Improvement of detection limit in gold-linked electrochemical immunoassay by investigation of the factors affecting on the sensitivity

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Abstract:
Gold-linked electrochemical immunoassay (GLEIA) is a promising method for bio-molecule measurement due to its high sensitivity, fast response, low cost, mass fabrication, and ability to be integrated into microsystems. The principle of this method is based on specific reaction between antibody and antigen. In order to improve the limit of detection of GLEIA, not only increasing electrochemical signal but also decreasing background is important. However, the origin of background is not known well, up to date. In our assumption, in GLEIA method, when preoxidation occurs, GNPs and electrochemically active substances present in the solutions will be oxidized, leading to increase in current signal, thus improve GLEIA sensitivity. Therefore, in this research, we investigated the effects of preoxidation with presence of first Ab, BSA and serum separately on GLEIA sensitivity and then we applied GLEIA for insulin detection.
In order to investigate the origin of sensitivity, we incubated separately solutions of first Ab, 1% BSA and serum on working electrode surface and measured DPV using 0.01mM $HAuCl_4$ in 0.1M HCl. The reason of using $HAuCl_4$ instead of GNPs is that we just want to focus on preoxidation of substances.
In the case of BSA/DEP chip, preoxidation time increases, both of background signal and current signal increase due to surface cleaning effect, and the signal/background ratio decreases, so preoxidation with presence of BSA affects negatively on the sensitivity. In the case of Ab/DEP chip, both of background signal and current signal increase due to surface cleaning effect, and the signal/background ratio is almost same, so preoxidation with presence of Ab has no significant effect on GLEIA sensitivity.
In the case of serum/DEP chip, when preoxidation time increases from 0s to 50s, both of background signal and current signal increase due to surface cleaning effect, but when preoxidation time increases from 50s to 100s, the current signal increases due to the surface cleaning effect but the background signal decreases due to the decomposition of background substances present in the serum solution (Fig. 2). As a result, signal/background ratio of serum/DEP chip increased with increase in preoxidation time. It can be said that high sensitivity of GLEIA comes mainly from the surface cleaning effect and partially from the decomposition of background substances.
Using GLEIA, we introduced a new sandwich immunoassay for insulin detection using disposable chip as a basic. EIS measurements proved successfully assemble on the working electrode surface for insulin detection. Optimal conditions for performance of immunoassay are proposed: capture Ab incubation time is 4 hours, 1% BSA blocking time of 12 hours, GNPs diameter of 15nm and DPV preoxidation potential of 1.2V. With this approach, a detection limit of 3.6ng/mL was achieved for insulin with the dynamic range of 3.6 – 50 $ngmL^{-1}$ (Fig. 1).

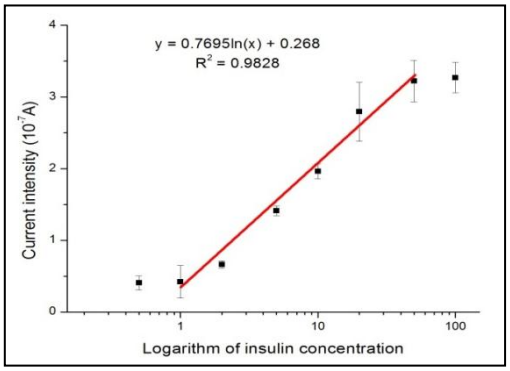

Figure 1: The calibration curve with insulin concentrations from 0.1$ngmL^{-1}$ to 100$ngmL^{-1}$. Detection limit of determination is 3.6$ngmL^{-1}$.

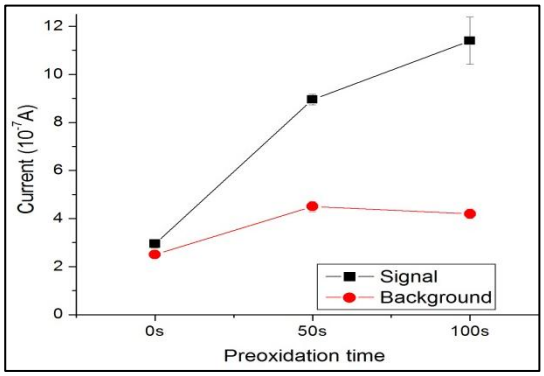

Fig 2: Signal intensity and Background of serum/DEP chip at different preconditioning time.

Keywords: electrochemical sandwich immunoassay, insulin determination, sensitivity.