Elucidation of Redox Behaviour of Ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone by Polarographic and Voltammetric Techniques

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The electrochemical behaviour of ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone has been studied in a wide range of pH by polarography, linear and cyclic sweep voltammetry, coulometry and spectral studies. The reduction of this compound takes place in a single 4e pH dependent irreversible step. In cyclic voltammetry one anodic peak is also observed, when the sweep was initiated towards positive potentials indicating thereby the possible electrooxidation of this compound. The products of electrolysis have been characterisded by tlc and gc.

ARYLHYDRAZONES have found wide application in the synthetic organic chemistry for the preparation of varieties of physiologically active compounds[1-4]. In analytical chemistry hydrazones have been used for identification and detection of large number of metal cations[5]. As from a polarographic point of view semicarbazones resemble the structurally related arylhydrazones, a large number of electrochemical investigations have been carried out on arylhydrazones and semicarbazones[6-8]. Nevertheless, most of these investigations were restricted to the polarography at dropping mercury electrode and no information is available on their behaviour at solid electrodes. For the last several years we are investigating the polarographic behaviour of biologically important compounds, however, most of our investigations were restricted to polarographic behaviour in want of other sophesticated instruments[9-11] and hence, we were unable to identify intermediates and products of electroreduction. In view of the great importance of hydrazones and semicarbazones, we have selected ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone, which possesses two polarographically reducible sites, viz. hydrazono and semicarbazono (1) to study its electrochemical behaviour at dropping mercury electrode and at rough pyrolytic graphite electrode (RPGE) using polarography, linear and cyclic sweep voltammetry and coulometry.

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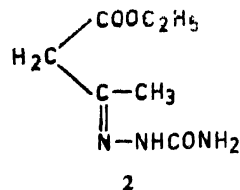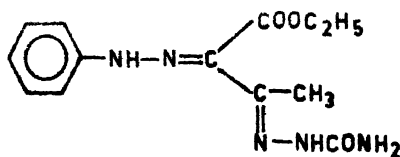
We initiated this investigation in the expection that a comparison of compound (1) with ethyl-3-oxobutyratesemicarbazone (2) and the mechanism observed electrochemically and products of electroreduction might throw some light on the biological redox reaction of this molecule. This paper describes our observations on the electrochemical behaviour of compound (1) using varieties of techniques. The products of electroreduction have been separated and identified by tlc and gas chromatography.

Experimental

Ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone was synthesised by the method reported in literature[12]. The stock solution of this compound (1 mM) was prepared in dimethylformamide (A.R., H_2O, 0.1%). Phosphate buffer[13] of ionic strength 0.5 M were prepared from reagent grade chemical in the pH range 2.2-11.8.

Polarographic curves were recorded on a Cambridge pen recording polarograph and capillary characteristics were 1.45 mg$^{2/3}$ sec$^{-1/2}$. Each curve was recorded at least twice to ensure reproducibility. Valtammograms were recorded at rough pyrolytic graphite electrode using Micronics cyclic voltam-

meter. The graphite electrode was rubbed gently on sand paper and washed with distilled water after each run. Controlled potentials during large scale electrolysis were applied with the help of a potentiostat[14] using platinum gauge as counter electrode. The characterisation of the intermediate and the product of electrode reaction were done by using uv-vis Specord spectrophotometer (C. Zeiss, Jena) and Aimil Nucon 5700 gas chromatograph equipped with a column of 3% SE-30 on chromosorb W and flame ionisation detector. The temperature of injector and detector were fixed at 190° and the column temperature was raised at a rate of 6° min⁻¹ from 80' until the final temperature 200° was reached.

Results

In the pH range 2.2-11.8, ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone reduced in a single, 4e, pH dependent step at dropping mercury electrode. To confirm whether this polarographic wave is due to the reduction of hydrazono (NH−N=C) group or semicarbazono group or a combined wave of both, a separate polarogram of ethyl-3-oxobutyratesemicarbazone (2) was also recorded in phosphate buffer of different pH. It was interesting to observe that this compound does not exhibit any wave indicating thereby that the wave is due to the reduction of hydrazono group of the molecule. It has been further reported that the reduction of hydrazono group is easier than semicarbazono group[16]. The limiting current was independent of pH and indicated a linear relation with $\sqrt{h}$ and concentration of depolariser (0.1 to 1.0 mM). The value of temperature coefficient was 1.2% deg⁻¹. All these observations indicated the diffusion-controlled nature of the electrode process. The half-wave potential was dependent on pH and shifted linearly towards more negative potential with increase in pH by 63 mV/pH. The value of P, number of protons, involved in the rate determining step together with other polarographic data is given in Table 1.

Linear and cyclic sweep voltammetry of compound 1, however, indicated an entirely different behaviour at rough pyrolytic graphite electrode. In cyclic voltammetry at scan speed of 50 mV s⁻¹ one well defined cathodic peak Ic is observed (Fig. 1).
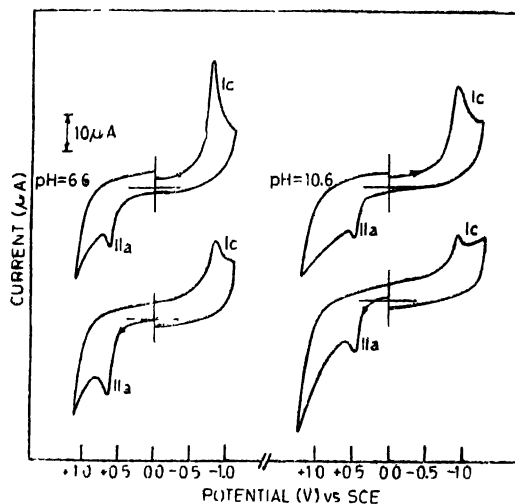

Fig. 1. Cyclic voltammograms of 0.1 mM ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone at a sweep rate of 200 mV s⁻¹. Upper curves are initiated towards negative direction and lower curves towards positive direction.

In the reverse sweep one well defined anodic peak (IIa) is also observed. The potentials of peak Ic and IIa were pH dependent and observed the relation.

$$-E_p(Ic) = [0.055\ pH + 0.38]V$$
$$E_p(IIa) = [0.970 - 0.052\ pH]V$$

Peak Ic and IIa were observed at a slow scan rate of 5mV s⁻¹ also. If the direction of sweep is initiated in the positive direction first (Fig. 1), peak IIa was observed very clearly and peak Ic had a tendency to become broad, indicating thereby that the electrode process for peak Ic and IIa are independent process and not related to one another. To decide whether the peak Ic is due to the reduction of hydrazono or semicarbazono group, cyclic voltammograms of ethyl-3-oxobutyratesemicarbazone (2) were also recorded at RPGE in phosphate buffers of various pH and it was found that this compound does not give peak Ic or IIa. This observation clearly reflects that peak Ic and IIa processes are due to reduction/oxidation of hydrazono group.

The value of n, number of electrons involved in the electroreduction of ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone at mercury pool cathode or at rough pyrolytic graphite indicated that 4.0 ± 0.2 (average ± maximum deviation) electrons are involved (Table 2). The progress of electrolysis was monitored by recording cyclic voltammograms at various intervals of times. A cyclic voltammogram of completely reduced solution did not exhibit any peak and thus indicated that no electroactive

TABLE 1—POLAROGRAPHIC AND VOLTAMMETRIC CHARACTERISTICS OF 0.1 mM ETHYL-2,3-DIOXOBUTYRATE-2-PHENYLHYDRAZONO-3-SEMICARBAZONE AT DIFFERENT pH

pH[a]	Polarographic characteristics			Voltammetric characteristics[b]	
	$-E_{1/2}$	I_d	P	$-E_p$ (Ic)	E_p (IIa)
	V	μA		V	V
2.2	0.34	0.95	0.95	0.50	0.85
3.0	0.40	0.95	1.01	0.54	0.82
3.6	0.44	0.90	0.98	0.55	0.80
4.6	0.50	0.90	0.97	0.60	0.75
5.7	0.56	0.85	1.02	0.67	0.68
6.6	0.60	0.90	1.01	0.72	0.65
7.2	0.66	0.95	1.10	0.77	0.58
8.6	0.74	0.85	1.08	0.82	0.51
9.2	0.84	0.85	0.99	0.86	0.48
10.6	0.90	0.90	0.89	0.93	0.44
11.2	0.95	0.85	0.98	0.97	0.38

[a] Phosphate buffers of μ = 0.5 M.
[b] Sweep rate 50 mV s⁻¹ and area of electrode 0.6 cm².

species remained in the solution after complete electroreduction.

TABLE 2—COULOMETRIC n VALUES OBSERVED FOR ELECTROREDUCTION AND ELECTROOXIDATION OF ETHYL-2,3-DIOXOBUTYRATE-2-PHENYLHYDRAZONO-3-SEMICARBAZONE AT DIFFERENT ELECTRODES

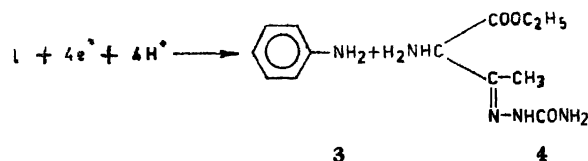| pH[a]	Concn. mM	Potential V	Experimental n value
Mercury-pool cathode			
3.0	0 8	−0.8	4.12
5.7	0.5	−1.0	3.98
7.2	1.0	−1.0	3.87
9.2	1.0	−1.1	4.08
Pyrolytic graphite[b]			
3.0	0.5	−0.8	4.16
5.7	1.0	−1.0	4.09
7.2	1.0	−1.0	3.81
9.2	0.8	−1.1	3.94
3.0	1.0	+1.0	0.98
5.7	1.0	+0.8	0.83
7.2	1.0	+0.8	0.92
9.2	1.0	+0.7	0.92

[a] Phosphate buffers of $\mu = 0.5$ M. [b] Area of electrode 4.5 cm^2.

The electro-oxidation of compound 1 at potential more positive than peak IIa at RPGE, indicated that close to one electron is involved in the oxidation of this compound (Table 2). Complete oxidised solution also did not exhibit any peak in cyclic voltammetry in buffers of different pH.

Characterisation of intermediate and products : For characterisation 3.0-4.0 mg of the compound was reduced at potentials more negative to peak Ic or at plateau potential. The change in spectra in the region 330-600 nm was monitored. A spectra of compound 1 before electrolysis is presented by curve 1 in Fig. 2A. It was observed that with progress of electrolysis the λ_{max} at 390 nm start decreasing systematically. The curves 2 to 9 were recorded at various intervals of time. After 100 min of electrolysis curve 10 was recorded and it was observed that the peak at 390 nm and shoulder at

420 nm disappeared completely. Thus the products of electrolysis does not absorb in the region 330-600 nm. As during electroreduction absorbance did not increase at higher or lower wave length (Fig. 2.) it was obvious that no intermediate capable of absorbing at shorter or longer wave length is generated. It was observed that if potential is turned off after curve 7 no change in spectra can be observed (Fig. 2B). Thus no intermediate with sufficient half-life was produced during the electro-reduction of compound 1. The electrolysed solution was concentrated and the phosphate was removed by extracting the slurry with solvent ether. The tlc of the ether extract in chloroform-ethanol (90 : 10) mixture gave two clear spots ($R_f \sim 0.42$ and 0.58) and gas chromatography gave two peaks ($R_t \sim 1.2$ and 1.5 min) (Fig. 3B).

To compare the products observed with possible end products an ether solution of aniline was analysed by tlc and gas chromatography. It was interesting to observe that aniline gave $R_f \sim 0.58$ and $R_t \sim 1.5$ (Fig. 3A), which corresponds to the values observed for electrolysed solution. Thus, aniline (3) was one of the major products of electroreduction. The other product (4) corresponding to $R_f \sim 0.42$ and $R_t \sim 1.2$ min could not be identified completely at this stage. However, a comparison with ethyl-3-oxobutyratesemicarbazone (2) which has one amino group less than the product 4, showed $R_f \sim 0.42$ and $R_t \sim 1.10$ respectively. Thns, the following overall mechanism can be proposed to account for coulometric value of 4.0.

$$1 \;+\; 4e^- \;+\; 4H^+ \;\longrightarrow\; \bigcirc\!\!-\!NH_2 + H_2NHC \begin{matrix} \diagup COOC_2H_5 \\ \diagdown C-CH_3 \\ \;\;\;\; \| \\ \;\;\;\; N-NHCONH_2 \end{matrix}$$

$$\qquad\qquad\qquad\qquad\qquad\qquad 3 \qquad\qquad 4$$

A similar mechanism has also been proposed by a large number of workers[16-18] for the electro-reduction of hydrazones.
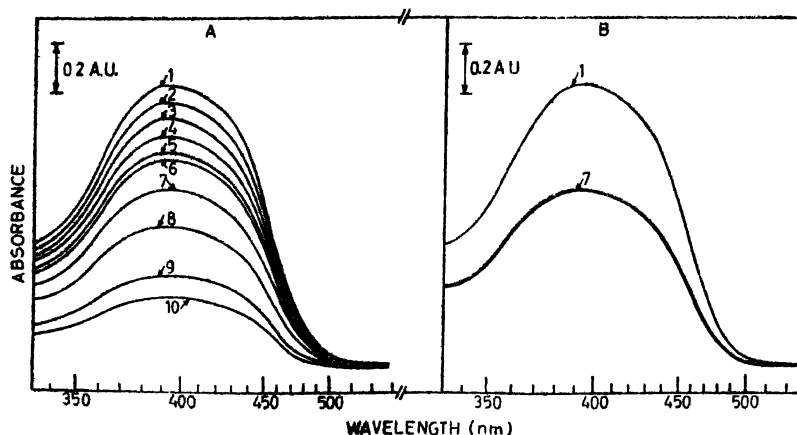

Fig. 2. Spectral changes observed during electroreduction of ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone at pH 7.0, applied potential 1.0 V at RPGE. (A) curves were recorded at (1) 0, (2) 5, (3) 10, (4) 20, (5) 30, (6) 35, (7) 50, (8) 65, (9) 75, (10) 100 min of electrolysis, (B) Electrolysis was stopped after 50 min corresponding to curve 7.
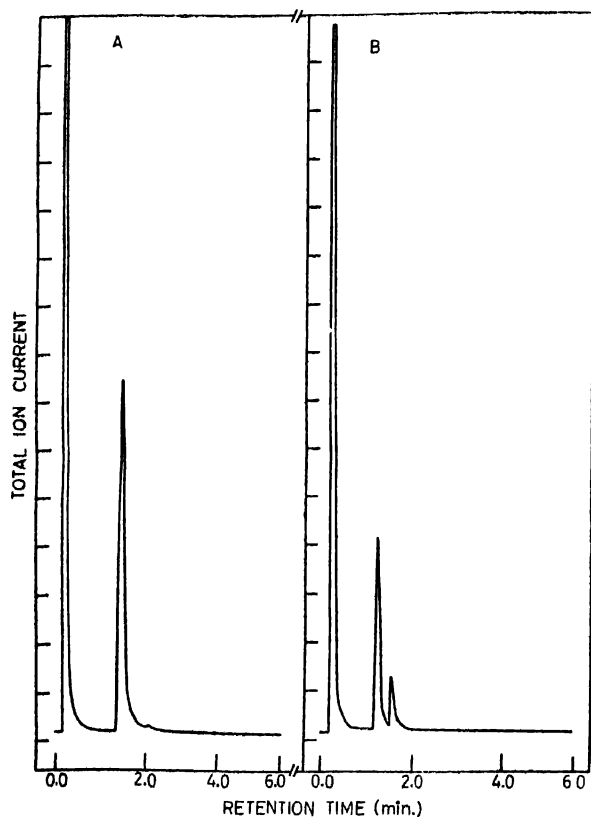
Fig. 3. A comparision of gas chromatogram of aniline (A) with ether extract of electroreduction products (B).

For the characterisation of intermediate and products during electrooxidation at RPGE, a procedure similar to electroreduction was applied. It was interesting to observe that oxidation at potential more positive to peak IIa also indicated a systematic change in the spectra in the range 330-600 cm^{-1} as have been observed for electroreduction (Fig. 2). The ether extract of the electroxidised solution gave only one spot in the tlc ($R_f \sim 0.4$) and did not exhibit any peak in gas chromatography, probably due to its non-volatile nature. The overall electrooxidation product based on one electron transfer will lead to the formation of a dimer. A similar mechanism for the autooxidation of hydrazones to give dimer have also been proposed by Pausacker[19] and Stanley[20].

Discussion

The evidence thus presented clearly indicates that in ethyl-2,3-dioxobutyrate-2-phenylhydrazono-3-semicarbazone reduction of hydrazono group occurs and semicarbazono group does not undergo reduction under these conditions. As the slope of $dE_{1/2}/dpH$ was practically similar to that of $\Delta E_p/dpH$, it is evident that the electroreduction is taking place by the same mechanism at dropping mercury electrode and at rough pyrolytic graphite electrode. A little difference of 8 mV observed may be due to

the different nature of the two electrodes. The observed spectral change during electroreduction clearly indicates that π chromophore system does not increase or decrease extensively and hence absorbance simply shows a systematic change. No change in absorbance after turning off the reduction potential, indicate that no intermediate capable of absorbing in the region 330-600 cm^{-1} is formed. A comparison with authentic aniline confirmed the mechanism of electroreduction.

The appearance of oxidation peak (IIa) in cyclic voltammetry clearly indicates that compound 1 can also undergo electrooxidation. Since most of the earlier observations have been carried out at dropping mercury electrode, this oxidation behaviour has not been observed earlier. As the number of electrons involved in the oxidation has been found as one, a mechanism similar to autooxidation of hydrazone is also suggested for the compounds. A detailed investigation on the electrooxidation of this compound is in progress.

Acknowledgement

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