

Innovative Instruments, Inc. <http://www.2in.com>

Dissolved Oxygen Meter

inO2

Instruction Manual

Serial No.....

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Introduction

inO2 and its associated OXY-2 sensor provide accurate and stable oxygen measurements. The recorder output is isolated from the electrode circuitry. This offers the important advantage that other electrodes may be used in the same sample as the OXY-2 without interfering with one another, and background noise is greatly reduced. The small tip diameter of just 2 mm and low oxygen consumption, allow the measurements in small sample volumes, in addition fast response time and a sturdy stainless steel sensor body.

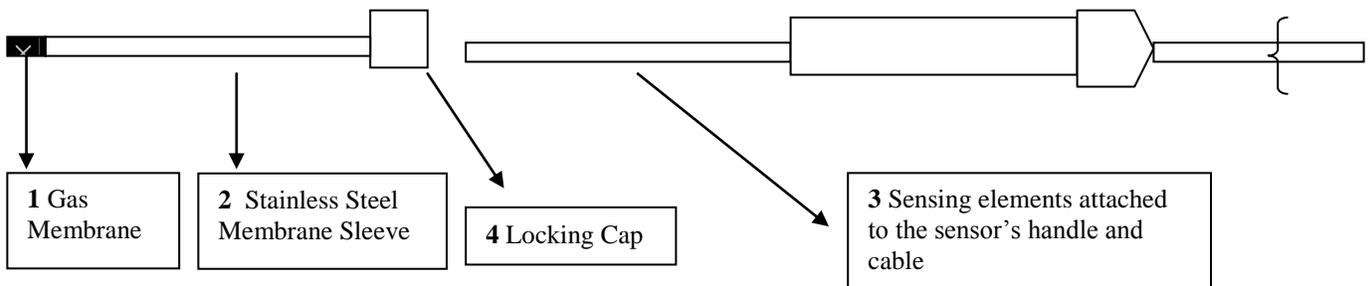
OXY-2 is an amperometric sensor. inO2 applies an electrical potential that is sufficient to reduce oxygen to water. The amount of electrical charge transferred during the reduction is measured as electrical current which is proportional to the amount of oxygen passed through the gas permeable membrane isolating the sensing elements from the sample solution. The rate of oxygen diffusion through the membrane is proportional to the concentration of oxygen or partial pressure outside the membrane, in the sample. Measurements can be displayed either as a percentage of atmospheric pressure, parts per million (ppm), or as a redox current in nanoamperes (nA). OXY-2 houses a platinum working electrode and a silver counter inside a stainless steel sleeve. The gas permeable polymer membrane fits over the end of the sleeve, which allows oxygen to pass while blocking liquids, ions and particulate matter.

inO2 is simple to use. Just attach OXY-2 to the meter, turn the power on and wait for the current to decay to a stable value. This usually takes few of hours if OXY-2 was disconnected from the meter for very long time. The current can be monitored by setting inO2 to the nA setting.

Sensor Structure and Assembly

Fig. 1 shows the principal components of an OXY-2 sensor. A gas permeable membrane (1) is attached to the tip of the outer stainless steel sleeve (2). The interior of the sensor is composed of the sensing element, a platinum cathode and silver counter electrode inside the sleeve. The sensing element is permanently mounted in the sensor’s plastic handle (3). After electrolyte has been added inside the sleeve (see below), the sensing element is slowly inserted into the sleeve and secured by screwing the sleeve cap (4) gently into the probe handle. If the membrane on the stainless sleeve becomes damaged, the entire sleeve must be replaced. The assembled sensor can be sterilized with alcohol or other chemicals. The membrane sleeves can be autoclaved separately from the probe should the user desire to do this and then place the sterilized membrane sleeve onto the sensing elements.

Figure 1: Structure of OXY-2



Calibration

Connect the power line adapter to the back of the meter.

Note: The Calibration should be performed as close as possible to the temperature of the sample to be measured.

OXY-2 should be kept connected to the meter all the time to provide a stable baseline (background current). The sensor can be kept immersed in distilled water when not in use.

Zero Point Calibration

When OXY-2 reaches a stable background, a calibration for zero oxygen may be carried out in pure nitrogen gas or in water saturated with nitrogen. With stirring, the complete saturation of water with nitrogen may take more than ten minutes. Calibration in pure nitrogen gas is much faster and generally considered more reliable. A plastic calibration bottle (Fig.2) is provided for this purpose. Connect a plastic tube from the side tube to a pure nitrogen gas source at a low pressure (less than 5 psi) and purge the bottle with nitrogen gas. Insert OXY-2 sensor into the bottle through the top vent hole on the bottle cap. The current should be observed to drop rapidly. If the current value is not zero after 10 minutes of nitrogen bubbling, use the Zero ADJ knob to adjust the nA reading to zero. Switch the selection knob to read %. It should read zero. If not, adjust to zero using a small screw driver to turn the screw labeled %.

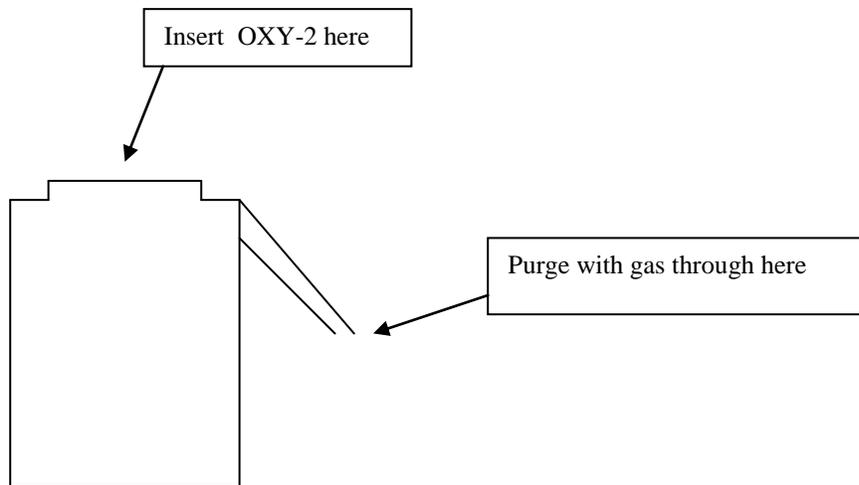


Figure 2; The Calibration Bottle

Gas Phase Measurements

Sensor calibration for gas phase measurements can be accomplished using the calibration bottle, as described above for zeroing the instrument with nitrogen, and using a tank of known oxygen composition, for example 100 % O₂.

Turn the selection knob to %. With the bottle purged with nitrogen the display should read 0%. With the bottle purged with oxygen the display should read 100%. If the display does not show 100%, use a small screwdriver to adjust the % adjustment screw so that the meter reads 100%.

Alternatively, air can be used as the calibration standard but since water vapor does affect the sensor reading it is best to use dry air unless the ambient humidity is accurately known. Dry air

can be obtained by passing room air through a column containing a solid drying agent such as calcium chloride and then into the calibration bottle for calibration. Turn the select knob to %. The display should read 21. If not, use a small screwdriver to adjust the % adjustment screw so that the meter reads 21. If ambient air is used to calibrate at 21%, ambient humidity may cause a calibration error of as much as 1% O₂. The user may decide if this is acceptable.

The physical interpretation of the % reading of the inO₂ is the percentage of atmospheric pressure that the oxygen present exerts. For example in a 100% oxygen environment the display will read 100 which means that the partial pressure of oxygen is 1atm (760 mm Hg). If the display reads 21, then this means that the partial pressure of oxygen is 0.21 atm (160 mm Hg).

Aqueous measurements

For aqueous calibration, fill the calibration bottle with distilled water to approximately one-half of its full volume. This water is assumed to have the proper amount of oxygen upon equilibration with air over long time. Immerse the sensor tip into the water via the top hole. Turn the selection knob to %. The scale reading should be allowed to stabilize. Dissolved oxygen calibration is corrected for the effect of water vapor by the following equations:

$$(1) \text{ pO}_2 = 21\% \times (1 - \text{pH}_2\text{O})$$

$$(2) \text{ pO}_2 = 21\% \times (1 - \text{P}'\text{H}_2\text{O}/760)$$

where pH₂O and p'₂O are the partial pressure of water vapor at standard atmospheric pressure in atmospheres and in mm Hg, respectively (see Table 3). For example, the pH₂O in water-saturated air at 24 deg is 22 mm Hg (see Table 3). Therefore the pO₂ = 21% x (1 - 22/760) = 20.4%. Note that for purposes of oxygen measurements liquid water is considered to be “water-saturated air”. The display should thus read 20.4%. If it does not, use a screwdriver to adjust the % adjustment screw so that the meter reads the correct calibration value. The values of water vapor pressures at different temperatures can be found in Table 3.

To measure dissolved oxygen in parts per million (ppm), switch the select knob to ppm. Refer to Table 1a. This table gives the solubility of oxygen in water at different temperatures at an ambient pressure of 1 atm. If the solution temperature is 25C, for example, the proper oxygen reading when the probe is in water should be 8.4 ppm. If the display does not show this value, adjust the ppm screw with a screwdriver so that the meter displays the correct value. No need for correction for the water-vapor effect for the ppm calibration since the values in Table 1a are obtained in “water-saturated air” at an atmospheric pressure of 760 mm Hg.

The unit ppm is equivalent to mg/l. This is illustrated as follows. The solubility of oxygen in water at 0 deg according to the Merck index is 4.889 ml per 100 ml. Using the ideal gas law we can calculate the number of moles of oxygen present in 100 ml:

$$\begin{aligned} PV &= nRT \\ n &= PV/RT \\ n &= (0.21)(4.889 \times 10^{-3}) / (0.0206)(273) \\ n &= 4.58 \times 10^{-5} \text{ moles} \end{aligned}$$

Where P is the partial pressure of oxygen, V is the volume of oxygen, n is the number of moles of oxygen, R is the universal gas constant and T is the absolute temperature. From the number of grams of oxygen:

$$4.58 \times 10^{-5} \text{ mole} \times 32 \text{ g/mole} = 0.00146 \text{ g}$$

Therefore there will be $(1.46 \times 10^{-3} \text{ g} / 0.1 \text{ L})$ 14.6 mg of oxygen per liter. Since 1 L of water has a mass of 1000g, and there are 1 million mg in 1000g, the concentration in ppm shall be:

$$(14.6 \times 10^{-3} \text{ g/L}) / (1000\text{g/L}) = 14.6 \text{ ppm}$$

Note that this value corresponds to that given in Table 1a.

For accurate results the temperature of the water sample and the fluid being tested should be identical. They should be continuously stirred using a magnetic stirrer. Redox current can be measured by switching the Select switch to nA.

Calibration Method for O₂ Measurements in Living Tissue or Blood

The inO₂ meter and OXY-2 sensor may be used extensively in applications involving O₂ measurements in vitro or in vivo in living tissue or fluids such as blood. You may still use the calibration procedure in this manual for these measurements since a membrane-covered amperometric oxygen electrode will always measure oxygen activity, not concentration, it is actually more appropriate to define oxygen in solution in terms of activity, since this is the “effective concentration”. For example, in distilled water the activity coefficient, Y_c, is close to unity; but in solutions with high salt concentration the activity coefficient is different from unity and concentration and activity of dissolved oxygen are no longer equal. For the membrane-covered oxygen electrode this is an important effect since an oxygen detector only responds to the difference in activity across the membrane rather than the concentration difference

Thus, if it is necessary to have a measure of dissolved oxygen in terms of concentration, then the calibration is somewhat more complicated since the relationship between activity and concentration may change with the change of salt concentration in the samples. The activity coefficient, a ratio of the activity to the concentration, generally cannot be predicted and one must rely on empirical determinations since the compositions of living fluids such as blood are extremely complicated. One may directly use the fluid to be tested as a “solvent” to prepare a calibration standard. Alternatively, one may use the Bunsen absorption coefficient, **a**, to calculate oxygen concentration in blood terms of the results with the oxygen electrode. The equation is:

$$C = \frac{\text{a}}{\text{Molar Volume} \times K} \times (\text{pt} - \text{pH}_2\text{O}) \times \text{pO}_2$$

Where K is a conversion factor depending on the unit of pressure chosen (1 for atm), pt and pH₂O are the total pressure of gas and the partial pressures of water, respectively. pO₂ is the partial pressure of oxygen in blood obtained from the measurements with the oxygen electrode. Bunsen Coefficients for solubility of oxygen in plasma and blood can be found in Table 4.

Cleaning the Electrode Surface

The reduction of oxygen causes a decrease in the surface activity of the working electrode, which will gradually over time “poison” it. This poisoning reduces the redox current from the reduction of oxygen. The poisoning can be inferred from low current value of the sensor in air (21%). If the current value of sensor in air drops below 30 nA, then the sensor needs re-activation.

Once the reduction current does drop below 30 nA, there is a CLEAN function, which may be used to restore activity so that the electrode can continue to be used. This is done by switching the Clean knob **up**. When this is done the potential applied to the platinum electrode is changed to a different value. This causes changes to occur to the surface, which help to restore the electrode activity. The user can leave the instrument on this CLEAN setting for a period up to 30 minutes. Note that the LCD display output in this setting should be ignored. After the thirty minutes have elapsed, switch the Clean knob **down**. Wait until the current reaches a stable value. This might take more than 1 hour, after which use the instrument as you normally would.

Changing the Membrane Sleeve

If the membrane at the tip of the stainless sleeve is broken, a new membrane sleeve should be used. The breakage of the membrane is indicated by electrolyte leakage from the sensor tip, current is close to zero and goes to a very high value when the sensor tip is immersed in ion buffer or other solutions. To change the membrane, unscrew the membrane sleeve from the handle and pull out the sensing element out of the sleeve. Remove the plastic screw cap from the sleeve and slide it into a new membrane sleeve. Wash the sensing elements with distilled water. Connect the sensor to the meter (without the membrane sleeve). Wipe the sensing element with a soft tissue to dry it. The reading should be zero. Using the supplied long tip syringe, inject few drops of electrolyte into the new membrane sleeve. Inject them by inserting the long tip all the way inside the new membrane sleeve. Insert the sensing element into the new membrane sleeve and fasten to the handle by screwing the plastic cap. The current reading should be high and decaying. Wait for a stable background and use the sensor normally.

Recorder output

The recorder output terminal is not electrically connected to the sensor structure. This offers an important recording advantage because other electrodes in the same test medium will not interact adversely with OXY-2. The output signal from the Recorder connector is 1 millivolt per nanoampere of electrode current regardless of whether the selector switch is in the %, ppm or nA range. Connect the meter through the provided double BNC cable to a recorder or any data acquisition system

Storage

For long term storage unscrew and remove the sleeve from the sensor handle, rinse the electrode tip and the sleeve with distilled water. Dry them and replace the sleeve to protect the sensing elements.

Trouble Shooting

| Problem | Solutions |
|---------|-----------|
|---------|-----------|

| | |
|---|---|
| Current is very low or zero | Open circuit in cable, check cable connections. |
| Current does not decrease after nitrogen bubbling | Electrolyte dried out. Disassemble and refill with fresh electrolyte solution according the procedure mentioned in “Changing the Membrane Sleeve”. |
| Current does not increase after oxygen bubbling | *The zero knob was wrongly adjusted. Disconnect the sensor and adjust the reading to zero. *Heavily contaminated or defective membrane. Replace stainless steel membrane sleeve. *The electrode surface needs to be cleaned using CLEAN function. Switch the Clean knob up for 30 min. |
| The current still very low after using the CLEAN Function | Remove the membrane, Connect to meter, immerse in 0.10 M sulfuric acid, switch to CLEAN function for 30 min, wash with distilled water, dry, fill with electrolyte, and place a new membrane sleeve. |

Table 1

Solubility of Oxygen in water-saturated air as a function of Temperature. 1 atm..

| Temperature, C | Solubility, ppm | Temperature, C | Solubility, ppm |
|----------------|-----------------|----------------|-----------------|
| 0 | 14.6 | 19 | 9.4 |
| 1 | 14.2 | 20 | 9.2 |
| 2 | 13.8 | 21 | 9.0 |
| 3 | 13.5 | 22 | 8.8 |
| 4 | 13.1 | 23 | 8.7 |
| 5 | 12.8 | 24 | 8.5 |
| 6 | 12.5 | 25 | 8.4 |
| 7 | 12.2 | 26 | 8.2 |
| 8 | 11.9 | 27 | 8.1 |
| 9 | 11.6 | 28 | 7.9 |
| 10 | 11.3 | 29 | 7.8 |
| 11 | 11.1 | 30 | 7.6 |
| 12 | 10.8 | 31 | 7.5 |
| 13 | 10.6 | 32 | 7.4 |
| 14 | 10.4 | 33 | 7.3 |
| 15 | 10.2 | 34 | 7.2 |
| 16 | 10.0 | 35 | 7.1 |
| 17 | 9.7 | 36 | 7.0 |
| 18 | 9.5 | 37 | 6.9 |

Table 1b

Solubility of Oxygen as a function of Chloride Concentration

Table 1b
Chloride Concentration of Seawater

| °C | 5 g/l | 10 g/l | 15 g/l | 20 g/l |
|----|-------|--------|--------|--------|
| 1 | 13.8 | 13 | 12.1 | 11.3 |
| 2 | 13.4 | 12.6 | 11.8 | 11 |
| 3 | 13.1 | 12.3 | 11.5 | 10.8 |
| 4 | 12.7 | 12 | 11.2 | 10.5 |
| 5 | 12.4 | 11.7 | 11 | 10.3 |
| 6 | 12.1 | 11.4 | 10.7 | 10 |
| 7 | 11.8 | 11.1 | 10.5 | 9.8 |
| 8 | 11.5 | 10.9 | 10.2 | 9.6 |
| 9 | 11.2 | 10.6 | 10 | 9.4 |
| 10 | 11 | 10.4 | 9.8 | 9.2 |
| 11 | 10.7 | 10.1 | 9.6 | 9 |
| 12 | 10.5 | 9.9 | 9.4 | 8.8 |
| 13 | 10.3 | 9.7 | 9.2 | 8.6 |
| 14 | 10.1 | 9.5 | 9 | 8.5 |
| 15 | 9.9 | 9.3 | 8.8 | 8.3 |
| 16 | 9.7 | 9.1 | 8.6 | 8.1 |
| 17 | 9.5 | 9 | 8.5 | 8 |
| 18 | 9.3 | 8.8 | 8.3 | 7.8 |
| 19 | 9.1 | 8.6 | 8.2 | 7.7 |
| 20 | 8.7 | 8.3 | 7.9 | 7.4 |
| 21 | 8.6 | 8.1 | 7.7 | 7.3 |
| 22 | 8.4 | 8 | 7.6 | 7.1 |
| 23 | 8.3 | 7.9 | 7.4 | 7 |
| 24 | 8.1 | 7.7 | 7.3 | 6.9 |
| 25 | 8 | 7.6 | 7.2 | 6.7 |
| 26 | 7.8 | 7.4 | 7.2 | 6.7 |
| 27 | 7.7 | 7.3 | 6.9 | 6.5 |
| 28 | 7.5 | 7.1 | 6.8 | 6.4 |
| 29 | 7.3 | 6.9 | 6.5 | 6.1 |

Table 1b:
Solubility of oxygen
(milligrams/ liters) in
seawater of different
salinities, in
equilibrium with air at
barometric pressure of
760 mm Hg (101.3 kPa)
and oxygen partial
pressure
of 159 mm Hg (21.2kPa)

Table 2

Table 2:

Oxygen solubility

Obtained from Table 1a

Table 1b should be corrected if barometric

pressure is different than 760 mm Hg or at altitudes other than sea

| Altitude' level | Feet | Pressure , mm Hg |
|--------------------|-----------|---------------------|
| Sea Level | Sea Level | 760 |
| | 500 | 746 |
| | 1000 | 732 |
| | 1500 | 720 |
| | 2000 | 707 |
| | 2500 | 694 |
| | 3000 | 681 |
| | 3500 | 668 |
| | 4000 | 656 |
| | 4500 | 644 |
| | 5000 | 632 |
| | 5500 | 621 |
| | 6000 | 609 |

Table 3: Water Vapor Pressure in mm Hg

| Temp, C | Pv, mm Hg | Temp. C | Pv, mm Hg |
|---------|-----------|---------|-----------|
| 0 | 5 | 22 | 20 |
| 2 | 5 | 24 | 22 |
| 4 | 6 | 26 | 25 |
| 6 | 7 | 28 | 28 |
| 8 | 8 | 30 | 32 |
| 10 | 9 | 32 | 36 |
| 12 | 11 | 34 | 40 |
| 14 | 12 | 36 | 45 |
| 16 | 15 | 38 | 50 |
| 18 | 16 | 40 | 55 |
| 20 | 18 | | |

**Table 4
Bunsen Coefficients (a) for Solubility of**

Oxygen in Plasma and Blood

| Temp °C | Blood g/100 ml Hb | | | | |
|------------|----------------------|--------|--------|--------|--------|
| | Plasma | 5g | 10g | 15g | 20g |
| 15 | 0.0302 | 0.031 | 0.0312 | 0.0316 | 0.0323 |
| 20 | 0.277 | 0.0282 | 0.0284 | 0.0287 | 0.0293 |
| 25 | 0.257 | 0.0261 | 0.0263 | 0.0265 | 0.0271 |
| 28 | 0.0257 | 0.0249 | 0.0251 | 0.0253 | 0.0259 |
| 30 | 0.0238 | 0.0241 | 0.0243 | 0.0245 | 0.0251 |
| 35 | 0.022 | 0.0226 | 0.0227 | 0.0229 | 0.0234 |
| 37 | 0.214 | 0.022 | 0.0221 | 0.0223 | 0.0228 |
| 40 | 0.0208 | 0.0221 | 0.0212 | 0.0214 | 0.0219 |

Specifications of inO2 and OXY-2

| | | | |
|-------------------|---------------|----------------------|------------|
| % | 0-199% | dimensions | 3x9x8 inch |
| ppm | 2-19.99 | Shipping weight | 5 lb |
| current | 0-1999 nA | Sensor | |
| resolution | 0.1 ppm | Sensors Tip Diameter | 2 mm |
| Accuracy | ±1.5% | Tip length | 75 mm |
| Output resistance | 1000 ohms | | |
| Power | 120/240 V, AC | | |

Accessories

Filling electrolyte solution (1)
 Filling syringe (1)
 Calibration bottle (1)
 Membrane Sleeves (2)
 Adapter (1)
 Double BNC cable (1)
 Grounding cable

Warranty

Innovative Instruments, Inc. warrants to the original purchaser that this equipment and its components shall be free of defects. The Electronic parts are warranted for one year part and labor. The sensor and consumable are warranted for 30 days.

This instrument and its accessories are not intended for human use.