Dissolved Si Analysis

Reagents:

Ammonium Molybdate: Dissolve 8g ammonium molybdate in 1000mL Nanopure water. When dissolved, add 24mL concentrated HCl. Store in plastic bottle. Reagent is stable for about one month. Clean bottle with NaOH and Nanopure before making new reagent.

Reducing reagent: This is made of a combination of the following FOUR solutions (see chemical compositions below). Mix 2mL Metol-sulfite reagent with 1.6mL Nanopure water, 1.2mL 50% H_2SO_4, and 1.2mL saturated. oxalic acid for EACH sample. Reagent is stable for one day, and so must be remade daily. MAKE ENOUGH FOR ALL YOUR SAMPLES + STANDARD CURVE + ANY DILUTIONS YOU MAY NEED TO MAKE (rule of thumb is 20% of your samples will need to be diluted).

1) Metol Sulfite Reagent: - dissolve 12g of sodium sulfite (Na_2SO_3) in 1000mL Nanopure water. When dissolved, add 20g para-Methylaminophenol Sulfate. Store in brown plastic bottle.
2) Ultrapure Water: - Use water from the Nanopure system for this and for mixing all reagents.
3) 50% H_2SO_4: - Place 500mL Nanopure water in a clean, thick-walled plastic bottle. While holding the bottle in an ice bath, slowly add 500mL concentrated H_2SO_4. BE CAREFUL! A tremendous amount of heat is generated, keep the bottle in the ice bath. Keep the cap on loosely if there is no danger of the bottle spilling, or cap tightly and "burp" the bottle occasionally so it does not collapse.
4) Saturated Oxalic Acid:- dissolve oxalic acid in Nanopure water. Saturation is approximately 10g per 100mL. Store in plastic bottle.

# Samples	Metol (ml)	Nanopure (ml)	Sulfuric Acid (ml)	Oxalic Acid (ml)	Total Volume (ml)
5	10	8	6	6	30
10	20	16	12	12	60
15	30	24	18	18	90
20	40	32	24	24	120
25	50	40	30	30	150
30	60	48	36	36	180
35	70	56	42	42	210
40	80	64	48	48	240
45	90	72	54	54	270
50	100	80	60	60	300
55	110	88	66	66	330
60	120	96	72	72	360
65	130	104	78	78	390
70	140	112	84	84	420
75	150	120	90	90	450
80	160	128	96	96	480
Cannot use dispenser for volumes below. Mix larger volume in beaker and transfer to dispenser.					
85	170	136	102	102	510
90	180	144	108	108	540
95	190	152	114	114	570
100	200	160	120	120	600

Dissolved Si Analysis:

1) Dispense 10ml of Artificial Seawater (for seawater samples) or Nanopure (for BSi samples) or HF/Boric Acid mix (for LSi samples) into the 12 bottles to make your reagent blanks and standard curve. The background matrix needs to be similar to the matrix you are measuring.
2) Pipette the following amounts of 2.5umol/ml Si standard into the 10ml liquid to make 8 of the following concentrations. Do not add any Si standard to 4 of the bottles – 2 reagent blanks and 2 0.0uM bottles.

Conc(uM)	Vol of Std (uL)	Conc(uM)	Vol of Std (uL)	Conc(uM)	Vol of Std (uL)	Conc(uM)	Vol of Std (uL)
0.625	2.5	5.625	22.5	20	80	37.5	150
1.25	5	6.25	25	22.5	90	40	160
1.875	7.5	7.5	30	25	100	42.5	170
2.5	10	10	40	27.5	110	43.75	175
3.125	12.5	12.5	50	30	120	45	180
3.75	15	15	60	31.25	125	47.5	190
4.375	17.5	17.5	70	32.5	130	50	200
5	20	18.75	75	35	140	56.25	225

3) Be certain that all bottles (samples and standard curve) have 10ml of liquid. Record what is in each bottle on the data sheet.

4) To the 2 reagent blank bottles ONLY, add 6ml reducing reagent, mix, immediately add 4ml of ammonium molybdate reagent. Mix well and set these 2 bottles aside so you don't add anything to them by mistake.

5) Loosen the caps on all the standard curve bottles and sample bottles, set the timer for 10 minutes and start it. Dispense 4ml of ammonium molybdate reagent into the bottle, tighten cap, mix by swirling/shaking, loosen cap and replace in tray. Loosening the caps saves a bit of time during the dispensing process.

6) After the timer goes off, add 6ml of reducing reagent to each bottle, mixing after the addition.

7) Turn on the spectrophotometer – it takes 1 hour to warm up. Look at the samples – if any look darker blue than the most concentrated standard curve bottle, dilute them. It's best to try and do this right away so that you don't end up having to make up another standard curve.

8) Wait 2.5-3.5 hours for the blue colour in the samples to develop. Set the spec to read percent transmittance (%T) at 810nm. Use the auto zero option to blank the spec with Nanopure water in the 1cm cell. Read all samples on the 1cm cell first, then zero with the 10cm cell and read the std curve and any samples that were >=90%T on the 1cm cell. DON'T FORGET TO MEASURE THE FILTER BLANKS, TUBE BLANKS AND REAGENT BLANKS IN THE 10CM CELL IF YOU HAVE TO READ ANY SAMPLES IN 10CM CELL.

Determining Sample Concentrations:

1) Determine sample absorption from the %T values as follows:

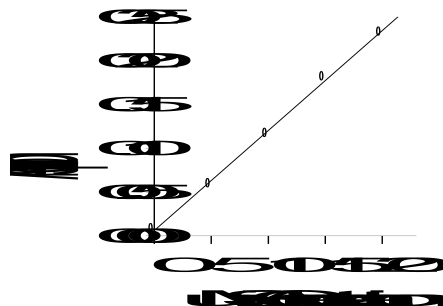
   %T/100 = T

   ABS = (-1) * log(T)

   All %T values MUST be translated to absorbance (ABS) prior to the following calculations. YES, it would be easier to measure ABS on the spectrophotometer but we lose accuracy that way so we DON'T do it!!!

2) Subtract the average absorbance of the reagent blanks from the absorbances of all samples and standards (see PSI template).

3) Do a regression on the standard curve data comparing the concentration (independent variable) (e.g.. 0, 5, 10, 15, and 20 uM) to the absorption (dependent variable) to get the slope of the line.



4) Divide the (sample absorbance – rgt blank) value by the slope to get the uM Si concentration in the sample. Multiply this value by 0.01 to get umol Si in 10ml.

5) Correct for dilutions (dilution factors are listed on the PSI template) to get umol Si on the filter.

6) AFTER you've corrected for dilutions, subtract the average value of the tube or filter blanks from the umol Si/filter value. If you remove the tube or filter blanks before the dilution correction, you will overcompensate for the blank. For example:

   average blank value is 0.001 umol Si in 10ml, 0.001 umol Si on filter (no dilution factor)

   sample value is 0.1 umol Si in 10ml, 0.3 umol Si on filter (dilution factor is 3)

   (0.1-0.001)*3=0.297 does not equal (0.1*3)-0.001=0.299

   It doesn't look much different but its a good habit to get into.

7) For BSi, divide the value by the volume filtered to get the final uM Si concentration. For LSi, the per filter value needs to be corrected for the rinsing step (see LSi procedure) before the final uM Si concentration can be calculated.