

전기화학면역센서 개발을 위한 탄소 전극의 특성 분석

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Introduction

During the past decade, immunosensors with molecular recognition system such as antigen-antibody recognition have been successfully developed and applied to many fields including food, environmental and biomedical processes [1,2]. In the development of immunosensors, the antibody immobilization process for stability and activity of antibody and the signal transfer process for sensing the specific antigen-antibody interaction at the receptor are two major processes. In general, the antibody immobilization on a substrate is achieved by chemisorption or physisorption [3,4]. The signal transfer process that is the most important part in the molecular recognition has been developed into the signal amplification or changing the optical signal at the antigen-antibody interaction to exploit the secondary antibodies. Therefore, level-free immunosensors without secondary antibodies is the most desirable and have gained considerable interest as bioanalytical devices because they are robust, are economical to mass production, and can achieve excellent detection limits with small analyte volumes.

In this study, level-free immunosensors for detection of *E.coli* O157:H7 was prepared by using graphite originally having conductive property. A carbon composite electrode was made by a sol-gel method having many advantages including low-temperature encapsulation of biorecognition elements, tenability of physical characteristics, and mechanical rigidity. Anti-*E.coli* O157:H7 antibodies were immobilized on a carbon composite electrode at the porous site. The immobilization of antibodies onto the carbon composite electrode surface and the binding of *E. coli* O157:H7 cells with antibodies were

observed by cyclic voltammetry (CV) in presence of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. Electron-transfer resistance of the electrode and the concentration of *E.coli* cells were investigated by cyclic voltammetry as the same principles.

Experimental

Materials. All solutions were prepared in doubly distilled deionized water. Affinity purified mouse anti-*E.coli* O157:H7 antibody was obtained from Biodesign International. A 1:1 dilution was prepared with 50% glycerin solution in water before use. Phosphate-buffered saline (PBS; 0.01M, pH 7.4), hexacyanoferrates, tetraethyl orthosilicate (TEOS), ethyl alcohol, graphite power and hydrochloric acid were purchased from Sigma-Aldrich. *E.coli* O157:H7 was obtained from American Type Culture Collection. All CV measurements were performed using 660a series from CH Instruments, Inc.

Film Fabrication of an Immuno-electrode and Immobilization of Anti-*E.coli* O157:H7 Antibodies. A sol-gel stock was prepared by mixing TEOS, ethanol, water, and HCl. One gram of graphite powder was subsequently dispersed into the 1.4mL sol-gel solution. The resulting sol-gel derived graphite composites were printed with $0.2 \times 3 \text{ cm}^2$ area on the slide glass for the working electrode. The diluted antibody solution ($20 \mu\text{l}$) was spread on the surface of the sol-gel derived graphite electrode. The electrode was then rinsed extensively with PBS (pH 7.4) containing 1% BSA and deionized water, and then it was dried with nitrogen. *E.coli* cultures with different cell numbers ($20 \mu\text{l}$) were dropped onto the surfaces of the sensors.

Results and Discussion

Cyclic voltammetry was used for confirmation of the stepwise changed of the sol-gel derived carbon composite electrode-based immunosensors. Before the antibodies were immobilized on the surface of electrode, the tendency of CV was measured by observing the same area ($0.2 \times 3 \text{ cm}^2$) and the different weight of carbon paste electrode. The voltammogram of Figure 1-a shows that, when the weight of carbon paste was increased from 5.1mg to 10.2mg, the resulting current was also increased 0.71mA to 0.94mA. The quantity of carbon has an effect on the peak current in the CV. In addition, two curves (7.6mg and 7.7mg) among these results are compared to confirm the characterization of the weight of carbon (Figure 1-b). Carbon paste electrode with same area and weight has the similar current of the electron transfer on the surface of the electrode.

Figure 2 shows that the voltammetric behavior of the redox probe is influenced by the electrode

surface modification. The permeability of ions through the sol-gel carbon composite electrode is so high that a redox couple can penetrate it (Figure 2-a). Immobilization of an anti-*E.coli* O157:H7 reduces the penetration of the redox couple (Figure 2-b). After the treatment of the antigen-antibody interaction was occurred, the electron transfer of the electrode decreased more (Figure 2-c). When the electrode was immobilized with antibodies, a decrease occurred in peak current (from 516.2 to 390.0 μ A). A decrease of $\sim 25\%$ in the separation of peak currents was clearly observed upon the binding of antibody immobilization to the electrode surface. The cell binding also resulted in a reduction in peak current (from 396.0 to 325.9 μ A). This indicates that the blocking of the interfacial electron transfer between the soluble redox probe and the electrode upon binding of the protein to the carbon paste surface is not efficient.

Figure 3 shows the cyclic voltammogram of detecting the *E.coli* O157:H7 with respect to the concentration. As the concentration of *E.coli* O157:H7 was increased, the peak current of the electrodes was also increased. When, the concentration of *E.coli* O157:H7 is 7.15×10^8 , 1.83×10^9 and 8.21×10^9 , the voltammogram is a, b and c in Figure 3, respectively. The decrease of the current by increasing the concentration of antigen is the same effect with the increase of the electron transfer resistance by adsorbing the antibody on the carbon paste electrode surface. From the above results, the carbon paste electrode derived sol-gel method was successfully applied to detection of the *E.coli* O157:H7 by electrochemical method.

In order to immobilize the antibody on the carbon paste electrode surface, sol-gel derived material was prepared and coated to slide glass. Antibodies were immobilized to the porous surface of sol-gel derived carbon paste electrode via physical adsorption. The detecting biomaterials, *E.coli* O157:H7, were immobilized on the carbon paste electrode using the antigen-antibody interaction. Each electrode was investigated by cyclic voltammetry at the presence of hexacyanoferrates as a redox probe. The results confirmed that the electron transfer was decreased by being the biomaterials between the carbon paste electrode surface and the soluble redox probe.

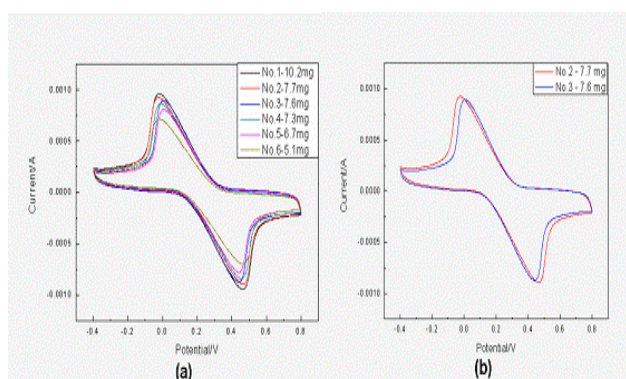


Figure 1. Influence of amount of graphite on cyclic voltammetry. In the presence of 5mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.01M PBS, pH 7.4; scan rate = 100mV/s; Ag/AgCl as a reference electrode.

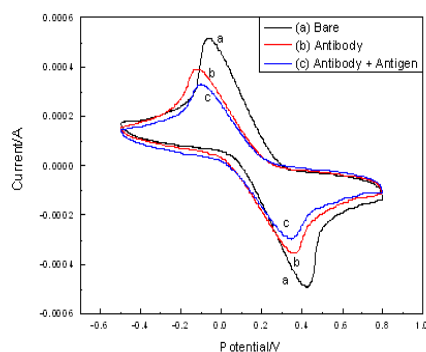


Figure 2. Cyclic voltammetry of the bare sol-gel derived carbon composite electrode (a), after antibody immobilization (b), and after *E.coli* cells binding (c) in the presence of 10mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.01M PBS, pH 7.4, Scan rate = 100 mV/s; Ag/AgCl as a reference electrode; platinum as a counter electrode; *E.coli* O157:H7 1.83×10^8 CFU/mL/

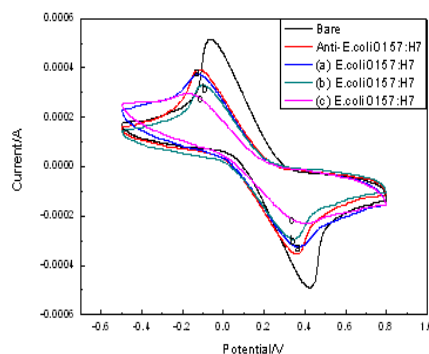


Figure 3. Effect of antigen concentration on CV curve. (a) 7.15×10^8 , (b) 1.83×10^9 , (c) 8.21×10^9 CFU/mL *E.coli* cells on their surfaces.

ACKNOWLEDGEMENTS

This research was supported by the Nano/Bio Science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Science and Technology (MOST).

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