# Fabrication and Characterization of a Bimetallic Nanomaterial Modified Aptasensor for Organophosphorus Pesticide Sensing

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Organophosphorus pesticide residues pose a considerable threat to both environmental and public health. Conventional analytical methods, while effective, are often hindered by high operational costs, extended analysis durations, and the need for sophisticated instrumentation [1]. Aptamers, synthetic single-stranded DNA or RNA oligonucleotides, have emerged as promising recognition elements due to their high affinity and specificity toward target analytes [2]. In this study, an aptasensor was designed for the selective and sensitive detection of Diazinon, a representative organophosphorus pesticide. The sensor was constructed by immobilizing a thiol-modified aptamer onto a screen-printed carbon electrode (SPCE) modified with gold–copper (Au@Cu) bimetallic nanomaterials. This surface modification enhanced the sensor's sensitivity, selectivity, and stability by improving both the electroactive surface area and electron transfer capabilities. Various Au:Cu molar ratios (1:1, 1:2, 2:1, 1:4, 4:1; M:M) were investigated to optimize the bimetallic composition. Optimal drop-casting volumes and concentrations were determined using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). After modification with Au@Cu bimetallic nanomaterials, a 1.20-fold increase in the anodic peak current was observed, attributed to the enhanced surface area of the nanomaterial. A 5 µM thiolated aptamer, prepared from a 100 µM stock in TE buffer (pH 8.0), was applied to the modified electrode surface and incubated at +4°C for 3 hours. Following immobilization, the electrode was rinsed with ultrapure water, blocked with 1% BSA for 30 minutes to prevent nonspecific interactions, and then washed with Tris-HCl buffer. The influence of aptamer immobilization time (1–18 hours) on electrochemical performance was evaluated using CV, differential pulse voltammetry (DPV), and EIS. A decrease in current was observed after aptamer immobilization, which is attributed to the intrinsic electron-transfer blocking effect of the aptamer molecules. EIS measurements conducted using the $[Fe(CN)_6]^{3-/4-}$ redox couple yielded charge transfer resistance ($R_{ct}$) values via equivalent circuit modelling. The binding site saturation was achieved within 3 hours, which was thus selected as the optimal immobilization time. To determine optimal aptamer concentration, responses across 0.5–10 µM were evaluated, with 5 µM yielding the best performance. Similarly, the aptamer–Diazinon interaction time was optimized by testing durations from 15 to 120 minutes, identifying 30 minutes as optimal. Increasing Diazinon concentrations were applied to the modified electrodes, followed by 30-minute incubation. The aptasensor's performance was then evaluated electrochemically. A linear calibration curve was obtained over the concentration range of 32.8 pM to 16.4 nM. The calculated limit of detection (LOD) and limit of quantification (LOQ) were 10.8 nM and 32.8 nM, respectively, confirming the sensor's high sensitivity. Selectivity studies demonstrated that the sensor selectively recognized Diazinon, with no significant interference from structurally similar pesticides. Furthermore, stability assessments revealed that the sensor retained its activity for at least 15 days, confirming its potential for practical use in long-term monitoring applications.

**References**

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