ANALYTICAL CHEMISTRY< Volume 74, Number 20, Pages 5410-5413

Comparison of $\delta^{18}O$ measurements in nitrate by different combustion techniques

Kinga Révész[*] *and John-Karl Böhlke*

U.S. Geological Survey. 431 National Center, Reston, Virginia 20192.

FAX: 703-648-5274. E-mail: Krevesz@usgs.gov,; JKbohlke@usgs.gov

Three different KNO3 salts with $\delta^{18}O$ values ranging from about –31 to + 54 per mill (‰) relative to VSMOW were used to compare three off-line, sealed-glass-tube-combustion methods (widely used for isotope studies) with a more recently developed on-line carbon-combustion technique. All methods yielded roughly similar isotope ratios for KNO3 samples with $\delta^{18}O$ values in the midpoint of the $\delta^{18}O$ scale near that of the nitrate reference material IAEA-NO-3 (around +21 to +25 ‰). This reference material has been used previously for one-point inter-laboratory and inter-technique calibrations. However, the isotope ratio scale factors by all of the off-line combustion techniques are compressed such that they are between 0.3 and 0.7 times that of the on-line combustion technique. The contraction of the $\delta^{18}O$ scale in the off-line preparations apparently is caused by O isotope exchange between the sample and the glass combustion tubes. These results reinforce the need for nitrate reference materials with $\delta^{18}O$ values far from that of atmospheric O2, to improve inter-laboratory comparability.

Since the paper of Rittenberg et al[1] was published in 1956, various techniques have been developed to analyze the oxygen isotope ratios of nitrate[2,3,4,5,6]. These techniques are used

---

[1] Rittenberg, D.; Ponticorvo, L., Appl. Radiat. Isotopes, 1956, 1, 208-214.

[2] Amberger, A.; Schmidt, H.L., Geochim. Cosmochim. Acta, 1987, 51, 2699-2705.

[3] Voerkelius, S. Ph.D. Thesis, Tech. Univ. Munchen, 1990.

[4] Silva, S.R.; Kendall, C.; Wilkison, D.H.; Ziegler, A.C.; Chang, C.C.; Avanzino, R.J., Journal of Hydrology, 2000, 228, 22-36.

extensively to study sources and fate of nitrate in hydrologic systems[7,8,9,10,11,12]. In general, the developers of these techniques used IAEA-NO-3 KNO_3 nitrogen isotope reference material or some other laboratory nitrate reagent for inter-laboratory and inter-technique calibration. All of these materials typically have oxygen isotope compositions close to that of atmospheric O_2: $\delta^{18}O = 23.8‰$[13,14 15]. The lack of international nitrate reference materials with a wide range of $\delta^{18}O$ values prevented more than one-point calibration. The proper calibration of the different techniques is essential so that oxygen isotope ratios of the same sample reported by different laboratories will be identical within analytical uncertainty. The purpose of this communication is to document some potential discrepancies among these techniques and the importance of the international distribution of reference materials with a wider range in oxygen isotopic composition than what is available now.

MATERIALS AND METHODS

In this experiment we used three different KNO_3 salts with greatly different $\delta^{18}O$ values. One of the three salts is a laboratory reagent (RSIL N-11), with a $\delta^{18}O$ value close to that of IAEA NO-3 and atmospheric O_2 [5]. The other two salts (N-34a and N-35a) have $\delta^{18}O$ values that are substantially lower and higher than that of IAEA-NO-3. In the experiment, we compared three off-line, sealed-glass-tube combustion methods[3,4,5] and one on-line carbon-combustion method[6]. We attempted to reproduce these methods as described in previous publications. The off-line methods are based on the reaction of KNO_3 or $AgNO_3$ with various C-bearing reducing reagents at different temperatures in sealed silica glass tubes. Varieties of glass tubing used in our experiment included high-purity "quartz" glass 99.995 % SiO_2 from two manufacturers (Hereaus

---

5 Révész, K.; Böhlke, J.K.; Yoshinari, T., Anal. Chem., 1997, 69, 4375-4380.

6 Kornexl, B.; Gehre, M.; Hoefling, R.; Werner, R.A., Rapid Communication in Mass Spectrometry, 1999,13, 1685-1693.

7 Böttcher, J.; Strebel, O.; Voerkelius, S.; Schmidt, H.L., J. Hydrol. 1990, 114, 4413-4424.

8 Durka, W.; Schulze, E.D,; Gebauer, G.;Voerkelius, S., Nature 1994, 372, 765-767.

9 Kendall, C;. Silva, S.R.; Chang, C.C.Y.; Burns, D.A.; Champbell, D.H.; Shanley, J.B., Extended Synopses International Symposium on Isotopes in water resources Manegement, , Vienna, Austria, 20-24. March, 1995; International Atomic Agency,: Vienna, 1995, 336.

10 Aravena, R; Evans, M.L.; Cherry, J.A., Ground Water, 1993, 31, 180-186.

11 Kendall, C. and Aravena, R. in Cook, P. G. and Herczeg, A. L., eds., Environmental Tracers in Subsurface Hydrology: Boston, Kluwer Academic Publishers, 2000, 261-297.

12 Böhlke, J.K.; Ericsen, G.E.; Révész, K., Chemical Geology, 1997, 136 135-152.

13 Kroopnick, P. and Craig, H., Science, 1972, 175, 54-55.

14 Coplen, T. B.; Hopple, J. A.; Böhlke, J. K.; Peiser, H. S.; Rieder, S. E.; Krouse, H. R.; Rosman, K. J. R.; Ding, T.; Vocke, R. D.Jr.; Révész, K. M.; Lamberty, A.; Taylor, P. and De Bièvre, P., U.S. Geological Survey Water-Resources Investigations Report 2002,01-4222.

15 Horibe, Y., Shigehara,K., and Takakuwa, Y., Journal of Geophysical Research, 1973, 78, number 15, 2625-2629.

Amersil; GE-214), "Vycor" 96% silica and Pyrex glass manufactured by Corning. The main combustion product is CO_2 that was analyzed for $\delta^{18}O$ by a DuPont double-collecting dual-inlet isotope-ratio mass spectrometer. The $\delta^{18}O$ values were calibrated with respect to Vienna Standard Mean Ocean Water [VSMOW] on a scale normalized to $\delta^{18}O$ of Standard Light Antarctic Precipitation [SLAP] = -55.5 ‰[16], and the oxygen isotope fractionation factor between water and CO_2 gas was taken to be 1.0412. The on-line method is based on the high-temperature reaction of KNO_3 in a glassy carbon reactor in a continuous He flow which was done by a ThermoQuest/Finnigan Thermal Conversion Elemental Analyzer (TC/EA) inlet system. The oxygen-bearing product was CO, which was cleaned by gas chromatograph and transferred in a He carrier gas to a Finnigan Delta Plus continuous-flow isotope-ratio mass spectrometer for $\delta^{18}O$ analysis. These analyses were calibrated by analyzing the sample KNO_3 against aliquots of IAEA-NO-3, which was assumed to have a $\delta^{18}O$ value of either +22.7 [5] or +25.3[6] ‰. In this case, no adjustments were made in the $\delta^{18}O$ scale factors.

Mercury cyanide off-line combustion method.[3] KNO_3 and $Hg(CN)_2$ [$KNO_3/Hg(CN)_2 = 3/4$ molar ratio] sufficient for 5 analyses were mixed and homogenized gently in an agate mortar. Aliquots containing 100 μmole of KNO_3 were weighed into 9-mm glass tubes, evacuated overnight at 100 °C, and sealed. The sealed tubes were baked at 560 °C for 6 h, then cooled by removing power from the oven, after which the temperature decreased by 60 °C in the first hour, 50 °C in the second hour, etc. to room temperature in approximately 24 hours. Gases from the combustion tubes were separated cryogenically; then the CO_2 gas was transferred by cryogenic distillation into a sample tube for $\delta^{18}O$ analysis.

Low temperature graphite off-line combustion method. [5] KNO_3 and catalyzed C sufficient for 5 analyses were mixed and homogenized in an agate mortar. The C/KNO_3 mole ratio was 4 and we used two different kinds of catalyzed carbon, containing approximately 1 weight percent of either Pd or Ni, respectively. Aliquots containing 100 μmole KNO_3 were weighed into 9-mm glass tubes, and a small slab of 0.0025 mm thick gold foil was added to each tube. The tubes were evacuated overnight at 100 °C, sealed, baked at 520 °C for 24 h, and then cooled slowly. Gases from the combustion tubes were separated cryogenically; the CO_2 gas was transferred by cryogenic distillation into a sample tube for $\delta^{18}O$ analysis. The remaining solid in the combustion tube (K_2CO_3) was reacted with phosphoric acid at 25 °C to release CO_2. The CO_2 yield was determined and the sample was analyzed for $\delta^{18}O$. The results for the CO_2 and K_2CO_3 were combined to obtain the $\delta^{18}O$ value of the original KNO_3 [5].

High temperature graphite off-line combustion method. [4] Because this technique requires $AgNO_3$, our KNO_3 samples were converted to $AgNO_3$ by AG MP-50 cation exchange resin in the Ag form. Amounts of $AgNO_3$ and pure spectrographic graphite (C) sufficient for 5 analyses were mixed and homogenized in an agate mortar. The $C/AgNO_3$ mole ratio was 4. Aliquots containing 100 μmole $AgNO_3$ were weighed into 9-mm glass tubes, which were evacuated

---

[16] Coplen, T.B., Chemical Geology, 1988,72, 293.

overnight, sealed, and heated in a programmable oven to 850 °C at its maximum rate, then cooled slowly. Gases from the combustion tubes were separated cryogenically, and the CO_2 gas was transferred into a sample tube for $\delta^{18}O$ analysis.

On line carbon combustion method.[6] KNO_3 samples were dried in a 90 °C oven overnight, then capped to prevent moisture contamination. Aliquots of 0.3 mg (about 3 μmole) were weighed into silver capsules and combusted in a ThermoQuest/Finnigan TC/EA unit. The samples were dropped into a reaction tube held at 1450 °C and a continuous He flow of 70 mL/min transported the product to a GC column (5Å molecular sieve, 80-100 mesh, 2 feet) held at 70 °C for separation of CO from other gases. From there, the reaction products, mainly N_2 and CO, were transferred to a Finnigan Delta Plus mass spectrometer via a ConfloII open- split interface for measurement of $\delta^{18}O$.

Sample size and silica additive experiments with low and high temperature graphite off-line combustion methods. Samples were prepared as described above, but aliquots containing 50 μmol KNO_3 or AgNO3 (rather than 100 μmole) were weighed into 9-mm quartz glass tubes. The set of aliquots was then divided into two subsets: one subset was finished as described above; the other subset received the addition of 20 mg quartz glass powder per aliquot, then was finished as described above. The quartz glass powder was prepared by crushing the same type of quartz glass tube (Hereaus Amersil) that was used for the sealed-tube combustions.

Pure CO_2 gas /quartz glass isotope exchange experiment. CO_2 gases with two different $\delta^{18}O$ values were introduced to two sets of 9 evacuated quartz glass tubes, around 10 μmole in each tube. The gas in each tube was frozen in the bottom by liquid N_2 while the tube was flame-sealed to avoid isotope fractionation during sealing. Three tubes were analyzed without further treatment, three after being baked at 520 °C for 24 hours and cooled slowly, and three after being heated to 850 °C and cooled slowly. This experiment was repeated with addition of reagents (C or catalyzed carbon) in the amounts that were used in the off line nitrate preparations.

RESULTS AND DISCUSSION

The results of experiments with nitrate (Tables 1 and 2, and Figure 1) indicate that the $\delta^{18}O$ values of the three nitrate samples covered a wide range from about –31 to + 54 ‰ with respect to VSMOW (assuming IAEA-NO-3 has $\delta^{18}O$ = +22.7 ‰[5]) when analyzed by the on–line carbon combustion technique (TC/EA). All methods yielded roughly similar results for the sample with $\delta^{18}O$ near the midpoint (sample N-11 in Table 1); therefore all techniques gave comparable data when only a single midpoint sample was used for calibration[3,4,5,6]. However, the results differ substantially for the samples with $\delta^{18}O$ values far from the midpoint. In every case, the apparent off-line $\delta^{18}O$ values for the endpoint samples are closer to the midpoint values than are the on-line $\delta^{18}O$ values. The relative $\delta^{18}O$ scale factor for each set of analyses is defined by the slope of a linear fit to the data: $\delta^{18}O_{\text{sealed tube combustion}} = m * \delta^{18}O_{\text{TC/EA}} + b$; where m is the slope (or scale factor) and b is the y intercept. The average $\delta^{18}O$ scale factors for the off-line sealed-tube combustion techniques are compressed such that they are between 0.33 and 0.68 times that of the on-line combustion technique, and the compression depends on the sample size and combustion temperature (Table 2). Consequently, the nitrate $\delta^{18}O$ values further from that of the midpoint value differ substantially from the TC/EA values and also have large uncertainties (Table 1; Figure 1). Because the on-line technique has the largest scale factor and is the only one that does not involve glass, we hypothesized, that an isotope exchange reaction between the product CO_2 and glass reaction tube could be mainly responsible for these discrepancies[17,18]. This hypothesis was tested in part by analyzing the same set of samples using half the amount of nitrate that was used before, and adding crushed quartz glass powder to enhance the effect of the possible oxygen exchange with the quartz glass. The isotope ratio scale is more compressed when 50 μmole nitrate was used than it is when 100 μmole of nitrate was used (Figure 1 and Tables 1 and 2). The SiO_2 additive enhanced the exchange reaction in the case of low temperature catalyzed carbon combustion so that the isotope ratio scale factor became even smaller (Table 2). The CO_2 exchange experiment yielded roughly similar, though somewhat subdued results: the CO_2 gas with $\delta^{18}O = 6‰$ (relatively close to the midpoint value) was changed only slightly by baking the tubes, whereas the CO_2 gas with $\delta^{18}O = 70$ ‰ was changed substantially, with a larger shift at 850 °C than at 520 °C. When this experiment was repeated with reagents in the tubes, the per mill scale factor changed from 0.8 to 0.5 (Figure 2 and Table 3) indicating that the oxygen exchange reaction between the CO_2 gas and SiO_2 was enhanced significantly. In contrast, the $\delta^{13}C$ of CO_2 gas was not altered significantly during the heating period. This indicates, that there was no carbon isotope exchange reaction between C reagent and CO_2 gas, and that the reagent carbon did not react with the glass oxygen to produce CO_2.

These experimental results indicate that the CO_2 produced from nitrate samples by off-line combustion reactions in glass tubes was altered by exchange of O with the glass. The mechanism of the exchange reaction cannot be determined from this series of experiments. Other studies indicate that isotope exchange can occur by diffusion of CO_2 molecules into the glass structure, followed by the exchange with the oxygen atoms between the CO_2 and the SiO_2[17]. The relatively small effect for pure CO_2 in comparison with the larger effects for nitrates and for CO_2 + reagents may indicate that the exchange is facilitated by the presence of the reagents (carbon) and the nitrate salts. The results indicate that the magnitude of the exchange effect is a function of the sample size, the SiO_2 surface area, and temperature, but there is no clear difference in the results from different types of glass tubing (Table 1). Because the deviations from the on-line results are symmetrical and generally disappear between about 10 and 20 ‰ (Figure 1), it may be inferred that the O components from the glass had $\delta^{18}O$ values lower than that of atmospheric O_2 and most nitrate chemical reagents. The increased uncertainty in case of samples far from the midpoint $\delta^{18}O$ value (20 ‰) indicates however, that the isotope exchange between the oxygen of CO_2 and the oxygen of SiO_2 is not in equilibrium, in agreement with published data[17].

It is possible that we have failed to reproduce exactly the procedures used in some laboratories, so the discrepancies illustrated here may not be typical. Nevertheless, we conclude that because of the potential for isotope exchange between CO_2 product and silica glass, nitrate

---

[17] Stolper, E. and Epstein, S. Stable Isotope Geochemistry: A Tribute to Samual Epstein, The Geochemical Society, Special Publication No. 3, 1991, Editors: H. P. Taylor, Jr., J.R. O'Neil and I. R. Kaplan.

[18] O'Neil, J.R.; Roe, L.J.; Reinhard, E.; Blake, R.E., Israel Journal of Earth-Sciences, 1994, 43, 203-212.

reference materials with widely varying $\delta^{18}O$ values are essential to allow laboratories to normalize their $\delta^{18}O$ scales. For this reason, large aliquots of nitrate salts similar to the ones used in this study have been prepared for distribution as O isotope reference materials [Böhlke et al., in prep.]. Future inter-laboratory comparisons using these reference materials will be useful for determining more quantitatively the magnitudes of the discrepancies among the techniques and reevaluate published data.

ACKNOWLEDGEMENTS

We thank Irene Hamblen, Stan Mroczkowski, and Janet Hannon for assistance in the laboratory, and Qi Haiping, Stan Mroczkowski, and Tyler B. Coplen for valuable discussions and manuscript review. Thanks also to the two anonymous reviewers of this Journal for valuable remarks, which helped improve this paper.

Received for review June 11, 2002. Accepted August 7, 2002. AC025854B

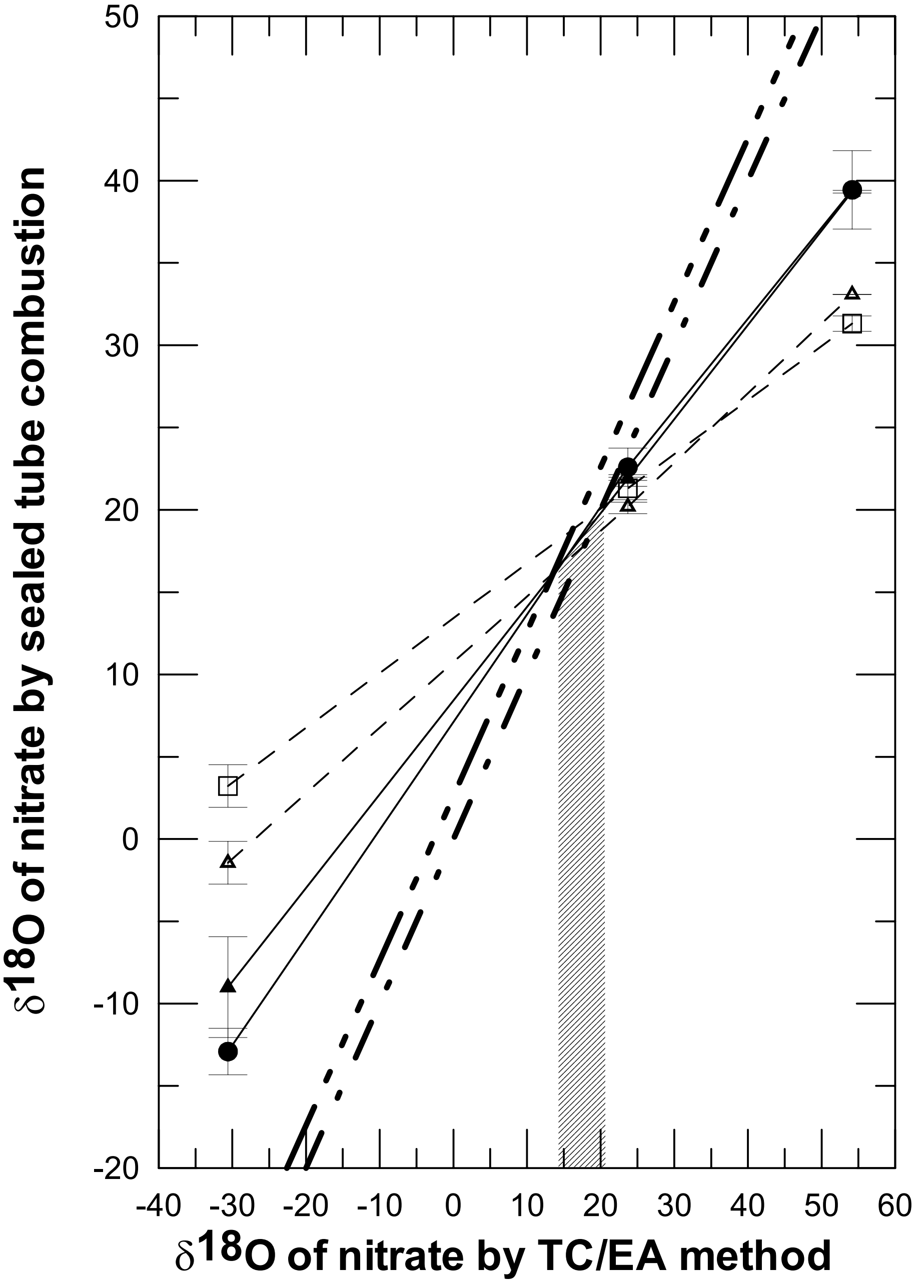

Figure 1: $\delta^{18}O$ values of nitrate samples prepared by different sealed-tube combustion techniques using different sample sizes, versus the $\delta^{18}O$ values derived from the on-line (TC/EA) combustion technique for the same sample set. $\delta^{18}O$ values are given relative to VSMOW. Error bars represent the standard deviation for the sealed-tube analyzes (typically n=3-6); standard deviations for TC/EA analyzes were ±0.2-0.3 **‰**. Dashed line with 3 dots represents analysis by TC/EA normalized to IAEA-NO-3=25.3 **‰**[6]; dashed line with one dot represents the analysis by TC/EA normalized to IAEA-NO-3=22.7**‰**[5]; squares represent high temperature carbon combustion [4], triangles represent low temperature catalyzed graphite combustion [5]; circles represent low temperature $Hg(CN)_2$ combustion[3]. Dashed lines represent 50 μmole sample sizes and solid lines are for 100 μmole sample sizes. Shaded area represents the estimated range of $\delta^{18}O$ of the glass exchange component.

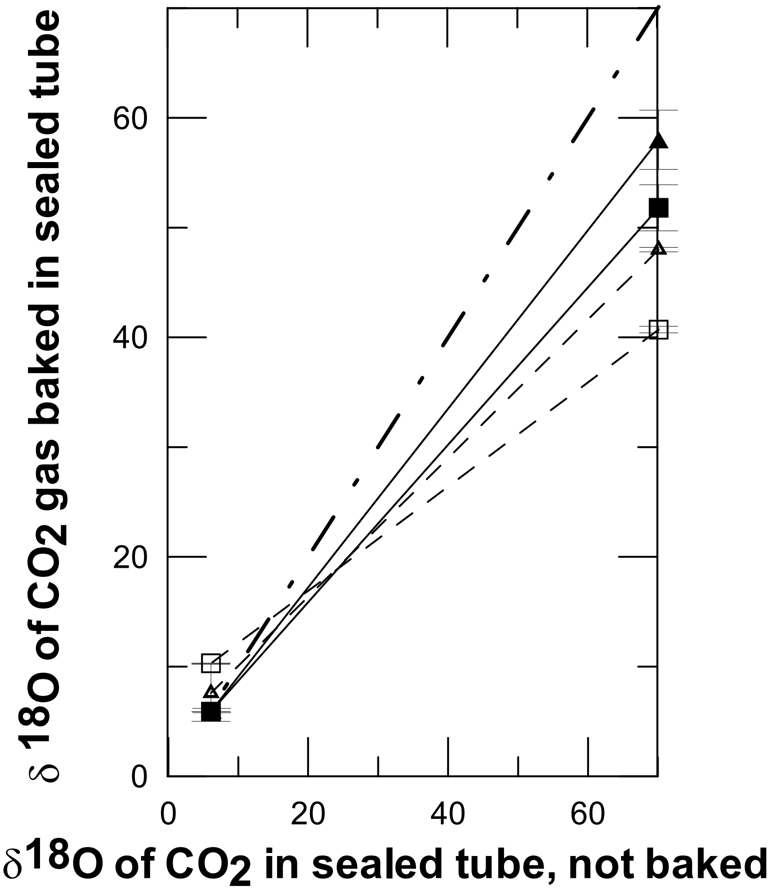

Figure 2. δ^{18}O values of CO$_2$ gases baked at temperatures and times corresponding to the different sealed-tube combustion techniques with or without reagents, versus the δ^{18}O values of original CO$_2$ gases. δ^{18}O values are given relative to VSMOW. Error bars represent the standard deviations for the baked samples (typically n=3), standard deviations for CO$_2$ samples not baked analyzes were ±0.2-‰. Dash dot line is the 1:1 line. Squares represent high temperature (850 °C) carbon combustion [4], triangles represent low temperature (520 °C) catalyzed graphite combustion [5]. Dashed lines and open symbols represent experiments with reagents (carbon or catalyzed carbon) in tubes.

Table 1. $\delta^{18}O$ values of three nitrates determined by using different combustion techniques

			$\delta^{18}O^{a}$	$\delta^{18}O^{a}$	$\delta^{18}O^{a}$	m*X+b
			N-34a	N-11	N-35a	
Type of Combustion:	**Type of glass**	**T oC**	**σ n=3**	**σ n=3**	**σ n=3**	
NiC	Quartz Hereaus	520	-5.3±2.7	22.0±0.3	36.9±0.4	0.50*X+10.0
PdC	Quartz Hereaus	520	-10.3±2.7	22.0±0.2	39.8±1.4	0.59*X+7.8
PdC	Quartz GE-214	520	lost	21.8±0.2	39.4±0.2	0.58*X+8.1
PdC	Pyrex	520	-16.6±2.8	22.7±0.6	40.4±0.9	0.69*X+4.8
C	Quartz Hereaus	850	-0.9±2.6	23.3±0.3	40.3±0.2	0.48*X+13.3
C	Quartz GE-214	850	-8.5±0.2	21.3±0.0	42.7±1.3	0.60*X+6.6
$Hg(CN)_2$	Quartz Hereaus	560	-12.9±3.4	22.6±1.0	39.4±0.8	0.62*X+6.6
$Hg(CN)_2$	Vycor	560	-14.9±2.8	21.0±0.2	42.8±0.7	0.67*X+5.6
CFIRMS	Glassy C	1450	-30.6^{b}±0.2	23.7^{b}±0.2	54.2^{b}±0.3	

a= The $\delta^{18}O$ values by sealed tube techniques are reported relative to Vienna Standard Mean Ocean Water [VSMOW] on a scale normalized to $\delta^{18}O$ of Standard Light Antarctic Precipitation [SLAP] = -55.5 **‰** [16].
b=$\delta^{18}O_{TC/EA}$calibrated to IAEA-NO-3 = 22.7 **‰**[5].

Table 2. Effects of varying sample size and SiO2 additive on the apparent values of δ^{18}O of nitrates.

Type of Combustion	Sample size μmole	T °C	δ^{18}O ‰ N-34a σ n=3	δ^{18}O ‰ N-11 σ n=3	δ^{18}O ‰ N-35a σ n=3	m*X+b
PdC	100	520	-10.3±2.7	22.0±0.2	39.8±1.4	0.57*X+9.4
PdC	50	520	-1.5±	20.2±0.4	33.1±1.3	0.41*X+14.9
PdC + SiO_2	50	520	-0.4±0.5	18.7±0.1	29.2±1.0	0.36+X+14.5
C	100	850	-0.9±2.6	23.3±0.3	40.3±0.2	0.53*X+12.1
C	50	850	3.2±1.3	21.3±0.8	31.3±0.5	0.33*X+14.0
C + SiO_2	50	850	5.1±0.5	24.3±0.5	44.1±0.9	0.45*X+18.2

a= The δ^{18}O values by sealed tube technique are reported relative to Vienna Standard Mean Ocean Water [VSMOW] on a scale normalized to δ^{18}O of Standard Light Antarctic Precipitation [SLAP] = -55.5 **‰** [16].

Table 3. Effect of baking, with or without reagents, on the apparent δ^{18}O values of CO_2 gas in quartz glass tubes.

T. °C	**δ^{18}O‰ heavy CO_2**	**δ^{18}O‰ light CO_2**	**m*X+b**	**δ^{13}C‰ heavy CO_2**	**δ^{13}C‰ light CO_2**
not baked	**70.1±0.2**	**6.2±0.2**		**-4.2**	**-40.9**
520	58.0±2.7	6.0±0.2	0.81*X+1.0	-4.2	-41.0
520 + PdC	48.0±0.2	7.6±2.1	0.65*X+2.1	-4.2	-40.8
850	51.8±2.1	5.9±0.0	0.71*X+1.5	no data	no data
850 + C	40.7±0.3	10.3±0.3	0.48*X+7.4	-3.2	-40.0

a= The δ^{18}O values are reported relative to Vienna Standard Mean Ocean Water [VSMOW] on a scale normalized to δ^{18}O of Standard Light Antarctic Precipitation [SLAP] = -55.5 **‰.**[16] **b**= The δ^{13}C values are reported relative to Vienna Peedee belemnite [VPDB], such that $\delta^{13}C_{NBS19/VPDB}$ = +1.95 **‰**[19]**.**

---

19 Hut, G., , Consultants' Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations, Vienna, 16 to 18 September 1985. Report to Director General, International Atomic Energy Agency, Vienna, 1987, 42 p.