Strathkelvin 782 2-Channel Oxygen System version 1.0

CONTENTS

1 Safety And Technical Information

As with all electrical equipment, care should be taken not to splash water, especially seawater, on to the meter or electrodes.

The 782 meter conforms to the protection requirements of the Electromagnetic Compatibility Directive as per generic standards EN50081 part 1 (emissions), and EN50082 part 1 (immunity).

If subjected to strong radiated radio-frequency interference, such as that generated by mobile telephones at close range, a degradation of performance, including apparent changes in electrode output, may be expected. (Permitted Performance Class A, Electromagnetic Compatibility Directive.)

The 782 meter conforms to the requirements of the Low Voltage Directive 73/23/EEC as per generic standard EN61010-1, part 1.

SPECIFICATION:

Inputs:	2 oxygen electrode connectors, bias voltage 650mV, maximum input current 2.6 nA.
Connection:	USB version 1.0.
Meter Power:	5V DC +/- 10 %.
Plug top power unit:	Input 100-240V AC, 47-63 Hz. Output 5V DC, 2.4A.
General:	
Operating Range:	+5ºC to +40ºC, 20% to 80% RH.
Storage Range:	-20ºC to +60ºC.
Environmental:	Indoor use at altitudes up to 2000m. Pollution Degree 1.
Safety:	Complies with EN61010-1.
EMC:	Complies with EN50081-1 and EN50082-1.
Size:	185 x 135 x 105 mm.
Weight:	Meter: 0.66 kg; Power unit: 0.18 kg.

2 The 782 System

2.1 The 782 System

Welcome to the 782 2-channel dissolved oxygen measuring system.

This system offers the flexibility to either use the meter connected to a computer or to use its datalogging facility to store your data until the meter can be attached to a computer. This eliminates the need to spend long hours keeping notebooks of data and analysing slopes from chart recorder traces. Everything is carried out automatically. You will be able to conduct the whole experiment on screen, from set-up right through to tabulation of normalised respiration rates, which can be copied to other Windows programs (eg. spreadsheet) for graphical display or statistical analysis. The facility to print the screen traces to a color printer is also available.

2.2 Minimum System Requirements

The 782 system is supplied as a meter, USB connecting cable, software and instruction manual. In addition, you will need up to 2 oxygen electrodes, an electrode service kit, and respirometer chambers available from Strathkelvin Instruments. In addition a constant temperature water bath to maintain the electrodes within the range of ± 0.05°C will be needed. The system has been designed for the Strathkelvin 1302 Clark-type microcathode oxygen electrodes, but should be compatible with some other low output electrodes.

Computer and Printer

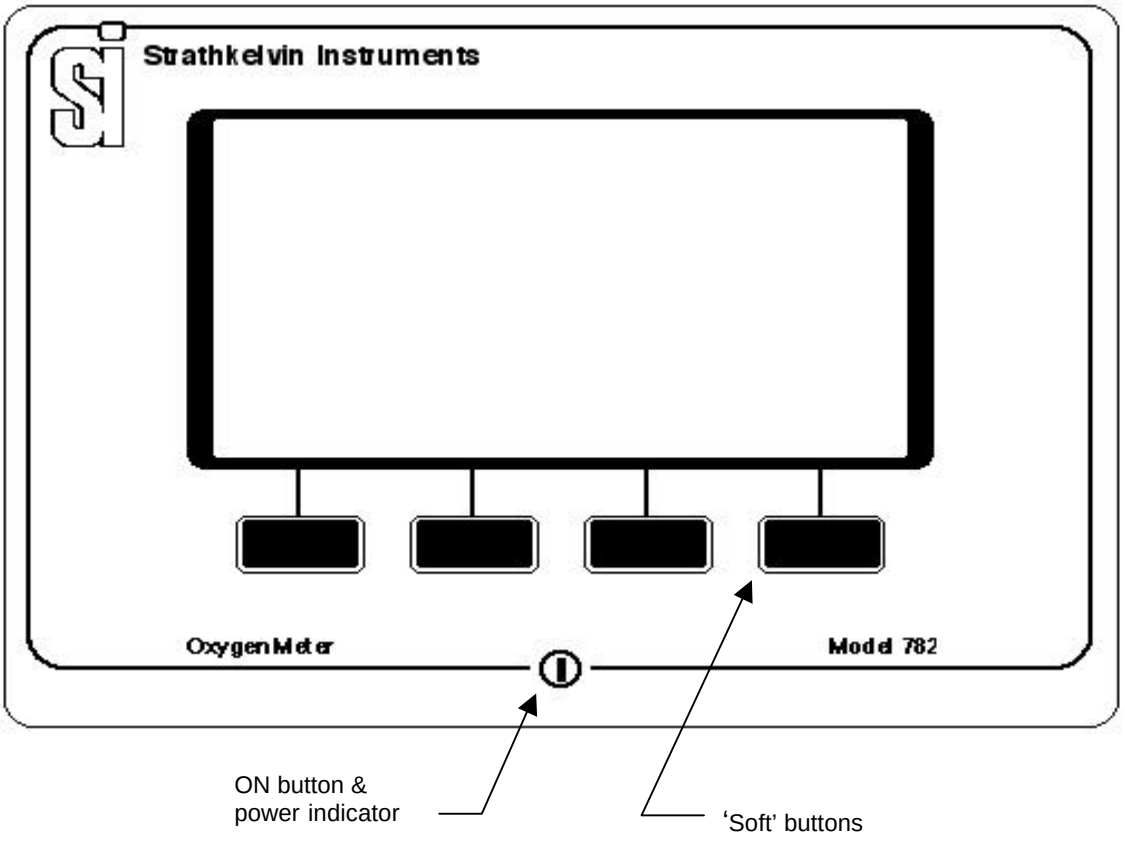
You will need a Pentium computer with a USB port and Windows 98, ME, 2000 or XP installed. You will also need at least 6 Mbytes of free hard disk space and at least 64 Mbytes of RAM.

For many applications a color printer will not be necessary since recordings will be saved as data files which can be recalled as required. However the facility is available for chart-recorder-like screen traces to be printed out as hard copy. Any standard color inkjet printer is suitable.

2.3 The 782 Meter

The meter contains the electronics needed to supply a fixed stable bias voltage to up to 2 oxygen electrodes, amplify the signal currents from them, convert them into digital values, display and store them and/or send them to the computer.

Front Panel

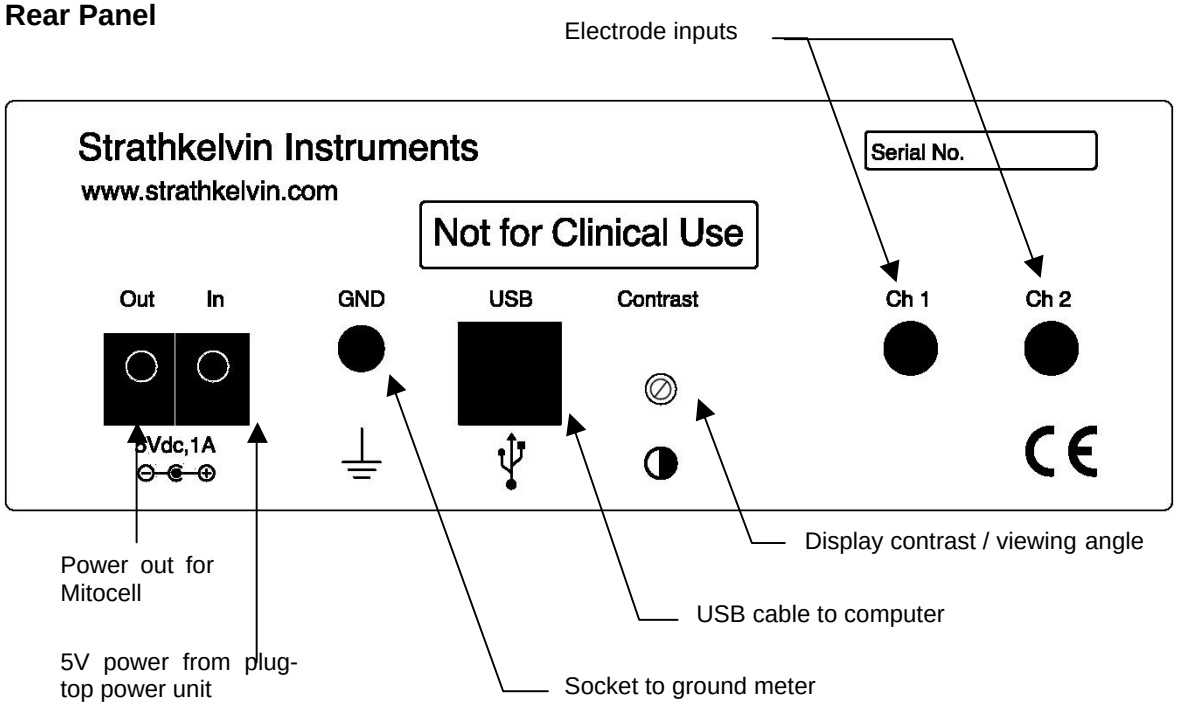


The ON button & power indicator illuminates to indicate that the meter is receiving 5V power. When it is pressed it switches the meter on. The electrode(s) are polarised when they are connected and the power indicator is illuminated.

Four 'soft' buttons are labelled immediately above on the display. Their functions change with the display. These only function when the meter is not connected to a computer running the Strathkelvin software.

The display also shows the oxygen concentration values with the units (selected in setup) plus difference (if selected in setup) and time and date (if selected in setup).

Rear Panel



The Power **Out** can be used to power one or two Mitocells (if purchased).

The Power **In** must be supplied from the 5V plug top power unit supplied.

The **GND** socket allows the meter to be grounded if necessary.

The meter is attached to the computer by a **USB** cable.

The display **contrast** adjuster is used to adjust the contrast and viewing angle of the front panel display.

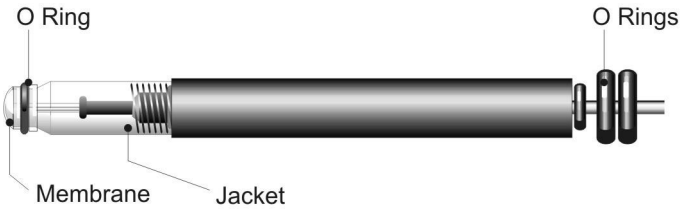
The Electrode input sockets (**Ch1** and **Ch2**) receive the plugs from the 1302 oxygen electrodes.

2.4 1302 Microcathode Oxygen Electrodes

These electrodes, which are in use worldwide with Strathkelvin's 781, 782 and 928 instruments, have the advantage of small size, high precision, and the ability to operate in either stirred or unstirred media. They are supplied with polypropylene membranes, but for fast speed of response, they can be fitted with FEP membranes. In this configuration, they cannot be used unstirred and require vigorous water movement at the electrode tip. In all applications, the electrodes have to be housed in special electrode holders or accessories (see the Strathkelvin website: www.strathkelvin.com). These have been designed so that only the membrane at the electrode tip comes into contact with the medium. Failure to protect the electrode in this way can result in anode depolarisation and a shortening of the useful life of the electrode.

The 1302 Electrode

A full description of the 1302 electrode, including theoretical considerations and details of care and maintenance, is given in Appendix 8.2.



2.5 Computer Software

The software has been written for Windows, and has been structured so that it is user-friendly and intuitive in use. The full power of the program is accessible to anyone with only introductory-level knowledge of Windows. Nevertheless, it has the flexibility and power typical of Windows programs which may be appreciated by more advanced Windows users.

From the opening screen you will be offered the choice of either recording an experiment or of analysing a previously recorded data set. If you choose to record, the screen changes to the **Recording Screen**, in the set-up mode. The menus bring up dialog boxes which allow you to specify the details of the experiment. These details will then be used to set the axes and scrolling rate and other features of the recording screen. These settings are saved and many of them will therefore not need to be set up again in subsequent experiments. The **Setup** screen is also used for the calibration of the oxygen electrodes.

When recording, traces of the oxygen measurements scroll across the screen, as on a chart recorder. The menu provides facilities for placing event markers on the screen to indicate any changes to the preparation, such as the addition of substances to the respirometer chambers. At the termination of recording, the data file is closed and you have the option of analysing the data immediately or of doing this at a later date.

To calculate respiration rates or to read oxygen values from the experimental traces, the data from the experiment are recalled from the data file to an **Analysis** screen. The traces can be scrolled across the screen and expanded or contracted on the *x* or *y* axis until the section of trace which is of interest is optimally displayed. In respiration experiments, the rate is automatically calculated on the selected section of trace, normalised to biomass if required, and tabulated with all the experimental details on a **Report** page. Hard copy of the results can be printed and/or the results can be exported direct to other Windows applications.

On-screen Help is always available.

2.6 Product Support

A trouble-shooting section is included in Appendix 8.4 to assist with any problems which might arise in the use of oxygen electrodes.

For any other problems contact Strathkelvin Instruments directly by e-mail: **info@strathkelvin.com**

2.7 Uses of the 782 System

The 782 has been designed to meet the need for replication of dissolved oxygen measurement, with provision for up to 2 oxygen electrodes. The main uses of the system are as follows:

Respiration Measurements

Respiration rate is measured in a wide variety of applications, with a variety of different procedures and units of oxygen measurement being used. The main procedures are:

Closed cell respirometry in which a decrease in oxygen with time is measured. This is probably the most commonly used method, so the Instruction Manual will focus most closely on this procedure.

Flow-through respirometry in which the respiration rate is determined from the rate of water flow, and from the difference in the oxygen concentration of water entering and leaving the respirometer. This method has the advantage of operating at a constant oxygen level and is particularly suitable for longer-term experiments.

Oxygen Monitoring In many situations there is simply a requirement for logging of oxygen levels, as in the outflow of blood or saline from organ preparations. The 782 is ideally suited to log data from the oxygen electrodes in such experiments.

3 Getting Started

3.1 Software Installation

You will need a Pentium computer with a USB port and Windows 98, ME, 2000 or XP installed. You will also need at least 6 Mbytes of free hard disk space and at least 64 Mbytes of RAM.

If you are installing to Windows 2000, you must have administrator privileges set.

Insert the installation CD into the CDROM drive in your computer.

1. If the setup program does not start automatically, click on the **Start** button and select **Run**. Windows will prompt you for the name of an application to run; type the following command:

   d:setup if the CDROM is in drive D:

2. Press **Enter**.

3. The setup program will then ask you if you wish to install the software to the \Strathk folder on the hard disk containing Windows. If you wish to install it to a different hard disk, change the drive letter; otherwise simply click on **Continue** or press **Enter**.

4. At the conclusion of the installation process, you will find a new icon on the desktop called '782 Oxygen System'.

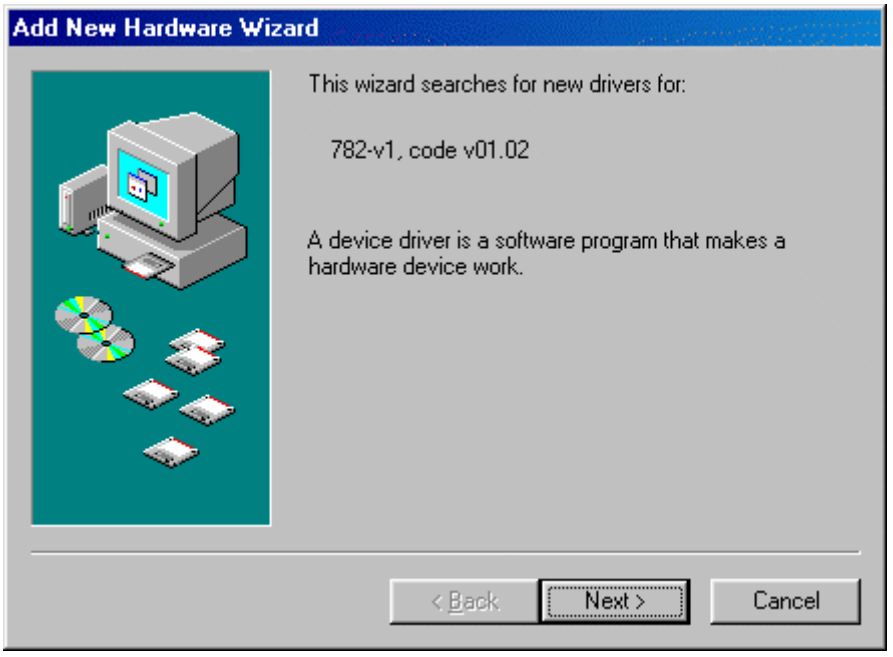
Leave the CDROM in the drive until the meter is running, connected to the PC and the driver has been installed (see below).

3.2 Setting up the 782 Meter

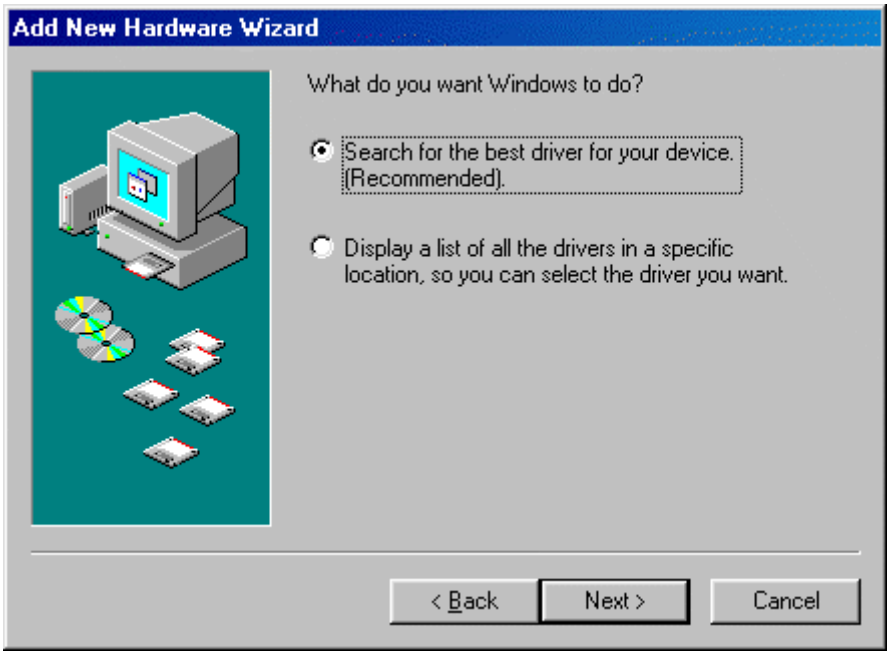
Place the meter where you intend to use it, ensuring that there is no risk of water or saline falling on to its case. ***The meter is not water resistant***. Plug the 5V power supply into your power outlet and push its output plug firmly into the **5V In** socket on the meter. Switch on the power at the outlet and leave it permanently on; thereafter the meter should be turned on and off with its front panel buttons.

Press the **ON** button indicator on the meter front panel to switch on. Check that the display is easily readable from your normal operating position. If not, use a small screwdriver through the Contrast hole in the rear panel to adjust the single-turn control until the display contrast is optimum for your viewing angle.

Connect the USB cable. On the PC, Windows will detect the connection and show this dialog to initiate the installation of the appropriate USB driver:



Click **Next** to display this dialog:



Select **Search for the best driver…** as shown and click **Next** to show:

Tick **CD-ROM drive** only and click **Next** to show:

Click **Next** to install the driver. A final dialog announcing that Windows has finished installing the driver should appear. Click **Finish** on this dialog and remove the CDROM from your computer.

3.3 Operation

Always switch the meter on before starting the 782 computer program.

When first switched on, the meter initialises its internal program and then displays the oxygen concentrations using the settings stored when it was switched off. The front buttons will be operational.

Once the computer program starts running, the meter display will show ‘ONLINE’ and continue to display the oxygen values for each channel selected on the computer. The buttons on the meter will cease to operate.

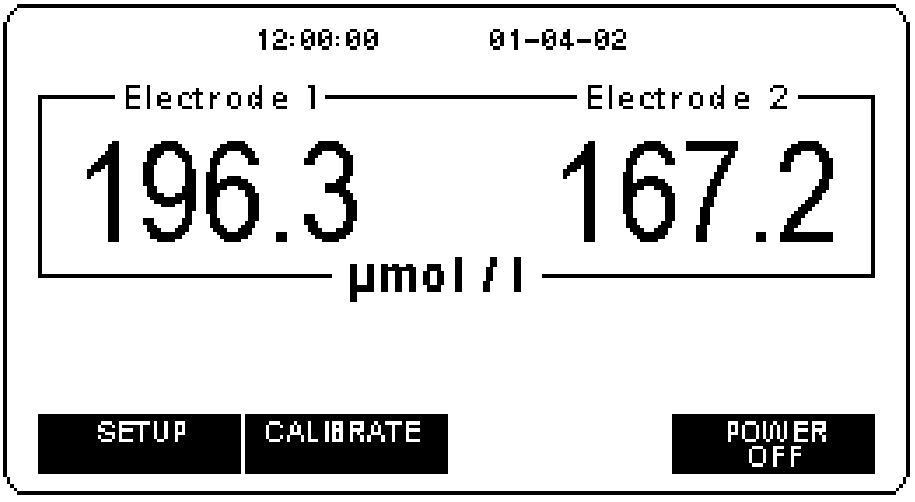
The meter can only be switched off with its panel buttons when it is **not** under computer control. This protects against accidental interruption of an experiment. By making the appropriate selection on the program menus the meter can be made to turn off when the program is closed down.

4.1 Introduction

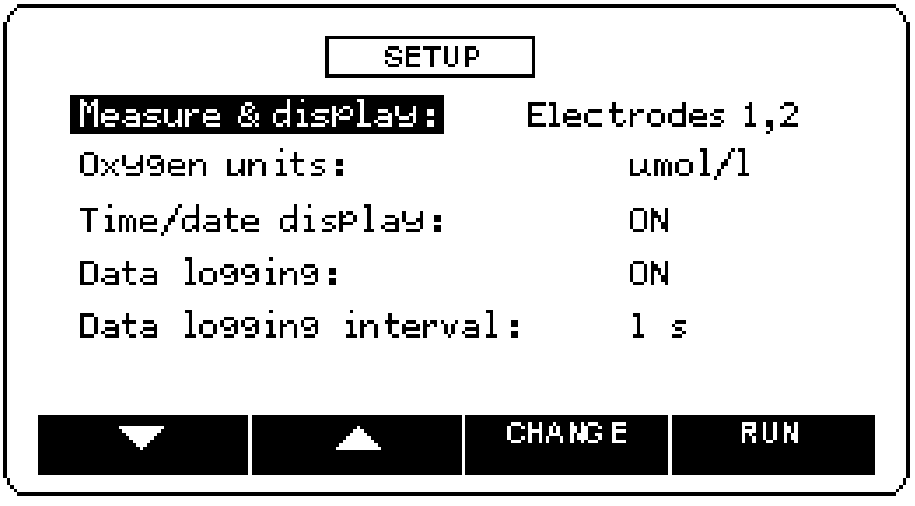
This chapter describes the setup and running of experiments when the instrument is used as a stand alone meter i.e. when it is not controlled by a computer. If the meter will always be used with a computer then the setup and running procedures will be carried out using the 782 System Software which is described in Chapter 5.

4.2 Setup

When the meter is switched on its display will look similar to that below.



To change to one electrode or to change the units or other settings press the **SETUP** button which will bring up this screen:



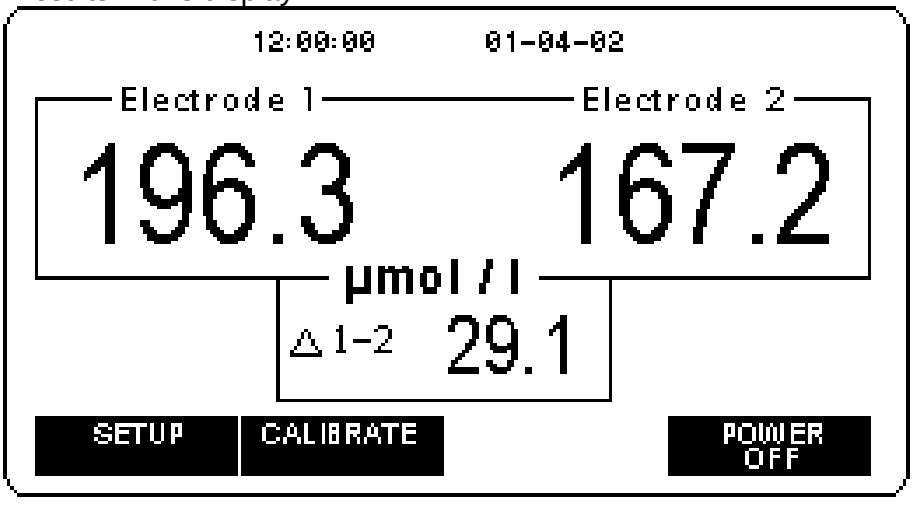
To change any of the **SETUP** options use either the ▼ or ▲ button and press the **CHANGE** button when the option to be changed is highlighted. You may need to press the **CHANGE** button a number of times to go though all the options that can be selected.

SETUP **Options**

Measure and Display: has three options

Electrode 1	will make measurements from the electrode plugged into Ch 1.
Electrode 1, 2	will make measurements from two electrodes plugged into Ch1 and Ch2.
Electrodes 1, 2, 1-2	will make measurements from two electrodes plugged into Ch1 and Ch2 and will display the difference between the readings by subtracting the reading of electrode 2 from that of electrode 1.

The last option results in this display.



Oxygen Units: You may choose to work in units of µg/ml; mg/l; µl/ml; ml/l; µmol/l; torr; kPa or %saturation.

Time/date display: ON/OFF

Note: The Time/date display is set to Windows time/date settings every time the meter is connected to the PC and the Strathkelvin software is run.

Data Logging: ON/OFF This enables a facility for saving oxygen measurements to the memory of the meter (see section 4.4)

When Data Logging is selected **ON** another option will be displayed.

Data logging interval: You may select Manual to save a reading each time the Save button is pressed, or a time interval to automatically save values at fixed time intervals. Intervals of 1s, 5s, 10s or 20s are available. Note that you cannot change between Manual and Timed intervals unless the data memory is empty.

To exit the **SETUP** screen, press the **Run** button.

4.3 Calibrating your Electrodes

The electrodes will normally need to be calibrated every day or when the measurements units have been changed. If the electrodes have just been connected to the meter, or the power to the meter has just been turned on, wait one hour to allow the electrode(s) to stabilise before calibrating. Subsequently, the electrodes can be kept connected to the meter with the power on ready for immediate use.

> **Note:** If you have changed the type of membrane (polypropylene, FEP), you must connect the meter to the PC and run the software to change the meter sensitivity. See Section 5.4.

During calibration, the meter displays the electrode output in picoamps. This is not used in the actual calibration procedure but is a useful check on the state of 'health' of the electrodes. See Appendix 8.3.

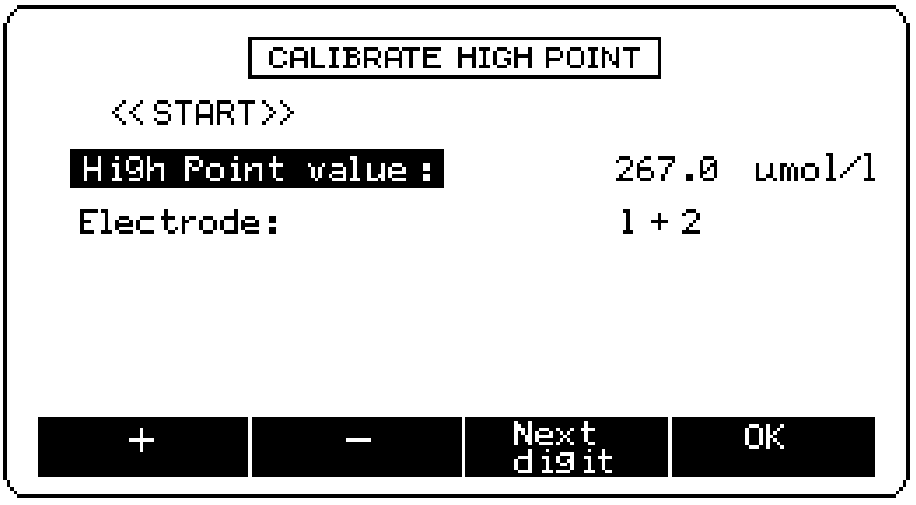
Before starting your calibration, make sure your calibrating solutions have been prepared (see Appendix 8.6).

Proceed through the following steps:

1. Set the high point calibration value. If your high-point calibration solution will be air-saturated water, this will be:

   The partial pressure of oxygen, if working in pressure units
   or
   100 % saturation, if working in percentage saturation units
   or
   the concentration of oxygen in an air-saturated solution which you will have obtained from tables (see Appendix 8.7), if using concentration units.

   The high point value can be changed by using the ▼ button to highlight the **High point value:** and then press **Change.** The meter will then display the following:

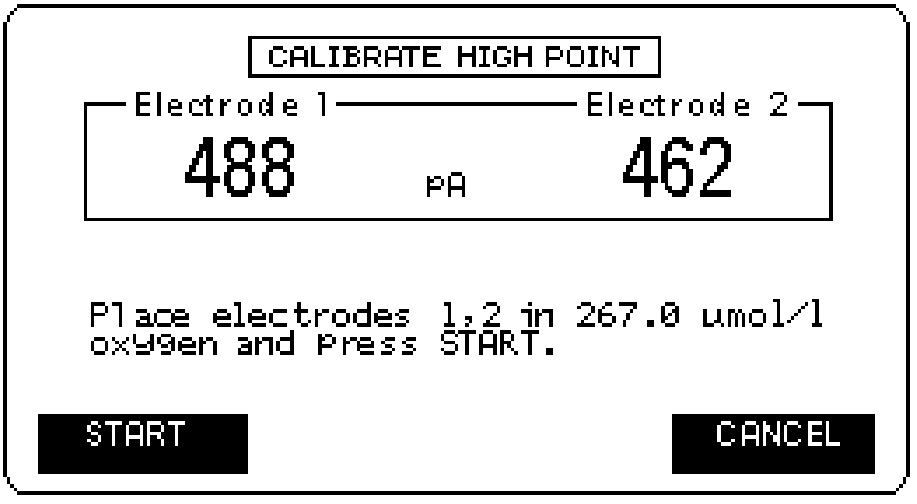


The furthest right digit will be flashing, in this case the 0. Use the **+** or **–** buttons to increase or decrease the number. Once it is correct, press the **Next digit** button so that the next digit left will flash and repeat the process.

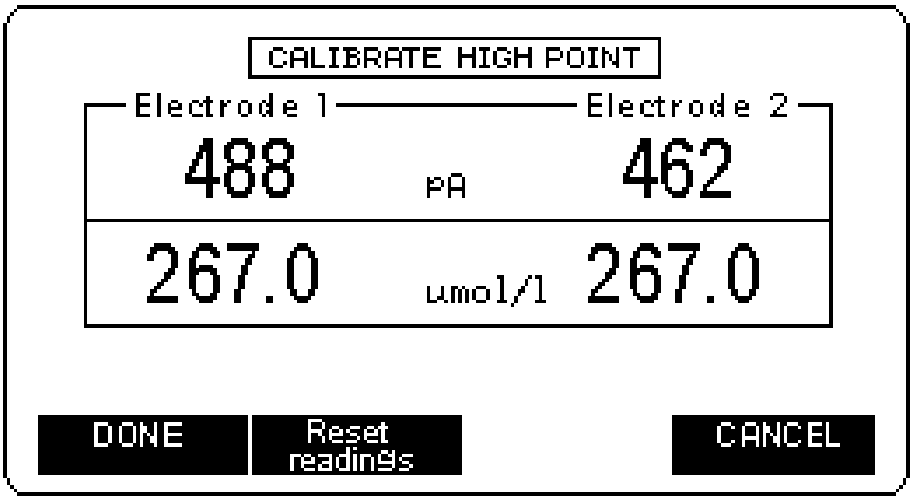
2. The electrodes may be calibrated together or singly. To change the electrode(s) selected highlight the **Electrode**: option using the ▼ button and keep pressing the **CHANGE** button until you have the required option. Press the **OK** button.

3. Use the▲ button to highlight **<<START>>** and then press the **OK** button.

4. The display below will appear, telling you to expose the electrode(s) to the high point calibration solution. After doing this, press the **START** button. There is also an option to **CANCEL** the calibration at this stage.



5. You will then be asked to wait while the electrode(s) stabilise. At the end of this one minute waiting period, the display below will appear.



6. Press the **Reset readings** button if any of the values drift away from the high point value. When the values are stable, press the **Done** button.

Note: It is important that you do **not** remove the electrode(s) from the calibrating solution before you press the **DONE** button.

7. The display will now change to **CALIBRATE ZERO**. Repeat steps 4 to 6, using the zero oxygen calibrating solution. If the **CANCEL** button is pressed at this stage the high point calibration will be saved.

4.4 Data Logging

The data logging facility allows readings to be saved when the meter is not attached to a computer. The meter can be set up to either take readings at fixed time intervals or to allow data to be manually saved.

On using the meter for the first time the data logging function will be turned off. To start data logging press the **SETUP** button. Use the ▼ button to highlight the **Data logging:** option. Press the **CHANGE** button to turn the Data logging ON. Another **SETUP** option will now be available titled **Data logging interval**. Using the down arrow button highlight the **Data logging interval** option and press the **CHANGE** button to go through the options available. Manual logging or Timed readings at fixed intervals can be selected.

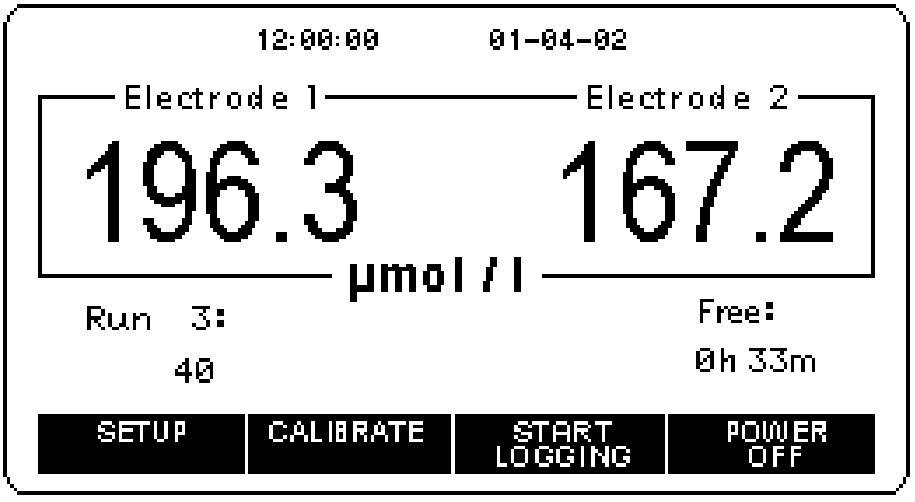
Timed readings

Timed readings are taken at fixed time intervals of 1s, 5s, 10s or 20s. Depending on the time interval chosen the display will show the amount of logging time remaining at the current sampling rate (amount of free memory). This will be displayed on the lower right-hand side of the display under the heading **Free**.

For each sample interval the total logging time is given below.

Sample Interval (seconds)	**Logging Time– 1 channel** (hours & minutes)	**Logging Time – 2 channels** (hours & minutes)
1s	4h 32 m	2h 16m
5s	22h 44m	11h 22m
10s	45h 28m	22h 44m
20s	90h 56m	45h 28m

The recorded data may be divided into a number of runs (maximum: 20 runs), a new run beginning each time the **START LOGGING** button is pressed. On the lower left-hand side of the display the run number and the number of readings saved in the run are displayed. An example of a display is given below showing Run 3 with 40 readings and a Free logging time of 33 minutes.



To start a logging run, press the **START LOGGING** button.

The options available will change to **SET MARK** and **STOP LOGGING**.

The **SET MARK** allows a marker to be set in the recording data. If you want to introduce a substance to the respirometer chamber, you can record this event by inserting an event marker. You must note manually the information corresponding to the marker as this cannot entered. The time the marker was set will be up loaded along with the data when the meter is connected to a computer. A maximum of 8 markers per run may be set. The **SET MARK** button label shows the number of the next marker to be stored.

At the end of a run press the **STOP LOGGING** button. The **Run** display will increase by one.

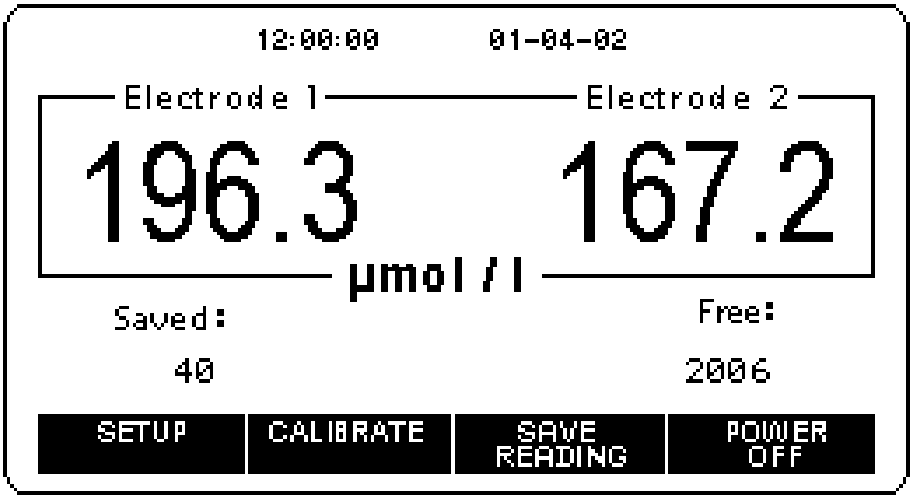
Manual readings

If automatic readings have been saved you will need to up load these and erase the memory before the manual option can be selected.

Change **Data logging interval** to the **Manual** setting.

The display will list the number of readings that can be saved in the lower right hand side under the heading **Free**. Before any readings have been saved it will show 3272 when 2

channels are selected. On the lower left hand side of the display the number saved will be given under the heading **Saved**. An example of this display screen is given below.



To save a reading press the **SAVE READING** button.

Notes:
1. The memory of the meter can only be erased with the computer program.
2. If manual data is stored, logging will be disabled if the number of channels is changed.

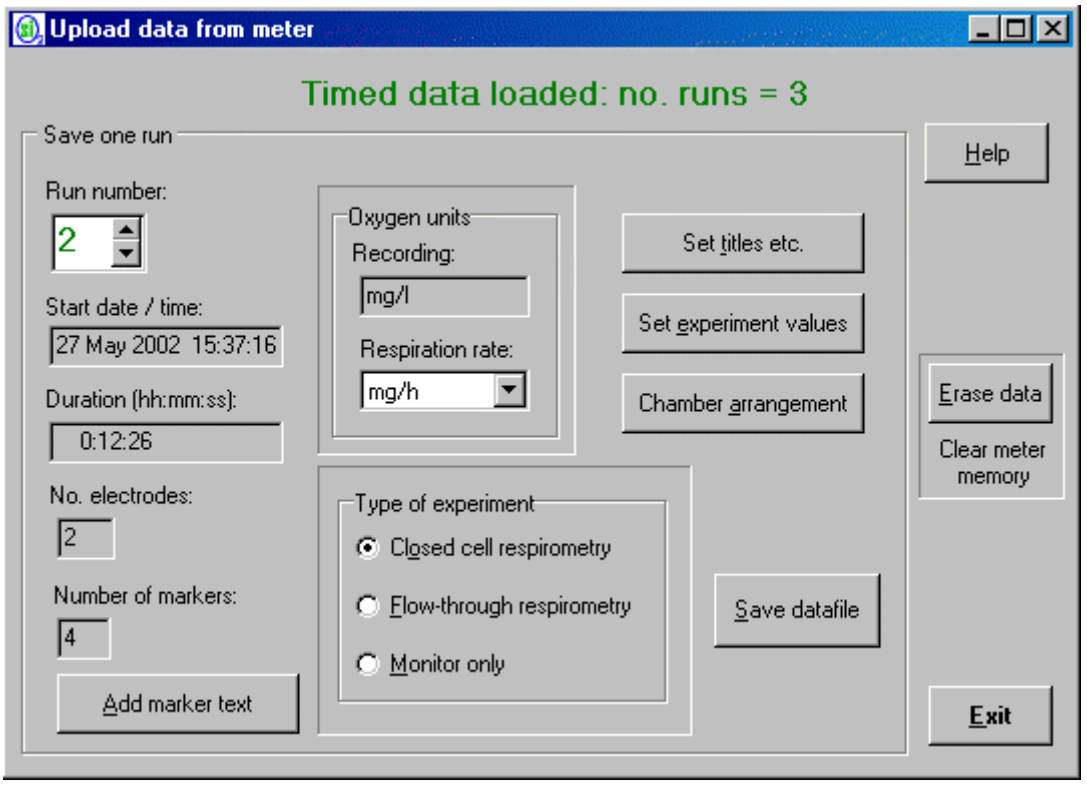
To switch off the meter press the **POWER OFF** button. A screen will display asking you to confirm power off. If you wish to continue, press the **OK** button if not press the **CANCEL** button.

4.5 Uploading Stored Data from the 782 Meter

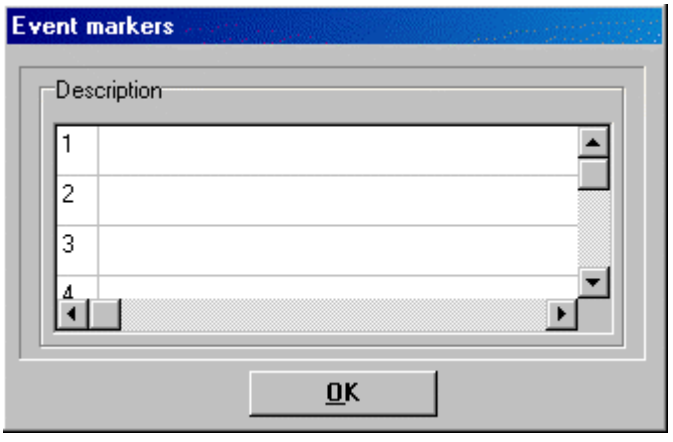
Connect the meter to the PC using the USB cable. Start the Strathkelvin software and press the **Upload data** button will cause the **Upload data from meter** dialog to appear. If no data is available, the dialog will display 'No data stored'. If data is loaded from the meter the dialog will appear in one of two forms depending on whether the stored data is timed or manual:

Timed readings

This may consist of a number of runs, each containing samples saved at fixed intervals of 1, 5, 10 or 20s. The time of start of each run and up to 8 event markers are also recorded. After adding details of the experiment, each run can be saved to disc, to be subsequently opened by the Analyse part of the Strathkelvin software.



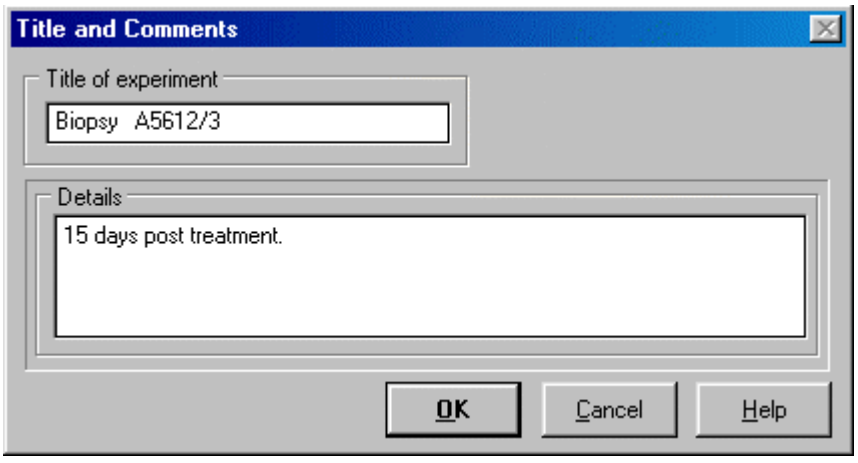
If the stored data includes markers, the **Add marker text** button will display a dialog to allow the entry of a description of each marker.



The units for **Respiration rate** can be chosen from the list box.

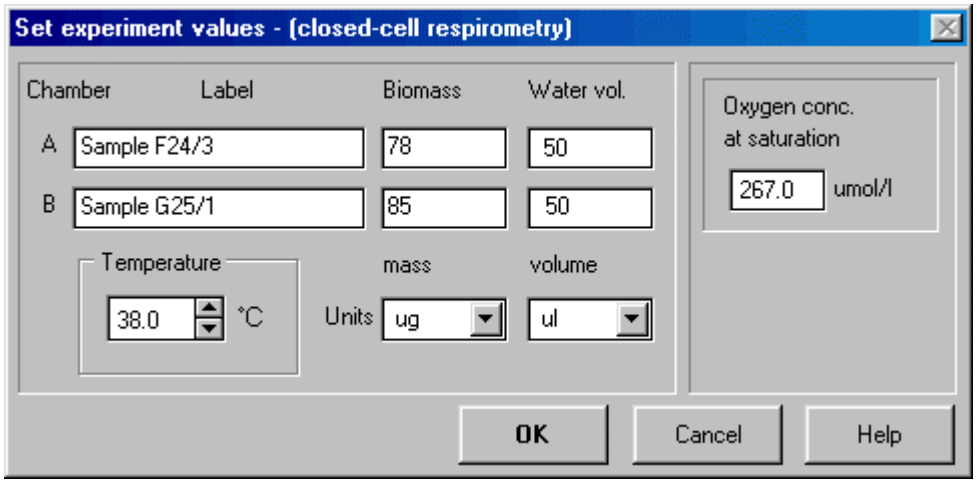
Select the **Type of experiment** by highlighting one of the three options.

Click **Set titles etc** to display the dialog which allows the entry of a title and description of the experiment. The text will be saved with the data file (recording).



Click the **Set experiment values** button to enter data required for analysis (you must have selected the experiment type first). Three different dialogs will display depending on the experiment type. The data to be entered includes chamber volume, biomass and flow rate.

1) If you selected **Closed cell respirometry** you will now see the following dialog:



This dialog box provides for the entry of details of the experiment. Since this is closed chamber respirometry, there will be one electrode to each respirometer chamber. We assume that electrode 1 will be used with chamber A, electrode 2 with chamber B.

Label For each of the electrodes in use you may record some information about the material in the respirometer chamber.

Biomass If the biomass is important, either for subsequent plotting of results or for normalising of respiration rate to biomass, the relevant values should be entered here. The units should be selected from the list box below.

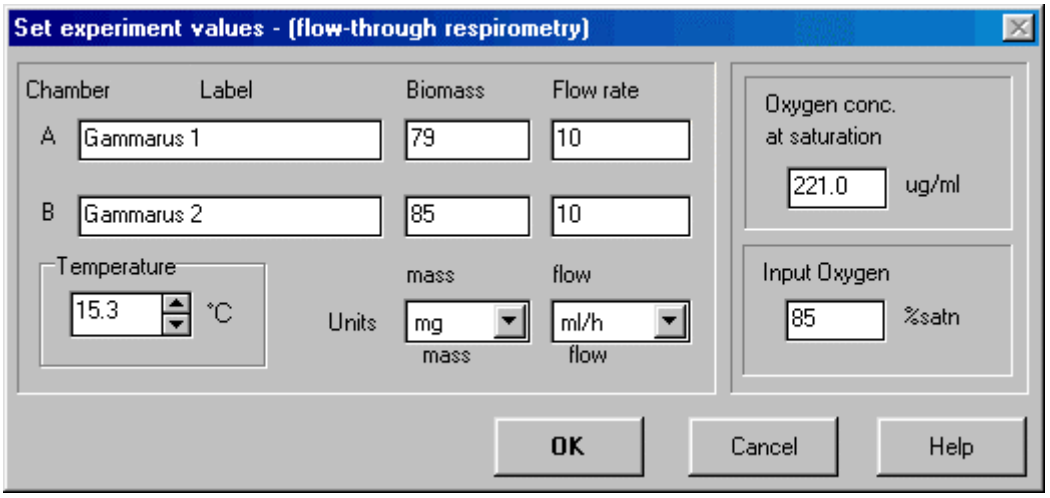
Water Vol. Enter the volume of water or solution in each chamber. The units should be selected from the list box below.

Oxygen conc. at saturation This box will appear on the dialog if you have measured in units of % saturation. Enter the oxygen

concentration of water or solution which is 100 % saturated with air. You can obtain these values from published tables (see Appendix 8.7).

Solubility factor This box will only appear if you have measured oxygen in units of oxygen partial pressure i.e. torr (mm Hg) or kPa. Enter the concentration of oxygen air saturation in the units which are specified. These values can be obtained from published sources (see Appendix 8.7).

2) If you selected **Flow-through respirometry** you will now see the following dialog:



This dialog provides for the entry of the details of the experiment. The arrangement of chambers and electrodes will need to be selected in the Chamber arrangement dialog.

Label For each of the electrodes in use you may record some information about the material in the respirometer chamber.

Temperature Use the up/down arrows to specify the temperature at which the experiment was run.

Biomass If the biomass is important, either for subsequent plotting of results or for normalising of respiration rate to biomass, the relevant values should be entered here. The units should be selected from the list box below.

Flow rate Enter the flow rate through each of the chambers. The units should be selected from the list box below.

Oxygen conc. at saturation This box will appear on the dialog if you have measured in units of % saturation. Enter the oxygen concentration of water or solution which is 100 % saturated with air. You can obtain these values from published tables (see Appendix 8.7).

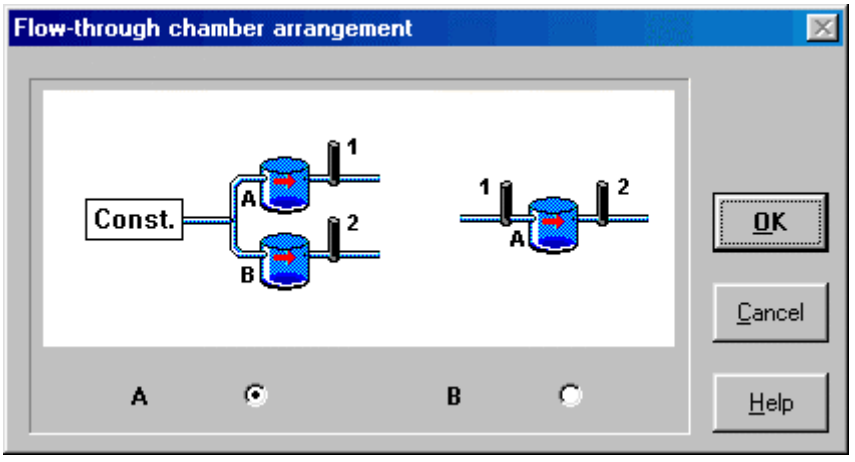
Solubility factor This box will only appear if you have measured oxygen in units of oxygen partial pressure i.e. torr (mm Hg) or kPa. Enter the concentration of oxygen air saturation in the units which are

specified. These values can be obtained from published sources (see Appendix 8.7).

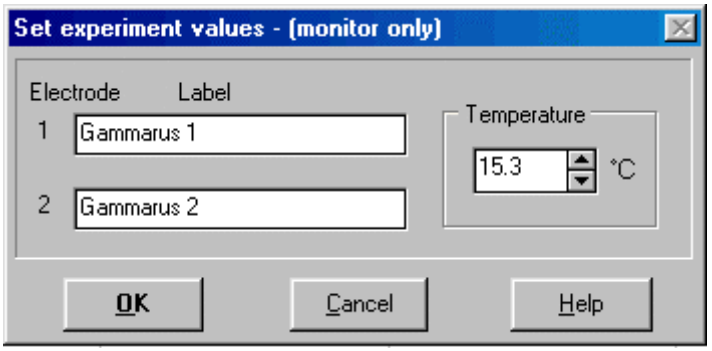
Input oxygen This box will only appear if you have chosen configuration A in chamber arrangements i.e. with a constant input oxygen concentration. Enter the oxygen concentration or partial pressure or percentage saturation of the inflow depending upon the recording units. If you have not chosen the chamber arrangement press OK and click **Chamber arrangement**. Remember to go back into **Set experiment values** to finish adding the data.

Chamber arrangement

Choose the arrangement which matches your experiment. The software automatically designates a letter (A, B) for each respirometer chamber. These chamber identifiers will be used subsequently in the Analysis part of the program. If you are using two electrodes it is important to check that they are connected to the 782 inputs so that their numbers match the positions shown.



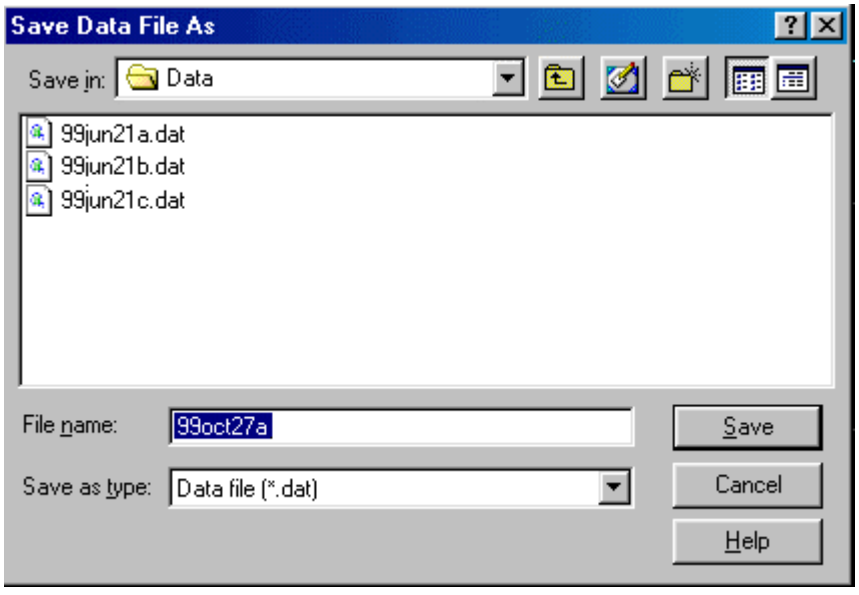
3) If you selected **Monitor only**, you will now see the following dialog:



Label For each of the electrodes in use you may record some information about the material in the respirometer chamber.

Temperature Use the up/down arrows to specify the temperature at which the experiment was run.

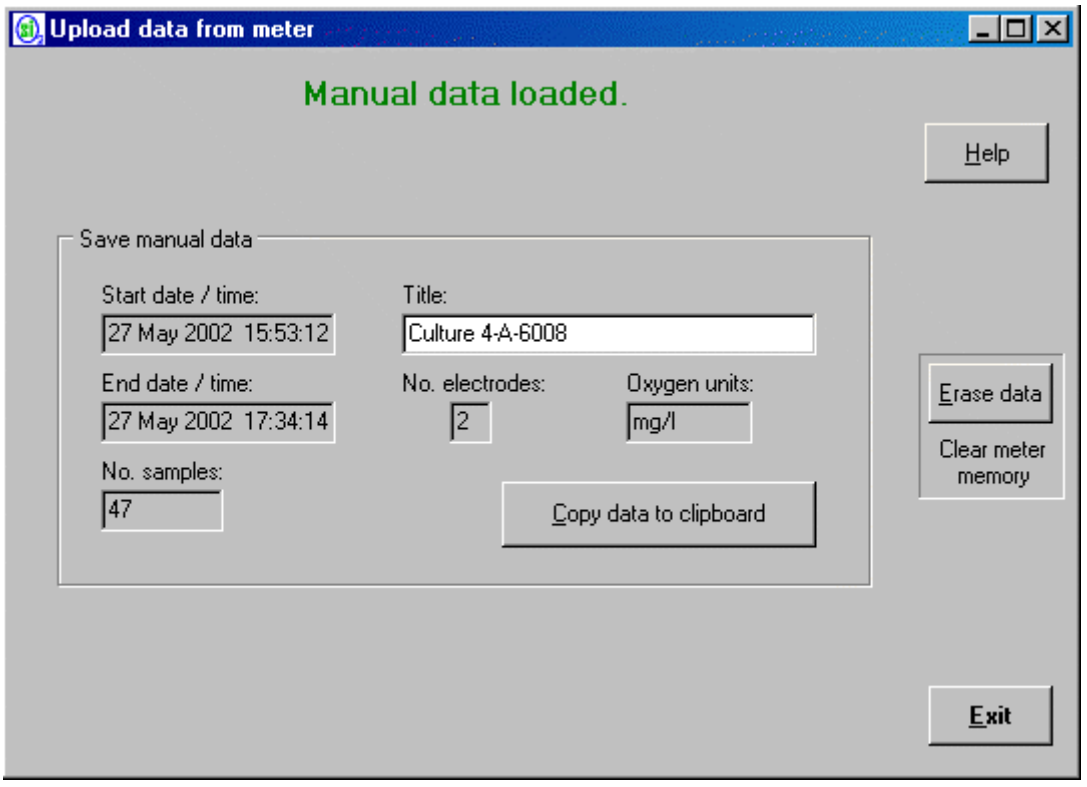
To save the data plus the information you have now entered click **Save datafile**. This will show a **Save Data File As** dialog where the file name can be entered and file destination chosen.



The saved file can later be opened in the Analyse part of the software (see Section 6 of the manual).

Manual readings

This consists of a single run of samples, each saved when the **SAVE READING** button on the meter was pressed. The time of each sample is also saved. Because the time intervals between samples are irregular, this data cannot be used by the Analyse part of the program and must be copied to an external spreadsheet (eg. Excel) for analysis and plotting.



The only additional data that can be entered is a title which will appear at the top of the spreadsheet.

The **Copy data to clipboard** places all the data on the clipboard from where it can be pasted into a spreadsheet. The columns that result are Date, Time and a column of values for each electrode.

Both versions of the dialog contain an **Erase data** button which empties the data memory of the meter.

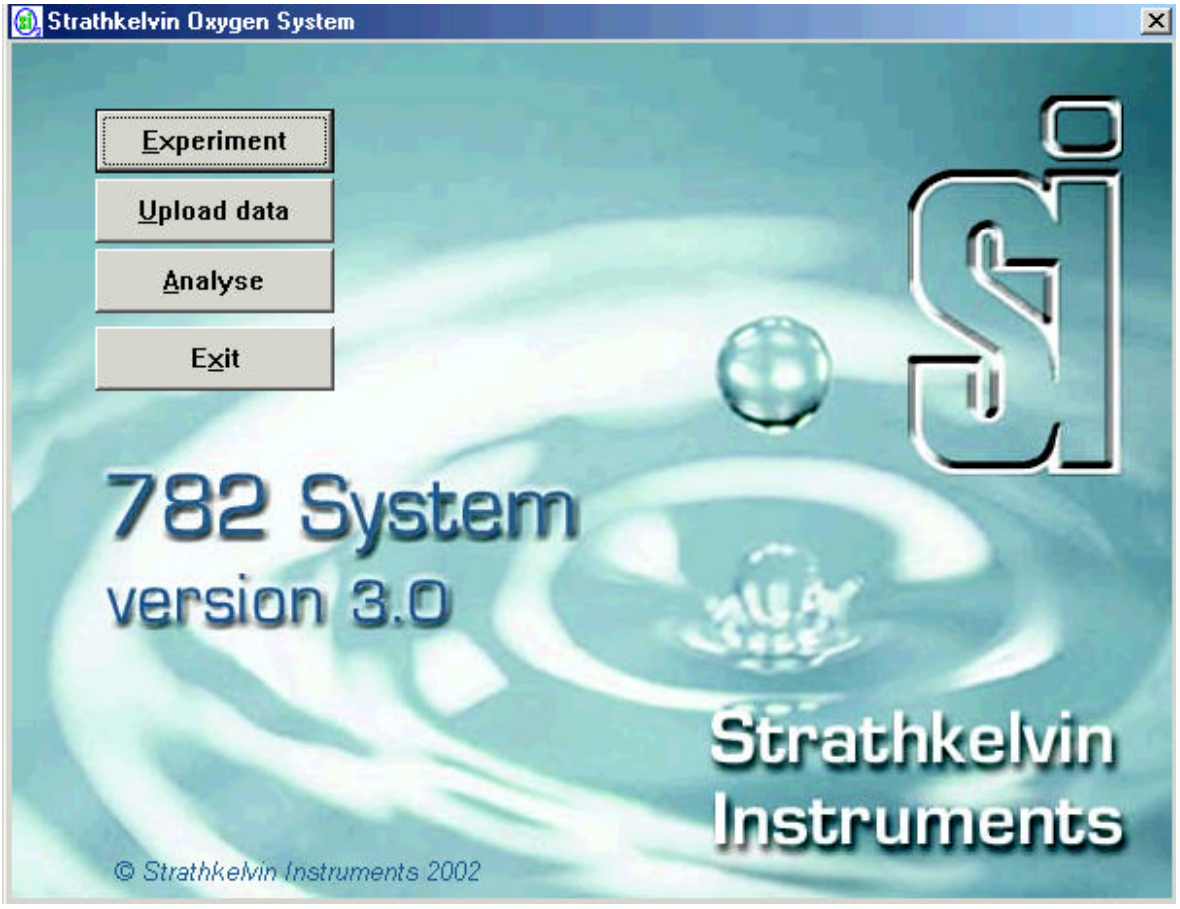
Caution: Once erased the data cannot be resurrected, so use the **Erase data** button only after you have uploaded and saved the data on the PC.

5 782 System Software

5.1 Introduction

In this and the next two chapters, we will go through the procedures of setting up, recording and analysing an experiment. Each of these procedures is based upon a different screen display. The layout of the screen, including the menus and other controls, will be explained. Then we will take you through the steps involved in using these to execute a respiration or monitoring experiment.

Switch the computer on. Switch the meter on using the power button on the front panel. The LCD displays the current electrode readings. On the desktop of the PC double click the 782 icon. This brings up the sign on dialog, offering three option buttons.



Experiment to prepare and run an experiment.

Upload data when the 782 has been used as a stand alone meter this allows the saved data within the meter to be saved into a database to be analysed and reported.

Analyse allows you to analyse a previously recorded experiment

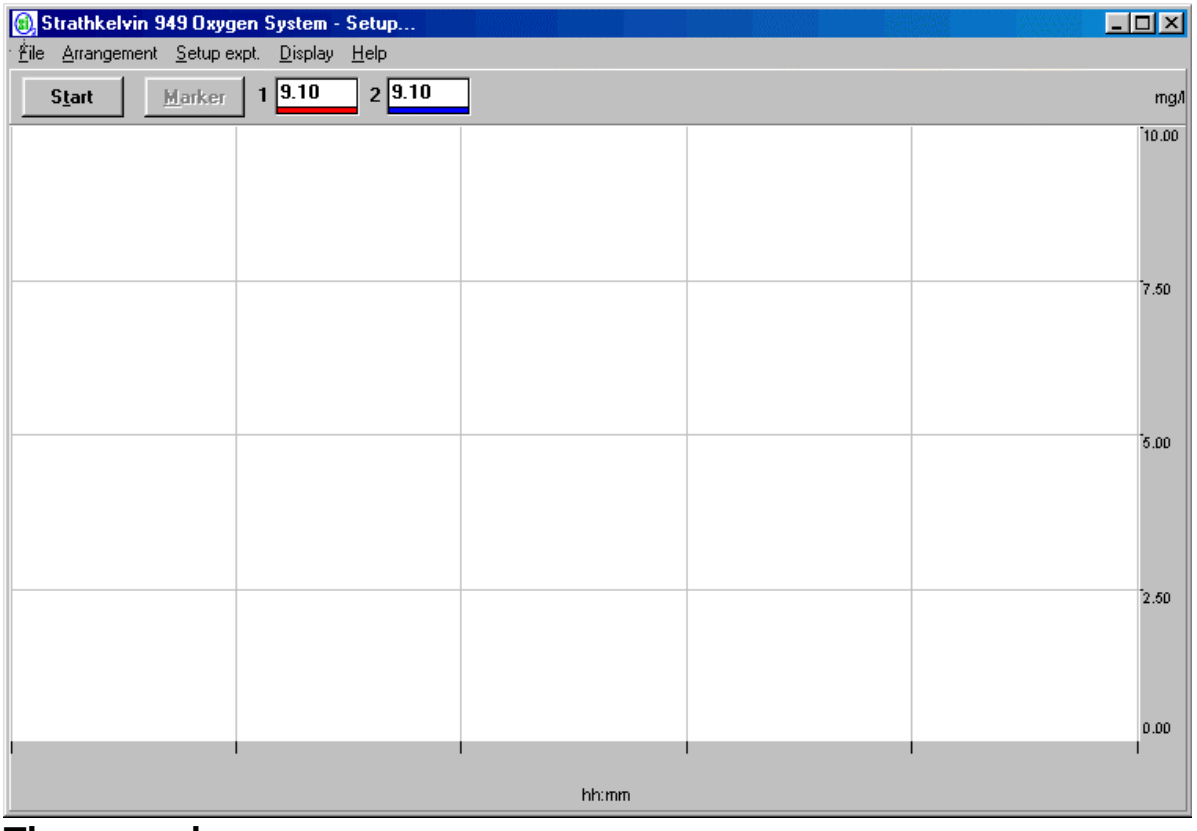
Exit to exit the program and return to the desktop.

If you have forgotten to switch on the meter, you will receive a warning message: **Communication with the 782 meter failed**. If this happens, press the power button (see Section 2.3).

5.2 The Setup Screen

On the Sign on screen, press **Experiment**. The meter will come under control of the software (displaying **ONLINE**) and the **Setup** screen will appear. This screen is used for all initial settings for the respirometry run. When you have completed the recording of an experiment this screen is redisplayed to allow you to choose whether to analyse the experiment straight away, to set up another experiment or to close down.

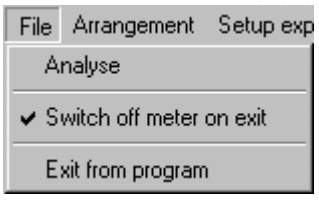
The main part of the screen charts the oxygen values from the electrodes. The *x* and *y* axes will have default units and values assigned to them. These will be changed during the course of setting up.



The menu bar

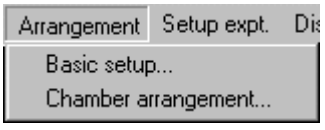
There are five menu items:

File has three options



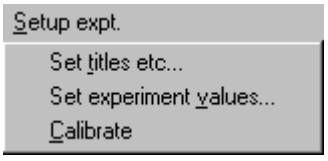
Analyse	allows you to analyse a previously recorded experiment.
Switch off meter on exit	issues instructions to this effect to the meter.
Exit from program	returns you to the **Sign On** screen.

Arrangement has three options



Basic setup...	allows you to set the number of electrodes, the type of experiment and the oxygen units to be used.
Chamber arrangement...	allows you to select the chamber configuration for flow through respiration experiments. It is greyed out if **closed-cell respiration** or **monitor** has been selected in **Basic setup**.

Setup expt. has three options



Set title etc...	allows you to enter a title and brief descriptive comments on the experiment.
Set experiment values...	allows you to insert detailed information about this particular experiment, including title.
Calibrate…	displays a dialog used to calibrate the oxygen electrode(s).

Display has four options

Set Scales...	allows you to set the scales and to specify the scrolling rate of the recording screen
Set trace colors...	allows you to change the colors of the recording traces.
Printer on	This should be ticked if you want to print traces from the **Recording** Screen. A screen width of traces will be printed as soon as each screen is filled.
Plot in setup	This should be ticked if you want the output from the electrodes to scroll across the screen whilst you are using the **Setup** screen (not recording data).

Toolbar

The **Start** button is clicked to start the recording of an experiment when Setup has been completed. Boxes 1 and 2 indicate the values being read from each of the electrodes in use. The colors assigned to their traces are indicated below the boxes.

5.3 Preparing the Experiment

We will now go through the steps of setting up an experiment. In certain places there will be slightly different procedures for closed cell respirometry, flow-through respirometry and monitoring experiments. These will be treated separately as they occur.

Switch on the constant temperature water bath connected to the respirometer chambers and wait for the temperature to stabilise. Prepare the respirometer chambers. Weigh the preparation if normalised respiration rates will be required. Alternatively this can be done after the experimental run, but before analysis.

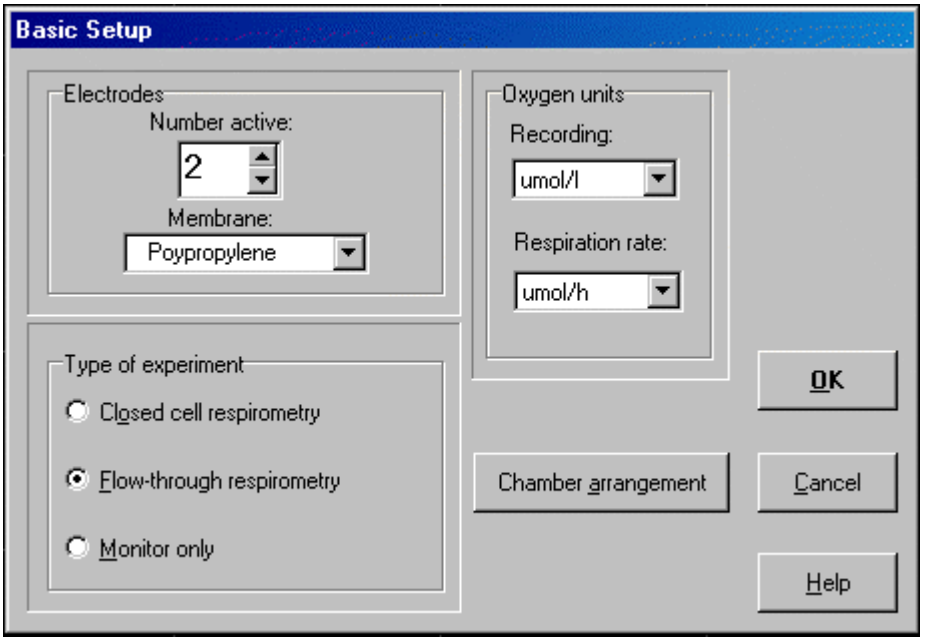
Call up the 782 program by double clicking the icon on the desktop. On the Sign On screen click **Experiment** to bring up the Setup screen.

When first used, the program will have some values set. These will be replaced when changes are made by you during Setup. This means that when setting up subsequent experiments, you will find that those settings which do not need to be changed from one experiment to the next will already be set. (You will learn which of the settings you need to change, but overall, the ability of the program to remember settings will result in significant time saving during the preparation of an experiment).

As a general rule you should move in sequence from left to right along the menu bar so that, for example, **Arrangement** which sets the basic experimental parameters, is set before **Setup expt** and **Display**. This is important because values and units which are designated at the initial stages are automatically transferred to the later stages in Setup.

5.4 Basic setup

Open the **Arrangement** menu. Notice that **Chamber arrangement** option is greyed out. This option is used to select the location of the electrodes in flow-through respirometry and is only available when **Flow-through respirometry** has been clicked in the **Basic setup** option. Now select **Basic setup**.



Note: you can use the tab key on your keyboard to step the highlight sequentially through the boxes on all dialogs in Setup.

With the **Electrodes, Number active** list box, set the number of electrodes in use.

With the **Electrodes, Membrane** list box, set either Polypropylene or FEP as the membrane material on your 1302 electrodes. This changes the sensitivity of the meter to suit. The meter must be operating with the software when you make this change, but it will be stored in the meter for offline use. You must recalibrate after changing the membrane type.
(There is also a third choice 'Not 1302 electrode' which gives the lowest sensitivity and may allow you to use other small cathode types of electrode.)

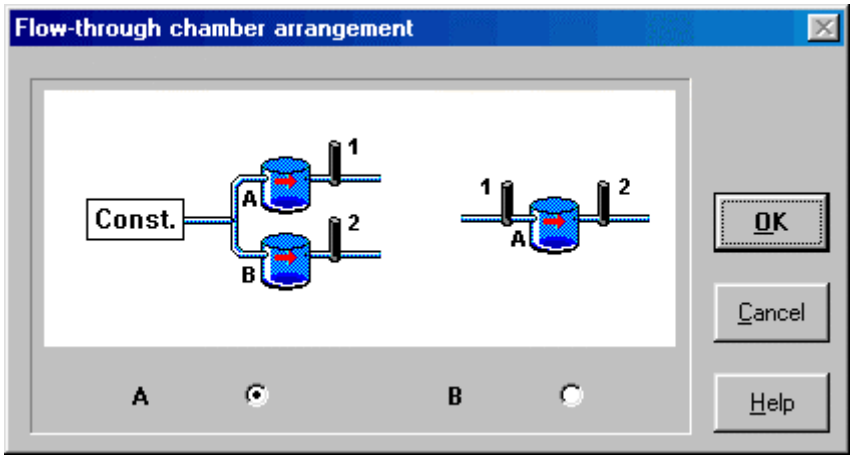
Click the **Flow-through** or **Monitor only** option button if the experiment is not closed cell respirometry.

Recording units. If you want to record oxygen values in units other than the default units shown, open the list box by clicking on the arrow and then select the units required.

Respiration rate units. If you want to express the respiration rate in units other than those shown, open the list box and select the required units. (If you are monitoring O_2, this box will be greyed out.) You must select Recording units first.

If you have selected **Closed cell respirometry** or **Monitor only**, click **OK** and proceed to Section 4.5.

If you have selected **Flow-through respirometry**, you should now click the **Chamber arrangement** button. This reveals a dialog to enable you to select the arrangement of electrodes and chambers which you are using.



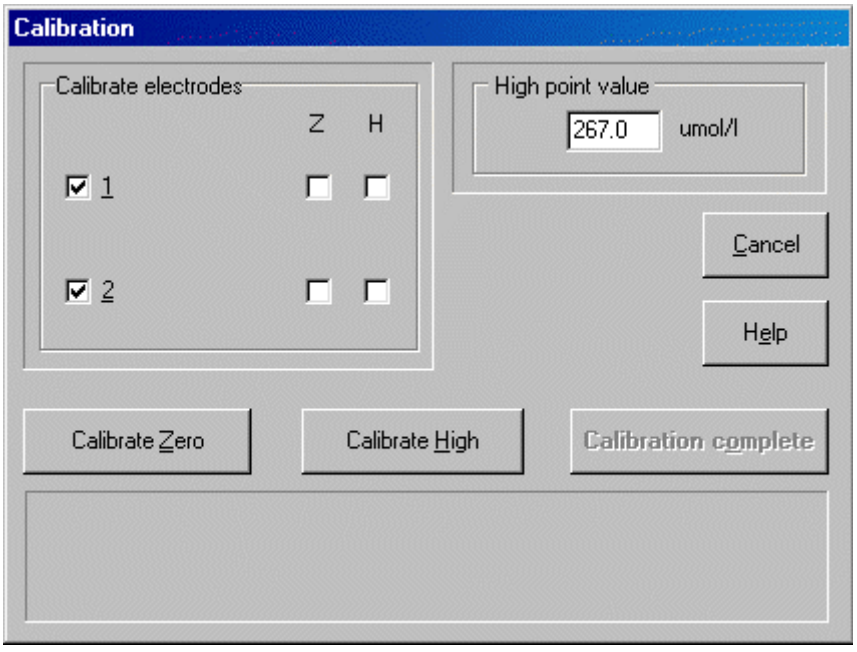
The computer will present different variants of this screen depending upon whether you have selected one or two electrodes in the **Basic Setup** dialog box. Select option button A if you have a constant oxygen level at the input (eg saturated water); B if you are measuring both input and output to the chamber. Note that the computer assigns the A - B chamber identification, according to the number of electrodes used and their configuration. It is important that you use these chamber and electrode configurations during your respiration experiment.

After making the selection, click on **OK**.

Note that this dialog can also be accessed directly from the **Arrangement** menu.

5.5 Calibration

Select the **Setup experiment** menu and then the **Calibrate** option. This brings up the following dialog:



When doing this for the first time, wait at least one hour after switching on the meter, to allow the electrode(s) to stabilise before calibrating. Subsequently, the electrodes can be kept connected to the meter with the power on ready for immediate use.

During calibration, the meter displays the electrode output in picoamps. This is not used in the actual calibration procedure but is a useful check on the state of 'health' of the electrodes. See Appendix 8.3.

Before starting your calibration, make sure your calibrating solutions have been prepared (see Appendix 8.6).

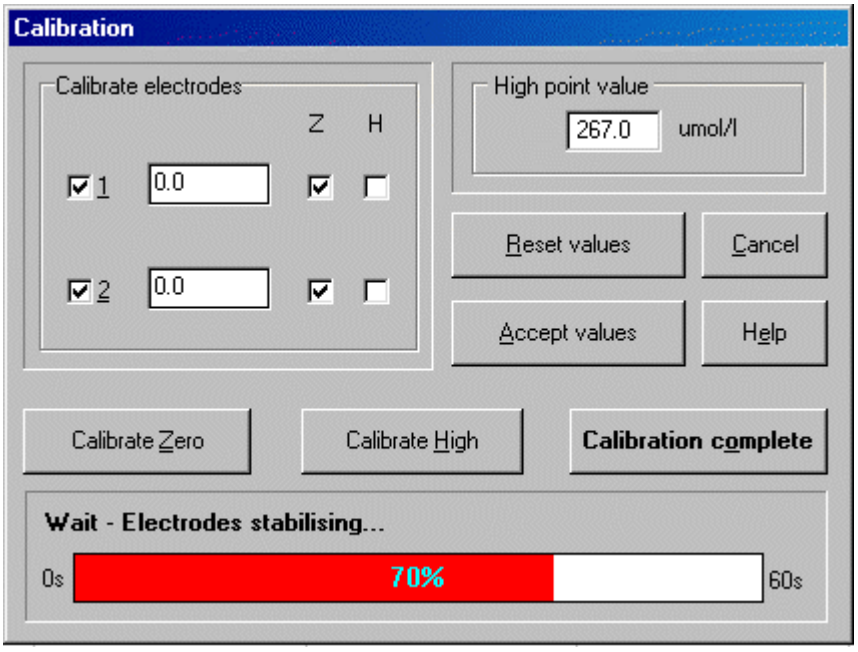
Proceed through the following steps:

1) The electrodes may be calibrated together or singly. If you do not wish to calibrate one electrode, click on its box. The tick will disappear from this box.

2) Type in the high point calibration value. If your high point calibration solution will be air-saturated water this will be:

   The partial pressure of oxygen, if working in pressure units,
   or
   100% if you are working in %satn units,
   or
   concentration of oxygen in an air-saturated solution which you will have obtained from tables (see Appendix 8.7), if using concentration units.

3) Click on the **Calibrate High** button. An information box will appear, telling you to expose the electrode(s) to the high point calibration solution. After doing this, click on **OK** and the information box will disappear.



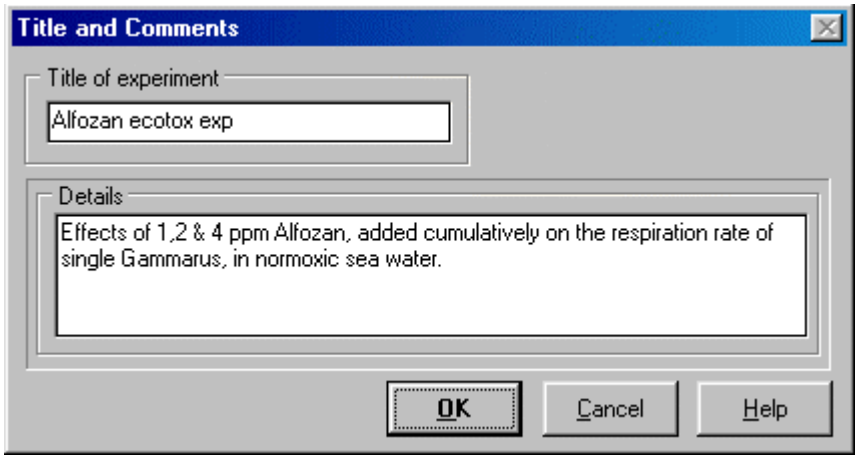
4) You will then be asked to wait while the electrodes stabilise. At the end of this one minute period, oxygen values will appear in the **Calibrate electrodes** boxes, a **Reset values** button and an **Accept values** button will appear. Click the **Reset values** button if any of the values drift away from the high point value. When the values are stable, click **Accept values**.

> Note: It is important that you **do not** remove the electrodes from the calibrating solution before you click **Accept values**.

5) Click on **Calibrate Zero** to set the second calibration point. Repeat steps 4 & 5, using the zero oxygen calibrating solution.

6) At the end of the calibration, ticks will appear in the boxes headed H and Z indicating that both highpoint and zero calibrations have been completed. Click on **Calibration complete**. If **Cancel** is clicked, the previous calibration will continue to apply.

Click the **Setup expt.** menu and then select **Set titles etc**…



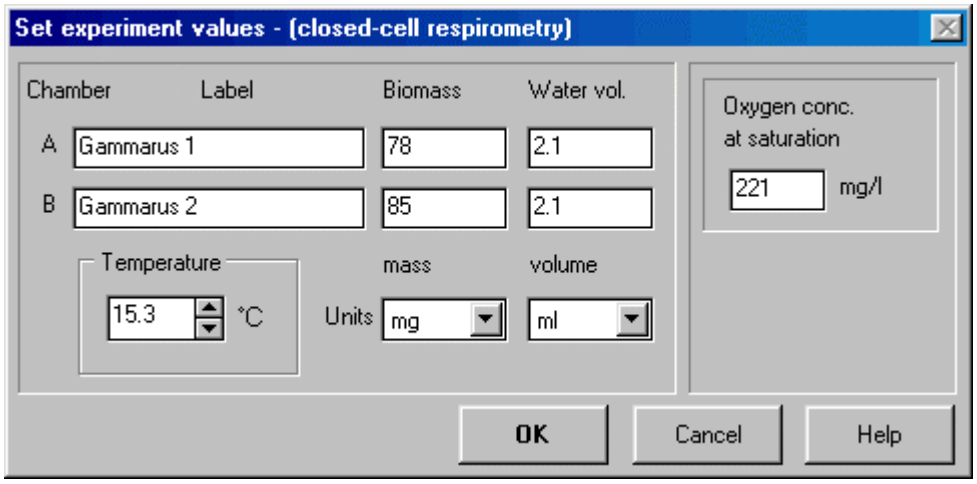
This dialog enables you to give a title to the experiment and to fill in some other experimental details. The information which you enter here will be incorporated, with the date and the water temperature, into the Calculated Results table when you have analysed the data at the end of the experiment.

5.7 Setting Experimental Values

Select **Setup experiment** from the menu bar, and then select **Set experiment values**. The dialog which is displayed depends on whether you have previously chosen **Closed cell**, **Flow-through respirometry** or **Monitor only**.

1. Closed Cell respirometry

If you have selected **Closed cell respirometry**, the following dialog will appear:



Now fill in the boxes as follows:

Label
For each of the electrodes in use you may record some information about the material in the respirometer chamber. Each box will accommodate 15 characters.

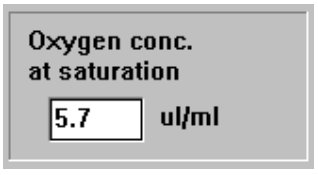
Biomass
If the biomass is important, either for subsequent plotting of results or for normalising of respiration rate to body weight, the relevant values should be entered here. Select the units of biomass that you want to use from the list box. Note that if you subsequently want to express respiration rate as the weight-normalised rate in the post-experiment analysis, normalisation will be made on the basis of the units specified here. Enter the biomass values in the respective boxes. Each box will accommodate 5 characters (including the decimal point).

Very often it happens that the weight of the organism is not measured until after the respiration run is over. To accommodate this situation, you can enter the values during Analysis.

Water volume
Select the units of volume for the water or solution in the respirometer chambers from the list box. Then enter the volume of water or solution in each chamber. Each box will accommodate 5 characters (including the decimal point).

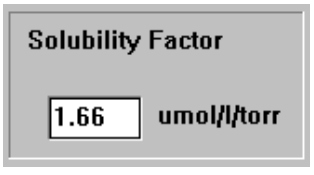
If the volume will not be measured until the experiment is over, these values may be entered during Analysis.

Oxygen concentration at saturation



This box will appear on the dialog box if you have elected to measure oxygen in units of % saturation in the **Basic setup** dialog box. Enter the oxygen concentration of water or solution which is 100% saturated with air. You can obtain these values from published tables. (See Appendix)

Solubility factor



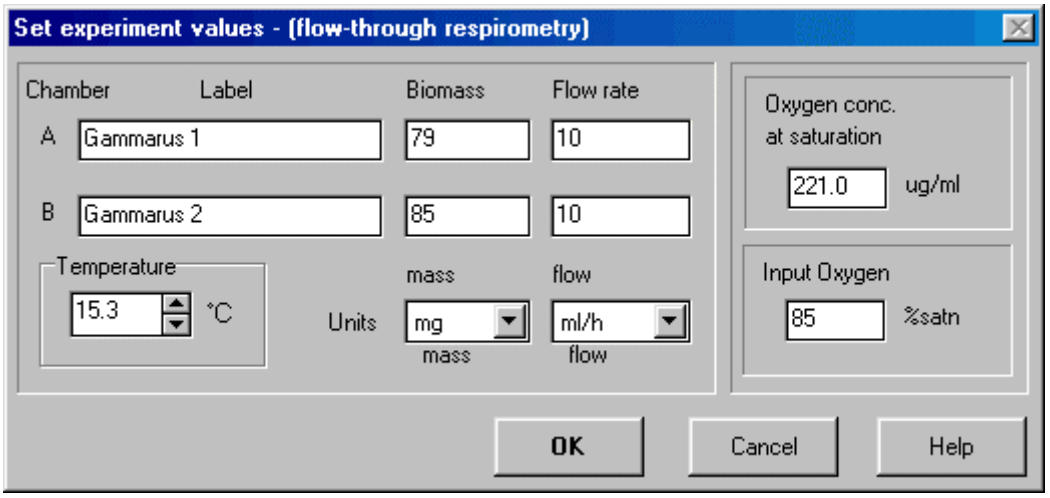
This box will only appear if you have elected to measure oxygen in units of oxygen partial pressure i.e. torr (mm Hg) or kPa in the **Basic setup** dialog box. Enter the concentration of oxygen at air saturation in the units which are specified (µl, µg or µmol/l/torr; or µl, µg or µmol/l/kPa). These values can be obtained from published sources. (See Appendix 8.7).

Temperature
Use the up/down arrows to specify the temperature at which the experiment will be run. This must be the same as the temperature during calibration.

Click on **OK** when complete.

2. Flow-through respirometry

If you selected **Flow-through respirometry** you will now see the following dialog:



Fill this in as follows:

Label
You may record information about the material in each respirometer chamber. Up to 15 characters are available in each box.

Biomass
If the biomass is important, either for subsequent plotting of results or for normalising of respiration rate to body weight, the relevant values should be entered here. Select the units of biomass that you want to use from the list box. Note that if you subsequently want to express respiration rate as the weight-normalised rate in the post- experiment analysis, normalisation will be made on the basis of the units specified here.

Enter the biomass values in the respective boxes. Each box will accommodate 5 characters (including the decimal point).

Very often it happens that the weight of the organism is not measured until after the respiration run is over. To accommodate this, you can enter the values during Analysis.

Flow rate
Select the units of water flow from the list box. Then enter the flow rate through each chamber. Each box will accommodate 5 characters (including the decimal point). ***Flow rate should not be changed during the experiment.*** If necessary, these values could be measured at the end of the experiment and then entered during Analysis.

Input oxygen
This box will only appear if you have chosen configuration A (i.e. with a constant input oxygen concentration). Enter the oxygen concentration or partial pressure or percentage saturation of the inflow water, depending upon the units specified in **Basic setup** (5 characters including the decimal point).

Input oxygen
This box will only appear if you have chosen configuration A. i.e. with a constant input oxygen concentration. Enter the oxygen concentration or partial pressure or percentage saturation of the inflow water, depending upon the units specified in 'Basic setup' (5 characters including the decimal point).

Oxygen Concentration at Saturation
This box will only appear if you have elected to measure oxygen in units of % saturation. Enter the oxygen concentration when the water or solution is 100% saturated with air. You can obtain these values from published tables. (See Appendix) Note that if you are using air saturated water as the inflow and are using configuration A, the value that you place in this box will be the same as in the box above.

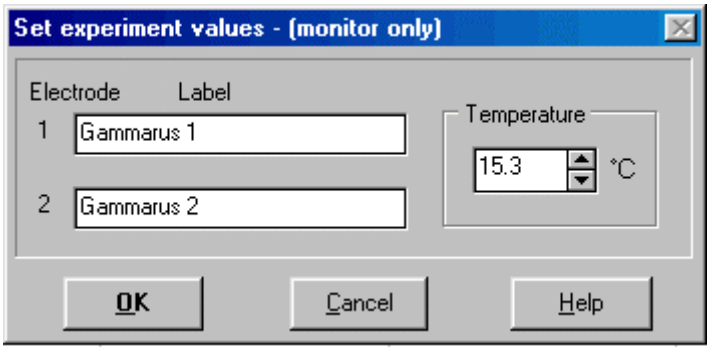
Solubility Factor
This box will only appear if you have elected to measure oxygen in units of oxygen partial pressure i.e. torr (mm Hg) or kPa. Enter the concentration of oxygen at air saturation in the units which are specified (µl, µg or µmol/l/torr; or µl, µg or µmol/l/kPa). These values can be obtained from published sources. (See Appendix 8.7).

Temperature
Use the up/down arrows to specify the temperature at which the experiment will be run. This must be the same temperature as in calibration.

Click on **OK** when complete.

3. Monitoring oxygen levels

If you selected **Monitor only**, you will now see the following dialog:



Fill this in as follows:

Label
For each of the electrodes in use, you may record some information about the material being monitored. Each box will accommodate up to 15 characters.

Temperature
Use the up/down arrows to specify the temperature at which the experiment will be run. This must be the same temperature as in calibration.

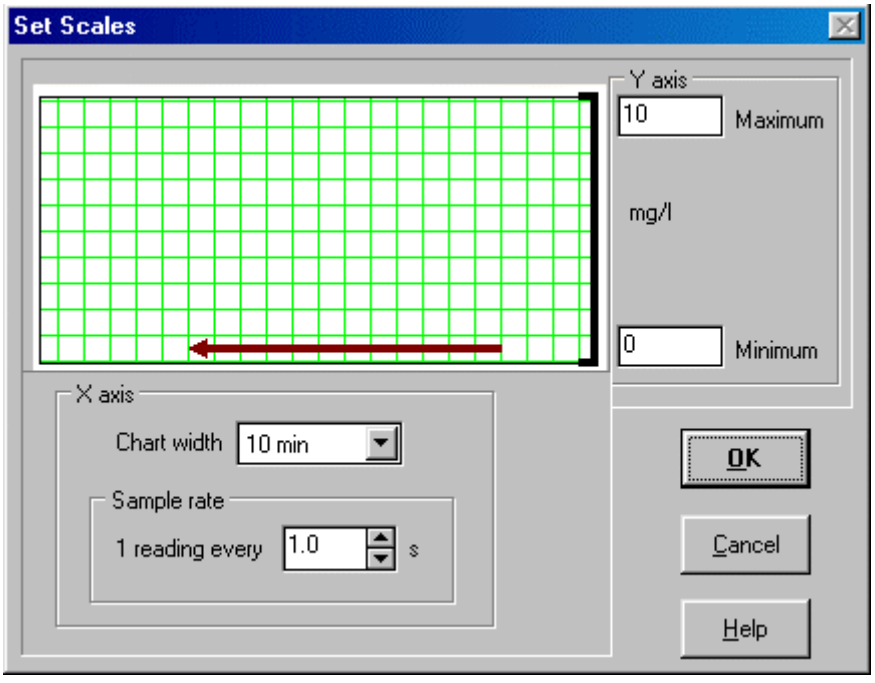
Experiment details

Enter here any further information about the experiment which you wish to appear on the Report.

Click on **OK** when complete.

5.8 Setting Recording Screen Parameters

Click the **Display** menu and then the **Set scales** option to bring up this dialog:



Fill in the maximum oxygen value that you would like on the recording display screen, by typing this in the **Maximum** box. Do the same for the **Minimum** box.

Set the scrolling speed (equivalent to setting the chart speed with a chart recorder) in the **Chart width** list box, by selecting the time to be taken for the trace to traverse one chart or screen width. For closed cell respirometry, this will relate to your expectation of the rate of oxygen depletion.

Note: you do not have to confine the whole experiment to one screen width. The traces will continue to scroll across the screen when the first screen has been filled.

From this information, the computer will calculate an optimal rate at which to read the electrode output and this will be displayed in the **Sample rate** list box. You can change the interval between electrode readings by opening this list box, if you wish.

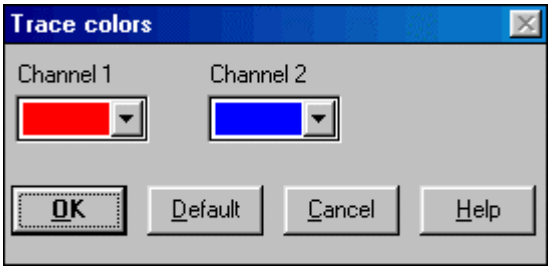
5.9 Printing Screen Traces

If **Printer on** is selected from the **Display** menu, a copy of each screen will be printed to the current Windows printer when the screen fills or when you press the 'stop' button to terminate recording. The printed traces are optimised for a color printer and each trace will be printed in the same color as it is displayed. A black and white printer can be used, but the traces will not be identified with a label and may have to be annotated by hand.

Details of the experiment, from information provided during Setup, together with information on markers used during Recording, are printed when the recording is saved at the end of the run.

5.10 Setting Trace Colors

Default colors have already been allocated to each trace. These appear below the value boxes on the toolbar. The colors can be changed by clicking the **Set Trace Colors** option of the Display menu. This brings up this dialog box:



Select whatever color is required from the list box of each channel, or select the default setting, using the **Default** button.

5.11 Plotting during Setup

If you want to see the values from the electrodes scrolling across the screen during the **Setup** procedures, select **Plot in setup** from the **Display** menu. If this is not selected, the screen will remain blank and the scrolling traces will not appear until the **Start** button is clicked to start the recording.

5.12 Preparing to Record

At this stage you will have completed the setup procedure. When you come to set up for the next experiment, you will find that the computer has saved the critical settings which will then appear as default. Subsequent setups will be much quicker, since you will probably not need to change many of the settings, and you may find that you only need to use the **Set Title**, **Set Experimental values** and **Calibrate** options.

You can now turn your attention to the respirometers. Re-insert the electrodes if they have been removed for calibration. Add the water, followed by the respiring preparation, and seal the respirometers (for closed chamber) or start the water flow (for flow-through). Click the **Start** button.

5.13 Recording an Experiment

The Recording screen

The main part of the screen is the same as in Set-up and displays the oxygen traces. However, you will notice that the time and date at which recording started has been inserted below the recording area. On the toolbar, the **Start** button has been replaced by a **Stop** button, and the previously greyed out **Marker** button is now enabled. The **Marker** button allows you to record events during a respiration run, such as the addition of an inhibitor to the respiration chamber.

The menu bar now offers only **Help** and **Display**.

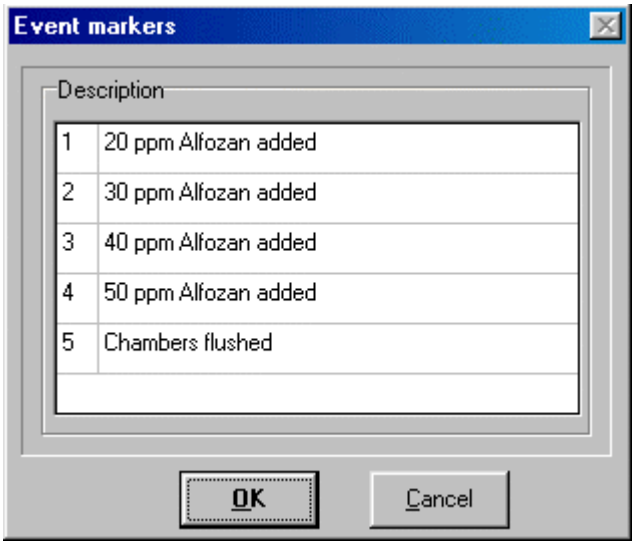
Printer on allows you to switch on the printer, if not done during setup, or to switch it off.

5.14 Procedures in Recording

When you click **Start**, the time axis resets to zero if you were plotting the traces during **Setup**. At the same time a vertical line is applied to the screen at the right hand side and the oxygen value traces scroll from right to left at a rate which you determined in the **Set Scales** dialog.

5.15 Adding Event Markers to the Recording Area

If you want to introduce a substance to the respirometer chamber or change some other experiment condition, you can record the event by inserting an event marker on the screen. Click on the **Marker** button at the exact moment that you add the substance to the chamber. Alternatively press **Alt** and **M** on the keyboard. This will result in a numbered vertical mark appearing at the top of the screen. The **Event marker** dialog appears:

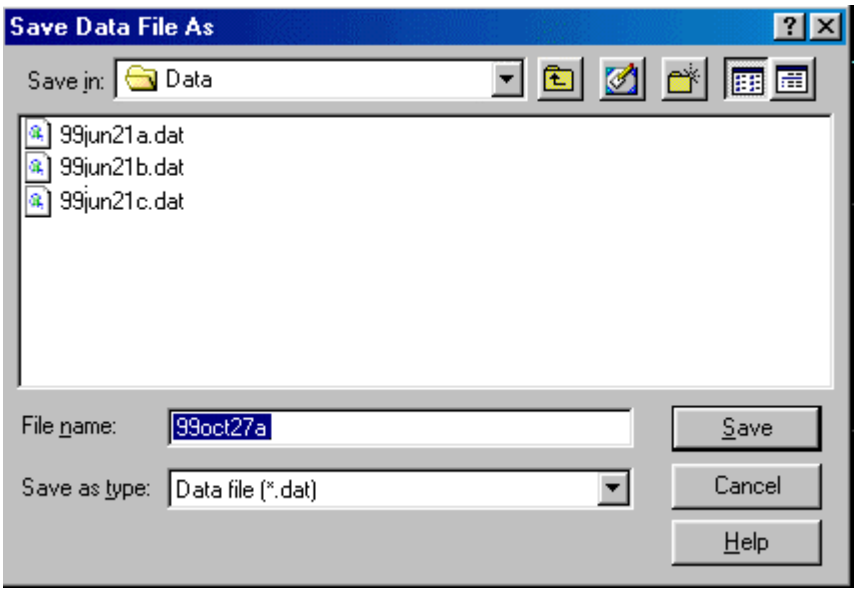


You can insert up to 42 characters of descriptive text. Then click **OK**.

If you have 2 chambers in operation, you may find it more convenient to record additions to each chamber with a separate event marker. The maximum number of events which you can record in this way is 15.

5.16 Ending Recording

At the end of the experiment, click **Stop**. Another vertical line will appear and you now have the opportunity to save the respiration run data in a datafile:



A default filename based upon the date and a letter is offered. The letter increments automatically with succeeding runs on the same day. Either accept this filename or insert another.

Click **OK**. You will then return to the **Setup** screen.

You now have two options:

1. To proceed immediately to analysis of the recorded data.

2. To exit the program and analyse the recording later.

Both of these options are available from the Setup screen **File** menu.

6 Analysis of Data

6.1 Introduction

This is a very powerful and versatile part of the program which will give tremendous time savings in comparison with traditional methods of data analysis. The features of the Analysis screen and the steps of analysis can be illustrated by the use of pre-recorded datafiles, which are supplied with the software. The pre-recorded files are called:

Cceldem2.dat an example of closed cell respirometry file.

Flowdem2.dat an example of flow-through respirometry file.

Mondem2.dat an example of oxygen monitoring file.

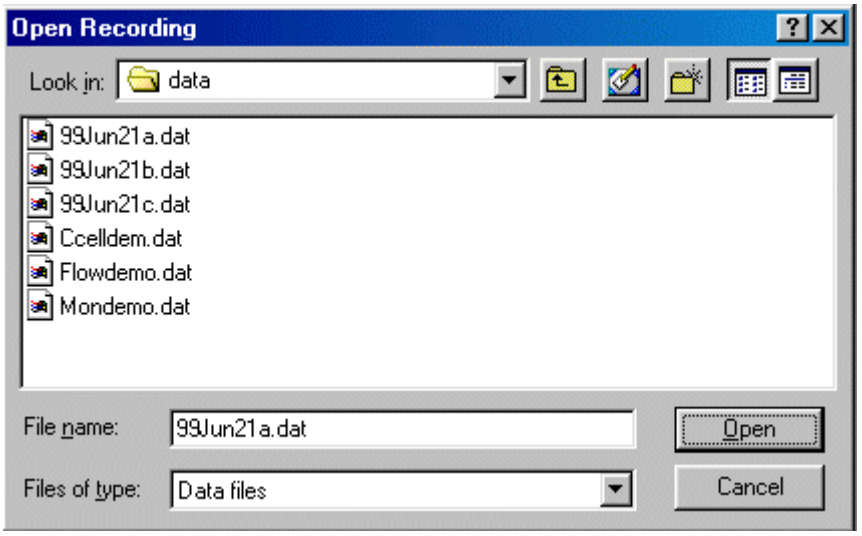
A closed cell respirometry file is used as an example in Section 6.3.

6.2 Accessing the Analysis Screen

There are three different ways of entering the Analysis section of the program.

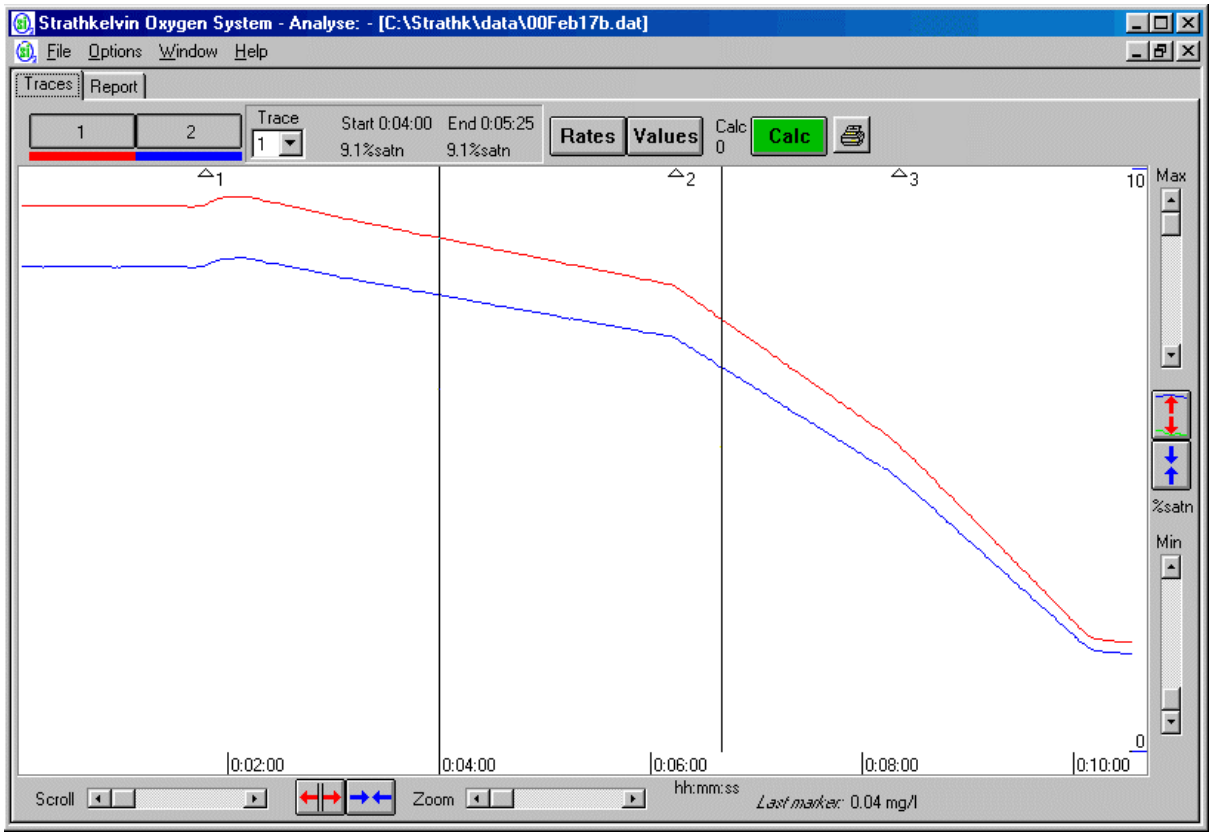
1) Immediately following the recording of an experiment (while the meter was connected to the PC). In this case you would click **File** on the Setup screen (to which you are returned from the Recording screen after clicking the **Stop** button). You would then select **Analyse** and the traces of the respiration experiment which had just been recorded would reappear on the Analysis screen.

2) At some other stage, after exiting the program or uploading data from the 782 meter memory. Very often you may wish to record several experiments successively and then return to the analysis perhaps on another day. In this case you would click **Analysis** on the Sign On screen. This brings up an **Open Recording** dialog with c:\strathk\data as the default directory:



3) After completing the analysis of a data file. Additional analysis windows can be opened on top of the current window by clicking **File** and then selecting the **Open Recording** option.

6.3 The Analysis Screen

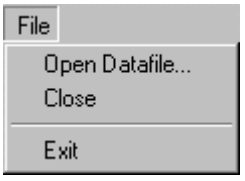


The Analysis screen comprises two separate tabbed pages. The one that you now see, with a **Traces** tab, and the **Report** page, beneath it and accessed by the **Report** tab.

The Menu Bar

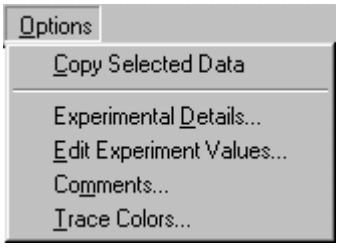
There are three menus (in addition to Help)

File has three options:



Open Datafile...	allows you to open a recording (A number may be opened in the same Analyse window).
Close	closes the displayed datafile.
Exit	closes the Analysis part of the program. If this was called from the sign-on screen or Setup, an error dialog will appear ('COM server warning…'). Click Yes to close or No, and switch back to Meter using the taskbar.

Options has five items:



Copy Selected Data	copies to the Windows clipboard the values corresponding to the traces between the selector lines. These can be pasted into another program (e.g. a spreadsheet) for further analysis.
Experimental Details ...	displays a dialog containing the details of the experiment entered in Setup.
Edit Experiment Values...	displays a dialog in which you can insert values for biomass or chamber volume (closed cell) or biomass or flow rate (flow-through) if these were not entered in Setup.
Comments…	allows you to enter comments on the experiment.
Trace Colors ...	allows you to change the trace colors.

Toolbar

The numbered buttons correspond to the numbered traces used in the recording. If you do not want to analyse both traces simultaneously, you can click on any button to temporarily remove its trace from the screen.

To the right of the trace buttons is a readout panel and a trace selector control. The readout gives the time and oxygen values at the points where the selector lines cross the selected trace.

On the right of the toolbar are three buttons: **Setup Report**, **Calc** and **Print** (icon).

Setup Report Click this button to select which type of calculation you would like to carry out (Rates or Values). You would select **Rates** if calculating respiration rates and regression lines would be fitted automatically to the selected parts of the trace in closed cell experiments. You would select **Values** to read oxygen values at selected times on the traces. You would normally use this in Monitor experiments, but it can also be used in respiration experiments. The **Setup Report** button also allows you to select which results you would like listed in the report.

Calc Each time this is clicked, the respiration rates are calculated for the data between the two selector lines if **Rates** has been selected. The value at the left hand selector line is calculated if **Values** has been selected. The calculated rates or values are automatically printed to the Report page.

Print This causes the Analysis screen to be printed.

In addition to the buttons there is a Calc number indicator. Each time you click the **Calc** button, the number is increased by 1, showing the number of rates or values which have been printed to the Report page. There is also a Calculate label which either reads ‘Rates’ or ‘Values’ as selected in the **Setup Report** dialog.

Recording Area

The **scroll** scrollbar can be used to scroll the trace and to view the traces of the later part of the experiment.

The two vertical selector lines may be moved either by moving the pointer to the left or right of one of them and then clicking, or by positioning the pointer on the line, holding down the left mouse button and dragging it to the desired position. Position the selector lines to start and end of the part of the trace you want to analyse and press **Calc**.

X-axis controls

Scroll slider bar enables you to scroll along the trace to find the sections of trace which you want to analyse.

Zoom slider bar allows you to expand or contract the traces about the midpoint of the *x* axis. You would use this, for instance, if you had a very long run that you wished to compress on to the one screen.

 allows you to expand the section of trace between the selector lines to horizontally fill the whole screen.

 restores the screen to its appearance before pressing the above button.

Y-axis controls

The **max** and **min** slider bars enable you to rescale the maximum and the minimum values on the *y* axis. Increasing the maximum value can be useful if the recording has gone off screen in photosynthesis or monitoring experiments. Similarly, resetting the minimum *y*-axis value to a higher value can give a useful expansion if only a small part of the available *y* scale has been used in a closed cell respirometry experiment.

 allows you to expand the scales so that the traces occupy the full screen height.

 restores the screen to its appearance before pressing the above button.

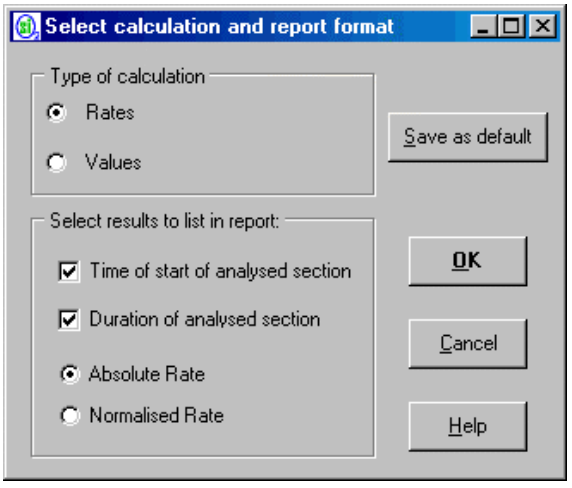
6.4 Analysing Closed Cell Respirometry Files

The file Cceldem2.dat can be opened as an example of a closed cell respirometry file.

Use the horizontal and vertical scroll slider bars to view the section of the trace you want to analyse.

If the traces are superimposed on one another so that you cannot see them clearly, you have the option of switching off one or more traces. This is done by clicking the appropriate button on the toolbar. For example, click button 1 on the toolbar. The red number 1 trace disappears. Click button 1 again to restore it.

Now click **Setup Report.** This brings up the **Select calculation and report format** dialog:



Click the relevant controls to determine what information you want to be printed to the Calculated Report window and click **OK**. If you select **Normalised rate** before any biomass values have been entered, you will be asked to enter them.

> **Note :** Absolute rate and Normalised rate are alternatives. You cannot calculate both at once.

To analyse you would simply drag the selector lines to enclose the section of the trace you wish to be analysed and click **Calc**.

For example, move the pointer to the left selector line, hold down the left mouse button and drag the line to the right of the first Event marker where the trace becomes linear. Move the right selector line to the end of this section of trace - i.e. just to the left of Event marker 2. Regression lines are fitted to the section of the trace selected and displayed. You should examine the regression lines to determine whether it might be advisable (if experimentally admissible) to move the selector lines to get a better fit.

When you clicked **Calc** the respiration rates were automatically calculated for the regression lines and transferred to the Report page.

Other sections of the trace can be analysed by repeating the above.

Remember that each time you click **Calc**, a line of data is printed to the Report page. If you click this button other than as indicated, or if you continue to click it, the Report screen could fill with unwanted data (which can be deleted manually.)

On pressing the print icon you will be given the option to either print the whole file or the current window. A preview of the printed page will be displayed with three option buttons; **Print**, **Close** and **Print trace numbers**. If you wish the traces to be identifiable on a black and white copy, click the **Print trace numbers** button and this will add the number to the right hand end of each trace.

6.5 Analysing Flow-through Respirometry Files

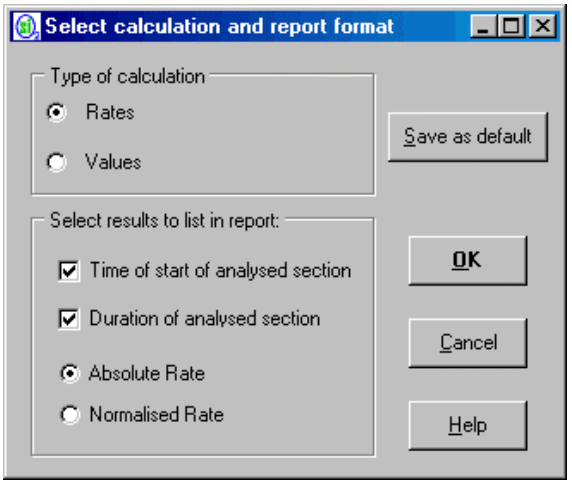
The file flowdem2.dat can be opened as an example of flow-though respirometry file.

This shows an experiment which measures the outflow PO_2 from an isolated organ preparation, for which there is a constant PO_2 flowing in, indicated by the top trace (blue). The respiration rate increases after the event marker.

Use the horizontal and vertical scroll slider bars to view the section of the trace you want to analyse.

If the traces are superimposed on one another so that you cannot see them clearly, you have the option of switching off one or more traces. This is done by clicking the appropriate button on the toolbar. For example, click button 1 on the toolbar. The red number 1 trace disappears. Click button 1 again to restore it.

Now click **Setup Report.** This brings up the **Select calculation and report format** dialog:



Click the relevant controls to determine what information you want to be printed to the Calculated Report window and click **OK**. If you select **Normalised rate** before any biomass values have been entered, you will be asked to enter them.

Note : Absolute rate and Normalised rate are alternatives. You cannot calculate both at once.

To analyse you would simply drag the selector lines to enclose the section of the trace you wish to be analysed and click **Calc**. For example to calculate the rate after the event marker, move the selector lines to a section of the trace after they have stabilised again. Click **Calc**.

At this stage, difference values between the inflow trace and the outflow trace are derived for all data points between the selector lines. A mean difference is calculated which is then used with the previously entered flow rate to calculate the respiration rate.

6.6 Monitoring experiments

The file Monodem2.dat can be opened as an example of oxygen monitoring file.

This experiment records the PO_2 in an organ bath. The PO_2 changes after 12 minutes, which is not currently on screen.

Click the **Setup Report** button and select **Values**. Move the left hand selector line - which is the only one from which values will be recorded in this mode – to, say 5 minutes, as read from the **Start** values in the box on the toolbar. Then click **Calc**. Repeat at 10 minutes. Then use the **Scroll** scrollbar to bring the second half of the experiment on to the screen. Repeat at say 13 minutes and 18 minutes. Then open the 'Report' window to view the selected values.

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7 Dealing with Reports

7.1 Introduction

The Report page for the experiment has several options available. You can save the results, print a hard copy or export them to a word processor, spreadsheet or statistical package.

The Report page is editable to the extent that you can delete any line by highlighting it and clicking **delete** or you can edit the label identifying each line.

7.2 The Report page

The Report page tabulates the results, together with the information about the experiment that you entered in **Setup** and perhaps also in **Analyse**, together with the date and filename. Unwanted lines of results can be deleted and the label for each line can be edited.

The page has three buttons on its top toolbar:

The **Print** icon button enables you to print a hard copy of the **Report**.

The **Copy** icon button enables you to Copy the **Report** to the Clipboard from where it can be pasted into a word processing program, spreadsheet etc.

The **Save** icon button enables you to save the results directly to the \Strathk\data folder as a spreadsheet file (normally Excel format, but other formats can be chosen).

To exit click **File** and choose **Close** option. This closes the Analyse screen and its Report page.

8 Appendices

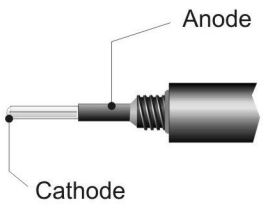
8.1 Appendix 1: Error displays

Error displays on the 782 meter

1. **ELECTRODE FAULT** This displays when the electrode output is too high for the meter. With the 1302 electrode this indicates a fault, which is normally due to either a short circuit in the electrode cable or connectors, or due to a damaged membrane.

8.2 Appendix 2: Oxygen electrodes

Principle of operation of Clark-type oxygen electrodes



The Clark-type oxygen electrode consists of a probe at whose tip is an exposed gold or platinum cathode and a silver or silver/silver chloride anode. When the anode and cathode are polarised so that the cathode is held at a voltage of -0.6 to -0.8 volts relative to the anode, and connected via a solution of electrolyte such as KCl, the following reaction will occur at the anode:

$$4Ag \rightarrow 4Ag^{+} + 4e \quad \text{and} \quad 4Ag^{+} + 4Cl^{-} \rightarrow 4AgCl$$

Simultaneously, at the cathode, any oxygen which is present is reduced:

$$O_2 + 2H_2O + 4e \rightarrow 4OH^{-}$$

Thus for each oxygen molecule reduced, 4 electrons of current flow in the circuit. Oxygen is therefore continually 'consumed' as it is reduced to OH^- at the cathode.

In practice, the anode and cathode are covered by an oxygen permeable membrane to exclude other species which would interfere. The KCl electrolyte is buffered to remove the OH^- produced in the cathode reaction.

As oxygen is removed at the cathode, a PO_2 gradient is set up which extends outwards into the surrounding medium. In unstirred water, oxygen therefore diffuses inwards along the PO_2 gradient. Because of the PO_2 gradient, the outside of the electrode membrane is effectively sensing a very much lower PO_2 than that in the surrounding water. For this reason, most Clark electrodes require the water to be stirred.
The size of the signal generated by the electrode is proportional to the flux of oxygen molecules to the cathode. This oxygen flux is proportional to:

1. The PO_2 of the water
2. The permeability of the membrane
3. The temperature of the water
4. The surface area of the cathode

Increase in any of these factors will therefore increase the size of the signal which is generated.

It is important to note that most Clark-type electrodes require the water to be stirred. Only in a microcathode electrode, fitted with a low permeability membrane, is stirring not required.

Principle of the 1302 Microcathode Electrode

The 1302 is a precision electrode with a very small diameter microcathode. Because of this, the rate of consumption of oxygen is extremely low, so that when used with the relatively low permeability polypropylene membranes, most of the resulting oxygen gradient is confined to the distance between the outside of the membrane and the cathode surface.Consequently there is no requirement for physical movement of the solution to replenish the oxygen at the outer surface of the membrane. There is a very small stirring effect, which would result in an error of 2 - 3% if the electrode has been calibrated in stirred solution and used in unstirred solution, and vice versa.

When used with fast-responding highly permeable FEP membranes, the flux of oxygen through the membrane is increased. In this case, the electrode behaves like a macrocathode electrode and it is then necessary to stir the solution. Regrettably, it is not possible to build an electrode with a fast response time but no stirring requirement.

Electrode Construction

The electrode consists of a glass tube containing a 20µ platinum wire whose exposed tip forms the cathode. A band of silver is wrapped around the glass a few centimetres behind the tip, which, when chlorided, forms the anode. Electrical connection between the anode and cathode is via an electrolyte of buffered KCl solution. The electrolyte is contained within a jacket over whose tip is stretched a thin polypropylene membrane which is kept taut by an 'O' ring. When assembled, the membrane makes a tight fit over the glass tip. The jacket is unscrewed from the body of the electrode in order to replace the membrane or electrolyte. A standard jacket is used with standard polypropylene membranes but when using fast responding FEP membranes, it is necessary to use a jacket with a very fine pressure relief hole. If a standard jacket is used with FEP membranes, the hydrostatic pressure produced as the jacket is tightened on the thread will cause the membrane to lift away from the cathode, thereby increasing the O_2 diffusion path and response time of the electrode.

Fitting the membrane

The electrode is normally supplied 'dry' and should be stored in this way if not in use for prolonged periods of time.

Unscrew the electrode jacket and lay the electrode carefully on a paper tissue on a safe part of the bench. It is very vulnerable to damage when the jacket has been removed. Pour off the electrolyte and carefully prise off the retaining 'O' ring with the points of fine forceps. Rinse the electrode and jacket with distilled water and blot them dry with soft paper tissue. Stand the electrode jacket with its broad end on the bench. Now take an 'O' ring and tension it by sliding it on to the conical applicator. Stand the applicator on the bench and carefully push the 'O' ring down to the farthest edge.

Take a membrane in the left hand and place over the tip of the jacket, pressing down a little to tension it **very slightly.** Now press the recessed end of the applicator over the membrane, and, with finger and thumb, press the 'O' ring downwards until it snaps into position in the 'O' ring groove in the jacket.

With fine scissors trim away excess membrane surrounding the 'O' ring, leaving a small frill. Check the appearance of the membrane. It should be taut but not deformed and there should be no holes or blemishes visible.

Invert the jacket and add electrolyte solution with a pasteur pipette up to the inscibed line. The jacket will then be about half filled. Tap the jacket to dislodge any bubbles and inspect under a strong light to **ensure that there are no air bubbles adhering to the inside of the membrane.**

Slowly insert the electrode into the jacket and screw down until tight, and the tip causes the membrane to bulge slightly. There will be an airbubble inside the jacket. Dry the outside of the electrode immediately with paper tissue.

Alternative Membranes

In order to use the microcathode electrode in unstirred solutions, it is necessary to use the polypropylene membranes which are supplied as standard. These are relatively slow (about 18 sec for 90% change). In applications where oxygen concentration changes rapidly (as with respiration of mitochondria), it will be necessary to use the fast responding, highly permeable FEP membranes. If working at very low temperatures, it may also be worth considering FEP membranes since speed of response varies inversely with temperature. The FEP membranes are only 12.5µ in thickness and have to be used with a special electrode jacket, Part No SI035. When using FEP membranes, remember that it is necessary to stir the medium.

Housing the electrode

The 1302 electrode is designed to operate with only the outer face of its tip in contact with medium. If therefore has to be housed in a special holder - such as those provided with the MT200, MC100, RC300, RC350, RC400 and TC500 accessories, or in an EH100 or FC100 electrode holder. Each of these has a precision-engineered tip to provide a face seal against the tip of the electrode. It is not possible to make a satisfactory seal using the membrane 'O' ring since this will often disturb the membrane tension and, in addition, solution will often penetrate past the 'O' ring via microchannels in the membrane folds.

The area behind the tip of the electrode must remain dry. This is to avoid earth leakage currents via the molecular film of liquid extending beneath the 'O' ring, which would lead to depolarisation of the anode. It follows from this that ***on no account should the electrode be immersed directly in the solution.***

Electrode Maintenance

When in regular use, the electrode should be kept in water saturated air or the tip immersed in saline solution, to prevent drying out of the electrolyte. If the electrode is in daily use, keep it connected and polarised. If the electrode will not be used for several weeks, rinse it and dry it and store in the electrode box. If it is to be stored for longer than that, remove the jacket and empty out the electrolyte. Rinse the inside of the jacket and electrode with distilled water, dry and refit the jacket and store dry in the electrode box.

Changing the membrane

The intervals at which this should be done varies with usage. Generally, the membrane becomes coated with organic materials with time, and its permeability, and hence the electrode output will fall and response time will increase. The membrane may be carefully wiped with a soft paper tissue and this could be done once a day. However, if there are

any abrasive particles on the membrane, it could become scratched and damaged and the electrode output will rise. If the electrode is used with blood or other solution which may deposit protein aggregates on the membrane, the electrode should be left with its tip in a mild proteinase solution overnight.

In normal usage, the membrane will last for several weeks. Fit a new membrane (Section 8.2) only if it is suspected that the electrode output has changed or the speed of response has increased. It is advisable to use a new 'O' ring if the membrane has not been changed for several weeks.

The electrode membrane will need several hours to stabilise each time the electrode is replaced in the holder/respiration cell. During this stabilisation period the measured oxygen values will drift slightly. Thus it may be a good idea to change the membrane at the end of the working day.

Cleaning the Cathode

Cleaning the cathode should be undertaken at intervals of about 3 - 4 weeks, if the electrode is in continuous use, or when changing the membrane.

Remove the electrode from its jacket, rinse with distilled water and dry the tip with paper tissue. Polish the tip with **a few light** strokes of the polishing paper provided in the service kit. Add new electrolyte to the jacket and screw the jacket back on to the electrode body. Take care not to finger the anode during this operation.

Bubbles in the electrolyte

Whilst it is undesirable to have an air bubble trapped on the membrane adjacent to the cathode, an air bubble elsewhere in the electrolyte will do no harm providing that there is good electrical connection between anode and cathode through the electrolyte. In fact it is quite normal for the electrolyte to lose water through the membrane and for air bubbles to appear.

Temperature and pressure effects

Ensure that the medium is not subjected to changes in pressure (as would happen, for instance, if a sample of blood or water is drawn vigorously into a syringe before injection into the MC100 microcell) nor exposed to bubbles of air. Remember that oxygen measurements have to be made at the same temperature as calibration and that the temperature control should be within +/- 0.05°C, since the signal from the electrode varies with temperature.

When working at a different temperature, it will be necessary to recalibrate the electrode. Allow the tip to come to temperature equilibrium (10 to 15 minutes) before calibrating. Do not expose the electrode to sudden increases in temperature particularly if there is a bubble in the electrolyte. The associated pressure change could cause the membrane to bulge and the response time of the electrode would increase. If an air bubble is present, it is best to top up the electrolyte level, before raising the temperature.

8.3 Appendix 3: Checking electrodes

Checking Electrode Output

It is possible to check the state of the electrodes by observing the electrode output in picoamps which is displayed on the meter during Calibration. Electrodes vary in their outputs (which is why they have to be calibrated) but the output may also change with time. There are no absolute values to work with, but observation of the outputs will alert you to potential problems.

If you want to check the state of an electrode at any time, connect it to channel 1 of the meter, either enter the program, click on **Calibrate** and **Calibrate Zero**, or on the meter front panel select **Calibrate** and **Start** and observe the outputs on the meter in zero oxygen and in air saturated solution.

High Point output With standard polypropylene membranes, the output at 20ºC in air saturated water is normally in the range of 200 - 600 pico amps. The output changes by 2 - 3% with each degree Centigrade of temperature change, increasing as the temperature increases.

Zero output In zero oxygen, there is usually a very small residual current, called the dark current. During Calibration, this signal is compensated for, so that a zero reading is obtained in zero oxygen. The interface displays this dark current during zero oxygen calibration. Normally the value shown will be between 0 and about 50 pico amps. Very high dark currents may indicate a short circuit within the electrode.

8.4 Appendix 4: Troubleshooting

High dark current

A high dark current displayed on the interface during Calibration with the electrode in zero oxygen solution could be due to your solution having become oxygenated again. So first check the solution by adding more sodium sulphite to it. If the problem persists, it may indicate an internal short circuit within the electrode. This cannot be repaired. Return the electrode to your distributor or to Strathkelvin Instruments for verification.

Very high output

This may indicate that you have an air bubble trapped between the cathode and the membrane. Remove the electrode jacket, add more electrolyte and tap vigorously to dislodge any air bubble. A high reading will also result if the jacket is not screwed on tightly, or if there is no air bubble in the electrolyte. The output could also increase as a result of plating on to the cathode, thereby increasing its surface area. This can be corrected by cleaning the cathode. (See Appendix Section 8.2)

Low output

The output will fall as a result of fouling of the membrane by bacteria or adsorbed proteins. Wipe the membrane from time to time with fine paper tissue.

Continual downward drift

When the electrode is first connected to the interface and thereby subjected to the bias voltage, there will be a very high reading - which will gradually decline to a stable value, providing that the electrode is at a constant temperature and exposed to a constant oxygen concentration. After changing the membrane, the electrode may take several

hours to settle down again. If the electrodes are going to be in daily use, keep the electrode connected to the interface which should be switched on. The electrodes will then be ready for immediate use as required.

The electrode might be expected to drift by no more than 0.5% over 12 hours. If the downward drift is greater than this, check the following:

1) is the electrolyte being maintained at constant temperature? A drop in temperature will cause the signal to fall.

2) is the electrode in an enclosed volume of water? In these circumstances, the reading can fall because the O_2 concentration in the chamber is declining due to bacterial respiration. If checking the electrode in a situation like this, make sure the chamber has been sterilised and that the solution is sterile. Even distilled water from a distilled water reservoir can have a significant population of bacteria. Ideally, use freshly boiled then cooled and re-aerated saline and keep a bacteriocidal solution in the chamber when not in use.

If you are using an EH100, the best check of electrode stability is to clamp the EH100 with the electrode tip just below the water surface of the constant temperature water bath and keep the bath water well aerated.

If you are certain that the drift is not due to either of these two causes, go to Section 8.5.

Continual upward drift

Check whether the temperature of the solution being measured has changed. This sometimes happens in respirometry experiments if full sunlight or other bright light is allowed to fall on the respiration chamber. The electrode output will change by about 3% for each °C change in temperature. If the temperature change can definitely be ruled out, go to section 8.5.

Unstable Display

The reading flickers erratically over several digits. This can be caused by trouble with the membrane, the cathode or the anode. Try the following in turn:

1) Wipe the membrane with a soft tissue, first dry, then moistened with acetone or alcohol. If no improvement, change the membrane.

2) Remove the electrode jacket and rinse the inner electrode with distilled water. Carefully dry with a soft paper tissue. Clean the anode or cathode with a soft tissue moistened with acetone or alcohol to remove any grease which may have got on to these surfaces. Add more electrolyte to the jacket and screw on tight again.

3) Clean the cathode – Appendix, Section 8.2.

4) Rechloride the anode – Appendix, Section 8.5.

Response time becomes excessively high

Response time varies with a number of factors including temperature, and membrane type and thickness. If the response time increases, it is probably because the membrane has become coated and is therefore less permeable . Try wiping the membrane very

carefully with a soft tissue. If you are measuring oxygen in high-protein solutions, dip the tip of the electrode (in its holder) into a proteinase solution when not being used.

Sometimes increased response time is due to an increase in the membrane to cathode distance. This could be due to the membrane becoming slack, in which case change the 'O' ring. It may also be due to the jacket not having been screwed on tightly.

Persistent malfunction

If there is a persistant malfunction in an electrode, send it, with a purchase order number and full description of the experimental set-up and the nature of the fault, to your distributor or to Strathkelvin Instruments. We will do our best to service and repair the electrode although where there is excessive dark current, repair is sometimes not possible.

8.5 Appendix 5: Rechloriding electrode anode

The anode of the 1302 is a silver band with a dark brown layer of silver chloride deposited upon it. If there has been any leakage to ground from the electolyte, the anode may become depolarised and will appear very light and silvery in colour due to loss of most of the silver chloride. The most likely cause of this is that the electrolyte has been in electrical contact with the grounded solution. This can happen if there is a minute hole in the membrane, or if water gets into the electrode holder. If the anode has been stripped, it is usually possible to rechloride it successfully.

You will need:

1. A 1.5v battery and battery holder.
2. About 5cm length of silver wire, soldered to a length of copper wire which is connected to the negative side of the battery holder.
3. Another length of copper wire connected to the positive side of the battery holder and terminating in a crocodile clip.
4. A small beaker of 5 - 6cm diameter, containing about 4cm depth of 0.1N HCl.

Method:

Position the silver wire vertically on one side of the beaker containing the 0.1N HCl. Unscrew the electrode jacket and rinse the electrode in distilled water. Dry the anode carefully with paper tissue and then carefully rub it with very fine (400 grit or finer) emery (or similar) abrasive paper until any residual silver chloride coating has been removed and it appears a uniform silver color. Take care not to finger the anode. Rinse in distilled water again and dry with soft paper tissue.

Place a light above the beaker, connect the crocodile clip to the outside of the Lemo connector and insert the electrode into the beaker as far as possible from the silver wire, to a depth which covers the anode.

Rotate the electrode in the solution for 15 seconds. You will see hydrogen bubbles on the silver wire and the anode will become a dark brown colour as silver chloride plates on to it. Remove the electrode from the solution and examine it. If the anode is not an even brown color, repeat the process again.

Rinse the electrode in distilled water, dry with paper tissue and reassemble.

8.6 Appendix 6: Calibration solutions

It is possible to calibrate the oxygen electrodes with gas mixtures, but most researchers tend to calibrate at the low end with a zero oxygen solution and at the high end with air-saturated water.

Air-saturated water This must be at exactly the same temperature (within 0.1 C) as the solution which will be measured in the experiment, since the electrode is temperature sensitive. To obtain air-saturated water, bubble air through it, or stir vigorously for 15-20 mins. Make sure that this does not alter the temperature of the water.

Zero oxygen solutions An oxygen-free solution can be produced by adding a pinch of sodium sulphite to distilled water or saline in a beaker and swirling to dissolve. Alternatively make up a solution of 2% sodium sulphite in 0.01M sodium borate (3.81 g sodium borate in 1 litre distilled water) and keep in a stoppered bottle. The borate acts as a weak buffer to stabilise the solution. Some early literature suggested the use of sodium dithionate solution. Do not use this, since its breakdown products can impair the functioning of the cathode.

Rinse all traces of the sulphite from the electrode after zero calibration.

8.7 Appendix 7: Oxygen solubility tables

The concentration of oxygen in a solution will vary with temperature and the concentration of solutes dissolved in it. The solubility coefficient or Bunsen coefficient a expresses the concentration of oxygen in solution when in equilibrium with oxygen gas at a pressure of 760 torr (mm Hg), in mls O_2 (S T P) per ml solution.

For freshwater and seawater, refer to the detailed tables in references (1) and (4).

Mammalian Ringer solution:

Concentration of oxygen at 100% saturation with air and values for a (ml O_2 per ml solution, at PO_2 of 760 torr).

Temperature	**Concentration** (air saturation)	**Solubility Coefficient (a)**
°C	ml $O_2.1^{-1}$	ml $O_2.ml^{-1}$
10	10.05	0.0480
15	7.12	0.0340
20	6.49	0.3100
25	5.97	0.0285
30	5.44	0.0260
35	5.13	0.0245
40	4.81	0.0230

From reference (7).

Mitochondrial medium:

Concentration of oxygen at 100% saturation with air.

Temperature	**Concentration** (air saturation)	**Concentration** (air saturation)
°C	µmol $O_2.l^{-1}$	µg atoms $O_2.ml^{-1}$
15	288	0.575
20	255	0.510
25	237	0.474
30	223	0.445
35	205	0.410
37	199	0.398
40	190	0.380

From reference (3).

8.8 Appendix 8: Conversion units

Conversion units

Pressure

1mm Hg = 1 torr = 0.133322 kilo Pascals = 0.001316 atm.

Oxygen concentration units

1mg = 0.700ml (at S T P) = 31.251 µmol = 0.0625 mg atoms.

8.9 Appendix 9: Electrolyte solution

Weigh out: 5.31g disodium hydrogen phosphate dihydrate
2.6g potassium dihydrogen phosphate
1.04g potassium chloride

Make up to 100ml with distilled water. Add a few crystals of silver chloride to give a saturated solution. Finally drop in a small crystal of thymol, to inhibit fungal and bacterial growth. Shake vigorously and leave for 12 hours for the thymol to go into solution. Filter into a stoppered bottle.

8.10 Appendix 10: References

1. Carpenter, J.H. (1966) New Measurement of oxygen solubility in pure and natural waters. Limnology and Oceanography 11, 267 - 277.

2. Christoforides, D. & J. Hedley-White (1969) Effect of temperature and hemoglobin concentration of solubility of O_2 in blood. J. appl. Physiol. 27, 592 - 596.

3. Clark, J.B. (1992) Electrochemical essays: the oxygen electrode. In Enzyme Assays (ed. Eisenthal, R. & Danson, M.J.). IRL Press, Oxford, 181 – 190.

4. Green, E.J. & D.E. Carritt (1967) New tables for oxygen saturation of seawater. J. mar. Research 25, 140 - 147.

5. Sendroy, J., R.T. Dillon & D.D. van Slyke (1934) Studies of gas and electrolyte equilibria in blood. XIX The solubility and physical state of uncombined oxygen in blood. J. biol. Chem, 105, 597 - 632. (Table II shows the effects of various electrolytes in reducing the solubility of oxygen in water.)

6. Van Slyke, D.D., J Sendroy, A Baird Hastings & J.M. Neill (1928) Studies of gas and electrolyte equilibria in blood. X The solubility of carbon dioxide at 38° in water, salt solution, serum and blood cells. J. biol. chem. 78, 765 - 799. (Table 1 shows the relative solubility of oxygen in a range of acids, bases and solvents.)

7. Umbreit, W.W. et al (1964). Manometric techniques (4th Edn), Burgess Publ Co. (Contains a wealth of information on tissue metabolism, using Warburg and similar techniques.)