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Full Paper

Development of Single Walled Carbon Nanotube-Molybdenum Disulfide Nanocomposite/Poly-ethylene Glycol Modified Carbon Paste Electrode as an Electrochemical Sensor for the Investigation of Sulfadiazine in Biological Samples

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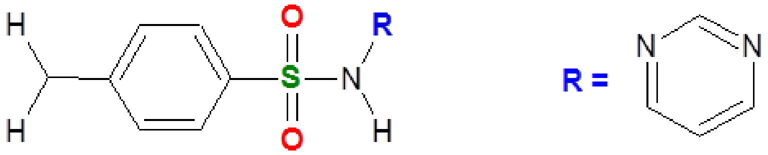
Abstract- A rapid electrochemical analysis of sulfadiazine (SFZ) has been carried out by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods by employing a sensitive single walled carbon nanotube-molybdenum disulfide nanocomposite/poly ethylene glycol modified carbon paste electrode (SWCNT-MoS_2/PEG/CPE). The SFZ shows anodic peak potential at 0.94 V (*vs.* Ag/AgCl) in 0.1 M PBS of pH 7.0. The SFZ has been examined at different scan rate from 50 to 300 mVs^{-1} in 0.1 M PBS of pH 7.0 and the nature of mass transfer route is diffusion-controlled process. The different pH from 5.6 to 8.0 was analyzed at SFZ and obtained the slope value 0.063 proves the equal number of electrons and protons were involved in the reaction. In DPV mode LOD of SFZ in the linear range of 2-90 μM was found to be 1.69 μM. The prepared electrode was examined by electrochemical impedance spectroscopy (EIS) using 5 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl. The effective surface area of SWCNT-MoS_2/PEG/CPE was found to be 0.262 cm^2. This method could be successfully employed to determine the concentration of SFZ in biological fluids.

Keywords- Carbon paste electrode, Cyclic voltammetry nanotubes, Nanoparticles, Sulfadiazine

1. INTRODUCTION

The discoveries of sulfonamide (sulfa) drugs were used for treating the bacterial infections and its effectiveness were improved by the modification of structure [1]. The sulfonamide drugs basically employed to treat urinary tract diseases, ear infections, skin burns bacterial meningitis, pneumonia. Sulfadiazine (SFZ) is one of drug used for treating the bacterial diseases. The SFZ half-life is 10 h and also known as short-acting sulfa drug. This short-operating drug bound to the serum protein very less and remaining drug will eagerly go by cerebrospinal fluids. The SFZ has found to be a good antibacterial drug and as best as sulfonamide to treat the meningitis. Of about 30% SFZ recapture in the urine and very less soluble than other sulfa drugs. So that one can take extraordinary care to make sure about superior hydration and urine discharge [2]. So the determination of the concentration of SFZ in human and veterinary system has got more importance. The SFZ was investigated by various techniques including, such as HPLC [3,4], capillary electrophoresis [5,6], enzyme-linked immunosrbent assay [7], gas chromatography [8,9], time resolved fluoroimmunoassay [10] etc. But these methods have need of high-cost instrumentation, time-consuming, difficulties in sample handling and preparation. The electrochemical methods play an important role to determine the drug samples over these techniques. The electrochemical techniques have been took the most preferable choice due to their high sensitivity, low-cost, easy operation, rapid analysis etc. In electrochemistry the characteristics of electrode purely depends on the nature of its size, composition and surface modifications. The metal nanoparticle composite electrode provides the appreciable unique advantages over the macroelectrodes with enrichment in mass transfer, active surface area and catalysis [11]. The conductive polymer modification of electrode surface also provides the good electron transport phenomena. Many literatures have been reported the nanocomposite modified electrode provides the better sensitivity and selectivity [12-15]. The carbon nanotube (CNT) have the enormous applications in medical, lithium ion battery, hydrogen storage, sensors technology, composite materials, gas sensors, super capacitor etc. [16]. The nanocomposite CNT provides superior activity towards the determination of drugs. The MoS_2 is paying attention due to their unique chemical and physical properties. MoS_2 is extensively used in the electrochemical catalysis and sensing field owing to its graphene-like two-dimensional configuration, excellent catalytic activity and other properties. In order to develop the properties and extend the application range, MoS_2 is usually combined with other functional materials, such as graphene, gold nanoparticles, polyaniline, etc. [17,18].

This work reports the development of molybdenum disulfide nanocomposite single walled carbon nanotube-ploy-ethylene glycol modified carbon paste electrode (SWCNT-MoS_2/PEG/CPE) as an electrochemical sensor for the investigation of sulfadiazine. The CV, DPV and EIS technique have been employed to determine SFZ.



Scheme 1. Chemical structure of sulfadiazine

2. EXPERIMENTAL

2.1. Apparatus

The CV, DPV, SWV and EIS studies were performed using an electrochemical workstation (Model: CH Instrument 660D, USA). All the voltammetric measurements have been carried out by three conventional electrodes system. In these experiments used the bare carbon paste (BCPE) or SWCNT-MoS_2/PEG/CPE as working, Ag/AgCl as reference and platinum wire as auxiliary electrode, respectively. The pH measurements have been carried out using pH meter model EQ-611 and all the analytical studies have under taken at lab temperature (28 °C).

2.2. Chemicals and Reagents

The pure drug Sulfadiazine (≥99%) and MoS_2 nanoparticles (99%) were procured from Sigma Aldrich (Germany), K_2HPO_4 (≥98%), KH_2PO_4 (≥99%), KCl (≥99%) were purchased from Merck (Mumbai, India). SWCNT was obtained from United Nanotech Innovations Pvt. Ltd. (Bangalore, India). The $K_3[Fe(CN)_6]$ (≥99%) and silicon oil have been obtained from Himedia (Mumbai, India). Phosphate buffer of pH 7.0 was used throughout the experiment. All the reagent solutions used in this experiment were prepared by doubly distilled water.

2.3. Procedure

2.3.1. Preparation of SWCNT-MoS_2 nanocomposite

The SWCNT-MoS_2 nanocomposite has been prepared by ultrasonication method. A 15 mg of SWCNT was taken in a 30 ml of ethanol and dispersed thoroughly for about 30 min. Soon after, 10 mg of MoS_2 nanoparticles were added and continued the sonication for further 30 minutes. The above obtained sample was dried under vacuum at 50 °C until the solvent evaporation takes place. The so prepared sample has been used for the electrode modification [19-21].

2.3.2. Electropolymerization on SWCNT-MoS_2/CPE

The electropolymerization of ethylene glycol has been carried out at SWCNT-MoS_2 electrode over the potential range between -1.6 to 1.6 V. The cathodic peak current was observed at -1.01 V and its shows the electrode got polymerized. The reduction peak current increases subsequently at the electrode surface indicate the formation of electroactive polymer species at the surface of SWCNT-MoS_2 nanocomposite. The number of cycles at the electrode surface has been optimized by running 5, 10, 15 and 20 cycles. In CV the 10th cycle has got maximum peak current at 1 mM SFZ so that it could be selected for further electrochemical analysis [22]. For the comparison purpose, the electropolymerization of ethylene glycol was carried out on SWCNT/CPE in absence of MoS_2 nanoparticles and on MoS_2/CPE in absence of SWCNT using the above procedure.

2.3.3. Fabrication of carbon paste electrode (CPE)

The working electrode has been prepared by hand mixing the graphite powder, silicon oil and SWCNT-MoS_2 composite in the ratio of 76:20:4 (w/w) respectively. The above mixture was thoroughly homogenized using motor and pestle by simple grounding method. The resultant mixture was tightly filled into glass tube (3 mm diameter) and electrical contact was made by inserting the copper wire at the other end. Exposed electrode surface has smoothened by rubbing on clean butter sheet for every start of new experiment. The above procedure has followed for the preparation of bare carbon paste electrode (BCPE) without the addition of modifier.

3. RESULTS AND DISCUSSION

3.1. Electrochemical response of $[Fe(CN)_6]^{3-/4-}$

The electrochemical performance of BCPE and modified electrodes have been examined using the redox couple $[Fe(CN)_6]^{3−/4−}$. From voltammogram, the redox behaviour of $[Fe(CN)_6]^{3−/4−}$ couple provides the of 297, 249, 198 and 125 mV at BCPE, SWCNT/CPE, SWCNT-MoS_2/CPE, SWCNT-MoS_2/PEG/CPE respectively (Fig. 1). The obtained values of potential difference clearly indicates the more reversible charge-transfer reaction takes place at SWCNT-MoS_2/PEG/CPE compared to other electrodes. The increase in peak current on SWCNT-MoS_2/PEG/CPE clearly indicates the larger surface area of the modified electrode. The active surface area of the electrodes has been determined by using Randles-Sevcik equation:

$$i_p = (2.69 \times 10^5)\, A\, n^{3/2} D_0^{1/2} C_0^{*} v^{1/2} \tag{1}$$

where i_p, A, ν indicates the peak current (μA), active surface area of electrode (cm^2) and scan rate (V s^{-1}) respectively, C_0^* and D_0 stands for the bulk concentration of $K_3[Fe(CN)_6]$ (mol cm^{-3}) and diffusion co-efficient ($cm^2 s^{-1}$) respectively. The diffusion co-efficient for 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl can be obtained by plotting I_{pa} *vs*. $ν^{1/2}$ (n=1, D_o=7.6×10^{-6} cm^2s^{-1}). On substituting the above values, effective surface area of BCPE, SWCNT/CPE, SWCNT-

MoS_2/CPE, SWCNT-MoS_2/PEG/CPE were found to be 0.001, 0.045, 0.087 and 0.262 cm^2, respectively.

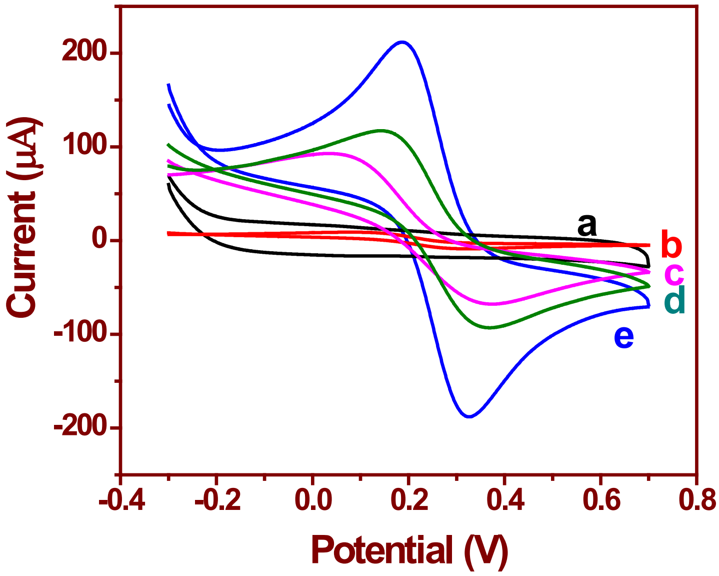


Fig. 1. CV's of (a) blank at SWCNT-MoS_2/PEG/CPE; (b) BCPE (c) SWCNT/CPE (d) SWCNT-MoS_2/CPE (e) SWCNT-MoS_2/PEG/CPE in 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl at scan rate of 100 mVs^{-1}

3.2. Electrochemical characterization of SWCNT-MoS_2/PEG

The electrochemical impedance spectroscopy (EIS) has been used to describe the electrode interfacial properties (adsorption, electrosorption, kinetics of homogeneous reaction) and geometric effect of electrode (linear, cylindrical or spherical mass transfer). Fig. 2 represents the Nyquist plot obtained for 5 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at BCPE, SWCNT/CPE, SWCNT-MoS_2/CPE, SWCNT-MoS_2/PEG/CPE in the frequency range from 0.1 to 10^3 kHz. From the semi-circle diameter, charge transfer resistance (R_{ct}) was calculated by fitting the equivalent circuit. The outcome of R_{ct} values at BCPE, SWCNT/CPE, SWCNT-MoS_2/CPE, SWCNT-MoS_2/PEG/CPE were found to be 990, 963.3, 253.4 and 42.4 kΩ respectively. The smaller semi-circle diameter has been observed at SWCNT-MoS_2/PEG/CPE (d) which indicates the fast-electron exchange. The low resistance value was observed and this may be owing to the effect of polymerization at SWCNT-MoS_2/CPE. The different R_{ct} values have been obtained at the modified electrode and it evidently proved that SWCNT, SWCNT-MoS_2, SWCNT-MoS_2/PEG has closely immobilized at the surface of CPE. The k_{app} has calculated by using the formula:

$$k_{app} = \frac{RT}{F^2 R_{ct} C} \quad (2)$$

where k_{app} electron transfer rate constant, R, T, F, are the universal gas constant, absolute temperature, Faraday's constant respectively. The R_{ct} is charge transfer resistance and C is the concentration of $K_3[Fe(CN)_6]$ (5 mM). The calculated values of k_{app} for BCPE, SWCNT/CPE, SWCNT-MoS_2/CPE and SWCNT-MoS_2/PEG/CPE were found to be 0.53×10^{-10}, 0.552×10^{-10}, 4.64×10^{-11} and 2.775×10^{-10}, respectively. Lesser values of R_{ct} and superior values of k_{app} suggest the rapid electron transfer process.

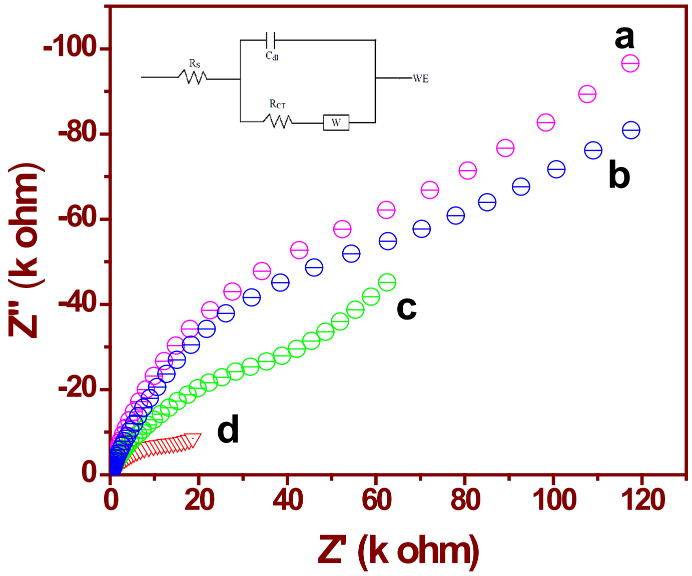


Fig. 2. Nyquist diagram of 5 mM $[Fe(CN)_6]^{3-/4-}$ including 0.1 M KCl at: (a) BCPE; (b) SWCNT/CPE; (c) SWCNT-MoS_2/CPE and (d) SWCNT-MoS_2/PEG/CPE (inset: Randle's equivalent circuit; frequency from 0.1 to 10^3 kHz)

3.3. Electrochemical performance of SFZ at BCPE and modified electrodes

The CV has been employed to analyze the electrochemical performance of SFZ at BCPE and modified electrodes. In SFZ the $-NH_2$ group is easily oxidizable but reduction of $-SO_2$ group is quite difficult. The reduction potential is depends on the nature of R group, but there is no appreciable influence of R group on the oxidation of $-NH_2$ (Scheme 1) [23]. The oxidation peak potential of SFZ for BCPE, SWCNT/CPE, SWCNT-MoS_2/CPE, MoS_2/PEG/CPE, SWCNT/PEG/CPE and SWCNT-MoS_2/PEG/CPE were found to be 0.999, 0.923, 0.913, 0.925, 0.9074 and 0.908 V respectively (Fig. 3). The enhancement in peak current with a negative shift in peak potential on SWCNT-MoS_2/PEG/CPE clearly signifies the good electrocatalytic activity towards the determination of SFZ in PBS of pH 7.0.

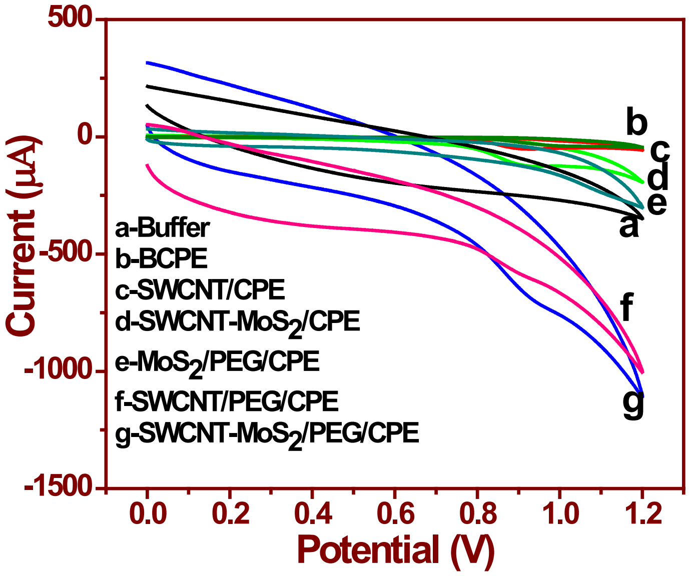


Fig. 3. Cyclic voltammograms of: (a) buffer at SWCNT-MoS_2/PEG/CPE; 1 mM of SFZ at (b) BCPE; (c) SWCNT/CPE; (d) SWCNT-MoS_2/CPE (e) MoS_2/PEG/CPE (f) SWCNT/PEG/CPE and (e) SWCNT/MoS_2/PEG/CPE at scan rate of 100 mVs^{-1} in 0.1 M PBS of pH 7.0

3.4. Influence of scan rate and pH

Voltammetric investigation of SFZ has been examined at SWCNT-MoS_2/PEG/CPE in 0.1 M PBS of pH 7.0 at different scan rate from 50 to 300 mVs^{-1} (Fig. 4A). The anodic peak current was found to be enhanced with increase in the scan rate. Regression equation was obtained by plotting the graph of I_p *vs.* $v^{1/2}$; I_p (μA)=$2.26\times10^{-4}+4.55\times10^{-5}\ v^{1/2}$ ($mV\ s^{-1}$), R^2=0.9878 (N=11) (Fig. 4B) and also plotting the graph of log I_p *vs.* log *v*; log I_p=-3.82+0.33 log *v* (mVs^{-1}), R^2=0.9784 (N=11) (Fig. 4C). The log I_p *vs.* log *v* gave slope value 0.33 and it confirmed the nature of mass transfer route and for the diffusion-controlled process the slope value is equal to be 0.5. For the irreversible reaction of SFZ, Laviron equation accounted and has been given below:

$$E_p = E_0 + \left[\frac{2.303RT}{\alpha nF}\right] \log\left[\frac{RTk^0}{\alpha nF}\right] + \left[\frac{2.303RT}{\alpha nF}\right] \log \nu \qquad (3)$$

where, E_0 is the formal potential and is obtained from intercept E_p *vs.* *v* (extrapolation along vertical axis, *v*=0), k^0 is standard rate constant obtained from slope (plotting the graph of E_p *vs.* log *v*) and R, is the universal gas constant (8.314 $JK^{-1}mol^{-1}$), T is temperature (298 K), an F is Faraday's constant (96480 C mol^{-1}). The k^0 was found to be 0.503.

The electrochemical reaction of SFZ has been studied at different pH from 5.6 to 8.0 at SWCNT-MoS_2/PEG/CPE (Fig. 5). From the previous report SFZ will undergo irreversible pH dependent $2e^-$ reaction [23]. DPV method was used to study the influence of pH on SFZ. The increase in anodic peak current observed that at pH 7.8 but due to the physiological importance pH 7.0 has been selected as optimum condition for further electrochemical studies. The oxidation potential has been shifted towards to negative side with increase in pH, which indicates the participation of equal number of H^+/e^- in the electrochemical reaction. The linear regression equation has been provided by $E_p/V=1.21–0.063$ pH ($R^2=0.977$) [24]. The slope value 0.063 proves the equal number of electrons and protons were involved in the reaction.

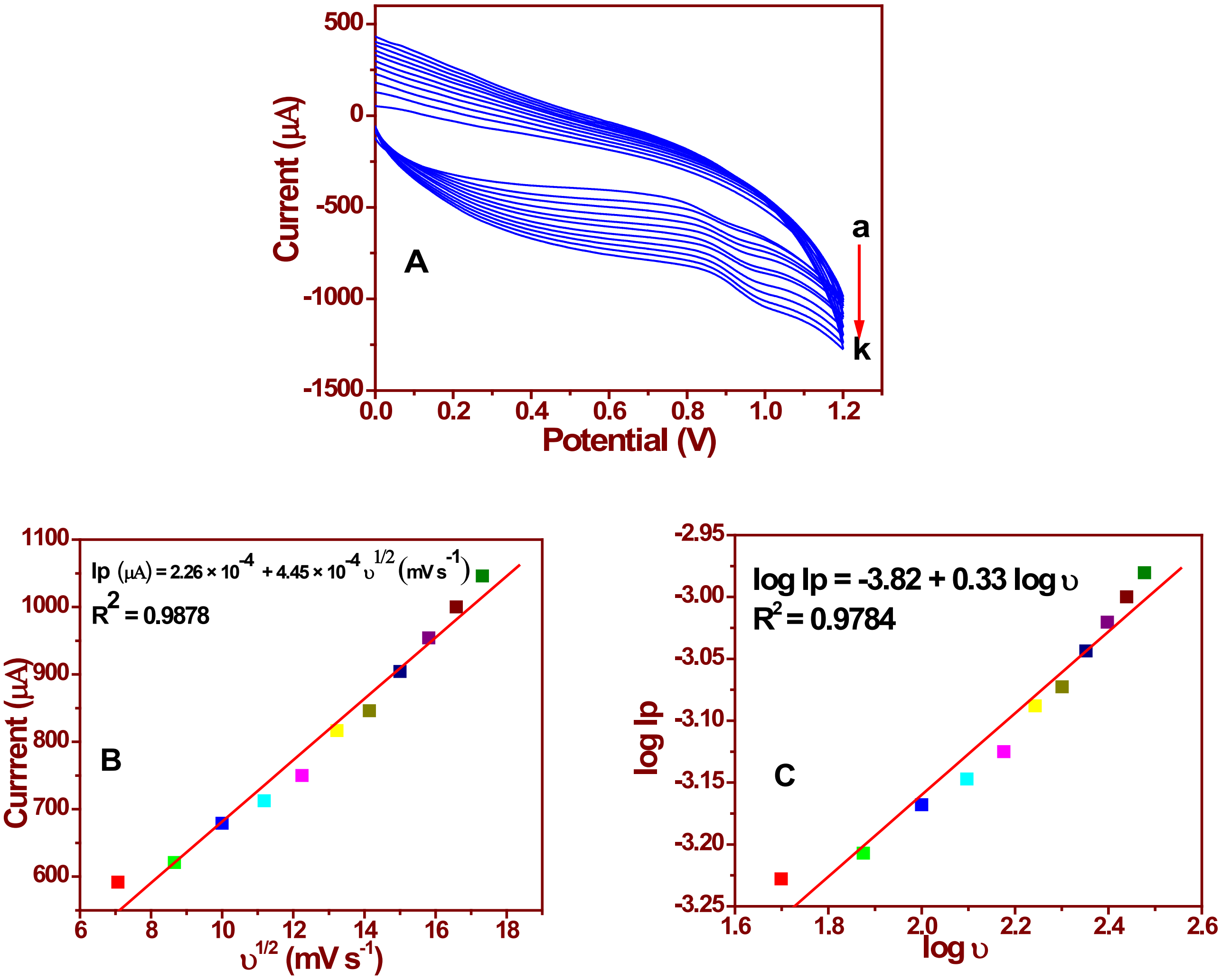


Fig. 4. **(A)** Cyclic voltammogram of 1 mM SFZ at different scan rate from 50 to 300 mVs^{-1} (a to k) in 0.1 M PBS at pH 7.0; linearity graph; **(B)** I_p *vs.* $v^{1/2}$ and **(C)** log I_p *vs.* log v

3.5. Studies of analytical factors at SWCNT-MoS_2/PEG/CPE

The concentration of SFZ has been analyzed by DPV mode at SWCNT-MoS_2/PEG/CPE in PBS of pH 7.0 (Fig. 6). The current has been enhanced with raise in the concentration from 2 to 120.0 μM. The plot of I_p *vs.* conc. has provided the equation: $I_p/\mu A=1.59\times10^{-5}-1.84\times10^{-7}$ C/μM (for blank; sd=1.04×10^{-7}; N=5) (Fig. 7). The limit of detection has found to be 1.69 μM in the linearity range from 2-90 µM and compared with previously reported modified electrodes, the SWCNT-MoS_2/PEG/CPE has got good sensitivity (Table 1).

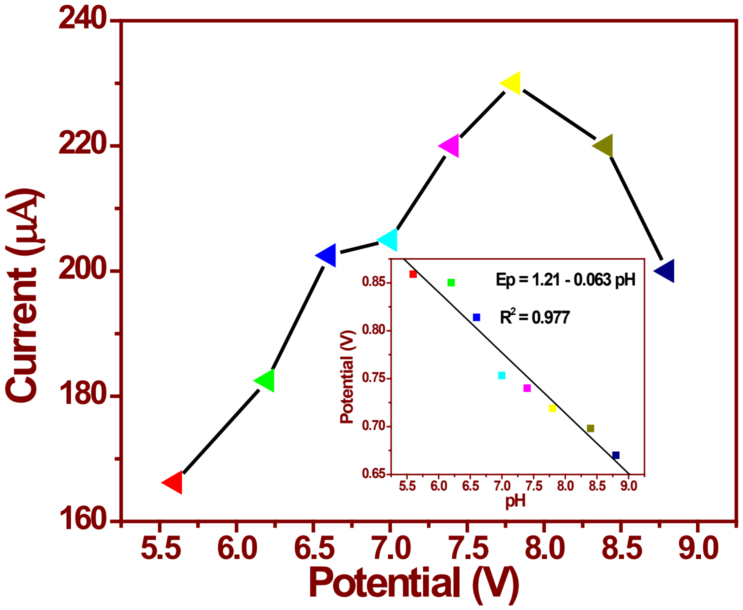


Fig. 5. Effect of pH (from 5.5 to 8.0) on DPV's of 50 µM SFZ (inset: linearity graph of pH *vs.* potential)

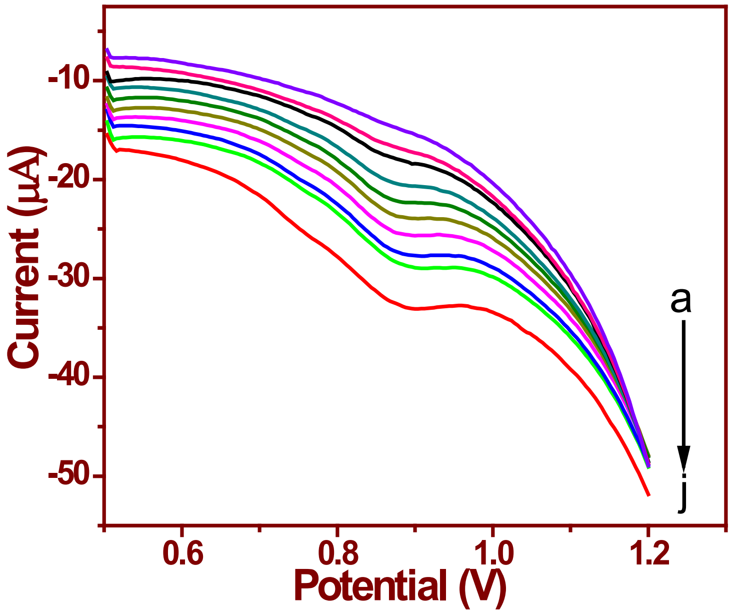


Fig. 6. DPV graphs of various concentrations from 2 to 120 µM (a to j; 2, 5, 10, 20, 30, 40, 50, 60, 70, and 120 µM) achieved at SWCNT-MoS_2/PEG/CPE in 0.1 M PBS of pH 7.0

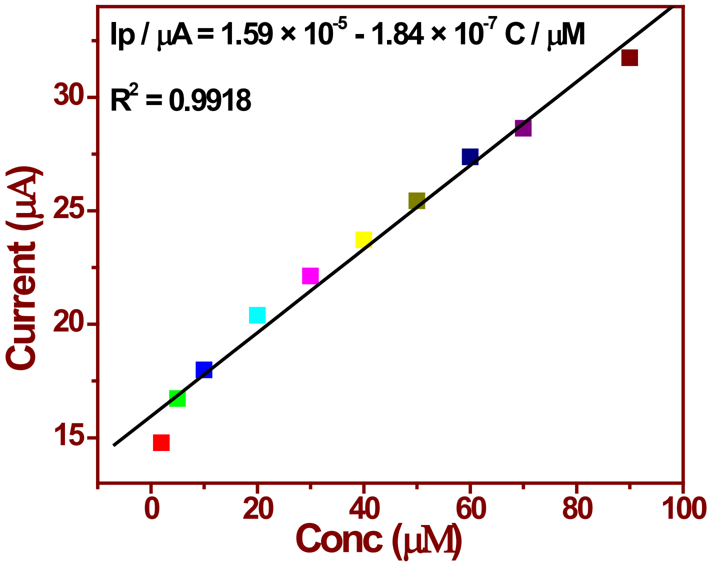


Fig. 7. Linearity graph of I_p *vs.* Conc from 2 to 90 µM

Table 1. Comparison with other reported modified electrodes

Electrode	**Reaction**	**Method**	**LOD=3s/m (µM)**	**Ref**
[a]BDDE	Oxidation	SWV	2.19	[23]
[b]SE	Oxidation	LSV	5.4	[25]
[b]SE	Oxidation	DPV	1.1	[25]
[c]BiFE	Reduction	DPV	2.1	[26]
[c]BiFE	Reduction	DPV	12.2	[26]
[d]GCE	Reduction	SWV	10.9	[27]
[e]CPE (SWCNT-MoS$_2$/PEG)	Oxidation	DPV	1.69	this work

a- Boron Doped Diamond Electrode **b**- Solid Electrode
c- Bismuth-Film Electrode
d- Glassy Carbon Electrode
e- Carbon Paste Electrode

The stability and reproducibility of SWCNT-MoS$_2$/PEG/CPE have been achieved by using SFZ analyte. The stability of modified electrode has been studied by preserving the electrode at lab temperature for 12 days and it retains its initial peak current with 94%. And also the reproducibility has been studied by preparing the five different electrodes and employing at 1 mM SFZ, attained the result of RSD value 3.6%. The optionality of the SWCNT-MoS$_2$/PEG/CPE at the electrochemical investigation of SFZ may impose several foreign substances present in real and pharmaceutical samples. So, a known concentration of SFZ in 0.1 M PBS of pH 7.0 has been analyzed in presence of trace quantities of many interfering excipients such as Cd^{2+}, Ca^{2+}, K^+, Mg^{2+}, Na^+, Cl^-, SO_4^{2-}, CO_3^{2-}, glucose, starch, ascorbic acid and folic acid. The obtained current signal clearly indicated that the interfering compounds didn't affect the electrochemical behaviour of SFZ [28-30].

3.6. Real sample analysis

3.6.1. SFZ Analytical applications in real sample (Urine and serum)

In order to corroborate the efficacy of prepared sensor for the investigation of SFZ present in the real samples, the spiked blood and urine samples have been examined. The determination of concentration and percent recovery of SFZ has been carried out by standard addition method (within the calibration range as shown in Fig. 7) at SWCNT-MoS_2/PEG/CPE. The obtained percent recovery was in between the 98.3 to 102.2 % (Table 2). This confirmed the modified electrode SWCNT-MoS2/PEG/CPE has good accuracy and could be effectively applied for the investigation of SFZ present in human biological fluids.

Table 2. SFZ analysis in urine and blood sample

Sample		Spiked (μM)	Found (μM)	Recovery (%)
Urine	1	00	50.69±1	98.30
	2	10	60.71±1	98.80
	3	20	79.10±1	101.0
Blood	1	00	10.10±1	99.00
	2	20	28.35±1	102.2
	4	30	60.34±1	99.43

4. CONCLUSION

The SWCNT-MoS_2/PEG/CPE was developed to analysis of SFZ. The prepared electrode has been characterized by CV, EIS techniques. The active surface area and charge transfer resistance were found to be 0.262 cm^2 and 42.4 kΩ, respectively. The limit of detection for SFZ was calculated in the linear range 2 to 90 μM and it was found to be 1.69 μM. The prepared electrode has good stability, reproducibility and it could be applicable for the investigation of SFZ present in biological fluids.

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Disclosure statement

No potential conflict of interest.

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