Sampling and preservation of seawater for CO$_2$ analyses

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GOAL

Without a change in CO\textsubscript{2} system
How can we achieve this reliably?
IMAGINE A SAMPLE OF SEAWATER IN A CONTAINER
How can its CO₂ composition change?

By changing the total dissolved inorganic carbon, the total alkalinity, or both.
**NEED TO AVOID:**

Biological activity in the water after sampling.

**WHY?**

*photosynthesis*

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \rightleftharpoons \text{C}_6\text{H}_12\text{O}_6 + 6\text{O}_2 \]

*respiration*

**HOW?**

Add small amount of mercury(II) chloride
Also need to avoid:

Exchange between the water and the air above.

A. Loss of water by evaporation
B. Gain / loss of CO₂ as gas
C. Further contamination by microorganisms

How?

Seal the container
Closed pyrex bottle with air-tight seal
We use a greased, ground-glass joint

Note: the bottle is not completely full

WHY?

An ideal head-space is about 1% of the total volume.
Too small, and the water may expand pushing out the stopper
Too large, and the gas exchange with the head-space may be significant.
Is there anything else?

Important to fill gently so that opportunities for exchange with the atmosphere are limited.

Can overflow the bottle to reduce effect of such exchange.
The container must be clean before being filled.

The seawater must not react with the material the container is made of.

The container must be impervious to CO₂ and H₂O.
Guide to Best Practices for Ocean CO₂ Measurements

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1. Scope and field of application

This SOP describes how to collect discrete samples, from a Niskin or other water sampler, that are suitable for the analysis of the four measurable inorganic carbon parameters: total dissolved inorganic carbon, total alkalinity, pH and CO$_2$ fugacity.

2. Principle

A sample of sea water is collected in a clean glass container in a manner designed to minimize gas exchange with the atmosphere (note: CO$_2$ exchange affects the various carbon parameters to differing degrees ranging from the very sensitive CO$_2$ fugacity, $f$(CO$_2$), to alkalinity which is not affected by gas exchange). The sample may be treated with a mercuric chloride solution to prevent biological activity, and then the container is closed to prevent exchange of carbon dioxide or water vapor with the atmosphere.
A seawater filtration method suitable for total dissolved inorganic carbon and pH analyses

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Abstract

High biomass and heavy particle loads may interfere with carbonate chemistry analyses of samples from experimental aquaria and cultures used to investigate the impact of ocean acidification on organisms, as well as from biologically productive coastal regions. For such samples, a filtration method is needed that does not change the dissolved CO$_2$ content, and consequently does not alter the total dissolved inorganic carbon and pH of the sample. Here, a filtration method is presented in which the sample seawater is pumped by a peristaltic pump through a replaceable 0.45 µm filter in a 50 mm polycarbonate filter holder and then into the sample bottle. Seawater samples of known carbonate composition were filtered to confirm that the filtration method did not alter the CO$_2$ content, and compromise the subsequent sample analysis and data usefulness. Seawater samples with added phytoplankton concentrations in the range of 1–5 x 10$^5$ cells mL$^{-1}$ were also filtered successfully. Finally, seawater with added biogenic CaCO$_3$ was tested to prove that the method could successfully filter out such particles and produce dependable results. This approach will help to ensure more consistent and reliable carbonate chemistry measurements in coastal environments and from ocean acidification aquaria and cultures, by providing a well-tested method for sample filtration.
Phytoplankton can contribute alkalinity

Fig. 4. Measured alkalinity values of unfiltered seawater (filled bars) containing each of the four cultured phytoplankton species (P. minimum, H. akashiwo, S. costatum, and P. micans) and of the same seawater passed through a 0.7-μm filter (open bars) as a function of POC concentration. For determination of the alkalinity caused by each phytoplankton species, cell population in each batch culture containing nearly a maximum cell population was decreased by 100% to 300% by diluting the batch culture with nutrient-depleted cell-free seawater. For direct comparison of alkalinity measurements, all alkalinity values were normalized to a mean salinity of 32.9.

Fig. 5. Measured alkalinity differences between unfiltered seawater containing each of the four cultured phytoplankton species and the same seawater passed through a 0.7-μm filter. The solid lines show linear regression plots between alkalinity (A_T) and the POC concentration for four phytoplankton species.

Contribution of bacterial cells to the measured alkalinity (A_T BACTERIA) — Previous findings have shown that bacteria are ubiquitous in the water column and are abundant in suspended particulate matter (Cho and Azam 1988). Bacterial populations range from 10^5 cells mL^-1 in oligotrophic waters to 5 × 10^6 cells mL^-1 in coastal waters (Fukuda et al. 1998). Similar to phytoplankton cell surfaces, negatively charged functional groups on bacterial cell surfaces reacted with protons during the titration of unfiltered seawater with hydrochloric acid, causing an increase in the measured alkalinity. This study indicates that, in the eastern coastal waters of Korea, the alkalinity bacteria in seawater ranges from 1 to as high as 6 mol kg^-1 (Fig. 6b). The contribution of bacteria appears to be inversely related to the contribution of phytoplankton, suggesting that the bacterial population could be more important in open ocean waters in which phytoplankton are less abundant. Our results indicate that more alkalinity measurements on unfiltered and filtered (a 0.45-μm filter) samples with low POC concentrations are needed to assess the contribution of bacterial cells to the measured alkalinity.

The seawater samples analyzed in our study probably contained bacterial cells that are small enough to pass through a 0.45-μm filter and therefore are not included in our estimate of the contribution of bacterial cells to the measured alkalinity. Nonetheless, we chose the 0.45-μm pore size for two reasons. First, our alkalinity measurements on a limited number of coastal seawater samples suggested that bacteria smaller than 0.45 but greater than 0.2 μm contributed less than 0.5 mol kg^-1 to measured alkalinity. We considered this contribution to be negligible because it is within the precision of our alkalinity measurements. Second, particulate matter is defined operationally as material that can be collected on a filter, usually one with a pore size of 0.45 μm. Therefore, the contribution to the measured alkalinity of biological particles larger than 0.45 μm is of particular concern to oceanographers. It is also important to note that our estimation of the contribution of bacteria to the measured alkalinity.

Whitings in the Bahamas
CaCO$_3$ particles can be a BIG problem!

Dissolution or precipitation of CaCO$_3$ will change both total alkalinity and total dissolved inorganic carbon.

Worse yet, methods for the determination of both total alkalinity and total dissolved inorganic carbon usually involve adding acid to the sample, guaranteeing dissolution of CaCO$_3$. This either increases the acid needed to measure alkalinity (biasing the result high), or releases extra CO$_2$, increasing the apparent total dissolved inorganic carbon.
BUT SIMPLE FILTRATION CAN BE A PROBLEM

WHY?

It increases the opportunity for gas exchange between the seawater sample and the atmosphere — thus changing the total dissolved inorganic carbon, and thus the pH and $p(\text{CO}_2)$. 
Filtration method for CO₂ analyses

Use a peristaltic pump thus minimizing gas exchange.

Use an enclosed, flow-through filter, again to minimize gas exchange. (0.45 µm; 47 mm)
FILTRATION METHOD FOR CO$_2$ ANALYSES
Table 1. Measured results for $C_T$, pH, and $A_T$ for the various experiments reported here. The certified values for CRM Batches 124 and 125 are included for comparison. In each case, values are expressed as mean ± one standard deviation (number of analyses).

<table>
<thead>
<tr>
<th></th>
<th>$C_T$ (µmol kg$^{-1}$)</th>
<th>pH (Total scale)</th>
<th>$A_T$ (µmol kg$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td><strong>Measurements of CRM Batch 124</strong></td>
<td></td>
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<tr>
<td>Batch 124 certified values</td>
<td>2015.72 ± 0.74 (9)*</td>
<td>7.8796 ± 0.0019 (18)*</td>
<td>2215.08 ± 0.49 (24)</td>
</tr>
<tr>
<td>Filtered samples</td>
<td>2016.18 ± 0.93 (12)</td>
<td>7.8799 ± 0.0006 (12)</td>
<td>2215.40 ± 0.76 (24)</td>
</tr>
<tr>
<td><strong>Measurements of CRM Batch 125</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch 125 certified values</td>
<td>2141.94 ± 0.37 (6)*</td>
<td>7.5541 ± 0.0020 (18)*</td>
<td>2216.26 ± 0.52 (18)</td>
</tr>
<tr>
<td>Filtered samples</td>
<td>2141.19 ± 1.07 (12)</td>
<td>7.5569 ± 0.0020 (12)</td>
<td>2216.30 ± 0.78 (24)</td>
</tr>
<tr>
<td><strong>Measurements of CRM Batch 124 with P. tricornutum</strong></td>
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<tr>
<td>Batch 124 certified values</td>
<td>2015.72 ± 0.74 (9)*</td>
<td>7.8796 ± 0.0019 (18)*</td>
<td>2215.08 ± 0.49 (24)</td>
</tr>
<tr>
<td>Filtered samples</td>
<td>2015.79 ± 0.61 (7)</td>
<td>7.8807 ± 0.0007 (7)</td>
<td>2215.53 ± 0.89 (14)</td>
</tr>
<tr>
<td>Unfiltered samples</td>
<td>2016.25 ± 0.98 (7)</td>
<td>7.8799 ± 0.0012 (7)</td>
<td>2218.45 ± 0.68 (14)</td>
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<tr>
<td><strong>Measurements of seawater with CaCO$_3$ particles</strong></td>
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<tr>
<td>Filtered samples</td>
<td>2000.17 ± 0.49 (7)</td>
<td>7.8860 ± 0.0006 (7)</td>
<td>2201.74 ± 1.07 (13)</td>
</tr>
<tr>
<td>Unfiltered samples</td>
<td>2071 ± 11 (7)</td>
<td>7.8855 ± 0.0007 (7)</td>
<td>2457 ± 145 (14)</td>
</tr>
</tbody>
</table>

*The certified $C_T$ values for reference materials were not measured using the SOMMA-Coulometric system used for the other measurements reported here. They were measured using a more involved vacuum extraction/manometric method.
†The assigned pH values for reference materials were measured by the same technique used here over a period of 4 months.
EVIDENCE FOR SAMPLE STABILITY

1. Duplicate samples collected at the Bermuda and Hawaii time-series stations for later analysis at Scripps agree closely even if not analyzed for ~2 years.

2. Reference materials which are treated the same way (filtered, and poisoned with mercury(II) chloride) have been shown to be stable for >3 years ($C_T$) and for 10 years ($A_T$).
Known problems

1. Samples are less stable when stored in bottles that are not scrupulously clean. (We clean by heating to 550 °C in oven.)

2. Glasses other than Pyrex (or with a similar low coefficient of expansion) react with seawater over time, increasing alkalinity.

3. Microorganisms exist that can tolerate mercury(II) chloride.

4. Plastic bottles are permeable to water and to CO₂.

5. It is difficult to make a perfect gas-tight seal with a screw-capped bottle.

6. To use serum bottles, use a solid aluminum crimp seal.
Has been demonstrated to be impermeable to water vapor. OK with CO₂
Conclusions

It is possible to take seawater samples, and to preserve them for later analysis on shore without compromising the overall measurement quality.

Nevertheless this requires the use of Pyrex bottles (which are quite expensive) and mercury(II) chloride (a known hazard) to ensure success.