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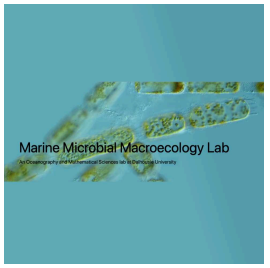
Measurement of biogenic silica from plankton V.3



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We use this protocol and it's working

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Keywords: biogenic silica, dissolution, silicomolybdic acid, measurement of biogenic silica, measuring biogenic silica, biogenic silica from plankton, biogenic silica, amorphous silica, molybdate measurement technique, plankton, diatomaceous earth, sodium carbonate, accuracy of the measurement, production of monosilicic acid

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Abstract

Here, we present a method for measuring biogenic silica from plankton. Biogenic silica is digested using a wet-alkaline method, in which 2 M sodium carbonate is used to hydrate and depolymerize amorphous silica, resulting in the production of monosilicic acid. The molybdate measurement technique is based on the method described by Shemesh et al. (1988) and follows the JGOFS protocols outlined by UNESCO (1994).
To ensure the accuracy of the measurement, Celite S diatomaceous earth is used as a check standard for the recovery of biogenic silica. Our method yields a recovery rate of 85% to 90%.

Citation

Shemesh, Aldo; Mortlock, Richard A; Smith, R J; Froelich, Philip N (1988)
. Determination of Ge/Si in marine siliceous microfossils: separation, cleaning and dissolution of diatoms and radiolaria.
Marine Chemistry.

https://doi.org/10.1016/0304-4203(88)90113-2 LINK

Protocol materials

- Ammonium molybdate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**09878-100G
- Oxalic acid dihydrate **VWR International (Avantor)** **Catalog #**BDH4556-500G
- Sodium hexafluorosilicate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**250171
- Sodium sulfite **Fisher Scientific** **Catalog #**S430-500
- 4-(methylamino)phenol hemisulfate salt **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**320013
- Methyl orange **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**1013230250
- Celite S diatomaceous earth **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**06858

Troubleshooting

Sample collection

1 Estimation:
The low limit of detection is approximately 0.6 uM silicate in the molybdate method. For siliceous plankton, sample requires no less than 4 ug PON (particulate organic nitrogen) per filter when using a 50 mL volumetric flask, or 2 ug PON per filter when using a 25 mL volumetric flask. The sampling volume for biogenic silica samples is approximately 10% of the PON sample volume. For seawater samples, the sampling volume for biogenic silica samples should be determined based on the community composition.

2 Filter blank media (without cells, same volume as plankton samples) through polycarbonate filter as blank

3 Transfer filter into 2 mL cryogenic vial

4 Flash freeze and store at -20 °C

5 Filter plankton sample in liquid media onto polycarbonate filters, using gentle vacuum pressure (130 mmHg)

Equipment

Filter forceps	NAME
blunt end, stainless steel	TYPE
Millipore	BRAND
XX6200006P	SKU

6 Rinse filter funnel with filtered artificial seawater without macronutrients

7 Fold the filter with two tweezers:
(1) Fold in half along its diameter, creating a semicircular shape;
(2) Fold once more in the same direction, resulting in a long strip;

(3) Fold once more, halving its length, so that sample is secured.

8 Transfer filter with sample into 2 mL cryogenic vial

9 Flash freeze and store at -20 °C

10 Transfer sample to 50 mL falcon tube with clean filter forceps (rinsed by 95% ethanol and air-dried), dry at 90 °C in the airforce oven. Prior to transferring:

(1) For filters folded into half-strip, unfold once to return to a strip.
(2) For filters folded into quarter-circles, unfold once to return to a half-circle shape, then fold once along the dimension to form a strip.
(2) For filters haphazardly into a compact mass, carefully unfold with two tweezers (avoiding losing biomass), fold once into a half-cricle shape, then fold once more along the dimension to form a strip

Equipment	
Forced air oven	NAME
VWR	BRAND
89511-410	SKU

Equipment

Falcon® Centrifuge Tubes	NAME
Polypropylene, Sterile, 50 mL	TYPE
Corning®	BRAND
352070	SKU

Standard primary solution and reagents

11 Molybdate reagent stock solution

Note

Require 100 uL per sample

11.1 Ammonium molybdate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**09878-100G

Ammonium paramolybdate:
[(NH4)6Mo7O24.4H2O]
CAS: 12054-85-2

11.2 Add 1.6 g ammonium paramolybdate into a 125 mL plastic bottle and top to 100 g with MilliQ.

11.3 Store out of direct sunlight. Discard if white precipitation forms.

12 HCl stock solution

Note

Require 100 uL per sample

12.1 Use graduated cylinder, measure 95 mL MilliQ and transfer into a 125 mL plastic bottle.

12.2 In the fume hood, add 5 mL 12 N HCl into the bottle, mix well.

13 Metol-sulfite solution

Note

Require 100 uL per sample

13.1 4-(methylamino)phenol hemisulfate salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**320013

Sodium sulfite **Fisher Scientific Catalog #**S430-500

13.2 Require:
(1) 50 mL syringe
(2) Syringe filter

Equipment

Syringe filter	NAME
0.2 um PES	TYPE
VWR	BRAND
28145-501	SKU

13.3 In a 100 to 250 mL plastic beaker, add 0.6 g sodium sulphite.

13.4 Add 1 g 4-(methyl amino)phenol hemisulfate.

13.5 Top to 50 g with MilliQ water.

13.6 Fill syringe with Metol-sulfite solution, filter through the syringe filter, collect filtrate into four 15 mL falcon tubes wrapped with foil, keep at room temperature.

13.7 Prepare fresh every month.

14 Oxalic acid solution

Note

Require 100 uL per sample.

14.1 Oxalic acid dihydrate **VWR International (Avantor) Catalog #**BDH4556-500G

14.2 In a 125 mL plastic bottle, add 6 g oxalic acid and top to 100 g.

14.3 Let the solution stand at room temperature overnight.

14.4 Decant the solution from the crystals into a plastic bottle.

14.5 Keep at room temperature.

15 Sulphuric acid (30%)

15.1

Note

Require 100 uL per sample

15.2 Mix 3 part concentrated sulphuric acid into 7 part of MilliQ
Cool down to room temperature

Note

This can be prepared on Day 2 prior to molybdate reaction

16 Primary silica standard solution (~ 1 mM Si)

16.1 Sodium hexafluorosilicate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**250171

16.2 Transfer 1 g sodium fluorosilicate in a plastic vial

16.3 Keep the vial in a vacuum desiccator overnight to remove excess water (do not heat or fuse)

16.4 In a one litre plastic volumetric flask, dissolve ~ 0.1881 g (log the actual mass) of dry sodium fluorosilicate in MilliQ water and top to 1 L with MilliQ water.

16.5 It takes about 30 min to complete the dissolution. This cannot be rushed.

16.6 Store in a plastic bottle at room temperature.

Day 1: Dissolution

17 [M] 2 Mass Percent Na_2CO_3 (18.69%)

Note

Need to be freshly prepared.
The old reagent can yield high blank possibly by leaching silicate from plastic material.

17.1 Each sample requires 10 mL 2 M Na_2CO_3

17.2 Weigh 186.9 g Na_2CO_3 in a weighing dish.
(CAS: 497-19-8, FW 105.99)

17.3 Tare a 1 L plastic erlenmeyer flask

17.4 Transfer Na_2CO_3 into the flask

17.5 Top to 1000 g with MilliQ and shake until all salt is completely dissolved.

17.6 Aliquot the solution into four 250 mL plastic bottles.

18 Turn on airforce oven to 85 °C

19 In the fume hood, transfer diatomaceous into a 5 mL plastic tube for weighing convenience (the original package is 1 kg).

Note

1. Diatomaceous is used as a check standard for the recovery of biogenic silica
2. Diatomaceous is hygroscopic, it needs to be stored in the vacuum desiccator

Safety information

Diatomaceous:
Upper respiratory irritant. May cause coughing or throat irritation. Breathing dust containing crystalline silica over a long period may cause lung damage.

Celite S diatomaceous earth **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**06858

19.1 Weigh 100~200 ug diatomaceous into 50 mL falcon tube, in triplicate. Log the actual weight.

Safety information

Do not open the container until the static charge of diatomaceous powder has been neutralized by ionization blower.

Note

Less than 100 ug sample might introduce more error amongst the replicates in recovery.

19.2 Prepare one empty 50 mL falcon tube as the reagent blank for diatomaceous.

20 Add 10 mL 2 M Na_2CO_3 to each tube, including:

- reagent blank for check standards
- check standards
- blank for samples
- samples

21 Vortex

22 Loose the caps and place all tubes into the airforce oven overnight (for example, from 5 pm to 9 am). 16h

Day 2: Acidification

23 Volume of 12 N HCl required:
about 3.5 mL X N

24 Transfer 12 N HCl into a 50 mL Falcon tube in the fume hood.

25 Work on one tube at a time, and leave other tubes in the oven.

26 In the fume hood, add 30 µL Methyl orange into the tube.

Methyl orange **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**1013230250

27 Add MilliQ until the volume of solution in the falcon tube is 10 mL.

Note

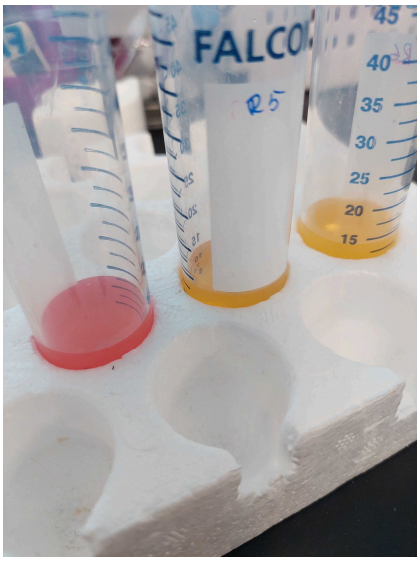
The original volume of Na2CO3 is reduced due to evaporation of water during 20-h dissolution.

28 Dropwise add 3 mL 12 N HCl by using 1000 uL pipet.

Safety information

Do it slowly. Swirl the tube until reaction stops and then add the next drop. The most vigorous reaction is at about 3 mL 12 N HCl.

29 Switch to a 100 uL pipette, add 100 uL at a time. Near the equivalence point, when the colour starts to change to pink more markedly but after mixing the orange colour returns, it is necessary to add HCl **drop by drop**. The first drop that causes a permanent colour change to pink determines the equivalence point. Stop adding HCl. Cap the tube, hold tube horizontally, gently invert the tube to wash residue at the inner side of the cap down to the solution. The color may change back to orange, add more drops of HCl until the color turns to permanent pink again (See the color of the left tube).



Note

We have found that the optimal pH for the reaction between silicate and molybdate to form silicomolybdic acid is 3 to 4. Too low or too high pH decreases recovery of biogenic silica.
The acidified solution yields pH at 2 to 3. It is diluted to 10% in the molybdate assay, which gives pH at 3 to 4.

30 Transfer resulted solution from falcon tube to 25 or 50 mL polypropylene volumetric flask.

Note

Be careful while transferring the solution and ensure that the filter does not fall out of the tube, which spills the solution and causes sample loss.

31 Use MilliQ to rinse the tube ***three times*** and transfer all samples into the volumetric flask.

Note

If a 50 mL volumetric flask is used, rinse the falcon tube with 5 mL of MilliQ at a time.
If a 25 mL volumetric flask is used, rinse the falcon tube with 1 mL of MilliQ at a time.

32 Use transfer pipet, top final volume to 25 or 50 mL with Milli-Q.

33 Shake and thoroughly mix the solution.

> Note
>
> Before mixing, check the cap to avoid leaking

34 Transfer solution from volumetric flask to a clean and labelled Falcon tube.

Day 2: Molybdate reaction

3h

35 Secondary standard solution (Freshly prepared prior to the assay)

50 uL primary stock solution
450 uL MilliQ

36 Standard working solutions (Freshly prepared prior to the assay)

	Standards	Secondary (uL)	MilliQ (uL)	Conc. (uM)
	S1	0	500	0
	S2	5	495	1
	S3	10	490	2
	S4	20	480	4
	S5	40	460	8
	S6	60	440	12
	S7	80	420	16
	S8	100	400	20

37 Vortex and then transfer 50 µL from (1) blank for check standards, (2) check standards, (3) blank for samples, and (4) samples into labelled 2 mL microtubes.

38 Add 450 µL MilliQ into each tube to obtain a 10% dilution.

39 Molybdate working solution

Note

Require 200 uL per sample

39.1 **1 part** Molybdate stock reagent
1 part HCl stock reagent

40

Safety information

The addition of reagent must be operated in the fume hood. Acidified sodium fluorosilicate may contain some hydrofluoric acid.

41 Add 200 µL Molybdate reagent into each tube.

42 Vortex each tube and then shake at Room temperature for 00:15:00 for the formation of silicomolybdic acid. 15m

43 Reducing solution

Note

Require 300 uL per sample

43.1 **1 part** Metol-sulfite solution
1 part oxalic acid solution
1 part sulphuric acid solution

44 Add 300 µL reducing solution into each tube.

45 Vortex each tube and then shake at Room temperature for 03:00:00 3h

46 ....Measure pH of each sample (in the Falcon tube)

	Sample code (example)	Sample code	pH
	Blank for check standards		
	Check standard 1		
	Check standard 2		
	Check standard 3		
	Blank for samples		
	Sample 1		
	Sample 2		
	...		

Day 2: Colorimetric measurement

3h

47 In the fume hood, vortex each tube and then load 250 µL of the sample into one well of the microplate. Vortex again and load the same sample into another well of the microplate as replicate.

Equipment

96-Well Microplates, Polystyrene, Clear,	NAME
Greiner Bio-One	BRAND
655101	SKU

48 Setup the layout.

49 Setup the program

	A	B
	Shake duration	00:00:05
	Shaking type	Continuous

	A	B
	Shaking force	High
	Shaking speed [rpm]	600
	Wavelength [nm]	812
	Use transmittance	No
	Pathlength correction	No
	Measurement Time [ms]	100

Equipment

Varioskan LUX Multimode Microplate Reader NAME

Thermo Fisher BRAND

VL0L00D0 SKU

50 Read the samples.

51 Export data sheet to excel.

Waste disposal 3h

52 Collect all solution with paramethylaminophenol sulphate and sodium fluorosilicate into the waste container.

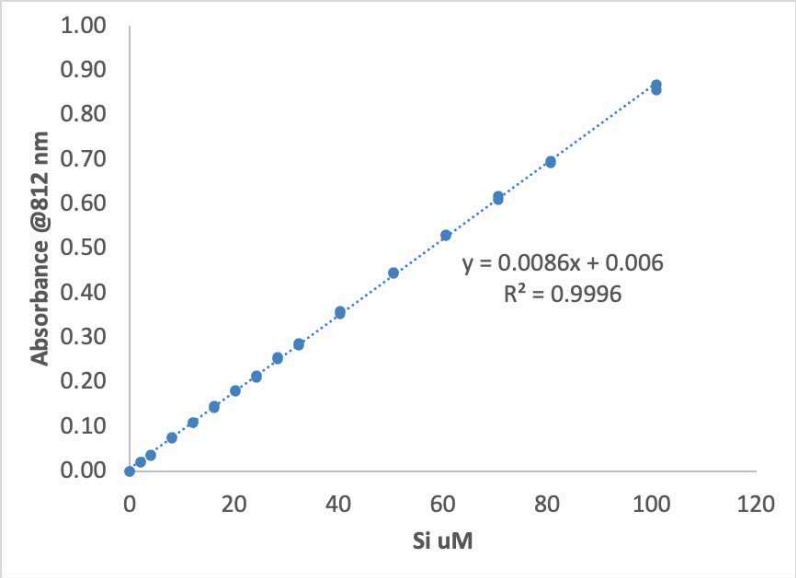
53 Rinse microtubes and microplate with tap water, dispose in blue recycling bin.

Day 2: Calculation 3h

54 Subtract the average absorbance at 812 nm of the blank standard replicates from the absorbance at 812 nm of all other standard working solutions.

55 Subtract the average absorbance at 812 nm of the blank sample (i.e. blank filter) replicates from the absorbance at 812 nm of all other individual samples.

56 Prepare a standard curve by plotting the average blank-corrected 812 nm absorbance for each standard working solution versus its concentration in uM.



57 Use the standard curve to determine the silicate concentration of each unknown sample by using its blank-corrected 812 nm absorbance.

58 Si per sample = Si X V X (0.001) X DF

	Variable	Unit	Definition
	Si per sample	umol	element Si in the sample collected
	Si	uM	silicate concentration calculated from the standard curve
	V	mL	volume of volumetric flask
	DF		From volumetric flask to the microtube, DF=10

59 % Diatomaceous recovery = 100 X Si X V X (0.001) X MW X DF /(M_ug X Purity X 0.01)

	Variable	Unit	Definition
	% Diatomaceous recovery		percentage recovery of diatomaceous
	Si	uM	silicate concentration calculated from the standard curve
	V	mL	volume of volumetric flask
	MW	ug/umol	molecular mass of SiO2, i.e. 60.08
	DF		From volumetric flask to the microtube, DF=10
	M	ug	actual mass of diatomaceous
	Purity		purity of SiO2 in Celite S diatomaceous earth (06858) is 90.2%

Note

The recovery should be around 85 to 90%.

Citations

Shemesh, Aldo; Mortlock, Richard A; Smith, R J; Froelich, Philip N. Determination of Ge/Si in marine siliceous microfossils: separation, cleaning and dissolution of diatoms and radiolaria
https://doi.org/10.1016/0304-4203(88)90113-2