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Miniaturized Redox Potential Probe for In Situ Environmental Monitoring

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The need for accurate, robust in situ microscale monitoring of oxidation−reduction potentials (ORP) is required for continuous soil pore water quality monitoring. We are developing a suite of self-contained microelectrodes that can be used in the environment, such as at Superfund sites, to monitor ORP in contaminated soils and sediments. This paper presents details on our development of microelectrode sensor arrays for ORP measurements. The electrochemical performance of these ORP electrodes was fully characterized by measuring redox potentials in standard solutions. It found that the newly developed integrated ORP microelectrodes produced a very stable voltage response (the corresponding rate of the integrated microelectrode potential change was in the range of 0.6−1.1 mV/min), even when the measurement was carried out outside of a Faraday cage where signals from most conventional microelectrodes are usually inhibited by external electrical nose. These new microelectrodes were easier to fabricate and were more robust than conventional microelectrodes. The tip size of the integrated ORP microelectrode was approximately 200 nm square, with a taper angle of approximately 20° and a length of 57 μm. The integrated ORP microelectrode exhibited better signal stability and substantially shorter response times (from less than a few milliseconds to 30 s, depending on the standard solution used) than the commercial millielectrode (a few minutes). Compared with the slope of the commercial millelectrode, the slope of the integrated microelectrode (61.5 mV/pH) was closer to the ideal slope against quinhydrone calibration solutions. Therefore, it is to be expected that the newly developed ORP microelectrode may have wider applications in contaminated soils, biofilms, and sediments.

Introduction

The determination of oxidation−reduction potential (ORP or redox potential) is of great importance in the field of water quality monitoring since solutions can be graded as oxidizing or reducing based on measurements of ORP values. There is an increasing demand for measurement of ORP in solutions, both in industry and in environmental research. Macroelectrodes have been shown to be valuable tools for direct detection of ORP in streams or lakes, wastewater treatment reactors, and water distribution systems due to the speed of analysis, low maintenance cost, and availability of necessary equipment. For example, it has previously been shown that on-line monitoring of ORP can be a reliable and effective technique for control of the activated sludge process, sludge digestion, biological nutrient removal, and chemical oxidation/reduction processes (*1*−*4*). ORP values can also provide significant information for disinfection in pool, spa, and potable water since they are related to the kill time of *Escherichia coli* bacteria in water (*5*). Similarly, electrolyzed oxidizing water has been reported to have strong bactericidal effects on many pathogenic bacteria (*6*). ORP measurements in microbial systems are also of interest since ORP is primarily determined by the energy-yielding reactions of bacterial cells and is, therefore, a parameter associated with a dynamic process (*7*).

Although many studies have already pointed out that ORP can be used as an indication of biological treatment efficiency and water quality, little work of relevance has been done on monitoring soil or sediment biofilm with ORP measurements (*8*−*10*). The reasons are that traditional monitoring techniques are still based on the laboratory analysis of representative field-collected samples: they require considerable efforts, the sample ORP may change before analysis, and the results are often not available in due time to allow on-line updating of the process controller. Furthermore, unfortunately, most of these macroelectrodes are relatively large in size, on the order of 1−3 cm in diameter. They can be used to monitor bulk liquid concentrations when there is sufficient volume to wet the electrode contacts, but they are often inappropriate for measurements in small volumes of liquids or in soils. Also, their size makes it impossible to make spatial measurements over small distances, as needed for biofilm monitoring. Because of these limitations, the continuous surveillance of hazardous areas is not possible. Remediation of Superfund and other hazardous waste sites, particularly those using bioremediation techniques, requires significant use of monitoring procedures. Rapid information feedback during waste site remediation for real time in situ monitoring capabilities is essential for the sustainable management of soils. The development of a fast, accurate, and robust in situ microscale monitoring technique, which can cope with these problems, would be highly desirable. From these, microscale in situ measurements of ORP at a specific locations where biodegradation of toxic organics is occurring in soil, biofilm, sediments, or other systems can be used to explain many of the complex reactions that take place in the system being tested because many chemical or biological reactions are correlated with the ORP−time relationship.

Microelectrodes have recently been proposed as an alternative for the measurement of ORP in extremely small volumes and for the determination of ORP gradients over micrometer distances. ORP microelectrodes with tip diameters of 1−10 μm have been utilized for environmental measurements in biofilms, in activated sludge floc particles, in soil columns, and in gel beads containing immobilized bacteria (*7*, *11*, *12*), but they are fragile and susceptible to electrical interference. In addition, they are difficult to manufacture and operate, limiting their use to specialized laboratories under highly controlled conditions. They are

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usually subject to electrical interferences because of the small signals and the length of necessary cable connecting the microelectrode to the signal processor. Typically, measurements must be done inside a well-grounded Faraday cage. This generally negates their use in the environment.

The objective of this research was to develop a new generation of microelectrodes to overcome the shortcomings of the conventional electrode systems mentioned previously. We are developing a suite of self-contained integrated microelectrodes intended to be used in the environment, such as at Superfund sites, to monitor ORP in contaminated soils and sediments. These devices consist of a microelectrode array intimately connected to an integrated circuit (IC) sensing stage and a signal processor. Finally, they can be connected to a transmitter to telemeter the information to a conventional data storage device. This paper presents detailed information on the electrochemical performance of these ORP microelectrode arrays (detailed fabrication is given elsewhere (*13*)), including comparison of the new ORP microsensor with conventional microelectrodes and commercial millielectrodes.

Materials and Methods

Generally, ORP measurements are based on the potential difference measured between a working electrode made of an inert metal (platinum or gold) and a reference (Ag/AgCl) electrode. In this study, we made two kinds of working microelectrodes, namely, a conventional microelectrode (designated as *conv*) and an integrated microelectrode (designated as *integ*) (Figure 1 b). The former was patterned after an ORP microelectrode described by Yu (*12*). The latter are a new breed of microelectrode arrays. An Ag/AgCl millielectrode (Microelectrodes Inc., Catalog No. MI-409) was used as a reference electrode. A 3 M KCl solution was used as the electrolyte solution in the reference electrode. Each set of electrodes (combination of working and reference electrodes) was connected to an Accumet Microprocessor Model 15 pH/mV meter (Fisher, Catalog No. 13-635-15A). The responses of the two ORP microelectrodes were also compared with that of a commercial combined redox potential millielectrode with an internal Ag/AgCl reference electrode (Microelectrodes Inc., Catalog No. MI-4156), which is designated as *com* in this study (Figure 1b). These electrodes were in turn immersed in each of the redox standard solutions.

Fabricating Conventional Redox Potential Microelectrodes. The conventional ORP microelectrode is a solid-state electrode made from a platinum wire melted into a lead glass micropipet with a tip diameter of 10 μm. The construction method consists of the following major steps: (a) pulling a glass micropipet (World Precision Instrument, No. PG10150-4) with a 1.50 mm o.d., 0.75 mm i.d., and 10 cm length using a small burner; (b) etching the platinum wire (Aldrich, No. 26 716-3), which is originally 0.127 mm o.d. and 99.99% pure; (c) inserting the etched platinum wire into the micropipet; (d) melting the glass to coat the tip of the platinum wire; (e) beveling the tip to expose the platinum wire surface at the tip using a beveller (Sutter Instrument Co., Catalog No. BV-10); and (f) assembling the microelectrode to connect the platinum wire and the electric wire connected to a BNC cable.

Fabricating Integrated Redox Potential Microelectrodes. The new integrated ORP microelectrode is fabricated from 175 μm thick borosilicate glass wafers. The process has four major steps: dicing, etching, metallization, and packaging. The device is schematically illustrated in Figure 1a.

Dicing. A dicing saw is used to form glass probes by cutting at 900 μm center-to-center spacing with a 10 millithick, 45 μm diamond grit resinoid blade. The diced probes are cleaned in a 7:3 mixture (v/v) of H_2SO_4/H_2O_2 and annealed at 550 °C for 10 min to relieve stress.

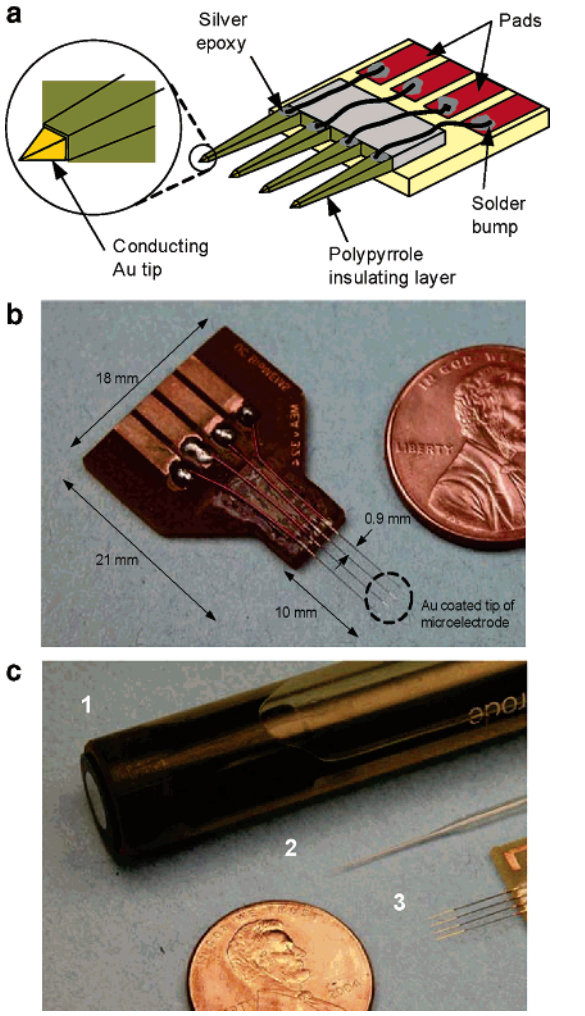

FIGURE 1. (a) Schematic diagram of the ORP microelectrode array packaged on printed circuit board (PCB) carrier; (b) fabricated and packaged microelectrode array; (c) comparison of electrode tip dimensions: commercial millielectrode named *com* (1), conventional microelectrode named *conv* (2), and integrated microelectrode array named *integ* (3).

Etching. The probes are transformed into the sharpened microelectrodes using an HF-based meniscus etching process first reported in 1984 by Turner (*14*) and recently applied by us to the fabrication of optic probes (*15*). The process uses an organic layer (e.g., vegetable oil) to modify the contact angle at the galss−etchant interface. In the first step of the etching process, the diced probes are immersed into a 10:7:33 solution (v/v/v) of $HF/HNO_3/H_2O$ for approximately 20 min at room temperature with 250 rpm agitation followed by a gradual withdrawal for 18 min to produce a taper terminating in 20 μm square microelectrode tips. In the second etch step, a 1 mm length of the tapered probe is immersed into the same etchant for further sharpening using meniscus etching at room temperature without agitation. Following etching, microelectrodes are cleaned with a 7:3 mixture (v/v) of H_2SO_4/H_2O_2.

Metallization. A 30 nm thick layer of Cr as a seed layer and 200 nm thick layer of Au as a conductive layer are deposited by evaporation to metalize individual microelectrodes. The gold sensing layer at the tip is formed by coating approximately 0.5−1 mm of the tip with low melting-temperature paraffin at 62 °C. An approximately 2 μm thick layer of polypyrrole is electrodeposited on the microelectrodes as a protection layer. The gold gives more reliable measurement of ORP than platinum for this application. It

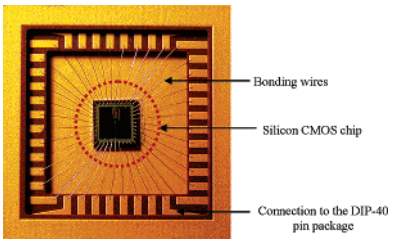

FIGURE 2. Picture of the CMOS chip packaged in a 40-pin Dual Inline Package (DIP).

was reported that platinum may catalyze some additional reactions at its surface (*16*) and is more subject to coating than gold. Polypyrrole electrodeposition is performed using a 150 mL aqueous solution of oxalic acid (2.7 g) and pyrrole (2.07 mL) as electrolyte and two stainless steel plates (3 × 5 cm) as cathodes (counter electrodes). The current density was 6 mA/cm^2. Following electrodeposition, the gold-sensing layer was exposed by dissolving paraffin in Opticlear (National Diagnostics, Inc.).

Packaging. In the final step, the microelectrodes are fixed to a printed circuit board (PCB) carrier for simpler handling and electrical connection. Conductive silver epoxy (Ablebond8700E, Emerson and Cuming) and conventional solder are used to establish electrical connections to individual microelectrodes.

Circuit Design and Measurement Method for Integrated Redox Potential Probes. The ability to perform an ORP measurement outside of the controlled laboratory environment of a well-grounded Faraday Cage is based in part on the integration of the microelectrodes with a Complementary Metal Oxide Semiconductor (CMOS) based integrated circuit (IC). The CMOS chip has been developed to perform both signal acquisition and processing. A number of circuit design and packaging features has been exploited to reduce electronic noise that could have a deleterious impact on the ORP measurement system. First, by reducing the signal detection and processing electronics to a single chip that can be connected to the microelectrodes with a short-shielded cable, it is possible to significantly reduce the amount of wiring that can act as a noise collecting antenna. Second, the input stage of the signal detection circuit inherently provides a low pass filtering operation that removes high-frequency noise from the signal before it passed through the amplification stages. Finally, common mode rejection capabilities associated with the use of differential amplification stages are employed to cancel out electronic noise that may have been coupled into the signal carrying traces on the CMOS chip.

The chip was designed using standard VLSI circuit layout tools and a mixed signal standard cell library available from Tanner Research, Inc. The chip was fabricated using the AMI 1.5 μm CMOS process that is available through the MOSIS foundry service. Figure 2 shows a photograph of the fabricated IC. The central part of the figure consists of a silicon CMOS chip connected to the external package with gold bonding wires. The package provides the connection to the external measurement domain.

As a design verification step, the circuit implemented in the IC was simulated using the analogue circuit simulation tool Simulation Program for Integrated Circuits Emphasis (SPICE). Comparison between simulation results and experimental results obtained by testing the fabricated chip indicated that the IC is operating as designed.

Figure 3 shows a block diagram of the measurement set up used to perform the benchtop experiments outside the Faraday cage. The arrows in the figure indicate the direction of the signal flow. The input signal from the probes is fed into the chip. At the same time, the signal is measured using

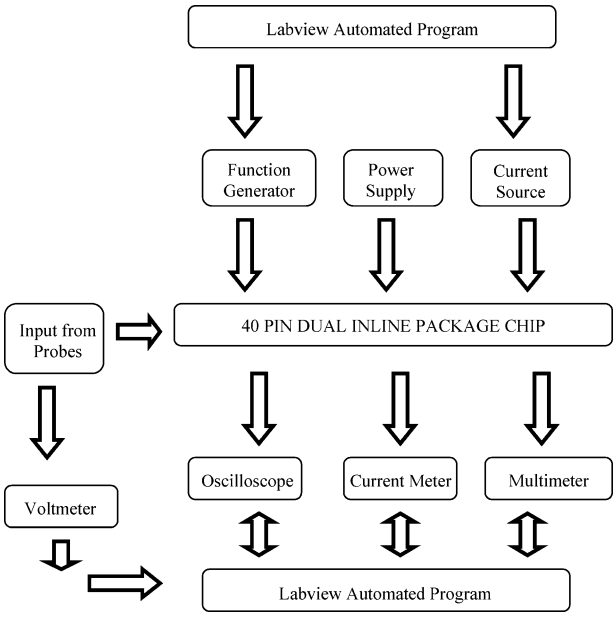

FIGURE 3. System level representation of the measurement setup.

a voltmeter. The characterization of the circuit is performed using the signal from the function generator and current source. The power supply to the chip is provided using 0−5 V and variable bias voltages needed to drive the circuitry. The output from the circuit is monitored by an oscilloscope, current meter, and a multimeter. All the output is monitored using the LABVIEW automated measurement software program that controls/monitors chip readings.

Characterizing the Redox Probes. Four calibration redox solutions, including a ferrous−ferric (FF) standard solution and pH 4, 7, and 10 buffer reference solutions saturated with quinhydrone (Aldrich, 28 296-0) were used to investigate the performance of the redox probes. When coupled with a Ag/AgCl reference electrode, redox potentials for the pH 7 and 4 reference solutions, as recommended by the ASTM (*17*), should be 92 and 268 mV, respectively, at 20 °C and 86 and 263 mV, respectively, at 25 °C. If the signal readings for the standard solutions showed them to be out of range (more than ±10 mV from the known potential values), the microelectrodes (integrated and conventional) made in our laboratory were discarded. Probes were rinsed with distilled water after use and stored on the shelf. The reference solutions saturated with quinhydrone are susceptible to air oxidation, so these reference solutions were prepared freshly daily before each use.

Results and Discussion

Integrated ORP Microelectrode Array (MEA). Potentiometric integrated microelectrode arrays for ORP measurements were successfully fabricated as shown in Figure 1b. The new microelectrode arrays consist of four 1 cm long microelectrodes at 900 μm center-to-center spacing. The size of the microelectrode tips was approximately 200 nm square, with a taper angle of 20° and a length of 56.7 μm. Approximately 0.5−1 mm length of the gold-coated tip was exposed. Integrated ORP microelectrodes with these dimensions are suitable for making environmental measurements in biofilms, in activated sludge floc particles, in soil columns, and in gel beads containing immobilized bacteria. Further, the four probe array permits increased reliability of measurements as data from each of the four microelectrodes can be recorded simultaneously as either individual measurements or averaged into a single measurement. Although meniscus etching has been used previously with only with single probes (*14*),

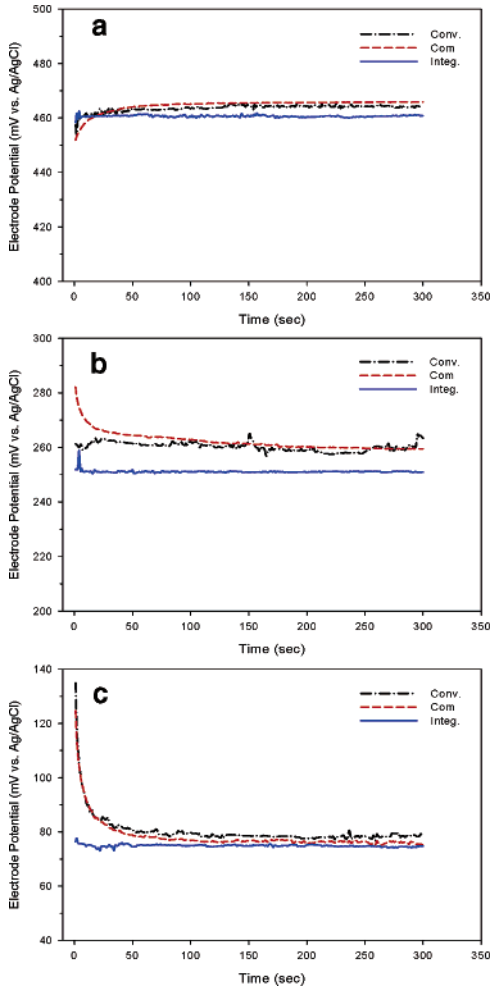

FIGURE 4. Comparison of performance of *conv*, *com*, and *integ* probes for a 5 min operation in FF standard solution (a), pH 4 (b), and pH 7 (c) reference solutions.

the results presented herein, and our recent work with cantilevered glass probes (*5*, *18*), are the first demonstrations of meniscus etching for fabrication of arrays of tapered glass probes. Figure 1c compares microelectrode array tips (integrated probe, *integ*) demonstrated in this work with the tips of a commercially available millielectrode (*com*) and a conventional microelectrode (*conv*). To optimize electrode fabrication, we fully characterized the roughness of vertical and horizontal surfaces with SEM and AFM and determined the vertical and horizontal etch rates, which were found to be 2.31 and 2.54 μm/min, respectively (data not shown). This information was important to the microelectrode array fabrication since a square tip at the end of the first etch-step was required to produce a symmetrical submicrometer tip.

Characterizing Performance of the Integrated ORP Microelectrode Arrays. It is shown in Figure 4 that the response times (the time for the electrode to reach 99% of the final stable reading) for the integrated redox potential probe were less than a few milliseconds for the ferrous−ferric (FF) standard solution, approximately 10 s for the pH 4 quinhydrone reference solution, and less than 30 s for the pH 7 quinhydrone reference solution. The longer response time for the pH 7 quinhydrone reference solution is partially due to the fact that the number of the final stable reading (74.91 ± 0.41 mV) is small as compared to that of the FF standard solution (460.68 ± 0.31 mV). Under the same conditions, the response times for the commercial millielectrode were 2 min for the FF standard solution, about 5 min for the pH 4 quinhydrone reference solution, and more

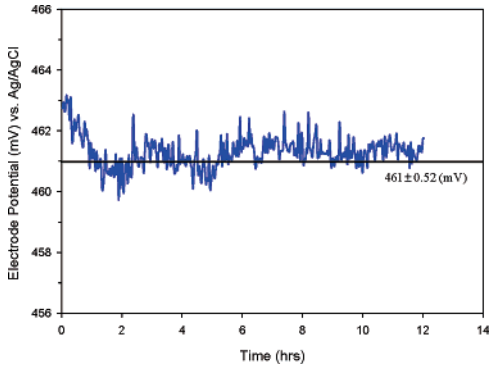

FIGURE 5. Performance of integrated microelectrode for 12 h measurement in FF solution.

than 10 min for the pH 7 quinhydrone reference solution. Therefore, the time for the redox potential microelectrode to reach 90% of the final stable reading was used. The time for the commercial millielectrode to reach 90% of the final stable reading for pH 7 quinhydrone reference solution was 2 min. Under the same conditions, the response times for the integrated microelectrode were only a few seconds. Therefore, these results indicate that the integrated redox potential microelectrode has a much shorter response time than either the conventional microelectrode or the commercial millielectrode.

Figure 4 also indicates that prior readings of integrated microelectrode, as compared to the both commercial electrode and conventional microelectrode, have no effect on the reading of the following sample. It was found that the gold sensing layer at the tip coated by low melting-temperature paraffin at 62 °C was inert enough that the microelectrode has no memory of the redox potential measured previously. This is also an important check to determine whether the ORP microelectrode is good (*19*).

To check the stability of the integrate microprobe for long time measurements, the FF standard solution was used since the quinhydrone reference solutions are not stable for more than a few hours. The profiles of the integrated redox potential in Figure 5 reveal that the measured ORP decreased gradually but showed a stable potential response with only a slight fluctuation (±1 mV) after 3 h. The most common problem reported with regard to ORP determination in environmental aqueous samples is that readings can differ by a significant margin (50−100 mV), even though the sensors are in the same solution. Here, though, the integrated ORP probe gave a very stable voltage response (the corresponding rate of the integrated microelectrode potential change was in the range of only 0.6−1.1 mV/min) even when the measurement was carried out outside the Faraday cage where signals could have been inhibited by external factors such as static electricity or vibrations. The integrated probe had a response voltage in the range of 461 ± 0.52 mV.

After selecting the stable probes, the following experiment was conducted. Unlike dissolved oxygen and other ion-specific electrodes that measure a current or potential that is proportional to the concentration of the chemical species in a solution, an ORP electrode only measures directly the potential (in millivolts) of the solution itself (a single point calibration). Thus, an ORP electrode merely measures the ratio of oxidized to reduced forms of all chemical species in solution. Therefore, an ORP electrode or microelectrode cannot be calibrated in the conventional meaning, like for pH measurement. However, it is standard practice to check the electrode response against standard and reference redox potential solutions for proper operation. In this study, to evaluate whether the fabrication procedure produces a good redox potential probe, the response of the redox potential

TABLE 1. Nominal Redox Potentials of the Standard or Reference Solutions and Measured Redox Potentials by the Redox Potential Microelectrodes and Commercial Redox Potential Millielectrode with Ag/AgCl Reference Electrode (3 M KCl)

	nominal redox potential[a] (mV)			measured redox potential[b] (mV)		
redox standard or reference solution	20 °C	23 °C	25 °C	conventional microelectrode	commercial millielectrdoe	integrated microelectrode
ferrous−ferric standard solution			463	463.62 ± 1.22	464.69 ± 2.15	460.68 ± 0.31
pH 4 quinhydrone reference solution	268	265	263	260.26 ± 1.55	262.20 ± 3.19	251.10 ± 0.49
pH 7 quinhydrone reference solution	92	88.4	86	80.20 ± 5.56	78.30 ± 3.94	74.91 ± 0.41

[a] Compiled from ASTM D1498-93. The values for 23 °C and 3 M KCl are derived by interpolation from Tables 2 and 3 in that document. ASTM (D1498-93) suggests two redox reference solutions: pH 4 and 7 quinhydrone reference solutions. [b] Each value of the measured redox potential is the average of the electrode potential readings within 1% of the final potential reading of that electrode. Ag/AgCl reference electrode with 3 M KCl was used during the calibrations. The temperature during the calibrations was 23 °C.

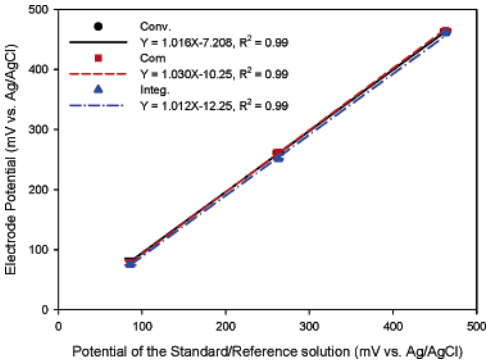

FIGURE 6. Redox potential readings against the ferrous−ferric standard solution (FF) or pH 4 quinhydrone (pH 4) and pH 7 quinhydrone (pH 7) reference solutions.

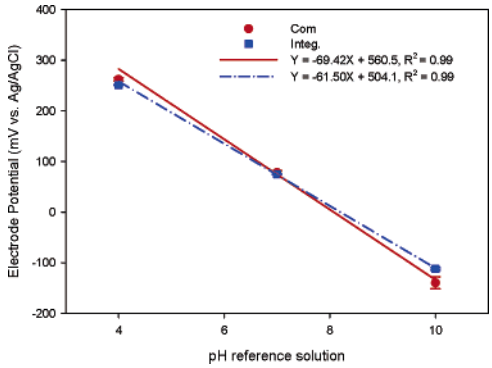

FIGURE 7. Performance of the integrated microelectrode and commercial redox potential millielectrode in FF solution with a change of pH.

microelectrode was first checked against three redox potential standard or reference solutions and then compared with the responses of both the conventional microelectrode and the commercial millielectrode. As shown in Table 1, the nominal redox potential of the FF standard solution with an Ag/AgCl reference electrode containing 3 M KCl at 25 °C is 463 mV. The nominal redox potentials of pH 4 and 7 quinhydrone reference solutions with a Ag/AgCl reference electrode at 25 °C are 263 and 86 mV, respectively. At 23 °C, these values should be slightly (approximately 1−2 mV) higher. The measured redox potentials of the integrated redox potential probe with respect to the Ag/AgCl reference electrode and 3 M KCl at 23 °C were 460.68 ± 0.31 mV for FF standard solution, 251.10 ± 0.49 mV for the pH 4 quinhydrone reference solution, and 74.91 ± 0.418 mV for the pH 7 quinhydrone reference solution, respectively. The measured ORPs using the three kinds of redox potential probes were typically slightly lower than those of the nominal redox potential. ASTM suggests that the measured redox potentials should be within 10 mV of the nominal redox potentials for a good redox electrode (*17*). Thus, all of the measurements should be deemed acceptable.

There are two ways to calibrate an ORP-measuring device. The two-point calibration procedure is the recommended procedure to set the slope of the ORP electrode, but sometimes it may be necessary to perform a single point standardization. Figure 6 shows the standardization curves for the three ORP probes against three redox standard or reference solutions. The slopes of the three curves (1.016 for the conventional microelectrode, 1.030 for the commercial millielectrode, and 1.012 for the integrated probe) are quite close to the theoretical value of 1.00. The comparison shows that the integrated microelectrode has the same or more accurate readings than the other two electrodes.

ASTM (*17*) suggests two redox reference solutions (pH 4 and 7 quinhydrone reference solutions); no redox reference solution with negative potential has been established, probably due to problems associated with air oxidation. Other chemicals for negative potential, such as sodium thioglycolate, have been suggested but have not been standardized (*20*). In this study, the reference solutions contain solid quinhydrone which, when added to the supplied buffers, yield three solutions (pH 4, 7, and 10) with well-defined, but different, ORP values. As shown in Figure 7, the ORP value was found to be correlated to the logarithm of the hydrogen concentration with a linear relationship. Error bars represent the standard deviation for data collected over the application times. The integrated ORP probe showed a log-linear ORP response down to a hydrogen concentration of 10^{-10} M. The redox potential of the integrated ORP probe and the commercial millielectrode were in the negative ORP range of −112.25 ± 2.4 and −139.5 ± 11.87 mV in a pH 10 reference solution, respectively. The redox potentials of the two reference solutions (pH 4 and 7 quinhydrone reference solutions) are listed in Table 1. Pang and Zhang (*19*) reported that the ideal slope (Δredox potential/ΔpH unit) calculated from these reference solutions was 59 mV/pH unit. The slope of the integrated microelectrode (61.5 mV/pH) was close to the ideal slope.

The effect of mixing on ORP measurements was also investigated. The experiment was carried out by sequentially inserting the integrated microelectrode into standard ORP solutions using five different stirring velocities. As shown in Figure 8, the redox potential profile exhibited a trend of a very gradual decrease, as the stirring intensity increased. The slightly unstable potential profile between 300 to ~500 rpm occurred when the stirring bar began bumping the beaker wall. Even with this additional turbulence, the measured ORP variability was less than 1 mV. Thus, it can be concluded that the signal was not influenced by stirring.

Temperature changes can cause variations in ORP measurements. This factor definitely needs to be taken into account for calibration and should be considered when reporting ORP values. The ORP measurement is

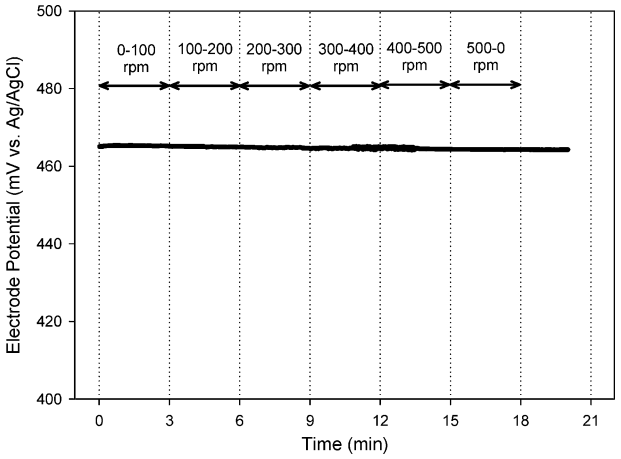

FIGURE 8. Performance of integrated microelectrode in FF solution with a change in mixing intensity.

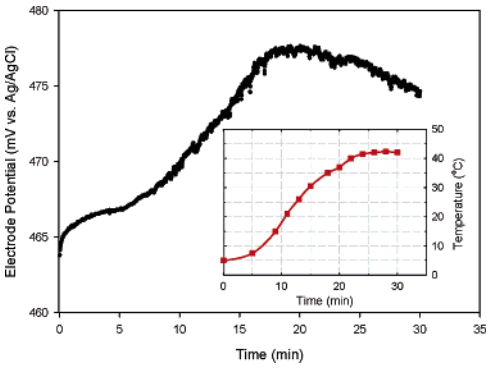

FIGURE 9. Performance of integrated microelectrode in FF solution with a variation in temperature.

governed by the Nernst equation

$$E = E^{o} + 2.3\frac{RT}{nF}\left(\log\frac{A_{ox}}{A_{red}}\right) \quad (1)$$

where E is the potential developed at the metal electrode surface coupled with an Ag/AgCl reference electrode (mV); E^{o} is the constant dependent on the reference electrode (mV); R is the universal gas constant; T is the absolute temperature in degrees Kelvin (K); n is the number of electrons involved in the equilibrium between the oxidized and reduced species; F is the Faraday constant (96 500 C); A_{ox} is the activity of the oxidized species; and A_{red} is the activity of the reduced species. As can be seen from examination of the Nernst equation, the ORP is dependent on temperature. The temperature of the FF standard solution for which the integrated ORP probe was determined was found to slightly affect the voltage output of the probe. It is shown in Figure 9 that the ORP changes were proportional to the increased temperature. In contrast, ORP profiles obtained under the steady or increasing temperature between 20 and 30 min showed a smaller ORP change (less than 2 mV). One possible reason was that the properties of the FF standard solution might be changed due to a higher temperature (35 °C). It was reported that increasing temperature affects ion activity, such as activity coefficients and interactions between ions in solution (*16*). Between 5 and 40 °C, the mean changes in the signal per °C were less than 3%. This result indicates that, although the effect of temperature on the integrated ORP probe is small, neglecting to record the temperature with every ORP measurement might lead to error and lack of reproducibility.

Potential Application of Integrated ORP Microelectrode Array. As described in Standard Methods for the Examination of Water and Wastewater (Section 2580 B) (*21*), the oxidation−reduction potential (ORP) is a potentiometric measurement of the tendency of a given system to donate or receive electrons (i.e., to become oxidized or reduced). Although some pioneering studies have already pointed out that ORP measurements can be powerfully used to control the aeration in biological treatment process and water quality monitoring, up to now, few reports can be found on the application of ORP electrode for the monitoring of soil or sediment pore water, groundwater, and other systems.

To date, many of these measurements have been made using traditional chemical electrodes that are relatively large, on the order of 1−3 cm in diameter. These large electrodes can be used to monitor bulk liquid concentrations when there is sufficient volume to wet the electrode contacts, but they are often inappropriate for measurements in small volumes of liquids or in soils. Further, their size makes it impossible to make spatial measurements over small distances, as needed for biofilm monitoring. It was reported that using separate reference and working microelectrodes was good for laboratory measurements and relatively easy to fabricate (*7*). However, the drawback of conventional separate microelectrodes is that their use requires good shielding and grounding systems to minimize electrical interference. To overcome the shortcomings of the conventional microelectrode systems described previously, a new redox potential microelectrode for in situ environmental monitoring has been successfully developed in this study. These new integrated microelectrodes were easier to fabricate and were more robust than the conventional microelectrodes.

The potential (or tendency) of the medium for electron transfer was sensed by a microelectrode made of an inert metal (gold) and read relative to a Ag/AgCl reference electrode that was immersed in the same standard solutions. The readout of the sensor versus the Ag/AgCl reference electrode was a voltage, with positive values indicating an oxidizing environment (ability to accept electrons) and negative values indicating a reducing environment (ability to furnish electrons). The functionality of a CMOS chip connected with the sensors was to detect small voltage changes outside of a Faraday cage by eliminating environmental and instrumental noise. The objective of building a robust system that is immune to noise is accomplished by taking care of the design issues both at the design level and at the circuit level. The input voltage signal from the microelectrode array (MEA) is fed into the low pass filter, which removes extraneous noise coupled with the signal to be measured. The filtered signal is an input to the isolation or buffer amplifier. The output signal of the buffer amplifier is sourced into a fully differential output instrumentation amplifier, which is the gain adjusting stage through a single variable resistor. This differential instrumentation amplifier is the design methodology used in minimizing the effects of environmental noise. Differential measurement rejects noise common to both the inputs. The high common mode signal rejection ratio of this amplifier improves the signal-to-noise ratio. The output voltage of the differential amplifier is passed through a buffer stage. This output voltage is a measure of ORP of the solution. The major advantage of this design is that the signal is amplified very close to the microelectrode and that the integrity of the signal is preserved. This system design approach used was able to stabilize the output signal and enhance the signal-to-noise ratio, which is one of the performance metrics in measuring low analogue signals.

The new microelectrodes were fully characterized using standard solutions and were shown to exhibit better signal stability. Moreover, the speed of ORP response time of the integrated microelectrode was sufficiently fast for rapid measurement or control. Provided that these new microelectrodes can be made robust enough to use in the

environment to evaluate in situ conditions, their accuracy, precision, freedom from electrical interference problems, and very small size could lead to the development of a major new monitoring technique.

With further development, it may be possible to use the new integrated microelectrode to obtain direct information from measurements inside heterogeneous biological systems (i.e., the distribution of organisms and kinetic parameters). In addition, remediation of Superfund and other hazardous waste sites, particularly those using bioremediation techniques, requires the significant use of monitoring procedures. This is necessary to ensure that environmental conditions required for bioremediation of specific toxicants are present and to verify that pollutant removal is occurring. In most cases, measurements made on samples extracted from the site are not acceptable. Microscale in situ measurements of various constituents in aqueous and soil environments are essential for proper monitoring of environmental conditions at a specific location and to determine impacts of environmental stressors. In situ monitoring is also required in laboratory reactors, both to determine existing environmental conditions and to properly control them.

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Note Added after ASAP Publication

Due to a processing error the labels were missing from panels A−C in Figure 1 in the version published ASAP July 16, 2005; the corrected version was published ASAP July 26, 2005.

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