Total Alkalinity Protocol
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Brand: Compact Titrator
Version: G20S

A. Materials:

- Distilled water
- Titrant HCl solution (0.1 N)
- plastic backers
- balance
- NBS buffers (4.01 and 7.00) to verify the pH sensor (22 °C)
- sea water buffer TRIS to calibrate the pH sensor (22 °C)
- Standard: batch of Dickson (CRM)
- waste bottle for junk CRM
- paper and gloves

B. Sampling and Starting procedure:

1. Make sure to collect enough seawater to make at least 2-3 measurements / replicates for each sample (1 bottle of 250 ml). As soon as possible, transfer seawater from Niskin bottle to your bottle using a small tube via siphon principle. For alkalinity measurements, bubbles are OK since CO_2 does not interfer with the measurement of alkalinity. Transport your samples in the dark and maintain it at a constant temperature, if possible.
2. Once in lab, put some parafilm around the bottle cap and store the samples in the fridge until run the analysis. If filtered, run the analysis within 15 days from the sampling. ~~add $HgCl_2$ (0.02% of final volume, saturated solution. Ex: 100 µl of saturated $HgCl_2$ for 250 ml). Use all the protective equipment. Do it under hood.~~
3. The number of samples that we can analyse depends on volume and alkalinity of samples. Generally, with 1L of HCl solution, we can analyse about 100 samples of 100g of seawater with alkalinity values about 2600 - 3000 µmol/kg.

C. Verification and calibration of the pH electrode (once a week).

1. Turn on the titrator (Mettler Toledo G20S, do not connect to LabX and login as Administrator) and the thermostat (set at 22 °C). Put the pH electrode in seawater for 30 mins before the start of the analysis. Open the little hole of the pH electrode to put in equilibrium with the air pressure and put some parafilm on the tube with KCl to preserve the pH sensor. Start the stirrer (STIRRER button on the Titrator screen). Leave the electrode in this solution until the Temperature is stable.
2. Put the buffers (NBS: 7.00-4.01 and TRIS) as well as the standard and your sample in the water bath. Everything should be warmed up before use.
3. Switch on the computer and the balance.
4. Once a week, the electrode should be checked using NBS buffers and calibrated using TRIS buffer.
5. The electrode must be put 5 min in pH 7.00 buffer before this step.
6. Use the method "pH sensor" to check the slope of the pH electrode with NBS buffers. Follow the instructions on the screen, the acid tip is not needed for those steps. Put first the electrode

in pH 7.00 buffer and after pH 4.01. Before and between the two buffers, rinse the pH electrode and the temperature sensor with distilled water and dry them well.

7. Rinse the electrode and temperature probe carefully with distilled water and gently dry it with soft paper.
8. Put the pH electrode in the TRIS buffer and select the method "TRISSSSS" on the desktop (select the first temperature sensor from the list e mV as unit). Wait for 5 minutes, until the mV and the temperature are stable and write it down. These values (together with pH of the TRIS – see the file 'ALK_slope_tris_pH_calculations.xlsx') will be necessary to run the R script. In the file 'ALK_slope_tris_pH_calculations.xlsx change only the temperature. Write these values (pH, mV and Temp) in the last raw of the csv file 'Calibration_TRIS_ischia.csv'.
9. The electrode is checked and calibrated, you will now run normal seawater before running the standard.

D. Measurement using Compact Titrator:

Before measuring your samples and in order to have reliable measurements, you must:
1. Run 2-3 "old" seawater. "Old" seawater is seawater that is not your sample.
2. Run 2-3 standards every about 20-25 samples. Your measured standard value must be within ± 4 μmol Kg^{-1} from the theoretical standard value.
3. Run your samples.

The following steps are the same for the 3 previous measurements (old, standards and samples). Only the seawater inside the beaker will be different (old, standards or samples)

1. **Connect the USB where save the data during the titration**
2. **Purge the burette to remove bubbles:** move up the acid pipe from the acid bottle (use gloves and paper) and unplug the burette. Put the acid tip in a waste beaker then hit on the RINSE button on the Titrator screen. The burette will flush and create a big bubble inside since the acid pipe is unplugged. Twist the unit up and down to remove little bubbles stuck at the bottom of the burette (hit the pipe with your fingers to remove bubbles). When done, plug back the unit and the pipe to the acid bottle. The burette is ready to use.
3. Plunge the pH electrode in the beaker containing seawater, open the little hole of the pH electrode to put in equilibrium with the air pressure: put some parafilm on the tube with KCl to preserve the pH sensor when it is not used. Start the stirrer (STIRRER button on the Titrator screen). Leave the electrode in this solution until the Temperature is stable.
4. Put the sensor of the temperature, the tube for acid and the stirrer in the right places (be sure that they do not touch each other). Put the sensor connected to the thermostat to maintain the temperature constant during the analyses.
5. Switch on the mixer under the HCl bottle.
6. Use the method already set (see point E**);
7. Tare the 100 ml baker on the balance and fill it with the 'old', standards (Batch di Dickson or CRM) or samples. Weight the sample (about 50 g) and note the value on the notebook. Connect the baker to the titrator and start the analysis of the alkalinity; make at least two replicates for each sample;
8. Between a sample and the following, clean with distilled water the pH electrode, temperature sensor and the acid tip. Dry them gently with clean paper and remove drops;
9. Throw away in the waste bottle with Hg or in the sink without HgCl2;
10. Data are automatically saved in the USB device with the sample name. These data (together with the volume of the sample, the temperature during the reaction, the sample salinity, the density and the concentration of the acid to make the titration) will be processed by using the R script – See point E;

11. Once the titrations are completed, rinse the acid tip, electrodes and dry them carefully. Close the little hole in the glass electrode and put it in the storage solution (N.B. Check always that there is enough liquid to maintain the pH sensor). Switch off the thermostat, save your data and turn off the computer, wash the beakers, clean up the table and put everything in the cupboard. Switch off the titrator e unplug it.

E. Set the method EQP (Equivalence Point Titration):

The method is already set on the display of the Titrator and ready to be used.
If you want check it, these are the characteristics of the method EQP.

Be carefully when measure the Batch of Dickson and the Sample, in this case you have to change the predispense and the termination volume of the acid.

Sample
Sample type: Sample
Number of IDs: 1
ID 1: (write the name of the sample)
Entry type: fixed volume
Volume: 100 ml
Density: 1.0 g/ml
Correction factor: 1.0
Temperature: 25.0 °C

Titration stand
Type: manual stand
Titration stand: manual stand 1

Titration (EQP)
Titrant: HCl
Concentration: 0.1 mol/l
Type of sensor: pH
Sensor: DG115-SC
Unit: mV
Temperature acquisition: DT1000
Unit: °C

Stir
Speed: 20%

5. **TITRATION -> Predispense** For about 50 g of seawater sample set the HCl predispense at 1.5 ml and the termination at 2 ml. For CRM set the predispense at 1.3 and the termination at 1.9 ml.
   Mode: volume
   Volume (ml): xxxx ml
   Wait time (s): 200

Control: user
Titrant addition: incremental
Dv (ml): 0.025
Mode: equilibrium controlled
dE: 0.1 mV

dt (s): 5 s
t(min): 15 s
t(max): 100 s

Evaluation and recognition
Procedure: standard
Threshold: 40.0 mV/mL
Tendency: none
Ranges: 1
Lower limit (mV): -100
Upper limit (mV): 0
Add EQP criteria No

Termination
At Vmax: xxxx ml

After number of recognized EQPs: No

F. Data computing with R (Samir method)

1. Once the titration is finished, the data will be saved in the USB. Copy and paste each new data file in the "Desktop/Total_Alkalinity_Samir/Titration_exports/" folder.
2. The exported file will be used for calculations using the R script. Open R and open the script “titration alkalinity jeremy” in the ‘Total_Alkalinity_Samir’ folder (on the desktop).
4. You must click on SOURCES to run the script in order to be prompted for the Salinity and Weight. If you select the script and click on "run" as usual, you will not be prompted. You must enter the S and W for each titration files.
5. Enter the Tris data from the screen in a CSV document each time you make a Tris calibration in Calibration_TRIS_ischia.csv (you will need the mV also). In this doc, you enter the voltage in mV, the temperature in C and the pH calculated via the function tris (salinity, temperature) from seacarb. Be careful, we want a stable measurement of the voltage, T of your TRIS and we don’t want the slope.
*** Note that the R script will always take the Tris informations of the last row (most recent one).

Wash bottles polluted with Hg:

- rinse 2 times with distilled water;
- rinse with HCl (1N) for 20 minutes;
- rinse 2 times with milliQ and 2 times with distilled water.

Samir tips:

Concerning TA:
We use Dickson CRM only for samples that are part of a big experiment in order to publish the results. This is to save CRM since they have a cost. When we are making some « little experiments » or if I am interested in an approximate value of TA, I use standards that I have prepared at lab: « lab CRM».

When I start a TA day analysis, after calibrating and checking the instrument once a week, I run 3 « old » seawater samples (that is normal seawater) in order to use the instrument to seawater.
When my 3 results are stable I run 2/3 CRM. The difference between your 2/3 measurements mean and theoretical value of CRM should be +/- 4 micromoles.
And after I generally run 10 samples before to run again CRM.
So, when Yui told you 15/20 it depends of the stability if the instrument I guess.

To prepare Lab CRM:
- sampling 15 litres of seawater in the bay
- filter it
- fill 11 (eleven) borosilicate bottles (500ml)
- poison 10 (ten) bottles, put grease and rubber bands and keep 1 (one) bottle without poisoning, ready to be analysed.
- analyse this bottle at least 10 times (10 replicates of the same bottle).
- when I do this one bottle analysis, I check my instrument with one CRM Dickson before, to be sure that my instrument is correct.

At the end, I have an accurate measure of TA from the 15 l stock seawater. And I can say that all the 10 bottles have the same TA with a standard deviation based on 10 replicates. This is my theoretical lab CRM.

I use those 10 lab CRMS time to time during the year when it is not necessary to use a real Dickson CRM.

Concerning pH:
I use TRIS only when I am making a batch of dye. I never use TRIS before or after a single sample. The quality and precision of my dye is checked the first day of the preparation of the dye and after like 5/6 months later when I am doing a new dye batch I check this new batch with a new TRIS.
Do you have more dye in stock?
You can check on the excel file that I put on the pH computer. There is a tab with calibration where you can see a TRIS measurement when I have done a dye batch in February.