National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2910a

Calcium Hydroxyapatite

This Standard Reference Material is intended primarily for use in evaluating the physical and chemical properties of calcium apatites of biological, geological, and synthetic origin. The Ca/P molar ratio for SRM 2910a is consistent with the theoretical Ca/P molar ratio of 1.667 for calcium hydroxyapatite with a compositional formula of $Ca_{10}(PO_4)_6(OH)_2$. A unit of SRM 2910a consists of a 2 g bottle of Hydroxyapatite.

Certified Values: This SRM is certified for the mass fractions of calcium and phosphorus, and the Ca/P molar ratio [1,2]. Calcium and phosphorus were determined by colorimetry and inductively coupled plasma atomic emission spectrometry (ICP-AES). Material homogeneity was assessed for each of the certified values by means of analysis of variance (ANOVA) [3]. Uncertainties were assessed by use of the CIPM approach. The certified and reference values are expressed as mean ± expanded uncertainty with an approximate confidence level of 95 % [4]. Note: The certified values for the mass fractions of Ca and P are given as percentages.

Reference Values: Additional X-ray diffraction measurements [1,2] and data were obtained to further characterize the material and are provided as reference values.

Expiration of Certification: The certification of SRM 2910a is valid, within the measurement uncertainty specified, until **01 January 2018**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Use"). This certification is nullified if the SRM is damaged, contaminated or otherwise modified.

Maintenance of SRM Certification: NIST will monitor representative samples from the SRM lot over the period of its certification. If substantive 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 SRM was prepared and analyzed by M. Markovic of the Paffenbarger Research Center, American Dental Association Foundation, assigned to the NIST Polymer Division.

Statistical analysis was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

The Inductively Coupled Plasma Atomic Emission Spectroscopy analysis was performed by S.A. Wilson and Dr. P.H. Briggs of the U.S. Geological Survey, Reston, Virginia.

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

Eric K. Lin, Chief
Polymers Division

Gaithersburg, MD 20899
Certificate Issue Date: 17 July 2008

Robert L. Watters, Jr. Chief
Measurement Services Division

NOTICE AND WARNING TO USERS

Source of Material: Calcium hydroxyapatite-standard reference material (SRM 2910a) was synthesized by solution reaction of calcium hydroxide and phosphoric acid in accordance with the preparation of McDowell et al. [5]. In brief, about 5 L of distilled water were boiled for 60 min in a 7.5 L pot equipped with an electric stirring paddle, a reflux condenser with a CO_2-absorbing NaOH trap to protect from atmospheric CO_2, and ports for introducing titrant and nitrogen gas. Calcium oxide (prepared from calcium carbonate heated for 3 h at 1100 °C) was added to the water. Phosphoric acid (concentration 2 mol/L) was added to the calcium oxide/calcium hydroxide slurry at a rate of 0.3 mL/min to 0.6 mL/min and to a final Ca/P molar ratio of 1.67. The reacting mixture was boiled for 2 d. The precipitated solid phase was allowed to settle, the supernatant decanted, and an equal volume of boiled distilled water was added. This suspension was boiled for another 2 d. These washing and boiling procedures were repeated four times until the pH of the supernatant was ≈ 6; at pH 6, any possible traces of anhydrous dicalcium hydrogenphosphate are converted into calcium hydroxyapatite. The precipitate was dried at 105 °C in a nitrogen atmosphere for 5 d.

Handling and Storage: This SRM should be stored in its original container with the cap tightly closed under normal laboratory conditions of temperature and humidity.

INSTRUCTIONS FOR USE

SRM 2910a can be used without any pretreatment when stored according to the above (see "Handling and Storage") procedures.

CERTIFIED VALUES

Calcium Content: Calcium was determined in two laboratories by (a) colorimetry as the arsenazo complex [6] and (b) inductively coupled plasma atomic emission spectroscopy [7]. Twelve replicate measurements were performed by each method. The certified mass fraction, based on results from two different laboratories using different analytical methods, is:

$$38.89\ \% \pm 0.85\ \%$$

The expanded uncertainty of the Ca mass fraction was computed using the formula $U = k \bullet u_c$, where $u_c = 0.33997$ % is the combined standard uncertainty of the certified value with $\nu = 5.41$ effective degrees of freedom and $k = 2.5131$ is the coverage factor used to obtain an approximate confidence level of 95 % [4].

Phosphorus Content: Phosphorus was determined in two laboratories by (a) colorimetry as the phosphovanadomolybdate complex [8,9] and (b) inductively coupled plasma atomic emission spectroscopy [7]. Twelve replicate measurements were performed by each method. The certified mass fraction, based on results from two different laboratories using different analytical methods, is:

$$18.029\ \% \pm 0.071\ \%$$

The expanded uncertainty of the P mass fraction was computed using the formula $U = k \bullet u_c$, where $u_c = 0.03356$ % is the combined standard uncertainty of the certified value with $\nu = 17.49$ effective degrees of freedom and $k = 2.1053$ is the coverage factor used to obtain an approximate confidence level of 95 % [4].

Ca/P Molar Ratio: The certified calcium to phosphorus molar ratio based on the determinations of calcium and phosphorus is:

$$1.667 \pm 0.037$$

The expanded uncertainty of the Ca/P molar ratio was computed using the formula $U = k \bullet u_c$, where $u_c = 0.014900$ is the combined standard uncertainty of the certified value with $\nu = 5.91$ effective degrees of freedom and $k = 2.4562$ is the coverage factor used to obtain an approximate confidence level of 95 % [4].

REFERENCE VALUES

X-Ray Powder Diffraction:[1] The X-ray diffraction (XRD) patterns of the powdered SRM samples were obtained in the range of 3° 2θ to 60° 2θ with a Rigaku DMAX 2200 diffractometer operating at 40 kV and 40 mA, producing graphite-monochromatized CuKα radiation with wavelength $\lambda = 0.15405945$ nm. For determination of diffraction line positions (2θ-values and $d_{2\theta}$ values), six samples were prepared by mixing the SRM with pre-ground silicon (Silicon Powder 2θ/d-Spacing Standard, NIST SRM 640b) that served as an internal standard to correct 2θ-values of the SRM. The samples contained mass fractions of 80 % SRM and 20 % silicon. Two separate scans with the speeds of 0.02 2θ/min, 0.04 2θ/min or 0.06 2θ/min were obtained for each sample. For each scan, the position of each SRM and silicon diffraction line was determined with MDI JADE 6.1 as the average of four measurements using pseudo-Voigt and Pearson-VII profile functions (two measurements for each profile function) [10]. The 2θ-values and $d_{2\theta}$ values and reported in Table 1.

The intensities, reported as relative intensities (I_{rel}) in Table 1 were determined for six SRM samples at scan speeds from 0.02 2θ/min to 0.5 2θ/min. The diffraction line heights relative to the most intense line normalized to the intensity of 100, were determined with the Materials Data, Inc., JADE 6.1 XRD Patterns Processing software (MDI JADE 6.1).

Crystallinity: For determination of sample induced diffraction line profile broadening, reported as Full Width Half Maximum (FWHM), the angular width at half the background subtracted height of at least four scans of the (200), (002) and (210) diffraction lines for each of six SRM samples were obtained by XRD at scan speeds of 0.02 2θ/min, 0.03 2θ/min or 0.06 2θ/min. For determination of diffraction line angular width at its half-height, the lines having hkl indices (200), (002) and (210) were recorded. The diffraction line angular width, B, at its half-height above background was measured with JADE 6.1 using pseudo-Voigt and Pearson-VII functions and expressed in ° 2θ. The angular width (B) was corrected for instrumental line broadening (b) caused by instrument imperfections [10]. A stoichiometric, highly crystalline monoclinic calcium hydroxyapatite prepared by solid-state thermal reaction [11] was used as a reference substance in determination of the value of b (the angular width at the half-height of diffraction lines). The b-values for highly crystalline calcium hydroxyapatite diffraction lines were determined for the same six lines as for SRM. The corrected value of the angular width (β) expressed in ° 2θ, was calculated from Warren's equation $\beta = (B^2 - b^2)^{1/2}$ [10]. The reciprocal of the β value ($1/\beta$) positively correlates to the crystallite size/perfection. The $1/\beta$ value for the (200) line denotes the size/strain along the a-axis perpendicular to b-c plane, the $1/\beta$ for the (002) denotes the size/strain along the c-axis perpendicular to a-b plane, and $1/\beta$ for (210) denotes the size/strain in the plane perpendicular to c-axis. The $1/\beta$ values for the (002), (200), and (210) diffraction lines are:

$$1/\beta(200) = 4.44\ (^\circ 2\theta)^{-1} \pm 0.15\ (^\circ 2\theta)^{-1}$$

$$1/\beta(002) = 7.75\ (^\circ 2\theta)^{-1} \pm 0.29\ (^\circ 2\theta)^{-1}$$

$$1/\beta(210) = 4.42\ (^\circ 2\theta)^{-1} \pm 0.11\ (^\circ 2\theta)^{-1}$$

[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.

Table 1. Reference Values of X-Ray Diffraction Measurements: 2θ-values and relative intensities (I_{rel}) observed from the XRD pattern of SRM, d-values determined from 2θ-values ($d_{2\theta}$), and corresponding indices (hkl).

2θ (°)	$d_{2\theta}$ (nm)	I_{rel}	Hkl
10.84	0.816	8	100
16.82	0.527	3	101
18.80	0.472	1	110
21.76	0.408	6	200
22.84	0.389	6	111
25.35	0.351	3	201
25.85	0.344	44	002
28.14	0.317	9	102
28.93	0.308	15	210
31.76	0.282	100	211
32.16	0.278	59	112
32.89	0.272	59	300
34.03	0.263	24	202
35.45	0.253	5	301
39.17	0.2298	6	212
39.80	0.2263	21	310
40.40	0.2231	2	221
41.96	0.2151	6	311
42.30	0.2135	1	302
43.83	0.2063	5	113
44.38	0.2040	2	400
45.28	0.2000	4	203
46.69	0.1944	28	222
48.07	0.1891	12	312
48.59	0.1872	3	320
49.45	0.1842	30	213
50.49	0.1806	13	321
51.27	0.1781	9	410
52.07	0.1755	11	402
53.14	0.1722	15	004
54.41	0.1685	1	104
55.87	0.1644	5	322
57.10	0.1612	3	313
58.01	0.1588	2	501
58.27	0.1582	2	412
58.75	0.1570	1	330
59.91	0.1543	4	420

Note: 2θ-values have expanded uncertainty (U) of ± 0.02° 2θ (n = 6).

REFERENCES

[1] Markovic, M.; Fowler, B.O.; Tung, M.S.; Lagergren, E.S.; *Composition and Solubility Product of a Synthetic Calcium Hydroxyapatite. Chemical and Thermal Determination of Ca/P Ratio and Statistical Analysis of Chemical and Solubility Data*, In: Mineral and Scale Formation, Proc. of ACS Symposium, Ed. Z. Amjad, Plenum, NY, pp. 271–282 (1995).

[2] Markovic, M.; Fowler, B.O.; Tung, M.; *Preparation and Comprehensive Characterization of a Calcium Hydroxyapatite reference Material Characterization of Calcium Hydroxyapatite Reference Material*, J. Res. Natl. Inst. Stand. Technol., Vol. 109; pp. 553–568 (2004).

[3] Montgomery, D.C.; *Design and Analysis of Experiments*, 2nd ed., John Wiley and Sons: NY (1976).

[4] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization: Geneva, Switzerland (1993).

[5] McDowell, H.; Gregory, T.M.; Brown, W.E.; *Solubility of $Ca_5(PO_4)_3OH$ in the System $Ca(OH)_2$-H_3PO_4-H_2O at 5, 15, 25 and 37 °C*, J. Res. Natl. Bur. Stand., Vol. 81A; pp. 273–281 (1977).

[6] Vogel, G.L.; Chow, L.C.; Brown, W.E.; *A Microanalytical Procedure for the Determinination of Calcium, Phosphate and Fluoride in Enamel Biopsy Samples*, Caries Res., Vol. 17; pp. 23–31 (1983).

[7] Taggart, J.E.; *Analytical Methods of Chemical Analysis of Geologic and Other Materials*, U.S. Geological Survey, U.S. Geological Survey Open-File Report 02-0223, Version 5.0 (2002).

[8] Gee, A.; Deitz, V.R.; *Determination of Phosphate by Differential Spectrophotometry*, Anal. Chem., Vol. 25; pp. 1320–1324 (1953).

[9] Markovic, M.; Fowler, B.O.; Brown, W.E.; *Octacalcium Phosphate Carboxylates. 2. Characterization and Structural Considerations*, Chem. Mater., Vol. 5; pp. 1406–1416 (1993).

[10] Klug, H.P.; Alexander, L.E.; *X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials*, 2nd ed., John Wiley and Sons, NY, pp. 618–708 (1974).

[11] Fowler, B.O.; *Infrared Studies of Apatites. II. Preparation of Normal and Isotopically Substituted Calcium, Strontium, and Barium Hydroxyapatites and Spectra-structure-composition Correlations*, Inorg. Chem., Vol. 13; pp. 207–214 (1974).

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 at http://www.nist.gov/srm.