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RESEARCH ARTICLE

Investigation of effective parameters on electrochemical aptasensor for detection of Penicillin antibiotic

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ABSTRACT

In this research, the fabrication of aptasensor prepared by immobilization of Penicillin aptamer on gold nanoparticles (AuNPs) electrodeposited onto electrospun carbon nanofiber (ECNF) mat was reported to detect Penicillin antibiotic in the milk. The AuNPs/ECNF mat electrode was firstly produced using the electrospinning, heat treatment and electrodeposition process, respectively. Then, the Penicillin aptamer (pDNA) was immobilized on the AuNPs/ECNF mat electrode. The effects of layer thickness of AuNPs, amount of immobilized pDNA, incubation period of pDNA and Penicillin, temperature and pH of experiment solution on the electrochemical response were studied by cyclic voltammetry (CV) technique. The results revealed that the suitable thickness of AuNPs was achieved at 3 mM $HAuCl_4$ concentration. An enhancement in the electrochemical current response was also observed with increasing pDNA in the electrolyte. Moreover, the increment of incubation time led to improvement of CV peak currents. Meanwhile, the maximum peak currents of CV were obtained in the solution with pH of 7 at 35 °C.

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INTRODUCTION

Penicillin as a group of β-lactam antibiotics is extensively used to prevent and cure different bacterial infections in dairy cattle. However, the excessive use of this antibiotic may cause the presence of residues in dairy product like raw milk which is a dangerous threat to human health. Therefore, it is necessary to develop a sensitive and cost effective method for identification of antibiotics residues in dairy products such as milk, butter, yoghurt and cheese [1, 2].

Up to now, several various methods have been introduced for the detection of antibiotics including high performance liquid chromatography (HPLC) [3], fluorescence [4], liquid chromatograph-mass spectrometry (LC-MS) [5] and colorimetric [6]. Although these methods have the advantage of being able to precise and reliable detection of antibiotics, they are often time-consuming process, require a complex sample preparation, professional user and costly instruments. Hence, it is of great significance to develop an alternative method for the detection of Penicillin residues in dairy products. In recent years, aptasensors classified as a group of biosensors have attracted great attention for Penicillin detection due to their valuable features such as low cost, high sensitivity, portability and versatility [7-9]. Aptamers used in aptasensors as biological recognition elements are artificial single-stranded DNA or RNA nucleic acids that can specifically bind to their target molecules with high affinity [10].

The platform utilized for the immobilization of aptamers has a significant effect on performance of biosensors. Among various platforms, gold electrode has been widely used in aptasensor

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because of its unique electronic properties [11]. the low specific surface area of this electrode may be insufficient for the immobilization of high amount of aptamers that results in attenuation of signal intensity. To overcome this problem, the use of nanomaterials for modification of electrode has been proposed. It was reported that aptasensor platform has been designed with nanomaterials such as gold nanoparticles, graphene, carbon nanotubes [12]. For example, Razdari et al. constructed an electrochemical aptasensor for the detection of Penicillin antibiotic using a graphite electrode modified with reduced graphene oxide and gold nanoparticles [13]. In another study, GR–Fe_3O_4NPs and PEDOT–AuNPs composite as a platform was used to immobilize the aptamer for detection of Penicillin antibiotic [14].

Another nanomaterial that has attracted considerable attention is carbon nanofiber mat used as platform and raises the surface sites for the immobilization of the biomolecules leading to signal amplification in biosensor [15]. One of the relative simple and inexpensive methods used for the fabrication of carbon nanofiber mat is electrospinning technique [16]. The use of electrospun carbon nanofiber mat electrode modified by gold nanoparticles (AuNPs) can developed an ultrasensitive aptasensor because of unique properties of this electrode like a good electrical conductivity and high electrochemical activity. However, to the authors' knowledge, no attempt has been made to assemble the aptamer on this electrode for Penicillin detection in raw milk. Hence, it is great challenge to fabricate the Penicillin aptamer/AuNPs/ECNF mat electrode for the detection of Penicillin in milk sample.

In this research, a novel Penicillin aptamer / AuNPs/ECNF mat aptasnsor for the rapid detection of Penicillin in milk samples was designed. For this purpose, the AuNPs was firstly electrodeposited on the ECNF mat electrode. Then, the Penicillin aptamer was immobilized on the AuNPs/ECNF mat electrode. During fabrication, in order to improve the aptasensor performance, the various parameters such as concentration of $HAuCl_4$ and aptamer in the electrolyte, incubation time of aptamer and Penicillin, temperature and pH of the electrolyte were optimized.

EXPERIMENTAL

Reagents and Materials

Commercial polyacrylonitrile (PAN) with a molecular weight of 150,000 g/mol was received from Polyacryl company (Iran). Dimethylformamide (DMF) used to dissolve the PAN was obtained from Merck. Potassium chloride (KCl), sodium chloride (NaCl), sodium phosphate dibasic (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4), hydrogen tetracholoroaurate ($HAuCl_4$), Sulfuric acid (H_2SO_4), bovine serum albumin (BSA), potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) were purchased from Sigma-Aldrich.

Penicillin aptamer sequence (5'-thiol-$(CH_2)_6$-CTG AAT TGG ATC TCT CTT CTT GAG CGA TCT CCA CA-3') was brought from Faza Biotech Co. (Iran). Ultra pure water was utilized to prepare all solutions.

Electrode fabrication

The fabrication of Penicillin aptamer/AuNPs/ECNF mat electrode consists of three steps, preparation of ECNF mat, electrodeposition of AuNPs and immobilization of Penicillin aptamer, which is explained in the following:

(1) The production of ECNF mat electrode was conducted according to the procedure described in details elsewhere [16-18] . Briefly, the spinning solution prepared by dissolving of 12 wt.% PAN in DMF at 40 °C for 10 h was ejected from a 18 gauge needle using a syringe pump at a flow rate of 1 ml/h. The stabilization of prepared PAN nanofiber mat was carried out at 290 °C for 4 hrs in air followed by carbonization at 1000 °C for 1 h in a nitrogen atmosphere. Finally, the ECNF mat was cut into circular disc with diameter of 5 mm using a metal hole punch.

(2) The gold nanoparticles were eletrodeposited potentiostatically at -0.4 V (versus Ag/AgCl) on the ECNF mat for 90 seconds in the electrolyte solution containing 0.5-7 mM $HAuCl_4$ and 0.1 M H_2SO_4.

(3) The Penicillin aptamer/AuNPs/ECNF mat electrode was prepared by dropping 4 µL of 1-16 µM Penicillin aptamer on the AuNPs/ECNF mat electrode surface. The modified electrode was then rinsed with ultra pure water to remove any un-immobilized aptamer. Thereafter, the residual binding sites were blocked by injecting 5 µL of phosphate buffered saline (PBS) solution containing 10% BSA onto the surface of electrode and holding at room temperature for 2 h. The electrode was washed with ultra pure water and dried at room temperature before further use.

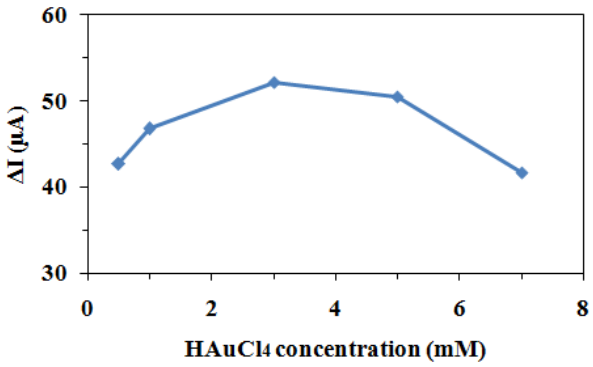

Fig. 1. The difference between peak currents of AuNPs/ECNF mat electrode and ECNF mat electrode versus $HAuCl_4$ concentration.

Preparation of milk sample for electrochemical evaluation

The milk bought from the local market was first centrifuged at 5000 rpm for 15 min to remove milk fat due to detrimental effect on measurements. Then, the fat free milk diluted with 20 % PBS was spiked with 10 µL Penicillin antibiotic.

Study of electrochemical behavior

The electrochemical behavior of aptasensor was investigated by cycle voltammetry (CV) in PBS solution containing 1 mM ferri/ferrocyanide ($[Fe(CN)_6]^{-3/-4}$). The tests were carried out using a µStat 400 potentiostat/galvanostat (DropSens, Spain) at temperatures of 15-65 °C and pH levels in range of 5-9. A conventional three-electrode system consisting of a modified electrode as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as reference electrode was used in these experiments. To investigate the aptasensor performance, the Penicillin aptamer/ AuNPs/ECNF mat electrode was laid in the solution prepared for different incubation times. The electrode was slowly washed with ultra pure water to remove the unbounded Penicillin antibiotic molecules. Electrochemical measurements was finally conducted by dipping the aptasensor in PBS solution containing $[Fe(CN)_6]^{-3/-4}$.

RESULTS AND DISCUSSION

The different fabricating parameters were presented in the following sections:

Amount of electrodeposited AuNPs

The CV peak current response is affected by the amount of electrodeposited AuNPs on ECNF mat. Therefore, the concentration of hydrogen tetracholoroaurate in the bath that is effective parameter on the amount of electrodeposited gold nanoparticles was investigated. Fig. 1 shows the difference of peak currents between AuNPs/ECNF mat electrode and ECNF mat electrode with change of concentration of hydrogen tetracholoroaurate. It can be shown that with increase in concentration of $HAuCl_4$ the difference of peak currents increase, reaches a maximum at 3 mM. Maximum difference of peak currents at 3 mM corresponds to enhancement of electron transfer owing to the increase in thickness of electrodeposited gold nanoparticles on ECNF mat which results from increase in the amount of $HAuCl_4$ in the electrolyte. The reduction in difference of peak currents beyond 3mM concentration can be attributed to the increase the gold nanoparticles coating thickness which destroy the electrode conduction properties. This finding is consistent with result reported by Liu et al. [19].

Amount of immobilized aptamer

Two critical factors affected the amount of immobilized aptamer on AuNPs/ECNF mat electrode are concentration of aptamer and incubation time. Hence, different concentrations of Penicillin aptamer and incubation times were investigated to obtain the optimum electrochemical response against Penicillin antibiotic. As seen in Fig. 2a, the difference of peak currents was enhanced with increasing in the aptamer concentration up to 8 µM. No significant enhancement in difference of peak currents was observed for the aptamer concentration more than 8 µM. Therefore, it can be concluded that the active sites on modified electrode was almost saturated at a concentration of 8 µM aptamer. The change

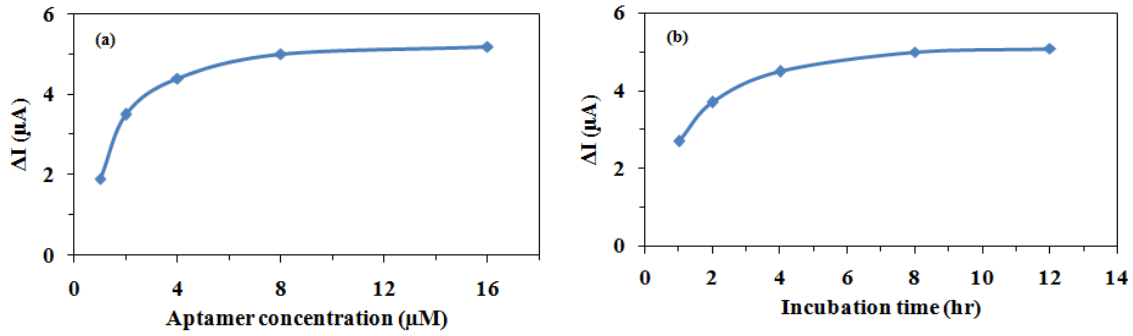

Fig. 2. The difference between peak currents of pDNA/AuNPs/ECNF mat electrode and AuNPs/ECNF mat electrode versus (a) aptamer concentration and (b) incubation time.

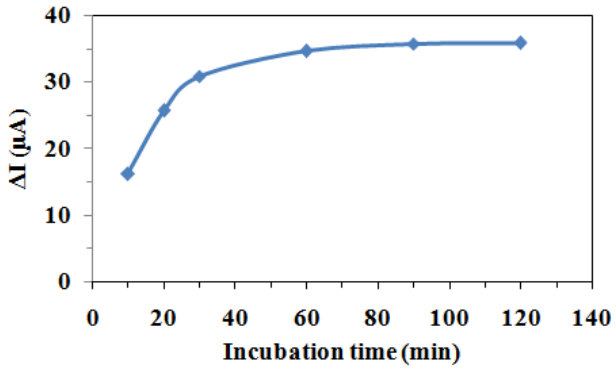

Fig. 3. Effect of incubation time of Penicillin antibiotic on difference of peak currents between and Penicillin/pDNA/AuNPs/ECNF mat electrode and pDNA/AuNPs/ECNF mat electrode.

of peak currents difference with incubation time is shown in Fig. 2b. The increasing trend of peak currents difference was similar to concentration of Penicillin aptamer.

Incubation time of Penicillin antibiotic

The difference between peak currents of Penicillin/pDNA/AuNPs/ECNF mat electrode and pDNA/AuNPs/ECNF mat electrode versus incubation time of penicillin antibiotic was depicted in Fig. 3. This trend indicates that with increase the incubation time up to 60 min the peak currents difference increase, but it stays approximately constant in further incubation time. That is, the saturation of binding sites between Penicillin antibiotic and pDNA was occurred beyond 60 min.

The pH and temperature of the electrolyte

The performance of aptasensor depends on the different parameters including pH and temperature of the electrolyte. The effect of the electrolyte pH on the peak currents difference of electrodes is shown in Fig. 4 (a). There is an increase in the peak currents difference of electrodes when the pH increases to 7. With further increase in pH, nevertheless, the peak currents difference of electrodes reduces. The reason of this trend can be attributed to relationship between pH and protonation or deprotonation process which leads to increase or decrease of the electrostatic interaction between the aptamer and penicillin antibiotic [20].

The trend of the change of the difference of peak currents with temperature was shown in Fig. 4(b). With increasing the temperature, the difference of peak currents between and Penicillin /pDNA/AuNPs/ECNF mat electrode and pDNA/AuNPs/ECNF mat electrode increased first and then decreased after reaching the maximum at 35 °C. The deactivation of aptamer can be the reason for decreasing the difference of peak currents [21].

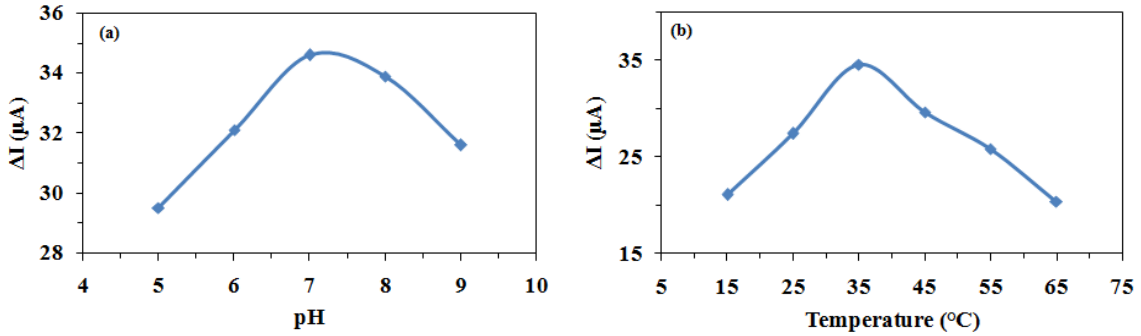

Fig. 4. The difference between and Penicillin antibiotic/pDNA/AuNPs/ECNF mat electrode and pDNA/AuNPs/ECNF mat electrode versus (a) pH and (b) temperature of the electrolyte.

CONCLUSION

A novel aptasensor for the detection of Penicillin antibiotic in milk sample was fabricated using immobilization of pDNA on AuNPs electrodeposited on ECNF mat electrode. The cyclic voltammetry method was used to optimize the fabrication steps of aptasensor. The results indicated that the optimum thickness of electrodeposited AuNPs for maximum electrochemical response was obtained at 3 mM concentration of $HAuCl_4$. The change of the difference of peak currents between and pDNA/AuNPs/ECNF mat electrode and AuNPs/ECNF mat electrode versus the aptamer concentration showed that with increase the aptamer concentration from 1 to 8 μM the difference of peak currents increased, but it remained approximately constant in further aptamer concentration. A similar trend was also seen in incubation times. Furthermore, the best electrochemical responses were achieved in the solution with pH of 7 at 35 °C.

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