

1-1-2018

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Application of a pencil graphite electrode for voltammetric simultaneous determination of ascorbic acid, norepinephrine, and uric acid in real samples

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Received: 07.08.2017

Accepted/Published Online: 06.12.2017

Final Version: 27.04.2018

Abstract: A pencil graphite electrode (PGE) was used for the simultaneous detection of ascorbic acid (AA), norepinephrine (NE), and uric acid (UA) by differential pulse voltammetry and cyclic voltammetry. The anodic peaks of AA, NE, and UA in their mixture can be well separated in 0.1 M Britton–Robinson buffer solution at pH 4.0. The effects of various experimental parameters such as pH, scan rate, and voltammetric parameters on the voltammetric response of these compounds were investigated. Under optimum conditions, linear calibration graphs were obtained from the AA, NE, and UA concentration ranges, which were 100–800 nM, 20–170 nM, and 40–175 nM, respectively. The detection limits for AA, NE, and UA were 27 nM, 4 nM, and 10 nM in the form of a mixture at the PGE. This electrode shows great analytical performance characteristics, corresponding repeatability and recovery for the simultaneous determination of these compounds. PGE, which was used for the first time in this method, has been successfully applied for the assay of UA in human urine samples with the aim of determining AA and NE in pharmaceutical drugs.

Key words: Pencil graphite electrode, ascorbic acid, norepinephrine, uric acid, simultaneous

1. Introduction

NE (1-(3,4-Dihydroxyphenyl)-2-aminoethanol) is an important derivative of catecholamines secreted in the adrenal medulla, which plays an important physiological role in the central nervous system.¹ Thus, the quantitative determination of NE in biological fluids provides important information on its physiological functions and the diagnosis of some diseases in medicine.²

Ascorbic acid (AA) is found in many biological systems³ and multivitamin preparations that are commonly used as supplements for malnutrition. However, it is widely used as an antioxidant in foods to stabilize color and aroma by extending the shelf life of the products. Thus, determining the AA content is particularly important in the pharmaceutical and food industries. It is generally accepted that the direct oxidation of AA is done by conventional electrodes, which are totally irreversible, and for this reason it requires a high overpotential, which is much higher than its standard redox potential.^{4,5}

Uric acid (UA) is synthesized in mammalian systems, the final product in purine metabolism. Abnormal levels of UA are symptoms of various diseases such as gout, hyperuricemia, and Lesch–Nyhan disease.⁶ Generally, UA can be irreversibly oxidized in aqueous medium because of electroactive properties.^{7,8} As UA and AA are kept together in pharmaceutical drugs and urine, it is important to develop a technique for the simultaneous determination of AA and UA in routine analysis. For this reason, developing fast, simple, and

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complicated methods for the determination of these compounds has been a focal subject in bioscience, biotechnology, medicinal chemistry, especially in neurochemistry,⁹ and several other methods such as chromatographic methods, spectroscopic methods, chemiluminescence, and capillary electrophoresis. Furthermore, the simultaneous determination of AA, UA, and NE is of critical importance not only in the field of biomedical chemistry and neurochemistry, but also for diagnostic and pathological research.¹⁰ In the literature, the simultaneous determinations of these compounds have been mostly carried out using modified electrodes in electrochemical methods.^{11–19} Among these methods in the literature, the most important advantage of electrochemistry is that it is fast and simple. However, there are two basic problems for the electrochemical simultaneous determination of these compounds, i.e. AA, NE, and UA on bare electrodes give a poor electrochemical response and oxidize in almost the same potential region. In order to solve these problems, using different modified electrodes in the literature has become a need.

Through reviewing the literature, no reports have been found on pencil graphite electrodes (PGEs) for the simultaneous determination of these compounds. In electrochemical studies, while working with plain and modified solid electrodes, the fundamental issue is that analytical response size decreases since the oxidation products of some compounds accumulate as a fine film over the electrode. Moreover, it is hard work; it takes too much time and repeatability loss occurs although electrode-cleaning procedures are implemented. Therefore, electrode cleaning is a crucial issue restricting the electrochemical techniques being widely used in analyses. Since the beginning of the late 1990s, the interest in PGEs has significantly increased because making single-use handmade pencil graphite is easy and it costs less than usual.^{20–23}

Similar to the previous studies^{24–32} on the electroanalytical application of pencil leads, this study aims to develop a new, rapid, and highly sensitive electroanalytical method for the simultaneous determination of AA, NE, and UA, a PGE using DPV that is possible to be adopted in both urine and pharmaceutical formulations.

2. Results and discussion

2.1. Electrochemical behaviors of AA, NE, and UA at the PGE

The electrochemical properties of AA (0.1 mM), NE (0.1 mM), and UA (0.1 mM) compounds were examined by CV and DPV techniques at a PGE singly or simultaneously. Figures 1a–1d show the electrochemical response obtained from the surface PGE at different scan rates in 0.1 M Britton–Robinson (BR) buffer (pH 4.0). As shown in Figures 1b–1d, the anodic peak potentials of AA, NE, and UA were observed at about +0.35 V, +0.52 V, and +0.63 V, respectively. Additionally, in the reverse scan, the cathodic peak potential of NE was observed at about +0.39 V distinctively.

The effect of scan rate was investigated in AA, NE, and UA by CV at the PGE under single or simultaneous conditions in Figures 1a–1d. At scan rates greater than 100 mV/s, the anodic peak potentials of the compounds were observed not to be well separated from each other (Figure 1a) while the compounds were simultaneous. It was even observed that these oxidation peaks turned into a single oxidation wave. Thus, the scan rate was increased to 100 mV/s for the simultaneous determinations. As seen in Figures 1b–1d, there is a linear relationship between the scan rate (25–400 mV/s) and the peak current for AA, NE, and UA; $ip (\mu A) = 60.173 v (V/s) + 9.08$, $r = 0.963$, $ip (\mu A) = 75.561 v (V/s) + 8.24$, $r = 0.986$, $ip (\mu A) = 90.848 v (V/s) + 2.84$, $r = 0.952$, respectively. At the same time, in terms of the relationship between the logarithms of the peak current and the scan rate, it can be said that the slope close to 0.5 is also effective in diffusion under the effect of absorption of the electrochemical oxidation reaction of the compounds,³³ AA; $\log ip (\mu A) = 0.483 \log v + 2.345$, $r = 0.892$, NE; $\log ip (\mu A) = 0.513 \log v - 1.78$, $r = 0.892$ and UA; $\log ip (\mu A) = 0.479 \log v + 7.145$,

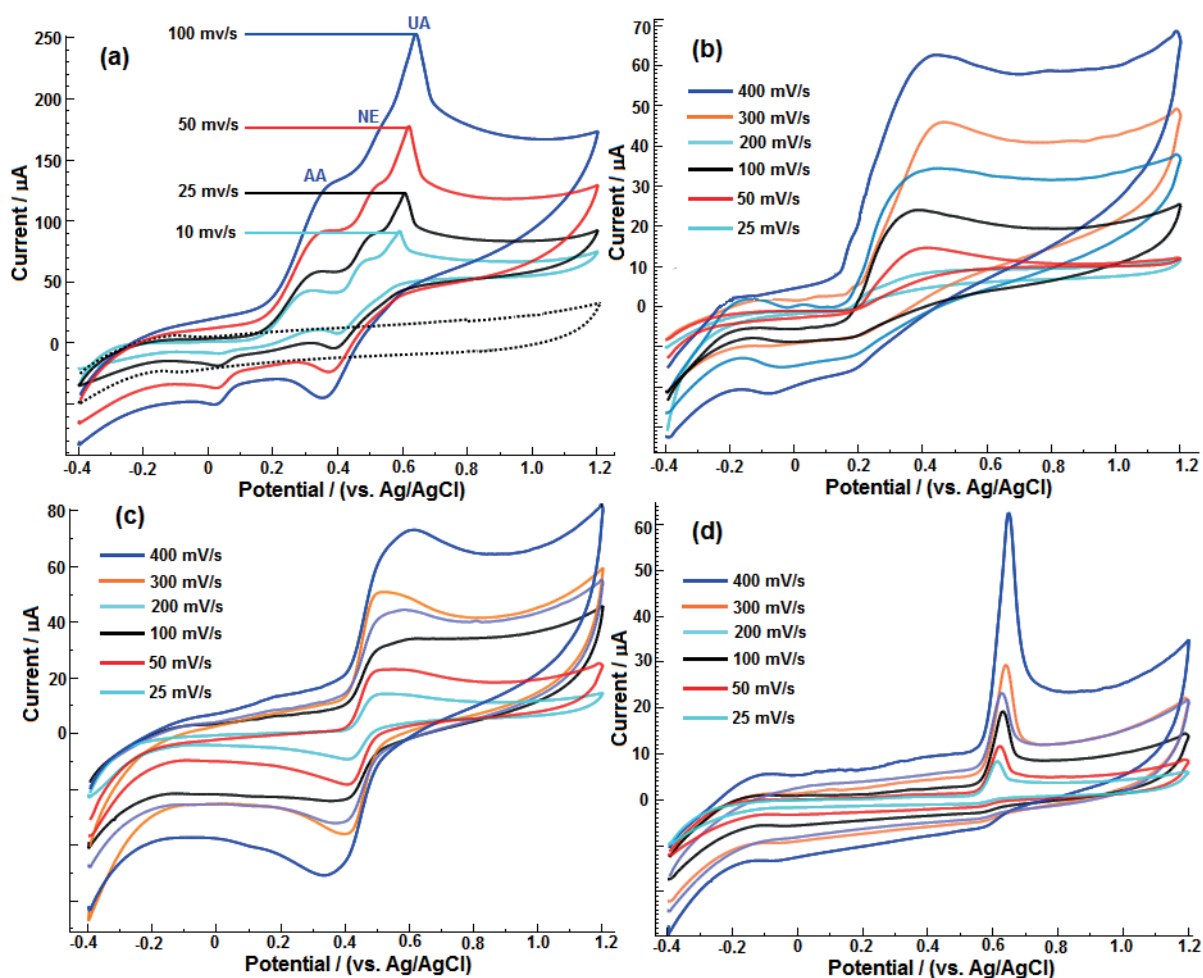


Figure 1. CVs of 0.1 mM AA, NE, and UA at PGE in BR (pH 4.0) at different scan rates. Dashed line represents supporting electrolyte, (a): Simultaneous, (b): AA, (c): NE, and (d): UA.

$r = 0.956$. In addition, the increase in the scan rate (v) in all the compounds shows that the oxidation peak potential of the compounds was shifted positively in Figures 1a–1d. In the irreversible electrochemical processes, the relationship between the oxidation peak potential (E_p) and v is given by $[E_p = E_0 + (2.303 RT/\alpha nF) \log(RT k_0/\alpha nF) + (2.303 RT/\alpha nF) \log v]$.³⁴ In this equation, α and n are the charge transfer coefficients and the number of electrons R , T , and F are known as constants. In this study, the relationship between the E_p and the v is AA: $[E_p (\text{V}) = -0.0601 \log v (\text{V/s}) + 37.05, r = 0.982]$, NE: $[E_p (\text{V}) = -0.0553 \log v (\text{V/s}) + 0.6073, r = 0.991]$, and UA: $[E_p (\text{V}) = -0.0575 \log v (\text{V/s}) + 0.6568, r = 0.899]$. From the calculations using the related relativities, the numbers of the electrons were found to be approximately 1.96, 2.13, and 2.05 for AA, NE, and UA, respectively. The number of protons accompanying the electrochemical oxidation reaction of the compounds, and the change in peak potentials with pH (3–10) are shown in Figures 2a and 2b. The relationship between the oxidation peak potential and pH was calculated as follows: AA: $E_p (\text{V}) = -53.32 \text{ pH} + 0.3705$, NE: $E_p (\text{V}) = -55.44 \text{ pH} + 0.6073$, and UA: $E_p (\text{V}) = -62.12 \text{ pH} + 0.8276$. According to these results, this electrochemical pathway using the PGE contributes to the protons equalized with the contributions of the number of electrons.

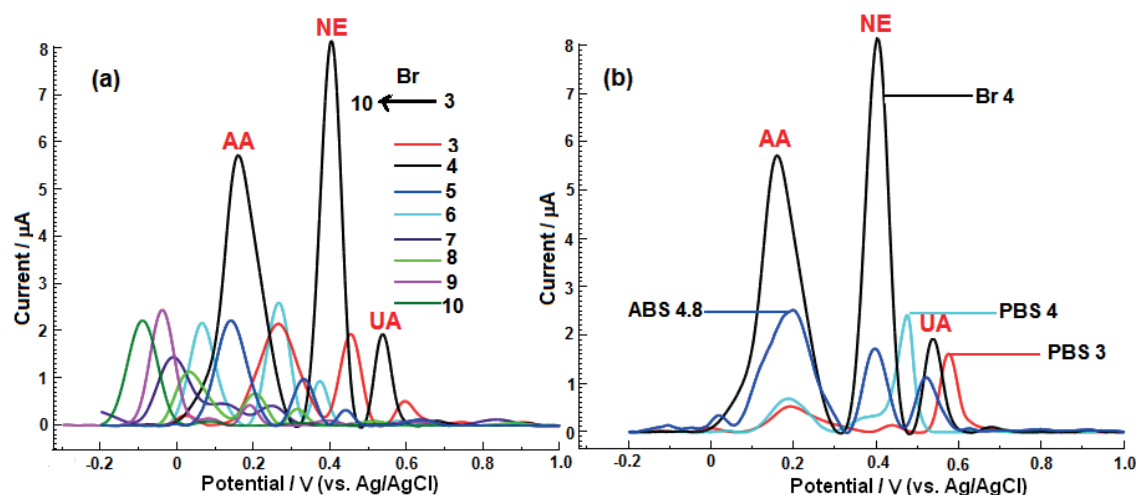


Figure 2. DP voltammograms of $2.5 \mu\text{M}$ AA, $0.75 \mu\text{M}$ NE, and $0.15 \mu\text{M}$ UA solutions in BR (pH 3–10) buffer (a) and in various supporting electrolytes (b) at the PGE. DPV parameters: step potential, 0.003 V; modulation amplitude, 0.07 V; modulation time, 0.02 s; interval time, 0.5 s; scan rate, 0.006 V/s.

2.2. Determination of optimum conditions

In order to perform the electrochemical simultaneous determination of AA, NE and UA for developing the sensitive and selective voltammetric method, well separated and sharper peaks were obtained with the DPV technique. DP voltammograms were recorded in the potential range -0.2 V to $+1.00 \text{ V}$ of the compounds prepared in the appropriate supporting electrolyte solution in order to investigate the effect of supporting electrolyte and pH on the voltammetric behavior of AA, NE, and UA compounds in Figures 2a and 2b. For this purpose, acetate buffer (AB, pH 4.7), phosphate buffer (PB, pH 3.0, 4.0, 7.4, 9.0) and BR (pH 3–10) buffer solutions were used. As seen in Figures 2a and 2b, the oxidation peaks of the compounds are better separated and have higher faradic current in the BR (pH 4.0) buffer when compared with the peaks of the other supporting electrolytes. When the pH is increased, the peak potentials of the compounds shift towards negativeness. In addition, the oxidation peak signal intensity of compounds decreases when it is $\text{pH} > 4.0$. Moreover, the simultaneous determination of these compounds is not possible if the pH is greater than 6.0. Since the signal responses in the voltammetric methods can be changed by the signal parameters of the device and the software used, the faradic peak current can be improved with optimizations of the software parameters in the potentiostat device. For this purpose, the voltammograms of the BR buffer (pH 4.0) containing AA (100 nM), NE (200 nM), and UA (100 nM) were performed, with the step potential 0.001–0.008 V, modulation amplitude 0.01–0.1 V, modulation time 0.01–0.08 s, and interval time 0.1–0.8 s. The best conditions for giving a high faradic current signal and well-marked peaks on the PGE are as follows: Step Pot: 0.03 V, Mod. Amp: 0.07 V, Mod. Time: 0.02 s and Int. Tim: 0.5 s.

2.3. Analytical applications

In studying the analytical performance of the developed voltammetric method using the PGE, the study range, accuracy, sensitivity, and reproducibility conditions of this method were investigated under optimum experimental conditions. With the help of this one, DP voltammograms were recorded at different concentrations; the oxidation peak current and potential effect of AA, NE and NA were studied under the same conditions. In this

method, which was developed for the electrochemical simultaneous determination of AA, NE, and UA using PGE with DPV in BR buffer (pH 4.0), it was seen that the oxidation peak potentials of the compounds were well separated from each other in Figures 3a–3c and 4. When the concentrations of NE (100 nM) and UA (60 nM) were kept constant, the oxidation peak current of AA (100–800 nM) linearly increased (Figure 3a). Similarly, if the concentrations of AA (250 nM) and UA (60 nM) were kept constant and the concentration of NE (20–170 nM) was gradually increased, it is clear that the peak current of NE was linearly increased (Figure 3b). By keeping the concentrations of AA (250 nM) and NE (100 nM) constant, it was seen that the oxidation peak current of the UA also increased linearly when the concentration of UA (40–175 nM) was gradually increased (Figure 3c). In addition, when the concentrations of the three compounds were gradually increased, it was seen that the oxidation peak currents of the compounds linearly increased (Figure 4). When the concentrations versus the current values were placed in the calibration graphs, it was seen that the linearity ranges were very good in calculations from the equations obtained (Table 1).

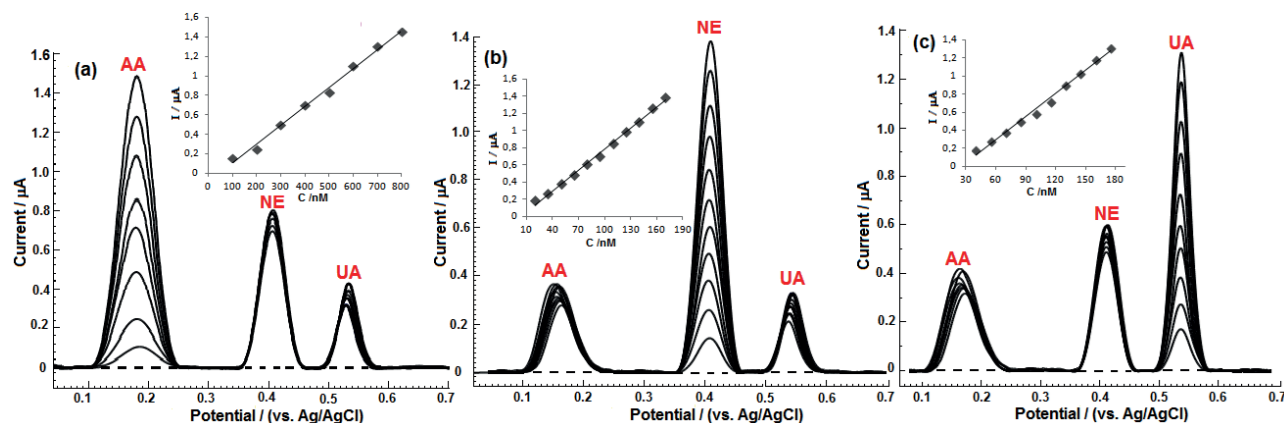


Figure 3. DP voltammograms obtained at different concentrations (a) AA (100, 200, 300, 400, 500, 600, 700, and 800 nM) in the presence of 100 nM NE and 60 nM UA, (b) NE (20, 35, 50, 65, 80, 95, 110, 125, 140, 155, and 170 nM) in the presence of 250 nM AA and 60 nM UA, (c) UA (40, 55, 70, 85, 100, 115, 130, 145, 160, and 175 nM) in the presence of 250 nM AA and 100 nM NE, in BR (pH 4.0) at the PGE. Dashed line represents supporting electrolyte. Insert corresponding calibration curves of AA, NE, and UA, DPV parameters: step potential, 0.001 V; modulation amplitude, 0.04 V; modulation time, 0.03 s; interval time, 0.4 s; scan rate, 0.0025 V/s.

In order to determine the analytical sensitivity of the developed method, the limit of detection (LOD) was calculated according to the equation $3s/m$. In the equation, "s" is the standard deviation of the smallest nine signals that can be read on the baseline of the support electrolyte solution and "m" is the slope of the calibration curve.

As a result of the calculations achieved by evaluating the calibration curve equation, the LOD values were found to be 27 nM, 4 nM, and 10 nM for AA, NE, and UA, respectively. Table 2 shows the comparison results of AA, NE, and UA electrochemical simultaneous determinations with previously reported electrochemical methods. In this study, these LOD levels calculated by the DPV method using the PGE for the electrochemical simultaneous determinations of AA, NE, and UA were much more sensitive than for CCA/GCE,¹¹ EB/GCE,¹² PAA-MWCNTs/SPCE,¹³ GME,¹⁵ Au-AuNPs/DMSA/CA/Au-NPs,¹⁶ EACPE,¹⁷ MCPE,¹⁸ and DSNPs-GCE¹⁹ electrodes but less sensitive than for the p-ATD/GC¹⁴ electrode (Table 2).

The precision of the developed voltammetric method was tested with the relative standard deviation

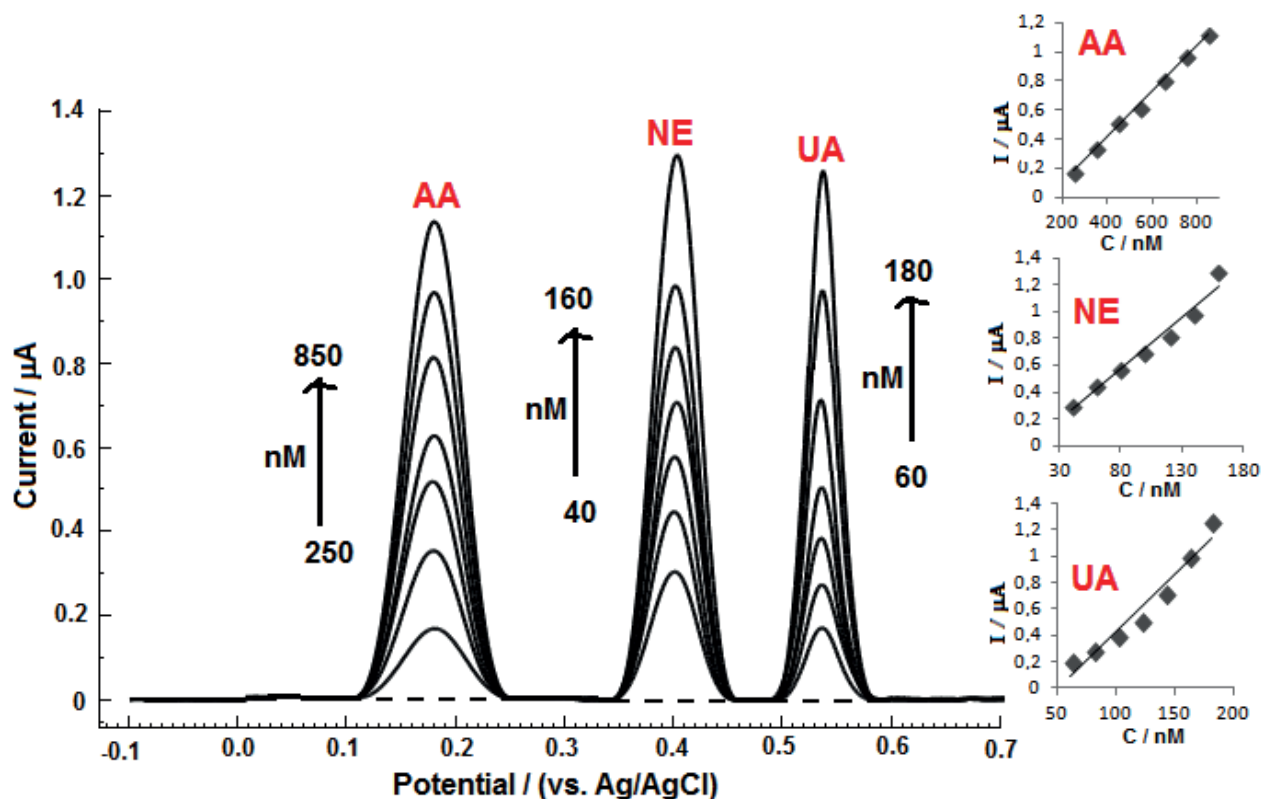


Figure 4. DP voltammograms of PGE in BR (pH 4.0) containing different concentrations of AA + NE + UA in nM from inner to outer: 250 + 40 + 60; 350 + 60 + 80; 450 + 80 + 100; 550 + 100 + 120; 650 + 120 + 140; 750 + 140 + 160; 850 + 160 + 180; respectively. Dashed line represents supporting electrolyte. Inserts: calibration plots of AA, NE, and UA. Other operating conditions as indicated in Figure 3.

(RSD) on day and interday reproducibility values of the oxidation peak current and peak potential. Repeatability results were replicated seven times for the DPV method in optimum conditions in five different solutions on the same day and on different days. The % RSD values of the oxidation peak currents and potentials of AA (250 nM), NE (100 nM) and UA (60 nM) were calculated as 3.36%, 3.22%, and 3.01%, respectively. According to the results obtained, it was seen that the reproducibility of the current and potential values of the oxidation peak was quite good.

Using the PGE, the electrochemical method developed for the simultaneous determination of AA, NE, and UA was successfully applied to urine and drug samples in order to test the accuracy and selectivity. The tolerance limit was taken as the maximum concentration of the interfering substances that caused an approximately $\pm 5\%$ relative error in the simultaneous determination of the three compounds. According to the measured results, it was observed that the Na^+ , K^+ , Ca^{2+} , Ca^{2+} , Zn^{2+} , Mg^{2+} , SO_4^{2-} , and NO_3^- did not interference on the height of the peak currents at peak potential at the PGE for the simultaneous determination of AA, EP, and UA. However, it was observed that folic acid, dopamine, and epinephrine interfered. As shown in Figure 5, simultaneous analyses of compounds in the urine sample can be applied in a highly selective manner. Through the analyses, UA in the urine sample was found in the recovery range of 92%–108% (Table 3). The detection of the Redox and Forefrin ampoules containing AA and NE was calculated as the averages of 94% and 95% recovery, respectively (Table 4).

Table 1. The calibration data of AA, NE and UA obtained by DPV using PG electrode.

Compound	Linear working range (nM)	Linear regression equation	r	LOD (nM)	RSD %***
AA*	100-800	$i_p (\mu A) = 0.0019 C (nM) - 0.0939$	0.991	27	3.7
NE*	20-170	$i_p (\mu A) = 0.0081 C (nM) - 0.0297$	0.997	4	3.5
UA*	40-175	$i_p (\mu A) = 0.0085 C (nM) - 0.2073$	0.994	10	3.4
AA**	250-850	$i_p (\mu A) = 0.0016 C (nM) - 0.2016$	0.998	72	4.1
NE**	40-160	$i_p (\mu A) = 0.0077 C (nM) - 0.0371$	0.991	9	3.8
UA**	60-180	$i_p (\mu A) = 0.0087 C (nM) - 0.4357$	0.989	16	3.7

*Statistical data for the concentration. One of the compounds changed while the other two were kept constant.

**Statistical data for all the compounds simultaneously.

***Results are the average of analyses.

It seems that the data obtained from the recovery studies of AA and NE in pharmaceutical drugs without any extractions or pre-concentration are in harmony with the amounts declared in the pharmaceutical form (Table 4). Therefore, the obtained results are close to real values, indicating that the developed method gives sensitive, accurate, reliable, and stable results. It is demonstrated that the developed method has successfully been applied to drug and urine samples with good recovery rates and % RSD of AA, NE, and UA, which, respectively, are 4.28, 4.04, and 3.8.

3. Conclusion

For the first time, an electrochemical method was proposed for the simultaneous determination of AA, NE, and UA in this work with a PGE. The results obtained with this voltammetric method were compared with the studies on the electrochemical determination of AA, NE, and UA outlined in Table 2. In the previous studies, the methods suggested for the simultaneous determination of these compounds were carried out using the modified electrode. However, the electrodes used in these methods were expensive, the modification procedure and the polishing and cleaning steps before modification were time-consuming, and not every modified electrode was prepared in the same manner, which affected the reproducibility of the results, making these methods disadvantageous. On the other hand, the use of the PGE as an electrode material in order to examine the electrochemical behavior of AA, NE, and UA and to determine these compounds in urine and pharmaceutical formulations as in the present study was advantageous in terms of both providing practicality and low cost and saving time for procedures such as cleaning the electrode surface on solid electrodes, removing the modification process, and cleaning the electrode in the ultrasonic bath. Moreover, this method, which was developed using PGE, gave more sensitive results than the methods used with the modified electrode. Considering the results of the present study, it is suggested that the developed voltammetric method using PGE has successfully been applied to the simultaneous determination of AA, NE and UA. The method is quite sensitive, practical, and

Table 2. Comparison of electrochemical methods in the literature with the DPV method for simultaneous determination of AA, UA, and NE.

Electrode	Comp.	Technique	pH	LOD (nM)	Ref.
CCA/GCE	AA	DPV	6 (PBS)	500	11
	NE			10	
	UA			500	
EB/GCE	AA	DPV	5 (PBS)		12
	NE			35	
	UA				
PAA-MWCNTs/SPCE	AA	DPV	7.5 (PBS)	49,800	13
	NE			131	
	UA			458	
p-ATD/GC	AA	DPV	5 (PBS)		14
	NE			0.17	
	UA				
GME	AA	CV	4 (PBS)	1200	15
	NE			100	
	UA			600	
Au-AuNPs/DMSA/CA/Au-NPs	AA	DPV	7 (PBS)	900,000	16
	NE			33	
	UA			700,000	
EACPE	AA	DPV	7.1 (PBS)	6000	17
	NE			70	
	UA			100	
MCPE	AA	CV	7.4 (PBS)		18
	NE			430	
	UA				
DSNPs-GCE	AA	DPV	7.0 (PBS)		19
	NE			400	
	UA				
PGE	AA	DPV	4.0 (BR)	27	This work
	NE			4	
	UA			10	

GCE: glassy carbon electrode, PGE: pencil graphite electrode, CCA: calconcarboxylic acid, EB: Evans Blue, DSNPs: delphinidin silver nanoparticles, MCPE: poly(glutamic acid) modified carbon paste electrode, PAA-MWCNTs: polyacrylic acid-coated multiwall carbon nanotubes, SPCE: screen printed carbon electrode, p-ATD: 2-amino-1,3,4-thiadiazole, GME: graphene modified glassy carbon electrode, Au-NPs: gold nanoparticles, DMSA: meso-2,3-dimercaptosuccinic acid, CA: cysteamine.

reproducible and it also involves low cost. Moreover, it has been proven to be applicable to the simultaneous determination in pharmaceutical products and urine.

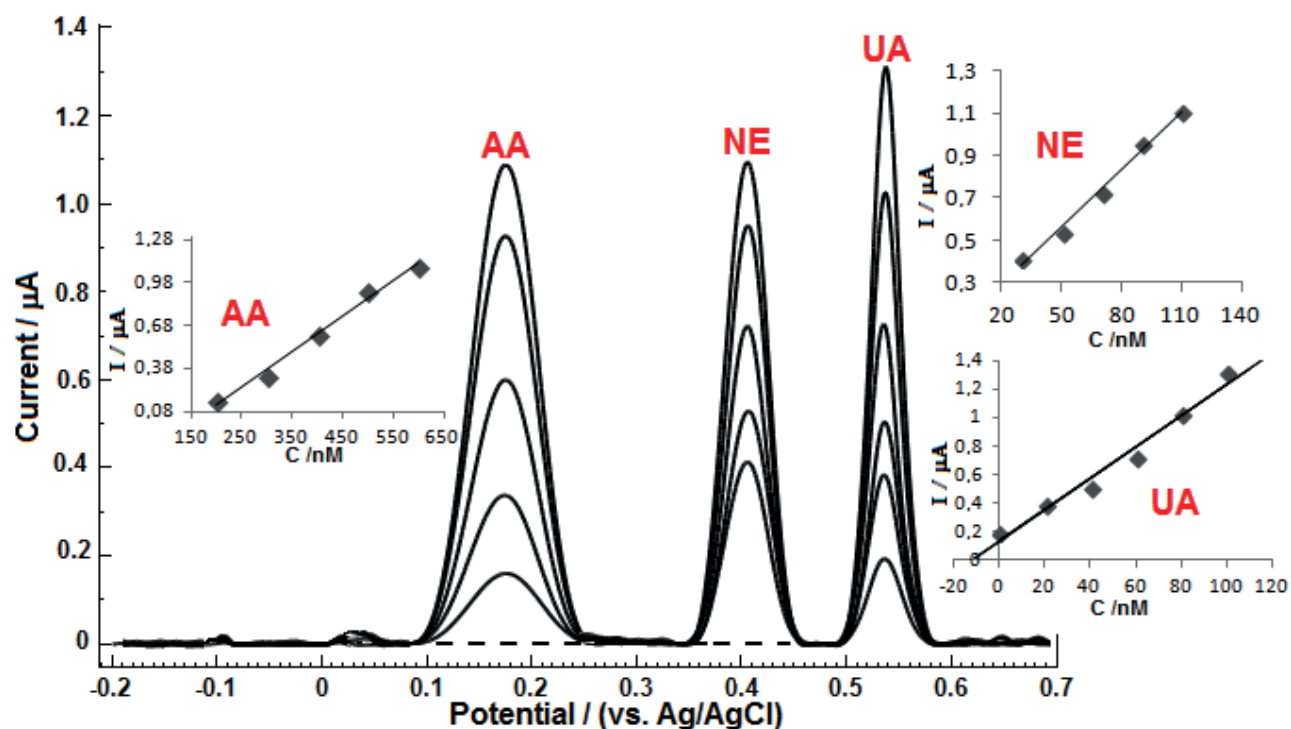


Figure 5. DP voltammograms obtained from the standard addition of AA (200, 300, 400, 500, and 600 nM), NE (30, 50, 70, 90, and 110 nM) and UA (0, 20, 40, 60, 80, and 100 nM) in male urine sample using PG electrode in BR (pH 4.0). Inserts: calibration plots of AA, NE, and UA. Dashed lines represent blank male urine. Other operating conditions as indicated in Figure 3.

4. Experimental

4.1. Apparatus

CV and DPV were taken using an Autolab PGSTAT 128N potentiostat (EcoChemie, the Netherlands). The bare DPVs were improved by using baseline correction of the Nova software, followed by the polynomial fixed order (polynomial order = 10) with snap to data. A conventional three-electrode electrochemical system was used for all electrochemical experiments consisting of a working electrode (PGE), a platinum wire counter electrode, and Ag/AgCl ($3.0 \text{ mol L}^{-1} \text{ KCl}$) as the reference electrode. In the experiments, a Rotring T 0.5 (Germany) mechanical pencil was used. Electric conductivity was provided with copper wire. About 10 mm of the pencil tip were immersed into 10 mL of analysis solution in an electrochemical cell. The electrochemical pretreatment of PG was exercised anodically at +1.40 V for 60 s in electrochemical studies to introduce the oxygen-containing functional groups on the electrode surface and to increase the effective surface area of the electrode by the oxidation of the graphite layer.³⁵

4.2. Reagents

All the reagents including UA, AA, and NE were purchased from Sigma–Aldrich/Merck. Stock solutions of NE and AA (1 mM) were prepared daily by dissolving appropriate amounts in water in a 10-mL volumetric flask. UA solution (1 mM) was prepared by dissolving the solid in a small volume of 0.1 M NaOH solution and diluted

Table 3. Results of the recovery analysis of AA, NE, and UA in male urine sample by DPV using the PGE.

Target	Added (nM)	Found (nM)*	Recovery (%) \pm RSD (%)
AA	0	ND**	
NE	0	ND	
UA	0	12.05	0 \pm 3.4
AA	200	210	105 \pm 4.1
NE	30	28.8	96 \pm 4.3
UA	20	33.65	108 \pm 3.8
AA	300	327	109 \pm 3.9
NE	50	46	92 \pm 3.5
UA	40	54.45	106 \pm 4.1
AA	400	388	97 \pm 3.8
NE	70	72.1	103 \pm 4.0
UA	60	67.85	93 \pm 4.2
AA	500	530	106 \pm 5.1
NE	90	94.5	105 \pm 3.5
UA	80	85.65	92 \pm 3.6
AA	600	618	103 \pm 4.5
NE	110	114.4	104 \pm 4.9
UA	100	115.05	103 \pm 3.3

*The values obtained are the average of three independent analysis of each spiked sample.

**ND: not detected.

Table 4. AA and NE content in pharmaceutical drugs by DPV using the PGE.

Sample	Target	Detected (mg/mL)	Recovery (%) \pm RSD (%)*
Drug ¹	AA	470	94 \pm 4.2
Drug ²	NE	3.8	95 \pm 3.8

*The values obtained are the average of five analyses

¹Redox-C, Bayer pharmaceutical Co. Ltd., Turkey, 500 mg 5 m/L ampoule

²Forefrin, Farma-Tek pharmaceutical Co. Ltd., Turkey, 4 mg 4 m/L ampoule

to the desired concentration. Three different supporting electrolytes were used in this work: AB (0.1 M, pH 4.8), BR buffer (0.1 M, pH 3–10), and PB (0.1 M, pH 3.0 and 4.0). Other working solutions were prepared by diluting in buffer solutions. All solutions were stored at +4 °C when not in use and protected from daylight during use. Aqueous solutions were made up with deionized water and next purified via Milli-Q unit.

4.3. Electrochemical procedure

At first, variables that influence the performance of the first working electrode such as the selection of the supporting electrolyte and voltammetric methods, ionic strength and pH, composition of the measurement as well accumulation potential and accumulation time and voltammetric waveform were investigated comprehensively through DPV.

Before the analyses began, the PGE was activated as described in section 4.1 in the electrochemical analysis at all stages of the study. In the measurements of this analysis, the electrochemical properties of AA, NE, and UA compounds were determined with CV (-0.4 V to +1.2 V) and DPV (-0.2 V to +1.0 V). The DPV parameters were as follows: step potential, 0.001 V; modulation amplitude, 0.04 V; modulation time, 0.03 s; interval time, 0.4 s; scan rate, 0.0025 V/s. Each measurement was performed using a new pencil surface in a 10-mL voltammetric cell, at a laboratory temperature (20 ± 5 °C).

4.4. Real sample preparation

Urine samples were obtained from a healthy male volunteer aged about 30 years; 5 mL of sample was integrated with 5 mL of acetonitrile. After the urine sample was mixed, it was centrifuged at 5000 rpm for 10 min. Pharmaceutical formulations were obtained from Turkish pharmacies. The samples that were ready for analyses were diluted with 10 mL of support. The analyses in real samples were performed using the standard addition method.

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