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Technical Note: Precise quantitative measurements of total dissolved inorganic carbon from small amounts of seawater using a gas chromatographic system

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Abstract. Total dissolved inorganic carbon (C_T) is one of the most frequently measured parameters used to calculate the partial pressure of carbon dioxide in seawater. Its determination has become increasingly important because of the rising interest in the biological effects of ocean acidification. Coulometric and infrared detection methods are currently favored in order to precisely quantify C_T. These methods however are not sufficiently validated for C_T measurements of biological experiments manipulating seawater carbonate chemistry with an extended C_T measurement range (~ 1250–$2400 \, \mu mol \, kg^{-1}$) compared to natural open ocean seawater (~ 1950–$2200 \, \mu mol \, kg^{-1}$). The requirement of total sample amounts between 0.1–1 L seawater in the coulometric- and infrared detection methods potentially exclude their use for experiments working with much smaller volumes. Additionally, precise C_T analytics become difficult with high amounts of biomass (e.g., phytoplankton cultures) or even impossible in the presence of planktonic calcifiers without sample pre-filtration. Filtration however, can alter C_T concentration through gas exchange induced by high pressure. Addressing these problems, we present precise quantification of C_T using a small, basic and inexpensive gas chromatograph as a C_T analyzer. Our technique is able to provide a repeatability of $\pm 3.1 \, \mu mol \, kg^{-1}$, given by the pooled standard deviation over a C_T range typically applied in acidification experiments. 200 μL of sample is required to perform the actual C_T measurement. The total sample amount needed is 12 mL. Moreover, we show that sample filtration is applicable with only minor alteration of the C_T. The method is simple, reliable and with low cumulative material costs. Hence,

it is potentially attractive for all researchers experimentally manipulating the seawater carbonate system.

1 Introduction

Oceanic absorption of anthropogenic carbon dioxide (CO_2) in the past and during the next decades leads to seawater acidification (Caldeira and Wickett, 2003), which will increasingly influence biological and biochemical processes in the open oceans (Doney et al., 2009; Houghton, 1995; Kroeker et al., 2010) and in coastal waters (Melzner et al., 2012). Due to this fact, scientific interest in precise measurements of C_T has increased. Among other parameters, such as total alkalinity and pH, C_T is used to describe the seawater carbonate system. The measurement range for C_T in experimental studies (~ 1250–$2400 \, \mu mol \, kg^{-1}$) (e.g., Thomsen et al., 2013; Yu et al., 2011; Melzner et al., 2011; and Lohbeck et al., 2012) usually exceeds the typically applied measurement range of C_T in open ocean water (~ 1950–$2200 \, \mu mol \, kg^{-1}$). Often only small sample volumes are available. Moreover, such experiments often contain non-negligible quantities of biomass (e.g., from phytoplankton cultures), which require sample filtration (Mueller et al., 2012; Matthiessen et al., 2012) before precise C_T measurement can be performed.

Several analytical methods have been established to quantify the C_T content in water samples. The commonly applied techniques (i.e., coulometric and infrared detection method) convert C_T into CO_2 by acid addition prior to quantification by a highly precise gas-detection system (Johnson et al., 1985; Wong, 1970). Today the coulometric C_T

Table 1. Comparison of established C_T measurement methods. Specific measures of precision from the literature are summarized as repeatability.

Method	Injected volume	Collected sample volume	Total sample volume required after SOP 1	Repeatability
Coulometric	~ 30 mL	150–500 mL	> 300 mL	$\pm 1.5 \, \mu\text{mol kg}^{-1}$[a]
NDIR	2.5–10 mL	50–250 mL	> 100 mL	$\pm 2 \, \mu\text{mol kg}^{-1}$[b]
Photometric	~ 0.5 mL	~ 50 mL	~ 100 mL	$\pm 3 \, \mu\text{mol kg}^{-1}$[c]
"Old GC"	~ 2 mL	~ 150 mL	~ 300 mL	$\pm 7 \, \mu\text{mol kg}^{-1}$[d]

[a] Johnson et al. (1993); [b] Goyet and Snover (1993); [c] Stoll et al. (2001); [d] Weiss and Craig (1973).

analysis is the most preferred method in oceanographic research with the highest precision of ± 0.06 %, which equals $\pm 1.5 \, \mu\text{mol kg}^{-1}$ (Table 1) for a typical open ocean water measurement range (see above) (Johnson et al., 1993). This precision is needed because present ocean acidification causes only a small increase of $\sim 1 \, \mu\text{mol kg}^{-1} \, \text{yr}^{-1}$, which is added to a C_T background of $\sim 2100 \, \mu\text{mol kg}^{-1}$ (Houghton, 1995). Disadvantages of this semi-automatic method are the necessity of relatively large total sample volumes (~ 0.3–1 L) and the requirement of highly toxic chemicals such as ethanolamine and hydroxyethylcarbamic acid (Dickson and Goyet, 1994; Johnson et al., 1985). The improved infrared detection method (non-dispersive infrared analysis, NDIR) (Kaltin et al., 2005) and a newer photometric method (continuous-flow analysis) (Stoll et al., 2001) for C_T measurements corrected for these drawbacks by a faster sample throughput, using less toxic chemicals and requiring only a small amount of sample. However, these new methods decreased the measurement precision by ± 0.08 % (Table 1).

Gas chromatography (GC) analysis of the C_T content from sea water samples was first tested in the early 1960s (Park and Catalfomo, 1964; Swinnerton et al., 1962), and improved for precision in the beginning of the 1970s (Weiss and Craig, 1973). The GC measurement process was divided into three temperature controlled parts. First, sample and acid addition into the stripping chamber was performed at ambient temperature. Second, released gases from the chamber were transferred by the carrier gas into the GC components at 40 °C. A sub-sample was collected using a 0.1 mL sample loop. Third, CO_2 was separated by a silica gel column at 45 °C and finally quantified by a thermal conductivity detector. Approximately 0.3–1 L of total sample volume was required for sampling, rinsing the analytical system and finally measuring a sample. The precision of the GC method was denoted with a 0.7–0.3 % relative standard deviation for the open ocean C_T range (Swinnerton et al., 1962; Weiss and Craig, 1973). However, this kind of GC method (hereafter referred to as "old GC method") has not been established as a common technique to quantify C_T.

In contrast, for non-quantitative stable isotope analysis of dissolved inorganic carbon (δ^{13}C; DIC) GC has become a standard method applying a head space technique (Capasso et al., 2005; Salata et al., 2000; St-Jean, 2003). For this purpose sealed vials containing < 20 mL of sample were acidified. After CO_2 equilibration (~ 24 h) between water and the head space a gas sub-sample was collected from the latter. The CO_2 was separated by a GC column and transferred to a stable isotope ratio mass spectrometer via an interface. δ^{13}C values for DIC measurements were determined with a good precision of ± 0.1 ‰ (Salata et al., 2000). However, this quantification of DIC has never been validated against a certified reference material (Dickson, 2010).

Here we present a gas chromatographic technique precisely quantifying C_T of measurement ranges typically emerging in experimentally manipulated acidification studies. A C_T measurement is performed by sample injection of only 200 µL taken from a 10 mL sample. 2 mL samples are required for rinsing the sampling tube. That is, a total sample volume of 12 mL for each sample. Moreover, we show that sample filtration leads only to minimal alterations of the C_T.

Our method was verified by certified reference material (Dickson, 2010) and tested using samples comprising high biomass of calcifying phytoplankton in low and high C_T concentrations.

2   Material and procedures

2.1   Instrumental setup

The analytical system consisted of a small gas chromatograph (8610C; 48 cm × 34 cm × 37 cm; SRI-Instruments, California, USA), a C_T measurement chamber (10 mL headspace crimp vial; 23 mm × 48 mm; sealed with a butyl rubber septum; Restek, Bad Homburg, Germany), and an external automated cooling trap (Thermo Fisher Scientific, Bremen, Germany) (Fig. 1a, b). Transfer lines between the C_T measurement chamber, gas chromatograph, and cooling trap were made from fused silica (Guard column, 0.53 mm ID; Restek, Bad Homburg, Germany). In order to penetrate
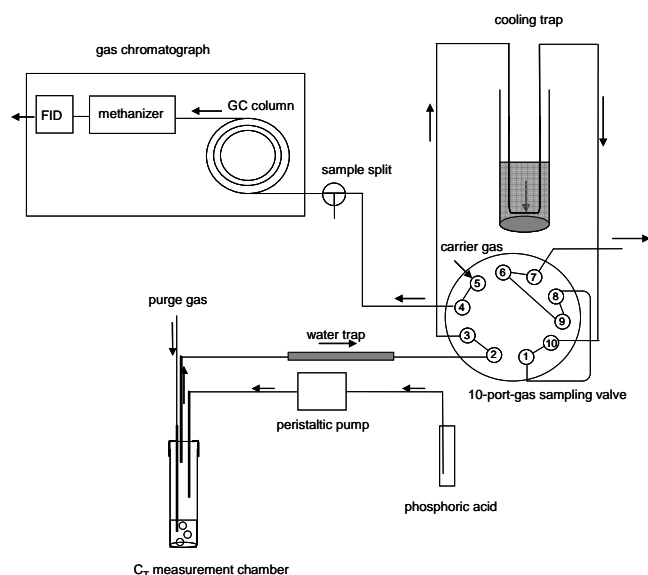
Fig. 1a. C_T analyzer setup in "sample load position" collecting released CO_2 from a sample.
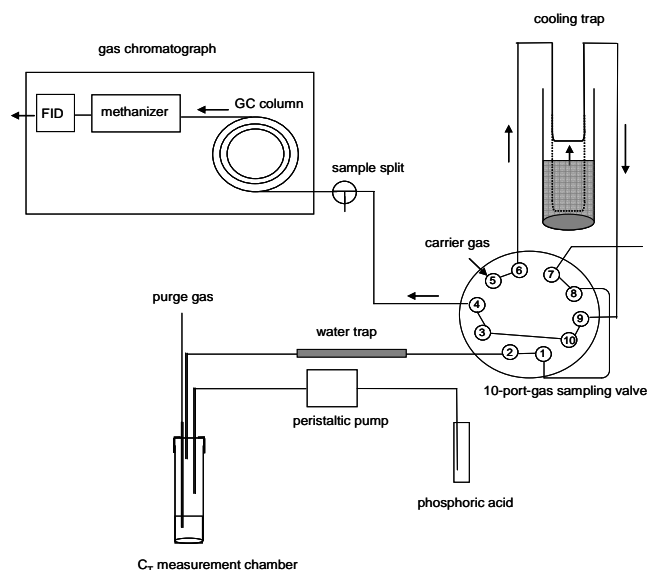
Fig. 1b. C_T analyzer setup in "sample injection position", CO_2 released from trap is loaded into the column for analysis.

the septum of the measurement chamber, stainless steal injection needles (0.80×80 mm; Unimed, Lausanne, Switzerland) were used. The gas chromatograph was equipped with a 10-port-gas sampling valve, a methanizer (nickel catalyst, 380 °C; SRI-Instruments, California, USA) and a flame ionization detector (FID) (SRI-Instruments, California, USA) (Fig. 1a, b). The catalytic efficiency was stable during all measurements. Phosphoric acid addition into the C_T measurement chamber to convert C_T into CO_2 was conducted by a peristaltic pump (IPC; Ismatec, Glattbrugg, Switzerland). CO_2 separation was performed by a micro packed GC column for permanent gases (Shincarbon®, 120 mm length, 1 mm inner diameter (ID); Restek, Bad Homburg, Germany) using the following temperature program: initial 45 °C held for 9.8 minutes (min), ramp 50 °C min^{-1} to 180 °C and held for 2 min. Moisture was removed by a water trap. The trap consisted of a glass tube (length ~ 20 cm, outer diameter 6 mm) that was filled with phosphorus pentoxide (P_2O_5) (SICAPENT® with indicator; Merck, Darmstadt, Germany). All connections were sealed by Swagelok reducing connectors of 1/8 to 1/16 in. (water trap connection) and straight connectors of 1/16 in. (Guard column connection) using graphite-vespel ferrules (1/8 in. to 6 mm ID, or 1/16 in. to 0.53 mm ID; Restek, Bad Homburg, Germany). Helium (purity 99.9996 %) used as carrier gas was set to ~ 30 mL min^{-1} with a column split flow ratio of 2.5. The helium purge flow was adjusted to 50 mL min^{-1}. Data processing, action of the 10-port-gas sampling valve, and activation of the cooling trap (SRI Instruments Europe GmbH, Bad Honnef, Germany) was operated by the GC software "Peak simple" (version 2.83; SRI-Instruments, California, USA).

2.2 Preparation of C_T measurement chambers

In order to prepare chambers for C_T measurements 10 mL glass vials and septa as described above were used. Prior to sealing, each vial was equipped with a small magnetic stir bar (10 mm × 6 mm). Subsequent additions and connections into and out of the vial were made by injection needles penetrating the sealing (see instrumental setup above). In the next step vials were pre-purged with nitrogen at a flow rate of ~ 30 mL min^{-1} for 20 s. Thereafter, 1 mL of deionized water (DI water) was added into each vial. These prepared vials, now defined as the C_T measurement chambers, were integrated into the analytical system by connection to the helium purge supply and a transfer line directing to the cooling trap (Fig. 1a, b).

In the following the same type of vials and sealing were used for sample collections and for preparation of C_T reference standards.

2.3 Preparation of a sodium carbonate solution used a s C_T lab standard

The C_T lab standard consisted of highly pure sodium carbonate (Suprapur®, 99.999 %; Merck, Darmstadt, Germany) and DI water. A defined amount of ~ 3 mg anhydrous sodium carbonate (SOP 2, Annexe B; Dickson and Goyet, 1994) was weighed into a small tin capsule (5 × 9 mm Hekatech, Wegberg, Germany) using a micro balance (SC2, readability: 0.1 µg; Sartorius, Göttingen, Germany), and subsequently transferred into a pre-weighed 10 mL vial. The vial was carefully filled up to the maximum with DI water using the same peristaltic pump described in the instrumental setup above. The occurrence of turbulences and air bubbles was avoided.
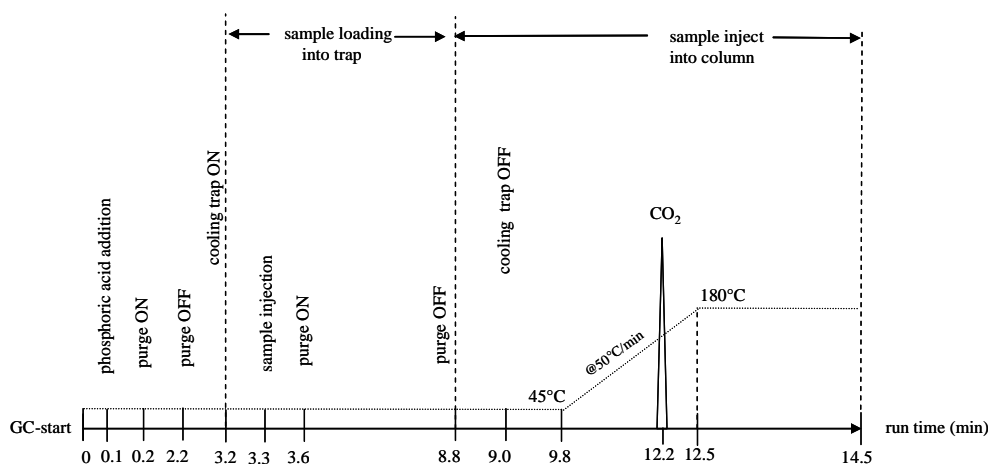
Fig. 2. Operating sequence of the GC-C_T measurement for one sample.

Afterwards the vial was sealed and weighed once again. The headspace was kept below 1 %. Finally, the exact C_T concentration was calculated.

2.4 Preparation of sub-samples and filtered sub-samples from certified reference material (CRM)

Sub-samples (sub-CRM) of certified reference material CRM (batch 108, $C_T = 2022.7 \pm 0.45\,\mu\mathrm{mol\,kg}^{-1}$) (Dickson, 2010) were carefully drawn from an original bottle containing 500 mL using a Tygon® plastic tube (1.65 mm ID). The sub-samples were forced into 10 mL vials by gravity. In order to initiate the flow, a 10 mL single-use syringe was used to draw the sample into the tube. Samples were immediately sealed to avoid gas diffusion. Approximately 20 sub-samples were prepared.

Filtered sub-samples (filtered sub-CRM) were taken with the peristaltic pump (see instrumental setup above) into 10 mL vials. The filtration was conducted using a single-use syringe filter (0.2 μm; Minisart RC25, Sartorius, Göttingen, Germany) which was connected to the intake tube (Tygon® plastic tube, 1.65 mm ID) of the pump. The flow rate was set to $\sim 6\,\mathrm{mL\,min}^{-1}$ in order to avoid turbulence and degassing during the filling process. Vials were immediately sealed after filling.

2.5 C_T sampling procedure from calcifying phytoplankton cultures

Test samples from cultures containing the planktonic calcifier *Emiliania huxleyi* were collected into 10 mL vials. In order to obtain samples with different C_T (i.e., low, medium, and high), samples were taken from three different cultures with non-manipulated (low) and enriched (medium and high) C_T respectively. C_T enrichment of the culture media was achieved by bubbling with an air-CO_2 mixture. In order to

remove biomass from the samples filtration was conducted as described above. The filtered samples were poisoned with 2 μL saturated $HgCl_2$ solution, and immediately sealed and stored at 4 °C in darkness until the measurement procedure.

2.6 GC-C_T measurement procedure and calibration

Following the time axis in Fig. 2, a C_T measurement was started by the GC program with an addition of 0.8 mL concentrated phosphoric acid (85 % p.a. (per analysi); Merck, Darmstadt, Germany) into the prepared C_T measurement chamber (Fig. 1a). In the next step the acidified and continuously stirred (magnetic stirrer, RCT basic; Staufen, Germany) DI water inside the chamber was pre-purged with helium for 2 min in order to reduce background carbon dioxide to a minimum. The purge gas flow was stopped and the cooling trap was activated (Fig. 1a). After ~ 1 min the gas pressure inside the C_T measurement chamber was close to ambient pressure. Now a sample injection of 200 μL into the C_T measurement chamber was performed manually using a calibrated digital syringe™ (DS80700; 250 μL; Hamilton, Nevada, USA). A few seconds later the gas purge flow was started once again and the released CO_2, which hereafter passed the P_2O_5 (water trap), was collected by the cooling trap (Wong, 1970) with liquid nitrogen (Fig. 1a). The purge flow was stopped after 5.2 min and the 10-port-gas sampling valve was set to "inject position" (Fig. 1b). After the cooling trap was deactivated, CO_2 was removed at ambient temperature and transferred into the column at 45 °C. Rapidly heating the column up to 180 °C desorbed the CO_2. The CO_2 passed the catalyst (methanizer) and was converted into methane. Finally, the methane was measured by the FID (Fig. 1b). Time for a complete analysis took 14.5 min (Fig. 2). C_T was determined from the methane peak area. The C_T analyzer was calibrated daily by measuring certified reference material (CRM; Dickson, 2010). Applying the rule of proportion, C_T values in $\mu\mathrm{mol\,kg}^{-1}$ were calculated by the measured
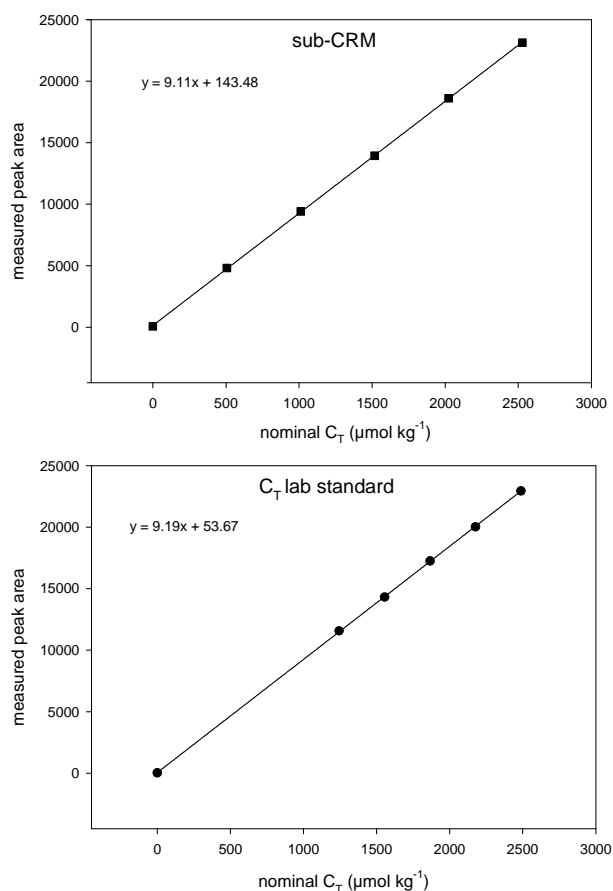
Fig. 3. Measurement linearity indicated by calculated nominal C_T in $\mu mol\,kg^{-1}$ of sub-CRM and C_T lab standard versus measured peak area, respectively.

peak areas of both the actual sample and the measured CRM or sub-CRM.

In order to account for background CO_2, the CO_2 amount obtained from a pre-purged and acidified C_T measurement chamber without sample injection was used as a blank measurement. Each measurement using the C_T lab standard contained an additional blank by the injection of $200\,\mu L$ DI water, which was used as the solvent to prepare the sodium carbonate solution. Consequently measurements with the C_T lab standard involved were blank corrected.

2.7 Method validation

All measurements were conducted under the same conditions adhering to repeatability, i.e., analysis was performed by the same operator in the same laboratory using the same equipment. The laboratory was air-conditioned and set to $20\,°C$.

In order to show the measurement linearity, we measured the C_T amount by injection of different volumes (range 100–$250\,\mu L$, $50\,\mu L$ steps) of the sub-CRM and C_T lab standard, respectively (Fig. 3). For the sub-CRM the nominal measurement range was ~ 500–$2500\,\mu mol\,kg^{-1}$. For the C_T lab stan-

dard a range of nominal ~ 1250–$2500\,\mu mol\,kg^{-1}$ was covered. Measured peak areas were plotted against nominal C_T concentrations.

In order to investigate potential effects on measurement quality of C_T by sub-sampling and sample filtration, we conducted C_T measurements taken from CRM ($500\,mL$), sub-CRM ($10\,mL$) and filtered sub-CRM ($10\,mL$). Additionally, as a reality check, natural samples of seawater, which contained calcifying phytoplankton and had different (low, medium and high), C_T were measured. Such samples represent the most difficult analytical conditions because they require filtration prior to C_T measurements. Each sample was measured three times, and mean and standard deviation (SD) were calculated, respectively. In the following the quality (repeatability) of C_T measurements is given by a pooled standard deviation S_p (IUPAC, 2006). S_p is given for each treatment separately and across all C_T measurements.

3 Results

The measurement linearity of our GC system could be determined ($r^2 = 0.9999$; $n = 6$ for both sub-CRM and C_T lab standard, respectively) following a linear regression model: $y = ax + b$; with y and x representing measured peak area and nominal C_T in $\mu mol\,kg^{-1}$, respectively. The regression slope (a) of the sub-CRM was 9.11, the peak area (intercept, b) accounted for 143.48. The regression slope of the C_T lab standard was 9.19, the peak area represented 53.67 (Fig. 3). Slopes of both standards differed 1 % from each other. Whereas the intercept of the C_T lab standard represented a relatively good agreement with obtained blank measurements (see below), the intercept derived from the sub-CRM was slightly increased. This was potentially due to slightly higher scatter in the measured sub-CRM. The most probable source of error was the imprecision of the digital syringe, which increased with decreasing injection volume (250–$100\,\mu L$, in $50\,\mu L$ steps) while implementing the nominal C_T values. However, linearity was not applied to calibrate the system, and hence to calculate or correct actual C_T values.

Blank measurements obtained from pre-purged and acidified C_T measurement chambers without sample addition during analysis resulted in $5\,\mu mol\,kg^{-1} \pm 1.8$ for all conducted measurements. C_T blank measurements conducted by the injection of only $200\,\mu L$ DI water were slightly higher with $\sim 7\,\mu mol\,kg^{-1}$.

Average measurements of sub-CRM and filtered sub-CRM were 2022.1 and 2024.3 $\mu mol\,kg^{-1}$, respectively (Table 2). The S_p of C_T measurements of both treatments were ± 2.8 and $\pm 4.1\,\mu mol\,kg^{-1}$, respectively (Table 2).

Results for test measurements conducted from filtered samples derived from phytoplankton cultures with low, medium and high C_T showed a S_p of ± 1.8, ± 5.5, and

Table 2. Comparison of the CRM with sub-sampled (sub-CRM), and filtered sub-CRM, respectively. Triplicate measurements are given as means ± standard deviations. S_P refers to pooled standard deviations per treatment.

	(CRM, 500 mL, batch 108, $C_T = 2022.7 \pm 0.45\,\mu mol\,kg^{-1}$)	
	Measured peak area of CRM $\pm 2.2\,\mu mol\,kg^{-1}$	
Sample	Sub-CRM ($\mu mol\,kg^{-1}$)	Filtered sub-CRM ($\mu mol\,kg^{-1}$)
1	2020.9 ± 3.6	2028.2 ± 4.2
2	2022.3 ± 1.5	2020.5 ± 5.5
3	2018.0 ± 1.9	2029.3 ± 3.7
4	2027.0 ± 3.6	2019.0 ± 2.6
Mean $\pm S_P$	2022.1 ± 2.8	2024.3 ± 4.1

Table 3. Comparison of measurements from low, medium and high C_T samples. Samples were derived from calcifying phytoplankton cultures (*Emiliania huxleyi*), and thus filtered prior to measurements. Triplicate measurements are given as means and standard deviations. S_P refers to pooled standard deviations per treatment.

Sample	Low C_T	Medium C_T	High C_T
1	1846.2 ± 2.6	2285.0 ± 4.5	2406.6 ± 1.0
2	1847.7 ± 1.6	2289.5 ± 5.8	2411.1 ± 2.6
3	1843.0 ± 0.6	2281.7 ± 6.2	2415.3 ± 2.6
Mean $\pm S_P$	1845.6 ± 1.8	2285.5 ± 5.5	2411.0 ± 2.2

$\pm 2.2\,\mu mol\,kg^{-1}$, respectively. The S_p across all samples was $\pm 3.1\,\mu mol\,kg^{-1}$.

4 Discussion

The here presented C_T quantification method using a GC system allowed for precise measurements with a sample volume of 10 mL (total required volume 12 mL) comprising a typical extended C_T measurement range (1250–2400 $\mu mol\,kg^{-1}$) used in biological experiments manipulating seawater carbonate chemistry. C_T measurements were only marginally affected by sample filtration.

Regarding the extended measurement range, the coulometric and NDIR techniques (Goyet and Snover, 1993; Goyet and Hacker, 1992) have solely been validated for the measurement linearity (0–2500 $\mu mol\,kg^{-1}$) using an artificial standard (sodium carbonate solution), but not for actual C_T sample measurements. Measurements for an extended C_T measurement range were neither verified by the "old GC" nor by the photometric method (Stoll et al., 2001; Weiss and Craig, 1973).

Additional difficulties in handling C_T samples arise under experimental conditions when calcified phytoplankton biomass requires sample filtration prior to measurements. The relatively good agreement of filtered samples with CRM and sub-CRM and the small S_p underline the reliability of the present analytical method as well as the applicability of sample filtration. However, differences in the average C_T values among filtered and unfiltered CRM and the slightly higher S_p in the filtered set of samples point to the fact that careless handling and filtration of such small volumes prior to measurements are prone to cause imprecision. The S_p of $\pm 5.5\,\mu mol\,kg^{-1}$ derived from filtered phytoplankton samples with medium C_T shows that the combination of high phytoplankton biomass, here especially calcifying phytoplankton, and increased C_T represents the most difficult analytical challenge.

Another critical point when measuring C_T from experimentally derived seawater samples is the typically small total sample amount. The requirement of small samples is particularly important when conducting microcosm experiments from which only a few mL are available for analysis. Whereas the coulometric method requires total sample volumes of $> 0.3\,L$, the NDIR and photometric method need considerably smaller amounts (Table 1). However, while commercial manufacturers of C_T analyzers with NDIR technique (Marianda, Kiel, Germany; Apollo SciTech Inc., Bogart, USA) report about the requirement of a ~ 10 mL sample to perform a C_T measurement, no detailed statements are given about (i) the total sample volume required during a sample collection (e.g., SOP 1, Dickson and Goyet, 1994) supplying this technique, and (ii) the volume necessary for the rinsing procedure between alternating high and low C_T measurements. Finally, no verified repeatability or precision is given for processing such samples. Hitherto, due to small sample sizes in this experimental scientific field, the photometric method (Stoll et al., 2001) was preferred.

The GC technique presented in this paper is not comparable with the "old GC method" described by Weiss and Craig (1973), because the new method applies a complete sample transfer instead of a gas sub-sample. Therefore, the complex analytical "old GC system", equipped with a gas sample loop for sub-sampling, is highly sensitive to carrier gas pressure conditions, which in turn requires a constant working temperature during C_T analysis (e.g., 40 °C; Weiss and Craig, 1973). Moreover, the non-separation of purge and

carrier gas lines tends to cause analytical imprecision by an increased exhaustion of the water trap and the slow accumulation of water in the separation column material. This reduces the gas flow and the separation efficiency during analysis. To get rid of the accumulated water a periodical heating of the separation column of up to 270 °C for about 4 h is necessary (Weiss and Craig, 1973).

A potential source of error of the photometric method might be that stored samples have to be opened before analyses (Stoll et al., 2001), which potentially causes CO_2 outgassing. This could be particularly critical when using extremely high C_T ranges, e.g., due to manipulated seawater. The other extreme would be particularly low C_T values due to inorganic carbon consumption through phototrophic growth, which potentially leads to gas diffusion into the sample. Opening of the samples is probably necessary due to the robustness of the used sealing, which does not allow for penetration with injection needles when using a common autosampler. Moreover, the photometric method configured as a continuous flow system is sensitive to temperature fluctuations, which causes a baseline drift and thus makes data correction necessary. Signal interferences can occur during analysis in the presence of sulfides, and the dependency on constant ion strength excludes a sample set of different salinities. In comparison, the butyl rubber septa for sample sealing in the present GC system has been proved as gas tight (Brandes, 2009; Spötl, 2004). Manual penetration of this sealing by a syringe in order to collect a sub-sample minimizes the contact to the atmosphere. Chemical interferences and disturbances by different salinities were not observed.

C_T quantification by basic infrared detection is potentially limited because IR measurements are extremely sensitive to water vapor. This requires a strict prevention of water entry into the detector during analysis (Wong, 1970). More sophisticated high precision NDIR detectors (e.g., LI-7000, LICOR-Biosciences, Germany) with water vapor correction overcome this problem; however, they are more expensive. In the GC system presented here, water vapor is not compromising the measurements, because P_2O_5 is used as strong water adsorbing material to protect the separation column.

Sample carryover effects were observed in NDIR and coulometric systems, which were equipped with automatic pipettes for sample addition (Kaltin et al., 2005). This effect can occur through insufficient rinsing between subsequent sample measurements and depends on the difference in C_T concentrations among samples. A carryover effect was not reported by authors using the old GC system. However, the usage of a sample loop for transfer into the stripping chamber may show the same problem when using different high C_T concentrations. In general, the quantity of repeated rinsing procedures between such C_T measurements will increase the required sample amount. Our GC method using a 250 μL digital syringe was always rinsed twice with the new sample before a new measurement was conducted. Carry over effects were not detected.

The complex manifolds of the NDIR , coulometric and the photometric analyzers as well as the old GC systems consist of a number of tubes, valves and connectors that get contaminated by the highly toxic preservative $HgCl_2$. Handling and operating such systems must be done with care. Handling of the toxic preservative $HgCl_2$ during analysis using the present method could be restricted to the C_T measurement chamber (single-use vials) and sample syringe.

Sample injection with a manual syringe applied in the present C_T quantification method was identified as the most error-prone process during analysis. However, using a digital syringe improved the repeatability of measurement to the here presented S_P of 3.1 μmol kg^{-1}. This result still does not meet precision requirements for oceanographic research but will be sufficiently precise for C_T samples derived from manipulated acidification experiments with large measurement ranges with a difference of up to ∼ 1000 μmol kg^{-1}. Moreover, the CRM (Dickson, 2010) that was used to calibrate the system had a pCO_2 close to ambient values. Thus, due to potential loss of CO_2 from high C_T samples and the unavailability of CRM containing high C_T, the imprecision for measurements of high C_T samples may be larger and should be subject to more extensive tests.

5 Conclusions

It can be concluded that the here presented GC-C_T quantification method offers a number of advantages compared to other methods. In particular the good repeatability of measurement along an extended C_T measurement range, the required very low sample volume and the applicability for sample filtration might lead to establishment of this method as an alternative for C_T quantification of samples from biological ocean acidification experiments.

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