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RESEARCH ARTICLE

# Optimization of Effective Parameters for an Aptamer-Based Electrochemical Sensor for Rapid Detection of Ampicillin in Hot Dog Samples

***Somayeh Ayoughi[1], Mohsen Adabi[2*], Kamran Pooshang Bagheri[3], Zahra Beigmohammadi[1], Zhaleh Khoshkhoo[1]***

*[1] Department of Food Science and Technology, NT. C., Islamic Azad University, Tehran, Iran*

*[2] Department of Metallurgy and Materials Science, Ro. C., Islamic Azad University, Roudehen, Iran*

*[3] Venom and Biotherapeutics Molecules Lab, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran*

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**ABSTRACT**

Aptamer-based electrochemical sensors offer a promising alternative to conventional chromatographic and immunoassay techniques for monitoring antibiotic residues in complex food matrices. In this work, an aptamer/AuNPs/electrospun carbon nanofiber (ECNF) sensing platform was engineered and applied to the rapid determination of ampicillin (AMP) in homogenized hot dog samples. ECNF working electrodes were prepared from electrospun and carbonized polyacrylonitrile mats, followed by potentiostatic deposition of gold nanoparticles to enhance the electroactive surface area and provide suitable anchoring sites for a thiolate ssDNA aptamer specific for AMP. Systematic optimization of key experimental parameters, including HAuCl4 concentration, gold electrodeposition time, and electrolyte temperature, identified 10 mM HAuCl4, 45 s electrodeposition at -400 mV, and an electrolyte temperature of 30 °C as the optimal conditions, with 30°C providing the most favorable conditions for the signal-off response toward AMP. Under these optimized conditions, the aptasensor exhibited a pronounced and reproducible voltammetric response to AMP in a 20% (w/v) hot dog matrix, enabling sensitive detection of residues in this complex, protein- and fat-rich environment. The proposed sensing platform, based on a simple electrode fabrication procedure and straightforward sample preparation, demonstrates the feasibility of developing cost-effective, rapid screening tools for β-lactam antibiotic residue analysis in processed meat products and underscores the critical role of parametric control in achieving reliable performance in real food samples.

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## INTRODUCTION

Intensive and often uncontrolled use of antibiotics in food-producing animals has heightened concern over the occurrence of drug residues in products that are consumed routinely. Ampicillin (AMP), a widely used broad-spectrum β-lactam, is frequently administered in livestock for prophylaxis and therapy, and misuse or inadequate withdrawal periods can leave measurable residues in edible tissues and processed meats. Hot dogs and related ready-to-eat meat products, typically manufactured from mixed animal sources, represent a particularly relevant matrix for residue monitoring because of their high consumption and heterogeneous composition. The presence of AMP in such products not only threatens consumer health but also contributes to the escalation of antimicrobial resistance [1, 2].

To safeguard public health, regulatory

* Corresponding Author Email: *mohsen.adabi@iau.ac.ir*

authorities have defined maximum residue limits (MRLs) for β-lactam antibiotics, including AMP, in foods of animal origin. Ensuring compliance with these limits demands analytical methods capable of detecting trace amounts of antibiotics in complex, protein- and fat-rich matrices. Established techniques such as HPLC, LC–MS/MS, and ELISA provide excellent sensitivity and selectivity, yet they rely on costly instrumentation, specialized operators, and extensive sample preparation, and are generally confined to centralized laboratories. These constraints limit their suitability for rapid, high-throughput screening or in-plant monitoring in food processing facilities [3, 4]. Consequently, there is a strong impetus to develop simpler, more affordable sensing platforms that can deliver timely information on AMP contamination in processed meats such as hot dogs.

Among emerging alternatives, aptamer-based biosensors have gained considerable attention for antibiotic detection owing to the favorable properties of aptamers as recognition elements [5, 6]. Aptamers are short single-stranded nucleic acids selected to bind specific targets with high affinity and specificity, and they offer key advantages over antibodies, including chemical synthesis, facile modification, thermal robustness, and good batch-to-batch reproducibility [7, 8]. When combined with electrochemical transduction, aptamer recognition enables the construction of compact, inexpensive aptasensors that exhibit rapid response and are amenable to miniaturization and on-site measurements. In these devices, immobilization of the aptamer on an electrode surface allows target binding such as interaction with AMP to manifest as changes in interfacial charge transfer or redox behavior, which can be monitored by techniques like DPV, CV, or EIS [7, 9].

Despite the promising performance metrics reported for many electrochemical aptasensors for antibiotics, their behavior in real food matrices and their dependence on experimental conditions have not been fully clarified. Sensor response is governed by numerous interrelated parameters, including aptamer surface coverage and orientation, immobilization conditions, incubation times for both aptamer and analyte, and the physicochemical properties of the supporting electrolyte [10]. Additional factors such as potential window, scan rate, and temperature further shape the quality and stability of the electrochemical signal, while matrix components in hot dog samples proteins, lipids, and other interferents add significant complexity. Systematic optimization of key fabrication parameters, including $HAuCl_4$ concentration, gold electrodeposition time, and electrolyte temperature, is essential for maximizing the electrochemical signal and ensuring reliable performance in complex food matrices. In this work, we undertake such an optimization by constructing a carbon nanofiber-based, AuNP-modified aptasensor for AMP and rigorously evaluating the effect of these parameters on the voltammetric response in homogenized hot dog samples.

## EXPERIMENTAL

### *Materials*

The polyacrylonitrile (PAN) used as the nanofiber precursor was supplied by Polyacryl Company (Iran) and had a molecular weight of 150,000 g/mol. Dimethylformamide (DMF), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), and hydrogen tetrachloroaurate ($HAuCl_4$) were obtained from Merck (Germany). Ampicillin (98% purity), bovine serum albumin (BSA), potassium phosphate monobasic ($KH_2PO_4$), sodium phosphate dibasic ($Na_2HPO_4$), and sodium chloride (NaCl) were purchased from Sigma-Aldrich. Phosphate buffer solutions (PBS) were prepared by dissolving 0.1 M $KH_2PO_4$ and 0.1 M $Na_2HPO_4$ in ultrapure water. All chemicals and reagents were used as received, without additional purification. The single-stranded DNA (ssDNA) aptamer sequence used in this work was 5'-thiol-$(CH_2)_6$-TTAGTTGGGGTTCAGTTGG-3'.

### *Electrode modification*

The aptamer/AuNPs/ECNF sensing electrode was prepared through a sequential fabrication process that integrated ECNF production, gold nanoparticle deposition, and aptamer functionalization into a single workflow. Initially, carbon nanofibers were obtained from a polyacrylonitrile (PAN) precursor. PAN (1.1 g) was dissolved in dimethylformamide (DMF, 8.9 mL) and magnetically stirred at 1000 rpm for 9 h at 45 °C to yield a clear, homogeneous solution. This solution was subsequently subjected to electrospinning using an Electroris setup (Fanavaran Nanomeghyas Ltd., Co., Iran). Under ambient conditions, the polymer solution was

delivered through an 18-gauge needle while a high voltage of 20 kV was applied between the spinneret and the collector, with a 10 cm tip-to-collector distance and a collector rotation speed of 100 rpm, resulting in uniform PAN nanofiber mats.

The electrospun PAN nanofibers were then converted into conductive ECNFs by thermal treatment. Stabilization was carried out in air at 290 °C for 4 h in a tube furnace, followed by carbonization at 1000 °C for 1 h under nitrogen atmosphere. The temperature was increased at controlled rates of 1.5 °C/min during stabilization and 4 °C/min during carbonization to ensure gradual structural transformation. After heat treatment, the ECNF mats were cut into circular pieces with a diameter of 5 mm and connected to a copper wire to provide a reliable electrical contact, forming the ECNF working electrodes.

To introduce gold nanostructures, the ECNF electrodes were subjected to potentiostatic electrodeposition of AuNPs at -400 mV for different times, generating a nanoscale gold coating that enhanced the electroactive surface and provided anchoring sites for biomolecular immobilization.

To prepare the aptamer/AuNPs/ECNF electrode, an adequate volume of aptamer solution (10 µM) was cast onto the AuNPs/ECNF surface. After immobilization, the electrode was rinsed with ultrapure water to remove unbound aptamers. Subsequently, 5 µL of phosphate-buffered saline (PBS) containing 10% bovine serum albumin (BSA) was deposited onto the electrode and incubated at ambient temperature to block residual binding sites. The electrode was then rinsed again with ultrapure water and dried, ready for further analytical measurements.

*Hot dog sample preparation*

A commercially available hot dog was obtained from a local outlet and homogenized thoroughly using a household blender. The resulting paste was diluted with PBS to yield a 20% (w/v) hot dog suspension. For analytical measurements, aliquots of this 20% suspension were spiked with AMP by adding 10 µL of the AMP solution.

*Electrochemical characterization*

The electrochemical behavior of the modified electrodes was evaluated by cyclic voltammetry (CV) in a solution containing 5 mM ferricyanide/ferrocyanide ($[Fe(CN)_6]^{3-/4-}$) and 0.1 M PBS (pH 7.4). The potential was scanned from -300 mV to 700 mV at a sweep rate of 50 mV $s^{-1}$. These measurements were used to probe the electron-transfer characteristics of the electrode, the extent of surface modification, and the interaction between the immobilized aptamer and its target, as described in previous studies.

## RESULTS AND DISCUSSION

In this study, we systematically examined how key experimental parameters, including hydrogen tetrachloroaurate ($HAuCl_4$) concentration, optimization of gold electrodeposition time, and electrolyte temperature affect the electrochemical current response.

*Hydrogen tetrachloroaurate concentration*

The amount of gold nanoparticles electrochemically deposited on the ECNF mat markedly influences the CV peak current response. Among the factors controlling AuNP loading, the concentration of $HAuCl_4$ in the electrolyte plays a critical role. The effect of $HAuCl_4$ concentration on the peak current of the AuNPs/ECNF mat electrode is presented in Fig. 1. As the $HAuCl_4$ concentration in the electrolyte was increased, the peak current initially rose from about 23 µA to 35 µA and subsequently decreased to approximately 31 µA. The initial increase can be attributed to facilitated electron transfer, resulting from the higher density of electrodeposited AuNPs on the ECNF mat when the $HAuCl_4$ concentration was raised to 10 mM. Beyond this concentration, further addition of $HAuCl_4$ produced a thicker AuNP layer, which adversely affected the electrical conductivity of the electrode [11, 12].

*Gold electrodeposition time on the ECNF*

The effect of gold electrodeposition time on the electrochemical response of the ECNF mat was investigated in the range of 15–75 s. As shown in Fig. 2, the peak current increased markedly when the deposition time was extended from 15 to 45 s, reaching a maximum at 45 s. This enhancement suggests that progressive growth of the gold layer within this interval improves the effective electroactive surface area and facilitates electron transfer, thereby promoting a more efficient interaction between the transducing surface and the redox probe.

When the deposition time was further increased beyond 45 s, a gradual decline in the current response was observed. This behavior can

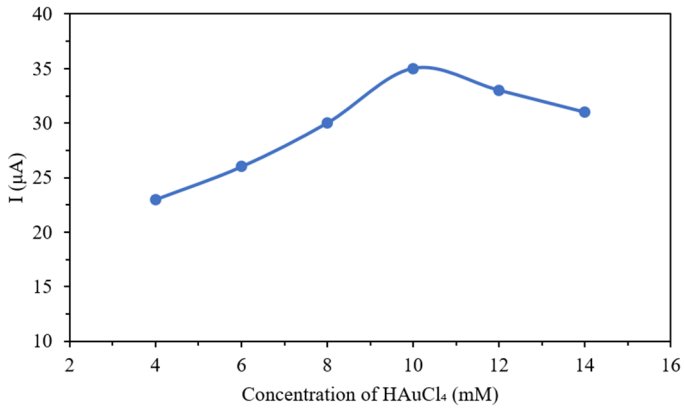


**Fig. 1.** Influence of $HAuCl_4$ concentration on the peak current of the AuNPs/ECNF electrode.

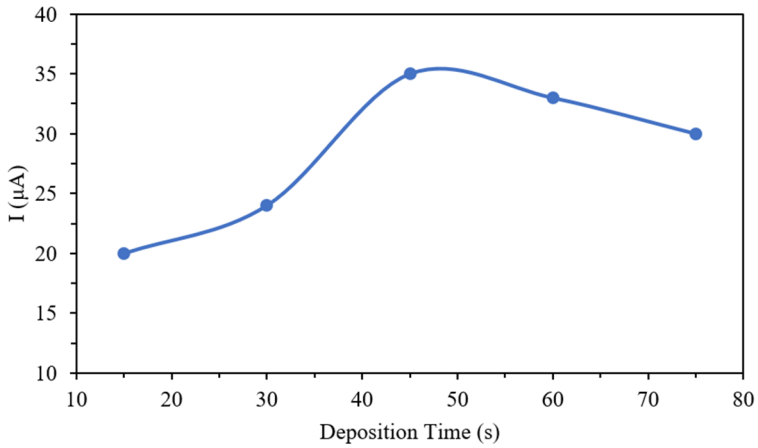


**Fig. 2.** Effect of gold electrodeposition time on the peak current of the AuNPs/ECNF electrode.

be attributed to excessive gold growth, which may lead to surface roughening, partial blockage of conductive sites, or the formation of a denser layer that hinders mass transport of electroactive species toward the electrode. Consequently, the balance between increased surface area and optimal morphology is disrupted at longer deposition times.

Overall, these results indicate that a deposition time of 45 s provides the most favorable gold coating for electrochemical signal generation, ensuring an adequate surface area while preserving suitable film architecture. Therefore, 45 s was selected as the optimal electrodeposition time for subsequent sensor fabrication and analytical measurements [13, 14].

*Influence of Temperature on Aptasensor Performance*

The analytical response of the aptasensor is strongly influenced by the temperature of the electrolyte solution. As shown in Fig. 3, the peak current of the electrode decreased progressively with increasing temperature from 20 to 30 °C, reaching a minimum at 30 °C. Beyond this temperature, a gradual increase in the peak current was observed.

The decrease in peak current can be attributed to the enhanced interaction between ampicillin (AMP) and the immobilized aptamer at moderately elevated temperatures. The binding of AMP to the aptamer recognition sequence can hinder electron transfer at the electrode/electrolyte interface, resulting in a decrease in the measured peak current. Thus, the decrease in current up to 30 °C indicates a more favorable AMP–aptamer binding interaction and a stronger signal-off response of the aptasensor.

When the temperature was increased above

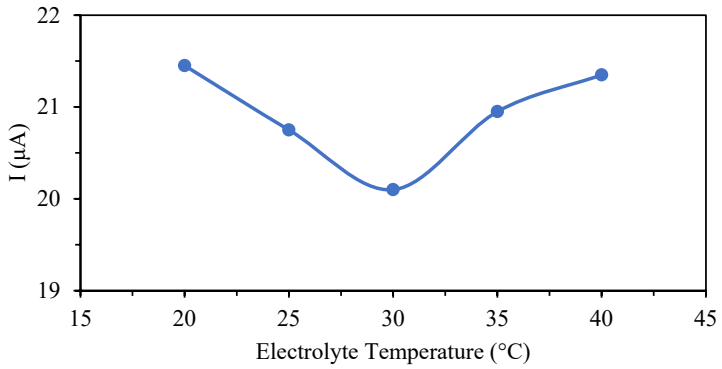


**Fig. 3.** Effect of the electrolyte temperature on the peak currents.

30 °C, the peak current gradually increased. This behavior may be associated with thermally induced changes in the conformation and stability of the immobilized aptamer. Excessive thermal energy may disturb the structural conformation required for efficient AMP recognition, thereby weakening the aptamer–AMP interaction. Consequently, a lower fraction of AMP may remain effectively bound to the aptamer, leading to partial recovery of the electrochemical signal and an increase in the peak current.

Overall, the temperature-dependent response exhibits a clear non-monotonic behavior, with the minimum peak current observed at 30 °C. This result suggests that 30 °C provides the most favorable conditions for AMP recognition by the immobilized aptamer and consequently produces the strongest signal-off response under the experimental conditions. Therefore, 30 °C was selected as the optimum operating temperature for subsequent experiments [11, 15-17].

## CONCLUSION

In this study, an aptamer-based electrochemical sensor was developed and systematically optimized for the rapid detection of ampicillin (AMP) residues in hot dog samples. The sensing platform was based on an electrospun carbon nanofiber electrode modified with electrodeposited gold nanoparticles (AuNPs), which increased the electroactive surface area and provided suitable sites for the immobilization of the thiolated ssDNA aptamer. Systematic investigation of the effects of $HAuCl_4$ concentration, gold electrodeposition time, and electrolyte temperature on the voltammetric response identified 10 mM $HAuCl_4$, a gold electrodeposition time of 45 s, and an operating temperature of 30 °C as the optimal conditions. Under these conditions, the aptasensor exhibited its strongest signal-off response toward AMP, with 30 °C providing the most favorable conditions for AMP–aptamer recognition, while the optimized gold electrodeposition conditions contributed to favorable electron-transfer characteristics.

The optimized aptasensor demonstrated a pronounced electrochemical response to AMP in a 20% (w/v) hot dog matrix, highlighting the importance of precise control over electrode surface modification and measurement conditions for reliable sensing in complex, protein- and fat-rich food matrices. The simplicity of electrode fabrication, straightforward sample preparation, and low-cost electrochemical readout further demonstrate the practical potential of the proposed platform for rapid screening applications. Overall, these findings highlight the importance of systematic parameter optimization in the development of electrochemical aptasensors and demonstrate the potential of the proposed sensor as a rapid and portable platform for monitoring β-lactam antibiotic residues in processed meat products and supporting food safety monitoring.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest related to the research, authorship, or publication of this manuscript. All authors have disclosed any financial or personal relationships that could potentially influence or bias the work presented.

## USE OF ARTIFICIAL INTELLIGENCE (AI) IN MANUSCRIPT PREPARATION

During the preparation of this manuscript,

the authors used artificial intelligence (AI) solely to improve the English language, grammar, and readability of the manuscript. All AI-generated suggestions were carefully reviewed, verified, and edited by the authors, who take full responsibility for the content and conclusions of this manuscript.

## REFERENCES


1. Arsène MMJ, Davares AKL, Podoprigora IV, Smolyakova LA, Souadkia S, Khelifi I, et al. The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. Vet World. 2022;15(3):662-671. https://doi.org/10.14202/vetworld.2022.662-671
2. Ghimpețeanu OM, Pogurschi EN, Popa DC, Dragomir N, Drăgotoiu T, Mihai OD, et al. Antibiotic use in livestock and residues in food-A public health threat: A review. Foods. 2022;11(10):1430. https://doi.org/10.3390/foods11101430
3. Sierra D, Sánchez A, Contreras A, Luengo C, Corrales JC, Morales CT, et al. Detection limits of four antimicrobial residue screening tests for β-lactams in goat's milk. J Dairy Sci. 2009;92(8):3585-3591. https://doi.org/10.3168/jds.2008-1981
4. Beltrán MC, Romero T, Althaus RL, Molina MP. Evaluation of the Charm maximum residue limit β-lactam and tetracycline test for the detection of antibiotics in ewe and goat milk. J Dairy Sci. 2013;96(5):2737-2745. https://doi.org/10.3168/jds.2012-6044
5. Niknam S, Dehdast SA, Pourdakan O, Shabani M, Koohi MK. Tungsten disulfide nanomaterials (WS2 NM) application in biosensors and nanomedicine: A review. Nanomed Res J. 2022;7(3):214-226. https://doi.org/10.22034/nmrj.2022.03.001
6. Heshteli RR, Paimard G, Adabi M, Esmaeili S. Advances in biosensors: A breakthrough in rapid and precise brucellosis detection. Anal Biochem. 2025;700:115782. https://doi.org/10.1016/j.ab.2025.115782
7. Niknam S, Shabani-Nooshabadi M, Adabi M. Nanoarchitectonics of aptamer-based electrochemical sensor using electrospun carbon nanofibers and Au nanoparticles for Cd(II) analysis. Sci Rep. 2026;16:9271. https://doi.org/10.1038/s41598-026-39085-3
8. Banan K, Niknam S, Ahmadi M, Tabasi S, Ghalkhani M, Adabi M, et al. Molecularly imprinted electrochemical sensor based on carbon nanofibers for Amiodarone determination. Microchem J. 2024;200:110365. https://doi.org/10.1016/j.microc.2024.110365
9. Adabi M, Rajabi Z, Adabi M. Nanomaterial-enhanced electrochemical immunosensor for sensitive detection of Staphylococcus aureus in milk. Microchem J. 2025;218:115189. https://doi.org/10.1016/j.microc.2025.115189
10. Bao W, Aodeng G, Ga L, Ai J. Aptamer-based electrochemical biosensors: Signal transduction mechanisms, application progress, and future trends. Sens Actuators Rep. 2025;10:100366. https://doi.org/10.1016/j.snr.2025.100366
11. Ebrahimi Vafaye SE, Rahman A, Safaeian S, Adabi M. Investigation of effective parameters on electrochemical aptasensor for detection of Penicillin antibiotic. Nanomed Res J. 2021;6(1):73-78. https://doi.org/10.22034/nmrj.2021.01.008
12. Behzad F, Samadi A, Rahimpour E, Adabi M, Jouyban A. Recent advances in gold nanoparticle-based colorimetric aptasensors for chemical and biological analyses. Bioanalysis. 2022;14(23):1509-1524. https://doi.org/10.4155/bio-2022-0209
13. Ariasena E, Wicaksono WP, Anshori I, et al. Evaluation of electrodeposition synthesis of gold nanodendrite on screen-printed carbon electrode for nonenzymatic ascorbic acid sensor. Sci Rep. 2024;14:22854. https://doi.org/10.1038/s41598-024-69970-8
14. Anshori I, Althof RR, Rizalputri LN, Ariasena E, Handayani M, Pradana A, et al. Gold nanospikes formation on screen-printed carbon electrode through electrodeposition method for non-enzymatic electrochemical sensor. Metals (Basel). 2022;12(12):2116. https://doi.org/10.3390/met12122116
15. Naghshbandi B, Adabi M, Pooshang Bagheri K, Tavakolipour H. Design of a new electrochemical aptasensor based on screen printed carbon electrode modified with gold nanoparticles for the detection of fumonisin B1 in maize flour. J Nanobiotechnology. 2022;20:534. https://doi.org/10.1186/s12951-022-01745-7
16. Mohammadi S, Mousavi Nadushan R, Pooshang Bagheri K. Investigation of effective parameters on aptamer-based electrochemical sensor for rapid detection of tetracycline residues in chicken ham. Nanomed Res J. 2024;9(4):432-436. https://doi.org/10.22034/nmrj.2024.04.010
17. Rahmani HR, Adabi M, Khanverdi Z, et al. Effect of processing variables on the performance of electrochemical aptasensor for determination of aflatoxin M1. Nanomed Res J. 2020;5(4):378-382. https://doi.org/10.22034/nmrj.2020.04.010