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A SIMPLE CO₂ EXTRACTION METHOD FOR RADIOCARBON ANALYSES OF DISSOLVED INORGANIC CARBON IN WATER SAMPLES WITHOUT A CARRIER GAS

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ABSTRACT. We developed a simple and cost-effective method for extracting carbon from dissolved inorganic carbon (DIC) in water samples without a carrier gas. This method only slightly modifies the existing vacuum line for CO₂ purification in radiocarbon research laboratories by connecting several reservoirs and traps. The procedure consists of repeated cycles of CO₂ extraction from water into the headspace of the reaction container, expansion of the extracted gas into the vacuum line, and cryogenic trapping of CO₂. High CO₂ yield (~98%) was obtained from a variety of water samples with a wide range of DIC concentrations (0.4–100 mmol·L⁻¹, in the case of 1.2 mgC). The δ¹³C fractionation depended on the CO₂ yield, while the ¹⁴C concentration was constant within the error range, regardless of the CO₂ yield. The average δ¹³C discrepancy between the results of this method and direct analyses made using the GC-IRMS was 0.02 ± 0.06‰. The standard deviations (1σ) in fraction of modern carbon (F¹⁴C) ranged from 0.0002 to 0.0004 for waters below 0.01 of F¹⁴C, and below 0.8% of F¹⁴C values for waters above 0.1. We conclude that this method is useful for effectively extracting CO₂ from DIC in water and yields accurate ¹⁴C data.

KEYWORDS: AMS, CO₂ extraction method, DIC, water.

INTRODUCTION

The carbon isotopes of dissolved inorganic carbon (DIC) in water are useful indicators for oceanographic and hydrologic studies of carbon behavior. Radiocarbon (¹⁴C) concentrations are used as indicators of water residence time, the atmosphere–ocean carbon budget, ocean circulation, and anthropogenic or magmatic carbon supplies (Elliot et al. 1999; Iwatsuki et al. 2001; Matsumoto 2007; Ostlund 1987; Rose and Davisson 1996; Sikes et al. 2000; Takahashi et al. 2013). International programs that focus on ocean water analyses, such as WOCE, GLODAP, and GEOTRACES (McNichol et al. 1994; Key 1996; Ge et al. 2016; Olsen et al. 2016), provide experimental protocols for analyzing carbon isotopes to obtain highly accurate data. For CO₂ extraction that is used to measure the ¹⁴C of DIC in water samples, the bubbling method (WOCE method) is the most widely used. An acidified seawater sample is bubbled with a carrier gas (usually N₂) to ensure the CO₂ is trapped until the DIC is completely stripped from the sample. Casacuberta et al. (2020) reduced the vial size to 120 mL (60 mL of water) and developed an automated experimental system coupled with an automated graphitization equipment (AGE) system (Wacker et al. 2010) to realize high-density observations. Because a bubbling container with a fixed volume is used, the bubbling method is suitable for samples with relatively constant DIC concentrations, such as seawater. To carry out the bubbling method, a certain volume of water is needed. Therefore, the bubbling method is difficult to adapt for water samples with wide ranges of DIC concentrations, such as groundwater and hot spring water, from below 0.5 mmol·L⁻¹ to over 100 mmol·L⁻¹, especially high-DIC-concentration waters. To adapt such samples to the bubbling method, extra-treatments of

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changing to a large volume bubbling container or diluting water samples with carbon-free water are needed.

The headspace gas extraction method has been employed instead of water bubbling in several studies (Molnár et al. 2013; Gao et al. 2014). This extraction method is commonly used for stable carbon isotope ($\delta^{13}\text{C}$) measurements (Atekwana and Krishnamurthy 1998; Torres et al. 2005; Miyajima et al. 2009; Mažeika et al. 2013; Meier and Sharma 2015; Sack and Sharma 2014; Takahashi et al. 2019a). A water sample is injected into an acid-containing vial filled with He or N_2 gas. CO_2 is released into the headspace of the vial, ensured by syringe extraction or carrier gas purging. Because small vials (usually ~ 12 mL) are used for $\delta^{13}\text{C}$ measurement in the headspace method, water samples that can be applied to this method are limited to those with relatively high DIC concentrations. Gao et al. (2014) and Molnár et al. (2013) established the headspace extraction method for ^{14}C measurement using 60-mL and 36-mL vials, but the volume are still smaller than those used in the bubbling method. When treating waters with low DIC concentration, CO_2 must be extracted multiple times from different vials of water. In fact, Takahashi et al. (2019c) treated ^{14}C water samples (~ 1 mmol $\cdot\text{L}^{-1}$) by combining CO_2 obtained through duplicate extractions from two or three different vials. The headspace method is suitable to treat small volumes of water, but it is difficult to treat large volumes of water. This means that the treatable volume of water differs between the bubbling and headspace methods, and thus various water samples with wide ranges of DIC concentrations are difficult to treat by both methods.

The precipitation method has also been widely used, especially in studies of groundwater (Carmi et al. 1971; Haynes and Haas 1980; Drimmie et al. 1991; Aucour et al. 1999; Alvarado et al. 2013; Burg et al. 2013; Mažeika et al. 2013; Minami et al. 2015). In this method, DIC is precipitated as carbonate material (BaCO_3 or SrCO_3) by adding BaCl_2 or SrCl_2 to the sample and controlling the pH using alkaline reagents. The precipitated carbonate is filtered or decanted and reacted with acid (normally H_3PO_4) to release CO_2 under a vacuum system. The precipitation method is satisfactory for water samples with a wide range of DIC concentrations and no carrier gas use and can provide reliable ^{14}C results. However, good agreements and disagreements of the ^{14}C results relative to those obtained using the bubbling or headspace methods have been reported (Takahashi et al. 2019c). Nakata et al. (2016) noted that modern carbon contamination was occurring from the NaOH reagent used for pH control. Although this risk of obtaining incorrect values can be prevented by using appropriate reagents and improved procedures, it is possible that the precipitation method may produce inaccurate results. The co-existing SO_4^{2-} in water can inhibit the precipitation of carbonates and affects carbon isotopic analyses (Kusakabe 2001; Takahashi et al. 2019c). Hence, we cannot reliably use the precipitation method as the dominant method.

The gas-permeable polymer membrane contactors have also been utilized to extract carbon from CO_2 in water (Gospodinova et al. 2016; Yokochi et al. 2018). The membrane contactor acts as an interface between the gas and liquid phases as a physical barrier that separates liquid water from the gas phase. Gas permeates from the liquid side using differential partial pressure, and CO_2 in the gas is trapped cryogenically. The membrane contactor extraction method developed by Yokochi et al. (2018) was originally designed for a large volume of water, from which radiokrypton was extracted. Another potential CO_2 extraction approach is the use of headspace equilibration and molecular sieve traps (Garnett et al. 2016). This method is focused on analyzing the ^{14}C of aquatic CO_2 in water

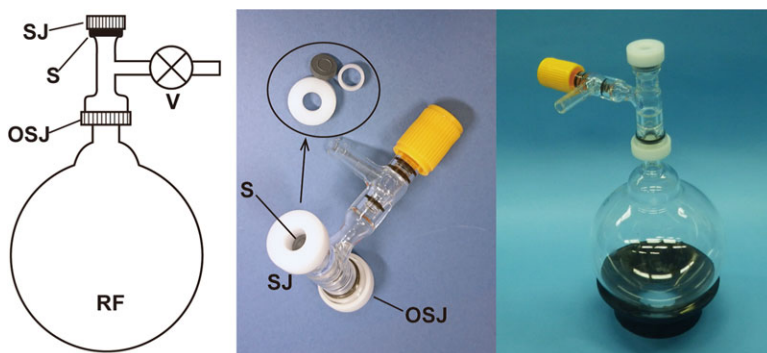


Figure 1 Reaction container assembled from a flask and connector. SJ: seal joint (Koshin Rika Ltd., Japan), S: pierceable septum (Labco grey chlorobutyl septum, Labco Ltd., UK), OSJ: O-ring seal joint (Koshin Rika Ltd., Japan), V: greaseless O-ring stopcock (SD valve; Koshin Rika Ltd., Japan), RF: reaction flask.

to investigate CO₂ evasion from inland water. Therefore, these methods do not include sample acidification and require large water samples (100–200 L or 2–5 L). As a result, they cannot extract carbon species of HCO₃⁻ and CO₃²⁻ from water because of the lack of pH control. When these degassing systems are adapted to analyze the ¹⁴C of total DIC, they require modifications that reduce their scale and add an acidification procedure. This would introduce additional complications and is difficult.

In this study, we tried to establish CO₂ extraction procedure that can treat water samples with a wide range of DIC concentrations. The required specifications for the developed procedure are as follows: (1) A variable water volume (1–250 mL) injected into the system, depending on the DIC concentration (0.4–100 mmol·L⁻¹ in the case of 1.2 mgC) of the sample. This requirement is compatible with extraction from terrestrial waters, as well as from seawater. (2) A simple procedure and a compact system (easy and inexpensive). The development of an automated carbon extraction system is above the required specifications for hydrological research, which measures two or three dozen samples per year, unlike oceanographic studies. Hence, the implementation cost of such an apparatus is not justified. (3) An acceptable time required to conduct a sample treatment (~30 min per sample). (4) A high extracted CO₂ yield and reliable ¹⁴C analysis.

METHODS AND SAMPLES

CO₂ Extraction Procedure (ReCEIT)

The bubbling method cannot treat a volume of 1 mL or less water since this amount of water cannot do bubbling. The method that satisfied our required specifications was the headspace method since it can treat a wide range of volumes. However, there were two problems in the existing headspace method: first was the limitation of treatable water volume, and second was the efficiency of CO₂ extraction from a large volume of water. In the method developed in this study, a glass flask was used for the extraction container (100, 300, and 500 mL), which was used to treat water samples with a wide range of DIC concentrations (Figure 1). In a previous study, the extraction container was sealed with a rubber septum and a screw nut or swaged aluminum cap, and CO₂ gas was extracted by a carrier gas through a needle. We changed the CO₂ extraction procedure using the carrier gas to carry it out under vacuum conditions.

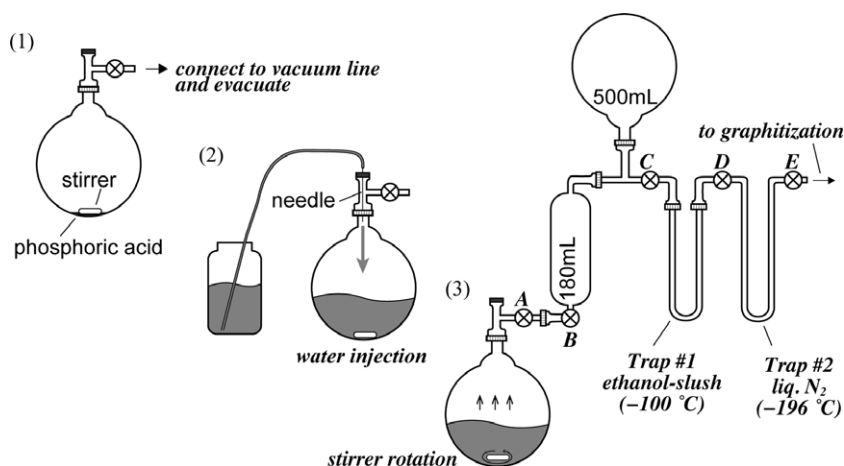


Figure 2 Schematic illustration of CO₂ extraction from DIC in water using the ReCEIT procedure. (1) Set up the reaction container. (2) Water sample injection. (3) Repeated cycles: Water vapor and CO₂ were ensured at cryogenic traps #1 and #2, respectively. The alternating opening of valves-A and -C can reduce amount of water condensed at trap #1 to be < ~1 mL. The tube of trap #1 is exchangeable for every sample to remove water.

The flask was connected to the vacuum line using O-ring fittings via a connector with a pierceable septum (Labco grey chlorobutyl septum, Labco Ltd., UK) sealed with a screw cap and a greaseless O-ring stopcock (SD valve; Koshin Rika Ltd., Japan) as shown in Figure 2. Hereafter, the assembled flask and connector (Figure 1) is referred to as the reaction container. By using the connector with a stopcock, the inside of the reaction container can be evacuated. A water sample was injected into the reaction container through the needle that pierced the rubber septum.

The operating procedure is characterized by repeated cycles of gas extraction into the headspace, extracted gas introduced into the vacuum line, and cryogenic trapping (Figure 2). We refer to this procedure as the ReCEIT (repeated cycles of extraction, introduction, and trapping). Step 1: A magnetic stir bar and 5 mL of phosphoric acid (85%, guaranteed grade) were placed into a flask. The flask was attached to the connector (Figure 1). The pierceable rubber septum was placed between the glass of the connector and the plastic spacer in the seal joint (SJ in Figure 1; Koshin Rika Ltd., Japan). The air inside the reaction container was evacuated for over an hour using a vacuum pump below 0.1 Pa. The seal joint was re-tightened slightly five minutes after starting the evacuation in order to ensure a reliable seal. This re-tightening is important because a hard tightening of the seal joint distorts the septum. Step 2: A water sample was injected by vacuum suction into the reaction container through a needle (20 G, 70 mm) connected to a plastic tube. Before injection, the needle and plastic tube were filled with the sample water to remove any air. The volume of water analyzed was calculated using the weights of the reaction container before and after sample injection. The maximum water volume is approximately half of the volume of the flask. If the volume of water injected into the reaction container was small, a gas-tight syringe was used. Step 3: An interval between Steps 2 and 3 is not required. CO₂ released into the headspace of the reaction container was introduced into the vacuum line and cryogenically purified. Gases in the headspace of the reaction container are introduced by expansion based on the pressure differential to valve-C in Figure 2. For effective gas introduction, two reservoirs (180 mL and 500 mL) were set

into the line. The reaction flask connector valve and the valve immediately before the cryogenic traps (valves -A and -C in Figure 2) were opened in an alternating order to prevent a large volume of water vapor reaching to traps. The expanded gases in the two reservoirs were collected by two cryogenic traps and un-trapped gases were removed by a vacuum pump. Step 4: The ensured gas to trap #2 in Figure 2 is purified to CO₂ cryogenically according to the procedures of each laboratory. After that, the CO₂ gas is quantified to proceed to the next step of making graphite target for ¹⁴C analysis.

When a high CO₂ yield is necessary, the cycle of expansion and cryogenic trapping (Step 2) should be repeated several times (e.g., 4 cycles), but we found in this study that one cycle is enough only for ¹⁴C analysis (see Results and Discussion). The treatment time depends on the number of cycles not on the water amount. One cycle of Step 3 takes about 3 to 4 minutes. The total times required for Steps 3 and 4 are ca. 20 minutes and ca. 30 minutes for 1 cycle and 4 cycles of Step 3, respectively.

¹⁴C and δ¹³C Measurements

The CO₂ extracted from the samples was purified cryogenically using an ethanol-slush trap (ca. -100°C) and liquid nitrogen traps in the vacuum line. The CO₂ was quantified using a capacitance manometer (Baratron® 722A low pressure type, MKS Instruments Inc., USA) to compute the yield. Purified CO₂ was sealed in a Pyrex tube for storage. For ¹⁴C analyses, CO₂ gas was treated by with Sulfix (8–20 mesh, Kishida Chemical Co., Ltd., Japan) as appropriate, and reduced to graphite with hydrogen gas to produce measurement targets for ¹⁴C analysis.

The ¹⁴C concentrations were measured using accelerator mass spectrometry (AMS; model 4130-AMS, HVEE, the Netherlands) at the Institute for Space-Earth Environmental Research at Nagoya University, Japan (Nakamura et al. 2000) relative to the NIST oxalic acid standard (SRM 4990C: HOx-II). Isotope fractionation was corrected using the ¹³C/¹²C ratio measured using AMS. The δ¹³C values of the extracted CO₂ were measured using an isotope ratio mass spectrometry (IRMS) with a dual inlet (Delta-V Advantage, Thermo Fisher Scientific, Inc., USA) at the Geological Survey of Japan. The δ¹³C values were determined with respect to the Vienna Pee Dee Belemnite (VPDB) standardized by NBS-19 (Coplen et al. 2006). The δ¹³C values and DIC concentrations of water samples were directly measured using a continuous-flow isotope ratio mass spectrometer coupled with gas chromatography (GC-IRMS; Delta-V Advantage with Gas Bench II, Thermo Fisher Scientific, Inc., USA) at the Geological Survey of Japan (Takahashi et al. 2019a). Samples measured using GC-IRMS are described in the respective sections. The DIC concentrations were calculated from the peak area of the GC-IRMS measurements. A calibration curve was generated using NaHCO₃ solutions with concentrations of 1–50 mmol·L⁻¹, prepared by diluting a 1 mol·L⁻¹ NaHCO₃ standard solution for ion chromatography (Kanto Chemical Co. Inc., Japan).

Repeated Cycles of CO₂ Extraction

The ReCEIT procedure contains several repeated cycles of CO₂ extraction into the headspace of the reaction container, expansion of the extracted gas into the vacuum line, and cryogenic trapping of CO₂ (Figure 2). To determine the optimal number of cycles, CO₂ extractions were carried out with repeated cycles of 1, 2, 3, and 4 times using NaHCO₃ solutions and some artificial water samples. The NaHCO₃ solutions were prepared from a 1 mol·L⁻¹ ion

chromatography standard (Kanto Chemical Co. Inc., Japan) by dilution with ultra-purified water (Milli-Q Direct 8 or Milli-Q Integral 3, Merck Millipore Co., USA) to 1.2 mmol·L⁻¹, 3 mmol·L⁻¹, and 6 mmol·L⁻¹. The ultra-purified water had an 18.2 MΩ·cm resistivity at 25°C, ≤ 5 ppb TOC, and < 1 particulate·mL⁻¹ (size > 0.22 μm). NaHCO₃ is known to have a very low ¹⁴C concentration (Takahashi et al. 2019b, 2019c). The artificial water samples (W09–W14) used in this study were made by Takahashi et al. (2019c) in order to compare inter-laboratory ¹⁴C concentrations. They were designed to have similar chemical contents as natural waters (Table 1). The fraction of modern carbon (F¹⁴C) values (Stenström et al., 2011) of samples W09–W14 were reported to be 0.0055–0.9918 (Takahashi et al. 2019c), but there is a possibility of secular changes in DIC concentration, ¹⁴C concentration, and δ¹³C. Takahashi et al. (2019c) reported such secular changes during the preservation period.

We measured the CO₂ yields, δ¹³C values, and ¹⁴C concentrations to compare the results of 1–4 extraction cycles. Water volumes injected into the reaction containers were 1.16–255 mL, corresponding to ~1–210 mmol·L⁻¹ DIC concentrations. Before injection samples W09–W14 were homogenized in a single container. Therefore, the results from samples W09–W14 can be compared for 1–4 extraction cycles. Meanwhile, NaHCO₃ solution concentrations were 1.2, 3, and 6 mmol·L⁻¹. Due to a flaw in the experimental design, the solutions at each concentration were not homogenized. Therefore, the solutions used in cycles 1 and 4 were a little different from the solutions used in cycles 2 and 3.

Blank Estimation

We estimated the system blank for all the operating processes (e.g., needle piercing to septum, leakage from connections, and acid background) in the ReCEIT procedure using the amount of CO₂ extracted from Milli-Q water. Although Milli-Q water has very low concentrations of dissolved materials, it might contain a gas component dissolved from the atmosphere. The amount of dissolved CO₂ is proportional to the volume of Milli-Q water. The system blank of the ReCEIT procedure, excluding the water itself, was estimated using several Milli-Q waters (43–248 mL) by extrapolating the data so that the amount of water is zero. The volume of the reaction flask used for the blank estimation analyses was 500 mL. Four extraction cycles were used for all treatments.

It is possible that carbon contamination may occur during water injection into the reaction flask if the injected water was exposed to the ambient air. Such contamination can be eliminated by injecting under CO₂-free conditions in a glovebox, where delicate operations are difficult. Moreover, it is difficult to eliminate all CO₂ from the box. If there is no effect on carbon contamination, water injection into the reaction flask without using a glovebox saves time and effort. Setting the water intake tube around the bottom of the sample container would prevent samples from being exposed to ambient air CO₂. We assessed the CO₂ contamination of the injection process with and without eliminating the ambient CO₂. A relatively low concentration NaHCO₃ solution (0.6 mmol·L⁻¹) was used to sensitively detect any CO₂ contamination. The NaHCO₃ solution was divided and poured into three plastic bottles outside a glovebox. Two bottles were placed in a glovebox, then the CO₂ in the glovebox was absorbed by an alkali reagent (Thomas® Ascarite II CO₂ Absorbent, Thomas Scientific, USA). The two NaHCO₃ solutions were injected into the reaction containers in the glovebox under CO₂-free conditions, while the other solution was injected without using the glovebox (hereafter referred to as NaHCO₃-free and NaHCO₃-normal, respectively). We consider that the CO₂ contamination of the NaHCO₃ during preservation

Table 1 Chemical composition and assumed types of natural water of six artificial water samples used in this study. The chemical data, except for DIC, were listed in the chemical composition at the end of the campaign of Takahashi et al. (2019c). DIC concentration was measured by GC-IRMS at the time of CO₂ extraction in this study.

Sample	Assumed water type	Na ⁺ (mg·L ⁻¹)	K ⁺ (mg·L ⁻¹)	Mg ²⁺ (mg·L ⁻¹)	Ca ²⁺ (mg·L ⁻¹)	Cl ⁻ (mg·L ⁻¹)	SO ₄ ²⁻ (mg·L ⁻¹)	DIC (mg·L ⁻¹)
W09	Shallow groundwater	9.2	0.5	3.7	11.9	11.1	3.5	12.2
W10	Hot spring (SO ₄ type)	240.3	1.8	17.9	153.4	104.2	501.9	79.1
W11	Hot spring (Cl type)	903.2	21.0	13.8	0.0	654.5	108.6	245.6
W12	Groundwater (Volcanic region)	2984	62.9	318.0	91.8	4870	31.5	306.5
W13	Seawater	12,098	407.9	720.5	133.1	19,132	2980	39.9
W14	Deep groundwater	13,448	376.1	196.3	0.0	14,009	17.6	2598

Table 2 CO₂ yields for 1–4 repeated extraction cycles. Samples A/B: 1.2 mmol·L⁻¹ NaHCO₃, C/D: 3 mmol·L⁻¹ NaHCO₃, E/F: 6 mmol·L⁻¹ NaHCO₃. Samples A, C, and E were used for 1 and 4 repeated cycles, B, D, and F were used for 2 and 3 repeated cycles. The relatively higher error in the CO₂ yields of samples W11 and W12 was derived from the large error in the DIC analyses.

Sample	DIC (mg·L ⁻¹)	Sample amount (mL)	Flask size (mL)	CO ₂ yield at each repeated cycle (%)			
				1	2	3	4
A/B	13.9/13.9	248.8–255.4	500	53.9 ± 0.9	84.8 ± 1.5	92.4 ± 1.6	96.2 ± 1.7
C/D	34.6/35.2	84.9–97.9	500	67.3 ± 2.2	92.2 ± 1.7	96.0 ± 1.7	100.4 ± 2.7
E/F	68.2/69.6	49.8–51.4	500	72.5 ± 1.3	92.0 ± 1.6	97.4 ± 1.7	101.3 ± 1.7
W09	12.2	213.8–240.5	500	56.5 ± 0.8	83.4 ± 1.2	93.8 ± 1.3	98.9 ± 1.3
W10	79.1	42.9–44.5	300	80.8 ± 2.4	97.4 ± 2.8	No data	101.5 ± 3.0
W11	245.6	13.1–13.9	100	88.4 ± 5.8	96.9 ± 6.0	97.6 ± 5.3	97.0 ± 5.6
W12	306.5	11.1–11.5	100	92.3 ± 5.1	99.4 ± 5.3	99.3 ± 5.3	99.7 ± 5.3
W13	39.9	63.8–73.5	300	76.4 ± 1.1	92.4 ± 1.3	No data	96.6 ± 1.2
W14	2598	1.2–1.3	100	95.0 ± 2.9	91.6 ± 2.8	94.0 ± 2.9	92.5 ± 2.8
Average (±1σ)				75.9 ± 14.8	92.2 ± 5.4	95.8 ± 2.8	98.2 ± 2.9

is negligible because it was stored in PAN (acrylonitrile butadiene methyl acrylate) bottles with a high performance of gas barrier (Takahashi et al. 2019b).

RESULTS AND DISCUSSION

Optimal Number of Repeated CO₂ Extraction Cycles

We compared the CO₂ yields with the number of repeated cycles (Table 2). Higher CO₂ yields were obtained when more cycles were used for almost all water samples, except W14. A CO₂ yield of close to 100% was obtained after 4 cycles of extraction. The δ¹³C values of these extracted CO₂ samples are almost identical to the δ¹³C values measured using GC-IRMS (Table 3). Sample W14 did not show a clear relationship between the CO₂ yield and the number of repeated cycles. In addition, the δ¹³C values of the CO₂ extracted from sample W14 were scattered among the number of cycles. The vacuum conditions inside the reaction container at sample injection splashes some of the sample water on the inside wall of the connector. The reaction container did not tilt over 90° to prevent wetting of the O-ring joint and septum. Therefore, the splashed water could not react with the acid in the flask. In cases where the injected water volume is small, as with sample W14, the influence of splashed and unreacted water on CO₂ released from the sample could increase and produce more widely scattered results. Therefore, the CO₂ yield and δ¹³C of sample W14 were not used for further discussion in this study.

When the water volume was small, the CO₂ was collected with sufficient yield, even when many cycles of CO₂ extraction were not carried out (Figure 3a, Table 2). The CO₂ yield changed with the sample volume, and the degree of this change decreased gradually with more repeated cycles. The increase in CO₂ yield was larger for the first few cycles, especially in the case of a large sample volume. When less than 100 mL was injected, the CO₂ yield reached 90% after 2 cycles, 95% after 3 cycles, and 98% after 4 cycles. Four cycles of extraction provided more than 95% CO₂ yields for all samples, and 98% yields for almost all of the

Table 3 $\delta^{13}\text{C}$ values of the water samples and extracted CO_2 from 1–4 repeated extraction cycles, and $\delta^{13}\text{C}$ discrepancies between the ReCEIT and GC-IRMS results ($\Delta\delta^{13}\text{C}$). The descriptions of sample A–E are same as Table 2. *: Data of sample W14 were not used for calculation.

Sample	$\delta^{13}\text{C}$ (‰) GC-IRMS	$\delta^{13}\text{C}$ at each repeated cycle (‰)				$\Delta\delta^{13}\text{C}$ at each cycle (‰)			
		1	2	3	4	1	2	3	4
A/B	No data	−3.50	−3.74	−3.80	−3.81	No data			
C/D	−4.00/−3.89	−3.66	−3.77	−3.80	−3.89	0.31	0.12	0.09	0.08
E/F	−3.85/−3.86	−3.71	−3.78	−3.78	−3.82	0.14	0.09	0.08	0.04
W09	2.08	2.46	2.22	2.13	2.07	0.37	0.13	0.05	−0.01
W10	−11.20	−11.09	−11.12	No data	−11.21	0.11	0.09	no data	−0.01
W11	−5.09	−4.94	−5.02	−5.00	−5.01	0.15	0.07	0.09	0.08
W12	−10.45	−10.46	−10.49	−10.47	−10.52	−0.02	−0.04	−0.02	−0.07
W13	No data	−0.67	−0.82	No data	−0.89	No data			
W14	−19.82	−19.93	−20.28	−20.03	−20.14	(−0.11)	(−0.46)	(−0.22)	(−0.32)
				Average of $\Delta\delta^{13}\text{C}^*$		0.18	0.08	0.06	0.02
				Standard deviation (1σ) of $\Delta\delta^{13}\text{C}^*$		0.14	0.06	0.05	0.06

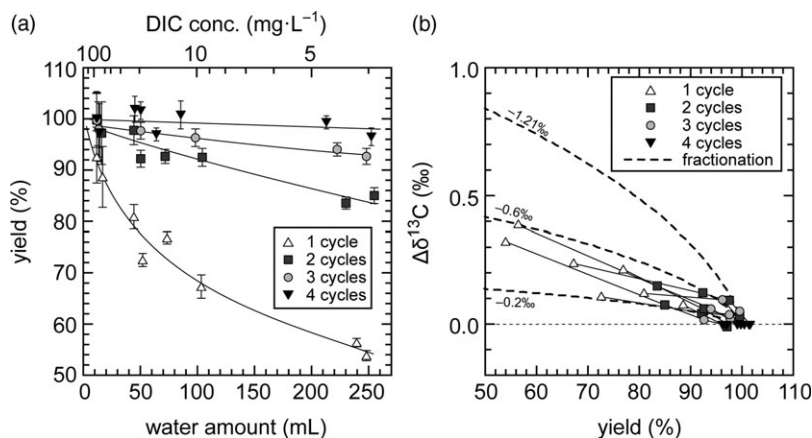


Figure 3 Results of assessments of 1–4 repeated extraction cycles. (a) The relationship between the CO₂ yield and the water volume for repeated cycles. DIC concentrations are shown as corresponding to 1 mg carbon at each respective water amount. (b) Changes in δ¹³C values of the extracted CO₂ (Δδ¹³C) along the CO₂ yield. The dashed lines: the isotopic change based on Rayleigh fractionation (fractionation factors: -1.21‰, -0.6‰, and -0.2‰).

samples with volumes of less than ~250 mL. The δ¹³C values of the extracted CO₂ decreased with increasing CO₂ yield and the number of cycles (Figure 3b). We postulate that the δ¹³C changes were caused by isotopic fractionation among the carbon species in water and the headspace CO₂. The dominant carbon species in the water is H₂CO₃ or CO₂ (aq) due to the low pH. The δ¹³C fractionation between the aqueous and gaseous CO₂ ($\epsilon_{\text{aq-g}}$) was calculated as -1.21‰ at an approximate room temperature of 20°C using the equation from Zhang et al. (1995). Hence, the δ¹³C of the CO₂ gas is higher than that of the equilibrium CO₂ (aq). The δ¹³C changes in the extracted CO₂ with CO₂ yield, shown in Figure 3b, can be explained mainly by Rayleigh fractionation, although they do not match exactly. If the δ¹³C fractionation is assumed to be -0.6‰ to -0.2‰, the calculated value matches well with the observed changes. The difference in fractionation values reported by Zhang et al. (1995) was -1.21‰, and the measured values were -0.6‰ to -0.2‰, which was likely caused by the fact that aqueous and gaseous CO₂ did not reach equilibrium in the reaction container. The δ¹³C changes in the extracted CO₂ were expected to be 0.15‰ for a CO₂ yield of 90%, 0.10‰ for a 95% yield, and 0.05‰ for a 98% yield, based on Rayleigh fractionation and assuming that the fractionation value was -0.6‰. The δ¹³C discrepancy between the results using the ReCEIT and GC-IRMS analytical methods decreased with an increasing number of cycles to 0.02 ± 0.06‰ on average (Table 3). The number of repeated cycles should be determined based on the required δ¹³C measurement precision by referring to the CO₂ yield and the respective water volumes shown in Figure 3.

With regards to F¹⁴C, the individual solutions had identical values for the 1–4 repeated CO₂ extraction cycles (Figure 4). This constant relationship is also observed in δ¹⁴C, which is not corrected for isotopic fractionation. The isotopic fractionation during CO₂ extraction observed in δ¹³C did not influence the ¹⁴C results because the fractionation factor is too small. Therefore, we can extract CO₂ gas for ¹⁴C measurements from one ReCEIT cycle if a sufficient amount of CO₂ gas can be obtained. However, we must pay attention to the fact that CO₂ extracted from one cycle does undergo isotopic changes, especially in δ¹³C, owing to fractionation.

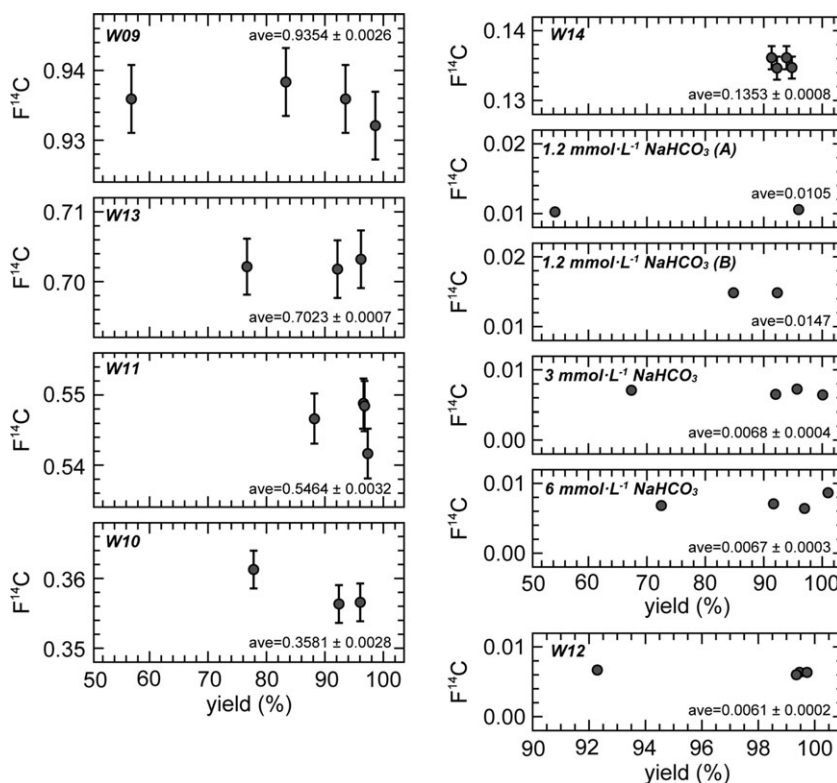


Figure 4 F¹⁴C of the extracted CO₂ for 1–4 repeated cycles. The “ave” indicates the average values with a standard deviation. CO₂ yield of W14 is for reference only (see text).

As mentioned above, two sets of NaHCO₃ solutions were independently prepared for 1 and 4 cycles, and 2 and 3 cycles. Two different NaHCO₃ solutions of 3 mmol·L⁻¹ and 6 mmol·L⁻¹ exhibited identical F¹⁴C values, but the 1.2 mmol·L⁻¹ exhibited different values. Other preliminary experiments using NaHCO₃ solutions (1, 2, 3, and 4 mmol·L⁻¹) showed F¹⁴C increases of 0.002–0.007 in solutions with concentrations below 2 mmol·L⁻¹. Casacuberta et al. (2020) reported that a ¹⁴C blank in Milli-Q water decreased after acidification, heating overnight, and finally flushing with He into Milli-Q water. They showed that the F¹⁴C of the Milli-Q containing IAEA-C1 decreased from ~0.02 to 0.0042 using this treatment. Another cause of the increase in F¹⁴C in NaHCO₃ solutions below 2 mmol·L⁻¹ is modern CO₂ contamination from ambient atmosphere during solution preparation (dilution of the NaHCO₃ solution). Such contamination does not become apparent for solutions with higher DIC concentrations. Because the Milli-Q water used to dilute the NaHCO₃ reagent in this study did not undergo such pre-treatment, we consider that the F¹⁴C results of the NaHCO₃ solutions cannot represent the ¹⁴C background using the ReCEIT method.

The standard deviations (1σ) in F¹⁴C of the NaHCO₃ solutions and sample W12, which have low F¹⁴C values (note: they are not ¹⁴C background), ranged from 0.0002 to 0.0004. Regarding the above 0.1 F¹⁴C values, the deviations (1σ) were below 0.8% of the F¹⁴C values. We therefore determined that the ReCEIT method can extract CO₂ from DIC in water with a high F¹⁴C precision. We recommend 4 repeated cycles if a high CO₂ yield from a large

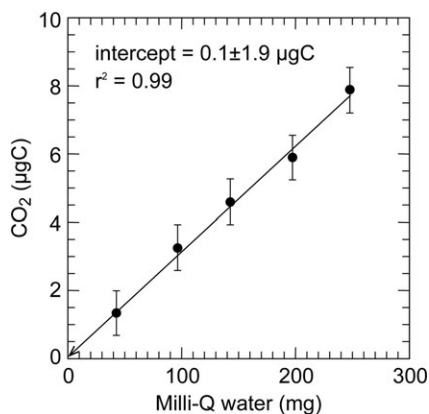


Figure 5 Relationship between CO₂ extracted from Milli-Q water and the analyzed water volume.

water volume is required. However, one cycle of extraction was sufficient to obtain CO₂ from a sample to measure the ¹⁴C when the DIC concentration was greater than ~10 mg·L⁻¹ and the sample volume was 250 mL that is the maximum water volume analyzed using the ReCEIT method at present. Because the maximum water volume is limited by the size of the reaction container, there is the potential to increase this volume. At the lower limit of analysis, a water sample of ~1 mL had uncertainties in the CO₂ yield and δ¹³C results, likely due to splashing of the sample in the reaction container at injection, while the F¹⁴C result was not influenced. Therefore, a water sample even below 1 mL can be treated by the ReCEIT method for the measurement of ¹⁴C. We conclude that the ReCEIT method can treat water samples with a wide range of DIC concentrations.

Blank Estimation of the ReCEIT Method

In order to check for air leaks inside the reaction container, which includes the flask and connector, the pressure changes inside the reaction container were measured for 10 days after evacuation using a capacitance manometer (Baratron® 722A low-pressure type, MKS Instruments, USA) connected to a glass joint with an O-ring fitting. The pressure changes were below 10 Pa, which is the detection limit of the capacitance manometer. If the interior pressure change and ambient CO₂ concentration are assumed to be 10 Pa and 400 ppm, respectively, the CO₂ contamination in the reaction container was estimated to be 0.013 µgC, even though the dead volume of the joint was considered. This value is the maximum amount of CO₂ contamination for a 10-day period because the assumed value of 0.01 kPa was overestimated. The actual amount of CO₂ contamination in the reaction container might be smaller. Therefore, we concluded that air contamination during the extraction processes may be negligible.

The amount of CO₂ extracted from the Milli-Q water increased as the water volume increased (Figure 5). The range shown in Figure 5 is much smaller than that of the dissolution amount of CO₂ at equilibrium with the air. This is likely caused by disequilibrium, because the Milli-Q water was used immediately after purification. The extrapolated value of 0.1 ± 1.9 µgC at a zero water volume was considered as the blank for the ReCEIT extraction system. Modern carbon contamination of 0.1 µgC increased to ~0.0001 in F¹⁴C, when we analyzed a water

Table 4 F¹⁴C and δ¹³C values of the NaHCO₃ solutions analyzed for carbon contamination from air during water injection.

F ¹⁴ C / δ ¹³ C of injection in air (NaHCO ₃ -normal)	F ¹⁴ C / δ ¹³ C of injection in CO ₂ -free atmosphere (NaHCO ₃ -free)
0.0253 ± 0.0006 / -3.94‰	0.0244 ± 0.0006 / -3.94‰
	0.0255 ± 0.0006 / -3.96‰

sample containing 1 mgC of dead carbon. In the case of the maximum error (2.0 µgC of blank), the ¹⁴C influence increased to ~0.002 in F¹⁴C. These estimated blanks of F¹⁴C are sufficiently low for analyzing water samples.

The results of the assessment of CO₂ contamination during sample injection are shown in Table 4. Although the assessment was carried out under harsh conditions such as low DIC concentrations and high solution pH, the ¹⁴C concentrations of the “NaHCO₃-normal” were between two of the “NaHCO₃-free” results and were identical to each other within error. We concluded that the influence of CO₂ contamination during sample injection into the reaction flask is negligible, even though the operation was carried out in air and without a glovebox. Practically, some natural samples are expected to have a F¹⁴C of zero and a low DIC concentration in hydrological studies. Therefore, we suggest the use of a glovebox when analyzing these samples by this CO₂ extraction method.

CONCLUSIONS

A method for extracting CO₂ from DIC in water was developed. This method is simple and cost-effective, as it requires only slight modifications to the vacuum line for CO₂ purification and graphitization of organic materials. The operating procedure was composed of repeated cycles of CO₂ gas extraction into the headspace of the reaction container, expansion of the extracted gas into the vacuum line, and cryogenic trapping of CO₂. We refer to this procedure as repeated cycles of extraction, introduction, and trapping (ReCEIT). The advantages of this method are the lack of a required carrier gas and that samples with a wide range of DIC concentrations can be analyzed. The ReCEIT method can treat various amounts of water with a wide range of DIC concentrations.

The cycle of CO₂ extraction and trapping was repeated several times to achieve a high CO₂ yield. When volumes under 100 mL were analyzed, their CO₂ yields reached 90% after 2 cycles and 95% after 3 cycles. The CO₂ yield reached ~98% when 4 cycles were carried out. The δ¹³C values of the CO₂ extracted from 4 repeated cycles and the GC-IRMS measurements were within 0.02 ± 0.06‰. ¹⁴C concentrations can be measured using CO₂ gas extracted from one cycle, if a sufficient amount of CO₂ gas was obtained, because the F¹⁴C values were constant within the error range regardless of the CO₂ yield.

The blank level of the ReCEIT system was estimated as 0.1 ± 0.9 µgC using ultra-purified water. This blank provided a ¹⁴C increase of ~0.0001 in F¹⁴C when 1 mg of dead carbon sample was analyzed. The exposure of water samples to the air during injection did not influence the ¹⁴C results, even if the ambient air was not treated to remove CO₂, with the exception of water samples with very low DIC and ¹⁴C concentrations. We conclude that the ReCEIT method is useful for effectively extracting CO₂ from DIC in water to obtain accurate ¹⁴C results.

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