Evaluation of precision and accuracy for carbon isotopic measurements of ^{13}C-labeled samples by an online elemental analyzer / isotope ratio mass spectrometer system

Nanako O. Ogawa[1], Rie Sato[2] and Naohiko Ohkouchi[1]

[1] Research Program for Paleoenvironment, Institute for Research on Earth Evolution (IFREE)
[2] Stable Isotope Analyses Center, Shoko Co.Ltd, Sugito, 345-0023 Japan

1. Introduction

For decades, stable isotope tracer method has been used as a conventional tool for understanding the elemental cycles in natural environments. For example, feeding experiments by using ^{13}C-enriched algae and bacteria were successfully done to trace the biogeochemical processes on the deep sea floor [*Blair et al*., 1996; *Levin et al*., 1999; *Nomaki et al*., 2005]. In these reports, isotope-ratio mass spectrometer (IRMS) coupled with an elemental analyzer (EA) or a gas chromatography was used for determination of the isotopic compositions up to several thousand per mil. However, to our knowledge, the accuracy and precision of these "mildly ^{13}C-enriched (up to +5000‰)" isotopic compositions have not carefully been evaluated so far. In this study, we determined seven differently ^{13}C-labeled glycine standards by the conventional EA/IRMS system to evaluate the analytical precision and accuracy.

2. Experimental

Seven glycine powders with variable ^{13}C contents were prepared and analyzed in this study. One out of these seven samples was a natural ^{13}C level (i.e., non-labeled) glycine, which was purchased from Nakalai Tesque Co. The other six samples were mildly ^{13}C-enriched glycines which were made by combining and mixing the non-labeled and 99% ^{13}C-labeled glycine powders purchased from Mass trace Co. To perfectly homogenize the mixtures, the mixtures of these glycine powders were dissolved in distilled water and then, freeze dried. Expected carbon isotopic compositions for these glycine mixture samples range from -29.0 to +3743‰, which correspond to the ^{13}C atomic fraction from 1.08 to 5.06% (Tab. 1).

Carbon isotope analyses were performed by using an online system of ThermoQuest Delta plus XL isotope ratio mass spectrometer coupled to an EA1110 automatic elemental analyzer through a ConFlo II continuous flow interface [*Brand*, 1996]. The sample powders were folded in pre-cleaned tin capsules and transferred to the combustion column of EA which was heated to 1050˚C by an autosampler. The carbon isotopic composition was expressed as conventional delta notation ($\delta^{13}C$, ‰) against VPDB (Vienna Pee Dee Belemnite);

$$\delta^{13}C = (R_{sample} / R_{VPDB} - 1) \times 1000$$

where R_{sample} and R_{VPDB} denote $^{13}C/^{12}C$ ratio for sample and VPDB standard, respectively.

Samples sizes introduced to the analytical system are 0.02, 0.04, 0.1, 0.15, 0.20, 0.25, and 0.35 mgC for each glycine mixture sample. Triplicate analyses were performed for each sample.

3. Results and Discussion

For non-labeled glycine sample (Glycine 1), the precise measurements of carbon isotope composition was successfully achieved with a wide range of sample sizes from 0.02 to 0.25 mgC. However, it was not the case for the ^{13}C-enriched glycine samples (Glycine 2 to 7), since the peak height of $^{13}C^{16}O_2^+$ ion (*m/z* 45) was over 40V which is out of the range of signal linearity in the 2nd MS detector in the conventional IRMS system. On the other hand, we empirically know that the peak height of $^{12}C^{16}O_2^+$ ion (*m/z* 44) should be higher than 4V to obtain a precision better than 0.1‰ [e.g., *Ohkouchi et al*., in press]. Therefore, it is expected that an optimum sample size should be carefully chosen to optimize the analytical condition of the conventional EA/IRMS system when analyzing the ^{13}C-enriched samples.

In Figure 1, we illustrated the relationship between measured and expected $\delta^{13}C$ values in our experiments. Although the measurement appears to slightly underestimate the $\delta^{13}C$ values for samples whose expected $\delta^{13}C$ values are higher than +2000‰, in terms of empirical sense, the measured $\delta^{13}C$ values correspond well with the expected values. The variation of triplicate measurements is the smallest (0.5‰) for the non-labeled sample (Glycine 1), whereas that of the most ^{13}C-enriched Glycine 7 exhibited the largest up to +149‰. Generally, the magnitude of the precision tends to be elevated along with the increase of the sample mass (Fig. 2). As expected, the precisions are substantially improved when the introduced carbon amount was carefully chosen (mostly between 0.02 and 0.1 mgC). If we ignore the analytical results which either *m/z* 44 or 45 ion peak is out of the optimal range (4 to 40V), the precisions (68% probability or 1s) for these samples are better than 5‰. Accuracy of the measurements is somewhat difficult to assess, because we are unaware of the precise isotopic composition of the ^{13}C-labeled glycine standard used for the sample preparation. However, the measured isotopic compositions of samples correlated well with the expected values especially for samples whose $\delta^{13}C$ are lighter than +2000‰, suggesting that the "99% ^{13}C labeled" should be close to the "real" value. Under the assumption that the ^{13}C atomic fraction of ^{13}C-labeled glycine standard was 99%, the accuracy is at most 28‰ for the most ^{13}C-enriched samples.

Overall, our experiments strongly suggest that the measure-

ments of stable carbon isotope ratio for mildly ^{13}C labeled samples can be achieved by a conventional online EA/IRMS system with sufficient precision and accuracy in terms of the isotope tracer experiments. We believe that the "mild" stable isotope tracer method less perturbs the natural environments relative to "heavy" stable isotope tracer methods and radioisotope tracer methods and will be on the right track in near future.

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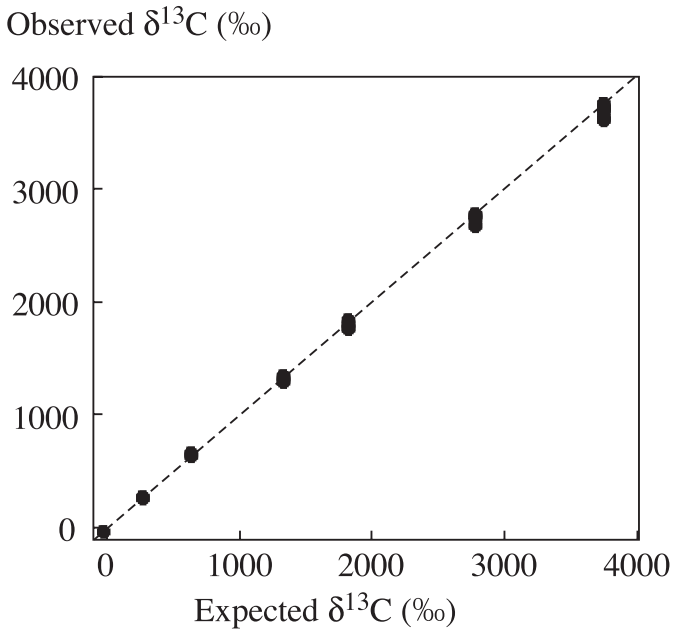

Figure 1. Comparison of observed $\delta^{13}C$ data against expected $\delta^{13}C$ values. Expected $\delta^{13}C$ values were provided from weight data. Each data point means an average value of triplicate analyses of samples. Dotted line indicates the line where exp-$\delta^{13}C$ = obs-$\delta^{13}C$.

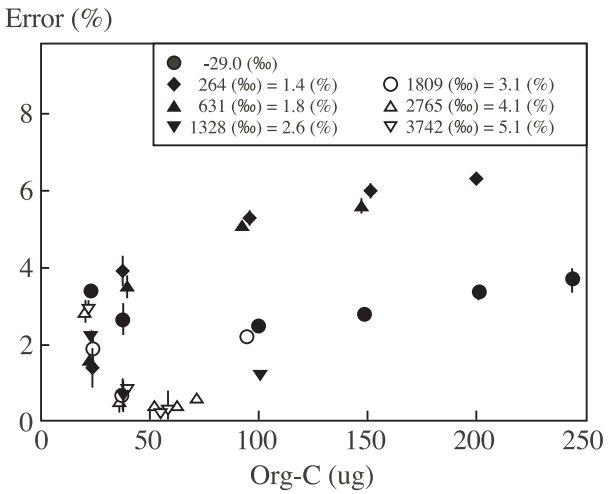

Figure 2. Distributions of error (%) between observed $\delta^{13}C$ and expected $\delta^{13}C$ against introduced carbon amount (Org-C μg). Error (%) = |(Exp-$\delta^{13}C$ – obs-$\delta^{13}C$) / exp-$\delta^{13}C$ | x 100. Org-C (μg) means total carbon mass introduced as glycine mixture. Each data plot reflects averaged value for three analyses with standard deviation (vertical bar).

Table 1. Actual $\delta^{13}C$ values for glycine mixture standards using in this study.

	$\delta^{13}C$ (‰)	F (Atom%)
Glycine 1	-29.0	1.08*
Glycine 2	264*	1.40
Glycine 3	631*	1.80
Glycine 4	1329*	2.55
Glycine 5	1809*	3.06
Glycine 6	2766*	4.06
Glycine 7	3743*	5.06

F = $^{13}C/(^{13}C + ^{12}C)$ x 100

* Calculated from F or $\delta^{13}C$ using 0.0112372 as $^{13}C/^{12}C_{VPDB}$.