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Voltammetric Determination of Sulfadoxine and Its Application in Pharmaceuticals and Urine Samples

N.M. Gokavi and S.T. Nandibewoor*

P. G. Department of Studies in Chemistry, Karnatak University, Dharwad-580 003, Karnataka, India

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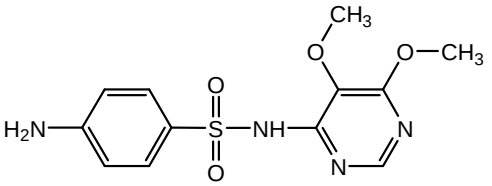
The voltammetric behaviour of Sulfadoxine (SDN**)** was studied at a glassy carbon electrode in 0.2 M phosphate buffer solutions using cyclic, differential-pulse (DPV) and square wave voltammetry (SWV). The dependence of the current on pH, concentration, and scan rate was investigated to optimize the experimental conditions for the determination of SDN. The oxidation process was shown to be diffusion controlled, irreversible over the pH range from 3.0-9.2. An analytical method was developed for the determination of SDN in phosphate buffer solution at pH 3.0 as a supporting electrolyte. A DPV method showed a good linear response as compared to SWV. The anodic peak current varied linearly with SDN concentration in the range 0.310-4.34 μg ml^{-1} of SDN with a limit of detection (LOD) of 0.01 μg ml^{-1}. The recovery was determined in the range from 95.6-100.1%. The proposed method was successfully applied to the quantitative determination of SDN in pharmaceutical formulations and an urine as real samples.

Keywords: Glassy carbon electrode, Sulfadoxine, Voltammetry, Phosphate buffer

INTRODUCTION

Sulfadoxine is chemically 4-amino-N-(5,6-dimethoxy-pyrimidin-4-yl)benzene-1-sulfonamide (Scheme 1) belonging to the class of drug known as Sulfanilamides. It is mainly used for the treatment of malaria and also used as anti-infective agent. Malaria and Pneumonia are major contributors to a global mortality of children under 5 years old and are the utmost cause of childhood deaths in sub-Saharan Africa [1]. The drug compliances often inadequate, which is usually explained by carers' delay in recognizing the disease in combination with poor access to health facilities [2]. Sulfadoxine-Pyrimethamine (FansidarTM) has been used extensively against chloroquine resistant *Plasmodium falciparum*. Recently Sulfadoxine-Pyrimethamine assumed greater significance because of its possible role in combination therapy with artemisinin derivatives [3] and the synergistic combination of Sulfadoxine (SD), a long-acting benzene sulphonamide, and



Scheme 1. Chemical structure of Sulfadoxine (SDN)

the dihydrofolate reductase inhibitor Pyrimethamine (PR) became a cheap and effective replacement for chloroquine [4], for *e.g.* Tanzania, the pyrimethamine/sulfadoxine combination has recently replaced chloroquine as the first-line drug for the treatment of uncomplicated malaria [5]. Due to the low solubility of both these drugs, their effectiveness depends on the bioavailability of both components after oral administration. The questions have been arisen on the quality, and bioavailability of the pharmaceutical formulations present on the African market. Determination of antimalarial drug concentrations during treatment has been proposed by the World Health Organization (WHO) for the definition and identification of

*Corresponding author. E-mail: stnandibewoor@yahoo.com

drug resistance. In developing countries the dearth of research funds and the high-technology analytical instruments or even the availability of most reagents recommended in official compendia has forced to scientist to develop alternative and sensitive methods of analysis of drugs. Hence, we attempt to develop a simple, rapid, inexpensive and sensitive voltammetric method for the determination of Sulfadoxine.

Several methods have been reported for the determination of SDN such as a Packed column supercritical fluid chromatography [6], spectrophotometric methods [7,8] as well as HPLC techniques [8,9]. The voltammetric techniques for determination of SDN with Pd [10] as well as UPLC coupled with G/PANI modified screen-printed carbon electrode [11] were also reported. However, these methods have some disadvantages such as high cost, long analysis of time, sample pre-treatment, low sensitivity and selectivity, which make them unsuitable for routine analysis. The development of a new method capable of determining drug amount in pharmaceutical and biological dosage forms are important. An electroanalytical techniques have been used for the determination of a wide range of drug compound with the advantages that there is no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques [12,13]. Additionally, an application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fates or their *in vivo* redox processes or pharmacological activity [14-17]. The main goal of this work was to develop a voltammetric method for direct determination of SDN in pharmaceutical dosage forms, raw materials and spiked human urine samples. This paper describes fully validated, simple, rapid, selective and sensitive procedures for the determination of SDN employing DPV, SWV and CV techniques at GCE.

EXPERIMENTAL

Materials and Reagents

The powdered form of SDN was obtained from Sigma Aldrich and used without further purification. A stock solution (1×10^{-3} M) of SDN was prepared in ethanol and double distilled water (10:90 v/v). The Phosphate buffers from pH 3-9.2 were prepared in double distilled water as described by Christian and Purdy [18]. All of the other employed chemicals were of analytical grade and solutions were prepared in double distilled water.

Instrumentation

The electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a 3 mm diameter glassy carbon electrode (GCE) as the working electrode. All the potentials are given against the Ag/AgCl (3 M KCl). All experiments were carried out at an ambient temperature of 25 °C ± 0.1 °C. The pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India).

At different scan rates, the area of the electrode was calculated using 1.0 mM $K_3Fe(CN)_6$ as a probe. For a reversible process, the Randles-Sevcik formula has been used [19].

$$i_{pa} = (2.69 \times 10^5)\, n^{3/2}\, A\, D_o^{1/2}\, C_o\, \upsilon^{1/2} \quad (1)$$

where, i_{pa} refers to the anodic peak current (A), n is the number of electrons transferred, A is the surface area of the electrode (cm^2), D_0 is diffusion coefficient ($cm^2\ s^{-1}$), υ is the scan rate ($V\ s^{-1}$) and C_0 is the concentration of $K_3Fe(CN)_6$ (M). For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n = 1, $D_0 = 7.6 \times 10^{-6}$ $cm^2\ s^{-1}$ [20], then from the slope of the plot of i_{pa} *vs.* $\upsilon^{1/2}$ relation, the surface area of electrode was calculated. In our experiment, the slope obtained was 2.59 and the surface area of glassy carbon electrode was calculated to be 0.035 cm^2.

Analytical Procedure

The GCE was carefully polished using 0.3 micron Al_2O_3 slurry on a polishing cloth before each experiment. After polishing, the electrode was thoroughly rinsed with water. After this mechanical treatment, the GCE was placed in buffer solution and various voltammograms were recorded until a steady state baseline voltammogram was obtained.

The GCE was first activated [21] in phosphate buffer

(pH 3.0) by cyclic voltammetric sweeps between 0.4 to and 1.4 V until stable cyclic voltammograms were obtained. Then electrodes were transferred into another 10 ml of Phosphate buffer (pH 3.0), it containing proper amount of SDN. The optimized accumulating potential and time were 100 mV and 10s respectively. The potential scan was initiated and cyclic voltammograms were recorded between 0.4 and 1.4 V, with a scan rate of 100 mV s^{-1}. All measurements were carried out at room temperature of 25 ± 0.1 °C.

Sample Preparation

The two pieces of SDN tablets (Amalar Micro labs limited, India) were powdered in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0 × 10^{-3} M was accurately weighed and transferred into a 100 ml calibrated flask and diluted with a water and was followed by sonication for 10 min for complete dissolution. An appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with the phosphate buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method.

RESULTS AND DISCUSSION

Cyclic Voltammetric Behavior of SDN

To understand the electrochemical behaviour of SDN, cyclic voltammetry was carried out at the glassy carbon electrode between pH 3.0 and 9.2 of phosphate buffer which produced a well defined oxidation peak. The cyclic voltammogram of SDN at pH 3.0 in phosphate buffer was as shown in Fig. 1 curve (i) and the blank solution without SDN was shown by curve (ii). An oxidation peak corresponding to SDN was appeared at 1.11 V.

It is shown that no reduction peak was observed in the reverse scan, it was suggesting that the electrochemical reaction was an irreversible process. Nevertheless, it was found that the oxidation peak current of SDN showed a remarkable decrease during the successive cyclic voltammetric sweeps. After every sweep, the peak current decreased continuously and finally remained unchanged. This phenomenon may be attributed to the consumption of adsorbed SDN on the electrode surface or due to the fact

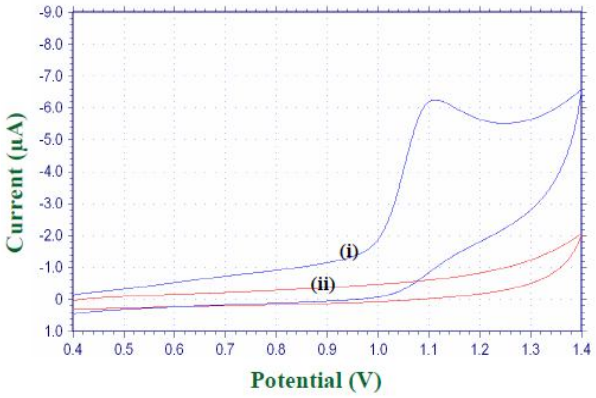


Fig. 1. Cyclic voltammograms obtained for 1.0 mM sulfadoxine (SDN) on GCE in pH 3.0 , 0.2 M buffer: (i) GCE with SDN (ii) GCE without SDN at scan rate 100 mV s^{-1}.

that the adsorption of oxidative product occurs at the electrode surface. Therefore, the voltammograms corresponding to the first cycle was generally recorded.

Influence of pH

The effect of solution pH on peak potentials of SDN at GCE was also investigated. Cyclic voltammograms at different pH values of 3-9.2 were carried. However, above pH 9.2 the peaks were not sharp. Hence, the study was restricted from pH 3.0-9.2 as shown in Fig. (2a). These data shown that an increase in pH of the solution caused shift in the oxidative peak potential to the negative direction, indicating that the electrode process was influenced by protonation reactions. A linear correlation between the peak potential and solution pH was obtained as shown in Fig. (2b) with a linear equation and correlation coefficient of:

Epa (V) = 1.228 - 0.041 pH; r = 0.991

The slope was found to be 41 mV/pH, which is close to the theoretical value of 30 mV/pH. This indicates that the number of protons transferred is half of the number of electrons transferred in the rate determining step [17,22-23].

The variation of peak current with pH is as shown in Fig. (2c). The peak current goes on decreasing from pH 3-9.2. From the experimental results, it is observed that highest peak current and better shape of the voltammogram was observed at pH 3.0, suggesting this pH is optimal pH

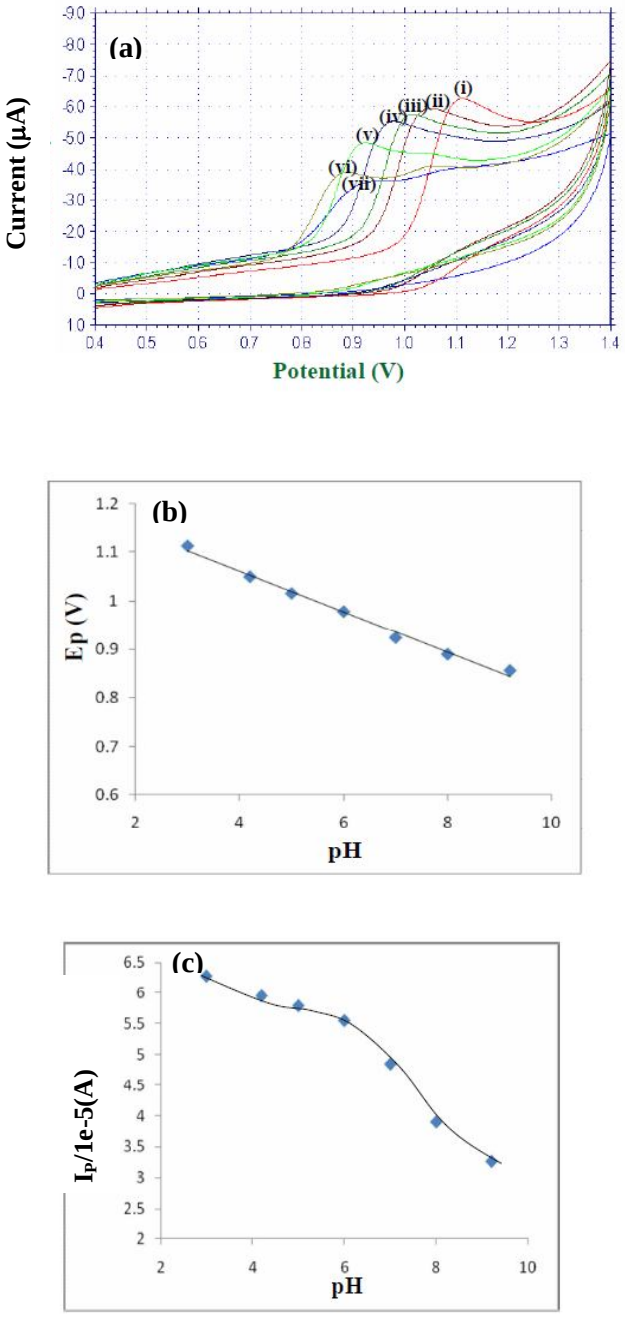


Fig. 2. (a) Cyclic voltammograms obtained for 1.0 mM SDN in buffer solution at (i) pH 3.0, (ii) pH 4.2, (iii) pH 5.0, (iv) pH 6.0, (v) pH 7.0, (vi) pH 8.0 and (vii) pH 9.2 with potential scan rate 100 mV s^{-1}. (b) Variation of peak potential with pH for 1.0 mM SDN. (c) Variation of peak current with pH for 1.0 mM SDN.

value.

Influence of Scan Rate

An electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behaviour of SDN has been studied at different scan rates from 100-400 mV s^{-1}, Fig. (3a). There was a good linear relationship between peak current and square root of scan rate and can be expressed as Ip = 0.670 $\nu^{1/2}$ - 0.191; r = 0.996 as shown in the Fig. (3b) which confirms the irreversibility of the process. In addition, there was a linear relation between logIp and logν corresponding to the equation: logIp = 0.516 logν - 0.222; r = 0.996 as shown in Fig. (3c). The slope of 0.516 was close to the theoretically expected value of 0.5 for a diffusion controlled process [24]. With an increase in scan rate, the peak potential shifted to more positive value. The linear relation between peak potential and logarithm of scan rate can be expressed as Ep = 0.033 logν + 1.043; r = 0.992 as shown in the Fig. (3d).

As for an irreversible electrode process, according to Laviron [25], Ep is defined by the following equation:

$$E_P = E^0 + \left[\frac{2.303RT}{\alpha nF}\right]\log\left[\frac{RTk^0}{\alpha nF}\right] + \left[\frac{2.303RT}{\alpha nF}\right]\log\nu \qquad (2)$$

where α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred, ν is the scan rate and E^0 is the formal redox potential. Other symbols have their usual meanings. Thus, the value of αn can be easily calculated from the slope of Ep *vs.* logν plot. In this system, the slope was 0.108, taking T = 298 K, R = 8.314 J K^{-1} mol^{-1} and F = 96,480 C, and α was found to be 1.792.

Again α was calculated using the Bard and Faulkner formula [24] in total irreversible electrode process.

$$\alpha = \frac{47.7}{E_P - E_{P/2}}\, mV \qquad (3)$$

where Ep/2 is the potential where the current is at half the peak value. So, the value of α determined was 0.59. So the number of electrons (n) transferred in the electro-oxidation of SDN was calculated to be 3.03 ≈ 3.

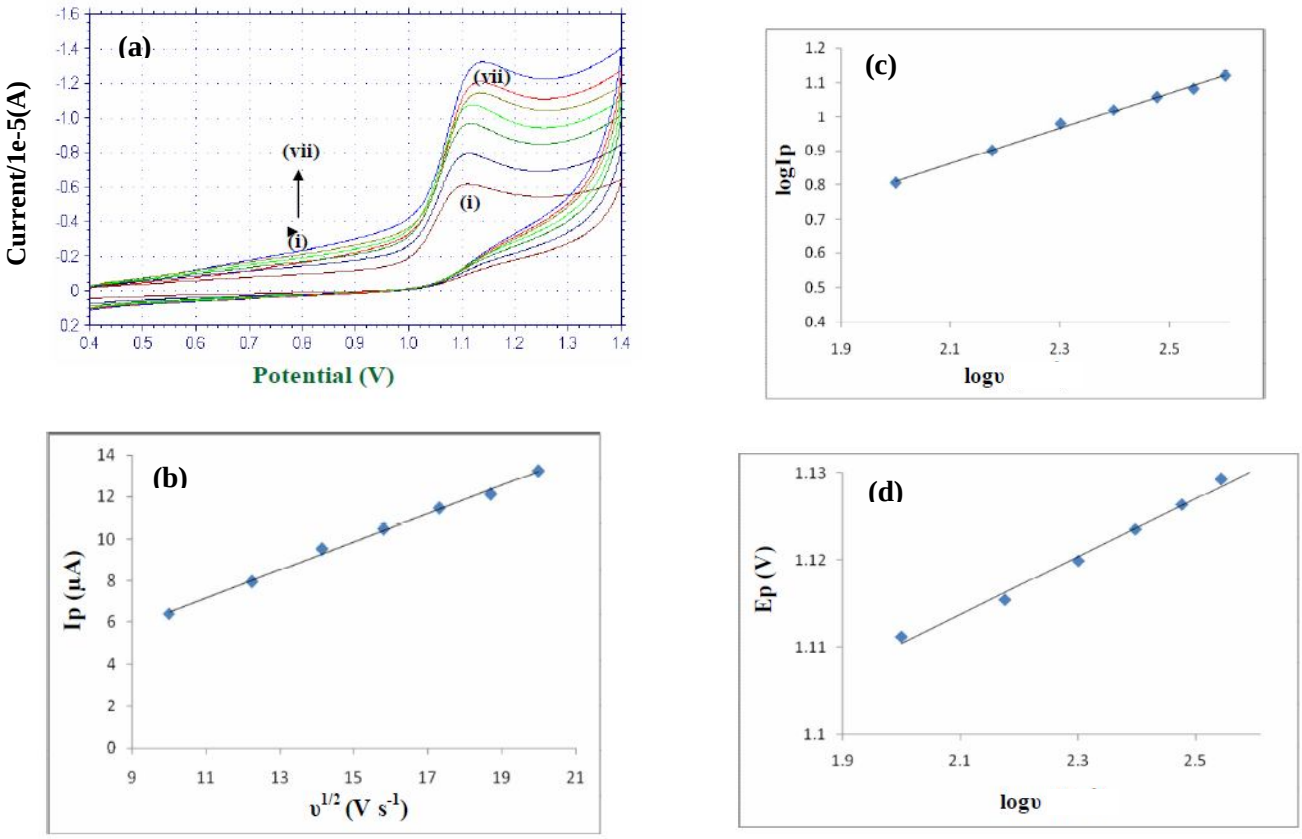
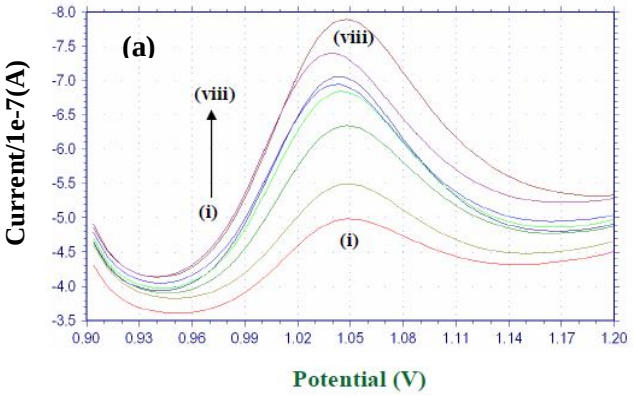


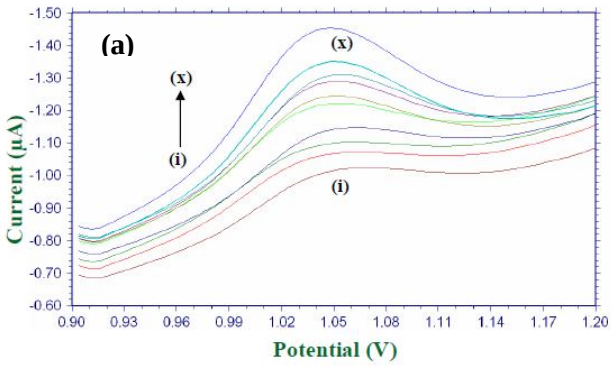
Fig. 3. (a) Cyclic voltammograms obtained for 1.0 mM SDN in buffer solution of pH 3.0 at scan rates of (i) 100, (ii) 150, (iii) 200, (iv) 250, (v) 300, (vi) 350 and (vii) 400 mV s^{-1}. (b). Linear relationship between the peak currents and the square root of scan rate. (c). Linear relationship between the logarithmic peak currents and the logarithmic scan rates. (d). Variation of peak potential with log**υ** for 1.0 mM SDN.

The value of k^0 can be determined from the intercept of the above plot if the value of E^0 is known. The value of E^0 in Eq. (2) can be obtained from the intercept of Ep *vs.* ν curve by extrapolating to the vertical axis at ν = 0 [26]. In our system the intercept for Ep *vs.* logν plot was 1.04 and E^0 was obtained to be 0.35 V, the k^0 was calculated to be 2.28×10^3 s^{-1}.

Calibration Curve

In order to develop a rapid and sensitive voltammetric method for determining the SDN, we adopted the differential pulse (DPV) and square wave voltammetric (SWV) methods, because the peaks were sharper and better defined at a lower concentration of SDN, than those obtained by cyclic voltammetry, with low a background current, resulting in an improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of SDN. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of SDN as it gives maximum peak current at pH 3.0. The peak at about 1.048 V in DPV was considered for the analysis. The differential pulse and square wave voltammograms are obtained with increasing amount of SDN showed that the peak current increased linearly with increasing concentration, as shown in Figs. (4a, b) and Figs. (5a, b).





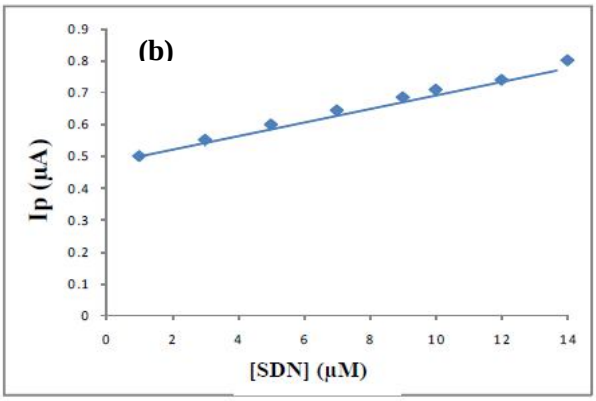


Fig. 4. (a) Differential pulse voltammograms of SDN at different concentrations at (i) 1, (ii) 3, (iii) 5, (iv) 7, (v) 9, (vi) 10, (vii) 12 and (viii) 14 µM. (b) Plot of the peak current against concentration of SDN.

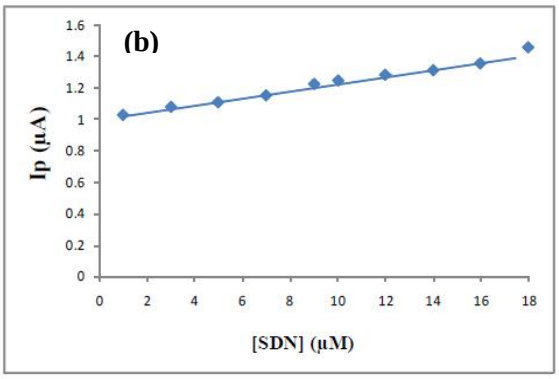


Fig. 5. (a) Square wave voltammograms of SDN at different concentrations at (i) 1, (ii) 3, (iii) 5, (iv) 7, (v) 9 , (vi) 10, (vii) 12 µM, (viii) 14, (xi) 16 and (x) 18 µM. (b) Plot of the peak current against concentration of SDN.

According to the procedure, two calibration graphs from the standard solution of SDN were constructed by using DPV and SWV techniques. A linear relation (Figs. 4b, 5b) in the concentration range between 0.310-4.34 µg ml^{-1} and 0.310-5.58 µg ml^{-1} by using DPV and SWV methods, respectively. The linear equations were

i_p (µA) = 0.022C (µM) + 0.485 (r = 0.994) in case of DPV and

i_p (µA) = 0.023C (µM) + 0.99 (r = 0.984) in case of SWV

The DPV presents a good linear response as compared to SWV in view of less intercept of linear plots of Ip against concentration of SDN. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product on the electrode surface [27]. It was also observed that the peak potential (Ep) was shifted towards more positive value suggesting that product undergoes adsorption at the surface of GCE. Related statistical data of the calibration curve was obtained from five different calibration curves. The limit of detection (LOD) is 0.01 µg ml^{-1} (DPV) and 0.07 µg ml^{-1} (SWV) and quantification limits (LOQ) is 0.03 µg ml^{-1} (DPV) and 0.23 µg ml^{-1} (SWV), respectively. The LOD and LOQ were

calculated using the following equations:

$$LOD = 3s/m, \quad LOQ = 10s/m$$

where, *s* is the standard deviation of the peak currents of the blank (five runs) and *m* is the slope of the calibration curve. Comparison with earlier methods except UPLC coupled with G/PANI screen-printed carbon electrode, the present method was better for the determination of SDN [6, 8,9,11] (Table 1).

Stability and Reproducibility

In order to study the stability and reproducibility of the electrode, a 1 μM SDN solution were measured with the same electrode (renewed every time) for every several hours within a day, the RSD of the peak current was 0.72% (number of measurements = 5). As to the reproducibility between days, it was similar to that of within a day repeatability, if the temperature was kept almost unchanged which could be attributed to the excellent stability and reproducibility of GCE.

Effect of Interferences

For the analytical applications of the proposed method, the effects of potential interferences that are likely to be in biological samples were evaluated under the optimum experimental conditions. The differential pulse voltammetric experiments were carried out for 1.0 μM SDN in the presence of 1.0 mM of each of the interferences (the overlay plot of DPV of (i) SDN and (ii) SDN in presence of 1 mM citric acid as shown in Fig. 6). The experimental results (Table 2) showed that thousand-fold excess of glucose, starch, sucrose, dextrose, gum acacia, citric acid and oxalic acid did not interfere with the voltammetric signal of SDN. Therefore, the proposed method can be used as a selective method.

Tablet Analysis and Recovery Test

In order to evaluate the applicability of the proposed method, the commercial medicinal sample containing SDN from 'Amalar'(Micro labs limited)India, was studied. The tablets were grounded to powder, dissolved in water and then further diluted so that SDN concentration falls in the range of calibration plot. The differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential pulse voltammograms for plotting calibration plot. It was found that SDN concentration determined for various tablets using this method are in good agreement with the reported values.The F and Student t tests were carried out on the data and the results are given in Table 3. The validity of the obtained results by spectrophotometric and voltammetric method were statistically examined. According to the Student's *t*-test, the calculated t was less than the theoretical values in either test at the 95% confidence level. This indicates that there was no significant difference between the accuracy of the proposed and reported methods.

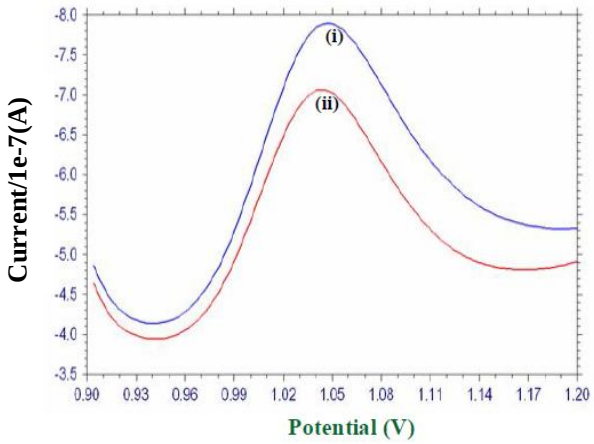


Fig. 6. Differential pulse voltammograms of (i) SDN and (ii) SDN in presence of 1mM citric acid.

Detection of SDN in Urine Samples

The applicability of the DPV to the determination of SDN in spiked urine was also investigated (Table 4). The recoveries from urine were measured by spiking drug free urine with known amounts of SDN. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pre-treatment. A quantitative determination can be carried out by adding the standard solution of SDN into the detect system of urine sample. The calibration graph was used for the determination of spiked SDN in urine samples. The detection results of four urine samples obtained are listed in Table 4. The recovery determined was in the range from 95.6-100.1% and the

Table 1. Comparison of some Methods for the Determination of SDN with the Proposed Method

Analytical method	Linearity range ($\mu g\ ml^{-1}$)	Detection limit ($\mu g\ ml^{-1}$)	Ref.
1) Packed column superficial fluid chromatography	(0.5-80)	(0.15)	[6]
2) Spectrophotometric method	(40-100)	(0.26)	[8]
3) HPLC method	(2.5-100)	(0.01)	[9]
4) UPLC coupled with G/PANI screen-printed carbon electrode	(0.01-10)	(0.0029)	[11]
4) Glassy Carbon Electrode	(0.31-4.34)	(0.01)	Present work

Table 2. Influence of Potential Interferents on the Voltametric Response of 1 µM SDN

Interferents	Concentration (mM)	Signal change (%)
Citric acid	1.0	-0.102
D-Glucose	1.0	-0.141
Gum acacia	1.0	0.012
Oxalic acid	1.0	-0.143
Starch	1.0	-0.187
Sucrose	1.0	-0.151

R.S.D. was 0.30%. Thus, satisfactory recoveries of the analyte from the real samples and a good agreement between the concentration ranges studied and the real ranges encountered in the urine samples when treated with the drug make the developed method applicable in clinical analysis.

CONCLUSIONS

The Voltammetric determination of SDN was investigated on GCE. The electro-oxidation mechanism of SDN at the electrode was diffusion controlled irreversible process involving the number of protons taking part in the electrode reaction is half of the number of electrons. The DPV and SWV are effective and rapid electrochemical techniques with well-established advantages and low detection limits. The DPV and SWV signals of SDN increased linearly over the concentration range 0.310-4.34 $\mu g\ ml^{-1}$ and 0.310-5.58 $\mu g\ ml^{-1}$, with a detection limit of 0.01 $\mu g\ ml^{-1}$ (DPV) and 0.07 $\mu g\ ml^{-1}$ (SWV) as well as quantification limit of 0.03 $\mu g\ ml^{-1}$ (DPV) and 0.23 $\mu g\ ml^{-1}$ (SWV), respectively. The proposed method was applied for the determination of SDN in pharmaceutical formulations and urine samples with satisfactory results making it practical for routine analysis. The common interfering substances in the real sample do not interfere.

Table 3. Results of the Assay and the Recovery Test of SDN in Pharmaceutical Preparations Using Differential Pulse Voltammetry

	Amalar forte	Spectroscopic method
Labeled claim (mg)	500.0	
Amount found (mg)[a]	494.0	
RSD (%)	0.96	
Bias (%)	1.20	
Added (mg)	5.00	5.00
Found (mg)[a]	4.94	4.92
Recovered (%)	98.8	98.4
RSD (%)	0.65	2.21
Calculated F	3.75	
Calculated t	2.84	$t_c = 2.94$
Bias (%)	1.20	

[a]Average of five determination. t_{th} = Theoritical t-value (2.7).

Table 4. Determination of SDN in Urine Samples

Urine	Spiked (µM)	Detected (µM)[a]	Bias (%)	Recovery (%)	SD ± RSD (%)
Sample 1	1	0.956	4.40	95.6	0.0021 ± 0.10
Sample 2	3	2.90	3.19	96.6	0.0114 ± 0.29
Sample 3	5	4.92	1.44	98.4	0.0184 ± 0.30
Sample 4	7	7.01	-0.14	100.1	0.0223 ± 0.03

[a]Average of five determination.

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