CHAPTER 4

Electrochemical Investigation and Applications of Carbon-Carbon Nanotubes-Silicone oil Composite Paste Electrodes
4.1 Introduction

Carbon electrodes, especially glassy carbon and carbon paste electrodes are extensively used in electrochemical investigations because of the availability of wide potential windows (both anodic and cathodic), low background current, chemical inertness, low cost and suitability for detection of various organic and biological compounds. Among these, carbon paste electrodes due to unique characteristics like renewability of the electrode surface, versatility of chemical modification and compatibility with various electron mediators, has extensively been used in these studies [1, 2].

Electrode surfaces are modified to enhance specificity and reactivity. Carbon nanotubes (CNTs) have attracted the fancy of many scientists world wide. The small dimensions, strength and the remarkable physical properties of these structures make them a very unique material with a whole range of promising applications. The CNTs with unique microstructures and properties have attracted much attention from a great variety of fields. CNTs have demonstrated to be extremely useful for the development of new electrode materials. Remarkable properties of CNTs include mechanical, structural and electrical properties with their catalytic effects in electrochemical processes. Large microscopic surfaces can be obtained using CNT modified electrodes which made CNTs as a new and interesting subject in the construction of very sensitive sensors and biosensors with excellent responses in connection with several compounds of clinical, biological and environmental interest [3].
4.2 Review of Simultaneous Determination of Adenine and Guanine

H. S. Wang et al detected the G and A simultaneously at electrochemically pretreated glassy carbon electrode [4]. Z Wang et al made β-cyclodextrin incorporated carbon nanotubes-modified electrodes for simultaneous determination of G and A +0.79 and +1.09 V [5]. Nafion–ruthenium oxide pyrochlore modified electrode by J.M. Zen et al for the simultaneous detection of G and A, the peaks obtained at +0.84 and +1.08 V respectively [6]. Boron-doped diamond electrode was developed by A. Apilux et al and it was practically applied for the simultaneous detection of G and A which were obtained at the potentials +1.1 and +1.3 V respectively [7]. R.N Goyal et al detected G and A simultaneously at C₆₀ modified glassy carbon electrode, the potential at which detection was +0.69 and +0.99 V respectively [8].

4.3 Review of Electrochemistry of carbon nanotube modified electrodes

The subtle electronic properties suggest that CNTs have the ability to promote electron-transfer reactions when used as an electrode in chemical reaction. Previous studies reveal that the first type of CNT electrodes constructed by multi-wall carbon nanotubes (MWCNTs) mixed with bromoform, mineral oil, or liquid paraffin and the resulting electrode could be used to probe the oxidative behavior of dopamine [9] and showed favorable reversible electrochemical responses to cytochrome c and azurin [10]. Secondly, it has been reported that a microelectrode was constructed from an individual SWCNT and its voltammetric response was characteristic of steady-state radial diffusion [11]. The third type of CNT electrode was constructed by casting films of SWCNTs on Pt and Au electrodes [12], but it did not show well-resolved voltammograms. A carboxylic SWCNT solution dispersed with N,N-dimethylformamide (DMF) was cast on a glassy
carbon electrode [13] and 3,4-dihydroxyphenylacetic acid [14]. The fourth type of MWCNT modified electrode was constructed by intercalating CNT on the graphite surface [15]. Wang and Musameh [16] have reported on a composite electrode. M. D Rubianes et al reported composite electrode obtained by dispersion of multiple wall carbon nanotubes within mineral oil [17]. Palleschi and co-workers [18] have reported on the electrochemical behavior of carbon nanotubes paste electrodes prepared by mixing single wall carbon nanotubes with mineral oil. Other reports on carbon nanotube paste electrodes are available [19 - 23]. Recently Saeed et al modified the electrode by mixing MWCNT and graphite powder with a 25:75 mass ratio in dichloromethane. After the evaporation of the solvent, an appropriate amount of the carbonic mixture with mineral oil (in 75:25 w/w) was transferred to the mortar and pestle for hand mixing [24].

There are excellent reports on the performance of graphite paste electrodes using silicone oil as binder. The preparation and aqueous electrochemistry of carbon paste electrodes modified by SWCNTs and C60 are reported [25]. To our knowledge, there are no reports on the modification of electrode by mixing MWCNT and graphite powder in silicone oil. Hence in the present work, the design and preparation of the carbon-paste electrodes modified with multi-walled carbon nanotubes (MWCNT/CPE) is described. In this work, the MWCNT/CP electrodes showed a very stable electrochemical behavior, so they can be used to study the electrochemistry of a wide range of molecules for promising biosensor applications. Keeping this in mind, we try to determine simultaneously adenine and guanine in a mixture. The features offer great promise for in vitro application of a carbon nanotube modified carbon paste electrode, and useful information for in vivo detection.
4.4 Experimental

4.4.1. Apparatus and Procedure

Voltammetric experiments were performed using a Electroanalyser model EA 201 from Chemilink Systems, Mumbai, India. The electrochemical cell was assembled with a conventional three-electrode system: MWCNT/CPE, C$_{60}$ modified carbon paste electrode (C$_{60}$/CPE) or a bare CPE acts as working electrode (1-mm diameter), a platinum wire and SCE were used as counter and reference electrodes, respectively. All potentials are referred to the latter. All experiments were carried out at room temperature. Cyclic voltammetry experiments were carried out at a scan rate of 100 mV/s, unless otherwise mentioned, over the relevant potential range. KNO$_3$ and phosphate buffer (pH 5) serves as supporting electrolytes.

4.4.2 Reagents.

Multi-wall carbon nanotubes were obtained from Chengdu Organic Chemicals Co. Ltd., (Chengdu, China). Graphite powder (particle size 50 mm, density 20-30g/100mL) obtained from Loba Chemie, (Mumbai, MH, India). Potassium ferricyanide, dopamine, adenine and guanine were obtained from HiMedia Laboratories Pvt Limited (Mumbai, MH, India). All chemicals from commercial sources were of analytical grade.

4.4.3 Preparation of CP, C$_{60}$/CP and MWCNT/CP Electrodes

The unmodified classical carbon (graphite) paste electrode was prepared by mixing graphite powder with an appropriate amount of silicone oil and thorough hand mixing in a mortar and pestle. In the present study the ratio of carbon powder to silicone oil was optimized to 1g: 0.33 mL. An increase in the volume of pasting liquid greatly affected the reversibility of the electrode. This was reflected in the greater peak separations in potassium ferricyanide model system used for the study. The lower volumes of the binder affected the stability of the electrode.
The modified electrode was prepared by mixing MWCNT and graphite powder with a given wt% ratio (wt% of MWCNT to graphite powder) in silicone oil was transferred to the mortar and pestle for hand mixing. The C$_{60}$/CPE was prepared in a similar way by mixing C$_{60}$ and graphite powder with silicone oil. A portion of the resulting paste was packed firmly into the cavity (3.0mm diameter) of a teflon tube. The electrical contact was provided by a copper wire connected to the paste in the inner hole of the tube. The paste was kept at room temperature in a desiccator until used. A new surface was obtained by smoothing the electrode onto a weighing paper.

4.4.4 Preparation of DNA samples

A gentle treatment of DNA with 1 mM HCl leads to the selective removal of its purine bases by cleavage of purine glycoside bonds. The calf thymus dsDNA was hydrolysed as follows for quantification of guanine and adenine. 3 mg of dsDNA was digested using 1 ml 1M HCl in a sealed 10 ml glass tube. After heating in a boiling water bath for 90 mins, 1 ml 1 M NaOH added to the solution. Then the solution was diluted in 10 ml using 0.2 M phosphate buffer (pH 5).

4.4.5 Voltammetric procedure of guanine and adenine

The electrochemical experiments were performed in 0.1 M pH 5 phosphate buffer solution with different concentrations of guanine and adenine. The adsorptive accumulation of guanine and adenine at working electrode was done in a stirred solution at open circuit for 5 min. after a 10 s quiet period, the anodic stripping voltammograms were recorded from +0.0 V to +1.3 V at 100 m V s$^{-1}$. The electrode can be stable upto 5 measurements.
4.5 Results and discussion

4.5.1 Characterization of the MWCNT/CPE and bare CPE

*Structural and Morphological Characterizations:* Scanning electron microscopic technique was used to characterize the carbon paste and modified carbon paste electrodes. Figure 4.1a compares the typical morphological features of a CPE and of a MWCNT/CPE before use. The carbon paste (Figure 4.1a) is characterized by a surface formed by irregularly shaped micrometersized flakes of graphite. The carbon nanotube modified carbon paste shows a surface topography, formed by the assembling of rather large CNTs (20-50 nm) (Figure 4.1b).

4.5.2 Choice of the CNT Composition

The C_{60}/CPE electrodes were prepared by mixing different weight percentages of C_{60}, classical carbon powder and silicone oil. The amount of fullerenes was kept as 2, 4, 6, 8, 10 and 15% the mass of carbon powder. The pasting liquid silicone oil is kept constant as optimized. Similarly the MWCNT/CPEs were prepared by mixing different weight percentages of MWCNTs, classical carbon powder and silicone oil. For all these electrodes, cyclic voltammetry was run in 1 mM K_{3}Fe(CN)_{6} in KNO_{3}, between 0.6 and -0.2 V and with a scan rate of 100 mV S^{-1}. The influence of the C_{60} and MWCNT composition upon the separation of the cyclic voltammetric peak potentials (\Delta E_{p}) and peak current (I_{p}) for K_{3}Fe(CN)_{6} is represented in Figure 4.2A. Results obtained with MWCNT/carbon powder ratios of 2/98 (w/w) and 15/85 (w/w) confirmed that the best reversibility in terms of \Delta E_{p} and I_{pa} was obtained for 10/90 (w/w) MWCNT/carbon powder composition. Figure 4.2B shows the multiple scans of ferricyanide showing the electrode stability. In fact, when the composition of the paste is similar to the dry carbon
powder (which means that the silicone oil percentage is diminished in the MWCNT/oil composition) the electroanalytical response improves (lower $\Delta Ep$ and higher current $Ip$). On the contrary, the electroactivity of the 2/98 (w/w) CNT/carbon paste electrode resulted in a higher resistance and, thus, a higher $\Delta Ep$ for $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4+}$ redox couple. This electrochemical behavior was explained because the amount of carbon nanotubes in the composition of the MWCNT/CPEs decreases while the silicone oil percentage increases, producing a very high resistance at carbon paste electrode surfaces. Results confirmed that the best reversibility in terms of $\Delta Ep$ and $Ip$ was obtained for a high ratio (w/w) CNT/carbon paste composition of 10/90 (w/w).

The graphite particles, having a lower surface area, are probably completely covered by a high amount of silicone oil. This hampers the electrical communications between graphite particles, resulting in a higher resistance and, thus, a higher $\Delta Ep$ for $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4+}$ redox couple. According to these results, the best paste composition, resulting in sharper voltammograms (lower $\Delta Ep$ and higher $Ip$) was the one prepared with 10 wt% carbon nanotubes, graphite particles and silicone oil. This composition also assured the construction of stable and robust graphite paste electrodes, which are essential to compare the electrochemical responses of new carbon nanotube-modified electrodes.

4.5.3 Electrochemical reactivity

Previous studies on the electrochemical behavior of MWCNT-modified electrodes have shown that these electrodes are capable to improve the kinetics of the electrode processes and sensitivity of the measurements [3, 17, 26-32].
The modified composite electrodes prepared with carbon nanotubes/graphite particles and mineral oil was then used to investigate the electrochemistry of a wide range of chemical species. Analysis of the ferricyanide faradaic current as a function of the scan rate resulted in a linear $I_p$ vs $\nu^{1/2}$ relationship over the 5-500 mV/s range (not shown; conditions as in Figure 4.2), indicating that the current is controlled by a semi-infinite linear diffusion.

For dopamine, such a large enhancement of anodic current revealed that by lowering the anodic overpotential of the electrode process, the kinetics of electron transfer improves remarkably at the MWCNT-modified carbon paste electrode. The effective catalytic role of the MWCNT-modified carbon paste electrode toward dopamine oxidation can be attributed to some oxygen functional groups, the large microscopic area, specific electronic structure and high electrical conductivity of MWCNT for the modified carbon paste electrode. Such a behavior has been previously reported for CNT modified electrode in the electrochemical oxidation of various organic compounds [32-34].

4.5.4 Effect of surfactant media

Keeping the concentration of ferricyanide at $1\times10^{-3}$ M, in KNO$_3$ and with a scan rate 0.05 Vs$^{-1}$, the voltammograms were recorded with further modifying the MWCNT/CPE electrode by immobilizing the surfactants, viz., CTAB, SDS and TX-100 on the electrode surface. Concentration of surfactants varied from $1\times10^{-5}$ M to $7\times10^{-5}$ M.

In the case of anionic surfactant SDS, initially the peak current increased with increase in the addition of SDS and no effect on peak potentials. Different factors affect the surfactant adsorption on solid/liquid interface [35]. Apart from this, it is also claimed that at higher concentrations of the surfactant SDS, the repulsive forces among the
surfactant molecules adsorbed at the interface of the solid (adsorbent)-solution are more effective [36]. By increasing the concentration of the surfactant, more and more anionic part of the surfactant gets adsorbed at the surface of the electrode resulting in decrease in the cathodic current. The reductive group of analyte now requires more potential on account of adsorption and hence potential shifts towards more negative region and the current decreases. The heterogeneous kinetic process of transfer of electrons between the electrode surface and molecules in the interfacial region can be significantly affected by the microstructure and blocking of active sites on the electrode surface by adsorbed materials.

In the case of cationic surfactant CTAB, the peak potential does not change with the increase in concentration of CTAB (up to $6.0 \times 10^{-3}$ M). Current decreases as the concentration of CTAB increases. The decrease in current could be due to the slower diffusion of the electroactive species as a result of higher viscosity of the medium. The cathodic peak was not observed at the higher concentration of CTAB. This could be due to the saturation capacity [37] of carbon for adsorption of the cationic surfactant due to the electrostatic interaction between the adsorbent and the surfactant.

The nonionic surfactant Triton X-100 affects the electrochemical behavior of analyte, i.e., the peak current increases in a greater extent and we observed slight shift in the peak potential which is shown in figure 4.3 and 4.4.

**4.5.5 Voltammetry of dopamine at CPE and MWCNT/CPE**

Modified and unmodified carbon paste electrodes were tested as dopamine probes. The two-electron oxidation of dopamine to dopaquinone showed an excellent reversibility in cyclic voltammetry which was significantly better than that observed at
carbon paste electrodes. Figure 4.5 shows the electrochemical data of dopamine carried out at both CPE and MWCNT/CPE electrode at pH 6.5 phosphate buffer aqueous solutions. Redox behavior observed for dopamine system at MWCNT/CPE, the difference in peak potential is 60 mV and current ratio is almost equal to unity. The peak current increases for the oxidation of dopamine at MWCNT/CPE when compared to bare carbon paste electrode due to the rate of electron transfer is quite good at modified electrodes.

4.5.6 Electrochemical impedance study

EIS experiments were carried out using AUTOLAB potentiostat with FRA module at the frequency range of 10 kHz–45 mHz at the perturbation amplitude of 5 mV peak to peak. Polarization resistance and the capacitance behavior of the electrochemical interface of CPE and the ones covered with CPE-C\textsubscript{60} and CPE-CNT had been studied. Impedance behavior of bare CPE and also, modified by MWCNT and C\textsubscript{60} are shown in Nyquist plot format in Figure 4.6. It is seen from the impedance plot at open circuit condition that in case of CPE polarization resistance was very high. Polarization resistance decreased on deposition of C\textsubscript{60} the CPE and further decreased for MWCNT/CPE. It had become almost four orders of magnitude lower compared to bare CPE in case of the MWCNT/CPE used for the present study. Double layer capacitance had also got similar changes like polarization resistance, i.e., very low value CPE and higher value at CPE modified with MWCNT.

4.5.7 Cyclic voltammetry of adenine and guanine

CV responses of adenine and guanine tested at bare CPE, modified C\textsubscript{60}/CPE and MWCNT/CPE where the C\textsubscript{60} or MWCNT and CPE compositions were varied. 10/90 mass ration of MWCNT/CPE electrode proved to be the best composition.
Figure 4.7a illustrates the CV response of 20 μmol L⁻¹ G and 20 μmol L⁻¹ A in 0.2 mol L⁻¹ phosphate buffer (pH 5) solution at bare graphite, C₆₀/CPE and MWCNT/CPE electrode. It can be seen that, in the case of bare graphite electrode, the voltammograms of adenine and guanine exhibit just a small hump peak with the overlapping potential. Compared to the electrochemical response at bare CP electrode and C₆₀/CPE, in the case of MWCNT/CPE, the peak current signals of adenine and guanine enhance significantly with their oxidative potential moving negatively, especially the oxidative potential of guanine moves negatively for about 300 mV which leads to the effective separation of the peak potentials of adenine and guanine. Guanine and adenine gives two well-defined oxidation peaks at about +0.67 and +0.99 V respectively. No cathodic peaks were observed on the reverse scan within the investigated potential range which indicated that adenine and guanine oxidation is an electrochemically irreversible process. Results confirmed that the best separation of guanine and adenine in terms of potential shift and peak potential was obtained for a high ratio (w/w) MWCNT/carbon paste composition of 10/90 and 15/85 (w/w). The oxidation of guanine and adenine is expected to follow a two-step mechanism involving the total loss of four electrons. In both cases, the first two-electron oxidation is rate-determining [4, 38 – 41] as shown in the following schemes 4.1 and 4.2.

![Scheme 4.1. The reaction mechanism for guanine.](image-url)
Scheme 4.2. The reaction mechanism for adenine.

We studied the reaction process at MWCNT/CPE, by effect of scan rate to guanine electrochemical response. The CV of MWCNT/CPE in the presence of 20 µmol L⁻¹ guanine in phosphate buffer is directly proportional to the scan rate over the range of 10–200 mV s⁻¹ further confirms that the guanine is absorbed on the surface of electrode. With increasing scan rate, the oxidation peak potential (Epa) shifts to more positive values and there is a linear correlation between the peak potential and the logarithm of scan rate, log (v). These results indicate that the electrode process was controlled simultaneously by diffusion and adsorption.

4.5.8 Adsorption of guanine and adenine

After immersing the modified electrode into a phosphate buffer solution (pH 5) containing guanine and adenine for 5 min, the CV shows poor responses. After a 5 min
stirring at open circuit, responses improved considerably. This suggested that the guanine and adenine can be adsorbed on the modified electrode. After subsequent cycles the peaks of guanine and adenine almost disappeared (not shown in figure). This phenomenon may be attributed to the consumption of adsorbed guanine and adenine and the adsorption of electrochemical oxidation products at the modified electrode.

4.5.9 Analytical application

In order to test the analytical applicability of the MWCNT/CP electrode, we selected acid denatured DNA for simultaneous determination of guanine and adenine. The acid-denatured DNA gives two well-defined peaks at the MWCNT/CP electrode due to the oxidation of guanine and adenine. Figure 4.7b shows the typical CV responses of guanine and adenine. The experimental conditions are same as explained before.

4.6 Conclusions

- Carbon nanotube paste-based modified electrodes have been prepared and characterized and compared with fullerene modified and classical carbon paste electrodes.

- Studies on the composition of the multi-wall carbon nanotubes in the MWCNT/CP electrodes showed a different behavior. The best ratio (w/w) proved that CNT/carbon paste composition of 10/90 (w/w).

- In fact, some molecules showed a similar electrochemistry at carbon paste electrodes without modification and the electrochemistry of ferricyanide, dopamine, improved significantly at modified electrodes.

- The MWCNT/CP electrodes constructed using silicone oil resulted in an effective electrode used for simultaneous detection of guanine and adenine.
• Guanine and adenine gives two well-defined oxidation peaks at about +0.67 and +0.99 V respectively which proves MWCNT/CPE advantageous than other conventional electrodes.

• The analytical applicability of the MWCNT/CP electrode was quite good for simultaneous determination of guanine and adenine which proved from the detection in acid denatured DNA, which gives two well-defined peaks.
Figure 4.1. SEM images of (a) bare carbon paste electrode and (b) MWCNT modified carbon paste electrode
Figure 4.2A. Cyclic voltammetry of ferricyanide at (a) CPE, (b) C60/CPE and (c) MWCNT/CPE, Supp electrolyte = KCl, Scan rate = 100 mV/s

Figure 4.2B. Cyclic voltammetry of ferricyanide at MWCNT/CPE, showing multiple scan rate (10 scans)
Figure 4.3A. Cyclic voltammetry of ferricyanide at MWCNT/CPE (a) and MWCNT/CP/TX-100 modified electrode (b). Scan rate = 100 mV/s.

Figure 4.3B. CV of ferricyanide showing effect of surfactant, TX-100 on scan rate at MWCNT/CPE, a=5, b=10, c=50, d=100, e=200 and f=500 mV/s
Figure 4.4 (a) Performance of modified electrode with 2, 4, 8, 10 wt % of C\textsubscript{60} and surfactant TX-100 shows increase in peak current.

Figure 4.4 (b) Performance of CPE modified with 10 wt% MWCNT and surfactant TX-100 on peak current, ferricyanide system.
Figure 4.5. CV of dopamine at (a) bare carbon paste electrode and (b) MWCNT/CPE in pH 6.5 phosphate buffer solutions, scan rate = 100 mV/s

Figure 4.6. Nyquist plot of the carbon paste electrode without modification (a), carbon paste electrode modified with $C_{60}$ (b) and carbon paste electrode modified with MWCNT (c).
Figure 4.7A. CV of guanine and adenine at bare carbon paste electrode, C_{60}/CPE and MWCNT/CPE, phosphate buffer pH 5. accumulation time, 5 min. a=bareCPE, b= C_{60}/CPE, c= CNT/CPE (8:92 wt%), d= CNT/CPE (10:90 wt%) and e= CNT/CPE (15:85 wt%).

Figure 4.7B. CV of guanine and adenine of acid denatured DNA at MWCNT/CPE, phosphate buffer pH 5 accumulation time, 5 min.
4.7 References


