

Detection of Prostate-Specific Antigen (PSA) with an Ultra High Performance Electrode Modified with Sol-gel Au Nanoparticles

Solomon W. Leung¹, Seyedeh M. Mozneb², James C. K. Lai³ and David Assan⁴

¹ Civil & Environmental Engineering Department and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA Fax: 208-282-4538; tel: 208-282-2524; email: leunsolo@isu.edu

² Civil & Environmental Engineering Department, Box 8060, Idaho State University, Pocatello, ID 83209, USA. Fax: 208-282-4305; tel: 208-282-2524; email: mosnseye@isu.edu

³ Biomedical and Pharmaceutical Sciences Department and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA Fax: 208-282-4305; tel: 208-282-2275; email: lai@pharmacy.isu.edu

⁴ Corresponding author, Civil & Environmental Engineering Department, Box 8060, Idaho State University, Pocatello, ID 83209, USA. Fax: 208-282-4305; tel: 208-282-2524; email: assadavi@isu.edu,

ABSTRACT

Prostate cancer is one of the leading cancer caused death in men, especially in western developed countries. Prostate-specific antigen (PSA) is one of the biomarker that can be used to diagnose prostate cancer, although using PSA as the sole diagnosis tool for prostate cancer remains a debatable issue.

Our research group is in the quest of developing a biosensor platform that is fast in response time and versatile that can be used to detect a variety of chemicals species and metabolites. In this study, we have modified a gold electrode with layers of sol-gel nanogold particle composite that could detect PSA concentrations below 10 fg/ml in aqueous solutions, that is at least an order of magnitude more superior than the literature reports. The sensitivity range and stability of the biosensor were investigated. This sensor platform can be adapted to simultaneously detecting multiple biomarkers which is believed to be the more sensible approach for cancer detections.

Keywords: PSA, prostate cancer, high performance electrode, nanogold particles, biosensor

1 INTRODUCTION

Recent advances in clinical diagnostics have necessitated the need for ultra high performance systems for early detection of diseases and efficient treatment regimes. Prostate cancer is the second leading cause of cancer death in men in the United States, exceeded only by lung cancer. Prostate cancer accounts for about 10% of cancer-related deaths in men [1]. Prostate cancer is currently being diagnosed by measuring the levels of Prostate-Specific Antigen (PSA) in blood serum, and if

needed, a biopsy conducted. Since PSA is present in other bodily fluids other than the prostate, there is the need for a sensitive and selective method of measuring PSA levels to prevent unnecessary and painful medical procedures.

This research team has developed an ultra-sensitive and high performing sensor for the detection of PSA in concentrations below 10 fg/ml by using a gold electrode coated with Au sol-gel and biocomposite. The standard minimum detection limit for PSA in prostate cancer diagnosis has been set between 0 and 4 ng/ml for a long time, recent studies have indicated concentration levels lower than 4 ng/ml could be related to cancer [2], our sensor reported in this study can provide warning of early cancer diagnosis by measuring PSA at extremely low concentrations so that early preventive measure can be considered.

2 MATERIALS AND METHODS

2.1 Materials

The gold electrode used in the preparation of the ultra-sensitive sensor was purchased from Tianjin Aida Heng Sheng Co, Tianjin, China. The electrode diameter was 0.2 cm. A platinum counter electrode with diameter of 0.1 cm and length of 0.5 cm was used. Cysteamine, Bovine Serum Albumen (BSA), AuCl₃HCl·4H₂O (Au % > 48 %) and Sodium Citrate were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA. Anti-PSA was purchased from Novus Biologicals, LLC, Littleton, CO, USA and PSA was purchased from Abcam, Cambridge, MA, USA. The electrochemical measurements conducted against a saturated calomel electrode (SCE) as a reference electrode, were carried out on the Gamry Instrument reference 600 potentiostat.

All the other chemicals were of analytical grade. The experiment was carried out in a deoxygenated 0.1M phosphate buffer solution (PBS) of pH 7.0. Electrodes were stored in PBS at 4 °C in the dark when not in use.

2.2 Methods

The gold electrodes as well as preparations of gold nanoparticles were largely modified according to previously reported procedure [3,4], except that the final biocomposite was coated with Anti-PSA and BSA. The highly modified gold electrode/Cyst/AuNP/Anti-PSA/BSA (sensor) was used in a three-cell arrangement for electrochemical determination.

3 RESULTS AND DISCUSSIONS

The electrochemical method employed was cyclic voltammetry. The voltammograms for a freshly prepared electrode that were recorded for stepwise increases of PSA concentrations from 10^{-16} to 10^{-6} g/ml are shown in Figure 1.

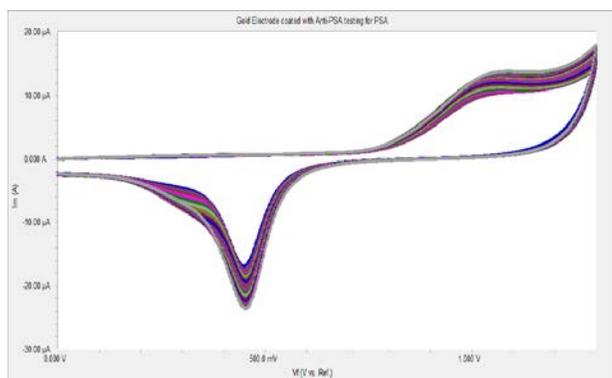


Figure 1: Voltammetric response of a freshly prepared Au electrode coated with Anti-PSA at pH 7.0 reacted with linear additions of PSA from 10^{-16} to 10^{-6} g/ml. Characteristic peaks observed at 1.06 V (oxidative) and 0.45V (reductive), respectively.

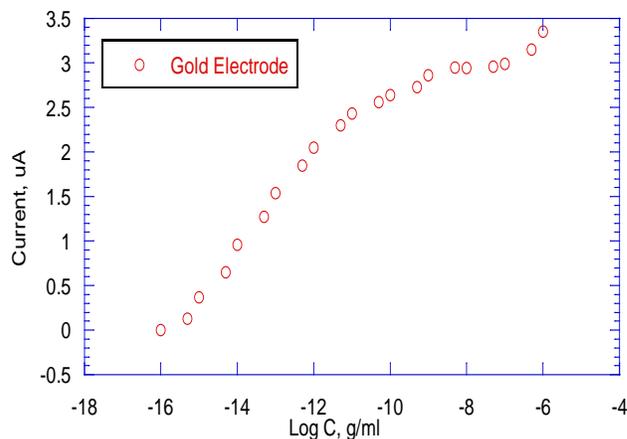


Figure 2: A plot of oxidative peak current with PSA concentrations from 10^{-16} to 10^{-6} g/ml at 1.06V.

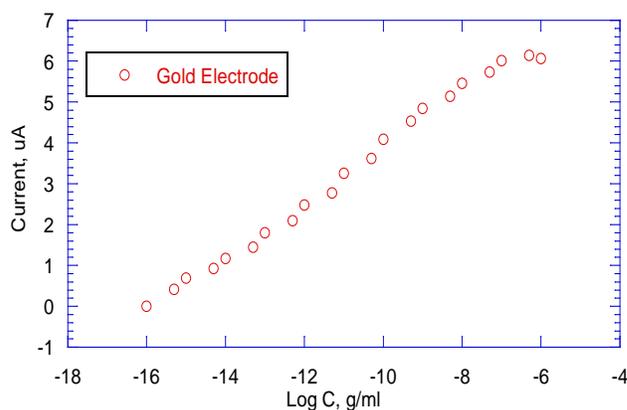


Figure 3: A plot of reductive peak current with PSA concentrations from 10^{-16} to 10^{-6} g/ml at 0.45V.

Figure 2 and 3 indicate increasing current response of Anti-PSA to PSA. As seen in figures 1, 2 and 3, the reductive peaks produced a much larger magnitude in current response compared to the oxidative peaks.

Figure 4 and 5 are the Lineweaver-Burk plot for the calculation of K_M (kinetic response) of the electrode to increasing concentrations of PSA from 5×10^{-16} to 1×10^{-13} g/ml for oxidative and reductive peaks respectively. The K_M value for the oxidative reaction was 2.7×10^{-15} g/ml (7.9×10^{-17} mol/L). For the reductive reaction, the K_M obtained was 1.5×10^{-15} g/ml (4.4×10^{-17} mol/L).

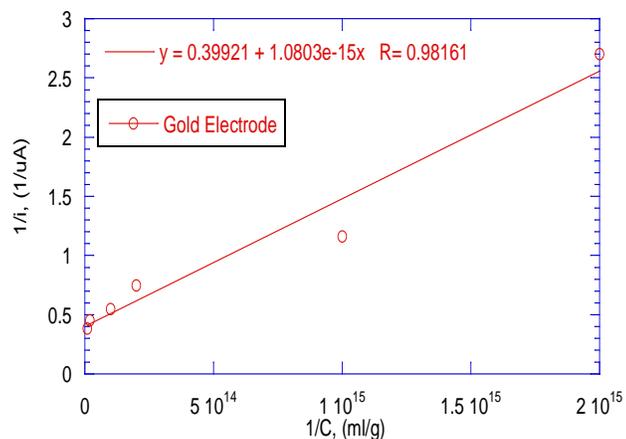


Figure 4: A Lineweaver-Burk plot for the calculation of KM for the oxidative peak from 5×10^{-16} to 1×10^{-13} g/ml.

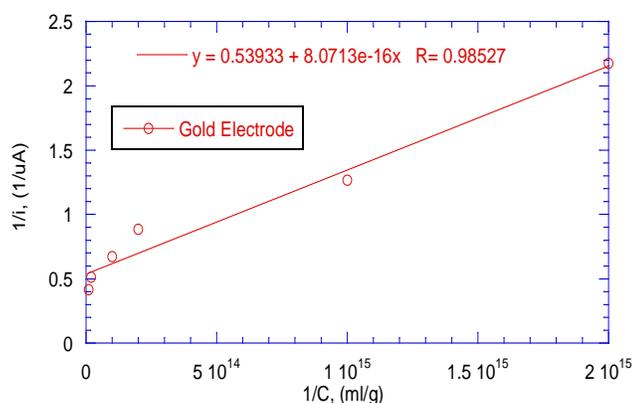


Figure 5: A Lineweaver-Burk plot for the calculation of KM for the reductive peak from 5×10^{-16} to 1×10^{-13} g/ml.

In Figure 6, 7 and 8, the selectivity and sensitivity of the modified electrode was tested with the addition of interferants commonly encountered as metabolite by products in blood serum. The electrode was applied to test H_2O_2 , NO_2^- and NH_4^+ and showed no response to increasing concentrations of the interferants in a potential range of 0 to 1.3V. This validates the selectivity of the PSA sensor in its ability to react to only PSA.

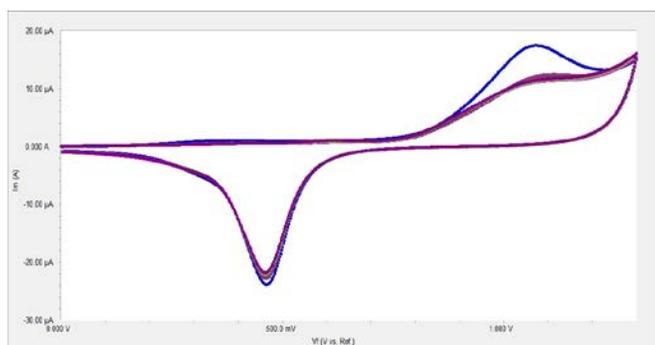


Figure 6: Voltammetric response of the modified electrode to H_2O_2 interference running from 10^{-16} to 10^{-6} g/ml.

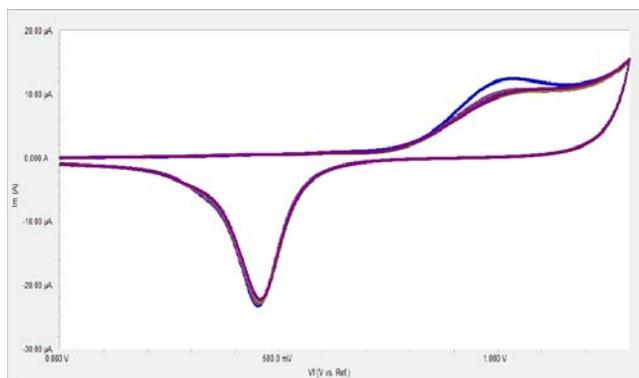


Figure 7: Voltammetric response of the modified electrode to NO_2^- interference running from 10^{-16} to 10^{-6} g/ml.

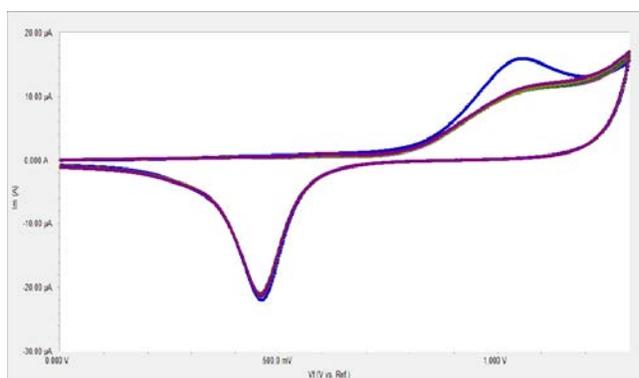


Figure 8: Voltammetric response of the modified electrode to NH_4^+ interference running from 1×10^{-16} to 1×10^{-6} g/ml.

4 CONCLUSION

The modified gold electrode was applied to the detection of PSA in aqueous solutions. The electrode was capable of detecting PSA concentrations from 10^{-16} to 10^{-6} g/ml. This is at least an order of magnitude better than those reported in literature. The electrode exhibited a distinct characteristic oxidative and reductive peak at 1.06 V and 0.45 V respectively against an SCE reference electrode.

The kinetic constant computed for both oxidative and reductive was 2.7×10^{-15} g/ml (7.9×10^{-17} mol/L) and 1.5×10^{-15} g/ml (4.4×10^{-17} mol/L) respectively.

The electrodes sensitivity and selectivity was tested with interferences such as H_2O_2 , NO_2^- and NH_4^+ and indicated that the electrode was very selective to PSA under the experimental conditions.

This sensor platform can be easily modified with other antibodies for simultaneous detection of cancer biomarkers.

5 ACKNOWLEDGMENTS

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