Chromium Reducible Sulfide (CRS) method

1. Preparation of 1000 ppm (0.0312M) stock sodium sulfide solution

1. De-gas 250 mL of MilliQ.
2. Fill a 250 mL VF with Argon.
3. Add some MilliQ and then 1.873 g $Na_2S{\cdot}9H_2O$ powder (MW: 240.18 g/mol) to a 250 mL VF containing 5 mL of 10 M NaOH under argon atmosphere. Cover with parafilm, mix to dissolve powder, then add MilliQ to the calibration mark.
4. Transfer the solution to a small brown bottle and cap it. Put the bottle inside a larger bottle filled with argon to reduce likelihood of interaction with light and oxygen.

2. Standardization of sulfide stock solution via potentiometric titration using Sulfide Ion Selective Electrode (ISE)

Basic ISE Operation

1. Remove the black protective caps from the ISE and the reference electrodes (note that the reference electrode cap contains liquid and must be kept upright) and insert the electrodes into the labelled sockets of the ELIT dual electrode head.
2. Install the electrode head in any standard electrode holder with an articulated arm, and connect the cable to the BNC socket of the mV/Ion meter.
3. Immerse the electrodes in the Preconditioning Solution (normally the 1000 ppm standard).
    a. Leave to stand (swirling occasionally) for at least 10 minutes, or until the millivolt output reaches a stable reading. Note that when first used or after prolonged storage this may take several hours - or even days in extreme cases.
4. When the mV reading is stable, remove the electrodes from the preconditioning solution, rinse with a jet of de-ionised water and dab dry with a paper tissue. WARNING: Do not leave the electrodes soaking in de-ionised water.
5. Wash and dry the electrodes, then place them in a beaker containing tap water for storage.
6. At the end of the analyses, and replace the caps to prevent drying out of the external filling solution of the reference electrode and to protect the ISE from mechanical damage or atmospheric oxidation/corrosion.
7. For optimum precision and accuracy, recalibrate after every ten samples, or if the temperature changes by >1°C.

Polishing the ISE head

1. Rinse electrode head with MilliQ.
2. Put some Aluminum Oxide powder on a paper towel, then add a drop of MilliQ.
3. Place electrode head on the paste and swirl it around.
4. Rinse electrode and head with MilliQ, then wipe with kimwipe.
    a. Dab the electrode head with the kimwipe to protect against mechanical damage.
5. Repeat steps 3 and 4 as necessary until the electrode head is shiny.

6. Immerse in preconditioning solution for at least 30 minutes before use.

Titration of the Na_2S stock with 1000ppm Cu^{2+} standard

1. Immerse the ISE and reference electrodes in a beaker containing 10 mL of sulfide stock solution and 10 mL of MilliQ; let sit until the mV reading becomes stable.
    a. The beaker should be covered in parafilm with holes made for the electrodes and pipette tip.
2. Add Cu^{2+} standard solution using micropipette and wait for mV reading to equilibrate after each addition. Note the Cu^{2+} volumes added and stable mV readings in a spreadsheet.
3. The endpoint occurs when the mV reading drops sharply for a small amount of titrant added.
    a. Add small amounts of titrant near the endpoint.
4. Conduct at least 2 precise trials and determine stock sulfide concentration using Gran Analysis.

3. Preparation of sulfide dilution series for Purge and Trap (PT) calibration

1. Obtain four clean 50mL and one 100mL Volumetric Flasks (VF), degassed MilliQ, parafilm, a Pasteur pipette, five 50mL Falcon tubes, gloves, and appropriate micropipettes and tips.
2. Prepare a fresh 100mL solution of 2000nM sulfide from the standardized stock.
    a. Add some MilliQ into the VF using the Pasteur pipette.
    b. Pipette an appropriate volume of Na_2S stock ($V_1 = \frac{C_2 \cdot V_2}{C_1}$) into the VF using a micropipette, then reseal the Na_2S stock in the argon bottle.
    c. Add MilliQ to the VF calibration mark, transfer half of the solution to a labelled Falcon tube and cap it, then place parafilm over the mouth of the VF.
3. Use the 2000nM solution from the falcon tube in preparation of the 10, 100, 500, and 1000nM sulfide solutions in 50mL VFs.
    a. Add some MilliQ and an appropriate volume of 2000nM sulfide, then top up with MilliQ.
    b. Transfer the solution to a labelled Falcon tube and cap it.
    c. Prepare each dilution to completion before starting another to ensure minimal exposure to oxygen; ensure 2000nM falcon tube remains capped as much as possible.
4. After dilutions are prepared, transfer the rest of the 2000nM sulfide solution from the VF to the falcon tube.

4. Conduct Purge and Trap (PT) procedure on Na_2S and CuS calibration samples then measure DOC samples against calibration curves

1. Remove reaction tubes, collection tubes, U-shaped connectors, septum connector pieces, and sample vials and lids from the acid baths. Wash them with MilliQ and set them to dry on paper towels on the benchtop or in a fume hood.
2. Obtain Na_2S or CuS dilution series or diluted DOC sample, de-gassed MilliQ, 50% HCl, 0.05N NaOH, acid syringe, and a 1-10mL micropipette along with three tips to have them ready.
3. Place clean and dry reaction tubes, a Styrofoam holder, $CrCl_2$ powder, parafilm, a small burette stand, a large pair of gloves, an electronic scale, a scoopula, and a weigh boat in inflatable glove bag.
    a. Typically 6 reaction tubes per set of experiments are needed – two references and two samples done in duplicate.
    b. The burette stand is there for structural support when the glove bag is deflated. Wrap some paper towel over the tip of the stand to ensure that it doesn't pierce the bag.
4. Close the glove bag and attach Argon gas line. Pump Ar through the glove bag until fully inflated. Open the glove bag slightly, squeeze it to force the gas out, then close it and re-inflate it.
    a. Fill, release and re-fill with gas at least three times to ensure that oxygen is not present.
    b. Your face may be near the opening of the bag; hold your breath briefly if the Argon is blowing in your face as it is being pushed out of the bag.
5. Insert hands into the built in gloves of the bag and place lab gloves over the plastic to improve manual dexterity.
6. Place ~0.6145 grams $CrCl_2$ powder into each reaction tube.
    a. Before Cr addition, place tube on scale and tare.
    b. Add ~0.62g $CrCl_2$ powder to each tube and seal with parafilm.
    c. Ensure the $CrCl_2$ powder bottle remains tightly closed when not in use. Do not raise the bottle too high within the glove bag; if there is any oxygen present inside the bag, it will be at the top.
7. Cover the reaction tube mouths with parafilm. Remove hands from the glove bag and prepare PT system in the Styrofoam holders.
    a. Attach septum connector pieces with capped septa to the Ar gas lines.
    b. Dispense 15mL of 0.05M NaOH into each trapping tube using a micropipette.
    c. Insert the U-shaped connectors into each trapping tube such that the gasses will flow down the tube through the NaOH solution. Fasten the connectors to the tubes using plastic clips.
8. Open the glove bag and remove the parafilm-sealed reaction tubes and

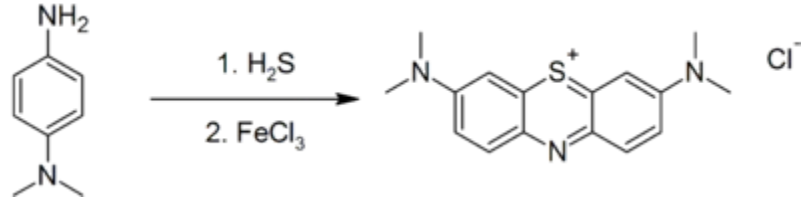
Styrofoam holder. Place them by the PT set-up.

9. Using a micropipette, add 30mL of calibration standards or samples on top of the $CrCl_2$ powder.
    a. Open the parafilm slightly and add standard/sample. Remove parafilm, then quickly attach reaction tube to the septum piece and close the PT system. Do this one at a time for each reaction tube to minimize air exposure. Parafilm should be removed before attaching to septum piece because it can interfere with the seal between glass pieces.
    b. Thoroughly mix the standards/sample container prior to sampling to ensure even distribution of analyte.
10. Turn on gas and Allow Ar to flow through the system for ~5 minutes – ensure all joints are sealed (it should be bubbling into the NaOH) and that the flow through rate is set at ~65 mL/min, then turn off gas.
11. Using the acid syringe and long needle, dispense 5 mL of 50% v/v HCl into each reaction tube through the septum; let the remaining acid drip down the center tube. Place parafilm around each septum of ensure seal.
12. Turn on the gas, adjust the flow rate of each dial to ~65 mL/min, and allow Ar to flow through for 30 minutes. Monitor the flow rate as fluctuations may occur.
13. After 30 minutes, turn off the gas and disconnect the receiving tubes, resting them slightly on the connection tubes. Some Ar may be needed to blow out the tips of the tubes.
14. Keep the solutions from the receiving tubes in part 5 – Methylene blue colourimetric analysis.
15. To clean up, the connecting tubes and receiving tubes can be put directly back into the acid (or rinse the connecting tubes to use them again). The sample tubes need to be washed and then put back into the acid bath. The N_2 tube-piece just gets rinsed off with MilliQ from a wash bottle – around where the Teflon is, the narrow tube, and then inside the larger part so that all of the chromium is gone. Then the gas lines are put onto another set of sample tubes, and lids are put back on for storage.

5. Methylene blue colourimetric analysis

1. Prepare the mixed diamine reagent (MDR).
    a. Mix Part A and Part B (in the fridge door) in a 1:1 ratio in the vial wrapped in tape and shake immediately.
    b. Only 0.5 mL is required for each sample so don't make more than 0.5 mL in excess.
    c. This is usually prepared during the 30 minutes while PT is running.
2. Remove six clean, dry sample vials and lids and label them.
3. Pour each sample into one of the vials, touching the lip of the receiving flask to the edge of the vial to help it drain (in particular, the drop that is just at the end of the ground glass connector tends to be difficult to coax out). Using a micropipette, add 5 mL MilliQ to the tube, swirling around the outside while adding it. Shake a bit to rinse and add this to the vial as well.
4. Add 0.5 mL MDR to each sample vial with a micropipette. Once it has been added, immediately put the lid on and shake the vial. After sitting on the bench for a few seconds it will turn pink. Then put the samples into the drawer to allow the methylene blue color to develop. It should be fully developed in ~3 hours.
5. Spectrophotometrically analyze methylene blue concentration in each vial by pipetting solution into a 10 cm Quartz cell and then measuring absorbance at

610.1nm.



Reagents

De-gassed MilliQ

1. Fill beaker with MilliQ.
2. Attach the aquarium stone to the free Ar gas line, rest it in the beaker of MilliQ, and cover with parafilm.
3. Turn on Ar gas and allow it to sparge for 15 – 20 minutes.
4. Cover the beaker with parafilm for temporary storage (make a new batch daily).
5. Allow the stone to dry (use a kimwipe if necessary), then store it tightly wrapped by a kimwipe.

1000 ppm (0.0312 M) Na_2S Stock Solution and Dilutions

1. De-gas 250 mL of MilliQ.
2. Fill a 250 mL VF with argon.
3. Add some MilliQ and then 1.873 g $Na_2S{\cdot}9H_2O$ powder (MW: 240.18 g/mol) to a 250 mL VF containing 5 mL of 10 M NaOH under argon atmosphere. Cover with parafilm, mix to dissolve powder, then add MilliQ to the calibration mark.
4. Transfer the solution to a 250 mL glass bottle and cap it. Wrap the bottle in aluminum foil and put it inside a larger clear bottle filled with argon to reduce likelihood of interaction with light and oxygen.

10M and 0.05M NaOH

1. Weigh out 100.0 g NaOH pellets and add to a 250mL VF containing some water.
    a. Let the solution cool and dissolve (may take up to an hour; very strongly basic solution)
    b. Fill up to the calibration mark with MQ to make a 10.0 M NaOH solution.
2. Add 1.25 mL 10.0 M NaOH to a 250 mL VF flask and fill to mark with MQ to make 0.05 M NaOH.

50% v/v HCl

1. Partially fill a 250 mL VF with MilliQ.
2. Add 125 mL [ ]'d HCl and allow it to cool before filling up to the mark with MQ.

~0.5 M HCl

1. Partially fill a 100 mL VF flask with MQ.
2. Add ~4.2 mL 12 M HCl to the flask and fill to the mark with MQ.

MDR – Part A

1. Weigh out 0.290 g N,N-dimethyl-p-phenylenediamine oxalate and pour into a clean, dry Nalgene bottle .
2. Add 50 mL [ ]'d HCl and 50 mL MQ.
3. Store in the fridge.

MDR – Part B

1. Weigh out 0.216 g $FeCl_3 \cdot 6H_2O$ and pour into a clean, dry Nalgene bottle.
2. Add 50 mL [ ]'d HCl and 50 mL MQ, and store in the fridge.

5 µM CuS colloid solution

1. Add 500 mL of MilliQ to a 1 L glass beaker using a volumetric flask. Insert the aquarium stone, cover with parafilm and de-gas.
2. Remove the gas line, add a magnetic stir bar and Na_2S stock to a 5 µM concentration (0.25 mL of 0.01M Na_2S). Re-seal parafilm covering and stir for about 5 minutes.
    a. Can add 20-50 µL of 0.05M NaOH to ensure the solution remains basic enough upon addition of acidic Cu^{2+}; aim for solution pH 7.5 – 8 after Cu^{2+} addition.
    b. It is possible make a small hole in the parafilm to insert a pH electrode and monitor pH.
3. Add Cu^{2+} standard to equimolar concentration (0.1589 mL of 1000ppm Cu^{2+}).
4. Stir continuously for 2 hours while covered and under argon atmosphere.
5. Remove parafilm and equilibrate with laboratory atmosphere.
    a. Check for smell of sulfide; if present, some sulfide got out since $Cu(NO_3)_2$ is acidic
6. Place perforated parafilm over the beaker during storage.
7. Thoroughly stir with a clean plastic rod before subsampling.

Glassware Storage

The various pieces of glassware are cleaned in separate plastic containers of 10% TMG HNO_3 (except for the pieces attached to the N_2 tubes). The connectors and developing vials are soaked in the large container, and the reaction and collection tubes both have their own (labeled) containers of HNO_3. To dry the glassware quickly, rinse acid off thoroughly with MQ, spray with acetone wash, and let dry in the oven (>100°C). Clean, dry glassware not in use can be stored in clear binds behind the gas tanks. The septum pieces (N_2 tubes) are stored on a set of reaction tubes in the styrofoam holders with glass stoppers. The acid bins are stored in the cupboard to the right side of the gas cylinders.

Replacing the acid baths (using conc TMG HNO_3)

Sample tubes: 6.25 L MilliQ + 0.6 L acid
10 cm cell/glass wool: 225 mL MilliQ + 25 mL acid
Connector tubes: 2.75 L MilliQ + 275 mL acid
NaOH tubes: 2L MilliQ + 225 mL acid

General Notes

- The tubing in the apparatus is Tygon.
- A piece of glass wool has been inserted in the Teflon tubing to filter the N_2. The gas is ultra-pure, but it still helps a lot.
- The syringes and needles are re-used (stored in a beaker on the first shelf).
- The test tube of water is designed to equalize the pressure of the Ar gas flow, which can be adjusted.
- Keep a clean paper towel on the lab bench to set things on, and cover everything with a kimwipe when not in use.
- The glass dispersion tube (for sparging MQ with Ar) is stored in a big ziploc bag (tip is wrapped with a kimwipe).

- The absorbance for the reference solutions should be < 0.009. If they aren't, then Russell suggested putting some glass wool pushed in from the male B14 joint of the connecting tube. Just push it up with a small glass rod (there is one in one of the small bins in the acid bath cupboard) to the point where the 4mm tube started, and slightly into the 4mm tube, so that the glass wool stuck when held vertical.