

Simultaneous Determination of Ascorbic Acid, Dopamine, and Uric Acid by Differential Pulse Voltammetry using Tiron Modified Glassy Carbon Electrode

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A highly selective voltammetric method was developed for the simultaneous determination of ascorbic acid (AA), dopamine (DA), and uric acid (UA) using Tiron polymer film modified on glassy carbon electrode. The modified electrode separated the anodic oxidation peaks potential of AA, DA and UA with a well-defined peak separation in the presence of each other to measure AA, DA and UA individually or simultaneously without any intermolecular effect. The calibration curves were obtained over the range of 4.0-792.0 $\mu\text{mol/L}$ AA, 0.2-45.8 $\mu\text{mol/L}$ DA, and 0.06-166.0 $\mu\text{mol/L}$ UA. Detection limits of 1.79 $\mu\text{mol/L}$ AA, 0.07 $\mu\text{mol/L}$ DA, and 0.021 $\mu\text{mol/L}$ UA were obtained at pH 3.0. The interference of potential interfering substances on the determination of AA, DA and UA were studied, and the results confirm the selectivity of the method. The modified electrode was used for the determination of AA, DA, and UA simultaneously in real samples such as drugs, urine, and synthesis samples, with satisfactory results.

Keywords: Electrochemical determination; Ascorbic acid; Dopamine; Uric acid; Voltammetry

1. INTRODUCTION

Dopamine (DA), Ascorbic acid (AA), and Uric acid (UA) are compounds of great biomedical interest, playing a potential role in human metabolism. Ascorbic acid (vitamin C) is a vital component in human diet. Among animal organs, the liver, leukocytes, and anterior pituitary lobe show the highest concentration of AA. It is widely used in foods and drinks as an antioxidant. 3,4-Dihydroxyphenylethylamine, also commonly known as dopamine, is an important neurotransmitter in the mammalian central nervous system. It is currently the subject of intensive research by neuroscientists and chemists and rapid and simple methods for the determination of its concentration are sought. DA can be determined by electrochemical methods because it is an electroactive

compound. The electrochemical oxidation of DA has been studied to show that it is a two-electron irreversible process with transfer of two protons. Uric acid is the primary end product of purine metabolism. Abnormal levels of UA are symptoms of several diseases such as gout, hyperuricemia, and Lesch-Nyan disease [1]. In general, electroactive UA can irreversibly oxidize in aqueous solutions with the major product being allantoin [2,3]. UA and AA are co-present in biological fluids such as blood and urine. It is important to develop a technique to selectively detect UA in the presence of AA conveniently in a routine assay. However, the direct electro-oxidation of UA and AA at bare electrodes requires high overpotentials. The oxidation potential of UA and AA are too close to be separately determined by the use of bare electrodes.

It is known that the oxidation peaks of DA and AA at a bare electrode are at nearly the same potential, which results in overlapped voltammetric responses making their discrimination greatly difficult [4,5]. Literature numbers of studies have reported on new modified electrodes employed for simultaneous determination of DA and AA [5-8]. Moreover, at bare electrodes, DA, AA, and UA are oxidized at nearly the same potential resulting in overlapped voltammetric responses.

To overcome the above problems, modified electrodes are reported to have been developed for the single detection of AA, DA, and UA by modifying bare glassy carbon electrode (GCE), as well as platinum and gold electrode [9-11]. In recent years, substantial efforts have been devoted to the development of electrochemical sensors based on electrodes modified by electro-synthesized polymeric films. Polymeric films of 3-methylthiophene [12], aniline and pyrrole [13] and *p*-nitrobenzene resorcinol [14] are reported to be useful in the selective detection of DA in excess of AA. Poly(cresol-red) modified electrodes [15] poly(oracet blue) modified electrode [16], poly(eriochrom black T) modified electrode [17], poly(naphthalene sulfonic acid) modified electrode [18], poly(evans blue) modified electrode [19], poly(vinyl alcohol) and poly(chromotrope 2B)-modified electrodes [20] are used to detect DA and/or DA, AA, and UA, simultaneously. Ruthenium oxide-modified electrode could attain the capability for simultaneous determination of AA and DA in the presence of UA [21]. More recently, poly(4-amino-1,1'-azobenzene-3,4'-disulfonic acid)-coated electrode has been reported for the selective detection of DA in the presence of AA, UA and NADH [22] while poly(acrylic acid) multiwall carbon nanotube composite-covered GCE [23], Pt-Au hybrid film-modified electrode [24], pyrolytic graphite electrode modified [25], zinc oxide composite film [26], palladium nanoparticle-loaded carbon nanofibers [27], poly (3-(3-pyridyl) acrylic acid) [28], and 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid [29] have been reported for simultaneous determination of AA, DA, and UA.

The electropolymerization of Tiron has not been reported in the literature, and the authors were not able to find any electrochemical studies of the Tiron. In this paper, we report for the first time on a polymer film of Tiron to modify GCE and describe the electrochemical behavior of AA, DA, and UA at the surface of the modified electrode. Based on the different electrocatalytic activities of the modified electrode toward these species, a sensitive and selective method will be established for simultaneous determination of DA in the presence of AA and UA. Comparisons of the proposed method with the similar electrochemical methods are presented in Table 1. As the results showed, the proposed method is comparable with the reported methods via linear dynamic range (wider range) and/or limit of detection.

Table 1. Comparison of some characteristics of the different modified electrodes for the determination of AA, DA and UA.

Sensitivity ($\mu\text{A } (\mu\text{mol L}^{-1})$)			Limit of detection ($\mu\text{mol L}^{-1}$)			Determination range ($\mu\text{mol L}^{-1}$)			Interferences	Reference
AA	DA	UA	AA	DA	UA	AA	DA	UA		
0.052	-	0.054	0.92	-	1.1	1-400	-	2-400	Not reported	[3]
-	0.048	-	-	1.2	-	-	1.8-460	-	Not reported	[5]
-	0.34	-	-	0.005	-	-	0.55-110	-	Glucose, Tartaric acid, Citric acid	[8]
0.89	0.96	3.11	7.6	1.4	0.6	10-250	2.0-70	2.0-50	Glucose, Lysine, Cysteine	[10]
-	1.18	-	-	0.02	-	-	0.1-100	-	Glucose, Cysteine	[11]
-	0.087	-	-	0.3	-	-	5-25	-	Not reported	[14]
-	-	-	0.5	1.5	-	50-500	10-100	-	Not reported	[15]
0.0022	0.147	0.0071	1.3	0.02	0.4	65-2000	0.22-7.0	12.5-400	Not reported	[16]
0.017	1.0728	0.091	10	0.02	1.0	150-1000	0.1-200	10-130	Not reported	[17]
-	1.0	-	-	0.5	-	-	2-10	-	Not reported	[18]
0.18	0.33	0.038	0.3	0.25	2.0	5-105	1-10	30-110	Lysine, Glucose, Cystein	[19]
0.0136	0.095	-	-	0.3	-	50-400	2-80	-	Lysine, Glucose, Cystein	[20]
-	0.796	-	-	0.1	-	-	1-100	-	Not reported	[23]
-	-	-	-	0.02	0.01	-	0.04-3	0.3-10	Not reported	[24]
0.008	0.05	0.016	103	24	21	103-165	24-384	21-336	Not reported	[25]
0.012	1.87	1.52	13	0.11	1.4	25-500	1-20	2.5-30	Not reported	[26]
-	0.25	-	1.4	0.7	4.5	15-240	6.0-960	50-800	Not reported	[27]
-	-	-	15	0.2	0.7	50-4000	0.5-160	2-200	Not reported	[28]
0.11	0.054	0.35	1.43	0.29	0.016	5-240	5-280	0.1-18	Asprin, Tyrosine, Copper(II)	[29]
0.16	-	-	2.52	-	-	5-50	-	-	Formaldehyde	[34]
5.285	-	-	0.51	-	-	6-800	-	-	Not reported	[35]
-	-	-	-	20	50	-	100-300	60-600	Not reported	[36]
-	0.0074	-	-	0.1	-	-	50-250	-	Not reported	[37]
0.007	0.33	0.093	1.78	0.075	0.021	4.0-792	0.2-45.8	0.06-166	Not found	This work

2. EXPERIMENTAL PART

2.1. Reagents and Apparatus

All chemicals were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments. Uric acid was purchased from Sigma-Aldrich

(Milwaukee, USA). Dopamine and ascorbic acid were obtained from Merck (Merck, Darmstadt, Germany). Phosphate buffer solutions (PBS) with different pH values were prepared by mixing 0.20 mol/L Na_2HPO_4 and 0.20 mol/L NaH_2PO_4 . pH values were adjusted by addition of 1.0 mol/L H_3PO_4 and/or NaOH solution.

The stock solutions of dopamine (0.010 mol/L) and ascorbic acid (0.010 mol/L) were prepared daily by dissolving dopamine hydrochloride and ascorbic acid in water. Uric acid solution (0.010 mol/L) was prepared by dissolving the solid in a small volume of 0.1 mol/L NaOH solution and diluted to desired concentration. Other dilute standard solutions were prepared by appropriate dilution of the stock solutions in PBS, pH 3.0.

A Corning pH-meter, Model 140 (Switzerland), with a glass electrode (conjugated with an Ag/AgCl reference electrode, Model 6.0232.100) was used to determine pH levels of the solutions.

Voltamograms were obtained using a Metrohm instrument, Model 797 VA (Switzerland) processor, with three electrodes consisting of a platinum wire as an auxiliary electrode, the poly-Tiron film modified glassy carbon electrode (PTFMGCE) as a working electrode, and with Ag/AgCl as a reference electrode.

2.2. Preparation of poly-Tiron Modified Glassy Carbon Electrode

Prior to each experiment, the glassy carbon electrode (GCE) was polished with 0.05- μm alumina in a water surry using a polishing cloth. The GCE was subsequently sonicated in a mixture of water/ethanol after each polishing step to be electrochemically pretreated later by cycling at a scan rate of 100 mV/s for 10 cycles in 0.1 mol/L H_2SO_4 solution (Yao et al. 2007) in the potential range of -0.40 to 1.50 V. Subsequently, the electrode was placed in a solution containing 0.2 mol/L NaOH and 1.0 mmol/L Tiron and cyclic potential sweep was applied in the range of +0.10 and +1.30 at a scan rate of 100 mV/s for 20 times. After the polymerization, the modified electrode was washed with water and scan cycled in pH 3.0 (PBS) between -0.30 and 0.80 for 20 times to increase its reproducibility.

2.3. Procedure

Ten milliliters of buffer solution (pH 3.0) was transferred into an electrochemical cell using the three-electrode system containing PTFMGCE. Then, the DP voltammogram was recorded from -0.10 to 0.60 V (with a pulse amplitude of 50 mV, a pulse time of 0.04 s, a voltage step time of 0.1 s, and a voltage step of 5 mV) with a potential scan rate of 60 mV/s. The peak current was measured and recorded as a blank signal (I_b). After the background voltammogram had been obtained, aliquots of the sample solution containing AA, DA, and/or UA were introduced into the cell. Then, the DP voltammogram was recorded as described above to give the sample peak current. The peak current was measured and recorded as a sample signal (I_s). All the data were obtained at 25 °C. The difference in current ($I_p_s - I_p_b$) was considered as a net signal (ΔI_p) for each of the species. Calibration graphs were prepared by plotting the net peak currents vs. AA, DA and/or UA concentrations in solution.

2.4. Real Sample Preparation

Ten tablets of vitamin C (labeled 500 mg vitamin C per tablet, 10 tablets) were completely ground and homogenized, 140 mg of which was accurately weighed and dissolved with ultrasonication in 25 mL of water. Then, 100 μL of the solution plus 5 mL of the buffer (pH 3.0) was diluted with water in a 10-mL volume flask and the resulting solution was used for analysis. Dopamine hydrochloride injection solution (40 mg mL^{-1}) was analyzed directly after it was diluted 100 times with water. 0.10 mL of the diluted solution was injected into a 10-mL volumetric flask and made up to volume with the buffer solution (pH 3.0). The test solution was then transferred into the electrochemical cell and the AA, DA, and UA contents were determined according to the recommended procedure. Urine samples were analyzed directly after dilution for 25 times with the buffer solution (pH 3.0) without any further pretreatment. Then, an aliquot 10 mL of this test solution was transferred into the electrochemical cell and the AA, DA, and UA contents were determined according to the recommended procedure.

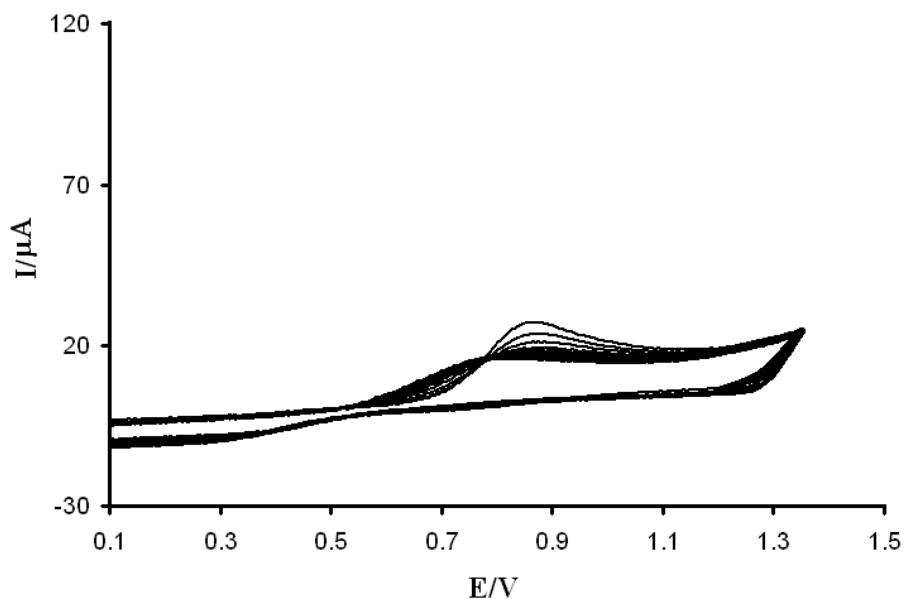


Figure 1. Cyclic voltammetry of electropolymerization of Tiron at 100 mV/s.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Properties Of Poly-Tiron Film Modified GCE

Figure 1 depicts the electrochemical polymerization of Tiron onto a GCE over the potential range of +0.10 to +1.30 V for 20 cycles at a scan rate of 100 mV/s in Tiron solution. As can be seen in the cyclic voltammogram, an anodic peak appeared at 0.86 V due to the oxidation of Tiron monomer gradually decreased with cyclic time increasing, whereas on the scan reversal, a cathodic wave formed

at a potential of 0.33 V. These anodic and cathodic peak potentials tended to be stable after 20 scans. These suggest that the initially-formed oligomer and/or Tiron film had a leaching process with the scan cycle increasing up to 20 times, which may imply a self-adjustment of the polymer film thickness at the GCE (Yao et al. 2007). During the polymerization of Tiron, Tiron was first deposited at the surface of GCE and oxidized to form quinone, whose structure was subsequently reduced to Tiron at the reverse scan.

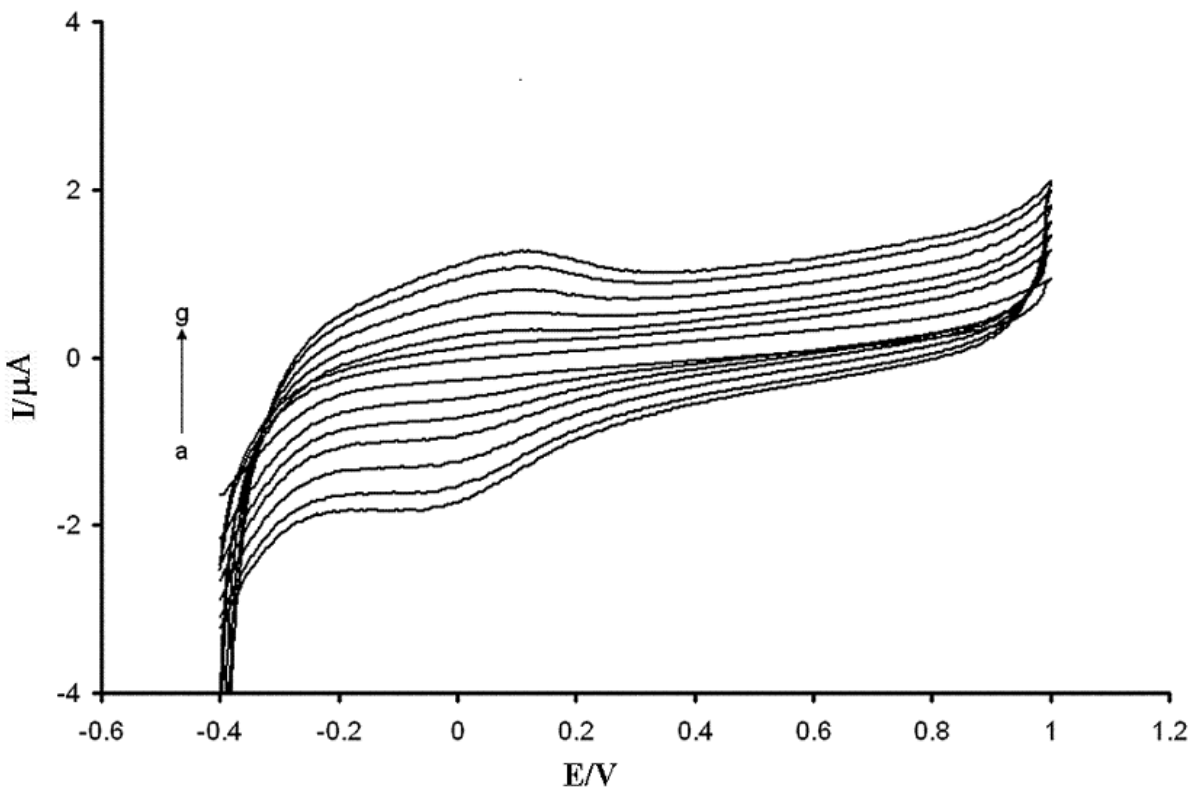


Figure 2. Cyclic voltammograms of poly-Tiron modified GCE in PBS (pH 3.0) at various scan rates; a) 10 mV/s, b) 30 mV/s, c) 50 mV/s, d) 70 mV/s, e) 90 mV/s, f) 110 mV/s, and g) 130 mV/s.

Cyclic voltammograms of PTFMGCE in PBS (pH 3.0) at different scan rates are shown in Fig. 2. Two pairs of reduction and oxidation peak currents obtained in each cycle. The anodic peak current (I_{pa}) was linearly dependent on the scan rate (ν) with the regression equation $I(\mu A) = 0.0209 + 12.42\nu$ ($V s^{-1}$) ($r^2 = 0.9996$) and the ratio of the anodic and cathodic peak current (I_{pa}/I_{pc}) being nearly equal to unity. These behaviors are consistent with diffusionless systems or with reversible electron transfer processes at low scan rates [30]. The separation of the peak potentials (ΔE_p) was 58 mV at a low scan rate (20 mV/s) although ΔE_p would not change with increasing scan rate. So, it is suggested that the poly-Tiron film modified electrode reaction could be a one electron transfer process ($n = 1$), because the oxidized form hold also aromaticity. From the behavior of the modified GCE with scan rate, we can conclude that the electrode reaction was a quasi-reversible electron transfer [31]. Therefore, the peak current must be related to the surface concentration of electroactive species, Γ , using

$$I_p = n^2 F^2 A \Gamma v / 4RT \quad (1)$$

where, n represents the number of electrons involved in the reaction ($n = 1$), A is the surface area of the electrode (0.0314 cm^2), I_p is the peak current, Γ represents the surface coverage concentration (mol cm^{-2}), and v is the scan rate. From the slope of the anodic peak currents vs. scan rate, the calculated surface concentration of Tiron is $4.2 \times 10^{-10} \text{ mol cm}^{-2}$.

The electrochemical response of PTFMGCE depends on the pH value of the supporting electrolyte solution. By increasing the pH level of the supporting electrolyte (from 2.0 to 8.0), the oxidation peak potentials shifted to negative values linearly. The slope of the linear equation was -54.7 mV pH^{-1} , implying the ratio of the precipitated protons to the transferred through the poly-Tiron film was 1:1.

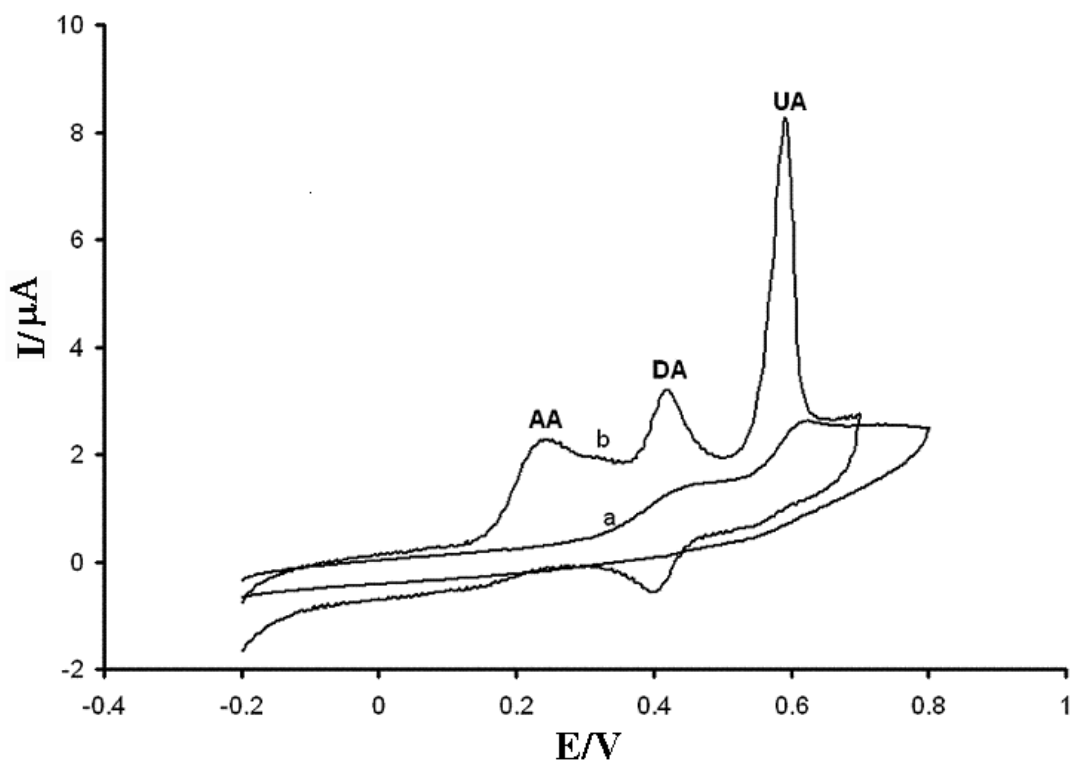


Figure 3. Cyclic voltammograms of $100.0 \mu\text{mol/L}$ UA, $200.0 \mu\text{mol/L}$ AA and $50.0 \mu\text{mol/L}$ DA at a) a bare GCE; b) the modified glassy carbon electrode in buffer solution (pH 3.0) with voltage step 5 mV and scan rate of 100 mV/s .

3.2. Electrooxidation of AA, DA and UA at the Surface of Poly-Tiron GCE

Figure 3 shows the oxidation of AA, DA, and UA at the surface of a bare GCE and PTFMGCE. The results showed that all three compounds were oxidized with well defined and distinguishable

sharp peak potentials. On the other hand, the indistinguishable and broad peak potentials at a bare GCE indicate a slow electron transfer kinetic. The oxidation peak potentials of AA, DA, and UA on the modified electrode separated completely into three well-defined peaks with 0.19, 0.41, and 0.59 V vs. Ag/AgCl, respectively. In addition, all the three peak potentials in PTFMGCE had positive potential shifts. These shifts in the oxidation peak potentials and enhanced currents of the oxidation peak potential with PTFMGCE indicate that the modified electrode plays a catalytic effect on the oxidation of AA, DA, and UA, but the catalytic role of PTFMGCE for AA is stronger than DA and UA. Our studied showed that the oxidation peak potentials were 0.41, 0.43, and 0.60 V at pH= 3.0 at the bare GCE for AA, DA, and UA, respectively.

The influence of the scan rate on the anodic peak current of AA was studied by cyclic voltammetry (Fig. 4). The results showed that the peak current increased by increasing the scan rate. The good linear relationship between $v^{1/2}$ and I_{pa} within the scan rate of 10 to 170 mV s^{-1} confirms a diffusion-controlled process on the modified electrode ($r^2 = 0.9993$).

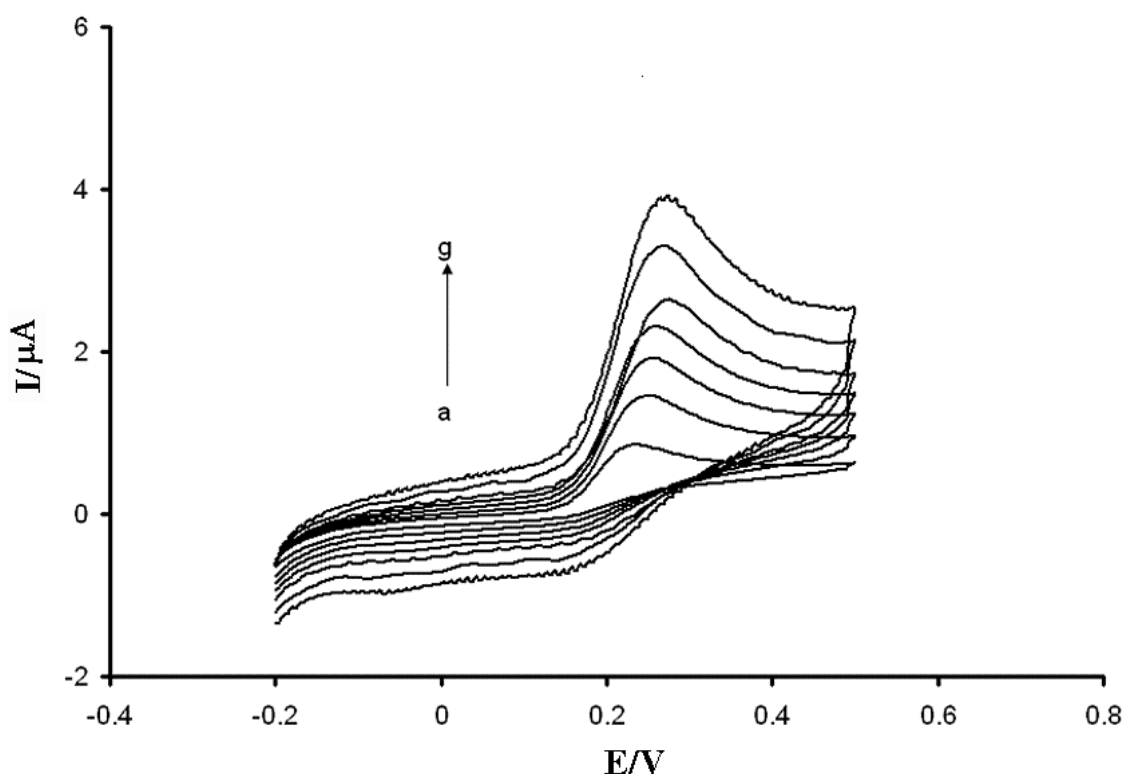


Figure 4. Cyclic voltammograms of 200.0 $\mu\text{mol/L}$ AA at the polymer modified electrode with various scan rates as: a) 10; b) 30; c) 50; d) 70; e) 90; f) 130; and g) 170 mV/s .

3.3. Experimental Variables

Since differential pulse voltammetry (DPV) has a much higher sensitivity and resolution than cyclic voltammetry, DPV was used for simultaneous determination of AA, DA, and UA. The influence

of the pH level of the electrolyte and DPV parameter on peak separation and peak current were studied. The results showed that the peak current of AA decreased from a pH level of 3.0 to higher pH values, whereas for DA and UA it decreased the signal by up to a pH level of 5.0. As is known, the pK_a values of R-SO₃H (R = aryl group) are usually about 4; therefore, the -SO₃Na of poly-Tiron film could dissociate favorably into a negative charge group -SO₃⁻ under this condition. The alkaline -NH₂ group of DA ($pK_a = 8.9$) could obtain a proton and form of a positive ion of DA. This has a great affinity toward the poly-Tiron film. In addition, for AA, the maximum current obtains at a pH level of 3.0 ($pK_1 = 4.17$) and for UA, the anodic current also reaches its maximum at a pH level of 3.0. Therefore, for simultaneous determination of these compounds a pH value of 3.0 (PBS, 0.05 mol/L) was selected for further study.

The DPV parameters including pulse amplitude, pulse time, and voltage step time changed when the concentration of AA, DA, and UA on the cell were 150, 160, and 20 $\mu\text{mol/L}$. The results showed that maximum peak current obtained when the pulse amplitude was 50 mV, the pulse time was 0.05 s, and the voltage step time was 0.1 s. These values were selected for further study.

4. SIMULTANEOUS DETERMINATION of AA, DA, and UA

As our results showed, the PTFMGCE possessed a higher active surface area and excellent electrocatalytic activity for AA, DA, and UA. The difference in the oxidation peak potentials for AA-DA and DA-UA were 0.21 and 0.18 V respectively, which were large enough separations to allow for the simultaneous determination of AA, DA, and UA in a mixture. The electrooxidation processes of AA, DA, and UA in the mixture evaluated by varying the concentration of the individual analytic species. The analytical parameters for the simultaneous determination of AA, DA, and UA are presented in Table 2.

Table 2. Calibration curves parameters for determination of DA, AA and UA.

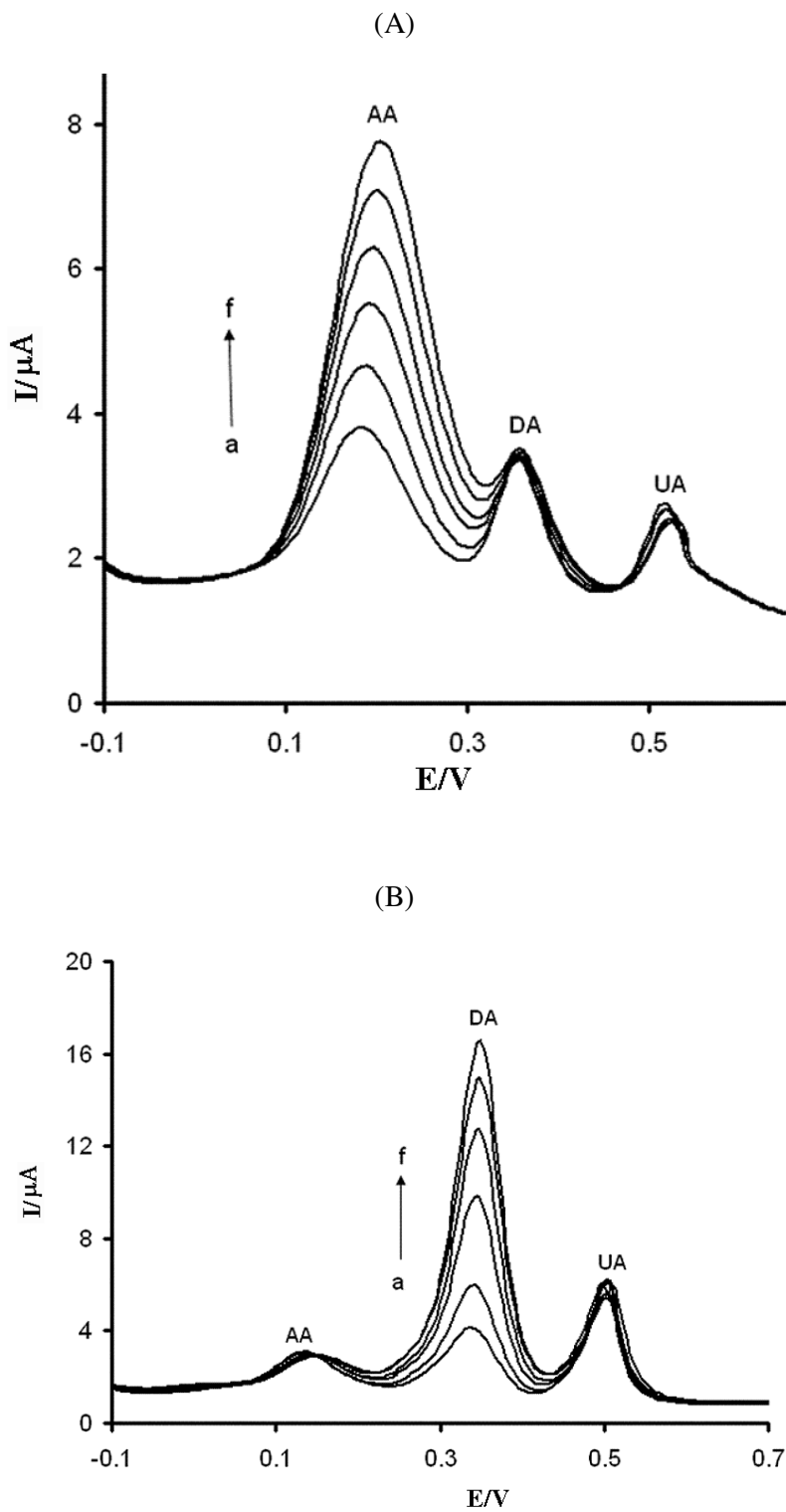
Analyte	Regression equation	r^2	RSD% ^a	LOD ^b ($\mu\text{mol L}^{-1}$)	LDR ^c ($\mu\text{mol L}^{-1}$)
AA	$I(\mu\text{A}) = 0.0073C + 0.5628$	0.999	1.8	1.78	4.0 - 792.0
DA	$I(\mu\text{A}) = 0.3316C + 2.5000$	0.991	2.3	0.075	0.20 - 45.8
UA	$I(\mu\text{A}) = 0.0929C + 0.3498$	0.992	5.3	0.021	0.06 - 166.0

^{a.} Relative standard deviation.

^{b.} Limit of detection.

^{c.} Linear dynamic range.

Detection limits of $1.78 \mu\text{mol L}^{-1}$ AA, $0.075 \mu\text{mol L}^{-1}$ DA, and $0.021 \mu\text{mol L}^{-1}$ UA were obtained at pH 3.0.



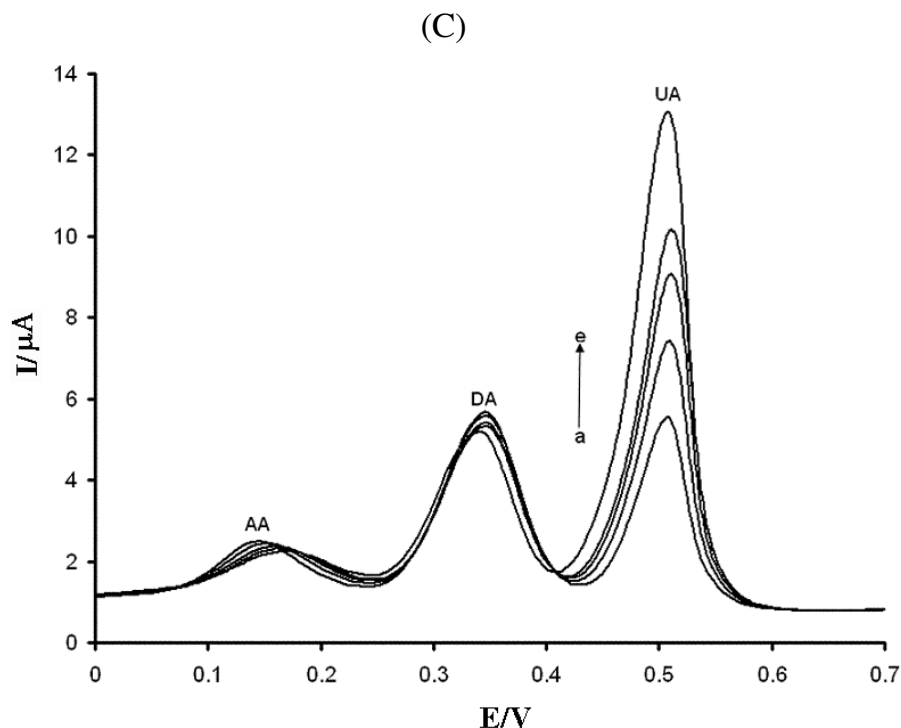


Figure 5. (A): DPV graphs of a) 196.0; b) 291.0; c) 384.0; d) 470.6; e) 566.0; and f) 655.0 $\mu\text{mol/L}$ AA, in the presence of 10.0 $\mu\text{mol/L}$ DA and 10.0 $\mu\text{mol/L}$ UA. (B): DPV graphs of a) 9.0; b) 11.8; c) 23.5; d) 29.0; e) 40.5; and f) 45.8 $\mu\text{mol/L}$ DA in the presence of 100.0 AA and 20.0 $\mu\text{mol/L}$ UA. (C): DPV graphs of a) 15.0; b) 30.0; c) 45.0; d) 55.0; and e) 85.0 $\mu\text{mol/L}$ UA in the presence of 100.0 AA and 4.0 $\mu\text{mol/L}$ DA in buffer solution (pH 3.0) at the PTFMGCE.

In order to check the intermolecular effects between AA, DA, and UA, three different experiments were carried out under optimum conditions at a pH 3.0. In each experiment, the concentration of one of the three compounds was changed while keeping the concentrations of the other two constant. The results are shown in Figs. 5A, 5B and 5C. The peak currents for AA, DA, and UA increase linearly with increases in their respective concentrations without remarkably affecting the other peak currents. They also indicate that the peak current of AA increased linearity with a correlation coefficient of 0.999 while AA concentration increased (Fig. 5A). In addition, different concentrations of DA in the presence of 100.0 $\mu\text{mol/L}$ AA and 20.0 $\mu\text{mol/L}$ UA exhibit excellent DPV responses to AA, DA, and UA without any obvious intermolecular effects among them, with a correlation coefficient of 0.991 when DA concentration increased (Fig. 5B).

We also examined the influence of AA and DA on the oxidation of UA under the optimum conditions at a pH 3.0 using the modified GCE. As shown in Fig. 5C, no obvious change was observed in the DA and AA peak currents while changing the UA concentration. These results confirm that the oxidation processes of AA, DA, and UA at PTFMGCE are independent from each other, and that they help the simultaneous determination of these three compounds without any interference.

5. INTERFERENCE STUDY

The influence of potential interfering substances on the determination of these compounds were investigated. The tolerance limit was defined as the maximum concentration of foreign substances, with a relative error of less than 5%. Interference studies were conducted by exposing the PTFMGCE in a solution containing 100.0 $\mu\text{mol/L}$ AA, 20.0 $\mu\text{mol/L}$ DA and 50.0 $\mu\text{mol/L}$ UA plus the interfering substance at a pH 3.0. The DPV responses resulting from the presence of interfering substances were obtained for AA and DA plus UA. The results are presented in Table 3.

Table 3. Interference of some foreign substances for 20.0 $\mu\text{mol/L}$ DA, 100.0 $\mu\text{mol L}^{-1}$ AA, and 50.0 $\mu\text{mol L}^{-1}$ UA.

Species	AA	DA	UA
Glycine	100	1000 ^a	600
Citric acid	80	30	150
Aspartic acid	300	250	50
Aspirin	30	25	30
Urea	150	400	200
Fructose, Sucrose, Glucose	300	300	400
Oxalate	50	30	180
Mg ²⁺ , Ca ²⁺	400	1000	600

^a. Maximum concentration of the substances used.

6. REAL AND SYNTHESIS SAMPLES ANALYSIS

In order to evaluate the applicability of the proposed method for the determination of AA, DA, and UA in real samples, the developed method was tested by determining these compounds in several model (mixed) samples. The results are summarized in Table 4. The utilization of the proposed method in real sample analysis was also investigated by direct analysis of urea and pharmaceutical samples without any pretreatments. DA and AA in injection solutions and in vitamin C tablets, and DA plus UA in urine samples were determined using the proposed method. The results are presented in Table 5. In addition, comparisons were also made between the results obtained from the proposed method and those from the official method [32,33] to confirm the lack of any significant differences between the two methods.

Table 4. Simultaneous determination of DA, AA and UA in mixtures synthesis samples^a.

Sample	Added ($\mu\text{mol L}^{-1}$)			Found ($\mu\text{mol L}^{-1}$)			Recovery (%)		
	AA	DA	UA	AA	DA	UA	AA	DA	UA
1	200.0	10.0	30.0	201.4(± 4.0)	9.7(± 0.6)	31.7(± 2.3)	100.9	97.1	105.6
2	600.0	30.0	60.0	568.0(± 6.0)	29.5(± 2.0)	61.1(± 5.6)	94.7	98.3	101.8
3	400.0	20.0	100.0	398.0(± 4.1)	21.0(± 0.7)	94.8(± 8.0)	99.5	105.1	94.8

^a. Number in the parenthesis show the standard deviation for n = 3.

Table 5. Determination DA, AA and UA in real samples.

Sample		Added ($\mu\text{mol L}^{-1}$)	Proposed method ($\mu\text{mol L}^{-1}$)	Recovery (%)	Standard method ($\mu\text{mol L}^{-1}$)
Vitamin C ^a	AA	-	101.23 \pm 2.3	-	100.3 \pm 3.3
Vitamin C ^a	AA	200	275.4 \pm 13.2	91.7	-
Urine 1	UA	-	5.25 \pm 0.35	-	5.34 \pm 0.16
	UA	10.0	14.73 \pm 0.75	96.0	-
	AA	100.0	104.3 \pm 3.25	104.3	-
	DA	10.0	10.3 \pm 0.56	103.3	-
Urine 2	UA	-	4.35 \pm 0.41	-	4.10 \pm 0.26
	UA	40.0	42.23 \pm 0.34	95.7	-
	AA	200.0	195.4 \pm 5.63	99.7	-
	DA	30.0	27.89 \pm 1.3	93.0	-
Dopamine ^b	DA	-	20.23 \pm 0.54	-	20.68 \pm 0.67
Dopamine ^b	DA	10.0	30.87 \pm 0.59	100.6	-
Vitamin C ^c	AA	-	300.89 \pm 7.8	-	301.65 \pm 15.0
Vitamin C ^c	AA	200.0	498.3 \pm 13.1	99.3	-

^a. Vitamin C: Swiss Natural Sources (500 mg).

^b. Dopamine ampoule, 200 mg/5mL, Caspian Tamin Pharmaceutical Co., Rasht, Iran.

^c. Vitamin C ampoule, 500mg/5mL, Darou Pakhsh Co., Iran.

7. CONCLUSION

This study has indicated that the poly-Tiron film modified glassy carbon electrode exhibits electrocatalytic activity to AA, DA, and UA oxidation. The modified electrode not only improved the

electrochemical catalytic oxidation of AA, DA, and UA, but also resolved the overlapping anodic peaks. The results in Table 1 confirmed that although the proposed method has a lower sensitivity for AA than the other electrochemical methods, its sensitivity and detection limit are better for DA and UA analysis. Moreover, the proposed method was successfully applied for the determination of these compounds in real samples.

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