Performance Comparison of Immunosensors Modified with Polymers, Nanoparticles and Antibody

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ABSTRACT

Our research group is in the quest of developing a biosensor platform that is fast in response time, versatile that can be used to detect a variety of chemicals species and metabolites, and ultra-sensitive that can detect concentrations below pico-level. In this report, we are using this biosensor platform to prepare immunosensors that can be used for detection of specific DNAs and biomarkers of cancers. As a demonstration of concept, we fabricated three immunosensors for the detection of human immunoglobulin G (HIgG) that were based on Pt, Au, and glassy carbon electrode (GCE) modified with biocomposite coatings. The biocomposite coating consisted of layers of polymers, nanogold particles, and anti-HIgG. The measurement method was cyclic-voltammetry. In general, all three immunosensors were capable to measure HIgG at concentration levels of 10^{-15} g/mL or lower. Performance comparison of these immunosensors based on sensitivity, durability, and selectivity are addressed.

Keywords: human immunoglobulin G (IgG), nanoparticle, immunosensor, antibody, electrodes

1 INTRODUCTION

For more than two decades, research into the uses, functionality, and fabrication of immunosensors have received considerable interest from the biochemical and biomedical communities. Different methods of fabrication and combination of reagents are being employed to obtain efficient biosensors for a variety of purposes. In particular, focus has been concentrated on biosensors for the early detection of diseases in the human body such as cancers and congenital disorders. With such, the need for a highly sensitive and selective biosensor is therefore the key to unlock this avenue of research.

Biosensors are generally composed of immobilized layers of biomolecules such as proteins that are attached to support materials, these biomolecules are selectively coupled with the targeted substrates, thus define the selectivity of the sensors. Sensitivity of the biosensors often depends on the efficacy of the availability of the biomolecules that will bind with the targeted substrates, thus modification of how the biomolecules are linked to the supporting materials and their (distribution) configurations can improve sensitivity of the biosensors. For example, implanting of nanogold particles between the supporting materials and biomolecules enhances the binding surface availability for the substrates, thus increases the sensitivity of the biosensors [1].

Human Immunoglobulin G (HIgG), one of five immunoglobulins, is the most common anti-infection antibody in serum (70-80%) in the human body and its concentration is an indicative parameter for many infections and immunity diseases [2]. Many immunoassay methods have been employed to develop immunosensors for their good sensitivity, low cost, small size, and ease in use [3].

In this report, we investigated the performance of three different electrodes that were coated with identical biocomposite that could be used for the detection of HIgG in solution. The feasibility of these immunosensors was evaluated based on their sensitivity, durability, and selectivity.

2 MATERIALS AND METHOD

2.1 Materials

The electrodes used in the modification were gold (Au), platinum (Pt), and 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, herring DNA, 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 [4, 5], 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.

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 4ºC in buffer solution in the dark when they were not used during experimentation.

3 RESULTS AND DISCUSSIONS

3.1 GCE, Au and Pt electrodes with HIgG

Cyclic voltammograms of GCE, Au and Pt electrodes coated with the same biocomposite were obtained for increasing concentrations of HIgG from 10^{-20} to 10^{-5} g/mL, as shown in Figure 1 to 3 for the freshly prepared electrode biosensors. Among these electrodes, GCE produced consistent responses to increasing HIgG concentrations at 1.1 V for the concentration range, Pt electrode produced the largest magnitude between 10^{-20} to 10^{-5} g/mL at 0.6 V as indicated in Figure 4. As the concentration of HIgG reached to about 10^{-6} g/mL, responses of the Pt electrode increased drastically for the freshly prepared electrode. This observation will be further addressed in another report. Au electrode appeared to be relatively non-responsive in this application over the potential we tested and thus is considered not a good anchoring material for this sensor application.

It should be noted that we measured the unique oxidative or reductive response given by the respective electrode that is selective, with which we term it the characteristic peak. This characteristic peak is related to the coupling reaction(s) of anti-HIgG and HIgG, but is not limited to just one reaction. There are multiple reactions/sites that the anti-HIgG can log on to the HIgG, hence that would result in multiple peaks at different potentials, which can be seen in the voltammograms shown in Figure 1 to 3. This also explains why we have different characteristic peak for different anchoring material in this study. These various characteristic peaks can be used for identification of HIgG, but not necessary be specific enough for chemical kinetics study due to the uncertainty of binding reaction sites.

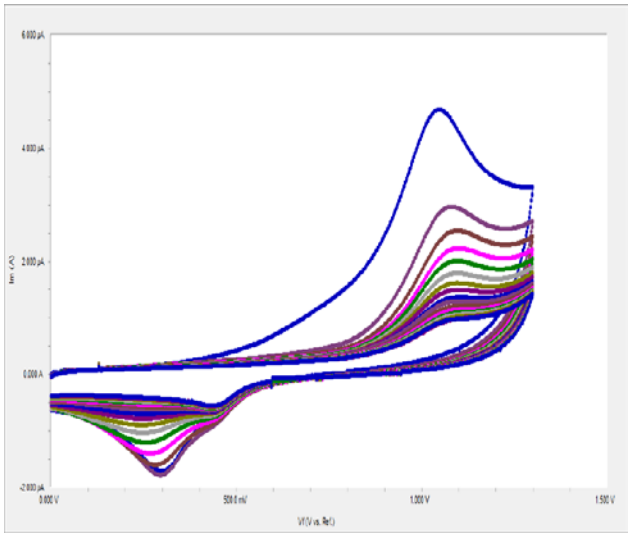

Figure 1: Voltammetric responses of a freshly prepared GCE coated with anti-HIgG at pH 7.0 reacting with linear additions of HIgG from 10^{-20} to 10^{-5} g/mL, characteristic reductive responses were monitored at 1.1 V.

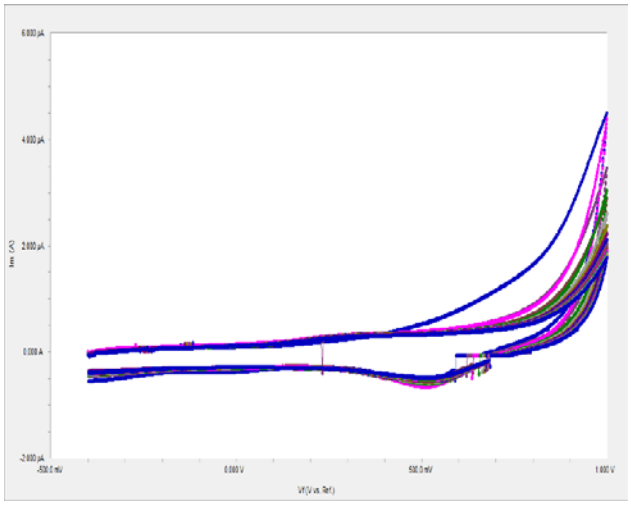

Figure 2: Voltammetric response of a freshly prepared (Day 1) Au electrode coated with anti-HIgG at pH 7.0 reacting with linear additions of HIgG from 10^{-20} to 10^{-5} g/mL, characteristic oxidative/reductive peaks were monitored at 0.6 V.

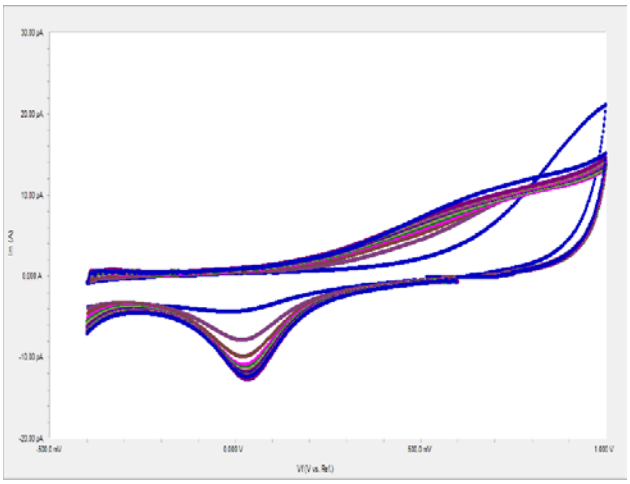

Figure 3: Voltammetric responses of a freshly prepared Pt electrode coated with anti-HIgG at pH 7.0 reacting with linear additions of HIgG from 10^{-20} to 10^{-5} g/mL, characteristic oxidative responses were monitored at 0.6 V.

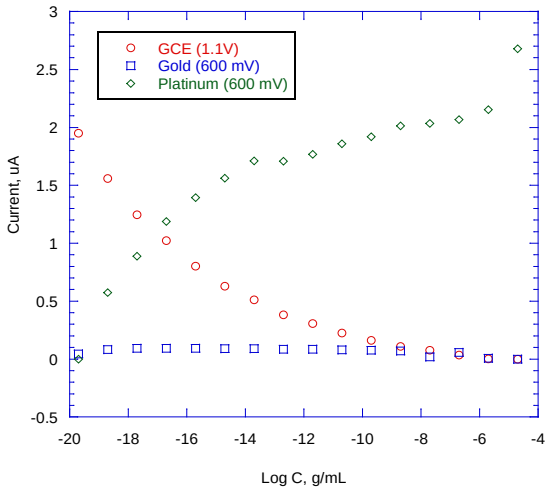

Figure 4: Comparison performance of GCE, Au and Pt electrode with increasing additions of HIgG from 10^{-20} to 10^{-5} g/mL on Day 1.

3.2 GCE, Au and Pt electrodes Performance with Time

Figure 5 shows the responses of GCE, Au and Pt electrodes to linearly increasing concentrations of HIgG from 10^{-20} to 10^{-5} g/mL after 28 days. While the magnitudes of the Au and GCE decreased, the response magnitude of the Pt electrode actually increased. This indicates that the integrity of the Pt sensor was preserved with no deterioration of the biomolecules within, or the anti-HIgG has changed to a more favorable binding configuration with HIgG. Thus, Pt would be the preferred anchoring materials if the immunosensor is to be used for a long time.

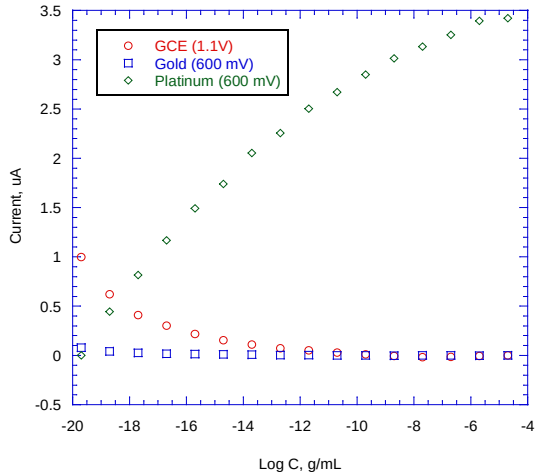

Figure 5: Comparison performance of GCE, Au and Pt electrode with increasing additions of HIgG from 10^{-20} to 10^{-5} g/mL after 28 days.

3.3 Pt electrodes Performance with Interferents

Anti-HIgG can bind with many redox reagents and thus the immunosensors can be affected by many interferents. Figure 6 shows the responses of the Pt electrode with the addition of an interferent initially (NO_2^-, ammonia, peroxide, and herring DNA individually at concentrations between 10^{-12} to 10^{-7} M) in the running solution before conducting HIgG measurements. Except with NO_2^-, the sensor was still functioning but the sensitivity was drastically reduced especially in HIgG concentrations above 10-14 g/mL. In addition, the characteristic peak at about 0.6 V for the Pt electrode has shifted as indicated in the legends of Figure 6. Nitrite affects the immunosensors immensely and the topic is addressed in a future article.

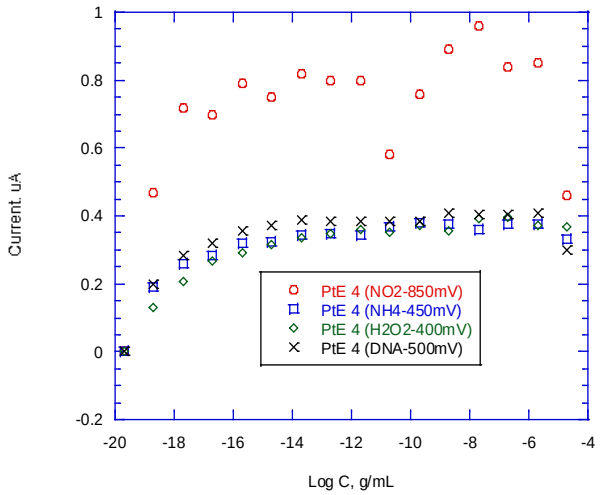

Figure 6: Responses of Pt electrode to inorganic and organic interferences running from 10^{-20} to 10^{-5} g/mL (after 35 days).

4 CONCLUSIONS

Three electrodes (GCE, Au, Pt) modified with polymers, NanoAu particles, and anti-HIgG were successfully employed in the electrochemical detection of HIgG, with detection lower limit as low as 10^{-20} g/mL.

Pt electrode coated with the biocomposite functioned best among the test group in the testing concentration range of 10^{-20} to 10^{-5} g/mL, even after 28 days. This demonstrates that the modified electrode is durable and can still be viable after considerable time of aging when stored at 4°C in the dark in buffer solution.

Testing the electrodes in the presence of interferents indicated that the modified electrodes responded to interferences due to the active nature of anti-HIgG. Further investigations are being conducted by this research group to determine the viability of the immunosensors in real-time scenarios.

5 ACKNOWLEDGMENT

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