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Multi-walled carbon nanotube-based nanobiosensor for the detection of cadmium in water

S.P. Sreekanth [a], Abdullah Alodhayb [b,c], Abdulaziz K. Assaifan [c], Khalid Eidah Alzahrani [b,c], Muthumareeswaran Muthuramamoorthy [c], Hend I. Alkhammash [d], Saravanan Pandiaraj [e], Abdullah M. Alswieleh [f], Quyet Van Le [g,*], R. Mangaiyarkarasi [a], Andrews Nirmala Grace [a,**], Vimala Raghavan [a,***]

[a] Centre for Nanotechnology Research, Vellore Institute of Technology, Vellore, India
[b] Research Chair for Tribology, Surface, and Interface Sciences, Department of Physics and Astronomy, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia
[c] King Abdullah Institute for Nanotechnology, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia
[d] Department of Electrical Engineering, College of Engineering, Taif University, P.O.Box 11099, Taif, 21944, Saudi Arabia
[e] CFY Deanship, King Saud University, Riyadh, Saudi Arabia
[f] Department of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
[g] Institute of Research and Development, Duy Tan University, Da Nang, 550000, Vietnam

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ABSTRACT

Industrial and agricultural processes have led to the prevalence of cadmium in the ecosystem. A successive build-up of cadmium in food and drinking water can result in inadvertent consumption of hazardous concentrations. Such environmental contamination of cadmium can pose a substantial threat to human and animal life. In humans, it is known to cause hypertension, cardiovascular diseases, DNA lesions, inhibition of DNA repair protein or disturb the functioning of lung, liver, prostate and kidney. The development of a reliable method for Cd (II) ions detection would reduce the exposure and complement existing conventional methods. In this study, a DNA based electrochemical method is employed for the detection of Cd (II) ions using ethyl green (EG) and multi-walled carbon nanotube (MWCNT). Glassy carbon electrode (GCE)/MWCNT forms the working electrode for differential pulse voltammetry (DPV) analysis for the detection of Cd (II) ions. The dsDNA is immobilized onto the working electrode. The indicator dye EG, preferably binds to ssDNA and its reduction peak current is noticeably less in the presence of dsDNA. The Cd (II) ions after interacting with dsDNA, unwinds the dsDNA to ssDNA, upon which the EG molecules bind to ssDNAs, giving a higher reduction peak current. The difference in the reduction peak currents in the presence and absence of Cd (II) ions is proportional to its concentration. The linear detection range achieved in this method is 2 nM–10.0 nM with a sensitivity of around 5 nA nM^{-1} and the limit of detection is 2 nM, which is less than the permissible limit of WHO for human exposure. This study considerably broadens the possible application of multi-walled carbon nanotube modified electrodes as biosensors and holds prospects for the detection of other heavy metals in environmental samples.

1. Introduction

Heavy metal ions are the major contributors to environmental pollution worldwide. Although the presence of heavy metal ions in the environment is also due to the naturally occurring weathering process, the main contribution has been from the industrial waste released into the environment, agricultural activities with the usage of pesticides, insecticides and chemical fertilizers. The mismanagement in the disposal of wastewater and lack of strict environmental regulations are the key reasons behind environmental pollution (Baghayeri et al., 2018).

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: levanquyet@dtu.edu.vn (Q. Van Le), anirmalagladys@gmail.com (A.N. Grace), vimala.r@vit.ac.in (V. Raghavan).

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Cadmium is one of the most toxic heavy metals and widely found in plastics, pesticides, fertilizers, combustion of fossil fuels, etc. Both short-term and long-term exposure to this heavy metal above the tolerance levels is detrimental to human health and can lead to serious diseases and several types of cancers (Flick et al., 1971). Due to the presence of Cd (II) ions, the DNA repair proteins are inhibited and DNA lesions are caused. It has been reported to cause renal dysfunction, osteoporosis and several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Zang et al., 2019; Liu et al., 2013, 2014; Mastouri et al., 2014; Wang et al., 2005). Based on the study on humans as well as on animals, cadmium is categorized as a carcinogen by the International Agency for Research on Cancer (IARC), particularly causing cancer in kidneys. According to the World Health Organization (WHO), the permissible limit of cadmium in drinking water is 3.0 μg/L (Ebrahimi et al., 2018).

With the knowledge of the extent of damage caused by Cd (II) ions, it becomes imperative to detect the presence of Cd (II) in water. Several traditional analytical techniques such as inductively coupled plasma (ICP) optical emission spectrometry (Kula et al., 2011), inductively coupled plasma mass spectrometry (ICP-MS) (Christopher and Thompson, 2013), atomic absorption (AA) spectroscopy (Jahromi et al., 2007), voltammetry (Locatelli 2007) and polarography (Zhao and Gao, 1988) have been widely used for detection of cadmium. In spite of being highly sensitive and selective, these dedicated instrumental methods suffer from several limitations viz. expensive instruments, complex sample preparation methods and cumbersome (Moyo and Okonkwo, 2014; Li et al., 2018). Nevertheless, an electrochemical method used for detection of cadmium apart from offering high sensitivity uses less complex procedure and equipment, and allows miniaturization, which is suitable for mass-production of sensors (Fan et al., 2016; Qu et al., 2015; El Mhammedia et al., 2009). Hence, it is indispensable to develop a prompt and sensitive screening method to determine the presence of metal ions in water. In general, electrochemical route has many advantages over the conventional methods like high selectivity, sensitivity, rapid analysis both quality and quantity, on-site detection (portable), inexpensive equipment and does not require pre-treatment of sample. DPV technique can provide improved selectivity for observing different redox processes compared with CV and LSV.

Though there are various ways for the detection of heavy metal ions, biosensing process offers a promising approach for rapid and precise detection of a wide range of analytes and has been a forthcoming research area in recent years. As they demand minimum sample pretreatment, less time for analysis, low cost and adequate selectivity and sensitivity they are considered to be more advantageous (Han et al., 2001; Rodriguez-Mozaz et al., 2006; Suwansa-ard et al., 2005; Ursula 2000; Yang et al., 2008). Biosensor involves specific biochemical reactions mediated by organelles, isolated enzymes, tissues, whole-cell or immune systems to detect chemical compounds by optical signals, electrical or thermal signals (Dede and Altay, 2018). A biosensor consists of two components; a biological element or bio-receptor and a physico-chemical transducer. A biological element can be DNA molecules, enzymes, antibodies, organelles and whole-cell microorganisms. The biological element recognizes or senses the analyte and it is chosen based on the analyte. A physico-chemical transducer converts the sensed signal to a readable signal and it can be any of mechanical, acoustic, optical, and electrochemical type (Gutiérrez et al., 2003). Nanobiosensors with large reactive surface area offer improved sensitivity, greater selectivity and improved response times (Moyo and Okonkwo, 2014). Various nanobiosensors were previously prescribed for the detection of Cd (II) in water. An amperometric glucose oxidase biosensor for detection of metal ions based on the inhibition effect on glucose oxidase by metal ions Ag^{+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Zn^{2+}, Ni^{2+}, CrO_4^{2-} and Mn^{2+} has been reported (Guascito et al., 2003). A new alternative biosensor was investigated for high sensitive Cd (II) ion quantification using cysteine functionalized single-wall carbon nanotube (SWCNT) (Gutierrez et al., 2017). Electrochemical detection of Cd (II) was studied using horseradish peroxidase and maize tassel-MWCNT as a composite biosensor (Moyo and Okonkwo, 2014). A reagentless and reusable electrochemical aptasensor was investigated for the detection of Cd (II) ion (Zhad et al., 2017).

Usage of DNA is an important class of biosensors for heavy metal ion detection. These methods prove to be sensitive, simple, reusable and high throughput (Qu et al., 2015). There are various DNA based biosensors using electrochemical methods prescribed previously for Cd (II) ion detection. An electrochemical biosensor for Cd (II) ion detection was demonstrated using DNA hybridization and ethyl green as the signal indicator (Ebrahimi et al., 2018). One of the studies utilized ssDNA modified gold electrodes for the electrochemical detection of Cd (II) ions, in which DNA is covalently immobilized on a gold electrode (Qu et al., 2015). The surface-immobilized ssDNA along with underpotential deposition of metal was used as a sensor for Cd (II) ions detection (Wong et al., 2007).

With scanty research reports available on electrochemical DNA-based biosensors, there is more scope for research on the detection of Cd(II) ion using this method. With their large surface area, nanomaterials increase the electron transfer from the electrolyte to the working electrode, thereby increasing the sensitivity of the sensor. In general, the use of multiwalled carbon nanotube for electrochemical analysis has enhanced properties like low charge transfer resistance, direct detection, high sensitivity, rapid response, excellent reproducibility and extreme simplicity.

Cd(II) ions upon binding to dsDNA, destabilizes it by cleaving the base pairs. This can be detected using DNA hybridization detector like ethyl green (EG), which prefers binding to ssDNA. EG provides an electrochemical signal, which increases with cadmium concentration (Ebrahimi et al., 2018). Glassy carbon electrode (GCE) is a form of the carbon electrode, which is electrically conductive, gas-impermeable and has a higher resistance to chemical attack. It is used as a working electrode in voltammetry studies. Compared to other solid electrodes like pyrolytic graphite electrode, GCE is favorable because of its ease of fabrication and maintenance (Zittel and Miller, 1965). In the present study, an electrochemical nanobiosensor was developed using a glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes (MWCNTs) and immobilized with DNA. Compared to bare electrodes, usage of MWCNT films improves the sensitivity by exhibiting the surface enhancement effects considerably (Sierra-Rosales et al., 2017).

2. Materials and methods

2.1. Chemicals and materials

Ethyl Green (EG), $Hg(Cl)_2$, $Cd(Cl)_2$ and $Pb(Cl)_2$ were purchased from Sigma Aldrich, India. Tris-HCl and EDTA were purchased from HIMEDIA. MWCNT was purchased from Sisco research laboratories private limited. Milli-Q-water (resistivity 18 MΩ cm^{-1}) was used in preparing all the solutions. For preparing dsDNA, two oligonucleotides of 10–mer were purchased from Eurofins Genomics. The sequences of required oligonucleotides are as follows:

ssDNA1: 5′-CCC CCC CCC C −3' (poly C DNA 100% cytosine bases)
ssDNA2: 5′-GGG GGG GGG G −3' (poly G DNA 100% guanine bases)

In the buffer solution of 10 mM Tris-HCl and 1.0 mM EDTA with pH = 8.00 probes stock solutions of 10^{-4} M were prepared and kept in freezing. A diluted stock solution of 10^{-6} M was prepared by using 20 mM NaCl with 50.0 mM, pH 7.40 Tris-HCl buffer solution. Hybridization of ssDNA1 and ssDNA2 was performed by placing the solution containing them for 10 min in a water bath at 93 °C. The formation of dsDNA can be observed at room temperature. The dsDNA samples were stored in a freezer until further use. The working principle of the nanobiosensor studied in this work is illustrated. (Fig. 1).

CHI660C Electrochemical Work Station was utilized for the

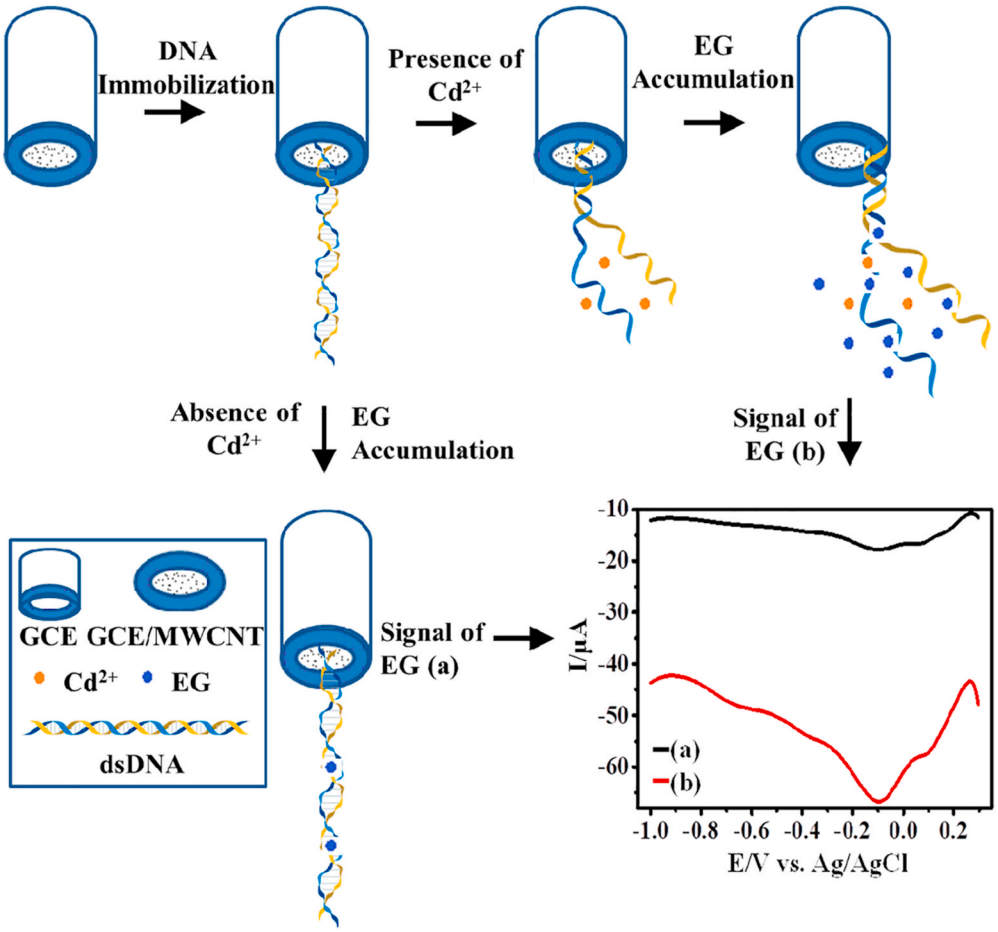

Fig. 1. A schematic of nanobiosensor for the detection of cadmium in water.

electrochemical studies. The conventional three-electrode system was followed where MWCNT dispersed GCE forms the part of the working electrode, the platinum wire forms the auxiliary electrode and Ag/AgCl (3M KCl) forms the reference electrode. All potentials were applied to this reference electrode.

2.2. Purification of MWCNT

To improve the quality of the purchased MWCNTs (99% pure) further, 20 mg of MWCNT was added to 2 mL of HNO_3 (65%) taken in a round bottom flask of 250 mL capacity. Along with a magnetic stirrer, reflux of the mixture was performed for 1 h at room temperature. The mixture was then diluted with distilled water and centrifuged followed by dilution with DI water until the solution reached neutral pH. After reaching to neutral pH, MWCNT were separated through the process of vacuum filtering using nylon membrane with 0.45 μm pore size and kept in a hot air oven at a maintained temperature of 80 °C for drying (Kim et al., 2006).

2.3. Functionalization of MWCNT

For the functionalization of MWCNT, purified MWCNT was mixed with the solution containing 1 mL of HNO_3 (65%) and 3 mL of H_2SO_4 (98%) (1:3 ratio). The mixture was sonicated for 3 h, followed by the addition of NaOH to remove acids and adjust the pH to 7.0. The aliquot was washed 2 to 3 times with DI water using centrifuge process. The functionalized MWCNT was separated from the solution using vacuum filtering using nylon membrane and kept drying at 80 °C in a hot air oven for 48 h (Kim et al., 2006; Wahab et al., 2017).

2.4. Preparation of MWCNT-dispersed/modified GCE

To disperse the MWCNT on GCE, ethanol was used as a dispersion agent. The procedure involves polishing GCE with 0.3 and 0.05 μm slurries of alumina, followed by washing thoroughly with DI water. The suspension of MWCNT was prepared by dispersing ultrasonically 2 mg of the MWCNT for 5 min in 2 mL of ethanol. 5 μL of the dispersed MWCNT/ethanol was cast on to the GCE and dried for 5 min at room temperature. The resulting modified electrode was denoted as MWCNT/GCE. The immobilization of dsDNA onto the working electrode followed by the interaction of Cd (II) ions with the dsDNA was carried out following the procedure described by Ebrahimi et al. (2018) (Ebrahimi et al., 2018). For the immobilization of dsDNA on to the working electrode, 0.50 V potential was applied to the electrode in a solution of DNA with 10^{-6} M with stirring for 5 min. For this process, "i-t amperometric" technique of CHI660 workstation was utilized. Following this, the electrode was rinsed with sterile deionized water.

2.5. Electrochemical detection of Cd (II) ions

Further, the dsDNA immobilized electrode (GCE/MWCNT/dsDNA) was made to interact with Cd (II) ions by incubating in 0.05 M Tris-HCl buffer solution of pH 7.40 containing Cd (II) ions solution for 5 min under stirring, while electric potential was not applied. This paves way for the interaction between the solute Cd (II) ions and the dsDNA. The dsDNA and Cd (II) ions interacted MWCNT/GCE modified electrode was immersed for 5 min into a buffer solution (pH 7.0) of 10 mM Tris-HCl containing 1.0 mM EG while stirring and without application of potential. EG was thus accumulated onto the working electrode and it acts

as an electroactive label. Finally, the electrode was rinsed with deionized water.

2.6. Characterization

Fourier transform infrared (FTIR) spectroscopic analysis of the functionalized MWCNT was carried out to understand the functional groups of the sensing materials (Shimadzu Affinity-1). The FTIR analysis was performed for both r-MWCNTs (raw) and f-MWCNTs (functionalized) in the range of 4000–400 cm^{-1} using KBr pellets and the results are depicted in Fig. 2. The chemical functionalization was confirmed using Raman spectroscopy (Horiba) by characterization of both r-MWCNTs and f-MWCNTs in the wavenumber range 0–3600 cm^{-1} and a laser of 532 nm wavelength as the excitation source (Fig. 3). All the electrochemical measurements were done using a CHI660C Electrochemical Work Station. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were studied for bare GCE and GCE/MWCNT working electrodes in the 0.05 M Tris-HCl (pH = 7.40) buffer solution with 20 mM NaCl, to understand the oxidation and reduction potential range. The CV and DPV curves for the bare GCE and GCE/MWCNT working electrodes are displayed (Figs. 4 and 5). For the electrochemical sensing of Cd (II) ion concentration, differential pulse voltammetry (DPV) method was used.

In the Tris-HCl buffer solution, the reduction peak current of EG in the presence of immobilized dsDNA was measured by scanning of cathodic potential from −1.1 V to 0.3 V vs. Ag/AgCl at 0.05 modulation amplitude, 0.06 s modulation time, 0.5 s time interval and 0.004 V step potential. In the absence of Cd (II) ion, more hybridized dsDNA is present on the electrode, which results in less attachment of EG molecules to DNA and in turn the reduction peak current measured is less. The CV and DPV curve in the absence of Cd (II) ions is shown in Figs. 4 and 5.

Upon the incubation of dsDNA immobilized electrode in Cd (II) ions solution, the Cd (II) $ions^{+}$ attach to dsDNA through the guanine bases N7 atoms of DNA. This leads to dsDNA destabilization and breaking of base pairs. The concentration of ssDNA formation on the working electrode depends on the concentration of the Cd (II) ion solution. Due to this, cathodic peak current of EG increases. The measurement of DPV peak current is performed after the incubation of Cd (II) ions, measured for different concentrations of Cd (II) ions in the 0.05M Tris-HCl, pH 7.40 buffer solution and 20 mM NaCl. The difference in the DPV peak currents before and after the incubation of Cd (II) ions is proportional to its concentration in the buffer solution. Peak current gradually increased with the increase of Cd (II) ions concentration. The CV and DPV curve for various levels of Cd (II) ions is as shown (Figs. 6 and 7).

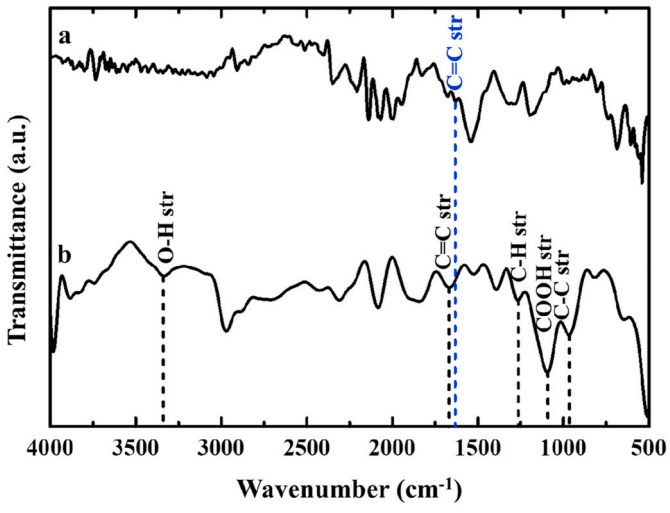

Fig. 2. FTIR spectra of chemical functionalization of MWCNT; (a) r-MWCNT, (b) f-MWCNT.

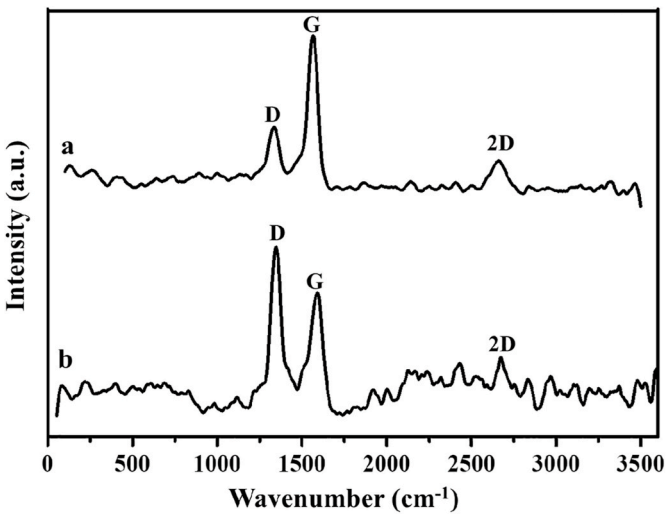

Fig. 3. Raman spectroscopy of (a) r-MWCNT, (b) f-MWCNT.

In this study, nafion membrane is incorporated for interference study. Nafion is a porous sulfonated fluoropolymer with a negatively charged surface. Nafion was coated on the working electrode to avoid the anionic interferences. The working electrode GCE/MWCNT was coated with 5 μL of 5 wt% nafion and DPV measurements for Cd (II) ion detection were carried out.

3. Results and discussion

3.1. Functionalization of MWCNT

The pristine MWCNTs are hydrophobic in nature, which agglomerates into bundles in many common solvents. The surface modification is required for dispersing in a solvent and to make them reactive for usage in nano-biosensor application. So, as the first step, the chemical functionalization of MWCNTs was performed. During this process, the side caps of the MWCNT are opened, the functional groups are covalently attached to the MWCNT opened ends and some defects are introduced into the structure. These defects are said to increase the reactivity of the carbon atoms. FTIR results for the raw and functionalized MWCNT are given (Fig. 2). The FTIR plot for r-MWCNT (Fig. 2a) indicates the presence of only the weak peak of the double bond between carbon atoms (C═C) around wavenumber of 1630.57 cm^{-1}. The chemical functionalization was confirmed from FTIR plot for f-MWCNT (Fig. 2b). The slight shift of weak peak between carbon atoms (C═C) to wavenumber 1667.67 cm^{-1} compared to r- MWCNT could be attributed to the acid treatment. The additional functional groups attached after the acid treatment are at wavenumbers 3333.62 cm^{-1} (O–H stretching vibration), 1264.06 cm^{-1} (C–H stretching band), 1095.95 cm^{-1} (-COOH carboxylic acid group) and 965.70 cm^{-1} (C–C). It is said that the HNO_3/H_2SO_4 (1:3) acid treatment should be performed for 2–3 h. Prolonging the process damages the sidewalls of the MWCNT and shorten the carbon nanotube length (Goyanes et al., 2007).

The sonication of MWCNT in strong acids, produces significant destruction. The Raman spectroscopy for f-MWCNT (Fig. 3b) has higher intensity D band compared to D band of r- MWCNT (Fig. 3a). This increase is attributed to oxygen functionalization which increases the number of carbon atoms in sp^3 hybridization in the nanotube framework (Murphy et al., 2006). Upshift of D (1331 cm^{-1}) and G (1567 cm^{-1}) bands to higher wavenumbers (1350 cm^{-1} and 1593 cm^{-1}) is observed (Fig. 3).

These phenomena confirm the chemical functionalization of MWCNT. After the functionalization, the MWCNT got dispersed in ethanol and aliquot was physically adsorbed onto the GCE in required

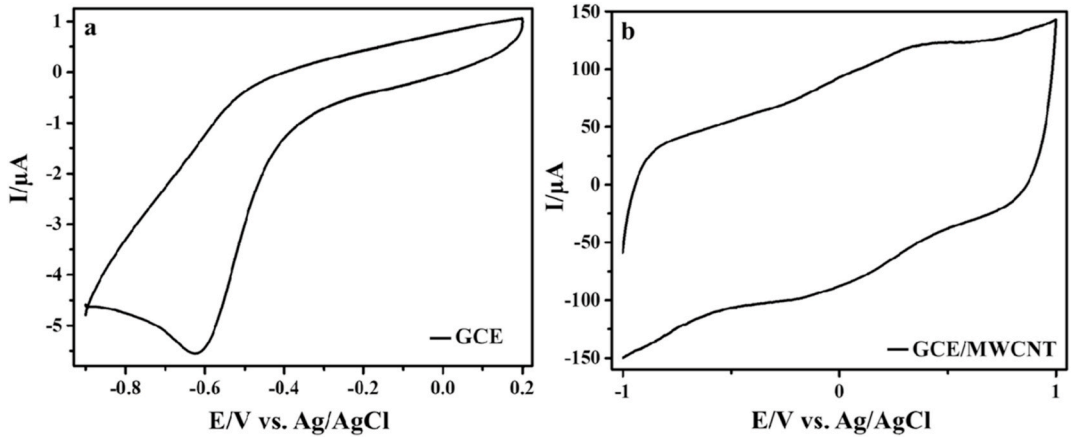

Fig. 4. CV curves for GCE and GCE/MWCNT working electrodes in 0.05M Tris-HCl (pH = 7.40) buffer solution and 20 mM NaCl; CV of (a) GCE, (b) GCE/MWCNT (scan rate – 100 mV/s).

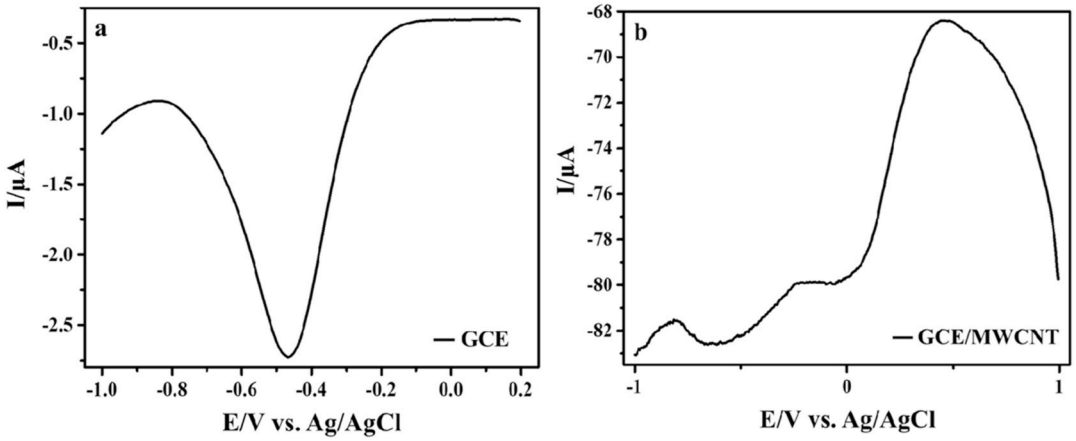

Fig. 5. DPV curves for GCE and GCE/MWCNT working electrodes in 0.05M Tris-HCl (pH = 7.40) buffer solution and 20 mM NaCl; CV of (a) GCE (b) GCE/MWCNT. (scan rate −100 mV/s).

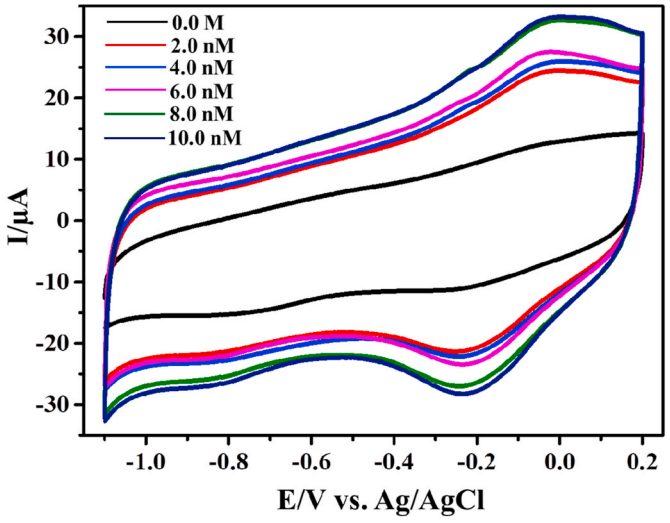

Fig. 6. CV of different concentrations of Cd (II) ions in 0.05M Tris-HCl (pH = 7.40) buffer solution with 20 mM NaCl for the potential range of −1.1 V–0.2 V vs. Ag/AgCl and −1.0 V–0.3 V vs. Ag/AgCl. (scan rate – 100 mV/s).

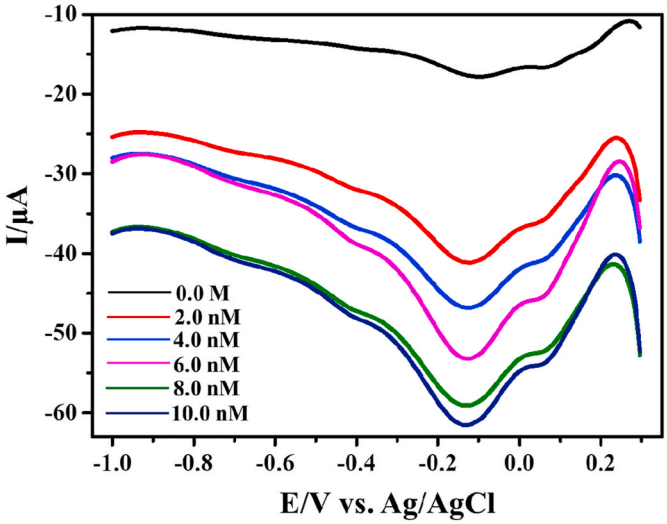

Fig. 7. DPV of different concentrations of Cd (II) ions in 0.05M Tris-HCl (pH = 7.40) buffer solution with 20 mM NaCl for the potential range of −1.1 V–0.2 V vs. Ag/AgCl and −1.0 V–0.3 V vs. Ag/AgCl.

quantity for electrochemical characterization.

3.2. Detection of Cd (II) ions

The Cyclic Voltammetry (CV) curves of bare GCE and GCE/MWCNT in 0.05M Tris-HCl buffer solution is indicated (Fig. 4a and b). With bare GCE as a working electrode (Fig. 4a), the CV current values produced are less against the applied potential. Oxidation peak is not generated and during the cathodic cycle, a reduction peak is observed around the applied potential of −0.6 V vs Ag/AgCl. Upon physically adsorbing MWCNT on to GCE, the electron transfer between the electrode and the electrolyte is increased, which resulted in a large increase in the voltammetry current (Fig. 4b). The significant increase in voltammetric current is attributed to the large surface area of MWCNT which resulted in enhanced electron transfer.

The GCE/MWCNT working electrode did not produce well-identified oxidation or reduction peak curve during CV, instead, broad curves are observed for both anodic and cathodic cycles. Similar behavior is observed during the cathodic scan of Differential Pulse Voltammetry (DPV) curves. Bare GCE generates a well-identified reduction peak with very less current (Fig. 5a) and GCE/MWCNT produces higher current without a well-identified peak (Fig. 5b).

For the detection of Cd (II) ions in this study, dsDNA with 10 mer G and C pairs is immobilized on to the GCE/MWCNT by applying 0.5 V vs. Ag/AgCl potential under stirring condition. One of the studies describes the immobilization of DNA on GCE and nature of the interaction between them (Pedano and Rivas, 2005). Experiments based on electrochemical studies and spectroscopy demonstrated that the interaction between DNA and glassy carbon surface is mainly hydrophobic. Even at high negative potentials applied, the immobilized DNA is said to be stable. Based on the concentration of DNA stock solution, the adsorbed layer on GCE can be thin or thick. Previous research papers have prescribed various methods to attach DNA with MWCNT (Abdullin et al., 2007). In this study, the attempt made is to utilize the higher surface area and large electron transfer capacity of MWCNT and attachment of DNA is expected to occur only with GCE. After the immobilization of dsDNA, the working electrode GCE/MWCNT/dsDNA has interacted with various concentrations of Cd (II) ions solutions under stirring. The CCd (II) ions from the solution bind the dsDNA through N7 atoms of guanine bases and this destabilizes the base pairs, which breaks to form ssDNAs. The Cd^{2+} interaction is followed by the interaction of working electrode GCE/MWCNT/dsDNA/ssDNA with EG. EG being a DNA hybridization indicator prefers binding to ssDNA compared to dsDNA. So, more EG molecules bind to ssDNAs formed on the working electrode.

The CV curves for the working electrode after EG interaction in 0.05M Tris-HCl buffer solution is displayed (Fig. 6) for the various concentrations of Cd (II) ion solution. The peak around the −0.2 V vs Ag/AgCl during the cathodic scan can be attributed to the reduction of EG (Liu et al., 1996). The same reduction is confirmed by the DPV curves (Fig. 7). For the estimation of Cd (II) ions concentration in the solution, the DPV technique is utilized in this study. As the concentration of Cd (II) ions interact with the working electrode is increased, destabilization of dsDNA increases, thereby unwinding of base pairs leads to a rise in the numbers of ssDNA on the working electrode. Upon this, more molecules of EG are expected to bind with ssDNA and increase the electrons generated. MWCNT, being a good electron transfer material with a greater surface area conducts these electrons to the surface of GCE, resulting in a greater flow of current. This is confirmed by a proportional increase in the current of CV and DPV (on the cathodic scan) for the corresponding increase in the Cd (II) ions concentration (Figs. 6 and 7), confirming that this nanobiosensor can be utilized for the detection of Cd (II) ion concentration in water.

The calibration curve of difference in the DPV (on the cathodic scan) currents for different concentrations of Cd (II) ions (Fig. 8) shows linearity in the range 2 nM–10 nM with some deviation. The line is represented regression x = 0.3666y-6.7363 and having a correlation

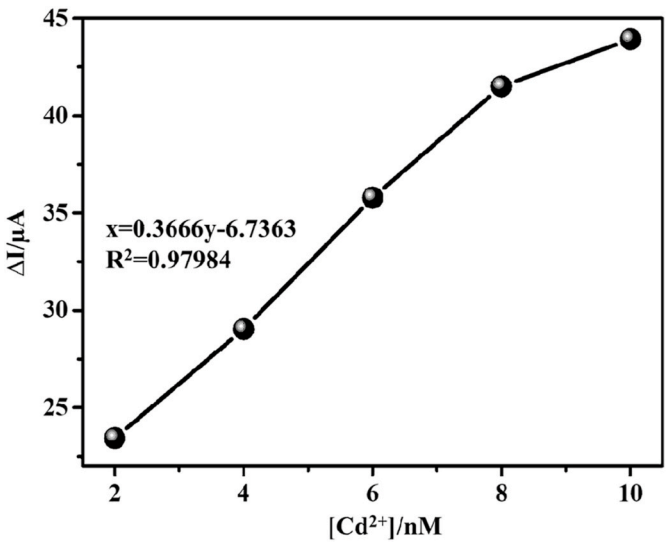

Fig. 8. The plot of difference in DPV responses vs. concentrations of Cd (II) ions (2 nM–10.0 nM).

coefficient of determination $R^2 = 0.97984$. The regression line obtained can be used to find the unknown concentration of Cd (II) ions in water. The limit of detection of nanobiosensor is 2 nM with a sensitivity of around 5 nA nM^{-1}. The sensor performance is compared with previously prescribed methods in Table 1.

In this study, Nafion membrane is incorporated for interference study. Nafion is a porous sulfonated fluoropolymer with a negatively charged surface. Nafion was coated on the working electrode in a study reported by Abrar et al., (2016) to avoid the anionic interferences. In this work, the working electrode GCE/MWCNT was coated with 5 μL of 5 wt % Nafion and DPV measurements for Cd (II) ion detection were performed and shown in Fig. 9. It was observed that with Nafion coating, the current value decreased for the 2 mM Cd (II) ions concentration compared to that of 1 mM. This phenomenon could be explained as follows: When the working electrode coated with Nafion is stirred in Cd (II) ions solution, Nafion allows the Cd (II) ions ions from the solution to interact with dsDNA. As the concentration of Cd (II) ions increased in the solution, more ions pass through porous polymer and block the way for the EG molecules, preventing them from binding to dsDNA and ssDNA. This lowered the EG reduction current as the concentration of Cd (II) ions increased. So, the usage of Nafion with DNA bio-sensor was not feasible.

3.3. Selectivity of the nanobiosensor

The buffer solution was incubated with possible interfering heavy metal ions like Pb^{2+}, Ca^{2+}, Cu^{2+}, Hg^{2+} with concentration of 1.0 μM. To understand the response of the sensor for these metal ions, the DPV

Table 1
Comparing the result of this work with previously prescribed methods.

Electrode	Determination Technique	LOD	Linear Range	Reference
CPE Electrode/ DNA	DPV	0.3 pM	1.0 pM–1.0 nM, 10.0 nM–10.0 μM	[2]
Gold Electrode	CV	0.3 ng L^{-1}	1 ng L^{-1} – 20 ng L^{-1}	[10]
Gold Electrode	CV and OSWV	10 pM	10 pM–500 nM	[11]
GCE/ MWCNT/ DNA	DPV	2 nM	2 nM–10 nM	This work

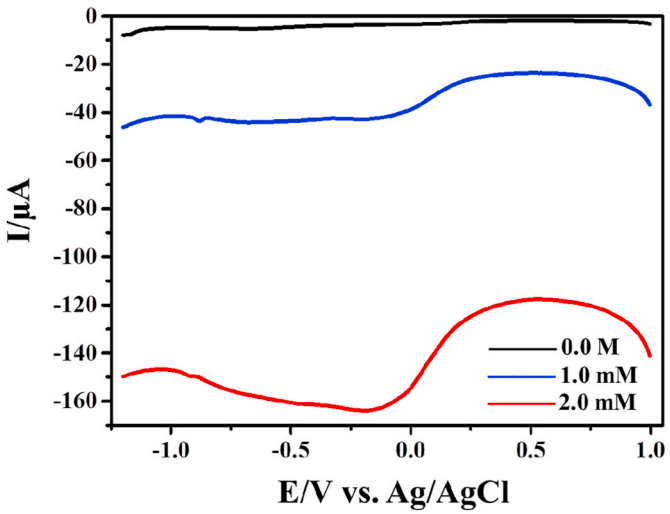

Fig. 9. The study of nano-biosensor with Nafion coated on working electrode.

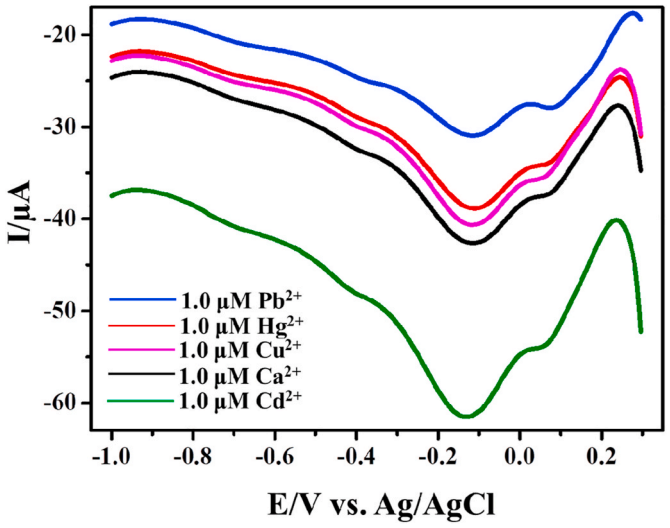

Fig. 10. The plot of selectivity study of nano-biosensor for Cd (II) ions with interfering heavy metal ions Pb^{2+}, Hg^{2+}, Cu^{2+} and Ca^{2+} at 1.0 μM concentration.

measurements were performed in buffer solution containing each of these metal ions individually. From the compiled DPV results (Fig. 10), which it can be inferred that the nanobiosensor is very selective to Cd (II) ions, as the DPV current is higher than interferences even at 10 nm Cd (II) ions concentration.

4. Conclusion

An electrochemical DNA-based nanobiosensor for the detection of Cd (II) ions in water was developed. It established an approach for sensing Cd (II) ions utilizing the functionalized multi-walled carbon nanotube as part of the working electrode with GCE along with DNA. This study considerably broadens the possible application of multi-walled carbon nanotube modified electrodes as biosensors and holds prospects for the detection of other heavy metals in environmental samples. The constructed electrodes display a lower detection limit, good sensitivity and selectivity, thus proving to have a potential for real water samples.

Credit author statement

Sreekanth S P1: Conceptualization, Methodology, Data curation, Writing- Original draft preparation. Abdullah Alodhayb, Abdulaziz K. Assaifan, Khalid Eidah Alzahrani, Muthumareeswaran Ramamoorthy, Hend I. Alkhammash, Saravanan Pandiaraj, Abdullah M Alswieleh, and Mangaiyarkarasi: Visualization, Investigation. Quyet Van Le, Andrews Nirmala Grace, and Vimala Raghavan: Supervision, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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