Comparison of Anchoring Materials for High Performance Sensors Modified with Nanoparticles and Enzymes

Xiang Gao[*], David Assan[**], James C.K. Lai[***], and Solomon W. Leung[****]

[*]Civil & Environmental Engineering Department, School of Engineering, Box 8060, Idaho State University, Pocatello, ID 83209, USA, gaoxian@isu.edu

[**]Civil & Environmental Engineering Department, School of Engineering, Box 8060, Idaho State University, Pocatello, ID 83209, USA, assadavi@isu.edu

[***]College of Pharmacy and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, lai@pharmacy.isu.edu

[****]Corresponding author, Civil & Environmental Engineering Department and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, leunsolo@isu.edu

ABSTRACT

We have previously developed some of the most sensitive biosensors/electrodes modified by Au nanoparticles and enzymes that are capable of detecting concentration levels below ppb. These sensors were fabricated with composite layers of nanomaterials and enzymes anchored on conductive but non-reactive materials, such as glassy carbon, Au, Ag, and Pt. Performance of these sensors varies depending on the anchoring materials as well as the composition of the biocomposite materials.

In this report, we investigated the performance of these ultra high performance sensors fabricated with identical biocomposite materials and procedures, except the anchoring conductive materials. The anchoring materials were glassy carbon, Pt, Au and Ag; the biocomposite layer consisted of polymer/Au nanoparticles/enzyme. The enzymes in the biocomposite layers are essential for the target species detection with which enable the coupling (detection) reactions occur. The enzymes used in this study were LDH, GDH, and hemoglobin. The specific target species for the detection included lactate, NO_2^-, peroxide, and NH_4^+. We also tested different combinations of the biocomposite layers for optimal performance of the sensors/electrodes. The testing solutions were buffered with 0.1 M of phosphate at pH 7.0.

Among the four tested anchoring materials, Ag generated the most noise and was relatively unstable that after the electrode was used multiple times (cycles), the electrode characteristic peak would deteriorate rather fast compared to other materials used in this study.

Glassy carbon electrode was the best anchoring material and the detection limit of this ultra high performance sensor platform could be as low as $1x10^{-17}$ M, depending on the target species to be detected.

Keywords: performance, anchoring material, nanoparticle, sensor, bioenzymes

1 INTRODUCTION

The modern era of chemical species detection is response time and detectable concentration. Due to the advances of computer and nanotechnology, the fourth generation of sensor should have almost instantaneous response time and ultra high detection sensitivity.

In this research, we were testing the detection limits of an ultra high performance sensor platform that was anchored by various conducting materials: Au, Ag, Pt and glassy carbon. These electrode sensors were then challenged by detecting various biological and environmental samples for their performance, the target species included lactate, ammonia, NO_2^-, and peroxide.

The principle of detection of our biosensors is by means of an enzymatic reaction as described in the following as an example: glutamate and NAD^+ can be hydrolyzed to form α-ketoglutarate, NADH, and ammonium ion with the enzyme, glutamate dehydrogenase. The equilibrium constant is in favor of the formation of glutamate and thus the reverse reaction is faster kinetically:

$$\alpha\text{-Ketoglutarate} + NH_4^+ + NADH + H^+ \xleftrightarrow{GDH} \text{Glutamate} + NAD^+ + H_2O$$

Detection of other chemical species are possible by using different enzymatic coupling reactions and enzymes.

2 MATERIALS AND METHOD

2.1 Electrodes

Au (GE), Ag (AE), Pt(PtE), and glassy carbon (GCE) electrode all had diameter of 0.2 cm. The platinum counter electrode had diameter of 0.1 cm and length of 0.5 cm. They all were purchased from Tianjin Aida Heng Sheng Co, Tianjin, China. These electrodes were coated with either glutamate dehydrogenase (GDH) or lactate

dehydrogenase depending on the target chemicals to be detected [1].

2.2 Materials

All enzymes and biochemicals were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA. All tother chemicals were of analytical grade or highest grade available. All experiments were carried out under deoxygenated condition in 0.1 M phosphate buffer solution at pH 7.0 unless otherwise specified.

2.2 Nanoparticles and Electrode Preparations

Nanoparticles and electrodes were prepared according to methods reported previously [1, 2].

2.3 Detections

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 GDH Coating on Pt Electrode and GCE

Fig. 1 and Fig. 2 show the modified Pt electrode and GCE with GDH. As it shown, the characteristic peak of α-ketoglutarate and NH_4^+ conversion to glutamate was detected at about 750 mV at pH 7.0. The responses of GCE were much preferred since they were "smoother" responses stepwise.

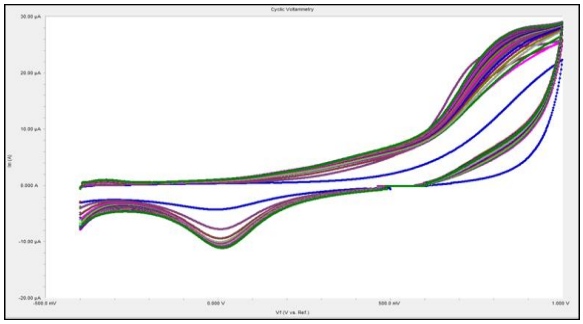


Fig. 1 Voltammetric responses of a Pt electrode coated with GDH at pH 7.0. Responses were stepwise additions of α-ketoglutarate with NH_4^+ from $5.0x10^{-17}$ mol/L to $1.0x10^{-6}$ mol/L at 760 mV. The concentration of NADH was $3x10^{-3}$ mol/L.

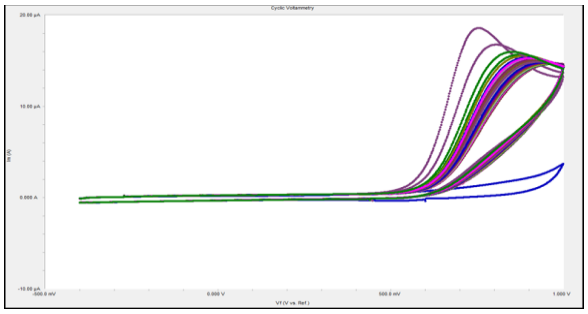


Fig. 2 Voltammetric responses of a glassy carbon electrode coated with GDH at pH 7.0. Responses were stepwise additions of α-ketoglutarate with NH_4^+ from $5.0x10^{-17}$ mol/L to $1.0x10^{-6}$ mol/L. The concentration of NADH was $3x10^{-3}$ mol/L.

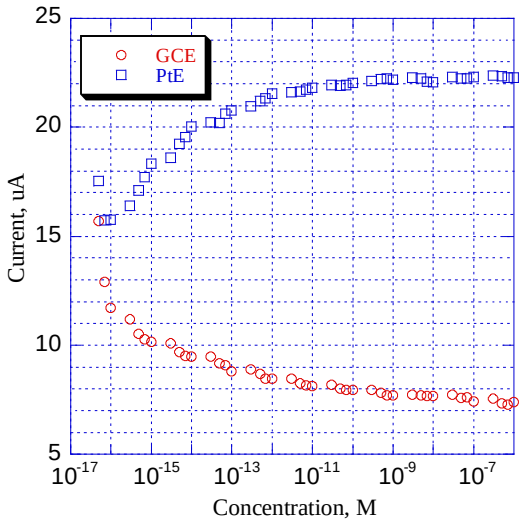


Fig. 3 Current measured with stepwise additions of ammonia and α-ketoglutarate corresponding to the responses from Fig. 1 and 2.

Fig. 3 shows the accumulative current responses with for GCE and PtE for the NH^+ detection. Current increased with concentration for the PtE which indicated oxidation while current decreased with concentration for the GCE which indicated reduction. Nevertheless, both electrodes can be used for sensing purpose as long as the responses are consistent. The concentration range used in this test was between $5x10^{-17}$ to $1x10^{-6}$ M.

3.2 LDH Coating on Au Electrode and GCE

Fig. 4 and Fig. 5 show the modified gold electrode (GE) and GCE with LDH coated for lactate detection. As it shown, the characteristic peak of lactate conversion to pyruvate was detected at about 310 mV at pH 7.0.

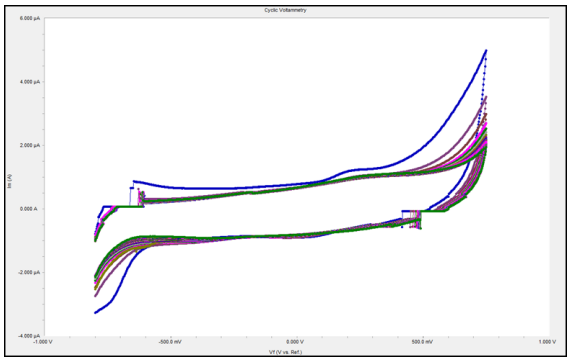


Fig. 4 Voltammetric responses of an Au electrode coated with LDH at pH 7.0. Responses were stepwise additions of lactate from 5.0x10 $^{-13}$ mol/L to 1.0x10 $^{-6}$ mol/L at 310 mV. The concentration of NAD^+ was 3x10 $^{-3}$ mol/L.

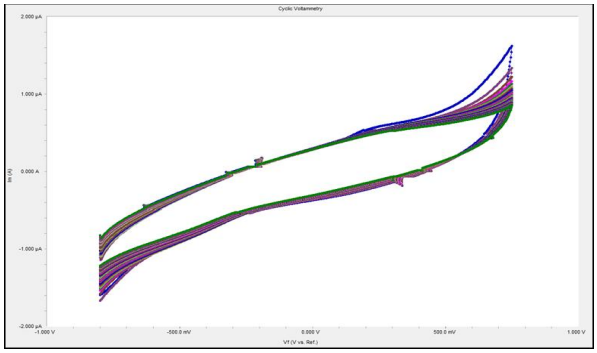


Fig. 5 Voltammetric responses of a GCE electrode coated with LDH at pH 7.0. Responses were stepwise additions of lactate from 5.0x10 $^{-13}$ mol/L to 1.0x10 $^{-6}$ mol/L at 310 mV. The concentration of NAD^+ was 3x10^{-3} mol/L.

Fig. 6 shows the accumulative current responses from lactate detection for the GE and GCE. Similar to the responses with GDH, current increased with lactate concentration for the GE and decreased with GCE. The concentration range used in this test was between 5x10 $^{-13}$ to 1x10 $^{-4}$ M.

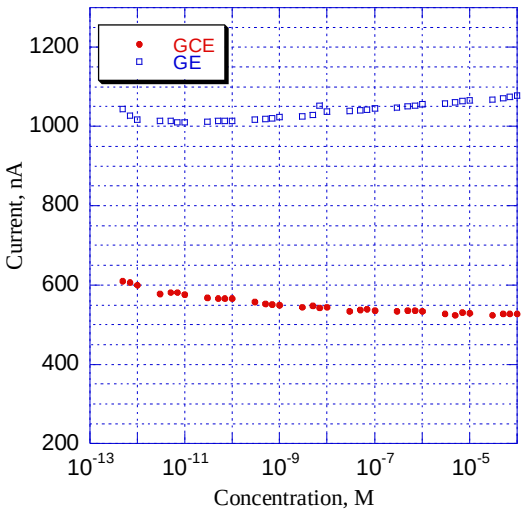
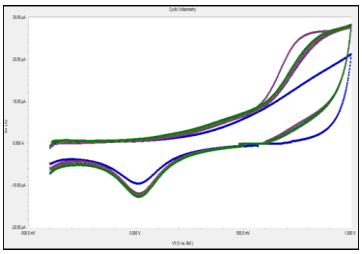


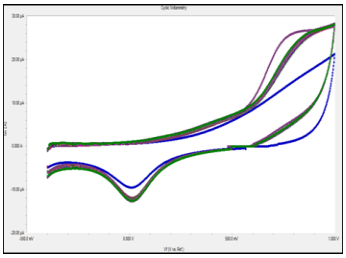
Fig. 6 The current measured at 310mV for both GE and GCE at pH 7.0

3.3 Specificity of the Ultra High Performance Electrode Sensor

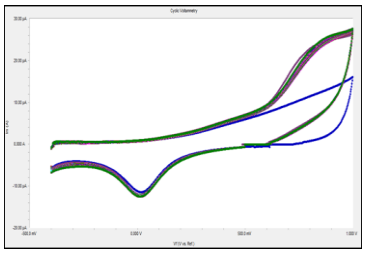
Fig. 7 shows the cyclic voltammetry responses of the selectivity test on GDH coated PtE. Lactate, NO_2^-, and peroxide were used to replace NH_4^+, and the concentration range used was between 5x10 $^{-17}$ and 1x10^{-6} M. Comparing with Fig. 1, Fig. 7 shows almost no separation due to concentrations at 750 mV, when lactate, NO_2^-, or peroxide were added in the testing solutions, which implied GDH coating has specific selectivity as expected.



(a)



(b)



(c)

Fig.7 Pt electrode coated with GDH at pH 7.0 with added α-ketoglutarate and (a) NO_2^-, (b) lactate, or (c) peroxide.

Out of the three metals used in the testing of anchoring materials, Pt was thought to be the most ideal metal due to its inertness to chemical reactions. However, Pt appeared not to bind well with our linker polymers that was part of the biocomposite layer and thus did not generate characteristic signals as strong as other metals for at least the coupling enzyme, LDH. Despite of this observation, Pt anchored electrode appeared to be functioning competitively with GDH as part of the composite layer for the corresponding coupling reactions, thus the detections of target species (e.g., $NH4^+$). Glassy carbon worked competitively as compared to the metal electrodes, but is the most expensive among all electrodes tested. The reliable detection limit of these sensors routinely was lower than $1x10^{-14}$ M.

4 CONCLUSIONS

We successfully modified the ultra high performance sensor platform with Au, Ag, Pt, and Glassy carbon as anchoring materials. These electrode sensors could detect target chemicals as low as $1x10^{-17}$ M, depending on the enzyme coupling and anchoring materials. Ag was the least expensive anchoring material but was prone to oxidation, thus less desirable. GCE was the best anchoring material in this study but was the most expensive to fabricate. GE and PtE were competitive performancewise, PtE may not link certain biocomposite coatings favorably thus its sensitivity may be compromised.

5 ACKNOWLEDGMENT

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