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Technical Note: Highly precise quantitative measurements of total dissolved inorganic carbon from small amounts of seawater using a common gas chromatographic system: an alternative method compared to established detection systems

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Abstract

Total dissolved inorganic carbon (C_T) is one of the most frequently measured parameters in order to calculate the partial pressure of carbon dioxide in seawater. Its measurement has become increasingly important because of the rising interest in the biological effects of acidification. The coulometric- and infrared detection methods are favoured to precisely quantify C_T. However, these methods were not validated for C_T samples from acidification experiments investigating biological responses to manipulated partial pressure of carbon dioxide (pCO_2), which need an extended C_T measurement range ($\sim$ 1250–2400 µmol kg^{-1}) compared to natural open ocean seawater samples ($\sim$ 1950–2200 µmol kg^{-1}). Additionally, the requirement of total sample amounts between 0.25–1 L seawater in the coulometric- and infrared detection methods exclude their use for experiments working with smaller volumes. Precise C_T analytics also become difficult with high amounts of biomass (e.g. phytoplankton cultures) or even impossible in the presence of planktonic calcifiers without sample pre-filtration. However, filtration can alter C_T concentration through gas exchange. Addressing these problems, we present precise quantification of C_T using a small, basic and inexpensive gas chromatograph as a highly sensitive C_T-analyzer. Our technique is able to provide a measurement precision of ± 3.7 µmol kg^{-1} and an accuracy of ± 1.2 µmol kg^{-1} in a C_T range typically applied in acidification experiments. It requires sample sizes of only 200 µL taken from 10 mL pre-filtered samples or from a 10 mL sub-sampled seawater reference (Dickson standard). Our method is simple, reliable and with low cumulative analytical costs. Hence, it is potentially attractive for all scientists 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

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influence biological- and biochemical processes in the open oceans (Doney et al., 2009; Houghton, 1995; Kroeker et al., 2010) and in costal waters (Melzner et al., 2012). Due to that 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 manipulated seawater carbonate systems in biological experiments. In such experimental studies the measurement range for C_T ($\sim$ 1250–2400 µmol kg^{-1}) usually exceeds the typically verified measurement range of C_T from natural open oceanic water ($\sim$ 1950–2200 µmol kg^{-1}). Moreover, often only small sample volumes are available which sometimes require pre-filtration due to high amounts of biomass (e.g. from phytoplankton cultures).

Several analytical techniques 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 (Brandes, 2009; Goyet and Snover, 1993; Johnson et al., 1993). However, C_T measurements using these techniques were only validated within a C_T range of natural open ocean seawater (see above).

Gas chromatography (GC) analyzing 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 (WLD). Approximately 0.25–1 L total sample volume was required for sampling, rinsing the analytical system and finally to performing a sample measurement. The precision of the GC-method was denoted with 0.7 %-0.3 % relative standard deviation for the open ocean C_T range. However, this

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kind of GC-method (hereafter referred to as old GC-method) has not been established as a common technique to quantify C_T.

Currently, methods such as the coulometric- and the infrared detection (Johnson et al., 1985; Wong, 1970) replaced the gas chromatographic C_T quantification due to its higher measurement precision. Today the coulometric C_T analysis is the most preferred method in oceanographic research with the highest precision of ± 0.06 % which equals ± 1.5–2.0 µmol kg^{-1} for a typical open ocean water measurement range (see above) (Goyet and Snover, 1993). This precision is needed because present ocean acidification causes only a small increase of ~ 1 µmol kg^{-1} per year which is added to a C_T background of ~ 2100 µmol kg^{-1} (Houghton, 1995). Disadvantages of this semiautomatic method are the necessity of relatively large total sample volumes (0.5–1 L) and the requirement of highly toxic chemicals such as ethanolamine and hydroxyethylcarbamic acid (Dickson, 1994; Johnson et al., 1985; Knap, 1994). The improved infrared detection method (non-dispersive infrared analysis, NDIR) and a newer photometric method (continuous-flow analysis) for C_T measurements corrected these drawbacks by a faster sample throughput, using less toxic chemicals and requiring only a few mL of sample (Johnson et al., 1993; Stoll et al., 2001). However, these new methods decreased the measurement precision to ± 0.08 %.

Until now, the option of gas chromatography as an alternative method to quantify C_T was neglected by the research community. In contrast, non-quantitative gas chromatographic stable isotope analysis of dissolved inorganic carbon (δ^{13}C -DIC) using smaller volumes has become an established method applying the head space technique (Capasso et al., 2005; Salata et al., 2000; St-Jean, 2003). For this purpose sealed vials containing < 20 mL sample were acidified. After carbon dioxide 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 ‰. However, this quantification of DIC has never been validated against an international accepted reference value (Dickson, 2010).

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Here we present a gas chromatographic technique precisely quantifying C_T from measurement ranges typically emerging in experimentally manipulated acidification studies. It requires low sample sizes of only 200 µL taken from 10 mL pre-filtered samples (total 12 mL).The method was verified by the international accepted reference value (Dickson, 2010) and tested using samples with high biomass of calcifying phytoplankton in low and high C_T concentrations. Moreover, the required amount of toxic chemicals is considerably reduced.

2 Material and procedures

2.1 Instrumental setup

The analytical system consists of a small GC (SRI-8610, 48 cm × 34 cm × 37 cm, Torrance, USA) which is connected to an external cooling trap (Thermo Fisher Scientific, Bremen, Germany). The GC is equipped with an automatic 10-port-gas sampling valve, a methanizer (nickel catalyst, 380 °C) and a flame ionization detector (FID). The catalytic efficiency was stable during all measurements. Phosphoric acid addition to convert C_T into CO_2 is conducted by a peristaltic pump (IPC, Ismatec, Schwitzerland) (Fig. 1a, b).

CO_2 separation is performed by a micro packed column for permanent gases (Shincarbon®, 120 mm length, 1 mm ID, Restek, Bad Homburg, Germany) using the following temperature program: initial 45 °C held for 8.9 min, ramp 50 °C min^{-1} to 180 °C and held for 2 min. Moisture is removed by a water trap. The trap consists of a glass tube (length ∼ 20 cm, outer diameter 6 mm) which is filled with phosphorus pentoxide P_2O_5 (SICAPENT® with indicator, Merck, Darmstadt, Germany). Pure helium (99.9996 %) with a flow rate of ∼ 12 mL min^{-1} is used as carrier gas. The split flow is set to a ratio of 2.5 with a helium purge flow of ∼ 50 mL min^{-1}. Transfer lines between measuring chamber, GC, and cooling trap are made of fused silica (0.53 mm ID, Restek). All connections are sealed by graphite-vespel ferrules (Swagelok connectors

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1/16″ or 3/8″, Singen, Germany). Data processing, action of the 10-port valve, and activation of the trap (Schambeck, Bad Honnef, Germany) are operated by the software "Peak simple" (version 2.83).

2.2 Preparation of a sodium carbonate solution used as internal lab standard

In order to control the performance of the GC-system for C_T-measurements an internal lab standard made from high pure sodium carbonate (Suprapur®, 99.999 %, Merck, Darmstadt, Germany) was used. A defined amount of ~ 4 mg anhydrous sodium carbonate (Dickson, 1994) was weighed into a small tin capsule (5 × 9 mm Hekatech, Wegberg, Germany) using a micro balance (SR2, Sartorius, Göttingen, Germany) and transferred into a pre-weighed 10 mL glass vial (Restek, Germany). The vial was carefully filled up to maximum with DI water using a peristaltic pump. Turbulences and air bubbles were avoided. The vial was sealed with a butyl rubber-septum and weighed once again. Subsequently, the exact C_T concentration was calculated.

2.3 Preparation of sub-samples from the international reference (sub-Dickson)

Sub-samples of the internationally accepted reference (Dickson 2010; batch 108, true value: $C_T = 2022.7\ \mu mol\ kg^{-1} \pm 0.4$) were carefully drawn from an original bottle (Dickson batch) using a Tygon® plastic tube. The samples were collected by hydrostatic pressure into 10 mL vials. Samples were immediately sealed as explained above to avoid diffusion of gases. Approximately 20 sub-samples could be obtained from a 500 mL Dickson-bottle.

2.4 C_T sampling procedure from calcifying phytoplankton cultures

We collected test samples from calcifying phytoplankton cultures with high and low C_T concentrations. Sampling was conducted using the same type of peristaltic pump as explained above. In order to remove biomass from the samples a single-use syringe filter

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(0.2 µm, Minisart RC25, Sartorius, Göttingen, Germany) was connected to the intake tube of the pump for sample-filtration. The flow rate was set to ~6 mL min^{-1} to avoid turbulence and degassing during filling process. The filtered samples were poisoned with 20 µL of a saturated $HgCl_2$ solution and immediately closed with a headspace < 1 % taking butyl rubber-septa and stored at 4 °C in darkness until usage.

2.5 Preparation of C_T measurement chambers

The same 10 mL glass vials and septa as described above were used. Prior to sealing, each vial was equipped with a small magnetic stirrer. Subsequent additions and connections into and out of the vial were made by injection needles penetrating the sealing. 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 DI water was added into each vial. These prepared vials are now defined as the measurement chambers and 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).

2.6 GC-C_T measurement procedure

Following the time axis in Fig. 2 measurement was started by the GC program with an addition of 0.8 mL concentrated phosphoric acid (Merck, Darmstadt, Germany) into the prepared measurement chamber. In the next step the acidified and continuously stirred DI water inside the chamber was pre-purged with helium for 2 min in order to reduce background carbon dioxide to a minimum. After the gas pressure inside the measurement chamber was close to zero the cooling trap (Fig. 1a, b) was activated. A manual sample injection into the measurement chamber followed by using a calibrated digital syringe™ (DS80700, Hamilton, Nevada, USA). A few seconds later the gas purge was started once again and the released CO_2, which hereafter passed the P_2O_5 (water trap) was collected by the cooling trap with liquid nitrogen (Fig. 1a, b). After the automated 10-port valve was set to "inject position" (Fig. 1b), CO_2 was removed at ambient

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temperature and transferred into the column at 45 °C. Rapidly heating the column up to 180 °C desorbed the CO_2. The gas passed the catalyst and was measured by a FID (Fig. 1a, b). Time for a complete analysis took 14.5 min.

2.7 Method validation

Method validation was done in agreement with the terminology for accuracy and precision (RSC, 2003). We conducted measurements of low and high C_T-concentrations under stable lab and system conditions in order to calculate an overall precision. The accuracy was defined as the closeness of agreement between measured results and the accepted true value. The CO_2 amount obtained from a pre-purged and acidified measurement chamber without sample addition during analysis was used as a blank measurement. An additional blank measurement was conducted with 200 µL of DI water which was used for the preparation of the sodium carbonate solution. C_T raw data were always blank corrected (5 µmol ± 1.8) and sample temperature and salinity were taken into account. Each C_T-sample was measured in triplicates.

2.7.1 GC-system performance

The analytical performance of our GC-method was tested over a C_T-measurement range of ~ 500–2600 µmol kg^{-1} by injection of different volumes of the internal standard (see above) and the internationally accepted reference (Dickson standard).

2.7.2 Test measurements of sub-sampled and filtered Dickson standard

We compared three samples of the Dickson standard with three samples of filtrated and unfiltered Dickson sub-samples (sub-Dickson) taken from the same batch, respectively. Triplicate measurements of each sample were conducted.

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2.7.3 Test measurements of phytoplankton samples

We performed C_T test measurements from cultures containing the planktonic calcifier *Emiliania huxleyi*. In order to obtain samples with low C_T concentration, three samples were collected from a non-manipulated *Emiliania huxleyi* culture. Samples with high C_T concentration were collected from a culture which was enriched with an air-CO_2 mixture. For the latter triplicate samples were taken from two different cultures, respectively.

From each of the samples (i.e. three samples for low C_T, and six samples for high C_T) triplicate measurements were conducted.

3 Results

The GC system performance using different C_T concentrations ($\sim$ 500–2600 µmol kg^{-1}) of sodium carbonate (internal standard) and different volumes of the Dickson standard indicated an excellent measurement linearity following a linear regression model: $y = a \times + b$; with y and x representing measured peak area and given C_T in µmol kg^{-1}, respectively. The regression is described by a mean slope (a) of 9.14 ± 0.04 and mean intercept (b) of 89.88 ± 47.36 peak area, $r^2 = 0.9999$ ($n = 19$) (Fig. 3). The overall precision for sodium carbonate standards in a range of 1250–2600 µmol kg^{-1} could be calculated with a standard deviation of ± 3.8 µmol kg^{-1} ($n = 9$).

An average precision and accuracy of ± 3.7 and ± 1.2 µmol kg^{-1} ($n = 27$) could be determined for C_T measurements of the Dickson standard, sub-Dickson and filtrated sub-Dickson, respectively. All measurements were compared among each other as well as to the true C_T Dickson value. The comparison of C_T measurements directly taken from the original bottle with collected sub-samples showed neither significant differences among each other (ANOVA: $F = 0.63$; $p > 0.05$) nor to the true Dickson value (t-test: $t \geq 2.21$; $p \geq 0.16$). However, precision and accuracy for the filtered Dickson standard were slightly lower (Fig. 4).

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Results for test measurements from phytoplankton cultures containing low C_T concentration showed an average value of 1845.6 µmol kg^{-1} ± 2.5 (n = 9) (Fig. 5). Whereas the triplicate measurements of sample 1 and 2 did not differ from each other, the third set of measurements was significantly lower compared to the second (ANOVA: $F = 6.2$; $p < 0.05$; Tukey's HSD post-hoc test between measurements of samples 2 and 3: $p < 0.05$). Though the third measurement showed a significant difference to the average C_T value (t-test: $t = -18.92$; $p < 0.05$), these measurements showed the best precision compared to previous data. The samples which contained two different high C_T concentrations showed average values of 2285.5 µmol kg^{-1} ± 5.5 (n = 9) and 2411.0 µmol kg^{-1} ± 2.5 (n = 9), respectively (Fig. 6). The triplicate measurements within sampling sets 1 and 2, respectively, did not significantly differ from each other (ANOVAs: $F < 2.24$; $p > 0.17$).

4 Discussion

Our C_T quantification method using a GC-system allowed for highly precise measurements with a sample volume of 10 mL (total required volume 12 mL) comprising a typical C_T measurement range (1250–2400 µmol kg^{-1}) used in biological experiments manipulating seawater carbonate chemistry. Conventional quantification techniques for C_T have been only validated for concentrations of ~ 1950–2200 µmol kg^{-1} which is a typical measurement range for oceanographic research analysing natural samples. Precision and accuracy for an extended C_T measurement range covering experimental acidification scenarios were not verified by the old gas chromatographic, coulometric, photometric, or NDIR- technique (Goyet and Snover, 1993; 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 analytical method for measurements of filtrated C_T samples derived from cultures with high and low C_T concentration is validated by the overall accuracy and precision. In general the high precision underlines the reliability of the

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analytical method. Differences in the average C_T values among samples points to the fact that careless handling of samples prior to measurements can cause inaccuracy.

The lower average precision in the high C_T phytoplankton culture samples shows that the combination of high phytoplankton biomass (here calcifying phytoplankton) and high C_T concentrations represents the most difficult analytical challenge.

The new 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 line 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 heat up of the separation column up to 270 °C for about 4 h is necessary (Weiss and Craig, 1973).

Another critical point in measuring C_T from experimentally derived seawater samples is the typically small total sample amount. Whereas the NDIR and the coulometric methods naturally require total sample volumes of ~ 0.25–1 L, the photometric method uses considerably smaller amounts. The requirement of small sample sizes is particular important when conducting microcosm experiments from which only a few mL of sample are available. Therefore, in this field hitherto the photometric method was preferred. However, a potential source of error of the photometric method might be that stored samples have to be opened before analysing (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 used sealing which does not allow for

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penetration with injection needles when using a common auto-sampler. 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 sulphides and the dependency on constant ion strength excludes a sample set of different salinities. In comparison, the butyl rubber septa for sample sealing in our GC-system has been proved as gas tight (Brandes, 2009; Spötl, 2005). Manual penetration of this sealing by a syringe 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 limited because IR-measurements are extremely sensitive to water vapour. 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 vapour correction overcome this problem; however, they are very expensive. In the GC-system presented here, water vapour 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 problematic 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 consists of a number of tubes, valves and connectors which get contaminated by the highly toxic preservative $HgCl_2$. Handling and operating with

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such systems must be taken with care. Handling of the toxic preservative $HgCl_2$ during analysis using our method could be restricted to the measurement chamber (single-use vials) and sample syringe.

Sample injection with a manual syringe applying the GC-C_T technique was identified as the most error-prone process during analysis. However, using a digital syringe improved the main measurement uncertainty to the here presented $\sim 3.8\,\mu mol\,kg^{-1}$ shown by the verification of the system performance. 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 up to $\sim 1000\,\mu mol\,kg^{-1}$.

A potentially extension of this GC-C_T analytical setup would be the connection to a stable isotope ratio mass spectrometer to additionally conduct $\delta^{13}C$ measurements avoiding a time consuming phase-equilibration, as described for GC-$\delta^{13}C$ stable isotope analysis (Assayag et al., 2006; Capasso et al., 2005) which would allow a simultaneous highly precise quantification of C_T and a $\delta^{13}C$–DIC measurement.

5 Conclusions

It can be concluded that our quantitative GC-C_T technique offers a number of advantages compared to other methods. Besides the precision along an extended C_T measurement range to natural seawater samples, the very low sample volume from prefiltrated samples, the cost effectiveness of sub-sampling of the Dickson standard, and the relatively high environmental acceptability due to reduced toxic chemicals demonstrate advantages which might lead to establishment of this method as an alternative for C_T quantification from biological experiments.

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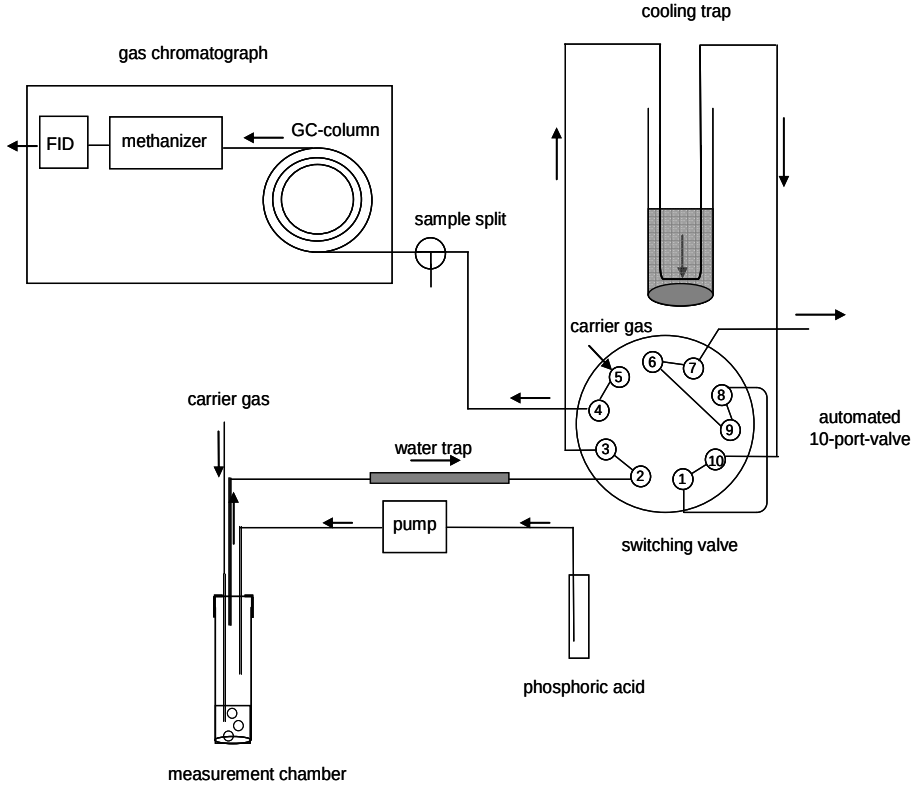

Fig. 1a. GC-C_T analyzer setup in "sample load position" collecting released CO_2 from a sample.

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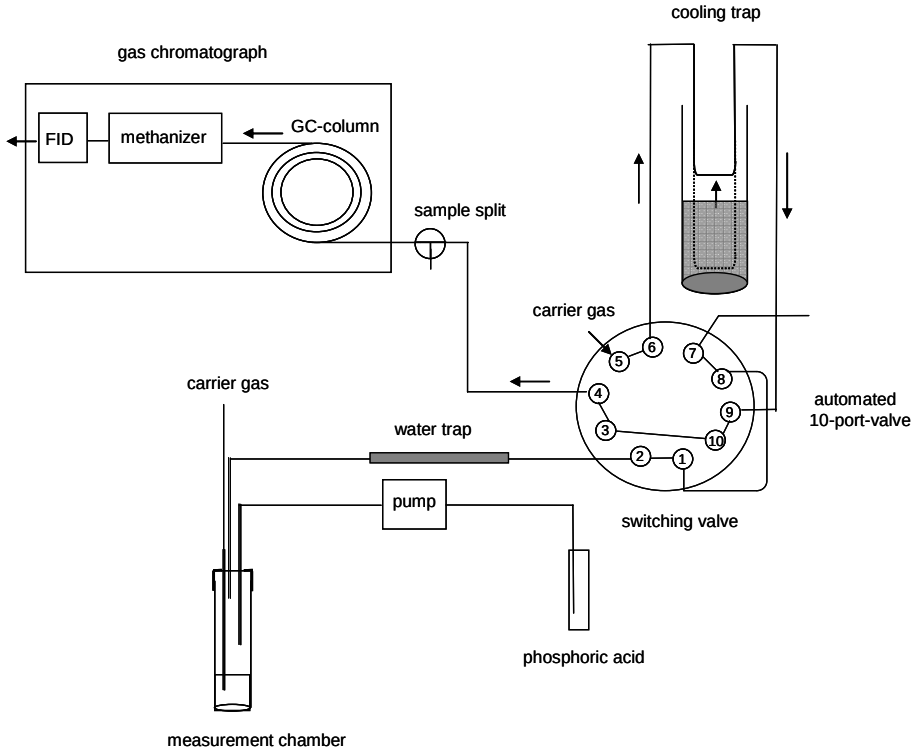

Fig. 1b. GC-C_T analyzer setup in "sample injection position", CO_2 released from trap is loaded into the column for analysis.

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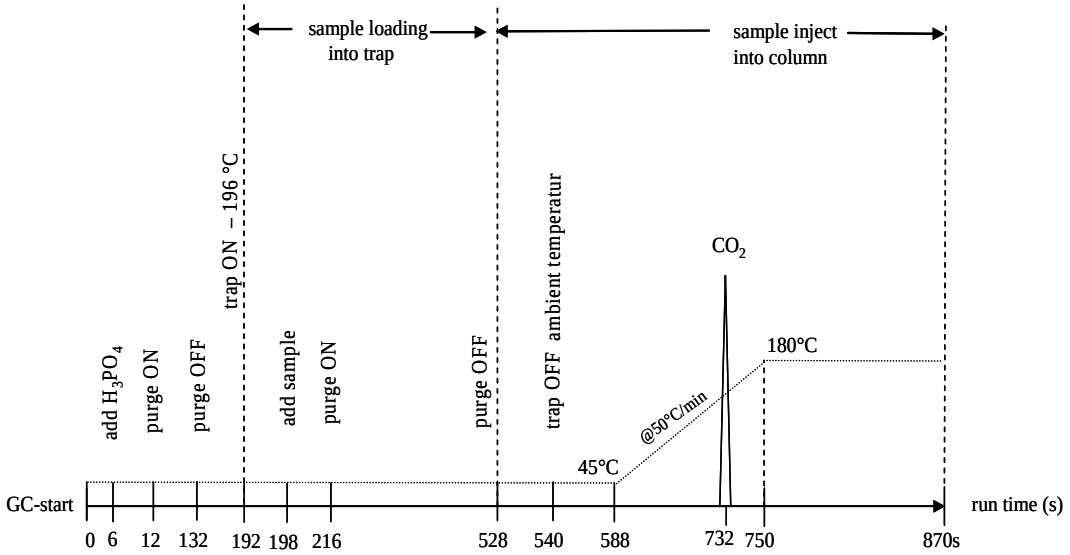

Fig. 2. Operating sequence of the GC-C_T measurement for one sample.

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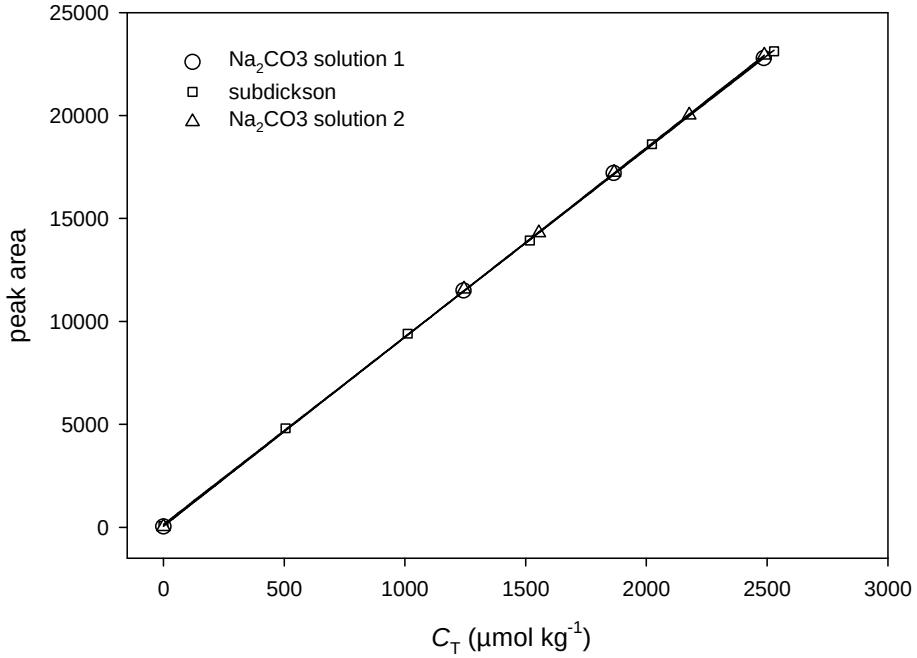

Fig. 3. GC-C_T performance verified by measurements of different injection volumes taken from sodium carbonate solutions (internal standard) and from the international reference standard (Dickson).

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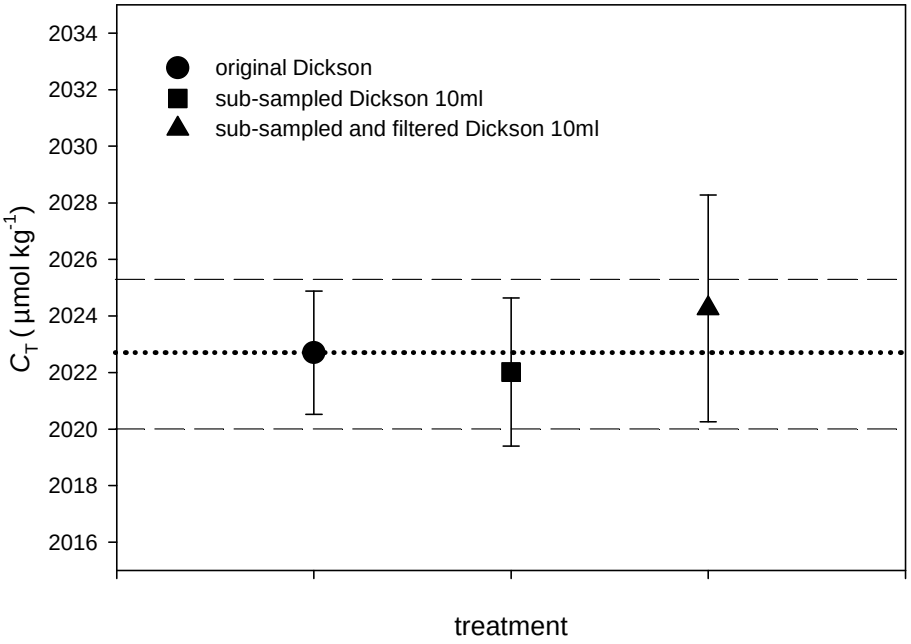

Fig. 4. Comparison of the Dickson standard measured versus sub-sampled and filtered Dickson, ($n = 27$). The dotted line gives the true Dickson value as reference for accuracy. The dashed lines give the average standard deviation of all measurements (i.e. Dickson, sub-Dickson, filtered sub-Dickson) as an overall indicator for precision.

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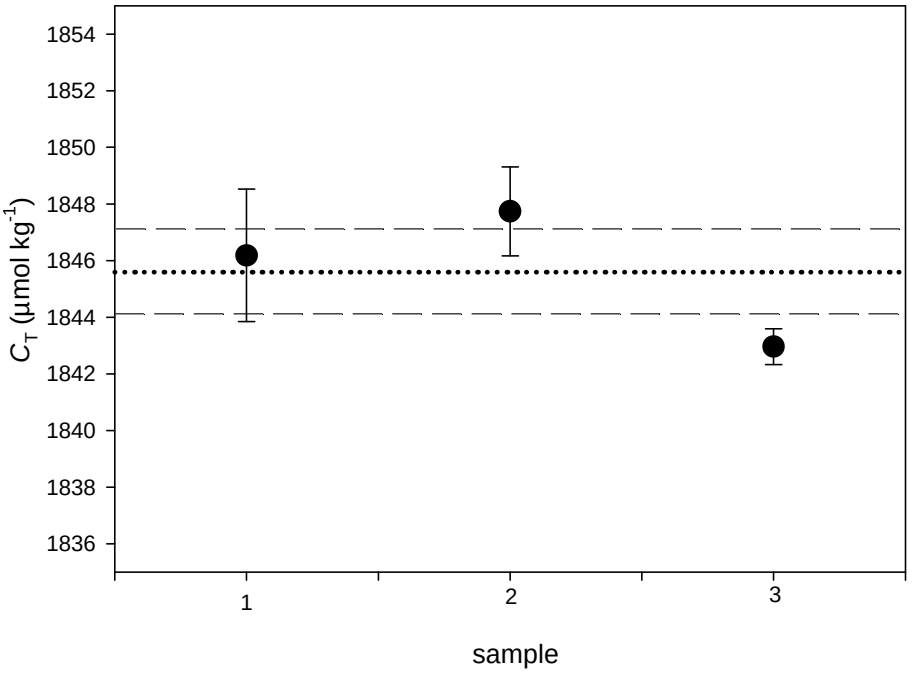

Fig. 5. Measurements of filtered sub-samples taken from low C_T samples derived from an *Emiliania huxleyi* culture ($n = 9$). The dotted line gives the average C_T value. The dashed lines give the average standard deviation of all measurements as an overall indicator for the measurement precision.

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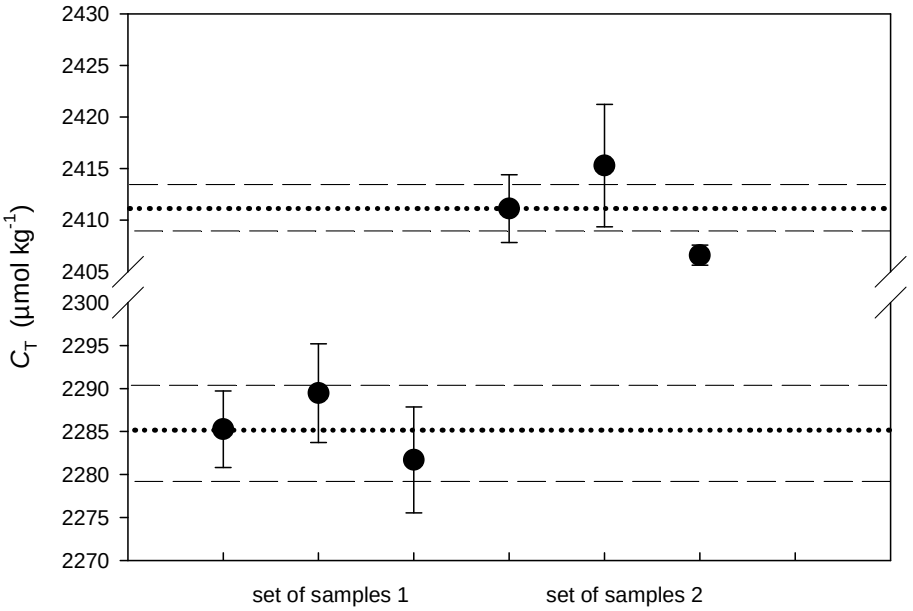

Fig. 6. Measurements of filtered sub-samples taken from two high C_T concentration *Emiliania huxleyi* cultures ($2 \times n = 9$).The dotted line gives the average C_T value for each set of samples. The dashed lines give the average standard deviation of the measurements as an overall indicator for the measurement precision.

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