UNISENSE

HYDROGEN SULFIDE SENSOR USER MANUAL

Hydrogen sulfide sensor manual

Version May 2020

HYDROGEN SULFIDE SENSOR MANUAL

UNISENSE A/S

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WARRANTY AND LIABILITY

Notice to Purchaser

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

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.

Warranty and Liability

The hydrogen sulfide microsensors are covered by a limited warranty. For the SULF-type the warranty period is 6 months and for the H_2S-type this is 3 months. 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 in 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.

Repair or 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.

CONGRATULATIONS WITH YOUR NEW PRODUCT!

Support, ordering, and contact information

The Unisense hydrogen sulfide microsensor is a miniaturized Clark-type hydrogen sulfide sensor designed for reliable and fast measurements in a large number of research applications.

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

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

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.*
- *The sensors are correctly packed for return to Unisense, in accordance with the note included in the sensor box.*

OVERVIEW

This manual covers all the Unisense hydrogen sulfide sensors. Identify your sensor type before connecting to a picoammeter.

IMPORTANT: The two types of sensors must be polarized differently. See Polarization section below. **Wrong polarization may destroy the sensor.**

IMPORTANT
Unisense sensors are neither intended nor approved for use of humans

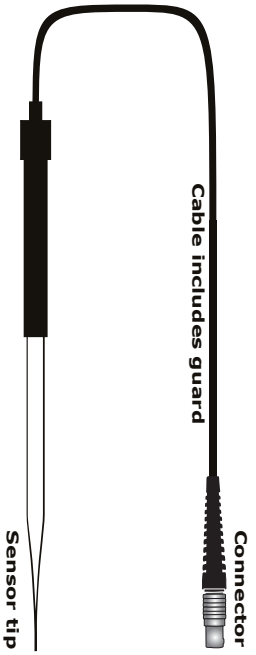
Type I: Label on sensor shaft: SULF-xxx-xxxxxx. The outer glass casing of the sensor is transparent.

Type II: Label on sensor shaft: H_2S-xxx-xxxxxx. The outer glass casing of the sensor is painted black.

The H_2S microsensors are designed for research applications within physiology, biotechnology, environmental sciences, biogeochemistry and related areas.

With their minute tip size, excellent response time, and minimal stirring sensitivity, the H_2S sensors makes it possible to make reliable and fast measurements at high spatial resolution.

Our H_2S microsensors are miniaturized amperometric sensors with internal reference, a sensing and a guard anode. The sensor is connected to a high-sensitivity picoammeter and the anodes are polarized against the internal reference. Driven by the external partial pressure, H_2S from the environment enters through the sensor tip membrane into the electrolyte where the H_2S is ultimately oxidised by the anode. This generates a current in the pA range which is measured by a high quality picoammeter e.g. the Unisense UniAmp series of amplifiers.



The connector contains connections for both reference, guard, and sensing cathode.

In the Type I sensor (SULF-xxx) the signal is generated by oxidation of H_2S directly on the anode in the tip of the sensor. The type I sensor is sensitive to hydrogen and should not be used in environments with high hydrogen concentrations. However, the type I sensor has a better signal to noise ratio, has a longer expected lifetime than the type II sensor and is not sensitive to light.

In the Type II sensor (H_2S-xxx) the H_2S, that enters through the membrane in the tip, is converted to HS^- ions in the alkaline electrolyte. This is immediately oxidized by ferricyanide, producing sulfur and ferrocyanide. The sensor signal is generated by re-oxidation of ferrocyanide at the anode in the tip of the sensor (Jeroschewski et al. 1996). The internal guard anode facilitates a constant ratio of ferri- to ferrocyanide in the electrolyte, thus minimizing the zero-current. The electrolyte in the type II microsensor is photo-degraded by high light intensities – especially UV and blue light. This is results in a higher signal in light than darkness for the same amount of H_2S. The sensors are painted black to protect them against light. However, light may still enter the sensor through the tip, which it is not possible to paint.

For calculation of total sulfide concentrations, it is necessary to know the pH. When measuring H_2S in pH gradients, pH should be measured along the same profile with a pH microsensor (e.g. pH-10/pH-25).

GETTING STARTED

Unpacking a new sensor

When receiving a new microsensor remove the shock-absorbing grey plastic net. **Do not remove the seal and protective tube before the following steps are successfully completed.**

Polarization

The signal from the H_2S sensor is generated in pA. Therefore, the H_2S sensor must be connected to a picoampere amplifier during measurements.
IMPORTANT**: Wrong polarization may destroy the sensor!**

Type I hydrogen sulfide sensors (label on the sensor shaft reads: SULF-xxx-xxxxxx) should be polarised with +200 mV.

Type II hydrogen sulfide sensors (label on the sensor shaft reads: H_2S-xxx-xxxxxx) should be polarised with +85 mV.

Polarization on the fx-6, fx-3 pA, x-5, and H_2S UniAmp
Polarization is automatically set correctly once the sensor is connected to the UniAmp instrument. The polarization is displayed and may be changed in the calibration tab in SensorTrace Suite and in the Unisense Service in the Windows Notification Area on the PC:
Click the Unisense Service icon and a pop-up window will show the channels to which sensors are connected. Click the three horizontal lines for the channel with the H_2S sensor connected. The polarization can now be seen and changed.

Polarization on the Field Microsensor Multimeter
Connect the sensor to a pA-channel (channel 1-5 on a standard Field Multimeter). Polarization is set to 0 mV when connecting the sensor*.
On the "Sensors" screen, highlight the sensor using the arrow keys.

WARNING

Do not remove the seal and protective plastic tube before polarization has been set correctly, the sensor has been pre-polarized as described for each type of sensor, and calibration has been successfully completed

NOTE:

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

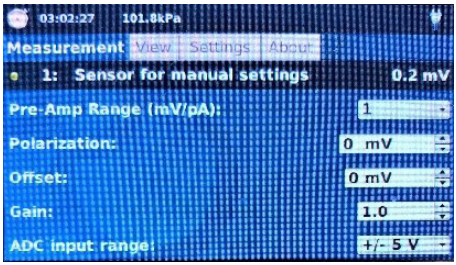
Press SELECT and choose EDIT SENSOR PARAMETERS (only available if a sensor is connected).
Highlight the "Polarization voltage (mV)" and enter the correct polarization.

NOTE: A manually set polarization will remain in effect until it is changed manually, even if the sensor is unplugged, or the instrument has been turned off. Therefore, make sure that the polarization of this channel is not set to a voltage that will damage the sensor. The EDIT SENSOR PARAMETERS menu is only accessible if a sensor is connected. Therefore, to be on the safe side it may be necessary to turn the instrument off and on before connecting the sensor. This will reset the polarization to 0 mV.

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.

Polarization on the Microsensor Multimeter and Monometer

- Connect the sensor to a pA-channel. Polarization is set to 0 mV when connecting the sensor.
- Highlight Sensor for manual settings using the arrow keys and press SELECT
- Go to the Polarization line with the arrow keys and press SELECT. Set the polarization using the up or down arrow keys. The selected polarization becomes active when SELECT is pressed.
- Once the correct polarization is entered, press SELECT.
  The sensor is now polarized.



Polarization on the PA2000

If you are using the legacy PA2000 please consult the PA2000 manual, which is available on www.unisense.com/manuals

If you are using another amplifier or sensor connection type, please check the polarization voltage before connecting a sensor, since incorrect polarization may destroy the sensor.
For details on how to set the polarization, consult the user manual of the amplifier that you are using.

Pre-polarization

A period of polarization is necessary before you can use the sensor. This is called the pre-polarization period. If the sensor is new or has not been operated for several days, it must be pre-polarized for at least 2 hours before it can be calibrated and used. After shorter periods without polarization, the sensor should be pre-polarized until it has shown a stable signal for at least ten minutes. When pre-polarization is initiated, the signal will be very high and then drop rapidly over the first few minutes. After that, the signal will drop slowly for up to two hours (for needle- and some custom-made sensors slightly longer). It takes normally between 10 minutes and an hour before a sensor is stable. The sensor signal depends on the specific sensor (see the delivery note which came with the sensor). If the signal does not stabilize or is too high or too low, refer to the 'Troubleshooting' section.

Calibration theory

The sensor detects the partial pressure of H_2S gas, which is only one component of the total sulfide equilibrium system. If the total sulfide concentration,
[S^{-2}tot], is defined as:

$$S_{tot}^{-2} = [H_2S] + [HS^-] + [S^{2-}]$$

the H_2S concentration will be defined as

$$[H_2S] = [S_{tot}^{-2}] / \left(1 + \frac{K_1}{[H_3O^+]} + \frac{K_1K_2}{[H_3O^+]^2}\right)$$

which can be simplified to

$$[H_2S] = [S_{tot}^{-2}] / \left(1 + \frac{K_1}{[H_3O^+]}\right)$$

for pH<9 (Jeroschewski et al. 1996). Thus it is necessary to know the pH (i.e. to know $[H_3O^+]$) of the sample/calibration solution to calculate $[S_{tot}^{-2}]$ (see Figure 1).

$$[H_3O^+] = [H^+] = 10^{-pH}$$

$$pH = -\log [H^+]$$

In solutions with a pH below 4 the equation can be simplified to:

$$[H_2S] \approx [S_{tot}^{-2}]$$

$$K_1 = 10^{-pK1}$$

pK_1 is dependent on temperature and salinity. The literature gives slightly different equations for calculating pK_1 in water as a function of temperature (T) and salinity (S).
The following equation is derived by Millero et al. 1988:

$pK_1 = -98.08 + 5765.4/T + 15.04555 * LN(T) + (-0.157 * (S^{0.5})) + 0.0135 * S$
(Temperature in Kelvin)

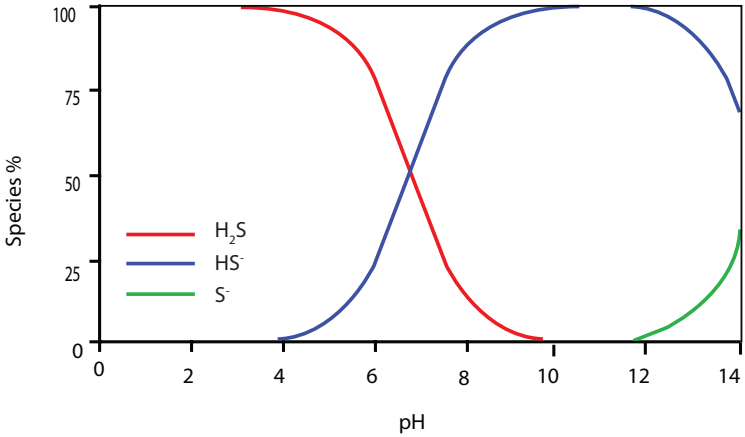


Figure 1. The species distribution of H_2S, HS and S^{2-} as a function of pH.

As H_2S sensors are sensitive to temperature, it is necessary to perform calibration and measurements at the same temperature.

Please note that, in contrast to oxygen, the relationship between salinity, temperature and solubility is not well known and therefore not tabled for sulfide gas

Calibration

It is recommended to use the Unisense H_2S Sensor Calibration Kit for both SULF- and H_2S-type sensors (https://www.unisense.com/calibration_kits/). This kit ensures accurate and simple calibration both in the lab and in the field. The calibration kit can be shipped as normal cargo and does not require dangerous goods shipping. Therefore, it is ideal also for shipping to field work, research cruises etc. The detailed calibration procedure can be found in the H2S Sensor Calibration Kit manual (https://www.unisense.com/manuals/)

The H_2S concentration in the calibration kit is verified against water saturated with a certified H_2S gas mixture. Therefore, the uncertainty in preparing a Na_2S stock solution at a known concentration is eliminated. In the alternative calibration described below, the preparation of the Na_2S stock solution at a known concentration is crucial. Due to the variation in the amount of crystal water in Na_2S x H_2O, the final concentration of the stock solution must be verified through analysis. Furthermore, the sulfide content of the Na_2S crystals may decrease over time due to oxidation of S^{2-} on the surface of the Na_2S crystals.

Alternative calibration

Calibration in the laboratory

Calibration must be performed after the sensor signal has stabilized during pre-polarization. The H_2S microsensor responds linearly over a certain range (e.g. 0-300 µM), above which the slope of the response curve decreases, but the response and resolution above the linear range may be sufficiently good for reliable measurements. We suggest that you establish the linear range by making a coarse calibration in the entire concentration range of

interest. If measurements of concentrations above the linear range are needed, a calibration with sufficient resolution has to be made in the non-linear range, whilst a 3-point calibration is sufficient in the linear range.

Please read the entire procedure below before the calibration is commenced. It is best to first prepare the Na_2S stock solution so it is ready. Then prepare the buffer and perform the calibration quickly thereafter in order to avoid reintroduction of oxygen.

NOTE: For field calibrations, an alternative method is recommended - this will enable you to prepare reagents to bring into the field for easy calibration.

IMPORTANT
It is important to perform calibration and subsequent measurements in solution with the same temperature and salinity.

Laboratory calibration

1. Prepare a stock solution

A stock solution of S^{2-} (≈ 0,01M total sulphide) is prepared anaerobically by dissolving 0,24 g Na_2S * 9 H_2O in 100 mL of N_2-flushed water in a closed container. The crystal water content of Na_2S may vary and the sulfide on the surface of the crystals may become oxidized over time. Therefore, the concentration of the stock solution is most likely lower than that calculated from the amount of Na_2S dissolved. It is recommended that the actual concentration is determined by standard analysis (e.g. Cline 1969 or Budd & Bewick 1952).
Furthermore, the concentration of S^{2-} in the stock solution may decrease over time due to oxidation by oxygen entering the flask either when aliquots are withdrawn from the flask or by diffusion through the stopper.

FIELD CALIBRATION
For field calibrations, please see alternative calibration procedure below.

2. Prepare the calibration buffer

Any standard pH buffer, with a pH value less than 4, can be used. Remove oxygen from a volume of the buffer. This can be done in two ways:

a. By vigorously bubbling with an oxygen-free inert gas (e.g. N_2) for at least 5 minutes. NOTE: Vigorously bubbling buffer with any gas may cause

IMPORTANT
Vigorous bubbling water with any gas may cause the water to cool considerably. Monitor the temperature to find a suitable bubbling rate, which does not cool the water significantly.

the water to cool considerably. Monitor the temperature to find a suitable bubbling rate, which does not cool the buffer significantly.

b. By adding a suitable reductant (e.g. Ti(III)Cl; MERCK supplies this in a 10% HCl solution) to the oxygen free buffer to a final concentration of 50 mM. Add a few glass beads (2-3 mm in diameter) to facilitate mixing. The transfer is preferably performed with a pipette to minimize mixing with oxygen, and a maximum of 10% of the vial volume should be left as headspace. Close the container with a gas tight lid and shake vigorously.

IMPORTANT: do not use (b) protocol with Ti(III)Cl with any of our Low Range SULF (Type 1) sensors!

3. Obtain zero reading

The signal at zero H_2S can be obtained by immersing the sensor tip into one of the vials with calibration buffer. Note the signal, which is the calibration value for zero H_2S partial pressure (S0). This signal should be 0-30 pA (otherwise see "Troubleshooting").

4. Obtain sulfide standard readings

Calibration points within the expected range of measurement are prepared by injecting suitable amounts of the S^{2-} stock solution anaerobically into the calibration buffer with a micro-syringe (the stock solution should be diluted at least 10 times). Mix the solutions. If you have not added reductant, oxygen that dissolve in the calibration solution will oxidize the sulfide, so the stock solution should be added immediately after the oxygen removal (e.g. N_2 bubbling) and the calibration should be done immediately after adding the stock solution.
For each calibration solution, measure the calibration values

by removing the rubber stopper and immerse the microsensor tip into the solution. Read the signal and plot it against the concentration.

The most precise calibration curve is obtained by fixing the calibration solutions with Zn-acetate and subsequently determining the total sulfide concentration using the Cline method (Cline 1969). This may, due to the nature of the method, be difficult if a reductant has been added previously.

Calibration in the field

For an easy and portable field calibration, prepare the following reagents in the lab. Bring the reagents and a 1 ml syringe + needle to the field.

Reagent 1:

- 1 mM Na_2S
- 1 mM NaOH

Prepare the solution in a membrane flask with gas-tight lid. After mixing, flush the solution with nitrogen gas to remove all oxygen and quickly place the lid on the flask.
During storage, practically all sulfide in Reagent 1 will be in alkaline form and since it is stored in an gas-tight vial, it cannot evaporate to any significant degree.

Reagent 2:

- 0.1 M HCl
- 50 mM TiCl

As the TiCl is a strong reducing agent, all oxygen will be reduced in the mixture, but it is still recommended that you avoid introducing too much oxygen during mixing.

Fill reagent 2 into a 10 ml glass vial with gas-tight membrane lid. Avoid head space. **Prepare as many vials as the number of calibrations you expect to make**. Bring reagent 1, the vials with reagent 2 and 1 ml syringe+needle with you to the field.

Perform the zero point determination in clean water (without H_2S) at field site temperature.
Prepare a 100 µM calibration standard. Perform the calibration quickly after mixing as the durability of the mix is short.

1. Loosen the lid of a reagent 2 vial and remove carefully 1 ml with a syringe + needle through the lid. Throw this away.
2. Take 1ml of reagent 1 and add it to the reagent 2 vial.
3. Tighten the lid and mix.
4. Remove the lid and place the sensor tip carefully in the vial and perform the 100 µM calibration.

For making higher or lower concentrated H_2S calibration solutions, make reagent 1 more or less concentrated with Na_2S.

IMPORTANT: do not use (b) protocol with Ti(III)Cl with any of our Low Range SULF (Type 1) sensors!

Re-calibration

Check the calibration at appropriate time intervals and repeat it, if the sensor exhibits significant drift. When the sensor is new, you may need to calibrate it every two hours. An older extensively used sensor may require calibration only every 24 hours or less. To minimize the need for calibration, keep the sensor polarized between measurements if possible (unless the time between measurements exceeds several days). If the signal does not stabilize or remains too high or too low, refer to the "Troubleshooting" section in this manual.
The response of the H_2S microsensor does change with time, and a decrease in signal of up to 50 % pr. month is to be expected. This does not affect the quality of the measurements as long as the sensor is regularly calibrated.

Approval of new sensor

If the sensor functions according to the criteria given in the delivery note, the seal and protective plastic tube can be carefully removed, and measurements can be started.

MEASUREMENTS

Mounting the sensors

Due to the small size of the microsensor tip and the steepness of H_2S gradients in many environments, even a few microns' displacement of the sensor tip may change its immediate H_2S environment. The sensor tip is quite flexible and can bend around physical obstacles. But coarse lateral movements of the sensor when its tip is in contact with a solid substrate may easily cause the tip to break.
Therefore measurements should be performed only in a stabilized set-up, fixed on a sturdy table free of moving or vibrating devices. We recommend our lab stand (LS) and the micromanipulator (MM33 or MM33-2) for this purpose.

WARNING

Measurements in a light gradient should be avoided with the H_2S-type sensor. Interpretation of measurements in a light gradient should be made with the light interference in mind. SULF-type sensors are not affected by light.

Electrical noise

The signal of the microsensor is very small (10^{-10} to 10^{-13} ampere). Although all our amplifiers and the H_2S microsensors are very resistant to electrical noise from the environment, electrical fields may interfere with the sensor signal. Therefore we recommend that unnecessary electrical/mechanical equipment is switched off and that the sensor or wires are not touched during measurements and signal recording.
On suspicion of sensor damage, repeat calibration and consult the Troubleshooting section.

WARNING

Always introduce and retract the microsensor axially using a micromanipulator and a stable stand when measuring in solid or semisolid substrate like sediment, tissue, biofilms, microbial mats etc.

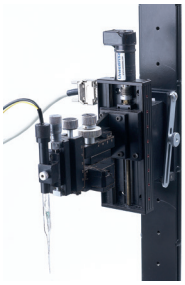
pH influence

As mentioned above, the H_2S concentration is a function of pH. If measurements of H_2S are performed in points along a pH gradient, pH should be measured in the same points with a pH microsensor (e.g. pH-10, pH-25, or pH-100) to allow the determination of the total sulfide concentration. Use the equations in the calibration section in order to calculate [S^{-2}tot].

Interference

Chemical interferences are give in the table below for both types of hydrogen sulfide sensors.

In addition, Type II sensors are sensitive to light because ferro cyanide reacts in light, especially blue (short-wave) light, which gives a false H_2S signal. This reaction causes the zero current to increase leading to an overestimation of the H_2S concentration in light. Thus, when measuring through a light gradient (e.g. when measuring into an illuminated biofilm) the signal will decrease slightly, when entering the biofilm. In many systems the light will be almost completely attenuated within the first few hundred microns.



Micromanipulator

The sensor has been painted black to reduce this phenomenon, however light may still enter the sensor through the tip, which is not possible to paint.

		Interferences for gases in gas phase (%)[1]		Interferences for gases dissolved in water (%)[2]	
Name	Formula	SULF	H_2S	SULF	H_2S
Methane	CH_4	0	0	0	0
Carbon dioxide	CO_2	0	0	0	0
Nitrogen	N_2	0	0	0	0
Oxygen	O_2	0	0	0	0
Air	O_2, N_2, Ar	0	0	0	0
Nitrous oxide	N_2O	0	0	0	0
Ammonia	NH_3	0	0	0	0
Hydrogen	H_2	0.8	0.03	96	4
Carbon monoxide	CO	0.6	4	77	487
Dimethyl sulfide	$(CH_3)_2S$	18	3	18	3
Methyl mercaptan	CH_3SH	174	117	44	30
Ethyl mercaptan	C_2H_6S	13	8	14	9
Sulfur dioxide	SO_2	40	34	1	1

[1]Given as signal for the interfering species in % of H_2S signal at equal partial pressures

[2]Given as signal for the interfering species in % of H_2S signal at equal molar concentrations

ADVANCED USE

Unisense can construct H_2S sensors for customer requested applications at additional costs. The most frequent construction options are described under sensor specification at our website. The options include e.g. customer specified dimensions, cable length etc.
If your specifications for a special H_2S sensor are not described at our web page please contact sales@unisense.com for further options.

STORAGE AND MAINTENANCE

Type I

Store the H_2S microsensor unpolarized in the protective plexiglass tube used for shipping. Due to the light sensitivity of the electrolyte H_2S, microsensors should be applied and stored at low to moderate levels of light. The sensor can be stored with the tip exposed to water or air. The room in which the H_2S microsensor is stored should be dry and not too hot (about 5-35°C).

Type II

Store dry on shelf at room temperature.

Cleaning the sensor

Standard procedure: Rinse with 96% ethanol, rinse with 0.01 M HCl and rinse with water.

Alternatively rinse with:
0.1 M NaOH
Isopropanol
Detergent

LIGHT SENSITIVITY

Type I

Due to the light sensitivity of the electrolyte H_2S microsensors should be applied and stored at low to moderate levels of light.

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TROUBLE SHOOTING

Problem	A high and drifting signal
Possible cause 1	Gas bubbles are present inside the sensor tip due to a 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.
Possible cause 2	The sensor tip is broken.
Solution	Replace the H_2S microsensor.

Problem	A slow response.
Possible cause	Insoluble compounds deposited on the sensor tip.
Solution	Rinse with 96% ethanol, rinse with 0.01 M HCl and rinse with water.

Problem	Measurements in two environments with equal H_2S concen trations exhibit different signals.
Possible cause	The light intensity differs between the two environments, causing different light interference.
Solution	Try to keep constant light conditions and interpret data with the light interference in mind.

Problem	Signal unstable fluctuatung if the set-up is touched or equipment is introduced in the medium
Possible cause	Electrical disturbance through the tip membrane
Solution	Ground the set-up by connecting the reference plug on the picoammeter (blue plug) with the medium you are measuring in. Use the provided blue grounding wire. Leave the other end with approximately 1 cm un-insulated wire (If you are measuring in e.g. a sediment, just put the open wire end in the water column).

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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