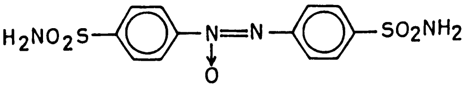
Bull. Chem. Soc. Jpn., ***62***, *2662—2667 (1989)* [Vol. 62, No. 8

Electrochemical Behavior of Azoxybenzene-4,4′-disulfonamide at Pyrolytic Graphite Electrode

R. N. Goyal* and Sandhya Bhargava
Department of Chemistry, University of Roorkee, Roorkee-247667, India
(Received August 29, 1988)

The electrochemical reduction of azoxybenzene-4,4′-disulfonamide has been studied in the Britton Robinson buffers of pH range 3.0—10.6 at a pyrolytic graphite electrode. The electroreduction was found to occur in a single well defined 4e, 4H$^+$ peak. The hydrazo compound has been identified as the main product of the electrode reaction.

The studies on the electrochemical behavior of azo compounds has attracted considerable attention[1,2] in the last decade due to their application in dyes and pharmacy. Azoxy compounds on the other hand have not attracted considerable attention in spite of the importance of azoxy group in enhancing the antibiotic as well as antifungal nature of an organic compound.[3,4] A well-known azoxy antibiotic Elaiomycin has been found very effective in vitro but not in vivo against Mycobacterium tubercuiosis.[5] The other antibiotic LL-BH 872a, having an azoxy group, has been reported as a new antifungal agent.[6] In view of the importance of azoxy compounds in medicine, it was considered interesting to study the redox behavior of azoxybenzene-4,4′-disulfonamide (**I**) claimed as peroxodisulfate oxidation product of sulfanilamide.[7] Since oxidation products of sulfonamides have been suggested[8] responsible for their bacteriostatic action, it is expected that the electrochemical behavior of this compound would throw light on the metabolic pathway of the bacteriostatic action of sulfanilamide.


(I)

Experimental

Azoxybenzene-4,4′-disulfonamide was synthesized in the laboratory by the reported method[9] and its purity was ascertained by repeated crystallization, mp and TLC.

Equipments used for voltammetric and coulometric studies has been described elsewhere.[10] The UV spectral studies were carried out using Specord (C, Zeiss Zena) spectrophotometer. IR spectrum of the product was recorded on Beckmann IR-20 spectrophotometer. Thin-layer chromatography was carried out on silica Gel-G plates with benzene-methanol (80:20) as developer. The pyrolytic graphite electrode was prepared by the method reported earlier[11] and had an area ca. 2.0 mm^2. All potentials are referred to a saturated calomel electrode (SCE).

Chromatographic Procedure. For the identification of the product, about 8—10 mg of azoxybenzene-4,4′-disulfonamide was electroreduced at potentials more negative to peak 1c, in B. R. buffer of desired pH using a three compartment cell. A pyrolytic graphite plate (6×1 cm^2) was used as working electrode, cylinderical platinum gauze and SCE as the counter and reference electrodes respectively. The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. The completely electrolyzed solution was transferred from the cell and lyophilized. The freeze-dried material was dissolved in 1—2 ml of distilled water and passed through a glass column packed with Sephadex G-10 (Sigma, bead size 40—120 μm) using doubly distilled water as eluant. This arrangement allowed separation of phosphate from the product. Fractions of 5 ml each were collected and their absorbance was monitored at 210 nm. The first peak to emerge out of the column (between volume 120—160 ml) was found to contain phosphate and rejected. The other peak between 200—230 ml was freeze-dried and analyzed by IR, melting point, and mass spectra.

The stock solution of compound **I** (2 mM; 1 M=1 mol dm^{-3}) was prepared in methanol (A. R.). The solutions for voltammetric studies were prepared by mixing 5.0 ml of azoxybenzene-4,4′-disulfonamide with 5.0 ml of B. R. buffers. The solutions were deaerated for 10—15 minutes and the curves were then recorded.

Results and Discussion

Linear sweep voltammetry of azoxybenzene-4,4′-disulfonamide at a scan rate of 10 mV s^{-1} exhibited a bump 1′c and a well defined reduction peak in B. R. buffers of pH range 3.0—10.6. The peak potential of the reduction peak was dependent on pH and shifted to more negative potential with increase in pH. The plot of E_p versus pH exhibited a break at around pH 6.8 (Fig. 1). The relationship between the peak potential and pH for the reduction peak (1c) is given by the equations:

$$E_p\ (\text{pH } 3.0\text{—}6.8) = [-0.52-0.057\ \text{pH}]\ \text{V vs. SCE}$$

$$E_p\ (\text{pH } 6.8\text{—}10.58) = [-0.82-0.013\ \text{pH}]\ \text{V vs. SCE}$$

Cyclic voltammetry of **I** at a scan rate of 100 mV s^{-1} exhibited a single reduction peak (1c) with a bump (1′c) in B. R. buffers of different pH when sweep was initiated in the negative direction. In the reverse sweep no peak was observed at pH<7.0. However, at pH>7.0 a bump closer to zero volt was occasionaly observed. Some of the typical cyclic voltammograms are presented in Fig. 2. The bump (1′c) was characterized as preadsorption peak due to strong adsorption

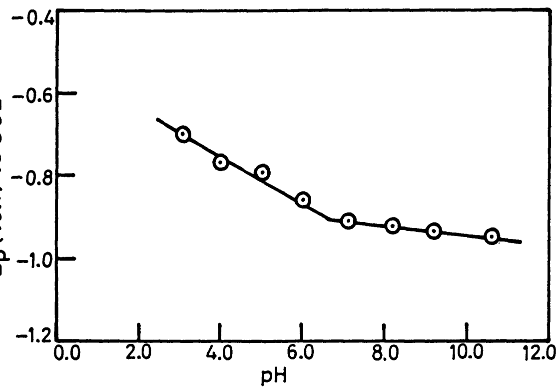


Fig. 1. Variation of E_p with pH for the voltammetric reduction peak lc of azoxybenzene-4,4′-disulfonamide.

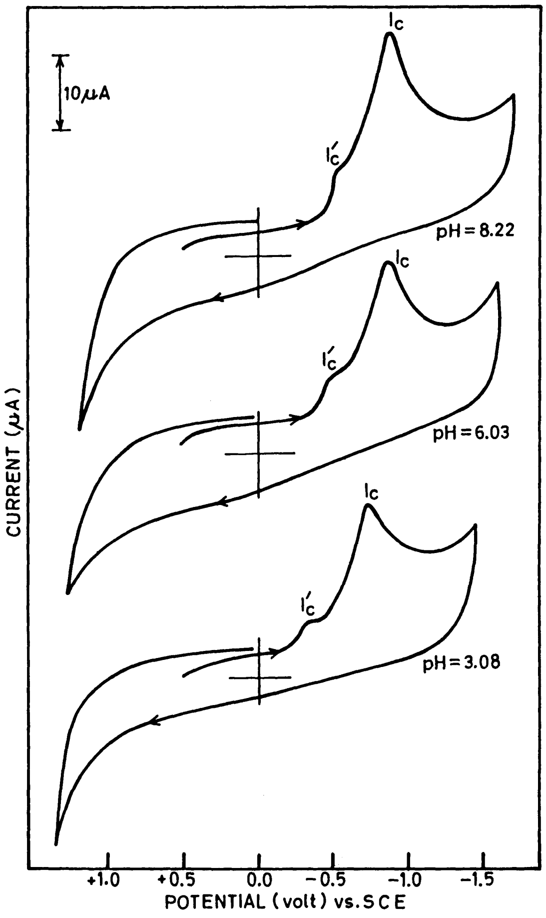


Fig. 2. Cyclic voltammograms of 1.0 mM azoxybenzene-4,4′-disulfonamide at the PGE in the phosphate buffers of different pH. Sweep rate 100 mV s^{-1}.

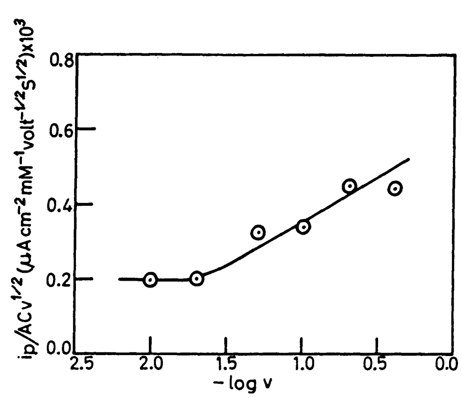


Fig. 3. Variation of peak current function with logarithm of sweep rate for peak l′c.

Table 1. Effect of Concentration of Azoxybenzene-4,4′-disulfonamide on the Ratio of Peak Currents for Peaks l′c/lc

Concentration of **I** (mM)	lc′/lc
0.2	0.91
0.4	0.78
0.6	0.57
0.8	0.06
1.0	0.56
1.2	0.52
1.4	0.58
1.6	0.59
1.8	0.66
2.0	0.48
2.2	0.33

Table 2. Effect of Sweep Rate on the Relative Height of Peaks l′c and lc

Scan rate mV s^{-1}	l′c/lc
20	0.30
50	0.45
100	0.45
200	0.62
500	0.69

of the product by monitoring the relative height of adsorption response (l′c) to diffusion response (lc) with increase in the concentration of compound **I**.[12] It was interesting to observe that the ratio of peak currents for peaks l′c/lc decreases with increase in concentration of compound **I** (Table 1) and thus indicated that peak l′c is preadsorption peak due to strong adsorption of the product.[12] A further confirmation was made by recording cyclic voltammograms of **I** at different sweep rates. It was found that the ratio of l′c/lc increases with increase in sweep rate (Table 2). Hence it was believed that peak l′c is due to the strong adsorption of the product. The peak current function ($i_p/ACV^{1/2}$) for peak l′c was also plotted against the sweep rate. It was observed that the peak current function increases with increase in sweep rate in the range 20—500 mV s^{-1} (Fig. 3), thereby further indicating peak l′c due to strong adsorption.

The peak lc was found to be diffusion controlled at low concentrations whereas at higher concentrations an indication of weak adsorption of the reactant was noticed. The peak current values (i_p) for the reduc-

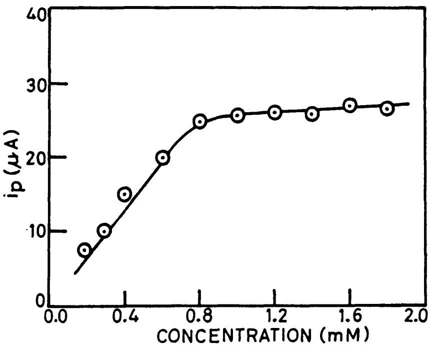


Fig. 4. Dependence of i_p on concentration for the reduction peak 1c at pH 7.26.

tion peak 1c increased linearly with increasing concentration of azoxybenzene-4,4′-disulfonamide upto a concentration of about 0.8 mM and at higher concentrations the peak current values had a tendency to become constant (Fig. 4). This behavior suggests complication due to the weak adsorption of the reactant.[13] Adsorption of the substrate at higher concentrations was further confirmed by the criteria proposed by Wopschall and Shain[14] for strong adsorption of product with weak adsorption of reactant. In such cases, at low scan rates (usually 10 to 500 mV s^{-1}) the adsorption peak current increases linearly with scan rate while diffusion peak current increases approximately as the square root of the sweep rate. A similar behavior was observed for peak 1′c and 1c in the case of azoxybenzene-4,4′-disulfonamide (Fig. 5). The strong adsorption of the product and the weak adsorption of reactant was further confirmed by plotting the ratio of adsorption (1′c) to diffusion (1c) peak current against $\sqrt{V}$. The dotted line shown in Fig. 6 indicates only adsorption of the product whereas the deviation of the observed behavior from straight line clearly pointed out that the reactant is weakly adsorbed and the product is strongly adsorbed in the present studies. This behavior is similar to that reported by Wopschall and Shain[15] for such systems.

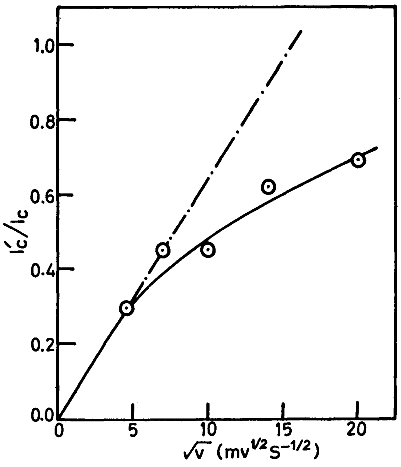


Fig. 6. Ratio of adsorption peak to diffusion peak (1′c/1c) as a function of square root of sweep rate. The dotted line indicates only adsorption of the product.

Table 3. Coulometric n-Values Observed for the Electroreduction of Azoxybenzene-4,4′-disulfonamide

pH	Potential V vs. SCE	Concn mM	Experimental n value[a]
3.08	0.9	0.5	4.12
	1.0	0.8	4.16
4.05	1.0	0.5	4.10
	1.0	0.8	3.88
5.06	1.1	0.5	3.92
6.03	1.1	0.5	4.16
	1.1	0.8	4.10
8.22	1.1	0.5	3.89
	1.1	0.8	3.90
10.58	1.1	0.5	4.04

a) Average of at least two replicate determination.

Controlled Potential Electrolysis. Controlled potential coulometry of azoxybenzene-4,4′-disulfonamide at pH values ranging from 3.0—10.58 at potentials more negative to peak 1c indicated that 4.0±0.1 electrons are transferred in the peak 1c process.

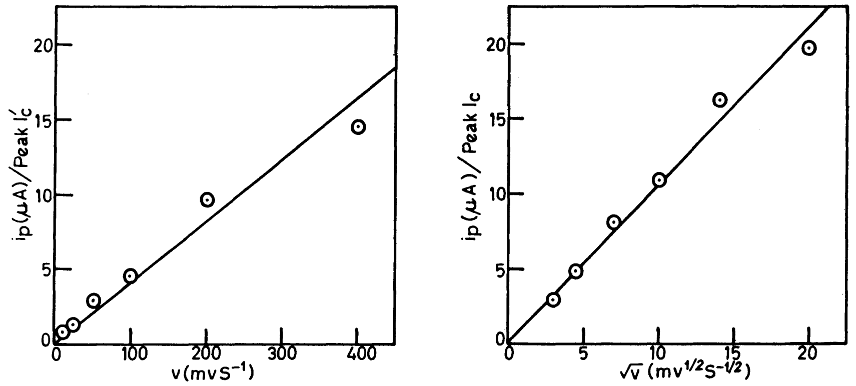


Fig. 5. Dependence of peak currents for peaks 1′c and 1c on V and $\sqrt{V}$ respectively.

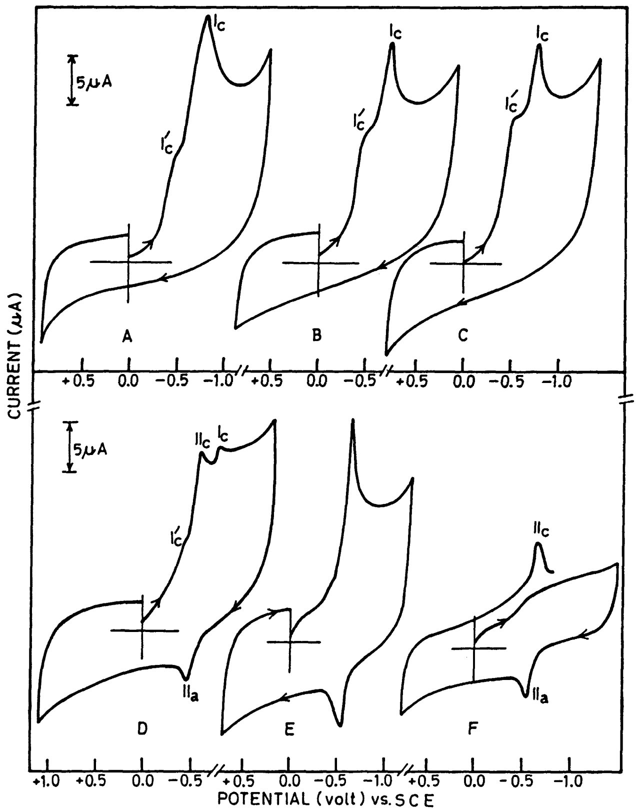
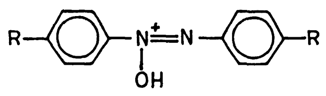


Fig. 7. Cyclic voltammograms of observed for 0.5 mM azoxybenzene-4,4′-disulfonamide undergoing electroreduction at −1.2 V. Curves were recorded at 0 (A), 10 (B), 30 (C), 60 (D), 90 (E), and 180 (F) min of electrolysis.

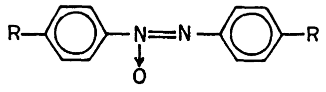
The values of *n* determined at various concentration of **I** and at different pH are presented in Table 3.

The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. The cyclic voltammogram of azoxybenzene-4,4′-disulfonamide before electrolysis at pH 7.06 is presented by the curve A in Fig. 7. With progress of electrolysis reduction peak 1c decrease systematically whereas a new cathodic peak 11c starts appearing at a less negative potential after about 60 minutes of electrolysis. Peak 11c formed a quasi-reversible couple with peak 11a observed in subsequent sweep towards cathodic potentials. On further electrolysis, peak current for the redox couple 11c/11a increases. After about 3 h, peak 11c practically disappeared when sweep was initiated in negative direction. However, peak 11a was clearly observed and peak 11c was observed only in the second cycle (Fig. 5). The orange solution turned to colorless at the end of the electrolysis.

Spectral Studies. The UV/visible spectra of azoxybenzene-4,4′-disulfonamide were recorded in B. R. buffers in the pH range 3.08—10.58 to determine the pK_a. It was observed that compound **I** exhibits λ_{max} at 330 nm with a shoulder around 270 nm in the UV region and at 468 nm in the visible region below pH 6.03. In the pH range 6.03 to 7.07, the λ_{max} at longer wavelength shifted to 472 nm. At pH>7.07 the λ_{max} value was 480 nm. The absorbances at 350 and 472 nm were plotted against pH and gave an inflection point at around pH 6.8. Thus it is clear that below pH 6.8 compound **I** exists as a protonated species and at pH>6.8, it mainly exists as a neutral molecule. It is reported[16] that azoxy compounds are weak base and therefore protonation occurs on the oxygen atom. Hence the following species must exist in the solution.



below pK_a



above pK_a

The observed value of pK_a for azoxybenzene-4,4′-disulfonamide is in the range observed for azoxybenzene and a large number of substituted azoxybenzene.[17]

The progress of the electrolysis of azoxybenzene-4,4′-disulfonamide was also monitored by recording the spectral changes during the electrolysis. The UV spectrum of 0.05 mM azoxybenzene-4,4′-disulfonamide at pH 7.26 before electrolysis exhibited λ_{max} at

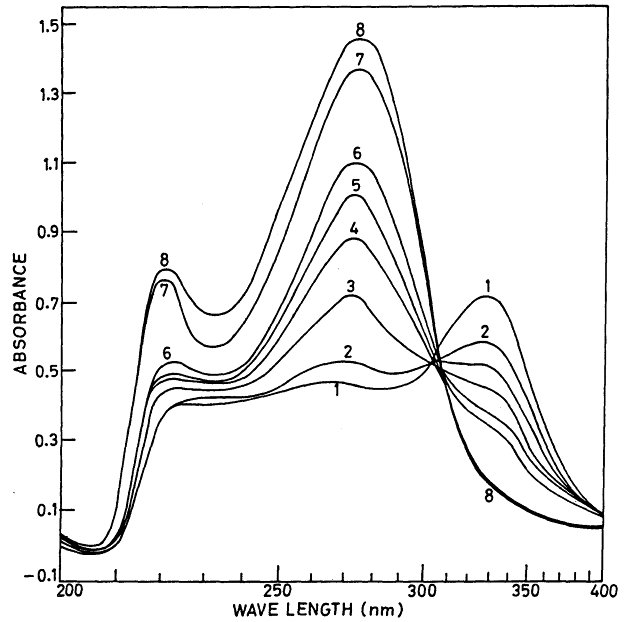


Fig. 8. Observed spectral changes for 0.5 mM azoxybenzene-4,4′-disulfonamide undergoing electroreduction at pH 7.26. Potential −1.2 V. Curves were recorded at 0 (1), 10 (2), 20 (3), 30 (4), 40 (5), 60 (6), 120 (7), and 180 (8) min of electrolysis.

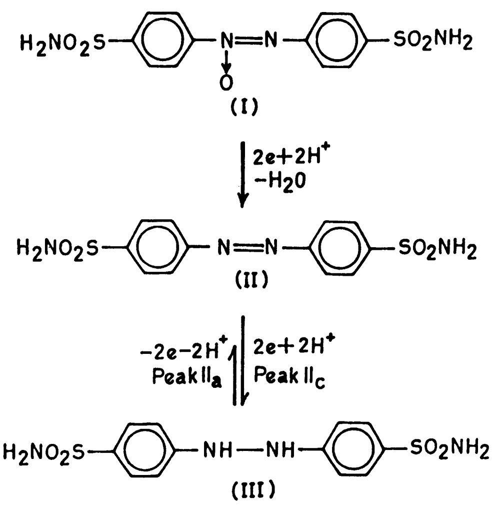
330 nm (Fig. 8, Curve 1). Application of a potential more negative to peak 1c, leads to decrease in absorbance at 330 nm and a systematic increase in absorbance in the region 250—300 and 210—225 nm. At the end of electrolysis a maximum at 276 nm and 220 nm was observed with isosbestic point at 310 nm. This clearly indicates that products of electroreduction of **I** absorbs at shorter wavelength in comparison to the starting material and hence π-chromophore system has been decreased. An almost similar behavior was observed at pH 3.08 and 10.58.

Characterization of the Product. In order to characterize the end product of the electrode reaction, the electrolysis was carried out at pH 3.08 and 7.26. The exhaustively electrolyzed solution was lyophilized and passed through a Sephadex column. The volume collected between 200—230 ml on freeze drying gave light yellow product which was analyzed by TLC, mp, IR, and mass spectra. TLC of the product exhibited only one spot with R_f=0.04 indicating thereby the formation of a single product during electroreduction of compound **I**. The product had a melting point of 158 °C. IR spectrum of azoxybenzene-4,4′-disulfonamide exhibited a strong band at 1450 cm^{-1} (N→O) in addition to other strong bands at 1610, 1400, 1320, 1300, 1245, 1080, 1010, and 910 cm^{-1}. The band at 1450 cm^{-1} disappeared in the product thereby clearly indicating that oxidation is occuring at N→O linkage. The mass spectrum of the product exhibited a clear molecular ion peak at 342. Some other high mass peaks were at 326 (7.3%); 325 (3.4%); 324 (1.1%); 262 (11.8%); 261 (7.3%); 260 (4.3%); 171 (17.1%); 170 (13.3%); 169 (9.2%). However, no attempt was made to explain the fragmentation pattern.

Redox Behavior. The experimental results indicate that reduction of azoxybenzene-4,4′-disulfonamide involves a 4e, $4H^+$ reaction to give a product which absorbs at a shorter wavelength in comparison with the starting material.

As there is no separation of peaks, it appears that 4e, $4H^+$ electrode reaction occurs in a single step. However, the formation of a quasi-reversible couple at the end of the electrolysis corresponding to azo derivative indicates that electrode reaction involves two 2e, $2H^+$ steps and since their potential is close to one another a single 4e, $4H^+$ step was observed in cyclic voltammetry. The following tentative mechanism can be suggested:

The first step in the reaction appears to be 2e, $2H^+$ reduction of azoxybenzene-4,4′-disulfonamide (**I**) to give azobenzene-4,4′-disulfonamide (**II**). As reduction of **II** is easier than **I**, it further reduces by taking 2e, $2H^+$ to give hydrazobenzene-4,4′-disulfonamide (**III**). Since the cleavage of N→O bond of azoxybenzene requires more negative potentials than electroreduction of the -N=N- bond in azobenzene,[18] therefore, **II** can not be formed as a stable intermediatory product and **III** is directly obtained (Scheme 1).



Scheme 1. Tentative mechanism proposed for the electroreduction of azoxybenzene-4,4′-disulfonamide.

To confirm whether the couple IIa/IIc is due to the azohydrazobenzene-4,4′-disulfonamide system, cyclic voltammograms of azobenzene-4,4′-disulfonamide were recorded in buffers of different pH. The identical dE_p/dpH values clearly indicate that the redox couple IIa/IIc is due to azobenzene-4,4′-disulfonamide. As hydrazobenzene-4,4′-disulfonamide (**III**) has less extensive π-conjugation system in comparison to **I**, the λ_{max} values in UV region shifted to shorter wavelengths.

Conclusion

It is apparent from the present studies that azoxybenzene-4,4′-disulfonamide is electroactive in nature and readily undergoes electroreduction in a 4e, $4H^+$ process to give hydrazo derivative as the final product. It has been suggested by Levitan et al.[19] that polarographic oxidation of sulfanilamide gives azoxybenzene-4,4′-disulfonamide together with other products. Since this compound is electroactive, it can undergo further reduction at higher negative potentials to give hydrazo compound as the ultimate product. Wawzonek[20] and others[21] have reported that electroreduction of azoxybenzene in acid media does not stop at hydrazo stage rather cleavage of NH-NH bond occurs to give aniline. However, in the present studies electroreduction of azoxybenzene-4,4′-disulfonamide gives hydrazo derivative as the final product. Thus it appears that the electron-withdrawing -SO_2NH_2 groups present in compound **I** inhibit the further reduction of -NH-NH- and only hydrazo product is formed. The nonreduction of -NH-NH- in presence of electron-withdrawing groups has been reported earlier by Florence[22] in azo/hydrazo benzene system. Azoxy compounds have also been reported to convert into azo compounds on prolonged electrolysis under controlled potential conditions.[23] Hence it is possible that azoxy compound produced by polarographic oxidation of sulfanilamide converts to azo and then hydrazo product on prolonged electrolysis under controlled potential conditions.

Burton,[24] Mayer,[25] and others[26] have proposed that oxidation products of sulfonamides may partly be responsible for their antibacteria activity. Thus it is concluded that azoxybenzene-4,4′-disulfonamide, a suggested polarographic and chemical oxidation product of sulfanilamide is electroactive in nature and gives hydrazobenzene-4,4′-disulfonamide as the final product. Hence the redox behavior involved with compound **I** is most likely responsible for the bacteriostatic action of sulfanilamide.

The financal assistance for this work was provided by the Indian Council of Medical Research, New Delhi.

References

1) J. P. Stradins and V. T. Glezer, "Encyclopedia of the Electrochemistry of the Elements," ed by A. J. Bard and H. Lund, Vol. XIII, Marcel Dekker, N. Y. (1978), p. 163.

2) H. Lund, "Organic Electrochemistry," ed by M. Baizer, Marcel Dekker, N. Y. (1973).

3) A. Kobayashi, *Agric. Biol. Chem.*, **26**, 203 (1962).

4) T. H. Haskell, A. Ryder, and Q. R. Bartz, *Antibiot. Chemother.*, **4**, 141 (1954).

5) K. Okuma, G. Nakamura, and S. Yamashita, *J. Antibio. Ser. A.*, **10**, 224 (1957).

6) A. G. Karlson, *Antibiot. Chemother.*, **12**, 446 (1962).

7) S. P. Srivastava, A. Kumar, and A. K. Mittal, *Indian J. Chem.*, **17**, 593 (1979).

8) R. L. Mayer, *Bull. Acad. de Med.*, **117**, 727 (1937).

9) M. K. Seikel, *J. Am. Chem. Soc.*, **62**, 1214 (1940).

10) R. N. Goyal, R. Bhushan, and A. Agarwal, *J. Electroanal. Chem.*, **171**, 281 (1984).

11) R. N. Goyal, S. K. Srivastava, and R. Agarwal, *Bull. Soc. Chim. Fr.*, **4**, 656 (1985).

12) E. R. Brown and R. F. Large, "Physical Methods of Chemistry," ed by A. Weissberger and B. W. Rossiter, Wiley Interscience, N. Y. (1972).

13) R. S. Nicholson and I. Shain, *Anal. Chem.*, **36**, 706 (1964).

14) R. H. Wopschall and I. Shain, *Anal. Chem.*, **39**, 1514 (1967).

15) R. H. Wopschall and I. Shain, *Anal. Chem.*, **39**, 1535 (1967).

16) C. S. Hahn and H. H. Jaffe, *J. Am. Chem. Soc.*, **84**, 949 (1962).

17) P. J. Krueger, "The Chemistry of Hydrazo, Azo and Azoxy Groups," ed by S. Patai, John Wiley, N. Y. (1975), p. 173.

18) L. Chuang, I Fried, and P. J. Elving, *Anal. Chem.*, **37**, 1528 (1965).

19) N. I. Levitan, I. M. Kolthoff, W. G. Clark, and D. J. Tenenberg, *J. Am. Chem. Soc.*, **65**, 2265 (1943).

20) S. Wawzonek and J. D. Frederickson, *J. Am. Chem. Soc.*, **77**, 3988 (1955).

21) G. Costa, *Gazz. Chim. Ital.*, **83**, 875 (1953).

22) T. M. Florence, *J. Electroanal. Chem.*, **52**, 115 (1974).

23) F. G. Thomas and K. G. Boto, "The Chemistry of the Hydrazo, Azo, and Azoxy Groups," Part I, Wiley, London (1975), p. 443.

24) R. L. Mayer and C. Oechslin, *Compt. Rend.*, **205**, 181 (1937).

25) A. C. Burton, H. J. White, and E. K. Marshall, *Proc. Soc. Expt. Biol. Med.*, **42**, 847 (1939).

26) M. G. Sevag, M. Shelburne, and M. Ibsan, *J. Biol. Chem.*, **144**, 711 (1942).