A sensitive electrochemical aptasensor based on single wall carbon nanotube modified screen printed electrode for detection of *Escherichia coli O157:H7*

M.R. Housaindokht[1*], A.Verdian[2], E. Sheikhzadeh[3], P. Pordeli[4], Z. Rouhbakhsh Zaeri[3], F. Janati-Fard[3], M. Nosrati[3] M. Mashreghi[4], A.R.Haghparast[1], A. Nakhaeipour[3], A. A. Esmaeili[1], S. Solimani[5]

[1]*Research and Technology Center for Biomolecules, Ferdowsi University of Mashhad, Mashhad, Iran*
[2]*Department of Food Nanotechnology, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran*
[3]*Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran*
[4]*Department of Biology, School of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*
[5]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[*]Corresponding author

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Abstract

Escherichia coli O157:H7 (E.coli O157:H7), is an extremely infectious and potentially fatal water and food-borne pathogen, responsible for numerous hospitalisations and deaths all over the world. Herein we reported the development of simple and label-free electrochemical aptasensor based on single wall carbon nanotube (SWCNT) modified screen printed electrode (SPE) to detect *E.coli O157:H7* bacteria. The electrochemical probe of methylene blue (MB) was used to investigate the interactions between aptamer and *E.coli O157:H7*. The resulting MB/Apt/SWNT/SPE layer exhibited good current response to detect *E.coli O157:H7*. The aptasensor detect *E.coli O157:H7* in the concentration range of 1.7×10^1 - 1.1×10^7 CFU mL^{-} and limit of quantification of 1.7×10^1 CFU mL^{-1}. The proposed aptasensor is rapid, convenient and low-cost for effective sensing of *E.coli O157:H7* with high selectivity over interference bacteria. The suitability of the aptasensor for real sample measurements was investigated by recovery studies in tap water sample. Copyright © 2018 VBRI Press.

Keywords: Escherichia coli O157:H7, aptamer, electrochemical aptasensor, label-free detection.

Introduction

Foodborne diseases are a growing public health problem in both developed and developing countries caused by ingestion of foodstuffs contaminated with microorganisms or chemicals [1].

Escherichia coli (E. coli) is a Gram-negative, rod shaped bacterium of Enterobacteriacea family. Although Most *E. coli* serotypes are harmless and exist normally in intestinal tract, some serotypes like *E. coli O157:H7* are pathogenic for human and animals[2]. According to the Center of Disease Control and Prevention (CDC) *E. coli O157:H7* is a foodborne and waterborne pathogen that cause 73,000 cases of infection and 61 associated deaths in the United States each year with the symptom of abdominal cramps, bloody diarrhea and kidney failure[3]. This bacteria produce a toxin called Shiga toxin which causes severe damage to the lining of the intestine and to other target organs such as kidneys [4]. Detection of this bacteria by traditional procedures are based on combination of pre-enrichment step, selective culturing and serological confirmation that take several days to obtain reliable result [5].

Polymerase chain reaction (PCR) techniques require long preparation steps such as cell lysis and DNA separation, without the capability to detect viable bacteria at the time of the sampling. Enzyme linked immunosorbent assays (ELISA) can detect *E.Coli* at concentration of 10^5cells mL; in order to detect lower concentration this techniques should be combine with preconcentration step that usually take about 16-24 hours [6, 7]. Therefore, in order to reduce the risk to public health, new methods for rapid and simple detection of *E. coli O157:H7* are needed.

Electrochemical detection methods are extensively used due to simple instrument, high sensitivity and the ability to miniaturize for portable application. For example, Xu et al., have developed an immunosensor based on screen-printed interdigitated microelectrode to detect *E.coli O157:H7* in ground beef [8]. Moreover, a disposable electrochemical immunosensor fabricated with Multi-walled carbon nanotubes/sodium alginate/ carboxymethyl chitosan composite film for voltammetric detection of *E.coli O157:H7* down to 3.27×10^3 CFUmL^{-1}. Finally, impedimetric immunosensor could recognize

E.Coli O157:H7 in food sample with detection limit of 1.5×10^3CFU mL^{-1}.

Aptasensors are latest generation of biosensors in which aptamer has been used as a reorganization element [9-11]. Aptamers are single-stranded oligonucleotides that have the ability to fold into particular conformations, which bind strongly and selectively to a specific target [12, 13]. Compared to antibody-based biosensors, aptasensor possess outstanding features, such as high productivity, affinity, selectivity, and stability [14].

Carbon nanotube CNT have found widespread application in different area because of various properties like thermal conductivity, stability and the capability to interact with variety of organic and inorganic analyte [15]. Moreover single-walled carbon nanotubes (SWCNTs) have outstanding charge-transfer and transducing ability and can readily use to modify electrode surface. Even more interestingly, π-π stacking interaction between the nucleic acid bases of aptamers and the (SWCNTs) walls can be used to facilitate the self-assemble of aptamer on electrode surface. [16]. Among various aptasensors, label-free electrochemical aptasensors vastly exploited because of their advantages such as simplicity, high sensitivity, low cost and high stability [17,18]. Here, we proposed a novel, simple, and label-free electrochemical aptasensors based on current response derived from MB tag to detect *E. coli O157:H7*. Moreover, with this approach unmodified aptamer was applied which highly reduce cost of proposed method. To the best of our knowledge, no work exists in electrochemical aptasensors for *E. coli O157:H7* detection.

The suitability of the aptasensor was determined by calibration, selectivity and recovery studies, which proved its sensitivity, specificity and applicability for the detection of an *E. coli O157:H7* in real sample measurement.

Experimental

Chemicals and reagents

Aptamer with ccg gac gct tat gcc ttg cca tct aca gag cag gtg tga cgg sequence was purchased lyophilized from Macrogen (Korea). [19] All sequences were dissolved in highly pure water (sterile Millipore water, 18.2 mΩ) as stock solutions; the concentrations of ordinary oligonucleotides was calculated by absorption spectra from the nearest-neighbor model of Cantor et al. [20]. Bovine serum albumin (BSA) was obtained from Sigma and dissolved in PBS (0.1 M, pH 7.4). SWCNTs with purity of 95% were obtained from Nanolab (Brighton, MA). Phosphate buffer solutions (PBS) were prepared with NaH_2PO_4 and Na_2HPO_4 and have been used as supporting electrolyte.

Bacteria and culture plating methods

The tested microorganisms were Gram-positive and Gram-negative bacteria. *E. coli O157:H7* (NCTC 12900), E. coli (PTCC 1399), Salmonella aureus (PTCC 1431), Bacillus subtilis (PTCC 1365), Pseudomonas aeroginosa (PTCC 1074) and Salmonella enterica (PTCC 1709) were recovered from long-term storage at -85 °C in 15% glycerol. Bacteria were refreshed in Nutrient Broth (Merck, Darmstadt, Germany) at 37°C for 20 h and inoculated on Nutrient Agar (Merck, Darmstadt, Germany) plates to check the purity. Bacterial strain were cultured overnight at 37°C in Nutrient Broth (NB, Merck) and Cells were harvested by centrifuge (5 min, 4000 rpm), then suspended in deionized water (1 ml), repeat the procedure with centrifuge and suspended with 5ml of deionized water to the concentration of 10^8 colony forming units per ml (CFU mL^{-1}) (according to Mc Farland turbidity standards) as stock solutions of bacteria. Dilution serials (10^1 - 10^7 CFU mL^{-1}) of standard stock solutions were prepared with PBS (0.1 M, pH 7.4). The number of viable cells was determined by conventional colony counting method on agar plate before use.

Apparatus

Voltammetry measurements were performed using a µstat 400 portable Bipotentiostat/Galvanostat, (DropSens, Spain) which equipped with Drop View 8400 Software. The screen-printed carbon electrodes also obtained from DropSense and used in conjunction with the µstat 400. Before using the unmodified SPE the surface was polished with filter paper and washed with deionized water.

Preparation of the MB/Apt/SWCNT/SPE electrode

The fabrication of the aptasensor includes three steps as follows:

The pretreatment of SWCNT/SPE, self-assembly of *E. coli O157:H7* aptamer, and accumulating MB into aptasensor. Firstly, the working surface area of a bare SPCE was modified by depositing uniformly a 7 µl drop of SWCNT suspension (1mg SWCNT/1ml THF), followed by an evaporating the solvent at room temperature for 24 h. Secondly, the prepared electrode was thoroughly rinsed with ultrapure water to remove excessive SWCNT. Before immobilization of probe, *the E. coli O 157:H7* aptamer was firstly denatured by heating at 90 °C for 10 min, slowly cooled at 25 °C to allow renaturation of aptamer to attain its most stable conformation, which is a perquisite condition for its binding to target molecule. Then, the specific aptamer solution of *E. Coli O157:H7* in 0.05 M PBS (7 µl, 10 µM) was drop-casted on the surface of the modified screen-printed carbon electrode and incubated on the electrode surface for 5 h at 25°C.Then the aptamer modified electrode was rinsed thoroughly with 1% PBS in order to remove any unbounded aptamers. Then, the procedure followed by adding 7 µl aliquot of 0.1 M PBS (pH 7.4) contain 1% BSA for 30 min on the modified electrode surface to reduce non-specific binding. Afterwards, the modified electrode was immersed in a stirring 0.1 M PBS solution have 50 µM methylene blue to accumulate MB for 10 min. The fabricated electrode (MB/Apt/SWCNT/SPE) was successively rinsed with ultrapure water and PBS and finally it was immersed into

bacterial solution with different concentrations. The schematic in **Fig. 1** represents the fabrication of *E. Coli O157:H7* aptasensor.

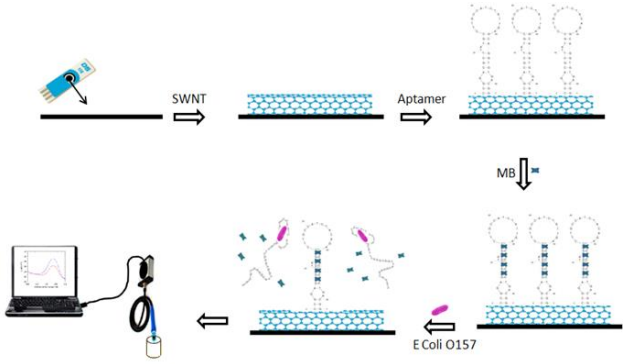

Fig. 1. Schematic outline of the principle for label-free electrochemical E. Coli O157 biosensing.

Electroanalytical measurements

The formation of aptamer-target complexes was performed by immersing the MB/Apt/SWCNT/SPE into PBS buffer (0.1 M, pH 7.4) has a given bacterial concentration for 35 minute at room temperature. All electrochemical measurements were performed in PBS (0.1 M, pH 7.4) solution. Cyclic voltammetry (CV) measurements were performed over a potential range from -0.4 to +0.05 V at scan rate of 100 mV s^{-1}. The electrochemical differential pulse voltammetry (DPV) measurements were carried out under the following conditions:

The voltage scanned from -0.52 to -0.02 V with a pulse width of 0.05s. The electrochemical data analysis was carried out and decrease in peak currents was measured before and after the sample treatment ($\Delta I = (I_0 - I)$). Where ΔI is relative current change, I_0 and I represent the peak current before and after bacteria treatment, respectively. All the experiments were repeated three times for the detection of bacteria. All measurements were carried out at room temperature.

Results and discussion

Design strategy of the aptasensor (Principle of E. Coli O157:H7 detection using MB/Apt/MWCNTs/ SPE electrode)

Fig. 1 depicts the mechanism of our strategy to fabricate the electrochemical aptasensor. The SPE electrode surface is first modified by a homogeneous layer of SWCNTs. This leads to increase the effective surface area of electrode and provides an appropriate loading surface for the subsequent immobilization of aptamer. On the basis of the strong π–π stacking effect between the nucleobases of the aptamer and the sp^2 atoms of SWNTs, the aptamer can be brought into close proximity to the SWNTs surface. MB was used as the electrochemical redox probe and intercalated into DNA to produce the electrochemical signal. This redox tag was used as electrochemical indicator in our proposed strategy due to its good redox characteristics and its low cost. The Apt/MWCNTs/ SPE electrode could not produce any electrochemical signal. A well-defined electrochemical signal could be observed upon addition of MB **(Fig. 2)**. The *E. Coli O157:H7* aptamer has 42 nucleotides that were non-covalently grafted on the SWCNT/SPE surface. It is formed a cluster of long arms on the electrode surface, which is a proper substrate for intercalation of redox probe MB. As shown in **Fig. 3,** the secondary structure of *E. Coli O157:H7* aptamer predicted by mfold web server has several positions for intercalation of MB.

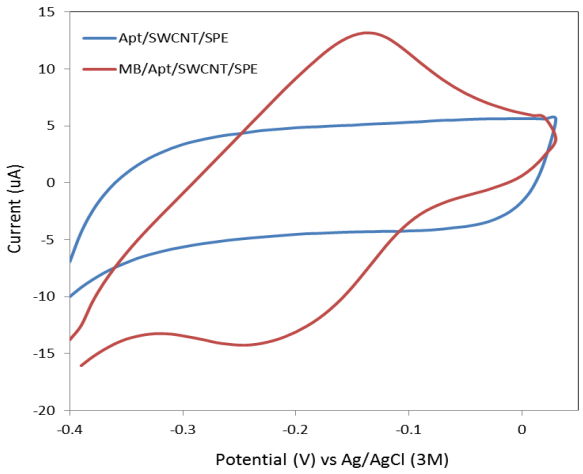

Fig. 2. CV responses of Apt/MWCNTs/ SPE electrode and MB/Apt/MWCNTs/ SPE electrode. Cyclic voltammetry (CV) measurements were performed over a potential range from -0.4 to +0.05 V at scan rate of 100 mV s^{-1}.

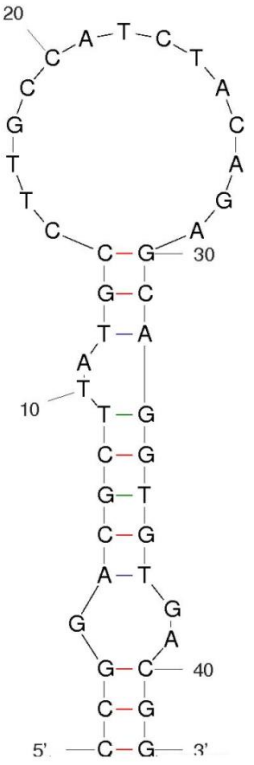

Fig. 3. Secondary structure of anti-E. Coli O157 aptamer predicted by mfold program (ΔG = -2.12 kcal/mol).

The competitive recognition and binding of *E. coli O157:H7* on the surface of electrode induced the release of aptamer from the electrode and decreased the peak current of MB for quantitative detection.

Optimization of E. coli O157:H7 incubation time

The dependence of incubation time on the decrease of current was studied to determine the optimum incubation time. As shown in **Fig. 4,** the electrochemical signal decreased rapidly with increasing the incubation time and then tended to level off after 35 min. The result showed that there is change in the conformational of aptamer induced by target binding which completed after 35 min. So, 35 min was selected as the *E. Coli O157:H7* incubation time.

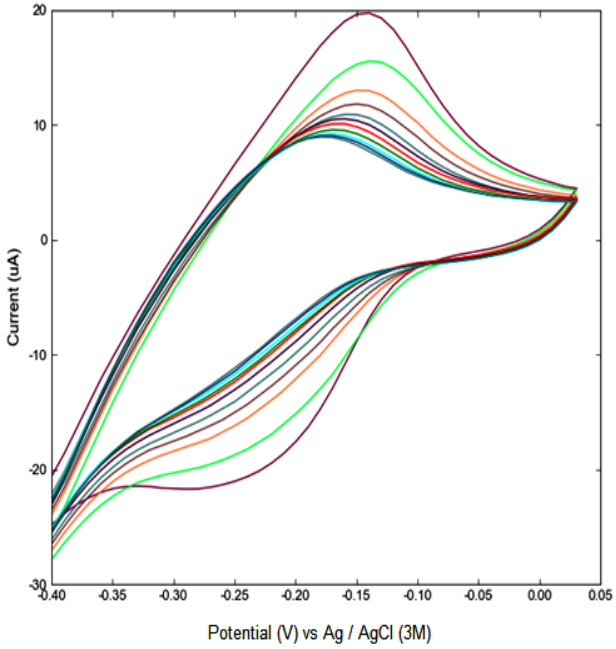

Fig. 4. CV responses of aptamer-modified electrode + methylene blue + E. Coli O157 (10^4 CFU/mL), in PBS (pH=7.5) as a function of incubation time (from top to bottom 0-60 min). Cyclic voltammetry (CV) measurements were performed over a potential range from -0.4 to +0.05 V at scan rate of 100 mV s^{-1}.

Performance of the aptasensor

Calibration curve of the aptasensor

The aptamer-modified electrodes were incubated with different concentrations of *E. Coli O157:H7* under the optimal conditions and the DPV responses of the proposed aptasensor were recorded. As can be seen in **Fig. 5 (a),** the DPV peak current of MB decreased with the increasing concentrations of bacteria. A calibration curve was obtained for E. Coli O157:H7 from 1.7×10^1 to 1.1×10^7 CFU mL^{-1} **(Fig. 5(b))** and the obtained correlation coefficient (R^2) was 0.998. The limit of quantification was 1.7×10^1 CFU mL^{-1}.

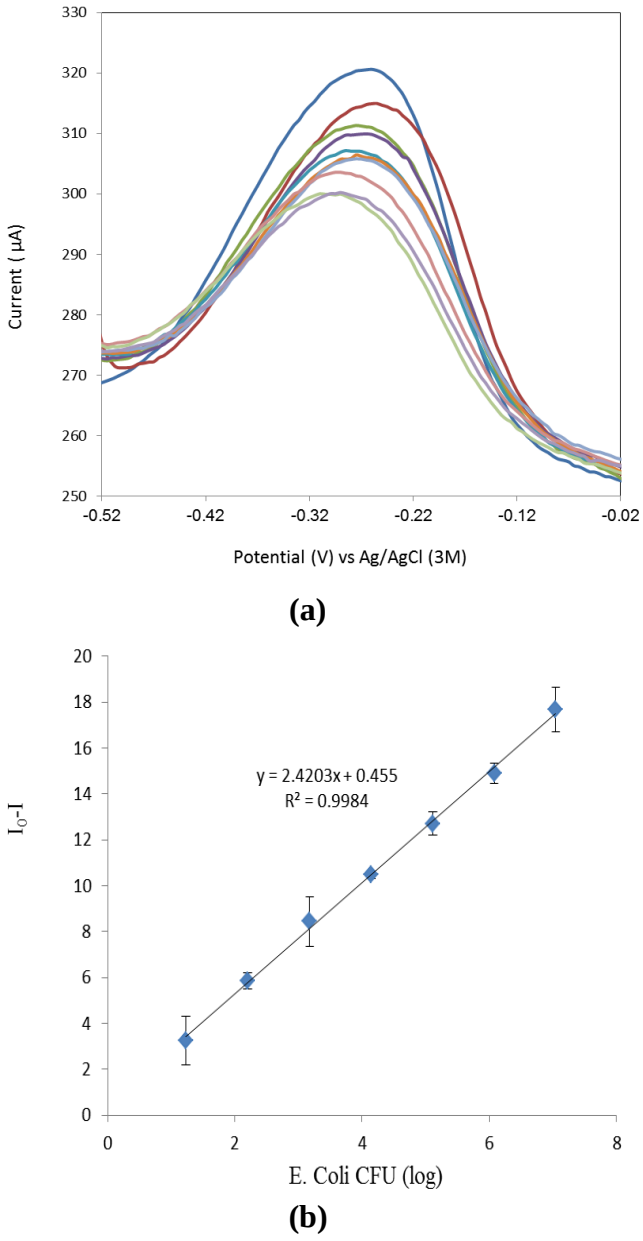

Fig. 5. (a) DPV peaks of the aptamer-modified electrode after incubation for 30 min with various concentrations of E. Coli O157 (from top to bottom 0-1.1×10^7 CFU/mL). (b) E. Coli O157 standard curve in PBS (pH 7.5). The voltage scanned from -0.52 to -0.02 V with a pulse width of 0.05 s.

Specificity of the aptasensor

To evaluate the selectivity of the proposed aptasensor for the detection of *E. Coli O157:H7*, experiments were conducted by using Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli O167, and Salmonella typhimurium bacteria as the interference bacteria with concentration of 10^7 CFU mL^{-1}.

The experimental results were shown in **Fig. 6**. The results of specificity experiments confirmed that there was insignificant interference of other bacteria and the developed aptasensor could be used to determinate *E. Coli O157:H7* with good specificity.

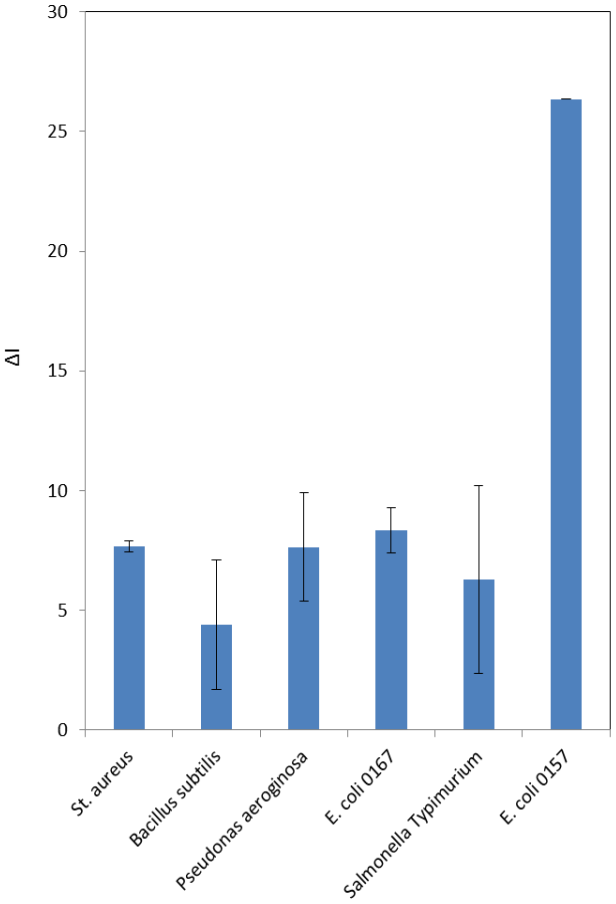

Fig. 6. Specificity of aptasensor detection over six model pathogen Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli O167, and Salmonella typhimurium.

Real sample measurement

Real sample measurement were carried out in spiked (10^3 and 10^5) tap water. The results of recovery data are outlined in **Table 1**. Concentration of bacteria was calculated by putting ΔI data calculated from equation 1 in the calibration curve of Fig 5(b).

Table 1. Summrey of recovery studies in tap water sample.

Sample number	Spiked (E. Coli CFU/ml (log))	Measured (E. Coli CFU/ml (log))	Recovery (%)
1	3	2.96	98.67
2	5	3.8	78

Conclusion

A simple, label-free and low cost electrochemical aptasensor for the determination of *E. Coli O157:H7* was developed. The aptamer was immobilized on the SWNT/SPE modified electrode as *E. Coli O157:H7* capture and MB used as redox probe. Binding of E. Coli O157:H7 caused DPV current to decrease, and consequently provided a basis for detection. This sensor showed excellent selectivity toward E. Coli O157:H7 with a limit of quantification as low as 1.7×10^1 CFU mL^{-1} and can be used for real sample measurement. More importantly, the proposed electrochemical aptasensor has been offered as a convenient, specific, and sensitive method not only for detecting *E. Coli O157:H7* as a model target but also it has potential to be applied for other targets detection.

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References

1. Organization WH (2014) WHO initiative to estimate the global burden of foodborne diseases: fourth formal meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG): Sharing New Results, Making Future Plans, and Preparing Ground for the Countries.
2. Esameili H, Khanjari A, Gholami F, (2015) D etection and characterization of E scherichia coli O157: H7 from feral pigeon in Qom province, I ran. Asian Pacific Journal of Tropical Disease 5 (2):116-118
3. Wang Y, Ye. Z, Si. C, Ying Y (2013) Monitoring of Escherichia coli O157: H7 in food samples using lectin based surface plasmon resonance biosensor. Food chemistry 136 (3):1303-1308
4. Smith J, Fratamico P, Launchi N (2014) Update on non-O157 Shiga toxin-producing E. coli as a foodborne pathogen: analysis and control. Advances in Microbial Food Safety 2:1
5. Dou W, Tang W, Zhao G (2013) A disposable electrochemical immunosensor arrays using 4-channelscreen-printed carbon electrode for simultaneous detection ofEscherichia coli O157:H7 and Enterobacter sakazakii. Electrochimica Acta 97:79– 85
6. E. Sheikhzadeh, M. Chamsaz, A.P.F. Turner, E.W.H. Jager, Beni V (2016) Label-free impedimetric biosensor for salmonella.Typhimurium detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer. Biosens Bioelectron 80: 194-200
7. Shih CM, Chang CL, Hsu M, .Y.,, Lin JL, Kuan CM, Wang HK, Huang CT, Chung MC, Huang KC, Hsu CE, Wang CY, Shen YC, Cheng CM (2015) Paper-based ELISA to rapidly detect Escherichia coli. Talanta in press
8. Xu M, Wang R, Li Y (2015) Rapid Detection of Escherichia coli O157:H7 and Salmonella Typhimurium in Foods Using anElectrochemical Immunosensor Based on Screen-printed Interdigitated Microelectrode and Immunomagnetic Separation. Talanta 148:200-208
9. Basta M, Karmali M, Lingwood C (1989) Sensitive receptor-specified enzyme-linked immunosorbent assay for Escherichia coli verocytotoxin. Journal of clinical microbiology 27 (7):1617-1622
10. Torres-Chavolla E, Alocilja EC (2009) Aptasensors for detection of microbial and viral pathogens. Biosensors and Bioelectronics 24 (11):3175-3182
11. Wu S, Duan N, Shi Z, Fang C, Wang Z (2014) Simultaneous aptasensor for multiplex pathogenic bacteria detection based on multicolor upconversion nanoparticles labels. Analytical chemistry 86 (6):3100-3107
12. Verdian-Doghaei A, Housaindokht M (2015) Spectroscopic study of the interaction of insulin and its aptamer–sensitive optical detection of insulin. Journal of Luminescence 159:1-8
13. Verdian-Doghaei A, Housaindokht M, Abnous K (2014) A fluorescent aptasensor for potassium ion detection-based triple-helix molecular switch. Analytical biochemistry 466:72-75
14. O'Sullivan CK (2002) Aptasensors–the future of biosensing? Analytical and bioanalytical chemistry 372 (1):44-48

15. Herrera-Herrera AV, González-Curbelo MA, Hernández-Borges J, Rodríguez-Delgado MA (2012) Carbon nanotubes applications in separation science: A review. Analytica Chimica Acta 734:1-30
16. Zelada-Guilln G, A,, Riu J, Duzgun A, Rius FX (2009) Immediate Detection of Living Bacteria at UltralowConcentrations Using a Carbon Nanotube Based Potentiometric Aptasensor. AngewChem Int Ed 48:7334-7337
17. Hianik T, Wang J (2009) Electrochemical aptasensors–recent achievements and perspectives. Electroanalysis 21 (11):1223-1235
18. Vidal JC, Bonel L, Ezquerra A, Hernández S, Bertolín JR, Cubel C, Castillo JR (2013) Electrochemical affinity biosensors for detection of mycotoxins: A review. Biosensors and Bioelectronics 49:146-158
19. Wu W-h, Li M, Wang Y, Ouyang H-x, Wang L, Li C-x, Cao Y-c, Meng Q-h, Lu J-x (2012) Aptasensors for rapid detection of Escherichia coli O157: H7 and Salmonella typhimurium. Nanoscale research letters 7 (1):1-7
20. Cantor CR, Warshaw MM, Shapiro H (1970) Oligonucleotide interactions. III. Circular dichroism studies of the conformation of deoxyoligonucleolides. Biopolymers 9 (9):1059-1077

