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RESEARCH ARTICLE

# Investigation of Effective Parameters on Aptamer-Based Electrochemical Sensor for Rapid Detection of Tetracycline Residues in Chicken Ham

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**ABSTRACT**

This study investigates the impact of key parameters, including aptamer concentration, aptamer incubation time, tetracycline (Tet) incubation time, pH, and temperature, on the peak current of an aptasensor designed for Tet detection. The aptasensor was developed by immobilizing a Tet aptamer onto gold nanoparticles (AuNPs) electrodeposited on an electrospun carbon nanofibers (CNFs). The integration of CNFs and AuNPs enhances the sensor's performance by increasing the surface area and improving conductivity. The results show that aptamer concentration, aptamer incubation time, incubation time of Tet, pH, and temperature affect the peak current of the aptasensor, demonstrating the importance as effective parameters in the preparation of biosensors.

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## INTRODUCTION

Tetracycline (Tet) is a broad-spectrum antibiotic widely utilized for treating various infections, including those caused by bacteria, parasites, and certain types of acne. Its therapeutic efficacy is primarily attributed to its ability to inhibit protein synthesis in bacteria, making it a critical tool in combating various infectious diseases. However, the emergence of Tet resistance in bacterial strains poses a significant challenge, as it limits treatment options and complicates infection management. Consequently, monitoring Tet residues in food products has become increasingly important to ensure food safety and public health[1].

Traditional methods for detecting tetracycline residues, such as high-performance liquid chromatography (HPLC) and mass spectrometry, provide precise quantification of antibiotic levels in food samples. Additionally, techniques like liquid chromatography-mass spectrometry (LC-MS)[2, 3] and gas chromatography-mass spectrometry (GC-MS)[4] are also employed to analyze complex matrices. Despite their accuracy, these methods are often time-consuming and require specialized laboratory environments operated by qualified personnel. This underscores the urgent need for the development of rapid, efficient testing methods that can be implemented in field settings to effectively monitor antibiotic residues.

DNA can find and stick to many different chemicals. This makes it useful for building biosensors that can find specific things. These biosensors often use DNA pieces called aptamers. Aptamers are like tiny, folded-up strings of DNA. They are made to grab onto certain target substances very tightly. This lets the biosensor find those substances, even in messy samples. Aptamers are very important for these biosensors because they recognize and grab the target[1, 5].

In response to this need, electrochemical biosensors have emerged as promising alternatives

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for rapid tetracycline detection. These devices offer portability and ease of use, making them suitable for on-site testing[6]. An electrochemical biosensor typically consists of a working electrode, a reference electrode, and a counter electrode that facilitate the electrochemical reactions necessary for detection[1]. The integration of nanomaterials such as electrospun carbon nanofibers and gold nanoparticles into these biosensors enhances their sensitivity and performance [7-9].

The utilization of gold nanoparticles (AuNPs) enables the formation of stable and high-density single-stranded DNA (ssDNA) monolayers on electrode surfaces, a technique frequently employed in the fabrication of biosensors. The incorporation of AuNPs contributes to enhanced electrical conductivity and improved stability of the resulting biosensor, thereby facilitating more sensitive detection of target biomolecules across a range of applications [10-12].

Furthermore, the integration of carbon nanofibers (CNFs) into these biosensor architectures provides additional performance enhancements. CNFs offer an increased surface area for biomolecule immobilization and promote accelerated electron transfer kinetics. The synergistic combination of AuNPs and CNFs not only augments the sensitivity of the biosensor but also broadens the spectrum of detectable analytes, rendering these devices increasingly valuable in diverse fields such as medical diagnostics, environmental surveillance, and food safety assurance [13-15].

This manuscript explores the approach of utilizing electrospun carbon nanofibers and AuNPs in conjunction with aptamer technology to develop highly sensitive electrochemical sensors for the detection of tetracycline in chicken ham. By advancing these methodologies, we aim to optimize the steps of prepration for aptasensor including aptamer concentration, aptamer incubation time, incubation time of Tet, pH, and temperature for the detection of Tet.

## MATERIALS AND METHODS

*Materials*

Polyacrylonitrile (PAN) with a molecular weight of 150,000 g/mol was purchased from Polyacryl Company (Iran). Dimethylformamide (DMF), which was used as a solvent for the PAN was obtained from Merck. Potassium chloride (Kcl), sodium chloride (NaCl), Tetracycline (98%), sodium phosphate dibasic ($Na_2HPO_4$, 0.1 M), potassium phosphate monobasic ($KH_2PO_4$, 0.1 M), were employed to prepare phosphate buffer solutions (PBS). Hydrogen tetracholoroaurate ($HAuCl_4$), sulfuric acid ($H_2SO_4$), potassium ferricyanide ($K_3$ [Fe (CN) $_6$]), and potassium ferrocyanide ($K_4$ [Fe (CN) $_6$]) was obtained from Sigma-Aldrich. Faza Biotech Co. (Iran) provided the Tet aptamer sequence (5′-thiol-$(CH_2)_6$CG TACGGAATTCGCTAGCCCCCCGGCAGG CCACGGCTTGGGTTGGTCCACTGCGCG TGGATCCGAGCTCCACGTG-3′). All solutions were prepared using ultrapure water.

*Electrode modification*

The aptamer/AuNPs/CNF electrode was fabricated through a three-step process: preparation of carbon nanofibers (CNFs), electrodeposition of gold nanoparticles (AuNPs) on CNF electrode, and immobilization of the aptamer on AuNPs/CNF electrode. Each step is detailed below.

(1) CNF Preparation: CNFs were synthesized following established methods [1, 8, 10]. Briefly, 1.1 g of polyacrylonitrile (PAN) was dissolved in 8.9 mL of DMF and stirred magnetically at 1000 rpm for 9 hours at 45°C to ensure complete dissolution. The resulting solution was electrospun using an Electrories device (Fanavaran Nanomeghyas Ltd., Co., Iran) under ambient conditions. The PAN solution was loaded into a syringe fitted with an 18-gauge needle, and a high-voltage power supply (19 kV) was applied to generate a potential difference between the spinneret and the collector, enabling the ejection of the solution toward the collector. The distance between the nozzle and the rotating collector was set at 10 cm, while the collector rotated at a speed of 100 rpm. To convert the electrospun PAN nanofibers into CNFs, sequential stabilization and carbonization processes were performed. Stabilization involved heating the PAN nanofibers in a tube furnace under an air atmosphere at 290 °C for 4 hours. This was followed by carbonization under an inert atmosphere at 1000 °C for 1 hour. The heating rates were controlled at 1.5 °C/min for stabilization and 4 °C/min for carbonization. The CNFs electrodes were spherically perforated with a diameter of 5 mm and attached to a copper wire to establish electrical contact.

(2) AuNPs Electrodeposition: AuNPs were electrodeposited onto the CNF electrode by applying a constant potential of -0.4 V for 45 seconds.

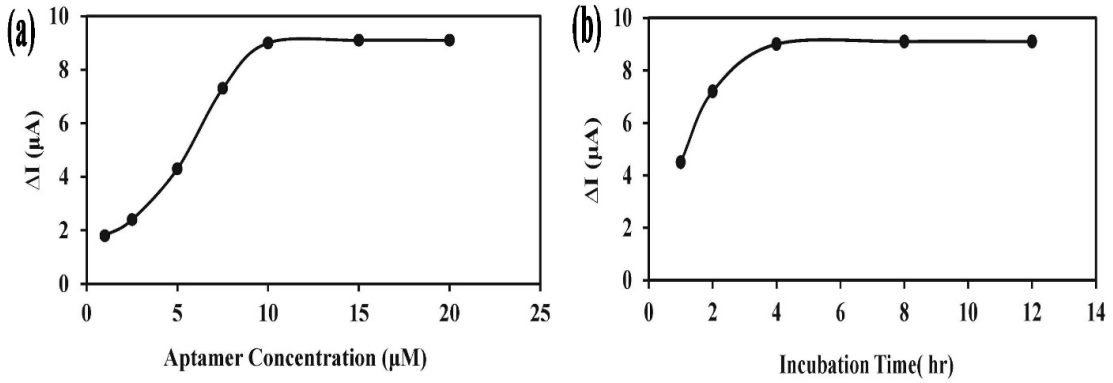


Fig. 1. The variation in peak current differences between the Aptameras a function and AuNPs/CNFs electrode (a) Different concentrations of aptamer, and (b) Incubation time. All studies used 0.1 M PBS in 0.5 M $[Fe(CN)_6]^{3-/4-}$.

(3) Aptamer Immobilization: To prepare the aptamer/AuNPs/CNF electrode, 5 µL of aptamer solutions was applied to the AuNPs/CNF electrode surface. The electrode was then rinsed with ultra-pure water to eliminate any unbound aptamer and dried at room temperature before use.

*Preparation of Chicken Ham Sample for Electrochemical Evaluation*

Chicken ham (70%) was obtained from a local market and first comminuted using a commercial blender. The comminuted sample was then diluted with 20% PBS. The chicken ham suspension was diluted with PBS to a concentration of 20% and then spiked with different concentrations of Tet antibiotics using a 10 µL volume.

*Study of electrochemical behavior*

In these experiments, CNF electrode was used the working electrode and a platinum wire served as the auxiliary electrode, while an Ag/AgCl electrode was used as the reference electrode. The electrochemical behavior of the aptasensor was studied using CV in a 0.1 M PBS solution containing 0.5 M $[Fe(CN)6]^{3-/4-}$. A µStat 400 potentiostat/ galvanostat (DropSens, Spain) was used to perform the tests, scanning the potential from -0.4 V to 0.6 V at a scan rate of 50 mV/s. CV was employed to evaluate the electron transfer kinetics and surface coverage of the modified electrodes, offering insights into the aptamer's binding affinity for the target molecule1. After washing the electrode with ultra-pure water to remove any unbound Tet, electrochemical measurements were taken by immersing the aptasensor in PBS solution containing $[Fe(CN)6]^{3-/4-}$.

## RESULTS AND DISCUSSION

*Electrochemical behavior*

The performance of the electrodes was assessed using CV with a 0.5 mM $[Fe(CN)_6]^{3-/4-}$. Redox probe in a pH 7.4 buffer solution. The CV responses were investigated for CNF electrode and modified electrodes the modification of CNF electrode with AuNPs enhanced the peak current. This improvement can be attributed to the unique properties of AuNPs including conductivity, large surface area which promote faster electron transfer [16] and electrocatalytic properties of the AuNPs[17].

*Aptasensor optimization electrode*

The concentration of the aptamer and the incubation time were identified as critical factors influencing the immobilization of aptamers on the AuNPs/CNFselectrode. An optimal electrochemical response for Tet detection was achieved at a 10 µM aptamer concentration. Higher concentrations did not yield improvements, indicating potential saturation of active sites [18, 19]. Furthermore, the peak current enhanced with increasing the incubation time from 1 to 4 hours. However, peak current did not increase considerably with increasing incubation time from 4 to 12 hours[19, 20].

*Incubation Time of Tet Antibiotic*

Fig. 2 illustrates how the difference in peak currents between the Tet/aptamer/AuNPs/CNFs electrode and the aptamer/AuNPs/CNF electrode varies with the incubation time for the Tet antibiotic. The data indicate that the peak current

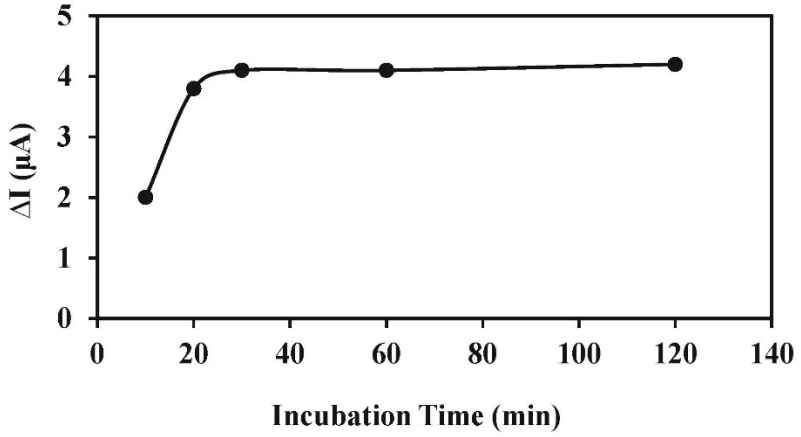


Fig. 2. The impact of incubation time of the Tet antibiotic on the peak current differences between the Tet/aptamer/AuNPs/CNFs electrode and the aptamer/AuNPs/CNFelectrode. All studies used 0.1 M PBS in 0.5 M $[Fe(CN)_6]^{3-/4-}$.

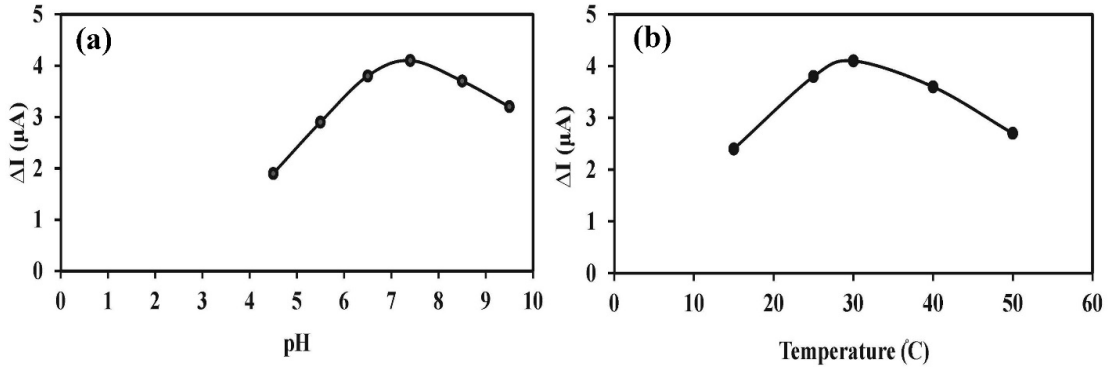


Fig. 3. Effect of electrolyte pH (a) and temperature (b) on the peak current difference between Tet/aptamer/AuNPs/CNF electrode and aptamer/AuNPs/CNF electrodes. All studies used 0.1 M PBS, in 0.5 M $[Fe(CN)_6]^{3-/4-}$.

difference increases as the incubation time extends up to 30 minutes. Beyond this point, the difference stabilizes, showing minimal change with longer incubation periods. This behavior suggests that the binding sites between the Tet antibiotic and the aptamer reach saturation after approximately 30 minutes[20].

*pH and Temperature Optimization of the Aptasensor*

The performance of the aptasensor is influenced by the electrolyte's pH and temperature. Fig. 3(a) illustrates the effect of pH on the peak current difference between electrodes. As the pH increases to 7.4, the peak current difference also increases. However, a further increase in pH leads to a reduction in the peak current difference. This trend may be attributed to the protonation or deprotonation processes, which modulate the electrostatic adsorption between the aptamer and the Tet antibiotic[21].

The effect of temperature on the peak current difference is shown in Figure 3(b). As the temperature increases, the peak current difference between the Tet/aptamer/AuNPs/CNFelectrode and the aptamer/AuNPs/CNFelectrode initially increases, reaching a maximum at 30 °C, and subsequently decreases. This decrease may be due to the deactivation of the aptamer at higher temperatures[21].

## CONCLUSION

A novel aptasensor was developed for detecting the Tet antibiotic in ham samples by immobilizing an aptamer onto an AuNPs/CNFs electrode. The modification using CNFs significantly enhanced electrochemical activity, leveraging their improved

conductivity, large surface area, and effective analyte adsorption to facilitate faster electron transfer. Furthermore, the incorporation of AuNPs further augmented the current, underscoring enhanced electron transport attributed to the AuNPs' high conductivity and electrocatalytic effects.

The analysis of peak current differences between the aptamer/AuNPs/CNF electrode and the AuNPs/CNF electrode to aptamer concentration revealed that as the concentration increased from 1 to 10 µM, the difference in peak currents also increased. However, this difference remained relatively stable at higher aptamer concentrations, suggesting active site saturation. A similar pattern was observed concerning incubation times, where longer times did not proportionally increase the signal. Additionally, optimal electrochemical responses were recorded at a pH of 7.4 and a temperature of 30 °C. The performance of such aptasensors is influenced by factors like pH and temperature, which can modulate the electrostatic interaction between the aptamer and the Tet antibiotic. Aptamer-based sensors are recognized for their simplicity, accuracy, cost-effectiveness, and selectivity in detecting antibiotics, making them suitable for environmental and food safety analysis.

## CONFLICT OF INTEREST

The authors of this article have no conflict of interest.

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