Electric potential microelectrode for studies of electrobiogeophysics

Lars Riis Damgaard1, Nils Risgaard-Petersen1,2, and Lars Peter Nielsen1,2

1Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus C, Denmark, 2Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus C, Denmark

Abstract Spatially separated electron donors and acceptors in sediment can be exploited by the so-called “cable bacteria.” Electric potential microelectrodes (EPMs) were constructed to measure the electric fields that should appear when cable bacteria conduct electrons over centimeter distances. The EPMs were needle-shaped, shielded Ag/AgCl half-cells that were rendered insensitive to redox-active species in the environment. Tip diameters of 40 to 100 μm and signal resolution of approximately 10 μV were achieved. A test in marine sediments with active cable bacteria showed an electric potential increase by approximately 2 mV from the sediment-water interface to a depth of approximately 20 mm, in accordance with the location and direction of the electric currents estimated from oxygen, pH, and H2S microprofles. The EPM also captured emergence and decay of electric diffusion potentials in the upper millimeters of artificial sediment in response to changes in ion concentrations in the overlying water. The results suggest that the EPM can be used to track electric current sources and sinks with submillimeter resolution in microbial, biogeochemical, and geophysical studies.

1. Introduction

Variations in soil surface electric potential (EP), referred to as self-potential (SP) anomalies, are found in nature and are proposed to be associated with electrokinetic, electrochemical, thermoelectric, redox, and piezoelectric effects [Jouniaux et al., 2009]. For instance, Sato and Mooney [1960] proposed that observed SP anomalies over ore bodies could be explained as spatially separated redox processes linked by electrically conducting mineral structures (“geobatteries” [Bigalke and Grabner, 1997]). Revil et al. [2010] coined the term “.biogeobatteries” for geobatteries, where biological components are responsible for performing the redox processes. In geophysical surveys, electric potential variations are mapped on the meter or kilometer scale with electrodes positioned on the soil surface or in boreholes, and the data are used to infer the position and direction of groundwater flow, mineral ores, and pollutions [Jouniaux et al., 2009].

Cable bacteria are filamentous Desulfovibulbaceae that can transport electrons in marine sediments, thereby coupling the reduction of oxygen in the top layer of the sediment to the oxidation of sulfide about 12–20 mm thereunder [Nielsen et al., 2010; Pfeffer et al., 2012; Risgaard-Petersen et al., 2012]. In these studies, minimum estimates of electric current density across the oxic-anoxic interface were obtained from microprofles of oxygen and pH measured with classical microelectrodes. A more detailed determination of the spatial distribution of electron sources and sinks is difcult to make from chemical data only, and the microscale distribution of electric potentials generated by the cable bacteria could be a more useful and direct parameter. For that purpose, we constructed a nondestructive electric potential microelectrode (EPM) that (a) could operate on a submillimeter scale, in contrast to the large stationary electrodes used in geophysical research; (b) was insensitive to redox-active compounds in the environment within experimental timeframes; and (c) had a signal resolution suficient to capture low currents over short distances in a highly conductive, saline sediment. The effect of different design parameters on the resistance to contamination by redox-active substances was evaluated. The applicability of the microelectrode in marine sediment was demonstrated by the measurements of EP profles that matched electrochemical reactions indicated by the measured microprofles of O2, pH, and H2S. The validity was further confirmed by measurements in defined electric fields and diffusion potential gradients.

2. Materials and Methods

2.1. EPM Construction

The electrode body (Figure 1) was made in glass, which, for other types of microsensors, has proved suitable in terms of malleability, structural strength, and electric insulation [N. P. Revsbech and Jørgensen, 1986]. An
Ag/AgCl half-cell was chosen as the element for converting the electric potential in the aqueous environment to an electronic potential in the measuring circuitry. To prevent signal fluctuations from the intrusion of redox-active substances, i.e., substances capable of reacting chemically with the Ag/AgCl half-cell, the Ag/AgCl half-cell was separated from the electrode tip by an at least 5 cm long electrolyte path. Tapering of the tip to a diameter of 40–100 μm provided further resistance to diffusing substances as well as enabling nondestructive penetration in sediment at submillimeter resolution. The electrode was filled with electrolyte, which in the tip was solidified with agar to prevent electrolyte flow.

Finally, to minimize electromagnetic noise interference, shielding was provided by adding an outer shield casing with electrolyte, which was connected to electric ground through an Ag/AgCl half-cell.

The construction procedure was as follows: A Ø6 mm glass tube (Schott, AR-Glas®) — the electrode capillary — was heated in a flame and pulled to taper down to approximately Ø1 mm and inserted into a shorter Ø8 mm glass tube — the shield casing — which had also been tapered, but to a slightly larger diameter, such that the electrode capillary protruded through the tip of the shield casing. A 5 cm long Ø0.25 mm silver wire was electrochlorinated for a few minutes in 1 M HCl at 1.5 V, rinsed in distilled water and air-dried to create an Ag/AgCl electrode, which was inserted into the space between the shield casing and the electrode capillary. A drop of UV-light-curing glue was applied to fix the silver wire, the electrode capillary, and the shield casing to each other. The tip opening of the shield casing was subsequently sealed around the electrode capillary with air-curing silicone sealant (Dow Corning® 734 Flowable Sealant). The tip of the EPM was formed by hanging the electrode in a micromanipulator by the thin end of the electrode capillary close to a small heating loop. By adjusting the heating loop temperature and the electrode position under microscope control, the tip was tapered down in diameter. The final length and diameter were made by cutting with a diamond knife and pinching with fine tweezers under a microscope. The size of the tip opening was adjusted by approaching a heating loop and melting the tip end slightly. For use in soft sediments, a relatively thin — and thus relatively fragile — shape with a diameter down to 40 μm was formed (Figure 2a), whereas for coarse sand, a thicker and more robust tip with a tip diameter of approximately 100 μm was chosen (Figure 2b).

As an alternative to an apical opening, which makes the electrodes somewhat sensitive to particle impacts as it is pushed into the substrate, a side port design was implemented. This was done by first making an oblique break (by breaking the tip by bending instead of by pinching) and subsequently touching the glass tip with the heating loop and heating very carefully. In this way, the final opening could be placed on the side 30–100 μm from the very tip (Figure 2a).

A few centimeters of the tip of the electrode capillary were filled with 1 M KCl electrolyte stabilized with 1.5% agar. This was done by dipping the tip in hot molten agar solution and applying suction strong enough to suck in the agar but not so strong that bubbles formed. One molar KCl electrolyte was then

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**Figure 1.** Electric potential electrode. Left: schematic drawing and right: photograph.
injected from behind in the electrode capillary onto the top of the solidified agar to fill the whole electrode capillary except the top few centimeters.

An internal Ag/AgCl electrode was prepared by electrochlorinating, rinsing, and drying 10 cm of a Ø0.25 mm silver wire, which had been spiraled for compactness. The Ag/AgCl electrode was soldered to the center lead of a coaxial cable and then inserted into the electrode capillary, such that the major part of the internal Ag/AgCl electrode was submerged in the KCl electrolyte, while the unshielded part of the cable was below the level of the top of the shield casing (Figure 1). In order to avoid an unstable competing half-cell potential between the solder material and the KCl electrolyte, care was taken to avoid submersing the solder point into the electrolyte. To minimize the risk of electric leak currents between the cable’s shield layer and its center lead, care was also taken not to contaminate the end of the coaxial cable with electrolyte. As a further measure to avoid leak currents through moisture films on cables and glass surfaces, mineral oil was injected down along the coaxial cable to immerse the solder point and the transitions between electrolyte, glass walls, and cable ends in oil. The needle used for injecting the oil was left in the opening of the electrode capillary which was closed with glue. The needle served as a pressure relief to prevent pressure differences that could cause displacement of the agar in the tip. Finally, the shield casing was filled with 1 M KCl electrolyte and closed with glue.

Ag/AgCl half-cells can be light sensitive. This should be tested for each electrode, and if the light conditions during measurements vary to a degree that will influence the signal, the internal Ag/AgCl electrode should be shielded against light, for instance with black tape or paint (Figure 1).

The tip of the EPM was always kept in aqueous solution to prevent evaporation of the electrolyte from the tip opening, which would lead to up-concentration of ions in the tip and thus temporary changes in tip diffusion potential. Furthermore, desiccation of the agar could lead to the formation of an air pocket in the tip, blocking the electric contact with the environment.

2.2. EPM Measurement Setup

The EPM and a reference electrode (REF201, Radiometer Analytical, Denmark) were connected to a custom-made millivoltmeter which had an input resistance of approximately $10^{14}$ Ω. The internal resistances of the
EPM and reference electrodes were determined in circuits at various voltages in a beaker with 1 M KCl (with a conductivity of approximately 10 S m⁻¹ [Pratt et al., 2001]) and were less than 1000 kΩ and 40 kΩ, respectively. Thus, the electrodes could not impose significant voltage drops in the measuring circuit.

In order to take better advantage of the digital resolution of the data acquisition system, a custom-made signal modifier was used to multiply the signal output of the millivoltmeter with a factor of 10 before it was fed into the 16-bit analog to digital converter (ADC-216, Unisense A/S, Denmark), which had a minimum input range of ±1 V.

2.3. Microsensors and Microelectrodes for O₂, H₂S, pH, and Redox Potential

Custom-made microsensors and microelectrodes for O₂, H₂S, and pH were made and calibrated as described elsewhere [Jeroschewski et al., 1996; Revsbech, 1989; Revsbech and Jørgensen, 1986]. The pH microelectrode data were used to calculate a total sulfide profile (S²⁻tot) from the H₂S microsensor data as described by Jeroschewski et al. [1996]. Where pH microprofiles were not as deep as H₂S microprofiles, conversion of H₂S data to S²⁻tot was done with the deepest measured pH value.

Redox microelectrodes were prepared by glass coating a 100 μm platinum wire tapered by cyanide etching to a 25 μm tip size. The glass was removed from the tip-most 25 μm by heating, thereby exposing the platinum surface. For electric shielding, a shield casing was mounted as described for the EPM. The redox microelectrode was connected to a millivoltmeter, and the potential was measured against a reference electrode as described for the EPM. Calibration was performed in quinhydrone solutions of pH 4 and 7, which at 15°C have a redox potential of 267.6 and 92.9 mV, respectively, against an Ag/AgCl, 3 M KCl reference, which in turn can be converted to SHE (standard hydrogen electrode) equivalents by adding 211 mV [Bier, 2009].

2.4. Electrode Positioning and Data Logging

A motorized microprofiling system (Unisense A/S, Denmark) was used for microsensor positioning and data logging. Before measuring each vertical microprofile—that is series of measurements taken at defined positions along a trajectory—the vertical position of the sediment surface was first established by lowering the microsensor with manual micromanipulator control while watching the very tip in a dissection microscope as it approached and touched the surface. The vertical surface position could be determined with an accuracy of approximately 100 μm. When measuring with several sensor types, each sensor tip was first brought in close proximity with the tip of a fine glass filament stuck into the sediment before moving a defined horizontal distance with the micromanipulator. This way it was possible to ensure that a set of sediment microprofiles could be measured in the same horizontal position with approximately 100 μm precision. To minimize disturbance from previous measurements in the same spot, microprofiles of O₂, H₂S, and pH were only measured deep enough to document the relative positions of the oxygen and sulfide fronts and the pH shifts in the oxic zone. Microprofiles were made with step sizes of 50 μm to 1 mm, depending on sensor type and the depth in the sediment.

In some sediment samples, the signal of the EPMs with apical tip opening exhibited erratic variations when the electrode was moved forward (i.e., in the direction of the tip), possibly due to particles blocking the tip opening. In these cases the variations were minimized by measuring the profiles backward, starting in the deeper layers and moving up in steps.

2.5. Test of the EPM in a Controlled Electric Field

A 3 mm wide, 5 mm deep groove in a plastic slab was filled with 1 mM KCl electrolyte, and two working electrodes made of 2 cm coils of electrochlorinated Ag/AgCl wire were inserted 200 mm apart. One of the electrodes also served as the reference electrode for the EPM, which was connected to the input of a millivoltmeter as described above. A series of DC voltage levels (−10, −5, −1, 0, 1, 5, and 10 mV) were applied between the electrodes using a Keithley 2401 SourceMeter (Keithley Instruments Inc., Cleveland, Ohio, USA). At each voltage level, the tip of the EPM was lowered into the groove at defined positions between the two working electrodes, and its signal was logged.

2.6. Test of the Effect of Electrode Design on Sulfide Interference

Test electrodes were produced as described above, except that in order to ensure a well-defined position of the internal electrode, it was insulated by glass except for the distal 30 mm, which was coiled to a small spiral,
approximately 3 mm in diameter and 1 mm high. Also, no shield casing was implemented. The effects on the signal of (1) the distance of the internal electrode to the tip opening (2, 5, 10, 20, or 50 mm), (2) tip shape (Ø8 mm parallel-sided versus conical with a tip angle of approximately 35° and a tip opening of 30 or 100 μm), and (3) electrochloriation (3 min) were investigated by inserting the test electrode through the rubber stopper of a closed container with a few millimolar sodium sulfide solution buffered to pH 7.3. This container was in electric connection with a beaker of 1 M KCl through a salt bridge. The reference electrode was placed in a second beaker with 1 M KCl, and a large Ag/AgCl electrode was placed in each beaker. These two large Ag/AgCl electrodes were connected through an adjustable voltage source in the form of a simple battery voltage divider. The voltage source allowed the evaluation of the electrodes’ ability to respond to externally imposed electric fields (5–100 mV). The test and reference electrodes were connected to a millivoltmeter, and the signal was logged. The first and second time derivatives of the signal are equal to the drift rate and the change in drift rate, respectively, and are thus good indicators of undesirable signal instability. To obtain these derivatives, the time course of the signal was fitted with a second-order Savitzky–Golay smoothing filter [Savitzky and Golay, 1964] using the R software package “signal” [Signal Developers, 2013].

Subsequent chemical analyses of samples from the test container showed variations in the sulfide concentrations between 4 and 8 mM. The EPM signals were not normalized to these variations as they should only change the half-cell potential by a few tens of millivolts according to the Nernst equation.

2.7. Test of Redox Potential Gradient Interference on the EPM Signal

A sediment system with a steep redox gradient and without salinity gradients or cable bacteria activity was established as follows: A slurry of sulfidic sediment and water from Aarhus Harbor was sieved and poured into a glass tube (Ø2 cm and 10 cm long) closed in the bottom with a rubber stopper. The top of the glass tube was sealed with a rubber stopper, excluding any headspace, and placed in an aquarium at 15°C for 24 h, allowing the sediment to settle. Then the top stopper was removed, and an airstream was applied on the water surface to ensure circulation and equilibration of the water above the sediment with atmospheric oxygen. After 1 h of aeration, microprofiles for H2S, O2, pH, electric potential, and redox potential were measured.

2.8. EP Signal Postprocessing and Drift Correction

All microprofiles included measuring points in the water phase, and if no significant drift had occurred in the water-phase signal between before and after the measurement of one microprofile, the water-phase signal value was subtracted from all signal values in the profile. If significant drift was observed, a second-order polynomial as a function of time was fitted to the water-phase signal values for several microprofiles and subtracted from all measurements in these profiles (see supporting information for details). In this way, the final processed profile data came out as drift-corrected values for the electric potential relative to that in the overlying water.

2.9. Application of EPM in Cable Bacteria Sediment

Sediment was sampled from Aarhus Harbor, Denmark (56.1388°N; 10.2140°E; water depth: 4 m) using a Kajak sampler. The top 5 cm of the sediment was discarded, and the underlying sulfidic sediment was passed through a 0.5 mm sieve and homogenized in a box. Glass tubes (inside diameter: 45 mm and 70 mm long) were then inserted into the sediment in the box and closed in the bottom with rubber stoppers. The filled glass tubes were left with a cover of approximately 5 cm sediment for 1 day in order to ensure settling and compaction of the sediment. Then the cores were retrieved, rinsed on the outside, and leveled with a blade to align the sediment surface with the upper rim of the glass tube. The sediment cores were then transferred to a 30 L Styrofoam-insulated temperature-controlled (15°C) glass aquarium with artificial seawater (dissolved Red Sea salts). To avoid the presence of salinity gradients, which would induce diffusion potentials, the salinity of the seawater was adjusted to the salinity of the sediment pore water (25‰); the latter being measured on pore water extracts.

Parallel sets (n = 3) of EP, O2, pH, and H2S microprofiles were measured in a microprofiling setup as described above after 4–5 and 19 days.

At the end of the experiment, sediment porosity of the upper 24 mm was gravimetrically determined with a depth resolution of 3 mm in three parallel cores. Sediment conductivity was estimated from sediment porosity and the conductivity of the water [Fofonoff and Milard, 1983] using the formulas of Ullman and Aller [1982] for muddy sediments.
The diffusive O₂ uptake was calculated from numerical modeling of the measured O₂ concentration profiles [Berg et al., 1998].

2.10. Measuring Diffusion Potentials in a Sand Column

Diffusion potentials were generated with NaCl solutions in artificial sediment column made from pure fine sand (50–70 mesh particle size, Sigma-Aldrich) with no biological or chemical activity. A 30 mm thick layer of sand settled out in a vessel with 0.5 M NaCl. EP microprofiles were measured as described above before and after the NaCl concentration in the overlying water was lowered to 0.45 M by diluting with pure water.

3. Results and Discussion

3.1. Test of the EPM in a Controlled Electric Field

Changing the applied voltage and EPM position in the Perspex groove resulted in corresponding changes in the EPM signal (Figure 3a). At distance 0 (close to the Ag/AgCl electrode used as reference), the voltage varied around 1 mV between different voltage levels, which can be attributed to a minor dependence of the potentials of the Ag/AgCl electrodes on the electrode current at the different voltage levels. When subtracting the zero position signal, the observed EPM signal change closely followed the change that could be calculated from applied voltage and position (Figure 3b), confirming that the EPM signal can be used for reliable measurements of the electric potential at the EPM tip.

3.2. Test of Interference From Sulfide and Redox Gradients

After the start of exposure of the test electrodes to sulfide, the signals settled at a level within ±50 mV with a stability of approximately 0.5 mV for each electrode after an initial settling period of a few minutes. After minutes to days, depending on the design, the signal started to drop, marked by distinct minima in the first and second derivatives of the signal. Subsequently, the signal gradually stabilized at a level several hundred millivolt lower (Figure 4a). The log₁₀ of the time for the first signal derivative minimum, i.e., maximum (absolute) drift, as a function of the log₁₀ of the electrode position (distance of internal electrode from tip) is plotted in Figure 4b for different designs as an indication of the time it takes for sulfide to affect the signal. The slope of the curve for the parallel-sided, nonelectrochlorinated design is approximately 2, confirming the theory stating that the diffusion time in general is a square function of the distance in such a one-dimensional system [Crank, 1975, equation 3.40]. Figure 4c is a plot of the log₁₀ of the negative of the minimum of the first signal derivative versus the log₁₀ of the electrode position for different designs. The slope of the curve for the parallel-sided, nonelectrochlorinated design is −2, indicating that the maximum drift is inversely related to the position squared. The slope for the second signal derivative is −4, which means an even stronger attenuation with distance (data not shown). Figures 4b and 4c show that the approximately 30° conical design prolongs the time for sulfide interference by about 1 order of magnitude and the maximum drift drops approximately 1 order of magnitude for the 100 μm tip and approximately 2 orders of magnitude for the 30 μm tip compared to the parallel-sided design. The overall electrode diameter prohibits a simple extrapolation of the effect of conicity observed at 5 and 10 mm to even longer distances, but implementing smaller tip openings is possible and would further enhance these effects. Furthermore, Figures 4b and 4c show that the 3 min electrochlorination has a pronounced effect on the
time it takes for sulfide to affect the sensor signal, delaying the response approximately 45 and 77 h for 5 mm and 10 mm distance, respectively, compared to the nonelectrochlorinated design. This can be explained as a protective effect of the layer of solid AgCl formed by electrochlorination. The potential of an Ag/AgCl electrode is governed by the Ag⁺ concentration, and as long as AgCl is the least soluble salt near the silver surface, the Ag⁺ concentration is governed by the Cl⁻ concentration. Ag₂S has a solubility many orders of magnitude lower than that of AgCl, but initially, the sulfide that reaches the outside of the AgCl layer on the internal electrode reacts chemically with the equilibrium concentration of Ag⁺ and precipitates as Ag₂Sa and does thus not reach the silver surface. But after a period of time, the diffusing sulfide has scavenged enough Ag⁺ to disrupt the AgCl layer, which allows sulfide to reach the silver surface where it will determine the Ag⁺ concentration—and thus the electrode potential—through the Ag₂S solubility equilibrium.

The experiments show that design parameters: long distance between electrode tip and internal electrode, conicity, and electrochlorination, work together to delay and reduce the extent to which sulfide in the sample can interfere with the signal. In addition, in real EPMs where the internal electrode is closer to the cable end, some oxygen can diffuse into the electrolyte from the cable end and react with sulfide or other reducing substances, impeding their flux to the internal electrode. Two electrodes of the normal standard design (right side of Figure 1) were left in the setup (concentration > 2 mM) for 7 days and did not exhibit any response that could be attributed to sulfide interference (drift less than 1 mV).

In all cases, both before and after a sulfide-induced signal drop, an imposed external voltage would cause an equal (within 1%) change in the electrode signal (data not shown), indicating that the ambient electric potential is directly additive to the electrochemical half-cell potential, so even in cases where sulfide does reach and affect the internal electrode, the electric potential can be evaluated from the total EPM signal by drift correction using the overlying water as reference.

As a further confirmation that the design makes an EPM insensitive to redox-active compounds within the time frame of a typical experiment, the EPM did not respond to a steep redox-potential gradient in a sediment system (Figure 5). In contrast, as intended, the redox microelectrode immediately reacted to redox-active species due to the direct exposure of its catalytic metal tip surface to the environment.

There has been some confusion and controversy about electric potentials and redox potentials, particularly in the debate of self-potential anomalies proposed by Sato and Mooney [1960] to indicate geobatteries.
in the subsurface. While some have ascribed the measured SP anomalies to gradients in redox potential \cite{Corry,1985;Nyquist and Corry,2002;Williams et al.,2007}, others maintain that large SP anomalies indicate the presence of geobatteries \cite[e.g., Bigalke and Grabner,1997;Ntarlagiannis et al.,2007]. In lack of detailed information on electrode design and operation, it is difficult to evaluate electrode sensitivities to intruding redox-active compounds in most of these studies. One exception is the study of Ntarlagiannis et al.\cite{2007}, who used Ag/AgCl electrodes to test if electrons were conducted from reduced layers to the oxic surface of saturated sand columns inoculated with *Shewanella oneidensis*. At first, the results were interpreted as a confirmation \cite{Ntarlagiannis et al.,2007}, but later it was recognized that rather than reflecting electric potential gradients from the establishment of a biogeobattery in the column, the 400 h time record of electrode signals probably indicated the accumulation and intrusion of reduced metabolites, reacting first with the reference electrode at the bottom and then successively with the measuring electrodes in the direction of the surface \cite[Atekwana and Slater,2009;Hubbard et al.,2011;Revil et al.,2010]. This interpretation is further supported by the direction of the final supposed electric potential gradient which was opposite of the expected, as exemplified in Figure 6.

The zone of increasing EP corresponded to a zone depleted in pore water sulfide, and a pH peak was observed in the oxic zone (Figure 6a). In contrast, none of these features were present in the beginning of the incubation (Figure 6b). Together, the potential change, the depletion of sulfide, and the pH peak after 19 days indicate electrogenic sulfide oxidation \cite{Malkin et al.,2014}, where bacterial filaments conduct electrons from sulfide-oxidizing filament cells in deeper layers to oxygen-reducing filament cells in the oxic layer as suggested by Pfeffer et al.\cite{2012}, Nielsen et al.\cite{2010}, and Risgaard-Petersen et al.\cite{2012}. This intrafilament electron transport gives rise to an electric potential of a magnitude sufficient to drive an ionic current in the pore water of the same size but opposite direction, closing the electric circuit. As described in the present study. In general, the value of past and coming studies of electrogeophysical phenomena depends on a clear distinction between redox potential and electric potential measurements, and, whenever possible, the design of SP/EP electrodes and the experimental conditions should be carefully evaluated and tested with respect to cross reactions with redox-active species.

### 3.3. Application of the EPM in Sediment

For three replicate EP microprofiles from the same spot on the sediment surface the signal standard variation was less than 0.1 mV at any depth (see example in the supporting information), confirming that microprofiling in sediments and biofilms is nondestructive if the sensors are sufficiently thin \cite{Kühl and Revsbech,2001}.

After 19 days of incubation, an electric field was observed in the pore water with the electric potential relative to the water phase increasing from zero at the surface to +1.85 ± 0.1 mV at 18.5 mm depth and below. The zone of increasing EP corresponded to a zone depleted in pore water sulfide, and a pH peak was observed in the oxic zone (Figure 6a). In contrast, none of these features were present in the beginning of the incubation (Figure 6b). Together, the potential change, the depletion of sulfide, and the pH peak after 19 days indicate electrogenic sulfide oxidation \cite{Malkin et al.,2014}, where bacterial filaments conduct electrons from sulfide-oxidizing filament cells in deeper layers to oxygen-reducing filament cells in the oxic layer as suggested by Pfeffer et al.\cite{2012}, Nielsen et al.\cite{2010}, and Risgaard-Petersen et al.\cite{2012}. This intrafilament electron transport gives rise to an electric potential of a magnitude sufficient to drive an ionic current in the pore water of the same size but opposite direction, closing the electric circuit. As described

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**Figure 5.** Test of redox interference. Microprofiles of $O_2$, pore water sulfide, electric potential (EP, average of 3 replicates and standard deviations), and redox potential (versus SHE) in a sample of sediment without cable bacteria activity or salinity gradients. Note the different scales for EP and redox potential.
by Risgaard-Petersen et al. [2014], EP profiles can be used to calculate pore water current densities—and thus the intrafilament current density of equal size—according to $J = \Phi E$, where $J$ is the current density, $E = \frac{dV}{dx}$ is the voltage gradient, and $\Phi$ is the electric conductivity in the pore water. The voltage gradient at the oxic-anoxic interface (i.e., the slope of the EP microprofile at approximately 0.6 mm depth) after 19 days was 0.17 ± 0.01 V m$^{-1}$ (Figure 6a), and with a porosity of 0.82 ± 0.016, a salinity of 25 ‰, and a temperature of 15°C, the pore water conductivity could be calculated to approximately 1.75 S m$^{-1}$. The observed electric field in the pore water at the oxic-anoxic interface thus corresponds to a current density of 298 ± 10 mA m$^{-2}$. The oxygen flux from the water phase was 67 ± 12 mmol m$^{-2}$ d$^{-1}$, which together with the oxic zone proton consumption rate estimated from the pH profile yields a minimum estimate of the current density of 180 ± 50 mA m$^{-2}$, according to the proton/oxygen mass balance model of Nielsen et al. [2010]. In addition to the effect of variable oxygen reduction stoichiometry and noncarbonate pH buffering described by Nielsen et al. [2010], the lower current density estimate from the proton/oxygen mass balance model can be due to limitations of the pH microelectrode in resolving the real steepness of the pH gradients.

3.4. Diffusion Potentials in Sand Sediment

Diffusion potentials arise from the charge separation in concentration gradients of ions with different mobility. Figure 7 shows a series of drift-corrected EP microprofiles before and after decreasing the concentration of NaCl in the water overlying the sand. Decreasing the NaCl concentration in the overlying water by 10% immediately caused an increase in electric potential inside the sand sediment because of the 1.52 times faster diffusion of Cl$^-$ ions compared to that of Na$^+$ ions [Barry and Lynch, 1991]. Over time, diffusion continuously decreased the concentration gradient at the sediment-water interface, resulting in a similar decrease in the gradient of the electric potential. A difference of 10% in the NaCl concentration should cause a diffusion potential of 0.55 mV according to the equation

$$-\Delta \psi = \frac{RT}{F} \sum_i t_i \frac{Z_i}{Z_i} \ln \frac{c_i(t)}{c_i(0)}$$
1. For measurements, where drift correction is not possible, a more stable electrode is desirable. This stability; the side port tip design enabled forward processing and contamination of the sample as observed by for instance Jougnot and Linde [2013]. To get a rough quantitative estimate of this phenomenon in the EPM, we added a potential relative to the overlying water in artificial sand sediment. Initially, the NaCl concentration was 0.5 M throughout the system; at t = 0, the concentration in the overlying water was changed to 0.45 M.

3.5. EPM Performance

The choice of glass for the electrode body enabled the shaping of the tip for different applications (Figure 2). The 90% response time of the total measuring circuit following step changes in the electric potential at the EPM tip was below 1 s when the EPM was stationary (data not shown). However, the movements of the EPM to a new position in sediment often caused transient dips or peaks in the signal, and data were therefore recorded after 5 s when the signal was stable. The reason for the instability is not resolved, but transient changes in diffusion potential at the sensor tip might be a possibility.

The potentials of a batch of seven test EPMs had an interelectrode standard deviation of 0.2 mV when measured in a beaker with 1 M KCl against a reference electrode. The maximum drift of electrochlorinated electrodes was below 2 mV for up to 7 days, and the described EPM and measurement system has a stability of typically less than 10 μV over a many minutes (see example in the supporting information). However, drift on the order of 1 mV/h can occur, especially right after the EPM is introduced to a new environment. The drift can be attributed to changes in the electric potential in different parts of the measuring circuit due to for instance temperature fluctuations. The electrochemical half-cells of both the internal electrode of the EPM and the reference electrode will have a temperature response in the order of ~1 mV/°C [Ives and Janz, 1961]. Another source of potential drift is a change in diffusion potential at the EPM tip when it is moved to a different salinity. With the drift correction procedure (see supporting information), we could use the multiple reference data points in the water phase between microprofiles to correct for drift and achieve a signal resolution of less than 10 μV in the laboratory without any electronic protection beyond the coaxial cable, shield casing, and mineral oil isolation of the EPM. As demonstrated in Figure 5 and Figure 6, the drift-corrected data are very reproducible; for instance, for the measurements on day 5 before cable bacteria activity is detectable, the variation in EP measurements with depth and across three replicates is on the order of 10 μV. With electrodes with the opening straight on the tip, backward profiling was necessary to maintain this stability; the side port tip design enabled forward profiling measurements with the same level of stability. For measurements, where drift correction is not possible, a more stable electrode is desirable. Petiau [2000] describes a PbCl₂ electrode with interelectrode variability in the order of a few microvolt and a temperature coefficient of ~20 μV/°C.

Electrolyte ions can diffuse through the tip opening of electrolytic electrodes, causing a decrease in electrolyte concentration and contamination of the sample as observed by for instance Maineult et al. [2004] and Jougnot and Linde [2013]. To get a rough quantitative estimate of this phenomenon in the EPM, we can consider the tip region a 100 μm long, 25 μm diameter pore, separating a 2 mL homogenous internal 1 M KCl electrolyte from a 2 mL sample of pure water. Using Fick’s first law, J = –D dC/dx, (where J is the flux, D is diffusion coefficient, dC is the concentration difference, and dx is the diffusion distance) with a KCl
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