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# Comparison of Biocomposite Electrode Sensor for the Detection of Oxidative Reactions

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**Abstract:** Hemoglobin has a very strong affinity with oxygen and thus is a good catalyst to carry oxygen for many oxidation reactions. Many researchers have explored this property of hemoglobin to develop sensors for the detection of nitrite and peroxide that are important in biomedical and environmental monitoring. We have previously reported the development of biosensors that can detect nitrite and peroxide with a lower detecting limit below $1\times10^{-12}$ M, that were among the best detectors at the time. With a similar sensor platform, we have now developed a biosensor that can detect the oxidation reaction orders of magnitude better than the previous sensors. The sensor comprises layers of biocomposite made up of a polymer, nanogold particles, and a complex protein on the surface of an electrode. The performance of this electrode biosensor is compared with the previously developed sensors, the durability and other factors that can affect the performance and fabrication of this biosensor are discussed. *Copyright © 2014 IFSA Publishing, S. L.*

**Keywords:** Oxidation, Nitrite, Peroxide, Nanogold particles, Biosensor.

## 1. Introduction

Rapid development of microelectronics and nanotechnology enable fabrication and functionality of immunosensors to territories that were inconceivable decades ago. Different methods of fabrication and combination of reagents are being studied to obtain optimal and efficient biosensors for a variety of purposes. Our research group has been developing a platform for electrochemical sensing that is versatile with high detection sensitivity [1-3], it can be used for detecting various chemical species and bioelectrolytes for environmental monitoring, as well as health care diagnostic testing.

In 2009, we reported a biosensor coated with hemoglobin (Hob) that could detect nitrite and peroxide well below parts per billions [2] which was one of the leading biosensors of the time for the testing of nitrite and peroxide. Since then, our research group has made strides towards the sensing

selectivity and sensitivity of this sensor platform. In this study, we have modified our sensor preparation by using a different coupling bioenzyme pair (human immunoglobulin) that can detect most bioredox reactions. In this report, we compared the performance this newly modified biosensor with the senor that we reported in 2009. Nitrite and peroxide were also used as surrogates of environmental pollutants and biometabolites for the testing comparisons of oxidative species (species production as a result of redox reaction). It should be noted that the performance of the sensor prepared with Hb in this report is far superior than the one we reported in 2009 due to progress in our sensor preparation. We also have tested the sensors fabricated with different anchoring materials (Au, Pt, and glassy carbon) for performance comparison. Table 1 and Table 2 list the major methodologies used in recent years in nitrite/nitrate and peroxide detection, with their detection limits and linear ranges, as well as the type of electrodes used.

## 2. Materials and Methods

### 2.1. Materials

The electrodes used in the modification were gold (Au), platinum (Pt) and glassy carbon (GCE), purchased from Tianjin Aida Heng Sheng Co, Tianjin, China. The electrodes had a diameter of 0.2 cm. The platinum counter electrode (in the electrochemical cell) had a diameter of 0.1 cm and length of 0.5 cm. The electrochemical measurements were carried out on a Gamry 600 potentiostat. Cysteamine, melamine, anti-HIgG (human immunoglobulin), HIgG, bovine serum albumen (BSA), hemoglobin (Hb), herring DNA, $AuCl_3HCl•4H_2O$ (Au %> 48 %), and sodium citrate were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA. All the other chemicals in the solution preparations were of analytical grade. All experiments were carried out in a deoxygenated 0.1 M phosphate buffer solution at pH 7.0.

**Table 1.** Summary of Recent Studies (1997-2012) of Detecting Nitrite/Nitrate by Electrochemistry.

| Material | Detection Coupling (Enzyme) | Linear Range | Detection Limit |
|---|---|---|---|
| Platinum Electrode | Nano-$TiO_2$/chitosan | 5.0 μM - 1.0 mM | $1.28 \times 10^{-6}$ M [4] |
| Gold Electrode | Cytochrome c/Nafion and a Cu-Mg-Al layered double hydroxide/GoldE | 0.75 μM - 123 μM | $2 \times 10^{-7}$ M [5] |
| Glassy Carbon Electrode | Hb/multiwall carbon nanotube-CdS/chitosan/GCE | 0.60 μM- 0.80 mM | [6] |
| | HB/$ZrO_2$/[BMIM]BF4/chitosan/GCE | | [7] |
| | AuNPs/Cu calcined layered double hydroxide/GCE | 1 μM - 191 μM | 0.5 μM [8] |
| | Hb/GCE | | 0.47 μM [9] |
| | Hb/nafion/carbon nanochips/GCE | | [10] |
| | Hb/RTILs/MWNTs | $4.0 \times 10^{-6}$ M - $3.2 \times 10^{-4}$ M | $8.1 \times 10^{-7}$ M [11] |
| | Hb/CNPs/polyvinyl alcohol/GCE | 0.2 mM - 1.8 mM | [12] |
| | Hb/CdS hollow nanospheres/GCE | 0.3 μM - 182 μM | 0.08 μM [13] |
| | AuNPs/ethylenediamine/GCE | $1.3 \times 10^{-4}$ M - $4.4 \times 10^{-2}$ M | $4.5 \times 10^{-5}$ M [14] |
| | Hb/hexagonal mesoporous silica(HMS)/GCE | 0.2 μM - 3.8 μM | $6.11 \times 10^{-7}$ M [15] |
| | Hb/GCE | $2.9 \times 10^{-4}$ M - $1.1 \times 10^{-2}$ M | $1 \times 10^{-4}$ M [16] |
| | Hb/gelatine/GCE | | [17] |
| | Catalase/MWCNTs/GCE | 5 μM - 10 μM | 1.35 μM [18] |
| Indium Tin Oxide (ITO) Electrode | Palladium nanoparticles/ITOE | | [19] |
| Edgeplane Pyrolytic Graphite (EPG) Electrode | Hb/konjak glucomannan/EPGE | | [20] |

**Table 2.** Summary of Current Studies about Detecting Hydrogen Peroxide by Electrochemistry.

| Material | Detection Coupling (Enzyme) | Linear Range | Detection Limit |
|---|---|---|---|
| Platinum Electrode | Copper oxide nanoparticles/nafion/PtE | 0.15 μM - 9.00 mM | $6 \times 10^{-8}$ M [21] |
| | Clay/Hb | | [22] |
| Gold Electrode | Hb/Chitosan-$Fe_3O_4$ nano-composite/GoldE | 2.3 μM - 9.6 mM | 1.1 μM [23] |
| | Fe-III-DETPA/polyallylamine-MWCNTs/Au substrate electrode | $10^{-8}$ M - 4.75 mM | $6.3 \times 10^{-9}$ M [24] |
| | Horseradish peroxidase/nafion/tt-MgO/GoldE | 1.0 μM - 450 μM | 0.3 μM [25] |
| | Cytochrome c/NiO-NPs/carboxylated MWCNT/polyaniline/GoldE | 3 μM - 700 μM | 0.2 μM [26] |
| | Hb/MesoZrO(2)/GoldE | $1.75 \times 10^{-7}$ M - $4.9 \times 10^{-3}$ M | $1.0 \times 10^{-7}$ M [27] |
| | Hb/AuNPs/HDT/GoldE | $5.0 \times 10^{-8}$ M - $1.0 \times 10^{-6}$ M | $1.0 \times 10^{-8}$ M [28] |
| Glassy Carbon Electrode | RuO2/AuNPs/nafion/GCE | 0.1 nM - 30 mM | $0.1 \times 10^{-9}$ M [29] |
| | HRP/AgNPs/cysteamine/p-ABSA/GCE | 1.2 μM - 9.8 mM | $1.1 \times 10^{-8}$ M [30] |
| | Hb/multiwall carbon nanotube-CdS/chitosan/GCE | 0.125 μM - 1.20 mM | [6] |
| | Hb/MWCNT/ZnO/GCE | | 0.02 μM [31] |
| | Hb/CS-SDS-CNT/GCE | | 4.2 μM [32] |
| | Cytochrome c/$ZrO_2$NPs/GCE | 40 μM - 270 μM | [33] |
| | Cytochrome c/graphene/PEDOT/GCE | 0.5 μM - 0.4 mM | $2.49 \times 10^{-7}$ M [34] |
| | Hb/Au-F127/GCE | 0.3 μM - 0.57 mM | $4.0 \times 10^{-8}$ M [35] |
| | HRP/GNPs-thionine/chitosan/GCE | 0.1 μM - 0.1 mM | $5.0 \times 10^{-8}$ M [36] |
| | Hb/carbon nanospheres/AgNPs/dopamine/GCE | 1.0 μM - 147.0 μM | 0.3 μM [37] |
| | Hb/chitosan/Au colloid/3-aminopropyl triethylene silane/Prussian blue/GCE | 2 μM - 480 μM | 0.1 μM [38] |
| | Hb/GCE | | 0.61 μM [9] |
| | Hb/nafion/carbon nanochips/GCE | 0.5 μM - 30 μM | 0.05 μM [10] |
| | Hb/CNPs/polyvinyl alcohol/GCE | 1.96 μM - 112 μM | [12] |
| | Catalase enzyme/nickel oxide nano-scale islands/GCE | 1 μM - 1 mM | [18] |
| | Hb/gelatine/GCE | | [17] |
| | Catalase/MWCNTs/GCE | 1 μM - 6 μM | 0.15 μM [39] |
| | Hb/hexagonal mesoporous silica(HMS)/GCE | 0.4 μM - 6.0 μM | $1.86 \times 10^{-9}$ M [15] |

## 2.2. Methods

The preparation of electrodes were the same as previously reported [2-3, 40], except that the biocomposite layers were either comprised of the anti-HIgG/BSA or Hb only. For the electrodes that were coated with anti-HIgG, an extra coating of BSA was added on the outmost surface for performance enhancement [41]. A successful coating of the biocomposite of the sensor electrode would have the spectra as shown in Fig. 1.

Detection of the oxidative species was conducted with a Gamry 600 potentiostat. The method of detection of the characteristic peaks of nitrite and peroxide by means of the enzymatic coupling were described in previous studies [1-3].

## 3. Results and Discussions

### 3.1. Detection of Nitrite/Nitrate

Fig. 2 shows a typical cyclic voltammogram of nitrite/nitrate by a Pt electrode. The characteristic peak for the measuring species chosen usually is the peak with the highest intensity (current), however, multiple peaks can be chosen if there are interferences. These peaks can be either oxidative or reductive, and direction of the redox coupling reaction is reversible depending on the concentration of the detecting species. This phenomenon will be discussed in later part of this section.

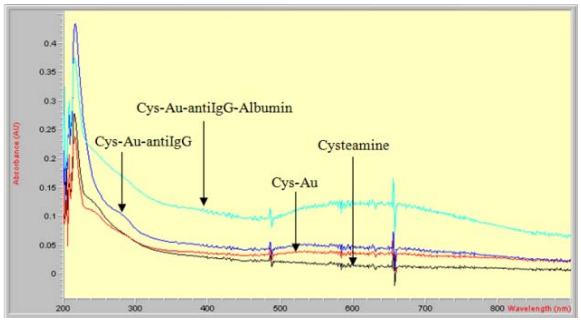


**Fig. 1.** An UV-Vis spectra of the biocomposite coated on a transparent plastic surface, the spectra of layer-by-layer coatings are the same as what were coated on the sensor electrode.

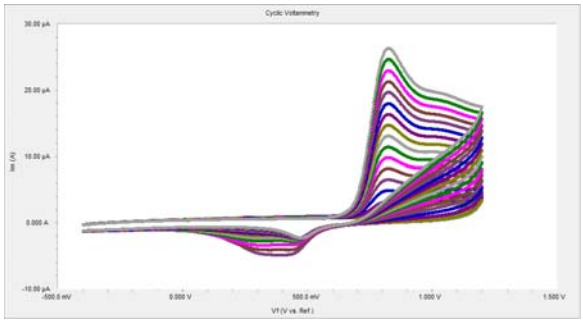


**Fig. 2.** Pt electrode sensor with anti-HIgG/HIgG for $NO_2^-$ detection from $1\times10^{-18}$ M to 0.0015 M.

### 3.2. Performance Comparison of the Hb (Old) and Anti-HIgG (New) Sensor

Fig. 3 is the performance comparison of the freshly prepared Au, Pt and GCE electrodes for the detection of nitrite. As shown in Fig. 3(a) and Fig. 3(b), both the Hb and anti-HIgG sensor possess extraordinary linear relationship of current versus concentration, especially in the concentration range of $1\times10^{-4}$ M to $1\times10^{-3}$ M in this study. The sensitivity (current in μA) of these sensors is excellent in this concentration range.

By comparison, sensors prepared by anti-HIgG/HIgG were slight better in all anchoring electrodes. Fig. 3 shows that these anti-HIgG/HIgG sensors were also very sensitive in extreme low concentrations below $1\times10^{-15}$ M.

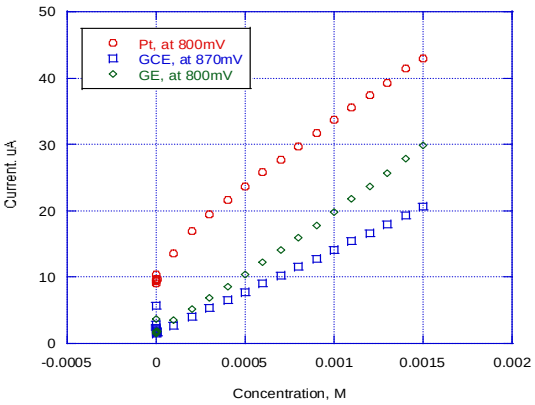


**Fig. 3 (a).** Nitrite measurements with Hb sensors (old) prepared with Pt, GCE, and Au electrode.

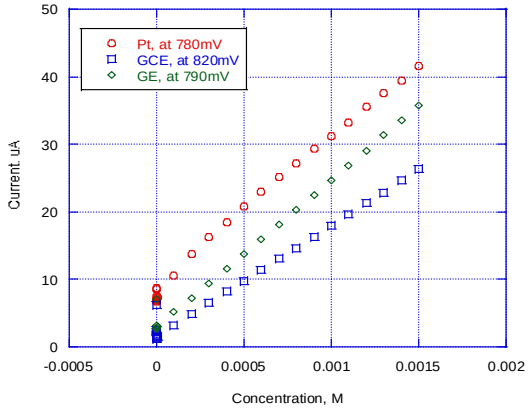


**Fig. 3 (b).**. Nitrite measurements with anti-HIgG/HIgG sensors prepared with Pt, GCE, and Au electrode.

Fig. 4 is a semi-log representation of the performance of the Pt, GCE, and GE electrode with running concentrations from $1\times10^{-18}$ M to 0.0015 M. As shown, all three sensors are capable of measuring nitrite at the concentration of $1\times10^{-18}$ M to 0.0015 M; however, the sensors are more sensitive at extreme low ($1\times10^{-18}$ M) and high (0.0015M) region.

The coupling reaction was reductive for all three electrodes at the low concentration region, with a transition to oxidation at concentration about $1.0\times10^{-9}$ M. The shifting from oxidative to reductive reaction or vise visa have been observed in many of our experiments, especially for experiments with a wide concentration range, and the rationale of such will not be further discussed in this report. Overall, the Pt electrode appears to be more superior compared to the other two electrodes as the anchoring material for the composite layer in the sensor preparation.

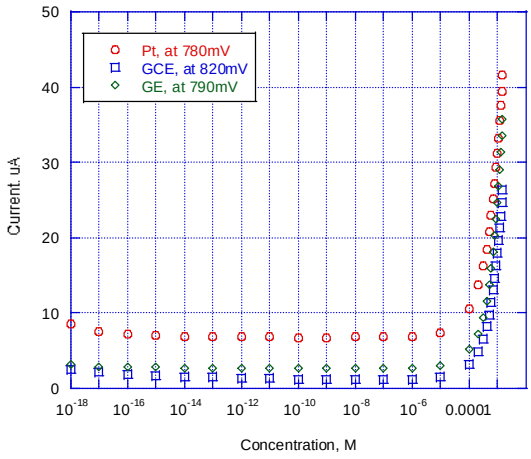


**Fig. 4.** Nitrite measurements with anti-HIgG/HIgG sensors freshly prepared with Pt, GCE, and Au electrode.

### 3.3. Durability of the Anti-HIgG/HIgG Sensor

Sensors prepared by anti-HIgG/HIgG were durable, as shown in Fig. 5, the sensors remained relatively sensitive after 3 weeks in buffer solution. Majority of the sensitivity loss was in the first week.

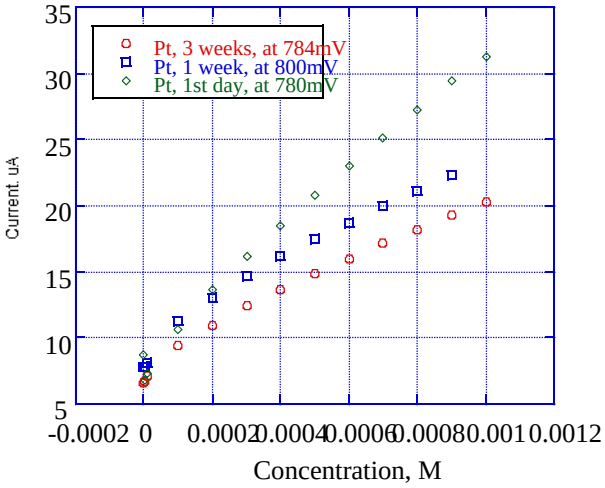


**Fig. 5.** Anti-HIgG/HIgG sensors performance with nitrate measurements after storing 3 weeks in a pH 7 buffer solution.

### 3.4. Peroxide Detection

As shown in Fig. 6 (a), the anti-HIgG/HIgG sensor is also an excellent sensor for peroxide detection in concentrations above $1\times10^{-4}$ M. Nearly perfect linearity was revealed in the testing concentrations of $1\times10^{-4}$ M to $1.5\times10^{-3}$ M.

Fig. 6 (b) is a semi-log representation of the performance of the Pt, GCE, and GE electrode with running concentrations from $1\times10^{-18}$ M to 0.0015 M. Similar to the measurements of nitrite/nitrate, the sensors are more sensitive at the extreme low ($1\times10^{-18}$ M) and high (0.0015 M) region. While the coupling reaction was oxidative through the whole concentration region for the Pt electrode, the GCE and GE electrode were having reductive reaction at the extreme low region but having an oxidative transition at about $1\times10^{-5}$ M. Likewise, Pt electrode was more superior among the three electrodes in peroxide detection.

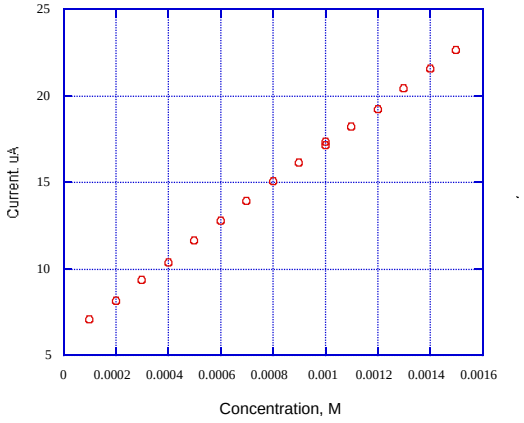


**Fig. 6 (a).** Performance of the anti-HIgG/HIgG sensor with $H_2O_2$ at concentrations from $1\times10^{-4}$ M to $1.5\times10^{-5}$ M.

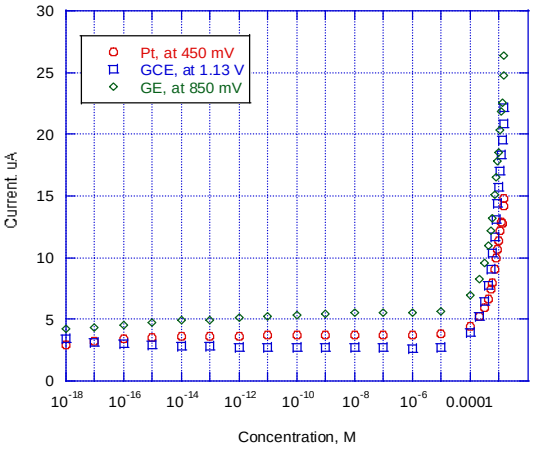


**Fig. 6 (b).** Performance of the anti-HIgG/HIgG sensor with $H_2O_2$ at concentrations from $1\times10^{-18}$ M to $1.5\times10^{-3}$ M.

### 3.5. Interferences and Selectivity of the Anti-HIgG Sensor

This high performance anti-HIgG biosensor is proven to be selective and relatively free of interferences. Fig. 7 (a) shows a typical measurement of the linear current increment with concentration of a GE electrode sensor. In general, the reproducibility of different sensors fabricated with the same materials would have a variation of less than ±15 %. General inorganic materials (e.g., $Cl^-$, $NO_3^-$, $SO_4^{2-}$) would create nearly no interference at dilute solution ($<1.0\times10^{-3}$ M) (Fig. 7 (b)); however, some organic materials may exert interference which is also concentration dependent. As shown in Fig. 7(c),

herring DNA distorted the current linear relationship with concentration and the interference was concentration dependent. On the other hand, glucose does not create any interference with this sensor in peroxide measurement (data not shown).

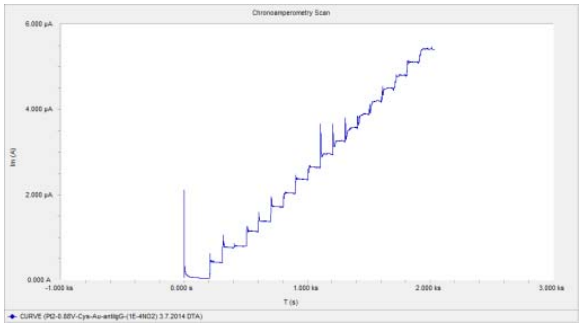

**Fig. 7 (a).** Measurement of current increment per dropwise addition of $1\times10^{-4}$ M per minute of nitrite using a GE electrode at 0.8 V.

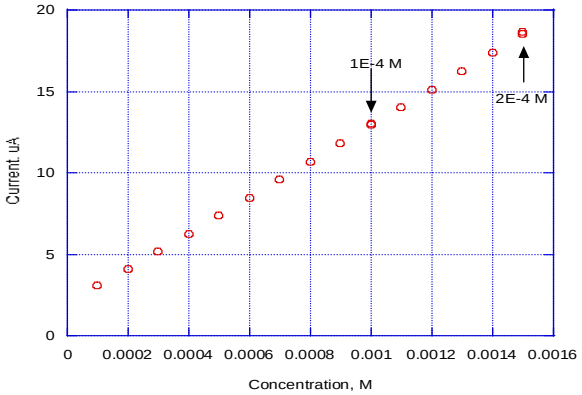


**Fig 7 (b).** Measurement selectivity test of peroxide by adding $1\times10^{-4}$ M of KCl solutions in the testing solution at the indicated concentrations (GE electrode).

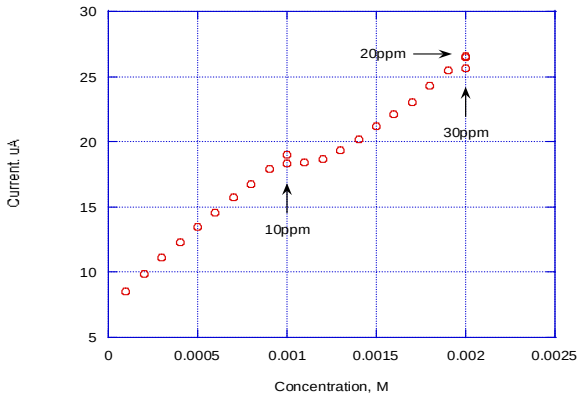


**Fig. 7 (c).** Measurement selectivity test of peroxide by adding various concentrations of DNA (herring) in the testing solution at the indicated concentrations (GE electrode).

## 4. Conclusions

Our newly developed anti-HIgG/HIgG sensor is proved to be very versatile and durable. Its performance is better than the Hb sensor previously developed for the same measurements of nitrite and peroxide [2]. Its high sensitivity at higher concentrations ($>1\times10^{-5}$ M) and extreme low concentrations ($<1\times10^{-15}$ M) (Fig. 4 and Fig. 6) would be very beneficial in detecting many redox reaction enzymes and chemicals (pollutants). The lowest detection limit of this sensor was at $10\times10^{-18}$ M that is many orders of magnitude lower than any electrochemical systems that were reported in the public domain (Table 1 and Table 2).

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