Particulate Si Analysis

In the Lab:

1. If tube blanks did not get prepared during the cruise, use new clean purple capped 15ml PP centrifuge tubes, place them in the drying oven overnight so they've been heated the same as the tubes with samples. Treat blanks as you do all other vials with filters.
2. Cover filter with 4ml of 0.2N NaOH. Cap and vortex. Be sure filter remains submerged. Loosen caps but be sure the top of the tube is completely covered.
3. Place in water bath at **95°C** for :
   - RoMP samples = 1 hr
   - BATS samples = 2hr
   - JGOFS = 40 min
   - Plumes and Blooms = 30 min
   - LTER = 40 min
   - EqPac = 45 min
   - HOT = 45 min
4. Cool in ice water bath immediately. Samples should be cool to the touch before adding acid.
5. Add 1.0 ml of 1 N HCl and vortex after each addition. This neutralizes the NaOH and along with the cooling stops the digestion. Work quickly to minimize the time difference between the first and last sample.
6. With a Teflon rod or long forceps, crunch the filter into bottom of tube. Rinse the rod/forceps with Nanopure between samples. Scrunching the filter makes it easier to withdraw your sample later, but you will need to remove the filter eventually so be sure it's not jammed into the bottom of the tube.
7. Centrifuge for 10 min at setting 6 (desktop clinical centrifuge) or program 4 (Thermo 20 tube centrifuge) to drive the lithogenic Si particles to the bottom of the tube.
8. Withdraw 4ml of the 5ml in the PMP tube and place in 50ml PP (polypropylene) tube – be sure to take the sample from the top of the liquid, don't push the pipette tip down into the filter. Add 6ml of Nanopure to the PP tube for a total of 10ml. This 10ml will be used for dSi analysis – the dilution factor for this sample will be 5/4=1.25.
9. The lSi sample needs to be transferred to a new 15ml PP tube in order to reduce the tube blanks. Label a new set of tubes and pour the remaining 1ml of digest and the filter ball into the new tube. Dispense 4ml of Nano into the old tube, vortex and pour it into the corresponding new tube. Repeat this two more times – that will give 3 rinses of the old tube. Cap and centrifuge the new tubes using the above settings. If filter does NOT spin to the bottom of tube, push it down with clean poker and spin again for 10 minutes (remember we are trying to rid ourselves of any remaining dissolved silica from the bSi digestion not the particulate lSi). Aspirate to 1ml (BE SURE to rinse slurper tip before you use it!!)
10. For the 2nd rinse, dispense 12ml of Nano into the new tube, vortex (make sure the filter lifts off the bottom), centrifuge and aspirate as above. Two rinses are necessary.
11. Loosely cap the centrifuge tubes and place in drying oven at 65°C until dry (takes approximately 48hours). Once dry, these filters can be stored at room temperature (cap tightly) until you have time to continue.

ONCE THE TUBES FOR lSi ARE IN THE OVEN AND THE bSi SAMPLES ARE READY TO ENTER THE dSi PART OF THE ANALYSIS YOU CAN STOP FOR THE DAY. THE bSi SAMPLES SHOULD BE PROCESSED WITHIN 48 HOURS.

12. When dry, remove filters from oven and let cool. Wear gloves when working with HF, and you may want to work in the hood but it's not necessary. Completely cover each filter with 0.2ml of 2.5M HF.

BE SURE FILTER IS DRY AND COOL, DO NOT ADD HF TO WARM FILTERS

13. Crunch the filter down into the bottom of the tube with the HF poker (teflon stirring rod or spatula), remove all air bubbles and completely submerge the filter ball under the HF. Rinse the poker well with Nanopure between samples. The filter will have to be removed from the tube later so you'll want to try and flip it over or lift it slightly off the bottom to make that removal easier!!.

DO NOT USE GLASS OR METAL STIR RODS / FORCEPS / SPATULAS WITH HF

14. Cap tubes tightly. Be sure to treat the tube and filter blanks with 0.2ml HF and the HF poker as well. Let samples and blanks sit covered with HF for **48 hours**.

15. A couple of hours before the 48hour waiting period is over, the saturated boric acid solution (~1M) needs to be filtered. The H_3BO_3 **MUST** be freshly filtered prior to use. A total volume of 14.8ml per sample is required, plus 110ml for the standard curve plus enough to make dilutions if necessary. The saturated boric acid should be filtered through a 0.6um 47mm PC membrane filter and collected in a clean bottle. Filter enough boric acid for all samples, standard curves and dilutions you might need – it doesn't hurt to filter too much.

16. Vortex the tube to release the HF inside the crunched filter. Set the dispensette on the filtered boric acid to 7.4ml. Dispense 7.4ml of filtered boric acid into the tube, vortex to resuspend filter and transfer the boric acid, HF and scrunched filter to a 30ml PP bottle. Dispense a second 7.4ml aliquot of boric acid into the tube, vortex to rinse the tube and transfer volume to same 30ml bottle. The total volume in the bottle is 15ml – 14.8ml of filtered boric acid and 0.2ml of 2.5M HF.

17. Withdraw 10ml of the 15ml in the PP bottle and transfer to a clean 30ml PP bottle for the dSi reaction. The dilution factor here is (15/10)=1.5.

18. The standard curve is prepared in a 0.2ml**:**14.8ml ratio of 2.5M HF**:**filtered saturated boric acid. Mix 3ml 2.5M HF and 222ml filtered boric acid in a beaker, transfer 10ml of this solution to each of the standard curve bottles. **DO NOT USE NANOPURE FOR THE LSi STANDARD CURVE**. Use this solution for dilutions as well. If you'll have many dilutions, make a larger volume.

19. Follow same protocol as for Dissolved Si Analysis - 4 ml of the Acid/Moly reagent, wait 10 minutes to form silicomolybdic acid, add 6 ml reducing reagent.

20. Tube blanks correct for signal generated by the effect of the NaOH & HF digestions on the tube. Filter blanks correct for signal generated by the effect of the digestions on the filter. A signal from the tube blank <90%T for 1cm cell, <80% for 10cm cell is high and may indicate a bad tube. The filter blank incorporates the tube blank, the signal from the filter blank is subtracted from the signal on all samples.

21. Calculate the lSi concentration in the original seawater sample (lSi). Correct the value based on the number of nanopure rinses in steps 12/13. The calculation is as follows:

$$lSi_{umol/filter\ corrected} = lSi_{umol/filter} - ((1/5)*(1/13)*(1/13)*bSi_{umol/filter}) = LSi - (0.00118*bSi_{umol/filter})$$