RESEARCH ARTICLE

Effect of processing variables on the performance of electrochemical aptasensor for determination of aflatoxin M1

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ABSTRACT

A novel aptasensor based on electrospun carbon nanofiber (ECNF) mat modified with gold nanoparticles (AuNPs) and aptamer was fabricated to detect aflatoxin M1 (AFM1) in the milk. The electrospinning and heat treatment technique were firstly used to construct the ECNF mat electrode. Then, this platform was electrodeposited by AuNPs and immobilized with a thiol-modified single stranded DNA (ss-HSDNA). The effect of processing parameters such as concentration of $HAuCl_4$ and ss-HSDNA in the electrolyte, incubation time of aptamer and AFM1, pH and temperature of the electrolyte on the performance of aptasensor was investigated using cyclic voltammetry (CV) experiments in the $[Fe(CN)_6]^{-3/-4}$ solution. The results showed that the optimum concentration of $HAuCl_4$ in the electrolyte was 4mM. An increment in ss-HSDNA aptamer of electrolyte led to improvement of the electrochemical current response. Furthermore, the peak currents of CV enhanced with increasing incubation time of aptamer or AFM1. By increasing temperature of the electrolyte, the CV peak currents increased and then decreased. This trend was also observed in pH of electrolyte.

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INTRODUCTION

There is always the possibility of human health hazard from existence of aflatoxins (AFs) in food and feed. Aflatoxins made by fungal species are one of the most important natural mycotoxins. AFB1, AFB2, AFG1 and AFG2 classified as group 1 human carcinogens are the most prevalent type of aflatoxin. Among these aflatoxins, AFB1 is known as the potent carcinogen commonly derived from two fungal strains, Aspergillus flavus and Aspergillus parasiticus [1]. These fungi which growth in the temperature range of 10-45°C and a relative humidity of 80% can develop on various of human food and animal feed materials during production, harvest or storage [2]. Exposure to high levels of AFB1 found in agricultural products can cause risks to humans and animals health [3].

AFB1 in contaminated feed is transformed to AFM1 by the action of liver enzyme and secreted into milk through the mammary gland of dairy cows [4]. The presence of this contamination in milk and dairy products due to its hepatotoxic and carcinogenic properties make a risk for health of humans. It should be noted that the pasteurization and other thermal treatments cannot remove this contamination [5]. Therefore, the maximum allowable levels of AFM 1 in milk and dairy products have been set by many countries worldwide. For instance, the United States of America regulations permit a maximum level of 500 ng/L AFM1 in

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milk, while it is 50 ng/L in the European Union [6].

There are different techniques such as high-performance liquid chromatography (HPLC), Enzyme-Linked Immunosorbent Assay (ELISA) and thin layer chromatography (TLC) for detection and measurement of AFM1contamination in milk [7-9]. However, these methods are not very attractive because they need expensive equipment and well-trained personnel in addition to long analysis time. Hence, various measurement techniques based on biosensors have been developed due to a low detection limit, high sensitivity, rapid response and low cost [10].

In recent years, electrochemical biosensors based on aptamer have appeared as sensitive method for analysis of AFM1. Aptamers originating from SELEX (Systematic Evolution of Ligands by Exponential Enrichment) are functional short oligonucleotides showing a very high affinity towards targets molecules through hydrogen bonding, van der waals forces and electrostatic interactions [11-13]. Moreover, a high coverage of aptamers on a substrate surface owing to small size of aptamer dimensions leads to measurement of dissociation constants in the range of picomolar (pM) to micromolar (μM) [14]. There are several investigations about the immobilization of aptamer on different sensors surface for detection of AFM1. The obtained results showed that these aptasensors had a good sensitivity and low detection limit [15, 16]. Further improvement in performance of aptasensor can be achieved using nanomaterials such as gold nanoparticles and carbon nanofibers as a suitable platform for immobilization of aptamer [17]. For example, Wang et al. exhibited that the assembly of DNA-functionalized gold nanoparticles (AuNPs) on electrospun carbon nanofibers (ECNFs) was utilized satisfactorily in nucleic acid detection [18].

The aim of this research was to fabricate ss-HSDNA/AuNPs/ECNF mat aptasnsor by the immobilization of ss-HSDNA at AuNPs electrodeposited on ECNF mat electrode and to investigate the effect of different parameters, e.g. concentration of $HAuCl_4$ and ss-HSDNA in the electrolyte, incubation time of aptamer and AFM1, pH and temperature of the electrolyte on the sensor performance.

EXPERIMENTAL

Materials

Polyacrylonitrile (PAN) (M_w = 150,000 g/mol) was provided by the Polyacryl company (Iran). Sulfuric acid (H_2SO_4) and Dimethylformamide (DMF) were bought from Merck. Hydrogen tetracholoroaurate ($HAuCl_4$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), sodium chloride (NaCl), potassium chloride (KCl), Sodium phosphate dibasic (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4) and Aflatoxin M1(fabricated by A. flavus) were purchased from Sigma-Aldrich. AFM1-aptamer 21-mer ss-HSDNA sequence (5'-thiol-$(CH2)_6$-ACT GCT AGA GAT TTT CCA CAT-3') was obtained from Faza Biotech Co. (Iran, Tehran). The ultra pure water was used for preparation of all solutions.

Steps of aptasensor preparation

Fabrication of the electrode based on ECNF mat and AuNPs

The details of the ECNF mat electrode preparation have been presented elsewhere [19]. Briefly, the homogeneous PAN/DMF solution with concentration of 10 wt.% obtained by stirring at 50 °C for 10 h was ejected through plastic syringe equipped with an 18-gauge needle at a flow rate of 1.1 ml/h. The PAN nanofiber mat collected on the thin aluminum foil was stabilized at 290 °C for 4 hrs in air and subsequently carbonized at 1000 °C for 1 h in a nitrogen atmosphere. The circle ECNF mat with 7 mm in diameter were then prepared and used as platform. After that, the electrodeposition of gold nanoparticles on the ECNF mat platform was carried out by applying constant potential of -0.4 V versus Ag/AgCl for 70 seconds in the 0.1 M H_2SO_4 electrolyte containing 1-6 mM $HAuCl_4$.

Immobilization of ss-HSDNA on AuNPs/ECNF mat electrode

The immobilization of ss-HSDNA was performed by dipping AuNPs/ECNF mat electrode in phosphate buffered saline (PBS) solution with different concentrations of ss-HSDNA for various incubation times. After immobilization, the electrode was washed with PBS solution and ultra pure water for several times.

Preparation of milk sample

The performance of aptasensor was evaluated by a milk sample purchased from a local market. Before electrochemical experiments, centrifugal separation was performed to remove fat from milk to prevent the fat effect on the measurements. Then, 30 μL of AFM1 toxin was dropped into the fat free milk diluted with 50 % PBS (0.1 M).

Electrochemical Measurements

Cyclic voltammetric measurements were obtained by a µStat 400 potentiostat/galvanostat (DropSens, Spain). A three-electrode electrochemical cell, with the ss-HSDNA on AuNPs/ECNF mat as a working electrode, an Ag/AgCl as reference and a platinum wire as counter electrode, was employed in the PBS solution containing 2 mM ferri/ferrocyanide ($[Fe(CN)_6]^{-3/-4}$) at temperatures of 10-60 °C. The electrochemical experiments were carried out at pH levels of 5-9.

It should be also noted that the performance of aptasensor was investigated by dipping of the ss-HSDNA/AuNPs/ECNF mat electrode into the solution prepared according to the procedure of 2.3 section. The aptasensor was then removed from the solution after different incubation times and rinsed with the ultra pure water to separate loosely adsorbed aflatoxin molecules from the electrode surface. The aptasensor was then dipped into PBS solution containing ($[Fe(CN)_6]^{-3/-4}$) for performing electrochemical measurements.

RESULTS AND DISCUSSION

In this research, the effect of experimental parameters including hydrogen tetracholoroaurate concentration, aptamer concentration, incubation time of aptamer and AFM1, pH and temperature on electrochemical current response was investigated.

Hydrogen tetracholoroaurate concentration

The amount of gold nanoparticles electrodeposited on ECNF mat affects the CV peak current response. One of the important parameters affected the amount of electrodeposited gold nanoparticles is a hydrogen tetracholoroaurate concentration in the solution. The effect of concentration of hydrogen tetracholoroaurate on difference of peak currents between and AuNPs/ECNF mat electrode and ECNF mat electrode was is shown in Fig. 1. With increasing the amount of $HAuCl_4$ in the electrolyte, the difference of peak currents increased from about 17 to 29 and then decreased to about 20. The increasing trend of curve can be related to promotion of electron transfer due to increment of electrodeposited gold nanoparticles on ECNF mat by increasing the amount of $HAuCl_4$ up to 4 mM into the electrolyte. However, further addition of $HAuCl_4$ led to increase the gold nanoparticles coating thickness and therefore reduce the conduction properties of the electrode. The similar result was reported by Liu et al. [20].

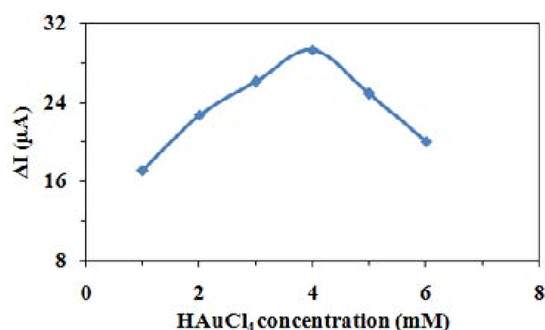

Fig. 1. Effect of $HAuCl_4$ concentration on difference between peak currents of AuNPs/ECNF mat electrode and ECNF mat electrode.

Aptamer concentration and incubation time

The amount of immobilized electrochemical response indicator depends on concentrations of ss-HSDNA and incubation time. Therefore, the influence of ss-HSDNA concentrations and incubation time on difference of peak currents between and ss-HSDNA/AuNPs/ECNF mat electrode and AuNPs/ECNF mat electrode was investigated. As shown in Fig. 2a, the difference current steadily increased from 0.1 to 1 µM of aptamer

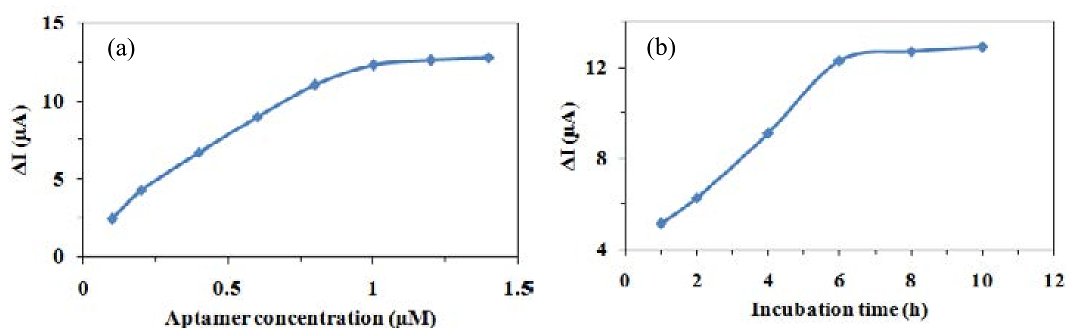

Fig. 2. Effects of (a) aptamer concentration and (b) incubation time on difference between peak currents of ss-HSDNA/AuNPs/ECNF mat electrode and AuNPs/ECNF mat electrode.

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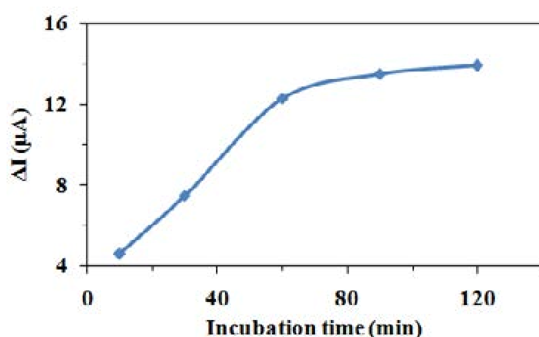

Fig. 3. Incubation time effect of AFM1 on difference of peak currents between and AFM1/ss-HSDNA/AuNPs/ECNF mat electrode and ss-HSDNA/AuNPs/ECNF mat electrode.

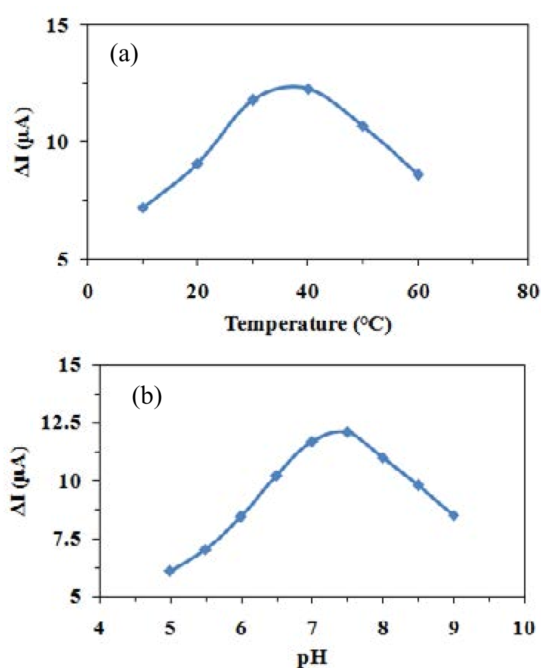

Fig. 4. Effects of (a) temperature and (b) pH of the electrolyte on difference between and AFM1/ss-HSDNA/AuNPs/ECNF mat electrode and ss-HSDNA/AuNPs/ECNF mat electrode.

concentration. Nevertheless, there was negligible increase in the difference current for a concentration of aptamer over 1 µM. The reason of this matter is that the active sites for immobilization of aptamer were saturated at concentrations more than 1 µM. The similar trend was also observed for incubation time (Fig. 2b). These results are consistent with the findings reported by Zhu et al. [21].

Incubation time of AFM1

The incubation time effect of AFM1 on difference of peak currents between and AFM1/ss-HSDNA/AuNPs/ECNF mat electrode and ss-HSDNA/AuNPs/ECNF mat electrode was evaluated as shown in Fig. 3. It can be seen that increasing incubation time up to 60 min increased the difference of peak currents, but no considerable increase in difference of peak currents was occurred over 60 min. This trend indicated that the binding sites between AFM1 and ss-HSDNA were approximately saturated after 60 min.

Temperature and pH of the electrolyte

The temperature and pH of the electrolyte was also optimized to increase the performance of aptasensor. As shown in Fig. 4a, the difference of peak currents between and AFM1/ss-HSDNA/AuNPs/ECNF mat electrode and ss-HSDNA/AuNPs/ECNF mat electrode was enhanced as the temperature increased from 10 to 40 °C and then reduced above 40 °C. The decreasing trend of curve can be due to the deactivation of aptmaer. The similar trend was also observed for pH (Fig. 4b). In fact, the pH of electrolyte can affect the protonation or deprotonation of the aptamer and AFM1, resulting in enhancement or reduction of the electrostatic interaction between the aptamer and AFM1 [22].

CONCLUSION

An electrochemical aptasensor based on ss-HSDNA/AuNPs/ECNF mat electrode has been successfully produced to analyze the AFM1 in the milk samples. The following conclusions can be extracted from the results:

1- The optimum concentration of $HAuCl_4$ in the electrolyte used for electrodeposition of AuNPs on the ECNF mat electrode was 4mM.

2- With increasing aptamer concentration, the difference of peak currents between and ss-HSDNA/AuNPs/ECNF mat electrode and AuNPs/ECNF mat electrode increased and then remained almost stable over 1µM concentration. The similar trend was also observed in incubation time of aptamer.

3- The difference of peak currents between and AFM1/ss-HSDNA/AuNPs/ECNF mat electrode and ss-HSDNA/AuNPs/ECNF mat electrode increased as incubation time of AFM1 increased up to 60 min and did not increase considerably above 60 min.

4- The optimum temperature and pH of electrolyte utilized in electrochemical measurements were 40 °C and 7.5, respectively.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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