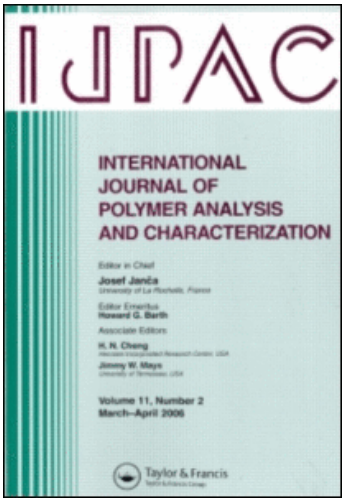

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

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Online publication date: 05 November 2010

To cite this Article Majidi, M. R. , Khoshkdaman, T. and Nazarpur, M.(2010) 'Simultaneous Determination of Ascorbic Acid and Uric Acid in Blood Serum Using an Overoxidized Polypyrrole Film Modified Glassy Carbon Electrode', International Journal of Polymer Analysis and Characterization, 15: 6, 351 — 359
To link to this Article: DOI: 10.1080/1023666X.2010.500529
URL: http://dx.doi.org/10.1080/1023666X.2010.500529

International Journal of Polymer Anal. Charact., 15: 351–359, 2010

ISSN: 1023-666X print
DOI: 10.1080/1023666X.2010.500529

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SIMULTANEOUS DETERMINATION OF ASCORBIC ACID AND URIC ACID IN BLOOD SERUM USING AN OVEROXIDIZED POLYPYRROLE FILM MODIFIED GLASSY CARBON ELECTRODE

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The present study reports the simultaneous determination of ascorbic acid (AA) and uric acid (UA) at pH = 7.0 using an overoxidized polypyrrole (OPPy) modified glassy carbon electrode (GCE). The bare GCE does not separate the voltammetric signals of AA and UA, whereas OPPy-modified GCE not only can completely separate the overlapped oxidation peaks of AA and UA but also dramatically enhances their oxidation currents compared with the bare GCE. Factors influencing determination including pH and scan rate were optimized. A linear response was observed in the concentration range from 7.7×10^{-7} to 2.1×10^{-5} M and 3.8×10^{-5} to 1.1×10^{-3} M with a correlation coefficient of 0.998 and 0.999 for UA and AA, respectively. The detection limit calculated was 7.2×10^{-7} for UA and 1.6×10^{-5} M for AA. The relative standard deviations (R.S.D.) for the oxidation of 7.5×10^{-4} M AA and 5.0×10^{-5} M UA are 3.5% and 1.1%, respectively. The application of the modified electrode was demonstrated by measuring the concentration of UA and AA in human blood serum.

Keywords: Ascorbic acid; Glassy carbon electrode; Human blood serum; Overoxidized polypyrrole; Uric acid

INTRODUCTION

Uric acid (UA) and ascorbic acid (AA) have been extensively studied because of their significance in bioelectrochemistry, neurochemistry, and clinical diagnostics applications.[1] UA is the primary end product of purine metabolism and abnormal levels of UA are symptoms of several diseases, such as gout, hyperuricemia, pneumonia, kidney damage, cardiovascular diseases, and Lesch-Nyhan syndrome.[2] AA, present in both animal and plant kingdoms, has been employed to prevent and treat common colds, mental illness, infertility, cancer, and AIDS.[3] So, their determination is very important and necessary. However, UA and AA often interfere with each other because they coexist at relatively high concentrations in biological

Submitted 19 March 2010; revised 24 April 2010; accepted 19 May 2010.
The authors gratefully acknowledge financial support from the Post Graduate Office of the University of Tabriz.
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fluids such as blood and urine.[4,5] Electrochemical technique is a common method for the sensitive determination of UA and AA owing to their electrooxidation properties. It is generally believed that direct redox reactions of these species at bare electrodes take place at very similar potentials, and high overpotentials are usually required for their electrochemical determination.[6] One promising approach for minimizing this effect is the use of chemically modified electrodes (CMEs).[7] Many different strategies have been employed for electrode modification such as electrochemical polymerization,[8] covalent bonding,[9] and mixing with carbon paste.[10]

Polymer-modified electrodes prepared by electropolymerization have received extensive interest in the detection of analytes.[11] Among polymers utilized for this purpose, polypyrrole (PPy) is especially promising for commercial applications because of its good environmental stability, facile synthesis, and higher conductivity than many other conducting polymers. Overoxidation of PPy is a destructive process that usually results in the addition of carbonyl functionality to the pyrrolic rings[12] with concomitant loss of conjugation and hence electronic conductivity. The high electron density of the carbonyl group acts as a barrier to hinder the diffusion of anions in the film.[13] A net electronegative character is imparted to the polymer film by overoxidation and cation permselective behavior for such overoxidized PPy (OPPy) films.[14]

In this study, we report on a glassy carbon electrode (GCE) modified with overoxidized polypyrrole (OPPy). Electrochemical behaviors of uric acid (UA) and ascorbic acid (AA) were explored on the modified electrode, which can effectively catalyze the oxidation of UA and AA in 0.1 M PBS (pH 7.0).

EXPERIMENTAL SECTION

Chemicals

Ascorbic acid, uric acid, lithium perchlorate ($LiClO_4$), and pyrrole were purchased from Merck. A 0.1 M phosphate buffer solution (PBS) (pH = 7.0) was prepared from NaH_2PO_4, Na_2HPO_4.

Apparatus

The experiments were performed using an Autolab modular electrochemical system (Eco Chemie, the Netherlands) equipped with a PGSTAT 20 module and driven by GPES (Eco Chemie) in conjunction with a three-electrode system. Glassy carbon (2 mm diameter) was used as the working electrode; all potentials reported in this article are referenced to a saturated calomel electrode (SCE). All electrodes were obtained from Azar Electrode Co. (Urmia, Iran).

Preparation of OPPy Film–Modified Glassy Carbon Electrode

Polypyrrole films were electropolymerized on the GCE in 0.05 M pyrrole and 0.1 M $LiClO_4$ with potential scanning between −0.2 and 0.85 V at a scan rate of 200 mVs^{-1} two times. Polypyrrole films on the surface of GCE were overoxidized in 0.5 M NaOH solution with potential scanning between −0.3 and 1.1 V at a scan

rate of $20\,mVs^{-1}$ seven times. The electrode was rinsed thoroughly with water and transferred into a 0.1 M phosphate buffer solution (pH = 7.0).

RESULTS AND DISCUSSION

Electrochemical Oxidation of AA and UA at Bare and OPPy-Modified Glassy Carbon Electrode Individually

Before carrying out the simultaneous determination of AA and UA, the electrochemical behavior of each was individually studied at the bare and OPPy-modified glassy carbon electrodes (OPPy/GCE) in 0.1 M PBS (pH 7.0). Figure 1 depicts the electrocatalytic behavior of 2×10^{-3} M AA (A) and of 1×10^{-4} M UA (B) at bare glassy carbon electrode (a) and the OPPy/GCE (b). As shown, at the bare GCE, AA shows a broad oxidation peak at 0.24 V, indicating a slow electron transfer kinetic, while the oxidation peak shifts to −0.046 V with a well-defined peak shape at the OPPy/GCE so that the peak current of AA is seven times higher than that at the bare GCE. About 0.286 V, negatively shifted potential and enhanced current of the anodic peak indicate that the OPPy/GCE shows strong electrocatalytic effect on the AA oxidation. Also, the oxidation of UA occurred at 0.33 V on the bare GCE, while UA was oxidized at 0.275 V on the OPPy/GCE. The oxidation current obtained for UA at the OPPy/GCE is nearly sixty-six-fold higher than that at the bare GCE.

The reason behind this can be that on the bare electrode, the electron transfer rate is rather slow owing to fouling of the electrode surface by the adsorption of the oxidation product of these species. AA and UA are both anionic species in neutral solution and their approach to the OPPy film should be restricted. However, chemical interactions between the OPPy surface and the approaching species may occur. The existence of dense carbonyl groups on the backbone of the OPPy film can provide a selective interface for molecular interaction, which may form hydrogen bonding with proton-donating groups such as NH-containing species like UA. The hydrogen bonding increases the surface concentration of these species to

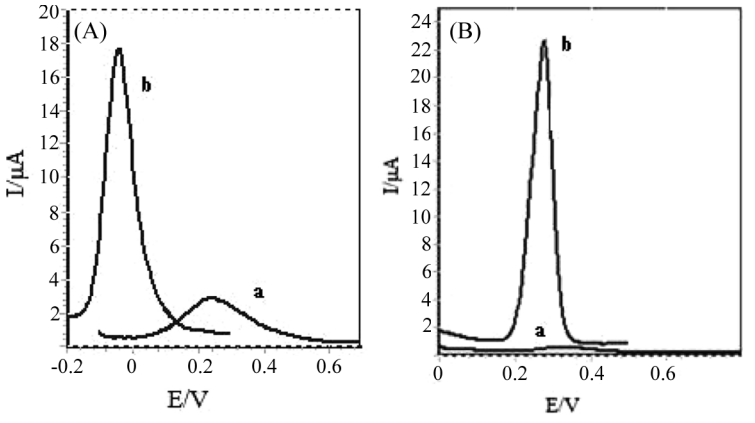
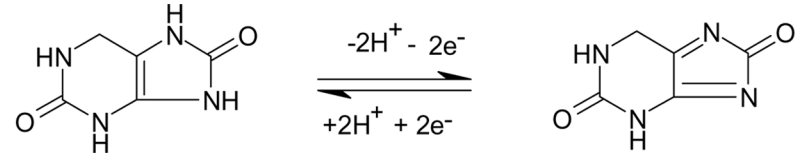


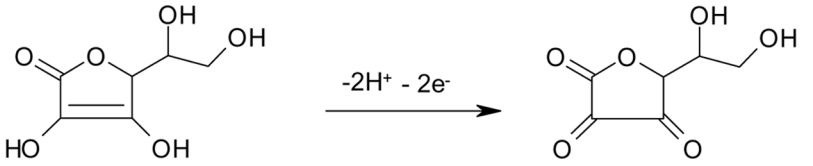
Figure 1. Differential pulse voltammograms of 2×10^{-3} M AA (A) and of 1×10^{-4} M UA (B) at bare GCE (a) and the OPPy/GCE (b) in 0.1 M phosphate buffer solution (pH = 7.0).

different extents; thus, remarkably, the signal at the modified electrode is attributed to the modification of the electrode surface by negatively charged OPPy film, which changes the overpotentials and the electron transfer rate as well. It is evident that electron transfer rate is enhanced and the modified electrode shows effective electrocatalysis for the oxidation of AA and UA.[4]

The probable electrocatalytic oxidation reaction of UA (Equation (1)) and AA (Equation (2)) on the OPPy/GCE was described as follows[5]:



(1)



(2)

Effect of Scan Rate

Cyclic voltammograms of AA and UA on the OPPy/GCE were obtained at different scan rates, and the results show that the anodic peak currents of AA and UA increased linearly with increasing of square root of the scan rates in the range from 10 to 200 mVs^{-1} for AA with correlation coefficient ($R^2 = 0.998$) and 400 to 1500 mVs^{-1} for UA with $R^2 = 0.9978$. According to these results, the electrode quasi-reversible reactions are controlled by the diffusion process.

Effect of pH on the Oxidation of AA and UA

The effect of solution pH on the electrochemical response of the OPPy/GCE towards the determination of AA and UA was studied in the pH range from 3.0 to 9.0. The plot of peak potentials for both AA and UA versus pH is constituted from two linear segments with different slopes. The plot slopes of AA and UA for pH values below 7.0 were obtained as 63.4 and 58.0 mV/pH unit, respectively. These indicate the oxidation of AA and UA are two-electron and two-proton processes, and for pH values more than 7.0, the slopes are 27 and 33 mV/pH unit, which are two-electron and one-proton processes. Also, at pH = 7.0 maximum current is obtained, thus this pH is selected for the simultaneous determination of these analytes in biological samples as optimum value.

Electrochemical Oxidation of Simultaneous AA and UA at the OPPy-Modified Glassy Carbon Electrode

In order to establish a sensitive and selective method for the quantification of AA and UA, the OPPy/GCE was utilized. As shown in Figure 2, the DPVgrams of the mixture of the AA and UA solutions show one broad and overlapped anodic peak

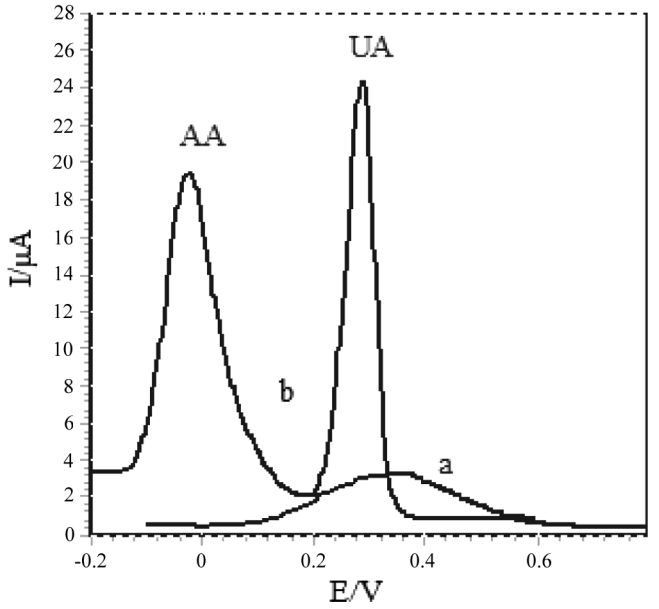


Figure 2. Differential pulse voltammograms of 2×10^{-3} M AA and 1×10^{-4} M UA at the bare GCE (a) and the OPPy/GCE (b) in 0.1 M phosphate buffer solution (pH = 7.0).

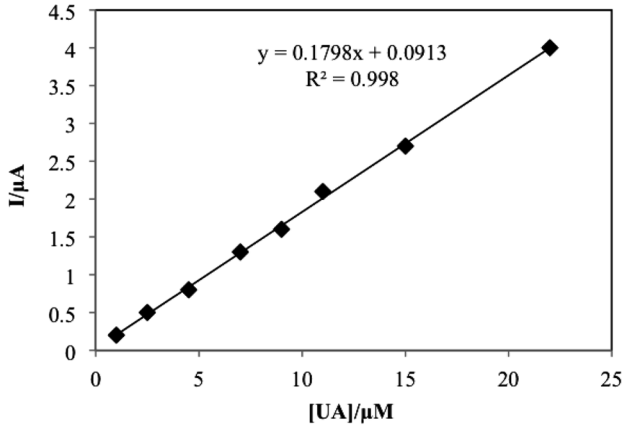
at the bare GCE (curve a). But at the OPPy/GCE, the overlapped voltammetric peak is resolved in two well-defined differential pulse voltammetry (DPV) peaks (curve b) at about −0.04 and 0.28 V, corresponding to the oxidation of AA and UA, respectively.

DPV Determinations of UA and AA Simultaneously

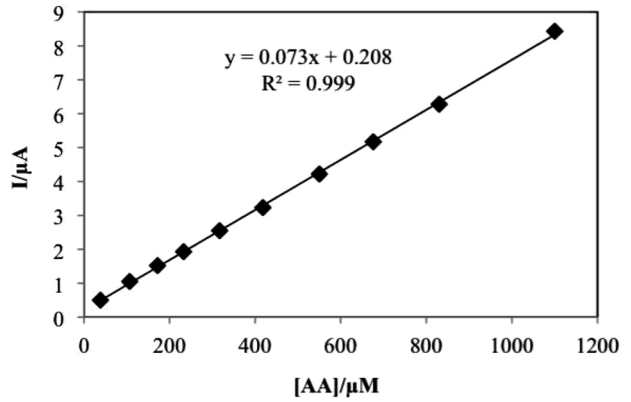
The determination of UA and AA concentrations using the OPPy/GCE was performed with the differential pulse voltammetry technique. In these measurements, the concentration of only one compound was varied, while the concentration of the other compound was kept constant. DPVgrams of different concentrations of UA in the presence of a constant concentration of AA and vice versa were recorded in PBS (pH 7.0). Figure 3a presents the calibration curves of different concentrations of UA in the presence of 4.0×10^{-4} M AA, and the results show that i_{pa} is proportional to the concentration of UA in the range of 7.7×10^{-7} to 2.1×10^{-5} M with a detection limit of 7.2×10^{-7} M (S/N = 3). A similar experiment was carried out with various concentrations of AA in the presence of 8.16×10^{-5} M UA where the i_{pa} is linear in the range of 3.8×10^{-5} to 1.1×10^{-3} M with a detection limit of 1.6×10^{-5} M (S/N = 3) (Figure 3b). The relative standard deviation of 10 successive replication measurements was 3.5% for 7.5×10^{-4} M AA and 1.1% for 5.0×10^{-5} M UA.

Amperometric Determination of AA

The hydrodynamic voltammograms obtained for 3×10^{-4} M AA and 9×10^{-5} M UA at the OPPy/GCE in phosphate buffer solution (pH = 7.0) show



(a)



(b)

Figure 3. Calibration curves for UA determination with the OPPy/GCE in 0.1 M phosphate buffer solution (pH = 7.0) containing 4×10^{-4} M AA and various concentrations of UA: 0, 0.771, 2.14, 4, 6.4, 8.2, 10.8, 14.6, and 22 μM (a) and for AA determination containing 8.16×10^{-5} M UA and various concentrations of AA: 0, 0.0384, 0.107, 0.172, 0.233, 0.317, 0.418, 0.55, 0.676, 0.83, and 1.1 mM (b).

that the current response starts at 0.05 V and increases quickly with increasing potential. It finally reaches a steady response at about 0.1 V. In this work, an applied voltage of 0.1 V was chosen for the amperometric determination of AA. Figure 4 shows the amperometric responses obtained at a potential of 0.1 V for successive additions of AA to the stirring 1×10^{-5} M UA solution. A linear response for AA in the concentration range of 9×10^{-7} to 3.95×10^{-5} M with a correlation coefficient of 0.9996 was obtained. The detection limit is 7.8×10^{-7} M for AA and the signal-to-noise ratio is 3. The modified electrode exhibited rapid response to changes in the concentration of UA and AA, producing steady-state signals, which indicated that the modified electrode is also an amperometric sensor of AA.

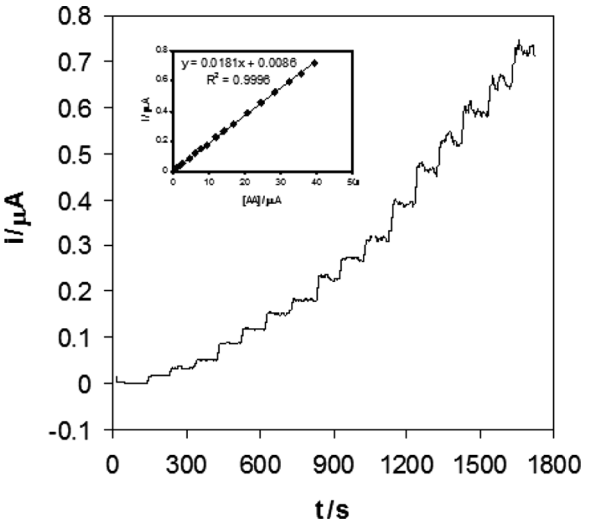


Figure 4. Current-time recording at the modified electrode with increasing AA concentrations at 0.1 V in phosphate buffer solution (pH = 7.0). Inset: calibration plot for AA determination.

Determination of UA and AA in Human Blood Serum Individually

Table I presents the UA concentrations determined in real samples using the differential pulse voltammetry and colorimetric-enzymatic methods. Results obtained from recovery tests indicate that matrix effects were not observed, and, therefore, it is possible to determine the UA concentration in blood without separation.

Determination of AA in human blood serum samples was also investigated. As shown in Table II, 12 human blood serum samples were determined. Samples with

Table I. Results obtained for UA determination at human blood serum ($n = 3$)

Sample number	Results obtained from proposed method $\overline{X}$ (mg/dL)	Results obtained from colorimetric-enzymatic as standard method $\overline{X}$ (mg/dL)	RSD (%)
1	7.25	7.13	2.34
2	5.25	5.30	2.10
3	4.17	4.25	0.719
4	4.92	5.11	1.63
5	5.54	5.76	5.78
6	6.22	6.18	2.25
7	7.11	6.98	1.27
8	2.35	2.50	4.68
9	4.76	4.84	4.09
10	6.50	6.55	4.00
11	4.38	4.32	3.42
12	6.91	7.02	1.74

Table II. Results obtained for AA determination at human blood serum ($n = 3$)

Sample number	Found by proposed method μM (mg/dL)	Spike (μM)	After spike (μM)	Recovery (%)
1	0.271 (0.477)	79.02	76.10	96.30
2	0.313 (0.551)	80.65	81.70	101.3
3	0.301 (0.53)	81.40	79.40	97.60
4	0.277 (0.488)	81.57	75.70	92.80
5	0.321 (0.565)	83.80	81.10	96.80
6	0.401 (0.706)	91.30	89.20	97.70
7	0.365 (0.643)	94.86	81.60	86.02
8	0.524 (0.923)	101.1	103.8	102.7
9	0.478 (0.84)	101.3	94.70	93.50
10	0.684 (1.2)	114.4	124.3	108.6
11	0.716 (1.26)	126.2	115.5	91.50
12	0.801 (1.41)	126.7	138.1	109.0

previously found content of UA were spiked with known standard concentration of AA.

CONCLUSION

An overoxidized polypyrrole (OPPy) modified glassy carbon electrode was fabricated by electropolymerization method and following overoxidation stage. The modified electrode exhibits highly electrocatalytic activity for the oxidation of AA and UA. A suitable separation of the UA and AA oxidation potential was 0.320 V, demonstrating that the proposed electrode could be used to detect UA and AA simultaneously and individually. The OPPy/GCE showed excellent selectivity, good sensitivity, and long-term stability. The proposed method has been applied to the simultaneous determination of UA and AA in healthy human blood. The reliability and stability of the modified electrode offers a good possibility for applying the technique to routine analysis of UA and AA in clinical tests and real sample analysis with satisfactory results.

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