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## Simultaneous Determination of Ascorbic Acid, Dopamine, and Uric Acid by Differential Pulse Voltammetry Using Poly(Chromazurol s) Modified Glassy Carbon Electrode

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### Abstract

The Chromazurol s has been electropolymerized on the glassy carbon electrode and the Poly- Chromazurol s modified glassy carbon electrode (PCSMGCE) has shown excellent electrocatalytic activity toward the oxidation of ascorbic acid in phosphate buffer solution (pH=3). It was used for the simultaneous determination of ascorbic acid (AA), dopamine (DA), and uric acid (UA). Analytical parameters were optimized and electrochemical behavior of modified electrode was studied. The calibration curves were obtained over the range of 3.0-2200.0  $\mu\text{mol L}^{-1}$  AA, 0.09-980.0  $\mu\text{mol L}^{-1}$  DA, and 0.05-900.0  $\mu\text{mol L}^{-1}$  UA. Detection limits of 1.7  $\mu\text{mol L}^{-1}$  AA, 0.05  $\mu\text{mol L}^{-1}$  DA, and 0.03  $\mu\text{mol L}^{-1}$  UA were obtained at pH 3.0. Prepared modified electrode had satisfactory results for determination of DA, AA and UA in dopamine injection solution, vitamin C tablets and human urine samples.

**Keywords:** Poly(Chromazurol s), Glassy Carbon Electrode, Ascorbic acid, Dopamine, Uric acid, Differential pulse voltammetry.

### 1. Introduction

In last decade, chemically modified electrodes have been used remarkably for determination of pharmacological and biological compounds [1-7]. Interaction between modifier and analyte, causes decreasing overpotential and increasing signal. In some cases a modifier has different interactions with different species. When an analyte has interaction with modifier and another analyte has no or weak interaction, potential shifting will be different and simultaneous determination of both would be possible. Electropolymerization is one of the methods for produce sensitive and selective electrocatalyst layer on the electrodes. In this method direct formation of polymer from monomers produces a thin electroactive polymeric film onto the surface of the electrode.

Ascorbic acid (AA), Vitamin C, is an electron donor in metabolic reactions and used for treatment of scurvy [8]. In addition, ascorbic acid is found in high concentration in some fruits and foods and commonly used as antioxidant food additives [9]. Electrochemical determination of AA by direct oxidation is difficult because of its large overpotential and fouling by the oxidation products [10].

Dopamine is a highly conserved catecholaminergic neurotransmitter in the mammalian brain and it has

important role in the control of movement, human behavior and brain pathological processes [11]. In addition to a wide variety of electrochemical techniques [12-14], other analytical methods such as HPLC-MS [15], Chemiluminescence [16], and Fluorimetry are also used for determination of DA in biological samples [17,18].

Ascorbic acid, uric acid and dopamine play important roles in physiological function of organisms. Several modified electrodes have been reported in the literature for simultaneous determination of DA, AA and UA. In table 1, the detection limit, linear dynamic range and the sensitivity for DA, AA and UA obtained in this work has been compared with several other modified electrodes.

The present paper is intended to describe a new method for simultaneous determination of Dopamine, Ascorbic Acid and Uric Acid based on electrocatalytic activity of Chromazurol s polymeric thin film on glassy carbon electrode. Characteristics of modified electrode and analytical parameters were optimized. Then electrochemical behavior of electrode was studied using cyclic voltammetry and chronoamperometry techniques. Finally, under the optimized conditions, prepared electrode was used for measurement of desired analytes in dopamine injection solution,

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

Linear Dynamic Range ( $\mu\text{mol L}^{-1}$ )			Detection limit ( $\mu\text{mol L}^{-1}$ )			Sensitivity ( $\mu\text{A } \mu\text{mol L}^{-1}$ )			Interferences	Ref.
AA	DA	UA	AA	DA	UA	AA	DA	UA		
5-105	1-10	30-110	0.3	0.25	2.0	0.18	0.33	0.038	Lysine, Glucose, Cystein	[19]
50-400	2-80	-	-	0.3	-	0.0136	0.095	-	Lysine, Glucose, Cystein	[20]
5-240	5-280	0.1-18	1.43	0.29	0.016	0.11	0.05	0.35	Aspirin, tyrosine, copper (II)	[21]
-	0.55-110	-	-	0.005	-	-	0.34	-	Glucose, Tartaric acid, Citric acid	[22]
65-2000	0.22-7.0	12.5-400	1.3	0.02	0.4	0.0022	0.147	0.0071	Not reported	[23]
150-1000	0.1-200	10-130	10	0.02	1.0	0.017	1.0728	0.091	Not reported	[24]
103-165	24-384	21-336	103	24	21	0.008	0.05	0.016	Not reported	[25]
25-500	1-20	2.5-30	13	0.11	1.4	0.012	1.87	1.52	Not reported	[26]
15-240	6.0-960	50-800	1.4	0.7	4.5	-	0.25	-	Not reported	[27]
5-1300	0.2-470.3	1-100	1.7	0.08	0.11	0.006	0.08	0.1	Aspirin, Oxalate	[28]
-	1.8-460	-	-	1.2	-	-	0.048	-	Not reported	[29]
10-250	2.0-70	2.0-50	7.6	1.4	0.6	0.89	0.96	3.11	Glucose, Lysine, Cysteine	[30]
-	0.1-100	-	-	0.02	-	-	1.18	-	Glucose, Cysteine	[31]
3-2200	0.09-980.0	0.05-900.0	1.7	0.05	0.03	0.006	0.01	0.02	Aspirin, Oxalate	This work

vitamin C tablets and human urine samples by differential pulse voltammetry.

## 2. Experimental

### 2.1. Apparatus and chemicals

Electrochemical techniques were carried out using a computerized potentiostat/galvanostat (Autolab PGSTAT101, Utrecht, The Netherlands). A Pentium IV computer controlled all settings and data processing of the system. All the electrochemical studies were performed at  $25 \pm 1$  °C. A conventional three-electrode cell was used which consists of the Poly(Chromazurol s) Modified Glassy Carbon Electrode, a platinum wire and a Ag/AgCl reference electrode as working, auxiliary and reference electrode, respectively. The pH of the solutions was controlled with a Metrohm pH meter (model 827).

Uric acid was purchased from Sigma-Aldrich. Dopamine and ascorbic acid were obtained from Merck. Other chemicals were of analytical grade. The water was deionized using a Milli-Q water purification system (resistance  $> 18 \text{ M}\Omega/\text{cm}$ ).

Phosphate buffer solutions (PBS) with different pH values were prepared by mixing  $0.20 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4$  and  $0.20 \text{ mol L}^{-1} \text{ NaH}_2\text{PO}_4$ . pH values were adjusted by addition of  $1.0 \text{ mol L}^{-1} \text{ H}_3\text{PO}_4$  and/or NaOH solution.

A  $0.010 \text{ mol L}^{-1}$  dopamine solution and a  $0.010 \text{ mol L}^{-1}$  ascorbic acid solution were prepared daily by dissolving adequate amounts of dopamine hydrochloride and ascorbic acid in water. Uric acid solution ( $0.010 \text{ mol L}^{-1}$ ) was prepared by dissolving the solid in a small volume of  $0.1 \text{ mol L}^{-1}$  NaOH solution and diluted to desired concentration. Other dilute solutions were prepared by appropriate dilution of these solutions in PBS, pH 3.0.

### 2.2. Preparation of Real sample

Dopamine hydrochloride injection solution ( $40 \text{ mg mL}^{-1}$ ) was diluted 100 times with PBS, pH 3.0 and an aliquot 10 mL of this solution was transferred into the electrochemical cell for analysis according to the recommended procedure

Ten tablets of vitamin C (labeled 500 mg vitamin C per tablet, 10 tablets) were completely ground and homogenized. Then 140 mg of prepared powder was accurately weighed and dissolved in water with help of ultrasonication in 25 mL. Afterward, 100  $\mu\text{L}$  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.

Urine samples were analyzed directly after dilution for 25 times with the buffer solution (pH 3.0) without any further pretreatment.

### 2.3. Preparation of Poly- Chromazurol s Modified GC Electrode (PCSMGCE)

In order to preparation of the surface of glassy carbon electrode (GCE), it was polished with  $0.05\text{-}\mu\text{m}$  alumina slurry on polishing cloth and rinsed with water. Then it was sonicated in a mixture of water/ethanol. Subsequently, the electrode was placed in a solution containing  $0.2 \text{ mol L}^{-1}$  NaOH and  $1.0 \text{ mmol L}^{-1}$  Chromazurol s and cyclic potential sweep was applied in the range of -0.10 and 1.20 at a scan rate of  $100 \text{ mV s}^{-1}$  for 25 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 10 times to increase its reproducibility.

### 2.4. Recommended Procedure

Ten milliliters of buffer solution (pH 3.0) was transferred into an electrochemical cell using the three-electrode system containing Poly- Chromazurol s

Modified GC Electrode. 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 voltage step of 5 mV with a potential scan rate of 60 mV s<sup>-1</sup>). 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 room temperature. The difference in current ( $I_p$  -  $I_b$ ) was considered as a net signal ( $\Delta I_p$ ) for each of the species. Calibration graphs were prepared by plotting the net peak currents vs. AA, DA and/or UA concentrations in solution.

Chronoamperometric studies were carried out using 10mL of the buffer (pH 3.0) and DA with a potential step number of 1, potentials of 0.40 V and a sampling current of 0.05 over 10 s. Poly-Chromazurol s Modified GC was used as a working electrode.

### 3. Results and discussion

#### 3.1. Formation of Poly-Chromazurol s thin film and electrochemical study of PCSMGCE

The electrochemical polymerization of Chromazurol s onto a GCE fall out when the potential range of -0.10 to +1.20V apply for 25 cycles at a scan rate of 100mVs<sup>-1</sup> in solution. Figure 1 shows that an anodic peak appeared at 0.48V due to the oxidation of Chromazurol s monomer. Experiments showed that after 22 cycles, the anodic peak potential and current tended to be stable and the peak current was nearly constant. Thus, 25 cycles was selected for further study. Decreasing anodic peak currents in polymerization process with increasing the number of cycles, may be due to leaching process in first steps and self-adjustment of the polymer film thickness at the GCE in further cycles [24].

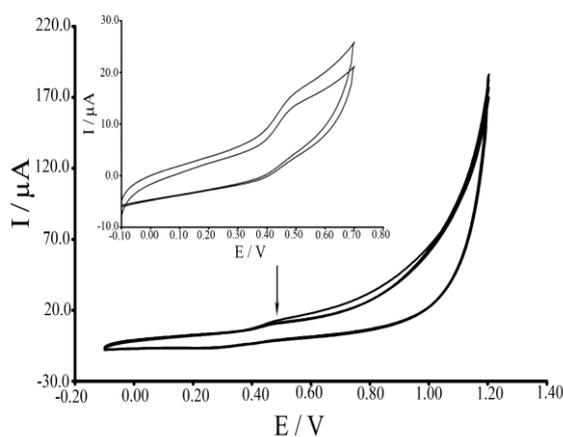


Figure 1. Cyclic voltammetry of electropolymerization of poly(Chromazurol s) at 100mVs<sup>-1</sup> (vertical arrow shows decreasing the peak current during cycles).

Cyclic voltammograms of Poly-Chromazurol s Modified GC Electrode in PBS (pH 3.0) at different scan rates have been shown in Figure 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 ( $v$ ) with the regression equation  $I (\mu A) = 1.69 (\pm 0.1) + 23.83 (\pm 0.8) v (V s^{-1})$  ( $r^2 = 0.997$ ) and the ratio of the anodic to cathodic peak currents ( $I_{pa}/I_{pc}$ ) being nearly equal to unity. These behaviors are consistent with diffusionless systems or with reversible electron transfer processes at low scan rates [32]. The separation of the peak potentials ( $\Delta E_p$ ) was 60mV at a low scan rate (30mVs<sup>-1</sup>) although  $\Delta E_p$  would not change with increasing scan rate. Poly (Chromazurol s) was first deposited at the surface of GCE and oxidized to form a quinone, whose structure (scheme 1) was subsequently reduced to Chromazurol s at the reverse scan. So, it is suggested that the poly(Chromazurol s) film modified electrode reaction could be a one electron transfer process ( $n = 1$ ), because the oxidized form hold also aromaticity. Based on behavior of the modified GCE with scan rate, reaction is maybe a quasi-reversible electron transfer [28]. Therefore, the peak current must be related to the surface concentration of electroactive species,  $\Gamma$ , 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<sup>2</sup>),  $I_p$  is the peak current,  $\Gamma$  represents the surface coverage concentration (mol cm<sup>-2</sup>), and  $v$  is the scan rate. From the slope of the anodic peak currents vs. scan rate, the calculated surface concentration of Chromazurol s is  $8.10 \times 10^{-10}$  mol cm<sup>-2</sup>.

The electrochemical response of electrode depends on the pH value of the supporting electrolyte solution. By increasing the pH level of the supporting electrolyte (from 2.0 to 6.0), the redox and oxidation peak potentials shifted negatively, and the anodic peak potential ( $E_{pa}$ ) depended linearly on pH level ( $I(\mu A) = 0.653 - 0.057 \text{ pH}$ ).

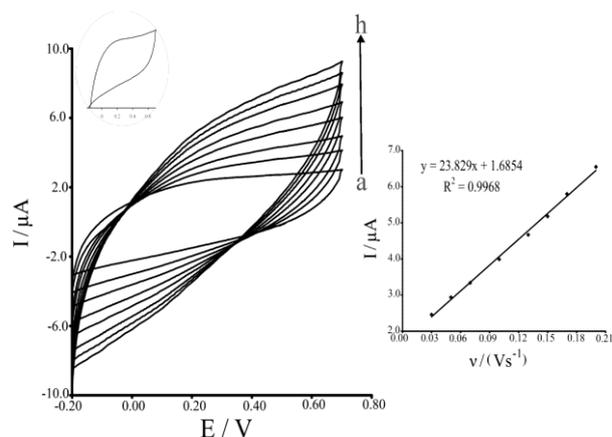
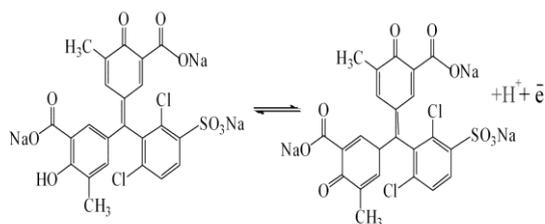


Figure 2. Cyclic voltammograms of poly(Chromazurol s) film modified glassy carbon in PBS (pH 3.0) at various scan rates a) 30; b) 50; c) 70; d) 100; e) 130; f) 150; g) 170; and h) 200 mV s<sup>-1</sup>. Inset, linear relationship between anodic peak current ( $I_{pa}$ ) and scan rate ( $v$ ).



Scheme 1. Electrooxidation of Chromazurol s at GCE.

### 3.2 Electrochemical behavior of AA, DA and UA at the Surface of PCSMGCE

AA, DA, and UA were oxidized at the surface of PCSMGCE with well defined and distinguishable sharp peak potentials with 0.14, 0.33, and 0.49 V vs. Ag/AgCl (Figure 3). But, at a bare GCE they had broad peak potentials indicate a slow electron transfer kinetic. In addition, all the peak currents at Poly(Chromazurol s)-GCE had positive potential shifts and enhancement in currents indicate that the modified electrode plays a catalytic effect on the oxidation of AA, DA, and UA.

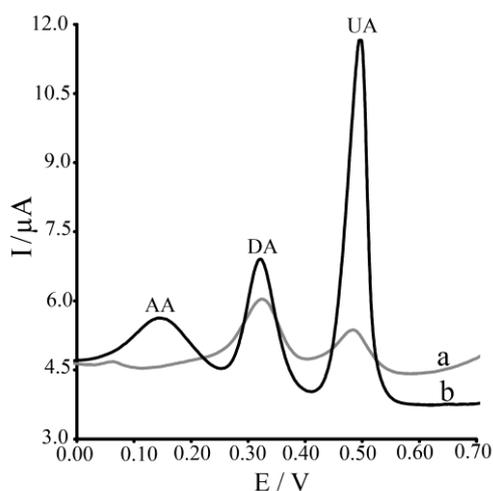
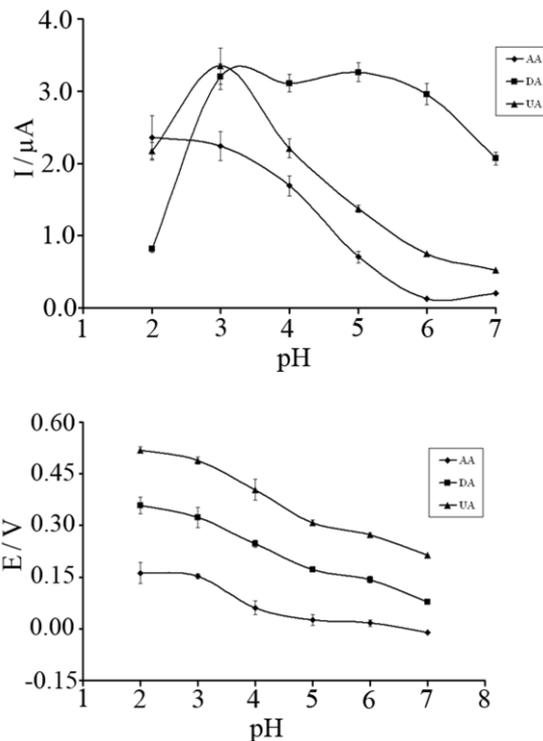


Figure 3. DPV graphs of 200.0  $\mu\text{mol L}^{-1}$  UA, 17.0  $\mu\text{mol L}^{-1}$  AA and 17.0  $\mu\text{mol L}^{-1}$  DA at a) a bare GCE; b) the modified glassy carbon electrode in the buffer solution (pH 3.0) with voltage step 5 mV and scan rate of 60  $\text{mVs}^{-1}$ .

### 3.3 Experimental parameters

In order to study the effect of pH on peak currents and potentials of AA, DA and UA, differential pulse voltammograms of their solutions have been obtained with different pH. Figure 4 shows the dependence of their peak currents and potentials on the pH level of the solution. Since all compounds have different pKa, changing in the pH has different effect on the peak currents. As can be seen in Figure 4, at pH 3.0 all compounds had maximum oxidation currents. Therefore, for simultaneous determination of these compounds a pH value of 3.0 (PBS, 0.05  $\text{mol L}^{-1}$ ) 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  $\mu\text{mol L}^{-1}$ . 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.

Figure 4. Effect of pH on the peak potential (A) and peak current (B) of 200.0  $\mu\text{mol L}^{-1}$  AA, 20.0  $\mu\text{mol L}^{-1}$  DA and 15.0  $\mu\text{mol L}^{-1}$  UA at scan rate of 100  $\text{mV s}^{-1}$ .

The influence of the scan rate on the anodic peak current of DA was studied by cyclic voltammetry (Figure 5). 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 230  $\text{mV s}^{-1}$  confirms a diffusion-controlled process on the modified electrode ( $r^2 = 0.996$ ).

### 3.4 Chronoamperometric studies

The rate constant for the chemical reaction between AA and Poly(Sulfonazo III) Modified Glassy Carbon Electrode can be evaluated according to Galus equation [32]:

$$I_c/I_L = \gamma^{1/2} [\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (2)$$

Where,  $I_c$  is the catalytic current of Poly(Chromazurol s)-GCE in the presence of DA,  $I_L$  is the limited current in the absence of AA, and  $\gamma = k_h C_b t$  ( $C_b$  is the bulk concentration of AA,  $\text{mol L}^{-1}$ ) is the argument of error function. In cases where  $\gamma$  exceeds 2,

the error function is almost equal to 1 and the above equation can be reduced to:

$$I_c / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (k_h C_b t)^{1/2} \quad (3)$$

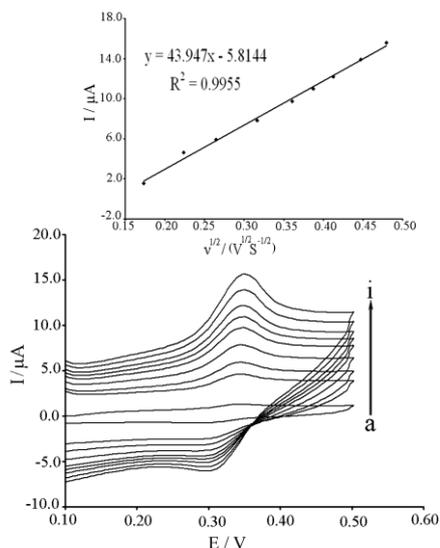


Figure 5. Cyclic voltammograms of  $40.0 \mu\text{mol L}^{-1}$  DA at the polymer modified electrode with various scan rates as: a) 10; b) 50; c) 70; d) 100; e) 130; f) 150; g) 170; h) 200; and i) 230  $\text{mV s}^{-1}$ . Inset, linear relationship between anodic peak current ( $I_{pa}$ ) and  $v^{1/2}$ .

Where,  $k_h$  and  $t$  are the catalytic rate constant ( $\text{M}^{-1} \text{s}^{-1}$ ) and time elapsed (s), respectively. Equation 2 can be used to calculate the rate constant of the catalytic process,  $k_h$ . From the slope of  $I_C/I_L$  vs.  $t^{1/2}$  plot the value of  $k_h$  can be simply calculated for a given concentration of the substrate (Figure 6). The calculated value of  $k_h$  is equal to  $3.04 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ . This value of  $k_h$  also explains the sharp feature of the catalytic peak observed for catalytic oxidation of AA at the surface of Poly(Chromazurol s)-GCE.

The catalytic oxidation of AA by Poly(Chromazurol s)-GCE was also studied by chronoamperometry at different concentrations of AA. The experimental plots

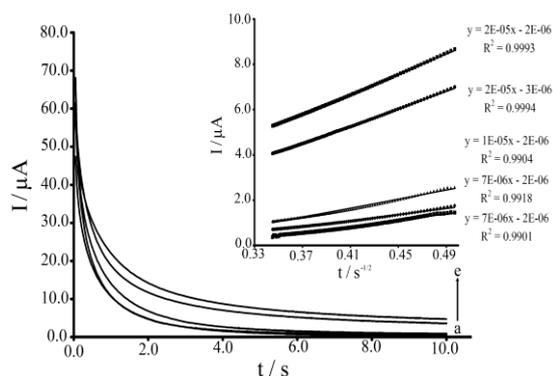


Figure 6. Chronoamperograms were recorded for different DA concentrations as a) 0; b) 25; c) 600; d) 1600 and e) 2100  $\mu\text{mol L}^{-1}$  of DA with one potential step, potential of 0.40 V, and sampling current of 0.05 with duration of 10 s

of  $I$  vs.  $t^{-1/2}$  were employed with the best fits for different concentrations of AA. The slopes of the

resulting straight lines were then plotted vs. the AA concentration. From these results, we calculated the diffusion coefficient,  $2.6(\pm 0.10) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ , for DA.

#### 4. Simultaneous Determination of AA, DA, and UA

The Poly(Chromazurol s)-GCE 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 0.19 and 0.16 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 have been evaluated by varying the concentration of the individual analytic species. The differential pulse voltammetry for the oxidation of AA, DA, and UA at different concentrations are shown in Figs. 7- 9 for these species at a pH value of 3.0 using Poly(Chromazurol s)-GCE oxidation. It can be seen that the peak current of individual analytes increase with increasing concentration. 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 under the optimum conditions.

Analyte	Regression equation	$r^2$	RSD <sup>a</sup>	LOD <sup>b</sup>	LDR <sup>c</sup>
AA	$Y = 0.006X + 6.29$	0.994	1.6	1.7	3.0 – 2200.0
DA	$Y = 0.012X + 4.31$	0.997	3.3	0.05	0.09 – 980.0
UA	$Y = 0.021X + 9.32$	0.996	1.5	0.03	0.05 – 900.0

<sup>a</sup>Relative standard deviation.

<sup>b</sup>Limit of detection.  $Y_{LOD} = Y_B + 3\sigma$

<sup>c</sup>Linear dynamic range

In order to study the intermolecular effects between AA, DA, and UA, three different experiments were carried out under optimum conditions at a pH level of 3.0. In each experiment, the concentration of one of the three compounds was changed, while the concentrations of two other compounds was kept constant. The results are shown in Figs. 7-9. The peak currents for AA, DA, and UA increase linearly with increases in their respective concentrations without remarkably affecting the other peak currents. The Figures confirm that no obvious changes were observed in the DA and UA oxidation currents while varying the concentration of AA. They also indicate that the peak current of AA increased linearly with a correlation coefficient of 0.994 while AA concentration increased (Figure 7). In addition, different concentrations of DA in the presence of  $300.0 \mu\text{mol L}^{-1}$  AA and  $30.0 \mu\text{mol L}^{-1}$  UA exhibit excellent DPV responses to AA, DA, and UA without any obvious intermolecular effects among them. The peak current of DA increased linearly with a correlation coefficient of 0.997 when DA concentration increased (Figure 8).

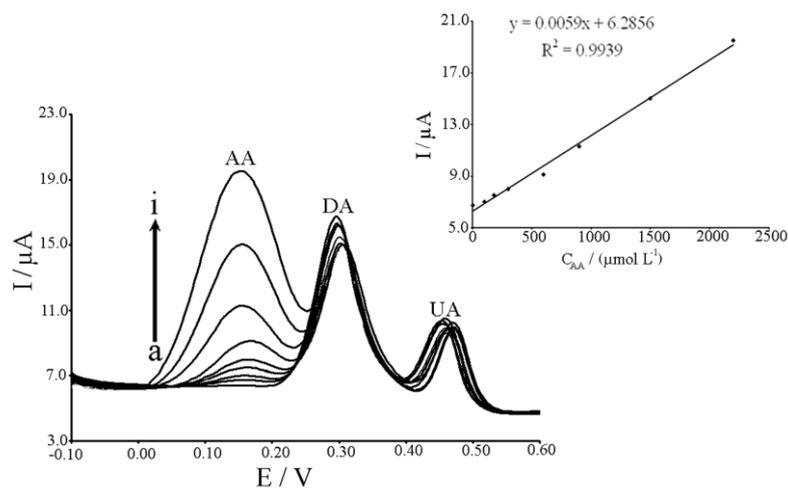


Figure 7. DPV graphs of a) 0.0; b) 3.0; c) 100.0; d) 180.0; e) 300.0; f) 600.0; g) 900.0, h) 1500.0 and i) 2200.0  $\mu\text{mol L}^{-1}$  AA in the presence of 60.0  $\mu\text{mol L}^{-1}$  DA and 10.0  $\mu\text{mol L}^{-1}$  UA. . Inset, Calibration curve for determination of AA.

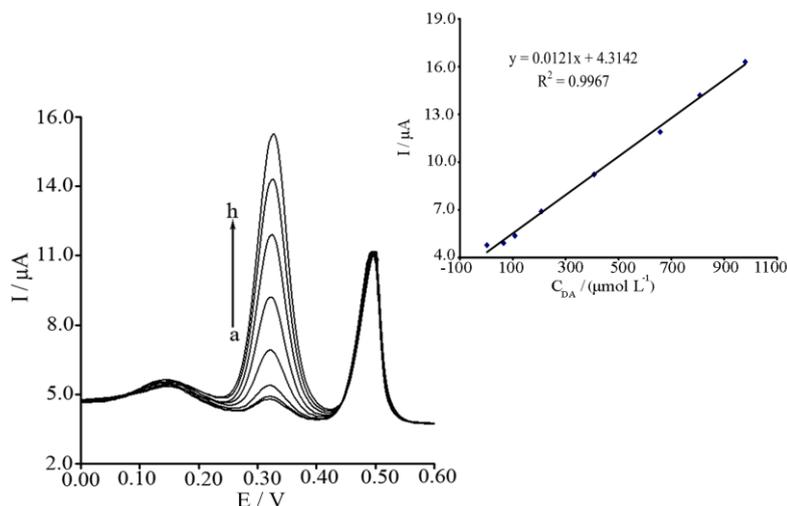


Figure 8. DPV graphs of a) 0.09; b) 65; c) 108; d) 208; e) 408; f) 658; g) 808; and h) 980.0  $\mu\text{mol L}^{-1}$  DA in the presence of 300.0 AA and 110.0  $\mu\text{mol L}^{-1}$  UA. Inset, Calibration curve for determination of DA.

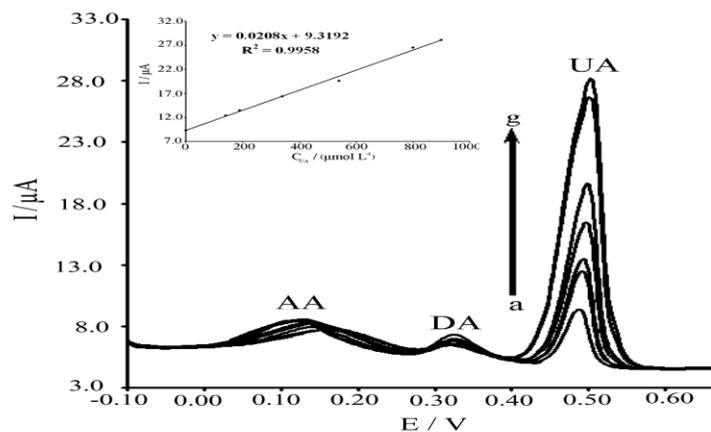


Figure 9. DPV graphs of a) 0.05; b) 140.0; c) 190.0; d) 340.0; and e) 540.0 f) 800.0; g) 900.0  $\mu\text{mol L}^{-1}$  UA in the presence of 100.0 AA and 40.0  $\mu\text{mol L}^{-1}$  DA in buffer solution (pH 3.0) at the poly(Chromazurol s) film modified glassy carbon. Inset, Calibration curve for determination of UA.

Table 3. Interference studied of some foreign substances for 10.0 DA, 50.0 AA and 30.0  $\mu\text{mol L}^{-1}$  UA.

Species	AA	DA	UA
Glycine	400	500	500
Citric acid	50	80	100
Aspartic acid	400	200	25
Aspirin	40	20	10
Urea	400	400	200
Nitrate	1000 <sup>a</sup>	1000	400
Fructose, Sucrose, Glucose	500	350	200
Oxalate	10	10	150
$\text{Mg}^{2+}$ , $\text{Ca}^{2+}$	500	1000	1000

<sup>a</sup> Maximum concentration of the substances used.

Table 4. Determination DA, AA and UA in real samples.

Sample	Added ( $\mu\text{mol L}^{-1}$ )	Proposed method ( $\mu\text{mol L}^{-1}$ )	Recovery (%)	Official method ( $\mu\text{mol L}^{-1}$ )
Vitamin C <sup>a</sup>	-	103.7±1.4	-	101.4±2.7
Vitamin C <sup>a</sup>	400.0	458.4±8.6	91.4	-
Urine 1	-	4.8±0.3	-	4.9±0.1
UA	10.0	15.1 ±0.5	101.3	-
DA	-	<DL	<DL	<DL
DA	200.0	198.7 ± 2.8	99.3	-
Urine 2	-	5.3 ±0.4	-	5.2±0.1
UA	100.0	104.2 ±0.3	99.0	-
DA	<DL	<DL	<DL	<DL
DA	30.0	30.9±1.3	103.0	-
Dopamine <sup>b</sup>	-	20.7±0.9	-	21.7±0.6
Dopamine <sup>b</sup>	10.0	29.7±2.0	93.7	-
Vitamin C <sup>c</sup>	-	301.3±10.1	-	307.6±13.0
Vitamin C <sup>c</sup>	600.0	864.3±20.7	95.2	-

<sup>a</sup> Vitamin C: Swiss Natural Sources (500 mg).

<sup>b</sup> Dopamine ampoule: Caspian tamin 200 mg/5mL. Pharmaceuticalco.Rasht.Iran.

<sup>c</sup> Vitamin C ampoule Darou pakhsh-iran 500mg/5.

The influence of AA and DA on the oxidation of UA under the optimum conditions at a pH level of 3.0 was examined using the modified GCE. As shown in Figure 9, no obvious change was observed in the DA and AA peak currents while changing the UA concentration. In addition, the oxidation peak current of UA increased linearly with a correlation coefficient of 0.996 while UA concentration increased. These results confirm that the oxidation processes of AA, DA, and UA at Poly(Chromazurol s)-GCE 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 various substances as potentially interfering compounds on the determination of AA, DA, and UA 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 PCSMGCE in a solution containing 80.0  $\mu\text{mol L}^{-1}$  AA, 17.0  $\mu\text{mol L}^{-1}$  DA and 17.0  $\mu\text{mol L}^{-1}$  UA plus the interfering substance at a pH level of 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. The results indicate that no interference was observed for common substance and ions such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , nitrate, glucose, fructose, aspartic acid, urea, and starch.

### 6. Determination of AA, DA, and UA in real samples

To investigate the applicability of the proposed method for the determination of AA, DA, and UA in real samples, the utility of the developed method was tested by determining these compounds in several model (mixed) samples. The results are summarized in Table 4. The good recoveries of the mixture samples indicate the successful applicability of the proposed method for simultaneous determination of AA, DA, and UA.

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 to confirm the lack of any significant differences between the two [32-35].

## 7. Conclusions

The results reported above show that, the poly(Chromazurol s) film modified glassy carbon electrode exhibits electrocatalytic activity to AA, DA, and UA oxidation. Effect of the solution pH on electrochemical behavior of the modified electrode was studied. The modified electrode separated the anodic oxidation peak potential of AA, DA, and UA with a well-defined peak separation in the presence of each of the other compounds to measure AA, DA, and UA severally or simultaneously without any intermolecular effects. Thus, using this modified electrode not only improved the electrocatalytic oxidation peak current of AA, DA and UA, but also resolved the overlapping anodic peaks. Proposed method has good linear dynamic ranges for determination of AA, DA and UA. The value of homogeneous rate constant ( $k$ ) and electron transfer coefficient ( $\alpha$ ) were found to be  $3.45(\pm 0.4) \times 10^3 \text{ cm s}^{-1}$  and 0.58, respectively. Potential interferences were investigated and the method has good selectivity. Moreover, the proposed method was successfully applied for determination of these compounds in real samples.

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# اندازه گیری همزمان آسکوربیک اسید، دوپامین و اوریک اسید به روش ولتامتری پالس تفاضلی با استفاده از الکتروُد گلاسی کربن اصلاح شده با

## Poly(Chromazurol s)

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چکیده:

در این روش Chromazurol s به روش الکتروشیمیایی بر روی الکتروُد گلاسی کربن پلیمریزه شده است. الکتروُد گلاسی که به این طریق اصلاح شده است، دارای اثر الکتروکاتالیزی برای اکسایش آسکوربیک اسید در بافر فسفاتی با pH برابر ۳/۰ می باشد. الکتروُد اصلاح شده برای اندازه گیری همزمان آسکوربیک اسید (AA)، دوپامین (DA) و اوریک اسید (UA) استفاده شده است. پارامترهای تجزیه ای روش بهینه شدند و رفتار الکتروُد اصلاح شده مورد مطالعه قرار گرفت. منحنی درجه بندی روش پیشنهادی در محدوده های ۳/۰-۲۲۰۰ میکرومولار AA، ۰/۰۹-۹۸۰ میکرومولار DA و ۰/۰۵-۹۰۰ میکرومولار UA دارای پاسخ خطی می باشد. حد تشخیص روش ۱/۷ میکرومولار AA، ۰/۰۵ میکرومولار DA و ۰/۰۳ میکرومولار UA بدست آمد. از الکتروُد ساخته شده برای اندازه گیری AA، DA و UA در در محلول تزریق، قرص ویتامین C و نمونه ادرار استفاده شد.

کلمات کلیدی: Poly(Chromazurol s)، الکتروُد گلاسی کربن، آسکوربیک اسید، دوپامین، ولتامتری پالس تفاضلی

