

Fabrication of Anodically Electrodeposited Iridium Oxide Film pH Microelectrodes for Microenvironmental Studies

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A new method for fabrication of anodically electrodeposited iridium oxide film pH microelectrodes has been developed in this study. Novel for its tip size (3–10- μ m tip diameter), the microelectrode is fabricated in a tapered glass micropipet filled with a low melting point alloy. The tapered end is recessed and platinized. Thereafter, iridium oxide is electrodeposited over the platinized end in the recessed part. The microelectrode has a very short response time ($t_{80} < 5$ s) in the pH range of 0–12 with an accuracy of 0.05 pH unit. The pH microelectrode is not affected by most ions and complexing agents of relevance in environmental and biological studies; it can be used in fluids over wide ranges of stirring speeds (0–55 rpm) and temperatures (~ 5 –40 °C). Redox agents such as dissolved oxygen and hydrogen peroxide have no effect on the pH response while quinhydrone, ferro- and ferricyanide, and sodium sulfide have marked effects. However, the microelectrode can still be used in any sample when calibration is done in standards having similar redox characteristics.

Microelectrodes are powerful tools for microscale measurements in various fields of physiology, medicine, microbial ecology, and environmental studies. In most microscale studies, pH is a very important parameter to characterize any microenvironment or to explain complex reactions taking place in a particular microenvironment. The increasing use of pH microelectrodes as a research tool has led to various technical developments for fabrication of pH microelectrodes.^{1–11}

There are three types of commercially available pH sensors,¹² viz., glass membrane electrodes, liquid ion-exchanger electrodes, and solid-state electrodes; pH microelectrodes are fabricated based on the principles involved in these commercial electrodes. pH sensing glass membrane microelectrodes have rapid ion-exchange capacity and are not sensitive to redox species¹³ and, hence, are often used.^{2,5,6,12} However, they suffer from monovalent cation interferences.¹³ Moreover, the fragility of glass microelectrodes is a drawback, which restricts their use in many situations.^{2–4,14}

Liquid ion-exchanger pH microelectrodes are also quite popular among researchers because of their established procedure for construction.^{7,10,15,16} However, it is very difficult to control the thickness of the pH-sensitive membrane with the front- or back-filling method.¹⁶ Furthermore, these pH microelectrodes have very short life span (< 1 day⁷), which limits their applications.

Solid-state electrodes include, among others, metal,¹⁷ metal-oxide,^{3,4,11,13,14,18–25} carbon fiber,^{8,11} and optical fiber types.^{26,27} Metal oxide electrodes and microelectrodes show very good pH responses and are more or less free from cation interferences.¹³ Oxides such as TiO₂, RuO₂, RhO₂, SnO₂, Ta₂O₅, IrO₂, and PtO₂,¹³ PdO₃, Hg₂O and HgO,^{18,19,24} and Sb₂O₃^{19,21,24} have been investigated. Among these metal oxides, the use of iridium oxide as the pH-sensing layer (e.g., iridium oxide deposited on electronic conductors such as stainless steel, iridium metal, platinum metal,

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and carbon fiber) has been advocated.^{4,11,13,22,25,28} Reasons for this may include the fact that iridium oxide is very stable and does not undergo dissolution.^{29,30} Iridium oxide electrodes show^{25,31} (a) potential stability over a wide range of temperature and pressure in aqueous solution, (b) a wide range of pH response, (c) fast responses, and (d) the lowest sensitivity to redox interferences and stirring.

To form a pH-sensing layer on a conducting metal, iridium oxide can be deposited^{4,22,23,32} (a) thermally (thermally oxidized iridium oxide film or TOIROF), (b) by sputtering (sputtered iridium oxide film or SIROF), or (c) electrochemically. Although (anhydrous) iridium oxide pH electrodes prepared thermally and by sputtering give slopes of ~ 59 mV/pH unit,^{4,22} the electrochemical method is often used. Observations suggest that the electrochemical method can be comparatively simple with the scope of readily regulating the growth rate and thickness of the oxide film.²³ Sputtering is a less practical method because it needs expensive iridium in a relatively larger quantity.²⁵ Electrochemical methods for iridium oxide formation include (a) potential cycling and (b) direct deposition of the oxide film. Anodic iridium oxide film (AIROF) formation involves cycling iridium in sulfuric acid at potential between -0.25 and $+1.25$ V.^{4,14,23} There are three different methods for direct deposition of an iridium oxide film. Yoshino et al.³³ proposed a method to form periodically reversed electrodeposited iridium oxide film (PRIROF) using acidic sulfatoiridium [commercial $\text{Ir}_2(\text{SO}_4)_3$] solution. Yamanaka²⁸ experimented with anodically and cathodically electrodeposited iridium oxide films (AEIROF and CEIROF) using a solution containing iridium tetrachloride, hydrogen peroxide, oxalic acid, and potassium carbonate. Wipf et al.¹¹ fabricated a carbon fiber–iridium oxide (CF/IrO_x) pH microelectrode. They used 10- μm -diameter carbon fiber and electrodeposited hydrous iridium oxide onto the carbon fiber surface using a solution of Na_3IrCl_6 in HCl.

Apparently it is possible to fabricate AIROF and CF/IrO_x pH microelectrodes in the laboratory. However, we need some instruments for these two microelectrodes that may not normally be available in a regular research laboratory. Also, potentially cycled AIROF undergoes fast dissociation or change.²⁵ On the other hand, the AEIROF is very stable with very good reversibility.²⁸ In addition, no sophisticated instrumentation is needed to deposit the oxide film by the AEIROF method.

The objective of this study was to develop a new procedure to fabricate pH microelectrodes for microenvironmental studies. It was decided to develop iridium oxide pH microelectrodes due to their aforementioned advantages. The AEIROF method was used because of its relative simplicity. This paper describes the processes of AEIROF pH microelectrode construction and its evaluation and discusses the possible mechanism involved.

AEIROF pH Microelectrode Construction. (a) Iridium Oxide Deposition Solution. The deposition solution for AEIROF was prepared as follows:^{25,28} (a) iridium chloride hydrate ($\text{IrCl}_4 \cdot$

H_2O , 0.075 g, Aldrich, Catalog No. 33,448-0) was dissolved into 50 mL of water in a 140-mL borosilicate beaker. The solution was stirred magnetically for 30 min; (b) 0.5 mL of aqueous hydrogen peroxide (30 wt %, VWR, Catalog No. VW3742-1) was added to the solution and stirred for 10 min; (c) 0.250 g of oxalic acid [$(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$, Aldrich, Catalog No. 24,753-7] was added to the solution and stirred again for another 10 min; (d) small amounts of Na_2CO_3 (Fisher, Catalog No. S263-500) were gradually added to the stirred solution to adjust its pH to 10.5 (others^{25,28} used anhydrous K_2CO_3); (e) the resulting yellow solution was covered and left standing at room temperature ($23 \pm 1^\circ\text{C}$) for at least 2 days to stabilize it. The solution changed from yellow to light violet and finally became blue. The solution was stored in a transparent borosilicate bottle at room temperature. During this study, the same deposition solution had been used up to three months after its preparation.

(b) Supplies and Equipment. All chemicals used in this study met American Chemical Society (ACS) requirements and were used as received unless otherwise specified. Aqueous solutions were prepared with Nanopure water (Series 550; Barnstead/Thermolyne Corp., Dubuque, IA). Universal pH buffers were used (buffers for pH 4, 7, and 10 from Corning, NY, and 5 and 9 from VWR Scientific, West Chester, PA). A glass pH electrode (Orion, Catalog No. 910600) and an oxidation–reduction potential probe (Orion, Catalog No. 9778BN) were used in combination with pH/ion meters (Model Accumet 925, Fisher Scientific, Pittsburgh, PA).

(c) Microelectrode Measurement System. Potential (mV) measurements were done using a chemical microsensor (model II, Diamond General, Ann Arbor, MI) inside a Faraday cage (Technical Measuring Corp., Peabody, MA). The pH microelectrode was bound together with a custom-made Ag/AgCl microelectrode (3 M KCl filling solution saturated with AgCl and solidified with 2% agar; tip diameter ≤ 10 μm) using plastic adhesive tapes and positioned in the test solution using a micromanipulator (World Precision Instrument, Sarasota, FL). In this paper, whenever the potential of iridium oxide microelectrode is referred to, it is always with respect to the Ag/AgCl microelectrode. The measurement system was connected to a ground line separated from the building grounding. In this study, a microelectrode potential reading is considered stable if the value did not change by >0.2 mV in 1 min.

(d) pH Microelectrode Fabrication. The new construction method consists of the following major steps.

(1) *Pulling Glass Pipets.* Single-barreled uncleaned and untreated borosilicate glass tubes (Sutter Instrument Co., Novato, CA, Catalog No. B150-86-15) with o.d. 1.50 mm and i.d. 0.86 mm are pulled to make the micropipets using a Sutter Instrument's micropipet puller (model P-30, Sutter Instrument Co.) with a heating index of 999 and a pulling index of 500. The tips of the pulled micropipets are broken using a forceps to get an outer tip diameter of 3–10 μm . Pipets with tip diameters of >10 μm are discarded.

(2) *Filling the Micropipet with Low Melting Point Alloy.*³⁴ A low melting point alloy (LMA, 44.7% Bi, 22.6% Pb, 19.1% In, 8.3% Sn, and 5.3% Cd; Belmont 2451, Belmont Metals Inc., Brooklyn, NY) wire (3–4 cm long) is inserted into the glass micropipet. The LMA

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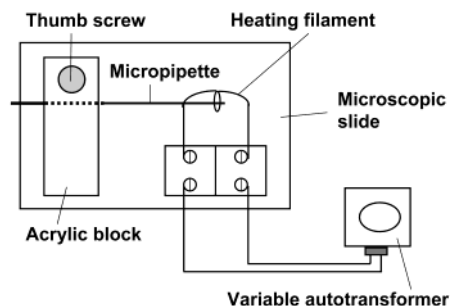


Figure 1. Schematic of the arrangement for filling LMA into the micropipet. Reproduced (with permission from the American Chemical Society, copyright 1998) from Pang and Zhang.³⁴

wire is made by sucking the molten alloy into a silicone tubing (Cole-Parmer, Vernon Hills, IL, Catalog No. 06411-60) of an inner diameter slightly smaller than that of the glass micropipet and then scraping the tubing off as the alloy solidifies. The micropipet is inserted into a loop of Nichrome heating filament (Figure 1), and the head of the alloy wire is then melted by applying a voltage of 0.5 V dc, supplied by a variable autotransformer (model 3 PN 1010, Staco Energy Products Co., Dayton, OH). Once the alloy is molten, the wire is pushed from the other end with a metal plunger to fill the alloy completely to the tip of the micropipet. The whole operation is conducted under a microscope.

(3) *Creating a Recess.* The other end of the LMA is then melted, and an insulated copper wire with a metal connector tip is pushed in for electrical connections. The alloy-filled tip of the micropipet is then electrocorroded for ~15 min to create a recess of ~15–25 μm as per the method described elsewhere.³⁵ The electrocorrosion unit consists of a custom-made 3-V battery-operated dc power source and a 40-mL borosilicate beaker filled with 2 M acidified ZnCl_2 . The LMA filled micropipet is connected to the positive side of the power supply, and the negative goes to a Nichrome wire. The recessed micropipet is kept immersed in water for some time to remove any residual corrosion solution.

(4) *Depositing Platinum.* The recess of the micropipet is filled completely with platinum using the electrodeposition method described by Pang and Zhang.³⁴ A platinum wire (0.076-mm diameter, 99.99% purity, Aldrich Catalog No. 35,737–5) is used as the anode (Figure 2). The plating solution is prepared by mixing 2 mL of 10% hydrogen chloroplatinate ($\text{H}_2\text{PtCl}_6 \cdot 4\text{H}_2\text{O}$, platinic chloride, Sigma, Catalog No. P-5775) and 0.2 mL of 1% lead acetate [$(\text{C}_2\text{H}_3\text{O}_2)_2\text{Pb} \cdot 3\text{H}_2\text{O}$, Sigma, Catalog No. L-3396] with 18 mL of water. A plating current of 0.3–0.6 μA at 0.5–0.8 V is applied from a dc power source (model 3015, Protek, Cole-Parmer, Chicago, IL) coupled with a picoammeter (model 485, Keithley Instruments, Cleveland, OH). The deposition process normally took ~15–20 min. The micropipet tip is washed with water to remove the attached plating solution and is air-dried before the next operation is performed.

(5) *Anodic Deposition of Iridium Oxide.* Electrodeposition of iridium oxide is done in a setup similar to that used for platinum deposition (Figure 2). About 2 mL of the deposition solution is used with additional drops added during the course of the deposition process. The deposition solution is discarded after the deposition on one microelectrode is completed; fresh solution is

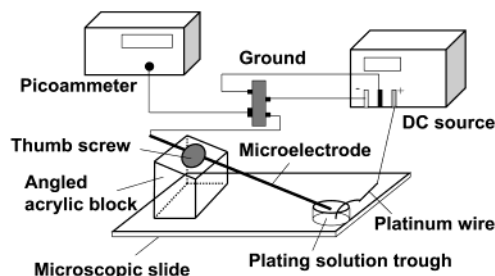


Figure 2. Schematic of the platinum plating arrangement. The same arrangement is used for anodic deposition of iridium oxide onto the plated platinum by reversing the connections (i.e., the micropipet used as the anode and the platinum wire as the cathode). Reproduced (with permission from the American Chemical Society, copyright 1998) from Pang and Zhang.³⁴

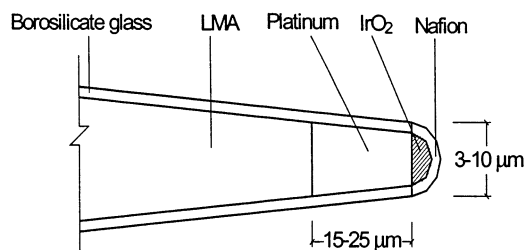


Figure 3. Schematic of the tip of an AEIROF microelectrode. The exact shape and thickness of the IrO_2 and the Nafion layers are not known. The diagram is not to scale.

added for the next microelectrode. The platinum wire is used as the cathode. A current of 0.03–0.13 pA (current density of 0.0002–0.0004 mA/cm^2) at 0.3–0.5 V is used for ~20 min. Once the deposition is complete, the microelectrode tip is washed with water and air-dried. Then the tip of the microelectrode is coated with proton-exchanging Nafion resin (perfluorosulfonic acid ion-exchange resin³²) by dipping it into a 5% Nafion solution (Aldrich, Catalog No. 27,470-4) for a few seconds; the resin is air-dried for ~10–20 min. The microelectrodes (Figure 3) are cured in pH 7 buffer for 2 days to stabilize the potential reading.²⁵

Characterization of Microelectrode pH Responses. (a)

Calibration. The pH microelectrodes were calibrated using pH buffers 4, 5, 7, 9, and 10 at room temperature under normal atmospheric pressure. Typically, a slope slightly higher than ~60 mV/pH was observed for freshly prepared microelectrodes after 2 days of curing in pH 7 buffer. It should be noted that, to be consistent with others, throughout this paper the negative slope of the Nernstian curve is reported as positive. Slopes of 59.6–74,¹⁴ 81.9,²³ and 70–80 mV/pH⁴ have been reported for AIROF macro- and microelectrodes. Marzouk et al.²⁵ obtained a slope of ~64 mV/pH for the AEIROF macroelectrode. In this study, any slope in the range of 50–65 mV/pH was considered good for our AEIROF pH microelectrodes provided the correlation coefficient (R^2) of the calibration curve was found satisfactory (≥ 0.96).

(b) Effective pH Range and Comparison with Glass pH Electrode. To establish its pH response range, the new AEIROF microelectrode together with a conventional glass pH electrode was positioned in a 140-mL borosilicate beaker with 100 mL of water. To get acidic or alkaline pH, 0.1 N HNO_3 or 0.1 N KOH was added dropwise to the beaker, and the solution was stirred continuously with a magnetic stirrer. The measured pH range

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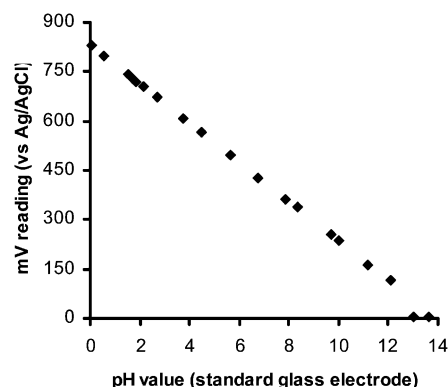


Figure 4. Plot of mV readings from an AEIROF pH microelectrode vs pH measured with a standard glass pH electrode. The test solution was Nanopure water, and the pH values were changed with 0.1 N HNO_3 and 0.1 N KOH.

(from the glass electrode) was between 0.04 and 13.63 (Figure 4).

To find out the optimal pH range for the new pH microelectrode, the best-fit lines were drawn for different ranges of pH–mV values. The best-fit line ($R^2 = 1.00$) was found for the pH range of approximately 0–12 (Figure 4). When we tried to fit a line for all the measured points, the R^2 decreased to 0.99. The difference in pH measured by the new iridium oxide microelectrode and that by a standard glass electrode was always ≤ 0.05 unit when measured pH was within 12. However, for the readings taken beyond pH 12, the difference was 0.28–0.90 unit. On the basis of these observations, we inferred that the effective range for our new pH microelectrode is 0–12. This is an improvement over the range of 2–10 reported for similar AEIROF macroelectrodes²⁵ and 2–12 reported for CF/ IrO_x microelectrodes.¹¹ Van-Houdt and Lewandowski⁴ reported a pH range of 1–12 in buffer solutions using AIROF microelectrodes; a linear response was also observed by Kinoshita et al.¹⁴ in the pH range of 2.5–8.5. IrO_2 (used as a macroelectrode) showed near-Nernstian behavior in the pH range of 2–12 in air-saturated buffer solutions.¹³

In this study, the new pH microelectrode has shown an error of ≤ 0.05 pH unit in the pH range of 0–12 when compared with the glass pH macroelectrode. It is important to note that the readings from the glass pH electrode have been assumed to be absolute in this study, which, however, may not be an exactly right assumption as standard pH glass electrodes suffer monovalent cation interferences.¹³ The present findings are in conformity with those of Hitchman and Ramanathan;²³ they reported that potentially cycled iridium oxide electrodes are comparable with glass pH electrodes with an accuracy of 0.2 pH unit. Accuracy up to ± 0.02 pH unit, however, has been reported for AEIROF macroelectrodes.²⁵

(c) Response Time. The response time for the new AEIROF pH microelectrode was evaluated in buffers of pH 4, 7, and 10. The new pH microelectrode has very short 90% response time (t_{90}) of 1–17 s for acidic, neutral, and alkaline pH, which is consistent with findings by other researchers.^{4,14,23,25} Marzouk et al.²⁵ observed that regardless of the pH, $\sim 80\%$ of the response of the AEIROF macroelectrode was observed within 5 s (t_{80}) in the pH range 2–10; however, they found t_{100} (with ± 0.5 -mV fluctuations) to be pH dependent, being 30 s for pH < 5 , 90 s in the pH

Table 1. Response Time of the AEIROF pH Microelectrode Tested in Different pH Buffers^a

buffer pH	t_{90} (s) ^b	t_{95} (s) ^b	t_{100} (s) ^b
4	1	12	45
7	9	17	35
10	17	110	290

^a The microelectrode was washed with Nanopure water before putting into a test buffer solution. ^b t_x means $x\%$ response time.

range 5–7, and 60 s for pH > 7 . Wipf et al.¹¹ reported a 30 s response time for their CF/ IrO_x microelectrodes irrespective of the pH value. In the present study, the response time of our pH microelectrodes increased with an increase in pH (Table 1). Similarly, for higher pH, the AIROF macroelectrode took longer time for 100% response.²³ However, Kinoshita et al.¹⁴ recorded shorter response time for the AIROF macroelectrode in alkaline solution as compared to that in acidic one.

(d) Effective Life. To determine the effective life of the microelectrodes under different storage conditions, three microelectrodes were stored in standard pH 7 buffer, three in water, and three in air. The microelectrodes were calibrated periodically to check their slopes (mV/pH). Most of the microelectrodes were found to be usable (slopes in the range of ~ 50 – 65 mV/pH with an R^2 value of ~ 0.99) up to ~ 2 months after their construction. Maximum decreases in slope of ~ 16 , ~ 10 , and ~ 12 mV/pH were observed in the microelectrodes preserved in pH 7 buffer (68 days), water (60 days), and air (63 days), respectively (Figure 5). The drift of the microelectrode slope was between 1 and 2 mV/pH per week depending on the storage method. The new pH microelectrodes have an improved effective life as compared to AIROF, CF/ IrO_x , and liquid ion-exchanger pH microelectrodes. A decrease in slope from ~ 77.3 to ~ 65.8 mV/pH was observed in only 1 week in AIROF microelectrodes.⁴ However, Hitchman and Ramanathan²³ did not observe any drift in slope for the AIROF macroelectrodes after 1 month of storing in water. CF/ IrO_x microelectrodes had a working life of 1–2 months irrespective of whether they were stored in air or pH buffer.¹¹ For AEIROF macroelectrodes stored in tris buffer (pH 7), the drift was ~ 2.5 mV/pH per month.²⁵

(e) Hysteresis of Microelectrode Response. The microelectrode hysteresis was tested using buffers of pH 4, 5, 7, 9, and 10. The microelectrode was calibrated 5 times using pH buffers from lower to higher and higher to lower values alternatively. The mV readings (data not shown) showed a maximum drift of 0.8 mV corresponding to an error of only 0.02 pH unit. Marzouk et al.²⁵ also found no hysteresis of the AEIROF macroelectrode response.

Effects of Different Environmental Factors on pH Microelectrode's Performance. (a) Effect of Stirring and Temperature. The AEIROF microelectrode can be used up to a temperature of $\sim 40^\circ\text{C}$ (Figure 6). Beyond this point the microelectrode itself will be damaged as the melting of the LMA used is 47.2°C (117°F). The mV readings changed by a maximum of 10 mV when

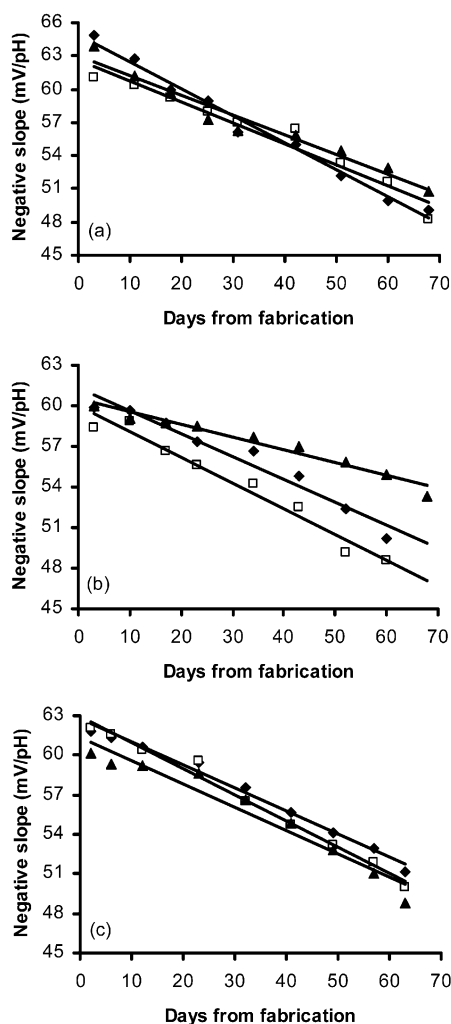


Figure 5. Effect of aging on the calibration slopes of the AEIROF microelectrodes when stored in (a) pH 7 buffer, (b) Nanopure water, and (c) air. Three individual microelectrodes (represented by \blacklozenge , \blacktriangle , and \square) were stored in each medium. Individual microelectrodes were discarded once the slope was <50 mV/pH or when the correlation coefficient (R^2) for the calibration data was not satisfactory (<0.96). All microelectrodes were cured in pH 7 buffer for 2 days after construction.

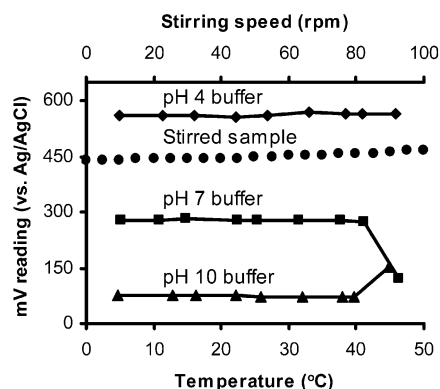


Figure 6. AEIROF microelectrode responses under different temperatures and stirring speeds. The test solution for the stirring test was Nanopure water (pH adjusted to 4.85 with 0.1 N HNO_3).

temperature was raised from ~ 5 to ~ 40 $^{\circ}\text{C}$. These changes may be due to the change in the temperature coefficient of the dissociation constant of the acid/base used in the buffer.

Table 2. Effect of Various Ions on the pH Response of the AEIROF Microelectrode

ion (1)	concn tested ^b (mg/L) (2)	concn in seawater ³⁶ (mg/L) (3)	microelectrode potential (vs Ag/AgCl) ^a	
			without ion (mV) (4)	with ion (mV) (5)
Na^+	10692.5	10561	278.1	268.9
K^+	590.9	380	278.1	268.2
Li^+	208.2		250.1	248.7
F^-	896.7		250.1	257.2
Cl^-	20496.5	18980	278.1	271.5
Br^-	8052.6	65	265.3	272.7
I^-	12797.5		250.1	249.6
NH_4^+ (as N)	60.2		278.1	281.4
Mg^{2+}	1286.5	1272	278.1	276.8
Ca^{2+}	416.4	400	278.1	271.8
NO_3^- (as N)	97.3		278.1	273.3
SO_4^{2-}	2731.5	2649	278.1	288.3
MoO_4^{2-}	4438.6		250.1	248.6

^a The potential reading was first taken in pH 7 buffer only (data in column 4), and then for the second reading (data in column 5) the selected ion was dissolved into pH 7 buffer. ^b Based on reported values or as in seawater; equal or higher concentrations were used.

For the stirring test, a jar test apparatus (model 300, Phipps & Bird, Richmond, VA) was used with the stirring speed between 0 and 100 rpm and the pH of the test solution (water) of 4.85 (adjusted with 0.1 N HNO_3). For a stirring speed of ≤ 55 rpm, the error in pH reading was <0.16 pH unit. However, at a higher speed the error increased almost linearly; a pH error of ~ 0.5 pH unit was observed at 100 rpm (Figure 6). Other workers, however, reported no interference due to stirring.^{13,25}

(b) Anion and Cation Interferences. Various ions typically present in environmental systems were tested for their possible interferences in pH measurement, and their concentrations were decided based on reported values in water and wastewater^{7,13} or by taking typical seawater³⁶ as the worst possible aqueous system. In each test, a specific amount of a salt corresponding to an ion (Table 2) was dissolved completely into 15 mL of buffer solution (pH 7) in a small disposable plastic beaker. The test solution was discarded after testing each ion, and the microelectrode was washed with water. To check for possible poisoning by each ion, the microelectrode potential was checked in pH 7 buffer after each ion was tested.

None of the tested ions (shown in Table 2) had any marked interference ($<3.7\%$) or poisoning effect on the pH response of the new microelectrode. The maximum drift was $+10.2$ mV for SO_4^{2-} (Table 2); this potential difference corresponds to an error of ~ 0.18 pH unit. Fog and Buck¹³ reported that IrO_2 electrodes were not affected by Li^+ , Na^+ , K^+ , F^- , and Cl^- in the pH range 2–10 and by Br^- in the pH range 2–7; however, I^- had a marked effect on the readings. Marzouk et al.²⁵ also reported that Li^+ , Na^+ , K^+ , NH_4^+ , Ca^{2+} , and Mg^{2+} had no adverse effect on the electrode response in the physiologically relevant pH of 7.

(c) Effect of Complexing Agents. The concentrations of the tested complex forming ligands that may exist in some biological fluids were selected on the basis of reported works on other pH electrodes.^{14,21} Microelectrode response was tested by dissolving

(36) Spiegler, K. S. *Saltwater purification*; John Wiley & Sons: New York, 1962.

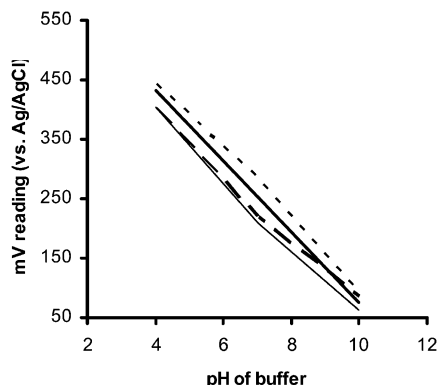


Figure 7. Effect of complexing agents on the AEIROF pH microelectrode calibration. The individual complexing agent was dissolved in buffers of pH 4, 7, and 10. The data points are not specifically shown to avoid overcrowding, and only the lines joining the data points are presented. All complexing agents were 0.03 M except borate and oxalate, which were 0.01 M. The calibration curves in the presence of phosphate, acetate, citrate, and oxalate are overlapped with the original one (only pH buffer solutions). Legend: —, only pH buffer, phosphate, acetate, citrate and oxalate; — —, borate; · · ·, EDTA; — ·, carbonate.

a definite amount of each complexing agent in buffers of pH 4, 7, and 10. Most of the agents did not have any marked effect on calibration of our microelectrodes (slopes ~ 59.32 mV/pH) (Figure 7). However, in the presence of borate, carbonate, and EDTA, the potential changed up to ~ 30 mV with the slope of the corresponding calibration curve being 52.45, 56.6, and 58.53 mV/pH, respectively. Kinoshita et al.¹⁴ studied the effects of ligands on AIROF electrode calibration and found that carbonate and oxalate affected the pH response. The presence of lactate was also found to have no effect on pH response of AEIROF macroelectrodes.²⁵

(d) Effect of Different Redox Systems. Metal/metal oxide electrodes are affected by different redox systems.^{13,14} In this study, several redox systems were selected on the basis of their environmental importance (e.g., oxygen, sulfide) or being standard redox systems [e.g., quinhydrone, $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$] or because they have been studied by others (e.g., H_2O_2). The effects of selected redox systems on the pH response of the new microelectrodes were evaluated in two sets of experiments. In the first set of tests, the mV readings of the pH microelectrodes were measured in pH 7 buffer with and without the selected redox systems. In the second set of tests, the mV readings of the pH microelectrodes were evaluated in pH buffers 4, 7, and 10 with the selected redox systems that showed strong interferences in the first set of tests.

The mV readings decreased by ~ 200 , ~ 100 , ~ 60 , ~ 50 , and ~ 30 mV when quinhydrone, ferrocyanide, sodium sulfide, ferri-/ferrocyanide (mixed together), and ferricyanide, respectively, were dissolved in pH 7 buffer solution, while H_2O_2 or oxygen did not have any major interference on the microelectrode behavior (Table 3). The effect of oxygen was also tested in 0.85% saline water, and the reading remained more or less constant with and without bubbling of oxygen gas. Fog and Buck¹³ and Marzouk et al.²⁵ also documented that iridium oxide electrodes are not affected by H_2O_2 in the pH range 2–10. Marzouk et al.²⁵ reported that iridium oxide is most advantageous as regards redox interferences; they found

Table 3. Redox Interference on pH Response of the AEIROF Microelectrode

substance (1)	amount/ condition (2)	microelectrode potential (vs Ag/AgCl) ^a	
		without substance (mV) (3)	with substance (mV) (4)
quinhydrone H_2O_2 (30 wt %)	saturated	445.2	235.6
	0.1 mL	356.7	358.6
	0.2 mL		358.6
	0.7 mL		361.8
	1.2 mL		366.3
oxygen	0%	287.1	285.9
	10%		286.8
	21%		287.4
	100%		288.5
ferricyanide	0.01 M	287.1	317.8
ferrocyanide	0.01 M	287.1	192.2
ferri-/ferrocyanide	0.01 M	287.1	236.0
sodium sulfide	0.01 M	287.1	223.1

^a The potential reading was first taken in pH 7 buffer only (data in column 3), and then for the second reading (data in column 4) the redox causing substance was dissolved into pH 7 buffer.

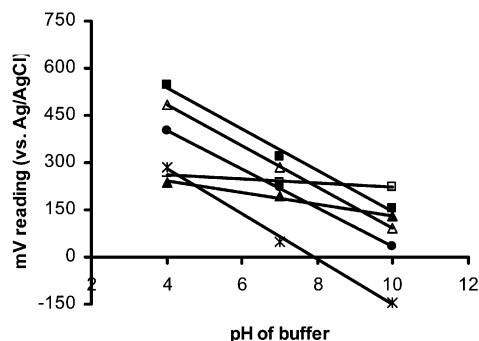


Figure 8. pH responses of AEIROF microelectrodes in various redox systems. 0.01 M solutions (in pH 4, 7, and 10 buffers) of ferro- and ferricyanide, ferro-/ferricyanide (mixed together), and sodium sulfide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) were used. For quinhydrone, saturated buffer solutions were used. Legend: ■, ferricyanide; △, only pH buffer; ●, sodium sulfide; *, quinhydrone; □, ferri-/ferrocyanide; ▲, ferrocyanide.

that ascorbic acid, uric acid, and dissolved oxygen had no major interference on AEIROF macroelectrodes.

The results of the present study indicate that different redox systems will affect the pH responses of the new AEIROF pH microelectrode differently. As shown in Figure 8, however, linear calibration curves could still be obtained in each case. Since the slopes (mV/pH) of these calibration curves are different corresponding to different redox systems, we can conclude that a calibration curve for the new AEIROF pH microelectrode in a specific redox system can only be used to interpret the pH of samples with the similar redox characteristics.

DISCUSSION

Possible Mechanism. Fog and Buck¹³ proposed five possible mechanisms to explain responses of pH electrodes. Since our microelectrode is not affected by cations, the mechanisms as in a standard glass electrode (simple ion exchange in a surface layer containing OH groups) and an electrode with a solid solution of hydrogen atoms in the oxide (the hydrogen content of this oxide

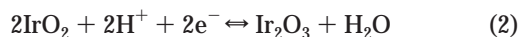
can be varied by passing current, and thus changing redox equilibrium) are not plausible.¹³ Fog and Buck¹³ also ruled out the mechanism involving steady-state corrosion of the electrode material for electrodes showing a Nernstian response. Therefore, the mechanism involving a redox equilibrium between two different solid phases (e.g., lower and higher valence oxides of iridium) and that involving a single-phase oxygen intercalation are the two possibilities for the present AEIROF microelectrode.

The complex nature of oxide formation and different response slopes obtained for iridium oxide electrodes made by different methods make it complicated to advocate a single theory (reaction) for the electrode behavior. Midgley²⁴ reported that the slope of the pH response curve of an iridium oxide electrode depends on the method of preparation. Hitchman and Ramanathan²³ showed that the slope depends on the charge storage capacity of the AIROF electrode, and the charge storage capacity depends on the thickness of the oxide layer formed. Based on their individual experience, researchers have proposed a number of possible mechanisms. Sputtering and thermal oxidation produce predominantly anhydrous iridium oxide (IrO₂), while electrochemical oxidation produces predominantly hydrated iridium oxide(s) [IrO₂·4H₂O, Ir(OH)₄·2H₂O, {IrO₂(OH)₂·2H₂O}²⁻·2H⁺].^{4,22,28}

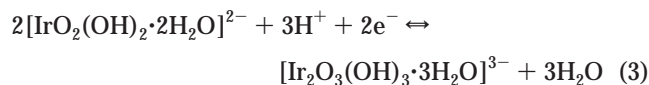
Anhydrous IrO₂ responds to pH changes with a slope of ~59 mV/pH,^{4,22} which can be explained using eq 1^{4,22} or eq 2^{4,22,23}



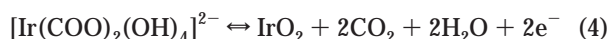
or



Hydrated iridium oxide presents super-Nernst responses²² with a slope of ~90 mV/pH, indicating 0.67 electron transferred per H⁺ (eq 3).

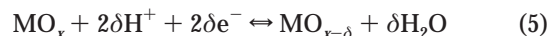


Kinoshita et al.¹⁴ found that response slopes for AIROF electrodes formed on mono- and polycrystalline iridium were ~69.7 and ~62–68 mV/pH, respectively, at 25 °C. Hitchman and Ramanathan²³ obtained a slope of 81.9 ± 1.5 mV/pH for the AIROF pH macroelectrode, which is very close to the value predicted by eq 3. Yamanaka²⁸ reported that CEIROFs contain reduced amorphous iridium oxide (IrO_{2-δ}) and the average valence of the iridium deposited is near 4.0. X-ray photoelectron spectrometer (XPS) measurements show that the average valence of iridium in AEIROFs is almost the same as that in CEIROFs (i.e., near 4.0). It is thought that the deposition of AEIROFs is mainly due to the fact that an iridium(IV) oxide is formed by anodic oxidation of an Ir(IV) compound, which exits in the deposition solution. The reaction mechanism in which IrO₂ is formed by anodic oxidation of ligands in an iridium complex compound is probably²⁸



Yamanaka²⁸ found the slope to be ~60 mV/pH for electrochromic iridium oxide films. Marzouk et al.²⁵ also reported that their AEIROF macroelectrodes showed near-Nernstian responses of 63.5 ± 2.2 mV/pH after 2 days of conditioning in tris pH 7 buffer. They compared their slope to that obtained from iridium oxide electrodes prepared by thermal oxidation or sputtering. AEIROF showed the characteristic electrochromic behavior of IrO₂.²⁸ Cyclic voltammetry results further indicated that the AEIROF is IrO₂.²⁵

In the present study, we obtained a slope of ~60 mV/pH unit for freshly prepared microelectrodes after 2 days of curing in pH 7 buffer. It is possible that an anhydrous IrO₂ layer was formed during anodic electrodeposition of the present AEIROF microelectrodes. The current density we used in electrodeposition of IrO₂ was ~0.0003 mA/cm², which is far lower than 0.5–1 mA/cm² used by Yamanaka²⁸ and Marzouk et al.²⁵ However, because the deposition time for the present AEIROF microelectrode was 3 times (~20 min) the 6 min used by them,²⁵ it is possible that the deposition was equally effective. It is also important to note that the exposed surface area was very high in the work reported by Yamanaka²⁸ (used for electrochromic display devices) and Marzouk et al.²⁵ (used for AEIROF macroelectrodes). Fog and Buck¹³ advocated the oxygen intercalation mechanism (eq 5)



for metal oxide electrodes. Comparing eq 5 with eq 2, it may be concluded that eq 2 is more likely to be the governing mechanism for the pH response of our AEIROF microelectrodes (where $x = 2$ and $\delta = 0.5$). Also, as the AEIROF deposited on the pH microelectrode tip is possibly anhydrous IrO₂, and the microelectrode behavior may be governed by eq 1 or 2 or both, the mechanism involving a single-phase intercalation¹³ is a distinct possibility.

Improvement. Fog and Buck¹³ discussed in detail the requirements of a pH sensor. In an ideal electrochemical ion-exchanger pH sensor, its interface with external electrolyte should provide nearly uniform composition surfaces that permit rapid exchange of protons, and the film or bulk phase should act as an ionic semiconductor without electronic conductivity. An ideal pH sensor should be stable over the pH range 0–14 and also over a wide temperature range. The reactive surface layer should reach equilibrium very rapidly, and ion exchange by other ions should not be energetically feasible. Also, it should be free from redox interferences.

While there are commercially available sensors for conventional pH measurements, microscale studies call for microelectrodes of tip diameters of <10 μm, and they need to be sturdy enough to resist breakage during use.^{2–4,14} So far, only a few kinds of pH microelectrodes have been reported, including AIROF iridium oxide,⁴ glass,^{1,2} liquid ion-exchanger,^{7,9,10,37} carbon fiber,⁸ and Pd–PdO³ microelectrodes. As a contribution, the present AEIROF pH microelectrodes have several improvements as compared to liquid ion-selective membrane or AIROF pH microelectrodes. The AEIROF deposition method is comparatively low

(37) Ammann, D.; Lanter, F.; Steiner, R. A.; Schulthess, P.; Shijo, Y.; Simon, W. *Anal. Chem.* **1981**, *53*, 2267–2269.

(38) Burke, L. D.; Whelan, D. P. *J. Electroanal. Chem.* **1984**, *162*, 121–141.

cost,²⁵ does not need any major instrumentation, and uses a very small amount of expensive platinum metal and iridium compound. From our experience, it can be said that one person can easily make 10–15 microelectrodes on average in an 8-h working day with almost 100% success. Our AEIROF microelectrodes have a very fast 90% response time (t_{90} of 4–17 s) with an accuracy of ± 0.05 pH unit; they are stable and usable over a period of ~ 2 months. However, two microelectrodes produced by the present method may show different mV/pH slopes when tested after 2 days of curing in pH 7 buffer. Fog and Buck¹³ also reported a typical potential difference between two sensors of the same construction to be of the magnitude of 100 mV.

There is no major interference from ions or complexing agents that may exist in most environmental and biological fluids. The AEIROF microelectrode is not affected by oxygen partial pressure, making it a better candidate for use in environmental and biological systems where dissolved oxygen (DO) is a major variable. The present microelectrode is very stable under stirred conditions (≤ 55 rpm) and over a wide temperature range (~ 5 – 40 °C), making it a candidate for use in most environmental and biological systems with flowing fluids. In addition, it may be

possible to construct AEIROF microelectrodes for pH measurement at higher temperature using other available LMAs (e.g., with melting point up to ~ 138 °C; Belmont Metals Inc., Brooklyn, NY). Although affected by different redox systems, the AEIROF microelectrodes can be effectively used to measure the pH of various solutions provided the calibration and the test solutions have similar redox characteristics. Overall, the present AEIROF microelectrode holds promise for use in various biological fluids, groundwater, wastewater, and surface water including seawater. The usefulness of AEIROF macroelectrodes in medical research has already been demonstrated by Marzouk et al.²⁵

ACKNOWLEDGMENT

We are thankful to Dr. S. A. M. Marzouk of United Arab Emirates University (UAE) and Dr. W. E. Cascio of North Carolina State University for their academic assistance during the course of this study.

Received for review May 14, 2002. Accepted August 27, 2002.

AC020326L