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Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Materials USGS88 and USGS89

(Hydrogen, Carbon, Nitrogen, Oxygen, and Sulfur Isotopes in Collagen)

These reference materials (RMs) are intended for normalization of stable hydrogen (δ^2H), carbon (δ^{13}C), nitrogen (δ^{15}N), oxygen (δ^{18}O), and sulfur (δ^{34}S) isotope measurements of unknown collagen and similarly behaving hydrogen-, carbon-, nitrogen-, oxygen-, and sulfur-bearing substances. A unit consists of 0.5g in a 4-mL glass vial vacuum sealed in a plastic pouch. There is no limit on distribution. These RMs were prepared by A. Schimmelmann (Indiana University, Bloomington, Indiana). These RMs are not safe for human consumption and are strictly intended for laboratory use only.

Recommended values: Stable hydrogen and oxygen isotopic compositions are expressed herein as delta values [1] relative to VSMOW (Vienna Standard Mean Ocean Water) on scales normalized such that the δ^2H and δ^{18}O values of SLAP (Standard Light Antarctic Precipitation) are –428 ‰ and –55.5 ‰, respectively [2,3]. Stable carbon isotopic compositions are expressed herein as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the δ^{13}C values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [4]. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous [5]. On this scale, the $\delta^{15}N_{AIR\text{-}N_2}$ value of USGS32 KNO_3 may be assigned a value of +180 ‰ [6]. Stable sulfur isotopic compositions are expressed relative to Vienna Cañon Diablo troilite (VCDT) by assignment of δ^{34}S of IAEA-S-1 silver sulfide = –0.3 ‰ [7]. On this scale, the $\delta^{34}S_{VCDT}$ values of IAEA-S-2 silver sulfide and IAEA-S-3 silver sulfide are +22.62 ± 0.08 ‰ and −32.49 ± 0.08, respectively [8]. Expanded measurement uncertainties at the 95 % confidence level are provided, and the coverage factors, k, used were (i) $k = 4$ for $\delta^{13}C_{VPDB\text{-}LSVEC}$ measurements, $\delta^{15}N_{AIR\text{-}N_2}$, and $\delta^{34}S_{VCDT}$, (ii) $k = 6$ for $\delta^{18}O_{VSMOW\text{-}SLAP}$ measurements, and (iii) $k = 9$ for $\delta^{2}H_{VSMOW\text{-}SLAP}$ measurements [9]. The presence of exchangeable organic hydrogen and adsorbed H_2O in collagens causes significant variance depending on sample pretreatment and the type of measurement; the indicated $\delta^{2}H_{VSMOW\text{-}SLAP}$ values were determined for bulk hydrogen according to a specific double-equilibration procedure [10,11, 12]. Stable hydrogen-, carbon-, nitrogen-, oxygen-, and sulfur isotope-delta values of USGS88 marine collagen from wild-caught fish and USGS89 porcine collagen with combined expanded uncertainties are given below.

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Name	$\delta^{2}H_{VSMOW\text{-}SLAP}$ (‰)	$\delta^{13}C_{VPDB\text{-}LSVEC}$ (‰)	$\delta^{15}N_{AIR\text{-}N_2}$ (‰)	$\delta^{18}O_{VSMOW\text{-}SLAP}$ (‰)	$\delta^{34}S_{VCDT}$ (‰)	Data source
USGS88	+20.1 ± 6.3	–16.06 ± 0.07	+14.96 ± 0.14	+15.91 ± 0.44	+17.10 ± 0.44	[9]
USGS89	–43.7 ± 7.8	–18.13 ± 0.11	+6.25 ± 0.12	+8.37 ± 0.40	+3.86 ± 0.56	[9]

Technical coordination for these RMs was provided by Arndt Schimmelmann of Indiana University and Haiping Qi of the U.S. Geological Survey Reston Stable Isotope Laboratory (RSIL).

Information Values: Hydrogen-, carbon-, nitrogen-, oxygen-, and sulfur-mass fractions are provided as information values and are method specific. Collagens were predried in a vacuum oven at 40 °C for at least 5 h before determination of hydrogen-mass fractions. Note that drying collagens at higher temperature under reduced pressure will decrease the moisture content and affect elemental mass fractions. A chromium-packed reactor was used in these measurements. Uncertainties are standard deviations.

Reference	Element	Mass fraction	Data source
USGS88 marine collagen from wild-caught fish	hydrogen	0.0632 ± 0.0017 (n = 6)	[9]
	carbon	0.4409 ± 0.0071 (n = 15)	[9]
	nitrogen	0.1666 ± 0.0045 (n = 8)	[9]
	oxygen	0.2584 ± 0.0123 (n = 12)	[9]
	sulfur	0.0046 ± 0.0002 (n = 12)	[9]
USGS89 porcine collagen	hydrogen	0.0617 ± 0.0001 (n = 6)	[9]
	carbon	0.4304 ± 0.0077 (n = 26)	[9]
	nitrogen	0.1578 ± 0.0019 (n = 6)	[9]
	oxygen	0.2516 ± 0.0123 (n = 11)	[9]
	sulfur	0.0028 ± 0.0001 (n = 12)	[9]

Expiration of Reference Values: The reference values for the isotopic compositions of USGS88 and USGS89 are valid until December 31, 2036, provided these RMs are stored in a freezer upon receipt and are handled in accordance with the instructions given in this Report of Stable Isotopic Composition (see “Instructions for Use”). A reference value is nullified if the RM is damaged, contaminated, or otherwise modified.

Source of the RMs: This information is taken from Schimmelmann and others [9]. Marine collagen powder (USGS88) from wild-caught fish was purchased in November 2018 and January 2019 as

unflavored powder from single lot #18129 distributed by Zen Principle via Amazon. The entire 5.44-kg amount of collagen powder was pooled in a single large polyethylene bag and homogenized by 5 minutes of vigorous tumbling prior to sealing of aliquots in multiple glass containers. Prior to sealing of glass flasks under vacuum, small aliquots of collagen from 10 flasks were selected for testing of homogeneity.

Seventy-three aliquots of 25 g each of porcine (pig) collagen powder (USGS89) from a previous European Framework 6 (TRACE) Integrated Project, which had been stored at −20 °C, were pooled in a common container and tumbled for 5 min vigorously for homogenization prior to sealing multiple aliquots in glass flasks under vacuum.

For long-term storage of more than 95 % of the supply in freezers at Indiana University and the USGS in Reston, Virginia, aliquots of the two collagen powders were stored in preannealed 100-, 250-, and 500-mL borosilicate round-bottom glass flasks. Filled glass flasks were individually attached to a vacuum line, each headspace was evacuated, and each glass flask was sealed under vacuum with a glass-blowing torch.

Maintenance of RM Report of Stable Isotopic Composition: The U.S. Geological Survey RSIL will monitor these RMs and will notify the purchaser if substantive technical changes occur that affect their isotopic compositions.

Distribution and Stability: A distribution unit is available in amounts of 0.5 g in a 4-mL glass vial, which is sealed in a plastic pouch. The shelf life of unopened glass vials of USGS88 and USGS89 stored in a freezer is five years.

Instructions for Use: USGS88 and USGS89 can be interspersed among every 10–15 unknowns. After opening any container of food matrix RMs, the remaining shelf life greatly depends on the handling and further storage of the material. These RMs should be stored in a freezer when not in use. Exposure to atmospheric oxygen decreases the shelf life of RMs.

Proteinaceous collagen powders offer large surface areas for adsorption of atmospheric moisture and contain isotopically exchangeable organic hydrogen linked to oxygen and nitrogen [9]. Bulk δ^2H and δ^{18}O values will depend on (i) the isotopic composition of the H_2O that was last in contact with the substrates, (ii) the time and temperature of the exchange (or equilibration), and (iii) the thoroughness and mode of drying prior to analysis.

Reproducible measurements of bulk δ^2H and δ^{18}O values of collagen powders make it necessary not only to control the amount of adsorbed moisture, but also to control its isotopic composition and to pre-equilibrate the isotopically exchangeable organic hydrogen in an isotopically reproducible fashion [9]. Numerous practical approaches have been published, yet their non-standardized choices of equilibration temperature, time, and drying conditions result in incompatible bulk δ^2H (and δ^{18}O) values [10,11,12,13,14,15,16]. Bulk δ^2H and δ^{18}O values of collagens reported in this study are meaningful only within the context of their applied analytical methodology reported here, and they represent information values, not consensus values [9].

Reporting of Stable-isotope-delta Values: The following recommendations are provided for reporting stable hydrogen-, carbon-, and oxygen-isotope data. It is recommended that:

- The δ^2H and δ^{18}O values of vegetable oils and similar hydrogen- and oxygen-bearing materials be expressed relative to VSMOW-SLAP on a scale where the δ^2H of SLAP = –428 ‰ exactly [3].

- The $\delta^{13}C$ values of all carbon-bearing substances be expressed relative to VPDB-LSVEC on a scale such that the $\delta^{13}C$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [4], even though LSVEC is no longer recommended as a RM for $\delta^{13}C$ measurement [17].
- The $\delta^{15}N$ values of all nitrogen-bearing substances be expressed relative to atmospheric nitrogen [5].
- The $\delta^{34}S$ values of all sulfur-bearing substances be expressed relative to VCDT on a scale such that the $\delta^{34}S$ value of IAEA-S-1 silver sulfide is –0.3 ‰ [7].
- Authors of new publications report delta values of international distributed (secondary) isotopic reference materials as though they had been interspersed among and used for normalization of unknowns, as appropriate for the measurement method. In this manner, measurement results can be adjusted in the future as analytical methods improve and consensus values of internationally distributed isotopic reference materials change.
- Reporting of delta values relative to PDB (Peedee belemnite) be discontinued [18].

REFERENCES

[1] Coplen, T. B., 2011, Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results: Rapid Communications in Mass Spectrometry, v. 25(17), p. 2538–2560. https://doi.org/10.1002/rcm.5129

[2] Gonfiantini, R., 1978, Standards for stable isotope measurements in natural compounds: Nature, v. 271, p. 534–536. https://doi.org/10.1038/271534a0

[3] Coplen, T. B., 1994, Reporting of stable hydrogen, carbon, and oxygen isotopic abundances: Pure and Applied Chemistry, v. 66(2), p. 273–276. https://doi.org/10.1351/pac199466020273

[4] Coplen, T. B., Brand, W. A., Gehre, M., Gröning, M., Meijer, H. A. J., Toman, B., and Verkouteren, R. M., 2006, New guidelines for $\delta^{13}C$ measurements: Analytical Chemistry, v. 78(7), p. 2439–2441. https://doi.org/10.1021/ac052027c

[5] Mariotti, A., 1983, Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements: Nature, v. 303, p. 685–687. https://www.nature.com/articles/303685a0

[6] Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M., Prohaska, T., 2014, Assessment of international reference materials for stable isotope ratio analysis 2013, Pure and Applied Chemistry, v. 86(3), p. 425-467. https://doi.org/10.1515/pac-2013-1023

[7] H. R. Krouse, T. B. Coplen, 1997, Reporting of relative sulfur isotope-ratio data: Pure and Applied Chemistry, v. 69(2), p. 293–295. https://doi.org/10.1351/pac199769020293

[8] Mann, J. L., Vocke, R. D., Jr., Kelly, W. R., 2009, Revised $\delta^{34}S$ reference values for IAEA sulfur isotope reference materials S-2 and S-3: Rapid Communication in Mass Spectrometry, v. 23(8), p. 1116−1124. https://doi.org/10.1002/rcm.3977

[9] Schimmelmann, A., Qi, H., Dunn, P. J. H., Camin, F., Bontempo, L., Potočnik, D., Ogrinc, N., Kelly, S., Carter, J. F., Abrahim, A., Reid, L. T., and Coplen, T. B., 2020, Food matrix reference materials for hydrogen, carbon, nitrogen, oxygen, and sulfur stable isotope-ratio measurements: Collagens, flours, honeys, and vegetable oils: Journal of Agricultural and Food Chemistry, v. 68(39), p. 10852–10864. https://doi.org/10.1021/acs.jafc.0c02610

[10] Qi, H., and Coplen, T. B., 2011, Investigation of preparation techniques for δ^2H analysis of keratin materials and a proposed analytical protocol: Rapid Communications in Mass Spectrometry, v. 25(15), p. 2209−2222. https://doi.org/10.1002/rcm.5095
[11] Qi, H., and Coplen, T. B., 2011, Erratum, Investigation of preparation techniques for δ^2H analysis of keratin materials and a proposed analytical protocol: Rapid Communications in Mass Spectrometry, v. 25(18), p. 2672−2672. https://doi.org/10.1002/rcm.5186
[12] Coplen, T. B., and Qi, H., 2013, Recognizing the potential pitfalls of hydrogen isotopic analysis of keratins with steam equilibration to infer origins of wildlife, food, and people: Rapid Communications in Mass Spectrometry, v. 27(22), p. 2569. https://doi.org/10.1002/rcm.6715
[13] Sauer, P. E., Schimmelmann, A., Sessions, A. L., and Topalov, K., 2009, Simplified batch equilibration for D/H determination of nonexchangeable hydrogen in solid organic material: Rapid Communications in Mass Spectrometry, v. 23, p. 949−956. https://doi.org/10.1002/rcm.3954
[14] Ruppenthal, M., Oelmann, Y., and Wilcke, W., 2013, Optimized demineralization technique for the measurement of stable isotope ratios of nonexchangeable H in soil organic matter: Environmental Science & Technology, v. 47(2), p. 949−957. https://doi.org/10.1021/es303448g
[15] Wassenaar, L. I., Hobson, K. A., and Sisti, L., 2015, An online temperature-controlled vacuum-equilibration preparation system for the measurement of δ^2H values of non-exchangeable-H and of δ^{18}O values in organic materials by isotope-ratio mass spectrometry: Rapid Communications in Mass Spectrometry, v. 29(5), p. 397−407. https://doi.org/10.1002/rcm.7118
[16] Oerter, E. J., Singleton, M., Thaw, M., and Davisson, M. L., 2019, Water vapor exposure chamber for constant humidity and hydrogen and oxygen stable isotope composition: Rapid Communications in Mass Spectrometry, v. 33(1), p. 89−96. https://doi.org/10.1002/rcm.8311
[17] Assonov, S., 2018, Summary and recommendations from the International Atomic Energy Agency Technical Meeting on the development of stable isotope reference products (21-25 November 2016): Rapid Communication in Mass Spectrometry, v. 32(10), p. 827−830. https://doi.org/10.1002/rcm.8102
[18] Coplen, T. B., 1995, Discontinuance of SMOW and PDB: Nature, v. 375, p. 285. https://doi.org/10.1038/375285a0