PARTICULATE BIOGENIC SILICA

1.   **SCOPE and APPLICATION**

1.1 Silicomolybdate is reduced in acid solution to "molybdenum blue" by ascorbic acid. Oxalic acid is added to eliminate interference from phosphates. Detection of the silicomolybdate complex is by colorimetry.

1.2 A Method Detection Limit (MDL) of 0.009 mg Si/L was determined using the Student's *t* value (3.14) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value. The Quantitation Limit for biogenic silica was set at 0.027 mg Si/L.

1.3 This procedure should be used by analysts experienced in the theory and application of particulate inorganic analysis. Three months experience with an analyst, experienced in the analysis of biogenic silica, is required.

1.4 This method can be used for all programs that require analysis of biogenic silica.

1.5 The effective date for Biogenic Silica analysis using the AquaKem 250 is December 2006.

1.6 This procedure is based on methods described in

       1.6.1 Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. 1. *Thalassiosira pseudonana (Cyclotella nana)* grown in a chemostat with silicate as limiting nutrient. Mar. Biol. 19:117-126.

       1.6.2 Technicon Industrial Method No. 811-86T. November 1986. Technicon Industrial Systems. Tarrytown, New York, 10591.

2.   **SUMMARY**

Particulate samples, collected on a filter pad, are dissolved in NaOH in a 100°C water bath, then cooled in an ice bath to terminate the reaction, and then neutralized with H_2SO_4 before being analyzed colorimetrically.

3.   **DEFINITIONS**

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range – 0 to 42.2 mg/L Si.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 300 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 10 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

    3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

    3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

    3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 15-20 field sample analysis.

3.12 Certified Reference Material –A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. We currently use LabChem Inc. LC22400-7 where 1ml=1 mg Si.(ISO 17025)

3.13 Photometer – The AquaKem 250 robotically mixes reagents with aliquots of sample, incubates, and then measures absorbance. Absorbance values are determined by comparing non-absorbing blank values with absorbing sample values.

3.14 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.15 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.16 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.17 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.18 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.19 External Standard (ES) – A pure analyte that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.20 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.21 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.22 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.23 Ice Bath – An ice-water mixture used to stop the digestion reaction.

3.24 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.25   Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.26   Laboratory Reagent Blank (LRB) – A blank matrix (i.e., DI water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.27   Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.28   Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.29   Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.30   Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.31   Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.32   May – Denotes permitted action, but not required action. (NELAC)

3.33   Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.34   Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.35   Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.36   Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.37   Quality Control Sample (QCS) – A sample of analyte of known and certified concentration. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It

is used to check laboratory performance with externally prepared test materials.

3.38   Re-Pipette- A calibrated dispenser of reagent to samples.

3.39   Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.40   Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.41   Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.42   Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.43   Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

3.44   Water Bath – A water bath, set to 100 °C, filled with water to a depth that will cover liquid in tubes for digestion.

4   INTERFERENCES

4.1 No glassware should be used as it might be a source of Si contamination.

5   SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Sodium Hydroxide	3	0	2	4	White Stripe
Sulfuric Acid	4	0	2	4	White
Ammonium Molybdate	2	0	1	2	Orange
Oxalic Acid	2	1	1	3	white
Ascorbic Acid	1	1	1	1	green
Potassium Phosphate	0	0	0	1	Orange

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
STORAGE

> Red – Flammability Hazard: Store in a flammable liquid storage area.
> Blue – Health Hazard: Store in a secure poison area.
> Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
> White – Contact Hazard: Store in a corrosion-proof area.
> Green – Use general chemical storage (On older labels, this category was orange).
> Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label.
> Proper storage must be individually determined.

6   EQUIPMENT AND SUPPLIES

6.1 Refrigerator 4°C.

6.2 100 °C Hot Water bath.

6.3 Ice bath

6.4 Lab ware – All reusable lab ware (NO glass, use polypropylene) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% NaOH.

6.5 2 digital timers

6.6 Polypropylene centrifuge tubes with caps, and racks

6.7 2 polypropylene re-pipettes

7   REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high

purity to permit its use without compromising the accuracy of the determination.

7.3 Digestion Reagents

7.3.1   NaOH, 0.2 N; 8 g up to 1000 mL with Deionized water
7.3.2   Sulfuric Acid, 1 N; 28 ml conc H_2SO_4 up to 1 L with Deionized water

7.4 Analytical Reagents

7.4.1   SULFURIC ACID SOLUTION (H2S SILCBL)
Sulfuric Acid (H_2SO_4 conc)   4.06 mL
Stock Phosphate solution   21.4 mL

Dilute the above to 1 L in plastic volumetric flask. Transfer to brown poly bottle and refrigerate. Make every 12 months

Phosphate solution is prepared by dissolving 0.4394 g potassium phosphate to 1 L with DI H_2O.

7.4.2   AMMONIUM MOLYBDATE (MOL SILCBL)
Ammonium Molybdate   3.0 g

Dissolve 3.0 g ammonium molybdate to 100 mL with DI H_2O in plastic volumetric flask. Make fresh daily.

7.4.3   OXALIC ACID SOLUTION (OXA SILCBL)
Oxalic acid   100 g

MAKE THE DAY PRIOR TO RUNNING, DISSOLUTION IS SLOW! Dissolve 100 g oxalic acid to 1L with DI H_2O in plastic volumetric flask. Transfer to brown poly bottle. Make every 12 months

7.4.4   ASCORBIC ACID SOLUTION (ASC SILCBL)
Ascorbic acid   100 g
Oxalic acid   5 g

Dissolve the above to 1 L with DI H_2O in plastic volumetric flask. Transfer to several poly bottles and freeze until needed. Store thawed bottle in the refrigerator for up to 6 months.

7.5 Standards

7.5.1   Stock Silica Standard
Sodium silicofluoride (Na_2SiF_6), dried at 45 °C, 1.88 g
Deionized water up to 1L
7.5.2   Secondary Silica Standard
Stock Silica Standard   25 mL

Deionized water up to   100 mL

7.5.3   Working Silica Standards
To labeled 50 mL polypropylene screw cap centrifuge tubes, add 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL of Secondary Silica Standard. Digest standards as samples. After digestion, standard tubes will contain 0, 6.94, 13.8, 20.6, 27.2, 33.75 and 40.3 mg Si/L as Si, respectively.

8   SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Filter a known volume (volume dependent on water source) of water through a 0.4 µm Nucleopore ™ polycarbonate filter.

8.2 Fold filter in half, sample inside, and place in a labeled 50 ml polypropylene centrifuge tube, cap, and refrigerate at 4 °C.

9   QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1   The initial demonstration of capability is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2   Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.

9.2.3   Method Detection Limits (MDLs) – MDLs should be established for biogenic silica using a low level ambient water sample. To determine the MDL values, analyze seven replicate aliquots of water. Perform all calculations defined in the procedure (Section 12.1) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = St_{(n-1, 1-\alpha=0.99)}$$

Where,

S = Standard deviation of the replicate analyses.
n=number of replicates

$t_{(n-1, 1-\alpha=0.99)}$ = Student's *t* value for the 99% confidence level with n-1 degrees of freedom (*t*=3.14 for 7 replicates.)

MDLs shall be determined yearly and whenever there is a significant change in instrument response, a significant change in instrument configuration or a new matrix is encountered.

9.3 Assessing Laboratory Performance

9.3.1   Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. For this analysis a blank filter is treated the same as a sample and used as the LRB. Analyte found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment.

9.3.2   Quality Control Sample (QCS)/ Standard Reference Material (SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3   The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4   Control Charts – The Accuracy Control Chart for QCS/SRM samples is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL = $\pm 2s$) and upper and lower control levels (CL = $\pm 3s$). These values are derived from stated values of the QCS/SRM. The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Set up an accuracy chart by using percent recovery since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5   Continuing Calibration Verification (CCV) – Following every 18-23 samples, one CCV of a duplicate standard is analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards, and are to be within TV $\pm 3\sigma$. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery - % Recovery

9.4.1   Analyte recovery is assessed through percent recoveries of laboratory spikes.

9.4.2   % Recovery = (Actual Value/Expected Value) X 100

9.5 Assessing Analyte Precision – Relative Percent Difference

    9.5.1   Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference.

    9.5.2   RPD = (Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 100

9.6 Corrective Actions for Out of Control Data

    9.6.1   Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

    9.6.2   Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

    9.6.3   Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.

    9.6.4   When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

    9.6.5   When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7 General Operation - To assure optimal operation and analytical results, the Reagent Blank and CCV are tracked daily in the raw data file, copied to Reagent Blank and CCV Control Charts.

10 CALIBRATION AND STANDARDIZATION

10.1   Calibration – Daily calibration must be performed before sample analysis may begin. Seven point calibrations are used with AquaKem 250. The reagent blank with no Si added is used as the zero standard.

10.2   Working Silica Standards: To labeled 50 mL polypropylene screw cap centrifuge tubes, add 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL of Secondary Silica Standard. Digest standards as samples. After digestion, standard tubes will contain 0, 6.94, 13.8, 20.6, 27.2, 33.75 and 40.3 µg Si, respectively.

11 PROCEDURE FOR SAMPLE PREPARATION

11.1   Fill water bath to depth that will cover liquid in tubes and heat to 100 °C.

11.2   Prepare ice bath.

11.3   Digest blanks and standards interspersed with samples. Digest 5% of samples in duplicate.

11.4 To the sample pad in the centrifuge tube, add 10 ml of 0.2 N Sodium Hydroxide from a polypropylene re-pipette. Make sure that the filter pad is covered by the Sodium Hydroxide. Cap the tube leaving it loosened ¼ turn.

11.5 Place the centrifuge tube in the 100 °C water bath for exactly 20 minutes.

11.6 After exactly 20 minutes, remove the tube from the hot water bath and place in ice bath for exactly 4 minutes.

11.7 After 4 minutes remove tube from ice bath and add 2.5 ml of 1 N Sulfuric Acid Solution to tube to neutralize. Cap and shake. The samples can now be stored at room temperature until analyzed.

11.8 To analyze, transfer extract to an AutoAnalyzer cup using a polyethylene Pasteur pipette. Avoid particulate pieces. Samples may need to be centrifuged at 3000 rpm for 10 minutes.

11.9 Analyze for silicon on AquaKem 250.

12 CALCULATIONS

12.1 Calculation for µg Si/L

$$\mu g \ Si/L = \frac{(AquaKem \ 250 \ Value - blank)}{Volume \ filtered \ in \ mls}$$

12.2 To convert from µg Si/L to mg Si/L, divide µg Si/L by 1000

12.3 When calculating spikes be sure to use proper filtered volume in liters

13 WASTE MANAGEMENT AND POLLUTION
Liquids generated by this method are safe to put down the sink drain.