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A DNA biosensor for molecular diagnosis of *Aeromonas hydrophila* using zinc sulfide nanospheres

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Abstract. Today, identification of pathogenic bacteria using modern and accurate methods is inevitable. Integration in electrochemical measurements with nanotechnology has led to the design of efficient and sensitive DNA biosensors against bacterial agents. Here, efforts were made to detect *Aeromonas hydrophila* using aptamers as probes and zinc sulfide (ZnS) nanospheres as signal enhancers and electron transfer facilitators. After modification of the working electrode area (in a screen-printed electrode) with ZnS nanospheres through electrodeposition, the coated surface of a modified electrode with ZnS nanospheres was investigated through scanning electron microscopy (SEM). The size of synthesized ZnS nanospheres was estimated at about 20–50 nm and their shape was in the form of porous plates in microscopic observations. All electrochemical measurements were performed using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and constant potential amperometry (CPA) techniques. The designed DNA biosensor was able to detect deoxyribonucleic acid (DNA) of *Aeromonas hydrophila* in the range 1.0×10^{-4} to 1.0×10^{-9} mol L^{-1}; the limit of detection (LOD) in this study was 1×10^{-13} mol L^{-1}. This DNA biosensor showed satisfactory thermal and pH stability. Reproducibility for this DNA biosensor was measured and the relative standard deviation (RSD) of the performance of this DNA biosensor was calculated as 5 % during 42 days.

1 Introduction

Bacterial infections cause heavy losses on fish farms in the aquaculture industry (Austin and Austin, 2007; Gauthier, 2015; Shoemaker et al., 2001; Sommerset et al., 2014). Among the factors causing these losses, *Aeromonas*, particularly *Aeromonas hydrophila*, is highly regarded (Harikrishnan et al., 2015; Huang et al., 2015; Liu et al., 2013; Ponnusamy et al., 2016). This bacterium is facultative anaerobes, gram-negative, oxidase, and catalase positive (Cumberbatch et al., 1979; Popoff and Véron, 1976). *Aeromonas hydrophila* is ubiquitous and opportunistic in that it is one of the important bacteria in the warm-water fish culture industry, and sometimes it causes disease in saltwater fishes (Cipriano et al., 1984; Viswanatan et al., 2015). *Aeromonas hydrophila* is transmitted to fish through contaminated water or infected animals, and this bacterium may also cause some human diseases such as gastroenteritis and diarrhoea (Blake et al., 1980; Daskalov, 2006; Ljungh et al., 1977). Many different methods have been used for detection and determination of pathogenic bacteria, including solid and aqueous culture media (Kiyohara et al., 1982; Xie et al., 2005), gram stain (Nugent et al., 1991), biochemical studies (coagulase, oxidase, and catalase) (Hjelm et al., 2004; Raus and Love, 1983; Sumner and Taylor, 1989), impedance measurement (Suehiro et al., 2003), flow cytometry (Gunasekera et al., 2000), adenosine triphosphate (ATP) assessment (Chen and Godwin, 2006), polymerase chain reaction (PCR) (Belgrader et al., 1999), enzyme-linked immunosorbent assay (ELISA), and other novel techniques (Mansfield and

Forsythe, 2000; Ruzicka et al., 2016). All conventional methods have at least one of these disadvantages: low detection accuracy, long time of detection, and the high detection cost (de Boer and Beumer, 1999; Gunasekera et al., 2000; Jorgensen and Turnidge, 2015; Megraud, 1996). Today, finding rapid and sensitive diagnostic techniques against pathogenic agents is very important. Since most of the time bacterial pollution is in low concentrations, and because of the worldwide prevalence of *Aeromonas hydrophila*, finding rapid and accurate diagnosis ways can prevent its prevalence; this matter is very important for world health (Ghali et al., 2016; Sebastian et al., 2016; Templier et al., 2016). Biosensors are analytical tools that can intelligently use biological materials to detect biochemical compound(s) and react to them (Ozsoz, 2012; Zhang et al., 2011); aptamers are used in conventional and optimized aptasensors based on their high affinity with a target and their specific function (Cosnier, 2015; Mascini, 2009; O'Sullivan, 2002; Radi et al., 2005). So far, several studies have been reported about the use of aptasensors to detect bacteria (Bagheryan et al., 2016; Hamidi-Asl et al., 2016; Kim et al., 2017; Li et al., 2016; Templier et al., 2016). Current research has shown new promising results about detection of bacterial pathogens at the gene level (Liébana et al., 2016; Lopez et al., 2016; Palchetti, 2016); accurate and rapid diagnostic methods against these pathogens are established through specific hybridization between aptamers and the single-stranded DNA (ssDNA) of bacteria (Jacobs and Bonham, 2016; Shachar et al., 2016; Sheikhzadeh et al., 2016). These biosensors that are capable of detecting the genes through hybridization of two strands of DNA are defined as a DNA biosensor (Beattie et al., 1995). A group of nanobiosensors can be produced by designing an interaction pathway between biological molecules and transducers; these nanobiosensors can be widely used for detection of biomolecules such as genes (Chiu and Huang, 2009; Pumera et al., 2007); producing these nanobiosensors via the construction of new nanomaterials and progressing their technological modifications is considered seriously in biosensing research (Chiu and Huang, 2009). Today, nanotechnology has affected various aspects of human life, and its applications domain is enhanced with the development of research in various fields (Brumfiel, 2003; Emerich and Thanos, 2003; Sahoo et al., 2007). Combining nanoparticles and biological components in a biosensor has been proposed as a powerful tool for pathogen detection (Deisingh and Thompson, 2004; Palchetti and Mascini, 2008; Zourob et al., 2008). Biosensors that use nanomaterials in their structure have a high efficiency and sensitivity due to the wide area of the immobilization surface in their bioreceptor. Thin layers of a ZnS semiconductor include one of the combinations from group II–VI semiconductors (Nanda et al., 2000). So far, ZnS nanoparticles have been used in several biomedical purposes (Malarkodi and Annadurai, 2013; Pawaskar et al., 2002; Wang and Hong, 2000). In this study a new molecular method has been introduced to detect *Aeromonas hydrophila* using electrochemical techniques in preparation of a sensitive and accurate DNA biosensor. Using molecular science and aptamers, detecting the DNA of *Aeromonas hydrophila* and accelerating the process of detection, using ZnS nanospheres, and designing the presented DNA biosensor were our important aims.

2 Experiments

2.1 Reagents and materials

Tris (hydroxymethyl) aminomethane (99 %), 6-Mercapto-1-hexanol (MCH, 97 %), dl-dithiothreitol (DTT) (1N), NaCl, $MgCl_2$, KCl, NaOH, KOH, HCl, ethylacetate solution, ethanol solution, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene glycol (PEG), zinc acetate dehydrate, sodium sulfide, sodium hydroxide, methylene blue (MB), blood agar (base), and deionized water (capacity 750 mL) were purchased from Sigma-Aldrich (USA). Synthetic oligonucleotides for *Aeromonas hydrophila* were purchased from the Bioneer Corporation (South Korea) as below:

- probe ssDNA (pssDNA): sequence 5'- thiolated -GAT CCG GGC CTC ATG TCG TTCGAA-3';
- target ssDNA (ssDNA): sequence 5'-AAC CTG GTT CCG CTC AAG CCG TTG-3'.

All other used materials were provided through certified companies.

2.2 Apparatus and experiment procedures

Several screen-printed carbon electrodes (ref. DRP-C110) were used during all experiments with the information below:

- working electrode: carbon (4 mm diameter);
- auxiliary electrode: carbon;
- reference electrode: silver;

and a cable connector as an interface between electrodes and a potentiostat device that was provided by DropSens (Spain). All electrochemical studies were performed through a computer-controlled μ-Autolab electrochemical potentiostat device that was equipped with General Purpose Electrochemical System (GPES) and Frequency Response Analyzer (FRA2) software (version 4.7) (Eco Chemie Ultecht, the Netherlands). All electrochemical experiments were done in a 10 ml voltammetric cell containing a Tris-HCl buffer as the binding solution (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4). In this research, some electrochemical measurement techniques such as electrochemical impedance spectroscopy (EIS) and

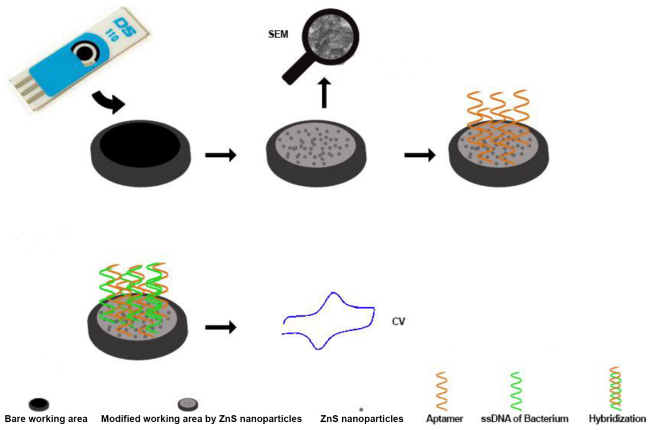

Figure 1. Schematic procedure to design and detect *Aeromonas hydrophila*; all actions were performed on a working area in a screen-printed electrode; the steps to design it were preparation of a bare electrode, modification with ZnS nanospheres, aptamer immobilization, hybridization for aptamers and *Aeromonas hydrophila* DNA and a product electrochemical redox signal respectively.

cyclic voltammetry (CV) were applied to detect related analytes. The size and morphology of coated ZnS nanospheres through electrodeposition (−0.6 V for 200 s) on the surface of the working area in a screen-printed electrode were investigated by SU3500 Premium VP-SEM scanning electron microscopes (Japan). All other used devices included a pH meter, refrigerator, digital scale, and DNA hybridization incubator that were prepared by valid companies. ZnS nanospheres were synthesized according to a previously used reported protocol by us (Dehghani et al., 2016); the summarized details for the used protocol are as below.

In this procedure, ZnS nanospheres were synthesized through a co-precipitation technique using PVP, PVA, and PEG (Bandaranayake et al., 1995; Cheng et al., 2004; Griffith, 1961). In this technique, precipitation occurred in involved metal ions with sulfide ions within production solution. Firstly, zinc acetate dihydrate (0.1 M) and sodium sulfide (0.1 M) were mixed together through capping agent solutions including PVP, PVA, and PEG. Then, other production steps were done and the achieved solution was inserted at 80 °C for 4 h to obtain ZnS nanospheres in a powder state; the achieved solution in previous steps was used for electrodeposition via a potentiostat device (−0.6 V for 200 s). The colour change in the working surface area in the screen-printed electrode was seen after successful electrodeposition, and this change was from black to grey; this schematic process is shown in Fig. 1. A sequence 5'- thiolated oligonucleotide probe (sequence 5'- thiolated -GAT CCG GGC CTC ATG TCG TTCGAA-3') was used to increment the covalent attachment affinity in the hybridization and detection process against *Aeromonas hydrophila* target single-stranded DNA (ssDNA) (5'-AAC CTG GTT CCG CTC AAG CCG TTG-3'). The disulfide protecting group in

Table 1. Various statuses of used electrodes in electrochemical experiments.

Electrode type status	Existence of ZnS nanospheres	Existence of pssDNA	Existence of tssDNA
Type 1	+	+	−
Type 2	+	+	+
Type 3	−	+	+
Type 4	+	−	+
Type 5	−	−	+

+ existence; − non-existence.

pssDNA was removed and broken using 10 µL of dithiothreitol (DTT) 100 mM buffer solution at pH 5.0 dropped in the aptamer's stock for 30 min at 25 °C and then, in order to remove excess DTT, the extraction aptamer's vial four times (each time with 300 µL of ethyl acetate solution). Due to the preparation protocols, first 380 µL of deionized water were added to each lyophilized primer stock; the achieved concentrations were 200 nmol μL^{-1} (0.2 M) and from these main stocks all needed concentrations were prepared and diluted (aptamer preparation solution properties: Tris-HCl buffer (30 mM Tris-HCl, 200 mM NaCl; pH 8.4). The main aptamer stock vials were kept frozen (−20 °C) when not used. Single-stranded and paired aptamers were heated at 95 °C and then cooled at room temperature (25 °C) for 20 min to find them in dehybridization status (in paired status) and the best conformational structure (in single-stranded status); this condition was frequently repeated during some experiments. In one of the first procedure steps, the surface of the screen-printed electrode was rinsed with deionized water and dried with nitrogen. Then 5 µL of 20 mM of pssDNA were filled on the surface of the working area as a dropping. To have the best aptamer sequence immobilization time on the surface of a modified working area with ZnS nanospheres in a screen-printed electrode, the open circuit potential (OCP) technique was applied and the best aptamer sequence immobilization time calculated as 3 h. The screen-printed electrode was stored at 4 °C during the immobilization process; after this time period, the electrode was washed regularly with deionized water and 6-Mercapto-1-hexanol to remove all unbounded aptamers from the surface of it. The hybridization process between pssDNA and tssDNA was performed in a DNA hybridization incubator (37 °C, 45 min) for each prepared concentration separately. The schematic procedure for designing and detecting *Aeromonas hydrophila* is shown in Fig. 1.

Before each electrochemical measurement 10 µL of MB (50 mM) were used and dropped onto the surface of the working area as a redox marker in all electrochemical experiments (the time for binding between MB and aptamer was 10 min); MB has a high strong interaction with guanine bases in single-strand DNA. Every time that the pssDNA aptamer was hybridized with another tssDNA aptamer, the possibil-

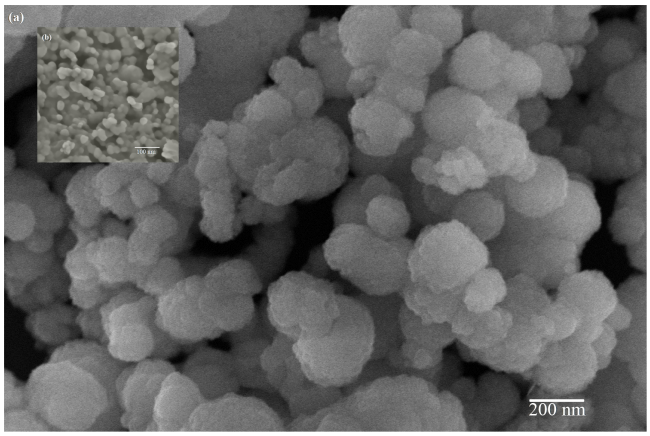

Figure 2. SEM images of synthesized ZnS Nps with different magnifications: **(a)** magnification was 40 000× and the scale bar was 200 nm; **(b)** magnification was 4000× and the scale bar was 100 nm.

ity of tagging and influencing MB molecules with the aptamer was decreased. This feature caused the creation of a shift in different peaks in various concentrations of the analyte. The maximum access state of MB and pssDNA aptamer molecules to each other was found in the absence of the tssDNA; the minimum access state of MB and pssDNA aptamer molecules to each other was found in the presence of the highest concentration of tssDNA; EIS and CV were performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4). Electrochemical experiments were performed in various statuses that are shown in Table 1; this test was performed to find the selectivity of the designed DNA biosensor.

Evaluation of real samples was performed using 10 salmon that suffered from *Aeromonas hydrophila*. Initially, biopsies of their kidneys and livers were performed; then, *Aeromonas hydrophila* was cultured and incubated for 24 h on blood agar at 22 °C for use in PCR. *Aeromonas hydrophila* DNA amplification was performed through the PCR technique via the Mastercycler nexus (Eppendorf, Germany). In the next step, *Aeromonas hydrophila* DNA extraction was performed using a PCR kit (MBS598131 – mybiosource-USA); to obtain the concentration of the extracted DNA, a UV-Vis spectrophotometer was used (Thermo Scientific NanoDrop, USA). Then, real samples (tssDNA) were hybridized with pssDNA and redox peaks were achieved to compare and validate the optimal performance of the designed electrochemical DNA biosensor. To find an optimized DNA biosensor, some other tests were also performed, such as regeneration, reproducibility, stability, interference effects, pH effect, and temperature effect.

3 Results and discussion

3.1 Scanning electron microscopy (SEM) investigation

Sample topography features, including surface properties, surface morphology of the sample (including shape, size, and located position of particles on the surface of materials), composition, and characteristics of the components, make it determined via SEM (Goldstein, 1992). In most recent studies on the properties of nanostructures, SEM was used to investigate the items including particle size, shape, structure, and surface crystallography (Goldstein et al., 2012). In this research, after modification of electrodes with ZnS nanospheres, the coated surface of electrodes was studied through SEM, and it is found that the particles size was about 20–50 nm. SEM images revealed that the shape of these particles was similar to porous plates with high roughness, and it was used as a nice feature for successful attachment of aptamers to the surfaces of electrodes. The specific morphology of ZnS nanospheres provided an increased surface for attachment of aptamers; in addition, high reactivity and expanded electron transfer capability were found on this surface based on special shapes of ZnS nanospheres. Figure 2 shows related images of ZnS nanospheres with different magnifications. In Fig. 2a, magnification was 40 000× and the scale bar was 200 nm, and in Fig. 2b, magnification was 4000× and the scale bar was 100 nm. At higher magnification it is shown that there is an appropriate surface for strong attachment of biological components such as aptamers.

3.2 Finding the best hybridization time for the *Aeromonas hydrophila* DNA biosensor

The prepared DNA biosensor was incubated 10 times consecutively (every 5 min) and during hybridization a constant concentration of tssDNA is used (1.0×10^{-6} mol L^{-1}). The results of this test showed that the best hybridization time for the *Aeromonas hydrophila* DNA biosensor occurred 45 min after first incubation and that the peak current was −110 µA at this time; the related calibration equation was $y = -2.0848x - 8.6667$, $R^2 = 0.9917$ (Fig. 3). This optimized time was used in all other hybridization experiments.

3.3 Main electrochemical measurements

The EIS technique is one of the useful electrochemical measurements that help to find the best probing features of surface-modified electrodes (Chang and Park, 2010; Orazem and Tribollet, 2011; Retter and Lohse, 2005). This technique also provides the investigation of the formation and quality of working electrode behaviour. In this research EIS was applied with a frequency of 0.05–100 kHz in various electrode types ($n = 1$–4). A constant concentration of tssDNA was used (1.0×10^{-6} mol L^{-1}) in all electrodes to perform this test. The test was performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM

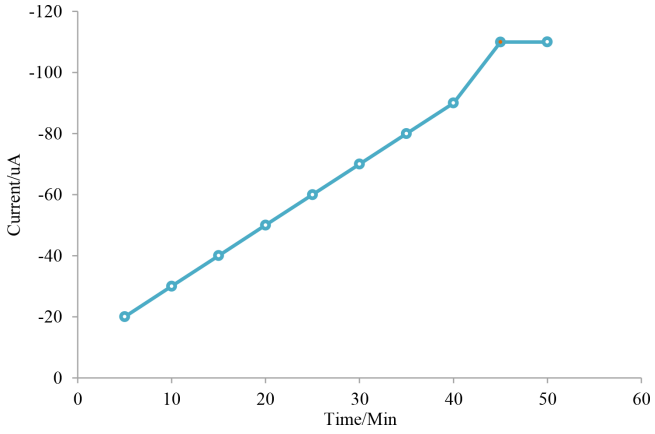

Figure 3. Best hybridization time for the *Aeromonas hydrophila* DNA biosensor in time range 5–50 min; the optimum was 45 min.

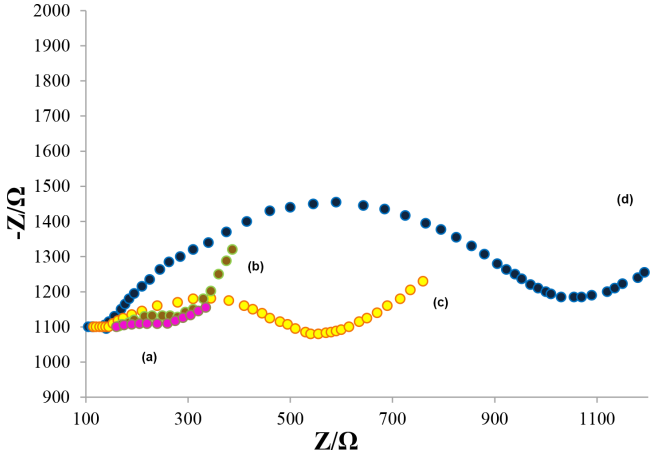

Figure 4. EIS; **(a)** electrode with tssDNA; **(b)** electrode with ZnS nanospheres and tssDNA; **(c)** electrode with pssDNA and tssDNA; **(d)** electrode with ZnS nanospheres, pssDNA, and tssDNA; electrolyte binding buffer (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4); the frequency for EIS was 0.05–100 kHz.

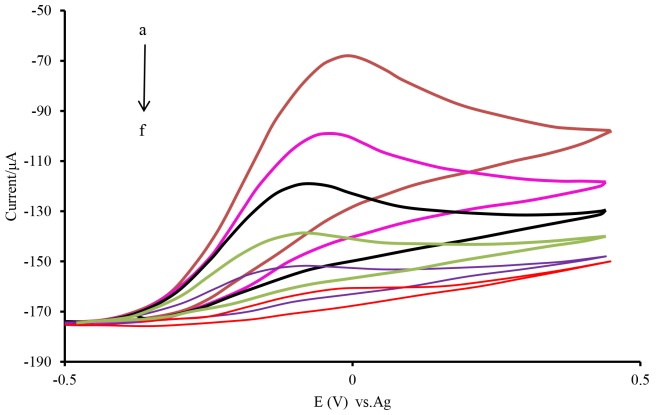

Figure 5. Cyclic voltammograms of hybridized pssDNA with various concentrations of *Aeromonas hydrophila* tssDNA (1.0×10^{-4} mol L^{-1} (curve a), 1.0×10^{-5} mol L^{-1} (curve b), 1.0×10^{-6} mol L^{-1} (curve c), 1.0×10^{-7} mol L^{-1} (curve d), 1.0×10^{-8} mol L^{-1} (curve e), and 1.0×10^{-9} mol L^{-1} (curve f)).

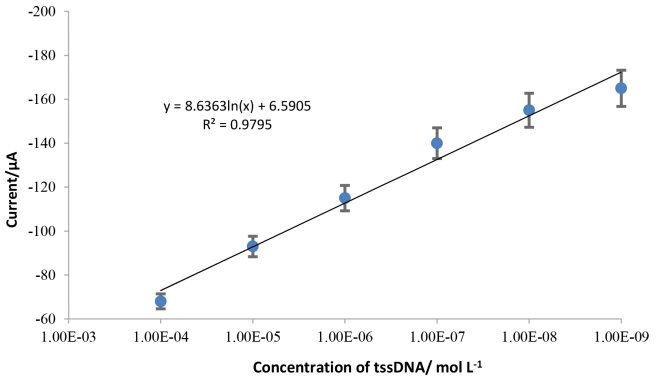

Figure 6. Calibration diagram of hybridized pssDNA with various concentrations of *Aeromonas hydrophila* tssDNA (1.0×10^{-4}, 1.0×10^{-5}, 1.0×10^{-6}, 1.0×10^{-7}, 1.0×10^{-8}, and 1.0×10^{-9} mol L^{-1}).

$MgCl_2$, and 5 % ethanol; pH 7.4). Output results were provided just for electrode type 2 (curve d), electrode type 3 (curve c), electrode type 4 (curve b), and electrode type 5 (curve a), and the Nyquist plots are shown in Fig. 4. The EIS test was not investigated for electrode type 1, because this electrode did not contain any tssDNA of *Aeromonas hydrophila*. The impedance response for electrode type 2 (curve c) was larger and showed more successful electron charge transfer than other electrodes. There was a little heterogeneous resistance to charge transfer (Rct) in the bare electrode (curve b), and this phenomenon strongly increased in electrode type 2 (curve c).

In another experiment the CV technique was applied for various concentrations of *Aeromonas hydrophila* tssDNA (Fig. 5). The used concentrations were 1.0×10^{-4} mol L^{-1} (curve a), 1.0×10^{-5} mol L^{-1} (curve b), 1.0×10^{-6} mol L^{-1} (curve c), 1.0×10^{-7} mol L^{-1} (curve d), 1.0×10^{-8} mol L^{-1} (curve e), and 1.0×10^{-9} mol L^{-1} (curve f). This test was performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4). The peak current was decreased while concentrations were increased continuously; the 1.0×10^{-9} mol L^{-1} concentration was considered the maximum response for the *Aeromonas hydrophila* DNA biosensor; peak current was constant for more concentrations than 1.0×10^{-9} mol L^{-1}.

The calibration diagram for mentioned concentrations of *Aeromonas hydrophila* tssDNA and their hybridization process with pssDNA are shown in Fig. 6. The relation between peak currents and various concentrations was calculated as a logarithmic equation ($y = 8.64\ln(x)+6.6$); in addition, R squared was investigated ($R^2 = 0.98$). These results

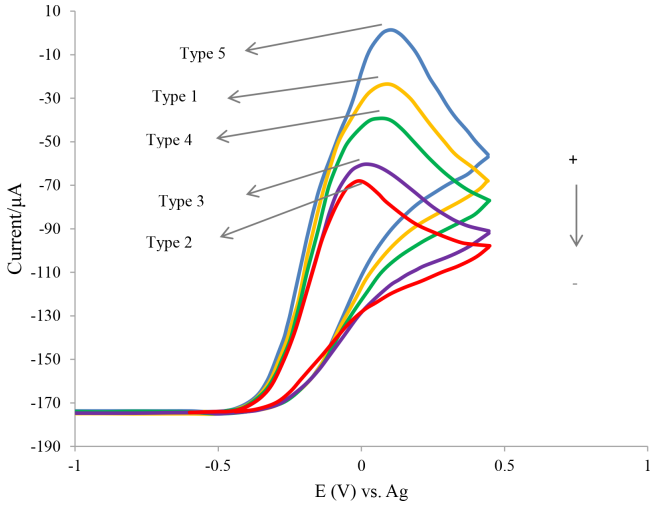

Figure 7. Selectivity test of the response of the *Aeromonas hydrophila* DNA biosensor through various electrodes. Electrode type 2: existence of ZnS nanospheres, pssDNA, and tssDNA; electrode type 3: existence of pssDNA and tssDNA; electrode type 4: existence of ZnS nanospheres and tssDNA; electrode type 1: existence of ZnS nanospheres and pssDNA; electrode type 5: existence of tssDNA.

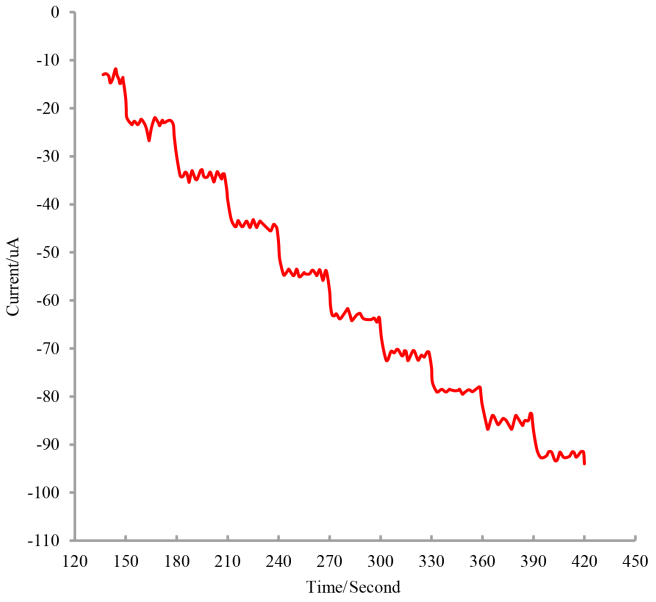

Figure 8. Constant potential amperometry responses of the *Aeromonas hydrophila* DNA biosensor during 137–415 s; rotating speed was 500 rpm. This test was performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4).

confirmed that there was a linear relation between analyte concentrations and peak current reductions.

3.4 Selectivity test of the response of the *Aeromonas hydrophila* DNA biosensor

A selectivity test of the response of the *Aeromonas hydrophila* DNA biosensor was performed using five electrodes with different statuses. A constant concentration of tssDNA was used (1.0×10^{-6} mol L^{-1}) to perform this test in all electrodes. This test was performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4). The maximum response was found in electrode type 2 that applied the existence of ZnS nanospheres, pssDNA, and tssDNA to it (Fig. 7). The coated surface of the working area in a screen-printed electrode with ZnS nanospheres provided a proper state for immobilization of aptamers on the surface of it; this nanoelectrode also contributed a successful redox reaction against other electrode statuses. Electrode type 2 was selected for all bioelectrochemical experiments. The results of this test showed that this DNA biosensor is applicable for electrode types 2 and 3. The other electrode types are not applicable for detecting *Aeromonas hydrophila*, because any hybridization between pssDNA and tssDNA did not occur in them. The difference between peaks in other electrodes was related to different tendencies of the MB redox agent versus each electrode, which led to the production of different electrochemical signals.

In the next experiment, the *Aeromonas hydrophila* DNA biosensor was investigated and its behaviour was followed by the CPA technique. This technique was applied to investigate the efficiency of the designed *Aeromonas hydrophila* DNA biosensor, and it is very useful to find real interactions among substrate concentration and biosensor results (Arrigan, 2015; Jiang, 2007; Wang, 2006). So far, the CPA technique has been considered a highly sensitive electrochemical technique. Here, CPA responses for the *Aeromonas hydrophila* DNA biosensor were followed during 137–415 s and the rotating speed was 500 rpm; this test was continued till a non–Faradaic current found a stable level. In Fig. 8, the step-wise array of time/second was versus current (µA). In this experiment several concentrations of tssDNA were used, the output results showed a good stable state, and DNA biosensor feedback was acceptable. This test was performed in a 10 mL electrolyte cell (50 mM Tris-HCl, 100 mM NaCl, 15 mM KCl, 10 mM $MgCl_2$, and 5 % ethanol; pH 7.4).

3.5 pH effect on the response of the *Aeromonas hydrophila* DNA biosensor

Bacteria indicate variable growth and activity at various pH values. Here the pH effect on the response of the *Aeromonas hydrophila* DNA biosensor was investigated in pH range 2–10 (Fig. 9). The optimum activity response of this designed DNA biosensor was found at pH 7 when the peak current was −140 µA. After the optimum point (pH 7), a severe decline was observed in the activity of this designed DNA biosensor,

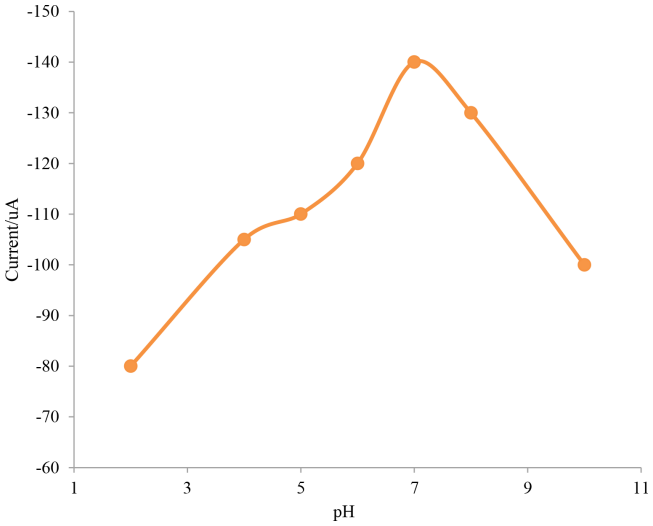

Figure 9. pH effect on *Aeromonas hydrophila* DNA biosensor response in pH range 2–10; optimum pH 7.

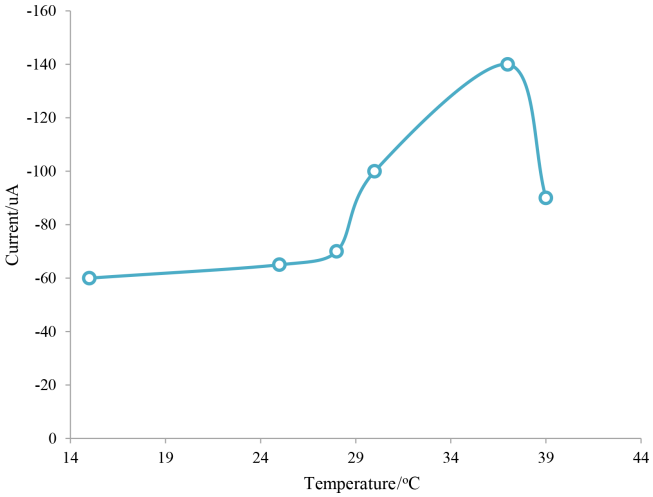

Figure 10. Temperature effect on *Aeromonas hydrophila* DNA biosensor response in temperature range 15–39 °C); optimum 37 °C.

with pH increasing. The possible mechanism for reduction in the activity of the *Aeromonas hydrophila* DNA biosensor was changes in the aptamer structure caused by pH changes that resulted in the possibility of a hybridization process. In addition, the changes in the activity and DNA structure of *Aeromonas hydrophila* in the DNA hybridization process should not be ignored.

3.6 Temperature effect on the response of an *Aeromonas hydrophila* DNA biosensor

Hybridization between pssDNA and tssDNA was performed at various temperatures (the temperature range was between 15 and 39 °C). The maximum response for a *Aeromonas hydrophila* DNA biosensor was found at 37 °C where the peak current was −160 µA (Fig. 10). Due to the influence of heat, hydrogen bonds in hybridized strands of two ssDNA were broken gradually and their bases were separated; this matter will lead to the separation of two strands of DNA. The separation event in hybridized DNA molecules at high temperatures led to an increase in their tagging possibility against redox agents and, due to this occurrence, changes in the redox peak height and their related peak current were observed.

4 Conclusions

Designing a fast and accurate method against pathogenic effects of *Aeromonas hydrophila* will lead to increased levels of health and hygiene in the environment and human societies. In this research, the designed DNA biosensor could detect *Aeromonas hydrophila* with high sensitivity and specificity. Detection of this pathogenic bacterium was provided through a combination of electrochemistry and nanotechnology (use of ZnS nanospheres) at very low concentrations and with little amounts of analyte. The results of this research also provided a precise quantitative method to detect disease severity caused by *Aeromonas hydrophila* in all tissues of animals or humans. This designed DNA biosensor also showed good specificity, stability, and sensitivity after examination under various conditions.

Data availability. No data sets were used in this article.

Competing interests. The authors declare that they have no conflict of interest.

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