Nitric Oxide Sensor

User Manual

UNISENSE
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Nitric Oxide Sensor User Manual

UNISENSE A/S

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1. WARRANTY AND LIABILITY

1.1 Notice to Purchaser

This product is for research use only. Not for use in human diagnostic or therapeutic procedures.

1.2 Warning

Microsensors have very pointed tips and must be handled with care to avoid personal injury and only by trained personnel. Unisense A/S recommends users to attend instruction courses to ensure proper use of the products.

1.3 Warranty and Liability

The NO microsensor is covered by a 90 days limited warranty. Microsensors are a consumable. Unisense will only replace dysfunctional sensors if they have been tested according with the instructions in the manual within 14 days of receipt of the sensor(s).
The warranty does not include repair or replacement necessitated by accident, neglect, misuse, unauthorized repair, or modification of the product. In no event will Unisense A/S be liable for any direct, indirect, consequential or incidental damages, including lost profits, or for any claim by any third party, arising out of the use, the results of use, or the inability to use this product.

Unisense mechanical and electronic laboratory instruments must only be used under normal laboratory conditions and a dry and clean environment. Unisense assumes no liability for damages on laboratory instruments due to unintended field use or exposure to dust, humidity or corrosive environments.

1.4 Repair and Adjustment

Sensors and electrodes cannot be repaired. Equipment that is not covered by the warranty will, if possible, be repaired by Unisense A/S with appropriate charges paid by the customer. In case of return of equipment please contact us for return authorization.

For further information please see the document General Terms of Sale and Delivery of Unisense A/S as well as the manuals for the respective products.

2. CONGRATULATIONS WITH YOUR NEW PRODUCT!

2.1 Support, ordering, and contact information

The Nitric Oxide (NO) microsensor is a miniturized sensor for measuring the partial pressure of NO gas in the nanomolar range.

If you wish to order additional products or if you encounter any problems and need scientific/technical assistance, please do not hesitate to contact our sales and support team. We will respond to your inquiry within one working day.

E-mail: sales@unisense.com
Unisense A/S
Langdyssen 5
DK-8200 Aarhus N, Denmark
Tel: +45 8944 9500

Further documentation and support is available at our website www.unisense.com.

3. REPLACEMENT OF SENSORS

Unisense will replace sensors that have been damaged during shipment provided that:

- The sensors were tested immediately upon receipt in accordance with the delivery note and the manual.
- The seal is still intact.
- the sensors are returned to Unisense for inspection within two weeks (contact the sales team before returning).
- the sensors are correctly packed for return to Unisense, in accordance with the note included in the sensor shipping box.

4. OVERVIEW

This manual covers the following sensors:

NO-15	Tip diameter 15-20 µm
NO-50	Tip diameter 40-60 µm
NO-100	Tip diameter 80-120 µm
NO-500	Tip diameter 400-600 µm
NO-MR	Designed for use with microrespiration system
NO-NP	Needle-type NO sensor for piercing
NO-ST-1/4	With ¼ inch stainless steel tube
NO-SL-1/4	With ¼ inch stainless steel flow cell (Swagelok)
NO-SL-1/8	With ⅛ inch stainless steel flow cell (Swagelok)
NO-PEEK-1/8	With ⅛ inch PEEK flow cell
NO-PEEK-1/16	With 1/16 inch PEEK flow cell
NO-FT-GLASS-6	With 6 mm OD glass flow cell
NO-FT-GLASS-8	With 8 mm OD glass flow cell

The Unisense nitric oxide (NO) microsensor is designed for research applications within physiology, biotechnology, environmental sciences, and related areas.

WARNING: Unisense sensors are neither intended nor approved for use in humans

The Unisense NO microsensor can be made with:

- a small tip size (15 and 50 µm), which ensures excellent response time and insignificant stirring sensitivity and makes it possible to make reliable and fast measurements with high spatial resolution
- a larger tip size (100 and 500 µm) which allows for low detection limit (approx. 2-3 nM)

For non-destructive measurements, the sensor should be designed to have a small tip; for other applications the sensor is designed for maximum sensitivity.

4.1 Working Principle

The NO sensor is a miniaturized Clark-type sensor which must be connected to a high-sensitivity picoammeter. The measuring anode inside the sensor tip is polarized against an internal reference electrode by the picoammeter. Driven by the external partial pressure, NO from the environment will penetrate the sensor tip membrane and reach the anode surface where it is oxidized. The picoammeter converts the resulting oxidation current to a voltage signal.
The Unisense NO sensor has an extremely low consumption compared to other standard NO sensors. The maximum consumption (i.e. for the NO100) is 935 femtomol/hour which corresponds to 0.1%/hour in a 1ml 0.1uM NO sample.

5. GETTING STARTED

5.1 Unpacking a new sensor

When receiving a new NO microsensor, remove the shock-absorbing grey plastic net. Please do not remove the seal and protective tube before the following steps are successfully completed.

WARNING: It is extremely important to ground the NO sensor before use. Otherwise the sensor is very susceptible to electrical disturbances and in some cases will be damaged. Please read the information and directions in this manual and contact Unisense if you are in doubt of what to do.

WARNING: Do not remove the seal and protective plastic tube before these steps and calibration are succesfully completed.

5.2 Grounding the set-up

Before connecting the NO sensor, it is important to ground the set-up, both the measurement media and the picoammeter. Grounding the set-up means to stabilize its electrical potential to avoid noisy signals and/or damage to the sensor. Grounding is done by connecting any media/liquids the sensor will touch to the ground connection of the picoammeter using the provided wire, and connecting the ground plug of the picoammeter to an earth line. An earth line can often be found in electrical outlets, alternatively a good electrical connection to a water pipe may be used. If an earth line is not available, at least keep media and picoammeter ground connected.

Technical details: The tip membrane of a microsensor serves as an electrical barrier which minimizes the effect of electrical disturbances. If, however, a sufficiently high electrical potential has built up across the membrane, the resistance is not high enough to prevent a discharge of electricity across the membrane. Often this happens when a person carrying electrical charges touches the set-up. The discharge across the membrane can cause a permanent breakdown of the electrical resistance of the membrane and subsequently, electrical noise e.g. 50/60 Hz noise from mains power installations can then affect the signal severely. As NO sensors have particularly thin membranes, they are more prone to this problem than other sensors. See also the Troubleshooting section.

5.3 Polarization

The signal from the NO sensor is a small current in the picoampere range. Therefore the NO sensor must be connected to a polar-izing picoammeter (e.g. a UniAmp instrument, Microsensor Monometer, a Microsensor Multimeter, or the Unisense PA2000).

NOTE: The conversion of sensor signal in pA to amplifier signal in mV is controlled by the Pre-Amp Range (mV/pA) setting in the software (UniAmp) or on the amplifer (not PA-2000)

The anode of the nitric oxide sensors should be polarized at +1250 mV relative to the cathode. This happens automatically on the Unisense UniAmp series instruments. On the Unisense Multimeter, Monometer and PA2000 instruments this must be set manually. Please consult relevant the instrument manual for how to adjust polarization.
If you are using a PA2000 amplifier, please check the polarization voltage before connecting the sensor, since incorrect polarization may destroy the sensor.

5.4 Pre-polarization

When the sensor is not in use, NO will build up inside the elec-trolyte. This must be removed order to obtain a stable zero current as background for calibrations and measurements. and thus stable operation of the sensor is possible. Therefore, a period of polar-ization is necessary before you can use the sensor. This is called the pre-polarization period.

When the NO sensor is connected to the picoammeter and polarization of the sensor is initiated, the signal will be very high, drop rapidly over the first few minutes, and then drop more slowly. For new sensors and sensors that have not been in use for several days, it takes up to 24 hours for the signal to stabilize. After shorter periods without polarization, the sensor should be polarized until it has exhibited a stable signal for 10 minutes.

If the signal does not stabilize below 30 pA at room temperature, please look in the 'Trouble-shooting' section of this manual.

5.5 Calibration

Before concentration measurements can be performed, a calibration should be performed.

IMPORTANT: Calibration must be performed after pre-polarization when the sensor signal has stabilized. Always use a calibration solution with the same temperature and salinity as the sample solution.

The NO sensor responds linearly to NO from zero up to a maximum linear concentration. Thus, a two-point calibration is sufficient. The maximum linear concentration depends on the application the sensor is designed for. The calibration standards should be within the linear range for the sensor in question and cover the expected concentration range to be measured, e.g. zero NO and 1 μM.

Using NO stock solution: Please see the following section "Stock solutions and NO donors" for instruction on preparation.

Zero reading: Record the signal, S0, in water without any NO (or interferents). (Note: Be careful if you try to use gas-sparged water which has contained NO. NO will convert to NO_2^- in the presence of oxygen and if the water is acidic, HNO_2 is formed. HNO_2 interferes on the NO sensor and is not easily removed by sparging the water).

High reading: NO will react spontaneously with oxygen, so an NO calibration solution should be made in an anoxic environment. A continuously anoxic solution can be maintained in the following manner: Get a vessel – preferably made of glass – with a lid with two holes in it (e.g. Unisense CAL300). One hole is for sparging with oxygen free gas (e.g. N_2 gas), the other hole is for inserting the NO sensor. The vessel is filled ¾ with neutral pH buffered water, and there is thus a headspace under the lid. The sensor is placed through the sensor hole in the lid with its tip a few cm inside the buffer.
The oxygen-free gas is led through the gas-sparging hole through a long pipe or needle that opens at the bottom of the vessel. Bubbling oxygen-free gas through the buffer solution will remove all oxygen. The gas should stream fast enough to effectively flush the headspace, preventing atmospheric oxygen from entering through any of the holes in the lid. For the Unisense calibration chamber CAL300, 5 minutes of bubbling at a rate of 0.5 L gas per minute is sufficient to drive out 99% of the oxygen.

WARNING: Bubbling of water with gas may cause the water to change temperature due to the gas temperature or evaporative cooling. Monitor the temperature to find a suitable bubbling rate, which does not change the water temperature significantly.

After the buffer is stripped of oxygen, the gas pipe is retracted somewhat such that it opens in the headspace.
A small volume of an NO stock solution (see Stock solution and NO donors section below) is injected into the buffer.
The buffer is stirred briefly to distribute the NO evenly. Stirring can be done with a glass rod through the gas flushing hole,

with a stirrer magnet with the vessel placed on a stirrer plate. The continued gas flow in the headspace will keep oxygen out, but will only slowly remove NO from the buffer (as opposed to bubbling which will remove NO quickly). The high calibration reading (S1) is recorded, corresponding to the concentration (C1) calculated from the stock concentration and the dilution ratio.
To convert a signal S from partial pressure to the corresponding concentrations of NO (C), perform a linear conversion:

$C = ((S - S0) \times C1)/(S1 - S0)$

If you use Unisense software, the calibration calculations can be performed by the software. Consult the software manual for details.
Check and repeat calibration at appropriate time intervals to ensure that all measurements can be calibrated to correct concentrations. When the sensor is new, you may need to calibrate more often, while an older extensively used sensor may require calibration only every 24 hours or less. To minimize the need for calibration, it is recommended to keep the sensor polarized between measurements.
The membrane permeability of NO microsensors changes with time, so a change in signal of up to 50% may occur over months. This does not affect the quality of the measurements as long as the sensor is regularly calibrated.

Using NO donors: Some researchers prefer making calibrations with so-called NO donors. We will not describe a procedure here. However, refer-ences can be found in the Stock solutions and NO donors section.

6. STOCK SOLUTIONS AND NO DONORS

There are several ways to prepare an NO stock solution:

1. Using NO gas:

    a. A sealed vial is half-filled with distilled water or buffer, flushed with argon to remove oxygen, and the headspace is finally vacuum-treated. NO gas from a pressure gas tank is bubbled through a washing bottle, containing 1 M NaOH. The bottle captures impurities in the form of N_2O_3 and N_2O_4 gas. The gas streams long enough to flush all oxygen out. After the washing bottles, the gas is lead through a needle. The needle is led to penetrate the septum in the vial, filling the headspace with a slight over-pressure of NO. Subsequently, the vial is shaken to equilibrate the headspace NO with the water/buffer, before the surplus pressure is removed by puncturing the membrane.
    Ref: Methods in Molecular Biology, Vol. 100, Nitric Oxide Protocols. Ed. Michael A. Thitheradge, Humana Press Inc., Totowa N.J., p.232:

    b. A researcher implements the above principle in the following way: bubbles NO gas from a pressure gas tank through two washing bottles in series, containing 5 M NaOH. These bottles capture impurities in the form of NO_2 and NO_3 gas. After the washing bottles, the gas is lead through a long needle that penetrates a septum in a vial. The vial has a few ml distilled water inside and the NO gas bubbles through this water. A second needle through the septum leads from the headspace to the exterior to allow the gas to escape. The set-up is kept in a fume hood. Bubbling for a approx. 15 minutes results in a clean saturated stock solution of NO in the vial. This stock is diluted for calibration standards. After the NO production, the system is flushed for 45 minutes with Argon, if NO is left in the system it will attack rubber tubing.

2. Chemical NO synthesis:

    Another alternative is NO synthesis from $NaNO_2$ with the following procedure: Concentrated H_2SO_4 is slowly added to a N_2-flushed mixture of saturated $NaNO_2$ and KI. This evolves NO gas which is passed through a wash bottle with 10 M NaOH to remove traces of NO_2. The purified NO gas is captured in a vial with a membrane which contains a few ml of distilled water. A second needle through the septum leads from the headspace to the exterior to allow the gas to escape.
    Ref: Diab et al. 2005. Electrochemical nitric oxide sensor preparation: a comparison of two electrochemical methods of electrode surface modification. Bioelectrochemistry 66:105-110.

As an alternative to NO stock solutions, some researchers use the a compound that can decompose and release NO (NO donor).

1. Ref: Richardson et al. 2006. The nitrosative stress response of Staphylococcus aureus is required for resistance to innate immunity. Molecular Microbiology (2006) 61(4), 927–939: "Nitric oxide donating compounds used in this study were obtained from Alexis Biochemicals (San Diego, CA). Diethylamine NONO-ate (DEA/NO, t1/2 = 2 min), Proline NONO-ate (ProliNO, t1/2 = 1.8 s), and Diethylethylenediamine NONO-ate (NOC-12, t1/2 = 100 min) were dissolved to 500 mM final concentration in 0.01 N NaOH and stored at -80°C. SNAP was resuspended to a final concentration of 500 mM in DMSO and aliquots were stored at -80°C. The conjugate donor compounds diethylamine (Sigma D-0806), proline (Sigma P-0380), diethylethylenediamine (Aldrich 126942), and NAP (Aldrich A19008) were diluted to same concentrations as cognate NONO-ates in like solvents and stored at -80°C."'

2. For further information on the SNAP method: Ref: Xhang et al. 2000. Novel Calibration Method for Nitric Oxide Microsensors by Stoichiometrical Generation of Nitric Oxide from SNAP. Electroanalysis 12(6):425-428.

7. MEASUREMENTS

Before doing microscale measurements, the seal must be broken and the protective tube removed. NOTE: this terminates Unisense's warranty.

IMPORTANT: Always introduce and retract the NO microsensor axially using a micro manipulator and a stable stand when measuring in solid or semisolid substrate like sediment, tissue, biofilms, microbial mats, etc.

7.1 Orientation of sensors

The microsensors cannot be used with the tip pointing upwards. This will make the air bubble in the electrolyte rise to the narrow parts of the sensor which will block the electrical conductivity in the electrolyte. If this happens, the signal will be very low. To solve the problem, shake the sensor as mentioned in Troubleshooting below.

7.2 Use of glass-tip microsensors

Although the Unisense microsensors are made of glass, the tip is flexible and can bend slightly, and the sensors are surprisingly **robust in use in coarse substrates**. However, lateral movements of the sensor when the tip is in contact with a solid substrate may easily cause the tip to break. Also, due to the small size of the microsensor tip and to the steepness of gradients in many environments, even a displacement of the sensor tip of few microns may change its environment. Therefore, we recommend that **measurements are performed only in a stabilized set-up free of moving or vibrating devices**. We recommend the Unisense lab stand LS and the Unisense micromanipulator MM-33 (or double MM33-2) for laboratory use. For in situ use we recommend our in situ stand (IS19) and a micromanipulator or our automated in situ profiling instruments.

WARNING: It is extremely important to ground the NO sensor before use. Otherwise the sensor is very susceptible to electrical disturbances and in some cases will be damaged. Please read the information and directions in this manual and contact Unisense if you are in doubt of what to do.

IMPORTANT: To resolve the very small concentrations that are most often of interest, it is very important to eliminate electrical noise as much as possible. This can be done by grounding the set-up (if you have a PA2000, you can place an analog filter on it.)

7.3 Use of sensors without visible glass-tip

Sensors with stainless steel tubes, needles, flow through cells, etc. do not have an exposed glass tip and are, therefore, less fragile. However, these sensors still contain a glass sensor inside which can be damaged by physical shock. To protect the sensor, do not let the sensor drop onto the table or floor. If the sensor has a needle, make sure that the needle does not bend or flex. This will break the glass sensor inside.

7.4 Electrical noise

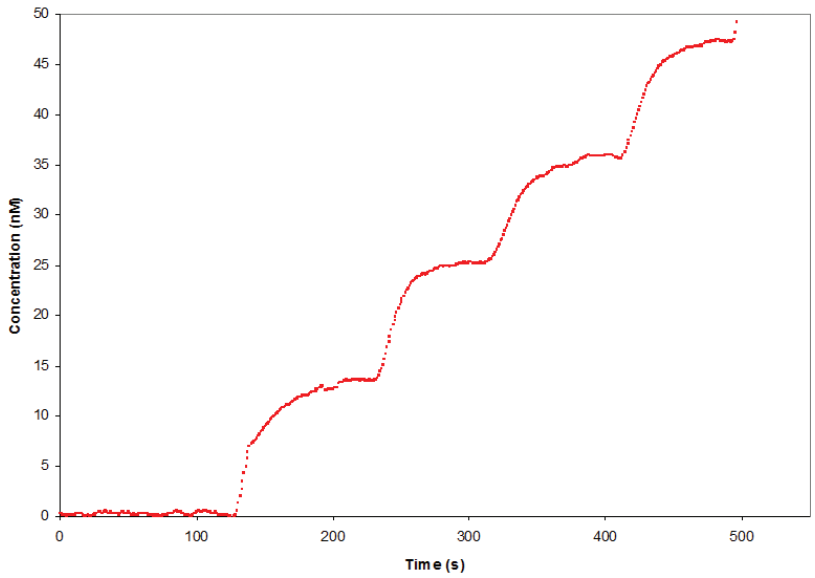
The signal of a NO microsensor is very small (10-14 to 10-10 ampere). Although both the Unisense amplifiers and microsensors in general are relatively resistant to electrical noise from the environment, electrical fields may interfere with the sensor signal.

NO microsensors are more susceptible to electrical noise than e.g. oxygen microsensors, so it is necessary to 'ground' the set-up. This is done by creating an electrical connection between the Ground connection of the picoammeter and the sample to be studied (tissue, sediment core water etc.). Also, unnecessary electrical/mechanical equipment should be switched off and touching components of the set-up during calibrations and measurements should be avoided.

On suspicion of sensor damage, repeat calibration and consult 'Trouble-shooting'.

7.5 Interference

The following compounds and physical parameters are known to exhibit interference on the NO microsensor: HNO_2, H_2S, light, temperature. Keep updated on reported interferents on our web page.



The graph shows a NO sensor response to sequential addition of 12nM of NO.
Data points were taken every second. Each of the shown points is the running average of 10 measured data points.

8. ADVANCED USE OF THE NO SENSORS

Unisense can construct NO sensors for customer requested applications at additional costs. The most frequent construction options are described at our website.

Options include customer specified modifications, response time, stirring sensitivity, pressure tolerance, range, and detection limit. If your requirements for a NO sensor are not described on our website, please contact sales@unisense.com for further options and prices.

Examples of advanced applications:

•Respiration/production rates in small samples in Unisense microrespiration chambers MR-CH
•Measurements of NO under high external pressure e.g. in closed pressurized systems or in the deep sea
•Flow-through cell measurements

9. STORAGE AND MAINTENANCE

8.1 Storage

Store the sensor in the protective plastic tube used for shipping. The NO microsensor can be stored with the tip exposed to water or air. The room in which the NO microsensor is stored should be dry and not too hot (10-30°C). If the sensor is used regularly it can be stored polarized connected to a Unisense Meter.

8.2 Cleaning the sensor

The sensor tip can be sterilized with ethanol.
During use, different compounds may deposit on the sensor tip, resulting in a less sensitive and slower sensor response. Depending on the substance, different solutes can remove the deposit. The standard procedure is to rinse with 96% ethanol, then rinse with 0.01 M HCl and then rinse with water; this will remove most substances.
Alternatively it is possible to rinse with 0.1M NaOH, isopropanol, or detergents.
In some cases the deposits need physical abrasion to be removed; contact Unisense for advice on this.

Solubility in water:

T (°C)	NO solubility (mM)
15	2,31
20	2,10
25	1,92
30	1,78
35	1,66

Reference: SOLUBILITY OF SELECTED GASES IN WATER
by L. H. Gevantman, CRC Handbook of Chemistry and Physics, 92nd Edition

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TROUBLESHOOTING

Problem	High and drifting signal.
Possible cause	Gas bubbles present inside the sensor tip due to short circuit or electrical shock.
Solution	Degas water by boiling and subsequent cooling or by 10 minutes of vacuum treatment. Immerse the sensor tip for 20 min in the degassed water. Repeated or prolonged treatment may be necessary.
Problem	Signal very low and no response to NO.
Possible cause	Gas bubble present in the narrow part of the sensor blocks the electrical conductivity in the electrolyte.
Solution	Shake the sensor gently like shaking an old mercury fever thermometer as shown in this video: unisense.com/video-guides/#troubleshooting
Problem	The sensor tip is broken.
Solution	Replace the NO microsensor.
Problem	Signal very low.
Possible cause	Inactivation of the anode surface.
Solution	Replace the NO microsensor.
Problem	Signal constantly very low and no response to NO.
Possible cause	Gas bubbles present inside the tip of the sensor causes a disruption in the electrolyte.
Solution	Degas water by boiling and *subsequent cooling* or by 10 min. of vacuum treatment. Immerse the sensor tip for 20 min in the degassed water. Repeated or prolonged treatment may be necessary.
Problem	Slow response.
Possible cause	Insoluble compounds deposited at the sensor tip.
Solution	Consult the "Cleaning" section.
Problem	Slow response. Especially needle sensors
Possible cause	A gas bubble is trapped at the needle tip.
Solution	Remove the gas bubble by gentle movements of the sensor.
Problem	Unstable signal or the signal fluctuates if the set-up is touched or equipment is introduced in the medium you are measuring in.
Possible cause	Electrical disturbance of the sensor through the tip membrane
Solution	Ground the set-up using the blue grounding cable supplied with the picoammeter. Connect the reference plug on the picoammeter (blue plug) with the medium you are measuring in. Please see directions on page 8 of this manual.

If you encounter other problems and need scientific/technical assistance, please contact sales@unisense.com for online support (we will answer you within one workday).

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