Electrochemical oxidation of sulphamethoxazole at pyrolytic graphite electrode: An example of free radical dimerization

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The electrochemical oxidation of sulphamethoxazole (I) has been studied in the pH range of 2.23-9.94 (phosphate buffers) at pyrolytic graphite electrode by linear and cyclic sweep voltammetry, coulometry and spectral studies. It is found that I is oxidized in $2e^- , 2H^+ $ step to give azobenzene-4, 4'-(N-3-methoxazolyl) disulphonamide as the major product.

Electrochemical investigations provide an invaluable insight into the redox behaviour of biologically important compounds$^1 - 3$. It was, therefore, considered interesting to elucidate the mechanism of electrooxidation of the well-known antibacterial drug, sulphamethoxazole (I) at pyrolytic graphite electrode.

Materials and Methods
Sulphamethoxazole was a gift from Roche Products Ltd, Bombay. Azobenzene-4, 4'-(N-3-methoxazolyl) disulphonamide was synthesised by the method reported in the literature and its purity checked by TLC. All experiments were carried out at 25 ± 1.0°C in phosphate buffers of ionic strength 0.5 M. The IR spectra were recorded on a Beckmann IR-20 spectrophotometer and PMR spectra on a Jeol JMS D 300 instrument. All potentials were referred to SCE.

Procedure
Voltammograms of deaerated solution of sulphamethoxazole (2.0 mM) in phosphate buffers (pH 2.23-9.94) were recorded. For controlled potential electrolysis a solution of sulphamethoxazole (8-10 mg) in appropriate buffer (25 ml) was electrooxidised in an H-type cell at potentials about 100 mV more positive than the peak potential using pyrolytic graphite plate (6 × 1 cm$^2$), platinum gauze cylinder and SCE as working, auxiliary and reference electrodes respectively. The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. When the oxidation peak $I_a$ completely disappeared, the electrolysed solution was removed from the cell and lyophilised. The product obtained at pH 7.3 gave a single spot ($R_t$ = 0.46) in TLC. However, two products were formed on carrying out electrolysis at pH 3.6 ($R_t$ = 0.27 and 0.48). These were separated as follows: The lyophilised material was dissolved in distilled water (1-2 ml) and passed through a glass column packed with Sephadex G-10 (Sigma, bead size 40-120 µ) using doubly distilled water as eluent, collecting 10 ml fractions. The absorbance of the fraction was monitored at 210 nm. The absorbance versus volume plot exhibited three peaks for the product obtained at pH 7.3 in GLC. The first peak obtained (fraction 6 to 15) corresponded to phosphate and was discarded. The fractions corresponding to other peaks were mixed, freeze-dried and characterized by m.p., IR, UV, PMR and mass spectra.

Results and Discussion
Linear sweep voltammetry of sulphamethoxazole (I) at a sweep rate of 10 mV s$^{-1}$ exhibited a single well-defined, pH-dependent oxidation peak at the pyrolytic graphite electrode (PGE). The $E_p$ versus pH plot exhibited two breaks at about pHs 2.7 and 6.5.
As sulphamethoxazole (I) possesses an aromatic amino group and a sulphonamide moiety, cyclic voltammogram of benzene sulphonamide at PGE was recorded. As benzene sulphonamide did not exhibit any peak at PGE, it was concluded that oxidation of the aromatic amino group not of sulphonamide group, occurred at PGE.

Cyclic voltammograms of I at a sweep rate of 100 mV s\(^{-1}\) exhibited a sharp oxidation peak \((I_a)\) when the sweep was initiated in the positive direction. In the reverse sweep two reduction peaks \((II_a, III_a)\) were observed which formed a quasi-reversible couple with peaks \(II_a\) and \(III_a\) observed in the subsequent sweep towards positive potentials (Fig. 2).

Peaks \(III_a/III_c\) were observed only below pH 4.0 at a sweep rate \(>50\) mV s\(^{-1}\), whereas the couple \(II_a/II_c\) was observed in the entire pH range (2.2-9.9). The peak potentials of peaks \(II_a/II_c\) and \(III_a/III_c\) were also dependent on pH and \(E_p\) versus pH plot exhibited a linear relation with \(dE_p/d\text{pH}\) values as 63, 70, 100, 110 mV/pH for peaks \(II_a/II_c\), respectively. The peak current of peak \(I_a\) was found to increase with increase in concentration of I in the range of 0.2 to 3.0 mM. Below concentration 1.5 mM the plot of \(i_p\) versus concentration was approximately linear whereas at concentration > 1.5 mM, the peak current more or less attained a constant value. This indicated complexity in the electrode reaction due to adsorption of the depolariser. This was further supported by occasional splitting of the peak \(I_a\) and the increase in the values of the peak current function \((i_p/A\text{CV}^{1/2})\) with increase in sweep rate \(n\).

The value of \(n\), i.e. the number of electrons involved in electrooxidation was determined by controlled potential coulometry of I at different pH values. Graphical integration of the current-time curve, reported by Lingane gave \(n = 2.0 \pm 0.1\) in the entire pH range studied.

A 1 mM stirred solution of I generally took 3 hr for complete electrooxidation and at the end solution became light yellow from colourless. The cyclic voltammograms recorded at different time intervals indicated that while peak \(I_a\) systematically decreased in the entire pH range, peaks \(II_a/II_c\) and \(III_a/III_c\) did not exhibit any significant change at pH < 4.0. At pH > 4.0, only peaks \(II_a/II_c\) were observed in the exhaustively electrolysed solution. Thus, it is clear that products of electrooxidation of sulphamethoxazole are electroactive in nature.

**Spectral studies**

The UV spectra of a 0.05 mM solution of sulphamethoxazole (I), in the pH range of 2.2-9.9, exhibited a well-defined band at 267 nm below pH 5.21 while at pH > 5.21, this band shifted to 256 nm region. The plot of absorbance versus pH at \(\lambda_{\text{max}}\) showed two inflexions
sections in the $E_p$ versus $pH$ plots, at around $pH$ 2.7 and 6.5. The $pK_a$ (1) at around $pH$ 2.7 corresponds to the equilibrium of $-N^+H_3^-\rightarrow NH_2^+H^+$, whereas the $pK_a$ (2) at $pH$ 6.5 suggests the acid dissociation of amide linkage. This is in accordance with the potentiometrically determined $pK_a$ value.

The progress of electrolysis of compound I at $pH$ values 3.6 and 7.3 was also monitored by recording the UV spectra at different time intervals. The absorbance of $\lambda_{max}$ of I at 256 nm after electrolysis at $pH$ 7.3 showed progressive decrease with time whereas the absorbance in the region 280 to 340 nm increased. After two-hour electrolysis another shoulder at around 250 nm appeared. Similarly the absorbance of $\lambda_{max}$ at 270 nm at $pH$ 3.6 also showed progressive decrease with time whereas the absorbances in the regions of 290 to 340 nm and 220 to 250 nm registered increases. At the end of electrolysis at $pH$ 3.6 the solution exhibited a broad band at 240 nm. These observations indicated that the products of electrooxidation of sulphamethoxazole (I) at $pH$s 3.6 and 7.3 are different.

**Product characterization**

For the identification of the products at $pH$s 3.6 and 7.3, the electrolysed solution of sulphamethoxazole was lyopholised. The TLC of lyopholised solution of $pH$ 7.3 exhibited a single spot indicating the formation of only one product during electrooxidation. The freeze-dried material obtained from GLC was analysed by IR, PMR and mass spectra.

Sulphamethoxazole (I) exhibits $\nuNH_2$ in the region of 3400-3120 cm$^{-1}$ besides other sharp bands at 680, 830, 1150, 1310 and 1480 cm$^{-1}$. The product of oxidation at $pH$ 7.3 did not exhibit the $\nuNH_2$, indicating that oxidation of I occurred at the amino group. Further, a strong band at 1610 cm$^{-1}$ indicated the presence of azo group in the product. The IR spectrum was superimposable over that of the authentic azo compound.

The PMR spectrum of sulphamethoxazole displayed signals at: 82.29 (−$CH_3$), 4.57 (−$NH_2$), 6.11 (−$CH=$), 6.63 and 7.66 (aromatic proton). The PMR spectrum of the product did not exhibit any signal for amino group suggesting the disappearance of amino group in the product. Finally, the azo structure of the product was confirmed by the molecular ion peak at $m/z$ 502 in its mass spectrum.

The formation of an azo compound as a product was further supported by the cyclic voltammograms of authentic azo compound at different $pH$ values, which were similar to those of the products. The UV spectrum of the authentic azo compound was similar to that of the exhaustively electrolysed solution of I at $pH$ 7.3.

The exhaustively electrolysed solution of I at $pH$ 3.6 on the other hand, exhibited two spots in TLC ($R_f$ 0.27 and 0.48), indicating the presence of two products. One of the products, corresponding to $R_f$ value 0.48 was found to be similar to the azo product obtained at $pH$ 7.3. The other product ($R_f$ 0.27) could not be characterized due to nonavailability of an authentic sample.

**Redox mechanism**

The evidences presented above clearly indicate that electrooxidation of sulphamethoxazole (I) involves a two-electron process. In order to account for the overall mechanism it seems reasonable to assume that $1e, 1H^+$ oxidation of sulphamethoxazole (I) gives a free radical species (II), which can be deactivated by a number of ways. Species (II) may further undergo $1e, 1H^+$ oxidation to give nitrene (III) which on dimerization results in the formation of an azo compound...
confirmed in the present studies by determining the slope of $E_p$ versus log $V$ plot. Such a plot is linear (Fig. 3) with a slope of 20 mV. Experimental slope and the theoretical value corresponding to free radical dimerization as suggested by Andrieux et al.\textsuperscript{9,10} were the same. It is, therefore, concluded that oxidation of sulphamethoxazole (I) proceeds through electrodimerization to give an azo product at the pyrolytic graphite electrode.

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References