Use of Nanoparticles for Improving Enzyme Stability to Prolong Biosensor Functionality

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Statement of Purpose: Most enzyme based biosensors lose functionality in a couple of weeks due to the instability of enzyme and other causes. Improving the stability of enzyme in a biosensor is crucial to prolonging its functionality. In this study, we examined how gold nanoparticles of different sizes can be used to enhance the stability of enzyme in an electrochemical based biosensor. Specifically, we focused on improving the stability of glucose oxidase when it is incorporated into a pyrrole/enzyme mixture for functionalizing electrodes made of 3D nanopillar structures for glucose detection.

Methods: Gold nanoparticles of various sizes were prepared by reduction of chloroauric acid with THPC (2-4 nm), citrate KBH_4 (8-10 nm) and sodium citrate (25-30 nm). 3D nanopillar electrodes with 50 nm nanopillars were prepared used the method reported earlier [1]. 3D nanopillars formed on glass substrates were first cleaned electrochemically and then functionalized via an electro-polymerization process in a mixture solution of glucose oxidase (GOx), polypyrrole and GNPs. The electro-polymerization was performed under a current density of 35 $\mu A/cm^2$ for 35 minutes in 0.1 M KCl solution containing 0.05 M pyrrole and 0.5 mg/ml GOx at pH7.2. The functionalized electrodes were characterized using amperometric measurements to examine the enzyme stability. Measurements were taken on Day 1, Day 3, Day 12 and Day 120. UV-vis and electrochemical voltammetry and impedance were measured for further structural characterizations.

Results and Discussions: Figure 1 shows two TEM images of some of the obtained nanoparticles prepared with KBH_4 (1A) and sodium citrate (1B). Figure 2 shows the amperometric current responses to stepwise addition of 2.5 mM of glucose and their corresponding sensitivity calibration curves. Of the three groups of GNPs tested, the largest ones (25-30 nm; Fig.2A &2B) showed the least sensitivity of 0.876 $\mu A/mM/cm^2$ with a steady trend of losing sensitivity over time. By Day 120, a loss of 70% in sensitivity was seen compared with that of Day 1. In the case of 8-10 nm GNPs, an increasing trend in sensitivity was seen in the first 12 days, and on Day 120 the sensitivity dropped some 38% from that of Day 12. The case of 2 -4 nm GNPs produced the best performance in terms of maintaining the enzyme stability. Similar to the case of 8-10 nm GNPs, these electrodes reached a peak sensitivity on Day 12 (8.87 $\mu A/mM/cm^2$) and lost about 23% percent of its peak value on Day 120 (6.78 $\mu A/mM/cm^2$).

Figure 3 shows some bar graphs summarizing the change in sensitivity over time for various GNP cases. Clearly, the results suggest that smaller nanoparticles produced better results in improving the sensitivity of the glucose biosensor and prolonging its functionality and stability. At this moment, it is not clear what caused the increase in sensitivity in the first several days, but we speculate that it might be attributed to favorable conformational changes in the enzyme when small GNPs are present, which may also prevent enzymes from leaching out of the polypyrrole matrix. The obtained UV-vis spectra seem to support this argument by showing enhanced contact between the smaller GNPs and the enzyme. Voltammetric and impedance results showed that the electrodes modified with smaller nanoparticles exhibited a lower electron transfer resistance.

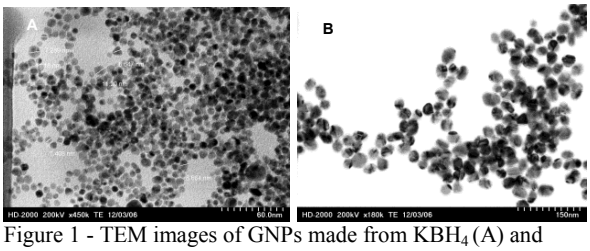

Figure 1 - TEM images of GNPs made from KBH_4 (A) and sodium citrate (B) reduction methods.

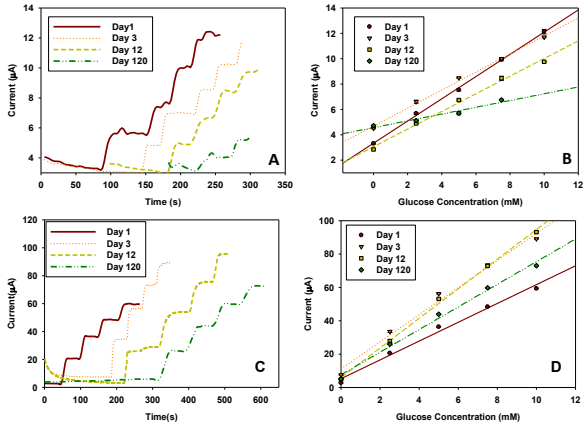

Figure 2 - Amperometric responses to stepwise addition of glucose for the 3D electrodes having the enzyme mixed with 25-30 nm GNPs (A) and with 2-4 nm GNPs (C), along with their corresponding sensitivity calibration curves (B & D).

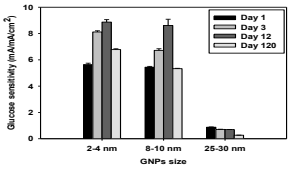

Figure 3 – Comparison of the sensitivity values for various GNP/enzyme modified 3D nanopillar electrodes.

Conclusions: In improving the stability of enzyme in electrochemical glucose biosensors using nanoparticles, we found that the size of the GNPs had a distinct effect. It seems the smaller the better. Nanoparticles with diameter of 2–4 nm provided the best result by prolonging the biosensor's functionality over a period of 120 days with only 38% loss in signal detection sensitivity. By contrast, GNPs larger than 25 nm appear to have no benefits.

References: Gangadharan R, Anandan V, Zhang A, Drwiega JC and Zhang G. Enhancing the performance of a fluidic glucose sensor with 3D electrodes. Sensors and Actuators B: Chemical, 160 (1), p.991-998, 2011.