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RESEARCH ARTICLE

# Investigation of the effective parameters on aptamer-based electrochemical biosensors for the detection of fumonisin B1 in maize flour

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**ABSTRACT**

This study examined the effective parameters on fabrication of an aptasensor to detect fumonisin B1 (FB1) in maize flour. For this purpose, gold nanoparticles (AuNPs) was firstly electrodeposited onto screen printed carbon electrode (SPCE). Then, a thiol-modified single stranded DNA (ss-HSDNA) was immobilized on the AuNPs/SPCE electrode. By applying the cyclic voltammetry (CV) technique, the effects of HAuCl4 and ss-HSDNA concentrations in the electrolyte, incubation time of aptamer and FB1, pH and temperature of the electrolyte on the peak current response were investigated. The findings indicated that the optimal concentration of $HAuCl_4$ was 5 mM. The peak current of CV decreased as the concentration of ss-HSDNA increased and the optimum ss-HSDNA concentration was chosen at 5 µM. In addition, the CV peak currents decreased with increasing incubation time of aptamer or FB1. The peak currents of CV first decreased and then increased as the electrolyte's temperature increased. The electrolyte's pH also showed this trend. Base on the results, this aptasensor could be a promising tool for FB1 detection in maize flour.

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## INTRODUCTION

The worldwide demand for agricultural products has risen over time, with an anticipated increase of 84% between 2000 and 2050. Food safety is a controversial issue all over the world and especially among progressed communities. Fumonisin is a fungal toxin that can be prepared by different species of fungi, such as *F. verticillioides* and *F. proliferatum* [1, 2]. There are at least 15 types of fumonisin, and among them, fumonisin B1 (FB1) is the most prevalent type and is the cause of 70% of fumonisin contamination in maize [3, 4]. It is estimated that each person ingests about 12-140 g of this poison every day, and unfortunately, in some areas, the intake reaches 2500 g/day. It has been proposed that FB1 has an effect on esophageal cancer and neural tube defects in human embryos [3], and studies have also shown that FB1 causes hepatotoxicity [5], immunotoxicity [6], and has an effect on oocyte quality in mice [7]. It is stated that FB1 exposure induces toxicities in these ways: Epigenetic changes such as severe DNA methylation and hypomethylation cause chromatin instability. The other mechanisms are a change in autophagy and oxidative stress [8].

Until now, several methods like enzyme-linked immunosorbent assays, liquid chromatography spectrometry, high performance liquid chromatography, and thin layer chromatography

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have been used for detecting FB1 in agricultural crops [4]. Although these approaches are reliable and accurate, their application is limited by the complex process, need for expertise, and expensive devices [9, 10]. Therefore, the need for an easy and fast technique to detect FB1 contamination still remains.

Biosensors have been extensively utilized in the detection of mycotoxins over the past decades because of their prompt response, ease of operation, and low cost raw materials [11]. The electrochemical biosensor, among different biosensors, has emerged as a popular choice for mycotoxins detection due to outstanding advantages such as simplicity of equipment, high sensitivity, and capability of miniaturization [12, 13].

The use of aptamer as a diagnostic biomarkers in biosensors has increased in recent years. Aptamers are short, single-stranded oligonucleotides that exhibit high affinity and specificity towards target molecules [14, 15]. So far, several aptamer-based biosensors, known as aptasensors, have been designed for the detection of FB1. For example, the aptamer based microcantilever array biosensor was successfully exploited by Zhang et al. for FB1 detection [16]. In another study, Wang et al. designed an electrochemical aptasensor to determine FB1 [17].

Screen-printed carbon electrode (SPCE) has been widely used as a platform in electrochemical aptasensor for highly sensitive and selective detection of a wide range of analytes. Reports indicate that the modification of SPCE with nanoparticles by increasing the surface area to volume ratio results in improvement of aptasensor performance. Gold nanoparticles (AuNPs) being one of the most popular nanoparticles for use in aptasensor due to high stability, less toxicity, and superior electron transfer [18].

Herein, the aptasensor based on ss-HSDNA/AuNPs/SPCE for the rapid detection of FB1 in maize flour was designed and various parameters such as concentration of $HAuCl_4$ and ss-HSDNA, incubation time of ss-HSDNA and FB1, temperature and pH of the electrolyte media were analyzed and optimized in order to boost the aptasensor performance.

## EXPERIMENTAL

*Materials and Reagents*

Screen-printed carbon electrodes being based on a classical three-electrodes approach with the carbon working electrode (WE), the carbon counter electrode, and the Ag/AgCl reference electrode (RE) were purchased from DropSens (Spain). Hydrogen tetracholoroaurate ($HAuCl_4$), sodium chloride (NaCl), potassium chloride (KCl), Tris–HCl, sulfuric acid ($H_2SO_4$), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium phosphate dibasic ($Na_2HPO_4$), methanol ($CH_3OH$), potassium phosphate monobasic ($KH_2PO_4$), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), and 2-mercaptoethanol (2-MCE) were bought from Sigma-Aldrich. FB1-aptamer ss-HSDNA sequence (5' $SH(CH_2)_6$AGCAGCACAGAGGTCAGATGCGATCTGGATATTATTTTTGATACCCCTTTGGGAGACATCCTATGCGTGCTACCGTGAA-3′) was obtained from Thermo Fisher Scientific. All solutions were made from ultrapure water.

*The ss-HSDNA/AuNPs/SPCE preparation*

The ss-HSDNA/AuNPs/SPCE was prepared during two stages:

1) Electrodeposition of AuNPs on SPCE

This process was carried out for the duration of 60 sec under a constant potential of -0.4 V versus Ag/AgCl in the 0.1 M $H_2SO_4$ electrolyte that contained 1-8 mM $HAuCl_4$.

2) Immobilization of ss-HSDNA onto AuNPs/SPCE

To immobilize ss-HSDNA aptamers, the AuNPs/SPCE was immersed in a solution containing 5 µM of the ss-HSDNA for 6 h at 36±2 °C. By heating the aptamer for 5 min at 94 °C and immediately cooling it for 15 min on ice, the aptamer folded correctly. Unimmobilized aptamers were removed using binding buffer (100 mM NaCl, 5 mM KCl, 2mM $MgCl_2$, 20 mM Tris–HCl and 1 mM $CaCl_2$). To block any remaining active sites, the modified electrode was then immersed in 10 µL of 2-MCE solution.

*Preparation of working solution*

The working solution of FB1 at different concentrations was prepared by dissolving the FB1 mycotoxin powder in acetonitrile/$H_2O$ (50/50, v/v).

*Preparation of the maize sample*

The maize flour was purchased from local markets. To ensure that the maize flour did not contain FB1, LC/MS analysis was performed on it. After milling the maize flour and passing it through 80 mesh sieve, 50 µL of FB1 working solution at different concentrations was spiked

into the mixture, followed by incubation at room temperature for 24 h. Then, 3 mL of methanol/water (20:80, v/v) was utilized to extract maize samples using a horizontal shaker at room temperature for 20 min and followed by centrifugal apparatus for 6 min at 4 °C and 5000 rpm. The supernatant was diluted with binding buffer after filtration through 0.22 µm syringe filter.

*Electrochemical measurements*

The aptasensor assay's response was achieved by putting the ss-HSDNA/AuNPs/SPCE into the solution prepared based on section 2.3. Then, the aptasensor was submerged in an electrolyte containing 0.1 M KCl and 5.0 mM ferri/ferrocyanide ($[Fe(CN)6]^{3-/4-}$). Cyclic voltammetry measurements were performed by means of µStat 400 potentiostat/galvanostat (DropSens, Spain) in the potential range of -0.4 - 0.7 V at the scan rate of 50 mV/s.

## RESULTS AND DISCUSSIONS

In the present study, the effect of $HAuCl_4$ concentration, aptamer concentration, incubation time of aptamer and FB1, temperature and pH of the electrolyte were investigated on the CV signal of the prepared aptasensors.

*Hydrogen tetracholoroaurate concentration*

In this research, CV technique was applied to optimize $HAuCl_4$ concentration. As shown in Fig. 1, the peak current had a biphasic behavior. That is, by increasing the $HAuCl_4$ concentration in the solution, the peak current response increased from 85 µA to a maximum value of 103 µA and then slowly dropped to 92 µA. Hence, it can be concluded that the concentration of 5 mM is the optimum to obtain the maximum response. The improvement of the peak current response can be attributed to enhancement of charge transfer resulting from promotion of electrodeposited AuNPs on SPCE by increasing the $HAuCl_4$ concentration up to 5 mM into the electrolyte. Nevertheless, as more $HAuCl_4$ was added to the electrolyte, the thickness of gold nanoparticles increased resulting in weaker electron conduction. This is in good agreement with the result reported by Du et al [19].

*Aptamer concentration and incubation time*

As shown in Fig. 2a, the variation of ss-HSDNA concentration influences the quantity of immobilized electrochemical response marker. In other words, by increasing the aptamer concentration to 5 µM, the peak current was decreased sharply followed by the decrement in a gentle slope Therefore, it can be argued that the 5 µM concentration of aptamer was the optimum concentration, and at concentrations higher than that, the efficiency of the aptasenor was reduced due to the aptamers immobilization resulting from spatial restriction and overload of the surface active regions. The saturation of the binding site of aptamer by the addition of aptamer concentration was also demonstrated in other studies [9, 20].

Figure 2 illustrates how the peak response is influenced by the duration of incubation. According to that with the enhancement of the incubation time from 1 h to 10 h, the peak current was slightly decreased. In fact, the best time for aptamer incubation was 6 h in our study. The plateau after 6 h could be due to saturation of immobilized aptamers found in other studies too [21, 22].

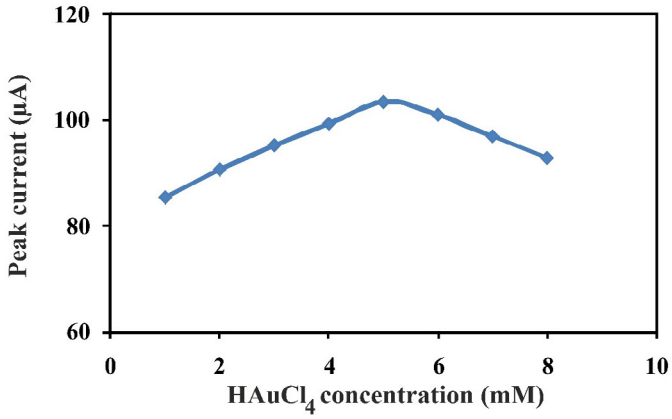


Fig. 1. Effect of $HAuCl_4$ concentration on the peak current of SPCE.

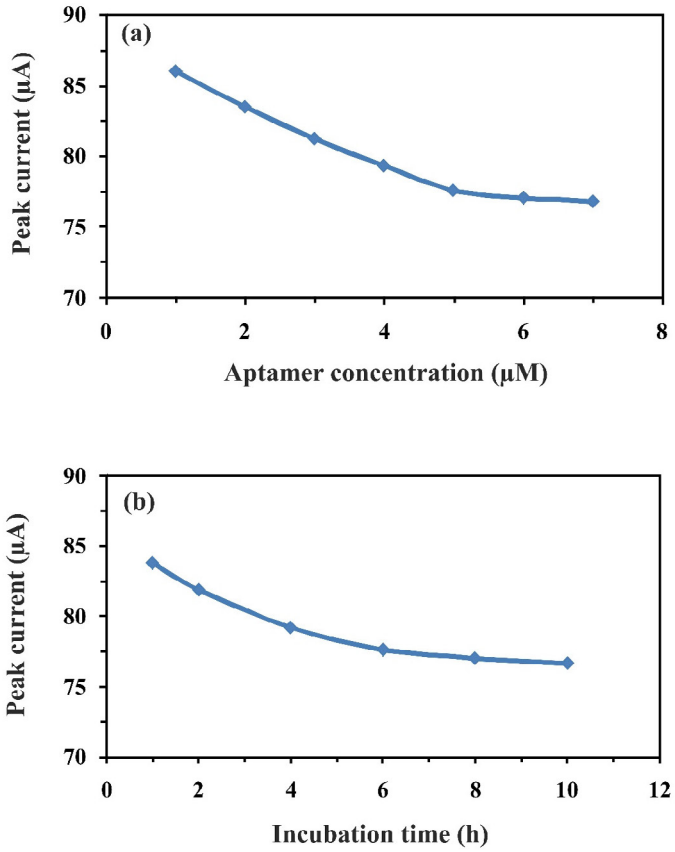


Fig. 2. Effect of a) aptamer concentration and b) aptamer incubation time on the peak current of ss-HSDNA/AuNPs/SPCE.

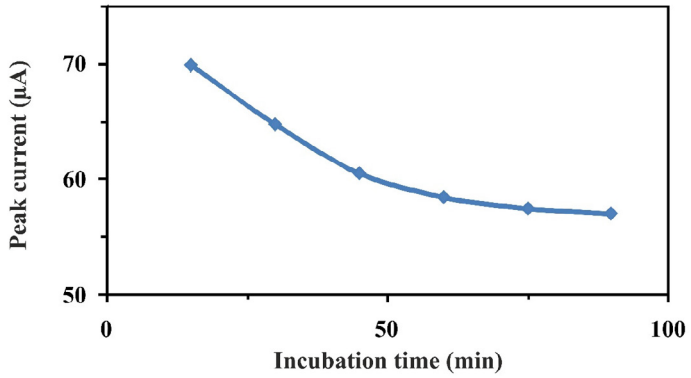


Fig. 3. Effect FB1 incubation time on the peak current of ss-HSDNA/AuNPs/SPCE.

*FB1 incubation time*

The effect of the FB1 incubation time on the peak current was depicted in Figure 3. It can be seen that by increasing incubation time, the peak current decreased and reached to the plateau and therefore, the time duration of 60 min was optimum for the incubation time of the FB1. It can be concluded that the saturation of binding sites between FB1 and ss-HSDNA aptamer was occurred beyond 60 min. The similar trend was also reported by liu et al. [21].

*Electrolyte temperature and pH*

The electrolyte's temperature and pH were also adjusted to improve the aptasensor's performance.

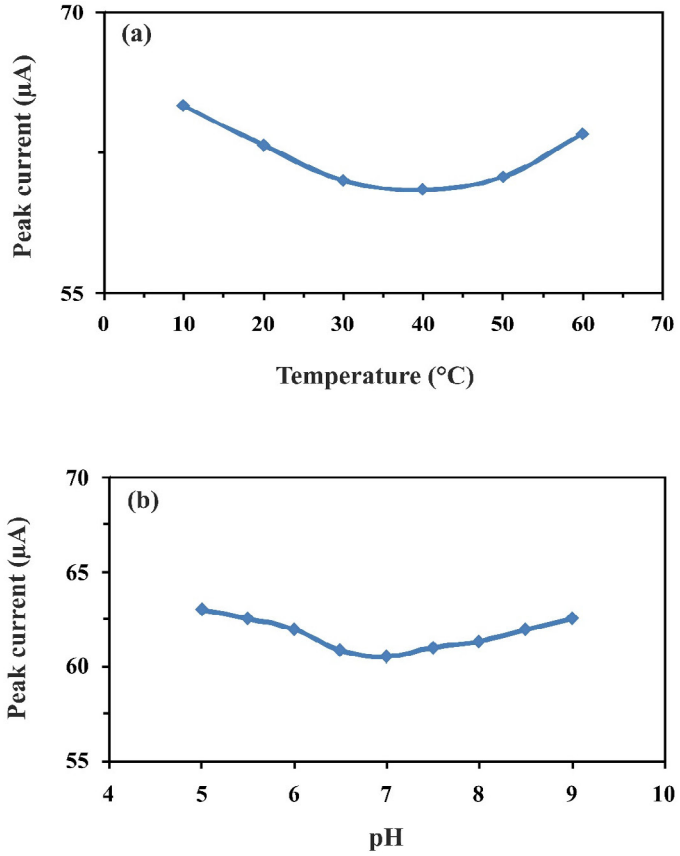


Fig. 4. Effect of a) temperature and b) pH on the peak current of ss-HSDNA/AuNPs/SPCE.

Figure 4a shows that the change in temperature resulted in the biphasic pattern of peak current response. That is, by increasing electrolyte temperature peak current response decreased, reaching a minimum and then increasing again. The increasing trend of curve could be attributed to the deformation of the aptamer's structure [22]. The pH also exhibited a similar trend (Fig. 4b). The relationship between pH and the protonation or deprotonation process, which affects how strongly the aptamer interacts with the FB1, is thought to be the cause of this tendency [23].

## CONCLUSION

In this study using ss-HSDNA aptamer immobilized on AuNPs electrodeposited onto SPCE electrode, a new aptasensor was designed to detect the FB1 in maize flour. The aptasensor manufacturing stages were optimized by cyclic voltammetry technique. The outcomes can be drawn as follows:

1- The optimum thickness of electrodeposited AuNPs was attained at a concentration of 5 mM of $HAuCl_4$.

2- The peak current response decreased with increasing aptamer concentration, but it then remained nearly constant at a concentration of 5 µM. Aptamer incubation time also showed a similar pattern.

3- No substantial change in the peak current response was found after 60 min of FB1 incubation.

4- For electrochemical measurements, the ideal electrolyte temperature and pH were 40 °C and 7, respectively.

## CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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