

Technical Notes

Accelerator Mass Spectrometry Targets of Submilligram Carbonaceous Samples Using the High-Throughput Zn Reduction Method

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The high-throughput Zn reduction method was developed and optimized for various biological/biomedical accelerator mass spectrometry (AMS) applications of mg of C size samples. However, high levels of background carbon from the high-throughput Zn reduction method were not suitable for sub-mg of C size samples in environmental, geochronology, and biological/biomedical AMS applications. This study investigated the effect of background carbon mass (m_c) and background ^{14}C level (F_c) from the high-throughput Zn reduction method. Background m_c was 0.011 mg of C and background F_c was 1.5445. Background subtraction, two-component mixing, and expanded formulas were used for background correction. All three formulas accurately corrected for backgrounds to 0.025 mg of C in the aerosol standard (NIST SRM 1648a). Only the background subtraction and the two-component mixing formulas accurately corrected for backgrounds to 0.1 mg of C in the IAEA-C6 and -C7 standards. After the background corrections, our high-throughput Zn reduction method was suitable for biological (diet)/biomedical (drug) and environmental (fine particulate matter) applications of sub-mg of C samples (≥ 0.1 mg of C) in keeping with a balance between throughput (270 samples/day/analyst) and sensitivity/accuracy/precision of AMS measurement. The development of a high-throughput method for examination of ≥ 0.1 mg of C size samples opens up a range of applications for ^{14}C AMS studies. While other methods do exist for ≥ 0.1 mg of C size samples, the low throughput has made them cost prohibitive for many applications.

Accelerator mass spectrometry (AMS) measures long-lived radioisotopes such as ^{14}C for geochronology, environmental, and biological/biomedical applications at attomole (amol, 10^{-18}) to zeptomole (zmol, 10^{-21}) level sensitivity,¹ which makes AMS

several orders of magnitude more sensitive over liquid scintillation counting.^{2–4} Preparation of carbonaceous samples for AMS involved oxidizing the carbon to CO_2 and reducing the CO_2 to graphite or graphitelike materials; the process is called “graphitization”, and the prepared sample is referred to as an AMS target. The CO_2 reduction method frequently used H_2 or Zn as the reductant.^{2,5–7} Biological/biomedical ^{14}C AMS applications were typically conducted on samples containing 1.0 mg of C for high-throughput at the expense of sensitivity. While radiocarbon dating, geo-chronology, or environmental applications of AMS were often performed on samples containing submilligram quantities of carbon (sub-mg of C), μg of C size samples were also analyzed at high sensitivity but at the expense of high throughput.^{7–11} However, the high-throughput Zn reduction method using a septa-sealed vial (HT Zn reduction method in present study) were not suitable for sub-mg of C size biological/biomedical/environmental samples of AMS due to background carbon from reagents (i.e., impurities in CuO, Zn dust, Fe, or Co)¹² and/or from quartz/Pyrex glassware¹² used in graphitization.

Modified graphitization methods that baked/cleaned the reagents and glassware minimized background carbon mass (m_c)

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and ^{14}C .^{6,13} Even after baking/cleaning reagents and glassware, the background m_c ranged from 0.001 to 0.011 mg of C, and background ^{14}C levels referred to as Fraction Modern of background carbon (F_c) ranged from 0.2 to 0.6.^{13–16}

To correct for background of sub-mg of C size samples, three correction formulas were frequently used.^{13,16,17} The correction formulas included background subtraction,^{13,16} two-component mixing,^{13,16} and the expanded formula.¹⁷

The HT Zn reduction method was first developed for biological/ biomedical applications of 1.0 mg of C size samples.⁷ The HT Zn reduction method was optimized for CO_2 reduction conditions to achieve reliable ion currents, accurate/precise measurement, and/or high-throughput.⁸ The HT Zn reduction method produced a mix of graphitizable carbon (lacking crystallinity) and Fe_3C rather than crystalline graphite.^{8,18} The HT Zn reduction method¹⁴ reliably measured samples containing ≥ 0.5 mg of C; furthermore, another study⁷ measured samples as small as 0.25 mg of C. Samples as small as 0.02 mg of C were measured using the Zn reduction method,¹ which modified the CO_2 transfer and reduction conditions. The longer pumping (≥ 1 h before the CO_2 gas transfer) and CO_2 reduction times (18 h) reduced sample throughput.¹

The use of the gas-fed AMS ion source has reduced samples down to 0.001 mg of C for radiocarbon dating and biological/ biomedical/environmental applications of AMS.^{2,19} However, the gas-fed ion source experienced sample to sample contamination and lower ion current compared to solid AMS targets.^{2,20}

The possibility of optimizing the balance between high-throughput and sensitivity/accuracy encouraged us to investigate the feasibility of sub-mg of C size samples using the HT Zn reduction method.⁸ We were also prompted to fill the gap that exists concerning isotopic fractionation, background levels, and background corrections of sub-mg of C size samples using the HT Zn reduction methods.⁸ The present work tested the feasibility of the HT Zn reduction method for biological/biomedical/ environmental ^{14}C -AMS applications on sub-mg of C size samples.

EXPERIMENTAL SECTION

Reagents. Australian National University sucrose (ANU, IAEA-C6) and oxalic acid (Ox, IAEA-C7) were from the International Atomic Energy Agency (IAEA), Vienna, Austria. The oxalic acids [Ox (IAEA-C7) and Ox-2 (NIST SRM 4990C, oxalic acid-II)] are very hygroscopic ($\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), so it takes ≈ 5.3 mg of Ox and Ox-2 to supply ≈ 1.0 mg of C. Aerosol standard material (NIST SRM 1648a) was from National Institute of Standards

and Technology, Gaithersburg, MD. The SRM 1648a (having 2.3% moisture) was stored in a desiccator for ≥ 2 days with magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$) to eliminate moisture. Prior to use, the SRM 1648a was shaken/mixed to obtain a representative mix of its carbonaceous components. All other reagents and supplies used in this study were the same as we have described previously.^{8,21}

Procedures. Seven carbonaceous samples containing 0.025–1.2 mg of C were converted to the AMS targets.⁸ Graphitization yield (%) and isotopic fractionation ($\delta^{13}\text{C}$, ‰) of solid samples were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20/20 isotope ratio mass spectrometer (EA-IRMS, Sercon Ltd., Cheshire, U.K.).²² Three laboratory standards (NIST 1547 peach leaves, glycine, sucrose) were included with every batch of 12 samples. NIST 1547 peach leaves, glycine, and sucrose were each calibrated against NIST SRM (IAEA-N1, IAEA-N2, IAEA-N3, IAEA-CH7, and NBS-22). Gas samples (CO_2) from the combustion step were cryogenically transferred to 12 mL soda glass vials (16.5 mm o.d. \times 101 mm height, Labco, Houston, TX), and the $\delta^{13}\text{C}$ of gas samples was measured by a Europa GEO 20/20 mass spectrometer fitted with a dual inlet system (Sercon Ltd., Cheshire, U.K.).

The graphitization yield was calculated as

$$\text{graphitization yield (\%)} = \frac{\text{mg of C by EA-IRMS}}{\text{mg of C by gravimetry}} \times 100$$

where gravimetry was performed using a Mettler Toledo MT5 microbalance (Mettler-Toledo Inc., Columbus, OH) with an accuracy of $\pm 0.0001\%$ and a precision of $\pm 0.00015\%$. The microbalance was calibrated using a reference calibrator (Troemner, no. 18723, Thorofare, NJ) prior to each use.

The $\delta^{13}\text{C}$ were calculated as

$$\delta^{13}\text{C}(\text{‰}) = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} \times 1000$$

where VPDB referred to the Vienna-Pee Dee Belemnite.²³

The ^{14}C level (F_m , see the Supporting Information) in samples was measured at the Center for Accelerator Mass Spectrometry (CAMS), Lawrence Livermore National Laboratory. The background m_c was calculated¹⁶ as

$$\frac{m_c}{m_s + m_c} = 1 - \left\langle \frac{R_{\text{OX-2}}(m_s)}{R_{\text{OX-2}}(1 \text{ mg of C})} - \frac{R_b(m_s)}{R_{\text{OX-2}}(1 \text{ mg of C})} \right\rangle$$

where m_c = background carbon mass; m_s = sample mass; R = measured $^{14}\text{C}/^{13}\text{C}$ (Fraction Modern or F_m); R_b = F_m of ^{14}C -free sample; $R_{\text{OX-2}}$ = F_m of Ox-2

The background F_c was calculated¹⁶ as

$$\frac{R_c^A}{R_{\text{OX-2}}^A} = \left\langle 1 + \left\langle \left\langle 1 - \frac{R_{\text{OX-2}}(m_s)}{R_{\text{OX-2}}(1 \text{ mg of C})} \right\rangle + \left\langle \frac{R_b(m_s)}{R_{\text{OX-2}}(1 \text{ mg of C})} \right\rangle \right\rangle^{-1}$$

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Table 1. Characteristics of ^{14}C -AMS Applications Using the High-Throughput Zn Reduction Method^b on Submilligram Size Carbonaceous Samples

carbonaceous sample mass, mg of C ^a	graphitization yield, % ^b	isotopic fractionation ($\delta^{13}\text{C}$), ‰ ^b	ion current, μAmps ^b		calcd background m_c , μg of C ^c	calcd background F_c , modern ^c
			$^{12}\text{C}^-$	$^{13}\text{C}^+$		
0.1–1.0	85–100 ($n = 218$)	–2.5 to 0.75 ($n = 245$)	≈ 140	≈ 0.55 ($n = 387$)	11.6 ± 5.0 ($n = 161$)	1.5445 ± 0.2223 ($n = 161$)

^a Carbon mass was measured by gravimetry (Mettler Toledo MT5 microbalance, Mettler-Toledo Inc., Columbus, OH). ^b See Figures 1–3 in the Supporting Information. ^c Values are mean \pm SD.

where R_c^A = calculated background ^{14}C level, F_c ; $R_{\text{Ox-2}}^A$ = accepted F_m of Ox-2 (1.3407); all remaining abbreviations are the same as above for background m_c . The background corrections were preformed using three different methods: (1) background subtraction,^{13,16} (2) two-component mixing,^{13,16} and (3) expanded formulas.¹⁷

RESULTS AND DISCUSSION

mg of C to Sub-mg of C Samples: Graphitization Yield, Isotopic Fractionation, and Ion Currents. The ratios of C/Fe, C/H₂, C/Fe/Zn (and/or TiH₂), catalyst type/size, reduction time, or temperature are well-known to affect graphitization yield, isotopic fractionation ($\delta^{13}\text{C}$), and/or the ion current of graphitized sample.^{6–8,13,24–26} With the use of about 1 mg of C, our HT Zn reduction method⁸ not only enabled up to 270 samples per day to be prepared but it also achieved graphitization yields of 84–93% and $\delta^{13}\text{C}$ shift of –1.6 to 0.3‰ (see Table 1 in the Supporting Information), which was consistent with previously published graphitization yields and $\delta^{13}\text{C}$ shift except for about a 5-fold lower throughput.^{5,8,11}

During the HT Zn reduction method, Zn dust served as a reductant to convert CO₂ to CO. The Zn dust also generated H₂ from water (H₂O_(g) + Zn_(s) \rightleftharpoons H_{2(g)} + ZnO_(s)). Finally, CO/CO₂ was reduced to C_{graphite} with H₂.^{8,27} Water must be completely eliminated or converted to H₂, to maximize carbon deposition,²⁸ i.e., graphitization yield. Excess H₂ thermodynamically favored CH₄ formation,^{5,25,26,28} consequently decreased graphitization yield. With the use of the HT Zn reduction method, sample to sample differences in elemental composition make it difficult to control for the amount of water and/or H₂.⁵ Differences in the amount of water and/or H₂ can affect graphitization yield for our short (3 h) graphitization period.⁸ However, in the present study, samples which had C/H ratios of 1:0–1:3 produced graphitization yield of 84–93%, which was suitable for HT ^{14}C -AMS measurement (see the Supporting Information, Table 1). Samples rich in sulfur (S) produced SO₂ which could also decrease the graphitization yield.²⁷ Although blood plasma or SRM 1648a had elements like S, graphitization yield was 84–85% which was also suitable for HT ^{14}C -AMS measurement (see Table 1 in the Supporting Information). Thus, water vapor and sulfur levels did not have a significant effect on graphitization

yields (84–93%) from about 1 mg of C, and therefore the HT Zn reduction method was suitable for HT ^{14}C -AMS measurement.

For sub-mg of C size samples, we tested the hypothesis that such samples could also be analyzed with good graphitization yield, $\delta^{13}\text{C}$, and reliable ion currents using the HT Zn reduction method. In the present study, samples above the cutoff of 0.1 mg of C (≥ 0.1 mg of C) poorly correlated with graphitization yield, $\delta^{13}\text{C}$, and ion currents (see Figures 1–3 in the Supporting Information). Thus, samples of ≥ 0.1 mg of C had graphitization yields of 85–100%, $\delta^{13}\text{C}$ shift of –2.5 to 0.75‰, and reliable ion currents ($^{12}\text{C}^-$ of $\approx 140 \mu\text{A}$ and $^{13}\text{C}^+$ of $\approx 0.55 \mu\text{A}$) (Table 1). In sharp contrast, samples below cutoff of 0.1 mg of C (< 0.1 mg of C) had highly variable graphitization yield, mass-dependent with large $\delta^{13}\text{C}$ shift, and mass-dependent with low ion currents (see Figures 1–3 in the Supporting Information).

With respect to graphitization yield, as carbon mass decreased at < 0.05 mg of C, the yield also decreased for lack of complete graphitization reactions⁹ caused by low CO₂ pressure which led to a less favorable kinetic condition. However, in the present study, graphitization yield at < 0.1 mg of C varied from 30 to 230% (not mass-dependent). The variable graphitization yield was due to incomplete graphitization reactions, large background m_c , or both (see Figure 1 in the Supporting Information).

With respect to isotopic fractionation ($\delta^{13}\text{C}$), the $\delta^{13}\text{C}$ (during the graphitization) varied with incomplete graphitization reactions, contaminants, or both.^{5,9,29,30} In the present study, the $\delta^{13}\text{C}$ shift of ≥ 0.1 mg of C was within –2.5 to 0.75‰ (Table 1), which was more precise than the $\delta^{13}\text{C}$ shift (–8 to –1‰) in the previous studies.^{5,6,26,27,31} This difference of $\delta^{13}\text{C}$ shift between the present study and previous studies^{5,6,26,27,31} resulted from different graphitization conditions such as reduction temperature/time, catalyst type/size, etc. Larger $\delta^{13}\text{C}$ shift was also experienced with an excess mix of TiH₂ and Zn dust (> 50 mg of Zn/mg of C) by Xu et al.⁵ Unlike the prior study,⁵ our HT Zn reduction method used 100 mg of Zn for ≥ 0.1 mg of C but did not experience a large $\delta^{13}\text{C}$ shift because our HT Zn reduction method used only Zn dust rather than an excess mix of TiH₂ and Zn dust. Furthermore, in the present study (see Figure 2 in the Supporting Information), $\delta^{13}\text{C}$ was mass-dependent at < 0.1 mg of C but not at ≥ 0.1 mg of C, which was comparable to the prior study of Hua et al.⁹

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Ion currents were mass-dependent at <0.1 mg of C (see Figure 3 in the Supporting Information), which was consistent with studies by other researchers.^{6,30} A long-lasting/reliable ion current from ≈ 1.0 mg of C (C^- of $>100 \mu A$) was usually required for HT biological/biomedical AMS measurement to have a precision of $\approx 1\%$.³² Because of the low ion currents of <0.1 mg of C, our HT Zn reduction method was not suitable for accurate/precise HT AMS measurements (see Figure 3 in the Supporting Information). In addition, the amount of Fe powder also affected ion currents, because Fe powder served as a metal binder (for transferring thermal/electrical energy between Cs^+ and the surface of the AMS target) during the ionization with Cs^+ sputter. Furthermore, our results (Table 1) were consistent with the prior observations that 4–5 mg of Fe powder favored the production of longer and more reliable ion currents than did 2 mg of Fe powder.^{5,6}

In summary, graphitization yield, $\delta^{13}C$, and ion currents from ≥ 0.1 mg of C were feasible for HT AMS measurements using the HT Zn reduction method. However, background correction for ≥ 0.1 mg of C may be needed to further improve the accuracy of HT AMS measurements.

Background Carbon Mass, ^{14}C Levels, and Background Corrections. In the present study, the correction curve using the graphite standard¹⁸ (GST, ^{14}C deficient sample, >46 600-year-old) was mass-dependent, so that the F_m of the GST (Figure 1A) was increased as carbon mass decreased, and this was consistent with prior studies.^{13,16} The correction curve using the Ox-2 (Figure 1B) was also mass-dependent; however, the pattern of the Ox-2 correction curve was opposite that in prior studies.^{13,16} Because the background F_c was higher than the accepted F_m of the Ox-2 (1.3407), the F_m of the Ox-2 in the present study was increased as carbon mass decreased.

With the use of the GST and Ox-2 correction curves (Figure 1), background m_c was calculated to be $11.6 \pm 5.0 \mu g$ of C, and background F_c was calculated to be 1.5445 ± 0.2223 (Table 1). Our HT Zn reduction method for biological/biomedical studies had 5–20-fold higher background m_c and ≥ 2 -fold higher background F_c than did the H_2 reduction method for radiocarbon dating or environmental studies.^{6,13,15,16} Furthermore, background F_c in the present study was ≈ 2.3 -fold higher than that (0.687 ± 0.046) in another HT Zn reduction method,¹⁴ although background m_c in the present study was similar to that ($10.7 \pm 0.6 \mu g$ of C) in the other study.¹⁴ Our high background F_c possibly originated from graphitization facilities, reagents, glasswares, or all of the above.

First, we monitored background ^{14}C levels in our graphitization facilities using the swipes (using tributyrin, product no. 40971, MP Biomedicals) and aerosol/vapor contaminants trap (using fullerene soot, product no. 40971, Alfa Aesar); according to the criteria,³³ our graphitization facilities were satisfactory (swipes, <50 amol of ^{14}C /mg of C, air trap, <2.5 amol of ^{14}C /mg of C).

Second, we measured background m_c , $\delta^{13}C$, and F_c of three reagents (CuO wire, Fe powder, Zn dust) used in graphitization. Among the three reagents, the CuO wire and the Fe powder

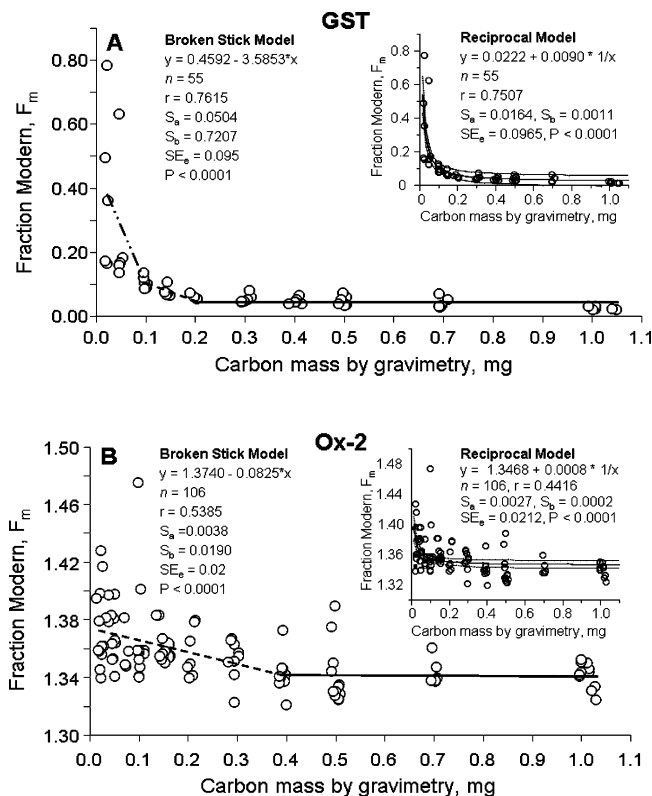


Figure 1. Correction curves using GST (A) and Ox-2 (B) each contained 0.025–1.0 mg of C. Carbon mass by gravimetry was determined using the Mettler Toledo MT5 microbalance (Mettler-Toledo Inc., Columbus, OH). The $\delta^{13}C$ by EA-IRMS was used to correct F_m . The broken stick model was a little better fit than the reciprocal model. With the use of the broken stick models, the cutoff mass was ≥ 0.2 mg of C in the GST or ≥ 0.4 mg of C in the Ox-2 for high throughput and accurate AMS measurements prior to the background correction. S_a , S_b , and SE_e refer to the standard error of the intercept, slope, and estimate, respectively.

Table 2. Background Carbon Mass (m_c), Background $\delta^{13}C$ Measured by EA-IRMS, and Background ^{14}C levels (F_c) Measured by AMS of Reagents Used in Graphitization^a ($n \geq 10$)

reagents	backgrounds		
	$m_c, \mu g^a$	$\delta^{13}C, \text{‰}^a$	$^{14}C, F_c^b$
CuO wire, baked, 500 °C, 2 h (500 mg)	3.0 ± 0.4	-28.1 ± 1.7	nonmeasurable ($n = 24$)
Fe powder, nonbaked (5 mg)	1.8 ± 0.3	-31.0 ± 2.4	for low ion current ($^{13}C^+$ of ≤ 30 nA)
Zn dust, nonbaked (100 mg)	12.6 ± 9.2	-17.5 ± 4.2	

^a Values are mean \pm SD. ^b After the combustion step, background m_c of ≈ 500 mg of CuO (no carbonaceous test sample) was cryogenically transferred into a septa-sealed vial that contained 5 mg of Fe and 100 mg of Zn dust.⁸ The F_c represented Fraction Modern of background carbons in the three reagents only (no carbonaceous test sample). The F_c was measured after graphitization using only the above three reagents as a blank.

together contributed $5 \mu g$ of C background m_c . The Zn dust contributed an additional $12.6 \pm 9.2 \mu g$ of C background m_c , for a total of background m_c of $\approx 18 \mu g$ of C (Table 2). Other studies reported that sample combustion process was the main contributor of contamination,²⁴ and background m_c from 500 mg of CuO ($m_c = 8.9 \mu g$ of C) was larger than that from 2 mg of

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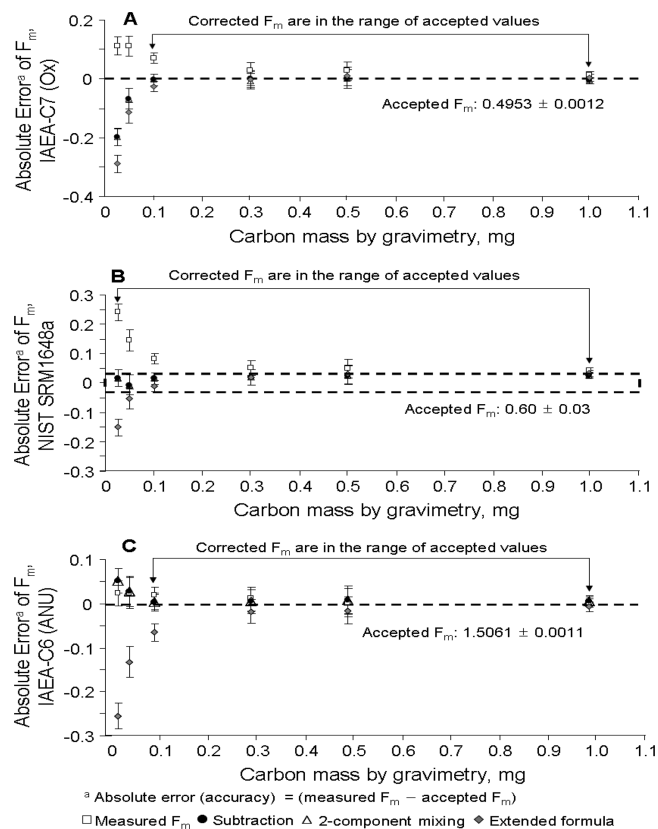


Figure 2. Comparison of measured F_m (empty square) versus corrected F_m of Ox ($n \geq 8$, part A), NIST SRM 1648a ($n \geq 6$, part B), and ANU ($n \geq 8$, part C) each containing 0.025–1.0 mg of C. Carbon mass by gravimetry was determined using the Mettler Toledo MT5 microbalance (Mettler-Toledo Inc., Columbus, OH). The horizontal rectangle with broken lines in parts A–C represented the accepted $F_m \pm$ SD of the three carbonaceous standards. A zero value on the y-axis means that accepted and measured F_m values were identical. Background corrections were performed with background subtraction (\bullet),^{13,16} two-component mixing (Δ),^{13,16} and expanded formula (\blacklozenge).¹⁷ The $\delta^{13}\text{C}$ by EA-IRMS was used to correct F_m . Error bars indicate standard deviation (absolute precision). An accuracy (relative error, RE = (absolute error/accepted F_m) \times 100) and a precision (% relative precision, % RSD = (SD of measured F_m /mean F_m) \times 100) were calculated using absolute error and standard deviation of measured F_m .

Fe ($m_c = 0.1\text{--}0.2 \mu\text{g}$ of C).¹² Therefore, background m_c seemed to be dependent on the sources of reagents. Besides, the calculated background m_c ($11.6 \pm 5.0 \mu\text{g}$ of C) using the GST and Ox-2 correction curves was $\approx 35\%$ less than the total of measured background m_c ($\approx 18 \mu\text{g}$ of C), indicating $\approx 65\%$ background m_c from three reagents was contributed by the AMS targets.

The $\delta^{13}\text{C}$ of three reagents ranged from -31.0 to -17.5% , and the $\delta^{13}\text{C}$ of Zn dust was higher than the $\delta^{13}\text{C}$ of the Fe powder and of the baked CuO wire. The F_c (reagents only) was typically nonmeasurable due to insufficient ion current; nevertheless, the reagents alone occasionally ($n = 6$ of 30) measured the F_c of 1.1999 ± 0.0262 due to their background m_c and F_c (Table 2).

Hence, because of high background m_c and F_c , the F_m of the sub-mg of C sample was increased. Effects of background m_c and F_c are significantly increased in sub-Modern/sub-mg of C samples (Figure 2A,B) compared to high-Modern/sub-mg of C

sample (Figure 2C). The F_m of sub-mg of C was more accurately corrected using subtraction and two-components mixing methods which are consistent with prior studies.^{13,16} The expanded formula was less accurate in correcting the F_m of sub-mg of C, and corrected F_m values were significantly lower than those using subtraction and two-component mixing methods. Our results seemed to be due to a high level of background contamination during graphitization compared to other studies.^{13,16}

With the use of subtraction and two-component mixing methods, the corrected F_m of the Ox had an accuracy (relative error, RE = (absolute error/accepted F_m) \times 100) of -1.39 to 0.45% and a precision (% relative standard deviation, % RSD = (SD of measured F_m /mean F_m) \times 100) of $2.23\text{--}6.38\%$ at ≥ 0.1 mg of C (Figure 2A). Although the corrected F_m of SRM1648a had less accuracy (-1.03 to 4.45%) and low and variable precision ($0.94\text{--}23.27\%$) at ≥ 0.025 mg of C (Figure 2B), the corrected F_m of SRM1648a was in the accepted F_m range to 0.025 mg of C, due to its greater uncertainty for the accepted F_m (0.60 ± 0.03). Furthermore, the corrected F_m of ANU had a good accuracy ($0.22\text{--}0.54\%$) and a good precision ($0.79\text{--}1.36\%$) at ≥ 0.1 mg of C (Figure 2C) compared to those of the Ox and SRM 1648a, because accepted F_m (1.5061) of ANU was similar to background F_c (1.5445).

After background corrections, the difference of carbon mass cutoff (0.1 versus 0.025 mg of C) was likely to be due to different and variable amounts of background m_c and F_c from reagents used in the graphitization. In the present study, the GST (Figure 1A), Ox-2 (Figure 1B), and SRM 1648a (Figure 2B) were analyzed using the Zn dust from the same bottle (but at different times), while the Ox (Figure 2A) and ANU (Figure 2C) were analyzed using the Zn dust from a new bottle (but at different times). Among Zn dust bottles, background m_c ranged from 5.0 to $25.0 \mu\text{g}$ of C. Therefore, background corrections of <0.1 mg of C of the Ox (Figure 2A) and ANU (Figure 2C) were less accurate than those of the SRM 1648a (Figure 2B). Thus, further cleaning (baking) of reagents and glasswares should minimize sample size of the HT Zn reduction method to 0.025 mg of C.

In conclusion, the HT Zn reduction method had a mean background m_c of 0.011 mg of C and a mean background F_c of 1.5445 . After background corrections, this method was applicable for accurate ^{14}C -AMS measurement of ≥ 0.1 mg of C size samples with the traditional subtraction and the two-component mixing correction formulas. The HT Zn reduction method produced $^{12}\text{C}^-$ of $\approx 140 \mu\text{A}$, graphitization yield of $85\text{--}100\%$, $\delta^{13}\text{C}$ of $-2.5\text{--}0.75\%$, and precision of $\leq 1.0\%$ at ≥ 0.1 mg of C size samples and throughput of up to 270 samples/day/skilled analyst.

Development of the HT Zn reduction method for examination of ≥ 0.1 mg of C size samples opened up a range of applications for ^{14}C AMS studies. While other methods existed for ≥ 0.1 mg of C size samples, the low throughput has made the cost prohibitive for many applications. The extreme sensitivity of ^{14}C -AMS allowed ^{14}C -labeled drug candidates and food components such as vitamins and phytochemicals to be studied *in vivo* in humans.^{34,35} Finally, the present study enabled acquisition of absorption, distribution, metabolism, and elimination data for

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¹⁴C-labeled drug candidates in phase 0 clinical trials and food components in order to construct kinetic and/or dynamic models of their metabolisms.

The lower cost of analysis for ≥ 0.1 mg of C size samples of the method opened up ¹⁴C-AMS to nonbiological applications. The ill effects of aerosol pollutants^{36–39} required an understanding of their sources and chemistries in order to regulate/control exposure to dangerous aerosols. ¹⁴C-AMS studies have been performed on atmospheric aerosols to determine fossil versus modern carbon sources.^{40–42} However, the smaller size samples and reduced cost of the presented method will allow for studies in which the aerosol is size fractionated prior to analysis and followed over an extended period of time, for example, the World Trade Center (WTC) study. The reduced cost and reduced sample size would allow for analysis of aerosols with shorter collection

times, yielding higher time resolution in the data. The reduced sample size of the presented method may open up applications in forensics, art validation, or other fields where sample size is limited. Thus, the present study is important because it optimized the balance between high throughput and sensitivity/accuracy after background corrections for sub-mg of C size samples.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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