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Accelerated Articles

Moving-Wire Device for Carbon Isotopic Analyses of Nanogram Quantities of Nonvolatile Organic Carbon

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We describe a moving-wire analyzer for measuring ^{13}C in dissolved, involatile organic materials. Liquid samples are first deposited and dried on a continuously spooling nickel wire. The residual sample is then combusted as the wire moves through a furnace, and the evolved CO_2 is analyzed by continuous-flow isotope ratio mass spectrometry. A typical analysis requires 1 μL of sample solution and produces a CO_2 peak ~5 s wide. The system can measure "bulk" $\delta^{13}C$ values of ~10 nmol of organic carbon with precision better than 0.2‰. For samples containing ~1 nmol of C, precision is ~1‰. Precision and sensitivity are limited mainly by background noise derived from carbon within the wire. Instrument conditions minimizing that background are discussed in detail. Accuracy is better than 0.5‰ for nearly all dissolved analytes tested, including lipids, proteins, nucleic acids, sugars, halocarbons, and hydrocarbons. The sensitivity demonstrated here for ^{13}C measurements represents a ~1000-fold improvement relative to existing elemental analyzers and should allow the use of many new preparative techniques for collecting and purifying nonvolatile biochemicals for isotopic analysis.

Subtle variations in the natural abundances of stable isotopes are widely exploited by earth scientists studying natural processes. For measurements of the $^{13}C/^{12}C$ ratio in organic materials, analyses fall into two broad categories. "Bulk" methods quantitatively convert all carbon in a sample to CO_2. In contrast, "compound-specific" methods employ chromatographic separation of analytes immediately prior to isotopic analysis, generally by coupling a gas chromatograph (GC) to the isotope ratio mass spectrometer (IRMS) via a combustion oven.[1,2] Unfortunately, these methods do not provide a general approach to the analysis of very small amounts of purified, nonvolatile biochemicals such as proteins, nucleic acids, polar lipids, and carbohydrates. Such molecules are too involatile for separation by GC and cannot easily be purified in quantities sufficient for bulk analyses—typically tens to hundreds of micrograms of carbon.[3] The ubiquity and information content of such molecules make them an obvious target for isotopic analysis, and this problem has captured much recent interest.[4−9]

Efforts reported thus far have focused mainly on coupling liquid chromatography (LC) directly with the IRMS in a manner analogous to the GC-combustion-IRMS. Approaches include a microwave-powered chemical reaction interface,[4,10] deposition of the LC eluent onto moving belts[11] or wires,[12,13] or inline chemical

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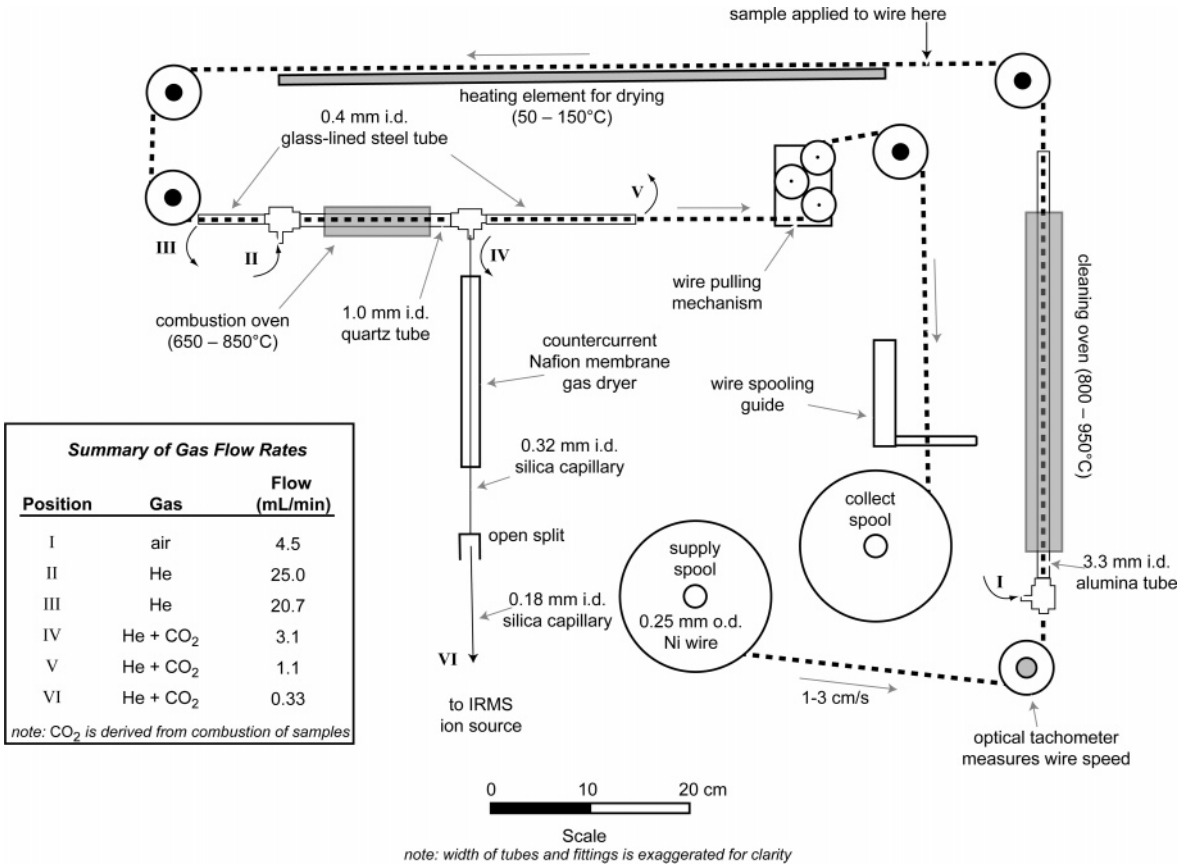

Summary of Gas Flow Rates

Position	Gas	Flow (mL/min)
I	air	4.5
II	He	25.0
III	He	20.7
IV	He + CO_2	3.1
V	He + CO_2	1.1
VI	He + CO_2	0.33

note: CO_2 is derived from combustion of samples

Figure 1. Schematic drawing of the moving-wire system. Operation of the device is described in the text. Curved arrows and Roman numerals correspond to gas flow rates listed in the box. Flow rates and oven temperatures are typical values and can be adjusted to suit various solvents and analytes. Drawing is to scale, and the entire device is mounted vertically on a metal panel that is 1 m wide × 61 cm tall.

oxidation of analytes.[14] These methods are limited to various degrees by low sensitivity, poor peak resolution, restrictions on chromatographic conditions and reagents, slow analyses, or large carbon backgrounds.

An alternative approach is to couple preparative ("offline") separation of analytes with bulk analyses of collected fractions to produce isotopic measurements of individual compounds. The idea is both obvious and old (e.g., ref 15), but it becomes significantly more appealing as sample-size requirements decrease and offline preparative methods are automated. A key development that we report here is the adaptation of a moving-wire device that was introduced previously by Brand and Dobberstein.[13] The earlier report used the moving-wire device exclusively as a continuous LC-combustion-IRMS interface (i.e., a compound-specific analyzer). Modifications in our laboratory, described below, have now optimized it for use with discrete liquid samples (i.e., a bulk analyzer) generated by a variety of preparatory methods. Samples ~1 μL in size can be analyzed every 30−40 s, with few memory effects, high sensitivity, and excellent accuracy and precision. Virtually all volatile solvents, including water, and all nonvolatile organic analytes can be accommodated. The moving-wire analyzer can be used to determine bulk $\delta^{13}C$ values of tens of nanograms of nonvolatile organic carbon with precision better than 0.2‰ and accuracy better than 0.5‰. This dramatic increase in sensitivity—roughly 3 orders of magnitude relative to existing elemental analyzers— opens the door to many preparative technologies that are otherwise impractical as a prelude to isotopic analysis. These include LC, electrophoresis, probe−capture,[9] precipitation or ultrafiltration, density gradient centrifugation, and many others. The relative speed and ease of isotopic analyses also suggest that this device could have uses in biochemistry and medicine as a rapid screening tool for the presence of ^{13}C label. This report describes the operating characteristics and performance limits of the device.

EXPERIMENTAL SECTION

Moving-Wire Analyzer. The device is described in Figure 1. Briefly, a small motor pulls a 0.25-mm nickel wire through a circuit of pulleys and ovens. Wire is used once and then discarded. The wire (a low-carbon, 99% Ni alloy, N02270, obtained from Ametek, Wallingford, CT) is first cleaned by heating to ~900 °C in air, and then a liquid sample droplet is deposited manually onto the wire using a standard, glass barrel syringe. Droplets up to ~2.0 μL will reliably cling to the wire due to surface tension. The sample droplet is carried over a heating element where the liquid solvent evaporates in air and then into a combustion furnace where nonvolatile organic compounds adhering to the wire are quantitatively combusted to CO_2, H_2O, and $N_2 + NO_x$. A fraction of the

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combustion gases flows through a countercurrent Nafion membrane dryer[16] to remove H_2O, through an open split to buffer pressure fluctuations, and then into the IRMS for carbon isotopic analysis.

The combustion interface consists of a quartz tube (285 mm long, 3.1 mm o.d. × 1.0 mm i.d.) inside a resistively heated tube furnace (100 mm long × 3.3 mm i.d.). The temperature of the furnace is monitored via a type-R thermocouple placed directly beneath the resistance wire coils. Three Cu wires (10 cm long × 0.25 mm diameter) are twisted together and threaded into the quartz tube prior to its installation. They are oxidized in place by flushing with 100% O_2 at ~500 °C, where they serve as the primary oxidant for combustion (no additional O_2 is added during routine sample analysis). No detectable improvements (in isotope ratio or combustion efficiency) were measured with the addition of Pt wire to the reactor, so none was used in the experiments reported here.

Gas flow rates are summarized in Figure 1. Wire speed and oven temperatures are adjusted for the volatilities of specific solvents and analytes to achieve complete drying, quantitative conversion to CO_2, and to minimize background signals as described below. The typical flow rates and temperatures shown in Figure 1 are compatible with many solvents including toluene, chloroform, methanol, acetonitrile, and water. Highly volatile solvents (e.g., acetone, dichloromethane) are problematic because they tend to evaporate before the sample droplet can be transferred from syringe to wire.

The moving-wire device described here is the same prototype instrument described by Brand and Dobberstein[13] with the following modifications. (1) The device was previously controlled via a custom-built microprocessor and now via LabView. (2) A new source of low-carbon Ni wire has been obtained. (3) The combustion furnace has been modified to include CuO to improve combustion. (4) The cleaning oven was formerly purged with pure O_2 and now with compressed air. (5) Wire speeds, gas flows rates, and oven temperatures have been optimized for discrete samples.

Isotopic Analyses. For the experiments reported here, the moving-wire system was interfaced to a Finnigan 252 IRMS controlled by IsodatNT v1.21 software. Ion currents corresponding to masses 44, 45, and 46 were recorded continuously, so that each sample aliquot appears as a discrete, time-resolved peak typically ~5 s wide (Figure 2). Carbon isotope ratios are calibrated relative to known standards as in isotope ratio monitoring (IRM)-GC/MS.[17] All isotopic abundances are reported here in the standard delta notation in units of permil relative to the VPDB standard.[18]

The carbon contents of analytes are calculated from the integrated area of the mass-44 ion current peak produced by combustion of each sample aliquot. Peak areas must then be compared to a calibration curve to yield results in concentration units.

Samples. Various organic compounds were used to test the system. Compounds were purchased in high purity (>98%), and stock solutions containing 1 (mg of analyte C)/(mL of solution) were prepared gravimetrically in appropriate solvents—water for polar, 1:1 water/methanol for slightly polar, and toluene for nonpolar analytes. Samples of lower concentration were then prepared by serial dilution in the same solvent. Aliquots of the same solvent were tested directly on the moving-wire system to estimate blank contributions.

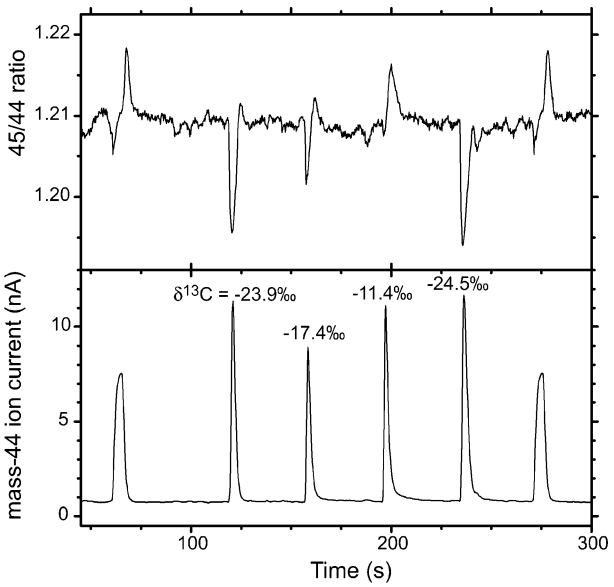

Figure 2. Typical signals recorded by the IRMS from combustion of organic samples. The lower trace is the mass-44 ion current, the upper trace is the mass-45/44 ion current ratio, proportional to the $^{13}C/^{12}C$ ratio. Peaks from left to right are as follows: CO_2 reference gas, lactate (6.6 nmol of C), albumin (5.0 nmol of C), phenylalanine (5.0 nmol of C), NIST 1649a (unknown concentration due to rapid settling), CO_2 reference gas. Delta values measured in this analysis are listed on the figure above each peak. Offline delta values for these compounds are in Table 2.

RESULTS AND DISCUSSION

Flow Rates and Split Ratios. The flow rates reported in Figure 1 were measured under typical operating conditions. The flow rate of He at location II (noted on the figure) is chosen to exclude atmospheric CO_2 from the combustion reactor. Conductances on the pathways leading to locations III, IV, and V are such that ~83% of the He exits at the upstream end of the combustion reactor (i.e., the left side of the reactor in Figure 1), 4.5% exits at the downstream end, and the remainder flows to the open split. Assuming that no sample-derived CO_2 is entrained by the He exiting at the upstream end of the reactor, 74% of the CO_2 [= 3.1/(3.1 + 1.1)] flows to the open split and 11% of that (= 0.33/3.1) is sent to the mass spectrometer. The overall efficiency with which sample carbon is transmitted from the moving wire to the mass spectrometer is thus ~8% (= 0.11 × 0.74). If gas is fully mixed within the combustion reactor, the first split ratio would be 3.1/25 = 0.12 and the overall efficiency would be 1% (= 0.11 × 0.12).

Sensitivity and Precision. The relationships between observed precision and sample size are summarized in Figure 3. The relative precision of measurements of C content was 5−10% and is independent of sample size (Figure 3a). For samples of ≥8 nmol of C, the precision of isotopic analyses was better than 0.3‰ (1σ) at natural abundances of ^{13}C. Lower uncertainties

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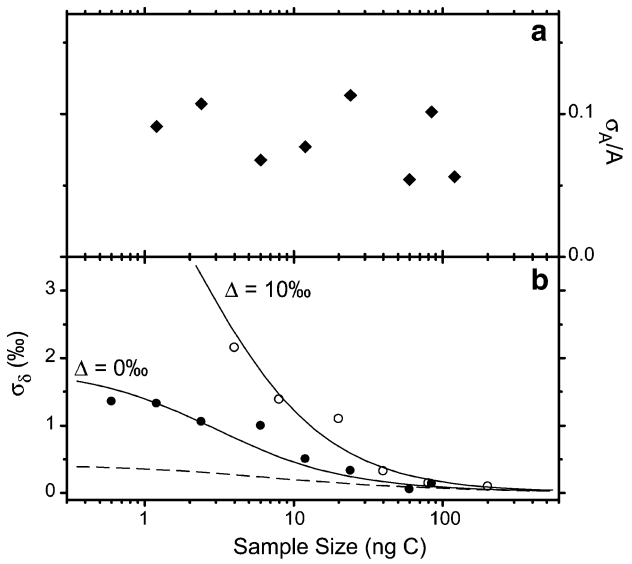

Figure 3. Attainable precision for C content (σ_A) and δ_T (σ_δ) versus sample size. All values represent the standard deviation of five replicate analyses without any blank correction. Solid and dashed lines represent the error model described in the text. *A* is the peak area of the mass-44 ion beam; Δ is the difference in $\delta^{13}C$ values between sample and blank carbon. Symbols: (●) cholesterol; (○) glucose.

can be obtained by averaging results from multiple aliquots, which is quite practical considering that analyses require less than 40 s. For samples containing 1 nmol of C, the isotopic precision is ~1‰.

Factors Limiting Precision. The precision with which C content and isotopic compositions can be measured is limited by signal/noise ratios and by the presence of carbon blanks. Precision for C content depends only on noise in the mass-44 ion current (σ_{44}). The precision of isotopic analyses is related to noise in the mass-45/44 ratio (σ_R). These noise levels in turn depend on the following: (1) the performance of signal-processing components, (2) "shot noise" related to the randomly spaced arrivals of ions at the collector, and (3) sample-associated blanks and nonstatistical fluctuations in CO_2 background that cannot be removed through background subtraction schemes. For modern IRMS instruments, noise associated with signal processing (factor 1 in the preceding list) is negligible in comparison to shot noise (factor 2).[19]

The importance of shot noise for the moving wire will be determined largely by the efficiency with which carbon is transferred from the wire to the ion source (i.e., the effects of the split ratios noted above). The "shot noise limit" (dashed line in Figure 3b) is based on an ionization efficiency of 10^{-3} (CO_2^+ ions collected)/(molecules introduced) and a transmission efficiency of 0.08 (CO_2 molecules to ion source)/(C atoms on wire). The failure of the system to approach this limit indicates that random variations in the CO_2 background and blanks (factor 3) must degrade the performance of the present system. In IRM-GC/MS systems, where CO_2 backgrounds (due mainly to column bleed) change slowly relative to peak widths, ultimate precision can approach the limit imposed by ion-counting statistics within a factor of ~2 (ref 19). In contrast, CO_2 backgrounds in the moving-wire system derive primarily from carbon in the wire, and background ion currents change rapidly relative to signals from sample peaks, leading to the observed limitations.

To assess quantitatively the factors limiting precision, it is necessary to consider both the carbon blank associated with each sample and the CO_2 background derived from the wire. The former appears as part of the sample CO_2 peak. Given a separate assessment of analytical blanks, it can be subtracted later. In contrast, the background CO_2 signal can be immediately subtracted based upon an estimate of its magnitude over a time window preceding the sample peak. Unfortunately, power spectra of background variations include significant components at frequencies near those of the sample peaks, and their presence must degrade attainable precision regardless of background subtraction algorithms.[19] Possible sources of these components include airborne particles landing on the wire, losses of the wire's surface oxide layer with associated exposure of uncleaned, deeper layers of the nickel, abrupt changes in wire speed due to kinks or slipping, and differences in carbon content along the length of the wire.

Figure 3 assesses precision as the standard deviation of measurements for multiple aliquots of the same sample. These measurements have the average CO_2 background subtracted but have not been corrected for blanks. The reported precision thus documents the detrimental effects of both sample-associated blanks and nonrandom deviations from the average CO_2 background. Because the two effects cannot be distinguished experimentally, we lump them together here under the label "blank" carbon.

Equation 1 describes the mixing of blank- and sample-derived CO_2

$$\delta_T = \frac{b}{T}\delta_b + \frac{T-b}{T}\delta_s \qquad (1)$$

where δ values are carbon isotopic compositions relative to the VPDB standard, b is the molar quantity of carbon in the blank, T is the total amount of carbon from the sample and the blank, and the subscripts T, b, and s refer to total carbon, blank carbon, and sample carbon, respectively.

Rearrangement of eq 1 yields

$$\delta_T = (b/T)\Delta + \delta_s \qquad (2)$$

where $\Delta = \delta_b - \delta_s$ is the isotopic difference between the sample and the blank. Consideration of the propagation of uncertainties then yields

$$\sigma_{\delta T}{}^2 = \Delta^2\left(\frac{1}{T^2} + \frac{b^2}{T^4}\right)\sigma_b{}^2 + \left(\frac{b}{T}\right)^2\sigma_{\delta b}{}^2 + \sigma_{SNL}{}^2 \qquad (3)$$

where the σ^2 terms indicate the variances in δ_T, in b, in δ_b, and the irreducible shot noise that would be observed even in the absence of variations in the amount and isotopic composition of the blank. The first term on the right, which represents the effects

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of variations in the amount of blank carbon, drops to zero if the isotopic compositions of the sample and blank are identical ($\Delta = 0‰$). The second term represents the effects of variations in the isotopic composition of the blank. The third, the shot noise limit, is given by[20]

$$\sigma_{\mathrm{SNL}}{}^2 = \frac{2 \times 10^6 (1 + R)^2}{ExTN_{\mathrm{A}}R} \tag{4}$$

where 2×10^6 is a coefficient required to express the result in permil units, R is the ion current ratio (~0.01 in the case of 45/46 and the analysis of ^{13}C), E is the efficiency of ionization (estimated at 10^{-3} in the preceding discussion), N_A is Avogadro's number, and x is the efficiency of carbon transfer in the moving-wire interface, estimated above to range between 0.01 and 0.08.

The unbroken lines in Figure 3b derive from eq 3 with $b = 0.25$ nmol of C, $\sigma_b = 0.12$ nmol of C, $\sigma_{\delta b} = 1.8‰$, $x = 0.01$, and $\Delta = 0$ (lower line) or 10‰ (upper line). The points clustering around the upper line derive from replicate analyses of glucose ($\delta = -9.9‰$). Those around the lower line derive from replicate analyses of cholestanol ($\delta = -25.3‰$). The error model is apparently realistic but imperfect. For $\Delta = 3‰$, effects of variations in b and in δ_b contribute equally to uncertainties in δ_T. For $\Delta < 3‰$, variations in the isotopic composition of the blank are more important than variations in the size of the blank. The situation is reversed for $\Delta > 3‰$.

Sources of Background CO_2. The size of the CO_2 background is strongly correlated with the bulk carbon content of the wire, indicating that it derives mainly from carbon *within*, rather than *on*, the wire. Even when the wire speed is regulated precisely, background ion currents related to CO_2 are noisy. The magnitude of that noise is strongly correlated with the size of the CO_2 background, leading to a correlation between the size of the CO_2 background and attainable precision (Figure 4; NB, σ_{44} must inevitably increase with $\sqrt{i}$, but the variations observed here exceed those expected from ion statistics by a factor of $>10^5$).

The precision of moving-wire analyses can be improved by minimizing (reducing b) and stabilizing (reducing σ_b and $\sigma_{\delta b}$) the CO_2 background. We thus examined the effects of various operating parameters on the size and variability of background signals. Figure 5 shows the relationship between wire speed and CO_2 background. As wire speed increases, more carbon is delivered to the combustion oven per unit time, and the CO_2 background increases in both magnitude and variability. Decreasing wire speed results in longer analyses and increasingly broad peaks, which lowers the signal/noise ratio. For analyses of narrow, 5-s peaks, the optimal compromise between these competing factors—using presently available materials—is at a wire speed of 1.5–2.0 cm/s. To translate the results of Figure 5 into more familiar form, a 1.0 nA collected mass-44 ion current corresponds to 1.0 nmol of C/s delivered to the combustion oven of the moving-wire device (assuming 0.01 split ratio and 10^{-3} ionization efficiency, as above). If this signal were obtained at a wire speed of 2.0 cm/s, it would correspond to a wire C content of ~1.4 ppm, significantly lower than the nominal C content of 27 ppm for uncleaned wire.

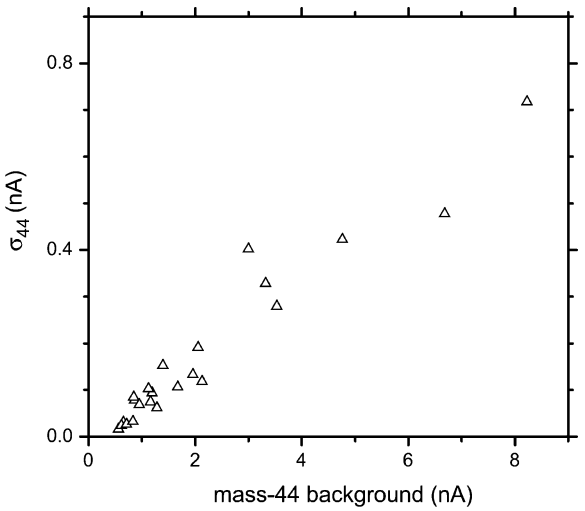

Figure 4. Ion current noise (σ_{44}) versus the magnitude of CO_2 background. Values were estimated by calculating the standard deviation of 100-s observations of mass-44 ion currents. The size of the CO_2 background was varied by changing the cleaning and combustion oven temperatures and the wire speed. Similar results are obtained when the CO_2 background varies due to carbon content of the Ni wire.

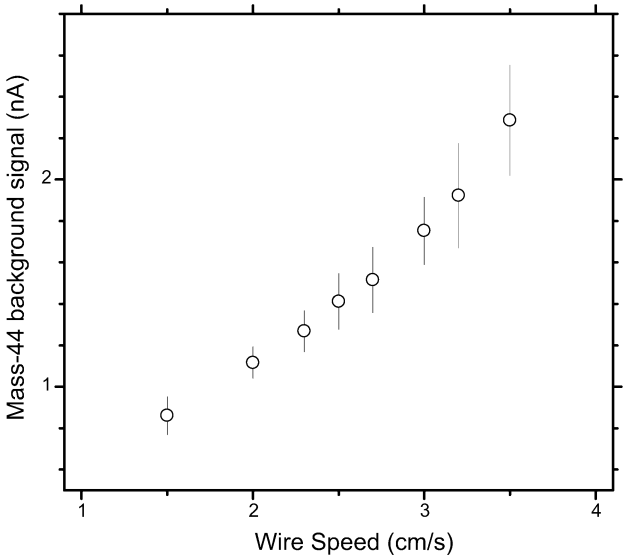

Figure 5. Relationship between CO_2 background signals and wire speed. CO_2 background signals increase with wire speed because more C is delivered to the combustion reactor per unit time. Vertical bars represent $\pm 1\sigma$ range of signal variations over a 100-s interval. Data were collected with cleaning oven at 900 °C and combustion oven at 800 °C.

Characteristics of the cleaning and combustion ovens affect the CO_2 background as well (Figure 6). Temperature is most important, and the best results are obtained when the cleaning oven is at least 100 °C hotter than the combustion oven. The highest temperature at which the cleaning oven can be operated without the wire breaking is ~950 °C. Combustion temperatures must also be sufficiently high to quantitatively convert organic

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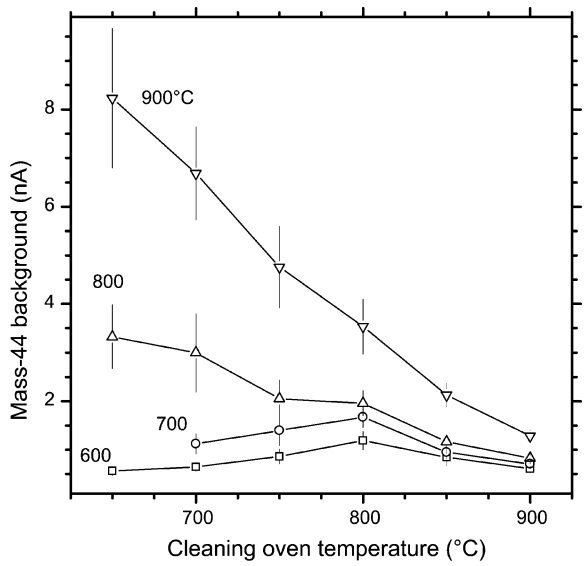

Figure 6. Relationships between CO_2 background signals and oven temperatures. Temperature of the wire-cleaning oven is indicated on the *x*-axis. Temperature of the sample combustion oven is indicated by numbers next to each curve. Vertical bars represent $\pm 1\sigma$ range of signal variations over a 100-s interval. Data were collected at a wire speed of 1.7 cm/s.

analytes to CO_2. The best results for a range of analytes are obtained with the cleaning and combustion ovens at temperatures of ~900 and ~750 °C, respectively. A second factor is the length of time during which the wire is exposed to high temperatures within the ovens, proportional to the physical length of the ovens. The current system is designed so that the cleaning oven is ~3 times longer than the combustion oven. Further increases in that ratio may yield a still lower background.

A third factor that influences the magnitude and variability of the CO_2 background is the formation of a brittle oxide coating on the nickel wire during cleaning. Irregular flakes of oxide fall off the wire as it passes around pulleys and through small orifices. This exposes unoxidized wire with higher carbon content. This process is probably responsible for a significant proportion of the observed variability in the CO_2 background and can explain why background noise actually increases when 100% O_2 instead of air is used to flush the wire-cleaning oven.

A final consideration is the choice of metal alloy for the wire. The requirements are (1) sufficient tensile strength, (2) resistance to oxidation at high temperature, (3) low carbon content, and (4) cost. The latter consideration arises because the wire cannot easily be reused, so hundreds of meters are consumed during a few months of normal operation. Noble metals are too expensive; copper and iron have inadequate tensile strength at high temperature and oxidize too rapidly; steel and Nichrome alloys contain too much carbon. The best compromise found to date is pure nickel wire.[13] Given difficulties with incomplete combustion of analytes (below), further design changes to enable the use of platinum might be worthwhile.

Blanks. With the high sensitivity of the moving-wire analyzer, many previously negligible sources of sample-associated blanks become important (Table 1). The injection blank, including contributions from the syringe, glass vials, and pipets (but not solvent), was estimated by standard additions of ethyl acetate (1−100 mL, all concentrated to 0.5 mL) as 100 ± 40 pmol of C, a value that was subtracted from all subsequent measurements to calculate the values in Table 1. Carbon blanks for various solvents were estimated by concentrating the solvents 20-fold under N_2 before measuring, and range from 3 to 44 pmol of C/μL solvent. A typical sample analysis uses 1 μL of solvent, so these values are small relative to the injection blank but become very significant in cases where the solvent is substantially concentrated by evaporation. The use of a sub-boiling still to condense solvents from a stream of N_2 carrier gas has proven to be the most effective means to reduce this solvent blank, typically by a factor of 2 but in some cases up to 1 order of magnitude. Laboratory materials often used for preparing organic samples (glass wool, anhydrous sodium sulfate, silica gel) were tested and yielded blanks ranging from 4 to 70 pmol of C. Finally, the C content of column bleed from a typical LC column was estimated by comparing solvents before and after flow through the column. Values ranged from <1 to 21 pmol of C/μL of solvent. Considering all these background sources, a typical analysis requiring no concentration of solvents must contend with a blank of roughly 0.1−0.3 nmol of C, and potentially much greater if solvents are concentrated. Thus, a practical limit to sample size, to achieve <10% blank contribution, is currently ~3 nmol of C/μL solvent. This analysis suggests that further progress toward improving detection limits is hindered more by sample-related blanks than by instrumental background noise.

Table 1. Typical Blank Sizes for the Moving-Wire System

blank	description	pmol of C
injection[a]	syringe + 2-mL vial	100 ± 40
glass wool	2 cm packed in glass pipet[b]	70
Na_2SO_4	anhydrous, 1.5 g in glass pipet[b]	50
silica gel	0.75 g in glass pipet[b]	4

		pmol of C/μL	
solvents	description	as received	redistilled[d]
water	Milli-Q system 1[c]	44	
water	Milli-Q system 2[c]	27	
acetonitrile	Fisher Optima grade	14	
methanol	Fisher GC Resolve	18	9
ethyl acetate	Fisher HPLC grade	4	3
methyl *tert*-butyl ether	Fisher HPLC grade	3	<1
chloroform	Fisher GC Resolve	35	5
toluene	Fisher Optima grade	7	
hexane	Fisher GC Resolve	3	<1

LC column bleed[e]	description	pmol of C/μL solvent
water	10 mL of eluant	4
acetonitrile	10 mL of eluant	<1
methanol	10 mL of eluant	21

[a] Measured by standard additions of ethyl acetate. [b] Combusted at 450 °C for 8 h prior to use. Material was rinsed with 10 mL of chloroform, which was concentrated 20-fold for analysis. [c] Millipore Milli-Q Ion free ultrapure cartridge kit CFIF01205 [d] Redistilled in sub-boiling still as described in text. [e] Tested column is a Zorbax eclipse XDB-C8, 4.6 mm × 150 mm. Column was precleaned with 50 mL of each solvent before collecting sample.

Operation as a Dropwise Detector. The moving-wire and other similar systems can be used as bulk analyzers, receiving discrete sample droplets, or as compound-specific analyzers that continuously receive a chromatographic effluent (e.g., ref 13). Given that liquid chromatographic peaks are typically >30 s wide, while discrete sample aliquots produce peaks ~5 s wide, there is a significant gain in signal/noise ratio when a chromatographic peak is collected, concentrated, and analyzed as a discrete sample ("dropwise" introduction). As a demonstration of this principle, we analyzed a chlorophyll *a* standard using reversed-phase, high-pressure liquid chromatography (HPLC) in two ways. With the HPLC column coupled directly to the moving wire in compound-specific mode,[13] chlorophyll peaks containing 77 nmol of C were >40 s wide, had signal/noise ratios of ~100, and produced measurements of δ^{13}C with a precision of 0.19‰ ($n = 5$). When the relevant chromatographic peak was instead collected in a vial, concentrated by evaporation, and then applied to the moving wire with a syringe, aliquots nearly 20-fold smaller, containing 4 nmol of C, produced peaks 5 s wide with a signal/noise ratio of >150 and comparable isotopic precision (0.17‰; $n = 9$). A minor disadvantage of this approach is that carbon blanks (from the HPLC, solvents, etc.) can no longer be subtracted as part of the background and must be accounted for by independent blank analyses.

Factors Affecting Accuracy. Numerous factors influence the accuracy of all continuous-flow isotope ratio measurements, including data processing,[20] standardization,[17] instrument tuning and linearity, and water background,[21]. These also affect the moving-wire analyzer but are not reviewed here. The element that is unique to the moving-wire system is combustion of analytes adsorbed on a metal wire surface that is heated only briefly. The system described by Brand and Dobberstein[13] used the oxide coating on the wire itself as the sole oxidant for combustion. Subsequent tests have revealed that the presence of inorganic salts in the sample solution can decrease the yield of CO_2 yield and increase the observed δ^{13}C value (Figure 7A). Such detrimental "matrix effects" increase with salt concentration (Figure 7B). This situation is unacceptable for many applications (such as preparative LC or electrophoresis) where inorganic buffers must be used.

Numerous organic fragments are present in the mass spectra of combustion gases from samples containing inorganic salts, indicating that the matrix effects are related to incomplete combustion of analytes. A prominent m/z 45 organic fragment is the direct cause of apparent changes in carbon isotopic ratio. The mechanism by which inorganic salts prevent complete combustion is currently unknown. Addition of O_2 gas to the combustion tube at a rate producing a ~3-V O_2^+ ion beam, a typical level for IRMS-GC/MS systems, provided little improvement. However, the addition of CuO as an oxidant to the combustion tube effectively eliminated all organic species from the combustion gases. Precise and accurate measurements of isotopic composition were possible with this modification, but the yield of CO_2 remained significantly lower than expected (Figure 7).

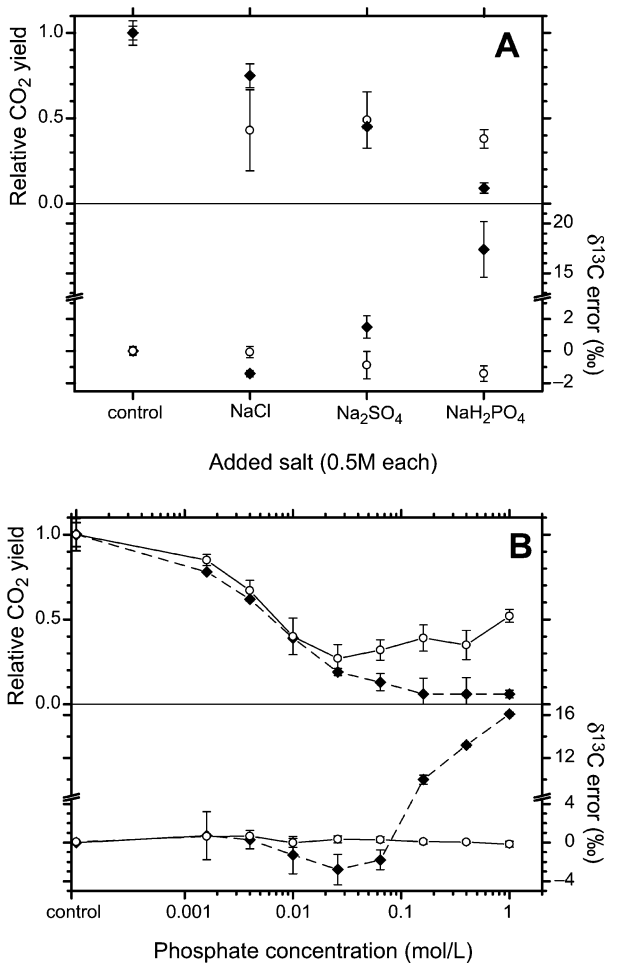

Figure 7. Matrix effects on the accuracy of C content and δ^{13}C measurements. Panel A: Changes in CO_2 peak area and δ^{13}C value relative to a control sample (in deionized water) with the addition of inorganic salts. Samples contained 100 ng/μL sodium lactate as the organic carbon source. Solid symbols indicate combustion with no supplemental oxidizing reagents; open symbols indicate combustion with CuO added to the combustion reactor. Panel B: Changes in CO_2 peak area and δ^{13}C value with changes in phosphate concentration. Samples contained 100 ng/μL sodium lactate as the organic carbon source. Solid vs open symbols are as above. Note breaks in scale for δ^{13}C values in both panels.

A second factor affecting combustion efficiency is the temperature of the combustion oven. For all analytes studied, both C yield (defined here as the collected mass-44 ion current per mass of introduced sample carbon) and δ^{13}C value increased systematically and reproducibly with combustion temperature (Figure 8). Delta values for all analytes except glucose were within 0.5‰ of offline values at combustion temperatures of 750−900 °C. Isotope ratio measurements for sugars, including glucose, were systematically less precise and accurate than those obtained from other types of analytes, perhaps due to elimination of CH_2O during the solvent evaporation step. Although higher combustion temperatures might normally be preferred, they also lead to higher CO_2 backgrounds. Combustion temperatures of ~750−800 °C represent an optimal compromise. Variations in combustion oven length were not tested here, but would presumably present the same compromise between increasing combustion efficiency and larger background signals. Wire speed also can affect combustion

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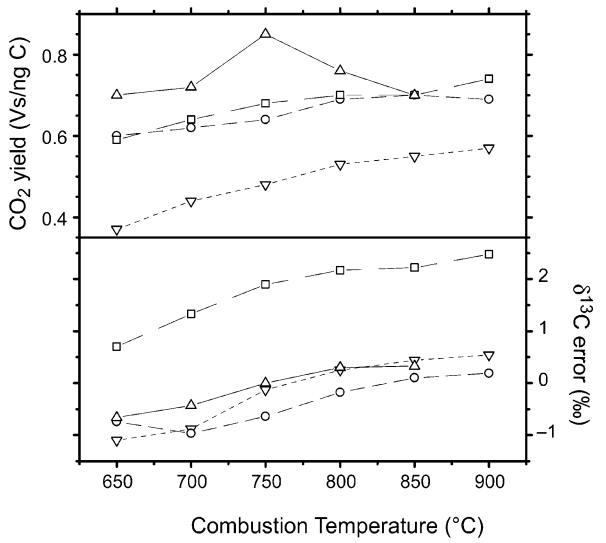

Figure 8. CO_2 yield and $\delta^{13}C$ value as a function of combustion temperature. CO_2 yield is reported as the integrated ion current per mass unit of sample carbon. Errors in $\delta^{13}C$ were determined by admitting CO_2 gas from offline combustion of the same compound into the inlet-system bellows of the IRMS and treating it as a reference peak with $\delta^{13}C = 0.0‰$. Symbols: (□) glucose; (○) epiandrosterone; (△) cholesterol; (▽) RNA.

Table 2. Comparison of Blank-Corrected Isotope Ratios Measured by EA and Moving Wire

analyte	EA $\delta^{13}C$[a]	MW $\delta^{13}C$ (1σ)[b]	EA anal. source[c]
adenosine	−7.20	−7.38 (0.21)	WHOI
adenosine-5-phosphate	−17.72	−17.12 (0.28)	WHOI
ribose	−19.80	−20.11 (1.2)	WHOI
ribose-5-phosphate	−13.47	−12.92 (0.37)	WHOI
yeast RNA	−22.69	−22.24 (0.23)	WHOI
glucose	−10.50	−9.93 (0.40)	MBL
cholestanol	−25.60	−25.51 (0.17)	MBL
sodium acetate	−31.95	−31.61 (0.21)	MBL
ribonuclease	−11.25	−10.92 (0.26)	MBL
cholesterol	−25.25	−25.47 (0.05)	UC Davis
perylene	−24.74	−24.27 (0.21)	UC Davis
hexachlorobiphenyl	−29.44	−29.3 (0.56)	UC Davis
phosphatidyl choline	−32.51	−32.32 (0.06)	UC Davis
phenylalanine	−11.36	−12.07 (0.61)	UC Davis
urban dust std	−25.45	−24.25 (0.18)	UC Davis
albumin	−16.08	−16.76 (0.33)	UC Davis

[a] Estimated uncertainty for offline EA analyses is ±0.2‰ based on replicate analyses of samples and standards. [b] Values in parentheses are $\pm1\sigma$ uncertainties for the blank-corrected delta values. [c] WHOI analyses by A. Gagnon, NOSAMS; MBL analyses by M. Otter, MBL Stable Isotope Laboratory; UC Davis analyses by David Harris, UC Davis Stable Isotope Facility.

efficiency, but only at speeds of >5 cm/s. Numerous other efforts to increase CO_2 yield, including addition of Pt catalyst and other solid oxidants to the combustion oven, or adjusting gas flow rates and composition, did not provide any appreciable improvement.

Carbon yields for different analytes vary significantly, both as a function of analyte composition and of combustion temperature. For example, RNA yields only 50−70% as much CO_2 per unit of sample carbon as does cholestanol. Because there is little associated isotopic fractionation, we hypothesize that the problem is desorption of molecules from the wire, rather than incomplete combustion of volatilized molecules. The net result is that C contents measured by the moving wire can depend on analyte composition, sample matrix (particularly inorganic salts), and instrumental operating parameters. Most of these effects can be minimized by carefully matching samples and calibration standards.

To demonstrate the accuracy of the moving-wire system for a range of sample compositions, 16 different standards were analyzed by EA at other laboratories to provide results that could be compared to those obtained using the moving wire (Table 2). Test compounds included sugars, lipids, nucleosides and nucleotides, amino acids and proteins, a salt of a carboxylic acid, a polycyclic aromatic hydrocarbon, and a polychlorinated biphenyl. One standard (NIST1649a, an urban dust reference material) tested the suitability of the moving wire for analyses of suspended, rather than dissolved, analytes. We interpret the relatively large error (1.2‰) for this sample as indicative of problems with differential settling in a heterogeneous population of particles. Further refinements in sampling technique will likely be needed to improve the reliability of the moving wire for suspensions of insoluble material. In general, results of EA and moving-wire analyses agree within 0.5‰. The root-mean-square error for all analytes is 0.46‰. This initial survey demonstrates that isotopic analyses with the moving wire are applicable to most, if not all, organic substances that can be dissolved in a volatile solvent. While the failure to achieve quantitative conversion of analytes to CO_2 remains unexplained, we have not yet identified any analytes for which it causes isotopic fractionation.

CONCLUSIONS

The moving-wire analyzer can provide rapid, precise, and highly sensitive measurements of $\delta^{13}C$ for most or all nonvolatile organic compounds. As such, it provides a new method for determining the ^{13}C content of individual biochemicals that have been purified preparatively by HPLC or other chromatographic methods. Characteristics of the moving-wire analyzer include the following:

1. Measurement of $\delta^{13}C$ values with a precision of <0.2‰ (1σ) for samples containing ∼10 nmol of C, and ∼1‰ for samples containing <1 nmol of C.

2. Rapid analyses of very small liquid samples (∼1 sample every 30−40 s).

3. Accuracy for $\delta^{13}C$ values better than 0.5‰ for all dissolved analytes tested, including lipids, sugars, nucleotides, proteins, and hydrocarbons. Suspended samples can be problematic if settling is rapid (time scales of minutes).

4. Measurements of sample C content, while reproducible (∼10% relative), are also strongly dependent on analyte composition and sample matrix. Quantitative estimation of carbon content is possible through careful standardization, though for most samples the moving wire should be considered semiquantitative.

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