Introduction

This note will provide information on the effect of silicate on ecosystems and/or organisms, the importance of silicate monitoring and detection, and the analysis of silicate in water using Turner Designs Trilogy Silicate Absorbance Module.

Background

Silica is a naturally occurring compound that can be found in many forms such as sand, quartz, opal, rock, and sea floor sediments. It also is an important element in animal and plant life. Specifically biogenic silica is extracted from water by plants, microorganisms, or invertebrates for building structural materials and growth. Primary producers such as Diatoms extract silica from water, which is required for growth, allowing blooms to occur and ultimately increasing the energy flow from lower to higher trophic levels in an ecosystem. Microorganisms called radiolarians, use silica to make glass tests, or structures, which are deposited after blooming events and can be used as markers to track past events such as volcanic eruptions and storms. Sponges are larger organisms that use silica from water to produce their housing structures and are important filter feeders that help balance ecosystems.

Why is measuring silicate important?

Estimating the silica content of water systems can be very helpful in determining community structure and ecosystem dynamics. Ecosystems with low silica concentrations are likely to have an abundance of flagellates, most of which may be toxic producing algae. Monitoring silica concentrations can be used to assess impacts of storms on aquatic systems. Storms increase silica concentrations in water and if this occurs in areas where silica is limiting there might be a rapid increase in zooplankton and fish populations.

Silicate Analysis

Overview
The following standard method for estimating silicate, using absorbance, is adapted from Strickland and Parsons (1968), "A Practical Handbook of Seawater Analysis", Determination of Reactive Silicate. This method allows the formation of silicomolybdate complex. The complex is then reduced, using metol and oxalic acid, to produce a blue solution that has an extinction coefficient at 810 nm wavelength, which is proportional to the color's intensity.

Apparatus
Absorbance measurements are made using the Turner Designs Trilogy Laboratory Fluorometer (P/N: 7200-000) with the silicate absorbance kit (P/N: 7200-072). The Trilogy is a solid state instrument that uses a Light Emitting Diode (LED), filter, and photodiode combination to measure silicate extinction coefficients at 810 nm. For greatest accuracy in determining silicate in water, spectrophotometric grade, 1-centimeter path length, 10x10, glass cuvettes are recommended because of the stability of signal at low micromolar concentrations. ***The following method is adapted from Strickland and Parsons (1968), Determination of Reactive Silicate.***

Reagents Required:

Ammonium Paramolybdate	$[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$
Oxalic Acid Dihydrate	$[(COOH)_2 \cdot 2H_2O]$
Magnesium Sulfate Heptahydrate	$[MgSO_4 \cdot 7H_2O]$
Anhydrous Sodium Sulfite	$[Na_2SO_3]$
p-methylaminophenol Sulfate	$[C_{14}H_{20}N_2O_6S]$
Hydrochloric Acid (12N)	[HCL]
Sodium Silicofluoride	$[Na_2SiF_6]$
Sulfuric Acid	$[H_2SO_4]$
Sodium Chloride	[NaCl]

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Reagent and Standard Preparation (Strickland and Parsons 1968)

Molybdate Reagent

Using a 500 ml (milliliter) volumetric flask, dissolve 4.0 g (grams) of ammonium paramolybdate in 300 ml of distilled water. Then add 12 ml of (12N) hydrochloric acid, mix and make to a volume of 500 ml using distilled water. Store in a polyethylene bottle **and keep out of direct sunlight**. This solution should be stable for many months. (*Note: if white precipitate forms on the sides of the container the reagent should be discarded*)

Metol-sulfite solution

Dissolve 6.0 g of anhydrous sodium sulfite in 500 ml of distilled water, then add 10 g of metol (p-methylaminophenol sulfate). When metol had dissolved, filter the solution through a No. 1 Whatman filter (dia. 42.5mm) and store the filtrate in a clean glass bottle that is tightly stoppered. This solution should be prepared fresh every month because it may deteriorate rapidly and erratically.

Oxalic acid solution

Shake for 10 minutes, in a 1-Liter container, 50 g of oxalic acid dihydrate with 500 ml of distilled water. Decant the solution from the crystals and store the solution in a clean glass bottle. This solution is stable indefinitely.

Sulfuric acid solution 50% v/v

Add 250 ml of distilled water to a 1-Liter beaker. **(Recommended: place beaker in ice bath.)** Slowly and carefully, while stirring add 250 ml of concentrated sulfuric acid to the beaker. The resulting reaction is **exothermic** so the solution will heat up quickly heating the beaker. Allow beaker and solution to cool in the ice bath. Once cooled carefully measure the volume using a 500 ml or 1-Liter graduated cylinder. A little extra, distilled, water may be required to make the volume 500 ml. This solution can be stored in a glass bottle and is stable indefinitely.

Reducing Reagent

Mix 100 ml of metol-sulfite solution with 60 ml of oxalic acid solution. Add slowly, with mixing, 60 ml of the 50% sulfuric acid solution and make the mixture up to 300 ml with distilled water. This solution is will deteriorate very quickly and should be prepared for immediate use each time.

Silicate solution (standard)

Weigh 0.960 g of sodium silicofluoride and dissolve in 100 ml of distilled water. Dilute to exactly 1000 ml, mix and immediately transfer the solution to a polyethylene container for storage. This solution is stable and has a concentration of 5000 micromoles/Liter and can be diluted to desired concentrations.

Synthetic seawater

Dissolve 25 g of sodium chloride and 8 g of magnesium sulfate heptahydrate in each Liter of distilled water. This water will have a salinity of 28‰ (parts per thousand) and must be stored in a polyethylene container. The silicon content of this solution should not exceed 2 micromoles/Liter.

Salinity Calculations

The regression equation from standard values is determined using calibration standards that have been diluted in synthetic seawater with a specific salinity. Therefore, the slope of the calibration curve is a function of that salinity.

Freshwater or Similar Salinity

If working in freshwater environments or systems where salinity does not vary by more than 10%, synthetic seawater can be made to desired salinity and used to dilute silicate standard solution for creating a calibration curve, which can be applied to all samples being analyzed.

Varying Salinity

If salinity of samples vary by more than 10%, samples can be grouped with respect to salinity. Synthetic seawater can be made per group and used for diluting silicate standard solution to create group specific calibration curves.

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Varying Salinity Corrected using Calcuations

If working in areas where the salinity variation is greater than 10%, synthetic seawater can be made to desired salinity and used for diluting standard solution creating one calibration curve. The following equation can be used to correct the slope of the calibration curve for each sample that has a greater than 10% variation in salinity from the synthetic seawater used.

$$m_s = \frac{m \ (1 + (0.003 \times \text{Salinity of sample}))}{1.08} \qquad \textbf{(Equation 1)}$$

Where m is the slope from the regression equation ($Y = mx + b$) for desired salinity (i.e. 28‰) and m_s is the salinity corrected slope.

Example of calculated m_s,

If regression equation ($Y = 40.587x + 0.4731$) is a function of synthetic seawater salinity 28‰, then samples with salinity of 35‰ would be corrected using equation 1:

$$m_s = \frac{40.587(1 + (0.003 \times 35‰))}{1.08} = \quad 41.527$$

would

Therefore the regression equation for salinity 35‰ would be: ($Y = 41.527x + 0.4731$)

Recommendations

Absolutely **NO glass vials** should be used to either store or collect silicate calibration standard solutions or samples for longer than a few minutes.

Calibration

1. Snap in the silicate absorbance module making sure that the correct filter paddle is being used and turn the Trilogy Fluorometer on

2. Choose the absorbance mode and verify that the correct snap in module has been loaded (i.e Silicate Absorbance Module) then press OK

3. Choose a working range, typical of samples, to calibrate (*Note: accuracy increases as your working range decreases*)

4. Dilute your silicate solution in synthetic seawater to make solutions (**Calibration Standards**) of known concentrations that can be used to create a calibration curve for your working range (*Note: accuracy increases as you increase the number of calibration points used*)

5. Prepare 2 blank solutions from synthetic seawater for analysis along with your calibration standards

6. Make sure all solutions are at room temperature before adding reagents

7. Pipette 1 ml of the molybdate solution into a clean, dry plastic screw cap vial or dry plastic stoppered vial

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8. Pipette 2.5 ml of your calibration standard or blank solutions to the vial, mix thoroughly and wait for 10 minutes (*Do not let mixed solution stand for longer than 25 minutes*)

9. Pipette 1.5 ml of the reducing reagent, mix immediately and let solution stand for **2-3 hours** to complete the reduction of the complex

10. After 2-3 hours, press the calibrate function on the touch screen, you will be prompted to insert a blank

11. Transfer a blank solution *(synthetic seawater with mixed reagent added)* from the plastic vial to a 1 cm path length, 10x10 glass cuvette

12. Fit cuvette into the module (*use the same cuvette and orientation for all standards or solutions in the batch)*

13. Close lid and press ok, the Trilogy has now been blanked

14. Remove the cuvette, properly dispose of the solution and refill the cuvette with the same blank solution

15. Press Measure Absorbance button and record absorbance value

16. Remove cuvette and dispose of solution

17. Rinse the cuvette with the next **blank** solution before filling

18. Fill the cuvette, fit into module using previous orientation, close lid and press measure absorbance

19. Record absorbance value of solution

20. Remove cuvette and dispose of solution

21. Rinse the cuvette with the next **standard** solution before filling

22. Fill the cuvette, fit into module using previous orientation, close lid and press measure absorbance

23. Record absorbance value of solution

24. Repeat steps 20-23 for all remaining standard solutions

25. Graph concentration of calibration standards vs. Absorbance units (Au) for each standard

26. Determine a regression using standard values, use the regression equation (**Y = mx + b**) to calculate the concentration of silicate in water samples that fall within that working range by plugging in the absorbance values for 'x' in the equation

27. Any samples that exceed the calibrated working range need to be diluted to fit the calibrated working range for accurate estimation of silicate concentration

Sample Processing

1. Seawater samples should be stored in plastic screw top or stoppered vials ***(no glass vials should be used for storage of samples)***

2. Make sure all samples are at room temperature before adding reagents

3. Pipette 1 ml of the molybdate solution to a clean, dry plastic screw cap vial or dry plastic stoppered vial

4. Pipette 2.5 ml of your seawater sample into the vial, mix thoroughly and wait for 10 minutes (*Do not let mixed samples stand for longer than 25 minutes*)

5. Pipette 1.5 ml of the reducing reagent, mix immediately and let samples stand for **2-3 hours** to complete the reduction of the complex

6. Transfer a mixed sample from its plastic vial to a 1 cm path length, 10x10 glass cuvette

7. Fit cuvette into the module (use the same cuvette and orientation for all samples in the batch)

8. Press Measure Absorbance button and record absorbance value of mixed sample

9. Remove cuvette and dispose of sample

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10. Rinse the cuvette with the next **sample** before filling
11. Repeat steps 6-10 for all remaining samples
12. Use the regression equation (**Y = mx + b**) from your calibration curve to calculate the concentration of silicate in your samples by plugging in the absorbance values for 'x' in the equation
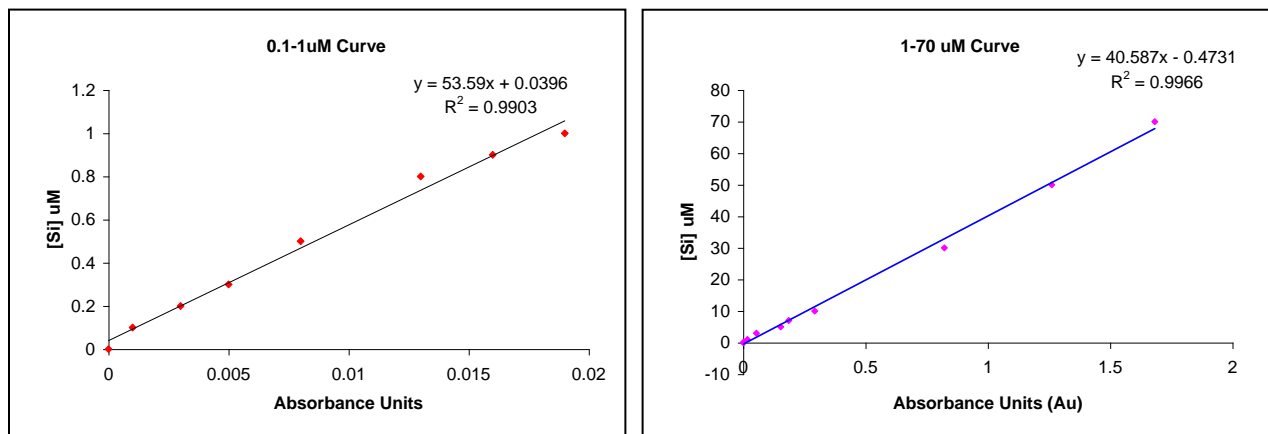

Figure 1: Calibration curves showing strong linear relationship for a broad range of μM concentrations. Regression equations are shown for two working ranges (0.1-1 & 1-70 μM concentrations).

Reference:

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