



Hydrogen Sensor

User Manual

Hydrogen 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 Hydrogen sensor is covered by a 90 days limited warranty. Microsensors are a consumables. 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.

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 Hydrogen microsensor is a miniturized sensor for measuring partial pressure of H₂ in the micromolar 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
Langdysen 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 UNDER WARRENTY

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 this 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 packing guide included in the sensor box.

4. OVERVIEW

This manual covers all the Unisense H₂ and H₂-X sensors.

For a complete list of sensors sizes and types please go to www.unisense.com.

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

The standard hydrogen sensor type, the H₂-type, is for use in environments where H₂S is not expected to occur. The H₂S insensitive type, the H₂-X-type, has an H₂S trap in front of the H₂ sensing part, allowing the sensor to be used in H₂S containing environments (see "**6.4 Interference**").



Figure 1: H₂ sensor

The Unisense hydrogen microsensor is designed for research applications within physiology, biotechnology, environmental sciences, and related areas. With the minute tip size, excellent response time, and good sensitivity the Unisense hydrogen sensor facilitates reliable and fast measurements with a high spatial resolution.

The H₂-X sensor has a slightly longer response time than the corresponding H₂ sensor.

The Unisense hydrogen microsensor is a miniaturized Clark-type hydrogen sensor with an internal reference electrode and a sensing anode. The sensor must be connected to a high-sensitivity picoammeter where the anode is polarized against the internal reference. Driven by the external partial pressure, hydrogen from the environment will pass through the sensor tip membrane and will be oxidized at the platinum anode surface. The picoammeter converts the resulting oxidation current to a signal.

5. GETTING STARTED

The H₂-type and H₂-X-type sensors are used in the same way. Only the sensitivity to H₂S and the response time differ between the two types of hydrogen sensors.

IMPORTANT: The H₂ sensor cannot be used with the tip pointing upwards. The sensor can be horizontal or the tip can point downwards. The sensor has a gas bubble inside and if the sensor points upwards, it will float into the narrow part of the sensor and block electrical connectivity in the electrolyte. This will result in no or very little response to H₂. See section 9. Troubleshooting for how to resolve this problem.

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

5.1 Unpacking a new sensor

When receiving a new microsensor remove the shock-absorbing grey plastic net.

5.2 Polarization

The signal from the hydrogen sensor is generated in picoampere. Therefore the hydrogen sensor must be connected to a polarizing picoammeter (e.g. a UniAmp series amplifier).

WARNING: Incorrect polarization may destroy the sensor.

The anode of the hydrogen sensors should be polarized at +100 mV relative to the cathode. This happens automatically on the Unisense UniAmp series instruments. On the Unisense Multimeter, Monometer and PA-2000 instruments this must be set manually. Please consult the relevant the instrument manual for how to adjust polarization. If you are using a PA2000, please check the polarization voltage before connecting the sensor, since incorrect polarization may destroy the sensor.

5.3 Connecting the microsensor

Insert the connector into a pA input terminal on the amplifier. The connector contains connections for both internal reference electrode and sensing anode.

*NOTE: The software converts the raw pA sensor signal into mV.
Standard setting is 1 mV = 1 pA.
This may be modified by changing the Pre-Amp setting (mV/pA) in the software (UniAmp)
or on the instrument (Multimeter, Monometer, and Field Multimeter).*

5.4 Pre-polarization

Just after connecting the sensor, the signal will be very high and unstable then drop rapidly over the first few minutes and then stabilize. Therefore, a short period of polarization is necessary before you can use the sensor. This is called the pre-polarization period.

IMPORTANT: Hydrogen sensors are sensitive to temperature and salinity.

The signal should stabilize at 0-10 mV (on the PA2000, the sign will be negative since sensor is positively polarized) for zero hydrogen concentration, depending on the specific sensor. If the sensor is new or has not been operated for several days, it will take a little longer to stabilize after being polarized.

The signal depends on the specific sensor type (see the value in the specifications that came with the sensor). If the signal does not stabilize or is too high or too low, refer to the 'Trouble-shooting' section of this manual.

5.5 Calibration

The calibration procedure is the same for the H₂ and H₂-X sensors.

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.

Hydrogen sensors respond linearly in the range of 0 to 100 % hydrogen (Low Range sensor from 0 - 10% H₂) and signals can be linearly converted to H₂ content in the unit you choose when starting an experiment file.

Calibration must be performed after the sensor signal has stabilized during pre-polarization.

5.5.1 Calibration in Liquid

Place/keep the sensor tip in water and read the signal. This signal is your calibration value for zero hydrogen conditions.

Hydrogen reading:

The hydrogen sensor responds linearly and consequently a two-point calibration is sufficient. Prepare water with a defined hydrogen concentration, which is slightly above the maximum expected concentration to be measured. A defined hydrogen concentration can be obtained by 2 different procedures:

1. Use a gas mixture controller to obtain a defined mixture of hydrogen and hydrogen free inert gas from a gas tank (e.g. N₂) as bulk carrier gas. For instance, to obtain a hydrogen concentration of 40,25 µM in the calibration chamber at 20°C, bubble the water in the calibration chamber vigorously with a gas mixture containing a 95 % N₂ and 5 % H₂. The hydrogen partial pressure is in this case 0.05 atm, and the Solubility is 805 µmol/L/atm. Multiplying the solubility with the partial pressure results in the concentration: 805 µmol/L/atm * 0,05 atm = 40,25 µM.

See **Table 1** for more values of the solubility, or use the H₂ calculator in the Unisense uSense Solutions software. Start the software, click "**Tools**" and select "**H₂ calculator**".

For a Unisense CAL300 calibration chamber, 5 minutes of bubbling at a rate of 5 l per minute is sufficient time to achieve 99 % of the concentration. If the equipment (gas mixture controller) is available, this method can be convenient, as you

WARNING: 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

can switch between different constant hydrogen conditions without changing the water. Use the solubility table (**Table 1**), or the H₂ calculator in the uSense software to find the correct mixture at temperatures other than 20°C.

To obtain correct concentrations, the headspace above the water in the calibration chamber must be closed except for a hole only slightly larger than the microsensor shaft. This effectively prevents ambient air from entering the vessel.

We recommend the CAL300 Calibration Chamber for calibrations.



Calibration chamber CAL300

2. Add a defined volume of hydrogen-saturated water to a defined volume of water in a calibration chamber. For instance, 1 ml of H₂ saturated water contains 0,805 μmol at 20°C (see **Table 1**), or the H₂ calculator in the uSense software, and to obtain water with a hydrogen concentration of 10 μM, 3.08 ml hydrogen-saturated water should be added to a total volume of 246,9 ml hydrogen free water in the calibration chamber. After the addition of hydrogen-saturated water to the calibration chamber mix it thoroughly by moving the sensor in its protection tube up and down for a few seconds and read the signal when it is stable. Do not stir bubbles into the water or mix by bubbling, as this will remove hydrogen from the water. A magnetic stirrer is not recommended as a mixing tool as a magnetic stirring can introduce electrical noise to the signal. The hydrogen in the water will slowly escape to the atmosphere and the concentration can only be considered constant for a few minutes.

5.5.2 Calibration in Gas

The H₂ sensor responds linearly to H₂, so a two-point calibration is sufficient. The two points are typically zero H₂ and one known H₂ content. This can be done by exposing the sensor to air for the zero point and a gas with a known H₂ content for the second calibration point.

When recording the sensor response for a gas with a known H₂ content, the calibration gas, it is important to create a system where the tip is exposed to 100% of the calibration gas. This setup will depend on the sensor configuration you have available. Some examples of how this can be done are given below.

1. Sensors with flow cells: Simply connect the tubing from the source of H₂ containing gas directly to the flow cell.
2. Sensors with protection tubes: Mount the black calibration cap on the protection tube (Figure 1). Adjust the flow rate so that all atmospheric air is expelled and the gas at the sensor tip is pure calibration gas. Make sure that overpressure doesn't build up.
3. All sensors: Use a closed container which is flushed with the calibration gas until the container contains pure calibration gas.



Figure 1

5.5.3 General calibration advice

1. For all calibrations that involve continuous flow of calibration gas it is important to test if the gas flow rate is sufficiently high to prevent dilution by atmospheric air. For calibration in gas, the gas flow must ensure that the sensor tip is exposed to pure calibration gas. For calibration in liquid, the gas flow must ensure that there is equilibrium of H₂ in gas and liquid. To test this, let the sensor signal stabilize at a given flow rate. Then increase the flow rate. If the sensor signal doesn't change, then the flow rate is high enough. If the sensor signal increases, then the previous flow rate was too low. Repeat this until the sensor signal remains constant at higher flow rates.
2. Rubber tubing is not 100% gas tight, and the composition of the calibration gas will change when passing through rubber tubing. This problem increases with gas permeability of the rubber tubing and length of the tubing. Therefore,

keep the tubing short and use rubber tubing that is relatively impermeable to gases. Silicone rubber is very permeable to gases, whereas Iso-Versinic, butyl rubber, or Viton tubing are much less permeable to gases.

3. Sensors respond to partial pressure of the gas, and the partial pressure of H₂ in the gas is linearly dependent on the total pressure in the gas. Therefore, it is important to avoid increase in total pressure in any of the gas calibration methods suggested above.
4. Check and repeat calibration at appropriate intervals to ensure that all measurements can be converted to correct concentrations. When the sensor is new, the appropriate interval may be every 2 hours; later it may be 24 hours or even less frequent. To minimize the need for calibrations, keep the sensor polarized between measurements, unless the time between measurements exceeds several days. The membrane permeability of hydrogen microsensors changes with time, so a change in signal of up to 10% per month may occur.

Hydrogen sensors respond linearly in the range of 0 to 100 % hydrogen (Low Range sensor from 0 - 10% H₂) and signals can be linearly converted to partial pressure.

Check and repeat calibration at appropriate intervals to ensure that all measurements can be converted to correct concentrations. When the sensor is new, the appropriate interval may be every 2 hours; later it may be 24 hours. To minimize the need for calibrations, keep the sensor polarized between measurements, unless the time between measurements exceeds several days or weeks. The membrane permeability of hydrogen microsensors changes with time, so a change in signal of up to 10% per month may occur.

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

6. MEASUREMENTS

The H₂-type sensor should be used in H₂S free environments. If H₂S is expected to be present, the H₂-X-type sensor should be used.

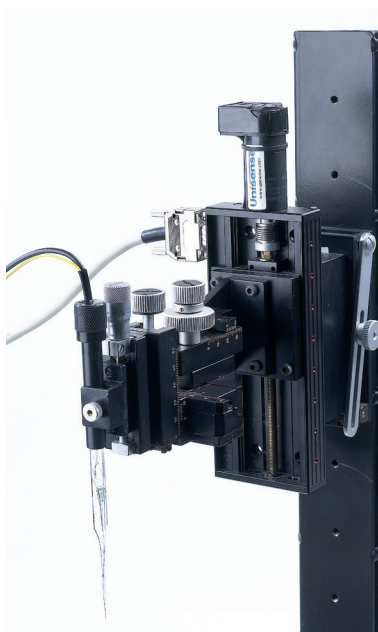
Hydrogen sensors can be used for a wide variety of measurements (see our website for further information unisense.com). The most common use of hydrogen sensors is for making profiles in e.g. sediment or animal tissue where a high spatial resolution is wanted, or for hydrogen measurements in water samples.

6.1 Use of glass-tip microsensors

Although the Unisense microsensors are made of glass, the tip is flexible and can bend slightly around physical obstacles. The sensor is thus rather sturdy in the longitudinal direction. However, large obstacles like stones or lateral movements of the sensor when the tip is in contact with a solid substrate may cause the tip to break.

Furthermore, 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 should be performed only in a stabilized set-up free of moving or vibrating devices. We recommend the Unisense lab stand LS and the Unisense micromanipulator MM33 (MM33-2 or MMS) for laboratory use. For in-situ use, we recommend our in situ stand (IS19) and a micromanipulator.



Micromanipulator

6.2 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.

6.3 Electrical noise

The signal of the microsensor is very small (10^{-13} to 10^{-10} ampere). Although both the Unisense amplifiers and the Unisense Hydrogen 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 the sensor or wires are not touched during measurements and signal recording.

6.4 Interference

Sulphide in the H₂S form may interfere with the H₂ measurements. The standard hydrogen sensor, the H₂-type, is very sensitive to H₂S and other reduced sulphur gases. It should, therefore, not be used in environments where H₂S and other reduced sulphur gases are present. The H₂-X sensor type is not sensitive to H₂S up to 100 µM in solution or 1000 ppm H₂S in gas. The H₂S trap on the H₂-X sensor works by removing protons from the H₂S and the ionized forms of sulfide cannot pass through the silicone membrane into the H₂ sensing part. Other sulphur gases where protons are less easily removed may still penetrate the silicone membrane. The H₂-X sensor may, therefore, still be sensitive to other reduced sulphur gases than H₂S. It is recommended to only expose the H₂S-X sensor to H₂S when needed, to maximize the lifetime of the H₂S trap.

The H₂-X sensor may be made even more resistant to H₂S. If you need a custom built sensor, contact sales@unisense.com

6.5 Interference from H₂ or H₂S entering the rear of the sensor

Avoid exposing the rear end of the sensor to H₂ or H₂S. This is especially important for sensors for In Situ use, mounted in the In Situ Connector. H₂ or H₂S entering the sensor from rear end will affect the potential of the reference electrode, which is placed here. This will cause signal drift and give negative values.

7. ADVANCED USE

Unisense can construct hydrogen sensors for customer requested applications at additional costs. The most frequently requested construction options are described on our website www.unisense.com.

The options include for instance customer specified dimensions, response time, stirring sensitivity, pressure tolerance, range and detection limit. If your specifications for a special hydrogen sensor is not described at our web page please contact sales@unisense.com for further options and prices.

7.1 Examples of advanced applications

- Consumption/production rates of hydrogen. E.g. during enzyme assays in small samples in Unisense microrespiration chambers MRCh
- Measurements of hydrogen under high external pressure e.g. in closed pressurized systems, underwater and deep sea applications
- Long-term hydrogen monitoring

If you have questions, please contact sales@unisense.com

8. STORAGE AND MAINTENANCE

Store the sensor in the protective plastic tube used for shipping.

The hydrogen microsensor can be stored with the tip exposed to water or air. The room in which the hydrogen microsensor is stored should be dry and not too hot (10-30°C). If the sensor is used regularly it can be stored polarized.

8.1 Cleaning the sensor

Depending on which substance is present on the sensor tip or membrane, the sensor can be cleaned with different solutes. The standard method is to rinse with 96 % ethanol (NOT in the protection tube), then rinse with 0.01 M HCl and rinse with water. This will remove most substances.

Alternatively it is possible to rinse with 0.1M NaOH, isopropanol or different detergents

TROUBLESHOOTING

NOTE! Always ensure that you are using the latest version of uSense Solutions. You can always download the latest version from www.unisense.com

Problem	High and drifting signal
Possible cause	The sensor tip is broken
Solution	Replace the hydrogen sensor
Problem	The signal is very low
Possible cause	Damage to internal working electrode
Solution	Replace the hydrogen sensor
Problem	Very low sensitivity to H ₂ and low signal
Possible cause 1	Bubble in the narrow parts of the sensor, often not visible to naked eye
Solution 1	Shake the sensor gently like shaking an old mercury fever thermometer. As shown in this video guide: unisense.com/video-guides/#troubleshooting
Possible cause 2	Bubble in the sensor tip, <i>not</i> visible to the naked eye
Solution 2	Soak the sensor in degassed water for at least 2 hours. Degas water by boiling it and subsequently cool it to room temperature without getting air into it
Problem	Slow response
Possible cause	Insoluble compounds deposited at the sensor tip
Solution	Rinse with 96 % ethanol, rinse with 0.01 M HCl and rinse with water
Problem	Unstable signal or the signal fluctuates if the set-up is touched or equipment is being 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 amplifier. Connect the reference plug on the amplifier (blue plug) with the medium you are measuring in.

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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APPENDIX

Salinity (parts per thousand)									
Temp. (°C)	0	10	20	30	32	34	36	38	40
-2			901,34	851,79	841,96	832,59	823,21	813,84	804,46
-1			890,18	841,52	832,14	822,77	813,84	804,46	795,54
0	982,59	929,46	879,46	832,14	822,77	813,84	804,46	795,98	787,05
1	969,64	917,86	869,2	822,77	813,84	804,91	795,98	787,5	778,57
2	957,59	907,14	858,93	813,84	804,91	796,43	787,5	779,02	770,98
3	945,54	896,43	849,55	805,36	796,43	787,95	779,91	771,43	763,39
4	934,38	886,16	840,18	796,88	788,39	780,36	771,88	763,84	755,8
5	923,66	876,34	831,7	789,29	780,8	772,77	764,73	756,7	748,66
6	912,95	866,96	823,21	781,7	773,66	765,63	757,59	749,55	741,96
8	893,3	849,11	807,14	767,41	759,38	751,79	744,2	736,61	729,46
10	875,45	833,04	792,41	754,02	746,43	739,29	731,7	724,55	717,41
12	858,93	817,86	779,02	741,96	734,82	727,68	720,54	713,39	706,7
14	843,75	804,02	766,52	730,8	723,66	716,96	710,27	703,13	696,43
16	829,46	791,52	755,36	720,54	713,84	707,14	700,45	693,75	687,5
18	816,96	779,91	744,64	711,16	704,46	698,21	691,52	685,27	679,02
20	805,36	769,64	735,27	702,68	695,98	689,73	683,48	677,23	671,43
22	794,64	759,82	726,34	694,64	688,39	682,14	676,34	670,09	664,29
24	785,27	751,34	718,75	687,5	681,7	675,45	669,64	663,84	657,59
26	776,79	743,3	711,61	681,25	675	669,2	663,39	657,59	651,79
28	768,75	736,16	704,91	675,45	669,64	663,84	658,04	652,23	646,88
30	762,05	729,91	699,55	670,09	664,29	658,48	653,13	647,32	641,96

Table 1: Equilibrium hydrogen concentrations ($\mu\text{mol/litre}$) at ambient hydrogen partial pressure of 1 atm. in water as a function of temperature.

Ref. Wiesenburg and Guinasso 1979. *J.Chem Eng. Data* 24(4):356-360

Note: The Unisense software has a built-in calculator, giving the solubility of H_2 at a given combination of temperature and salinity ("Tools - H_2 Calculator" or the " H_2 table" button in the calibration dialog).

For conversion between units go to the Unit Converter on Unisense website: unisense.com/unit-converter

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