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Comparative Voltammetric Analysis of Adenine and Xanthine on a Pencil Graphite Electrode in the Presence of Copper Ions

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This work compares voltammetric responses of adenine (Ade) and xanthine (Xan) on a pencil graphite electrode (PeGE) in the presence of copper ions. For electroanalytical monitoring linear sweep voltammetry (LSV) in connection with elimination procedure (EVLS – elimination voltammetry with linear scan), differential pulse voltammetry (DPV), and constant current potentiostatic stripping analysis (CPSA) were used. Both substances are oxidized on PeGE providing simple oxidation peaks in the absence of Cu(II) at about 0.8 V (Xan) and 1.1 V (Ade). Electrochemically produced Cu(I) ions give rise to new oxidation peaks corresponding to the oxidation of a sparingly soluble Cu(I)-purine complex at less positive potentials (~0.45 V) while the former oxidation purine peaks are increased. Compared to Ade the oxidative signal of Xan is split into two peaks depending on scan rate. Peak-counterpeak EVLS signals reflect the charge transfer processes proceeding in an adsorbed state, and this fact confirms the suggested mechanism of both electrode processes. The splitting of xanthine oxidation signal was tested by means of a potential jump from -0.15 V to 0.65 V. The effect of scan rate, pH and concentration of Cu and ligands was studied in order to evaluate not only the nature of the additional Xan oxidation peak but also to optimize the best conditions for electroanalytical determination of Ade and Xan in mixtures.

Keywords: Oxidation of purines; Pencil graphite electrode; Linear sweep voltammetry; Differential pulse voltammetry; Elimination voltammetry with linear scan

1. INTRODUCTION

Purine derivatives play a significant role in human metabolism, and hence they are of great biochemical and biomedical interest. Many analytical methods were developed for their determination

in solutions or for monitoring of their concentration levels in biological fluids such as human blood, urine, or blood serum. Most of these compounds are electrochemically active and can be determined by electrochemical techniques.

Oxidation of some biologically important xanthines was studied by cyclic voltammetry (CV) or differential pulse voltammetry (DPV) in connection with a pyrolytic graphite electrode (PGE) [1-3] or a pretreated carbon paste electrode (CPE) [4]. Later, electrochemical analysis appeared using carbon fiber ultramicroelectrodes [5] and boron-doped diamond electrodes [6]. A general reaction scheme for the oxidation of xanthines was suggested [7,8].

Sensitive detection of purine nucleobases, their derivatives, and also oligonucleotides containing purine nucleobases was performed in the presence of copper ions [9-11]. Formation of purine complexes with metals, including copper, was studied extensively by electrochemical methods [12-18]. The species Cu(II) can be reduced to Cu(I) and, in the presence of purines such as adenine or xanthine, Cu(I) reacts with them to form insoluble complexes. It was found out that these structures can be accumulated at the carbon electrode surface and subsequent anodic stripping results in a sensitive increase of oxidation signals [16,17,19,20]. A change in electrode polarization to positive potentials results in a complex dissolution giving characteristic signals on the current-voltage curve. This approach was applied even for the detection of oligonucleotides (ODNs) after acid hydrolysis which releases purine bases from the ODN chain [16,19,21].

Recently we confirmed that the Cu(I) complex was formed also by aminopurines (adenine, 2-aminopurine, 2,6-diaminopurine) [22]. The anodic process of the sparingly soluble Cu(I)-aminopurine complex dissolution, corresponding to the oxidation of Cu(I) to Cu(II), takes place in the potential range between 0.4 and 0.5 V vs. Ag/AgCl/3M KCl. At more positive potentials, the aminopurines provide voltammetric peaks resulting from the oxidation of the purine ring [23,24]. The appropriate complex of Cu(I)-aminopurine has a synergic effect on the heights of these peaks. The stability of the accumulated complex layer was investigated by the adsorptive transfer stripping (AdTS) technique [16]. The elimination voltammetry with linear scan (EVLS) analysis using the elimination function E4, eliminating the kinetic and capacitive current components and conserving the diffuse current component, provides the possibility of increasing current sensitivity and changing peaks into well-readable peak–counterpeaks. Our measurements confirmed that according to both presented and previous results it can be assumed that the complex formation involves not only nitrogen in position 9, but also the nitrogen atom of the pyrimidine ring [25]. Recent work showed that the barrier to complex formation is not even the substitution at N6 position because the complex of Cu(I)-purine is formed also in the case of 6-benzylaminopurine (6-BAP). Voltammetric data for this adenine-type cytokinin obtained with a pencil graphite electrode (PeGE) allowed to propose the stoichiometry of the possible complexes formed [26].

Xanthine is of critical biological importance, because it plays a prominent role as an intermediary in purine degradation to uric acid in the human body. An electrochemical anodic stripping procedure for ultra-trace assay of xanthine in Cu(II) solution at a glassy carbon electrode was described with respect to various experimental and instrumental conditions [14], and the authors declare a detection limit within nanomolar concentrations. Quite recently, modified electrodes were prepared for simultaneous determination of xanthine and other purine derivatives in human urine. The

fabrication of these electrodes was based either on the single-wall nanotubes modification [27] or on the functionalized multi-walled carbon nanotubes composite film-modified electrode [28].

An elimination procedure was developed for improving voltammetric results (LSV, CV) through the investigation of the effects of diffusion (I_d), kinetic (I_k) and charging currents (I_c) on the total voltammetric current [26,29-34] The elimination or conservation of the current components chosen from the total voltammetric current is achieved by the elimination function using their different scan rate dependence. Based on the ratio ½ and 2 between the reference scan rate and further chosen scan rates, e.g. 1/2 $v_{ref,}$ v_{ref}, and 2 v_{ref} , the six elimination functions were calculated. These EVLS functions are listed in Table 1 (I_d – diffusion current, I_k – kinetic current, and I_c – charging current).

Table 1. Types of EVLS functions

f(I)	Characteristics	EVLS equation
E1	$I_d \neq 0;\ I_k = 0\ (I_c\ dist.by 1.707)$	$f(I) = -3.4142\, I_{1/2} + 3.4142\, I$
E2	$I_d \neq 0;\ I_c = 0\ (I_k\ dist.by 2.414)$	$f(I) = 4.8284\, I_{1/2} - 2.4142\, I$
E3	$I_d = 0;\ I_k \neq 0\ (I_c\ dist.by -0.707)$	$f(I) = 3.4142\, I_{1/2} - 2.4142\, I$
E4	$I_d \neq 0;\ I_k = 0;\ I_c = 0$	$f(I) = -11.657\, I_{1/2} + 17.485\, I - 5.8284\, I_2$
E5	$I_d = 0;\ I_k \neq 0;\ I_c = 0$	$f(I) = 6.8284\, I_{1/2} - 8.2426\, I + 2.4142\, I_2$
E6	$I_d = 0;\ \ I_k = 0;\ I_c \neq 0$	$f(I) = 4.8284\, I_{1/2} - 8.2426\, I + 3.4142\, I_2$

*dist. - the current component is distorted ([32])

In the case of adsorbed electroactive analytes such as biologically important compounds, EVLS yields sensitive electroanalytical signals (peak-counterpeak) [34-39]. It was found that EVLS supplies more detailed information about the process on the electrode, including not only adsorption but also structural changes of an electroactive species at an electrode [39].

In this paper we report on adsorptive stripping and adsorptive elimination voltammetric analysis of Ade and Xan at PeGE in the presence of copper ions. The advantages of PeGE over the existing carbon electrodes are its high electrochemical reactivity, commercial availability, good mechanical rigidity, and low cost. We compare their electrochemical behavior depending on pH, concentrations of components forming the Cu(I)-purine complex, polarization potential, and its scan rate. The results obtained by means of LSV with EVLS, DPV are completed by chronopotentiometric stripping analysis (CPSA). Based on our previous research it is expected that the common feature of the microdetermination of both purine derivatives is as follows: (a) formation of a slightly soluble complex of Cu (I)-purine and its accumulation on the graphite electrode surface, (b) oxidation of the complex signal and its corresponding base, and (c) using adsorptive stripping (AdS) in connection with EVLS enhances the sensitivity and selectivity of voltammetric measurements. On the other hand, the different features, such as the position and height of oxidative signals and their different dependence on pH, scan rate, and/or concentration of complex components, are helpful for the separation and determination of Ade and Xan oxidation signals in mixtures.

2. EXPERIMENTAL

2.1. Electrochemical measurements

Voltammetric and chronopotentiometric measurements were performed with an AUTOLAB analyzer (Metrohm, Ecochemie, The Netherlands) connected with a VA-Stand 663 (Metrohm, Zurich, Switzerland). A standard cell with three electrodes was used. The working electrode was a pencil carbon electrode (PeGE) with a surface area of 1.67 mm^2 (Tombo 05 HB, Japan). The PeGE was electrochemically pretreated by scanning the potential between -0.1 V and 1.4 V with a scan rate of 100 mV/s for 100 cycles in 0.1 M acetate or phosphate buffer (if not stated otherwise, pH 5.1). The electrode Ag/AgCl/KCl (3 M) as a reference electrode and platinum wire as an auxiliary electrode were used. The measurements were performed under the following parameters: LSV or CV - starting potential -0.15 V, reference scan rate 400 mV/s, potential increment 5 mV, vertex potential 1.2 V; for the EVLS procedure total voltammetric currents were recorded at scan rates of 200, 400, and 800 mV/s; DPV - starting potential -0.15 V, pulse amplitude 50 mV, pulses were inserted in 0.5 s intervals; CPSA - starting potential -0.15 V, potential positive limit 1.4 V, constant current 10 μA, time of accumulation 120 s in 0.1 M phosphate buffer (pH 6.4). All experiments were carried out at room temperature. The advantages of PeGE over the existing carbon electrodes are its high electrochemical reactivity, commercial availability, good mechanical rigidity, and low cost. As the holder for the graphite lead a Noki pencil Model 2000 (Japan) was used. The electrical contact with the lead was made by soldering a metallic wire to the metallic part.

2.2. Chemicals

Adenine, xanthine, and buffer solution reagents were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). The Ade and Xan concentrations were determined spectrophotometrically. UV-Vis spectra were measured with an HP 8452 spectrophotometer at an absorption maximum of 260 nm (absorption molar coefficient 13.4 M^{-1} cm^{-1} and 10.2 M^{-1} cm^{-1} for Ade and Xan, respectively).

2.3. Procedure

In voltammetric and chronopotentiometric measurements purine derivatives were exposed to a deposition potential of -0.15 V from a stirred solution (rod-like stirrer, 1520 rpm) for a deposition time of 120 s in the supporting electrolyte. Then PeGE was polarized from -0.15 V to more positive potentials and *I-E* or inversed derivative *E-t* curves were recorded. Each measurement was performed three times and the averaged value was taken for further evaluation. The solutions were deoxygenated by passing a stream of argon through them and during the measurements over their surface.

The voltammetric data were treated using a Savitzky and Golay filter (level 2) implemented in the General Electrochemical System software 4.9 (Ecochemie). Voltammetric curves measured at three different scan rates were taken into the EVLS procedure. The files with ocw extension were directly exported in Macra (Microsoft Excel) and the elimination functions were calculated.

3. RESULTS AND DISCUSSION

Recently we described the advantages of PeGE on the example of electrochemical oxidation of some aminopurines [22]. We found that an electrochemically activated electrode with Cu(I) was able to shift their oxidation potential by about one hundred millivolts towards negative potentials and enlarged the potential window for electrochemical measurements in comparison with the carbon paste electrode. In the present study we used the same type of electrode and the same activation procedure to find differences between the oxidative behavior of Ade and Xan in the presence of Cu(II) ions. In agreement with the earlier published results [3,14,40] Xan is oxidized at PeGE much more easily compared to some other purines including Ade. Oxidation proceeds in a wide interval of pH with peak height maximum in acetate buffer about pH 5. Further, the addition of Cu(II) ions into the solution resulted in the appearance of a Cu(I)-purine oxidation signal with simultaneous enhancement of the original oxidation signals. Under certain experimental conditions (see below) Xan yields a split oxidation signal. To explain this phenomenon we applied not only LSV and EVLS methods but also additional voltammetric and chronopotentiometric measurements.

3.1. LSV, DPV, and CPSA responses

The peak currents of Ade and Xan were directly proportional to the scan rate, which indicates that the electrode processes were limited by adsorption [41]. Figure 1 demonstrates the linear sweep voltammograms of 20 μM Ade or Xan in 0.1 M acetate buffer solution (pH 5.1) and shows the voltammetric responses of Ade or Xan at three different scan rates (used for EVLS) in the presence or absence of Cu(II).

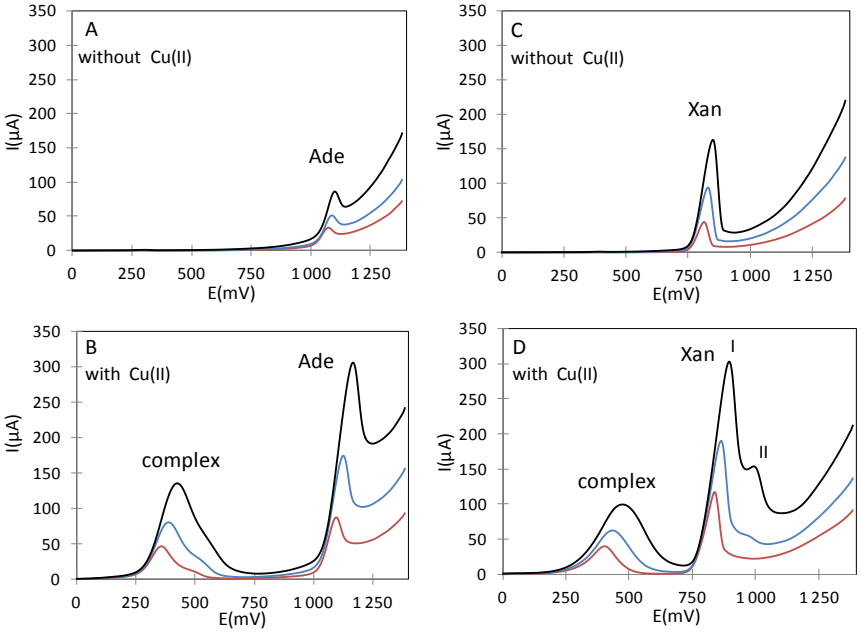

Figure 1. (A-D) Linear sweep voltammograms of Ade and Xan at three different scan rates: 200 mV/s (red line), 400 mV/s (blue line), and 800 mV/s (black line) in the absence **(A,C)** or presence **(B,D)** of Cu(II) ions. Acetate buffer pH 5.1; c_{Ade} = 20 μM, c_{Xan} = 20 μM, $c_{Cu(II)}$ = 20 μM.

Both substances are oxidized on PeGE providing simple oxidation peaks in the absence of Cu(II) at about 0.8 V (Xan) and 1.1 V (Ade). Addition of Cu(II) gives rise to finding new oxidation peaks corresponding to the Cu(I)-purine complex at less positive potentials (~ 0.45 V) due to oxidative decomposition of their complexes with Cu(I), while the former oxidation peaks are increased. In the case of Xan, addition of Cu(II) resulted in doubling the more positive peak (Figure 1D, Xan II). It appears at higher scan rates and, with decreasing the scan rate to a value of 200 mV/s, this peak disappears.

The redox responses of the PeGE in the presence of Xan in combination with Cu(II) are dependent on the method used. When we used the DPV method in which the electrode polarization is relatively slow, we observed a single oxidation signal of Xan (Figure 2A). On the other hand, the fast CPSA method resulted in a splitting of this signal (Figure 2B, Xan II). It is obvious that this phenomenon is strongly dependent on time because we are able to monitor it only at LSV or CPSA. We suppose that the most positive signal at Xan is related to the Xan oxidation pathway, which is different when compared to the Ade pathway, and comprises the formation of unstable oxidation products including uric acid [14], which is immediately oxidized at these high positive potentials.

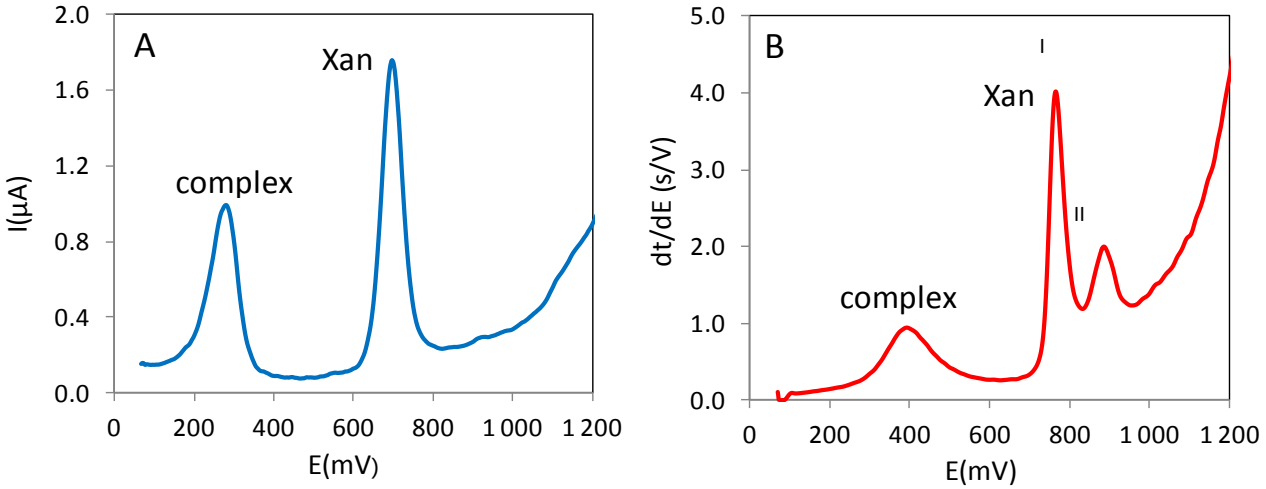

Figure 2. (A) Differential pulse voltammogram and **(B)** chronopotentiogram of Xan in the presence of Cu(II) ions. Acetate buffer pH 5.1; c_{Xan} = 20 μM, $c_{Cu(II)}$ = 20 μM. Other conditions are given in Experimental.

3.2. EVLS responses

EVLS functions (Table 1) were applied to the linear sweep voltammetric data of Xan with a reference scan rate of 400 mV/s (Figure 3). Unlike EVLS functions eliminating only one current component (E1, E2, E3), EVLS functions (E4, E5, E6) eliminating two current components indicate clearly an additional oxidation process at higher positive potentials (~1 000 mV, Xan II). EVLS transformations into the form peak-counterpeak (E4) or counterpeak-peaks (E5, E6) [35] confirm the adsorptive state of both electroactive species, Cu(I)-Xan complex, and Xan. Furthermore, the course of these EVLS curves (E4, E5 and E6) was in accordance with theoretical predictions [35]. According to the course of EVLS functions E1 and E4 compared to functions E2, E3, E5 and E6 (from the graph

$\beta_{EVLS} = f(x)$, where x is the scan rate exponent [30]) it follows that kinetics plays a significant role in the whole oxidation process. In the case of Ade we observed similar EVLS curves without an additional positive peak [22]. For both Ade and Xan the EVLS E4 function yielded the highest signal.

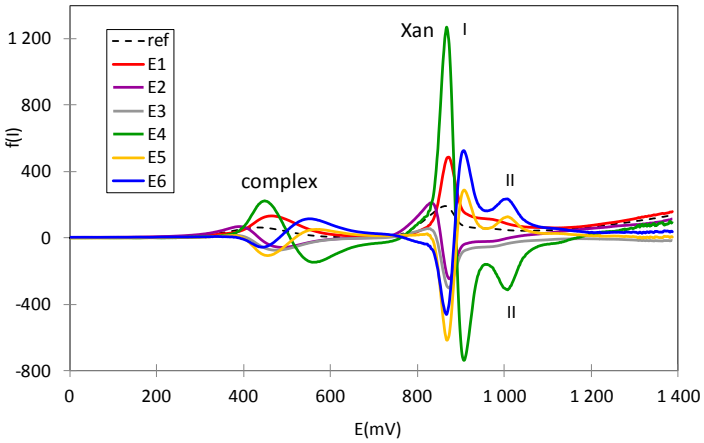

Figure 3. Elimination voltammograms of Xan for all EVLS functions presented in Table 1 (reference scan rate 400mV/s - dashed line) in the presence of Cu(II) ions. Acetate buffer pH 5.1; c_{Xan} = 20 μM, $c_{Cu(II)}$ = 20 μM.

3.3. LSV and EVLS responses of Ade and Xan mixture

To find out how to change the oxidation signals of both purine derivatives and their complexes in mixtures we measured LSV signals and calculated E4 EVLS functions. The results are shown in Figure 5. Worth noting is the fact that the oxidation peaks of Cu(I)-Ade and Cu(I)-Xan merge into one broad peak and the position of oxidation peaks of the corresponding purines is shifted to more positive potentials (Figure 4). A similar shift is observed in the case of Ade and Xan oxidation peaks.

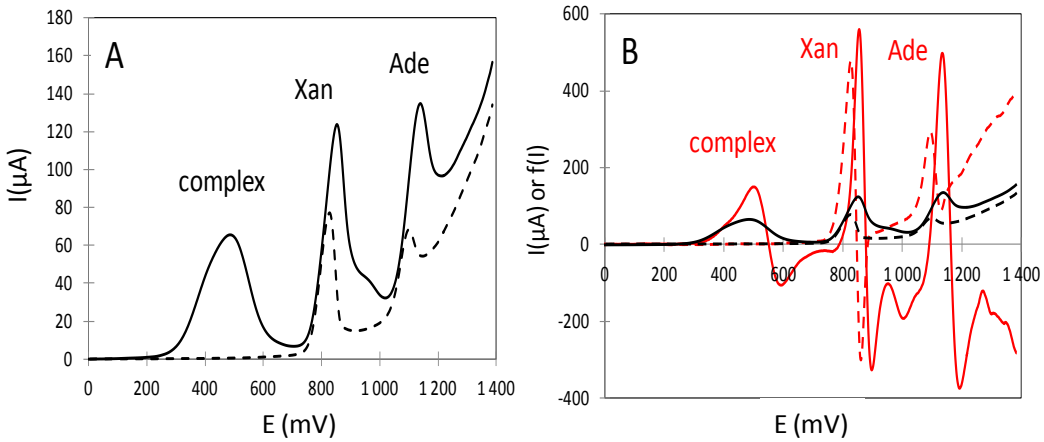

Figure 4. **(A)** Linear sweep voltammograms of Ade and Xan mixture in the absence (dashed black line) and presence (full black line) of Cu(II) ions recorded at a scan rate of 400 mV/s. **(B)** Elimination voltammograms of Ade and Xan mixture in the absence (dashed red line) and presence (full red line) of Cu(II) ions. To compare LSV and EVLS heights, LSV curves recorded at a reference scan rate of 400 mV/s are shown too. Acetate buffer pH 5.1; c_{Ade} = 20 μM, c_{Xan} = 20μM, $c_{Cu(II)}$ = 20 μM.

From Figure 4B it is quite clear that the EVLS Xan oxidation signal is also split in a mixture with Ade.

To compare the peak heights of Xan, Ade and their mixtures in dependence on the scan rate, presence or absence of Cu(II), LSV and EVLS, the results were summarized in a diagram (Figure 5).

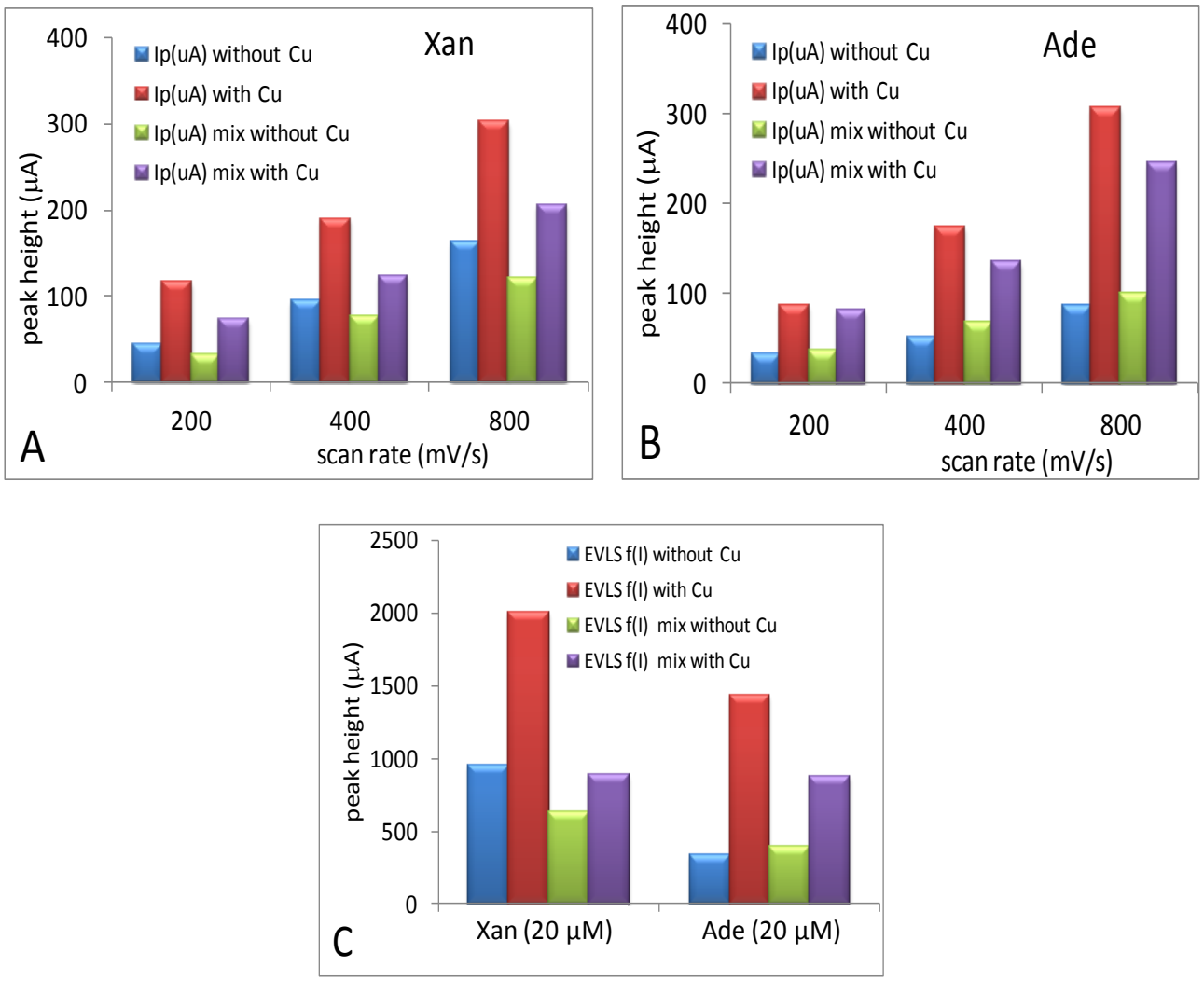

Figure 5. **(A, B)** LSV peak heights of Ade (20 µM), Xan (20 µM) and their mixture in the absence and presence of Cu(II) ions (20 µM) recorded at a scan rate of 400 mV/s. **(C)** EVLS peak heights of Ade, Xan and their mixture in the absence and presence of Cu(II) ions (reference scan rate 400 mV/s). Acetate buffer pH 5.1. The results were obtained as a mean value of three independent measurements.

We found that increased sensitivity for the determination of purine derivatives is guaranteed not only by the addition of Cu(II) ions and by increasing scan rate but also by the application of EVLS in an adsorptive mode. This finding is not entirely true for complex oxidation signals. The results

concerning complex oxidation responses are not included in these diagrams due to slight changes compared to the data presented in Figure 5.

3.4. Effect of Cu(II) ions and Xan concentration

The peak current was found to be dependent either on Cu(II) concentration or on the concentration of Ade or Xan. Figure 6 demonstrates the dependence of Xan peak height on the concentration of Cu(II) at different scan rates.

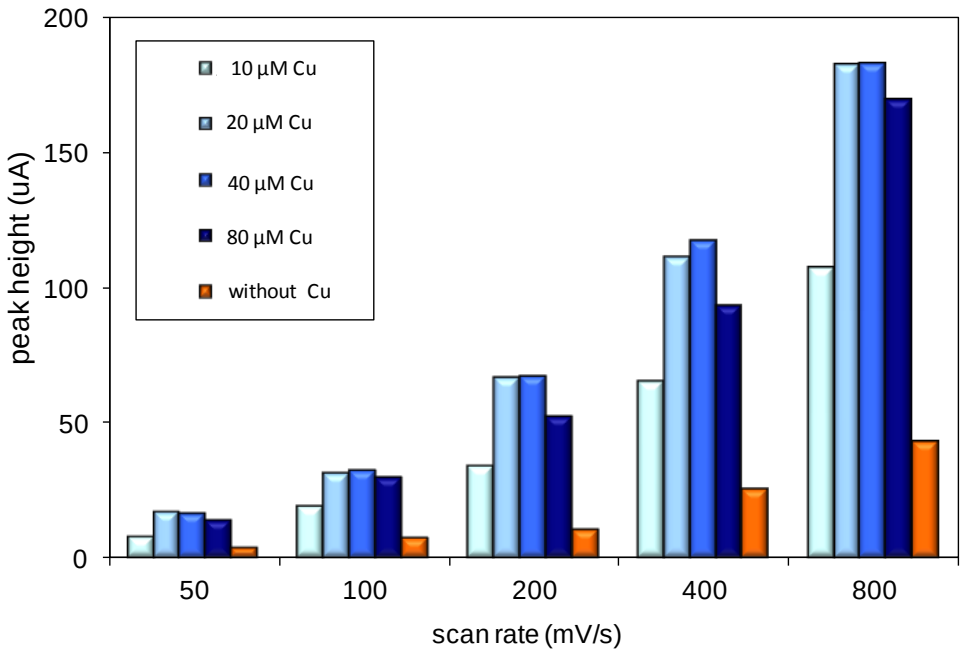

Figure 6. Dependence of Xan I voltammetric peak heights on Cu (II) concentration (0, 10, 20, 40, 80 µM) for different scan rates (50, 100, 200, 400, 800 mV/s). c_{Xan} = 20 µM. Acetate buffer pH 5.1.

The concentration of Xan was kept at 20 □M, that of Cu(II) was changed within 0 □M and 80 □M, and measurements were performed with scan rates from 50 to 800 mV/s. It is clear that the current-concentration graph at every scan rate exhibits nonlinear dependence with a maximum at concentrations with Xan:Cu(II) ratios between 1:1 and 1:2. At higher Cu(II) concentrations Xan peak height decreased. Similar results were obtained with Ade (not shown). We suppose that under our experimental conditions Cu(I)-purine complexes are formed at the PeGE surface in these component ratios. On the other hand, when the concentration of Cu(II) was kept constant and the concentration of the purine derivative was changed, the concentration dependences were similar with a maximum roughly at the above-mentioned concentration ratios. A large excess of one component results into smaller voltammetric signals.

3.5. Effect of potential jump

There are several possibilities of monitoring the stability of the Cu(I)-purine derivative complex on the electrode. One of them is based on the adsorptive transfer technique in which the electrode with the accumulated product (complex) on the surface is transferred into a solution where no component is present with a subsequent convenient measurement. Depending on the time after electrode transfer we can estimate approximately the stability of the complex. We used this approach earlier [16,42,43]. The possible effect of air oxygen and a specific skill in manipulating the electrode belong to the disadvantages of this approach. Here we chose another procedure based on a rapid change of the electrode potential (potential jump) with electrode dipped in the same solution. We tried to find how the oxidation signal of xanthine changed depending on potential jump. This potential jump resulted in current changes which can be related either to indirect proof of complex formation or to monitoring of signals splitting. We measured changes in oxidation peak depending on time (after jumping) in a region from 0 s to 300 s. Voltammetric measurements showed a decrease of the voltammetric signal and missing split depending on time, and after a period of 60 s the peak height and shape of xanthine oxidation signal was similar as in absence of copper ions.

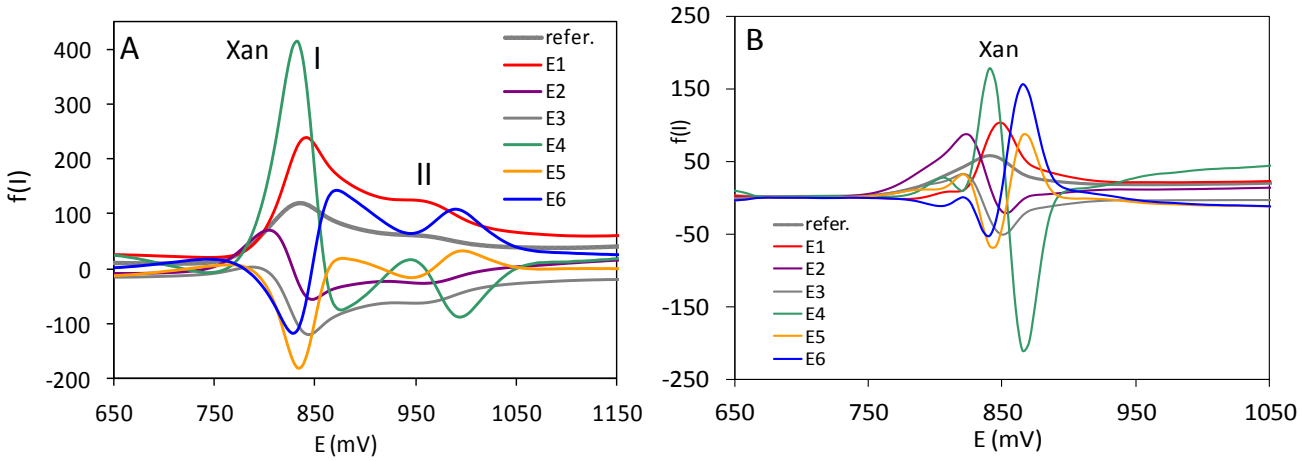

Figure 7. Elimination voltammograms of Xan for all EVLS functions presented in Table 1 (reference scan rate 400mV/s, gray line) in the presence of Cu(II) ions; jumping time (A) 0 s, (B) 300 s. Acetate buffer pH 5.1; c_{Xan} = 20 μM, $c_{Cu(II)}$ = 20 μM.

In Figure 7 we show EVLS responses of Xan at a jumping time of 0 s and 300 s. The above-mentioned deductions are supported by all EVLS functions indicating the EVLS Xan II peak at a jumping time of 0 s and its missing at a time of 300 s.

4. CONCLUSIONS

Electrochemical oxidation of Ade and Xan in the presence and absence of cuprous ions at an activated pencil graphite electrode (PeGE) was investigated. For comparative analysis voltammetric

methods including elimination voltammetry with linear scan (EVLS) and current potentiometric stripping analysis (CPSA) were used. The measurements were based on the formation of a complex between cuprous ions and Ade or Xan molecules, adsorption of the complex on the electrode surface, and its dissolution by oxidative stripping. These processes resulted in two voltammetric peaks; the former corresponds to the oxidation of the complex at a lower positive potential and the latter to the oxidation of the corresponding Ade or Xan oxidation signal. We found that voltammetric signals are dependent on scan rate, concentration of individual components, pH, and changes connected with time dependence at the potential jump. It was found that the PeGE shows an excellent electrochemical activity toward the analyzed compounds in a wide pH range.

Linear sweep voltammetric and chronopotentiometric measurements on PeGE modified by the Cu(I)-Xan complex revealed an additional voltammetric peak concerning the Xan oxidation process, which appeared either on LSV curves at higher scan rates or on CPSA curves. This phenomenon was studied also by EVLS which (a) indicates an additional oxidation process of Xan, (b) confirms electron transfer in the adsorptive state for both the complex and the corresponding purine, (c) shows that kinetics is significant in both oxidation processes, and (d) is able to increase the sensitivity of voltammetric signals by one order of magnitude using EVLS function E4.

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