

# Characterization of Modified Gold Electrode Sensor with Nanoparticles and Applications

S. W. Leung<sup>\*</sup>, Y. Wang<sup>\*\*</sup>, H. Gu<sup>\*\*\*</sup>, and J.C.K. Lai<sup>\*\*\*\*</sup>

<sup>\*</sup>Civil and Environmental Engineering Department, College of Engineering, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, leunsolo@isu.edu

<sup>\*\*</sup>Civil and Environmental Engineering Department, College of Engineering, Idaho State University, Pocatello, ID 83209, USA, yuanwang@isu.edu

<sup>\*\*\*</sup>Department of Hygiene Analytical Chemical, School of Public Health, Nantong University, Nantong, Zhangshu, 226007, P.R. China, hygu@ntu.edu.cn

<sup>\*\*\*\*</sup>Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, lai@otc.isu.edu

## ABSTRACT

In the recent development of biosensor, response time of the sensor is drastically improved by using direct coupling of enzymes onto the surface of electrode. The drawback of the direct coupling of enzymes is denature of enzymes on the surface of electrode during the cycles of redox reactions

In order to develop sensors that are useful with predictable performance, the sensors must be well characterized under working conditions so that the performance is ensured.

We are developing a biosensor with gold electrode modified by surface coating of cysteamine coupled with nanoparticles, then with hemoglobin (Hb). Due to the high chemical affinity of hemoglobin with nitrite, this Au-cysteamine-nanoparticle-Hb biosensor can be used to detect nitrite/nitrate by means of a cyclic voltammographic technique.

The detection limit of nitrite/nitrate of this biosensor was well below mM and its response time was almost instantaneous. The reproducibility of performance was tested, and the chemical kinetics of the nitrite/nitrate redox reaction on the surface of the modified electrode was studied. This sensor was further characterized by the oxidation of peroxide and the results were reported.

**Key words:** biosensor, nanoparticle, hemoglobin, nitrite, nitrate, electrode, peroxide.

## 1 INTRODUCTION

In the recent development of biosensor, response time of the sensor is drastically improved by using direct coupling of enzymes onto the surface of electrode. The response time of biosensors has improved from minutes to less than a second. The drawback of the direct coupling of enzymes is decomposition or denature of enzymes, especially when the

enzymes are assembled on the surface of an electrode, the redox potential associated with the detection mechanism can alter the structural configuration of the enzymes easily when these enzymes are no longer anchored by the surrounding biological fluids that have stabilizing effect naturally to the enzymes.

In order to develop sensors that are useful with predictable performance, the sensors must be well characterized under working conditions so that the performance is ensured. In our research group, we are developing a biosensor with gold electrode modified by surface coating of cysteamine coupled with nanoparticles (Au and Ag), then with hemoglobin (Hb). Due to the high chemical affinity of hemoglobin with nitrite, this Au-cysteamine-nanoparticles-Hb biosensor can be used to detect nitrite/nitrate by means of a cyclic voltammographic technique.

In this study, we reported factors that would affect the performance of the modified electrode, the detection limits, and other applications that were associated with the electrode.

## 2 MATERIALS AND METHODS

### 2.1 Electrode Preparation

A clean gold electrode was immersed in cysteamine solution for 2 hours in the dark, then it was dipped into colloidal gold or silver nanoparticles for 24 hours, finally the electrode was dipped into Hb solution for 20 hours before it was used for testing.

### 2.2 Nanoparticles Solution Preparations

Nanoparticles of Ag was prepared by reaction of AgNO<sub>3</sub> with citric acid; nanoparticles of Au was prepared by HAuCl<sub>4</sub> with citric acid.

All chemical reagents used in this study were analytical grade or the highest grade available, water was double deionized distilled water. All the experiments were carried

out under deoxygenated condition in 0.1 M phosphate buffer solution.

### 2.3 Detections

UV-VIS spectrophotometry was carried out by an Agilent diodearray spectrophotometer; cyclic voltammetry was conducted by using a Gamry 600 Potentiostat. Voltammetric potential was measured against a saturated chloride electrode (SCE).

## 3 RESULTS AND DISCUSSIONS

### 3.1 Coating of Hb on Modified Au Electrode

There are various ways to verify if the Hb is properly coated onto the Au electrode: As shown in Figure 1, an UV-VIS photospectrograph, showed different peaks for cysteamine (black), Hb (red), gold colloid (blue) in solution, and cysteamine-Au particles/Hb on plastic (blue green).

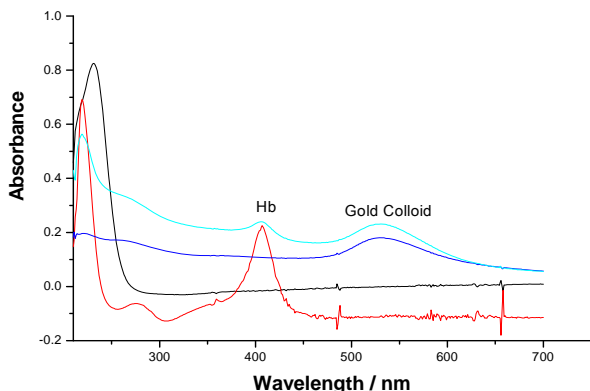


Figure 1: UV-VIS spectrometric method to identify cysteamine, Hb, gold colloid in solution and plastic-cysteamine-Au particles-Hb.

An alternative way to verify that an electrode is coated with Hb properly can be shown by electrochemical impedance spectroscopy (EIS); an electrode coated with different materials would possess different electrical impedance. As shown in the Nyquist diagram in Figure 2, an Au electrode coated with cysteamine and Ag nanoparticles had the highest impedance at low frequencies (red line, Bode diagram not shown); the same electrode coated with cysteamine, Ag nanoparticles, and Hb had lower electrical impedance at low frequencies, which also indicated that the electrode could be more efficient in current conduction thus required less energy for reactions to take place on the surface of the electrode when Hb was coated with the Ag nanoparticles (green line). The blue line was measured after the same Au-cysteamine-Ag particle-Hb electrode had been used as a nitrite sensor with many redox cycles, it had the lowest electrical impedance at low

frequencies among the three electrodes. This impedance reduction phenomenon was observed often in similar experiments that we had conducted and is speculated that the redox cycles streamline the molecular arrangements that is not done in the self-assembly process during the electrode preparation. Eventually, all the electrodes had about the same electrical impedance at high frequencies. The EIS measurements were conducted in Fe(II)/Fe(III) solution.

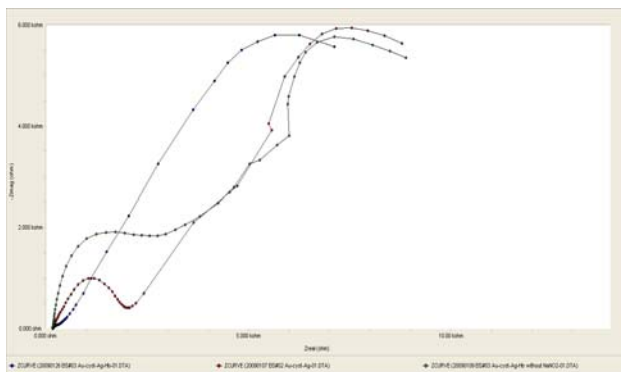


Figure 2: EIS of three electrodes with a) Au-cysteamine-Ag particles, b) Au-cysteamine-Ag particles-Hb, c) Au-cysteamine-Ag particles-Hb after numerous redox cycles.

A cyclic voltammogram of Hb at pH 7 is shown in Figure 3. The characteristic Hb reductive peak was at about 0.25 V. The height (resolution) of the peak changed with the scanning rate of the voltammogram and our measurement was comparable with other investigations in literature [1].

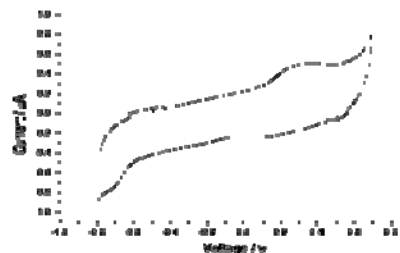


Figure 3: Cycle voltammogram of a modified Au electrode with Hb attached (Au-cysteamine-Au particles-Hb) at pH 7.0 in 0.1 M phosphate buffer solution.

### 3.2 Detection of Nitrite

As shown in Figure 4, the modified Au electrode can be used to detect oxidation of nitrite to nitrate, the reductive peaks (current) at about 0.88 V increased linearly with added nitrite concentrations. It should be noted other researchers have used peaks at different locations for nitrite identification [2]. Certainly, the locations of peaks are also a function of reference electrode used in the potential measurements.

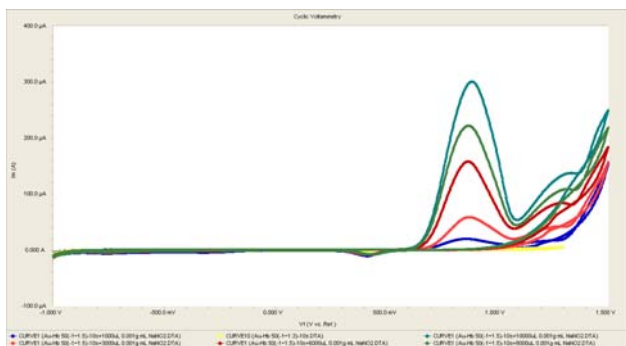


Figure 4: Linearly relationship of current with nitrate concentration for the electrode.

Step responses of the electrode with nitrite additions at a fixed voltage (0.85 V) is shown in Figure 5. It can be seen that the responses of the electrode were rather stable, however, these responses would shift with increase of measuring cycles.

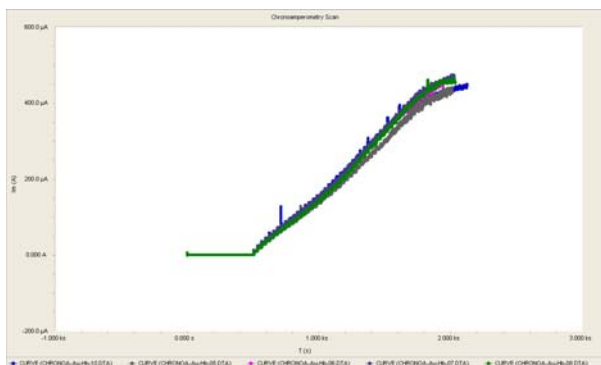


Figure 5: Step responses (current) of the electrode with nitrite additions

Limit of detection of nitrite for the electrode is about  $1.0 \times 10^{-6}$  M for the lower limit and about  $1.0 \times 10^{-3}$  M for the upper limit. As it is shown in Figure 6, when the concentration to be detected exceeds the limit, the current linearity with concentration was no longer valid.

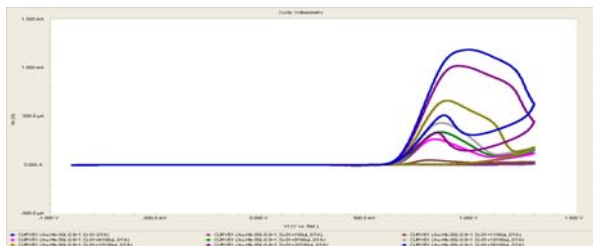


Figure 6: Responses of the electrode with that were above the concentration limit of  $1.0 \times 10^{-3}$  M.

### 3.3 Effect of pH

Higher pH in solution shifted the potential requirement for the oxidation of nitrite catalyzed by Hb to lower voltage. It appeared that the heme in Hb became more efficient in the energy transfer at higher pH. Since our body functions operate between pH 6.5 to 7.4, if this

observation has any biological implications remains to be studied.

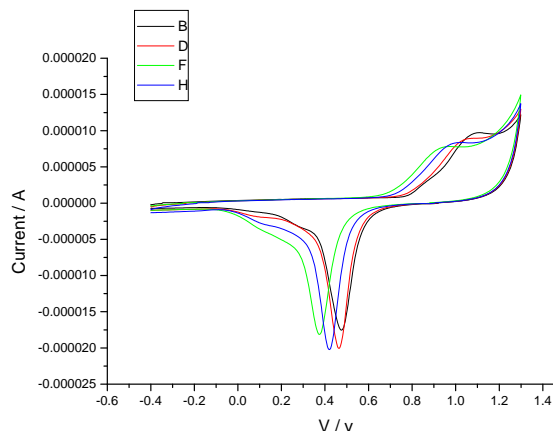


Figure 7: Higher pH shifted the reaction potential to lower voltage requirement for the oxidation of nitrite. Green: 7.8; blue: 7.5; red: 7.0; black: 6.5.

### 3.4 Effect of ionic strength and other inorganic salts

Chloride, nitrate, and sulfate salt did not seem to create any detection interferences for the nitrite oxidation catalyzed by Hb for the modified electrode, but the increase of ionic strength appeared to shift the potential requirement for the reaction higher, thus less energy efficient compared to that of lower ionic strength in the solution, as it is shown in Figure 8.

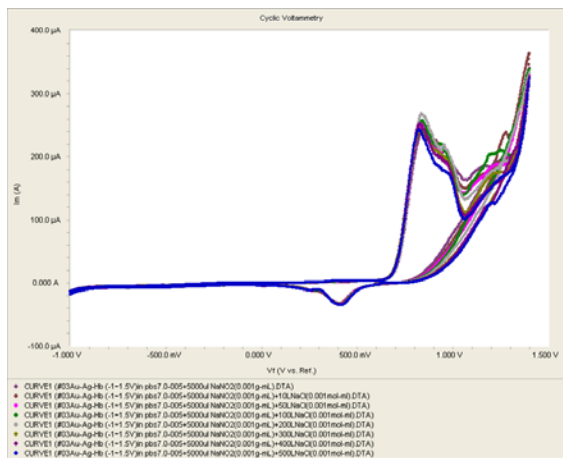


Figure 8: Increase of ionic strength shifts nitrite oxidation to higher reaction potential. The blue line is the initial peak before additions of NaCl.

### 3.5 Other Applications:

When the modified Au electrode with Hb was used to detect the reduction of  $H_2O_2$ , the lowest detection limit was about 0.24 ppb and the upper limit was about 0.24 ppm, as shown in Figure 9. Other enzymes can also be attached to the modified electrode for various biological applications,

however, handling of these enzymes has to be cautious due to their unstable nature under dilute condition.

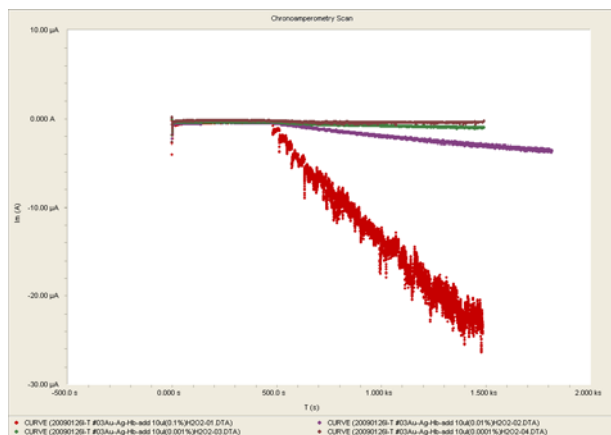


Figure 9: Detection limit of  $\text{H}_2\text{O}_2$ . Lowest limit: 0.24 ppb (purple), upper limit: 0.24 ppm (red).

## 4 CONCLUSIONS

The modified Au electrode can have many biomedical applications because of its fast responses to the detection targets and the lower detection limit can be in ppb and lower. When the electrode was modified with either Au or Ag nanoparticles, the results were similar, in some experiments, the responses of the electrode modified with Ag nanoparticles were better than those when Au nanoparticles were used. Stability and consistency are still issues to be resolved if the electrode is to be used for widespread applications

## 5 ACKNOWLEDGMENT

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