Biological/Biomedical Accelerator Mass Spectrometry Targets. 1. Optimizing the CO₂ Reduction Step Using Zinc Dust

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Biological and biomedical applications of accelerator mass spectrometry (AMS) use isotope ratio mass spectrometry to quantify minute amounts of long-lived radioisotopes such as ¹⁴C. AMS target preparation involves first the oxidation of carbon (in sample of interest) to CO₂ and second the reduction of CO₂ to filamentous, fluffy, fuzzy, or firm graphite-like substances that coat a ~400-mesh spherical iron powder (~400MSIP) catalyst. Until now, the quality of AMS targets has been variable; consequently, they often failed to produce robust ion currents that are required for reliable, accurate, precise, and high-throughput AMS for biological/biomedical applications. Therefore, we described our optimized method for reduction of CO₂ to high-quality uniform AMS targets whose morphology we visualized using scanning electron microscope pictures. Key features of our optimized method were to reduce CO₂ (from a sample of interest that provided 1 mg of C) using 100 (1.3 mg of Zn dust, 5 (0.4 mg of ~400MSIP, and a reduction temperature of 500 °C for 3 h. The thermodynamics of our optimized method were more favorable for production of graphite-coated iron powders (GCIP) than those of previous methods. All AMS targets from our optimized method were of 100% GCIP, the graphitization yield exceeded 90%, and δ¹³C was −17.9 ± 0.3‰. The GCIP reliably produced strong ¹²C⁻ currents and accurate and precise Fm values. The observed Fm value for oxalic acid II NIST SRM deviated from its accepted Fm value of 1.3407 by only 0.0003 ± 0.0027 (mean ± SE, n = 32), limit of detection of ¹⁴C was 0.04 amol, and limit of quantification was 0.07 amol, and a skilled analyst can prepare as many as 270 AMS targets per day. More information on the physical (hardness/color), morphological (SEMs), and structural (FT-IR, Raman, XRD spectra) characteristics of our AMS targets that determine accurate, precise, and high-throughput AMS measurement are in the companion paper.

Accelerator mass spectrometry (AMS) is known for its exquisite sensitivity in quantifying ¹⁴C. AMS uses tandem mass spectrometry to quantify long-lived radioisotopes with attomolar sensitivity at a precision of ±1%. This ability is especially useful in conducting ¹³C tracer studies in biological/biomedical systems.¹–³ In order for AMS to measure ¹³C in biologic specimens, they were converted to graphite or possibly a mix of closely related forms of carbon such as amorphous carbon, iron carbide, and graphite (that we characterize in part 2). AMS target preparation begins by first converting sample carbon into carbon dioxide gas and then reducing the CO₂ to graphite that coats over metal catalysts. Previously used reduction methods include thermal and radiofrequency (rf) cracking of hydrocarbon⁴,⁵ and a glow-discharge (DC) in CO/H₂,⁶ but now they are not widely used for AMS.

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Currently popular reductants for AMS are \(\text{H}_2\) or Zn (\(\text{Zn} + \text{CO}_2 = \text{ZnO} + \text{CO}\), \(\text{Zn} + \text{H}_2\text{O} = \text{ZnO} + \text{H}_2\)) because either reductant produces improved AMS measurements. Use of Zn as a reductant was first reported for carbon dating.\(^7\)\(^8\) Time required to reduce \(\text{CO}_2\) to graphite using Zn as reductant ranged from 2 to 10 h depending on the sample size, the temperature, and the type and the size of catalyst used. AMS applications in geochronology and archeology typically require great sensitivity and precision but not necessarily a high throughput, while biological/biomedical archeology typically require less sensitivity but accuracy and high throughput are essential.\(^9\)\(^10\)\(^11\)

The method for biological/biomedical AMS application\(^12\) used flame-sealed quartz tube containing a mix of Zn dust plus titanium hydride (\(\text{TiH}_2\)) as reductant. The method also used a disposable \(\text{CO}_2\) gas-transfer system to minimize sample-to-sample contamination. High throughput was considered to be \(\sim 60\) samples per d/skilled analyst.\(^12\) A recent method used a mix of prebaked \(\text{TiH}_2\) and Zn dust to increase sensitivity for radiocarbon dating, but throughput was 40 samples per d/analyst.\(^13\) The flame-sealed quartz tube was replaced with a septa-sealed vial, which increased the throughput to 150 samples per d/skilled analyst.\(^14\) The method\(^14\) used 2–3 mg of \(-400\) mesh spherical Fe powder (\(-400\)MSIP), a temperature of 500 °C for 4 h, and the AMS targets made by this method produced a satisfactory ion current but the fraction modern (\(F_m\)) values were still variable in our experience. So, we modified the method\(^14\) to mitigate variability in the \(F_m\) values by raising the amount of \(-400\)MSIP to 10 mg, the temperature to 525 °C, and the time to 6 h. We refer to the modified method as “our previous method”.\(^15\) Eighty percent of the time our previous method\(^15\) produced AMS targets of graphite-coated Fe (GCI) and 20% of the time it produced AMS targets of a gray-colored iron—carbon material (ICM) instead of GCI. AMS targets of GCI produced a reliable ion current of \(-115\) μA \(^{13}\text{C}/\text{mg}\) of C while AMS targets of ICM produced an ion current that was only \(87\)% as strong as AMS targets of GCI. Furthermore, AMS targets of ICM and of GCI were hard and consequently were difficult to tamp into AMS target holders. AMS targets of metal carbide, especially cobalt carbide, produced low ion currents with a variability of 5–10% in the accompanying \(14\text{C}^{13}\text{C}^+\) ratios.\(^12\) The reliability and variability of ion currents and \(F_m\) values along with the limited throughput prompted us to further optimize the preparation of AMS targets\(^12\)\(^14\)\(^15\) for biological/biomedical applications.

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**EXPERIMENTAL SECTION**

**Reagents.** The reductant (Zn dust, CAS No. 7440–66–6, \(<10\) μm, 98+% ) was purchased from Sigma-Aldrich (St. Louis, MO). The five catalysts (three of Fe and two of Co) differed from one another in type, particle size, and particle shape. The \(-400\)MSIP was from Sigma-Aldrich (CAS No. 7439–89–6, 99.99+% ); it was used as the reference catalyst.\(^13\) The \(-325\) mesh, irregular Fe, \(\text{H}_2\) reduced (Catalog No. FE-110, 99.8%) and the \(-50\)/+100 mesh, irregular \(\text{FeH}_2\) (Catalog No. FE-114, 99.8%) were from Atlantic Equipment Engineers (AEE, Bergenfield, NJ). The two Co catalysts included, the \(-325\) mesh irregular Co (Catalog No. 10456, 99.5%) from Alfa-Aesar (Ward Hill, MA), and the \(-100\) mesh cracked egg shell-like Co (CAS No. 7440–48–4, \(<150\) μm, 99.9+% ) was from Sigma-Aldrich. The four sources of solid/sample C included oxalic acid powder (Ox-2, SRM 4990C, Australian National University sucrose (ANU sucrose), tributyrin (TRIB), and fasting human plasma. The Ox-2 SRM was from National Institute of Standards and Technology (NIST), Gaithersburg, MD. The accepted \(F_m\) value for the Ox-2 SRM was 1.3407.\(^16\) Four milligrams of the Ox-2 SRM supplied 1 mg of solid C. The ANU sucrose was from the Lawrence Livermore National Laboratory (LLNL), 2.38 mg supplied 1 mg of solid C. The methanol (\(\text{MeOH}, \text{CAS No. 67–56–1, HPLC grade}\) was from Fisher-Scientific (Santa Clara, CA). The TRIB (CAS No. 60–01–5, sub-Modern carbon carrier) was from MP Biomedicals (Solon, OH). The TRIB was diluted with MeOH (TRIB/\(\text{MeOH}, 1:24, v:v\) so that each 50 \(\mu\)L of TRIB–\(\text{MeOH}\) solution supplied 1.19 mg of solid C. Fastig human plasma (baseline) was from each of four healthy nonsmoking persons whose ages ranged from 25 to 67 y. A 25-\(\mu\)L aliquot of plasma supplied \(~1\) mg of solid C.\(^17\)

**Procedure.** In optimizing the reduction step of our previous method,\(^15\) Ox-2 was used as the AMS standard rather than the ANU whose supply was limited. Oxidation of the 1-mg aliquots of solid C (which has traces of \(\text{H}_2\text{O}\) vapor) to \(\text{CO}_2\) was conducted as described by our previous method.\(^15\) Transfer of the \(\text{CO}_2\) to septa-sealed vials that had an inner vial containing a catalyst that sat atop glass beads that sat atop a reductant (Zn dust) was also the same as by our previous method\(^18\) except that several amounts of redundant, catalyst, types of catalysts, heating temperatures, and heating durations were evaluated as summarized in Table 1.

Criteria for evaluating the AMS data included a high, reliable, and stable \(^{13}\text{C}^+\) current along with an accurate \(F_m\) value. The redundant levels ranged from 1 to 300 mg of Zn dust in increments of 25 mg. The five catalysts included three of iron and two of cobalt, each at only one level, 10 mg. Then, six levels of the best performing (defined by ion current) of the five catalysts that ranged from 1 to 10 mg were evaluated. Three reduction temperatures that ranged from 500 to 550 °C in increments of 25 °C and 12 reduction durations that ranged from 0.5 to 6 h in increments of 0.5 h were tested. Graphitization yield and isotopic fractionation were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).\(^18\) The resulting graphite was tamped into AMS target holders and analyzed at the LLNL as by our previous method.\(^15\) The \(^{13}\text{C}^+\) current of the AMS target of interest was normalized by the \(^{13}\text{C}^+\) current of the Ox-2 SRM and referred to as normalized \(^{13}\text{C}^+\) (\(\text{n}^{13}\text{C}^+\) current).
The $F_m$ of a sample of interest was the ratio of $\frac{^{14}C/^{13}C}_{\text{sample}} / \frac{^{14}C/^{13}C}_{\text{Ox-2}}$, each normalized for $\delta^{13}C$. The $\delta^{13}C$ was calculated as $\left[ \frac{^{13}C/^{12}C}_{\text{sample}} - \frac{^{13}C/^{12}C}_{\text{PDB}} \right] / \frac{^{13}C/^{12}C}_{\text{PDB}} \times 1000$, where PDB referred to the Cretaceous belemnite formation at Peedee, SC. The $\delta^{13}C$ value used in this study was $-25$‰ for biological/biomedical application and $-17.8$‰ for Ox-2 SRM. Four C sources were compared using our previous method\textsuperscript{15} and our optimized method.

Graphite quality was also examined using scanning electron microscopy (SEM) with a Philips XL30 (FEI Co., Hillsboro, OR) set at 15-kV accelerating energy and 5 spot size. The graphite-coated catalysts (Fe or Co) were sputtered 3 times with a thin
RESULTS

Figure 1 presented an overview of the second step of AMS target preparation. The left panel shows the setup and appearance of the zinc dust reductant and the −400MSIP catalyst before the CO2 was reduced. The right panel shows the appearance of the zinc and the −400MSIP after the CO2 was reduced using our previous method.15 After the CO2 was transferred to the septa-sealed vial but before it was reduced, the Zn dust consisted of loose spherical particles, while after reduction, the Zn dust was deformed and sintered. During the reduction, Zn dust was converted to (1) two Zn bands in the coolest regions of the septa-sealed vial (Zn band-1 at 260 °C and Zn band-2 at 380 °C), (2) two Zn mirrors in a warmer region (Zn mirror-1 at 480 °C and Zn mirror-2 at 525 °C), and (3) a hard Zn cake at 560 °C that stuck to the bottom of the septa-sealed vial. Zn band-1 appeared fibrous (rubbery), while Zn band-2 appeared plasticized or plastic-like. Zn mirror-2 appeared to have a crystal structure and stuck to the septa-sealed vial. Finally, the −400MSIP appeared as a fuzz of GCI rather than being covered with a fluff of graphite which upon shaking fell off.

Figure 2 plotted the n13C+ current according to the quantity of Zn dust being used (per mg of C) for the reduction step. The highest n13C+ current was experienced when the quantity of Zn dust was in the range of 100–250 mg of Zn dust, so we considered the 100 mg of Zn dust as optimal. When ≥150 mg of Zn dust was used, the excess graphite began to coat over the Zn as Zn bands and mirrors (instead of the −400MSIP), thereby decreasing the graphitization yield and increasing isotopic fractionation. The Zn band-1 and Zn band-2 tended to fall atop the AMS target in the borosilicate inner vial, inadvertently contaminating the AMS target, and thereby reducing the n13C+ current. When <100 mg of Zn dust/mg of solid source C was used, ICM was formed depending on the temperature and mass of iron catalyst.

Figure 3 summarized SEM pictures before and after the reduction of CO2, the n13C+ current, and the Fm values after the reduction of CO2, when 10 mg each of three Fe catalysts or two Co catalysts was used along with 100 mg of Zn at 525 °C for 6 h. The −400MSIP had a uniform coat of graphite, the strongest and most reliable n13C+ current, and the most accurate and precise Fm values (histograms), so we ranked the −400MSIP as the best catalyst. The −325 mesh H2-reduced Fe, the −50+/+100 mesh H2-reduced Fe, and the −100 mesh Co (cracked egg shell-like-Co) were loosely covered with a fluff of graphite. The −325 mesh irregular Co was covered with filamentous graphite (FG). Because all five catalysts, except for the −400MSIP, did not coat uniformly with graphite, their respective n13C+ current and Fm values were weak and variable.

Figure 4 summarized the SEM pictures and n13C+ current to quantity the −400MSIP used. In general, quantities of the −400MSIP in the range of 5–8 mg yielded the strongest n13C+ current and were uniformly coated with a fuzz of graphite, so we choose 5 mg of −400MSIP as optimum. When a small amount of −400MSIP (1 mg) was used, it was heavily (and loosely) coated with graphite (left SEM picture), and the excess graphite appeared as Zn band-1 and Zn band-2 that contaminated the AMS target thereby lowering the n13C+ current. When a large quantity (10 mg) of −400MSIP was used, it was coated with too little GCI or ICM. ICM was possibly an iron carbide (Fe3C) instead of graphite (right SEM picture).

Figure 5 summarized SEM pictures, n13C+ current, and Fm values according to the three reduction temperatures/mg of C listed. The left SEM picture showed that the 500 °C temperature produced a uniform coat of a fuzz of graphite, while the 525 or 550 °C temperatures produced a crustlike cover of graphite. At 500 and 525 °C, the n13C+ currents were reliable and Fm values were accurate and precise. At 500 °C, a uniform coat of graphite was produced 100% of the time while 525 °C produced ICM 20% of the time instead of graphite. As reduction temperature increased, more sinter of the −400MSIP occurred, which led to isotopic fractionation, thus underestimating the Fm values.20,21 Also, septa-sealed vials begin to melt at 600 °C. Finally, at 550 °C, the Fm value of the Ox-2 SRM was underestimated (1.3151 ± 0.0128 versus 1.340719). Therefore, we considered 500 °C as optimal.

Figure 6 summarized SEM pictures and n13C+ current according to duration (up to 6 h) of heating at 525 °C. In general, the

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n$^{13}$C$^+$ current was independent of the duration of heating under these conditions (bottom panel). When the duration, the temperature, or both of the reduction step were insufficient or when the mass of the $-400$MSIP was not optimal, inaccurate $F_m$ values were experienced apparently because the CO$_2$ reduction step was incomplete. Heating for 6 h at 525 °C (broken rectangle) was needed to minimize isotopic fractionation while heating for only 3 h at 500 °C (solid rectangle) also minimized isotopic fractionation with increased throughput, so it was considered as optimal.

Figure 7 compared the AMS targets’ qualities using our previous$^{15}$ and our optimized methods. Our optimized method used 100 ± 1.3 mg of Zn dust, 5 ± 0.4 mg of $-400$MSIP, and 3 h reduction time at 500 °C, whereas our previous method$^{15}$ used ~100 mg of Zn dust, ~10 mg of $-400$MSIP, and 6 h reduction time at 525 °C. Our optimized method produced exclusively graphite-coated Fe powder (GCIP) 100% of the time. The GCIP consisted of a mix of a graphite fuzz plus small pieces of “graphite sheet” (right SEM picture), whereas our previous method$^{15}$...
produced GCI, which was a nonuniform coat of graphite fuzz, only 80% of the time (left SEM picture). The GCIP produced higher $^{13}\text{C}^+$ currents (∼3%) and the $F_m$ values that matched those of the Ox-2 SRM more closely than did our previous method15 ($F_m$ values that best matched the theoretical value (1.3407) of the Ox-2 (NIST SRM 4990C).16 The lowest temperature was important because at ∼600 °C the septa-sealed vial begins to soften and melt.

**Figure 5.** Before and after CO₂ reduction SEM pictures and the corresponding $^{13}\text{C}^+$ currents and $F_m$ values of the Ox-2 SRM according to reduction temperature. [The $^{13}\text{C}^+$ currents are represented by full bars and the $F_m$ values by empty bars (means ± SE). The 500 °C temperature was optimal (abscissa solid rectangle) because it was the lowest temperature at which the −400MSIP received a uniform coat of graphite fuzz (left SEM) with the $F_m$ values that best matched the theoretical value (1.3407) of the Ox-2 (NIST SRM 4990C). The lowest temperature was important because at ∼600 °C the septa-sealed vial begins to soften and melt.]

produced GCI, which was a nonuniform coat of graphite fuzz, only 80% of the time (left SEM picture). The GCIP produced higher $^{13}\text{C}^+$ currents (∼3%) and the $F_m$ values that matched those of the Ox-2 SRM more closely than did our previous method15 ($F_m$ values that best matched the theoretical value (1.3407) of the Ox-2 (NIST SRM 4990C). The lowest temperature was important because at ∼600 °C the septa-sealed vial begins to soften and melt.

**Figure 6.** SEM pictures and the $^{13}\text{C}^+$ currents according to the duration of heating (reduction) time (up to 6 h) using 100 mg of Zn dust, 10 mg of −400MSIP, and 525 °C used to reduce CO₂ to graphite (regression and 95% confidence interval belt). Under these conditions, reduction time was not a significant factor and the previous method16 needed 6 h (abscissa broken rectangle). But we confirmed that 3 h was optimal (abscissa solid rectangle) based on the uniformity of the graphite coat when CO₂ was reduced at 500 °C (data not shown).
values are detailed in Table 2). In addition, our optimized method avoided formation of the ICM, which produced n^{13}C^{+} currents and F_{m} values that were low and variable. Finally, the GCIP was also much easier to tamp into AMS target holders. Figure 8 summarized the relationship of ^{13}C current and of n^{13}C^{+} current to the F_{m} values. The "y axis" represented the

Figure 7. SEM pictures, the n^{13}C^{+} currents, and F_{m} according to our previous method\textsuperscript{15} that used 100 mg of Zn dust, 10 mg of -400MSIP, and 525 °C for 6 h to reduce CO_{2} to GCI. The n^{13}C^{+} currents are represented by full bars and the F_{m} values by empty bars (means ± SE, SE < line thickness). Our optimized method (abscissa solid rectangle) differed by using only 5 mg of -400MSIP, and 500 °C for 3 h. Our optimized method ensured the CO_{2} (from 1 mg of solid C) was uniformly and reliably converted to GCIP, which loaded easily into the AMS target holder. It maximized n^{13}C^{+} current for accurate F_{m} values of solid C from many sources. The 500 °C temperature for 3 h assured the septa-sealed vials and Teflon cap did not soften/melt.

Table 2. F_{m} Values of Four Carbon Sources by Our Previous and Optimized Graphitization Methods\textsuperscript{a}

| carbon source                        | mean of F_{m} ± SE n = number of samples | our previous method\textsuperscript{15} | our optimized method  | p-value |
|--------------------------------------|------------------------------------------|------------------------------------------|-----------------------|---------|
| oxalic acid II (Ox-2, NIST SRM 4990C)\textsuperscript{b} | accepted F_{m} value 1.3407\textsuperscript{16} | 1.3432 ± 0.0024 n = 32                  | 1.3410 ± 0.0027 n = 32 | 0.5401  |
| sucrose (ANU) consensus F_{m} value 1.5061 ± 0.001\textsuperscript{15} |                                         | 1.5091 ± 0.0032 n = 25                  | 1.5034 ± 0.0032 n = 25 | 0.2259  |
| TRIB used to fortify HPLC fractions with C |                                         | 0.0472 ± 0.0016 n = 40                  | 0.0497 ± 0.0019 n = 40 | 0.3161  |
| fasting human plasma                 |                                         | 1.0482 ± 0.0050 n = 63                  | 1.0609 ± 0.0023 n = 35 | 0.0716  |

\textsuperscript{a} The limit of detection (LOD) and limit of quantification (LOQ) were determined using the tributryn.\textsuperscript{22} The LOD was 0.04 amol and LOQ was 0.07 amol. The accuracy and precision between our previous and our optimized methods were compared using the Ox-2. The accuracy [relative error = (measured F_{m} − accepted F_{m})/accepted F_{m} × 100] was 0.022% using our optimized method compared to 0.187% using our previous method, while the precision (RSD (relative standard deviation) = SD of measured F_{m}/mean of measured F_{m}× 100) was 1% for both methods.\textsuperscript{b} The 1 mg of C from Ox-2 or ANU sucrose was oxidized to CO_{2} and then reduced to graphite by our previous\textsuperscript{15} and optimized methods.

The values are detailed in Table 2. In addition, our optimized method avoided formation of the ICM, which produced n^{13}C^{+} currents and F_{m} values that were low and variable. Finally, the GCIP was also much easier to tamp into AMS target holders. Figure 8 summarized the relationship of ^{13}C current and of n^{13}C^{+} current to the F_{m} values. The "y axis" represented the

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Our previous method\textsuperscript{15} produced AMS targets of GCI 80% of the time and of ICM 20% of the time. AMS targets of GCI produced intense ion currents of $\sim$115 $\mu$A $^{12}$C$^-$/mg of solid source C. On the other hand, AMS targets of ICM produced $^{12}$C$^+$ currents of only 100 $\mu$A $^{12}$C$^+/mg$ of solid source C, which led to low and variable $F_m$ values. A possible reason why ICM produced weaker $^{12}$C$^+$ current was that the ICM might be iron carbide (mostly Fe$_3$C with traces of FeC$_2$). Cobalt carbide produced lower ion current than did graphite, which resulted in a 5–10% error in the $F_m$.\textsuperscript{12} AMS targets of ICM were also difficult to tamp into the AMS target holder. Therefore, our objective was to examine the experimental conditions of the second step of AMS target preparation where CO$_2$ transferred to a septa-sealed vial was reduced to high-quality graphite for the intense ion current required for accurate measurements of $F_m$ with high throughput for biological/biomedical applications of AMS. Biological/biomedical applications involved $F_m$ values in the range of 0.01–100 or isotope ratios in the range of $10^{-14}$–$10^{-10}$ compared to ratios of $10^{-15}$–$10^{-12}$ for carbon dating.\textsuperscript{19,24} Adequate quantities of solid C source are almost always present in samples for biological/biomedical AMS, whereas small quantities of solid C source are present in samples for carbon dating.

**Effect of the amount of Zn dust.** The amount of Zn dust was one of the critical factors for the formation of high-quality graphite because it generated a H$_2$ reductant and converted CO$_2$ to CO during the reduction step (Table 3, eqs 1 and 2). Generally, excess Zn was undesirable because it led to the formation of thicker Zn bands and Zn mirrors on the inside of the septa-sealed vial during the reduction of CO$_2$. Higher reduction temperature and longer reduction duration produced thicker bands and mirrors.\textsuperscript{25}

Some of the Zn bands and the Zn mirrors fell atop the AMS targets in the borosilicate inner vial of the septa-sealed vial and were inadvertently transferred onto (contaminating) the AMS target and decreased sputtering yields in the AMS ion source. Furthermore, when the ratio of the mg of Zn dust/mg of solid C source was in excess, graphite began to coat over the Zn instead of the Fe, thereby decreasing the graphite yield, and increasing isotopic fractionation. It was reported previously that 75–150 mg of Zn/mg of solid C source maximized the $^{13}$C$^+$ current.\textsuperscript{14} While ~175 mg of Zn dust maximized the n$^{13}$C$^+$ current (Figure 2), it also resulted in a thicker Zn band, which reduced the inside diameter of the septa-sealed vials, which in turn made it more difficult to retrieve the inner vial (containing the AMS target). At 100 mg of Zn dust, the n$^{13}$C$^+$ current was still reliable, the $F_m$ measurement was still accurate and precise, and the thickness of the Zn band allowed for the inner vial (containing the AMS target) to be easily retrieved from the septa-sealed vial. At <100 mg of Zn dust/mg of solid source C, ICM was formed depending on the quantity of iron catalyst or the reduction temperature used.

The n$^{13}$C$^+$ current obtained by using 100 mg of Zn dust confirmed the previous findings\textsuperscript{15} that the 100 mg of Zn dust was optimal because it produced reliable n$^{13}$C$^+$ current, so we considered 100 mg of Zn dust as optimal. Once the quantity of $F_m$ in biological/biomedical applications of AMS.\textsuperscript{11,25}
Zn dust was optimized, the type, particle size, and quantity of catalysts were then optimized.

**Effect of Catalyst Type and Particle Size.** During the reduction step using Zn dust, the CO or CO2 was reduced to C. At the catalyst interface was detrimental to carbon deposition onto graphite, and extreme ratios (Fe/C of 15:1) fused the graphitized graphite, and production of hydrocarbon passes from 400MSIP as optimal because this smaller ratio of Fe/C minimized production of ICM.29

An evaluation of catalyst suitability for AMS revealed that Alfa-Aesar’s −325 mesh Fe formed FG, which did not affect the C− current intensity or background and was easy to pack into the AMS target holders.30 Furthermore, the SEM pictures in Figure 3 were consistent with prior SEM pictures of graphite formation when −325 mesh H2-reduced Fe or −325 mesh Co but not when −400MSIP (left SEM picture in Figure 3) was used.31 The difference may be associated with the formation of solid graphite on the −400MSIP that occurred with increased Fe sintering when the reduction step was conducted at temperatures of >525 °C.

**Effect of Catalyst Quantity per Milligram of Solid Source C.** A prior study has shown that a 15:1 ratio of Fe/solid C source fused the graphitized iron into a bead.31 Our previous method15 formed GCI 80% of the time, which produced reliable ion currents and accurate \( F_m \) values for various solid C sources. Our previous method15 also produced some ICM 20% of the time. The occasional production of ICM may be due to the use of the larger ratio of Fe/C compared to prior studies.27,28 Powder X-ray diffraction (PXRD) analysis of the ICM revealed a crystal structure characteristic of iron carbide (Fe3C) rather than graphite (see Figure 5 in part 2), whereas XRD analysis of the GCI and GCIP also revealed a Fe3C crystal and possibly a disordered carbon structure. Furthermore, FT-IR analysis revealed a C=O bond stretching for GCI and GCIP but not for ICM (see Figure 3 in part 2). Therefore, we considered the 5 mg of −400MSIP as optimal because this smaller ratio of Fe/C minimized production of ICM.

**Effect of Reduction Temperature and Time.** Above 750 °C, graphite formation did not occur because the −400MSIP was deformed (inactivated). Below 500 °C, production of hydrocarbon by Fischer−Tropsch reaction (Table 3, eqs 8 and 9) was favored over graphite especially at \( \text{H}_2/\text{CO} \) ratios >3:1.27,31,32 As the temperature of the reduction step increased, the balance of the forward reactions in eqs 4−6 in Table 3 became less favorable thermodynamically but more favorable kinetically.31 The data in Table 3, eqs 1 and 5, confirmed prior work that the most favorable (thermodynamically) reduction occurred at 600 °C when using the modified Zn reduction method.34 When the H2 or the Zn reduction method was used, the rate of carbon deposition passed from 0.2 to 0.4 mg of C/h within the temperature range of 500−650 °C.7,26 Formation of graphite in 3 h at 500 °C by our optimized method was consistent with prior reports that graphite formation can be >95% completed in 1 h at 600 °C25 to 4 h at 500 °C.14 The formation of graphite in 3 h at 500 °C was also important for two reasons. First, at ≥600 °C, our septa-sealed Pyrex vials and Teflon (caps) melt, and second, at <450 °C, carbon samples were converted to methane.31 Although 525 °C was preferred for analytical purposes, previous methods43,44 used a lower temperature (180 °C).

### Table 3. Chemical Reactions during the Reduction Portion of Graphitization Process by Zn Reduction Method

| eq no. | reduction reaction of CO2 | \( \Delta G_{\text{temp}} \) (kJ·mol\(^{-1}\)) |
|--------|--------------------------|-----------------------------------------------|
| 1      | \( \text{CO}_2(g) + \text{Zn}(g) \rightleftharpoons \text{CO}(g) + \text{ZnO}(s) \) | 298 K (25 °C) 723 K (450 °C) 773 K (500 °C) 798 K (525 °C) 823 K (650 °C) 873 K (600 °C) |
| 2      | \( \text{H}_2\text{O}(g) + \text{Zn}(g) \rightleftharpoons \text{H}_2(g) + \text{ZnO}(s) \) | −61.2 | −55.6 | −54.9 | −54.6 | −54.3 | −53.5 |
| 3      | \( \text{CO}_2(g) + \text{H}_2(g) \rightleftharpoons \text{CO}(g) + \text{H}_2\text{O}(g) \) | −89.7 | −65.7 | −62.9 | −61.5 | −60.1 | −57.3 |
| 4      | \( \text{CO}(g) + \text{H}_2(g) \rightleftharpoons \text{C}_{\text{gr}}(s) + \text{H}_2\text{O}(g) \) | −28.5 | +10.4 | +8.3 | +7.2 | +6.1 | +4.0 |
| 5      | \( 2\text{CO}(g) \rightleftharpoons \text{C}_{\text{gr}}(s) + 2\text{CO}_2(g) \) | −91.3 | −34.3 | −27.5 | −24.2 | −20.8 | −14.1 |
| 6      | \( \text{CO}_2(g) + 2\text{H}_2(g) \rightleftharpoons \text{C}_{\text{gr}}(s) + 2\text{H}_2\text{O}(g) \) | −119.8 | −44.6 | −35.8 | −31.4 | −27.0 | −18.1 |
| 7      | \( \text{CO}_2(g) + \text{H}_2\text{O}(g) \rightleftharpoons \text{H}_2(g) + \text{CO}_2(g) \) | −62.8 | −23.9 | −19.3 | −17.0 | −14.7 | −10.1 |
| 8      | \( 2\text{CO}(g) + 2\text{H}_2(g) \rightleftharpoons \text{CH}_4(g) + \text{CO}_2(g) \) | −28.5 | −10.4 | −8.3 | −7.2 | −6.1 | −4.0 |
| 9      | \( \text{CO}(g) + 3\text{H}_2(g) \rightleftharpoons \text{CH}_4(g) + \text{CO}_2(g) \) | −170.6 | −61.1 | −48.23 | −41.8 | −35.4 | −22.5 |

\( ^a \) Graphite-coated Fe (called graphite). \( ^b \) The thermodynamics of CO2 reduction was predicted using the following equation (\( \Delta G^o/T \)) derived from the Gibbs–Helmholtz equation. (See Supporting Information available.)
thermodynamically in our previous method,\textsuperscript{15} it still needed 6 h to avoid larger isotopic fractionation for accurate AMS measurement. Therefore, we concluded that 500 °C for 3 h was optimal to maximize sample throughput with reliable \(^{13}\text{C}^-\) current and accurate \(F_m\) measurement.

**Comparison of our Previous\textsuperscript{15} and Our Optimized Methods.** Key factors that influence the formation and quality of graphite include the catalyst size, the mass ratio of Fe/solid source C, and the reduction temperature and time. In general, \(<500 \, ^\circ\text{C}\) was the preferred temperature for conversion of CO\(_2\) to CO whereas \(>500 \, ^\circ\text{C}\) was preferred for deposition of CO as graphite.\textsuperscript{31,34} The change of Gibbs’s energy with respect to temperature can be estimated using Gibbs–Helmholtz equation \([\partial (\Delta G/T)/\partial T]_p = -\Delta H/T^2\) and thermodynamic data at 1 bar and 298 K, whereas kinetic energy must be experimentally determined. Results of this calculation confirmed prior estimates\textsuperscript{31,34} except in eq 3 (Table 3) where all forward reactions were spontaneous, although the kinetics was slow at low temperatures. Methane formation was not a problem at 500 to 600 °C with lower H\(_2\) concentration whereas formation of iron carbonyl (Fe(CO)\(_5\)) was not spontaneous in that temperature range. Graphite formation was less favored thermodynamically at high temperature in eqs 4–6 (Table 3) because of entropy but became more kinetically favored due to diminished activation energy of reactions \((k = Ae^{-E_a/RT})\). In our present study, 500, 525, and 550 °C were thermodynamically favored, but CO\(_2\) reduction time at 500 °C was shorter than at 525 and 550 °C because it had less sinter of the Fe catalyst (\(-400\text{MSIP}\)). Use of 1–5-\(\mu\)m-sized powders did not increase the reaction rate due to sintering;\textsuperscript{28} instead, our optimized method produced a uniform GCIP (<37 \(\mu\)m, i.e., \(~5 \, \mu\)m size). Our optimized method had less sinter of the \(-400\text{MSIP}\) by using a combination of 500 °C and 5 mg \(-400\text{MSIP}\) per mg of solid C source indicating that our optimized method at 500 °C was more favorable thermodynamically for graphitization over our previous method.\textsuperscript{15} Ninety percent of the carbon in the sample of interest was recovered as graphite using our previous\textsuperscript{15} and optimized methods. The \(\delta^{13}\text{C}\) of 1-mg aliquots of solid C was \(-18.4 \pm 0.2\%\) by our previous method\textsuperscript{15} and was \(-17.9 \pm 0.3\%\) by our optimized method, and the \(\delta^{13}\text{C}\) especially by the optimized method was within the accepted range \((-17.8 \pm 0.05\%)\).\textsuperscript{16} Samples with 0.3–1 mg of C consistently produced high-quality graphite with high throughput using our optimized method.

Graphite quality (uniform coat of fuzz or filamentous graphite) affected the ionization efficiency and ion currents. We focused on high-quality graphite to consistently produce reliable \(^{13}\text{C}^+\) current, \(^{12}\text{C}^-\) current, and \(F_m\) values for the Ox-2 SRM that were accurate and precise. The 1-mg aliquots of solid C source produced a reliable \(^{13}\text{C}^+\) current of 0.35–0.5 \(\mu\)A, which corresponded to a \(^{12}\text{C}^-\) current of 0.9–1.1 or to a \(^{12}\text{C}^-\) current of \(~115\) \(\mu\)A. Intense ion currents in these ranges guaranteed accurate \(F_m\) values with high throughput for biological/biomedical applications of AMS\textsuperscript{11,23} despite a recent report\textsuperscript{36} that the ratio of \(^{14}\text{C}/^{12}\text{C}\) was independent of ion currents.

**CONCLUSIONS**

We optimized the reduction step by using 100 mg of Zn dust, 5 mg of \(-400\text{MSIP}\), and 500 °C for 3 h to produce exclusively high-quality AMS targets for biological/biomedical application of AMS. Our optimized CO\(_2\) reduction method was thermodynamically more favorable than our previous method. Consequently, throughput was doubled, graphitization yield was \(\geq 90\%\), \(\delta^{13}\text{C}\) of Ox-2 SRM was \(-17.9 \pm 0.3\%\), LOD was 0.04 amol, LOQ was 0.07 amol, relative error was 0.022%, and precision was 1%.

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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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