
A Novel Electrochemical Sensor Based on Multi Walled Carbon Nano Tubes/Poly (Evans Blue) Composite for the Determination of Dopamine in Presence of 5-HT and FA

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Abstract:

A new sensor was fabricated by electrochemical polymerization of Evans blue (EB) followed by deposition of multi walled carbon nano tubes (MWCNTs) on glassy carbon electrode (GCE). The developed electrode was characterized by using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The fabricated electrode was employed for analyzing neurotransmitter Dopamine (DA) drug and its simultaneous determination in presence of serotonin (5-HT) and folic acid (FA) with the help of differential pulse voltammetry (DPV) technique. The effect of pH of phosphate buffer solution (PBS) was studied and pH - 5.0 was found to be an optimum pH value. In the present investigation the kinetic parameters, such as charge transfer coefficient, number of electrons and rate constant values were evaluated. The voltammetric peak currents showed a linear response for DA concentrations from 0.5 to 100 µM and from the linear relationship the limit of detection (LOD) and limit of quantification (LOQ), values were as evaluated as 0.54 µM and 1.8 µM respectively. The fabricated electrode showed good stability, reproducibility and sensitivity. The practical application of the developed sensor in determining DA levels in pharmaceutical formulation was successfully monitored.

***Keywords**: Dopamine (DA), Evans blue (EB), Multi walled carbon nano tubes (MWCNTs), Voltammetry, Electrochemical impedance spectroscopy (EIS).*

Introduction:

Dopamine is a precursor required by the brain. DA is a very important catecholamine in the biological system and its imbalance levels causes Parkinson's disease [1-2]. Dopamine is a metabolic precursor used for the treatment of Parkinson's disease. The Parkinson's disease associated with low levels of dopamine in brain, causes reward and learning [3-4]. Many people suffering from Parkinson's disease have depleted levels of dopamine, which causes muscle stiffness, termor and slowness of movement. DA is used for the treatment of hypertension bronchial asthma, heart disease and myocardial infection [5]. Several investigators have reported the chronic administration of DA in patients with Parkinson's disease [6-7]. Serotonin (5-HT) is a biogenic monoamine neurotransmitter found in the human brain, which plays a crucial role in controlling and regulating various physiological functions such as eating disorder, sleep muscle contraction, sexual activity and thermo regulation [8]. 5-HT is involved in several gastrointestinal disorders including secretion, irritable bowel syndrome, food hypersensitivity and inflammatory bowel disease and low levels associated with various disorders, such as depression, migraine, bipolar disorder and anxiety [9-10]. Folic acid (or) vitamin-B_9 help the cells in producing heat and is an agent for cancer prevention by antioxidant activity [11- 12]. The variation in the concentration levels of FA in body fluids leads to several health problems, such as gigantocytic anemia, devotion of mentality and heart attack [13-14].

The dopamine produced from DA is mediated by the serotonin neurons and increase of dopamine levels cause oxidative stress and damages serotonin neurons [15-16]. The concentration of 5-HT in the animal brain is reported to be decreased with DA injection [17-18]. According to clinical evidence, DA interferes with the metabolism of 5-HT in humans, which may be due to displacement of 5-HT in the human body. In particular various tumors of the symapathoadrenal system are diagnosed by determination of the catecholamine's and their metabolites in urine and blood samples. Hence, it is of great importance to develop analytical method for the simultaneous determination of the DA and 5-HT. In addition, it shows significant interferences with folic acid, since it is electro active and coexists with DA and 5-HT in biological systems. Several methods have been reported for the determination of DA, such as spectrophotometer [19-20] flow injection analysis [21-22], High-performance liquid-chromatography (HPLC) [23], gas chromatography and voltammetry [24-26]. In the present electro chemical studies, the two-hydroxyl group present in DA oxidizes at the electrode surface, which is the basis for its investigation. As mentioned above DA, 5-HT and folic acid are of great importance in the biomedical field and folic acid shows serious interference with DA determination. Hence, it is of great challenging task for the researchers to determine DA in presence of 5-HT and FA.

The electro chemical methods have attracted extensive consideration due to their several benefits, such as fast response, good sensitivity, ease in handling, likelihood of efficiency and relatively low cost. The electro polymerization of dyes on the electrode surface increases the selectivity and sensitivity of the method [27-28]. CNTs have been extensively used as a signal-amplifying molecule to enhance the sensitivity of a sensor owing to its numerous advantages like elevated surface areas, excellent biocompatibility; low back ground currents and reduced over potentials [29-32].

2. Experimental

2.1 Instruments:

The Cyclic voltammetry (CV), electrochemical impendence spectroscopy (EIS) and differential pulse voltammetry (DPV) techniques were carried out by using CH Instruments model CHI 660D, Texas, Austin, USA which was controlled by an individual computer. The pH meter are used for preparing buffer solutions. The MWCNTs homogeneous suspension was prepared by using toshiba ultra-sonication bath made in India.

2.2 Preparation of MWCNTs/Poly(EB)/GCE

The GCE was polished on polishing pads with 1.0, 0.3 and 0.05 microns of alumina slurry to get a mirror shine and then thoroughly cleaned with distilled water. The uninterrupted 5 CV cycles at bare GCE was made with 1mM Evans blue aqueous solution (PBS pH - 5.0) between the potential scan windows from -0.4 to 2.0 V. The achieved effective polymerization on the electrode surface (Poly (EB)/GCE) was washed with distilled water to remove the physically adsorbed material. The resulted electrode was further modified by drop containing of 5 µL of homogeneous suspension of 1 mg MWCNTs dispersed in 1 mL of ethanol. The resultant electrode was here after denoted as MWCNTs/Poly(EB)/GCE and used as a working electrode.

2.3 Analytical procedure

The MWCNTs/Poly(EB)/GCE was dipped in 0.1 M PBS of pH 5.0, containing 1mM DA solution. The electrolytic solution was stirred and recorded CV or DPV in the potential window -0.2 to 0.8V at a potential scan rate of 0.1 V/sec. All the experiments were recorded at room temperature.

3. Results and Discussion

3.1 Electrochemical polymerization of Evans blue on GCE

Evans blue, a dye was electro polymerized on GCE by scanning between the potential windows -0.4 to 2.0 V through CV for 5 multiple cycles. The thickness of the film on the electrode surface was enhanced by increasing the number of cycles of polymerization. The peak currents were found to decrease after 5 cycles; this was due to the fact that the active electrode surface area does not change significantly after 5 cycles and the increases in thickness of the poly (EB) film stable the electron transfer rate. Therefore, the window between -0.4 to 2.0 V was considered to be more effective polymerization zone for EB on the surface of GCE [33].

3.2 Voltammetric characterization of modified electrodes

The developed sensor was characterized by using 1mM $[Fe(CN)_6]^{-3/-4}$ in 0.1 M KCl with the help of CV technique. Fig. 1 shows the CV's of various electrodes, such as bare/GCE (a), poly(EB)/GCE (b), MWCNTs/GCE (c) and MWCNTs/Poly(EB)/GCE (d) towards response of 1mM $[Fe(CN)_6]^{-3/-4}$. It can be seen from the figure that the peak to peak separations (ΔEp) for the respective electrodes were 126 mV, 103 mV, 57 mV and 56 mV. The ΔEp values were found to decrease gradually upon respective modifications. The conventional electrode has large separation value (ΔEp) compare with other modified electrodes, the reduced ΔEp values were due to the presence of large surface area and high electric conductivity properties of poly (EB) and MWCNTs. The minimum value of peak to peak separation (ΔEp) was observed at MWCNTs/Poly(EB)/GCE, suggesting, the best electrochemical reaction ability and fast electron transfer kinetics at this modified electrode. The electron transfer rate is an important factor that increases current response and decrease the ΔEp value. At the bare GCE, anodic and cathodic peak currents ($I_{pa} = 1.328 \times 10^{-5}$A, $I_{pc} = 1.323 \times 10^{-5}$ A) were less and after modification with poly (EB), the peak currents ($I_{pa} = 1.539 \times 10^{-5}$A, $I_{pc} = 1.591 \times 10^{-5}$ A) were increased, suggesting better conductive nature of Poly (EB). Further, on modification with MWCNTs, the anodic and cathodic peak currents ($I_{pa} = 3.129 \times 10^{-5}$A, $I_{pc} = 3.069 \times 10^{-5}$ A) were gradually increased, which was due to the increase in the surface area and better conductive nature of MWCNTs [34].

$I_p = 2.69 \times 10^5 n^{3/2} A C D^{1/2} \upsilon^{1/2}$ ------ (1)

Where Ip is the peak current, 'n' is the number of electrons involved in the oxidation process, 'C' is the initial concentration of $[Fe(CN)_6]^{-3/-4}$, 'υ' is the scan rate 'A' is the area of the electrode and 'D' is the diffusion coefficient of the $[Fe(CN)_6]^{-3}$.

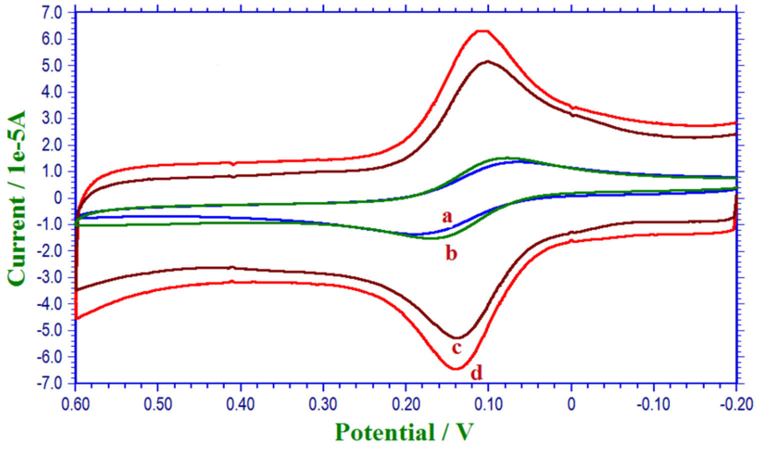

Fig: 1 Cyclic voltammograms of 1 mM $[Fe(CN)_6]^{-3/-4}$ in 1 M KCl solution with a scan rate of 0.1V s^{-1} at a) bare/GCE, b) Poly(EB)/GCE, c) MWCNTs/GCE and d) MWCNTs/Poly(EB)/GCE.

3.3 Electrochemical impedance spectroscopy analysis

The electrochemical impendence spectroscopy (EIS) is an effective method for examining the interface properties of a modified electrode surfaces. The EIS spectra are of two types i.e. nyquist plots and bode plots. The nyquist plot indicates the resistance of the electrode surface casted with chemical modifications. The bode plots provides, the information about the kinetic phenomena occurring at the electrode interface at different range of applied frequencies. At high frequencies, a semi-circular part was observed, which corresponds to the electron transfer process and at low frequencies a linear part was observed, which corresponds to the diffusion limited process [35]. The nyquist plots are shown in Fig. 2, where it shows the examination of different modified electrodes in 0.1 M KCl solution containing 0.5 mM $[Fe(CN)_6]^{-3/-4}$. The bare GCE (a) shows a large semi-circular part indicating large electron transfer resistance, indicating low level electron transfer rate at the bare GCE surface. The poly (EB)/GCE (b) shows reduced semi-circular part indicating reduced resistance and increased electron transfer rate at the surface. The MWCNTs/GCE (c) showed better electron transfer rate at the electrode surface, this was due to the better conductive nature of MWCNTs. Further the MWCNTs/Poly(EB)/GCE (d) exhibited almost a linear part, suggesting less resistance and good electron transfer rate at the electrode surface in comparison with all the three above electrodes[36].

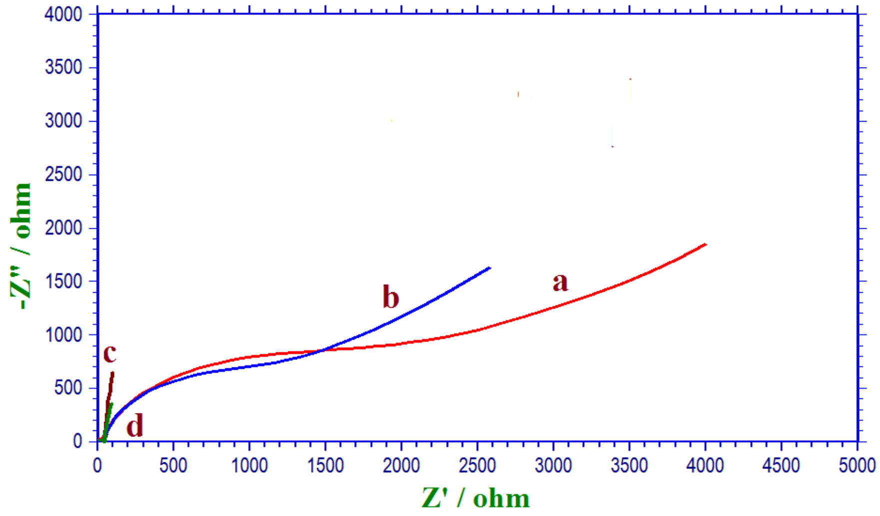

Fig: 2 Nyquist plots for a) Bare/GCE, b) Poly(EB)/GCE, c) MWCNTs/GCE d) MWCNTs/Poly(EB)/GCE.

3.4 Electrochemical behavior of Dopamine

The electrochemical behavior of DA was investigated with CV technique in 0.1 M PBS at pH 5.0. Fig. 3 shows the response of DA at different electrodes. Curve ‘a’ shows the response of bare GCE, were a weak response and low electron transfer rate was observed for DA. On electrochemical polymerization of bare GCE with EB (Poly(EB)/GCE) (curve ‘b’), an increased redox peak currents and decreased oxidation over potentials were noticed. Same to as on modification of bare GCE with MWCNTs (curve ‘c’), an increased redox peak currents was noticed in comparison to bare GCE and Poly(EB)/GCE with almost no change in the peak potentials). Lastly on bi-modification of bare GCE with poly (EB) and MWCNTs (curve‘d’) i.e., MWCNTs/Poly(EB)/GCE, showed an excellent increase in peak currents in comparison with all the above three electrodes with slight positive shift in the peak potentials. The shift of peak potentials to less positive potentials in comparison to bare GCE indicates the catalytic activity of EB and MWCNTs towards the oxidations of DA. The fabricated MWCNTs/Poly(EB)/GCE showed well resolved redox peaks (‘O’ and ‘R’ peaks) in comparison with remaining three electrodes [37].

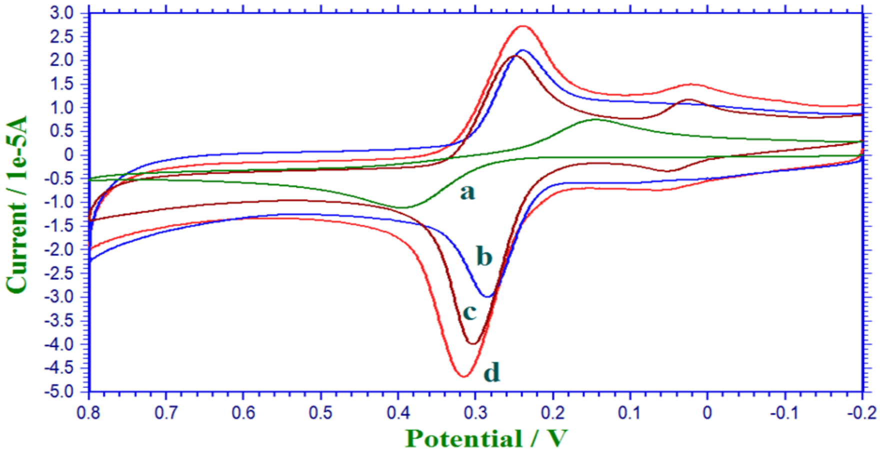

Fig: 3 cyclic voltammograms for the electrochemical response of DA at different electrodes in PBS of pH 5. 0, a) Bare GCE, b) Poly(EB)/GCE, c) MWCNTs/GCE and d) MWCNTs/Poly(EB)/GCE.

3.5 Effect of pH

The response of DA at various pH values ranging from 4.5 to 7.5 was observed in 0.1 M PBS at the fabricated MWCNTs/Poly(EB)/GCE. The pH of the supporting electrolyte was greatly influencing the voltammetric response of DA. Fig. 4a shows the DPV response of DA at different pH values. From the figure it can be seen that the peak potentials shifted towards negative side with increase in pH of the solution, indicating the involvement of protons in the electrode reaction process. Fig. 4b (A) shown the plot for peak currents and peak potentials versus pH of PBS towards the determination of DA. The maximum peak current response was observed at pH 5.0 therefore, pH 5.0 was chosen as an optimal pH for further electrochemical studies [38].

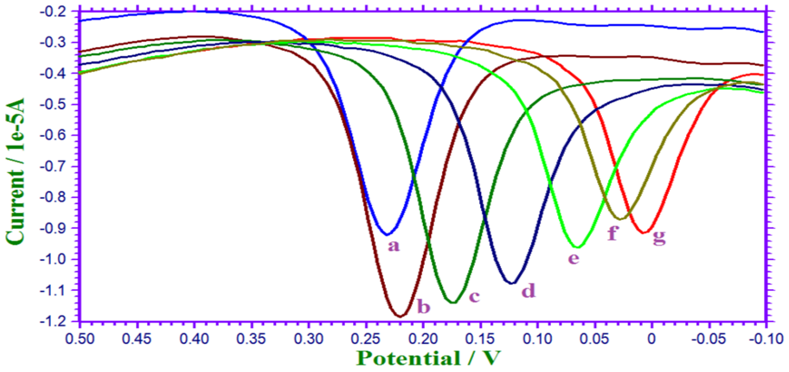

Fig: 4 DPV obtained for DA, at MWCTs/Poly(EB)/GCE (1 mM DA) in PBS of different pH values [(a) 4.5 (b) 5.0 (c) 5.5 (d) 6.0 (e) 6.5 (f) 7.0 (g) 7.5].

3.6 Effect of potential scan rate

The electrochemical oxidation of DA (1 mM) in PBS (pH 5.0) at various scan rates (10-120 mVs^{-1}) was studied with the help of CV. The CV responses of DA at different scan rates, predicting the peak current response was gradually increased with increase in the scan rate. Fig. 5 shows the good linearity's between the peak currents and scan rates with a linear regression equations of Ip_a (10^{-5} A) = 1.641 ν (V) + 0.0271 (10^{-5} A) and Ip_c (10^{-6} A) = 0.823 ν (V) + 0.02929 (10^{-6} A) with correlation co-efficient values of R^2 = 99715 and R^2 = 99948 respectively. From the obtained linearity between scan rates and peak currents, it was clearly understood that the electrochemical redox behavior of DA was controlled by adsorption process at MWCNTs/poly(EB)/GCE. From the following equations 2, 3 the charge transfer coefficient, number of electrons and heterogeneous rate constant values were calculated and was found to be as 0.308, 0.929 (≈ 1) and 3.328 × 10^{-6} S^{-1} respectively [39].

$$E_p = E^0 - m\,[0.78 + \ln(D^{1/2}/k_s) + (m/2)(\ln m)] + m/2 \ln \upsilon \quad \text{------ (2)}$$

$$m = RT/(1-\alpha) \quad \text{----- (3)}$$

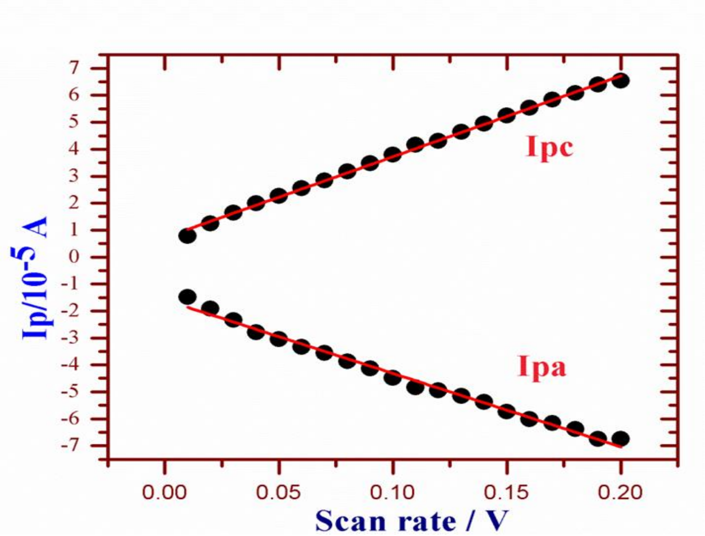

Fig: 5 Calibration plots for the scan rate versus redox peak currents

3.7 Calibration curve for determination of DA

The fabricated electrochemical sensor (MWCNTs/poly(EB)/GCE) was employed to study the electrochemical response of DA at different concentrations (5 µL to 200 µL) with the help of DPV technique (Fig. 6). It can be seen from the figure, that as the concentration of DA was increased, the increase in the peak currents was observed. Inset of Fig.6, shows the plot between the concentration of DA against the peak currents, a good linearity was observed with an excellent linear regression equation of Ip (10^{-7}A) = 1.8016 ± 0.0799 (10^{-7} M) (A) and correlation coefficient value of R^2 = 0.9948 respectively. From these results and by using equations 4 and 5, the limit of detection and limit of quantification values were calculated as 0.53 µM and 1.7 µM respectively.

$$LOD = 3S / m \ldots\ldots\ldots\ldots\ldots\ldots.. (4)$$

Where 'S' is the standard deviation of the peak currents, 'm' is the slope of the working curve.

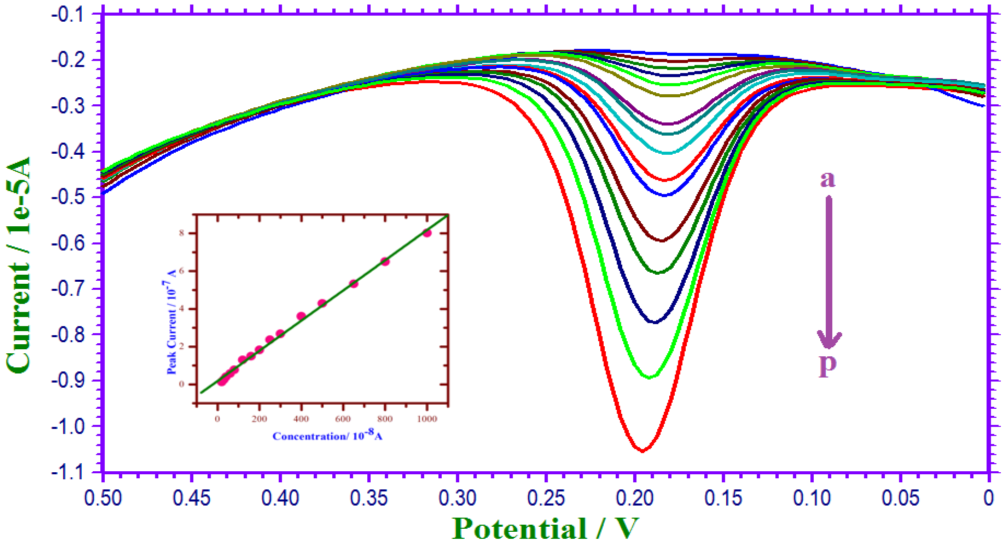

Fig: 6 DPVs for DA of different concentrations (20 µm - (a) to 1000 µm – (p)) at MWCTs/Poly (EB)/GCE in PBS of pH 5.0. Inset: calibration plot for the peak currents versus concentrations of DA.

3.8 Simultaneous determination of DA, 5-HT and FA:

The major objective of the present work was to employ the fabricated electrode towards the simultaneous determination of DA in presence of 5-HT and FA. The simultaneous investigation of DA in presence of 5-HT and FA is a challenging task, because always the oxidation potentials of the above three compounds overlap with each other at the bare GCE. To overcome this problem the chemical sensor fabricated (MWCNTs/Poly(EB)/GCE) in the present investigation was successfully employed. The simultaneous resolution of DA in the presence of 5-HT and FA with regular increase in DA concentrations and keeping remain two constant. The linear plot obtained between the different concentrations of DA verses peak currents with a linear regression equation of I_p DA = -0.76623 + 0.02124 C DA $_($mM) (R^2 = 0.99661). Similar increase in concentrations of 5-HT and FA by keeping remaining two compounds constant. The linear plot diagrams between concentrations of 5-HT and FA against peak currents with their linear regression equations and correlation values of $I_{p5\text{-}HT}$ = 0.03166 + 0.03739 $C_{5\text{-}HT}$ (mM) (R^2 = 0.97953) and $I_{p\ FA}$ = -8.797 + 0.21647 C_{FA}(mM) (R^2 = 0.99316) respectively.

3.9 Interference, stability and Reproducibility of MWCNTs/Poly(EB)/GCE

An important parameter for a sensor was its ability to discriminate between the interfering species and the target analyte. We are testing for DA, its related inorganic elements such as Cu^+, Ni^+ and Na^+. All of them which were might be present in the solution of DA. The obtained results 50 folds concentration of all compounds did not interfere with the oxidation peak currents of 50 × 10^{-6} M DA. The results suggested that the MWCNTs/Poly(EB)GCE prepared sensor had a good selectivity and no interference ability.

In order to examine the stability of the fabricated chemical sensor (MWCNTs/Poly(EB)/GCE), CV’s of 1 mM DA in PBS (pH 5.0) were recorded in several time intervals. During the intervals there was no apparent disturbance in the redox system and stable behavior of DA was observed. The fabricated (MWCNTs/Poly(EB)/GCE) electrode showed good stability. The reproducibility of the fabricated chemical sensor was investigated by fabricating three more MWCNTs/Poly(EB)/GCE chemical sensors of same composition and tested its CV response over 1 mM DA.

4. Recovery of DA in pharmaceutical formulation:

The practical application of the developed sensor was studied by monitoring DA in pharmaceutical formulation with the help of DPV technique. The tablet sample equivalent to 250 mg of DA was carefully grinded and dissolved in 100 mL of 0.1 M HCl to reach the calibration rang of DA. Similarly, standard solution of DA equivalent to the tablet sample was prepared and dissolved in 100 ml of 0.1 M HCl. The oxidation peak current of real sample solution of DA was noticed and to it the standard solution of DA was added in a regular interval.

5. Conclusion

The fabricated chemical sensor (MWCNTs/Poly(EB)/GCE) showed good detection of DA with a low LOD value, good sensitivity and enhanced charge transfer rate. The Kinetic parameters, such as charge transfer coefficient, heterogeneous rate constant values were evaluated. The fabricated (MWCNTs/Poly(EB)/GCE) sensor also displayed high selectivity and stability towards the simultaneous determination of DA in presence of 5-HT and FA. Further, it showed satisfactory recoveries of DA in pharmaceutical formulation.

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