Introduction

Method Overview
Alkalinity is the measure of how much acid it takes to lower the pH of a water sample enough to convert all bicarbonate (HCO$_3^-$) and carbonate (CO$_3^{2-}$) to carbonic acid (H$_2$CO$_3$). Although total alkalinity is equal to the stoichiometric sum of all bases in solution, ~97% of alkalinity in seawater is due to carbonates.

Method Theory
To measure alkalinity, a pore water sample is titrated with an acid to an endpoint at which carbonate is converted to bicarbonate and bicarbonate is converted to carbonic acid. In seawater, this endpoint occurs at about pH = 4.2.
The alkalinity determination in this method (Gran titration) relies on a mathematical evaluation of the second equivalence point of carbonate titration in seawater using the most stable part of the titration curve (i.e., the part beyond the equivalence point on the low pH side). In essence, the Gran method linearizes the titration curve by means of a simple function:

$$ F = (v + V_0) \times 10^{E/A}, $$

where:

- $F$ = Gran factor,
- $v$ = volume of acid added to the solution in the titration vessel,
- $V_0$ = original volume of the sample,
- $E$ = EMF (millivolts) at $v$, and
- $A$ = slope of electrode determined on the basis of the electrode calibration.

Generally, the slope is ~59 mV at 25°C. Slope is determined during calibration (see Calibrating the Electrode).

The function $F$, when plotted as a function of the volume of acid added ($v$), is linear when sufficiently removed from the equivalence point. We measure mV instead of pH to determine the endpoint because this method offers better precision. The optimum range of millivolts for linearity is 220–240 mV. The value of $v$ at $F = 0$ is the equivalence point from which the alkalinity is evaluated.

The slope of the $F$ vs. $v$ plot changes with variations in the sulfate content of the samples. This is because at lower pH values the following reaction

$$ H^+ + SO_4^{2-} \rightarrow HSO_4^- $$

plays an important role in establishing the pH of the solution through a buffering effect. This change in slope, however, has no effect on the Gran extrapolation intercept with the $y$-axis and is not accurate enough to estimate sulfate concentrations.

**Alkalinity System**

![Meterhm Autotitrator with Workstation](image)

**Figure 1.** Meterhm Autotitrator with Workstation (Note: dispenses 0.1 M HCl).
Apparatus, Reagents, & Materials

Laboratory Apparatus

Hardware and Software
- Metrohm Titrino 794 autotitrator
- LabVIEW Alkalinity program version 340T
- Laboratory oven
- Desiccator
- water bath
- Analytical balance system

Supplies and Materials
- Pipettes, Class A: 0.5, 2, 3, 5, 8, 10, and 20 mL
- Adjustable pipettor, 100–500 µL
- Volumetric flasks, Class A: 100 mL, 1 L
- Combination electrode (Metrohm, combined pH glass electrode, model 6.0234.100)
- pH paper, to bracket pH 7 (pH 1–12 paper)
- 5 mL snap-cap vials
- Magnetic stir bars

Laboratory Reagents
- Hydrochloric acid (HCl), ultrapure
- Potassium chloride (KCl), certified ACS
- Borax (Na₂B₄O₇·10H₂O)
- Sodium bicarbonate (NaHCO₃), certified ACS
- Sodium carbonate (Na₂CO₃), certified ACS

Reagent Solutions
- 0.1 N HCl solution (premade from Fisher, AMS# CH5009)
- 0.7 M KCl solution (52 g KCl in 1 L reagent water)

Stock Standard Solutions (1 L)
- 0.1 M borax solution (38.1 g borax in 1 L reagent water)
- 0.5 M NaHCO₃ (42 g sodium bicarbonate in 1 L reagent water)
- 0.1 M Na₂CO₃ (10.6 g sodium carbonate in 1 L reagent water)
- 0.5 M Na₂CO₃ (53 g sodium carbonate in 1 L reagent water)

Standard Solutions (100 mL)
- 5 mM Na₂CO₃ (pipet 2.5 mL 0.1 M Na₂CO₃ into 97.5 mL 0.7 M KCl)
- 20 mM Na₂CO₃ (pipet 10 mL 0.1 M Na₂CO₃ into 90 mL 0.7 M KCl)
- 40 mM Na₂CO₃ (pipet 20 mL 0.1 M Na₂CO₃ into 80 mL 0.7 M KCl)
- 50 mM NaHCO₃ (pipet 10 mL 0.5 M NaHCO₃ into 90 mL 0.7 M KCl)
- 100 mM Na₂CO₃ (pipet 10 mL 0.5 M Na₂CO₃ into 90 mL 0.7 M KCl)
- IAPSO standard seawater (alkalinity ~2.325 mM)
Reagent Water
18.2 MΩ millipore deionized water

Buffer Solutions

NBS buffers: commercially obtained low ionic strength solutions at pH 4.00, 700, and 10.00 (stable indefinitely; store in the chem lab refrigerator when not in use).

Tris buffer: tris(hydroxymethyl)amino methane or 2-amino-2-(hydroxymethyl)-1,3-propanediol

Bis buffer: bis(hydroxymethyl)methylamino methane or 2-amino-2-methyl-1,3-propanediol (not commercially available because of its highly hygroscopic nature; prepare as follows):

1. Heat reagent water in a glass beaker while adding Bis and stirring.
2. When precipitation is observed (saturation), add HCl until pH = 7 as tested with pH paper.
3. Cool solution (a gel forms in the beaker), then decant the water and place in a warm oven until dry.
4. Scrape dry remains from the bottom of the beaker and place in a vacuum desiccator to cool.
5. Take first weight quickly upon removal from desiccator when making up the buffer. See ODP Tech Note 15 for complete instructions on making up the powdered buffer.
6. Redry in oven and cool in desiccator each time prior to weighing.
7. Make new Bis buffer at the beginning of each expedition.

Instrument Calibration

Overview

Before the titrator can be used to measure samples, the electrode must be calibrated against pH buffers in the same range expected in samples. To ensure accuracy of sample measurements, the titrator settings must also be optimized. Calibration of the titrator includes the following steps: Calibrate the electrode, Select or create a dispensing rate program, and Calculate the standard ratio. Each of these steps are discussed in the following sections.

Calibrating the Electrode

The pH electrode must be standardized for linearity across the pH range of the samples. Generally, calibration at pH 4, 7, and 10 covers the necessary range. The electrode is calibrated at the beginning of each expedition as follows (calibration dataflow is shown in pH Dataflow).

1. Select the Calibration tab from the main menu of the Alkalinity program (Figure 2).
2. In the Buffers fields, enter pH of at least 2 buffers to bracket the expected sample pH values (generally 4, 7, and/or 10).
3. Place enough buffer solution in the titration vessel to cover the electrode tip and add a stir bar.
4. Immerse the electrode tip in the buffer solution (not touching the bottom of the vessel).
5. Set water bath temperature to 25°C, and activate stirrer.
6. Set the Drift Span field (below plot) to a value >0 (30 readings is recommended), and click Measure 1.
7. Measure with stirring until drift (mV) vs. time (sec) on the plot is near 0.0 (this may take an hour or longer).
8. When the drift is nearly 0.0, click Done.
9. Repeat Steps 2–8 with each calibration buffer, selecting Measure 2 and Measure 3, as applicable.
10. Click Calibrate to view the regression curve. The slope value should be close to −59 pH/mV.
11. Click Save Calibration to accept values.
Setting the Dispensing Rate

The rate at which the titrator dispenses the acid into the sample can be adjusted according to expected alkalinity value. Based on experience, for samples with alkalinity <20 mM, a 3 mV rate is usually appropriate. A 3 mV rate dispenses as follows:

<table>
<thead>
<tr>
<th>Volume of 0.1 N HCl (µL)</th>
<th>Until what voltage (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>220</td>
</tr>
<tr>
<td>1</td>
<td>220–240: through the linear Gran portion of the titration curve</td>
</tr>
</tbody>
</table>

Higher alkalinitities may require faster dispensing rates. The dispensing rate can be selected from a list of predefined rate programs or a new dispensing rate program can be created.

Selecting a Predefined Dispensing Rate Program

1. Open the Rates & Standard Corrections tab in the Alkalinity main menu (Figure 3).
2. Under Rates, select a predefined rate from the list. These rates are multistep rate programs such as the 3 mV rate example in the previous section.
3. Click Load This Rate to use the predefined rate program.
Figure 3. Selecting a Predefined Dispensing Rate Program.

Creating a New Dispensing Rate Program

1. Open the Alkalinity/Standard tab in the Alkalinity main menu (Figure 4).
2. Edit the Rates fields for each step in the rate program as follows:
   - Level (mV) up to which this rate is applicable (150 in the example shown below).
   - Acid (mL) to add in each increment (0.030 mL = 30 µL as below).
   - Drift tolerance (mV/s) for acid increment (0.02 as below). Measurement continues until drift tolerance is satisfied.
   - Seconds until rate program times out (timeout(sec)) if drift tolerance is not satisfied (180 as below).
3. Click Save This Rate.
4. Enter a rate program name in the dialog box and click OK.

Drift Span

A drift span of 30 (default) indicates that a minimum of 30 measurements will be taken after each addition of titrant (acid). The difference between the first and last measurements is compared to the drift tolerance specified in the rate program (see Creating a New Dispensing Rate Program). Generally, drift tolerance starts at 0.10 mV/s. Drift tolerance acts as follows:

   - If the difference between the first and last measurements is smaller than the drift tolerance the next increment of acid will be dispensed.
   - If the difference between the first and last measurements is larger than the drift tolerance measurement will continue at that increment until the drift tolerance is satisfied.

In Figure 5 the red arc on the plot is the signal coming directly from the electrode in real time. The y-axis is the mV reading, and the x-axis is time in seconds. The red arc/readings will continue until the drift tolerance is satisfied, which provides a final mV reading.

The blue trend on the plot on the right side of the figure is the trend of mV readings vs. acid additions in mL. The mV readings come from the final value reached in the red arc after satisfying the drift tolerance.
Calculating Standard Ratio

Calculating the standard ratio (estimated vs. actual alkalinity) for the anticipated range of alkalinity values accounts for measurement error in acid strength. Standard ratio can be calculated using borax solution, sodium bicarbonate solution, or IAPSO standard seawater, as necessary, to most closely match alkalinity values (within 5 mM, to preserve the first-order transfer function) of the unknown samples. Generally, IAPSO standard seawater is used to establish this ratio, and additional calibration standards are used if samples deviate >5 mM from the alkalinity of IAPSO (~2.325 mM). The measurement is repeated until at least 3 consistent measurements are obtained within 5% of actual value for each standard:

- IAPSO = 2.21–2.44 mM
- 20 mM standard = 19–21 mM
- 40 mM standard = 38–42 mM

Obtain measurements for the standard ratio as follows:

1. Open the Alkalinity/Standard tab and select Standard Ratio at the bottom of the window (Figure 6).
2. Check to make sure the reservoir is full with 0.1 N HCl. Set the water bath to 25°C, pipet standard into titration vessel, and add stir bar.
3. Ensure no air bubbles are present in the acid dispensing line. Press DOS on the body of the titrator to push acid through the line and remove air bubbles.
4. Remove the electrode from the storage solution, rinse with DI water, and blot dry with a Kimwipe. Do not rub the electrode, as this can cause a static charge. Insert the electrode tip into the titration vessel (not touching the bottom of the cup), make sure Drift Span = 30, and click Measure.
5. Enter the given Alkalinity and Sample volume (e.g., 3 mL) and click GO.
6. Once pH measurement is done and value is OK (>7.0), follow the onscreen instructions to complete the titration and measurement. If pH value is wrong, abort the measurement and go back to Step 4.
7. Repeat Steps 4–6 until you have at least 3 consistent measurements per standard.
8. Highlight the measurements to include in the average ratio and click Save Standard.
9. Name the ratio so it can be retrieved later from the Rates & Standard Corrections tab.
Sample Preparation and Analysis

Sample Storage and Preservation

Use 3 mL of interstitial water sample. Store residual sample in a 5 mL snap cap tube for additional shipboard analysis.

Sample Preparation and Analysis

The laboratory technician receives a whole-round sample at the catwalk to squeeze for interstitial water, which is passed through a 0.45 µm filter before analyzing pH and alkalinity.

The general procedure for analyzing pH and alkalinity on interstitial water samples is as follows:

1. Calibrate the electrode (see Instrument Calibration).
2. Pipette a 3 mL sample into the titration vessel and add stir bar.
3. Take an initial pH reading (see Measuring pH).
4. Titrate to determine total equivalent alkalinity value (see Measuring Alkalinity).
5. Upload data to LIMS (see LIMS Integration).
6. Store titrated sample in a sealed polytube with a new label indicating volume and concentration of acid added.

Measuring pH

The titrator measures and records the pH value for each sample before titration for alkalinity begins.

Measuring Alkalinity

1. On the Alkalinity/Standard tab, ensure dispensing rate, drift span, and standard ratio are set correctly (Figure 7).
2. Ensure the water bath temperature is set to 25°C and place sample in titration vessel.
3. After rinsing the electrode with DI water and gently blotting dry with a Kimwipe, insert the electrode tip (not touching the bottom of the cup).
4. Select the Alkalinity tab at the bottom of the screen and click Measure.
5. Select measurement type from the list, enter operator name (this must be the same as LIMS user ID), text_ID, and sample volume (Figure 8). Click OK.
6. After taking the initial mV and pH readings, the software prompts the user to insert the acid dispensing tip and click GO (Figure 9). If you make a mistake, click ABORT and no acid will be added to the sample. After clicking GO, the sample will be titrated to completion.
7. Click Done/Save to upload data (see LIMS Integration).
**Figure 7. Measuring Alkalinity.**

**Figure 8. Select Measurement Type.**

**Figure 9. Insert Acid Tip Prompt.**
Cleaning up after Measurement

1. Remove electrode from cup.
2. Rinse electrode with DI water in squeeze bottle.
3. Blot the electrode dry with a Kimwipe. Do not rub the electrode, which could cause static charge buildup.
4. Place the electrode in a storage container containing 3 M KCl.
5. Rinse and dry the acid dispensing tip and stir bar.
6. Pipette or pour the titrated alkalinity IW sample into a container to ship to repository or scientist.
7. Write on label how much HCl was added during titration.
8. Rinse the titration vessel cup with DI water and dry.

Data Handling

Editing Gran Factor Points

Outlier Gran factor points (Figure 10) can be deleted from the linear portion of the curve to improve the accuracy of sample results as follows.

1. When the titration is complete, the Alkalinity Calculation screen opens. Unlock the Gran factor axis.
2. Zoom in on the Gran factor points.
3. Select the cursor and move to the point to be deleted.
4. Click Delete Data Point and then click Done/Save when finished.
Figure 10. Gran Factor Points (blue = Gran factor F, red = optimum mV range for linearity: 220–240 mV).

Data Reports

Data reports are not available at the instrument, but data can be viewed on the Data Log tab (Figure 11). Also, the software appends the alkalinity results to the DAT file \texttt{C:\ALKALINITY\LOG\ALKALINITYSAMPLES.DAT}. The best way to view the alkalinity values to be used in graphs and reports is via LIMS Reports.
Quality Assurance/Quality Control

Overview

A quality assurance/quality control (QA/QC) program ensures that a measurement system is performing within control limits and therefore provides high-quality data. The QA/QC program for this system includes instrument calibration, calibration verification, and accuracy and precision monitoring.

Instrument Calibration

The instrument is calibrated by the onboard laboratory specialist at the beginning of the expedition. Calibration is verified routinely during operation. (See Instrument Calibration.)

Analytical Batch

An analytical batch is a group of samples run together in one sequence, sharing a calibration curve, blanks, reference materials, and verification samples. The alkalinity batch size is 10 samples. Each batch of 10 unknown samples contains a sample to verify precision and a sample to verify accuracy (see Precision and Accuracy).

Blanks

Blanks are not run for this method because DI water has no buffering capacity and would therefore fail the slope program. Thus, blanks are not applicable to this chemistry.

Calibration/Calibration Verification

- pH buffers are calibrated at the beginning of the cruise
- Borax/IAPSO standard correction factors are calibrated at the beginning of the cruise
- Recalibration is done if the precision or accuracy is greater than 5%
Precision

Precision is the degree to which further measurements will show the same or similar results.

- Duplicates are not run on samples because that would require using 6–10 mL of the interstitial water, which is usually too large a sample amount to justify. Instead, duplicate calibration verification samples (duplicate IAPSO standard measurements) are compared to calculate precision.
- Precision is measured with every batch of 10 samples.
- Precision control limit allows a difference of ±5%.
- If the precision control limit is exceeded, the system must be recalibrated and all samples run since the previous in-control precision measurement must be repeated.

Accuracy

Accuracy is the degree of closeness of a measured value to the actual (true) value.

- Standards are run with every batch of 10 samples.
- Accuracy control limit allows a difference of ±5% from true standard value.
- If the accuracy control limit is exceeded, the system must be recalibrated and all samples run since the previous in-control accuracy measurement must be repeated.

Limits of Detection and Quantitation

- Samples are not reported as less than the detection limit for alkalinity. The only way a sample could not be analyzed is if its initial pH (before acid addition) is <4.2, which is rare.
- Results are reported to three decimal places.
- The titration uncertainty is ±0.003 mL. When carried through the alkalinity calculation, this uncertainty results in the alkalinity difference being 50 µM, though it is also dependent on the starting pH.

Software Dataflow

Two primary data types are generated by this system: pH and alkalinity. The alkalinity measurement depends on a series of pH measurements, so sample pH is determined before alkalinity titration begins.

pH Dataflow

Calibration

User-configurable variables include the following (Figure 12). Refer to Drift Span for more information about these values:

$\text{mVSpan}$: drift span; number of samples to determine the slope (default = 30); the slope calculation is

\[ \text{Ncurrent sample} - \text{N[(current sample) – } \text{mVSpan}] \]

$\text{mVThreshold}$: slope threshold; maximum calculated value for the reading to be considered stable; also “drift tolerance.”
Alkalinity Dataflow

User-defined variables (Figure 13), with values from the example given in the Setting the Dispensing Rate section:

- $MV1$: rate 1 mV threshold (150 mV)
- $MV2$: rate 2 mV threshold (220 mV)
- $MV3$: rate 3 mV threshold (240 mV)
- $Rate1$: rate for first mV threshold (30 µL)
- $Rate2$: rate for second mV threshold (4 µL)
- $Rate3$: rate for third mV threshold (1 µL)
- $SlopeSpan$: number of samples used to calculate the slope (default = 30)
- $StabilityThreshold$: maximum slope value to ensure a stable reading (also referred to as drift tolerance)
LIMS Integration

In LIMS terminology, we perform two types of alkalinity analyses: ALKALINITY and ALK_QAQC:

- **ALKALINITY** analyses include pH and alkalinity tests performed on interstitial water samples.
- **ALK_QAQC** includes the same physical tests performed on a calibration standard or standard sample.

**ALK_QAQC** analysis has three different variations:

- **CALIBRATE**: tests done to calibrate the autotitrator
- **STANDARD**: standard analyses
- **REPLICATE**: duplicate sample analyzed to check precision and/or accuracy

LIMS stores several components of each analysis, which are detailed in the chart below.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Component</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALKALINITY</td>
<td>alkalinity</td>
<td>mM</td>
<td>Ability of a solution to neutralize acid to the equivalence point of carbonate</td>
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<tr>
<td></td>
<td>pH</td>
<td>NA</td>
<td>Acidity or basicity of a solution (–log[H⁺])</td>
</tr>
<tr>
<td></td>
<td>acid_quantity</td>
<td>µL</td>
<td>Amount of 0.1 M HCl added to the sample during titration</td>
</tr>
<tr>
<td></td>
<td>correction_factor</td>
<td>—</td>
<td>Correction factor for non-ideal behavior of samples to adjust calibration</td>
</tr>
</tbody>
</table>
## Analysis

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<th>Analysis</th>
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</tr>
</thead>
<tbody>
<tr>
<td>ALK_QAQC</td>
<td>alkalinity</td>
<td>mM</td>
<td>Ability of a solution to neutralize acid to the equivalence point of carbonate</td>
</tr>
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<td></td>
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<td>correction_factor</td>
<td>—</td>
<td>Correction factor for non-ideal behavior of samples to adjust calibration</td>
</tr>
<tr>
<td></td>
<td>slope</td>
<td>—</td>
<td>Slope of the calibration equation</td>
</tr>
<tr>
<td></td>
<td>intercept</td>
<td>—</td>
<td>Intercept value of the calibration equation</td>
</tr>
<tr>
<td></td>
<td>corr</td>
<td>Rho</td>
<td>Correlation coefficient of the calibration</td>
</tr>
</tbody>
</table>

### Uploading Data to LIMS

When the alkalinity titration finishes, the Alkalinity Calculation screen shows the slope information, correction factors, and final alkalinity value (Figure 14).

Edit outlier Gran factor points, if necessary (see Editing Gran Factor Points). If satisfied with the results, click Done/Save to load the values into LIMS.

![Figure 14. Load Data to LIMS.](image)

### Health, Safety, & Environment

#### Safety

Wear personal protective equipment including close-toed shoes, lab coat, gloves, and safety glasses when working with acids of any strength.

Use a fume hood when making solutions from concentrated acids.

#### Pollution Prevention

Make reagent and standard solutions in quantities no larger than needed to complete sample analysis.
Waste Management
Neutralize acid solutions before disposal.

Maintenance/Troubleshooting

Common Issues
- Make sure the probe storage container is filled with 3 M KCl solution and the reservoir is filled with 0.1 M HCl solution.
- Ensure no air bubbles are present in the acid dispensing line. Press DOS on the body of the titrator to push acid through the line and remove air bubbles.
- Change out the Drierite trap when ~50% of the color turns from blue to pink.

Consumables
- Electrode: Metrohm 6.0234.100 or equivalent.
- 0.1 M HCL, Fisher CH5009
- Microvalve buret tip, Metrohm 020683244, CM5129