APPENDIX VII

NOTE: The First nine pages of this appendix contain several drawings that could not be easily reproduced for the WWW. This Appendix also contains chemical formulas that can not be correctly presented on the Web. Every effort has been made to present the information in a form that accurately presents the data or formula. Subscripts are italicized Copies of the complete appendix can be obtained from the UNOLS Office.

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An Easy-to-Construct Automated Winkler Titration System
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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 endpoint 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 1). A disk containing the program needed to operate the new device is also included. (This disk is not available from the UNOLS Office.)

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 5 ml dispensing unit was used while developing the current software. Using a l ml 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 the UNOLS Office for a copy of this diagram.)

The connection between the Dosimat and a standard 25 pin RS232 computer port is:(Contact the UNOLS Office for a copy of this diagram.)

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.

(Contact the UNOLS Office for a copy of this figure)

A top view of the internal layout of the base unit: (Contact the UNOLS Office for a copy of this diagram.)

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. (Contact the UNOLS Office for a copy of this diagram.)

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. (Contact the UNOLS Office for a copy of this diagram.)

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 Ml 141 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. (Contact the UNOLS Office for a copy of this diagram)

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:

$lWE	(write enable)
$lSU3lA2OlC2	(setup string)
$lWE	(w-rite enable)
$lRR	(remote reset)

For details on this procedure refer to chapters 1 and 5 in the Mlle/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.

<u>Major Components List</u>

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

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 mm
2. Near zero transmission between 450 run and 1 100 mn
3. Greater than 30% transmission at 350 mm

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 VIIa, 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

(Note: This section contains chemical and other formulas that can not be correctly presented on a Web page. All of the data is presented for interpretation.)

Manganous Chloride: Dissolve 600 gm of reagent grade Mn Cl_2 $4H_2O$ 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 1 ml 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 l ml 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 Na2S2O3.5H20 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° C) 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°C. 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 H2SO4 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 microenviromments 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 procedure 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.
	Accepts a menu choice

Enter	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, canceling 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 edit 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 [Fl] or enter the File menu and choose Create File.
2. Enter a disc drive name or press Enter to choose the cuffent 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 [FlO] 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:
   - $X = (9000\text{-detector output in mv})\text{-(Incremental Rate)}$
   - Volume in ml $= (X + X(\text{superscript}1.3)/2000)\text{-}(0.0001\text{-volume of Dosimat unit})$
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 fired 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 unformed ASCII text files. Printing uses the LPT1 port. Exporting requests a destination directory that cannot be the same as 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.

Acknowledgments

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