Monterey Bay Aquarium
Research Institute

Developing Methods for Quantifying the Contribution of Organic Carbon in Total Alkalinity

George Austin Fabian, University of Arizona

Mentors: Dr. Lenka O'Connor Sraj and Dr. Yui Takeshita

Summer 2022

Marine Chemistry, Seawater Chemistry, Total Alkalinity, Organic Alkalinity, Titrations

ABSTRACT

Accurately measuring the concentration of carbon dioxide in the ocean is required in order to understand and predict how anthropogenic CO_2 will impact global change. Autonomous vertical profiling float data can be used to estimate the partial pressure of carbon dioxide (pCO_2) in seawater, which, is an important parameter in measuring the concentration of CO_2 that absorbs into the ocean from the atmosphere. However, this estimate, calculated by using the inorganic carbon system, contains discrepancies with direct pCO_2 measurements. One potential source of uncertainty in this estimate is hypothesized to come from organic acid-base species, with pK values around 4 and 6, contributing to the measurement of total alkalinity. To determine the potential contribution that this organic alkalinity adds to total alkalinity, two total alkalinity titration methods were compared, the modified gran function-open cell titration method and the spectrophotometric single-step titration method. The open cell titration method potentially titrates both inorganic and organic acid-base species contributing to total

alkalinity, whereas, the spectrophotometric titration method potentially titrates just inorganic acid-base species contributing to total alkalinity. The difference in the two titration methods' total alkalinity values could potentially represent organic alkalinity. A comparison of the two titration methods could not have been done, due to uncertainty in the data analysis. Further experiments lowering the titrated endpoint pH of the spectrophotometric titration method resulted in a potential identification of organic alkalinity at a pH range between 4 and 5.

1. INTRODUCTION

The rise in anthropogenic carbon dioxide emissions since the Industrial Revolution has led to consequences unimagined by many over the years. Current research as to the impacts that anthropogenic carbon dioxide (CO_2) will have on global change largely depends on accurate measurements of CO_2 concentrations. Spectroscopic analysis of CO_2 via satellites provides high-accuracy measurements of atmospheric CO_2 concentrations (Guo et al., 2012), however, the atmosphere is not the only CO_2 sink on our planet. The ocean is a net sink for about 25% of cumulative anthropogenic CO_2 emissions (Watson et al., 2020), therefore it is important to accurately constrain the CO_2 flux between the atmosphere and the ocean.

The air-sea CO_2 flux can be expressed as:

$$F = k * K_H * \Delta pCO_2,$$

where F represents the net CO_2 flux, k is the gas transfer velocity of CO_2, K_H is the solubility constant of CO_2 in seawater, and ΔpCO_2 is the difference between the partial pressure of CO_2 in the ocean and atmosphere (Zhai et al., 2005). Accurately measuring the partial pressure of CO_2 in the ocean has been done historically by shipboard underway systems and mooring pCO_2 systems (Bakker et al. 2016). These measurements have high accuracy, but the temporal and spatial resolutions are low since these systems require research vessels to go out and take samples. The research vessels follow repeated

transects and usually sample for less than 4 months per trip, covering select ocean transects (Katsumata, 2022).

Autonomous profiling floats, such as from the BGC-Argo network (Claustre et al. 2020), are capable of estimating pCO_2 (Williams et al. 2017) and have a higher temporal and spatial resolution (Claustre et al. 2020). The floats use sensors to directly measure pH, conductivity (salinity), and temperature (along with other parameters such as O_2, Chl-a fluorescence, optical backscatter, nitrate, and downwelling irradiance). With these direct measurements, Total Alkalinity, the difference between Hydrogen ion acceptors and donors (Yao and Byrne, 2015), can be estimated. By measuring pH and estimating Total Alkalinity (TA), the rest of the carbonate system (e.g. pCO_2 and Dissolved Inorganic Carbon (DIC)) can be estimated (Yang et al, 2015) (Liu et al, 2015) (Fong and Dickson 2019). Autonomous profiling float pCO_2 estimates contain discrepancies to the direct pCO_2 measurements (Fong and Dickson, 2019).

The purpose of this study is to focus on identifying and quantifying one contributing variable that adds uncertainty to the estimates of pCO_2. Fong and Dickson 2019 compared the possible contributions of uncertainty from multiple parameters in the calculations for pH using the carbonate system. In their paper pH substitutes pCO_2 as the variable with the discrepancy between direct measurements and estimate calculations using the carbonate system. The parameters adding potential uncertainty to the estimated calculated pH were pK_1 and pK_2, for the dissociation constants of carbonic acid, the ratio between total boron and salinity, measurement biases in TA and DIC, as well as the contribution of other acid-base species dissociating in seawater. Incorporating the higher bound of potential uncertainty from each of the parameters did not adjust the calculated pH to be equal to the measured pH (fig. 1). A difference of zero in measured and calculated pH was achieved when about 4 μmol/kg excess of alkalinity, contributed from other acid-base species, was introduced as well as all the other parameters potential uncertainty (fig. 1). It was hypothesized that this excess in alkalinity is attributed to organic conjugate base species of pK values between 4.5 to 6. Developing analytical methods for identifying and quantifying the potential contribution of organic alkalinity in TA calculations is the focus of this internship project to further improve pCO_2 estimates from float data used to calculate the carbon dioxide flux between the air and sea.
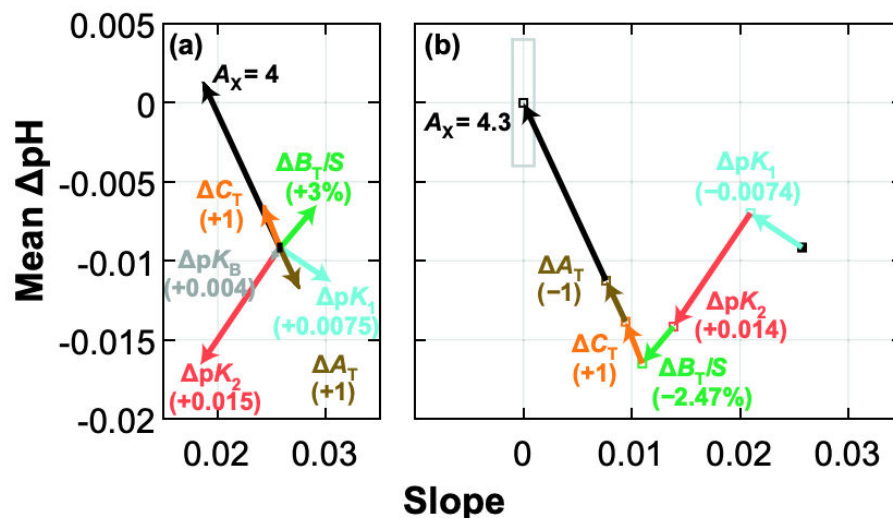
fig. 1. Potential uncertainty affects from pk values for the dissociation constants of carbonic acid, the ratio between total boron and salinity, calculations in TA and TIC, and alkalinity of other acid-base species dissociating in seawater onto the calculations for pH using the carbonate system. These effects are plotted as vectors on the axis ΔpH (the difference between measured and calculated pH) vs slope (ΔpH divided by the measured pH), where measured pH results from hydrography data from a 2015 cruise. This figure comes from the paper by Fong and Dickson, 2019.

2. THEORY

The basis for identifying organic alkalinity comes from how TA is measured. TA is measured by titrating seawater with acid, typically hydrochloric acid to the desired end pH and then using the salinity and temperature to calculate the TA (Sharp and Byrne, 2020). There are several characterized methods to calculate TA using titrations, but two, in particular, could potentially resolve the organic alkalinity signal. The open cell TA method uses a multi-step titration in an open cell to potentiometrically calculate TA (Sharp and Byrne, 2020). The open cell method uses a modified Gran fit to modify nonlinear plots of the electrical potential of a solution vs the amount of acid added into linear plots meeting together at the titration equivalence point (Sharp and Byrne, 2020). This TA method titrates seawater solutions purged of dissolved inorganic carbon to end pH points around 3.5 to 3.0, hypothetically titrating inorganic alkalinity acid-base species and organic alkalinity acid-base species (Sharp and Byrne, 2020). The other method, the

spectrophotometric TA method, uses a single-step titration in which one addition of the acid is delivered to titrate the seawater to an estimated pH (Liu et al, 2015) (Yao and Byrne, 1998). This method uses the absorbance ratio between the maximum absorbance of the deprotonated acidified seawater and the maximum absorbance of the protonated acidified seawater to calculate the pH of the titrated seawater using the equation,

$$pH_T = 5.8182 + 0.00129(35 - S) + ...$$

$log((R(25) - 0.00381)/(2.8729 - 0.05104\,R(25)))$ (Liu et al, 2015) (Yao and Byrne, 1998). In this equation, S is the salinity of the seawater, and R(25) is given by the equation $R(25) = R(t)(1 + 0.01869(25 - t)$ (Liu et al, 2015) (Yao and Byrne, 1998). In this equation, t is the temperature of the seawater, and R(t) is the ratio of the absorbance mentioned earlier. The TA is then calculated using the equation,

$$A_T = (([HCl]_A M_A - [H^+]M_{ASW})/M_{sw}) * 1000000, \text{ where } [HCl]_A \text{ is the}$$

concentration of the acid, M_A is the mass of the acid in grams, $[H^+]$ is the concentration of the hydrogen ion, M_{ASW} is the mass of the acidified seawater in grams, and M_{sw} is mass of the seawater in grams (Liu et al, 2015) (Yao and Byrne, 1998). The concentration of Hydrogen ion is calculated by pH = -log$[H^+]$. This method for calculating TA titrates to an end pH of about 4.2, hypothetically titrating only inorganic alkalinity (Sharp and Byrne, 2020). Taking the difference between the TA calculated using the open cell TA method and the TA calculated using the spectrophotometric TA method will hypothetically resolve the organic alkalinity signal. This relies on the open cell TA method titrating the organic and inorganic alkalinity and the spectrophotometric TA method titrating just the inorganic alkalinity. The difference would potentially represent organic alkalinity.

3. MATERIALS AND METHODS

3.1 REAGENTS

The titrant acid, used in both the single-step titration and the open cell gran titration, was prepared by adding sodium chloride to a standardized solution of 0.1N hydrogen chloride to a final concentration of 0.6M NaCl. The exact concentration of the acid solution was calculated by multiplying the certified molarity and the density of the solution. Each acid solution was stored in a borosilicate media bottle, with a screwed-on cap. Indicator dye, Bromocresol Green (BCG) is prepared by following the methods in Yao and Byrne, 1998. Both dyes were contained in a 150 mL borosilicate media bottle with a screw-on cap. The indicator containers were covered in aluminum foil to prevent degradation by ambient light.

3.2 SEAWATER COLLECTION + FILTRATION & CERTIFIED REFERENCE MATERIALS

Both single-step titrated solutions and open cell titrated solutions used filtered seawater. The seawater was collected offshore MBARI at 17 meters depth and was filtered in the Seawater Lab at MBARI. The seawater was filtered using a Whatman Polycap TC filter and stored in a 25 L Nalgene® Carboy with a spigot. The salinity for filtered seawater was measured using a Mettler Toledo DM45 Density Meter. Seawater samples were also collected during a cruise on a research vessel, the Western Flyer, from June 21, 2022, to June 25, 2022. Thirty-nine samples were collected from a 12 Niskin bottle CTD-Rosette system at various locations and depths from 0 to 2000 meters. About 800 mL were collected from each Niskin bottle into a one-liter plastic bottle. The seawater was then filtered using a Masterflex L/S Analog Variable-Speed Console Drive (model number 7555-00) with an attached Masterflex Easy-Load II pump head (model number 77201-62). Thirty-four of the samples were filtered using a 0.2 µm polycarbonate membrane filter (PCTE). The last five samples were filtered using a 0.7 µm GF/A microfiber filter. The filtered seawater samples were contained in 500 mL glass bottles. The bottlenecks were dried and the stoppers were greased before sealing with a wide rubber band and grip. These seawater samples were not analyzed during this study and are set to be analyzed in the future. Certified reference materials were purchased

from the Dickson lab (Dickson, 2003) Samples were preserved using standard protocols (Dickson 2007).

3.3 ANALYTE PREPARATION FOR 1-SHOT TITRATION

Masses for the filtered seawater and acid titrant were measured gravimetrically using a Master Toledo XPE504 analytical balance. Filtered seawater was poured into a tarred 250 mL glass bottle as close to the desired mass, with an acceptable margin ± 0.005 grams. The bottle was tarred again and the acid titrant was added using a Metrohm Dosino syringe pump at 75 dosing velocity and 75 filling rate. The syringe pump was attached to a borosilicate media bottle housing the acid titrant where the syringe would collect the acid amount and deliver it through a dispensing tube. The volume/mass of the acid titrant was estimated using a regression curve fitted onto the plot of preliminary single-step titrated samples and their measured pH values. By knowing the ratio of acid titrant mass to seawater mass and the measured pH after degassing for 5 minutes, the regression curve could estimate the needed mass or volume required to get an estimated desired pH. A magnetic stir bar was added to the seawater plus acid solution and stirred using a Thermo Scientific Stirring Plate with a stirring velocity of about 400 rpm. N_2 gas was inserted into the sample using a sparging tube to release dissolved inorganic carbon species residing in the solution.

3.4 SPECTROPHOTOMETRIC ANALYSIS FOR 1-SHOT TITRATION

Spectrophotometric analysis was conducted on an Agilent 8453 spectrophotometer with a 5cm flow cell. The sample was pumped into the flow cell using a Norgen Kloehn V6 48K syringe drive pump with an attached 6-port injection port. The spectrophotometer was tilted on one side so aid in bubble reduction. Each full run of samples was initiated with three full rinses of the syringe with deionized water. Three junk samples were run at the start with careful attention to bubbles to assess proper system performance.

3.5 OPEN CELL METHOD TITRATION

The open cell titration was conducted in a thermally stable titration cell using a Metrohm 850 auto titrator. It was comprised of a 50 mL beaker inside a water jacket that was being held stable at 21.3°C by using a Thermo Haake ARCTIC AC200 A25 Immersion Bath Circulator. A thermometer was inserted into the titration cell to check that the water bath temperature was correct. A tube inserted in the cell was connected to a Metrohm Dosino syringe pump, which injected the acid titrant. This is the same syringe pump used to inject acid titrant into the 1-shot samples. Another Metrohm Dosino syringe pump was used to inject filtered seawater from the samples in the 855 Robotic Titrosampler "Basic" (1T/2P). Tubing connected to a 25 L Nalgene® carboy filled with MilliQ water was used to rinse the cell before running and in between runs. A waste tube in the cell allowed for MilliQ and sample seawater to be ejected into waste bins. N_2 gas was introduced to the titration cell through an Alicat mass flow controller, and a magnetic stir bar on a stirring plate was used to release dissolved inorganic carbon from the cell. A glass pH electrode inserted in the cell was connected to the Metrohm 850 to measure voltage from the titration cell. Each sample was contained in a 70 mL plastic vial and sealed with parafilm to minimize evaporation. To run a complete set of samples, the first ten slots were filled with filtered seawater junks, the next five consisted of certified reference materials, then the samples of interest with a certified reference material after 8-10 samples, and lastly 3-5 certified reference material at the end.

3.6 GRAVIMETRIC PRECISION OF ACID ADDITION ON SINGLE-STEP TITRATED SAMPLES

The addition of the acid titrant to the filtered seawater samples was tested on two methods: a micropipette or a Metrohm Dosino syringe pump. Ten single-step titrated solutions were prepared by adding 150.000 g of filtered seawater and 3.5887 g of acid titrant using 10 mL, 1 mL, and 10 μL micropipettes. Exact masses for the filtered sea water and acid titrant were recorded. Each micropipette was primed three times with the acid titrant before adding the acid to the sample bottles. Ten single-step titrated solutions

were prepared as section 2.3 explains with 150.000 g of filtered seawater and 3.504 mL of acid titrant delivered via the Metrohm Dosino Syringe Pump. The 20 single-step titrated solutions were all degassed with N_2 for five minutes and then analyzed using the spectrophotometric TA method.

3.7 SPECTROPHOTOMETER PH MEASUREMENT PRECISION

A single-step titrated solution was prepared in a one-liter borosilicate media bottle with a screw-on lid with 600.00g of filtered seawater and 14.42 g of acid titrant. The solution was then degassed with N_2 for about 7 minutes. The solution was analyzed with the spectrophotometric TA method using Bromocresol Green indicator dye. Ten replicates were taken, each with 100 µl of BCG used for analysis.

3.8 RESIDUAL DISSOLVED INORGANIC CARBON AS A FUNCTION OF TIME

To determine the most efficient time to release a single-step titrated solution of DIC species, 13 different solutions were prepared using 150.000g of filtered seawater and 3.558 g of acid titrant. The single-step titrated solutions were degassed for different times: 0, 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 15, and 20 minutes. Once finished degassing, the sample was covered with parafilm and placed into a water bath set to 20°C. After about 10 minutes the DIC was analyzed using a custom DIC analyzer, based on the design from O'Sullivan and Millero, 1998.

3.9 DYE PERTURBATION EFFECTS ON TOTAL ALKALINITY

A one-liter beaker was filled with about 800 mL of filtered seawater and degassed with N_2 gas for 14 minutes. An absorbance spectrum was manually taken from the degassed seawater to serve as a blank spectrum. Bromocresol green indicator dye was then added to the seawater and a new blank spectrum was manually taken. The acid titrant solution was then added to the seawater in small increments and more absorbance spectra were taken. A total of 5 absorbance spectrums were taken for incremental

additions of the acid titrant. The isosbestic point for Bromocresol Green was then estimated by observing the 5 absorbance spectrums and the wavelength in between the acidic and basic max absorbance peaks. The double dye addition samples were prepared using single-step titrated solutions with seawater mass of about 500g. The mass of the acid titrant was estimated based on previous experiments using 150 g of seawater and obtaining final pH values between 4 and 5. A total of 10 single-step titrated solutions with final pH values ranging from 3.8 to 5 were used for the dye additions. The Spectrophotometer TA method was run on these 10 solutions. For each solution, three replicates were performed, and for each replicate, a low dye addition and a high dye addition were added. The low dye addition amounts were 65 µL, 67.5 µL, and 75 µL. The high dye addition amounts were 75 µL, 77.5 µL, 85 µL, and 95 µL. For both the low and high dye additions, the absorbance values for the peak acid absorbance and the peak basic absorbance were kept between 0.1 and 1.0.

3.10 TOTAL ALKALINITY AS A FUNCTION OF ENDPOINT PH FOR DIFFERENT SEAWATER

Thirteen single-step titrated solutions were prepared with 150.000 g of filtered seawater and varying amounts of acid titrant. Acid titrant volumes were calculated to estimate pH values of 4.0 to 5.0 in 0.1 increments as well as pH of 3.8 and 5.2. All samples were degassed with N_2 for five minutes. The samples were analyzed with the spectrophotometric TA method using BCG indicator dye. The amount of dye changed with each sample, but the maximum absorbance peak was kept at an absorbance interval between 0.8 and 1.2. The experiment was repeated using three different filtered seawater batches, one from May 2022, June 2022, and July 2022.

RESULTS

4.1 GRAVIMETRIC PRECISION OF ACID ADDITION ON SINGLE-STEP TITRATED SAMPLES

The average mass of the acid titrant delivered via micropipette was 3.590 ± 0.001 g (1σ). The titrated seawater solution had an average mass of 153.591 ± 0.001 g (1σ). TA measurements for samples with acid titrant delivered with micropipette had an outlier with a total alkalinity of 2,245.39 μmol/kg (Fig. 2). The outlier is attributed to an air bubble caught in the flow cell. With the outlier removed from the dataset, the average TA was 2,263.9 ± 2.6 μmol/kg. For samples with acid delivered via a syringe pump, the average mass of the acid was 3.584 ± 0.002g (1σ). The titrated seawater solutions for this acid addition had an average mass of 153.585 ± 0.003 g (1σ). The average TA for samples with acid titrant delivered via syringe pump was 2,253.3 ± 1.9 μmol/kg (Fig. 2).
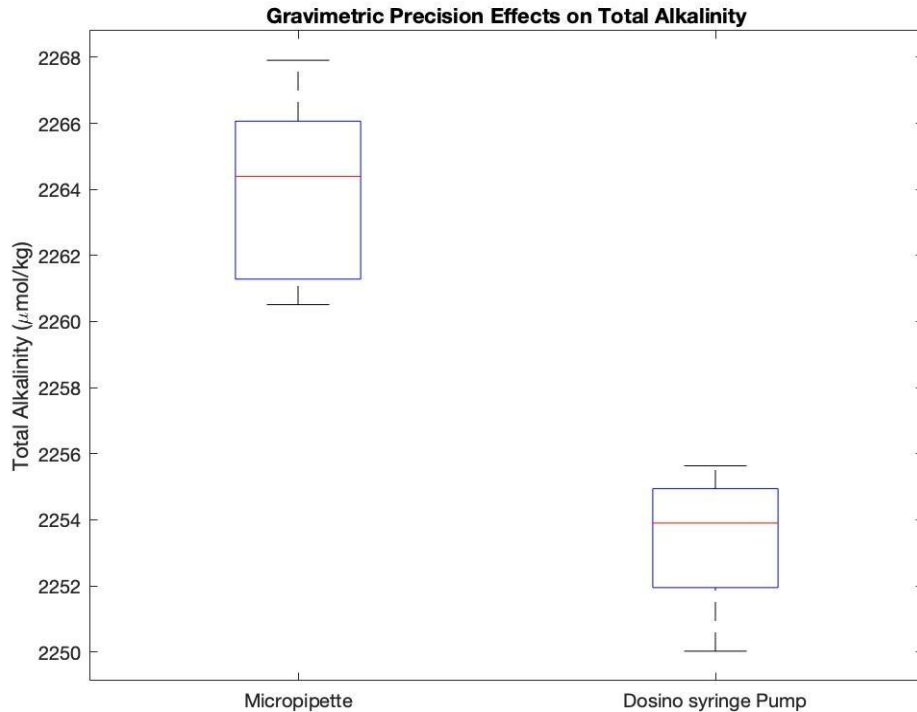
Fig. 2. Boxplots comparing the distribution spread on total alkalinity for samples with acid titrant delivered with a micropipette and delivered with a Metrohm Dosino syringe pump.

4.2 SPECTROPHOTOMETER PH MEASUREMENT PRECISION

The average pH measurement given by the spectrophotometer using Bromocresol Green Indicator Dye was 4.123 ± 0.001 (1 σ). The TA calculated with those measured pH replicates was on average $2{,}267.5 \pm 0.2$ µmol/kg (1σ). TA values stayed within a 1 µmol/kg range as seen in fig. 3.
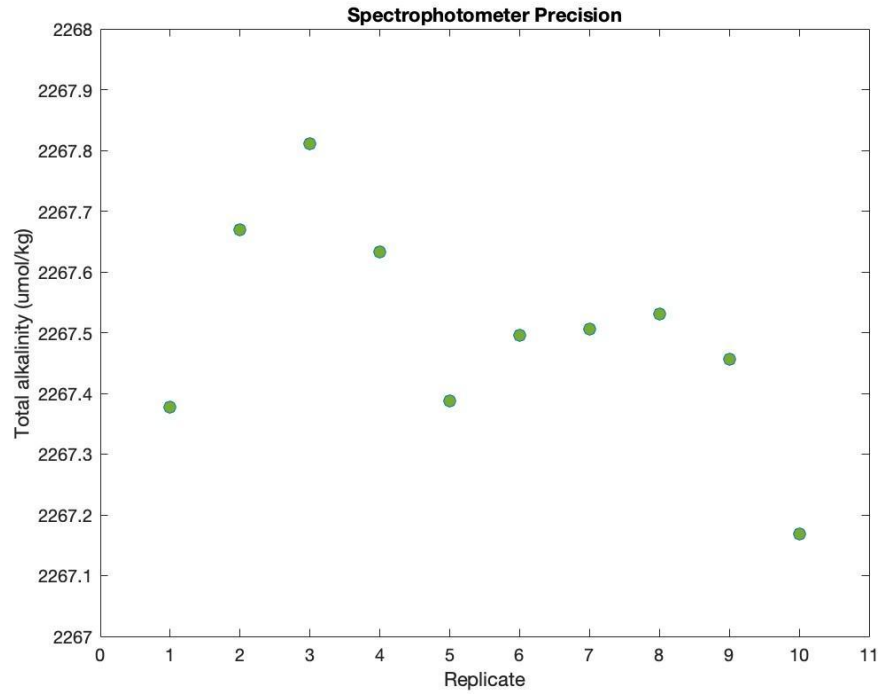
fig. 3. TA values measured using the spectrophotometric single-step titration method with one solution measured ten times.

4.3 RESIDUAL DISSOLVED INORGANIC CARBON AS A FUNCTION OF TIME

For a 153 g single-step titrated sample without any N_2 degassing, the Total Inorganic Carbon (TIC) is about 1,587 µmol/kg (fig. 4). After three minutes of N_2 degassing, the TIC starts to plateau at less than 10 µmol/kg (fig. 4).
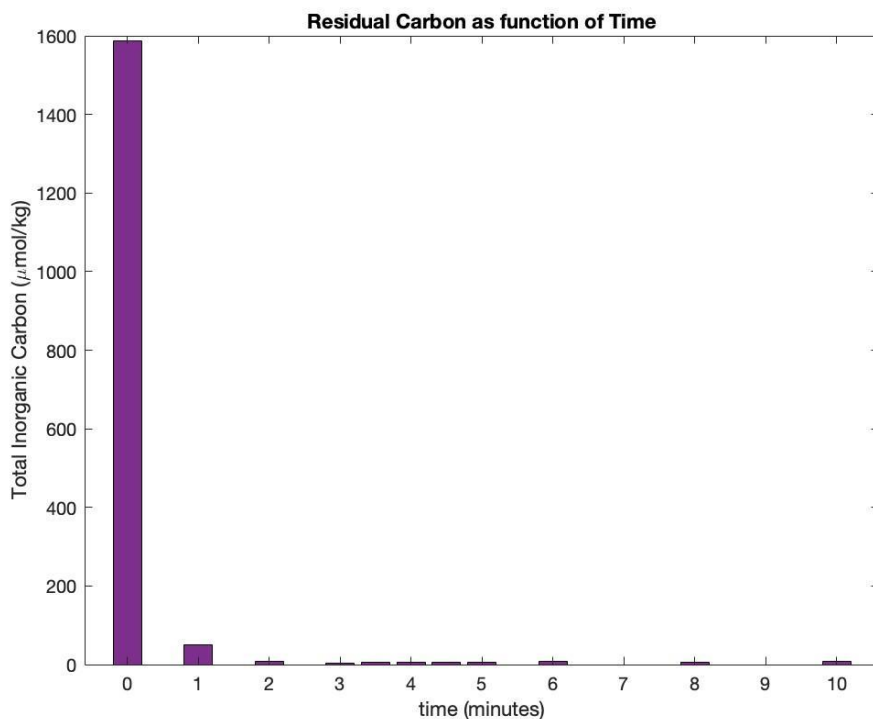
fig. 4. TIC for 11 single-step titrated solutions degassed with N_2 for different time intervals between 0 and 10 minutes.

In the region between 3 and 10 minutes, the average pH measured with the spectrophotometer was 4.24 ± 0.05 (1σ) (fig. 5). For this same time region, the TA averaged $2,259.7 \pm 4.4$ μmol/kg (1σ) (fig. 5).
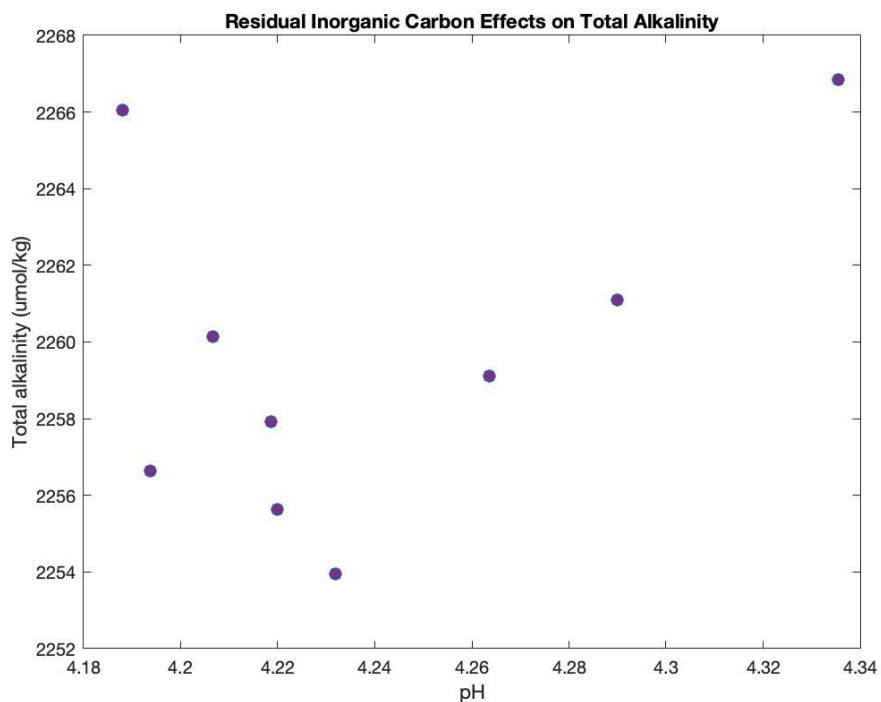
fig. 5. TA as a function of pH for single-step titrated solutions with TIC less than 10 μmol/kg, corresponding to 3 to 10 minutes degassing with N_2 gas.

4.4 Spectrophotometric TA Method and Open Cell TA Method Comparison

Certified reference material with a TA of 2,254.09 ± 0.7 μmol/kg (1σ) was used to test the accuracy of the open cell TA method. The average TA for 7 replicates of the certified reference material was 2,254.34 ± 0.9 μmol/kg (1σ). The percent error for the average TA of the CRM was 0.0112%, based on the certified TA measurement. The multi-step titrated samples for 06/10/2022 FSW had an average TA of 2,259.52 ± 1.1 μmol/kg (1σ) (fig. 6). Single-step titrated samples analyzed with the spectrophotometric TA method were titrated to final pH values between 3 and 7, however, comparison between the spectrophotometric TA method and the open cell TA method was only done for samples between pH of 3 and 5. A full comparison between the two titration methods was not possible because of uncertainty due to a calibration factor. The calibration factor for the open cell TA method comes from the calibration of the open cell system to the certified reference materials. The calibration factor is applied to the concentration of the

acid titrant, and this value was initially applied to the concentration of the acid titrant for the single-step titrated solutions (fig. 6). When applied the TA values are much higher than the TA values for the open cell TA method. When the calibration factor was not applied to the concentration of the acid titrant, the TA values for the single-step titrated solutions were lower and much closer to the TA values of the open cell TA method (fig. 6).
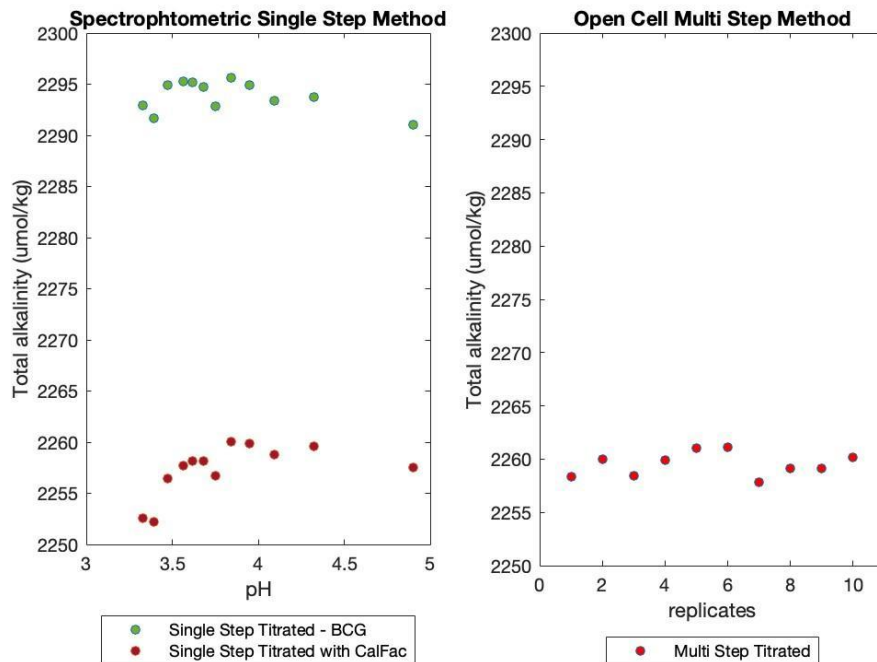

fig. 6. Left: TA values for single-step titrated solutions analyzed with the spectrophotometric TA method for final pH values between 3 and 5. The green data points represent TA measurements that were calculated using the calibration factor. The red data points represent TA measurements calculated without the calibration factor. Right: TA values for multi-step titrated solutions analyzed with the open cell TA method. There were 10 replicates of the same filtered seawater sample.

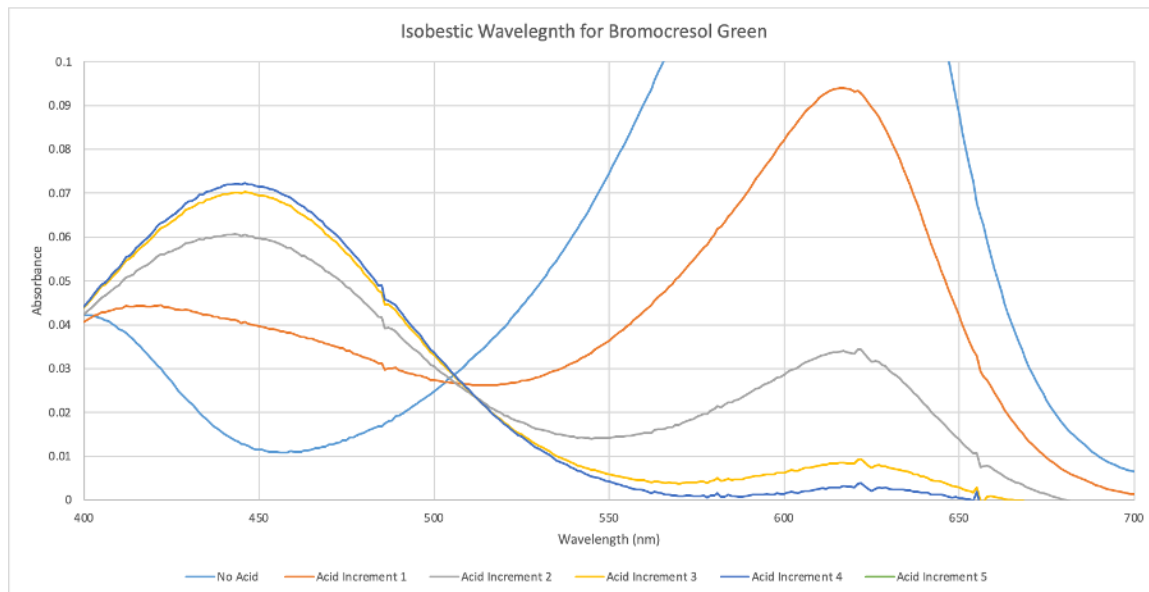## 4.5 DYE PERTURBATION EFFECTS ON TOTAL ALKALINITY

fig. 7. Absorbance spectra of bromocresol green in filtered seawater with over a large range of pH.

The isosbestic wavelength for the solution of bromocresol green was about 506 nm (fig. 7). For the dye addition plot, outliers were excluded when fitting the regression line onto the plot of fig. 8. These outliers include: solutions analyzed without consistent dye addition, solutions with a final measured pH greater than 5, absorbance spectrum influenced by a bubble, and dye additions with a $\Delta pH/\Delta A_{iso}$ greater than +0.05. A 2nd order polynomial regression curve was fit to the dye additions at varying pH. The equation of the curve is $y = -0.4232x^2 + 3.3347x - 6.5572$, where x is the pH measured by the spectrophotometer.
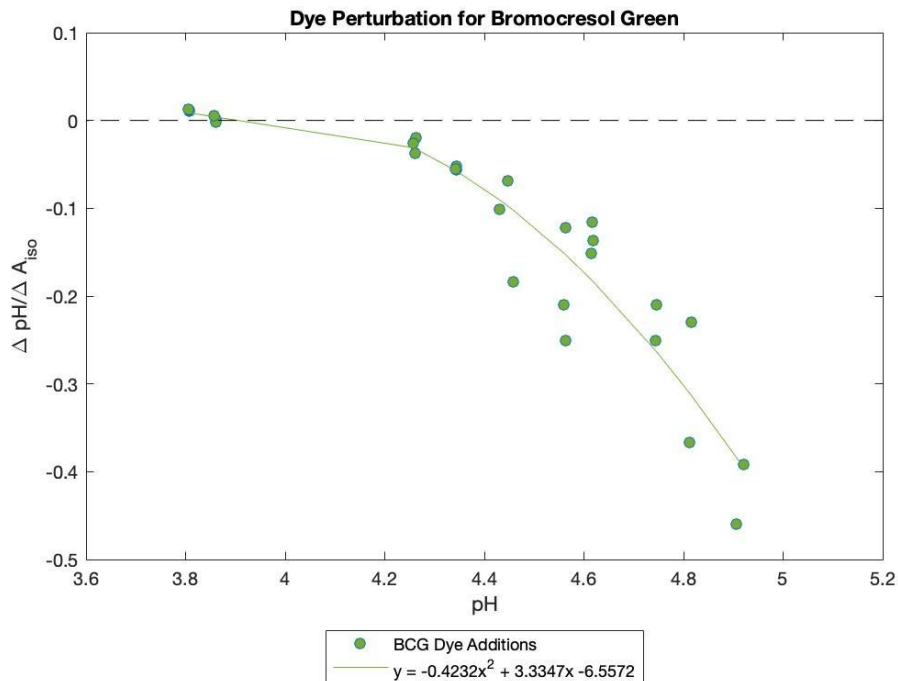
fig. 8. The green data points plot the slope $\Delta pH/\Delta A_{iso}$ against measured pH. ΔpH is the pH difference between two dye additions at different dye volumes, and ΔA_{iso} is the absorbance of the isosbestic wavelength difference between the two dye additions. The green curve is a 2nd order polynomial regression curve for the dye additions at varying pH.

The dye perturbation equation was applied to a data set of TA as a function of endpoint pH. The absorbance at the isosbestic wavelength for our bromocresol green dye solution (506 nm) was multiplied by the dye perturbation equation output. This pH adjustment was then added to the measured pH and the results are shown in fig. 9. The slope, TA/pH, was -0.03318 for pH measurements not adjusted with the dye perturbation equation. The slope became more negative (-2.6742) when pH was adjusted for dye perturbation (fig. 9).
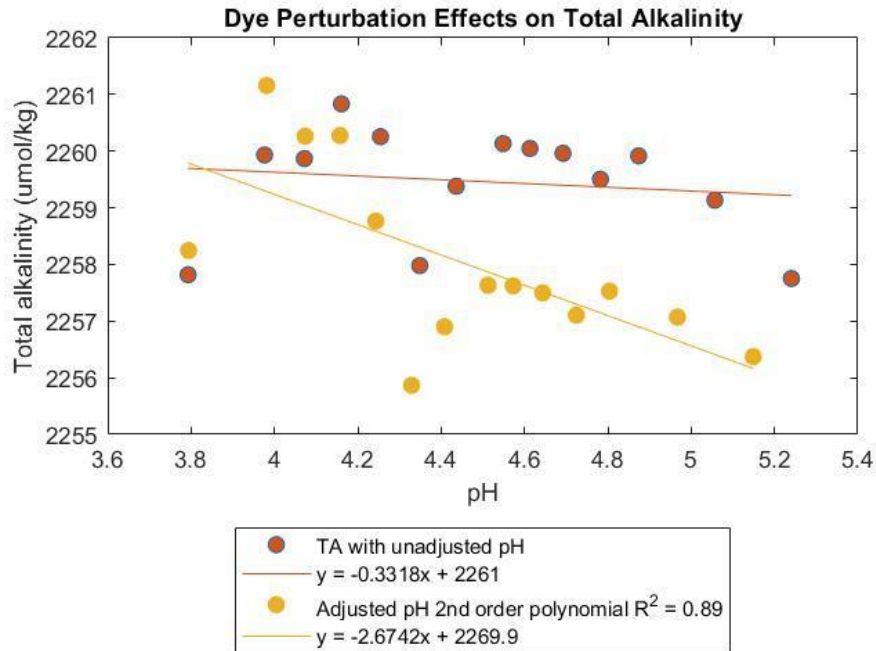
fig. 9. TA as a function of pH for single-step titrated solutions analyzed with the spectrophotometer TA method. The red data points are TA values calculated with pH measurements not adjusted using the dye perturbation equation. The red line plots a linear regression onto the TA values not adjusted with the dye perturbation equation. The yellow data points are TA values for the same solutions as the points in red with adjusted pH measurements using the dye perturbation equation. The yellow line plots a linear regression for the adjusted TA values.

4.6 TOTAL ALKALINITY AS A FUNCTION OF PH FOR DIFFERENT SEAWATER

Single-step titrated solutions using FSW obtained in June 2022 had a negative regression in TA with increasing pH. The slope of the regression was -2.6742, and had an R-Squared value of 0.439 (fig. 10). The single-step titrated solutions using FSW obtained in July 2022 had a negative regression in TA with increasing pH. The slope of this regression was -20.0660, and had an R-Squared value of 0.773 (fig. 11). The single-step titrated solutions using FSW obtained in May 2022 had a negative regression in TA with increasing pH. The slope of the regression was -2.3170 and had an R-Squared value of 0.352 (fig. 12).
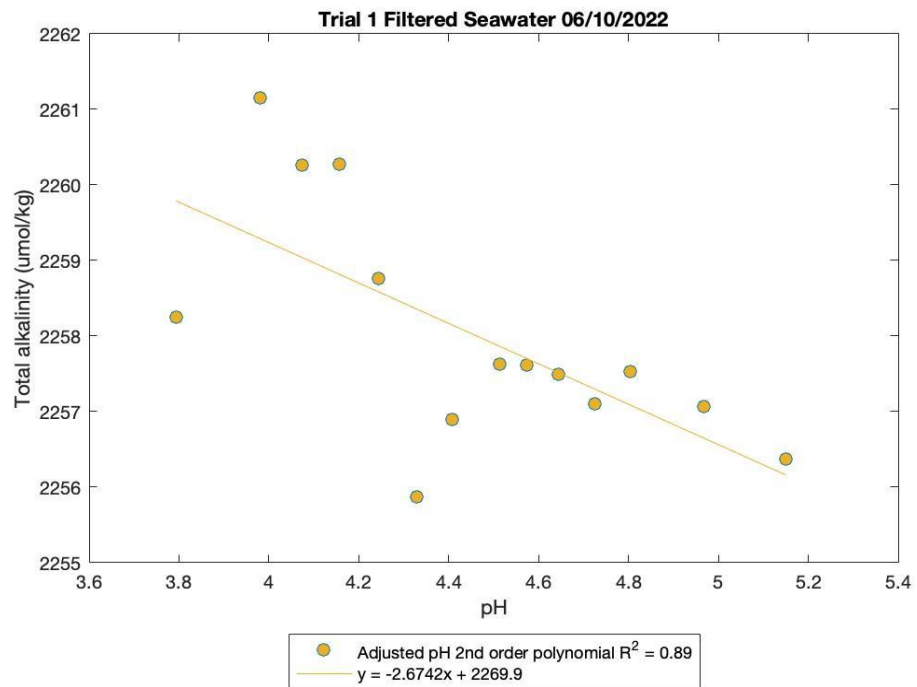
fig. 10. TA as a function of pH for single-step titrated samples using filtered seawater obtained June 2022. The TA values were adjusted using the dye perturbation equation.
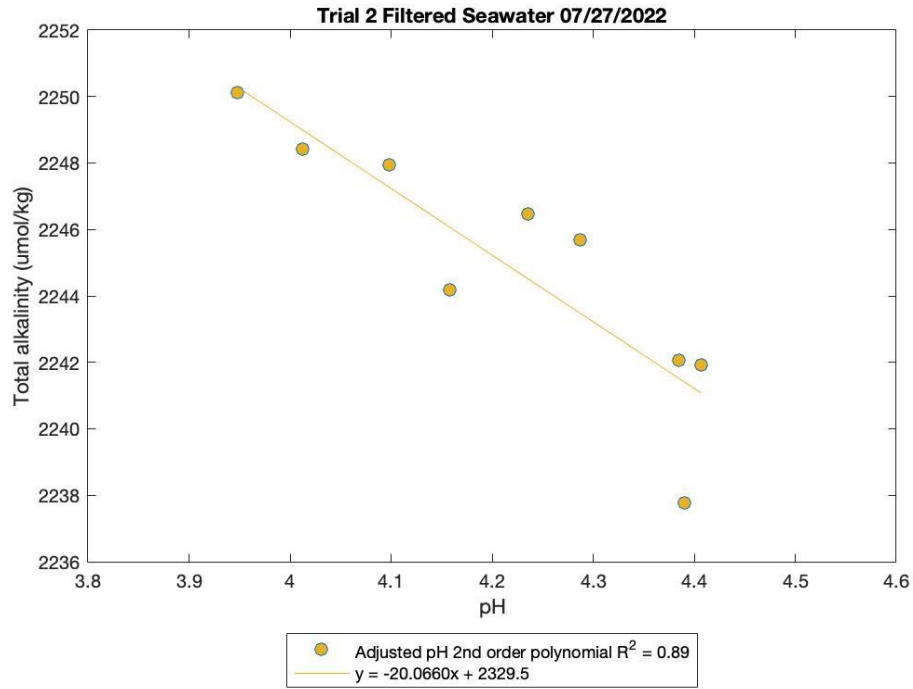
fig. 11. TA as a function of pH for single-step titrated samples using filtered seawater obtained July 2022. The TA values were adjusted using the dye perturbation equation.
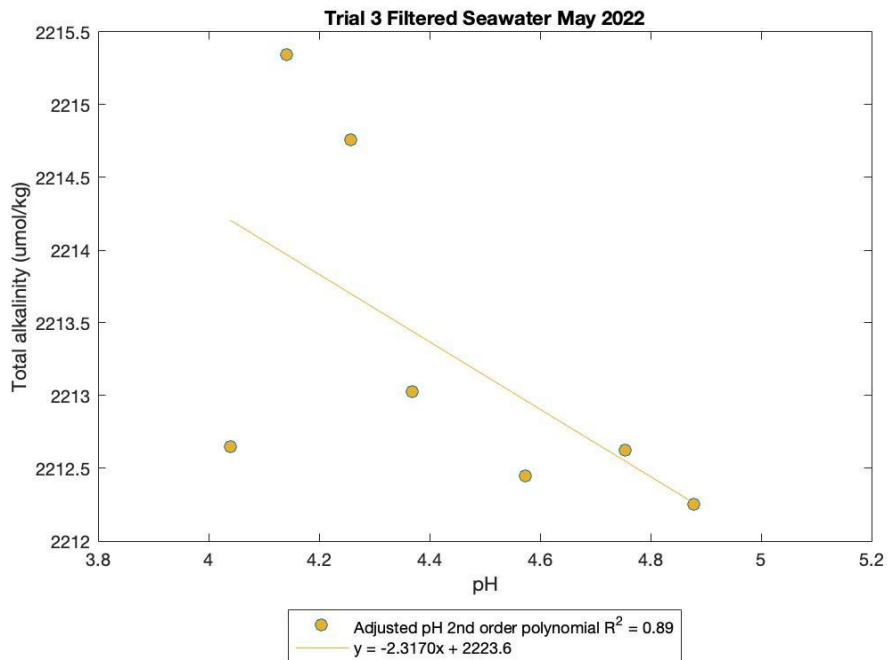
fig. 12. TA as a function of pH for single-step titrated samples using filtered seawater obtained May 2022. The TA values were adjusted using the dye perturbation equation.

DISCUSSION

For the precision of the spectrophotometric single-step TA method, a standard deviation less than 1μmol/kg is accepted, as it falls in line with previous literature (Yoa and Byrne, 1998). Acid titrant delivery via the Metrohm Dosino syringe pump had a closer standard deviation to 1 μmol/kg, however, acid titrant delivery via micropipette offered a lower percent error to its estimated mass of acid titrant. The possibility of obtaining a standard deviation less than or equal to 1 μmol/kg is likely for both micropipette and syringe pump delivery of acid titrant, as was observed in experiments post the gravimetric precision experiment, however, the results of these later experiments will not be presented in this study. The amount of time needed to prepare the single-step titrated samples via micropipette was longer in comparison to the time it took using a syringe pump, therefore, future experiments were conducted with the Metrohm Dosino syringe pump as a delivery for the acid titrant. Instrumental precision for the spectrophotometer pH measurement was well within the accepted range being that the coefficient of variation was 0.024%, which is less than 1%. The standard deviation of the TA using these pH measurements was less than 1 μmol/kg, showing that the spectrophotometer was not a significant contributor to the precision of TA measurements.

It is unclear whether the DIC instrument has a level of uncertainty in its DIC measurement, or if N_2 degassing can not remove all DIC contents from the single-step titrated samples. Regardless of the uncertainty, it was assumed that less than 10 μmol/kg of TIC is negligible and the solution is approximately removed of inorganic carbon. Although 3 minutes of N_2 degassing for 153 g of solution was enough for there to be less than 10 μmol/kg of TIC, future single-step titrated samples would be degassed for about 4 to 5 minutes to ensure removal of DIC. When observing the TA for single-step titrated samples degassed between 3 to 10 minutes the standard deviation was not less than or equal to 1 μmol/kg. The standard deviation for these samples was around 4 μmol/kg,

21

which would be considered high for samples that otherwise should have given very similar TA measurements. Possible sources of variation in pH measurements are thought to be from the evaporation of the sample while the single-step titrated samples were bubbling in N_2, and/or possible CO_2 dissolving in the sample while sitting out.

Understanding whether or not the calibration factor must be applied to the concentration of acid titrant is needed, to compare the spectrophotometric TA method and open cell TA method. TA values for the multi-step titrated method should be higher compared to the single-step titrated method values if organic alkalinity is being titrated for pKa values between 4 and 5 (Sharp and Byrne, 2020). TA values of the spectrophotometric TA method calculated with the calibration factor did have a stable region where the TA was comparative to the open cell TA values, but the further comparison of the two TA methods was not possible due to the uncertainty in the calibration factor.

Not being able to compare the two different TA methods to potentially find an organic alkalinity signal, it was decided to continue using the spectrophotometric single-step TA method to find the potential signal. To do this several single-step titrated solutions would be analyzed at different pH endpoints for TA. A similar trend in TA would be sought out for, whether there is higher TA at a lower end pH, such as pH of 4, and higher TA at a higher pH such as pH 5. The first trial of this experiment did not give a clear distinction between the pH regions 4 to 5, but it was asked whether dye perturbation could influence the TA trend since the potential signal is hypothesized to be of the order of max 20 μmol/kg (Fong and Dickson, 2019). The dye perturbation equation does appear to resolve a TA difference between pH 4 to 5 of the order between 2 to 20 μmol/kg. The dye addition solutions, however, did have a lot of outliers that were excluded from the data set. This could add potential bias to the TA as a function of pH trends in favor of a negative trend from lower to higher pH.

CONCLUSIONS/RECOMMENDATIONS

During my time at MBARI, we have been able to set up and characterize the spectrophotometric TA method. The method can produce consistent TA measurements with a standard deviation of less than 1 μmol/kg, similar to a paper that characterized their own spectrophotometric TA system (Yao and Byrne, 1998). The spectrophotometric TA method has potentially identified an organic alkalinity signal in seawater, but further experiments on other batches of seawater with replicates are ideal to further support these findings. It is also beneficial to perform more dye addition experiments to have a dye perturbation equation with more data points included to correct for any potential bias that may influence TA measurements. The MBARI Carbon Lab is currently developing a new TA system that combines the single-step titration and multi-step titration in one system, by using an ISFET pH electrode that will further experiment to identify a potential organic alkalinity signal and quantify the contribution of the organic alkalinity. This system uses a jacketed glass beaker to mimic the open cell, including a temperature sensor, N_2 degasser tube, ISFET pH electrode, and water bath. This system will be set up to run the single-step titration first to get an end pH of about 5 and then a multi-step titration to have an end pH of about 3. The new Durafet TA method would distribute any potential instrumental uncertainty to both the single-step titrated TA and the multi-step titrated TA so that the errors cancel out. The organic signal is the difference between the two TA values.

ACKNOWLEDGEMENTS

References:

Bakker, D. C., Pfeil, B., Landa, C. S., Metzl, N., O'Brien, K. M., Olsen, A., Smith, K., Cosca, C., Harasawa, S., Jones, S. D., Nakaoka, S.-ichiro, Nojiri, Y., Schuster, U., Steinhoff, T., Sweeney, C., Takahashi, T., Tilbrook, B., Wada, C., Wanninkhof, R., … Xu, S. (2016). A multi-decade record of high-quality $f$co2 Data in version 3 of the Surface Ocean co2 Atlas (SOCAT). *Earth System Science Data*, *8*(2), 383–413. https://doi.org/10.5194/essd-8-383-2016

Claustre, H., Johnson, K., & Takeshita, Y. (2020). Observing the global ocean with biogeochemical-argo. *Annual Review of Marine Science*, *12*(1), 23–48. https://doi.org/10.1146/annurev-marine-010419-010956

Fong, M. B., & Dickson, A. G. (2019). Insights from go-ship hydrography data into the thermodynamic consistency of CO2 system measurements in seawater. *Marine Chemistry*, *211*, 52–63. https://doi.org/10.1016/j.marchem.2019.03.006

Guo, M., Wang, X., Li, J., Yi, K., Zhong, G., & Tani, H. (2012). Assessment of global carbon dioxide concentration using MODIS and GOSAT Data. *Sensors*, *12*(12), 16368–16389. https://doi.org/10.3390/s121216368

Katsumata, K., Purkey, S.G., Cowley, R. *et al.* GO-SHIP Easy Ocean: Gridded ship-based hydrographic section of temperature, salinity, and dissolved oxygen. *Sci Data* 9, 103 (2022). https://doi.org/10.1038/s41597-022-01212-w

Liu, X., Byrne, R. H., Lindemuth, M., Easley, R., & Mathis, J. T. (2015). An automated procedure for laboratory and shipboard spectrophotometric measurements of seawater alkalinity: Continuously monitored single-step acid additions. *Marine Chemistry*, *174*, 141–146. https://doi.org/10.1016/j.marchem.2015.06.008

O'Sullivan, D. W., & Millero, F. J. (1998). Continual measurement of the total inorganic carbon in surface seawater. *Marine Chemistry*, *60*(1-2), 75–83. https://doi.org/10.1016/s0304-4203(97)00079-0

Sharp, J. D., & Byrne, R. H. (2020). Interpreting measurements of total alkalinity in marine and estuarine waters in the presence of Proton-binding organic matter. *Deep Sea Research Part I: Oceanographic Research Papers*, *165*, 103338. https://doi.org/10.1016/j.dsr.2020.103338

Watson, A.J., Schuster, U., Shutler, J.D. *et al.* Revised estimates of ocean-atmosphere CO2 flux are consistent with ocean carbon inventory. *Nat Commun* 11, 4422 (2020). https://doi.org/10.1038/s41467-020-18203-3

Williams, N. L., Juranek, L. W., Feely, R. A., Johnson, K. S., Sarmiento, J. L., Talley, L. D., Dickson, A. G., Gray, A. R., Wanninkhof, R., Russell, J. L., Riser, S. C., & Takeshita, Y. (2017). Calculating surface ocean PCO 2 from biogeochemical Argo floats equipped

with ph: An uncertainty analysis. *Global Biogeochemical Cycles*, *31*(3), 591–604. https://doi.org/10.1002/2016gb005541

Yang, B., Byrne, R. H., & Lindemuth, M. (2015). Contributions of organic alkalinity to total alkalinity in Coastal Waters: A spectrophotometric approach. *Marine Chemistry*, *176*, 199–207. https://doi.org/10.1016/j.marchem.2015.09.008

Yao, W., & Byrne, R. H. (1998). Simplified seawater alkalinity analysis. *Deep Sea Research Part I: Oceanographic Research Papers*, *45*(8), 1383–1392. https://doi.org/10.1016/s0967-0637(98)00018-1

Zhai, W., Dai, M., Cai, W.-J., Wang, Y., & Hong, H. (2005). The partial pressure of carbon dioxide and air–sea fluxes in the northern South China Sea in spring, Summer and Autumn. *Marine Chemistry*, *96*(1-2), 87–97. https://doi.org/10.1016/j.marchem.2004.12.002