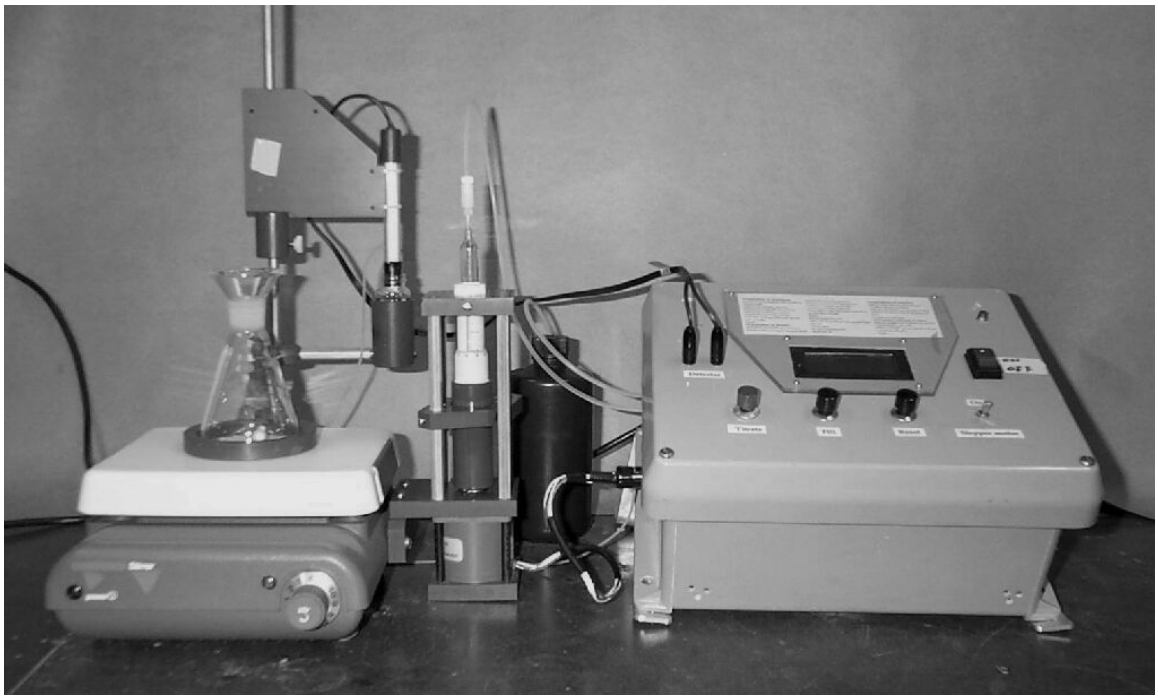
The Amperometric Oxygen Titrator: Assembly and Procedure



The Lamont-Doherty Earth Observatory
of Columbia University

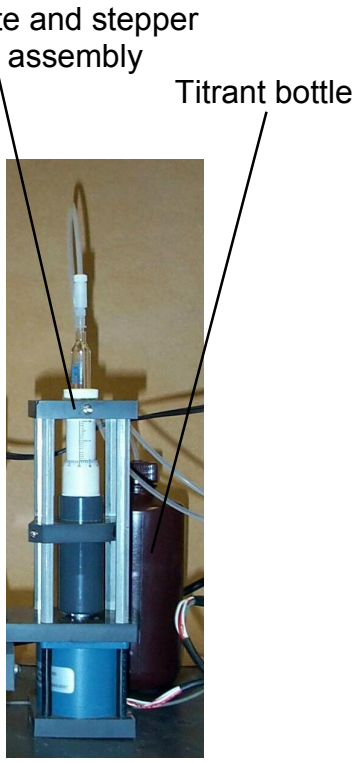
Introduction

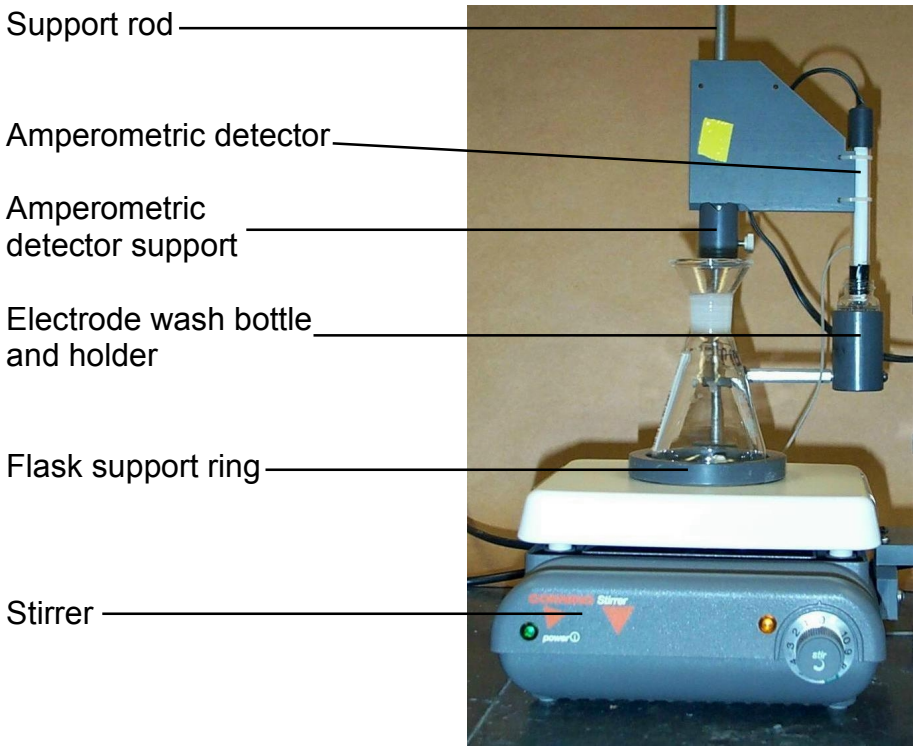
This document describes the assembly and procedures for operating the amperometric oxygen titrator designed by Dr. Chris Langdon.

Assembly should take less than one hour. Following assembly the burette needs to be filled with the thiosulfate solution and any bubbles removed from the lines. Next, the document goes over the procedures for running standards, blanks and samples. Finally, there is a section on common problems. The appendices go over matters that will be useful for the experienced user.

The instrument outputs the endpoint of a sample, standard or blank titration in units of μliters of titrant. The system has been optimized to work with a 0.16 M thiosulfate solution, 25g/L of anhydrous thiosulfate or 40 g/L of the pentahydrate form. This concentration gives good precision in all waters from the poles to the tropics. It is strongly advised that first time users employ the recommended concentration. An excel spreadsheet has been created to perform the calculations that convert the endpoint to oxygen concentration in units of μmoles/*l* or ml/*l*. The spreadsheet is named "O2 calculations.xls" and is included on the diskette. The spreadsheet contains a worksheet for the bottle volumes, a worksheet for standards and blanks and a worksheet for performing the oxygen concentration calculations. Use the entries given as an example. Enter your values for the standard and blank values. Copy down the cells that contain the bottle volume lookup and the oxygen concentration calculation formulas. The other columns can be changed to meet the users needs.

Parts





(Optional computer with software such as TxTools to download the titrator output data.)

Assembly

Secure the support rod to the back of the stirrer. Slide onto the support rod, in order: the flask support ring, the electrode wash bottle holder, the detector support and the detector. The exact placement will depend upon flask size. The detector support should be adjusted so that the wires of the detector but not the glass body are immersed in the titration flask. Inserting the the detector too deeply into the flask can result in poor performance of the titrator due to poor mixing of the solution in the neck of the flask. Now adjust the height of the wash cup so that when the detector is rotated away from the titration flask the tip of the detector is immersed in DIW contained in the wash cap and gets a good rinse between samples.

Attach the burette and stepper motor assembly to the right side of the stirrer.

Attach the electronics box to the aluminum angle supports.

Insert the stepper motor power cable / interface into the left side of the electronics box. Insert the detector pin plugs into the top left of the electronics box.

Attach the rigid plastic tube with the burette adaptor to the glass burette tip, a light application of stopcock grease may be applied to the tip to ensure an airtight fit. Insert the plain rigid plastic titrant uptake tube into the titrant bottle through one of the holes in the cap to within 1/2 inch of the bottom. Attach the third c-flex plastic titrant dispensing tube to the detector with tape or by snaking it through the space behind the glass electrode casing. The tube should extend about 1/2 inch below the electrodes.

A computer may be connected to the back of the electronics box via an RS232 port in order to communicate with the titrator and to download data. The computer connection is required to capture the titration data to disk. A Windows program called Txtools4.14 has been provided for

this purpose. The communication protocol is baud 19.2K, 8 data bits, 1 stop bit, no parity and XON/XOFF handshaking. You can capture data to disk by clicking on the Capt Off button on the bottom command bar. A box will open for you to enter a filename. This is useful for diagnostic purposes and is good practise. However, please note that the titration data written to the file is not particularly useful for routine data reduction. For that you need to record the sample ID and endpoint on a log sheet and manually enter into the "O2 calculations.xls" spreadsheet. This sounds like a pain but really isn't. If you keep the spreadsheet open you can enter the information as it comes out. With occasional power failures and computer crashes I have found it is much safer to keep a hand written log of the data. Txtools is also needed if you need change one of the parameters controlling how quickly the titrator approaches the endpoint. The parameter values stored in your titrator have been optimized and only a experienced user should consider altering the values (Appendix A). Txtools is also needed to upload software updates (Appendix B). Again only experienced users should consider this and only after consulting with C. Langdon. Misuse of this procedure can wipe out the titration program stored in the titrator and put you out of business.

Initialization

Fill the electrode wash bottle with distilled water. Replace daily.

The titrator should be off. Zero the burette by hand. Make sure the stepper motor switch is in the off position. Turn on the titrator. Switch stepper motor switch to the on position.

Place a waste beaker in the flask support ring and position the detector with the titrant dispensing tube above the beaker.

Push the *FILL* button to begin the burette fill cycle.

Examine the burette and the two teflon tubes for bubbles. Dislodge any bubbles by tapping with a finger.

When the burette is full and all the tubing is clear of bubbles, push *FILL* when the burette piston is on a down stroke to end the fill cycle. Discard the titrant collected in the waste beaker.

Store the electrodes in the bath.

Fill all the reagent dispensers after they have been rinsed a few times with small aliquots of the solutions.

Titration

Prepare a log sheet to record the sample and flask information, results, reagent additions and observations. It is not advisable to depend solely on a computer file for the data.

Check the level of the titrant to ensure enough solution to finish the samples. Standards should be run each day samples are run. Over the course of the cruise that standard value will slowly decrease. This is normal. A typical standard value is 700 µL.

> The titrant, sodium thiosulfate $Na_2S_2O_3$, degrades in light and must be stored in brown containers. Glass is recommended for use with the titrator as it allows easy determination of the titrant level and the position of the uptake tube.
> The titrator is optimized for sample flask size of 120-140 ml and titrant concentration of 0.16 molar. The titrator uses a slope factor (m) to anticipate the endpoint. If either the flask size or the titrant concentration are changed the slope factor must be changed. See Appendix A for instructions on the calculation and change of the slope and the speed.

Check the level of the other reagents. It is not necessary to run standards when any of the other reagent bottles are refilled. Blanks should be run for each batch of $MnCl_2$ and NaI-NaOH. Note any change of reagents on the log sheet. A typical blank is ±2 µL or less. Many people have trouble getting good blanks. If the blank is much larger than ±2 µL it should be considered suspect.

If a computer is being used to download the titrator output, initiate the program that will save it before turning on the titrator power to ensure the download of the speed and slope factors which are output only when the titrator is first turned on. Devise a naming procedure for the files, such as 09NOV01A.DAT, 09NOV01B.DAT, 10NOV01A.DAT, etc.

Swirl the titrant bottle to ensure the titrant is well mixed, especially if the titrant bottle has been refilled. Flush the burette 2 to 3 times. Discard the initial few *ml*s of the titrant, after which the titrant dispensing tube may be placed in the air hole of the titrant bottle cap to conserve titrant, wiping off any distilled water on the outside of the tube from the wash bottle. Check for bubbles, tapping with a finger to dislodge any.

Standards

Standards should be run once a day, more often if there is significant variation in the results. Run standards until achieving at least three replicates in close agreement i.e. ±1-2 μL. If it is necessary to run many standards due to large variability, check all equipment. The standard should be around 700 μL if the thiosulfate is 0.16 M. If it is much different there is a problem.

Standards must be run whenever the titrant bottle is refilled. It is not unusual for there to be small variations in standard values with different titrant batches.

If a new batch of the standard solution is used, drain the standard reagent bottle and thoroughly rinse it a few times with small aliquots of the new solution. Note the normality of the standard batch on the log sheet.

Reserve several sample flasks and teflon coated magnetic stirring rods for the determination of standards and blanks. Scrub the flasks and rinse well with distilled water. It is very important to thoroughly clean the flasks and stirrers to remove any residual Mn ions.

Flush the standard solution into a waste container until the tubing is clear of any bubbles. Flush the other reagent dispensers at least once, either back into the reagent bottle or a waste container; this will depend on the type of dispenser used.

Rinse a flask and stirrer with deionized water.

Fill the flask about 1/3 with deionized water.

Add 1*ml* of H_2SO_4 solution and mix well.

Add 1*ml* of NaOH-NaI solution and mix well.

Note the reagent order for standards and blanks is the reverse as for sampling.

Add 1*ml* of $MnCl_2$ solution and mix well.

If there is any indication of precipitate formation, discard, as the standard has been contaminated. This will occur if the sample is not well mixed after the addition of each reagent.

Add 10*ml* of KIO_3 standard solution and mix well.

The volume of the standard solution dispensed is extremely critical. There must be no bubbles in the dispenser tip. Do not permit the standard dispenser to clear into the sample.

Fill the flask with deionized water to the bottom of the neck.

Place the flask on the stirrer in the flask support ring. Set the stirrer speed so that bubbles are not entrained or a large vortex forms in the solution.

Position the electrodes with the titrant dispensing tube in the solution. The electrodes should be fully immersed but not the glass body of the electrode.

Push the *TITRATE* button. Push the *TITRATE* button a second time after approximately two seconds to signify that you are running a sample a standard. The titrator will go past the endpoint and determine the endpoint as the intersection of line segments passing through the data just before and just after the endpoint.

The titrator will *beep* when the titration is complete. Record the value of the titrant dispensed on the log sheet.

Rotate the detector into the wash cup.

Push the *RESET* button to refill the burette.

If the burette is not RESET, the titrator will start from that position on the next titration and it may exceed its capacity. The plunger may burst the glass burette tip.
Do not remove until the fill cycle is complete, the titrator will *beep*.

Scrub the flask well and rinse with distilled water.

Blanks

Blanks should be run whenever any of the reagents is changed. Run 2 or 3, more if there is no close agreement.

Flush the reagents if they have not already been flushed.

Rinse a flask and stirrer with deionized water.

Fill the flask about 1/3 with deionized water.

Add 1*ml* of H_2SO_4 solution and mix well.

Add 1*ml* of NaOH-NaI solution and mix well.

Note the reagent order for blanks and standards is the reverse as for sampling.

Add 1*ml* of $MnCl_2$ solution and mix well.

If there is any indication of precipitate formation, discard, as the blank has been contaminated. This will occur if the sample is not well mixed after the addition of each reagent.

Add 1*ml* of the KIO_3 standard solution and mix well.

Note that for blanks the amount of standard dispensed is 1*ml*, not 10*ml* as for standards.

Fill the flask with deionized water to the bottom of the neck.

Wait a few minutes.

Position the electrodes with the titrant dispensing tube in the solution. The electrodes should be fully immersed.

Push the *TITRATE* button. Push the *FILL* button after approximately two seconds to titrate.

Note that for blanks the *FILL* button is the second button pushed, not the *TITRATE* button as for standards and samples.

Record the result (R1) on the log sheet.

Rotate the detector into the bath.

Push the *RESET* button to refill the burette.

Add an additional 1*ml* of the standard solution.

Wait a few minutes.

Push the *TITRATE* button. Push the *FILL* button after approximately two seconds to titrate.

Record the result (R2) on the log sheet.

Rotate the detector into the bath.

Push the *RESET* button to refill the burette.

Scrub the flask well and rinse with distilled water.

The blank value is calculated as: R1 – R2.

Sample Titration

Open an oxygen sample flask and drop in a stirring rod. If samples have been stored with the flask neck filled with water, dry well.

Add 1*ml* of H_2SO_4 solution.

Place the flask on the stirrer in the support ring. Set the stirrer speed so that bubbles are not entrained or a large vortex forms in the solution. All of the precipitate should dissolve leaving the solution a clear medium to dark straw yellow, add more H_2SO_4 as necessary to dissolve all the precipitate adding additional acid does not have any effect on the endpoint.

Position the electrodes for titration, fully immersed.

Push the *TITRATE* button. Push the *TITRATE* button a second time after approximately two seconds.

The titrator will *beep* when the titration is complete. Record the titration value on the log sheet with the sample flask number.

Rotate the detector into the bath.

Push the *RESET* button to refill the burette.

If the burette is not RESET, the burette may exceed its capacity. The plunger may burst the glass burette tip. Do not remove until the fill cycle is complete, the titrator will *beep*.

Rinse the flask thoroughly.

Storage

Flush the system thoroughly with distilled water before storage. This is critical. If this is not done the titrator will dry out in the tubing and solenoid valve and may result in a blockage.

There are several variations of sampling techniques. This section is included for completeness and is geared toward sampling at sea.

Setup for Sampling

Store the plastic ends of the oxygen sampling tubes in seawater to soften the plastic. This will reduce bubble formation during sampling, and is best started about two days before sampling is to begin. Replace the seawater twice a week to prevent fouling.

Find a location near were the sampling will be done to mount the pickling reagent rack. If sampling will not be done on deck, it is advisable to mount some type of catch container with an absorbent under the rack as NaOH-NaI may leak from the dispenser tips due to capillary action. Mount the catch container so as not to interfere with pickling.

Store the reagent dispensers in the oxygen lab when not sampling. Note the room temperature.

Check that the reagent dispensers contain enough solution before each station. Swirl the reagent bottles and dispense at least 2 to 3 times from each, or until there are no bubbles in any of the tubes. The solutions may be flushed back into the reagent bottle or a waste container; this will depend on the type of dispenser used. Check that the dispensers are set to 1*ml*. The NaOH/NaI dispenser typically stiffens up after a week or so and must be disassembled and cleaned. Be very careful not to lose any of the small pieces. In particular the ruby bead that serves as a check valve. Wear safety glasses during this operation. I learned the hard way.

Sampling

Select an oxygen sample flask. Record the flask number and the rosette bottle number on the log sheet.

Attach the sample tube to the water sample bottle and open the stopcock. Squeeze the sample tube as necessary to remove any air bubbles. Rinse the sample flask.

> New oxygen sample flasks may have a chronic bubble formation problem. Stopper the flask with 1/4 to 1/2 water and shake vigorously. This will break the surface tension on the inside of the flask and greatly reduce the formation of bubbles. Rinse the flask very well as the water has become supersaturated with oxygen.

Insert the glass end of the sample tube into the flask and allow it to fill slowly in order to prevent bubble formation, squeezing the sample tube to regulate the water flow. Once the flask is full, the rate of flow can be maximized. Use the glass tube to work loose any bubbles. When there are no bubbles adhering to the side, allow the sample flask to overflow 2 to 3 times its volume. Rinse the stopper with the overflow. The end of the sampling tube should be close to the bottom of the flask.

Pickle the sample. Insert the $MnCl_2$ reagent dispenser tube to within 1/2 inch of the sample flask bottom and dispense. Dispense the NaOH-NaI solution in the same manner. Dispensing the reagents close to the bottom of the flask will ensure that no precipitate forms from the water in the neck of the flask which will be displaced by the stopper.

> Dispensers clear the tip by ejecting a small amount of reagent when the plunger is raised. Do not allow the reagent to clear into a sample. Check the type of dispensers used; some dispensers clear the tip before the sample is delivered and some clear after.

Stopper the flask carefully without trapping bubbles. Shake the sample very well; a milky yellow brown precipitate should be evenly dispersed throughout the sample.

Post Sampling

Return the reagent bottles to the lab after the rosette sampling is finished.

Reshake the flasks when the precipitate has settled by 1/2, about 20 minutes to one hour.

> If the samples are not reshaken, a crust may form on the bottom of the flask. This crust may not totally dissolve during the titration procedure, resulting in an artificially low value. Furthermore, the crust may remain and contaminate future samples in that flask. If a flask has a crust on the bottom, fill it about 1/4 with water and add 1*ml* of H_2SO_4. Swirl the flask until the crust is dissolved and rinse well.

Samples may be titrated when the precipitate has settled the second time. Samples should be titrated within 1 to 2 days for optimal results.

If the samples will not be titrated within one day, fill the rims of each flask with distilled water and cover with damp paper towels or newspaper. This will prevent the sample from drying out and air being sucked into the top of the sample flask, contaminating it. Check the flasks regularly for water in the rims, this will ensure viability of the samples. The flask rims must remain filled with water as long as the samples remain untitrated. Alternatively, all the flasks may be submersed in a water bath. It is recommended to store the flasks away from light.

Suggested Readings:

Anonymous, 1971. Marine Technicians Handbook: Oxygen Analysis. Scripps Institution of Oceanography Reference No. 71-8.

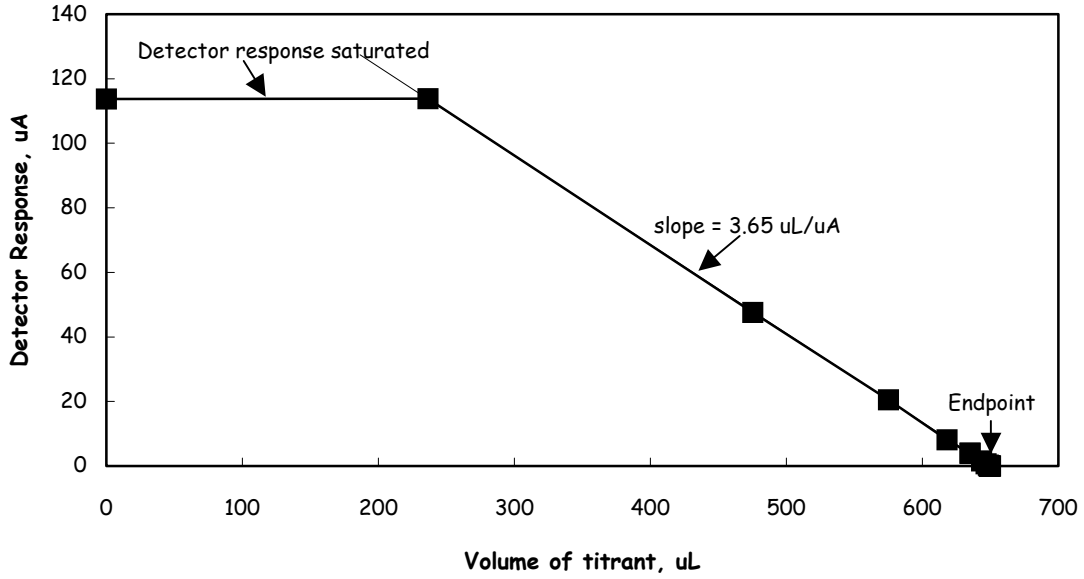
Carpenter, J. H., 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. *Limnol. Oceanogr*. **10**, 141-143.

Culberson, C. H. and S. Huang, 1987. Automated amperometric oxygen titration. *Deep-Sea Research* **34**, 875-880.

[WOCE…]

Appendix A: Determination of the titration slope and optimal titrator speed.

Write down the volume dispensed and detector current from a representative titration. If a computer is used to capture the output, the data from the file can be used. Plot the volume of titrant versus the detector current. The plot should look like the figure below.



Calculate the reciprocal of the slope of the linear portion of the titration curve by dividing the change in volume of titrant by the change in detector current. The slope will depend on the concentration of the thiosulfate and the volume of the bottle. The titrator controller uses the slope to determine how much titrant to add. Each time the titrator prepares to make an addition it makes the following computation:

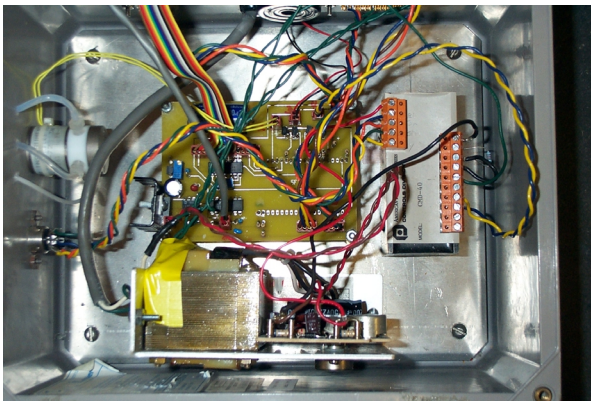
Volume of titrant = m*speed*Detector current

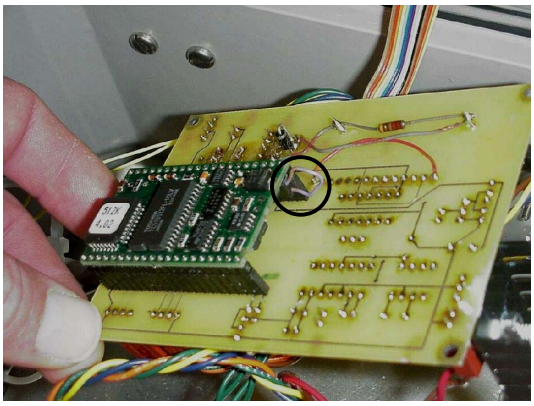
Where m is the slope, speed is a unitless number between 0 and 1. Typically speed is set to 0.6. This means that each time the titrator computes how much titrant to add to reach the end point and then adds 60% of that to ensure that it approaches the endpoint quickly but does not overshoot it. If the value used for m is too large the titrator will approach the endpoint too quickly. This can result in collecting too few data points to accurately determine the endpoint. If the value of m is too low the titrator will take a long time to reach the endpoint. Optimal operation is achieved when the value m closely matches the actual slope of the curve for your situation. Once m is set correctly, the value of speed can be tweaked upwards to improve speed. As you do, however, the number of points will drop. There should be at least nine data points during the final stage (stage 3) of the titration.

Appendix B: Software Update Procedure.

Before updating the software, download the current program and save it to disk. Start txtools and turn on the titrator, # should be the prompt. Enter Ctrl+c, select tattletale, select remind eprom. Enter a filename, use .HEX as the extension. To upload a program, select file, select open. Locate the file, such as O2TIT140.TXB, press enter or select open. The program code should appear on the screen. Enter Alt+r to upload the program. The program will remain in memory and be active as long as the titrator remains on. When the titrator is turned off, the next time it is turned on, the previous version will initiate.

In order to burn a new program into eprom, it must first be loaded to compile it. The program is then downloaded and saved to disk with an extension of .HEX. Follow the procedure above. Power off the titrator and unplug it to avoid electrical shock. Open the electronics box. Remove the printed circuit board, the yellow board in these figures.





Insert a solid core jumper wire (as shown in the circle in the figure on the right) into the Onset model 5F board on the bottom of the circuit board. Plug in the titrator and turn on the power. In txtools, enter the mini monitor, ! will appear as the system prompt. Enter Shift+x to erase the old program. Select com port, enter enter to 'send ascii file'. Enter the filename, such as O2TIT140.HEX and select OK. After the program has uploaded, turn off the titrator power and unplug it. Remove the jumper, secure the circuit board and shut the electronics box. Plug in the titrator and turn on the power. Check that the version number is as expected.

Appendix C: Notes

Txtools is basically a simple terminal program that displays characters that are sent to it by the titrator. The titrator contains a microprocessor with the titrator software burnt into its EEPROM.

The detector electrodes are fragile, handle with care.

The software is optimized to operate with a 0.16 M thiosulfate solution, 25g/*l* of anhydrous thiosulfate. Thiosulfate-pentahydrate will require 40g/*l* to achieve a 0.16 M solution.

The titrant, sodium thiosulfate, degrades in light and must be stored in brown containers. It is highly recommended that a glass bottle be used with the titration apparatus in order to best monitor the titrant level and the position of the intake tube. The bottle cap should have two small holes drilled in the top, one for the intake tube and a second as a vent.

NaOH is very corrosive. The NaOH-NaI reagent is very dense and prone to leak from the dispenser tip due to capillary action.

Dispensers clear the tip by ejecting a small amount of reagent when the plunger is raised. Do not allow the reagent to clear into a sample. Check the type of dispensers used; some dispensers clear the tip before the sample is delivered and some clear after.

Good standard replication will be affected by uneven dispensing. Familiarize yourself with the proper operation of the standard dispenser; when it clears, if the tip should be wiped, etc.

The titration sequence is *TITRATE TITRATE* for standards and samples, and *TITRATE FILL* for blanks.

If the burette is not RESET, it may exceed its capacity. The plunger may burst the glass burette tip. If a second sample is titrated without a reset, the operator has two choices. If the oxygen concentration can be estimated and will be low enough to allow the sample to titrate without exceeding the capacity of the burette, the first value can be subtracted from the total to yield the value for the second. If it is likely that the capacity of the burette will be exceeded, the titration must be stopped with a loss of the sample. Remove the electrodes from the sample to stop the titration and reset.

If the samples are not reshaken a second time, a crust may form on the bottom of the flask. This crust may not totally dissolve during the titration procedure, resulting in an artificially low value. Furthermore, the crust may remain and contaminate future samples in that flask. If a flask has a crust on the bottom, fill it about 1/4 with water and add 1*ml* of H_2SO_4. Swirl the flask until the crust is dissolved and rinse well.

A light application of stopcock grease to the burette tip is advised if bubbles appear regularly in the tubing.

If a bubble is lodged in the tubing and is undetected, the first sample titrated may be off if the bubble is dispensed into the sample. It is advisable to start titration in the middle of a station to prevent consistent offsets in the first or the last sample.

New oxygen sample flasks may have a chronic bubble formation problem. Stopper the flask with 1/4 to 1/2 water and shake vigorously. This will break the surface tension on the inside of the flask and greatly reduce the formation of bubbles. Rinse the flask very well as the water has become supersaturated due to the shaking.

The volume of the oxygen sampling flasks is carefully calibrated. Each flask is paired with a stopper when calibrated. If a flask loses its stopper, it must be recalibrated with another stopper before use.

Flush the burette well with distilled water before storing.

Standards Quick Reference:

- Fill a flask with stirrer 1/3 with deionized water.
- Add 1*ml* H_2SO_4 and mix well.
- Add 1*ml* NaOH-NaI and mix well.
- Add 1*ml* $MnCl_2$ and mix well.
- Add 10*ml* KIO_3 standard solution and mix well.
- Fill the flask with deionized water to the bottom of the neck.
- Place the flask on the stirrer.
- Position the electrodes, fully immersed.
- Push *TITRATE*. Push *TITRATE*.
- Record the value.
- Rotate the detector into the bath.
- Push *RESET*.
- Scrub the flask and rinse.

Blanks Quick Reference:

- Fill a flask with stirrer 1/3 with deionized water.
- Add 1*ml* H_2SO_4 and mix well.
- Add 1*ml* NaOH-NaI and mix well.
- Add 1*ml* $MnCl_2$ and mix well.
- Add 1*ml* of the KIO_3 standard solution and mix well.
- Fill the flask with deionized water to the bottom of the neck.
- Position the electrodes, fully immersed.
- Push *TITRATE*. Push *FILL*.
- Record the value (R1).
- Add an additional 1*ml* of the KIO_3 standard solution.
- Push *TITRATE*. Push *FILL*.
- Record the value (R2).
- Rotate the detector into the bath.
- Push *RESET*.
- Scrub the flask and rinse.