

BIOGENIC SILICA ANALYSIS

Safety Precautions

1. When preparing reagents, take care to avoid spills, skin contact, eye contact, inhalation, or ingestion.
2. You will be working with strong acids and bases. Use protective gear, such as safety goggles, gloves, etc. When handling strong acids, work in the fume hood.
3. During sample digestions, the hot water bath will be hot and will contain hot liquid. The digestion tubes will contain hot NaOH. Take appropriate safety measures.
4. Label 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.

Purpose

To determine the percent biogenic silica in sediments.

Part 1: Sample Preparations

1. Sample cores at a volume that will produce at least .03 grams of DRY sediment. This is the required amount for 1(one) sample. If replicates are needed, sample the core in multiples of .03 grams.
2. Freeze the sediment samples, and place them into the lyophilizer (freeze-dryer) for at least 24 hours, until completely dry.
3. Grind the samples to a fine powder with a mortar & pestle. You are now ready to do the analyses:

PART 2: Preparing Reagents

SODIUM HYDROXIDE SOLUTION

1000 ml Nalgene volumetric flask
Milli Q water
20 grams Sodium Hydroxide

Fill approximately 500 ml Milli Q water in a 1000-ml volumetric flask. Add and dissolve 20 grams sodium hydroxide. Add enough Milli Q water to bring to a volume of 1000 ml.

MOLYBDATE SOLUTION

500 ml Nalgene volumetric flask
Milli Q water
4 grams Ammonium Paramolybdate
12 ml 12N. HCL

Fill approximately 300 ml Milli Q water in a 500 Nalgene volumetric flask. Add and dissolve 4 grams Ammonium Paramolybdate. Work under the fume hood, add 12 ml 12N. HCL. Mix and add enough Milli Q water to bring to a volume of 500ml. Store in a dark plastic bottle.

METOL-SULFITE SOLUTION

500 ml Nalgene volumetric flask
Milli Q water
6 grams Anhydrous Sodium Sulfite
10 grams Metol (P-methylaminophenol Sulfate)
No. 1 Watman filter paper

Fill approximately 300 ml Milli Q water in a 500-ml Nalgene volumetric flask. Add and dissolve 6 grams Anhydrous Sodium Sulfite. Add and dissolve 10 grams Metol (P-methylaminophenol Sulfate). Add enough Milli Q water to bring volume to 500 ml. Filter solution through a No. 1 Watman filter paper and store in a clean, dark glass bottle. Record the date on the bottle. This solution deteriorates rapidly and should be prepared fresh at least every month.

OXALIC ACID SOLUTION

1000 ml Nalgene volumetric flask
500 ml Milli Q water
50 grams analytical grade reagent oxalic acid dehydrate

Measure 500 ml Milli Q water and pour it into a 1000-ml Nalgene volumetric flask. Add and dissolve 50 grams analytical grade reagent oxalic acid dehydrate. Let stand overnight. Decant the solution from the crystals. This solution may be stored in a glass bottle and is stable indefinitely.

50%V/V SULFURIC ACID FROM CONCENTRATED SULFURIC ACID To be prepared under the fume hood

500 ml Nalgene volumetric flask
250 mls of concentrated analytical reagent quality sulfuric acid
Milli Q

Fill approximately 200 mls of Milli Q water in a 500 ml Nalgene volumetric flask. Add 250 mls of concentrated analytical reagent quality sulfuric acid. Cool to room temperature and bring volume to 500 mls with Milli Q water. Store in polyethylene bottle. Alternatively, order 50% V/V Sulfuric Acid directly from Fisher or Baxter.

REDUCING SOLUTION

To be prepared and dispensed under the fume hood
For a batch size of 4 samples

250 ml wide-mouth Nalgene reagent polyethylene bottle
50 ml Metol Sulfate solution
30 ml Oxalic Acid solution
30 ml 50% Sulfuric Acid solution
40 ml Mili Q water (make to a volume of 150 ml)

For 12 samples

500 ml Nalgene volumetric flask
100 ml Metol Sulfate solution
60 ml Oxalic Acid solution
60 ml 50% Sulfuric Acid solution
80ml Milli Q water (make to a volume of 300 ml)

Using the Eppendorf Repeater pipette (a separate labeled combitip for each reagent), measure and add to bottle, Metol Sulfate solution. Add Oxalic Acid solution and mix. Add slowly with mixing Sulfuric Acid solution. Add Milli Q water and mix. This should be made daily just before using.

For 24 samples

1 Liter Nalgene volumetric flask
200 ml Metol Sulfate solution
120 ml Oxalic Acid solution
120 ml 50% Sulfuric Acid solution
160 ml Milli Q water (make to a volume of 600 ml)

Use Nalgene volumetric flasks to accurately measure the reagents. Measure Metol solution and add to the 1L volumetric flask. Add Oxalic Acid and mix. Add slowly with mixing the Sulfuric Acid. Add Milli Q water and mix. Reducing Solution should be made just before using.

Part 3: STANDARD PREPARATION

Standards are run each time that samples are analyzed in order to set up a calibration curve.

Place a small quantity of Sodium Fluosilicate (Na_2SiF_6) in an open plastic vial and place in desiccator overnight to remove excess water. Do not heat or fuse.

Dissolve 0.5642 g of the Sodium fluosilicate in a one-liter Nalgene volumetric flask. Dissolution is slow so allow at least 30 minutes. This cannot be rushed. Use deionized silica-free water (Milli Q water).

The concentration of this standard is 3000 microgram-atoms/L.

Store in a 500-ml polyethylene bottle. The standard is stable indefinitely.

Dilutions from primary standard - When making dilutions use silica-free water and store in polyethylene bottles.

Using a 50-ml volumetric flask, begin with the following amounts of primary standard. Bring each to 50mls total volume.

| mls of primary standard | Concentration microgram-at/L |
|-------------------------|------------------------------|
| .5 | 30 |
| 1 | 60 |
| 2 | 120 |
| 4 | 240 |
| 6 | 360 |
| 8 | 480 |
| 10 | 600 |
| 15 | 900 |
| 20 | 1200 |
| 40 | 2400 |

Perform analysis procedure as in Part 5. Generate a concentration (y) vs absorption (x) graph. Perform a polynomial line fit to the second power to arrive at an equation. For example, $y=18.123+724.12x+105.90x^2$. To calculate % Biogenic Silica use the equation: % Silica = $((Y*\text{dilution factor}*\text{NaOH volume in L}*60.1)/1000)/\text{wt.mg})*100$

Plug in the standards equation for the value of Y into the % Silica equation. X = absorption values for your individual samples. In an excel spreadsheet to calculate " % Biogenic Silica", enter the equation. E.g.: $((((18.123+724.12*\text{Abs}+105.90*(\text{Abs}*\text{Abs}))*\text{dilution factor} *\text{NaOH volume in L}*60.1)/1000)/\text{Wt mg})*100$

Standards are to be run each day that analyses are done. Use the equation generated to calculate the % Biogenic Silica.

Part 4: SAMPLE DIGESTIONS

IMPORTANT: Set up must be completed before doing digestions. The amount of samples that can be run at one time is limited by the space in the hot water bath. At this time, we can run 24 samples per batch.

SET UP FOR SMALL SAMPLE SIZE:

For each sample:

1. Measure 40 ml NaOH solution into a 50-ml centrifuge tube, cap, and place in the rack in the hot water bath. Each tube cap should be assigned and labeled with a number. (1 to 24)
2. Fill the hot water bath to just below the neck of the tubes.
1. Turn on bath to heat to 85° C (This takes approximately 2 hours).
4. Weigh approximately 0.03 grams of sample into a 1-dram shell vial cap or similar container and cover with a clean, white scintillation bottle cap or similar device.
5. Note the core name, depth, and weight in the notebook.
6. Set up 7 bottles for each sample, each labeled for the time of the aliquot extraction. Extractions are to be taken at 5, 15, 30, 60, 90, 120, and 200 minutes. Label each bottle with the sample number and aliquot extraction time.
7. Using the Eppendorf Repeater pipette (combitip) measure into each bottle 4-ml Milli Q water. Cap to prevent evaporation.
8. Choose a beginning time for samples 1 to 12 and a beginning time 20 minutes later for samples 13 to 24. Calculate the time for each aliquot extraction.

DIGESTIONS:

1. Place the caps of sediment into the appropriate NaOH digestion tubes ahead of time. Place them gently on top of the solution so that they float. Recap each tube. Ensure that NaOH does not contact the sample before the appropriate time.
2. Begin the digestion process by shaking each digestion bottle at exactly the pre-planned time.
3. Timing is very important. At exactly the assigned aliquot extraction time, extract a 1-ml aliquot from each digestion bottle and add it to the corresponding bottle of 4 ml Di Si free water.
4. After extracting aliquots for each extraction time shake and replace each digestion bottle as quickly as possible.
5. Shake each sample tube to homogenize its contents.

SET UP FOR LARGE SAMPLE SIZE:

For each sample:

1. Measure 200 ml NaOH solution into a 250-ml nalgene bottle, cap, and place in the rack in the hot water bath. Each tube cap should be assigned and labeled with a number. (1 to 12)
2. Fill the hot water bath to just below the neck of the tubes.
3. Turn on bath to heat to 85° C (This takes approximately 2 hours).
4. Weigh approximately 0.3 grams of sample.
5. Note the core name, depth, and weight in the notebook.
6. Set up 7 bottles for each sample, each labeled for the time of the aliquot extraction. Extractions are to be taken at 5, 15, 30, 60, 90, 120, and 200 minutes. Label each bottle with the sample number and aliquot extraction time.
7. Using the Eppendorf Repeater pipette (combitip), measure into each bottle, 9-ml Di Si free water. Cap to prevent evaporation.
8. Choose a beginning time for samples 1 to 12 and a beginning time 20 minutes later for samples 13 to 24. Calculate the time for each aliquot extraction.

DIGESTIONS:

1. Place the caps of sediment into the appropriate NaOH digestion tubes ahead of time. Place them gently on top of the solution so that they float. Recap each tube. Ensure that NaOH does not contact the sample before the appropriate time.
2. Begin the digestion process by shaking each digestion bottle at exactly the pre-planned time.
3. Timing is very important. At exactly the assigned aliquot extraction time, extract a 1-ml aliquot from each digestion bottle and add it to the corresponding bottle of 9 ml Di Si free water.
4. After extracting aliquots for each extraction time shake and replace each digestion bottle as quickly as possible.
5. Shake each sample tube to homogenize its contents.

Part 5: ANALYSIS PROCEDURES- Step by step

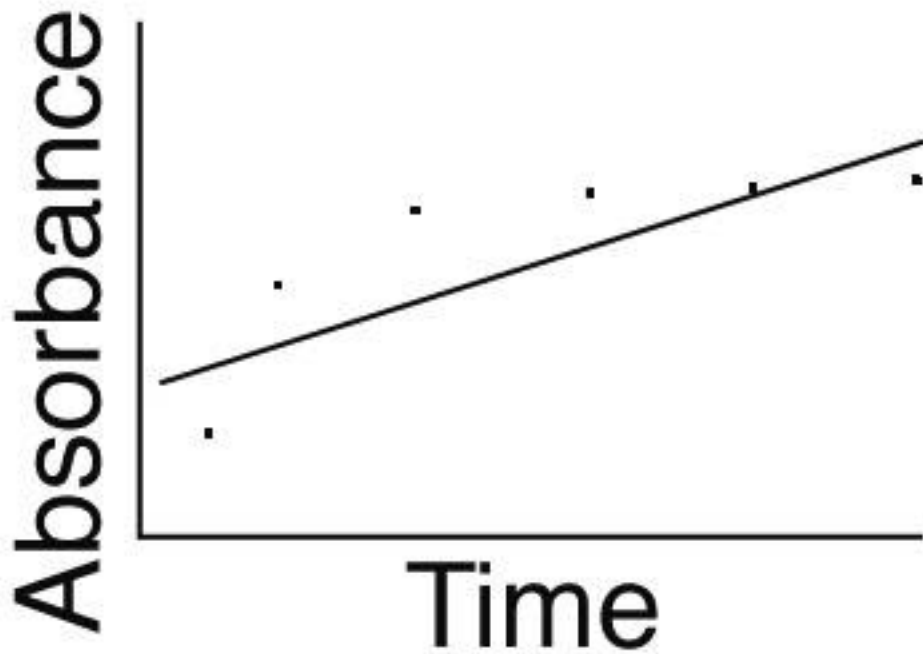
1. Have all reagents prepared ahead of time. You will need Milli Q water, Molybdate solution and reducing solution. Prepare reducing solution fresh on the day of analysis.
2. Label and set up 3-dram plastic vials and caps - one for each of your bottles above and one for each primary standard concentration.
3. Pipette 2.0 mls of Molybdate solution, using an Eppendorf repeater pipette, into each of the plastic vials.
4. Pipette 0.5 ml of sample into respective vials with Eppendorf microliter pipette. Take note of the order in which the sampling was done and record the time.
5. Pipette 4.0 ml of silica free distilled water (Milli Q water) into each vial during the 15-minute waiting period begun upon pipetting the last of the samples in step 4. (Use the Eppendorf Repeater pipette)
6. After 15 minutes, (time required for yellow color to fully develop) add 3 mls of reducing solution to the vials in the same order in which the sampling was done. NOTE TIME.
7. Cap vials to reduce evaporation, shake lightly, and wait 3 hours for blue color to develop.
8. Measure absorbance at a wavelength of 812.

Working with your data:

Working with Your Graphs

NORMALIZATION:

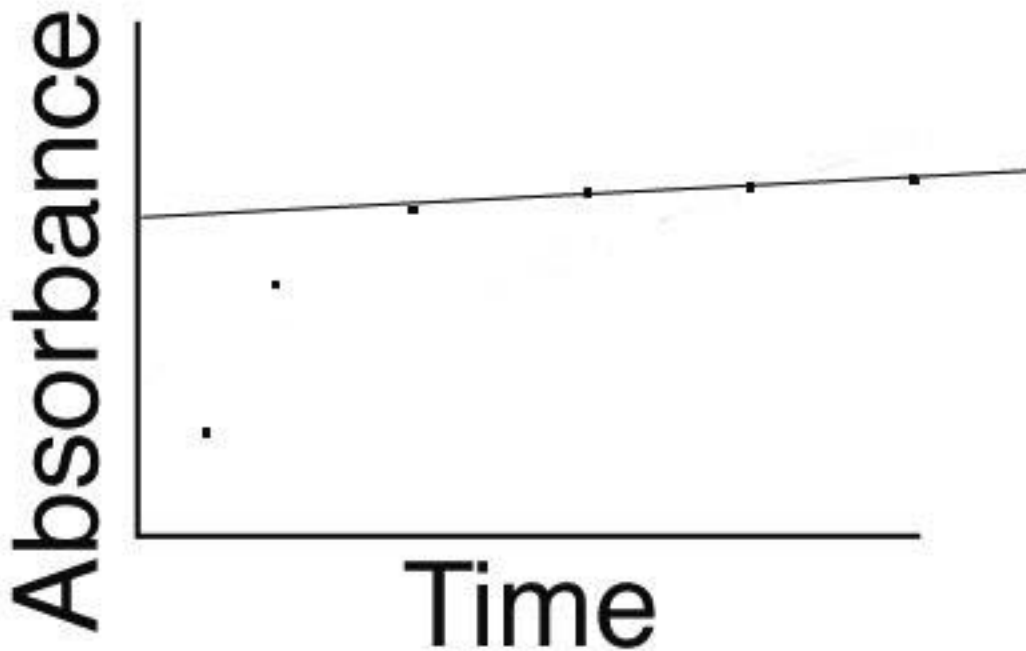
Create a scatter graph of sample absorption vs. time. In excel, estimate and draw a best-fit line. Your graph should look something like this:



The initial steep slope is due to the actual biogenic silica. The shallower slope represents background silica digested from the clay materials in the sediments.

In order to estimate the absorption value due to biogenic silica; run the regression through only the points that define the shallowly sloping line at the end of the sample digestion. Get the y-intercept of this regression from the regression equation in excel, which is the actual absorption value that was added to the normal background of clays by the biogenic opal.

In other words, the graph without biogenic silica would look like this:



The Y-intercept is the Absorbance value with which to calculate %BSi, as it is the value corrected for Si contributions from inorganic silicates.

THE MATH

Take the normalized value you just arrived at, and put it in place of the variable x in your standard curve's equation. You will arrive at some number, y , which is the micro-moles per liter or micro-gram.atm/L

Multiply this number by the dilution factor (10 for aliquots diluted to 10 ml and 5 for aliquots diluted to 5 ml) to account for dilution from the original concentration in the digestion bottles. Multiply the subsequent number by 0.2 for samples digested in 200 ml of NaOH or 0.04 for samples digested in 40 ml NaOH) to arrive at the total micro moles of SiO₂ in your original sample.

Multiply the total micro moles of SiO₂ by 60.1 (the formula weight of SiO₂) and this will give you micro grams of SiO₂ in your original sample. Divide micro grams of SiO₂ by 1000 to arrive at your total mg of SiO₂, and divide that by the weight of dry sediment in mg (DW), used in the

digestion. Finally, multiply that number by 100 to arrive at percent biogenic SiO₂.

% BSi =

$$\left(\frac{Y \cdot \text{dilution factor} \cdot \text{NaOH volume in L} \cdot 60.1}{1000}\right) / \text{wt.mg} \cdot 100$$

NOTES:

Use nalgene products unless otherwise noted. Use of glass in many cases is sure to contaminate the samples.

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

Use silica free water for all mixtures (Water from a Milli Q system).

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

If your particular spectrophotometer cannot be set at 812 (near infrared), a wavelength of 725 nanometers has been suggested. A small reduction of sensitivity should be expected however.

All tubes and utensils must be cleaned, given a final rinse in silica free water, and dried before use. Tubes and vials may be dried in the drying oven. **Measuring utensils are not to be dried in the drying oven.**

