



The Dissolved Oxygen Handbook

a practical guide to dissolved oxygen measurements



YSI.com/weknowDO

CONTENTS

Introduction	1
Dissolved Oxygen Sensors	3
Optical Sensors	5
YSI Optical Dissolved Oxygen Instruments	6
Optical Sensing Element.....	7
How an Optical Sensor Measures Dissolved Oxygen	9
Electrochemical Sensors	10
YSI Electrochemical Instruments	10
Electrochemical Membranes	12
How an Electrochemical Sensor Measures Dissolved Oxygen	15
Advancements in Steady-state Electrochemical Sensors.....	20
Comparing Steady-state Polarographic and Galvanic Sensors.....	21
Comparing Optical and Electrochemical Sensing Technologies	25
Measurement Accuracy	25
Approved Methodology	28
Response Time.....	29
Flow Dependence	32
Warm-Up Time.....	34
Calibration Frequency	34
Measurement Interferences	35
Maintenance Requirements.....	35
Power Consumption.....	35
Summary	35
Measuring Dissolved Oxygen with Either Sensor Type.....	37
Variables that Affect Dissolved Oxygen Measurements.....	38
Temperature	39

Salinity.....	40	Electrode Maintenance	67
Correcting for Salinity	42	Probe Care and Maintenance for Optical Sensors	69
Barometric Pressure	43	Storage	70
Using Barometric Pressure for DO Calibration	44	Final Words.....	70
Local DO % Measurements.....	46	Appendix A - Oxygen Solubility Table	72
Calibration	46	Appendix B – Calibration Values for Various Atmospheric Pressures and Altitudes.....	74
Calibration Frequency	46	References	76
Calibration Methods	47		
Winkler Titration Calibration.....	48		
Air-saturated Water Calibration	49		
Water-saturated Air Calibration	49		
Two Point Calibration.....	54		
Calibration Musts	55		
Calibration Musts for Electrochemical Sensors	55		
Calibration Musts for Optical Sensors	56		
Errors During Calibration	57		
GLP (Good Laboratory Practices) File.....	58		
Taking Measurements.....	59		
BOD Measurements	60		
Measurement Precautions and Interferences	61		
Biofouling.....	61		
Coating Materials	61		
Probe Attacking Liquids	62		
Interfering Gases	63		
Membrane and Sensing Element Integrity	63		
Probe Care and Maintenance.....	64		
Probe Care and Maintenance for Electrochemical Sensors	64		
Changing a Membrane.....	64		

THIS PAGE LEFT INTENTIONALLY BLANK

INTRODUCTION

YSI has a long history in developing and manufacturing sensors that measure dissolved oxygen in aqueous solutions and has had many firsts over the years including the invention and commercialization of the first portable dissolved oxygen instrument in 1963. This instrument utilized a membrane-covered Clark Polarographic sensor, commonly referred to as a Clark electrode, which was developed in 1956 by Dr. Leland Clark (figure 1), a researcher at Antioch College who was working in collaboration with YSI scientists. Before the introduction of the Clark electrode, methods for measuring dissolved oxygen were laborious, time-consuming and highly susceptible to interference. Today the world continues to benefit from Dr. Clark's invention as the Clark electrode is still used by many manufacturers and in several YSI instruments. In addition to the variety of Clark electrodes offered, YSI also manufactures optical based dissolved oxygen sensors for laboratory, spot sampling and long term monitoring applications. See figure 2 for a brief overview of other YSI milestones in dissolved oxygen measurement technologies over its 60 year history.

This booklet describes in detail the different types of dissolved oxygen sensing technologies available. It also covers, in general terms, recommended calibration methods, regular maintenance procedures that can be performed by the user and how to take a measurement in order to obtain accurate data. For instrument specific instructions and recommendations, please refer to the instrument's instruction manual.

YSI offers seminars on the topic of dissolved oxygen measurement technologies which may apply to continuing education units depending on the certifying agency. If you would like to schedule a seminar for your group or organization, please contact YSI at environmental@ysi.com, 1-800-897-4151 or +1 937-767-7241.

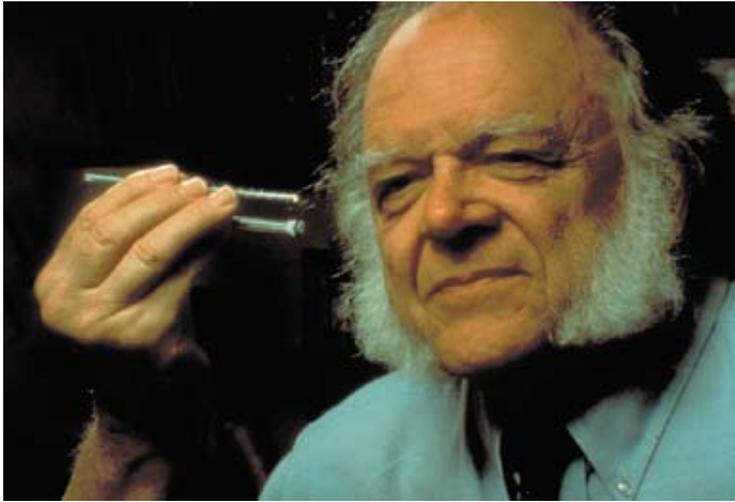


Figure 1. Dr. Leland Clark, inventor of the Clark polarographic electrode.

Notable Events in YSI's History of Developing Sensing Technologies for Measuring Dissolved Oxygen

1956 – Dr. Leland Clark invents the membrane covered Polarographic electrode while working with YSI Scientists.

1965 – YSI develops the first biological oxygen monitor. Considered a breakthrough for modern medicine and surgery, this instrument enabled physicians to perform open-heart surgery for the first time because immediate blood oxygen measurements could be taken real-time in the operating room rather than having a sample drawn and taken to a lab for analysis.

1993 – YSI patents first long-term, in-situ, stirring independent oxygen sensor (Rapid Pulse™ DO) and packages it with multiparameter instruments.

1993 – YSI patents first stirring independent micro-electrode oxygen sensor (Micro-Electrode Array or MEA) for spot sampling applications.

2002 – YSI releases polyethylene membranes for use on polarographic dissolved oxygen sensors. This advancement in membrane material lowered the stirring dependence and quickened the sensor's response time over traditional Teflon® membranes.

2006 – YSI releases the ROX® optical dissolved oxygen sensor. The sensor has a dedicated wiper for long term monitoring on multi-parameter sondes.

2007 – YSI releases a galvanic electrochemical sensor for use on the Pro Series handheld product family.

2008 – YSI releases the ProODO® optical dissolved oxygen instrument for spot sampling and laboratory applications.

Figure 2. YSI's Dissolved Oxygen Time line.

DISSOLVED OXYGEN SENSORS

There are two primary types of dissolved oxygen sensing technologies available: the optical based sensing method which is commonly referred to as luminescent and the Clark electrochemical or membrane-covered electrode. Within these two types of technologies, there are slight variations available. For example, there are two types of optical sensors. Both types of optical sensors measure luminescence as it is affected by the presence of oxygen; however, one sensor measures the lifetime of the luminescence while the other sensor measures the intensity of the luminescence.

The two types of Clark electrochemical sensors available are Polarographic and Galvanic. Additionally, YSI manufactures two types of Polarographic sensors: Steady-state and the patented Rapid Pulse sensor. Refer to figure 3 for a diagram of the various sensor types.

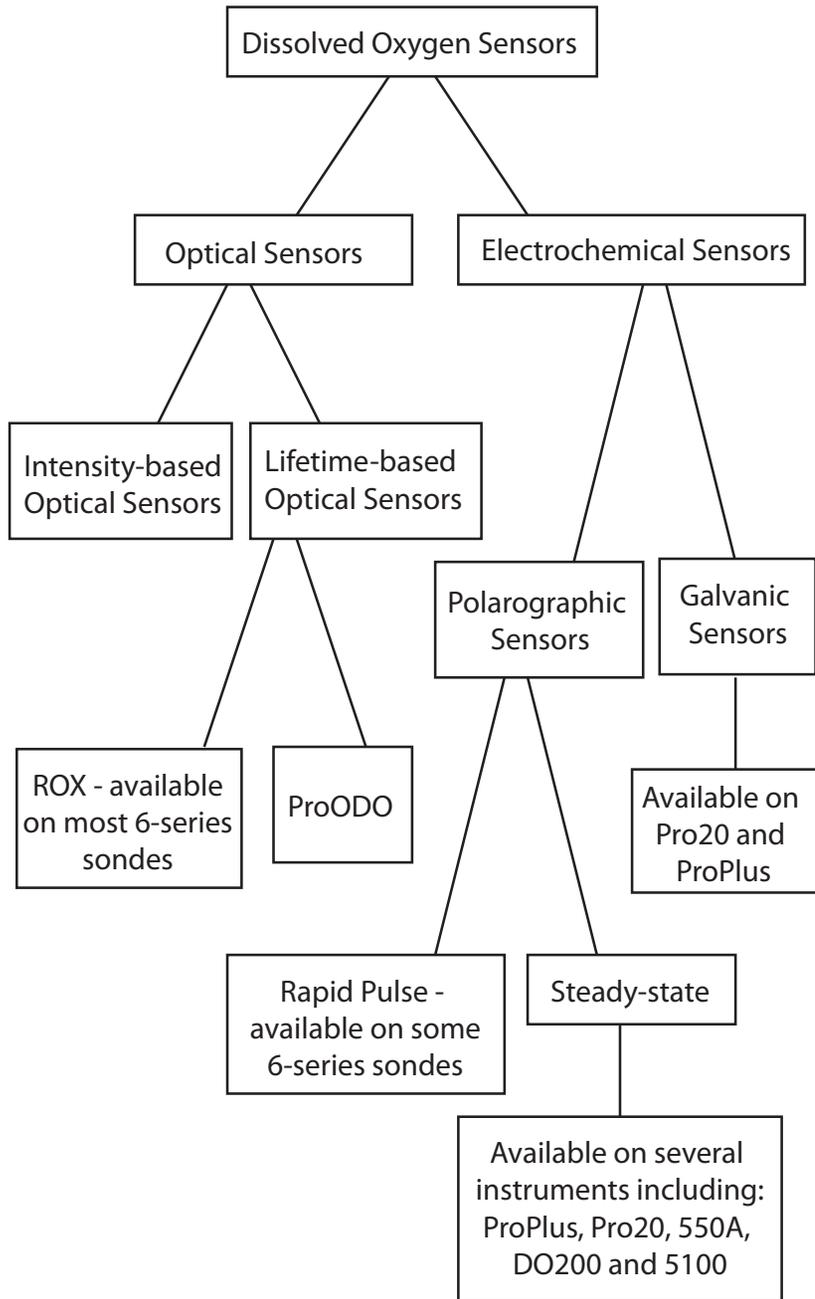


Figure 3. Diagram of Dissolved Oxygen Sensors.

OPTICAL SENSORS

Lifetime and intensity optical measurement methods detect dissolved oxygen based on the well documented principle that dissolved oxygen quenches both the lifetime and intensity of the luminescence associated with carefully-chosen chemical dyes. When there is no oxygen present, the lifetime and intensity of the signal are at their maximum. As oxygen is introduced to the sensing element, both the lifetime and intensity of the luminescence become shorter. Therefore, the lifetime and intensity of the luminescence are inversely proportional to the amount of oxygen present. The relationship between the oxygen pressure outside the sensor and the lifetime or intensity of the luminescence in the dye layer of the sensing element can be generally quantified by the Stern-Volmer equation (figure 4). However, the Stern-Volmer equation implies an inversely linear relationship which is not strictly true especially at higher oxygen concentrations; therefore, YSI employs the use of a 3rd order polynomial to correct for this non-linearity and to obtain the desired range of dissolved oxygen readings.

The Stern-Volmer Relationship

$$I_0/I = 1 + k_q t_0 * O_2$$

Where:

I_0 = Intensity or lifetime of luminescence without the quenching molecule (O_2).

I = Intensity or lifetime of luminescence with the quenching molecule (O_2).

k_q = Is the quencher rate coefficient.

t_0 = Is the luminescence lifetime of the chemical (the dye) to be quenched.

O_2 = The concentration of oxygen.

Figure 4. Stern-Volmer equation.

Given that the sensing elements of the two optical sensor types are identical, the primary advantage of the lifetime method over the intensity method is that a lifetime sensor will be more stable in the long term. This is because the

degradation of the dye in the sensing element has less effect on the lifetime based measurement than the intensity based measurement. Therefore, the intensity method will require more frequent calibrations - particularly at zero oxygen.

YSI OPTICAL DISSOLVED OXYGEN INSTRUMENTS

YSI offers two lifetime optical sensors: the ProODO (figure 5) sampling instrument and the 6150 ROX[®] (figure 6) sensor which can be used on most 6-series sondes that have an optical port. In addition, there will be an optical BOD-style sensor available for use on the ProODO in early 2010.



Figure 5. The ProODO is a compact, handheld instrument designed to withstand the harshest field conditions yet is accurate enough for use in a laboratory.



Figure 6. The ROX sensor's dedicated wiper and anti-fouling accessories help extend deployment times while protecting data integrity making it ideal for unattended, remote and real time monitoring applications.

OPTICAL SENSING ELEMENT

YSI's two optical dissolved oxygen sensors utilize sensing elements that are similar in function but slightly different in design. The ProODO's sensing element is referred to as a Sensor Cap due to its screw on cap design (figure 7). The ROX's sensing element is referred to as a ROX Membrane (figure 8) and is held in place by 3 screws.



Figure 7. ProODO Sensor Cap.

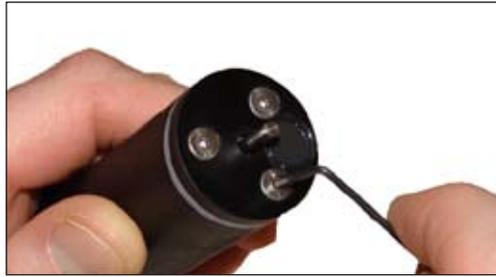
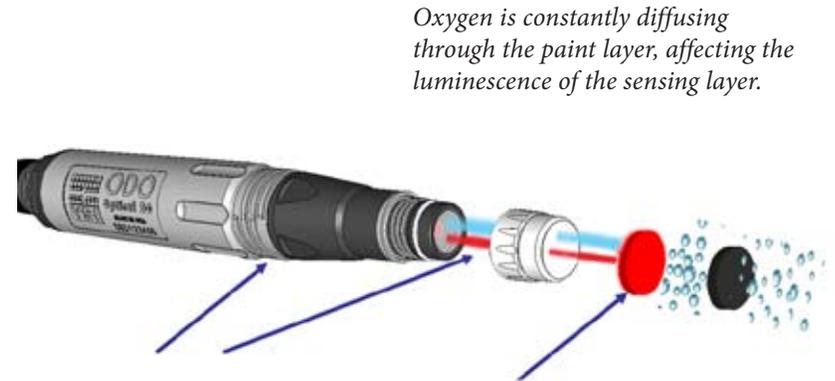


Figure 8. ROX membrane.

Each sensing element has two layers. The outer layer is a paint that acts as an oxygen permeable diffusion layer which allows oxygen molecules to pass through while protecting the dye layer. The sensing layer is an immobilized polystyrene dye layer that luminesces when excited with light of a proper wavelength (figure 9). The degradation of this dye layer over time is what causes the sensor cap to need replacement and all lifetime based optical sensors require that this dye layer be replaced periodically. YSI sensing elements are warranted for 1 year but may last much longer. The working life of a sensing element may be extended by keeping it clean and properly stored between uses. See the **Probe Care and Maintenance** section of this booklet for more information on cleaning and storage.

The sensing elements are factory calibrated at YSI and a calibration code specific to each individual sensing element is determined during the manufacturing process. The calibration code consists of coefficients that are preloaded into the sensor at the factory for increased measurement accuracy. Replacement sensing elements are supplied with their unique calibration codes which can easily be entered into the instrument and probe without the need to return it to the factory. The unique codes and instructions for entering them into the instrument can be found on the instruction sheet provided with the replacement sensing element.



The lifetime of the luminescence is measured by the sensor and compared against a reference.

The amount of oxygen passing through to the sensing layer is inversely proportional to the lifetime of the luminescence in the sensing layer.

Figure 9. Illustration of how a YSI optical sensor measures oxygen.

HOW AN OPTICAL SENSOR MEASURES DISSOLVED OXYGEN

The probe measures dissolved oxygen by emitting a blue light of the proper wavelength that causes the dye in the sensing element to luminesce or glow red. Oxygen dissolved in the sample continually passes through the diffusion layer to the dye layer, affecting the luminescence of the dye both in intensity and lifetime. The YSI sensor measures the lifetime of the dye's luminescence as it is affected by the presence of oxygen with a photodiode (light detector) in the probe and compares that reading to a reference (figure 9).

To increase the accuracy and stability of the measurement, the sensor also emits a red light that is reflected by the dye layer back to the photodiode in the sensor. The sensor measures the reflected light and uses that reading as the reference value for comparison to the previously measured lifetime luminescent value. The lifetime of the luminescence from excitation by the blue light is compared to that of the reference value (red light) and a stable dissolved oxygen concentration is calculated by the probe.

Although the accuracy of an optical sensor's measurement is not dependent on flow, it is dependent on temperature. This temperature dependence

is removed by proprietary algorithms in the system software. As for any oxygen probe, the mg/L concentration is calculated from the sensor's % saturation reading (temperature compensated), temperature, and salinity after the calibration of the system using barometric pressure. The effects of these factors on dissolved oxygen readings are described in the **Measuring Dissolved Oxygen with Either Sensor Type** section of this booklet.

ELECTROCHEMICAL SENSORS

YSI offers three types of field rugged electrochemical sensors: steady-state galvanic, steady-state polarographic, and Rapid Pulse polarographic (figure 3). In addition to several different field probes, YSI offers a BOD-style laboratory polarographic probe with a built in stir bar.

Dr. Leland Clark first invented the Polarographic electrode in 1956 and variations of this electrode are still used by many manufacturers today. Figure 10 shows a picture of a YSI 5739 polarographic sensor which is currently offered by YSI and is similar in design to the original Clark electrode.



Figure 10. Model 5739 polarographic sensor.

YSI ELECTROCHEMICAL INSTRUMENTS

YSI offers numerous instruments that utilize an electrochemical sensor. Most notably are the Professional Plus multiparameter and the Pro20 dissolved oxygen and temperature instruments (figure 11 and 12). These two models are the most versatile YSI handheld dissolved oxygen instruments since they can use either a polarographic or galvanic sensor. Deciding between a polarographic and galvanic sensor depends on the application and user preference. See the **Comparing Steady-state Polarographic and Galvanic Sensors** section to understand the advantages and disadvantages of using one type of sensor over the other.

The Professional Plus and Pro20 as well as other YSI instruments can be equipped with either a field or lab BOD-style sensor (figure 11). This flexibility allows for the convenience and cost-savings of having one instrument for both applications. Additionally, these two instruments utilize a screw-on cap membrane which makes membrane changes simple and easy to perform. The third electrochemical sensor type, the Rapid Pulse sensor, can be used on several of the 6-series multiparameter sondes (figure 13).



Figure 11. The Professional Plus multiparameter instrument with a BOD-style sensor. The Professional Plus can be outfitted with several different single and multi-parameter cables including the Quatro which can measure dissolved oxygen, temperature, conductivity, and two ISEs at the same time and on the same cable.



Figure 12. The Pro 20 as well as the Pro Plus can be outfitted with a polarographic or galvanic sensor.



Figure 13. Rapid Pulse sensor for use on some 6-series sondes.

ELECTROCHEMICAL MEMBRANES

An electrochemical membrane is a thin semi-permeable material, stretched over the sensor that isolates the electrodes from the environment while allowing gases to enter. YSI offers several different types of dissolved oxygen membranes of varying design, thickness and material.

The two types of membrane designs include the traditional, sheet-like stretch membrane that is held in place by an o-ring and the plastic screw-on cap membrane that has the membrane material pre-stretched at the factory.

The membrane's material and thickness affect the sensor's flow dependence and response time. The table in figure 14 shows the various membrane

thicknesses and materials offered by YSI and their corresponding response times, flow dependences, and required flow rates. For tips on how to overcome flow dependence, see the **Taking Measurements** section. The topics of flow dependence and response time are discussed further in the **Comparing Optical and Electrochemical Sensing Technologies** section.

Membrane Comparison Table

Sensor	Membrane Material Color of Cap	Response Time 100 to 0% (T-95)	Flow Dependence after 4 minutes	Required Flow Rate
Optical	Diffusion layer and sensing element	40 seconds*	0 (zero) Non-consumptive	0 (zero)
Steady-state Galvanic or Polarographic	1.0 mil Teflon Black Cap	18 seconds	45%	12 inches per second
Steady-state Galvanic or Polarographic	1.25 mil PE Yellow Cap	8 seconds	25%	6 inches per second
Steady-state Galvanic or Polarographic	2.0 mil PE Blue Cap	17 seconds	18%	3 inches per second

Figure 14. Newer membrane caps are made of polyethylene notated as PE in the table. The response time in this table is listed as T-95 which is the amount of time it takes for the sensor to get to 95% of the desired reading when moved from a 100% saturated sample to a 0 oxygen environment.

*YSI studies have shown that stirring an optical sensor can lower its response time. For example, using a magnetic stirrer or stir bar could result in an optical response time of 22 seconds or less for T-95.

The type of membrane that can be used with a particular instrument is dictated by the sensor type in use and by the instrument's microprocessor. Using an incorrect membrane could result in erroneous readings so care

should be taken when ordering replacement membranes. Refer to figure 15 for a guide to ordering the proper membrane for a specific instrument and sensor. If you have an instrument that can use more than one type of membrane, refer to figure 14 to determine which option is best for your application based on the response times and required flow rates of the membranes available for use on your instrument. For instruments that can use more than one membrane, it should be noted that the instrument must be properly configured for the membrane installed in order to obtain accurate readings.

Membrane Selection Guide

Membrane Model # (Item #)	Type, Thickness, Material	Instrument and probes it is used with
5680 (060745)	Stretch Membrane, 2.0 mil Teflon	Probes: 5719, 5739, and 5750 when used with a model 58 instrument only.
5775 (098094)	Stretch Membrane, 1.0 mil Teflon	Instrument: 55 Probes: 5719, 5739, 5750, and Rapid Pulse
5776 (098095)	Stretch Membrane, 0.5 mil Teflon	Probes: 5719, 5739, and 5750 when used with a model 58 instrument only.
5906 (059880)	Black Cap Membrane, 1.0 mil Teflon	Instrument: 85, 550, 556 MPS, and Pro Plus with a Polarographic sensor. Probes: 5239, 5905 and 5010
5908 (059881)	Yellow Cap Membrane, 1.25 mil Polyethylene (PE)	Instruments: DO200, 550A, and 556 Probes: Pro Series Polarographic sensors

Membrane Model # (Item #)	Type, Thickness, Material	Instrument and probes it is used with
5909 (059882)	Blue Cap Membrane, 2.0 mil Polyethylene (PE)	Instruments: 556 Probes: Pro Series Polarographic sensors
5912 (605912)	Black Cap Membrane, 1.0 mil Teflon	Pro Series Galvanic sensor when used with a Pro Plus instrument only
5913 (605913)	Yellow Cap Membrane, 1.25 mil Polyethylene (PE)	Pro Series Galvanic sensors
5914 (605914)	Blue Cap Membrane, 2.0 mil Polyethylene (PE)	Pro Series Galvanic sensors

Figure 15. Newer membrane caps are made of polyethylene notated as PE in the table.

HOW AN ELECTROCHEMICAL SENSOR MEASURES DISSOLVED OXYGEN

Electrochemical sensors, both polarographic and galvanic, consist of an anode and a cathode that are confined in electrolyte solution by an oxygen permeable membrane. Oxygen molecules that are dissolved in the sample diffuse through the membrane to the sensor at a rate proportional to the pressure difference across it. The oxygen molecules are then reduced at the cathode producing an electrical signal that travels from the cathode to the anode and then to the instrument. Since oxygen is rapidly reduced or consumed at the cathode, it can be assumed that the oxygen pressure under the membrane is zero. Therefore, the amount of oxygen diffusing through the membrane is proportional to the partial pressure of oxygen outside the membrane.

For example, in air or air-saturated water at sea level, the oxygen partial pressure is approximately 160 mmHg (21% of 760 mmHg), while the pressure under the membrane is zero. This difference in oxygen pressures produces the current that is read by the instrument. As the oxygen pressure varies, so does the oxygen diffusion through the membrane which causes

the probe current to change proportionally. Figure 16 is a dissection of a Clark electrode and illustrates how an electrochemical sensor, either polarographic or galvanic, works.

It is important to recognize that oxygen dissolved in the sample is consumed during the measurement with a steady-state electrochemical sensor. This results in a measurement that is dependent on flow. It is therefore essential that the sample be continuously stirred at the sensor tip. If stagnation occurs, the readings will be artificially low. The flow dependence and therefore the rate of stirring required for an accurate measurement varies by membrane, see figure 14. The topics of flow dependence and response time are discussed further in the **Comparing Optical and Electrochemical Sensing Technologies** section. For tips on how to overcome flow dependence, see the **Taking Measurements** section.

Electrochemical dissolved oxygen measurements are also affected by barometric pressure and the temperature and salinity of the sample. These three factors and how they affect dissolved oxygen readings are described in detail in **Measuring Dissolved Oxygen with Either Sensor Type** section.

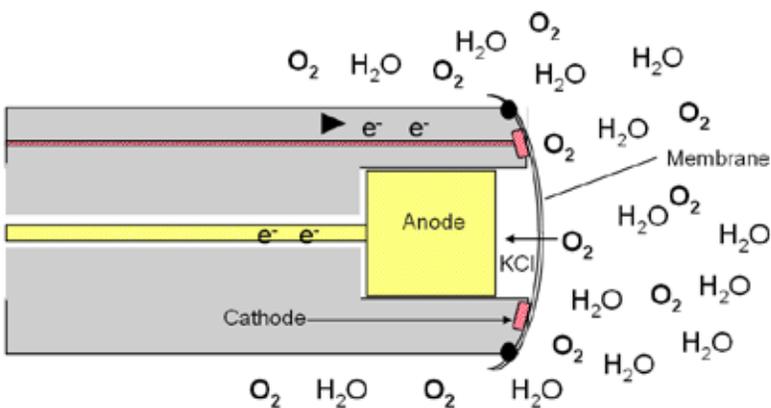


Figure 16. An illustration of an electrochemical sensor.

Steady-State Polarographic Sensors

In a polarographic sensor, the cathode is gold and the anode is silver. The system is completed by a circuit in the instrument that applies a constant voltage of 0.8 volts to the probe, which polarizes the two electrodes, and a meter to read the dissolved oxygen response from the sensor.

The electrolyte held under the membrane allows the electrical signal to travel from the cathode to the anode. The signal continues down to the meter as shown by the basic circuit diagram in figure 17. The polarographic sensor operates by detecting a change in this current caused by the variable pressure of oxygen while the potential is held constant at 0.8 V. The more oxygen passing through the membrane and being reduced at the cathode, the greater the electrical signal (current) read by the probe. As oxygen increases, the signal increases and, conversely, as oxygen decreases, the signal decreases. Chemically, this is described as the oxidation of the silver and reduction of oxygen at the gold cathode as follows:



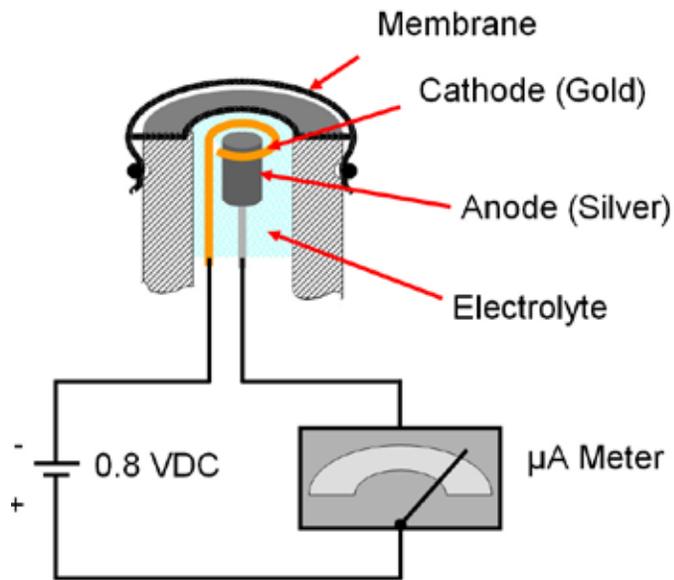


Figure 17. A simplified diagram of a polarographic sensor and circuit.

Steady-State Galvanic Sensors

In the YSI galvanic sensor, the cathode is silver and the anode is zinc. The anode may be a different material, such as lead, in different manufacturers' sensors. Figure 18 is an illustration of a galvanic sensor.

A circuit completes the measurement, but, unlike the polarographic sensor, the galvanic sensor does not have or need a constant voltage applied to it. In the Galvanic sensor, the electrodes are dissimilar enough to self-polarize and reduce oxygen molecules without an applied voltage. It is similar in function to a battery.

A galvanic dissolved oxygen system uses a meter to read the electrical signal coming back from the probe and this signal is proportional to the amount of oxygen passing through the membrane. Oxygen passing through the membrane and being reduced at the cathode increases the electrical signal (current) read by the probe. As oxygen increases, the signal increases and as oxygen decreases, the signal decreases. Chemically, this is described as the oxidation of the zinc and reduction of oxygen at the silver cathode as follows:

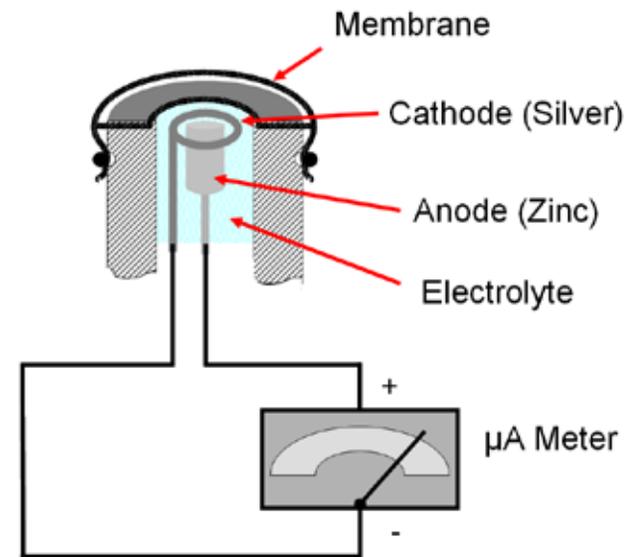
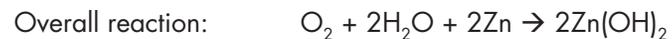
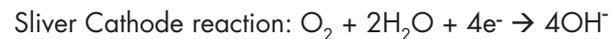


Figure 18. A simplified diagram of a galvanic sensor and circuit.

Rapid Pulse Polarographic Sensors

The third type of electrochemical sensor offered by YSI is the patented Rapid Pulse polarographic sensor. This sensor is like the steady-state polarographic sensor in that it has a gold cathode, silver anode and utilizes electrolyte solution that is held in place by an oxygen permeable membrane. Also, similar to the traditional polarographic sensor, a voltage of 1.0 V is applied to the electrodes and oxygen passing through the membrane is reduced at the cathode. This reduction causes a change in the electrical signal read by the instrument. The amount of oxygen passing through the membrane and being reduced at the cathode is directly proportional to the electrical signal sent back to the instrument.

The Rapid Pulse sensor differs from a steady-state sensor in that it pulses on and off during the measurement allowing the oxygen to replenish at the membrane surface. The voltage is only applied for 40 milliseconds; it is then turned off for 3960 milliseconds for a total measurement cycle of 4 seconds. This results in a sensor with almost zero flow dependence and therefore does not require stirring or sample movement in order to obtain accurate dissolved oxygen readings. It also uses digital electronics for processing data as opposed to analog electronics like the two steady-state electrochemical sensors previously discussed.

The Rapid Pulse sensor is also unique in the fact that it uses a third electrode, see figure 19. The third electrode is a silver reference electrode that is separate from the silver anode as opposed to the steady-state polarographic and galvanic sensors where the reference electrodes and anodes are combined. The three electrode configuration is required to polarize and de-polarize the electrodes consistently and for a very short period of time, the feature that makes the Rapid Pulse sensor function as a flow independent sensor.

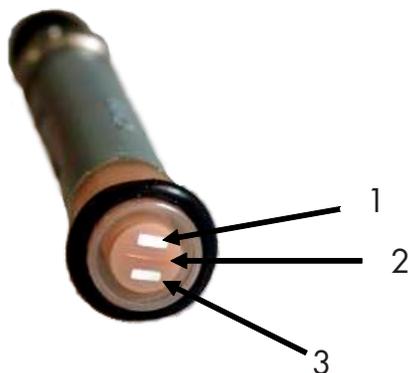


Figure 19. Illustrating the 3 electrodes of a Rapid Pulse Sensor.

ADVANCEMENTS IN STEADY-STATE ELECTROCHEMICAL SENSORS

The design of the electrochemical sensor has undergone many enhancements over the years. The first YSI advancement in electrochemical sensors was the redesign of the electrodes and membrane so that the membrane could be pre-stretched into a plastic cap at the factory for easier replacement

by the user. Most YSI electrochemical sensors manufactured today utilize this screw-on membrane cap design. Since the cap has a membrane that is pre-stretched at the factory, there is no need to worry about stretching the membrane too much or not enough when installing it on a sensor. Further, the cap is filled with electrolyte and then screwed on to the sensor as opposed to filling the sensor and then stretching the membrane over the solution. This design minimizes the chances of an air bubble getting trapped underneath the membrane which could result in inaccurate readings. See the **Probe Care and Maintenance Section** for more information on changing a membrane.

The next advancement in the steady-state sensor was the release of polyethylene membranes in 2002. This new membrane material lowered the stirring dependence and reduced the response time of a sensor when compared to traditional Teflon membranes. This resulted in a faster, more accurate dissolved oxygen measurement.

Additionally, the dissolved oxygen sensor and cable are now manufactured to be detachable so the sensor can be replaced without affecting the cable, lowering the overall total cost of ownership. Further, no tools are required to change sensors on YSI's newest instruments, the Professional Plus and Pro20, making the sensors truly field replaceable. Newer instruments also include sensor diagnostics and error messages to help the user determine when to change a membrane or recondition the electrodes, see the **GLP (Good Laboratory Practices) File** section for more information on sensor diagnostics.

COMPARING STEADY-STATE POLAROGRAPHIC AND GALVANIC SENSORS

In terms of physical configuration and general performance, YSI galvanic dissolved oxygen sensors are very similar to the polarographic sensors which YSI pioneered and has sold for 40 years. The main advantage of using galvanic sensors is convenience. Galvanic sensors provide an instant-on sensor without the need for a warm-up time but this factor also adversely affects the life of the sensor. Polarographic sensors last longer and have a longer warranty but require a 5-15 minute warm-up time before use or calibration. This section describes the similarities and differences between these two technologies. These points are also summarized in figure 20.

Similarities

First, both the polarographic and galvanic sensors are 'steady-state'. This means that when the instrument is turned on in the instance of a polarographic sensor or, in the case of a galvanic sensor without the instrument being turned on, the electrodes are continuously polarized or in 'steady-state'.

Second, both sensor types reduce or consume oxygen at the cathode and are therefore dependent on flow across the sensor tip. Flow dependence is caused by the reduction of oxygen at the cathode which depletes oxygen at the membrane surface in the sample. The oxygen depletion at the membrane surface results in the need for sample movement across the membrane in order to get accurate dissolved oxygen measurements. If enough sample movement is not supplied by the natural flow of the medium, manual agitation of the sensor by the user, a mechanical stirrer, or some combination of these factors, then the sensor will continue to deplete oxygen at the membrane surface which will result in artificially low DO readings. The flow dependence is dictated by the membrane in use. The flow dependence and required flow for each type of membrane is listed in figure 14. The topics of flow dependence and response time are discussed further in the **Comparing Optical and Electrochemical Sensing Technologies** section. Tips on how to overcome flow dependence to obtain accurate readings can be found in the **Taking Measurements** section.

Lastly, both sensors utilize electrolyte solution that allows an electrical current to flow between the cathode and anode and is held in place by an oxygen permeable membrane.

Differences

A major difference between polarographic and galvanic sensors is that the galvanic sensor does not require a polarizing voltage to be applied in order to reduce the oxygen that has passed through the membrane. Rather, the electrode materials for the galvanic sensor have been chosen so that their electrode potentials are dissimilar enough to reduce oxygen without an applied voltage. Thus, the cathode and anode of the galvanic sensor are made from silver and zinc (a highly reducing metal), respectively, while the cathode and anode for the polarographic sensor are gold and silver (a much less reducing metal), respectively.

Given that the galvanic sensor does not require an external voltage for polarization, it therefore does not require the "warm-up" polarization time that the polarographic sensor requires. Since a galvanic sensor doesn't require a "warm-up" time, the instrument is ready to measure when it is powered on and, therefore, users are not required to wait to calibrate or to take readings when using a galvanic sensor. Conversely, when an instrument with a polarographic sensor is turned on, the polarizing potential is applied to the sensor and a substantial "warm-up" period is required before the sensor is stable enough for calibration and measurements. The polarization warm-up time for a polarographic sensor is between 5-15 minutes depending on the age and condition of the electrodes. Thus, there is an advantage of galvanic sensors over polarographic sensors in terms of convenience of use.

Another difference between the two sensor types is in the oxidation of their anodes. To balance the reduction reaction associated with oxygen at the cathode, there is a corresponding oxidation reaction at the anode which converts the zinc to zinc hydroxide (for galvanic sensors) or the silver to silver chloride (for polarographic sensors). The practical difference between the two types of anode oxidations is that the silver chloride remains attached to the anode in a polarographic sensor while much of the zinc hydroxide detaches from the anode in a galvanic sensor and forms a suspension of white solid in the electrolyte. Thus, visual inspection of the electrolyte at the tip of a galvanic sensor is likely to show the presence of a white solid while the electrolyte for a polarographic sensor will be clear. The amount of solid which is visible in the electrolyte for a galvanic sensor and attached to the anode for a polarographic sensor is directly proportional to the total current from the reduction of oxygen. Eventually, the formation of oxidized anode material, either on the anode for polarographic sensors or in the electrolyte for galvanic sensors, will cause degradation of sensor performance. This degradation usually is indicated by low sensor output and/or slightly jumpy readings. When these symptoms occur, the user must remove the old membrane, clean the sensor anode if necessary, and install a new membrane and electrolyte to restore proper performance. The presence of the solid in galvanic sensors is likely to require a somewhat more frequent membrane/electrolyte replacement than for polarographic sensors due to degradation of overall system performance, giving an advantage to polarographic sensors in this area. However it should be

noted that (a) YSI has demonstrated 2-3 months of good galvanic sensor performance even when there is considerable solid present in the electrolyte and (b) the membrane/electrolyte replacement is very simple and relatively inexpensive. Thus, the polarographic advantage is likely to be minor. See **Probe Care and Maintenance** for more information on routine maintenance procedures for electrochemical sensors and the recommended intervals for performing these actions.

The last main difference between the two sensors is their theoretical sensor life. Because the galvanic sensor's electrodes are continuously polarized, it is always 'on' even when the instrument is turned off. This results in a degradation of the consumable anode which could result in a theoretically shorter sensor life. Since the Polarographic sensors are not self-polarizing, the sensor is off when the instrument is off and, therefore, the consumable anode is not being continuously degraded which results in a longer theoretical sensor life. Therefore, theoretically, the polarographic sensor may have some advantage over galvanic in overall sensor longevity. In practical terms, the anode of a YSI galvanic sensor should last for several years so the advantage is minor; however, it should be noted that a YSI polarographic sensor is warranted for 1 year while a YSI galvanic sensor is warranted for 6 months.

Steady-State Polarographic versus Steady-State Galvanic Sensors

Similarities:

- Both sensors are Steady-state.
- Both reduce oxygen, thus they require stirring or sample movement for accurate readings.
- Both use electrolyte that is held in place by an oxygen-permeable membrane.

Steady-State Polarographic versus Steady-State Galvanic Sensors

Differences:

- Galvanic sensors do not require a warm-up time and are ready for immediate use after being powered on.
- Polarographic sensors require warm up period before use.
- Galvanic sensors continually consume the anode, even when the instrument is off. Thus, they have a shorter theoretical sensor life and are warranted for 6 months
- The consumption of polarographic sensors stops when the instrument is turned off. Thus, they have a longer theoretical sensor life and are warranted for 1 year.

Figure 20.

COMPARING OPTICAL AND ELECTROCHEMICAL SENSING TECHNOLOGIES

There are many advantages and disadvantages associated with each sensing technology and these should be considered when selecting an instrument. This section describes how the two technologies compare in accuracy, approval for compliance purposes, and general use.

MEASUREMENT ACCURACY

Electrochemical and optical dissolved oxygen sensing technologies produce nearly identical results across a wide measurement range as long as you provide enough sample movement when using a steady state electrochemical sensor.

Refer to the graphs in figure 21, the top graph represents dissolved oxygen measurements from a stirred steady state electrochemical sensor at six different dissolved oxygen concentrations. The bottom graph represents measurements of the same samples taken with a lifetime optical sensor. Each concentration was measured by each sensor seven times. As shown by the data in the graphs, the two technologies measured dissolved oxygen equivalently across the wide measurement range.

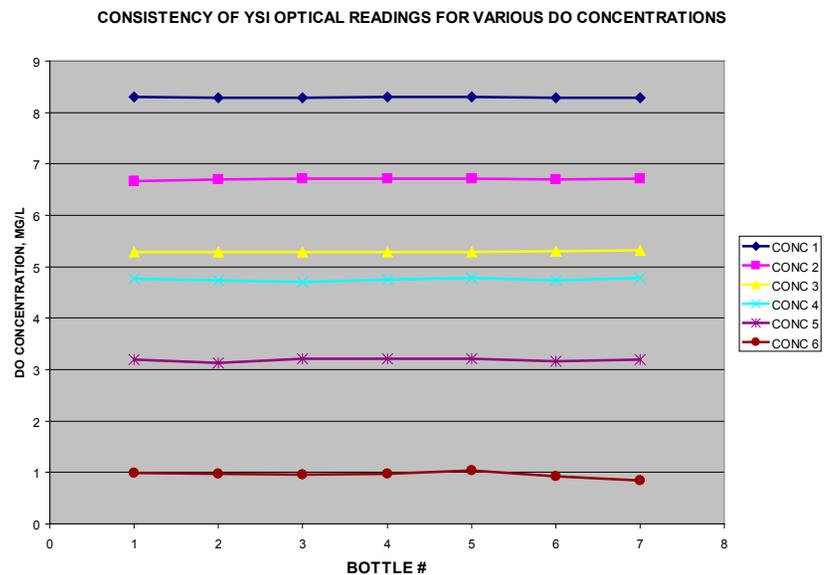
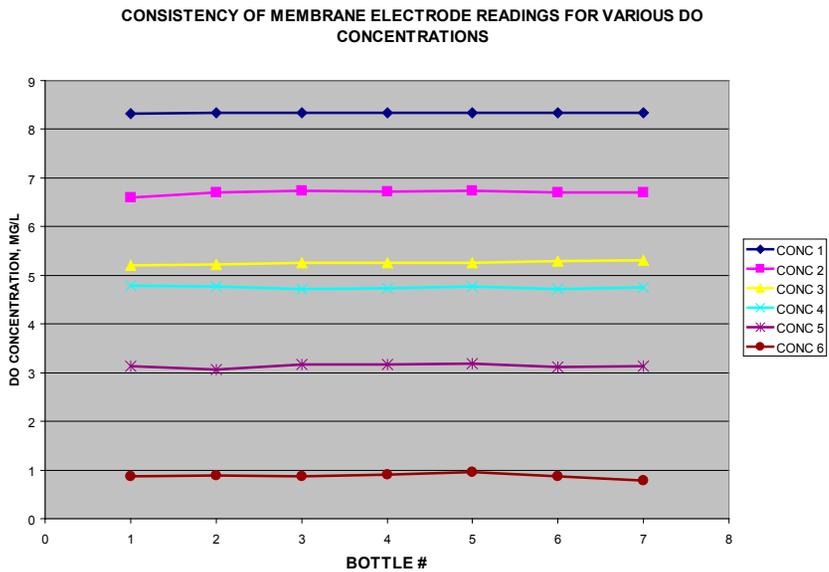


Figure 21. Studies were performed by YSI Scientists for the Standard Methods Review Panel.

Figure 22 further illustrates that the two sensor technologies perform equivalently. In this example, a ROX optical sensor and a Teflon covered Rapid Pulse polarographic sensor were deployed continuously for 52 days. Since the Rapid Pulse sensor was used, stirring or sample movement was not required. Both sensors were equipped with wipers to minimize the effects of bio-fouling. The data between the two sensors (blue circles for the Rapid Pulse sensor and red circles for the ROX sensor) tracked consistently with each other even during a low DO event. A manual spot check of DO was periodically taken during the test and is represented on the graph with black triangles. There are a few apparent discrepancies for the manual readings, which could be the result of insufficient sample movement or improper calibration of the instrument used to take the manual reading. In general, the three types of measurements track well.

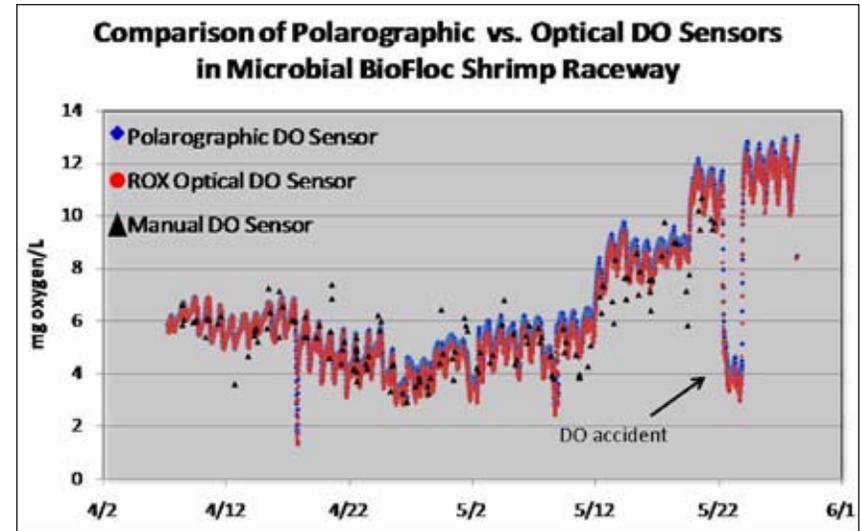


Figure 22. Source: South Carolina Department of Natural Resources – Waddell Mariculture Center; Research funded by National Institute of Standards and Technology¹.

Even though the two technologies perform equivalently in the field, optical sensors are slightly more accurate than electrochemical sensors under controlled conditions in the 0-20 mg/L measurement range as is shown in YSI's published specifications, see figure 23.

Optical	<i>Range</i>	<i>Resolution</i>	<i>Accuracy</i>
% DO Saturation	0-500%	0.1%	<u>0 - 200%: ±1%</u> of reading or 1% Saturation, whichever is greater. <u>200 - 500%: ±15%</u> of reading.
mg/L	0-50 mg/L	0.01 mg/L	<u>0 - 20 mg/L: ±0.1 mg/L</u> or 1% of reading, whichever is greater. <u>20 - 50 mg/L: ±15%</u> of reading.
Electrochemical	<i>Range</i>	<i>Resolution</i>	<i>Accuracy</i>
% DO Saturation	0-500%	0.1%	<u>0 - 200%: ±2%</u> of reading or 2% Saturation, whichever is greater. <u>200 - 500%: ±6%</u> of reading.
mg/L	0-50 mg/L	0.01 mg/L	<u>0 - 20 mg/L: ±0.2 mg/L</u> or 2% of reading, whichever is greater. <u>20 - 50 mg/L: ±6%</u> of reading

Figure 23.

APPROVED METHODOLOGY

At the time of publication, the optical sensing method is not nationally approved by the United States Environmental Protection Agency (US EPA) for compliance monitoring and reporting. It has gained interim approval in many EPA regions, but you must contact your Regional EPA ATP (Alternative

Test Protocol) Coordinator for individual approval. Submitting an application to your Regional ATP Coordinator is typically all that is required but, in some cases, you may have to submit data in order to get approval to use this method for compliance reporting. ASTM International has approved the optical method for measuring dissolved oxygen in water, method D888-05, but it has not yet been approved by Standard Methods for the Examination of Water and Wastewater. A Standard Method will likely be adopted in the near future.

The electrochemical method has been used for decades and is therefore a proven technology. It is approved for monitoring and compliance reporting by the US EPA and can be used without contacting a regional US EPA office. This factor gives it a slight advantage over the optical method.

RESPONSE TIME

The response time of a sensor should be considered when selecting an instrument since this feature will dictate the amount of time required to conduct a sampling session. A faster responding sensor could save time and therefore money if used instead of a slower responding sensor.

The table in figure 24 shows the typical response times of optical and steady-state electrochemical sensors. The table further illustrates the response time of an electrochemical sensor when used with various types of membranes. The response time is expressed as T-95 which is the length of time it takes to reach 95% of the final (true) reading when a sensor is transferred from a fully saturated sample to a zero oxygen environment. As shown, an optical sensor's response time is about twice as long as a Teflon covered electrochemical sensor and five times as long as a polyethylene (PE) covered electrochemical sensor. The varying sensor response times are also demonstrated in the graph in figure 25 where you can see the polyethylene covered electrochemical sensor responded much quicker than the other two sensors.

Although 40 seconds is a relatively short period of time to take one reading, the table in figure 26 illustrates how much longer it would take to measure dissolved oxygen in 500 samples with an optical sensor versus a polyethylene or Teflon covered electrochemical sensor. If using a polyethylene covered

sensor, 500 samples could be measured in about 1 hour where it would take over 5 hours to measure 500 samples with an unstirred optical sensor. It should be noted that YSI studies have shown that stirring an optical sensor can lower its response time. For example, using a magnetic stirrer or stir bar could result in an optical response time of 22 seconds or less for T-95. Therefore, reading 500 samples with a stirred optical BOD sensor (OBOD) could take about 3 hours to perform.

Sensor Type	Membrane Material	Response Time 100 to 0% (T-95)
Optical	Sensing element with diffusion layer	40 seconds**
Galvanic or Polarographic	1.0 mil Teflon Black cap	18 seconds
Galvanic or Polarographic	1.25 mil PE Yellow Cap	8 seconds
Galvanic or Polarographic	2.0 mil PE Blue Cap	17 seconds

Figure 24. A table defining the measurement response time of various sensors and membranes.

** YSI studies have shown that stirring an optical sensor can lower its response time. For example, using a magnetic stirrer or stir bar could result in an optical response time of 22 seconds or less for T-95.

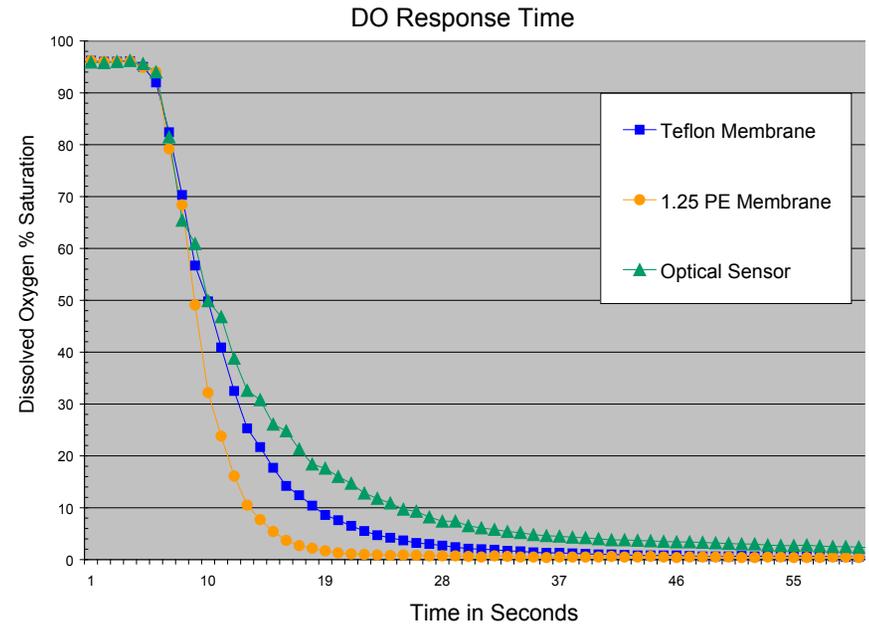


Figure 25. A graph displaying DO measurements over time for three sensors that were transferred from 100% saturated water to a zero oxygen environment.

Sensor Type	Number of Samples	T-95 Response in seconds	Total Time in hours
Polarographic with 1.0 mil Teflon membrane	500	18	2.5
Polarographic with 1.25 mil PE membrane	500	8	1.1
Optical	500	40	5.6

Figure 26. The data in the table demonstrates the amount of time it would take to measure 500 samples with three different sensors.

FLOW DEPENDENCE

As mentioned, steady-state electrochemical sensors consume oxygen during measurement and therefore require sample movement or the readings will be artificially low. This is commonly referred to as flow dependence since the sensor is dependent on flow or water movement across the membrane in order to obtain accurate readings. Optical sensors, however, use a non-consumptive method for dissolved oxygen measurements resulting in a sensing method with zero flow dependence or stirring requirement.

The graphs in figure 27 and 28 illustrate this advantage of the optical sensor. Figure 27 is a graph of data measured with a steady-state polarographic sensor in an air-saturated water sample where adequate sample movement was provided by a mechanical stir bar. When the stirring mechanism was turned off, the readings began to fall resulting in artificially low dissolved oxygen measurements. Figure 28 is a graph of data measured with an optical sensor in the same air-saturated water sample where sample movement was provided by a stir bar. When the stirring mechanism was turned off for the optical measurements, the readings remained constant and accurate proving the optical sensor is not dependent on flow. This is a considerable advantage of the optical sensor especially for low flow applications or applications where probe stirring or sample movement is difficult such as down well.

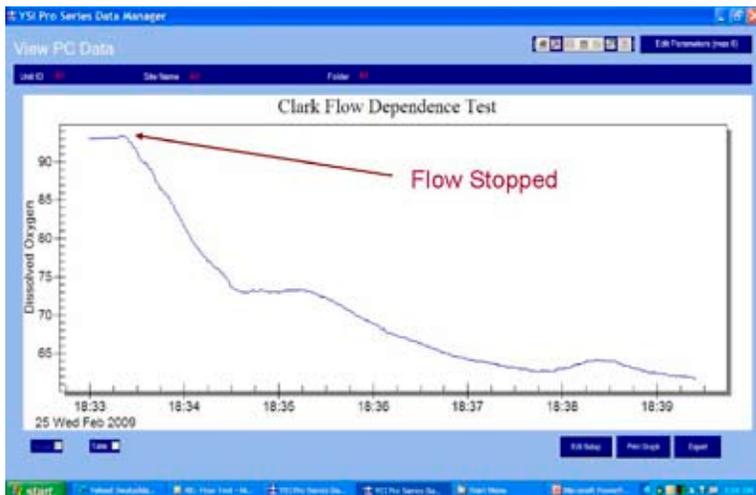


Figure 27. A graph, created in YSI's Data Manager software program, illustrating the flow dependence of a steady state electrochemical sensor.

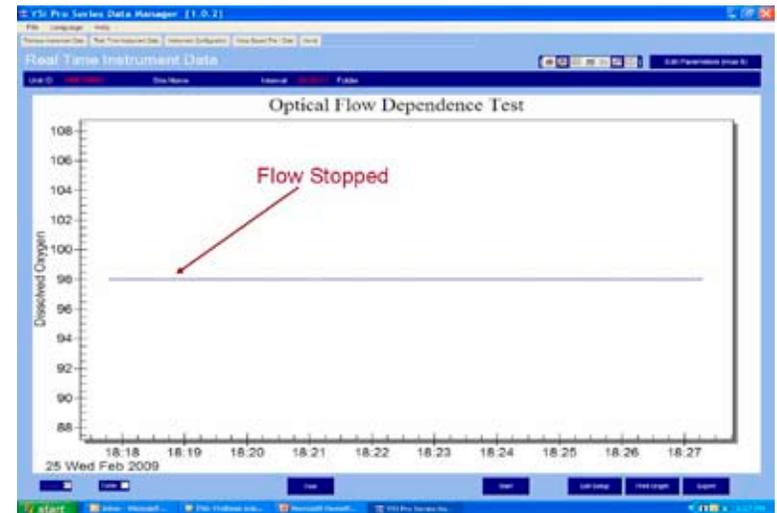


Figure 28. A graph, created in YSI Data Manager software program, illustrating the flow independence of an optical sensor.

For steady-state electrochemical sensors, the membrane material and thickness dictates the degree of the sensor's flow dependence. For example, polyethylene membranes, frequently notated as PE, require less movement or flow than traditional Teflon membranes as illustrated by the graph in figure 29. In this graph, three different sensors were placed in fully aerated water with a stir bar. Once the stirring was ceased, the steady-state electrochemical readings began to fall. Notice how the Teflon covered sensor fell further and more rapidly than the PE covered sensor. The stirring dependence of each sensor and membrane type, along with the recommended stirring rates, is listed in figure 14. For tips on how to overcome flow dependence, see **Taking Measurements**.

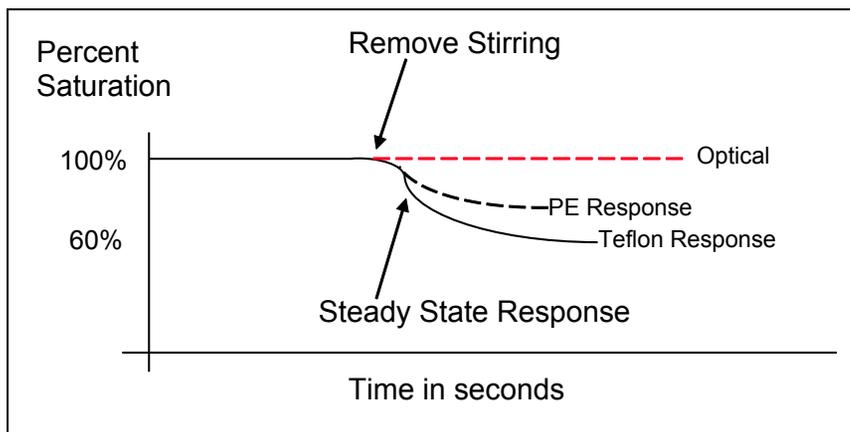


Figure 29. Flow dependence comparison graph.

WARM-UP TIME

A key advantage of the optical sensor for sampling applications is that it does not have a warm up time since there are no electrodes to polarize. So, similar to a galvanic sensor, an optical sensor can be calibrated and used immediately after being turned on. A polarographic sensor, however, requires 5-15 minutes for the electrodes to polarize before a calibration or measurement can be performed.

CALIBRATION FREQUENCY

The optical measurement method experiences very little calibration drift when compared to electrochemical sensors and this advantage results in less frequent calibrations. In fact, optical sensors are so stable they are capable of holding their calibration for many months, although YSI recommends verifying the calibration regularly to ensure the highest data accuracy. Steady-state electrochemical sensors require a calibration each day they are used in a spot sampling application. Although this can be considered a disadvantage of the electrochemical method, it must be noted that a dissolved oxygen calibration is simple and easy to perform. See the **Calibration** section for additional information.

MEASUREMENT INTERFERENCES

Some gases, including hydrogen sulfide, can permeate the membrane of an electrochemical sensor interfering with the electrodes and the readings. A list of these gases can be found in the **Measurement Precautions and Interference** section. Optical sensors, on the other hand, are unaffected by the presence of hydrogen sulfide and other gasses that interfere with the traditional electrochemical method. This gives the optical sensor the ability to sample in environments with hydrogen sulfide like wastewater, lake bottoms and wetlands.

MAINTENANCE REQUIREMENTS

The optical sensor requires much less maintenance than traditional electrochemical sensors. With the optical sensor, the sensing element only needs to be replaced about once per year where an electrochemical sensor usually requires a membrane change every 2-8 weeks. Further, there is no need to clean or resurface electrodes in an optical sensor as is required when using an electrochemical sensor. For more information on performing routine maintenance tasks, see the **Probe Care and Maintenance** section.

POWER CONSUMPTION

Optical sensors have a higher power consumption rate than the electrochemical sensors. This results in a shorter battery life which could be a disadvantage for the optical sensor in some sampling applications.

SUMMARY

As you can see, there are several advantages and disadvantages associated with each sensing method and these factors as well as some additional points have been summarized in figure 30. It is important to analyze your specific application needs to help determine which sensor is best for your specific application. See the figure 31 for a table that outlines the best suited applications for each sensing method.

Electrochemical Sensors	
<i>Advantages</i>	<i>Disadvantages</i>
Proven technology. Approved by the US EPA for compliance monitoring and reporting.	Steady-state sensors are flow dependent and therefore require sample movement for accurate readings.
Faster measurement response time than an optical sensor.	Requires more frequent calibrations than an optical sensor.
Lower power consumption than an optical sensor.	The measurement is susceptible to interferences from gases such as hydrogen sulfide.
Lower initial acquisition cost than an optical sensor.	The electrodes require periodic maintenance.
	The membrane requires regular changes.

Optical	
<i>Advantages</i>	<i>Disadvantages</i>
Instant on – no warm up time required.	Not approved by the US EPA for compliance monitoring and reporting.
Exhibits very little calibration drift and can hold a calibration for several months.	Higher initial acquisition cost.
Not susceptible to interferences like hydrogen sulfide.	Slower measurement response time than traditional electrochemical sensors.
Non consumptive method – no need to stir or provide sample movement.	Higher power consumption than traditional electrochemical sensors.
Less maintenance than traditional electrochemical sensors.	

Figure 30. Summary of advantages and disadvantages associated with each sensor type.

Best Suited Application for each Dissolved Oxygen Sensor Type		
<i>Sensor Type</i>	<i>Membrane Material</i>	<i>Best Application (in general)</i>
Optical	Sensing element with diffusion layer	<ul style="list-style-type: none"> • Deep water profiling • Shallow water where stirring is difficult • Low flow applications • Samples that are small in volume • Samples with hydrogen sulfide • Continuous long-term deployments with the ROX sensor
Galvanic or Polarographic	1.25 mil PE	Sampling applications that require the fastest response time
Galvanic or Polarographic	2.0 mil PE	Sampling applications that require a lower flow dependence

Figure 31. Best suited application per sensor type.

MEASURING DISSOLVED OXYGEN WITH EITHER SENSOR TYPE

Dissolved oxygen sensors, both electrochemical and optical, do not measure the concentration of dissolved oxygen in mg/L or ppm (parts per million which is equivalent to mg/L). Instead, the sensors measure the pressure of oxygen that is dissolved in the sample. To simplify the readings displayed by an instrument, the pressure of the dissolved oxygen is expressed as DO % Saturation. The instrument converts the dissolved oxygen pressure value from the sensor to % Saturation by dividing the sensor output in mmHg by 160*** (the pressure of oxygen in air at 760 mmHg) and then multiplying

by 100. Thus, a measured oxygen pressure of 150 mmHg would be displayed by a YSI instrument as 93.8 % Saturation ($150/160 * 100$).

The fact that the sensor measures oxygen pressure and not dissolved oxygen concentration is known to be true because a sample of fresh water can dissolve more oxygen than a sample of sea water at the same temperature and at the same altitude (or under the same barometric pressure); however, the sensor's output signal is identical in both samples since the oxygen pressure is identical in both media. See figure 32 for an example of this concept.

***The pressure of oxygen at sea level is 160 mmHg because oxygen is about 21% of the earth's atmosphere and 21% of 760 (average sea level barometric pressure) is about 160 mmHg.

DO Sensors Measure % Saturation

If two samples, one of fresh water and one of sea water, are fully saturated with oxygen the dissolved oxygen concentration will be:

Fresh water at 25°C = 8.26 mg/L

Sea water (36 ppt) at 25°C = 6.27 mg/L

However, the signal output from either sensor type will be identical in the two samples. Since both are 100% saturated, % saturation (or oxygen pressure) is what both sensors are measuring.

Figure 32. DO sensors measure % saturation.

VARIABLES THAT AFFECT DISSOLVED OXYGEN MEASUREMENTS

There are several factors that affect the measurement of dissolved oxygen. These variables include temperature, salinity, flow or stirring dependence, and barometric pressure.

Temperature and salinity are compensated for during instrument calibration

and field use with the use of additional sensors and/or instrument software settings. The effect of flow dependence has been overcome by advances in measurement technologies. Newer, optical dissolved oxygen sensors have no flow dependence. For electrochemical based sensors, users need to provide sample movement to overcome flow dependence or the measurements could be artificially low. The stirring dependence and recommended stirring rates of each sensor and membrane type are listed in figure 14. For tips on how to overcome flow dependence, see the **Taking Measurements** section.

Barometric pressure primarily affects the calibration of dissolved oxygen sensors as it defines the pressure of oxygen in the calibration environment. Generally, there is no need to be concerned about changes in barometric pressure that take place after calibration, except if reporting DO% Local with a YSI 556 or 650 as explained in the **Barometer** section.

TEMPERATURE

The most significant variable for dissolved oxygen measurements is temperature; therefore, it is important to ensure the temperature sensor on the instrument is measuring accurately. Temperature affects DO measurements in two ways.

First, due to the increase or decrease in molecular activity, diffusion of oxygen through the membrane of an electrochemical probe or sensing element of an optical probe changes with temperature. The change in diffusion rate based on temperature can be up to approximately 4% per degree Celsius depending on the membrane material for steady-state electrochemical sensors, 1% per degree Celsius for Rapid Pulse sensors, and is approximately 1.5% per degree Celsius for optical sensors. For example, if the temperature of a sample changes from 20°C to 15°C, the probe signal would decrease by varying rates depending on the sensor in use, giving a lower DO % saturation reading even though the % saturation of the water has not changed. Therefore, the sensor signal must be compensated for changes in temperature. This is done by adding a thermistor to the circuit of older, analog instruments. For newer, digital instruments, the software compensates for temperature changes with proprietary algorithms that use the temperature readings from the probe's thermistor.

The adjustment described so far only compensates for temperature's effect on the oxygen diffusion rate through a membrane or sensing element. In addition to this effect, temperature also affects the ability of water to dissolve oxygen. It is a scientific fact that the solubility of oxygen in water is directly proportional to temperature; see Oxygen Solubility Table in **Appendix A**. Warmer water cannot dissolve as much oxygen as colder water. For example, in an oxygen saturated sample of water at sea level (exposed to 760 mmHg of barometric pressure), the % saturation value will be 100% regardless of the temperature because it is fully saturated. However, the dissolved oxygen mg/L concentration will change with temperature because the solubility of oxygen in water changes with temperature. For instance, at 15 °C water can dissolve 10.08 mg/L while 30 °C water can only dissolve 7.56 mg/L of oxygen even though the % saturation value is 100% in both samples. Therefore, we must compensate the mg/L concentration reading per the temperature of the sample.

Both of these temperature effects are factored into the conversion of the probe signal to a mg/L concentration. For newer, digital instruments, the software compensates for both of these temperature-related factors after instrument calibration and during readings. The temperature compensation for the % saturation reading is empirically derived, while the conversion from % saturation, temperature and salinity to a mg/L concentration is automatically carried out by the instrument's firmware using formulae available in Standard Methods for the Examination of Water and Wastewater². The calculation for converting % Saturation to mg/L and an example is provided in figure 33.

SALINITY

The second variable that affects DO concentration is the salinity of the water sample. While the % saturation reading is not a function of the salinity (or dissolved solids content) of the water, the mg/L concentration changes significantly with salinity. As the salinity of water increases, its ability to dissolve oxygen decreases. For example, oxygen saturated freshwater with 0 salinity at 25°C contains 8.26 mg/L of oxygen while oxygen saturated sea water (36 ppt) at the same pressure and temperature contains only 6.72 mg/L of dissolved oxygen.

Thus, salinity (along with temperature) must be factored into the instrument's calculation of mg/L. This calculation is based on the % saturation reading, temperature reading, and the measured or entered salinity value using formulae found in Standard Methods for the Examination of Water and Wastewater². The calculation for converting % to mg/L and an example is provided in figure 33.

Determining DO mg/L from % Saturation

The following explains how to convert % Saturation to mg/L (also referred to as ppm).

In order to perform this conversion, the temperature and salinity of the sample must be known.

Step one: Determine the % saturation, temperature, and salinity of the sample.

Step two: Multiply the % saturation reading by the value in appropriate column (depends on salinity) and row (depends on temperature) of the Oxygen Solubility Table in Appendix A.

Example:

Step one: Sample is measured to have: 80% DO saturation
0 salinity
20° C

Step two: Multiply .80 (which is the DO %) by 9.09 (value from oxygen solubility table at 0 salinity and 20° C) = 7.27 mg/L.

Result: 7.27 is the mg/L value that corresponds to an 80% DO-Saturation reading of a sample with zero salinity at 20° C.

Figure 33. Calculating mg/L from % saturation, temperature and salinity.

CORRECTING FOR SALINITY

The salinity value used by the instrument in the calculation of mg/L is obtained one of two ways, depending on the instrument being used. For YSI dissolved oxygen instruments that also measure conductivity, the salinity value measured by the conductivity sensor is used for the mg/L calculation. Therefore, it is important to ensure the conductivity sensor is calibrated and reading accurately in order to obtain accurate DO mg/L readings.

For YSI dissolved oxygen instruments that do not have a conductivity sensor, the salinity value of the sample must be manually entered by the end user. See the salinity guide in figure 34 for a list of typical salinity values for various types of water.

Salinity Guide - Average salinity by water type³	
<u>Water Type</u>	<u>Average Salinity</u>
Fresh water	<0.5 ppt****
Brackish water	0.5 to 30 ppt
Sea water	33 to 37 ppt
Saline water	30 to 50 ppt
Brine	> 50 ppt

Figure 34. Average salinity of different types of water.

****Salinity is a unitless measurement determined from conductivity and temperature readings according to the Practical Salinity Scale which can be found in Standard Methods for the Examination of Water and Wastewater². Historically, salinity values determined via the Practical Salinity Scale were given the designation “ppt” because these values were very close to those determined by the previously used method where the mass of dissolved salts in a given mass of water (parts per thousand) were reported. Today, ppt is commonly replaced by psu (Practical Salinity Units) as the preferred unit to describe salinity calculated by the Practical Salinity Scale; however, these values are equivalent since they are determined by the same method.

When sampling water of varying salinity, for example in brackish waters such as estuaries or coastal wetlands, it is recommended that you use a

dissolved oxygen instrument that also measures conductivity for highest data accuracy. A dissolved oxygen instrument that also has a conductivity sensor will use the real-time salinity readings from the conductivity sensor for every mg/L calculation. This will make sampling easier since it will not be necessary to manually change the correction factor at each sampling new site.

BAROMETRIC PRESSURE

The final factor with regard to dissolved oxygen calibration and measurement is barometric pressure. Barometric pressure affects the pressure of oxygen in a sample of air or water. For example, the percentage of oxygen in air is always 21%, but the actual pressure of oxygen varies with changes in barometric pressure. At sea level, the pressure of oxygen is 160 mmHg (.21 x 760 mmHg). In a fully aerated sample, under these conditions, the % saturation measured by a sensor would be 100% (160/160 x 100%, see Measuring Dissolved Oxygen with Either Sensor Type). If the temperature of the sample is 25 °C, the instrument would calculate the dissolved oxygen concentration as 8.26 mg/L based on the Oxygen Solubility table. As the sample is moved up in altitude and kept air-saturated, the barometric pressure would decrease and so would the pressure of oxygen in the sample. At 1126 ft of elevation, the pressure of oxygen would be 153 mmHg (.21 x 730 mmHg) and the % saturation relative to sea level read by the probe would be 95.6% (153/160 x 100%) in the fully aerated sample. If the temperature of the sample is 25 °C, the instrument would calculate a dissolved oxygen concentration of 7.92 mg/L or 96% of 8.26 based on the Oxygen Solubility table.

The effect of barometric pressure is overcome by proper sensor calibration. Barometric pressure is used in the majority of dissolved oxygen sensor calibrations as described in the **Calibration** section since it determines the absolute pressure of oxygen in a sample of air or water at the time of calibration and it is this pressure which is measured by all oxygen sensors. When calibrating oxygen sensors, the sensor’s output is set to this known pressure of oxygen. If the sensor output changes after calibration, then the instrument would calculate a % saturation based on a simple linear regression calculation. Thus, as long as the system does not drift, the sensor’s output can always be used to define the oxygen pressure in any

medium after performing a proper calibration and the use of the barometric pressure (or altitude) at the time of calibration is the key factor in setting the proper calibration coefficient. Therefore, it is not necessary to correct for changes in barometric pressure after performing a proper calibration in order to obtain accurate readings in the field.

In summary, as barometric pressure changes due to a change in altitude or local weather front, the pressure of oxygen changes. However, there is never any reason to compensate for this change if a proper calibration has already been performed and the sensor has not drifted.

Note: If DO% Local is being measured with a YSI 6-series sonde or 556 it may be necessary to recalibrate the instrument after extreme changes in barometric pressure or altitude in order to keep the DO% Local value at 100% in a fully saturated environment. This is not a requirement if only mg/L values are being recorded since these values will remain accurate without recalibrating Local DO %. If reporting DO% Local with newer instruments such as the Professional Plus, ProODO, or Pro20, it is not necessary to recalibrate after a significant barometric pressure change in order to report an accurate DO% Local since these instruments have an on-board barometer that is read by the instrument continuously. See the **Local DO % Measurement** section for more information on this reporting option.

USING BAROMETRIC PRESSURE FOR DO CALIBRATION

The method for calibrating dissolved oxygen sensors using the local barometric pressure depends on the instrument in use and the calibration technique.

If performing a % air calibration on newer instruments, like the Pro20 and ProODO, a barometer is built into the instrument and the reading from that barometer is used to define the oxygen pressure during the calibration. Because this value determines the accuracy of future DO field readings, it is recommended to periodically verify the barometer reading's accuracy and to recalibrate the barometer to the local "true" barometric pressure as necessary. See figure 35 for information on obtaining the local "true" barometric pressure.

When performing a % air saturation calibration on instruments without an internal barometer, the user inputs either the local "true" barometric pressure or local altitude into the instrument during the calibration process. Local altitude is used on some of YSI's older instruments and barometric pressure is used on newer instruments. Although entering altitude as opposed to barometric pressure during calibration is a little less accurate (up to .1 mg/L), it should provide enough accuracy for most applications. In either situation, the calibration of the instrument in % saturation is handled automatically by the system's software or microprocessor based on either the internal barometer reading or the value entered by the user at the time of calibration.

A third method for calibrating DO to barometric pressure is used on older, box-style instruments that only report mg/L and not % saturation. In this method, the operator places the probe in either air-saturated water or water-saturated air and manually corrects for barometric pressure by calculating the pressure adjusted dissolved oxygen mg/L concentration and entering this value into the instrument. This value is calculated from the Oxygen Solubility table and the barometric pressure calibration correction table found on the back of older instruments or in the instrument's manual (**Appendix A and B**).

"True" Barometric Pressure

When entering the local barometric pressure or calibrating an instrument's internal barometer, be sure to use "true" barometric pressure and not a barometer reading that has been corrected to sea level. Laboratory barometer readings are usually "true" (uncorrected) values of air pressure and can be used "as is" for DO % calibrations and for calibrating the barometer in a YSI instrument. Weather service readings are usually not "true", i.e., they are corrected to sea level, and therefore cannot be used until they are "uncorrected". An approximate formula for this "uncorrection" is below:

True BP =

$$[\text{Corrected BP mmHg}] - [2.5 * (\text{Local Altitude in ft. above sea level}) / 100]$$

Figure 35. Information on "True" Barometric Pressure.

LOCAL DO % MEASUREMENTS

When calibrating a YSI instrument in DO % saturation, the instrument compensates for the local barometric pressure or altitude and calibrates the % reading to a value corresponding to the current barometric pressure (altitude). Therefore, the calibration value is only 100% when at 760 mmHg of barometric pressure (sea level). To determine the DO % calibration value for other barometric pressures/altitudes, refer to the table in Appendix B or divide the barometer reading by 760 and then multiply that number by 100:

Example: $750 / 760 = 0.9868 \times 100 = 98.68\%$

Some users may wish to report "Local DO" where the calibration value is 100% regardless of the barometric pressure at the time of calibration. The 100% calibration value for this convention reflects the fact that the calibration environment is at 100% oxygen pressure for that specific location. There are several YSI instruments that are capable of reporting Local DO, such as the Pro Plus, Pro20, and ProODO to name a few. These three instruments calculate the barometric pressure effect on each Local DO % and mg/L reading as it is measured in the field or lab.

CALIBRATION

The most important thing that can be done in order to obtain accurate dissolved oxygen readings is to perform an accurate calibration. As a general rule, the data collected is only as accurate as the calibration performed prior to data collection so care should be taken to ensure that the calibration is performed correctly. This section describes how to calibrate a dissolved oxygen sensor in general terms. For instructions specific to your instrument, consult the instrument's manual.

CALIBRATION FREQUENCY

Electrochemical sensors require more frequent calibrations than optical sensors. Steady-state galvanic and polarographic sensors should be calibrated every day that they are used in a spot sampling application. In general, it is best to calibrate frequently at first and, as experience dictates,

you may reduce calibration frequency.

Optical sensors have greater stability and are less susceptible to drift than traditional electrochemical sensors. Experience and scientific studies have shown that optical sensors can hold their calibration for many months. However, for the highest data accuracy, YSI recommends verifying the optical sensor's calibration on a regular basis. To verify the instrument's calibration, place the sensor in its calibration environment and check to see that the instrument's DO% reading matches the calibration value based on the barometric pressure. Refer to **Appendix B** for the DO% calibration values based on barometric pressure and altitude.

NOTE: It may be useful to check the DO calibration before and after an unattended monitoring study to ensure high data quality.

When calibrating DO % saturation to barometric pressure/altitude, it is not necessary to recalibrate the instrument due to a change altitude or barometric pressure because the DO % saturation and mg/L readings will remain accurate. However, if Local DO% is being reported with a 556 or 650 it may be desirable to recalibrate after a significant change in altitude or barometric pressure in order to keep the % saturation value at 100% in the calibration environment. This is not a requirement if only mg/L values are being recorded since these values will remain accurate without recalibrating Local DO %. It is also not required to recalibrate instruments that have an on-board barometer for accurate Local DO% readings, like the Pro Plus and ProODO, since the barometer will automatically assure that the Local DO% value remains at 100% in a fully saturated sample. For more information on Local DO, refer to the **Local DO % Measurements** section of this booklet.

CALIBRATION METHODS

In general, calibration consists of exposing the sensor to a sample of known oxygen content and calibrating the instrument to read that value. There are three primary methods for calibrating a dissolved oxygen instrument:

- Winkler titration
- Air-saturated water
- Water-saturated air

The Winkler Titration method calibrates the mg/L concentration value while the air-saturated water and water-saturated air methods both calibrate the % saturation reading.

The calibration environment in which the sensor is placed for calibration differs for each method. All three methods are reliable and are described in this booklet in general terms. For greatest ease and highest accuracy, YSI recommends the water-saturated air method. However, if conducting long-term, unattended monitoring with a ROX sensor on a 6-series sonde, YSI suggests that you calibrate in air-saturated water for greatest accuracy.

Note: It is only necessary to perform one type of calibration. Since mg/L is calculated from the % saturation reading, calibrating % saturation automatically calibrates the mg/L reading and vice versa. Therefore, it is only necessary to calibrate one or the other no matter what you plan to measure in the field.

WINKLER TITRATION CALIBRATION

If calibrating in mg/L, it is necessary to calibrate to a solution of known dissolved oxygen. The only way to determine the dissolved oxygen concentration of a sample without using a sensor is by performing a Winkler Titration. The Winkler Titration method was developed by Lajos Winkler in 1888⁴. In general, this method is cumbersome and time consuming.

To calibrate against a Winkler Titration, a sample of water should be divided into four parts. If the instrument being calibrated does not have a conductivity sensor or salinity correction option, the water sample should be of the same salinity as the water that will be tested. Titrate three of the samples. If any one of the samples' titrated value varies more than +/- 0.5 mg/L, it should be discarded. The remaining results are then averaged. Next, place the sensor into the fourth sample and allow the temperature and dissolved oxygen readings to stabilize. Adequate sample movement must be supplied if using a flow dependent sensor. Next, enter the averaged mg/L value into the instrument's calibration menu.

The success of this method depends on how the samples are handled and is highly subjective to human error. At all stages of the titration, steps must

be taken to ensure that oxygen is neither introduced to nor is lost from the sample. Furthermore, the water sample must be free of any solutes that will oxidize iodide ions or reduce iodine. For these reasons, YSI does not recommend calibrating against a Winkler.

AIR-SATURATED WATER CALIBRATION

The second calibration technique is air-saturated water. The air-saturated water method is the preferred method for calibrating ROX DO sensors for long-term unattended studies. Like the Winkler technique, this method is also time consuming and could introduce error if not done properly. To calibrate an instrument against air-saturated water, use an aquarium air pump and air stone to aerate a sample of clean tap water for 1 hour. Then place the sensor in the sample and allow the temperature and dissolved oxygen readings to stabilize. If using a flow dependent sensor, ensure that there is adequate sample movement to the sensor. Next, select DO% as the calibration method and enter the local altitude or "true" barometric pressure depending on the instrument in use. Refer to figure 35 for information on how to obtain the local "true" barometric pressure. Then, if using an instrument without a conductivity sensor, enter the salinity value of the water that will be tested and press enter to confirm the calibration.

Unless Local DO% is enabled in the instrument, the % calibration value will only be 100% at sea level or 760 mmHg. To determine the calibration value based on altitude or barometric pressure, refer to **Appendix B** or simply divide the local "true" barometric pressure in mmHg by 760 and then multiply that number by 100. For example, if the local true barometric pressure is 700 mmHg, the % calibration value will be $700/760 * 100\% = 92\%$. For more information on Local DO, refer to the **Local DO % Measurements** section of this booklet.

WATER-SATURATED AIR CALIBRATION

So far, the two calibration techniques described have some drawbacks. Winkler Titrations are cumbersome and time consuming. Air-saturated water requires some preparation time. The third technique, water-saturated air, overcomes these shortcomings and generally is YSI's recommended calibration method. As previously noted, however, if conducting long-term, continuous monitoring with a ROX sensor on a 6-series sonde, YSI

recommends that you calibrate in air-saturated water.

Depending on the sensor and storage method being used, this procedure should only take a few minutes to perform. First, dry the sensing element or membrane and temperature sensor by dabbing it with a lint free cloth or by shaking off excess water droplets. Next, place the sensor into a water-saturated air environment (air with 100% humidity). The probe must be vented to the atmosphere while in its calibration environment to ensure a 100% water-saturated air environment. Be sure the dissolved oxygen and temperature sensors are in water-saturated air only and not immersed in water! The calibration environment can be accomplished several different ways depending on the style of the sensor. See figure 36 for a table of calibration environment options.

Examples of Calibration Environments for Water-saturated Air Calibration:

<i>Calibration Environment</i>	<i>Description and Tips</i>
<p>Example 1: Clear storage chamber of older YSI sensors.</p> 	<ul style="list-style-type: none"> Moisten the sponge in the clear storage chamber with clean water. Chamber is vented to the atmosphere without any additional preparation. Ensure the sponge is clean because bacterial growth may consume oxygen and affect the calibration. Do not allow sunlight to heat the calibration chamber since that could keep the membrane and the thermistor from reaching thermal equilibrium.

<i>Calibration Environment</i>	<i>Description and Tips</i>
<p>Example 2: Moist sponge in gray calibration/storage sleeve. Included with some Pro Series instruments.</p> 	<ul style="list-style-type: none"> Calibration sleeve is vented to the atmosphere without any additional preparation. Be sure to use clean water. Do not allow sunlight to heat the calibration chamber since that could keep the membrane and the thermistor from reaching thermal equilibrium.
<p>Example 3: Clean, moist sponge in the calibration/storage chamber that is built into some handheld instruments.</p> 	<ul style="list-style-type: none"> Built-in storage chambers are vented to the atmosphere without any additional preparation. Be sure to use clean water. Do not allow sunlight to heat the calibration chamber since that could keep the membrane and the thermistor from reaching thermal equilibrium.
<p>Example 4: BOD Bottle</p> 	<ul style="list-style-type: none"> Place a small amount (1/8 inch or 3 mm) of clean water in a BOD bottle if using a BOD-style sensor. This is vented to the atmosphere without any additional preparation.

Calibration Environment	Description and Tips
<p>Example 5: Calibration/storage cup</p> 	<ul style="list-style-type: none"> Place a small amount of clean water in the calibration cup and place this over the sensor. Be sure to only engage one or two threads so the cup is vented to the atmosphere. Do not fully thread the calibration cup onto the probe! Do not allow sunlight to heat the calibration chamber since that could keep the membrane and the thermistor from reaching thermal equilibrium. Small amount of water = <ul style="list-style-type: none"> 1/8 inch or 3 mm for small calibration cups 1 inch or 2.5 cm for larger calibration cups
<p>Example 6: Wet towel wrapped around sensor guard.</p> 	<ul style="list-style-type: none"> Be sure to use clean water to wet the towel. Install the sensor guard and then wrap the towel around the sensor guard.

Figure 36. Examples of water-saturated air calibration environments.

After placing the probe in the calibration environment, turn the instrument on and wait 10 minutes for the calibration chamber to become completely saturated. Next, ensure the temperature and dissolved oxygen readings have stabilized. Unlike the previous two methods, it is not necessary to provide sample movement for flow dependent sensors with this method.

Enter the % calibration menu on the instrument and enter or confirm the local true barometric pressure or altitude. For YSI instruments that calibrate to the local altitude, enter your local altitude to the nearest 100 feet. Next, if using an instrument without a conductivity sensor, enter the salinity value of the water that will be tested and press enter to confirm the calibration.

Unless Local DO% is enabled in the instrument, the % calibration value will only be 100% at sea level or 760 mmHg. To determine the calibration value based on altitude or barometric pressure, refer to **Appendix B** or simply divide the local true barometric pressure by 760 and then multiply that number by 100. For example, if the local true barometric pressure is 700 mmHg, the % calibration value will be $700/760 * 100\% = 92\%$. For more information on Local DO, refer to the **Local DO % Measurements** section of this booklet.

Validity of Water-Saturated Air Calibration

It is a well documented fact that the pressure of oxygen in water-saturated air is the same as in air-saturated water. For example, air-saturated water and water-saturated air at sea level both have an oxygen pressure of 160 mmHg, see figure 37. Therefore, we would expect an oxygen sensor to give the exact same reading in either media. Since it is valid to calibrate in air-saturated water because it contains a known amount of oxygen, it must also be valid to calibrate in water-saturated air. In the case of the air-saturated water calibration, the concern was whether or not the water was completely oxygen-saturated. Since air is, by definition, oxygen saturated, this uncertainty has been removed.



Figure 37. The oxygen pressure in water-saturated air and air-saturated water is 160 mmHg at sea level.

TWO POINT CALIBRATION

Many newer instruments allow the user to perform a two point calibration with one point at zero and the other at full saturation. YSI recommends performing a two-point calibration 1.) if you are planning on taking readings in a low oxygen environment and you are certain that the sensor does not meet your accuracy requirements at low DO values or 2.) to verify your instrument's operation across its entire measurement range.

A zero oxygen solution can be made by dissolving approximately 2 grams of sodium sulfite (Na_2SO_3) and into 1 liter of tap water or DI water. Optionally, add a trace of cobalt chloride (CoCl_2) to the solution to speed up the reaction. Mix the solution thoroughly and then wait for the solution to be oxygen free which may take up to 60 minutes. Another method for making a zero oxygen environment is by dissolving 5-7 grams active dry yeast in 350 mL of distilled water and allowing the yeast to consume the oxygen which could take approximately 15 minutes. Or, a third method is to place the sensor in an inert gas such as nitrogen gas.

After creating an oxygen-free environment, place the sensor in the zero medium and allow the readings to stabilize. Stir the sensor if placed in a liquid and using a stirring dependent sensor

Select the two point calibration method within the instrument's menu and enter zero for the first calibration point. After completing the zero calibration point, thoroughly rinse the sensor of the oxygen-free solution and place it in a moist environment in order to follow up with a second point at full saturation.

NOTE: If you have calibrated a ROX sensor in a zero oxygen solution, remove the wiper from the probe to avoid sensing element contamination in the saturated environment.

CALIBRATION MUSTS

Regardless of the type of calibration performed, there are several common requirements for all three options. The lists below summarize the important points to be mindful of when performing a calibration. As stated before, your data is only as good as your calibration.

CALIBRATION MUSTS FOR ELECTROCHEMICAL SENSORS

1. First, inspect the membrane surface and probe solution. If there are air bubbles under the membrane, if the membrane is torn or dirty, or if the electrolyte solution looks contaminated, replace both the membrane and solution before calibration.
2. Second, if calibrating in air, remove water droplets from the membrane and thermistor by shaking the probe before inserting it into the calibration environment.
3. Third, if using a steady-state polarographic sensor, turn the instrument on and allow it to stabilize for 5-15 minutes before calibrating. This allows for the polarographic electrodes to polarize. Note that galvanic electrochemical and optical sensors do not require a warm up period for polarization.
4. Next, allow the temperature to stabilize completely in the calibration environment.

5. Be sure to provide enough sample movement if calibrating in liquid.
6. If calibrating in % mode, in either water or air, enter your altitude or "true" barometric pressure.
7. Next, input the salinity value of the water you will be testing into the instrument's software if using an instrument that does not measure conductivity.
8. For electrochemical sensors in spot sampling applications, it is required to calibrate daily, but not between samples.

CALIBRATION MUSTS FOR OPTICAL SENSORS

1. First, visually inspect the sensor cap for biofouling or large scratches in the paint surface. Biofouling could consume or produce oxygen thereby affecting the calibration. Large scratches in the paint surface could affect the calibration as well.
2. Make sure your sensor cap is properly hydrated and has not dried out.
3. Ensure that there aren't any water droplets on the sensing element or thermistor if calibrating in water-saturated air.
4. Allow the temperature to stabilize in the calibration environment.
5. If calibrating in % mode, in either water or air, enter your altitude or "true" barometric pressure.
6. Input the salinity value of the water you will be testing into the instrument's software if using an instrument that does not measure conductivity.
7. Lastly, optical sensors have shown to hold a calibration for many months. However, YSI recommends that you verify your calibration regularly.

ERRORS DURING CALIBRATION

If an error message such as 'Out of Range' or 'Questionable Results' appears during the calibration procedure, do not accept or proceed with the calibration. Instead, cancel or abort the calibration and try to determine what is causing the error. A list of potential error sources and remedies are listed in figure 38. For instrument specific recommendations, see the instrument's manual.

<i>Source of Error</i>	<i>Recommendation</i>
Bad electrochemical membrane	Inspect the membrane for contamination and clean with mild detergent if necessary. If damaged or if air bubbles are present under the membrane, replace it.
Dirty electrodes on steady-state polarographic and galvanic sensors	If a new membrane does not resolve the error, inspect the electrodes for tarnishing and, if necessary, perform a cleaning following the sensor-type specific recommendations in the Probe Care and Maintenance section of this booklet. Tarnishing of the electrodes is a natural result of the chemical reaction that takes place under the membrane and occasional maintenance of the electrodes is required to remove this build up.
Burn-in not performed on a Rapid Pulse membrane	For Rapid Pulse sensors, perform a proper burn-in as described in the 6-Series Calibration Tips document.
High DO charge (a sensor diagnostic value) for Rapid Pulse sensor	Resurface the electrodes and install a new membrane per the instructions for the Rapid Pulse in the Probe Care and Maintenance section of this booklet and in the 6-series manual.

<i>Source of Error</i>	<i>Recommendation</i>
Low DO charge (a sensor diagnostic value) for a Rapid Pulse sensor	There may be insufficient electrolyte under the membrane, an air bubble under the membrane, or a hole in the membrane. Replace the membrane with fresh electrolyte.
Bad optical sensing element	Inspect the sensing element for contamination and clean with mild detergent if necessary. If the sensing element is damaged or past its expected life (1+ year), replace it.
Dry optical sensing element	If the sensing element has been allowed to dry out, try performing the rehydration procedure described in the instrument's manual.
Bad Temperature reading	Verify the temperature is reading accurately. If erroneous, contact YSI Technical Support.
Bad calibration inputs	Ensure the values entered during the calibration process are accurate, i.e. "true" barometric pressure, local altitude, salinity correction value, DO concentration value of titrated samples, etc.

Figure 38.

GLP (GOOD LABORATORY PRACTICES) FILE

Newer YSI instruments contain a GLP (Good Laboratory Practices) file that records each calibration to the instrument's non-volatile memory. In general, the calibration record will contain the date and time the calibration was performed, the sensor's value, and whether or not the calibration was successful. Viewing the sensor's value in the GLP file can be useful for verifying the accuracy of the calibration and the condition of the sensor which can help with sensor diagnostics. For example, a sensor value that is out of the desired range could indicate that it is time to change the membrane or recondition the electrodes. For information on the sensor

value and its optimal range for your particular instrument, see the instrument specific manual.

TAKING MEASUREMENTS

To take a measurement, place the sensor in the sample to be measured.

If using a steady-state electrochemical sensor, be sure there is enough sample movement to overcome the sensor's flow dependency. As mentioned, the required flow rates vary by membrane type and are listed in figure 14. The flow requirement can be overcome by the sample's own movement, stirring the probe in the sample, a combination of the two, or by using a mechanical stirrer such as a stir bar or stir plate. A good rule to follow is to first stir the probe at the rate you think is required. Then, increase that movement. If the readings go up with the addition of more sample movement then you were not providing enough sample movement to begin with. If the readings remain the same, then you were providing enough movement. Continue with this process until no additional movement results in an increase in the DO readings. If you are using an optical or Rapid Pulse sensor, you do not have to stir.

After you are confident that there is sufficient sample movement to overcome the flow dependence of your sensor, wait for the temperature and DO readings to stabilize. The response time or time to stabilization varies by sensor and membrane material as shown in figure 14. It is important to keep stirring while you wait for probe stabilization. Once the readings have stabilized, you can record or save the reading.

If taking a measurement in an aeration basin or some other media that has a large quantity of air bubbles, you can suspend the probe upside down to allow the bubbles to flow past it as shown in figure 39. Large air bubbles can be a problem when they burst against the surface of the probe since this is sensed as 100% saturation. Inverting the probe as shown in figure 39 will keep bubbles from bursting on the membrane surface and help avoid erroneous measurements.



Figure 39. DO sensor inverted to avoid air bubbles bursting on the membrane surface.

BOD MEASUREMENTS

The BOD (Biochemical Oxygen Demand) test consists of taking an initial dissolved oxygen reading of a sample and then incubating it in a full, airtight BOD bottle in the dark at 20°C for either 3 or 5 days depending on the test protocol. The sample is measured again after the incubation period and the BOD is calculated from the difference between the two measurements along with the dilution and seed factors. Since most wastewater contains more oxygen-demanding matter than the amount of dissolved oxygen available in air-saturated water, BOD test samples are diluted to appropriately balance oxygen demand and supply. Because bacterial growth requires such nutrients as nitrogen, phosphorous and trace metals, these are added as seed to the dilution water, which is also buffered to ensure a suitable pH.

A variety of strategies are employed to deal with specific sample types. These include varying dilutions and diluent seeding. It is often desirable to

distinguish between carbonaceous and nitrogenous demand, in which case a nitrification inhibitor is used. Toxic and chlorinated samples also need special handling. The operator must be familiar with standard methods and with the technical literature on the subject.

When properly used, the BOD test provides a reliable characterization of wastewater. It can be expected to be a standard for regulatory agencies for many years even though its use as a control tool is limited by the 3 or 5 day wait required for the test. Various methods (based on short-term monitoring and extrapolation) of quickly estimating the probable results of the BOD test on a sample have been devised and the interested reader is advised to consult the appropriate literature.

MEASUREMENT PRECAUTIONS AND INTERFERENCES

There are a few potential interferences to be aware of when taking readings in order to get the best data possible.

BIOFOULING

A few of the potential interferences that should be considered mainly deal with keeping the sensing area clean. Always keep the sensor free from biofouling such as bacteria or algae growth. These organisms may not only prevent oxygen permeation through the membrane, but may also generate or consume oxygen resulting in erroneous readings for both optical and electrochemical sensors.

If biofouling is an issue for your application, try rinsing the probe between readings in order to keep the sensor clean. If deploying an instrument for continuous monitoring, consider using a mechanical wiper and anti-fouling protection to reduce the effects of biofouling and extend deployment times.

COATING MATERIALS

Keep the sensor free of oils that could clog the membrane or sensor cap and prohibit oxygen from diffusing to the sensor. The best advice if measurement cannot be avoided in these types of environments is to frequently rinse the probe. If necessary, periodically clean the membrane with a mild detergent to remove any residue. It may also be necessary to increase the frequency of membrane changes.

PROBE ATTACKING LIQUIDS

There are some substances that can attack the probe's materials and could potentially cause permanent damage. If using an optical sensor, avoid exposing the probe to alcohol and other organic solvents. Alcohol could cause deterioration of the paint layer that protects the dye layer and other organic solvents may deteriorate the dye layer. A list of solvents that may damage an optical probe are listed in figure 40. Obviously the concentration of the substance will also be a factor in whether any damage to the sensing element will occur.

Optical Probe Attacking Liquids

The following will remove the paint layer of the sensing cap:

Alcohols

The following will remove the paint and dye layer of the sensing cap and will attack the optical probe housing as well:

Toluene

Benzene

Carbon tetrachloride

Chloroform

Methylene chloride

Acetone

Methyl ethyl ketone,

Other organic solvents

Figure 40. Substances that could damage an optical sensing element.

For electrochemical sensors, care should be taken to avoid exposing the DO probe to strong acids, caustics or solvents. Probe materials that come in contact with liquid include polyethylene, polyurethane, Teflon, EPR rubber, epoxy, ABS plastic and stainless steel.

INTERFERING GASES

Certain gases, if dissolved in water, can cause interference for polarographic and galvanic sensors if they exist in high enough concentrations. An optical sensor's reading, however, is generally not affected by the presence of these gases. The gases that will cause interferences with DO measurements taken with electrochemical sensors include: hydrogen sulfide, sulfur dioxide, halogens, neon, nitrous and nitric oxides. Some of these gases and others have been tested by YSI and the response in apparent oxygen readings are listed below:

100% Carbon monoxide less than 1%

100% Carbon dioxide around 1%

100% Hydrogen less than 1%

100% Chlorine 2/3 O₂ response

100% Helium none

100% Nitrous oxide 1/3 O₂ response

100% Ethylene none

100% Nitric oxide 1/3 O₂ response

MEMBRANE AND SENSING ELEMENT INTEGRITY

While it is important to look for coating or fouling on the membrane or sensor cap, it is also important to check the integrity of the membrane or sensor cap by visually inspecting it. For an electrochemical membrane, look to make sure the membrane is still stretched properly and that there are no air bubbles trapped underneath. For an optical sensing element, check for large scratches. While small scratches in the paint layer will not affect the reading, large scratches may and you should replace your cap if you see large scratches and are having problems calibrating. Also, be sure to keep your membrane or optical sensing element hydrated.

PROBE CARE AND MAINTENANCE

An understanding of dissolved oxygen measurement would not be complete without discussing probe care and maintenance, much of which can be performed by the user.

PROBE CARE AND MAINTENANCE FOR ELECTROCHEMICAL SENSORS

This section describes the general care and maintenance recommendations for electrochemical sensors. For more detailed instructions that are specific to your instrument, please refer to the instrument's manual.

CHANGING A MEMBRANE

By far, the most frequent maintenance task with an electrochemical sensor is replacing the membrane. In general, membranes should be changed every 2-8 weeks depending on the application. If measuring in a high fouling environment or in water with high hydrogen sulfide content, it will be necessary to change the membrane at least every two weeks.

As discussed, YSI probes have one of two membrane styles – either stretch or cap. Older probes use a sheet-like membrane that is stretched over the probe and held in place by an o-ring. This is referred to as a stretch membrane. Figure 41 shows a sequence of drawings that demonstrate how this type of membrane is changed. After removing the old membrane, rinse the electrode with distilled or de-ionized water and then dry it. Next, secure a membrane between your thumb and the probe body. Add electrolyte to the probe until a large meniscus completely covers the gold cathode. Handle the membrane material with care, touching it at the ends only. With the thumb and forefinger of your other hand, grasp the free end of the membrane. With a continuous motion, stretch the membrane up, over, and down the other side of the sensor. Stretching forms the membrane to the contour of the sensor tip. Secure the end of the membrane under your forefinger while continuing to hold the probe. Roll the o-ring over the end of the probe, being careful not to touch the membrane surface. There should be no wrinkles in the membrane or trapped air bubbles under the membrane. Trim off excess membrane with scissors or a sharp knife. Shake off any excess KCl and then rinse the probe with clean water.

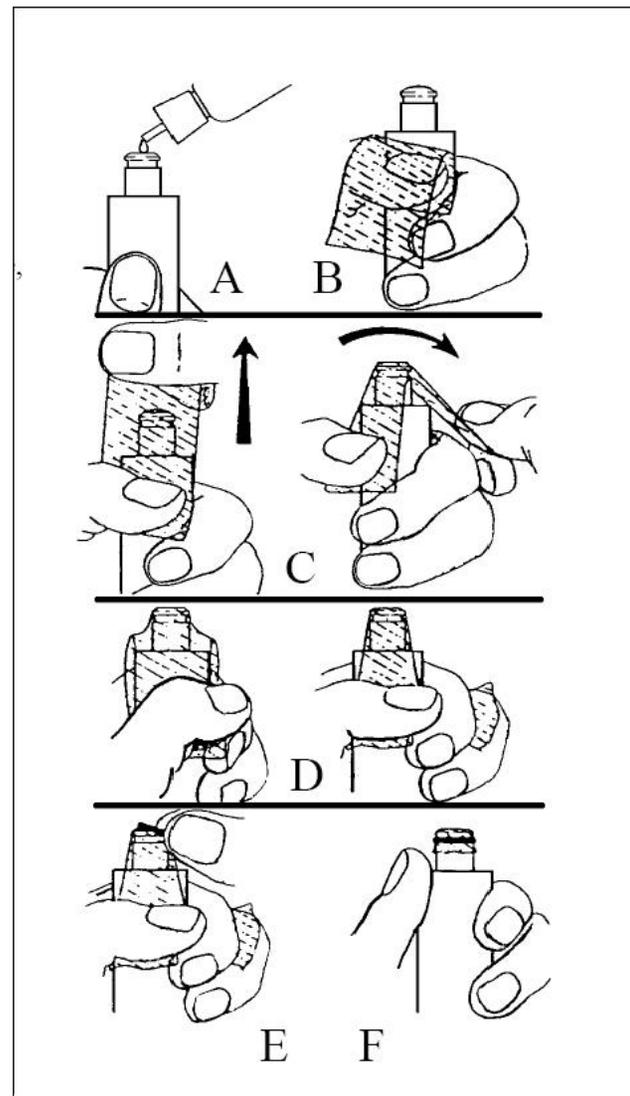


Figure 41. Sequence of drawings demonstrating a stretch membrane installation.

Newer, cap-style membranes are much easier to change, as shown in the series of drawings in figure 42. After removing the old membrane, rinse the electrode with distilled or de-ionized water and then dry it. Next, fill a new cap a little more than half way full with fresh electrolyte. Then, with the probe facing down, insert the probe into the membrane cap. Some electrolyte will spill over; this is normal. Continue with threading the

membrane cap onto the probe. Shake off any excess KCl and then rinse the probe with clean water. If you would like to see a video of this procedure, visit YSI's YouTube channel - www.Youtube.com/YSIIInc

NOTE: Always re-calibrate an instrument after changing a membrane.

In between membrane changes, check the membrane regularly to be sure it is still tightly stretched, not fouled, and that there are no air bubbles under it. A loose or wrinkled membrane will introduce measurement errors, make calibration difficult, and cause the electrodes to require more frequent maintenance.

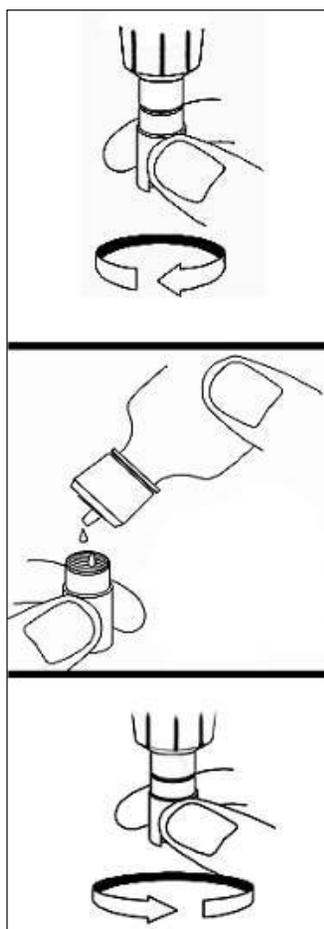


Figure 42. Sequence of drawings demonstrating a cap membrane replacement.

ELECTRODE MAINTENANCE

Requiring far less attention than the membrane of an electrochemical sensor are the electrodes, or more specifically the cathode and the anode. It is important to note that tarnishing of the electrodes is a natural by-product of the reaction taking place under the membrane due to the presence of oxygen. In other words, it is unavoidable. If using an electrochemical sensor, it will eventually require a cleaning. The frequency of the cleaning depends on use and the type of sensor. In general, electrode maintenance should only be necessary about once per year for steady-state polarographic sensors and about once every 3 months for steady-state galvanic sensors. Do not perform the following maintenance procedures just because a sensor 'looks' dirty. Only perform the following procedures if the instrument will not calibrate or if the readings are unstable and a regular membrane change did not correct either of these problems.

Steady-state Polarographic Anode Maintenance

A tarnished polarographic silver anode can be cleaned by soaking the probe in household ammonia cleaner overnight. Alternatively, the electrode may be soaked in 14% Ammonium Hydroxide for 2 to 3 minutes. After soaking the probe, rinse it thoroughly with clean water. This will brighten the anode. If you are using the style of electrode that uses a cap membrane, you may need to wet sand the anode with 400 grit sand paper after performing the ammonia soak. This can be done by wrapping the sanding disc around the anode and twisting as shown in figure 43.



Figure 43.

Always install a new membrane with fresh electrolyte after servicing the electrodes and allow the probe to stabilize before calibration.

NOTE: Do not perform the ammonia chemical soak on galvanic or Rapid Pulse sensors.

Steady-state Polarographic Cathode Maintenance

The cathode on most YSI probes can be restored to its normal polish by wet sanding the electrode. If there is a heavy build up of deposits, this procedure works best after the ammonia chemical soak described in the previous section. To sand the cathode, place a 400 grit wet/dry sanding disc, face-up in the palm of your hand. Next, touch the cathode to the sanding disc and twist the probe, see figure 44. After sanding, rinse the electrode with clean water.



Figure 44.

Always install a new membrane with fresh electrolyte after servicing the electrodes and allow the probe to stabilize before calibration.

Galvanic Maintenance

To remove the white deposits from the galvanic electrodes, perform a wet sanding procedure with 400 grit wet/dry sand paper on both the anode and cathode. After removing the membrane, rinse the electrode with clean water. Next, wrap the wet sanding disc around the anode and twist as shown in figure 43. To sand the cathode, place the wet sanding disc, face-

up in the palm of your hand. Next, touch the cathode to the sanding disc and twist the probe as shown in figure 44. After sanding, rinse the electrode with clean water. Always install a new membrane with fresh electrolyte after servicing the electrodes and allow the probe to stabilize before calibration. Do not perform the ammonia soak on a galvanic sensor.

Rapid Pulse Maintenance

The electrodes for the Rapid Pulse sensor can be serviced by performing a wet sanding procedure with 2400 grit wet/dry sand paper. For this procedure, place the sanding disc between your thumb and forefinger and sand parallel to the electrodes 10-15 times like you are striking a match, see figure 45. Then, rinse the electrodes with clean water and install a new membrane with fresh electrolyte. Do not perform the ammonia soak or use 400 grit sand paper on a Rapid Pulse sensor.

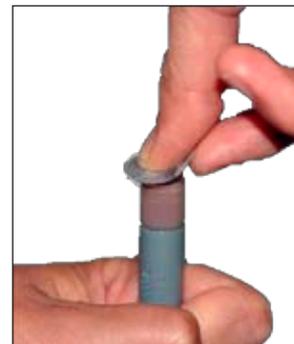


Figure 45.

PROBE CARE AND MAINTENANCE FOR OPTICAL SENSORS

The maintenance requirements for an optical sensor are less laborious than electrochemical sensors.

To clean the probe and sensing element, rinse it with clean water and a lint free cloth. If necessary, use a mild detergent. Do not use alcohols or other organic solvents that may deteriorate the sensing element.

The sensing element should be replaced about once per year but may last longer. It should also be replaced if it is cracked or damaged. While

changing the sensing element, rinse the optics with clean water and then wipe the optics with a lint free cloth or lens tissue. The instruction sheet that is shipped with the replacement sensing element includes calibration coefficients specific to the sensing element. For highest accuracy, these coefficients should be entered by the user into the instrument following the instructions provided. See the instrument specific manual for detailed instruction on how to change a sensing element.

STORAGE

For short term storage, electrochemical probes should be stored in a moist environment to keep the membrane from drying out and needing replaced. Do not store it directly in water since that could encourage algae growth on the probe. For long-term storage of the steady-state sensors, remove the membrane, rinse and dry the electrode and then install a dry membrane to protect it from dirt and dust during storage.

The Rapid Pulse sensors are a bit different in that they should not be stored dry as this could shorten their usable life. Instead, keep a membrane installed on a Rapid Pulse sensor and store in a moist environment for both short-term and long-term storage.

Optical sensors should not be allowed to dry out either. For storage, keep the sensing element installed and place the probe in a moist environment but not directly in water since that could encourage algae growth on the probe. If it dries out, you must re-hydrate it following the instructions in the instrument's manual.

To store the instrument for long periods of time, remove the batteries. Clean the case and cable with a mild detergent if necessary. Then, store the instrument in a cool, dry place.

FINAL WORDS

YSI has been manufacturing and distributing dissolved oxygen sensors for over 40 years and that experience has made the company a leader in dissolved oxygen measurement technologies. For more information on the measurement of dissolved oxygen, including the latest on the optical

method's EPA approval status and information on how to contact your US EPA ATP Coordinator, please visit YSI's We Know DO web page at www.ysi.com/weknowdo. The We Know DO web page is free and serves as a valuable resource center for those interested in learning more about dissolved oxygen.

YSI offers several different dissolved oxygen instruments to meet a wide variety of application requirements that range from spot sampling to continuous monitoring, from the field to the lab and from single parameter to multiparameter. YSI's knowledgeable Technical Support Staff is available to assist you in selecting the right instrument for your application. You can reach a Technical Support staff member by calling 800-897-4151 (+1 937-767-7241) or sending an email to environmental@ysi.com. In addition, YSI has offices located around the world ready assist you with selecting an instrument, ordering replacement parts or providing repair services. Please visit www.ysi.com for a list of our global offices.

APPENDIX A - OXYGEN SOLUBILITY TABLE

Solubility of Oxygen in mg/L in water exposed to water-saturated air at 760 mmHg pressure³.

Temp °C	Chlorinity: 0	5.0 ppt	10.0 ppt	15.0 ppt	20.0 ppt	25.0 ppt
	Salinity: 0	9.0 ppt	18.1 ppt	27.1 ppt	36.1 ppt	45.2 ppt
0.0	14.621	13.728	12.888	12.097	11.355	10.657
1.0	14.216	13.356	12.545	11.783	11.066	10.392
2.0	13.829	13.000	12.218	11.483	10.790	10.139
3.0	13.460	12.660	11.906	11.195	10.526	9.897
4.0	13.107	12.335	11.607	10.920	10.273	9.664
5.0	12.770	12.024	11.320	10.656	10.031	9.441
6.0	12.447	11.727	11.046	10.404	9.799	9.228
7.0	12.139	11.442	10.783	10.162	9.576	9.023
8.0	11.843	11.169	10.531	9.930	9.362	8.826
9.0	11.559	10.907	10.290	9.707	9.156	8.636
10.0	11.288	10.656	10.058	9.493	8.959	8.454
11.0	10.027	10.415	9.835	9.287	8.769	8.279
12.0	10.777	10.183	9.621	9.089	8.586	8.111
13.0	10.537	9.961	9.416	8.899	8.411	7.949
14.0	10.306	9.747	9.218	8.716	8.242	7.792
15.0	10.084	9.541	9.027	8.540	8.079	7.642
16.0	9.870	9.344	8.844	8.370	7.922	7.496
17.0	9.665	9.153	8.667	8.207	7.770	7.356
18.0	9.467	8.969	8.497	8.049	7.624	7.221
19.0	9.276	8.792	8.333	7.896	7.483	7.090
20.0	9.092	8.621	8.174	7.749	7.346	6.964
21.0	8.915	8.456	8.021	7.607	7.214	6.842
22.0	8.743	8.297	7.873	7.470	7.087	6.723
23.0	8.578	8.143	7.730	7.337	6.963	6.609
24.0	8.418	7.994	7.591	7.208	6.844	6.498

Temp °C	Chlorinity: 0	5.0 ppt	10.0 ppt	15.0 ppt	20.0 ppt	25.0 ppt
	Salinity: 0	9.0 ppt	18.1 ppt	27.1 ppt	36.1 ppt	45.2 ppt
25.0	8.263	7.850	7.457	7.093	6.728	6.390
26.0	8.113	7.711	7.327	6.962	6.615	6.285
27.0	7.968	7.575	7.201	6.845	6.506	6.184
28.0	7.827	7.444	7.079	6.731	6.400	6.085
29.0	7.691	7.317	6.961	6.621	6.297	5.990
30.0	7.559	7.194	6.845	6.513	6.197	5.896
31.0	7.430	7.073	6.733	6.409	6.100	5.806
32.0	7.305	6.957	6.624	6.307	6.005	5.717
33.0	7.183	6.843	6.518	6.208	5.912	5.631
34.0	7.065	6.732	6.415	6.111	5.822	5.546
35.0	6.950	6.624	6.314	6.017	5.734	5.464
36.0	6.837	6.519	6.215	5.925	5.648	5.384
37.0	6.727	6.416	6.119	5.835	5.564	5.305
38.0	6.620	6.316	6.025	5.747	5.481	5.228
39.0	6.515	6.217	5.932	5.660	5.400	5.152
40.0	6.412	6.121	5.842	5.576	5.321	5.078
41.0	6.312	6.026	5.753	5.493	5.243	5.005
42.0	6.213	5.934	5.667	5.411	5.167	4.993
43.0	6.116	5.843	5.581	5.331	5.091	4.861
44.0	6.021	5.753	5.497	5.252	5.017	4.793
45.0	5.927	5.665	5.414	5.174	4.944	4.724
46.0	5.835	5.578	5.333	5.097	4.872	4.656
47.0	5.744	5.493	5.252	5.021	4.801	4.589
48.0	5.654	5.408	5.172	4.947	4.730	4.523
49.0	5.565	5.324	5.094	4.872	4.660	4.457
50.0	5.477	5.242	5.016	4.799	4.591	4.392

APPENDIX B - CALIBRATION VALUES FOR VARIOUS ATMOSPHERIC PRESSURES AND ALTITUDES

This table is also used to correct mg/L for the local barometric pressure of older, box style instruments that did not report % Saturation.

Pressure				Altitude		Calibration value
<i>Inches Hg</i>	<i>mm Hg</i>	<i>kPA</i>	<i>mbar</i>	<i>Feet</i>	<i>Meters</i>	<i>% Saturation</i>
30.23	768	102.4	1023	-276	-84	101
29.92	760	101.3	1013	0	0	100
29.61	752	100.3	1002	278	85	99
29.33	745	99.3	993	558	170	98
29.02	737	98.3	983	841	256	97
28.74	730	97.3	973	1126	343	96
28.43	722	96.3	963	1413	431	95
28.11	714	95.2	913	1703	519	94
27.83	707	94.2	944	1995	608	93
27.52	699	93.2	932	2290	698	92
27.24	692	92.3	923	2587	789	91
26.93	684	91.2	912	2887	880	90
26.61	676	90.1	901	3190	972	89
26.34	669	89.2	892	3496	1066	88
26.02	661	88.1	881	3804	1106	87
25.75	654	87.2	872	4115	1254	86
25.43	646	86.1	861	4430	1350	85
25.12	638	85.1	851	4747	1447	84
24.84	631	84.1	841	5067	1544	83
24.53	623	83.1	831	5391	1643	82
24.25	616	82.1	821	5717	1743	81

Pressure				Altitude		Calibration value
<i>Inches Hg</i>	<i>mm Hg</i>	<i>kPA</i>	<i>mbar</i>	<i>Feet</i>	<i>Meters</i>	<i>% Saturation</i>
23.94	608	81.1	811	6047	1843	80
23.62	600	80.0	800	6381	1945	79
23.35	593	79.1	791	6717	2047	78
23.03	585	78.0	780	7058	2151	77
22.76	578	77.1	771	7401	2256	76
22.44	570	76.0	760	7749	2362	75
22.13	562	74.9	749	8100	2469	74
21.85	555	74.0	740	8455	2577	73
21.54	547	73.0	729	8815	2687	72
21.26	540	72.0	720	9178	2797	71
20.94	532	71.0	709	9545	2909	70
20.63	524	70.0	699	9917	3023	69
20.35	517	69.0	689	10293	3137	68
20.04	509	67.9	679	10673	3371	67
19.76	502	66.9	669	11058	3371	66

REFERENCES

- (1) South Carolina Department of Natural Resources – Waddell Mariculture Center; Research funded by National Institute of Standards and Technology.
- (2) *Standard Methods for the Examination of Water and Wastewater* (1998), 20th Edition. Published jointly by American Public Health Association, American Water Works Association, and Water Environmental Federation, Washington, D.C.
- (3) *Wikipedia, The Free Encyclopedia*. (2009, September 1). Wikimedia Foundation, Inc. Retrieved September 17, 2009, from <http://en.wikipedia.org/wiki/Freshwater>.
- (4) Winkler, L.W. (1888). Die Bestimmung des in Wasser gelösten Sauerstoffes. *Berichte der Deutschen Chemischen Gesellschaft*, 21, page 2843.

The Dissolved Oxygen Handbook
©2009 YSI Incorporated
www.ysi.com/weknowdo
environmental@ysi.com
September 2009
W39 0909
\$20.00