Preparation before the cruise:

Order chemicals. Prepare reagents. Ensure titrators are working properly. Calibrate dispensers and burettes if needed. Update any bottle volumes that need updating. Pack supplies and equipment.

Amperometric Oxygen Titrator Assembly:

The program is optimized for 120-140 mL flasks and 0.16 M concentration of thiosulfate (thio). Connect the support rod to the back of the stirrer. In order from bottom to top, connect the flask support ring, electrode wash bottle holder, detector support, and detector holder. Place accordingly in relation to size of flask. The detector holder should be placed so the electrodes are immersed in the flask, but the glass body is not submerged. Place the electrode wash bottle holder away from the flask, so the electrodes can be submerged in either 10% 5M H_2SO_4 or 10% HNO_3 in between samples (replace daily). Handle the electrodes carefully as they are fragile. Place burette and stepper motor to the right of the stirrer and the electronics box behind and slightly to the right of the burette and stepper motor. Insert the power and serial communications on the back of the electronic box, the stepper motor control cables on the left side of the electronic box, and the pair of electrode black plugs on top of the electronic box (there is no order). Place the thio y-splitter tubing into the inner slot (right side) of the valve and the sample y-splitter tubing into the outer slot (left side) of the valve on the electronics box (this orientation can change depending on firmware). Attach the rigid tube or "noodle" coming off the y-splitter tubing to the top of the glass burette tip by loosening the red plastic threaded cap on top of the burette to fix the burette tube (for airtight fit, may apply a light amount of stopcock grease to the tip). Attach the flexible "noodles" coming off the other side of the y-splitter tubing to the detector using an o-ring or electrical tape (1 cm to 1/2 inch below electrodes) and into the thio solution (1/2 inch of the bottom). Thio bottle should have a cap or parafilm with a hole in it to allow "noodle" to pass into bottle.

Initialization:

Ensure titrator and the stepper motor is off. Check the zero position of the piston. If not at zero, manually by hand zero the burette. Turn the titrator and stepper motor on. Using a waste beaker in the flask support ring, press the "Fill" button to fill up the burette with the thio solution. Ensure no air bubbles remain in the circuit by flicking the tubbing or CAREFULLY bumping the burette on the table. Once the burette is full and has cycled through a few times, hold the "Fill" button down for a few seconds during a down stroke of the piston. Place the electrodes in the DIW and acid bath. Discard titrant waste. Execute "Fill" procedure if thio has been sitting (exposed to light) in burette/tubing for extended period of time. Start the program on the computer, set up the configuration tab with serial communications, the µL per step and µL offset (these should match the stepper motor – piston bundle), slope, speed, wait, date, and time by clicking connect and set. Before processing the O2 samples always check the flask volume file is the correct for the box. These text files containing the flask volumes are separated by the color of the boxes. Check the ZERO position of the piston. Click reset&save in the program and the piston should match both zero marks. If they don't match, turn off the titrator and stepper motor, and manually rotate to the correct position. Check this position often along the sampling process.

Sampling:

Sampling from the Niskin should be done immediately once back on deck starting with the Niskin that has undergone the greatest change in pressure and temperature: first triggered (deepest). Once sampled from, a headspace is created in the Niskin causing gas exchange between the headspace gas and water. Warming of water sample can also cause outgassing and loss of oxygen. Take 10% duplicates of all bottles (ex: full rosette of 24 bottles has 2 duplicates and half rosette of 12 bottles has 1 duplicate).

A. Setup:
   Pickling reagent rack should be mounted near where sampling will take place. When not sampling, store reagent dispensers in air-controlled lab where samples will be processed. Before using dispensers, ensure no bubbles are stuck in the spout. Dispensers should be set to 1 mL. Clean dispensers when needed to avoid sticking (especially NaOH/NaI). Be careful not to lose/break any pieces of the dispenser when cleaning.
B. Procedure:
   1. Ensure flask and stopper (joined by rubber leash) are a pair (volume of flasks will be inaccurate if flasks and stoppers are mixed).
      a. The volume of these flasks is carefully calibrated. Recalibration is necessary if a flask or stopper is broken/misplaced (refer to flask volume calibration procedure).
   2. Note flask-Niskin pairs.
   3. Connect sampling “noodle” to the nipple of the petcock on the Niskin. Push in nipple. If water comes out of “noodle” when you push it, the bottle is considered leaking and the sample could be contaminated. Turn grey knob at top of Niskin to start the flow of water.
   4. Push bubbles out of “noodle” by squeezing tubing. Deeper Niskin samples will have more bubbles form in “noodle”.
   5. Rinse flask. Fill flask while tapping “noodle” on bottom of flask and moving the tube in a circular motion up the flask’s inner walls to dislodge any bubbles. Repeat 3 times.
      a. Break surface tension of new oxygen sample flasks by shaking a ½ full flask. This will reduce formation of bubbles. Rinse flask to ensure no oxygen saturated water remains in flask.
   6. Fill sample flask with water sample. Allow flask to overflow three full volumes. Note the draw temperature using a digital temperature meter inserted into flask. Pinch “noodle” to stop flow of water. Remove “noodle” (at an angle). Final sample should be filled all the way to the top. Do not insert stopper into flask. Stop the flow of water from Niskin.
      a. Draw temperature is used to compute seawater density at time of sampling. The density is used to convert oxygen concentration from µmol/L to µmol/kg. Knowing the temperature is also used to indicate if the Niskin bottle closed at the correct depth.
   7. As soon as possible (to avoid sample from oxygenating), slowly and not forcefully add the pickling reagents (in order): 1 mL of MnCl2 and 1 mL of NaI/NaOH.
      a. Ensure no bubbles in the tip of the dispenser.
      b. Do not place sample flask under dispenser until the barrel has been drawn up. Dispensers often discharge a small amount of reagent when lifting the barrel.

c. Fully immerse the tip of the dispenser into the sample flask to limit the amount of mixing in the flared neck of the flask (avoid reagent being lost when flask stopper is inserted).

8. Stopper the flask, ensuring no air bubbles have been trapped in sample.
    a. When stopper is inserted, 2 mL of sample will be lost to the volume of reagents added. This is reflected in the calculations.
9. Shake vigorously. Secure the stopper with your thumb and invert the flask using a "snapping of the wrist" motion. Agitating the flask this way is necessary in order to properly mix the reagents. Invert ~20 times. A milky yellow brown precipitate should form homogeneously throughout the sample.
10. Prevent air from entering the flask by adding water to the neck of the flask to create a water tight seal. Bubbles may form in flask, ruining the sample if this step is skipped.
11. Optional: to ensure all the oxygen has reacted with the reagents, it is recommended that the flasks are shaken again ~30 min later. Create water tight seal around the neck of the flask again.
12. Store the flasks in their designated covered crate long enough for the precipitate to lower to the bottom third of the flask. Samples can be stored for many days before analyzing if water tight seal is present.

Titration:

A. Reagent blank determination – Carpenter (1965) method.
Blanks should be performed at minimum at the beginning of the cruise, if any instrumentation is changed, and if a different batch of reagents is used. Blanks can optionally be performed before the first sample is analyzed, at the beginning of each day, at the same temperature as samples are analyzed, and at the end of the cruise. Designate flasks and magnetic stir bars that will only be used for standards and blanks. Rinse these flasks and stir bars multiple times with tap water then deionized water to ensure any residual Mn ions are removed. Wear goggles and gloves if possible.
    1. Fill a standard flask approximately half full with DIW. Add a stir bar and place on stirrer.
    2. Slowly and carefully add 1 mL of H_2SO_4 solution. Rinse the neck of the flask with DIW and mix.
        a. Remember: Ensure no bubbles in the tip of the dispenser. Do not place sample flask under dispenser until the barrel has been drawn up. Dispensers often discharge a small amount of reagent when lifting the barrel. This will be true anytime you use a dispenser.
    3. Slowly and carefully add 1 mL of NaI/NaOH solution. Rinse the neck of the flask with DIW and mix.
    4. Slowly and carefully add 1 mL of $MnCl_2$ solution. Rinse the neck of the flask with DIW and mix.
        a. If precipitate forms, this indicates that the standard has been contaminated.
        b. Solution should be clear like water.
    5. Slowly and carefully add **1 mL** of KIO_3 solution. Rinse the neck of the flask with DIW and mix.
    6. Fill flask up to neck with DIW.

7. Position flask and detector so that the electrodes are aligned, separate, and fully immersed, the glass body of the detector is not submerged, bubbles are not created, and a vortex does not form.
    a. This can be done by changing the speed of the stirrer and the height of the detector.
8. Titrate the blank.
    a. Piston zero marks match.
    b. Speed = 0.4 - 0.8 (at discretion of analyst).
    c. Click blank.
    d. Record endpoint.
    e. Click reset&save with the electrodes **outside** the flask. Allow the fill cycle to complete until you hear a beep.
    f. If the burette is not reset, the plunger may exceed its capacity and cause the glass tip to burst.
9. Slowly and carefully add **1 mL** of KIO_3 solution to the same flask.
10. Titrate a second time.
    a. Piston zero marks match.
    b. Speed = 0.4 - 0.8 (at discretion of analyst).
    c. Click blank.
    d. Record endpoint.
    e. Click reset&save with the electrodes **outside** the flask. Allow the fill cycle to complete until you hear a beep.
    f. If the burette is not reset, the plunger may exceed its capacity and cause the glass tip to burst.
11. The endpoint values will probably be around 60s or 70s. The difference of the replicates should be <2 µL. **Enter the difference in the Blk, µL field.**
    a. If large variability in blank endpoints, check equipment.
12. Rinse flask and stir bar multiple times with tap water then DIW.

B. Standardization of thiosulfate – Carpenter (1965) method.

Standardization should be performed at minimum at the beginning of the cruise, when the titrant bottle is refilled, if any instrumentation is changed, and if titrant has been sitting unused for several days. Standards can optionally be performed before the first sample is analyzed, at the beginning of each day, and at the same temperature as samples are analyzed. Standard value should be ~700 µL if thiosulfate is 0.14 M, but will likely vary amongst different batches. When changing the titrant to a new solution of thiosulfate, rinse the bottle a few times with small amounts of the new batch. Note the normality of each batch. Designate flasks and magnetic stir bars that will only be used for standards and blanks. Rinse these flasks and stir bars multiple times with tap water then deionized water to ensure any residual Mn ions are removed. Wear goggles and gloves if possible.

1. Fill a standard flask approximately half full with DIW. Add a stir bar and place on stirrer.
2. Slowly and carefully add 1 mL of H_2SO_4 solution. Rinse the neck of the flask with DIW and mix.
3. Slowly and carefully add 1 mL of NaI/NaOH solution. Rinse the neck of the flask with DIW and mix.

4. Slowly and carefully add 1 mL of $MnCl_2$ solution. Rinse the neck of the flask with DIW and mix.
    a. If precipitate forms, this indicates that the standard has been contaminated.
    b. Solution should be clear like water.
5. Slowly and carefully add **10 mL** of KIO_3 solution. It is of utmost importance that the KIO3 is dispensed accurately and consistently. Rinse the neck of the flask with DIW and mix.
    a. Solution should be yellowish.
6. Fill flask up to neck with DIW.
7. Position flask and detector so that the electrodes are aligned, separate, and fully immersed, the glass body of the detector is not submerged, bubbles are not created, and a vortex does not form.
    a. This can be done by changing the speed of the stirrer and the height of the detector.
8. Titrate the standard.
    a. Piston zero marks match.
    b. Speed = 0.4 - 0.8 (at discretion of analyst)
    c. Click sample.
    d. Record endpoint.
    e. Click reset&save with the electrodes **still in the flask**. Allow the fill cycle to complete until you hear a beep.
    f. If the burette is not reset, the plunger may exceed its capacity and cause the glass tip to burst.
9. Repeat this process for at least 3 replicates with endpoints within ±0.3% of each other (±1-2 µL). Average the replicates and calculate the standard deviation (<1 is acceptable but aim for <0.5). **Enter the average in the STD field (bottom left of the program).**
    a. If large variability in standard endpoints, check equipment.
10. Rinse flask and stir bar multiple times with tap water then DIW.

C. Samples

It is recommended to not solely depend on a computer file for data recording. Record data by hand on a log sheet as well. On the main and configuration tab, set the fields to their appropriate readings for each individual sample (refer to Oxygen Titration Program Tabs' Breakdown). STD and Blk, µL should already be set.

1. Dump water from neck flask and remove excess with a Kimwipe.
2. Remove the stopper and add a stir bar.
3. Add 1 mL of H_2SO_4 and mix, allowing all the precipitate to dissolve.
    a. Solution should be a clear medium to dark straw yellow color.
    b. If all precipitate doesn't dissolve, add another 1 mL of H_2SO_4.
    c. Position flask and detector so that the electrodes are aligned, separate, and fully immersed, the glass body of the detector is not submerged, bubbles are not created, and a vortex does not form.
4. Titrate the sample.
    a. Piston zero marks match.
    b. Click sample.
        i. Sample will eventually turn clear.
    c. Record endpoint, O2 µM.l, and O2.µM.kg.

d. Click reset&save with the electrodes still in the flask or collected in an excess flask/beaker.
    i. If excess is collected in a separate flask/beaker, you can ensure there are no air bubbles or blockages by watching the rest&save.
e. Place electrodes in electrode wash bottle holder.
f. Clean electrodes and "noodle" with Kimwipe before placing in next sample. Be careful with the electrodes.

5. Rinse flask and stir bar with tap water.

Notes:

- Broad
    - Dissolved oxygen refers to the amount of oxygen gas present in water, while discrete oxygen refers to the measurement of dissolved oxygen at specific points in time or locations.
    - Reason for samples: hysteresis correction for the seabird sensors (CTD calibration).
        - Pressure causes more of an offset after 1000m.
- Titration program
    - Darker samples = more precipitate = more oxygen = more titrant = higher slope.
        - If sample needs more titrant, set a higher slope.
        - Needs at least 10 point to create a good plot.
            - If too few points, set a lower slope.
        - System times out at 30 points and won't find an endpoint if slope is set too low.
            - Set a higher slope.
    - This is an example of a good plot:
        - The red line should be as horizontal as possible, meaning that the solution's conductivity is not changing anymore. That's a clear signal that the endpoint was passed after a sharp change in the conductivity.
        - This is a helpful visualization but not something to live by. The software plots don't always look "good" but may look "good" if plotted using another program.
    - Based on the flask size and thiosulfate concentration, the program uses a slope factor to anticipate the endpoint.
    - Number explanations on program as titration occurs:
        1) That thio is being dispensed every 8 seconds.
        2) Amount of titrant being dispensed (µL).
        3) Current of the sample (microamps).
            - Current is directly correlated to amount of oxygen in sample still so when current goes down to zero, no oxygen remains in the sample and we know much titrant it took to remove the oxygen in a known volume of the sample.
        4) Number of steps in titration.
        5) Once the software detects <0.5 amperage, it will go 9 more steps.
    - If flask volume file needs to be updated:

        - Titrator program – disconnect.
        - Go to volume file.
        - Update volume.
        - Save.
        - Got to titrator program – connect – double check that the update is seen.
    - Titration apparatus
        - Flush the burette with distilled water before storing.
        - Sometimes the electronics box needs to be reset:
            - Turn box off.
            - Turn stepper motor off.
            - Turn box on.
            - Twist stepper motor to zero by hand.
            - Turn stepper motor on.
            - Reset and save.
            - Configuration and reset stepper motor values, slope, wait, and speed.