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ORIGINAL ARTICLE

Rapid detection of *Salmonella enterica* in raw milk samples using *Stn* gene-based biosensor

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Abstract
In this study, a DNA-based nanosensor using specific NH_2 labeled single standard probe was developed against *stn* gene of *Salmonella enterica* in milk samples. The single-stranded DNA probe was immobilized on carboxylated multiwalled carbon nanotube and gold nanoparticle (c-MWCNT/AuNP) electrode using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC): *N*-hydroxy succinimide-based cross-linking chemistry. Electrochemical characterization was performed using cyclic voltammetry (CV) and Differential Pulse Voltammetry (DPV) techniques. The electrode surface at each step of fabrication was characterized using scanning electron microscopy. The sensitivity and lower limit of detection were found to be 728.42 ($\mu A/cm^2$)/ng and 1.8 pg/6 µl (0.3 pg/ml), respectively, with regression coefficient (R^2) of 0.843 using DPV. The sensor was further validated using raw and artificial milk samples, and results were compared with conventional methods of detection. The developed sensor was found to be highly sensitive and stable up to 6 months, with only 10% loss of initial peak current in CV analysis on storage at 4 °C.

Keywords *Salmonella enterica* · Foodborne illness · *stn* gene · Electrochemical biosensor

Introduction

Foodborne illness is one of the significant concern governing high rates of mortality worldwide. *Salmonella* is most commonly responsible for the foodborne illness and causes salmonellosis and gastroenteritis (Dong et al. 2013). In developed countries *Salmonella enterica* is frequently known bacteria responsible for 40–80% of food poisoning (Ozdemir and Acar 2014; Parvej et al. 2016). *Salmonella* alone causes 93.8 million human infections and 155,000 deaths annually across the world and this bacteria is commonly found in egg, milk, food industries, kitchen area, animal feces, meat, and seafood etc. (Hendriksen et al. 2011). The various virulent genes are responsible for pathogenicity of *Salmonella.* Out of these *Salmonella* enterotoxin (*Stn*) gene has been reported as the most dominant virulent gene encoding for 29-kDa protein (*Stn*) and is also a causative agent of gastroenteritis (Xu et al. 2010). In human population *Salmonella* infection enters through intestine into the bloodstream and cause diarrhea, fever, and abdominal cramps within 12–72 h after consumption (Alocilja et al. 2013). The *Salmonella* infection may become life-threatening if not treated immediately with antibiotics (Alocilja et al. 2013).

The conventional approach used for the detection of *Salmonella* includes various immunological method (ELISA, immune radiometric labeled antibody, latex agglutination) and DNA-based assay using PCR (Taha et al. 2010). However, these procedures are slightly sensitive, less specific, time-consuming, and require large sample volume (Pashazadeh et al. 2016). Electrochemical biosensor using gold nanoparticles on screen-printed carbon electrodes (SPCE) have already been reported for the detection of insertion element gene (*Iel*) of S*almonella enteritidis* with lower limit of detection (LOD). In this method, the probe was hybridized with the targeted DNA, and electrochemical analysis was carried out using differential pulse voltammetry. The reported detection time and LOD for the biosensor was 1 h and 0.7 ng/mL, respectively (Alocilja et al. 2013). However, to render the standard of food safety, a more accurate and rapid method for the detection of *S. enterica* is utmost required.

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The present study was aimed to develop simple, rapid, and sensitive electrochemical nanosensor for the detection of *S. enterica*. Validation studies of the developed nanosensor were carried out using raw milk and artificially spiked milk samples.

Experimental

Materials and methods

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), methylene blue (MB), RNase, Proteinase K were purchased from Sigma Aldrich USA. Tris-hydrochloric acid, phenol chloroform isoamylalcohol, Ethylenediaminetetraacetic acid (EDTA), ethanol, sodium chloride (NaCl), sodium dihydrogen orthophosphate (NaH_2PO_4) disodium hydrogen orthophosphate (Na_2HPO_4), and other chemicals were obtained from Hi-Media, India. Brain heart infusion broth was procured from Hi-Media, India. The Gram-negative *S. enterica* culture (MTCC-9844) was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. 5′-NH_2 modified *stn* gene-specific single-stranded DNA probe (5′-GTCCGGGTCAGC CTGAAT-3′) was procured from Eurofins, Bengaluru, India. Screen-printed c-MWCNT/AuNP-based screen-printed electrode was purchased from DropSens, Spain, and modified in the lab.

Apparatus

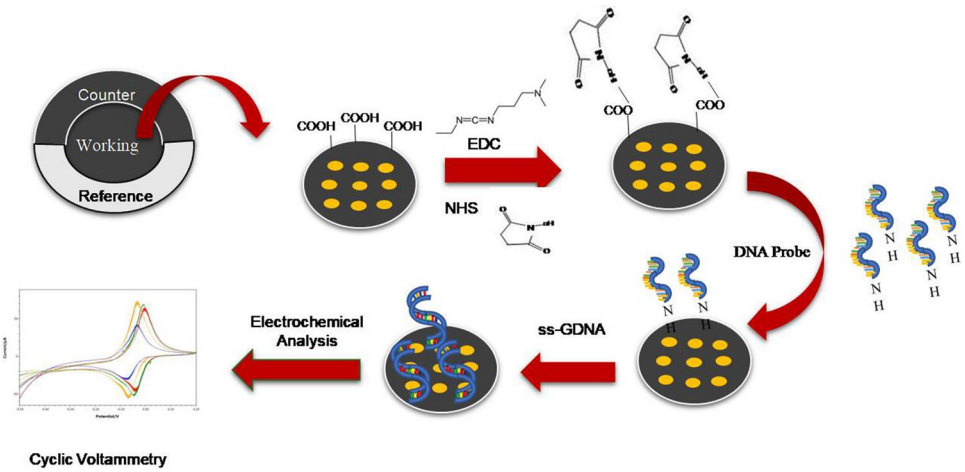
The electrochemical response in the form of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was studied using potentiostat (Palmsens 4, The Netherlands). The electrochemical measurements were analyzed with the PSTrace software. DNA was quantified using nanodrop spectrophotometer (QIAxpert System, USA). Electrode surface characterization analysis was carried out using Scanning Electron Microscopy (SEM), JEOL (Tokyo, Japan) 6510F.

Isolation of genomic DNA from culture and artificially spiked milk samples

The genomic DNA (GDNA) was isolated from *S. enterica* (MTCC 9844) grown in Brain heart infusion broth at 37 °C for 24 h using the phenol–chloroform method (Kaushal et al. 2012). Twenty raw milk samples were collected from different places of Soaln, HP, India and three milk samples were artificially spiked with standard *S. enterica* strain and incubated at 37 °C overnight. From each sample, 1 ml of spiked milk was centrifuged at 12,000 rpm for 10 min. The pellet obtained was dissolved in 100 µl of TE buffer and heated at 95 °C for 5 min. After heating, all the tubes were centrifuged at 8000×*g* for 3 min, and the pellet was washed with autoclaved water (centrifugation) and finally dissolved in 10 µl of TE buffer. The purity (A 260/280) and quantity of DNA (A 260) was measured using Nanodrop spectrophotometer.

Fabrication of the sensor

The schematic illustration of the fabrication of genosensor is shown in Scheme 1. The electrode (c-MWCNT/AuNP) surface was washed with Milli Q water for 2 min at room temperature. The electrode was further washed with TE buffer (pH 8) followed by drying at room temperature. The working surface of c-MWCNT/AuNP electrode was treated with a mixture of 10 mM EDC and 10 mM NHS (1:1, v/v in PBS, pH 7.4) for 1.5 h to activate the carboxyl groups (–COOH) on the surface. The electrode was then washed with PBS (pH 7.4) to remove excessive reagent on the surface and dried at room temperature. The 5′-NH_2 labeled 10 µM DNA probe (6 µl) was incubated overnight onto the working surface of the electrode at 4 °C. This treatment allows the formation of amide bond between the NH_2 group of the probe and



Scheme 1 Fabrication of electrochemical sensor using immobilization of 5′-NH_2 labeled ss-DNA probe of *stn* gene on c-MWCNT and hybridization with ssG-DNA of *S. enterica*

COOH group of the c-MWCNT (Zhu et al. 2008, Nagraik et al. 2019). The unbound probe was removed by repeated washing with TE buffer (pH 8) before performing electrochemical detection.

Hybridization of sample ss-DNA with ss-DNA probe of *S. enterica*

The different concentration of single-stranded genomic DNA obtained after denaturation by heating at 95 °C for 5 min was hybridized with single-stranded probe fabricated on the surface of working electrode for 10 min. This was followed by washing with TE buffer (pH 8). The electrode was properly dried at room temperature before electrochemical measurements. using CV and DPV.

Surface characterization of DNA biosensor

Morphological changes in the surface of biosensor at each step of fabrication in the bare electrode (c-MWCNT/AuNP), immobilized probe (c-MWCNT/AuNP/ss-DNA) and after hybridization with 100 ng/6 µl ssG-DNA of *S. enterica* (c-MWCNT/AuNP-dsDNA) were studied using scanning electron microscopy. In this study, the modified electrode surface was coated with gold (6 nm thickness) using sputter coater under vacuum. The surface of gold coated electrodes with different modifications was then analyzed by scanning electron microscopy.

Specificity and stability of fabricated DNA biosensor

To check the specificity of the developed DNA biosensor, ssG-DNA of different pathogens (*L. monocytogenes, E. coli, K. pneumoniae,* and *S. enterica*) present in food samples were allowed to hybridize with the immobilized ss-DNA probe of *S. enterica*. The CV peak current (Ip) in each case was compared with the peak current (Ip) recorded with the immobilized probe of *S. enterica*. The stability of the developed DNA biosensor was assessed through CV at a regular interval of 30 days up to 6 months.

Validation of DNA biosensor

The developed DNA biosensor was validated with twenty raw milk samples collected from Solan, HP, India and three milk samples artificially spiked with *S. enterica* (positive control). The peak current (Ip) values inspected from CV after hybridization with ssG-DNA of milk samples were compared with the probe, positive control (milk sample with *S. enterica)* and negative control (milk sample without *S. enterica*), respectively.

Results and discussion

In present study, an electrochemical nanosensor was developed for the detection of *S. enterica* in milk samples using *stn* gene as a marker. The results of various parameters studied are discussed in the following sections:

Cyclic voltammetry studies

The CV (Fig. 1) peak current values (Ip) of c-MWCNT/AuNP, c-MWCNT/AuNP/ss-DNA and, c-MWCNT/AuNP/dsDNA were recorded using 1 mM methylene blue (50 mM PBS, pH 7.4) as a redox Probe. The Cyclic voltammograms (CV) of electrodes were obtained between − 1.0 and 0.5 mV. The Ip of c-MWCNT/AuNP/ss-DNA was found to be 36 µA (curve a) which is higher than that of a bare electrode (not shown) due to the increased binding of methylene blue molecule (redox Probe) to ss-DNA probe. After hybridizing the electrode with ss-DNA of different concentrations (0.0976, 0.195, 0.39, 0.781, 1.5, 3.1, 6.25, 12.5, 25, 50, 100 ng/6 µl), the Ip was found to be in between 46–109 µA, respectively, as shown in Fig. 1 (curve a–l). This was attributed to the increased binding of methylene blue at each step of hybridization with increased concentration of ss-GDNA, that results in more oxidation of methylene blue molecules on the surface of the electrode which is responsible for high current generation and hence resulting in an increase in the current (Singh et al. 2014).

The plot between the ss-DNA concentrations and relative Ip values for probe (zero) was found to be hyperbolic (Fig. 1, inset [A]) with a regression coefficient (R^2) of 0.9878 (Fig. 1, inset [B]). The sensitivity (S) of the reported DNA sensor was 716.19 (µA/cm^2)/ng as calculated using the formula S = m/A, where m is the slope of linear equation, and A is the area of working electrode (0.126 cm^2). The LOD of the sensor was 3.3 pg/6 µl (0.5 pg/ml) and was calculated using the formula LOD = 3 (σ/S), where σ is the standard deviation, and S is the sensitivity (Fig. 1 inset [B]).

Differential pulse voltammetry studies

The DPV (Fig. 2) peak current values (Ip) of c-MWCNT/AuNP, c-MWCNT/AuNP/ss-DNA and, c-MWCNT/AuNP/dsDNA were recorded using 1 mM methylene blue (50 mM PBS, pH 7.4) as a redox probe (Dash et al. 2014). The differential pulse voltammograms (DPV) of electrodes were obtained between − 1.0 and 0.5 mV. The Ip of c-MWCNT/AuNP/ss-DNA was found to be 104 µA (curve a) which is higher than that of the bare electrode (not shown) due to the increased binding of methylene blue molecule (redox probe) to ss-DNA probe. After

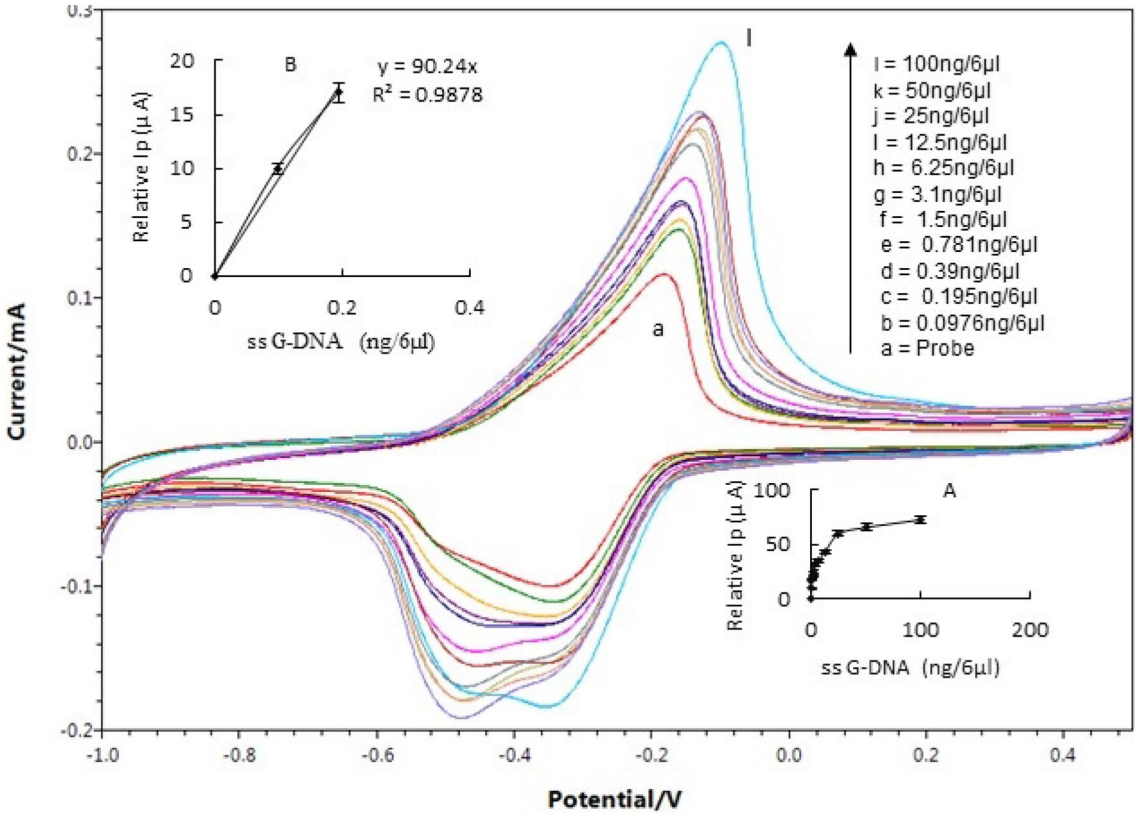


Fig. 1 CV of (a) ss-DNA (probe) (b–l) hybridization with 0.0976–100 ng/6 µl of *S. enterica* ssG-DNA at 50 mVs-1 using 1 mM MB in 50 mM PBS, pH 7.4. The inset-**A** shows a hyperbolic curve from 0 to 100 ng/6 µl with linear peak current (Ip) 0–0.19 ng/6 µl of ssG-DNA of *S. enterica*. Inset-**B** shows the linear plot from 0 to 0.19 ng/6 µl ssG-DNA for the calculation of sensitivity and LOD

hybridizing the electrode with ss-DNA of different concentrations (0.05, 0.11, 0.234, 0.468, 0.937, 1.875, 3.75, 7.5, 15, 30 ng/6 µl), the Ip was found to be in between 111 and 126 µA, respectively, as shown in Fig. 1 (curve a–k). This was attributed to increased binding of the methylene blue at each step of hybridization with increasing concentration of ss-GDNA and more oxidation of methylene blue molecules on the surface of the electrode and hence resulting in an increase in the current. The plot between the ss-DNA concentrations and relative Ip values concerning probe (zero) was again found to be hyperbolic (Fig. 1 inset [A]) with a regression coefficient (R^2) of 0.843 (Fig. 1 inset [B]). The sensitivity (S) of the reported DNA sensor was 728.42 (µA/cm^2)/ng as calculated using the formula S = m/A mentioned earlier. The LOD of the sensor was observed to be 1.8 pg/6 µl (0.3 pg/ml) (Fig. 1 inset [B]). The sensitivity of this sensor improved with synergetic effects of different properties of nanoparticles like conductivity, enhanced surface area to volume ratio and ability to separate the oxidation potentials of different analytes. All these attributes plays a major role in increasing the sensitivity and maintaining the lower limit of detection. Several metal nanoparticle/carbon nanotube substrate hybrid nanostructures have been used earlier as sensitive modified electrodes for different analytes (da Silva et al. 2018).

SEM study of the fabricated biosensor

The changes in surface morphology at each step of fabrication were studied using SEM and results of this study are shown in Fig. 3. The SEM image of the bare electrode (Fig. 3a) exhibits net-like, thin thread-like intercrossed, smooth and uniform structure of carbon nanotubes (Dash et al. 2014). After immobilization of ss-DNA (probe) on to the electrode, a transition from smooth to rough surface was recorded that probably exhibited the immobilization of ss-DNA probe on to the surface of carbon nanotubes (Fig. 3b). After hybridization of ssG-DNA of *S. enterica* onto the electrode, surface morphology further changed with an increase in roughness, thus confirming the binding of DNA onto the probe fabricated electrode surface (Fig. 3c).

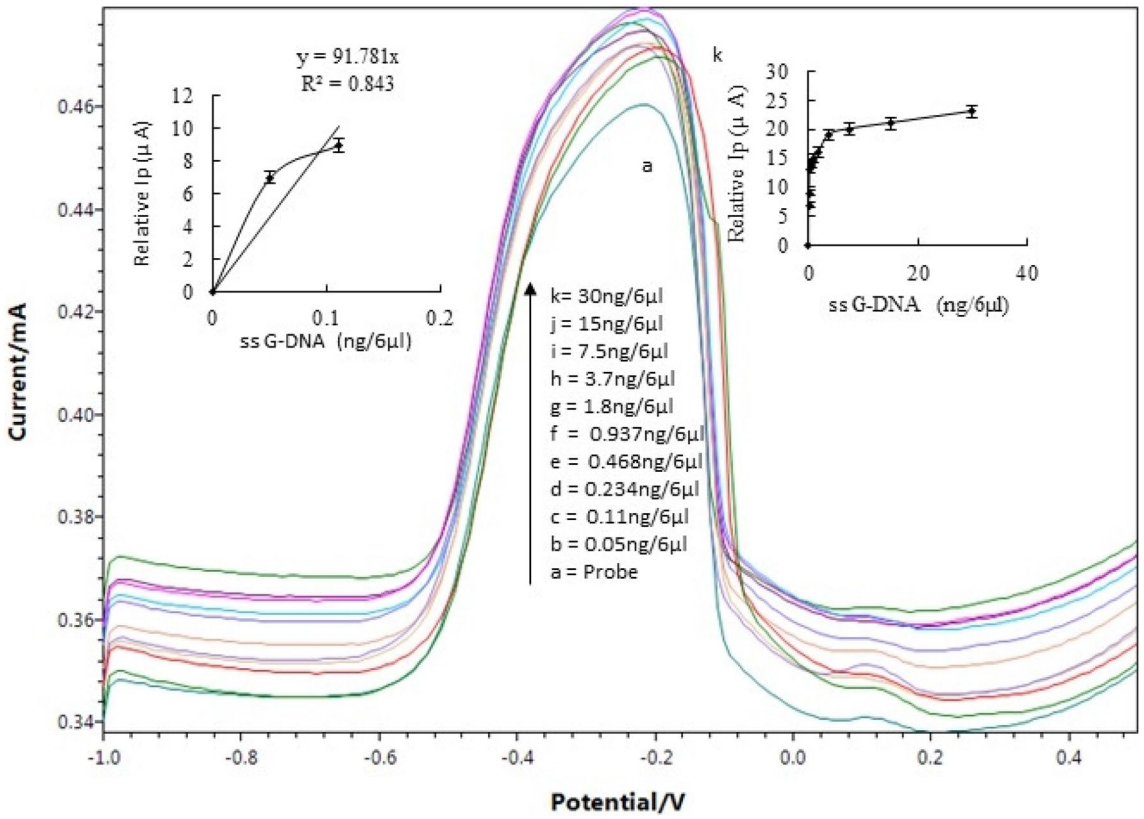


Fig. 2 DPV of (a) ss-DNA (probe) (b–k) hybridization with 0.05–30 ng/6 µl of *S. enterica* ssG-DNA at 50 mVs-1 using 1 mM MB in 50 mM PBS, pH 7.4. The inset-**A** shows a hyperbolic curve from 0 to 30 ng/6 µl with linear peak current (Ip) 0.05–0.11 ng/6 µl of ssG-DNA of *S. enterica*. Inset-**B** shows the linear plot from 0.05–0.11 ng/6 µl ssG-DNA for the calculation of sensitivity and LOD

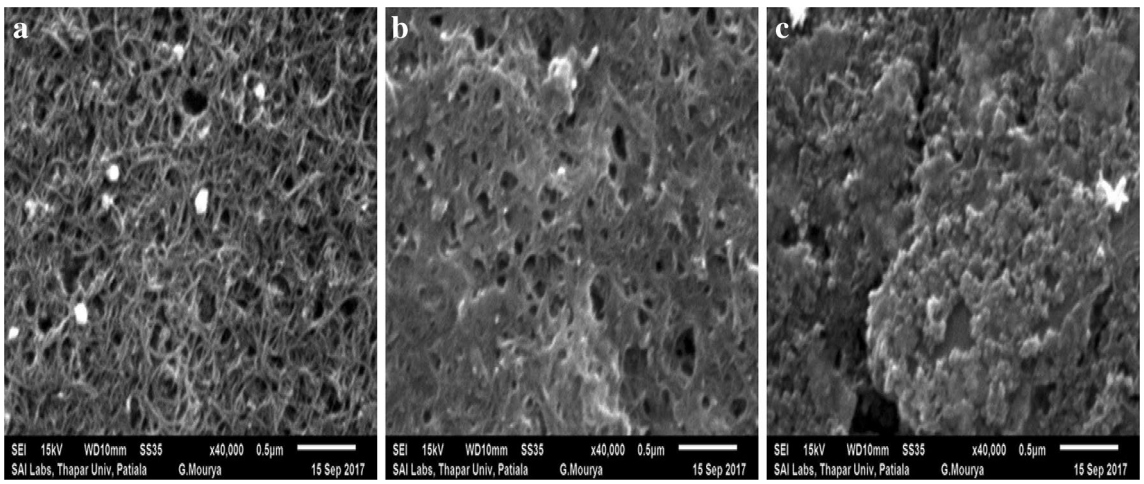


Fig. 3 Surface characterization of electrodes used in electrochemical biosensor fabrication for *S. enterica.* SEM micrograph of **a** c-MWCNT/AuNP **b** ss-DNA/c-MWCNT/AuNP and **c** dsDNA/c-MWCNT/AuNP after hybridization with 100 ng/6 µl ss-GDNA of *S. enterica*

Specificity and stability study of the sensor

The specificity of the developed sensor was further checked with *S. enterica* and other selected foodborne pathogens causing foodborne illness (Fig. 4). The relative peak current of the sensor after hybridization with ssG-DNA of other pathogens causing food poisoning or spoilage was found to be almost same as observed with

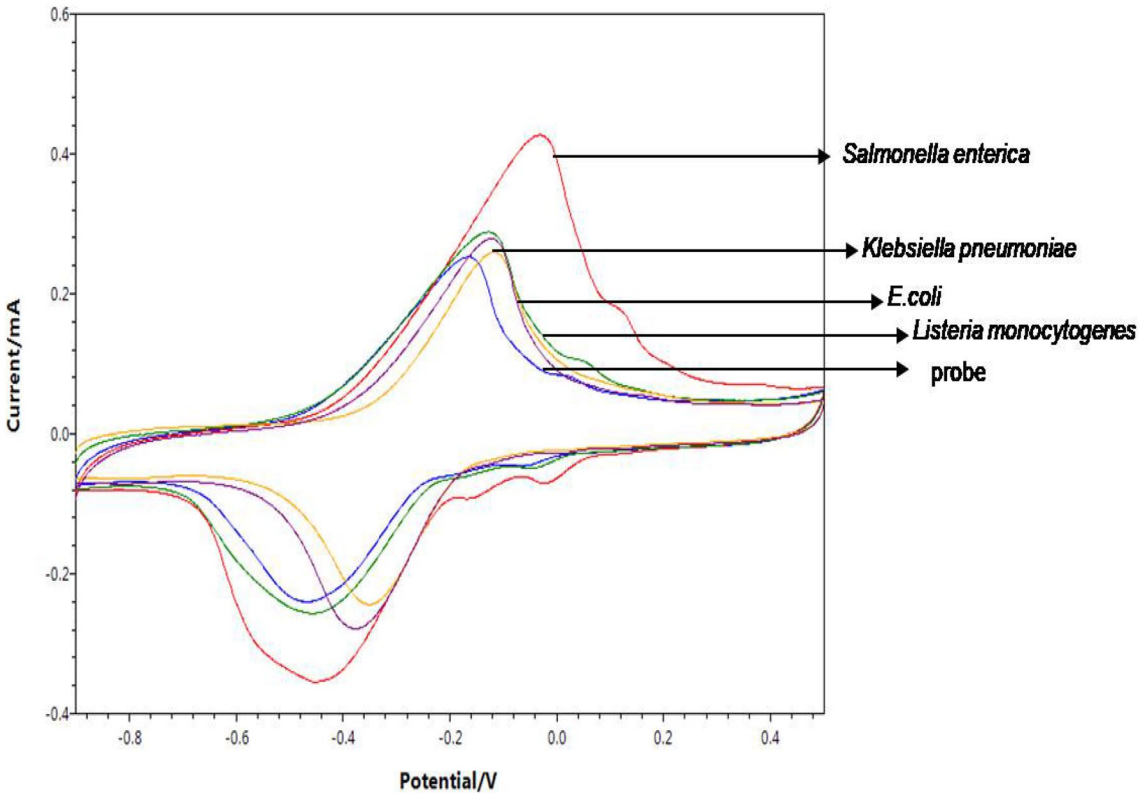


Fig. 4 Specificity of the fabricated c-MWCNT/AuNP sensor for *S. enterica* and other foodborne pathogens. The relative Ip value of CV (concerning immobilized probe) and after hybridization with 1.0 ng/6 µl ssG-DNA of *S. enterica* and other foodborne pathogens

immobilized probe using CV. However, a high peak current was recorded only with *S. enterica* thus, confirming the specificity of the developed sensor to *S. enterica* only.

The stability of ss-DNA probe immobilized electrode was further studied by measuring change in CV peak current at a regular interval of 30 days each on storage at

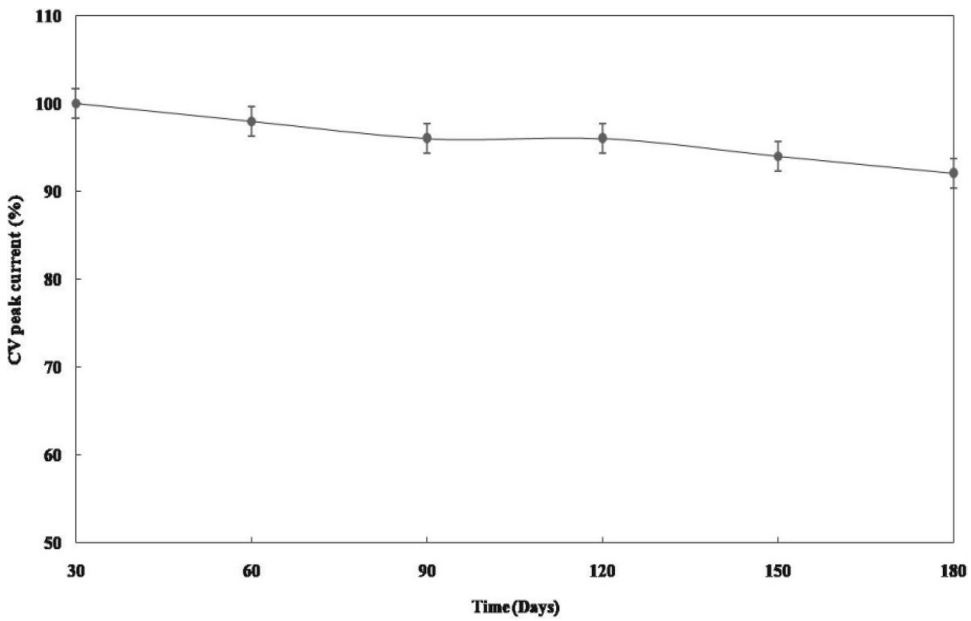


Fig. 5 Stability of c-MWCNT/AuNP electrochemical sensor measured as % relative peak current (for probe as zero using CV) after a regular interval of 30 days up to 6 months on storage at 4 °C

Table 1 Comparison of the c-MWCNT/AuNP-based DNA sensor with earlier reported methods for the detection of *S. enterica* in food samples

Type of biosensor	Organism	The matrix used in the study	Detection method used	Analytes	Limit of detection	Detection time	References
Electrochemical Biosensor	*Salmonella*	Polymer modified GCE	SWV	DNA	0.55 mM	–	Díaz-Serrano et al. (2011)
Electrochemical Biosensor	*Salmonella*	Gold electrode	EIS/SWV/SPR	DNA	0.5 pM	3.5 h	Li et al. (2012)
Electrochemical Biosensor	*Salmonella Enterica*	GCE/GO/AuNPs	CV/EIS	DNA	3 cfu/mL	–	Ma et al. (2014)
Electrochemical Biosensor	*Salmonella*	GCE	CV/EIS	DNA	25 cfu mL^{-1}	–	Jia et al. (2016)
Electrochemical Biosensor	*Salmonella typhimurium*	Sensor chip	–	DNA	0.94 nM	–	Savas et al. (2018)
Electrochemical Biosensor	*Salmonella typhimurium*	AuNP modified SPE	–	DNA	50 (±2.1) pM	1.3 h	Das et al. (2014)
Electrochemical Biosensor	*Salmonella Enterica*	c-MWCNT electrode	CV/DPV	DNA	1.8 pg/6 µl (0.3 pg/ml)	30 min	Present study

4 °C. During this study the probe immobilized electrode was found to be stable for 6 months with only 10% loss in initial Ip value of the CV (Fig. 5).

The analytical performance (detection time and limit of detection) of the developed nanosensor was also compared with the earlier reported methods used for the detection of *S. enterica* (Table 1). In earlier studies a electrochemical DNA biosensor was developed for the detection of *invA* gene of *Salmonella* using hybridization with a detection time of 3.5 h and LOD of 0.5 pM ((Li et al. 2012). However, in the present reported study, the sensor using c-MWCNT/AuNP was found to be better than above reported methods in terms of detection time, higher sensitivity, limit of detection, specificity, and stability. The detection time for the developed nanosensor in present study was found the minimum, i.e., 30 min among the compared methods.

Detection of *Salmonella enterica* in different milk samples

The developed nanosensor was also validated using twenty raw milk samples and three milk samples artificially spiked with *S. enterica* (Fig. 6). The change in current concerning the probe, (milk sample without *S. enterica*) and positive control (milk sample with *S. enterica*) serves as a threshold for the test milk samples. In Fig. 6, the *y*-axis denotes the relative peak current in CV with respect to the unhybridized probe (as negative control) without ssG-DNA in the sample to hybridize the probe (no increase in peak current), positive control with ssG-DNA of *S. enterica* hybridized with probe and showing an increase in the peak current as compared to the control (unhybridized probe). The ssG-DNA of the milk sample containing *S. enterica* was hybridized with the probe, and relative peak current (Ip) was monitored using CV. The positive and negative sample peak currents were plotted against the controls mentioned above. Negative controls showing absence of *S. enterica* (no complementary ssG-DNA) may have other pathogens (non complementary DNA or even no DNA) causing no significant increase in the peak current, whereas in the positive samples (with same ssG-DNA hybridizing concentration as used in ssG-DNA positive control), the peak current corresponds to the positive control with > 10% variation in average peak current.

Conclusion

In the present study, an amperometric nanosensor based on c-MWCNT/AuNP was developed for specific detection of *S. enterica.* The sensitivity of the sensor was observed to be 728.42 (µA/cm^2)/ng, and the LOD was 1.8 pg/6 µl (0.3 pg/ml) with a regression coefficient (R^2) of 0.843 using DPV. The developed sensor is highly specific for *S. enterica* and can detect the organism within 30 min.

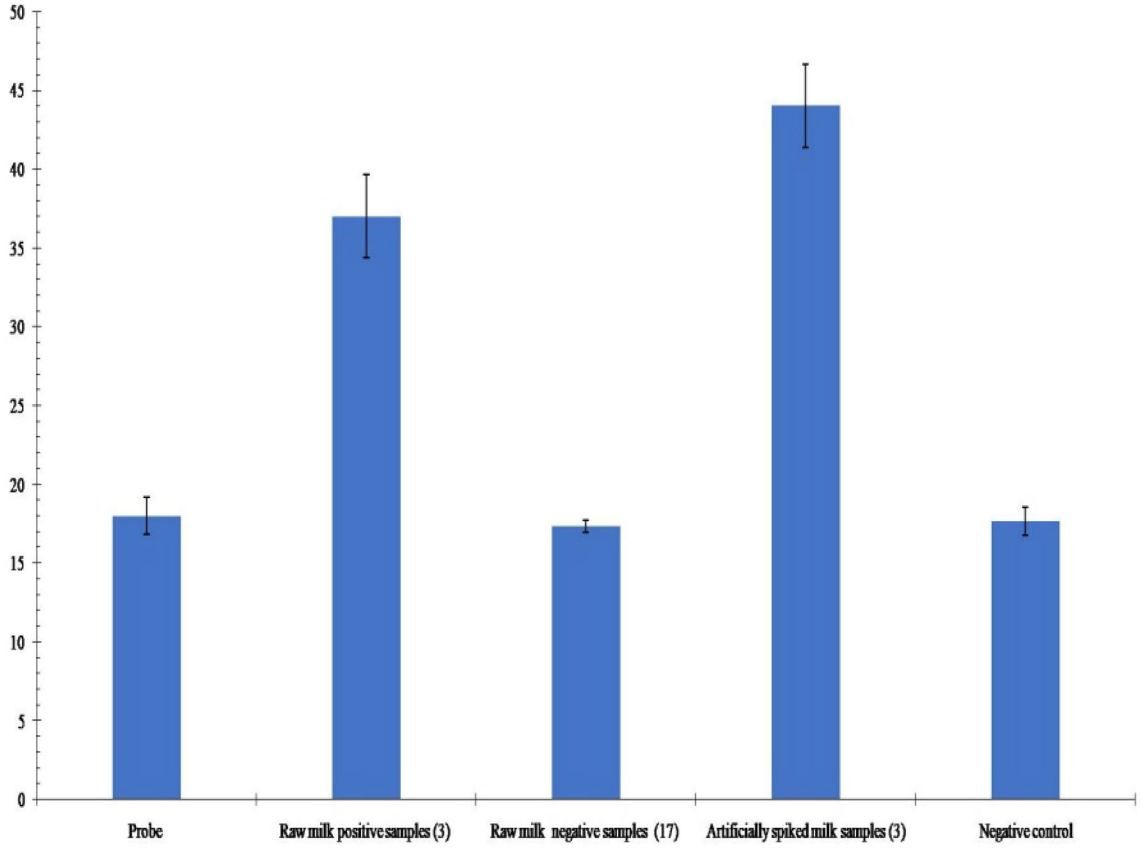


Fig. 6 Validation studies of c-MWCNT/AuNP electrochemical sensor by measuring relative peak current using CV after hybridization with ss-GDNA isolated microorganisms in raw milk samples

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Compliances with ethical standards

Conflict of interest There is no conflict of interest for authorship or related to any other context between authors.

Ethical standards The authors have complied and worked within standard ethical norms.

References

Alocilja EC, Zhang D, Shi C (2013) AuNP-DNA biosensor for rapid detection of *Salmonella enterica* serovar enteritidis. ACS Symp Ser 1143:43–53. https://doi.org/10.1021/bk-2013-1143.ch003

Das R, Sharma MK, Rao VK (2014) An electrochemical genosensor for *Salmonella typhi* on gold nanoparticles-mercaptosilane modified screen-printed electrode. J Biotechnol 188:9–16. https://doi.org/10.1016/j.jbiotec.2014.08.002

Dash SK, Sharma M, Khare S, Kumar A (2014) Carbon-mercaptooctadecane/carboxylated multi-walled carbon nanotubes composite based genosensor for detection of bacterial meningitis. Indian J Microbiol 54(2):170–177. https://doi.org/10.1007/s12088-013-0435-7

Díaz-Serrano M, Rosado A, Del Pilar J (2011) A Polymer-based electrochemical DNA biosensor for *Salmonella*: preparation, characterization, and calibration. Electroanal 23(8):1830–1841. https://doi.org/10.1002/elan.201000698

Dong J, Zhao H, Xu M (2013) Label-free electrochemical impedance immunosensor based on AuNPs/PAMAM-MWCNT-Chi nanocomposite modified glassy carbon electrode for detection of *Salmonella typhimurium* in milk. Food Chem 141(3):1980–1986. https://doi.org/10.1016/j.foodchem.2013.04.098

Hendriksen RS, Vieira AR, Karlsmose S et al (2011) Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis 8(8):887–900. https://doi.org/10.1089/fpd.2010.0787

Jia F, Duan N, Wu S et al (2016) Impedimetric *Salmonella* aptasensor using a glassy carbon electrode modified with an electrode deposited composite consisting of reduced graphene oxide and carbon nanotubes. Microchim Acta 183(1):337–344. https://doi.org/10.1007/s00604-015-1649-7

Kaushal A, Kumar D, Khare S, Kumar A (2012) *speB* gene as a specific genetic marker for early detection of rheumatic heart disease in human. Mol Cell Biol 58(1):50–54. https://doi.org/10.1170/T920

Li Q, Cheng W, Zhang D et al (2012) Rapid and sensitive strategy for *Salmonella* detection using an *InvA* gene-based electrochemical DNA sensor. Int J Electrochem Sci 7(1):844–856

Ma X, Jiang Y, Jia F et al (2014) An aptamer-based electrochemical biosensor for the detection of *Salmonella*. J Microbiol Methods 98:94–98. https://doi.org/10.1016/j.mimet.2014.01.003

Nagraik R, Kaushal A, Gupta S et al (2019) Optimized DNA-based bioassay for *Leptospira interrogans* detection: a novel platform for leptospirosis diagnosis. 3 Biotech 9:284–291. https://doi.org/10.1007/s13205-019-1815-4

Ozdemir K, Acar S (2014) Plasmid profile and pulsed-field gel electrophoresis analysis of *Salmonella enterica* isolates from humans in Turkey. PLoS One 9(5):e95976. https://doi.org/10.1371/journal.pone.0095976

Parvej MS, Nazir KH, Rahman MB (2016) Prevalence and characterization of multi-drug resistant *Salmonella enterica* serovar Gallinarum biovar Pullorum and Gallinarum from chicken. Vet World 9(1):65–70. https://doi.org/10.14202/vetworld.2016.65-70

Pashazadeh P, Mokhtarzadeh A, Hasanzadeh M (2016) Recent advances in materials and methods for sensing *Salmonella* infections. Biosens Bioelectron 87:1050–1064. https://doi.org/10.1016/j.bios.2016.08.012

Savas S, Ersoy A, Gulmez Y et al (2018) Nanoparticle enhanced antibody and DNA biosensors for sensitive detection of *Salmonella*. Materials 11:1541. https://doi.org/10.3390/ma11091541

Silva Da, Ghica ME, Brett CM (2018) Gold nanoparticle decorated multiwalled carbon nanotube modified electrodes for the electrochemical determination of theophylline. Anal Methods 10(47):5634–5642. https://doi.org/10.1039/c8ay02150c

Singh S, Kaushal A, Khare S et al (2014) Mga genosensor for early detection of human rheumatic heart disease. Appl Biochem Biotechnol 173:228–238. https://doi.org/10.1007/s12010-014-0836-z

Taha EG, Mohamed A, Srivastava KK et al (2010) Rapid detection of *Salmonella* in chicken meat using immunomagnetic separation, CHROMagar, ELISA and real-time polymerase chain reaction (RT-PCR). Int J Poult Sci 9:831–835. https://doi.org/10.3923/ijps.2010.831.835

Xu H, Lee HY, Ahn J (2010) Growth and virulence properties of biofilm-forming *Salmonella enterica* serovar Typhimurium under different acidic conditions. Appl Environ Microbiol 76(24):7910–7917. https://doi.org/10.1128/AEM.01508-10

Zhu L, Zhao R, Wang K et al (2008) Electrochemical behaviors of methylene blue on DNA modified electrode and its application to the detection of PCR product from NOS sequence. Sens J 8(9):5649–5660. https://doi.org/10.3390/s8095649