CHAPTER 4

Conclusion

HIgG is an antibody in the body. The high concentration of HIgG indicated various diseases so HIgG can be used as biomarkers for some diseases and determination of HIgG is necessary. In this work, the SIA system was developed with electrochemical immunosensor for determination of HIgG.

The electrochemical immunosensor was prepared by deposition of GO on SPCE surface and immobilized with anti-HIgG that acted as a bioreceptor of the immunosensor. The current signal was evaluated before and after immunointeraction with HIgG by measurement of redox current of $[Fe(CN)_6]^{3-/4-}$ in PBS solution. Bared SPCE and modified SPCE were characterized for the redox response and morphology by using CV and SEM techniques, respectively. The optimum analytical conditions were determined in term of decreasing current percentage. The results from CV shows that the anti-HIgG/GO/SPCE provided high electrical current from $[Fe(CN)_6]^{3-/4-}$ probe but after immunointeraction between HIgG and anti-HIgG the current was decreased proportionally to HIgG concentration due to the blockage of mass transfer by the proteins as confirmed by SEM and EDS. The optimum parameters of SIA system were found as follows: flow rate of 2 mL min^{-1}, applied potential of +350 mV, concentration of $[Fe(CN)_6]^{3-/4-}$ of 10 mM, and immunointeraction time of 10 min. Reduction of current peak is proportional to HIgG concentration and can be employed for determination of HIgG by using a calibration graph. Under the optimum condition of SIA system, the linear calibration graph for determination of HIgG was obtained in the range of 2-100 ng mL^{-1} with 1.70 ng mL^{-1} of detection limit. The immunosensor provided good repeatability and reproducibility in percentage of relative standard deviation (%RSD) value of 2.60% (N=11) and 3.55% (N=7), respectively, for determination of 50 ng mL^{-1} HIgG. The SIA system was applied to determine HIgG in real urine samples. Four urine samples were spiked with certain HIgG concentration and calculated from calibration

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graph. The results show 97.00 - 104.93 % of recoveries. Moreover, the samples were analyzed by standard addition method. The results showed amount of HIgG was in the range of 0.119 - 0.453 ng mL^{-1}.

Therefore, the proposed system can determine HIgG in real samples and offered convenient manipulation of various solutions, good repeatability and reproducibility, using smaller amount of reagents than batch analysis, and using simple instrument.

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