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An Easy-to-Construct Automated Winkler Titration System

by

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Introduction

The instrument described in this report is an updated version of the high precision, automated Winkler titration system described by Friederich *et al.* (1984). The original instrument was based on the work of Bryan *et al.* (1976) who developed a colorimetric end-point detector and on the work of Williams and Jenkinson (1982) who produced an automated system that used this detector.

The goals of our updated version of the device described by Friederich *et al.* (1984) were as follows:

1) Move control of the system to the MS-DOS environment because HP-85 computers are no longer in production and because more user-friendly programs could be written using the IBM XT or AT computers that control the new device.

2) Use more "off the shelf" components and reduce the parts count in the new system so that it could be easily constructed and maintained.

This report describes how to construct and use the new automated Winkler titration device. It also includes information on the chemistry of the Winkler titration, and detailed instructions on how to prepare reagents, collect samples, standardize and perform the titrations (Appendix I). A disk containing the program needed to operate the new device is also included.

Hardware Description

The titration and end-point detection apparatus consists of three interactive components:

1) IBM-PC/AT or IBM-PC/XT compatible computer
2) 665 Dosimat dispenser
3) Light source/ detector module

Items 1&2 are unmodified commercial devices and item 3 can be easily built from a few readily available components. The details of the configuration are given below.

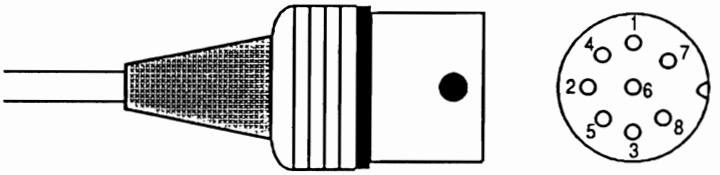
Computer

The system should have at least 512K of RAM available, otherwise problems may be encountered when manipulating large data files. Support of either CGA or EGA graphics are also necessary. Communications to the Dosimat and light detector are via a RS232 interface operating at 9600 baud, even parity, 7 data bits, 1 stop bit. During serial communications a line feed character is sent after a carriage return. The Data Carrier Detect line (DCD), the Clear To Send line (CTS) and the Data Set Ready line (DSR) are ignored.

665 Dosimat

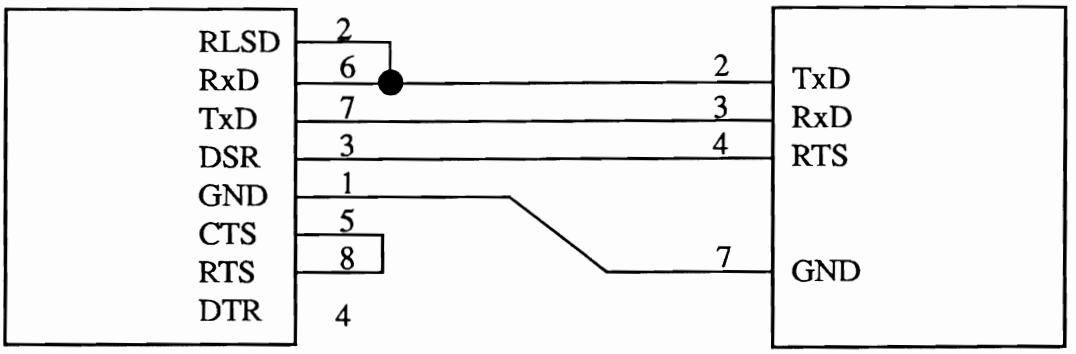
A 5ml dispensing unit was used while developing the current software. Using a 1ml unit with more concentrated thiosulfate would decrease precision slightly while a 10 ml unit with more dilute thiosulfate may exceed the capacity of the titration flasks. The software recognizes the particular dispensing unit and loads an appropriate set of default parameters. Dispensing units should not be changed while the program is in the Titrate option since the configuration of the Dosimat is only checked upon entering this mode. The diagram for the Dosimat RS232 connection is given below.

Contact location at the plug for Dosimat socket B:



1) Signal ground
2) Received Line Signal Detector (RLSD)
3) Data Set Ready (DSR)
4) Data Terminal Ready (DTR)
5) Clear To Send (CTS)
6) Received Data (RxD)
7) Transmitted Data (TxD)
8) Request To Send (RTS)

The connection between the Dosimat and a standard 25 pin RS232 computer port is:

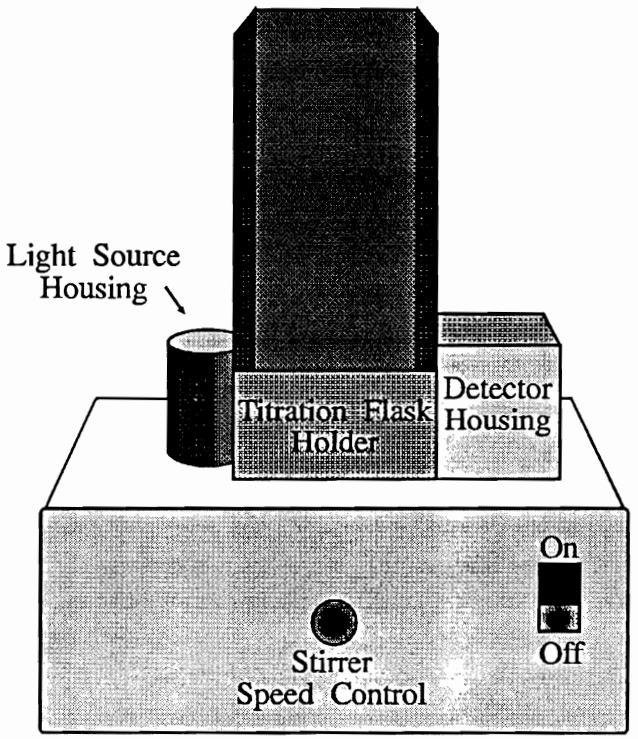


Dosimat socket B

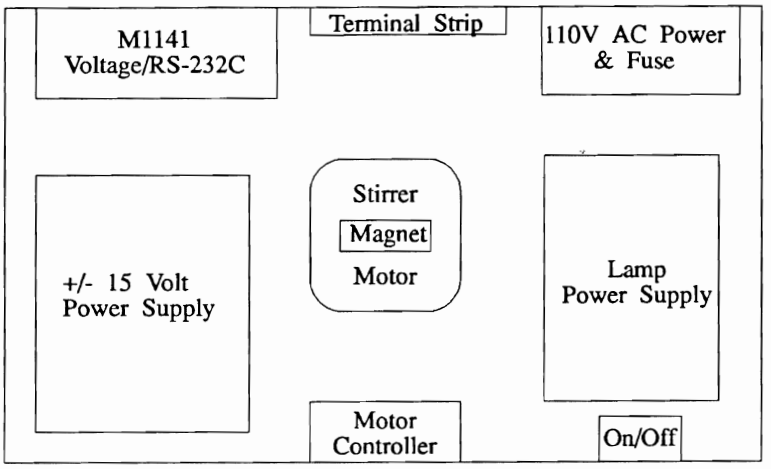
Computer 25 pin RS232 port

Light source\detector unit

The light source, light detector, analog to digital converter\RS232 interface, titration flask holder, magnetic stirrer and all associated power supplies are housed in a single unit. This unit has a footprint of 26 by 16 cm , an overall height of 30 cm and weighs about 5 kg. An approximate layout is given below and detailed descriptions of the individual components follows.



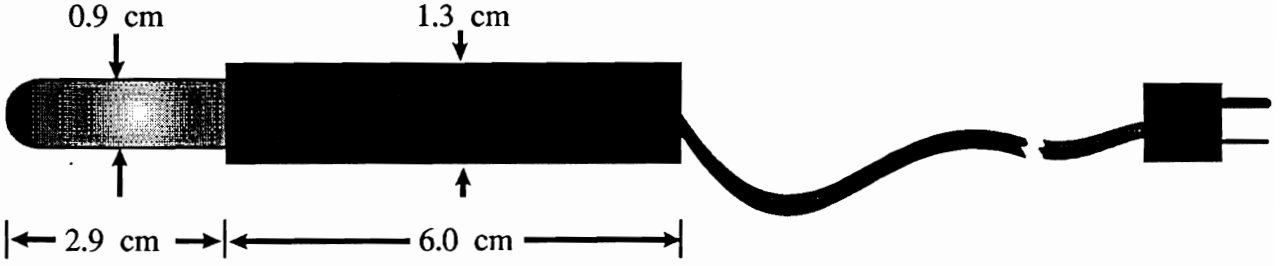
A top view of the internal layout of the base unit :



Note: The lamp power supply in this diagram consists only of the transformer. Electrical connections should be made identical to those in the original controller box supplied by the manufacturer.

Light Source:

The light source is an Analamp Model 80-1025-01\351 low pressure mercury vapor lamp with a phosphor coating. The lamp has an emission peak at 351 nm with a band width of 41 nm, thus closely matching the tri-iodate absorbance peak at 352 nm. The lamp has a starting voltage of 800 V and requires a current of 18 ma. The actual supply voltage from the power supply is 1600 V. The physical dimensions are shown below.



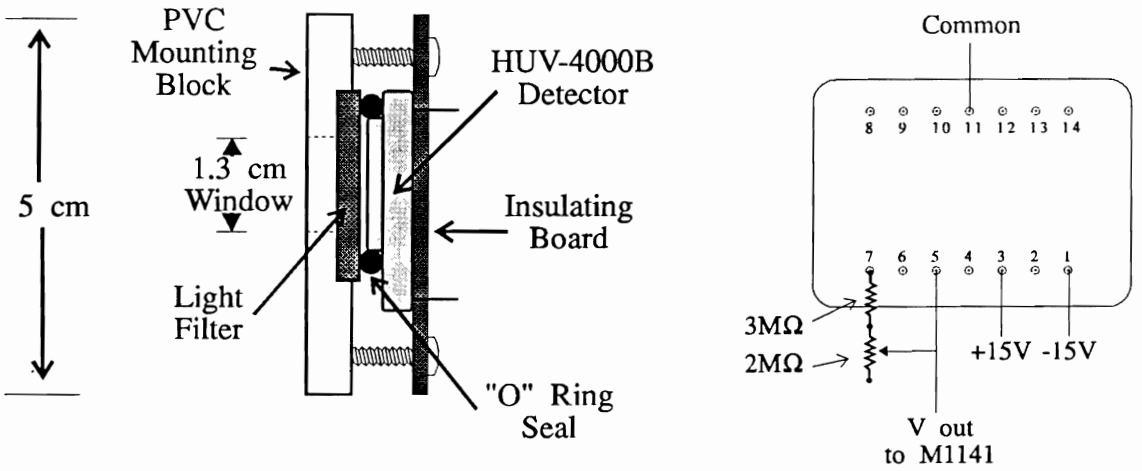
This lamp is mounted upright in the apparatus so that the center of the light emitting region is located at a level that is approximately 3 cm above the bottom of the titration flasks. A housing is used in order to shield the light from air currents that might cause rapid temperature excursions and to prevent excessive UV radiation from escaping. The side of the housing that is directly in line with the titration flask and the detector has a 1.3 cm diameter open window. The mounting and housing are made of PVC.

Caution should be used with these lamps. Although they are shielded and do not produce ozone they do produce radiation at 254 nm. In order to eliminate the 254 nm radiation a piece of Pyrex glass tubing must be placed over the light emitting portion of the lamp.

Note: The handle of the lamp is made of steel and is connected to the neutral line of the high voltage power supply which is connected directly to earth ground.

Light Detector:

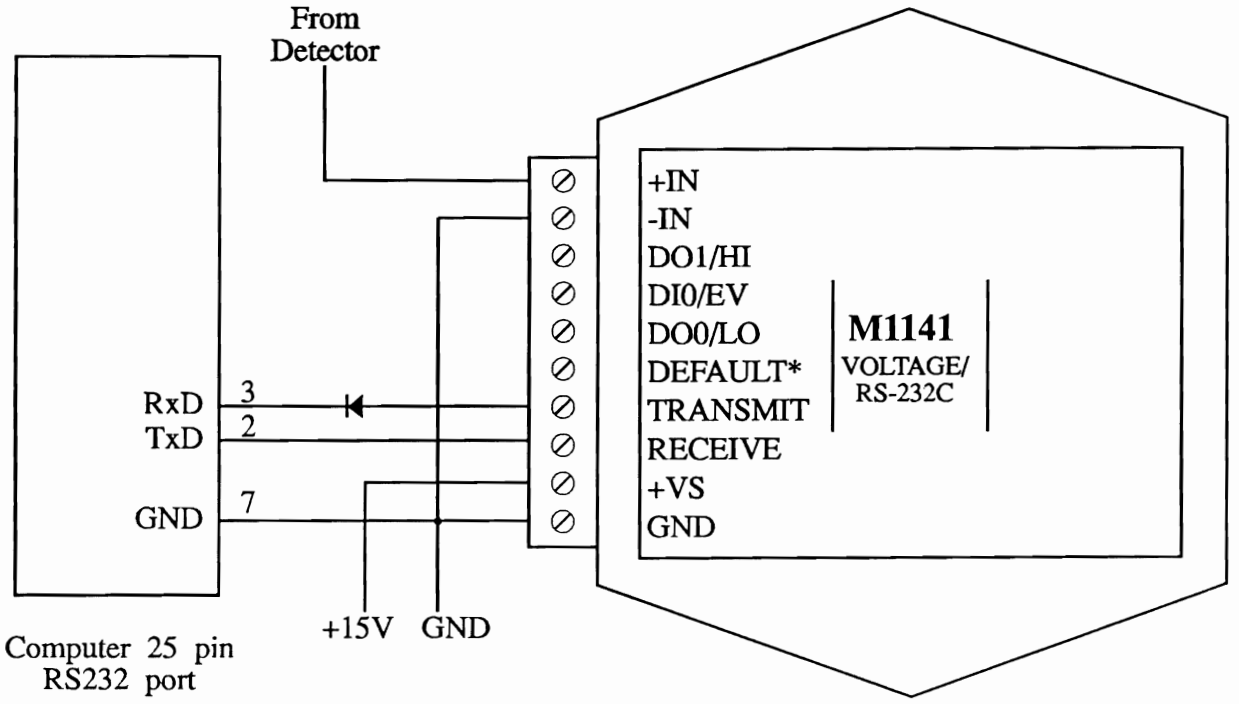
The detector is an EG&G model HUV-4000B operational amplifier\photodiode combination mounted behind a Corning type 7-37 glass filter. An external feedback resistance of about 4.5 megohms is required to obtain the proper output. The gain of the detector is adjusted using a fixed resistor in series with a 2 megohm trimpot accessible from the outside of the detector housing. The resistance required to obtain the proper gain may be different from the values given here due to changes or differences in the physical parameters of this unit. Power requirements for the detector are 2.2 ma at +/- 15 V. A power supply should be chosen to also supply the needs of the analog to digital converter(50 ma at 15V). Mounting details are shown below.



The above unit is mounted in such a way that the center of the window is at a level that is 3 cm above the bottom of the titration flask on the opposite side and directly in line with the light source.

Analog to Digital Converter:

The output from the detector is sent to the computer via a MetraByte Model M1141 RS232 compatible digital converter. The converter receives its power from the same supply as the detector and should require less than 50 ma of current at 15 V. In order to be able to communicate with this device and the Dosimat on a single RS232 port, the Transmit line of the converter must be equipped with a diode in order to allow the Dosimat to transmit. A connection diagram is given below.



These modules contain an EEPROM that stores all setup information. When shipped from the factory the setup includes a channel address of 1, 300 baud rate, no linefeeds, no parity, alarms off, no echo, 2 character delay, no large filter, 0.5 second small filter. To match communications protocol with the Dosimat the setup needs to be changed to 9600 baud, linefeed, and even parity. The remaining parameters do not need to be changed. The following series of commands need to sent to the module at 300 baud before it can be used in the titrator:

$1WE	(write enable)
$1SU31A201C2	(setup string)
$1WE	(write enable)
$1RR	(remote reset)

For details on this procedure refer to chapters 1 and 5 in the M1000/M2000 User Guide that is shipped with the module.

Stirring Motor:

The stirring motor can be any small motor whose speed can be controlled in the 600 to 1000 RPM range and has a magnet mounted on its shaft. In the titrator currently in use, the motor is a Bodine KCI-26 with a 1.8K 25W resistor in series with a 5K rheostat for speed control.

Major Components List

Description	Part no.	Supplier
Dosimat 665 Digital Buret 5ml Buret Unit 552-5BC	20 75 010-3 20 68 941-2	Brinkman Instruments Cantiague Road Westbury, NY 11590 ph: 800-645-3050
Mercury Lamp Lamp Power Supply	80-1025-01/351 90-0001-01	BHK Inc. 2855 Metropolitan Pl. Pomona, CA 91767 ph: 714-593-6590
Operational Amplifier/ Photodiode Combination	HUV-4000B	EG&G Judson 345 Protero Av. Sunnyvale, CA 94086 ph: 408 738 4266
Analog to Digital Converter 10V Input/ RS232 Output	M1141	Keithley Metrabyte 440 Myles Standish Blvd. Taunton, MA 02780 ph: 508-880-3000
+/-15 Volt 75 ma Dual Output Power Supply		Any manufacturer

Note: Any dual output power supply with an output between +/- 12 Volt and +/-18 Volt can be used.

Light Filter — Corning 7-37 glass

Note: Glass filters of this type can be obtained from various manufacturers as 2.54 cm diameter disks. If a filter other than Corning type 7-37 glass is used, it should have the following properties:

1) Maximum transmission at 350 nm
2) Near zero transmission between 450 nm and 1100 nm
3) Greater than 30% transmission at 350 nm

Software Information

The operations manual describes how to use the oxygen titration software; this section merely gives technical information about the software. A complete source code listing is given on the enclosed disc. The program was written in Microsoft BASIC version 7.1 and compiled to be used under MS-DOS. The libraries and tools that are utilized are combined in the file OXY.LIB and are listed blow.

Date/Time Functions	DTFMER.LIB
Format Functions	DTFMTER.LIB
User Interface Toolbox	MENU.BAS, MENU.BI
	WINDOW.BAS, WINDOW.BI
	MOUSE.BAS, MOUSE.BI
	GENERAL.BAS, GENERAL.BI
	UISAM.LIB

Details on these procedures can be found in the Microsoft BASIC Language Reference Version 7.0. The oxygen titration program consists of four modules listed below.

OXY1.BAS	Setup, file creation, file finding
OXY2.BAS	Data listing and data export
OXY3.BAS	Data editing
OXY4.BAS	Titrations
OXYGEN.BI	Variable declarations

All data files are stored in binary random access format and can be printed or exported as standard ASCII text files using the routines included in the program.

Operations Manual

The general sample collection and preparation techniques are based on those of Carpenter (1965). Details of these procedures are given in Appendix I, which also applies to manual methods and should be read by anyone not thoroughly familiar with this method. The information below with the exception of the recipes for the pickling reagents is specific for the hardware and software in this report.

Bottles

Although the titration system will work with various bottles, it is optimized for 125 ml Pyrex brand iodine determination flasks (Corning 5400). Each flask must be gravimetrically calibrated with its stopper. First record the empty dry weight of each flask to the nearest 0.01 gm then fill the flasks with room temperature deionized water that is free of any bubbles. Replace the stopper. Remove any moisture from the outside of the flask including the area around the stopper. Now record the full weight to the nearest 0.01 gm and the temperature to the nearest degree Celsius. When these values are entered in the bottle volume files, the volume is calculated according to the equations of Kell 1967. The uncertainty of the bottle volumes is about 0.02%.

Note: If other types of bottles are used, nonuniformity in optical characteristics may require the following procedure. Place a bottle filled with deionized water in the light path and rotate it until a region is found in which the light transmission is reasonably stable. Using the gain adjustment set the light transmission to ~90%. Place each bottle in the light path and rotate it until a light transmission of about 90% is obtained and place some type of line-up mark on each bottle. This mark can then be used in subsequent titrations to position the bottles.

Reagents

Manganous Chloride: Dissolve 600 gm of reagent grade Mn $Cl_2 \cdot 4H_2O$ in deionized water. Adjust the final volume to 1 liter. Manganous sulfate may be substituted for the chloride, but the chloride is suggested because of its solubility and its freedom from higher valence manganese compounds. This reagent should be delivered by a 1ml repeating dispenser that has a precision of about 1% and has been gravimetrically calibrated.

Alkaline Sodium Iodide: Dissolve 320 gm of reagent grade NaOH and 600 gm of reagent grade NaI in deionized water. Dilute to 1 liter. In order to prevent photochemical reactions, this reagent should be kept in dark bottle. This reagent should be delivered by a 1ml repeating dispenser that has a precision of about 1% and has been gravimetrically calibrated.

Sulfuric Acid: Slowly add 280 ml of concentrated reagent grade H_2SO_4 to about 700 ml of deionized water. Make up to 1 liter with deionized water. USE CAUTION AS A GREAT DEAL OF HEAT IS LIBERATED! This reagent should be delivered by an adjustable 1 ml dispenser. Adjust the volume of acid dispensed such that the final pH of the sample is

between 2 and 2.5.

Sodium Thiosulfate: Dissolve 10 gm of reagent grade $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter of deionized water. Add 1 pellet of NaOH and 2 drops of chloroform. Let stand (preferably for a few days) before using.
Note: When using bottles that have a significantly different volume or a Dosimat with a 1 ml or a 10 ml dispensing unit, the thiosulfate concentration should be adjusted accordingly.

Primary Standard: Dissolve 0.3567 gm of oven dried (105-110 ^oC) KIO_3 or 0.3250 gm of $KH(IO_3)_2$ dried in a vacuum desiccator in deionized water. Make up to exactly 1 liter. This solution is 0.01000N. KIO_3 is preferred because of its stability during drying. $KH(IO_3)_2$ (potassium bi-iodate) decomposes at temperatures above 100^oC. It may be more convenient to weigh out standards that are not exactly 0.01000 N.

Standardization

The thiosulfate solution should be standardized under the same conditions as the actual procedure for the determination of dissolved oxygen. To about 40 ml of deionized water add 1 ml of the H_2SO_4 reagent. Mix thoroughly. Add 1 ml of the NaOH·NaI solution. Mix thoroughly. The solution should be distinctly acid, clear and colorless. If any basic microenvironments exist at this point due to insufficient washing of the reagent into the bottle and insufficient stirring, poor results will be obtained. Add 1 ml of $MnSO_4$ reagent and mix. Pipet a precisely known quantity of the standard iodate solution into the above flask. Fill the flask to the neck with deionzed water and titrate. Repeat this proceedure using a range of standard volumes. The slope of the relationship between the standard volume and the thiosulfate volume will be used as the calibration factor and the intercept will be the blank. Two independent standards should be used. One may be kept in a 10 ml Repipet and the other in a 1 ml Repipet. The Repipets need to be gravimetrically calibrated and have to be recalibrated periodically to ascertain that their settings have not changed.

Titration and Data Manipulation

Turn on the computer, the Dosimat, and the light source\detector unit. The light source requires an initial warm-up time of about 15 minutes. Since the computer's clock will be used to timestamp all titration data, it should be set to the correct date and time. Load the program called OXYGEN.EXE. When not using a mouse use the keystrokes given in the following table

Alt	Activates the menu bar. Press the highlighted characters in the menu names to open menus and to choose commands from a menu. Or use the Left Arrow and Right Arrow keys to highlight a menu title, and then press the Up Arrow and Down Arrow keys to choose a command.
Enter	Accepts a menu choice Accepts a dialog box choice During data and standard editing displays a plot of the endpoint During bottle volume editing moves to the next field (same as Tab)
Esc	Closes menus and dialog boxes, cancelling any entries.
Tab	Moves between choices in a dialog box. Moves between edit fields during data editing.

In order to start, existing files must be chosen or new ones created. Three types of files are used:

1) Data files (filename.oxy) are used to store titration end point information for each titration. They also contain a header that holds information about reagent volumes, standard factors and blanks that will be used when this data is calculated. Therefore only data that will share this information can be included in a given file. Choose or create one of these files if you are going to titrate samples, edit data, list data or export data.
2) Standard files (filename.std) are used to store endpoint information for standards. The header in these files contains only comments. Choose or create one of these files if you are going titrate standards, edit standards, calculate factors or export standards.
3) Bottle volume files (filename.vol) are used to store bottle calibration data. Choose or create one of these files if you are going to enter bottle calibrations or wish to list calculated data.

To select a file:

1) Press [F2] or enter the File menu and choose Select File.
2) Enter a disc drive name or press Enter to choose the current drive.
3) Enter a valid path or press Enter to choose the default.
4) Enter a file selection argument or accept the default (note: you must change the file extension to find the standard and bottle volume files).
5) Select a file.

This file now remains the default of its type until you exit the program or it is changed using the above procedure. Repeat for each type of file that you need. An example of each file type is included on the program disc.

To create a file:

1) Press [F1] or enter the File menu and choose Create File.
2) Enter a disc drive name or press Enter to choose the current drive.
3) Enter a valid path or press Enter to choose the default.
4) Enter a file name making certain to use the proper extension.
5) Enter any available header data when it is requested. The headers can be edited at a later date if the information is not available. A newly created file becomes the selected file.

Titrations

If samples will be titrated press [F9] or select via the menu. For standards use [F10] or select via the menu. At this point, the computer attempts to establish communications with the Dosimat. Up to three attempts are made if it is not successful initially. The type of dispenser unit found and the default titration parameters for that unit are displayed. The titration parameters can be edited at this point.

1) Dispensing Rate (ml/min) is the rate at which continuous dispensing of thiosulfate occurs during the initial part of the titration.
2) Switch to Incremental Addition (%) is the light level above which dispensing of thiosulfate becomes incremental.
3) Incremental Rate is a factor applied to the size of the incremental additions of thiosulfate. Note: The increments become smaller as the light level increases and regardless of the rate factor take on the smallest possible value when the light level is 90% or greater. At light levels below 90% the volume of the increment that the Dosimat delivers is given by the relationships below:

$$X = (9000 - \text{detector output in mv}) \cdot (\text{Incremental Rate})$$

$$\text{Volume in ml} = (X + X^{1.3}/2000) \cdot (0.0001 \cdot \text{volume of Dosimat unit})$$

4) Maximum Slope at Endpoint is the slope (change in light/change in volume) that is considered acceptable due to noise once the endpoint has been reached. If there were no instrumental noise this value would zero.

You may now enter the appropriate identification parameters for the first sample or standard. Before titrating the first sample check the following:

1) Place a titration flask filled with deionized water free of bubbles in the light path and adjust the light transmission output to read about 90% using the potentiometer on the detector housing. (The reading should now be about 40% when the bottle is removed.)
2) Press R to enter the rinse mode. Enter the desired number of rinses for the dispenser.
3) Adjust the stirring rate. While rapid stirring is desirable, a deep vortex or a central column of bubbles must be avoided.
4) Press the Spacebar to clear the pipet tip before inserting it into the sample. Be sure to remove any pendant drops.

To titrate a sample:

1) Remove the stopper from the flask and add the acid.
2) Add a magnetic stirring bar to the flask and place it in the titration stand.
3) Clear the pipet tip by pressing the Spacebar and remove any pendant drops.
4) Place the pipet tip in the flask and press Ctrl + Enter to start the titration.

The progress of the titration is displayed graphically and numerically. When the endpoint is reached, the data can be accepted by pressing Enter or it can be edited using the cursor keys. Except for the addition of acid, a standard is run in the same manner. If Esc is pressed, the data is also saved but the program returns to the main menu rather than to the titration routine.

Notes:

1) If the light transmission at the endpoint is low then precision is decreased since the size of the incremental thiosulfate additions is controlled by light intensity. A warning message is displayed if the endpoint is found at a transmission value less than 80%.
2) If the light transmission at the endpoint is 100% then the actual endpoint was probably not reached and the oxygen concentration may be underestimated. Since the endpoint is determined by a low slope in the volume versus light relationship and value of 100% is the maximum output of the detector, a slope of 0 results once the light transmission reaches 100%. If this occurs then the gain of the detector must be reduced using the potentiometer on the detector housing.

Data Manipulation

Editing Data and Standards: Besides the sample identification and the endpoint, the last portion of the titration curve is also saved in the data and standard files. When entering the edit mode either via the menu or [F6] (data) or [F7] (standards), the current file becomes available for editing. Choose an editing field using the Tab key to move horizontally and the Up Arrow and Down Arrow keys to move vertically; if using a mouse you can use the scroll bar. The Station and Bottle fields are alphanumeric and the Depth field is a single precision number. To edit the endpoint, press Enter to display a plot of the titration. Use the cursor keys to choose a different endpoint. The date and time of titration can not be edited. In order to exclude a standard from the determination of the calibration factor; place a * in front of its ID. Upon exiting the edit mode, you have the choice of accepting the changes or discarding them.

Editing Headers; The headers of the above files can accessed via the menu and [F5] (data header). Besides identifying information the data header also contains the thiosulfate calibration factor and the reagent volumes, both of which must be present in order to view or export calculated data. Choose an edit field using the Up Arrow and Down Arrow keys. The baud rate cannot be edited in this version since the baud rate of the Dosimat cannot be changed.

Editing and Adding Bottle Volumes: Use the menu or [F8] to edit the current bottle volume file. Choose an editing field using the Tab or Enter keys to move horizontally and

the Up Arrow and Down Arrow keys to move vertically; if using a mouse you can use the scroll bar. If the Empty Weight, the Full Weight or the Temperature are modified and none of these fields is zero, then the volume is automatically updated. If the volume of a bottle is known and there is no calibration data, it can be entered. To add new bottles move to the bottom of the listing and enter new data. The bottle identifier can consist of numbers and letters. To remove a bottle delete its name. When exiting the edit mode you have the choice of accepting the changes or discarding them. When the data is saved, it is sorted numerically and alphabetically by bottle name or number.

Calculating Calibration Factors and Blanks: Thiosulfate calibration factors are calculated by choosing Standards in the Results menu. A list of all standards in the current standards file is displayed. When Enter is pressed a least squares linear regression of standard volume versus thiosulfate volume is calculated and a plot of the data is shown. Any standards that have an ID starting with * are ignored during the calculation. The Factor (slope) and Blank (intercept) are recorded in the header of the standard file. When acceptable values are obtained they should be entered into the headers of all the associated data files.

Displaying Results: To display calculated results, choose Data from the Results menu. The current bottle volume file will be searched for matches by bottle name and the data will be calculated with the factor, blank and reagent volumes in the data file header. Data will be displayed but not calculated if any of the above are missing.

Exporting and Printing Files: To export or print any file use File menu or [F3] (export) or [F4] (print). Then select which type of file. Files are sent as standard unformatted ASCII text files. Printing uses the LPT1 port. Exporting requests a destination directory that cannot be the same as the the directory containing the original data. If the bottle and calibration information is available, this information is combined with the data files to calculate oxygen concentrations before the information is exported or printed.

Acknowledgements

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APPENDIX I

ON THE DETERMINATION OF DISSOLVED OXYGEN IN SEA WATER

Lou Codispoti

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INTRODUCTION

With the exception of salinity determinations, dissolved oxygen is probably the most commonly measured chemical oceanographic variable. Historically, dissolved oxygen has been determined by some modification of the classic Winkler (1888) method. Increasingly, electrodes are being used to measure dissolved oxygen, but even when this is the case, the electrodes are often calibrated with Winkler titrations.

RANGE OF OCEANIC DISSOLVED OXYGEN CONCENTRATIONS

Dissolved oxygen concentrations are generally expressed in millimolar, mMoles/kg, mg-atoms/liter or ml/liter (mg-atoms of O_2/liter = 0.08931 x ml/liter). Values from 0 to 13 ml/liter are encountered in the ocean. The equilibrium solubility values for dissolved oxygen increase with decreasing temperature, and salinity, and they range from about 4 to 9 ml/liter throughout most of the ocean. Generally, biological processes are responsible for the wide range of values which are encountered. Phytoplankton growth, for example, can cause oxygen supersaturations while respiratory processes can remove all of the oxygen originally present in some regions.

CHEMISTRY OF THE WINKLER METHOD

Manganous sulfate (or chloride) solution is added to a known quantity of sea water and is immediately followed by the addition of an alkaline sodium hydroxide-sodium iodide solution. Manganous hydroxide precipitates and reacts with the dissolved oxygen in the water with the formation of a hydrated tetravalent oxide of manganese.

$$Mn^{++} + 2OH^{-} \longrightarrow Mn(OH)_2\,(S)$$

$$2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2\,(S)$$

Upon acidification, the manganese hydroxides dissolve. In the acid solution, the tetravalent manganese in $MnO(OH)_2$ acts as an oxidizing agent and liberates free iodine from the iodide ions.

$$Mn(OH)_2 + 2H^+ \longrightarrow Mn^{++} + 2H_2O$$

$$MnO(OH)_2 + 4H^+ + 2I^- \longrightarrow Mn^{++} + I_2 + 3H_2O$$

The liberated iodine, equivalent to the dissolved oxygen present in the water, is then titrated with a standardized sodium thiosulfate solution and the dissolved oxygen present in the sample is calculated. The reaction involved is as follows:

$$I_2 + 2S_2O_3^{=} \longrightarrow 2I^- + S_4O_6^{=}$$

(thiosulfate) (tetrathionate)

ACCURACY OF THE WINKLER METHOD

Under ideal conditions, the Winkler method is quite accurate. Carpenter (1965a), for example, has described one modification which has an accuracy of 0.1% at concentrations of ~5 ml/l, and using the Winkler method he (Carpenter, 1966) has produced a set of oxygen solubility tables which appear to be accurate within ~0.01 ml/liter (Murray and Riley, 1969). The field adaptation of Carpenter's technique (1965b) should have an accuracy of better than 0.05 ml/liter when carefully performed. (This estimate includes standardization, and "pickling" errors. Precision should be better than 0.05 ml/l). Automated titrating systems that will be described later on have the potential to attain precisions of ~$\pm$ 0.1% in the field (at concentrations of ~ 5 ml/l, page12).

Unfortunately, much of the historical oxygen data has been obtained using unsuitable variations of the Winkler method and/or by unskilled analysts. A study conducted several years ago indicated that errors approaching 0.5 ml/liter may be common in the historical data (Carritt and Carpenter, 1966). Such errors sometimes make it impossible for oceanographers to use existing oxygen data. Wüst (1964), for example, could not compare oxygen data from different cruises in his study of the Caribbean Sea.

THE CARPENTER MODIFICATION OF THE WINKLER METHOD

Carpenter's (1965b) modification of the Winkler method was designed to reduce the following errors:

1. Iodine Volatilization. Loss of the iodine produced after the sample has been acidified has been shown to be significant in some previous techniques. One way in which Carpenter's method reduces this error is by eliminating sample transfers which could contribute to iodine losses. Because the entire sample is titrated in the original collection flask, no transfers are necessary. The collection flasks can easily be calibrated for volume to $\pm$ 0.02%.

In Carpenter's method, loss of iodine during exposure to the atmosphere is also minimized by encouraging the formation of the less volatile complex (I_3^-). This is done by using a high concentration of sodium iodide which encourages the formation of the complex according to the following formula:

$$I_2 + I^- \rightleftharpoons I_3$$

Comparison experiments indicate that the error introduced by not using the glassware necessary to eliminate transfers is less than 0.05 ml/liter if aliquots are drawn with care and the Carpenter reagents are employed. Knapp et al. (1991) discuss an aliquot technique in which the error due to iodine loss may be negligible.

2. Air Oxidation of Iodide. Iodide reacts with oxygen in acidic solution:

$$4I^- + 4H^+ + O_2 \longrightarrow 2I_2 + 2H_2O$$

To minimize this source of error the optimum pH to permit the proper reaction between thiosulfate and iodine and prevent the above reaction was determined. The optimum pH range was found to be 2.0-2.5 and the reagent concentrations were adjusted to attain a final pH in the appropriate range.

3. Improper Blank Determinations. In most previous methods, allowance was made only for positive blanks, but negative blanks caused by reducing impurities are also possible. Consequently, a method for determining positive and negative blanks was devised.

4. Improper Standards. It was found that potassium dichromate which had been used in some methods was not a suitable standard. The best standard is potassium iodate but potassium biiodate can also be used if it is dried by vacuum dessication. Potassium iodate can be dried in an oven at -110°C, potassium biiodate decomposes at these temperatures.

Preparation of Reagents:

Reagent Bottles. The reagent bottles used for the manganous sulfate (or chloride), potassium hydroxide-iodide, and sulfuric acid should be such that automatic filling of the pipets is possible. In order to prevent photochemical reactions, the alkaline iodide reagent bottles should be of brown glass.

Manganous Chloride. Dissolve 600g of reagent grade $MnCl_2 \cdot 4H_2O$ in distilled water. Adjust final volume to 1 liter. Manganous sulfate may be substituted for the chloride, but the chloride is suggested because of its solubility and its freedom

from higher valence manganese compounds. When substituting Manganous sulfate or a form of MnCl with more or fewer waters of hydration than in $MnCl_2.4H_20$ adjust for differences in formula weight.

Alkaline Sodium Iodide. Dissolve 320g of reagent grade NaOH and 600 g of reagent grade NaI in distilled water. Dilute to 1 liter.

Sulfuric Acid. Slowly add 280 ml of concentrated reagent grade H_2SO_4 to about 700 ml of deionized water. Make up to 1 liter with deionized water. USE CAUTION AS A GREAT DEAL OF HEAT IS LIBERATED!

Starch Solution. Dissolve 1-2 g of starch indicator in 100 ml of distilled water, or boil a similar amount of potato starch in distilled water for about 5 minutes. The indicator should be made up fresh every day if possible since bacterial action degrades it. One sign of a degraded indicator is a reddish tinge to the normally blue color when the starch is added to the sample. It is permissible to "play around" with the starch concentration a little bit to produce an indicator color intensity that meets your personal preferences! **NOTE that some of the automated techniques discussed later on do not require a starch indicator solution.**

Sodium Thiosulfate Solution. Dissolve 1.6 g for the Aliquot method described here or 35 g for the Carpenter method, of reagent grade $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter of distilled water. Add 1 pellet of NaOH and 2 drops of chloroform.

NOTES:

a. The sodium hydroxide is added to negate the influence of any dissolved CO_2 as the presence of carbonic acid causes the following reaction:

$$S_2O_3^{=} + 2H^{+} \longrightarrow S + SO_2 + H_2O$$

b. Sodium thiosulfate is degraded by the action of bacteria. Chloroform is added to inhibit bacterial growth.

Primary Standard. Dissolve 0.3567 g of oven dried (~105-110°C) KIO_3 or 0.3250g of $KH(IO_3)_2$ dried in a vacuum desiccator in distilled water. Make up to exactly 1 liter. This solution is 0.01000N.

STANDARDIZATION OF THE THIOSULFATE

The thiosulfate solution should be standardized under the same conditions as the actual procedure for the determination of dissolved oxygen. By so doing, possible errors are compensated. The best practice is to run standards and blanks before and after every sample run until you are sure that the interval between standard runs can be extended.

To about 40 ml of distilled water add 1 ml of the H_2SO_4 reagent. Mix thoroughly. Add 1 ml of the NaOH-NAI solution. Mix and wash down the sides of the flask thoroughly. The solution should be distinctly acid, clear and colorless. If any basic microenvironments exist at this point due to insufficient washing of the reagents into the bottle and insufficient stirring, you will obtain poor results. Make good use of your wash bottle and stirring motion before going on! Add 1 ml $MnCl_2$ reagent and after mixing, pipet into the flask 10 ml of the standard Iodate solution. Stir for ~5 sec. Then, titrate with thiosulfate in the same manner described below for samples.

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$\frac{10\text{ml } KIO_3 \times 0.010000N}{[\text{ml } Na_2S_2O_3 - B]} = \text{Normality of } Na_2S_2O_3$$

Where 0.010000 is the normality of the iodate solution, and B = Blank. Often it is preferable to weigh out standards that are not exactly 0.01000 N because striving for an exact weight takes extra time and tends to make the weighing process less exact. It is, of course, a simple matter to substitute a different normality when performing your calculations.

NOTE: An alternate method for obtaining a standardization and blank reading that we prefer is to construct a standard "curve" by dispensing different amounts of standard from a calibrated (glass) Re-pipet with a nominal dispensing volume of ~1 ml. These re-pipets when properly used and calibrated appear to be capable of accuracies of about $\pm$ 0.1%· Typically, we pipet volumes of standard ranging from ~1 to 12 ml (e.g., 1,2,3,5,8, and 10 mls), and obtain R^2's of 0.9999 or better with the automated system described in Friederich and Codispoti (1991). With this method the intercept corresponding to 0 standard addition is the blank. Each standard must, of course, be made up separately "from scratch" since pipetting more standard into an already titrated standard as done for the blank described by Carpenter (1965b) will invalidate this technique. Typically, we accumulate sufficient data for constructing the curve over ~day, and have found that the curve does not vary significantly over several day periods. We employ a ~10 ml re-pipet for the ~10 ml standard addition and for "running" checks between calibration

curves. We fill this repipet with a totally independent (different lot) primary standard to guard against systematic errors.

Blank Determination. The Carpenter (1965b) procedure is the same as the standardization procedure except that exactly 1 ml of the standard KIO_3 is added instead of 10 ml, and, after the first titration, another 1 ml of standard is added to the same sample and the solution is titrated again. The blank is equal to the ml of thiosulfate needed for the first titration minus the ml of thiosulfate needed for the second titration. The blank may be either positive or negative.

SAMPLE COLLECTION AND TREATMENT

Samples for the determination of dissolved oxygen should be taken from the water sampling bottle immediately upon its arrival on deck. The sample should be collected in a calibrated, ground glass-stoppered container. Two types may be used - brown glass bottles approximating 250 ml and clear flasks or bottles approximating 125 ml. In the case of the latter, the entire content of the flask is used for titration while with the former, an aliquot is taken. We use 125 ml (approx.) clear borosilicate (Pyrex) glass bottles or flasks that are calibrated to contain with an accuracy of $\pm$ 0.03 ml and titrate the entire bottle as suggested by Carpenter (1965b).

To fill the oxygen bottle from the oceanographic sampling bottle (e.g. a Niskin bottle), you will need a length of amber surgical tubing (or similar material) with an inside diameter of approximately 1/4". The length of this tubing should be approximately 12", but keep the length as short as you can without sacrificing ease of movement while filling the oxygen bottle. To one end of this tubing attach a length of stiff plastic tubing or glass tubing that is a bit longer than the oxygen bottle. You are now ready to begin the challenging task of drawing a high quality oxygen sample without contamination while bathed in an atmosphere that is about 21% oxygen!! Believe it or not it can be done, and with a bit of practice you can do it!

A method that works well will be described next, but remember the principal which is to minimize contact of the seawater sample with the atmosphere. With this basic principle in mind, you can modify the technique to fit your particular blend of motor skills. Proceed as follows:

1) Attach the soft tubing to the spigot of the Niskin (or other type of bottle), and open the air vent on the Niskin bottle. If an appreciable amount of water comes out before opening the spigot, the bottle leaks! Make sure you note leaky bottles in the log sheet! Now open the air vent.

2) Flush the sampling tubing so that all air bubbles are removed. The easiest way to do this is to hold the tubing in a straight line (more or less) and point the tubing downward while letting water flow through the tubing at

maximum velocity for a brief period. At this point, you control the velocity of flow by pinching the soft tubing with your fingers. If the tubing is not completely free of bubbles after the first brief period of maximum velocity, repeat the process one or more times by turning the flow on and off by pinching and unpinching the rubber tubing. Some investigators prefer to try to remove the bubbles from the tubing by holding it in a "U" shape with the open end up and letting the water flow gently through the tubing. Any method or combination of methods that removes all of the bubbles from the tubing is okay Pre-wetting the tubing in a weak detergent may help to reduce the presence of "sticky" bubbles.

3) The next step is to rinse the oxygen bottle/flask, while minimizing contact with the atmosphere. You can do it by allowing the water to flow at a moderate pace out of the bubble-free tubing, inserting the tubing so that the stiff portion touches the bottom of the bottle, and then inverting the bottle. While in the inverted position, move the stiff part of the tubing around so that a moderate "sheet flow" rinses the entire bottle with minimal turbulence.

4) Next, momentarily stop the flow by pinching the tubing and invert the bottle quickly. Then, start a moderate flow again and let the bottle fill as quickly as possible without forming a lot of turbulence while keeping the stiff part of the tubing near the bottom of the bottle. As the bottle begins to overflow, let the overflow water rinse the ground glass stopper. After about one and a half bottle volumes has overflowed, begin to withdraw the tubing from the bottle by steadily raising while allowing another bottle volume (approx.) to overflow and continue to rinse the stopper. Let two to three volumes overflow before raising when taking samples from low oxygen (<~2 ml/l) water.

5) Now, immediately add 1 $\pm$ 5% ml of the manganous reagent by placing the tip of the delivery pipet just below the surface of the sample in the oxygen bottle. This step is immediately followed by the addition (in like manner) of the alkaline-iodide reagent. Both reagents are very much denser than sea-water, and they sink to the bottom and displace the upper ~2 ml of sea-water in the bottle which is helpful since the upper water has been in contact with the atmosphere. It is best not to immerse more than about 1/8" of the tips of the delivery pipets since this should help to cut down on contamination. Not immersing the tips at all increases the possibility of contamination from the atmosphere.

6) Now place the stopper in the bottle without trapping bubbles. The easiest way is also the way that works the best. Just drop the stopper into the bottle from a height of about one inch! If you place the stopper in the bottle slowly and carefully, you are more likely to trap a bubble. The stopper displaces the upper few ml of liquid in the neck of the bottle

thereby removing most of the atmospheric contamination that may have accumulated.

7) Now push down on the stopper to make sure that it is tight, and closely inspect the bottle to make sure that it is free of bubbles. A bubble the size of a pencil eraser will completely invalidate any sample, and much smaller bubbles can also totally ruin a sample depending on ambient concentrations and the accuracy that you desire. The best rule of thumb is to start over again if you can see a bubble in your sample even though there exist exceptional situations in which heating or the addition of the first two reagents can cause bubbles to form within the sample.

8) Now invert the bottle several times to mix the first two reagents with the sample. A precipitate will form. Allow the precipitate to settle, and repeat the mixing process one time. Shake vigorously during the second mixing to help break-up precipitate. Wait at least 15 minutes before repeating this mixing process. If you are using the Carpenter method (which we prefer), your sampling bottle will be a calibrated (for volume) bottle/flask made out of clear glass. Because of the possibility of undesirable photochemical reactions, it is important that you keep the bottle in the dark as much as possible. You can accomplish this by placing the bottles in a carrying case (which is painted black on the inside) in between manipulations. Also keep the samples cool (i.e., in the shade).

9) The next step is to add the acidic reagent. Some prefer to do this immediately after the precipitate in the oxygen bottle has settled for the second time, and others prefer to do it just before beginning the oxygen titration. No convincing evidence favors one technique over the other. In the Carpenter version of the Winkler method, the reagents are adjusted so that the final pH is between 2 and 2.5. This range of pH minimizes unwanted side reactions, but is just barely acid enough to dissolve the precipitate. If the precipitate is not dissolved when you are about to start your titration, add another drop of acid.

NOTES:

a. Occasionally after the addition of sulfuric acid, a gas bubble will appear. This bubble is composed largely of carbon dioxide and a little nitrogen which may have been liberated from the solution. The former results from changes in the carbonate system from the low pH. The presence of the nitrogen is accounted for by the reduced solubility of this gas upon the addition of the reagents and the possible increased temperature of the sample.

b. In running determinations of dissolved oxygen on fresh or slightly brackish waters, considerable difficulty may be experienced in

obtaining complete solution of precipitate. Solution may readily be affected by the addition of a few crystals of sodium chloride to the acid solution.

c. In fresh water studies the use of hydrochloric acid may be substituted for the sulfuric acid. (Concentrated HCL is 12N and concentrated H_2SO_4 is 36N.)

TITRATION OF SAMPLES

A measured volume or an entire flask is titrated with the standardized thiosulfate solution. Thiosulfate is added until the solution sample is a pale yellow color. Then ~1 ml of the starch indicator is added. This produces a blue color that is detected well by the human eye.

It is difficult to describe how to detect the visual end-point on the printed page, and the best way is to have an experienced analyst demonstrate this for you. In case you cannot find an experienced and competent analyst, here is a method that should work. 1) As the blue color begins to disappear, start to add the thiosulfate in small increments and begin to record the buret readings. 2) If your eyes are like mine, you will not see too much color change near the end-point. Instead the solution will become progressively clearer and/or brighter. 3) Keep on adding thisulfate until you no longer see changes in clarity and/or brightness upon the addition of more thiosulfate. 4) The reading that corresponds to the last thiosulfate addition that caused a visible change is the end-point! 5) Do not let your desire to obtain the best end-point cause the titration to take too much time! See note "b" below.

With the Aliquot method, a sample or samples of known volume are drawn from the brown glass bottle. The dissolved oxygen concentration may be calculated from the following equation:

$$DO = \frac{[R - R_{blk}]V_{IO_3} \times N_{IO_3} \times E}{[R_{std} - R_{blk}]\left[V_s - V_{reg} \times \frac{V_s}{V_b}\right]} - DO_{reg} \times \frac{140\text{ml}}{V_b}$$

where

DO = dissolved oxygen (ml/liter)

R = sample titration burette reading

R_{blk} = difference between blank titration burette readings (i.e. the blank value)

R_{std} = standardization burette reading

V_{IO_3} = volume of primary standard (ml)

V_b = volume of sample bottle (ml)

V_s = volume of titration aliquot (ml)

V_{reg} = volume of sample displaced by reagents (~2 ml for this method)

N_{IO_3} = normality of primary standard

E = 5,598 ml O_2 /equivalent

DO_{reg} = 0.018 ml/liter, the amount of oxygen added with the reagents if you were using the 125 ml flasks recommended by Carpenter (1965b) that actually contain ~140 ml.

NOTES:

a. Since the precipitate containing the dissolved oxygen has settled before the addition of the H_2SO_4, only ~2 ml of sample are displaced by reagent additions.

b. After completing the titration, if the solution is permitted to stand for a period of time, the blue starch-iodine color may again become evident. This should be ignored. Titrations should be carried out as quickly as possible consistent with accuracy.

If the entire sample is to be titrated, the amount of dissolved oxygen may be calculated from the following equation which applies to the "no-transfer" Carpenter method that we prefer.

$$DO = \frac{[R - R_{blk}]V_{IO_3} \times N_{IO_3} \times E}{[R_{std} - R_{blk}][V_b - V_{reg}]} - DO_{reg}$$

where

DO = dissolved oxygen (ml/liter)

R = sample titration burette reading

R_{blk} = blank value

R_{std} = standardization burette reading

V_{IO_3} = volume of KIO_3 standard (ml)

V_b = volume of sample bottle (ml)

V_{reg} = volume of sample displaced by reagents (ml)

N_{IO_3} = normality of KIO_3 standard (equivalents/liter)

E = 5,598 ml O_2 /equivalent, and

DO_{reg}= oxygen added with reagents when 1 ml of Manganous and 1 ml of alkaline iodide reagents are added to a 140 ml bottle/flask.

For the procedure described by Carpenter (1965b) in which 10.00 ml of 0.01000 N standard are employed,

$$DO = \frac{[R - R_{blk}]559.8}{[R_{std} - R_{blk}][V_b - 2]} - 0.018 \text{ ml / l}$$

NOTES: a) As methods for the determination of dissolved oxygen become increasingly accurate, the nominal value of 0.018 ml/l for the oxygen added with reagents should receive more scrutiny. Obviously, this value can change with factors such as temperature and the exact composition of the pickling reagents. It might be wise to do some experiments with reagents that have been purged with pure nitrogen to check this value for your particular experimental conditions. It may also eventually prove desirable to determine "Blanks" on sea-water samples that are treated like the blanks described in Carpenter's (1965) paper. Finally, calibrating the delivery of the devices that dispense the first two pickling reagents (Mn^{++} and alkaline NaI) becomes more and more important. Errors of ~5% in the volume of pickling reagents dispensed can cause errors of 0.1% in the final dissolved oxygen concentration and some of the new automated methods (see below) may have precisions of better than 0.1 %.

b. If you prefer to use the standard curve method for calibrating that we prefer, substitute the slope of the linear regression for:

$$\frac{V_{IO_3} \times N_{IO_3}}{[R_{std} - R_{blk}]}$$

SOURCES OF ERROR IN THE WINKLER DETERMINATION CAUSED BY SUBSTANCES FOUND ONLY OCCASIONALLY IN SEA WATER

a. If nitrite is present, high values for oxygen may be obtained because of the following chemical reaction:

$$2NO_2^- + 2I^- + 4H^+ \longrightarrow 2NO + I_2 + 2H_2O$$

With the exception of oxygen deficient regions where O_2 is less than ~ 0.1 ml/l the concentrations of nitrites in sea water are usually< 1 µg-atom/l.

b. If hydrogen sulfide is present, the Winkler method for determining oxygen is not applicable. Hydrogen sulfide will react with the dissolved oxygen and with iodine.

c. In waters polluted by industrial waste or containing relatively large concentrations of reducing material, Winkler's method is not applicable because any iodine liberated may react with the reducing substances or produced by oxidants.

LOW CONCENTRATION DISSOLVED OXYGEN METHOD

Broenkow and Cline (1969) have described a colorimetric dissolved oxygen method which is more suited to low concentrations (< 0.4 ml/liter) than the more normal methods described above. The normal Winkler methods described above cannot resolve the 0 to ~ 0.15 ml/l dissolved oxygen range, and high nitrite levels often occur in low oxygen waters.

AUTOMATED METHODS

A number of investigators (Williams and Jenkinson, 1982; Friederich, Sherman and Codispoti, 1984; Culberson and Huang, 1987; Friederich and Codispoti, 1991) have automated the dissolved oxygen titration. These methods reduce eye-strain, improve precision and allow the data to be directly acquired by computer. They do not require

the addition of starch since the yellow iodine color that is poorly perceived by the human eye can be detected with great precision electronically.

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