Method 366.0

Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

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1.0 Scope and Application

1.1 This method provides a procedure for the determination of dissolved silicate concentration in estuarine and coastal waters. The dissolved silicate is mainly in the form of silicic acid, H_4SiO_4, in estuarine and coastal waters. All soluble silicate, including colloidal silicic acid, can be determined by this method. Long chain polymers containing three or more silicic acid units do not react at any appreciable rate[1], but no significant amount of these large polymers exists in estuarine and coastal waters.[2,3] This method is based upon the method of Koroleff,[4] adapted to automated gas segmented continuous flow analysis.[5-7]

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Silicate	12627-13-3

1.2 A statistically determined method detection limit (MDL) of 0.0012 mg Si/L has been determined by one laboratory in seawaters of three different salinities.[8] The method is linear to 6.0 mg Si/L using a Flow Solution System (Perstorp Analytical Inc., Silver Spring, MD).

1.3 Approximately 60 samples per hour can be analyzed.

1.4 This method should be used by analysts experienced in the use of automated gas segmented continuous flow colorimetric analyses, and familiar with matrix interferences and procedures for their correction. A minimum of 6-months experience under supervision is recommended.

2.0 Summary of Method

2.1 An automated gas segmented continuous flow colorimetric method for the analysis of dissolved silicate concentration is described. In the method, β-molybdosilicic acid is formed by reaction of the silicate contained in the sample with molybdate in acidic solution. The β-molybdosilicic acid is then reduced by ascorbic acid to form molybdenum blue. The absorbance of the molybdenum blue, measured at 660 nm, is linearly proportional to the concentration of silicate in the sample. A small positive error caused by differences in the refractive index of seawater and reagent water, and negative error caused by the effect of salt on the color formation, are corrected prior to data reporting.

3.0 Definitions

3.1 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2 Dissolved Analyte (DA) -- The concentration of analyte in an aqueous sample that will pass through a 0.45 μm membrane filter assembly prior to sample acidification or other processing.

3.3 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether method performance is within acceptable control limits, and whether the laboratory is capable of making accurate and precise measurements.

This is basically a standard prepared in reagent water that is analyzed as a sample.

3.4 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all labware, equipment, 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 apparatus.

3.6 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.7 Method Detection Limit (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[8]

3.8 Reagent Water (RW) -- Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing and Materials (ASTM). Reverse osmosis systems or distilling units followed by Super-Q Plus Water System that produce water with 18 megohm resistance are examples of acceptable water sources.

3.9 Refractive Index (RI) -- The ratio of velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as estuarine or sea water versus reagent water. The correction for this difference is referred to as refractive index correction in this method.

3.10 Stock Standard Solution (SSS) -- A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

3.11 Quality Control Sample (QCS) - A solution of method analyte of known concentrations which is used to fortify an aliquot of LRB or sample matrix. 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.

3.12 SYNC Peak Solution - A colored solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by most data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

4.0 Interferences

4.1 Interferences caused by hydrogen sulfide, such as occur in samples taken from deep anoxic basins can be eliminated by oxidation with bromine or stripping with nitrogen gas after acidification. Interferences of phosphate at concentrations larger than 0.15 mg P/L is eliminated by the use of oxalic acid in the color development step of this method. Interferences of fluoride at concentrations greater than 50 mg F/L can be reduced by complexing the fluoride with boric acid.[4]

4.2 Glassware made of borosilicate glass should be avoided for use in silicate analysis. Plastic labware such as polyethylene volumetric flasks and plastic sample vials, should be used.

4.3 Sample turbidity and particles are removed by filtration through a 0.45 µm non-glass membrane filters after sample collection.

4.4 This method corrects for refractive index and salt error interferences which occur if sampler wash solution and calibration standards are not matched with samples in salinity.

4.5 Frozen samples should be filled about 3/4 full in the sample bottles. The expansion of water on freezing will squeeze some of the brine out of the bottle if the bottle was overfilled. The overfill of the sample bottle during freezing will drastically alter the nutrient concentrations in the sample that remains.

5.0 Safety

5.1 Water samples collected from the estuarine and coastal environment are generally not hazardous. However, the individual who collects samples should use proper technique.

5.2 Good laboratory technique should be used when preparing reagents. A lab coat, safety goggles, and gloves should be worn when preparing the sulfuric acid reagent.

6.0 Equipment and Supplies

6.1 Gas Segmented Continuous Flow Autoanalyzer Consisting of:

6.1.1 Autosampler.

6.1.2 Analytical cartridge with reaction coils for silicate analysis.

6.1.3 Proportioning pump.

6.1.4 Monochromator or spectrophotometer equipped with a tungsten lamp (380-800 nm) and a low refractive index flowcell.

6.1.5 Strip chart recorder or computer based data acquisition system.

6.2 Glassware and Supplies

6.2.1 All labware used in the analysis must be low in residual silicate to avoid sample or reagent contamination. Soaking with lab grade detergent, rinsing with tap water, followed by rinsing with 10% HCl (v/v) and thoroughly rinsing with reagent water was found to be effective.

6.2.2 Glassware made of borosilicate glass should be avoided for storage of solutions for silicate analysis. Plastic containers are preferable for silicate analysis.

6.2.3 Non-glass membrane filters with 0.45 µm nominal pore size. Plastic syringes with syringe filters, pipets, 60 mL polyethylene bottles, and polyethylene volumetric flasks, plastic sample vials.

6.2.4 Drying oven, desiccator and analytical balance.

7.0 Reagents and Standards

7.1 Stock Reagent Solutions

7.1.1 Sulfuric Acid Solution (0.05 M) - Cautiously add 2.8 mL of concentrated Analytical Reagent Grade sulfuric acid (H_2SO_4) to approximately 800 mL of reagent water, mix then bring up to 1 L with reagent water.

7.1.2 Ammonium Molybdate Solution (10 g/L) - Dissolve 10 g of ammonium molybdate (VI) tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in approximately 800 mL of 0.05 M sulfuric acid solution and dilute to 1000 mL with 0.05 M sulfuric acid solution. Store in an amber plastic bottle. This solution is stable for one month. Inspect the solution before use. If a white precipitation forms on the wall of container, discard the solution and make a fresh one.

7.1.3 Stock Silicate Solution (100 mg Si/L) - Quantitatively transfer 0.6696 g of pre-dried (105°C for 2 hours) sodium hexafluorosilicate (Na_2SiF_6) to a 1000 mL polypropylene flask containing approximate 800 mL of reagent water, cover with plastic film and dissolve on a stir plate using a Teflon-coated stirring bar. Complete dissolution usually takes 2-24 hours. Dilute the solution to 1000 mL in polyethylene volumetric flask with reagent water. Store the stock solution in a plastic bottle. This solution is stable for one year if care is taken to prevent contamination and evaporation.

7.1.4 Low Nutrient Sea Water (LNSW) - Obtain natural low nutrient seawater from surface seawater in the Gulf Stream or Sargasso Sea (salinity 36 ‰, < 0.03 mg Si/L) and filter through 0.45 µm pore size non-glass membrane filters. In addition, commercially available low nutrient sea water (< 0.03 mg Si/L) with salinity of 35 ‰ (Ocean Scientific International, Wormley, U.K.) can be used.

7.2 Working Reagents

7.2.1 Dowfax Start-up Solution - Add 2 mL of Dowfax 2A1 surfactant (Dow Chemical Company) to 1000 mL reagent water and mix gently.

Note: Dowfax 2A1 contains (w/w) 47% benzene, 1,1-oxybis, tetrapropylene derivatives, sulfonate, sodium salt, 1% sodium sulfate, 3% sodium chloride and 49% water.

7.2.2 Working Molybdate Reagent - Add 0.5 mL Dowfax 2A1 to 250 mL of ammonium molybdate solution, mix gently. Prepare this solution daily. This volume of solution is sufficient for an 8-hour run.

7.2.3 Ascorbic Acid Solution - Dissolve 4.4 g of ascorbic acid ($C_6H_8O_6$) in 200 mL of reagent water and 12.5 mL of acetone(C_3H_6O), dilute to 250 mL with reagent water. Store in a plastic container. This solution is stable for one week if stored at 4°C. Discard the solution if it turns brown.

7.2.4 Oxalic Acid Solution - Dissolve 50 g of oxalic acid ($C_2H_2O_4$) in approximately 800 mL of reagent water and dilute to 1000 mL with reagent water. Store in a plastic container. This solution is stable for approximately 3-months.

7.2.5 Refractive Index Matrix Solution - Add 0.5 mL Dowfax 2A1 to 250 mL of 0.05 M sulfuric acid solution and mix gently.

7.2.6 Colored SYNC Peak Solution - Add 50 µL of blue food coloring solution to 1000 mL reagent water and mix thoroughly. The solution should give a peak of between 25 to 100 percent full scale, otherwise the volume of food coloring added needs to be adjusted.

7.2.7 Calibration Standards - Prepare a series of calibration standards (CAL) by diluting suitable volumes of Stock Silicate Solution (Section 7.1.3) to 100 mL with

reagent water or low nutrient seawater. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards with equal increments in concentration should be used to construct the calibration curve.

When working with samples of a narrow range of salinities (± 2 ‰), it is recommended that the CAL solutions be prepared in Low Nutrient Seawater (Section 7.1.4) diluted to the salinity of samples, and the Sampler Wash Solution also be Low Nutrient Seawater (Section 7.1.4) diluted to that salinity. If this procedure is performed, it is not necessary to perform the salt error and refractive index corrections outlined in Sections 12.2 and 12.3.

When analyzing samples of varying salinities, it is recommended that the calibration standard solutions and sampler wash solution be prepared in reagent water and corrections for salt error and refractive index be made to the sample concentrations (Section 12.2 and 12.3).

7.2.8 Saline Silicate Standards - If CAL solutions will not be prepared to match sample salinity, then saline silicate standards must be prepared in a series of salinities in order to quantify the salt error, the change in the colorimetric response of silicate due to the change in the ionic strength of the solution. The following dilutions prepared in 100 mL volumetric flasks, diluted to volume with reagent water, are suggested.

Salinity (‰)	Volume of LNSW(mL)	Volume(mL) Si stock std	Conc. mg Si/L
0	0	1.5	1.5
9	25	1.5	1.5
18	50	1.5	1.5
27	75	1.5	1.5
35	98	1.5	1.5

8.0 Sample Collection, Preservation and Storage

8.1 Sample Collection - Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods: hydrocast or submersible pump systems.

8.1.1 A hydrocast uses a series of sampling bottles (Niskin, Go-Flo or equivalent) that are attached at fixed intervals to a hydro wire. These bottles are sent through the water column open and are closed either electronically or via a mechanical messenger when the bottles have reached the desired depth.

8.1.2 In a submersible pump system, a weighted hose is sent to the desired depth in the water column and water is pumped from that depth to the deck of the ship for sample processing.

8.1.3 For collecting surface samples, an acid - cleaned plastic bucket or a large plastic bottle can be used as convenient samplers. Wash the sampler three times with sample water before collecting samples.

8.1.4 Samples must be filtered through a 0.45 µm non-glass membrane filters as soon as possible after collection.

8.1.5 60-mL high density polyethylene bottles are used for sample storage. Sample bottles should be rinsed 3 times with about 20 mL of sample, shaking with the cap in place after each rinse. Pour the rinse water into the cap to dissolve and rinse away salt crusts trapped in the threads of the cap. Finally, fill the sample bottle about 3/4 full, and screw the cap on firmly. The expansion of water on freezing will squeeze some of the brine out of the bottle if the bottle was overfilled.

8.2 Sample Preservation - After collection and filtration, samples should be analyzed as soon as possible. If samples will be analyzed within 24 hours then keep refrigerated in tightly sealed, high density polyethylene bottles in the dark at 4°C until analysis can be performed.

8.3 Sample Storage - If samples are to be frozen for long-term storage ensure that none of the sample bottles are filled more than 3/4 full and the cap is firmly screwed on. Place the bottles upright on a rack and store in the freezer (-20°C).

Before analysis, frozen samples must be taken out of the freezer and allowed to thaw in a refrigerator at 4°C in the dark. Thawing times depend upon sample salinities. It was found that the frozen low salinity estuarine water took 4 days to thaw. After completely thawing, take samples out of the refrigerator and mix thoroughly. Keep samples in the dark at room temperature overnight before analysis.

Effects of thawing conditions on the recoveries of frozen samples are more pronounced in low salinity estuarine

waters than high salinity coastal waters as shown in following results:

Day	Recovery (%)			Remark
	S=35.85	S=18.07	S=2.86	
0	100.00	100.00	100.00	
7	102.44	102.65	89.37	a
14	98.59	101.06	86.49	a
21	99.51	99.30	83.49	a
27	98.86			a
		98.86	91.43	b
35	98.70			b
		98.66	92.98	b
42	100.87			b
49		102.44	79.12	c
		103.92	79.10	d
		99.92	89.68	e
56	103.47			c
	104.12			d
	99.35			e
84		100.80	91.71	f
		99.90	93.81	g
91	100.65			f
	100.22			g

S = Salinity
a, overnight thawing at room temperature
b, 20 hours thawing at room temperature
c, 24 hours thawing at room temperature
d, 8 hours thawing at room temperature then heating at 80°C for 16 hours
e, 24 hours thawing at room temperature in the dark
f, 4 days thawing at room temperature in the dark
g, 4 days thawing at 4°C in a refrigerator in the dark

To ensure a slow process of depolymerization of polysilicate to occur, thawing the frozen samples in the dark at 4°C for 4 days is critical condition for obtaining high recoveries of silicate in frozen samples. A maximum holding time for frozen estuarine and coastal waters is two months.[9-11]

9.0 Quality Control

9.1 Each laboratory using this method is required to implement a formal quality control(QC) program. The minimum requirements of this program consists of an initial demonstration of performance, continued analysis of Laboratory Reagent Blanks (LRB), laboratory duplicates and Laboratory Fortified Blanks (LFB) with each set of samples as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The Initial demonstration of performance is used to characterize instrument performance by determining the MDL and LDR and laboratory performance by analyzing quality control samples prior to analysis of samples using this method.

9.2.2 Method Detection Limits (MDLs) should be established using a low level seawater sample containing, or fortified at, approximately 5 times the estimated detection limit. To determine MDL values, analyze at least seven replicate aliquots of water which have been processed through the entire analytical method. Perform all calculations defined in the method and report concentration in appropriate units. Calculate the MDL as follows:

$$MDL = (t)(S)$$

where, S = the standard deviation of the replicate analyses

t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; t = 3.143 for six degrees of freedom.

MDLs should be determined every 6-months or whenever a significant change in background or instrument response occurs or a new matrix is encountered.

9.2.3 The LDR should be determined by analyzing a minimum of eight calibration standards ranging from 0.03 to 5.00 mg Si/L across all sensitivity settings (Absorbance Units Full Scale) of the detector. Standards and sampler wash solutions should be prepared in low nutrient seawater with salinities similar to that of samples, therefore a correction factor for salt error, or refractive index, will not be necessary. Normalize responses by multiplying the response by the Absorbance Units Full Scale output range setting. Perform the linear regression of normalized response vs. concentration and obtain the constants m and b, where m is the slope and b is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration C_C, that is within 100 ± 10% of the known concentration, C, where $C_C = (R-b)/m$. That concentration

defines the upper limit of the LDR for the instrument. Should samples be encountered that have a concentration that is 90% of the upper limit of LDR, then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) - A laboratory should analyze at least one LRB with each set of samples. LRB data are used to assess contamination from the laboratory environment. Should an analyte value in the LRB exceed the MDL, then laboratory or reagent contamination should be suspected. When the LRB value constitutes 10% or more of the analyte concentration determined for a sample, duplicates of the sample must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank (LFB) - A laboratory should analyze at least one LFB with each set of samples. The LFB must be at a concentration that is within the daily calibration range. The LFB data are used to calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90 -110%, the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90 -110%. When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

Upper Control Limit = x + 3S
Lower Control Limit = x - 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix (LFM)

9.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the total number of samples or one sample per sample set, whichever is greater. The analyte added should be 2-4 times the ambient concentration and should be at least four times greater than the MDL.

9.4.2 Calculate percent recovery of analyte, corrected for background concentration measured in a separate unfortified sample. These values should be compared with the values obtained from the LFBs. Percent recoveries may be calculated using the following equation:

$$R = \frac{(C_S - C)}{S} \times 100$$

where, R = percent recovery

C_S = measured fortified sample concentration (background + addition in mg Si/L)

C = sample background concentration (mg Si/L)

S = concentration in mg Si/L added to the environmental sample.

9.4.3 If the recovery of the analyte falls outside the required control limits of 90-110%, but the laboratory performance for that analyte is within the control limits, the fortified sample should be prepared again and analyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related and the sample data should be flagged.

10.0 Calibration and Standardization

10.1 At least five calibration standards should be prepared daily for system calibration.

10.2 A calibration curve should be constructed for each run by analyzing a set of calibration standard solutions. A run should contain no more than 60 samples.

It is suggested that a large set of samples be analyzed in several sets with individual calibration curves.

10.3 Place the calibration standards before samples for each run. All the calibration solutions should be analyzed in duplicate.

10.4 The calibration curve containing five data points or more should have a correlation coefficient 0.995.

10.5 Place a high standard solution cup and follow by two blank cups to quantify the **carry-over** of the system. The difference in peak heights between two blank cups is due to the carry over from the high standard cup. The carry-over coefficient, k, is calculated as follows:

$$k = \frac{P_{b1} - P_{b2}}{P_{high}} X\ 100$$

where, P_{high} = the peak height of the high silicate standard

P_{b1} = the peak height of the first blank sample

P_{b2} = the peak height of the second blank sample.

The carry over coefficient, k, for a system should be measured in seven replicates in order to obtain a statistically significant number. The k should be remeasured when a change in the plumbing of the manifold or replacement of pump tube occur.

The carry over correction (CO) on a given peak, i, is proportional to the peak height of the preceding sample, P_{i-1}.

$$CO = kxP_{i-1}$$

To correct a given peak height reading, P_i, one subtracts the carry over correction.[12,13]

$$P_{i,c} = P_i - CO$$

where $P_{i,c}$ is corrected peak height. The correction for carry over should be applied to all the peak heights throughout a run. The carry over should be less than 2%.

10.6 Place a high standard solution at the end of a run to check sensitivity drift. The sensitivity drift should be ± 5% during the run.

11.0 Procedure

11.1 If samples are frozen, thaw the sample at 4°C in the dark as outlined in Section 8.3. Mix samples thoroughly prior to analyses.

11.2 Turn on the continuous flow analyzer and PC components and warm up at least 30 minutes.

11.3 Set up the cartridge and pump tubes as shown in Figure 1.

Note: Fluctuation of ambient temperature can cause erratic results due to the effect of temperature on kinetics of color development. The laboratory temperature should be maintained as close to a constant temperature as possible. The cartridge should be away from the direct path of air flow from a heater or air conditioner. In cases such as on a ship where the fluctuation of temperature can be extreme, the temperature effect can be minimized by increasing the length of mixing coil 1 (Figure 1) to bring the formation of silicomolybdic acid reaction to completion.

11.4 Set the wavelength at 660 nm on the spectrometer/monochrometer.

Note: The absorption spectra of silicomolybdeum blue complex has two maxima at 820 nm and 660 nm with 820 nm higher than 660 nm. This method measures absorbance at 660 nm because the detector works in the range of 380 to 800 nm. The sensitivity of the method is satisfactory at 660 nm. The sensitivity, however, can be improved by using 820 nm if this wavelength is available on the detector.

11.5 On the monochromator, set the Absorbance Unit Full Scale at an appropriate setting according to the highest concentration of silicate in the samples. The highest setting used in this method was 0.2 for 6 mg Si/L.

11.6 Prepare all reagents and standards.

11.7 Begin pumping the Dowfax start-up solution (Section 7.2.1) through the system and obtain a steady baseline. Place the reagents on-line. The reagent baseline will be higher than the start-up solution baseline. After the reagent baseline has stabilized, reset the baseline.

NOTE: To minimize the noise in the reagent baseline, clean the flow system by sequentially pumping the

sample line with reagent water, 1 N HCl solution, reagent water, 1N NaOH solution for a few minutes each at the end of the daily analysis. Make sure to rinse the system well with reagent water after pumping NaOH solution to prevent precipitation of $Mg(OH)_2$ when seawater is introduced into the system. Keep the reagents and samples free of particulate. Filter the reagents and samples if necessary.

If the baseline drifts upward, pinch the waste line for a few seconds to increase back pressure. If absorbance drops down rapidly when back pressure increases, this indicates that there are air bubbles trapped in the flow cell. Attach a syringe at the waste outlet of the flowcell. Air bubbles in the flowcell can often be eliminated by simply attaching a syringe for a few minutes or, if not, dislodged by pumping the syringe pistion. Alternatively, flushing the flowcell with alcohol was found to be effective in removing air bubbles from the flowcell.

For analysis of samples with a narrow range of salinities (± 2 ‰), it is recommended that the wash water in the sampler be prepared in Low Nutrient Seawater diluted to the salinity of samples in place of reagent water. For samples with varying salinities, it is suggested that reagent waters and procedures in Sections 12.2 and 12.3 be employed.

11.8 A good sampling rate is approximately 60 samples per hour with 40 seconds of sample time and 20 seconds of wash time.

11.9 Use 10% HCl followed by reagent water to rinse sample cups. Place CAL solutions and saline standards (optional) in sampler. Complete filling the sampler tray with samples, laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Place a blank every ten samples and between samples of high and low concentrations.

11.10 Commence analysis.

11.11 If the reagent water is used as wash solution instead of Low Nutrient Seawater and an operator wants to quantify the refractive index correction due to the difference in salinities between sample and wash solution, the following procedures are used. Replace ammonium molybdate solution (Section 7.1.2) with refractive index matrix solution (Section 7.2.5). All other reagents remain the same. Replace the synchronization cup with the colored SYNC peak solution (Section 7.2.6). Commence analysis and obtain a second set of peak heights for all standards and samples. The peak heights obtained from these measurements must be subtracted from the peak heights of samples analyzed with color developing reagent pumping through the system. If a low refractive index flowcell is used, the correction for refractive index is negligible. This procedure is optional.

12.0 Data Analysis and Calculations

12.1 Concentrations of silicate are calculated from the linear regression, obtained from the standard curve in which the concentrations of the calibration standards are entered as the independent variable, and their corresponding peak heights are the dependent variable.

12.2 Refractive Index Correction for Estuarine and Coastal Samples (optional)

12.2.1 Obtain a second set of peak heights for all standards and samples with refractive index matrix solution being pumped through the system in place of color reagent (ammonium molybdate solution). All other reagents remain the same. The peak heights for the refractive index correction must be obtained at the same Absorbance Unit Full Scale range setting and on the same monochromator as the corresponding samples and standards.

12.2.2 Subtract the refractive index peak heights from the peak heights obtained from the silicate determination.

12.2.3 An alternative approach is to measure the relationship between the sample salinity and refractive index on a particular detector.

First analyze a set of silicate standards in reagent water with color reagent and obtain a linear regression from the standard curve.

Prepare a set of different salinity samples with LNSW. Analyze these samples with refractive index matrix solution being pumped through the system in place of color reagent (ammonium molybdate solution). All other reagents remain the same. The peak heights for the refractive index correction must be obtained at the same Absorbance Unit Full Scale setting and on the same monochromator as the corresponding standards.

For each sample, the apparent silicate concentration due to refractive index is then calculated from its peak height obtained with refractive index reagent and the regression of silicate standards obtained with color reagent pumping

through the system. Salinity is entered as the independent variable and the apparent silicate concentration due to refractive index in that detector is entered as the dependent variable. The resulting regression allows the operator to calculate apparent silicate concentration due to refractive index when the salinity is known. Thus, the operator would not be required to obtain refractive index peak heights for all samples.

12.2.4 Refractive index correction can be minimized by using a low refractive index flowcell. An example of typical results using a low refractive index flowcell follows:

Salinity (‰)	Apparent silicate conc. due to refractive index (mg Si/L)
4.5	0.0003
9.0	0.0005
18.0	0.0016
27.0	0.0027
36.0	0.0042

12.2.5 An example of a typical equation is:

Apparent silicate (mg Si/L) = $0.00001953*S^{1.5}$

where S is sample salinity. The form of fitted equation might vary as the design of flowcell, so the operators are advised to obtain the appropriate equation which has the best fit of their own data with the least fitting coefficients.

12.3 Correction for Salt Error in Estuarine and Coastal Samples

12.3.1 When calculating concentrations of samples of varying salinities from standards and wash solution prepared in reagent water, it is usual to first correct for refractive index errors, then correct for the change in color development due to the differences in ionic strength between samples and standards (salt error). The refractive index correction is negligible, so is optional, but correction for salt error is necessary.

12.3.2 Plot the salinity of the saline standards (Section 7.2.8) as the independent variable, and the apparent concentration of silicate (mg Si/L) from the peak height (corrected for refractive index) calculated from the regression of standards in reagent water, as the dependent variable for all 1.50 mg Si/L standards. The resulting regression equation allows the operator to correct the concentrations of samples of known salinity for the color suppression due to salinity effect, e.g., salt error. An example of typical results follows:

Salinty (‰)	Peak height of 1.50 mg Si/L	Uncorrected Si conc.calculated from standards in reagent water
0	2503	1.50
9	2376	1.32
18	2282	1.27
27	2250	1.25
36	2202	1.23

12.3.3 An example of a typical equation to correct for salt error is:

$$\text{Corrected mg Si/L} = \frac{\text{Uncorrected mg Si/L}}{1 - 0.02186 \times \sqrt{S}}$$

where S is salinity.

12.3.4 Results of sample analyses should be reported in mg Si/L or in µg Si/L.

mg Si/L = ppm (parts per million)
µg Si/L = ppb (part per billion)

13.0 Method Performance

13.1 Single Laboratory Validation

13.1.1 Method Detection Limit - A method detection limit (MDL) of 0.0012 mg Si/L has been determined by one laboratory in seawaters of three different salinities.

Salinity (‰)	SD (µg/L)	Recovery (%)	MDL (µg/L)
36	0.3924	105	1.233
36	0.4980	107	1.565
27	0.2649	104	0.832
27	0.3362	104	1.056
27	0.4671	100	1.468
18	0.3441	101	1.081
18	0.2809	105	0.883
18	0.2432	104	0.764
3	0.3441	101	1.081
3	0.2331	102	0.733
3	0.1963	98	0.617
3	0.2809	99	0.883

13.1.2 Single Analyst Precision - A single laboratory analyzed three samples collected from the Miami River and Biscayne Bay areas of Florida. Seven replicates of

each sample were processed and analyzed with salinities ranging from 2.86 to 35.85. The results were as follows:

Sample	Salinity (‰)	Concentration (mg Si/L)	RSD (%)
1	35.85	0.097	1.2
2	18.07	1.725	1.4
3	2.86	3.322	0.9

13.1.3 Laboratory Fortified Sample Matrix - Laboratory fortified sample matrixes were processed in three different salinities ranging from 2.86 to 35.85 and ambient concentrations from 0.095 to 3.322 mg Si/L with three fortified levels at each salinity. Seven replicates of each sample were analyzed and the results were as follows:

Salinity (‰)	Concentration (mg Si/L)		RSD (%)	Recovery (%)
	Ambient	Fortified		
35.85	0.095	0.1647	0.82	99.37
35.85	0.095	0.2196	1.34	100.61
35.85	0.095	0.2747	1.74	99.62
18.07	1.725	0.5517	1.11	107.18
18.07	1.725	1.1008	0.77	104.69
18.07	1.725	1.6508	0.98	103.62
2.86	3.322	0.5421	0.99	101.03
2.86	3.322	1.0801	1.26	103.22
2.86	3.322	1.6188	0.98	100.59

13.2 Multi-Laboratory Validation

Multi-laboratory validation has not been conducted for this method and, therefore, multi-laboratory data is currently unavailable.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

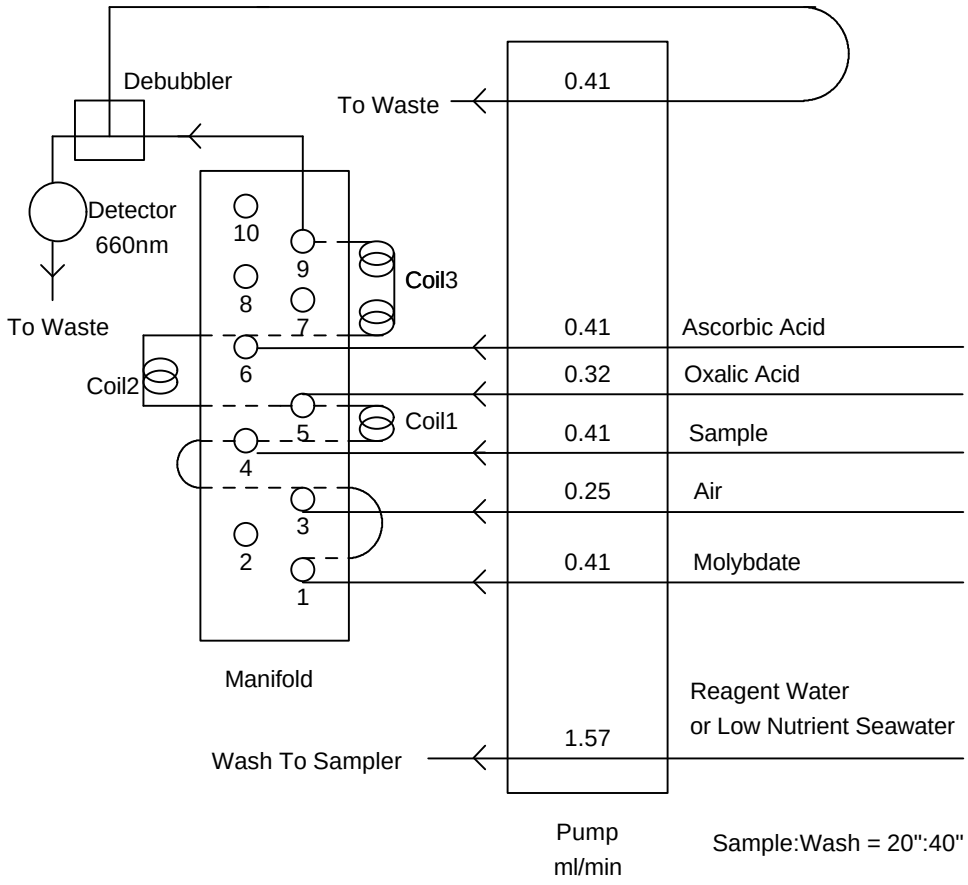

Figure 1. Manifold Configuration for Silicate Analysis.