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New Insight into the EC' Mechanism of Uric Acid Regeneration in the Presence of Ascorbic Acid on a Poly(3,4-ethylenedioxythiophene) Modified Gold Electrode

Cédric Crosnier de Lassichere,^[b] Laure Latapie,^[a] David Evrard,^[a] and Pierre Gros*^[a]

Abstract: A gold electrode surface was functionalized by means of an electropolymerized conductive poly(3,4-ethylenedioxythiophene) (PEDOT) organic layer. This modified electrode was used for the electrochemical detection of ascorbic (AA) and uric (UA) acids in an aqueous mixture with a selectivity around 340 mV. The electrochemical reactions kinetics were limited by AA diffusion and UA adsorption at the electrode surface,

Keywords: modified electrode • PEDOT film • ascorbic and uric acids • EC' catalytic mechanism • adsorption

respectively. Following a previous study ([*Electrochem Comm.* **2011**, *13*, 423–425]) cyclic voltammetry was used to provide a better understanding of the EC' mechanism of regeneration of UA by AA. Experiments particularly showed that allantoin (i.e. the final product of UA oxidation) is not actually involved in the synergic mechanism but rather the oxidized UA product diimine which is adsorbed at the electrode surface.

1 Introduction

The evaluation of the antioxidant properties of real media represents a growing interest in various application fields with respect to oxidative stress [1]. It is for instance used in biology or clinical analysis for diagnostic purpose [2–3]. Pharmaceuticals screening and food quality analysis are also of great importance in the case of reasonable supplementations of antioxidants [4–5]. This evaluation requires the simultaneous detection and assay of all coexisting antioxidant species, as well as the determination of their kinetic properties. Concerning the former two application fields, ascorbic (AA) and uric (UA) acids represent the major hydrophilic antioxidants not only because they are the highest concentrated compounds in several human biological fluids (serum, urine, tears, cerebrospinal fluid) but also due to their intrinsic antioxidant properties [6–7]. Both AA and UA are therefore considered as relevant biochemical markers in many pathologies (neonatal hypoxia and coronary heart diseases, amid several others) in which oxidative stress is involved [8–9].

Among the numerous methods dedicated to the evaluation of antioxidant properties, electrochemical techniques present several advantages, such as low-cost materials, simple experimental protocols, short-time analysis and good accuracy [10]. Furthermore, electrochemistry allows the determination of both antioxidant activity (related to oxidation potentials of biomarkers) and capacity (through the current values or electric charge consumed during their oxidation) [11]. However, the simultaneous electrochemical detection of AA and UA on unmodified electrodes results in poor selectivity since their oxidation takes place at very close, high overpotentials [12].

To overcome this problem, numerous works have been devoted to the design of chemically modified electrodes. Several electrode modification procedures have been successfully tested which reduced the anodic overpotential required and significantly improved the selectivity of the amperometric sensor response [13–14]: electrochemical pretreatment of electrode surface, electrogenerated redox and conducting polymers, chemically synthesized or electrodeposited metallic nanoparticles, carbon nanotubes and the use of (bio) electrocatalysts. However AA and UA were considered in most of these studies as potential interferences for the detection of dopamine [15–16] or epinephrine [17–18], or were introduced in large excess [19]. In comparison, only a few papers dealt with the simultaneous detection and determination of AA and UA under physiological concentrations, i.e. 34–79 μM and 180–420 μM for AA and UA, respectively [20–21].

We recently developed a voltammetric microsensor dedicated to the simultaneous determination of AA and UA concentration in aqueous standard solution [22] and in healthy human blood serum [23]. The electrode surface was modified using an electropolymerized poly(3,4-ethylenedioxythiophene) (PEDOT) film which strongly adheres on most electrode materials, shows high conductiv-

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ity in its oxidized state and presents a good stability in aqueous electrolytes and a biocompatibility with biological media [24]. We also demonstrated for the first time that the UA oxidation current increased in the presence of AA, in agreement with a coupled electrochemical / chemical catalytic mechanism [25]. This EC' mechanism is of great importance for clinical analysis in normal physiological conditions as it induces a systematic error in UA concentration. It has also to be taken into account to quantify the antioxidant capacity amplification considering the synergic effect resulting from the spontaneous regeneration of UA by AA [26]. In this work cyclic voltammetry was used in order to provide a better understanding of this EC' mechanism. Thermodynamic and kinetic considerations allowed to describe the species involved in the mechanism but also the nature of the reaction steps.

2 Materials and Methods

2.1 Chemicals

All reagents were of analytical grade and used as received. 3,4-ethylenedioxythiophene (EDOT, $C_6H_6O_2S$), ascorbic acid (AA, $C_6H_8O_6$, 99% powder), uric acid (UA, $C_5H_4N_4O_3$, 99% powder) and bovine serum albumin (BSA, 96% powder) were supplied by Sigma Aldrich. Tetrabutylammonium perchlorate (TBAPC, Bu_4NClO_4), potassium dihydrogenophosphate (KH_2PO_4), dipotassium hydrogenophosphate (K_2HPO_4), allantoin ($C_4H_6N_4O_3$, 98% powder) and extra dry acetonitrile (ACN) were purchased from Acros Organics. Sulfuric acid (H_2SO_4 95%) was obtained from VWR. All the solutions were prepared in ultrapure water (Milli-Q, Millipore, $18.2 M\Omega cm$). Nitrogen 4.5 was used for deaeration.

2.2 Materials

All the electrochemical experiments were performed with a Metrohm μ -Autolab II potentiostat (Metrohm) interfaced to a personal computer and controlled with the GPES 4.9 software (Metrohm). A three-electrode water-jacketed cell (thermostated at $20^\circ C$ using a Fisher Scientific Isotemp thermoregulator) was used for all the experiments. A 2 mm diameter gold disk electrode was used as working electrode. A platinum grid was used as auxiliary electrode. All potentials reported in the text are referred to a saturated calomel reference electrode (SCE) connected to the cell by a Luggin capillary.

2.3 Preparation of PEDOT-modified Microelectrodes

The working electrode was carefully pretreated before PEDOT electrodeposition. First the gold disk electrode was polished successively using $1 \mu m$ and $0.3 \mu m$ diamond slurries (Presi) during 2 min for both sizes. Between each polishing step, the surface was cleaned with Milli-Q water. Finally, the electrode was rinsed three times for 10 min in

an ultrasonic 96% ethanol bath and cleaned with Milli-Q water. Second, the freshly polished gold surface was activated by cycling the electrode potential between $-0.2 V$ and $1.6 V$ at $100 mVs^{-1}$ in a deaerated $0.5 molL^{-1} H_2SO_4$ solution. Experiments showed that 30 cycles were necessary to obtain a reproducible cyclic voltammogram. PEDOT electrodeposition was then achieved by cycling the electrode potential between $-0.88 V$ and $1.5 V$ at a scan rate of $250 mVs^{-1}$ in deaerated acetonitrile containing $0.1 molL^{-1} TBAPC$ as supporting electrolyte and $2.5 mmolL^{-1} EDOT$ monomer. The amount of polymer synthesized was previously optimized to maximize both sensitivity and selectivity of the amperometric response with respect to AA and UA oxidation [22]. It corresponded to an amount of anodic charge of $12 mCcm^{-2}$ and was controlled by means of the number of potential cycles during electropolymerization. The modified electrode (hereafter referred as Au-PEDOT) was finally rinsed with acetonitrile and distilled water to remove any physically adsorbed monomer.

2.4 Electrochemical Reactivity of AA and UA

Experiments were performed with the Au-PEDOT in 10 mL deaerated $0.1 molL^{-1}$ phosphate buffer solution (PBS) pH 7.0. The electrochemical reactivity of AA and UA was studied by cyclic voltammetry in the potential range from $-0.2 V$ to $0.45 V$. The potential scan rate was $50 mVs^{-1}$ unless otherwise indicated.

3 Results and Discussion

Figure 1 shows cyclic voltammograms (CVs) recorded with an Au-PEDOT modified electrode in $0.1 molL^{-1}$ PBS (pH 7.0) for different solution compositions. It has

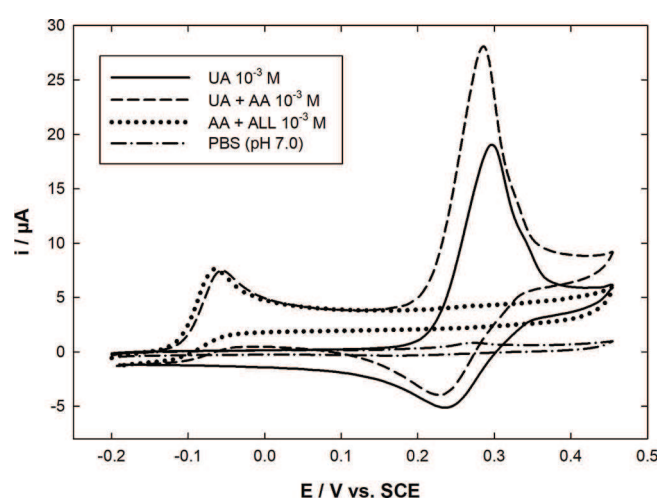
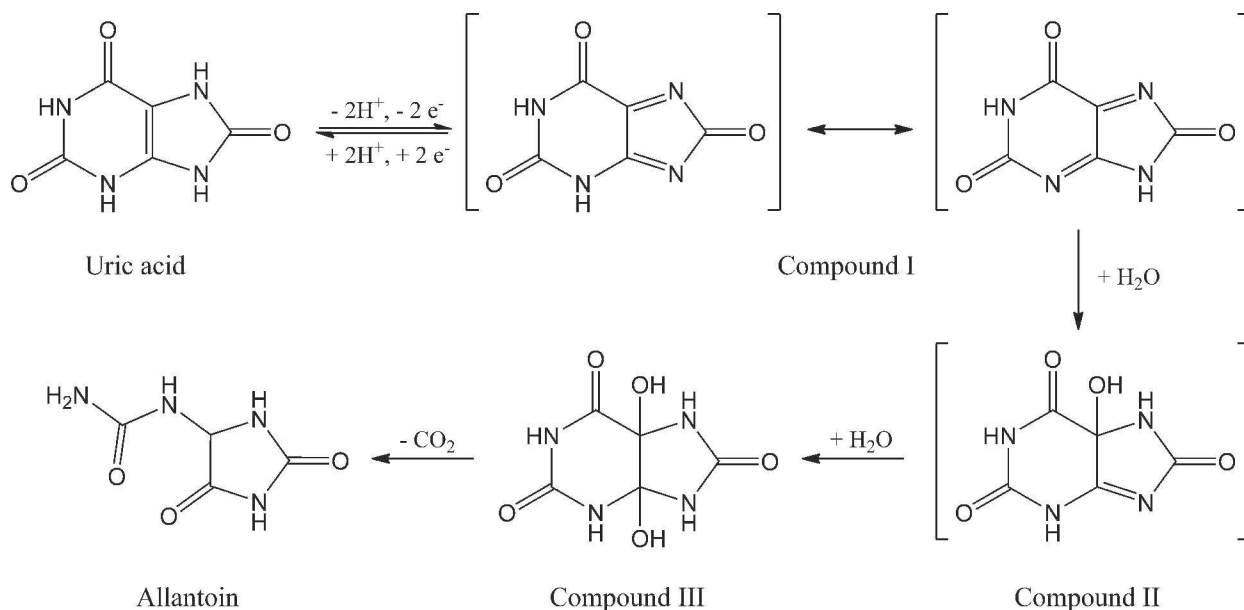


Fig. 1. Cyclic voltammograms recorded with a 2 mm diameter Au-PEDOT modified electrode in deaerated $0.1 molL^{-1}$ PBS pH 7.0 (dashed-dotted line) and containing $10^{-3} molL^{-1}$ UA (solid line); $10^{-3} molL^{-1}$ UA + AA (dashed line); $10^{-3} molL^{-1}$ UA + ALL (dotted line). Potential scan rate: $50 mVs^{-1}$.



Scheme 1. Reaction scheme for the electrochemical oxidation of uric acid to allantoin (from [44]).

been verified that no significant response was observed in PBS blank in the potential range used (dashed-dotted line). In the presence of $10^{-3} \text{ molL}^{-1}$ UA, the CV exhibited an oxidation – reduction signal centered at a potential close to 0.28 V (solid line) as previously reported [22]. It has to be noted that the cathodic peak current was lower than the anodic one. However, the diffusion of the oxidized product in solution during potential scan cannot be the only reason explaining this discrepancy taking account the relatively fast potential scan rate adopted (50 mVs^{-1}). Obviously the shape of this voltammogram makes evidence that the UA oxidation product is unstable and partially consumed during the experiment. The addition of $10^{-3} \text{ molL}^{-1}$ AA in the previous solution induced not only the appearance of an additional irreversible oxidation peak located at -0.06 V corresponding to AA oxidation, but also a significant amplification of the previous anodic peak current (dashed line), getting from 18.75 to 24.2 μA . This enhanced signal agreed well with an EC' mechanism resulting from a chemical reaction coupled to the electrochemical step as previously highlighted [25], where UA is regenerated at the vicinity of the electrode surface by reaction between its oxidized form and AA.

To the best of our knowledge no other paper dealing with the simultaneous assay of AA and UA focused on this EC' mechanism. Most papers addressing their electrochemical analysis showed the variation of AA oxidation current in the presence or the absence of UA [27]. For those studying the influence of AA concentration on UA amperometric response, either the concentration of AA was kept constant [28–31] and/or the AA concentration range adopted was generally high compared to that of UA [32–35]. In such conditions it was therefore not possible to highlight any electrocatalytic effect even if the EC'

mechanism was effective. Some other workers have even ignored the phenomenon although their experimental results (variation of the UA anodic peak current or calibration slope with and without AA) clearly showed it [36–38]. Only in a few cases no mutual interference was shown, the sensitivity of the modified electrode for UA detection being the same with or without AA [39–41].

The electrochemical oxidation of uric acid is nowadays well documented [42–44]. The primary step proceeds by a $2\text{e}^-/2$ protons mechanism to yield a diimine (Scheme 1, compound I). This diimine is unstable and is hydrated two times to give an imine-alcohol (compound II) and uric acid-4,5-diol (compound III) successively, this later being decomposed at neutral pH to allantoin (ALL) and CO_2 . Consequently, it was supposed that the spontaneous chemical reaction of the EC' mechanism involved the ALL/UA and the ascorbyl radical/AA ion redox systems considering their apparent standard potential, i.e. 0.59 V and 0.28 V at pH 7.0, respectively [45–46].

In order to strengthen this assumption a third CV was recorded in PBS containing $10^{-3} \text{ molL}^{-1}$ equimolar amounts of AA and ALL (Figure 1, dotted line). Mixing both species would have produced UA, this later being expected to induce an oxidative peak current close to 0.28 V. However, no signal was observed in this potential range and only the peak at -0.06 V corresponding to AA oxidation was recorded. This last result thus demonstrates that ALL is actually not involved in the spontaneous chemical reaction that leads to UA regeneration but rather an intermediate compound produced in the UA oxidation mechanism.

UA is known to be mostly oxidized into ALL at neutral pH [44]. Nevertheless, the electrochemical oxidation of UA produces protons at the electrode (as indicated in Scheme 1). Consequently, the solution is acidified in the

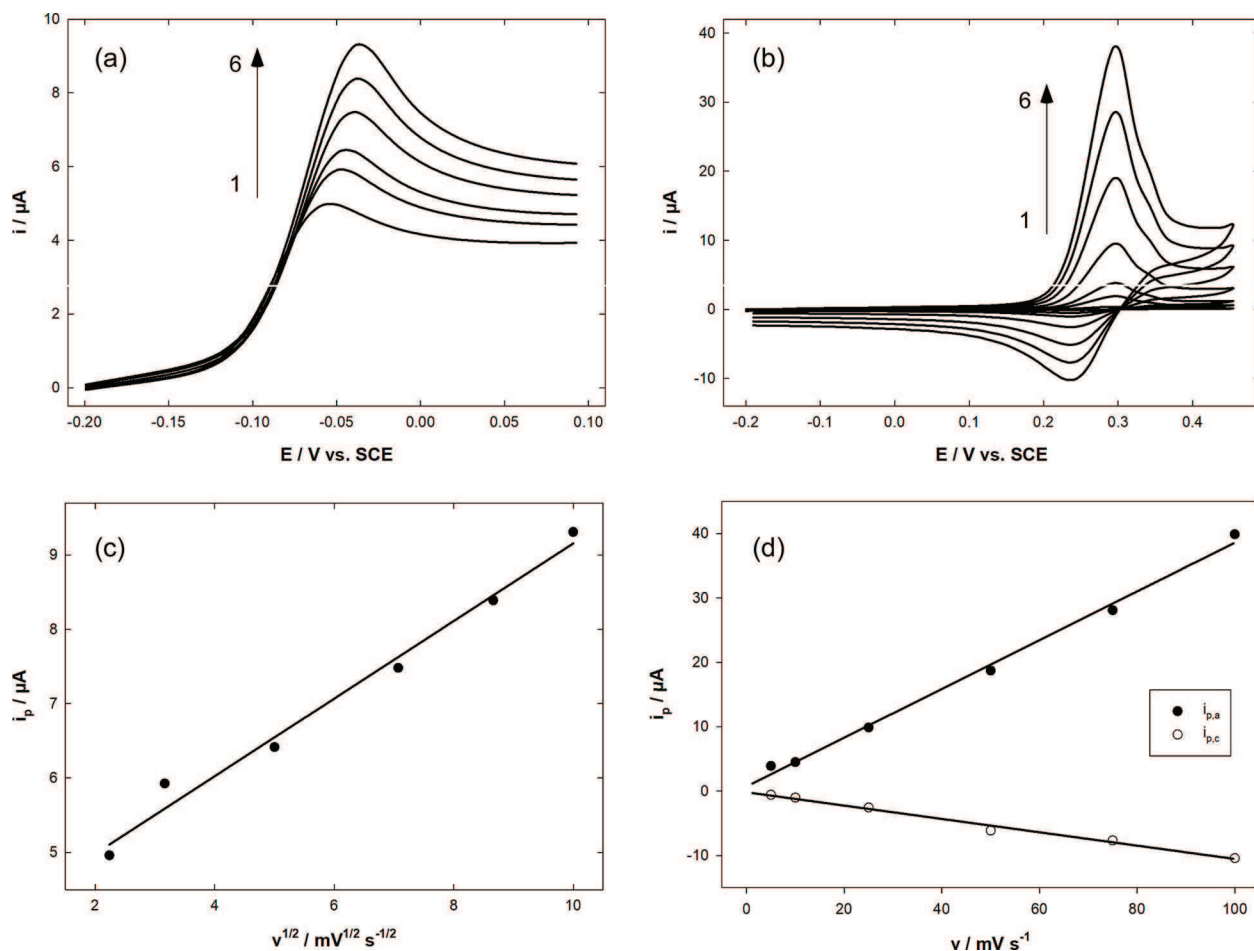


Fig. 2. Linear (Figure 2.a.) and cyclic (Figure 2.b.) voltammograms recorded with Au-PEDOT electrode in 0.1 molL^{-1} PBS pH 7.0 containing $10^{-3} \text{ molL}^{-1}$ AA (Figure 2.a.) and $10^{-3} \text{ molL}^{-1}$ UA (Figure 2.b.). Variation of the potential scan rate: (1) 5; (2) 10; (3) 25; (4) 50; (5) 75 and (6) 100 mV s^{-1} . Influence of the potential scan rate on AA oxidation peak current (Figure 2.c) and on both oxidation/reduction UA peak currents (Figure 2.d).

solution volume close to the electrode surface. Similar experiments were thus performed by replacing ALL successively by urea, alloxan and parabanic acid which are the major final products of UA oxidation under strongly acid conditions [42]. Unfortunately, no regeneration phenomenon was observed with all these compounds.

To go further into the understanding of the EC' mechanism a series of CVs was recorded in 0.1 molL^{-1} PBS containing $10^{-3} \text{ molL}^{-1}$ AA (Figure 2.a) or UA (Figure 2.b) by varying the potential scan rate. In the first case the anodic peak current was proportional to the square root of the scan rate (Figure 2.c) indicating that the reaction rate is under diffusional limitation. At the same time the peak potential increased slightly. On the other hand, both anodic and cathodic peak currents related to UA increased linearly with the potential scan rate (Figure 2.d) whereas the peak potential was almost unchanged. The electrochemistry of UA redox system thus clearly implies adsorbed species. All these results are coherent with the shape of the curve obtained by mixing both species (Figure 1, dashed line): the current corresponding to AA oxidation decreased after the

peak with time following a Cottrell evolution (i.e. I is function of $t^{-1/2}$). Comparatively the UA oxidation peak shows a symmetrical shape as can be obtained in thin layer electrochemistry. All these results are in accordance with several previous works [32] but surprisingly are in contradiction with others [47]. The influence of UA concentration on UA anodic peak current was also investigated. A saturation behavior of the current was expected due to the adsorptive phenomenon. Unfortunately, the current increased linearly with UA concentration as previously shown [22–23]. It was actually not possible for the concentration to exceed 1 mmolL^{-1} because of the relatively low solubility of UA in aqueous solution.

In order to confirm the role of adsorbed UA in the EC' mechanism, another experiment was performed: the Au-PEDOT modified electrode was first immersed during 30 minutes in PBS containing $10^{-3} \text{ molL}^{-1}$ UA to favor its adsorption onto the electrode surface. Then a CV was recorded in PBS containing only $10^{-3} \text{ molL}^{-1}$ AA (Figure 3, dashed line). Compared to the CV obtained in a $10^{-3} \text{ molL}^{-1}$ equimolar mixture of AA and UA (solid

line), the peak corresponding to AA oxidation shifted to more positive potential values in the presence of adsorbed UA, the difference between the two peak potentials being 95 mV. This certainly results from a decrease in the AA oxidation kinetics due to the presence of the adsorbed UA layer. It is noteworthy that the kinetics was not modified when the CV was recorded just after the modified electrode was immersed in the mixture solution. Consequently, the adsorption of UA seems to be not spontaneous. Furthermore, both UA oxidation peak potential and current were in the same order in both experimental conditions. This last result makes once again evidence that UA oxidation at the PEDOT-modified electrode is mainly due to adsorbed species.

Finally, a last experiment was performed by first immersing for 30 minutes the Au-PEDOT modified electrode in a 4 gL^{-1} BSA solution. BSA was used to coat the electrode surface, thus reducing drastically the active UA adsorption sites. The BSA/Au-PEDOT electrode was further immersed in a $10^{-3} \text{ molL}^{-1}$ UA solution during 30 minutes, then transferred in a $10^{-3} \text{ molL}^{-1}$ AA solution. The corresponding CV (Figure 3, dotted line) highlighted a strong decrease in the whole amperometric signal. It was particularly significant for the oxidation / reduction of UA redox system, the anodic current decreasing from 21 to $4.5 \mu\text{A}$. Actually BSA blocks all the active sites and therefore prevents drastically UA adsorption phenomenon. This result confirms once again that the EC' mechanism involves UA adsorbed at the Au-PEDOT electrode surface. Comparatively only a slight reduction of the AA oxidation peak current was observed. This might suggest that the BSA layer induced a slight additional diffusion barrier. Considering that the diimine

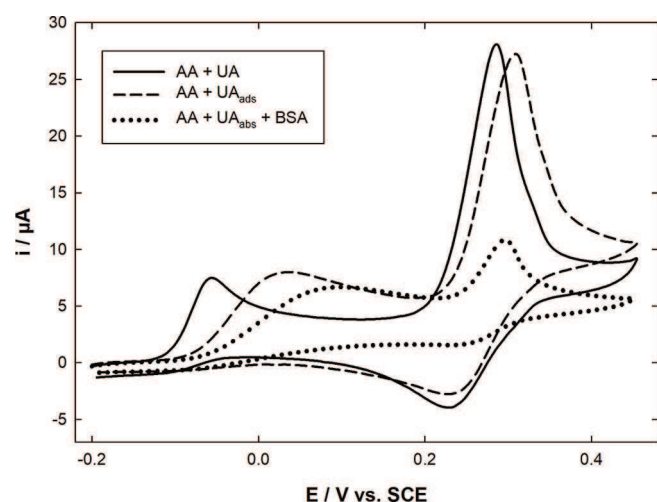


Fig. 3. Cyclic voltammograms recorded on Au-PEDOT electrode in 0.1 molL^{-1} PBS pH 7.0 containing (dashed and dotted lines) $10^{-3} \text{ molL}^{-1}$ AA and (solid line) $10^{-3} \text{ molL}^{-1}$ UA+AA. In the two former cases the modified electrode was previously immersed during 30 minutes in $10^{-3} \text{ molL}^{-1}$ UA solution (dashed line). The electrode was also previously immersed during 30 minute in 4 gL^{-1} BSA solution (dotted line).

intermediate can be strongly adsorbed at a rough electrode surface and that this adsorbed state is known to be more stable than in solution [44], it can reasonably postulate from all these results that this compound is actually the oxidized form of UA involved in the EC' mechanism.

4 Conclusions

This study definitively made evidence the spontaneous regeneration reaction of UA by AA on a PEDOT modified gold electrode, which results in an amplification of the UA anodic current. Experiments showed that allantoin is actually not involved in this EC' mechanism but rather an intermediate compound produced during electrochemical oxidation of UA which is strongly adsorbed at the electrode surface, probably the diimine intermediate. Works are now in progress to determine the kinetics of this catalytic reaction.

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