A Chip-Based Biosensor for Detecting Cortisol Biomarker

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Statement of Purpose: Adrenal insufficiency is a disease that occurs in nearly 60% of septic patients[2]. Immunoassay is the current clinical standard technique to detect cortisol levels. This can take 6-8 hours. Not being able to measure the concentration timely and accurately could cause serious delay in providing proper patient treatments. This research is aimed to develop a portable in-vitro biosensor that can accurately and rapidly detect cortisol levels. This procedure is carried out using a three-electrode electrochemical biosensor that has been developed and tested to detect cortisol by a means of running cyclic voltammetry tests.

Methods: Detection of cortisol is achieved by bio-functionalizing a three-electrode sensor. Three-electrode sensor chips consisting of a working, counter, and reference electrode are used. The screen-printed sensor chips are purchased from Metrohm USA. They have a gold working and counter, as well as a silver reference. Bio-functionalization of the working electrode beings with creating a self-assembled monolayer (SAM). Fig. 1 shows the protein monolayer that is created by binding the sulfide group of dithiobis (succinimidyl propionate) (DTSP) to the surface of the gold working electrode.

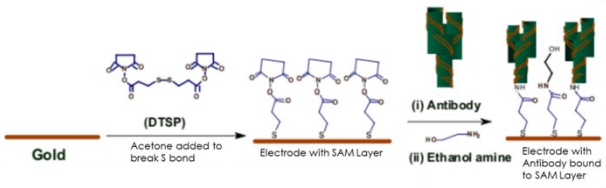
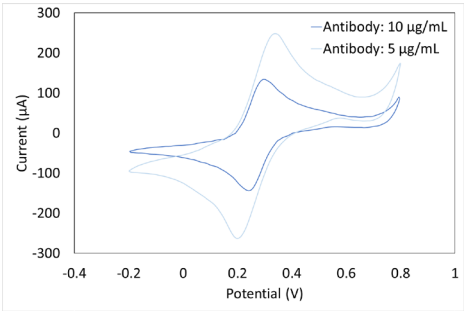
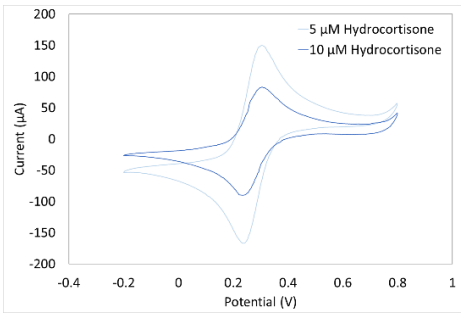


Fig.1 - Schematic of EA/C-Mab/DTSP/Au bio-electrode fabrication. Modified from reference 1.

Next, anti-cortisol antibody (C-Mab) is bound to the succinimidyl group of the SAM through the anti-body's amine group. [2]A solution containing phosphate buffered saline (PBS), 100% ethanol, and 2 mg of hydrocortisone (cortisol) is heated in a warm bath to dissolve the antigen. The prepared sensor is then exposed to a hydrocortisone solution diluted in PBS to a specific concentration for testing. Measurement of sensors' responses to cortisol is done by running cyclic voltammetry (CV) testing using a Potentiostat. These tests were run within a potential range from -0.2V to 0.8V and at a scanning rate of 150 mV/s for 20 cycles. To facilitate the electron transfer during the redox reaction, the CV tests were performed in a PBS supporting electrolyte solution containing 5 mM potassium hexacyanoferrate as the redox species.

Results: Fig. 2 and Fig. 3 show some typical CV curves obtained during experiments using the two types of sensor chips (AT and BT). Fig. 2 shows the CV curves for two BT sensors that were prepared with different concentrations of antibody, 5 µg/mL and 10 µg/mL. Fig. 3 shows the CV curves for two AT sensors that were exposed to 5 µM and 10 µM concentrations of hydrocortisone. In all cases, an oxidation current peak is reached at a potential of 305 mV and a reduction current peak at 237 mV. The change in peak current locations (Fig.2) and in peak current values (Fig.2 and Fig.3) indicate a success in the immobilization of antibody to the electrode and the binding of cortisol antigen to the anti-body. For varying antibody concentration, in the case of 5 µg/mL, the oxidation current reaches a peak of 248 µA and in the case of 10 µg/mL the peak is much lower at 134 µA. For varying cortisol concentration, in the case of 5 µM, the oxidation current peaks at 151 µA and in the case of 10 µM it is at 83.2 µA.





Moreover, the current levels for the AT and BT types of sensors are visibly different. This is likely owing to the physical difference existing in AT and BT sensor chips. The electrodes on an AT chip have more porous structure than that of a BT chip, creating a larger surface area for the functionalization steps.

Conclusions: The obtained results demonstrate a success in establishing the feasibility of detecting trace amounts of cortisol in solution. The peak currents obtained reflect the underlying kinetic, as well as mass transport mechanisms. As the concentration of hydrocortisone increases, the current level of the CV curves decreases, showing that there is less surface area on the working electrode available to participate in the electron transfer to facilitate the redox reactions, which serves as the sensing transduction mechanism.

References: [1] Arya, S. K., Chornokur, G., Venugopal, M., & Bhansali, S. *Antibody modified gold micro array electrode based electrochemical immunosensor for ultrasensitive detection of cortisol in saliva and ISF*. Procedia Engineering, Vol. 5, pg. 804-807.