UNISENSE

ELECTRICAL POTENTIAL MICROELECTRODE USER MANUAL

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Version January 2021

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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 Electrical Potential Microelectrode is covered by a 3 months limited warranty.
Microelectrodes 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.

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 Electric Potential Microelectrode is a specialized product. It is handmade and characteristics such as physical dimensions vary between individual sensors.

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.

3: REPLACEMENT OF ELECTRODES UNDER WARRANTY

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 **5.4: Testing the EP Microelectrode**
- 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

4.1: The Electric Potential Microelectrode

The Unisense Electric Potential Microelectrode is a slightly modified version of the originally published microelectrode (Damgaard et al., 2014). The design is only changed for shipping purposes and the functionality is exactly the same as the published version. With a tip diameter of 100 µm this microelectrode may be used for measurements of electric potential at very high spatial resolution. It may be used at salinities from freshwater to full ocean seawater.

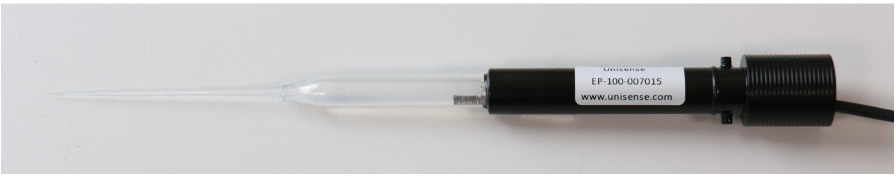

Figure 1: Electric Potential Microelectrode

4.2: Measuring principle

The Electric Potential Microelectrode will measure the difference in electric potential between the tip of the microelectrode and the reference electrode. The raw value of the difference in electrical potential between the Microelectrode and the reference electrode will depend on the type of reference electrode used and a zeroing is necessary (see 6 Calibration).

The potential of the Ag/AgCl electrode inside the Electric Potential Microsensor depends on the pressure inside the electrode. To avoid any change in potential due to pressure change in the sensor, the inside of the sensor is connected to the atmosphere through a glass capillary.

4.3: Reference electrode

For laboratory use, we recommend using the macro reference electrode, REF-RM, from Radiometer Analytical (please see **11: Manual for ref-rm (REF321)** at the end of this manual.

5: GETTING STARTED

WARNING

Do not remove the seal and protective plastic tube before the electrode has been tested to perform satisfactorily. (See **5.4: Testing the EP Microelectrode)**

5.1: Unpacking a new EP Microelectrode

1. When receiving a new micro electrode, remove the shock-absorbing grey plastic net.

5.2: Unpacking a new REF-RM reference electrode

1. Remove the protection cap from the electrode and any seals covering the filling hole.
2. Before starting a measurement, remove the clip which closes the electrode filling hole. Remember to put the clip back in place at the end of measurements.
3. Check the level of the filling solution. It should be approximately 0.5 cm below the filling hole. If necessary refill the KCl-Ag 3M KCl solution, saturated with AgCl.

NOTE: The filling hole on the REF-RM must be open during measurements and the level of electrolyte must be approximately 0.5 cm below the filling hole. This will ensure that there is a very slow flow of electrolyte through the porous white plug at the tip of the electrode which prevents the build-up of tip potentials, that might otherwise affect the potential of the reference electrode.

5.3: Connecting the microelectrode and setting up the software

5.3.1: Connecting the EP Microelectrode

1. Connect the Electric Potential Microelectrode to the amplifier by plugging the connector into a mV channel on the amplifier (a channel labelled mV on a Unisense laboratory instrument, channel 6 or 7 on a Field Microsensor Multimeter).

2. Connect the Reference electrode to the connector on the Electric Potential Microelectrode cable (Figure 2).

Figure 2: Connector for Unisense amplifier with a banana connector on the cable for connecting the reference electrode

5.3.2: Setting up the software

1. Launch the SensorTrace software.
2. Select *EP* as the sensor **Type** and *mV* as the **Unit** in the *Settings* window (Figure 3) for the EP Microelectrode.
3. Click the Start Experiment button to create a file.

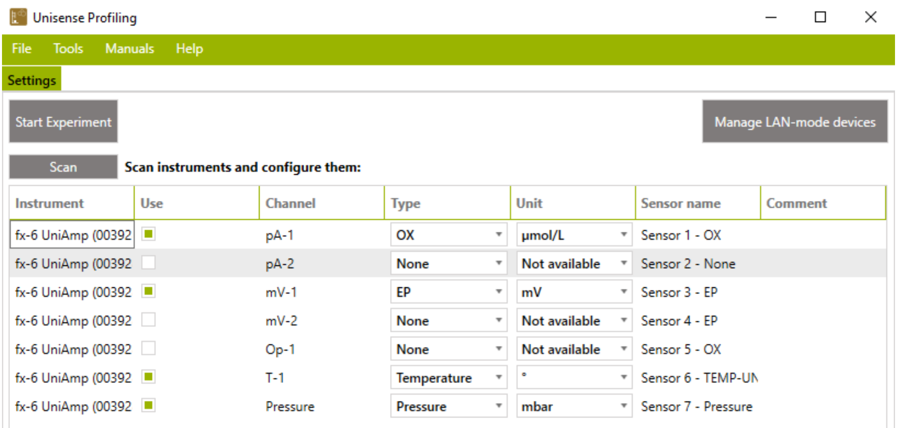

Figure 3: Settings window in SensorTrace Profiling. Electric Potential Microelectrode connected to channels 3.

5.4: Testing the EP Microelectrode

The Microelectrode must be tested within 2 weeks of receipt to maintain the warranty (**1: Warranty and liability**).

NOTE:

- Do not remove the plastic protection tube before the microelectrode has passed the test.
- Do not remove the seal (white sticker: Do not remove before test) before the microelectrode has passed the test.

5.4.1: Test the microelectrode

1. Connect the Electric Potential Microelectrode and start up the software as described in **5.3** above.
2. Remove the tape covering the hole on the side of the protection tube just below the nut. This will expose a small hole in the protection tube and allow the storage liquid to run out and the water to run in during testing.
3. Remove the lower piece of tape and the rubber stopper from the protection tube and pour the storage liquid into a vial or beaker. This liquid is 1 M KCl and may be saved for later use.
   NOTE: The Electric Potential Microelectrode must always be stored with the tip immersed in 1 M KCl.
4. Place the Electric Potential Microelectrode in a beaker with tap water.
5. Place the Reference electrode in the same beaker.
6. Check the signal.

If the microelectrode is used with a Unisense REF-RM the signal should stabilize at a level between -100 mV and +100 mV.

If this is the case, the seal and protective plastic tube can be carefully removed, and measurements can be started.

The level of the signal depends on the reference electrode used, as different types of reference electrodes have different half-cell potentials. Therefore, if the reference electrode used is not a Unisense REF-RM, the signal may stabilize at a different level. The microelectrode is then approved if the signal is stable even if the signal is outside the limits mentioned above.

6: CALIBRATION

The idea of electric potential (EP) measurement is to quantify the difference in EP between the tip of the Electric Potential Microelectrode and the reference electrode. This does not require calibration as such, because this potential is directly displayed. However, there is a difference in half-cell potential between the Electric Potential Microelectrode and the reference electrode which will give an offset in the signal. This offset must be corrected, so that the measured signal is zero if the tips of the two electrodes are exposed to the same electric potential.

Figure 4: Detailed view of the top of the sensor shaft showing the glass capillary that should be broken off before use.

6.1: Quantifying and correcting offset

1. Start by breaking off the glass capillary (Figure 4). This will open the connection between the interior of the electrode and the atmosphere and prevent any over- or under-pressure inside the electrode.
2. Place the Electric Potential Microelectrode in a beaker with water of the same ionic composition as the environment where the measurements will be made.

For example, for measuring in a marine sediment, use the seawater overlying the sediment for this.

3. Place the Reference electrode in the same beaker.
4. Let the sensor signal stabilize and note the Sensor Signal in mV (**Figure 5**).
5. Enter the sensor signal with opposite sign in the Offset (mV) field in the UniAmp Channel Configuration frame and press Enter.
6. After pressing Enter, the Sensor signal (mV) will show the offset-corrected sensor signal.

   NOTE: The Sensor signal shown is corrected for the Offset. To see the raw value without offset correction, enter 0 in the Offset field and press Enter.

6.1.1: Correcting the offset in the measuring environment

It is important to correct for the offset in water of the same composition as where the measurements will be done. This may be obtained with the microelectrode and reference electrodes mounted in the measuring setup with the electrodes in a position where the expected difference in electric potential between the two electrodes is zero. For sediment core measurements, place the electrodes in the water above the sediment where no difference in electric potential is expected between the two electrode tips.

6.2: Correction for the offset after measurements

It is possible to correct for the offset after the electric potential data have been recorded. The requires that one data set is recorded with the two electrodes positioned in a way where the expected difference in potential between the two is zero. This value may then be subtracted from the measured data during post processing.

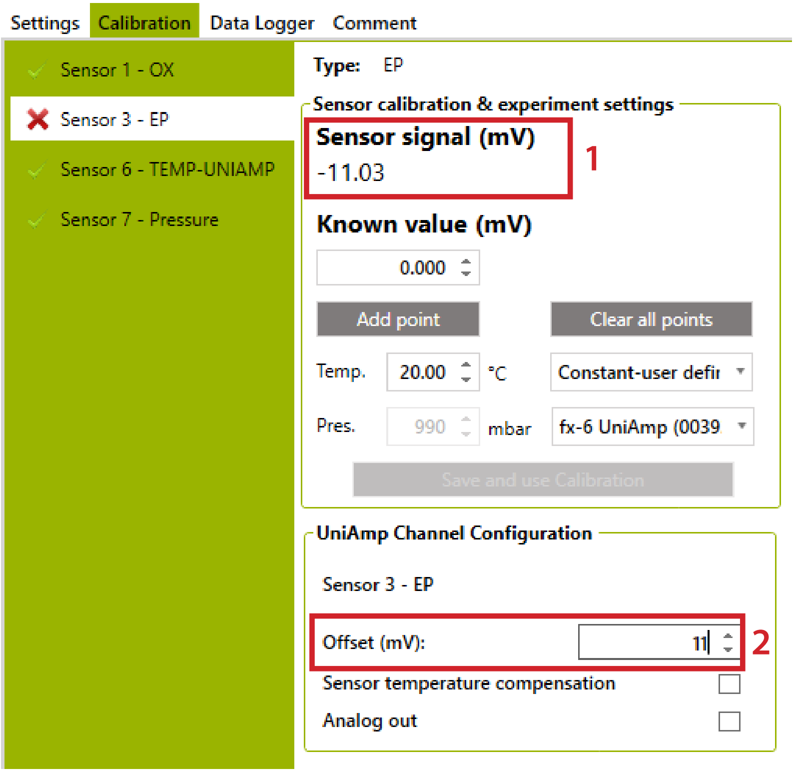

Figure 5: Calibration window for the EP microelectrode. The current sensor signal is shown (1). Enter the sensor signal with the opposite sign to set the sensor signal to zero.

7: WORKING WITH THE ELECTRICAL POTENTIAL MICROELECTRODE

7.1: Mounting of the electrode

To mount the electrode for measurements, unscrew the nut, breaking the white paper seal. When the screw is loose, the microelectrode can be drawn from the protective tube and used normally. Although the Unisense micro electrodes are made of glass, the tip is flexible and can bend slightly around physical obstacles. However, large obstacles like stones or coarse lateral movements of the electrode when the tip is in contact with a solid substrate may cause the tip to break.

Also, due to the small size of the microelectrode tip and to the steepness of gradients in many environments, even a displacement of the electrode tip of few micrometer may change its environment. Therefore, measurements should be performed only in a stabilized set-up, fixed on a sturdy table free of moving or vibrating devices. We recommend the Unisense Lab Stand LS and the Unisense micromanipulator MM-33 (MM33-2 double) for laboratory use. For in-situ use we recommend our in situ stand (IS19) and a micromanipulator.

7.2: Electrical noise

The electrical potential generated by the micro electrode is small. Although the Unisense Electric Potential Microelectrodes are very resistant to electrical noise from the environment, electrical fields may interfere with the electrode signal. Therefore, we recommend that unnecessary electrical or mechanical equipment is switched off and the electrode and wires are not touched during operation.

7.3: Measuring electric potential gradients in sediments

1. Place the reference electrode tip in the water overlying the sediment.
   a. If working with sediment cores in an aquarium,

the reference can be placed anywhere within the aquarium.

   b. If working directly in a sediment core, the reference electrode must be placed in the overlying water in the core.

2. Mount the Electric Potential Microelectrode in the micromanipulator and position the tip in the overlying water above the sediment.
3. Quantify and correct for the offset as described in **6.1 or 6.1.1** above.
4. Now measure the electric potential profile.

7.3.1: Recommended control for electrical potential gradients in sediments

If a gradient in electric potential is measured, and this is argued to be due to cable bacteria (Damgaard et al., 2014; Risgaard-Petersen et al., 2015), it is important with an appropriate control, i.e. what is the gradient with and without the effect of cable bacteria.
It has been shown that the effect of cable bacteria will disappear immediately if a thin wire is pulled across in the sediment core below the oxic and the nitrate containing zone This will cut the connection between top and bottom of the cable bacteria.
The difference in electrical potential gradient measured before and after such a cut will show how much of the gradient that was due to cable bacteria (Risgaard-Petersen et al., 2015).
Furthermore, the electric potential gradient created by cable bacteria depends on O_2 and/or NO_3- being available in the water above the sediment. Therefore, removing O_2 and NO_3- will remove the effect of cable bacteria on the electric potential and a profile measured under these conditions will show the gradient without influence of cable bacteria (Risgaard-Petersen et al., 2014).

8: STORAGE AND MAINTENANCE

8.1: Storage

WARNING
The tip of the EP microelectrode should be kept in aqueous solution at all times. It can however, tolerate up to 10 minutes of exposure to air.

8.1.1 Electrical Potential Microelectrode

The microelectrode should be stored in the protective tube. Always keep the tip moist. For long-term storage, the electrode tip most be immersed in 1 M KCl in the protection tube. For short-term storage (<10 min) the electrodes can be stored in air. The room in which the microelectrodes are stored should be dry and not too hot (5-35 °C).

8.1.2: Ref-RM

Between measurements, leave the electrode in 3M KCl solution. Overnight or longer: seal the filling hole with paraffin film or with the electrode clip and mount the protection cap filled with the 3 M KCl solution.

8.1.3: Cleaning the electrode

The Electric Potential Microelectrode can be cleaned by exposing it to 70 % ethanol HCl for a couple of minutes. After this, rinse with 1 M KCl.
The Ref-RM electrode should be rinsed with distilled water after measurements. Check the level of filling solution often. In case of deposits, please consult section **11 (Manual for Ref-RM).**

9: TROUBLE SHOOTING

Problem	The mV signal does not stabilize
Possible cause 1	The tip of the Electric Potential Microelectrode or the reference electrode does not touch the water
Solution	Make sure that the tips of the EP microelectrode and the reference electrode are immersed in the water and that there is liquid contact between the two. For example, dipped into the same beaker with water.
Possible cause 2	The conductivity of the water is too low
Solution	Add a little NaCl (1 - 10 g/l) to the water
Possible cause 3	The tip of the Electrical Potential Microelectrode has dried out which has caused air bubbles in the tip. This disrupts the electrical connection.
Solution	Boil water for 5-10 min, cool it to room temperature without mixing air into it, and place the sensor in this water for 1 hour. When water is boiled, all gases evaporate. After cooling the gas content will still be low and air bubbles in the sensor tip will diffuse out of the sensor into the water.

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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REFERENCES

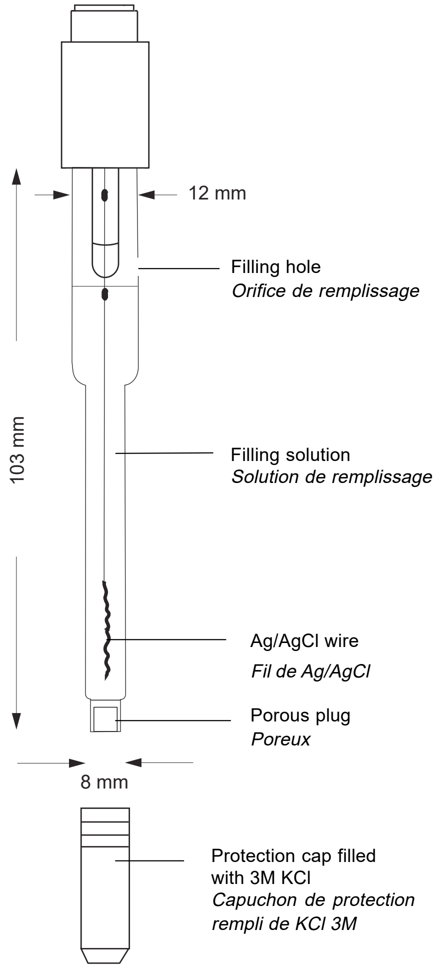
- Damgaard, L. R., N. Risgaard-Petersen, and L. P. Nielsen. 2014. Electric potential microelectrode for studies of electrobiogeophysics. J. Geophys. Res. Biogeosciences 119: 1906–1917. doi:10.1002/2014JG002665
- Risgaard-Petersen, N., L. R. Damgaard, A. Revil, and L. P. Nielsen. 2014. Mapping electron sources and sinks in a marine biogeobattery. J. Geophys. Res. G Biogeosciences 119: 1475–1486. doi:10.1002/2014JG002673
- Risgaard-Petersen, N., M. Kristiansen, R. B. Frederiksen, and others. 2015. Cable Bacteria in Freshwater Sediments. Appl. Environ. Microbiol. 81: 6003–6011. doi:10.1128/aem.01064-15

11: MANUAL FOR REF-RM (REF321)

REF321

Reference Electrode

Electrode de Référence



Radiometer analytical

REF321
Reference Electrode

Introduction

The REF321 is a general purpose Ag/AgCl reference electrode, fitted with a screw cap.

Preparation for measurement

1. Remove the protection cap from the electrode and any seals covering the filling hole.
2. Before starting a measurement, remove the clip which closes the electrode filling hole. Remember to replace the clip at the end of measurements.
3. Check the level of the filling solution. It should be approximately 0.5 cm below the filling hole. If necessary, refill with KCl•Ag 3 M KCl Solution, saturated with AgCl.

If desired, the concentration of KCl can be changed. However, it is advisable to use a high concentration. Remember always to saturate the solution with AgCl. For nonaqueous applications such as measurements in acetic acid. Empty the electrode and fill it up again with a saturated KCl solution in acetic acid. Saturate the solution with AgCl.

4. In order to remove air bubbles trapped inside the electrode, shake the electrode holding it at its head with the porous plug down.

Maintenance

1. Electrode contamination is a major cause of faulty measurements.
2. The electrode should be rinsed with distilled water after measurements.
3. Check frequently the level of filling solution.
4. In case of deposits which cover the electrode, clean the electrode with:

- a solution of acid (0.1M HCl, 0.1M HNO_3): mineral salt deposits, etc...
- KS400 Pepsin in HCl Solution or RENOVO•X Xtra Strong Cleaning Solution: protein deposits (milk, cheese, serums...). Duration of treatment 1 to 2 hrs.
- KS410 Thiourea Solution: for porous plugs contaminated with sulphides or blocked by an AgCl precipitate. Duration of treatment, a few hours until the porous plug turns white.
- RENOVO•N Normal Cleaning Solution: greasy or oily deposits...

The porous plug of the electrode can be cleaned using a fine abrasive paper.

Storage

Between measurements: leave the REF321 in KS110 3M KCl Solution.
Overnight or longer: seal the filling hole with paraffin film or with the electrode clip and fit back in place the protection cap filled with the KS110 3M KCl Solution.

Accessories

KS110 3M KCl Solution, 500 ml	C20C320
KCl•Ag 3M KCl Solution saturated with AgCl, 100 ml	S21M004
RENOVO•N Normal Cleaning Solution, 250 ml	S16M001
RENOVO•X Xtra Strong Cleaning Solution, 250 ml	S16M002
KS400 Pepsin in HCl Solution, 250 ml	C20C370
KS410 Thiourea Solution, 250 ml	C20C380
CL111 Electrode cable with banana plug	A94L111

Specifications

Reference potential when filled with 3M KCl (in mV)						
Temperature (C °)	0°	10°	20°	25°	30°	40°
vs. std. hydrogen elec.	225	219	212	208	204	196
vs. sat. Hg/Hg_2Cl_2elec.	-35	-36	-36	-36	-37	-38

Temperature range: 0 to 80 °C
Note: an electrode chain comprising pHG301 or XG100/200 glass electrode and a REF321 filled with 3M KCl will give a zero pH of approx. pH 7.25.

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