UNISENSE

HYDROGEN SENSOR USER MANUAL

Hydrogen sensor user manual

Version May 2021

HYDROGEN SENSOR USER MANUAL

UNISENSE A/S

TABLE OF CONTENTS

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 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.

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 H_2 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

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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.*

6

3: OVERVIEW

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

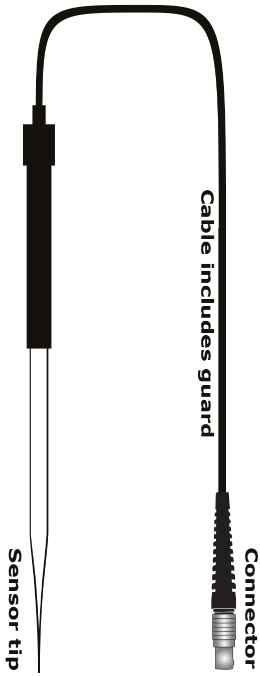
This manual covers all the Unisense H_2 and H_2-X sensors. For a complete list of sensors sizes and types please go to www.unisense.com.

The standard hydrogen sensor type, the H_2-type, is for use in environments where H_2S is not expected to occur. The H_2S insensitive type, the H_2-X-type, has an H_2S trap in front of the H_2 sensing part, allowing the sensor to be used in H_2S containing environments (see **"5:3 Interference"**).

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 H_2-X sensor has a slightly longer response time than the corresponding H_2 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.



Schematic view of a hydrogen sensor with a LEMO plug.

4: GETTING STARTED

The H_2-type and H_2-X-type sensors are used in the same way. Only the sensitivity to H_2S and the response time differ between the two types of hydrogen sensors.

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

4:1 Unpacking a new sensor

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

4:2 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.

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)

4:3 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.

4:4 Pre-polarization

Just after connecting the sensor, the signal will be very high and unstable then drop rapidly over the first few minutes. After that the signal will drop slowly for up to 1 hour. Therefore, a period of polarization is necessary before you can use the sensor. This is called the pre-polarization period.

The signal should stabilize at 0-10 picoampere (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 must be polarized for at least 1 hour before it can be calibrated and used. After shorter periods without polarization, the sensor should be polarized until it has exhibited a stable signal for 10 minutes.

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.

IMPORTANT

Hydrogen sensors are sensitive to temperature and salinity.

4:5 Calibration

The calibration procedure is the same for the H_2 and H_2-X sensors.

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

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.

Zero hydrogen reading

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. N_2) 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 % N_2 and 5 % H_2. 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 H_2 calculator in the Unisense SensorTrace Suite 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 can switch between different constant hydrogen conditions without changing the water. Use the solubility table (**Table 1**), or the H_2 calculator in the SensorTrace 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

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.

2. Add a defined volume of hydrogen-saturated water to a defined volume of water in a calibration chamber. For instance, 1 ml of H_2 saturated water contains 0,805 µmol at 20°C (see **Table 1**), or the H_2 calculator in the SensorTrace 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.

Hydrogen sensors respond linearly in the range of 0 to 100 % hydrogen (Low Range sensor from 0 - 10% H_2) 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 unless the picoammeter batteries are running out. The membrane permeability of hydrogen microsensors changes with time, so a change in signal of up to 50 % may occur over months.

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.

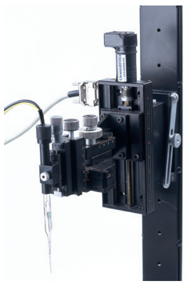
5: MEASUREMENTS

The H_2-type sensor should be used in H_2S free environments. If H_2S is expected to be present, the H_2-X-type sensor should be used.

Hydrogen sensors can be used for a wide variety of measurements (see our website for further information www.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.

5:1 Mounting of the sensors

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

5:2 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.

5:3 Interference

Sulphide in the H_2S form may interfere with the H_2 measurements. The standard hydrogen sensor, the H_2-type, is very sensitive to H_2S and other reduced sulphur gases. It should, therefore, not be used in environments where H_2S and other reduced sulphur gases are present. The H_2-X sensor type is not sensitive to H_2S up to 100 µM in solution or 1000 ppm H_2S in gas. The H_2S trap on the H_2-X sensor works by removing protons from the H_2S and the ionized forms of sulfide cannot pass through the silicone membrane into the H_2 sensing part. Other sulphur gases where protons are less easily removed may still penetrate the silicone membrane. The H_2-X sensor may, therefore, still be sensitive to other reduced sulphur gases than H_2S. It is recommended to only expose the H_2S-X sensor to H_2S when needed, to maximize the lifetime of the H_2S trap.

The H_2-X sensor may be made even more resistant to H_2S. If you need a custom built sensor, contact sales@unisense.com

6: 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.

6:1 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

7: 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.

7:1 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

8: REFERENCES

- Revsbech, N. P., and B. B. Jørgensen. 1986. Microelectrodes: Their Use in Microbial Ecology, p. 293-352. In K. C. Marshall (ed.), Advances in Microbial Ecology, vol. 9. Plenum, New York.
- Itoh, T., et al. 2009. Molecular Hydrogen Suppresses FcepsilonRI-Mediated Signal Transduction and Prevents Degranulation of Mast Cells. Biochem. Biophys. Res. Commun. 389:651-656.
- Kajiya, M. et al. 2009. Hydrogen From Intestinal Bacteria Is Protective for Concanavalin A-Induced Hepatitis. Biochemical and Biophysical Research Communications 386:316-321.
- Kajiya, M. et al. 2009. Hydrogen Mediates Suppression of Colon Inflammation Induced by Dextran Sodium Sulfate. Biochemical and Biophysical Research Communications 386:11-15.
- Vopel, K., et al. 2008. Modification of Sediment-Water Solute Exchange by Sediment-Capping Materials: Effects on O2 and PH. Marine and Freshwater Research 59, 1101-1110.

9: TROUBLESHOOTING

Problem	High and drifting signal.
Possible cause	The sensor tip is broken.
Solution	Replace the hydrogen microsensor.

Problem	The signal is very low.
Possible cause	Damage to internal working electrode.
Solution	Replace the hydrogen microsensor.

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
Possible cause 2	Bubble in the sensor tip, not visible to the naked eye
Solution2	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.

TROUBLE SHOOTING

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)

Table 1: Equilibrium hydrogen concentrations (µmol/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

	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	869,20	822,77	813,84	804,91	795,98	787,50	778,57
2	957,59	907,14	858,93	813,84	804,91	796,43	787,50	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	755,80
5	923,66	876,34	831,70	789,29	780,80	772,77	764,73	756,70	748,66
6	912,95	866,96	823,21	781,70	773,66	765,63	757,59	749,55	741,96
8	893,30	849,11	807,14	767,41	759,38	751,79	744,20	736,61	729,46
10	875,45	833,04	792,41	754,02	746,43	739,29	731,70	724,55	717,41
12	858,93	817,86	779,02	741,96	734,82	727,68	720,54	713,39	706,70
14	843,75	804,02	766,52	730,80	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	687,50
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	687,50	681,70	675,45	669,64	663,84	657,59
26	776,79	743,30	711,61	681,25	675,00	669,20	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

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