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Simultaneous Assay of Ascorbate and Urate Antioxidants in Human Blood Serum Using PEDOT- Based Electrochemical Microsensor

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Abstract: An electrochemical microsensor has been developed for the simultaneous assay of ascorbate (AA) and urate (UA) antioxidants in human blood serum. The electrode surface was modified by means of electropolymerized conductive poly(3,4-ethylenedioxythiophene) PEDOT organic films. Highly selective responses (detection potential difference more than 250 mV) were obtained for both biomarkers by optimizing the PEDOT thickness. Contrary to most previous studies the analytical performances were recorded by testing the microsensor directly in blood serum without any pretreatment of the samples. Using differential pulse voltammetry (DPV) the sensitivity and detection limit were 0.481 $\mu A\ \mu M^{-1}\ cm^{-2}$ and 4.2 $\mu mol\ L^{-1}$ for AA and 1.815 $\mu A\ \mu M^{-1}\ cm^{-2}$ and 2.0 $\mu mol\ L^{-1}$ for UA. The calibration curves were linear in the concentration range 5–200 $\mu mol\ L^{-1}$ for AA and 3-700 $\mu mol\ L^{-1}$ for UA. The accuracy of the sensor was satisfactorily compared with the reference analytical methods provided that the synergic effect between both antioxidants was considered. The sensor response was unmodified in the presence of the major biochemical interfering species.

Keywords: Antioxidants, ascorbic and uric acids, blood serum analysis, EC' mechanism, electrochemical microsensor, interfering species, PEDOT film.

1. INTRODUCTION

Oxidative stress has been one of the most widely studied biochemical processes for several years. It is the consequence of an excessive production of highly reactive oxygen (ROS) and nitrogen (RNS) species like superoxide anion $O_2^{\bullet-}$, hydrogen peroxide H_2O_2, hydroxyl radical $OH^{\bullet}$, nitric oxide NO, or peroxynitrite $ONOO^-$ [1] and leads to the oxidation of lipids, proteins and nucleic acids, which contributes to cellular dysfunction and death [2]. Oxidative stress is known to be involved in aging processes and has been proposed as one of the potential causes for cataract, cancers, cardiovascular and degenerative diseases (Parkinson, Alzheimer) and even infertility in men [3-5]. One of the main antioxidant defence systems involves low molecular weight antioxidants, i.e. ascorbic acid (AA), uric acid (UA), glutathione, β-carotene, α-tocopherol, coenzyme Q_{10}... which reduce ROS and RNS by oxido-reduction reactions [6]. Among them, AA and UA are present in highest concentrations in most biological fluids (plasma, serum, urine, tears, cerebrospinal fluids...) and may thus be considered as biochemical markers in a lot of pathologies (preeclampsia desease, neonatal hypoxia, coronary heart diseases..) where oxidative stress is involved [7-9]. Particularly AA is a powerful water soluble antioxidant at physiological levels. In synergic action with other circulating antioxidants, vitamin C plays a key role in protecting living cells against oxidative injury and prevents lipid peroxidation [10]. It is currently used clinically for the treatment and prevention of scurvy, common cold, mental illness, cancer, AIDS or coronary artery desease [11]. AA deficit (less than 11 $\mu mol\ L^{-1}$ in blood serum) is the principal cause of scurvy whose incidences increase because of the societal and economic current living conditions. For instance the prevalence of serum hypovitaminosis C was found to be only 0.95% among French healthy volunteers [12] but arise to 16 and 25% in UK low income/materially female and male respectively [13]. It also concerned 47.3% of hospitalized adult patients [14] and 70% of children with cancer upon diagnosis [15]. Consequently a biological assay is of great interest to quickly determine the diagnosis and thus avoid additional expensive examinations. In contrast, the role of UA in conditions associated with oxidative stress is not entirely clear. While commonly regarded as an indicator of gout, epidemiological studies suggest that high UA levels in serum represent also a risk factor for cardiovascular diseases [8] or Lesch-Nyhan syndrome [16]. Thus the selective and convenient detection of AA and UA is very important for biological researches as well as for routine analysis.

Numerous analytical methods have been developed for the quantitative determination of AA and UA in biological samples. They are generally based on enzymatic methods [17], spectrofluorometry [18], HPLC analysis [19] or electrophoretic microship [20]. However these methods suffer from costly materials and complex experimental protocols, require sample pretreatment and are generally time consuming. For instance the reference method for biological assay of AA involved HPLC coupled with a fluorometric detection and requires the serum to be previously deproteinized (see section 2.5 below). Comparatively electrochemical techniques present several advantages such as simplicity, low cost, fast analysis and

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good selectivity [21]. Moreover, analysis can be realized in short time scales and in relative complex and resistive media by using microelectrodes. However, a major problem is that AA and UA require generally high overpotentials on usual non-modified bare electrodes. Furthermore both antioxidants are in these conditions oxidized at very close potentials which make their simultaneous detection and quantitative determination difficult [22].

Chemically modified electrodes have been largely developed to solve these problems and to discriminate between the electrochemical responses of AA and UA oxidation. Several electrode modification processes have been proposed, using highly oxidized metal electrodes [23], metallic films [24] and nanoparticles [25, 26] electrodeposition, metal complexes [27], electrochemically reduced diazonium salts [28], single [29] or multiwalled carbon nanotubes [30-33], carbon-surface [34-37] and carbon-paste [38] electrodes, self-assembled thiol monolayer [39] or conducting polymers [40-43]. In this latter case, attention has been devoted since the mid-1990s to poly(3,4-ethylenedioxythiophene) (PEDOT) [44] which induces uniform and adherent polymer film on most electrode materials, shows quite high conductivity in its oxidized state, presents good stability in aqueous electrolyte and above all biocompatibility with biological media [45]. Moreover PEDOT was found to be resistant to fouling by the AA oxidation products [46], thus indicating PEDOT to be very promising in the design of amperometric sensors [47-49]. Nevertheless the analytical performances of all these sensors were systematically determined in model solutions whatever the modification procedure chosen. Moreover the analysis of real biological samples like human urine and blood serum, when considered, was mostly presented as a feasibility study realized in particular experimental conditions, i.e. considering AA and UA as interfering species for the assay of neurotransmitters like dopamine, or in presence of AA in large excess which does not reflect the healthy human situation (the concentration of AA in blood serum being one order of magnitude lower than that of UA in physiological conditions [50]). Consequently AA was either assayed separately to UA, or just detected and not assayed while its quantification represents a task of interest as well important as UA in clinical analysis. Finally AA was in other papers not detected in the original sample but added amounts were assayed by means of standard adding method.

In our previous studied [51] we evaluated PEDOT-modified electrodes to selectively detect and simultaneously assay ascorbic and uric acids under similar concentrations in aqueous solution. Particularly we reported the influence of PEDOT electropolymerization parameters on the amperometric response of the resulting microsensor. However most results were obtained in synthetic aqueous solutions and only one test was realized in blood serum. It is now essential to evaluate such modified electrode in biological medium in order to validate its analytical performances in real conditions and to apply the resulting sensor in the field of clinical biology analysis. The present paper experiences the voltammetric sensor in blood serum analysis. The analytical performances have been determined and discussed by considering the matrix effects of such complex medium. The accuracy of the sensor was evaluated by comparison with the reference analytical methods (HPLC for AA and enzymatic assay for UA). The influence of the major interfering species present in blood was also studied. Finally the efficiency of the microsensor was proved by analyzing 14 human blood serum samples.

2. MATERIALS AND METHODS

2.1. Chemicals

3,4-ethylenedioxythiophene (EDOT), potassium ferricianide $K_3Fe(CN)_6$ and metaphosphoric acid HPO_3 were purchased from Aldrich. Ascorbic acid, uric acid, dopamine, xanthine, hypoxanthine, glutamic acid, cysteine, N-Acetyl-L-cysteine (NAC), β-D glucose, albumin, uricase (E.C. 1.7.3.3) from *Bacillus fastidiosus* and peroxidase (E.C. 1.11.1.7) from horseradish were purchased from Sigma. Tetrabutylammonium perchlorate (TBAPC), potassium dihydrogenophosphate KH_2PO_4, di-potassium hydrogenophosphate K_2HPO_4, acetonitrile and N,N-bis(4-sulfobutyl) 3,5-dimethylaniline (MADB) were purchased from Acros. All reagents were of analytical grade and used as received. The aqueous solutions were prepared with doubled distilled water. High pure nitrogen was used for deaeration. Human blood serum samples were kindly given by the Laboratoire de Biochimie from Hôpital Rangueil – Toulouse and stored at -20°C before being used.

2.2. Materials

All electrochemical experiments were performed with an Autolab II Metrohm potentiostat (Eco-Chimie) interfaced to a microcomputer and using the GPES 4.9 software. A three-electrode system was used. A 50 μm diameter gold wire purchased from Goodfellow (Lille, France) was chosen as working microelectrode. The fabrication process involved glass capillaries from Clark Electromedical Instruments (Phymep, Paris, France), a microelectrode puller (Model PC 10) and a microgrinder (Model EG44) purchased from Narishige (London, UK). A 1 mm diameter platinum wire (1 cm length) was used as auxiliary electrode. All potentials reported in the text are referred to a saturated calomel reference electrode (SCE) ($Hg/Hg_2Cl_2/KCl_{sat}$) connected to the cell by a Luggin capillary. AA analysis was performed with a Dionex liquid chromatograph coupled to a fluorimetric detector. An isocratic pump and an autosampler were also used. Enzymatic assay of UA used an automate Olympus AU 2700 spectrophotometer.

2.3. Fabrication and Preparation of PEDOT-Modified Gold Microelectrodes

Microelectrodes were fabricated and characterized according to the procedure described by Ruffien-Ciszak *et al.* [52]. Briefly, the gold wire was inserted in the glass capillary. The capillary was pulled in two microelectrodes with the microelectrode puller. A tapered end was produced and warmed up again in order to consolidate the cohesion between glass and gold. Then this tip was polished on the diamond particle whetstone microgrinder at an angle of 90° for several minutes. The resulting microelectrodes presented disk geometry with total radius less than 120 μm.

The PEDOT electrodeposition process was similar to that previously reported [51]. A gold disk microelectrode was

polished with alumina slurry and rinsed with distilled water. The polished surface was then pretreated by cycling the electrode potential between -0.88 V and 1.5 V for 10 min at 10 mV s^{-1} in 20 mL deaerated acetonitrile containing 0.1 mol L^{-1} TBAPC as supporting electrolyte. Deaeration was obtained by purging the solution with nitrogen during 10 min; moreover a nitrogen atmosphere was maintained over the solution during the experiments. The electropolymerization was performed by cycling the electrode potential between -0.88 V and 1.5 V at 250 mV s^{-1} in the previous electrolytic solution also containing 2.5 mmol L^{-1} EDOT monomer. The amount of polymer synthetized corresponded to an anodic charge of 12 mC cm^{-2} (about 6.10^{-8} mol cm^{-2}) and was previously optimized for the simultaneous detection of AA and UA in synthetic aqueous solution [51]. The modified electrode was then rinsed with acetonitrile and distilled water successively to remove any physically adsorbed monomer. This modified electrode is hereafter referred to as μAu-PEDOT.

2.4. Electrochemical Detection of AA and UA

The μAu-PEDOT was immersed in a three-electrode cell where a nitrogen flux was constantly maintained. Experiments were performed in 250 μL unstirred and undiluted blood serum without adding any supplementary electrolyte. The sample was left to come at room temperature before analysis. Electrochemical measurements were performed by differential pulse voltammetry (DPV) since this electrochemical method induced higher currents than those recorded by cyclic voltammetry [46]. The experimental conditions were optimized with respect to the determination of AA [53]: pulse amplitude 50 mV, step potential 6 mV, pulse time 119 ms, interval time 1s, potential scan rate 6 mV s^{-1}. AA and UA assays were performed by standard addition method.

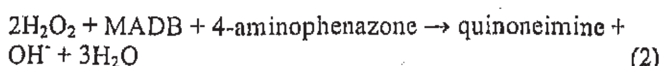
2.5. AA and UA Assay Using Reference Analytical Methods

AA concentration was determined indirectly from the assay of its oxidized form dehydroascorbic acid (DHA) obtained after addition of $K_3Fe(CN)_6$ 0.3 mmol L^{-1}. Serum samples were previously deproteinized with 0.5 mol L^{-1} metaphosphoric acid/methanol (75/25 v/v) and centrifugated at 3500 rpm at 4°C during 10 min. DHA analysis was accomplished in the supernatant by chromatography [54] using an isocratic elution on a Phenomenex Onyx C18 100*4.6 mm column thermoregulated at 25°C. The mobile phase consisted of 0.05 mol L^{-1} phosphate buffer solution (PBS)/methanol (90/10 v/v) pH 7.8 whose flow rate was kept constant at 1.6 mL min^{-1}. The fluorimetric detection was performed after addition of 4 mmol L^{-1} orthophenylenediamine in the sample with an exciting and emission wavelength set at 350 nm and 420 nm, respectively. Each assay involved two chromatographic runs, the first one to determine the initial concentration of DHA in blood serum and the second one after oxidative treatment of the sample. The concentration of ascorbate in serum was calculated by subtraction of DHA to the total vitamin C. The calibration and intralaboratory assays were estimated by using calibrator and internal standards delivered by CromSystem Instruments. The limit of quantification was 5 μmol L^{-1}. The repetability and reproductibility for two levels of total vitamin C (36.9 and 119.23 μmol L^{-1}) were 2.5 and 9%, and 3.6 and 8%, respectively.

UA assay was performed as previously described [55]. Briefly UA was oxidized into allantoïn in a first reaction catalyzed by uricase:

$$UA + O_2 + 2H_2O \text{ Alloxan} + CO_2 + H_2O_2 \quad (1)$$

Hydrogen peroxide was then reduced by MADB in a second reaction catalyzed by peroxidase to give the blue color quinoneimine:



$$2H_2O_2 + \text{MADB} + \text{4-aminophenazone} \rightarrow \text{quinoneimine} + OH^- + 3H_2O \quad (2)$$

All reactions were realized at 37°C. 25 μl of serum sample was added to 1 ml of phosphate buffer solution (PBS) 0.042 mol L^{-1} pH 7.5 containing 0.15 mmol L^{-1} MADB, 0.3 mmol L^{-1} 4- aminophenazone, 5.9 kU L^{-1} peroxidase and 0.25 kU L^{-1} uricase. Detection of the quinoneimine chromophor was performed spectrophotometrically at 660 nm. Calibration was obtained by the external standard method. The limit of quantification was 2 μmol L^{-1}. The repetability and reproductibility were 1.11 and 1.71% for a concentration of UA equal to 171 μmol L^{-1}, 1.55 and 2.44% for 388 μmol L^{-1} and 0.73 and 2.12% for 1362 μmol L^{-1}.

3. RESULTS AND DISCUSSION

3.1. DPV in Blood Serum

Fig. (1) shows differential pulse voltammograms (DPVs) recorded with a bare-gold microelectrode (dashed line) and the μAu-PEDOT modified electrode (solid line) immersed in 250 μL undiluted blood serum sample.

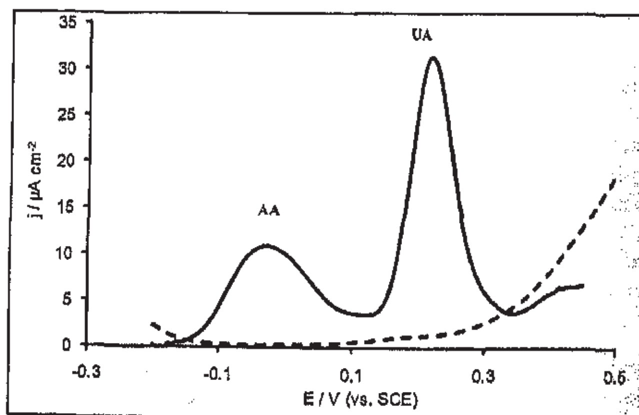


Fig. (1). DPVs recorded with a 50 μm Au microelectrode (dashed line) and a μAu-PEDOT (solid line) in blood serum sample containing 13.8 μmol L^{-1} AA and 225 μmol L^{-1} UA. PEDOT film was electropolymerized between -0.88 V and 1.5 V *vs* SCE at 250 mV s^{-1} in 0.1 mol L^{-1} TBAPC-acetonitrile containing EDOT 2.5 mmol L^{-1}. Electrochemical parameters are indicated in section 2.4.

Whatever the electrode, it has been previously verified that no significant signal was observed in PBS pH 7.0 except for the electro-oxidation of gold starting at about 0.8 V (result not shown). The oxidation of AA and UA on the unmodified electrode resulted in a broad and non exploitable wave starting at about 0.2 V. This result is in agreement with

previous works making evidence that both antioxidant species cannot be detected selectively on non-modified electrodes [22]. In contrast, two well-defined and distinct anodic peaks were recorded with the μAu-PEDOT electrode. By adopting the experimental conditions previously described for PEDOT electrodeposition (section 2.3) and for DPV parameters (section 2.4), it was possible to shift the peak potentials to lower values, i.e. -0.03 V and 0.23 V for AA and UA oxidation, respectively. This potential shift thus makes the sensor less sensitive to possible interfering species present in blood sample (see below). The potential difference was more than 250 mV, thus allowing the simultaneous detection of both species with the same modified electrode. The peak separation was attributed to different surface accumulations of each antioxidant on the polymer surface. It is known that the interactions between PEDOT and AA are essentially of electrostatic type (AA being anionic and the polymer backbone being positively charged at the pH used) [56] whereas UA is concentrated at the polymer mainly by means of hydrophobic interaction [57]. These interactions certainly explain also why the currents recorded at μAu-PEDOT were strongly higher compared to those obtained on the unmodified electrode, thus improving the sensitivity of the sensor. On the other hand both potential difference and amperometric responses were lower than those obtained previously in synthetic model solutions [51]. In the present case the voltammogram was plotted in blood serum without any dilution or pretreatment of the sample. The matrix effect certainly explains these results. It is noteworthy that the current depletion is more important for UA than for AA. In the aqueous solution UA was the most hydrophobic species whereas several other hydrophobic compounds (like lipids) are present in blood. These latter could adsorb on the polymer surface and thus prevent UA from being efficiently oxidized at the modified electrode. Anyway the PEDOT modified electrode clearly demonstrates a catalytic activity for the oxidation of both antioxidant species.

3.2. Analytical Performances

Fig. (**2A, B**) exhibit the DPVs recorded with the μAu-PEDOT electrode in blood serum under increasing concentrations of AA and UA, respectively. Although using high concentrated AA (resp. UA) stock solutions, the successive additions in such a low (250 μL) sample volume induced not only an increase in the corresponding peak current but also a decrease in the UA (resp. AA) oxidation signal. The resulting calibration curves are also shown (inset). Both exhibited a linear variation of the peak current as a function of the concentration of the corresponding antioxidant species. Complementary experiments showed that the linearity for AA was observed in the concentration range 5-200 μmol L^{-1}. The detection limit was 4.2 μmol L^{-1} (S/N=3). Similarly the UA oxidation current density varied linearly with the concentration in the range 3–700 μmol L^{-1} and a detection limit of 2.0 μmol L^{-1} (S/N=3) was reached. It is interesting to notice that both limits of quantification are very close to those of the reference methods (see section 2.5). The sensor sensitivities were 0.481 μA μM^{-1} cm^{-2} and 1.815 μA μM^{-1} cm^{-2} for AA and UA, respectively. It is noteworthy that the sensitivity was sensibly higher for UA than for AA. This difference resulted not only from the specific interactions of each antioxidant with the polymer as previously discussed; it was also the consequence of an EC' mechanism resulting from a chemical reaction in solution coupled to the electrochemical step [58] as demonstrated previously in our laboratory with synthetic aqueous solutions containing a mixture of AA and UA [59]. The apparent standard potential of the (ascorbyl radical/ascorbate ion) redox system being lower than that of the (alloxan/urate) one (0.28 V and 0.59 V at pH 7.0, respectively [60, 61]), a spontaneous oxidoreduction reaction between AA and the UA oxidation product takes place, thus regenerating the reduced form of UA at the vicinity of the modified electrode surface. Consequently an obvious change was observed in the sensitivity of the microsensor for UA oxidation current depending on whether AA was present in the sample or not [59]. This phenomenon was never considered before certainly because AA was in most studies in excess compared to UA. Consequently the regeneration process was constant and the calibration curve corresponding to UA assay was the same whatever the AA concentration. In contrast it is of particularly importance when AA and UA concentrations are in the same order of magnitude since even a little variation of AA concentration would modify sensibly the UA anodic current. For all real samples AA has thus to be assayed before UA, the calibration curve of UA taking account of the presence of AA and the consecutive synergic effect between both antioxidants. The relative standard deviations (R.S.D.) were 5.2 % and 4.8 % (n = 3 successive measurements) for AA and UA, respectively. The reproducibility was estimated at 7.4% for AA and 5.4% for UA on the basis of 4 independent measurements. It is noteworthy that these analytical performances are worse than those recorded in aqueous synthetic solutions. This is particularly true for the sensitivity which was found to be equal to 0.87 μA μM^{-1} cm^{-2} for AA and 4.05 μA μM^{-1} cm^{-2} for UA in the latter case [51]. This significant decrease in the sensor sensitivity is no doubt the consequence of the modifications of the electrode surface state during measurements which are induced by matrix effects almost systematically present with real complex samples. However all these performances are well suited to the assay of both antioxidants in biologic fluids like blood serum, as well as under physiological conditions (the physiological concentrations in blood serum are between 11 and 79 μmol L^{-1} for AA and between 180 and 420 μmol L^{-1} for UA [50]) as in the presence of one of the two acids at high concentrations. Table **1** indicates the analytical performances of recently reported electrochemical microsensors based on modified electrodes which were applied to the assay or detection of AA and UA in biological fluids. From these data one could rapidly conclude that the microsensor developed in this work presents the worse analytical performances. Comparison is not so easy since we have previously shown that PEDOT-modified electrode provided amongst the best results when used in model aqueous solution [51].

Actually most results reported in Table 1 were obtained in synthetic buffered solutions and the sensors were tested once in urine or blood serum. Furthermore works devoted to the simultaneous assay of AA and UA are very scarce. In these studies AA were very often considered as an interfering species or introduced in large excess, this latter

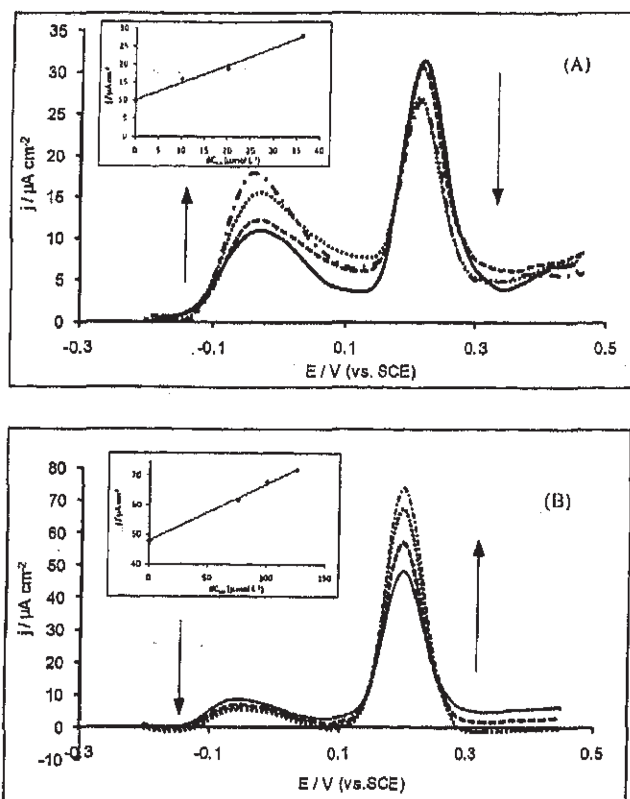


Fig. (2). DPVs recorded with the µAu-PEDOT in blood serum sample initially containing 13.8 µmol L^{-1} AA and 225 µmol L^{-1} UA (solid line) and after successive additions of AA (Fig. **2A**) or UA (Fig. **2B**). **(A)** addition of AA : 10 µmol L^{-1} (dashed line); 20 µmol L^{-1} (dotted line); 36 µmol L^{-1} (dashed dotted line). **(B)** addition of UA : 75 µmol L^{-1} (dashed line); 100 µmol L^{-1} (dotted line); 125 µmol L^{-1} (dashed dotted line). Inset: calibration curves.

case not reflecting the healthy human conditions. Consequently AA was generally only detected but not assayed. In our study the analytical performances of the microsensor were exclusively evaluated in blood serum and both antioxidant concentrations were determined simultaneously as it is currently the case in clinic biology analysis with other costly techniques. To the best of our knowledge it is the first time that such study was performed exclusively in real biologic sample with PEDOT-modified electrode.

The sensor accuracy was evaluated by comparing the results of the voltammetric sensor (taking account of the calibration curves) with those obtained by using the reference methods (see section 2.5 for experimental protocols). Fig. (**3**) shows the results recorded with several blood samples for AA (Fig. **3A**) and UA (Fig. **3B**) assay. In both cases an excellent correlation was observed in the physiological concentration range studied as both slopes (0.951 and 0.993 for AA and UA, respectively) and correlation coefficients (0.997 and 0.998) were close to 1. The electrochemical microsensor thus provides analytical results very similar to those of the reference methods while implying relatively cheaper apparatus. Furthermore results are obtained in quasi real time (DPVs are recorded in less than 1 min).

3.3. Interfering Species

The analytical performances of the voltammetric microsensor have also been evaluated in the presence of the main common co-existing substances which could interfere with the detection of AA and UA in blood serum. In this respect the effect of dopamine, xanthine, glutamic acid, cysteine, glucose and albumin was examined. The concentrations were chosen in order all the electroactive species to be clearly identified in the voltammogram. Supplementary addition of interfering species was realized if necessary. Fig. (**4**) shows the DPVs obtained with a blood sample containing all these species. Compared to the curve obtained with only AA and UA (Fig. **1**, solid line) no significant change was observed in the oxidation peak potentials of both antioxidant species (-0.065 V instead of -0.030 V for AA and 0.260 V instead of 0.230 V for UA). In this potential range, glutamic acid was not detected and glucose presented no electrochemical activity.

Table **2** reports AA and UA anodic currents recorded in the absence and in the presence of the different compounds. The values were not affected except in the presence of xanthine. In this latter case the peak current density corresponding to UA oxidation increased during the second potential sweep when the potential reached 0.70 V in the first scan, thus allowing the electrochemical oxidation of xanthine into uric acid [57]. In consequence, the potential range of the µAu-PEDOT voltammetric sensor was reduced from -0.2 V to 0.4 V in order to obtain selective and reliable results. Finally the presence of albumin induced a slight negative shift of about 30 mV in the oxidation peak potentials of all the electrochemically detected species and a decrease in both AA and UA anodic currents of about 10%.

3.4. Analysis of Blood Serum Samples

The voltammetric sensor has been tested with 14 blood serum samples and the results compared with the reference analytical methods. Data are reported in Table **3**. In all cases the electrochemical assays of AA and UA were confirmed by referent procedures. It is noteworthy that the microsensor allowed the detection of both antioxidants in a single measurement without any pretreatment or dilution of the sample. Furthermore, it was possible to proceed with the calibration using the standard addition method and a single calibration point per antioxidant species as the responses of the sensor were linear in a large concentration range.

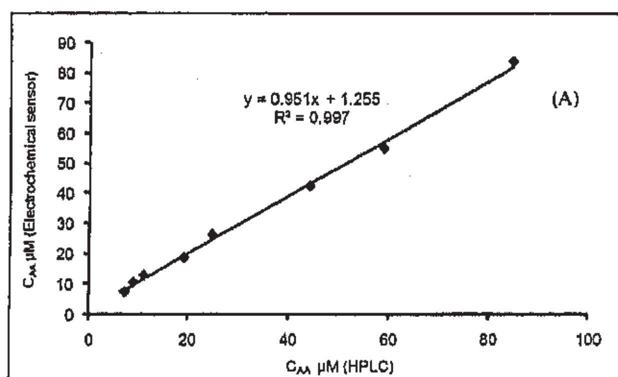
4. CONCLUSION

Modification of gold microelectrode surface by means of PEDOT electropolymerization has proved to be efficient to elaborate a voltammetric sensor for the assay of AA and UA in human blood serum. The µAu-PEDOT electrode exhibited an efficient electrocatalytic behavior which makes possible to detect both antioxidant species in human blood serum without any pretreatment of the sample nor dilution or addition of any electrolyte. Contrary to most previous studies the analytical performances of the microsensor were adapted to the simultaneous determination of both concentrations in physiological conditions. Even if the sensitivity of the sensor was significantly lower in blood serum than in aqueous

Table 1 Comparison of the Analytical Performances of Different Electrochemical Microsensors for the Determination of Ascorbate (AA) and Urate (UA) Antioxidants in Physiological Samples

Modification Method	ΔE_p/ mV	Linear Range/µM		Detection Limit/µM		Sensitivity/µA μM^{-1} cm^{-2}		Sample		Ref.
		AA	UA	AA	UA	AA	UA	AA	UA	
PVA	130	10-250	2-50	7.6	0.6	0.89	3.1	NR	urine	[34]
PMPy/Pd_{nano}	440	50-1000	0.5-20	7	0.027	0.006	0.28	NR	urine	[40]
AgHCFNPs	400	4-78	2-150	0.42	0.08	0.152	1.22	NR	serum	[25]
GNP/LC	320	3-2500	0.5-300	1	0.2	0.36	-	-	serum	[26]
PEDOT	372	500-3500	20-130	7.4	1.4	0.004	0.136	NR	serum	[41]
PG-activated	375	25-500	2.5-30	13	1.4	0.012	1.52	NR	serum	[35]
MWCNT	355	15-800	0.55-90	7.7	0.42	0.041	0.34	AA tablets	serum	[30]
PMT/Pd_{nano}	520	10-160	NR	7	NR	0.013	NR	serum	NR	[43]
ETPG	390	NR	0.05-10	NR	0.001	NR	32.75	NR	serum	[37]
TNCPE	240	1-100	0.4-100	0.5	0.08	NR	NR	AA tablets	serum	[38]
CILE	295	50-7400	2-220	20	1	NR	NR	NR	urine	[36]
Fe(III)P/MWCNT	460	14-2500	6-1300	3	0.3	0.081	0.059	ND	serum	[31]
PPD	345	2-2000	50-1600	0.4	2.5	0.007	0.005	ND	serum	[42]
Baelbp/Cu	223	5-160	5-300	2	2	0.49	0.51	serum	serum	[27]
PEDOT	403	5-300	2-600	2.5	1.5	0.875	4.05	synthetic solutions		[51]
PEDOT	250	5-200	3-700	4.2	2.0	0.481	1.815	serum	serum	This work

Note. PVA: poly (vinyl alcohol); Pt/PMPy/Pd_{nano}: poly (N-methylpyrrole)/Pd-nanoclusters; AgHCFNPs: silver hexacyanoferrate nanoparticles; GNP/LC: gold nanoparticles self-assembled; PEDOT: poly(3,-4ethylenedioxythiophene); PG-activated: pyrolytic graphite activated; MWCNT: multi walled carbon nanotubes; PMT/Pd_{nano}: poly(3-methylthiophene)/Pd nanoparticles; ETPG: electrochemically treated pencil graphite; TNCPE: thionic-nafion carbon paste modified electrode; CILE: carbon ionic liquid electrode; Fe(III)P/MWCNT: Non-covalent iron(III)-porphyrin functionalized multi-walled carbon nanotubes; PPD: para-phenylenediamine; Baelbp/Cu: 2-[bis(2-aminoethyl)amino]ethanol, 4,40-bipyridine bridged dicopper(II); ND: not determined; NR: not reported.



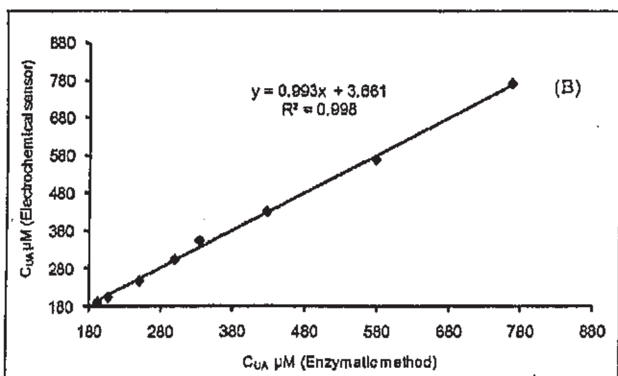


Fig. (3). Comparison of the analytical results obtained with the µAu-PEDOT microsensor with those recorded with the reference methods for AA (Fig. **3A**) and UA (Fig. **3B**) assays (see section 2.5. for experimental details).

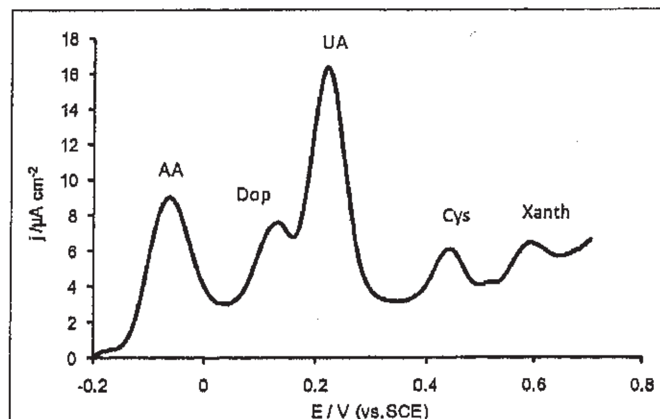


Fig. (4). DPV recorded with the µAu-PEDOT in blood serum sample containing ascorbic acid 10.7 µmol L^{-1}, dopamine 50 µmol L^{-1}, uric acid 19.2 µmol L^{-1}, cysteine 200 µmol L^{-1}, xanthine 100 µmol L^{-1}, glutamic acid 500 µmol L^{-1}, glucose 5.5 mmol L^{-1} and albumin 2 g L^{-1}.

solution due to matrix effects, the amperometric responses were not modified by the presence of the major biochemical interfering species. The sensor thus provided results in good agreement with those obtained with reference methods with an analytical procedure involving only one calibration point per antioxidant. Disposable sensors being preferable in clinical biology in order to avoid contamination between samples, works are in progress in our laboratory to evaluate the stability of the sensor under storage conditions.

Table 2. Effect of Interfering Species on AA and UA Anodic Peak Currents Recorded at -0.05 V and 0.23 V, Respectively. Blood Serum Initially Containing 10.7 μmol L^{-1} AA and 19.2 μmol L^{-1} UA

Interfering Species	Concentration	Peak Current (μA cm^{-2})	
		AA	UA
No interfering	-	10.4	20
Dopamine (μM)	50	10.2	19.85
Cysteine (μM)	200	10.3	20.1
N-acetyl-cysteine (μM)	100	10.4	20.1
Glucose (mM)	5.5	10.5	19.97
Glutamic acid (μM)	500	10	20.2
Xanthine (μM)	100	10.4	20
			22*
Hypoxanthine (μM)	100	9.98	20.1
Albumin (g/L)	2	9.46	18.2

*UA oxidation current recorded during the second DPVs.

Table 3. Determination of AA and UA Concentration in Blood Serum Samples with the Voltammetric Microsensor and the Reference Analytical Methods. AA and UA were Detected on the μAu-PEDOT at -0.05 V and 0.23 V, Respectively

Sample	AA/μmol L^{-1}		UA/μmol L^{-1}	
	μAu-PEDOT	Ref. Method	μAu-PEDOT	Ref. Method
1	42.5	43.9	431	429
2	18.7	19.2	205	208
3	22.0	25.8	192	193
4	13.8	12.3	248	251
5	42.3	44.2	569	579
6	7.4	7.3	355	335
7	26.2	24.8	336	304
8	4.2	5.6	304	301
9	83.7	84.8	442	413
10	18.4	16.3	769	769
11	10.7	9.0	368	365
12	55.0	58.9	228	232
13	34.2	32.6	436	433
14	15.9	17.2	252	251

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest by any means with respect to the instant research manuscript.

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