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Performance enhancement of polyaniline-based polymeric wire biosensor

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ABSTRACT

We demonstrate here the performance enhancement of polyaniline-based biosensor using screen-printing technology and pulse mode measurement technique. Screen-printed silver electrodes were made on a nitrocellulose membrane and the distance between the two electrodes was approximately 550 μm. Resistance of the electrodes had an average of 1.4 Ω with a standard deviation of ±0.4 Ω. The surface of nitrocellulose membrane was modified by glutaraldehyde to immobilize streptavidin. Biotinylated anti-mouse IgG was conjugated with polyaniline-coated magnetic nanoparticles. Formation of polyaniline-coated magnetic nanoparticles was confirmed by a transmission electron microscope image. The polyaniline was used as an electric signal transducer for the monitoring of the biospecific binding event. An electrical response induced by the streptavidin–biotin interaction was measured by pulse mode measurement. This measurement method reduced the resistance caused by interfacial capacitance. Dose-dependent resistance changes were also successfully analyzed by the pulse mode polymeric wire biosensor. Results showed that the pulse mode measurement technique enhanced the performance of the polyaniline-based polymeric wire biosensor by reducing the interfacial effects. This approach could be helpful in samples with high interfering background materials, such as food and clinical specimens.

1. Introduction

Recently, safety in water and food has become an essential issue for protecting human health so that the demand for a rapid, cheap, simple and on-site detection device has dramatically increased (Wutor et al., 2007; Stobiecka et al., 2007; Alocilja and Radke, 2003). Several biosensors were developed based on the electrochemical, optical, and piezoelectric detection principles (Kim et al., 2000; Yuk et al., 2006; Vaughan et al., 2001). A disposable polyaniline-based biosensor measuring an electrical response by using conducting polyaniline was developed to fulfill the above-mentioned demands (Muhammad-Tahir and Alocilja, 2003a). The polyaniline was doped with protonic acids, such as aqueous hydrochloric acid, to give its conductivity. This property could be used as an electrochemical transducer in reporting binding events of biological materials. Polyaniline compounds are generally soluble, environmentally stable polymers that are fabricated by a one-step synthesis involving inexpensive raw materials (Angelopoulos, 2001). Especially, polyaniline is recognized as the only air-stable conducting polymer with very high electrical conductivity in the protonated state (Syed and Dinessan, 1991) and can be bound easily to most proteins (Imisides et al., 1996). Due to the advantages of polyaniline, it has been used as the transducer of electrical biosensors.

However, the polyaniline-based biosensor has suffered from irreproducibility and unreliability due to manually made electrodes and signal processing (Muhammad-Tahir and Alocilja, 2003b). Screen-printing technology can be an alternative proposal to fabricate homogeneous and reproducible electrodes. Screen-printed electrodes are produced by printing inks of various types, such as gold, platinum, silver, palladium and graphite. Polyester screens are generally used for printing with patterns designed by the analyst in accordance with the analytical purpose. Thus, the screen-printing technology can provide high-volume production of inexpensive and highly reproducible and disposable low-cost electrodes (Renedo et al., 2007; Tudorache and Bala, 2007).

In this study, we present performance enhancement of the polyaniline-based polymeric wire biosensor based on screen-printing technology and pulse mode measurement technique by analyzing streptavidin–biotin interactions as a model system. Streptavidin has extraordinarily high affinity toward biotin ($K_d \approx 10^{-14}$ to 10^{-16} M). In addition, they are also unusually stable against heat, denaturants, extreme pH and to the activity of proteolytic enzymes (Laitinen et al., 2007). Screen-printed silver electrodes were fabricated on nitrocellulose (NC) membranes, which had a uniform space between electrodes. The NC membranes were modified with glutaraldehyde for use as a crosslinker

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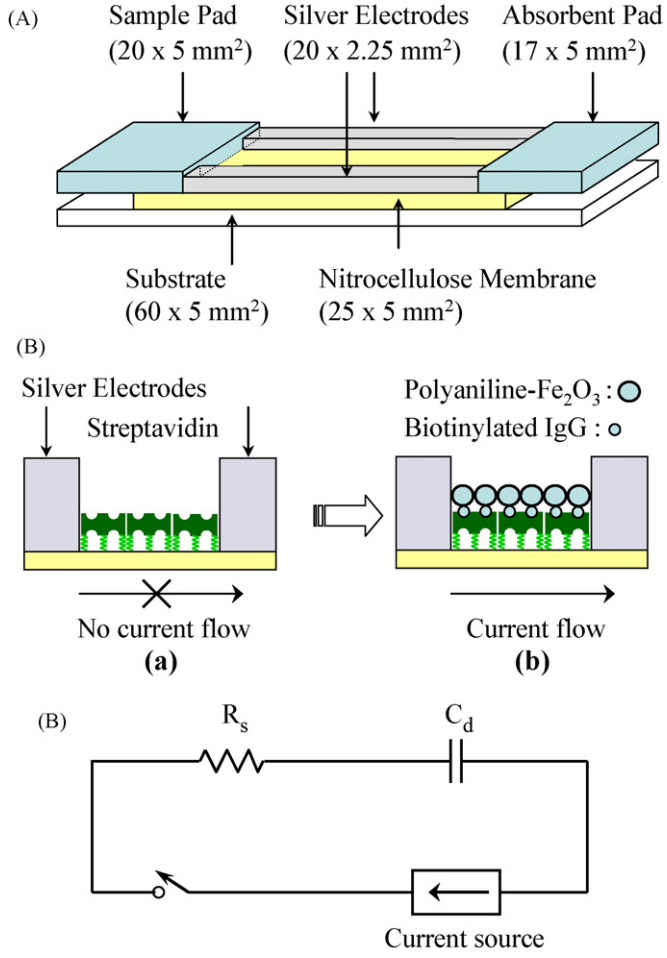

Fig. 1. Schematic diagram of polyaniline-based polymeric wire biosensor. (A) Components of the biosensor: (1) sample pad of cellulose membrane; (2) nitrocellulose membrane with screen-printed silver electrodes; (3) absorbent pad of cellulose membrane. (B) Cross section of the biosensor to illustrate electric signal generation: (a) before and (b) after applying the analyte onto the sample pad. (C) An equivalent RC circuit model when the electrical response was measured using the biosensor.

in the chemical immobilization. Biotinylated anti-mouse IgG was conjugated with polyaniline-coated magnetic nanoparticles. Resistance changes induced by the streptavidin–biotin interaction were investigated by pulse and non-pulse mode measurements. Dose-dependent responses according to various concentrations of biotinylated IgG conjugated with polyaniline coated magnetic nanoparticles were analyzed by the pulse mode measurement technique.

2. Working principle of the polyaniline-based biosensor

The principle of signal generation of conducting polyaniline-based biosensors is illustrated in Fig. 1(B), which is a cross section of the biosensor in Fig. 1(A) (Muhammad-Tahir and Alocilja, 2003a). When a liquid analyte is applied to the sample pad, the analyte containing the polyaniline–biotin complex flows by capillary action along the NC membrane with immobilized streptavidin. Streptavidin–biotin interaction occurs on the NC membrane and then the conducting polyaniline forms a polymeric wire and bridges the two silver electrodes (Fig. 1(B)). Thus, it is possible to flow current between the electrodes and the electrical response is measured.

The electrode–solution interface has been shown experimentally to behave like a capacitor, and a model of the interfacial region somewhat resembling a capacitor can be given (Bard and Faulkner, 2000). Thus, Fig. 1(B) can be approximated by an equivalent RC circuit model as shown in Fig. 1(C). When the equivalent RC circuit is charged by a constant current, then the voltage across the resistor and the capacitor is expressed as

$$V = iR_s + \frac{i}{C_d}\int_0^t dt, \quad (1)$$

or

$$V = i\left(\frac{R_s + t}{C_d}\right), \quad (2)$$

where R_s represents the solution resistance and C_d represents the capacitance at the electrode–solution interface. Resistance is calculated using Ohm's law; the resistance is obtained by dividing the DC voltage measured across the device by the DC stimulus current.

3. Experimental

3.1. Chemicals and reagents

Aniline, ammonium persulfate, iron(III) oxide, glutaraldehyde, streptavidin and biotin conjugate goat anti-mouse IgG (whole molecule) were purchased from Sigma–Aldrich (St. Louis, MO). NC membranes (8–10 μm pore size and flow rate of 135 s/4 cm, with polyester backing), cellulose fiber sample pads, and absorbent pads were purchased from Millipore (Bedford, MA). All other chemical reagents were of analytical grade.

3.2. Fabrication and characterization of electrodes

Nitrocellulose membrane (60 mm × 301 mm) with a backing support was washed with 10% (v/v) methanol and then dried in the air. A silver polymer ink (Gwent Electronic Materials Ltd., UK) was printed on the NC membrane through a patterned screen of two electrode system with a 500 μm space (Ryonet Corporation, WA, USA) and baked at 60 °C for 30 min. The screen-printed NC membrane was cut into 5 mm size using Matrix 2360 (Kinematic Automation Inc., CA, USA). Resistance of the silver screen-printed electrodes was measured by a 4192A LF Impedance Analyzer (Agilent Technologies Inc., CA, USA) with two-point probe measurement technique for a distance of 20 mm. The distance between the two screen-printed silver electrodes to be used as a current path was confirmed by a standard laboratory microscope BX41 (OLYMPUS, Germany).

3.3. Preparation of polyaniline-coated magnetic nanoparticles and their conjugation with biotinylated anti-mouse IgG

The water-soluble polyaniline was synthesized based on a standard procedure of oxidative polymerization of aniline monomer in the presence of ammonium persulfate as oxidizing agent (Sergeyeva et al., 1996). Hydrochloric acid (HCl) was used as the protonating acid, and iron(III) oxide nanopowder to be coated with polyaniline was added into the polymerization vessel in a weight ratio of 1:0.6 (γ-Fe_2O_3:aniline monomer) as optimized in previous studies (Pal et al., 2007). The morphology of polyaniline-coated magnetic nanoparticles (hereafter referred to as magnetic composite Fe_2O_3/polyaniline particles) was investigated by a transmission electron microscope (TEM) (JEOL JEM-100CX II, Japan).

The magnetic composite Fe_2O_3/polyaniline particles fractions (0.2 g) obtained as a result of filtration were incubated with 1 ml of 0.1% glutaraldehyde for 30 min. The magnetic composite Fe_2O_3/polyaniline particles were separated using a magnetic separator and the liquid was discarded. Four hundred microliter

aliquots of the biotinylated IgG (200 μg/ml) were added to the magnetic composite Fe_2O_3/polyaniline particles–glutaraldehyde complex. The incubation procedure was performed with shaking for 30 min at room temperature and 500 μl of 100 mM Tris buffer containing 0.1% casein (pH 7.4) was added to the reaction mixture in order to inactivate non-reacted aldehyde groups. Finally, conjugated biotinylated IgG with magnetic composite Fe_2O_3/polyaniline particles was separated by the magnetic separator and was mixed with 1 ml of phosphate buffer (8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, pH 7.4). The magnetic composite Fe_2O_3/polyaniline particles–biotinylated IgG was serially diluted with phosphate buffer (pH 7.4) in 3 dilutions and then stored at 4 °C until use.

3.4. Preparation of polyaniline-based biosensor

In preparing the polyaniline-based biosensor, the surface of NC membranes (20 mm × 0.5 mm) was modified with 1% (v/v) glutaraldehyde solution for 30 min and then rinsed with deionized water for 15 min and flushed with N_2 gas to dry. After drying, 500 μg/ml of streptavidin was prepared in phosphate buffer (8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, pH 7.4) and 12 μl of the protein solution was applied by using a micro-pipette to the surface of glutaraldehyde modified NC membrane. The NC membrane was washed with 0.05% Tween 20 in phosphate-buffered saline (8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 138 mM NaCl, 2.7 mM KCl, pH 7.4) (PBS) for 15 min, rinsed with deionized water and flushed with N_2 gas to dry. The NC membrane was then incubated with 10 mg/ml of bovine serum albumin in PBS for 30 min to reduce nonspecific interactions. After incubation, the NC membrane was washed with 0.05% Tween 20 in PBS for 15 min, rinsed with deionized water, dried under N_2 gas, and immediately assembled with sample and absorbent pads on the NC membrane as shown in Fig. 1 and installed to an etched copper printed circuit board (PCB). Interactions of streptavidin with biotinylated IgG conjugated with magnetic composite Fe_2O_3/polyaniline particles were analyzed after applying 80 μl of the analyte onto the sample pad.

3.5. Electrical measurement system

The electrical two-point probe measurement consisted of a measurement cell, a multimeter model 2000 (Keithley Instruments, Inc., OH, USA) connected with a general purpose interface bus (GPIB) cable, and a personal computer. To monitor the electrical response of the polyaniline-based biosensor, we measured the voltage generated between the two electrodes and displayed as resistance using Ohm's law. When the DC resistance was measured with a pulse mode voltammetry, DC current of 10 μA was applied every 1 s interval. Data acquisition and display were performed by a customized program based on LabVIEW.

4. Results and discussion

In order to achieve performance enhancement of the disposable biosensor for field test, we used screen-printing technology for the fabrication of electrodes and pulse mode measurement technique. Initially, the distance of the electrodes and resistance of screen-printed electrodes fabricated on the NC membrane were characterized by a microscope image and *I–V* measurements. The distance between the two electrodes was one of the major factors affecting electrical properties of the polyaniline-based biosensor because of a current path (Muhammad-Tahir and Alocilja, 2003a) so that the uniformity of the distance between electrodes was very important to obtain a reproducible electrical response. The distance between screen-printed electrodes was approximately

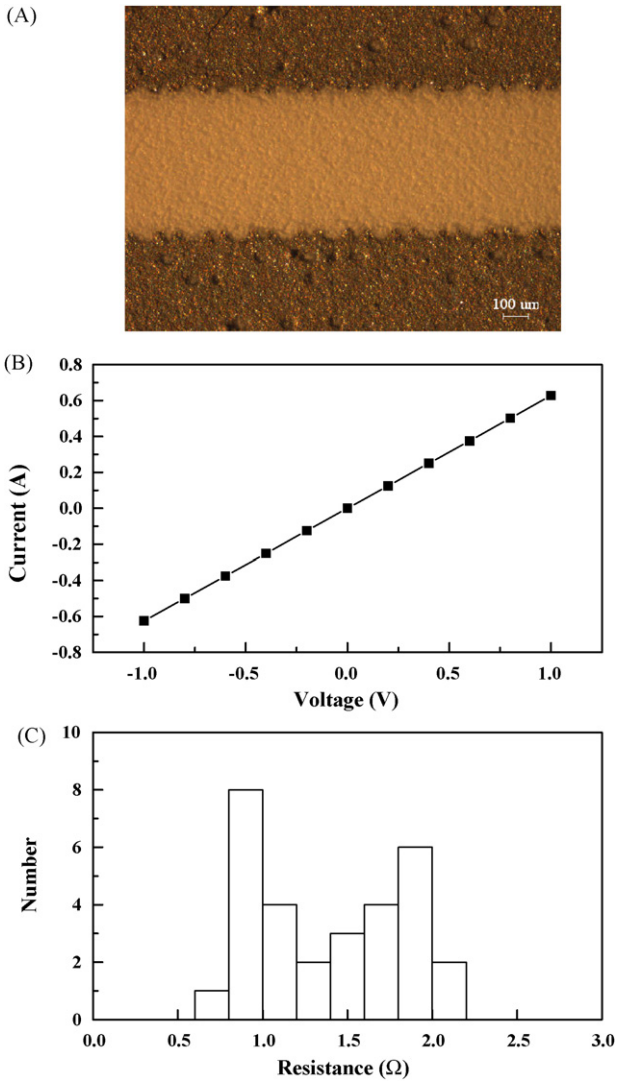

Fig. 2. Characteristics of screen-printed silver electrodes on the NC membrane. (A) Photograph of the screen-printed electrode. (B) *I–V* characteristic of the silver electrode measured by the 4192A LF impedance analyzer. (C) Histogram of resistances for the number of 30 samples.

550 μm and more uniform compared to that of the manually fabricated electrodes (data not shown) as shown in Fig. 2(A). Electrical characteristics of the electrodes fabricated with a silver polymer ink on NC membranes were studied by *I–V* measurements. Silver has a pure crystal structure that provides low residual currents and high signal-to-noise ratio (Ashcroft and Mermin, 1976; Ivnitski et al., 1999) so that silver was chosen as the electrode of the electrical biosensor. The *I–V* characteristics of the silver electrode showed an ohmic behavior as shown in Fig. 2(B), which means that the electrode would work well as a good conductor with a constant resistance (Halliday et al., 2001). Resistance of the silver electrode was calculated as 1.6 Ω from the *I–V* graph. To investigate the resistance fluctuation of screen-printed electrodes, we measured the resistance of 30 strips that were randomly selected. Fig. 2(C) shows the histogram of measured electrodes and the resistance spread out from 0.5 to 2.5 Ω with an average of 1.4 Ω. From the above results, it is found that the screen-printed electrodes have a uniform distance.

Next, we investigated whether magnetic composite Fe_2O_3/polyaniline particles was formed by a TEM image. Magnetic nanoparticles (<50 nm) were mixed with aniline monomer in

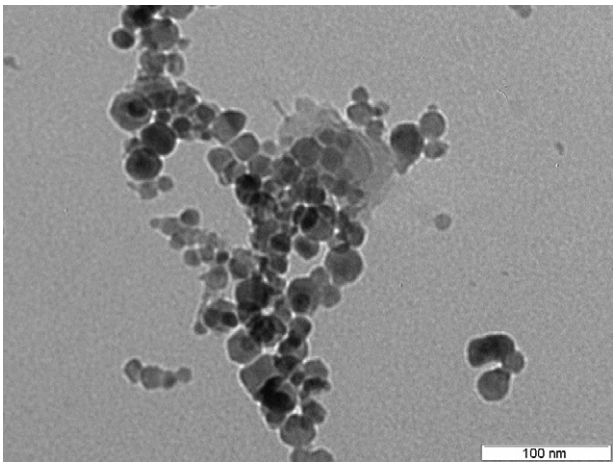

Fig. 3. TEM image of polyaniline-coated magnetic nanoparticles. The size of iron(III) oxide magnetic nanoparticles was less than 100 nm, which were connected with each other by the medium of the polyaniline.

a weight ratio of 1:0.6 to synthesize the magnetic composite Fe_2O_3/polyaniline particles. The polyaniline was polymerized based on a standard procedure of oxidative polymerization of aniline monomer in the presence of ammonium persulfate as oxidizing agent. Fig. 3 shows that polyaniline-coated magnetic nanoparticles have an average size of less than 100 nm. A polymeric wire was formed by a string of polyaniline-coated magnetic nanoparticles. The polyaniline has very high electrical conductivity in the protonated state and can be bound easily to most proteins. It has a promising electrical material because of its thermal and chemical stability in the doped form (Granholm et al., 1997). The polyaniline will be used as an electric signal transducer in our biosensor. It is well known that small iron oxide nanoparticles exhibit superparamagnetism having zero hysteresis while the larger ones (in μm) display ferromagnetism (Bean and Livingston, 1959; Dubois et al., 1998). One of the attractive advantages of the magnetic nanoparticles is its ability to be used in concentrating method using a magnetic separation technique thus eliminating the need for sample pretreatment, thus shortening the sample handling time and preventing shear stress-induced damage to biological materials (Hsing et al., 2007).

In order to investigate whether the pulse mode measurement technique effectively reduced the resistance related to the interfacial capacitance (to enhance reliability of the biosensor), we analyzed streptavidin–biotin interactions with two different measurement techniques. As a model system, streptavidin–biotin interaction was used. Streptavidin was immobilized on the NC membrane. The NC membrane was the porous nitrocellulose material with good adsorption properties for immobilized proteins and allowed non-target proteins to flow through. However, the NC membrane was modified by using the crosslinking reagent glutaraldehyde before applying the streptavidin because non-specific binding to nitrocellulose could be a significant problem and proteins adsorbed on hydrophobic surfaces tended to denature (Cretich et al., 2006). After biotinylated IgG conjugated with the magnetic composite Fe_2O_3/polyaniline particles solution was applied onto the sample pad, a resistance change was measured by pulse mode voltammetry. Resistance decreased with time and became stable after about 2 min with a reading of approximate 4.3 kΩ (solid line in Fig. 4). The signal generation could be explained by current flow induced by the streptavidin–biotin interaction. The analyte carrying the biotin molecules flowed into the NC membrane with immobilized streptavidin and the streptavidin–biotin complex was formed. The polyaniline in the complex bridged the two electrodes and provided a current path. The unbound analyte at the liquid-solid interface was subsequently washed away to the absorbing pad by capillary action so that an electrical response due to the pure streptavidin–biotin interaction was measured by the DMM.

To compare the resistance change measured by pulse mode with that by non-pulse mode, the same experiment was repeated. Except for the initial ramping, the signal response of the non-pulse mode (dashed line) was similar to the pulse mode (solid line) as shown in Fig. 4. However, the resistance obtained by the non-pulse mode was higher than the resistance obtained by the pulse mode. This might be attributed to the resistance related to the interfacial capacitance (t/C_d). The interfacial capacitance (Eq. (2)) might be less affected by the pulse mode measurement compared to the non-pulse mode measurement. The resistance of R_s expressed in Eq. (2) was generated by the streptavidin–biotin interaction on the biosensor. From the above results, it was found that the pulse mode measurement could more reliably measure the electrical response induced by the biospecific interaction by reducing the resistance related to the interfacial capacitance.

We also investigated whether resistance changes by interaction of various concentrations of magnetic composite Fe_2O_3/polyaniline particles–conjugated biotinylated IgG could be analyzed by the pulse mode measurement. Resistance of phosphate buffer (pH 7.4) was used as a reference signal for monitoring resistance changes induced by the streptavidin–biotin interaction in the polyaniline-based biosensor. After preparing the various concentration of analyte with 3 serial term fold dilutions, each analyte was applied onto the sample pad and the resistance change was monitored in real-time. Resistance measured at 3 min was used to investigate a dose-dependent response. Fig. 5 shows that resistance increased and then saturated according to the concentrations of the analyte. Increment of resistance at the diluted concentrations means that the biotin complex with polyaniline was less bound with streptavidin so that the voltage across the electrodes increased. The dose-dependent response obtained by pulse mode could be explained by the relative amount of magnetic composite Fe_2O_3/polyaniline particles–biotinylated IgG complex bound on the streptavidin surface of the biosensor. Thus, this study

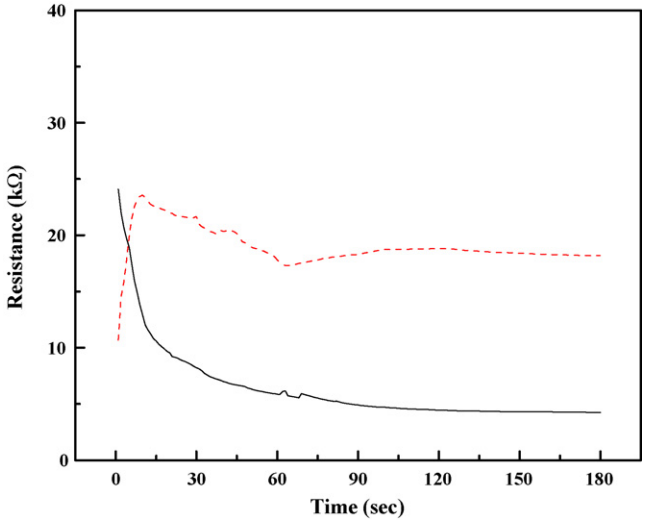

Fig. 4. Analysis of streptavidin-biontin interactions with pulse mode and non-pulse mode measurement. Resistances were measured every 1 s after applying the analyte with pulse current (10 μA) (solid line) and non-pulse current (dashed line) applied. Both of them, electrical responses became stabilized approximately after 2 min after applying the analyte.

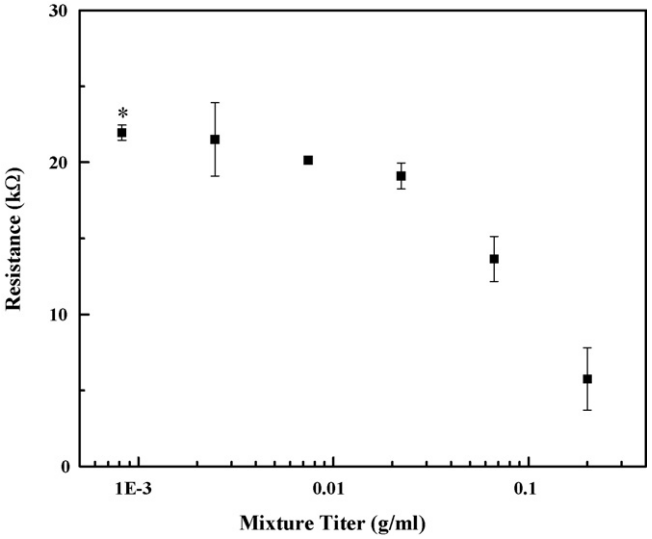

Fig. 5. Dose-dependent response according to the concentrations of the analyte. Streptavidin was immobilized on the NC membrane modified with 1% glutaraldehye and incubated with the indicated mixture concentrations of the magnetic composite Fe_2O_3/polyaniline particles–biotinylated IgG. Then, the polyaniline-based biosensors were analyzed by the pulse mode voltammetry. Sign of * is represented as a reference signal of the phosphate buffer to monitor resistance changes.

demonstrated that the reproducibility and reliability performance of polyaniline-based polymeric wire biosensor could be enhanced by using screen-printed silver electrodes and pulse mode measurement.

5. Conclusions

We have demonstrated that the reproducibility and reliability of polymeric wire biosensor was enhanced by using screen-printed silver electrodes and pulse mode measurement. The screen-printed electrodes had a uniform distance of 550 μm between two electrodes and an average resistance of 1.4 Ω. Formation of polyaniline-coated magnetic nanoparticles was confirmed by a TEM image. The polyaniline was used as an electric signal label of the biotin for the biospecific binding counterpart of streptavidin. The pulse mode polymeric wire biosensor showed more reliable electrical response induced by streptavidin–biotin interactions by reducing the resistance related to the interfacial capacitance. A dose-dependent response was also successfully measured by the pulse mode polymeric wire biosensor. Thus, it is suggested that the performance enhanced disposable polyaniline-based biosensor has great potential for field test with real-time measurements.

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