.
[b] A standard deviation *s* = 2 mV was obtained for 22 measurements.

Table 7: Information values for crystalline fraction for CNCD-1

Method	Value
Solid state NMR[a]	0.59[b]
X-ray diffraction[c]	0.88[d]

[a] The crystalline fraction is measured by ^{13}C cross polarization magic angle spinning solid state NMR and is the ratio of the broad signal from disordered C4 to the total C4 signal (disordered + crystalline); note that surface glucose units are included in the disordered fraction [3].
[b] A standard deviation *s* = 0.01 was obtained for 5 measurements, each on a different sample.
[c] A thin film sample was prepared from a CNCD-1 suspension. The crystalline fraction is obtained as the ratio of the total intensity of crystalline regions divided by the sum of crystalline and amorphous regions in the X-ray diffraction spectrum, with deconvolution using Ruland-Rietveld analysis [4].
[d] A standard deviation *s* = 0.02 was obtained for 5 measurements, each on a different subsample.

Table 8: Information values obtained by thermogravimetric analysis for CNCD-1

Peak ID[a]	Temperature, °C[b]	Mass loss, g/(100 g)
1	88	1.8
2	296	52.7[c]
3	305	
4	350	23.2[d]
5	454	
6	734	2.5
Residual mass		19.8

[a] Samples were heated between 25 and 800 °C in an argon atmosphere. Data were obtained by fitting the thermograms to a 6 asymmetric peak model and are mean values for 5 samples.
[b] The temperatures correspond to the maxima of the deconvoluted asymmetric Fraser-Suzuki peaks of the differential thermogravimetric curves. Standard deviations (°C) for the temperatures are 1, 2, 2, 2, 7 and 1 for peaks 1-6.
[c] Mass loss for peaks 2 and 3.
[d] Mass loss for peaks 4 and 5.

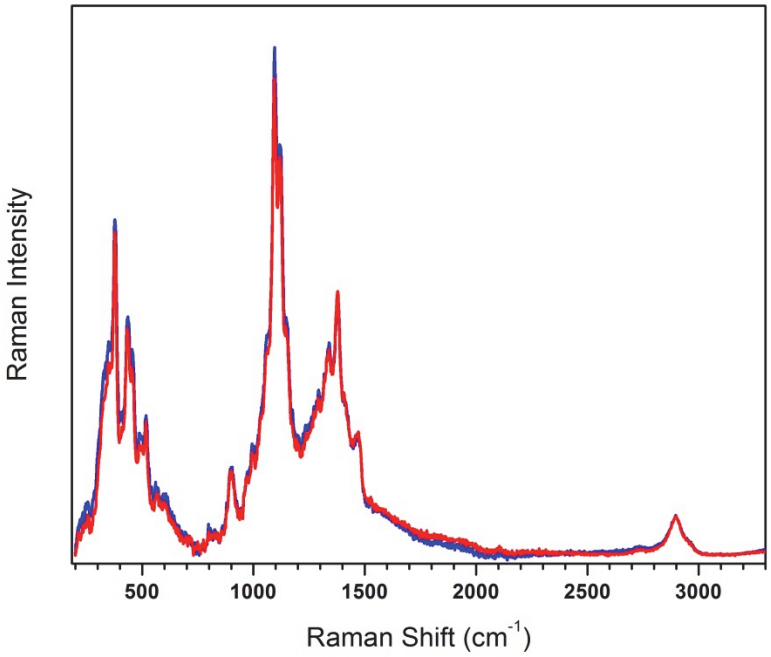

Figure 1. Raman spectrum for CNCD-1 measured with 785 nm excitation. Spectra for two samples are shown (red and blue) and each spectrum is the average from 3 different areas.

Certified values

Certified values are considered to be those for which the National Research Council Canada (NRC) has the highest confidence in accuracy and that all known and suspected sources of bias have been taken into account and are reflected in the stated expanded uncertainties. Certified values are the best estimate of the uncertainty and mean (Table 1).

Reference values

Reference values are non-certified values for which insufficient data are available to provide a comprehensive estimate of uncertainty to permit their full certification (Tables 2-4).

Information values

Information values are those for which insufficient data are available to provide any estimate of uncertainty (Tables 5-8, Figure 1).

Intended use

This certified reference material is primarily intended for use in the validation of procedures and the development of methods for the characterization of cellulose nanocrystals. It also serves as a stable test material to foster development and validation of international consensus-based standards for CNC. CNCD-1 has been gamma sterilized and is suitable for studies of environmental health and safety. A minimum sample mass of 250 mg is recommended for elemental sulfur, 50 mg for dispersion of CNCD-1 and 150 mg for the conductometric titration.

Storage and sampling

It is recommended that the material be stored in sealed containers at +4 °C. The bottle contents should be well mixed by rotation and shaking prior to use, and tightly closed immediately after use. Certified values are based on a minimum 250 mg sub-sample from the bottle. Once opened, the material should be placed in a desiccator until reused. Suspensions prepared by sonication of CNCD-1 may also be stored at +4 °C for up to 4 weeks and should not be frozen.

Instructions for drying

Determination of dry mass should be performed on a separate sample to avoid contamination. CNCD-1 can be dried to constant mass by storage in a desiccator over anhydrous magnesium perchlorate for 8 days. Alternatively, the material may be air dried in a 105 ^{o}C oven for at least 4 hours or until constant mass has been attained.

Preparation of material

This reference material was obtained as dry powder from Celluforce, Windsor, QC and was produced by sulfuric acid hydrolysis of softwood pulp, followed by neutralization and sodium exchange, purification and spray drying. The material was homogenized, sub-sampled in nominal 2 and 10 g amounts into pre-cleaned screw-capped glass bottles and gamma-irradiated to a minimum dose of 10 kGy at the Canadian Irradiation Centre (Laval, Quebec, Canada). Individual bottles were then packaged into heat sealed trilaminate foil bags.

Dispersion of CNCD-1 for size determination and zeta potential

Methods for size determination, zeta potential and sulfate half ester content require that the sample be dispersed in water. Dispersion of CNCD-1 was accomplished using a standard protocol that involves preparation of a 2 mg/g suspension that is sonicated to a total energy of 4850 J/g. The energy transfer efficiency of the sonicator was measured calorimetrically. For experiments requiring more dilute samples, the 2 mg/g suspension was diluted immediately prior to use to give final suspensions in either deionized water or 5 mM NaCl. Variation in sonication is included in the between bottles uncertainty for DLS. However, uncertainty due to the sonication energy transfer efficiency has not been included. Details on the dispersion and sonication procedure are available in the Characterization Method Report.

Stability

The short term stability of dry CNCD-1 and 2 mg/g suspensions was evaluated by dynamic light scattering. Long term stability was monitored by DLS and conductometric titration for 15 months for material stored at 5 °C. The long term stability of similar wood-pulp-derived CNC has been assessed for previous CNC reference materials (CNC-1 and CNCS-1) and in published studies. Uncertainty components for long and short term stability were considered negligible and are not included in the uncertainty budget.

Homogeneity

The homogeneity of the material was evaluated by dynamic light scattering of suspensions prepared using a standard protocol and by measuring total sulfur content by inductively coupled plasma atomic emission spectroscopy. The contribution to within and between bottle homogeneity is included in the expanded uncertainty for DLS and ICP-AES data.

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of established purity, CRMs, nano reference materials and international measurement intercomparisons. As such, CNCD-1 serves as a suitable reference material for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

Quality System (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented NRC Measurement Science and Standards (MSS) Quality System, which conforms with the requirements of ISO/IEC 17025 and ISO Guide 34.

The MSS Quality System supporting NRC calibration and measurement capabilities, as listed in the Bureau international des poids et mesures (BIPM) key comparison database (http://kcdb.bipm.org/), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM) and found to be in compliance with the expectations of the Comité international des poids et mesures (CIPM) Mutual Recognition Arrangement. The SIM certificate of approval is available upon request.

Updates

Users should ensure that the certificate they have is current. Our website at www.nrc.gc.ca/crm will contain any new information.

References

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Authorship

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CNCD-1

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This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our qualified vendors.

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