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Carbonate Precipitation Methods for the Measurement of Stable Isotope Ratio of Dissolved Inorganic Carbon

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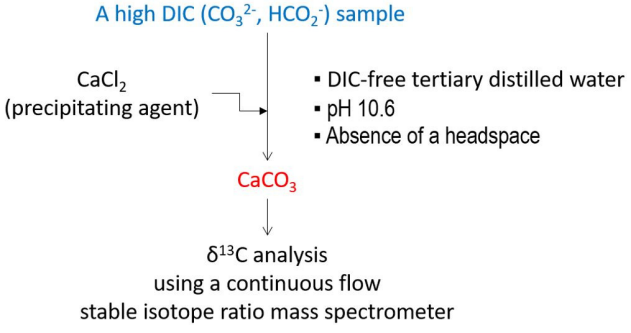
A B S T R A C T

The stable carbon (C) isotope ratio (δ^{13}C) of dissolve inorganic carbon (DIC) in environmental samples including soil extracts and water samples provides insight into the biogeochemical C cycling. The δ^{13}C of DIC can be measured in gas and solid (carbonate precipitates) states, and precipitates measurement of δ^{13}C of DIC is more convenient compared to the gases measurement for analyzing a large number of samples. However, it is still uncertain what precipitation conditions, such as pH, headspace, and distilled water, are suitable. To address the question, we conducted a series of precipitation experiments, followed by δ^{13}C, under different conditions of pH, headspace, and distilled water as well as precipitating agents ($SrCl_2$, $BaCl_2$, and $CaCl_2$). Our results demonstrated that DIC (depleted in ^{13}C) contaminant in double distilled water is the major error factor to decrease the δ^{13}C. By combining the results from the experiments, we suggest that reliable δ^{13}C data can be obtained by precipitation under the conditions of using DIC-free tertiary distilled water, samples with a high DIC concentration, $CaCl_2$ addition as a precipitating agent, pH 10.6, and the absence of a headspace produces a reliable δ^{13}C measurement.

Keywords: Barium carbonate, Calcium carbonate, pH adjustment, Stable isotope ratio, Strontium carbonate



Methods and experimental conditions of DIC precipitation for the analysis of δ^{13}C.

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Introduction

The stable carbon (C) isotope ratio ($\delta^{13}C$) of dissolve inorganic carbon (DIC), such as carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-), in soil extracts and water samples provides valuable information on the sources and biogeochemical C cycling (Atekwana and Krishnarmurthy, 1998). The natural background value of $\delta^{13}C$ in uncontaminated environment (e.g., groundwater) ranges from −15‰ to −6‰ (Arneth and Hoefs, 1988; Mook, 2000; Grossman et al., 2002). However, increases in carbon dioxide (CO_2) in soil and water environment through aerobic decomposition of C3 organic matter (around −27‰) leads to decrease in the $\delta^{13}C$ of DIC (Breukelen et al., 2003). Under anaerobic fermentation conditions, however, the evolved CO_2 is enriched in ^{13}C (range: between −10 and +20‰) due to ^{13}C isotope fractionation during methane (CH_4) generation, which produces ^{13}C-depleted CH_4 (range: −110 and −50‰) (Grossman et al., 1989; Conrad, 2005; Mohammadzadeh and Clark, 2011; Wimmer et al., 2013).

There are typically two methods available for the measurement of $\delta^{13}C$ of DIC; 1) gases DIC (i.e., CO_2) extraction after acidification of the sample solution followed by direct injection of CO_2 into stable isotope ratio mass spectrometer (SIRMS) (Atekwana and Krishnamurthy, 1998) and 2) precipitation of DIC as divalent metal-carbonate complexes followed by running on SIRMS equipped with elemental analyzer (EA) (Harris et al., 1997; Wimmer et al., 2013). Traditionally, the gaseous measurement $\delta^{13}C$ of DIC requires a special headspace gas collection line, and thus it is not feasible for a large number of samples (Gillikin and Bouillon, 2007; Torres et al., 2005). Meanwhile, measurement of $\delta^{13}C$ of DIC in precipitates using EA-SIRMS allows a direct combustion of the precipitates to liberate CO_2 into SIRMS through continuous gas flow; therefore, it is a more convenient method compared to the gases measurement for analyzing a large number of samples (Harris et al., 1997).

The carbonate precipitates for the analysis of $\delta^{13}C$ of DIC are obtained by reacting the sample solution with addition of $CaCl_2$, $BaCl_2$, or $SrCl_2$ after raising the pH to shift the carbonate equilibrium ($H_2CO_3 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$) to the right, which facilitates the formation of carbonate precipitates such as $CaCO_3$, $BaCO_3$, and $SrCO_3$ (Szynkiewicz et al., 2006). However, the effects of 1) pH adjustment (uncontrolled pH vs. raised pH) of the sample solution, 2) headspace (presence vs. absence of headspace) in the precipitation vessels, and 3) DIC in distilled water on the accuracy of the measurement of $\delta^{13}C$ of DIC are not clearly investigated yet. As the contamination of the sample with atmospheric CO_2 and CO_2 dissolved in distilled water ($\delta^{13}C$ below −10‰) in laboratories are critical concerns of the precipitation procedure, these factors need to be investigated.

This study was conducted to advance the carbonate precipitation method for precise analysis of $\delta^{13}C$ of DIC by addressing the pH, headspace, and distilled water issue as mentioned above. We hypothesized that 1) raised pH may facilitate carbonate precipitation but it may fix atmospheric CO_2, resulting in a lowered $\delta^{13}C$, 2) the presence of headspace may also lower the $\delta^{13}C$ due to contamination by atmospheric CO_2 depleted in $^{13}CO_2$, and 3) DIC in distilled water may also contribute to the erroneous measurement of the $\delta^{13}C$.

Materials and Methods

Overall experiment setting In this experiment, we did not conduct mutual exclusive experiments by setting paired experiments to investigate the effects of pH, headspace, and distilled water separately. Rather than, we started the experiment following the results of Harris et al. (1997), who reported that $SrCO_3$ is more suitable for analysis the $\delta^{13}C$ of DIC compared to $BaCO_3$ and $CaCO_3$. However, unfortunately, we have not obtained reliable results, and thus we tested the potential factors such as pH, headspace, and distilled water sequentially through a series of three experiments to achieve a reliable procedure (Table 1). All experiments were replicated three or five.

Materials In this experiment, a reference DIC solution (100 mg C L^{-1}) was prepared by dissolving analytical grade (99.8%, Kanto Chemical, Japan) $NaCO_3$ in either double and tertiary distilled water purified with a water purification system (Human Power 1+, Human corporation, Korea). The $\delta^{13}C$ of Na_2CO_3 determined using a continuous-flow stable isotope ratio mass spectrometer (SIRMS) (VisION, Isoprime Ltd, Cheadle Hulme, UK) was −7.45 ± 0.04‰ (n=5). The concentrations of DIC, dissolved organic C, and total C of distilled water were measured with a total organic C analyzer (Sievers 5310 C, GE Analytical Instruments, Boulder, CO, USA), and double distilled water had a higher ($p < 0.001$) C concentration than tertiary distilled water (Table 2). For carbonate precipitation, $CaCl_2$ (95.0%, Junsei, Japan), $BaCl_2$ (99.0%, Junsei, Japan), and $SrCl_2$ (99.0%, Junsei, Japan) were used. For the experiment, Na_2CO_3 solution (5 and 10 mg C L^{-1}) and precipitating agent solution (1 N concentration) was prepared immediately before (< 30 min) the experiment, and stored in air-tight bottles. To raise pH of the reaction solution, 1 N NaOH solution prepared from granular NaOH (97.0%, Junsei, Japan) was also prepared with other chemical reagents.

Experiment 1: headspace and double distilled water In the Experiment 1 (Table 1), all the chemical reagents were prepared in double distilled water, and the mixture of $SrCl_2$ (2 mL) and $BaCl_2$ (2 mL) was used as a

Table 1. Summary of experimental conditions for analysis of the $\delta^{13}C$ of dissolved inorganic carbon (DIC).

Experiment	pH treatment (pH)	Headspace	Grade of distilled water used	Precipitating agent
#1	Raised (12.2)	Presence	Double	Mixture of $SrCl_2$ and $BaCl_2$
#2	Control (10.2) vs. Raise (12.2)	Absence	Double	$CaCl_2$
#3	Control (10.2) vs. Raise (12.2)	Absence	Tertiary	$CaCl_2$

Table 2. The concentration of dissolved inorganic (DIC), organic (DOC), and total carbon (TC) of distilled water used in the experiments.

Distilled water	DIC (mg C L^{-1})	DOC (mg C L^{-1})	TC (mg C L^{-1})
Double	1.420 (0.100)	2.417 (0.043)	3.837 (0.143)
Tertiary	0.039 (0.002)	0.489 (0.001)	0.529 (0.001)

Values are the means with standard errors (n=3) in the parentheses.

precipitating agent for each experiment vessel containing Na_2CO_3 solution. Initially, we have added 4 mL of $SrCl_2$ to the vessel, but precipitates were not formed, and thus we used the mixture of $SrCl_2$ (4 mL) and $BaCl_2$ (4 mL), which produced visible precipitates immediately after the addition. The Na_2CO_3 solution (200 mL) were transferred into a 250-mL centrifuge bottle (total volume was 282 mL) and the precipitating agent (4 mL) and 1 N NaOH (2 mL) was added. Therefore, there was approximately 76 mL of headspace (including bottle top space) in the bottle. The number of samples was 10 (two levels of Na_2CO_3 solution × five replicates).

Experiment 2: pH and double distilled water In the Experiment 2 (Table 1), we changed the conditions of pH adjustment and headspace to solve the errors caused in the Experiment 1. Though precipitating agent was not initially considered, we have also changed the precipitating agent. All the chemical reagents were prepared in double distilled water, but $CaCl_2$ was used as a precipitating agent. A total of 12 centrifuge bottles were prepared (two levels of Na_2CO_3 solution × two pH treatments × three replicates). The Na_2CO_3 solution (250 mL) were transferred into a 250-mL centrifuge bottle and the precipitating agent (5 mL) was added. 1 N NaOH (2 mL) was added to half of the bottles to raise to pH from 10.2 to 12.2. After that, the remaining headspace were filled with the Na_2CO_3 solution to eliminate any headspace in the bottle.

Experiment 3: pH and tertiary distilled water In the Experiment 3 (Table 1), we repeated the Experiment 2 by using tertiary distilled water to eliminate the potential interference caused by DIC of the double distilled water.

Precipitation and chemical analyses The centrifuge bottle containing the samples were left at room temperature for 7 days to allow precipitation. The precipitation time was longer than 2 days (Bishop, 1990) and 6 days (Szynkiewicz et al., 2006) recommend by others. The bottles centrifuged at 5,000 rpm for 10 minutes, and a portion of supernatant (20 mL) was collected and the remaining was discarded. The precipitates were washed again by adding the corresponding precipitating agent (50 mL) followed by centrifugation.

The collected supernatant samples were analyzed for DIC concentration using the TOC analyzer. The precipitates were transferred a 50-mL conical tube, and dried under infra-red lamp. The dried precipitates were crushed to fine powder using a spatula. The $\delta^{13}C$ of the powder was analyzed using the SIRMS.

Calculation The recovery of carbonate (CO_3^{2-}) in precipitates (i.e., precipitation efficiency) was calculated as

$$\text{Carbonate recovery (\%)} = [(DIC_i - DIC_s)/DIC_i] \times 100$$

where DIC_i and DIC_s are the concentration of DIC in the initial solution used and in the supernatant solution after precipitation reaction, respectively.

The $\delta^{13}C$ were calculated as $\delta^{13}C$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$

where R is the atom % of ^{13}C, and the standard was the Vienna Pee Dee Belemnite standards (R=1.12372%). Accuracy of the measurement by the SIRMS tested using IAEA-C6 (sucrose, −10.8‰) was better than 0.2‰.

Statistical analysis Experimental data were tested for homogeneity of variance and normality of distribution with Levene's test and Kolmogorov-Smirnov test, respectively, and results showed that the variance was homogeneous and the distribution was normal. The effects of experimental treatments (e.g., DIC level and pH adjustment) were assessed with the analysis of variance (ANOVA) using the general linear model using the IBM SPSS Statistics 23 (IBM Corp., Ammonk, New York, USA) (Table 4). When ANOVA is significant, pairwise comparisons of the means were conducted using the Duncan's multiple range test. Statistical significance was set at α=0.05.

Results and Discussion

Precipitation efficiency Precipitation efficiency of DIC was different across the three experiments (Fig. 1), and within each experiment the precipitation efficiency was affected by DIC concentration and/or pH adjustment (Table 3). At a given experimental condition, the precipitation efficiency (85.8 and 96.2% in low and high DIC concentrations, respectively) in the Experiment 1, which was processed by addition of mixture of $SrCl_2$ and $BaCl_2$ under raised pH in the presence of headspace, was greater than those in the Experiments 2 and 3 (37.4 - 70.0% at pH 10.2 and 77.0 - 94.1% at pH 12.2) (Fig. 1). This is due to the lower solubility of $SrCO_3$ and $BaCO_3$ compared to $CaCO_3$ (Stenger, 1996); for example, the water solubilities (at 20°C) of $SrCO_3$, $BaCO_3$, and $CaCO_3$ are 0.0011, 0.0022, and 0.0013 g 100 g^{-1}, respectively (Seidell, 1940).

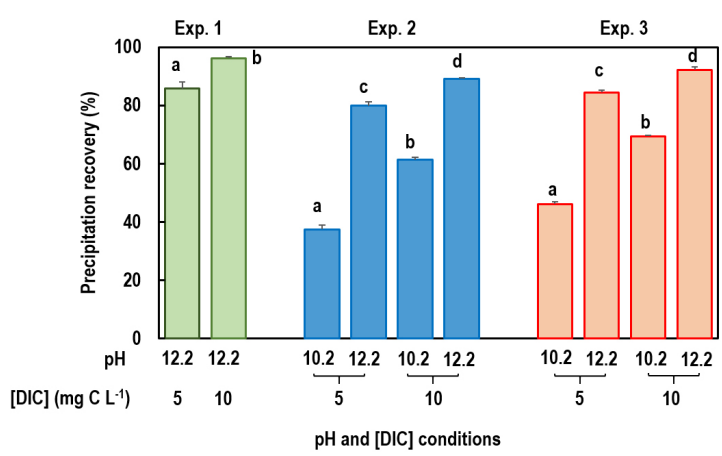

Fig. 1. Recovery of carbonates in the precipitates (precipitation efficiency) under different experimental conditions. Vertical bars are standard errors of the mean (n=5 for Experiment 1 and 3 for Experiments 2 and 3). Different lowercase letters indicate that the means are statistically different among the treatments within each experiment (see Table 2).

Across the Experiments 1–3, the precipitation efficiency was greater under high DIC concentration (Fig. 1 and Table 3) due to increased precipitation reaction through nucleation and crystal growth (Montes-Hernandez et al., 2009). An increase of pH from 10.2 to 12.2 in the Experiments 2 and 3 obviously increased the precipitation efficiency through a shift of carbonate equilibrium ($H_2CO_3 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$) to the right (Szynkiewicz et al., 2006; Lim et al., 2012). Therefore, these results suggest that precipitates yield of DIC may be more efficient for samples with a high DIC concentration and by addition of $SrCl_2$ and $BaCl_2$ rather than $CaCl_2$.

Precision of $\delta^{13}C$ Though the precipitation efficiency was high enough for the Experiment 1, the $\delta^{13}C$ of the $SrCO_3 \cdot BaCO_3$ was negative by more than 9‰ compared to the reference (−7.45‰) (Fig. 2). In addition, the $\delta^{13}C$ was not affected by DIC concentration (Table 4). Such errors could be ascribed primarily to the effect of ^{13}C-depleted atmospheric CO_2 remained in the headspace and dissolved in the double distilled water as previously mentioned; Szynkiewicz et al. (2006) reported that the $\delta^{13}C$ of contaminant DIC was −24.3‰. It may be also possible that the requirement of a high temperature for thermal decomposition of $SrCO_3$ and $BaCO_3$ causes C isotope fractionation, which produces $^{12}CO_2$ faster than $^{13}CO_2$ (Harris et al., 1997). Weast (1973) reported that thermal decomposition of $SrCO_3$ and $BaCO_3$ occurs at 1,100 and 1,300°C, respectively, while $CaCO_3$ thermally decomposes at 830°C.

In the Experiment 2, which was conducted by addition of $CaCl_2$ as a precipitating agent using double distilled water in the absence of a headspace, however, the precision of $\delta^{13}C$ measurement was not improved (Fig. 2). This result strongly suggests that DIC dissolved in the double distilled water is the main reason of the poor precision of $\delta^{13}C$ analysis. In the Experiment 3, which was conducted using tertiary distilled water under the same experimental

Table 3. The results (*P* value) of analysis of variance on the effects of DIC level and pH adjustment on the recovery of carbonate in precipitates (precipitation efficiency) for three experiments.

Effects	Experiment 1	Experiment 2	Experiment 3
DIC level (C)	0.001	< 0.001	< 0.001
pH adjustment (pH)	NA†	< 0.001	< 0.001
C × pH	NA	< 0.001	< 0.001

Details of the experiment are described in Table 1.
†Not applicable as pH adjustment was not included in the treatment.

Table 4. The results (P value) of analysis of variance on the effects of DIC level and pH adjustment on the $\delta^{13}C$ of carbonate in precipitates for three experiments.

Effects	Experiment 1	Experiment 2	Experiment 3
DIC level (C)	0.233	0.673	0.026
pH adjustment (pH)	NA†	0.315	0.275
C × pH	NA	0.241	0.328

Details of the experiment are described in Table 1.
†Not applicable as pH adjustment was not included in the treatment.

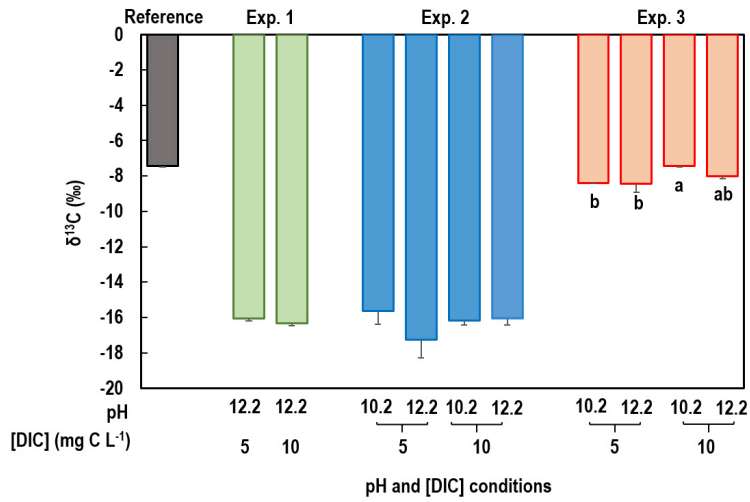

Fig. 2. $\delta^{13}C$ of the reference (Na_2CO_3) and the precipitates obtained from the Experiments 1–3. Vertical bars are standard errors of the mean (n=5 for the reference and Experiment 1 and 3 for Experiments 2 and 3). Different lowercase letters indicate that the means are statistically different among the treatments in the Experiment 3, and for the Experiments 1 and 2, there was no statistical significance (see Table 3).

conditions as the Experiment 2, the precision of $\delta^{13}C$ measurement was greatly improved. The differences in the $\delta^{13}C$ between the reference and the precipitates was less than 1.0‰, and the $\delta^{13}C$ of the precipitates produced from the solution with a high DIC concentration (10 mg C L^{-1}) by adding $CaCl_2$ without pH adjustment (pH=10.6) in the absence of a headspace was the same as that of reference (Fig. 2). The slightly decreased $\delta^{13}C$ of precipitates under a high pH (12.6) should be ascribed to the interference of the contaminant DIC from atmosphere; Szynkiewicz et al. (2006) reported that $\delta^{13}C$ of DIC decreased by 0.03, 0.07, and 0.22 at pH 10.26, 11.38, and 12.11, respectively. Therefore, these results together with the precipitation efficiency data imply that pH increases may facilitate DIC precipitation, but there is a possibility of DIC contamination.

Conclusion

Our results showed that DIC contaminant in double distilled water may cause unacceptable errors in the analysis of $\delta^{13}C$ of DIC, highlighting the strong necessity of using DIC-free tertiary distilled water. In this context, though not directly examined in the present study, CO_2 in a headspace may also cause similar errors. However, as the effects of pH, headspace, and distilled water on the precision of $\delta^{13}C$ analysis were not systematically investigated in the present study, we still lack of data on the quantitative errors caused by the conditions of pH, headspace, and distilled water for precipitation. Nevertheless, our results clearly showed that precipitation conditions for reliable $\delta^{13}C$ measurement are DIC-free tertiary distilled water, samples with a high DIC concentration, $CaCl_2$ addition as a precipitating agent, pH 10.6, and the absence of a headspace.

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