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Article

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ELECTROCHEMICAL GENOSENSOR TO DETECT PATHOGENIC BACTERIA (*E.COLI O157:H7)* AS APPLIED IN REAL FOOD SAMPLES (FRESH BEEF) TO IMPROVE FOOD SAFETY AND QUALITY CONTROL

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ABSTRACT

The electrochemical genosensor is one of the most promising methods for rapid and reliable detection of pathogenic bacteria. In the previous work, we perform an efficient electrochemical genosensor for detection of *Staphylococcus aureus* by using lead sulfide nanoparticles (PbSNPs). As a continuation of this study, in the present work, the electrochemical genosensor was used to detect *E. coli* O157:H7. The primer and probes were designed using NCBI data base and Sigma Aldrich primer and probe software. The capture and signalizing probes were modified by thiol (SH) and amine (NH_2), respectively. Then, the signalizing probe was connected using cadmium sulfide nanoparticles (CdSNPs) which show well defined peak after electrochemical detection. The genosensor was prepared by immobilization of complementary DNA on the gold electrode surface which hybridizes with a specific fragment gene from pathogenic to make a sandwich structure. The conductivity and sensitivity of the sensor were increased by using Multi-walled carbon nanotubes (MWCNT) that had been modified using chitosan deposited as a thin layer on the GCE surface, followed by a deposit of bismuth. The peak currents of *E. coli* O157:H7 correlated in a linear fashion with the concentration of tDNA. The detection limit was 1.97×10^{-14} M and the correlation coefficient was 0.989. A poorly-defined current response was observed as

the negative control and baseline. Our results showed high sensitivity and selectivity of the electrochemical DNA biosensor to the pathogenic bacteria *E. coli* O157:H7. The biosensor was also used to evaluate the detection of pathogen in real beef samples contaminated artificially. Compared with other electrochemical DNA biosensors, we conclude that this genosensor provides for very efficient detection of the pathogenic bacteria. Therefore, this method may have potential application in food safety and related field.

KEY WORDS

Electrochemical genosensor, Sandwich structure, Cadmium sulfide nanoparticle, *E. coli* O157:H7, Beef sample.

▪ INTRODUCTION

Foodborne bacterial infections and diseases are a major cause of illness and death worldwide[1] *E. coli* is common bacteria in both of food and human and predominant species of facultative anaerobes in the gut of animals. *E. coli* O157:H7 (designated by its somatic, O, and flagellar, H, antigens) was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982[2, 3]. There is an enterohemorrhagic strain of the bacterium and gram negative pathogen that has been responsible for several diseases, such as hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS), caused by the presence and expression of Shiga toxins that can occur with very low exposure (less than 10 cells) to the bacterium [3,4-5]. Some of the *E. coli* strains found in human feces also are themselves pathogenic.

E. coli is associated with infection in both food and humans, and it has been associated with food-related outbreaks in many countries and often involves contaminated beef and fresh produce. It exists in soils, fruits and raw vegetables, and it is a significant foodborne pathogen

carried in the intestinal tract of cattle and other animals. The bacteria may be transmitted to humans through fecal contamination of food[5]. The common source of infection is the consumption of contaminated food, especially meats, untreated water, unpasteurized milk and foods that have been in direct contact with animals. *E. coli* O157:H7 is commonly found in the intestinal tract of warm-blooded animals and the contamination depends on interaction with gut tissues where the pathogens adhere to the host cells. Consumption of foods contaminated with this pathogen can cause gastroenteritis as well as life-threatening diseases. Therefore, food safety becomes an issue of worldwide interest when we have repeated outbreaks of bacterial pathogens. Food safety in many countries requires monitoring, and more consumers and organizations now pay attention to food safety issues which may prevent complication.

Testing of food and food raw materials for the presence of pathogenic microorganisms and toxin producers is classically done using traditional method that such as plate samples. This is widely used and relies on a series of selective or non-selective media, each of which allows for growth of a certain group of pathogens. The detection of microorganisms using this traditional method (conventional plate count) is time-consuming, taking at least 2-5 days for results and up to 7–10 days for the final confirmation. Therefore, polymerase chain reaction (PCR) has been used to amplify the target DNA. However this method is time consumuming and requires expensive instrument [6]. In recent years, research has developed new methods[7]. However, these methods often fail to differentiate pathogenic species from nonpathogenic ones. Presently, the electrochemical method for DNA detection of pathogenic microorganisms in food safety has been receiving more attention because of its advantages of speed, sensitivity, selectivity and lower cost. In a method based on nanoparticles, the specific gene can be detected[8]. Another method developed is the biosensor. Much literature has focused on the biosensor, specially the

genosensor in particular due to high specificity which avoids false results as happen in traditional method and biochemical method. This approach is crucial in connection with research efforts directed at gene analysisas well as in detection of genetic disorders and tissue matching. Electrochemical sensors have received considerable attention due to their quickly response, remarkably higher sensitivity, good selectivity and strong operability for the detection of pathogenic microorganisms[9, 10]. The target DNA must be hybridized with a fixing probe (which is conjugated with nanoparticles). Another signalizing probe must be used that is fixed on a gold electrode surface that has been modified by a thiol group. To be clear, the most critical step in the preparation of DNA electrochemical biosensors is the immobilization of DNA strands on the surface of the gold electrode[11]. If the hybridization step is successful and creates a sandwich structure, the signal can be measured electronically. The development of nanotechnology and nanoscience has produced different nonmaterial that can be used for sensor design to development sensitivity and selectivity. The use of a heavy metal to develop electrochemical biosensors is done in order to differentiate signals from the sandwich hybridization using a stripping method[12]. The nanoparticle was used as a marker to label a sequence known as the oligonucleotide[13]. For example, one study used the Cds nanocrystal based electrochemi luminescence biosensor, cadmium sulfide (CdS) is one of the most important binary semiconductors that we have, has been extensively studied in the field of sensors[14]. Another study[15] used MWCNT to increase the sensitivity of the electrode. The carbon nanotube (CNT) is one of the most widely studied nonmaterials. Numerous researchers have investigated the biocompatibility of functional CNTs, both in vitro and in vivo. However, the toxicological effects of MWCNT have not been thoroughly investigated, and some of the findings remain controversial[16]. MWCNT provide a large surface area, significant mechanical strength, efficient

electro-catalytic activity, and high electrical conductivity that enhance electron transfer with the electrode surface. In addition, they may serve as catalysts[17]. Specific nucleic acid sequences were detected by single, stranded DNA probes complementary to the target DNA [15]. The key step in setting up such systems is the employment of high-efficiency testing methods, which must be accurate and sensitive enough to detect even low levels of contamination in a given commodity in order to prevent pathogens from being transmitted through the food chain to the consumer. In present study, we measure a highly sensitive electrochemical genosensor for the rapid detection of *E. coli* H157:H7. We used complementary probes to construct a sandwich structure, CdNPs, and GCE decorated with MWCNT-Chi. Figure 1 show the preparation of the sandwich structure on a GE surface and depicts electrochemical DNA detection using CdSNPs labels.

▪ MATERIAL AND METHODS

Materials and reagents

Cadmium chloride ($CdCl_2$), sodium sulfide (Na_2S), sodium hydroxide (NaOH), 3-mercaptoacetic acid were used for the synthesis of cadmium sulfide nanoparticles (CdS NPs). Tri (2-carboxyethyl) phosphinehydrochloride (TCEP), and 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) purchased from sigma –Aldrich were used as carboxyl activating agents for the coupling of primary amines to create amide bonds with carboxylic group on the surface of NPs. Salmon sperm DNA was used as blocking agent during the hybridization. Sodium dodecyl sulfate (SDS) was used to remove the unhybridized DNA after immobilization and hybridization. Multi-walled carbon (purity > 95%, diameter 50 nm, length 10- 20 μm) was obtained from Nanjing Xianfeng Nanomaterials Technology, China. Bismuth nitrate obtained from Scharlau Chewwice S.A. (Spain) was applied to the surface of the electrode

to increase the sensitivity. All the oligonucleotides used in the sensor were purchased from Takara Co (Dalian, China) and Sangon Biotechnology Co., Ltd (Shanghai, China) with HPLC purification. All the solutions were prepared using ultrapure water from a Milipore Mili-Q system.

Apparatus

A centrifuge was used to concentrate and wash the NPs. A vortex mixer was used to combine the amine group with the CdSNPs. An incubator was used for the hybridization reaction and a refrigerator for the preservation of the CdSNPs. Nanodrop 1000 Spectrophotometer (Thermo Scientific, Ashville, NC, USA) was used to measure concentration and purity DNA. Electrochemical experiments were performed on potentiostat/galvanostat (Shanghai CH Instruments Co.) The three-electrode electrochemical detection system consisted of a GCE as a working electrode, an Ag/AgCl (saturated KCl) as a reference electrode and platinum wire as a counter electrode were used. A scanning and transmission electron microscope (TEM, SEM) were used to images and morphologies of the CdSNPs and GCE surfaces respectively.

Bacteria culture and genomic DNA extraction

E. coli O157:H7 (Accession number JX206444.1) was obtained from the Zhangjiagang Entry-Exit Inspection and Quarantine Bureau (Suzhou, Jiangsu province, China). *Aeromonas hydrophila* (Accession number, M84709) was obtained from the Freshwater Fisheries Research Center (Wuxi, China) and used as a negative control in this biosensor. The others strains were obtained from Jiangnan university laboratory. *A. hydrophila* was grown in Ampicilin starch agar phenol red while *E. coli* O157:H7 was grown in MacConkey Agar (CM908) obtained from Beijing Land Bridge Technology Co., Ltd. Inoculums media strains were grown aerobically and

incubated at 37° C for 24 h in Tryptic Soy Broth media (TSB). Genomic DNA was extracted after the incubated for 18 h. Then, the DNA was extracted using a modified boiling method [18]. Briefly, the culture was boiling for 10 min to kill all pathogenic bacteria, 1 ml of the culture was transferred in to an Eppindrof tube and centrifuged it at 13000 rpm for 5 min at 4° C to pellet the cells. The supernatant was discarded and pellet obtained was suspended in 500 µL of sterile deionized water with a vigorous vortex. The cell suspension was boiled in a water bath for 15 min and immediately cooled at -20° C for 10 min to cause a shock to the cells. Then, the sample was centrifuged a second time at 12000 rpm for 3 min. Finally the supernatant containing genomic DNA was harvested into another sterile Eppindorf and used as the DNA template for the PCR amplification.

Oligonucleotides, PCR condition and DNA purification

All oligonucleotides used in this study were synthesized using the integrated DNA technology of Takara Co, (Dalian, China) and were purified by HPLC. The DNA probes for the *E. coli* were established by designing the primers used for the insertion element gene of *E. coli* to be complementary with the probes. For the amplification of the DNA samples, two primers (forward and reverse) were used. The pairs of primers were designed according to the target bacterial gene using the databases of the National Center for Biotechnology Information (NCBI), and the Basic Local Alignment Tool (BLAST) software (http://www.ncbi.nlm.nih.gov/)[19] DNA calculator from Sigma-Aldrich. The specificity of the primer sequences was further verified using software (http://www.sigmagenosys. com/calc/DNACalc.asp). All primer and probe sequences used in this study are listed in Table 1.

The reaction solution for PCR was performed in a total reaction volume of 25 µl, containing 2.5 µl of 10×PCR buffer, 2 µl of 10 mM of each dNTP (10 mM of each dNTP), 1µl of each of the forward and reverse primers (0.4 µM concentration), 0.25 µl of Qiagen Taq, 17.25 DNAase- free deionized water and 1µl tDNA. *A. hydrophila* (ATCC 7966) was used as a negative control. The amplifications were carried out in a thermal cycler (TAkaRa, GRADIENT PCR, Japan) using the following conditions: an initially denaturation at 94º C for 2 min, then followed by 30 cycles of denaturation at 94° C for 30 s, annealing at 45-53° C for 30 s for *E. coli* O 157:H7 and *A. hydrophila*, respectively. Finally extension at 72° C for 5 min and then held at 4° C. PCR amplifications were run on 2 % agarose gel electrophoresis. Then, the bands were stained with Andy Safe ^{TM} and the images were obtained using ultra violet light using the BIO-RAD Gel doc 1000. A DNA fragment of 100 bp was used as the molecular size. A fragment (752 pb) of the *E. coli* O157:H7 (Accession number JX206444.1) was used, along with one (161 pb) of *A. haydrophila*. A PCR purified kit purchased from Sangon Biotech company (Shanghai, China), was set-up to purify the DNA products after PCR amplification, and used according to the manufacturer's guidelines.

Synthesis of nanoparticles

Glassware used in the procedure was cleaned in a bath of freshly prepared 3:1 HCl-HNO_3, rinsed thoroughly in twice-distilled water and dried in an oven. Cadmium sulfide nanoparticles (CdS NPs) were prepared according to the literature[20, 21] with a slight modification. Specifically, 0.023 g of cadmium chloride ($CdCl_2$) was dissolved into 100 mL of deionized water to yield an aqueous solution at a concentration of 0.1 mM. Then 2 µL mercaptoacetic acid ($C_2H_4O_2S$) was added as a stabilizer using vigorous stirring. The pH was adjusted to a final level of pH 11 using additional concentrated 0.5 mol L^{-1} NaOH. The solution was bubbled with nitrogen for 30 min

and then 50 ml of 1.34 mmol L^{-1} freshly-prepared sodium sulfide (Na_2S) was added dropwise. Solutions were filtered prior to use with a 0.22 μm microporous membrane filter. The mixture turned yellow immediately due to the formation of CdS, after additional stirring, the reaction was carried out for 24 h under bubbled nitrogen. The prepared colloid CdSNPs were stored in brown glass bottles at 4° C until being used.

Transmission and scanning electron microscopy (TEM, SEM):

Transmission electron microscopy (TEM) has been found to be an excellent tool for characterizing the morphology of nanoparticles. A drop of the mixed solution was put on a TEM specimen and lit it until it dried; then, a TEM and SEM image were performed. This equipment was chosen to characterize the morphology of the electrode surface.

Conjugation between CdS NPs and NH_2-modified oligonucleotides:

Cadmium sulfide (CdS) was conjugated to the NH_2- modified oligonucleotides by using imidazole and EDC according to[22], with slight modifications. Briefly, 2 O.D. of NH_2 - modified oligonucleotides were mixed with 200 μL of 0.1 M imidazole buffer and stirring for 30 min. Then100 μL freshly-prepared 1-ethyl- (3-dimethylaminopropyl) carbodiimide EDC (0.1 M) was added. Washed CdSNP was added and incubated at room temperature for 24 h with gentle stirring. Afterwards, the solution was centrifuged at 10000 rpm for 30 min to purify the CdNP-NH_2-modified oligonucleotide. The precipitate was washed and centrifuged at 13000 rpm, the pellet was re-suspended and stored at -20° C.

DNA immobilization

Prior to the immobilization step, the gold electrode was heated in a piranha solution (3:7 H_2O_2/concentrated H_2SO_4), rinsed with DD water and dried under nitrogen flow. Then, the gold electrodes were polished to a mirror-like finish using a microcloth with alumina powder of grain sizes 0.03 μm and 0.05 nm. Then, the electrodes were washed with water and 95 % ethanol respectively for 5 min to remove the residual alumina powder and adsorbed impurities. To achieve the maximum cleaning process, the bare of gold electrode was cleaned electrochemically in a 0.5 mol/L H_2SO_4 until a repeatable cyclic voltammogram was obtained. The clean electrodes were washed with ultra-pure water and dried under a pure nitrogen stream. The dried electrodes were initially immersed in a fixing probe modified with thiol group at 4° C overnight to allow the adsorption on to the gold surface. Finally, the electrodes modified with fixing probes were thoroughly rinsed with 10 mM PBS solution (1 M NaCl, pH 7.4) to remove unbounded fDNA[8, 11, 23].

DNA hybridization

Prior to hybridization, the purified tDNA was denatured in a water bath (95ºC) and immediately chilled in ice for 5 min. The product was diluted with a PBS buffer solution (pH 7.0) and serial dilution. The first hybridization was performed by immersing the gold electrodes in tDNA and incubating at 45º C for 1 h to allow the hybridization between the fixing probe and the tDNA. Then the electrodes were immersed in 0.1% sodium dodecyl sulfate (SDS) for 10 min to remove the unhybridized tDNA. In the second hybridization, the electrodes were incubated again for 1 h with a signalizing probe containing sDNA- CdSNPs. Then, the sandwich structure (fDNA- tDNA- sDNA- CdSNPs) was constructed. The electrochemical response was performed using electrochemical analyzer at room temperature [8, 23, 24].

225 **Dissolution of CdS and electrochemical measurements**

226 After immobilization and hybridization, the gold electrode with a sandwich structure was
227 immersed in 150 μL of 1.0 mol L^{-1} HNO_3 solution for 5 min to dissolve the CdSNPs fixed on the
228 surface of the electrode. The released CdSNPs ions were then ready for electrochemical
229 measurement. For the decoration step, MWCNT–Chi was dropped on to the GCE surface and
230 dried at room temperature. Then bismuth layer was deposition electrochemically by immerging
231 modified electrode into a solution containing 0.1 M acetate buffer solution and 100 μL of
232 bismuth ions, and a negative potential for 100s was applied [25]. Electrochemical measurements
233 including DPV, clean electrode, cyclic votammetry (CV) and electrochemical impedance
234 spectroscopy (EIS) were performed using an electrochemical analyzer (CHI Inc. Shanghai,
235 China) and a three-electrode system at room temperature. All experiments were carried out at
236 room temperature, and all measurements were replicated three times. All curves were the
237 average of at least three test results. Different bacteria concentrations were used, and each
238 bacterial concentration was plotted against the current peak. The deposition potential used in this
239 study was -1.2 Vvs for 100 s, while DPV potential was -1.2 Vvs.

240 **Real application of sensor in food matrix**

241 Fresh beef meat was purchased from a local market in Wuxi, China. The sample was
242 prepared and examined according to the literature[26]. Briefly, the sample was divided into
243 different parts and sterilized with radiation. Then, 10 grams of beef sample were inoculated with
244 100 μL of different bacteria concentration (*E. coli* O157:H7) ranging from 10 to 10^5 CFU/ml
245 onto the surface of the beef sample and stored at 4° C for 4 h. I the same manner, controls
246 consisted of un-inoculated samples were used. Afterwards, each beef sample was transferred to a

sterile 250 mL Erlenmeyer flask and ninety milliliters of TSB was added to obtain the proportion 1:10 (sample weight/total weight). Then, a 1.5 mL portion of rinse fluid was removed from each bottle and placed in a sterile 2 mL micro centrifuge tube for DNA extraction. The DNA of the pellet was extracted using a boiling method, and the PCR products were checked as above.

▪ RESULTS AND DISCUSSION

Characterization of nanoparticles and Electrode

The morphologies and size of the CdSNPs were estimated using the TEM, Figure 2. The image of the CdSNPs shows the average length ranged from 8 to 11 nm with good dispersion. Furthermore, the image shows that the particles did not aggregate after synthesis and storage. The size of the NPs affected the quality and connection with the signalizing probe [27]. We found that the size of the cadmium sulfide (CdS) was approximately 7–53 nm, which agrees with research [28] documenting using of CdSNPs with size greater than 10 nm. After electrode polish, the GE was cleaned electrochemically by scan in 0.5 mol/L H_2SO_4, Figure 3 shows the scan of electrode. The modified GCE were characterized by SEM in different steps. Figure (4-A) shows the image of the bare GCE (blank control) and shows only a blurred background of the electrode surface. Figure (4-B) shows the GCE covered with MWCNT-Chi after being dried. There was an electrochemical deposition of bismuth to the GCE modified by the MWCNT, as Figure (4-C) confirms.

PCR amplification

A pair of primer was designed to be specific to *E. coli* O157:H7. The specificity of the PCR products of the pure culture using positive and negative bacteria is shown in Figure 5. The results indicate that the primer pair was specific for the corresponding target organisms. It used the

amplification reactions for the PCR detection of the target genes of *E. coli* O157:H7 at approximately 752 bp, and at 161 bp for *A. hydrophila.* It produced a high yield of specific DNA target sequences. This yield was verified by gel electrophoresis, which revealed a band that did not appear in any PCR reaction with another DNA template (negative control). The bands intensity was different when genome was extracted from pure culture and real sample. The PCR product had double-stranded DNA oligonucleotides with a complementary sequence of both fDNA and sDNA.

Eectrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV)

EIS experiments were performed to characterize a modified electrode surface in different steps. It was extensively used for the study of the electron transfer on the modified surface. It also measured the sensitive electrode surface phenomena and changes in bulk properties [10]. EIS data were measured at 100 kHz to 1Hz at a wave amplitude of 5 mV and an electrode potential of 0.20 V in $Fe(CN)_6$ 3−/4−. Figure 6 shows the EIS in various modified GE steps. In this study, we selected thiol- terminated fDNA because of the strong affinity of thiol-metal (SH-Au) linkages, which provides connection with the gold surface[11]. The bare electrode showed a straight line. The R_{et} increased after being modified and hybridized, which indicated that electron transfer became more difficult. This phenomenon resulted from the negative charge on the sDNA that made the electron transfer of ferricyanide probes difficult due to electrostatic repulsion[8] and interaction between the material and frrecyanide anion.

Figure 7 shows characterized of the modified GCE measure by EIS. The bare GCE showed a clear well-defined reversible redox peaks corresponding to $[Fe\ (CN)_6]^{3-/4-}$ electron transfer process. When GCE was modified with MWCNT-Chi the semicircle curve was observed. The bismuth layer formed during electro deposition showed the slight decreasing in peak currents and

a negligible was observed. The bismuth was slightly inhibits the electron transfer process in solution. The combination of MWCNT-Chi-Bi was enhances the sensitivity, adherence and electro deposition of released Cd^{2+}. GCE decorated by MWCNT- Chi, this modification can allow to use several times. Prior to the another detection, a potential of + 0.3 V for 30 s can applied to remove the Bi - CdSNPs from the electrode surface, and then a new bismuth layer could be generated by electro deposition.

The effect of the scan rate on the electrochemical performance (redox reaction) of modified GCE (MWCNT–Chi–Bi) was investigated, using CV at different scan rates from 10 to 150 mV, to measure the current response of a ferrcyanide solution exposed to different potential. It was found that the sharp increase in peak currents was observed with an increase of scan rate, and the relationship was linearly related to the scan rate, indicating that the sensor has a good electrochemical and conductivity. After the deposition of bismuth, the charge transfer decrease which indicate that the electron transfer of $[Fe(CN)_6]3^-/4^-$ was blocked. Bismuth has poor electric conductivity, so that improve the electrical characteristics by using MWCNT in GCE surface to enhance the efficiently. Figure 8 shows the scan rate in a different current.

Electrochemical

The DNA detection process was performed in two main steps. The first was immobilization to allow the modified fixing probe on the GE surface to connect thiol group with GE[15]. Prior to immobilization, fDNA was mixed with tri (2-carboxyethyl) phosphine hydrochloride at a proportion of 1:2 and incubated at room temperature for 1 h to allow the thiol group bond to be free. This procedure facilitated the attachment to the GE surface. Bovine serum albumin (BSA) blocking was carried out after the probe immobilization and washed with PBS. Then, hybridization was accomplished by first adding serial concentrations of tDNA to connect with

fDNA, and then adding signalizing DNA containing CdSNPs. To conjugate and complete the sandwich structure, sDNA modified by CdSNPs with EDC was used to cross link the CdSNPs and the amine group [22], and washed with GE to remove any un-hybridized DNA. In the next step, the GE was submerged in 1M HNO_3 solution[20] to release the sandwich structure content CdNPs to be measured by DPV. Glass carbon electrode (GCE) was decorated with MWCNT–Chi –Bi, which improves the deposition released of cadmium ions on the electrode surface. CNT accelerates electron transfer due to the high conductivity. Therefore, it increases the sensitivity of MWCNT and characterizes the different steps, as shown in Figure 4. The electrochemical behavior of the CdSNPs ion was measured to investigate the sandwich using differential pulse voltammetry at the GCE surface and taking measurements of peak current. The DPV responses of the sensor to various concentrations of *E. coli* O157:H7 is showed in Figure 8. The negative control *A. hydrophila* was measured, and both steps (immobilization and hybridization) were included in tests.

Electrochemical DPV measurements

DPV is a useful method for the sensitive detection of micro amounts of metal ion. Therefore, it is used in the measurement of (Cd^{+2}) [20]. The electrochemical behavior of the sandwich structure of the genosensor was detected as showed in Figure 9. The peak current of various tDNA concentrations was observed, and we observed that the signal intensities increased dynamically with the increase of the concentration of tDNA. The calibration plot Figure 10 shows that the signals of released Cd^{2+} had a good linear relationship with the peak currents and serial dilution of tDNA, with a correlation coefficient of (0.981) and the linear equation and of ($y = 113.9x + 5.86$), where (y) is the concentration of *E. coli* O157:H7 and (x) is the current. However, the peak appears at approximately -0.85 V. The detection limit of this study was

338 (1.97×10^{-14} M) with the ratio of signal to noise (s/n) equal to 3. The hybridization selectivity of
339 the genosensor was investigated by the negative control sample (non-complementary sequence),
340 and the results not showed response at 0.8 to 0.9 V (CdSNPs). The results indicate that the
341 MWCNT had a remarkable enhancement effect on the electrochemical response. The synergistic
342 effect of the electronic conductivity and electro catalytic activity of the MWCNT enhanced ionic
343 conductivity and ion-exchange capacity[17]. Overall, these results showed that this genosensor has
344 good specificity and that only the complementary DNA sequence could form a sandwich
345 structure. Therefore, the DNA genosensor sensitivity for the detection of *E. coli* O157:H7 has a
346 limit of (1.97×10^{-14} M), while in the previous study[29], the limit detection was (3.17×10^{-14} M)
347 using *S. aureus* and PbNPs. The sensor is highly sensitive and has high specificity compared to
348 other studies. Table 3 shows the detection limits of other studies and shows that this method
349 performs better in sensitivity and specificity regarding tDNA. Positive correlation was observed
350 when comparing the signal generated by CdSNPs to the positive sample. The peaks showed a
351 slight shift when using different tDNA concentrations. This phenomenon occurred in the
352 interaction between the deposited materials, and the metals formed during electro-deposition.

353 **Application of genosensor in food matrix**

354 The application of the present system is tested for enumeration of bacteria in the 6 samples.
355 The time between sampling and analysis has to be controlled in order to prevent changes in the
356 concentration of the target microorganism which occur naturally over time [4]. The counts ranged
357 from (6.00 ± 0.24) $\times$ 10^2 to (2.47 ± 0.75) $\times$ 10^4 cfu/mL for *E. coli* O157:H7. Although the
358 infectious dose varies among pathogen types, it is generally believed that most bacterial
359 pathogens are able to cause infection when more than 10^3 cfu/mL is ingested [26]. There were
360 significant growth increases for different dilutions. Table 2 shows the enrichment for bacteria

inoculated onto a beef sample. The DNA was extracted and amplified as described above. The gel electrophoresis detection of PCR products for serial dilution inoculums in beef samples is shown in Figure 11. The PDV obtained for the beef samples is shown in Figure 12. Measurement of the electrochemical response of the positive samples was indicated at −0.85 V, and the peak increased with the increase in concentration. On the other hand, the negative control *A. hydrophila* produced a negligible current. The electrochemical genosensor detection method has many advantages, it is quick, specific and sensitive for pathogenic bacteria detection. In the present study, the electrochemical genosensor detection method was used to detect *E. coli* O157:H7 and showed high precision in *E. coli* O157:H7 detection. It could detect a very small amount of *E. coli* O157:H7. The genosensor was also applied in real samples (beef samples contaminated artificially) and showed good sensitivity and selectivity to pathogenic bacteria. The fDNA modified by a thiol group was enhanced and immobilized on GE. CdSNPs were synthesized using chemical methods and characterized by TEM; they were then conjugated with an amine group on the modified probe. Modification of MWCNT- Chitosan with bismuth on the GCE surface increased electrochemical detection capacity. The signal of the released cadmium ions increased with increasing tDNA concentration. Therefore, this method will be very useful in food safety and quality control as an efficient and quick method for monitoring pathogenic bacteria in samples.

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406 ▪ **REFERANCE**

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519 **Table 1 Oligonucleotides sequences used in amplify, immobilization and hybridization**
520 **experiments specific for the *E. coli* O157:H7.**

Target	Accession No.	Oligonucleotide	Sequence (5'-3')	Length (bp)	Amplicon size (bp)
Aeromonas hidrophyla	M84709	Forward primer	5`-CCAATATGTCGGTGAAGA-3`	18	161
		Reverse primer	5`-CATGTTTGAAGCTGTCAG-3`	18	
E. coli O157:H7	JX206444.1	Forward primer	5`-GATAAATCGCCATTCG-3`	16	752
		Reverse primer	5`-GTCACAGTAACAAACC-3`	16	
E. coli O157:H7	JX206444.1	Capture probe	5`-GGAACCTCACTGACGC-SH-3`	16	--
		Detection probe	5`-$NH_2$-TGTGGCAAGAGCGATG-3`	16	

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536 **Table 2 Enumeration of *E. coli* O157:H7 in artificially contaminated (fresh beef sample),**
537 **and after samples inoculated, the number was counted after incubation for 24 h at 37 ° C.**
538 **Data are expressed as mean ± standard deviation (average of three replications for *E. coli***
539 **O157:H7) PCR analysis was carried out after overnight culture.**

Dilution factor	E. coli O157:H7 ± S.D. (cfu/mL)
10^1	$(2.47\pm0.75)\times10^4$
10^2	$(1.08\pm 0.38)\times10^4$
10^3	$(7.09\pm1.45)\times10^3$
10^4	$(2.09\pm0.52)\times10^3$
10^5	$(6.00\pm 0.24)\times10^2$

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553 **Table 3 The limit detection sensitivity and comparison performance of the genosensor with**
554 **other electrochemical DNA biosensor.**

Target	Methods	Detection limit	Ref
Synthesized oligonucleotide	DNA BIosensor	1.0×10^{-10} moll^{-1}	12
Low-Density Lipoprotein	CdS Nanocrystal – Based Electrochemi luminescence Biosensor	0.006 ng mL-1	21
Synthesized DNA	The elongated hexagonal- pyramid CdS (eh-CdS) modified	3.9×10^{-15} M.	14
syntheses (NOS) terminator gene sequence	Differential pulse anodic stripping voltammetric method.	2.75×10^{-12}M	20
staphylococcus aureus nuc gene	chitosan-Co_3O_4 nanorod- graphene	4.3×10^{-13} M	30
staphylococcus aureus	Electrochemical genosensor based on multiwalled carbon nanotubes, chitosan and bismuth	3.17×10^{-14} M	29
E. coli O157:H7	Electrochemical genosensor based on multiwalled carbon nanotubes, chitosan and bismuth	1.97×10^{-14} M	This study

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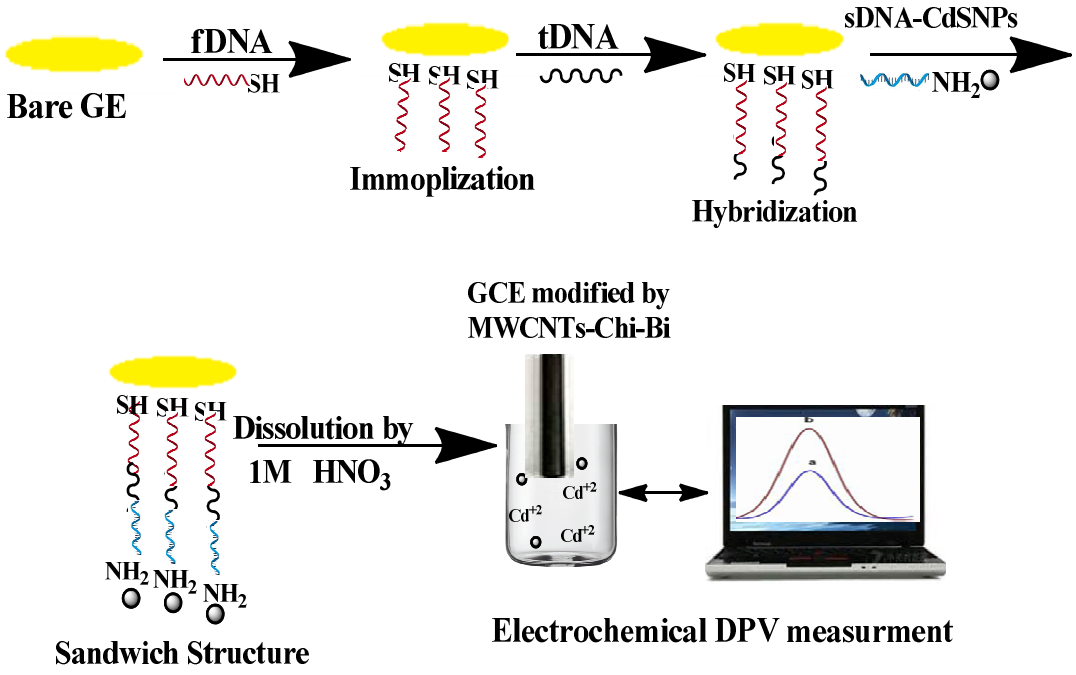

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560 **Figure 1. Schematic performance of the electrochemical detection of target DNA based on**
561 **CdSNPs labeled oligonucleotides DNA probes.**

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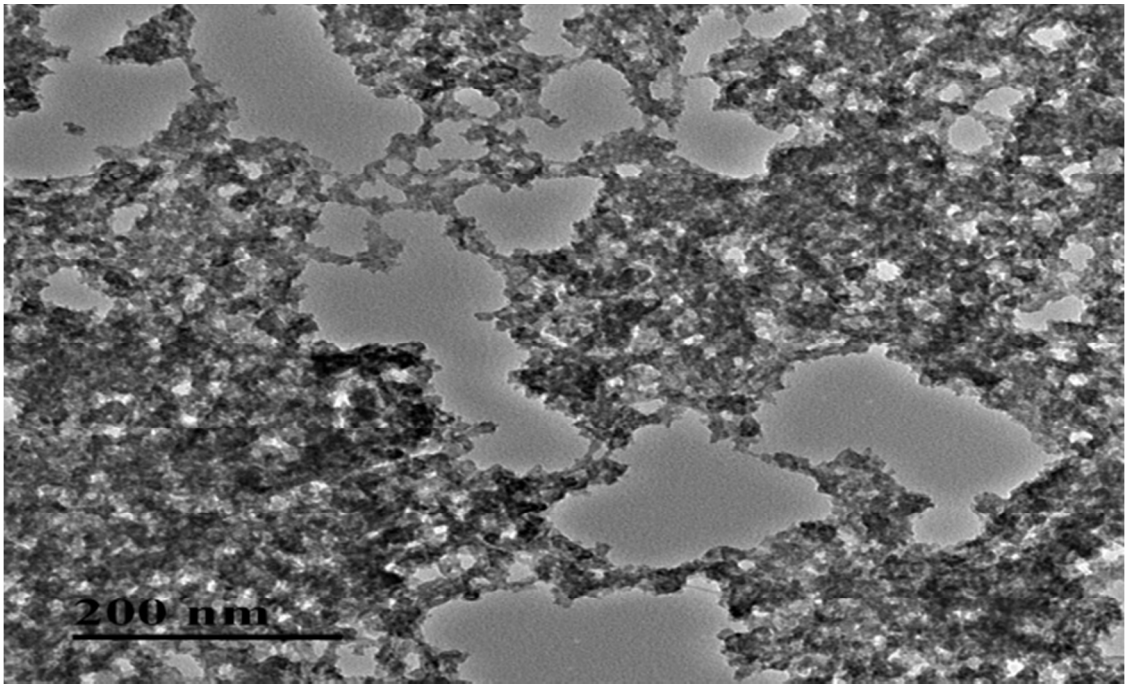
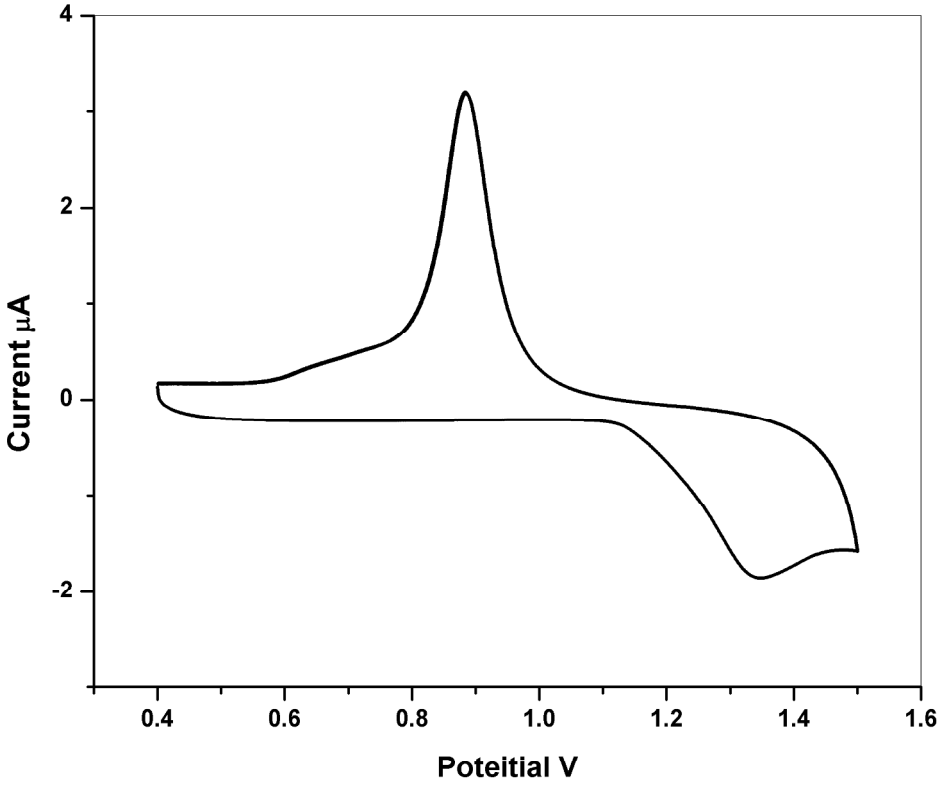


Figure 2. Transmission electron microscopic (TEM) micrographs of the synthesized CdSNPs



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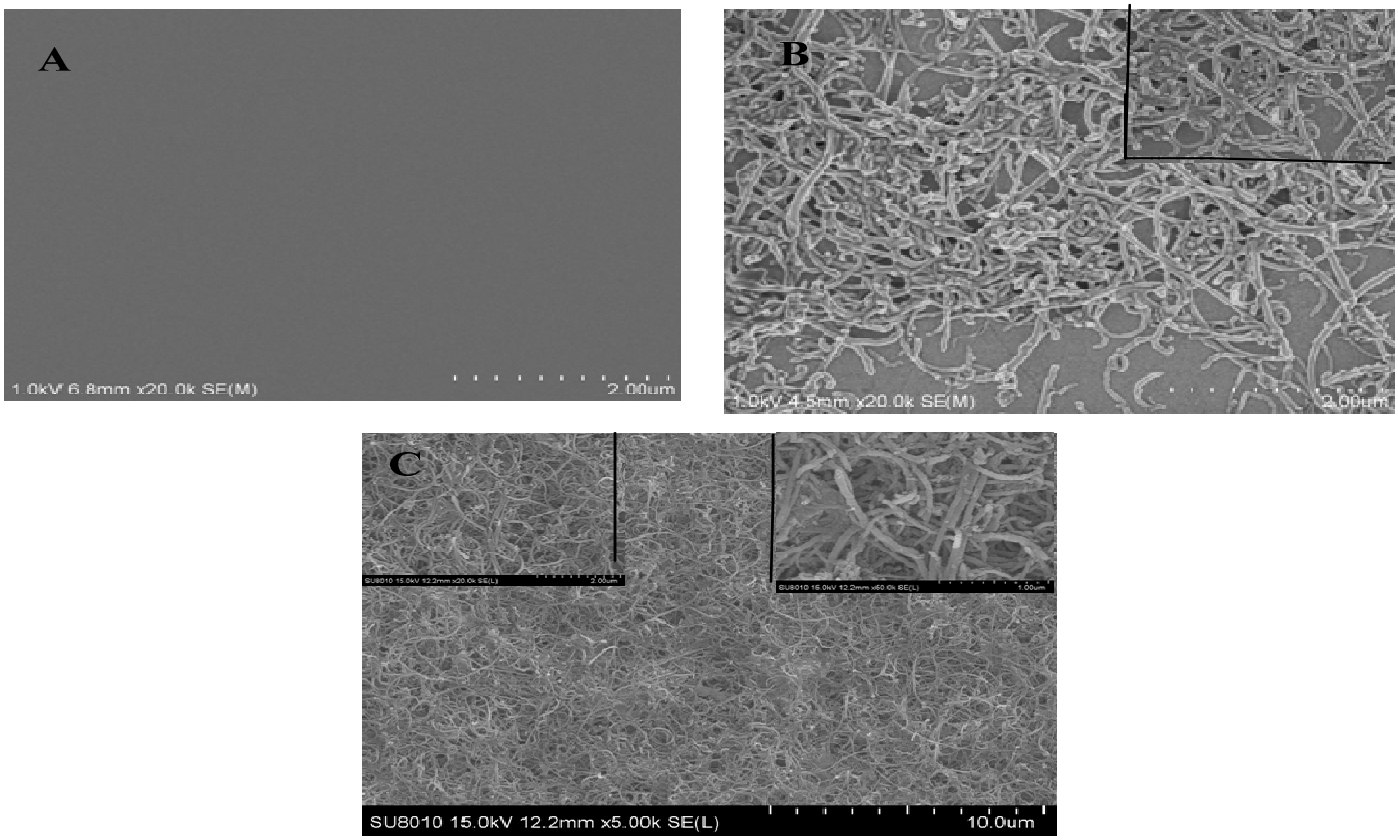
572 **Figure 3. Cleaning gold electrode by use 0.5 mol/L H_2SO_4**



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575 **Figure 4. Surface scan Electronic Microscopic (SEM) image of GCE morphological**
576 **changes in deferent resolutions: (A) bare electrode (B) GCE covered with MWCNT-Chi**
577 **(C) MWCNT-Chi-Bi**

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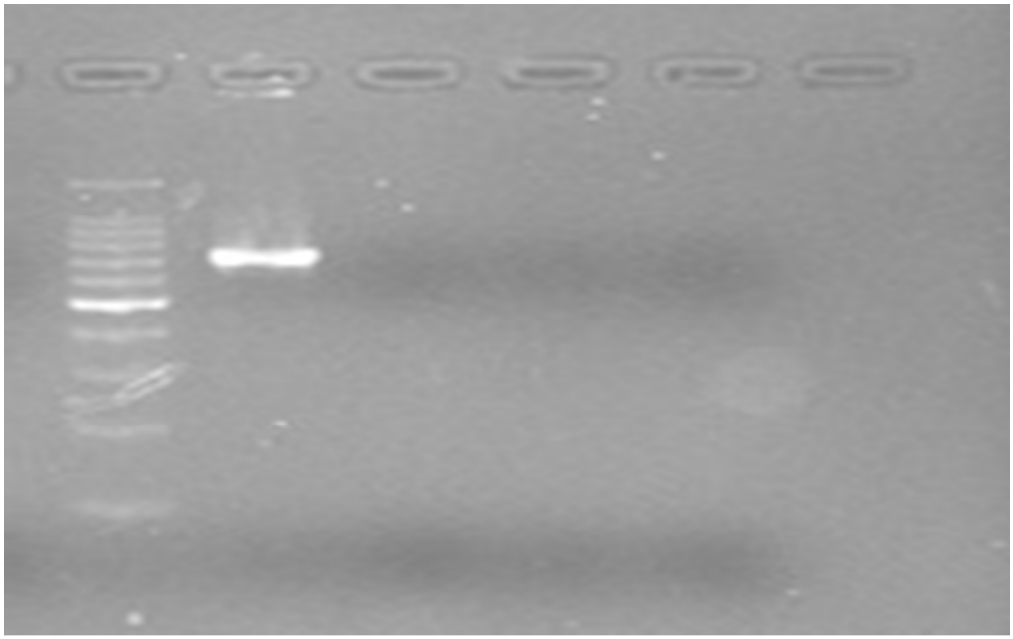


Figure 5. Agarose Gel Electrophoresis analysis (2 %) of PCR amplicon. Lane 1 and molecular marker (100 bp), Lane 2, positive test sample *E. coli* 157:H7 (accession number JX206444.1), Lane 3 negative control containing PCR mixture with *Aeromonas hydrophyla* (ATCC 7966), Lane 4, Negative control containing PCR mixture with *Staphylococcus aureus* (accession number EF529607.1).

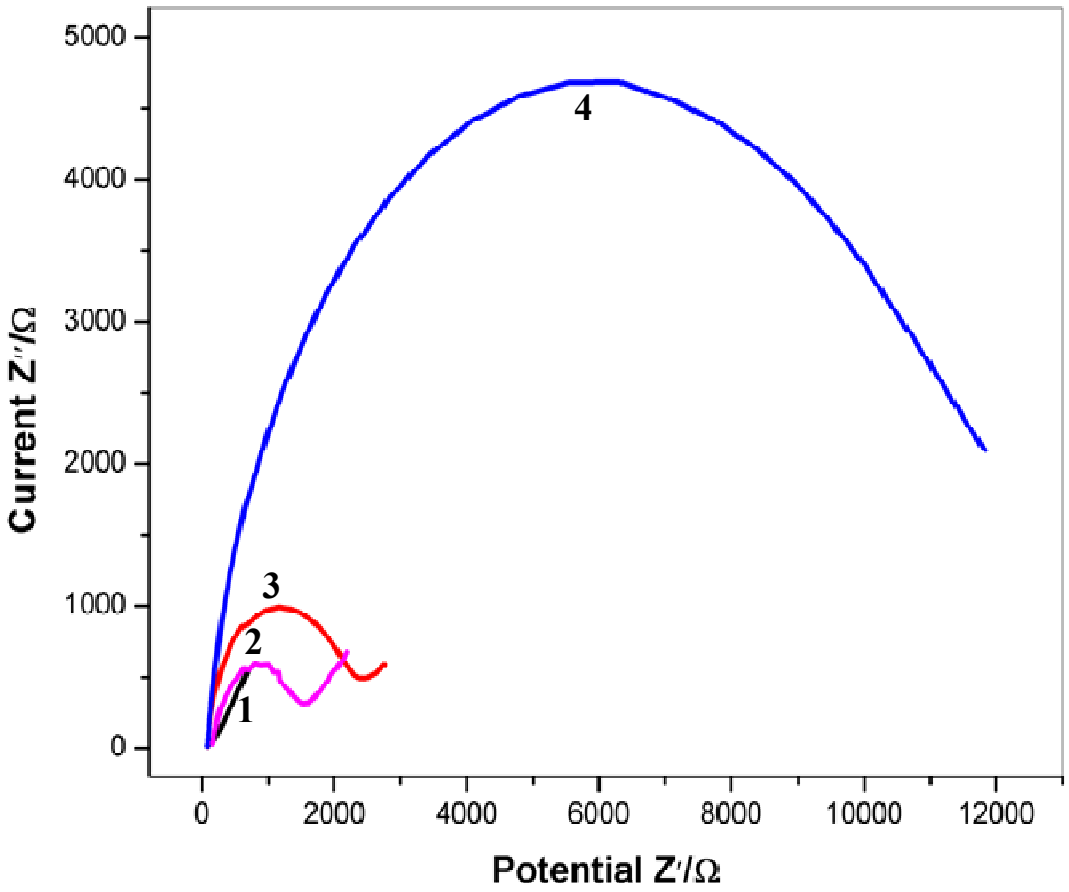
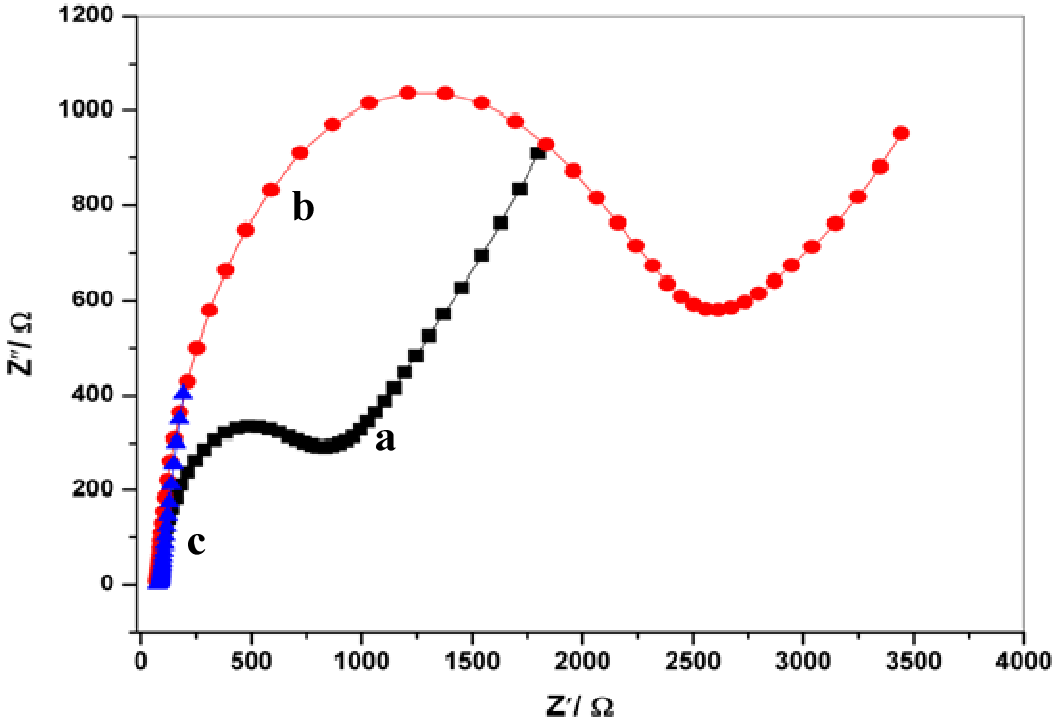


Figure 6. EIS of the electrode at different stages (different film modified electrodes) in Potassium ferricyanide [$K_3OFe(CN)_6$]. (1) On the bare gold electrode surface. (2) Gold electrode and fixing probe. (3) Gold electrode, fixing probe and tDNA. (4) Gold electrode, fixing probe, tDNA and capture probe.



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598 **Figure 7. Impedance spectroscopy EIS for GCE in different modifications steps obtained in**
599 **1mM $K_3Fe(CN)_6$ solution. (a). Bare GCE, (b). GCE modified by MWCNT–Chi, (c). GCE**
600 **modified by MWCNT– Chi–Bi.**
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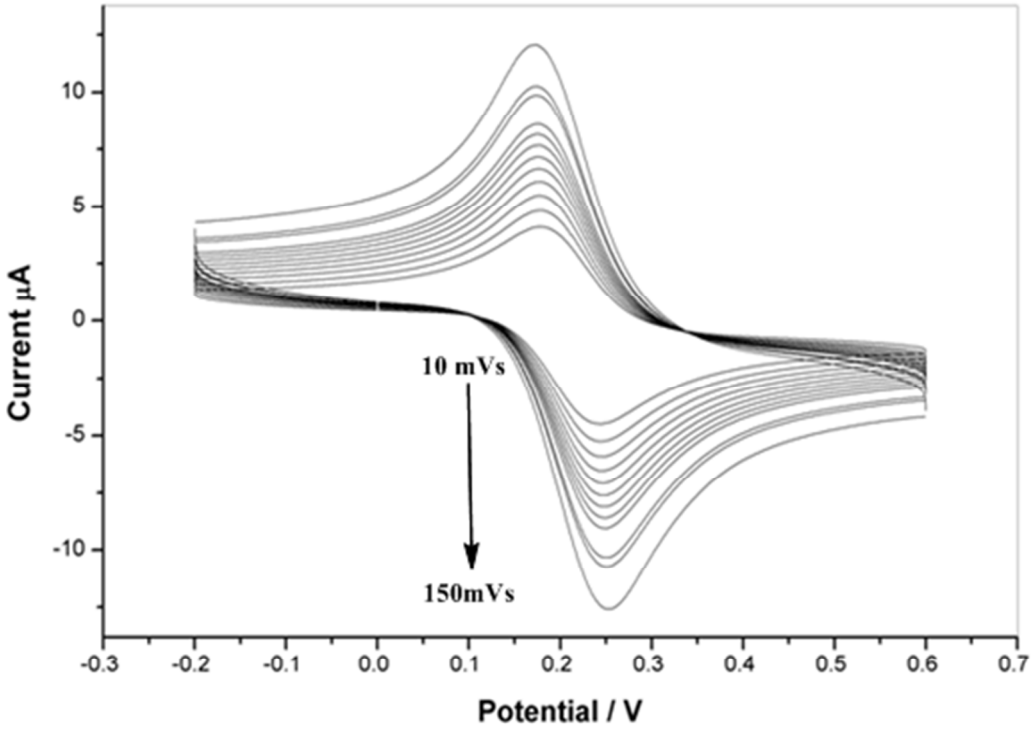

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603 **Figure 8. Cyclic voltammetry acquired on modified GCE in a solution containing**
604 **$Fe(CN)_6^{3-/4-}$, at different scan rates from 10 to 150 mVs-1. Scan range between − 0.2 to 0.6**
605 **V.**

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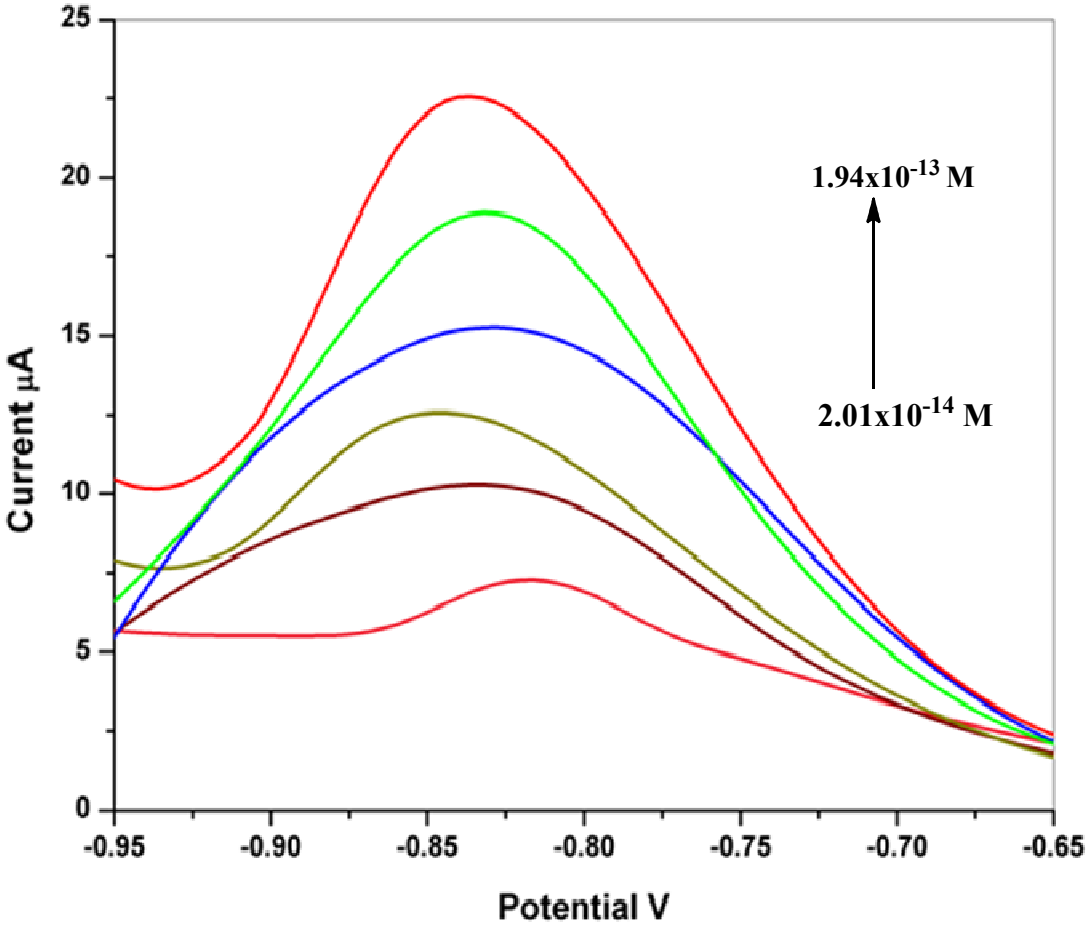

Figure 9. DPV analysis of electrochemical DNA sensors to detect tDNA with different concentration using CdSNPs for *E. coli* O157:H7 genomic DNA labeling, with sandwich structure fDNA – tDNA – cDNA – CdSNPs. The tDNA concentration range between 1.94×10^{-13} to 2.01×10^{-14} M

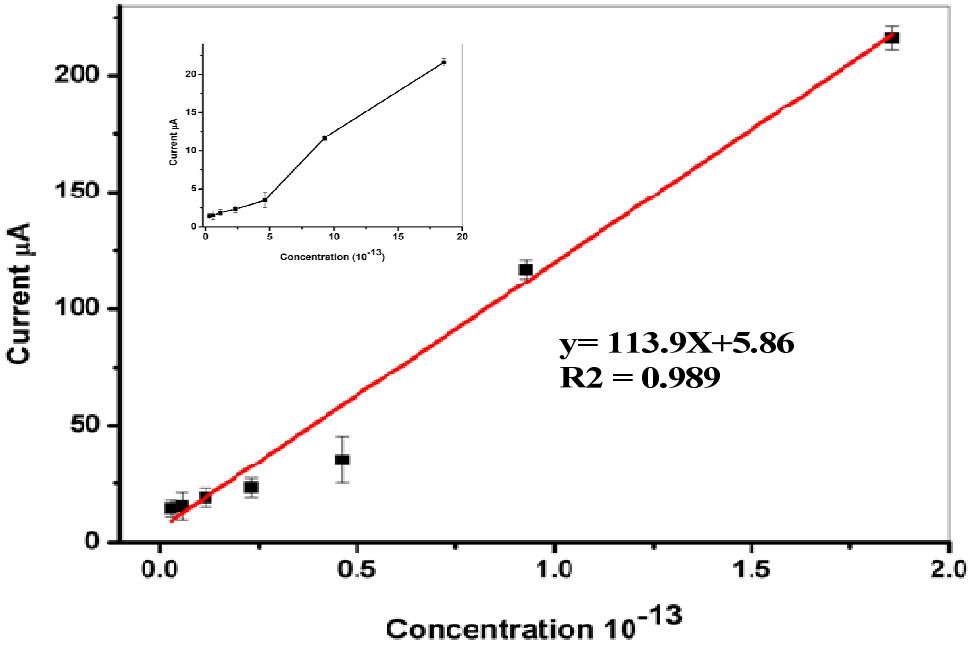

Figure 10. Corresponding calibration plots of peak current versus the concentration of *E. coli* O157H7 genomic DNA detection. The presented values are an average of triplicates with standard deviation. The resulting standard calibration curve of different target DNA concentration.

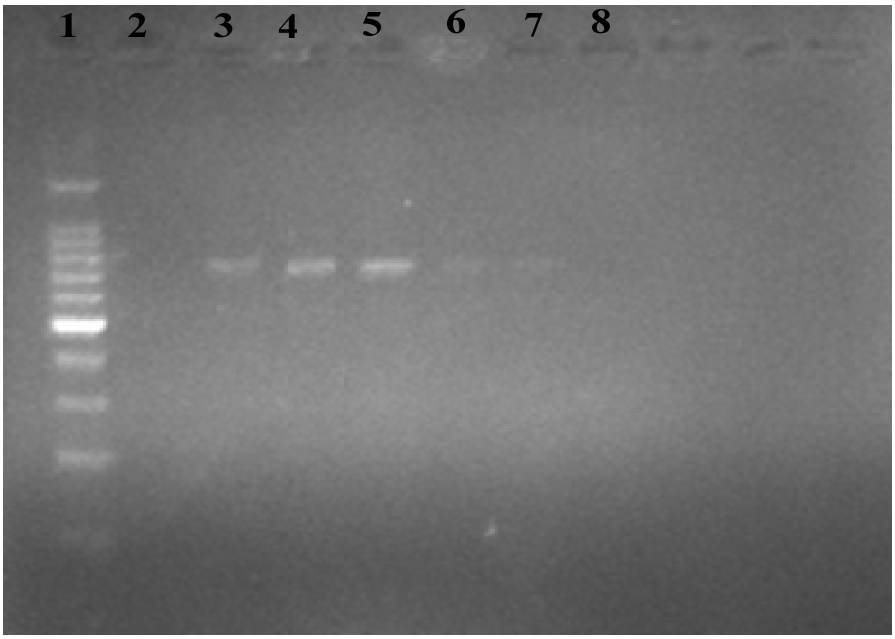

Figure 11. Agarose Gel Electrophoresis analysis (2 %) of PCR amplicon extraction from fresh beef sample. Lane 1 molecular marker (100 bp), Lane 2 negative test sample *Areomonas hydrophylla* (M84709), Lane 3 to 8 positive sample containing PCR mixture with *E. coli* (JX206444.1) in deferent concentrations.

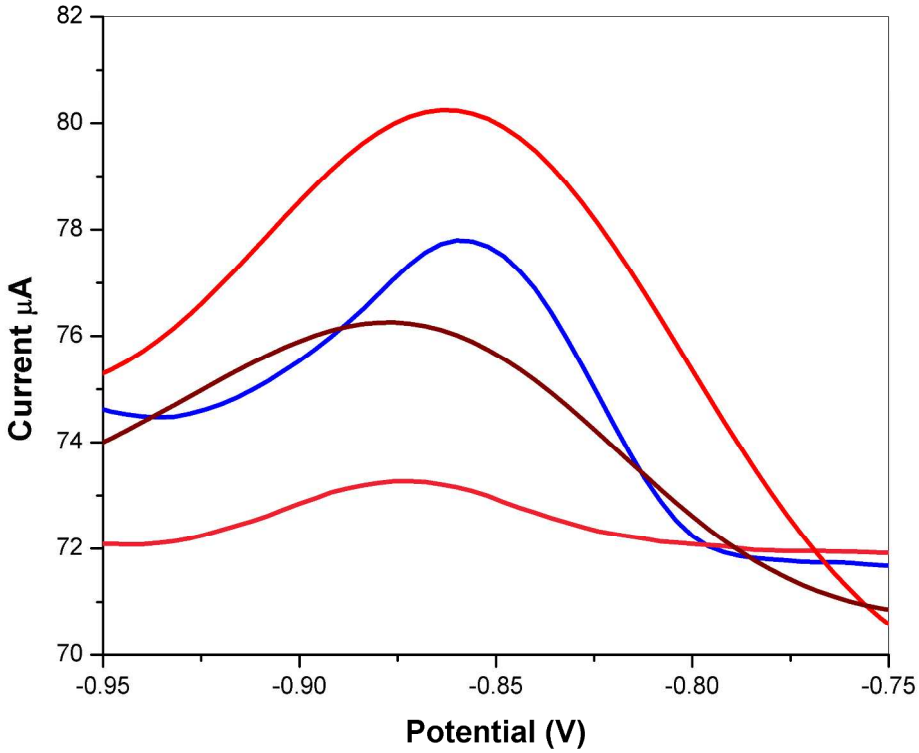

Figure 12. Differential pulse voltammograms response for real sample (beef sample) in different concentration.

672 **Toc Graphic**

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