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Electroanalysis of dapsone, an anti-leprotic drug

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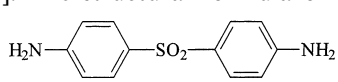
Abstract

The electrochemical oxidation and adsorption of dapsone, an anti-leprotic drug were studied in aqueous alcohol medium at a stationary glassy carbon electrode. Cyclic voltammetry studies showed one well-defined oxidation peak in the potential range 1.2–1.9 V at pH conditions 1.0, 4.0, 7.0, 9.2 and 13.0. The oxidation was irreversible and exhibited diffusion controlled adsorption. Controlled potential coulometry revealed one electron oxidation of the amino group in the molecule. A systematic study of the experimental parameters that affect the squarewave stripping response was carried out and the optimized experimental conditions were arrived at. A calibration plot was derived for the determination of the compound in solution. This method was used for the determination of dapsone in tablets and urine. The limits of determination was 0.0036 and 3.56 mg/ml and the relative standard deviation ($n = 10$) was 4 ppt (0.4%) at a concentration level 0.100 mg/ml.

Keywords: Dapsone; Cyclic voltammetry; Squarewave voltammetry; Tablets; Urine

1. Introduction

Dapsone, 4,4-diamino-biphenyl-sulphone (DDS) is one of the most important and main drug available for the treatment of leprosy [1]. It is also used in the treatment of malarial diseases and as an anti-inflammatory [2] in acute ileitis. Dapsone with clofazimine prevents the inhibitory effect on neutrophil motility [3]. With ethambutol and rifampin it is used for the treatment of human eye disease [4]. The structural formula of dapsone is:



Perusal of literature reveals the availability of methods like HPLC [5,6], LC [7], spectrophotometry and polarography for the determination of DDS in tablets, blood and urine [1–11]. The cleavage of DDS using a mercury cathode and tetraalkyl ammonium salts in both protic and aprotic media has also been reported [12]. A number of procedures were available for the determination of dapsone using HPLC technique. HPLC, at its current stage of development, is clearly not a method for analytical problems with a high repetition rate because the receptive condition of the system requires 24–36 h. On the other hand, electroanalysis is a manageable method, which is suitable for various problems [13]. Hence the development of electrochemical determination

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assumes importance. No electroanalytical data based on stripping voltammetry are available for the determination of this compound. Stripping voltammetry is a simple, highly sensitive and selective technique for the determination of electroactive compounds at ppm and ppb levels. Determination of drugs in pharmaceutical formulations and urine samples using differential pulse polarography was already reported [8]. Mercury is unjustly marked as one of the most insidious hazards within the laboratory. Solid electrode is better than the liquid electrode. Hence we selected glassy carbon as a working electrode. The present paper reports the cyclic voltammetric behaviour of DDS and square wave stripping voltammetric procedures for the determination of this drug. The analytical procedure proposed for the determination of DDS is easy to adopt and the lowest concentration level possible is better than the existing LC and polarographic methods. This method can be adopted for the determination of DDS in pharmaceutical formulations and urine samples.

2. Experimental

2.1. Apparatus and reagents

The voltammetric experiments and stripping analysis were performed using a Bioanalytical Systems (BAS 100A) electrochemical analyser. A glassy carbon working electrode (BAS Model MF 2012; geometric area = 0.0774 cm^2), a silver–silver chloride reference electrode (BAS Model MF2021), a platinum counter electrode and a standard one-compartment three-electrode cell of 15 ml capacity were used in all experiments. The compound DDS was prepared [14] by the action of thionyl chloride on acetanilide followed by the oxidation with CrO_3 to its sulphone. Then it was hydrolysed to 4,4′-diamino-biphenyl-sulphone (DDS). DDS was recrystallized from ethanol to white crystals (m.p. 178–179 °C). The stock solution was prepared in alcohol. The supporting electrolytes solutions, 10% H_2SO_4 in 50% aqueous alcohol (pH 1.0), B.R. buffer in 50% aqueous alcohol (pH 4.0 and 9.2), 0.1 M KCl in 50% aqueous alcohol (pH 7.0) and 0.1 M NaOH in 50% aqueous alcohol (pH 13.0) were prepared and used for the voltammetric studies.

2.2. Procedure

Under the experimental conditions, a solution of DDS was placed in the electrochemical cell and purified nitrogen gas was passed for 20 min to remove the dissolved oxygen under stirred conditions. The glassy carbon electrode was pretreated in two ways to get reproducible results: (1) mechanical polishing over a velvet micro-cloth with alumina suspension, and (2) electrochemical treatment by applying a potential of + 1.5 V for 4 s in the same solution in which the measurements was carried out. The electrode cleaning procedures were carried out for each and every experiment and this pretreatment requires only 5 min. The suitability of glassy carbon electrode was checked after every 100 stripping analysis by running cyclic voltammogram of potassium ferricyanide and measuring the potential difference between reversible peaks. If the potential difference was greater than 60 mV, then the electrode was boiled in 10% potassium hydroxide solution for 10 min, washed with water and the usual treatments mentioned above were done. The electrode was fairly stable for long periods. The Ag/AgCl reference electrode and the platinum counter electrode were used. The analyte solution was prepared in the ratio of 1:9 using the analyte and appropriate amount of the buffer solution and the total volume was kept constant (10 ml). Measurements were made using cyclic voltammetry, squarewave voltammetry, chronocoulometry and controlled potential coulometry for the reaction mechanism study. Squarewave stripping voltammetry was employed for the analytical study at pH 1.0. Exactly 9 ml 10% sulphuric acid in 50% aqueous ethanol and 1 ml of the ethanolic solution of DDS were taken in a 15 ml undivided cell for normal studies. There was no change observed in the stripping response when the volume of the pH solution was reduced to 8 ml and the substrate volume was increased to 2 ml. The total analyte volume was always kept at 10 ml. All the three electrodes were inserted, deaerated and the experiment was car-

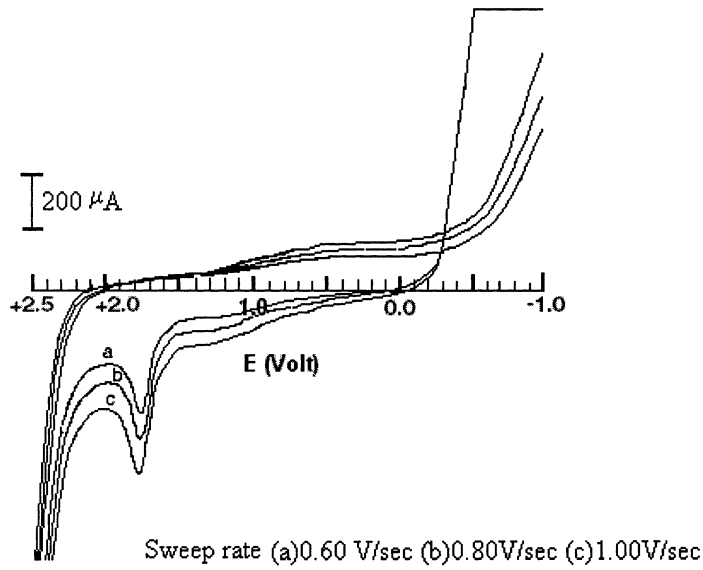


Fig. 1. Cyclic voltammogram of 7.7 mM DDS at various scan rates in 0.1 M H_2SO_4 in 50% aqueous ethanol.

ried out depending upon the necessity of the medium volume. Background values were recorded and subtracted.

3. Results and discussion

3.1. Cyclic voltammetric behaviour

Cyclic voltammograms of DDS at all the five pH conditions exhibited one distinct and well-defined anodic peak in the potential range 1.2–1.9 V at concentrations ranging from 3.2 to 29.4 mM and sweep rate from 0.1 to 1.0 V/s (Fig. 1). Apart from this, a cathodic peak with low peak current is observed at pH 7.0, 9.2 and 13.0. However, it does not satisfy the criteria for reversibility [15]. The cyclic voltammetric study was not considered beyond the concentration 29.4 mM of DDS in this medium due to the precipitation of substrate in the solution.

As the sweep rate is increased from 0.10 to 1.00 V/s at a fixed concentration of DDS: (i) the peak potential shifts anodically, (ii) the peak current increases steadily, (iii) the peak current function, $i_p/ACv^{1/2}$ exhibits almost constancy. If the polishing was not done between two sweep rate changes then a decrease in the peak current is observed. This indicates the possibility of adsorption. A straight line is obtained when i_p is plotted against $v^{1/2}$ (Fig. 2). This reveals that the anodic oxidation is diffusion controlled. A straight line ($E_p = 0.088 \log v + 0.0017$) is observed when E_p is plotted against log v at a particular concentration in pH 1.0 medium. From the slope of the straight line ($\Delta E/\Delta \log v$), the α_{na} value is calculated by using the formula, $\Delta E/\Delta \log v = -30/\alpha_{na}$. The α_{na} value is found to be 0.34 and is taken for further calculation for the number of electron transferred.

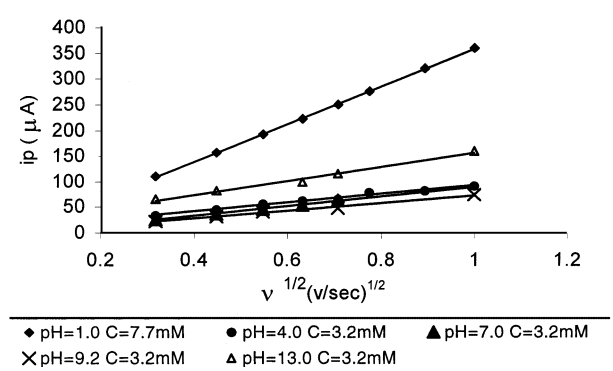


Fig. 2. Dependance of the peak current on the square root of scan rate.

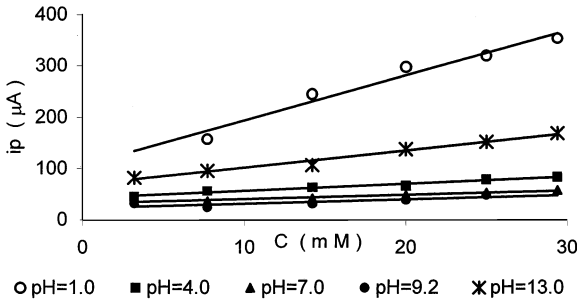


Fig. 3. Dependence of the peak current on the various concentrations of DDS.

Similar correlations were made for other pH conditions. With the increase of concentration from 7.7 to 29.4 mM: (i) the peak potential shifts anodically, (ii) the peak current increases, (iii) the peak current function decreases steadily. As the compound has the character of adsorption during the cycle itself on electrode surface, the polishing was given for every concentration changes. As a result of linear variation between i_p and concentration C, a straight line is obtained when i_p is plotted against C (Fig. 3). However, the peak current function exhibits a decrease with increase of concentration. This reveals the blocking effect due to adsorption of the substrate on the electrode surface.

The peak current, i_p and the peak current function, I_p values almost decrease gradually from pH 1.0 to 13.0 except in basic medium (pH 13.0) where an increase in the peak current is observed. The peak potential value decreases from pH 1.0 to 4.2 and then slight increase is observed at pH 7.0. Then it is decreased up to alkaline pH conditions (Fig. 4). This shows that the mechanism is different at acid, neutral and basic conditions. Since, no cathodic peak is observed when the potential scan direction is reversed the anodic oxidation is irreversible reaction. The fractional α_{na} value confirms the irreversible oxidation of DDS. After the first cycle, the peak current decreases tremendously and comes closer to the background current as the number of cyclic voltammetric cycles increased from 2 to 10 for 20 mM and sweep 0.300 V/s (Fig. 5). This indicates and confirms the adsorption behaviour of substrate.

3.2. Controlled potential coulometric behaviour

By using controlled potential coulometry, the number of electrons transferred, n values were found out from the charge consumed by 1×10^{-9} M concentration of the compound DDS. The charge consumed for every electrolysis was found as 774×10^{-7} C in acid medium. The coulometric n was calculated by using the equation, $Q = nFN$, where Q is charge in coulombs, F is Faraday's constant and N is number of moles of the substrate. The n value is found to be one (rounded

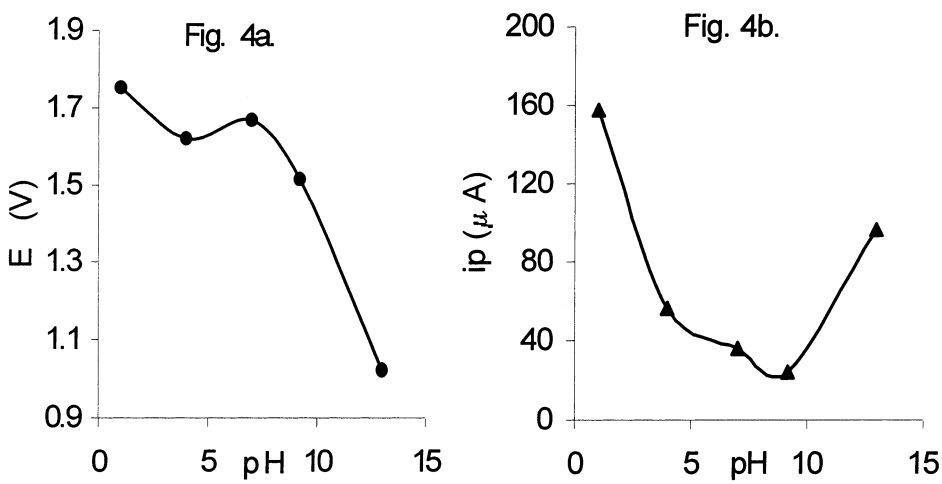


Fig. 4. (a) Plot of peak versus pH; (b) plot of peak potentials versus pH.

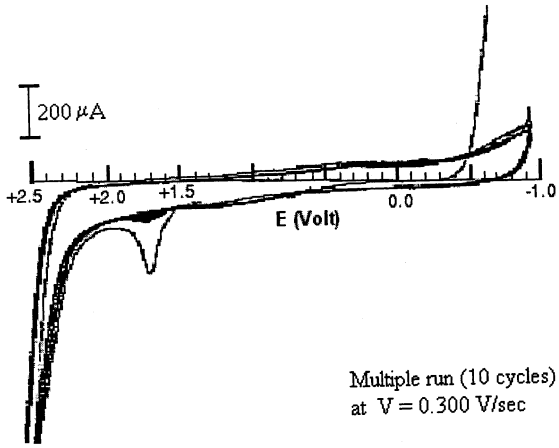


Fig. 5. Ten continuous cycles voltammagram of 7.7 mM DDS in pH 1.0 at scan rate 0.3 V/s.

value) for anodic peak of the compound DDS in all mediums (Table 1).

3.3. Chronocoulometric behaviour

In chronocoulometry, a plot of charge versus square root of time, Q versus $t^{1/2}$ transforms the data into a linear relationship whose slope was given as $\Delta Q/\Delta t^{1/2} = 2\ nFACD^{1/2}/\pi^{\frac{1}{2}}$. The number

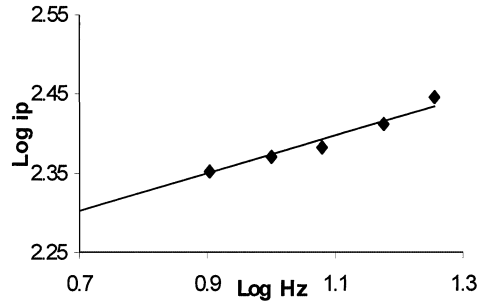


Fig. 6. Plot of log i_p versus log Hz.

of electrons transferred, n is one, taken from the controlled potential coulometric studies. By using forward slope and above equations, diffusion coefficient of DDS was calculated. It exhibits almost constancy except in basic medium (Table 2).

3.4. Square wave voltammetric behaviour

To confirm the results found in the cyclic voltammetry studies, the same experiments were carried out in pH 1.0 with the squarewave technique, maintaining the pulse amplitude at 25 mV and modifying the frequency used. The logarithm of the peak current was directly proportional to the logarithm of the frequency (Fig. 6).

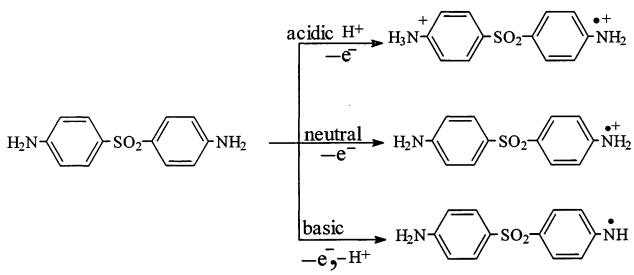
Table 1
Controlled potential coulometry

pH	Electrolytic potential (mV)	Current (A)	Charge (C)	Number of electrons transferred
1.0	1800	2023×10^{-8}	774×10^{-7}	0.80
4.0	1725	1994×10^{-8}	781×10^{-7}	0.81
7.0	1750	1983×10^{-8}	789×10^{-7}	0.82
9.2	1600	2017×10^{-8}	799×10^{-7}	0.83
13.0	1200	2239×10^{-8}	895×10^{-7}	0.93

Table 2
Chronocoulometry

pH	Concentration (mol/cm^3)	Forward slope (μC/s)	Intercept	Correlation coefficient	Diffusion coefficient (cm^2/s)
1.0	3.2×10^{-6}	8.5456	37.7749	0.9986	1.238×10^{-5}
4.0	9.1×10^{-6}	8.5290	−39.7049	0.9975	1.230×10^{-5}
7.0	3.2×10^{-6}	2.6976	4.9286	0.9994	1.001×10^{-5}
9.2	9.1×10^{-6}	8.0956	−80.8418	0.9999	1.113×10^{-5}
13.0	9.1×10^{-6}	15.1921	11.6859	0.995	3.918×10^{-5}

All the above facts reveal that the anodic oxidation of DDS at a glassy carbon electrode in acid medium is an irreversible one-electron transfer, the overall reaction is diffusion-controlled and the compound is having adsorptive behaviour. At acid medium, the NH_2 group is protonated and hence the loss of electron becomes difficult. This is understood from higher Ep values observed in acid medium. In alkaline medium the oxidation becomes easier because of the easy availability of electrons from the NH_2 group and removal of H^+ from NH_2 as H_2O. Thus hydroxide present in the medium facilitates the oxidation. Hence lowest E_p values are observed here. In neutral, slightly acidic and basic medium, intermediate values are possible because of the maximum possible oxidation of neutral substrate. The formed intermediates, radical cations or radical may undergo some coupled chemical reactions. Further deelectronation is not realized because of the absence of second anodic peak. On the basis above discussion the probable oxidation mechanism is depicted as follows:



3.5. Squarewave stripping voltammetric behaviour

Since highest peak current with no complication from other peaks was observed in pH 1.0, the acid medium was chosen for stripping. The test solution was purged with nitrogen for about 20 min and an accumulation potential of 0.0 V was applied to the electrode. The stirring was stopped after accumulation time of 15 s. After a 10 s rest period, Osteryoung squarewave stripping pulse sweep varying a.c. amplitude and frequency was carried out.

The set conditions given in the instrument were square wave amplitude = 50 mV, frequency = 15 Hz, quiet time = 10 s, sampling point = 256, step potential = 4 mV and deposit potential = 0.0 V. To carry out the square wave stripping voltammetry the optimum conditions of various instrumental parameters which lead to well-defined peaks with maximum peak current were fixed by varying them over a range (Table 3). A lower concentration of 1.42 mM was chosen to establish optimum conditions (deposition time 10 s, a.c. amplitude 50 mV, frequency 15 Hz, quiet time 5 s, step potential 15 mV and deposition potential 50 mV). Under optimum conditions the square wave stripping voltammograms were recorded for various concentrations varying from 0.0142 to 14.2 mM. (0.0036–3.56 mg/ml). As an illustration, the squarewave stripping voltammogram recorded for DDS under optimum conditions is presented in Fig. 7. The peak currents obtained for various concentrations were plotted against concentration, which give a straight line, $i_p(\mu A) = 60.534\ C$ where i_p is the peak current in μA and C is the concentration in mg/ml with good correlation ($r^2 = 0.9923$).The relative standard deviation for 10 measurements ($n = 10$) was 4 ppt (parts per thousand; 0.4%) at a concentration level 0.100 mg/ml.

4. Determination of DDS in pharmaceutical sample

One tablet of dapsone is dissolved in 100 ml of 50% aqueous alcohol and 1.5 ml of dapsone solution was pipetted out into a cell containing 5.5 ml of buffer solution (pH 1.0). By using optimum conditions, the squarewave stripping voltammogram was recorded (Fig. 7) and the peak current value was noted. The corresponding amount of DDS present in 1 ml of the tablet solution was

found out from the calibration equation. From this the amount of DDS present in the whole of the 100 ml of solution was calculated. The experimentally calculated value of DDS present in one tablet was 109.1 mg. The theoretical calculated value is 110.0 mg. Deviation in percentage is 0.82. This experiment was repeated 5 times to get concordant value. Similarly by pipetting out 0.5, 1.0 and 2.0 ml of tablet solution and keeping the buffer volume at 7.0 ml, the stripping voltammograms were recorded. By employing the same procedure the amount of DDS present was calculated. The results are presented in Table 4. A minimum of 3.6 ppm (0.0036 mg/ml) concentration of DDS was determined by this procedure.

5. Determination of DDS assay in urine

Measurement of DDS in an urine sample collected after 8 h from administration was demonstrated and 0.5 ml of the urine sample was mixed

Table 3
Square wave stripping voltammetry

Constant parameters	Varied parameter		E_p (mV)	i_p (μA)
C 1.42	Deposition Time (DT, s)	10	1724	36.23
ACA 50		20	1624	29.73
FR 15		30	1628	26.53
QT 10		40	1620	31.01
SP 04		50	1636	27.90
DP 0.0		60	1632	28.54
C 1.42	a.c. Amplitude (ACA, mV)	25	1572	29.98
DT 10		50	1568	44.43
FR 15		75	1532	34.73
QT 10		100	1504	28.12
SP 04		125	1460	36.93
DP 0.0		150	1452	40.53
		175	1400	38.42
		200	1344	33.70
C 1.42	Frequency (FR, Hz)	5	1508	16.01
DT 10		7	1512	19.73
ACA 50		9	1532	17.23
QT 10		11	1508	25.89
SP 04		13	1508	27.84
DP 0.0		15	1516	28.21
C 1.42	Quiet time (QT, s)	5	1516	30.74
DT 10		10	1524	30.62
ACA 50		15	1508	28.88
FR 15		20	1544	28.82
SP 04		25	1512	29.37
DP 0.0		30	1544	26.35
C 1.42	Step potential (SP, mV)	5	1530	31.53
DT 10		10	1560	34.00
ACA 50		15	1495	60.17
FR 15		20	1500	54.22
QT 05, DP 0.0		25	1525	30.04
C 1.42	Deposition potential (DP, mV)	0	1465	63.61
DT 10		50	1495	116.05
ACA 50		300	1525	44.28
FR 15		500	1495	42.63
QT 05, SP 15		600	1525	32.17

C = concentration (mM).

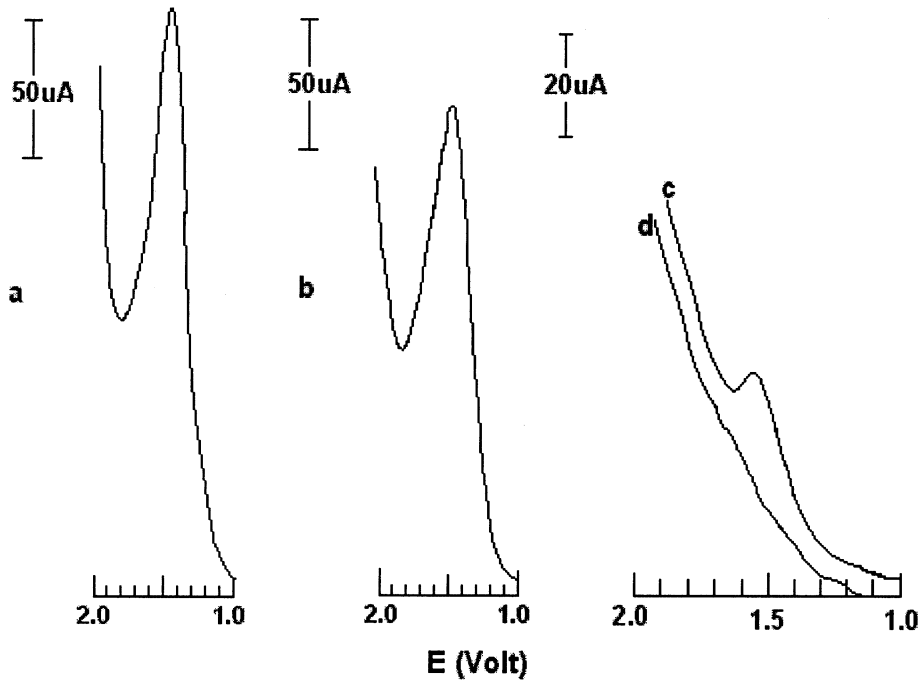


Fig. 7. Square wave voltammogram of DDS under optimum conditions for: (a) 11.8 mM; (b) tablet; (c) urine sample; (d) urine sample before DDS dosage.

Table 4
Determination of DDS in dapsone tablet

Volume (ml)	E_p (mV)	i_p (μA)	Experimental weight per tablet (mg)	Theoretical weight per tablet (mg)	Deviation (%)
0.5	1568	35.40	109.0	110	0.91
1.0	1555	66.61	109.1	110	0.82
1.5	1554	96.84	109.1	110	0.82
2.0	1574	127.39	109.1	110	0.82

with 6.5 ml of the supporting electrolyte solution and the pH was brought to 1.0. The squarewave stripping voltammogram was recorded under the optimum experimental conditions. The square-wave voltammogram was presented in Fig. 7. This experiment was repeated 5 times to get concordant value. There is no appreciable interference due to the presence of a small amount urine present in the electrolyte hence the same calibration plot can be used. By employing the calibration plot the amount of DDS present in 1.0 ml of the urine sample was calculated. Similar experiments were carried out with varying amount of urine sample, 1.0, 1.5 and 2.0 ml. In all the cases the suitability of the procedure was verified. The results are presented in Table 5.

There is no degradation of the analyte in solution during experiment. Only electrooxidation of the amino group of DDS is taking place. The other matters present in tablets and urine samples are not interfering with the study. This method is a stable method. Repetition rate is found to be high. Hence the proposed method can be used as a stable method like spectrophotometric or chromotographic methods.

Table 5
Determination of DDS in urine sample

Volume (ml)	E_p (mV)	i_p (μA)	Concentration (mg/ml)	Experimental weight per ml of urine (mg)
0.5	1551	9.26	0.1531	0.3061
1.0	1556	23.51	0.3067	0.3067
1.5	1560	27.64	0.4569	0.3046
2.0	1564	37.01	0.6117	0.3059

6. Conclusion

DDS is anodically oxidized irreversibly on glassy carbon electrode in the potential range of 0.10–0.18 V at all pH conditions. The oxidation is diffusion-controlled adsorption and loss of one electron is observed. Effect of pH leads to the conclusion that pH 1.0 is suitable for analytical studies. The adsorptive stripping voltammetric studies are carried out by employing Osteryoung squarewave stripping voltammetry. Optimum conditions are arrived at. The concentration effect was studied and a calibration plot was obtained. This is taken as a standard and used to find out the unknown concentration of DDS present in the pharmaceutical tablet and urine samples. Dapsone is determined over the range of 0.5–20.0 mg/ml by HPLC [6]. Compared to this a better limit of determination of DDS between 0.0036 and 3.56 mg/ml is obtained in this stripping voltammetry method. This technique is simple and easy to carry out. Once the instrument is set, just by changing the analyte and polishing the electrode, within a few minutes, the amount of DDS can be determined. Hence stripping voltammetry is a better technique over chromatography techniques.

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