

SQUARE WAVE VOLTAMMETRY AT A GLASSY CARBON ELECTRODE: THE ANODIC OXIDATION OF URIC ACID IN PHOSPHATE BUFFER pH 6.9

HELENA M. CARAPUÇA AND JOÃO E. J. SIMÃO

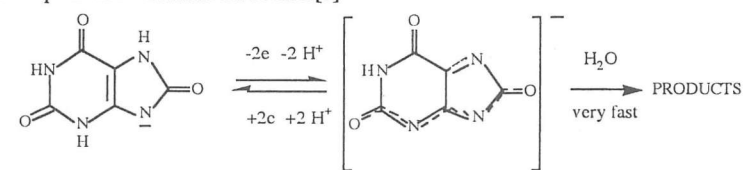
DEPARTMENT OF CHEMISTRY, UNIVERSITY OF AVEIRO,
3800 AVEIRO, PORTUGAL

SUMMARY

Square wave voltammetry (SWV) is applied to the anodic reaction of uric acid at a carbon electrode in aqueous solution. The electrochemical oxidation and adsorption characteristics of uric acid under these conditions are presented. In order to evaluate the analytical potentialities of this technique on the oxidative mode on a carbon electrode, reproducibility and sensitivity tests were carried out.

INTRODUCTION

Uric acid, a purine derivative of great biological importance, undergoes at $\text{pH} > \text{pK}$ (5.75) a 2 electron/2 proton electrochemical oxidation to an unstable intermediate with a proposed quinonoid diimine structure:[3]



The peak potential for this oxidation is given by the relation

$$E_p = (0.685 - 0.055 \text{ pH}) \text{ V vs. SCE at } 5 \text{ mVs}^{-1} \quad (1)$$

Concerning the role played by uric acid as a major catabolic product in man, its analytical determination has engaged the attention of the investigators. Several methods are proposed, such as HPLC with spectrophotometric[6] or electrochemical detection [9], flow-coulometry [7] and enzyme sensors [8].

The SWV is one of the most advanced electroanalytical techniques, which has been applied to several biologically significant molecules. However, this technique has been used only in reduction studies at mercury electrodes; if the reactant molecule adsorbs at the mercury electrode, the SW technique allows to reach nanomolar, or even

less, detection limits. On the other hand, the theory of SWV for the case of reactant and/or product adsorption is well established. Given a reaction mechanism for the reversible reduction of A to B, involving the adsorption of the reactant;



the theoretical results can be summarized as [5]:¹

$$i_p = \text{const } n^x \Delta E f a^y t_0^{1/2} C \quad (1 < x < 2; y < 1) \quad (3)$$

$$\delta E_p / \delta \log(f) = -2.3 RT / 2nF \text{ mV/d.u.} \quad (4)$$

As uric acid gives no reduction wave on the mercury electrode under normal conditions in aqueous solution, square wave voltammetry can not be applied directly to this molecule on the reductive mode.

EXPERIMENTAL

The Electrochemical Analyzer BAS 100B was used with a conventional three electrode system: the working electrode was glassy carbon (geometric area $7.07 \times 10^{-2} \text{ cm}^2$, supplied by BAS inc.), the auxiliary electrode a platinum wire and the reference electrode was Ag/AgCl(sat).

All experiments were carried out at room temperature.

The chemicals were reagent grade. Stock solution of uric acid (1mM) was stored in a refrigerator. The supporting electrolyte was $\text{KH}_2\text{PO}_4 / \text{Na}_2\text{HPO}_4$ (pH= 6.9, I=0.1M).

The glassy carbon electrode was subjected to a polishing and cleaning procedure before each voltammogram. At least three replicate voltammograms were recorded for each experimental condition and all the voltammograms were background subtracted.

RESULTS AND DISCUSSION

Cyclic voltammetric experiments on the glassy carbon electrode (GCE) showed that for $v = 5 \text{ mVs}^{-1}$ uric acid displays an anodic peak at $E_p = 0.316 \text{ V}$. For this sweep rate no cathodic peak is observed. But for 140 mVs^{-1} a small reduction peak appears on the reverse sweep (Fig.1) in agreement with earlier published work [1-3]. Such voltammetric behaviour indicates that the primary oxidation product of uric acid is unstable.

The net square wave current for $5.37 \times 10^{-6} \text{ M}$ uric acid in phosphate buffer is shown in Fig.2. As the baseline does not coincide with the zero value, which can be indicative of changes in the capacitive current probably due to adsorption of uric acid

¹ Γ_A is the surface concentration of A, i_p is the peak current (A), n the number of electrons, C the bulk concentration of the reactant, E_p the peak potential (V), T the temperature (K), R and F the gas and the Faraday constants respectively. ΔE , f, a and t_0 are the square wave parameters.

and/or of an oxidation product on the GCE, an interpolated baseline was drawn to estimate peak currents (dotted line in Fig.2).

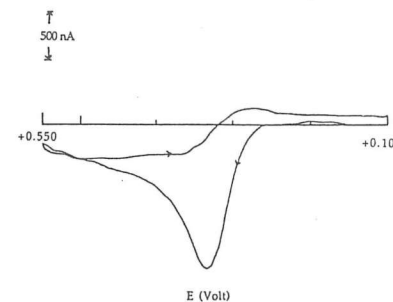


Fig.1. Cyclic voltammogram of $2.75 \times 10^{-5} \text{ M}$ uric acid. Sweep rate: 140 mVs^{-1} ; delay time: 10s

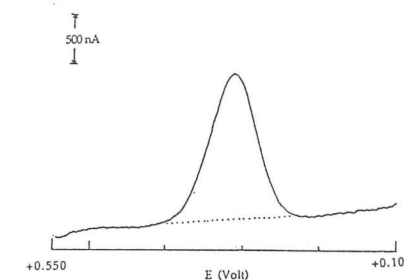


Fig.2. Square Wave voltammogram of $5.37 \times 10^{-6} \text{ M}$ uric acid. $a = 35 \text{ mV}$; $f = 70 \text{ s}^{-1}$; $\Delta E = 2 \text{ mV}$; $t_0 = 10 \text{ s}$; $E_{in} = 0.100 \text{ V}$

The presence of a surfactant (Triton X-100) in the solution decreases the peak current of uric acid (Fig.3) and shifts the peak potential slightly towards more positive values. This may be due to the displacement of adsorbed uric acid from the electrode surface by the larger surfactant molecule.

Assuming that the primary oxidation of uric acid is a quasi-reversible process controlled by reactant adsorption, equations 3 and 4 should apply. In fact, the dependence of i_p on the SW frequency is linear, in accordance with equation 3 (TABLE 1).

To cope with possible deviations due to different scanning rates, the normalized peak current was also plotted, resulting a slightly improvement of the linear regression.

A plot of E_p vs. $\log(f)$ is linear ($r = 0.998$; intercept = 0.29 V ; slope = 14.2 mV/d.u.). The slope, $\partial E_p / \partial \log(f)$, gives $n = 2.1$ (equation 4). Similar plots were drawn with solutions of different uric acid concentrations. A mean value of $n = 2.0 \pm 0.2$ (6 samples) was calculated, which is in accordance with the 2 electron electrochemical oxidation reaction of uric acid.

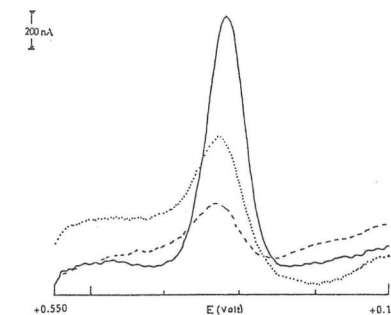


Fig.3. SW voltammograms of $5.00 \times 10^{-6} \text{ M}$ uric acid in the presence of Triton X-100: solid line - without Triton; dotted line - with 0.0007% Triton; dashed line - the same, after 40 minutes. SW parameters: $a = 20 \text{ mV}$, $f = 70 \text{ Hz}$, $\Delta E = 2 \text{ mV}$.

TABLE 1. Linear regression data for peak current vs. frequency and normalized peak current ($i_p/(t_{ac})^{1/2}$) vs. frequency.

	i_p vs. f	$i_p/(t_{ac})^{1/2}$ vs. f	$\log(i_p)$ vs. $\log(f)$	$\log[i_p/(t_{ac})^{1/2}]$ vs. $\log(f)$
r (N=6)	0.994	0.994	0.990	0.995
intercept	-2.2×10^{-8} A	-2.9×10^{-8} As	-7.6	-8.3
slope	1.9×10^{-8} As	6.0×10^{-9} As ^{1/2}	0.9	1.0

$$(t_{ac} = t_0 + (E_p - E_{in})/\Delta E \cdot f)$$

Further evidence for adsorption is the dependence of the peak current on the square root of the accumulation time, t_{ac} (Fig.4); the longer the accumulation time before the oxidation step, the larger i_p , since more uric acid is allowed to adsorb on the electrode. Moreover, the higher the concentration, the lower the accumulation time needed for i_p values to reach a horizontal plateau. From these data we can expect that saturation of the GCE surface by adsorbed uric acid would occur for $C \times (t_{ac})^{1/2}$ values higher than approximately 4×10^{-5} moldm⁻³s^{1/2}.

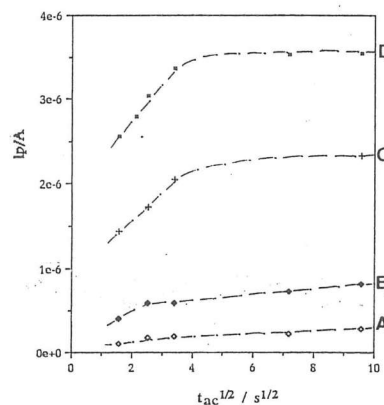


Fig.4. Peak current vs. $t_{ac}^{1/2}$ for different uric acid concentrations; (A) 9.7×10^{-7} , (B) 2.9×10^{-6} , (C) 7.2×10^{-6} and (D) 9.7×10^{-6} M. SW parameters: $a=35$ mV, $f=70$ Hz, $\Delta E=2$ mV, $E_{in}=0.100$ V.

Plotting the SW peak current vs. uric acid concentration (Fig.5), it can be seen that deviation from linearity begins for concentrations above approximately 1×10^{-5} M. At this concentration limit the product $C \times (t_{ac})^{1/2}$ equals 3.3×10^{-5} moldm⁻³s^{1/2} ($10 \mu\text{M} \times (10)^{1/2}$), which agrees with the previous value. This agreement is once more indicative of a process controlled by adsorption of the reactant.

From an analytical point of view, the linear part of the i_p vs. C curve (Fig. 5) shows that the detection limit at the proposed experimental conditions would be 1.0×10^{-6} M (twice the X-intercept for $t_0=10$ s). In order to maximize the ratio sensitivity / resolution of the SW voltammogram (experimentally given by $i_p/\Delta E_{p/2}$), the frequency and amplitude of the square wave were changed between 10-110 Hz and 2-50 mV, respectively, with the

² see Fig 4, in which this value corresponds to $\approx (4 \text{ s}^{1/2}) \times (9.7 \times 10^{-6} \text{ moldm}^{-3})$ for the higher concentration curve or $\approx (6 \text{ s}^{1/2}) \times (7.2 \times 10^{-6} \text{ moldm}^{-3})$ for the next one; the saturation value should not be reached for the lower concentrations.

SW step height ΔE of 2 mV. Those results led to an optimized SW parameters of $f=70$ Hz and $a=50$ mV, and a estimated detection limit of 8.8×10^{-7} M (for $t_0=10$ s) [10].

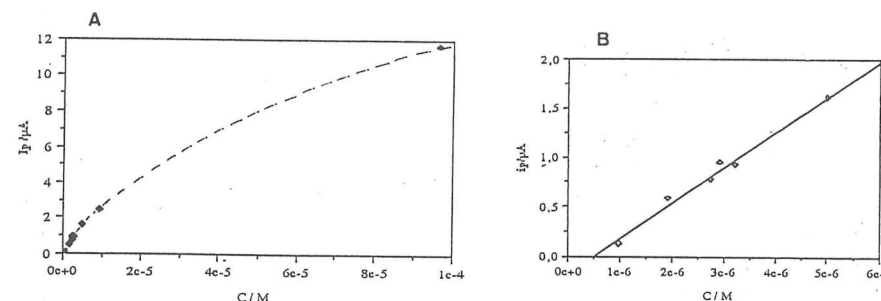


Fig.5. A) SW peak current vs. concentration of uric acid. B) Linear concentration range. Experimental parameters: $f=70$ Hz; $\Delta E=2$ mV; $a=50$ mV, $t_0=10$ s.

However, it should not be possible to lower this detection limit because the signal to noise ratio in the micro molar range becomes unfavourable. This is due to the intrinsic characteristics of the working electrode surface, namely the roughness caused by the cleaning and polishing procedure, and the porosity of the glassy carbon matrix itself.

REFERENCES

- [1] G. Dryhurst, "Electrochemistry of Biological Molecules", Academic Press, N. Y., 1977
- [2] R. N. Goyal, A. Brajter-Toth and G. Dryhurst, *J. Electroanal. Chem.*, **131**, 239 (1988)
- [3] G. Dryhurst, *Bioelectrochem., Bioenerg.*, **14**, 251 (1985)
- [4] K. McKenna, S. E. Boyette and A. Brajter-Toth, *Anal. Chim. Acta*, **206**, 75 (1988)
- [5] M. Lovric and S. Komorsky-Lovric, *J. Electroanal. Chem.*, **248**, 239 (1988)
- [6] G. Barja de Quiroga, M. López-Torres, R. Pérez-Campo and C. Rojas, *Anal. Biochem.*, **199**, 81 (1991)
- [7] S. Uchiyama, F. Umesato, S. Suzuki and T. Sato, *Anal. Chim. Acta*, **230**, 195 (1990)
- [8] M. Nanjo and G. G. Guilbault, *Anal. Chem.*, **46**, 1769 (1974)
- [9] M. Jänchen, G. Grünig and K. Bertermann, *Anal. Lett.*, **18**, 1799 (1985)
- [10] H. M. Carapuca and J. E. J. Simão, "Application of square wave voltammetry to the electrochemical oxidation of uric acid on the glassy carbon electrode", poster presented on the 4th European Conference on Electroanalysis, Noordwijkerhout, The Netherlands, 1992

ACKNOWLEDGMENTS

The financial support provided by INSTITUTO NACIONAL DE INVESTIGAÇÃO CIENTÍFICA (INIC), Lisbon, is acknowledged.