DO Theory Guide

Applications
Practical Hints
Theory

Laboratory and
Environment

Dissolved Oxygen Measurement

Theory and Practice of DO Applications

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Table of Contents

Introduction

This guide provides a clear and simple description of the measurement principles of Dissolved Oxygen (DO). The theory of DO is explained as well as the practical aspects of choosing the right sensor for an application, calibrating it, and carrying out DO and BOD measurements.

Purpose of This Guide

This guide presents general information and selected application examples. The information is based on the current state of our knowledge.

The application experiments were conducted with the utmost care using the instruments specified in the description of each application. The results were evaluated according to the current state of our knowledge. This does not however absolve you from personally testing the suitability of the examples for your own methods, instruments and purposes. Since the transfer and use of an application is beyond our control, we cannot of course accept any responsibility.

When chemicals, solvents and gases are used, general safety rules and the instructions given by the manufacturer or supplier must be observed.

1 Theory of Dissolved Oxygen (DO)

Oxygen is the third most common element in the universe and the most common in the Earth's crust (49%). However, because oxygen is highly reactive, most of it is bound to other elements to form chemical compounds. Elementary oxygen in its two allotropes O_2 and O_3 (ozone) only exists in relevant concentrations on our planet. That is because it stems from biological processes that pertain to Earth (mainly photosynthesis).

Thus, elementary oxygen and its occurrence are linked to life and its chemical activities. Due to its reactive nature, it also contributes to less desirable processes, such as corrosion or fire hazards.

Measuring concentrations of oxygen dissolved in water can be very important for monitoring habitats (such as lakes, oceans or aquariums), production processes (such as beer or cheese fermentation), treatment of wastewater, or corrosion-sensitive processes.

1.1 Dissolved Oxygen and Partial Pressure

Measuring the dissolved oxygen concentration in a sample is done by submerging the sensor in it. In the classical measurement principle, oxygen can enter and leave the sensor through a selectively permeable membrane, which leads to an equilibrium of oxygen activity (concentration) between the sample and the sensor. The oxygen concentration is then measured inside the sensor (for details see chapter 2). A consequence of this measuring principle is that it does not measure the concentration of dissolved oxygen directly, but rather its partial pressure.

Partial pressure can be visualized as the tendency of oxygen to leave a solution and can be expressed in percentage of air saturation. For example, a stable solution in air is 100% air-saturated. If the concentration were above 100%, the excess

would leave the solution and enter the air. Were it below 100%, the solution would slowly absorb oxygen from the air.

Similarly, if the partial pressure in the solution is higher than in the sensor, oxygen will enter the sensor, and vice versa.

At equilibrium, the partial pressure inside the sensor is equal to that in the solution. However, this does not mean that the concentrations are equal. Which concentration corresponds to a partial pressure of 100% saturation, depends on the solubility of oxygen in the solution. For water, the most common solvent, this solubility is well known and the saturation can be converted into concentration in mg/L.

1.2 Solubility of Oxygen in Water

DO is majorly measured in water samples. The solubility of O_2 in water depends on the solution's salinity and temperature and the atmospheric pressure (see table 1.1 and figure 1.1). These values are needed, in order to convert the partial pressure (that the sensor measures) into the DO concentration in mg/L.

	Salinity/g/kg								
T/°C	0	5	10	15	20	25	30	35	40
0	14.62	14.12	13.64	13.17	12.71	12.28	11.85	11.45	11.05
1	14.22	13.73	13.27	12.82	12.38	11.96	11.55	11.15	10.77
2	13.83	13.36	12.91	12.48	12.06	11.65	11.26	10.88	10.51
3	13.46	13.01	12.58	12.16	11.75	11.36	10.98	10.61	10.25
4	13.11	12.67	12.26	11.85	11.46	11.08	10.71	10.35	10.01
5	12.77	12.35	11.95	11.55	11.18	10.81	10.45	10.11	9.774
6	12.45	12.04	11.65	11.27	10.91	10.55	10.21	9.872	9.550
7	12.14	11.75	11.37	11.00	10.65	10.30	9.970	9.647	9.335
8	11.84	11.47	11.10	10.74	10.40	10.07	9.744	9.431	9.128
9	11.56	11.19	10.84	10.50	10.16	9.839	9.526	9.223	8.930
10	11.29	10.93	10.59	10.26	9.934	9.621	9.318	9.024	8.739
11	11.03	10.68	10.35	10.03	9.715	9.412	9.117	8.832	8.556
12	10.78	10.44	10.12	9.808	9.505	9.210	8.925	8.645	8.379
13	10.54	10.21	9.901	9.597	9.302	9.017	8.739	8.470	8.210
14	10.31	9.993	9.689	9.394	9.108	8.830	8.561	8.300	8.046
15	10.08	9.780	9.485	9.198	8.921	8.651	8.389	8.135	7.888
16	9.870	9.575	9.289	9.010	8.740	8.478	8.223	7.976	7.737
17	9.665	9.378	9.099	8.829	8.566	8.311	8.064	7.823	7.590
18	9.467	9.188	8.917	8.654	8.399	8.151	7.910	7.676	7.449
19	9.276	9.005	8.742	8.486	8.237	7.995	7.761	7.533	7.312
20	9.092	8.828	8.572	8.323	8.081	7.846	7.617	7.395	7.180
21	8.914	8.658	8.408	8.166	7.930	7.801	7.579	7.262	7.052
22	8.743	8.493	8.250	8.014	7.785	7.785	7.561	7.344	6.929
23	8.578	8.334	8.098	7.867	7.644	7.426	7.214	7.009	6.809
24	8.418	8.181	7.950	7.725	7.507	7.295	7.089	6.888	6.693
25	8.263	8.032	7.807	7.588	7.375	7.168	6.967	6.771	6.581
26	8.113	7.888	7.668	7.455	7.247	7.045	6.849	6.658	6.472
27	7.968	7.748	7.534	7.326	7.123	6.926	6.734	6.548	6.366
28	7.827	7.613	7.404	7.201	7.003	6.810	6.623	6.441	6.263
29	7.691	7.482	7.278	7.079	6.886	6.698	6.515	6.337	6.164
30	7.558	7.354	7.155	6.961	6.772	6.589	6.410	6.236	6.066
31	7.430	7.230	7.036	6.846	6.662	6.483	6.308	6.137	5.972

	Salinity/g/kg								
T/°C	0	5	10	15	20	25	30	35	40
32	7.305	7.110	6.920	6.735	6.555	6.379	6.208	6.042	5.880
33	7.183	6.993	6.807	6.626	6.450	6.278	6.111	5.948	5.790
34	7.065	6.879	6.697	6.520	6.348	6.180	6.017	5.857	5.702
35	6.949	6.767	6.590	6.417	6.248	6.084	5.924	5.768	5.617
36	6.837	6.659	6.485	6.316	6.151	5.991	5.834	5.681	5.533
37	6.727	6.553	6.383	6.218	6.056	5.899	5.746	5.597	5.451
38	6.619	6.449	6.283	6.121	5.963	5.810	5.660	5.513	5.371
39	6.514	6.348	6.186	6.027	5.783	5.722	5.575	5.432	5.292
40	6.412	6.249	6.090	5.935	5.783	5.636	5.492	5.352	5.215

Table 1.1: Solubility of O_2 in water in mg/L at different temperatures and salinities (at standard pressure).

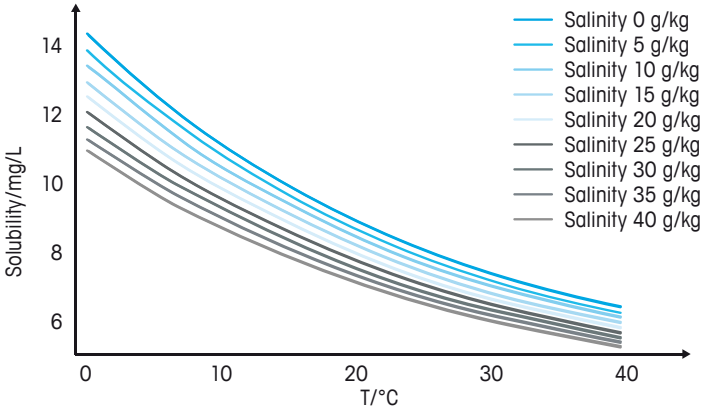


Figure 1.1: Temperature and salinity dependency of oxygen's solubility in water (at standard pressure).

The data in table 1.1 and figure 1.1 can be summarized as follows: at standard pressure, the solubility of oxygen in water increases as temperature and salinity decrease. With increasing atmospheric pressure, the solubility of oxygen in water increases (data not shown).

1.3 Biological Importance of Dissolved Oxygen

The concentration of oxygen in water has a large impact on how biologically active the water body is. Oxygen is usually generated by water plants and algae but it can also be absorbed from the air. All animals and fungi depend on oxygen to live and thrive. To name a few examples: bottom feeders, crabs, oysters, and worms need minimal amounts of oxygen (1–6 mg/L), while shallow water fish need higher levels (4–15 mg/L). It is generally accepted that fish cannot live in water with DO concentrations below ~3 mg/L. Most fish will not even survive at levels between 4 and 5 mg/L. In addition, eggs and growing fish require more oxygen than adult fish, so that DO levels that are sufficient for adult fish survival can still lead to extinction due to the unsustainable conditions for embryo/juvenile development. Thus, shifting long-term DO concentrations can drastically alter aquatic ecosystems.

As most aquatic animals are cold-blooded, temperature also plays a role in oxygen consumption. For example, a trout consumes up to six times more oxygen at 24 °C than at 4 °C due to its increased metabolism.

As the solubility of oxygen in water depends on temperature, reduced DO levels may be caused by heating the water. This is a relevant environmental side-effect of many industrial processes pumping warm water (that was used for cooling) into streams and lakes.

Likewise, high concentrations of fertilizers from agriculture will cause a highly increased aquatic plant growth. When these plants die, their decomposition by bacteria consumes large amounts of oxygen, causing the DO levels to drop drastically. The resulting biotope is called a dead zone.

Because of these factors, measuring DO is an indispensable tool to water monitoring programs.

Due to the importance of water quality, several official bodies have reviewed the impact of DO levels (amongst other parameters) on freshwater quality in terms of fish survival, growth and reproduction. Subsequently, DO concentration guidelines for the protection of aquatic life and its uses have been published. Such bodies include the US Environmental Protection Agency (US EPA 1986, US EPA SESD) and the European Inland Fisheries Advisory Commission (EIFAC 1973).

1.4 Industrial Importance of Dissolved Oxygen

Because oxygen is a reactive molecule, its concentration in a solution has a strong impact on the oxidizing properties. Especially metal parts can deteriorate quickly due to corrosion. For example, iron can oxidize rapidly in the presence of oxygen and water to form iron oxide (i.e. rust). This application of DO measurement is almost exclusively of preventive nature, in order to preserve metal parts for as long as possible.

A similar, preventive application is controlling the shelf life of food items (and other biological products). Because most decay processes of such products consist of aerobic fermentation (i.e. they consume oxygen), ensuring low levels of DO is a necessary step for guaranteeing long shelf lives.

2 Types of DO Sensors

O_2 is a highly reactive molecule, so reactive in fact that it only exists in our atmosphere because it is continuously produced by photosynthesis. It reacts as an oxidizing agent—i.e. in a chemical reaction it receives electrons. Most DO sensors utilize this property of electrochemical reactions for measuring oxygen concentrations. These types of sensors are well established as conventional methods for measurement. They can be categorized as galvanic or polarographic sensors.

Optical sensors (utilizing fluorescence quenching, see below) constitute a modern alternative to these electrochemical methods.

2.1 Introduction to Redox Chemistry

This sub-chapter gives a simple overview of the terms and concepts used in electrochemistry because they are relevant to understand how electrochemical DO sensors work.

Electrochemistry is based on the **redox** principle (i.e. **red**uctions and **ox**idizations). It relates to the proneness of different elements and compounds to give or receive electrons (in reaction equations electrons are denoted by e^-). In order for this electron exchange to happen, an oxidation and a reduction have to take place simultaneously.

A reaction, in which a substance gives electrons, is called an **oxidization**, for example:

$$Zn \rightarrow Zn^{2+} + 2\,e^-$$

A reaction in which a substance receives electrons, is called a **reduction**, for example:

$Ag^+ + e^- \rightarrow Ag$

A **reducing agent** reduces another substance. Since it gives electrons to another substance, a reducing agent is oxidized in a reaction. Vice versa, an **oxidizing agent** oxidizes (takes electrons from) another substance and is reduced in a reaction.

Almost all elements or compounds can undergo redox reactions. But they are not equally likely to do so. A substance that easily participates in redox reactions is called redox active. Metals that are easily oxidized are traditionally called **ignoble metals**, whereas metals that are more resistant to oxidization are named **noble metals**. Amongst other things, this means that noble metals are rarely corroded.

Electrochemical measurements detect the electrons' flow through a wire between a cathode and an anode. The cathode releases electrons because an oxidization occurs and the anode accepts them in a reduction reaction (figure 2.1). To avoid a direct reaction between cathode and anode, they have to be physically separated.

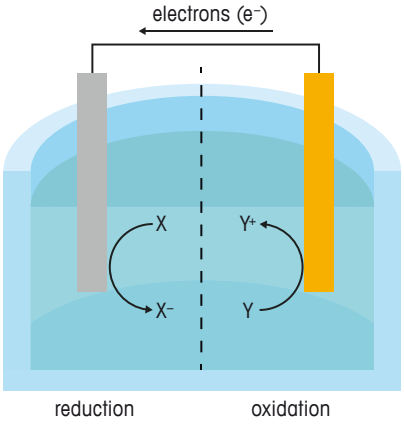


Figure 2.1: The principle of an electrochemical measurement: physically separated reduction (cathode) and oxidation (anode) reactions are connected through a wire, where the electrons' flow is measured.

If the metals the cathode and anode are made of differ in nobility, then an electrical potential builds up on its own, a process called **self-polarization**. If however, the nobility of these two metals is similar, they can be **polarized** by applying an external voltage.

2.2 Galvanic Dissolved Oxygen Sensors

A galvanic DO sensor contains two electrodes made of different metals (of different nobility) in an electrolyte solution. The electrodes are interconnected by wires, which allow a current to flow between them. These components are encompassed in a shaft, which is sealed off by a membrane that is selectively permeable to oxygen (figure 2.2). Because this type of membrane ages, some galvanic DO sensors are produced with exchangeable membrane modules.

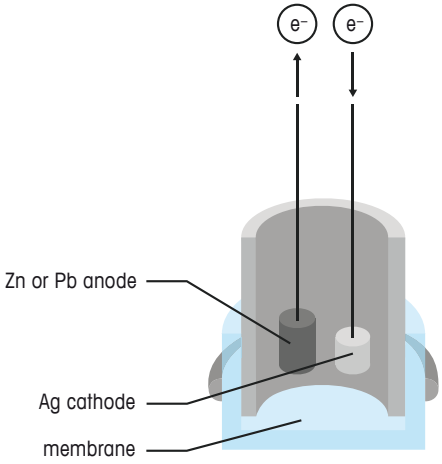


Figure 2.2: Schematic cross-section of a galvanic DO sensor.

The anode is usually made of zinc or lead (in rare cases, a different ignoble metal), while the cathode is usually made of silver or another noble metal. The electrolyte has to be aqueous and alkaline.

Due to the difference in their standard reduction potential (how readily they accept electrons), the system self-polarizes—a static electrical potential is generated between the two electrodes. Since this self-polarization is an internal property of the galvanic DO sensor, it does not require any warm-up time.

The entry of oxygen into the sensor enables a chemical reaction in which the anode is oxidized (donates electrons) and consumed. In contrast, the cathode is noble and does not participate in the reaction: it exists as a reaction surface onto which oxygen is reduced. The electrons transported from the anode to the cathode through the wire generate a current, which can be measured in the DO meter. The more oxygen enters the system, the more current is generated.

Typical reaction equations are:

Anode reaction	$2\ Zn$	$\rightarrow$	$2\ Zn^{2+} + 4\ e^-$
Cathode reaction	$O_2 + 4\ e^- + 2\ H_2O$	$\rightarrow$	$4\ OH^-$
	$2\ Zn^{2+} + 4\ OH^-$	$\rightarrow$	$2\ Zn(OH)_2$
Overall reaction	$O_2 + 2\ Zn + 2\ H_2O$	$\rightarrow$	$2\ Zn(OH)_2$

The zinc hydroxide that is produced by these reactions is precipitated in the electrolyte solution. A well-designed sensor is not affected by this byproduct of its function. The precipitate neither coats the anode nor consumes the electrolyte, and thus does not affect the performance until the quantity becomes excessive. Some galvanic sensors can be opened and emptied if necessary.

While the membrane keeps most gases out (except for oxygen), it still allows some gases to enter the sensor. Redox reactive gases (such as H_2S or NO_x) can cause additional current and therefore false results, and even the destruction of the electrodes. Such gases are only present in specific applications, which lead to their formation.

As oxygen is consumed during the measurement (see reaction equations above), after some time the concentration of oxygen in the vicinity of the sensor is reduced. Therefore, stirring the sample is essential to redistribute the dissolved oxygen.

The principle of self-polarization means that the oxidation of the anode will continue even if the instrument is not in use. As long as reducible compounds are inside the sensor, the redox reaction will occur. This effect can be minimized by storing the sensor in highly concentrated NaCl solution, which prevents oxygen from passing the membrane. Such a measure not only prolongs the sensor's lifetime, but also averts the accumulation of byproducts.

2.3 Polarographic Dissolved Oxygen Sensors

As a galvanic DO sensor, a polarographic one (sometimes referred to as a Clark-type sensor after Leland Clark) consists of an anode and a cathode in an electrolyte solution. A membrane, which is selectively permeable to oxygen, separates it from the sample (figure 2.3).

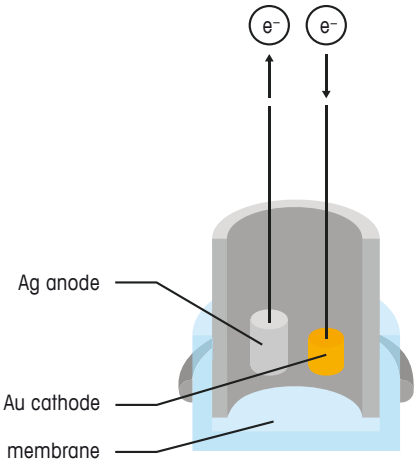


Figure 2.3: Schematic cross-section of a polarographic DO sensor.

The anode is made of silver and the cathode is a noble metal, such as gold or platinum. These electrodes are polarized by a constant voltage, provided by the instrument. As a consequence the anode acquires a positive charge and the cathode a negative one. This process takes a certain amount of time, which is why a polarographic DO sensor has to be polarized prior to the actual measurement.

The sensor elements are separated from the sample by an oxygen-permeable membrane. When oxygen enters the sensor, the oxygen molecules are reduced at the cathode to form hydroxide ions. Because the polarization potential is held constant, the oxygen reaction increases the electrical signal. This effect is proportional to the partial pressure of oxygen in the sample.

The sensor uses a chemical reaction in which the silver anode is oxidized and consumed. In contrast, the cathode is noble and does not participate in the reaction. Instead, it provides a surface onto which oxygen is reduced by electrons transported from the anode through the wire.

Anode reaction	$4\ Ag \rightarrow 4\ Ag^+ + 4\ e^-$
	$4\ Ag^+ + 4\ KCl \rightarrow 4\ AgCl + 4\ K^+$
Cathode reaction	$O_2 + 4\ e^- + 2\ H_2O \rightarrow 4\ OH^-$
	$4\ K^+ + 4\ OH^- \rightarrow 4\ KOH$
Overall reaction	$O_2 + 4\ Ag + 4\ KCl + 2\ H_2O \rightarrow 4\ AgCl + 4\ KOH$

Because silver chloride dissolves poorly in water, the anode reaction results in a tarnished surface, i.e. coating of the electrode with AgCl. Over time, this coating accumulates and slowly reduces the performance of the sensor. However, if there is the possibility to clean the interior, the problem can be minimized.

Like in the measurements with the galvanic sensor, it is essential to ensure an equal oxygen distribution, therefore the sample should be stirred.

The similarities and differences between a galvanic and a polarographic DO sensor are summarized in Table 2.1.

Characteristic	Galvanic DO Sensor	Polarographic DO Sensor
Anode Material	Zinc or lead	Mostly silver
Cathode Material	Silver	Gold or platinum
Polarization	Self-polarization	Instrument-promoted polarization
Polarization Time	None	Some hours (approx. 6)
Anode Depletion	All the time	Only during measurement
Oxidation Product's Fate	Precipitates in the electrolyte	Accumulates on the anode
Inherent Consequences of the Working Principle	• Precipitate covers the membrane and reduces its permeability to oxygen • Anode is depleted regardless of whether sensor is in use or not	• Oxidation product coats the anode and reduces the sensitivity of the sensor • Needs several hours of polarization time prior to measurement

Table 2.1: Summary of the characteristics of galvanic and polarographic DO sensors.

2.4 Optical Dissolved Oxygen Sensors

When a molecule absorbs light of sufficient energy, it can be excited into a higher energy state. However, it cannot remain in this excited state forever, and so it relaxes into the ground state by emitting light. This process is called fluorescence (the more in-depth details are presented in the box).

Fluorescence is a phenomenon linked to the interaction of light with molecules. A molecule in its electronic ground state (called S_0) is excited to a higher vibrational state of the first excited electronic state (S_1) by energy absorption.

Almost immediately, it relaxes to the vibrational ground state of S_1 in a process called vibrational relaxation, which is not detectable. From this excited state, the molecule relaxes further by emitting light—a process called fluorescence.

Fluorescence has a lower energy than the excitation energy, which means a different wavelength and therefore a different color of the absorbed and emitted lights.

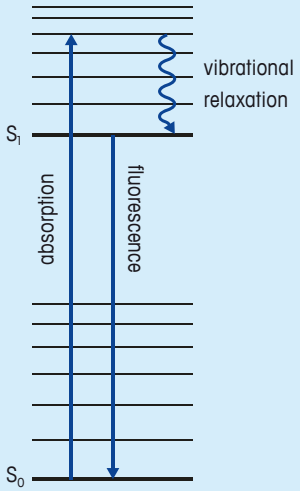


Figure 2.4. The electronic (horizontal thick lines) and vibrational (thin lines) states of a molecule and the process causing fluorescence. The straight arrows indicate light-driven transitions, the wavy arrow an invisible vibrational transition.

The emission of light is not the only way for a molecule to lose the excess of energy. It can also transfer this energy onto another molecule (e.g. by colliding with it and transferring vibrational energy). This is called quenching.

An optical DO sensor uses a special dye embedded in a membrane at the tip of the sensor (figure 2.5). This dye can be excited by absorbing blue light emitted internally by the sensor. As the

excited dye returns to its ground state, it fluoresces by emitting red light, which is measured by a photodetector inside the sensor. When oxygen molecules are present on the outer surface of the membrane, they can absorb the excess energy of the excited dye. By doing so, they reduce (quench) the amount of fluorescence that reaches the photodetector. The more oxygen present in the sample, the more fluorescence quenching, and the lower the measured signal.

The sensor also contains a red light source. This light does not excite the dye—and thus does not cause fluorescence—but is merely reflected by the dye and measured by the photodetector. The red light is used as a reference to account for a decrease in the detected light that is not related to oxygen quenching, e.g. decay of the dye or temperature-dependent sensitivity of the detector.

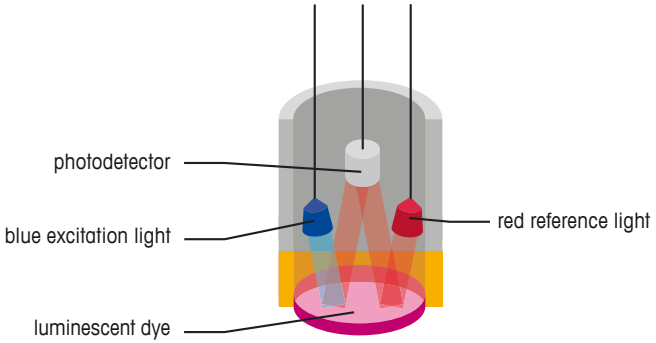


Figure 2.5: Schematic cross-section of an optical DO sensor.

The influence of oxygen on the fluorescence intensity can be described by the Stern-Volmer equation (equation 2.1). The instrument measures the fluorescence in the presence of oxygen and uses this equation for calculating the oxygen concentration:

$$\frac{I_0}{I} = 1 + k_q \times \tau_0 \times [O_2]$$ Equation 2.1

I_0 → fluorescence intensity in the absence of oxygen
I → fluorescence intensity in the presence of oxygen
k_q → quencher rate coefficient of O_2 (temperature-dependent)
τ_0 → lifetime of the excited state of the dye
$[O_2]$ → concentration of oxygen

Equation 2.1 is only an approximation. For higher oxygen concentrations, the relation between the oxygen concentration and the intensity is no longer linear. Thus, the equation has to be adapted with three non-linearity parameters (equation 2.2). These parameters are specific for the dye and are empirically determined by the manufacturer of the sensor.

$$\frac{I_0}{I} = 1 + A \times [O_2] + \frac{B \times [O_2]}{1 + b \times [O_2]}$$ Equation 2.2

I_0 → fluorescence intensity in the absence of oxygen
I → fluorescence intensity in the presence of oxygen
A, B, b → non-linearity parameters of the quencher (of O_2)
$[O_2]$ → concentration of oxygen

As no chemical reaction is involved in the measurement, optical sensors offer a number of advantages over electrochemical sensors:

- No oxygen consumption, no need for stirring.
- No interference of redox reactive gases.
- No electrode consumption, no precipitates inside the sensor.

In addition, higher accuracy at low oxygen levels can be reached. On the other hand, optical DO sensors are more expensive and usually require more power during operation.

3 Handling the Equipment

This chapter summarizes some hints and recommendations for the daily use of DO sensors. They are based on generally accepted handling and operational rules.

3.1 DO Sensor Preparation

Optical DO sensors do not require any preparation prior to their usage. Electrochemical sensors on the other hand, must be checked for membrane integrity. Additionally it must be guaranteed that the electrolyte is properly replenished, if electrolyte refilling is applicable. When using a polarographic sensor, the proper polarization of the sensor has to be ensured.

3.2 DO Sensor Calibration

The calibration of a DO sensor can be carried out as a 1-point or a 2-point calibration. A measurement in water-saturated air should be used as the first point (this corresponds to 100% oxygen saturation). If the sensor is calibrated with just one point, the meter

can only adjust the slope of the calibration curve by assuming a lack of signal at 0% (figure 3.1, left). For determining the offset, a second calibration point is required. Because the offset of most DO sensors does not deviate much from zero, a 1-point calibration is sufficient for many applications.

For a second calibration point, an oxygen-free standard solution should be prepared (this corresponds to 0% oxygen saturation). For this purpose, Zero Oxygen tablets are dissolved in water in order to eliminate all the dissolved oxygen in it. With this second point, the offset can be determined (figure 3.1, right). It is recommended to perform a 2-point calibration when measuring samples with an oxygen saturation below 10% or an oxygen concentration below 1 mg/L.

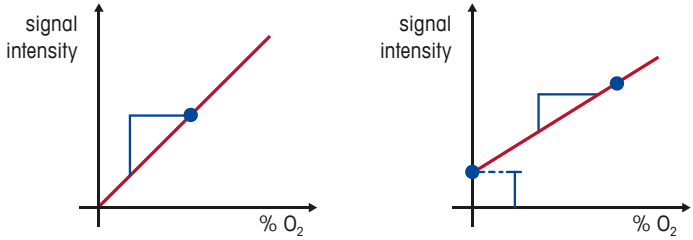


Figure 3.1: While a single calibration point only enables adjusting the slope (left), the offset can be adjusted with two points (right). For the sake of clarity, the difference between a 1- and 2-point calibration is wildly exaggerated.

3.3 Carrying out a DO Measurement

For most electrochemical DO sensors, stirring is inevitable because the sensors consume oxygen while measuring. The stirring should be kept at a constant speed. In contrast, optical sensors do not require it because they do not consume oxygen.

In order to reduce the measurement duration, the sensor tip should be submerged into the sample before starting the measurement. This procedure will allow the oxygen concentration and temperature to equilibrate.

Air bubbles at the sensor tip must be avoided. Otherwise the oxygen concentration of the air bubbles will be measured, leading to false results. In general, any coating of the membrane will affect the readings. Thus, sample residues on the sensor (such as oils, algae, or slurries) should be immediately removed after the measurement. Scratching the membrane should be avoided, as it would damage it permanently.

The presence of oxidizing gases, such as chlorine, nitrous oxide, and nitric oxide, can also interfere with the measured oxygen concentration. In addition, sulfur-based molecules like H_2S and SO_2 are also known to interfere with DO measurements. While these compounds only directly influence DO measurements of electrochemical sensors, all DO sensor types are indirectly affected because the gases' oxidizing potential can damage the sensor material.

3.4 Converting Units

An electrochemical DO sensor measures the amount of oxygen inside itself. Thus, the sensor can only measure how strongly the dissolved oxygen is pushed into the sensor through the selective membrane. As such, the primary result of the measurement is the partial pressure of oxygen in solution.

This value can be converted to oxygen concentration if the solubility of oxygen in the used solvent is known. For water, the most common solvent, this relationship is well understood. Because it depends on the temperature and the salinity of the sample, these two values also have to be determined or known.

The meter uses equation 3.1 for converting the measured value into an oxygen concentration:

$$c = \frac{I - I_0}{S_L} \times \frac{[c_s(p_n) - (Sal \times F(T))]}{X_{O_2} \times (p_n - p_w)}$$ Equation 3.1

- c → O_2 concentration (mg/L)
- I → measured current (nA)
- I_0 → measured current at zero O_2 (assumed to be 0 nA)
- S_L → sensor slope (nA/mbar)
- $c_s(p_n)$ → solubility of O_2 in water at standard pressure p_n (mg/L)
- Sal → salinity of sample solution (mg/g)
- F(T) → salinity correction factor at temperature T (mg/L, see table 3.1)
- X_{O_2} → molar ratio of O_2 in calibration gas (in air equal to 0.2095)
- p_n → standard pressure (mbar)
- p_w → water vapor pressure at calibration temperature (mbar)

Temperature/°C	Salinity correction factor F(T)/mg/L	Temperature/°C	Salinity correction factor F(T)/mg/L
0	0.0875	31	0.0365
1	0.0843	32	0.0353
2	0.0818	33	0.0345
3	0.0789	34	0.0339
4	0.0760	35	0.0331
5	0.0739	36	0.0323
6	0.0714	37	0.0316
7	0.0693	38	0.0309
8	0.0671	39	0.0302
9	0.0650	40	0.0296
10	0.0632	41	0.0289
11	0.0614	42	0.0283
12	0.0593	43	0.0277
13	0.0582	44	0.0272
14	0.0561	45	0.0266
15	0.0545	46	0.0261
16	0.0532	47	0.0256
17	0.0514	48	0.0251
18	0.0500	49	0.0247
19	0.0489	50	0.0242
20	0.0475	51	0.0238
21	0.0464	52	0.0234
22	0.0453	53	0.0231
23	0.0443	54	0.0228
24	0.0432	55	0.0225
25	0.0421	56	0.0222
26	0.0407	57	0.0220
27	0.0400	58	0.0218
28	0.0389	59	0.0216
29	0.0382	60	0.0215
30	0.0371		

Table 3.1: Salinity correction factor F(T).

3.5 Maintenance and Storage

After a measurement, the sensor should be cleaned with water and wiped with a soft tissue. Especially when measuring biological samples, microbiological growth should be carefully avoided. For an optimal performance, a sensor should be stored in a safe environment at temperatures between 5 and 45 °C and fast temperature changes should be avoided.

For short-term storage, a galvanic DO sensor should be rinsed with deionized water and stored in storage solution. For long-term storage, it should also be short-circuited (to prevent deterioration due to continuous self-polarization) and stored in a cool place. To avoid the 6-hour polarization requirement of a polarographic DO sensor, it can be left connected to the instrument. For long-term storage, it should be detached from the instrument, because continuous polarization will gradually reduce its lifetime. Provided the sensor is filled with inner electrolyte and the protective cap is placed over the membrane, it can be stored for several months. However, to use the sensor again after more than 3 months of storage, the electrolyte should be replaced. If storage of more than 6 months is intended, the electrolyte should be removed.

Exposure to H_2S or SO_2 can tarnish the electrode of electrochemical DO sensors. Additionally, polarographic cathodes become coated with AgCl over time as a consequence of the chemical reactions involved in their function. The coating of an electrode can be removed mechanically. After washing with distilled water and drying with a soft tissue, the probe can be refilled with new electrolyte to be fully operational.

In general the frequency of electrolyte renewal depends on the concentration of oxidizing gases during measurements and how often the sensor is used.

An optical sensor should be stored dry. Sensors with a replaceable membrane module should have it exchanged as soon as the sensor shows signs of reduced performance.

4 Biological Oxygen Demand (BOD)

Due to their similar names, **Biological Oxygen Demand** (BOD) and **Chemical Oxygen Demand** (COD) are sometimes confused. In essence, COD describes the amount of oxygen a sample could chemically react with. COD is not determined by a DO measurement but by titration.

A great variety of monitoring programs (e.g. for drinking water, waste water, sewage water, groundwater of fertilized fields, etc.) depend on measuring the amount of nutrients in the water. As nutrients stimulate the growth of aquatic plants, they can indirectly lead to an increase in organic material as the plants decay, which subsequently promotes the growth of aerobic microorganisms.

Because of the risk they can pose to human health as well as to the stability of ecosystems, monitoring the ability of a water sample to support microbial life is crucial. However, directly measuring the concentration of nutrients is challenging and counting microbes in a sample is time-demanding. In addition, the number of microbes is not a strong indicator of the nutrient concentration in the sample, as different microbes exhibit different metabolisms and consume different amounts of nutrients.

The oxygen demand of these biological systems, on the other hand, constitutes a better water quality indicator because it is much easier to measure and it is directly related to the microbiological consumption of nutrients. This correlation stems from the fact that most organisms need oxygen for their metabolism, which is used proportionally to their activity level. Hence, if the water is overpopulated by microbes, they will consume a high percentage of all the dissolved oxygen, leading to detrimental consequences for the other cohabitating species, namely fish.

In order to determine the BOD, the amount of dissolved oxygen is measured at two points in time in the same sample (Fig. 4.1), most commonly day zero and day five. In between these time points, the sample is incubated in a sealed bottle, in complete darkness and at a constant temperature of 20 ± 2 °C, thus avoiding any replenishment of oxygen either by diffusion or by photosynthesis. To allow for proper biodegradation, the sample is normally seeded with microorganisms that consume oxygen during the decomposition of organic compounds. Nutrients can also be added. In parallel to the sample, the BOD of control

samples has to be determined; they are prepared in the same way as the sample and incubated under the same conditions.

The oxidation of inorganic materials such as sulfides as well as nitrogenous compounds is considered an interference. In most cases the BOD is determined as carbonaceous BOD (CBOD). This is achieved by adding an inhibitor that prevents the oxidation of nitrogenous compounds. With this setup, the amount of consumed oxygen relates directly to the amount of organic matter that was present in the water.

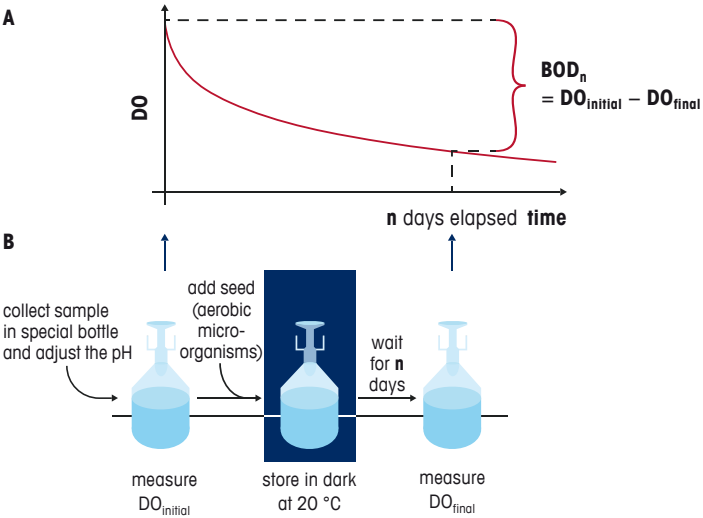


Figure 4.1: General principle of a BOD measurement: (A) The BOD value of a sample is calculated as the difference between the initial DO concentration ($DO_{initial}$) and the final one, measured after n days (DO_{final}). (B) In between those measurements, the sample is stored in a closed container, in the dark and at 20 °C.

For an accurate, reliable and compliant measurement, a number of parameters should be controlled, such as the pH of the sample (see Table 4.1). Additionally special bottles (such as Karlsruher or Wheaton bottles) should be used and in some cases, to achieve an oxygen consumption within the required range, the sample has to be diluted.

	DIN/EN standards	**APHA standard**
pH	6 to 8	6.5 to 7.5
Elapsed time BOD5	120 h ± 2 h	120 h ± 4 h
Elapsed time BOD7	168 h ± 2 h	168 h ± 4 h
BOD bottle types	Karlsruher or Wheaton bottles	Karlsruher or Wheaton bottles
Seed	should cause less than 1 mg/L BOD	should cause less than 1 mg/L BOD

Table 4.1: Requirements for a BOD sample, according to DIN/EN and APHA standards.

For a BOD measurement of an undiluted sample, the BOD_n can be easily calculated using equation 4.1.

$$BOD_n = c(t_0) - c(t_n) \qquad \text{Equation 4.1}$$

$c(t_0)$ → O_2 concentration at time zero
$c(t_n)$ → O_2 concentration after n days

In case of a diluted sample, the calculation has to be adjusted (equation 4.2). A blank sample, consisting of dilution water only, has to be analyzed simultaneously. In table 4.2, dilution factors are suggested depending on the expected BOD value.

$$BOD_n = [(c(t_0) - c(t_n)) - \frac{V_{total} - V_{sample}}{V_{total}} \times (c_{blank}(t_0) - c_{blank}(t_n))] \times \frac{V_{total}}{V_{sample}} \qquad \text{Equation 4.2}$$

$c(t_0)$ → O_2 concentration in the sample at time zero
$c(t_n)$ → O_2 concentration in the sample after n days
$c_{blank}(t_0)$ → O_2 concentration in the blank at time zero
$c_{blank}(t_n)$ → O_2 concentration in the blank after n days
V_{sample} → sample volume
V_{total} → total volume of the sample (undiluted sample + dilution water)

Expected BOD_n mg/L of oxygen	Dilution factor
3 to 6	between 1 and 2
4 to 12	2
10 to 30	5
20 to 60	10
40 to 120	20
100 to 300	50
200 to 600	100
400 to 1200	200
1000 to 3000	500
2000 to 6000	1000

Table 4.2: Suggested dilution factors for BOD analysis.

An example of a diluted sample: A bottle with a volume of 250 mL is filled with 100 mL of sample and 150 mL of dilution water. The initial DO values are 7.5 mg/L for the sample and 7.4 mg/L for the blank. After five days the sample contains 5.6 mg/L dissolved oxygen and the blank 7.3 mg/L.

The resulting BOD is calculated in equation 4.3.

$$BOD_5 = [(7.5\frac{mg}{L} - 5.6\frac{mg}{L}) - \frac{250\ mL - 100\ mL}{250\ mL} \times (7.4\frac{mg}{L} - 7.3\frac{mg}{L})] \times \frac{250\ mL}{100\ mL} = 4.6\ mg/L$$

Equation 4.3

Some examples for typical BOD values for water quality and for sewage water are given in tables 4.3 and 4.4, respectively.

BOD in ppm	Water quality
1–2	Very Good: Low levels of organic waste in the water.
3–5	Fair: The water is only moderately clean.
6–9	Poor: The water is polluted to some extent. It is expected to contain bacteria and other microorganisms that are decomposing the organic material present in it.
>10	Polluted: The water contains high amounts of organic material. There is a chance that manure is being discharged in this water body.

Table 4.3: BOD levels for water quality according to the International Organization for Standardization, 2003.

BOD in ppm	Sewage
20	For municipal sewage after a three-stage treatment.
600	For untreated sewage in Europe.
200	For untreated sewage in the USA (which is more diluted).

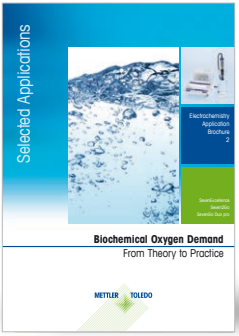
Table 4.4: Typical BOD levels in sewage water.

5 For More Information

For more details about BOD measurement, please consult our BOD application brochure:

Biochemical Oxygen Demand
Application Brochure

One important analysis to assess water quality is determining the BOD (biochemical oxygen demand). With this comprehensive application brochure and appropriate METTLER TOLEDO equipment, you will be capable of setting up your own BOD determination process within the shortest time.



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