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Short Communication

Development of a Nanotechnology-Based Screen-Printed Biosensor for Detection of *Schistosoma Mansoni* Antibodies

Mohamed Shohayeb[1,2,*], *Hassan Arida*[1,3], *Gaber A. M. Mersal*[4,5], *Mohamed El-Badawy*[1,6]

[1]College of Pharmacy, Taif University, 888 Hawiya, Taif, Saudi Arabia.
[2]Faculty of Pharmacy, Tanta University, Tanta, Egypt.
[3]Hot Laboratories Center, Atomic Energy Authority, 13759- Cairo, Egypt.
[4]Materials and Corrosion Lab, Department of Chemistry, Faculty of Science, Taif University, 888 Hawiya, Saudi Arabia.
[5]Chemistry Department, Faculty of Science, South Valley University, Qena, Egypt.
[6]Microbiology and immunology Department, Faculty of Pharmacy, Misr University for Science and Technology, sixth of October, Cairo, Egypt.
[*]E-mail: Shohayeb@hotmail.com

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Schistosomiasis comes after malaria as the second most important fatal parasitic disease. In Saudi Arabia *Schistosoma mansoni* is endemic in Jazan, Bishah, Aseer, Madina, Al-Bahah and Taif. The methods used for diagnosis of schistosomiasis are complex, time-consuming, and require extra instruments and/or need skillful persons; therefore, there is a need for the development of a sensitive easy method. In this study, for the first time, a novel screen-printed immunosensor for detection of *S. mansoni* antibodies (ABs) was developed. Soluble worm antigens (SWA) were fixed onto the nano-carbon working area of a screen-printed electrode using glutaraldehyde-chitosan cross-linkers. The ability of *S. mansoni* ABs to bind to the antigen-loaded screen-printed electrode was evaluated by cyclic and differential pulse voltammetry. A calibration curve for *S. mansoni* ABs binding to the SWA-loaded screen-printed electrode produced a reproducible linear relationship at a concentration ranging between 0.038 to 20 ng/ml. The quantitative response obtained at nano-level amounts of ABs suggests that this method can be used in the future to develop a disposable screen-printed electrode for diagnosis of schistosome infections.

Keywords: *Schistosoma mansoni*, biosensor, screen-printed electrode, soluble worm antigens, anti-schistosome antibodies

1. INTRODUCTION

Schistosomiasis is one of the most fatal and drastic diseases of humans which represents the second most important parasitic disease after malaria [1]. It is an antique parasitic disease since its eggs were recovered from several thousand years old Egyptian and Chinese mummies [2, 3]. It is

estimated that the number of infected people with schistosomiasis is 270 million, of whom 120 million are symptomatic and 20 million have severe symptoms [4]. Six hundred million people are at risk of schistosome infection [5]. Schistosomiasis has been reported in 54 countries [4], amongst which are some Arab countries like Iraq, Sudan, Egypt, Yemen, and Saudi Arabia [2, 6-8].

The main causative agent of schistosomiasis in humans is *S. mansoni, S. haematobium* and *S. japonicum* [9, 10]. In the Kingdom of Saudi Arabia both *S. mansoni*, *S. haematobium* are endemic with a prevalence of 2.9/100,000 persons [11]. Schistosomiasis has been reported in Saudi Arabia in the areas of Jazan, Bishah, Aseer, Baha and Taif. *S. mansoni* is more prevalent in Taif, Bahah, Aseer, Bishah, Najran, Makkah and Medina [11], where it is presumably, transmitted by rodents, baboon monkeys and infected humans [12-14].

Diagnostic tools for schistosomiasis are far from ideal [17]. For example, identification of eggs in feces, which is the most commonly used method, is time-consuming and has a poor positive test rate. Furthermore, it is difficult to apply fecal sampling methods for screening in areas with relatively less severe infection levels [18, 19]. Immunoassays may be used for detection of antigens (AGs) released by the parasite in blood or urine, or the detection of ABs produced by the infected individuals [18, 20, 21]. However, these methods are complex and time-consuming procedures, require extra instruments and need skillful persons [22]. Although recently, dipsticks were developed for rapid detection of cathodic antigens of *Schistosoma mansoni*, however, dipsticks are presently too expensive to be cost-effective for wide scale use [23].

On the other hand, screen-printed electrode biosensors are attracting allot of interest in clinical medicine, food and drug analysis, agriculture, environmental studies, fermentation process control and in military and aerospace fields [25, 26]. These sensors can determine small amounts of the analyte level through detecting changes in potential, current, conductance, or impedance caused by the immunoreaction. They have the advantage of allowing for a wide diversity in the selection of the electrode shape and material. They also offer the advantages of simple, fast, miniaturization and low price [26].

With the introduction of nanomaterials in the preparation of nano-sensors, their sensitivity has been enhanced so that they can detect biomolecules at relatively low detection limit and high sensitivity [27]. Therefore, amperometric immune-sensors are especially promising in medical diagnostics.

Although, recently, there have been several trials to fabricate immune-sensors for detection of *S. japonicum* ABs or antigens [28, 30], to our knowledge from the literature, no sensor so far has been developed for detection of *S. mansoni*. In this study, an amperometric immune-sensor for detection of *S. mansoni* ABs was constructed by immobilization of worm soluble antigens on a printed electrode biosensor.

2. EXPERIMENTAL

2.1. S. mansoni *soluble worm antigens:*

Schistosome AGs were prepared by Theodor Bilharz Research Institute, Egypt. SWA was prepared from crude S. mansoni AGs by an immunoaffinity fractionation column, in which ABs

obtained from chronic S. mansoni-infected human sera were bound to CNBr-activated Sepharose 4B. After passing the crude worm antigens through the column, the pure SWA was eluted by glycine-HCI-NaCl [31].

2.2. Fabrication of nanocarbon-screen-printed electrode (NCE) based S. mansoni AGs:

A solution of chitosan (0.5 %) was prepared in 100 ml acetic acid (1%) and stirred for 3 h at room temperature. Aliquots of 89 µl of chitosan solution were added and thoroughly mixed with1 µl of AGs (1000 µg/L). To these mixture aliquots of 10 µl glutaraldehyde (1% solution) was added and mixed. The obtained solution was used as a sensitive element. A screen printed electrodes obtained from Suzhou Deltabiotech (Ltd, China) were used as electrode substrates. Aliquots of 2 µl of the AGs-chitosan-glutaraldehyde solution were dropped onto the NCE working areas. The fabricated screen-printed electrodes were incubated for 40 min at 37 ℃ and washed with phosphate buffered saline (PBS). The surface bare spots on the nanocarbon working area were blocked by adding 5 µl PBS containing 1% bovine serum albumin onto the working electrode for 30 min at room temperature before washing with PBS and drying in air at room temperature [30]. This assembly was used as working electrode in the quantification of *S. mansoni* ABs.

2.3 Detection of S. mansoni ABs by NCE-AGs:

S. mansoni ABs were obtained from Aviva System Biology, San Diego, USA. The ABs were serially in PBS. Five µl of ABs were dropped onto NCE-AGs, and allowed to dry for 40 min, washed with PBS, and dried at room temperature. Finally, all immune-sensors were subjected to electrochemical measurement. The impedance spectra were recorded for strips immersed in5 mM $[Fe(CN)_6]^{-3/-4}$ and 1 M KCl at a scan rate of 50 mV/s [30].

2.4. Electrochemical measurements:

The electrochemical characterization of the new electrode was performed by cyclic voltammetry (CV) and differential pulse voltammetry using an Autolabpotentiostat PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) driven by the General Purpose Electrochemical Systems Data Processing Software (GPES, software version 4.9, Eco Chemie).

3. RESULTS AND DISCUSSION

Accurate and precise detection of *S. mansoni* represent a very important challenge for many scientists. Methods in current use of *S. mansoni* detection are expensive, complex and time-consuming. These including immunoassay diagnostic methods which have been employed for screening for schistosomiasis like precipitin test, indirect hemagglutination test, and enzyme-linked immunosorbent assay (ELISA) [19–21]. In addition, they require extra instruments and skillful personnel [22, 23].

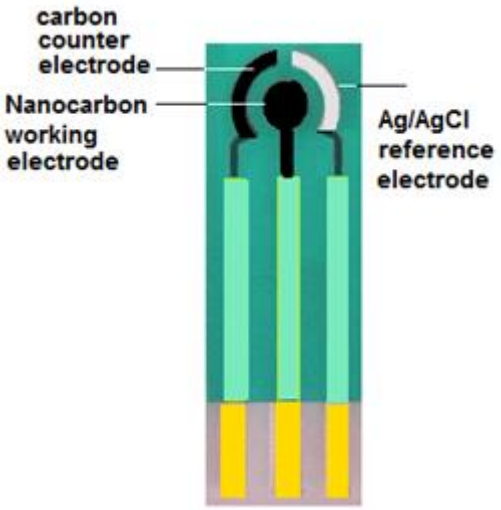

Figure 1. Nanocarbon screen-printed electrode.

This electrode assembly was fabricated and electrochemically characterized according to IUPAC recommendations. In order to investigate the degree of insulation of electrodes after assembly of AGs and ABs, the cyclic voltammogram of the working electrode, immersed in 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl, was performed. The results obtained are presented in figure 2.

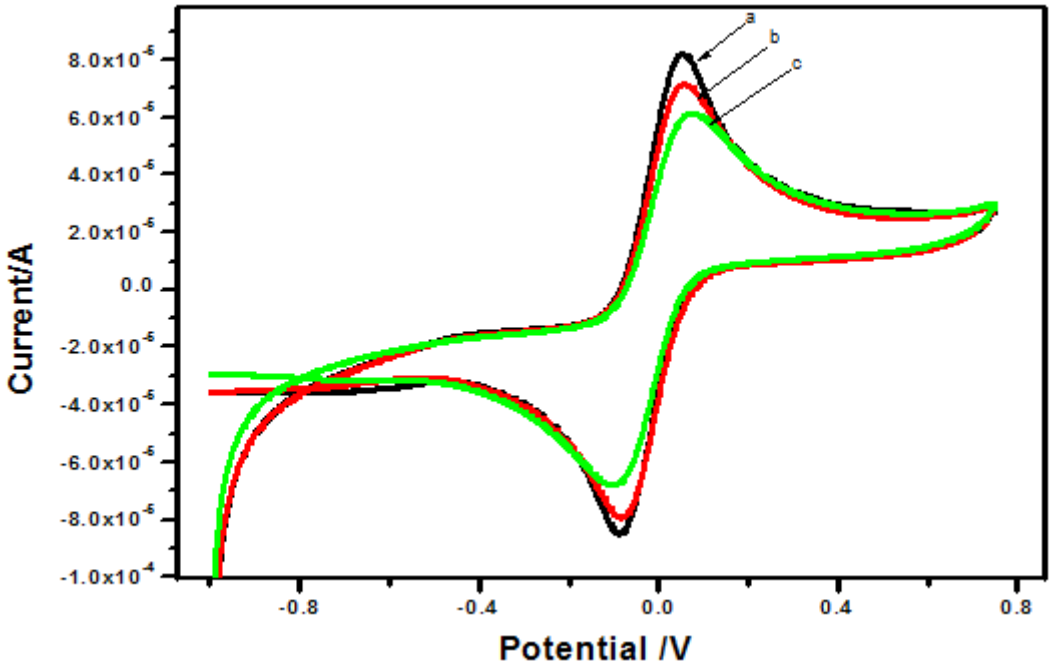

Figure 2. Cyclic voltammograms of nano-carbon electrode (NC) obtained with 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl. The scan rate was 50 mV/s. a, unmodified NCE; b, NCE-AGs; c, NCE-AGs-ABs.

On the other hand, the printed electrode biosensors have been used in clinical medicine. They have the advantage of simple, fast, miniaturization and low price [26]. With the introduction of nano-materials in the preparation of nano-sensors, their sensitivity has been enhanced [27].

In this study, we have constructed an amperometric immunosensor in which *S. mansoni* soluble worm antigens were immobilized on NCE by glutaraldehyde and chitosan (Fig. 1).

The electrochemical behavior of NCE was examined in a potential ranging from +0.75 to -1.0 V, using a potential scan rate of 50 mV s^{-1}. As it can be seen for the NCE, there was a characteristic quasi-reversible redox cycle. The quasi-reversible redox system is due to Fe(II)/Fe(III) couple and the anodic peak current is due to the electrode process:

$$[Fe(CN)_6]^{-4} \longrightarrow [Fe(CN)_6]^{-3} + e^-$$

The cathodic peak current is due to the following electrode process:

$$[Fe(CN)_6]^{-3} + e^- \longrightarrow [Fe(CN)_6]^{-4}$$

The ratio of the anodic-to-cathodic peak currents (I_{pa}/I_{pc}) is close to unity, the anodic peak current appeared at +0.056V and the cathodic peak current appeared at -0.082 V (Fig. 2-curve a). The separation of the anodic and cathodic peak potentials ($\Delta E = E_{pa} - E_{pc}$) was 138 mV, and the half-wave potential ($E_{1/2} = E_{pa} + E_{pc}/2$) was approximately -13mV.

By the addition of soluble antigen, the anodic and cathodic peak currents decreased and a small shift for anodic and cathodic peak potentials was observed. The anodic peak was observed at +55 mV and the cathodic peak at +79 mV with an anodic-to-cathodic peak current ratio of approximately one (Fig. 2-curve b). The separation of the anodic and cathodic peak potentials (ΔE) was 134 mV, and the half-wave potential ($E_{1/2}$) was approximately -12 mV.

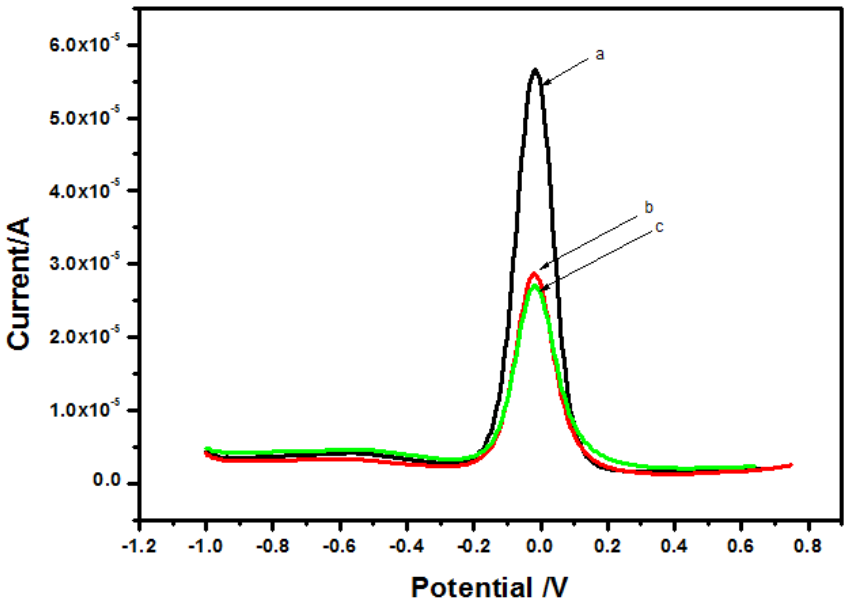

Figure 3. Deferential pulse voltammograms of nc-electrode obtained with 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl at a scan rate of 50 mV/s. a, NCE; b, NCE-AGs; c, NCE-AGs-ABs.

In the presence of ABs (Fig. 2-curve c), there was even more decrease in the anodic and cathodic peak currents. The anodic peak potential shifted to a more positive value (E_a = +77 mV), and the cathodic peak potential shifted to a more negative value (E_p = -95 mV), with an anodic-to-cathodic peak current ratio of approximately one (Fig. 2-curve c). The separation of the anodic and cathodic peak potentials (ΔE) was 172 mV, and the half-wave potential ($E_{1/2}$) was approximately -9 mV. The decreased in the anodic and cathodic peak currents by the addition of ABs may be attributed to the blocking of the electron transfer by the formation of more AGs-ABs complexes.

The same behavior was also observed using differential pulse voltammetric technique. Figure 3 shows a differential pulse voltammogram (DPV) for NCE oxidation peak potential which appeared at -15 mV for 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl at a scan rate of 50 mV/s (Fig 3a). By the addition of antigen the peak current decreased sharply (Fig 3b), and on the addition of ABs the oxidation peak current decreased even more due to the formation of AGs-ABs complex further decline in the peak current values (Fig 3c).

The decrease in the peak current values by the addition of ABs might be attributed to the formation of AGs-ABs complex film which increases the resistance to $[Fe(CN)_6]^{3-/4}$ molecule to cross through this film and decreases the number of its molecules which react with the electrode surface.

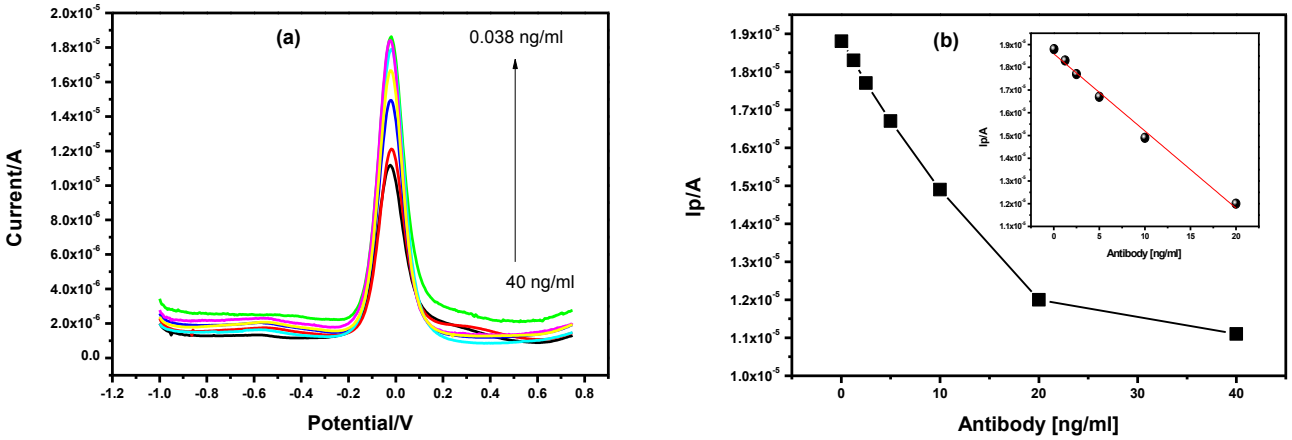

Figure 4. a, Cyclic voltammograms for different ABs concentrations ranging from 0.038 to 40 ng/ml; b, Calibration curve of current response to different ABs concentrations ranging from 0.038 to 40 ng/ml using differential pulse voltammetry.

The effect of different *S. mansoni* ABs concentrations ranging from 0.038 to 40 ng/ml was examined on the DPV response for NCE-AGs. By increasing the concentrations of ABs of *S. mansoni*, the peak current decreased (Fig. 4).

The analysis of ABs of *S. mansoni* (from 0.038-40 ng/ml) using nanocarbon electrode in the presence of fixed AGs produced a linear dynamic range for DVP response at ABs concentrations ranging between 0.038 to 20 ng/ml (Figure 4b). The linear relation between the concentration and ABs concentrations can be represented by the following equation:

$I(A) = 1.86x10^{-5} - 3.39x10^{-7}$ x [C]

Where *I* is the peak current in A and [C] is the ABs concentration in ng/ml. The regression coefficient (R) of the above equation is about 0.998, indicating a good linear relationship between anodic DVP peak currents and ABs concentrations. This demonstrates the sensitivity of the electrode and a concentration-dependent response. This sensitivity is high enough to detect ABs which exist at levels of a fraction of microgram/ml in people suffering from *S. mansoni* infection [32]. We are currently evaluating the designed NCE in comparison with already commercially available immunoassays.

4. CONCLUSION

In this study, a nano-carbon based screen-printed electrode for the detection of *S. mansoni* ABs was designed for the first time. The quantitative response and the high sensitivity of designed NCE electrode to ABs of *S. mansoni* as low as 38pg/ml, suggest that it could be developed as a disposable one-use, cheap, and rapid electrochemical immunosensor. This sensor could be used for both diagnosis *S. mansoni* infections and for surveys in rural areas where schistosomiasis is endemic.

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