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# Detection of *Aeromonas hydrophila* DNA oligonucleotide sequence using a biosensor design based on Ceria nanoparticles decorated reduced graphene oxide and Fast Fourier transform square wave voltammetry

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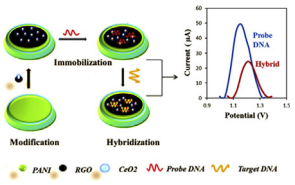
## HIGHLIGHTS

- New DNA biosensor is designed for sub-femtomolar detection of *Aeromonas hydrophila* DNA sequence.
- Reduced graphene oxide decorated Ceria nanoparticles was used as a new immobilization platform.
- Biosensor was successfully used to detect *A. hydrophila* DNA sequence in fish pond water.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A new strategy was introduced for ssDNA immobilization on a modified glassy carbon electrode. The electrode surface was modified using polyaniline and chemically reduced graphene oxide decorated cerium oxide nanoparticles ($CeO_2$NPs-RGO). A single-stranded DNA (ssDNA) probe was immobilized on the modified electrode surface. Fast Fourier transform square wave voltammetry (FFT-SWV) was applied as detection technique and $[Ru(bpy)_3]^{2+/3+}$ redox signal was used as electrochemical marker. The hybridization of ssDNA with its complementary target caused a dramatic decrease in $[Ru(bpy)_3]^{2+/3+}$ FFT-SW signal. The proposed electrochemical biosensor was able to detect *Aeromonas hydrophila* DNA oligonucleotide sequence encoding aerolysin protein. Under optimal conditions, the biosensor showed excellent selectivity toward complementary sequence in comparison with noncomplementary and two-base mismatch sequences. The dynamic linear range of this electrochemical DNA biosensor for detecting 20-mer oligonucleotide sequence *of A. hydrophila* was from $1 \times 10^{-15}$ to $1 \times 10^{-8}$ mol $L^{-1}$. The proposed biosensor was successfully applied for the detection of DNA extracted from *A. hydrophila* in fish pond water up to 0.01 μg $mL^{-1}$ with RSD of 5%. Besides, molecular docking was applied to consider the $[Ru(bpy)_3]^{2+/3+}$ interaction with ssDNA before and after hybridization.



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## 1. Introduction

Recently, the research area of DNA hybridization biosensors has found an important place in the diagnosis of genetic and infectious diseases, genotyping, bacterial and viral analysis and food safety monitoring [1]. Generally, a DNA biosensor comprises a single-stranded oligonucleotides (ssDNA) immobilized on a transducer surface, which can then recognize its complementary sequence via hybridization event [2]. Many selective and sensitive detection techniques, based on different signal transduction, including optical, piezoelectric and electrochemical read outs have been developed [3]. Among them, electrochemical methods attract more attentions for use in point-of-care clinical diagnostic devices due to their fast, simple, sensitive, low cost detection and ease of miniaturization [4,5].

The configuration of the recognition layer is a critical step in designing a DNA biosensor [6,7]. One of the important approaches for immobilization of DNA sequence on the electrode surface (especially gold or gold nanoparticles modified electrodes) is using thiolated ssDNA. However, this process is expensive and labor consuming. Biocompatible nanomaterial is largely used to modify the electrode surfaces in order to obtain high hybridization efficiency and decrease non-specific adsorption of ssDNA [8,9]. The proposed nanocomposite in this study can be a new and easy approach for immobilization of ssDNA on the electrode surface without using any functionalization or mediators.

Polyaniline (PANI) is a highly conductive polymer with good environmental stability and biocompatibility. Its application in DNA biosensor design is becoming increasingly popular since it can provide a large surface area and excellent electrical conductivity, which can facilitate the signal transduction. Moreover PANI can interact with graphene sheets through Π-Π stacking [10,11]. Graphene is single-atom sheets of $sp^2$ Carbons which has an extremely large surface area, excellent thermal and electrical conductivity [12,13]. It has been used for immobilizing of DNA probe via non-covalent interaction (Π-Π stacking) between its conjugated interface and DNA bases [14,15]. Further modification strategies were using graphene-metal nano-composites [16,17]. Among metal nanoparticles $CeO_2$ (called Ceria) is particularly attractive because of its high catalytic activity and biocompatibility [18,19]. Also, it has been found that $CeO_2$ can adsorb DNA through an electrostatic attraction, specifically binds with DNA backbone phosphate groups [20].

Square wave voltammetry (SWV) is a sensitive electrochemical technique that has been extensively used as detection technique in designing biosensors [21–23]. In SWV measurements, the detection limits can be drastically improved. SWV measures the current response while rapid alternating potentials are applied during a staircase scan. Moreover, Fast Fourier transform (FFT) in combination with SWV technique has shown excellent sensitivity [24,25]. The approach is designed to separate the voltammetric signal and background signal in frequency domain using discrete Fast Fourier transformation (FFT) method. Therefore, some of the environmental noises was digitally filtrated that could improve the detection limit [24–26].

The ssDNA used in this work is a 20-mer oligonucleotides sequence that has been previously verified and reported to have high selectivity and specificity to *Aeromonas hydrophila* aerolysin gene [27]. *A. hydrophila* is a highly epidemic pathogen, mostly distributed in aquatic environments and food. This opportunistic pathogen is a gram-negative, rod-shaped bacterium that is classified as an emerging pathogen [28]. The pathogenicity of *A. hydrophila* was believed to be mediated mainly by an extracellular protein, aerolysin, which its encoding gene is used for identification of this bacterium by polymerase chain reaction (PCR) [29,30]. The microgravimetric (QCM, quartz crystal microbalance) biosensor coupled with PCR was used for detection of aerolysin gene fragments [31]. Although these methods provide efficient detection of *A. hydrophila*, they were time consuming, complicated and does not have low detection limit in practice. Recently, electrochemical DNA biosensors have been also used to identify aerolysin gene (with various sequences). In all of them labeled ssDNA were used which has a difficult and expensive biochemical preparation [27,32,33]. In this study, we used a new methodology for immobilization of ssDNA sequence on glassy carbon electrode. Reduced graphene oxide decorated cerium oxide nanocomposite/Polyaniline/glassy carbon electrode [$CeO_2$NPs-RGO/PANI/GCE] was fabricated as a platform for immobilization of ssDNA sequence. By employing this method, the interaction of captured ssDNA on the electrode surface got much stronger. The hybridization process was monitored by the FFT-SW redox current signal of $[Ru(bpy)_3]^{2+/3+}$. Under optimal experimental condition, the proposed DNA biosensor showed high sensitivity and selectivity toward the detection of a 20-mer oligonucleotides sequence related to *A. hydrophila.* Besides the experimental studies, molecular docking was applied to explain the interaction of $[Ru(bpy)_3]^{2+/3+}$ with ssDNA before and after hybridization.

## 2. Materials and methods

### 2.1. Apparatus

Voltammetric measurements (CV and FFT-SW) were performed using a homemade potentiostat connected with a three-electrode system consists of a glassy carbon electrode (GCE) modified, an Ag/AgCl reference electrode and a platinum wire as an auxiliary electrode. The potentiostat was connected to a PC equipped via a data acquisition board (PCL-818H, Advantech Co.), used to control the potential of potentiostat and acquire current readings. Software was developed using Delphi 6.0 to apply repeatedly a waveform to the working electrode and synchronously acquire, analyze, and store the current data. The controlling program was accompanying dynamic link libraries allowed waveform generation and current sampling to be synchronized, which was essential in interpreting SWV current response [24–26]. Electrochemical impedance measurements were carried out on AUTOLAB PGSTAT 30. Phase compositions of the samples were characterized by X-ray diffraction (XRD) on a Philips PW-1730 X-ray diffractometer using Cu Kα radiation (λ = 1.5405 Å). Scanning electron microscopy (SEM) measurement was carried out on a Zeiss SIGMA VP scanning electron microscope. The pH values of all solutions were measured by a model SAT-2100 pH meter (Sabalan Azmai Tehran Co., Ltd, Iran).

### 2.2. Materials & reagents

All the chemical reagents were of analytical grade and all solution were prepared using deionized water. All oligonucleotides were purchased from Generay Biotech CO., Ltd. (Shanghai, China). The DNA stock solutions were prepared with TE buffer (10 mmol $L^{-1}$ Tris–HCl, 1 mmol $L^{-1}$, EDTA, pH 8.00) and kept frozen. More dilute solutions were prepared with 10 mmol $L^{-1}$ Tris–HCl buffer containing 10 mmol $L^{-1}$ NaCl pH 7.40. ssDNA probe: 5′- GTGGTGGGCT GGGCGATCAA-3′

Target DNA (fragments of *A. hydrophila* aerolysin previously reported) [27]:

5′-TTGATCGCCCAGCCCACCAC -3′

Mismatch (MM): 5′- TTGATCGCTCAGCACACCAC -3′

Non-complementary DNA:

5′- GCATGACGTTATTTATGAGAT-3′