Instruction Manual

OXY-10

10-Channel Fiber-Optic Oxygen Meter

Instruction Manual
OXY-10

Software Version OXY10v3_33FB

April 2005

Table of Contents

1 Preface

Congratulations!

You have chosen a new, innovative technology for measuring oxygen!

The OXY-10 is a compact, easy to transport and completely PC-controlled fiber-optic oxygen meter. The data evaluation is PC-supported as well.

The OXY-10 was specially developed for small fiber-optic oxygen sensors, flow-through cells and integrated sensor systems. It is based on a novel technology, which creates very stable, internally referenced measured values. This allows a more flexible use of oxygen sensors in many different fields of interest.

Optical oxygen sensors (also called optodes) have important advantages over common Clark type electrodes:

- They are small
- They do not consume oxygen
- Their signal does not depend on the flow rate of the sample
- They have an excellent long-term stability
- They can be physically divided from the measuring system which means a contactless measurement
- They can be autoclaved and γ-sterilized.

Therefore, they are ideally suited for the examination of small sample volumes, long-term measurements in difficult samples, and for biotechnological applications.

A set of different oxygen minisensors, flow-through cells and integrated sensor systems is available to make sure you have the sensor which is ideally suited to your application.

Please feel free to contact our service team to find the best solution for your application.

Your Loligo Team

2 Safety Guidelines

PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS INSTRUMENT!

This device has left our works after careful testing of all functions and safety requirements.

The perfect functioning and operational safety of the instrument can only be ensured if the user observes the usual safety precautions as well as the specific safety guidelines stated in these operating guidelines.

- Before connecting the device to the electrical supply network, please ensure that the operating voltage stated on the power supply corresponds to the mains voltage.
- The perfect functioning and operational safety of the instrument can only be maintained under the climatic conditions specified in Chapter 10 "Technical Data" in this operating manual.
- If the instrument is moved from cold to warm surroundings, condensate may form and interfere with the functioning of the instrument. In this event, wait until the temperature of the instrument reaches room temperature before putting the instrument back into operation.
- Balancing, maintenance and repair work should exclusively be carried out by a suitable, qualified technician, trained by us.
- Especially in the case of any damage to current-carrying parts, such as the power supply cable or the power supply itself, the device must be taken out of operation and protected against being put back into operation.
- If there is any reason to assume that the instrument can no longer be employed without a risk, it must be set aside and appropriately marked to prevent further use.
- The safety of the user may be endangered, e. g., if the instrument
    - is visibly damaged
    - no longer operates as specified
    - has been stored under adverse conditions for a lengthy period of time
    - has been damaged in transport.
- If you are in doubt, the instrument should be sent back to the manufacturer for repair and maintenance.
- The operator of this measuring instrument must ensure that the following laws and guidelines are observed when using dangerous substances:
    - EEC directives for protective labor legislation
    - National protective labor legislation
    - Safety regulations for accident prevention
    - Safety data-sheets of the chemical manufacturer.

The OXY-10 is not protected against water spray

The OXY-10 is not water-proof

The OXY-10 must not be used under environmental conditions which cause water condensation in the housing

The OXY-10 must not be opened.

We explicitly draw your attention to the fact that any damage of the manufactural seal will render all guarantee warranties invalid.
Any internal operations on the unit must be carried out by personnel explicitly authorized by Loligo, and under antistatic conditions.

The OXY-10 may only be operated by qualified personnel.

This measuring instrument was developed for use in the laboratory. Thus, we must assume that, as a result of their professional training and experience, the operators will know the necessary safety precautions to take when handling chemicals.

Keep the OXY-10, power supply and optical sensors out of the reach of children!

As the manufacturer of the OXY-10, we only consider ourselves responsible for safety and performance of the device if

- the device is strictly used according to the instruction manual and the safety guidelines
- the electrical installation of the respective room corresponds to the DIN IEC/VDE standards.

The OXY-10 and the sensors must not be used in vivo examinations on humans!

The OXY-10 and the sensors must not be used for human-diagnostic or therapeutical purposes!

3 Description of the OXY-10 Device

3.1 Instrument

The **OXY-10** is a 10-channel oxygen meter for use with fiber-optic oxygen minisensors based on a 2 mm polymer optical fiber (POF). The OXY-10 system detects oxygen (the oxygen partial pressure) in both solutions (dissolved oxygen) as well as in the gaseous phase.

For operation, a PC/Notebook with RS-232 interface is required. The OXY-10 is controlled using a comfortable software, which also saves and visualizes the measured values.

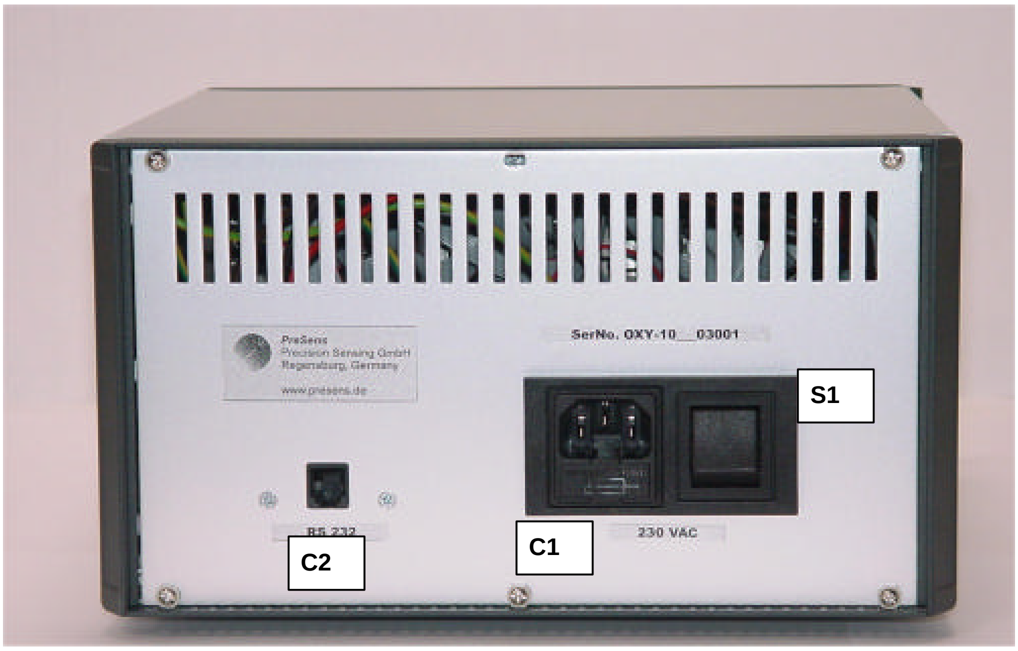
OXY-10 **does not** contain temperature sensors; temperature changes during the measurement are not compensated by the software.

3.2 Front Panel of the OXY-10 Device

ELEMENT	DESCRIPTION	FUNCTION
CH1 – CH10	SMA fiber connector	Connect the fiber-optic oxygen minisensor here
Line	Blue Control LED	on: instrument on off: instrument off

3.3 Rear Panel of the OXY-10 Device

The electrical specifications of all rear panel connectors are given in the technical specification sheet. Please read also the technical notes to avoid mistakes.



ELEMENT	DESCRPTION	FUNCTION
S1	ON/OFF switch	Switches the device ON and OFF
C1	Line adapter for power supply	Connector for 230 V AC power supply
C2	RS-232 interface (male)	Connect the device with a RS-232 data cable to your PC/Notebook here

4 Required Basic Equipment

- Oxygen meter OXY-10*
- Software for OXY-10*
- PC / Notebook
  (System requirements: Windows 98/2000/XP/Millenium/NT 4.0; Pentium processor, at least 166 MHz, 64 MB RAM; a RS 232 port or a USB port and a USB-serial adapter is needed)
- RS 232 Cable *
- Line adapter (110 - 220 V AC, 12 V DC) *
- Oxygen-sensitive minisensor
  The minisensors are mounted into different types of housings
- Vessels for calibration standard 100 (water vapor-saturated air ≡ 100 % air saturation) and calibration solution 0 (oxygen-free water)

We recommend Schott laboratory bottles with a thread which can be obtained by Merck Eurolab (ordering number: 215L1515)

* *scope of supply*

5 Planar Oxygen Minisensors

5.1 Sensor Characteristic of the Oxygen-Sensitive Minisensors

The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. Figure 5.1 shows a typical response curve of an oxygen-sensitive sensor. In the presence of oxygen the signal - in our case the phase angle Φ - decreases. The phase angle Φ can be related to the oxygen content as shown in Figure 5.2. The theoretical aspects are explained more detailed in the appendix.

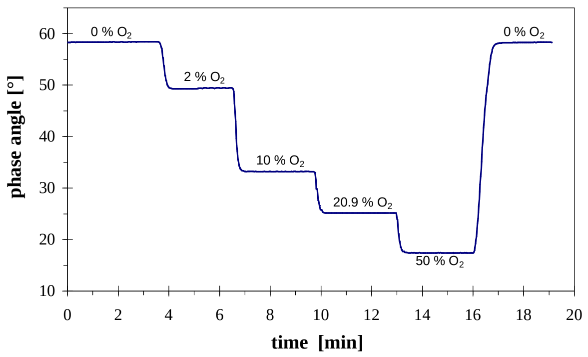


Figure 5.1 Response of minisensor **PSt3** toward changes in the oxygen concentration.

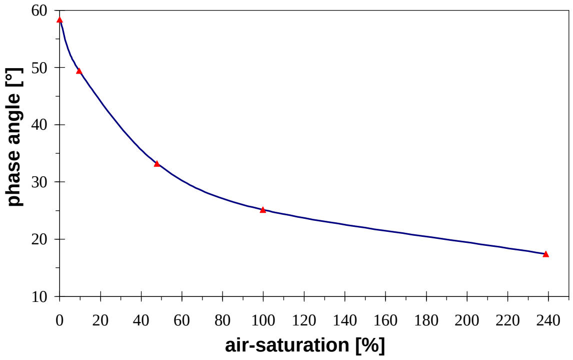


Figure 5.2 Effect of the phase angle of minisensor **PSt3** on different oxygen contents.

Measuring range and limit of detection

We offer an oxygen sensor membrane called **PSt3** which is tailored for oxygen measurements up to 250 % air-saturation. The measuring ranges and the limit of detection of this sensor are given in Table 5.1.

***Table 5.1** Measuring range and limit of detection of the **PSt3 oxygen sensor membrane**.*

	Dissolved Oxygen	**Gaseous & Dissolved Oxygen**
Measurement range	0 - 22 mg/L (ppm) 0 - 700 µmol	0 - 250 % air-sat. 0 - 50 % oxygen-sat. 0 - 380 Torr 0 - 500 hPa
Limit of Detection (LOD)	0.15 % air-saturation, 15 ppb dissolved oxygen	0.31 hPa, 0.23 Torr

Resolution and accuracy

Since the oxygen calibration plot displays a non-linear behavior, the oxygen resolution is given for four different partial pressures at 20 °C, the accuracy for two different partial pressures. The resolution in oxygen is also transformed in different oxygen units.

Table 5.2. *Oxygen resolution and accuracy of the* ***PSt3 oxygen sensor membrane*** *at different oxygen contents at 20 °C and 1013 mbar.*

Resolution **at 20 °C and 1013 hPa**	0.09 ± 0.005 mg/L (ppm) 2.72 ± 0.01 mg/L (ppm) 9.06 ± 0.05 mg/L (ppm) 22.65 ± 0.15 mg/L (ppm)	1 ± 0.05 % air-sat. 30 ± 0.1 % air-sat. 100 ± 0.5 % air-sat. 250 ± 1.7 % air-sat.
		0.21 ± 0.01 % oxygen 6.3 ± 0.02 % oxygen 20.9 ± 0.1 % oxygen 52.4 ± 0.35 % oxygen
	2.83 ± 0.14 µmol 85.0 ± 0.28 µmol 283.1 ± 1.4 µmol 798.0 ± 4.7 µmol	1.55 ± 0.08 Torr 46.7 ± 0.2 Torr 155.5 ± 0.75 Torr 388.8 ± 2.6 Torr
		2 ± 0.1 hPa 60 ± 0.3 hPa 200 ± 1 hPa 500 ± 0.3 hPa
Accuracy (20 °C)	± 1% at 100 % air-saturation; ± 0.15% at 1 % air-saturation	

Temperature

The oxygen sensors can be used in the temperature range of 0 to 50 °C. The OXY-10 **does not** contain temperature sensors. Temperature changes during the measurement are not compensated by the software.

Cross sensitivity:

No cross sensitivity exists for ***carbon dioxide*** (CO_2), ***hydrogen sulfide*** (H_2S), ***ammonia*** (NH_3), ***pH***, any ionic species like ***sulfide*** (S_2^-), ***sulfate*** (SO_4^{2-}), ***chloride*** (Cl^-) or ***salinity***. Turbidity and changes in the stirring rate have no influence on the measurement.

The sensors can also be used in ***methanol***- and ***ethanol-water*** mixtures as well as in ***pure methanol*** and ***ethanol***.

We recommend to avoid other organic solvents, such as acetone, chloroform or methylene chloride, which may swell the sensor matrix.

Interferences were found for gaseous sulfur dioxide (SO_2) and gaseous chlorine (Cl_2). Both of them mimic higher oxygen concentrations.

Response time

The response time (t_{90}, 90 % of the signal change has occurred) of the **PSt3** oxygen sensor is less than 30 s in solution (non-stirred) and even less than 8 s in the gas phase.

The response time (t_{90}) of the oxygen sensor is dependent from the diffusion rate of oxygen through the sensor layer, and, hence, on the thickness of the sensor layer and the stirring rate. A typical oxygen response curve of sensor membrane **PSt3** in a non-stirred and stirred sample solution is shown in Figure 5.3 below. The response times (t_{90}) of sensor membrane **PSt3** are listed in Table 5.3 below.

Unlike electrodes, optical sensors do **not** consume oxygen and the signal is independent of changes in flow velocity which means that stirring decreases the response time, but has no effect on the measured value.

Optical isolation of the oxygen-sensitive layer which is applied to exclude ambient light and improve chemical resistance will slow down the sensor response.

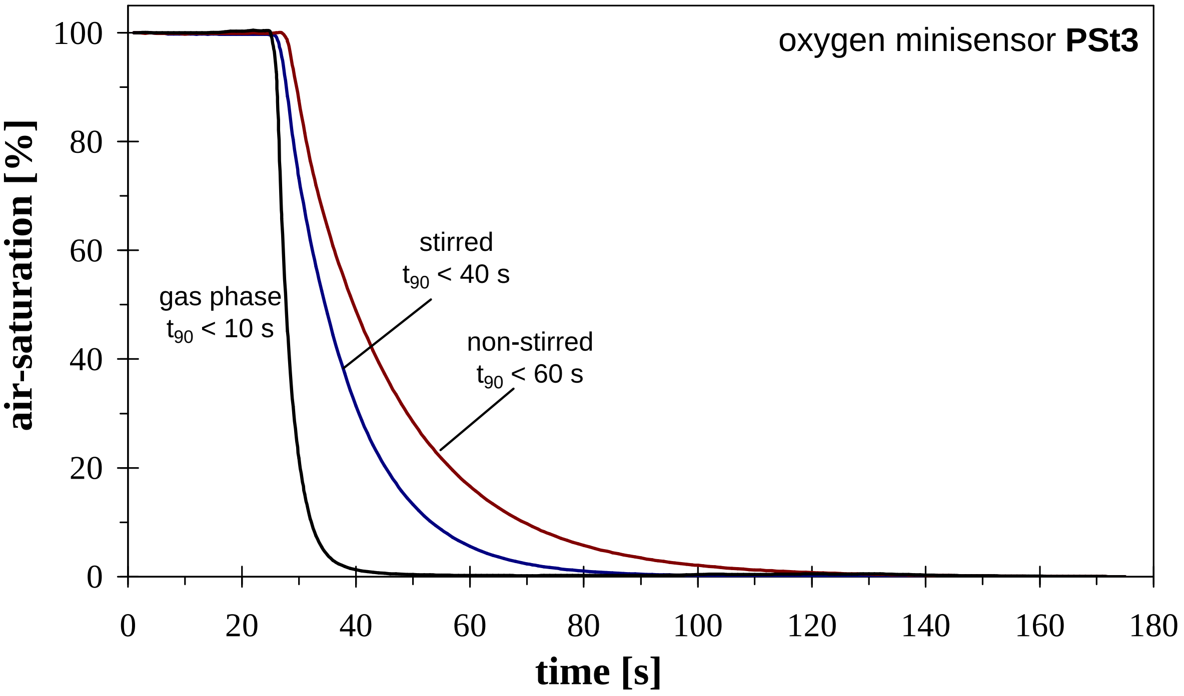


Figure 5.3. *Response characteristic of an optical isolated oxygen sensor* ***PSt3*** *in a stirred and a non-stirred sample solution and in the gas phase.*

Table 5.3. *Response times (t_{90}) of the* ***oxygen sensors membrane PSt3****.*

	dissolved oxygen		**gaseous oxygen**
	stirred	**not stirred**	
oxygen sensor PSt3			
t_{90} without optical isolation	< 20 s	< 40 s	< 6 s
t_{90} with optical isolation	< 30 s	< 60 s	< 10 s

Optical isolation

Optical isolated sensor tips are required if your sample shows intrinsic fluorescence between 540 - 700 nm. Consequently, an optical isolation is recommended measuring in whole blood, urine or chlorophyll-containing samples. Using optical isolated sensors excludes the impact of colored samples and ambient light on measurements. Furthermore, the optical isolation layer is applied to exclude strong ambient light, to improve chemical resistance especially against oily samples as well as to reduce bio-fouling on the sensor membrane.

Optical isolated sensor tips of oxygen sensors enable measurement in photosynthetically active samples, since stimulation of photosynthesis due to emission of blue-green light from the fiber tip is avoided.

We offer additional optical isolation for all types of oxygen sensors.

Sensor Stability

The oxygen-sensitive membrane stands gamma-sterilization, sterilization by ethylene oxide, steam autoclavation (140 °C, 1.5 atm), CIP conditions (cleaning-in-place, 5 % NaOH, 90 °C), as well as a 3 % H_2O_2 solution.

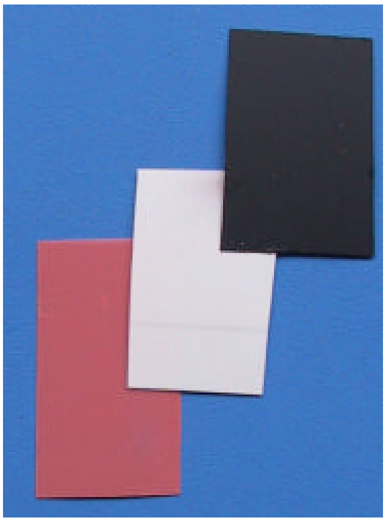
The oxygen-sensitive material may be subject to photodecomposition resulting in a signal drift. Photodecomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light.

Table 5.4. *Drift in % air-saturation at 100 % air-saturation when illuminating the oxygen sensor* ***PSt3*** *for 1, 12 and 24 hours in the continuous mode (1 sec mode).*

	Drift per hour	Drift per 12 hours	Drift per 24 hours
PSt3	---	---	< 0.4 % air-saturation

5.2 Housings of Oxygen-Sensitive Minisensors

The fiber-optic oxygen sensors are based on 2 mm polymer optical fibers (**POF**). Depending on the respective application, we offer a set of different standard designs.



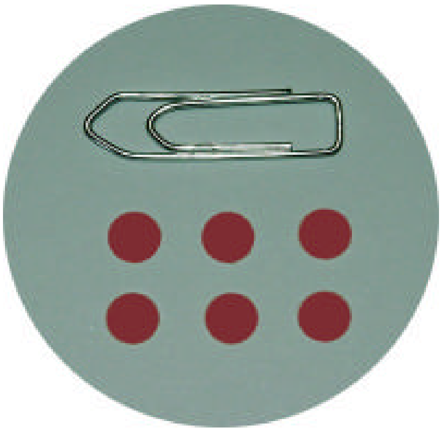
Planar oxygen-sensitive **sensor foils (SP)**

Flow-through cell design connected to a 2 mm POF **(FTC)**

2 mm **Dipping Probe (DP)** with coated sensor foil

Of course, it is possible to build ***customer-specific*** designs. Please feel free to contact our service team to find the best solution for your application.

5.2.1 Planar Oxygen-Sensitive Foils (SP-PSt3)

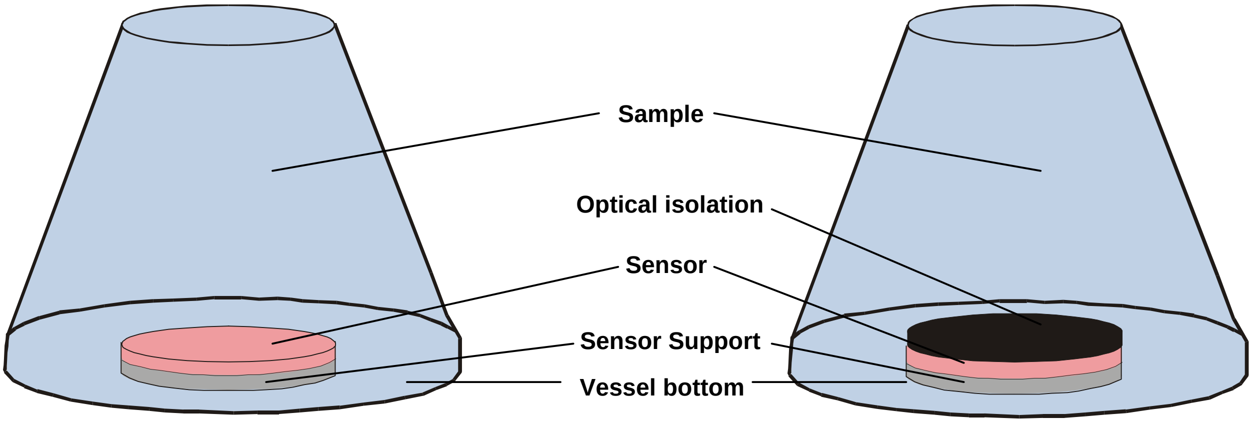


Planar oxygen sensors **SP-PSt3** immobilized onto different supports (polyester, glass) are available for customer- specific applications. Sensors based on a polyester support can be easily cut into small pieces using a razor blade. Round spots (sensor spots) of 3 mm in diameter can be punched.

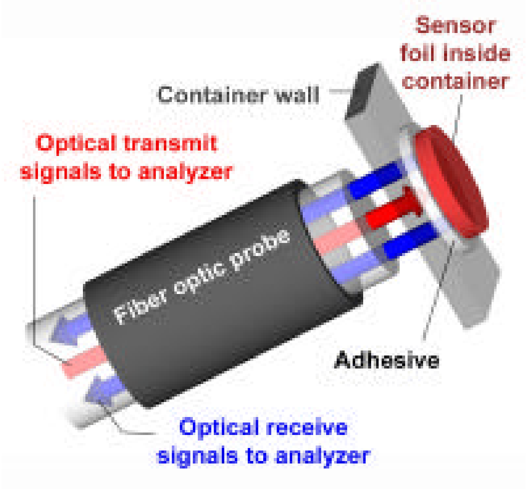
The **sensor spots** can be glued, for example, inside glass vials such as cell culture flasks, bags, and disposables. The oxygen concentration can be measured **non-invasively** and **non-destructively** from outside through the wall.

Only prerequisite: The wall has to be transparent and non-fluorescent.

Please note: Be sure to glue the sensor spots onto your vessel with the proper side! The sensor support (polyester foil or glass) on which the sensor is spotted (identifiable by its faint reflection) is glued to the vessel, while the sensor itself must look toward the sample. The figure below shows how the (highly enlarged) sensor spot must be glued to the vessel.



Sensor spot (SP) glued into a vessel (no optical isolation). Optical isolated sensor spot (SP) glued into a vessel.



Features

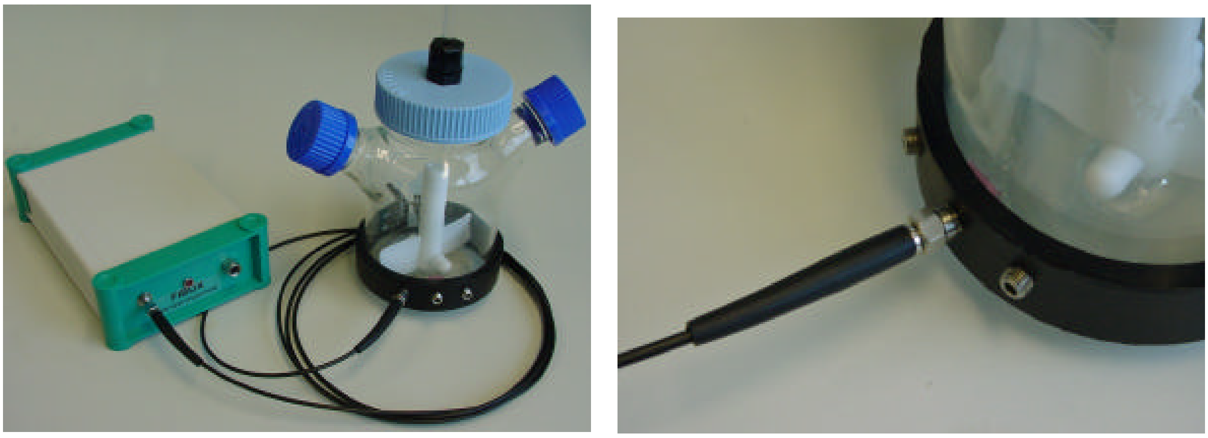
- non-invasive and non-destructive measurement from outside through the wall of the flask
- excellent mechanical stability and long-term stability (more than 100000 data points without drift)
- online monitoring
- response time (t_{90}) in the order of 30 s
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Oxygen sensor immobilized onto a glass support

- stands CIP (Cleaning In Place) conditions
- sterilizable (autoclave (130 °C, 1.5 atm), ethanol, ethylene oxide, H_2O_2)

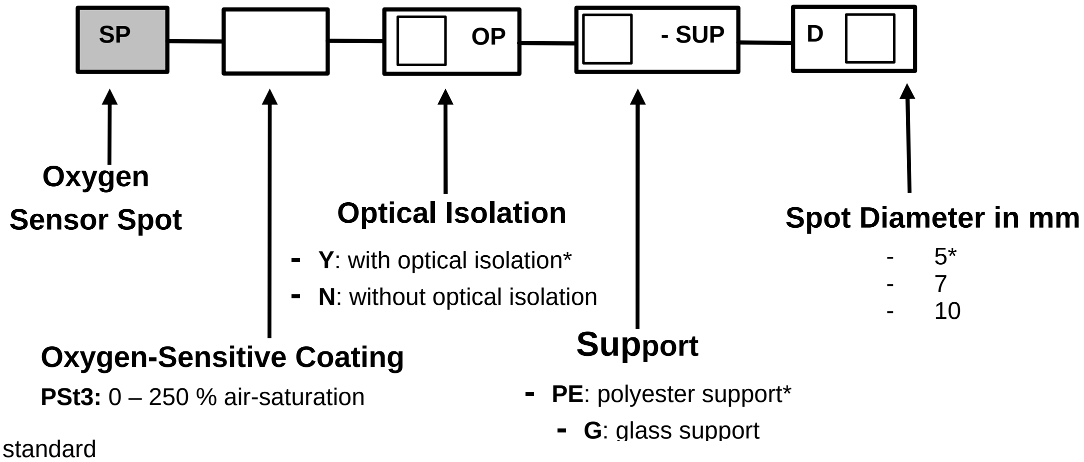
Oxygen sensor immobilized onto a polyester support

- stands CIP (Cleaning In Place) conditions
- sterilizable (ethanol, ethylene oxide, H_2O_2)
- **not** autoclavable
- flexible



A polymer optical fiber is used as a light guide between the OXY-10 oxygen meter and a sensor foil (SP-PSt3) which was glued inside a glass vial to read out the analyte concentration non-invasively and non-destructively **from outside through the transparent wall of the flask**.

Ordering information



Oxygen Sensor Spot

Oxygen-Sensitive Coating

PSt3: 0 – 250 % air-saturation

Optical Isolation

- **Y**: with optical isolation*
- **N**: without optical isolation

Support

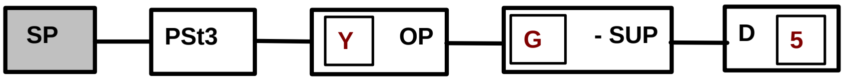
- **PE**: polyester support*
- **G**: glass support

Spot Diameter in mm

- 5*
- 7
- 10

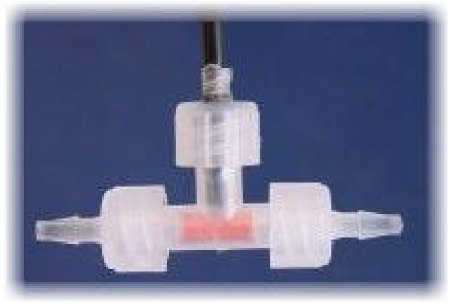
* standard

Example



With this code you will order a planar oxygen sensor spots, type ***PSt3*** (0 - 250 % air-saturation) with optical isolation (***YOP***) immobilized onto a glass support (***G-SUP***). The spot diameter is 5 mm.

5.2.2 Flow-Through Cell with Integrated Planar Oxygen Sensor (FTC-PSt3)

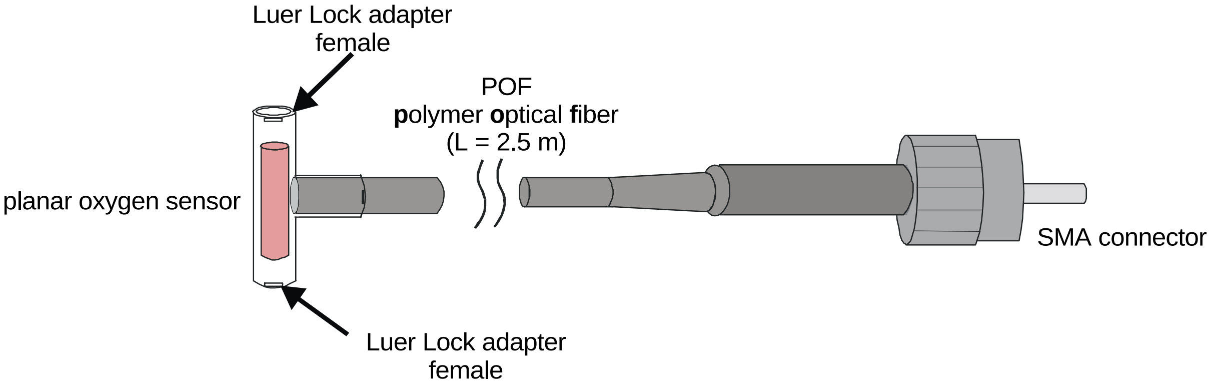


The flow-through oxygen minisensor **(FTC-PSt3)** is a miniaturized fiber-optic chemical sensor integrated in a T-shape flow-through cell.

The flow-through cell is connected to the oxygen meter OXY-10 by a polymer optical fiber with 2 mm diameter as a light guide. A glass tube with 2 mm inner diameter (4 mm outer diameter) is coated with oxygen-sensitive dye at its inner wall. The volume for liquid inside the FTC cell is about 100 (± 10) µl.

The standard T-shape flow cell can be easily connected via Luer-Lock adapters to external tubings. Liquids (like water, blood, etc.) can be pumped through the cell.

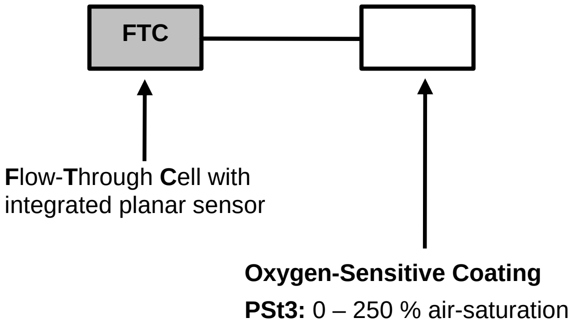
Schematic drawing of flow-through cell oxygen sensors



Features

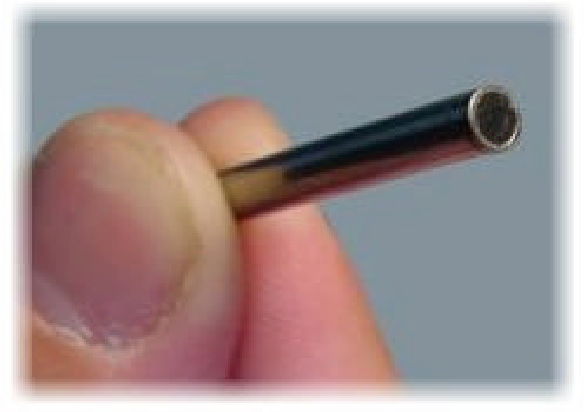
- very robust sensor with an excellent long-term stability (more than 100000 data points without drift)
- online monitoring
- sterilizable (autoclave (130 °C, 1.5 atm), ethanol, ethylene oxide)
- response time (t_{90}) in the order of 1 minute
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.
- stands CIP conditions (cleaning-in-place, 5 % NaOH, 90°C)

Ordering Information



Order code for the Flow-Through Cell: FTC-PSt3

5.2.3 Oxygen Dipping Probe (DP-PSt3)

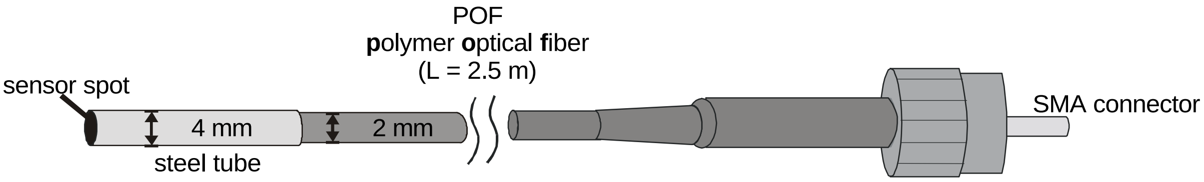


This oxygen sensor consists of a polymer optical fiber with a polished distal tip which is coated with a planar oxygen-sensitive foil.

The end of the polymer optical fiber is covered with a high-grade steel tube, to protect both the sensor material and the POF.

Usually, the fiber is coated with an optical isolated sensor material in order to exclude ambient light from the fiber tip.

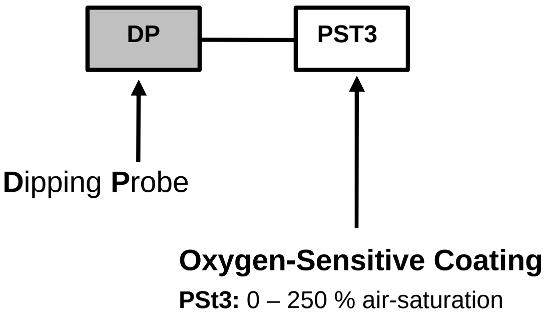
Schematic drawing



Features

- usable for process application
- very robust sensor with an excellent long-term stability (more than 100000 data points without drift)
- sterilizable (H_2O_2, ethanol, ethylene oxide)
- **not** autoclavable (POF does not stand autoclaving conditions (130 °C, 1.5 atm))
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

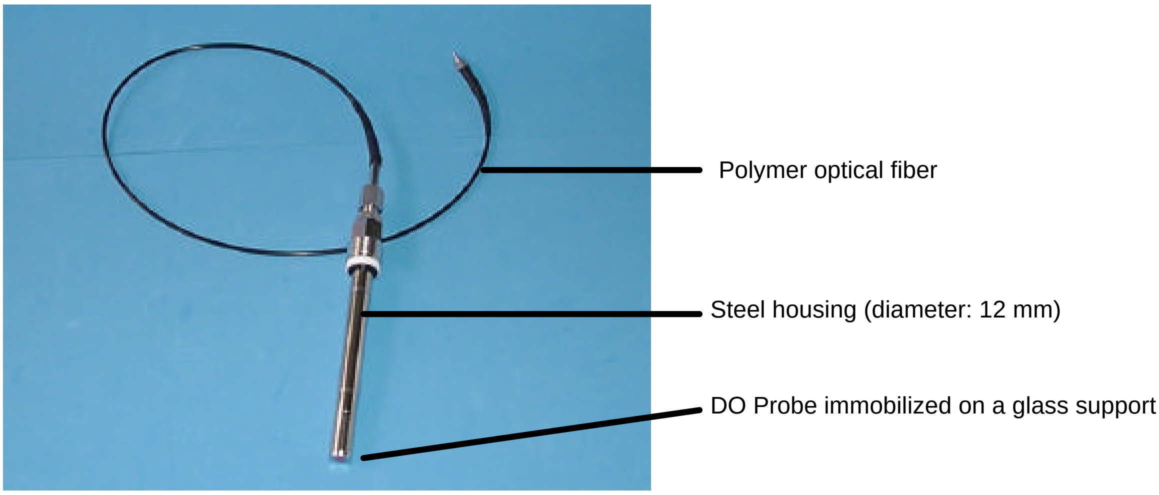
Ordering information



Order code for the Oxygen Dipping Probe: DP-PSt3

5.2.4 Oxygen Probe for Inline Measurements in Fermenters (OIM)

OIM consists of a fitting made from stainless steel. The oxygen sensor is integrated in the top of the metal fitting (as shown below). The metal fitting is connected to the instrument via a polymer optical fiber. The standard fiber cable length is 2.5 m. OIM is available in different sizes (12 mm, 25 mm) and standard OIM fits to B. Braun Biostat B and B. Braun Biostat C fermenters.

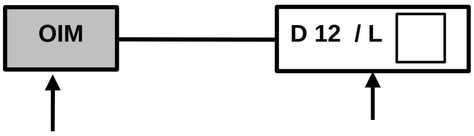


This chemo-optical DO probe has outstanding properties:

- The system can be used after autoclavation without recalibration
- The system is fully autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life

Please note: The OIM is only ready for use together with the Optical Exchange Cap (OEC, see page 16) containing the oxygen sensor membrane.

Ordering information



Oxygen **I**nline **M**easurements

Steel Tube

Diameter [mm] / Length [mm]

- 12 / 120
- 12 / 140
- 12 / 215
- 12 / 325
- 12 / 425

5.2.5 OIM Exchange Cap (OEC-PSt3)

Applications:

The **O**IM **E**xchange **C**ap (**OEC**) is the sensitive coating in a metal cap. It is used to replace the old sensitive coating and has outstanding properties:

- The PSt3-coated OEC can be used after autoclavation without recalibration
- The PSt3-coated OEC is autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- The PSt3-coated OEC can be easily exchanged
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life
- Optical isolation prevents cross-sensitivity of the sensor towards turbid or fluorescent samples.
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

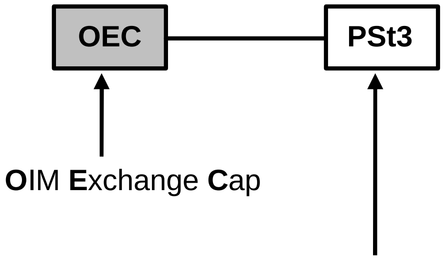
The OEC is coated with the oxygen-sensitive material **PSt3**. It has to be integrated into the Oxygen Probe OIM.

The sensor signal is transmitted to the instrument via a polymer optical fiber. The standard fiber cable length is 2.5 m. If you need a cable length of more than 2.5 m (up to 15 m available), please contact our service team.

Response times of the oxygen sensor OEC-PSt3

Response time	Stirred	Not stirred	Gaseous Oxygen
t_{90}	< 60 s	< 120 s	< 20 s

Ordering information



Order code for the OIM Exchange Cap: OEC-PSt3

5.2.6 OxyFinger Chemo-Optical DO Probe for Mini-Fermenters (OFG-PSt3)

OxyFinger consists of a glass test tube (glass finger) which is coated with an oxygen-sensitive foil. The sensor signal is transmitted to the instrument via a polymer optical fiber.

Cable lengths between 2 and 15 meters are available.

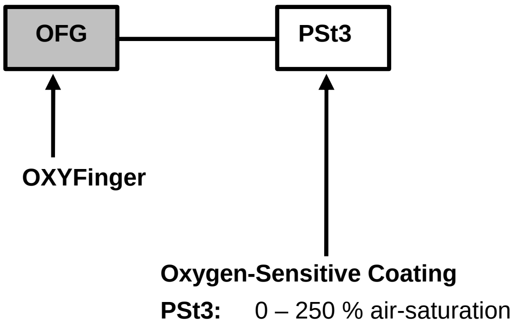
OxyFinger will be manufactured following your specifications. Please specify both length and diameter of the glass finger and the size of the vessel closure.

Please contact our service team.

The OxyFinger Chemo-Optical DO Probe for Mini-Fermenters has outstanding properties:

- The system can be used after autoclavation without recalibration
- The system is fully autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Ordering information

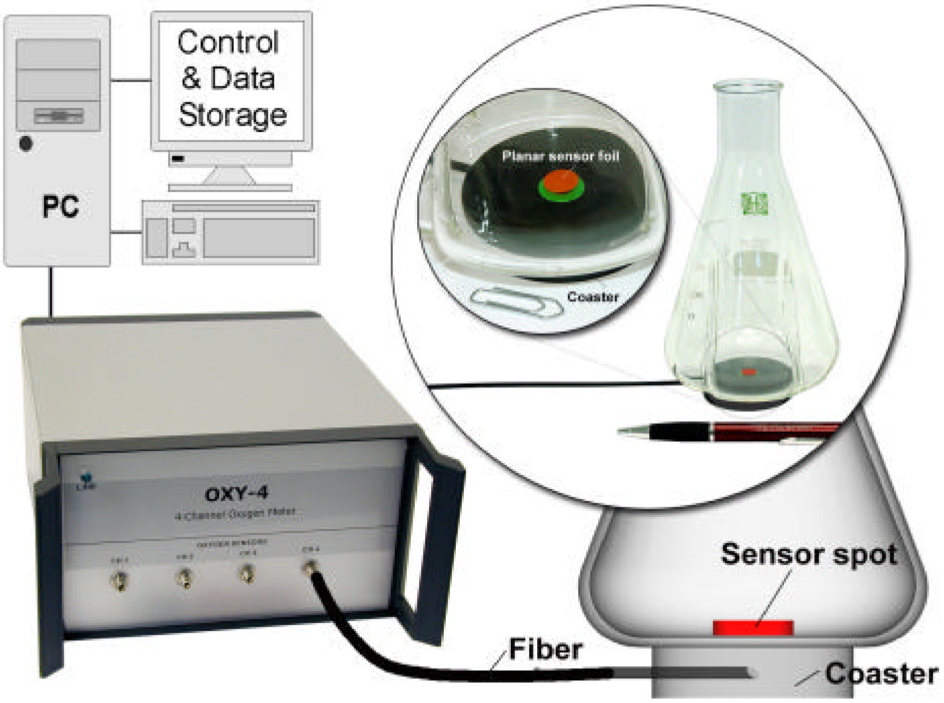


Order code for the OxyFinger: OFG-PSt3

5.2.7 Coaster for Shaking Flasks and Spinner Flasks (CSF)

Application:

Online control of oxygen in shaking flasks and spinner flasks.



System set-up for online DO measurement in shaking flasks

Specifications:

The coaster for shaking flasks and Spinner flasks is a tool for online monitoring of dissolved oxygen concentration in shaking flasks. The coaster for shaking flasks does not contain a sensor. It redirects the light so that oxygen sensors in shaking flasks can be read out.

The coaster has a colored circle which makes it easy to position it right under the sensor. The position of the optical read-out is flexible and can be adjusted to different sizes of shaking flasks.

The standard cable length is 2.5 m. The cable has an outer diameter of 2.6 mm.

Please note:

The coaster for shaking flasks and spinner flasks does not contain a sensing layer. It is designed to read out sensor foils which are attached to the inner side of a shaking flask, spinner flask or a similar vessel (e.g. beaker).

Ordering Information:

Order code for the Coaster for Shaking Flasks: CS

6 Software

6.1 Software Installation

This software is compatible with Windows 98/2000/Millenium/NT4.0/XP.

1. Insert the supplied disc/CD into the respective drive. Start the file *setup.exe* in folder *installer* and install the program to the desired folder.
2. Thereafter, Labview® runtime engine is installed. Please click Next in the following three windows without changing the chosen options.
3. Finally click Finish in the next window.

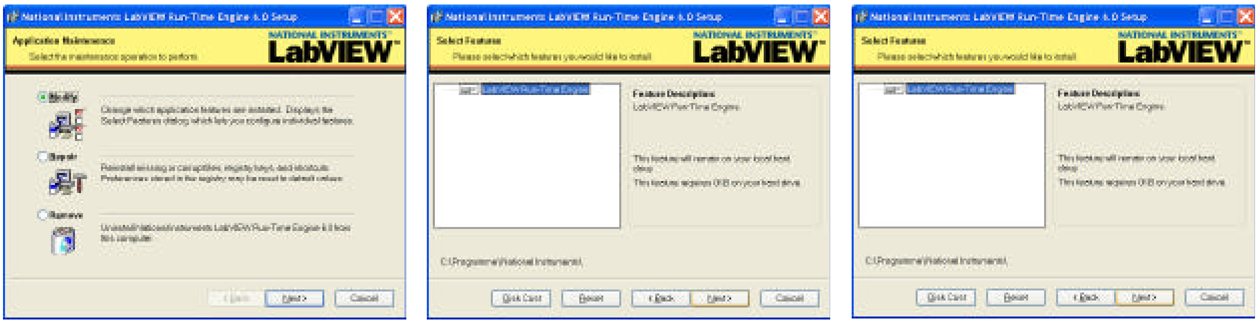
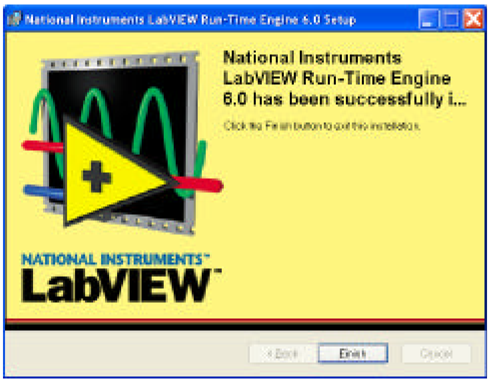


Figure 6.1 **Screenshots of the windows appearing during the installation process of Labview® Run-time Engine.**



National Instruments LabVIEW Run-Time Engine 6.0 has been successfully i...

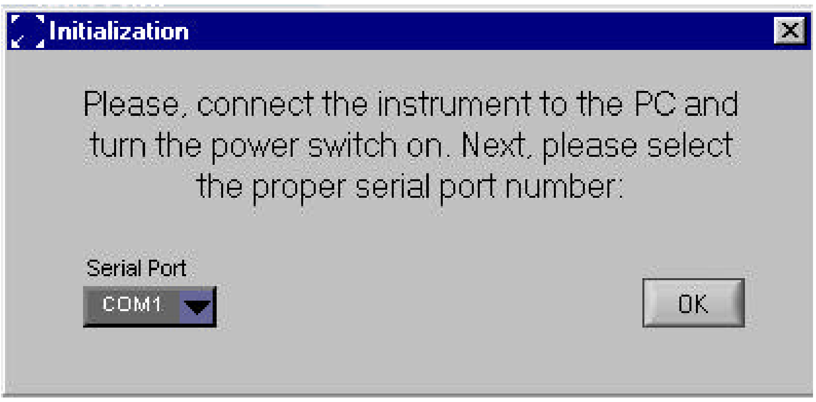
Click the Finish button to exit this installation.

Figure 6.2 **Screenshot of the last window of the installation of Labview® Run-Time Engine.**

Now the Software is properly installed and you will find a link in your Start menu.

6.2 Starting OXY-10 and its Software

1. Connect the OXY-10 via the supplied serial cable to a serial port of your computer. Tighten the cable with the screws on your computer and on the OXY-10.
2. Connect the power supply.
3. Please close all other applications as they may interfere with the software. Start the program *OXY10v3_22* in the Start menu. The following information window appears:

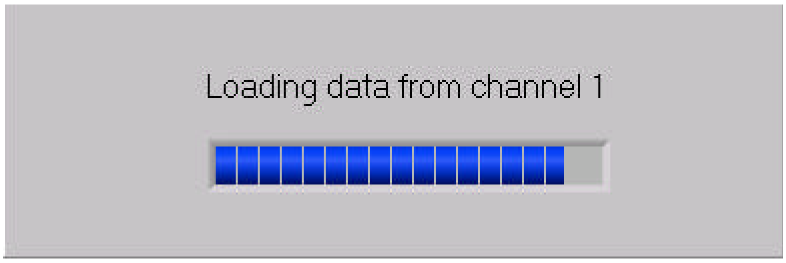


Initialization

Please, connect the instrument to the PC and turn the power switch on. Next, please select the proper serial port number:

Serial Port: COM1

4. Choose the right com port with a left mouse click on ‘Serial Port’. Then press OK. The following window appears and all 10 channels are initialized.



Loading data from channel 1

If the wrong com port is adjusted the software gives an error message. Please change the com port and press OK.

6.3 Function and Description of the OXY-10 Software

The window shown below is displayed after starting the software OXY-10 software:

The program has 5 main sections:

1. Measurement
2. Calibration
3. Logging
4. All Channels: Graphical and numerical display of all channels
5. Graphical and numerical display of the respective channel

6.3.1 Measurement

With this software it is possible to start the measurement scanning all channels by clicking the ‘**All Channel**’ button or selected channels by clicking the required ‘**single channel**’ button. Before starting the measurement, you have to choose the ‘**oxygen unit**’ (% air-saturation, hPa, Torr, mg/L or µmol/L), the **‘sampling rate’** (fastest sampling (= max): 15 s), and enter the measurement ‘**temperature**’.

The button ‘**Warnings**’ has the possible captions.

- *WARNINGS ON*

  This caption indicates that the software will warn if no sensor is connected or if the sensor intensity is low.

  If sensor intensity is low the *Intensity check* should be done by the user. If the sensor intensity process fails the sensor must be replaced.
- *WARNINGS OFF*

  This caption indicates that the software will show no warnings. This is only recommended for ‘advanced’ users.

On the right side you can find the version number of the software. If you have questions regarding the OXY-10 software, please contact our service team and have the software version number ready.

Furthermore, the actual ‘**date/time**’ are presented on the right side.

6.3.2 Calibration

The detailed calibration process of oxygen sensors you can find in chapter 7.

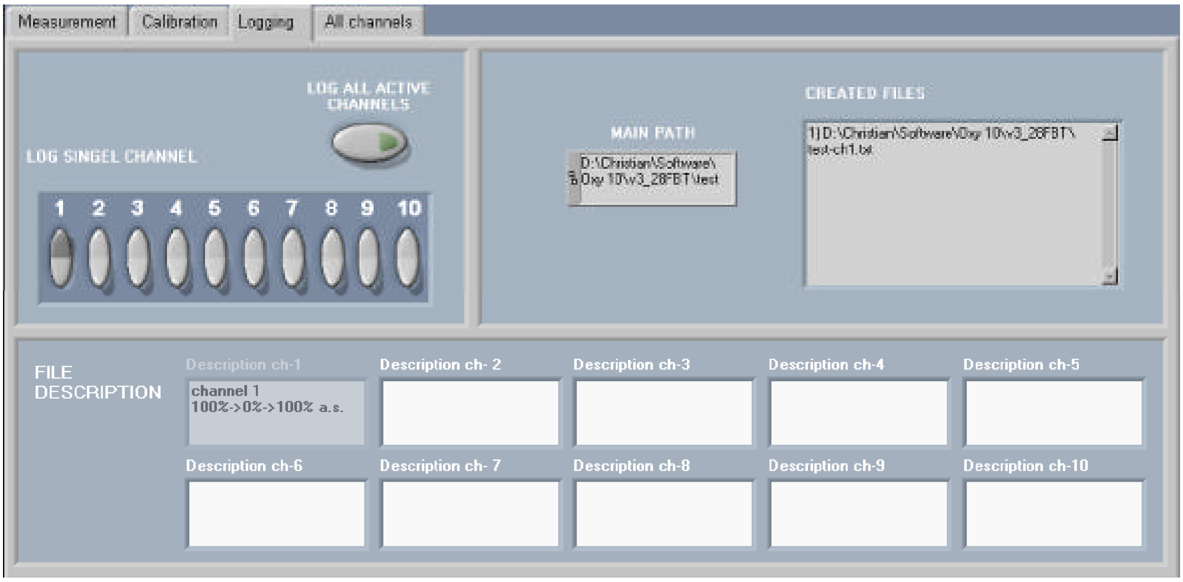
6.3.3 Logging

Measured data are logged during the measurement. Add contents to the description fields in the *File description* section before starting logging (max. 3 lines). This is not necessary but helpful as the description is later stored in the data file.

1. Depending on the scan mode click on ***All channels*** for logging all channels or ***Single channel*** for single channel logging.
2. In the appearing “save as” window specify filename **without extension** and its location.

The PC software automatically creates one to ten different files. The filename consists of the specified filename and the extensions “-ch1.txt”, “-ch2.txt”, to -ch10.txt”, respectively (-ch stands for channel). All filenames are displayed in the section *Created files*.

Please note: Logging must be enabled **before** starting the measurement. After the measurement it is not possible to store the measured values. Starting logging during the measurement is also possible, but stores only the values from the beginning of logging on.



MAIN PATH: D:\Christian\Software\Oxy 10\v3_28FBT\test

CREATED FILES: 1) D:\Christian\Software\Oxy 10\v3_28FBT\test-ch1.txt

Description ch-1: channel 1
100%->0%->100% a.s.

6.3.4 All Channels: Graphical and numerical of all channel

NOTE: The *All channels* window is only enabled when scanning times for all adjusted channels are measured with the same scanning time.

The *graphical display* shows all data since the measurement was started. The oxygen unit is the same as specified in the window *Measurement* at the beginning of the measurement. It is not possible to change the unit during the measurement.

With the *data choice button* it is possible to switch between the data

- a) oxygen concentration in the unit specified in the window *Measurement* at the beginning of the measurement
- b) phase (see appendix for description of phase and amplitude)
- c) amplitude (see appendix for description of phase and amplitude)

The *Clear graph button* is used to clear the graphical window from all former data.

The scale of the y-axis and the starting point of the x-axis can be set to different modes by clicking on the *Autoscale* button. Two options are implemented:

1. Autoscale On

   The software determines the minimum and maximum values to display all data.

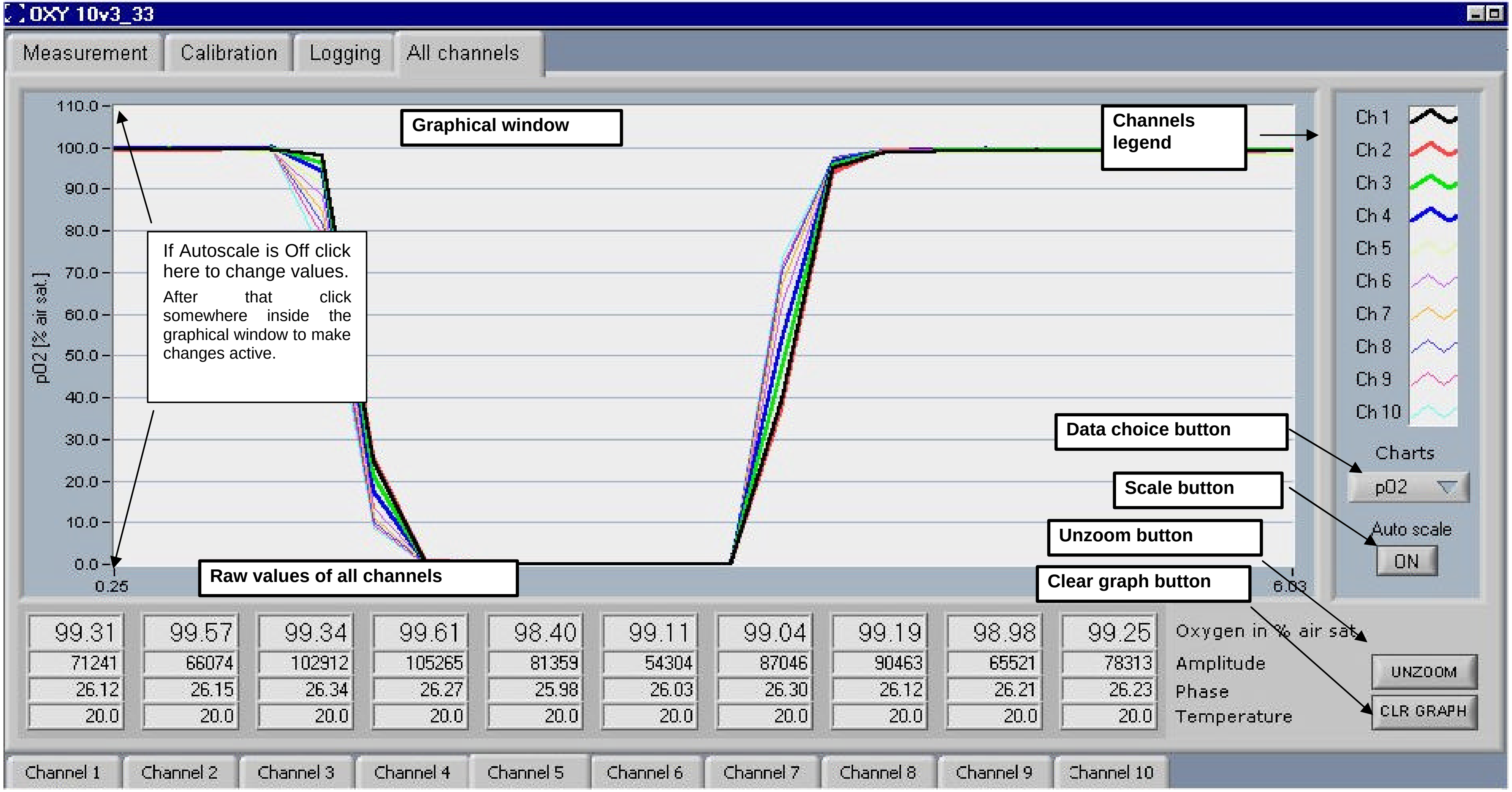
2. Autoscale Off

   The maximum and minimum value of the y-axis can be defined by the user. By clicking on the current value the minimum and/or maximum value can be changed. After typing the new value one has to click in the graphical window again to realize the change. For the x-axis, only the starting value can be changed.

Zoom Function:

1. Press the left mouse button and drag from left to right to enlarge a certain area of the graphical window. The graphical window displays the selected data points and is not actualized with new data.
2. Press the left mouse button and drag from right to left to recover the original display, or click the '**Unzoom**' button.

The oxygen content is displayed in the chosen unit, the temperature in °C. The raw values (the phase angle in degrees and the sensor amplitude in mV) are also displayed (see Appendix for description of phase and amplitude).



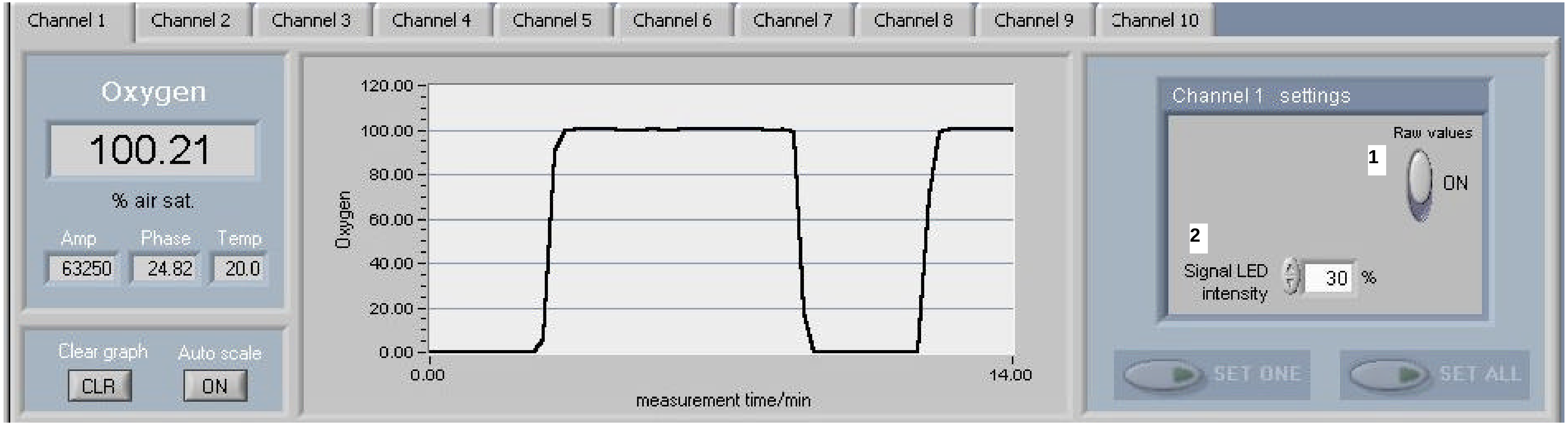
If Autoscale is Off click here to change values.

After that click somewhere inside the graphical window to make changes active.

99.31	99.57	99.34	99.61	98.40	99.11	99.04	99.19	98.98	99.25	Oxygen in % air sat
71241	66074	102912	105265	81359	54304	87046	90463	65521	78313	Amplitude
26.12	26.15	26.34	26.27	25.98	26.03	26.30	26.12	26.21	26.23	Phase
20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	Temperature

6.3.5 Graphical and numerical display of the respective channel

The measuring values and all raw values are displayed for each channel separately. By clicking on the respective channel, the actual measured oxygen concentration is shown in the unit you have adjusted in the menu bar ‘measurement’. The raw values amplitude and phase value can be displayed by clicking on ‘Raw values on’ in the **Channel settings** on the right side, or they can be hidden by clicking on ‘off’.



Oxygen 100.21 % air sat.

Amp 63250

Phase 24.82

Temp 20.0

Clear graph CLR

Auto scale ON

Raw values ON

Signal LED intensity 30 %

Channel settings:

In the channel settings the user is able to configure each channel separately and to optimize the instrument performance.

1. **Raw values**:

The raw values (the phase angle in degrees and the sensor amplitude in mV) can be displayed/hidden by clicking the ‘On/Off’ button. If the raw values are not displayed, the signal amplitude is shown via a bar; the longer the bar, the higher the signal.

2. **Signal LED intensity**

Note: After changing the intensity any sensor calibration for the changed channel has to be repeated.
With the current of the LED you can adjust the amount of light illuminating the sensor spot. The LED current can be set manually between 10 and 100 %. If you increase the LED current, the signal amplitude increases, since a higher light density illuminates the sensor spot.

The channel settings can be adjusted for the respective chosen channel by clicking ‘set one’. However, it is also possible to set all channels with the same value by clicking ‘set all’.

Please note:

By increasing the light intensity you increase the amplitude of the oxygen microsensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

Ten windows are used to display the data of each channel separately. The windows named *channel 1* to *channel 10* show the data of the respective channel. The oxygen unit is the same as specified in the window *Measurement* at the beginning of the measurement. It is not possible to change the unit during the measurement. The *last measured oxygen value* is displayed in the marked field on the left.

The scale of the y-axis and the starting point of the x-axis can be set to different modes by clicking on the *Scale button*. Two options are implemented:

1. Autoscale On

   The software determines the minimum and maximum values to display all data.

2. Autoscale Off

   The maximum and minimum value of y-axis and the minimum of the x-axis can be defined by the user. By clicking on the current value the minimum and/or maximum value can be changed.

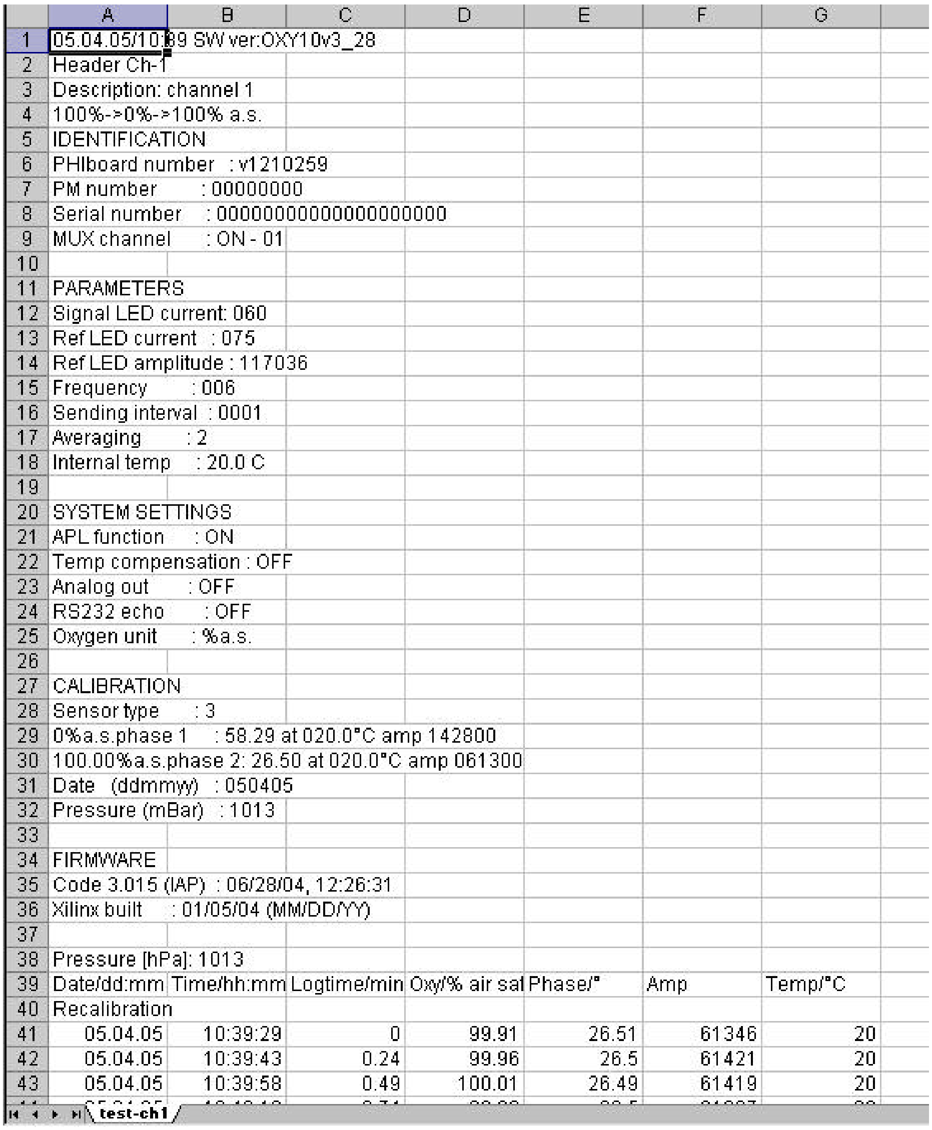
The *Clear graph button* is used to clear the graphical window from all former data.

6.4 Data Handling

In the head of the ASCII file, you find

Line 1	time and date when the measurement has started; software version
Line 2	number of measured channel
Line 3 - 4	description of the measurement if a content is added to the *File description* section (max. 3 lines)
Line 5 - 9	Identification information of the hardware (only useful for service)
Line 11 - 18	Set parameters of the hardware (only useful for service)
Line 20 - 25	System settings (only useful for service)
Line 27 – 32	Information about the calibration which was used for the measurement (useful if a mistake during calibration is supposed)
Line 34 – 36	Information about the firmware (only useful for service)
Line 38	Pressure at which calibration was performed
Line 39 - xx	The following rows, separated by semicolons, list the measuring data. The first two rows contain the **date** and **time**, the third the **log-time** in minutes, the fourth the **oxygen content** in the chosen unit. The raw data - **phase angle** in [°] and the **amplitude** in [mV] - are stored in the fifth and sixth row, respectively. The seventh row contains the **temperature** in °C as given by the user. Raw data can be used for user-defined recalculations according to the formulas and tables listed in the appendix.

The data can be processed with any text editor. The use of Microsoft® Excel® is possible by importing the file.



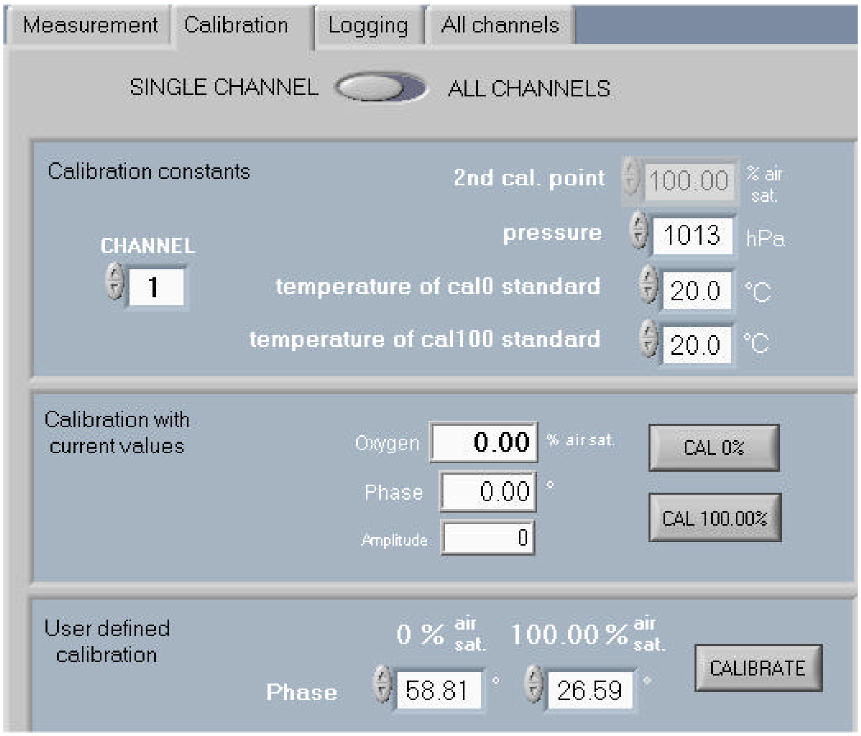
	A	B	C	D	E	F	G
1	05.04.05/10:39 SW ver:OXY10v3_28						
2	Header Ch-1						
3	Description: channel 1						
4	100%->0%->100% a.s.						
5	IDENTIFICATION						
6	PHIboard number : v1210259						
7	PM number : 00000000						
8	Serial number : 00000000000000000000						
9	MUX channel : ON - 01						
10							
11	PARAMETERS						
12	Signal LED current: 060						
13	Ref LED current : 075						
14	Ref LED amplitude : 117036						
15	Frequency : 006						
16	Sending interval : 0001						
17	Averaging : 2						
18	Internal temp : 20.0 C						
19							
20	SYSTEM SETTINGS						
21	APL function : ON						
22	Temp compensation : OFF						
23	Analog out : OFF						
24	RS232 echo : OFF						
25	Oxygen unit : %a.s.						
26							
27	CALIBRATION						
28	Sensor type : 3						
29	0%a.s.phase 1 : 58.29 at 020.0°C amp 142800						
30	100.00%a.s.phase 2: 26.50 at 020.0°C amp 061300						
31	Date (ddmmyy) : 050405						
32	Pressure (mBar) : 1013						
33							
34	FIRMWARE						
35	Code 3.015 (IAP) : 06/28/04, 12:26:31						
36	Xilinx built : 01/05/04 (MM/DD/YY)						
37							
38	Pressure [hPa]: 1013						
39	Date/dd:mm	Time/hh:mm	Logtime/min	Oxy/% air sat	Phase/°	Amp	Temp/°C
40	Recalibration						
41	05.04.05	10:39:29	0	99.91	26.51	61346	20
42	05.04.05	10:39:43	0.24	99.96	26.5	61421	20
43	05.04.05	10:39:58	0.49	100.01	26.49	61419	20

Figure 6.3 Logged data of channel 1 displayed in Microsoft® Excel®

7 Calibration

The second window in the menu bar of the OXY-10 software is named *Calibration*. It consists of two sub windows. The sub window ***SINGLE CHANNEL*** (Figure 7.1) is used to calibrate each channel individually. The sub window ***ALL CHANNELS*** (Figure 7.2) calibrates all channels with one command. Use this option if you want to calibrate all channels in one calibration procedure.

Please note:
For calibrating the sensor, it is necessary to stop the measurement. Calibration during measurement is not possible. To stop the measurement, go to the window '***Measurement***' and click the '***All channel***' button, if you perform a measurement with all channels, or the respective active channel button. During a measurement the calibration buttons are not active.



Calibration constants

CHANNEL 1

2nd cal. point 100.00 % air sat.

pressure 1013 hPa

temperature of cal0 standard 20.0 °C

temperature of cal100 standard 20.0 °C

Calibration with current values

Oxygen 0.00 % air sat.

Phase 0.00 °

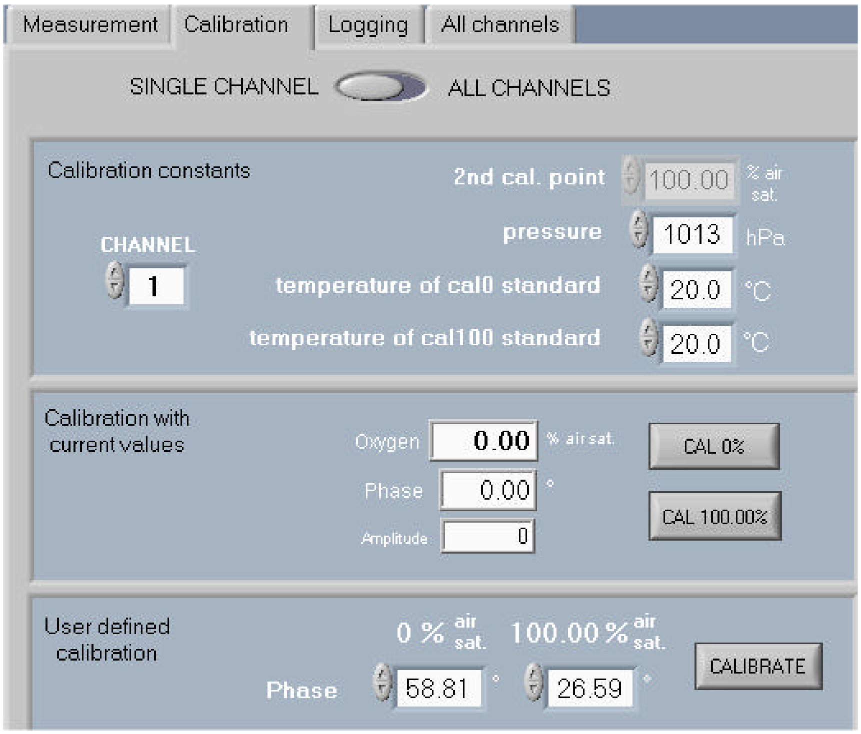
Amplitude 0

User defined calibration

0 % air sat. 100.00 % air sat.

Phase 58.81 ° 26.59 °

Figure 7.1 Screenshot of the sub window *SINGLE CHANNEL* of the window *Calibration.*



Calibration constants

CHANNEL 1

2nd cal. point 100.00 % air sat.

pressure 1013 hPa

temperature of cal0 standard 20.0 °C

temperature of cal100 standard 20.0 °C

Calibration with current values

Oxygen 0.00 % air sat.

Phase 0.00 °

Amplitude 0

User defined calibration

0 % air sat. 100.00 % air sat.

Phase 58.81 ° 26.59 °

Figure 7.2 Screenshot of the sub window *ALL CHANNELS* of the window *Calibration.*

7.1 Calibration of Oxygen Dipping Probe DP-PSt3

There are two ways to calibrate the optical sensors. One is the use of two calibration standards, the other one is to type in known constants. The first way needs two calibration standards. Their preparation is described in chapter 7.1.1. The second possibility is described in chapter 7.1.2.

7.1.1 Calibration with two Calibration Standards

For the calibration with current values you need calibration standards and your mounted sensor.

7.1.1.1 Preparation of the Calibration Standards

Calibration of oxygen minisensors is performed using a conventional two-point calibration in *oxygen-free water* **(cal 0)** and *water vapor-saturated air* or *air-saturated water* **(cal 100)**. In the sub window *Calibration constants*, cal 100 is denoted as 2^{nd} cal point.

Preparation of calibration standard cal 0 (oxygen-free water)

1. Add 1 g sodium sulfite (Na_2SO_3) to a vessel and label it ***cal 0***.
2. Dissolve Na_2SO_3 in 100 mL water.

   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination. The shelf life of ***cal 0*** is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard cal 100 (water vapor-saturated air)

1. Place wet cotton wool in the vessel with the label cal 100.
2. Drill a hole for inserting the minisensor in the screw top and close the vessel. If you want to calibrate several minisensors simultaneously, drill as many holes as there are minisensors.
3. Wait about 2 minutes to ensure that the air is water-vapor-saturated

Alternatively: Preparation of calibration standard cal 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch off the air-pump and stir the solution for further 10 minutes to ensure that water is not supersaturated.

7.1.1.2 Mounting the Oxygen-Sensitive Minisensors

1. Remove the oxygen sensor carefully from the protective cover.
2. Carefully remove the protective plastic cap covering the oxygen-sensitive sensor spot.
3. Fix the oxygen sensor with a clip to a laboratory support or a similar stable construction.
4. Remove the protective cap from the male fiber plug and connect it to one of the SMA plugs of the OXY-10 device. The safety nut must be carefully attached while turning slightly clockwise.

7.1.1.3 Performing the calibration

1. Switch on the OXY-10 and the PC software following chapter 6.
2. Go to window ***Calibration*** and enter the actual **atmospheric pressure** in hPa and the **temperature** of the calibration standards cal 0 and cal 100. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L µmol/L).

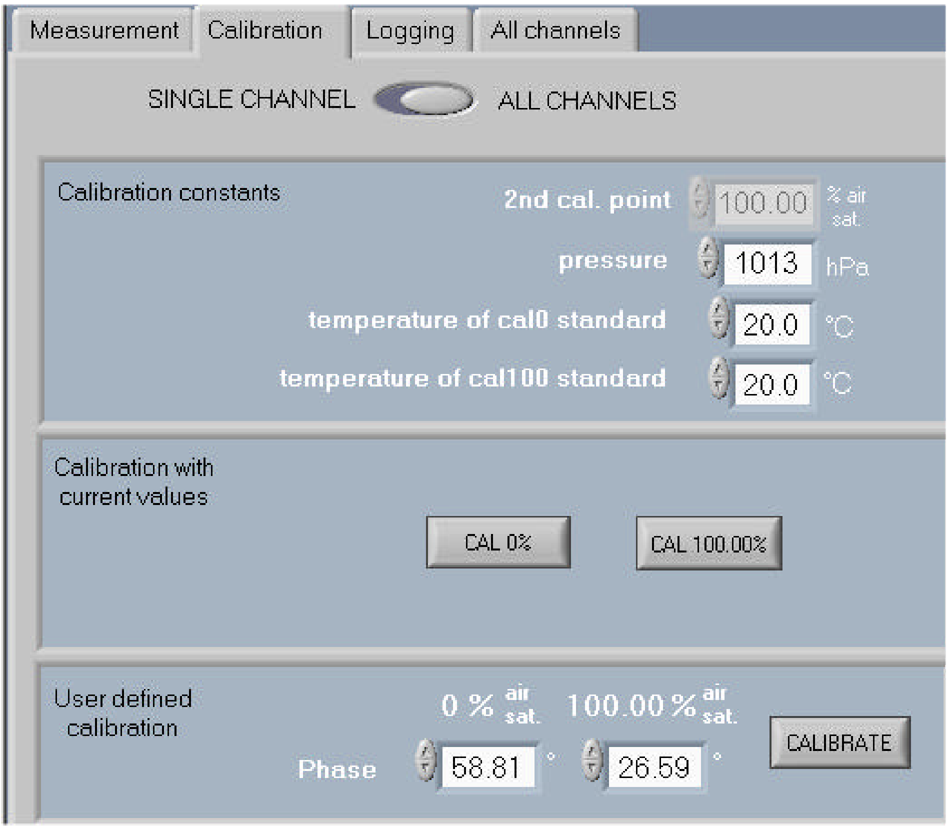
   Please ensure that there are no temperature changes during the calibration of the oxygen sensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.
3. Select the channel to be calibrated in the section *Channel* of sub window ***SINGLE CHANNEL*** or switch to the sub window ***ALL CHANNNELS***.

Calibrate All Channels:

4. Place the calibration standard 100 (***cal 100***), containing wet cotton wool, underneath the oxygen minisensors. The vessel with the label "***cal 100***" has to be closed with the screw top containing the holes.

   Insert the plastic fibers carefully through the holes without touching the oxygen-sensitive spots until they are about 3 cm deep inside the vessel.

   Make sure that the plastic fibers with their sensor spots cannot touch the vessel and the cotton wool.



2nd cal. point 100.00 % air sat.

pressure 1013 hPa

temperature of cal0 standard 20.0 °C

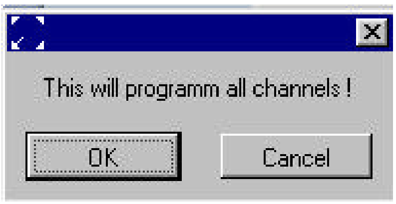
temperature of cal100 standard 20.0 °C

Phase 0 % air sat. 58.81 °

Phase 100.00 % air sat. 26.59 °

5. Wait about 3 minutes until the phase angles are constant and press the '***CAL 100%'*** button to store the 100 % air-sat. calibration values at the adjusted temperature.

   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.


This will programm all channels !

6. To record the second calibration values, oxygen-free water (***cal 0***), place the vessel with the label "***cal 0***" underneath the oxygen minisensors.

   Insert the plastic fibers about 2 cm deep into the ***cal 0*** solution.

   Make sure that the plastic fibers with their sensor spots cannot touch the vessel.

   To increase the response time, stir the ***cal 0*** solution. Wait about 3 minutes until the phase angles are constant (the variation of the phase angles should be smaller than ± 0.05°) and click the '***CAL 0%***' button to store the 0 % calibration values at the adjusted temperature.

   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.

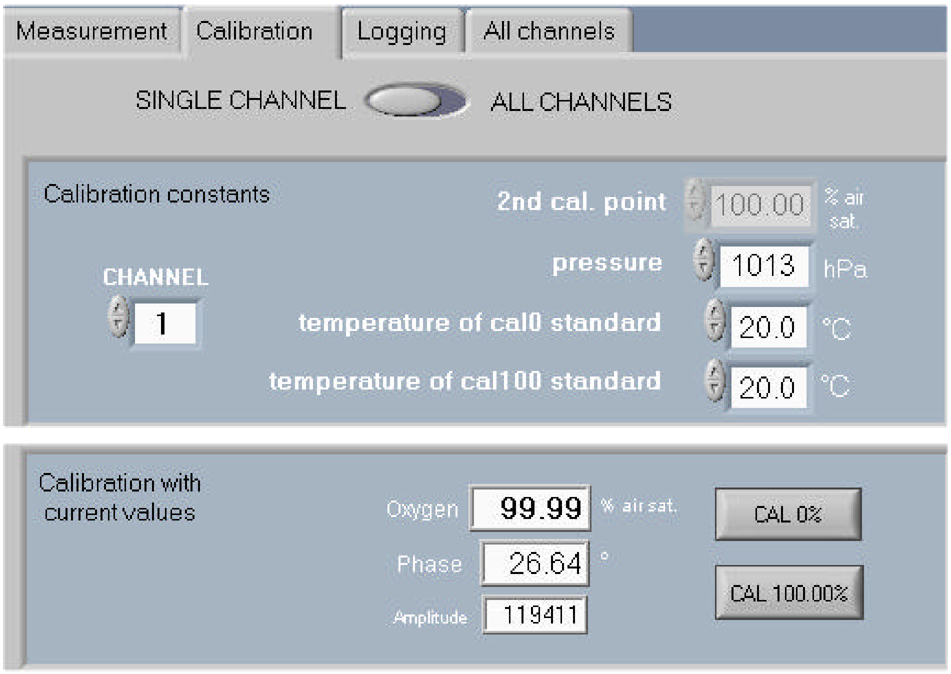
7. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

Calibrate Single Channels:

4. Place the calibration standard 100 (***cal 100***), containing wet cotton wool, underneath the oxygen minisensor. The vessel with the label "***cal 100***" has to be closed with the screw top containing the hole.

   Insert the plastic fiber carefully through the hole without touching the oxygen-sensitive spot until it is about 3 cm deep inside the vessel.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel and the cotton wool.



Calibration constants

CHANNEL 1

2nd cal. point 100.00 % air sat.

pressure 1013 hPa

temperature of cal0 standard 20.0 °C

temperature of cal100 standard 20.0 °C

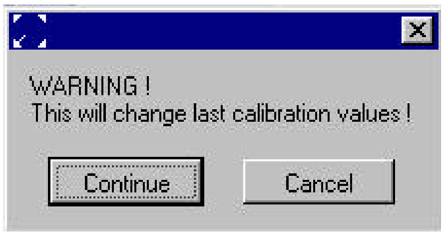
Calibration with current values

Oxygen 99.99 % air sat.

Phase 26.64 °

Amplitude 119411

A dialogue appears informing you that you will change the last calibration values. Click the '***Continue***' button to store the new calibration data.



WARNING !
This will change last calibration values !

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the cal 100 % value.

5. To record the second calibration value, oxygen-free water (***cal 0***), place the vessel with the label "***cal 0***" underneath the oxygen minisensor.

   Insert the plastic fiber about 2 cm deep into the **cal 0** solution.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel.

   To increase the response time, stir the ***cal 0*** solution. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '***CAL 0%***' button to store the 0 % calibration value at the adjusted temperature.

   A message window opens and informs you that you will change the last calibration values. Click the '***OK***' button to store the new calibration data.

   Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the oxygen-free calibration value (cal 0).

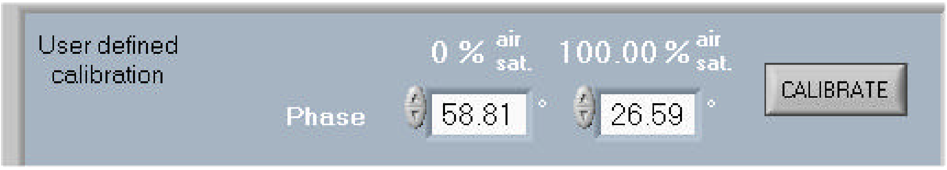
6. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

7.1.2 User-defined calibration

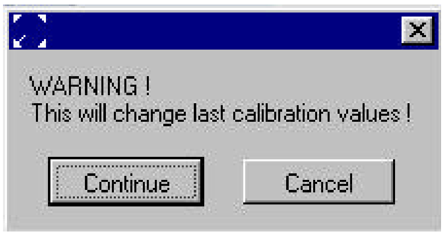
If temperature and phase angle of a former calibration of the same sensor are known, the *Calibration with constant values* is possible.

1. Switch on the OXY-10 and the PC software following chapter 6.
2. Go to the window *Calibration.* Select the channel to be calibrated in the section *Channel* of sub window *Single channel* or switch to the sub window *All channels*.
3. Enter the respective calibration values
    a. phase at 0 % air saturation
    b. temperature at 0 % air saturation
    c. phase at 100 % air saturation
    d. temperature at 100 % air saturation
    e. pressure at which values a. – d. were measured

    The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L).


User defined calibration
Phase 0 % air sat.: 58.81 °
Phase 100.00 % air sat.: 26.59 °

4. To store the calibration values click the *'Calibrate'* button. A dialog appears and informs you that you will program all channels in case the sub window all channels is chosen. If sub window single channel is chosen a dialogue appears informing you that you will change the last calibration values. Click the '*Continue*' button to store the new calibration data.


WARNING !
This will change last calibration values !

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record user-defined calibration values.

7.2 Calibration of Flow-Through Cell FTC-PSt3

There are two ways to calibrate the optical sensors. One is the use of two calibration standards, the other one is to type in known constants. The first way needs two calibration standards. Their preparation is described in chapter 7.2.1. The second possibility is described in chapter 7.2.2.

7.2.1 Calibration with two calibration standards

For the calibration with current values you need calibration standards and your mounted sensor.

7.2.1.1 Preparation of the Calibration Standards

Calibration of oxygen minisensors is performed using a conventional two-point calibration in *oxygen-free water* **(cal 0)** and *water vapor-saturated air* or *air-saturated water* **(cal 100)**. In the sub window *Calibration*, cal 100 is denoted as 2nd cal point.

Preparation of calibration standard cal 0 (oxygen-free water):

1. Add 1 g sodium sulfite(Na_2SO_3) to the vessel and label it ***cal 0***.
2. Dissolve Na_2SO_3 in 100 mL water.

   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination.

To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of ***cal 0*** is about 24 hours provided that the vessel has been closed with the screw top.

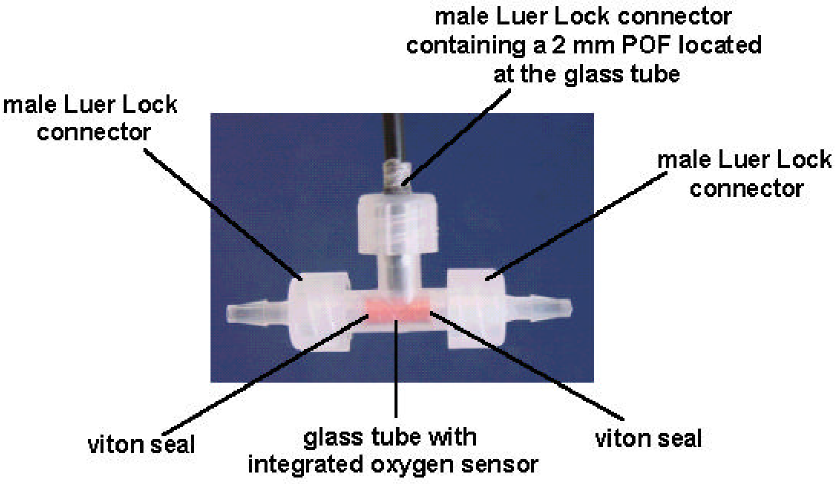
Preparation of calibration standard cal 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch of the air-pump and stir the solution for further 10 minutes to ensure that the water is not supersaturated.

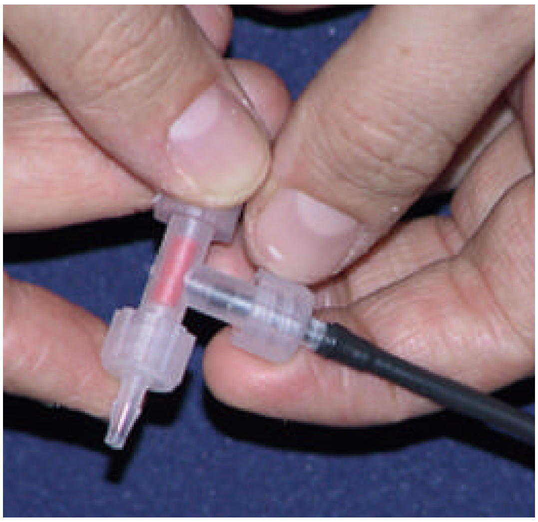
7.2.1.2 Mounting the Oxygen-Sensitive Minisensors

1. Remove the flow-through cell oxygen sensor carefully from the protective cover. The oxygen-sensitive material is immobilized to a glass tube which is located in a T-connector with two female Luer-Lock adapters. The glass tube is tightened with a viton seal and two male Luer-Lock connectors fix the glass tube in the T-connector. On request the seal can also be out of silicone.

   Don't remove the two male Luer-Lock adapters from the T-connector. You may loose the viton seal and the glass tube may be dislocated.



2. Fix the male Luer-Lock adapter with the integrated 2 mm POF (polymer optical fiber) to the Luer T-connector and ensure that the fiber is located close to the glass tube.



3. Fix the flow-through cell with a clip to a laboratory support or a similar stable construction.
4. Connect the two male Luer-Lock connectors with the tubings of your flow-through system.
5. Remove the protective cap from the male fiber plug and connect it to the SMA plug of the OXY-10 device. The safety nut must be carefully attached while turning slightly clockwise.

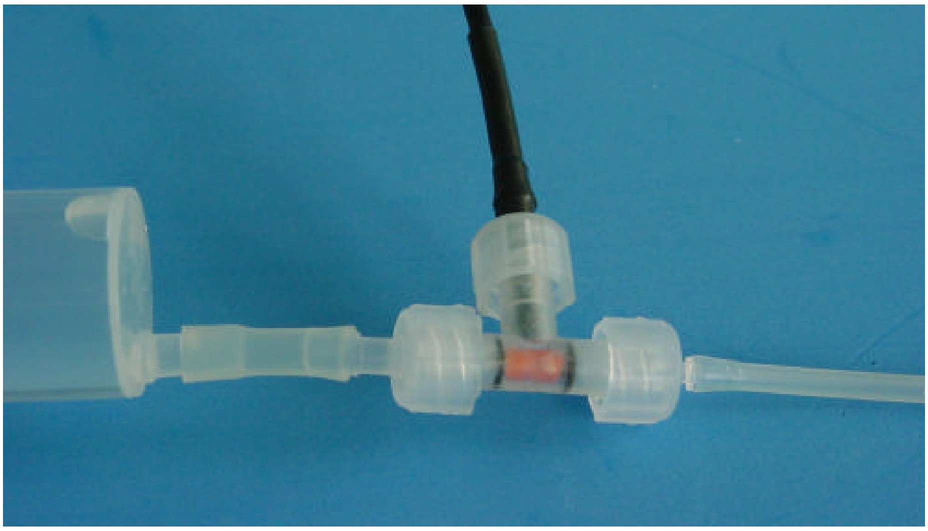
7.2.1.3 Performing the calibration

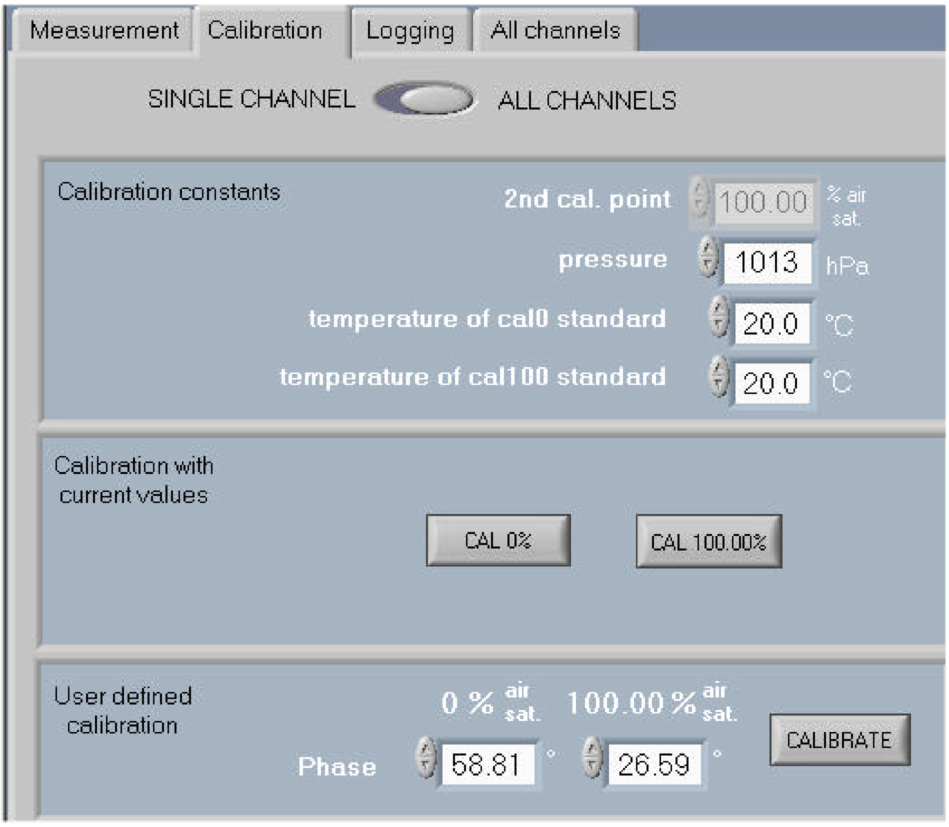
1. Switch on the OXY-10 and the PC software following chapter 6.
2. Go to window ***Calibration*** and enter the actual **atmospheric pressure** in hPa and the **temperature** of the calibration standards cal 0 and cal 100. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L µmol/L).

   Please ensure that there are no temperature changes during the calibration of the oxygen sensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.
3. Select the channel to be calibrated in the section *Channel* of sub window ***SINGLE CHANNEL*** or switch to the sub window ***ALL CHANNNELS***.

Calibrate All Channels:

1. For each sensor, connect one plastic tubing with a syringe, the other dip into the vessel containing the calibration solution 100, ***cal 100***. Fill the syringes slowly with calibration standard cal 100. Please ensure that there are no air bubbles located in the glass tubes of the flow-through cells.





Calibration constants

2nd cal. point 100.00 % air sat.

pressure 1013 hPa

temperature of cal0 standard 20.0 °C

temperature of cal100 standard 20.0 °C

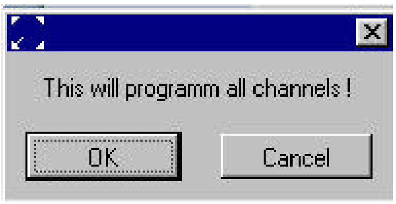
Calibration with current values

User defined calibration

	0 % air sat.	100.00 % air sat.
Phase	58.81 °	26.59 °

2. Wait about 3 minutes until the phase angles are constant (the variation of the phase angles should be smaller than ± 0.05°) and press the **CAL100%** button to store the 100 % air-sat. calibration values at the adjusted temperature. Afterwards, press the calibration solutions into the waste.

   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.



This will programm all channels !

3. To record the second calibration values, oxygen-free water, dip the plastic tubings into the vessel containing the calibration solution 0, ***cal 0*** and fill the syringes slowly with it. Please ensure that there are no air bubbles located in the glass tubes of the flow-through cells.

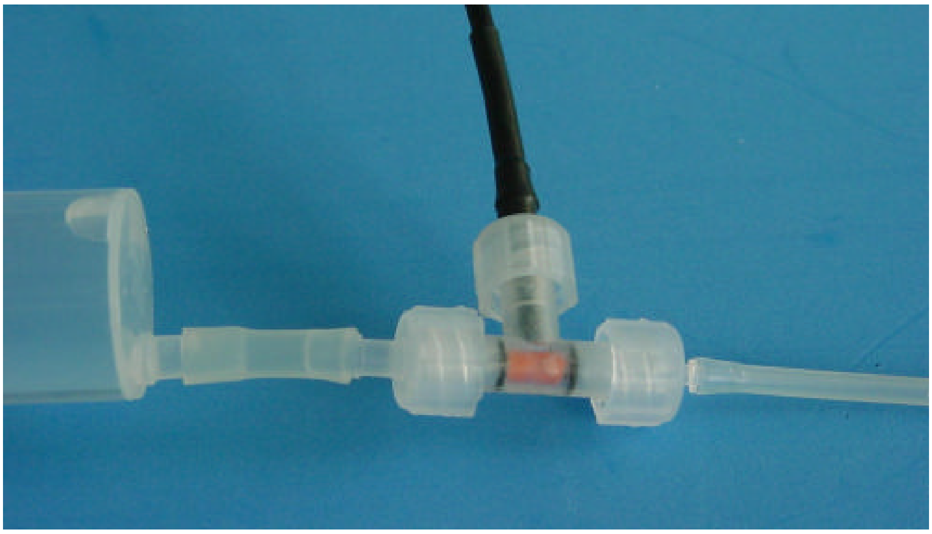
   Wait about 3 minutes until the phase angles are constant (the variation of the phase angles should be smaller than ± 0.05°) and click the **CAL 0%** button to store the 0 % air-sat. calibration values at the adjusted temperature. Afterward, press the calibration solutions into the waste.

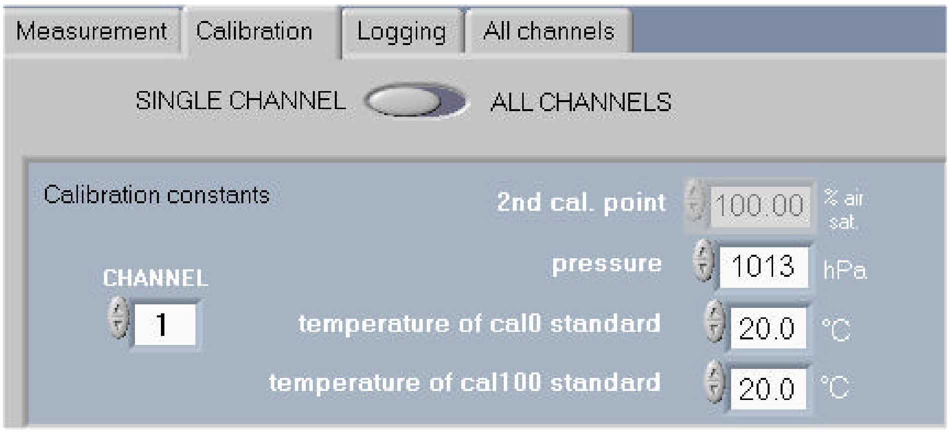
   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.

4. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

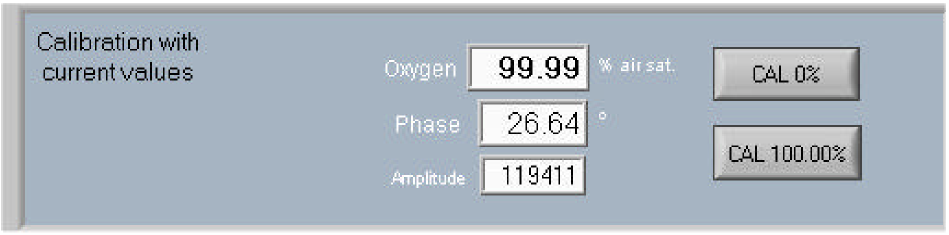
Calibrate Single Channels:

1. Connect one plastic tubing with a syringe, the other dip into the vessel containing the calibration solution 100, ***cal 100***. Fill the syringe slowly with calibration solution cal 100. Please ensure that there are no air bubbles located in the glass tube of the flow-through cell.





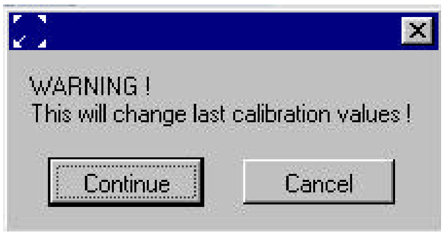
2nd cal. point 100.00 % air sat.
pressure 1013 hPa
CHANNEL 1
temperature of cal0 standard 20.0 °C
temperature of cal100 standard 20.0 °C



Oxygen 99.99 % air sat.
Phase 26.64 °
Amplitude 119411

2. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the **CAL 100.00%** button to store the 100% air-sat. calibration value at the adjusted temperature. Afterwards, press the calibration solution into the waste.

   A dialogue appears informing you that you will change the last calibration values. Click the '***Continue***' button to store the new calibration data.



WARNING !
This will change last calibration values !

3. Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the 100 % air-saturation calibration value (cal 100%).
4. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, ***cal 0*** and fill the syringe slowly with it. Please ensure that there are no air bubbles located in the glass tube of the flow-through cell.

Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the **CAL 0%** button to store the **0% air-sat.** and **temp. at 0%** values. Afterwards, press the calibration solution into the waste.

A message window opens and informs you that you will change the last calibration values. Click the '***OK***' button to store the new calibration data.

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the oxygen-free calibration value (cal 0%).

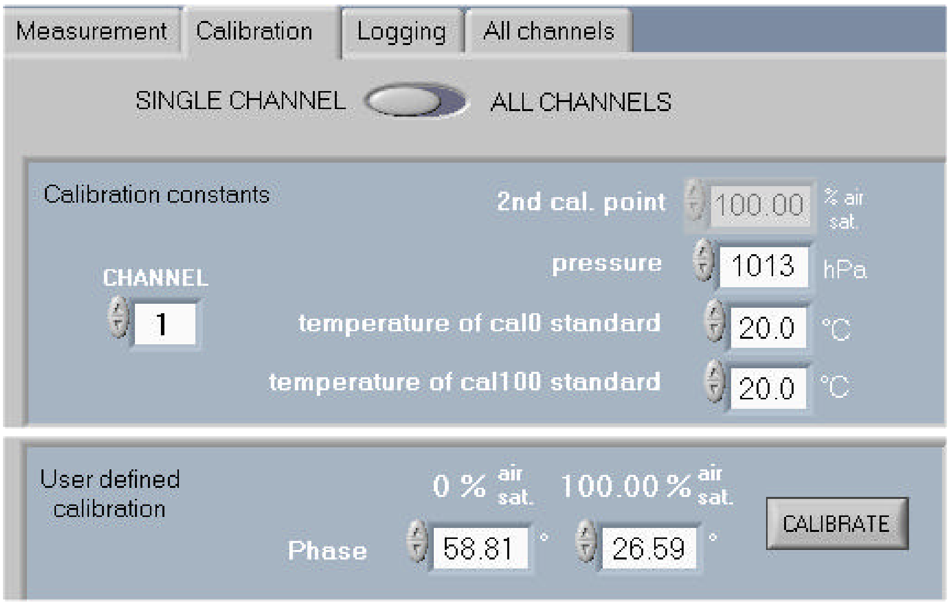
5. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

7.2.2 User-defined calibration

If temperature and phase angle of a former calibration of the same sensor are known, the *Calibration with constant values* is possible.

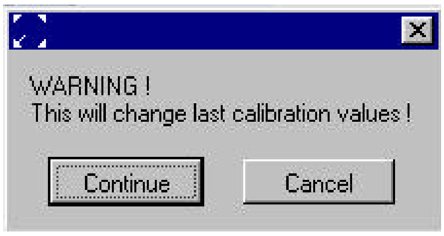
1. Switch on the OXY-10 and the PC software following chapter 6.
2. Go to the window *Calibration.* Select the channel to be calibrated in the section *Channel* of sub window *Single channel* or switch to the sub window *All channels*.
3. Enter the respective calibration values
    a. phase at 0 % air saturation
    b. temperature at 0 % air saturation
    c. phase at 100 % air saturation
    d. temperature at 100 % air saturation
    e. pressure at which values a. – d. were measured

    The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L).



2nd cal. point 100.00 % air sat.
pressure 1013 hPa
CHANNEL 1
temperature of cal0 standard 20.0 °C
temperature of cal100 standard 20.0 °C
Phase 0 % air sat. 58.81 °
Phase 100.00 % air sat. 26.59 °

4. To store the calibration values click the calibrate button. A dialog appears and informs you that you will program all channels in case the sub window *All channels* is chosen. If sub window *Single channel* is chosen a dialogue appears informing you that you will change the last calibration values. Click the '*Continue*' button to store the new calibration data.


WARNING !
This will change last calibration values !

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record user-defined calibration values.

7.3 Calibration of Planar Oxygen-Sensitive Foils PSt3 Integrated in Glass Vials

There are two ways to calibrate the optical sensors. One is the use of two calibration standards, the other one is to type in known constants. The first way needs two calibration standards. Their preparation is described in chapter 7.3.1. The second possibility is described in chapter 7.3.2.

7.3.1 Calibration with two calibration standards

For the calibration with current values you need calibration standards and your mounted sensor.

Calibration of the minisensors is performed using conventional two-point calibration in *oxygen-free water* (**cal 0**) and *water vapor-saturated air* or *air-saturated water* (**cal 100**). In the sub window *Calibration*, cal 100 is denoted as 2nd cal point.

7.3.1.1 Preparation of the Calibration Standards

Preparation of calibration solution 0 (oxygen-free water):

1. Add 1 g sodium sulfite(Na_2SO_3) to the vessel and label it **cal 0**.
2. Dissolve Na_2SO_3 in 100 mL water.

   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is eliminated (removed) by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water the is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination.

To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of **cal 0** is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water vapor-saturated air)

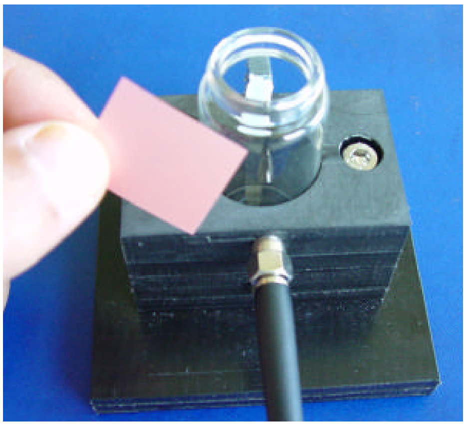
1. Place wet cotton wool in the vessel and label it **cal 100**.
2. Drill two holes for inserting the minisensor and the temperature sensor in the screw top and close the vessel with it.
3. Wait about 2 minutes to ensure that air is water vapor-saturated

Preparation of calibration solution 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch off the air-pump and stir the solution for further 10 minutes to ensure that the water is not supersaturated.

7.3.1.2 Mounting the Oxygen-Sensitive Minisensors

1. Remove the oxygen-sensitive foil carefully from the protective cover.
2. Glue small spots of the oxygen-sensitive foil into the desired glass vessel using transparent silicone. The transparent silicone can be purchased from Loligo.



left: **Oxygen-sensitive foil glued on the inner surface of a glass vial**
right: **Glass vial with integrated oxygen-sensitive foil in appropriate adapter**

Please note: Be sure to glue the sensor spots onto your vessel with the proper side! The sensor support (polyester foil or glass) on which the sensor is spotted (identifiable by its faint reflection) is glued to the vessel, while the sensor itself must look toward the sample. The figure on page 11 shows how the (highly enlarged) sensor spot must be glued to the vessel.

3. Remove the protective cap from the male fiber plugs of the delivered fiber cable and connect it to the SMA plugs of the OXY-10 and the holding device. The safety nut must be carefully attached while turning slightly clockwise.

If the oxygen-sensitive foil is used in shaking flasks, we recommend the use of coasters for shaking flasks. They are shown below. During calibration and measurement the sensor spot has to be placed on top of the fiber optic of the coaster. The fiber optic of the coaster is located in the middle of the green positioning ring. See also page 18.

left: **Set of four coasters for shaking flasks**
right: **Coaster with green positioning ring**

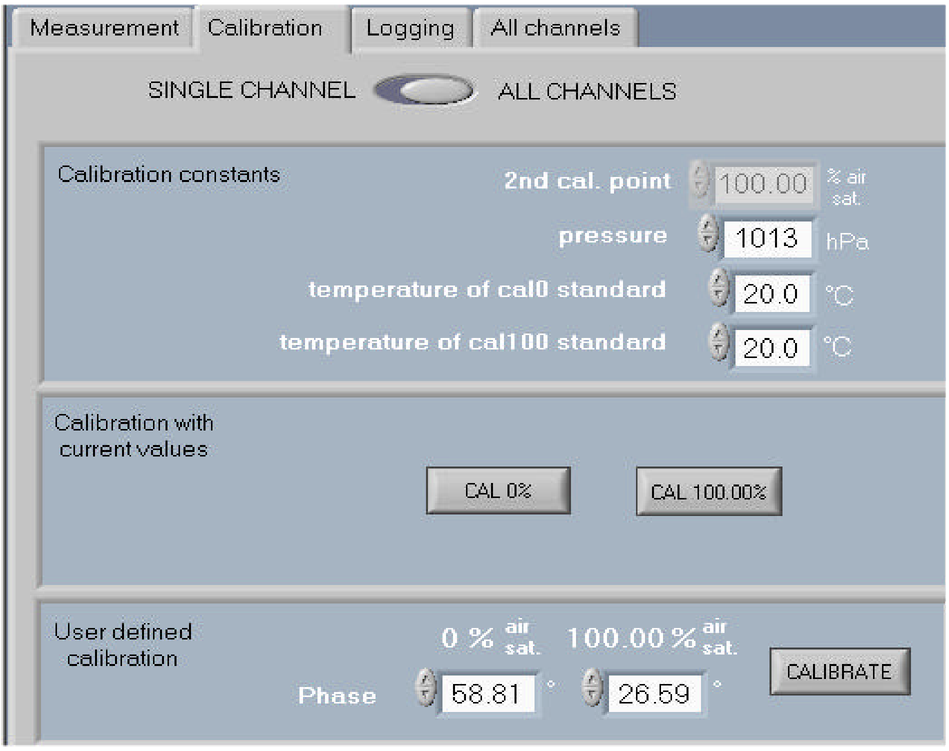
7.3.1.3 Performing the calibration

1. Switch on the OXY-10 instrument and the PC software following chapter 6.
2. Go to window ***Calibration*** and enter the actual **atmospheric pressure** in hPa and the **temperature** of the calibration standards cal 0 and cal 100. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L µmol/L).

   Please ensure that there are no temperature changes during the calibration of the oxygen sensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.
3. Select the channel to be calibrated in the section *Channel* of sub window ***SINGLE CHANNEL*** or switch to the sub window ***ALL CHANNNELS***.

Calibrate All Channels:

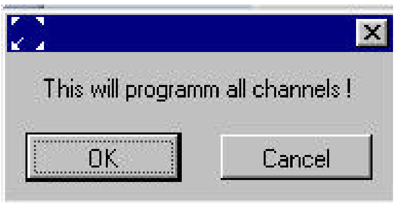
1. Place the calibration solution 100 (**cal 100**), air-saturated water (or water vapor-saturated air), into the glass vessels. To minimize the response time, slightly stir the solution. Please ensure that the **cal 100** solution completely covers the sensor foils.



2nd cal. point 100.00 % air sat.
pressure 1013 hPa
temperature of cal0 standard 20.0 °C
temperature of cal100 standard 20.0 °C
0 % air sat. 100.00 % air sat.
Phase 58.81 ° 26.59 °

2. Wait about 3 minutes until the phase angles are constant (the variation of the phase angles should be smaller than ± 0.05°) and click the **CAL 100%** button to store the 100 % air-sat. calibration values at the adjusted temperature.

   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.



This will programm all channels !

3. To record the second calibration values, oxygen-free water (**cal 0**), remove the ***cal 100*** solution from the vessels and fill them with the calibration standard 0, **cal 0**.

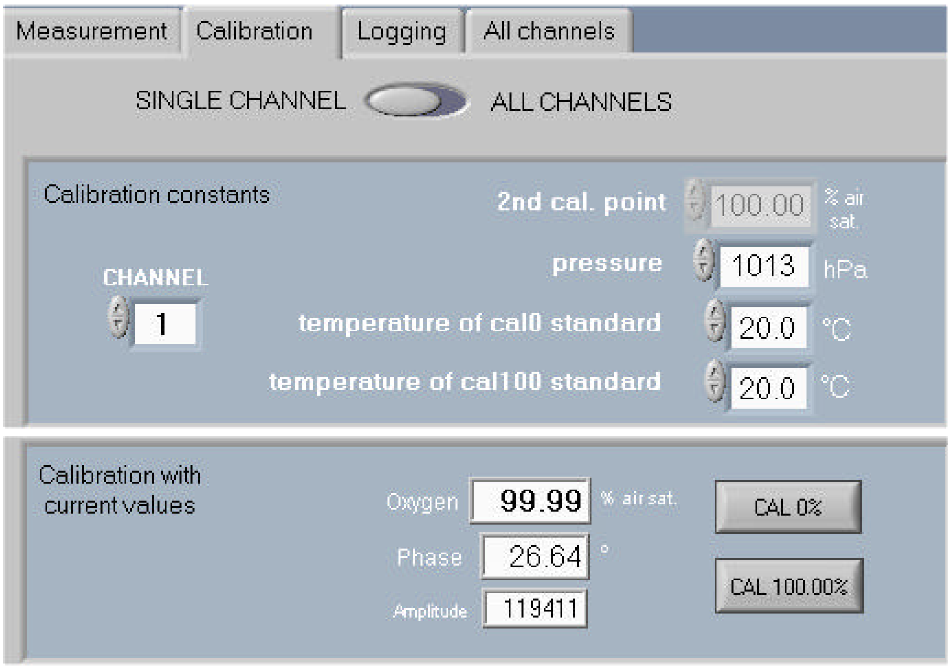
   To minimize the response time, **slightly** stir the solution.
   Please note: Vigorous stirring can lead to an oxygen contamination of the **cal 0** solution.

   Wait about 3 minutes until the phase angles are constant (the variation of the phase angles should be smaller than ± 0.05°) and press the **CAL 0%** button to store the 0 % air-saturation calibration values at the adjusted temperature.

   A message window opens and informs you that you will program all channels with the actual calibration values. Click the '***OK***' button to store the new calibration data.
4. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

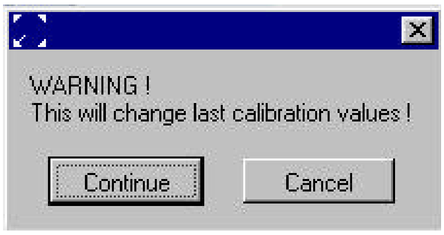
Calibrate Single Channels:

1. Place the calibration solution 100 (**cal 100**), air-saturated water (or water vapor-saturated air), into the glass vessel. To minimize the response time, slightly stir the solution. Please ensure that the **cal 100** solution completely covers the sensor foil.



2nd cal. point 100.00 % air sat.
pressure 1013 hPa
CHANNEL 1
temperature of cal0 standard 20.0 °C
temperature of cal100 standard 20.0 °C
Oxygen 99.99 % air sat.
Phase 26.64 °
Amplitude 119411

2. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the **cal 100%** button to store the 100 % air-sat. calibration value at the adjusted temperature. A message window opens and informs you that you will change the last calibration values. Click the '***OK***' button to store the new calibration data.



WARNING !
This will change last calibration values !

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the 100% calibration value (cal 100%).

3. To record the second calibration value, oxygen-free water (**cal 0**), remove the ***cal 100*** solution from the vessel and fill it with the calibration standard 0, **cal 0**.

   To minimize the response time, **slightly** stir the solution.
   Please note: Vigorous stirring can lead to an oxygen contamination of the **cal 0** solution.

   Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the **CAL 0%** button to store the 0% air-sat. calibration value at the adjusted temperature.

   A message window opens and informs you that you will change the last calibration values. Click the '***OK***' button to store the new calibration data.

   Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record the oxygen-free calibration value (cal 0%)

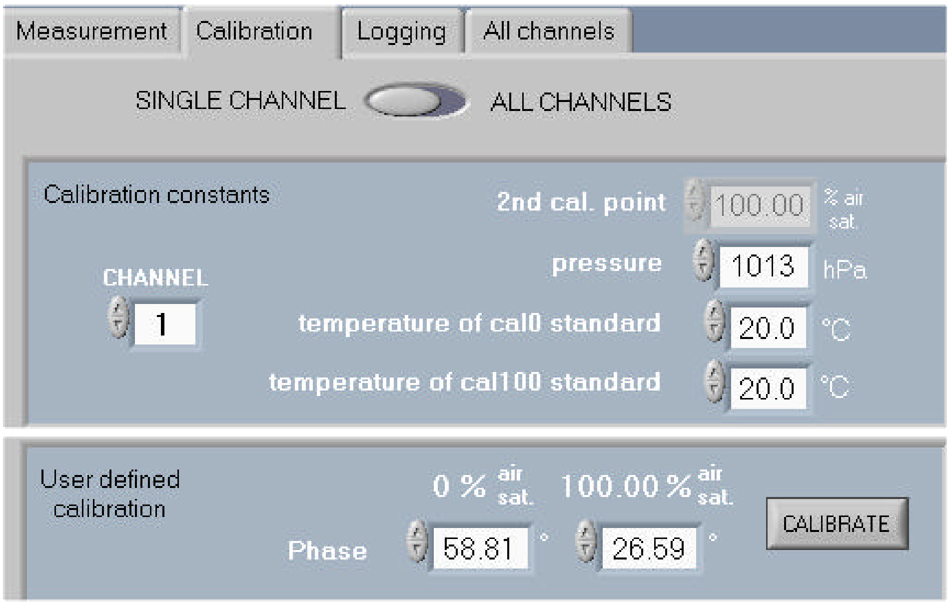
4. Rinse the sensor membranes gently with water after calibration to remove sulfite remaining on the surface.

7.3.2 User-defined calibration

If temperature and phase angle of a former calibration of the same sensor are known, the *Calibration with constant values* is possible.

1. Switch on the OXY-10 and the PC software following chapter 6.
2. Go to the window *Calibration.* Select the channel to be calibrated in the section *Channel* of sub window *Single channel* or switch to the sub window *All channels*.
3. Enter the respective calibration values
    a. phase at 0 % air saturation
    b. temperature at 0 % air saturation
    c. phase at 100 % air saturation
    d. temperature at 100 % air saturation
    e. pressure at which values a. – d. were measured

    The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L).



2nd cal. point 100.00 % air sat.

pressure 1013 hPa

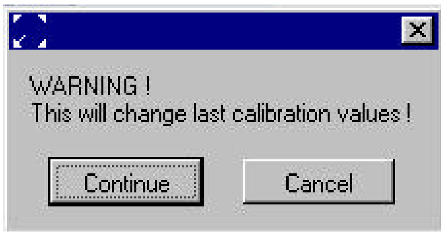
CHANNEL 1

temperature of cal0 standard 20.0 °C

temperature of cal100 standard 20.0 °C

Phase: 0 % air sat. 58.81 °; 100.00 % air sat. 26.59 °

4. To store the calibration values click the *'Calibrate'* button. A dialog appears and informs you that you will program all channels in case the sub window *All channels* is chosen. If sub window *Single channel* is chosen a dialogue appears informing you that you will change the last calibration values. Click the '*Continue*' button to store the new calibration data.



WARNING !
This will change last calibration values !

Repeat this procedure for all other channels by typing in the respective channel into the Channel Window to record user-defined calibration values.

8 Measurement with Oxygen Sensor PSt3

Calibration of the sensor is recommended before each measurement (see chapter 7). If you don't want to recalibrate a sensor, you can use the calibration values of your last measurement. These values are stored in the hardware of OXY-10.

Each calibration is only valid for the corresponding sensor. Especially after longer measurements (more than 10000 measure points or 3 h continuous mode) the sensor should be re-calibrated.

Ensure that the temperature of the sample is known and is constant during measurement.

8.1 Starting the measurement

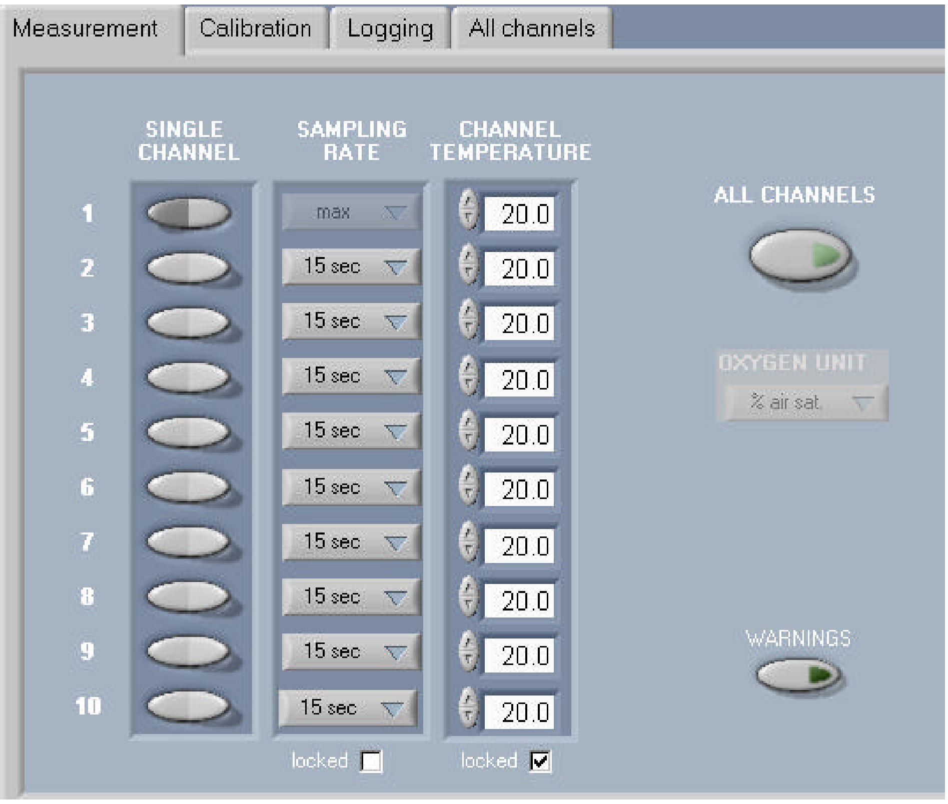
Note: Saving of data is explained in chapter 6.3.3(page 22) and in chapter 8.1.3 (page 44) (Logging).

Measurement is started either for selected one to ten channels or for all channels. In both cases the scanning rate can be defined for each channel separately. In both cases the channels are scanned sequentially.

Click the ‘Measurement’ menu bar to adjust the measurement conditions.

8.1.1 Scanning all channels

1. Switch on OXY-10 and PC-software following chapter 8.1.3.
2. Go to window *Measurement*.
3. Choose oxygen measurement unit from button *Oxygen unit*.
4. Enter the sampling rates and the temperature for each channel. By clicking ‘**locked’** the adjusted sampling rate of the first channel is also set for all other channels.
5. Start the measurement by clicking button *All channels*.



	SAMPLING RATE	CHANNEL TEMPERATURE
1	max	20.0
2	15 sec	20.0
3	15 sec	20.0
4	15 sec	20.0
5	15 sec	20.0
6	15 sec	20.0
7	15 sec	20.0
8	15 sec	20.0
9	15 sec	20.0
10	15 sec	20.0
	locked ☐	locked ☑

OXYGEN UNIT: % air sat.

8.1.2 Scanning selected channels

1. Switch on OXY-10 and PC software following chapter 6.
2. Go to window *Measurement*.
3. Choose oxygen measurement unit from button *Oxygen unit*.
4. Enter the sampling rates and the temperature for each channel.

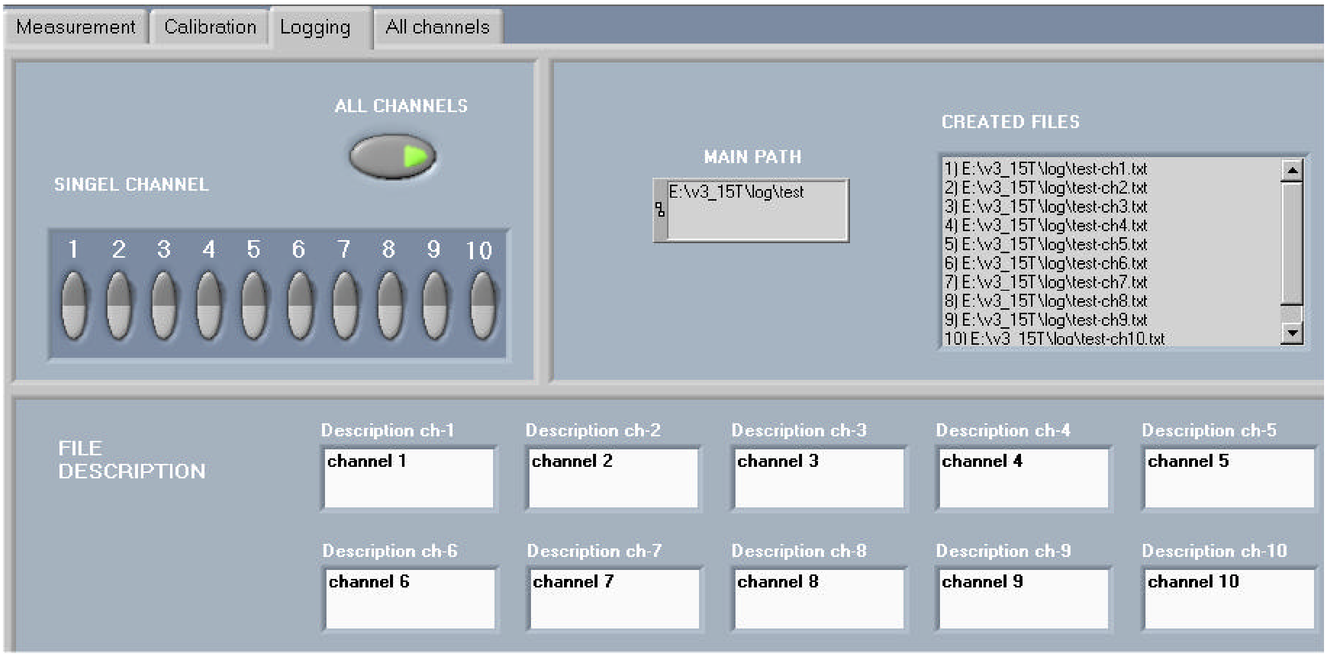
   Please note: If you want to adjust different scanning rates for the activated channels, ensure that the 'locked' field is not activated.
5. Start the measurement by clicking the required channel button.

8.1.3 Logging

Measured data are logged during the measurement. Add contents to the description fields in the *File description* section before starting logging. This is not necessary but helpful as the description is later stored in the data file.

1. Depending on the scan mode click on ***All Channels*** for logging all the selected channels in the measuring window or ***Single channel*** for single channel logging.
2. In the appearing "save as" window specify filename **without extension** and its location.

The PC software automatically creates one to ten different files. The filename consists of the specified filename and the extensions "-ch1.txt", "-ch2.txt", to -ch10.txt", respectively. All filenames are displayed in the section *Created files*.



MAIN PATH
E:\v3_15T\log\test

CREATED FILES
1) E:\v3_15T\log\test-ch1.txt
2) E:\v3_15T\log\test-ch2.txt
3) E:\v3_15T\log\test-ch3.txt
4) E:\v3_15T\log\test-ch4.txt
5) E:\v3_15T\log\test-ch5.txt
6) E:\v3_15T\log\test-ch6.txt
7) E:\v3_15T\log\test-ch7.txt
8) E:\v3_15T\log\test-ch8.txt
9) E:\v3_15T\log\test-ch9.txt
10) E:\v3_15T\log\test-ch10.txt

Description ch-1: channel 1
Description ch-2: channel 2
Description ch-3: channel 3
Description ch-4: channel 4
Description ch-5: channel 5
Description ch-6: channel 6
Description ch-7: channel 7
Description ch-8: channel 8
Description ch-9: channel 9
Description ch-10: channel 10

8.2 Some Advice for Correct Measurement

8.2.1 Signal drifts due to oxygen gradients

Please, keep in mind, that the sensor only measures the oxygen content near its surface. In unstirred solutions an oxygen concentration gradient often occurs.

Please check if air bubbles are on the sensor tip whenever unexpected drifts, gradients or unstable measurement values occur. Critical conditions for bubble formations are, for example, purging with air or other gases and increasing temperature during measurement.

The formation of a bio-film during long-term measurements or the accumulation of other sample components like oil or solid substances may lead to an oxygen gradient.

8.2.2 Signal drifts due to temperature gradients

A further source of imprecise measurement is insufficient temperature compensation. Please bear in mind, that the OXY-10 only measures correctly if the sample temperature is constant during measurement and the temperature is the same as you typed in at the beginning of the measurement. Please also refer to Chapter 12.5 "Temperature Compensation of the Response of Oxygen Sensors".

8.2.3 Signal drift due to photodecomposition

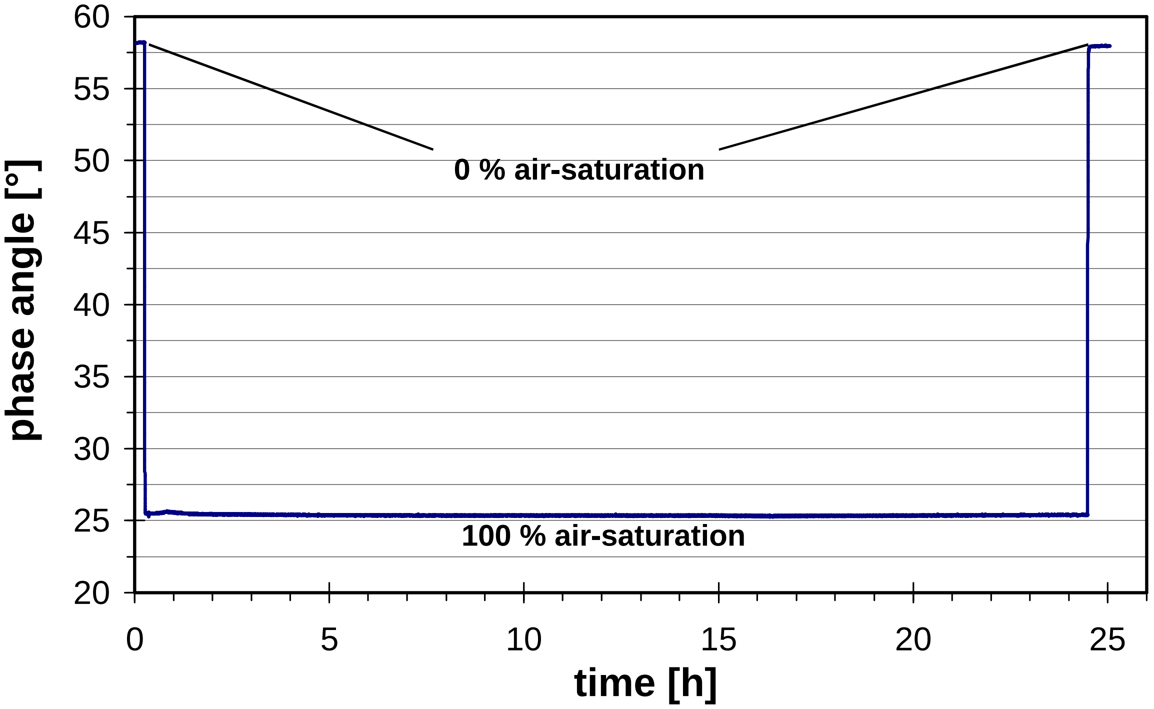
The oxygen-sensitive material may be subject to photodecomposition resulting in a signal drift. Photodecomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light. Therefore, the excitation light was minimized.

Continuous illumination of a **DP-PSt3** oxygen sensor over a period of 24 hours may lead to a phase drift of up to + 0.4 % air-saturation measured at 100% air-saturation at 20°C. However, this effect of photodecomposition can even be minimized by changing the measuring mode to the second or minute interval mode. In these modes, the software switches off the excitation light after recording the data point and switches it on after the interval you have chosen.

Please use the interval method whenever it is possible to increase the shelf life of the sensor.

Drift in % air-saturation at 100% air-saturation when illuminating the oxygen sensor ***PSt3*** *for 1, 12 and 24 hours in the continuous mode.*

	Drift per hour	Drift per 12 hours	Drift per 24 hours
PSt3	---	---	< 0.4 % air-saturation



Photodecomposition test of **PSt3**, continuously illuminating the sensor membrane for 25 hours.

8.2.4 Signal drift due to too much ambient light

A source of error is the detector overload due to too much ambient or sensor light. A detector overload can be recognized with appearing warning, prospective the warnings are activated.

Please notify that your measurement is not reliable if the warning light overload is shining red. A detector overload can affect both the amplitude and the phase angle.

Please decrease the LED intensity to reduce the amounts of photons reaching the photodetector.

8.2.5 Performance proof

If you want to prove the performance during the past measurement, please check the calibration values by inserting the sensor tip in the '**cal 0**' and '**cal 100**' calibration standards when you have finished your measurement. If the device shows 0% air-saturation immersing the sensor tip into the '**cal 0**' solution and the value of the second calibration point measuring the '**cal 100** ' standard, the sensor worked perfectly during the whole measurement.

8.2.6 Correction for air pressure variations

The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L µmol/L). The partial pressure and the oxygen concentration units are calculated from % air-saturation by the software. Consequently, changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

9 General Instructions:

9.1 Warm-Up Time

The warm-up time of the electronic and opto-electronic components of the OXY-10 is 5 min. Afterwards stable measuring values are obtained.

9.2 Maintenance

The instrument is maintenance-free.

The housing should be cleaned only with a moist cloth. Avoid any moisture entering the housing! Never use benzine, acetone, alcohol or other organic solvents.

The SMA fiber connector of the minisensor can be cleaned only with lint-free cloth. The sensor tip may be rinsed only with distilled water.

9.3 Service

Balancing, maintenance and repair work may only be carried out by the manufacturer:

Loligo Systems ApS
Niels Pedersens Allé 2
DK-8830 Tjele
Denmark

Phone: +4589992545
E-mail: mail@loligosystems.com
Internet: www.loligosystems.com

Please contact our service team should you have any questions. We look forward to helping you and are open for any questions and criticism.

10 Technical Data

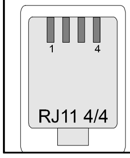
10.1 General Data

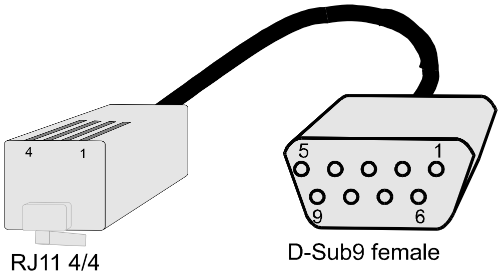
MODES	
PSt3 oxygen sensor	range: 0 - 250 % air-saturation resolution: 1 ± 0.05 % air -saturation 30 ± 0.1 % air -saturation 100 ± 0.5 % air-saturation 250 ± 1.7 % air-saturation accuracy: ± 1 % air-saturation at 100 % air-saturation

CALIBRATION PROCEDURE	
PSt3 oxygen sensor	2-point calibration in oxygen-free water and humidified air or air-saturated water

OPTICAL INTERFACE	
Optical Connector	SMA-compatible, 2 mm polymer optical fiber
Channels	10
Wavelength	505 nm

POWER INPUT	230VAC, 50/60Hz, 0.5A max.

DIGITAL INTERFACE	
Communication Protocol	serial interface RS-232 38400 Baud, Databits 8, Stoppbits 1, Parity none, Handshake none
Instrument output:	on RJ11 4/4 plug  1 ... TXD 2 ... RXD 3 ... n.c. 4 ... GND

Interface cable to PC:	RJ11 4/4 to DSub9:  RJ11 4/4: 1 ... TXD 2 ... RXD 3 ... n.c. 4 ... GND D-Sub9 female: 2 ... RXD 3 ... TXD 5 ... GND 1,4,6,7,8,9 ... n.c.

ENVIRONMENTAL CONDITIONS	
Operating temperature	0 to +50 ºC
Storage temperature	-10 to +65 ºC
Relative humidity	up to 95 %

OPERATION CONTROL	LED at the front panel

DIMENSIONS	length: 300 mm width: 250 mm height: 140 mm weight: 3600 g

10.2 Technical Notes

RS-232 Interface

The unit uses a special interface cable. Another cable can cause the unit’s malfunction.

Optical Output (SMA)

The SMA connector is a high-precision optical component. Please keep it clean and dry. Always use the rubber cap to close the output when not in use.

10.3 Operation Notes

Oxygen Measurement

To achieve the highest accuracy the OXY-10 should be warmed-up for 5 min before starting the measurement. Please see the details of the measurement process described in the OXY-10 manual.

11 Concluding Remarks

Dear customer,
With this manual we hope to provide you with an introduction to work with the OXY-10 fiber-optic oxygen meter.

This manual does not claim to be complete. We are endeavored to improve and supplement this version.
We are looking forward to your critical review and to any suggestions you may have.

You can find the newest version at www.loligosystems.com.

With best regards,

Your Loligo Team

12 Appendix

12.1 Basics in Optical Sensing of Oxygen

12.1.1 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

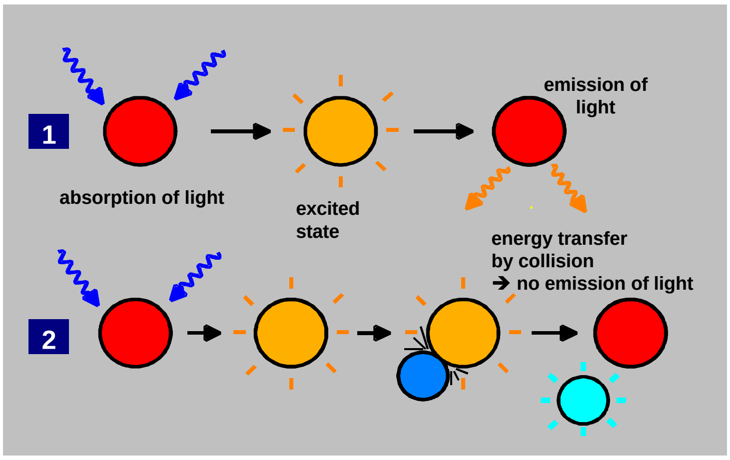


Figure 12.1 *Principle of dynamic quenching of luminescence by molecular oxygen*

(1) Luminescence process in absence of oxygen

(2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.

A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant.

$$\begin{aligned} \frac{I_0}{I} &= \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \\ I &= f([O_2]) \\ \tau &= f([O_2]) \end{aligned} \qquad (\mathbf{1})$$

I: Luminescence intensity in presence of oxygen
I_0: Luminescence intensity in absence of oxygen
τ: Luminescence decay time in presence of oxygen
τ_0: Luminescence decay time in absence of oxygen
K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)
$[O_2]$: Oxygen content

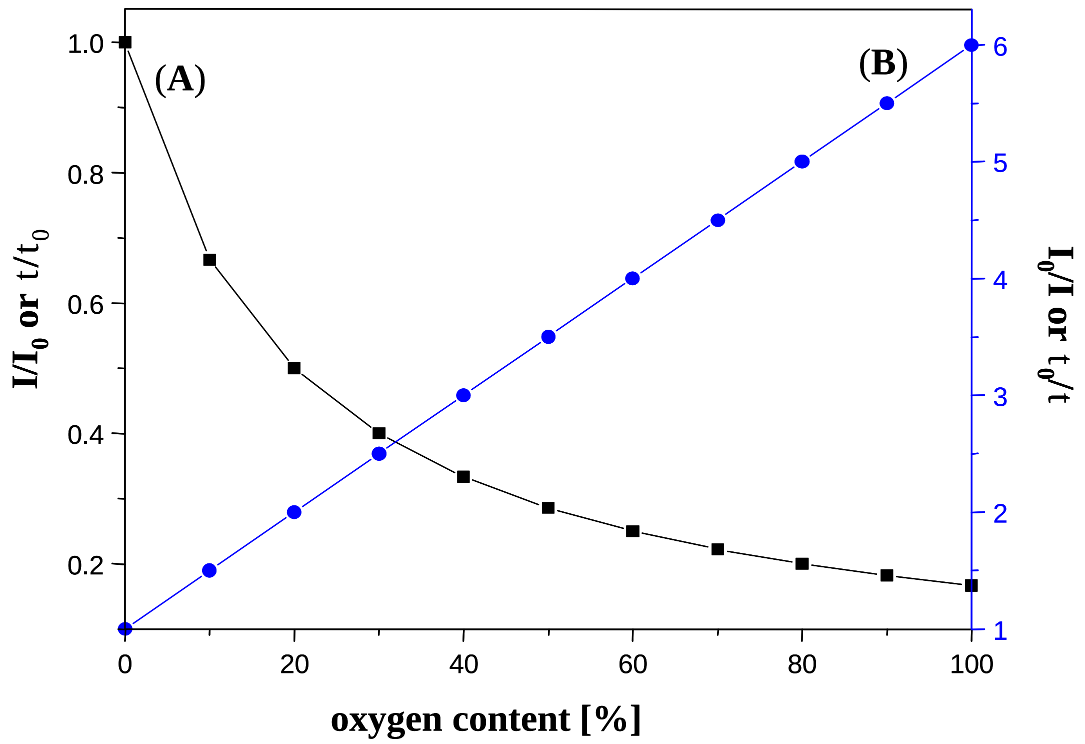


Figure. 12.2 *(**A**) Luminescence decrease in the presence of oxygen. (**B**) Stern-Volmer plot.*

Indicator dyes quenched by oxygen are, for example, polycyclic aromatic hydrocarbons, transition metal complexes of Ru(II), Os(II) and Rh(II), and phosphorescent porphyrins containing Pt(II) or Pd(II) as the central atom.

12.1.2 Major Components of Fiber-Optic Minisensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarization). Light acts as the carrier of the information.

The major components of a typical fiber-optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilized in a solid matrix)

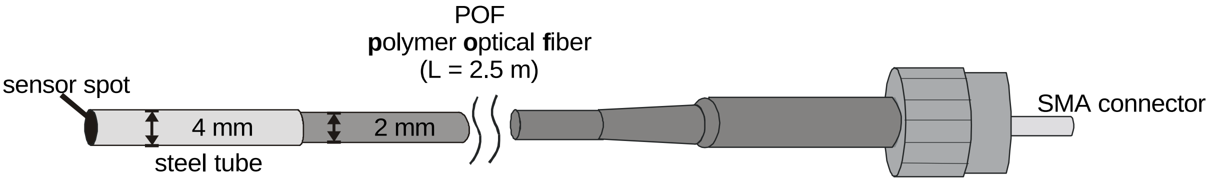


Figure 12.3 *Scheme of a minisensor.*

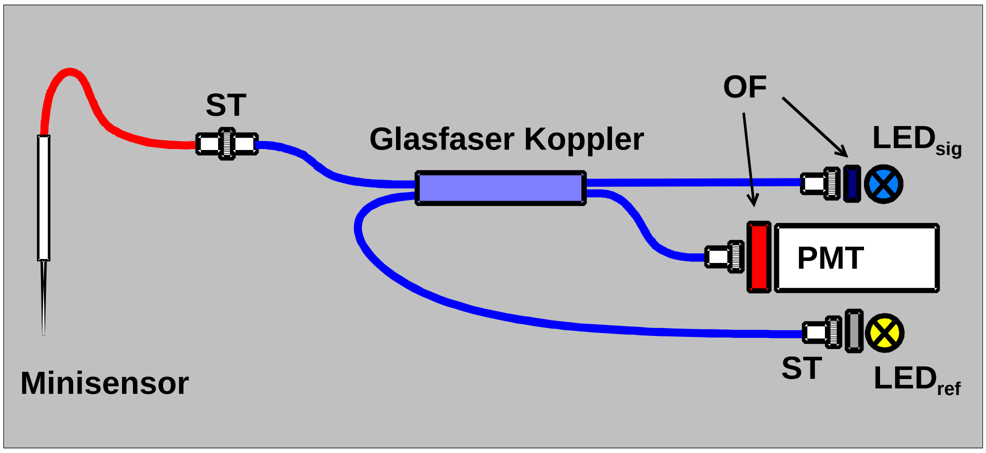


Figure 12.4 Schematic drawing of the optical setup of a measuring system with minisensors (LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector).

12.1.3 Advantages of Optical Oxygen-Sensitive Minisensors

- no oxygen is consumed during the measurement;
- the signal is independent of changes in flow velocity;
- they are able to measure the oxygen content in dry gases
- they are insensible towards electrical interferences and magnetic fields;
- they are more sensitive than conventional electrodes (up to ppt-range);
- long-term stability and low drift;
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light-conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarization, information such as decay time or delayed fluorescence);

12.1.4 Luminescence Decay Time

The OXY-10 measures the luminescence decay time of the immobilized luminophore as the oxygen-dependent parameter.

$$\tau = f([O_2]) \tag{2}$$

The OXY-10 uses the phase modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is excited with light with sinusoidally modulated intensity, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exciting and emitted signal. This phase angle is shifted as a function of the oxygen concentration. The relation between decay time τ and the phase angle Φ is shown by the following equation:

$$\tau = \frac{\tan\Phi}{2\pi \cdot f_{mod}} \tag{3a}$$

$$\tan\Phi = 2\pi \cdot f_{mod} \cdot \tau \tag{3b}$$

$$\tau \equiv \tan\Phi \equiv \Phi \equiv f([O_2]) \tag{3c}$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency

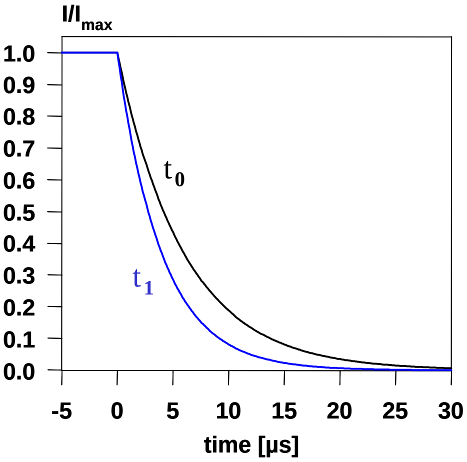


Figure 12.5 *Schematic of the single exponential decay ($t_0 > t_1$).*

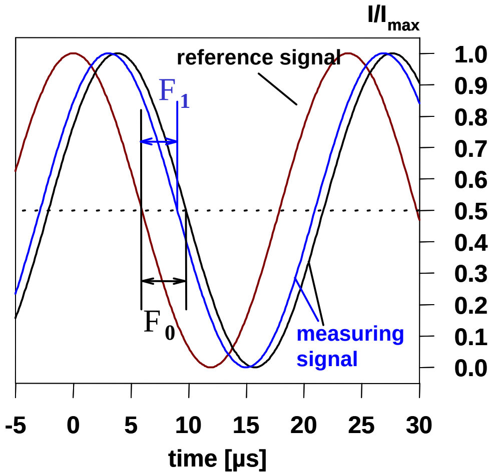


Figure 12.6 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement:

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;
- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;
- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ photobleaching and leaching of the indicator dye has no influence on the measuring signal;
- The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

12.1.5 Literature

If you want to find out more about this subject, we recommend the following publications.

- Wolfbeis O.S. (Ed.), ***Fiber Optic Chemical Sensors and Biosensors***, Vol. **1**&**2**, CRC, Boca Raton (1991).
- Klimant I., Wolfbeis O.S., ***Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes***, Anal. Chem., **67**, 3160-3166 (1995).
- Klimant I., Kühl M., Glud R.N., Holst G., ***Optical measurement of oxygen and temperature in microscale: strategies and biological applications***, Sensors and Actuators B, **38-39**, 29-37 (1997).
- Holst G., Glud R.N., Kühl M., Klimant I., ***A microoptode array for fine-scale measurement of oxygen distribution***, Sensors and Actuators B, **38-39**, 122-129 (1997).
- Klimant I., Meyer V., Kühl M., ***Fiber-optic oxygen microsensors, a new tool in aquatic biology***, Limnol. Oceanogr., **40**, 1159-1165 (1995).
- Klimant I., Ruckruh F., Liebsch G., Stangelmayer A., Wolfbeis O.S., ***Fast Response Oxygen Microsensors Based on Novel Soluble Ormosil Glasses***, Mikrochim. Acta, **131**, 35-46 (1999).

12.2 Determination of the Oxygen Concentration Using a Modified Stern-Volmer Equation

The Stern-Volmer equation (**4**) displays a linear correlation between $\tan\Phi_0/\tan\Phi$ or τ_0/τ and the oxygen concentration $[O_2]$.

$$\frac{\tan\Phi_0}{\tan\Phi} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \qquad (\mathbf{4})$$

Φ_0	phase angle of oxygen-free water
Φ	measured phase angle
K_{SV}	Stern-Volmer constant
$[O_2]$	oxygen content in % air-saturation

The Stern-Volmer plots of all our sensors show a distinct non-linearity in their response behavior which is also observed for many other oxygen sensors described in literature.

This non-linear response behavior can be described with a **modified** Stern-Volmer equation (**5**).

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1 + K_{SV_1} \cdot [O_2]} + \frac{1 - f_1}{1 + K_{SV_2} \cdot [O_2]}) \right)^{-1} \qquad (\mathbf{5})$$

This model is based on the assumption that the indicator is distributed in the polymer matrix at two different sites and each fraction (f_1, 1-f_1) shows a different quenching constant (K_{SV1}, K_{SV2}).

For practical use this model is not very convenient since it has too many parameters which have to be calibrated. Therefore, two simplified models based on equation 5 can be used.

In the first model, one fraction of the indicator is assumed to be non-quenchable (K_{SV2}=0).

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1 + K_{SV} \cdot [O_2]} + (1 - f_1) \right)^{-1} \qquad (\mathbf{6})$$

The oxygen content in % air-saturation can be calculated according to equation 7.

$$[O_2] = \frac{1 - \dfrac{\tan F}{\tan F_0}}{K_{SV} \cdot \left(\dfrac{\tan F}{\tan F_0} - (1 - f_1) \right)} \qquad (\mathbf{7})$$

A second model, which also is based on equation 5, can be used for describing the oxygen calibration plot. In this model, K_{SV2} is set to be $m{*}K_{SV1}$.

$$\frac{\tan F_0}{\tan F} = \left(\frac{f_1}{1 + K_{SV_1} \cdot [O_2]} + \frac{1 - f_1}{1 + m \cdot K_{SV_1} \cdot [O_2]}) \right)^{-1} \qquad (\mathbf{8})$$

The oxygen content in % air-saturation can be calculated according to equation 9.

$$[O_2] = \frac{-B + \sqrt{B^2 - 4 \cdot A \cdot C}}{2 \cdot A} \qquad (\mathbf{9})$$

with the coefficients

$$A = \frac{\tan F}{\tan F_0} \cdot m \cdot K_{SV}^2 \qquad (9a)$$

$$B = \frac{\tan F}{\tan F_0} \cdot K_{SV} + \frac{\tan F}{\tan F_0} \cdot m \cdot K_{SV} - f_1 \cdot m \cdot K_{SV} - K_{SV} + f_1 K_{SV} \qquad (9b)$$

$$C = \frac{\tan \Phi}{\tan \Phi_0} - 1 \qquad (9c)$$

12.3 Oxygen Conversion Formulas

Please note:

These conversion formulas are only valid in aqueous solutions and humidified air. These formulas have to be modified if measurements have to be performed in organic solvents or solutions with high salinity.

% saturation

% air-saturation

Default setting of the instrument (see equation 9 in 12.2)

% oxygen-saturation

$$\%\, O_2 = \%\ \text{air-saturation} \cdot \frac{20.95}{100} \qquad (10)$$

0.2095: volume content of oxygen in air

ppm in the gaseous phase:

$$\text{ppm}[O_2] = \%\ \text{air} - \text{saturation} \cdot \frac{20.95}{100} \cdot \frac{1}{10000} = \frac{\%\, O_2}{10000} \qquad (11)$$

$$1\text{ppm} = \frac{1}{1000000} = \frac{1\text{mg}}{1\text{kg}} = \frac{1\mu\text{L}}{1\text{L}} = \frac{1}{10000}\%$$

Partial pressure of oxygen

in hPa

$$p_{O_2}[\text{hPa}] = \left(p_{atm}[\text{hPa}] - p_W(T)[\text{hPa}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (12)$$

in mbar

$$p_{O_2}[\text{mbar}] = \left(p_{atm}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (13)$$

in Torr

$$p_{O_2}[\text{Torr}] = \left[\left(p_{atm}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095\right] \cdot 0.75 \qquad (14)$$

Please note:

1 mbar = 1 hPa = 0.750 Torr

Oxygen Concentration

in mg/L

$$c_{O_2}[\mathrm{mg/L}] = \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \qquad (\mathbf{15})$$

in ppm = mg/L

$$c_{O_2}[\mathrm{ppm}] = c_{O_2}[\mathrm{mg\ /\ L}] = \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot a(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \qquad (\mathbf{16})$$

in µmol/L

$$\begin{aligned} c_{O_2}[\mu\mathrm{mol/L}] &= c_{O_2}[\mathrm{mg/L}] \cdot \frac{1000}{M(O_2)} = c_{O_2}[\mathrm{mg/L}] \cdot 31.25 \\ &= \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot a(T) \cdot 1000000 \cdot \frac{1}{V_M} \end{aligned} \qquad (\mathbf{17})$$

p_{atm}: actual atmospheric pressure

p_N: standard pressure (1013 mbar)

0.2095: volume content of oxygen in air

$p_W(T)$: vapor pressure of water at temperature T given in Kelvin

$\alpha(T)$: Bunsen absorption coefficient at temperature T; given in $cm^3(O_2)/cm^3$

$M(O_2)$: molecular mass of oxygen (32 g/mol)

V_M: molar volume (22.414 L/mol)

12.4 Temperature-Dependent Constants Affecting the Oxygen Content

12.4.1 Water Vapor Pressure

As shown in equation 12 - 17, the water vapor pressure p_w influences the oxygen partial pressure of air-saturated water and water vapor-saturated air.

Oxygen partial pressure in dry air:

$$p(O_2) = p_{atm} \cdot 0.2095 \qquad (\mathbf{18})$$

$p(O_2)$: oxygen partial pressure in dry air at a barometric pressure p_{atm}
0.2095: volume content of oxygen in air.

Oxygen partial pressure in air-saturated water and water vapor-saturated air:

$$p(O_2)' = (p_{atm} - p_W(T)) \cdot 0.2095 \qquad (\mathbf{19})$$

Temperature variations strongly affect water vapor pressure, and thus influence the oxygen partial pressure as shown in equation 19.

Table 12.1 Variation of water vapor pressure $p_W(T)$ with temperature.

q [°C]	0	5	10	15	20	25	30	35	40	50
T [K]	273	278	283	288	293	298	303	308	131	323
$p_W(T)$ [mbar]	6.1	8.7	12.3	17.1	23.3	31.7	42.4	56.3	73.7	123.3

A convenient fitting function is given by the Campbell equation 20:

$$p_W(T) = \exp\left(A - \frac{B}{T} - C \cdot \ln T \right) \qquad (\mathbf{20})$$

where T is the temperature in Kelvin and A, B and C constants given in Figure 12.7

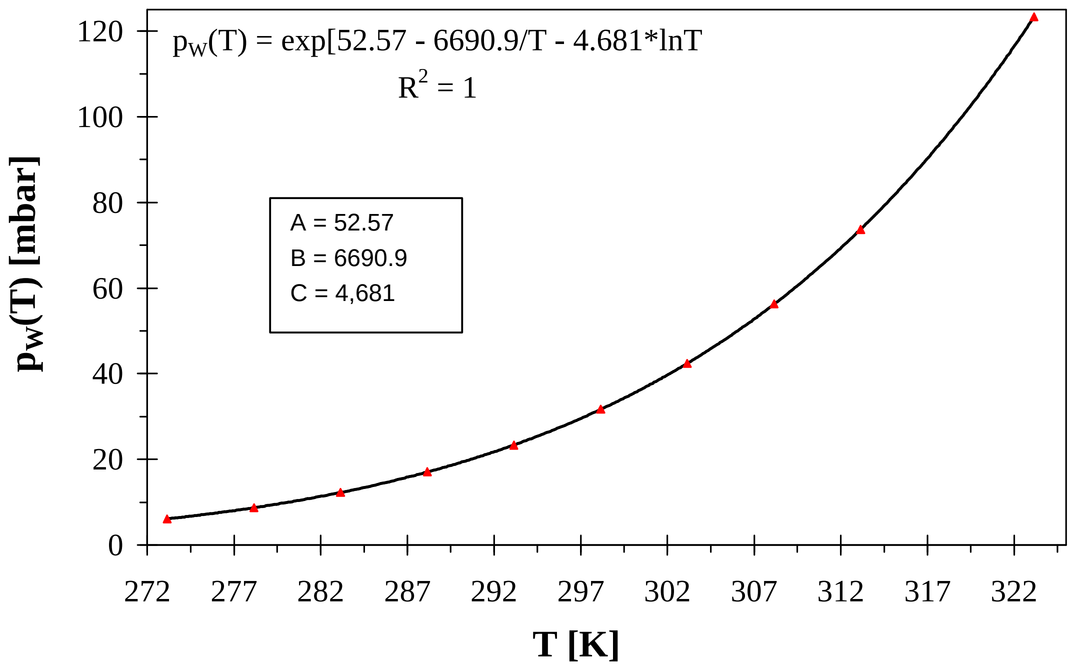


Figure. 12.7 *Variation of water vapor pressure with temperature. R^2 is the square of the correlation coefficient.*

12.4.2 Bunsen Absorption Coefficient

The solubility of oxygen in water is temperature-dependent and can be described using the Bunsen absorption coefficient α(θ) and the oxygen partial pressure $p(O_2)$ according to equation 21. With increasing temperature, the solubility of oxygen in water decreases.

$$c_S(p,?) = \frac{p(O_2) - p_W(T)}{p_N} a(?) \qquad \textbf{(21)}$$

$c_S(p,\theta)$: temperature-dependent solubility of oxygen in water, given in (cm^3 (O_2) / cm^3)

$p(O_2)$: oxygen partial pressure

p_N: standard pressure (1013 mbar)

$\alpha(\theta)$: Bunsen absorption coefficient, given in (cm^3 (O_2) / cm^3)

Table 12.2 Variation of Bunsen absorption coefficient α(θ) with temperature.

q **[°C]**	0	5	10	15	20	25	30	35	40	50
a(q)·**10^3**	49.01	42.94	38.11	34.17	31.01	28.43	26.30	24.63	23.16	20.85

The data in Table 12.2 can be described by two forms of equations.

The first form of equation to describe the temperature-dependent variation of the Bunsen absorption coefficient α(θ) is obtained by fitting a general power series to the values in Table 12.2. A fourth degree polynomial fit can be chosen, yielding equation 22.

$$10^3\alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4 \qquad \textbf{(22)}$$

where θ is the temperature in °C and a - e the coefficients calculated by standard curve fitting procedures as given in Figure 12.8.

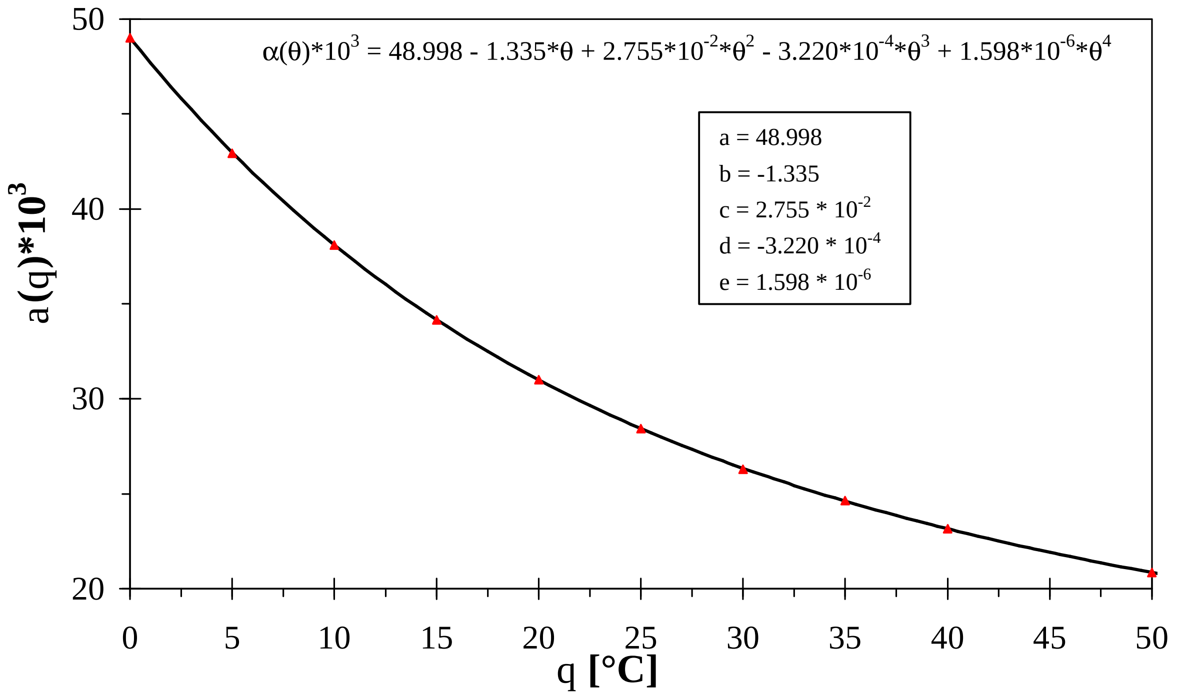


***Figure 12.8** Variation of Bunsen absorption coefficient a(q) with temperature. R^2 is the square of the correlation coefficient.*

The other form of equation to describe the variation of α with temperature can be derived from a thermodynamical correlation and gives an equation of the form

$$\ln 10^3 \alpha = \frac{A}{T} + B \cdot \ln T + C \qquad (23)$$

where A, B and C are constants and T is the temperature in K. For oxygen dissolved in water we find by fitting the equation to the values of α in Table 12.2 that A = 8.553 * 10^3, B = 2.378 * 10, and C = -1.608 * 10^2.

Values of α calculated from eqns. 22 and 23 for the same temperature agree within ± 0.5 %.

The Bunsen absorption coefficient, however, is not a very practical measure. Values of α(θ) have therefore to be converted to mg/L, and the method for doing this is best illustrated by an example.

Example: Calculation of the oxygen content ($c_S(p_{atm},\theta)$) in air-saturated water at a temperature θ of 20°C.

Equation 21 allows the solubility of oxygen in air-saturated fresh water to be calculated for any temperature and pressure provided that the values of the Bunsen absorption coefficient α(T) and the vapor pressure $p_W(T)$ at the particular temperature are known. Equation 22 or 23 can be used to obtain α, and p_W can be calculated from equation 20. The oxygen content c_S of air-saturated water can be calculated according to

$$c_S(p_{atm},\theta) = \frac{p_{atm} - p_W(\theta)}{p_N} \cdot 0.2095 \cdot \alpha(\theta) \cdot \frac{M_{O_2}}{V_M}. \qquad (24)$$

In equation 24, p_{atm} is the actual atmospheric pressure corrected for the contribution of the water vapor pressure p_W and related to standard pressure p_N. The corrected pressure is multiplied by 0.2095, the volume content of oxygen in air, by α(θ) and by the molecular mass of oxygen (M_{O2}) divided by the molar volume V_M.

At a given atmospheric pressure of 1013 mbar (p_{atm} = p_N) and a temperature of 20 °C the oxygen content can be calculated according to equation 24 and results in

$$c_S(1013\text{mbar},20°\text{C}) = \frac{1013 - 23.3}{1013} \cdot 0.2095 \cdot 0.031 \cdot \frac{32 \cdot \text{g/mol}}{22.414\text{mol/L}} = 0.009\text{g/L} = 9.06\text{mg/L} \qquad (25)$$

Table 12.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C. The calculated value for c_S at a temperature of 20.0 °C agrees with the tabulated value of 9.08 mg/L.

Figure 12.9 shows the temperature-dependent oxygen solubility in air-saturated fresh water.

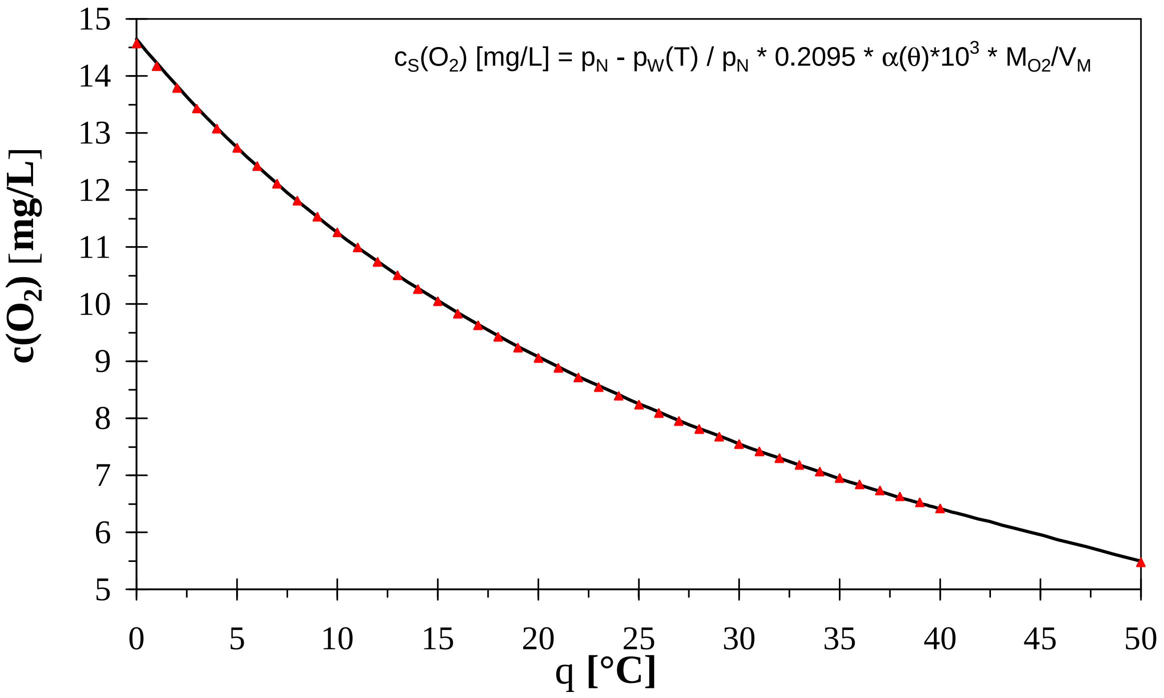


Figure 12.9 *Dependence of the oxygen solubility in air-saturated fresh water on temperature.*

Table 12.3 Oxygen solubility in air-saturated fresh water [mg/L].

T [°C]	c_S(T)	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
0	**14.**	64	60	55	51	47	43	39	35	31	27	23
1		23	19	15	10	06	03	99	95	91	87	83
2	**13.**	83	79	75	71	68	64	60	56	52	49	45
3		45	41	38	34	30	27	23	20	16	12	09
4		09	05	02	98	95	92	88	85	81	78	75
5	**12.**	75	71	68	65	61	58	55	52	48	45	42
6		42	39	36	32	29	26	23	20	17	14	11
7		11	08	05	02	99	96	93	90	87	84	81
8	**11.**	81	78	75	72	69	67	64	61	58	55	53
9		53	50	47	44	42	39	36	33	31	28	25
10		25	23	20	18	15	12	10	07	05	02	99
11	**10.**	99	97	94	92	89	87	84	82	79	77	75
12		75	72	70	67	65	63	60	58	55	53	51
13		51	48	46	44	41	39	37	35	32	30	28
14		28	26	23	21	19	17	15	12	10	08	06
15		06	04	02	99	97	95	93	91	89	87	85
16	**9.**	85	83	81	70	76	74	72	70	68	66	64
17		64	62	60	58	56	54	53	51	49	47	45
18		45	43	41	39	37	35	33	31	30	28	26
19		26	24	22	20	19	17	15	13	11	09	08
20		08	06	04	02	01	99	97	95	94	92	90
21	**8.**	90	88	87	85	83	82	80	78	76	75	73
22		73	71	70	68	66	65	63	62	60	58	57
23		57	55	53	52	50	49	47	46	44	42	41
24		41	39	38	36	35	33	32	30	28	27	25
25		25	24	22	21	19	18	16	15	14	12	11
26		11	09	08	06	05	03	02	00	99	98	96
27	**7.**	96	95	93	92	90	89	88	86	85	83	82
28		82	81	79	78	77	75	74	73	71	70	69
29		69	67	66	65	63	62	61	59	58	57	55
30		55	54	53	51	50	49	48	46	45	44	42
31		42	41	40	39	37	36	35	34	32	31	30
32		30	29	28	26	25	24	23	21	20	19	18
33		18	17	15	14	13	12	11	09	08	07	06
34		06	05	04	02	01	00	99	98	97	96	94
35	**6.**	94	93	92	91	90	89	88	87	85	84	83
36		83	82	81	80	79	78	77	75	74	73	72
37		72	71	70	69	68	67	66	65	64	63	61
38		61	60	59	58	57	56	55	54	53	52	51
39		51	50	49	48	47	46	45	44	43	42	41
40		41	40	39	38	37	36	35	34	33	32	31

Example:: c_S(**20.0°C**) = **9.08 mg/L**

12.4.3 Dependence on the Salt Concentration

Table 12.4 gives values of the concentration of dissolved oxygen at several temperatures in solutions with various chloride concentrations. Increasing the salt concentration leads to a decrease in oxygen solubility. This behavior is characteristic for the solubility of many nonelectrolytes - it is the phenomenon known as the ***salting-out effect***.

Instead of chlorinity $[Cl^-]$ - the amount of chloride in parts per thousand - which was used as a measure of the amount of salt in water, the term salinity is often used. If salinity is preferred as a measure of salt concentration, then the conversion from g/L can be readily made using equation 26.

$$S = 1.805[Cl^-] + 0.03 \qquad (\mathbf{26})$$

where S is the salinity in [‰] or [g/1000g].

Table 12.4 Solubility of oxygen in water as a function of temperature and salt concentration (Total pressure = 760 torr)

T [°C]	***Oxygen solubility [mg/L]***					
$[Cl^-]$ (g/1000g)	**0**	**4**	**8**	**12**	**16**	**20**
0	14.5	13.9	13.3	12.6	12.0	11.3
10	11.3	10.8	10.4	9.9	9.5	9.0
20	9.1	8.8	8.5	8.1	7.8	7.4
30	7.5	7.3	7.0	6.7	6.4	6.1

The effect of increasing the salt concentration on the vapor pressure is negligible small as shown in Table 12.5.

Table 12.5. Variation of solution vapor pressure (p_W) with salt concentration

T [°C]	***Vapor pressure of solution (torr)***			
$[Cl^-]$ (g/1000g)	**0**	**9**	**18**	**26**
0	4.6	4.5	4.4	4.4
10	9.2	9.1	8.9	8.8
20	17.5	17.3	17.0	16.7
30	31.8	31.4	30.9	30.4

The dependence of oxygen solubility on salt concentration can also be obtained from equation 21 except that now values calculated from either equation 27 or 28 have to be used for calculation of the Bunsen absorption coefficient. Equation 27 differs from equation 22 by an additional forth degree polynomial term for chlorinity.

$$10^3 \cdot \alpha = a + b \cdot \theta + c \cdot \theta^2 + d \cdot \theta^3 + e \cdot \theta^4 - [Cl^-] \cdot (p + q \cdot \theta + r \cdot \theta^2 + s \cdot \theta^3 + t \cdot \theta^4) \qquad (\mathbf{27})$$

where θ is the temperature in °C, a - e are the coefficients used in equation 22 and p - t are new constants given in Table 12.6. The values of these new constants are obtained by fitting the polynomial to experimental data in the ranges $0 \le \theta \le 30°C$ and $0 \le [Cl^-] \le 20‰$. To obtain an oxygen solubility from the Bunsen absorption coefficient, the same procedure as described previously is used (s. equation 24, page 60).

An alternative equation to compensate the Bunsen absorption coefficient by the salt concentration displays equation 28.

$$10^3 \cdot a = \exp\left[\left(A + \frac{B}{T} + C \cdot \ln T + D \cdot T\right) - [Cl^-] \cdot \left(P + \frac{Q}{T} + R \cdot \ln T + S \cdot T\right)\right] \qquad (\mathbf{28})$$

where T is the temperature in Kelvin, and A - D and P - S are the coefficients given in Table 12.6. They are based on measurements for $273.1 \le T \le 308.18$ K and $0 \le [Cl^-] \le 30‰$ and is therefore more extensive than equation 27. Both equations give values of $10^3 \cdot \alpha$ which agree to better than ± 1 %.

Table 12.6 Values of the coefficients in equations **27** and **28**.

Eqn. 27	a	$4.900 * 10$	p	$5.516 * 10^{-1}$
	b	-1.335	q	$-1.759 * 10^{-2}$
	c	$2.759 * 10^{-2}$	r	$2.253 * 10^{-4}$
	d	$-3.235 * 10^{-4}$	s	$-2.654 * 10^{-7}$
	e	$1.614 * 10^{-2}$	t	$5.362 * 10^{-8}$
Eqn. 28	A	-7.424	P	$-1.288 * 10^{-1}$
	B	$4.417 * 10^{3}$	Q	$5.344 * 10$
	C	-2.927	R	$-4.442 * 10^{-2}$
	D	$4.238 * 10^{-2}$	S	$7.145 * 10^{-4}$

Seawater has a typical salinity of 35 ‰ (35 g / 1000 g) or a chloride content of about 19 ‰, and therefore falls within the scope of both equations.

12.5 Temperature Compensation of the Response of Oxygen Sensors

A typical oxygen response characteristic at different temperatures is shown in Figure 12.10. The phase angle Φ is a function of the oxygen content ($\Phi = \tan\Phi = f(O_2)$) and decreases with increasing the oxygen content.

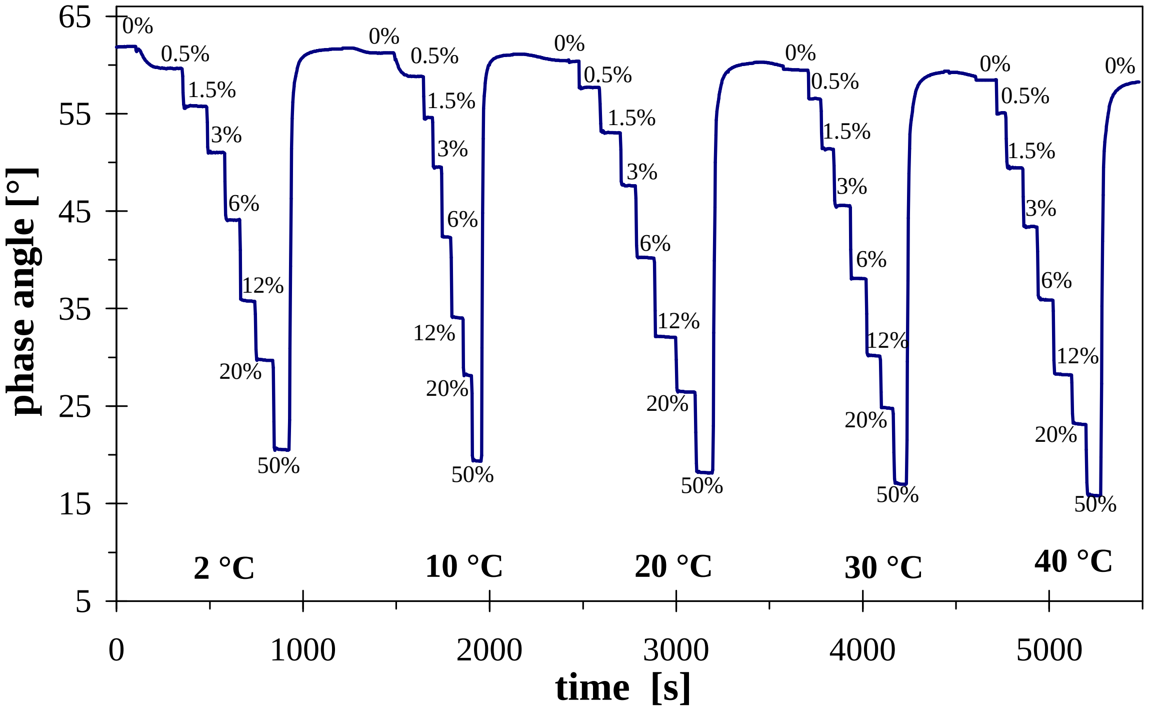


__Figure 12.10__ Oxygen response characteristics at different temperatures.

Figure 12.11 displays the oxygen dependence of the phase angle at different temperatures and Figure 12.12 the respective Stern-Volmer plots. These two figures show that both the phase angle in absence of oxygen (Φ_0; Figure 12.11) and the Stern-Volmer constant (K_{SV}; starting slope in Figure 12.12 according to equation 4) are temperature-dependent. Φ_0 decreases with increasing temperature, while K_{SV} increases with increasing temperature.

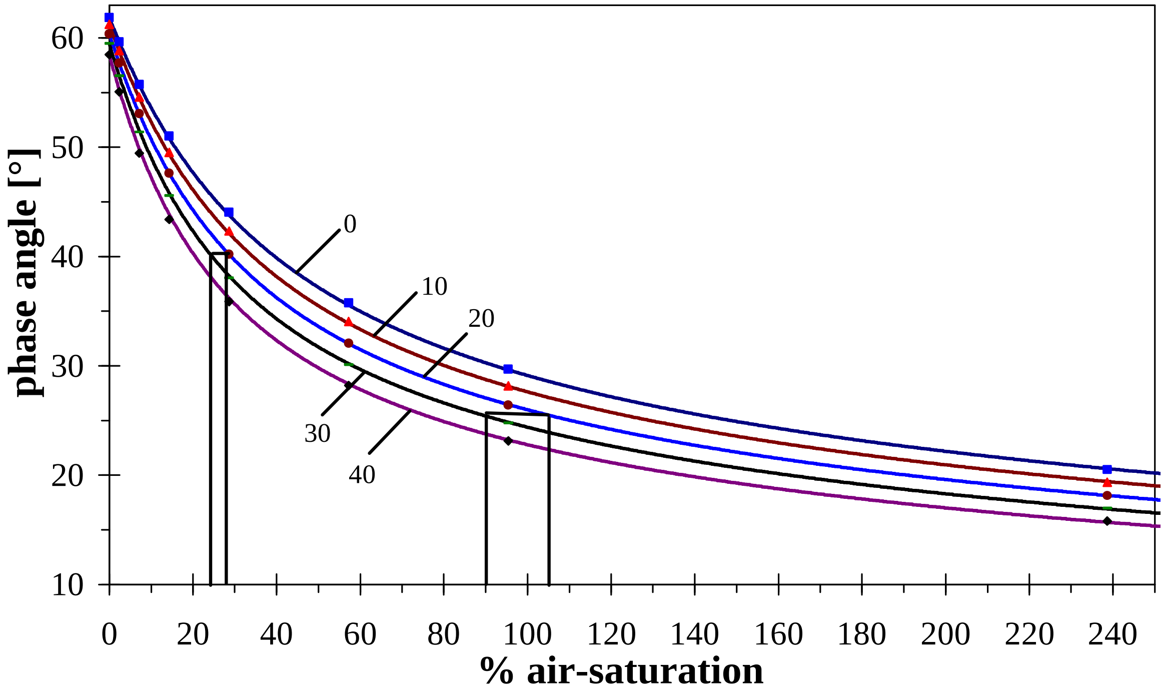


Figure 12.11 *Effect of the temperature on the phase angle at different oxygen contents given in % air-saturation.*

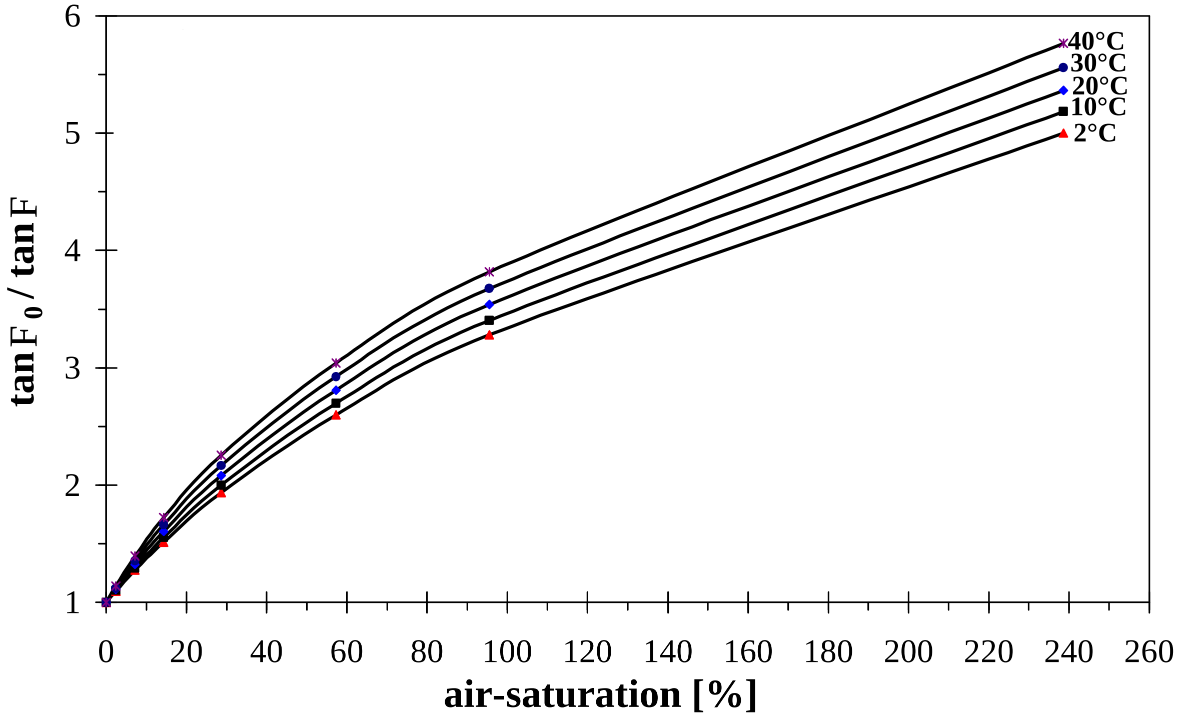


Figure 12.12 *Effect of the temperature on the Stern-Volmer constant.*

Figure 12.13 displays the temperature dependence of Φ_0 and K_{SV}. From Figure 12.13, decreases in Φ_0 of about 0.09° can be calculated by increasing the temperature by 1 K. On the other hand, the Stern-Volmer constant (K_{SV}) increases about $5.0*10^{-4}$ [% air-sat.]$^{-1}$ by increasing the temperature by 1 K.

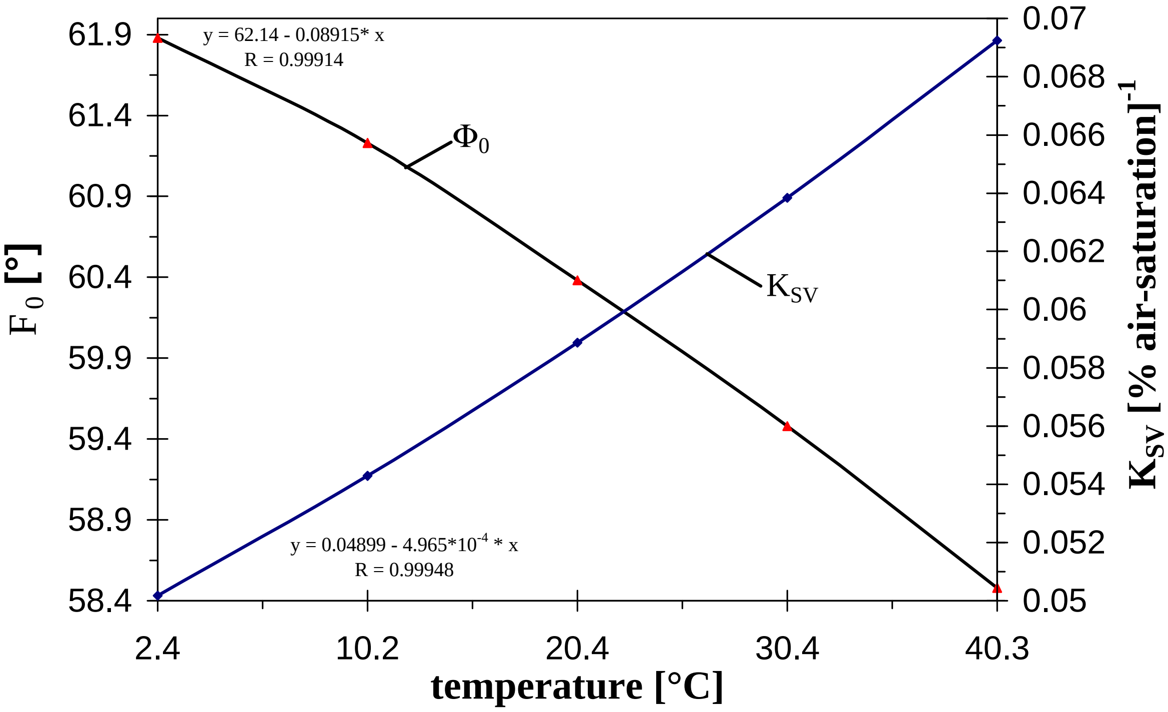


Figure 12.13 *Effect of the temperature on F_0 and the Stern-Volmer constant (K_{SV}).*

Consequently, variations in the temperature cause variations in the measuring value at a constant oxygen content (see Figure 12.11). Table 12.7 displays the deviation of the measuring value from the real oxygen content depending on temperature and the oxygen content.

Table 12.7 Deviations in the measured oxygen content (Δ % air-saturation) at a constant oxygen content and variations in temperature by ± 1K.

% air-saturation	25 % air-sat. (50.9 hPa)	100 % air-sat. (203.6 hPa)	250 % air-sat. (508.9 hPa)
Δ% air-saturation /K	± 0.43 (0.88 hPa)	± 1.62 (3.30 hPa)	± 3.91 (7.96 hPa)

Example:

If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100 % air-saturation of 100 ± 0.7 air-saturation.