GÜ, Gazi Eğitim Fakültesi Dergisi, Cilt 31, Sayı 3 (2011) 775-791

Dopamin ve Ürik Asitin Birinin Varlığında Diğerinin Kare Dalga Voltametrisi ile Tayini

The Determination of Dopamine and Uric Acid with the Presence of Other Using Square Wave Voltammetry

Erdoğan HASDEMİR[1], Tuğba TABANLIGİL[2]

[1]*Gazi University, Science Faculty, Chemistry Department, hasdemir@gazi.edu.tr*
[2]*Gazi University, ATAMYO, Chemistry Department, ttabanligil@gazi.edu.tr*

ÖZET

Bu çalışmada çeşitli destek elektrolit ortamlarında DA ve ÜA' nın yükseltgenme davranışları incelenmiş, pH 2,5 H_2SO_4 ve pH 7,2 fosfat tamponunun uygun olduğuna karar verilmiştir. pH 2,5 H_2SO_4 ortamında DA ve ÜA' nın sırasıyla +0,480 V, +0,620 V' da ki, fosfat tamponunda da sırasıyla +0,140 V, +0,270 V' da ki yükseltgenme pikleri kullanılmıştır. Her iki ortamda farklı [DA] / [ÜA] oranları çalışılmıştır. Sentetik numune için, pH 2,5 H_2SO_4 ortamında DA ve ÜA için % hata sırasıyla -0,41 ve 0,27, pH 7,2 fosfat tamponu ortamında aynı numune için % hata sırasıyla -0,61 ve -0,14 olarak bulunmuştur. pH 2,5 H_2SO_4 ortamında DA ve ÜA için gözlenebilme sınırı (LOD) sırasıyla 2,16 × 10^{-8} M ve 4,14 × 10^{-8} M, tayin sınırı (LOQ) ise 6,48 × 10^{-8} M ve 1,24 × 10^{-7} M olarak bulunmuştur. pH 7,2 fosfat tamponu ortamında DA ve ÜA için gözlenebilme sınırı (LOD) sırasıyla 2,28 × 10^{-8} M ve 2,13 × 10^{-8} M, tayin sınırı (LOQ) 6,84 × 10^{-8} M ve 6,39 × 10^{-8} M olarak bulunmuştur. Bu yönteme çeşitli anyon ve katyonların girişim etkileri incelenmiştir.

Anahtar Kelimeler: *Kare dalga voltametrisi, Tayin, Dopamin, Ürik asit.*

ABSTRACT

Initially the electrochemical behavior of dopamine (DA) and uric acid (UA) in different supporting electrolytes was investigated. It was found that the most suitable supporting electrolytes were pH 2.5 H_2SO_4 and pH 7.2 phosphate buffer. The analysis of DA and UA in acidic medium was carried out by the use of their oxidation peaks observed at +0.480 V and +0.620 V, respectively. The analysis of the phosphate buffer medium were carried out by the use of the oxidation peaks of DA and UA appeared at +0.140 V and +0.270 V, respectively. The studies carried out with different [DA] / [UA] ratios gave acceptable results for both media. The synthetic sample was analyzed. The error was -0.41 % for DA and 0.27 % for UA in acidic media and -0.61 % for DA and -0.14 % for UA in basic media. The limits of detection (LOD) were found as 2.16 × 10^{-8} M and 4.14 × 10^{-8} M in acidic media, 2.28 × 10^{-8} M and 2.13 × 10^{-8} M in basic media for DA and UA, respectively.

Keywords: *Square wave voltametry, Determination, Dopamine, Uric acid.*

INTRODUCTION

Dopamine hydrochloride (DA) is an important neurotransmitter and extracellular messenger in biological systems. Determination of DA and related catecholamine compounds is significant for neurochemistry and brain-science studies (Wightman *et al.* 1988). A loss of DA-containing neurons may result in some serious disease, such as Parkinson's disease. Therefore, detailed information regarding its reaction at the molecular level and a determination of the concentration of DA are important (Lane and Blaha 1990). In addition, a significant problem for dopamine determination is the fouling effect due to accumulation of reaction products which, form electro-polymerized films on the electrode surface (Wang *et al.* 2002).

The fact that DA and other catecholamines are easily oxidizable compounds makes their detection possible by electrochemical methods based on oxidation reaction. Several studies of electrochemical processes of DA have been carried out (Hawley *et al.* 1967, Bishop and Hussein 1984).

Uric acid (2, 6, 8-trihydroxypurine, UA), the end metabolic product of purine through the liver, is present in blood and urine. Monitoring UA in the blood or urine is important because it can be used as a powerful indicator for an early warning sign of kidney diseases. Abnormal UA level in a human body could be caused by several diseases such as gout, hyperuricemia, Lesch–Nyan syndrome, cardiovascular and chronic renal disease. Thus, UA concentration determination is of paramount importance, due to its crucial role in health assessment and monitoring. There are some electrochemical methods for determination of UA (Shahrokhian and Zare-Mehrjardi 2007, Ardakani *et al.* 2006, Gilmartin and Hart 1992, Tatsuma and Watanabe 1991). The normal UA levels in human serum ranges from 240 to 520 mM and in urinary excretion from 1.2 to 4.4 mM (Pachla *et al.* 1987). Various methods such as colorimetric (Pileggi *et al.* 1972, Dilena *et al.* 1986), enzymatic (Tatsuma and Watanabe 1991, Pachla *et al.* 1987, Pileggi *et al.* 1972, Dilena *et al.* 1986, Gilmartin and Hart 1992, Miland *et al.* 1996),

electrophoretic (Guan *et al.* 2005, Lee and Chen 2004), and chromatographic (Ross 1994, Lykkesfeldt 2000) have been reported for the analysis of UA in the human body fluids. Electrochemical methods offer an analytical platform which can exhibit a higher selectivity and sensitivity than other commonly employed methods and have an inherent advantage of lower cost and rapid sensing (Dutt *et al.* 2005, Bravo *et al.* 1998, Rivas *et al.* 2007).

The aim of this study is to develop a new and simple method in the determination of DA or UA with the presence of other molecule, using square wave voltammetry.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical reagent grade. Solution of $1.0 \cdot 10^{-3}$ mol/dm^{-3} DA was prepared by dissolving 0.0189 g DA (Merck) in water and diluting to 100 mL in volumetric flaks. Double distilled water was used throughout. Solution of $1.0 \cdot 10^{-3}$ mol/dm^{-3} UA was prepared by dissolving 0.0176 g UA (Merck) in 20 mL $1.0 \cdot 10^{-2}$ mol/dm^{-3} NaOH solution and diluting to 100.0 mL with water. pH 7.2 Na_2HPO_4/NaH_2PO_4 buffer solutions were prepared by dissolving 2.18 g $NaH_2PO_4.2H_2O$ (Merck) and 9.65 g $Na_2HPO_4.7H_2O$ (Merck) in water, diluting to 250 mL in a volumetric flask. Argon gas with % 99.99 purity was used to de-aerate solutions. pH 1-3.5 solutions were prepared by H_2SO_4 solution.

Aparatus

Square-wave voltammetry (SWV) experiments were performed using a Electrochemical Work Station CHI 660B electroanalyzer. Measurements were carried out with a glassy carbon electrode (GCE) in a three electrode arrangement. GCE as the working electrode (BAS MF-2012), a saturated Ag/AgCl/KCl as the reference and a platinum wire as the counter electrode with a conventional three-electrode system. All the experiments were performed at room temperature and at an atmosphere of argon. Inolab WTW-720 digital pH meter was used for pH measurements.

Procedure

Before the experiment, the GCE working electrode was polished with alumina powder (0.3 and 0.05 μm), then washed in an ultrasonic bath for 5 min to obtain a mirror face. The polished electrodes were treated by Piranha solution (concentrated H_2SO_4:H_2O_2(30%)=3:1) for 10 min, then ultrasonically cleaned by distilled water and absolute ethanol for 5 min, respectively.

The solutions were purged with water-saturated argon for 5 min in the first cycle and 30 s for each successive cycle.

Working in acidic medium

After the sample of DA and UA mixture was added to H_2SO_4 support electrolyte in voltammetric cell, standard DA solutions were added. Standard addition graphic was made and unknown concentration DA in the sample was determinated.

Standard UA solution were added on H_2SO_4 support electrolyte and a portion of DA in voltammetric cell, consecutively. Then, calibration graphic was made for UA and unknown concentration UA in the sample was determinated.

Working in basic medium

After the sample of DA and UA mixture was added to phosphate buffer support electrolyte in voltammetric cell, standard DA solutions were added. Standard addition graphic was made for DA and unknown concentration DA in the sample was determinated. In the same way was followed for determination of UA in phosphate buffer support electrolyte.

RESULTS and DISCUSSION

Voltammograms of the UA and DA molecules

Preliminary experiments were carried out to identify the general features that characterize the behavior of the DA and UA systems on glassy carbon electrode. Fig.1

displays the voltammograms of different concentration DA and UA in H_2SO_4, pH 2.5 medium. Fig.2. displays the voltammograms DA and UA in phosphate buffer (pH 7.2).

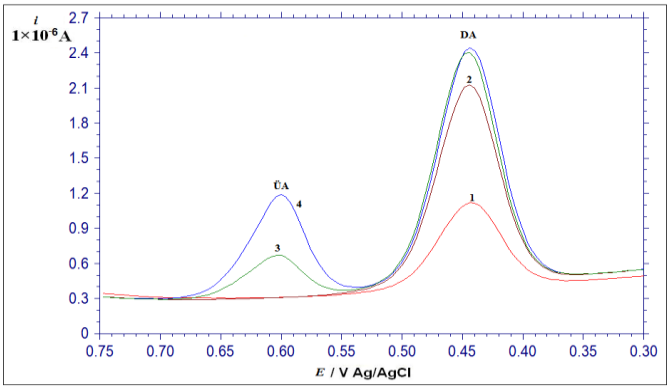

Figure 1. Voltammograms of DA and UA in H_2SO_4, pH 2.5, 1. 10 mL support electrolyte + 0.05 mL 10^{-3} M DA, 2. 1 + 0.10 mL 10^{-3} M DA, 3. 2 + 0.10 mL 10^{-3} M UA, 4. 3 + 0.10 mL 10^{-3} M UA

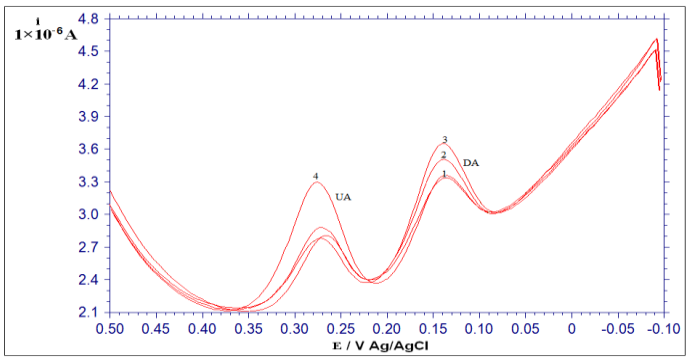

Figure 2. Voltammograms of DA and UA in phosphate buffer (pH 7.2), 1. 10 mL support electrolyte + 0.05 mL 10^{-3} M DA + 0.10 mL 10^{-3} M UA, 2. 1 + 0.05 mL 10^{-3} M DA, 3. 2 + 0.05 mL 10^{-3} M DA, 4. 3 + 0.10 mL 10^{-3} M UA

Optimization of Parameters

Effect of pH

The influence of pH on the oxidation peaks current of DA and UA was studied in the pH range of 1.0–3.5 for H_2SO_4. It was seen from Table 1 the peaks potential of DA and UA shifted toward more negative values and distance between them decreased with

increasing pH of the mixture of DA and UA solution. Therefore, pH 2.5 was selected for subsequent uses, because at pH 2.5 DA's oxidition peak current was very big.

Table 1. Effect of pH on peaks potential and separation

H_2SO_4					
	DA		ÜA		
pH	E_p (V)	i_p 1×10^{-7} (A)	E_p (V)	i_p 1×10^{-7} (A)	ΔE_p (V)
1.0	+0.516	2.070	+0.660	6.625	0.144
1.5	+0.472	9.125	+0.624	5.641	0.152
2.0	+0.444	11.280	+0.600	4.240	0.156
2.5	+0.445	54.770	+0.605	4.972	0.160
3.5	+0.364	13.900	+0.520	2.230	0.156

Influence of [DA]/[UA] ratios

In first study, [DA]/[UA]= 1 and 9.80×10^{-6} M in pH 2.5 H_2SO_4 solution was investigated (Figure 3 and Figure 4). After than have worked with samples which presented different [DA]/[UA] ratios. All results are shown in Table 2.

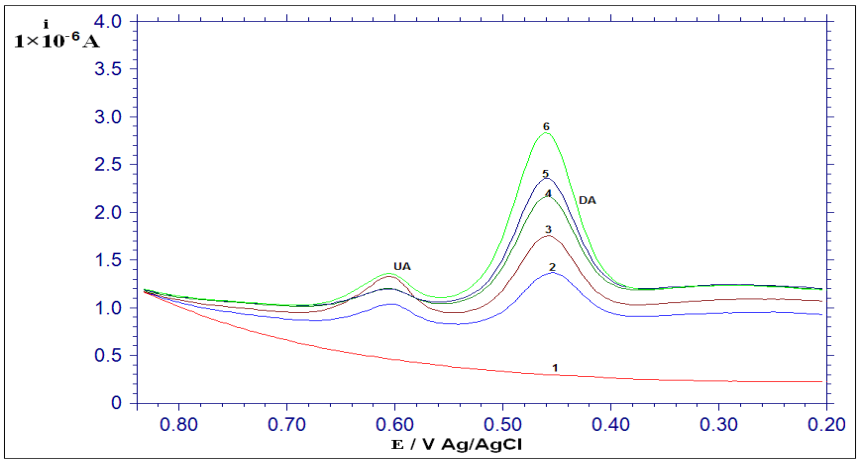

Figure 3. SWV's for increaments of DA amounts on H_2SO_4 (pH 2.5) and DA+UA sample, 1. 10 mL support electrolyte, 2. 1 + 0.10 mL 10^{-3} M DA + 0.10 mL 10^{-3} M UA, 3. 2 + 0.05 mL 10^{-3} M DA, 4. 3 + 0.05 mL 10^{-3} M DA, 5. 4 + 0.05 mL 10^{-3} M DA, 6. 5 + 0.10 mL 10^{-3} M DA

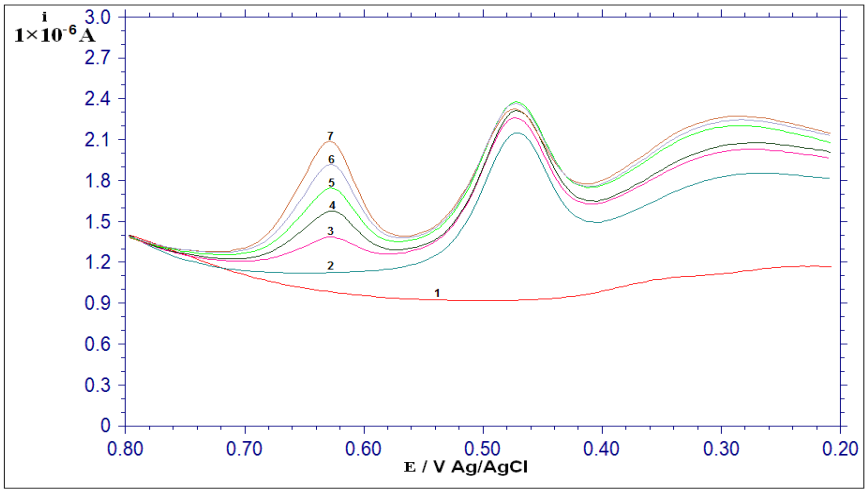

Figure 4. SWV's for increaments of UA amounts on H_2SO_4 (pH 2.5) and DA sample, 1. 10 mL support electrolyte, 2. 1 + 0.10 mL 10^{-3} M DA, 3. 2 + 0.05 mL 10^{-3} M UA, 4. 3 + 0.05 mL 10^{-3} M UA, 5. 4 + 0.05 mL 10^{-3} M UA, 6. 5 + 0.10 mL 10^{-3} M UA, 7. 6 + 0.10 mL 10^{-3} M UA.

Table 2. Determination of DA and UA in pH 2.5 H_2SO_4 (n=3)

[DA]/[ÜA]	Added (10^{-6} M)		Found (10^{-6} M)		Standard Deviation (10^{-6})		$\bar{x} \pm \frac{t*s}{\sqrt{N}}$ (10^{-6})		% Error	
	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA
1:1	9.81	9.81	9.88	9.51	0.28	1.06	9.88±0.47	9.51±1.78	0.81	-2.96
1:2	4.92	9.85	4.82	9.20	0.21	0.69	4.82±0.36	9.20±0.17	-2.07	-6.61
1:3	4.90	14.70	4.92	14.37	0.19	0.31	4.92±0.32	14.37±0.52	0.41	-2.24
1:4	2.47	9.88	1.84	8.92	0.02	0.13	1.84±0.03	8.92±0.21	2.56	9.75
1:5	4.85	24.27	4.78	23.70	0.05	2.62	4.78±0.08	23.70±4.41	-1.40	-2.35
2:1	19.42	9.71	19.27	9.22	0.25	0.74	19,27±0.42	9.22±0.74	0.78	-2.48
3:1	14.70	4.90	14.50	4.90	0.16	0.75	14,50±0.27	4.90±0.13	-1.36	4.59
4:1	19.51	4.88	19.50	5.18	0.56	0.22	19,50±0.94	5.18±0.37	-0.05	6.23
5:1	24.27	4.85	24.03	4.93	0.61	0.68	24,03±1.03	4.93±1.15	-0.98	1.61

*90 % confidence level

When, [DA]/[UA]= 1 and 9.80×10^{-6} M in pH 7.2 phosphate buffer solution was investigated (Figure 5 and Figure 6). After than have worked with samples which presented different [DA]/[UA] ratios. The results are shown in Table 3.

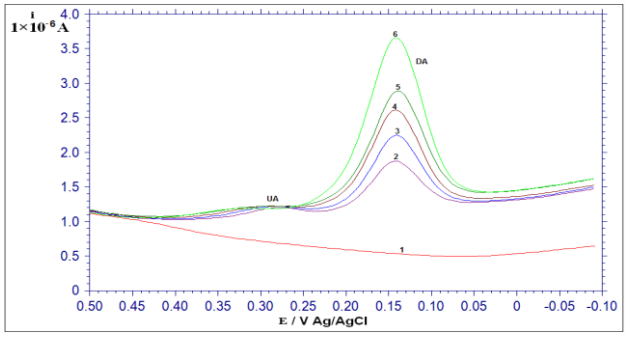

Figure 5. SWV's for increaments of DA amounts in phosphate buffer (pH 7.2) and UA sample, 1. 10 mL support electrolyte, 2. 1 + 0.10 mL 10^{-3} M DA + 0.10 mL 10^{-3} M UA, 3. 2 + 0.05 mL 10^{-3} M DA, 4. 3 + 0.05 mL 10^{-3} M DA, 5. 4 + 0.05 mL 10^{-3} M DA, 6. 5 + 0.10 mL 10^{-3} M DA

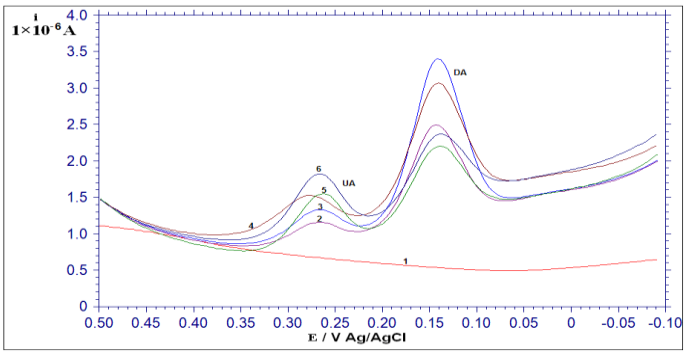

Figure 6. SWV's for increaments of UA amounts in phosphate buffer (pH 7.2) and DA sample, 1. 10 mL support electrolyte, 2. 1 + 0.10 mL 10^{-3} M DA + 0.10 mL 10^{-3} M UA, 3. 2 + 0.05 mL 10^{-3} M UA, 4. 3 + 0.05 mL 10^{-3} M UA, 5. 4 + 0.05 mL 10^{-3} M UA, 6. 5 + 0.05 mL 10^{-3} M UA

Table 3. Determination of DA and UA in pH 7.2 phosphate buffer (n=3)

DA]/[Ü	Added (10^{-6} M)		Found (10^{-6} M)		Standard Deviation (10^{-6})		$\bar{x} \pm \frac{t * s}{\sqrt{N}}$ (10^{-6})		% Error	
	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA
1:1	9.81	9.81	9.86	9.74	0.21	0.16	9.86±0.36	9.74±0.26	0.55	-0.71
1:2	4.92	9.85	4.87	9.86	0.04	0.08	4.87±0.06	9.86±0.14	-0.98	0.11
1:3	4.90	14.70	4.76	14.40	0.07	0.17	4.76±0.12	14.40±0.29	-2.94	-2.04
1:4	2.47	9.88	2.47	9.50	0.20	0.32	2.47±0.34	9.50±0.54	-0.08	-3.81
1:5	4.85	24.27	5.10	24.80	0.28	0.82	5.10±0.47	24.80±1.38	5.09	2.18
2:1	19.42	9.71	19.60	9.64	0.36	0.10	19.60±0.61	9.64±0.18	0.93	0.71
3:1	14.70	4.90	14.40	4.81	0.16	0.06	14.40±0.27	4.81±0.10	-2.04	-1.76
4:1	19.51	4.88	20.27	5.25	1.16	0.50	20.27±1.95	5.25±0.84	3.88	7.54
5:1	24.27	4.85	23.60	5.18	0.50	0.28	23.60±0.84	5.18±0.48	-2.76	6.87

*90 % confidence level

Detection limits (LOD) and Limit of Quantification (LOQ) of Method

To verify the linear relationship between peaks current, DA and UA concentrations 3 standart addition calibration graphs were constructed under optimum conditions (pH 7.2 phosphate buffer, frequency 10 Hz, pulse amplitude 0.025 V, potential increment 0.004 V). The limits of detection (LOD) were 2.16×10^{-8} M and 4.14×10^{-8} M and limits of quantification (LOQ) were 6.48×10^{-8} M and 1.24×10^{-7} M for DA and UA in acidic media. These values were found to be 2.28×10^{-8} M and 2.13×10^{-8} M for LOD and 6.84×10^{-8} M and 6.39×10^{-8} M for LOQ in basic media, respectively.

Table 4. LOD and linear working range values for DA and UA in some literatures and this working

	LOD		**Linear working range**	
References:	**DA (M)**	**UA (M)**	**DA (M)**	**UA (M)**
(Deletioğlu at al. 2010)	$1.99x10^{-6}$	$4.99x10^{-6}$	$1.00x10^{-6}$-$1.00x10^{-4}$	$1.00x10^{-6}$-$1.00x10^{-4}$
(Ardakani at al. 2008)	$8.70x10^{-8}$	$1.50x10^{-5}$	$1.00x10^{-7}$-$9.00x10^{-4}$	$2.00x10^{-5}$-$6.50x10^{-4}$
(Huang at al. 2008)	$2.40x10^{-7}$	$1.70x10^{-7}$	$1.00x10^{-6}$-$3.50x10^{-5}$	$1.00x10^{-6}$-$3.20x10^{-5}$
(Wang at al. 2007)	$1.20x10^{-7}$	$6.00x10^{-7}$	$2.00x10^{-7}$-$8.00x10^{-5}$	$1.20x10^{-6}$-$1.00x10^{-4}$
(Liu at al. 2006)	$8.00x10^{-7}$	$7.00x10^{-7}$	$2.50x10^{-6}$-$5.00x10^{-4}$	$1.00x10^{-6}$-$1.00x10^{-4}$
(Cao at al. 2009)	$5.00x10^{-8}$	$4.00x10^{-7}$	$4.00x10^{-7}$-$8.00x10^{-5}$	$4.00x10^{-6}$-$8.00x10^{-4}$
(Yu and Lin 2008)	$3.60x10^{-8}$	$1.00x10^{-7}$	$1.10x10^{-7}$-$3.80x10^{-5}$	$3.00x10^{-7}$-$5.70x10^{-5}$
(Thiagarajan at al. 2009)	-	-	$1.97x10^{-6}$-$9.88x10^{-6}$	$1.97x10^{-5}$-$9.88x10^{-5}$
(Ye at al. 2008)	$6.00x10^{-8}$	-	$5.00x10^{-7}$-$3.00x10^{-5}$	-
(Zhao at al. 2006)	-	$2.00x10^{-6}$	-	$1.00x10^{-5}$-$8.00x10^{-5}$
(Popa at al. 2000)	-	$1.50x10^{-8}$	-	$5.00x10^{-8}$-$1.00x10^{-6}$
(Liu at al. 2007)	$2.00x10^{-8}$	$1.10x10^{-8}$	$4.00x10^{-8}$-$3.00x10^{-6}$	$3.00x10^{-7}$-1,$00x10^{-5}$
(Wang at al. 2004)	$1.14x10^{-9}$	-	$4.00x10^{-9}$-$4.00x10^{-7}$	-
(Yang at al. 2009)	-	$3.02x10^{-7}$	-	$1.52x10^{-6}$-$1.54x10^{-3}$
This working	$2.16x10^{-8}$ (H_2SO_4)	$4.14x10^{-8}$ (H_2SO_4)	$1.00x10^{-7}$-$1.00x10^{-5}$	$3.00x10^{-7}$-$1.00x10^{-5}$
	$2.28x10^{-8}$ (Buffer)	$2.13x10^{-8}$ (Buffer)	$8.00x10^{-8}$-$1.00x10^{-5}$	$1.00x10^{-7}$-$1.00x10^{-5}$

It is seen that in terms of detection limit (LOD) and linear working range, better results were obtained from this study, which was made for the detection of DA and UA, when compared to the results of the other studies given in Table 4. LOD is well and range that can be accepted as a good linear range. The detection was made by forming a modified electrod in most of the studies, even in those given in Table 4. Considering the modification process of an electrot, it can be said that it is a process which needs much more time, effort and money as well, compared to that of bare electrot. Combined all together, this study can be preferred for the analysis of DA and UA concentrations.

Interference Studies

Some interference ions by other ionic species and molecul in the square wave voltammetric determination of DA and UA was investigated by the addition of an interfering ions to a solution containing 4.88×10^{-6} M of DA or UA using the optimized conditions. The addition of interfering ions into the solution was gone to with DA or UA peaks were overlapped and/or peaks currents were decreased. These points were called tolerance ratio. The results of this study are summarized in Table 5.

Table 5. Interference study of determination of DA and UA under the optimum condition.

Species	Tolerance ratio[a]
NH_4^+ , Cl^-	1000
Zn^{2+}, SO_4^{2-}	400
Na^+, CO_3^{2-}	300
NO_3^-	200
Ca^{2+}	10
Ascorbic Acid	3

[a]Maximum ratio of ions tested

Analyses of Synthetic Sample

The studies were realized where [DA] / [UA] ratio was equal to 2/3 (Table 6). The synthetic sample which is containing 9.76×10^{-6} M DA and 1.46×10^{-5} M UA were analyzed.

Table 6. Determination of DA and UA in synthetic samples (n=3).

	pH 2,5 H_2SO_4												pH 7,2 phosphate buffer											
	Added (10^{-6} M)		Found (10^{-6}M)		Average (10^{-6}M)		Standard Deviation (10^{-6})		$\bar{X} \pm \frac{t*s}{\sqrt{N}}$		% Error		Found (10^{-6} M)		Average (10^{-6} M)		Standard Deviation (10^{-6})		$\bar{X} \pm \frac{t*s}{\sqrt{N}}$		% Error	
	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA
1	9,76	14,63	9,67	14,60	9,72	14,67	0,06	0,06	$(9,72\pm0,10)\times10^{-6}$	$(14,67\pm0,10)\times10^{-6}$	-0,41	0,27	9,69	14,51	9,70	14,61	0,02	0,10	$(9,70\pm0,04)\times10^{-6}$	$(14,61\pm0,18)\times10^{-6}$	-0.61	-0.14
2	9,76	14,63	9,71	14,70									9,69	14,60								
3	9,76	14,63	9,78	14,70									9,73	14,72								

* 90 % confidence level

Analysis of Dopamine and Uric Acid in Real Sample

In this method for DA and UA determination was tested by the determination of DA in drug using the recommended procedure (Table 7). The concentration of DA and UA was determined by the standart addition method. Solutions of known concentration of DA and UA were added to drug samples. Three determinations were made each addition.

Table 7. Determination of DA and UA in real samples (n=3)

	pH 2,5 H_2SO_4						pH 7,2 phosphate buffer					
	Sample (10^{-6} M)		Found (10^{-6} M)		Recovery (%)		Added (10^{-6} M)		Found (10^{-6} M)		Recovery (%)	
	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA	DA	UA
1	4.98	19.61	4.94	19.60	99.20	99.95	14.78	24.39	14.70	24.60	99.46	100.86
2	4.98	19.61	4.93	19.10	98.99	97.40	14.78	24.39	14.80	25.00	100.13	102.50
3	4.98	19.61	5.01	19.70	100.60	100.50	14.78	24.39	14.90	24.60	100.80	100.86

CONCLUSION

The present study demonstrates that square wave voltammetry of DA and UA based on the oxidation of on the glassy carbon electrode can be used for the determination of trace amounts of both molecules in the presence of each other. The method offers a practical separation of oxidation potentials for simultaneous determination of trace amount of DA and UA in a single scan with high selectivity and sensitivity, simplicity and speed.

The ratio of [DA] and [UA] was changed afterwards and the best results were obtained when [DA] / [UA] = 5 at pH 2,5. In phosphate buffer medium the most satisfying results were observed when [DA] / [UA] > 1. However the studies carried out with different [DA] / [UA] ratios gave acceptable results for both media. The interference of various anions and cations was also investigated.

REFERENCES

Ardakani, M. M., Akrami, Z., Kazemian, H., Zare, H.R. (2006). Electrocatalytic characteristics of uric acid oxidation at graphite–zeolite-modified electrode doped with iron (III). *J. Electroanal. Chem.*, 586, 31–38.

Ardakani, M. M., Beitollahi, H., Ganjipour, B., Naeimi, H., Nejati, M. (2008). Electrochemical and catalytic investigation of dopamine and uric acid by modified carbon nanotube paste electrode. *Bioelecterochemistry*, 75, 1-8.

Bishop, E. and Hussein, W. (1984). Anodic voltammetry of dopamine, noradrenaline and related compounds at rotating disc electrodes of platinum and gold. *Analyst*, 109, 627-632.

Bravo, R., Hsueh, Ch., Jaramillo, A., Brajter-Toth, A. (1998). Possibilities and limitations in miniaturized sensor design for uric acid. *Analyst*, 123, 1625-1630.

Cao, X., Luo, L., Ding, Y., Yu, D., Gao, Y., Meng, Y. (2009). Simultaneous determination of dopamine and uric acid on nafion/sodium dodecylbenzenesulfonate composite film modified glassy carbon electrode. *J. Appl. Electrochem.*, 39, 1603–1608.

Deletioğlu, D., Hasdemir, E., and Solak, A. O. (2010). Simultaneous Determination of dopamine and uric acid in the presence of ascorbic acid at the indole-3-

carboxaldehyde modified glassy carbon electrode. *Current Analytical Chemistry*, 6(3), 203-208.

Dilena, B.A., Peake, M.J., Pardue, H.L., Skoug, J.W. (1986). Direct ultraviolet method for enzymatic determination of uric acid, with equilibrium and kinetic data-processing options. *Clin. Chem.*, 32, 486-491.

Dutt, J.S.N., Cardosi, M.F., Livingstone, C., Davis, J. (2005). Diagnostic implications of uric acid in electroanalytical measurements. *Electroanalysis*, 17, 1233-1243.

Gilmartin, M.A.T., Hart, J.P. (1992). Voltammetric and amperometric behaviour of uric acid at bare and surface-modified screen-printed electrodes: studies towards disposable uric acid sensor. *Analyst*, 117, 1299–1303.

Gilmartin, M.A., Hart, J.P. (1994). Amperometric biosensor for uric acid based on a chemically modified screen-printed carbon electrode coated with cellulose acetate and uricase. *Analyst*, 119, 833-840.

Guan, Y., Wu, T., Ye, J. (2005). Determination of uric acid and *p* -aminohippuric acid in human saliva and urine using capillary electrophoresis with electrochemical detection: Potential application in fast diagnosis of renal disease. *J. Chromatogr. B*, 821, 229-234.

Hawley, M.D., Tatawawadi, S.V., Piekarski, S., and Adams, R. N. (1967). Electrochemical studies of the oxidation pathways of catecholamines. *J. Am. Chem. Soc.*, 89, 447- 450.

Huang, X., Li, Y., Wang, P., Wang, L. (2008). Sensitive Determination of Dopamine and Uric Acid by the Use of a Glassy Carbon Electrode Modified with Poly(3-methylthiophene)/Gold Nanoparticle Composites. *Anal. Sci.*, 24, 1563-1568.

Lane, R.F., Blaha, C.D. (1990). Detection of catecholamines in brain tissue: surface-modified electrodes enabling in vivo investigations of dopamine function. *Longmuir*, 6, 56–65.

Lee, H.L., Chen, S.C. (2004). Microchip capillary electrophoresis with electrochemical detector for precolumn enzymatic analysis of glucose, creatinine, uric acid and ascorbic acid in urine and serum. *Talanta*, 64, 750-754.

Liu, C., Lu, G., Jiang, L., Jiang, L., Zhou, X. (2006). Study on the Electrochemical Behavior of Dopamine and Uric Acid at a 2-Amino-5-mercapto-[1,3,4] Triazole Self-Assembled Monolayers Electrode. *Electroanalysis*, 18, 291 – 297.

Liu, A., Honma, I., Zhou, H. (2007). Simultaneous voltammetric detection of dopamine and uric acid at their physiological level in the presence of ascorbic acid using poly(acrylic acid)-multiwalled carbon-nanotube composite-covered glassy-carbon electrode. *Biosensors and Bioelectronics*, 23, 74–80.

Lykkesfeldt, J. (2000). Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction

methods: reliable reduction with tris[2-carboxyethyl] phosphine hydrochloride. *Anal. Biochem.*, 282, 89-93.

Miland, E., Ordieres, A.J.M., Blanco, P.T., Smyth, M.R., Fagain, C.O. (1996). Poly(o-aminophenol)-modified bienzyme carbon paste electrode for the detection of uric acid. *Talanta*, 43, 785-796.

Pachla, L.A., Reynolds, L.D., Wright, S., Kissinger, P.T. (1987). Analytical methods for measuring uric acid in biological samples and food products. *J. Assoc. Offic. Anal. Chem.*, 70, 1-14.

Perello, J., Sanchis, P., Grases, F. (2005). Determination of uric acid in urine, saliva and calcium oxalate renal calculi by high-performance liquid chromatography/mass spectrometry. *J. Chromatogr. B* 824, 175-180.

Pileggi, V.J., Wybenga, D.R., Digiorgi, J. (1972). A one-tube serum uric acid method using phosphotungstic acid as protein precipitant and color reagent. *Clin. Chim. Acta*, 37, 141-149.

Popa, H., Kubota, Y., Donald, A. T., Fujishima, A. (2000). Selective Voltammetric and Amperometric Detection of Uric Acid with Oxidized Diamond Film Electrodes. *Anal. Chem.*, 72, 1724-1727.

Rivas, G.A., Rubianes, M.D., Rodrígues, M.C., Ferreyra, N.F., Luque, G.L., Pedano, M.L., Miscoria, S.A., Parrado, C. (2007). Carbon nanotubes for electrochemical biosensing. *Talanta*, 74, 291-307.

Ross, M.A. (1994). Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography. *J. Chromatogr. B,* 657, 197-200.

Shahrokhian, S., Zare-Mehrjardi, H.R. (2007). Simultaneous voltammetric determination of uric acid and ascorbic acidusing a carbon-paste electrode modified with multi-walled carbon nanotubes/nafion and cobalt (II) nitrosalophen. *Electroanalysis*, 19, 2234–2242.

Tatsuma, T., Watanabe, T. (1991). Oxidase/ peroxidase bilayer-modified electrodes as sensors for lactate, pyruvate, cholesterol and uric acid. *Anal. Chim. Acta*, 242, 85–89.

Thiagarajan, S., Tsai, T. H., Chen, S. M. (2009). Easy modification of glassy carbon electrode for simultaneous determination of ascorbic acid, dopamine and uric acid. *Biosensors and Bioelectronics*, 24, 2712–2715.

Wang, X., Jin, B., Lin, X. (2002). In-situ FTIR spectroelectrochemical study of dopamine at a glassy carbon electrode in a neutral solution. *The Japan Soc. Anal. Chem.*, 12, 931- 933.

Wang, S., Du, L., Wang, L., Zhuang, H. (2004). Flow injection with inhibited chemiluminescence method for the determination of dopamine hydrochloride. Analytical Sciences, 20, 315-317.

Wang, P., Li, Y., Huang, X., Wang, L. (2007). Fabrication of layer-by-layer modified multilayer films containing choline and gold nanoparticles and its sensing application for electrochemical determination of dopamine and uric acid. *Talanta*, 73, 431–437.

Wightman, R.M., May, L.J., Michael, A.C. (1988). Detection of dopamine dynamics in the brain. *Anal. Chem.*, 60, 769-779.

Yang, G., Tan, L., Shi, Y., Wang, S., Lu, X., Bai, H., and Yang, Y. (2009). Direct Determination of Uric Acid in Human Serum Samples Using Polypyrrole Nanoelectrode Ensembles. *Bull. Korean Chem. Soc.*, 30(2), 454-458.

Ye, F., Nan, J., Wang, L., Song, Y., Kim, K. B. (2008). The ultrasonic electropolymerization of an 5-[*o*-(4-bromine amyloxy)phenyl]-10,15,20-triphenylporphrin (*o*-BrPETPP) film electrode and its electrocatalytic properties to dopamine oxidation in aqueous solution. *Electrochimica Acta.*, 53, 4156–4160.

Yu, Z., Lin, X. Q. (2008). Simultaneous Detection of Dopamine and Uric Acid under Coexistence of Ascorbic Acid with DNA/Pt Nanocluster Modified Electrode. *Chinese Journal of Chemistry*, 26, 898-904.

Zhao, Y., Bai, J., Wang, L., XuHong, E., Huang, P., Wang, H., Zhang, L. (2006). Simultaneous electrochimical determination of uric acid and ascorbic acid using L-Cysteine self-assembled gold electrode. *Int. J. Electrochem. Sci.*, 1, 363-37

GENİŞ ÖZET

Bu çalışmada, kare dalga voltametrisi kullanılarak dopamin (DA) ve ürik asit (ÜA) tayini için metot geliştirilmeye çalışılmıştır. Bunun için ilk önce çeşitli destek elektrolit ortamlarında Dopamin ve Ürik asitin yükseltgenme davranışları incelenmiştir. Buna göre H_2SO_4 ve fosfat tamponunun analiz için uygun olduğuna karar verilmiştir. Daha sonra H_2SO_4 ortamında pH taraması yapılarak DA ve ÜA' nın aynı derişimine karşılık en yüksek pik akımının elde edildiği pH 2,5 değeri analiz çalışmaları için uygun bulunmuştur. Diğer analiz ortamı olan fosfat tamponu için, kan pH sına uyumlu olması gerekçesiyle, ön çalışmalar yapılmadan doğrudan pH 7,2 değeri kullanılmıştır. pH 2,5 H_2SO_4 ortamında analiz yapılırken DA ve ÜA' nın sırasıyla +0,480 V, +0,620 V' da ki yükseltgenme pikleri kullanılmıştır. ÜA varlığında DA analizi standart ekleme metodu ile yapılırken, DA varlığında ÜA analizi ise kalibrasyon grafiği metodu ile yapılmıştır. Daha sonra diğer bir destek elektrolit ortamı olan fosfat tamponu ile çalışmalara devam edilmiştir ve DA ve ÜA' ya ait sırasıyla +0,140 V, +0,270 V' da ki yükseltgenme

pikleri kullanılmıştır. Bu ortamda ise her iki tür standart ekleme metodu ile tayin edilmiştir. Her iki ortamda da ilk önce [DA] / [ÜA] = 1 iken çalışılmıştır. Daha sonra bu oran ([DA] / [ÜA]) değiştirilerek çalışmalar yapılmış ve pH 2,5 H_2SO_4 ortamında özellikle [DA] / [ÜA] oranının 5 olduğu durumda, çok daha düşük hatalar ile tayinler gerçekleşmiştir. Fosfat tamponu ortamında ise özellikle [ÜA] / [DA] > 1 olduğu durumda çok küçük hatalar ile analiz yapılabilmiştir. Bununla birlikte her iki ortamda da yapılan DA ve ÜA tayinlerinde, her bir oran çalışması için % hata değerleri, kabul edilebilir düzeydedir. Daha sonra, hazırlanan sentetik numune ile çalışılmıştır. Bu sentetik numune için, pH 2,5 H_2SO_4 ortamında DA derişimi 9,76 x 10^{-6} M ve ÜA derişimi 14,63 x 10^{-6} M iken sonuçlar DA ve ÜA için sırasıyla (9,72 ± 0,10) ×10^{-6} M ve (14,67 ± 0,10) × 10^{-6} M olarak bulunmuştur. Bu çalışma için % hata DA tayini için -0,41, ÜA tayini için 0,27 olarak bulunmuştur. pH 7,2 fosfat tamponu ortamında aynı numune için sonuçlar DA ve ÜA için sırasıyla (9,70 ± 0,04) × 10^{-6} M ve (14,61 ± 0,18) × 10^{-6} M olarak bulunmuştur. Bu çalışma için % hata DA tayini için -0,61, ÜA tayini için - 0,14 olarak bulunmuştur. pH 2,5 H_2SO_4 ortamında DA ve ÜA için gözlenebilme sınırı (LOD) sırasıyla 2,16 × 10^{-8} M ve 4,14 × 10-8 M, tayin sınırı (LOQ) ise sırasıyla 6,48 × 10^{-8} M ve 1,24 × 10^{-7} M olarak bulunmuştur. pH 7,2 fosfat tamponu ortamında DA ve ÜA için gözlenebilme sınırı (LOD) sırasıyla 2,28 × 10^{-8} M ve 2,13 × 10^{-8} M, tayin sınırı (LOQ) ise sırasıyla 6,84 × 10^{-8} M ve 6,39 × 10^{-8} M olarak bulunmuştur. Analize girişim yapması muhtemel anyonlar, katyonlar ve askorbik asit için girişim çalışmaları yapılarak, bu türlerin dopamin ve ürik asidin yükseltgenme pikleri üzerine girişime başladığı derişim değerleri belirlenmiştir.