Standard Operating Procedure (SOP) 3.1.1.2

By the Coyote Creek Riparian Station

Measurements of Dissolved Oxygen with the Modified Winkler Titration

The following instructions assume the use of the all-liquid reagent kit for measuring dissolved oxygen in the field, as provided by LaMotte (other field kits would present small variations in the way reagents are packaged and dispensed, and in minute details of the titration equipment).

It is assumed that the water sample has been collected with appropriate devices. Sampling techniques for collecting a water sample without contact with air are available in this Compendium (SOP-2.1.1.2 Water sampling using the Kemmerer Bottle, and SOP-2.1.1.3 Water sampling using the syringe pump apparatus.)

Measurement of Dissolved Oxygen

The procedure is composed of several steps, broken into two phases:. In the first phase the oxygen in the sample is "fixed" by reagents that we add to the sample bottle, to form a chemical complex between the free oxygen and some of the reagents. In the second phase the amount of the complex (which reflects the original concentration of dissolved oxygen in the sample) is quantified by slowly adding a “neutralizing agent” until all the complex molecules disappear. This process is called “titration”, the neutralizing agent is called “titrant”, and the amount of titrant used reflects the original oxygen concentration in the sample.

Phase 1: Fixing the sample

1.1 Once you have collected the sample in the D.O. bottle, examine the full bottle to make sure no air bubbles are trapped inside. (An air bubble will produce false, high readings. If an air bubble is present, another sample must be taken from the creek). Fix the sample immediately after collection (see steps 1.2 and 1.3 below).

1.2 Add **8 drops of Manganous Sulfate Solution** *(white cap)* and **8 drops of Alkaline Potassium Iodide Azide *(white cap).*** Some of the sample will overflow as chemicals are added, but sufficient amounts of the oxygen-reacting chemicals will fall to the bottom of the bottle. These concentrated reagents are added in excess, so if you put 7 or 9 drops it is probably still accurate. **Cap** the D.O. bottle immediately after adding the second reagent. The overflow assures that when the sample bottle is closed, the cone inside the cap will push excess liquid out and no air will be trapped inside. **Mix** the content of the D.O. bottle by inverting several times. The liquid will turn cloudy, and then a precipitate (chunks

of solid flakes) will form. Allow the precipitate to settle below the shoulder of the bottle before proceeding. Proceeding too quickly may result in an incomplete reaction and produce false low readings.

1.3 Add **8 drops of Sulfuric Acid, 1:1** *(red cap).*
Cap the bottle, wipe the excess liquid from the external surface of the bottle *(this stuff is nasty!)* and gently mix the content until all the precipitate has dissolved. At first you will see the fluffy precipitate turn into brown flakes, looking very different from the particulates in the original sample, and you need to wait until these flakes dissolve. If the brown flakes are still there after a few minutes, add a few more drops of acid. A clear- yellow (low D.O.) to brown orange color (high D.O.) will develop, depending on the oxygen content of the sample. The sample is now fixed. Following completion of this step, contact between the water sample and the atmosphere will not affect the test results, and you may store the fixed sample for a few hours (not overnight).

Phase 2: Titrating the complex

2.1 Rinse the titration vial and the direct-reading titrator syringe with deionized water (DI).

2.2 Fill the direct reading titrator (*syringe)* with the titrant solution, **Sodium Thiosulfate 0.025N.** To do this, first **insert** the titrator into the plastic fitting of the titrant solution bottle (hold it close to the tip when you do this). **Invert** the bottle and slowly **withdraw** the plunger until the bottom of the plunger is opposite the zero mark on the scale (Figure 1). Small air bubbles may appear in the titrator barrel. Expel the bubbles by partially filling the barrel and pumping the titration solution back into the inverted reagent container, or by tapping the barrel of the syringe with your finger to dislodge clinging air bubbles. Repeat these actions until the bubbles disappear.) Turn the bottle right-side-up and remove the titrator.

2.3 **Transfer** 20 ml of the fixed water sample from the D.O. bottle into the titration vial. Remember to read the measurement from the bottom of the meniscus.

2.4 Insert the full titrator syringe into the center hole of the titration vial cap. While swirling the vial, slowly press the plunger until the water sample becomes pale yellow. **Do not titrate beyond a pale yellow tinge**.

2.5 Add **8 drops** of the **Starch Indicator Solution** to the titration vial. The liquid should turn blue. **Cap** the vial.

2.6 **Insert** the full titrator syringe into the center hole in the cap of the titration vial. While swirling the vial, slowly press the plunger until the water sample

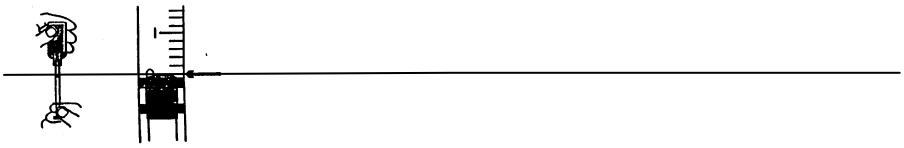
solution is less blue. Continue to press the plunger to add one drop of titrant at a time**. Swirl** to mix THOROUGHLY after each drop. Continue until the blue color instantly turns colorless. Color changes will occur where the drop first contacts the sample, but the drop must be dispersed throughout the sample. The entire solution will turn clear and remain that way for 1-2 minutes when the entire sample is at the endpoint.

If the plunger tip reaches the bottom line of the titrator before endpoint color change, record the volume already used, and then refill the titrator and continue the titration.

2.7 Read the test results where the plunger tip meets the scale (Figure 1). Each minor division on the scale equals 0.2 mg/l (parts per million, or ppm). If the titrator was refilled, add the first 10 mg/l to the last reading to reflect the total amount of reagent dispensed. (See Note below)

2.8 Record the results as mg/l dissolved oxygen in the "Value" column in your field data sheet. If you repeat the titration of the same fixed sample, you are conducting a "replicate measurement". In this case, record the second titration in the rep/dup/range column and add "rep" (this means it was a replicate titration). If you take a new sample form the creek within a few minutes and repeat both phases 1 and 2, it is considered a "field duplicate". In that case, record the result of the second DO test in the rep/dup/range column and add "dup".

Figure 1. Filling and Reading the Titrator in the Dissolved Oxygen Measurement.



Filling the syringe. Note tip of syringe is at "0" line of glass barrel.

Reading the results of the titration on the glass syringe barrel.

After you have completed all the titration steps and recorded the results, discard the titrated solution from the vial into the waste bucket. Thoroughly rinse the titration vial with distilled water and discard in waste bucket.
After all D.O. titrations are complete, thoroughly rinse the D.O. bottle, the titration vial, and the titrator syringe with distilled water, putting the waste into the waste bucket for proper disposal.

Note: Check with your monitoring team leader that your result is reasonable. If there is any concern that the D.O. value is not correct, repeat your measurement. For example, if the titration was overrun, do not record the data, but redo the titration. There is enough volume of fixed sample for three repeats. If the entire procedure (sampling, fixing, titrating) was done correctly and the value is unusually low, another water sample may also be taken and the entire preparation repeated.

What to expect:

Cool, fast flowing turbulent water is expected to contain D.O. at saturation levels (9-10 mg/l, depending on the temperature). The fixed sample is orange, and a nearly a full syringe of titrant is needed. Algae and aquatic plants will add D.O. to the water in the light, as a result of photosynthesis. Pools may have supersaturation (levels above saturation) of oxygen if there are algae or plants in them or immediately upstream, even if the water is turbid. In that case the fixed sample will appear very orange, and a few drops of acid may be needed to dissolve the brown flakes. Refill of the titrator syringe will be necessary, and should be done as quickly as possible. Turbid, smelly water in stagnant pools may be depleted of oxygen. The fixed samples will be yellow or pale yellow, and the titrant should be added careful (drop by drop) immediately at the beginning of the titration. If the D.O. of the sample is below 4.0 mg/l, another sample needs to be collected and the D.O. test should be repeated.

Accuracy Checks

Because you cannot really obtain an oxygen "Standard", you need to conduct accuracy checks often. One of these checks is to measure D.O. in clean water (e.g., deionized water, DI) at dissolved oxygen saturation at a given temperature. Please refer to the Information Paper on dissolved oxygen for further information. Essentially, you can use clean water that has been at ambient temperature, not in the refrigerator, and prepare a saturated DI sample by transferring about a glass of water, from cup to cup back and forth, about 20 times, creating turbulence and ample contact with air. Then you use this sample to fill a D.O. bottle, measure temperature, and fix the sample for titration.

You can also buy and use a "standard" solution, prepared by the manufacturer specifically for the Winkler titration, to test the strength of your sodium

thiosulfate titrant. This standard represents a phase equivalent to fixed and acidified sample (i.e., the orange solution in the D.O. bottle after reagents 1, 2 and 3 have been added).

Monitoring Tips

For the Winkler titration - sulfide interferes with the reagents and may mask the presence of low D.O. levels. Sulfide and D.O. usually do not co-exist, but if it is desirable to detect traces of D.O., the sulfide must be precipitated with zinc acetate and the supernatant be used for the Winkler titration.

Sources and Resources

This SOP was created by personnel of the Coyote Creek Riparian Station (CCRS 1993) and was later revised by Woodward Clyde Consultants (WCC 1996) and by SFEI (1996) It was refined for this Compendium by the Clean Water Team implementing the Citizen Monitoring Program of the State Water Resources Control Board in 2000.

References

Coyote Creek Riparian Station (CCRS) 1993. The Santa Clara County Stream Inventory. Progress Report and Protocols prepared by CCRS, Alviso, CA. November.

San Francisco Estuary Institute (SFEI) 1996. Volunteer Monitoring Protocols. Guidance document prepared for the State Water Resources Control Board, Sacramento, CA.

Woodward-Clyde Consultants (WCC). 1996. Watershed Monitoring by Volunteers, FY 94-95 Pilot Study. Report prepared for the Alameda Countywide Clean Water Program, Hayward, CA, May.