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Electroanalytical behaviour of 5-nitroorotic acid

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Abstract. The electroanalytical behaviour of 5-nitroorotic acid has been studied at several pH values, using several techniques (DC and DP polarography and CV).

The 5-nitroorotic acid undergoes five irreversible diffusion-controlled reduction waves over entire pH range considered. The optimum conditions for determination of 5-nitroorotic acid–with the above technique are also studied.

Keywords. 5-Nitroorotic acid; polarography; voltammetry.

1. Introduction

Pyrimidines in various guises appear in all living cells and are vitally involved in many biological processes.

Cavaliery and Lowry (1953) subsequently used a polarographic method to study the tautomerism of a large number of hydroxy and amino-substituted pyrimidines. Pyrimidine itself gave only a single wave at pH 1·2 and 5·8.

Smith and Elving (1962) suggested that electrochemical reduction of 2-oxypyrimidine is a $1e$-$1H^+$ process giving a free radical that dimerizes.

Janik and Palecek (1964) subsequently proposed that 2-oxypyrimidine is reduced in a $2e^-$ reaction with formation of 3,4-dihydro-2-oxypyrimidine.

The polarographic behaviour of 5-nitroorotic acid has been studied before by other authors (Jain and Kapoor 1968; Cripta *et al* 1971). These articles show very different results.

The reduction of the nitro group has been studied extensively. Nitro compounds are not particularly common among pharmaceuticals, but examples can be found in many classes of drugs, when nitration is often used as a preliminary step in polarographic determination, due to the facility of reduction of the nitro group.

2. Experimental

2.1 *Chemicals and solution*

An aqueous solution of 5-nitroorotic acid (5×10^{-3}M) was prepared. Britton-Robinson buffers were used and NaCl was used as the supporting electrolyte

* For correspondence

adjusted to 0·1 mol.l^{-1} ionic strength. All the reagents used were of analytical grade.

The samples were deaerated by bubbling nitrogen gas through it for 10 min.

2.2 *Apparatus*

A Metrohm Herisau E-506 was used, the dropping time was kept mechanically at 1 s, the flow of Hg being $m = 2{\cdot}20\,\text{mg.s}^{-1}$, Ag/AgCl was used as the reference electrode with a platinum auxiliary-electrode (DC and DPP).

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

The values of experimental variables utilized until the optimization of each one of them have been the following: temperature = 25 ± 0·1°C, pulse amplitude −40 mV, scan rate potential 5 mVs^{-1}.

3. Results

3.1 *Influence of the pH*

The influence of the pH was studied in the interval between 0·50 and 11·00, using different buffers.

Two waves (I and III) were observed both in DC and in DP polarography from pH 0·50 to 4·00. In that interval of pH, the two waves are very well defined and very sensitive.

At pH > 6·10 wave (I) disappears and at pH > 3·80 wave (II) and at pH > 4·3 wave (IV) appear.

3.2 *Effect of the other variables*

There was good linearity in the plots of i_l and i_p vs. concentration of 5-nitroorotic acid for the four waves, while $E_{1/2}$ and E_p were independent of the 5-nitroorotic acid concentration.

The best waves for the determination of the reagent are waves (I) and (III); these were very well defined and reproducible.

The limit of determination was 1×10^{-5} mol.l^{-1} for the classic polarography and 1×10^{-6} mol.l^{-1} for DP polarography.

The variation of I_l for the four waves with the square root of mercury height was linear.

The variation of i_p for the four waves with $h^{2/3}$ was also linear.

$E_{1/2}$ and E_p were independent of the mercury height.

The variation with temperature of I_l and I_p for the waves studied was linear between 15°C and 45°C.

There was good linearity between E_{dc} and $\log(i_d - i/i)$.

We obtained a good linearity between i_p and ΔE for the four waves studied up to a pulse amplitude of −90 mV. The criterion of Birke confirms the irreversibility of all these processes.

The good linearity obtained between i_p and $t_d^{2/3}$ for the four waves confirm its diffusion characteristics.

3.3 *Cyclic voltammetry*

The cyclic voltammetric behaviour of 5-nitroorotic acid was studied for the four waves, using HMDE. In all cases, we obtained curves, in which only 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 waves I, II and IV, but wave III only has good linearity when using small concentrations of the reagent.

4. Discussion

The results obtained in the electrochemical study of 5-nitroorotic acid show that its reduction is diffusion controlled through several irreversible processes, as has been observed for other pyrimidines and nitrocompounds.

The four waves clearly observed in DC and DPP (I, II, III and IV) may be assigned to waves I and II the reduction of the nitro group and the waves III and IV the reduction of the pyrimidine ring.

In acid media (pH < 4) there are several irreversible processes of reduction (wave I) in which six electrons are involved (chronocoulometry). As the acidity decreases, a change is observed which can be related to the dissociation of the amino group (pK = 4·20). Simultaneously, wave II appears, which corresponds to an irreversible reduction process involving four electrons. The wave II is independent of pH.

The best conditions for the determination of the reagent are: pH = 0·8 ($HClO_4$), $\Delta E = -90$ mV, $t_g = 2$ s, $T = 25$°C. Under these conditions we have obtained two methods for determination (waves I and III) of the reagent between 2×10^{-7} M and $1{\cdot}1 \times 10^{-5}$ M. Statistical calculations on several series of eleven identical samples containing 5-nitroorotic acid 1×10^{-6} M, give relative standard deviation of 4·21% for the first method and of 4·02% for the second method.

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