Research Article

Rapid Communications in Mass Spectrometry

Received: 5 April 2012 Revised: 29 April 2012 Accepted: 17 May 2012 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2012**, *26*, 1776–1782
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6285

Elimination of nitrogen interference during online oxygen isotope analysis of nitrogen-doped organics using the "NiCat" nickel reduction system

William M. Hagopian* and A. Hope Jahren

Department of Geology and Geophysics, University of Hawaii, Honolulu, HI 96822, USA

RATIONALE: Accurate online analysis of the $\delta^{18}O$ values of nitrogen-bearing organic compounds is of interest to several emergent fields, including ecology, forensics and paleontology. During online analysis, high-temperature conversion (HTC) of nitrogen-bearing organics produces N_2 gas which creates isobaric interference with the isotopic measurement. Specifically, N_2 reacts with trace amounts of oxygen in the mass spectrometer source to form $^{14}N^{16}O$ (*m/z* 30), which prevents accurate evaluation of the sample $^{12}C^{18}O$ peak (*m/z* 30).

METHODS: We present an alternative system to the conventional HTC, which uses a nickel-catalyzed ("NiCat") reduction furnace to convert HTC-produced CO into CO_2, allowing for $\delta^{18}O$ measurement using signal intensities at *m/z* 44 and 46.

RESULTS: This system yields identical $\delta^{18}O$ values for nitrogen-doped and undoped sucrose and cellulose compounds up to molar yield ratios of N_2:CO = 0.22. In contrast, our conventional HTC system configured to factory recommendations with the stock gas chromatography (GC) column produced a discrepancy of ~5‰ between nitrogen-doped and undoped samples.

CONCLUSIONS: Because of its ability to eliminate isobaric interference, the NiCat system is a viable alternative to conventional HTC for $\delta^{18}O$ measurement, and can be constructed from relatively inexpensive and readily available materials. As an additional advantage, the CO_2 analyte produced by NiCat may be cryofocused, to allow for oxygen-isotope determinations on very small amounts of sample substrate. Copyright © 2012 John Wiley & Sons, Ltd.

The oxygen isotope analysis ($\delta^{18}O$ values) of organic substrates has been primarily applied to nitrogen-free compounds (e.g. sugars,[1,2] cellulose,[3–5] and oils[6]). In recent years, the application of stable isotope techniques to forensics and environmental research has led workers to determine the $\delta^{18}O$ value of several nitrogen-bearing organic compounds including hair,[7–9] phenylglucosazone,[10] and nitro-explosives.[11] Solid samples are traditionally analyzed for their $\delta^{18}O$ value using a High-Temperature Conversion (HTC) continuous flow system. Within the HTC system, samples are introduced to a highly reducing reactor (maintained at 1080–1450 °C) where oxygen within the substrate is converted into carbon monoxide (CO). After gas chromatography (GC) separation from other gaseous products (i.e., H_2 and N_2), CO gas flows to an Isotope Ratio Mass Spectrometry (IRMS) instrument where its $\delta^{18}O$ values are determined by measuring the signal intensities of the CO^+ ions at *m/z* 28 and 30 and calculating the isotopic ratio. HTC results in highly precise and accurate measurement of the $\delta^{18}O$ values of samples that do not contain nitrogen (~ ±0.2‰).[12] Nitrogen-bearing substrates have, however, been shown to be susceptible to isobaric interferences caused by the N_2 peak in the IRMS source. Reaction with trace amounts of oxygen forms $^{14}N^{16}O$ (*m/z* 30), giving rise to an elevated *m/z* 30 baseline prior to the sample $^{12}C^{18}O$ peak (*m/z* 30).[12–14] The interfering *m/z* 30 signal decreases over the course of the sample peak measurement, resulting in the removal of a portion of the sample *m/z* 30 peak when the area below the horizontal baseline determination is subtracted for isotope ratio calculations. The result is an under-representation of mass 30 contribution to the sample isotopic ratio, skewing the $\delta^{18}O$ measurement of the sample to lower values by as much as ~13.0‰.[14] Researchers employ two strategies to mitigate the interference of N_2 with the results of $\delta^{18}O$ analyses. The first involves optimization of N_2 and CO peak separation to prevent N_2 from reaching the source.[12,14–19] The second strategy relies upon modified baseline corrections to the raw data post-analysis.[12,17] Here we propose a fundamentally different approach to analyzing solid nitrogen-bearing organics. Instead of trying to minimize the effect of N_2 reaching the IRMS source, we convert the CO produced by HTC into CO_2 using a nickel-catalyzed ("NiCat") disproportionation. By measuring the signal intensities of the CO_2^+ ions at *m/z* 44 and 46, we eliminate the isobaric interference at *m/z* 30 that causes the complications described above.

We performed two experiments designed to test whether measuring $\delta^{18}O$ values using the NiCat system could produce the same precision and accuracy for nitrogen-bearing organic materials as conventional HTC provides for nitrogen-free

* *Correspondence to:* W. M. Hagopian, 1680 East–West Road, 701 POST Building, University of Hawaii, Honolulu, HI 96822, USA.
E-mail: william8@hawaii.edu

compounds. In the first experiment, the NiCat system was compared directly with the conventional HTC system for a suite of nitrogen-free reference materials (four celluloses: $C_6H_{10}O_5$; one sucrose: $C_{12}H_{22}O_{11}$; three benzoic acids: $C_7H_6O_2$), which represented a large range in $\delta^{18}O$ value (~17‰ to 71‰). Batch runs were structured identically for both systems, with blank capsules and appropriate reference materials included to normalize the data and to assess and correct for both drift and blank contributions. During the HTC step, all samples were decomposed under identical conditions. The only difference between the two analytical sequences was the conversion of CO into CO_2 for the NiCat measurements, and subsequent IRMS analysis using *m/z* 44 and 46 instead of *m/z* 28 and 30, as is the case for conventional HTC. Within our second experiment, we compared the results of NiCat analyses on nitrogen-doped oxygen-bearing compounds against undoped oxygen-bearing compounds. Specifically, we compared pure sucrose ('IAEA-CH6': $C_{12}H_{22}O_{11}$) and cellulose ('SigCell': $C_6H_{10}O_5$) reference materials with those that had been doped with three different nitrogen-bearing compounds (adenine: $C_5H_5N_5$; imidazole: $C_3H_4N_2$; and 2-aminopyrimidine: $C_4H_5N_3$). For this experiment, SigCell and IAEA-CH6 samples were weighed into 3.3 × 5 mm silver capsules with amounts tailored to obtain a yield of 32 µg O (±10%). The amount of doping compound was adjusted to obtain N:O molar ratios up to 3.3, to encompass the range of nitrogen-bearing organic compounds that are of interest for $\delta^{18}O$ analysis (e.g. nitro-organic explosives = 0.2 to 1.0, phenylglucosazone = 1.0, caffeine = 2.0). Undoped and doped samples were analyzed together and randomized within each analytical batch run. Finally, to confirm the interference effect of the N_2 peak within our conventional HTC system, the $\delta^{18}O$ values of a series of SigCell samples, doped with adenine, were measured using only our HTC system.

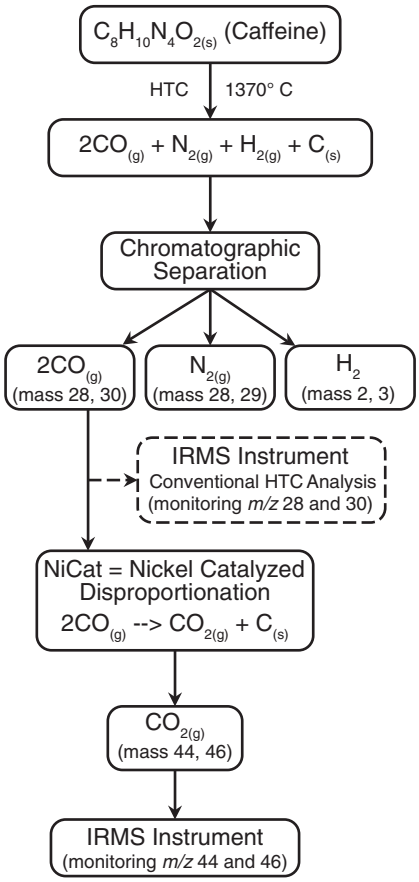

Figure 1. Flow chart depicting the fate of oxygen from the example compound caffeine ($C_8H_{10}N_4O_2$) through both the conventional HTC system and the NiCat system. N_2, H_2, and C conversions are not necessarily quantitative, with the yields dependent on substrate and instrumental conditions.[14,16]

EXPERIMENTAL

The NiCat system is comprised of three principal components: (1) a conventional HTC unit; (2) a nickel reactor; and (3) an IRMS instrument. The primary difference between the NiCat configuration and the conventional HTC system is the incorporation of the nickel reactor that catalytically converts CO flowing from the HTC unit into CO_2, which then flows to the IRMS instrument for analysis of $\delta^{18}O$ using *m/z* 44 and 46. Figure 1 illustrates the fate of oxygen as it proceeds through each analytical system.

The nickel reactor setup is a modified version of the design used by Hagopian and Jahren[20] to analyze C, H, O compounds, which was originally modeled after the approach of Loader and Buhay[21](Fig. 2). The furnace is a standalone unit (Applied Test Systems Inc., Butler, PA, USA) powered by a 115 V variable autotransformer, which provides manual control of the furnace temperature. The reactor is lightly packed with 10 cm of nickel powder (stock #10255; Alpha Aesar, Ward Hill, MA, USA) centered within the reactor tube. In the NiCat analytical mode, CO from the HTC unit is diverted through the nickel reactor, which is set to the optimal conversion temperature of 600 °C (±5 °C).[20,21] The nickel reactor was packed and replaced prior to each NiCat batch run. It was necessary to reduce the nickel reagent with hydrogen gas (99.999% pure) prior to use. Four-way valves (A and B, Fig. 2) are set such that H_2 flows through the nickel reactor at 8 mL/min at the operating temperature for 6 h with H_2 exhaust vented to a hood for safety. The valves are then switched so that outflow from the HTC unit flows through the nickel reactor and onto the IRMS instrument. This provides an oxygen-free environment at all times and assures complete reduction of the nickel reagent.

For both the conventional HTC and the NiCat systems, the first step of the analytical sequence is thermal decomposition of the sample in an HTC unit. For our tests, we used the same HTC unit for both configurations: a High-Temperature Conversion Elemental Analyzer (TC/EA; Thermo Fisher, Bremen, Germany). It was set up in a similar manner as previously published methods[2,20] with the following operating parameters. The reduction reactor comprised a ceramic outer tube with an inner glassy carbon tube. The glassy carbon tube was filled with glassy carbon chips up to the hot zone of the furnace (set to 1370 °C), where a graphite crucible was positioned to collect ash and silver residue. The helium (99.9999% purity) flow rate was set to 90 mL/min and the GC temperature to 100 °C. We opted to use the factory-installed GC column (5 Å molecular sieve, 0.6 m long) rather than replace it with an extended one, a common practice to decrease nitrogen interferences.[15,18] We felt it would be a better test of our system if high-precision analyses on nitrogen-bearing

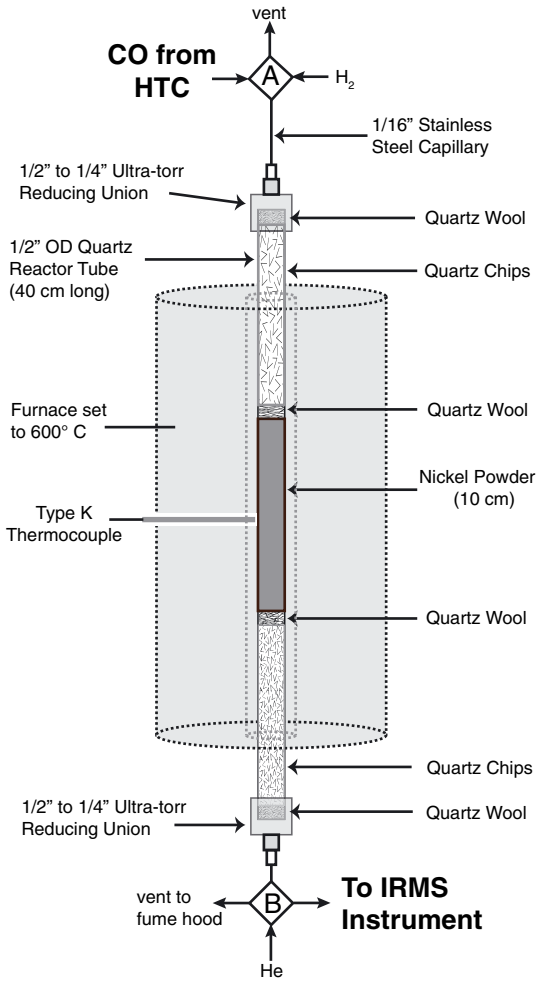

Figure 2. Diagram of the NiCat system that catalytically converts CO into CO_2. Four-way valves (A and B) can be set to either divert the CO from the HTC unit through the nickel reactor, or to introduce H_2 gas into the reactor for the pre-analysis reduction step. The furnace and reactor are supported with an aluminum rod framework and lab clamps. Ultra-torr unions and reactor tube must be securely held in place with clamps.

substrates could be obtained without taking additional measures to reduce nitrogen from entering the IRMS ion source. A Carbosorb (Elementar Americas, Mt. Laurel, NJ, USA) and magnesium perchlorate trap was installed between the reactor and the GC column to remove potential trace amounts of CO_2, H_2O, and acid gases generated during thermal decomposition. Samples were introduced into the reactor furnace *via* a zero blank autosampler (Costech Analytical, Valencia, CA, USA), which prevented atmospheric air from entering the system during the sample drop sequence and provided a moisture-free helium atmosphere prior to analysis. For all analyses in this study, samples were wrapped in silver capsules.

RESULTS

The results of the first experiment, which compared NiCat with conventional HTC, are presented in Table 1. All samples except one (KWD02) showed a difference in $\delta^{18}O$ values of ≤0.04‰ between NiCat and conventional HTC analysis. The data is presented graphically in Fig. 3. A regression of the conventional HTC versus NiCat data yielded $R^2 = 1.00$. Student's t-test and covariance test of the resulting slope versus a slope of 1 revealed no significant difference from a 1:1 relationship for these data ($p = 0.328$). Because the yield of N_2 has been shown to be variable for different substrates,[16,19] and between different HTC set-ups,[14] we plotted the $\delta^{18}O$ values of nitrogen-doped oxygen-bearing compounds data against their actual N_2:CO molar yield ratios as determined from IRMS peak areas (assuming equal ionization efficiencies). However, we note that the maximum yield ratio (N_2:CO = 0.22) was generated by SigCell doped with adenine to obtain an N:O = 3.3 molar ratio of starting material, our largest molar ratio used. There was no correlation between $\delta^{18}O$ value and N_2:CO for any of the N-doping treatments (Table 2; Fig. 4). The means of three of the individual doping treatments showed a statistically significant difference between doped and undoped means, with a maximum difference of $\delta^{18}O = 0.20‰$. Figure 4 shows that the implementation of our conventional HTC system

Table 1. Comparison of $\delta^{18}O$ values for a suite of nitrogen-free compounds analyzed using a conventional HTC configuration and the NiCat system

			Conventional HTC			NiCat			
Sample	Material	Formula	$\delta^{18}O^a$ [‰]	1σ	n	$\delta^{18}O^a$[‰]	1σ	n	$\Delta\delta^{18}O^b$ [‰]
BPRO1	α-cellulose	$C_6H_{10}O_5$	17.68	0.09	5	17.72	0.23	7	−0.04
KWD02	α-cellulose	$C_6H_{10}O_5$	19.19	0.16	5	19.46	0.11	5	−0.27
SigCell	α-cellulose	$C_6H_{10}O_5$	28.96	0.09	29	28.97	0.20	32	−0.01
IAEA-CH3	α-cellulose	$C_6H_{10}O_5$	32.70	0.29	5	32.67	0.23	11	0.03
IAEA-CH6	Sucrose	$C_{12}H_{22}O_{11}$	36.73	0.07	4	36.76	0.14	15	−0.03
Tbenz	Benzoic acid	$C_7H_6O_2$	21.01	0.05	5	21.00	0.08	11	0.01
IAEA-601	Benzoic acid	$C_7H_6O_2$	22.68	0.13	10	22.70	0.15	19	−0.02
IAEA-602	Benzoic acid	$C_7H_6O_2$	70.93	0.45	10	70.91	0.49	16	0.02

[a] $\delta^{18}O$ values were normalized to the VSMOW-SLAP scale using waters sealed in silver tubing[25] obtained from the USGS, Reston, VA, USA ('W102721' with $\delta^{18}O = -3.15‰$; and 'W102760' with $\delta^{18}O = 71.05‰$).
[b] $\Delta\delta^{18}O = \delta^{18}O$ (Conventional HTC) − $\delta^{18}O$ (NiCat).

Rapid Communications in Mass Spectrometry

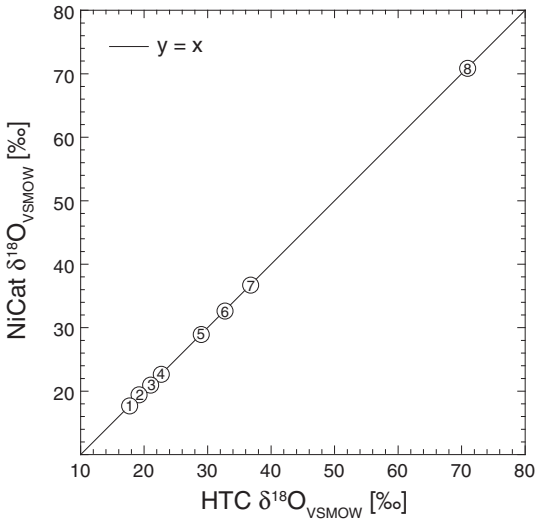

Figure 3. $\delta^{18}O$ values for a suite of compounds measured using the NiCat system plotted against $\delta^{18}O$ values for the same compounds measured using a conventional HTC system. Numbered data points correspond with the following samples: 1. BPR01 (α-celllulose); 2. KWD02 (α-celllulose); 3. Tbenz (benzoic acid); 4. IAEA-601 (benzoic acid); 5. SigCell (α-celllulose); 6. IAEA-CH3 (α-celllulose); 7. IAEA-CH6 (sucrose); 8. IAEA-602 (benzoic acid). Analytical uncertainty is smaller than the size of the symbols.

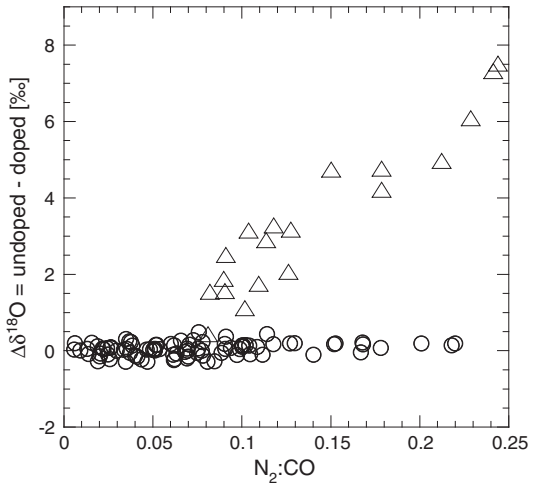

Figure 4. The difference between undoped and nitrogen-doped analyses versus N_2:CO ratios (measured using IRMS peak areas). Circles represent NiCat analyses of SigCell cellulose ($C_6H_{10}O_5$) or IAEA-CH6 sucrose ($C_{12}H_{22}O_{11}$) doped with adenine ($C_5H_5N_5$), imidazole ($C_3H_4N_2$), or 2-aminopyrimidine ($C_4H_5N_3$). Triangles represent conventional HTC analyses doped with adenine. Because the NiCat-doped samples were analyzed using CO_2, N_2:CO is inferred from separate conventional HTC analyses. Analytical uncertainty for individual doped measurements is estimated to be ≤0.21‰ based on 1σ of the replicate analyses of undoped SigCell. Error bars for N_2:CO are smaller than the size of the symbols.

alone resulted in differences between the $\delta^{18}O$ values of doped and undoped SigCell ranging from 0.4 to ~5‰ (at N_2:CO yield ratios of 0.08 and 0.22, respectively).

DISCUSSION

Workers have developed many different strategies in order to use conventional HTC to adequately determine the $\delta^{18}O$ values of nitrogen-bearing organic compounds. Some workers have attempted to remove or minimize the N_2 peak by installing an extended GC column in order to achieve more complete N_2 and CO peak separation,[15,18] by diverting or diluting the N_2 peak with helium in order to minimize the amount of N_2 available for NO^+ formation in the source,[13,14,17–19] and by trapping the CO with a chemical adsorbent while diverting N_2 to waste, then releasing the CO for analysis.[16] There are also baseline subtraction protocols that rely upon subtraction of the NO^+ *m/z* 30 interference signal from the raw *m/z* 30 sample peak area that is used to calculate the $\delta^{18}O$ value.[12,17]

Several of the factors that control the magnitude of the interfering N_2 peak vary between instrumental configurations,[14] and within the same instrument over time,[18]

Table 2. NiCat results for SigCell (α-cellulose) and IAEA-CH6 (sucrose) doped with nitrogen-bearing compounds up to a maximum N:O molar ratio = 3.3

Material(s)	Formula	N:O [molar]	N_2:CO (pk area)	$\delta^{18}O$[a] [‰]	1σ	n	$\Delta\delta^{18}O$[b] [‰]	*P*-value[c]
SigCell (undoped)	$C_6H_{10}O_5$	0	0	28.70	0.21	10		
SigCell + Adenine	$C_6H_{10}O_5 + C_5H_5N_5$	0.6 to 3.3	0.05 to 0.22	28.60	0.16	15	0.10	0.208
SigCell + Imidazole	$C_6H_{10}O_5 + C_3H_4N_2$	0.6 to 2.5	0.02 to 0.08	28.87	0.11	15	−0.17	0.014
SigCell + 2-Aminopyrimidine	$C_6H_{10}O_5 + C_4H_5N_3$	0.6 to 3.2	0.02 to 0.11	28.50	0.15	15	0.20	0.011
IAEA-CH6 (undoped)	$C_{12}H_{22}O_{11}$	0	0	35.86	0.07	10		
IAEA-CH6 + Adenine	$C_{12}H_{22}O_{11} + C_5H_5N_5$	0.2 to 3.2	0.01 to 0.22	35.84	0.11	14	0.02	0.619
IAEA-CH6 + Imidazole	$C_{12}H_{22}O_{11} + C_3H_4N_2$	0.2 to 3.3	0.01 to 0.11	35.77	0.07	15	0.09	0.027
IAEA-CH6 + 2-Aminopyrimidine	$C_{12}H_{22}O_{11} + C_4H_5N_3$	0.2 to 3.2	0.01 to 0.11	35.78	0.13	15	0.08	0.084

[a]Mean $\delta^{18}O$ values of all reps in treatment (using raw data values).
[b]$\Delta\delta^{18}O = \delta^{18}O$ (undoped) – $\delta^{18}O$ (doped).
[c]*P*-values are reported for a two-tailed t-test comparing doped and undoped means, assuming equal covariance.

rendering it difficult to effectively eliminate the interference across a wide range of substrates and analytical conditions. These factors include the oxidation state of nitrogen and the types of functional groups comprising the sample material,[14,16,19] reactor conditions influencing nitrogen intermediates,[14,16] IRMS source conditions such as oxygen availability,[12,14] and the condition of the GC column.[18] In contrast, the NiCat system circumvents each of these issues by converting the CO into CO_2 and measuring $\delta^{18}O$ values using *m/z* 44 and 46, thus eliminating the fundamental problem of using CO as the analyte. Our results show that NiCat successfully measures the $\delta^{18}O$ values of nitrogen-bearing organics up to molar yield ratios of N_2:CO = 0.22 without interference from N_2. In comparison, SigCell doped to obtain N_2:CO = 0.22 levels and analyzed on our conventional HTC system (i.e. our HTC configured to factory recommendations with the stock 0.6 m GC column) produced a discrepancy of ~5‰ between nitrogen-doped and undoped samples (Fig. 4). Although the means of three of the individual NiCat doping treatments showed a statistically significant difference from their undoped counterparts (*P*-values <0.05), the maximum difference was just 0.2‰ (Table 2). In addition, there was no systematic effect across all treatments, or with increasing N_2:CO, confirming that the differences were not the result of N_2 interference.

In order to evaluate each doping compound for an oxygen blank, we analyzed an amount identical to that used for our maximum molar doping levels (N:O = 3.2 or 3.3). Impurities in the doping compounds resulted in the following contributions of oxygen to the sample IRMS signal (expressed as a percentage of total peak area): 0.5%, 0.8%, and 0.4% for 2-aminopyrimidine, adenine, and imidazole, respectively. Due to the small size of the blanks, and the inability of the IRMS instrument to measure $\delta^{18}O$ values accurately at such low signal intensities (blanks were all <8 mV), we were unable to determine the $\delta^{18}O$ values of the blank contributions. As the doping amounts increase, the blank contribution should also increase, systematically skewing the measured $\delta^{18}O$ values of the doped material towards the $\delta^{18}O$ value of the blank. The effect would be greater the further the blank $\delta^{18}O$ value was from the sample $\delta^{18}O$ value. For all six treatments of our doping experiment, we did not detect any systematic changes in the $\delta^{18}O$ values as the doping amounts increased. In addition, the magnitude of the changes in $\delta^{18}O$ values for the doping treatments compared with undoped was not consistent with what we would expect if the differences were due to a blank contribution. For example, SigCell ($\delta^{18}O$ = 28.70‰) doped with 2-aminopyrimidine resulted in a 0.20‰ decrease, whereas IAEA-CH6 sucrose ($\delta^{18}O$ = 35.86‰) resulted in a 0.08‰ decrease. In order to result in these changes, the blank $\delta^{18}O$ value would need to be −9‰ for SigCell, and +21‰ for IAEA-CH6, which is unlikely considering that the same batch of 2-aminopyrimidine was used for both. It is more likely that the $\delta^{18}O$ values of the blanks were somewhere between those of the SigCell and IAEA-CH6, imparting less than a 0.05‰ effect on the sample measurement at the highest doping level.

We surmise that the minor differences between doped and undoped treatments could be the result of interactions within the HTC reactor or the nickel reactor, or a combination of both. For example, the higher availability of carbon provided by the doping compound could have altered the reducing conditions in the HTC reactor. Alternatively, the addition of hydrogen from the doping compound could have affected the reactivity of the nickel catalyst prior to the catalytic conversion of CO into CO_2. Under either scenario, the net implications for the resultant $\delta^{18}O$ measurement are within the uncertainty (±0.2‰) commonly reported for conventional HTC analysis.[12,16]

Based on our results, organic substrates with <0.22 stoichiometric N_2:O should be fully analyzable for $\delta^{18}O$ value using the NiCat system given a maximum possible N_2:CO yield ratio <0.22. Substrates with higher stoichiometric N_2:O may also be amenable to analysis *via* NiCat if the HTC unit to be employed determines that N_2:CO peak area ratios are <0.22. Table 3 lists the N_2:CO yields of a variety of nitrogen-bearing organics; most of them meet this criterion, suggesting the NiCat system has the potential for widespread application of nitrogen-bearing compounds of analytical interest. We were prevented from exploring doped compounds with N_2:CO >0.22 by the increased blank contribution from the doping compounds; however, some HTC systems may be able to overcome this by producing higher yields of N_2 (e.g. a

Table 3. Conventional HTC N_2 yields reported for several substrates of interest

Substrate	Formula	Reference	N_2:O[a]	N_2:CO[b]
IAEA-600 caffeine	$C_8H_{10}N_4O_2$	Brand[14]	1.00	0.02 to 1.0
IAEA-600 caffeine	$C_8H_{10}N_4O_2$	Hunsinger[19]	1.00	0.10
Glycine	$C_2H_5NO_2$	Hunsinger[19]	0.25	0.16
Pentaerythritol tetranitrate	$C_5H_8N_4O_{12}$	Hunsinger[19]	0.17	0.18
4-Nitroacetanilide	$C_8H_8N_2O_3$	Hunsinger[19]	0.33	0.21
Cyclotrimethylene trinitramine	$C_3H_6N_6O_6$	Hunsinger[19]	0.50	0.52
Urea	CH_4N_2O	Sieper[16]	1.00	0.22 (0.05)[c]
Formylhydrazide	CH_4N_2O	Sieper[16]	1.00	0.32 (0.06)[c]
Acetanilide	C_8H_9NO	Sieper[16]	0.50	0.03
p-Aminoacetophenone	C_8H_9NO	Sieper[16]	0.50	0.03
Sucrose/Benzotriazole	$C_{12}H_{22}O_{11}/C_6H_5N_3$	Sieper[16]	0.14	0.05

[a]Stoichiometric yield assuming quantitative conversion.
[b]Ratio of gas yields measured with IRMS peak areas.
[c]Value in parentheses indicates N_2:CO yields when using a polyethylene $(C_2H_4)_n$ additive to minimize N_2 formation.

system similar to the USGS or ANU units described in Brand *et al.*[14]). Evidence of an elevation of the *m/z* 30 baseline prior to the CO peak[18,19] has been used to demonstrate the successful removal of N_2 interference from modified conventional HTC systems. If we apply similar reasoning to the NiCat system, we note that we did not observe any baseline shift in the *m/z* 44 or 46 signal prior to the CO_2 sample peak for any of the N-doping tests, suggesting a lack of isobaric interference. This same approach could be used to assess substrates that generate N_2:CO peak area ratios above 0.22 (Table 3), if doping is not practical.

Another advantage of the NiCat system is that it produces CO_2, which can be effectively cryofocused for very small sample analysis.[22,23] We have previously used the "CryoNiCat" system to analyze the $\delta^{18}O$ value of $C_7H_6O_2$ (benzoic acid) down to 1.3 μg oxygen, lowering the detection limit by a factor of ten.[20] The results that we present here suggest that CryoNiCat could be successfully extended to the $\delta^{18}O$ analysis of very small nitrogen-bearing samples, such as nitro-explosive residues,[11] chitinous microfossil fragments,[24] and the incremental analysis of single strands of hair.[7] The NiCat system may also be preferable for nitrogen-bearing compounds that have proven exceptionally difficult to analyze *via* conventional HTC. For example, published values for the international reference material IAEA-600 caffeine ($C_8H_{10}N_4O_2$) vary widely, from −0.58 to −4.63‰.[14,16,19] The difficulty in analyzing caffeine might be the result of slow-elution of N_2 formed from the breakdown of precursor molecules such as paracyanogen ($(NC\text{-}CN)_x$) during the HTC reaction, giving rise to a variable *m/z* 30 background that is difficult to predict and correct.[14,16] During our comparison of nitrogen-doped and undoped compounds using the NiCat system, we did not observe baseline shifts in *m/z* 44 or 46; therefore, we do not expect unpredictable contributions of N_2 eluting from the GC column to interfere with the analysis.

CONCLUSIONS

The NiCat system is a viable alternative to conventional HTC systems for the measurement of the $\delta^{18}O$ values of nitrogen-bearing organics, and can provide a standardized instrumental configuration that is not susceptible to isobaric interference. The precision and accuracy for nitrogen-doped organic substrates are as good as those obtained by conventional HTC analysis of nitrogen-free organic compounds (better than ~0.2‰). We have also shown that CO into CO_2 conversion using the NiCat system is reproducible and reflects the $\delta^{18}O$ value of the CO source. When analyzing very small samples, the adaptation of conventional HTC to a NiCat system may be of particular advantage, and requires relatively inexpensive and readily available materials.

Acknowledgements

We thank the Reston Stable Isotope Laboratory at USGS for providing aliquots of sealed water samples for scale normalization. We thank L. A. Stern for helpful advice. This work was supported by the National Science Foundation (Grant No. NSF/EXE-CBET08-54754).

REFERENCES

[1] G. Houerou, S. D. Kelly, M. J. Dennis. Determination of the oxygen-18/oxygen-16 isotope ratios of sugar, citric acid and water from single strength orange juice. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1257.
[2] J. Koziet. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. *J. Mass Spectrom.* **1997**, *32*, 103.
[3] J. S. Roden, J. R. Ehleringer. Hydrogen and oxygen isotope ratios of tree ring cellulose for field-grown riparian trees. *Oecologia* **2000**, *123*, 481.
[4] M. N. Evans, D. P. Schrag. A stable isotope-based approach to tropical dendroclimatology. *Geochim. Cosmochim. Acta* **2004**, *68*, 3295.
[5] L. D. L. Sternberg, in *Modern Methods of Plant Analysis*, vol. *10*, (Eds: H. F. Linskens, J. F. Jackson). Springer-Verlag, Heidelberg, **1989**; p. 89.
[6] O. Breas, C. Guillou, F. Reniero, E. Sada, F. Angerosa. Oxygen-18 measurement by continuous flow pyrolysis/isotope ratio mass spectrometry of vegetable oils. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 188.
[7] J. R. Ehleringer, G. J. Bowen, L. A. Chesson, A. G. West, D. W. Podlesak, T. E. Cerling. Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2788.
[8] K. J. Petzke, B. T. Fuller, C. C. Metges. Advances in natural stable isotope ratio analysis of human hair to determine nutritional and metabolic status. *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 532.
[9] D. M. O'Brien, M. J. Wooller. Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2422.
[10] L. D. L. Sternberg, M. C. Pinzon, P. F. Vendramini, W. T. Anderson, A. H. Jahren, K. Beuning. Oxygen isotope ratios of cellulose-derived phenylglucosazone: An improved paleoclimate indicator of environmental water and relative humidity. *Geochim. Cosmochim. Acta* **2007**, *71*, 2463.
[11] D. Widory, J. J. Minet, M. Barbe-Leborgne. Sourcing explosives: A multi-isotope approach. *Sci. Justice* **2009**, *49*, 62.
[12] M. Gehre, G. Strauch. High-temperature elemental analysis and pyrolysis techniques for stable isotope analysis. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1497.
[13] J. K. Bohlke, S. J. Mroczkowski, T. B. Coplen. Oxygen isotopes in nitrate: new reference materials for O-18 : O-17 : O-16 measurements and observations on nitrate-water equilibration. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1835.
[14] W. A. Brand, T. B. Coplen, A. T. Aerts-Bijma, J. K. Bohlke, M. Gehre, H. Geilmann, M. Groning, H. G. Jansen, H. A. J. Meijer, S. J. Mroczkowski, H. P. Qi, K. Soergel, H. Stuart-Williams, S. M. Weise, R. A. Werner. Comprehensive inter-laboratory calibration of reference materials for $\delta^{18}O$ versus VSMOW using various on-line high-temperature conversion techniques. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 999.
[15] G. D. Farquhar, B. K. Henry, J. M. Styles. A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1554.
[16] H.-P. Sieper, H.-J. Kupka, L. Lange, A. Rossmann, N. Tanz, H.-L. Schmidt. Essential methodological improvements in the oxygen isotope ratio analysis of N-containing organic compounds. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2849.
[17] F. Accoe, M. Berglund, B. Geypens, P. Taylor. Methods to reduce interference effects in thermal conversion elemental analyzer/continuous flow isotope ratio mass spectrometry $\delta^{18}O$ measurements of nitrogen-containing compounds. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 2280.

[18] H. Qi, T. B. Coplen, L. I. Wassenaar. Improved online $\delta^{18}O$ measurements of nitrogen- and sulfur-bearing organic materials and a proposed analytical protocol. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2049.
[19] G. B. Hunsinger, L. A. Stern. Improved accuracy in high-temperature conversion elemental analyzer $\delta^{18}O$ measurements of nitrogen-rich organics. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 554.
[20] W. M. Hagopian, A. H. Jahren. Minimization of sample requirement for $\delta^{18}O$ in benzoic acid. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2542.
[21] N. J. Loader, W. M. Buhay. Rapid catalytic oxidation of CO to CO_2 – On the development of a new approach to on-line oxygen isotope analysis of organic matter. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1828.
[22] B. Fry, R. Garritt, K. Tholke, C. Neill, R. H. Michener, F. J. Mersch, W. Brand. Cryoflow: Cryofocusing nanomole amounts of CO_2, N_2, and SO_2 from an elemental analyzer for stable isotopic analysis. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 953.
[23] P. J. Polissar, J. M. Fulton, C. K. Junium, C. C. Turich, K. H. Freeman. Measurement of C-13 and N-15 isotopic composition on nanomolar quantities of C and N. *Anal. Chem.* **2009**, *81*, 755.
[24] Y. V. Wang, D. M. O'Brien, J. Jenson, D. Francis, M. J. Wooller. The influence of diet and water on the stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. *Oecologia* **2009**, *160*, 225.
[25] H. Qi, M. Groening, T. B. Coplen, B. Buck, S. J. Mroczkowski, W. A. Brand, H. Geilmann, M. Gehre. Novel silver-tubing method for quantitative introduction of water into high-temperature conversion systems for stable hydrogen and oxygen isotopic measurements. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1821.

