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Selective detection of Hg (II) over Cd(II) and Pb(II) ions by DNA functionalized CNT

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Abstract— **This work describes the selective detection of Hg(II) ions over Cd(II) and Pb(II) ions by DNA functionalized carbon nanotube (DFC) resistive network. The nucleobases, especially adenine and thymine, which are bound to the nanotubes through π—π stacking interactions, have strong affinities for Hg(II) ions and bind to the Hg(II) ions at suitable ligand sites forming a stable chelate complex. The coordinate ligand-Hg(II) bond possesses higher covalent character than either the ligand-Cd(II) or the ligand-Pb(II) bond. The higher the covalent character of the coordinate bonds in the chelate complex formed on the DFC network more is the e-scattering at the metal binding sites and thus greater is the reduction in the conductance of the DFC network. The amount of covalent character of the metal-ligand bond is measured by the stability factor of the complex.**

Index Terms— **heavy metals, sensor, bio-functionalization, carbon nanotubes,**

I. INTRODUCTION

The interactions between metal ions and DNA strands can be either non-specific or specific[1]. Cations exhibit non-specific binding with the negatively charged phosphate back bone of the DNA but undergo specific binding with naturally occurring ligands like nucleobases through pseudo-first order kinetic interaction. This leads to the generation of duplex moieties formed by the replacement of imino protons of the nucleobases by metal ions [2]. Metal ions are classified into hard and soft ions based on their affinities for the ligand binding sites of the nucleobases. The soft ions are more polarizable than the hard ions and the interactions between soft ions and ligands possess more covalent character than their counterpart. In this work we study the interaction between soft ions of Hg (II), Cd(II) and Pb(II) and the nucleobases and compare the covalent character of their interactions.

Since the nucleobases of the DNA constitutes naturally occurring multisite ligand, they are used to trap Hg(II) ions through chelate complex formation. Adenine and thymine are best suited to form complexes with Hg(II) due to their higher stability constant with Hg(II) [3]. Driven by negative enthalpy change and positive entropy change, the Hg(II) ions selectively stabilize mismatched T-T and A-T base pairs and unbound thymine and adenine bases[4]. When both adenine and thymine coexist the formation of chelating complex is governed by inter-ligand interactions including electrostatic, hydrophobic and stacking interactions[5]. The coordinate bonds in the chelate complex at the metal binding sites are not purely covalent but labile covalent bond with significant degree of ionic character [6]. Experimental analysis have shown that protons are liberated when Hg(II) binds to nuclebases in DNA [7, 8] to form complexes. The reaction scheme for the formation of T-Hg(II)-T complex is represented in Figure 1 (a). A-Hg-A and A-Hg-T complexes, shown in Figure 1(b) and (c) respectively, are formed following the same scheme. Due to the formation of such complexes the nucleobases undergo conformational changes which in turn influence the specific rotation of the molecule.

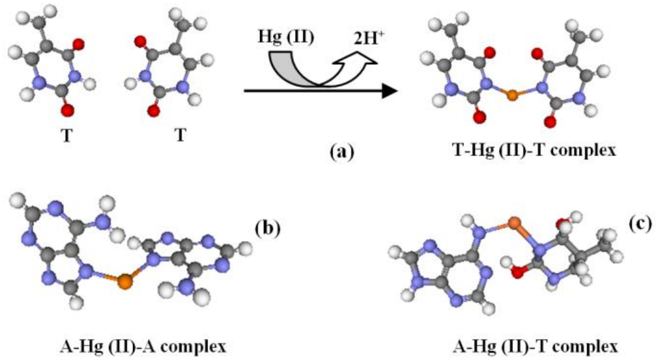

Figure 1: (a) Reaction scheme for T-Hg(II)-T pair formation. Molecular structures of cross linked (b) A-Hg-A and (c) A-Hg-T complexes. The C, H, O, N and Hg atoms are depicted by grey, white, red, blue and orange spheres

Hg(II) ion sensors with low detection limits have been developed based on the chelation chemistry between nucleobases and Hg(II) ions[9-11]. Wang et al. [12] devised a micro-fluidic chip-based near-infrared fluorescence detection set-up for specific analysis of Hg(II) ions with a detection limit as low as 1.5 nM. The mechanism behind the detection is the formation of T-Hg (II)-T mismatches followed by loosening of van der Waals bonds between the nucleobases and the side-walls of the nanotubes. The device utilizes multipart fabrication process flow for the development of micro-fluidic channels and suffers from higher time of detection. Liu et al. [13] constructed DNA modified electrode based electrochemical detection scheme for Hg(II) ion which achieves a low detection limit of 0.32 nM with good selectivity and reusability.

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This paper provides an electrical platform for selective detection of Hg(II) ions through ligand chemistry route using DNA functionalized carbon nanotube (DFC) network. The shortcomings of colorimetric, fluorescence-based and electrochemical methods (namely, unstable chemical and fluorescence signals, and signals from contaminating colorants) are avoided in this work. Comparative measurements with Hg (II), Cd(II) and Pb(II) ions in this work show that the nucleobases respond strongly to Hg(II) ions due to the formation of chelate complex between Hg(II) ions and ligand sites. The ssDNA strands unbinds from the surface of DFC as the Hg(II) produces weakening of the π—π stacking interaction between nanotubes and nucleobases which forms chelate complex with the Hg(II) ions. The nucleobase-Hg (II)-nucleobase coordinate bond possesses high covalent character and imparts greater stability to the complex than those with Cd(II) and Pb(II) ions. Selective detection of Hg(II) ions for concentration as low as 0.05 nM was achieved by the device.

II. EXPERIMENTS

A. *Materials*

The SWNT and the ssDNA samples were purchased from Sigma Aldrich, USA as detailed in [14]. The ssDNA sample constitutes 41.9% G-C and 58.1% A-T content. Analytical grade Hg (II) acetate, Cd(II) acetate and Pb(II) acetate were purchased from Sigma Aldrich, USA.

B. *Instruments*

FESEM investigations on DFC film were performed with SUPRA 40 FEG-SEM (Carl Zeiss SMT AG, Germany). CD investigations were performed using JASCO J-810 (Tokyo, Japan) spectropolarimeter in the wavelength range of 220 nm-330 nm at room temperature. Electrical characterization of the device was performed in the probe station (PLV 50, Cascade Microtech, Germany) by semiconductor parameter analyzer (SPA), (Model: 4155C Agilent Technologies, USA), remotely connected to a computer through which data acquisition takes place. The schematic representation of the experimental set-up is shown in Figure 2.

C. *Synthesis of DFC*

The DFC solution was prepared as follows. First, the ssDNA solution was prepared by adding 0.1 mg of ssDNA in 5 ml tris buffer solution and allowing the ssDNA to dissolve overnight in the sample mixer (HulaMixer™ from Life Technologies-Invitrogen, USA). Following this, 0.2 mg SWNT was then added to the ssDNA solution, and subjected to 4 hours of alternate cycles of (i) low intensity sonication for 30 mins in an ice bath and (ii) mixing in sample mixer for 30 mins. The resultant solution was centrifuged at 2000 r/min to eliminate the large bundles of unmodified SWNT.

The supernatant (stock) solution was collected and preserved for drop casting between Au-electrodes. The Au electrodes of thickness 150 nm, width 20 µm, and separated by gap length of 25 µm were fabricated by conventional photolithography on thermally grown SiO_2 layer. The DFC solution was drop cast between Au electrodes by micro-cantilever based drop dispensing technique using Nano-eNabler system (Bioforce Nanoscience, USA) as shown in Figure 3a. This drop casting technique produces DFC film of maximum thickness 90 nm as characterized by Dektak surface profilometer. Figure 3b shows the Dektak profilometer image of the tested device. The mounds on the DFC network are due to remnant agglomerates in the solution. The steps of DFC synthesis and the process of device fabrication are discussed in detail in our earlier work [15].

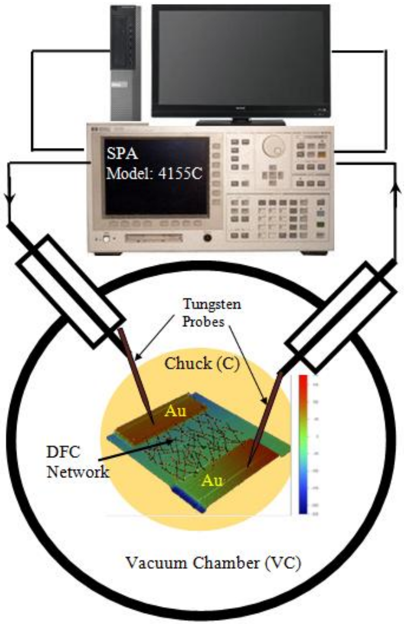


Figure 2: Schematic representation of the experimental set-up

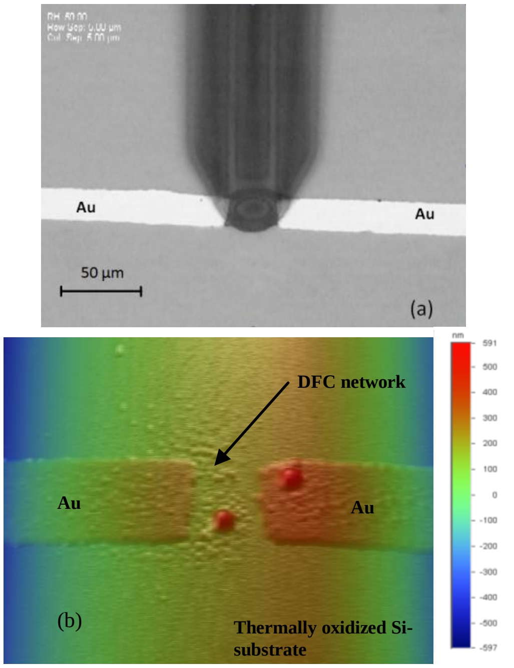


Figure 3: a) Photograph of solution dispensing of DFC solution between Au-electrodes separated by a gap of 25 µm by micro-cantilever based drop dispensing technique (b) Dektak profilometer image of the tested device

III. RESULTS

A. *Electron Microscopy Characterization*

Figure 4 (a) and (b) show the FESEM images of DFC network on SiO_2 substrate before and after addition of Hg (II) ions respectively. Before addition of metal ions the DFC network shows thick wrapping of DNA around CNTs. After addition of Hg(II) ions there is a distinct change in morphology of the DFC network. The non-covalent van der Waals interactions between DNA strands and surface of the nanotubes are weakened on exposure to Hg (II) ions as a result of which the strands are loosened and unwrapped to form sparse agglomerates of chelate complexes on the nanotube surface as shown in Figure 4 (b).

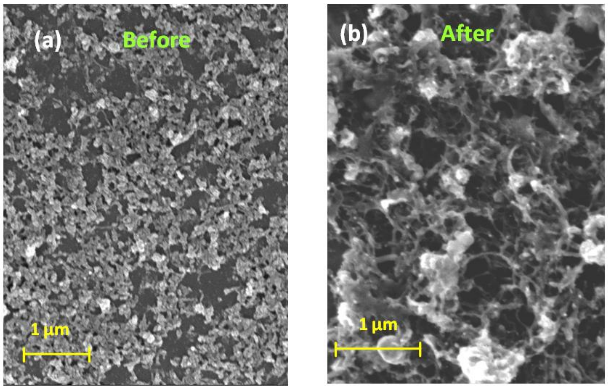


Figure 4: FESEM image of DFC network before (a) and after (b) Hg (II) ion treatment

B. *Electrical characterization*

Electrical characterization of DFC resistive network was performed in response to different concentrations of Hg(II), Cd(II) and Pb(II) ions at temperature 22 °C, 40% RH and under normal atmospheric pressure. The chemical stability of co-ordinate bonds between metal (M) ions and nucleobases in the chelate complex was studied by I-V semi-log plots of M-ions treated DFC network at different concentrations of M-ions. The thick wrapping of ssDNA over SWNT in DFC (as shown in Figure 4 a) provides schottky character to the nanotube- nanotube and Au-nanotube contacts of the DFC network which gives rise to exponential behavior of the I-V curves. I-V measurements were performed on separate devices for each concentrations of 0.05 nM, 0.5 nM, 5 nM, 50 nM and 500 nM for Hg(II), Cd(II) and Pb(II) ions as shown in Figure 5, 6 and 7 respectively . All the I-V characteristics for different concentrations of respective M-ion were compared with the I-V curve obtained from DI water which was considered as a control of the experiment.

The blank response of DFC network towards DI water is represented by

$$\eta = \sigma_{DFC} - \sigma_{DI} \; \ldots\ldots\ldots\ldots\ldots\ldots \; (1)$$

where σ_{DFC} and σ_{DI} are the slopes of the I-V semi-log curves for DFC network before and after addition of DI water respectively.

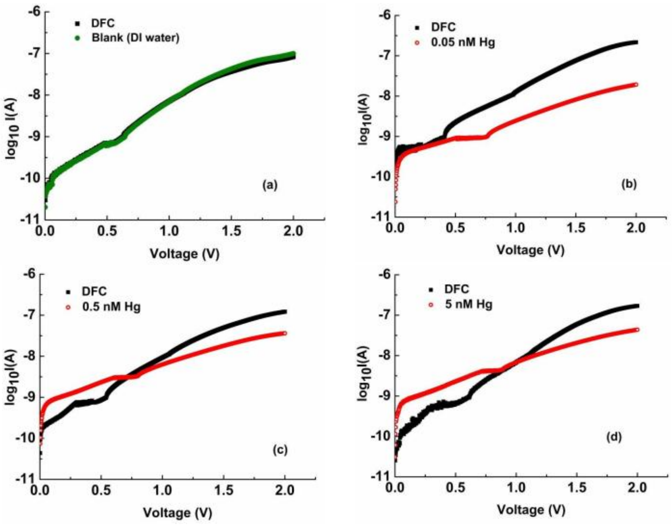
DFC based resistive devices are known to suffer from reproducibility error in output signal current due to variation in chirality of the nanotubes in the network. Additionally we found differences in contact areas between DFC network and Au electrodes contribute to the error. This problem has restricted us to calibrate the devices in terms of output signal current for different concentrations of the M-ions. Therefore we calibrated the device against different concentrations of M-ions with the stability factor, Δ :

$$\Delta = \frac{\sigma_{DFC} - \sigma_{M-ion}}{\eta} \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2)}$$

where σ_{M-ion} = conductance of M-ion treated DFC network, σ_{DFC} = conductance of the untreated DFC network, and η is given by (1). σ_{M-ion} decreases in response to different concentrations of M-ions,: it is found to be strong for Hg (II) ions and weak for Cd (II) and Pb (II) ions. Δ represents the number and strength of the coordinate covalent bonds between the central M-ions and the ligand in the chelate complex. Figure 8 shows Δ vs. concentration of Hg(II), Cd(II) and Pb(II) ions in the range of 0.05-500 nM. The nucleobases-M coordinate bonds possess mixed covalent and ionic character. The amount of covalent character depends of the affinity of the respective nucleobases for different metal ions. For a fixed concentration of M-ion, Δ depends on the covalent character of the coordinate bonds upon chelation which increases the stability of the complex. Thus Δ is considered as the stability constant of the nucleobase-M-nucleobase chelate complex formed on the surface of DFC.

In our study, the detection limits are determined as the concentration of M-ions at which the limiting criteria $\Delta = 3$, is satisfied. The detection limit of Cd(II) and Pb(II) of our device is graphically determined to be 1 nM and 10 nM respectively. However, since the experiments were conducted in the M-ion concentration range of 0.05-500 nM, the detection limit of Hg(II) was determined to be 1 pM by extrapolating the Δ vs. concentration plot to $\Delta = 3$.

The hydrophobic nucleobases of the ssDNA bind themselves on the nanotube wall through non-covalent van der Waals interactions, whereas the phosphate backbone of the DNA, being hydrophilic, is in contact with the aqueous medium. The metal (M)-ions interacts with the ligand nucleobases to form chelate complexes on the surface of DFC where the



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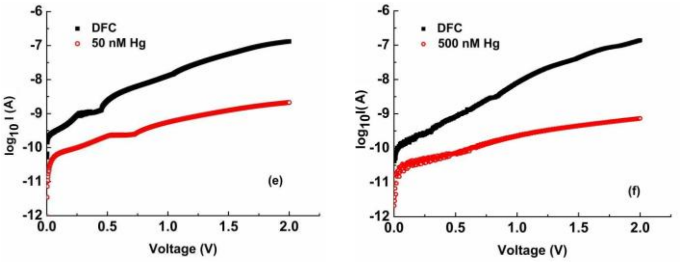
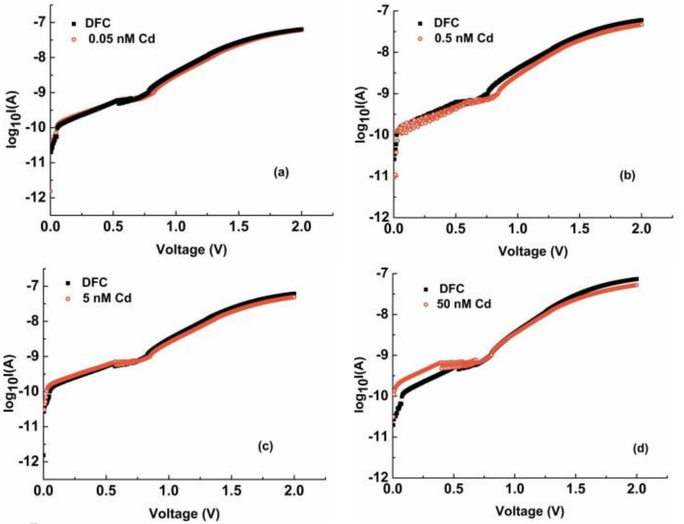


Figure 5: (a) I-V characteristics of control experiment with DI water (b) – (f) Semi-log I-V plots of untreated and Hg(II) ion treated DFC network for concentrations of 0.05 nM, 0.5 nM, 5 nM, 50 nM and 500 nM respectively



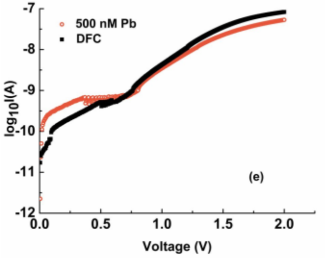
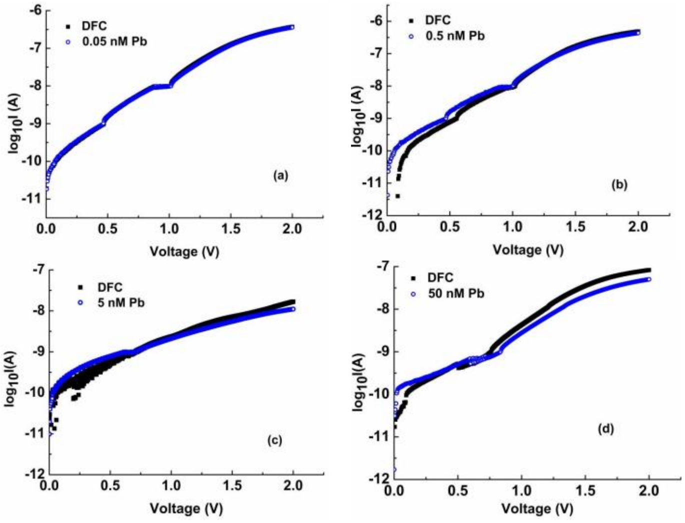


Figure 6: (a)-(e) Semi-log I-V plots of untreated and Cd(II) ion treated DFC network for concentrations of 0.05 nM, 0.5 nM, 5 nM, 50 nM and 500 nM respectively



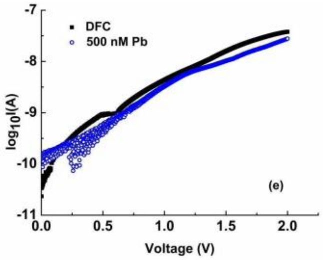


Figure 7: (a)-(e) Semi-log I-V plots of untreated and Pb (II) ion treated DFC network for concentrations of 0.05 nM, 0.5 nM, 5 nM, 50 nM and 500 nM respectively

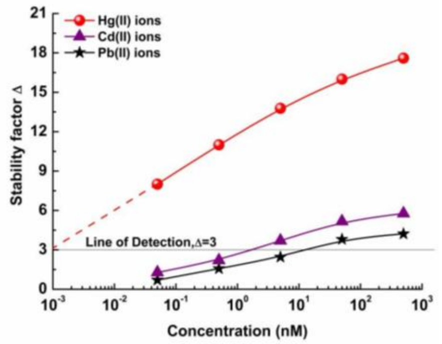


Figure 8: Stability factor vs. concentration plot for Hg (II), Cd(II) and Pb(II) ions

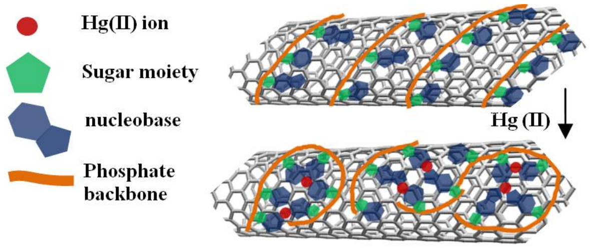


Figure 9: Schematic representation of chelation of nucleobases with Hg(II) ions on the surface of DFC network

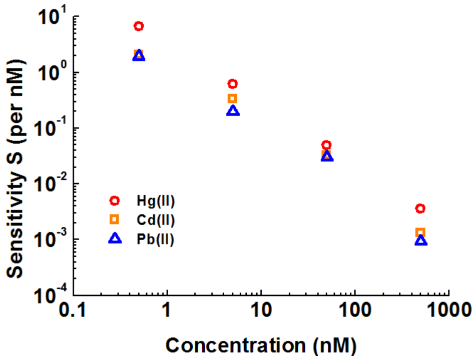


Figure 10: Sensitivity vs. concentration plot for Hg(II), Cd(II) and Pb(II) ions

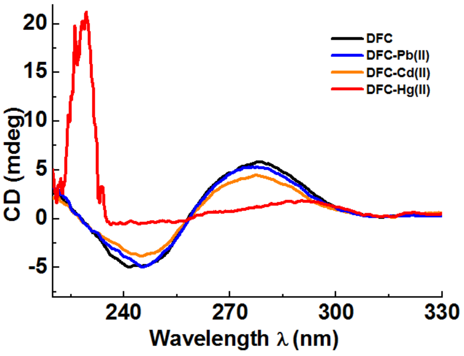


Figure 11: CD spectrograph of untreated and metal ion treated DFC solution

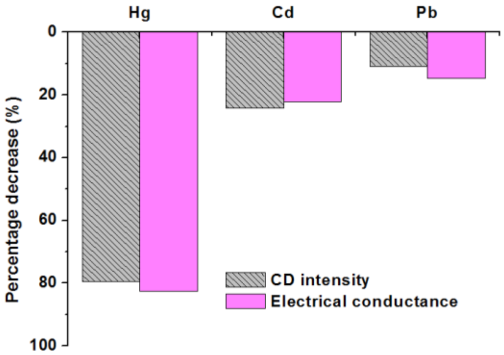


Figure 12: Comparative percentage decrease in CD intensity and electrical conductance of DFC after addition of different M-ion solutions of concentration 5 nM

stability of the nucleobase-M-nucleobase coordinate covalent bond in the complex depends on different metal ion affinities of respective nucleobases. The formation of chelate complex by Hg (II) ions on the DFC surface is schematically shown in Figure 9.

Figure 10 shows the log-log plot of sensitivity($S = \frac{d\Delta}{dC}$) vs. concentration for Hg(II), Cd(II) and Pb(II) ions and are determined from the slope of the piecewise linear regions between two adjacent data points in Figure 8. The plot shows that for different concentrations of Hg (II), the corresponding sensitivities were one order higher than those obtained for Cd(II) and Pb(II) ions. The metal ions become less sensitive to nucleobases at high concentrations due to exhaustion of the ligand binding sites on the nucleobases.

C. *Optical characterization*

The interaction between DFC network and M-ions were further investigated by circular dichroism (CD) spectroscopy which confirmed the establishment of strong coordinate covalent bond between Hg (II) ions and DFC. Measurements were performed after separately adding 5 nM solution of metal ions to 1/20 parts of the stock solutions of DFC in the volumetric ratio of 1:3. CD spectra of untreated and M-ion treated DFC solution are shown in Figure 11. The CD spectrum of untreated DFC solution shows positive and negative peaks at 278 nm and 245 nm respectively with a crossover at 260 nm. The CD intensities in both positive and negative sides are highest for untreated DFC solution and decreases when the DFC solution was treated with` M-ions. The larger the reduction in CD intensity the stronger is the covalent bonds established between the M-ions and the nucleobases of the DFC. Both positive and negative CD peaks are drastically reduced on addition of Hg (II) ions as compared to that of Cd(II) and Pb(II) ions. The large reduction in intensity for Hg(II) ions is attributed to the weakening of the π—π stacking interactions between the nanotubes and the nucleobases on chelation as a result of which DNA strands loosen from the surface of the nanotubes. The Hg(II) ions, with high affinity for the nucleobases, attacks the ligand sites to form most stable chelate complex as compared to that with Cd(II) and Pb(II) ions. Owing to the low affinity of the nucleobases for Cd(II) and Pb(II) ions, no significant reduction in intensity is observed on interaction with DFC. However, the positive and the negative peaks for Cd(II) and Pb(II) ion treated DFC solution suffer red and blue shifts of 3 nm and 4 nm respectively. This is due to the conformational changes in nucleobases and variations in wrapping pitch upon interaction with M-ions. As the CD intensity is heavily reduced for Hg (II) ion treated DFC solution this peak shift could not be ascertained. The steep positive peak at 230 nm for Hg (II) ion is attributable to the formation of duplex and multiplex chelate complex. Percentage decrease in electrical conductance and CD intensities for different M-ion solutions of concentration 5 nM are compared in Figure 12.

IV. DISCUSSIONS

The nucleobases undergo chelation with Hg (II) ions forming highly stabilized coordinate covalent bonds between ligand and the central Hg (II) ion which lead to higher Δ than formed by the Cd(II) and Pb(II) ions. When Hg(II) ions bind covalently to the nucleobase the strength of π-π stacking interactions between the surface of the SWNT and the nucleobase are weakened [16]. The repulsive cationic interactions stretch the DNA strands resulting in partial or complete detachment of the DNA strands from the nanotube surface. Thus the pitch of wrapping of DNA strands around the nanotube increases after binding with the Hg (II) ions. The nucleobase thus, selectively targets the Hg (II) ions in the aqueous medium at specific ligand sites forming chelating complexes.

In contrast, due to the lower metal ion affinity of the nucleobase for Cd(II) and Pb(II) ions, they establish irreversible partial coordinate covalent bonds with these metal ions. These bonds with Cd(II) and Pb(II) ions exhibit lower covalent character than that with Hg(II) ions and therefore imparts less stability to their corresponding chelate complexes. The more the covalent character in the nucleobases-M coordinate bond, the more stable is the complex. The stability of the nucleobase-M bond studied in our work can be expressed as nucleobase-Hg(II) > nucleobase-Cd(II) > nucleobase-Pb(II).

The M-ions bound to the nucleobases through partial coordinate covalent bond set up scattering sites for electron transport on the surface of the DFC network. This electron scattering at the metal binding sites is pronounced when the covalent character of the coordinate bond is high leading to greater stability of the nucleobases-M chelating complex for Hg (II) ions. The e-scattering at the metal binding sites decreases the overall conductance of the DFC network. Moreover, cross-linking of the nucleobases through Hg (II) ions forming a complex structure releases protons which recombine with the electrons causing reduction in the availability of electrons contributing to charge transport in the DFC network, thereby decreasing the overall conductance of the DFC network. The nucleobases are less sensitive towards Cd(II) and Pb(II) ions which inhibit the formation of stable chelate complexes on DFC surface resulting in less decrease in DFC network conductance. However Cd(II) and Pb(II) as well as the Hg(II) ions undergo unspecific binding at the phosphate backbone of the type -P-O-M-O-P-, which decreases the mutual electrostatic repulsion between adjacent strands [17].

V. CONCLUSIONS

The work describes the development of DFC resistive network based selective detection scheme for Hg (II) ions. Comparative studies with Hg (II), Cd(II) and Pb(II) ions reveal that the conductance of the DFC network suffer significant drop when Hg(II) ions interact with the nucleobases to form chelate complex on the surface of the DFC. The formation of chelate complex between the Hg(II) and the nucleobases imparts maximum covalent character into the coordinate bond at the Hg(II) binding sites of the nucleobases. The nucleobases are less sensitive to Cd(II) and Pb(II) ions and induce comparatively less covalent character to the coordinate bond forming a less stable chelate complex. The covalent character of the coordinate bond with the ligand is measured in terms of the stability factor Δ which is highest for Hg (II) ions. Since nucleobases selectively targets the Hg (II) ions forming a highly chelate stable complex. DFC network shows promise for selective and fast electronic detection of Hg (II) ions. Concentration as low as 0.05 nM of Hg (II) ions was successfully detected by the DFC resistive network.

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