

Polarographic behaviour of orotic acid (vitamin B₁₃). Cathodic processes

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Abstract. The electroanalytical behaviour of orotic acid in cathodic processes has been studied in HClO₄, at several pH values in Britton–Robinson buffers, using DPP and CV techniques.

Orotic acid undergoes three cathodic waves over the entire pH range considered. Optimum conditions for determination of orotic acid using the DPP technique are also studied.

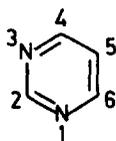
Keywords. Orotic acid (vitamin B₁₃); polarography; voltammetry.

1. Introduction

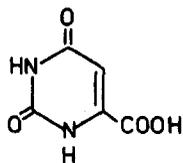
Pyrimidines occur in all living cells and are vitally involved in many biological processes. Like purines (which by themselves can be considered pyrimidine derivatives), they are rarely found in the free state, being rather found as constituents of much larger molecules.

Pyrimidine has a six-membered unsaturated ring containing two heterocyclic nitrogen atoms separated by a single carbon atom. For this reason pyrimidines are occasionally known as *m*-diazines. Structure I illustrates the pyrimidine ring structure and the numbering system that will be employed here.

The spectra, ionization constants, syntheses, theoretical aspects and general chemistry of pyrimidines have been reviewed exhaustively (Neyer and Jacobson 1923; Johnson and Hahn 1933; Marshall and Walher 1951; Janik and Palecek 1953; Brown *et al* 1955; Brown 1962; Manson 1962). It can be seen from these data that in general the oxygen-containing pyrimidines exist predominantly in the keto form. Heath (1923) was probably the first to examine the polarographic reducibility of pyrimidine



Structure I



Structure II

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derivatives. He reported that cytidine, cytidilic acid and uracil are not reducible at the DME in 0.1 M ClO_4 .

Icha (1959) has described orotic acid (structure II) as polarography-active.

Toshio and Soichiro (1975) observed three reduction waves for orotic acid in the pH range 2 to 12, whereas Shizuko and Susumo (1976) indicate two waves in the pH range 1.12 to 8.00 using the DC technique. The present work was carried out in order to study its DP polarography behaviour in detail.

2. Experimental

2.1 Chemicals and solutions

An aqueous solution (2×10^{-3} M) of orotic acid (Sigma) was prepared by dissolving the appropriate amount of acid in distilled water. The solution used for analysis was prepared by dilution of the stock solution to the desired concentrations. All other reagents used were of analytical grade.

2.2 Apparatus

The polarograms were recorded using a Metrohm Herisau E-506 Polarograph. The dropping time was mechanically kept at 1 s. The characteristics of the DME used, in 0.1 M KClO_4 and at 70 cm height of the mercury column, are $t = 2.03$ s and $m = 3.50$ mg s^{-1} . The Ag/AgCl/saturated KCl electrode was utilized as the reference electrode and the auxiliary electrode was a platinum wire (DPP).

A potentiostatic Metrohm E-611, with a scan generator with functions similar to the Metrohm E-612, was used with an HMDE as working electrode (CV).

Experimental variables utilized as optimum values have been the following: temperature = $25 \pm 0.1^\circ\text{C}$, pulse amplitude (ΔE) = -90 mV, scan rate = 4 mV s^{-1} . Purified nitrogen was used to eliminate dissolved oxygen.

3. Results

3.1 The influence of pH

The influence of pH was studied in the interval between 0 and 12, using HClO_4 and Britton–Robinson buffers, and NaClO_4 as support electrolyte.

Figure 1 shows the DP polarograms of the three waves.

Wave I occurs at pH 0.90–4.50 with a constant peak current until pH 3.50. The peak potential, E_p , moves linearly towards more negative values as the pH increases. A slope of 0.108 V/pH unit was obtained.

Wave II is present in the pH range 4.5–7.0 with a maximum peak current at pH 4.5. Its E_p is constant. Above pH 5.0 wave II appears and shows peak current constant at pH > 7.0 .

3.2 The influence of the pulse amplitude

The study of the influence of the pulse amplitude (ΔE) for a solution of orotic acid

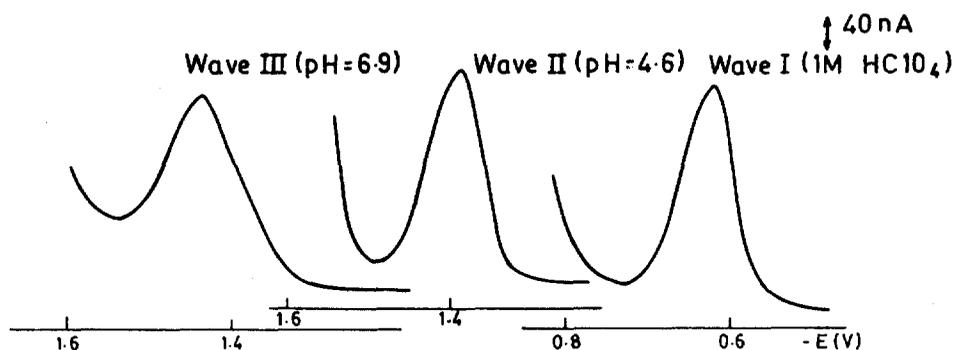


Figure 1. DP polarograms of orotic acid at different pH values. Orotic acid concentration = 1×10^{-5} M, sweep rate = 4 mV/s, $T = 25^\circ\text{C}$, pulse amplitude = -100 mV, drop time = 1 s and $h_{\text{Hg}} = 70$ cm.

(1.5×10^{-5} M) at different pH values [1 M HClO₄ (wave I), pH 5.22 (wave II) and 9.80 (wave III)] has been carried out.

A linear relation between $(\Delta_i)_{\text{max}}$ and (ΔE) was found when ΔE was changed between -10 mV and -100 mV.

3.3 The influence of the drop-time

The effect of the drop-time (t_g) on the $(\Delta_i)_{\text{max}}$ was investigated by recording the polarograms of orotic acid solution (1.5×10^{-5} M) in 1 M HClO₄, at two pH values (5.22 and 9.80).

A linear relation between $(\Delta_i)_{\text{max}}$ and $t_g^{2/3}$ was found for t_g values permitted for the apparatus and capillary used.

3.4 The influence of the temperature

The effect of the temperature on the $(\Delta_i)_{\text{max}}$ was investigated by recording the polarograms of orotic acid solution (1.5×10^{-5} M) in 1 M HClO₄ and at two pH values (5.22 and 9.80). A temperature coefficient of $1.25\%^\circ\text{C}^{-1}$ was obtained.

3.5 Study of the reversibility of the process

In order to check the reversibility of the electrode reactions we have applied the following criteria:

- Birke criterium (Birke *et al* 1981),
- peak width ($W_{1/2}$) vs. ΔE ,
- E_p vs. ΔE ,
- $(\Delta_i)_{\text{max}}$ vs. $(1 + \sigma)/(1 - \sigma)$,

($\sigma = \exp(nFE/RT^2)$).

The application of these four criteria indicate the irreversible nature of electrodic processes.

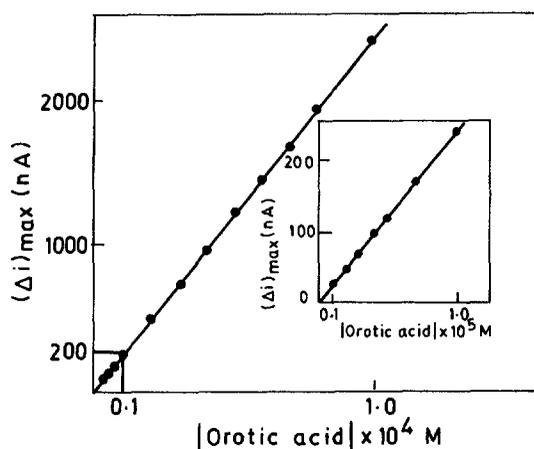


Figure 2. Influence of orotic acid concentration on $(\Delta i)_{\max}$ in DPP (HClO_4 1 M). All other conditions are as in figure 1.

3.6 Effect of the concentration of orotic acid

There was good linearity in the plots of $(\Delta i)_{\max}$ vs. concentration of orotic acid for the three waves, while E_p was independent of the orotic acid concentration.

The best wave for the determination of the reagent is the wave I, which is very well defined. Figure 2 shows the linear range of concentration between 1×10^{-6} M and 1×10^{-4} M ($r = 0.9996$).

The best conditions for the determination of the reagent are: HClO_4 1 M, $\Delta E = -90$ mV; $t_g = 1$ s; $T = 25^\circ\text{C}$. Statistical calculations on several series of eleven identical samples containing orotic acid (5×10^{-6} M) give a relative standard deviation of 3.5%.

3.7 Cyclic voltammetry

The cyclic voltammetric behaviour of orotic acid was studied for the three waves, using HMDE. In all cases, we obtained curves in which only the cathodic peaks appeared, without the corresponding anodic ones, as expected for an irreversible chemical process.

The study of the influence of the scan rate, v , in i_p gave good linearity between i_p and $v^{1/2}$ for the three waves.

4. Discussion

All the processes are diffusion-controlled, as can be deduced from the study of the influence of temperature, the effect of the height of the mercury column and the influence of the concentration.

The effect of experimental variables on the cathodic waves indicates that all the reduction processes involved are irreversible.

In acid media ($\text{pH} < 3.5$) a reduction process (wave I) is obtained, in which two electrons are involved. When the acidity decreases, a change is observed, which can be

related to the dissociation of the protonated acid group (pK_1). Simultaneously, wave II appears, which corresponds to a reduction process that involves the same number of electrons. In basic media, wave III appears, which corresponds to the second dissociation of orotic acid (pK_2).

The three waves clearly observed in DPP may be assigned to the reduction of the double bond C(1) = C(6).

$(\Delta_i)_{\max}$ for the three waves are pH-dependent. The E_p for the waves I and III is pH-dependent.

A new method for the determination of orotic acid in the range of concentration between 1×10^{-6} and 1×10^{-4} M is proposed. For this determination, a concentration of 1 M of HClO_4 is recommended.

References

- Birke R, Kimrad M H and Strassfield M 1981 *Anal. Chem.* **53** 852
Brown D J (ed.) 1962 *The pyrimidines* (New York: Wiley Interscience)
Brown D J, Hoerger E and Mason S F 1955 *J. Chem. Soc.* 1035
Heath J C 1923 *Nature (London)* **158** 23
Icha F 1959 *Pharmazie* **14** 684
Janik B and Palecek E 1953 *Arch. Biochem. Biophys.* **47** 272
Johnson T B and Hahn D A 1933 *Chem. Rev.* **13** 193
Manson S F 1962 in *The pyrimidines* (ed.) D J Brown (New York: Wiley Interscience)
Marshall J R and Walther J 1951 *J. Chem. Soc.* 1004
Neyer V and Jacobson P 1923 *Lehrbuch der Organischen Chemie* (Berlin: Walter de Gruyter and Co.) vol. II, part 3, p. 1172
Shizuko K and Susumo K 1976 *Utsonomiya Daigaku Kyoikugakubu* **26** 27
Toshio I and Soichiro M 1975 *Bull. Chem. Soc. Jpn.* **48** 435