pH-Dependent response of a hydrogen peroxide sensing probe

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The response of a fiber-optic hydrogen peroxide (H2O2) sensing probe is investigated under a range of pH conditions. The H2O2 optrode is prepared by depositing a thin layer of Prussian blue (PB) onto the tip of a multimode optical fiber, and its detection mechanism is based on the PB/Prussian white (PW) redox reaction. PW is oxidized to PB by H2O2, leading to changes in the absorption of visible light. The sensor can be restored after usage by reducing the PB to PW with ascorbic acid. This optrode was previously shown to have good repeatability and sensitivity to H2O2 concentrations at room temperature and a pH of 4. For practical applications, the sensor must be capable of detecting H2O2 in environments spanning a range of pH conditions, including biological and industrial environments which together span pH values from 7 to less than 3. We present experiments using multiple PB optrodes, at pH values ranging from 7 to 2, and for different concentrations of H2O2. The work demonstrates that the optrode remains functional and provides stable and reproducible measurements of H2O2 under a range of pH conditions that correspond, in particular, to those in an operating PEM fuel cell.

**Introduction**

Hydrogen peroxide (H2O2) is a by-product of oxidative metabolisms [1,2]. It is involved in many biological processes and plays a leading role in a variety of damage mechanisms. For example, H2O2 induces cellular damage in human cells [3,4] and its presence in exhaled breath and breast cancer cells can be used to diagnose illnesses such as asthma and human breast cancer, respectively [5,6]. On the other hand, H2O2 is also believed to play a central role in chemical degradation of polymer membranes in PEM fuel cells [10]. A versatile H2O2 sensor that functions in environments spanning a range of pH values is therefore of practical importance in various fields. In this study we investigate the functionality of an H2O2 optrode at different pH values, and in particular, at pH value corresponding to those in an operating fuel cell, to assess its suitability for eventual in situ monitoring of H2O2 in PEM fuel cells.

H2O2 sensors are usually based on the electrochemical and spectroscopic techniques [11–17], with spectroscopic techniques being preferred for many biochemical and industrial applications because of their immunity to electromagnetic interference. On the other hand, Prussian blue (PB), ferric ferrocyanide, is widely used for making sensors and biosensors for detection and measurements of H2O2 [11,18]. In particular, optrodes based on PB have shown reliable response to H2O2 concentration [19,20]. These sensors were prepared by depositing PB onto the tip of multimode optical fibers, with PB serving as a spectroscopic indicator of H2O2. Because optical fibers are made of non-conductive materials, the chemical deposition of PB has been preferred for constructing PB-based fiber-optic sensors. Among chemical deposition methods, single-source precursor and layer-by-layer (LbL) techniques have been successfully used to prepare PB-based H2O2 optrodes [14,20]. However, the performance of PB-based H2O2 sensors prepared with LbL is limited in environments spanning a range of pH values and although the performance of this type of sensors is slightly improved by adding capping bilayers on the LbL-based PB film, long-term stability is poor.

The single-source precursor technique is a synthesis approach in which all the elements required in the final product are incorporated into one compound. In this technique, the precursor may participate in several consecutive reactions to arrive at the final product. The single-source precursor technique was selected in this
work to deposit PB onto the optical fiber because this approach leads to sensors with better long-term stability in aqueous solutions at pH 4 [20]. The single-source precursor technique is a more straightforward process than the LBL technique and results in more stable PB film on the tip of the optical fiber. This is likely due to the different structure of PB that is afforded though the single-source precursor technique compared to the structure that is obtained through the LBL technique. It has been observed that the pH stability of the PB film also depends on the deposition process [18].

A potential application of such a sensor is integration into a PEM fuel cell for in situ detection and measurement of H2O2 concentrations. The sensor is well suited for this application because of its small size, immunity to electromagnetic interference, and compatibility of the fiber material (i.e. silica glass) with the electrochemically active environment inside a fuel cell. Since H2O2 is a product of glucose oxidation in the presence of glucose oxidase and oxygen, the proposed optrode has also potential for indirect measurement of glucose content [21].

The H2O2 spectroscopic detection mechanism relies on the redox reactions of the PB/PW system, and the evaluation of corresponding changes of the optical properties of the compound. The optrode is first reset by reducing PB to PW in the presence of a reducing agent, such as ascorbic acid, as shown in Eq. (1). When the PW-modified fiber is exposed to an oxidizing agent, such as H2O2, the reverse reaction (i.e. PB formation) occurs, as shown in Eq. (2). PB has a strong intervalence charge transfer absorption band near 700 nm due to a transition from Fe^{III}Fe^{II} to Fe^{II}Fe^{III} states. On the other hand, PW does not have any distinct absorption bands in the visible range [22]. As a result, the increasing absorbance in the visible range as PW oxidizes to PB, in the presence of H2O2, can be used to detect the presence of this oxidant. Moreover, the initial PW state can be recovered by exposing PB to ascorbic acid. Fig. 1 illustrates the detection mechanism of H2O2 using the optrode based on chemically deposited PB/PW system onto the tip of an optical fiber.

\[
\text{Fe}_4[\text{FeCN}_6]_{3(PB)} + 2\text{C}_6\text{H}_8\text{O}_6(\text{Ascorbic acid}) + 4\text{K}^+ = 4\text{KFe}_4[\text{FeCN}_6]_{3(PW)}+ 2\text{C}_6\text{H}_8\text{O}_6 + 4\text{H}^+ \quad (1)
\]

\[
\text{K}_4\text{Fe}_4[\text{FeCN}_6]_{3(PW)} + 2\text{H}_2\text{O}_2 = \text{Fe}_4[\text{FeCN}_6]_{3(PB)}, 4\text{OH}^- + 4\text{K}^+ \quad (2)
\]

In this study we investigate the functionality of an H2O2 optrode at different pH values, and in particular, at pH value corresponding to those in an operating fuel cell, to assess its suitability for eventual in situ monitoring of H2O2 in PEM fuel cells. An H2O2 optrode was prepared using the single-source precursor technique for deposition of PB onto the tip of multimode optical fibers. Sensing measurements in H2O2 buffered solutions with pH values, ranging from 7 to 2, were performed to investigate the functionality of the optrode. The sensing behavior of the probe, including stability and reproducibility, was investigated at the operating pH of PEM fuel cells (i.e. 2). Furthermore, sensing experiments were performed at three additional pH values, (i.e. 2, 5, and 6) and in each pH with different concentrations of H2O2 to study the pH dependency of the sensor response.

2. Experimental

2.1. Materials

Potassium hexacyanoferrate (III) (product No. 13746-66-2) from Sigma-Aldrich was used as a single-source precursor in an aqueous solution of hydrochloric acid (HCl) (37%) for synthesizing PB onto the tip of an optical fiber. L-ascorbic acid from Aldrich, (catalog No. 25,556-4), was used as the reducing agent for the PB to PW reaction. H2O2 (30 wt%) from ACP Chemicals was used to prepare the oxidizing solutions at different concentrations.

Potassium phosphate monobasic (product No. 7778-77-0) from Caledon, sodium phosphate dibasic (product No. 7558-79-4) from EM science, sodium phosphate monobasic (product No. 13472–35-0) from EM science, and Phosphoric acid (product No. 7664-38-2) from Caledon were used to prepare phosphate buffer solutions (PBS) at a range of pH values (i.e. 2, 3, 5, 6, and 7). The reducing solution is prepared in PBS with 0.04 mol L⁻¹ of L-ascorbic acid. Sodium hydroxide and phosphoric acid were used to adjust the pH of the solutions to the desired values.

All chemicals were used as received with no further purification. The water used in the experiments was purified with a four-cartridge purification system (Super-Q Plus, Millipore, Billerica, MA) and has a resistivity of 18.2 MΩ cm. The optical fiber is a multi-mode AF50/125Y from Thorlabs (Newton, NJ), with core and cladding diameters of 50 μm and 125 μm, respectively.
2.2. Sensor fabrication

The PB-based optrode was prepared using chemical deposition of PB film onto the tip of a multimode optical fiber. As reported in our previous work [20], 10 mmol L\(^{-1}\) of potassium ferricyanide, as a single-source precursor, is added to 25 mL of an aqueous solution of 0.1 mol L\(^{-1}\) HCl. The optical fiber is then immersed in this prepared mixture, and held at 40 °C under continuous stirring at 300 rpm for 10 h. The synthesis process is performed with exposure to fluorescent lamp light (Philips Alto, F28T5 25W). Finally, the optical fiber is left in the solution to cool to room temperature. The prepared optrode is then removed from the solution and left at room temperature for one day. We have explored a range of annealing temperatures and times and found that annealing at 100 °C for 15 min leads to the best performance of the optrode. Therefore, the sensor was annealed under these conditions.

2.3. Sensing test procedure and instrumentation

The sensing test procedure comprises three steps: First, the sensor response to aqueous solutions with different pH values (i.e. 2, 3, 5, 6, and 7) and containing 100 μmol L\(^{-1}\) of H\(_2\)O\(_2\) is evaluated. Then, the sensor behavior in concentrations of H\(_2\)O\(_2\) ranging from 5 μmol L\(^{-1}\) to 400 μmol L\(^{-1}\) is examined in solutions with a pH of 2.0 ± 0.1, which is the typical operating pH of a PEM fuel cell. Additional sensing measurements are performed at pH of 2.0 ± 0.1 with multiple sensors to study the performance of the sensors in terms of repeatability, durability, and reproducibility. Finally, several sensing tests are carried out in solutions containing various concentrations of H\(_2\)O\(_2\) at a range of pH values (i.e. 2, 5, and 6). Ascorbic acid and H\(_2\)O\(_2\) solutions are prepared in PBS at each pH. The pH of all of the prepared solutions are determined using a digital pH-meter (AR255, Accumet, Fisher Scientific, Hampton, NH).

Each sensing test is performed by alternately immersing the optrode to an ascorbic acid and H\(_2\)O\(_2\) solutions, for the recovery and oxidation steps of the PB/PW system. When the intensity of reflected light from the optrode plateaus to a minimum level, it is considered to have reached the PW state. The optrode is then removed from the ascorbic acid and placed in the H\(_2\)O\(_2\) solution and kept there until the intensity plateaus to a maximum, indicating the oxidation from PW to PB.

Fig. 2 illustrates the experimental setup used for collecting and measuring the reflected light from the optrode. The light from the white light source (LS-1, OceanOptics, Dunedin, FL), is carried to the optrode through arm 1 of a bifurcated optical fiber (BIF200-UV–vis, OceanOptics) and the common arm. Part of the scattered light from the PB/PW film on the tip of the optrode is collected by the optical fiber and guided back through the common arm and arm 2 of the bifurcated optical fiber into an optical fiber spectrometer (USB2000, Ocean Optics), which measures the intensity of the light in a wavelength range from 370 nm to 1048 nm. The reflected spectrum is sampled at a frequency of 1 Hz using the software, SpectraSuite (Ocean Optics, Dunedin, FL). The intensity data is integrated over the full range of the spectrometer (i.e. 370–1048 nm) and is used to study the response of the PB/PW system during both the oxidizing and the reducing steps.

3. Results and discussion

3.1. pH-dependent response

The pH-dependent response of the optrode is investigated in 100 μmol L\(^{-1}\) H\(_2\)O\(_2\) solutions at different pH values. Ascorbic acid and H\(_2\)O\(_2\) test solutions are prepared in PBS with pH of 2, 3, 5, 6, and 7. Fig. 3 illustrates the intensity of the light reflected from the optrode at different pH values. The circles and squares in Fig. 3 indicate the times at which the optrode is immersed in either the ascorbic acid or H\(_2\)O\(_2\) test solutions, respectively. The PB at the fiber tip is reduced to PW when immersed in ascorbic acid, and the PW is oxidized to PB when immersed in the H\(_2\)O\(_2\) test solutions. The reactions are time dependent because of the diffusion of the reducing/oxidizing agent into the PB/PW structure.

As shown in Fig. 3, the optrode is first immersed in an ascorbic acid solution with pH 7 to recover the sensor to the PW state and the subsequent detection step was done using an H\(_2\)O\(_2\) test solution with pH 7. Afterwards, the recovery step in ascorbic acid and the subsequent detection step in H\(_2\)O\(_2\) test solutions are performed at lower pH values down to pH 2, and thereafter at higher pH values up to 7. It is observed in Fig. 3 that the optrode detects H\(_2\)O\(_2\) at a wide range of pH (from pH 2–7). The ability of this optrode to detect H\(_2\)O\(_2\) across this pH range suggests that it can be applied in a variety of physiological and industrial environments. As shown in Fig. 3, pH influences the intensity of the reflected light at minimum plateaued state, achieved during the recovery step in ascorbic acid. A similar behavior was observed before by Del Villar et al. [13].

The precise reason for the increased intensity when the film is in PW state at lower pH values shown in Fig. 3 is unknown as several factors are involved, such as the various equilibria involved (e.g. ionization equilibrium of acid ascorbic) and even the microstructured environment of the film. The reduction potential of ascorbic acid also decreases with increasing pH, according to the Nernst equation, which should also contribute to this effect. A similar trend has been observed in another study [23] on reduction of PB nanoparticles in different reaction media with different pH values. In that study, the intensity of the absorbance of PB nanoparticles after reduction in different pH values showed no significant
change between pH 2 and 3 while the intensity changed significantly between pH 3 and 5.

The intensity response of the optrode when it is at equilibrium in the presence of H$_2$O$_2$ is similar for all tests, regardless of the pH of the test solution, as shown in Fig. 3. This suggests that the extent of PW oxidation to PB in the presence of H$_2$O$_2$ is also similar at all pH values. However, the intensity response of the optrode at equilibrium in the presence of either H$_2$O$_2$ or ascorbic acid decreases slightly after several tests, which can be attributed to leaching or possible alterations in the microstructure of the deposited PB/PW film. Possible leaching of the PB film would reduce the durability of the optrode. Therefore, the durability of the optrode has been investigated and discussed in Section 3.2.3.

To investigate the optrode response to test solutions, the time constant of the optrode was extracted from the intensity response of each test. For this calculation, the moment of immersion in each H$_2$O$_2$ solution is defined as t = 0, and the data is fit to an inverse exponential function (Eq. (3)):

$$I(t) = I_0 + A \left[1 - \exp \left(-\frac{t}{\tau}\right)\right]$$

where I(t) and I$_0$ are the intensities at time t and the instant the optrode was initially immersed into the H$_2$O$_2$ solutions, respectively. $\tau$ is a constant equal to the time elapsed when the intensity reaches 63% of the intensity at equilibrium and is the parameter used to evaluate the response of the optrode. A is a constant related to the geometry of the film, the diffusion conditions through the PB structure, and the oxidation of PW to PB. This inverse exponential behavior is consistent with the diffusion mechanism underlying the operating principle of the optrode [19].

Fig. 4 depicts the average time constant of the optrode versus pH of the test solutions containing 100 μmol L$^{-1}$ H$_2$O$_2$, on a log-log scale. It is apparent that pH affects the time constant of the optrode. The time constant is smaller for the test H$_2$O$_2$ solutions at lower pH values.

3.2. Sensing behavior at pH corresponding to PEM fuel cell operating conditions

Sensing H$_2$O$_2$ and understanding the underlying conditions that promote its formation within a fuel cell are keys to understanding the mechanism of PEM fuel cell membrane degradation. Therefore, the sensing behavior of the optrode for detection and measurement of H$_2$O$_2$ has been studied at a pH typical of an operating PEM fuel cell (i.e. pH 2).

**Fig. 4.** Time constant of the optrode versus pH of the test solutions with 100 μmol L$^{-1}$ of H$_2$O$_2$.

Fig. 5 shows the intensity response of the optrode to different H$_2$O$_2$ concentrations at pH 2. The intensity of the reflected light reaches approximately the same value after each immersion at different concentrations of H$_2$O$_2$. Using the technique described in Section 3.1, the time constant, $\tau$, is extracted from Fig. 5 and plotted versus H$_2$O$_2$ concentration in Fig. 6.

Fig. 6 shows that the time constant changes linearly (i.e. $R^2 = 0.99$) with H$_2$O$_2$ concentration, on a log-log scale. This linear behavior allows us to detect and quantify the concentration of H$_2$O$_2$. Fig. 6 also shows that the time constant is smaller for the test solutions with higher concentration of H$_2$O$_2$. This behavior is governed by the increased diffusion rates associated with the higher concentration potentials.

3.2.1. Repeatability

To investigate the repeatability of the measurements, four experiments with 50, 100, 200, and 400 μmol L$^{-1}$ of H$_2$O$_2$ and with each one comprising three sensing tests were performed using the same optrode. Fig. 7 illustrates the intensity response of the optrode during these four experiments.

Fig. 7 shows that the intensity levels at minimum and maximum plateaued states are essentially the same and it is only the slope that increases following immersion into higher concentration H$_2$O$_2$ test solutions, leading to smaller time constants. The slope of intensity variation for each concentration of H$_2$O$_2$ is calculated during the first 60 s after the probe immersion into test solutions and presented in Table 1. The slopes for each concentration of H$_2$O$_2$ are close to each other, particularly at higher concentrations of H$_2$O$_2$, which indicates the repeatability of the results. On the other hand, the slope of intensity variation during the probe immersion in ascorbic acid solutions is always the same with an average of negative 23 counts per seconds and standard deviation of 1.09 (i.e. slopes were calculated during the first 180 s after probe immersion.
3.2.2. Repeatability

Two optrodes are prepared in the same manner at different times and multiple sensing experiments are performed with each. The time constants of both optrodes are plotted versus H$_2$O$_2$ concentration in Fig. 9. Both optrodes exhibit a linear behavior and a lower time constant with higher H$_2$O$_2$ concentration. The slopes for both optrodes are similar, −0.97 and −1.00 s mol$^{-1}$ L. However, the extrapolation of the linear fits do not intercept the vertical axis at the same point. This suggests that each sensor will require an initial calibration with known concentrations of H$_2$O$_2$. The different intercepts can be attributed to variations in sensing film thickness or microstructure which affect diffusive transport and lead to different diffusion times. However, the similarity of the slopes for the different optrodes suggests that this parameter depends mainly on the nature of the deposited Pb/PW film under given experimental conditions (i.e. the temperature and pH of the sensing environment) and that is independent of the film thickness and structure.

3.2.3. Durability

The durability of an optrode is evaluated by performing similar sensing experiments five, six, and nine months after fabrication. Fig. 10 presents the response of the optrode versus H$_2$O$_2$ concentration. The time constant of the optrode varies linearly with H$_2$O$_2$ concentration for all tests after five, six, and nine months, as shown in Fig. 10. The response and the slopes are similar with R$^2$ values of 0.97, 0.92, and 0.99 and slopes of −0.97, −1.04, and −1.07 for the best linear fits after five, six, and nine months, respectively. These features indicate acceptable durability of the optrode and a long-term stability of the Pb film deposited on the tip of the optical fiber through the single-source precursor technique.

3.3. pH-dependent sensitivity

The optrode time constant depends on the pH of the H$_2$O$_2$ test solutions, see Fig. 4. To further evaluate the dependency of the sensor response on the pH of the test solution, tests were performed at three different pH values (i.e. pH 2, 5, and 6) with different concentrations of H$_2$O$_2$. Fig. 11A–C illustrate the response of an optrode versus H$_2$O$_2$ concentration at pH 6, 5, and 2, respectively. The sensor was found to be functional in all three pH values with a linear response to H$_2$O$_2$ concentration on a log-log scale. However, the slope of the best linear fit to the time constant of the sensor varies with pH value (i.e. −0.6, −0.78, and −1.07 at pH 6, 5, and 2 respectively). The sensor sensitivity (s mol$^{-1}$ L) increases with decreasing pH of the H$_2$O$_2$ test solution. This suggests that the H$_2$O$_2$ reduction rate increases with increasing H$_2$O$_2$ concentration at lower

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![Graph](image)

**Table 1**

<table>
<thead>
<tr>
<th>H$_2$O$_2$ concentration (µmol L$^{-1}$)</th>
<th>Slope of intensity variation (counts/seconds)</th>
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<tbody>
<tr>
<td></td>
<td>First response</td>
</tr>
<tr>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>100</td>
<td>23</td>
</tr>
<tr>
<td>200</td>
<td>63</td>
</tr>
<tr>
<td>400</td>
<td>118</td>
</tr>
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Note: Slopes were calculated during the first 60 s after probe immersion into H$_2$O$_2$ test solution.

![Graph](image)

Fig. 7. Successive intensity responses of the optrode to red) 50 µmol L$^{-1}$ of H$_2$O$_2$, green) 100 µmol L$^{-1}$ of H$_2$O$_2$, blue) 200 µmol L$^{-1}$ of H$_2$O$_2$, and orange) 400 µmol L$^{-1}$ of H$_2$O$_2$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

![Graph](image)

Fig. 8. Time constant of the optrode versus H$_2$O$_2$ concentration.

![Graph](image)

Fig. 9. Time constant of two different optrodes versus H$_2$O$_2$ concentration at pH 2.
across a wide range of pH values (i.e. from pH 2 to pH 7) demonstrated. The pH dependency was quantified as the time constant of the optrode. Shorter time constants (i.e. faster response) were obtained at lower pH values. The linear response of the optrode to H$_2$O$_2$ concentration allows us to detect and quantify the concentration of H$_2$O$_2$ with good precision (i.e. R$^2$ of 0.99). The optrode shows rapid and reliable response to H$_2$O$_2$ concentrations with improved reliability at higher concentrations. Sensing experiments with different optrodes exhibit the similar linear behavior with different time constants which indicates that each sensor requires individual calibration. The optrode also shows reliable responses five, six, and nine months after fabrications with the R$^2$ values of 0.97, 0.92, and 0.99, respectively, for the best linear fits on the time constant. This verifies the durability of the probe and long-term stability of the PB film deposited on the tip of the optical fiber through the single-source precursor technique. Sensing tests at three different pH values (i.e. pH 2, 5, and 6) with different concentrations of H$_2$O$_2$ reveal that the optrode remains functional at all pH values with a linear response to H$_2$O$_2$ concentration on a log-log scale. Moreover, the sensor sensitivity (s mol$^{-1}$ L$^{-1}$) increases with decreasing test solution pH.

Although this work was primarily motivated by the need to develop in situ detection of H$_2$O$_2$ in an operating PEM fuel cell, where low pH values (~2) prevail, the optrode described in this paper can be used in a variety of industrial and environmental systems and could potentially be adapted to biological applications and physiological environments once appropriately calibrated.

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**References**

Biographies

Hamed Akbari Khorami is a PhD student in the Department of Mechanical Engineering at the University of Victoria (Canada). He received his B.Sc. in Materials Science and Engineering from Sharif University of Technology (Iran) in 2008 and his M.Sc. in Materials Science and Engineering-Nanomaterials from Materials and Energy Research Center, MERC (Iran) in 2010. He is currently working on development of fiber-optic based H$_2$O$_2$ sensors for in operando detection of H$_2$O$_2$ within PEM fuel cells. Hamed’s research interests include fiber-optic sensors, nanotechnology and advanced materials: emphasis on sensors and biosensors, and fabrication of energy conversion devices.

Peter Wild is a Professor in the Department of Mechanical Engineering at the University of Victoria. He holds the Natural Sciences and Engineering Research Council (NSERC) Chair in Sustainable Energy Systems Design and is the Director of the Institute for Integrated Energy Systems at UVic (IESVic). Dr. Wild’s research interests include: optical sensors for industrial, environmental and biomedical applications; impacts of integration of renewable energy generation into existing grids; and renewable energy generation technologies.

Alexandre G. Brolo is a chemistry professor at the University of Victoria in British Columbia, Canada. He obtained his M.Sc. from the University of Sao Paulo (Brazil) in 1992 and his Ph.D. from the University of Waterloo (Canada) in 1998. Following a 2 years postdoctoral tenure at the University of Western Ontario (Canada), he then joined the Department of Chemistry of the University of Victoria in 2001. Dr. Brolo’s research interest are of the fabrication of nanostructured materials; the investigation of their optical properties; and their application in analytical chemistry.

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