

Biogenic Silica (BSi) Analysis

Modified Mortlock and Froelich procedure

Purpose

To determine the percent biogenic silica in sediments using sodium hydroxide dissolution of silica and molybdate blue spectrophotometry.

Principle

A freeze dried sediment sample is heated in sodium carbonate to dissolve the silica in the sample. The dissolved silica comes from biogenic sources, i.e. diatoms, and non-biogenic sources, i.e. clays. One timed aliquot is extracted from the sodium carbonate and dyed molybdate blue. Spectrophotography is used to determine the samples absorption at 812 nm. The absorption percent is then used to calculate the percent biogenic silica in the sample.

A more accurate measurement is made if multiple timed aliquots are removed from the sodium carbonate solution, allowing for differentiation of rapidly soluble and slowly soluble silica.

Safety Precautions

When preparing reagents, take care to avoid spills, skin contact, eye contact, inhalation, or ingestion.

You will be working with strong acids and bases. Use protective gear, such as safety goggles, vinyl gloves, lab coat, closed-toe footwear, long pants, etc.

When handling strong acids you must work in the fume hood.

During sample digestions, the water bath will be hot and will contain hot liquid. The digestion tubes will contain hot Na_2CO_3 . Wear protective eyewear and latex gloves.

Label and date everything! Do not pour reagents from their original bottles into unlabeled or mislabeled bottles. Reagents prepared by you must be stored in properly labeled containers. Label water bottles appropriately.

Notes

When using the microliter pipette, it is critical that the amount of sample you draw into the pipette and the amount expelled from the pipette is always exactly the same. Pay attention to the level of liquid in the tip, and start over if the amount of liquid doesn't look quite right or if bubbles are present. Problems commonly arise from improperly seating the tip on the pipetter and from failing to depress the plunger completely.

Use polyethelene products unless otherwise noted. Use of glass may contaminate the samples.

Have all materials needed pre-labeled and set up ahead of time prior to beginning the digestions.

Use silica free water for all mixtures ("18.2 M Ω " water from the recirculating water purifier). This is often referred to as ddH₂O.

Always add the sample to the Molybdate (never the reverse). This will help to keep the mixture above a certain pH.

Getting ready

1. Analyze **54 samples, 4 standards** and **2 blanks** simultaneously (60 50-mL tubes). About 5 samples should be duplicates. 1 cm³ samples should provide plenty of samples for duplicate runs. When you are proficient with the method, increase the number of samples to 80.
2. Record your observations (e.g., possible loss of sediment, overflow of solution, etc.) in the "Biogenic Silica" notebook or your sample list. Enter data in spreadsheet as collected, and back up spreadsheet onto the Paleo server daily.
3. Unless you have already measured sediment bulk density, BSi analysis should begin with volumetric samples (e.g., 0.5 or 1.0 ml) for bulk-density calculation. Bulk density data are necessary for calculating accumulation rates.

Part 1. Sample drying and weighing

1. Sample 1 cm³ of sediment and place in a ½ dram shell vial. Cover shell vial in tissue paper held on with rubber band. To freeze dry without a freeze-dryer: place in automatically defrosting freezer for several weeks. If samples are not completely dry, finish drying in a drying oven at 40°C. To freeze dry with a freeze dryer: Put a small hole pushed through the plastic cap. Freeze the sediment samples, and then freeze dry for at least 24 hours, until completely dry.
2. Gently grind sample within shell vial to mix sample.
3. Weigh total amount of sediment to determine bulk density of your sample (g/cm³). *(Note: If samples are stored in a cold room, they should be taken out 24 hours prior to extraction to equilibrate to room temperature. It's usually helpful to weigh the samples the day before. Zero the scale and write down the weight until the reading is **stable**. If weight continuously increases, it is absorbing moisture, and the lowest weight should be used).*
4. Weigh the appropriate amount (typically 0.06 ± 0.01 g) of 44 samples into auto-analyzer vial caps. Make sure to add duplicates as shown in the spreadsheet. Do not touch the cap with your fingers, use tweezers so that the oil from your fingers does not change the weight of the cap. For standards (Eleanor Lake standard, ca. 40% BSi), use 40 mg. Note: scale is in units of grams, so 40 mg = 0.040 grams. Record core, **depth, weight**, and **sample number** (1-60) in the biogenic silica spreadsheet. It is handy to mark the cap of the vial with a dot for each time you take a sample from it so that you know which samples have been duplicated. This step should not be done more than a day or two in advance. When done in advance, weighed samples must be kept in a desiccator. Keep remainder of sediment in ½ dram vials with caps for storage.
5. Label 50-ml test tubes 1-60 (or however many samples are run). Tubes should be labeled on caps as well. Place samples in empty 50 ml test tubes, then grind gently with a glass stir rod to break up clumps. Wipe stir rod with a dry kim-wipe between samples. Note: be sure to not lose any sample on the stir rod.

Part 2. Extracting biogenic silica from bulk sediment

Sodium carbonate filled digestion tubes	Samples
Hot water bath	50 mL glass repipet

NOTE: This is a 6-hour procedure, with lots of free time available between tasks. Plan your day accordingly.

NOTE: Timing is very important. When loading samples or extracting aliquots you need to spend the same amount of time on each sample. Use a timer to keep track of elapsed time.

NOTE: If samples are high in carbonate, dissolve carbonates in 10% HCl, centrifuge at highest setting possible (5300 rpm) for 15 minutes, and fully decant liquid.

1. Turn on the water bath (85° C) and allow to warm up to 85°C. (about 1 hour).
2. Start timer (or note time on accurate clock). Add **exactly** 40.0 mL of 10% Na₂CO₃ solution using the 50 ml glass Repipet to the first 20 tubes. Work fairly quickly but accurately. (Be sure air intake is uncapped on the side of the glass repipet). Cap tubes and shakes vigorously (be sure to shake all the test tubes the same amount of time). Unscrew the caps on the test tubes just enough so air can escape during the dissolving process, and place tubes in the racks inside water bath at 85° C.
3. At 15 minutes, add 10% Na₂CO₃ solution to tubes 21-40. Repeat as in step 2. At 30 minutes, add 10% Na₂CO₃ solution to tubes 41-60. Etc. etc. etc.
4. After 100 minutes, screw on cap tightly and shake test tubes 1-20. Uncap loosely and place back in water bath. At 115 mins repeat this step for test tubes 21-40. At 130 mins repeat his step for test tubes 41-60, etc.
5. After 200 minutes, screw on cap tightly and shake test tubes 1-20. Uncap loosely and place back in water bath. At 215 mins repeat this step for test tubes 21-40. At 230 mins repeat his step for test tubes 41-60, etc.
6. At 300 minutes, remove samples 1-20 from bath, cap tightly. Centrifuge for 10 minutes at highest setting.
7. At 315 minutes, remove samples 21-40 from bath, cap tightly. Centrifuge for 10 minutes at highest setting.
8. While samples 21-40 are centrifuging, use a plastic syringe (we have 80+ of them) to remove ca. 20 mL from each 50 mL tube and add this sample to a 20 mL (labeled) scintillation vial. Work relatively quickly through the samples, because you have about 10 mins to do this step.
9. At 330 minutes, remove samples 41-60 from bath, cap tightly. Centrifuge for 10 minutes at highest setting.
10. Repeat removing 20 mL from each 50 mL tube after centrifuging until all are done.
11. Store 20 mL scintillation vials in refrigerator for long-term storage.

Part 3: Preparing samples for determination of BSi concentrations

30-mL reaction bottles (n=202).
Molybdate working solution in 4L plastic bottle
Reducing working solution in 2L plastic bottle
200 μ L pipetter
50 mL glass re-pipetter
10 mL pipetter (Oxford Benchmate)

NOTE: Check the solutions needed. Make new solution if needed (refer to end of the protocol).

NOTE: When using the microliter pipette, it is critical that the amount of sample you draw into the pipette and the amount expelled from the pipette is always exactly the same. Pay attention to the level of liquid in the tip, and start over if the amount of liquid doesn't look quite right or if bubbles are present. Problems commonly arise from improperly seating the tip on the pipette and from failing to depress the plunger completely, or depressing it too far.

1. Arrange 80 of the 30 mL reaction bottles in trays as done for 5 mL vials. Bottles should be labelled with sample number for the batch. Uncap bottles.
2. Dispense 17.5 mL of molybdate working solution in each reaction vessel (i.e., 30 ml plastic bottle). Use graduated cylinder or the 50 mL glass pipet.
3. At 30-second intervals, add 125 μ L of sample (or standard or blank) using the 200 μ L pipetter. Swirl reaction vessel to mix solution inside. Do not reuse pipet tips. If working alone, it is best to only do 40 samples at a time.
4. Cap vessel and allow reaction to continue for 20 minutes.
5. At 30-second intervals, add 7.5 ml of reducing working solution to reaction vessel using the green "pipette pump". Cap and mix the vessel (note: do not let the solution touch cap).
6. Allow reaction to continue overnight (over 12 hours).

Note: Part 3 may be completed directly following Part 2, or on a later date.

Part 4: Measuring absorbance with a spectrophotometer

NOTE: Cuvettes are delicate and fairly expensive. Handle gently and do not insert anything but kim-wipes and cut sponges within the cuvette to clean it. Use only lens cleaning paper or fine cloth to wipe the optical surfaces, most paper products contain wood fibers which may scratch or damage the cell face or surface.

19. Turn on the power switch of the spectrophotometer (GENESYS 8). Allow to warm up for at least 5 minutes. Display should read "Abs" and 812 λ .
20. Using glass cuvettes (hold only on frosted sides), fill one with ddH₂O and cap.
21. Place in spectrophotometer. Press "Zero"
22. Fill four remaining glass cuvettes using glass pipettes with bulb. Cap, take readings, and enter in notebook. Rinse pipettes as needed (we have ~40 of them). Note: Some days the spectrophotometer is more finicky than others. To make sure that the zero has not drifted between sample readings, always check the zero (cuvette with ddH₂O) between each sample reading.
23. Empty cuvettes into beaker, and when done pour into labeled waste container.
24. Rinse cuvette using squirt bottle of ddH₂O, be sure exterior is clean. Dry inside of cuvette gently with compressed air. Fill next four samples.

- *Input data into Excel spreadsheet and plot the data. If you have multiple batches, adjust the sample values according to standards.*
- *Clean up utilities used (tubes, racks, bottles, cuvettes, graduated cylinders, repipets etc.)*
- *Check all the chemicals and refill or order new items if necessary.*
- *After spectrophotometer readings are entered and backed-up, discard reagents (working solutions) into a labeled container (see below for labeling). Have EHS take away. Clean out reaction bottles. Save the 20 mL extractions in a refrigerator until several batches from same are run and the data appears robust.*

Preparing stock solutions

Measure large volumes of water using tall graduated cylinder. Read off of the bottom of the meniscus. Wear safety glasses, gloves, lab coat, closed-toe footwear.

1. Molybdate reagent

- Dissolve 16.0 g of ammonium paramolybdate – $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$ in 1000 mL of ddH₂O.
- Store solution in a tightly capped polyethylene bottle out of direct sunlight. The solution is stable indefinitely, but should be discarded if it forms a white precipitate or turns faintly blue.

2. Metol-sulfite reagent

- 6 g of Na₂SO₃ (sodium sulfite) in 500 mL of ddH₂O.
- Add 10 g of metol (4-(Methylamino) phenol sulfite)
- Dissolve everything
- Filtering through no.1 whatman filter paper
- Store in an amber-glass stoppered bottle
- Stable for 1 – 2months

3. Oxalic acid reagent

- Dissolve 60 g of oxalic acid dihydrate – $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ in 1000 ml of ddH₂O
- Store in polyethylene. The solution is stable indefinitely

4. Sulfuric acid reagent

Work in the fume hood.

- Slowly add 300 ml of concentrated sulfuric acid into 770 ml of ddH₂O. Work in the fume hood.
- Cool to room temperature and store in polyethylene

5. Hydrochloric acid (for Molybdate Working solution)

Work in the fume hood.

- Add 100 mL of concentrated (12N) HCl to 1100 mL of ddH₂O → 1200 ml 1N HCl

Mixing 10% sodium carbonate solution : See last page

Preparing working solutions from stock solutions

Estimate the amount of solution needed.

Molybdate working solution: 17.5 mL/sample. So, 3500 mL for 200 samples.

Reducing working solution: 7.5 mL/sample (so, 1500 mL for 200 samples).

1. Molybdate Working Solution

Mix in this ratio (Add in order listed)

1 ml Molybdate Stock Solution
5 ml ddH₂O
1 ml HCl (HCl stock) = 1 : 5 : 1

Calculate amount needed for your batch. For example:

To make 3500 mL of molybdate working solution in a 4 L plastic bottle:

- Add 500 mL Molybdate Stock Solution
- Add 2500 mL of ddH₂O (measure in 1 L volumetric flask and 250 mL graduated cylinder).
- Add 500 mL of HCl stock solution.

To use solution, pour in increments into small plastic bottle (use a funnel) in the fume hood. Use pipetter out of small bottle.

2. Reducing Working Solution

Mix in this ratio (Add in order listed)

1 ml Metol-Sulfate Stock Reagent
1 ml Oxalic Acid
1 ml Sulfuric Acid Stock

Calculate amount needed for your batch. For example:

To make 1500 mL of reducing working solution in a 2 L plastic bottle:

- add 500 mL Metol-Sulfate Stock Reagent
- add 500 mL Oxalic Acid
- add 500 mL Sulfuric Acid Stock

3. 10% sodium carbonate solution (10% weight/volume).

- Mix cold...it may take an hour or more to dissolve.
 - Divide total volume desired by 9. This is the weight in grams of Na₂CO₃ crystals to use.
 - To make 4 liters: 4000/9=444.4 grams.
 - Clean carboy by rinsing with ddH₂O.
 - Add 4 L of ddH₂O
 - Add 444.4 grams of Na₂CO₃ crystals. Mix until dissolved.
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- Or, order Sodium carbonate solution 10% W/V (weight by volume). Fisher Catalog 7160-32. Order in 4L volumes via Chem Stores.

Materials for Biogenic Silica

- 30 mL bottles (we have a large supply, please re-use. Fisher 03-700-30.)
- 20 ml scintillation vials.
- ½ dram glass shell vials. Fisher 03-339-26A
- Plastic vial cartons (holds 100 ½ dram vials). USA Scientific.

Dry chemicals:

- 4-(methylamino) phenol hemisulfate salt 99% ACS reagent (100 g quantities is sufficient)
- Ammonium molybdate (para) tetrahydrate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (99%)
(250 g quantities is sufficient, order from www.alfa.com, item A13766)
Note that Ammonium molybdate (para) hydrate puratronic $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$ was used in recent past (2008-2010). This is much more expensive (highly pure, >99.99%) and has a different molecular weight. In 2011, switching to tetrahydrate form (less pure, as well).
- Sodium sulfite anhydrous granular (500 g quantities is sufficient)
- Oxalic acid $(\text{H}_2\text{C}_2\text{O}_2 \cdot 2\text{H}_2\text{O})$ crystals (500 g quantities is sufficient)

Waste disposal

Store Na_2CO_3 extracts in refrigerator until project is done (and published).

Pour extra Na_2CO_3 down drain with abundant tap water on if $\text{pH} < 10$. Otherwise pour into waste container.

Reaction vessel chemicals should be poured into large waste bottles. (Large empty bottles can be found under the sink in PAC on the left hand side. Be sure to label what waste is being placed into the container. Obtain empty waste bottles from environmental health and safety services if needed).

Hazardous waste tag:

Ammonium paramolybdate: 0.2%

Hydrochloric acid: 1%

Sodium sulfite: 0.1%

Oxalic acid: 0.6%

Sulfuric acid: 2.8%

metol (4-(Methylamino) phenol sulfate: 0.3%

Water: 95%

Check **corrosive acid**
