National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 917b

D-Glucose (Dextrose)

This Standard Reference Material (SRM) is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for glucose determinations employed in clinical analysis, and for routine critical evaluation of the daily working standards used in these procedures. It is also intended for use as a saccharimetry standard in calibrating polarimetric systems. A unit of this SRM consists of one bottle containing 50 g of crystalline D-glucose.

Certified Purity and Uncertainty: The certified chemical purity values presented in Table 1 were determined by measuring the mass fractions of impurities, including water, other saccharides, and residue from ashing, summing the impurities, and subtracting this sum from 100 %. The mass fractions of α-D-glucopyranose and β-D-glucopyranose were determined from proton NMR and ^{13}C NMR measurements.

Reference Values and Uncertainties: Reference values for the specific optical rotation of SRM 917b at two wavelengths are provided in Table 2 in both milliradians and degrees. Analyses for value assignment were performed at NIST and at one collaborating laboratory, using the same method. Reference values for the mass fractions of water and total oligosaccharides are shown in Table 3. Reference values are provided for analytes or properties for which (1) results have not been confirmed by an independent analytical technique as required for certification, (2) the disagreement among the methods was greater than expected for certified values, and/or (3) analyses have not been performed at NIST.

Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of SRM Certification: The certification of this SRM is valid until **31 December 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the original technical measurements leading to certification were under the chairmanship of B. Coxon of the NIST Center for Analytical Chemistry. The direction and coordination of the technical activities concerning the revision of this certificate were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 06 January 2006
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

The analytical measurements leading to the original certification were performed in the NIST Center for Analytical Chemistry, Organic Analytical Research Division, by A. Cohen, B. Coxon, D.K. Hancock, S.A. Margolis, and L.T. Sniegoski.

Optical rotation measurements in 1999 were made using state-of-the-art polarimeters at JASCO, Inc., Easton, MD.

The statistical analysis of the optical rotation measurements made in 1999 was performed by N.F. Zhang of the NIST Statistical Engineering Division. The statistical analysis of the original NIST data was performed by R. Paule of the NIST National Measurement Laboratory.

NOTICE AND WARNING TO USERS

SRM 917b IS INTENDED FOR IN VITRO DIAGNOSTIC USE ONLY.

Storage: The SRM should be stored in its original bottle at temperatures between 20 °C and 25 °C. The bottle must be tightly re-capped after use and protected from heat, excessive moisture and direct sunlight. Refrigeration in a desiccator is recommended for prolonged storage. However, the bottle and contents should be allowed to warm to room temperature before opening.

INSTRUCTIONS FOR USE

Drying Instructions: For laboratory environments where the relative humidity is below 75 %, there are no special drying requirements before use. *For laboratory environments where the relative humidity is 75 % or above, the sample must be dried under vacuum at 60 °C for 24 hours before use.* The surface of the material absorbs a significant amount of moisture when exposed to a relative humidity of approximately 75 %. Because the certified purity is based on a moisture content of 0.17 %, any added moisture will lower the purity. NIST experience indicates that moisture gain is not a significant problem at a relative humidity of approximately 50 %.

Instructions for Preparation of Solutions for Optical Rotation: Samples should be weighed in air immediately after withdrawal from the bottle, which should be resealed tightly without delay. Transfer 10.0000 g (mass in air) of the SRM into a preweighed 50 mL volumetric flask, reweigh flask to verify complete transfer, dilute to approximately 50 mL with sterile water (0.20 μm filtered Milli-Q®[1] water), equilibrate at 20.00 °C ± 0.05 °C for at least 1 hour, and adjust to volume. *Prepared solutions must be allowed to stand overnight for equilibration of the α and β anomers before optical rotation measurements are made.* For very accurate measurements, the optical rotation measurements should be made soon after equilibration.

Instructions for Use as a Standard in Clinical Applications: A 1 % standard solution of glucose may be prepared by transferring 1.000 g (mass in air) of SRM 917b into a 100 mL volumetric flask, filling to approximately 100 mL with a 0.2 % benzoic acid solution (a preservative) and swirling to dissolve. Adjust to volume with the 0.2 % benzoic acid solution. The benzoic acid should be ACS Reagent grade. The final concentration of this solution contains 10 mg/mL of glucose.

SOURCE AND ANALYSIS[1]

Source: The D-glucose used for this SRM was obtained from Pfanstiehl Laboratories, Inc., of Waukegan, IL.

NIST Analysis for Oligosaccharides, Moisture, and Ash: The contents of α-D-glucopyranose and β-D-glucopyranose were determined from freshly prepared solutions of D-glucose in dimethylsulfoxide-d6 by ^{2}H NMR spectroscopy at 400 MHz and by ^{13}C NMR spectroscopy at 100.6 MHz. These methods revealed up to four isomers of D-glucose at various levels, two of which (furanose anomers), were formed on dissolution. No non-glucose impurities were detected. Moisture was determined by the Karl Fischer method. No impurities were revealed by thin-layer chromatography. Ash content was found to be below 0.02 %.

[1]Certain commercial equipment, instruments, or materials are identified in this certificate in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

The total oligosaccharide content of the material was measured by high performance liquid chromatography. The possible presence of another impurity was indicated by a very weak ultra-violet absorption maximum at approximately 280 nm. However, the intensity of this absorption was substantially less than that of SRM 917, which was used as a control material.

External Laboratory Measurements for Optical Rotation: Recent measurements made in 1999 for optical rotation were performed by JASCO, Inc., Easton, MD, in order to confirm the stability of the optical rotation values for this SRM. Two 10.00 g samples from each of three bottles of the SRM were precisely weighed into a pre-calibrated 50 mL volumetric flask and ultra pure water was added to a total volume of 50.00 mL. Optical rotation measurements were carried out after allowing the solutions to stand overnight at 20.0 °C. The precision of the Model P-1030 Polarimeter by JASCO was verified to have a relative error of 0.05 % or less by calibrating with SRM 17e Sucrose, and then comparing the result against International Commission for Uniform Methods of Sugar Analysis (ICUMSA) (1994) [1]. Optical rotation measurements were made in a 100.00 mm water jacketed cell at 546 nm (mercury lamp) and 589 nm (sodium lamp) at 20.0 °C ± 0.2 °C.

Original NIST Analysis for Optical Rotation: The original optical rotation measurements were performed initially at NIST. From each of five bottles of this SRM, a single 10.0000 g sample was weighed into a sterile, preweighed 50 mL volumetric flask, diluted to approximately 50 mL with sterile water (0.20 μm filtered Milli-Q® water), equilibrated at 20.00 °C ± 0.05 °C for at least 1 hour, adjusted to volume and weighed. Each solution was allowed to stand overnight for equilibration of the α and β anomers. The optical rotation measurements were made in a 100.00 mm water jacketed polarimetric cell at 20.00 °C ± 0.05 °C using a commercial polarimeter. Sequential measurements were made against air, the aqueous glucose solutions, and the internationally measured standard quartz control plate, QCP1727A. The mean quartz control readings were used to calibrate the polarimeter. Optical rotation measurements of the aqueous sucrose solutions were made at both 546 nm (mercury lamp) and 589 nm (sodium lamp).

Additional measurements at NIST were of the optical rotation in dimethylsulfoxide (DMSO). From each of four bottles of the SRM, a 5.000 g sample was taken and dissolved in DMSO up to a volume of 50.00 mL at 20.00 °C ± 0.05 °C. These solutions were prepared as quickly as possible and the optical rotation measured immediately at 20.00 °C ± 0.05 °C at 589.44 nm in a 100.00 mm water jacketed cell. Sequential measurements were also made against air, the dimethylsulfoxide-glucose solutions and the standard quartz control plate.

The (initial) optical rotation of the glucose in DMSO is useful in that it is a characterization of the crystalline material as is, before it has had a chance to interconvert to a mixture of other anomers and ring forms. This value reflects essentially the content of the major amount of alpha pyranose anomer in the crystalline material modified only very slightly by the small content of beta pyranose anomer. The optical rotation of the glucose in dry, pure DMSO changes only slowly with time and makes it very easy to extrapolate the values at $t > 0$ back to $t = 0$. The optical rotation of equilibrated solutions does not reflect the original composition of the crystalline material.

Table 1. Certified Values and Associated Uncertainties for D-Glucose

Constituent	Mass Fractions (%)
Purity[(a)]	99.7 ± 0.2
α-D-Glucopyranose[(b)]	96.7 ± 0.2
β-D-Glucopyranose[(b)]	3.0 ± 0.2

[(a)] The purity of SRM 917b, as total D-glucose, is based on allowance for the measured moisture and total oligosaccharide contents, and on the absence of impurities detectable by other methods. The uncertainty for purity is based on scientific judgment and is meant to approximate two standard deviations of the certified value.

[(b)] The uncertainties for the two glucopyranose compounds are two standard deviations of the certified values, and include a contribution for any observed sample variability.

Table 2. Reference Values and Associated
Uncertainties for the Specific Optical Rotation at 20.00 °C [a]

Wavelength, λ	$[\alpha]_{\lambda}^{20}$, Specific Optical Rotation	
In vacuo, nm	mrad	degrees
546.23	1097.92 ± 0.24[b,c]	62.906 ± 0.014[b,c]
589.44	929.25 ± 0.21[b,c]	53.242 ± 0.012[b,c]
589.44	1943.2 ± 2.3[d,e]	111.34 ± 0.13[d,e]

[a] The specific optical rotation measurements were made without spiking with ammonia.

[b] The reference value is the weighted mean of the means of results obtained at NIST and JASCO, Inc., with the weights being the number of observations. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO and NIST Guides [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined uncertainty due to material variability and measurement uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the 14 degrees of freedom and 95 % confidence.

[c] Measurements were made in water, in a 100.00 mm cell, and are at a concentration of 10.000 g of SRM 917b in 50 mL.

[d] Measurements were made in dimethylsulfoxide, in a 100.00 mm cell, at a concentration of 5.000 g of SRM 917b in 50 mL.

[e] The reference value is the mean of results obtained at NIST. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO and NIST Guides [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined uncertainty due to material variability and measurement uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the 3 degrees of freedom and 95 % confidence.

Table 3. Reference Values for Moisture and Total Oligosaccharide [a]

Constituent	Mass Fraction (%)
Moisture	0.17 ± 0.02
Total Oligosaccharide	0.16 ± 0.02

[a] The uncertainties are two standard deviations of the mean values, and include a contribution for any observed sample variability except for the total oligosaccharide. The listed uncertainty for the total oligosaccharide content is based on scientific judgment and is meant to approximate two standard deviations of the mean value.

REFERENCES

[1] *International Commission for Uniform Methods of Sugar Analysis (ICUMSA) Methods Book*, *The 100 °Z Point of the International Sugar Scale*, Vol. 3; Conley, Norwich, England (1994).

[2] ISO; *Guide to the Expression of Uncertainty in Measurement*, ISBN 72-67-10188-9, lst ed., International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*, NIST Technical Note 1297; U.S. Government Printing Office, Washington, DC (1994); available at http://physics.nist.gov/Pubs.

Certificate Revision History: 06 January 2006 (This technical revision reports a change in the expiration date); 28 February 2002 (This certificate revision reflects clarification of the 'Instructions for Preparation of Solutions for Optical Rotation'.); 19 July 2001 (This revision reflects a correction of the concentration of benzoic acid used as a preservative in solutions for clinical applications from 2 % to 0.2 %.); 25 October 2000 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.