

POLAROGRAPHY OF 5-NITRO-OROTIC ACID*

S. L. GUPTA, N. KISHORE and P. S. RAGHAVAN

Chemistry Department, Birla Institute of Technology & Science, Pilani,
Rajasthan, India

Abstract—Polarography of 5-nitro-orotic acid in aqueous medium has been carried out in the pH range 1–10, using different buffer systems. There are two well-defined steps up to pH 9.0; the first step is purely diffusion-controlled (6 e) reduction at all pH values, and the second step (4 e) is purely diffusion-controlled in the acidic range and shows adsorption characteristics in the alkaline. Above pH 9, the compound is reduced in three steps: the first is purely diffusion-controlled (4 e); the second (4 e) and third (2 e) steps have adsorption character. At pH > 11, the reduction involves three steps, but the wave heights are not reproducible. The number of electrons involved in the diffusion-controlled steps was determined by comparing the wave heights with that of the first step of nitrobenzene reduction under identical conditions, which was confirmed by finding the diffusion coefficient D with the help of the McBain–Dowson cell. Kinetic parameters (αn_a and $-\log k^\circ$) have been computed for the diffusion-controlled steps, using Koutecky's method. The probable reduction mechanism is discussed.

Résumé—Polarographie en milieu aqueux de l'acide 5-nitro-orotique, dans un domaine de pH compris entre 1 et 10 et dans différents systèmes tampons. Il y a deux étapes bien définies jusqu'à pH = 9,0; la première est une réduction (6 e) purement contrôlée par la diffusion quelque soit le pH, et la seconde (4 e) l'est également dans le domaine acide, mais révèle les caractéristiques de l'adsorption dans la zone alcaline. Au dessus de pH 9 le composé est réduit en trois étapes: la première est purement contrôlée par la diffusion (4 e) la seconde (4 e) et la troisième (2 e) ont un caractère d'adsorption. A pH > 11, la réduction se fait aussi en trois étapes, mais les hauteurs de vague ne sont pas reproductibles. Le nombre d'électrons impliqués dans les étapes contrôlées par la diffusion est déterminé en comparant les hauteurs de vague à celle du premier palier de la réduction du nitrobenzène dans les mêmes conditions; il a été confirmé par la détermination du coefficient de diffusion D à l'aide de la cellule de McBain-Dowson. Les paramètres cinétiques (αn_a et $\log k^\circ$) ont été calculés, lorsque la diffusion est régulatrice, par la méthode de Koutecky et le mécanisme probable de la réduction est discuté.

Zusammenfassung—5-Nitro-6-Uracilcarbonsäure wurde in wässriger Lösung verschiedener Puffer (pH 1–10) polarographiert. Unterhalb pH 9,0 findet man zwei definierte Wellen, die erste ist diffusionskontrolliert (6 e), die zweite Stufe (4 e) ist im sauren Gebiet diffusionskontrolliert, im alkalischen Bereich weist sie Adsorptionscharakteristiken auf. Oberhalb pH 9 wird die Verbindung in 3 Stufen reduziert: Die erste ist rein diffusionskontrolliert (4 e), die zweite (4 e) und die dritte (2 e) haben Adsorptionscharakter. Oberhalb pH 11 fand man drei Stufen, deren Höhe jedoch nicht reproduzierbar war. Die Anzahl Elektronen in den diffusionskontrollierten Stufen wurde durch Vergleich der Stufenhöhen mit derjenigen der ersten Stufe der Reduktion von Nitrobenzol unter identischen Bedingungen erhalten, dies wurde durch Ermittlung des Diffusionskoeffizienten mit einer McBain/Dowson-Zelle bestätigt. Die kinetischen Parameter der diffusionskontrollierten Stufen wurden nach der Methode von Koutecky bestimmt. Ein wahrscheinlicher Reduktionsmechanismus wird diskutiert.

KAPOOR and Jain,¹ in polarographic studies of some nitropyrimidines, reported that 5-nitro-orotic acid undergoes a single step (6 e) reduction between pH 7 and 9 in Britton–Robinson buffers and that above pH 9, the reduction process splits into two waves, one corresponding to 4 e followed by another wave of 2 e. They explained this as due to the retardation of the reduction of 5-nitro-orotic acid, possibly owing to the negative charge of the ionized carboxyl group.

While studying the effect of surface-active substances on the reduction of the nitro group in certain aromatic nitro-compounds, we observed two well-defined steps for the reduction of 5-nitro-orotic acid at pH 8, in the absence of surfactants. This led

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us to repeat the polarographic study of 5-nitro-orotic acid, the results of which are now reported.

EXPERIMENTAL TECHNIQUE

A stock solution of a purified sample of 5-nitro-orotic acid (potassium salt, anhydrous; Sigma Chemicals Co., U.S.A.) was prepared in redistilled air-free water. AnalaR reagents were used to prepare HCl-KCl, Na_2HPO_4 -citric acid, boric acid-NaOH and Na_2HPO_4 -NaOH buffer systems of constant ionic strength ($\mu = 0.54 \text{ M}$) adjusted by KCl. Triton X-100 (0.0004 to 0.001 %) was used as maximum suppressor. Temperature was maintained with an accuracy of $\pm 0.1^\circ\text{C}$. The capillary used for the dme had $m = 2.931 \text{ mg/s}$, $t = 3.05 \text{ s}$ in 0.54 M KCl (open circuit) at $h = 40.0 \text{ cm}$ (uncorrected for back pressure). The mercury used in the dme was first purified chemically and subsequently distilled under reduced pressure. A manual set-up² was used to record the current/voltage curves.

RESULTS AND DISCUSSIONS

Current/voltage curves at various pH values

5-nitro-orotic acid gave a two-step cathodic wave between pH 1 and 9. Above pH 9 the wave split into three steps (Fig. 1). The half-wave potential ($E_{0.5}$) for both steps shifted to more negative values with increased pH from 1-9. To clarify the nature of these reduction steps, the effect of concentration of the depolarizer and the height of the mercury column on these reduction steps were examined at pH 1, 9 and 10. These observations indicated that the first step (pH 1-10) was purely diffusion-controlled. The second step too was diffusion-controlled in highly acidic medium, but exhibited adsorption characteristics in alkaline medium. The third step at pH 10 also had adsorption characteristics.

The effect of concentration of depolarizer and that of height of mercury column on $E_{0.5}$, as well as the slopes of the linear plots of $\log i/(i_a - i)$ vs E at pH 1, 9 and 10 showed that it was an irreversible reduction.

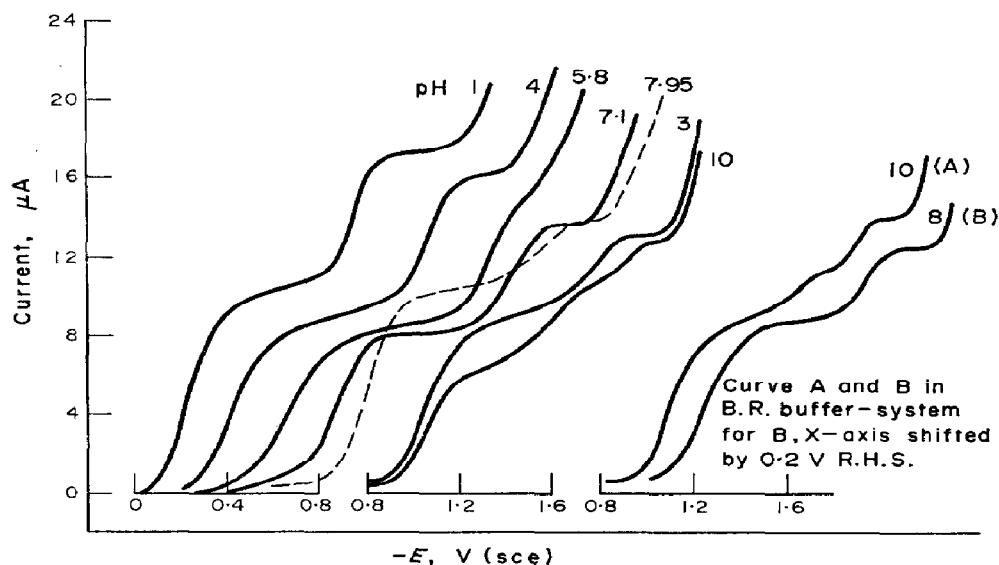


FIG. 1. Current/voltage curves for 0.4 mM depolarizer at various pH values.

The number of electrons involved in the electrode process, at various pH values, was determined by comparing the limiting current values of 5-nitro-orotic acid with those of the known wave (4 e) of nitrobenzene under identical experimental conditions. These were further supported by determining the values of diffusion coefficient D at pH 1, 9 and 10 with the help of the McBain–Dowson cell,³ using the King–Cathcart⁴ equation.

TABLE 1. NUMBER OF ELECTRONS, FROM MCBAIN–DOWSON CELL

pH	$D \times 10^6$ cm ² /s	Step	Number of electrons for diffusion-controlled step
1.0	10.2	First	6.24 \approx 6
		Second	3.93 \approx 4
9.0	9.315	First	5.6 \approx 6
10.0	10.15	First	4.15 \approx 4

From these observations it was concluded that 5-nitro-orotic acid was reduced in two steps up to pH 9, the first step corresponding to a 6-e and the second one to a 4-e process. However, above pH 9, the first and the second steps were 4-e each and third step a 2-e reduction process.

Values of kinetic parameters (αn_a and $-\log k^\circ$) were determined by using Koutecky's methods,⁵ for the diffusion-controlled first step at pH 1–10. The results are tabulated in Table 2. The plots of $-\log k^\circ$ vs pH, and $E_{0.5}^1$ vs pH (pH 1–9) resulted in identical S-shaped curves, whereas, the $E_{0.5}^2$ vs pH (pH 1–9) was a linear plot.

TABLE 2. VALUES OF αn_a AND $-\log k^\circ$ AT VARIOUS pH. 0.4 mM DEPolarizer; 30°C; HEIGHT = 40.0 cms; IONIC STRENGTH OF BUFFER, $\mu = 0.54$ M

pH	i_d μA	$-E_{0.5}$ V(sce)	αn_a	$-\log k^\circ$	Concn. Triton X-100 %
1.0	10.45 ^a 7.50 ^b	0.250 0.928	0.3413 —	2.954 —	0.001
2.0	10.25 ^a 6.60 ^b	0.328 1.052	0.420 —	3.485 —	0.001
3.0	9.40 ^a 6.80 ^b	0.395 1.130	0.353 —	3.7585 —	Nil
4.0	9.2 ^a 6.60 ^b	0.450 1.250	0.2943 —	3.945 —	Nil
5.8	8.3 ^a 6.25 ^b	0.700 1.530	0.2775 —	5.037 —	0.0004
7.1	7.7 ^a 5.6 ^b	0.880 1.60	0.374 —	6.8943 —	0.0004
7.95	10.0 ^a 3.4 ^b	1.010 1.73	0.3812 —	7.732 —	Nil
9.0	8.6 ^a 3.85 ^b	1.080 1.745	0.3458 —	7.721 —	0.001
10.0	6.0 ^a 4.4 ^b 2.1 ^c	1.065 1.560 1.90	0.4165 — —	8.582 — —	0.001

^a, first step; ^b, second step; ^c, third step.

The $-\log k$ vs E plot for the first step (pH 1–10) Fig. 2, and the same plot for the second step (pH 1.0) yielded straight lines, thus showing a single rate-determining step. Values of αn_a showed no regularity, but $-\log k^\circ$ increased with pH, indicating that the electrode process becoming more irreversible with increase in pH. This may be

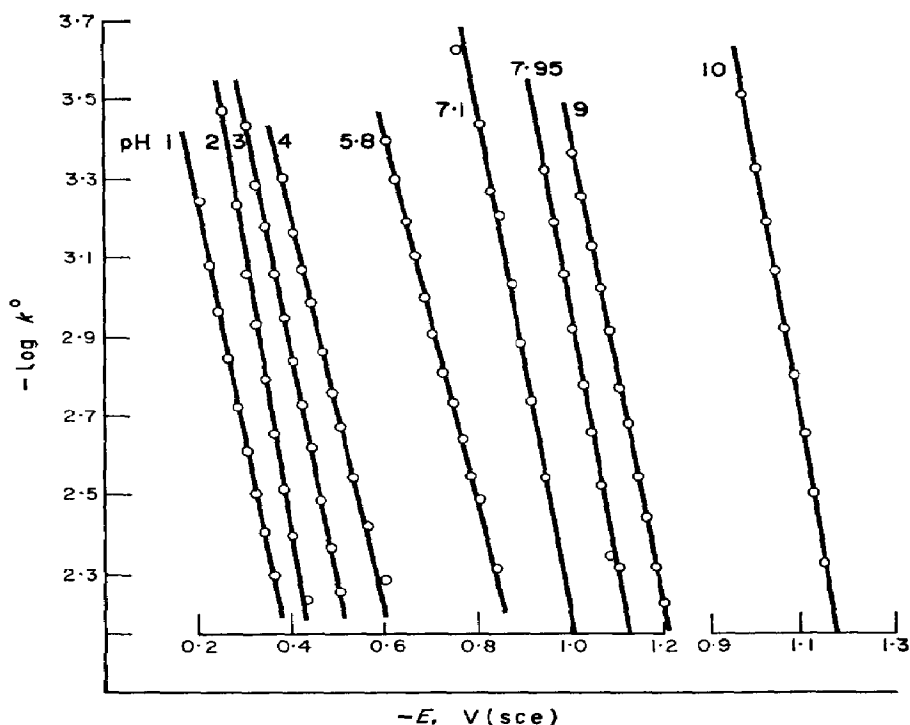
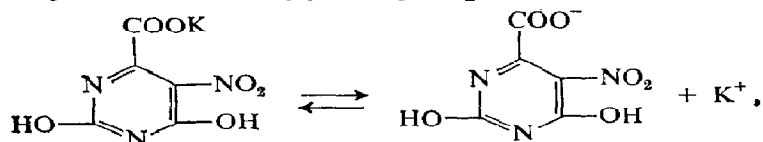


FIG. 2. $-\log k'$ vs E plot for the first step at various pH values.

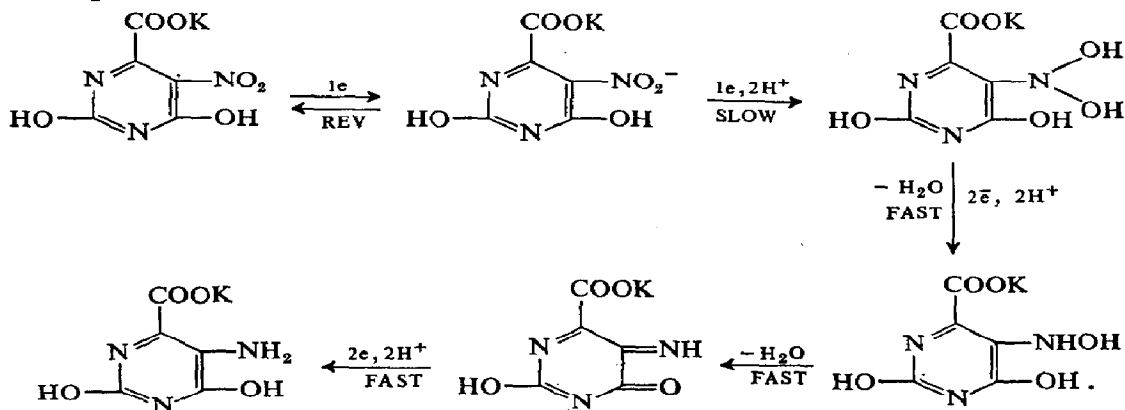
due to the decreased availability of H^+ ions, which play an important role in the reduction process.

The probable reduction mechanism of 5-nitro-orotic acid below pH 9, where two steps (6 e, 4 e) are obtained, is as follows. The equilibrium, given below, may exist below pH 9, involving neutral and singly charged species.

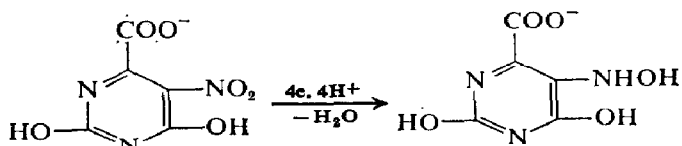


The neutral molecule is reduced at the dme in a single step, 6 e, through the quinonoid form; the singly charged anion then undergoes, 4 e, single-step reduction.

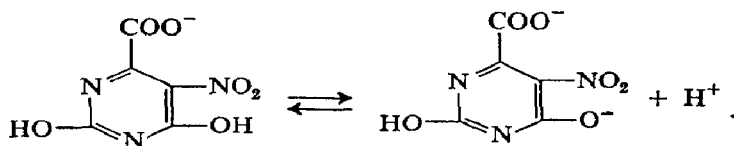
First step, 6 e



Second step, 4 e



However, above pH 9, the species present in equilibrium may be singly- and doubly-charged as shown below:



Here, the singly- and doubly-charged species undergo 4-e reduction leading to the corresponding hydroxylamines, through first and second steps respectively. The third step may correspond to the further reduction of singly-charged hydroxylamine to the amine stage:

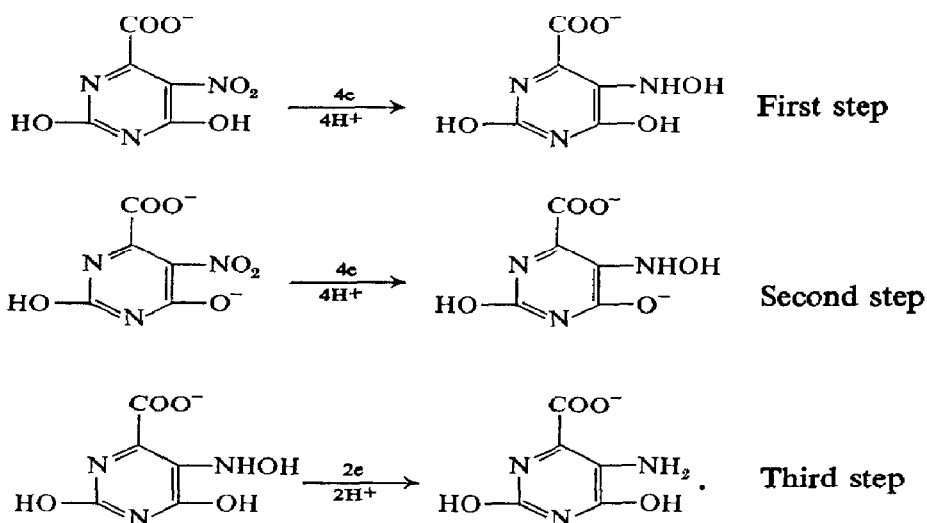


Table 2 shows that the half-wave potentials of the first and the second waves become more negative with increase in pH from 1-9. However, at pH 10, this sequence breaks and the half-wave potentials for the first and the second waves are more positive as compared to values at pH 9. This further shows that the reduction mechanisms at pH 9 and 10 are different and involve different species. The adsorption character of the second wave at pH 9, and the second and the third wave at pH 10, may be due to the formation of a complex between the reduced form of the depolarizer and the mercury, as the complex of 5-amino-orotic is more stable than 5-nitro-orotic acid.¹

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REFERENCES

1. P. C. JAIN and R. C. KAPOOR, *J. Polarogr. Soc.* **14**, 145 (1968).
2. S. L. GUPTA and N. KISHORE, *J. Inst. Chemists, India* **61**, 210 (1969).
3. J. W. MCBAIN and C. R. DOWSON, *Proc. R. Soc. A* **152**, 32 (1935).
4. C. V. KING and W. H. CATHCART, *J. Am. chem. Soc.* **58**, 1639 (1936).
5. J. KOUTECKY, *Colln Czech. chem. Commun.* **18**, 597 (1953); **21**, 836 (1956).