Ultra-High Performance Immunosensor Prepared by Glassy Carbon Electrode Modified with Nano Gold Particles and Antibody

David Assan[*], Maedeh Mozneb[*], James C.K. Lai[**], and Solomon W. Leung[***]

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

In previous reports, we have successfully fabricated biosensors that could measure chemical species that are important in environmental monitoring, as well as metabolites that are indicators of the proper functioning of our body. The detection lower limits of these biosensors are well below the measuring limits of methods used in the public domain. We continue to expand our non-toxic sensor platform based on prior reports to develop biosensors that are capable to measure metabolites at extremely low limits for biomedical applications, such as DNAs and biomarkers for cancer detections. As a demonstrative model, we are reporting an immunosensor for the detection of human immunoglobulin G (HIgG) which is the smallest but most common anti-infection antibody that makes up of majority (75-80%) of all antibodies in human body. HIgG is also the only type of antibody that can cross the placenta in a pregnant woman to help protect the fetus.

The immunosensor consists of a carbon glassy electrode modified with a layer of biocomposite sol-gel material, the biocomposite sol-gel comprises coatings of polymer (cystamine/melamine), nano Au particles, and anti-HIgG. The detection range of 10^{-5} to 10^{-20} g/ml of this immunosensor is reported. The detection limit of this immunosensor is orders of magnitude more superior than any measurements reported in the literature. In addition, selectivity and stability of this biosensor are discussed.

Keywords: human immunoglobulin G (IgG), performance, nanoparticle, sensor, antibody, glass carbon electrode (GCE)

1 INTRODUCTION

An immunosensor is basically made up of three major components: the biological agent, a transducer and an output display device. Specific biosensors and immunosensors have been fabricated to identify specific biological, chemical or environmental events. There have been many assay methods proposed over the years to satisfy a particular need: such as lithography, chemical etching, microfabrication and printing, sequential conjugation, and layer-by-layer self-assembly deposition [1, 2]. Immunomagnetic separation (IMS) and immunoassays are widely used for pathogen detection. Newly developing technology platforms with highly selective antibodies are essential to improve detection sensitivity, specificity and performance, one example is the application of porous silicon-carbon (pSi-C) composite [3, 4].

Currently, Fluorescence-enhancement immunoassay (FEI), quartz crystal microbalance (QCM), surface plasmon resonance (SPR), electrochemistry, flow injection analysis (FIA) are the most commonly used measurement methods being studied. Electrochemistry has been known to be widely preferred for detecting specific analytes due to its excellent sensitivity, wide linear range and cost of operation [40, 41].

This study is to report the performance of an electrochemical immunosensor comprised of a biocomposite layer with a glassy carbon electrode (GCE) as anchoring material which can detect HIgG at ultra-low concentration levels of 10^{-20} g/ml. How does this immunosensor's performance compare with other sensors prepared with similar fashion but with different anchoring materials, such as Au and Pt, is reported in another parallel article [7].

2 MATERIALS AND METHOD

2.1 Materials

The electrodes used was a glassy carbon electrode (GCE) purchased from Tianjin Aida Heng Sheng Co., Tianjin, China. The electrodes had a diameter of 0.2 cm. the platinum counter electrode had a diameter of 0.1 cm

and length of 0.5 cm. Cysteamine, melamine, anti-HIgG, HIgG, bovine serum albumen (BSA), $AuCl_3HCl.4H_2O$ (Au%>48%) and sodium citrate were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade. All experiments were carried out in a deoxygenated 0.1M phosphate buffer solution at pH 7.0 prepared with double deionized water.

2.2 Methods

Preparation of the biosensors were similar to the procedures outlines previously [8, 9], excepted that the biomolecules in this case were anti-HIgG immobilized on the electrodes. To ensure the anti-HIgG would only couple with HIgG at the right configuration/sites, the electrodes were submerged into BSA solution for 24 hours to saturate all other possible binding sites of anti-HIgG except those that would only couple with HIgG to ensure the efficacy of the electrodes. Slightly different biocomposites with different linker polymers (cysteamine and melamine) [8] were used to prepare the GCE sensor for performance comparison.

Detection of HIgG was carried out by cyclic voltammetry with a Gamry 600 Potentiostat. Voltammetric potential was measured against a saturated calomel electrode (SCE) at constant room temperature. Electrodes were stored at 4°C in buffer solution in the dark when they were not used during experimentation.

3 RESULTS AND DISCUSSIONS

3.1 GCE Detection of HIgG

The immunosensor could detect HIgG at extremely low concentrations. As it is shown in Figure 1a, a freshly prepared GCE sensor responded satisfactorily with a wide concentration range from 10^{-20} to 10^{-5} g/mL (at 1.1 V). As the sensor aged from Day 1 to Day 5, it lost about 65% of its sensitivity and remained the same with time afterward (at Day 28) (Figure 1b and Figure 2). So, it suggested that the sensor should be utilized soon after it is constructed.

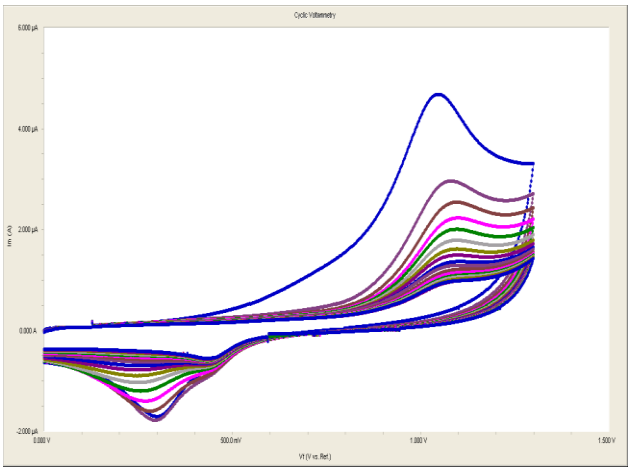

Figure 1a: Voltammetric responses of a freshly prepared GCE coated with anti-HIgG at pH 7.0 reacting with linear additions of HIgG from 10^{-20} to 10^{-5} g/mL, characteristic reductive responses were monitored at 1.1 V

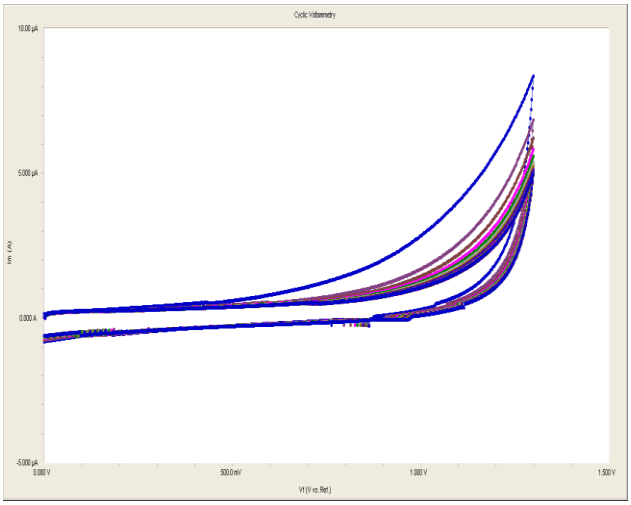

Figure 1b: Voltammetric responses of a Day 28 GCE coated with anti-HIgG at pH 7.0 reacting with linear additions of HIgG from 10^{-20} to 10^{-5} g/mL (same electrode of Figure 1b)

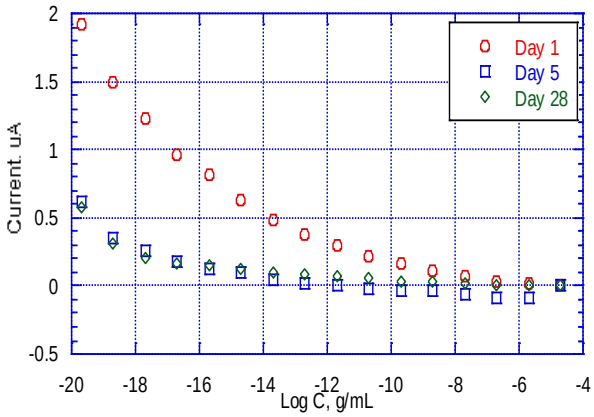

Figure 2: Performance of the same electrode in 28 days (Figure 1) for the same concentration measurements.

3.2 GCE with Different Linking Polymer

Many of our sensors were constructed with cysteamine as linker to connect the anchoring material and the sol/gel biomolecules (nanoAu and HIgG). Cysteamine was used due to its good binding property with GCE and the sol/gel. In this study, melamine was tried to replace cysteamine because melamine is less expensive and perhaps can provide with larger reactive surface for the anti-HIgG/HIgG coupling due to its branch-like structure.

Figure 2 is the Day 1 responses of the freshly prepared Pt immunosensor with melamine replacing cysteamine in the biocomposite. A point-by-point comparison for the sensor used in Figure 1 with the sensor used in Figure 2 suggested that the performance of the 2 sensors was nearly identical, and their performance with time (up to 20 days) was also very similar.

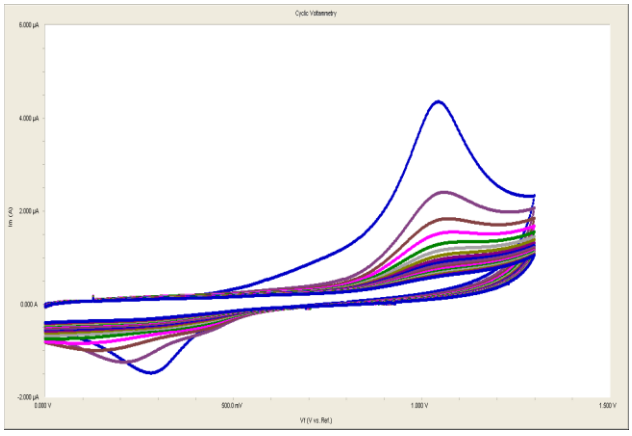

Figure 3: Voltammetric responses of a freshly prepared GCE with a different polymer linker (melamine) from the one in Figure 1 in sensor preparation.

4 CONCLUSIONS

We have successfully prepared an ultra-high performance immunosensor with a GCE modified with a biocomposite sol-gel that could detect HIgG from concentrations of 10^{-20} to 10^{-5} g/mL. The sensitivity of this sensor was reduced to about one third after 28 days at a pH 7 buffer solution at 4°C in the dark. Melamine, a less expensive alternative, can be used to replace cysteamine in the preparation of the biocomposite sol-gel with nearly identical performance

5 ACKNOWLEDGMENT

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