Electrochemical Determination of Zn\(^{2+}\) ion using Diphenylamine/Single Walled Carbon Nanotube/Cetyltrimethylammonium Bromide Modified Glassy Carbon Electrode

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Abstract — Diphenylamine encapsulated inside a composite film of single walled carbon nanotube (SWCNT) and cationic cetyltrimethylammonium bromide (CTAB) on the surface of Glassy Carbon (GC) electrode can be converted into diphenylbenzidine on application of a potential of +0.600 V versus Ag-AgCl reference electrode for 60 seconds. This modified electrode, in tris buffer solution (pH 7.0), shows reversible cyclic voltammogram due to diphenylbenzidine / diphenylbenzidine violet redox couple. This cyclic voltammogram is observed in pure CTAB film but not observed inside the film of pure SWCNT, polyvinyl pyrollidone or TX-100. In the SWCNT + CTAB film the redox potential is ca. +0.430 V. The oxidation and reduction currents of the modified electrode decreases with the increase in the concentration of Zn\(^{2+}\) ion in the electrolytic solution. The relative decrease is found to be 0.60 to 0.65. The ions such as Na\(^+\), K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\), do not affect the redox currents. Diphenylbenzidine / SWCNT / CTAB modified GC electrode can determine Zn\(^{2+}\) ion concentration in the range 20 × 10\(^{-6}\) mol L\(^{-1}\) to 200 × 10\(^{-6}\) mol L\(^{-1}\) while diphenylbenzidine/CTAB modified GC electrode can determine between 0.2 × 10\(^{-6}\) mol L\(^{-1}\) to 2 × 10\(^{-6}\) mol L\(^{-1}\). A mechanism for the electrochemical inactivation of the electrode by Zn\(^{2+}\) ions has been proposed.

Keywords : Diphenylamine, zinc, cyclic voltammetry, surfactant, glassy carbon electrode, indium doped glass electrode.

INTRODUCTION
Zinc ions play a number of critical roles in life processes like function of metalloenzyme, gene expression, synaptic neurotransmission, neuronal excitotoxicity,
etc. [1]. Decrease in the concentration of zinc ions in human brain leads to diseases like epilepsy, Alzheimer’s and Parkinson’s diseases [2]. The experimental techniques commonly used for Zn\(^{2+}\) ion estimation is fluorescence spectroscopy [3,4,5] where a fluorescent probe increases its intensity on binding with Zn\(^{2+}\). The chelate moieties of these probes contain aliphatic tertiary amines which are significantly protonated at physiological pH hindering the Zn\(^{2+}\) detection. Biosensors based on the enzyme, carbonyl anhydrase are known for Zn\(^{2+}\) estimation [6], which is difficult to fabricate and handle. Recently carbon nanotube (CNT) based electrochemical sensors have generated considerable interest due to high electron transfer rate and high electrocatalytic effect of CNT [7]. Prussian blue incorporated CNT modified electrode is reported for glucose determination [8]. Electrodes modified with CNT dispersed on a chitosan matrix have been fabricated as galactose biosensor [9]. CNT based electrochemical biosensor is also known for monitoring concentration of cholesterol in blood [10] and other biologically important molecules such as NADH, neurotransmitters, ascorbic acid, etc, [11]. Surfactants also can be adsorbed on solid surface to form surfactant films [12, 13]. The adsorption is through hydrophobic surfactant tail on the electrode surface with the polar head groups directing towards the bulk water phase [14]. Recently we have shown that electrochemical experiment can conveniently be carried out inside a surfactant film on electrode surface and its redox potential is influenced significantly by the charged nature of the film [15].

There are few reports on adsorptive stripping voltammetry [16–18] for probing Zn\(^{2+}\) ions and Square Wave Voltammetric study to understand transfer of Zn\(^{2+}\) ions [19]. But to our knowledge till date there is no sensor based on cyclic voltammetric technique which can selectively detect Zn\(^{2+}\) in presence of other metal ions of physiological importance. There may be two reasons for this— (i) Zn\(^{2+}\) is electro inactive in common potential range and (ii) ligands which binds well to Zn\(^{2+}\) are not generally redox active. As the electrochemical technique, compared to fluorescent method, is more cost effective and easy to handle, there is an urgent need to develop an electrochemical sensor for Zn\(^{2+}\) ion.

In this paper we report that diphenylamine, while inside cetyltrimethylammonium bromide (CTAB) film on Glassy Carbon electrode surface, can be oxidized into diphenylbenzidine violet by controlled application of potential. Diphenylbenzidine violet can be reversibly reduced into colorless diphenylbenzidine giving rise to reversible cyclic voltammogram. This modified electrode can determine Zn\(^{2+}\) without any interference at all from ions such as Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) while Cu\(^{2+}\) and Fe\(^{3+}\) interfere a very little.
EXPERIMENTAL

Materials: All the chemicals were from Loba Chemie and used without further purification. SWCNT (120–150 nm) was purchased from Aldrich, USA.

Methods: CHI 660B Electrochemical Analyzer (USA) with a three-electrode cell assembly was used for electrochemical studies. Electrochemical experiments were carried out under a blanket of nitrogen gas after passing the gas through the solution for ten minutes. The working electrode is Glassy Carbon (GC) disc, reference electrode is Ag-AgCl and sodium nitrate (0.1M) is the supporting electrolyte. In Osteryoung Square Wave Voltammetry (OSWV) experiments, the square wave amplitude was 25 mV. The working electrode was cleaned, by polishing with 0.05 micron alumina powder using a polishing kit (CHI) followed by sonication.

Preparation of modified electrodes —
A. Diphenylamine/CTAB film modified electrode: 0.025 g diphenylamine and 0.025 g CTAB were dissolved in 5 mL of dichloromethane. 10 μL of this solution was placed onto the surface of a pre cleaned GC electrode and dichloromethane was allowed to evaporate at room temperature for 30 minutes.

B. Diphenylamine / SWCNT / CTAB film modified electrode: 0.025 g SWCNT was taken in 5 mL of dichloromethane and sonicated for 15 minutes when a suspension is obtained. 0.025 g diphenylamine was dissolved into this suspension and 10 μL of this solution was placed onto the surface of the pre-cleaned GC electrode and allowed to evaporate at room temperature for 30 minutes. 10 μL of a solution prepared from 0.025 g CTAB in 5 mL of dichloromethane is placed on the surface of the SWCNT modified electrode and allowed to dry.

RESULTS AND DISCUSSIONS

Characterization of electrodes
Tip of the diphenylamine/CTAB film modified electrode was placed into a tris buffer solution (pH 7.0) containing 0.1 M NaSO₄ and + 0.600 V potential (versus Ag-AgCl) was applied for 60 seconds. A potential scan from +0.600V to –0.200V found to give a well defined cyclic voltammogram with reduction and oxidation peaks at –0.140 V and +0.090 V, respectively at a scan rate, 20 mV s⁻¹. Fig. 1 shows the cyclic voltammogram of this electrode in tris buffer solution at various scan rates. The redox potential of the electrode is found to be –0.025 V at a scan rate, 0.020 V s⁻¹ and the peak separation potential is 0.230 V. The redox potential is further confirmed by the Osteryoung square wave voltammetry.
When the potential applied to the electrode is +0.600 V or above, the electrode was found to show two irreversible reduction peaks at +0.515 V and −0.385 V, on the other hand, if the pre-anodization potential is lower than +0.600 V, no cyclic voltammogram is observed at all. Hence the value of the pre-anodization potential is very crucial.

When the cyclic voltammogram is recorded for the diphenylamine/SWCNT/CTAB modified electrode with pre-anodization potential +0.600 V for 60 seconds, reversible cyclic voltammogram is observed with reduction and oxidation peak positions at +0.380 V and +0.435 V, respectively at the scan rate, 20 mV s⁻¹. Fig. 2 shows the cyclic voltammogram of the electrode at various scan rates. Redox potential is +0.407 V and peak separation is only 0.055 V. This peak separation is markedly low compared to that of 0.230 V for diphenylamine/CTAB film modified electrode due to the high electron transfer ability of SWCNT. The redox potential of the diphenylamine/SWCNT/CTAB electrode is 0.432 V, more positive than the diphenylamine/CTAB film modified electrode. The redox potential is further confirmed by Osteryoung square wave voltammetry.
In case of diphenylamine/SWCNT/CTAB film modified electrode the pre-anodization potential (+0.600V) is applied for different times and the cyclic voltammogram is recorded. It has been observed that both the oxidation and the reduction peak currents increase with pre-anodization time and the peak current is maximum when the potential is applied for 60 seconds. It is well known that diphenylamine can be oxidized chemically into diphenylbenzidine (I) which can further be reversibly oxidized into diphenylbenzidine violet (II) (Scheme 1) [20]. The observed reversibility of the cyclic voltammogram of the modified electrode means that the redox couple involved must be I/II (scheme 2). Hence at pre-anodization potential of +0.600 V, diphenylamine oxidizes to II probably through the formation of free radical as shown in scheme 1. When the applied potential on the electrode is more than +0.600V, II is further oxidized irreversibly into some unknown compound, which cannot be reduced back to II [20].

The probable mechanism for the reversible oxidation of I into II involving free radical is shown in Scheme 2. The electrode at potential +0.600 V, takes up one
electrons each from the two lone pairs on the two amine groups of I giving a di-cationic free radical. This di-cationic radical gives up two protons and the free radical is formed. A simple electronic arrangement forms the oxidized product II. During reduction process, first protonation on II leads to a dication and addition of two electrons from the electrode gives rise to the free radical, which on electronic rearrangement leads to the reduced product I. When the electrode surface was
modified with diphenylamine/SWCNT film (without CTAB), no cyclic voltammogram could be recorded even for pre-anodization time much higher than 60 seconds. The bromide ions of CTAB present in the film should stabilize the diphenylbenzidine dicaticonic free radical, formed on application of +0.600 V potential at the modified electrode. This stabilization of the intermediate is crucial in facilitating the further steps of scheme 2. In absence of CTAB in the film there is no corresponding anion to stabilize the dicaticonic radical and hence it is likely to break down back into diphenylamine and the cyclic voltammogram is not observable. To verify the role of anion, dipheylamine was incorporated in neutral Triton X-100 (TX-100) and polyvinylpyrrollidone (PVP) film on GC electrode and the cyclic voltammogram could not be obtained. On the other hand when the lipid, L-α phosphatidylcholine (PL) is used in place of CTAB, cyclic voltammogram can be recorded only when pre-anodization is done for at least 200 seconds and the potential scan rate is less than 0.020 V s⁻¹. Above this scan rate no cyclic voltammogram can be recorded. PL is zwitterionic and therefore should be able to stabilize the dication to some extent.

**Effect of Zn²⁺ ion concentration on the redox process of the electrode**

Fig. 3 shows the effect of Zn²⁺ ion concentration on the peak currents of the cyclic voltammogram of diphenylamine/SWCNT/CTAB modified electrode. Both the oxidation and reduction currents were found to decrease with increasing Zn²⁺ ion concentration in the electrolytic solution till it becomes 200 μM when the peak currents remain the same (Fig. 3, inset). The relative decrease in reduction current defined as the actual decrease in current (current obtained when no zinc ion is added minus the current at a given zinc ion concentration) divided by the initial current [21], at Zn²⁺ ion concentration 200 μM, is found to be 0.60. No change in peak positions of the redox couple is observed indicating formation of simple electro-inactive compounds between Zn²⁺ ion and I and/or II. In the case of diphenylamine/CTAB-modified electrode (without SWCNT), the peak currents remain the same at Zn²⁺ ion concentration above 2 μM in the electrolytic solution (Fig. 4, inset). The relative decrease in current at 2 μM concentration of Zn²⁺ ion is 0.65. Hence the presence of SWCNT in the film increases the detection range of the electrode by 102 times. The plot of peak currents versus Zn²⁺ ion is found to be linear upto 2 μM (Fig 4, inset). Fig. 5 shows the plot of the relative decrease in reduction currents for both the modified electrodes as a function of Zn²⁺ ion concentration. For both the modified electrodes the peak currents were found not to be affected by Na⁺, K⁺, Ca²⁺ and Mg²⁺. In our experiment when the concentrations of Na⁺, K⁺, Ca²⁺ and Mg²⁺ ions are gradually increased up to 0.5 mM, redox currents of both diphenylamine/CTAB and diphenylamine/CTAB/SWCNT modified
Das GC electrode are found to remain the same. In presence of Cu\(^{2+}\) and Fe\(^{3+}\) ions, at a concentration, 100 times the Zn\(^{2+}\) ion concentration, for which the maximum relative decrease in current is obtained, the relative decrease in currents are found to be 0.04 and 0.05 respectively for both the electrodes. The decrease in current (due to Cu\(^{2+}\) and Fe\(^{3+}\)) may be considered negligible compared to the maximum relative decrease of 0.60 and 0.65 imparted by Zn\(^{2+}\) ions for the electrodes, diphenylamine/CTAB/GC and diphenylamine/CTAB/SWCNT/GC, respectively. The detection limit for diphenylamine/CTAB/GC is 0.1 μM and for diphenylamine/CTAB/SWCNT/GC electrode is 1.0 μM.

Zn\(^{2+}\) ion is unique among the metal ions considered in this work in two aspects; it is considerably soft due to polarizable d\(^{10}\) configuration and it has high charge to radius ratio making it significantly electrophilic [22]. These two properties in combination makes Zn\(^{2+}\) ion selective over the other ions towards this modified electrode. The high electrophilicity makes Zn\(^{2+}\) ion bind to the electron rich imine group of II and the resulting co-ordinated positive ions are stabilized by the soft

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Fig. 3. Cyclic voltammogram of the diphenylamine /CTAB/SWCNT modified GC electrode when Zn\(^{2+}\) ion is added into the electrolytic solution. Both the oxidation and reduction currents decrease with Zn\(^{2+}\) ion concentration till 200 μM. Inset: Plot of reduction current versus Zn\(^{2+}\) concentration. (RE, Ag-AgCl, Tris buffer, pH 7.0).
bromide ions. Once Zn$^{2+}$ binds to one of the two imine N atom of II (Scheme 2), it stops the protonation step described in scheme 2 and the redox process is hindered. Since Cu$^{2+}$ is moderately soft and Fe$^{3+}$ has high charge, for these two ions a small interaction with the electrode has been observed. If this is true, addition of Zn$^{2+}$ should lock the deep blue colored II and to verify this we have done the following experiment. It is known that I is white while II is deep blue and we have used Indium doped tin oxide (ITO) glass (0.5 cm$^2$ area) attached to a platinum wire as an electrode to verify the formation of I and II on electrode surface. When this electrode was coated with diphenylamine incorporated CTAB film, in tris buffer, an application of 0.6 V for 60 seconds makes the film bluish green. When the potential was scanned to ~0.2 V, the color of the film was found to vanish slowly with the potential approaching ~0.2 V and finally becomes colorless at ~0.2 V. On the reverse scan towards +0.6 V, the color slowly develops and regains the bluish green color at +0.6 V. This color change with the variation of applied potential, visible with naked eye, was found to repeat when more than one cycle was allowed to take place. We have
observed this color change up to twenty cycles. When we tried to do this potential cycle with Zn$^{2+}$ ions (20 mM) in the electrolytic solution, the color of the film remains locked into bluish green. Application of $-0.200$ V potential for 300 seconds can not make the film white. This confirms that Zn$^{2+}$ strongly binds to II through the lone pair on imine N.

We can determine the composition of electro-inactive adduct between diphenylbenzidine violet (DBV) and Zn$^{2+}$ with the following argument based on the original proposal by Zhu and Li [23] for some other systems. Suppose one mole of DBV combines with m mol of Zn$^{2+}$, the equilibrium may be written as:

$$DBV + m \text{Zn}^{2+} \rightleftharpoons DBV.m\text{Zn}^{2+}$$

The equilibrium constant, $\beta = [DBV.m\text{Zn}^{2+}]/[DBV][\text{Zn}^{2+}]^m$ (1)

DBV is considered to be adsorbed as a film on electrode surface and then the initial current ($I_{\text{init}}$) at zero concentration of Zn$^{2+}$ will be given by [24],

$$I_{\text{init}} = (n^2F^2vA/4RT) \ C_{\text{DBV}} = k \ C_{\text{DBV}}$$

(2)

where n is the number of electrons involved in the redox process which is 2 here, F is the Faraday constant, $v$ is the scan rate (20 mV s$^{-1}$), R, the gas constant and T, the absolute temperature. $C_{\text{DBV}}$ is the surface concentration of DBV in the film.

$$C_{\text{DBV}} = [DBV] + [DBV.m\text{Zn}^{2+}]$$

(3)
Determination of Zn$^{2+}$ ion using Modified Glassy Carbon Electrode

The current at any added amount of Zn$^{2+}$ will be the product of $k$ and free DBV in the film.

\[
\text{Hence } I = k [\text{DBV}] \tag{4}
\]

(2) – (4) implies $I_{\text{init}} - I' = \Delta I = k [\text{DBV}.m\text{Zn}^{2+}] \tag{5}$

(2) – (5) implies $I_{\text{init}} - \Delta I = k[\text{DBV}] \tag{6}$

Introduction of equation (5) and (6) into equation (1) leads to -

\[
\beta = \frac{\Delta I}{(I_{\text{init}} - \Delta I).[\text{Zn}^{2+}]^m} \tag{7}
\]

or $\ln \frac{\Delta I}{(I_{\text{init}} - \Delta I)} = \ln \beta + m \ln[\text{Zn}^{2+}]$

which on rearrangement gives rise to the following relation,

\[
\frac{1}{\Delta I} = \frac{1}{I_{\text{init}}} + \left( \frac{1}{\beta I_{\text{init}}} \right) (1/[\text{Zn}^{2+}]^m) \tag{7}
\]

According to the above equation a plot of $1/\Delta I$ versus $1/[\text{Zn}^{2+}]^m$ should be linear. We have plotted $1/\Delta I$ versus $1/[\text{Zn}^{2+}]^m$ for $m = \frac{1}{2}, 1$ and 2 and found that the plot is most linear for $m = 1$ (Fig. 6) indicating that one Zn$^{2+}$ ion gets bound to diphenylbenzidine violet. It is quite obvious from scheme 2 that binding of one Zn$^{2+}$ ion is sufficient to stop the redox process at the electrode.

![Plot of 1/ΔI versus 1/[Zn$^{2+}$]$^m$ for m = ½ (▲), 1 (●)and 2 (●). The plot for m =1 is found to approach the straight line the most, confirming that one Zn$^{2+}$ ion binds to each diphenylamine molecule.](image)
Analytical application

We have tested both the electrodes in determining Zn$^{2+}$ ion concentration in multi mineral tablet (supra cal, MMC Health Care, H.P., India) containing 280 mg Ca$^{2+}$, 100 mg Mg$^{2+}$, 200 IU vitamin D3 and 4 mg Zn$^{2+}$ per tablet. One tablet was dissolved in 50 mL tris buffer (pH 7.0) solution and a standard solution of Zn$^{2+}$ ion was prepared by dissolving 0.4 g in 50 mL tris buffer solution. Two electrodes each for diphenylamine/CTAB/SWCNT/GC and diphenylamine/CTAB/GC were prepared as described in the experimental section. The redox currents of one (diphenylamine/CTAB/SWCNT/GC) of the two electrodes have been measured adding 100, 200, 300, 400 and 500 $\mu$L of the standard Zn$^{2+}$ solution into 5 mL of the electrolytic solution. The redox current of the other electrode is measured after similar addition of the prepared tablet solution. When the Zn$^{2+}$ ion concentration in the electrolytic solution was calculated, the dilution effect has been taken into account. Fig. 7 compares the relative decrease in reduction currents at the same Zn$^{2+}$ ion concentration for the standard and tablet solution for the diphenylamine/CTAB/SWCNT/GC electrode. From the figure it is clear that the current obtained for a particular concentration of Zn$^{2+}$ ion in case of the tablet solution is in excellent agreement with the current obtained.

![Fig. 7](image)
Determination of Zn$^{2+}$ ion using Modified Glassy Carbon Electrode at the same concentration of the standard Zn$^{2+}$ ion solution. Good agreements between the currents for the same concentration of Zn$^{2+}$ ion for standard and tablet solution were obtained for diphenylamine/CTAB/GC electrode also.

CONCLUSION

Diphenylamine in SWCNT/CTAB or in pure CTAB film on GC electrode can be converted into diphenylbenzidine violet on controlled application of potential. This modified electrode was found to show reversable cyclic voltammogram due to diphenylbenzidine violet/diphenylbenzidine redox couple. The redox potential is +0.407 V with peak separation value 0.055 V for SWCNT/CTAB modified electrode and ~0.025 V with peak separation 0.270 V for CTAB modified (without SWCNT) electrode. The current of oxidation and reduction processes were found to decrease linearly with addition of Zn$^{2+}$ ion in the electrolytic solution, for both the modified electrodes. Diphenylbenzidine/SWCNT/CTAB modified GC electrode can determine Zn$^{2+}$ ion concentration in the range 20 $\times$ 10$^{-6}$ mol L$^{-1}$ to 200 $\times$ 10$^{-6}$ mol L$^{-1}$ while diphenylbenzidine/CTAB modified GC electrode can determine between 0.2 $\times$ 10$^{-6}$ mol L$^{-1}$ to 2 $\times$ 10$^{-6}$ mol L$^{-1}$. Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ ions were found not to interfere the detection of Zn$^{2+}$ ion at all while Cu$^{2+}$ and Fe$^{3+}$ were found to interfere very little. The electrode has been found to be good for determination of Zn$^{2+}$ ion concentration in multi mineral tablet containing Zn$^{2+}$, Ca$^{2+}$, Mg$^{2+}$ and vitamin D3.

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