Electrochemical investigations on some potential antibacterials, I

Rajeev Jain and M. Damodharan

Abstract: Electrochemical behaviour of the medicinally important 4-(4′-sulphonamoyl)hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones has been studied at d.m.e. and glassy carbon electrodes. At d.m.e., all six compounds exhibited a single, well-defined, four-electron wave in the pH range 2.5–12.0. Polarographic four-electron wave was found to be diffusion-controlled and irreversible. Similarly, cyclic voltammetry of these compounds at glassy carbon electrode exhibited a single peak. Peak potential shows shifts towards negative potential with pH, with linear segments up to pH 8.2 and are practically pH independent at higher pH values. An anodic peak at far-off positive potential was observed in the reverse scan, indicating the irreversible nature of the electrode process. Controlled potential electrolysis and coulometric studies gave the value of n as 4.0 ± 0.1 in the pH range 2.5 to 10.0. Out of the two major end products formed, one was identified as sulphanilamide and the other as 1-phenyl-3-methyl-4-amino-2-pyrazolin-5-one on the basis of IR and NMR studies. On the basis of DCP, LSV, CV, CPE, coulometry and spectral analysis, a mechanism has been postulated for the reduction of these compounds at d.m.e. and glassy carbon electrodes.

Key words: polarography, cyclic voltammetry, antibacterials, pyrazolin-5-ones.

Résumé : On a étudié le comportement électrochimique de 4-(4′-sulfonamoyl)hydrazono-1-phényl-3-méthyl-2-pyrazolin-5-ones au niveau d'électrodes d.m.e. et de carbone vitreux. Avec les électrodes d.m.e., à des pH allant de 2,5 à 12,0, les six composés choisis présentent tous une seule vague, bien définie, de quatre électrons. On a établi que cette vague polarographique est contrôlée par la diffusion et qu'elle est irréversible. De la même manière, la voltampérométrie cyclique de ces composés au niveau d'une électrode de carbone vitreux présente un seul pic. Le potentiel du pic présente un déplacement vers le potentiel négatif avec une variation du pH; les segments sont linéaires à des pH allant jusqu'à 8,2 et ils sont pratiquement indépendants du pH à des valeurs de pH plus élevées. Lors du balayage inverse, on a observé un pic anodique à un potentiel extrêmement positif; ceci suggère une nature irréversible pour le processus. Une électrolyse à potentiel contrôlé et des études coulométriques indiquent que, à des pH allant de 2,5 à 10,0, la valeur de n est égale à 4,0 ± 0,1. En se basant sur des études de rmn et d'ir, on a identifié les deux products formés au cours des réactions; l'un est le sulfanilamide et l'autre est la 1-phényl-3-méthyl-4-amino-2-pyrazolin-5-one. En se basant sur les DCP, LSV, CV, CPE, la coulométrie et les analyses spectrales, on propose une mécanisme pour la réduction de ces composés aux électrodes de d.m.e. et de carbone vitreux.

Mots cles : polarographie, voltampérométrie cyclique, antibactériens, pyrazolin-5-ones.
[Traduit par la rédaction]

A review (1, 2) of the electrochemistry of some biologically important organic molecules illustrates very well how modern electrochemical techniques can provide biologically significant information. Electrochemical studies of hydrazones have attracted many workers (3–7) because of their medicinal importance (8, 9). The reduction of hydrazones has been reviewed in detail by Turyan (10). Fundamental contribution in this field has been made by Lund (11) and Kitajev et al. (12–14). The reduction mechanism has been reviewed by Kitajev and Buzykin (15). A four-electron reduction is usually observed in an acidic solution (11–14) involving breaking the N—N bond during the reduction. More recently a four-electron reduction of the hydrazones has been reported (16–18) in acidic as well as in alkaline media with the formation of amino compounds. On the other hand, Zuhri et al. (5) have found a two-electron reduction followed by a hydrolysis that resulted in the formation of the corresponding carbonyl compound. Hydrolysis of the reduced product has also been observed by Lund (7) for similar hydrazones. However, Fahmy et al. (19) have reported further reduction of the amino compound formed after the four-electron reduction to ammonia and ketone in a two-electron wave. However, only a few reports are available on the electroreduction of hydrazones at solid electrodes (20, 21).

An underlying rationale for our studies on the electrochemical reduction of biologically important *N*-heterocyclic molecules is that such studies can lead to information on the reaction routes and mechanisms of biological redox reactions. One of the reasons to investigate the electrochemical behaviour of biologically important compounds is the development of possible correlation based on the mechanistic paths involved and the associated characteristic potentials; e.g., half-wave potentials are a function of electron density and other factors, which are simply related to some biological,

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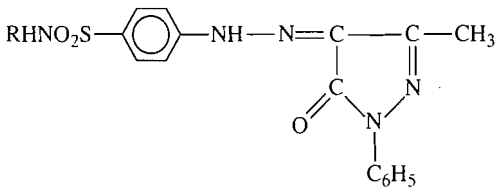
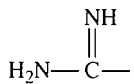
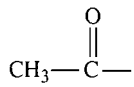
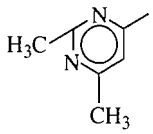
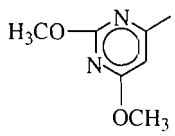
Table 1. Electrochemical characteristics of 4-(4'-sulphonamoyl) hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones, concentration 1.0×10^{-4} M, at pH 6.5.



S.No.	R	$-E_{1/2}$, V	i_d, μA	E_p, V	i_p, μA
1	H—	0.93	1.22	0.93	14.4
2		1.02	1.04	0.95	11.2
3		1.06	1.04	0.98	10.02
4		1.06	1.04	1.04	16.20
5		1.10	1.00	1.05	10.52
6		1.10	1.06	1.00	10.4

clinical, physical, or chemical property and activity. Furthermore, to broaden our knowledge on electrochemistry and to probe the possibilities of electroanalytical assays, we thought it worthwhile to study in a comprehensive manner the electroreduction behaviour of the medicinally important (22) 4-(4'-sulphonamoyl)hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones at d.m.e. and glassy carbon electrodes.

Experimental

Polarographic curves were recorded on an ELICO DC CL 25 polarograph with capillary characteristics $m^{2/3}t^{1/6}$, 3.62 $mg^{2/3}$ $s^{1/2}$, drop time 3.20 s^{-1} at h = 65 cm in 1.0 M KCl solution (0.0 V SCE). Saturated calomel electrode was used as the reference electrode. Temperature coefficient was determined by Nejedly's (23) method. Triply distilled mercury was used for the d.m.e. All the polarograms were recorded at 298 ± 0.1 K.

Voltammograms were recorded on a cyclic voltammograph (BAS, CV-27) with an *x–y/t* recorder (Digital, Model Omnigraph). A three-electrode system was used, glassy carbon as the working electrode, Ag/AgCl in 1.0 M KCl as the referenced electrode, and a platinum wire as the counter electrode. The GC electrode was polished with fine grade emery paper followed by polishing alumina (0.5 μm), washed, and activated by triangular voltage sweeps from +1.0 to −1.4 V at the rate of 5 and 200 mV/s for 15 min. The activated electrode was tested (24) for its activity in ferricyanide–ferrocyanide in 0.1 M KCl solution.

The compounds listed in Table 1 were prepared by the reported (22) procedure. Britton-Robinson buffers (25) were used throughout the electrochemical study. pH of the solutions were measured with a glass electrode. In a typical electrochemical study, e.g., when studying the influence of pH on the cyclic voltammetric behaviour, 1.0×10^{-4} M solutions of these compounds were prepared by mixing 1.0 mL compound (1.0×10^{-3} M in DMF), 3.0 mL DMF (which was necessary to keep the compound in solution), 5.0 mL appropriate buffer, and 1.0 mL (1.0 M) KCl as the supporting electrolyte. Polarograms and voltammograms were recorded after removal of oxygen.

As the products of controlled potential electrolysis (CPE) have been known to differ depending on the nature of the electrode employed (26), controlled potential electrolysis (CPE) was done at d.m.e. and GC electrodes in a microcell (27). For coulometric determination (27) of the number of electrons transferred in the electrode process 2.0 mL of the solution of the depolarizer (1.0×10^{-3}) having 4.0 mL of buffer, 3.0 mL of dimethyl formamide, and 1.0 mL of potassium chloride was electrolysed by applying a potential corresponding to the limiting current of the polarographic wave. From the decrease in current with time during electrolysis, the number of electrons transferred was calculated. Progress of the electrolysis was monitored by recording the polarograms at different intervals of time, until only the background current is attained. Coulometry was done at the GC electrode, using a BAS CV-27 coulometer, the value of Q was directly read, and by applying

$$Q = nFN$$

n was calculated; here N is the number of moles.

Results and discussion

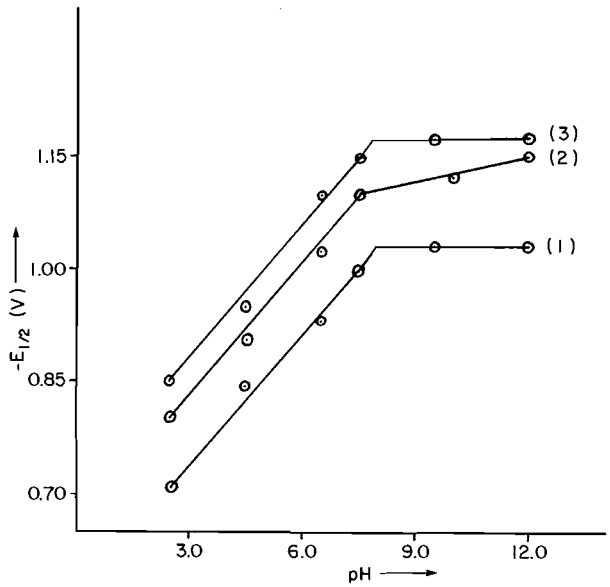
Polarographic behaviour

All 4-(4'-sulphonamoyl)hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones (Table 1) exhibited a single, well-defined, four-electron wave in the pH range 2.5–12.0. A comparison of limiting currents of different pyrazolin-5-ones indicates that most of the compounds studied are reduced by the same number of electrons. The nature of the wave was established by recording the polarograms at various heights of mercury reservoir (55 to 95 cm) as well as by studying the effect of concentration ($(1.0–5.0) \times 10^{-4}$ M) on the limiting current. It was observed that the limiting current is directly proportional to the square root of the mercury reservoir height and the concentration of the depolarizer. These facts confirmed that the waves are controlled by diffusion (28). The half-wave potentials were dependent on pH and shifted towards more negative potentials with an increase in pH. The plots of $E_{1/2}$ vs. pH were (Fig. 1) linear up to 8.2 and after that there was a little change in $E_{1/2}$.

Cyclic voltammetry

Cyclic voltammograms of 4-(4'-sulphonamoyl)hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones in buffer solutions of pH 2.5–12.0, at different scan rates (50–200 mV/s) and concen-

Fig. 1. Plot of $-E_{1/2}$ vs. pH (*1*) 4-[4′-sulphonamoyl]-, (*2*) 4-[4′-guanylsulphonamoyl]-, (*3*) 4-[4′-(4″,6″-dimethylpyrimidinyl)sulphonamoyl]hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-ones at concentration 1.0×10^{-1} M.



trations ((1.0–5.0) $\times 10^{-4}$ M), exhibited not very well-defined, single four-electron peaks. Some typical voltammograms are shown in Fig. 2. This behaviour is in agreement with that observed from polarographic measurements. Peak potentials show shifts towards negative potential with pH, with linear segments up to pH 8.2, and are practically pH independent at higher pH values. An anodic peak at far off positive potential was observed in the reverse scan indicating the irreversible (29) nature of the electrode process. The values of peak potential and peak currents are given in Table 1.

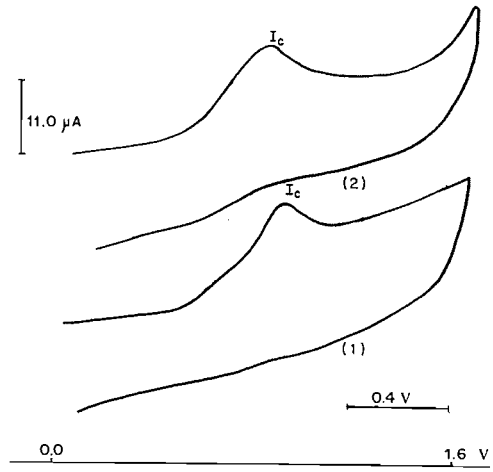
Coulometry and product analysis

Controlled potential electrolysis of these compounds at the plateau potential (−1.0 V) using the mercury pool cathode consumed 4.0 ± 0.2 electrons. The dark yellow colour of the starting solution almost disappeared at the end of the electrolysis. Coulometric studies at slightly more negative potential than the peak potential I_c, using GC electrodes, also gave the value of n as 4.0 ± 0.1 in the pH range 2.5–10.0. A cyclic voltammogram of the solution after exhaustive electrolysis did not exhibit any reduction peak. To characterise the major end products, electrolysis was carried out at pH 2.5, 6.5, and 10.0. The electrolysis resulted in two products on separation by TLC. One of the products was identified as sulphanilamide by comparison with the authentic sample. The other product was identified as 1-phenyl-3-methyl-4-amino-2-pyrazolin-5-one on the basis of IR and NMR studies (ν_{max}^{Nuzol} 3320 (NH_2), 1665 (C=O cyclic), 1600 (C=N), 1650 (heterocyclic five-membered 2-pyrazolin-5-one), δ ($CDCl_3$): 2.1 (1H, CH), 3.17 (3H, CH_3), 4.2 (2H, NH_2), and 7.0–8.0 (5H′, C_6H_5)).

Mechanism

Keeping in view the results of DCP, LSV, CV, CPE, and coulometry, the scheme on the facing page may be proposed for the reduction of these compounds at d.m.e. and GC electrodes.

Fig. 2. Cyclic voltammograms of 4-[4′-sulphonamoyl]hydrazono-1-phenyl-3-methyl-2-pyrazolin-5-one at (*1*) pH 4.5, (*2*) pH 2.5; concentration 1.0×10^{-4} M.



Alternatively, the radical (**I**) could also combine with an electron instead of a proton, but this possibility is ruled out by the fact that such a reduction would involve only two electrons, whereas polarographic data clearly indicate a four electron transfer reaction at the d.m.e.

The above mechanism is supported by a shift of $E_{1/2}$ towards more negative values with pH as protons are consumed in the reduction. As the equilibrium shifts towards the unprotonated form, the $E_{1/2}$ tends to become constant. Dependence of $E_{1/2}$ on pH is described by the equation (28):

$$E_{1/2} = \text{constant} + \frac{RT}{\alpha nF} \ln\left[1 + \frac{[H^+]}{K_a}\right]$$

The plot of $E_{1/2}$ vs. pH is composed of two intersecting linear segments and the pH at the point of intersection of its two linear parts is $\approx pK_1$. Similar steps of the reduction of the hydrazono group involving cleavage of the —N—N— bond have also been proposed by other workers (30–33). Due to an acid–base equilibrium between the structural forms of the compound (NH—N=C— ⇌ NH—NH=C—),[+] the wave height remains pH independent as long as the formation of the acidic form from the basic form is fast enough. As the pH increases and the rate of the protonation decreases, the wave height decreases as well. Ultraviolet–visible spectra of the solution during electrolysis were recorded at different time intervals; at 425 nm the peak due to —NH—N=C— grouping showed a decrease in the peak height after each interval of time and finally the peak disappeared, confirming the above reduction mechanism. The value of P is determined in the usual manner (28).

It was observed that the half-wave potential shifted to more negative potential (34) with increasing concentration of DMF, whereas the limiting current decreases.

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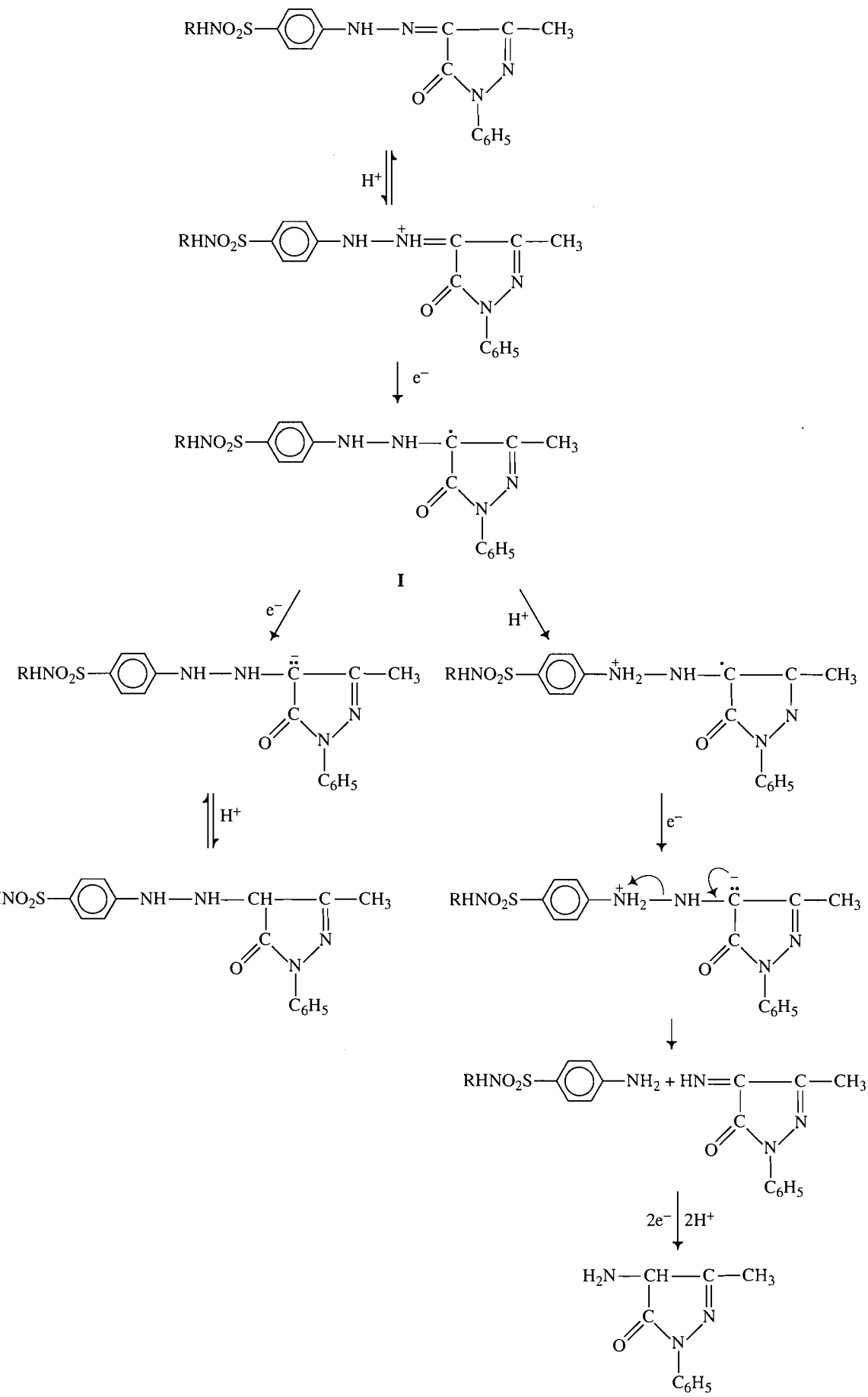

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