

Construction and calibration of a rapidly responding pH mini-electrode: application to intertidal sediments*

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ABSTRACT: The construction and calibration is described of a new type of pH glass electrode with a measuring bulb of 1.0 mm in diameter. The lower one-fourth zone of the bulb, a 15 to 70 μm thick membrane of pH glass, contributed at least 70 % to the final reading, hence allowing a spatial resolution down to 100 μm . The 90 % response time depended on temperature and varied from 20 s at 0°C to 5 s at 36°C in buffer solutions and from 5 to 15 s in sediment at 18°C. A maximal deviation of 4 % resulted from the use of standard buffers for calibration instead of saline buffers. Over the temperature and pH range tested (0 to 36°C and pH 7 to 10) the mini-electrode showed a linear response. Responses varied from 91 to 98 % of those calculated from the Nernstian equation. In melting ice 75 % of the Nernstian response was still found. The sturdy bulb of the mini-electrode allows its use in muddy as well as sandy sediments. The short response time and high spatial resolution permit the measurement of vertical pH-gradients at microscale, parallel to such gradients in oxygen, measured with oxygen microelectrodes. An example is given of a combined measurement of the photosynthetic activity of benthic microalgae, pH, and oxygen in an estuarine sediment.

INTRODUCTION

During the last decade, microelectrodes have been developed for use in marine and estuarine sediments. The use of these electrodes has increased our insight into the microbiological and geochemical processes in a variety of biotopes. Ecological processes in the oxic layer of intertidal flats cannot be understood without knowledge of the fluctuations of oxygen and pH. Recent studies have shown large daily fluctuations in oxygen and pH due to the activity of bacteria and microalgae (Revsbech et al. 1983, Revsbech & Ward 1984, Jørgensen et al. 1983, Rasmussen et al. 1983). Ultrafine pH microelectrodes of glass developed for intracellular measurements were described by Carter & Pucacco (1978) and Thomas (1978). The latter description was used by Revsbech and coworkers for their pH microelectrodes. The electrical resistance of scaled-down pH microelectrodes increased the time needed for stabilization from 15 to 180 s (Carter & Pucacco, 1978). This implies that a suitable compromise has to be found between tip size and adequate response time,

depending on the aim of the research. Application in estuarine and marine sediments requires rapidly responding electrodes as pH may change during the measuring procedure. The construction of a rapidly responding, sturdy pH glass mini-electrode is described here as well as the method used for calibration. The properties of this mini-electrode are discussed with respect to spatial resolution and response time. An example of in situ application in an intertidal flat is presented.

MATERIAL AND METHODS

Construction. The tip of the mini-electrode is made of Corning 0150 pH glass. This glass is composed of SiO_2 (72 %), Na_2O (22 %) and CaO (6 %), and exhibits a Nernstian response to H^+ ions up to pH 11 to 12 (Lakshminarayanaiah 1976). A tube of Corning 0150 glass, 2.5 cm in length and 2 mm wide, is pulled to a fine capillary with a micropipette puller to a final outer diameter of 0.2 to 0.5 mm; the thickness of the wall is then ca 0.1 mm. The open end of the capillary is closed by heating with a circular microforge heating element

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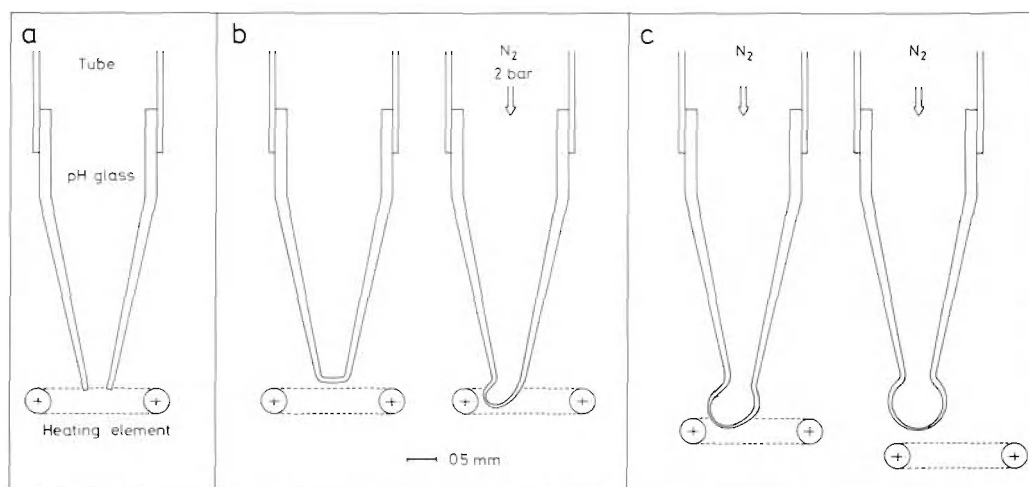


Fig. 1. Construction of the pH bulb. (a, b) Pulling of glass capillary with a circular microforge heating element. (b, c) Blowing of the bulb with N_2 at pressure 2 atm. The heating element is first moved towards the bulb to make one side thinner. Afterwards, the element is moved down and to one side to move the partially thinned glass to the lower part of the bulb

(Fig. 1a). The transformation of the closed end into a rounded bulb results from the interaction of the heating at the tip and the blowing of N_2 -gas into the open end of the tube (Fig. 1b). The heating element is first moved towards one side to create a thinner wall. This side is moved downwards by moving the heating element relative to the bulb (Fig. 1c). With experience, perfect spheres can be formed with bulbs varying in diameter 0.1 to 1.0 mm; we preferred 0.5 to 1.0 mm bulbs for measurements in sediments as will be discussed later. The glass thickness of the side and lower parts depends on the distance between the heating element and the tip. For a 1 mm bulb it decreased from 100 μm in the upper part to 15 μm in the lower part. The electrode is glued into a pasteur pipette (WU, Mainz, FRG) with a 2-component glue based on epoxy resins (Uhu/Fismar, FRG). The connection must be watertight to ensure correct measurements. The total length of the protruding pH tip varies from 5 to 15 mm.

The electrode is filled under vacuum to prevent formation of micro gas bubbles. The internal electrolyte is a mixture of 0.1 M sodium citrate and 0.1 M sodium chloride. To prevent microbial growth inside the electrode, silver nitrate is added to a final concentration of 1 %. On top of the internal solution a drop of paraffin oil is added to close the electrode and prevent evaporation.

A 24 h period of stabilization is needed for the equilibration of the pH glass and its internal solution. The electrodes are stored at 4°C in a tube containing the internal electrolyte solution. The lifetime is then about 6 mo. For longer storage the electrodes should be left unfilled. Between daily measurements the pH mini-electrode is kept in seawater of ambient temperature and salinity. In this way large pH changes are pre-

vented, prolonging its lifetime. Decline of the potential difference per pH-unit or sudden shifts in potential readings indicate malfunctioning.

RESULTS AND DISCUSSION

Calibration

The response of each newly-made pH mini-electrode is checked for its response time at a pH interval of 7 to 10 before use. This interval includes all pH values encountered in measurements in sediments of the Oosterschelde estuary (The Netherlands). Earlier tests showed responses close to theoretical values between pH 4 to 11.

The buffer solutions used are commercially available (Merck). Saline buffer solutions were made by adding sodium chloride (Merck p.a.) to obtain a final salinity of 30 ‰.

The experimental set-up consisted of a sturdy metal rack with a motor-driven micromanipulator (Ull/Assler, FRG) holding the pH mini-electrode. The mini-electrode was linked to a pH/mV meter (PHM 82 Radiometer Copenhagen, Denmark) by a silver wire connecting the internal solution with a shielded cable. The high electrical resistance of the mini-electrode, 10^{10} to $10^{12} \Omega$, makes it very sensitive to interferences of static electricity or short-circuiting by moisture. Therefore, proper insulation and shielded cables are required. A single junction reference electrode with ceramic grit (Radiometer Copenhagen, Denmark) was connected to the pH/mV meter, which was stored in a waterproof housing.

We tested the pH mini-electrode in 4 standard buffer

solutions over a temperature range of 0 to 36°C. At each temperature the corresponding pH values were corrected according to the manufacturer's information or by additional calibration with a pH macro-electrode (GK 2421C, Radiometer Copenhagen, Denmark) coupled to a similar reference electrode as used for the mini-electrode. Linear regression analysis always showed high correlation coefficients between pH and potential difference, ranging from $r = 0.999$ at 0.0°C to $r = 1.000$ at 35.6°C, $n = 4$. The lowest sensitivity was found in melting ice. The theoretical relationship between slope and temperature is defined by the Nernst equation. This equation predicts sensitivities of 58.2 and 60.1 mV pH⁻¹ at 20 and 30°C, respectively (Fig. 2). Measured slopes were between 95 and 98 % of

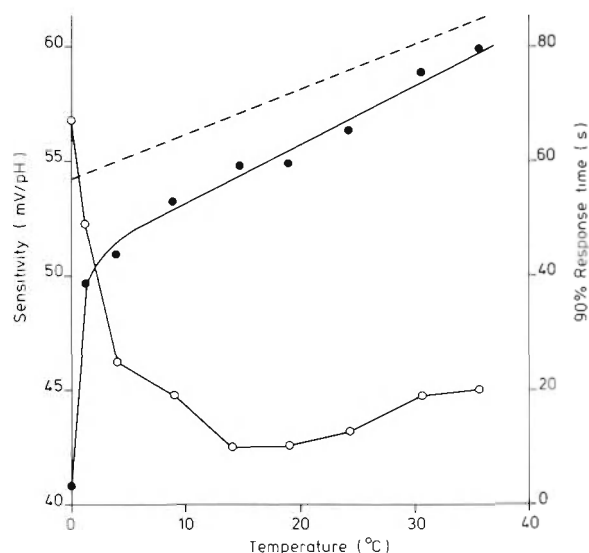


Fig. 2. Sensitivity (●; mV pH⁻¹) and 90 % response time (○; s) measured at steps of 1 pH-unit in $S = 30$ buffer solutions with temperature ranging from 0 to 36°C. Dashed line represents the sensitivity calculated according to the Nernst equation, slope = 0.198. A slope of 0.269 ($n = 8$, $r = 0.988$) was calculated from the measured sensitivities (solid line) for the pH mini-electrode tested, excluding the 'melting ice' point

the Nernstian slopes over the range of 9 to 36°C decreasing to between 91 and 93 % at 1 to 9°C. In melting ice 75 % of the Nernstian response was still found. The non-ideal response at temperatures lower than 2°C is probably due to retarded diffusion and the presence of ice in the solution. Field measurements in ice-covered sediments showed unreliable and unstable signals, indicating slow exchange over the pH glass membrane.

The accuracy of pH measurements is subject to discussion. Differences in set-up or sets of electrodes introduce several sources of error. The main technical error results from the use of different junctions between

pH and reference electrode. Although the free diffusion liquid junction provides the best alternative, its application causes problems in sediment. We used a reference electrode with a permeable ceramic grit for calibration and in situ measurements. The deviation can be reduced to 0.01 pH-unit when the same pair of pH and reference electrode are used throughout a series of measurements (Whitfield et al. 1985).

A second source of errors arises from physical factors, such as temperature and salinity. In our tests we calculated the effect of temperature to be 0.27 mV pH⁻¹ °C⁻¹ between 1 and 36°C, somewhat larger than that predicted from the Nernstian equation, 0.20 mV pH⁻¹ °C⁻¹ (Fig. 2). The effect of salinity was a shift in the reading of the potential of the buffer solutions due to changed conductivity which decreased the original pH value. The phosphate-based pH 7 buffer shifted from 7.0 to 6.62, whereas the 3 borate-based buffers of pH 8, 9 and 10 each decreased 0.16 pH-unit. However, only slight changes in sensitivity (1 to 4 %), compared to standard fresh buffer solutions, were found. In other studies salt shifts varied from 0.01 to 0.09 pH-unit (Schwabe 1974, Butler et al. 1985, Whitfield et al. 1985, Millero 1986). Millero stated that for pH determination over a salinity range of 5 to 40, any set of buffers at a fixed salinity can be used, introducing a shift of 0.01 pH-unit at most. The transition of $S = 0$ to $S = 5$, however, may cause larger errors, up to 0.09 pH-unit.

90 % response time and drift

The 90 % response time is defined as the time needed to reach 90 % of the stabilized value (Revsbech et al. 1983). These times were estimated from the recorder readings. An average was calculated from each series of 4 buffers. The most rapid responses were found between 15 and 25°C (Fig. 2). Over the range 9 to 36°C, response times varied little, 10 to 20 s. At temperatures lower than 2°C the time needed for 90 % stabilization was significantly longer, 49 to 67 s, applying in this case to shifts of 1 pH-unit. On moving the pH mini-electrode to buffer solutions with similar pH, stabilization was seen within a few seconds.

The 90 % response time gives only a rough indication of the sensitivity. Even a very sensitive electrode exhibits response times of 5 to 50 s due to changes in temperature and hence diffusion velocities. We used a standard 90 % response time of maximal 10 s at 20°C at a shift of 1 pH-unit as a testing norm; electrodes with slower response times were not used as their bulb walls were too thick to allow fast diffusion.

The electrode drift was very small, 1 to 12 mV h⁻¹ at 20°C, which corresponds to a maximal drift of 0.004 pH-unit min⁻¹.

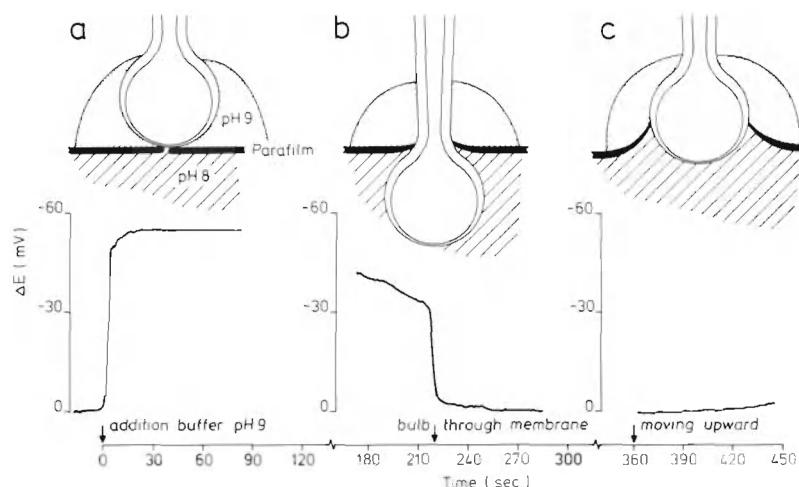


Fig. 3. Test procedure used to determine the actual measuring zone of the bulb of a pH mini-electrode. The bulb was separated from 2 different buffers by a thin parafilm with a hole of 50 μm . (a) At time = 0 s a drop of buffer solution (pH 9.0) was added to the upper side of the parafilm. (b) At time = 180 s the bulb was pushed gradually through the parafilm membrane. After 220 s the whole bulb was situated below the parafilm membrane. (c) At time = 360 s the electrode was gently moved upwards. The situation illustrated was reached after 450 s

Spatial resolution

A specific test was carried out to determine the actual measuring zone of the tip and thereby its spatial resolution. The method used allowed discrimination between slow- and rapid-responding areas of the bulb. We took a 1 mm bulb-sized electrode exhibiting maximal response time at 20°C in order to make a maximal estimation of the measuring error involved in measuring sub-millimeter intervals. The electrode was held with the tip touching a stretched parafilm membrane (Fig. 3a). This membrane separated 2 buffer solutions of pH 8.0 and 9.0. A hole of approximately 50 μm was made to provide contact between electrode and underlying solution. The liquid contact through the small connecting hole was sufficient to establish a stable recording signal. When a drop of pH 9 buffer was added the electrode signal showed a shift of 55 mV within 10 s. The electrode was then moved through the membrane while recording the signal continuously together with microscopic observation. A gradual decrease of the electrode signal was seen as the bulb moved downwards through the membrane (Fig. 3b). As soon as half of the bulb had passed the membrane, the membrane closed at the neck and the signal dropped almost instantly to the original pH 8.0 value. This means that the pH is measured by the bulb-formed tip of the mini-electrode. Moving the tip upwards gave further information about the actual measuring zone (Fig. 3c). A small drift of 2 mV was measured when $\frac{3}{4}$ of the bulb had passed the membrane. Additional microscopic study of the bulb showed that the thickness of the glass membrane narrowed from 70 to 15 μm in the lower $\frac{1}{4}$ part. Over the lowest 100 μm zone the 15 to 25 μm thick glass favoured exchange of H^+ ions in a 5:2 ratio relative to the adjacent 200 μm zone above. This means that at least 70 % of the response was obtained

via the thinnest part of the bulb. It also implied a minimal spatial resolution of 100 μm at 1 mm bulb-size.

Application to intertidal sediments

An in situ measurement in estuarine sediment was carried out with an oxygen microelectrode and a pH mini-electrode. The oxygen microelectrodes were constructed according to the description given by Revsbech (1983). Photosynthesis was measured in 50 μm intervals at in situ irradiance (1180 $\mu\text{E m}^{-2} \text{s}^{-1}$) and temperature (18.2°C), using the darkening technique described by Revsbech & Jørgensen (1983). Calibration of the oxygen microelectrode was done in seawater of ambient temperature by Winkler titration direct after the measurement. Calibration of the pH mini-electrode took place directly after measuring in 3 buffers covering the pH range measured, 7 to 9. The buffers were kept at ambient temperature without exposure to direct sunlight. A sediment sample was taken with a plexiglass corer of 20 cm length and 6 cm width from an emersed intertidal sandflat in the Oosterschelde estuary, southwestern Netherlands, on 11 June 1986. Oxygen and photosynthesis profiles were measured within 10 min after sampling under in situ conditions of irradiance and temperature at the sampling site; this took 10 min for 2 profiles. The first measured profile of photosynthesis and oxygen is shown in Fig. 4. The pH profile was measured immediately after the photosynthesis measurements. Intervals of 0.1 mm were measured up to 1.5 mm depth, and 0.5 mm intervals to a depth of 5.0 mm (Fig. 4). The whole profile was completed in 8 min.

The 90 % response time was maximally 5 s when moving over 100 μm intervals with a pH change of 0.05 to 0.50. At greater depth intervals longer response

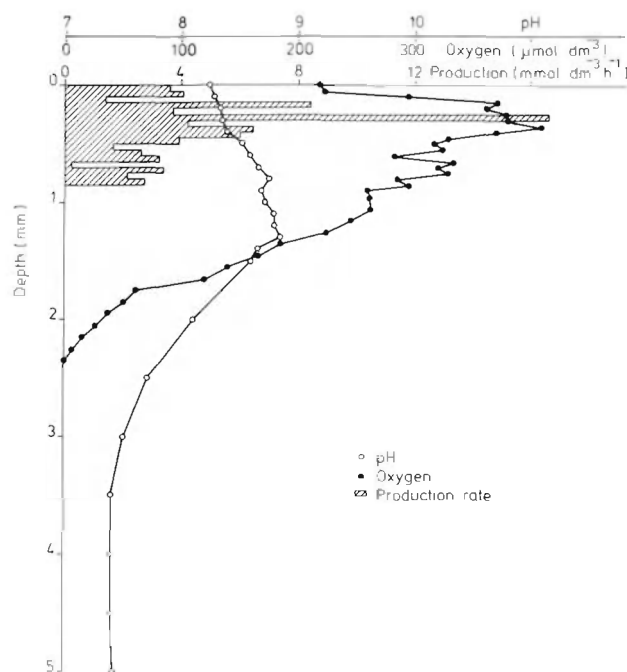


Fig. 4. Depth profiles of pH (\circ), oxygen (\bullet ; $\mu\text{mol dm}^{-3}$ interstitial water) and photosynthetic rate (bars; mmol dm^{-3} wet sediment h^{-1}) measured in a sandy emerged intertidal flat, 11 June 1986. Measurements took place at in situ conditions of irradiance ($1180 \mu\text{E m}^{-2} \text{s}^{-1}$), temperature (18.2°C), and salinity ($S = 34.8$) immediately after sampling (10:50 h). Calibration was done with 3 standard buffers (7.0, 8.0 and 9.0) with a calculated sensitivity of 54.2 mV pH^{-1} ($n = 3$, $r = 1.000$)

times were recorded, 10 to 15 s at correspondingly higher pH changes. The in situ response times, corrected to 1 pH-unit change, were similar to those measured in buffer solutions at 20°C .

As can be seen from the sediment profile, pH changes are less extreme than those in the oxygen profile. A maximum pH of 8.9 was reached at 1.3 mm depth, while at the surface a pH value of 8.2 was recorded. Below the oxic zone the pH stabilized at 7.5. Photosynthetic activity of benthic diatoms, which dominated in these sediments, was measured to a depth of 0.85 mm with maximal values at 0.3 mm.

An in situ pH profile records the $\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$ equilibrium. This chemical equilibrium shifts due to photosynthetic activity of benthic microalgae (Fig. 4). Therefore, measuring should take place under in situ conditions and should avoid sudden changes in irradiance or temperature so that the microalgae maintain their steady state. Coupling of photosynthetic activity to pH gradients can be achieved by simultaneous measurements or by a very short time lapse requiring electrodes with short response times.

The contribution of adjacent layers to the pH is maximally 30 % at a bulb-size of a 1 mm diameter when measuring 0.1 mm intervals. At this spatial resolution the pH measured is an average over the pH gradient existing in this interval. Peak values of pH tend to be slightly underestimated as both the upper and lower adjacent 0.1 mm layer will affect the stabilized value. In an increasing or decreasing pH gradients this error will be much smaller as the effects of the upper and lower adjacent layers will tend to cancel out.

Table 1 summarizes the specific properties of the described pH mini-electrode compared with 2 types of pH-microelectrodes and 1 type of commercially available pH mini-electrode. Except for the commercial pH mini-electrode, all types showed high sensitivity, low drift, and minimal response times of 5 to 15 s. The choice of the electrode type depends strongly on the structure of the sediment and its infauna. Until now, measuring sub-millimeter pH changes was restricted to fine-grained sediments where microelectrodes could be used at a reduced risk of breaking (Revsbech 1983, Revsbech et al. 1983, 1984, Jørgensen et al. 1983). Meyers et al. (1987) were unable to use pH microelectrodes in their study of microdistribution of interstitial fauna in sandy sediments. The larger-sized and more sturdy pH mini-electrodes performed very well in sandy sediments without breaking (Fisher & Matisoff 1981, this study). We used the same electrode type in river sediments with salinities below 3 ‰ and found values close to Nernstian values up to $\text{pH} = 11$. Therefore, this rapidly responding pH mini-electrode can

Table 1. Comparison of specific properties of 4 pH electrodes

Electrode characteristic	Mini-electrodes		Microelectrodes	
	This study	Fisher & Matisoff (1981)	Carter & Pucacco (1978)	Revsbech et al. (1983)
Tip size	500–1000 μm	800 μm	3–150 μm	50 μm
Sensitivity	95–98 %	97.5 %	>97 %	>95 %
Response time	5–15 s	30–60 s	15–180 s	10–60 s
Drift	<1 %	~1 %	<10 %	Unknown
Resolution	$\geq 100 \mu\text{m}$	$\geq 800 \mu\text{m}$	$\geq 10 \mu\text{m}$	$\geq 100 \mu\text{m}$

make a useful contribution toward the understanding of the biological and chemical processes occurring in sandy sediments.

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