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<td><strong>Author(s)</strong></td>
<td>Wong, Lai Peng; Wei, Yanyan; Toh, Chee-Seng</td>
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Accepted Manuscript

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PII: S1572-6657(12)00067-7
DOI: 10.1016/j.jelechem.2012.02.017
Reference: JEAC 797

To appear in: Journal of Electroanalytical Chemistry

Received Date: 30 September 2011
Revised Date: 11 February 2012
Accepted Date: 13 February 2012

Please cite this article as: L.P. Wong, Y. Wei, C-S. Toh, Self-powering amperometric sensor and biosensor, Journal of Electroanalytical Chemistry (2012), doi: 10.1016/j.jelechem.2012.02.017

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Self-powering amperometric sensor and biosensor

Lai Peng Wong, Yanyan Wei, Chee-Seng Toh*

Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 637371.

*Corresponding author. Tel.: +65 65922553

E-mail address: cstoh@ntu.edu.sg (C.S. Toh).

ABSTRACT

A unique Prussian blue nanotubes sensor using a two-compartment cell derives the current signal from the chemical energy of the hydrogen peroxide analyte, without input of electrical potentials, is described. The Prussian blue reduces hydrogen peroxide and is itself reduced by electron flow from the counter reaction at the auxiliary electrode. The concentrations of the Prussian blue (PB) and Everitt’s salt (ES) forms of the Prussian blue are maintained at steady-state values, by the hydrogen peroxide reduction and the galvanic cell reaction. This strategy gives low detection limit of 0.1 µM \( \text{H}_2\text{O}_2 \) with linear range up to 80 µM and is further demonstrated in a model glucose biosensor. The simple design to reduce energy usage opens up the study of amperometric sensor development by selecting anodic and cathodic reactions with suitable thermodynamic potentials with consideration of reactions at the sensing and auxiliary electrodes modified with appropriate mediators or enzymes.
Keywords: Prussian blue, enzyme, hydrogen peroxide, glucose, membrane, biofuel cell

1. Introduction

Typically, electrochemical sensors and biosensors detect analytes by monitoring electron flow through the sensor under an applied electrical potential which provides an equivalent power magnitude of $I.V$ (current.voltage) to drive the redox reaction between the analyte and electrode, mediator or electrocatalytic species including enzymes. The first demonstration on using alternative chemical energy in biosensors has been reported ten years ago [1], based on a biofuel cell design which measured the changes in the open-circuit potential in response to the biofuel concentration. Subsequent works in similar direction have included analytes such as lactate [1], glucose [2], cyanide [3] and Hg$^+$ [4]. Conversely, there are few reports on self-powering amperometric method, which have been demonstrated particularly for the detection of methanol in fuel cells [5-6].

The chemical reaction driven sensor design comprises a porous alumina membrane coated with ~50 nm thick platinum working electrode layer in direct contact with the sensing solution. The membrane separates a sensing solution from the reference solution which contains the reference and auxiliary electrodes as shown in Scheme 1. In this way, the analyte added in the sensing solution reacts at the working electrode while the counter reaction occurs in the reference solution to give electrical current flow between the working and auxiliary electrodes. This is unlike analyte detections in one or two-compartment cells using nanoporous membranes [7-9] which rely on applied electrical potential to drive the sensing reactions [10-14]. Herein, hydrogen peroxide is selected as the analyte of interest because of its significance as waste in groundwater, in cell metabolism, signal transduction, as stress indicators of living cells, as clinical markers for diseases and is the product of several hundreds of oxidases enzymes, commonly used in many enzyme based biosensors [15-21].
To enhance the electrochemical activity of the porous metal-coated membrane towards hydrogen peroxide, Prussian blue with known high activity for H\textsubscript{2}O\textsubscript{2} reduction, was deposited onto the nanoporous platinum membrane using potential cycling method which forms nanotubes structures embedded within the membrane (Scheme 1). It is well known that Prussian blue (PB) can be reduced to the Everitt’s salt (ES) at $E^\ddagger \approx +0.24\text{V}$ (vs. Ag/AgCl, 1 M KCl) or oxidize to Berlin green (BG) at more positive potential of $E^\ddagger \approx +0.90\text{V}$ (vs. Ag/AgCl, 1 M KCl) [22-24] and has been utilized in sensors and biosensors [25-27]. Apparently, these two half-cell reactions could be combined with the half-cell reactions for the oxidation of water or reduction of oxygen at the counter electrode to give a cell reaction with theoretical driving force of 0.55 V. This paper describes a hydrogen peroxide amperometric sensor design constructed from Prussian blue nanotubes coupled to a Galvanic cell which gives the sensing signal derived from the Galvanic current flow.

Scheme 1

2. Materials and methods

2.1 Chemicals

Nanoporous alumina membranes (Anodisc\textsuperscript{TM}, 13 mm diameter, 0.02 μm pore size) from Whatman (Maidstone, Kent, U.K.). 37% HCl from Analar Normapur, 30% H\textsubscript{2}O\textsubscript{2} and KCl from Scharlau, potassium hexacyanoferrate(III) and D(+)-glucose anhydrous from Merck, anhydrous ferric chloride from GCE Laboratories, 1 M Tris buffer pH 7.0 from 1st Base. Glucose oxidase enzyme (GOx) from Aspergillusniger (EC 1.1.3.4, ~200 units/mg), was purchased as lyophilized powder from Sigma and stored at -20°C. Stock solutions of glucose in 1 M Tris buffer were left overnight and then stored at 4°C before used. All solutions were prepared in ultrapure water (Sartorius Ultrapure Water System).
2.2 Instrumentation

JEOL JFC-1600 Auto Fine Coater was used to prepare platinum-coated porous membrane electrode. Electrochemical experiments were studied with a CHI750D electrochemical workstation, an e-corder 401 (eDAQ) and a potentiostat (eDAQ EA161) and performed in a 2-compartment cell with 3-electrodes system with porous PB-nanotubes (nt) membrane electrode as the working electrode, a Ag/AgCl (1 M KCl) reference electrode and a platinum mesh as the counter electrode were used.

2.3 Sensor fabrication

Fabrication of the Prussian blue nanotubes sensor followed previous report [28]. Conductive platinum layer sputtered on one side of the membrane was subsequently electrodeposited with a Prussian blue layer to give the porous PB-nt membrane. The electrodeposition of PB-nt was achieved by a potential sweep from -0.5 to +0.6 V at 50 mV s⁻¹ for 30 cycles in a solution containing 5.0 mM K₃Fe₃(CN)₆, 5.0 mM FeCl₃, 0.1 M KCl and 0.01 M HCl. The porous PB-nt membrane was subsequently rinsed with ultrapure water and dried overnight at room temperature.

2.4 Determination of H₂O₂ and glucose

35% H₂O₂ solution was used to prepare the stock hydrogen peroxide solutions. Sensing solution refers to the solution that was in direct contact with the porous PB-nt membrane while reference solution refers to the solution where both reference and counter electrodes were placed. Stock solution of glucose in 1 M Tris was added successively into a 0.1 M KCl in 1 M Tris solution containing 4.0 mg mL⁻¹ glucose oxidase for glucose sensing. No external electrical potential was applied during the amperometric detection of H₂O₂ and glucose.
3. Results and discussion

3.1 Oxygen and hydrogen peroxide reduction at porous PB-nt and Platinum membrane electrode

To study the electrochemical behaviors of the PB-nt membrane electrode, current-potential curve for H$_2$O$_2$ reduction is obtained in a de-aerated solution with comparison to oxygen reduction under ambient and air-saturated conditions. Fig. 1a shows the voltammogram of PB-nt membrane under slow potential sweep rate of 1 mV s$^{-1}$, carried in order to achieve near steady-state condition so the typical voltammetric waves of PB are absent (see typical voltammogram of PB-nt in Supporting Information Fig. S2-S1). Fig. 1a clearly shows that hydrogen peroxide reduction at the PB-nt commences at $\sim$+0.05 V (vs. Ag/AgCl, 1 M KCl), $\sim$200 mV overpotential compared to a control voltammetric experiment carried out using porous platinum (Pt) membrane electrode (Fig. 1b). In addition, the Pt membrane gives lower reduction current towards H$_2$O$_2$ compared to the PB-nt membrane (Fig. 1a,b). These observations are consistent with the more rapid electrochemical rate constant for H$_2$O$_2$ reduction at PB compared to Pt [25]. Furthermore, at potentials positive of -0.1 V vs. Ag/AgCl, the Pt membrane electrode shows an oxidation peak ascribed to chloride reaction [29]. In contrast, the PB-nt membrane electrode shows negligible oxidation current, which indicates a significantly reduced Pt activity when the Pt membrane is coated with PB (Fig. 1a,b). Under ambient condition, reduction current towards oxygen occurs at $\sim$0 V vs. Ag/AgCl is observed for the Pt membrane electrode (Fig. 1b). However, at the PB-nt membrane, the oxygen reduction current is negligible when compared to H$_2$O$_2$ reduction (Fig. 1a).

Overall and important to this work, the PB-nt does not suffer significant interferences from the underlying Pt layer nor reduction of solution oxygen under ambient condition.

Figure 1
3.2 Effect of adding H₂O₂ on the open-circuit and closed-circuit potentials of PB-nt membrane electrode

Fig. 2a shows the change in the open-circuit potential of the PB-nt membrane electrode versus the auxiliary electrode arranged in the 2-compartment cell design (Scheme 1), when aliquots of H₂O₂ are added. Under this condition when no current flows between the working and auxiliary electrodes, a sigmoidal shape titration curve is obtained, as expected for exhaustive depletion of ES in increasing H₂O₂ concentration (Fig. 2a). In contrast, Fig. 2b shows incremental change in the driving force (in volts) between the PB-nt membrane and auxiliary electrodes under closed-circuit condition, measured from the potential difference between the nanotubes and auxiliary electrode when aliquots of H₂O₂ are added to the sensing solution and current is allowed to flow between electrodes. This incremental increase in the driving force is evident of a second reaction that can regenerate the reduced form of PB even in excess amount of H₂O₂. Fig. 2b inset shows the driving force of the sensor reaches steady-state values in ~30-60 s, after addition of each aliquot of H₂O₂ when the sensor is challenged with 10 µM to 5 mM H₂O₂. The plotting of these steady-state potential data versus the logarithm of H₂O₂ concentration in Fig. 2c gives an average slope close to 59/2 mV. This is consistent with the steady-state model derived from the Nernst relation (see Eq. (6) in Supporting Information S3-S2), thus indicates steady-state condition for the H₂O₂-powered sensor can be somewhat achieved within a particular concentration range. However, the poor correlation between the experimental and fitted data suggests that the steady-state potential values measured under current discharge condition, is not analytically useful.

It is noteworthy to mention herein, that the maximum power density of the sensor using slow scan linear sweep voltammetry [30] is ~30 µWcm² at cell voltage of 0.105 V (vs auxiliary electrode). This small power output is expected since the self-powering sensor operates with very small amount of analyte (fuel) concentrations unlike conventional fuel and biofuel cells.
3.3 Effect of $K^+$ ions concentration

A typical amperometric response of the sensor shows the increase in steady-state current towards increasing concentration of $H_2O_2$ (Fig. 3). To study the effect of the intercalating $K^+$ ion on the steady-state current of the $H_2O_2$-powered sensor, three different arrangements of cell solutions are used: (a) $Pt|xM KCl||H_2O_2, xM KCl|PB$ (b) $Pt|1 M Tris||H_2O_2, xM KCl|PB$ (c) $Pt|xM KCl||H_2O_2, 1M Tris|PB$. $Pt|KCl$ or $Pt|Tris$ refer to the auxiliary electrode placed in KCl or Tris reference solution while $H_2O_2, KCl|PB$ or $H_2O_2, 1M Tris|PB$ refer to PB- or membrane electrode placed in the $H_2O_2$ sensing solution with KCl or Tris as background electrolyte. A control containing 1 M Tris buffer without KCl in both reference and sensing solutions is found to give lower currents compared with the cells above. In the absence of $K^+$, the sensor gives least sensitive response towards $H_2O_2$ and in contrast, the sensor sensitivity (response slope) improves significantly with increasing $K^+$ concentration. This sensitivity attenuation by $K^+$ ion can be explained by the $K^+$ dependent conversion reaction between PB and ES which influences its electrochemical reduction and oxidation rate. Taking into consideration the dependence of the steady-state current signal $i$ on this electrochemical rate, coupled to the 2 e$^-$ reduction of $H_2O_2$, the steady-state model (see Eq.(8) in Supporting Information S3-S2) gives excellent fits to the experimental data (Fig.3).

The sensor gives similar analytical signal responses when $K^+$ is added in either the reference or sensing solution (Fig.3). Placement of high $K^+$ concentration in the reference solution is the more favourable cell arrangement to avoid the additional procedure of adding $K^+$ ion to the sample solution and to reduce interference from unknown amount of $K^+$ ion when present in some samples. This demonstration of a high sensitivity $H_2O_2$ sensor without use of applied electrical potential opens up the development of analytical sensors with low energy needs by simple change
from a single compartment to a two-compartment cell comprising a porous electrocatalytic electrode as sensor and a counter anodic (or cathodic) element of a Galvanic cell.

3.4 Determination of H$_2$O$_2$ and glucose

One significant advantage of using PB-$nt$ membrane is the high loading amount of PB nanotubes of $\sim$1 x 10$^7$ mol cm$^{-2}$, determined from charge passed during potential cycling in 0.5 M KCl solution (see Supporting Information Fig. S2-S1), which is 10-100 times higher than thin PB films [22]. Thus this presents the possibility of higher amperometric signal when applied in the self-powering mode.

Under optimized conditions, Fig. 4 shows the sensor’s amperometric signal response towards H$_2$O$_2$ with rapid response time of 30 s, excellent linearity at low H$_2$O$_2$ concentrations up to 80 $\mu$M, a low detection limit of 0.1 $\mu$M and sensitivity of 48 mA M$^{-1}$ cm$^{-2}$ (Fig.4a). Its detection limit is comparable to potential controlled PB sensors [27, 31, 32]. Reasonably reproducible responses with standard deviation of $\sim$20% can be obtained from three different sensors by normalizing the sensors’ signals against the amount of charges passed during charging of PB-$nt$ upon closing the circuit in the absence of H$_2$O$_2$. For individual sensors, the typical standard errors are in the range of 1-3% and can be used up to four sets of 15 measurements each.

To demonstrate the general utility of this new sensor design as an enzyme biosensor, the reduction of H$_2$O$_2$ at PB-$nt$ membrane is coupled to the glucose oxidase (GOx) catalysis of glucose, selected for its well-studied reaction, high stability of GOx and significance of glucose [33, 34]. Fig.4b shows the enzyme sensor response during successive addition of glucose to an air-saturated 1 M Tris pH 7.0 buffer, 0.1 M KCl solution containing 4.0 mg mL$^{-1}$ GOx enzyme. Apparent Michaelis Km(glucose) value of 25(±3.4) mM derived from non-linear curve fitting of
the biosensor response is similar to reported value (33 mM) of the free enzyme, thus indicates the sensor response is controlled by homogeneous enzyme kinetics [35]. The sensor linear working range from 1-25 mM and rapid response time of ~60 s are typical of homogeneous GOx sensors for measuring physiological concentration of glucose in blood [36].

Figure 4

The practical use of this proposed biosensor is tested against redox species that often co-exist with glucose in blood samples. Fig. 5 shows the amperometric response of the proposed biosensor to glucose and the interference species such as uric acid and ascorbic acid. As shown, 1 mM uric acid and ascorbic acid induce negligible responses while 1 mM glucose gives a significant change in the self-powering current response. Importantly, the physiological concentrations of uric acid and ascorbic acid in human blood samples are much lower than 1 mM, thus suggests potential development into low-power sensing devices for the monitoring of chemicals and biochemicals by incorporating appropriate oxidase enzymes [21].

Figure 5

4. Conclusions

In conclusion, we describe a self-powering sensor and biosensor for the detection of hydrogen peroxide and glucose based on a porous PB-nt membrane electrode. The unique Galvanic cell sensor configuration does not utilize any electrical potential, thus requires minimal energy input. Potential controlled voltammetric experiments and measurement of the sensor electrochemical potentials, indicate the PB-nt are of similar characteristics as usual PB films and that the sensing reaction proceeds via reduction of hydrogen peroxide. Excellent curve fits of the potential and
current data to theory indicates the spontaneous reduction of hydrogen peroxide at the PB-nt membrane together with the Galvanic cell regeneration of the reduced form of PB, proceeds under steady-state condition. Outstanding analytical performance of the self-powering sensor towards hydrogen peroxide is comparable to potential-controlled PB sensors. Proof-of-concept glucose biosensor derived from the hydrogen peroxide sensor is demonstrated in the presence of a homogeneous solution of glucose oxidase enzyme, indicates possibility for other oxidase enzyme-based biosensors.

Acknowledgements

The authors thank NTU for research grant (M58110084) and PhD research scholarship for WYY.

Supporting Information

Typical cyclic voltammogram of PB-nt membrane electrode in KCl solution (Fig.S2-S1) and detailed explanation of the steady-state model for the self-powering sensor and biosensor responses (Note S3-S2).

References

Captions to Figures

**Scheme 1.** Construction of the porous PB-nt membrane electrode by (A) sputter-coating an 50 nm thick platinum layer on one side of a 60 μm thick nanoporous alumina membrane with 20 nm nominal pore size, followed by (B) electrodeposition of PB onto the porous Pt membrane electrode. (C) The optimized sensor design comprises the nanoporous PB-nt membrane which separates two solutions of a 2-compartment sensor cell. Analyte H₂O₂ oxidizes PB-nt in the sensing solution, followed by Galvanic current flow between the porous PB-nt membrane electrode in the sensing solution and auxiliary electrode in the reference solution, which gives the sensing signal.

**Figure 1.** Current-potential curves for solution oxygen reduction under ambient condition (— - —), oxygen reduction in air-saturated solution (----), and hydrogen peroxide (8 mM) reduction in nitrogen-saturated solution (—) at (a) porous PB-nt membrane (or porous Pt membrane coated with PB); (b) porous Pt membrane. Conditions: 1 mV s⁻¹ scan rate; 0.5 M KCl solutions.

**Figure 2.** (a) Effect of adding H₂O₂ on the potential difference between PB-nt and counter electrodes under open-circuit condition when no current flows through the 2-compartment cell. (b) Effect of adding H₂O₂ on the driving force measured from the potential difference between PB-nt and auxiliary electrode during closed-circuit condition when current flows through the 2-compartment cell. Inset: magnified view of Fig. 2(b) shows the driving force reaches steady-state values in ~30-60 s, upon addition of each aliquot of H₂O₂. (c) Plot of steady-state values of the driving force obtained from (b) versus the logarithm of H₂O₂ concentration. Conditions: Sensing solution contains 1 M Tris buffer, reference solution contains 0.5 M KCl.
Figure 3. Effect of increasing K⁺ concentration in the reference or sensing solution during the addition of H₂O₂ to the sensing solution. The following three arrangements of cell solutions are used: (a) Pt|xM KCl||H₂O₂, xM KCl|PB; (b) Pt|1M Tris||H₂O₂, xM KCl|PB; (c) Pt|xM KCl||H₂O₂, 1M Tris|PB. Lines are non-linear curve fits using Eq. 8 of Supporting Information S3-S2. A control containing 1 M Tris buffer without KCl in both reference and sensing solutions gives linear response for comparison with the rest.

Figure 4. Self-powering chronoamperometric responses of (a) H₂O₂ sensor during successive additions of H₂O₂ (indicated by arrows), and (b) glucose sensor during successive additions of glucose (indicated by arrows) into quiescent sensing solution. Insets: Best fitted calibration plot of sensor signal toward (a) H₂O₂ or (b) glucose with Eqs. 9 and 10 of Supporting Information S3-S2, using three different sensors with normalization to initial charges passed upon closing the circuit but before addition of analyte. Conditions: all signals are filtered at 1 Hz, (a) Sensing solution contains 1 M Tris buffer (pH = 7), reference solution contains 0.5 M KCl; (b) Sensing solution contains 1 M Tris buffer (pH = 7) with 4 mg mL⁻¹ glucose oxidase, reference solution contains 0.1 M KCl in 1 M Tris buffer (pH = 7).

Figure 5. Amperometric response of the self-powering glucose sensor to 1 mM glucose (Glu) and interference species, 1 mM uric acid (UC) or 1 mM ascorbic acid (AC). Conditions: all signals are filtered at 1 Hz, quiescent sensing solution contains 1 M Tris buffer (pH = 7) with 4 mg mL⁻¹ glucose oxidase, reference solution contains 0.1 M KCl in 1 M Tris buffer (pH = 7). After each addition, the mixture is stirred rapidly for ~3 s, resulting in the sharp transient current peaks.
The manuscript describes:

An analyte-powered amperometric sensor and biosensor driven by spontaneous cell potential.

Unique Prussian-blue nanotubes embedded within membrane support which functions as sensor.

Performances of the self-powering H₂O₂ sensor and glucose biosensor are comparable to the potential driven ones.
Scheme 1
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5