Needle-Type Environmental Sensors

Accessed on: 05 Oct 2023

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Needle-Type Environmental Sensors

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   Tip Formation
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7.1 Introduction

The development of environmental microsensor techniques is a revolutionary advancement in the measurement of both absolute levels and changes in chemical species in engineered and natural aquatic systems. This advancement has utilized microscopic monitoring to contribute a greater understanding of biological mechanisms in many environmental processes for decades. Currently, microelectromechanical systems (MEMS) microfabrication technology, which is intimately connected to a microelectronic circuit for amplifying, processing, and transmitting microelectrode (ME) signals, is being successfully applied to the development of multianalyte sensor systems for in situ monitoring.

The needle-type electrochemical microsensor is one of the most prominent, novel methods for studying biofms. Biofms are colonies of microbial cells in an organic polymeric matrix (usually polysaccharides) attached to a surface in water systems. They have complex structures consisting of highly stratified microbial communities. The ability of bacteria to attach to surfaces and to form biofms from biofilm is an important competitive advantage for them over bacteria growing in suspension. Biofms allow for mixed microbial communities, concentration of nutrients, and protection from antibiotics. The formation of biofms has been associated with a broad range of industrial problems at an annual cost of billions of dollars. For example, biofms are ubiquitous in water distribution systems,
and the control of their growth has been a great challenge, with many water utilities in the United States reporting biof l m survival in water distribution systems despite the continuing presence of disinfectants. Biof l m may also harbor various types of microorganisms including opportunistic pathogens and thus can threaten public health. On the other hand, biof l m plays a major role in many water</p>
Amperometric sensors: electrode output is a current (pA)

Control $E$
Measure $I$ ($i \neq 0$)

- Dissolved oxygen (O$_2$)
- Nitrite (NO$_2^-$)
- Nitrous oxide (N$_2$O), Nitric oxide (NO)
- Hydroxylamine (NH$_2$OH)
- Monochloramine (NH$_2$Cl), Free chlorine (HOCl), Chlorine dioxide (ClO$_2$)
- Sulfide (H$_2$S, HS$^-$, and S$^{2-}$)

Potentiometric sensors: electrode output is a voltage (mV)

Control $I$ ($i = 0$)
Measure $E$

- pH (Hydrogen ion)
- Phosphate (HPO$_4^{2-}$ and H$_2$PO$_4^-$)
- Ammonium (NH$_4^+$), Nitrate (NO$_3^-$)
- Redox potential
- Carbon dioxide (CO$_2$)
- Sulfide (H$_2$S, HS$^-$, and S$^{2-}$)
- Specific ions (e.g., Ca$^{2+}$, Mg$^{2+}$, Cu$^{2+}$, Cd$^{2+}$)

**FIGURE 7.1** Principles of needle-type electrochemical environmental microsensor operation. $E$, potential (mV); $I$, current (pA).

A number of microsensors (e.g., oxygen, sulf de gas, hydrogen, nitrous oxide [N$_2$O], nitric oxide, redox, and nitrite) are commercially available from Unisense A/S (www.unisense.com) or other manufacturers. Typical environmental microsensors have diameters of about 3–15 $\mu$m (Figure 7.2), while a specific combined Clark-type microsensor, which needs an outer chamber (or casing), has a diameter of 50–80 $\mu$m. Smaller sensors have the advantage of causing less disturbance when introduced, but there is some decrease in sensitivity [2].

### 7.3 Amperometric Microsensors

The most well-known amperometric microsensor is the oxygen sensor (Figure 7.2a) because oxygen has been used as a critical indicator for microbial activity in many environmental processes [3,6,9,24–26]. The method used for the sensor fabrication depends on the type of microsensor being constructed. Oxygen (or dissolved oxygen [DO]) MEs are made by f lling a micropipette with a low-melting-point alloy; the tip is then electroplated with a thin layer of gold (Au) [27]. It measures DO amperometrically based on oxygen reduction in the cathode reaction [6]. The current is proportional to the bulk oxygen concentration (Equation 7.1):

$$I = \frac{nFADP_{O_2}}{Z} = kP_{O_2}$$

(7.1)

where

- $I$ is the current
- $n$ is the number of electrons involved in oxygen reduction
- $F$ is the Faraday number (96,487 °C/mol)
- $A$ is the cathode area (cm$^2$)
- $P_{O_2}$ is the oxygen partial pressure
- $Z$ is the oxygen diffusion layer thickness
- $S$ and $D$ are solubility coefficient and oxygen diffusion viscosity, respectively
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Type</th>
<th>Analytical Methods/ Mechanisms</th>
<th>Slope, Response Time</th>
<th>Conc. Range (LOD)</th>
<th>Key Materials/Compound/ Procedures</th>
<th>References</th>
</tr>
</thead>
</table>
| Oxygen (O₂)          | Clark, Solid⁠ | • Amperometry  
• Oxygen reduction in the cathode reaction at Au | 13 pA/(mg O₂/L), <5 s  
0-8.3 mg O₂/L (23°C) |                                  | Low-melting-point (47°C) bismuth alloy  
Au electroplating  
Polarization | [3-5]        |
| pH                   | ISE⁠ | • Potentiometry  
• Nernstian electrode response from neutral carrier | 45-60 mV/pH  
pH 6-10 |                                  | Salinization of the tip  
Electrolyte pH 7 buffer solution  
Liquid membrane: Fluka 95293  
Cellulose acetate and protein layer at the tip | [3]         |
|                      |        |                                                                                           | 55-59 mV/pH  
pH 1.3-9.8 |                                  | Salinization of the tip  
Electrolyte: NaCl  
(100 mmol/L), KH₂PO₄  
(250 mmol/L), and Na₂HPO₄  
(250 mmol/L)  
Liquid membrane: Fluka 95297  
Iridium wire  
IrOx formed by potential cycling in 0.5 M sulfuric acid  
H₂S interference | [6]         |
| Ammonium (NH₄⁺-N)    | ISE⁠ | • Potentiometry  
• Nernstian electrode response (NH₄⁺-N) from neutral carrier | 54.5 mV/dec  
10⁻⁴-1⁻¹ M (10⁻⁵-8 M) |                                  | Salinization of the tip  
Electrolyte: 0.01 M NH₄Cl  
Liquid membrane: Fluka 09879  
Cellulose acetate and protein layer at the tip | [8]         |
| Nitrate (NO₃⁻-N)     | ISE⁠ | • Potentiometry  
• Nernstian electrode response (NO₃⁻-N) from neutral carrier | 55.6 mV/dec  
10⁻³-1⁻¹ M (20 μM) |                                  | Salinization of the tip  
Electrolyte 0.05 M KNO₃ +  
0.05 M KCl  
Liquid membrane: Fluka 72549  
Cellulose acetate and protein layer at the tip | [9]         |
<table>
<thead>
<tr>
<th>Substance</th>
<th>Method</th>
<th>Detection Limit</th>
<th>Time Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite ($\text{NO}_2^-$)</td>
<td>Solid Amperometry</td>
<td>242 pA/(mg N/L)</td>
<td>0–20 mg N/L (0.02 mg N/L)</td>
</tr>
<tr>
<td></td>
<td>Direct electrochemical oxidation of nitrite ($\text{NO}_2^-$) on solid electrode surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric oxide (NO)</td>
<td>Clark Amperometry</td>
<td>2 pA/μM, ~1 s</td>
<td>0–4 μM (~30 nM)</td>
</tr>
<tr>
<td></td>
<td>Oxidation of nitric oxide at electrocatalytic surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{N}_2\text{O}$</td>
<td>Clark Amperometry</td>
<td>0.2 pA/μM, 40–50 s</td>
<td>0–1.2 mM (1 μM)</td>
</tr>
<tr>
<td></td>
<td>$\text{N}_2\text{O}$ reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (P)</td>
<td>Solid Potentiometry</td>
<td>33–84 mV/dec, &lt;1 min</td>
<td>$10^{-4}$–$10^{-3}$ M ($2.2 \times 10^{-6}$ M)</td>
</tr>
<tr>
<td></td>
<td>Oxidation corrosion potential (mV) measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reaction between CoO and phosphate ion ($\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$) between pH 6.7 and 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free chlorine (Cl$_2$)</td>
<td>Solid Amperometry</td>
<td>1 pA/μM, &lt;1 s</td>
<td>0–7.5 mM</td>
</tr>
<tr>
<td></td>
<td>Reaction between PtOH and HOCI (and/or ClO$^-$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbon fiber Polarization</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Type</th>
<th>Analytical Methods/Mechanisms</th>
<th>Slope; Response Time</th>
<th>Conc. Range (LOD)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Key Materials/Compound/Procedures</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH&lt;sub&gt;3&lt;/sub&gt;Cl</td>
<td>Solid</td>
<td>• Amperometry</td>
<td>18 pA/(mg Cl&lt;sub&gt;-&lt;/sub&gt;/L), &lt;5 s</td>
<td>0–10 mg Cl&lt;sub&gt;-&lt;/sub&gt;/L (0.3 mg Cl&lt;sub&gt;-&lt;/sub&gt;/L)</td>
<td>• Pt wire</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reaction between PtOH and NH&lt;sub&gt;3&lt;/sub&gt;Cl</td>
<td></td>
<td></td>
<td>• Polarization</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td>• Controlled condition where predominant chlorine species are NH&lt;sub&gt;3&lt;/sub&gt;Cl (i.e., Cl&lt;sub&gt;-&lt;/sub&gt;N =41, pH 8.0)</td>
<td></td>
</tr>
<tr>
<td>Redox potential</td>
<td>Solid</td>
<td>• Potentiometry</td>
<td>1 mV/mV, &lt;1 min</td>
<td>—</td>
<td>• Pt wire</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Nernstian electrode response</td>
<td></td>
<td></td>
<td>• Etching in 2 M KCN solution</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Within 10 mV of the nominal redox potentials (ASTM D 1496-93)</td>
<td></td>
</tr>
<tr>
<td>Sulfide (S&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>Solid</td>
<td>• Potentiometric measurement of sulfide (S&lt;sup&gt;-2&lt;/sup&gt;) at alkaline pH and total dissolved sulfide (S&lt;sub&gt;-&lt;/sub&gt; = H&lt;sub&gt;-&lt;/sub&gt;S + HS&lt;sup&gt;-&lt;/sup&gt; + S&lt;sup&gt;-&lt;/sup&gt;) at neutral pH</td>
<td>31 mV/dec (pH &gt;13.5) 51 mV/dec (pH 7.2)</td>
<td>5 × 10&lt;sup&gt;-7&lt;/sup&gt;–5 × 10&lt;sup&gt;-3&lt;/sup&gt; M (pH &gt;13.5) 5.8 × 10&lt;sup&gt;-6&lt;/sup&gt;–5.8 × 10&lt;sup&gt;-3&lt;/sup&gt; M (pH 7.2)</td>
<td>• Silver wire</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Nernstian response</td>
<td></td>
<td></td>
<td>• Etching in 2M KCN solution</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Chemical treatment of Hg&lt;sup&gt;2+&lt;/sup&gt; (HgCl&lt;sub&gt;2&lt;/sub&gt;) to form the sulfide sensing layer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Sulfidation of the silver surface with Na&lt;sub&gt;2&lt;/sub&gt;S</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Sulfidation of the silver surface with Na&lt;sub&gt;2&lt;/sub&gt;S</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Required simultaneous pH measurement</td>
<td></td>
</tr>
<tr>
<td>Clark</td>
<td></td>
<td>• Amperometry</td>
<td>1.2–0.6 pA/μM H&lt;sub&gt;2&lt;/sub&gt;S, &lt;400 ms</td>
<td>2–300 μM H&lt;sub&gt;2&lt;/sub&gt;S (2 μM)</td>
<td>• Polarization (&lt;85 to &lt;150 mV)</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Oxidation of H&lt;sub&gt;2&lt;/sub&gt;S by K&lt;sub&gt;4&lt;/sub&gt;[Fe(CN)&lt;sub&gt;6&lt;/sub&gt;]&lt;sub&gt;3&lt;/sub&gt;, resulting in the formation of elemental sulfur and ferrocyanide</td>
<td></td>
<td></td>
<td>• Internal electrolyte ferrocyanide (K&lt;sub&gt;4&lt;/sub&gt;[Fe(CN)&lt;sub&gt;6&lt;/sub&gt;]&lt;sub&gt;3&lt;/sub&gt;) as redox mediator</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Si membrane</td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide (CO&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>ISE, Clark</td>
<td>• Potentiometry</td>
<td>100 mV/dec, ~10 s (or 2-5 min)</td>
<td>0–2 mM (3 μM)</td>
<td>• LIX membrane (pH measurement)</td>
<td>[6, 20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Nernstian response from changes in pH (buffer action)</td>
<td></td>
<td></td>
<td>• Internal electrolyte 2 mM NaHCO&lt;sub&gt;3&lt;/sub&gt; + 0.5 M NaCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Si membrane</td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>Type</td>
<td>Electrode</td>
<td>Potentiometry</td>
<td>pH</td>
<td>Sensitivity *</td>
<td>Conditioning</td>
</tr>
<tr>
<td>-------------</td>
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<td>-----------</td>
<td>---------------</td>
<td>----</td>
<td>---------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Copper (Cu⁺)</td>
<td>Solid</td>
<td>Potentiometry</td>
<td>29 mV/dec, 10 s</td>
<td>10⁻⁶–10⁻¹ M (10⁻⁷ M)</td>
<td>Copper wire (75 μm diameter)</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reaction of selenium with the copper surface as adsorbed hydrogen reduces HSeO₃ ions to selenium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni-TMPP: 2-Nitrophenyl</td>
<td>ISE</td>
<td>Potentiometry</td>
<td>33.1 mV/dec</td>
<td>10⁻²–10⁻⁴ M (2 × 10⁻⁶ M)</td>
<td>Membrane containing ionophore NaTFPB, o-NPOE, and PVC</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nernstian electrode response (Cu²⁺) from neutral carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium (Cd²⁺)</td>
<td>ISE</td>
<td>Potentiometry</td>
<td>25.2–27.7 mV/dec, 15–30 s</td>
<td>&gt;0.001 mM (10⁻¹⁰ M)</td>
<td>Salinization of the tip</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nernstian electrode response from neutral carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Slope of linear regression indicating sensitivity.

** Limit of detection.

† The Clark-type microsensor is a combined microsensor. In this microsensor, the sensing cathode (or anode) is placed behind an electrically insulating gas-permeable Si membrane, which is extremely permeable to oxygen. ✦ The cathode (or anode) is bathed in an electrolyte solution into which a Ag/AgCl reference electrode is immersed [24].

§ The solid-type microsensor is a single-barrel microsensor (i.e., single-anode or cathode-type ME) with the solid metal tip sealed with a glass micropipette and with external reference electrode placed in the sample.

¶ ISE: Ion-selective electrode that contains LIX membrane.

* Ni-TMPP: nickel (II) tetrakis (3-methoxy-4-hydroxyphenyl) porphyrin.

* 2-Nitrophenyl octyl ether (o-NPOE).

* Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate (NaTFPB).

* Tetrahydrofuran (THF).

* Tetraethylammonium nitrate (Et₄NNO₃).

\[77\]
Both platinum (Pt) and Au exhibit sensitivity to oxygen at negative potential (i.e., $-0.75$ V vs. Ag/AgCl), but Au has a higher sensitivity than Pt. As Au wire has different heat expansion coefficients and it is too soft to insert into the glass micropipette, Au electroplating methods have been used [3,5]. For the use of a bare Au wire (50 µm), a modified procedure is needed: the etched Au wire connected to a copper wire (for electric connection) is inserted into the glass micropipette and then pulled out using a micropipette puller (P-1000, Sutter Instrument) with an appropriate setting (e.g., heat: 460, pull: 120, vel: 100, delay: 1, pressure: 500, and ramp: 445). The response time of an amperometric microsensor is very short (within few seconds), and the shelf-life time of a solid-state microsensor (e.g., oxygen microsensor, free chlorine microsensor) is typically 1–2 years, depending on its contamination status.

In amperometric sensing, each microsensor must operate at a specific applied potential (or polarization potential) (e.g., $-0.75$ V for oxygen sensor, $+0.15$ V for monochloramine [NH$_2$Cl] sensor, and $+1.2$ V for nitrite sensor) depending on the electrochemical reaction between the sensing material and the analyte to be measured. Cyclic voltammetry (CV) has been used to evaluate the applied potential of the microsensors (Figure 7.3). Generally, oxygen reduction in a noble metal occurs at a negative potential; therefore, most microsensors, except oxygen sensors, have positive potentials for their operation unless they have a special treatment to remove the oxygen interference (e.g., ascorbate chamber in front of the microsensor [2]).
### Table 7.2: Materials and Devices Necessary for Needle-Type Environmental Microsensor Fabrication

<table>
<thead>
<tr>
<th>Item</th>
<th>Use</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal wires</td>
<td>Sensing materials for solid-state working electrodes</td>
<td>99.99% pure 50-100 μm diameter · Noble metals (Pt or Au) · Other metals (Co, iridium, or silver)</td>
<td>California Fine Wire (Pt: 100-896, Ag: 100-183, Au: 100-542) · Alfa Aesar (Au wire: 0.1 mm, 10968) · Sigma-Aldrich (Pt wire: 26716-3, Co wire: 52344-5, discontinued)</td>
</tr>
<tr>
<td>Carbon fiber</td>
<td>Nitrite microsensor · Nitric oxide microsensor</td>
<td>PAN-based carbon fiber (7 μm) · Carbon fiber (30 μm)</td>
<td>Cytec Industries, Inc. (T 300 12K) · WPI b</td>
</tr>
<tr>
<td>Nichrome wire</td>
<td>Heating filaments for meting glass pipettes</td>
<td>Nichrome wire, 22 gauge</td>
<td>Fisher Scientific (G49085) · VWR (66258-066)</td>
</tr>
<tr>
<td>Silver wire</td>
<td>Reference electrode</td>
<td>99.99% pure, 0.25 mm diameter</td>
<td>Sigma-Aldrich (32703-4) · Sigma-Aldrich (49654-5)</td>
</tr>
<tr>
<td>Graphite rods</td>
<td>Counter electrodes for etching process</td>
<td>High density, nonporous (e.g., glassy graphite)</td>
<td>Sigma-Aldrich (13-678-20C)</td>
</tr>
<tr>
<td>Glass pipette</td>
<td>Outer casing of combined sensors</td>
<td>Disposable Pasteur pipettes</td>
<td>Warner Instruments (G86150T-4, G75150T-4) · Schott (8533) · Macalaster Bicknell Co.</td>
</tr>
<tr>
<td>Glass micropipette</td>
<td>Glass capillary tubing</td>
<td>Low electric conductivity glass · Borosilicate glass · Corning 8161 (potash rubidum lead): amperometric microsensor · Corning 7056: LI X microsensor</td>
<td>Fisher Scientific (B12069-15) · WPI (PG10150-4)</td>
</tr>
<tr>
<td>Au solution</td>
<td>Au electroplating</td>
<td>H AuCl₅·3H₂O · Au plating solution</td>
<td>Sigma-Aldrich (G4022-5G) · Sifco Metachemical (Dalic 3020)</td>
</tr>
<tr>
<td>KCN</td>
<td>Electrochemical etching</td>
<td>Potassium cyanide ACS grade</td>
<td>Sigma-Aldrich (31252-250G) · Sigma-Aldrich (180955)</td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>Membrane used on amperometric microsensor</td>
<td>Cellulose acetate solution 5%-10% (w/v) in acetone</td>
<td></td>
</tr>
<tr>
<td>Bismuth alloy</td>
<td>Conductive filling material for electroplating Au for DO microsensors</td>
<td>117°F low-melting-point alloy: 44.7% bismuth, 22.2% lead, 19.1% indium, 8.3% tin, and 5.3% cadmium</td>
<td>Belmont Metals, Inc. (2451A)</td>
</tr>
<tr>
<td>Si</td>
<td>Membrane for DO or N₂O microsensor</td>
<td>Silastic medical adhesive Si type A</td>
<td>Dow Corning (Silastic or 732)</td>
</tr>
<tr>
<td>Injection needle</td>
<td>Backfilling of internal reference solution</td>
<td>Fine injection needle</td>
<td>WPI (M F28G-5)</td>
</tr>
<tr>
<td>Liquid membrane</td>
<td>LI X⁻ membranes for ion-selective microsensors</td>
<td>Hydrogen ionophore · Ammonium ionophore · Nitrate ionophore</td>
<td>Fluka, Sigma-Aldrich (95293, 95297, 90879, or 72549)</td>
</tr>
<tr>
<td>Salinization solution</td>
<td>Used to make glass pipette surface hydrophobic</td>
<td>Salinization solution · N,N,N-dimethyltrimethylsilylexylamine</td>
<td>Fluka, Sigma-Aldrich (85120, 41716-5ml)</td>
</tr>
<tr>
<td>Puller</td>
<td>Pulling glass micropipette</td>
<td>Flaming/brown micropipette puller</td>
<td>Sutter Instrument (P-1000)</td>
</tr>
</tbody>
</table>

(continued)
TABLE 7.2 (continued)  Materials and Devices Necessary for Needle-Type Environmental Microsensors Fabricationa

<table>
<thead>
<tr>
<th>Item</th>
<th>Use</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illuminator</td>
<td>Light source</td>
<td>NOVAFLEX fiber optic illuminator</td>
<td>WPI (NOVA)</td>
</tr>
<tr>
<td>AC power supply</td>
<td>Etching process</td>
<td>Variable transformer</td>
<td>Staco (3PN1010B)</td>
</tr>
<tr>
<td>DC power supply</td>
<td>Electroplating</td>
<td>Variable single output dc power supply (0-18 V dc at 0–3 A)</td>
<td>Omnitron Electronics (HY1803D)</td>
</tr>
<tr>
<td>Micromanipulators</td>
<td>Micromovement during construction</td>
<td>Manual micromanipulator</td>
<td>WPI (M 3301)</td>
</tr>
<tr>
<td>Micropipette beveler</td>
<td>Grinding ME tips</td>
<td>Grinder, microscope, micromanipulator, and light source</td>
<td>Sutter Instrument (BV-10)</td>
</tr>
<tr>
<td>Faraday cage</td>
<td>Screen electromagnetic fields</td>
<td>Bench top Faraday cage</td>
<td>Technical Manufacturing (81-334-06)</td>
</tr>
<tr>
<td>Vibration-free platform</td>
<td>Prevent vibrations</td>
<td>Vibration-free platform</td>
<td>WPI (VFP)</td>
</tr>
<tr>
<td>Stereomicroscope</td>
<td>Monitor heat element</td>
<td>Precision stereo zoom binocular microscope</td>
<td>WPI (PZM IV-BS)</td>
</tr>
<tr>
<td>Upright light microscope</td>
<td>Monitoring and inspection of ME tips</td>
<td>Profession grade microscope: 4×, 10×, 40×</td>
<td>WPI (W 305)</td>
</tr>
</tbody>
</table>

*a The information in the table is for reference purposes only. We do not promote any of the products specified in the table.
*b World Precision Instruments (WPI).
*c There is a need for a special type of glass with high insulation properties (i.e., Schott 8533 green glass) in the fabrication of oxygen microsensors in order to achieve a low background current.
*d LIX, liquid ion exchange.

PalmSens is delivered with a shielded cable with connections for a working (gray) electrode, a counter (hatched) electrode, and a reference (solid black) electrode by means of 2 mm banana connectors with mini-crocodile clips.

```plaintext
- Scan rate: 100 mV/s
- Potential range: –1000 (beginning potential) to +1000 mV (end potential)
- Step potential: 20 mV
```

FIGURE 7.3 Schematic drawings of a CV test of amperometric microsensors. An example of the operation range and the connection is described.
7.4 Potentiometric Microsensors

Most ion-selective microsensors are potentiometric sensors where the voltage (mV generated vs. Ag/AgCl) is theoretically dependent on the logarithm ionic activity, according to the Nernst equation (Equation 7.2):

\[
E_{\text{cell}} = E_{\text{external ref.}} - E_{\text{internal ref.}} + E_{\text{membrane}} + E_{\text{junction}}
\]

\[
= \Delta E_{\text{ref}} + \frac{RT}{ZF} \ln \frac{A_{\text{(external)}}}{A_{\text{(internal)}}} + E_{j}
\]

\[
= k + \frac{RT}{ZF} \ln A_{i(\text{sample})} = k' + \frac{0.059}{Z} \log C_{i(\text{sample})}
\]

where

- \( E_{\text{cell}} \) is the potential difference between the ion-selective microsensor and the reference electrode
- \( E_{\text{membrane}} \) is the membrane potential
- \( E_{\text{external ref.}} \) and \( E_{\text{internal ref.}} \) are potentials of the external and internal reference electrodes, respectively
- \( E_{\text{junction}} \) is the liquid junction potential
- \( A_i \) is the ionic activity (\( A(\text{activity}) = \gamma(\text{activity coefficient}) \times C(\text{concentration}) \))
- \( z \) is the number of electrons involved in the reaction
- \( F \) is the Faraday constant
- \( R \) is the gas constant
- \( T \) is the absolute temperature

The response time of a potentiometric microsensor depends on the concentration of the target ions. Therefore, it varies from a few seconds (at high concentration) to several minutes (low concentration). For example, the response time of a copper ion-selective microsensor varies from less than 60 s (\( \geq 10^{-6} \text{ M Cu}^{2+} \)) to 10 min (\( < 10^{-6} \text{ M Cu}^{2+} \)) [22].

Liquid-ion-exchange (LIX) membrane ion-selective microsensors (Figure 7.2c) have been fabricated and used to measure specific ions such as pH, ammonium, and nitrate in biological samples. The key to the ion-selective microsensor is the LIX membrane, which contains a nitrogen neutral carrier for the target ion. Neutral carrier makes it possible to measure the potential of the target ion’s activity in the presence of other ions. Therefore, it can detect the target ion without any ion interference. Figure 7.2c shows the LIX membrane of the microsensor that is separated from the electrolyte. Liquid membrane length is generally 300 (50–1000) \( \mu \text{m} \), and its lifetime depends on the length of the membrane, varying from 1 day to several weeks. A typical lifetime of an ion-selective microsensor for pH, ammonium, and nitrate is 2–3 days with a 500–1000 \( \mu \text{m} \) length of liquid membrane. Most ionophores for ion-selective MEs can be purchased from Fluka, Sigma-Aldrich (Switzerland) under the trademark Selectophore™ (http://www.sigmaaldrich.com/analytical-chromatography/analytical-reagents/sensoric-applications.html). Recently, ion-selective microsensors with a built-in reference electrode have been introduced to remove outer environmental interferences (e.g., low conductivity) during microprof le measurements [28]. Detailed fabrication procedures are described elsewhere [5,24]. One of the important steps is the silanization step. If LIX is not contained at the tip of the microsensor, this indicates that the silanization step was not done correctly. One possible reason is the contamination of the furnace (i.e., if the furnace is a general use one, organics may be deposited and the silanization will fail. Avoid any dirt, grease, or organics on the case before silanization).

7.5 Data Acquisition and Processing

The microsensor is positioned above the sample (e.g., biofilm) perpendicular to its surface when microprof le is measured using a microsensor. Then it is moved to the bottom of the biofilm and is used to measure concentration prof les at a microscale (Figure 7.4). From the measured concentration prof les, we can
understand transport processes in the biofilm by calculating the rates and net fluxes of constituents (e.g., oxygen, ammonia, and nitrate) and/or diffusion coefficients using Fick’s first law of diffusion (Equation 7.3):

$$J = -D_s \frac{dC}{dx}$$

where

- $J$ is the net flux (nmol/cm$^2$ s)
- $D_s$ is the molecular diffusion coefficient of compound $C$ in water
- $dC/dX$ is the concentration gradient

The general experimental setup for microprofile measurements [27] includes a flow chamber (custom-made), stereo microscope (Nikon, SM Z-2T; PZM TIV-BS-CCTV, WPI, Sarasota, FL) with CCD camera (Model JE-3662 HR, Javelin Elec., Torrance, CA; 501381, WPI, Sarasota, FL), video display (501385, WPI, Sarasota, FL), data acquisition system (Diamond General Corp, Product 1231 for amperometric sensors; mV meter [Model 215, Denver Instruments] for potentiometric sensors; microsensor multimeter [Unisense A/S, Denmark] with SensorTrace Pro 2.0 software [Unisense A/S, Denmark] for multisensors), 3-D micromanipulators (Model MO-203, Narishige, Japan; MMS, Unisense A/S, Denmark), Faraday cage (Technical Manufacturing Co., Peabody, MA), vibration isolation table, and peristaltic pump systems to flow the medium.

### 7.6 MEMS Sensor Arrays

As discussed earlier, MEMS can be fabricated in a number of ways [29,30], most commonly by pulling a glass micropipette, inserting a noble metal wire such as Pt or Au, and then filling with a low-melting-point alloy [3,4]. Although these MEM fabrication methods are well established, there are a number of inherent disadvantages including low success rate, poor reproducibility, fragility, and difficulty in making a multianalyte sensor device [31–33]. Further, these MEM sensors are susceptible to electric interference and have to be operated in specialized laboratories under highly controlled conditions. Therefore, there is a critical need for robust, sensitive, and easy-to-fabricate sensors for in situ measurements.

MEMS miniaturization and integration using MEMS technologies can offer many advantages for overcoming current difficulties for fabrication and integration of sensor components [16,27,33–43].
The key advantages of MEMS microfabrication technologies are (1) the reduction of costs due to batch fabrication, (2) an increase in integration, (3) reduction in power consumption due to smaller size, (4) reduction in complexity, and (5) increased reproducibility, thus producing a large number of uniform sensors. However, the most important advantage of using MEMS fabrication is the increase in sensor reliability due to redundancy and better process control.

Many researchers that apply MEMS technologies to solve sensor problems have focused on microfluidic lab-on-a-chip (LOC) systems [44–46]. Such systems typically contain microfluidic channels for sample collection, preparation, or transport with planar sensing areas for specific target analytes. Nevertheless, the key drawback of such systems is that samples still must be extracted from the site of interest, which often is not acceptable for in situ measurements. MEMS technologies have also been used to develop penetrating 3D MEMS sensors for neuroscience applications [47–50]. However, all of these needle-type MEMS electrodes have been designed for neural recording and can only measure potential (e.g., neuronal activity).

The recently developed needle-type environmental MEMS sensors, as summarized in Table 7.3, were tapered using the HF etch process, which is a typical MEMS fabrication process. The basic sensing principles are the same as described in Figure 7.1 for these miniaturized MEMS sensors. Figure 7.5 shows schematic diagrams and SEM images of various needle-type environmental MEMS sensors. There are currently no environmental MEMS sensors that are commercially available. The representative materials and fabrication equipment for needle-type environmental MEMS sensors are summarized in Table 7.4.

### 7.7 Amperometric MEMS Sensor Arrays

The DO MEMS sensor has a recess created at the tip of the ME, as shown in Figure 7.5b [27,36,37,40,43]. The fabrication process has five major steps:

- Dicing to form the array structure
- Etching to sharpen the probes
- Metal deposition for the conductive layer
- Packaging for electric connection
- Sensor tip formation for recessed DO MEMS sensor applications

The following sections are a brief description of the MEMS sensor fabrication process.

#### 7.7.1 Dicing to Form the Array Structure

Glass cover slips are diced to form the array structure of the glass probes. The probe length is 2 cm with 900 μm center-to-center spacing between each glass probe. The cut wafer is then annealed at 550°C for 10 min to relieve stress from the dicing process.

#### 7.7.2 Etching to Sharpen Probes

The probes are first etched in the HF-based etchant solution to smooth the diced surface and reduce the probe dimensions from 175 μm in thickness × 190 μm in width to 85 μm × 90 μm. Next, the glass probes are gradually pulled out to taper them down to 20 μm × 20 μm at the tip. In the final etching step, the glass probes are sharpened to ~200 nm tips using chemical meniscus etching, which forces surface tension at the glass–etchant interface. The process is schematically illustrated in Figure 7.6a.

#### 7.7.3 Metal Deposition for Conductive Layer

The sharpened probes are deposited with a conductive layer, Ti/Au (20 nm/200 nm thick), on all sides by thermal evaporation. Figure 7.6b shows that twelve ME arrays containing 48 MEs were all metalized at the same time.
### Table 7.3 Overview of Needle-Type Environmental MEMS Sensors

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Type</th>
<th>Analytical Methods/Mechanisms</th>
<th>Slope(^a), Conc. Range (LOD(^b))</th>
<th>Key Materials/Compound/Procedures</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redox potential</td>
<td>Solid</td>
<td>Potentiometry, Electrode potentials between a metal electrode and a standard reference electrode given by the Nernst equation, Measures the oxidized and reduced forms of all chemical species in the solution (nonselective)</td>
<td>1 mV/mV, &lt;1 s, 0–8.3 mg O(^2)/L (23°C)</td>
<td>Core material is a glass or a Si, Au thin film as a working electrode, Meniscus etching (chemical sharpening process) to sharpen penetrating needle-type ME, American Society for Testing and Materials (ASTM) recommends four calibration redox standard and reference solutions within 10 mV of the nominal redox potentials (ASTM D 1498-93)</td>
<td>[27,35,42]</td>
</tr>
<tr>
<td>Oxygen (O(_2))</td>
<td>Recessed(^c)</td>
<td>Amperometry, Diffusion using DO is reduced at the cathode to create a measurable electric current, Linear relationship between the oxygen concentration and the electric current</td>
<td>~147 pA/mg/L, DO, &lt;20 s</td>
<td>Parylene C layer (1.5 μm thick) to insulate individual MEs, T recess process: Parylene C deposition, beveling tip end, and etch glass/Au, Au thin film as a working electrode, Apply ~750 mV polarization voltage</td>
<td>[27,36,37,40,41]</td>
</tr>
<tr>
<td>Phosphate (P)</td>
<td>Solid</td>
<td>Potentiometry, Co metal electrodes detecting the inorganic and organic phosphate, Oxidation corrosion potential (mV) measurement on CoO surface, Reaction between CoO and phosphate ion depending on the solution pH</td>
<td>96 mV mV/dec, ~1-30 s, 10^-6–10^-3 M (2.2 × 10^-6 M)</td>
<td>Co electroplating using Co plates as anodes, Oxidize by immersing in DI water along with a Ag/AgCl reference electrode to generate CoO layer at the tip, Oxygen affects activity of phosphate ions</td>
<td>[16,38,40–42]</td>
</tr>
<tr>
<td>pH</td>
<td>Solid</td>
<td>Potentiometry, InOx electrode without an ion-selective membrane, Electrode potentials given by the Nernst equation</td>
<td>~62 mV/pH, pH 2–12, &lt;5 s</td>
<td>Electroplate InOx using InOx electrolyte solution, Plasma oxygen etch to remove Parylene C insulate layer</td>
<td>[27,41,42]</td>
</tr>
</tbody>
</table>

\(\text{a}\) Slope of linear regression indicating sensitivity.

\(\text{b}\) Limit of detection.

\(\text{c}\) Types of DO-ME are used for DO measurements: the Clark-type, the open-type, and recessed DO-ME. The Clark-type DO-ME consists of a working electrode with a gas-permeable membrane and an internal reference electrode in the electrolyte. The open-type and recessed DO-MEs use an external reference electrode, thus, the miniaturization of these sensor types can be achieved easily with simpler fabrication and smaller working electrode size.

\(\text{d}\) Inorganic phosphate including \(\text{H}_2\text{PO}_4^-\), \(\text{HPO}_4^{2-}\), and \(\text{PO}_4^{3-}\).

\(\text{e}\) Organic phosphate including di-sodium adenosine 5'-triphosphate (ATP), di-sodium adenosine 5'-diphosphate (ADP), and di-sodium adenosine 5'-monophosphate (AMP).
7.7.4 Packaging for Electric Connection

To establish electric connections with individual sensors, MEMS array are packaged on a printed circuit board (PCB) with conductive silver epoxy. Parylene C is coated over the entire substrate to insulate individual MEMs.

7.7.5 Sensor Tip Formation

To create the recess, the HF-based etchant is used to etch the glass core, and the Ti is exposed by beveling. The beveling and etching steps permit precise control of the recess opening size and depth to relocate the Au sensing area inside the formed recess.

The finished MEA sensor consists of four 2 cm long sharpened MEMs packaged on a PCB carrier as is shown in Figure 7.6d. The tip sizes and the overall dimensions of the MEMS sensor array are compared with those of commercially available millielectrodes (COMs) and MEM sensors in Figure 7.6c. On, the DO MEMS sensors are polarized with an applied −0.75 V and calibrated with a commercial Ag/AgCl reference electrode. The completed MEMS sensor has a high sensitivity of ~147 pA/mg/L DO, with less than 20 s required reaching 90% response (Table 7.3).

7.8 Potentiometric MEMS Sensor Arrays

Table 7.3 shows three potentiometric MEMS sensors including redox potential (oxidation-reduction potential [ORP]), phosphate, and pH sensors. These MEMS sensors are good examples for potentiometric sensors with/without an ion-selective membrane. The ORP sensor is the most basic electrode
<table>
<thead>
<tr>
<th>Item</th>
<th>Use</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borosilicate glass wafer</td>
<td>Core structure material for working electrode</td>
<td>175 μm thick, 45 mm × 50 mm</td>
<td>Erie Scientific, Pittsburgh, PA</td>
</tr>
<tr>
<td>Dicing saw</td>
<td>Cut individual glass probes</td>
<td>45 μm diamond grit resinoid blades (MicroSwiss)</td>
<td>K&amp;S (740)</td>
</tr>
<tr>
<td>Programmable box furnace</td>
<td>Anneal glass wafers to relieve stress from the dicing process</td>
<td>Programmable target temperature and hold time</td>
<td>Thermo Scientific, Norwood, MA (Lindberg/Blue M)</td>
</tr>
<tr>
<td></td>
<td>Use cooling steps to reduce thermal shock</td>
<td>Reliable uniform temperature inside furnace</td>
<td></td>
</tr>
<tr>
<td>Motorized linear translation stage</td>
<td>Control glass probes to pull out gradually from glass etchant</td>
<td>Computer-controlled (via LabView v.7) translation stage</td>
<td>Newport Corp., Irvine, CA</td>
</tr>
<tr>
<td></td>
<td>Taper glass probes</td>
<td>Minimum step size is 1.57 μm</td>
<td></td>
</tr>
<tr>
<td>Organic layer</td>
<td>Modify the contact angle at the glass-etchant interface</td>
<td>Vegetable oil</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td></td>
<td>Prevent vaporizing toxic HF-based etchant solution</td>
<td>Paraffin oil</td>
<td></td>
</tr>
<tr>
<td>Metal targets</td>
<td>Evaporation targets</td>
<td>99.99% pure</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td></td>
<td>Titanium (Ti), chromium (Cr), Au, Pt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-beam evaporator</td>
<td>Metal deposition</td>
<td>Evaporation Ti, Cr, Au, Pt</td>
<td>Airco Temescal (FC-1800)</td>
</tr>
<tr>
<td>PCB</td>
<td>Establish electric connections with individual sensors</td>
<td>Copper-clad laminate glass epoxy</td>
<td>D&amp;L Products, Inc.</td>
</tr>
<tr>
<td></td>
<td>Carriers for easier handling</td>
<td>Lamine (790 μm thick)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copper (35 μm thick layer) and dry film negative photoresist (33 μm thick layer)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Photolithographically pattern and etch in ferric chloride to define designed traces on the PCB surface</td>
<td></td>
</tr>
<tr>
<td>UV-cured epoxy</td>
<td>Fix MEs on PCB carriers</td>
<td>Epoxy bond</td>
<td>Loctite, Rocky Hill, CT (3301)</td>
</tr>
<tr>
<td>Conductive silver epoxy</td>
<td>Conductive filling material for electroplating Au for DO microsensors</td>
<td>Conductive silver-based low curing temperature (120 ºC)</td>
<td>Emerson &amp; Cuming, Billerica, MA (Ablebond 8700E)</td>
</tr>
<tr>
<td>Parylene C</td>
<td>Insulate individual MEs</td>
<td>A well-known biocompatible polymer material</td>
<td>Specialty Coating Systems, Indianapolis, IN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inert and optically transparent material with the pinhole-free surface</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 7.4 (continued) Materials and Devices Necessary for Needle-Type Environmental MEMS Sensor Array Fabrication

<table>
<thead>
<tr>
<th>Item</th>
<th>Use</th>
<th>Description</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parylene coater</td>
<td>Deposit Parylene C easily and uniformly on any substrate</td>
<td>Simple vapor deposition</td>
<td>Specialty Coating Systems (PDS 2010 Parylene Lab)</td>
</tr>
<tr>
<td>Micropipette beveler</td>
<td>Bevel ME tips</td>
<td>Beveler, microscope, micromanipulator, and light source</td>
<td>Sutter Instrument (BV-10)</td>
</tr>
<tr>
<td>Potentiostat</td>
<td>Electroplate metal layers</td>
<td>Galvanostatic mode</td>
<td>Gamry, Inc. (Reference 600)</td>
</tr>
<tr>
<td></td>
<td>Supply the polarization voltage and current</td>
<td>Control current and voltage</td>
<td>Diamond General Development Corp. (No 1231)—Chemical Micsensors II potentiostat</td>
</tr>
<tr>
<td>pH/mV meter</td>
<td>Measure pH and potential</td>
<td>Data acquisition system</td>
<td>Denver Instrument (model 225)</td>
</tr>
<tr>
<td>IrOx</td>
<td>Use solid-state working electrode for pH sensor</td>
<td>Electroplate IrOx f lm</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>IrOx electrolyte solution</td>
<td>Pulling glass micropipette</td>
<td>Mixture of iridium tetrachloride (IrCl₄), hydrogen peroxide (H₂O₂), oxalic acid (C₂H₂O₄), and potassium carbonate (K₂CO₃) in DI water</td>
<td>Sutter Instrument (P-1000)</td>
</tr>
<tr>
<td>Cobalt (Co) electrolyte solution</td>
<td>Grinding ME tips</td>
<td>Mixture of 33 g CoSO₄ and 3 g H₂BO₃ in 100 mL of DI water</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Piranha etchant</td>
<td>Clean glass surface</td>
<td>H₂SO₄ and H₂O₂ in a 7:3 (v/v) ratio</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>HF-based etchant solution</td>
<td>Glass etchant</td>
<td>HF, HNO₃, and H₂O in a 10:7:33 (v/v/v) ratio</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Au etchant</td>
<td>Etch Au</td>
<td>Mixtures of I₂, KI, and H₂O (1:4:40 (m/m/v)) (1:4:40 (m/m/v))</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Ag/AgCl reference electrode</td>
<td>Construction of Ag/AgCl</td>
<td>Commercial Ag/AgCl reference ME</td>
<td>Microelectrodes Inc. (M1-401)</td>
</tr>
<tr>
<td>T ick photore sist buffer</td>
<td>Buffer for beveling and plasma ashing</td>
<td>5-8 μm</td>
<td>Clariant Corporation (AZ 4562)</td>
</tr>
<tr>
<td>Plasma oxygen ashing</td>
<td>Etching tip of electrodes</td>
<td>50 μm</td>
<td>March CS-1701 RIE</td>
</tr>
</tbody>
</table>

and is used to measure the oxidized and reduced forms of all the chemical species (nonselective) in the solution. The working electrode just needs to have its exposed conductive area in contact with a solution without the need for an ion-selective membrane, as shown in Figure 7.5a [35,42]. ORP electrodes directly measure the potential of a solution between a metal electrode and a standard reference electrode, given by the Nernst equation (Equation 7.2).
For phosphate MEA sensors, cobalt (Co) is electroplated to form a Co film as an ion-selective membrane filling the empty recessed area, as depicted in Figure 7.5b [38]. Two Co plates are used as anodes in the prepared Co electroplating electrolyte solution. Co is electrodeposited to a ~0.2 \( \mu \)m thickness on the Au tips. The MEMS phosphate sensor measures oxidation corrosion potential (mV) on the cobalt oxide (CoO) surface, in conjunction with a reference electrode.

The MEMS pH sensor uses an electroplated iridium oxide (IrOx) film as a working electrode in place of an ion-selective membrane [42]. To achieve a more durable sensor for penetrating into a sample, the IrOx film is used in a solid-state electrode without the attachment of an ion-selective membrane. The fabrication process for the needle-type pH sensor is composed of six major steps: dicing, etching, metalization, IrOx electroplating, packaging, and sensor tip formation. The first three steps are the same as the batch fabrication processes previously described in Section 7.3. The final three steps to form a solid-state electrode for the pH MEMS sensor are discussed in detail later.

### 7.8.1 IrOx Electroplating

After the Ti/Au conductive layer deposition, IrOx is electroplated on the entire Au seed layer. Dark blue IrOx films are obtained with a thickness of 100–150 nm. Fresh IrOx-coated sensors are stabilized in pH 7 buffer for 2 days to reduce potential drift during measurements.
7.8.2 Packaging for Electric Connection

ME arrays are packaged on a PCB, followed by IrOx electrodeposition. Parylene C is coated over the entire substrate to insulate MEs.

7.8.3 Sensor Tip Formation

Two steps are used to expose a ~50 μm long IrOx sensing tip. The first step is to cover the entire sensing tip using photoresist, and then ~50 μm long sensing tips are stripped by acetone. Secondly, the plasma oxygen process is used to etch Parylene C on the exposed sensor tip end. The schematic diagram of the MEMS pH sensor is illustrated in Figure 7.5c.

7.9 Multianalyte MEMS Sensor Arrays

The individual needle-type MEA sensor for measuring ORP, DO, and phosphate, respectively, can be integrated into a single multianalyte sensor array as shown in Figure 7.6d, exhibiting higher sensitivity, faster response time, and higher stability with a smaller tip size than the conventional sensors. The major advantages of these new MEMS sensors include the ability to penetrate samples to perform measurements, the small tip size for in situ measurements, array structure for higher robustness, and the possibility of multianalyte detection. These sensors demonstrate the ability to monitor local concentration changes in small structures with a high spatial resolution and offer the versatility of the ME technique as well as the capability for repetitive measurements.

The integration process was aimed at easier and simpler fabrications for mass production with a minimum number of steps. Following the metallization step, the integration process begins with the application of Parylene C insulation to the ME tips. All four MES in an array were beveled at the same time to expose glass and Ti/Au layers. The recess etching process was used to form the recessed structure. Since Au exposed to the solution enables ORP measurements, the recessed structure can measure not only ORP but also DO. For phosphate sensors, Co was electrodeposited on the exposed Au tips. Ultimately, the multianalyte MEMS sensor array will enable in situ measurements in a wide variety of small sample applications in environmental engineering and life sciences.

7.10 Data Acquisition and Processing

MEMS sensors can use the same general experimental setup for microprofile measurement described in the Section 7.2. However, the ability to perform an in situ measurement outside of the controlled laboratory environment of a well-grounded Faraday cage is based on the integration of the MEs with a complementary metal oxide semiconductor (CMOS)-based integrated circuit (IC) for noise cancellation.

The CMOS chip has been developed to perform both signal acquisition and processing [35] and then integrated with the MEMS sensor. The circuit for potentiometric measurements with MEMS sensors is based on the CMOSASIC design. The circuit block is divided into three stages: a low pass filter, an isolation amplifier, and an instrumentation amplifier. The built-in noise cancellation design eliminates both environmental and instrumental noise, permitting measurement of voltages in the 10⁻³–10⁻⁶ V range outside the Faraday cage.

7.11 Application for Environmental Processes

Figure 7.7 shows the areas where microsensors are or could be applied in engineered and natural water systems. Biofilm control issues in drinking water have been one of the high-priority research areas. A NH₂Cl microsensor has been used to evaluate biofilm control strategies and nitrification in
chloraminated drinking water distribution systems, by combining with environmental microbiological approaches (e.g., confocal laser scanning microscopy [CLSM], cryo-cross sectioning, fluorescent in situ hybridization [FISH]) and other microsensors (e.g., oxygen, free chlorine, ammonium, pH, and nitrate) [25,26]. Microsensor applications can extend to the characterization and evaluation of corrosion and biocorrosion (e.g., localized corrosion and electrochemical activity distribution on pipe surfaces in real time with high spatial resolution) during disinfection in water infrastructure (e.g., ductile/cast iron pipe, copper, lead, and concrete pipe materials) and also provide significant impact on other industrial water infrastructures (e.g., ship hulls, ballast water disinfection, and cooling water systems), which experience corrosion and biocorrosion. One example of future applications is an envisioned field-deployable version of real-time monitoring of chloramine species in drinking water distribution systems (including premise plumbing).

In the bioremediation processes, it is important to investigate the dynamics of soil biofilm structure and function, related kinetics, and reaction mechanisms for better understanding of bioremediation processes such as degradation of hydrophobic petroleum compounds [51]. The appropriate modeling kinetic parameters can be determined using microsensors for better biological processes.

In wastewater treatment plants, environmental microsensors have been used for decades to understand the mechanics of relative biological processes and/or lead to the improvement of modeling, design, and operation of existing biological water/wastewater treatment processes including membrane processes for nutrient removal [3,8,13]. Microsensor applications for membrane biofilm processes [52] and microbial fuel cells (MFCs) [28,53] have also increased to improve engineering processes that produce renewable energy while reducing the energy requirements for a treatment plant. In situ monitoring using microsensors also prevents possible contamination of natural systems and/or treatment systems, and thus we can readily and continuously monitor water quality and prevent an impending event such as toxicity breakthrough or operational malfunction.

Microsensors have also been used in the fields of ecology and environmental science, such as to study the microenvironments in lake (or marine) sediments [54], with zebra mussel issues (e.g., nitrite/fication in shell biofilm) [55], with invertebrates (e.g., denitrification in the gut) [56], with rhizosphere soil [57], and with roots of submerged plants [58] where important constituents' transport
(e.g., oxygen, phosphate) or nitrogen cycling occurs. The sensor techniques can be extended to in-field integrated water quality monitoring systems (e.g., monitoring of global climate change and eutrophication control) over a large area, providing real-time water quality data by combining with geographic information system (GIS) technology.

7.12 Outlook of Needle-Type Environmental Sensors

Needle-type environmental microsensors have been used to monitor environmental conditions and contaminant profiles in situ in various environmental samples (e.g., biofilm, pore water, and sediments). Their use for protection of human health and the environment has increased with the advanced fabrication techniques and improvements for stable data acquisition. In this chapter, we focused on needle-type microsensors for environmental monitoring, but they can also be applied to industrial and biomedical areas. Consequently, the development and application of microsensors have a great potential for collaboration with a wide range of disciplines. Potential research areas for new environmental microsensors would be hydroxylamine (NH₂OH), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metal (e.g., lead, mercury, and arsenic) monitoring sensors. For example, NH₂OH is a key intermediate during the process of the nitrogen cycles (e.g., ammonia oxidizing bacteria) and the production of N₂O, a major greenhouse gas. Therefore, a sensor for quantitative determination of NH₂OH will be very important, both in studies of biological processes and for industrial purposes. Antifouling methods (e.g., functionalized and controlled surface design and biomimetics) are also challenging areas to prevent biofouling during in-situ monitoring and will provide more reliable sensor surface technology. Furthermore, there is a critical need for highly sensitive and selective low-power chemical sensors for a broad range of applications from environmental monitoring, food safety, health diagnostics, as well as global security and global warming. Although many sensitive transducer platforms have been developed, including microsensors, the long-standing fundamental problem of sufficient sensitivity (sub-ppm) and selectivity has not yet been solved. The key component for accomplishing ultrahigh sensitive sensors is an innovative improvement of sensor technology. Nanoelectromechanical systems (NEMS) technologies can permit the integration of different mechanical elements, sensors, and electronics in a small device and therefore can be suitable for the development of multianalyte sensors. The current roadblock to the implementation of such ultrahigh sensitive multianalyte sensors is the lack of nanotransducer platforms, which can be integrated with various reliable membrane materials.

Acknowledgments

The authors gratefully acknowledge financial support of this work by grants from the National Science Foundation (BES-0228603, BES-0529217), the National Institute of Environmental Health Sciences (P42ES04908-16/Project 5), the USEnvironmental Protection Agency (EP-C-05-056), and the University of Cincinnati Institute for Nanoscale Science and Technology.

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