National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Reference Material

BNNT-1

BNNT-1 is a boron nitride nanotube reference material of known provenance as well as stable composition and morphology intended for development of applications and characterization, purification, and processing methods. Each hermetically sealed bottle contains minimum 100 mg of boron nitride nanotube powder sterilized by gamma irradiation.

Reference values

The mean nanotube diameter, x_h, the standard deviation of the diameter distribution, s_h, and the corresponding expanded uncertainties, $U_c = ku_c$, where k is the 95 % confidence level coverage factor and u_c is the combined standard uncertainty, are presented below rounded up to the first decimal place as reference values. The reference values are the best estimate of the true value where the sources of uncertainty may have not been fully investigated or evaluated. Nanotube diameter was measured by atomic force microscopy (AFM) as a height of isolated nanotubes dispersed on a substrate.

$x_h \pm U_{c,x(h)}$ (nm)	3.1±0.3
$s_h \pm U_{c,s(h)}$ (nm)	1.2±0.4

Informational values

Presented below as informational values are results of additional BNNT-1 characterization: the mean nanotube length and standard deviation of the length distribution, elemental composition with atomic fraction of five most abundant elements, and X-ray photoelectron, infrared attenuated total reflectance, and solid state nuclear magnetic resonance (ssNMR) representative spectra. Characterization results are reported as informational values when they may be of interest to the reference material users and either limited analyses were conducted or there is insufficient information to adequately evaluated measurement uncertainties.

Mean nanotube length, x_l, and standard deviation of length distribution, s_l, measured by AFM for isolated nanotubes dispersed on a substrate.

x_l (nm)	530
s_l (nm)	310

Elemental composition (normalized atomic fraction, x) rounded to the first decimal place, measured by X-ray photoelectron spectroscopy (XPS) for five most abundant elements.

	boron	nitrogen	oxygen	carbon	chlorine
x	42.5 %	40.7 %	12.8 %	3.8 %	0.3 %

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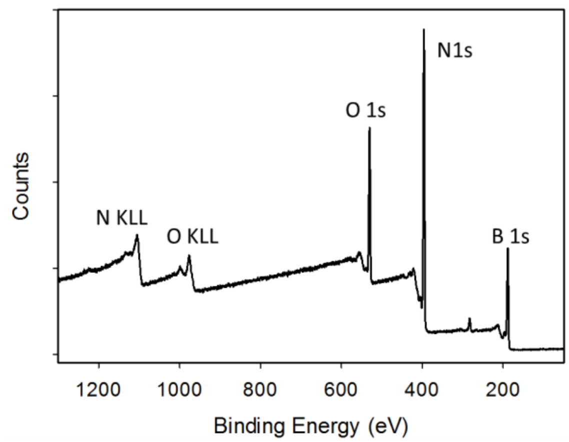

Figure 1: A representative X-ray photoelectron spectrum of BNNT-1.

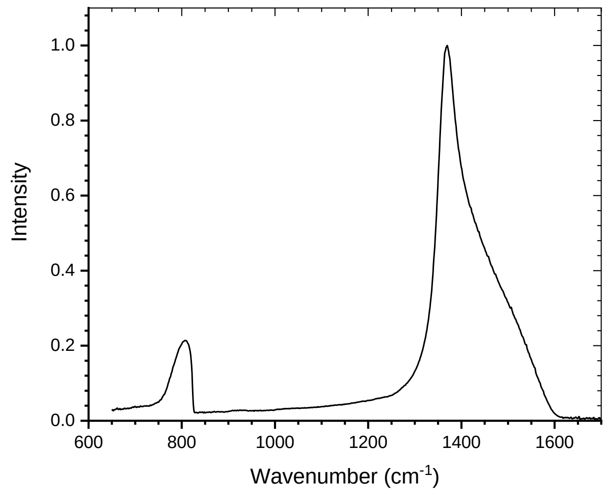

Figure 2: A representative attenuated total reflectance infrared spectrum of BNNT-1.

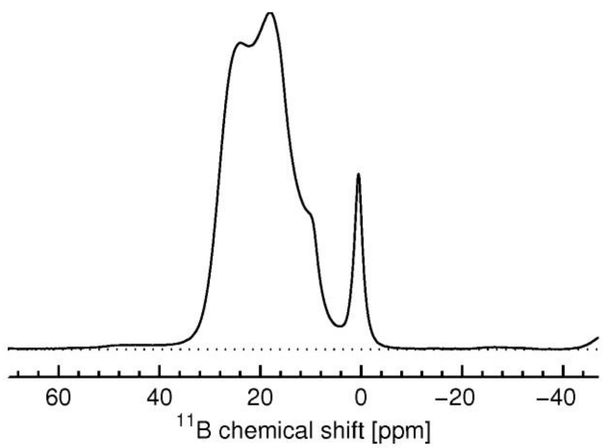

Figure 3: A representative ^{11}B solid state nuclear magnetic resonance spectrum of BNNT-1.

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Period of validity

If stored unopened at room temperatures, the BNNT-1 reference values will be valid for the period of one year from the date of sale.

Storage conditions

BNNT-1 is intended to be used as is. The reference material should be stored at room temperatures, (21±3) °C. Presently, there are no other storage restrictions or recommendations.

Use

A BNNT-1 bottle should be opened in a clean lab environment. Personal protection equipment should be used. Once opened, the bottle can be re-closed and the reference material can be used over extended period of time provided the material is not exposed to dust, chemicals, high humidity, or otherwise contaminated.

Safety Instructions

Standard safety precautions for handling nanostructured powder materials should be followed. Ensure adequate ventilation. Avoid inhalation of dust or contact with skin and eyes. Wear suitable protective clothing and respiratory track and eye protection.

There exists a limited and inconclusive research regarding cytotoxicity of BNNTs with controversial and sometimes contradictory results attributed to use of BNNT materials of varying purity and morphology. Availability of BNNT-1 is expected to facilitate comparative research on BNNT toxicity.

Recent study conducted with the post-processed BNNT-1 reference material showed size-dependent cytotoxicity, with longer tubes being more cytotoxic, as well as concentration and cell-line dependent cytotoxic effects (1).

Source and processing

Boron nitride nanotube raw material was synthesized at the National Research Council Canada (NRC) from hexagonal boron nitride (h-BN) powder by the hydrogen-assisted BNNT synthesis process (2, 3). As-produced material was chlorine treated at 750 °C to remove elemental boron impurities. The BNNT powder was subsequently homogenized with a powder grinder, bottled in a class 10000 clean room environment, and stored at room temperatures. Prior to use, the bottles were cleaned following the EPA protocol B. The sealed bottles containing BNNT reference material were sterilized with (10.8 to 14.4) kGy gamma radiation (Nordion Gamma Center of Excellence, Laval, QC). A total of 200 bottles, each containing minimum 100 mg of BNNT powder, were produced.

Homogeneity

Homogeneity of BNNT-1 was assessed by XPS elemental analysis with samples from ten randomly selected bottles, one sample per bottle, and three areas of interest per sample randomly selected for analysis. Normalized atomic fraction was determined for five most abundant elements (boron, nitrogen, oxygen, carbon, and chlorine) and analyzed by ANOVA for within bottle and between bottles effects. ANOVA showed that there was no between bottles effect at 0.05 level for boron, nitrogen, and carbon. However, a between bottles effect was detected for oxygen and chlorine fractions at 0.05 level. Sidak test indicated that atomic fraction means were significantly different for one pair of bottles for oxygen and two pairs of bottles for chlorine. At 0.01 level neither oxygen nor chlorine showed a bottle effect. Since the magnitudes of the between bottles effect are negligible in relation to the intended use of the reference material and in comparison with the method precision, we concluded that BNNT-1 bottles are homogeneous.

Additional homogeneity assessment was conducted by AFM analysis of nanotube diameter (measured as a height of nanotubes dispersed on a substrate) and length distributions. Three randomly selected and independently prepared samples were imaged. Of the total of 907 tubes analyzed, 306, 304, and 297 were from the samples 1, 2, and 3, respectively. The three diameter and length distributions were analyzed by ANOVA. The mean values and variances were found not significantly different at the 0.05 level.

Stability

Boron nitride nanotubes in the BNNT-1 material are considered morphologically and chemically stable when stored at room temperature in the original vial. Small diameter, few wall BNNT were previously found to be thermally stable and resistant to oxygen up to at least 800 °C (4, 5). However, boron-containing and other impurities may potentially evolve with time. Chemical stability monitored by XPS has been initiated and will be continued. The preliminary 6 month interval stability study indicated no instability for boron, nitrogen, and chlorine. However, linear fit shows that oxygen and carbon fractional contents may not be stable, as the slopes are significantly different than zero at the 0.05 level.

Value assignment and uncertainty

The BNNT mean diameter and length value and corresponding standard deviation of distributions were assigned as the average values of the measurement results for the three samples independently analyzed by AFM. The combined uncertainties of the mean diameter and the standard deviation of the diameter distribution were obtained via multiplying standard uncertainties by coverage factors calculated as a *t* multiplier corresponding to v_{eff} (effective degrees of freedom) at the 95% confidence level. Standard uncertainties were calculated by combining all reasonable sources of error related to the measurement process and data analysis.

The normalized atomic fraction for each of the five elements (B, N, O, C, Cl) was assigned as an average of two rounds of measurements, one including 10 randomly selected units and the other 5 units.

Metrological Traceability

Mean diameter and standard deviation of diameter distribution are traceable to the SI definition of meter through a chain of step height standards.

Quality System (ISO/IEC 17025, ISO 17034)

This material was produced in compliance with the documented National Research Council Canada (NRC) Metrology Quality System, which conforms to the requirements of ISO/IEC 17025 and ISO 17034.

The Metrology Quality System supporting NRC calibration and measurement capabilities 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.

Analytical Methods

The reference value for nanotube mean diameter and standard deviation of diameter distribution were determined by AFM. The nanotube diameter was measured as the height of nanotubes dispersed on a substrate. Indicative values and properties assigned to BNNT-1 reference material were determined for nanotube length by AFM and elemental composition by XPS. In addition, typical attenuated total reflectance infrared spectrum (Figure 2) and ssNMR (Figure 3) have been reported. All measurements were conducted at the National Research Council Canada.

For height and length distribution measurement by AFM, BNNT-1 was processed as follows. The as-bottled material was repeatedly washed with acetone, redispersed in 0.01 wt% PEI (MW 750 kD) at 0.5 mg/mL in 18.2 MΩ cm deionized water, and further diluted with 18.2 MΩ cm deionized water to 0.2 mg/mL. Next, it was centrifuged for 1 h at 1500g to remove undispersed impurities. Finally, 75 μL of the supernatant was spin coated onto a freshly cleaved mica substrate (1). Three BNNT-1 samples were prepared independently and imaged with a NanoWizard II BioAFM (JPK Instruments, Berlin, Germany) and a MultiMode NanoScope V with a PeakForce QNM mode (Bruker Nano Surfaces Division, Santa Barbara, CA, USA). A total of 907 tubes were measured with 306, 304, and 297 counts for the samples 1, 2, and 3, respectively.

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This Certificate is only valid if the corresponding material was obtained directly from NRC or an Authorized Reseller.

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